This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 952152.

Emerging Social Issues on Targeted Drug Delivery

Prof. Aristidis M. Tsatsakis, Dr. Maria Vliora,
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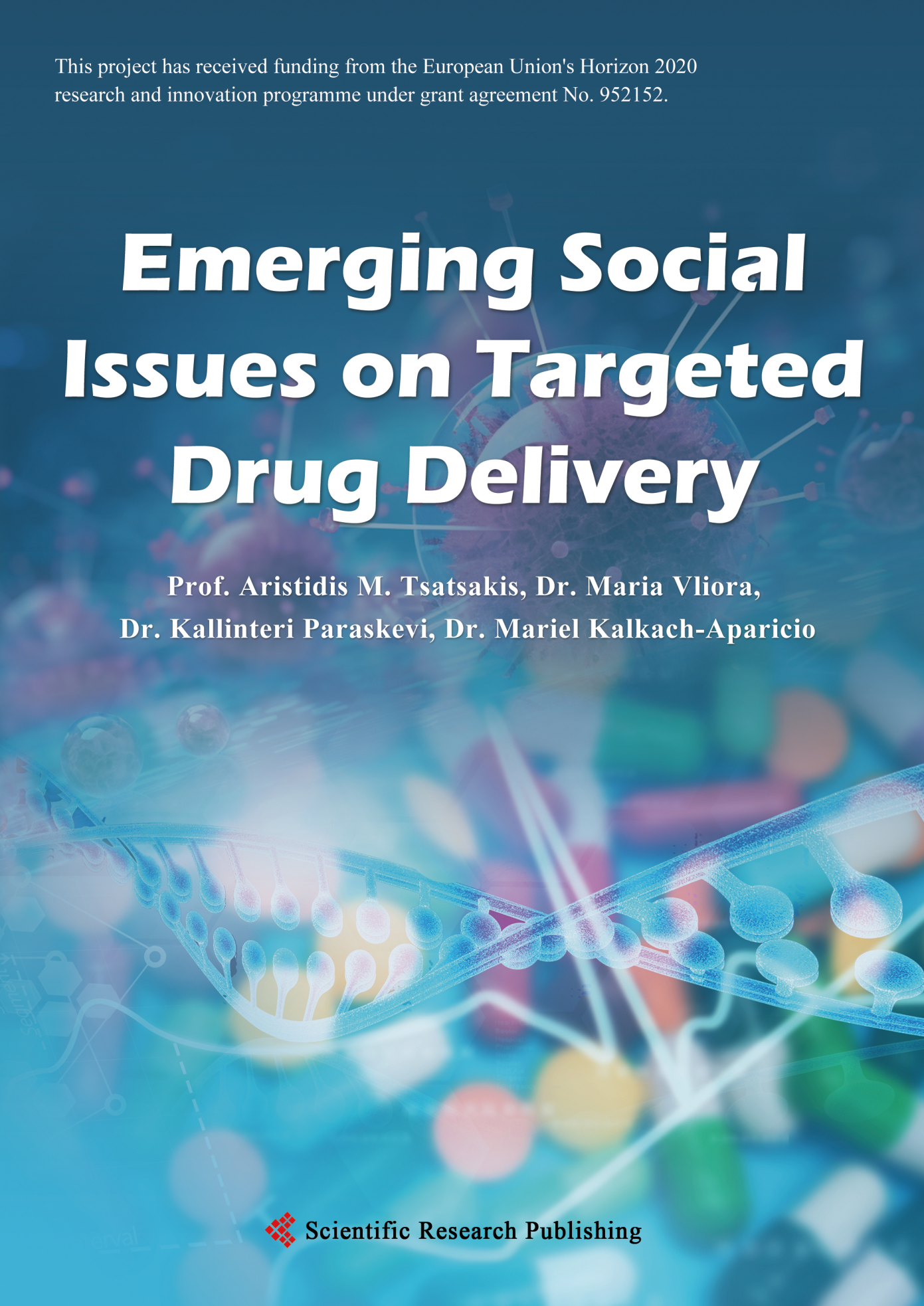

Scientific Research Publishing

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Published by
Scientific Research Publishing, Inc.
ISBN: 979-8-89507-901-0
Word Count: 341,000 words
Publication Date: Dec. 31, 2024
http://www.scirp.org

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This book will be co-edited by Professor Aristides Tsatsakis, Dr Maria Vliora. Dr. P Kallinteri, and Dr. M. Kalkach.

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Dr. P. Kallinteri is a pharmacist with a 13-year academic career in Nanotechnology. She studied Pharmacy at the University of Patras (Greece). She also did an MSc in Industrial Pharmaceutics and a PhD on liposome targeting to hepatocytes using asialofetuin conjugates as targeting moieties at the same university. Then, she moved to the UK, where she worked initially as a researcher at the University of Nottingham in a project related to nanoparticles made of novel polymers for drug delivery to brain tumors. In 2006, she was appointed as a lecturer at the University of Kent and her main research was focused on arsonoliposome delivery to

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Contents

Chapter 1
Emerging Social Issues on Targeted Drug Delivery

Maria Vliora, Paraskevi Kallinteri, Mariel Kalkach Aparicio, Aristides M. Tsatsakis

The world of targeted drug delivery (TDD) represents an incredible leap forward in how we treat and manage diseases [1]. It's a frontier where science converges with hope, precision, and, in many ways, a reimagining of healthcare itself. As we move closer to realizing a future where therapies can target specific cells or tissues with minimal side effects, the excitement is tempered by a growing recognition of the technical, ethical, and societal challenges that come with this revolutionary shift in medical practice [2]. In this drawing from the insights of researchers and thinkers who are deeply engaged in navigating this transformative field. Together, the following chapters, illustrate how the evolving landscape of TDD holds both immense promise and significant complexity.

At the heart of many of these advances lies nanomedicine, which has opened up new pathways for treating diseases, particularly cancer [3]. In Chapter 2, Catalin Zaharia and Ionut-Cristian Radu present a compelling argument for the use of nanoparticles in drug delivery, especially in oncological settings. Nanoparticles, tiny carriers that can be engineered to navigate the body's complex environments, offer a level of precision that was once the stuff of science fiction. These particles can be functionalized with targeting moieties—biological or chemical structures that allow them to "home in" on cancer cells, leaving healthy tissue unharmed. Traditional cancer therapies, such as chemotherapy, though effective in destroying cancer cells, often cause significant damage to normal cells. This leads to debilitating side effects for patients, most commonly nausea, fatigue, anemia, hair and skin problems, and immune suppression, which can diminish quality of life and limit treatment options.

The beauty of nanoparticle-based delivery systems lies in their ability to

mitigate these side effects by delivering chemotherapy agents directly to the tumor site [4]. Nanoparticles can be designed to recognize markers on the surface of cancer cells, ensuring that they release their drug payload only when they reach their intended target. However, as Zaharia and Radu point out, while this technology holds enormous potential, its implementation is fraught with challenges. One of the primary difficulties is scalability. Manufacturing nanoparticles consistently and at a commercial scale while maintaining precise control over their size, shape, and surface chemistry is a complex process. Any variability in these parameters can affect the behavior of the nanoparticles in the body, leading to inconsistent results or, worse, unintended side effects.

Another challenge is the long-term safety of nanoparticles. While they are often designed to degrade within the body over time, the breakdown products of these particles may interact with the body in unpredictable ways [5]. The body's immune system might also react to the nanoparticles themselves, treating them as foreign invaders and mounting an immune response that could undermine their effectiveness. Zaharia and Radu emphasize that more research is needed to understand these interactions and to ensure that nanoparticles are safe not only in the short term but also over the long haul. The regulatory landscape for nanomedicine is still developing, and the introduction of these new technologies requires a rethinking of how we evaluate safety and efficacy in drug delivery systems.

Societal implications are also at the forefront of Souhaila El Moukhtari's and Maria Blanco-Prieto examination of nanomedicine's broader impact in Chapter 3. While nanotechnology offers unprecedented opportunities to improve healthcare, it also presents significant risks, particularly in terms of healthcare access [6]. Blanco-Prieto and El Moukhtari argue that the high costs associated with the development and commercialization of TDD systems could exacerbate existing inequalities in healthcare. As these technologies become more integrated into treatment protocols, there is a danger that they will be available only to those who can afford them, leaving underserved populations without access to potentially life-saving therapies. This could create a two-tiered system of healthcare, where the rich benefit from the latest advancements while others are left behind.

Moreover, nanotechnology presents environmental challenges that are often overlooked in discussions about its benefits [7]. The production of nanoparticles

typically involves the use of rare or hazardous materials, and their disposal raises concerns about environmental contamination. Nanoparticles are small enough to bypass many of the body's natural defense mechanisms, and there is a growing concern that they could have similar effects in the environment, accumulating in ecosystems and potentially affecting the food chain. For instance, if nanoparticles are released into water systems, they could be ingested by aquatic organisms, leading to bioaccumulation and potentially impacting human health [8]. El Moukhtari and Blanco-Prieto emphasize the need for sustainable practices in nanotechnology development, advocating for green manufacturing processes and careful consideration of the environmental impact of these innovations.

Ethical considerations extend far beyond cost and environmental concerns. Andreea-Iulia Someşan and Ion Copoeru provide a nuanced exploration of the ethical landscape surrounding wireless drug delivery systems in Chapter 4. These systems, which allow for remote monitoring and control of drug release, offer an unprecedented level of personalization in treatment. Imagine a patient whose medication dosage can be adjusted in real-time, based on changes in their body's response to treatment, without the need for frequent clinic visits. For many, this could improve quality of life and reduce the burden of managing chronic illnesses. However, as Someşan and Copoeru point out, these advances come with significant ethical challenges, particularly in terms of data privacy and security.

Wireless drug delivery systems rely on the collection and transmission of vast amounts of personal health data. This data, if not adequately protected, could be vulnerable to hacking or unauthorized access, leading to breaches of patient privacy [9]. Furthermore, patients may not fully understand the risks associated with these technologies, particularly the implications of sharing sensitive health information. Informed consent, a cornerstone of ethical medical practice, becomes more complicated in this context. Patients must be provided with clear, accessible information about the potential risks and benefits of these technologies so they can make informed decisions about their care [10]. Someşan and Copoeru advocate for a transdisciplinary approach to nanoethics, one that involves collaboration between bioethicists, technologists, and legal experts to develop guidelines that protect patient rights while allowing for the responsible use of wireless technologies.

João Leitão, Dina Pereira, and Ana Cristovão expand on this discussion in Chapter 5, by examining the challenges of drug delivery to the brain in patients with cognitive impairments. Individuals suffering from neurodegenerative diseases such as Alzheimer's may not have the capacity to provide informed consent, complicating the ethical landscape of treatment [11]. The dynamics of caregiver involvement become crucial in these scenarios, where family members or legal guardians often play a key role in decision-making. Leitão and his colleagues emphasize the necessity of involving caregivers in the consent process, ensuring that they understand the treatment options and the potential benefits and risks involved. This approach not only respects the autonomy of the patient but also recognizes the vital role that family members play in supporting individuals with cognitive challenges.

Moreover, the authors highlight the importance of developing clear guidelines and protocols that can aid healthcare providers in navigating these complex ethical waters. As neuro-nanotechnology becomes more mainstream in clinical settings, the healthcare community must be equipped with strategies to handle consent and patient involvement effectively. This may involve training for healthcare professionals to recognize when a patient may lack the capacity to consent and how to facilitate discussions with caregivers. Leitão and his colleagues advocate for a collaborative approach, where researchers, clinicians, and ethicists work together to create frameworks that prioritize patient rights and welfare.

As we delve deeper into the specific applications of TDD in cancer treatment, Chandraiah Godugu and his team shed light on the role of tumor-penetrating peptides, such as the iRGD peptide, in enhancing drug delivery effectiveness. Traditional chemotherapy often faces challenges with drug penetration, as solid tumors can create dense environments that obstruct therapeutic agents from reaching their intended targets [12]. Godugu *et al.* discuss how tumor-penetrating peptides can facilitate the movement of drugs into the tumor microenvironment, ensuring more efficient delivery of anticancer agents.

The authors underscore in Chapter 6 the importance of understanding the unique biology of tumors, particularly how the tumor microenvironment can influence drug delivery. The complexity of tumors, which often includes a heterogeneous population of cells and a unique extracellular matrix, can lead to uneven

distribution of chemotherapy agents. By leveraging peptides that can bind to receptors overexpressed in tumors, researchers are developing methods to enhance drug uptake and retention within the tumor [13]. This advancement could significantly improve treatment efficacy and reduce the doses required, thereby minimizing systemic side effects.

However, as with any emerging technology, the introduction of peptide-based delivery systems raises new safety concerns. Godugu and his colleagues note that the body's immune system may recognize these peptides as foreign entities, potentially leading to adverse reactions. The authors argue that public perception can serve as a barrier to the acceptance of these new therapies; patients may feel uneasy about the idea of engineered peptides being introduced into their bodies. To overcome these challenges, they stress the importance of transparent communication and public education campaigns. These initiatives should aim to provide clear information about the safety and benefits of peptide-based therapies, as well as the rigorous testing that these treatments undergo before reaching patients. By fostering a better understanding of the science behind these innovations, healthcare providers can build trust and confidence among patients.

The issue of sex differences in drug response is also pivotal, as highlighted in Chapter 7 by Ashique Sumel in his examination of lung cancer treatment. Research has increasingly shown that men and women can experience different disease progressions, treatment responses, and drug toxicities. For example, studies have indicated that women with lung cancer often present with distinct molecular characteristics compared to men, which can influence their response to certain therapies [14] [15]. Sumel emphasizes the need for a more personalized approach to TDD that considers these sex-specific differences.

Understanding the biological and hormonal differences between sexes can significantly enhance treatment efficacy. For instance, variations in metabolism and the pharmacokinetics of drugs can result in differential responses to chemotherapy agents. Asique advocates for a model of TDD that incorporates sex as a critical variable, thereby allowing for the development of tailored treatment protocols that optimize outcomes for both men and women. By ensuring that clinical trials account for sex differences, researchers can generate more representative data, leading to more effective treatment strategies that cater to the unique needs of

diverse patient populations.

In tandem with these considerations, Dimitris Pnevmatikos and his colleagues discuss the importance of promoting nano-literacy across educational levels in Chapter 8. As nanotechnology becomes increasingly prevalent in medical and consumer applications, fostering a better understanding of these technologies among students and the general public is essential. Pnevmatikos argues that integrating nanoscience concepts into science curricula can help equip future generations with the knowledge and skills necessary to engage with these innovations critically.

Nano-literacy goes beyond simple understanding; it empowers individuals to make informed decisions about their health and engage in meaningful discussions about the implications of nanotechnology in society. By cultivating a scientifically literate public, we can foster a culture of inquiry that encourages dialogue about the ethical, social, and health-related challenges posed by emerging technologies [16]. Education should also address the potential risks associated with nanotechnology, ensuring that students are aware of the benefits and limitations of these innovations.

The COVID-19 pandemic has provided a unique lens through which to view the role of computational modeling and simulation in drug development [17]. In Chapter 9, Romina Fucà and João Leitão reflect on the impact of predictive simulations during the crisis, particularly in the rapid development of mRNA vaccines. The ability to model viral behavior and vaccine responses has allowed researchers to optimize vaccine formulations and streamline the clinical trial process. This not only reduced the time required for vaccine development but also minimized the reliance on traditional animal testing methods.

However, as Fucà and Leitão note, the integration of computational methods into drug development is not without challenges. While simulations can provide valuable insights, they must be validated against empirical data to ensure accuracy. The authors emphasize the need for a balanced approach that combines computational modeling with rigorous in-vivo testing to verify safety and efficacy. The lessons learned from the pandemic have highlighted the importance of flexibility in research and development processes, as well as the need for robust public

health infrastructure to support the rapid deployment of new therapies.

Looking ahead, in the last chapter, Vittorio Bava explores how wireless nanomedical devices can facilitate continuous monitoring and controlled drug release, offering patients unprecedented levels of personalization in their treatment. In his exploration of the future of wireless nanomedical devices, Vittorio Bava delves into the entrepreneurial landscape and the burgeoning market opportunities surrounding targeted drug delivery. The convergence of nanotechnology, biotechnology, and digital health has paved the way for a new generation of TDD solutions, offering precision and personalization that were previously unimaginable. For entrepreneurs, this emerging market represents a fertile ground for innovation, where the demand for advanced, effective, and patient-centered therapies is steadily growing. Bava highlights that the development of wireless nanomedical devices—capable of continuous monitoring and controlled drug release—provides an exciting prospect for startups and established companies alike. These devices offer not only enhanced patient outcomes but also the potential to streamline healthcare delivery, reduce costs, and transform how chronic diseases are managed. As healthcare shifts toward more personalized and remote treatment options, the demand for such devices is poised to rise, creating a unique window of opportunity for those entering the field.

Bava emphasizes the importance of adaptability and foresight for entrepreneurs who wish to succeed in this dynamic sector. The TDD market is characterized by rapid technological advancements, and companies must be able to respond swiftly to regulatory changes, evolving patient needs, and emerging scientific discoveries. Entrepreneurs must also navigate complex challenges, including securing funding for research and development, building partnerships with healthcare providers, and complying with stringent regulatory standards. However, the potential rewards are substantial. The global market for TDD is expected to grow significantly in the coming years, driven by increased demand for innovative treatments for diseases such as cancer, neurodegenerative disorders, and cardiovascular conditions [18]. Bava notes that companies capable of developing effective, safe, and scalable solutions will not only capture significant market share but also contribute to shaping the future of healthcare. By embracing a forward-thinking approach and fostering collaborations across disciplines, entrepreneurs

can capitalize on this opportunity to bring cutting-edge TDD technologies to market, ultimately improving patient outcomes and redefining the therapeutic landscape.

Addressing the aforementioned issues requires a collective effort—one that involves researchers, clinicians, ethicists, educators, entrepreneurs, and policymakers working in concert to navigate the complexities of TDD. By fostering open dialogue and collaboration, we can ensure that the benefits of these technologies are harnessed responsibly and equitably. It is crucial to establish clear regulatory frameworks and ethical guidelines that prioritize patient education, rights, safety, and well-being.

Moreover, we must remain vigilant in our commitment to public education and engagement. As nanotechnology and targeted drug delivery systems become increasingly integrated into healthcare, equipping the public with knowledge and understanding will be essential to fostering trust and acceptance. By promoting nano-literacy and encouraging informed discussions, we can empower individuals to navigate the complexities of emerging technologies and make choices that align with their values and needs.

Ultimately, the future of targeted drug delivery is bright, filled with promise and opportunity. Yet, it is our collective responsibility to ensure that this future is shaped by principles of ethics, equity, and compassion. By addressing the emerging challenges head-on and embracing a holistic approach to innovation, we can unlock the full potential of TDD, paving the way for a healthier, more equitable world for all.

In summary, the unfolding narrative of targeted drug delivery invites us to reflect on the intersection of science, ethics, and society. As we venture further into this uncharted territory, let us do so with a commitment to integrity, responsibility, and a shared vision for a future where the benefits of medical innovation are accessible to all.

References

[1] Tewabe, A., *et al.*, *Targeted Drug Delivery - From Magic Bullet to Nanomedicine: Principles, Challenges, and Future Perspectives.* J Multidiscip Healthc,

2021. **14**: p. 1711-1724.

[2] Gerke, S., T. Minssen, and G. Cohen, *Ethical and legal challenges of artificial intelligence-driven healthcare*. Artificial Intelligence in Healthcare. 2020: 295-336. doi: 10.1016/B978-0-12-818438-7.00012-5. Epub 2020 Jun 26.

[3] Xu, M., *et al.*, *Cancer Nanomedicine: Emerging Strategies and Therapeutic Potentials.* Molecules, 2023. **28**(13).

[4] Elumalai, K., S. Srinivasan, and A. Shanmugam, *Review of the efficacy of nanoparticle-based drug delivery systems for cancer treatment.* Biomedical Technology, 2024. **5**: p. 109-122.

[5] Domb, A.J., *et al.*, *Safety Evaluation of Nanotechnology Products.* Pharmaceutics, 2021. **13**(10).

[6] Malik, S., K. Muhammad, and Y. Waheed, *Emerging Applications of Nanotechnology in Healthcare and Medicine.* Molecules, 2023. **28**(18).

[7] Forest, V., *Combined effects of nanoparticles and other environmental contaminants on human health - an issue often overlooked.* NanoImpact, 2021. **23**: p. 100344.

[8] Xuan, L., *et al.*, *Nanoparticles-induced potential toxicity on human health: Applications, toxicity mechanisms, and evaluation models.* MedComm (2020), 2023. **4**(4): p. e327.

[9] Cartwright, A.J., *The elephant in the room: cybersecurity in healthcare.* J Clin Monit Comput, 2023. **37**(5): p. 1123-1132.

[10] van der Boon, R.M.A., *et al.*, *Risks and benefits of sharing patient information on social media: a digital dilemma.* Eur Heart J Digit Health, 2024. **5**(3): p. 199-207.

[11] Shabani, L., *et al.*, *Neuro-nanotechnology: diagnostic and therapeutic nano-based strategies in applied neuroscience.* Biomed Eng Online, 2023. **22**(1): p. 1.

[12] Chehelgerdi, M., *et al.*, *Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation.* Mol Cancer, 2023. **22**(1): p. 169.

[13] Saggar, J.K., *et al.*, *The tumor microenvironment and strategies to improve*

drug distribution. Front Oncol, 2013. **3**: p. 154.

[14] May, L., *et al.*, *Sex Differences in Lung Cancer.* Cancers (Basel), 2023. **15**(12).

[15] Rakshith, H.T., *et al.*, *Sex differences in drug effects and/or toxicity in oncology.* Curr Res Pharmacol Drug Discov, 2023. **4**: p. 100152.

[16] Arabeyyat, Z.H., M.M. Jamaliah, and M.A. Khalaf, *Public Awareness of Nanotechnology and Its Implications for Health in Jordan.* Sustainability, 2022. **14**(10): p. 5786.

[17] Sharma, P.P., *et al.*, *Computational methods directed towards drug repurposing for COVID-19: advantages and limitations.* RSC Adv, 2021. **11**(57): p. 36181-36198.

[18] Palanisamy, C.P., *et al.*, *New strategies of neurodegenerative disease treatment with extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs).* Theranostics, 2023. **13**(12): p. 4138-4165.

Chapter 2

Nanomedicine and Nanoparticle Drug Delivery Systems. Recent Strategies and Future Implications in Cancer Management

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2.1. Introduction

Nanotechnology has catalyzed the emergence of innovative solutions and products across diverse fields by amplifying material properties and enabling manipulation at the nano-level. From revolutionizing the electronics sector to discovering exciting applications in agriculture, food, textiles, defense, cosmetics, and environmental industries, its contributions are remarkable.

In medicine, nanotechnology has introduced the concept of nanomedicine, which focuses on the manipulation and manufacturing of materials and devices with at least one dimension in the 1 - 100 nanometer range. The ultimate goal is to enhance various aspects of disease prevention, imaging, diagnosis, monitoring, treatment, and regeneration. Nanomedicine has emerged as a rapidly growing field of study, offering promising opportunities for advancing clinical investigation and improving healthcare by providing novel approaches to disease treatment.

A significant amount of research in the biomedical field is currently directed towards nanomedicine, encompassing a wide range of areas. Novel nano-sized materials and devices, which form the cornerstone of nanomedicine advancements, are being applied across various levels of medical intervention. Particularly in the realms of imaging and diagnosis, nanomedicine is making significant progress. Early detection is recognized as crucial in preventing the progression of

numerous diseases and maximizing available treatment options. In this realm, numerous nano-based materials have been developed as remarkable imaging tools, including magnetic resonance imaging (MRI) contrast agents [1], luminescent nanoparticles [2], carbon-based nanomaterials [3], and fluorescent nanomaterials for bioimaging [4]. Combining diagnostic and therapeutic approaches is made possible with theranostic nanoparticles, representing another exciting application of nanomedicine with great potential, particularly in cancer treatment. Nanotheranostics plays a pivotal role in predictive patient stratification by assessing the accumulation of nanomaterials at the diseased site. It provides real-time monitoring and outcome prediction while simultaneously enhancing imaging precision and targeted therapeutic delivery [5].

An additional avenue of research lies in the fields of tissue engineering and regenerative medicine. Here, nanomaterials hold potential for facilitating heightened levels of specific interactions at the cellular level, leading to more efficient formation of new tissue. Nanomaterials can be utilized to functionalize scaffolds, enhancing their properties, and providing multifunctionality. Additionally, they can serve as carriers for targeted release and delivery of specific growth factors, therapeutic agents, or genes to damaged tissues [6] [7].

Nanomedicine is also advancing gene therapy and vaccine preparation, with nanomaterials finding application in immunization and vaccine development. The concept of nanovaccines has been introduced to enhance targeted delivery and antigen presentation, thereby reducing the occurrence of side effects post-vaccination. Moreover, nanovaccines stimulate the body's innate immunity, require smaller volumes and fewer doses, and elicit a robust T cell response, all while maintaining safety standards to effectively combat infectious diseases [8] [9].

The use of nanomedicine in treating major diseases stands as one of the prominent applications of nanotechnology in the medical realm, with nano-sized drug delivery systems serving as a cornerstone in this regard. Broadly, a drug delivery system can be defined as a formulation or device functioning as a carrier, facilitating the introduction of therapeutic substances or drugs into the body. Such a system could be engineered to release the substance of interest in a controlled manner, managing the rate, timing, and specific site of release. The notion of drug delivery systems emerged as a potential solution to enhance patient compliance,

mitigate fluctuations in drug concentration within the body, reduce the risk of adverse effects, and decrease the frequency of dosing, thereby overcoming the limitations associated with conventional administration systems [10].

Nano-sized drug delivery systems represent a substantial leap forward in drug delivery technology, standing as a pivotal innovation in the pharmaceutical sector. Often termed as nanocarriers, these nano-based drug delivery systems boast enhanced solubility of encapsulated drugs owing to their minute size and expansive surface area. This advancement leads to heightened bioavailability, enabling greater deposition at designated sites within the body. Moreover, such systems hold promise in enhancing drug stability, facilitating transportation across biological barriers, augmenting penetration and retention in solid tumors, and prolonging circulation times. Ultimately, these advancements contribute to increased safety and efficacy in drug delivery [11]-[13].

There are drug delivery systems which have from either the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA) for treating various forms of cancer, schizophrenia, iron deficiency, hepatitis, and infections, among others. Additionally, many nano drug delivery systems are currently undergoing clinical phase trials for a wide array of diseases. While researchers continue to explore nanoparticle applications for diseases such as cardiovascular disease, diabetes, infectious diseases, and neurodegenerative disorders, cancer research presently holds a dominant position in the field. In fact, over half of all nanoparticle-based clinical trials are focused on cancer treatment [14]-[16].

This book chapter presents the most important types of nanoparticle drug delivery systems and their implications in cancer management. It explores the mechanisms of action, the advantages over traditional delivery methods, and the latest advancements in nanoparticle technology. Additionally, the chapter discusses the challenges and prospects of using nanoparticles in oncology, including their role in personalized medicine and potential for reducing side effects.

2.2. Nanoparticle Drug Delivery Systems

The primary components of nanosized drug delivery systems are nanoparticles. Nanoparticles have emerged as a highly promising approach for delivering pharmaceutical agents, thanks to their distinct characteristics, including small

dimensions, high surface area, and the capacity for functionalization to enable specific targeting. Consequently, diverse systems for drug delivery have been developed using nanoparticles as vehicles, each with its own set of advantages and limitations.

Nanoparticle-based drug delivery systems can be classified based on various parameters, including size, morphology, composition, surface properties, drug release and encapsulation mechanisms, targeting strategies, ability to respond to stimuli, and route of administration. Each parameter plays a crucial role in engineering an ideal system tailored to a specific therapeutic application. For instance, the size of nanoparticles can significantly impact therapeutic effectiveness by influencing their circulation time, tissue penetration capability, and cellular uptake efficiency. Size emerges as a pivotal determinant, directly shaping the pharmacokinetic profile and biological distribution of drug delivery systems within the body. Moreover, the variety of shapes, including nanospheres, nanocages, nanorods, nanotubes, and nanowires, can also impact cellular uptake and in vivo drug distribution. Additionally, the surface charge of nanoparticles, serving as carriers, is another key feature influencing biodistribution profiles, potential interactions with various biological systems, and cellular uptake efficiency [17] [18].

Nanoparticle-based drug delivery systems can be further classified based on two additional major aspects: drug loading and drug release mechanisms. Concerning drug loading, three primary strategies are utilized: conjugation via covalent bonding, encapsulation, and electrostatic interaction. Regarding drug release mechanisms, nanoparticles can release drugs through various pathways such as diffusion, solvent-controlled release, degradation, or triggered mechanisms, depending on their specific properties [19] [20].

In terms of targeting mechanisms, the three main categories are passive targeting, active targeting, and physical targeting. Passive targeting of nanoparticles exploits inherent physiological or pathological phenomena, such as the enhanced permeability and retention effect in tumors, to selectively accumulate drug-loaded nanoparticles in specific tissues. Active targeting involves the use of ligands to enhance the affinity between nanoparticles and cellular receptors, thereby enhancing selectivity. Nanoparticles can be designed to target specific sites by modifying their surface by targeting ligands such as proteins, peptides, vitamins,

or aptamers. Physical targeting relies on external forces like magnetic fields to direct nanoparticles to the target site and regulate drug release [21] [22].

Stimuli-responsive nano-sized delivery systems have been established as effective smart delivery carriers. Changes in their compositions, structures, or conformations can appear in response to chemical, biochemical or physical stimuli, resulting in the release of encapsulated active substances. Furthermore, stimulated release can considerably regulate the site and duration of drug release since it could be generated externally by a variety of stimuli that impact the response of intracellular carriers. Hence, nanodrug delivery systems can be categorized according to the stimuli to which they are sensitive, including pH, glucose, temperature, electrical fields, magnetic fields, ultrasound, light, or enzymatic activity [23].

Regarding the route of administration, nanoparticle-based systems can be administered via various routes including intravenous, oral, transdermal, ocular, nasal, pulmonary, or intramuscular. Each route necessitates specific design considerations [24].

Engineering nanodrug delivery systems involves multiple considerations, including material selection, surface modification to enhance targeting, characteristics of the encapsulated drug such as loading and release mechanisms, implementation techniques, and meeting specific clinical application requirements.

Figure 1 illustrates these key considerations, focusing on four primary attributes: surface chemistry, size and shape, ability to respond to stimuli, and material properties.

Lipidic, polymeric, and inorganic nanoparticles are now utilized in commercial nanodrug delivery systems or clinical trials. Among these, lipid-based formulations are the most frequent, with liposomes being the most commonly used for the development of nanodrug delivery systems [25].

Lipidic and polymeric nanoparticles fall under the organic category due to their composition. **Figure 2** shows the different types of organic nanoparticles employed in nanodrug delivery systems development.

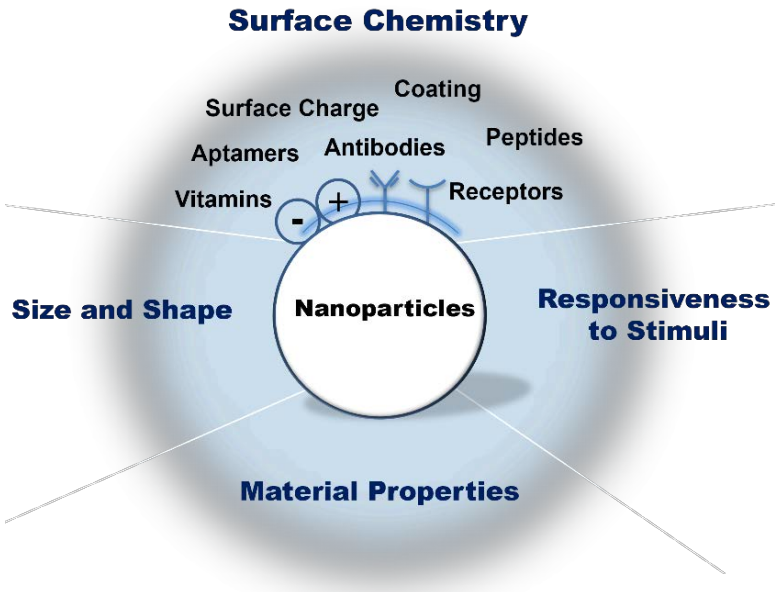

Figure 1. Key Considerations in Nanoparticles Engineering.

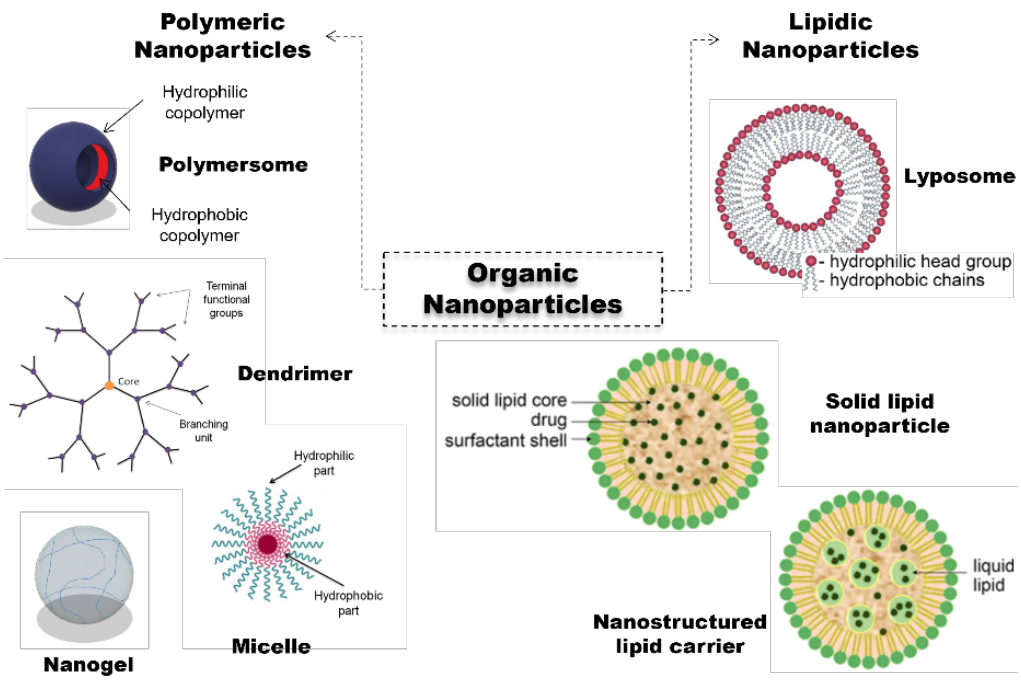

Figure 2. Types of organic nanoparticles [26] [27].

2.2.1. Lipid-Based Nanoparticles

Lipid nanoparticles (LNPs) have emerged as a solution to the complex challenges of drug delivery, demonstrating outstanding pharmacological performance and prospective therapeutic effects, resulting in significant attention in preclinical and clinical studies.

LNPs are spherical vesicles typically composed of four primary lipid constituents: phospholipids and cholesterol, which are crucial for particle formation and stability; cationic or ionizable lipids, enabling interaction with negatively charged nucleic acids and enhancing drug loading capacity; and polyethylene glycol (PEG)-modified lipids, which enhance particle stability and prolong circulation time within the biological milieu. Different categories of lipid-based nanocarriers, such as liposomes, lipid nanoemulsions, solid lipid nanoparticles, nanostructured lipid carriers, and lipid-polymer hybrid nanoparticles, may exhibit distinctive characteristics and functionalities [28]. Several synthesis methods have been proposed for obtaining of LNPs, including solvent-based emulsification, nonsolvent emulsification, nanoprecipitation, microfluidic-based approaches, coacervation, and supercritical fluid technologies [29].

As nanoengineered platforms, LNPs can be designed to encapsulate, protect, and regulate the release of bioactive compounds, offering the potential to enhance bioavailability, drug stability, and targeting precision. LNPs have already been introduced into clinical use, with numerous others in various stages of development. They enable the treatment of previously unaddressed diseases and mitigate side effects, ultimately enhancing the quality of life for patients undergoing treatment.

Lipid nanocarriers played a pivotal role in the development of mRNA vaccines utilized during the COVID-19 pandemic. Engineered formulations incorporating ionizable cationic lipid, cholesterol, PEGylated lipid, and distearoylphosphatidylcholine (DSPC) were utilized to encapsulate mRNA encoding the instructions for producing the spike protein. These mRNA vaccines were developed in record time, altering the trajectory of the COVID-19 pandemic, and marking an unprecedented milestone during a time of urgent medical necessity [30].

Another significant domain of utilization involves using LNPs for delivering cancer treatments, representing the foremost application of LNPs in drug delivery.

Numerous therapeutics delivered via LNPs have received clinical approval for cancer treatment. Examples of clinically approved LNPs include Doxil, a liposome-based chemotherapy drug approved to treat ovarian and breast cancer; Myocet, a cytotoxic chemotherapy used in metastatic breast cancer; and Lipusu, approved for delivering paclitaxel in squamous non small-cell lung cancer and esophageal cancer. LNPs are also utilized in gene therapy applications, such as delivering double-stranded small interfering RNA (siRNA), as demonstrated by the approved formulation Onpattro for treating polyneuropathy caused by hereditary transthyretin-mediated conditions. Additionally, LNPs are acknowledged as promising delivery systems for transdermal drug delivery [27].

2.2.1.1. Liposomes

Liposomes are self-assembling spherical vesicles consisting of one or more concentric phospholipid bilayers surrounding an aqueous core. They can be categorized based on their size and the number of bilayers they possess. For instance, small unilamellar vesicles (SUVs) have a size ranging from 20 to 100 nm, while large unilamellar vesicles (LUVs) exceed 100 nm. Moreover, giant unilamellar vesicles (GUVs) have a size greater than 1000 nm, oligolamellar vesicles (OLVs) range from 100 to 500 nm, and multilamellar vesicles (MLVs) have a size less than 500 nm [31]. In addition to size classification, liposomes can also be classified based on their compositions. This includes conventional liposomes, charged liposomes, stealth stable liposomes, actively targeted liposomes, stimuli-responsive liposomes, and bubble liposomes [32].

The advantages of liposomes as drug delivery vehicles include their tunable properties, encapsulation abilities for both hydrophilic and hydrophobic drugs, protection of the encapsulated drugs against degradation, and targeted delivery. Drugs can be loaded into liposomes through passive or active methods. Passive loading involves dispersing drugs and lipids in an aqueous buffer during liposome formation, leading to entrapment. Active loading involves drugs being trapped in the bilayer or aqueous core of liposomes based on the properties of the drug and lipids. Using liposomes to encapsulate anticancer agents and deliver them has proven to be a promising option in cancer treatment. Furthermore, liposomal drug delivery has demonstrated effectiveness for various targets and disease conditions. Liposomal-based formulations have been approved for treating neoplastic

meningitis, fungal infections, and other medical conditions [33].

2.2.1.2. Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are formed from solid lipids such as mono-, di-, and triglycerides, fatty acids, waxes, and steroids, along with a stabilizing agent, typically a surfactant. These components form a surfactant shell surrounding a core matrix of solid lipids, resulting in spherical particles ranging in size from 10 to 1000 nm. Additionally, co-surfactants and solvent/co-solvents may be optionally included, along with charge modifiers, coating materials, antioxidants, preservatives, cryoprotectants, or viscosity enhancers [34].

SLNs possess several advantages as a drug delivery platform, including maintaining therapeutic drug concentrations and circulatory time at the target site, protecting drugs, improving pharmacokinetics, solubility, bioavailability, and stability, reducing toxicity through targeted delivery via surface modification, reducing dose frequency, and enhancing patient compliance.

Several drugs, including small drug molecules, large biomacromolecules, nucleic acids, vaccine antigens, antineoplastic, and antimicrobial drugs, can be incorporated into solid lipid nanoparticles (SLNs) in three distinct ways: along the length of the particle structure, in the shell, or in the core. They are predominantly released from the SLNs through erosion, degradation, and diffusion [28] [35].

2.2.1.3. Nanostructured Lipid Carriers

Nanostructured lipid carriers (NLCs) represent the next generation of LNPs compared to similar SLNs. Unlike SLNs, NLCs incorporate a lipid blend containing both solid and liquid lipids in their core, alongside surfactant. This enhanced structure allows for the development of nanoparticles ranging from 50 to 500 nm, enabling increased drug loading capacity and improved stability during storage, while maintaining low toxicity levels [36].

The proportion between the solid lipid, liquid oil, and surfactant is a crucial factor that affects the efficiency of trapping and the stability of therapeutic agents. Three distinct categories of NLCs are defined based on the composition of the lipid mixture. The first category, known as the imperfect type, is distinguished by an imperfect lipid matrix resulting from the mixing of lipids with different chemical

properties. The second category, referred to as the amorphous type, possesses a structureless solid amorphous lipid matrix. Lastly, the third category, known as the multiple type, encompasses a solid lipid matrix with nanosized liquid oil droplets.

NLCs offer versatile delivery options for both lipophilic and hydrophilic drugs through multiple administration routes, including oral, pulmonary, transdermal, nasal, parenteral, and ophthalmic routes. They have demonstrated potential in enhancing oral drug bioavailability and have undergone clinical trials for a range of pharmaceuticals, including mRNA vaccines for COVID-19, anticancer drugs, antioxidants, and antiviral agents [28, 37].

2.2.2. Polymeric Nanoparticles

Polymeric materials play an essential role across many areas of modern life, including medicine. Their growing adoption is driven by attributes like biocompatibility, non-toxicity, biodegradability, and versatile physical and mechanical properties. In medicine, both natural and synthetic polymers are employed, contributing to a wide array of applications ranging from medical devices like syringes and catheters to advanced biomedical uses such as tissue engineering scaffolds, drug delivery systems, and biosensors [38].

Polymers have emerged as pivotal components in nanodrug delivery systems, representing versatile materials in the development of new drug delivery strategies. Polymeric nanocarriers have been extensively investigated as systems for controlled, precise, sustained, and continuous release of drugs. They enhance the bioavailability of drugs, facilitate targeted delivery to specific sites, and improve drug solubility.

Polymers can be easily functionalized with a wide range of active targeted ligands, and passive targeting can be adjusted by varying their size and structure. Polymeric nanoparticles often demonstrate superior loading efficiency compared to other drug delivery approaches, thereby improving the mass ratio of encapsulated drug to carrier nanomaterials [41].

Polymeric nanoparticles can be obtained from preformed polymers through methods such as emulsification-solvent evaporation, emulsification/solvent diffusion, emulsification/reverse salting-out, nanoprecipitation, dialysis, and

supercritical fluid techniques. Alternatively, they can be synthesized from monomers using processes like emulsification, miniemulsion, microemulsion, controlled radical polymerization, and interfacial polymerization. Additionally, hydrophilic polymers can be utilized to form nanoparticles through methods such as ionic gelation or coacervation [39] [40]. The flexibility in synthesis of polymer-based nanocarriers permits the simultaneous delivery of therapeutic agents within a single system, irrespective of their hydrophilic properties or molecular weight. This adaptability, combined with the capacity to engineer nanoparticles to react to different stimuli, facilitates precise targeting and biodistribution of drugs. Researchers are also exploring the development of multifunctional drug delivery systems capable of responding to multiple stimuli and loading and releasing multiple drugs.

Various nanometric polymeric structures including micelles, vesicles, gels, capsules, and dendrimers, have been studied extensively as carriers for therapeutic delivery.

Examples of synthetic polymers used in drug delivery systems development include poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), poly(caprolactone) (PCL), poly(glycolic acid) (PGA) and poly(N-isopropylacrylamide) (PNIPAM). Natural based polymers explored in this direction are albumin, agarose, alginate, carrageenan, dextran, chitosan, hyaluronic acid, gelatin.

In addition to polymer-based nanocarriers, research in drug delivery also extends to the investigation of polymer nanocomposites and polymer hybrid nanoparticles. For instance, polymer nanocomposites incorporate nanoparticles such as metal oxides, carbon nanotubes, or clays into a polymeric matrix to enhance drug loading capacity, stability, and controlled release profiles. Similarly, polymer hybrid nanoparticles combine the advantageous properties of polymers with other materials, such as lipids leading to advanced materials such as polymer–lipid hybrid nanoparticles [41].

2.2.2.1. Polymersomes

Polymersomes, also known as polymeric vesicles, are vesicular nanostructures formed through the self-assembly of amphiphilic block copolymers. These

structures feature a bilayer membrane with a hollow vesicular interior. The hydrophilic cavity within the polymersome bilayers provides a suitable environment for encapsulating and protecting hydrophilic components such as proteins, RNAs, and enzymes. These components can reside within the aqueous core of the polymersome, shielded from the surrounding environment. Additionally, the hydrophobic segments of the polymersome membrane bilayers enable the loading of hydrophobic cargoes, such as emissive agents and chemotherapeutic drugs. These hydrophobic molecules can be accommodated within the lipid bilayers through hydrophobic-hydrophobic interactions, effectively entrapping them within the polymersome structure [42].

Polymersomes can be formed from a wide range of copolymers with different structural and physicochemical properties using methods such as thin film hydration, electroformation, pH switch method, nanoprecipitation, single and double emulsion methods, microfluidics synthesis, and polymerization-induced self-assembly.

Polymersomes share essential similarities with liposomes, particularly in regards with the structure, formed by layered membranes to create vesicles. However, it has been stated that polymersomes offer superior stability and enhanced protection for encapsulated therapeutic agents compared to liposomes. Moreover, polymersomes are recognized for their versatility, making them highly suitable for modification. This includes the attachment of functional groups or specific ligands for targeted delivery and the engineering of stimuli-triggered release mechanisms, enabling accurate liberation of the intended cargo at designated sites. By attaching targeting ligands, polymersomes can enhance their specificity towards particular targets, such as immune or cancer cells. Moreover, adjusting the structural properties of polymers allows for the obtaining of stimuli-responsive polymersomes, facilitating controlled release of payloads upon exposure to stimuli. Polymersomes show promise as drug delivery systems due to their adjustable stability, selective permeability, prolonged drug release, and targeted delivery, finding applications in delivering chemotherapeutic agents, nucleic acids, in immunotherapy or photodynamic therapy [43] [44].

2.2.2.2. Dendrimers

Dendrimers, also called dendritic polymers, are monodisperse macromolecules,

characterized by a special globular architecture. These highly defined structures feature a three-dimensional nanostructure, typically ranging in size from 1 to 15 nanometers [45]. The component elements of a dendrimer are the core, the interior layers formed of repetitive branches, and the terminal functional groups.

Dendrimers exhibit a range of remarkable properties. They are compounds distinguished by a high degree of branching, macromolecules with a narrow molecular mass distribution, symmetric nanostructured molecules with a uniform shape, and a monodisperse, homogeneous structure. Their unique architecture resembles a tree-like structure with symmetrical branches, anchored by either a small-molecular compound or a linear polymer nucleus. Furthermore, they possess a reactive polyfunctional surface and engage in interactions through both ionic and covalent bonds. Additionally, the terminal functional groups play a pivotal role in influencing reactivity and enhancing biocompatibility.

Two main strategies are employed for dendrimer synthesis: divergent and convergent methods. In the divergent approach, successive generations of dendrimers are grown from a central reactive core outward by modifying peripheral molecules. With each generation, the molar mass of the dendrimer doubles, yielding a significant number of dendrimers. The convergent method involves dendrimers with a multifunctional core that reacts with multiple dendrons, leading to the attachment of dendrons and yielding a final hyperbranched product.

Polyamidoamine (PAMAM) and polypropyleneimine (PPI) dendrimers are well-known representatives among dendritic families, alongside dendrimers based on polyamide, polyether, polyester, and phosphorus [46].

Interaction between drug molecules and dendrimers occurs through various mechanisms, including covalent conjugation, physical encapsulation, and electrostatic interaction. Their capacity to conjugate or encapsulate high molecular weight drugs, along with their efficient cellular uptake, controlled and targeted delivery, make them ideal candidates as drug carriers. Dendrimers have versatile applications in photodynamic therapy, gene delivery, vaccine delivery, as well as in delivering anti-inflammatory and anticancer agents [47].

2.2.2.3. Nanogels

Nanogels, also known as hydrogel nanoparticles, are aqueous dispersions of

hydrogel particles formed by physically or chemically cross-linked polymeric networks at nanoscale dimensions, typically ranging between 20 and 200 nm [48]. Various types of nanogels have been developed based on their structure, including simple, hollow, core-shell, hairy, multilayer, and functionalized nanogels [49]. Common polymers employed in nanogel synthesis include PLA, PLGA, PNIPAM, PEG, poloxamer, chitosan, and hyaluronic acid. These polymers can be obtained through physical self-assembly, polymerization of monomers in a homogeneous phase or nanoscale heterogeneous system, cross-linking of preformed polymeric chains, or template-assisted nanofabrication [50].

Nanogels offer several advantages, including higher drug loading capacity, reduced carrier material, enhanced control over drug release, and improved efficacy and safety. These attributes have sparked considerable interest in nanogels for drug delivery, driven by their increased surface area, drug-loading capacity, and tunability. Drug loading methods encompass covalent conjugation, physical entrapment, and passive or diffusion-based loading. Controlled and sustained drug release can be achieved through diffusional release, nanogel degradation, and/or in response to environmental stimuli. Nanogels can be engineered to react to stimuli like temperature, pH, and light, enabling precise control over drug release. Triggered by stimuli, the nanogel network can either swell or deswell, leading to the release of the encapsulated therapeutic cargo as it interacts with aqueous media. Nanogels have been studied in the context of cancer treatment, neurological disease treatment, anti-inflammatory therapy, and transdermal drug delivery [51].

2.2.2.4. Polymeric Micelles

Polymeric micelles are nano-sized colloidal dispersions that self-assemble in aqueous solutions, typically ranging in size from 10 to 100 nm. They consist of amphiphilic block copolymers, forming a hydrophobic core surrounded by a hydrophilic shell. In diluted solutions, polymers exist as dispersed units. However, when the concentration reaches a critical level known as the critical micelle concentration (CMC), polymers organize into ordered micellar structures. The CMC is a crucial parameter determining the thermodynamic stability of micelles [52] [53].

Polymeric micelles can be obtained through a variety of techniques, including

thin film hydration, solvent evaporation, dialysis, and direct dissolution. The commonly utilized polymers for micelles synthesis include amphiphilic di-block copolymers, such as polystyrene and poly(ethylene glycol), as well as triblock copolymers like poloxamers. Additionally, graft copolymers such as G-chitosan and ionic copolymers like poly(ethylene glycol)-poly(ε-caprolactone)-g-polyethyleneimine are also employed.

Polymeric micelles show significant potential for enhancing drug delivery systems, leading to improved therapeutic outcomes and minimized side effects. Drugs can be encapsulated within the micelles either during their formation or through a subsequent step, depending on the preparation method and the drug's physicochemical properties. Drug release from polymeric micelles can occur through either drug diffusion from intact micelles or micelle disassembly [26].

Polymeric micelles offer a significant advantage in their capability to solubilize poorly water-soluble or hydrophobic drugs within their core, thereby enhancing their bioavailability. They have garnered attention as potential drug delivery systems for addressing a wide range of conditions, including cancer, autoimmune and cardiovascular diseases, dementia, microbial infections, as well as eye and skin diseases [54].

2.2.3. Inorganic Nanoparticles

Inorganic nanoparticles exhibit promising potential in the realm of drug delivery systems development. They can be engineered to possess specific dimensions, shapes, chemical compositions, and surface characteristics, facilitating the encapsulation of targeted drugs. A defining attribute of these materials lies in their physicochemical properties, encompassing magnetic, thermal, optical, and catalytic properties [55].

A wide array of inorganic nanoparticles, including quantum dots, mesoporous silica nanoparticles, gold nanoparticles, silver nanoparticles, superparamagnetic iron oxide nanoparticles, carbon nanotubes, graphene, and fullerenes, has been synthesized and investigated for their utility in drug delivery.

Figure 3 highlights these types of inorganic nanoparticles, providing a visual representation of their various forms and illustrating the diversity of materials available for engineering advanced drug delivery systems.

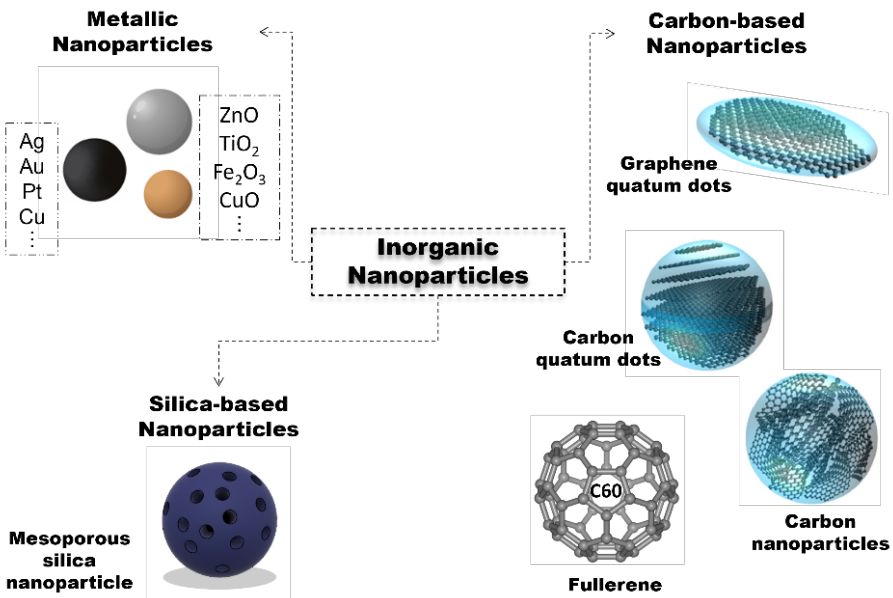

Figure 3. Types of inorganic nanoparticles [56].

Each type of nanoparticle possesses distinct physicochemical properties and can be functionalized with diverse ligands to improve drug delivery efficacy.

The incorporation of inorganic nanoparticles into drug delivery systems offers numerous advantages, including improved stability and solubility of encapsulated payloads, prolonged circulation time, and enhanced transport across biological membranes.

Inorganic nanoparticles have been extensively researched in fields such as iron-replacement therapy and antibacterial treatments. Particularly delivery and have emerged as promising diagnostic and therapeutic tools for various applications, such as tumor imaging, targeted drug delivery, and enhancing radiotherapy effectiveness. Some nanoparticle-based delivery systems have already received approval from the FDA [57].

2.2.3.1. Metallic Nanoparticles

Metal-based nanoparticles can be synthesized by either top-down approaches, where the nanoparticles are formed by size reduction method where bulk materials are broken down into small materials or by bottom-up methods, where

nanostructures are fabricated atom by atom or particle by particle to build up nanostructure. Top-down methods include lithography, laser ablation, sputtering deposition, vapour deposing, pulsed electrochemical etching, while bottom-up methods involved sol-gel synthesis, chemical vapour deposition, flame spraying, laser pyrolysis and micro emulsion [58].

An important example of metallic nanoparticles is gold nanoparticles, extensively researched for their biocompatibility and the ability to precisely control their size distribution and shape, including nanorods, nanoplates, and nanostars. The surface of these nanoparticles can be functionalized, leading to significant changes in their behavior, allowing for the fine-tuning of magnetic and optical properties. Gold nanoparticles exhibit the capability to deliver various payloads such as recombinant proteins, nucleotides, vaccines, and multiple drug molecules. They also enable controlled release mechanisms triggered by external stimuli, making them valuable in applications such as cancer chemotherapy and gene delivery. One method of drug loading involves conjugating drug molecules directly to the gold nanoparticles [55] [59].

Another significant member of metal-based nanoparticles category is iron oxide nanoparticles, which are widely utilized in the biomedical sector due to their biocompatibility and versatile magnetic properties. These nanoparticles serve as valuable candidates, as contrast agents in MRI scans and as components of drug delivery systems. Their notable magnetic attributes enable the development of intelligent delivery systems capable of being guided by an external magnetic field towards specific locations within the body, thereby enhancing the targeted delivery of therapeutic compounds to their intended sites of action. In particular, magnetite (Fe_3O_4) nanoparticles are the most common and valuable iron oxides, serving as MRI contrast agents and in photothermal therapy [60] [61].

Silver, platinum, palladium, copper, zinc oxide, metal sulfide and nanometal organic frameworks nanoparticles are also studied in the realm of drug delivery systems.

2.2.3.2. Carbon-Based Nanoparticles

Carbon-based nanomaterials hold significant promise for biomedical applications, owing to their appealing characteristics. Ranging in size from 1 to 100 nm

depending on the type, this class of nanomaterials exhibit diverse structures with distinctive properties, including electrical conductance, unique optical properties, thermal conductivity, and mechanical stiffness. Furthermore, their surfaces can be easily modified, enhancing solubility and biocompatibility under physiological conditions. Moreover, through the conjugation of specific ligands, carbon nanomaterials can precisely target specific types of cells, tissues, and organs, amplifying their potential for targeted biomedical applications [62].

Within the realm of carbon nanomaterials, carbon dots have emerged as promising nano-vehicles for drug delivery. Central to their appeal are their distinctive optical properties, notably absorption and photoluminescence. With dimensions smaller than 10 nm the category of carbon dots can be classified as graphene quantum dots (GQDs), carbon nanodots (CNDs), and polymer dots (PDs). Moreover, CNDs that exhibit a spherical form can be further divided into two subcategories: carbon nanoparticles (CNPs), distinguished by their amorphous structure, and carbon quantum dots (CQDs), which possess a crystalline structure. Carbon dots show promise in delivering drugs to treat various conditions such as cancer, brain disorders, eye diseases, and infections. They are also useful for delivering genes, vaccines, and antiviral drugs. Using carbon dots as nanocarriers for anticancer drugs offers several advantages, including the ability to carry a high amount of drugs, target specific areas, use lower drug doses, control drug release, which can be coupled with photothermal therapy [56] [63] [64].

Another significant carbon-based nanomaterial is fullerene, renowned for its exceptional properties as photosensitizers in photodynamic therapy and photothermal therapy. Fullerene, also referred to as buckminsterfullerene, encompasses a series of hollow carbon molecules that can adopt either a closed cage structure known as buckyballs, or a cylindrical form called carbon nanotubes. These inorganic nanoparticles possess a hydrophobic core and are characterized by their small size, typically around 1 nanometer. The most prevalent type of fullerene is C60. Fullerenes possess the potential for targeted delivery systems of anticancer drugs, thereby finding utility in diverse therapeutic approaches including photodynamic therapy and cancer vaccines [65] [66].

2.2.3.3. Silica Nanoparticles

Silica nanoparticles, while less well-established than other drug delivery systems

described earlier, have proven to be promising in drug delivery due to their valuable characteristics such as high surface area, controllable size and shape, customized surface properties, stability, and biocompatibility. Silica nanoparticles are in clinical trials for a range of biomedical uses, including oral medication administration, diagnostics, plasmonic resonance, and photothermal ablation treatment [66].

One type of silica nanoparticle that has emerged as an interesting approach for drug delivery is mesoporous silica nanoparticles (MSNs). MSNs possess several advantages in this direction such adjustable pore size within the range of 2-50 nm, high surface area, biocompatibility, high loading capacity, and tunable surface properties. MSNs have been explored for their potential as stimuli-responsive drug release systems, capable of releasing loaded drug molecules in response to pH changes, redox reactions, or enzymatic activity. These versatile nanoparticles find applications in cancer treatment, bioimaging, biosensors, and photodynamic therapy [67] [68].

2.3. Cancer Management

Despite advancements in medicine, cancer remains a significant global health challenge, being a leading cause of death worldwide. The estimated incidence of new cancer cases in 2020 was 19.3 million, compared to 10.9 million in 2002, representing a 77% increase [69]. In 2023, it was projected that there would be 1,958,310 new cancer cases and 609,820 cancer deaths in the United States [70]. Efforts in cancer management are centered on various fronts, including prevention, early detection, accurate diagnosis, and effective treatment. Moreover, attention is also devoted to enhancing the quality of life for both cancer patients and survivors.

The traditional treatment options for cancer patients include chemotherapy, radiotherapy, and surgery [71] [72]. The selection of a treatment method depends on various factors, such as the stage and location of the cancer and the patient's overall health status, which is often impaired by the disease and may deteriorate further with each successive treatment over time [73] [74].

Nanomedicine holds tremendous promise in advancing cancer treatments and early diagnosis. The true impact of nanomedicine in cancer lies in bridging research findings to clinical applications, aiming to enhance disease diagnosis and

treatment. While nanotechnology's role in cancer diagnosis and treatment is primarily in the developmental stage, numerous nanocarrier-based drugs have already received approval, with many more undergoing clinical trials. Nanomedicine strategies for cancer treatment involve engineering nanomaterials to obtain innovative therapies and devices, potentially minimizing toxicity while maximizing treatment effectiveness and delivery.

The integration of imaging methodologies into cancer diagnostics and therapeutic monitoring has been significantly enhanced by the strategic incorporation of nanoparticles. Nanoparticles present the exciting potential to deliver treatments with exceptional specificity. By functionalizing nanoparticles with ligands designed to selectively target cancer cells, the potential damage to healthy tissues is reduced, significantly enhancing the overall efficacy of therapeutic interventions. Nanoparticles are known for their ability to penetrate deep tissues, enhancing the enhanced permeability and retention (EPR) effect. Additionally, their surface characteristics influence bioavailability and half-life by effectively crossing epithelial openings.

Extensive research has been conducted on both inorganic and organic nanoparticles as potential nanocarriers for delivering anticancer drugs. However, despite the numerous advantages outlined for each type of nanocarrier, several significant challenges persist, limiting their widespread clinical application.

Table 1 compiles recent research studies from the literature, specifically focusing on nanoparticle-based drug delivery systems developed within the past year for cancer treatment. By synthesizing findings from these studies, it provides a comprehensive overview of the latest advancements in nanoparticle technology, offering valuable insights into emerging trends and innovations in cancer therapy.

Table 1. Nanoparticle-Based Cancer Drug Delivery Systems in research.

Cancer Type	NDDS	Encapsulated Drug/Molecule	Responsiveness to Stimuli	Applicability	Ref.
Breast cancer	PCL-PEG nanoparticles	Simvastatin	-	Breast cancer treatment	[75]
	Gold nanoparticles	Galangin	-	Antiangiogenesis therapy	[76]

	Platinum nanoparticles coated with alginate	-	X-ray radiation	Radiosensitizers for improving radiotherapy outcomes	[77]
	PEG-PLG nanoparticles	Paclitaxel Curumin	-	Overcoming multidrug resistance in breast cancer	[78]
	Self-responsive nanoparticles	Docetaxel	pH Reactive oxygen species	Cancer immunotherapy	[79]
	Mesoporous silica hybrid- based nanoparticles	Cytarabine Doxorubicin	pH Redox	Promising beneficial agent for improving breast cancer treatment	[80]
	PLGA-based nanoparticles	Perfluoropentane Paclitaxel Anti-miR-221 inhibitor	Ultrasound	Potential treatment for triple genitive breast cancer	[81]
Brain cancer	Lipid-based nanoparticles	Temozolomide	Alternating magnetic fields	Glioblastoma multiforme treatment	[82]
	Silica-based nanoparticles	Temozolomide	-	Glioblastoma treatment	[83]
	Albumin nanoparticles	CCF642 - protein disulfide isomer-ases (PDI) inhibi-tor	-	Overcome chemoresistance in glioblastoma	[84]
Lung cancer	Chitosan nanoparticles	Gefitinib	-	Enhanced lung cancer therapy	[85]
	PLGA-based nanoparticles	Cisplatin Up conversion nanoparticles	-	Promising lung cancer therapy	[86]
	Silk fibroin-based nanoparticles	Quercetin	-	Potential pulmonary drug delivery system	[87]
	Mesoporous silica -based nanoparticles	Metformin	-	Non-small cell lung cancer	[88]

	Iron oxide nanoparticles	Fucoidan	-	Potential multimodal anticancer therapeutics for lung cancer	[89]
Pancreatic cancer	Solid lipid nanoparticles	Herniarin	-	Potential therapeutic target against Panc-1 cell line	[90]
	Superparamagnetic iron oxide-based nanoparticles	Small interfering RNAs	-	Potential tool for diagnosis and treatment of pancreatic cancer	[91]
	Copper oxide nanoparticles	Cisplatin	pH	Potential treatment for pancreatic cancer	[92]
Ovarian Cancer	Methoxy PEG-PLA nanoparticles	Gefitinib Doxorubicin	pH reduction stimuli	Potential multiple anticancer drugs delivery tool	[93]
	Mn-based metal-organic framework nanoparticles	Cisplatin Niraparib	-	NDDS to reverse cisplatin resistance in patients with ovarian cancer	[94]
	Moringa gum-magnesium oxide nanoparticles	-	-	Potential antioxidant against ovarian cancer	[94]

The trend in the development of nanodrug delivery systems is directed towards a multifaceted approach aimed at improving the efficacy, specificity, and safety of cancer treatments. This involves delivering multiple drugs or molecules simultaneously to enhance treatment outcomes, engineering responsive nanoparticles that can respond to stimuli, and modifying surfaces to target specific cells or organs.

2.4. Conclusions

In conclusion, we can say that nanomedicine holds the potential to transform the diagnosis and treatment of diseases. In recent years, the use of nanoparticles in

drug delivery has gained significant attention due to their unique properties and potential applications in cancer treatment. Nanoparticles can deliver therapeutic agents directly to the tumor site, effectively bypassing the barriers posed by the tumor microenvironment. Further research and development are essential to optimize targeted therapy delivery. Enhancing the precision and efficiency of these therapies can significantly improve patient outcomes, reduce side effects, and provide more effective treatment options. Additionally, exploring novel nanoparticle designs and understanding their interactions with biological systems will be crucial for advancing the field of targeted cancer therapy. Ongoing collaboration between researchers, clinicians, and industry partners will be crucial in translating these advancements from the laboratory to clinical practice.

Acknowledgement

"This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI - UEFISCDI, project number PN-III-P4-ID-PCE-2020-1448, within PNCDI III" and from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952152.

References

[1] J.C.L. Chow, 9 - Magnetic nanoparticles as contrast agents in magnetic resonance imaging and radiosensitizers in radiotherapy, in: C.M. Hussain, K.K. Patankar (Eds.), Fundamentals and Industrial Applications of Magnetic Nanoparticles, Woodhead Publishing2022, pp. 291-316.

[2] T.K. Krishnapriya, M.K. Jayaraj, A.S. Asha, Chapter 6 - Luminescent nanoparticles for bio-imaging application, in: M.K. Jayaraj, P.P. Subha, S. Thomas (Eds.), Nanomaterials for Sensing and Optoelectronic Applications, Elsevier2022, pp. 107-128.

[3] S.Y. Lee, M. Kwon, I.S. Raja, A. Molkenova, D.W. Han, K.S. Kim, Graphene-Based Nanomaterials for Biomedical Imaging, Adv Exp Med Biol 1351 (2022) 125-148.

[4] G. Kalyan Sundar, S. Anchal, Fluorescent Nanomaterials for Cellular Imaging, in: G. Natalia (Ed.), Fluorescence Methods for Investigation of Living Cells and Microorganisms, IntechOpen, Rijeka, 2020, p. Ch. 17.

[5] P.J. Gawne, M. Ferreira, M. Papaluca, J. Grimm, P. Decuzzi, New opportunities

and old challenges in the clinical translation of nanotheranostics, Nature Reviews Materials 8(12) (2023) 783-798.

[6] R. Singla, S.M.S. Abidi, A.I. Dar, A. Acharya, Nanomaterials as potential and versatile platform for next generation tissue engineering applications, Journal of Biomedical Materials Research Part B: Applied Biomaterials 107(7) (2019) 2433-2449.

[7] F. Habibzadeh, S.M. Sadraei, R. Mansoori, N.P. Singh Chauhan, G. Sargazi, Nanomaterials supported by polymers for tissue engineering applications: A review, Heliyon 8(12) (2022) e12193.

[8] A. Das, N. Ali, Nanovaccine: an emerging strategy, Expert Review of Vaccines 20(10) (2021) 1273-1290.

[9] A. El-Sayed, M. Kamel, Advances in nanomedical applications: diagnostic, therapeutic, immunization, and vaccine production, Environmental Science and Pollution Research 27(16) (2020) 19200-19213.

[10] K.K. Jain, An Overview of Drug Delivery Systems, in: K.K. Jain (Ed.), Drug Delivery Systems, Springer New York, New York, NY, 2020, pp. 1-54.

[11] S.A.A. Rizvi, A.M. Saleh, Applications of nanoparticle systems in drug delivery technology, Saudi Pharmaceutical Journal 26(1) (2018) 64-70.

[12] C. Guzmán, G.M. Mercedes, G.F. Marta, V. Gloria, Nanoparticles as Drug Delivery Systems, in: V.P. Phuong (Ed.), 21st Century Nanostructured Materials, IntechOpen, Rijeka, 2021, p. Ch. 11.

[13] M.J. Mitchell, M.M. Billingsley, R.M. Haley, M.E. Wechsler, N.A. Peppas, R. Langer, Engineering precision nanoparticles for drug delivery, Nature Reviews Drug Discovery 20(2) (2021) 101-124.

[14] S. Sindhwani, W.C.W. Chan, Nanotechnology for modern medicine: next step towards clinical translation, Journal of Internal Medicine 290(3) (2021) 486-498.

[15] X. Shan, X. Gong, J. Li, J. Wen, Y. Li, Z. Zhang, Current approaches of nanomedicines in the market and various stage of clinical translation, Acta Pharmaceutica Sinica B 12(7) (2022) 3028-3048.

[16] M. Germain, F. Caputo, S. Metcalfe, G. Tosi, K. Spring, A.K.O. Åslund, A. Pottier, R. Schiffelers, A. Ceccaldi, R. Schmid, Delivering the power of nanomedicine

to patients today, Journal of Controlled Release 326 (2020) 164-171.

[17] J. Di, X. Gao, Y. Du, H. Zhang, J. Gao, A. Zheng, Size, shape, charge and "stealthy" surface: Carrier properties affect the drug circulation time in vivo, Asian Journal of Pharmaceutical Sciences 16(4) (2021) 444-458.

[18] T. Liang, Z. Xing, L. Jiang, J.-J. Zhu, Tailoring nanoparticles for targeted drug delivery: From organ to subcellular level, VIEW 2(5) (2021) 20200131.

[19] M. Chamundeeswari, J. Jeslin, M.L. Verma, Nanocarriers for drug delivery applications, Environmental Chemistry Letters 17(2) (2019) 849-865.

[20] G.-H. Son, B.-J. Lee, C.-W. Cho, Mechanisms of drug release from advanced drug formulations such as polymeric-based drug-delivery systems and lipid nanoparticles, Journal of Pharmaceutical Investigation 47(4) (2017) 287-296.

[21] A.V.V. Nikezić, A.M. Bondžić, V.M. Vasić, Drug delivery systems based on nanoparticles and related nanostructures, European Journal of Pharmaceutical Sciences 151 (2020) 105412.

[22] F. Salahpour Anarjan, Active targeting drug delivery nanocarriers: Ligands, Nano-Structures & Nano-Objects 19 (2019) 100370.

[23] Z. Shariatinia, Big family of nano- and microscale drug delivery systems ranging from inorganic materials to polymeric and stimuli-responsive carriers as well as drug-conjugates, Journal of Drug Delivery Science and Technology 66 (2021) 102790.

[24] N.K. Shah, E.A. Torrico Guzmán, Z. Wang, S.A. Meenach, Chapter 6 - Routes of administration for nanocarriers, in: E.J. Chung, L. Leon, C. Rinaldi (Eds.), Nanoparticles for Biomedical Applications, Elsevier2020, pp. 67-87.

[25] R.K. Thapa, J.O. Kim, Nanomedicine-based commercial formulations: current developments and future prospects, Journal of Pharmaceutical Investigation 53(1) (2023) 19-33.

[26] M. Ghezzi, S. Pescina, C. Padula, P. Santi, E. Del Favero, L. Cantù, S. Nicoli, Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions, Journal of Controlled Release 332 (2021) 312-336.

[27] R. Tenchov, R. Bird, A.E. Curtze, Q. Zhou, Lipid Nanoparticles—From

Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement, ACS Nano 15(11) (2021) 16982-17015.

[28] M. Mehta, T.A. Bui, X. Yang, Y. Aksoy, E.M. Goldys, W. Deng, Lipid-Based Nanoparticles for Drug/Gene Delivery: An Overview of the Production Techniques and Difficulties Encountered in Their Industrial Development, ACS Materials Au 3(6) (2023) 600-619.

[29] L. Xu, X. Wang, Y. Liu, G. Yang, R.J. Falconer, C.-X. Zhao, Lipid Nanoparticles for Drug Delivery, Advanced NanoBiomed Research 2(2) (2022) 2100109.

[30] B. Wilson, K.M. Geetha, Lipid nanoparticles in the development of mRNA vaccines for COVID-19, J Drug Deliv Sci Technol 74 (2022) 103553.

[31] A.A. Khafoor, A.S. Karim, S.M. Sajadi, Recent progress in synthesis of nano based liposomal drug delivery systems: A glance to their medicinal applications, Results in Surfaces and Interfaces 11 (2023) 100124.

[32] H. Nsairat, D. Khater, U. Sayed, F. Odeh, A. Al Bawab, W. Alshaer, Liposomes: structure, composition, types, and clinical applications, Heliyon 8(5) (2022) e09394.

[33] S. Pande, Liposomes for drug delivery: review of vesicular composition, factors affecting drug release and drug loading in liposomes, Artificial Cells, Nanomedicine, and Biotechnology 51(1) (2023) 428-440.

[34] V. Harish, S. Mohd, D. Tewari, N.K. Pandey, S. Vishwas, M.R. Babu, M.A. Salkini, Z.u. Rehman, J.T. Alotaibi, R.F. Alotaibi, F.A. Alrashed, P. Prasher, N. Sharma, G. Gupta, V. Jakhmola, Y. Singh, T.d.J.A. Pinto, K.R. Paudel, N. Mittal, T.G. Singh, P. Arora, K. Dua, S.K. Singh, Unravelling the role of solid lipid nanoparticles in drug delivery: Journey from laboratory to clinical trial, Journal of Drug Delivery Science and Technology 85 (2023) 104616.

[35] M. Munir, M. Zaman, M.A. Waqar, M.A. Khan, M.N. Alvi, Solid lipid nanoparticles: a versatile approach for controlled release and targeted drug delivery, Journal of Liposome Research 1-14.

[36] S. Khan, A. Sharma, V. Jain, An Overview of Nanostructured Lipid Carriers and its Application in Drug Delivery through Different Routes, Adv Pharm Bull 13(3) (2023) 446-460.

[37] M. Elmowafy, M.M. Al-Sanea, Nanostructured lipid carriers (NLCs) as drug

delivery platform: Advances in formulation and delivery strategies, Saudi Pharmaceutical Journal 29(9) (2021) 999-1012.

[38] J. Premkumar, K. SonicaSree, T. Sudhakar, Polymers in Biomedical Use, in: C.M. Hussain, S. Thomas (Eds.), Handbook of Polymer and Ceramic Nanotechnology, Springer International Publishing, Cham, 2021, pp. 1329-1355.

[39] Y. Herdiana, N. Wathoni, S. Shamsuddin, M. Muchtaridi, Scale-up polymeric-based nanoparticles drug delivery systems: Development and challenges, OpenNano 7 (2022) 100048.

[40] C.I.C. Crucho, M.T. Barros, Polymeric nanoparticles: A study on the preparation variables and characterization methods, Materials Science and Engineering: C 80 (2017) 771-784.

[41] R. De, M.K. Mahata, K.-T. Kim, Structure-Based Varieties of Polymeric Nanocarriers and Influences of Their Physicochemical Properties on Drug Delivery Profiles, Advanced Science 9(10) (2022) 2105373.

[42] Y. Zhu, S. Cao, M. Huo, J.C.M. van Hest, H. Che, Recent advances in permeable polymersomes: fabrication, responsiveness, and applications, Chemical Science 14(27) (2023) 7411-7437.

[43] M. Fonseca, I. Jarak, F. Victor, C. Domingues, F. Veiga, A. Figueiras, Polymersomes as the Next Attractive Generation of Drug Delivery Systems: Definition, Synthesis and Applications, Materials 17(2) (2024) 319.

[44] I. Meerovich, A.K. Dash, Chapter 8—Polymersomes for drug delivery and other biomedical applications, in: A.-M. Holban, A.M. Grumezescu (Eds.), Materials for Biomedical Engineering, Elsevier2019, pp. 269-309.

[45] P. Mittal, A. Saharan, R. Verma, F.M.A. Altalbawy, M.A. Alfaidi, G.E.-S. Batiha, W. Akter, R.K. Gautam, M.S. Uddin, M.S. Rahman, Dendrimers: A New Race of Pharmaceutical Nanocarriers, BioMed Research International 2021 (2021) 8844030.

[46] J. Wang, B. Li, L. Qiu, X. Qiao, H. Yang, Dendrimer-based drug delivery systems: history, challenges, and latest developments, Journal of Biological Engineering 16(1) (2022) 18.

[47] M. Nikzamir, Y. Hanifehpour, A. Akbarzadeh, Y. Panahi, Applications of Dendrimers in Nanomedicine and Drug Delivery: A Review, Journal of Inorganic

and Organometallic Polymers and Materials 31(6) (2021) 2246-2261.

[48] F. Pinelli, F. Ferracin, G. Perale, F. Rossi, Chapter Two—Synthesis and applications of nanogels via covalent cross-linking strategies, in: E. Mauri, Z.J. Zhang (Eds.), Advances in Chemical Engineering, Academic Press2023, pp. 35-58.

[49] A. Sharma, T. Garg, A. Aman, K. Panchal, R. Sharma, S. Kumar, T. Markandeywar, Nanogel—an advanced drug delivery tool: Current and future, Artificial Cells, Nanomedicine, and Biotechnology 44(1) (2016) 165-177.

[50] F. Pinelli, M. Saadati, E.N. Zare, P. Makvandi, M. Masi, A. Sacchetti, F. Rossi, A perspective on the applications of functionalized nanogels: promises and challenges, International Materials Reviews 68(1) (2023) 1-25.

[51] N.K. Preman, S. Jain, R.P. Johnson, "Smart" Polymer Nanogels as Pharmaceutical Carriers: A Versatile Platform for Programmed Delivery and Diagnostics, ACS Omega 6(8) (2021) 5075-5090.

[52] A. Bose, D. Roy Burman, B. Sikdar, P. Patra, Nanomicelles: Types, properties and applications in drug delivery, IET Nanobiotechnol 15(1) (2021) 19-27.

[53] S. Perumal, R. Atchudan, W. Lee, A Review of Polymeric Micelles and Their Applications, Polymers 14(12) (2022) 2510.

[54] O.I. Guliy, S.A. Staroverov, A.S. Fomin, E.G. Zhnichkova, S.V. Kozlov, L.G. Lovtsova, L.A. Dykman, Polymeric Micelles for Targeted Drug Delivery System, Applied Biochemistry and Microbiology 58(6) (2022) 726-737.

[55] G. Unnikrishnan, A. Joy, M. Megha, E. Kolanthai, M. Senthilkumar, Exploration of inorganic nanoparticles for revolutionary drug delivery applications: a critical review, Discover Nano 18(1) (2023) 157.

[56] D. Ozyurt, M.A. Kobaisi, R.K. Hocking, B. Fox, Properties, synthesis, and applications of carbon dots: A review, Carbon Trends 12 (2023) 100276.

[57] H. Huang, W. Feng, Y. Chen, J. Shi, Inorganic nanoparticles in clinical trials and translations, Nano Today 35 (2020) 100972.

[58] V. Chandrakala, V. Aruna, G. Angajala, Review on metal nanoparticles as nanocarriers: current challenges and perspectives in drug delivery systems, Emergent Materials 5(6) (2022) 1593-1615.

[59] W. Paul, C.P. Sharma, 13 - Inorganic nanoparticles for targeted drug delivery,

in: C.P. Sharma (Ed.), Biointegration of Medical Implant Materials (Second Edition), Woodhead Publishing2020, pp. 333-373.

[60] T. Vangijzegem, D. Stanicki, S. Laurent, Magnetic iron oxide nanoparticles for drug delivery: applications and characteristics, Expert Opinion on Drug Delivery 16(1) (2019) 69-78.

[61] Y.P. Yew, K. Shameli, M. Miyake, N.B.B. Ahmad Khairudin, S.E.B. Mohamad, T. Naiki, K.X. Lee, Green biosynthesis of superparamagnetic magnetite Fe_3O_4 nanoparticles and biomedical applications in targeted anticancer drug delivery system: A review, Arabian Journal of Chemistry 13(1) (2020) 2287-2308.

[62] S. Pattnaik, Y. Surendra, J.V. Rao, K. Swain, 18 - Carbon family nanomaterials for drug delivery applications, in: M. Mozafari (Ed.), Nanoengineered Biomaterials for Advanced Drug Delivery, Elsevier2020, pp. 421-445.

[63] J. Qi, R. Zhang, X. Liu, Y. Liu, Q. Zhang, H. Cheng, R. Li, L. Wang, X. Wu, B. Li, Carbon Dots as Advanced Drug-Delivery Nanoplatforms for Antiinflammatory, Antibacterial, and Anticancer Applications: A Review, ACS Applied Nano Materials 6(11) (2023) 9071-9084.

[64] H. Kaurav, D. Verma, A. Bansal, D.N. Kapoor, S. Sheth, Progress in drug delivery and diagnostic applications of carbon dots: a systematic review, Frontiers in Chemistry 11 (2023).

[65] N.B. Fernandes, R.U.K. Shenoy, M.K. Kajampady, C.E.M. Dcruz, R.K. Shirodkar, L. Kumar, R. Verma, Fullerenes for the treatment of cancer: an emerging tool, Environmental Science and Pollution Research 29(39) (2022) 58607-58627.

[66] Jyotsna, L. Stanley Abraham, R. Hanumant Singh, R.C. Panda, T. Senthilvelan, Biomedical Applications of Carbon-Based Nanomaterials, in: T.S. Santra, L. Mohan (Eds.), Nanomaterials and Their Biomedical Applications, Springer Singapore, Singapore, 2021, pp. 157-174.

[67] H. Kirla, D.J. Henry, S. Jansen, P.L. Thompson, J. Hamzah, Use of Silica Nanoparticles for Drug Delivery in Cardiovascular Disease, Clinical Therapeutics 45(11) (2023) 1060-1068.

[68] B. Murugan, S. Sagadevan, A.L. J, I. Fatimah, K.N. Fatema, W.-C. Oh, F.

Mohammad, M.R. Johan, Role of mesoporous silica nanoparticles for the drug delivery applications, Materials Research Express 7(10) (2020) 102002.

[69] C. Cainap, N. Crisan, Advances in Cancer Therapy from Research to Clinical Practice-Surgical, Molecular or Systemic Management of Cancer, Medicina (Kaunas) 59(7) (2023).

[70] R.L. Siegel, K.D. Miller, N.S. Wagle, A. Jemal, Cancer statistics, 2023, CA Cancer J Clin 73(1) (2023) 17-48.

[71] R. Baskar, K.A. Lee, R. Yeo, K.W. Yeoh, Cancer and radiation therapy: current advances and future directions, Int J Med Sci 9(3) (2012) 193-199.

[72] L. Wills, D. Nagarwalla, C. Pearson, S. McPhail, R. Hinchliffe, B. Sharpless, F. Fardus-Reid, L. Ambler, S. Harrison, J. Shelton, Estimating surgery, radiotherapy and systemic anti-cancer therapy treatment costs for cancer patients by stage at diagnosis, The European Journal of Health Economics 25(5) (2024) 763-774.

[73] M. Sell, A.R. Lopes, M. Escudeiro, B. Esteves, A.R. Monteiro, T. Trindade, L. Cruz-Lopes, Application of Nanoparticles in Cancer Treatment: A Concise Review, Nanomaterials (Basel) 13(21) (2023).

[74] E.A.E. Reijneveld, P. Bor, J.J. Dronkers, N. Argudo, J.P. Ruurda, C. Veenhof, Impact of curative treatment on the physical fitness of patients with esophageal cancer: A systematic review and meta-analysis, Eur J Surg Oncol 48(2) (2022) 391-402.

[75] M. Dadashpour, M. Ganjibakhsh, H. Mousazadeh, K. Nejati, Increased Pro-Apoptotic and Anti-Proliferative Activities of Simvastatin Encapsulated PCL-PEG Nanoparticles on Human Breast Cancer Adenocarcinoma Cells, Journal of Cluster Science 34(1) (2023) 211-222.

[76] M.H. Qaddoori, H.S. Al-Shmgani, Galangin-Loaded Gold Nanoparticles: Molecular Mechanisms of Antiangiogenesis Properties in Breast Cancer, International Journal of Breast Cancer 2023(1) (2023) 3251211.

[77] H. Rashidzadeh, F. Seidi, M. Ghaffarlou, M. Salehiabar, J. Charmi, K. Yaray, H. Nosrati, Y.N. Ertas, Preparation of alginate coated Pt nanoparticle for radiosensitization of breast cancer tumor, International Journal of Biological Macromolecules 233 (2023) 123273.

[78] X. Lin, Q. Wang, S. Du, Y. Guan, J. Qiu, X. Chen, D. Yuan, T. Chen, Nanoparticles for co-delivery of paclitaxel and curcumin to overcome chemoresistance against breast cancer, Journal of Drug Delivery Science and Technology 79 (2023) 104050.

[79] Y. Wang, Q. Wang, X. Wang, P. Yao, Q. Dai, X. Qi, M. Yang, X. Zhang, R. Huang, J. Yang, Q. Wang, P. Xia, D. Zhang, F. Sun, Docetaxel-loaded pH/ROS dual-responsive nanoparticles with self-supplied ROS for inhibiting metastasis and enhancing immunotherapy of breast cancer, Journal of Nanobiotechnology 21(1) (2023) 286.

[80] L. Zhu, S. Wang, A convergent fabrication of pH and redox dual-responsive hybrids of mesoporous silica nanoparticles for the treatment of breast cancer, Journal of Biomaterials Science, Polymer Edition 34(2) (2023) 147-165.

[81] L. Zhang, Z. Ren, J. Lü, X. Mo, J. Lin, Y. Li, W. Ma, P. Liu, Y. Shen, Q. Zhao, L. Qian, X. Cheng, Z. Yu, B. Zhang, Nanoparticles carrying paclitaxel and anti-miR-221 for breast cancer therapy triggered by ultrasound, Cell Death Discovery 9(1) (2023) 298.

[82] L. Beola, N. Iturrioz-Rodríguez, C. Pucci, R. Bertorelli, G. Ciofani, Drug-Loaded Lipid Magnetic Nanoparticles for Combined Local Hyperthermia and Chemotherapy against Glioblastoma Multiforme, ACS Nano 17(18) (2023) 18441-18455.

[83] T.I. Janjua, Y. Cao, A. Ahmed-Cox, A. Raza, M. Moniruzzaman, D.T. Akhter, N.L. Fletcher, M. Kavallaris, K.J. Thurecht, A. Popat, Efficient delivery of Temozolomide using ultrasmall large-pore silica nanoparticles for glioblastoma, Journal of Controlled Release 357 (2023) 161-174.

[84] K.M.-Y. Kiang, W. Tang, Q. Song, J. Liu, N. Li, T.-L. Lam, H.C. Shum, Z. Zhu, G.K.-K. Leung, Targeting unfolded protein response using albumin-encapsulated nanoparticles attenuates temozolomide resistance in glioblastoma, British Journal of Cancer 128(10) (2023) 1955-1963.

[85] H. Amin, M.A. Amin, S.K. Osman, A.M. Mohammed, G. Zayed, Chitosan nanoparticles as a smart nanocarrier for gefitinib for tackling lung cancer: Design of experiment and in vitro cytotoxicity study, International Journal of Biological Macromolecules 246 (2023) 125638.

[86] B. Yadav, M. Chauhan, S. Shekhar, A. Kumar, A.K. Mehata, A.K. Nayak, R. Dutt, V. Garg, V. Kailashiya, M.S. Muthu, Sonali, R.P. Singh, RGD-decorated PLGA nanoparticles improved effectiveness and safety of cisplatin for lung cancer therapy, International Journal of Pharmaceutics 633 (2023) 122587.

[87] Y. Tang, L. Zhang, R. Sun, B. Luo, Y. Zhou, Y. Zhang, Y. Liang, B. Xiao, C. Wang, Pulmonary delivery of mucus-traversing PF127-modified silk fibroin nanoparticles loading with quercetin for lung cancer therapy, Asian Journal of Pharmaceutical Sciences 18(4) (2023) 100833.

[88] F. Zhang, W. Liu, Y. Long, H. Peng, Targeted Delivery of Metformin Against Lung Cancer Cells Via Hyaluronan-Modified Mesoporous Silica Nanoparticles, Applied Biochemistry and Biotechnology 195(7) (2023) 4067-4083.

[89] T.-L. Ho, C. Mutalik, L. Rethi, H.-N.T. Nguyen, P.-R. Jheng, C.-C. Wong, T.-S. Yang, T.T. Nguyen, B.W. Mansel, C.-A. Wang, E.-Y. Chuang, Cancer-targeted fucoidan-iron oxide nanoparticles for synergistic chemotherapy/chemodynamic theranostics through amplification of P-selectin and oxidative stress, International Journal of Biological Macromolecules 235 (2023) 123821.

[90] A.M.D. Delkhah, E. Karimi, S. Farivar, Herniarin-loaded solid lipid nanoparticles: promising molecular mechanism and therapeutic potential against pancreatic cancer line, Molecular Biology Reports 50(8) (2023) 6469-6479.

[91] Y. Pu, H. Ke, C. Wu, S. Xu, Y. Xiao, L. Han, G. Lyu, S. Li, Superparamagnetic iron oxide nanoparticles target BxPC-3 cells and silence MUC4 for the treatment of pancreatic cancer, Biochimica et Biophysica Acta (BBA) - General Subjects 1867(9) (2023) 130383.

[92] F. Danışman-Kalındemirtaş, E. Sert, E. Tan, E. Akyüz, S. Karakuş, Potential multifaceted applications of cisplatin-loaded Camellia sinensis extract/CuO nanoparticles in cytotoxic and apoptotic effects, Biomass Conversion and Biorefinery (2023).

[93] H. Zhang, Y. Yang, Y. Chen, X. Zhang, X. Chen, A convergent fabrication of programmed pH/reduction-responsive nanoparticles for efficient dual anticancer drugs delivery for ovarian cancer treatment, Journal of Experimental Nanoscience 18(1) (2023) 2193400.

[94] Y. Liu, Y. Wang, X. Guan, Q. Wu, M. Zhang, P. Cui, C. Wang, X. Chen, X. Meng,

T. Ma, Reversal of Cisplatin Resistance in Ovarian Cancer by the Multitargeted Nanodrug Delivery System Tf-Mn-MOF@Nira@CDDP, ACS Applied Materials & Interfaces 15(22) (2023) 26484-26495.

Chapter 3
The Societal Impact of Nanomedicines

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Abstract: Nanomedicines are nanometric systems that can enable therapeutic molecules transport toward their target, so to treat, diagnose or prevent diseases. Among many known applications, these drug delivery systems can avoid the degradation of fragile compounds as nucleic acids or limit the toxicity of other drugs such as chemotherapeutics. Nanotechnology has been part of the clinics since the 90s, when the first liposomal nanoformulation loaded with doxorubicin (Doxil®) was approved, successfully reducing the well-known cardiotoxicity of this molecule while increasing its efficacy. Much has been written since this first formulation and over 70 nanomedicines have been commercialized up to this day for an increasing number of indications. The recent pandemic has brutally shown us the impact of nanotechnology worldwide. The rapid development of lipid nanoparticles-based COVID-19 vaccines and their high efficacy in preventing severe forms of the disease have demonstrated the significance of nanomedicine on a larger scale. Nevertheless, nanotechnology still has to face many old challenges such as regulatory impairments but also new ones, including their environmental print and the limitation of resources. This chapter will overview and discuss the major impact of nanotechnology on society.

Keywords: Nanomedicine, Societal Impact, Toxicity, Cancer, Infectious Diseases

3.1. Introduction

In the field of medical advancements, nanotechnology has played a pivotal role. [1]. Nanomedicines, nanometric drug delivery systems designed for precise

transportation of therapeutic molecules, represent a significant aspect of this evolution [2]. The emergence of nanotechnology in medicine in the 1990s hinted at its potential to reshape healthcare [3]. The approval of the liposomal nanoformulation, Doxil® [4], marked the initial chords of this transformative journey, effectively harmonizing enhanced drug delivery with reduced cardiotoxicity. Since then, approximately 70 nanomedicines have entered the market in Europe and the US [5], contributing to an expanding range of therapeutic interventions. These applications include safeguarding the integrity of nucleic acids [6], reducing the toxicity of potent agents like chemotherapeutics [7], enhancing the solubility of hydrophobic drug molecules, and serving as valuable tools in medical imaging. Beyond the laboratory and clinic, nanomedicines have a profound impact on society.

The rapid and decisive response of nanomedicine developers during the recent pandemic underscored their global significance [8]. Notably, there was only a two-month gap between the genetic sequencing of SARS-CoV-2 and the start of clinical trials for mRNA vaccines [9]. The use of lipid nanoparticles (LNPs) in COVID-19 vaccines quickly altered the course of the pandemic worldwide [10]. Their effectiveness in mitigating severe forms of the disease serves as a clear example of nanotechnology's impact on a large scale. As nanomedicines gain prominence, it's important to recognize both their successes and the challenges they face.

However, the clinical translation of nanomedicines lags significantly behind the substantial investment and large number of scientific publications in the field [2] [11] [12]. Out of the 50,000 research articles published between 2018 and 2022, only nine nanoproducts made it to commercialization [11]. Despite their intended benefits over other dosage forms, nanomedicines face several biological, technical, industrial and economical challenges, contributing to their low clinical translation [2] [11] [12]. This disparity is evident when comparing the success of nanotherapeutics in preclinical studies (both *in vitro* and *in vivo*) to the outcomes in clinical trials [13] [14].

This book chapter explores the impact of nanomedicines on our current society. Societal impact is defined as the positive or negative impact on a group of the population resulting from actions, policies, or projects (**Figure 1**). In that sense, the positive outcomes of nanomedicines are evident: improving both therapy and

diagnosis, thus enhancing the overall health of the population. However, only a few studies have assessed the real interaction of nanomedicines with society, considering the nature of this interaction and potential negative outcomes.

Figure 1. Illustration of the societal impact of nanomedicines, depicting the positive outcomes (green arrow) and the negative outcomes (red arrow) that arise from the development, implementation, and commercialization of nanomedicines in our society.

In this chapter, we will examine different aspects of the phenomenon of nanomedicines. This includes discussing societal improvements attributed to nanomedicines, analyzing regulatory developments and challenges, addressing safety and environmental considerations, and exploring novel perspectives and increased awareness. Our goal is to provide insights into the progress made by nanomedicines in shaping society. Throughout our exploration, we will focus on understanding the impact of nanomedicines on healthcare, examining both challenges and opportunities that arise.

3.2. The Societal Improvements Attributed to Nanomedicine

3.2.1. Strength of the Interaction Nanomedicine-Society

The integration of nanotechnology in the field of medicine has led to significant

advancements in healthcare, thereby impacting society. Nanomedicine primarily concentrates on the delivery of active pharmaceutical ingredients (API) through both untargeted and targeted approaches [15], disease diagnosis or theragnosis [16] and radiotherapy sensitization [17]. However, the primary focus of nanomedicines currently in clinical trials lies in the domains of cancer and infectious diseases, constituting 53% and 14% of ongoing trials, respectively [5] (**Figure 2**). This emphasis suggests that the most substantial impacts are observed within these two medical fields.

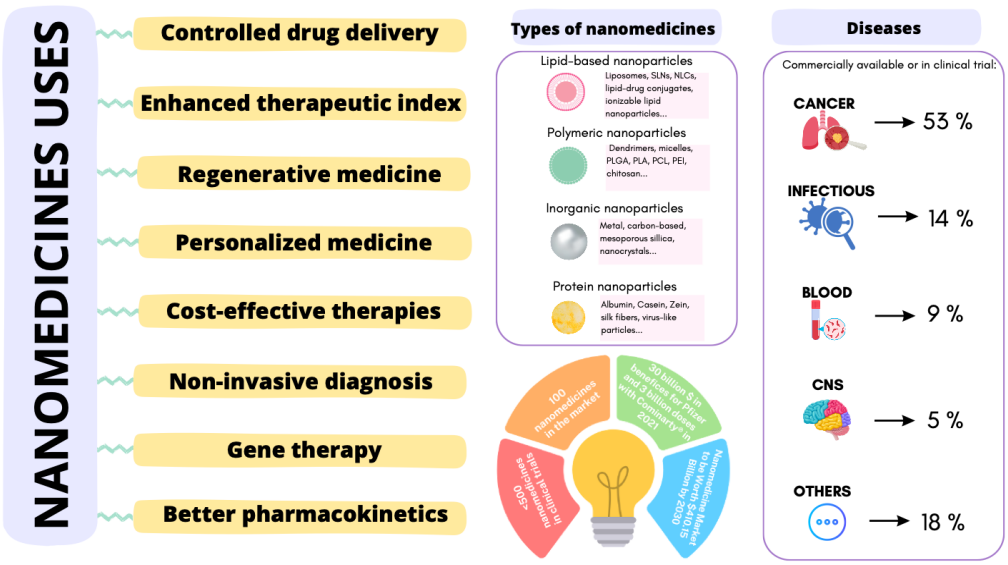

Figure 2. Overview of the status of nanomedicines. The figure depicts different users of nanomedicine, the percentage distribution of the most common available types of nanomedicines and an overview of nanomedicines that are commercially available or in clinical trials, as reported by Shan *et al.* [18]. *Abbreviations: CNS, central nervous system; NLC, nanostructured lipid carrier; PCL, Polycaprolactone; PEI, Polyethylenimine; PLA, Polylactic acid; PLGA, poly(lactic-co-glycolic acid); SLN, solid lipid nanoparticle.*

3.2.1.1 Main Categories of Nanomedicines

Nanomedicines were originally formulated as lipid-based nanoparticles (NPs) but now, a wide range of materials are employed including polymers, inorganic materials, proteins, etc. (**Figure 2**). Nevertheless, lipid-based NPs represent 33% of available nanomedicines in the market and are the most commonly developed

type [5], pointing out the major relevance of these types of carriers in the field.

1) Lipid-based nanomedicines

The first generation of liposomes, exemplified by Doxil® and Ambisome®, was formulated using the film-hydration method [8]. However, contemporary techniques, including advanced methods such as microfluidics [19], have emerged, facilitating the large-scale production of high-quality nanomedicines. Liposomes consist of a lipid layer and a water-soluble core, making them versatile carriers for both hydrophilic and hydrophobic molecules. Other lipid-based carriers and techniques have been swiftly adopted. Solid lipids, for instance, are employed to form solid lipid nanoparticles, eliminating the need for organic solvents [8] and enabling routes such as topical [20] and oral [21] administration. The latest generation of lipid-based NPs has been marked by the incorporation of ionizable cationic lipids, leading to the formation of cationic LNPs capable of efficiently transporting oligonucleotides, as observed in Onpattro® [22], Comirnaty® [23] and Spikevax® [24]. Overall, lipid-based NPs serve as excellent tools for extending the half-life and reducing clearance without increasing the toxicity of the active ingredient. An illustrative example is liposomal cytarabine (DepoCyt®), which exhibits a 40 times higher half-life than free cytarabine [25], significantly enhancing patient comfort and reducing the need for healthcare professional intervention. Due to their versatility and significant market presence, lipid-based NPs stand out as the most influential type of nanomedicines in terms of societal impact.

2) Polymeric nanomedicines

Polymer based nanomedicines account for approximately 10-15% of commercialized NPs [5]. Various polymeric systems have been utilized in the market or clinical trials, including dendrimers, protein-polymer conjugates, and polymeric micelles. In nanomedicine, the preference is typically for biodegradable and biocompatible polymers. Polymeric nanoparticles can be formulated using a variety of techniques, such as emulsification, nanoprecipitation, and even microfluidics [26]. Notably, due to their significant diversity, not all employed polymers have achieved considerable success. For instance, polymers like poly(lactic-co-glycolic) acid (PLGA), extensively researched for three decades, have not gained significant market success [9] [27], possibly attributed to a lack of comprehensive characterization. Eligard® is an exception, a polymeric nanomedicine used in palliative care

for prostate cancer patients, FDA-approved in 2002 [26]. This nanomedicine forms a solid implant after subcutaneous injection, achieving sustained release of leuprolide through its polymeric matrix made of PLGA [28]. This administration method enables over a month of sustained leuprolide release, improving the quality of life for palliative patients and demonstrating clear societal benefits for both patients and caregivers. Polymer nanomedicines, comprising a significant portion of NP innovations, encompass a diverse array of formulations currently under clinical evaluation. However, success rates vary due to differences in thorough characterization and the wide variety of polymers being evaluated.

3) Inorganic nanomedicines

Among inorganic nanoparticles, three major groups should be considered: carbon-based nanoparticles, metal nanoparticles, and mesoporous silica nanoparticles. In the case of carbon-based nanoparticles, graphene oxide nanoparticles have been extensively explored due to their stimuli-responsive properties [29] [30]. Other nanomaterials are gaining relevance, such as quantum dots, semiconductor crystals that enable both imaging and direct targeting [31]. Quantum dots are gaining exponential relevance, exemplified by the Nobel Prize in Chemistry awarded in 2023 to Moungi Bawendi, Louis Brus, and Alexei Ekimov for their discovery and research in this area. On the other hand, metal nanoparticles may not seem to be optimal drug carriers due to their physicochemical nature, but they possess excellent features that make them effective anticancer and antimicrobial agents (via ROS mechanisms) [32]. Metal nanomedicines can also be used as photo-thermal therapies [33] and even in combination with other materials as stimuli-responsive nanomedicines [34]. For example, superparamagnetic iron oxide nanoparticles (SPIONs) can be used for imaging, iron deficiency [35] and theragnosis [36]. In fact, several commercialized iron nanomedicines are available: Dexferrum® (American Regen, 1996), Venofer® (Luitpold Pharmaceuticals, 2000), and Hensify® (Nanobiotix, 2019) [5] [26]. Another major category involves the use of nanocrystals, extensively explored by Elan Nanosystems. For instance, the nanocrystal form of aprepitant (Emend®) successfully addressed the solubility issue of the drug while enhancing its gastrointestinal absorption [37]. This technology was also applied successfully to rapamycin, resulting in Rapamune®, a potent immunosuppressor. In conclusion, there are many inorganic nanomedicines that can influence population health in diverse ways. Although this significant

diversity highlights the interest in these technologies, it also underscores the difficulties in correctly characterizing them and predicting their fate once administered. Major concerns about the toxicity and bio-persistence of some inorganic products are among the reasons that their development might face challenges in the future.

4) Protein nanomedicines

With the increase of the number of known protein-structures in the last 20 years, the research on protein nanomedicines has significantly improved [38]. Protein-based nanomedicines can come from two different areas, first from mimicking virus structures or extracted from viruses, as for virus like particles [39]. Secondly, these types of nanomedicines can be obtained by the self-assembly of protein sources [40]. The advantages of protein nanomedicines include their highly ordered surface patterns, good geometry, and effectively cell internalization [38]. The rise of protein nanomedicines begun with the development of albumin-based nanomedicines containing paclitaxel (Abraxane®) [41]. Proteins such as gelatin, elastin, zein, casein have also been explored as potential nanocarriers using different techniques including coacervation, emulsion/solvent extraction or self-assembling [42]. Other examples include the use of heat shock proteins and more complex structures, providing the opportunity to utilize specific proteins as targeting agents [43]. Heat shock proteins, have been studied as tumor microenvironment-targeting nanomedicines due to their stimuli-responsive properties, exhibiting specific tumor biodistribution properties and promising antitumor effects in preclinical mouse models. In conclusion, the surge in known protein structures and the advancements in biotechnology over the past decades have driven research in protein nanomedicine. However, due to the diversity in this field, much research is yet to be done.

3.2.1.2. Development of Nanomedicines: The Economical Perspective

The nanomedicine market is undergoing substantial expansion, with more than 500 nanomedicines currently undergoing clinical trials [18]. Projected benefits are expected to increase from the 180 reported in 2018 to 400 billion dollars in the next few years [24] [44]. These data unequivocally illustrate the growing interest in the utilization of nanomedicines, a trend attributed to the factors outlined in **Figure 2**. Similar to other pharmaceuticals, the development of nanomedicines

requires a minimum of 10 years, and typically, the patents granted to industrial developers last only for 20 years, leaving a brief window for ensuring economic profitability. This imparts significant pressure on nanomedicine developers, potentially influencing them to avoid high-risk situations or to sidestep the treatment of rare diseases, thereby constraining the development of pioneering ideas.

The economic cost of nanomedicine development stands as the second most significant factor in the nanomedicine-society interaction, following health considerations. Research and development costs for a drug candidate have been reported to exceed 2.5 billion dollars [45]. Notably, costs in the clinical phase, particularly in Phase III, are logically higher [45]. Estimates reveal a 164% increase for drugs successfully applying for new drug applications and an 83% increase for those seeking biologic license applications, compared to drugs that fail in Phase III clinical trials. This underscores the critical role of economic investment in the success of clinical trials. However, it is essential to acknowledge that the source revealing these statistics, is supported by pharmaceutical and biotechnological companies [37], raising concerns regarding potential conflicts of interest. Additionally, the lack of availability of data regarding the drugs selected for the study and the raw numbers on which the analysis was based introduces skepticism regarding these reported costs [46]. Nevertheless, the costs associated with nanomedicine development remain a paramount concern for pharmaceutical companies, significantly influencing the societal impact of these therapeutic approaches. Typically, small companies involved in nanomedicine development secure funding through investors, capital markets, and partnerships with larger pharmaceutical entities. The survival of start-ups and innovative small companies hinges on the success of clinical trials. Indeed, failure in clinical trials can result in the termination of small companies or bankruptcy [47], as exemplified by the case of BIND Therapeutics with docetaxel-loaded polymeric NPs [48]. Clinical trial failures may arise due to the inability to demonstrate improved efficacy compared to commercially available forms, with unpredicted toxicity often emerging as the most limiting factor, especially in Phase I clinical trials [47]. This leads us to the conclusion that not only do marketed nanomedicines impact our society, but the failure of a nanoproduct in clinical trials can also have repercussions for society, necessitating consideration as part of the overall impact assessment of nanomedicines.

In conclusion, the societal impact of nanomedicine development is characterized

by promising growth, as evidenced by the substantial number of ongoing clinical trials and the projected increase in potential benefits. However, the intricate interplay between economics, pharmaceutical interests, and societal implications poses challenges. The stringent timeline imposed by patent expiration can limit innovation and discourage endeavors in the treatment of rare diseases. The exorbitant costs associated with drug development underscore the crucial role of financial input, although concerns about transparency in this matter persist. It is evident that the impact of nanomedicine on society extends beyond successful market entries; the failures in clinical trials also bear significant consequences for patients, workers, and the economic stability of markets. This emphasizes the complex and multifaceted impact that nanomedicines have on populations.

3.2.2. Examples of Societal Improvements Attributed to Nanomedicines: The Example of Cancer and Infectious Diseases

3.2.2.1. Cancer

The pioneering development of liposomes marked the inception of commercialized nanomedicines designed to combat cancer with unprecedented precision and efficacy. Over the years, an array of novel nanomedicines, including Daunoxome®, Abraxane®, Vyxxeos®, and many others, have emerged as powerful tools in the oncologist's arsenal. The introduction of Doxil® has left a lasting legacy, ushering in a new era of precision medicine in the battle against cancer. Researchers continue to explore and innovate in the field of nanomedicine, building on the efficacy and reduced cardiotoxicity demonstrated by this liposomal doxorubicin. This effectiveness is underscored by the findings of Xing *et al,* who conducted a comprehensive meta-analysis of 10 randomized controlled trials [49]. The high demand for Doxil® and the growing shortages [50] prompted the swift approval of the generic form (Lipo-Dox®) by Sun Pharma Global FZE in 2013 [51]. These shortages are a cause for concern among oncological patients, leading to the importation of foreign liposomal doxorubicin into the US under "exercise enforcement discretion", implying that the drug did not require advanced approval from the FDA. According to a market analysis report in 2015, the market size of liposomal doxorubicin reached 814.6 million dollars and was expected to grow due to its clear benefits over free doxorubicin and the rising prevalence of breast and ovarian

cancers [52]. Clearly, this drug holds significant implications for both patients and the market, and the shortage of liposomal doxorubicin is a major concern that should be considered in the near future.

Recognized as an essential medicine by the World Health Organization (WHO), the absence of liposomal doxorubicin has prompted swift political decisions to ensure its supply, highlighting the robust interaction between this specific nanomedicine and society.

The transition from the free-form Taxol® paclitaxel formulation to the albumin-based nanoparticle Abraxane® has significantly influenced the interaction between nanomedicine and society. Abraxane® has demonstrated enhanced safety compared to classic paclitaxel formulations, notably allowing for an increased maximum tolerated dose—49% higher than paclitaxel without the need for corticosteroid preventive treatment [53]. The reduction in toxicity is primarily attributed to the absence of Cremophor EL® in the formulation, aligning with the recognized knowledge that such excipients can induce severe hypersensitivity reactions. Since its approval in the US in 2005 and in Europe in 2008, Abraxane® has progressively gained market dominance over Taxol, with estimated sales reaching around 1 billion dollars in recent years [41]. Despite this remarkable success, the development of various paclitaxel nanoformulations by different companies may appear unproductive. However, concerns have arisen due to shortages of Abraxane®, as reported by the Japanese Medical Association in 2021 [54] and by the European Medicines Agency (EMA) in 2023.

These two prominent examples underscore the pivotal role of nanomedicines in the landscape of cancer treatment. The increasing shortage of these nanomedicines poses a significant new challenge in the era of nanomedicine that needs prompt attention to ensure the ongoing societal benefits of nanomedicines. One of the challenges faced in nanomedicine lies in the technical aspects of production. Ensuring a continuous production of high quality is a hurdle that must be overcome to meet the Good Manufacturing Practices (GMP) required for releasing a batch from a manufacturing plant [47]. In this context, the promising future of nanomedicine in cancer treatment hinges on the correct and sufficient production necessary to consistently meet the needs of patients.

3.2.2.2. Infectious Diseases

Until March 2020, many Western societies held the belief that infectious diseases no longer held the potential to alter the course of history. However, the subsequent events have highlighted the importance of remaining vigilant against unforeseen challenges. The emergence of antimicrobial nanomedicines coincided with the development of anticancer nanomedicines. An early example is the liposome formulation carrying amphotericin B, known as AmBisome®, designed for the treatment of serious fungal infections in febrile neutropenic patients [55]. This formulation offered the advantage of a reduced volume of distribution and a distinct biodistribution pattern, notably decreased distribution to the kidneys, resulting in significantly reduced toxicity [56].

In the context of bacterial infections, antibiotic resistance stands as a pressing challenge confronting contemporary society. As bacteria continuously evolve, developing resistance to commonly employed antibiotics, our capacity to effectively treat infections faces escalating limitations. This phenomenon not only jeopardizes individual health but also extends its ramifications to healthcare systems, economies, and public well-being on a global scale. Nanomedicine contributes to addressing this challenge by enhancing the delivery of conventional antibiotics, as exemplified by ARD-3150 [57] (liposomal ciprofloxacin for the management of bronchiectasis by inhalation). Furthermore, nanomedicine plays a role in disrupting bacteria's protective biofilms [58]. Biofilms shield bacteria, and certain NPs possess the ability to penetrate and disrupt these biofilms, facilitating the accessibility of antibiotics to eliminate bacterial cells.

Nanomedicines, in addition, hold promise in overcoming evolving resistance patterns in bacteria, providing a crucial flexibility in combating constantly changing bacterial threats. However, it's important to note that nanomedicines themselves are not immune to resistance. For instance, silver nanomedicines, employed as broad-spectrum antibacterial agents in topical ointments, are encountering the emergence of silver-resistant bacteria [59]. This raises questions about whether the benefits outweigh the risks and costs associated with pursuing the path of broad-spectrum nanomedicines for infectious diseases. Perhaps a more focused approach targeting specific vulnerabilities could mitigate the risk of antibioresistance. Regardless, nanomedicine is poised to play a pivotal role in the

ongoing battle against antibio-resistance, shaping the landscape of our societies in this regard.

Vaccine challenges were successfully addressed with the help of nanomaterials-based delivery systems [60]. In terms of their preclinical evaluation, the EMA's "Note for guidance on preclinical pharmacological and toxicological testing of vaccines" was published in 1997 and has not been updated since [61]. As a result, the EMA committee for medical products for human use recommends adhering to the guidance published by the WHO in 2005, given its crucial global role in vaccine development [62] [63]. The WHO guidelines on the nonclinical evaluation of vaccines do not specifically mention any considerations for nanoparticles (NPs). The document covers both therapeutic and prophylactic vaccines for infectious diseases. The guidelines recommend conducting immunogenicity studies, a toxicity assessment measuring local inflammatory reactions and potential effects on the draining lymph nodes, systemic toxicity, and the immune system. Local tolerance studies and other assessments are recommended on a case-by-case basis. The first generation of nano-based vaccines involved the encapsulation of inactivated viruses within NPs, typically administered intramuscularly. For example, the hepatitis A vaccine Epaxal® consisted of the RG-SB strain deactivated with formalin and contained in liposomes called virosomes [64]. In the case of influenza, another virosome carried its immune activity by containing inactivated hemagglutinin proteins [65]. All types of nanomedicine-based vaccines have been extensively described, culminating in the latest generation—the COVID-19 vaccines [66]. Much has been discussed and written about these RNA-loaded ionizable lipid NPs [67] and significant societal changes are anticipated due to the RNA-lipid NP revolution. Ongoing clinical trials, such as the phase II/III trials by Moderna on the mRNA-1345 vaccine against Respiratory Syncytial Virus (RSV) (NCT05127434) in patients aged ≥ 60 years, or the mRNA-1010 vaccine against seasonal influenza in healthy adults (NCT05827978), illustrate the breadth of current research. Another notable example is the recent phase I study (NCT05414786), evaluating the safety and immunogenicity of the eOD-GT8 60mer mRNA Vaccine (mRNA-1644) in HIV-1 Uninfected Adults in Good General Health by Moderna. This prophylactic vaccine against HIV-1 effectively activated B cell precursors of VRCO1-class broadly neutralizing antibodies (bnABs) in 97% of vaccine recipients.

In conclusion, the evolution of infectious disease treatment and prevention

through nanomedicine is a pivotal response to the challenges posed by evolving infections and resistances. While presenting promising advancements and solutions, the balance between benefits and risks remains essential in shaping the future of infectious disease management and societal well-being.

3.3. Impairments and Regulatory Evolutions

3.3.1. Limitations Encountered by Nanomedicines

One of the main challenges faced by nanomedicine researchers and industrials is the proper characterization and biological evaluation of the developed nanocarriers (**Figure 3**) [68].

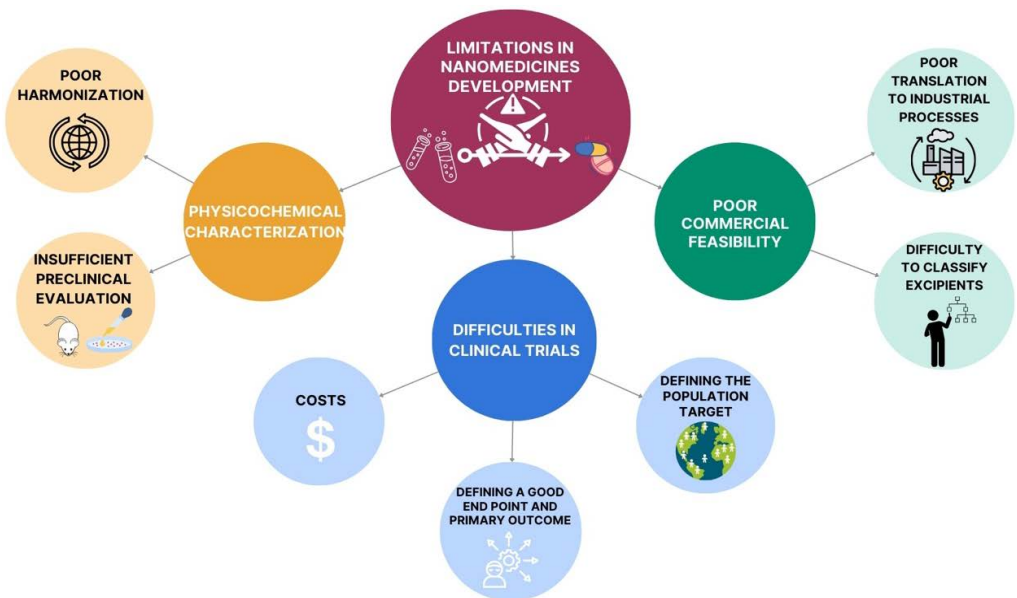

Figure 3. Limitations encountered by nanomedicines to reach the market. This figure outlines the primary obstacles faced by nanomedicines on their path to market entry. The key challenges depicted include issues related to proper physicochemical characterization (highlighted in the yellow circle), challenges encountered during clinical trials (indicated in the blue circle), and concerns regarding commercial feasibility (outlined in green). Each of these overarching limitations is further illustrated with specific examples in the smaller circles for clarity and emphasis.

Reproducibility limitations and complex nanoscale behaviors pose significant hurdles to the clinical translation of certain nanomedicines. The intricacies and

unpredictability at the nanoscale contribute to variations in drug encapsulation, release profiles, and biological interactions. In terms of biological challenges, a notable gap exists in understanding the specific mechanisms of action for some nanomedicines [2] [11] [12] [69]. The fate of a nanomedicine post-administration hinges on its surface interaction with the organism's biological environment, ultimately reaching the target tissue Initially, nanomedicines must surmount various biological barriers, including crossing the gastrointestinal barrier, traversing the blood-brain barrier, and navigating hostile action sites such as the tumor microenvironment [70]. Once in the bloodstream, potential challenges such as aggregation, adsorption to plasma proteins, premature release, and recognition as exogenous antigens leading to allergic or inflammatory responses can occur [12] [13]. To achieve its therapeutic action, a nanomedicine must overcome additional hurdles, including membrane permeability, endosomal and lysosomal escape, proper intracellular processing, and effective trafficking [2] [14] [70]. Given these complexities, a robust understanding of preclinical characterization is crucial when seeking marketing authorization. A lack of such understanding can lead to direct failure, even if clinical trials are successful. A case in point is Opaxio® (paclitaxel-loaded nanomedicine), which was withdrawn from its marketing application due to the inability of CTI Life Sciences Ltd. to address concerns raised by the Committee for Medicinal Products for Human Use (CHMP) from the EMA. One of the key concerns was the insufficient understanding of paclitaxel release from the nanomedicine and its biodistribution profile [71].

The development of nanomedicines encounters a myriad of challenges in ensuring high-quality production processes and reproducibility [47]. Consistency in manufacturing processes is hindered by the absence of standardized protocols, impeding regulatory approval and widespread adoption. Additionally, the sensitivity of nanomedicines to environmental conditions during production and storage poses a significant hurdle, requiring precise control over parameters such as temperature or humidity. The complexity of nanomedicine formulations and the necessity for rigorous characterization further exacerbate these challenges. For instance, concerns have been raised regarding the classification of excipients and active ingredients for nanomedicine [72]. General impairments in the development of nanomedicines include the lack of harmonized methods to characterize the safety and efficacy of these innovative formulations.

Clinical trials also pose significant challenges in nanomedicine development, as illustrated in **Figure 3**. Firstly, inherent costs associated with the trial, as mentioned in previous sections, contribute to the complexity of the process. Additionally, selecting a study population that statistically aligns with the target population for treatment is crucial. In some cases, especially during the early stages of clinical trials, researchers may exclude individuals with advanced conditions or older patients. This exclusion can result in a poor correspondence between the study population and the real-life population. Such practices have historically influenced health disparities. In the 20th century, clinical trials for certain drugs were predominantly conducted on middle-aged white men [73], leading to suboptimal drug outcomes in minorities and women [74]. Despite ongoing efforts, minority representation in clinical trials remains a significant issue, particularly in countries like the US [75]. Nanomedicine developers need to consider this matter when designing clinical trials. Beyond ethical considerations, LaVeist *et al.* argued that eliminating health disparities for minorities could reduce medical care expenditures by more than 1.2 trillion dollars [76], underscoring the societal impact of this issue.

In conclusion, maintaining a favorable benefit-risk balance in the interaction between nanomedicines and society is paramount. It is crucial to identify and address the limitations encountered by these treatments during preclinical assessment, GMP production, and clinical trials. As the market share of nanomedicines continues to grow, their societal impact will also increase. Therefore, these challenges should receive heightened attention in the coming years. Addressing these issues will not only ensure the continued advancement of nanomedicine but also enhance its positive impact on society.

3.3.2. Regulatory Evolutions

Regulatory agencies, such as the FDA and the EMA, serve as gatekeepers in managing the interaction between nanomedicines and society, with ever-increasing demands on their oversight. Examining the FDA's case, a report from 2020 revealed that since 1970, the agency received 600 applications for nanomedicines, half of which were submitted in the last decade, illustrating the exponential growth of the nanomedicine market. This surge suggests that regulations may be playing catch-up and are lagging behind the rapid pace of technological advances [77].

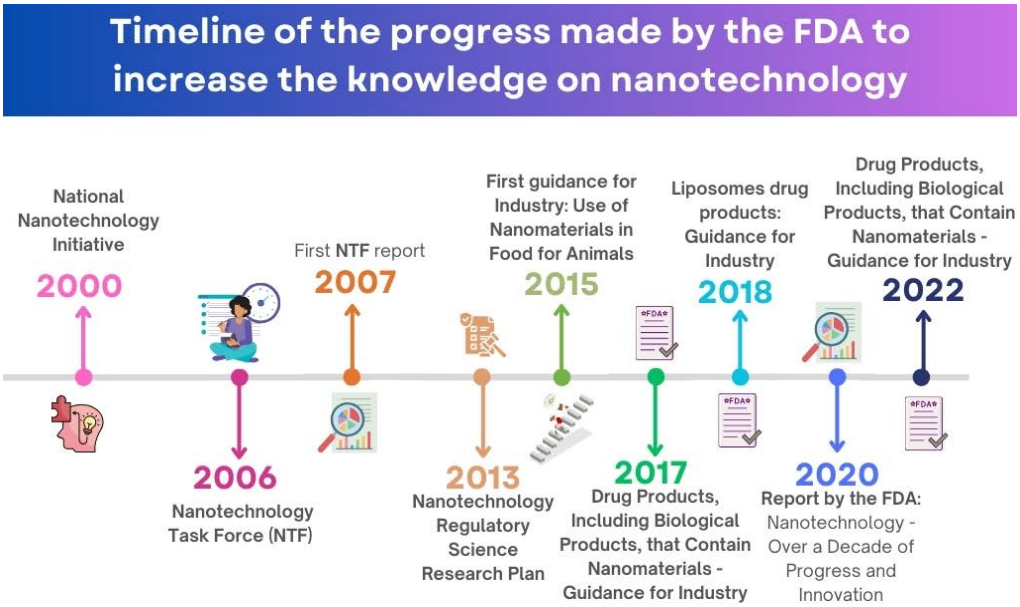

Figure 4. FDA's Regulatory Progress in Nanomedicine (2000-2022). This non-exhaustive timeline delineates various measures undertaken by the FDA from 2000 to 2022 to enhance regulatory comprehension of nanomedicines.

As illustrated in **Figure 4**'s timeline, the establishment of the National Nanotechnology Initiative in the US in 2000 led to significant advancements in understanding nanomedicines, although it did not introduce any accompanying regulations. Subsequently, in 2006, the formation of the Nanotechnology Task Force (NTF) aimed at improving comprehension, particularly to foresee the advancements in nanomedicines and potential requirements during FDA submissions. This effort resulted in the publication of the first report in 2007.

However, even in the 2010s, International Council for Harmonisation (ICH) guidelines encountered challenges in providing specific evaluations for nanomedicines. For instance, the "ICH guideline S9 on nonclinical evaluation for anticancer pharmaceuticals," designed for anticancer therapies, is applicable to small molecules and biopharmaceuticals [78]. This guideline only briefly addresses certain aspects of liposome evaluation. It specifies that "a complete evaluation of the liposomal product is not warranted if the unencapsulated material has been well characterized." Nevertheless, the safety assessment should encompass the final product along with some data regarding the unencapsulated material and the

carrier.

In 2013, the FDA published the Nanotechnology Regulatory Science Research Plan, providing a framework for nanomedicine regulation. A significant milestone occurred in 2015 when the FDA issued guidance on determining whether an FDA-regulated product involves nanotechnology. Despite this, the approval process for nanomedicines follows the same steps as other drug formulations. The latest guideline, titled "Drug Products, including biological products that Contain Nanomaterials," was released in April 2022 [79]. Notably, these recommendations are nonbinding, serving as guidance for manufacturers in developing novel nanomedicines. The document introduces a risk-based framework, emphasizing the importance of proper nanomaterial characterization and a thorough understanding of its intended use and application for ensuring safety. **Table 1** provides an overview of the nanomedicine, aligning with this regulatory approach. FDA's commitment to nanotechnology research is evident, with a cumulative investment exceeding $133 million since 2009 [80].

Table 1. Recommendations extracted from the FDA document: Drug Products, Including Biological Products, that Contain Nanomaterials Guidance for Industry [79].

Recommended Factors for Assessment of the Nanomaterial (non-comprehensive list by the FDA in the 2022 Guidance for industry)
• Adequacy of characterization of the material structure and its function • Complexity of the material structure • Understanding of the mechanism by which the physicochemical properties of the material impact its biological effects (effect of particle size on pharmacokinetic properties) • Understating the in vivo release mechanism based on the material's physicochemical properties • Predictability of *in vivo* release based upon established *in vitro* release methods • Physical and chemical stability • Maturity of the nanotechnology including manufacturing and analytical methods • Potential impact of manufacturing changes, including in-process controls and the robustness of the control strategy on critical quality attributes (CQAs) of the drug product • Physical state of the material upon administration • Route of administration • Dissolution, bioavailability, distribution, biodegradation, accumulation, and their predictability based or physicochemical parameters and animal studies*

*According to the FDA's recommendation on the application of the principles of the 3Rs for animal use in testing when appropriate.

Although rare nowadays, regulatory differences between the FDA and EMA can complicate tasks for drug developers. The lack of harmonized applications to regulatory agencies can result in varying approval timelines globally, independent of patient needs. An example is highlighted by Hemmrich and McNeil [72], concerning mRNA-LNPs for COVID-19. Moderna's submission for Spikevax® saw differences in how EMA and the FDA classified ionizable lipid SN-102 and the PEG-lipid (PEG200-DMG); EMA considered them excipients, while the FDA deemed them "starting materials for the drug substance." Furthermore, the distinction between active ingredients and excipients has become ambiguous, as excipients are not expected to exert therapeutic or diagnostic effects. For instance, silver NPs can carry drug molecules while actively influencing treatment. Additionally, modifications in the fate of the active compound can significantly impact efficacy and toxicity, exerting a therapeutic effect indirectly. In lipid NPs, for example, ionizable lipids are directly linked to therapeutic efficacy, playing a crucial role in the endosomal escape of nucleic acids, a critical step for their effectiveness.

In parallel, various organizations are actively promoting the development of nanomedicines to tackle the new challenges facing our society in healthcare. In the US, for instance, the National Nanotechnology Initiative has received significant federal funding over the years, as mentioned earlier [37]. In Europe, the European Technology Platform on Nanomedicine (ETPN), established in 2005, is an association led by both the industry and the European Commission (EC). It aims to address the implications of nanomedicines in the diverse healthcare systems of the 27 European Union member states [81]. The ETPN endeavors to engage all stakeholders, including academia, industry, healthcare professionals, policymakers, and more, in harnessing the benefits of nanomedicines for society. However, the dynamic landscape of nanomedicine underscores the increasing challenge faced by regulators. Despite considerable efforts to catch up, the pace of regulation struggles to keep up with rapid advancements, resulting in a disparity in global approvals and adding complexity to drug developers' endeavors.

3.4. Nanotoxicology and Environmental Implications

3.4.1. Nanomedicines and Nanotoxicology

The utilization of nanomedicines has increased in recent years, necessitating a focused examination of their toxicity implications for human health [70]. Indeed,

nanotoxicology has evolved into an independent research field [82]. Like any product intended for medical use, nanomedicines must undergo rigorous safety and efficacy assessments both before and after commercialization [83]. Notably, some nanomedicines, after successfully completing clinical trials, were subsequently withdrawn from the market due to efficacy and/or toxicity concerns, such as Feruglose® or Resovist® [84]. Despite being evaluated in accordance with international and national regulatory agencies, clear safety guidelines specific to NPs are yet to be established [83] [85]. The absence of specific safety regulations necessitates decisions to be made through a case-by-case evaluation, a process that is time-consuming and requires high-level expert input [14] [70].

In 2006, the experts of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) within the European Commission conducted a review of the safety methodologies for risk assessment of nanomaterials [86]. Emphasis was placed on the lack of knowledge concerning the characterization, detection, and fate of nanomaterials in humans and the environment, posing challenges for the comprehensive safety evaluation of these materials. It was noted that while existing eco-toxicological methods may be suitable for assessing some hazards related to NPs, they might not be sufficient for a thorough evaluation. Nevertheless, nanomedicines approved and commercialized by the EMA to date have undergone evaluation under the General Medicinal Product legislation, specifically the European Directive 2001/83/EC of the European Parliament and the Council of November 6, 2001, on medicinal products for human use [14] [85]-[88]. According to this directive, authorization for a medicinal product requires the submission of results from physiochemical, biological or microbiological, toxicological, and pharmacological tests, as well as clinical trials. Certain exceptions are outlined for information that may not be necessary to provide, especially when similar medicines are already approved and marketed—a section amended in 2004 In the absence of specific or adapted guidelines for the safety evaluation of nanomedicines, the recommended strategy is to adhere to International Council for Harmonization (ICH) guidelines. For instance, the ICH guideline M3 (R2) *on non-clinical safety studies provides guidance for conducting human clinical trials and obtaining marketing authorization for pharmaceuticals* [89]. However, the applicability and limitations of this guideline concerning the testing of nanomedicines remain uncertain.

The EMA has issued reflection papers for specific nanomedicine products, each structured differently for safety evaluation. Firstly, a concise "Reflection Paper on Nanotechnology-based Medicinal Products for Human Use" was published in 2006 [86]. This document underscores the knowledge gap concerning the characterization of NPs for effective risk assessment, noting that existing eco-toxicological evaluation methods may not be suitable for NPs. In 2011, the "Reflection Paper on Non-clinical Studies for Generic Nanoparticle Iron Medicinal Product Applications" was published [90]. It discusses the influence of the physicochemical properties of a generic product compared to the reference product on safety and efficacy. Similarly, the "Reflection Paper on Surface Coatings: General Issues for Consideration Regarding Parenteral Administration of Coated Nanomedicine Products," published in 2013, addresses the impact of coating on the physicochemical properties of nanomedicines, consequently influencing their efficacy and safety [91]. Two more reflection papers were published in 2013: the "Reflection Paper on the Data Requirements for Intravenous Liposomal Products Developed with Reference to an Innovator Liposomal Product," providing advice on generating relevant data for quality assessment, non-clinical studies, and clinical studies [92], and the "Reflection Paper on the Development of Block Copolymer Micelle Medicinal Products," offering suggestions for quality and non-clinical evaluation and the conduct of first-in-human studies [93]. Both reflection papers refer to relevant ICH guidelines for consultation, which, originally developed for the assessment of conventional medicines, still have unknown limitations concerning the evaluation of nanomaterials.

During the preclinical development of a drug, the FDA mandates toxicological and pharmacological *in vitro* and *in vivo* testing, encompassing genotoxicity screening, information on absorption, metabolism, and excretion, and an assessment of metabolite toxicity. As mentioned previously, the April 2022 FDA guideline for nanomaterials emphasizes evaluating both the safety and effectiveness of a product in light of its intended use [79]. Notably, the guideline does not prescribe a specific toxicity testing strategy.

In conclusion, it is imperative to promptly address the specific human health toxicity concerns associated with nanomedicines through comprehensive safety evaluations. While regulatory efforts have been made, the lack of specific guidelines

necessitates a case-by-case approach, underscoring the ongoing requirement for precise safety standards in the development of nanomedicines.

3.4.2. Nanotoxicology and Nanomaterials Used in Medical Devices

To our knowledge, the most comprehensive guidelines currently available, outlining a safety assessment strategy, are specific to medical devices containing nanomaterials rather than encompassing all nanomedicines. In 2015, the SCENIHR published the Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices [84]. This guidance not only offers suggestions for test batteries but also references corresponding International Organization for Standardization (ISO) or Organization for Economic Co-operation and Development (OECD) guidelines, detailing the applicable methods for each. It presents a comprehensive toxicology strategy, even specifying a battery of genotoxicity tests to be conducted. Importantly, the document acknowledges potential limitations in applying these tests to nanoparticles. Emphasis is placed on the critical role of accurate physicochemical characterization as the initial step in the evaluation, providing parameters to be assessed and the methods to acquire them.

Similarly, in 2017, the ISO issued a comprehensive guide titled "Biological evaluation of medical devices, Part 22, Guidance on Nanomaterials for the characterization, evaluation of the toxicokinetic and toxicology, and risk assessment of medical devices containing nanomaterials intended for human use" (ISO/TR 10993-22:2017). This detailed guide encompasses information on various aspects: 1) the essential physicochemical properties to be characterized along with recommended methods; 2) instructions for preparing materials for testing; 3) evaluation of release; 4) recommendations for toxicokinetic assessment, citing the relevant guide and providing information on factors influencing the assessment; 5) detailed instructions for conducting toxicological evaluation, referencing corresponding ISO or OECD guidelines; 6) guidance on performing risk assessment.

3.4.3. OECD Guidelines in the Evaluation of Nanotoxicities

The OECD compiles globally relevant testing methods widely used by governments, industry, and independent laboratories, providing internationally agreed Guidelines for the Testing of Chemicals and Good Laboratory Practice. The primary goal is to streamline the safety assessment of chemicals, fostering harmonization

and ensuring the generation of high-quality, reliable data [86]. The OECD guidelines, organized into five sections—Physiochemical Properties, Effects on Biotic Systems, Environmental Fate and Behavior, Health Effects, and Other Test Guidelines—are pivotal in regulatory safety testing. Regulatory agencies consistently turn to these guidelines when evaluating the safety of a product, necessitating that the assessment assays adhere to these standardized procedures.

The OECD guidelines are currently undergoing a comprehensive review to assess their applicability to nanomaterials. Initiated in 2017, the Health Effects section has seen adaptations in only two test guidelines thus far: the Test Guideline for Subacute Inhalation Toxicity, 28-Day Study [87], and the Test Guideline for Subchronic Inhalation Toxicity, 90-Day Study [88]. These guidelines have been tailored to test particle aerosols, including nanoparticles, introducing specific requirements like additional lung effect measurements, preliminary studies, and new standards tailored to nanoscale dimensions. The ongoing review includes four more documents in this section: the Guidance Document on Inhalation Toxicity Studies [89], the *In Vitro* Mammalian Cell Based Genotoxicity TGs, the In Vitro Skin Sensitization Guideline [90], and the Integrated In Vitro Approach for Intestinal Fate of Orally Ingested Nanomaterials. Additionally, a guidance manual for testing manufactured nanomaterials under the OECD sponsorship program [91] provides sponsors with a comprehensive plan for developing a Dossier Development Plan for a specific nanomaterial. This manual encompasses various endpoints, including identification, characterization, environmental fate, eco-toxicological effects, environmental toxicity, mammalian toxicity, and material safety, with each section referencing the corresponding OECD guidelines for the required assays.

Within the Health Effects section of the OECD guidelines, ongoing adaptations for nanomaterial testing are in progress. For instance, the TG 487 *in vitro* micronucleus test has undergone review, with a published report assessing its applicability to nanomaterials [94]. While certain *in vivo* assays appear more straightforward to adapt, as evidenced by already reviewed OECD TGs [95] [96], certain *in vitro* assays, such as the widely used Ames test for chemical evaluation, are deemed impractical for nanomaterials. This is due to challenges in nanomaterial penetration of bacterial cell walls [107]-[110]. Consequently, the Mouse Lymphoma

Assay (MLA) [111] is often employed as an alternative for nanomaterial assessment [108]. Insights from the OECD Expert Meeting on Genotoxicity of Manufactured Nanomaterials support this adaptation [112]. Although not directly applicable to nanomedicines, the European Food Safety Agency (EFSA) has updated its genotoxicity strategy, specifically recommending the MLA for detecting gene mutations in nanomaterials [113]. While OECD TGs continue to undergo review and adaptation for nanomaterials, seeking or developing alternatives becomes crucial when certain assays prove unsuitable. In other sections of the OECD guidelines, revisions are also underway. For example, in the Environmental Fate and Behavior section, OECD TG 318 (Dispersion Stability of Nanomaterials in Simulated Environmental Media) has already been updated [105]. Furthermore, the European Chemicals Agency (ECHA) integrated nanomaterials into the existing REACH regulation in 2020, introducing specific information requirements and revising annexes concerning characterization, safety assessment, registration requirements, and user obligations [69] [114] [115]. The annexes, encompassing characterization (annex VI), safety assessment (annex I), registration requirements (annexes III and VII-XI), and user obligations (annex XII), could be explored for potential applicability to nanomedicines.

3.4.4. Physicochemical Properties and Safety

As described throughout the text, a consensus among regulatory agencies is that the physicochemical properties inherent to the “nano” nature of nanomedicines directly influence their efficacy and safety. It is well-established that even slight changes in these properties can lead to a reduction in nanomedicine efficacy or an increase in toxicity, resulting in unintended effects and posing risks to human health [69] [98] [99]. These characteristics profoundly impact biocompatibility, biodistribution, interactions with tissues and membranes, toxicity, accumulation, and clearance [69] [98]. Given that nanomedicines are complex three-dimensional products composed of multiple components, rigorous characterization of each component and their interactions is imperative to ensure the success of clinical translation [58] [69].

Within the extensive list of characteristics to be determined for a nanomaterial, the most crucial ones include composition, purity, size, size distribution, surface area, shape, aspect ratio, surface coating, surface charge, stability, polydispersity,

and drug loading and release [98]-[101]. An illustrative example of how these physicochemical characteristics impact safety is as follows: the specific composition of each material directly influences its toxicity; size and surface area play vital roles in bio interactions, distribution, deposition, and elimination; the size of a particle dictates its ability to traverse bodily barriers and its duration in the bloodstream; as the size of a particle decreases, the surface area increases, resulting in heightened reactivity and toxicity; increased reactivity can trigger inflammatory responses; size, shape, and aspect ratio are interconnected with penetration, tissue distribution, and alteration of intracellular processes; surface characteristics such as coatings and charge may influence corona formation, circulation time, blood clearance, interaction with target cells, uptake, and permeability through membranes; and morphology influences the interactions of the nanomedicine with cells.

As observed, the mentioned physicochemical properties are interconnected, impacting various effects of a nanomedicine. Altering one property can affect others, creating a cascade of variations. Therefore, tailoring these properties is crucial for controlling nanotoxicity. The urgent need for common and validated protocols in the physicochemical characterization of nanomedicines becomes evident as a vital step in their development. It is not only essential to characterize each component of the materials and their interactions but also to understand their interactions with the organism and the changes that may occur upon administration. Currently, the OECD has published guidelines for the characterization of NPs, including TG No. 124: Volume Specific Surface Area of Manufactured Nanomaterials, TG No. 125: Nanomaterial Particle Size and Size Distribution, TG No. 126: Determination of Hydrophobicity Index, and TG 318: Dispersion Stability of Nanomaterials in Simulated Environmental Media. [102]-[105].

3.4.5. Environmental Implications of Nanomedicines

Describing the environmental impact of nanomedicines poses a challenge, emphasizing the need for immediate action to reduce their ecological footprint and ensure sustainable practices in this field. While initial assessments suggest lower risks due to minimal exposure [97], the larger-scale use, such as during the COVID-19 vaccination, reveals significant concerns. Data on the environmental repercussions of widespread nanomedicine use remains scarce, despite existing evaluation

systems like REACH, which currently exclude pharmaceutics, including nanomedicines [98]. Other frameworks, such as BIORIMA, aim to evaluate the risks of biomaterials, including nanomedicines [99] funded by the European Union and involving a consortium of entities from academia, private companies, and technological centers.

While newly marketed nanomedicines often include non-toxic components, preclinical research involving potentially hazardous materials, such as metals [100] [101], and fluoropolymers [100] [102], raises concerns about environmental contamination. Embracing newer technologies like DNA origami [103] or nanobots [104] introduces uncertainties regarding their ecological impact and patient safety. Addressing the environmental crisis requires researchers to advocate for optimized laboratory practices and reduced waste production. Leveraging computer tools, Quality By Design (QBD), and Design of Experiments (DOE) can optimize research practices, preserving our planet's resources. With global concerns about water scarcity, transitioning workflows becomes imperative to mitigate future challenges. It is evident that, despite the positive health advances, nanomedicines have a significant negative impact on the environment, and research practices should be optimized to minimize this impact. The future will reveal whether researchers have been cautious in preserving health by also preserving the environment.

3.5. Towards New Perspectives and Awareness

In the coming decades, the relationship between nanomedicines and society will continue to expand, fostering increased awareness to avert potential negative outcomes. The harmonization of nanomedicine characterization is not just an important goal; it's a concrete fact, demonstrated by actions such as those taken by the European Technology Platform on Nanomedicine (ETPN). The European Nanomedicine Characterization Laboratory (EUNCL) is one such action, aiming to aid the translation of nanomedicines to the clinic by providing a "comprehensive and integrated set of preclinical characterization assays". However, establishing a common strategy has been challenging due to the diversity among nanomedicines. Presently, regulatory bodies evaluate and approve individual nanomedicines on a case-by-case basis [82]. An improvement in this area involves the EMA suggestions through its nanomedicine group evaluation approach. A possible inter-

agency harmonized approach might involve categorizing nanomedicines into groups, striving to create tailored assessments for each category, akin to the EMA's recommendations by its nanomedicine group.

Harmonized nanomedicine characterization and regulation should be implemented in conjunction with good translation to the clinics. Liu *et al.* stated that more than 100,000 articles on nanomedicines were found between 1999 and 2022, with 80% published between 2015 and 2022 [105]. The substantial volume of research suggests that a significant portion of nanomedicines remains confined to preclinical phases. The challenges hindering the transition of most publications into clinical applications are mainly attributed to limitations in formulation methods, the materials employed, or the excessive reliance on organic solvents. To avoid unforeseen setbacks in later developmental stages, it is crucial to incorporate considerations for clinical translation when designing the formulation process for nanomedicine development (**Figure 5**). Furthermore, a proactive assessment of potential risks in the early stages of the development process can aid in overcoming limitations encountered during preclinical toxicity studies or in phase I clinical trials.

In conjunction with the aforementioned principles, the adoption of green nanotechnology aligns with green chemistry, representing the design of nanomedicines aimed at minimizing or eliminating human health hazards and environmental contamination [106]. There are concerted efforts to reduce the ecological footprint associated with the development of nanomedicines. Ultimately, the judicious optimization of resources by avoiding excessive and unnecessary experimentation would significantly enhance the environmental impact of nanomedicine development [107] [108]. For instance, Jane Kilcoyne reported that her laboratory achieved a 23% reduction in chemical waste, more than a 95% reduction in non-chemical waste, and a 69% decrease in single-use plastic consumption within one year by implementing changes within the laboratory [109]. A recent viewpoint in the Journal of the American Medical Association (JAMA) has even suggested the imperative need to consider the potential benefits and risks of a clinical trial in light of climate change, acknowledging its measurable impact on patients [110]. This underscores the necessity for a shift in attitudes within the research and industry community to bring about meaningful changes.

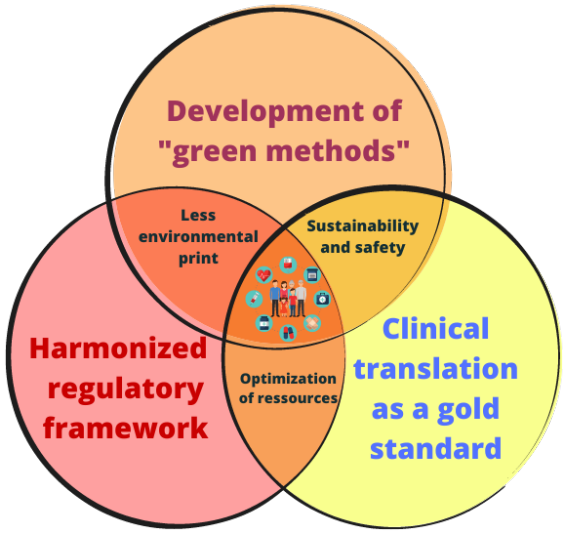

Figure 5. New principles for a better interaction of nanomedicines and societal improvements. The figure underscores the significance of resource optimization, positioning clinical translation as the gold standard in nanomedicine development. Additionally, it highlights the importance of a collective effort towards establishing a harmonized regulatory framework for the characterization of nanomedicines.

In the realm of advancing nanomedicine translation, new tools are emerging to facilitate the process. One noteworthy tool is artificial intelligence (AI), which has already demonstrated its utility in nanomedicine discovery and physicochemical prediction through molecular dynamics simulations [111] and machine learning [112]. AI's potential extends beyond nanomedicine development to enhance patient stratification during clinical trials. For instance, AI can rapidly profile biomarkers, a crucial step in identifying specific patient subgroups, significantly impacting the success of clinical trials [113]. Additionally, AI can play a role in optimizing dosages based on individual patient responses. An example is the platform CURATE.AI, which has undergone validation in clinical trials, highlighting its significance for the future of drug development and, more specifically, for nanomedicine advancement [114].

In conclusion, embracing the principles outlined in **Figure 5**, including a unified regulatory framework, resource optimization, and a focus on clinical translation and risk management, has the potential to significantly alleviate adverse consequences associated with the intersection of nanomedicines and society. This

strategic approach stands to improve the benefit-risk ratio in nanomedicine development, fostering advancements in public health while concurrently minimizing environmental impact.

3.6. Concluding Remarks

The burgeoning field of nanomedicine stands as a transformative force with far-reaching implications for society. Its advent has not only revolutionized therapeutic approaches but has also ushered in a new era of precision medicine, particularly in the domains of oncology and infectious diseases. Despite encountering substantial challenges in the clinical translation phase, nanomedicines have undeniably left an indelible mark on societal well-being. Their pivotal role in cancer treatment and substantial contributions to infectious disease management underscore their significance in addressing pressing health concerns. However, the absence of specific regulatory frameworks tailored to nanomedicines poses a notable challenge, necessitating urgent advancements in this aspect. As we navigate the dynamic landscape of technological advancements and societal shifts, the harmonized physicochemical characterization of nanomedicines emerges as a crucial prerequisite for ensuring their continued success and safe integration into medical practice. The symbiotic relationship between nanotechnology and society, marked by both triumphs and challenges, underscores the pressing need for ongoing interdisciplinary collaboration and the cultivation of a regulatory framework that aligns with the evolving landscape. In this context, the ever-evolving realm of nanomedicine not only reflects the progress of society but also imparts a mandate for researchers and industry professionals to proactively address emerging challenges, including the imperative to establish specific regulations. Seizing newfound opportunities and advocating for tailored regulatory measures will ensure a promising and impactful future for this groundbreaking field.

References

[1] M.L. Etheridge, S.A. Campbell, A.G. Erdman, C.L. Haynes, S.M. Wolf, J. Mccullough, J. Mccullough, The Big Picture on Small Medicine: The State of Nanomedicine Products Approved for Use or in Clinical Trials HHS Public Access, Nanomedicine. 9 (2013). https://doi.org/10.1016/j.nano.2012.05.013.

[2] R. van der Meel, E. Sulheim, Y. Shi, F. Kiessling, W.J.M. Mulder, T. Lammers, Smart cancer nanomedicine, Nat. Nanotechnol. 14 (2019) 1007-1017. https://doi.org/10.1038/s41565-019-0567-y.

[3] C.M. Dawidczyk, C. Kim, J.H. Park, L.M. Russell, K.H. Lee, M.G. Pomper, P.C. Searson, State-of-the-art in design rules for drug delivery platforms: Lessons learned from FDA-approved nanomedicines, J. Control. Release. (2014). https://doi.org/10.1016/j.jconrel.2014.05.036.

[4] S. Tran, P.-J. DeGiovanni, B. Piel, P. Rai, Cancer nanomedicine: a review of recent success in drug delivery., Clin. Transl. Med. 6 (2017) 44. https://doi.org/10.1186/s40169-017-0175-0.

[5] R.K. Thapa, J.O. Kim, Nanomedicine-based commercial formulations: current developments and future prospects, J. Pharm. Investig. 53 (2023) 19-33. https://doi.org/10.1007/s40005-022-00607-6.

[6] S.H. El Moukhtari, E. Garbayo, A. Amundarain, S. Pascual-Gil, A. Carrasco-León, F. Prosper, X. Agirre, M.J. Blanco-Prieto, Lipid nanoparticles for siRNA delivery in cancer treatment, J. Control. Release. 361 (2023) 130-146. https://doi.org/10.1016/j.jconrel.2023.07.054.

[7] J. Shi, P.W. Kantoff, R. Wooster, O.C. Farokhzad, Cancer nanomedicine: progress, challenges and opportunities, Nat. Rev. Cancer. 17 (2017) 20-37. https://doi.org/10.1038/nrc.2016.108.

[8] R. Tenchov, R. Bird, A.E. Curtze, Q. Zhou, Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement, ACS Nano. 15 (2021) 16982-17015. https://doi.org/10.1021/acsnano.1c04996.

[9] H. Park, A. Otte, K. Park, Evolution of drug delivery systems: From 1950 to 2020 and beyond, J. Control. Release. 342 (2022) 53-65. https://doi.org/10.1016/j.jconrel.2021.12.030.

[10] Nanomedicine and the COVID-19 vaccines, Nat. Nanotechnol. 15 (2020) 963. https://doi.org/10.1038/s41565-020-00820-0.

[11] Y. Jia, Y. Jiang, Y. He, W. Zhang, J. Zou, K.T. Magar, H. Boucetta, C. Teng, W. He, Approved Nanomedicine against Diseases, Pharmaceutics. 15 (2023) 774. https://doi.org/10.3390/pharmaceutics15030774.

[12] M.A. Younis, H.M. Tawfeek, A.A.H. Abdellatif, J.A. Abdel-Aleem, H. Harashima, Clinical translation of nanomedicines: Challenges, opportunities, and keys., Adv. Drug Deliv. Rev. 181 (2022) 114083. https://doi.org/10.1016/j.addr.2021.114083.

[13] J. Gonzalez-Valdivieso, A. Girotti, J. Schneider, F.J. Arias, Advanced nanomedicine and cancer: Challenges and opportunities in clinical translation, Int. J. Pharm. 599 (2021) 120438. https://doi.org/10.1016/j.ijpharm.2021.120438.

[14] J.B. Hertig, V.P. Shah, B. Flühmann, S. Mühlebach, G. Stemer, J. Surugue, R. Moss, T. Di Francesco, Tackling the challenges of nanomedicines: are we ready? Am. J. Heal. Pharm. 78 (2021) 1047-1056. https://doi.org/10.1093/ajhp/zxab048.

[15] B. Pelaz, C. Alexiou, R.A. Alvarez-Puebla, F. Alves, A.M. Andrews, S. Ashraf, L.P. Balogh, L. Ballerini, A. Bestetti, C. Brendel, S. Bosi, M. Carril, W.C.W. Chan, C. Chen, X. Chen, X. Chen, Z. Cheng, D. Cui, J. Du, C. Dullin, A. Escudero, N. Feliu, M. Gao, M. George, Y. Gogotsi, A. Grünweller, Z. Gu, N.J. Halas, N. Hampp, R.K. Hartmann, M.C. Hersam, P. Hunziker, J. Jian, X. Jiang, P. Jungebluth, P. Kadhiresan, K. Kataoka, A. Khademhosseini, J. Kopeček, N.A. Kotov, H.F. Krug, D.S. Lee, C.-M. Lehr, K.W. Leong, X.-J. Liang, M. Ling Lim, L.M. Liz-Marzán, X. Ma, P. Macchiarini, H. Meng, H. Möhwald, P. Mulvaney, A.E. Nel, S. Nie, P. Nordlander, T. Okano, J. Oliveira, T.H. Park, R.M. Penner, M. Prato, V. Puntes, V.M. Rotello, A. Samarakoon, R.E. Schaak, Y. Shen, S. Sjöqvist, A.G. Skirtach, M.G. Soliman, M.M. Stevens, H.-W. Sung, B.Z. Tang, R. Tietze, B.N. Udugama, J.S. VanEpps, T. Weil, P.S. Weiss, I. Willner, Y. Wu, L. Yang, Z. Yue, Q. Zhang, Q. Zhang, X.-E. Zhang, Y. Zhao, X. Zhou, W.J. Parak, Diverse Applications of Nanomedicine, ACS Nano. 11 (2017) 2313-2381. https://doi.org/10.1021/acsnano.6b06040.

[16] M.S. Muthu, L. Mei, S.-S. Feng, Nanotheranostics: advanced nanomedicine for the integration of diagnosis and therapy, Nanomedicine. 9 (2014) 1277-1280. https://doi.org/10.2217/nnm.14.83.

[17] X. Song, Z. Sun, L. Li, L. Zhou, S. Yuan, Application of nanomedicine in radiotherapy sensitization, Front. Oncol. 13 (2023). https://www.frontiersin.org/articles/10.3389/fonc.2023.1088878.

[18] X. Shan, X. Gong, J. Li, J. Wen, Y. Li, Z. Zhang, Current approaches of nanomedicines in the market and various stage of clinical translation, Acta Pharm. Sin. B. 12 (2022) 3028-3048. https://doi.org/10.1016/j.apsb.2022.02.025.

[19] K. Osouli-Bostanabad, S. Puliga, D.R. Serrano, A. Bucchi, G. Halbert, A. Lalatsa, Microfluidic Manufacture of Lipid-Based Nanomedicines, Pharmaceutics. 14 (2022). https://doi.org/10.3390/pharmaceutics14091940.

[20] R.H. Müller, M. Radtke, S.A. Wissing, Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations, Adv. Drug Deliv. Rev. 54 (2002) S131-S155. https://doi.org/10.1016/S0169-409X(02)00118-7.

[21] S.H. El Moukhtari, C. Rodríguez-Nogales, M.J. Blanco-Prieto, Oral lipid nanomedicines: Current status and future perspectives in cancer treatment, Adv. Drug Deliv. Rev. 173 (2021) 238-251. https://doi.org/10.1016/j.addr.2021.03.004.

[22] I. Urits, D. Swanson, M.C. Swett, A. Patel, K. Berardino, A. Amgalan, A.A. Berger, H. Kassem, A.D. Kaye, O. Viswanath, A Review of Patisiran (ONPATTRO®) for the Treatment of Polyneuropathy in People with Hereditary Transthyretin Amyloidosis, Neurol. Ther. 9 (2020) 301-315. https://doi.org/10.1007/s40120-020-00208-1.

[23] S. Shapiro Ben David, S. Baruch Gez, D. Rahamim-Cohen, N. Shamir-Stein, U. Lerner, A. Ekka Zohar, Immediate side effects of Comirnaty COVID-19 vaccine: A nationwide survey of vaccinated people in Israel, December 2020 to March 2021, Eurosurveillance. 27 (2022). https://doi.org/10.2807/1560-7917.ES.2022.27.13.2100540.

[24] W.-C. Wang, J.C.-Y. Fann, R.-E. Chang, Y.-C. Jeng, C.-Y. Hsu, H.-H. Chen, J.-T. Liu, A.M.-F. Yen, Economic evaluation for mass vaccination against COVID-19, J. Formos. Med. Assoc. 120 (2021) S95-S105. https://doi.org/10.1016/j.jfma.2021.05.020.

[25] S. Kim, E. Chatelut, J.C. Kim, S.B. Howell, C. Cates, P.A. Kormanik, M.C. Chamberlain, Extended CSF cytarabine exposure following intrathecal administration of DTC 101., J. Clin. Oncol. 11 (1993) 2186-2193. https://doi.org/10.1200/JCO.1993.11.11.2186.

[26] S. Wang, K. Cheng, K. Chen, C. Xu, P. Ma, G. Dang, Y. Yang, Q. Lei, H. Huang, Y. Yu, Y. Fang, Q. Tang, N. Jiang, H. Miao, F. Liu, X. Zhao, N. Li, Nanoparticle-based medicines in clinical cancer therapy, Nano Today. 45 (2022) 101512. https://doi.org/10.1016/j.nantod.2022.101512.

[27] K. Jani, N. Kaushal, Chapter 16 - Clinical translation of PLGA nanoparticles into market—From benchside to breakthrough therapy, in: P.B.T.-P. acid) (PLGA) N. for D.D. Kesharwani (Ed.), Micro Nano Technol., Elsevier, 2023: pp. 433-456. https://doi.org/10.1016/B978-0-323-91215-0.00013-3.

[28] O. Sartor, Eligard: leuprolide acetate in a novel sustained-release delivery system, Urology. 61 (2003) 25-31. https://doi.org/10.1016/S0090-4295(02)02396-8.

[29] Y. Zhu, S. Murali, W. Cai, X. Li, J.W. Suk, J.R. Potts, R.S. Ruoff, Graphene and Graphene Oxide: Synthesis, Properties, and Applications, Adv. Mater. 22 (2010) 3906-3924. https://doi.org/10.1002/adma.201001068.

[30] E. Mari, S. Mardente, E. Morgante, M. Tafani, E. Lococo, F. Fico, F. Valentini, A. Zicari, Graphene Oxide Nanoribbons Induce Autophagic Vacuoles in Neuroblastoma Cell Lines, Int. J. Mol. Sci. 17 (2016). https://doi.org/10.3390/ijms17121995.

[31] Y. Volkov, Quantum dots in nanomedicine: recent trends, advances and unresolved issues, Biochem. Biophys. Res. Commun. 468 (2015) 419-427. https://doi.org/10.1016/j.bbrc.2015.07.039.

[32] L. Xu, Y.-Y. Wang, J. Huang, C.-Y. Chen, Z.-X. Wang, H. Xie, Silver nanoparticles: Synthesis, medical applications and biosafety, Theranostics. 10 (2020) 8996-9031. https://doi.org/10.7150/thno.45413.

[33] T. Vangijzegem, V. Lecomte, I. Ternad, L. Van Leuven, R.N. Muller, D. Stanicki, S. Laurent, Superparamagnetic Iron Oxide Nanoparticles (SPION): From Fundamentals to State-of-the-Art Innovative Applications for Cancer Therapy, Pharmaceutics. 15 (2023). https://doi.org/10.3390/pharmaceutics15010236.

[34] F. Abbas, T. Jan, J. Iqbal, M.S. Haider Naqvi, I. Ahmad, Inhibition of

Neuroblastoma cancer cells viability by ferromagnetic Mn doped CeO2 monodisperse nanoparticles mediated through reactive oxygen species, Mater. Chem. Phys. 173 (2016) 146-151. https://doi.org/10.1016/j.matchemphys.2016.01.042.

[35] R. Jin, B. Lin, D. Li, H. Ai, Superparamagnetic iron oxide nanoparticles for MR imaging and therapy: design considerations and clinical applications, Curr. Opin. Pharmacol. 18 (2014) 18-27. https://doi.org/10.1016/j.coph.2014.08.002.

[36] E. Luque-Michel, L. Lemaire, M.J. Blanco-Prieto, SPION and doxorubicin-loaded polymeric nanocarriers for glioblastoma theranostics, Drug Deliv. Transl. Res. 11 (2021) 515-523. https://doi.org/10.1007/s13346-020-00880-8.

[37] F. Farjadian, A. Ghasemi, O. Gohari, A. Roointan, M. Karimi, M.R. Hamblin, Nanopharmaceuticals and nanomedicines currently on the market: challenges and opportunities, Nanomedicine. 14 (2019) 93-126. https://doi.org/10.2217/nnm-2018-0120.

[38] N. Habibi, A. Mauser, Y. Ko, J. Lahann, Protein Nanoparticles: Uniting the Power of Proteins with Engineering Design Approaches, Adv. Sci. 9 (2022) 2104012. https://doi.org/10.1002/advs.202104012.

[39] W. Shan, C. Wang, H. Chen, L. Ren, Rational Design of Virus-like Particles for Nanomedicine, Accounts Mater. Res. 4 (2023) 814-826. https://doi.org/10.1021/accountsmr.3c00050.

[40] L. Cappelli, P. Cinelli, F. Giusti, I. Ferlenghi, S. Utrio-Lanfaloni, N. Wahome, M.J. Bottomley, D. Maione, R. Cozzi, Self-assembling protein nanoparticles and virus like particles correctly display β-barrel from meningococcal factor H-binding protein through genetic fusion, PLoS One. 17 (2022) e0273322. https://doi.org/10.1371/journal.pone.0273322.

[41] A.M. Sofias, M. Dunne, G. Storm, C. Allen, The battle of “nano” paclitaxel, Adv. Drug Deliv. Rev. 122 (2017) 20-30. https://doi.org/10.1016/j.addr.2017.02.003.

[42] S. Hong, D.W. Choi, H.N. Kim, C.G. Park, W. Lee, H.H. Park, Protein-Based Nanoparticles as Drug Delivery Systems, Pharmaceutics. 12 (2020).

https://doi.org/10.3390/pharmaceutics12070604.

[43] K. Shi, Y. Wang, X. Zhou, H. Gui, N. Xu, S. Wu, C. He, Z. Zhao, Tumor microenvironment targeting with dual stimuli-responsive nanoparticles based on small heat shock proteins for antitumor drug delivery, Acta Biomater. 114 (2020) 369-383. https://doi.org/10.1016/j.actbio.2020.07.031.

[44] A.E.M. Nel, Transformational Impact of Nanomedicine: Reconciling Outcome with Promise, Nano Lett. 20 (2020) 5601-5603. https://doi.org/10.1021/acs.nanolett.0c02738.

[45] J.A. DiMasi, H.G. Grabowski, R.W. Hansen, Innovation in the pharmaceutical industry: New estimates of R&D costs, J. Health Econ. 47 (2016) 20-33. https://doi.org/10.1016/j.jhealeco.2016.01.012.

[46] J. Avorn, The $2.6 Billion Pill—Methodologic and Policy Considerations, N. Engl. J. Med. 372 (2015) 1877-1879. https://doi.org/10.1056/NEJMp1500848.

[47] H. He, L. Liu, E.E. Morin, M. Liu, A. Schwendeman, Survey of Clinical Translation of Cancer Nanomedicines—Lessons Learned from Successes and Failures, Acc. Chem. Res. 52 (2019) 2445-2461. https://doi.org/10.1021/acs.accounts.9b00228.

[48] H. Ledford, Bankruptcy filing worries developers of nanoparticle cancer drugs, Nature. 533 (2016) 304-305. https://doi.org/10.1038/533304a.

[49] M. Xing, F. Yan, S. Yu, P. Shen, Efficacy and Cardiotoxicity of Liposomal Doxorubicin-Based Chemotherapy in Advanced Breast Cancer: A Meta-Analysis of Ten Randomized Controlled Trials, PLoS One. 10 (2015) e0133569. https://doi.org/10.1371/journal.pone.0133569.

[50] A. McBride, L.M. Holle, C. Westendorf, M. Sidebottom, N. Griffith, R.J. Muller, J.M. Hoffman, National survey on the effect of oncology drug shortages on cancer care, Am. J. Heal. Pharm. 70 (2013) 609-617. https://doi.org/10.2146/ajhp120563.

[51] V. Gadekar, Y. Borade, S. Kannaujia, K. Rajpoot, N. Anup, V. Tambe, K. Kalia, R.K. Tekade, Nanomedicines accessible in the market for clinical interventions, J. Control. Release. 330 (2021) 372-397.

https://doi.org/10.1016/j.jconrel.2020.12.034.

[52] Liposomal Doxorubicin Market Analysis by Product (J&J [Doxil/Caelyx], Sun Pharma [Lipodox], Teva [Myocet], Others), By Application (Multiple Myeloma, Kaposi Sarcoma, Ovarian, Breast, Kidney Cancer), And Segment Forecasts, 2018-2024, 2015. https://www.grandviewresearch.com/industry-analysis/liposomal-doxorubicin-market#.

[53] W.J. Gradishar, S. Tjulandin, N. Davidson, H. Shaw, N. Desai, P. Bhar, M. Hawkins, J. O'Shaughnessy, Phase III Trial of Nanoparticle Albumin-Bound Paclitaxel Compared with Polyethylated Castor Oil-Based Paclitaxel in Women With Breast Cancer, J. Clin. Oncol. 23 (2005) 7794-7803. https://doi.org/10.1200/JCO.2005.04.937.

[54] M. Oura, H. Saito, Y. Nishikawa, Shortage of Nab-paclitaxel in Japan and around the World: Issues in Global Information Sharing, JMA J. 6 (2023) 192-195. https://doi.org/10.31662/jmaj.2022-0179.

[55] G.W. Boswell, D. Buell, I. Bekersky, AmBisome (Liposomal Amphotericin B): A Comparative Review, J. Clin. Pharmacol. 38 (1998) 583-592. https://doi.org/10.1002/j.1552-4604.1998.tb04464.x.

[56] B. Ihor, F.R. M., D.D. E., L.J. W., B.D. N., W.T. J., Pharmacokinetics, Excretion, and Mass Balance of Liposomal Amphotericin B (AmBisome) and Amphotericin B Deoxycholate in Humans, Antimicrob. Agents Chemother. 46 (2002) 828-833. https://doi.org/10.1128/aac.46.3.828-833.2002.

[57] C.S. Haworth, D. Bilton, J.D. Chalmers, A.M. Davis, J. Froehlich, I. Gonda, B. Thompson, A. Wanner, A.E. O'Donnell, Inhaled liposomal ciprofloxacin in patients with non-cystic fibrosis bronchiectasis and chronic lung infection with Pseudomonas aeruginosa (ORBIT-3 and ORBIT-4): two phase 3, randomised controlled trials, Lancet Respir. Med. 7 (2019) 213-226. https://doi.org/10.1016/S2213-2600(18)30427-2.

[58] C. Sahli, S.E. Moya, J.S. Lomas, C. Gravier-Pelletier, R. Briandet, M. Hémadi, Recent advances in nanotechnology for eradicating bacterial biofilm, Theranostics. 12 (2022) 2383-2405. https://doi.org/10.7150/thno.67296.

[59] O. McNeilly, R. Mann, M. Hamidian, C. Gunawan, Emerging Concern for Silver

Nanoparticle Resistance in Acinetobacter baumannii and Other Bacteria, Front. Microbiol. 12 (2021). https://www.frontiersin.org/articles/10.3389/fmicb.2021.652863.

[60] A.S. Cordeiro, Y. Patil-Sen, M. Shivkumar, R. Patel, A. Khedr, M.A. Elsawy, Nanovaccine Delivery Approaches and Advanced Delivery Systems for the Prevention of Viral Infections: From Development to Clinical Application, Pharmaceutics. 13 (2021) 2091. https://doi.org/10.3390/pharmaceutics13122091.

[61] EMA, CPMP, Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines, 1997.

[62] EMA, CHMP, EMA/CHMP/CVMP/3Rs/742466/2015. Reflection paper providing an overview of the current regulatory testing requirements for medicinal products for human use and opportunities for implementation of the 3Rs, 2018.

[63] WHO, WHO guidelines on non clinical evaluation of vaccines, WHO Tech. Rep. Ser. No. 927 (2005).

[64] A.P. Lea, J.A. Balfour, Virosomal Hepatitis A Vaccine (Strain RG-SB), BioDrugs. 7 (1997) 232-248. https://doi.org/10.2165/00063030-199707030-00006.

[65] R. Zurbriggen, Immunostimulating reconstituted influenza virosomes, Vaccine. 21 (2003) 921-924. https://doi.org/10.1016/S0264-410X(02)00541-8.

[66] A.C. Anselmo, S. Mitragotri, Nanoparticles in the clinic: An update post COVID-19 vaccines, Bioeng. Transl. Med. 6 (2021) e10246. https://doi.org/10.1002/btm2.10246.

[67] H.N. Jung, S.-Y. Lee, S. Lee, H. Youn, H.-J. Im, Lipid nanoparticles for delivery of RNA therapeutics: Current status and the role of *in vivo* imaging, Theranostics. 12 (2022) 7509-7531. https://doi.org/10.7150/thno.77259.

[68] J.M. Metselaar, T. Lammers, Challenges in nanomedicine clinical translation, Drug Deliv. Transl. Res. 10 (2020) 721-725. https://doi.org/10.1007/s13346-020-00740-5.

[69] P. Satalkar, B.S. Elger, P. Hunziker, D. Shaw, Challenges of clinical translation

in nanomedicine: A qualitative study, Nanomedicine Nanotechnology, Biol. Med. 12 (2016) 893-900. https://doi.org/10.1016/j.nano.2015.12.376.

[70] L.-P. Wu, D. Wang, Z. Li, Grand challenges in nanomedicine, Mater. Sci. Eng. C. 106 (2020) 110302. https://doi.org/10.1016/j.msec.2019.110302.

[71] EMA, CTI Life Sciences Ltd. withdraws its marketing authorisation application for Opaxio (paclitaxel poliglumex), (2009). Idnbmnnnibpcajpcglclefindmkaj. https://www.ema.europa.eu/en/documents/press-release/cti-life-sciences-ltd-withdraws-its-marketing-authorisation-application-opaxio-paclitaxel-poliglumex_en.pdf.

[72] E. Hemmrich, S. McNeil, Active ingredient vs excipient debate for nanomedicines, Nat. Nanotechnol. 18 (2023) 692-695. https://doi.org/10.1038/s41565-023-01371-w.

[73] M. Hussain-Gambles, K. Atkin, B. Leese, Why ethnic minority groups are under-represented in clinical trials: a review of the literature, Health Soc. Care Community. 12 (2004) 382-388. https://doi.org/10.1111/j.1365-2524.2004.00507.x.

[74] N. Duma, J. Vera Aguilera, J. Paludo, C.L. Haddox, M. Gonzalez Velez, Y. Wang, K. Leventakos, J.M. Hubbard, A.S. Mansfield, R.S. Go, A.A. Adjei, Representation of Minorities and Women in Oncology Clinical Trials: Review of the Past 14 Years, J. Oncol. Pract. 14 (2017) e1-e10. https://doi.org/10.1200/JOP.2017.025288.

[75] M.A. Ma, D.E. Gutiérrez, J.M. Frausto, W.K. Al-Delaimy, Minority Representation in Clinical Trials in the United States: Trends Over the Past 25 Years, Mayo Clin. Proc. 96 (2021) 264-266. https://doi.org/10.1016/j.mayocp.2020.10.027.

[76] T.A. LaVeist, D. Gaskin, P. Richard, Estimating the Economic Burden of Racial Health Inequalities in the United States, Int. J. Heal. Serv. 41 (2011) 231-238. https://doi.org/10.2190/HS.41.2.c.

[77] M.A. Hamburg, FDA's Approach to Regulation of Products of Nanotechnology, Science (80-.). 336 (2012) 299-300. https://doi.org/10.1126/science.1205441.

[78] EMA, EMA/CHMP/ICH/646107/2008. ICH guideline S9 on nonclinical evaluation for anticancer pharmaceuticals, 2010.

[79] FDA, Drug Products, Including Biological Products, that Contain Nanomaterials Guidance for Industry, 2022.

[80] FDA, Nanotechnology - A Decade of Progress and Innovation: A Report by the U.S. Food and Drug Administration, 2020. https://www.fda.gov/science-research/fda-grand-rounds/nanotechnology-over-decade-progress-and-innovation-fda-08132020-08132020.

[81] M. Germain, F. Caputo, S. Metcalfe, G. Tosi, K. Spring, A.K.O. Åslund, A. Pottier, R. Schiffelers, A. Ceccaldi, R. Schmid, Delivering the power of nanomedicine to patients today, J. Control. Release. 326 (2020) 164-171. https://doi.org/10.1016/j.jconrel.2020.07.007.

[82] R.L. Maynard, Nano-technology and nano-toxicology, Emerg. Health Threats J. 5 (2012) 17508. https://doi.org/10.3402/ehtj.v5i0.17508.

[83] T.I. Ramos, C.A. Villacis-Aguirre, K. V. López-Aguilar, L. Santiago Padilla, C. Altamirano, J.R. Toledo, N. Santiago Vispo, The Hitchhiker's Guide to Human Therapeutic Nanoparticle Development, Pharmaceutics. 14 (2022) 247. https://doi.org/10.3390/pharmaceutics14020247.

[84] D. Bobo, K.J. Robinson, J. Islam, K.J. Thurecht, S.R. Corrie, Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date, Pharm. Res. 33 (2016) 2373-2387. https://doi.org/10.1007/s11095-016-1958-5.

[85] S. Đorđević, M.M. Gonzalez, I. Conejos-Sánchez, B. Carreira, S. Pozzi, R.C. Acúrcio, R. Satchi-Fainaro, H.F. Florindo, M.J. Vicent, Current hurdles to the translation of nanomedicines from bench to the clinic, Drug Deliv. Transl. Res. 12 (2022) 500-525. https://doi.org/10.1007/s13346-021-01024-2.

[86] EMA, CHMP, Reflection Paper on Nanotechnology-based Medicinal Products for Human use, 2006.

[87] EC, Commission recommendation of 18 October of 2011 on the definition of nanomaterial (2011/696/EU), Off. J. Eur. Union. (2011).

[88] EC, Directive 2001/83/EC of the European parliament and of the council of 6 November 2001 on the Community code relating to medicinal products

for human use, Off. J. Eur. Communities. (2001).

[89] Step, ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals, 2009.

[90] EMA, CHMP, EMA/CHMP/SWP/100094/2011. Reflection paper on non-clinical studies for generic nanoparticle iron medicinal product applications Draft Agreed by Safety Working Party, 2011.

[91] EMA, CHMP, EMA/325027/2013. Reflection paper on surface coatings: general issues for consideration regarding parenteral administration of coated nanomedicine products, 2013.

[92] EMA, CHMP, EMA/CHMP/806058/2009/Rev. 02. Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product Final, 2013.

[93] EMA, CHMP, EMA/CHMP/13099/2013.Reflection paper on the development of block copolymer micelle medicinal products, 2013.

[94] OCDE, Test No. 487: In Vitro Mammalian Cell Micronucleus Test, 2023. https://doi.org/https://doi.org/10.1787/9789264264861-en.

[95] OECD, OECD GUIDELINES ON THE TESTING OF CHEMICALS No.412: 28-day (subacute) inhalation toxicity study, Oced/Ocde. 39 (2018) 1-23.

[96] OECD, OECD guidelines for the testing of chemicals. No. 414: prenatal developmental toxicity study., 2001.

[97] I. Mahapatra, J.R.A. Clark, P.J. Dobson, R. Owen, I. Lynch, J.R. Lead, Expert perspectives on potential environmental risks from nanomedicines and adequacy of the current guideline on environmental risk assessment, Environ. Sci. Nano. 5 (2018) 1873-1889. https://doi.org/10.1039/C8EN00053K.

[98] S. Berkner, K. Schwirn, D. Voelker, Too advanced for assessment? Advanced materials, nanomedicine and the environment, Environ. Sci. Eur. 34 (2022) 71. https://doi.org/10.1186/s12302-022-00647-7.

[99] V. Cazzagon, E. Giubilato, L. Pizzol, C. Ravagli, S. Doumett, G. Baldi, M. Blosi, A. Brunelli, C. Fito, F. Huertas, A. Marcomini, E. Semenzin, A. Zabeo, I. Zanoni, D. Hristozov, Occupational risk of nano-biomaterials: Assessment of nano-enabled magnetite contrast agent using the BIORIMA Decision Support

System, NanoImpact. 25 (2022) 100373. https://doi.org/10.1016/j.impact.2021.100373.

[100] C. Jacobasch, C. Völker, S. Giebner, J. Völker, H. Alsenz, T. Potouridis, H. Heidenreich, G. Kayser, J. Oehlmann, M. Oetken, Long-term effects of nanoscaled titanium dioxide on the cladoceran Daphnia magna over six generations, Environ. Pollut. 186 (2014) 180-186. https://doi.org/10.1016/j.envpol.2013.12.008.

[101] W. Liu, S. Mirzoeva, Y. Yuan, J. Deng, S. Chen, B. Lai, S. Vogt, K. Shah, R. Shroff, R. Bleher, Q. Jin, N. Vo, R. Bazak, C. Ritner, S. Gutionov, S. Raha, J. Sedlmair, C. Hirschmugl, C. Jacobsen, T. Paunesku, J. Kalapurkal, G.E. Woloschak, Development of Fe3O4 core-TiO2 shell nanocomposites and nanoconjugates as a foundation for neuroblastoma radiosensitization, Cancer Nanotechnol. 12 (2021) 1-25. https://doi.org/10.1186/s12645-021-00081-z.

[102] R. Lohmann, I.T. Cousins, J.C. DeWitt, J. Glüge, G. Goldenman, D. Herzke, A.B. Lindstrom, M.F. Miller, C.A. Ng, S. Patton, M. Scheringer, X. Trier, Z. Wang, Are Fluoropolymers Really of Low Concern for Human and Environmental Health and Separate from Other PFAS? Environ. Sci. Technol. 54 (2020) 12820-12828. https://doi.org/10.1021/acs.est.0c03244.

[103] E.-C. Wamhoff, G.A. Knappe, A.A. Burds, R.R. Du, B.W. Neun, S. Difilippantonio, C. Sanders, E.F. Edmondson, J.L. Matta, M.A. Dobrovolskaia, M. Bathe, Evaluation of non-modified wireframe DNA origami for acute toxicity and biodistribution in mice, BioRxiv. (2023) 2023.02.25.530026. https://doi.org/10.1101/2023.02.25.530026.

[104] F. Soto, J. Wang, R. Ahmed, U. Demirci, Medical Micro/Nanorobots in Precision Medicine, Adv. Sci. 7 (2020) 2002203. https://doi.org/10.1002/advs.202002203.

[105] Q. Liu, J. Zou, Z. Chen, W. He, W. Wu, Current research trends of nanomedicines, Acta Pharm. Sin. B. 13 (2023) 4391-4416. https://doi.org/10.1016/j.apsb.2023.05.018.

[106] E. Mostafavi, D. Medina-Cruz, A. Vernet-Crua, J. Chen, J.L. Cholula-Díaz, G. Guisbiers, T.J. Webster, Green nanomedicine: the path to the next generation of nanomaterials for diagnosing brain tumors and therapeutics? Expert Opin. Drug Deliv. 18 (2021) 715-736.

https://doi.org/10.1080/17425247.2021.1865306.

[107] G. Bistulfi, Reduce, reuse and recycle lab waste, Nature. 502 (2013) 170. https://doi.org/10.1038/502170a.

[108] M.A. Urbina, A.J.R. Watts, E.E. Reardon, Labs should cut plastic waste too, Nature. 528 (2015) 479. https://doi.org/10.1038/528479c.

[109] J. Kilcoyne, Y. Bogan, C. Duffy, T. Hollowell. Reducing environmental impacts of marine biotoxin monitoring: A laboratory report, PLOS Sustain. Transform. 1 (2022) e0000001. https://doi.org/10.1371/journal.pstr.0000001.

[110] J. D'Souza, G. Samuel, Clinical Research Risks, Climate Change, and Human Health, JAMA. 330 (2023) 2247-2248. https://doi.org/10.1001/jama.2023.23724.

[111] D. Ho, P. Wang, T. Kee, Artificial intelligence in nanomedicine, Nanoscale Horizons. 4 (2019) 365-377. https://doi.org/10.1039/C8NH00233A.

[112] D. Reker, Y. Rybakova, A.R. Kirtane, R. Cao, J.W. Yang, N. Navamajiti, A. Gardner, R.M. Zhang, T. Esfandiary, J. L'Heureux, T. von Erlach, E.M. Smekalova, D. Leboeuf, K. Hess, A. Lopes, J. Rogner, J. Collins, S.M. Tamang, K. Ishida, P. Chamberlain, D. Yun, A. Lytton-Jean, C.K. Soule, J.H. Cheah, A.M. Hayward, R. Langer, G. Traverso, Computationally guided high-throughput design of self-assembling drug nanoparticles, Nat. Nanotechnol. 16 (2021) 725-733. https://doi.org/10.1038/s41565-021-00870-y.

[113] P. Tan, X. Chen, H. Zhang, Q. Wei, K. Luo, Artificial intelligence aids in development of nanomedicines for cancer management, Semin. Cancer Biol. 89 (2023) 61-75. https://doi.org/10.1016/j.semcancer.2023.01.005.

[114] A. Blasiak, J. Khong, T. Kee, CURATE.AI: Optimizing Personalized Medicine with Artificial Intelligence, SLAS Technol. 25 (2020) 95-105. https://doi.org/10.1177/2472630319890316.

Chapter 4
From Blindness to Awareness in Nanoethics: The Wireless Drug Delivery Case

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Abstract: Nanomedicine, with its goal of expanding therapeutic options and enhancing medical safety, introduces both promise and apprehension. In this chapter, we delve into the nanoethical dimensions of wireless drug delivery, drawing insights from existing literature. Beyond classical ethical considerations—such as equity, autonomy, privacy, and data protection—the authors underscore the intricate challenges and potential hazards posed by these novel technologies. To transcend from blindness to moral awareness, the application of nanotechnology in clinical contexts necessitates a transdisciplinary approach to the multifaceted ethical landscape of nanomedicine. The developmental stages of ethical awareness, in this context, could be the following: common morality, ethical expertise and transdisciplinary approach to the complex ethical issues of nanomedicine.

Turning our focus to oncology, a specific domain of application, we find that public concerns center on informed consent, transparency, and equitable treatment access. In contrast, healthcare professionals grapple with their own lack of transdisciplinary ethical expertise. Addressing these ethical dilemmas requires embracing the concept of responsible risking and fostering collaborative efforts within the multidisciplinary team engaged in wireless drug delivery procedures.

Keywords: Nanomedicine, Nanoethics, Moral Awareness, Ethical Blindness, Wireless Drug Delivery

4.1. Introduction

Nanomedicine, an emerging field aiming at the exploration of advanced biotechnological possibilities, seeks to harness the potential of nanotechnology—manipulating and manufacturing materials and devices at the scale of approximately 1

to 100 nanometers (nm)—to revolutionize medicine. The term "nanomedicine" itself reflects the essence of this kind of therapies: the fusion of "medicine" with the prefix "nano-" denoting its scale. Unlike traditional medicine, nanomedicine holds the promise of personalized and regenerative approaches, allowing tailored therapies for individual patients [1]. At its core, nanomedicine revolves around several key aspects: Nanoparticles in Diagnostics and Therapy where these tiny particles play a pivotal role in enhancing diagnostic accuracy and targeted therapeutic interventions; Implant Surfaces where nanotechnology enables the design of implant surfaces that promote better integration with the body, improving patient outcomes; Smart Devices where materials incorporated into smart medical devices—such as drug-delivery systems—benefit from nanoscale precision; Scaffolds for Tissue Engineering where nanomaterial-based scaffolds facilitate tissue regeneration, offering hope for personalised medicine.

However, the complexity of nanomedicine potentially raises critical social, ethical, and regulatory questions [1]. Nanoethics encompasses "distributive justice, human and civil rights, autonomy, doctor-patient relations, workplace, occupational medicine and public safety and the role and scope of public health systems in the future health and medical landscape." [2].

4.2. Foundations of the Nanoethics

4.2.1. Philosophical Questions

In addition to the technical challenges that nanotechnology brings to attention, Alpert (2008) identifies four distinct categories of philosophical issues [3]. Let us delve into each of these thought-provoking types of questions:

1) Purpose and Application: The very purpose of nanotechnology in research and its practical applications raises fundamental questions: How will the technologies currently under development or already in use align with the overarching main goal of nanotechnology? Furthermore, we must critically evaluate whether allocating resources toward this purpose represents the most optimal and ethical investment approach.

2) Humanity and Technology Integration: As we incorporate technology into our bodies, we confront existential questions about our humanity, the essence of our existence being profoundly challenged by this integration. What does it mean

to be human when our physical selves merge with artificial constructs?

3) Human-Environment Nexus: Nanotechnology's impact extends beyond individual bodies, also impacting our environment. Numerous philosophical questions arise concerning our relationship with the natural world: How does our manipulation of matter at the nanoscale affect ecosystems and biodiversity on Earth?

4) Distributive Justice: In a world where nanotechnology is used, disparities may emerge between those who benefit from its advancements and those who lack access. How can we ensure equitable distribution of these new technologies?

In clinical implementation of nanotechnology with a great impact on the human body, and on shaping society and the environment, we must acknowledge the substantial gaps in our knowledge. The field remains shrouded in uncertainties even at a philosophical level, and these unknowns prompt further introspection in defining the purposes of their use. What are the involved concepts and what is their main meaning? What ethical compass should guide our intentions and hopes as we harness nanotechnology's potential?

4.2.2. Uncertainty as a Source of Ethical Challenges in Nanomedicine

Nanorobots and nanodevices represent cutting-edge technologies in the medical field. However, the literature exposes that their development occurs without significant public input and in the absence of a robust legal framework. Consequently, a multitude of uncertainties surrounds the risks associated with the implementation of these engineered products [4]. A high number of debates delve into the widespread development and implementation of nanotechnology particularly within the context of nanomedicine. Nanomaterials pose unique challenges in risk assessment in the clinical context [5], especially when dealing with engineered nanoparticles. The literature [1] [3]-[7] characterizes nanomaterials as exhibiting unpredictable behaviour, rendering them neither entirely safe nor inherently dangerous. The difficulty lies in assessing the potential risks associated with these novel materials. Unlike traditional substances, nanomaterials defy straightforward categorization. The uncertainty regarding the use of engineering in nanomedicine concerns three aspects:

1) Future Research Directions: The first aspect of uncertainty pertains to the trajectory of nanomedicine research. The field is rapidly evolving, and predicting

its future directions remains elusive. Researchers grapple with questions about the impact of implementing nanotechnology in clinical practice [6].

2) Equitable Distribution of Benefits: A second concern revolves around the fair distribution of nanotechnology benefits. Will these advancements be accessible only to developed nations or restricted to higher social classes? Addressing this disparity is crucial for ethical and equitable deployment [9].

3) Lack of Universally Accepted Exposure Metrics: The third challenge lies in establishing universally accepted exposure metrics for the professionals to nanomaterials. Without standardized measures, assessing safety becomes convoluted, leading to blurred ethical standards [7].

The papers highlight two directions in addressing the risks posed by the uncertainties related to nanotechnology. Firstly, the uncertainty surrounding nanomaterial and nanodevice safety underscores the need for clear ethical guidelines. As nanomedicine gains prominence, ethical standards must evolve alongside it. Secondly, a few researchers advocate for a systematic approach to quantifying uncertainty. Constructing scenarios related to nanoparticle exposure can help evaluate risk levels effectively.

4.2.3. An Interdisciplinary Exploration of Ethical Dimensions in Nanoethics

Nanotechnology, a burgeoning field at the intersection of science, engineering, and medicine, presents a complex landscape fraught with ethical challenges. As this field matures, it draws attention to a spectrum of ethical issues that demand thoughtful consideration from an interdisciplinary approach. These issues can be broadly categorized into several categories: “legal and regulatory issues; research funding and priorities; equity; environmental, safety and health issues; privacy; medicine” [8].

Because of the multifaceted nature of nanotechnology in addressing the ethical dilemmas raised, two contrasting viewpoints emerge regarding the ethical implications of nanotechnology. The first perspective contends that nanotechnology merely amplifies existing ethical concerns encountered in other technological domains. In this view, nanotechnology does not introduce fundamentally novel ethical issues but rather manifests new instances of certain well-known ones in new

contexts. Van de Poel, I. [17] highlights that many of the ethical questions raised by nanotechnology are already known from other contexts of ethical reflection (see also: [3] [6] [8]-[11]). However, an alternative stance asserts that nanotechnology indeed gives rise to distinctive ethical challenges by bringing together the complexities of the intertwined fields in nanomedicine, that require fresh normative frameworks and analytical tools.

The last view became the main one in approaching the ethical challenges brought by nanotechnology since the ethical implications of nanomedicine—particularly concerning human enhancement—loom large. As we delve into this realm, we encounter novel questions that transcend conventional bioethical boundaries [12]. Nanoethics, therefore, demands not only a deep understanding of existing ethical principles but also the cultivation of new ethical competencies and standards.

4.2.4. Dangers of Ethical Pitfalls

In his work, Dupuy [9] sheds light on the ethical complexities surrounding nanotechnology, emphasizing potential pitfalls that arise from misguided approaches. Let us delve into these challenges:

1) Prudence is Rational Risk Management: Dupuy contends that ethics cannot be reduced to mere prudence because, in the context of nanotechnology, prudence is often equated with rational risk management. However, this approach lacks a moral compass since agents act not out of a desire for moral correctness but rather to avert undesirable consequences.

2) Cost-Benefit Analysis: A second pitfall lies in the prevalent tendency to evaluate actions solely through a cost-benefit approach. Nanoethics discussions often adopt a strict utilitarian or consequentialist perspective, emphasizing economic analyses of risk while downplaying fundamental human values.

3) Ethics of Technique vs. Ethics of Technology: The third challenge emerges from the distinction between technique and technology. The former refers to practical know-how, while the latter encompasses broader discourse, including "symbolic or imaginary representations, with conceptions of the world, but also with institutions, rules and norms" [13]. Therefore, focusing solely on the ethics of technique risks overlooking the larger societal implications of nanotechnology.

4) Human Nature and the Unanswered Questions: Dupuy remarks confusion between human nature and the human condition in the discourses on nanoethics. Nanoethics debates frequently grapple with the potential impact of nanotechnology on human nature, an aspect that remains without answers. Dupuy suggests that a parallel reflection should also consider the human condition within the world.

Navigating the ethical landscape of nanotechnology requires a nuanced understanding that transcends the issues highlighted in debates at first stance. By addressing these pitfalls, we can foster responsible nanotechnological advancements that align with our shared values and aspirations as humanity.

4.2.5. Key Ethical Principles in the Nanotechnological Applications

Kermisch's paper [13] outlines four distinct stages in the development of nanotechnologies, drawing from Roco's typology. The initial stage, referred to as "first-generation nanotechnologies correspond to passive nanostructures" [13] designed to enhance materials properties, that may be used even in biomedicine. However, ethical concerns arise due to uncertainties surrounding the behaviour of nanomaterials within the human body.

The subsequent phase in their development, termed "second-generation nanotechnologies" [13], introduces active nanostructures that dynamically adapt to their environment. Notably, targeted cancer therapies fall within this category, demonstrating the ability to precisely deliver drugs to specific body regions. Despite these advancements, it is considered that most of the ethical debates emerge during the second stage of nanotechnological development.

Moving forward, the third generation will be focused on constructing artificial organs from the nanoscale. Anticipating the future, the last generation envisions heterogeneous molecular nanosystems, where each molecule serves distinct functions.

However, ethical considerations in nanoethics exhibit specificity based on the application field. Thus, the existing literature [1] [14] [15], identifies seven areas with unique ethical challenges: surveillance, diagnosis, targeted drug delivery, personalized medicine, nanosurgery, human enhancement and military applications.

Medical surveillance [1], based on nanotechnology, observes various organs and bodily functions. The ethical challenges faced include privacy concerns, respect for autonomy, human dignity, and Intellectual Property (IP) rights.

Nanotechnology also plays a crucial role in the field of diagnostics [14]. During medical investigations, a serious diagnosis may become relevant during the medical investigation, being pertinent to consider serious diagnoses even before the disease manifests. This proactive approach raises ethical questions regarding a patient's right to be informed or remain unaware. Additionally, the utilization of nanotechnology for diagnosis brings privacy concerns to the forefront, particularly when devices rely on digital data storage and wireless transmission.

Targeted drug delivery [14] aims to administer specific treatments to precise locations within the body, facilitated by wireless guidance using magnetic nanocarriers. Ethical considerations related to targeted drug delivery encompass several critical aspects: ensuring equity and accessibility, assessing the risk-benefit balance, addressing control risks (aiming the dangers of losing control of the nanodevices and their impact on the body) and autonomy concerns, safeguarding privacy rights, and navigating Intellectual Property (IP) rights.

The implementation of nanotechnology is poised to enhance the feasibility of personalized medication [1] that tailors treatments to the patient's particularities. Regrettably, clinical efforts in this direction may inadvertently impact the doctor-patient relationship by increasing the demands of the patients to improve the personalisation of the therapy even when it is not possible. However, amidst this transformation, there is a potential positive outcome by empowering patient autonomy since nanomedicine is focused on increasing the adaptation of the treatments to the particularities of each patient. Ethical considerations in this context extend to principles of justice (since personalised medicine based on nanotechnology is very expensive) and privacy, ownership or control over patient data related to diagnosis and treatment is regarded as vulnerable because of cloud storage.

A significant application of nanotechnology in the medical field is nanosurgery [14], where surgical procedures are performed at the nanometric scale. Ethical considerations related to nanosurgery include assessing the risk-benefit ratio of this medical intervention and ensuring equitable access to such procedures. Additionally, the affordability of nanosurgery compared to other surgical methods

should be considered in the decision-making process.

The medical applications mentioned earlier aimed to cure or observe certain medical conditions. However, there is a novel approach in the medical field: *Human Enhancement* procedures [14]. These procedures aim to enhance specific human abilities or acquire new ones (like improving sensorial abilities or cognition), being designed even for healthy individuals. This medical perspective raises profound ethical questions due to the unknown or hypothetical ways that this might be achieved and because of the dangers of entraining undesirable side effects. Firstly, is it morally acceptable for humans to "play God" by genetic manipulation using drug delivery systems to target changes in particular cells in a particular body area? This question stems from concerns about "slippery slope risks." Essentially, performing less controversial enhancements today might pave the way for future enhancements that are currently forbidden. Additionally, social pressure could drive individuals to undergo unnecessary enhancing procedures. Such a context raises issues related to human dignity, equity, and the principles of justice. Furthermore, the emphasis on enhancement may shift the focus from curing to constant improvement. Other ethical considerations include longevity, the ageing process, and the patentability of nanoproducts involving human beings.

Nanotechnological products find extensive applications in the military field [15], constituting a pivotal driver for their development. However, the utilization of nanotechnology for military purposes carries the inherent risk of creating substantial geopolitical and military disparities globally. Ethically, the deployment of nanotechnology in warfare raises profound concerns due to the potential impact of an unseen enemy that is highly feared by the population.

In this section, we summarized the applications of nanotechnology mentioned in the literature, highlighting their potential to spark serious ethical debates. However, to approach this topic ethically, we should analyse the fundamental principles and values involved. Several papers [2] [3] [5] [6] [10] [14]-[17] have analysed these principles and values within the context of nanotechnology, as we will summarize them below:

A. Ethical Principles:

- Autonomy: Respecting individuals' right to make informed decisions.
- Beneficence: Promoting well-being and maximizing benefits.

- Dignity: Upholding human dignity and treating all parties with respect.
- Equity or Justice: Ensuring a fair distribution of benefits.
- Informed Consent: Obtaining voluntary and informed agreement.
- Non-Malfeasance: Avoiding harm.
- Precaution: Anticipating and minimizing risks.
- Privacy and Data Protection: Safeguarding sensitive information.
- Sustainability: Considering long-term environmental impact.

B. Key Values [17] in Nanotechnology Development and Implementation:

- Safety: Prioritizing the well-being of users and the environment.
- Solidarity: Fostering collaboration and shared responsibility.
- Truthfulness and Trust: Upholding transparency and trustworthiness.
- Vulnerability: Protecting vulnerable populations.
- Responsibility: Acknowledging the consequences of technological choices.

As highlighted by Patenaude *et al.* [16] (compare this paper with Swierstra, & Rip, A. T. [10]), ethical debates surrounding nanotechnology often revolve around its implications for human nature and the human condition, human dignity, the pursuit of a good life, utility, equity, autonomy, and human rights.

4.3. Raising Awareness Concerning the Ethical Challenges in Wireless Targeted Drug Delivery

The second part of our paper focuses on the ethical considerations related to the clinical implementation of wireless targeted drug delivery. This innovative therapy has the potential to enhance clinical interventions in oncology and address vascular accidents caused by blood clots. A key advantage of drug vectorization lies in its ability to reduce systemic toxicity, thereby minimizing side effects while enhancing efficacy [18].

In the subsequent sections, we will delve into the development of skills necessary for an ethically sound approach to targeted drug delivery. Additionally, we will examine the specific ethical challenges associated with implementing this procedure in cancer therapy.

4.3.1. The Ethical Challenges in Dealing with the Issues of Wireless Targeted Drug Delivery

The first section of this paper sheds light on the ethical debates surrounding the

clinical implementation of nanotechnology. Consequently, a crucial question arises: *What underlies these ethical challenges, and to what extent will nanotechnology give rise to additional ethical dilemmas?*

Van de Poel *et al.* asserts that nanotechnology and nanoscience are still in an early developmental stage, making it challenging to predict all the ethical issues that may emerge within various clinical applications. While we can make a taxonomy of the types of ethical concerns that might arise, this classification alone does not provide a comprehensive framework for navigating the debates and establishing suitable guidelines. Given that nanotechnology is "new and innovative and still in development" [13], it becomes essential to develop a methodology that enables the discernment of specific ethical questions arising from its medical implementation.

As previously mentioned, a significant concern regarding the clinical use of nanotechnology pertains to the uncertainty surrounding the equitable distribution of benefits and the identification of major risks associated with its integration into clinical settings. Furthermore, Ebbesen and Jensen argue that although nanomedicine presents ethical complexities beyond classical medicine, the existing knowledge base in the field of bioethics can be extended to address nanomedicine's unique challenges [14].

Kermisch's classification of nanotechnology types provides a valuable framework for addressing these ethical concerns regarding wireless targeted drug delivery. In the second generation of nanotechnology, to which these nanodevices belong [12], nanometric structures are designed to adapt their behaviour based on the surrounding environment, allowing for precise transport of therapeutic agents and localized release within specific areas of the body. Therefore, they are regarded as having an extensive clinical application in cancer therapy.

Unfortunately, this kind of nanotechnological application is likely to threaten the following ethical principles: equity, autonomy, privacy and data protection, safety and responsibility. The clinical introduction of wireless targeted drug delivery prompts questions about affordability and equitable access to treatment. Disparities in affordability between developed and developing countries must be addressed. Since distributive justice, which seeks fairness in resource allocation, is crucial. How can we ensure that nanotherapies are accessible to all, regardless

of economic status?

Performing procedures involving nanocarriers on patients necessitates informed consent. However, the nanometric size of these devices complicates matters, being susceptible to raising doubts concerning the possibility of performing a procedure with nanocarriers on the patient without having informed consent since they are unseen to the naked eye. But why there may be a greater risk of bad faith in performing a therapy based on nanodevices than in a case of classical medical intervention? This concern arises within the population due to the nanometric dimensions of these devices, which are visible to the patient's eyes, an issue that may occur with a higher probability in the context of inadequate legal frameworks and ethical guidelines concerning the implementation of wireless targeted drug delivery. Wireless targeted drug delivery often involves cloud-based storage of patient information. Therefore, privacy concerns arise due to the potential accessibility of sensitive medical data by unauthorized entities.

In this context, the principle of autonomy is closely tied to the one of human dignity. Second-generation nanotechnology, whose movement within the body can be mechanically manipulated using external devices specifically designed for the intended application, involves a transition between local and cloud storage of certain medical data, which raises questions about patients' freedom to exercise their autonomy [19]. Who should have access to this medical data and records—employers or insurance companies? Furthermore, the emergence of implantable drug delivery nanochips and nanoparticles capable of on-demand pharmaceutical release, as well as nanochips for early diagnosis, will inevitably give rise to safety and responsibility concerns.

However, one critical ethical implication arises from the cost of nanodrugs. As these therapies become available, questions of equity and justice emerge. The unaffordability of nanodrugs for a significant portion of the population will affect society at different levels, leading to higher discrepancies between citizens and countries.

As we could observe in the previous lines, the clinical implementation of nanotherapies, like targeted drug delivery raises significant ethical questions, necessitating an amelioration of existing guidelines. Researchers, including Allon *et al.* (2016), emphasize the distinct ethical and social dilemmas posed by nanomedicine

[1]. These challenges demand adaptive responses and the establishment of novel professional, legal, and regulatory frameworks. Schuurbiers *et al.* (2009) advocate for an ethical amelioration of thinking and action through information and proactive engagement. Their approach underscores the need for ongoing ethical reflection. Building on these ideas, it becomes evident that ethics is a dynamic enterprise that demands "better collaborations among ethicists, scientists, social scientists, and technologists" and the development of a "more sophisticated ethical analysis" [6]. The author states that the "ethical reflection should become more tightly integrated with the R&D process itself, and requires increased collaborations through new multidisciplinary engagements between nanoscientists and nanoethicists" [6]. Contrary to the notion of creating entirely new ethical principles, Van de Poel (2008) asserts that nanotechnology's ethical challenges can be addressed by applying existing ethical norms to this novel field. However, the assessment of the ethical issues is far from straightforward; it involves a complex and long process rather than rigid adherence to established principles.

4.3.2. The Question of Ethical Blindness

In their study, Allon *et al.* (2016), identifying several factors contributing to ethical blindness, shed light on a critical risk associated with professionals who lack awareness of the ethical implications of their actions: "The absence of an ethical prism could lead to epistemic and moral blindness, which might lead to abusive research and the violation of human rights." [1] The lack of adequate ethical training and practice and the novelty of wireless targeted drug delivery in the medical field often cause professionals to overlook specific ethical issues. In contrast to ethical blindness, ethical awareness represents "the eagerness and ability to designate moral situations and dilemmas; critically analyze, evaluate, and additionally change one's own moral esteems; and look up the effects of one's own attitude for the lives of others." [20] But how can we cultivate ethical awareness in this new medical intervention? What are the key stages involved in acquiring the necessary skills for a well-balanced approach to addressing ethical issues related to wireless targeted drug delivery?

First and foremost, achieving ethical awareness requires moral maturity and a common moral competence. [21] In the realm of biomedicine, an approach based on common morality plays a pivotal role, standing as the basis for the four

principles of bioethics [14]. Common morality refers to the implicit norms that exist within a community [10]. These norms are often expressed through a "Cold" discourse [10], which avoids active debate. The central question in the common morality approach is: What action should I take to behave morally? When we approach ethical issues from the standpoint of common morality, we are essentially examining the established moral routines and orders [10]. However, it's essential to acknowledge that common moral norms can vary significantly across communities, and cultures, being influenced by personal belief systems and moral routines [10]. Therefore, professionals must navigate this variability while upholding ethical standards in their practice.

Beyond this foundational condition, the journey toward ethical expertise in nanoethics involves two key stages: basic ethical expertise in a specific professional field at theoretical and practical levels [22]; and individual and organizational awareness of ethical issues having an impact at different levels [3]. The development of ethical awareness as an expert involves the ability to engage in expert discourse regarding ethical issues. Professionals should not only grasp ethical principles but also apply them effectively in their work. This discourse is based on normative ethics, which entails an explicit understanding of ethical principles and values. However, unlike common morality, which seeks moral consensus within a community, there is no universal agreement on normative standards in moral philosophy [13]. Consequently, experts approach ethical issues through "hot" debates [10] aimed at addressing the question: *Which moral norms and values are in conflict?* Moreover, professionals must recognize that ethical considerations extend beyond individual patients to broader contexts, including organizational policies, community well-being, and environmental sustainability. They must be cognizant of ethical issues in the field concerning various levels—individual, organizational, community-wide, and even global. However, merely possessing expertise in the medical field is insufficient for gaining profound insights into nanoethical issues. True expertise in nanoethics requires transdisciplinary comprehension, encompassing ethical considerations related to health, society, and future implications [3]. Understanding the impact of their decisions on these different scales and areas is crucial within multidisciplinary teams.

In the subsequent pages, we will explore the intricate ethical challenges posed by cancer therapies, which are further compounded by the integration of

nanotechnology.

4.3.3. Ethical Challenges in Classical Cancer Therapies

In their paper, Zahedi and Bagher [23] expose the most common ethical challenges faced by Canadian clinicians in classical cancer therapies, highlighting ten key challenges faced by healthcare professionals, and emphasizing the need for contextual decision-making processes. The first ethical aspect centres around the disagreement between patients (and their families) and healthcare providers concerning treatment decisions. Peppercorn [24] (see also Swierstra, T., & Rip, A. [10]) highlights that some patients are not aware of the dangers of their situation, leading them to prefer natural treatments over surgical interventions. This divergence necessitates a delicate balance considering not only medical efficacy but also patient autonomy and informed consent. However, inadequate patient information may also lead to an overestimation of potential drug benefits, potentially compromising their ability to make informed decisions [25] [26]. Because of the complexity of the decision-making process in oncology, the authors highlight the existence of a real ethical challenge in obtaining informed consent which is crucial in cancer therapy since significant risks and benefits are associated with ongoing care.

Another ethical concern aims at the participation of oncological patients in research. Often, these patients have high expectations regarding experimental drugs. Additionally, the drug approval process raises significant ethical issues, as it aims to come with a profit for pharmaceutical companies and government regulations [27]. The integration of surgical innovations and new technologies into patient care also gives rise to a complex ethical dilemma since the legal frameworks and ethical guidelines do not consistently keep pace with the rapid advancements in medical procedures in oncology.

However, the multifaceted nature of cancer treatment extends beyond clinical considerations. Socio-economic, political, and cultural influences play a crucial role in the decision-making process [27]-[29] demanding community-based principles and procedures as essential tools in navigating this complex landscape. For example, in the case of religious individuals, it becomes imperative to consider their faith-based perspectives since their decisions, whether accepting or declining oncological treatments, often hinge on religious convictions. Another ethical challenge highlighted by Zahedi and Bagher pertains to the criteria for

establishing waiting lists [23]. Additionally, many cancer patients grapple with other chronic illnesses. Consequently, there is a pressing need to allocate healthcare resources effectively for the elderly, chronically ill, and mentally ill populations. Unfortunately, the shortage of family physicians or primary care teams in both rural and urban areas poses significant challenges in delivering appropriate care. But even when specialized clinicians are available, the risk of medical errors remains a concern. Thus, in the context of terminal illness or serious medical conditions, decisions regarding withholding or withdrawing life-sustaining treatments may give rise to numerous ethical questions.

4.3.4. Ethical Considerations in Wireless Targeted Drug Delivery for Cancer Therapy

In cancer therapies, various types of nanocarriers are employed. In this discussion, we will specifically focus on wireless targeted nanocarriers that possess magnetic properties, like Metal-based nanopharmaceuticals [30]. These nanocarriers are composed of metal-based nanopharmaceuticals. Notably, Ranade and Hollinger (2003) mention that in the 1960s, the first trials were conducted to explore the potential use of magnetically controlled drug carriers. These trials regarded the therapeutic vascular occlusion of an intracerebral aneurysm using carbonyl iron suspended in an albumin solution, as well as the treatment of renal cell carcinoma using carbonyl iron mixed with liquid silicone. Initially, research on magnetically controlled drug nanocarriers was met with optimism. However, subsequent trials encountered significant challenges associated with these devices since:

> "First, this approach requires an electromagnet that generates magnetic fields sufficient for extracorporeal control of the carriers in deep organs. Second, the magnet is expected to permit simultaneous fluoroscopic monitoring for arterial catheterization. However, frequent or continuous generation of strong magnetic fields may interfere with fine fluoroscopic monitoring and produce severe problems in the x-ray generator." [31]

The authors emphasize the need for additional studies to evaluate the toxicity of strong magnetic fields. Consequently, a critical concern in establishing fundamental ethical principles and necessary regulatory frameworks for the clinical application of magnetically controlled drug nanocarriers lies in assessing their safety profile and potential toxic effects. This evaluation is essential to determine

the risk/benefit ratio associated with their use [30].

However, assessing the risk/benefit ratio also involves considering the impact of computerized systems in the context of wireless targeted drug delivery. This issue is explored through the concept of Value Sensitive Design (VSD), which originates in the field of Human-Computer Interaction (HCI) and "supports hands-on development of high-tech products while taking social and ethical issues into account" [19]. VSD recognizes that technological applications are inherently non-neutral or value-laden, exerting, however, both political and morally relevant effects on individuals and society. The technology facilitates or constrains the agency depending on fundamental moral values such as freedom, equality, trust, autonomy, privacy and justice. The VSD approach aims to reconcile divergent and opposing values within engineering design and innovation processes. Notably, this methodology is particularly well-suited for addressing the dynamic and rapidly evolving landscape of uncertainty and ethical concerns in fields like nanopharmacy [19]. Over time, VSD has extended its influence to various specialized branches of information and communication technology (ICT), including Affective Computing and Augmented Reality, providing a framework to address responsibility in these domains [19].

In the context of emerging cancer therapies that rely on wireless targeted drug delivery, several ethical questions come to the forefront. These questions revolve around the responsible use of this innovative approach. Specifically, we need to consider the following six categories of ethical issues:

1) *Informed Consent and the right to discontinue the treatment:* What information should patients receive about this novel therapy? How will healthcare providers assess informed consent and ensure that patients fully understand the risks, benefits, and alternatives? What instruments will be used to evaluate patient comprehension, unrealistic hopes, or dystopian fears related to wireless drug delivery in cancer therapy? Given the complexity and the novelty of nanodevice-based therapies, patients often lack a comprehensive understanding of these treatments, their implementation procedures, and potential side effects. How will patients' right to discontinue treatment be respected, especially when nanocarriers may persist in the body?

2) *Privacy and Data Security:* Given the wireless nature of drug delivery, how do

we protect patient privacy and prevent unauthorized access to sensitive medical data? How will data access be managed, and what security requirements will be established given the system's vulnerability to cybernetic attacks? How will the healthcare system safeguard privacy in the context of medical surveillance based on wireless nanocarriers that might inadvertently reveal patient health information unrelated to the purpose of the clinical exam [1]? What impact will the use of a procedure involving cloud storage of personal information have on the doctor-patient relationship [7] and patient trust [5]? This question is particularly significant in the context of the evolving medical profession, where advancements in nanotechnology are shifting it from an individualistic art of execution to a collaborative team and technology-based service [5].

3) *Resource Allocation:* As this technology becomes more widespread, how do we allocate resources fairly? Should certain patient groups receive priority access?

4) *The overall toxicity* [31] *and the personalized treatment:* How do contractors assess the overall risk/benefit ratio? In the context of wireless drug delivery for cancer therapy, what does "personalized medicine" [1] entail?

5) *Long-Term Effects on the body and environment:* What are the potential long-term effects of wireless drug delivery? How do we monitor and address any unforeseen consequences? What is the duration of nanocarrier presence in the body, and how will it behave after drug release at the targeted site? Although this concern is not explicitly addressed in the literature, it warrants consideration due to questions about nanocarrier toxicity and privacy risks. In what ways might the use of wireless targeted drug delivery influence the relationship of humanity with the environment [30]?

6) *Equitable Access:* How will this procedure's affordability impact society, especially considering that it may not be accessible to patients with low income? How can we ensure that wireless targeted drug delivery is accessible to all patients, regardless of socioeconomic status or geographical location? How will the principles of equity [5] and autonomy be upheld regarding the objective of personalized medicine, considering the scarcity and costliness of the procedure?

As we delve into the critical ethical questions surrounding wireless targeted drug delivery in cancer therapy, it becomes evident that these inquiries arise from

different perspectives. On one hand, patients express concerns about nanocarrier safety, potential toxicity, data management, transparency, and the affordability of these therapies [5]. On the other hand, providers also grapple with ethical considerations related to delivering such treatments. The subsequent SWOT analysis will further illuminate patient-related aspects in this context:

Strengths	**Weaknesses**
• Safety: Overall reduction in toxicity. • Targeted Delivery: The ability to direct the nanocarrier to specific regions within the body.	• Toxicity: Refers to the potential adverse effects of the materials used in nanocarriers, as well as the impact of the magnetic field. • Uncertainty Regarding Therapeutic Response: The individual's body response to therapy remains uncertain. • Cost Considerations: The high expenses associated with these procedures. • Decision-Making Challenges: Decisions are often based on ambiguous foundations, and calculating risks for specific cases and patient types proves difficult. • Off-Label Drug Use and Personalized Medicine: Balancing off-label drug usage with personalized medicine objectives may be difficult [32]. • Data Vulnerability: Concerns related to cloud storage, including mobility and accessibility of databases. • Wireless Behavioural Monitoring: Surveillance of patient behaviour using wireless technology.
Opportunities	**Threats**
• Responsibility: Uphold ethical standards and consider consequences. • Informed Consent: Respect autonomy and protect participants' rights. • Public Debates: Foster informed decision-making through diverse perspectives. • Transparency: Disclose data security, usage, and accessibility. • Post-Market Monitoring: Continuously assess safety and efficacy.	• Patient's Incomprehension of the Procedure: Lack of understanding can jeopardize patient safety. • Patient's Suspicions: Concerns about magnetic fields, data security, and misuse posing risks. • Patient Vulnerability and Cloud Data Threats: The potential risks to patient vulnerability posed by cloud data storage. • Unfair Access to Treatment: Disparities hinder equitable healthcare delivery.

The SWOT Analysis highlights one of the most critical ethical principles to be considered during the clinical implementation of wireless targeted drug delivery: responsibility. Therefore, the medical team must prioritize the best interests of the patient, even when faced with a substantial amount of unknown data associated with this novel therapy. Consequently, this emerging clinical therapy can be approached through the lens of the 'responsible risking' concept.

Within the clinical setting, unforeseen situations may arise, placing the medical team in a position of responsibility for outcomes they could not predict. To act responsibly, it becomes essential to focus on a limited number of relevant scenarios that are both '"reasonably morally and probabilistically relevant." [33]. In other words, responsible action involves aligning with a substantive idea of responsibility and behaving in a morally sound manner that responds to descriptive responsibility: "To act and risk responsibly is to live up to some substantive idea about being responsible and acting in a way that morally responds to descriptive responsibility in a morally good way." [33]. This concept of 'responsible risking' necessitates the assignment of specific roles and obligations related to responsibility. These roles can be established by addressing the following four fundamental questions:

"(1) Who is in charge of A (where A is some action or domain)?'' [33]—it assigns the role of responsibility;

"(2) Who is to blame for O (where O is some outcome of an action)?" [33]—it assigns the role of blameworthiness;

"(3) Who will fix O or compensate for O (where O is some outcome of an action)?" [33]—it assigns the role of responsibility to repair;

"(4) Why did you do A (where A is some action)?" [33]—it assigns the role of answerability;

Therefore, clinicians should consistently adhere to three essential conditions. Firstly, they must legitimately maintain control over matters within their domain of responsibility. Secondly, they should avoid actions that would exceed their ability to make necessary repairs. Lastly, clinicians must also refrain from behaviours that might render them incapable of adequately explaining and justifying their actions to those directly impacted [33].

The second type of ethical challenge related to the clinical application of

wireless targeted drug therapy centres around the complexity and transdisciplinarity inherent in implementing this therapy for patients with cancer. It is crucial to emphasize that a significant drawback in clinical practice arises from the lack of robust ethical guidance and a well-defined legal framework within the field of nanomedicine [3]. In our subsequent SWOT analysis, we will delve into the implications of requiring an interdisciplinary approach in the clinical practice of this therapy, juxtaposed against the existing deficiencies in regulatory frameworks:

Strengths	**Weaknesses**
• Interdisciplinarity: The interdisciplinary approach to wireless targeted drug delivery therapy enhances the team's ability to perform the therapy effectively, thereby facilitating their duties.	• Poor Regulatory Frameworks and Terminological Clarification: The existing regulatory frameworks lack robustness, and there is a need for clearer terminological definitions. • Inadequate Internal Protocols for Interdisciplinary Work: The internal protocols related to interdisciplinary collaboration are insufficient, hindering effective teamwork. • Deficiency in Interdisciplinary Competencies: Addressing complex ethical issues observed in practice requires stronger interdisciplinary competencies.
Opportunities	**Threats**
• Ethical Debates within the Medical Team: These discussions, engaging with diverse perspectives within the team, aim to identify the key ethical questions related to wireless targeted drug delivery in cancer therapy. • Continuous Evaluation of the Risk-Benefit Ratio: Regularly assessing the risk-benefit ratio ensures maintaining a balance between potential risks and therapeutic benefits. • *Ethical Training and Coaching: Providing comprehensive training on ethical issues specific to wireless targeted drug delivery in cancer therapy is crucial. The focus should be on emphasizing the responsibility of each professional within the medical team.*	• Lack of Organizational Competencies in Ethics: This refers to the deficiency in the organization's ability to effectively address ethical matters. It implies that the organization lacks the necessary skills, knowledge, and practices related to ethical decision-making and conduct. • The Absence of an Ethical Expert in Medical Institutions: Having an expert who specializes in ethical matters of the field can significantly contribute to informed decision-making, policy development, and maintaining ethical standards in implementing innovative therapies.

In this SWOT analysis, we shed light on the need for a paradigm shift in recognizing the status of ethical experts within the biomedical field. Specifically, we explore the implications of implementing nanomedical therapies in cancer treatment—a multifaceted process that necessitates the collaboration of experts across diverse scientific domains. Traditionally, biomedical proficiency sufficed as a basis for bioethical expertise. However, the emergence of nanoethics introduces a transformative dimension. Mere competence within the biomedical field no longer guarantees mastery in this specialized arena. Instead, a holistic understanding that transcends disciplinary boundaries becomes essential and as we venture into clinical applications of therapies based on nanodevices, we encounter an intricate web of interdisciplinary expertise since "Progression in the field of nanotechnology causes transformation of the medical profession from being an art of execution to a team and technology service." [5]. Therefore, our central question arises: Who should be considered responsible and an expert in the emerging field of nanoethics? As we delve into the clinical deployment of nanodevices for targeted drug delivery, we encounter the need for a convergence of expertise—from medicine and biology to materials science and engineering. Nanoethics, therefore, emerges as a distinct field where traditional approaches dissolve, and interdisciplinary collaboration thrives. As clinicians, engineers, and designers converge, the traditional boundaries of responsibility and liability blur. This convergence shifts responsibility and liability toward service providers and product developers, resulting in a profound transformation of patient-physician relationships.

The introduction of the concept of Value Sensitive Design (VSD) marks a departure from the exclusive responsibility of medical professionals. Instead, it heralds a new era where designers and engineers play pivotal roles in healthcare ethics. This shift invites a reveille—an awakening—to embrace a design-centric approach in shaping the ethical landscape of medicine: "The developments outlined indicate a shift towards the designer or engineer as a responsible agent in the healthcare at the expense of traditional medical practitioner, calls for a new reveille: a design turn in ethics." [19].

4.4. Conclusions

In contrast to classical medicine, nanomedicine introduces a more intricate clinical approach to patient care. This paradigm shift involves close collaboration

between medical professionals and experts from diverse scientific fields. While nanomedicine holds great promise, it also harbours uncertainties.

Practically, the integration of nanomedication into clinical practice faces challenges that can erode patient trust in healthcare providers, especially when outcomes fail. Furthermore, accurately assessing the impact of nanomedicine-based therapies on individual health, community well-being, and environmental biodiversity remains elusive [34].

The abundance of unknown data surrounding nanomedicine necessitates an extensive ethical exploration—one that may encounter both philosophical and ethical pitfalls. As we pioneer this frontier, striking a balance between innovation and caution becomes crucial, equally important as engaging in public debates on the ethical implications of these groundbreaking therapies.

Addressing the challenges posed by nanotechnology requires interdisciplinary collaboration, robust regulatory frameworks, and a steadfast commitment to ethical decision-making. While it is evident that nanotechnology will inevitably raise certain issues, mere awareness is insufficient for discerning the specific ethical dilemmas that emerge during its ongoing development: “Obviously we have reason to expect that nanotechnology will raise certain issues, but that knowledge alone is not enough to discern the concrete ethical issues as they arise during the further development of nanotechnologies.” [13].

One such critical area is wireless targeted drug delivery in cancer therapy. Medical professionals, accustomed to conventional challenges in cancer treatment, may inadvertently overlook the unique complexities associated with wireless drug delivery. In this context, the scarcity of comprehensive data impedes accurate estimation of the procedure’s impact. Furthermore, their ethical competencies may not align with the novel demands of this cutting-edge approach in cancer therapy.

To navigate these challenges effectively, the medical team should adopt the concept of “responsible risking.” This involves thoughtful reflection on various scenarios and the assignment of clear responsibilities. Additionally, enhancing cooperation skills within a transdisciplinary team is essential for resolving ethical dilemmas.

By fostering interdisciplinary dialogue and embracing ethical reflexivity, we can pave the way toward responsible and transformative advancements in nanotechnology.

References

[1] Allon, I., Ben-Yehudah, A., Dekel, R., Solbakk, J.-H., Weltring, K.-M., & Siegal, G., (2016). Ethical issues in nanomedicine: Tempest in a teapot? *Medicine, Health Care and Philosophy*, 20(1), 3-11, p. 1. https://doi.org/10.1007/s11019-016-9720-7

[2] Yasri, S., & Wiwanitkit, V., (2017). Important ethical issues for nanomedicine. *Journal of Research in Medical Sciences: the Official Journal of Isfahan University of Medical Sciences*, *22*, 138. https://doi.org/10.4103/jrms.JRMS_856_17

[3] Alpert, S. (2008), Neuroethics and Nanoethics: Do We Risk Ethical Myopia? *Neuroethics,* 1. p. 62. https://doi.org/10.1007/s12152-007-9001-5

[4] Miah, A. (2017). Nanoethics, Science Communication, and a Fourth Model for Public Engagement. *Nanoethics*, *11*(2), 139-152. p. 144. https://doi.org/10.1007/s11569-017-0302-9

[5] Gökçay, B., & Arda, B., (2015). Nanotechnology, nanomedicine; ethical aspects. *Revista Romana de Bioetica*, 13(3). p. 6. http://www.bioetica.ro/index.php/arhiva-bioetica/article/view/829/pdf

[6] Schuurbiers, D., Sleenhoff, S., Jacobs, J. F., & Osseweijer, P., (2009). Multidisciplinary Engagement with Nanoethics Through Education-The NanoBio-RAISE Advanced Courses as a Case Study and Model. *Nanoethics*, 3(3), 197-211. https://doi.org/10.1007/s11569-009-0073-z

[7] Biroudian, S., Abbasi, M., & Kiani, M., (2019). Theoretical and Practical Principles on Nanoethics: A Narrative Review Article. *Iranian Journal of Public Health*, *48*(10), 1760-1767. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6908913/

[8] Bacchini, F., (2013). Is Nanotechnology Giving Rise to New Ethical Problems? *NanoEthics*, 7(2), 107-119. p. 111. https://doi.org/10.1007/s11569-013-0179-1

[9] Dupuy, J.-P. (2007). Some Pitfalls in the Philosophical Foundations of Nanoethics. *Journal of Medicine and Philosophy*, 32(3), 237-261. p. 241. https://doi.org/10.1080/03605310701396992

[10] Swierstra, T., & Rip, A., (2007). Nano-ethics as NEST-ethics: Patterns of Moral Argumentation About New and Emerging Science and Technology. *NanoEthics*, 1, 3-20. https://doi.org/10.1007/s11569-007-0005-8

[11] Gordijn, B. & Cutter, A. M., (2014). *In Pursuit of Nanoethics*. Dordrecht: Springer.

[12] Kermisch, C., (2012). Do new Ethical Issues Arise at Each Stage of Nanotechnological Development? *Nanoethics*, 6, 29-37. https://doi.org/10.1007/s11569-011-0137-8

[13] Van de Poel, I., (2008). How Should We Do Nanoethics? A Network Approach for Discerning Ethical Issues in Nanotechnology. *NanoEthics*, 2(1), 25-38. pp. 31-34. https://doi.org/10.1007/s11569-008-0026-y

[14] Ebbesen, M., & Jensen, T. G., (2006). Nanomedicine: techniques, potentials, and ethical implications. *Journal of biomedicine & biotechnology*, 2006(5), 51516. https://doi.org/10.1155/JBB/2006/51516

[15] Gupta, N., Fischer, A. R. H., & Frewer, L. J., (2015). Ethics, Risk and Benefits Associated with Different Applications of Nanotechnology: A Comparison of Expert and Consumer Perceptions of Drivers of Societal Acceptance. *Nanoethics*, *9*(2), 93-108. https://doi.org/10.1007/s11569-015-0222-5

[16] Patenaude, J., Legault, G. A., Béland, P., Parent, M., & Boissy, P. (2011). Moral Arguments in the Debate over Nanotechnologies: Are We Talking Past Each Other? *Nanoethics*, *5*(3), 285-293. https://doi.org/10.1007/s11569-011-0132-0

[17] Myskja, B. K., (2011). Trustworthy Nanotechnology: Risk, Engagement and Responsibility. *Nanoethics*, *5*, 49-56. https://doi.org/10.1007/s11569-011-0116-0

[18] Bensaude Vincent, B., & Loeve, S. (2013). Metaphors in Nanomedicine: The Case of Targeted Drug Delivery. *NanoEthics,* 1, 1-17. p. 1. https://doi.org/10.1007/s11569-013-0183-5

[19] Timmermans, J., & Zhao, Y. (2011). Ethics and Nanopharmacy: Value

Sensitive Design of New Drugs. *Nanoethics*, *5*(3), 269-283. https://doi.org/10.1007/s11569-011-0135-x

[20] Turegun, N. (2018). "Ethical Awareness, Ethical Decision Making, and Transparency: A Study on Turkish CPAs in Istanbul," Chapter, In Salman, A., & Abdul Razzaq, M. G., (Eds.), Accounting from a Cross-Cultural Perspective, *IntechOpen*. https://doi.org/10.5772/intechopen.7686

[21] Pleşu A., (2017), *Minima Moralia*, Humanitas. pp. 20-21.

[22] Frunză, M., "Despre expertiza etică şi Expertul în Etică în România. Elemente pentru un profil etic adaptat contextului românesc", In Haţegan, V. (Ed.) (2019) *Consilierea Filosofică şi etică*. *Reflecţii şi practici în România*, Editura Eikon. p. 183.

[23] Zahedi, F., & Bagher, A.L. (2007). Cancer Ethics from the Islamic Point of View. *Iran J Allergy Asthma Immunol,*6 (Suppl. 5), 17-24.

[24] Peppercorn, J., (2012). Ethics of ongoing cancer care for patients making risky decisions. *Journal of Oncology Practice*, 8(5), e111-e113. https://doi.org/10.1200/JOP.2012.000622

[25] Ryan, M., (1979). Ethics and the patient with cancer. *British Medical Journal*, *2*(6188), 480-481. https://doi.org/10.1136/bmj.2.6188.480

[26] Cuyler, A. J. (2017). Ethics, priorities and cancer. *Journal of Cancer Policy*, 11, 6-11. https://doi.org/10.1016/j.jcpo.2016.09.007

[27] Wise, P. H., (2016). Cancer drugs, survival, and ethics, *BMJ*, 355, i5792. p. 2. https://doi.org/10.1136/bmj.i5792

[28] Ghose, S., Radhakrishnan, V., & Bhattacharya, S., (2019). Ethics of cancer care: beyond biology and medicine. *Ecancer*, *13*, 911. p. 2. https://doi.org/10.3332/ecancer.2019.911

[29] Jolley, M.G., (1988). Ethics of cancer management from the patient's perspective. *Journal of Medical Ethics,*14(4), 188-190. https://doi.org/10.1136/jme.14.4.188

[30] Farjadian, F., Ghasemi, A., Gohari, O., Roointan, A., Karimi, M., & Hamblin, M. R., (2018). Nanopharmaceuticals and nanomedicines currently on the market: challenges and opportunities. *Nanomedicine* (London, England), 14(1), 93-126. p. 4. https://doi.org/10.2217/nnm-2018-0120

[31] Ranade, V. V. & Hollinger, M. A., (2003) *Drug Delivery Systems, Second Edition Pharmacology and Toxicology Basic and Clinical Aspects, 2nd ed.* CRC Press. p. 318.

[32] Toader, E., (2015) "Implicaţii etice privind deciziile medicale în indicaţii terapeutince neaprobate (terapia off-label)" In Buta M. G. (Ed.) *Influența valorilor creştine asupra bioeicii Europene*. Editura Renaşterea. p. 323-330.

[33] Hayenhjelm, M., (2023) "Responsible Risking, Forethought, and the Case of Human Gene Editing", In *Risk and Responsibility in Context*, Routledge. p. 155.

[34] Arnold, A. M., Bradley, A. M., Taylor, K. L., Kennedy, Z. C., & Omberg, K. M. (2022). The Promise of Emergent Nanobiotechnologies for In Vivo Applications and Implications for Safety and Security. *Health security*, 20(5), 408-423. p. 415. https://doi.org/10.1089/hs.2022.0014.

Chapter 5

Drug Delivery to the Brain: State of the Art and Challenges

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Abstract: The recent increase in research efforts into pathological conditions associated with ageing is related not only to the phenomenon of the inversion of the base of the age pyramid in most developed countries but also to the need to accelerate the achievement of global targets set within the framework of the United Nations' Sustainable Development Goals (SDGs), namely Good Health and Well-being (SDG3) and Reduced Inequalities (SDG10). Certain pathological conditions, such as Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis, acquired immunodeficiency syndrome (AIDS), diabetes, seizures, stroke, hypertensive encephalopathy, and traumatic brain injuries, necessitate the exploration and development of new drug delivery methods to effectively reach the central nervous systems. This chapter adds to the reference literature by detailing the ways used to transport medications into the central nervous systems, all of which deal with high degrees of complexity and problems. It discusses the benefits and drawbacks of the methods used to target the brain and highlights the most often utilized administration routes for brain targeting medications (e.g., invasive and non-invasive). Among the intraosseous injection of medication into the skull are approaches such as interstitial drug delivery, targeted ultrasound technologies, and pulsed electrical field technologies. Legal and ethical considerations related

to the distribution techniques are also addressed and debated.

Key-words: Ageing, Brain, Central Nervous Systems, Drugs delivery, Neuroscience

5.1. Introduction

The Central Nervous Systems (CNS) encompasses the spinal cord and the brain. As the brain performs vital functions essential to the human body, it is very well protected by the blood-brain barrier (BBB) [1] [2].

The BBB is a biological barrier that provides a controlled microenvironment by regulating the exchange of ions and molecules between the bloodstream and the brain parenchyma. It is composed by specialized endothelial cells, which line the cerebral capillaries, connected to each other through tight junctions, by pericytes that encircle the endothelial cells and can regulate capillary blood flow, by basement membrane, embedding the pericytes, and by astrocyte end-feet [3]-[5]. This barrier regulates the exchanges between the circulatory system and the CNS, allowing the passage of essential nutrients that provide a healthy neuronal function and homeostasis within the brain environment, while protecting the brain against potentially harmful substances and pathogenic agents present in the blood [2] [6] [7]. The tight junctions formed by the endothelial cells prevent paracellular diffusion from blood to brain, as they seal the aqueous pathways between the endothelial cells [3] [8]. Also, they result in high electric resistance, restricting penetration of large molecules with high electric charge, hydrophilicity, and polarity [5].

As so, the selective permeability of the BBB is essential for maintaining brain homeostasis but hampers the delivery of therapeutic compounds. Additionally, the presence of efflux transporters further impedes drug penetration, resulting in low brain concentrations and suboptimal therapeutic outcomes. With varying degrees of success, numerous strategies have been investigated to overcome the BBB and improve brain drug delivery, including direct surgical administration, transitory BBB disruption, and BBB permeability increase [9].

Under certain pathological conditions, such as Parkinson's disease (PD), Alzheimer disease (AD), multiple sclerosis, acquired immunodeficiency syndrome (AIDS), diabetes, seizures, stroke, hypertensive encephalopathy, and traumatic

brain injuries, the BBB shows a certain level of disruption, the junctions are compromised and, therefore, the BBB may become hyper-permeable [10]. However, the duration and scale of such permeability is not fully understood, and it can be associated with complications, once influx of blood-borne substances may interfere with the normal solute diffusion to the brain parenchyma [10] [11]. For instance, early-stage tumor cells in brain tumors, such as gliomas, develop their own BBB, so that, when they reach a certain growth level, new brain tumor capillaries form the blood-brain tumor barrier (BBTB). In the center of the tumor, the BBTB shows high permeability, and, on the other hand, the permeability is lower on the periphery. The combination of the BBB and the BBTB results in a major difficulty when trying to reach the brain in order to treat brain tumors [10] [12].

Although the BBB presents itself as a defense mechanism to the brain, the same characteristics that protect the brain can also be limitative and challenging while treating CNS diseases, once they create an obstacle to deliver drugs to target the brain, being only approximately 5% of candidate drugs able to effectively penetrate the BBB [7] [13].

The present chapter contributes to the reference literature by presenting the strategies in use to deliver drugs into the CNS, all dealing with high levels of complexity and challenges, such as methods to assure temporary BBB disruption, approaches to use a range of biomaterials and therapeutic delivery systems, the use of nanoparticles to penetrate into the hyper-permeable BBB, or Viral vectors, Exosomes, or Prodrugs, and Convection-Enhanced drug delivery.

Following, the chapter theorizes about the advantages and disadvantages concerning the methods used to target the brain and highlights the most accepted administration routes for brain targeting drugs (from one side the invasive ones, such as Intrathecal and intraventricular administration route, and the non-invasive, namely oral administration and intranasal drug delivery).

Subsequently, the chapter discusses the novel methods to drug delivery to the brain. Approaches like the interstitial drug delivery, or the focused ultrasound technologies, and the pulsed electrical field technologies, among the intraosseous administration of medication into the skull are herein presented.

Legal or ethical issues associated with the delivery methods in used or

proposed to be used are summarized and the chapter ends with the major conclusions.

5.2. Current Strategies Used to Deliver Drugs to the Brain

Transporting drugs across the blood-brain barrier (BBB) is still a significant problem for the treatment of neurological and neurodegenerative illnesses, brain cancers, and many other diseases. On the brain side of the BBB, the capillary vessels and the non-neuronal and neuronal cells forms a highly selective security system that permits waste products, such as carbon dioxide, to be removed from the brain and nutrients and oxygen to enter from the bloodstream. In contrast, infection agents, neurotoxic compounds, and the majority of drugs—including small molecule, antibody, and anti-sense oligonucleotide therapeutics—are actively prevented from entering the brain parenchyma by the BBB. The selective permeability of the BBB is essential for maintaining brain homeostasis but hampers the delivery of therapeutic compounds. Less than 1% of a drug's bloodstream dose typically reaches the brain. This low drug penetration capability governed by the BBB, results in low brain concentrations and suboptimal therapeutic outcomes.

Drug administration to the brain is now made easier by a range of biomaterials and therapeutic delivery systems that have recently been created [14]. For instance, by designing or altering the physiochemical properties of therapeutic compounds to allow for transport across the BBB, by circumventing the BBB by administering drugs via alternate routes, and also by temporarily disrupting the BBB (BBBD) using biophysical therapies, these technologies have addressed many of the limitations imposed by the BBB. The authors discuss the key components of BBB structure and function that are the mechanistic targets of these approaches, as well as colloidal drug carrier delivery systems, intranasal, intrathecal, and direct interstitial drug delivery techniques, focused ultrasound BBBD, and pulsed electrical field induced BBBD.

Several methods have been implemented to increase the accumulation of therapeutic agents in the brain parenchyma by disrupting the BBB. Those methods mainly explore the paracellular (between cells), transcellular (across cells), and transcellular leakage involving vesicles (transcytotic) pathways [15]. For the use of the transcellular pathway therapeutic molecules are chemically modified to cross the BBB and been transported through the endothelial cells via passive

diffusion. In contrast, the use of the paracellular pathway the tight junction and adherent junctions are chemically or physically weakened to allow therapeutic molecules to move between cells and bypass the BBB [16]. Some of the methods to deliver medicines to the brain via temporary BBB disruption, either by chemical or physical mechanisms, are considered invasive [12]. For instance, the administration of hyperosmotic solutions, such as mannitol, is used to reduce endothelial intracellular volume and decrease the expression of tight junctions' proteins, which leads to a disruption in the BBB and opens an opportunity to target the brain [10] [12] [17] [18]. The use of vasoactive compounds ultimately also resulted in increased BBB permeability [19]. Despite their advantages, the chemical disruption of the BBB may lead off-target effects and tissues damaging, which limits its clinical application [20] [21]. As for the physical mechanisms used to increase BBB permeability, the focus is to change the BBB integrity by destabilizing the tight junctions, which can recover with time after stimulation has ended [16]. Various external stimulations, such as light and ultrasound have been used to physically disrupt the BBB integrity [22].

To overcome the BBB obstacle to treat CNS diseases, there have been developed novel engineered materials for brain-targeted drug delivery approaches to efficient and safely release drugs into the brain. Nanoparticle-based drug delivery have been highly explored and developed to be used as a potentially enhanced method to drug delivery to the brain. Engineered nanoparticles can be functionalized with ligands that specifically bind to receptors expressed on BBB endothelial cells, such as receptor-mediated transcytosis or disruption of tight junctions enabling active transport across the barrier [22]. As the name suggests, nanoparticles are very small and can penetrate the hyper-permeable BBB found under disease conditions, being used as carriers for therapeutics [10] [23]. Small lipophilic molecules can diffuse across the BBB, and Solid-Lipid Nanoparticles (SLN), which are mainly constituted by lipids or modified lipid nanostructures, have a hydrophobic lipid core in which both lipophilic and hydrophilic drugs can be dispersed [24].

Viral vectors also gained attention within the therapeutics for neurodegenerative disorders, once they have the ability to infect host cells with their genetic material, such ability can be applied to gene therapy, transferring the desired genes to individuals suffering from said diseases [23] [25]. Herpes simplex virus (HSV),

lentivirus, adenovirus, and adeno-associated virus (AAV) vectors have reached gene transduction in the brain [10] [25]. For instance, different clinical studies showed promising results with the potential to revolutionize the treatment of PD using viral vector as carrier of gene-therapy [26].

Exosomes are vesicles that are released to the extracellular fluid by all cells and have the capability to deliver small molecules past the BBB. Moreover, they also function as delivery vehicles for proteins (larger molecules) and nucleic acids (RNA and DNA) [27]. PD is associated with low levels of antioxidant proteins like catalase, that helps in inhibition of neurodegeneration and oxidative stress. A study showed that catalase incorporated in exosomes was effectively delivered through the BBB and improved the disease state in PD patients [28]. As exosomes are naturally carriers of nucleic acids, they also can work in gene therapy and alter brain tumor expression [10] [28].

An alternative strategy involves the use of prodrug approaches, where the therapeutic agent is chemically modified to improve its stability, lipophilicity, and BBB permeability, and subsequently converted into its active form within the brain. Prodrugs are inactive drug molecules that after administration are metabolized within the brain into active drugs, they derivate from molecules that must go under biotransformation to turn active [29]. This method aims to overcome delivery limitations such as weak solubility, instability, systemic toxicity, poor absorption, and fast presystemic metabolism [29]. The modification of drugs/prodrugs aims to increase its brain penetration by chemical modifications, such as lipidation or conjugation with brain-targeting ligands, which can improve their lipophilicity and receptor affinity, facilitating their transport across the BBB [30]-[32].

Convection-Enhanced drug delivery (CED) consists of the infusion of drugs directly to the brain interstitial spaces through catheters by applying a pressure gradient in a way that convective forces influence the diffusion of the drugs [33]-[35]. This strategy allows to overcome the BBB penetration drug limitations and at the same time to directly infuse the drug in a specific brain region, avoiding putative off-target effects and possible toxicity. It has been mostly developed for brain cancer treatments, but holds a tremendous potential to be used for other neurological disorders, such as for the delivery of gene therapy-based strategies for PD [36]-[38].

5.3. Methods Used to Target the Brain: Advantages and Disadvantages

All the strategies previously described and the methods that are used to deliver drugs to target the brain, present advantages and disadvantages.

The blood-brain barrier disruption techniques have the advantages of enhancing the drug delivery to the brain, increase the therapeutic efficacy, and to potentiate non-invasive treatment. Nevertheless, it is known for its invasive nature, potential risk of adverse effects (such as edema or hemorrhage), and limited availability of specialized equipment. Besides the BBB disruption using chemical agents being capable of achieving therapeutic concentrations on the brain, it has some safety concerns once it may compromise irreversibly the integrity and functioning of the BBB by leveraging brain uptake of blood-borne substances with neurotoxic properties, causing neuronal injury [7] [12].

Nanoparticle-based drug delivery systems present advantages characteristics such as bioavailability, specific tissue targeting, controlled release, non-immunogenic nature, cost effectiveness, improved drug stability, enhanced brain penetration, targeted delivery, controlled release and they are able to deliver pharmacologic formulations. On the other hand, these systems depict several putative disadvantages such as potential toxicity, storage and administration stability, challenges in scale-up production, and regulatory concerns [24] [39].

With regard to viral vectors, their use as carriers for gene-therapy shows to be the most effective in achieving high efficiency gene transduction, it can target a larger number of cells, has the innate ability of cellular tropism, among other features. AVV viral vectors have shown a safety profile in humans as well as the capacity of gene delivery to the brain [40]. Still disadvantages on the use of virus have been pinpointed over the years, such as the fact they have a high production cost and may trigger severe immune response, cause mutagenesis by inserting their exogenous DNA in the host genome, and raised safety concerns, as patients have died in clinical trials using this carrier system [10] [25] [41] [42]. Being the route for administration of viral vectors the intrathecal injection, which on one hand it is very specific, on the other hand it entails risks from being an invasive procedure [10] [12].

For exosomes it has been shown that they potentially can bypass through the

BBB and that they have the ability to deliver genes to the brain, but the choice of donor cells, loading methodologies, pharmacokinetics, and toxicity still represent a challenge for its use as therapy carriers to the brain [10] [43].

Prodrug design shows improved pharmacokinetic properties, enhanced brain penetration, and increased drug stability within the bloodstream, has the potential for reduced side effects due to localized drug activation and can convert inactive drugs into active drugs within the brain [29] [44]. As to disadvantages and limitations, additional steps are required for prodrug activation, potential for off-target effects, and challenges in designing suitable prodrug candidates, it requires specific knowledge of drugs metabolism, it may not be applicable to all drugs, and it brings a risk of an incomplete conversion or conversion in an unintended area [29] [44].

In theory, CED as the advantages of bypassing the BBB, delivery of therapeutic concentrations, limitation of side effects, and the facility of concentrating the drug in a specific region of the brain, nonetheless, this method has physical limitations such as air bubbles (can disrupt the flow of the technique), backflow or reflux along the catheters, tumors and BBB disruption, and putative flow rate instability [33] [34].

5.4. Administration Routes for Brain Targeting Drugs

5.4.1. Overview

Determining the acceptance of specific drug delivery methods by patients involves considering factors such as ease of use, convenience, comfort, and patient preference.

Intrathecal administration route consists of the direct injection of drugs into the cerebrospinal fluid, which surrounds the brain. This method promptly increases cerebrospinal fluid drug concentration and is the simplest way to circumvent the BBB [2] [45].

Intracerebroventricular (ICV) injection is widely used to study the delivery of viral vectors and nanoparticles into the CNS, the drugs are directly injected into the brain ventricles, escaping the obstacle of the BBB. Unlike other methods, both small and large molecules can reach the brain and low drug concentrations is used diminishing toxicity [2] [46]. Thus, this is an invasive approach and whereas there

are elevated drug concentrations at the administration sites, the concentrations on outlying sites are low [46].

Some consideration must be taken in the use of the intrathecal and ICV administration routes, because they require skilled healthcare professionals for administration and holds the risk of infection or other complications associated with invasive procedures.

As the two methods above are invasive approaches, options for non- or less-invasive routes to administrate drugs to target the brain are oral administration and intranasal drug delivery, among others, such as transdermal, subcutaneous and intramuscular administration routes.

Oral administration is a convenient and commonly used route for drug delivery, however the effectiveness of reaching the brain depends on various factors, and overcoming the BBB remains a key consideration in the development of drugs targeting the central nervous system. Besides being non-invasive, oral administration for drug delivery comes with low costs and easy dosage of drugs. However, for the delivery to be effective there are some boundaries, such as the formulations must have enhanced gut absorption and the molecules have to reach certain plasma levels to cross the BBB and reach the brain [47]. Some drugs are designed to include specific chemical structures or characteristics that promote its transportation through the BBB, such as the previously mentioned prodrugs. Nanoparticles lipid-based formulations may be good candidates for efficient and safe oral administration to target the brain, especially in chronic disease [47] [48]. Besides its convenience as non-invasive and easy administration route, the oral administration imposes some disadvantages that need to be taken under consideration such as the potential gastrointestinal degradation and variable drug absorption rates.

Intranasal drug delivery has gained considerable attention as a promising strategy for targeting the brain due to its ability to bypass the BBB and directly access the CNS. Intranasal drug delivery takes advantage of two key routes: the olfactory and trigeminal pathways, that connect nasal cavity to the brain, and overcomes the BBB to reach the brain directly [2]. The olfactory region of the nasal mucosa is richly vascularized and highly permeable, allowing molecules to directly enter the CNS through the olfactory epithelium and olfactory nerves. Additionally, the

trigeminal nerve endings present in the nasal mucosa can facilitate drug transport to the brain by following the trigeminal nerve pathway [49] [50]. Previous studies have investigated the feasibility and efficacy of intranasal drug delivery for various neurological disorders. For instance, intranasal delivery of insulin-like growth factor-I (IGF-I) has shown promise in enhancing cognitive function and promoting neuroprotection in experimental models of Alzheimer's disease [51]. Antiretrovirals have low permeability across the BBB, but when incorporated in nanoparticle systems, increasing bioavailability and brain uptake, and administrated via intranasal route, the concentrations detected in the brain were higher than when orally administrated [52]. The major obstacle of this route is that the volume of the drug to be administrated cannot be elevated in order to be able to pass through the nasal cavity [10].

Both oral and intranasal administration come with the advantage of self-administration by the patients and, consequently, high compliance with the treatment.

5.4.2. Novel Methods

Regarding the mechanistic approaches targeted at improving drug delivery to the central nervous system (CNS) comprise the following: (i) designing or altering the physicochemical characteristics of substances or encapsulate it, to enable their transcellular pathways of transport into the CNS; (ii) subjecting the brain parenchyma to high concentrations of agents; (iii) avoiding the natural CNS barriers by using locoregional drug delivery techniques; and (iv) enhancing paracellular transport through the BBB [53]-[56].

Apart from intrathecal, oral and intranasal delivery methods, the other approaches are still considered exploratory treatments in people and animals and do not yet have recognized therapeutic usage, namely the Interstitial drug delivery (such as biodegradable implants, convection enhanced delivery, devices and drugs), or the focused ultrasound technologies (devices and drugs), and the pulsed electrical field technologies (devices and drugs).

The viability of several new strategies to bypass the BBB has been demonstrated in animals with naturally occurring brain diseases, opening the door for more widespread clinical applications of these techniques. These strategies involve designing novel therapeutic compounds or combining new drugs with non-

conventional routes of administration, such as via transient modulation of BBB permeability or via direct interstitial delivery.

For instance, according to recent studies myeloid and lymphoid cells can travel through direct vascular channels that exist between the meninges and the skull bone marrow. Currently, work is being done using as premise the fact that direct vascular channels can additionally enable the transfer of drugs to the brain from the bone marrow in the skull [9]. This work uses the intraosseous administration of medication into the skull (intracalvariosseous [ICO]), securing an innovative method for delivering drugs into the brain, bypassing the BBB. Such work was performed through the administration of nine compounds on mouse skulls, in order to improve ICO and to measure the brains' entry capacity, contrasting to systemic administration. This novel approach showed that the skull is somehow permeable to medication penetration into the brain, achieving the brain tissue in a higher percentage when contrasting with systemic application, and represents an innovative strategy to tackle the challenges of brain drug delivery.

These results imply that, in addition to the systemic route, the novel BBB bypassing pathway from skull to brain can be a potential approach for drug's entrance into the brain following ICO, presenting an interesting methodology to the successful treatment of brain disorders.

5.5. Legal or Ethical Issues

Legal and ethical issues can arise in the context of drug delivery methods, particularly concerning patient safety, informed consent, regulatory compliance, and equitable access to healthcare.

Starting in clinical trials, it may be a challenge to obtain the informed consents from patients who have neurological/neurodegenerative disorders that compromise their cognitive abilities. Ethically, it must be ensured that the patients or their legal representatives fully understand the benefits and risks.

It is necessary to assess effectiveness and safety of the drug delivery strategies and methods, especially when talking about the brain, which is the most important organ in the human body [57]. Patients should be fully informed about the risks, benefits, and potential side effects of the procedures. Genetic and all type of putative toxicity needs to be evaluated as well, in order to assess the potential of novel

drugs to produce mutations or other types of genetic damage [57].

The use of specific approaches, such as the use of nanoparticle-mediated delivery to the brain pose some concerns for ethics, as the brain surgery is only an option for mid- and late-stage patients of neurological diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD), because of the unethical condition of invasive treatments when targeting early-stage patients. For Parkinson's Disease, this is a huge constraint, as it endangers the efficacy of these delivery approaches, being that with the evolution of the disease, the less the efficacy of the therapy [58].

Other legal concerns are the long-term effects on the brain, the off-label use, as physicians should not use these methods for conditions for which they are not approved by regulatory authorities, and industrial property issues.

Ethically, there may be concerns about equal access to such drugs and methods and also about animal welfare and regulations once animal testing is widely used in these approaches.

Addressing legal and ethical considerations is essential to ensure the responsible and ethical advancement of drug delivery technologies, balancing innovation with the protection of individual rights and societal well-being. Collaboration between researchers, healthcare providers, policymakers, and ethicists are vital in navigating these complex issues.

5.6. Conclusions

In conclusion, the delivery of drugs to the brain, particularly in the context of CNS diseases, presents both challenges and opportunities. The BBB plays a crucial role in protecting the brain from harmful substances, but it can also limit the effectiveness of drug delivery to the brain. Several methods and strategies have been developed to overcome these challenges, each with its own set of advantages and limitations.

Disrupting the BBB using chemical agents or physical mechanisms can achieve drugs therapeutic concentrations in the brain, but it poses safety concerns by compromising BBB integrity and potentially causing neuronal injury. Nanoparticle strategies offer controlled drug release and tissue targeting but face challenges

in administration stability. Viral vectors show high gene transduction efficiency but come with safety concerns and high production costs. Exosomes have the potential to cross the BBB and deliver genes, but face challenges related to donor cell selection, loading methodologies, pharmacokinetics, and toxicity. Prodrug design enhances drug stability but requires specific knowledge and may not be applicable to all drugs. CED bypasses the BBB and deliver therapeutic concentrations but has physical limitations and may entail risks.

Novel approaches are being developed to surpass certain limitations to efficacy, and are constrained by disease stage and ethical considerations, such as the interstitial drug delivery, or the focused ultrasound technologies, as well as the pulsed electrical field technologies, and the intraosseous administration of medication into the skull.

The choice of drug delivery method and routes depends on the specific requirements of the disorders and the drugs being used. Intrathecal injection, ICV injection, oral, and intranasal administration routes offer distinct levels of invasiveness and effectiveness in delivering drugs to the brain, and their continuous improvement will contribute to reduce the existing challenges in treating neurological disorders.

Legal and ethical issues surrounding these drug delivery methods include obtaining informed consent from patients, assessing the effectiveness and safety of the strategies, addressing genetic toxicity concerns, preventing off-label use, and addressing industrial property issues. Additionally, ethical considerations include ensuring equal access to these treatments and animal testing. Such ethical limitations, for instance the unethical condition of invasive treatments when targeting early-stage patients can slow down or impede the efficacy of therapeutic treatment of patients.

In terms of implications, further research is required concerning the development of innovative approaches and administration roots, using a patient centric design and experience.

In summary, while drug delivery methods to target the brain is a challenging endeavor, the ongoing development of novel approaches and the consideration of legal, ethical, and regulatory aspects will contribute to advancing treatments for

CNS diseases and neurodegenerative disorders.

References

[1] S. C. Shinde, N. B. Mahale, S. R. Chaudhari, and R. S. Thorat, "RECENT ADVANCES IN BRAIN TARGETED DRUG DELIVERY SYSTEM: A REVIEW," 2015. [Online]. Available: www.wjpr.net

[2] M. Raghav, V. Gupta, R. Awasthi, A. Singh, and G. T. Kulkarni, "Nose-to-brain drug delivery: Challenges and progress towards brain targeting in the treatment of neurological disorders," *J Drug Deliv Sci Technol*, vol. 86, p. 104756, Sep. 2023, doi: 10.1016/j.jddst.2023.104756.

[3] D. J. Begley, "Delivery of therapeutic agents to the central nervous system: the problems and the possibilities," *Pharmacol Ther*, vol. 104, no. 1, pp. 29-45, Oct. 2004, doi: 10.1016/j.pharmthera.2004.08.001.

[4] A.-C. Luissint, C. Artus, F. Glacial, K. Ganeshamoorthy, and P.-O. Couraud, "Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation," *Fluids Barriers CNS*, vol. 9, no. 1, p. 23, Dec. 2012, doi: 10.1186/2045-8118-9-23.

[5] Y. Zhou, Z. Peng, E. S. Seven, and R. M. Leblanc, "Crossing the blood-brain barrier with nanoparticles," *Journal of Controlled Release*, vol. 270, pp. 290-303, Jan. 2018, doi: 10.1016/j.jconrel.2017.12.015.

[6] N. J. Abbott, A. A. K. Patabendige, D. E. M. Dolman, S. R. Yusof, and D. J. Begley, "Structure and function of the blood-brain barrier," *Neurobiol Dis*, vol. 37, no. 1, pp. 13-25, Jan. 2010, doi: 10.1016/j.nbd.2009.07.030.

[7] L. Guo, J. Ren, and X. Jiang, "Perspectives on Brain-Targeting Drug Delivery Systems," *Curr Pharm Biotechnol*, vol. 13, no. 12, pp. 2310-2318, Oct. 2012, doi: 10.2174/138920112803341770.

[8] H. Gao, "Progress and perspectives on targeting nanoparticles for brain drug delivery," *Acta Pharm Sin B*, vol. 6, no. 4, pp. 268-286, Jul. 2016, doi: 10.1016/j.apsb.2016.05.013.

[9] J. H. Kang and Y. T. Ko, "Intraosseous administration into the skull: Potential blood-brain barrier bypassing route for brain drug delivery," *Bioeng Transl Med*, vol. 8, no. 2, Mar. 2023, doi: 10.1002/btm2.10424.

[10] X. Dong, "Current Strategies for Brain Drug Delivery," *Theranostics*, vol. 8, no. 6, pp. 1481-1493, 2018, doi: 10.7150/thno.21254.

[11] L. Han and C. Jiang, "Evolution of blood-brain barrier in brain diseases and related systemic nanoscale brain-targeting drug delivery strategies," *Acta Pharm Sin B*, vol. 11, no. 8, pp. 2306-2325, Aug. 2021, doi: 10.1016/j.apsb.2020.11.023.

[12] R. I. Teleanu *et al.*, "Current Strategies to Enhance Delivery of Drugs across the Blood-Brain Barrier," *Pharmaceutics*, vol. 14, no. 5, p. 987, May 2022, doi: 10.3390/pharmaceutics14050987.

[13] H. Kadry, B. Noorani, and L. Cucullo, "A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity," *Fluids Barriers CNS*, vol. 17, no. 1, p. 69, Dec. 2020, doi: 10.1186/s12987-020-00230-3.

[14] B. Partridge *et al.*, "Advancements in drug delivery methods for the treatment of brain disease," *Front Vet Sci*, vol. 9, Oct. 2022, doi: 10.3389/fvets.2022.1039745.

[15] M. A. Erickson and W. A. Banks, "Transcellular routes of blood-brain barrier disruption," *Exp Biol Med*, vol. 247, no. 9, pp. 788-796, May 2022, doi: 10.1177/15353702221080745.

[16] M. E. M. Stamp, M. Halwes, D. Nisbet, and D. J. Collins, "Breaking barriers: exploring mechanisms behind opening the blood-brain barrier," *Fluids Barriers CNS*, vol. 20, no. 1, p. 87, Nov. 2023, doi: 10.1186/s12987-023-00489-2.

[17] A. Burgess and K. Hynynen, "Drug delivery across the blood-brain barrier using focused ultrasound," *Expert Opin Drug Deliv*, vol. 11, no. 5, pp. 711-721, May 2014, doi: 10.1517/17425247.2014.897693.

[18] B. J. Umlauf and E. V Shusta, "Exploiting BBB disruption for the delivery of nanocarriers to the diseased CNS," *Curr Opin Biotechnol*, vol. 60, pp. 146-152, Dec. 2019, doi: 10.1016/j.copbio.2019.01.013.

[19] Q. He *et al.*, "Towards Improvements for Penetrating the Blood-Brain Barrier—Recent Progress from a Material and Pharmaceutical Perspective," *Cells*, vol. 7, no. 4, p. 24, Mar. 2018, doi: 10.3390/cells7040024.

[20] F. Gosselet, R. A. Loiola, A. Roig, A. Rosell, and M. Culot, "Central nervous

system delivery of molecules across the blood-brain barrier," *Neurochem Int*, vol. 144, p. 104952, Mar. 2021, doi: 10.1016/j.neuint.2020.104952.

[21] J. Li *et al.*, "Development of Novel Therapeutics Targeting the Blood-Brain Barrier: From Barrier to Carrier," *Advanced Science*, vol. 8, no. 16, Aug. 2021, doi: 10.1002/advs.202101090.

[22] D. Wu, Q. Chen, X. Chen, F. Han, Z. Chen, and Y. Wang, "The blood-brain barrier: structure, regulation, and drug delivery," *Signal Transduct Target Ther*, vol. 8, no. 1, p. 217, May 2023, doi: 10.1038/s41392-023-01481-w.

[23] J. Ahlawat *et al.*, "Nanocarriers as Potential Drug Delivery Candidates for Overcoming the Blood-Brain Barrier: Challenges and Possibilities," *ACS Omega*, vol. 5, no. 22, pp. 12583-12595, Jun. 2020, doi: 10.1021/acsomega.0c01592.

[24] M. K. Satapathy *et al.*, "Solid Lipid Nanoparticles (SLNs): An Advanced Drug Delivery System Targeting Brain through BBB," *Pharmaceutics*, vol. 13, no. 8, p. 1183, Jul. 2021, doi: 10.3390/pharmaceutics13081183.

[25] S. J. Gray, K. T. Woodard, and R. J. Samulski, "Viral vectors and delivery strategies for CNS gene therapy," *Ther Deliv*, vol. 1, no. 4, pp. 517-534, Oct. 2010, doi: 10.4155/tde.10.50.

[26] M. Kozłowska *et al.*, "Parkinson's disease gene therapy: a comparative effectiveness review of completed clinical trials in terms of their possible implementation in treatment," *Medical Science Pulse*, vol. 16, no. 4, pp. 1-7, Feb. 2023, doi: 10.5604/01.3001.0016.2849.

[27] D. Ha, N. Yang, and V. Nadithe, "Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges," *Acta Pharm Sin B*, vol. 6, no. 4, pp. 287-296, Jul. 2016, doi: 10.1016/j.apsb.2016.02.001.

[28] M. J. Haney *et al.*, "Exosomes as drug delivery vehicles for Parkinson's disease therapy," *Journal of Controlled Release*, vol. 207, pp. 18-30, Jun. 2015, doi: 10.1016/j.jconrel.2015.03.033.

[29] J. Rautio, K. Laine, M. Gynther, and J. Savolainen, "Prodrug Approaches for CNS Delivery," *AAPS J*, vol. 10, no. 1, pp. 92-102, Mar. 2008, doi: 10.1208/s12248-008-9009-8.

[30] L. Bohn Thomsen *et al.*, "Brain Delivery Systems via Mechanism Independent of Receptor-Mediated Endocytosis and Adsorptive-Mediated Endocytosis," *Curr Pharm Biotechnol*, vol. 13, no. 12, pp. 2349-2354, Oct. 2012, doi: 10.2174/138920112803341842.

[31] B. R. Green *et al.*, "Introduction of lipidization-cationization motifs affords systemically bioavailable neuropeptide Y and neurotensin analogs with anticonvulsant activities," *Journal of Peptide Science*, vol. 16, no. 9, pp. 486-495, Sep. 2010, doi: 10.1002/psc.1266.

[32] N. Kato *et al.*, "Development of an apolipoprotein E mimetic peptide-lipid conjugate for efficient brain delivery of liposomes," *Drug Deliv*, vol. 30, no. 1, Dec. 2023, doi: 10.1080/10717544.2023.2173333.

[33] N. U. Barua, S. S. Gill, and S. Love, "Convection-Enhanced Drug Delivery to the Brain: Therapeutic Potential and Neuropathological Considerations," *Brain Pathology*, vol. 24, no. 2, pp. 117-127, Mar. 2014, doi: 10.1111/bpa.12082.

[34] A. M. Mehta, A. M. Sonabend, and J. N. Bruce, "Convection-Enhanced Delivery," *Neurotherapeutics*, vol. 14, no. 2, pp. 358-371, Apr. 2017, doi: 10.1007/s13311-017-0520-4.

[35] S.-K. Wu, C.-L. Tsai, Y. Huang, and K. Hynynen, "Focused Ultrasound and Microbubbles-Mediated Drug Delivery to Brain Tumor," *Pharmaceutics*, vol. 13, no. 1, p. 15, Dec. 2020, doi: 10.3390/pharmaceutics13010015.

[36] A. I. Mehta *et al.*, "Convection Enhanced Delivery of Macromolecules for Brain Tumors," *Curr Drug Discov Technol*, vol. 9, no. 4, pp. 305-310, Oct. 2012, doi: 10.2174/157016312803305951.

[37] P. S. Larson, "Improved Delivery Methods for Gene Therapy and Cell Transplantation in Parkinson's Disease," *J Parkinsons Dis*, vol. 11, no. s2, pp. S199-S206, Sep. 2021, doi: 10.3233/JPD-212710.

[38] R. S. D'Amico, M. K. Aghi, M. A. Vogelbaum, and J. N. Bruce, "Convection-enhanced drug delivery for glioblastoma: a review," *J Neurooncol*, vol. 151, no. 3, pp. 415-427, Feb. 2021, doi: 10.1007/s11060-020-03408-9.

[39] S. Scioli Montoto, G. Muraca, and M. E. Ruiz, "Solid Lipid Nanoparticles for Drug Delivery: Pharmacological and Biopharmaceutical Aspects," *Front Mol*

Biosci, vol. 7, Oct. 2020, doi: 10.3389/fmolb.2020.587997.

[40] F. Mingozzi and K. A. High, "Immune responses to AAV vectors: overcoming barriers to successful gene therapy," *Blood*, vol. 122, no. 1, pp. 23-36, Jul. 2013, doi: 10.1182/blood-2013-01-306647.

[41] E. Check, "Gene therapy put on hold as third child develops cancer," *Nature*, vol. 433, no. 7026, pp. 561-561, Feb. 2005, doi: 10.1038/433561a.

[42] S. Tanabe *et al.*, "The use of an optimized chimeric envelope glycoprotein enhances the efficiency of retrograde gene transfer of a pseudotyped lentiviral vector in the primate brain," *Neurosci Res*, vol. 120, pp. 45-52, Jul. 2017, doi: 10.1016/j.neures.2017.02.007.

[43] T. Yang *et al.*, "Delivery of Small Interfering RNA to Inhibit Vascular Endothelial Growth Factor in Zebrafish Using Natural Brain Endothelia Cell-Secreted Exosome Nanovesicles for the Treatment of Brain Cancer," *AAPS J*, vol. 19, no. 2, pp. 475-486, Mar. 2017, doi: 10.1208/s12248-016-0015-y.

[44] K. Prokai-Tatrai and L. Prokai, "Prodrug Design for Brain Delivery of Small- and Medium-Sized Neuropeptides," in *Neuropeptides*, vol. 789, A. Merighi, Ed., 2011, pp. 313-336. doi: 10.1007/978-1-61779-310-3_21.

[45] M. M. Miyake and B. S. Bleier, "The Blood-Brain Barrier and Nasal Drug Delivery to the Central Nervous System," *Am J Rhinol Allergy*, vol. 29, no. 2, pp. 124-127, Mar. 2015, doi: 10.2500/ajra.2015.29.4149.

[46] A. Sarkar *et al.*, "Nanoparticles as a Carrier System for Drug Delivery Across Blood Brain Barrier," *Curr Drug Metab*, vol. 18, no. 2, pp. 129-137, Mar. 2017, doi: 10.2174/1389200218666170113125132.

[47] A. Brookes *et al.*, "Is oral lipid-based delivery for drug targeting to the brain feasible?" *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 172, pp. 112-122, Mar. 2022, doi: 10.1016/j.ejpb.2022.02.004.

[48] S. Bertoni, D. Tedesco, M. Bartolini, C. Prata, N. Passerini, and B. Albertini, "Solid Lipid Microparticles for Oral Delivery of Catalase: Focus on the Protein Structural Integrity and Gastric Protection," *Mol Pharm*, vol. 17, no. 9, pp. 3609-3621, Sep. 2020, doi: 10.1021/acs.molpharmaceut.0c00666.

[49] F. Erdő, L. A. Bors, D. Farkas, Á. Bajza, and S. Gizurarson, "Evaluation of intranasal delivery route of drug administration for brain targeting," *Brain Res*

Bull, vol. 143, pp. 155-170, Oct. 2018, doi: 10.1016/j.brainresbull.2018.10.009.

[50] S.-H. Jeong, J.-H. Jang, and Y.-B. Lee, "Drug delivery to the brain via the nasal route of administration: exploration of key targets and major consideration factors," *J Pharm Investig*, vol. 53, no. 1, pp. 119-152, Jan. 2023, doi: 10.1007/s40005-022-00589-5.

[51] R. G. Thorne, G. J. Pronk, V. Padmanabhan, and W. H. Frey, "Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration," *Neuroscience*, vol. 127, no. 2, pp. 481-496, Jan. 2004, doi: 10.1016/j.neuroscience.2004.05.029.

[52] S. Gupta, R. Kesarla, N. Chotai, A. Misra, and A. Omri, "Systematic Approach for the Formulation and Optimization of Solid Lipid Nanoparticles of Efavirenz by High Pressure Homogenization Using Design of Experiments for Brain Targeting and Enhanced Bioavailability," *Biomed Res Int*, vol. 2017, pp. 1-18, 2017, doi: 10.1155/2017/5984014.

[53] W. M. Pardridge, "Blood-Brain Barrier and Delivery of Protein and Gene Therapeutics to Brain," *Front Aging Neurosci*, vol. 11, Jan. 2020, doi: 10.3389/fnagi.2019.00373.

[54] B. R. Partridge *et al.*, "High-Frequency Irreversible Electroporation (H-FIRE) Induced Blood-Brain Barrier Disruption Is Mediated by Cytoskeletal Remodeling and Changes in Tight Junction Protein Regulation," *Biomedicines*, vol. 10, no. 6, p. 1384, Jun. 2022, doi: 10.3390/biomedicines10061384.

[55] S. Pathan *et al.*, "CNS Drug Delivery Systems: Novel Approaches," *Recent Pat Drug Deliv Formul*, vol. 3, no. 1, pp. 71-89, Jan. 2009, doi: 10.2174/187221109787158355.

[56] A. P. Spencer *et al.*, "Breaking Barriers: Bioinspired Strategies for Targeted Neuronal Delivery to the Central Nervous System," *Pharmaceutics*, vol. 12, no. 2, p. 192, Feb. 2020, doi: 10.3390/pharmaceutics12020192.

[57] S. C. Gad, "Safety and regulatory requirements and challenge for CNS drug development," *Neurobiol Dis*, vol. 61, pp. 39-46, Jan. 2014, doi: 10.1016/j.nbd.2013.09.017.

[58] O. Bondarenko and M. Saarma, "Neurotrophic Factors in Parkinson's

Disease: Clinical Trials, Open Challenges and Nanoparticle-Mediated Delivery to the Brain," *Front Cell Neurosci*, vol. 15, Jun. 2021, doi: 10.3389/fncel.2021.682597.

Chapter 6
Role of iRGD Peptide in Cancer Therapy and Targeted Drug Delivery

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Abstract: Chemotherapy is a commonly used therapeutic approach for various cancers. However, many chemotherapeutic agents raise significant safety concerns, contributing to increased mortality and morbidity in cancer patients. Despite advanced targeted therapies like monoclonal antibodies, kinase inhibitors, and immune checkpoint blockers developed in recent decades, substantial success in mitigating adverse effects remains challenging.

One major factor behind these adverse effects is the inadequate intra-tumoral distribution of therapeutics, whether in free drug form or as nanoparticles, due to the versatile nature of the tumor microenvironment (TME). Tumor-penetrating peptides offer a promising solution to enhance drug penetration into tumors. Among them, the iRGD peptide has proven effective in improving the distribution of anticancer drugs within tumor tissues. This peptide, when conjugated with anticancer drugs or nanoparticle formulations or co-injected with them, enhances therapeutic distribution within tumors. It simultaneously reduces the typical pattern of drug distribution in normal tissues, resulting in increased anticancer effects.

This chapter explores into targeted therapies, examining the drawbacks of traditional chemotherapy and emphasizing the pivotal role of the TME in the constraints of anticancer treatments. Following this, we thoroughly explore the role demonstrated by iRGD in the delivery of anticancer drugs.

6.1. Introduction

Cancer is the rapid growth of aberrant or transformed cells that proliferate

beyond their typical limits and can invade nearby organs called metastasis and it is the major cause of death from cancer [1]. GLOBOCAN 2023 estimates worldwide 19.3 million new cancer cases and almost 10.0 million cancer deaths suggesting possible increase in their number in upcoming years [2]. According to the report released on 1 February 2024 by the International Agency for Research on Cancer (IARC) over 35 million new cancer cases are expected in 2050, a 77% rise from 20 million in 2022. Global cancer rates are rising due to population ageing and exposure to risk factors, especially those associated with socioeconomic development [2].

Surgery has been the most common approach in cancer management over the years, but due to cancer's recurrence, therapeutics have become crucial. Though chemotherapy was supposed to be effective, it wasn't effective in all cancer cases [3]. Alterations in the physiology of tumor microenvironment could be one of the primary factors [4]. The other potential approach in cancer treatment could be radiotherapy, which involves high doses of radiation to destroy cancer cells. However, limiting harm to healthy tissue is yet to be solved [4]. Limitations of these conventional therapies have led to rise in novel approaches like cancer immunotherapy and target-based therapies. Relative to the earlier discussed approaches (surgery, chemotherapy and radiotherapy), cancer immunotherapy has significantly improved patient survival and quality of life. To date, seven distinct kinds of immune checkpoint inhibitors have been approved by FDA for cancer therapy [5]. Monoclonal antibodies are one of a kind, and examples include Elrexfio (elranatamab), a bispecific antibody designed by Pfizer and Talvey (Talquetamab) developed by Johnson and Johnson, which achieved FDA approval in August 2023. Currently, CAR (Chimeric antigen receptor) T-cell therapy has become more effective in cancer management [6]. Immunotherapies are constrained by acquired resistance and immune-related adverse effects which might harm healthy tissues/organs, for which immunosuppressants are recommended [7]. Personalised cancer vaccines and novel immune check point inhibitors could be the future of potent cancer immunotherapy.

Target based therapies altering tumor microenvironment could be effective cancer therapies. Designing new class of cancer drugs to interfere with target proteins specifically to have a crucial role in the tumor progression or growth is referred to be targeted therapies [8]. Basically, targeted therapies are less toxic and

more tumor specific as they use small molecule inhibitors or therapeutic antibodies [9].

6.2. Targeted Therapies for Cancer

Targeted therapies comprise of direct and indirect methods for tumor targeting specifically. Direct approaches include targeting of tumor antigens which leads to altering signalling through interfering the target proteins by small molecule inhibitors or monoclonal antibodies. Indirect approaches include the expression of tumor antigens on the cell surface which acts as a target device for ligands and different effector molecules. Development of effective immunotherapies, ligand-targeted therapies and antibody therapies rely on molecular identification of tumor antigens as depicted in **Figure 1** [9].

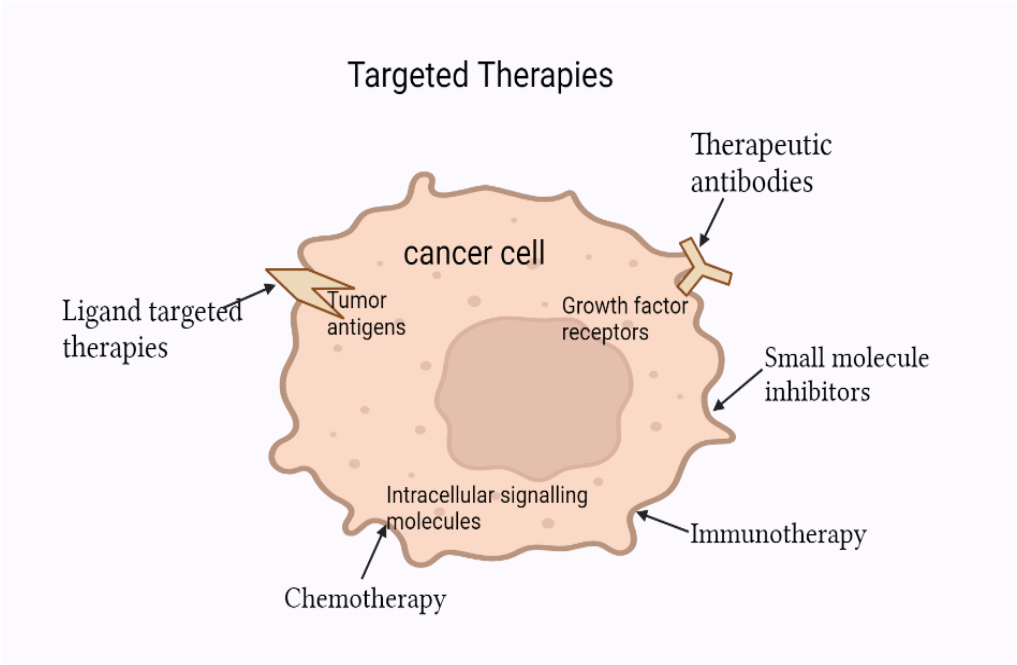


Figure 1. The frontiers of cancer treatment with a comprehensive overview of novel targeted therapies.

6.2.1. Antibody-Based Targeted Therapies

For various malignancies, monoclonal antibodies (mAbs) play a major key role in

the treatment. mAbs are effective and well tolerated and consequently approved by FDA because of their high affinity, long serum half-life and specificity for the treatment of different cancers [10]. The ability of mAbs to target tumors improved the selectivity compared to other anticancer agents [11].

Novel antibody-targeted therapies with numerous antigen recognition sites, effector domains and different sizes and structures will influence the ability to target tumor. Therapeutic antibodies are effective alone when conjugated with toxins, radionuclides or other chemotherapeutic agents which show promising applications in cancer therapy [11].

Rituximab (Rituxan; Genentech/Biogen Idec), the first therapeutic chimeric antibody was approved in the year 1997 by FDA for B-cell non-Hodgkin's lymphoma [12]. Trastuzumab (Herceptin; Genentech) in 1998 was approved for use in patients with metastatic breast cancer by FDA [13]. Bevacizumab (Avastin; Genentech) is used for the treatment of metastatic colorectal cancer which was approved in 2004 [14]. Approximately, 25-30% of biotechnological products which are been developed are mAbs among which several are approved for cancer as shown in **Table 1** [10].

Table 1. Therapeutic antibodies approved for oncological therapy by FDA till 2022.

Drug name and Company	Mab	Target	Type of cancer	Side effects	Year	Reference
Rituxan, Genentech	Rituximab	CD20	B cell lymphoma	Vision changes, memory problems	1997	[15]
Herceptin, Genentech	Trastuzumab	Her2	Breast cancer	Febrile Neutropenia	1998	[16]
Campath, Genzyme	Alemtuzumab	CD52	Chronic myeloid leukaemia	Thrombocytopenia	2001	[17]
Zevalin, Biogen	Ibritumomab tiuxetan	CD20	Non-Hodgkin's lymphoma	Neutropenia	2002	[18]
Bexxar, GSK	Tositumomab	CD20	Non-Hodgkin's lymphoma	Hypotension, vasodilation	2003	
Erbitux, ImClone (EliLilly)	Cetuximab	EGFR	Colorectal cancer	Belching burning, eye itching	2004	[19]

Avastin, Genentech	Bevacizumab	VEGF	HER2-breastcancer	Blurry vision, eye redness	2004	[20]
Vectibix, Amgen	Panitumumab	EGFR	Colorectal cancer	Insomnia, discolouration of skin	2006	[19]
Arzerra, Novartis	Ofatumumab	CD20	Chroniclymphocytic leukemia	Tumor lysis syndrome, skin thinning	2009	[19]
Yervoy, Medarex	Ipilimumab	CTLA-4	Melanoma, renal cell carcinoma	Pruritus, diarrhea	2011	[20]
Gazyva, Genentech	Obinutuzumab	CD20	Chroniclymphocytic leukemia,	Pneumonia	2013	[19]
Keytruda, Merck & Co.	Pembrolizumab	PD-1	Non-small cell lung cancer	Encephalitis, yellowing of skin	2014	[20]
Unituxin, United Therapeutics	Dinutuximab	GD2	Neuroblastoma	Upper airway swelling, irritated nerve cells	2015	[19]
Lartruvo, ImClone	Olaratumab	PDGRFa	Soft tissue sarcoma	Tingling and numbness	2016	[19]
Bavencio, Pfizer	Avelumab	PD-L1	Merkel-cell carcinoma	Bone pains and chest discomfort	2017	[19]
Lumoxiti, Innate Pharma,	Moxetumomab pasudotox	CD22	Hairy cell leukemia	Heamolytic uremic syndrome	2018	[21]
Danyelza, Y-mAbs	Naxitamab	GD2	Neuroblastoma	Blurred vision, neuropathic pain	2020	[22]
Rybrevant, Genmab	Amivantamab	EGFR, cMET	Non-small cell lung cancer	Blurred vision, skin lesions	2021	[19]
Imjudo, MedImmune	Tremelimumab	CTLA-4	Hepatocellular carcinoma	\Abdominal pain and diarrhea	2022	[23]

6.2.2. Targeted Therapy Through Small Molecules

Understanding molecular and the signalling events underlying the pathology of different cancers which leads to tumor growth and progression has increased the opportunities for the development of novel anticancer agents. Small molecule inhibitors for target proteins are identified through molecular screening [24].

Phosphorylation of proteins plays a major role in various cellular processes to maintain cell life. Abnormal phosphorylation is a key cause for the progression of disease in cancer biology such as angiogenesis, uncontrolled proliferation, anti-apoptosis and metastasis [13]. Tyrosine kinases are the enzymes which are involved in phosphorylation and their altered expression causes tumor formation and growth [25]. Therefore, small molecule inhibitors for tyrosine kinases have been developed. Around 30 small molecules are in clinical trials and two of the well-known anticancer drugs Glivec and Geftinib got FDA approval [8].

Glivec (imitanib mesylate, Gleevec, Novartis) is the first selective tyrosine kinase small molecule inhibitor which has been approved for cancer in 2001. It is chemically a 2-phenylaminopyrimidine, inhibits the ATP binding site of the Bcr-Abl kinase, a genetic change encoding abnormal protein in human cancer [26]. Gefitinib is another small molecule inhibitor which is approved by the FDA in 2003 for the treatment of non-small cell lung cancer (NSCLC) [27].

In the domain of monoclonal antibodies, immunotherapy, and small molecules, prevalent hurdles include issues like heterogeneity, bystander effects, protein aggregation, suboptimal internalization, and limited penetration into tumor cells. Challenges also encompass a narrow therapeutic index, and the emergence of resistance, among others. This chapter delineates these current impediments while highlighting recent breakthroughs and prospective opportunities for advancing next-generation Antibody-Drug Conjugates (ADCs) with enhanced therapeutic benefits [28].

6.2.3. Challenges of Targeted Therapies

In the year 1998, the approval of Trastuzumab marked a significant milestone as the inaugural monoclonal antibody (mAb) sanctioned for the treatment of solid cancers, specifically employed in HER2-positive breast cancer (BC) and subsequently in HER2-positive gastric cancer. The amplification of HER2 is observed in 15-20% of breast cancers, initially correlated with an unfavourable prognosis and elevated recurrence rates [29].

Following the initial endorsement of trastuzumab for HER2-positive breast cancer (BC), diverse treatments showcasing distinct mechanisms of action and safety profiles have received approval for managing both early-stage and metastatic

disease. However, limitations have been succinctly outlined, including the cardiac toxicity stemming from the inadequate penetration of trastuzumab and gastrointestinal issues observed during therapy [30].

Cetuximab, introduced in 2004, finds application in treating metastatic colorectal carcinoma and head-and-neck squamous cell carcinoma by targeting the Epidermal Growth Factor Receptor (EGFR), a pivotal factor in numerous oncogenic signalling pathways. Subsequently, two additional EGFR-targeting monoclonal antibodies (mAbs), namely Panitumumab and Necitumumab, secured FDA approval for metastatic colorectal carcinoma (Panitumumab) and NSCLC patients (Necitumumab). These mAbs differ in their isotype and level of humanization. Despite comparable efficacy, toxicity profiles, and signalling-based resistance mechanisms, challenges such as poor penetration to the target site advocate for the exploration of novel strategies in cancer therapy [29].

Belantamab mafodotin recently gained FDA approval as a monotherapy for treating patients with relapsed or refractory multiple myeloma, who have undergone at least four prior therapies, including an anti-CD38 monoclonal antibody, a proteasome inhibitor, and an immunomodulatory agent. The commonly reported adverse events encompass keratinopathy, decreased visual acuity, blurred vision, infusion-related reactions, anaemia, and thrombocytopenia. However, owing to its limited penetration, there is a recommendation to explore its use in conjunction with chemotherapy, an aspect currently under investigation in clinical trials [31].

Pembrolizumab, a humanized monoclonal IgG4-κ isotype antibody, selectively binds to the PD-1 receptor, hindering its interaction with PD-L1 and PD-L2. Employed in the treatment of NSCLC, Pembrolizumab monotherapy exhibits enduring antitumor activity and achieves high five-year Overall Survival (OS) rates in both treatment-naive patients and those previously treated for advanced NSCLC. Particularly noteworthy is the observation that the five-year OS rate surpasses 25% in patients with a PD-L1 tumor proportion score of 50% or greater. Pembrolizumab demonstrates a well-tolerated long-term safety profile, characterized by minimal evidence of late-onset or new toxicity, albeit with limitations in penetration [32].

Ipilimumab, a fully human IgG1 monoclonal antibody targeting CTLA-4, achieved a groundbreaking milestone as the first FDA-approved Immune

Checkpoint Inhibitor (ICI) in 2011 for advanced melanoma patients. It operates by averting T-cell suppression, thereby promoting the activation and proliferation of effector T cells. On a parallel front, Sintilimab, a humanized IgG4 anti-PD-1 monoclonal antibody, stands out as a prominent PD-1 inhibitor, although it awaits FDA approval despite compelling clinical outcomes. Similarly, Tislelizumab (BGB-A317), another humanized IgG4 anti-PD-1 monoclonal antibody, exhibits pronounced antitumor potential, particularly at 5 mg/kg dose, showcasing efficacy in advanced solid tumors. Despite promising results, ongoing studies are diligently exploring the safety and efficacy of these agents, unveiling diverse side effects. Notably, the contemporary concern revolves around poor tumor penetration, propelling the pursuit of novel cancer therapies [33].

Nevertheless, despite the advancements in antibody-based immunotherapy, it faces inherent limitations in pharmacokinetics, such as inadequate tissue and tumor penetration, minimal or absent oral bioavailability, and prolonged half-lives, along with pharmacodynamic challenges like immunogenicity. In stark contrast, small molecule immuno-oncology agents hold the potential to overcome these drawbacks associated with antibodies. Notably, therapeutic antibodies face limitations in penetrating cell membranes to influence intracellular targets like STimulator of Interferon Genes (STING) and Indoleamine 2,3-dioxygenase 1 (IDO1), whereas small molecules exhibit the capacity to act on intracellular targets. Additionally, small molecules offer greater flexibility for structural modifications, facilitating improved pharmacokinetics, including enhanced oral bioavailability [34].

While notable advancements in cancer therapy have addressed issues related to side effects, bioavailability, and shortened half-lives, the persistent challenge lies in effectively reaching the target site and achieving a concentration sufficient for pharmacological action in advanced therapies. A recently unveiled peptide, iRGD, has exhibited promising outcomes in enhancing drug penetration when co-administered or conjugated. This chapter explores the chemical attributes of iRGD, elucidates its mechanisms, explores diverse applications, and addresses the associated challenges.

6.3. The Role of Tumor Stroma in the Ineffectiveness of Cancer Therapy

The tumor stroma shows unique characteristics such as higher infiltration of

immune cells, high Interstitial Fluid Pressure (IFP), and myofibroblast formation from the fibroblasts. This change is associated with extensive depositing of extracellular matrix (ECM) that might result in matrix hardening (35). As shown in **Figure 2**, the altered stroma, cytokines and growth factors can enable this abnormal cell proliferation and immunologic escape, thus contributing to the emergence of a significantly changed microenvironment [36].

The main challenge of Nanoparticles (NPs) based therapy within the tumor stroma is caused by the complex extracellular matrix and inaccessible tumor tissue, a network of proteins, which is represented by a dense matrix that makes the tumor tissue impenetrable for NPs. In order to overcome the limitations within the restrictive tumor microenvironment (TME), logical ingestion of the stroma is essential. This requires a breakdown of the thick ECM to maintain an antitumor activity [37].

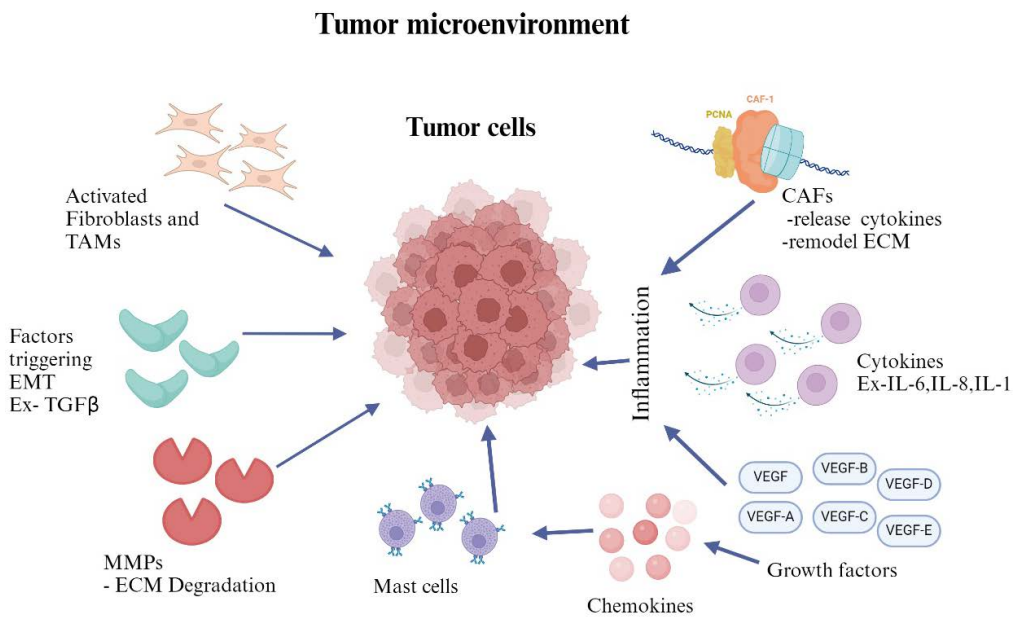


Figure 2. Impact of Tumor Microenvironment on Therapeutic Delivery. The figure illustrates how the interplay between cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and excessive extracellular matrix (ECM) deposition creates a barrier within the tumor microenvironment, hindering the effective delivery of therapeutics. This barrier results in erratic distribution of drugs, leading to treatment resistance and potential tumor relapse.

Several approaches to ECM targeting have been reported, which all search for ways to enhance the therapeutic impacts. The agents in this category are Relaxin analogues, angiotensin receptor blockers, and matrix modifiers [37]. Collagen analogues are prospective in shaping the dense ECM, thus boosting the overall transpired of more efficient drugs. Angiotensin receptor blockers are employed to attenuate the imbalanced cytokine profiles and growth factors, ultimately engaged in ECM alteration [38]. Matrix modifiers are studied for remodeling of the ECM either in the composition or the structure, which aids drugs and NPs distribution into tumor stroma. These attempts, however, have yielded diverse results in making the approaches more effective as therapy, underscoring the need to continue research and development of the strategies targeting ECM [39].

The widely accepted research hypothesizes the diffusion of NPs through tumors to be a very challenging task considering the distinct tumor-related obstacles [40]. Within the tumor microenvironment, problems arise as a result of non-cancerous cells, the infiltration of the immune cells, desmoplasia, acidic pH, poor oxygenation, complex blood vessels, cancer-associated fibroblasts (CAFs), altered signalling pathways, receptors, cytokine levels, rigid matrix, and increased IFP [41] [42].

Nanotherapies designed for tumor targeting often face challenges in reaching their intended destination, often localizing near neighbouring blood vessels due to these hindrances. Upon overcoming these initial obstacles, nanotherapeutics encounter a subsequent challenge represented by the cell membrane of tumor cells, limiting the delivery of payloads intracellularly [43]. Successful delivery and subsequent cytotoxic effects depend on factors such as the expression of cell surface receptors and membrane permeability [44]. Nevertheless, tumor cells are skilled in circumventing the uptake of drugs and NPs, establishing resistance to different drugs and nanotherapies, resulting in inefficacy of the therapeutic approach.

Elevated fluid pressure in tumors, stemming from reduced lymphatic drainage and increased vessel permeability, contributes to heightened IFP, resulting in increased hydrostatic pressure and irregular intratumoral distribution [45]. Desmoplasia, marked by connective tissue deposition, acts as a physical obstacle to drugs and NPs within specific solid tumors. This impaired accumulation is facilitated by the enrichment of the tumor stroma with CAFs, essential elements

within the TME. CAFs, also known as "myofibroblasts," play a central role in secreting excess ECM proteins, promoting cancer progression and invasion, fuelling tumor growth, and facilitating metastasis [46].

The expansion of CAFs is triggered by inflammatory signalling molecules, specifically the transforming growth factor-beta 1 (TGF-β1). TGF-β1, a robust cytokine with profibrotic properties, initiates the proliferation of CAFs and fosters the process known as epithelial-mesenchymal transition (EMT). Activation of EMT is associated with enhanced ability to migrate, invasive traits, increased resilience against programmed cell death, and elevated exhibition of ECM components [47]. Reports connect EMT to cancer metastasis, portraying the transformation of normal epithelial cells into a dynamic mesenchymal phenotype.

6.4. iRGD (Internalizing RGD Peptide) as a Promising Targeted Therapy Approach

Standard chemotherapeutic drugs aim to eliminate tumor cells by reaching the affected tissues. Nevertheless, their efficacy is frequently hindered by insufficient penetration into the inner areas of solid tumors. The concentration of these agents beyond the penetration depth often falls below the required threshold, resulting in treatment relapse or the emergence of drug resistance [48]. It demonstrates a strong affinity for αvβ3 and αvβ5 integrins, prevalent in tumor vasculature [49]. iRGD exhibits superior tumor-targeting capabilities compared to RGD as stated in **Table 2**, as it can selectively bind to integrins and neuropilin-1 (NRP-1) receptors, commonly overexpressed in various tumors [50]. The pivotal regulatory step in iRGD's mechanism involves proteolytic cleavage, revealing the CendR motif. This process results in the loss of affinity for integrins while gaining NRP-1-binding activity, thereby initiating extravasation, and facilitating cell and tumor penetration [51]. Due to increased deposition of ECM in the TME, compartmentalization of tissues resulting in some part of the tumor tissue becoming very hard to reach inaccessible portion. These areas achieve poor and inadequate anticancer drugs (either free drug form or through different delivery systems) distribution, which contribute to the therapeutic failure and tumor recurrence.

Various strategies have been explored to overcome the TME and ECM barriers and to increase intratumoral penetration and distribution of drugs either in free drug or NPs forms. Among all these strategies, this review discusses the potential

applications of tumor penetrating iRGD peptide in management different cancer conditions.

6.4.1. iRGD Peptide Structure and its Mechanism of Action

Peptides designed for tumor penetration, such as iRGD and LyP-1, are composed of three essential modules: a vascular homing motif, an R/KXXR/K tissue penetration motif, and a protease recognition site. These elements collaboratively orchestrate a stepwise targeted approach to tumor homing and penetration. The prototype tumor-penetrating peptide, iRGD, is defined by the sequence CRGDR/KGPDC [52].

The tumor-targeting mechanism of iRGD initiates with integrin-mediated binding [53]. Integrin, a transmembrane glycoprotein, comprises α- and β-subunits and facilitates cellular adhesion to the extracellular matrix (ECM) [54]. Expression levels of integrins closely correlate with cell type and microenvironment [55].

Eight distinct integrin heterodimers, namely αvβ1, αvβ3, αvβ5, αvβ6, αvβ8, α5vβ1, α8β1, and αIIbβ3, acknowledge the RGD motif within extracellular matrix (ECM) proteins [56]. Ruoslahti and collaborators initially identified αvβ3, which is heightened in various cancer types [57]. Initially recognized in various cancers, including gastric glioma, non-small-cell lung cancer, pancreatic cancer, and prostate cancer, αvβ3 was later joined by αvβ5 as a crucial regulator in these cancer types. The expression of integrins plays a pivotal role in the action of iRGD [58].

6.4.2 Overview of iRGD-Mediated Tumor Penetration and Targeting

iRGD functions through following three key steps:

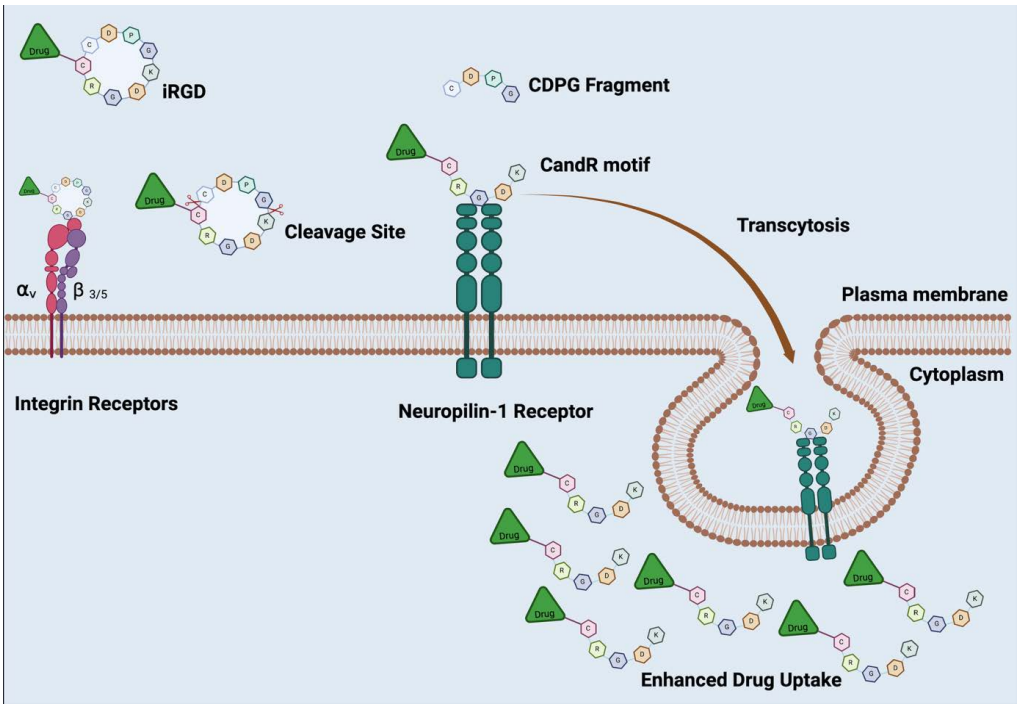
1) iRGD selectively targets tumors by interacting with αv integrins, predominantly found on the endothelium of tumor vessels. Integrin, essential for cellular adhesion to ECM, demonstrates strong correlation with cell type and microenvironment. The αv integrins ($\alpha v\beta 3$ and $\alpha v\beta 5$) are overexpressed in cancer vasculature, binding to peptides with the RGD motif.

2) Within the tumor, iRGD undergoes proteolytic cleavage to yield CRGDK/R. The cleaved CRGDK fragment exhibits significantly higher binding affinity to neuropilin-1 (NRP-1) than integrins, approximately 50- to 150-folds.

3) The shortened peptide, with diminished integrin-binding capacity, exhibits an increased attraction to NRP-1 as a result of the exposure of a C-terminal

conditional C-end Rule (CendR) motif as depicted in **Figure 3** (R/KXXR/K) [51].

Integrins have been noted for their interaction with NRPs, benefiting from NRPs' structural versatility and array of soluble ligands. The engagement with NRP-1 triggers tissue penetration, a phenomenon exclusive to tumors, given that cleavage is dependent on the preceding attachment of the peptide to integrins. These peptides enable the conveyance of both co-administered and conjugated payloads into the tumor parenchyma.



Uptake in the cell and distribution in tumor tissues

Figure 3. A concise elucidation of iRGD's mechanism of action involves the cleaved peptide facilitating the formation of a transport pore mediated by NRP-1. This process enhances the tissue penetration and intracellular uptake of co-administered or conjugated payloads.

Integrins have been noted for their interaction with NRPs, benefiting from NRPs' structural versatility and array of soluble ligands. The crucial interaction between the CendR motif and NRP-1 plays a decisive role in surmounting biological barriers. Recognized as a mediator for cellular and tissue penetration, the R/KXXR/K

motif follows the C-end Rule (CendR) with a strict requirement for C-terminal positioning. Essential features include the recognition motif R/KXXR/K, the necessity for C-terminal motif exposure, proteolytic cleavage transitioning internal CendR motifs to active C-terminal ones, and dependence on NRP-1 for both recognition and penetration functions.

iRGD, a newly identified cyclic 9-amino peptide with sequences CRGD[K/R] GP[D/E]C, exhibits similarities to conventional RGD. iRGD binds to cancer cell surfaces with high αv integrin expression levels. The enhanced penetration facilitated by iRGD is grounded in recognizing NRP-1, a transmembrane receptor abundantly expressed in various tumors. The process begins with iRGD initially binding to integrin receptors, followed by protease-dependent cleavage, revealing the CendR motif, CRGDK/R, and a CDPG fragment. This exposed CendR motif orchestrates binding to NRP-1, ultimately amplifying cell and tissue penetration.

The vesicular transport mechanism initiated by the C-terminal end of iRGD binding to NRP-1 is implicated in bulk transcytosis, leading to enhanced permeability. In tumor blood vessels, neuropilin-1 (NRP-1) and neuropilin-2 (NRP-2) play pivotal roles in regulating tumor tissue [59]. **Table 2** explains the major differences between RGD and iRGD peptides. These unique features, make iRGD potential agent for tumor targeting via co-administration and conjugation.

Table 2. Comparison of RGD and iRGD peptide interactions with Neuropilin-1.

	RGD Peptide	**iRGD**	**References**
Definition	RGD (Arg-Gly-Asp) is a short peptide sequence derived from extracellular matrix proteins like fibronectin and vitronectin.	iRGD (CRGDK/RGPD/EC) is a modified version of RGD that includes additional amino acids to enhance tumor penetration.	[59] [60]
Affinity for αv integrins	High nanomolar range	Mid to low nanomolar range	[59]
Affinity for neuropilin-1	Low	Stronger affinity due to C-terminal exposure of CendR motif	[50]

Tumor specificity	Lower	Increased due to shift from integrins to neuropilin-1	[50]
Homing biological characteristics	Moderate	Greater due to RGD-directed specific homing	[51]
Activation of tumor penetration	Not dependent on integrin interaction	Recruitment to cell surface likely necessary for proteolytic cleavage	[61]
Cell penetrating capability	Moderate	Far better than conventional RGD peptides	[62]
Cytotoxicity on healthy cells	Potential adverse effects	No adverse effects or cytotoxicity	[63]
Tumor penetration mechanism	Not well understood	Relies on the CendR motif and neuropilin-1 interaction	[51]
Carrying Capacity of Drug Load	Carries lesser pay load than iRGD	10X times more than conventional iRGD	[51]
Potential applications	Cell adhesion, angiogenesis, and targeted therapy	Enhanced tumor targeting and drug delivery	[64] [65]

6.5. Successful evidence of the use of iRGD in pre-clinical studies for cancer

6.5.1. Breast Cancer

Targeted therapy has proven effective in treating tumors, offering better compatibility with treatments and causing minimal toxicity to healthy cells. Since the discovery of iRGD's effectiveness in tumor targeting, a significant body of research has emerged, focusing on this peptide. Various strategies, such as utilizing exosomes and employing RNA interference for delivery, have been explored to enhance treatment outcomes by improving the distribution and penetration of therapeutic molecules.

Triple-negative breast cancer (TNBC) represents the most aggressive subtype,

marked by rapid metastasis, high recurrence rates, and a challenging prognosis. The lack of estrogen receptors, progesterone receptors, and Her-2 receptors on TNBC cells hinder the use of clinically targeted therapy drugs [66]. The strategic exploitation of the interaction between iRGD and the increased expression of NRP-1 in endothelial cells facilitates the penetration of tumor cells in targeted drug delivery systems [67]. The increased expression of αvβ3 and αvβ5 integrins, coupled with elevated NRP-1 levels in TNBC, is harnessed by iRGD, positioning it as a promising candidate for predicting favorable treatment outcomes [66].

Injecting iRGD systemically enhanced the effectiveness of different drugs, encompassing small molecules like doxorubicin, nanoparticles such as nab-paclitaxel and doxorubicin liposomes, and a monoclonal antibody called trastuzumab against breast tumors (BT474) and prostate cancer (22Rv1) in mouse models. The observation indicated that when administered together with iRGD, the drug penetrated and spread more effectively throughout the tumor site compared to when it was conjugated to therapeutics. The strategy of co-administering iRGD with drugs demonstrates potential in enhancing their efficacy while mitigating side effects, representing a significant objective in cancer research [67].

Using different specific ligands to target nanoparticles have displayed promising therapeutic potential in nanomedicine. However, the challenge of inadequate penetration of anti-tumor drugs into solid tumors persists as a significant hurdle. A targeted approach for delivering antitumor drugs involved combining a cross-linked multilamellar liposomal vesicle (cMLV) formulation with a tumor-penetrating peptide, iRGD. Research by Liu et al demonstrated the ability of iRGD peptides to facilitate the binding and cellular uptake of drug-loaded cMLVs, thereby enhancing the effectiveness of antitumor treatment in breast tumor cells, including those resistant to multiple drugs. Colocalization data analysis revealed that iRGD-conjugated cMLVs (iRGD-cMLVs) entered cells through the clathrin-mediated pathway, followed by transport through endosomes and lysosomes, facilitating efficient drug delivery. In vivo studies indicated that iRGD-cMLVs efficiently delivered anticancer drugs, resulting in significant tumor suppression [68].

A nano-delivery system, iRGD-PSS@PBAE@JQ1/ORI NPs, was developed using iRGD-modified polysaccharides for co-delivery of JQ1 (BET inhibitor) and oridonin (ORI) in breast cancer treatment. This system enhanced tumor targeting and

cellular uptake of both compounds, where JQ1 reversed immune tolerance by reducing PD-L1 expression, and ORI exhibited various antitumor effects, including anti-proliferation and inhibition of ROS and lactic acid. iRGD significantly improved cellular uptake and tumor penetration of the nanotherapeutic system, while the synergy of JQ1 with ORI demonstrated enhanced antitumor effects [69]. Gao *et al.* compared free isoliquiritigenin (ISL) with ISL-iRGD NPs and found that ISL-iRGD NPs exhibited increased cytotoxicity and cell apoptosis in various breast cancer cell types. This was attributed to enhanced cellular accumulation facilitated by iRGD-integrin recognition and the nanoscale effect. Additionally, ISL-iRGD NPs, benefiting from active accumulation in tumor tissue via iRGD peptides and extended circulation in vivo due to their stealthy nanostructure, demonstrated higher efficiency in inhibiting tumor growth in mouse bearing 4T1 breast tumors [70].

A groundbreaking approach was devised to enhance tumor penetration efficiency by co-administering iRGD with a size-shrinkable, tumor-microenvironment-responsive multistage nanodelivery system (DOX-AuNPs-GNPs). This strategy initially leveraged iRGD to heighten the permeability of both tumor vasculature and tissue, resulting in increased leakage of DOX-AuNPs-GNPs from the tumor vasculature. Subsequently, the multistage system passively accumulated in the tumor tissue, undergoing size reduction from 131.1 to 46.6 nm and facilitating penetration into deeper tumor regions. In vitro analyses demonstrated elevated cellular uptake and apoptosis ratios with the coadministration of iRGD and DOX-AuNPs-GNPs. In vivo experiments confirmed enhanced penetration and accumulation within the tumor when iRGD was combined with DOX-AuNPs-GNPs, showcasing superior antitumor efficacy in a 4T1 tumor-bearing mouse model [71].

A novel approach using iRGD peptide-functionalized polymersomes (PS) carrying Tamoxifen (iRGD-PS-Tam) showed enhanced penetration of estrogen receptor-positive (ER+) breast cancer cells in both 2D and 3D cultures. iRGD-PS-Tam treatment inhibited proliferation and heightened the sensitivity of fibronectin (FN)-cultured cells to Tamoxifen, reducing ER functionality and the population of breast cancer stem cells. Notably, in animal models, iRGD-PS-Tam selectively accumulated at tumor sites. iRGD-guided PS-Tam delivery can be a promising strategy for treating breast tumors expressing high FN levels [72].

The study investigated iRGD's utility as an imaging tool in TNBC, synthesizing 99mTc-labeled iRGD (99mTc-HYNIC-iRGD) for SPECT imaging. In vitro, iRGD displayed biocompatibility and specific binding to TNBC cells. The developed 99mTc-HYNIC-iRGD exhibited high radiochemical purity and stability. SPECT imaging in TNBC mouse models demonstrated notable tumor accumulation, quick blood clearance, and favorable biodistribution. These results emphasize the potential of iRGD as an effective imaging approach for TNBC [73].

In recent years, gene therapy has emerged as a promising avenue for tumor treatment, with a focus on developing safe and efficient gene delivery systems beyond traditional viral vectors. A novel approach involves ultrasound-mediated gene therapy, which enhances safety and efficiency by utilizing microbubbles as carriers for plasmid DNA. In a groundbreaking study by Zhu *et al.*, cationic microbubbles decorated with iRGD peptides and magnetic Fe_3O_4 nanoparticles (MBiM) were designed for targeted ultrasound contrast imaging guided gene therapy of tumors. The study demonstrated a significant increase in ultrasound image intensity at the tumor site, leading to enhanced gene transfection efficiency compared to non-targeted microbubbles [74].

Overcoming challenges related to limited penetration of nanocarriers into tumors and slow drug release, Wang *et al.* introduced a drug delivery carrier derived from mesoporous silica. This carrier, dually modified with the tumor-homing cyclic peptide iRGD and the pH-responsive polymer PEOz, exhibited selective binding to the αvβ3 integrin receptor, specific to MDA-MB-231 breast cancer cells and vessels. The pH-responsive nature of the carrier facilitated rapid drug release in the acidic cytoplasmic environment, offering a potential solution to the issues of drug release rate [75].

The intersection of proteolysis-targeting chimeras (PROTACs) and tumor-penetrating peptides was explored by He *et al.* In their study, an iRGD-PROTAC conjugation strategy was employed to deliver a bromodomain-containing protein 4 (BRD4) PROTAC deep into breast cancer tissues. The resulting iRGD-PROTAC conjugate demonstrated enhanced water solubility, tumor-targeting capability, and penetration within tumor tissues, showcasing increased anti-breast cancer efficacy [76].

Addressing postoperative breast cancer metastasis, Pan *et al.* investigated the

role of siS100A4-loaded iRGD-modified extracellular vesicles (siS100A4-iRGD-EVs). These nanoparticles, engineered for enhanced siRNA protection, cellular uptake, and compatibility, exhibited potent anti-metastasis effects by suppressing S100A4 expression in the lung tumor tissues [77].

Advancing drug delivery through exosomes, Tran *et al.* engineered exosomes from human embryonic kidney cells with dual-tumor-penetrating peptides, iRGD and tLyp1. The dual-targeting exosomes demonstrated superior drug uptake and potency in breast cancer cell lines compared to single-targeting exosomes [78].

In nanotheranostics, Zhang *et al.* introduced targeted peptide-conjugated gold nanocages (iRGD-PEG/AuNCs@FePt NPs) for mild photothermal/radiation therapy in breast cancer. This ternary metallic nanoparticle system, offering photoacoustic and magnetic resonance imaging guidance, showed potential for enhanced tumor inhibition through synergistic therapy, involving ferroptosis-augmented apoptosis [79].

Genomic DNA sequences, with high druggability value (likelihood to be targeted by a drug, to produce therapeutic effect), were targeted by Alipour *et al.* to induce apoptosis in breast cancer cells. They developed a nanohybrid using a peptide-based carrier functionalized with iRGD for targeting the BCL-2 oncogene. This nanohybrid efficiently delivered DNA inhibitor against BCL-2, showcasing robust antineoplastic potential with minimal impact on normal cells [80]. Li *et al.* proposed an innovative approach for sensitizing low-temperature photothermal therapy in breast cancer using an engineering multifunctional nanoplatform. This platform, involving MoS2 nanoparticles, DPA, CPT-11, and iRGD, demonstrated efficient tumor cell death through a combination of sensitized low-temperature photothermal therapy, ferroptosis, and chemotherapy [81].

In a study by Marin *et al.*, a unique strategy combining a tumor-penetrating peptide (TPP) with a peptide interfering with PP2A/SET interaction showed promise against breast cancer growth. Bifunctional peptides, resulting from the combination of the interfering peptide and either LinTT1 or iRGD, effectively inhibited tumoral growth in xenograft models. The in vivo results demonstrated the efficacy of the TPP-interfering peptide strategy as a therapeutic approach against breast cancer [82].

6.5.2. Lung Cancer

Lung cancer stands as the foremost contributor to cancer-related fatalities globally, and its incidence continues to rise [83]. Non-small cell lung cancer (NSCLC) represents the predominant form of lung cancer, constituting around 75-80% of all lung cancer cases [84]. Cisplatin remains the most efficacious treatment for solid tumors such as NSCLC, but its usage is constrained due to its toxicity, particularly in the renal system [85]. The integration of cisplatin into methoxypoly (ethylene glycol)-block-poly(L-glutamic acid) (mPEG-b-PLG) micelles, along with concurrent iRGD co-administration, exhibits significant promise for NSCLC chemotherapy. These micelles demonstrate sustained cisplatin release, dose- and time-dependent inhibition of HeLa and A549 cell proliferation, and minimal hemolytic activity. In vivo experiments using subcutaneous NSCLC xenograft models (A549) reveal a pronounced anti-tumor effect for both free cisplatin and cisplatin-loaded micelles. However, the toxicity associated with cisplatin is markedly diminished in the case of CDDP-loaded micelles co-administered with iRGD, leading to a more than 30% extension in survival time. This suggests that mPEG-b-PLG-loaded cisplatin, in conjunction with iRGD, presents a promising therapeutic avenue for NSCLC [86].

Another study aimed to enhance Gemcitabine's therapeutic efficacy for NSCLC using co-administered iRGD peptide. Tumor accumulation of Evans Blue+iRGD was 2.5 times that of Evans Blue alone. Tumors treated with Gemcitabine+iRGD exhibited 86.9% growth inhibition, decreased PCNA expression by 71.5%, and a 2.2-fold increase in apoptosis compared to Gemcitabine alone. The study suggests that iRGD enhances Gemcitabine's tumor-penetrating ability and therapeutic efficacy, proposing a novel strategy for NSCLC treatment [87]. A study explored the tumor-penetrating capacity of iRGD in combination with thymosin alpha 1 (Tα1). Tα1, a 28-amino acid hormone approved for cancer treatment, faces limitations in clinical application due to the lack of tumor targeting. The iRGD fragment notably enhanced Tα1's inherent ability to inhibit cancer cell proliferation in vitro, specifically in B16F10 mouse melanoma and H460 human lung cancer cell lines [88].

Among the innovative strategies, an internalizing iRGD modified liposome loaded with curcumin (CUR) and piperine (PIP) (iRGD-LP-CUR-PIP) has been developed for targeted therapy. This system demonstrated enhanced tumor targeting,

cellular internalization, and notable antitumor efficacy both in vitro and in vivo, highlighting its potential as a targeted therapeutic system for lung cancer [89]. Immunotherapy has emerged as a promising avenue for lung adenocarcinoma (LUAD), yet challenges persist with poor responses in some tumors. The upregulation of Cyclin-Dependent Kinase 7 (CDK7) promoting an immunosuppressive macrophage phenotype in LUAD inspired the development of an iRGD-conjugated gold nanoparticle (AuNP) system carrying siCDK7. This system exhibited excellent tumor targeting and photothermal effects, inducing tumor cell necroptosis. Furthermore, it improved the immunosuppressive microenvironment, enhancing the efficacy of anti-PD-1 treatment. The iRGD-conjugated AuNP/siCDK7 system presents a potential treatment strategy for LUAD, combining tumor cell necroptosis and immunotherapeutic responses [90]. In the area of nanomedicine, a polymeric conjugate named P-DOX-iRGD has demonstrated a dual impact on inhibiting primary tumor growth and substantially inhibiting pulmonary metastasis in an orthotopic mouse model of breast cancer. This polymeric form of doxorubicin exhibited contrasting results to its free form, emphasizing the significance of the enhanced permeability and retention (EPR) effect. The study contributes to the understanding of managing tumor metastasis using polymeric conjugates [91]. The potential of milk-derived exosomes in delivering paclitaxel for lung adenocarcinoma treatment has been explored. By modifying exosomes with the iRGD peptide, this nanoplatform exhibited effective killing of lung adenocarcinoma cells, demonstrating promise for targeted therapy. The study emphasizes the potential of milk exosome-based nanoplatforms in the treatment of lung adenocarcinoma [92].

In the context of icariin (ICA), a Chinese medicine monomer, a bionic targeted nano-preparation (iRINPs) has been developed by integrating iRGD with red blood cell membrane (RBCM). iRINPs demonstrated improved biocompatibility, stability, and therapeutic efficacy against lung cancer, showcasing its potential as a promising nano-platform for precise lung cancer therapy [93]. Gene delivery systems play a crucial role in cancer gene therapy, and the redox-responsive poly(amido amine) (PAA) with iRGD conjugation presents a promising candidate. This system, delivering siRNA specific to epidermal growth factor receptor (EGFR), exhibited enhanced gene silencing ability and inhibited lung tumor growth in vivo. PAA-iRGD represents a potential gene delivery system for cancer therapy, combining biocompatibility, biodegradation, and targeting ability [94]. In

exploring the therapeutic potential of the thymosin α1 (Tα1) peptide, a fusion protein Tα1-iRGD has been designed, introducing tumor homing capabilities. Tα1-iRGD demonstrated enhanced immunomodulatory activity and antitumor effects in melanoma and lung cancer models, positioning it as a superior antitumor drug compared to Tα1 alone [95]. Combining the therapeutic potential of cetuximab with the tumor-penetrating properties of iRGD has been investigated for NSCLC. Co-administration of cetuximab and iRGD resulted in increased tumor penetration, leading to significant tumor reduction in NSCLC xenograft models. This combined application presents a novel strategy to enhance the clinical therapeutic efficacy of cetuximab for NSCLC treatment [96]. The development of innovative 3D cell culture models is crucial for advancing cancer research. A novel air-grown multicellular spheroid (MCS) model for lung cancer has been established, providing a platform for evaluating aerosol anticancer therapeutics. This model, treated with iRGD and paclitaxel, demonstrated enhanced tumor-penetration and reduced tumor growth. The study signifies the potential of combining iRGD with therapeutic agents in lung cancer treatment [97]. In the pursuit of enhancing IL-24 therapy for NSCLC, a fusion protein IL-24-iRGD has been developed. This recombinant protein demonstrated increased production of proinflammatory cytokines, suppressed NSCLC cell growth, and exhibited superior antitumor effects in vivo. IL-24-iRGD represents a promising strategy for improving the efficacy of IL-24 in NSCLC treatment [98]. These diverse studies underscore the potential of iRGD-modified systems in advancing targeted therapies for lung cancer, offering insights into innovative strategies that combine nanotechnology, immunotherapy, and gene therapy for improved treatment outcomes.

6.5.3. Hepatocellular Carcinoma

Liver cancer poses a significant worldwide health burden, projected to exceed 1 million cases by 2025. Hepatocellular carcinoma (HCC) represents the predominant type of liver cancer, constituting approximately 90% of diagnosed cases [99]. Activated cytokine-induced memory-like natural killer (CIML NK) cells, enhanced by IL-12, IL-15, and IL-18, demonstrated potent cytotoxicity against HCC. iRGD modification improved their infiltration into tumor spheroids, yielding targeted cytotoxicity. In vivo, iRGD-modified CIML NK cells significantly impeded HCC tumor growth. These findings highlight the potential of iRGD-modified CIML NK

cells in HCC immunotherapy [100].

In one of the investigations of hepatocellular carcinoma (HCC) therapy, the study explored iRGD's potential in enhancing sorafenib and doxorubicin delivery. Using mouse models, iRGD demonstrated a threefold improvement in substance delivery to HCC tumors, selectively enhancing signal intensity in Gd-DTPA-enhanced MRI. The coadministration of iRGD significantly amplified the antitumor effects of sorafenib and doxorubicin without increasing systemic toxicity. These preclinical findings propose iRGD as a promising strategy for widening the therapeutic window in HCC chemotherapy [101]. Endostatin, a 20 kDa C-terminal fragment derived from collagen XVIII, possesses anticancer properties and is utilized in cancer treatment [102]. iRGD-modified endostatin exhibits heightened efficacy in liver cancer therapy, demonstrating enhanced inhibition of cell proliferation and migration, along with improved distribution within tumors. The antitumor effects and suppression of liver cancer growth are attributed to endostatin's neovascularization blockade facilitated by iRGD binding to αVβ integrins [103].

A cell-penetrating peptide-aptamer dual-modified nanocomposite (USILA NPs), designed for targeted and synergistic HCC treatment. Comprising sorafenib, ursolic acid, and indocyanine green, USILA NPs exhibited heightened cellular uptake and cytotoxicity in HepG2 and H22 cells. This nanocomposite selectively accumulated at H22 tumor sites in mice, detecting ICG fluorescence and demonstrating enhanced effects with the co-administration of iRGD peptide or PD-L1 antibody [104].

Adding to the arsenal of precision theranostics for HCC, a multifunctional ultrasound molecular probe (iRGD-ICG-10-HCPT-PFP-NPs) was engineered. This nanodevice showcased efficient targeting, coupled with ultrasound/photoacoustic dual-modality imaging and deep tumor penetration. In vitro and in vivo assessments demonstrated notable antiproliferative and proapoptotic effects, suggesting a promising strategy for HCC theranostic applications [105].

Focusing on the emerging frontier of ferroptosis-based therapy for HCC, sorafenib-loaded MIL-101(Fe) nanoparticles (MIL-101(Fe)@sor NPs) were explored. Co-administration with iRGD enhanced ferroptosis induction, effectively inhibiting tumor progression in vivo. This study positions MIL-101(Fe)@sor NPs as a promising strategy for HCC ferroptosis [106].

A critical cornerstone in HCC therapy, transcatheter arterial chemoembolization (TACE), faced challenges due to poor drug distribution. The integration of iRGD coadministration with TACE in a rabbit VX2 liver tumor model demonstrated enhanced antitumor efficacy, underscoring the potential of iRGD in HCC treatment [107].

In the radiofrequency ablation of liver tumors, a tumor-penetrating peptide iRGD-integrated thermally sensitive liposomal doxorubicin was developed. iRGD conjugation not only improved intratumoral doxorubicin accumulation but also heightened the activity of TSL-DOX in radiofrequency ablation of liver tumors [108]. As the quest for synergy in drug co-delivery gains momentum, iRGD-decorated lipid-polymer hybrid nanoparticles were designed to carry doxorubicin and sorafenib. These nanoparticles exhibited synergistic cytotoxicity, pro-apoptotic ability, and enhanced internalization rate in HCC cells, providing a promising avenue for nanoparticulate drug co-delivery in HCC [109].

A promising stride in systemic administration unfolded with iRGD-decorated polymeric nanoparticles designed for vandetanib delivery. These NPs significantly enhanced the potency of vandetanib against HCC in xenograft mouse models, showcasing promise in the field of nanomedicine [110]. Culminating this comprehensive approach is the development of iRGD-conjugated DSPE-PEG2000 nanomicelles for the targeted delivery of salinomycin to both liver cancer cells and cancer stem cells. M-SAL-iRGD demonstrated superior penetration tumor efficacy and potent antitumor activity, presenting a potential nanomedicine against liver cancer [111].

Pancreatic Cancer

Pancreatic cancer poses a significant and persistent global health challenge, marked by an overall 5-year survival rate below 5%. Traditional systemic administration of chemotherapy exposes the entire body to the therapeutic agents, affecting both tumors and healthy organs. Localized interventions, however, focus on retaining chemo-agents specifically at the tumor site, reducing unwanted toxicity. Consequently, there is increasing interest in exploring novel localized interventions as alternatives to systemic therapy [112]. Akashi *et al.* established pancreatic cancer models. In a subgroup of pancreatic cancer models characterized

by elevated NRP1 expression, responsiveness to iRGD co-administration was observed. The combination therapy of gemcitabine (GEM) with iRGD peptide significantly reduced tumor size compared to GEM monotherapy in cell line-based xenografts, but the effect was less prominent in tumor grafts. Cases with NRP1 overexpression (IHC-2+/3+) were identified as potential targets for iRGD, constituting 45.8% of the clinical specimens [113].

The engineered L30-iRGD-nanocage, featuring 30 amino acid linkers (GGS)10, exhibits enhanced binding affinity to pancreatic cancer cells compared to other known linkers. Incorporating the moderately hydrophobic anticancer drug, OSU03012, into the L30-iRGD-nanocage enhances its effectiveness in inducing cell death through the activation of the caspase cascade. This innovative iRGD-nanocage holds significant promise as a novel nanocarrier for targeted drug delivery in pancreatic cancer treatment [114]. In a KRAS-induced orthotopic model of Pancreatic Ductal Adenocarcinoma (PDAC), the combined use of iRGD facilitated the absorption of irinotecan-loaded silicasomes, resulting in enhanced survival and diminished metastasis. Ultrastructural imaging revealed a vesicular transport pathway induced by iRGD. These findings suggest iRGD's potential as an adjuvant in nanoparticle-based PDAC treatments [115]. Engineered peptide-based nanocomplexes have been developed to tackle the challenges of systemically treating pancreatic cancer. These nanocomplexes, specifically designed for PDAC, deliver siRNA targeting key genetic mutations. Leveraging iRGD for tumor penetration, the nanocomplexes demonstrated both penetrating properties and therapeutic efficacy in various PDAC models. This approach holds promise for translating genetic insights into effective PDAC treatments. [72].

Pancreatic ductal adenocarcinoma (PDAC) poses significant therapeutic challenges due to its immunosuppressive microenvironment and resistance to conventional treatments. In an innovative approach, Suzuki *et al.* identified αvβ5 integrin as a marker expressed by immunosuppressive regulatory T cells (Tregs) in PDAC tissue. These Tregs, susceptible to the iRGD tumor-penetrating peptide targeting αv integrin and neuropilin-1, underwent tumor-specific depletion upon long-term iRGD treatment. This strategy enhanced the efficacy of immune checkpoint blockade, offering a potential avenue for improving PDAC therapy [116].

Complementing this, Geng *et al.* introduced a combination chemotherapy strategy for pancreatic cancer utilizing bioactive black phosphorus (BP) and gemcitabine (GEM). The iRGD-modified zein nanoparticles co-loaded with BP quantum dots and GEM demonstrated excellent tumor targeting and prolonged circulation, resulting in synergistic killing of pancreatic cancer cells. The study showcased the potential of this targeted nanoplatform as a promising combination chemotherapy for pancreatic cancer, emphasizing the importance of innovative therapeutic approaches [117]. Addressing the challenges in PDAC therapy, Lo *et al.* proposed a nucleic acid delivery system based on the cyclic peptide iRGD for targeted delivery of siRNA. The iRGD-guided tumor-penetrating nanocomplexes (TPNs) efficiently delivered anti-Kras siRNA to PDAC in murine models, overcoming physical barriers and achieving significant tumor growth delay. The modular construction of this delivery platform allows for adaptability to future genetic targets, presenting a versatile strategy for PDAC treatment [118]. Furthermore, Ray *et al.* presented a pH-responsive nanoparticle platform for combination therapy in pancreatic cancer. Utilizing PEG-b-poly(carbonate) block copolymers, pH-responsive nanoparticles demonstrated efficient drug release in response to acidic conditions mimicking the tumor microenvironment. This approach holds promise for spatiotemporal control over drug accumulation, addressing the challenges associated with pancreatic cancer treatment [119]. Tsang *et al.* explored a novel oligonucleotide-mediated gene silencing technology targeting KRAS and MYC in pancreatic cancer. U1 Adaptors coupled with tumor-targeting peptides demonstrated potent antitumor activity in xenograft models. The study validated the therapeutic potential of simultaneously targeting KRAS and MYC, showcasing the adaptability of the U1 Adaptor technology for nanoparticle-free delivery systems [120]. Liu *et al.* emphasized the significance of transcytosis in nano drug delivery to pancreatic cancer, utilizing iRGD to trigger transcytosis and improve the efficacy of chemotherapeutics. The study highlighted the potential of iRGD-mediated transcytosis as a major mechanism to enhance drug delivery, paving the way for personalized approaches in implementing this technology [121]. To address cancer relapse, Karandish *et al.* developed a stimuli-responsive, polymeric nanocarrier targeting neuropilin-1 in pancreatic and prostate cancer stem cells. The iRGD-targeted polymersomes encapsulating the cancer stemness inhibitor napabucasin demonstrated selective internalization and significant reduction in cancer stem cell

viability. This approach opens new avenues for overcoming drug resistance and inhibiting cancer stemness in pancreatic cancer therapy [122]. In a groundbreaking study, Hurtado de Mendoza *et al.* identified carcinoma-associated fibroblasts (CAFs) as efficient drug delivery targets in PDAC. CAFs induced β5 integrin expression in tumor cells, enhancing the tumor-penetrating capacity of iRGD. This strategy significantly potentiated chemotherapy in mice with high β5 integrin expression, offering a targeted approach for aggressive PDAC subpopulations [123].

Lastly, Järveläinen *et al.* conducted a comprehensive assessment of the pharmacokinetics and tumor-targeting properties of CEND-1 (iRGD). The study demonstrated favorable in vivo pharmacokinetics, sustained tumor homing, and penetrability of CEND-1, suggesting its potential to enhance the therapeutic index of co-administered anticancer agents. Even a single injection of CEND-1 showed long-lasting tumor pharmacokinetic improvements, emphasizing its utility in combination therapies for cancer treatment [124].

Gastric cancer

Gastric cancer ranks as the fifth most commonly identified cancer and stands as the third principal contributor to global cancer-related fatalities [125]. Enhancing drug delivery to tumor tissues through improved vascularity and penetrability holds promise for advancing therapeutics. Zhou et al focused on addressing radiation resistance in radiotherapy (RT) by employing tumor-specific targeted sensitizers. Utilizing 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-polyethylene glycol-iRGD (DSPE-PEG-iRGD), red blood cells (RBCs) were modified for tumor targeting and RT enhancement. In vivo experiments demonstrated enhanced tumor targeting, reduced liver and spleen accumulation, and improved radiosensitivity in subcutaneous gastric tumor mice. This innovative approach holds promise for overcoming radiation resistance in RT [126]. The in vivo investigation revealed that co-administering Gambogic acid-NPs and iRGD via hydrogels exhibited superior antitumor efficacy compared to GA-loaded hydrogels and free GA combined with iRGD. The free GA group demonstrated minimal antitumor effects. Notably, there were no significant changes in body weight among groups, and leukocyte and hemoglobin count slightly decreased compared to the control. The iRGD and GA-NP-loaded hydrogel demonstrated effective antitumor activity, possibly attributed to retention, local administration, and sustained iRGD release,

offering a promising, low-toxicity strategy for enhancing anti-gastric cancer efficacy [127].

Another study unveils a ground-breaking role for the tumor-penetrating peptide iRGD in enhancing lymphocyte infiltration within 3D tumor spheroids and xenograft mouse models. This novel function, combined with iRGD modification and PD-1 knockout lymphocytes, exhibits superior antitumor efficacy. Mechanistically, iRGD's binding to neuropilin-1 induces tyrosine phosphorylation of VE-cadherin, promoting the opening of endothelial cell contacts and facilitating transendothelial lymphocyte migration. These findings highlight the iRGD's potential to address the challenge of limited lymphocyte infiltration, overcoming a critical barrier in adoptive cell immunotherapy for solid tumors [128]. In pursuing effective antitumor immunotherapy, the challenge lies in optimizing lymphocyte trafficking to the tumor microenvironment. With a foundation in the bispecific tumor-penetrating protein, anti-EGFR-iRGD, comprising the variable heavy chain region of anti-EGFR antibody and iRGD, the study established its prowess in enhancing drug penetration. The innovation extends to a co-administration method involving T cells and anti-EGFR-iRGD, presenting a comprehensive strategy to fortify T-cell trafficking, penetration, and antitumoral efficacy. These insights propose a preclinical translational avenue for anti-EGFR-iRGD as a therapeutic modifier in cancer immunotherapy, promising improved clinical outcomes [129].

Anti-EGFR iRGD, a bispecific protein, shows promising synergies in boosting gastric cancer radiotherapy. It hinders proliferation, suppresses EGFR upregulation, and induces apoptosis. Following radiation, the protein enhances tumor penetration, establishing it as a compelling candidate for integrated EGFR-targeted therapy and radiotherapy in gastric cancer. These findings hold substantial implications for both preclinical and clinical applications [130]. A targeted drug delivery system for gastric cancer was devised using erythrocyte membrane-derived vesicles, incorporating 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(maleimide[polyethylene glycol]-3400) (DSPE-PEG-MAL) for tumor-specific insertion. Employing the tumor-penetrating bispecific recombinant protein, anti-EGFR-iRGD, a novel nano-system (PRP) delivering paclitaxel (PTX) was created. PRP exhibited stability, uniformity, and efficient PTX release, displaying comparable cytotoxicity in vitro. In vivo, PRP efficiently accumulated in tumors, reducing

tumor volume by 61% without severe side effects. This innovative erythrocyte membrane-based nano-system holds promise for cancer treatment and synergistic anticancer approaches [131]. A novel integrin-targeted drug delivery system, iRGD-heparin nanocarrier (iHP), was synthesized to address the limitations of cisplatin in gastric cancer therapy. iHP exhibited biodegradability and biocompatibility, with precise integrin-targeting demonstrated in vitro and in vivo. Synthesis of targeted nanoparticles (iHDDP) yielded superior antitumor efficacy compared to untargeted counterparts (HDDP), without weight loss or organ damage in tumor-bearing mice. iHDDP offers a promising platform for enhancing cisplatin's therapeutic efficacy in gastric cancer treatment [132].

sTRAIL-iRGD, a recombinant protein uniting tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) with the integrin-targeting motif CRGDKGPDC, displays selective internalization into gastric cancer cells. This fusion protein exhibits potent antitumor effects in vitro, in multicellular spheroids, and in vivo. Notably, sTRAIL-iRGD, when combined with paclitaxel, demonstrates enhanced efficacy without inducing liver toxicity in treated mice. This promising agent presents a targeted and low-toxicity approach for combating gastric cancer, offering new avenues for therapeutic exploration [133].

Elevated NRP1 expression in gastric cancer tissues is linked to aggressive tumor characteristics. Functionally, NRP1 intensifies cancer cell proliferation, migration, and invasion. Notably, the tumor-targeting peptide iRGD enhances the efficacy of 5-Fluorouracil (5-FU) chemotherapy through NRP1, presenting a promising strategy for refining gastric cancer treatment. This study identifies NRP1 as a potential therapeutic target, highlighting the iRGD-5-FU combination as a valuable approach to enhance patient prognosis in gastric cancer therapy [134].

To overcome the limited tumor penetration of the pro-apoptotic peptide KLA, a recombinant protein, KLA-iRGD, was innovatively designed. This fusion protein combines the apoptosis-inducing KLA peptide with iRGD (CRGDKGPDC), a high-penetrating tumor-homing peptide. Upon internalization into tumor cells, KLA-iRGD, activated by the neuropilin-1 receptor, effectively disperses throughout the tumor mass. In vivo and in vitro studies demonstrated potent antitumor activity, positioning KLA-iRGD as a promising and selective anticancer agent with minimal systemic toxicity. This breakthrough holds potential for advancing targeted

anticancer therapies [134].

A novel tumor-targeting contrast agent for magnetic resonance imaging (MRI) was developed, coupling gadolinium diethylene triamine pentaacetate (Gd DTPA) with a bispecific recombinant anti-EGFR iRGD protein. Extracted from Escherichia coli, the protein efficiently targets EGFR and integrin αvβ/β5, displaying high cellular penetration. This Gd-labeled anti-EGFR iRGD exhibits superior T1 relaxivity and enhanced tumor targeting compared to conventional contrast agents, suggesting its potential as an improved MRI contrast agent for tumor localization [135].

Addressing the challenge of drug penetration into solid tumors, the bispecific protein anti-EGFR-iRGD was previously developed, showcasing efficacy in inhibiting gastric cancer cell proliferation. This study explores the synergistic potential of anti-EGFR-iRGD in combination with the widely used chemotherapeutic paclitaxel (PTX) in epidermal growth factor receptor highly expressing gastric cancer. The findings, spanning monolayer cells, multicellular spheroids, and tumor-bearing mice, underscore the therapeutic promise of this combination, offering a compelling avenue for enhanced gastric cancer therapy in clinical settings [136].

The recombinant protein anti-EGFR-iRGD, comprising anti-EGFR VHH fused with the tumor-specific binding peptide iRGD, emerges as a promising therapeutic for gastric cancer. Demonstrating extensive penetration into multicellular spheroids and tumors, anti-EGFR-iRGD exhibits potent antitumor activity across various models. Notably, it enhances the permeability and efficacy of anticancer drugs like doxorubicin (DOX) and bevacizumab within multicellular spheroids. This study underscores the significance of iRGD in optimizing therapeutic strategies, positioning anti-EGFR-iRGD as a potential standalone or adjunctive candidate for cancer treatment [137]. We have covered majority of the studies demonstrated application of iRGD in different forms to treat different types of cancers. There are more studies, that are not covered in this chapter.

6.6. Diagnostic and Theranostic Applications of iRGD

Theranostic nanoparticles are gaining prominence as powerful instruments for the non-invasive diagnosis, treatment, and monitoring of solid tumors. Given its multifaceted performance, iRGD has been integrated with various nanomaterials,

presenting itself as a valuable theranostic agent. Numerous studies have explored its potential applications in diagnostics and therapy, highlighting its adaptability and effectiveness across diverse contexts. The study conducted by Yan et al introduces a novel theranostic nanoparticle, iRGD-ICG-LPs, showcasing remarkable efficacy in near-infrared (NIR) molecular imaging and phototherapy. By targeting the overexpressed αvβ3 integrin in tumor angiogenic blood vessels, iRGD-ICG-LPs enable real-time molecular imaging, offering insights into early-stage tumor genesis and growth. The specificity of binding enhances the potential for advanced diagnostics and therapeutic interventions in oncology [138]. A research team has successfully engineered a dual-labeled multifunctional porous silicon nanoparticles (PSi NPs) nanosystem designed for cancer theranostic applications. The UnTHCPSi nanoparticles underwent modification to incorporate a ^{111}In radiolabel, fluorescent Alexa Fluor 488 dye, and iRGD peptide. This configuration allowed for the observation of nanocarrier biodistribution in vivo using single photon emission computed tomography (SPECT)/X-ray computer tomography (CT), along with tissue-level localization ex vivo through fluorescence microscopy. Additionally, the system demonstrated integrin targeting in a mouse xenograft model of prostate cancer [139]. A dual-channel fluorescent cyclic iRGD (TAMRA-iRGDC-Cy5.5) was utilized to monitor the simultaneous tumor internalization of N- and C-terminal fragments. Co-internalization of both fragments facilitated the development of a novel theranostic peptide platform (Cy5.5-iRGDC-Pt(IV)), integrating a fluorescent dye and a cisplatin prodrug on each terminus of cyclic iRGD for cancer-targeted imaging and therapy. In comparison to a non-iRGD control, Cy5.5-iRGDC-Pt(IV) demonstrated superior tumor imaging contrast and induced tumor-specific apoptosis, showcasing potential for theranostic applications with minimal systemic toxicity [140]. A new biomimetic nanoplatform, drawing inspiration from high-density lipoproteins (HDLs), was engineered to possess profound tumor-penetrating abilities. This innovative platform seamlessly incorporated the clinical imaging agent indocyanine green (ICG), paving the way for synergistic phototherapy. Through conjugation with the tumor-homing iRGD peptide, the HDL-protein formed a similar α-helix structure, maintaining the lipid nanoparticle's organization. The resulting nanoparticles exhibited nanosized diameters, superior biostability, and efficient encapsulation of ICG. Upon exposure to near-infrared (NIR) light, the liberated ICG triggered localized heat, serving the purpose

of photothermal therapy (PTT), and concurrently produced reactive oxygen species (ROS) for photodynamic therapy (PDT). Additionally, ICG fluorescence facilitated effective diagnosis. Administered intravenously, these HDL-mimicking nanoparticles demonstrated deep tumor penetration, enhancing phototherapy (PTT and PDT) under NIR laser irradiation [141].

A separate research team designed liposomes encapsulating indocyanine green (ICG) modified with internalized RGD (iRGD) for imaging-assisted photothermal therapy (PTT) and photodynamic therapy (PDT) in the management of laryngeal carcinoma. The lipid modification involving iRGD-PEG-DSPE conferred a strong attraction to tumor vascular targeting, facilitated tumor penetration, and enhanced targeting of tumor cells. In-vivo findings illustrate remarkable blood circulation and tumor accumulation of indocyanine green (ICG) encapsulated liposomes (iLIPICG). When exposed to an 808 nm laser, iLIPICG achieves regulated spatial and temporal responses, generating hyperthermia, ROS, and fluorescence-guided effects via ICG. These effects contribute to the efficient elimination of laryngeal carcinoma cells. The iLIPICG system represents a potential strategy for precise imaging and efficacious phototherapy in the treatment of laryngeal carcinoma [142]. Hollow mesoporous silica-coated MnO nanoparticles, conjugated with tumor homing peptide iRGD (MnO@mSiO2-iRGD NPs), were designed for pH-responsive, biodegradable, and tumor-specific immunotherapy. Leveraging manganese's role in immune activation via the cGAS-STING pathway, these NPs enabled MRI-guided tumor immune-chemodynamic combination therapy. The mSiO2 shell facilitated cellular uptake, and NPs dissociated under acidic conditions for MRI specificity and Mn^{2+} release. The results demonstrated synergistic effects with α-PD-1 antibody, promoting cytotoxic T lymphocyte infiltration and effectively restraining melanoma progression and metastasis. The MnO@mSiO2-iRGD NPs hold promise for tumor theranostic application [143]. A unique magnetic nanocatalyst, ipGdIO-Dox, was developed for MRI-guided chemo- and ferroptosis-based cancer therapy. The gadolinium-enhanced nanocatalysis, responding to weak acid conditions, releases Fe(II) ions within cancer cells, catalyzing H_2O_2 into highly toxic OH• for ferroptosis induction. The iRGD-PEG-ss-PEG coating allows controlled doxorubicin (Dox) release, intensifying anticancer effects. Systemic administration of ipGdIO-Dox provides precise T1- and T2-weighted MRI signals for accurate tumor recognition, presenting a promising approach for MRI-guided

chemo- and ferroptosis-based theranostic system [144].

6.7. Challenges Associated with iRGD for Clinical Translation

While iRGD has shown considerable promise in addressing inadequate penetration, there are persistent concerns that have not yet been resolved. The complete mechanism behind iRGD's ability to penetrate tumors has not been thoroughly investigated. Studies suggest that the protease-dependent cleavage of iRGD, revealing the CendR motif, triggers alterations in the internalization process. This initiation sets the stage for the development of a transport system that aids in delivering the attached or co-administered payload into the tumor cells. One study demonstrated that the tumor accumulation of iRGD-conjugated abraxane is marginally lower than that of abraxane co-administered with iRGD in breast and prostate cancer. However, the authors did not achieve statistical significance between the two groups [67]. An alternative study revealed that in mice bearing Kras-mutated or patient-derived pancreatic cancer and receiving an intravenous (iv) injection of a silica-based nanocarrier, the co-administration strategy demonstrated roughly 2.5 times greater effectiveness compared to the conjugation method [115]. It is essential to emphasize that the coadministration approach resolves a key limitation of the alternative delivery mechanism where the peptide is attached to the nanocarrier. This limitation is linked to the transport capacity of the carrier system, which is dependent on the availability of NRP-1 receptors, whereas administering the unconjugated peptide separately triggers substantial transportation to the tumor site in bulk [145]. The inclusion of covalently attached peptides might complicate the surface characteristics of nanocarriers, potentially leading to unintended effects within the complex in vivo system [146]. A major challenge with peptide attachment to nanoparticles is their removal by the reticuloendothelial system (RES) due to opsonization. It is implied that size plays a role in both clearance and distribution. Once the particle size surpasses 100 nm, significant alterations occur in their pharmacokinetics and biodistribution, leading to their presence in blood and various organs such as the spleen, lungs, liver, and kidneys [147]. A more efficient uptake by the body's RES will shorten the circulation period, leading to reduced tumor accumulation [148].

Considering translational feasibility, employing the free peptide is a more feasible and cost-effective option for clinical application when compared to the

conjugation peptide. This process inevitably increases both the cost and complexity of the nanocarrier. Similarly, the co-administration approach has demonstrated greater effectiveness than the conjugated method, potentially attributed to the unchanged nature of the co-administered drug [149]. To ensure the co-administered drug's efficacy, it needs to be near the iRGD peptide to utilize the transport pore it forms. While iRGD can bind to and penetrate cells, the precise method by which it enhances drug delivery is not fully understood. Extensive research is necessary to uncover the specific pathways through which iRGD facilitates drug penetration into tumor sites. Given the essential role of integrin expression and its interactions in enhancing drug penetration within the tumor, iRGD's efficacy relies on the presence of key receptors such as αvβ3 integrin and NRP-1 in this collaborative approach. iRGD selectively attaches to cancer cells abundant in αv integrin expression. When administered intravenously, iRGD undergoes proteolytic processing to form the CRGDK fragment. This fragment targets αv integrins, revealing the CendR motif at its C-terminus, activating the RXXR/K sequence motif. This interaction with NRPs triggers a substantial delivery process, enabling profound penetration of anticancer drugs into tumor tissues either by direct conjugation or simultaneous administration with iRGD [59]. Healthy epithelial tissues exhibit low expression levels of αvβ3 integrin, whereas tumor tissues showcase heightened levels of this integrin receptors [150].

Given the comparatively scarce population of tumor cells relative to the extensive number of normal cells in the body, the prevalence of the targeted receptor might not manifest markedly higher than its incidence in healthy cells, even with a minimal expression on normal cells. Additionally, the expression of a particular targeted receptor or protein is not consistently uniform, fluctuating based on the tumor stage and evolving over time. Similarly, it should be acknowledged that the level of αvβ3 integrin expression can vary among different tumors. These findings cast uncertainties on the efficacy of iRGD across diverse cancer types where integrin expression is not conspicuously elevated [151]. Furthermore, the extrapolation of findings from studies involving animals to human populations represents a critical phase, wherein inter-species differences in targeted protein expression often result in challenges when applying promising preclinical outcomes to clinical success [152].

Therefore, it would be prudent to initially validate integrin expression in each tumor before contemplating the use of this peptide in clinical trials. Additionally, the precise capacity of iRGD to aid drug distribution in challenging, deeper regions of tumors, particularly in desmoplastic tumors, remains uncertain. The dense fibrous tissue surrounding the tumor presents a substantial barrier, hindering drug delivery by establishing an impenetrable TME that sustains, fosters, and nurtures the tumor. Given the formidable impermeability of these tumors and their robust resistance to pharmacological interventions, optimizing the effectiveness of therapeutic interventions have consistently posed a significant challenge [153].

Upon thorough examination of the data, it appears improbable that iRGD would boost the infiltration of payloads in desmoplastic tumors. Akashi *et al.* have raised doubts about iRGD's suitability for these tumors. They observed that the drug accumulation and anticancer effects enhanced by iRGD didn't show significant results in certain tumor graft models. This lack of impact could be attributed to the modified stroma of the tumor graft model, which displayed fewer blood vessels and denser stromal tissue. It is reasonable to suggest that the densely collagenous features of tumor tissue countered the penetration effects of iRGD, thereby constraining its effectiveness in fibrous tumors. Thus, the potency of iRGD may be restricted to tumors exhibiting abundant vascularity and minimal fibrous reactions [113].Widespread studies have confirmed that the spreading of primary tumors, known as metastasis, stands as a primary cause of patient mortality. While iRGD's ability to improve drug distribution within cancerous regions has been acknowledged, there's also a concern that it might enhance metastasis by prompting tumor cells to move in the opposite direction into the bloodstream (antidromic tumor cell dissemination) [52].

Yet, numerous investigations indicate that in animal models, iRGD might actually decrease the occurrence of metastasis [154]. More studies and specific research are needed to precisely determine the mechanisms and effectiveness of iRGD in either triggering or suppressing metastasis. Presently, there's ongoing exploration into the safety of the peptide in human subjects, but uncertainties persist due to numerous factors involved in this assessment. The practical application of iRGD-based nanomaterial in clinical settings poses considerable challenges. Ensuring consistency remains a significant worry when using iRGD in combination

with other treatments [67].

There could be several reasons explaining the differences observed, with some significant possibilities being the varied expression of NRP-1 receptors, the type and stage of the tumor, limited correlation between laboratory and real-life conditions, and the use of different research methods. iRGD is considered a reliable and safe peptide for tumor penetration. However, while it enhances the absorption and infiltration of anti-tumor substances, it also raises concerns about elevated concentrations of therapeutic drug molecules in the body. Despite the absence of reported toxicity issues with iRGD so far, asserting its complete safety would not be warranted without rigorous studies [155].

6.8. Conclusion

In the context of overcoming chemotherapeutic resistance, the tumor stroma's role is pivotal. While ligand-based targeting therapies aim to concentrate drugs at tumor sites, the outcomes of active targeting approaches have been less promising. iRGD emerges as a promising strategy, enhancing intratumoral distribution of nanomaterials through transcytosis, overcoming barriers to drug distribution. Despite its potential, iRGD remains in the early stages, with unanswered questions requiring further exploration for successful clinical translation. This chapter concludes by highlighting the potential and challenges of iRGD in tumor management.

6.9. Future Perspectives

- iRGD alone or in combination with other traditional approaches like chemotherapy, radiotherapy and immunotherapy are offering novel ways to increase the efficacy of cancer treatment, reduces off-target toxicity and to overcome the therapeutic obstacles [156].
- iRGD can be co-administered with therapeutics and can also be conjugated to various therapeutic moieties like nanoparticles and antibodies to make them target specific [157].
- Thus, enhanced delivery of chemotherapeutic agents by iRGD into tumor site increases its concentration in the tumor site and reduces the systemic toxicity [158].

- Such enhanced cellular uptake could potentially sensitize the resistant cells and may help in overcoming the resistance to traditional cancer treatments. Cilengitide and Abegrin are available cyclic RGD peptides which were found to be potent anticancer agents. In pre-clinical studies, cilengitide was effective in treating glioblastoma by targeting αvβ3 and αvβ5 integrins. But its efficacy was limited in clinical trials and it triggered immunogenicity in some patients. Abegrin binds to αvβ3 integrins and proven to be effective in treating various cancers like breast and prostate cancers [159].
- RGD peptides were also shown to be powerful diagnostic agents, recently clinical studies (Phase I & II) were performed for 68Ga-FAP-RGD PET/CT as dual targeting PET radio tracer and observed to be efficacious imaging agent in various cancers [160].

There are more similarly modified peptides being examined in clinical trials for their diagnostic efficiency in various cancers. Thus, RGD functionalised carriers were found to be efficacious in cancer diagnosis and treatment. More pre-clinical and clinical research is required to tackle the challenges like immunogenicity and development of resistance, as in the case of traditional cancer treatments. If researchers could successfully translate the pre-clinical findings of iRGD peptide carriers into clinical investigations, it would be a significant step forward in the development of cancer diagnostics and treatment.

6.10. Public Perception of iRGD as a Targeted Drug Delivery Technology

The adoption and diffusion of approved technologies, particularly in drug delivery, often face challenges marked by slow uptake, disruptions, and disparities. Safety concerns and perceived risks associated with usage commonly contribute to uncertainty among potential users. The perception of the general public regarding the safety of targeted drug delivery technologies, particularly those involving iRGD co-administered or conjugated with anti-cancer drugs or their nano or other formulations, can be influenced by various factors [161].

Concerns about the safety and risks associated with the use of innovative drug delivery systems may arise due to limited familiarity and understanding of these technologies [161]. Lack of accessible information and evidence can exacerbate

these concerns, with individuals seeking reassurance from trusted healthcare professionals [162].

Perceptions of the effectiveness of iRGD-based drug delivery systems may vary based on familiarity with the healthcare system and trust in institutions. Branded drugs incorporating iRGD technology may be perceived more favourably than generic ones, especially when labelled as “new” or “innovative” [163]. Conversely, older drug delivery methods may be viewed as safer and more effective, particularly in the context of cancer treatments [164].

Psychological factors also play a role in shaping public perceptions, with attitudes toward novelty and innovation influencing acceptance of iRGD-based drug delivery technologies. Trust in institutions and concerns about corporate influence in medicine further impact public perceptions of these innovative approaches [165].

6.11. Combatting Bias Against Emerging Technologies

To address potential biases against the application of iRGD-based drug delivery technologies, it is essential to implement strategies that foster trust and provide transparent, accessible information to the public. This can include:

1) Education and Awareness Campaigns: Comprehensive information about the safety and efficacy of iRGD-based drug delivery systems should be disseminated through public education campaigns to dispel misconceptions and build trust.

2) Transparency and Communication: Open communication about the development, testing, and regulatory approval processes of iRGD-based drug delivery systems are crucial for enhancing public confidence in their safety and reliability.

3) Engagement with Stakeholders: Collaboration with patient advocacy groups and involvement of patient representatives in discussions about iRGD-based drug delivery technologies can provide valuable insights and address concerns.

4) Regulatory Oversight: Adherence to rigorous regulatory standards and guidelines for the development and deployment of iRGD-based drug delivery technologies is essential to ensure safety and efficacy.

5) Long-Term Monitoring: Robust post-market surveillance and monitoring mechanisms can track safety outcomes and address emerging concerns promptly, contributing to building trust in these innovative technologies.

Overall, fostering trust, providing clear information, and engaging with stakeholders are key to addressing bias against iRGD-based drug delivery technologies and promoting their acceptance and utilization in cancer treatment.

References

[1] Brown JS, Amend SR, Austin RH, Gatenby RA, Hammarlund EU, Pienta KJ. Updating the definition of cancer. Molecular Cancer Research. 2023; 21(11): 1142-7.

[2] Workie MS, Jalali R, Cheng Z. Background: Cancer is a leading cause of death in the world, and the estimated new cancer cases were 19 million and the estimated cancer deaths were around 10 million worldwide in 2020. Proton therapy (PT) is a promising treatment for cancers; however, only few patients with cancer received PT due to limited number of PT centers worldwide, especially in low-and middle-income countries. Universal Health Coverage and Global Health in Oncology. 2023; 17.

[3] Yildizhan H, Barkan NP, Turan SK, Demiralp Ö, Demiralp FDÖ, Uslu B, *et al.* Treatment strategies in cancer from past to present. In: Drug targeting and stimuli sensitive drug delivery systems [Internet]. Elsevier; 2018 [cited 2024 Feb 16]. p. 1-37. Available from: https://www.sciencedirect.com/science/article/pii/B978012813689800001X

[4] Behranvand N, Nasri F, Zolfaghari Emameh R, Khani P, Hosseini A, Garssen J, *et al.* Chemotherapy: a double-edged sword in cancer treatment. Cancer Immunol Immunother. 2022 Mar; 71(3): 507-26.

[5] Patwekar M, Sehar N, Patwekar F, Medikeri A, Ali S, Aldossri RM, *et al.* Novel immune checkpoint targets: A promising therapy for cancer treatments. International Immunopharmacology. 2024; 126: 111186.

[6] Albelda SM. CAR T cell therapy for patients with solid tumours: Key lessons to learn and unlearn. Nature Reviews Clinical Oncology. 2024; 21(1): 47-66.

[7] Esfahani K, Roudaia L, Buhlaiga NA, Del Rincon SV, Papneja N, Miller WH. A review of cancer immunotherapy: from the past, to the present, to the future. Current Oncology. 2020; 27(s2): 87-97.

[8] Zhong L, Li Y, Xiong L, Wang W, Wu M, Yuan T, *et al.* Small molecules in targeted cancer therapy: Advances, challenges, and future perspectives. Signal transduction and targeted therapy. 2021; 6(1): 201.

[9] Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nature reviews cancer. 2012; 12(4): 237-51.

[10] Singh S, Tank NK, Dwiwedi P, Charan J, Kaur R, Sidhu P, *et al.* Monoclonal antibodies: a review. Current clinical pharmacology. 2018; 13(2): 85-99.

[11] Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. Nature biotechnology. 2005; 23(9): 1147-57.

[12] Storz U. Rituximab: How approval history is reflected by a corresponding patent filing strategy. mAbs. 2014 Jul; 6(4): 820-37.

[13] Amiri-Kordestani L, Blumenthal GM, Xu QC, Zhang L, Tang SW, Ha L, *et al.* FDA approval: ado-trastuzumab emtansine for the treatment of patients with HER2-positive metastatic breast cancer. Clinical cancer research. 2014; 20(17): 4436-41.

[14] Garcia J, Hurwitz HI, Sandler AB, Miles D, Coleman RL, Deurloo R, *et al.* Bevacizumab (Avastin®) in cancer treatment: A review of 15 years of clinical experience and future outlook. Cancer treatment reviews. 2020; 86: 102017.

[15] Pierpont TM, Limper CB, Richards KL. Past, present, and future of rituximab—the world's first oncology monoclonal antibody therapy. Frontiers in oncology. 2018; 8: 163.

[16] Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, *et al.* Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. Journal of clinical oncology. 2023; 41(9): 1638-45.

[17] Robak T. Alemtuzumab for B-cell chronic lymphocytic leukemia. Expert Review of Anticancer Therapy. 2008 Jul;8(7):1033-51.

[18] Witzig TE. Efficacy and safety of 90Y ibritumomab tiuxetan (Zevalin)

radioimmunotherapy for non-Hodgkin's lymphoma. In: Seminars in oncology [Internet]. Elsevier; 2003 [cited 2024 Feb 20]. p. 11-6.

[19] Lu RM, Hwang YC, Liu IJ, Lee CC, Tsai HZ, Li HJ, *et al.* Development of therapeutic antibodies for the treatment of diseases. J Biomed Sci. 2020 Jan 2; 27: 1.

[20] Psilopatis I, Damaskos C, Garmpi A, Sarantis P, Koustas E, Antoniou EA, *et al.* FDA-Approved Monoclonal Antibodies for Unresectable Hepatocellular Carcinoma: What Do We Know So Far? Int J Mol Sci. 2023 Jan 31; 24(3): 2685.

[21] the Study 1053 investigators, Kreitman RJ, Dearden C, Zinzani PL, Delgado J, Robak T, *et al.* Moxetumomab pasudotox in heavily pre-treated patients with relapsed/refractory hairy cell leukemia (HCL): long-term follow-up from the pivotal trial. J Hematol Oncol. 2021 Dec; 14(1): 35.

[22] Lee EQ. Neurologic Complications of Cancer Therapies. Curr Neurol Neurosci Rep. 2021 Dec; 21(12): 66.

[23] Keam SJ. Tremelimumab: First Approval. Drugs. 2023 Jan;83(1):93-102.

[24] Gacche RN, Meshram RJ. Targeting tumor micro-environment for design and development of novel anti-angiogenic agents arresting tumor growth. Progress in biophysics and molecular biology. 2013; 113(2): 333-54.

[25] Paul MK, Mukhopadhyay AK. Tyrosine kinase-role and significance in cancer. International journal of medical sciences. 2004; 1(2): 101.

[26] Capdeville R, Buchdunger E, Zimmermann J, Matter A. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. Nature reviews Drug discovery. 2002; 1(7): 493-502.

[27] Singh M, Jadhav HR. Targeting non-small cell lung cancer with small-molecule EGFR tyrosine kinase inhibitors. Drug discovery today. 2018; 23(3): 745-53.

[28] Maiti R, Patel B, Patel N, Patel M, Patel A, Dhanesha N. Antibody drug conjugates as targeted cancer therapy: past development, present challenges and future opportunities. Arch Pharm Res. 2023 May; 46(5): 361-88.

[29] Tsao LC, Force J, Hartman ZC. Mechanisms of therapeutic antitumor monoclonal antibodies. Cancer research. 2021; 81(18): 4641-51.

[30] Swain SM, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: Advances and future directions. Nature Reviews Drug Discovery. 2023; 22(2): 101-26.

[31] Hafeez U, Parakh S, Gan HK, Scott AM. Antibody-drug conjugates for cancer therapy. Molecules. 2020; 25(20): 4764.

[32] Tang S, Qin C, Hu H, Liu T, He Y, Guo H, *et al.* Immune checkpoint inhibitors in non-small cell lung cancer: progress, challenges, and prospects. Cells. 2022; 11(3): 320.

[33] Naimi A, Mohammed RN, Raji A, Chupradit S, Yumashev AV, Suksatan W, *et al.* Tumor immunotherapies by immune checkpoint inhibitors (ICIs); the pros and cons. Cell Commun Signal. 2022 Apr 7; 20(1): 44.

[34] Wu Y, Yang Z, Cheng K, Bi H, Chen J. Small molecule-based immunomodulators for cancer therapy. Acta Pharmaceutica Sinica B [Internet]. 2022 [cited 2024 Feb 16].

[35] Beeghly GF, Shimpi AA, Riter RN, Fischbach C. Measuring and modelling tumour heterogeneity across scales. Nature Reviews Bioengineering. 2023; 1(10): 712-30.

[36] Turley SJ, Cremasco V, Astarita JL. Immunological hallmarks of stromal cells in the tumour microenvironment. Nature reviews immunology. 2015; 15(11): 669-82.

[37] Saifi MA, Sathish G, Bazaz MR, Godugu C. Exploration of tumor penetrating peptide iRGD as a potential strategy to enhance tumor penetration of cancer nanotherapeutics. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2023; 188895.

[38] Izci M, Maksoudian C, Manshian BB, Soenen SJ. The Use of Alternative Strategies for Enhanced Nanoparticle Delivery to Solid Tumors. Chem Rev. 2021 Feb 10; 121(3): 1746-803.

[39] Huang J, Zhang L, Wan D, Zhou L, Zheng S, Lin S, *et al.* Extracellular matrix and its therapeutic potential for cancer treatment. Signal Transduction and Targeted Therapy. 2021; 6(1): 153.

[40] Barua S, Mitragotri S. Challenges associated with penetration of nanoparticles across cell and tissue barriers: a review of current status and future

prospects. Nano today. 2014; 9(2): 223-43.

[41] Anderson NM, Simon MC. The tumor microenvironment. Curr Biol. 2020 Aug 17; 30(16): R921-5.

[42] Thakkar S, Sharma D, Kalia K, Tekade RK. Tumor microenvironment targeted nanotherapeutics for cancer therapy and diagnosis: A review. Acta Biomaterialia. 2020 Jan 1; 101: 43-68.

[43] Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nature biotechnology. 2015; 33(9): 941-51.

[44] Yang NJ, Hinner MJ. Getting Across the Cell Membrane: An Overview for Small Molecules, Peptides, and Proteins. Methods Mol Biol. 2015; 1266: 29-53.

[45] Azzi S, Hebda JK, Gavard J. Vascular permeability and drug delivery in cancers. Frontiers in oncology. 2013; 3: 211.

[46] Pramanik N, Gupta A, Ghanwatkar Y, Mahato RI. Recent advances in drug delivery and targeting for the treatment of pancreatic cancer. Journal of Controlled Release. 2024; 366: 231-60.

[47] Fergatova A, Affara NI. The cellular triumvirate: fibroblasts entangled in the crosstalk between cancer cells and immune cells. Frontiers in Immunology. 2024; 14: 1337333.

[48] Tunggal JK, Cowan DS, Shaikh H, Tannock IF. Penetration of anticancer drugs through solid tissue: a factor that limits the effectiveness of chemotherapy for solid tumors. Clinical cancer research. 1999;5(6):1583-6.

[49] Thirumalai A, Girigoswami K, Pallavi P, Harini K, Gowtham P, Girigoswami A. Cancer therapy with iRGD as a tumor-penetrating peptide. Bulletin du Cancer [Internet]. 2023 [cited 2024 Feb 9].

[50] Kang S, Lee S, Park S. iRGD peptide as a tumor-penetrating enhancer for tumor-targeted drug delivery. Polymers. 2020; 12(9): 1906.

[51] Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, *et al.* Tissue-penetrating delivery of compounds and nanoparticles into tumors. Cancer cell. 2009; 16(6): 510-20.

[52] Teesalu T, Sugahara KN, Ruoslahti E. Tumor-penetrating peptides. Frontiers

in oncology. 2013; 3: 216.

[53] Mezu-Ndubuisi OJ, Maheshwari A. The role of integrins in inflammation and angiogenesis. Pediatric research. 2021; 89(7): 1619-26.

[54] Duro-Castano A, Gallon E, Decker C, Vicent MJ. Modulating angiogenesis with integrin-targeted nanomedicines. Advanced Drug Delivery Reviews. 2017; 119: 101-19.

[55] Arosio D, Casagrande C. Advancement in integrin facilitated drug delivery. Advanced drug delivery reviews. 2016; 97: 111-43.

[56] Ludwig BS, Kessler H, Kossatz S, Reuning U. RGD-binding integrins revisited: how recently discovered functions and novel synthetic ligands (re-) shape an ever-evolving field. Cancers. 2021; 13(7): 1711.

[57] Pytela R, Pierschbacher MD, Ruoslahti E. Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. Cell. 1985; 40(1): 191-8.

[58] Böger C, Warneke VS, Behrens HM, Kalthoff H, Goodman SL, Becker T, *et al.* Integrins αvβ3 and αvβ5 as prognostic, diagnostic, and therapeutic targets in gastric cancer. Gastric Cancer. 2015 Oct; 18(4): 784-95.

[59] Zuo H. iRGD: a promising peptide for cancer imaging and a potential therapeutic agent for various cancers. Journal of Oncology [Internet]. 2019 [cited 2024 Feb 9]; 2019.

[60] Bellis SL. Advantages of RGD peptides for directing cell association with biomaterials. Biomaterials. 2011; 32(18): 4205-10.

[61] Hall SW, Humphries JE, Gonias SL. Inhibition of cell surface receptor-bound plasmin by alpha 2-antiplasmin and alpha 2-macroglobulin. Journal of Biological Chemistry. 1991; 266(19): 12329-36.

[62] Pellinen T, Ivaska J. Integrin traffic. Journal of cell science. 2006; 119(18): 3723-31.

[63] Zuo HD, Yao WW, Chen TW, Zhu J, Zhang JJ, Pu Y, *et al.* The effect of superparamagnetic iron oxide with iRGD peptide on the labeling of pancreatic cancer cells in vitro: a preliminary study. BioMed research international [Internet]. 2014 [cited 2024 Feb 9]; 2014.

[64] Yin L, Li X, Wang R, Zeng Y, Zeng Z, Xie T. Recent Research Progress of RGD Peptide-Modified Nanodrug Delivery Systems in Tumor Therapy. Int J Pept Res Ther. 2023 May 16; 29(4): 53.

[65] Qian J, Zhou S, Lin P, Lei J, Zheng S, Xu W, *et al.* Recent advances in the tumor-penetrating peptide internalizing RGD for cancer treatment and diagnosis. Drug Development Research. 2023 Jun; 84(4): 654-70.

[66] Huang J, Lai W, Wang Q, Tang Q, Hu C, Zhou M, *et al.* Effective Triple-Negative Breast Cancer Targeted Treatment Using iRGD-Modified RBC Membrane-Camouflaged Nanoparticles. IJN. 2021 Nov; Volume 16: 7497-515.

[67] Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Greenwald DR, *et al.* Coadministration of a Tumor-Penetrating Peptide Enhances the Efficacy of Cancer Drugs. Science. 2010 May 21; 328(5981): 1031-5.

[68] Liu Y, Ji M, Wong MK, Joo KI, Wang P. Enhanced therapeutic efficacy of iRGD-conjugated crosslinked multilayer liposomes for drug delivery. BioMed research international [Internet]. 2013 [cited 2024 Feb 9]; 2013.

[69] Chen B, Liu X, Li Y, Shan T, Bai L, Li C, *et al.* iRGD Tumor-Penetrating Peptide-Modified Nano-Delivery System Based on a Marine Sulfated Polysaccharide for Enhanced Anti-Tumor Efficiency Against Breast Cancer. IJN. 2022 Feb; Volume 17: 617-33.

[70] Gao F, Zhang J, Fu C, Xie X, Peng F, You J, *et al.* iRGD-modified lipid-polymer hybrid nanoparticles loaded with isoliquiritigenin to enhance anti-breast cancer effect and tumor-targeting ability. IJN. 2017 Jun; Volume 12: 4147-62.

[71] Cun X, Chen J, Ruan S, Zhang L, Wan J, He Q, *et al.* A Novel Strategy through Combining iRGD Peptide with Tumor-Microenvironment-Responsive and Multistage Nanoparticles for Deep Tumor Penetration. ACS Appl Mater Interfaces. 2015 Dec 16; 7(49): 27458-66.

[72] Diaz Bessone MI, Simón-Gracia L, Scodeller P, Ramirez MDLA, Lago Huvelle MA, Soler-Illia GJAA, *et al.* iRGD-guided tamoxifen polymersomes inhibit estrogen receptor transcriptional activity and decrease the number of breast cancer cells with self-renewing capacity. J Nanobiotechnol. 2019 Dec; 17(1): 120.

[73] Yu B, Su H, Zhao L, Yang J, Zhu M, Zhao J. 99mTc-labeled iRGD for single-positron emission computed tomography imaging of triple-negative breast cancer. Frontiers in bioengineering and biotechnology. 2022; 10: 1001899.

[74] Zhu Y, Arkin G, Zeng W, Huang Y, Su L, Guo F, *et al.* Ultrasound image-guided cancer gene therapy using iRGD dual-targeted magnetic cationic microbubbles. Biomedicine & Pharmacotherapy. 2024; 172: 116221.

[75] Wang X, Zhao G, Yan M, Liang X, Zhao N, Lu T. iRGD mediated pH-responsive mesoporous silica enhances drug accumulation in tumors. European Journal of Pharmaceutical Sciences. 2024; 106725.

[76] He S, Fang Y, Wu M, Zhang P, Gao F, Hu H, *et al.* Enhanced Tumor Targeting and Penetration of Proteolysis-Targeting Chimeras through iRGD Peptide Conjugation: A Strategy for Precise Protein Degradation in Breast Cancer. Journal of Medicinal Chemistry. 2023; 66(24): 16828-42.

[77] Pan R, He T, Zhang K, Zhu L, Lin J, Chen P, *et al.* Tumor-Targeting Extracellular Vesicles Loaded with siS100A4 for Suppressing Postoperative Breast Cancer Metastasis. Cel Mol Bioeng. 2023 Apr; 16(2): 117-25.

[78] Tran NH, Nguyen DD, Nguyen NM, Tran C, Nguyen Thi NT, Ho DT, *et al.* Dual-targeting exosomes for improved drug delivery in breast cancer. Nanomedicine. 2023 Mar; 18(7): 599-611.

[79] Zhang Z, Lo H, Zhao X, Li W, Wu K, Zeng F, *et al.* Mild photothermal/radiation therapy potentiates ferroptosis effect for ablation of breast cancer via MRI/PA imaging guided all-in-one strategy. J Nanobiotechnol. 2023 May 8; 21(1): 150.

[80] Alipour M, Sheikhnejad R, Fouani MH, Bardania H, Hosseinkhani S. DNAi-peptide nanohybrid smart particles target BCL-2 oncogene and induce apoptosis in breast cancer cells. Biomedicine & Pharmacotherapy. 2023; 166: 115299.

[81] Li K, Xu K, Liu S, He Y, Tan M, Mao Y, *et al.* All-in-One Engineering Multifunctional Nanoplatforms for Sensitizing Tumor Low-Temperature Photothermal Therapy In Vivo. ACS Nano. 2023 Oct 24; 17(20): 20218-36.

[82] Marin GH, Murail S, Andrini L, Garcia M, Loisel S, Tuffery P, *et al.* In Silico and

In Vivo Studies of a Tumor-Penetrating and Interfering Peptide with Antitumoral Effect on Xenograft Models of Breast Cancer. Pharmaceutics. 2023; 15(4): 1180.

[83] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA: a cancer journal for clinicians. 2014; 64(1): 9-29.

[84] Kim IY, Kang YS, Lee DS, Park HJ, Choi EK, Oh YK, *et al.* Antitumor activity of EGFR targeted pH-sensitive immunoliposomes encapsulating gemcitabine in A549 xenograft nude mice. Journal of Controlled Release. 2009; 140(1): 55-60.

[85] Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. European journal of pharmacology. 2014; 740: 364-78.

[86] Song W, Li M, Tang Z, Li Q, Yang Y, Liu H, *et al.* Methoxypoly(ethylene glycol)-*block*-Poly(L-glutamic acid)-Loaded Cisplatin and a Combination with iRGD for the Treatment of Non-Small-Cell Lung Cancers. Macromolecular Bioscience. 2012 Nov; 12(11): 1514-23.

[87] Zhang Q, Zhang Y, Li K, Wang H, Li H, Zheng J. A novel strategy to improve the therapeutic efficacy of gemcitabine for non-small cell lung cancer by the tumor-penetrating peptide iRGD. PLoS One. 2015; 10(6): e0129865.

[88] Lao X, Liu M, Chen J, Zheng H. A tumor-penetrating peptide modification enhances the antitumor activity of thymosin alpha 1. PLoS One. 2013; 8(8): e72242.

[89] Wang Y, Huang X, Chen H, Wu Q, Zhao Q, Fu D, *et al.* The Antitumour Activity of a Curcumin and Piperine Loaded iRGD-Modified Liposome: In Vitro and In Vivo Evaluation. Molecules. 2023; 28(18): 6532.

[90] Cai R, Wang M, Liu M, Zhu X, Feng L, Yu Z, *et al.* An iRGD-conjugated photothermal therapy-responsive gold nanoparticle system carrying siCDK7 induces necroptosis and immunotherapeutic responses in lung adenocarcinoma. Bioengineering & Transla Med. 2023 Jul; 8(4): e10430.

[91] Peng ZH, Jogdeo CM, Li J, Xie Y, Wang Y, Sheinin YM, *et al.* Tumor Microenvironment-Responsive Polymeric iRGD and Doxorubicin Conjugates Reduce Spontaneous Lung Metastasis in an Orthotopic Breast Cancer Model. Pharmaceutics. 2022; 14(8): 1725.

[92] Chen J, Cao F, Cao Y, Wei S, Zhu X, Xing W. Targeted therapy of lung adenocarcinoma by the nanoplatform based on milk exosomes loaded with paclitaxel. Journal of Biomedical Nanotechnology. 2022; 18(4): 1075-83.

[93] Ji Y, Zhang Z, Hou W, Wu M, Wu H, Hu N, *et al.* Enhanced antitumor effect of icariin nanoparticles coated with iRGD functionalized erythrocyte membrane. European Journal of Pharmacology. 2022; 931: 175225.

[94] Guo Z, Li S, Liu Z, Xue W. Tumor-Penetrating Peptide-Functionalized Redox-Responsive Hyperbranched Poly(amido amine) Delivering siRNA for Lung Cancer Therapy. ACS Biomater Sci Eng. 2018 Mar 12; 4(3): 988-96.

[95] Wang F, Li B, Fu P, Li Q, Zheng H, Lao X. Immunomodulatory and enhanced antitumor activity of a modified thymosin α1 in melanoma and lung cancer. International journal of pharmaceutics. 2018; 547(1-2): 611-20.

[96] Zhang Y, Yang J, Ding M, Li L, Lu Z, Zhang Q, *et al.* Tumor-penetration and antitumor efficacy of cetuximab are enhanced by co-administered iRGD in a murine model of human NSCLC. Oncology letters. 2016; 12(5): 3241-9.

[97] Gupta SK, Torrico Guzmán EA, Meenach SA. Coadministration of a tumor-penetrating peptide improves the therapeutic efficacy of paclitaxel in a novel air-grown lung cancer 3D spheroid model. Intl Journal of Cancer. 2017 Nov 15; 141(10): 2143-53.

[98] Yang J, Wei Y, Yin H, Fang L, Chai D, Li H, *et al.* Modification of IL-24 by tumor penetrating peptide iRGD enhanced its antitumor efficacy against non-small cell lung cancer. International immunopharmacology. 2019; 70: 125-34.

[99] Baretti M, Kim AK, Anders RA. Expanding the immunotherapy roadmap for hepatocellular carcinoma. Cancer cell. 2022; 40(3): 252-4.

[100] Dong Y, Huang Y, Zhang Z, Chen A, Li L, Tian M, *et al.* iRGD-modified memory-like NK cells exhibit potent responses to hepatocellular carcinoma. J Transl Med. 2023 Mar 17; 21(1): 205.

[101] Schmithals C, Koeberle V, Korkusuz H, Pleli T, Kakoschky B, Augusto EA, *et al.* Improving drug penetrability with iRGD leverages the therapeutic response to sorafenib and doxorubicin in hepatocellular carcinoma. Cancer research. 2015; 75(15): 3147-54.

[102] Folkman J. Antiangiogenesis in cancer therapy—endostatin and its

mechanisms of action. Experimental cell research. 2006; 312(5): 594-607.

[103] Hai-Tao Z, Hui-Cheng L, Zheng-Wu L, Chang-Hong G. A tumor-penetrating peptide modification enhances the antitumor activity of endostatin in vivo. Anti-cancer drugs. 2011; 22(5): 409-15.

[104] Le JQ, Song XH, Tong LW, Lin YQ, Feng KK, Tu YF, *et al.* Dual-drug controllable co-assembly nanosystem for targeted and synergistic treatment of hepatocellular carcinoma. Journal of Colloid and Interface Science. 2024; 656: 177-88.

[105] Li H, Shi S, Wu M, Shen W, Ren J, Mei Z, *et al.* iRGD Peptide-Mediated Liposomal Nanoparticles with Photoacoustic/Ultrasound Dual-Modality Imaging for Precision Theranostics Against Hepatocellular Carcinoma. IJN. 2021 Sep; Volume 16: 6455-75.

[106] Liu X, Zhu X, Qi X, Meng X, Xu K. Co-Administration of iRGD with Sorafenib-Loaded Iron-Based Metal-Organic Framework as a Targeted Ferroptosis Agent for Liver Cancer Therapy. IJN. 2021 Feb; Volume 16: 1037-50.

[107] Liu X, Xie Y, Qi X, Xu K. Transcatheter arterial chemoembolization (TACE) with iRGD peptide in rabbit VX2 liver tumor. Journal of Cancer Research and Therapeutics. 2020; 16(7): 1703-9.

[108] Yan F, Wang S, Yang W, Goldberg SN, Wu H, Duan WL, *et al.* Tumor-penetrating Peptide-integrated Thermally Sensitive Liposomal Doxorubicin Enhances Efficacy of Radiofrequency Ablation in Liver Tumors. Radiology. 2017 Nov; 285(2): 462-71.

[109] Zhang J, Hu J, Chan HF, Skibba M, Liang G, Chen M. iRGD decorated lipid-polymer hybrid nanoparticles for targeted co-delivery of doxorubicin and sorafenib to enhance anti-hepatocellular carcinoma efficacy. Nanomedicine: Nanotechnology, Biology and Medicine. 2016; 12(5): 1303-11.

[110] Wang J, Wang H, Li J, Liu Z, Xie H, Wei X, *et al.* iRGD-Decorated Polymeric Nanoparticles for the Efficient Delivery of Vandetanib to Hepatocellular Carcinoma: Preparation and in Vitro and in Vivo Evaluation. ACS Appl Mater Interfaces. 2016 Aug 3; 8(30): 19228-37.

[111] Mao X, Liu J, Gong Z, Zhang H, Lu Y, Zou H, *et al.* iRGD-conjugated DSPE-PEG2000 nanomicelles for targeted delivery of salinomycin for treatment of

both liver cancer cells and cancer stem cells. Nanomedicine. 2015 Sep; 10(17): 2677-95.

[112] Bazeed AY, Day CM, Garg S. Pancreatic cancer: challenges and opportunities in locoregional therapies. Cancers. 2022; 14(17): 4257.

[113] Akashi Y, Oda T, Ohara Y, Miyamoto R, Kurokawa T, Hashimoto S, *et al.* Anticancer effects of gemcitabine are enhanced by co-administered iRGD peptide in murine pancreatic cancer models that overexpressed neuropilin-1. British journal of cancer. 2014; 110(6): 1481-7.

[114] Murata M, Narahara S, Kawano T, Hamano N, Piao JS, Kang JH, *et al.* Design and Function of Engineered Protein Nanocages as a Drug Delivery System for Targeting Pancreatic Cancer Cells via Neuropilin-1. Mol Pharmaceutics. 2015 May 4; 12(5): 1422-30.

[115] Liu X, Lin P, Perrett I, Lin J, Liao YP, Chang CH, *et al.* Tumor-penetrating peptide enhances transcytosis of silicasome-based chemotherapy for pancreatic cancer. The Journal of clinical investigation. 2017; 127(5): 2007-18.

[116] Suzuki K, Kunisada Y, Miyamura N, Eikawa S, Hurtado de Mendoza T, Mose ES, *et al.* Tumor-resident regulatory T cells in pancreatic cancer express the αvβ5 integrin as a targetable activation marker. bioRxiv. 2023; 2023-05.

[117] Geng S, Zhang X, Luo T, Jiang M, Chu C, Wu L, *et al.* Combined chemotherapy based on bioactive black phosphorus for pancreatic cancer therapy. Journal of Controlled Release. 2023; 354: 889-901.

[118] Lo JH, Hao L, Muzumdar MD, Raghavan S, Kwon EJ, Pulver EM, *et al.* iRGD-guided tumor-penetrating nanocomplexes for therapeutic siRNA delivery to pancreatic cancer. Molecular cancer therapeutics. 2018; 17(11): 2377-88.

[119] Ray P, Confeld M, Borowicz P, Wang T, Mallik S, Quadir M. PEG-b-poly (carbonate)-derived nanocarrier platform with pH-responsive properties for pancreatic cancer combination therapy. Colloids and Surfaces B: Biointerfaces. 2019; 174: 126-35.

[120] Tsang AT, Dudgeon C, Yi L, Yu X, Goraczniak R, Donohue K, *et al.* U1 adaptors suppress the KRAS-MYC oncogenic axis in human pancreatic cancer xenografts. Molecular cancer therapeutics. 2017; 16(8): 1445-55.

[121] Liu X, Jiang J, Nel AE, Meng H. Major effect of transcytosis on nano drug

delivery to pancreatic cancer. Molecular & Cellular Oncology. 2017 Jul 4; 4(4): e1335273.

[122] Karandish F, Froberg J, Borowicz P, Wilkinson JC, Choi Y, Mallik S. Peptide-targeted, stimuli-responsive polymersomes for delivering a cancer stemness inhibitor to cancer stem cell microtumors. Colloids and Surfaces B: Biointerfaces. 2018; 163: 225-35.

[123] Hurtado de Mendoza T, Mose ES, Botta GP, Braun GB, Kotamraju VR, French RP, *et al.* Tumor-penetrating therapy for β5 integrin-rich pancreas cancer. Nature communications. 2021; 12(1): 1541.

[124] Järveläinen HA, Schmithals C, von Harten M, Kakoschky B, Vogl TJ, Harris S, *et al.* Assessment of the Pharmacokinetics, Disposition, and Duration of Action of the Tumour-Targeting Peptide CEND-1. International Journal of Molecular Sciences. 2023; 24(6): 5700.

[125] Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. Gastroenterology Review/Przegląd Gastroenterologiczny. 2019; 14(1): 26-38.

[126] Zhou C, Liu Q, Meng F, Ding N, Yan J, Liu B. Modification of erythrocytes by internalizing Arg-Gly-Asp (iRGD) in boosting the curative effect of radiotherapy for gastric carcinoma. Journal of gastrointestinal oncology. 2022; 13(5): 2249.

[127] Zhang D, Chu Y, Qian H, Qian L, Shao J, Xu Q, *et al.* Antitumor Activity of Thermosensitive Hydrogels Packaging Gambogic Acid Nanoparticles and Tumor-Penetrating Peptide iRGD Against Gastric Cancer. IJN. 2020 Jan; Volume 15: 735-47.

[128] Ding N, Zou Z, Sha H, Su S, Qian H, Meng F, *et al.* iRGD synergizes with PD-1 knockout immunotherapy by enhancing lymphocyte infiltration in gastric cancer. Nature Communications. 2019; 10(1): 1336.

[129] Zhu A, Sha H, Su S, Chen F, Wei J, Meng F, *et al.* Bispecific tumor-penetrating protein anti-EGFR-iRGD efficiently enhances the infiltration of lymphocytes in gastric cancer. American journal of cancer research. 2018; 8(1): 91.

[130] Ji F, Sha H, Meng F, Zhu A, Ding N, Zhang H, *et al.* Tumor-penetrating peptide fused EGFR single-domain antibody enhances radiation responses

following EGFR inhibition in gastric cancer. Oncology reports. 2018; 40(3): 1583-91.

[131] Chen H, Sha H, Zhang L, Qian H, Chen F, Ding N, *et al.* Lipid insertion enables targeted functionalization of paclitaxel-loaded erythrocyte membrane nanosystem by tumor-penetrating bispecific recombinant protein. IJN. 2018 Sep; Volume 13: 5347-59.

[132] Ai S, Zhen S, Liu Z, Sun F, He X, Chu F, *et al.* An iRGD peptide conjugated heparin nanocarrier for gastric cancer therapy. RSC advances. 2018; 8(52): 30012-20.

[133] Huang Y, Li X, Sha H, Zhang L, Bian X, Han X, *et al.* sTRAIL-iRGD is a promising therapeutic agent for gastric cancer treatment. Scientific Reports. 2017; 7(1): 579.

[134] Huang Y, Li X, Sha H, Zhang L, Bian X, Han X, *et al.* Tumor-penetrating peptide fused to a pro-apoptotic peptide facilitates effective gastric cancer therapy. Oncology Reports. 2017 Mar; 37(4): 2063-70.

[135] Xin X, Sha H, Shen J, Zhang B, Zhu B, Liu B. Coupling Gd-DTPA with a bispecific, recombinant protein anti-EGFR-iRGD complex improves tumor targeting in MRI. Oncology reports. 2016; 35(6): 3227-35.

[136] Sha H, Li R, Bian X, Liu Q, Xie C, Xin X, *et al.* A tumor-penetrating recombinant protein anti-EGFR-iRGD enhance efficacy of paclitaxel in 3D multicellular spheroids and gastric cancer in vivo. European Journal of Pharmaceutical Sciences. 2015; 77: 60-72.

[137] Sha H, Zou Z, Xin K, Bian X, Cai X, Lu W, *et al.* Tumor-penetrating peptide fused EGFR single-domain antibody enhances cancer drug penetration into 3D multicellular spheroids and facilitates effective gastric cancer therapy. Journal of Controlled Release. 2015; 200: 188-200.

[138] Yan F, Wu H, Liu H, Deng Z, Liu H, Duan W, *et al.* Molecular imaging-guided photothermal/photodynamic therapy against tumor by iRGD-modified indocyanine green nanoparticles. Journal of Controlled Release. 2016; 224: 217-28.

[139] Wang CF, Sarparanta MP, Mäkilä EM, Hyvönen ML, Laakkonen PM, Salonen JJ, *et al.* Multifunctional porous silicon nanoparticles for cancer theranostics.

Biomaterials. 2015; 48: 108-18.

[140] Cho HJ, Park SJ, Lee YS, Kim S. Theranostic iRGD peptide containing cisplatin prodrug: Dual-cargo tumor penetration for improved imaging and therapy. Journal of Controlled Release. 2019; 300: 73-80.

[141] Sheng Y, Wang Z, Neubi GMN, Cheng H, Zhang C, Zhang H, *et al.* Lipoprotein-inspired penetrating nanoparticles for deep tumor-targeted shuttling of indocyanine green and enhanced photo-theranostics. Biomaterials science. 2019; 7(8): 3425-37.

[142] Wu D, Zhao Z, Wang N, Zhang X, Yan H, Chen X, *et al.* Fluorescence imaging-guided multifunctional liposomes for tumor-specific phototherapy for laryngeal carcinoma. Biomaterials Science. 2020; 8(12): 3443-53.

[143] Sun Z, Wang Z, Wang T, Wang J, Zhang H, Li Z, *et al.* Biodegradable MnO-Based Nanoparticles with Engineering Surface for Tumor Therapy: Simultaneous Fenton-Like Ion Delivery and Immune Activation. ACS Nano. 2022 Aug 23; 16(8): 11862-75.

[144] Zhu L, Wang J, Tang X, Zhang C, Wang P, Wu L, *et al.* Efficient Magnetic Nanocatalyst-Induced Chemo- and Ferroptosis Synergistic Cancer Therapy in Combination with T_1-T_2 Dual-Mode Magnetic Resonance Imaging Through Doxorubicin Delivery. ACS Appl Mater Interfaces. 2022 Jan 26; 14(3): 3621-32.

[145] Ruoslahti E. Peptides as Targeting Elements and Tissue Penetration Devices for Nanoparticles. Advanced Materials. 2012 Jul 24; 24(28): 3747-56.

[146] Kim SW, Lee YK, Kim SH, Park JY, Lee DU, Choi J, *et al.* Covalent, non-covalent, encapsulated nanodrug regulate the fate of intra-and extracellular trafficking: impact on cancer and normal cells. Scientific Reports. 2017; 7(1): 6454.

[147] Chenthamara D, Subramaniam S, Ramakrishnan SG, Krishnaswamy S, Essa MM, Lin FH, *et al.* Therapeutic efficacy of nanoparticles and routes of administration. Biomater Res. 2019 Dec; 23(1): 20.

[148] Li Z, Zhu Y, Zeng H, Wang C, Xu C, Wang Q, *et al.* Mechano-boosting nanomedicine antitumour efficacy by blocking the reticuloendothelial system with stiff nanogels. Nature Communications. 2023; 14(1): 1437.

[149] Liu X, Jiang J, Ji Y, Lu J, Chan R, Meng H. Targeted drug delivery using iRGD

peptide for solid cancer treatment. Molecular systems design & engineering. 2017; 2(4): 370-9.

[150] Kariya Y, Oyama M, Suzuki T, Kariya Y. αvβ3 Integrin induces partial EMT independent of TGF-β signaling. Communications biology. 2021; 4(1): 490.

[151] Max R, Gerritsen RRCM, Nooijen PTGA, Goodman SL, Sutter A, Keilholz U, *et al.* Immunohistochemical analysis of integrin αvβ3 expression on tumor-associated vessels of human carcinomas. Intl Journal of Cancer. 1997 May 2; 71(3): 320-4.

[152] Mak IW, Evaniew N, Ghert M. Lost in translation: animal models and clinical trials in cancer treatment. American journal of translational research. 2014; 6(2): 114.

[153] Dewhirst MW, Secomb TW. Transport of drugs from blood vessels to tumour tissue. Nature Reviews Cancer. 2017; 17(12): 738-50.

[154] Sugahara KN, Braun GB, de Mendoza TH, Kotamraju VR, French RP, Lowy AM, *et al.* Tumor-penetrating iRGD peptide inhibits metastasis. Molecular cancer therapeutics. 2015; 14(1): 120-8.

[155] D'Amore VM, Donati G, Lenci E, Ludwig BS, Kossatz S, Baiula M, *et al.* Molecular View on the *i* RGD Peptide Binding Mechanism: Implications for Integrin Activity and Selectivity Profiles. J Chem Inf Model. 2023 Oct 23; 63(20): 6302-15.

[156] Qian J, Zhou S, Lin P, Lei J, Zheng S, Xu W, *et al.* Recent advances in the tumor-penetrating peptide internalizing RGD for cancer treatment and diagnosis. Drug Development Research. 2023 Jun; 84(4): 654-70.

[157] Chen B, Liu X, Li Y, Shan T, Bai L, Li C, *et al.* iRGD Tumor-Penetrating Peptide-Modified Nano-Delivery System Based on a Marine Sulfated Polysaccharide for Enhanced Anti-Tumor Efficiency Against Breast Cancer. IJN. 2022 Feb; Volume 17: 617-33.

[158] Kang S, Lee S, Park S. iRGD peptide as a tumor-penetrating enhancer for tumor-targeted drug delivery. Polymers. 2020; 12(9): 1906.

[159] Javid H, Oryani MA, Rezagholinejad N, Esparham A, Tajaldini M, Karimi-Shahri M. RGD peptide in cancer targeting: Benefits, challenges, solutions, and possible integrin-RGD interactions. Cancer Medicine. 2024 Jan; 13(2):

e6800.

[160] NCT05515783, C.g.I. U.S National Library of Medicine.... Google Scholar [Internet]. [cited 2024 Mar 21]. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=NCT05515783%2C+C.g.I.+U.S+National+Library+of+Medicine.+Clinical+Trails.gov+2023+September+21%2C+2023+%5BFirst+posted%3A+August+25%2C+2022%5D%3B+Available+from%3A+https%3A%2F%2Fclassic.clinicaltrials.gov%2Fct2%2Fshow%2FNCT05515783&btnG=

[161] Schultz É, Mignot L, Ward JK, Bomfim DB, Chabannon C, Mancini J. Public perceptions of the association between drug effectiveness and drug novelty in France during the COVID-19 pandemic. Therapies. 2022; 77(6): 693-701.

[162] Eyal G. The crisis of expertise [Internet]. John Wiley & Sons; 2019 [cited 2024 Mar 21]. Available from: https://books.google.com/books?hl=en&lr=&id=Wwu5DwAAQBAJ&oi=fnd&pg=PP2&dq=Eyal+G.+Polity+Press%3B+Cambridge,+United+Kingdom:+2019.+The+crisis+of+expertise%3B+p.+208.+%5BISBN:+978-0-745-66577-1%5D+&ots=ge_OyjNeQZ&sig=CDbT3ZbNNOWZv4EjmjZqNVIfw0c

[163] Colgan S, Faasse K, Martin LR, Stephens MH, Grey A, Petrie KJ. Perceptions of generic medication in the general population, doctors and pharmacists: a systematic review. BMJ open. 2015; 5(12): e008915.

[164] Jie Y. Older is better: Consumers prefer older drugs. Psychology and Marketing. 2020 Nov; 37(11): 1498-510.

[165] Russo S, Jongerius C, Faccio F, Pizzoli SF, Pinto CA, Veldwijk J, *et al.* Understanding patients' preferences: a systematic review of psychological instruments used in patients' preference and decision studies. Value in Health. 2019; 22(4): 491-501.

Chapter 7

Evolving Landscape on Sex Specific Status on Lung Cancer Management: Moderating Effects, Risk Assessment

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Abstract: The literature hasn't given enough consideration to the connection between sex and the onset, prognosis, and course of treatment of lung cancer. Research on diseases associated with tobacco use has predominated, even though lung cancer that is not tied to smoking is on the rise and disproportionately affects women. However, depending on a patient's sex, systemic therapy can have different effects and results, particularly when immunotherapy and chemotherapy are used. Further research is needed to fully comprehend these variances and address the rapidly changing lung cancer demographics worldwide. These results also highlight the growing awareness of the need for individualized therapies that account for variations in response and outcome related to sex.

Keywords: Sex, quality of life, lung cancer, risk, targeted approach

7.1. Introduction

The sex-specific aspects of lung cancer treatment represent a vibrant and exciting

area of study that integrates medical science, social science, and biology. Sex holds a significant role in the onset, course, and prognosis of lung cancer, as the subtleties of the illness become apparent [1]. In contrast to the "one size fits all" method, sex-specific methods of lung cancer care take into account the various ways that the illness can manifest itself in males and females [2]. By examining moderating effects, we investigate the variables influencing the many behaviors associated with lung cancer in both men and women, opening up new avenues for targeted and effective treatment [3]. Risk assessment, a cornerstone of healthcare, takes an even greater importance as we explore how sex-related characteristics impact vulnerability, therapeutic response, and overall prognosis. Understanding these nuances will result in more individualized and fair healthcare practices, in addition to being a scientific endeavor [4]. As we embark on this journey, the potential to apply sex-specific insights to transform the way we treat lung cancer holds the potential to fundamentally alter our methods and pave the way for a more sophisticated and effective approach to combating this aggressive disease [5].

7.2. Gender and Sex Differences

It is through comprehending the nuances of sex differences in lung cancer that we can make sense of this complex environment [6]. These differences manifest themselves in several ways when it comes to lung cancer. Genetic polymorphisms and physiological variations in hormone levels can cause variations in treatment responses and risk profiles [7]. For example, a woman's estrogen receptors may influence the course of her lung cancer and the degree to which it reacts to specific therapies [8]. Socially constructed gender roles can also have an effect.

Social standards based on gender regularly influence habits including smoking, seeking medical care, and working in particular settings. The kinds of lung cancer that are discovered and the stages at which they manifest may vary because of these variations. Understanding the differences between gender and sex is crucial for early detection systems, prevention programs, and treatment regimen customization [9]. In addition to acknowledging these distinctions, we also need to apply this knowledge to ensure that medical processes are more efficacious, inclusive, and attentive to the unique needs of every patient, irrespective of their gender. The first step in delivering individualized and fair care as we navigate the evolving field of lung cancer medicines is acknowledging and addressing these disparities.

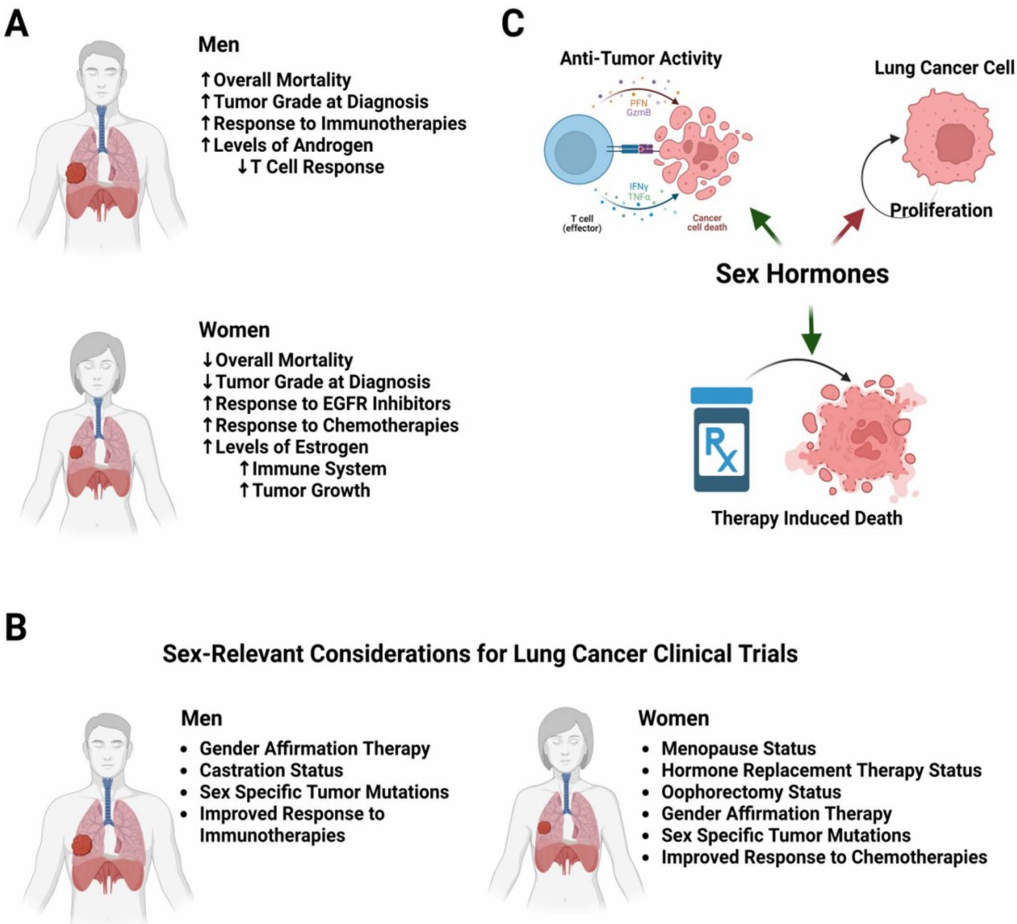

Figure 1. Sex differences in lung cancer growth and treatment [10] (This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

7.3. Sex-Dependent Drug Susceptibility in LC

The intricate connection between sex and drug sensitivity in lung cancer proposes a promising new avenue for the development of more effective treatments [11]. For instance, some targeted medications' efficacy and potential side effects may be influenced by how they interact with the hormone receptors in men and women [12].

In lung cancer research, the hormone receptors in question primarily refer to estrogen receptors (ER), androgen receptors (AR), and progesterone receptors

(PR). Estrogen and androgen receptors, in particular, have garnered significant attention because lung cancer cells often express these receptors, and their activation or suppression can influence tumor progression and drug response differently in men and women. Estrogen is known to play a role in lung cancer biology. Research has shown that estrogen can stimulate the growth of certain lung cancer cells, especially in women, by binding to ERs. This can affect the efficacy of anticancer treatments, making drugs that target the estrogen pathway potentially more effective in female patients. Androgens, or male hormones, also interact with lung cancer cells, particularly in men. Variations in AR expression may influence how male patients respond to certain therapies, especially those targeting pathways influenced by androgens. Though less extensively studied in lung cancer than ERs and ARs, PRs may also contribute to lung cancer progression and impact drug response in a sex-specific way. Sex variations in drug metabolism can also impact the rate at which a drug is metabolized and excreted from the body [13]. The fact that this strategy acknowledges that a patient's sex may change a drug's effectiveness goes beyond a one-size-fits-all approach [14]. As we navigate this terrain, our goals are to enhance treatment outcomes and elevate the caliber of care for patients with lung cancer [15].

7.4. Variations in the Descriptive Epidemiology of Lung Cancer by Sex

There are sex alterations in lung cancer that go beyond statistical variations when the descriptive epidemiology of the disease is studied [16]. The differences between male and female rates of lung cancer are impressive and thought-provoking as are the total statistics [17]. Firstly, there is a blatant sex bias in lung cancer incidence with men historically having higher rates. Although smoking patterns vary between the sexes, this tendency is shifting, with some differences narrowing. [18]. Furthermore, the various histological patterns of lung cancer in men and women add to the complexity of the epidemiological mosaic [19]. The knowledge of sex disparities in descriptive epidemiology improves our comprehension of the illness and has important implications for early detection, prevention, and treatment efforts. It emphasizes the need for sex-sensitive lung cancer management techniques, recognizing that better outcomes require a deeper understanding of the unique challenges and characteristics that each sex presents.

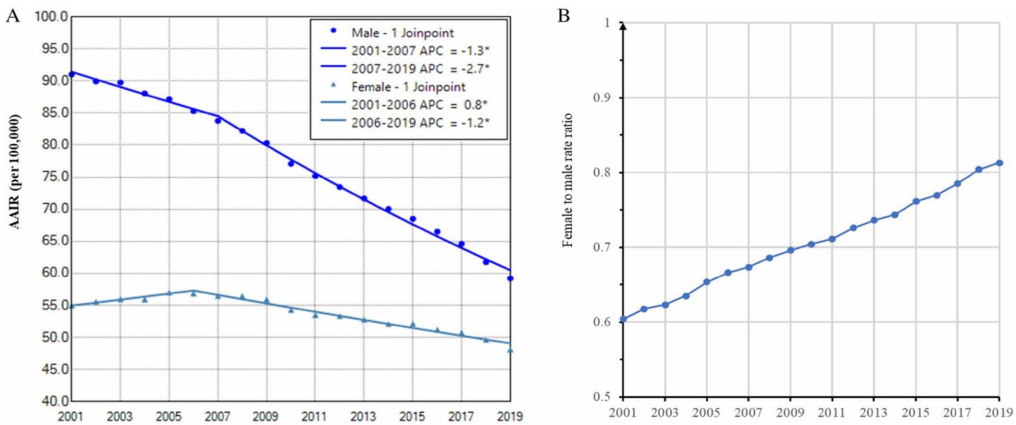

Figure 2. Temporal change of lung cancer incidence by sex during 2001-2019. (**A**). Change in AAIR by sex. (**B**). Female to male rate ratios of lung cancer incidence rate. AAIR: age adjusted incidence rate; APC: annual percentage change. *Indicates a significant change in APC [20]. (This article is licensed under a Creative Commons Attribution 4.0 International License, http://creativecommons.org/licenses/by/4.0/).

7.4.1. Mortality/Incidence

The correlation between lung cancer incidence and death shows a clear picture of the challenges and complexities associated with the disease's epidemiology [21]. Lung cancer is prevalent in males as opposed to women based on historical trends, and higher smoking rates are typically associated with this finding [22]. However, there has been a noticeable shift in this area, with a higher incidence of lung cancer among women nowadays [23].

Historically, lung cancer was predominantly a male disease, largely due to higher smoking rates among men. However, as smoking rates among women have increased, particularly in the late 20th century, the incidence of lung cancer in women has also risen significantly. While men still have higher overall lung cancer rates, the gap is narrowing. For instance, from 1975 to 1998, lung cancer incidence among women increased by 133%, while it decreased by 42% among men since their peak in the mid-1980s. This shift indicates a changing landscape where women's smoking habits are catching up with those of men [24].

It is important to have a deeper comprehension of risk variables that go beyond traditional smoking habits, as women who have never smoked may still be at high risk for lung cancer. This entails improving early detection methods, focusing on

high-risk individuals, and creating more personalized and effective treatment modalities. A more comprehensive and successful lung cancer treatment approach is guided by the intricate link between incidence and death [25].

7.4.2. Histologic Types

Lung cancer's histologic subtypes contribute to the disease's intricate terrain. The wide range of histological characteristics affects prognosis and treatment strategies in addition to adding to the illness's heterogeneity [26]. Owing to its diverse molecular properties, customized medications are currently accessible, offering a more targeted therapeutic approach [27]. Squamous cell carcinoma, which commonly starts in the central airways and may react favorably to certain treatment modalities, is often associated with smoking [28].

In the context of lung cancer treatment, "treatment modalities" refers to the various therapeutic approaches or strategies employed to manage the disease. This encompasses a range of interventions that can include chemotherapy agents, targeted therapies, and immunotherapies specifically designed for different histologic subtypes of lung cancer. Procedures aimed at removing tumors or affected lung tissue. The use of high-energy radiation to kill cancer cells or shrink tumors. Utilizing a mix of the above methods to enhance treatment efficacy based on the tumor's characteristics and patient-specific factors. The term emphasizes a comprehensive approach to treatment, taking into account the unique histological subtype of lung cancer and its molecular properties, which can significantly affect prognosis and response to therapy. Thus, treatment modalities are not limited to just one type of intervention but represent a spectrum of options tailored to individual patient needs and tumor biology [29]. Conversely, SCLC is less common yet well-known for its aggressiveness and early metastasis tendency. Treatment for this neuroendocrine subtype must be tailored and often involves chemotherapy and radiation. The development of customized immunotherapies and improvements in diagnostic methods are guided by the expanding understanding of lung cancer histologic types. It highlights how important it is to treat each patient uniquely, considering their unique histologic subtypes and designing suitable treatment plans. Notable differences in genetic mutations and alterations between male and female patients with SCLC. For example, males are often found to have higher rates of mutations in the TP53 gene, which is critical for tumor

suppression, while females may exhibit different profiles of mutations that could influence treatment responses. Estrogen has been implicated in the development and progression of lung cancer, particularly in women. Studies suggest that hormonal factors may contribute to the differences in tumor biology and aggressiveness between sexes, potentially leading to variations in how SCLC manifests and responds to therapies. There is evidence that sex-based differences can affect the efficacy of certain treatments for SCLC. For instance, women may respond differently to chemotherapy regimens compared to men, possibly due to variations in metabolism and drug clearance rates influenced by hormonal differences. Some studies have shown that female patients with SCLC may have better overall survival rates compared to their male counterparts, despite similar stages of disease at diagnosis. This could be linked to biological differences in tumor behavior or responses to treatment. By integrating these points into their analysis, the authors would not only clarify the complexities associated with lung cancer treatment but also emphasize the importance of personalized medicine approaches that consider sex as a significant factor in treatment planning. This specificity would help readers grasp the nuances of lung cancer epidemiology and its implications for clinical practice, thereby fostering a more comprehensive understanding of the disease's impact across different populations. The goal is to comprehend the molecular and genetic underpinnings of the various histologic classifications to create more effective and personalized treatment plans for lung cancer [30].

7.4.3. Survival Rate

Survival rates from lung cancer are important indicators of the challenges posed by the illness and the progress made in treating it. Because lung cancer patients typically have higher survival rates when the disease is discovered in its early stages, early identification is essential [32]. Since the introduction of immunotherapies and specialist medications, survival rates have increased, especially for patient subgroups with certain genetic defects. The survival rates have gradually increased over time. This rise could be explained by advancements in early diagnosis methods, medications, and the growing emphasis on individualized treatment. A comprehensive approach including state-of-the-art immunotherapies, radiation therapy, chemotherapy, and surgery is needed to increase survival rates [33].

7.5. Sex-Involved Factors

Sex-related factors significantly influence the complex landscape of lung cancer, affecting both susceptibility to the disease and treatment outcomes. These factors can be categorized into hormonal, behavioral, and biological elements, each contributing uniquely to the lung cancer experiences of men and women. Estrogens may play a crucial role in lung cancer development, particularly in women. Hormonal fluctuations, especially those occurring during menopause, can influence lung cancer incidence and progression [34]. Research suggests that postmenopausal hormonal changes may be linked to increased lung cancer risk due to altered cellular responses to carcinogens found in tobacco smoke. Studies have indicated that women undergoing HRT may experience an elevated risk of developing lung cancer. The interaction between exogenous hormones and tobacco carcinogens could exacerbate this risk, particularly among younger women or those with a history of smoking. Historically, smoking has been the primary cause of lung cancer, but trends in smoking prevalence differ by sex [35]. While men have traditionally had higher smoking rates, recent years have seen an increase in smoking among women, particularly in the younger population. This shift is crucial as it directly correlates with rising lung cancer incidence in women, including those who are nonsmokers [36]. Behavioral differences in healthcare-seeking patterns between men and women can impact early detection and treatment outcomes. Women are often diagnosed at younger ages and may present with different histological types of lung cancer, such as adenocarcinoma, compared to men. Genetic variations can influence how men and women metabolize carcinogens from tobacco smoke. For instance, polymorphisms in genes related to the cytochrome P450 family can affect the activation or detoxification of tobacco-related carcinogens differently in men and women. Women may exhibit higher levels of DNA adducts from tobacco exposure than men, indicating a greater susceptibility to lung cancer despite lower smoking rates. There is evidence suggesting that the immune response to lung cancer differs between sexes. Women may have a more robust immune response due to higher levels of certain immune cells in the tumor microenvironment, which could contribute to better survival rates despite similar disease stages. Understanding these immunological differences is critical for developing tailored immunotherapies that consider sex-based variations [37]. The way individuals respond to treatment can vary significantly between the sexes due to several factors, such as the effect of hormones on drug metabolism and efficacy. For

example, estrogen may affect how certain chemotherapy agents are processed in the body, potentially leading to different outcomes between male and female patients. Genetic differences can also dictate how patients metabolize medications. Variants in drug-metabolizing enzymes may lead to variations in drug efficacy and toxicity between sexes. Women often metabolize drugs differently than men due to physiological differences such as body composition and hormonal levels. This can lead to variations in treatment responses and side effects. In summary, understanding the interplay of hormonal, behavioral, and biological factors is essential for comprehensively addressing lung cancer's impact on different sexes. This knowledge is crucial for developing targeted prevention strategies and personalized treatment plans that consider the unique experiences of men and women affected by lung cancer [38].

7.5.1. Tobacco-Related Carcinogens Metabolism in Both Sexes

The metabolism of carcinogens associated with tobacco smoking may be an interesting factor in explaining the variations in lung cancer dynamics between sexes. There are noticeable differences in how men's and women's bodies respond to tobacco smoking, even though men and women metabolized these toxins differently [39] [40]. These differences could impact the body's capacity to either activate or detoxify carcinogens [41]. Comprehending the disparities in tobacco-related carcinogen metabolism between sexes is crucial in customizing interventions and preventive measures. It emphasizes how crucial it is to take behavioural and biological aspects into account when assessing the risk of lung cancer brought on by tobacco use.

The cytochrome P450 family of enzymes, particularly CYP1A1, CYP1A2, and CYP3A4, play a crucial role in the metabolism of tobacco carcinogens. Variations in these enzymes can lead to differences in how effectively individuals detoxify harmful substances found in tobacco smoke. CYP1A1 is associated with the activation of procarcinogens found in tobacco, leading to the formation of DNA adducts that can initiate carcinogenesis. Women have been found to have polymorphisms in CYP1A1 that increase their risk for lung cancer compared to men, especially among smokers. CYP2A6 is another enzyme that metabolizes nicotine. Research indicates that women generally exhibit higher activity levels of CYP2A6, which may lead to more efficient nicotine clearance but could also result in

increased exposure to harmful metabolites. These enzymes are involved in the detoxification of reactive metabolites. Variants such as GSTM1 and GSTT1 can influence an individual's ability to detoxify carcinogens from tobacco smoke. Studies suggest that women with certain GST polymorphisms may have a higher risk of lung cancer due to reduced detoxification capacity. Genetic differences affecting DNA repair mechanisms also play a role. For example, mutations in the p53 tumor suppressor gene are more frequent in women with lung cancer, which may impair their ability to repair DNA damage caused by tobacco carcinogens. Metabolizing tobacco carcinogens differently can inform tailored smoking cessation programs and preventive measures. For instance, interventions targeting specific metabolic pathways might be more effective for one sex over the other. By comprehending how variations in these enzymes affect lung cancer susceptibility, healthcare providers can better assess individual risk profiles based on genetic testing. Insights into sex-specific differences in enzyme activity could also guide treatment decisions, particularly when considering therapies that might interact with these metabolic pathways. In summary, discussing the specific enzymes involved in tobacco carcinogen metabolism such as cytochrome P450s and GSTs—along with their implications for lung cancer risk and treatment strategies would provide a clearer understanding of the biological underpinnings behind sex differences in lung cancer dynamics. This approach emphasizes the importance of integrating behavioral and biological factors when evaluating lung cancer risk associated with tobacco use, ultimately contributing to more effective prevention and cessation programs [42].

7.5.2. Involvement of Genetic Factors

Lung cancer is largely shaped by genetics. These characteristics also affect treatment outcomes and elements like susceptibility and progression [43]. Lung cancer is a multifactorial genetic disorder that involves both acquired abnormalities and inherited. Less common family predispositions and certain genetic illnesses, such Li-Fraumeni syndrome and hereditary non-polyposis colorectal cancer, have been linked to an elevated hazard cancer of lung [44]. Genetic variations within an individual can impact on how they metabolized and respond to cancer treatments. Pharmacogenetic factors affect how pharmaceuticals are metabolized and can affect how well targeted medications and chemotherapy work as well as their

negative effects [45]. Genetic factors found in the tumor microenvironment and changes within cancer cells are the reasons behind the progression of lung cancer. Genes associated with immunity, for instance, may influence the body's response to immunotherapies. The amount of information regarding the genetic complexity of lung cancer is always growing, which aids in the development of specialist treatment regimens and pharmaceuticals [46].

7.5.3. Hormonal Factors in Both Sexes

Lung the tumor's complicated interactions among men and women, are significantly influenced by hormonal factors. These elements influence the risk and response to therapy. Estrogen receptors are present in the lung tissue of women. The existence of estrogen receptors can change the rate of development and susceptibility of lung malignancies to treatment. Both estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) are expressed in lung tissues, with ERβ being predominantly found in lung cancer cells. The presence of these receptors allows estrogen to exert its effects on lung cancer cells, influencing their growth and behavior. Research indicates that ERβ is overexpressed in 60-80% of lung cancer tissues, particularly in adenocarcinomas, which is the most common subtype of NSCLC. Estrogen can promote the proliferation of lung cancer cells through both genomic and non-genomic pathways. When estrogen binds to its receptors, it activates signaling pathways that lead to increased cell division and survival. For instance, estrogen has been shown to enhance the secretion of hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), which are involved in tumor growth and metastasis. Estrogen signaling interacts with other critical pathways, such as the epidermal growth factor receptor (EGFR) pathway. This interaction can exacerbate tumor progression, as both ERs and EGFR are involved in regulating cell growth and survival. Studies have suggested that combining ER antagonists with EGFR inhibitors may provide therapeutic benefits by targeting both pathways simultaneously. Estrogen can affect the immune microenvironment within tumors, potentially influencing how the body responds to cancer. There is evidence that hormonal factors may alter immune cell populations and their activity, which can impact tumor progression and response to therapies. The risk of developing lung cancer may also be influenced by hormonal changes associated with menopause. Postmenopausal women experience a shift in estrogen

production that can affect lung tissue characteristics and susceptibility to carcinogenesis. Studies have indicated that hormone replacement therapy (HRT) might increase the risk of lung cancer in women due to its effects on estrogen levels [35].

Hormonal factors, mainly estrogen, have been connected to the beginning and spread of non-small cell lung cancer, the most prevalent variety of the condition. Estrogen receptor expression in malignancies may have an impact on sex-specific variances in NSCLC cancer incidence and prognosis. Studies have assessed the probable impact of menopausal HRT on a woman's risk of lung cancer [47]. Lung cancer risk has been linked to HRT, more especially to estrogen with progestin. This shows how hormonal factors, and the expansion of lung cancer are intricately linked. One's chance of developing lung cancer can be increased by smoking habits along with hormonal changes that occur at various life stages, such as women going through menopause [48]. Understanding these relationships is essential for a thorough assessment of the risks particular to sex. While most research focuses on estrogen, lung tissue also has androgen receptors, which are linked to hormones associated with men such as testosterone [49].

7.5.4. Viral Factors

Viral origins further complicate the already complex picture of lung cancer, as specific viruses have been linked to the start of the condition. Viruses linked to lung cancer include HPV, HTLV-1, and possibly EBV. HPV is widely recognized for its association with cervical cancer [50]. Studies have revealed HPV DNA in lung cancers, suggesting a potential connection between HPV and the emergence of certain lung cancers [51]. Research on the connection between HPV and lung cancer is underway. Lung cancer may appear and spread more quickly if the virus promotes genetic instability and immune evasion. Certain types of lymphomas have been associated with a retrovirus known as HTLV-1. Although the relationship between HTLV-1 infection and the development of lung cancer has not been as well studied as it has with other viruses and lung cancer [52].

7.5.5. Immune Factors

Lung cancer and the immune system have a complex interaction that includes immunological elements [53]. Through a variety of complex mechanisms, the immune system and cancer interact dynamically to either promote or inhibit the

growth of tumors. The immune system possesses the means to identify abnormal cells and eliminate them, including those that have the potential to develop into cancer [54]. For instance, the body's fight against cancer cells depends on the lymphocytes that infiltrate tumors. Lung cancer cells that function as immunological checkpoints could leverage the immune system's homeostasis, which is maintained by these regulating substances, to evade detection and killing. Immune checkpoint inhibitors are a novel therapeutic approach for lung cancer because they disrupt these inhibitory signals and strengthen the immune system's capacity to combat malignancies. In particular, checkpoint inhibitors have proven very effective in treating a subpopulation of lung cancer patients [55]. Understanding these immune escape mechanisms is vital to designing customized medicines that can overcome resistance. Advances in recognizing the immunological landscape of lung cancer have facilitated tailored immunotherapy. Treatment outcomes can be enhanced by detecting unique immune signatures present in malignancies by tailoring immunotherapy approaches to each patient. Research on the complicated link between immunological variables and lung cancer is being done as this field of study grows fast. Using the immune system to target lung cancer is a prospective approach for the creation of more effective and concentrated treatment alternatives [56].

7.5.6. Healthy Dietary Habits

Maintaining good eating habits is crucial for general wellbeing and minimizes the incidence of several ailments, including lung cancer. Antioxidant-rich foods enhance the defense against oxidative stress, which has been connected to the initiation of cancer. Eat a variety of fruits and vegetables as they are strong in antioxidants. Berries, vibrant vegetables, and leafy greens are all fantastic possibilities [57]. Brussels sprouts, kale, cauliflower, and broccoli are cruciferous vegetables that are rich in compounds that may have anti-cancer benefits [58]. Brown rice, quinoa, and whole wheat are a few of these. Because processed meals are heavy in chemicals, preservatives, and bad fats, they may raise your chance of contracting cancer and other illnesses. Eat a diet rich in whole, minimally processed foods as your main focus. It's crucial to remain adequately hydrated for general wellness. Digestion and the removal of toxins are two biological processes that are helped by water. Even if there is a correlation between consuming excessive amounts of

red and processed meats and various diseases, including lung cancer, moderation is still vital. Consider lean protein options include poultry, fish, and plant-based alternatives [59]. Reducing or giving up alcohol is a smart decision. Sustaining appropriate serving sizes can aid with weight management, which is essential for lowering the risk of cancer and improving general health. To maximize health advantages, treating food selections as part of a holistic lifestyle that also includes regular exercise and stopping smoking is essential. Additionally, seeking advice from dietitians or other medical specialists may result in recommendations that are particularly tailored to each person's requirements and concerns [60].

7.5.7. Daily Life Exercise

In addition to improving physical health, regular exercise also has positive effects on emotional and mental well-being [61]. Strive for a well-rounded program that incorporates strength, flexibility, and aerobic exercises. The small daily actions you take have a big influence on your total level of activity. Taking part in fun pursuits like hiking [62], sports, dancing, or other activities may help you sustain a healthy lifestyle over time. If a large portion of your work is spent sitting down, consider desk exercises, stretches, and quick breaks to get up and move about. Exercise, even little quantities of it, can improve overall health and lung health. Remember that you need to be consistent. To be sure that the exercise regimen you've chosen is appropriate for your particular requirements and circumstances, get medical advice. This is particularly crucial if you already have health issues [63].

7.5.8. Occupational and Residential Factors

Our comprehension of lung cancer is further hampered by the illness's heterogeneous nature and the complex interaction between residential and occupational variables [64]. Workers routinely come into contact with materials including asbestos, radon, silica, and industrial chemicals in industries like construction, mining, manufacturing, and healthcare. Strict safety laws and the availability of protective gear in these environments are necessary for reducing exposure [65]. Radon is one of the most important factors in homes and a major source of danger. This naturally occurring radioactive gas can seep into structures, especially those constructed on uranium-rich soil [66]. To reduce this danger, regular testing and mitigation measures are crucial. Although occupational settings are often linked

to asbestos exposure, houses, particularly older ones with materials that historically contained asbestos, can also be affected. Lung cancer is known to be exacerbated by secondhand smoking, which may enter both homes and workplaces. Inadequate ventilation and exposure to pollutants, whether from indoor or industrial sources, can lead to respiratory problems and an elevated risk of lung cancer. Worker safety regulations must be properly maintained since diesel exhaust contains carcinogens and is commonly encountered in industries like mining and transportation [67]. Variations in geography and residential status also impact lung cancer risk. Risks associated with proximity to industrial regions and environmental pollution might be increased, underscoring the necessity of public health campaigns and legislative actions to provide cleaner air and improved living circumstances. A complete approach to tackling occupational and residential challenges includes workplace safety standards, legislation that supports improved living conditions, public awareness campaigns, and routine air quality monitoring. By taking proactive steps and giving careful thought to these variables, settings that promote lung health and help lower the incidence of lung cancer may be established [68].

7.5.9. Psychosocial Factors

Developing strong coping mechanisms is essential to handle effectively these difficulties. Two examples of this include reaching out for social support and participating in therapeutic activities. One important psychological component that comes to light is social support networks. The stigma associated with lung cancer, which is frequently connected to smoking, can cause psychological problems [69]. To reduce stigma and create conditions that are supportive for people who are impacted, it is imperative to clarify misunderstandings and spread awareness throughout communities. Important psychological elements include cooperative decision-making and communication between patients, families, and healthcare professionals. If people are given knowledge and the chance to make decisions, their sense of power and psychological suffering could be diminished. Lung cancer patients may also have psychological difficulties due to financial burden. When it comes to addressing the financial aspects of psychological well-being, campaigning for cheap healthcare, insurance support, and access to financial resources are crucial. In addition to medical therapy, practical, social, and emotional support networks are necessary to enhance the lung cancer patient quality of life. A more

comprehensive and patient-centered approach to lung cancer care is offered by medical professionals and support networks that recognize and address these psychological aspects [70].

7.6. Consequences of Sex Differences

Differences in sex have an influence on monitoring, therapeutic comeback, and outcome.

7.6.1. On Screening

The death rate decrease for females was twenty-six percent in the NLST (National lung screening trial) experiment and sixty-one percent in the NELSON (Nederlands-leuvens longkanker screenings onderzoek (Dutch-belgian lung cancer screening trial [71], despite the fact that women were removed from the core target population, which resulted in an inadequate subgroup evaluation [72]. Women were removed from the German lung cancer screening trial after the initial rounds, potentially skewing results by limiting insights on sex-specific responses to screening and treatment outcomes [72].

The NLST cohort's prolonged follow-up research revealed that women's risk ratio (RR) for lung cancer mortality was lower (RR = 0.86) than that of men (RR = 0.97). In the German lung cancer screening intervention research, Becker *et al.* identified a substantial decline in lung cancer mortality among women (HR = 0.31) but not among men (HR = 0.94). No, the trial results may not be entirely reliable due to inadequate subgroup evaluation, which could obscure sex-specific differences in lung cancer mortality and treatment efficacy. The researchers came to the conclusion that both chance and the histological heterogeneity of cancer in both sexes might account for this odd discrepancy [73]. [74]. Previously, anybody with a smoking history of at least 30 pack years and who was between the ages of 55 and 80 who was still smoking or had quit within the preceding 15 years was eligible to be screened [75].

7.6.2. Regarding Prognosis and Reaction to Therapy

Several studies indicate that there are sex differences in the cytotoxic effects and toxic after-effects of chemotherapy [76]. Wakelee *et al.* evaluated the possible consequence of sex on life expectancy in the Eastern Cooperative Oncology Group E1594 study. Randomly assigned treatment arms based on platinum-based

chemotherapy were given to patients with stage IIIB or IV. Despite similar response rates and higher toxicity, women's median survival rose sharply by 1.9 months relative to men's [77]. Men's and women's differences in anticancer treatment toxicity and susceptibility can also be explained by the impact of sex dimorphism on the microbiota's makeup [78]. The differences in anticancer treatment toxicity and susceptibility between men and women can be explained by the impact of sex dimorphism on the gut microbiota's makeup, which influences immune responses and drug metabolism. Differences in anticancer treatment toxicity and susceptibility between men and women can be attributed to sex dimorphism in microbiota composition, hormonal influences, and pharmacokinetics. Women generally experience greater toxicity due to higher drug exposure and slower drug metabolism, while men may benefit more from certain therapies [79] [80].

7.7. Genetic Epidemiology Research on Sex Variations

Studies on the topic of sex distinctions in lung cancer risk from tobacco use via the lens of genetic epidemiology will be considerably easier by recent advancements in molecular biology [81]. Experts now have a better understanding of the molecular mechanisms underpinning resistance to lung cancer thanks to research on molecular markers of lung cancer spreading in the population and their effect on an individual's chance of acquiring lung cancer [82]-[85].

7.8. Targeted Therapy and Sex

7.8.1. Inhibitors of the Epidermal Growth Factor Receptor

The EGFR has been overemphasized in NSCLC and a few other malignancies [86]. A favorable link was found between females and a better incidence of treatment response in a phase III research comparing erlotinib versus placebo in patients with previously treated non-small cell lung cancer [87]. Additionally, relative to other histological subtypes, erlotinib significantly improved the outcomes in people with adenocarcinoma (14% vs. 4%, $P < 0.001$). Patients with these clinical features are more likely to have activating mutations of EGFR at exons 19 or 21 of the EGFR gene when EGFR inhibitors are given, and these mutations have been associated with higher response rates [88]. The same results occurred in the Iressa survival evaluation in lung cancer (ISEL) investigation, which included 1,692 patients with previously treated NSCLC, where gefitinib was distinguished to be the

best supportive therapy in a phase III trial [86]. Patients were randomized to receive either gefitinib (250 mg/day) or placebo. Notably, a significant portion of participants were female, with subset analyses highlighting improved response rates in women. Although gefitinib did not show statistically significant superiority over chemotherapy in the overall population, it demonstrated a notable PFS at 12 months (25% for gefitinib versus 7% for carboplatin/paclitaxel, $P < 0.001$). Additionally, responders using gefitinib were higher (43%) compared to previous studies. Despite the lack of convincing proof for pharmacological superiority, subset analysis revealed that women receiving gefitinib had greater response rates than those receiving supportive treatment. Out of the 1,217 patients that were enrolled, almost 80% of them were female. Since gefitinib appeared to be more successful than chemotherapy, the study's main goal to show that it was not less effective than carboplatin/paclitaxel was accomplished. Progression-free survival (PFS) at 12 months was 25% for those treated with gefitinib and 7% for those treated with carboplatin/paclitaxel ($P < 0.001$). Furthermore, a greater proportion of responders (43%) used gefitinib in a study compared to previous studies conducted in an unselected sample [86]. The sex disparities in lung cancer made it imperative to look at how hormones affect the tumor. Hormone replacement therapy (HRT) has been associated in the past with worse results for women with lung cancer. A retrospective study including 498 women who received a lung cancer diagnosis at one facility assessed the impact of hormone replacement therapy on results [89]. A documented history of HRT use was present in 36 individuals (17%). The women who had taken HRT and the non-users differed less in age (63 vs 68 years, $P < 0.0001$). The overall survival rate was lower for women who had received hormone replacement therapy (39 vs 79 months, $P < 0.02$). There is a tremendous lot of curiosity on how estrogen affects lung cancer because of these types of clinical data [90] [91].

7.8.2. Chemotherapy with Endothelial Growth Factor

Angiogenesis contributes significantly to the development and metastasis of malignancies. Defective angiogenesis in the context of neoplasia has been demonstrated to need vascular endothelial growth (VEGF) [86]. Studies have shown that inhibition of the VEGF receptor reduces angiogenesis, which has antitumor properties [92]. Bevacizumab is one VEGF antibody that is monoclonal and has been

recommended for the treatment of certain tumors. Patients with advanced non-squamous NSCLC revealed a statistically significant improvement in the median overall survival (OS) when bevacizumab was administered along with both paclitaxel and carboplatin in a phase III study (ECOG 4599) [92]-[99].

7.9. Lung Cancer: Risk Assessment Analysis in Both Sexes

A lot of research has examined the differences in lung cancer risk between males and females, offering insight into the distinct mechanisms that cause the disease to manifest. Hormonal effects, particularly those involving estrogen, may be beneficial for premenopausal women, while postmenopausal women may be more vulnerable due to hormonal swings. Genetic susceptibility genes and predispositions also impact sex-specific risk profiles; specifically, certain genetic variations affect males more than females when it comes to lung cancer susceptibility. Understanding these sex-specific risk factors is crucial to tailoring screening and prevention programs. Targeted therapies to support more effective ways of reducing the incidence and death from lung cancer may be developed by addressing the unique challenges that each sex experiences [102].

7.10. Targeted Therapeutic Techniques of the Future

Lung cancer treatment options are rapidly evolving, with a shift toward more advanced customized therapeutic techniques that allow patients more precise and effective alternatives [103]-[108]. The identification of specific molecular alterations in lung cancer has made targeted medications, providing a realistic route for improved patient outcomes. One area of study that shows promise is the use of tailored medications that focus on genetic abnormalities and alterations. Within subsets of lung cancer patients, genetic anomalies such as EGFR mutations, ROS1 fusions, ALK rearrangements, and others have been discovered. TKIs have revolutionized the treatment of these subtypes, since they have proven to be more successful and less harmful than traditional chemotherapy [109]. This facilitates the prompt adjustment of treatment methods in response to evolving tumor profiles [110]. Future study into the intricate molecular landscape of lung cancer is expected to lead to more customized and sophisticated treatment options. Immunotherapy, tailored medications, and precision medicine together are expected to transform lung cancer treatment and provide patients safer, more effective options [111].

7.11. Conclusion

In conclusion, a significant frontier in the search for more efficient and individualized treatment is the expanding field of sex-specific factors in lung cancer management. The identification of moderating factors, including genetic variants and hormonal fluctuations, highlights the necessity of tailored therapies that account for the particular challenges and possibilities that each sex presents. The improved awareness of sex-specific traits which is crucial for good management is causing a drastic shift in risk assessment. A comprehensive approach is required for risk classification since behavioral, environmental, and biological factors are interdependent. Precision medicine projects must identify and incorporate these complex elements into evaluation models to tailor therapies to the unique situations of each patient. The potential for customized medicine in the treatment of lung cancer is highlighted by the development of immunotherapy and targeted medications. However, a deep comprehension of sex-specific responses is necessary for these improvements to be effective. Understanding the complex relationships between sex-specific characteristics and treatment approaches will, in the long run, improve patient outcomes and provide new therapeutic avenues. This chapter provides a road map for negotiating the challenging terrain of sex-specific factors to be taken into account in the treatment of lung cancer. It seeks to give medical professionals the information they need to deliver more complex, individualized, and efficient treatment by bridging the knowledge gap between research findings and clinical applications. Adopting a sex-sensitive paradigm assures that, as we continue to unravel the secrets of lung cancer biology, our approach is not just state-of-the-art but also compassionate and customized to each patient's unique requirements.

References

[1] Srivastava S, Jayaswal N, Kumar S, Sharma PK, Behl T, Khalid A, *et al.* Unveiling the potential of proteomic and genetic signatures for precision therapeutics in lung cancer management. Cell Signal [Internet]. 2024; 113: 110932.

[2] de Castro G, Souza FH, Lima J, Bernardi LP, Teixeira CHA, Prado GF. Does multidisciplinary team management improve clinical outcomes in Non-

Small Cell Lung Cancer? A systematic review with meta-analysis. JTO Clin Res Reports [Internet]. 2023;100580.

[3] Mena E, Yanamadala A, Cheng G, Subramaniam RM. The Current and Evolving Role of PET in Personalized Management of Lung Cancer. PET Clin [Internet]. 2016; 11(3): 243-59

[4] Imyanitov EN, Iyevleva AG, Levchenko E V. Molecular testing and targeted therapy for non-small cell lung cancer: Current status and perspectives. Crit Rev Oncol Hematol [Internet]. 2021; 157: 103194.

[5] Roy M, Padda SK. Chapter 10—Epidermal growth factor receptor-mutated non-small cell lung cancer: a clinical approach. In: Gillaspie EA, Cass AS, Horn LBTLC, editors. St. Louis (MO): Elsevier; 2024. p. 217-52.

[6] Vasconcelos de Matos L, Volovat S, Debiasi M, Cardoso F. Unfolding the role of the PI3K/AKT/MTOR pathway in male breast cancer: A pragmatic appraisal. The Breast [Internet] 2023; 72: 103576.

[7] van Dam PA, Huizing M, Mestach G, Dierckxsens S, Tjalma W, Trinh XB, *et al.* SARS-CoV-2 and cancer: Are they really partners in crime? Cancer Treat Rev [Internet]. 2020; 89: 102068.

[8] Plazas JG, Arias-Martinez A, Lecumberri A, Martínez de Castro E, Custodio A, Cano JM, *et al.* Sex and gender disparities in patients with advanced gastroesophageal adenocarcinoma: data from the AGAMENON-SEOM registry. ESMO Open [Internet]. 2022; 7(3): 100514.

[9] Gallage S, García-Beccaria M, Szydlowska M, Rahbari M, Mohr R, Tacke F, *et al.* The therapeutic landscape of hepatocellular carcinoma. Med [Internet]. 2021; 2(5): 505-52.

[10] Kiyohara C, Ohno Y. Sex differences in lung cancer susceptibility: a review. Gender medicine. 2010 Oct 1;7(5):381-401. https://doi.org/10.3390/cancers15123111

[11] Thompson T, Ketcher D, Gray TF, Kent EE. The Dyadic Cancer Outcomes Framework: A general framework of the effects of cancer on patients and informal caregivers. Soc Sci Med [Internet]. 2021; 287: 114357.

[12] Virani SS, Newby LK, Arnold S V, Bittner V, Brewer LC, Demeter SH, *et al.*

2023 AHA/ACC/ACCP/ASPC/NLA/PCNA Guideline for the Management of Patients with Chronic Coronary Disease: A Report of the American Heart Association/American College of Cardiology Joint Committee on Clinical Practice Guidelines. J Am Coll Cardiol [Internet]. 2023;82(9):833-955.

[13] Yadav D, Patil-Takbhate B, Khandagale A, Bhawalkar J, Tripathy S, Khopkar-Kale P. Next-Generation sequencing transforming clinical practice and precision medicine. Clin Chim Acta [Internet]. 2023; 551: 117568. Available from: https://www.sciencedirect.com/science/article/pii/S0009898123003704

[14] Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, *et al.* The Immune Landscape of Cancer. Immunity [Internet]. 2018; 48(4): 812-830. e14.

[15] Nadeau C, Kerr AR. Evaluation and Management of Oral Potentially Malignant Disorders. Dent Clin. NorthAm [Internet]. 2018; 62(1): 1-27.

[16] Rodriguez Alvarez AA, Yuming S, Kothari J, Digumarthy SR, Byrne NM, Li Y, *et al.* Sex disparities in lung cancer survival rates based on screening status. Lung Cancer [Internet]. 2022; 171: 115-20.

[17] Ruiz Tsukazan MT, Vigo Á, Da Silva VD, Vieira A, Rosenthal R, Cabral F, *et al.* P1.01-053 Lung Cancer in Brazil: Men and Women Differences: Topic: Descriptive Epidemiology. J Thorac Oncol [Internet]. 2017; 12 (1, Supplement): S482.

[18] Piñeros M, Sierra MS, Forman D. Descriptive epidemiology of lung cancer and current status of tobacco control measures in Central and South America. Cancer Epidemiol [Internet]. 2016; 44: S909.

[19] Pasquinelli MM, Tammemägi MC, Kovitz KL, Durham ML, Deliu Z, Guzman A, *et al.* Addressing Sex Disparities in Lung Cancer Screening Eligibility: USPSTF vs PLCOm2012 Criteria. Chest [Internet]. 2022; 161(1): 248-56.

[20] Fu Y, Liu J, Chen Y, Liu Z, Xia H, Xu H. Gender disparities in lung cancer incidence in the United States during 2001-2019. Scientific Reports. 2023 Aug 3; 13(1): 12581. https://doi.org/10.1038/s41598-023-39440-8

[21] Guerreiro T, Forjaz G, Antunes L, Bastos J, Mayer A, Aguiar P, *et al.* Lung cancer survival and sex-specific patterns in Portugal: A population-based

analysis. Pulmonology [Internet]. 2021;

[22] Domingo-Relloso A, Joehanes R, Rodriguez-Hernandez Z, Lahousse L, Haack K, Fallin MD, *et al.* Smoking, blood DNA methylation sites and lung cancer risk. Environ Pollut [Internet]. 2023; 334: 122153.

[23] Zhao J, Shi YL, Wang YT, Ai FL, Wang XW, Yang WY, *et al.* Lung Cancer Risk Attributable to Active Smoking in China: A Systematic Review and Meta-Analysis. Biomed Environ Sci [Internet]. 2023; 36(9): 850-61.

[24] de Groot PM, Wu CC, Carter BW, Munden RF. The epidemiology of lung cancer. Translational lung cancer research. 2018 Jun;7(3):220.

[25] Frias-Gomez J, Alemany L, Benavente Y, Clarke MA, de Francisco J, De Vivo I, *et al.* Night shift work, sleep duration and endometrial cancer risk: A pooled analysis from the Epidemiology of Endometrial Cancer Consortium (E2C2). Sleep Med Rev [Internet]. 2023; 72: 101848.

[26] Olesen TB, Rasmussen TR, Jakobsen E, Engberg H, Hilberg O, Møller H, *et al.* Diagnosis and treatment of lung cancer in Denmark during the COVID-19 pandemic. Cancer Epidemiol [Internet]. 2023; 85: 102373.

[27] Feng X, Muller DC, Zahed H, Alcala K, Guida F, Smith-Byrne K, *et al.* Evaluation of pre- diagnostic blood protein measurements for predicting survival after lung cancer diagnosis. eBioMedicine [Internet]. 2023; 92: 104623.

[28] Alexander DD, Pastula ST, Riordan AS. Epidemiology of lung cancer among acrylonitrile-exposed study populations: A meta-analysis. Regul Toxicol Pharmacol [Internet]. 2021; 122: 104896.

[29] Tang M, Abbas HA, Negrao MV, Ramineni M, Hu X, Hubert SM, Fujimoto J, Reuben A, Varghese S, Zhang J, Li J. The histologic phenotype of lung cancers is associated with transcriptomic features rather than genomic characteristics. Nature communications. 2021 Dec 6;12(1): 7081. 2023; 175: 107686. Available from: https://www.sciencedirect.com/science/article/pii/S0091743523002669

[30] Galli G, Rossi G. Lung cancer histology-driven strategic therapeutic approaches. Shanghai Chest. 2020 Jul 10; 4.

[31] Wainer Z, Wright GM, Gough K, Daniels MG, Russell PA, Choong P, *et al.* Sex-Dependent Staging in Non-Small-Cell Lung Cancer; Analysis of the Effect of

Sex Differences in the Eighth Edition of the Tumor, Node, Metastases Staging System. Clin LungCancer [Internet]. 2018; 19(6): e933-44.

[32] Kiyohara C, Ohno Y. Sex differences in lung cancer susceptibility: A review. Gend Med [Internet]. 2010; 7(5): 381-401.

[33] Zhong S, Borlak J. Sex disparities in non-small cell lung cancer: mechanistic insights from a cRaf transgenic disease model. eBioMedicine [Internet]. 2023; 95: 104763.

[34] Sponagel J, Devarakonda S, Rubin JB, Luo J, Ippolito JE. De novo serine biosynthesis from glucose predicts sex-specific response to antifolates in non-small cell lung cancer cell lines. iScience [Internet]. 2022; 25(11): 105339.

[35] Florez N, Kiel L, Riano I, Patel S, DeCarli K, Dhawan N, *et al.* Lung Cancer in Women: The Past, Present, and Future. Clin Lung Cancer [Internet]. 2023;

[36] Rodriguez-Lara V, Hernandez-Martinez JM, Arrieta O. Influence of estrogen in non-small cell lung cancer and its clinical implications. Journal of thoracic disease. 2018 Jan;10(1):482.

[37] Xu L, Wang L, Cheng M. Identification of genes and pathways associated with sex in Non-smoking lung cancer population. Gene [Internet]. 2022; 831: 146566

[38] Sarnaik KS, Bassiri A, Poston LM, Gasnick A, Sinopoli JN, Tapias Vargas L, *et al.* Lymph Node Yield in Lung Cancer Resection is Associated With Demographic and Institutional Factors. J Surg Res [Internet]. 2024; 293: 175-86

[39] Barquín M, Calvo V, García-García F, Nuñez B, Sánchez-Herrero E, Serna-Blasco R, *et al.* Sex is a strong prognostic factor in stage IV non-small-cell lung cancer patients and should be considered in survival rate estimation. Cancer Epidemiol [Internet]. 2020; 67: 101737.

[40] Faida P, Attiogbe MKI, Majeed U, Zhao J, Qu L, Fan D. Lung cancer treatment potential and limits associated with the STAT family of transcription factors. Cell Signal [Internet]. 2023; 109: 110797.

[41] Varghese R, Efferth T, Ramamoorthy S. Carotenoids for lung cancer chemoprevention and chemotherapy: Promises and controversies. Phytomedicine [Internet] 2023; 116: 154850.

[42] Olak J, Colson Y. Gender differences in lung cancer: have we really come a

long way, baby? The Journal of Thoracic and Cardiovascular Surgery. 2004 Sep 1; 128(3): 346-51.

[43] Gemine RE, Davies GR, Lanyon K, Rees SE, Campbell I, Lewis KE. Quitting smoking improves two-year survival after a diagnosis of non-small cell lung cancer. Lung Cancer [Internet]. 2023; 186: 107388.

[44] Bassiri A, Badrinathan A, Alvarado CE, Boutros C, Jiang B, Kwak M, *et al.* Uncovering Health-Care Disparities Through Patient Decisions in Lung Cancer Surgery. J Surg Res [Internet]. 2024; 293: 248-58.

[45] Bassiri A, Badrinathan A, Alvarado CE, Kwak M, Sinopoli J, Tapias Vargas L, *et al.* Evaluating the Optimal Time Between Diagnosis and Surgical Intervention for Early-Stage Lung Cancer. J Surg Res [Internet]. 2023; 292: 297-306.

[46] Sehgal A, Bhat MA, Dogra D, Rawat S, Dhatwalia SK. EGCG: The antioxidant powerhouse in lung cancer management and chemotherapy enhancement. Adv Redox Res [Internet]. 2023; 9: 100085.

[47] Wojcik R, Morris A. Aiming to Improve Equity in Lung Health: Sex and Gender. Clin Chest Med [Internet]. 2023; 44(3): 613-22.

[48] Stapelfeld C, Neumann KT, Maser E. Different inhibitory potential of sex hormones on NNK detoxification in vitro: A possible explanation for gender-specific lung cancer risk. Cancer Lett [Internet]. 2017; 405: 120-6.

[49] Mederos N, Friedlaender A, Peters S, Addeo A. Gender-specific aspects of epidemiology, molecular genetics and outcome: lung cancer. ESMO Open [Internet]. 2020; 5: e000796. Available from: https://www.sciencedirect.com/science/article/pii/S2059702920326831

[50] Davuluri S, Bajpai AK, Thirumurugan K, Acharya KK. The molecular basis of gender disparities in smoking lung cancer patients. Life Sci [Internet]. 2021; 267: 118927.

[51] Sodhi A, Pisani M, Glassberg MK, Bourjeily G, D'Ambrosio C. Sex and Gender in Lung Disease and Sleep Disorders: A State-of-the-Art Review. Chest [Internet]. 2022; 162(3): 647-58.

[52] Tolwin Y, Gillis R, Peled N. Gender and lung cancer—SEER-based analysis. Ann Epidemiol [Internet]. 2020; 46: 14-9.

[53] Yu Z, Qin L, Yu G. The progresses of relevant factors on the efficacy of

immune checkpoint inhibitors in the non-small cell lung cancer patients. Cancer Treat Res Commun [Internet]. 2023; 37: 100758.

[54] Wang Z, Ren D, Chen S, Duan G. Pan-immune-inflammation value is an independent prognostic factor in patients with non-small cell lung cancer with an established nomogram prognostic model. Asian J Surg [Internet]. 2023; 46(11): 4999-5000.

[55] Cousin F, Desir C, Ben Mustapha S, Mievis C, Coucke P, Hustinx R. Incidence, risk factors, and CT characteristics of radiation recall pneumonitis induced by immune checkpoint inhibitor in lung cancer. Radiother Oncol [Internet]. 2021; 157: 47-55.

[56] Dall'Olio FG, Maggio I, Massucci M, Mollica V, Fragomeno B, Ardizzoni A. ECOG performance status ≥2 as a prognostic factor in patients with advanced non small cell lung cancer treated with immune checkpoint inhibitors—A systematic review and meta- analysis of real world data. Lung Cancer [Internet]. 2020; 145: 95-104.

[57] Dardzińska JA, Wasilewska E, Szupryczyńska N, Gładyś K, Wojda A, Śliwińska A, *et al.* Inappropriate dietary habits in tobacco smokers as a potential risk factor for lung cancer: Pomeranian cohort study. Nutrition [Internet]. 2023; 108: 111965.

[58] Drareni K, Dougkas A, Giboreau A, Laville M, Souquet PJ, Nazare JA, *et al.* Loss of smell in lung cancer patients undergoing chemotherapy: Prevalence and relationship with food habit changes. Lung Cancer [Internet]. 2023; 177: 29-36.

[59] Alessi A, Trevisan C, Citron A, Ceolin C, Bordignon A, Zoccarato F, *et al.* Dietary inflammatory index is associated with lung function in healthy older adults. Nutrition [Internet]. 2022; 99-100: 111653.

[60] Shi J, Shao X, Guo X, Fang W, Wu X, Teng Y, *et al.* Dietary Habits and Breast Cancer Risk: A Hospital-Based Case-Control Study in Chinese Women. Clin Breast Cancer [Internet]. 2020; 20(5): e540-50.

[61] Voorn MJJ, Driessen EJM, Reinders RJEF, van Kampen-van den Boogaart VEM, Bongers BC, Janssen-Heijnen MLG. Effects of exercise prehabilitation and/or rehabilitation on health-related quality of life and fatigue in patients

with non-small cell lung cancer undergoing surgery: A systematic review. Eur J Surg Oncol [Internet]. 2023; 49(10): 106909.

[62] Leimbacher AC, Villiger P, Desboeufs N, Aboouf MA, Nanni M, Armbruster J, *et al.*Voluntary exercise does not always suppress lung cancer progression. iScience [Internet]. 2023; 26(8): 107298.

[63] Harman N, Lazio M, Hayward R. Exercise training-induced adaptations in lung cancer patients who have undergone a lobectomy. Exp Gerontol [Internet]. 2021; 155: 111587.

[64] Liu Y, Wen H, Bai J, Sun J, Chen J, Yu C. Disease Burden and Prediction Analysis of Tracheal, Bronchus, and Lung Cancer Attributable to Residential Radon, Solid Fuels, and Particulate Matter Pollution Under Different Sociodemographic Transitions From 1990 to 2030. Chest 2023

[65] Loomis D, Dzhambov AM, Momen NC, Chartres N, Descatha A, Guha N, *et al.* The effect of occupational exposure to welding fumes on trachea, bronchus and lung cancer: A systematic review and meta-analysis from the WHO/ILO Joint Estimates of the Work- related Burden of Disease and Injury. Environ Int [Internet]. 2022; 170: 107565.

[66] Su Z, Jia XH, Fan YG, Zhao FH, Zhou QH, Taylor PR, *et al.* Quantitative evaluation of radon, tobacco use and lung cancer association in an occupational cohort with 27 follow-up years. Ecotoxicol Environ Saf [Internet]. 2022; 232: 113233.

[67] Markevych I, Zhao T, Fuertes E, Marcon A, Dadvand P, Vienneau D, *et al.* Residential greenspace and lung function decline over 20 years in a prospective cohort: The ECRHS study. Environ Int [Internet]. 2023; 178: 108036.

[68] Christian WJ, Walker CJ, Huang B, Levy JE, Durbin E, Arnold S. Using residential histories in case-control analysis of lung cancer and mountaintop removal coal mining in Central Appalachia. Spat Spatiotemporal Epidemiol [Internet]. 2020; 35: 100364.

[69] Manangama G, Gramond C, Audignon-Durand S, Baldi I, Fabro-Peray P, Gilg Soit Ilg A, *et al.* Occupational exposure to unintentionally emitted nanoscale particles and risk of cancer: From lung to central nervous system - Results from three French case-control studies. Environ Res [Internet]. 2020; 191:

110024.

[70] Celen YY, Oncul S, Narin B, Gunay O. Measuring radon concentration and investigation of it's effects on lung cancer. J Radiat Res Appl Sci [Internet]. 2023; 16(4): 100716.

[71] Mederos N, Friedlaender A, Peters S, Addeo A. Gender-specific aspects of epidemiology, molecular genetics and outcome: lung cancer. ESMO open. 2020 Jan 1; 5: e000796.

[72] Ragavan M, Patel MI. The evolving landscape of sex-based differences in lung cancer: a distinct disease in women. European Respiratory Review. 2022 Mar 31; 31(163).

[73] Becker N, Motsch E, Trotter A, Heussel CP, Dienemann H, Schnabel PA, Kauczor HU, Maldonado SG, Miller AB, Kaaks R, Delorme S. Lung cancer mortality reduction by LDCT screening—results from the randomized German LUSI trial. International journal of cancer. 2020 Mar 15; 146(6): 1503-13.

[74] Vu C, Lin S, Chang CF. Gender gaps in care: lung cancer screening criteria in women. Chest. 2019 Oct 1; 156(4): A407.

[75] Henschke CI, Yankelevitz DF, Reeves AP, Yip R. Evolution of Lung Cancer Screening Management. Oncology (Williston Park, NY). 2019 Jul 16; 33(7).

[76] Wheatley-Price P, Blackhall F, Lee SM, Ma C, Ashcroft L, Jitlal M, Qian W, Hackshaw A, Rudd R, Booton R, Danson S. The influence of sex and histology on outcomes in non- small-cell lung cancer: a pooled analysis of five randomized trials. Annals of oncology. 2010 Oct 1; 21(10): 2023-8.

[77] Wakelee HA, Wang W, Schiller JH, Langer CJ, Sandler AB, Belani CP, Johnson DH, Eastern Cooperative Oncology Group. Survival differences by sex for patients with advanced non-small cell lung cancer on Eastern Cooperative Oncology Group trial 1594. Journal of Thoracic Oncology. 2006 Jun 1; 1(5): 441-6.

[78] Conforti F, Pala L, Bagnardi V, De Pas T, Martinetti M, Viale G, Gelber RD, Goldhirsch A. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta- analysis. The Lancet Oncology. 2018 Jun 1; 19(6): 737-46.

[79] Conforti F, Pala L, Bagnardi V, Viale G, De Pas T, Pagan E, Pennacchioli E, Cocorocchio E, Ferrucci PF, De Marinis F, Gelber RD. Sex-based heterogeneity in response to lung cancer immunotherapy: a systematic review and meta-analysis. JNCI: Journal of the National Cancer Institute. 2019 Aug 1; 111(8): 772-81.

[80] Albain K, Darke A, Mack P, Redman M, Cheng T, Moon J, Holland W, Borczuk A, Chay C, Morris P, Vallieres E. OA06. 01 Case-series study in Ever-and never-smoking females and males with NSCLC: exposures, tumor factors, biology and survival (SWOG S0424). Journal of Thoracic Oncology. 2018 Oct 1; 13(10): S333.

[81] Kiyohara C, Ohno Y. Sex differences in lung cancer susceptibility: a review. Gender medicine. 2010 Oct 1; 7(5): 381-401.

[82] Tang DL, Rundle A, Warburton D, Santella RM, Tsai WY, Chiamprasert S, Hsu YZ, Perera FP. Associations between both genetic and environmental biomarkers and lung cancer: evidence of a greater risk of lung cancer in women smokers. Carcinogenesis. 1998 Nov 1; 19(11): 1949-53.

[83] Whibley C, Pharoah PD, Hollstein M. p53 polymorphisms: cancer implications. Nature reviews cancer. 2009 Feb; 9(2): 95-107.

[84] Kabat GC, Miller AB, Rohan TE. Reproductive and hormonal factors and risk of lung cancer in women: a prospective cohort study. International journal of cancer. 2007 May 15; 120(10): 2214-20.

[85] Begg CB, Zhang ZF, Sun M, Herr HW, Schantz SP. Methodology for evaluating the incidence of second primary cancers with application to smoking-related cancers from the surveillance, epidmiology, and end results (SEER) program. American journal of epidemiology. 1995 Sep 15; 142(6): 653-65.

[86] Harichand-Herdt S, Ramalingam SS. Gender-associated differences in lung cancer: clinical characteristics and treatment outcomes in women. InSeminars in oncology 2009 Dec 1 (Vol. 36, No. 6, pp. 572-580). WB Saunders.

[87] Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Critical reviews in oncology/hematology. 1995 Jul 1; 19(3): 183-232.

[88] Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ,

Lindeman N, Boggon TJ, Naoki K. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004 Jun 4; 304(5676): 1497-500.

[89] Liu SV, Gitlitz BJ. Predictive Markers in Lung Cancer. Biomarkers in Oncology: Prediction and Prognosis. 2012 Sep 18: 43.

[90] Márquez-Garbán DC, Chen HW, Fishbein MC, Goodglick L, Pietras RJ. Estrogen receptor signaling pathways in human non-small cell lung cancer. Steroids. 2007 Feb 1; 72(2): 135-43.

[91] Traynor AM, Schiller JH, Stabile LP, Kolesar JM, Eickhoff JC, Dacic S, Hoang T, Dubey S, Marcotte SM, Siegfried JM. Pilot study of gefitinib and fulvestrant in the treatment of post-menopausal women with advanced non-small cell lung cancer. Lung cancer. 2009 Apr 1; 64(1): 51-9.

[92] Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. Annals of surgery. 1972 Mar;175(3): 409.

[93] Saltz LB, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser, Yang TS, Rivera F, Couture F. Bevacizumab in Combination with Oxaliplatin-Based Chemotherapy As First-Line Therapy in Metastatic Colorectal Cancer: A Randomized Phase III Study. Journal of Clinical Oncology. 2023 Jul 20; 41(21): 3663-9.

[94] Schabath MB, Wu X, Vassilopoulou-Sellin R, Vaporciyan AA, Spitz MR. Hormone replacement therapy and lung cancer risk: a case-control analysis. Clinical cancer research. 2004 Jan 1; 10(1): 113-23.

[95] Blackman JA, Coogan PF, Rosenberg L, Strom BL, Zauber AG, Palmer JR, Langenberg P, Shapiro S. Estrogen replacement therapy and risk of lung cancer. Pharmacoepidemiology and drug safety. 2002 Dec; 11(7): 561-7.

[96] Donington JS, Colson YL. Sex and gender differences in non-small cell lung cancer. InSeminars in thoracic and cardiovascular surgery 2011 Jun 1 (Vol. 23, No. 2, pp. 137-145). WB Saunders.

[97] Chlebowski RT, Schwartz AG, Wakelee H, Anderson GL, Stefanick ML, Manson JE, Rodabough RJ, Chien JW, Wactawski-Wende J, Gass M, Kotchen JM. Oestrogen plus progestin and lung cancer in postmenopausal women (Women's Health Initiative trial): a post-hoc analysis of a randomised

controlled trial. The Lancet. 2009 Oct 10; 374(9697): 1243-51.

[98] Chlebowski RT, Anderson GL, Manson JE, Schwartz AG, Wakelee H, Gass M, Rodabough RJ, Johnson KC, Wactawski-Wende J, Kotchen JM, Ockene JK. Lung cancer among postmenopausal women treated with estrogen alone in the women's health initiative randomized trial. Journal of the National Cancer Institute. 2010 Sep 22; 102(18): 1413-21.

[99] Vavalà T, Catino A, Pizzutilo P, Longo V, Galetta D. Gender differences and immunotherapy outcome in advanced lung cancer. International Journal of Molecular Sciences. 2021 Nov 4; 22(21): 11942.

[100] Conforti F, Pala L, Bagnardi V, De Pas T, Martinetti M, Viale G, Gelber RD, Goldhirsch A. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta- analysis. The Lancet Oncology. 2018 Jun 1; 19(6): 737-46.

[101] Dafni U, Tsourti Z, Vervita K, Peters S. Immune checkpoint inhibitors, alone or in combination with chemotherapy, as first-line treatment for advanced non-small cell lung cancer. A systematic review and network meta-analysis. Lung cancer. 2019 Aug 1; 134: 127-40.

[102] Ten Haaf K, Jeon J, Tammemägi MC, Han SS, Kong CY, Plevritis SK, Feuer EJ, de Koning HJ, Steyerberg EW, Meza R. Risk prediction models for selection of lung cancer screening candidates: a retrospective validation study. PLoS medicine. 2017 Apr 4; 14(4): e1002277.

[103] Isla D, Majem M, Viñolas N, Artal A, Blasco A, Felip E, Garrido P, Remón J, Baquedano M, Borrás JM, Die Trill M. A consensus statement on the gender perspective in lung cancer. Clinical and Translational Oncology. 2017 May; 19: 527-35.

[104] Reguart N, Remon J. Common EGFR-mutated subgroups (Del19/L858R) in advanced non-small-cell lung cancer: chasing better outcomes with tyrosine kinase inhibitors. Future Oncology. 2015 Apr; 11(8): 1245-57.

[105] Fan L, Feng Y, Wan H, Shi G, Niu W. Clinicopathological and demographical characteristics of non-small cell lung cancer patients with ALK rearrangements: a systematic review and meta-analysis. PloS one. 2014 Jun 24; 9(6): e100866.

[106] Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, Felip E, Cappuzzo F, Paolini J, Usari T, Iyer S. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. New England Journal of Medicine. 2014 Dec 4; 371(23): 2167-77.

[107] Chen D, Zhang LQ, Huang JF, Liu K, Chuai ZR, Yang Z, Wang YX, Shi DC, Liu Q, Huang Q, Fu WL. BRAF mutations in patients with non-small cell lung cancer: a systematic review and meta-analysis. PloS one. 2014 Jun 30; 9(6): e101354.

[108] Michels S, Scheel AH, Scheffler M, Schultheis AM, Gautschi O, Aebersold F, Diebold J, Pall G, Rothschild S, Bubendorf L, Hartmann W. Clinicopathological characteristics of RET rearranged lung cancer in European patients. Journal of Thoracic Oncology. 2016 Jan 1; 11(1): 122-7.

[109] De Mello RA, Neves NM, Tadokoro H, Amaral GA, Castelo-Branco P, Zia VA. New target therapies in advanced non-small cell lung cancer: a review of the literature and future perspectives. Journal of clinical medicine. 2020 Nov 3; 9(11): 3543.

[110] Majeed U, Manochakian R, Zhao Y, Lou Y. Targeted therapy in advanced non-small cell lung cancer: current advances and future trends. Journal of hematology & oncology. 2021 Dec; 14(1): 1-20.

[111] Kumar Prashant, Pandey Nath Surya, Ahmad Farman, Verma Anurag, Sharma Himanshu, Ashique Sumel, Bhattacharyya Prakash Subhra, Bhattacharyya Indrani, Kumar Shubneesh, Mishra Neeraj and Garg Ashish, Carbon Nanotubes: A Targeted Drug Delivery against Cancer Cell, Current Nanoscience 2024; 20.

Chapter 8

Nanoscience-Nanotechnology Education for All: Promoting Nano-Literacy across Educational Levels

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Abstract: Nanoscience and nanotechnology (NST) are rapidly advancing fields with far-reaching applications in daily life, from superhydrophobic materials to advanced water purification systems and innovative sports equipment. This chapter focuses on integrating NST concepts in educational curricula from preschool to lower secondary education, with a dual focus on enhancing scientific understanding and promoting ethical awareness. It explores how the interdisciplinary nature of NST can stimulate student interest and cultivate nanoliteracy through innovative teaching methodologies and hands-on experiments. Following the nine "Big Ideas" for effective NST education, this chapter reviewed current literature regarding the pedagogical approaches applied in the NST field. It provides international case studies that reveal both successful approaches and common challenges. It underscores the pivotal role of interactive digital tools and Responsible search and innovation (RRI) in shaping the future of NST education. Finally, the chapter synthesizes insights for educators and policymakers to foster a well-informed, critically engaged student body prepared to navigate and contribute to the nano-enabled world of tomorrow.

8.1. Introduction

Nanoscience and nanotechnology (NST) have emerged as pivotal fields with profound implications for various aspects of modern life, from medicine and electronics to environmental sustainability. Recognizing the significance of NST, there is a growing movement to integrate these concepts into the early phases of education.

This chapter focuses on embedding NST concepts into primary and lower secondary education and in public, examining the content taught, the educational approaches employed, and the effectiveness of these interventions. The chapter explores how NST concepts can be effectively introduced to compulsory education, fostering a foundational understanding that could pave the way for more advanced studies in the future. By investigating different educational strategies and their outcomes, this chapter provides insights into the benefits and challenges associated with early NST education. It offers practical recommendations for educators and policymakers looking to incorporate NST into their curricula.

Although our review primarily addresses NST education at the primary and lower secondary levels and the public, we acknowledge that upper secondary education is crucial in deepening students' understanding and guiding them towards specialized STEM careers. Nonetheless, this chapter focuses on the foundational stages, emphasizing the importance of early exposure to NST concepts. Furthermore, emphasizing public understanding ensures that nanoscience and nanotechnology literacy is not limited to formal education but reaches a broader audience, fostering an informed society capable of engaging with these emerging technologies' ethical and societal implications.

A crucial component of effectively teaching NST is the emphasis on "big ideas" within the field. Gilbert and Lin [1] introduced the concept of achieving a "functionally nano-literate" understanding, which involves grasping nine essential concepts identified by Stevens *et al.* [2]. The nine "big ideas" proposed in the book '*Big Ideas in Nanoscale Science and Engineering: A Guidebook for Secondary Teachers*' [2] formed the roadmap for integrating NST concepts into secondary education. The 9 Big Ideas in NST described by Stevens and colleagues [2] are (1) Size and Scale, (2) Structure of matter, (3) Forces and interactions, (4) Quantum effects, (5) Size-dependent properties, (6) Self-assembly, (7) Tools and instrumentation, (8) Models and simulations, (9) Science, technology, and society. The book thoroughly presents NST concepts, outlines corresponding learning goals, describes their relationship to the curriculum, and provides illustrative phenomena suitable for instruction.

These big ideas serve as a scaffold for students, helping them make sense of nanoscience's complex and abstract principles. By focusing on these fundamental

themes, educators can provide a structured learning experience highlighting the relevance and applications of NST. Building on these concepts, several researchers have developed educational interventions and teaching materials for compulsory education. Aspects of these big ideas have been transformed to be suitable for instruction even at the primary education level and kindergarten. Integrating these big ideas not only aids in conceptual clarity but also ensures that students appreciate the broader implications of nanotechnology, fostering a deeper, more cohesive understanding of the subject and enlightening educators and policymakers about the key aspects of NST education.

The chapter is structured into three main sections, each focusing on a distinct aspect of NST education at different educational levels:

1) *Understanding NST Concepts in Primary Education*: This section addresses primary school children's unique cognitive capabilities and learning characteristics, typically aged 6-11 years. Given their developmental potential, introducing NST to this age group requires pedagogical approaches that simplify the complex NST ideas through didactic transformation without compromising their essence. This section explores various content areas within NST, such as NST-related phenomena, basic nanomaterials, their properties, and simple applications. The section highlights innovative teaching methods, including hands-on experiments, interactive storytelling, and digital tools like animations and simulations to make abstract NST concepts more tangible. Case studies from different countries illustrate the diversity of approaches and the contextual factors influencing their effectiveness.

2) *Understanding NST in Lower Secondary Education*: As students progress to lower secondary education (typically aged 12-15), their cognitive and analytical skills become more refined, enabling them to employ principles and deductive reasoning [3], which allows a deeper and more systematic exploration of NST. This section examines how NST is introduced at this educational level, often by integrating it with existing science curricula. Topics such as nanoscale dimensions, molecular structures, and nanotechnology applications in real-world scenarios are explored in greater detail. The section discusses various pedagogical approaches, including inquiry-based learning, project-based assignments, and advanced digital tools such as virtual labs and augmented reality. Studies from

different educational systems highlight the successes and challenges of implementing NST concepts in lower secondary education. The effectiveness of these interventions is assessed through various qualitative and quantitative approaches, including student engagement, understanding, and retention of NST concepts, as well as their ability to apply these concepts in problem-solving scenarios.

3) *Public Understanding of Nanotechnology*: While the primary focus is formal education, this section acknowledges the importance of fostering a broader public understanding of nanotechnology, which can influence and support educational efforts. Public engagement with NST is crucial for creating a well-informed citizenry that can actively participate in discussions about nanotechnology's ethical, social, and environmental implications. This section explores initiatives to raise public awareness and understanding of NST, such as science fairs, museum exhibits, outreach programs, and media campaigns. It also examines the role of informal education and lifelong learning in promoting NST literacy among diverse age groups. This section underscores the importance of a holistic approach to NST education that extends beyond the classroom by bridging the gap between formal education and public understanding.

The chapter concludes with a summary of the key findings and a discussion of the overall benefits of early NST education. It discusses and emphasizes the positive impact a solid foundation in understanding NST can have on students' future academic and career prospects and its potential to inspire the next generation of scientists, engineers, and informed citizens. The conclusion also provides practical recommendations for educators, policymakers, and curriculum developers on how to integrate NST into primary and lower secondary education effectively. By synthesizing the insights and lessons learned from various studies and initiatives, the chapter aims to contribute to the ongoing dialogue about the best practices for NST education and to support the development of effective educational strategies that can prepare young learners for the challenges and opportunities of the 21st century.

8.2. Understanding NST concepts in Primary Education

Several researchers argue that NST concepts should be introduced at all levels of education, starting in the early grades in primary schools [4]-[6]. Thirteen studies published between 2012 and 2024 in international journals and books examined

NST topics in three areas: Taiwan (China), South Africa, and Greece. These studies highlighted the successful integration of NST concepts into primary education, explored various educational approaches for teaching these concepts, and evaluated the effectiveness of these interventions on students' learning.

8.2.1. NST Big Ideas

Attempts to introduce NST Big Ideas to young children in kindergarten are very promising. An educational intervention was implemented focusing on the sizes of nano-objects (related to Big Idea 1) and the tools used to visualize them (related to Big Idea 7), as well as the gecko effect (e.g. the adhesive property of the gecko lizard, related to Big Idea 5) [7]. By the end of the lesson, students could identify how various objects appear when observed through different tools. For example, they could describe how a tree leaf looks to the naked eye, how it appears through a magnifying lens (where the veins are visible), and how the leaf's fibers and cells become visible under a microscope. Regarding the gecko effect, students were able to illustrate the structure of gecko feet by depicting the numerous hair-like structures (known as setae) that branch into even smaller structures (called spatulae), along with the extensive contact surface area [7].

In lower primary school, a teaching intervention focused on the size of objects (*i.e.*, Big Idea 1), specifically on qualitatively grouping and ordering objects of different scales [8] [9]. The authors encouraged students to solve problems related to nanoscale entities arising from the COVID-19 pandemic. Specifically, students were asked to describe how an entity from the nanoworld, such as viruses, affects the microworld, such as cells, and how these, in turn, can impact entities in the macroworld, such as the human body. This approach was used to clarify the interactions between the three size scales, as proposed by Spyrtou *et al.* [10].

In upper primary school, the concept of the size of objects was included in all the related studies [11]-[16]. The term "nanometer" was highlighted as a unit of length measurement, representing an extremely small length [16], used to identify the size of objects in the nanoworld and to compare their size with objects of other scales [13]. Other studies implemented quantitative approaches. They included the concept of powers of ten (e.g. one billionth = 0.000000001 = 1×10^{-9}) [15], or they focused on the size range of nanoworld (*i.e.*, 1-100 nanometer [12] as well as measuring and transforming the length of macroworld objects in

nanometers using a nano ruler [13] [15].

Qualitative approaches to the size of objects focus on grouping and ordering objects in the macroworld, microworld, and nanoworld [13] [14]. Specifically, students were asked to categorize objects into the macroworld, microworld, and nanoworld using, as a qualitative criterion, the observation tool that provides access to each world, *i.e.*, the naked eye, the optical microscope, and the electron microscope, respectively (related to Big Idea 1 & 7). This classification is crucial for NST education since it is the basis for understanding that nanoworld objects possess unique properties that differ significantly from larger objects. It can further scaffold students to recognize that the nanoworld profoundly impacts the macroworld. In addition, students are asked to order different-sized objects based on their relative size, *i.e.*, based on the criterion "which objects fit into other objects or are part of them" [14]. The skill of ordering is essential, as it can help students explain NST-related processes, such as water nanopore filters, by comparing the size of the filters' pores to the size of the excluded objects. These studies used either simple observation tools or where the content either focused solely on their visible characteristics and examples of objects that can be seen with them [14] [17] or included more complex information in museum exhibits, such as the fact that an electron microscope uses a beam of electrons to examine objects, while an optical microscope uses light and lenses to magnify them [15] (related to Big Idea 7).

Another concept introduced to upper primary school students is the size-dependent properties (*i.e.*, Big Idea 5). The most common example was the superhydrophobicity known as the lotus effect [11]-[14] [16] [17]. Students were asked to recognize the lotus effect on different surfaces by observing how water behaves. For surfaces that exhibit the lotus effect, students described the water as "rolling", while for surfaces that do not, they should describe it as "sliding") [11]. After experiencing this, students were engaged in inquiry activities and discussions aiming to explain the lotus effect. In their effort to explain the lotus effect, students were familiarized with the nanoscale structures responsible for the superhydrophobicity, the self-cleaning property of the lotus leaves, and other surfaces that exhibit the phenomenon [12] [13] [16].

Furthermore, research on content transformation related to size-dependent

properties, particularly the lotus effect, suggested that, from a conceptual change perspective [18], this content should be approached as an emergent process. This means that the effect is caused by the collective interaction of multiple agents rather than by a single, identifiable causal agent, *i.e.* direct process [14]. Researchers proposed helping students shift away from their preconceptions that explain the lotus effect as a direct process (e.g., attributing it to visible characteristics of the leaf) [19] towards understanding it as an emergent process. This shift from explaining the lotus effect as a direct process to explaining it as an emergent process involves recognizing two causal agents for primary school students: (a) the leaf's nanostructures and (b) the trapped air in the interstitial spaces that form the spherical shape of the droplet [14]. The researchers argued that learning about the concept of the nanostructure could form the idea that the lotus effect emerges from non-visible agents, helping students shift their conceptions in a transitional state from a direct towards an emergent process [14] [19].

Moreover, Mandrikas *et al.* [13] concentrated on explaining the superhydrophobic properties of the contact surface structure by utilizing a water absorbance and flow model. They created a simulation where wooden straws were arranged on a styrofoam base to mimic surfaces with varying degrees of roughness. A ball, representing a water droplet, rolled across these surfaces. The ease with which the ball moved depended on the spacing between the straws, thereby simulating hydrophilic, hydrophobic, and superhydrophobic materials. Expanding on size-dependent properties, the change in properties that matter exhibits at the nanoscale, caused by the increased surface area-to-volume ratio, has been introduced. Students, after conducting a simple experiment, were able to compare the reaction rates of whole and crushed effervescent tablets placed in two tubs, each half-filled with water, in order to observe how the reaction rate changes depending on the size of the pieces [13].

Another approach to understanding NST started from the applications of NST in ordinary products (related to Big Idea 9). Students were exposed to market products that mimic the lotus effect, such as textiles and wood treated with nanocoatings. They were asked to recognize the contributions of these products to daily life and explain the underlying science, particularly focusing on their nanostructured surfaces [12] [14] [16] [17]. Additionally, nanoporous filters for

water purification were used to investigate NST concepts. Students reflected on the benefits of such filters, especially when clean water is scarce, and became enthusiastic about their understanding of the science behind the filters' effectiveness because of their nanostructure [14] [17].

Ethical issues (related to Big Idea 9) concerning the NST have also been addressed to primary school children. Mandrikas *et al.* [13] included critical views and concerns about the NST products, focusing on Responsible Research and Innovation (RRI) issues in their educational intervention. The researchers argued that students should be aware about the production and use of nanomaterials and accept the need for ethical standards in scientific research [13]. Likewise, Pnevmatikos and Christodoulou [20] [21] implemented an educational intervention using the Values and Knowledge Education (V*a*KE) method [22] to introduce NST topics such as size and scale (*i.e.*, Big Idea 1), and size-dependent properties (*i.e.*, Big Idea 5) in primary school teachers. This approach incorporated a moral dilemma related to nanotechnology's impact on human health, promoting students' understanding of the scientific concepts and reflecting on the moral responsibilities associated with NST advancements. The authors emphasized the need for students to reflect on the societal consequences of nano products, thus reinforcing the integration of RRI in primary science education. Furthermore, the study challenges the reluctance of teachers to go beyond scientific knowledge and to integrate ethical topics strongly related to real-life decision-making, encouraging a holistic approach to problem-solving [20].

8.2.2. Educational Approaches

The studies used several educational approaches to teach NST concepts to kindergarten and primary education students. These approaches included inquiry-based activities. Most of the researchers used hands-on experimental activities focused on size-dependent properties. For example, Mandrikas *et al.* [13] asked students to compare whole and crushed effervescent tablets to demonstrate the effect of surface area on reaction rates. Mandrikas *et al.* [13] and Peikos *et al.* [14] asked students to examine the behavior of water on different surfaces to identify surfaces that exhibit the lotus effect on different leaves and textiles. Chen *et al.* [11] and Lin *et al.* [12] used a paper cup smoked at the bottom by a candle to coat it with carbon particles. After turning it upside down, water droplets were poured

onto the surface, allowing students to observe the rolling behavior of the water, demonstrating the lotus effect. Observation tools, such as magnifying lenses and microscopes, have been used, allowing students to observe small objects in greater detail [7] [14] [15].

Models were also a common instructional practice for introducing science [23] [24]. The use of models was included in the Big Ideas (*i.e.*, Big Idea 8). They were found to be a useful and effective strategy for representing nanoscale structures. Models were used to represent the lotus effect [13] [14], water purification using nanoporous filters [14], the surface to volume ratio [13] as well as nanotubes (related also to Big Idea 2) [15]. In addition to using or creating models, one study also addressed the epistemological aspects of models, specifically their nature and role. For example, students should understand that models are not replicas of reality but representations with specific purposes. Literature suggests that explicit instruction on these epistemological aspects can help students grasp abstract concepts more effectively [14] [24].

Several studies included digital tools to support students' learning of NST concepts. Digital games using Scratch [25] have been created to visualize the interaction of nano, micro, and macroworlds in the case of viral infection [8] [9]. Augmented reality applications have been used to support students in exploring NST concepts such as the lotus effect, viral infection, and water nano filters [14]. Moreover, digital scenarios in the Go-Lab platform have been implemented to support students exploring NST concepts [7]. Other multimedia resources, such as videos, have introduced students to NST topics [13]-[15].

Furthermore, non-formal education settings have been developed to introduce NST topics. Museum-based science centers have incorporated various nano-based activities for children, including workstations, posters, 3D models, and nano-products, allowing them to engage in hands-on experimentation [15]. Moreover, through camp activities, the Nanotechnology-based Popular Science Education Promotion and Teaching (NPSEPT) program provided children with hands-on experiences, including visits to a nanotechnology exhibition space featuring models and NST products useful in daily life [12]. In addition, students have been allowed to contact nanotechnology researchers via teleconference to discuss nanotechnology and RRI issues [13].

Values and Knowledge Education (V*a*KE) [22] addresses ethical issues in knowledge acquisition. In the context of NST education, Pnevmatikos and Christodoulou [20] [21] integrated a moral dilemma concerning the impact of nano-coatings on human health. Nano-coatings involve applying nanoscale materials onto surfaces to provide enhanced properties, such as increased durability, resistance to water, anti-bacterial effects, or UV protection. Due to their small particle size, nano-coatings are often used in various consumer products, where they can improve product longevity or hygiene. However, these tiny particles also raise questions about potential health risks, especially if inhaled or absorbed through the skin. For students to be competent and offer viable arguments in the discussions, engaging in additional inquiry and hands-on activities was imperative to promote their knowledge acquisition of NST concepts. Engaging in inquiry-based activities (e.g., experimentation with physical manipulatives and models) enabled students to gather concrete scientific evidence about nano-coatings, allowing them to argue about their impact on health.

8.2.3. Effectiveness of Educational Interventions

The studies conducted to instruct NST in primary school students showed positive results regarding the effectiveness of the educational interventions.

In kindergarten, based on pre- and post-interviews and students' drawings, the researchers categorized students' answers into four levels of success. They highlighted that, before instruction, most students' answers reflected alternative views or were vague or denoted ignorance (*i.e.*, "Do not Know" answers). After the instruction, most students provided scientific, or partly scientific views, demonstrating a medium learning gain based on the Hake gain. For instance, regarding the gecko effect, before instruction, a student attributed the gecko effect to suction cups (alternative view). In contrast, after instruction, a student's drawings depicted the abundance of setae that branch into spatulae and the large contact surface [7].

In lower primary school, a quasi-experimental study explored whether young children's understanding of NST concepts, such as the size of objects and viral infections, improved after using educational software on tablets (experimental group) compared to an alternative experiential teaching approach, specifically theatre play (control group). The difference in the children's performance in each

group across the pre-post measurements was statistically significant. Further analysis revealed that the mean improvement in the experimental group was significantly higher than in the control group [9]. Along the same line, an experimental study explored the effects of digital technology on understanding NST concepts (related to the size of objects and the viral infection) [8]. The first experimental group used computers with educational software to teach NST-related concepts, while the second used tablets with the same software. The control group, in contrast, employed theatre play as a teaching method. The researchers found that all groups improved the children's understanding of NST concepts after the educational interventions. However, students in the tablet-based experimental group showed significantly higher learning outcomes than those in the other groups. This suggests that the interactive environment provided by tablets, including visualizations and touchscreen features such as the ability to manipulate nanoscale objects and receive haptic feedback, could foster students' engagement and active participation. A multimedia environment that allows young children to engage cognitively in ways that resemble everyday life experiences and represent information in multiple forms can significantly enhance their level of nano-literacy in their early years [8] [9].

Studies in upper primary school have shown promising results regarding students' understanding of NST concepts after participating in educational interventions. In non-formal education, the research found that students who visited a museum exhibition related to NST concepts performed significantly better when comparing group mean to those who did not attend the exhibition. The interactive exhibits, which encouraged play and hands-on activities, helped simplify the complexity of NST and supported young students' kinaesthetic needs, making the learning process more enjoyable and appropriate for a primary school level [15]. Researchers investigated the effectiveness of an NST-related camp program for elementary school students. Based on pre-post posttests, the researchers found a significant difference in students' academic outcomes in NST-related tasks. In particular, the topics "Nanophenomena in nature" and "Definition, characteristics, and applications of nanotechnology" were the topics where the students achieved the highest post scores. The researchers attribute this finding to the relevance of the concepts to the students' lives, as they involve nanoscale structures and phenomena that exist in nature and are linked to commercial products [15].

Classroom implementations of educational interventions on NST examined the effectiveness of the expositive-teaching and the experiential-teaching approaches to students' learning based on a quasi-experiment design. Experiential teaching proved more effective, as the hands-on activities helped students understand the lotus effect and recognize related commercial products. However, other concepts, such as nanoparticles and nanotubes (related to Big Idea 2), were more challenging for students to grasp [11]. Yu and Jen [16] explored the effectiveness of an NST educational program in enhancing the learning of gifted primary school students. They found a significant improvement in students' knowledge of NST concepts compared to their initial understanding. For example, after the program, students correctly identified the lotus leaf surface as "super-hydrophobic". They understood that rolling water droplets carry away dust particles due to the leaf's self-cleaning properties. Additionally, they identified products that mimic the lotus effect, showcasing their improved comprehension of NST applications [16]. Mandrikas *et al.* [13] implemented a Teaching Learning Sequence (TLS) in primary students to investigate whether primary school students can understand basic NST concepts and determine which Responsible Research and Innovation (RRI) dimensions students primarily focus on when introduced to NST ideas. Based on students' worksheets and group interviews following the implementation of the TLS, the researchers concluded that the TLS effectively enhanced students' understanding of NST concepts [13]. For example, most students distinguished between the macroscale, microscale, and nanoscale and accurately ordered objects according to their size. Additionally, they correctly classified materials as hydrophilic, hydrophobic, or superhydrophobic and explained the change of properties in the nanoscale based on surface area/volume ratio. Concerning the RRI issues, the researchers observed that primary students better understood Ethics, Engagement, and Science Education. However, issues like Gender Equality, Open Access, and Governance received less attention [13].

Another TLS, designed within the Framework Theory [18] and conceptual change approach, explored whether it is feasible to teach non-visible entities at the nanoscale and processes to primary school students [14]. The researchers analyzed students' conceptions of NST concepts before the implementation of the TLS, after the implementation, and three months later. They found that, concerning the size of objects, students were aware of both visible and non-visible objects but could

not classify them into clear categories. The TLS helped students develop the lateral categories of macro, micro, and nano world objects based on the observation tools that make each world accessible (*i.e.*, the unaided eye, the optical microscope, and the electron microscope, respectively). Regarding size-dependent properties, specifically the lotus effect, students' preconceptions indicated that they viewed the effect as a direct process (*i.e.*, a single identifiable agent causing an outcome) and focused primarily on perceptual-based explanations. The TLS helped students approach the lotus effect either as an emergent process caused by multiple agents with equal importance, such as the leaf's nanostructure and the trapped air in the interstitial spaces, or as a transitional state between direct and emergent processes, where students identified a correct NST related agent such as the nanostructure. Regarding explaining the nanoporous water filtration process, after instruction, students shifted from linear causal reasoning based on inaccurate agents to relational causal reasoning, incorporating correct information, such as linking the size of the nanostructures to the size of the objects being excluded. In all the NST concepts mentioned, the improvement in students' understanding after implementing the TLS was significant and, in most cases, remained evident three months later [14].

Aside from exploring students' understanding of NST concepts, a study also examined the educational significance of NST [17]. Specifically, after participating in NST-related lessons, students were interviewed and asked questions such as, "How would you feel about other students participating in the nanotechnology course?" and "What did you think about the nanotechnology course?". The students recognized NST as an innovative subject relevant to everyday life as there are already available NST applications that could improve quality of life. For example, a student mentioned, "*I liked that we learned about the nanofilter. If there were no nonscientists, the children from Africa would not have clean water. I don't say that now they all have clean water, but efforts are being made*" [17] (p. 7). Moreover, students valued their participation in the NST courses because they were informed about potential future careers. As one student stated, "*Because it can be something that may gain my interest at this age and will make me continue when I grow up. Maybe I want to become a scientist in the field of nanotechnology*" [17] (p. 8).

8.3. Understanding NST in Lower Secondary Education

Eight studies published between 2009 and 2024 in international journals examined NST topics in six areas: Greece, Israel, Spain, Taiwan (China), Turkey, and the USA. The earlier appearance of interventions on NST in Secondary education shows that scholars had higher expectations for the effectiveness of NST instruction in Secondary (students aged 12-15) than in Primary Education settings. The interventions were focused on the size scale notion and surface-area-to-volume ratio and their role in ordinary applications. Similarly, these papers highlighted the successful integration of NST concepts into secondary education.

8.3.1. NST Big Ideas

Similarly to the studies conducted with primary school children, the concept of size and scale, namely the Big Idea 1, was identified in most of the interventions with secondary school students [26]-[31]. Students often find it challenging to comprehend nano-sized objects and the nanoscale, so even in middle school, it is a concept that requires focus.

Students were tasked with understanding that the nanoscale is much smaller than even the smallest visible objects (*i.e.*, Big Idea 1), that unique devices are required to cut and work with nanoscale objects, and qualitatively group and order objects of different scales. For example, in their intervention, Blonder and Sakhnini [27] tasked students to cut the smallest piece of paper they could and secretly write the name of a nanoscale object. This activity helped them understand and categorize nanometric sizes and scales. Another task involved repeatedly cutting a 150 mm × 5 mm strip of paper in half, illustrating how the size exponentially decreases, though they could not reach the nanoscale. Students also watched a video where two kids explained nanotechnology, size, and scale, using human hair as a hands-on model to demonstrate the concept of nanoscale and its significance in a relatable and visual way. Similar activities were included in the intervention implemented by Stavrou and colleagues [31]. Students are tasked with arranging in descending order pictures of various objects by size, from 1 meter to 10^{-9} meters. Another activity required them to cut a 1-meter paper strip into 10 equal pieces and then cut each of those pieces into 10 smaller parts, continuing this process. Additionally, students were asked to build structures using blocks while wearing kitchen gloves and to understand the importance of using appropriately

sized tools.

Closely linked to size and scale is the understanding of the surface-area-to-volume (SA/V) (related to Big Idea 5) ratio motion, as many of the properties that matter exhibit on the nanoscale are due to changes in the surface-area-to-volume ratio as size decreases. This concept was addressed in studies carried out by Taylor and Jones [32], Blonder and Sakhini [27], and Stavrou and colleagues [31]. Taylor and Jones [32] utilized raw, granulated, and powdered sugar so that students could study the impact of surface area on sugar adhesion to surfaces. A group discussion explored the plausibility of creatures, such as King Kong or giant insects, existence in reality. Additionally, paper and potato cubes were employed to examine how the surface area-to-volume ratio serves as a limiting factor in cell size. Lastly, a competition was held where participants constructed giant insects using materials like straws and paper clips to demonstrate how mass can restrict size. Blonder and Sakhini [27] asked students to relate the concept of surface-area-to-volume ratio (SA/V) to familiar situations, such as the heat radiators (*i.e.*, the larger surface areas allow for more efficient heat distribution in a room) and the way elephants regulate their body temperature (*i.e.*, they have large ears with a high surface area relative to their volume). This connection helped students model the SA/V concept to real-world scenarios, enhancing their comprehension and retention of the nanomaterial. Stavrou and colleagues [31] asked students to observe the behavior of a steel nail, steel wire, and steel wool when subjected to burning. Further, they examined how a whole potato and a similar potato cut into smaller pieces reacted with hydrogen peroxide (H_2O_2). They cut a cube into eight smaller cubes and then into 27 smaller cubes to further support students' explanations.

Another concept addressed in Lower Secondary is the size-dependent properties of various materials (*i.e.*, Big Idea 5). Stavrou *et al.* [31] and Guasch *et al.* [33] clarified how material properties change with size at the nanoscale. Stavrou *et al.* [31] examined the behavior of textiles (hydrophilic vs. hydrophobic), with the discussion drawing upon examples that enhance students' understanding of how these materials interact with moisture. Further, the phenomenon of color changes on the nanoscale was addressed, specifically gold colloids, with students investigating how varying colors result from differences in the particles' size at the

nanoscale. Guasch and colleagues [33] tasked students with various activities to identify and memorize its main properties and applications and design new applications that take advantage of them.

Another central focus in NST education for Lower Secondary education is the tools and instrumentation (*i.e.*, Big Idea 7), like the Atomic Force Microscope (AFM), that is appropriate to observe the nanoscale and nano-sized objects, which was exploited to facilitate the introduction and understanding of the size and scale concept (see [30] [31]). The study by Stavrou and colleagues [31] employed the "black box" analogy to engage students in exploring the concept of indirect observation, which is essential for understanding nanotechnology and tools like the AFM. Placing objects of varying heights inside a cardboard box with holes allowed students to identify and illustrate the contents using tools such as straws and markers. With this activity, students had to infer the characteristics of hidden objects based solely on their interactions with the box. Following this initial exploration, a model of the AFM was introduced, constructed with a magnet, a CD, and a laser pointer. This transition illustrated the principles of the AFM, which also operates on indirect observation, using a laser beam to scan surfaces and produce images of nanoscale structures. By graphing the reflected laser beam from a metal-capped surface, students gained practical experience in how the AFM functions, reinforcing their understanding of how nanotechnology can be utilized to analyze materials at the nanoscale. In the study by Laszcz and Dalvi [30], the AFM was introduced through a LEGO model, which demonstrated the movement of its internal components. This hands-on experience was enhanced by a computer interface that allowed students to visualize an image of the scanned sample surface. LEGO made the tool accessible and relatable, enabling students to see how different parts work together. Following the demonstration, students constructed their own AFM models using various materials, fostering a deeper understanding of its components and operations.

Only one study addressed the concept of ethics and the impact of NST in society (*i.e.*, Big Idea 9). Stavrou and colleagues [31] also targeted the potential risks of nanotechnology (NST). Students discussed the possible risks and benefits of NST applications, emphasizing that as an emerging science, the effects of nanotechnology may not be immediately evident and that research on its risks is still in its

early stages.

It is worth mentioning that studies aiming at NST in Lower Secondary Education focus on applications of nanotechnology in everyday life and nature (*i.e.*, Big Idea 9). Blonder and Sakhnini [27] highlighted that by connecting nanoscience and technology (NST) concepts to products and innovations that students are already familiar with—such as the iPhone's battery longevity, self-cleaning materials, and Nano X-ray cancer therapies—educators can enhance students' understanding of the underlying scientific principles driving these advancements. Cheng and colleagues [28] investigated the Lotus effect (related to Big Idea 5) and its application in nature, the nano-magnetic particle and biological navigation, and the change of color on the wings of butterflies and other insects. Further, Cavdar and colleagues [26] employed common and everyday items with water-repellent and dirt-resistant properties while sometimes incorporating energy generation. Solutions included a water-repellent roof that generates energy from water flow, a dirt-resistant dress for easier clothing care, and a durable water-repellent notebook. They also developed water and dirt-resistant glass for improved home furnishings or windows. For outdoor enthusiasts, a water-repellent tent with built-in energy generation was designed. These innovations demonstrate how nanotechnology can improve daily life by making objects more resistant to environmental factors, more durable, and, in some cases, capable of producing their own energy. This approach demystifies complex scientific concepts and emphasizes their practical implications in everyday life. For instance, discussing how nanoscale innovations improve the efficiency of common devices or how they contribute to advancements in healthcare (e.g., [27]) allowed students to see the relevance of their studies beyond the classroom. Such contextualization fosters a deeper engagement with the educational material, as students are more likely to appreciate the significance of NST when they can relate it to their personal experiences and observations. Moreover, by integrating nanotechnology into the curriculum, educators can significantly improve students' awareness of the importance of science and technology in addressing real-world challenges.

8.3.2. Educational Approaches

Interventions in secondary education implemented a wide range of instructional methods and strategies to present NST concepts. These methods are usually

combined to create rich, engaging learning experiences. Some studies included more active learning approaches, engaging students directly with the material through hands-on activities and experiences. Specifically, we identified hands-on learning [26]-[28] [30]-[32], interactive learning [33], inquiry-based learning [32], project-based learning [26] [27] [29] and learning through participatory design [33]. For instance, Stavrou and colleagues [31] used hands-on learning through experimentation to realize that the surface-to-volume ratio increases as the size of an object diminishes. Blonder and Sakhnini [27] employed project-based learning, as students were guided in developing their final projects, which included presentations and posters. They chose one application to present to an audience of education stakeholders. These projects aimed to enhance students' understanding of nanotechnology concepts while encouraging self-learning. Guasch and colleagues [33] followed a participatory design approach for the ideation and design of a new application for graphene. Students, along with the facilitator of the intervention, collaborated to create a new product, illustrating it on a map and highlighting the product's key features.

Another group of teaching approaches included approaches relevant to technology-enhanced learning. In these studies, the researchers employed digital tools and media to support and enhance the learning process, such as visualizations and multimedia [27], movies [27], simulations [27] [29] and power-point presentations [28]. In the study by Blonder and Sakhnini [27], students watched five movies. One movie provided an overview of Nanotechnology, and the second discussed Nanotechnology's dimensions, applications in industry, and importance. The third showcased the mechanical properties of nanomaterials (*i.e.*, carbon nanotubes), the fourth was about the self-cleaning properties of nanomaterials, and the last one was an explanation of the mechanism behind self-cleaning materials and their everyday applications. Delgado and colleagues [29] employed custom-made simulations in their study. Such an example was the simulation that iterated small objects across a pinhead at 10 per second while a 2000× microscope projected images of human hair and bacteria, helping students estimate bacterial sizes.

Along with technology-enhanced learning approaches, researchers frequently combined the implementation of models [27] [29]-[31], which allow students to

represent and explore the NST concepts fostering conceptual understanding. An example of this is the study by Laszcz and Dalvi [30]. The authors employed a LEGO-built Atomic Force Microscope (AFM) model to help students visualize and understand how scientists can see and manipulate objects at the nanoscale. Stavrou and colleagues [31] implemented model-based learning in their study to help students understand how the AFM was constructed and functioning.

Further, in some studies, we identified game-based methods, such as game-based learning [27], gamification [33], storytelling, and narratives [27]. Such approaches aimed at enhancing students' engagement and motivation. Blonder and Sakhnini [27] incorporated three game-based activities. In the first one, students repeatedly cut a strip of paper until it was as small as a nanometer, experiencing the challenge of reaching the nanoscale. The second game-based activity was "Nanoquest," a 3D computer game where students chose characters to navigate a crystal landscape, collecting parts to build a nano car, making the learning process both fun and engaging. In the last one, students used a model of atoms arranged carbon atoms on a magic ball, observing how unstable configurations led to gathering and collapsing due to surface area energy. Also, the researchers implemented the storytelling and narrative approach in one of the intervention modules by having the teacher share her research experience in a nano lab.

Finally, some studies combined traditional instructional approaches with previous instructional approaches, such as lectures (e.g., [26]) and oral presentations and demonstrations (e.g., [28]) with other instructional approaches. Cavdar and colleagues [26] used lectures, although not explicitly named as such, to inform the students about the structure of matter, nanoscale, nanomaterials, and nanotechnology over two weeks.

8.3.3. Effectiveness of Educational Interventions

Scholars implementing NST in secondary education reported improvements in students' conceptual understanding. Blonder and Sakhnini [27] revealed notable advancements in students' understanding of nanotechnology through three data collection methods. In the Nano Scraps, students initially struggled to identify nano-scale objects without examples. After the intervention, 86% successfully named nano-scale objects, indicating significant improvement. Interviews with eleven students showed a shift in attitude towards the topic. Initially fearful of its

complexity, students found nanotechnology engaging as they progressed. High achievers reported positive experiences, while average achievers acknowledged initial difficulties with concepts like size and Surface-Area-to-Volume Ratio yet recognized the value of varied teaching methods. Below-average students also transitioned from viewing the subject as unreasonable to appreciating diverse instructional approaches. Finally, the content analysis of the final projects revealed that students effectively integrated learned concepts. Most of the 36 posters included presentations on nanotechnology applications, explanations of size and scale, and Surface-Area-to-Volume Ratio concepts. All groups utilized multiple teaching methods in their presentations, reinforcing those various instructional strategies that greatly enhanced students' comprehension of complex nanotechnology topics.

Cavdar and colleagues [26] customized the TRIZ-STEM activities for nanotechnology education. They demonstrated that the activities significantly improved participants' views on the nature of engineering, as evidenced by a large effect size (Cohen's d = 0.82). Further, qualitative results showed enhanced understanding and positive perceptions of nanotechnology, with participants generating more ideas and applications related to nanotechnology despite facing challenges in product development and material acquisition.

Cheng and colleagues [28] revealed that after the intervention, students in the experimental group (Integrated Nanotechnology Science Curriculum) showed significantly higher understanding of integrating nanotechnology knowledge than the control group (Standard Curriculum). Additionally, situational interest was significantly higher in the experimental group. Qualitative data from interviews indicated that hands-on experiments and the novelty of nanoscale phenomena were pivotal in fostering students' situational interest. For example, one student noted, "*Experiment is to understand new things, is to inquire and impress"*, highlighting the meaningfulness of hands-on activities. Another remarked, "*Hands-on is fun, more interesting*", emphasizing enjoyment. Additionally, a student stated, "*I just like the feelings of hands-on and experiment; I can't see the phenomena of nanoscale in the book before",* which reflects the novelty and engagement that experiments provided.

Moreover, Delgado *et al.* [29] reported that the learning gains of their

intervention were statistically significant for the concept of size and scale (smallest object, smallest unit, ordering, absolute size) with a large effect size (Cohen's $d = 0.79$). Compared to high school peers, participants closed gaps in knowledge of the smallest object and absolute size. They also excelled in small measurement units.

Guasch and colleagues [33] examined students' perceived understanding of NST concepts rather than their actual understanding. Perceived understanding refers to how well students believe they grasp the material, which can differ significantly from their actual comprehension. They reported that 42.6% of students had a positive view of NST regarding knowledge acquisition. Notably, the aspects that students appreciated most were their insights into graphene and nanomaterials and the information's suitability to their learning level.

Laszcz and Dalvi [30] reported that before the implemented module, 78% of students denoted ignorance (*i.e.*, "*I do not know*" answer) to their crucial question about why nanotechnology is important. After the module, 85% of students provided answers that mentioned the importance of size and scale, with many also mentioning specific examples and applications related to NST. The authors considered this a significant shift, showing that students had a better conceptual understanding after the implementation. Moreover, the modeling task using the LEGO AFM prototypes fostered collaboration and understanding of subsystems, with students describing components through gestures when they struggled with terminology. This hands-on approach was vital, as one student noted the model was '*kid-friendly and helped me visualize the real thing'.*

Furthermore, Stavrou *et al.* [31] described their results as encouraging. Specifically, they mention that students struggled to understand the size of non-visible objects, except for molecules and atoms, and had difficulty grasping the relative sizes of items between atoms and visible objects. Regarding the tools and instrumentation, initially, students believed everyday tools could handle nanoscale objects. After the implemented activities, they recognized the need for specialized tools to observe nanoscale phenomena, such as AFM models. Furthermore, students initially thought the properties of objects were fixed. However, according to the authors, with guidance, they began to understand that properties can change with size, particularly the surface area to volume ratio and optical properties in

nanoscale materials. Finally, regarding risk assessment, students displayed polarized views on nanotechnology. However, they ultimately agreed on the necessity of a balanced, informed approach to assess its benefits and risks, emphasizing the importance of foundational knowledge.

Finally, Taylor and Jones [32] highlighted a robust link between proportional reasoning and the understanding of surface area to volume relationships, suggesting that solid reasoning skills enhance conceptual understanding of this concept. The increased correct responses in practical applications indicated that students retained knowledge and could transfer it to real-world scenarios. However, the small sample size limits the generalizability of these findings.

8.4. Public Understanding of Nanotechnology

Understanding and public perception of nanotechnology are pivotal for its successful integration into society. The research underscores that nanotechnology is yet to achieve comprehensive public understanding or trust. This research, conducted in countries such as Canada, France, Norway, and Taiwan (China), provides a view of the current landscape concerning media representations, citizen understanding and attitudes, and expectations from nanotechnology.

8.4.1. NST Big Ideas

The studies demonstrated that citizens do not understand basic nanotechnology concepts. For example, a survey in Taiwan (China) [34] indicated a significant gap, with approximately 70% of respondents struggling to grasp basic concepts of nanotechnology, such as size and scale, the structure of matter, the size-dependent properties, forces and interaction at the nano level, and tools and instrumentation. Familiarity with nanotechnology was notably low in French [35] and Canadian [36] studies. These findings underscore the urgent need for public education on nanotechnology. Gilbert and Lin [1] suggested that a realistic expectation from intervention in adults could be at the “functionally nano-literate” level, corresponding to the accepted understanding of the nine “big ideas” [2] with an effort to deconstruct the relevant misconceptions about them, and meaningful discussions of a limited range of nanophenomena.

Despite the lack of comprehension, participants expressed both high perceived benefits and risks associated with nanotechnology [34]. On the other hand, the

French public exhibited ambivalence and skepticism towards nanofood and nanotechnology in food packaging [35]. Increasing knowledge about food risks and nanotechnology could enhance support for nanofood packaging, but it proved insufficient for the broad acceptance of nanofood. Their skepticism does not derive from their deep understanding of nanotechnology as an innovative, scientific achievement with potential risks [34] nor from the media, which feeds their skepticism towards nanotechnology. On the contrary, [37], analyzing representations of nanotechnology in Norwegian newspapers, revealed that over 60% of articles depicted it positively, focusing on beneficial consumer products and promising future applications. The newspapers emphasized a future-oriented perspective, often portraying nanotechnology as revolutionary and its advancement as inevitable while assuring audiences that scientists possess precise control over potential societal impacts.

However, public skepticism toward NST is influenced by factors extending beyond the realm of nanotechnology itself. A notable finding is that a general distrust in government and industry significantly impacts the relationship between trust levels and risk perception. According to [34], individuals distrusting these entities tend to perceive greater risks associated with nanotechnology. This suggests that fostering trust in governmental and industrial stakeholders may be essential to mitigating public concerns and promoting a more positive acceptance of nanotechnology advancements. Conversely, positive attitudes toward science were associated with greater support for its applications [35]. This suggests that fostering a favorable view of science can enhance public acceptance and enthusiasm for scientific advancements, including those in nanotechnology.

These findings illustrate the vital role of social factors, demonstrating that trust in government and individual attitudes toward science and technology is critical for fostering public acceptance of nanotechnology products. This underscores the idea that public perception is influenced not just by the scientific merits of nanotechnology but also by the broader societal context in which it exists.

8.4.2. Approaches to NST Understanding in Public

A participatory research study in Edmonton, Canada [36] explored the local responses of 104 participants representing the local public, experts, policy, and industry elites focusing on fostering innovation and collaboration within the

nanotechnology sector within a city-region context.

Researchers followed a participatory research approach, including focus groups, a Citizen Summit, walking tours, and an interactive science museum exhibition. The study showed a lack of awareness and understanding of nanoscience within the Edmonton community, particularly among those outside research institutions. Moreover, the study identified significant barriers to collaboration among stakeholders and highlighted the importance of place-based solidarity. The study emphasizes the importance of public engagement and deliberation in nanotechnology-involved research networks, commercial entities, and community organizations collaborating on nanotechnology advancements.

The nanotechnology networking in Edmonton, Canada, underscores that engaging the public is essential for developing and accepting nanotechnology innovations. This engagement can occur through various methods involving community members and stakeholders in the innovation process. It suggests that place-based initiatives aiming to involve the public in the research process actively (e.g., focus groups, Citizen Summits, walking tours, interactive science museum exhibitions where visitors can interact with nanotechnology displays) can effectively foster a shared understanding of nanotechnology's opportunities and challenges.

These methods facilitate dialogue among researchers, developers, policymakers, and the public, ensuring that the innovation process is integrated with the broader community. This approach cultivates a sense of shared purpose and connection within the local community while enhancing support for and understanding of nanoscience products. Conscious brokering involves mediating and facilitating interactions between different stakeholders to build trust and integrate diverse perspectives into the development of nanotechnology.

However, for meaningful public involvement, presenting a broader range of perspectives, including potential risks and ethical considerations, is essential to stimulate critical reflection and debate. Ethical communication must ensure transparency and accountability. As Kjølberg [37] noticed, the dominant positive narratives in media may discourage public participation by fostering a sense of security and inevitability, thereby reducing the perceived need for engagement. Many scholars have stressed ethical issues across various applications, necessitating thorough and continuous ethical deliberation. For instance, the potential health

risks associated with nanomaterials and unpredictable ecosystem effects require thorough long-term studies to understand their implications [38]. Moreover, the potential for enhanced surveillance capabilities through nano-enabled devices raises significant ethical issues and privacy concerns [38].

The public's demand to be informed about their choices' social and ethical consequences is crucial. The ethical aspects of nanotechnology should be shared with the public to assess their benefits and risks, ensuring the public is fully informed about the presence and potential risks of nanomaterials, especially in sensitive areas like food [35] and medicine and other consumer products [39]. This demand cannot be attributed to irrational emotionalism or poor communication regarding their work on the part of scientists [40]. There is a critical need for balanced media representations of nanotechnology to support ethical public engagement, preventing misinformation and undue fear. Addressing these ethical issues involves transparent, balanced, and inclusive communication strategies that could build public trust and engagement in nanotechnology developments.

These strategies are justified by the evidence that public misconceptions and distrust can hinder nanotechnology's acceptance and responsible use. Addressing these through targeted education (even from primary and secondary education), balanced information, ethical considerations, and active public engagement is imperative.

8.5. Discussion of Nanoscience and Nanotechnology (NST) Education

Introducing NST concepts in early education is beneficial, as it fosters an early interest in science and improves understanding of complex concepts. **Figure 1** summarizes the number of studies implementing concepts related to Big Ideas found in studies involving educational interventions in primary and lower secondary education.

Interventions focused more on the concepts of Size and Scale (Big Idea 1), Size-dependent Properties (Big Idea 5) and Science, Technology, and Society (Big Idea 9). Size and Scale is fundamental for understanding the other Big Ideas [2], as it introduces students to the scale at which NST is performed [41]. Additionally, Science, Technology, and Society (Big Idea 9), related to NST applications, provides a

meaningful context for students to explore how these applications function, making the underlying science more relevant and meaningful [42]. Blonder and Yonai [41] suggest that these two concepts are well-suited for introduction in any NST-related program where students are not yet familiar with NST concepts. The size-dependent properties concept introduces a novel perspective within the NST Big Ideas framework [2], which is crucial for students to understand the unique properties of matter at the nanoscale [41].

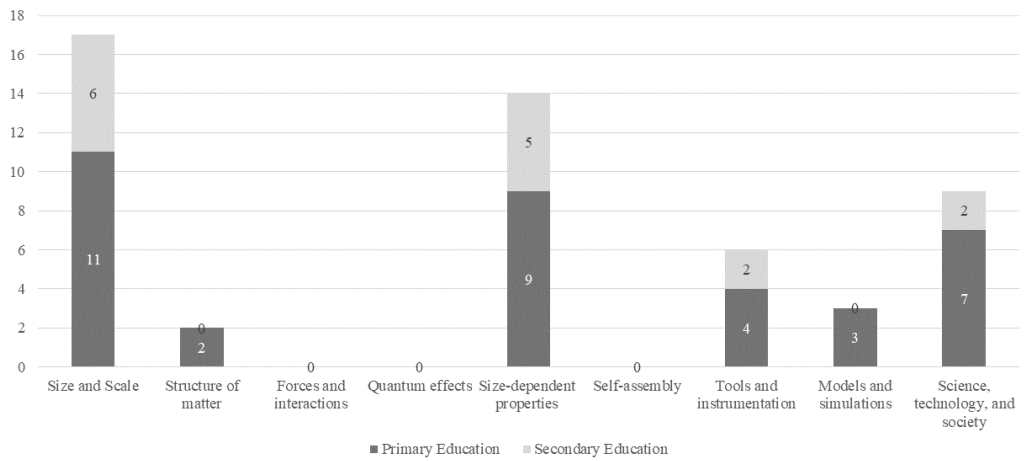

Figure 1. Number of Concepts Related to Big Ideas in Studies Involving Educational Interventions in Primary and Lower Secondary Education.

The Big Idea "Tools and Instrumentation" (Big Idea 7) appears frequently in studies, as it supports students in exploring the NST field using tools to measure and observe at the nanoscale [41]. "Models and Simulations" (Big Idea 8) and Structure of Matter (Big Idea 2) were addressed less frequently. Three of the Big Ideas, namely "Forces and Interactions" (Big Idea 3), "Quantum Effects" (Big Idea 4), and "Self-Assembly" (Big Idea 6), were not approached at these two educational levels. These Big Ideas, along with the "Structure of Matter" (Big Idea 2), are recognized by scholars as challenging to implement in educational settings due to their highly complex content [43].

Across all three levels, the concept of size and scale at the nanoscale is central. Primary education focuses on qualitative understanding (grouping and ordering objects by size) and introducing concepts like the lotus effect and its underlying

nanostructures [8] [9] [11]-[16]. In contrast, lower secondary education builds upon this with a more quantitative approach, including the surface-area-to-volume ratio and its implications [27] [31] [32]. Public understanding, however, reveals a significant knowledge gap, with many individuals struggling to grasp even basic NST concepts such as size and scale [34].

Studies showing promising results from interventions focusing on the sizes of nano-objects and visualization tools, as well as the gecko effect, demonstrate the feasibility of teaching NST even in kindergarten [7]. These early interventions helped students identify how objects appear differently under varying observation tools, highlighting nano-objects scale compared to macro and micro-world objects [7].

Various teaching approaches, primarily hands-on and digital tools, significantly enhance learning outcomes. Primary education utilizes hands-on experiments and digital tools like animations and simulations to make abstract concepts tangible [8] [9] [12]-[14] [16]. Lower secondary education incorporates various methods, project-based assignments, and advanced digital tools such as visualizations and multimedia [27] and movies [27]. Public understanding initiatives include science fairs, museum exhibits, and media campaigns to bridge the gap between formal education and public awareness [36] [37]. Future educational strategies should continue to adapt and incorporate innovative methods to maintain and build on these successes. Promoting NST literacy from a young age can lay a solid foundation for future scientific learning and ethical considerations, potentially influencing career paths and addressing societal challenges associated with emerging technologies.

A key scaffolding technique in approaching NST concepts at all levels is the strategic use of models and simulations. In primary education, these tools help represent complex nanostructures, like those responsible for the lotus effect, and illustrate processes such as water purification using nanoporous filters [13]-[15]. This approach effectively bridges the gap between abstract concepts and tangible representations, making NST concepts accessible to young learners. The progression from qualitative [28] to quantitative understanding [26] [28] [29] through scaffolding ensures a solid foundation for more advanced learning in secondary education. Digital tools like games and augmented reality applications greatly

enhance scaffolding by increasing engagement and enabling interactive exploration of nanoscale phenomena. These technologies allow users to visualize and manipulate complex concepts, making learning more dynamic and enjoyable [27] [29] [30] [33].

Studies on primary education show positive results in improving students' understanding of NST concepts, particularly when using experiential learning and digital tools (e.g., [9] [11] [13] [14]). Similar improvements in understanding are reported in lower secondary education, with interventions leading to statistically significant learning gains [26] [28] [29] [32]. However, public understanding interventions show that while public awareness may be raised through various means, a comprehensive understanding of basic NST concepts remains limited [34]-[36].

Nevertheless, in evaluating the success of the interventions, it is crucial to consider the effect size of these findings. Notably, only a few studies reported effect sizes, which are essential for quantifying the impact of the findings. This gap highlights a significant shortcoming in documenting the practical significance of NST interventions. While improvements in students' conceptual understanding have been shown, the absence of reported effect sizes—or the failure to provide information that could be calculated—makes it challenging to assess the magnitude of these improvements in a standardized and comparable manner. Therefore, we emphasize the necessity for future research to consistently report effect sizes, enabling a more robust interpretation of the practical significance of educational interventions.

Ethical considerations are explicitly addressed in primary education, focusing on Responsible Research and Innovation (RRI) issues, such as the production and use of nanomaterials and the need for ethical standards in scientific research [13], while the V*a*KE method is used to combine knowledge about NST and addressing ethical issues connected with this knowledge [20] [21]. Lower secondary education also touches upon nanotechnology's ethical and societal implications, showing that improved understanding enhances awareness of broader consequences. Public understanding discussions emphasize the crucial role of public trust in government and industry in shaping perceptions of nanotechnology risks, alongside the need for balanced media representation and transparent communication

regarding the potential risks and benefits [40]. Ethical concerns regarding health risks, environmental impacts, and privacy implications of nanotechnology are also highlighted [38].

8.6. Conclusion

This chapter has demonstrated the feasibility and benefits of integrating nanoscience and nanotechnology (NST) concepts into primary and lower secondary education and fostering public understanding. Across all three levels, the foundational concept of size and scale at the nanoscale proved central, though the approach—from qualitative to quantitative—varied depending on the age and cognitive abilities of the learners. While primary education successfully employed hands-on experiments [11]-[14] and digital tools [7]-[9] [14] [15] [25] to build foundational understanding, lower secondary education built upon this with inquiry-based learning and advanced digital tools (e.g., [27]). While raising awareness, public understanding initiatives revealed a significant knowledge gap in basic NST concepts [35] [36], highlighting the need for targeted and sustained communication strategies. Importantly, ethical considerations—particularly around responsible research and innovation (RRI) and the societal implications of nanotechnology—were integrated into both educational levels, underscoring the importance of responsible development and application [31]. The findings underscore the need for a holistic approach to NST education, encompassing formal and informal settings, to cultivate informed citizens who can engage critically and responsibly with the opportunities and challenges of nanotechnology in the 21st century.

References

[1] Gilbert, J. K., & Lin, H. S. (2013). How Might Adults Learn About New Science and Technology? The Case of Nanoscience and Nanotechnology. *International Journal of Science Education, Part B, 3*(3), 267-292. https://doi.org/10.1080/21548455.2012.736035

[2] Stevens, S.Y., Sutherland, L.M., & Krajcik, J.S. (2009). *The big ideas of nanoscale science & engineering: A guidebook for secondary teachers*. Arlington, VA: National Science Teacher Association Press.

[3] Demetriou, A., Spanoudis, G., Greiff, S., Panaoura, R., Vainikainen, M. P., Kazi, S., & Makris, N. (2024). *Educating the developing mind: A developmental*

theory of instruction. Taylor & Francis.

[4] Dorouka, P., Papadakis, S., & Kalogiannakis, M. (2021). Nanotechnology and mobile learning: perspectives and opportunities in young children's education. *International Journal of Technology Enhanced Learning*, *13*(3), 237-252. https://doi.org/10.1504/IJTEL.2021.10036680

[5] Ghattas, N. I., & Carver, J. S. (2012). Integrating nanotechnology into school education: a review of the literature. *Research in Science & Technological Education*, *30*(3), 271-284. https://doi.org/10.1080/02635143.2012.732058

[6] Winkelmann, K., & Bhushan, B. (2017). Global Perspectives of Nanotechnology Education. In B. Bhushan (Ed.), *Springer Handbook of Nanotechnology* (pp. 1603-1624). Springer.

[7] Manoloudi, M., & Lefkos, I. (2023). Nanotechnology in Kindergarten. Is there any Learning Gain using an ICT-based Approach? *International Journal for Digital Society*, *14*(1), 1868-1872. https://doi.org/10.20533/ijds.2040.2570.2023.0234

[8] Dorouka, P., & Kalogiannakis, M. (2023). Teaching nanotechnology concepts in early-primary education: an experimental study using digital games. *International Journal of Science Education*. https://doi.org/10.1080/09500693.2023.2286299

[9] Dorouka, P., Kalogiannakis, M., & Blonder, R. (2024). Tablets and Apps for Promoting Nanoliteracy in Early Childhood Education: Results from an Experimental Study. *Journal of Science Education and Technology*. https://doi.org/10.1007/s10956-024-10132-w

[10] Spyrtou, A., Manou, L., Peikos, G., & Papadopoulou, P. (2018). Inquiring the Nanoscale secrets (in Greek). Gutenberg.

[11] Chen, Y. Y., Lu, C. C., & Sung, C. C. (2012). Inquire learning effects to elementary school students' nanotechnology instructions. In *Asia-Pacific Forum on Science Learning and Teaching* (Vol. 13, No. 1, pp. 1-18). The Education University of Hong Kong, Department of Science and Environmental Studies.

[12] Lin, S.-Y., Wu, M.-T., Cho, Y.-I., & Chen, H.-H. (2015). The effectiveness of a popular science promotion program on nanotechnology for elementary school students in I-Lan City. *Research in Science & Technological Education*,

33(1), 22-37. https://doi.org/10.1080/02635143.2014.971733

[13] Mandrikas, A., Michailidi, E., & Stavrou, D. (2020). Teaching nanotechnology in primary education. *Research in Science & Technological Education*, *38*(4), 377-395. https://doi.org/10.1080/02635143.2019.1631783

[14] Peikos, G., Spyrtou, A., Pnevmatikos, D., & Papadopoulou, P. (2022). A teaching learning sequence on nanoscience and nanotechnology content at primary school level: evaluation of students' learning. *International Journal of Science Education*, *44*(12), 1932-1957. https://doi.org/10.1080/09500693.2022.2105976

[15] Saidi, T., & Sigauke, E. (2017). The Use of Museum Based Science Centres to Expose Primary School Students in Developing Countries to Abstract and Complex Concepts of Nanoscience and Nanotechnology. *Journal of Science Education and Technology*, *26*(5), 470-480. https://doi.org/10.1007/s10956-017-9692-2

[16] Yu, H.-P., & Jen, E. (2020). Integrating Nanotechnology in the Science Curriculum for Elementary High-Ability Students in Taiwan: Evidenced-Based Lessons. *Roeper Review*, *42*(1), 38-48. https://doi.org/10.1080/02783193.2019.1690078

[17] Spyrtou, A., Manou, L., & Peikos, G. (2021). Educational Significance of Nanoscience-Nanotechnology: Primary School Teachers' and Students' Voices after a Training Program. *Education Sciences*, *11*, 724. https://doi.org/10.3390/educsci11110724

[18] Vosniadou, S. (2013). Conceptual change research: An introduction. In International Handbook of Research on Conceptual Change (pp. 1-7). Routledge.

[19] Peikos, G., Spyrtou, A., Pnevmatikos, D., & Papadopoulou, P. (2023). Nanoscale science and technology education: primary school students' preconceptions of the lotus effect and the concept of size. *Research in Science & Technological Education*, *41*(1), 89-106. https://doi.org/10.1080/02635143.2020.1841149

[20] Pnevmatikos, D., & Christodoulou, P. (2018). Promoting conceptual change through Values and Knowledge Education (VaKE). In A. Weinberger, H. Biedermann, J-L. Patry, & S. Weyringer (Eds.). *Professionals' Ethos and*

Education for Responsibility, (pp. 63-74). Brill-Sense. https://doi.org/10.1163/9789004367326_005

[21] Pnevmatikos, D., & Christodoulou, P. (2022). Values and Knowledge Education meets conceptual change for Science Education. In S. Weyringer, J-L. Patry, D. Pnevmatikos, and F. Brossard - Borhaug (Eds.), *The VaKE Handbook: Theory and Practice of Values and Knowledge Education* (pp. 111-119). Brill.

[22] Weyringer, S., Patry, J-L., Pnevmatikos, D., Brossard Børhaug, F. (2022). *The VaKE Handbook: Theory and Practice of Values and Knowledge*. Brill.

[23] Gilbert, J. K., & Boulter, C. J. (Eds.). (2000). *Developing Models in Science Education*. Springer.

[24] Zoupidis, A., Pnevmatikos, D., Spyrtou, A., & Kariotoglou, P. (2016). The impact of the acquisition of Control of Variables Strategy and nature of models in floating-sinking phenomena reasoning and understanding of density as property of materials. *Instructional Science, 44*(4), 315-334. https://doi.org/10.1007/s11251-016-9375-z

[25] Maloney, J., Resnick, M., Rusk, N., Silverman, B., & Eastmond, E. (2010). The scratch programming language and environment. *ACM Transactions on Computing Education (TOCE)*, *10*(4), 1-15. https://doi.org/10.1145/1868358.1868363

[26] Cavdar, O., Yıldırım, B., Kaya, E., & Akkus, A. (2024). Exploring the Nanoworld: Middle School Students Use TRIZ-STEM in Nanotechnology Education. *Journal of Chemical Education*, *101* (3), 1049-1061. https://doi.org/10.1021/acs.jchemed.3c01031

[27] Blonder, R., & Sakhnini, S. (2012). Teaching two basic nanotechnology concepts in secondary school by using a variety of teaching methods. *Chemistry Education Research and Practice*, *13* (4), 500-516. https://doi.org/10.1039/C2RP20026K

[28] Cheng, J. C., Hung, J. F., & Huang, T. C. (2014). Promoting middle school students' understanding and situational interest in integrating nanotechnology into science curriculum. *US-China Education Review, 4(1)*, 48-53.

[29] Delgado, C., Stevens, S. Y., Shin, N., & Krajcik, J. (2015). A middle school instructional unit for size and scale contextualized in nanotechnology. *Nanotechnology Reviews*, *4* (1). https://doi.org/10.1515/ntrev-2014-0023

[30] Laszcz, M., & Dalvi, T. (2021). Studying the affordances of a technology-based nanoscience module to promote student engagement in learning novel nanoscience and nanotechnology concepts at the middle school level. *Research in Science & Technological Education*, *41* (2), 700-716. https://doi.org/10.1080/02635143.2021.1931833

[31] Stavrou, D., Michailidi, E., Sgouros, G., & Dimitriadi, K. (2015). Teaching high-school students nanoscience and nanotechnology. *Lumat: International Journal of Math, Science and Technology Education*, *3*(4), 501-511. https://doi.org/10.31129/lumat.v3i4.1019

[32] Taylor, A., & Jones, G. (2009). Proportional Reasoning Ability and Concepts of Scale: Surface area to volume relationships in science. *International Journal of Science Education*, *31*(9), 1231-1247. https://doi.org/10.1080/09500690802017545

[33] Guasch, B., González, M., & Cortiñas, S. (2020). Educational toolkit based on design methodologies to promote scientific knowledge transfer in secondary schools: A graphene-centered case study. *Journal of Technology and Science Education*, *10* (1), 17. https://doi.org/10.3926/jotse.787

[34] Lin, S. F., Lin, H. S., & Wu, Y. Y. (2013). Validation and exploration of instruments for assessing public knowledge of and attitudes toward nanotechnology. *Journal of Science Education and Technology, 22*, 548-559. https://doi.org/10.1007/s10956-012-9413-9

[35] Vandermoere, F., Blanchemanche, S., Bieberstein, A., Marette, S., & Roosen, J. (2011). The public understanding of nanotechnology in the food domain: the hidden role of views on science, technology, and nature. *Public Understanding of Science, 20*(2), 195-206. https://doi.org/10.1177/0963662509350139

[36] Shields, R., & Jones, K. E. (2024). Place and community responses to opportunity: an example from nanoscience innovation. *Innovation: The European Journal of Social Science Research, 37*(1), 152-166. https://doi.org/10.1080/13511610.2020.1822154

[37] Kjølberg, K. L. (2009). Representations of nanotechnology in Norwegian newspapers—implications for public participation. *Nanoethics, 3*, 61-72. https://doi.org/10.1007/s11569-008-0053-8

[38] Pilarski, L. M., Mehta, M. D., Caulfield, T., Kaler, K. V., & Backhouse, C. J. (2004). Microsystems and nanoscience for biomedical applications: a view to the future. *Bulletin of Science, Technology & Society, 24*(1), 40-45. https://doi.org/10.1177/0270467604263120

[39] Pouliot, C. (2015). What students and researchers in nanoscience and nanotechnology should know about PUS and STS: a look at Fages and Albe's viewpoint on social issues in nanoscience and nanotechnology Master's degrees. *Cultural Studies of Science Education, 10*, 459-467. https://doi.org/10.1007/s11422-014-9591-2

[40] Kent, J. (2003). Lay experts and the politics of breast implants. *Public Understanding of Science, 12*, 403-421. https://doi.org/10.1177/0963662503124005

[41] Blonder, R., & Yonai, E. (2020). Exposing school students to nanoscience: A review of published programs. In K. Sattler (Ed.), 21st century nanoscience-a handbook: Public policy, education, and global trends (Vol. 10, ch.9). CRC Press. https://bookshelf.vitalsource.com/

[42] Sakhnini, S., & Blonder, R. (2016). Nanotechnology applications as a context for teaching the essential concepts of NST. International Journal of Science Education, 38(3), 521-538. https://doi.org/10.1080/09500693.2016.1152518

[43] Manou, L., Spyrtou, A., Hatzikraniotis, E., & Kariotoglou, P. (2022). What does "Nanoscience-Nanotechnology" mean to primary school teachers? *International Journal of Science and Mathematics Education, 20*(6), 1269-1290. https://doi.org/10.1007/s10763-021-10199-6

Chapter 9

Post-COVID-19 Advancing Targeted Drug Delivery (TDD): Literature Insights and Market Dynamics

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Abstract: This chapter investigates the evolving role of targeted drug delivery (TDD) in the biotech and pharmaceutical industries, emphasizing the importance of predictive accuracy and interdisciplinary collaboration in optimizing drug delivery systems (DDSs). We explore various nanocarriers, including liposomes and dendrimers, and their structural features, highlighting their interactions within physiological barriers, such as the blood-brain barrier (BBB). Emphasizing the multifaceted applications of nanotechnology in TDD, we also address scalability challenges that arise when transitioning from lab-scale research to industrial-scale production. The COVID-19 pandemic accelerated the need for innovative DDSs, and our discussion underscores the importance of collaborative partnerships and regulatory compliance. We leverage the Analytic Hierarchy Process (AHP) to evaluate socioeconomic factors affecting TDD market expansion, highlighting the integration of predictive models with experimental data. Moreover, Eroom's Law is considered within the context of declining pharmaceutical R&D efficiency, arguing that TDD's advancements in personalized medicine and precision drug targeting are key to overcoming these inefficiencies. This paper concludes by reflecting on ethical considerations in in-vivo and in-vitro evaluations

and suggests that AI-driven methods are pivotal in advancing TDD research.

Keywords: Targeted Drug Delivery (TDD), Nanocarriers, Liposomes, Dendrimers, Predictive Accuracy, Pharmacokinetics (PK), Pharmacodynamics (PD), Scalability, Industrial Scale-Up, Blood-Brain Barrier (BBB), COVID-19, Analytic Hierarchy Process (AHP), Eroom's Law, Personalized Medicine, Precision Drug Targeting, AI-Driven Drug Delivery

9.1. Introduction

The biotech industry's niche, particularly companies specializing in targeted drug delivery (TDD), has undergone profound transformations over the past decade. The COVID-19 pandemic from 2020 to 2022 accelerated many of these changes, intensifying competition as new players entered the market. The pandemic's global economic impact was significant, with a sharp decline in services trade, which fell by over 20% in 2020, as noted by the **International Monetary Fund's (IMF) World Economic Outlook (April 2021)** [1]. This decline, compounded by supply chain disruptions and rising inflation, heightened the urgency for innovation within the healthcare sector, particularly in TDD.

The **World Economic Forum's (WEF) 2023 Annual Report** [2] further underscores global healthcare systems' critical challenges. Persisting inequities, barriers to innovation, and global health risks like climate change threaten societal well-being. In response, the WEF's Centre for Health and Healthcare has prioritized transforming health systems and improving healthcare access. These goals align with the broader objectives of TDD technologies, which aim to enhance precision in drug delivery while addressing cost-effectiveness and system resilience. The center's collaboration with global organizations such as the **Coalition for Epidemic Preparedness Innovations (CEPI)** and the **World Health Organization (WHO)** highlights the importance of partnerships in advancing personalized medicine and targeted therapies.

As part of its commitment to health equity, the WEF's **Zero Health Gaps Pledge**, signed by over 100 global leaders, seeks to drive investment in research and development, particularly in underserved regions. This initiative creates a favorable environment for the development of cutting-edge health technologies like TDD, which have the potential to reduce healthcare disparities by offering more

efficient and accessible treatment options.

Europe's response to these challenges has been multifaceted. As **Luis de Guindos**, Vice-President of the **European Central Bank (ECB)** [3]**, emphasized**, Europe's economic recovery hinges on three key goals: recovery, renewal, and resilience. These goals mirror the needs of the biotech sector, particularly in fostering innovation in TDD. The exceptional fiscal and monetary stimulus provided by the ECB has played a crucial role in stabilizing the market, allowing sectors like biotech to continue innovating despite inflationary pressures and supply chain challenges. However, Guindos also cautions against the potential systemic risks of prolonged monetary accommodation, which could impact long-term economic stability.

In line with these broader economic trends, the **European Union (EU)** has implemented several policies to support the biotech industry throughout the pandemic. While specific fiscal measures targeting TDD have been limited, the **Pharmaceutical Strategy for Europe** (2020) and establishing the **European Innovation Council (EIC)** in 2021 have laid the groundwork for innovation across healthcare sectors. These initiatives aim to foster cross-sectoral collaboration, particularly in digital and green technologies, which are relevant to the future of TDD.

The economic impact of the pandemic has also revealed significant weaknesses in the EU's conventional economic governance, as **Luo (2022)** [4] **argued**. The introduction of the **EU Recovery Fund** marks a paradigm shift, moving towards deeper fiscal integration within the Eurozone. This shift has potential implications for the biotech industry, where increased financial resources and institutional support can foster innovation, particularly in TDD. Luo warns, however, that failure to implement the recovery fund effectively could lead to further fragmentation and rising anti-EU populism [4]. This underscores the importance of aligning industry innovations with broader European goals for the TDD sector, ensuring that health-sector advancements contribute to national and EU objectives.

In this context, the EU's ongoing green transition, as outlined in the **Future of Jobs Report 2023** [5], further shapes the healthcare landscape. The shift toward sustainability—driven by investments in renewable energy, ESG standards, and climate adaptation—is expected to create significant new opportunities within the biotech and pharmaceutical sectors. Integrating green principles into drug

development and delivery systems, including TDD, aligns with these global trends toward sustainability. By reducing environmental impact and improving cost-efficiency, TDD technologies could play a key role in fostering more sustainable healthcare practices in the years to come.

9.2. Innovation in Drug Delivery (DD) Technologies

The development of drug delivery systems (DDSs) has significantly advanced in recent years, driven by the need to improve drug administration's efficacy, precision, and sustainability. Nanotechnology has emerged as a cornerstone in these advancements, as **Jadhav *et al.* (2024)** [6] noted, highlighting the unique physicochemical properties of nanomaterials that make them ideal for use in TDD systems. Nanomaterials facilitate the encapsulation of drugs, allowing for controlled release and targeted delivery, which is particularly beneficial in minimizing adverse effects and increasing therapeutic efficacy. In this context, bioeconomic strategies emphasize fostering innovation in DDS technologies, including integrating carbon-based, silica-based, and iron oxide nanostructures.

The rise of nanomaterials in DDS development aligns with the broader trends in the pharmaceutical industry, where interdisciplinary collaboration and technological innovation are essential. Synthetic nanomaterials offer superior biocompatibility, easy preparation methods, and storage stability, making them preferable in designing new drug carriers [6]. These advancements are particularly relevant to bioeconomic strategies in TDD, where the emphasis is on creating novel, efficient, and environmentally sustainable drug delivery systems.

Bioeconomic strategies in TDD bring together experts from various fields, such as medicine, biotechnology, engineering, and computational science, to collaborate on cutting-edge projects. Interdisciplinary partnerships are essential in advancing TDD technologies, particularly in the context of nanotechnology. The ability of nanomaterials to carry therapeutic molecules—ranging from small drugs to nucleic acids and proteins—has opened new avenues for collaboration across scientific disciplines, fostering innovation in targeted therapies. Nanomaterials—lipids and polymers to inorganic particles—have become vehicles for functional RNAs. These include messenger RNA (mRNA), small interfering RNA (siRNA), single guide RNA (sgRNA), and microRNA. Nanomaterials can enhance RNA stability and precisely guide it to its destination [7]. Partnerships between research

institutions, pharmaceutical companies, and technological firms are instrumental in accelerating the development of novel DDSs, such as antibody-drug conjugates (ADCs), which combine the selectivity of monoclonal antibodies with the potency of cytotoxic agents.

Sustainability is a critical consideration in TDD bioeconomic strategies. Using biodegradable materials in drug delivery systems and green synthesis methods ensures that DDSs minimize environmental impact. Drawing on insights from **Curran (2013)** [8], the life cycle assessment (LCA) approach can help evaluate the environmental impact of TDD systems, from raw material extraction to disposal. Incorporating biodegradable or recyclable materials into DDSs aligns with the principles of bioeconomics, promoting a circular economy and reducing waste. Additionally, sourcing materials locally and utilizing renewable resources contribute to more sustainable practices in producing nanomaterials for drug delivery [8].

Nanomaterial-based DDSs provide promising solutions in TDD by offering controlled release mechanisms and reducing drug toxicity [6]. However, challenges remain, particularly in scaling production and ensuring consistent quality. Bioeconomic strategies can address these challenges by promoting sustainable resource utilization and encouraging the adoption of eco-friendly manufacturing processes.

Integrating computational science into TDD strategies is vital for optimizing drug delivery systems. Predictive simulations and computational models allow researchers to evaluate DDS performance before clinical trials, reducing the need for extensive in-vivo testing [9]. These models can predict the interaction of nanomaterials with biological systems, thereby refining the design of DDSs for better therapeutic outcomes [9]. In line with the bioeconomic approach, leveraging computational tools enhances the efficiency of drug development and reduces resource consumption and waste generation.

9.3. Multidrug Loading in Innovative Nanomedicines and Nano Vectors

Integrating **artificial intelligence (AI)**, **data science**, **rapid diagnostics**, **telehealth**, and **computational simulations** has marked a transformative phase in Targeted Drug Delivery (TDD) development. These advancements significantly improve the precision, personalization, and efficiency of Drug Delivery Systems

(DDSs), directly enhancing patient outcomes. As noted by **Ashique and Sandhu (2021)** [10], TDD focuses on improving the delivery of medications to specific target sites in the body, minimizing side effects, reducing the incidence of dose dumping, and preventing dosage form failure. This selective targeting ensures that drugs interact only with their intended sites of action, sparing non-target organs, tissues, or cells from undesirable effects.

Recent trends in TDD development have focused on several innovative drug delivery methods [10], including:

- Nanotubes
- Bioadhesive systems
- Osmotic controlled delivery
- Prodrugs
- Pulsincap
- Solid lipid nanoparticles
- Folate targeting
- Gene delivery
- Brain targeting
- Colon targeting

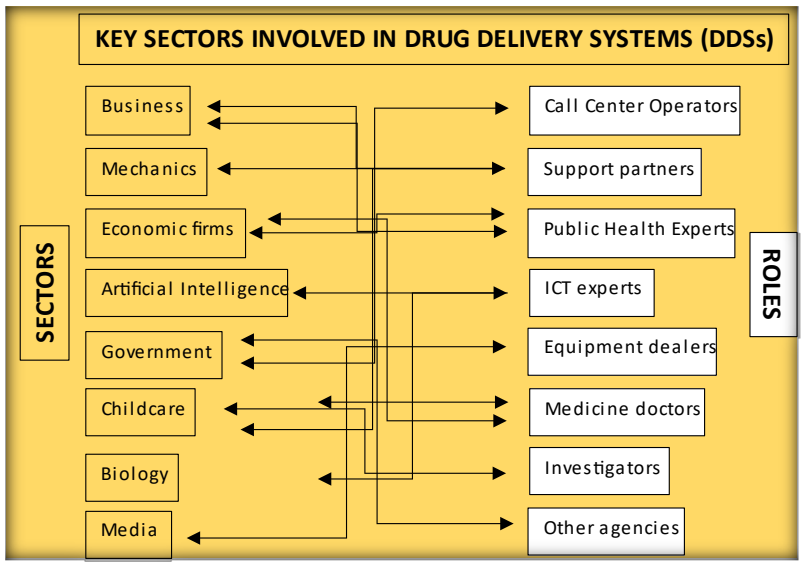
Initially, TDD relied heavily on **in-vivo** methodologies to assess the efficacy of various delivery systems. As **Tewabe *et al.* (2021)** [11] point out, early TDD research involved testing in live organisms—through methods like tissue sampling and animal models—to understand the pharmacokinetics (PK) of drugs and their interactions with target tissues. These in-vivo studies were essential in establishing baseline knowledge for TDD systems, but they often involved time-consuming trial-and-error procedures.

In recent years, however, the rise of AI and computational simulations has revolutionized the development and evaluation of TDD techniques. **Choudhary *et al.* (2022)** [12] emphasize that AI, mainly through **Machine Learning (ML)** and **Deep Learning (DL)**, is transforming how drug delivery systems are designed and tested. These technologies enable researchers to predict drugs' efficacy and potential side effects more accurately, reducing reliance on in-vivo testing. Moreover, AI algorithms help optimize drug delivery routes, minimizing errors and speeding up therapy procedures, as noted by **Wilson *et al.* (2022)** [13].

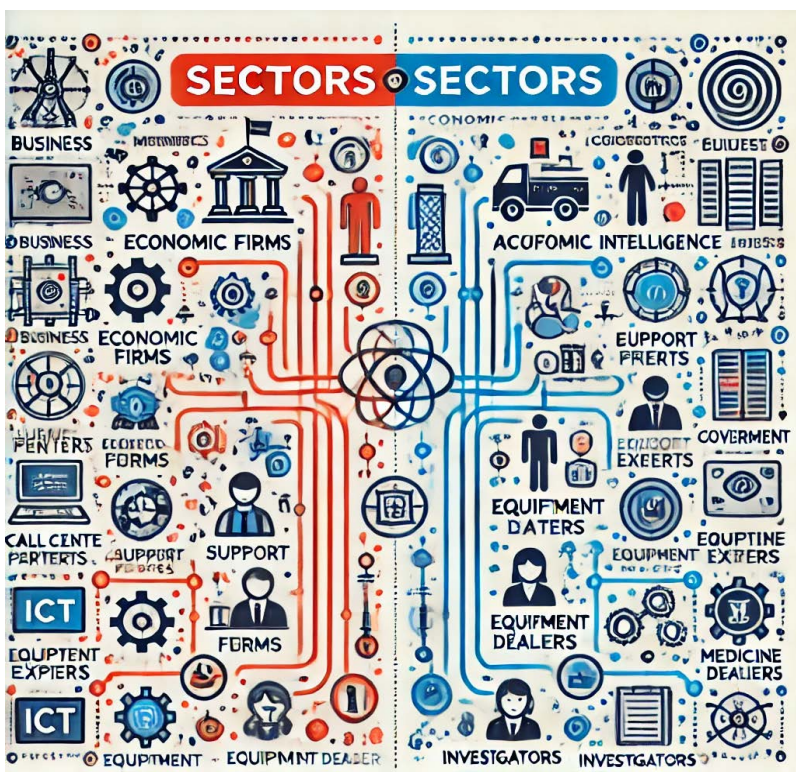
One of the most transformative computational methods applied to TDD is **Computer-Aided Drug Design (CADD)**. As **Shukla and Tripathi (2021)** [14] described, CADD accelerates drug discovery by allowing compounds to pass through preclinical testing phases more efficiently. At the heart of this approach is **Molecular Dynamics (MD) simulation**, a tool that simulates the behavior of drug-target complexes in real-time. MD simulations offer critical insights into molecules' conformational flexibility and interactions with biological targets, providing researchers with a virtual platform to optimize drug designs. This method has significantly improved the accuracy of predicting how drugs perform in clinical settings.

In the evolving landscape of TDD, the incorporation of (i) data science, (ii) rapid diagnostic tests, (iii) mobile-first telehealth, and (iv) computational simulations have become indispensable for biotech laboratories. These advancements have expanded the possibilities for optimizing drug delivery and enhancing patient care:

- **Data Science**: As noted by **Greener *et al.* (2022)** [15], data science is crucial in analyzing large datasets related to patient demographics, clinical information, and genetic profiles. This allows for creating personalized treatment plans, including tailored drug delivery mechanisms.
- **Rapid Diagnostic Tests**: According to **Manzari *et al.* (2021)** [16], rapid diagnostic tests enable quick and accurate diagnosis, allowing healthcare providers to tailor TDD protocols based on the patient's specific medical condition, improving the overall effectiveness of treatments.
- **Mobile-First Telehealth**: **Haleem *et al.* (2021)** [17] emphasize the role of mobile-first telehealth platforms in enhancing TDD by enabling real-time communication between patients and healthcare providers. This reduces the need for in-person consultations and accelerates the development of effective treatment plans.
- **Computational Science**: **Hossain *et al.* (2019)** [18] highlight the importance of computational models and simulations in predicting the efficacy of targeted drug delivery. These models simulate drug-target interactions, helping researchers fine-tune dosage and delivery timing parameters to optimize treatment outcomes.



(a)



(b)

Figure 1. Sectors and roles involved in DDSs (drug delivery systems).

These advancements in TDD have led to a more data-driven and patient-centered approach to drug development, as visualized in **Figure 1(a) & Figure 1(b)** above. The figure outlines the key sectors and roles involved in DDSs, demonstrating the critical interplay between technology, data analysis, and drug delivery optimization. At the top (1a) is the original drawing by the authors, and below (1b) is the elaboration by DALL·E, an AI image generation model developed by OpenAI.

By incorporating these advanced computational simulations, researchers are better equipped to refine drug delivery parameters and ensure that therapeutic agents are delivered precisely to their intended targets, maximizing efficacy and minimizing adverse effects.

9.2.1. Nanocarriers in Biomedicine

Nanocarriers play a crucial role in biomedicine by offering advanced drug delivery systems (DDSs) with various applications. **Nanoparticles**, **nanostructures**, and **carbon nanotubes (CNTs)** bring unique advantages, extending their use beyond drug delivery into biomaterials, catalysis, tissue regeneration, and biosensors. Functionalization, a critical aspect of nanocarrier design, enhances biocompatibility and allows for specific drug delivery (DD) targeting [19].

Types of Nanocarriers:

- **Nanoparticles**: Particles between 1-100 nm, composed of metals, polymers, or ceramics, can be used for drug delivery, biomaterials, and catalysis.
- **Nanostructures**: These include a variety of nanoscale forms such as thin films, quantum dots, nanowires, and nanofibers, each offering unique surface properties [19].
- **Carbon Nanotubes (CNTs)**: Cylindrical carbon tubes exhibiting exceptional mechanical strength, surface-to-volume ratio, and high aspect ratio, making them attractive for drug delivery applications [19].

Applications in Biomedicine:

- **Nanoparticles and Nanostructures**:
  - *Surface Modification*: Enhancing cellular uptake and biocompatibility.

- *Drug Protection*: Protecting drugs from degradation within the bloodstream [19].

- **CNTs**:
  - *Functionalization*: Improving biocompatibility through surface modification.
  - *Targeted Drug Delivery*: Facilitating direct drug delivery to target cells [19].

Differences Between Nanoparticles, Nanostructures, and CNTs:

- **Nanoparticles**:
  - Defined by their nanometer-scale size.
  - Composed of materials such as metals, polymers, and ceramics.
  - Applications include drug delivery and catalysis [19].
- **Nanostructures**:
  - Broad class of nanoscale forms, including thin films and nanowires.
  - Exhibits enhanced surface area and quantum effects [19].
- **CNTs**:
  - Allotropes of carbon, presenting as cylindrical tubes.
  - Used in drug delivery, tissue regeneration, and biosensors [19].

Biomedical Applications of CNTs:

CNTs are particularly useful in **drug delivery** for their ability to deliver drug molecules to diseased tissues and cells selectively. Their surface chemistry can be tailored to increase biocompatibility and reduce immunogenicity. CNTs are also employed in tissue regeneration, biosensor diagnostics, and biochemical extraction [19].

Importance of Functionalization:

- **Nanoparticles and Nanostructures**: Surface modifications enhance biocompatibility and control over drug release [19].
- **CNTs**: Functionalization allows targeted drug delivery by attaching bioactive molecules or using other modifications like polyethylene glycol (PEG) [19].

9.2.1.1. Polymers

Polymers are large molecules composed of repeating monomer units divided into **natural** and **synthetic** categories. Biopolymers, derived from microbial systems or extracted from plants, have wide-ranging applications, from medical materials to bioplastics. Examples of natural biopolymers include proteins like silk and collagen, which are used in drug delivery and reconstructive surgeries due to their high tensile strength [20].

9.2.1.2. Carbon Nanotubes (CNTs) in Drug Delivery and Beyond

CNTs have revolutionized drug delivery (DD) due to their high surface area, mechanical strength, and ability to deliver drugs directly into cells. **CNTs** selectively deliver molecules to diseased cells while minimizing side effects. Their unique properties also make them ideal for **imaging technologies** in biomedicine. Key advancements include:

- **Improved Purification Methods**: Techniques such as acid treatment and sodium cholate-assisted dispersion help purify CNTs by removing impurities and increasing yield [20].
- **Enhanced Synthesis Methods**: Chemical vapor deposition (CVD), arc discharge, and laser ablation allow precise control over CNT properties [20].
- **Surface Functionalization**: Functionalizing CNTs with molecules like PEG enhances biocompatibility and reduces toxicity [20].

Due to their excellent electrical and mechanical properties, CNTs have been used in field-effect transistors, conductive films, transparent electrodes, and sensors [20].

CNTs are expensive for large-scale applications despite their many benefits, primarily due to raw materials and production costs. Other limitations include potential **chemical and physical incompatibilities** and side effects from dosage interactions [21].

9.2.1.3. Limitations and Toxicity of CNTs

CNTs have been associated with toxicity due to their **non-biodegradability**, which can lead to adverse cellular interactions [22]. Recent studies on **chiral carbon dots (CCDs)**—a new class of carbon-based nanomaterials—suggest they may

offer a **non-toxic** alternative to CNTs in biomedical applications like **drug delivery** and **imaging** [22].

Advancements in Drug Delivery:

- CNT-based drug delivery systems are advancing rapidly, particularly in **photodynamic therapy (PDT)**, **photothermal therapy (PTT)**, **chemodynamic therapy (CDT)**, and **sonodynamic therapy (SDT)** [23].
- Magnetic nanoparticles target tumors and enhance drug efficacy, with potential applications in dual imaging and therapeutic probes [24] (Salata, 2004).

CNTs are effective carriers for antimicrobial agents, contributing significantly to **tissue engineering** and **regenerative medicine** [25].

9.2.1.4. Nanoparticles in Targeted Drug Delivery (TDD)

Nanotubes used as nanocarriers are generally categorized into organic and inorganic. **Organic nanotubes** are widely studied for their potential in drug delivery (DD) and other biomedical applications. Examples include:

- **Porphyrazine Nanotubes**: These organic molecules form tubular structures through self-assembly and are promising for drug delivery and gene therapy.
- **Peptide Nanotubes**: Composed of short chains of amino acids, peptide nanotubes self-assemble and show potential in drug delivery, tissue engineering, and regenerative medicine.
- **DNA Nanotubes**: DNA molecules can be engineered to form nanotubes with defined shapes and sizes, offering potential DD and diagnostic imaging applications.
- **Carbon Nanotubes (CNTs)**: These cylindrical structures of carbon atoms arranged in a lattice possess unique electrical and mechanical properties with applications in electronics, energy storage, and DD.
- **Fulfillment Nanotubes**: Organic molecules that self-assemble into tubular structures and have potential applications in nanomedicine [26].

Inorganic nanocarriers, such as Super Paramagnetic Iron-Oxide Nanoparticles (SPIONs), Mesoporous Silica Nanoparticles, and Gold/Silver Nanoparticles, have limited use in drug delivery. This is largely due to their poor drug-loading

efficiency and potential peripheral toxicity, posing significant health risks [26]. Organic-based nanocarriers, by contrast, are composed of lipids, including micelles, liposomes, solid lipid nanoparticles (SLN), and archaeosomes. These lipid-based drug delivery systems are commonly used to transport hydrophobic drugs, encapsulating them to protect against degradation and avoid peripheral organ toxicity [26].

Despite the promising advancements in nanostructures, toxicity remains a significant concern. As **Ahmad *et al.* (2021)** [26] note, safer, practical implementations of these materials require careful design, processing operations, and packaging material development. Addressing these toxicity concerns, alongside understanding biological complexities and genetic factors, is critical to developing safe and effective drug delivery systems.

The ongoing research is focused on optimizing drug loading strategies and synthesis methods to overcome drug discovery and development challenges. **Lombardo *et al.* (2019)** [27] highlight that while various smart nanocarriers have been developed, the intrinsic complexity of biological environments significantly influences the functionality of nanomaterials, often complicating their practical use in treatments. The design of nanocarriers—precisely their size, shape, material substrate, and surface chemistry—is crucial for ensuring efficient drug delivery. **Chamundeeswari *et al.* (2019)** [28] emphasize that identifying these critical design factors is fundamental to deciphering the complexity of biological processes and creating effective nanocarriers.

9.2.1.5. Nanocellulose Materials in TDD

Cellulose is a renewable biopolymer found in various natural sources such as trees, tunicates, and bacteria. It is non-toxic, biodegradable, and thermally stable, making it suitable for various industrial and biomedical applications. In its most common form, cellulose is a linear polysaccharide with β-1,4-D-linked glucose chains, often derived from plants, algae, fungi, bacteria (bacterial cellulose), and animals (e.g., tunicin from marine animals) [26].

The extensive use of cellulose includes the production of textiles, pharmaceuticals, energy drinks, and industrial sugar. Its typical applications are found in products such as wood for construction, paper, cotton, linen, rayon for clothing, and

cellulose acetate for packaging films.

Structurally, cellulose consists of d-glucopyranose ring units linked by β-1,4-glycosidic bonds. It exists in two polymorphs: **cellulose I (native cellulose)** and **cellulose II**, which result in semicrystalline structures stabilized by hydrogen bonds. Although insoluble in water, cellulose can absorb water (8-14% at 60% relative humidity, 20°C) and react with concentrated acids and strong bases. **Cellulose Nanocrystals (CNCs)**, produced via acid hydrolysis of cellulosic materials, possess properties like high tensile strength, colloidal stability, and ease of modification, making them promising for drug delivery applications [26].

Chitosan, another natural polysaccharide derived from marine crustaceans, is also biocompatible and biodegradable. Its cationic character at neutral and physiological pH, modified solubility, and mechanical properties make it useful for various biomedical and pharmaceutical applications, including drug delivery systems [26].

9.2.1.6. Phytochemicals as Antimicrobial Agents

Phytochemicals are natural compounds in plants, widely recognized for their antimicrobial properties. Historically, phytochemicals have been used in traditional medicine, and today, they are increasingly being studied for their ability to inhibit or kill microorganisms such as bacteria, fungi, and viruses [29]. Phytochemicals present a more comprehensive range of antimicrobial activities with fewer side effects than traditional antibiotics.

Nanocarriers containing phytochemicals have enhanced bioavailability, stability, and targeting efficiency. These nanocarriers include liposomes, solid lipid nanoparticles, polymeric nanoparticles, and metallic nanoparticles, which improve the delivery of phytochemicals to specific cells, tissues, or organs. Using colloidal systems in these nanocarriers increases the effectiveness of phytochemicals by improving their bioavailability and stability [26].

Mechanisms of Phytochemical Antimicrobial Action:

- Inhibition of microbial cell division and growth.
- Damage to microbial cell membranes.
- Interference with microbial metabolism.

- Prevention of microbial adherence to human cells [29].

Phytochemicals offer promising potential for combating microbial resistance. They provide a broader antimicrobial activity spectrum and have fewer side effects than conventional antibiotics. Phytochemicals with significant antimicrobial properties include **alkaloids**, **organosulfur compounds**, **phenolic compounds**, **coumarins**, and **terpenes** [29].

Antimicrobial Mode of Action:

- **Alkaloids** inhibit cell division and DNA synthesis in bacteria such as *Escherichia coli* and *Listeria spp.* [29].
- **Phenolic compounds** inhibit bacterial fatty acid biosynthesis, with Minimum Inhibitory Concentrations (MICs) for *Enterococcus faecalis* ranging from 128 to 512 μg/mL [29].
- **Organosulfur compounds** inhibit sulfhydryl-dependent enzymes and bacterial DNA synthesis [29].
- **Coumarins** inhibit DNA gyrase in *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [29].

Phytochemicals have demonstrated efficacy against waterborne pathogens, including *Salmonella*, *Vibrio cholerae*, *Shigella spp.*, *E. coli*, and *Yersinia enterocolitica*. They also show potential as alternative antibiotic-based growth promoters in livestock farming due to their lower risk of inducing antimicrobial resistance (AMR) [29].

Nanocarrier-based phytochemicals, particularly those using lipid-based nanocarriers, have shown enhanced stability, slower delivery, and improved cell permeability. These nanoformulations offer great potential in enhancing the efficacy of phytochemicals as antimicrobial agents [26].

9.2.1.7. Liposomes in TDD

1) Introduction to Liposomes

Liposomes are widely used as nanocarriers in Targeted Drug Delivery (TDD) for infectious diseases. These synthetic vesicles, composed of a lipid bilayer, can encapsulate hydrophilic and hydrophobic drugs. Due to their amphiphilic nature,

liposomes can incorporate various therapeutics, including antimicrobials, peptides, and proteins. They encapsulate the drug within the lipid bilayer, enabling controlled release and targeted delivery to infected cells or tissues [30].

Liposomes can address infectious diseases through two main mechanisms:

- **As Drug Delivery Systems (DDSs)**: Liposomes can encapsulate antimicrobial agents such as antibiotics and antivirals, ensuring targeted drug delivery to the infection site while protecting the drug from degradation or clearance in the body.
- **As Antimicrobial Agents**: Functionalized liposomes can interact specifically with pathogenic microorganisms, for instance, cationic liposomes that disrupt bacterial cell membranes through electrostatic interactions [30].

2) Liposomes for Infectious Diseases

Liposomes possess favorable **physicochemical properties** such as stability, biocompatibility, and biodegradability, enhancing their therapeutic potential. Several **Phytochemical Oral Delivery Systems (PODS)**, including micelles, emulsions, solid lipid nanoparticles, and liposomes, are now available for encapsulating phytochemicals. Each system has specific advantages and must be carefully optimized for compatibility with the final product matrix, considering factors such as pH, ionic strength, and thermal stability [30].

Additionally, liposomes can be modified to improve their in vivo performance. For example, **PEGylation** (attachment of polyethylene glycol chains) increases stability and circulation time, while **ligand-targeted liposomes** can be functionalized with antibodies, peptides, or carbohydrates to enable specific targeting [31].

Liposomes have been successfully used to encapsulate various drugs for treating infectious diseases, with notable examples including:

- **Liposomal Amphotericin B** for fungal infections.
- **Liposomal Doxycycline** for bacterial infections.
- **Liposomal Ganciclovir** for viral infections.

In veterinary medicine, liposomal formulations have also been developed to treat fungal, bacterial, and parasitic infections in animals, highlighting their broad

utility [31].

Liposomes offer several key benefits for drug delivery, including:

- Targeted drug delivery to specific cells or tissues.
- Enhanced drug stability and bioavailability.
- Reduced systemic side effects by ensuring drugs are delivered directly to the infection site.

Nanostructured liposomes, such as **PEGylated liposomes**, exhibit improved properties, including enhanced stability, circulation time, and the ability to carry a higher drug payload. **Theranostic liposomes**, which combine therapeutic and diagnostic capabilities, have also emerged as promising multifunctional systems for drug delivery in infectious diseases and cancer treatments [32].

Liposomes have also been employed in **Phytochemical Oral Delivery Systems (PODS)**, where they improve the stability and bioavailability of phytochemicals. **Nanostructured liposomes** provide enhanced delivery and retention of these compounds, which are often unstable in their natural forms. Phytochemicals such as **polyphenols**—widely studied in cancer models—are encapsulated in liposomes for their potential therapeutic effects, with early trials showing promising outcomes and minimal side effects [32].

9.3. COVID-19 Crisis and Biotech Laboratories in Drug Delivery (DD)

9.3.1. The EU Pandemic Response

9.3.1.1. Is Nanotechnology Good for Health?

Nanotechnology-based drug delivery systems (DDSs) have emerged as a promising alternative to conventional methods, offering several advantages, such as enhanced efficacy and targeted drug delivery [33]. While nanoparticles (NPs) are central to these nano-drug delivery systems, it is essential to note that not all NPs are designed exclusively for drug delivery. NPs have broader applications, including imaging, diagnostics, and therapeutic interventions. In the context of nano-DDSs, the focus is on the controlled and targeted delivery of therapeutic agents, which involves a complex interaction between the nanoparticles, drugs, and their behavior within the body.

One of the key goals of **intelligent nanoparticles** is to improve drug delivery, particularly in precision medicine. **Precision medicines** are tailored to individual patient characteristics, and optimizing their delivery can significantly enhance therapeutic outcomes. Accelerating the delivery of precision medicines from the laboratory to clinical settings is essential for swiftly bringing innovative treatments to patients, as highlighted by **Mitchell *et al.* (2021)** [34].

Despite these advancements, there is a notable gap in the regulatory guidelines for nano-drug delivery systems, stimuli-responsive delivery systems, and next-generation biomaterials. Developing and harmonizing regulatory standards is critical to ensuring the safety and efficacy of these advanced DDSs. Furthermore, continued research is required to understand better stimuli-responsive systems' behavior, toxicity, and specificity. The field can advance toward more precise and safer delivery systems by addressing these gaps.

Incorporating a diverse array of model-performance combinations in TDD within biotechnology is vital for:

1) Advancing precision medicine,
2) Optimizing drug delivery strategies, and
3) Ensuring the safety and efficacy of novel therapies.

Figure 2 provides a schematic illustration of a target drug release system in the cytosol and cell membrane, as depicted by **Sultana *et al.* (2022)** [33].

Nanotechnology is pivotal in advancing bioeconomic strategy, particularly in fields such as medicine, agriculture, and environmental sustainability. Nanotechnology has facilitated the development of targeted drug delivery systems, diagnostic tools, and imaging techniques in medicine. In agriculture, nanotechnology enhances crop production and pest control, aligning with the bioeconomic principle of utilizing sustainable and renewable resources.

While promising, these bioeconomic innovations face real-world challenges in market adoption and interdisciplinary collaboration. Understanding these challenges and opportunities comprehensively requires integrating perspectives from different fields, including chemistry, biology, medicine, and engineering. As noted by **Bröring *et al.* (2020)** [35], four key innovation trajectories (ITs) are specific to the bioeconomy, and they are directly applicable to sustainability-oriented innovations (SOIs) [35].

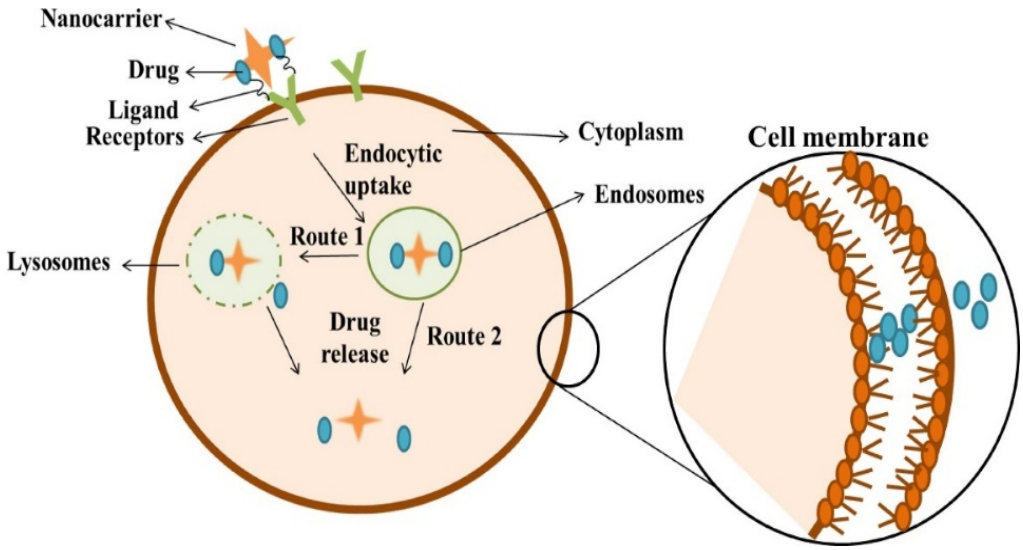


Figure 2. Schematic illustration of target drug release system in the cytosol and cell membrane. Source: Sultana *et al.* (2022), "Nano-based drug delivery systems: Conventional drug delivery routes, recent developments and future prospects," *Medicine in Drug Discovery*, May 2022, 100134, p. 4. Reprinted with permission.

Achieving these innovative trajectories in drug delivery involves collaboration across various disciplines. This interdisciplinary approach is particularly important when developing drug-loaded nanoparticles that prioritize using bio-based materials and sustainable production methods. For example, liposomes and dendrimers offer sustainable alternatives to traditional drug delivery systems that rely on non-renewable resources.

1) Systemic Routes and Communication Dynamics of Nanocarriers

Liposomes and **dendrimers** are essential nanocarriers in drug delivery, classified under "macromolecular structures." However, while "polymers" often refer to linear or branched macromolecules composed of repeating monomer units, liposomes and dendrimers have distinct structures. These unique architectures distinguish them from traditional polymers.

Polymers can be classified based on their charge characteristics, directly influencing their interactions with biological systems. For example, **cationic polymers** like polyethyleneimine (PEI) carry a net positive charge, allowing them to interact with negatively charged cell membranes. Conversely, **anionic polymers** like

sodium alginate have a net negative charge and can interact with positively charged surfaces. Such charge characteristics are crucial in drug delivery as they help stabilize nanoparticles and facilitate controlled drug release by forming electrostatic interactions with drug molecules [36].

Cationic polymers are often employed in **gene delivery**, interacting with negatively charged DNA to form complexes that cells can take up. This is particularly relevant in gene therapy applications where introducing genetic material into cells is necessary. Similarly, polymers are used in **tissue engineering** to influence cell adhesion and proliferation based on their surface charge [36].

2) Challenges for Drug Delivery (DD) in the Brain

The **Blood-Brain Barrier (BBB)**, a crucial protective barrier, presents significant challenges for drug delivery to the brain. The central nervous system (CNS) consists of the brain and spinal cord, and its protection is reinforced by barriers like the **choroid plexus epithelium** and the **avascular arachnoid epithelium** [36]. **Liposomes**, with their lipid bilayer structure mimicking cell membranes, can be modified (e.g., PEGylation) to enhance circulation time and evade immune recognition, thereby improving drug delivery across the BBB.

The BBB becomes more permeable under inflammatory conditions, such as **Alzheimer's Disease (AD)**, where immune responses alter the barrier's integrity, allowing immune cells and signaling molecules to access the brain. Understanding these communication dynamics is essential for developing effective therapeutic strategies targeting neurological disorders [36].

Introducing polymers or other nanomaterials into the body poses the risk of eliciting a **foreign body response**, leading to inflammation or forming fibrous capsules around the polymer. This can affect the polymer-based device's functionality and lifespan. Therefore, polymers used in drug delivery must be designed to minimize immunogenicity and unwanted immune responses. **Surface modifications**, such as **biocompatible** or **stealth coatings**, can help reduce immune system recognition and improve their efficacy [36].

PEGylated liposomes, for instance, improve BBB penetration by evading immune detection and promoting receptor-mediated endocytosis through ligands attached to the liposome surface. These ligands target receptors on BBB endothelial

cells, facilitating efficient drug delivery to the brain. However, safety and efficacy concerns remain, requiring careful consideration in developing nanoparticle-based drug delivery systems [36].

Dendrimers' highly branched, tree-like structures offer precise control over size and functionalization, making them ideal for drug encapsulation and targeted delivery. **Poly(amidoamine) (PAMAM) dendrimers** have been investigated for their ability to traverse the BBB, especially when coated with specific ligands that enhance receptor-mediated transport [37]. Dendrimers and liposomes differ significantly in their structure, with liposomes being lipid-based vesicles and dendrimers being synthetic macromolecules.

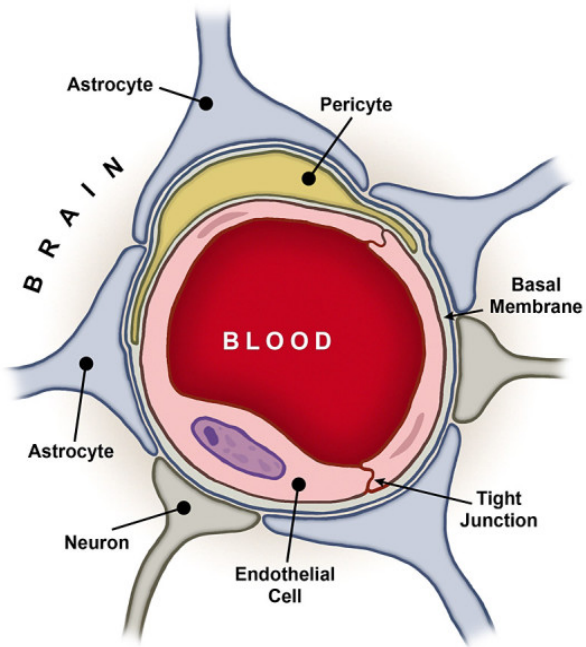


Figure 3. Brain Capillary Cross-Section. Source: Illustrations for the Bauer-Hartz Laboratory, University of Minnesota College of Pharmacy. Retrieved from: Capillary Cross-Section. With permission.

The challenge of delivering drugs to specific tissues extends beyond the CNS. For example, **bone-targeted drug delivery** faces difficulties in achieving

sufficient drug uptake. Nanoparticles and liposomes with **bone-targeting ligands** (such as bisphosphonates) are being explored to improve drug delivery to bone tissues. Similarly, the **Blood-Testis Barrier (BTB)**, which protects developing sperm cells, poses challenges in delivering drugs to testicular tissues. Nanoparticle-based systems and targeted drug conjugates are being investigated to overcome these barriers and achieve specific delivery [36].

Finally, the neurovascular unit (NVU) regulates intercellular communication within the CNS, consisting of endothelial cells, pericytes, astrocytes, and neurons. Various signaling molecules, including **cytokines** and **neurotransmitters**, play a crucial role in regulating drug delivery to the brain. Figure 3 above illustrates a cross-section of a brain capillary, showcasing the interconnected endothelial cells that form the BBB [36].

9.3.1.2. The Dual Role of TDD in Therapeutic-Theragnostic Medicine

Nanomaterials can serve dual roles in diagnostic (imaging) and therapeutic applications, known as **theragnostic medicine**. This approach integrates diagnostics and therapy into a single platform, enhancing the precision of treatments. Due to their nanoscale size and structure, nanomaterials offer a high surface area-to-volume ratio, allowing for a significant payload of imaging agents, such as contrast-enhancing molecules or nanoparticles, to be attached or encapsulated [38]. Nanomaterials can also be engineered to have multimodal capabilities, meaning they can support multiple imaging techniques simultaneously, such as **MRI (Magnetic Resonance Imaging)** and fluorescence imaging, providing complementary diagnostic information.

Nanomaterials can be **functionalized** by targeting ligands that selectively bind to specific biomolecules or cells, enhancing imaging specificity. This reduces background noise and improves the visualization of specific biological processes or locations [38].

Strategies for Targeted Drug Delivery (DD):

- **Nanoparticle-Based Systems**: Nanoparticles can encapsulate drugs and overcome biological barriers, enabling targeted delivery to specific tissues.
- **Ligand Targeting**: Attaching ligands to drug carriers allows them to bind to specific receptors on target cells or tissues, facilitating selective drug delivery.

- **Antibody-Drug Conjugates (ADCs)**: ADCs link a drug to a monoclonal antibody that recognizes specific antigens on target cells, commonly used in cancer therapy.
- **Localized Delivery Systems**: Implantable or localized systems allow sustained drug release at the target site, minimizing systemic exposure.
- **Surface Modification**: Modifying drug carrier surfaces with specific molecules or coatings can enhance their ability to cross biological barriers [39].

Biomarkers and **personalized medicine** are also gaining prominence, with drug delivery systems (DDSs) increasingly being tailored to individual genetic and molecular profiles, improving both efficacy and safety. Key elements that enhance the drug development process include:

1) Pharmacokinetic (PK) Models: Provide insights into drug absorption, distribution, metabolism, and excretion (ADME).

2) Dose Optimization: Ensures the most effective dose is delivered with minimal side effects.

3) In Vitro Models: Used for early drug formulation screening before advancing to in vivo studies.

4) In Vivo Models: Animal studies offer a more comprehensive view of drug behavior within living organisms, predicting how the DDS will interact with human physiology.

Computational Models: Utilize mathematical and computational techniques to simulate drug behavior in the body.

Technologies and Concepts Encountering Biological Barriers:

- **Chimeric Antigen Receptor (CAR)**: Often used in immunotherapy, this technology genetically modifies immune cells to target cancer cells better.
- **Epidermal Growth Factor Receptor (EGFR)**: A cell growth and division receptor frequently targeted in cancer treatments.
- **Enhanced Permeation and Retention (EPR)**: A phenomenon where nanoparticles accumulate in tumors due to their leaky vasculature, improving the targeting of cancer therapies.

- **Guide RNA (gRNA)** and **Ribonucleoprotein (RNP)**: Both are associated with CRISPR technology, and these components allow for precise genome editing and targeted genetic modifications [34].

Figure 4 below illustrates the effects of nanoparticles (NPs) in cancer environments, highlighting their potential in modulating tumor biology and influencing the tumor stroma—the supportive tissue surrounding tumors.

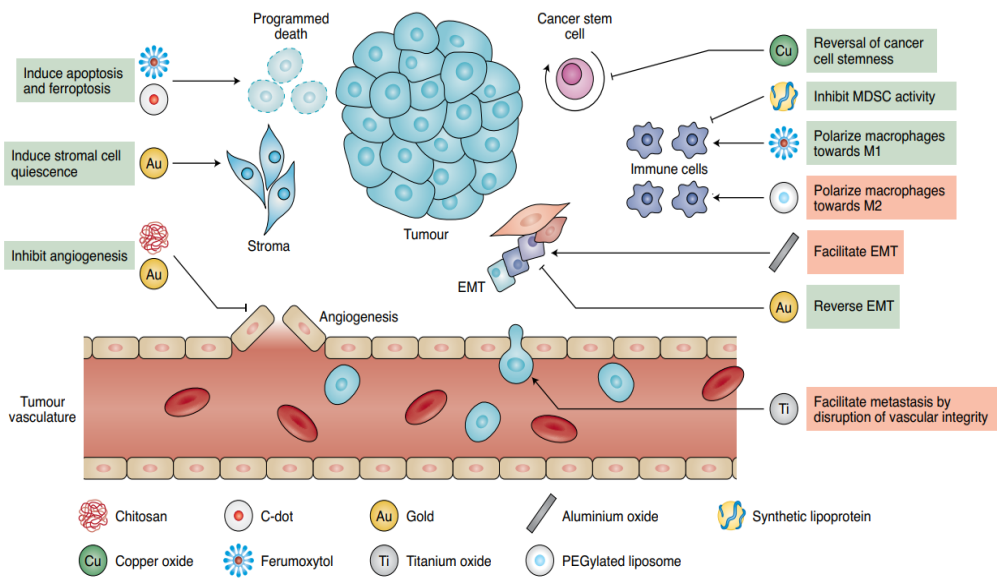


Figure 4. NP effects in cancer environments. Source: **Stater *et al.* (2021)** [40], "The ancillary effects of nanoparticles and their implications for nanomedicine," *Nature Nanotechnology*, 16, 1180-1194, p. 1187.

NPs can influence immune responses in the tumor microenvironment, potentially modulating the balance between pro-tumor and anti-tumor immune activities. This modulation can affect cancer progression, invasion, and metastasis.

Despite their promise, nanoparticles also pose challenges related to safety, toxicity, and unintended side effects. Major concerns include:

- **Nanoparticle-Induced Toxicity and Inflammation**: NPs can interact with the **reticuloendothelial system**, leading to inflammation in organs such as the liver, lungs, and brain due to oxidative stress. Although NPs can cross the

Blood-Brain Barrier (BBB), which is beneficial for treating brain diseases, this poses risks of neurotoxicity when targeting other organs [34]**-**[40].

- **Inorganic Mesoporous Nanoparticles (IMNPs)**: These nanoparticles are gaining attention for **Controlled Drug Delivery (CDD)** due to their structured mesopores and ease of surface modification. While IMNPs may take advantage of the **EPR effect** in tumors, not all tumors exhibit significant EPR. Additionally, cellular uptake and intracellular barriers must be overcome for effective drug release [40].
- **Stimuli-Responsive Polymers**: These polymers can provide **spatio-temporal control** over drug release, enhancing precision. However, improving their accuracy, precision, and repeatability remains challenging, particularly when using external stimuli like electric fields, magnetic fields, light, or heat. Stimuli-responsive systems must also avoid damaging healthy tissues, underscoring the need for precise control [40].

Nanomaterials in theragnostic medicine are advancing the integration of diagnostics and therapies in a single platform. Their multifunctionality allows for enhanced precision, but safety concerns, including nanoparticle-induced toxicity and unintended side effects, remain significant. As the field evolves, efforts to improve nanoparticle designs, including **stimuli-responsive systems**, will be critical to overcoming current limitations and enhancing the efficacy of nanomedicine.

9.3.1.3. Is Nanotechnology Good for the Economy?

Artificial Intelligence (AI) has revolutionized biomedical research and is pivotal in addressing critical healthcare challenges, particularly during the COVID-19 pandemic. AI-based forecasting and predictive tools have significantly improved the rapid diagnosis of irregular symptoms, provided real-time updates on patient conditions, and empowered healthcare professionals to prescribe accurate treatments [41]. This impact has been particularly beneficial in **Low- and Middle-Income Countries (LMICs)**, where AI applications, such as **Machine Learning (ML)** and **Artificial Neural Networks (ANNs)**, have been instrumental in predicting COVID-19 peaks and implementing effective containment measures [42, p. 9].

AI's potential extends far beyond pandemic management, with applications in biomedical research, pharmaceutical supply chain management, and remote

patient monitoring. These technologies promise to enhance healthcare delivery in LMICs and contribute to the growth of **predictive** and **preventive healthcare** [42].

AI can analyze vast amounts of **nanotechnology research, development, and commercialization data**, including scientific publications, patents, funding records, and market trends. By identifying patterns and correlations, AI offers insights into the economic implications of nanotechnology, such as:

1) Growth trajectory of the sector,

2) Investment trends, and

3) Emerging applications [43].

This information is critical for businesses and policymakers to make strategic decisions that align with economic goals. AI can also assist in **economic impact assessments** by analyzing job creation, GDP contribution, and the overall economic value generated by nanotechnology-related industries. Moreover, **scenario analysis** enables the evaluation of potential long-term economic outcomes under various conditions, helping stakeholders assess nanotechnology's broader impact on the economy [43].

AI can also be used to build **predictive models** that forecast the economic impact of advancements in nanotechnology, such as market demand, job creation, and the economic contributions of specific nanotechnology applications. Furthermore, **Natural Language Processing (NLP)** algorithms allow AI to extract meaningful insights from unstructured data sources like research papers, news articles, and policy documents. AI-powered NLP tools can analyze:

1) Public sentiment,

2) Regulatory landscapes, and

3) Societal attitudes toward nanotechnology, providing a more comprehensive understanding of its economic context [44].

AI also assists in assessing the potential **risks** associated with nanotechnology, including environmental, health, and safety concerns. By evaluating these risks, policymakers can make informed decisions that ensure the responsible development and adoption of nanotechnology. **AI-driven optimization algorithms** can

further enhance the efficiency of nanotechnology supply chains, improving production processes, minimizing waste, and optimizing resource allocation [44].

However, implementing AI in these contexts requires a diverse skill set across disciplines like computer science, chemistry, physiology, and physical-mathematical sciences. The specialized expertise required for AI and nanotechnology challenges employers in finding qualified individuals. Addressing these challenges requires investments in training programs, flexible work arrangements, and continued education opportunities [45].

Multidisciplinary Approach in TDD Systems:

Targeted Drug Delivery (TDD) systems rely on a multidisciplinary approach, requiring expertise in:

- **Computer Science**: To develop modeling and simulation techniques to optimize drug delivery and understand pharmacokinetics (PK) and pharmacodynamics (PD).
- **Chemistry**: For developing drug compounds and designing delivery systems.
- **Physiology**: To understand the biological mechanisms underlying drug delivery and the effects of drugs on the body.
- **Physical-Mathematical Sciences**: For understanding the physics of drug delivery and the mathematical modeling of drug interactions with cells and tissues.

EU biotech laboratories engaged in TDD can leverage **computational science predictive simulations** to enhance business resilience and competitiveness. Key benefits include:

1) Improved Drug Design: Computational simulations help model drug interactions, creating more effective drugs with fewer side effects.

2) Faster Drug Development: Predictive simulations accelerate the drug design process, reducing the time and costs associated with traditional trial-and-error methods.

3) Cost Savings: Accurate predictions reduce the need for costly physical testing.

4) Regulatory Compliance: Predictive simulations ensure TDD systems meet regulatory requirements, strengthening business resilience [45].

Europe is home to several significant research institutions involved in **AI R&D in cheminformatics and bioinformatics**, such as:

- **European Bioinformatics Institute (EBI)**: A UK-based institution known for its work in bioinformatics, genomics, and drug discovery.
- **German Cancer Research Center (DKFZ)**: Based in Heidelberg, Germany, DKFZ focuses on cancer research and AI-driven bioinformatics for developing new therapies.
- **ETH Zurich Department of Chemistry and Applied Biosciences**: This Swiss university is a leading institution in AI-based drug discovery and chemical synthesis research.

These institutions showcase Europe's economic strength in AI and nanotechnology, contributing to **drug discovery** and **biomedical advancements**. Combining AI models with **physiologically based pharmacokinetic (PBPK)** and **population pharmacokinetic (popPK)** models is crucial for maximizing predictive power in drug delivery system research [46; p.11]. However, concerns about the **reproducibility of results in humans** compared to animal models and the biases in large datasets underscore the need for caution.

9.3.2. EU Pandemic Response and TDD

The COVID-19 pandemic triggered a swift and coordinated response from global health authorities. After the World Health Organization (WHO) declared it a **Public Health Emergency of International Concern (PHEIC)** in January 2020, a variety of **non-pharmaceutical countermeasures (NPCs)** were recommended worldwide. These measures included proper ventilation, disinfection, medical face masks, hand hygiene, and physical distancing [47].

Governments worldwide, including the **European Union (EU)**, initiated clinical trials to identify effective treatments for COVID-19. These trials explored repurposed drugs, plasma therapy, stem cells, biologics, monoclonal antibodies, gene-silencing molecules (RNA interference), DNA plasmids, viral vectors, recombinant proteins, and nanotechnology-based solutions [48; p.649].

Through initiatives like **Horizon Europe**, the EU provided significant funding for COVID-19 research, including clinical trials involving **Targeted Drug Delivery (TDD)** technologies. Some notable examples include:

- **SOLIDARITY trial**: An international trial funded by the EU and WHO to evaluate various COVID-19 treatments on a global scale.
- **REMAP-CAP trial**: Sponsored by the EU, this trial focuses on treating critically ill COVID-19 patients across Europe.
- **RECOVERY trial**: A large randomized controlled trial supported by the UK government with EU collaboration, testing treatments for COVID-19 patients.

Horizon Europe, the successor to Horizon 2020, founded multiple COVID-19 research projects, emphasizing TDD in clinical trials. One example is the **EU-funded COVID-19 Therapeutics Accelerator**, a partnership between the European Commission, the Bill & Melinda Gates Foundation, and the Wellcome Trust, aimed at accelerating the development of effective therapeutics for COVID-19 by repurposing existing drugs and creating new treatments [49].

The EU also launched initiatives fostering collaboration in data sharing, surveillance, genome sequencing, and clinical outcomes related to COVID-19, including TDD-based approaches—these efforts aimed to accelerate treatment and vaccine development while creating new market opportunities for biotech companies. Though vaccines are not traditional TDD products, advancements in nanotechnology and DDSs have been crucial for enhancing vaccine efficacy and targeted delivery. For instance, several **nanocarrier-based vaccine delivery systems**, including **liposomes, emulsions, polymer-based particles, and carbon-based nanomaterials**, have shown promise in in-vivo vaccine applications [50; Table 1, p. 229].

Additionally, **biocompatible calcium phosphate (CaP) nanoparticles** have shown the potential to induce balanced immune responses, including **Th1 and Th2**, critical for effective immune reactions. When functionalized with **Toll-like receptor (TLR) ligands** and viral antigens, these nanoparticles effectively induce the maturation of human antigen-presenting cells (APCs) [51; p.4].

The EU's pandemic response has also emphasized cross-border collaboration

in TDD technologies, especially in treatments for COVID-19 and other viral infections. The pandemic highlighted the importance of **biomedical innovation** and the dual forces of **scientific globalism** (collaborative science) and **scientific nationalism** (focusing on national scientific advancement), as described by **Jit *et al.* (2021)** [52]. This tension reflects the balance between global scientific collaboration and prioritizing national interests in economic competitiveness and foreign policy [52; p.3].

The pandemic also underscored the need for **closer multi-country collaboration** to facilitate data sharing and accelerate scientific progress. From the early days of COVID-19, the international research community, including academic, health, and industry professionals—collaborated extensively. This open-science approach helped expedite the global response to the pandemic and fostered knowledge-sharing across borders [52; idem].

However, after 2021, political disagreements and debates over the virus's origins posed challenges to sustaining international cooperation. Despite these tensions, **TDD techniques** were crucial in developing efficacy-specific therapies for COVID-19. For example, **liposome nanoparticles** have been instrumental in mitigating side effects for patients treated with **Gallium**, a competitor to zinc, which interferes with the Zn-dependent cell entry of RNA viruses, such as SARS-CoV-2. Liposomes enhanced the precision of drug delivery and improved treatment efficacy by reducing side effects [53; p.2].

TDD has also been pivotal in developing **lipid nanoparticle (LNP)** technology used in **mRNA vaccines**, such as the Pfizer-BioNTech and Moderna COVID-19. LNPs protect the drug from degradation, control release, and target specific cells to generate an efficient immune response. Additionally, **antibody-conjugated polymer nanoparticles** enhance drug delivery specificity by recognizing and binding to receptors on target cells [54].

Beyond vaccines, TDD systems have been vital in developing **antiviral drugs** with specific dosages, effectively reducing viral load and disease severity. **Nanosystems** for respiratory drug delivery, such as those developed for **hydroxychloroquine**—a drug investigated for COVID-19 treatment—have demonstrated promising results [54].

9.3.3. Current Challenges for Biotech Laboratories

The dynamic nature of biotech and pharmaceutical companies underscores their continuous advancements and contributions to healthcare, agriculture, and technology. **Pharmaceutical companies** are primarily known for developing drugs that diagnose, cure, treat, or prevent diseases. With a global market value nearing one trillion U.S. dollars, the pharmaceutical industry plays a significant role in the global economy. In 2022 alone, the **research-based pharmaceutical industry** invested an estimated **€44,500 million** in **R&D** in Europe. This investment generates direct and three times more indirect employment along the supply chain, reflecting the industry's broader economic impact. However, pharmaceutical companies face several **regulatory hurdles**, including stringent approval processes for new drugs and evolving compliance requirements. The **increasing costs associated with R&D** also pose significant challenges, affecting the industry's financial resources.

According to **EFPIA (European Federation of Pharmaceutical Industries and Associations)** data for 2024 [55], the pharmaceutical industry experienced substantial **trade balance and export growth**, indicating a stronger global market presence and enhanced competitiveness.

From 2010 to 2020, **R&D expenditure** grew by **41%**, from 27,920 units in 2010 to 39,442 units in 2020. During the same period, **R&D employment** increased slightly, from 116,253 units in 2010 to 121,717 units in 2020, reflecting a **5%** growth. Additionally, healthcare systems saw a **12% increase in payments for pharmaceuticals**, indicating a moderate rise in pharmaceutical-related healthcare spending [55].

However, fiscal austerity measures implemented across Europe have also affected the pharmaceutical sector since 2010. These **austerity measures**—including budget cuts and reduced public spending—have impacted healthcare systems, pricing, and market access for pharmaceutical products [55]. Despite these challenges, the pharmaceutical industry remains a critical player in driving economic growth in Europe and ensuring the region's **global competitiveness**. **Figure 5** below highlights the **pharmaceutical R&D expenditure growth rate** across various regions.

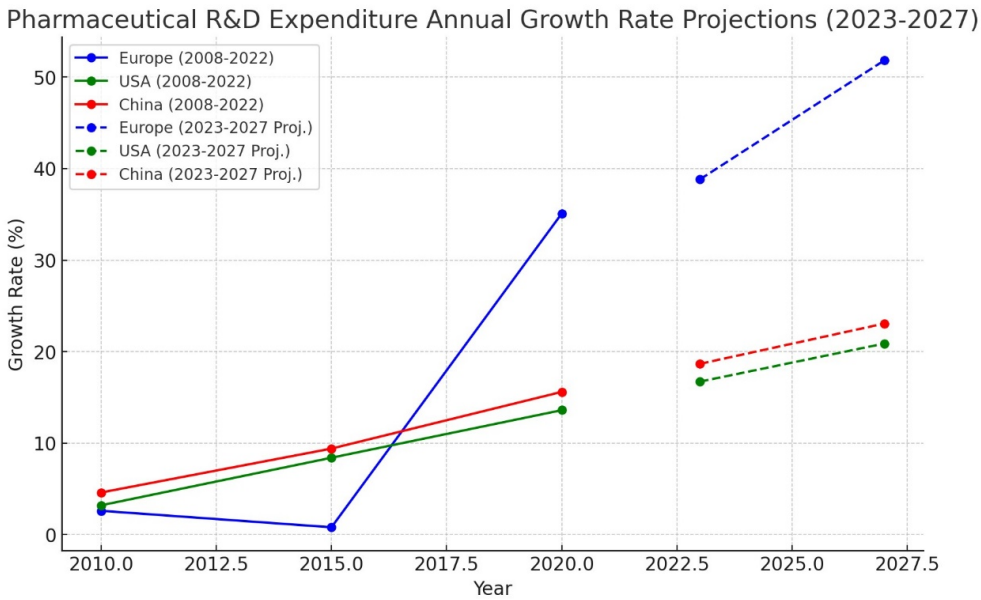


Figure 5. Pharmaceutical R&D Expenditure Annual Growth Rate (%). *Note*: Based on EFPIA key data 2024, page 9 with projections for 2023-2027.

While **Europe** experienced a decline in growth rates from **2.6%** (2008-2012) to **0.8%** (2013-2017), the annual growth rate surged to **35.1%** in the 2018-2022, reflecting a renewed focus on R&D investments. In contrast, the **USA** demonstrated a steady growth trajectory, with growth rates of **3.2%** (2008-2012), **8.4%** (2013-2017), and **13.6%** (2018-2022). **China** exhibited strong growth, with annual growth rates of **4.6%**, **9.4%**, and **15.6%** over the same periods, signaling its growing prominence in the global pharmaceutical R&D landscape [55].

The significant increase in **Europe's R&D growth rate** between 2018 and 2022 suggests an intensified focus on pharmaceutical innovation, likely driven by policy changes, increased industry investment, or evolving research priorities. The **USA's** continued leadership in **pharmaceutical and biotechnology R&D** remains evident. At the same time, **China's** rapid growth underscores its emergence as a major player in the sector, aligned with its broader goals in **life sciences** and **healthcare** [55].

The **pharmaceutical industry's emphasis on R&D** and innovation aligns with efforts to foster economic growth and maintain a competitive edge in the global

market. However, these advancements come with challenges, including the need for supportive **regulatory policies**, **fiscal strategies**, and **investment in innovation** to address ongoing industry challenges.

Notable pharmaceutical companies such as **Pfizer**, **Merck**, **Johnson & Johnson (U.S.)**, **Novartis**, **Roche (Switzerland)**, and **Sanofi (France)** are heavily involved in various sectors, including medical technology, consumer health products, and vaccines. Many leading pharmaceutical companies also have **biopharmaceutical divisions** dedicated to biotechnology products. For instance, **Roche** generates significant revenue from biotechnology products, while **Amgen** remains the world's largest **pure-play biotech company (Morningstar, 2024)**. Beyond pharmaceuticals, **biotechnology** extends into **agriculture** and **food manufacturing**, with companies like **Monsanto** (now part of **Bayer CropScience**) playing a key role in **agricultural genetic engineering**.

The pharmaceutical industry faces numerous challenges, from rising R&D costs to navigating complex regulatory environments. However, the industry's commitment to innovation and sustained investment in R&D highlights its role as a key driver of economic growth and competitiveness.

9.3.3.1. Predictive Accuracy in TDD Optimization

In drug delivery (TDD), predictive accuracy is crucial for ensuring that a system can target specific cells or tissues with minimal off-target effects [56]. Efficient models and strategies in TDD perform optimally across all objectives, whereas inefficient ones exhibit trade-offs. Improving predictive accuracy makes it possible to assess and compare different models or strategies quantitatively.

Predictive accuracy in TDD hinges on parameters like nanoparticle size, charge, geometry, and composition, which influence drug localization, cell penetration, and payload release [57] [58]. For instance, the irregularities in tumor vasculature require accurate predictions in pharmacokinetics (PK), pharmacodynamics (PD), and biodistribution to ensure an effective TDD system.

Regulatory agencies like the US Food and Drug Administration (FDA) emphasize predictive accuracy to provide evidence of safety and efficacy in drug approvals. Additionally, predictive accuracy protects TDD systems from unintended consequences, such as adverse side effects. Multi-objective optimization [59] in TDD

balances several goals—predictive accuracy, drug efficacy, safety, and cost-effectiveness—to identify systems that maximize performance while minimizing risks [60].

One approach to multi-objective optimization addresses the socioeconomic data from six European laboratories, including preferences for TDD systems concerning drug delivery precision, safety, efficacy evaluation, predictive system behavior in silico, and forming partnerships in TDD's economic landscape [61]. These preferences help tackle the challenges of balancing predictive accuracy with drug efficacy and safety.

9.3.3.2. Building the Bridge between Biomedical Engineering and Pharmaceutical Industry

Despite challenges in TDD research, collaboration between biomedical engineering and the pharmaceutical industry can significantly enhance drug delivery. Focusing on integrating PK and PD principles and understanding and overcoming physiological barriers is essential for advancing TDD and achieving more personalized drug delivery systems (DDSs).

Here are the advantages and disadvantages of TDD:

Advantages:

- Precise Targeting: Enables TDD systems to deliver drugs directly to the disease site, improving efficacy.
- Personalized Therapies: Offers the potential for therapies tailored to complex medical conditions, enhancing patient quality of life.
- Comprehensive Understanding: Improves knowledge of the disease state, contributing to more targeted therapeutic strategies.
- Improved Diagnostic Certainty: Facilitates better disease identification and understanding of its specific characteristics.
- Design of Effective Therapeutic Regimes: Helps customize treatment plans based on precise disease knowledge.
- Integration of PK and PD Principles: Optimizes drug absorption, distribution, metabolism, and elimination to improve drug safety and efficacy.

- Understanding Drug Bioavailability Barriers: Focuses on overcoming barriers like physiological, biochemical, and metabolic challenges that limit drug bioavailability.
- Localized Treatment: Develops strategies for localized treatment of stomach and colorectal cancers, emphasizing optimizing oral drug delivery for the gastrointestinal tract.
- Improved Multi-Layer Imaging: Enhances understanding of multiple layers (gastric juice, pericellular matrix, and mucous-rich layers) to optimize drug delivery.
- Simulation of Drug Transit: Utilizes simulations to understand drug dosage transit, residence time, and passage rate, factoring in the influence of food on drug absorption.
- Importance of Biopharmaceutical Classification System (BCS): BCS helps detect drug properties like polarity, charge, and lipophilicity, optimizing DDSs.

Challenges:

- Physiological Complexity: Ignoring physiological complexities can lead to inaccuracies in predictive simulations.
- Lack of Experimental Data: Simulations without experimental data risk compromising their reliability.
- Variability Across Populations: Failing to account for population variability can result in inaccurate predictions, especially in personalized medicine.
- Insufficient Resolution: Low-resolution simulations may result in inaccuracies at the molecular scale.
- Tumor-Specific Challenges: Factors like tumor vasculature, extracellular matrix heterogeneity, and drug resistance make drug delivery to solid tumors difficult to predict.

To effectively address these challenges, integrating PK and PD principles, understanding physiochemical barriers to drug bioavailability, and examining localized treatment of pathological conditions are crucial. Moreover, assessing socioeconomic factors influencing the TDD market's expansion can be guided by the

Analytic Hierarchy Process (AHP)—a decision-making tool developed by Saaty in 1977 that breaks down complex factors into a hierarchical structure for analysis and prioritization.

9.4. Engineering Optimal DDSs through Microfluidic Techniques

9.4.1. The Structured Nanocomposites

Microfluidic techniques have emerged as a promising tool for engineering optimal drug delivery systems (DDSs), offering precise control over drug formulation and delivery. By leveraging these techniques, pharmaceutical researchers and manufacturers can create advanced, targeted, and efficient DDSs that benefit personalized medicine and therapeutic outcomes [62]. By integrating micofluidic platforms, it is possible to produce drug delivery systems on a micro-scale with remarkable precision. Continuous manufacturing techniques enabled by microfluidics ensure that the drug delivery systems produced are uniform and consistent in their properties [63].

Microfluidic techniques are especially valuable in pharmaceutical processes, which span all stages of drug development, manufacturing, and delivery. These techniques are increasingly being applied in the early stages of drug development, where they are used for research purposes such as studying drug formulations, optimizing drug delivery systems, and improving therapeutic efficacy [62]. Microfluidics can also be employed in the formulation stage, where they allow precise mixing, encapsulation, and modification of drugs within carriers, ultimately resulting in novel drug delivery systems with enhanced properties [62].

During the manufacturing stage, microfluidic platforms can be integrated into pharmaceutical processes to produce drug delivery systems on a micro-scale. Continuous manufacturing techniques enabled by microfluidics offer high precision, enabling the production of uniform drug delivery systems with consistent properties. This precision is particularly advantageous when creating complex formulations that require carefully manipulating multiple components [62].

Furthermore, quality control processes ensure that DDSs meet regulatory and safety standards. Analytical techniques can be employed to assess the quality and performance of formulations produced using microfluidic methods, ensuring they meet the required standards for therapeutic use. However, scaling microfluidic

research to large-scale production remains one of the critical challenges, as adapting these precise techniques to produce larger volumes while maintaining the desired properties of the drug delivery system requires significant optimization.

Microfluidics provides precise control over fluid behavior, enabling accurate manipulation of drug formulations and creating drug carriers (e.g., nanoparticles, liposomes) with specific properties. These platforms enable continuous and scalable manufacturing processes that are efficient and reproducible, offering several significant advantages for drug delivery:

- Enhanced Bioavailability: Microfluidic techniques can improve the bioavailability of drugs by optimizing their formulation and delivery methods, ensuring that a higher proportion of the drug reaches its target site in the body.
- Targeted Delivery: One of the key benefits of microfluidic DDSs is the ability to target specific cells or tissues, thereby reducing side effects and enhancing therapeutic outcomes.
- Encapsulation: Microfluidic techniques allow for the encapsulation of drugs within carriers, protecting them from degradation and enabling controlled release kinetics over time.
- Complex Formulations: Microfluidic platforms allow the creation of complex drug formulations with multiple active components that can work synergistically to improve treatment efficacy.

Despite the advantages, several challenges remain in scaling and integrating microfluidic techniques within pharmaceutical manufacturing processes:

- Scalability: While microfluidic techniques offer precise control and enhanced performance at a microscale, scaling these techniques to meet commercial production demands can be challenging. The transition from small-scale research to large-scale production often requires significant optimization to maintain the consistency and quality of the drug delivery systems.
- Integration into Manufacturing: Integrating microfluidic systems into existing pharmaceutical manufacturing workflows may require adaptations to current processes, as the unique requirements of microfluidics differ from traditional methods. These adaptations could involve significant changes in equipment,

staff training, and validation processes.

- Standardization: Establishing standardized protocols for developing and manufacturing microfluidic DDSs is an ongoing challenge. Standardization is necessary to ensure these advanced systems meet regulatory requirements and can be consistently reproduced across different production environments.

9.4.2. Industrial Scale-up Validation for Nanotechnology in TDD

Industrial scale-up validation is critical in transitioning TDD systems from small-scale testing to large-scale production. This phase ensures that TDD technologies maintain their effectiveness, safety, and quality when produced at an industrial scale. The validation process is pivotal for commercializing TDD products, guaranteeing that they meet regulatory standards and are ready for widespread use in clinical trials and beyond.

The industrial scale-up process involves transitioning a TDD system from laboratory or pilot-scale testing to industrial-scale production. While a drug delivery system may function effectively in a laboratory setting, scaling up for mass production can present new challenges that must be addressed systematically.

- Key Elements of Industrial Scale-up Validation for TDD:

Laboratory Scale: At the initial stages, TDD technologies are developed and tested on a small scale in laboratory settings. It is essential to replicate these controlled conditions as closely as possible during larger-scale manufacturing. Researchers work to optimize DDS designs for precision, effectiveness, and safety at this stage. As noted, "lab-scale process development must be conducted under conditions that mimic, as close as possible, the intended large-scale manufacturing process" [60] (Crater J.S. & Lievense J.C., 2018).

Pilot Scale: Once laboratory tests are successful, the TDD system moves to a pilot-scale setting. This stage provides a bridge between lab-scale development and full-scale industrial production. Pilot-scale testing allows researchers to produce larger batches and gain insights into potential challenges, enabling them to adjust processes for large-scale production.

Industrial Scale-Up: The final transition involves pilot-scale testing to full-scale industrial production. This step ensures that the TDD system can be produced in

large quantities, making it suitable for clinical trials or commercial distribution. During this phase, parameters such as manufacturing processes, equipment, and quality control measures are optimized to maintain the system's integrity.

Validation: The validation process ensures that large-scale production consistently produces TDD systems that meet predefined quality, safety, and efficacy specifications. Extensive testing, documentation, and verification are required to confirm that the system's performance matches its intended design.

Quality Assurance: Rigorous quality assurance measures are crucial to guarantee that each unit of the TDD system adheres to established safety, efficacy, and reliability standards. This includes monitoring the manufacturing process and final product testing, ensuring compliance with regulatory guidelines.

Regulatory Compliance: As TDD systems transition to industrial production, regulatory compliance becomes critical. Manufacturers must meet relevant regulatory standards, secure approvals, and ensure the product meets safety and quality requirements.

Economies of Scale: The industrial scale-up process aims to capitalize on economies of scale, reducing the cost per unit as production volumes increase. Efficient manufacturing processes are essential to the commercial viability of TDD products.

9.4.3. Scalability Challenges in TDD

Scalability challenges refer to the difficulties encountered when expanding a process, technology, or system from a small or pilot scale to a more significant, industrial-scale or mass-production level. In the **TDD context**, scalability challenges are particularly significant because what works in a controlled, small-scale laboratory setting may not easily translate into large-scale manufacturing without issues. Here is a breakdown of the key scalability challenges in TDD:

1. Reproducibility and Consistency

- **Challenge**: Achieving consistent results when scaling up from a lab-scale or pilot project to total industrial production is challenging. The precise control and accuracy needed for producing TDD systems (e.g., nanoparticles, liposomes) on a small scale often become harder to maintain on a large scale.

- **Impact**: Variability in drug formulation, dosage, and delivery mechanisms may occur, leading to ineffective or unsafe products. Reproducibility is key to ensuring that each unit produced has the same quality and characteristics as those tested in smaller-scale trials.

2. Process Optimization

- **Challenge**: Scaling up requires optimizing each step of the drug delivery system's production, from synthesis to formulation and packaging. What might be easily controlled at the micro-level (e.g., microfluidics or nanotechnology) can become complex and inefficient at larger scales due to differences in equipment, process times, and environmental conditions.
- **Impact**: Failure to properly optimize production processes can result in higher costs, lower yields, and product inconsistencies.

3. Materials and Resource Management

- **Challenge**: The availability and handling of raw materials, such as polymers, lipids, or nanomaterials used in TDD systems, can present challenges at industrial scales. Certain materials may behave differently when processed in bulk, leading to difficulties controlling properties like particle size or drug encapsulation efficiency.
- **Impact**: Potential risks include material wastage, higher production costs, or the need for **alternative** materials that may compromise product quality.

4. Equipment and Infrastructure Requirements

- **Challenge**: Scaling up production often requires specialized equipment that can maintain the same level of precision and control achieved in the lab. When working with complex systems like TDD, industrial-scale machinery is often less flexible and more prone to errors.
- **Impact**: Purchasing or modifying equipment can significantly increase costs. Additionally, setting up new production lines or adjusting existing ones can take time, leading to delays in bringing new drugs to market.

5. Regulatory Compliance and Quality Assurance

- **Challenge**: As production scales up, regulatory requirements become more

stringent. Ensuring that large-scale manufacturing processes meet FDA, EMA, or other regulatory standards for quality, safety, and efficacy is challenging. Each batch must meet the same specifications as the ones produced in the initial testing phases.

- **Impact**: Non-compliance can lead to delays in regulatory approval, additional testing requirements, or even failure to launch the product. Achieving Good Manufacturing Practices (GMP) compliance at scale is particularly challenging for new technologies like nanomedicine.

6. Cost Management and Economies of Scale

- **Challenge**: While scaling up can theoretically reduce the per-unit cost due to economies of scale, the initial investment in infrastructure, equipment, materials, and regulatory compliance can be enormous. Moreover, operational inefficiencies at larger scales can offset the cost benefits expected from scaling up.
- **Impact**: If not managed correctly, cost overruns can make the drug delivery system too expensive for commercial viability, limiting its use in real-world healthcare settings.

7. Time and Process Validation

- **Challenge**: Validating the production process at an industrial scale is time-consuming. Every step, from synthesis to packaging, must be validated to ensure the product's safety and efficacy. Scaling up requires this process to be repeated on a larger scale, which adds significant time to the overall development timeline.
- **Impact**: Extended time for process validation may delay product launches, increasing R&D costs and potentially missing market opportunities, particularly in competitive areas like pharmaceuticals.

8. Supply Chain and Logistics

- **Challenge**: Scaling up production also requires scaling up the supply chain, including the sourcing of raw materials, transportation, and storage. Managing a global supply chain for sensitive materials like those used in TDD (e.g., temperature-sensitive liposomes) can be complex.
- **Impact**: Disruptions in the supply chain, storage issues, or delays in materials

delivery can significantly impact production schedules and costs.

9. Safety and Environmental Concerns

- **Challenge**: When scaling up the production of nanomaterials or other TDD technologies, safety and environmental considerations become more pressing. These materials may have different health and environmental impacts at larger scales, requiring stringent handling, disposal, and safety protocols.
- **Impact**: Failing to address these concerns can lead to safety risks for workers, patients, and the environment. Additionally, regulatory authorities may impose restrictions or fines for non-compliance with safety standards.

10. Product Stability and Shelf Life

- **Challenge**: Maintaining TDD products' stability and shelf life, particularly nanomedicines, can become more difficult as production scales up. Nanoparticles, for example, maybe more prone to aggregation or degradation in larger batches, which can compromise their efficacy.
- **Impact**: This can lead to higher product failure rates, additional quality control costs, and reduced marketability if stability and shelf life do not meet industry standards.

Scaling up TDD technologies from lab-scale experiments to industrial-scale production presents several challenges. These include maintaining consistency, ensuring regulatory compliance, optimizing processes, managing costs, and addressing safety concerns. Successfully navigating these challenges is crucial for bringing TDD innovations to market efficiently and at a scale that can impact healthcare globally.

9.4.4. Eroom's Law: TDD in Transition

Eroom's Law underscores the need for innovative approaches in drug development, and TDD plays a pivotal role in addressing the inefficiencies that Eroom's Law highlights. The term, a reversal of "Moore's Law," refers to the declining productivity in pharmaceutical R&D despite increasing investments. While Moore's Law describes the exponential growth in computing power, Eroom's Law illustrates the decreasing efficiency of drug discovery and development over time.

- Key Points Related to Eroom's Law and TDD:

Decreasing Efficiency: Eroom's Law highlights that despite significant increases in R&D investment, the number of new drugs approved has steadily declined. This decreasing efficiency is a significant challenge for the pharmaceutical industry, exacerbating the cost and complexity of developing new drugs.

Complexity in Drug Development: Drug development is an inherently complex and time-consuming process involving stages like discovery, preclinical testing, clinical trials, and regulatory approval. Identifying viable drug targets, understanding complex diseases, and overcoming regulatory hurdles all contribute to the inefficiencies observed in drug development.

Transition in Drug Development Models: Eroom's Law has prompted rethinking traditional drug development models. The pharmaceutical industry increasingly focuses on innovative approaches like precision medicine, personalized therapies, and TDD to improve efficiency and effectiveness.

Role of TDD in Transition: Targeted Drug Delivery (TDD) offers the potential to improve the precision and efficacy of drug therapies, aligning with the shift toward more efficient drug development models. By delivering drugs directly to specific sites of action, TDD minimizes systemic side effects and enhances therapeutic outcomes. These advantages are critical in overcoming some of the inefficiencies highlighted by Eroom's Law.

Advancements in TDD: TDD leverages nanotechnology, biomaterials, and advanced drug delivery systems to enhance drug targeting and delivery. These advancements help address common challenges in drug development, such as poor bioavailability and limited drug efficacy.

Individualized Approaches: TDD enables more individualized treatments, aligning with the growing focus on precision medicine and personalized healthcare. By tailoring drug delivery to individual patient characteristics, TDD improves the chances of successful clinical outcomes, particularly in complex diseases like cancer.

Eroom's Law reveals the increasing inefficiencies in drug development and highlights the need for a paradigm shift. TDD represents a vital component of this transition, offering more precise, individualized, and efficient therapeutic approaches to improve the drug development process's overall success rate.

9.5. Methods: Analytic Hierarchy Process (AHP) and TDD Criteria

9.5.1. The Main Criteria for TDD

Several criteria are pivotal for decision-making in developing and optimizing TDD systems, particularly in the biotech industry. Applying the Analytic Hierarchy Process (AHP) [64] is a systematic approach to evaluating and prioritizing these criteria, offering a structured decision-making process widely used in complex, multi-criteria problems. Although no empirical study is presented here, the following outlines the criteria that could be considered for future research using AHP tools.

9.5.1.1. Sub-criteria and Relative Magnitudes for Interpretation

Seven main criteria were identified that encapsulate the essential aspects of TDD in biotech industries. These criteria, along with their sub-criteria, could be applied to assess the economic, procedural, and societal implications of TDD systems:

1) Ec (General Economic data): Economic considerations encompassing cost-benefit analysis, time horizons, and regulatory frameworks in implementing TDD projects.

2) GuComp (Guidelines Compliance data): Regulatory compliance, ensuring alignment with local and international guidelines and safety standards.

3) CosMet (Cost Methodology data): A framework for assessing the methodologies employed in determining the costs associated with TDD development, manufacturing, and distribution.

4) StoPro (Storage Procedures data): Optimization of storage and handling procedures to maintain drug efficacy and safety throughout the supply chain.

5) OptPro (Optimization Procedures data): Methods for enhancing the efficiency and precision of TDD systems, including drug formulation and delivery techniques.

6) InfoMa (Information Management data): The management and dissemination of critical data, ensuring transparency and the integrity of TDD processes.

7) PubAw (Public Awareness data): The role of public awareness and education in fostering trust in TDD systems and addressing ethical and social concerns.

- **Potential Sub-criteria for AHP Analysis**

For a more granular analysis, several sub-criteria could be examined for each main criterion. For instance, under Ec (General Economic data), the following sub-criteria might be explored:

- Cost Elements: Analyzing the key components that comprise the total TDD implementation cost.
- Benefit Identification: Assessing TDD systems' direct and indirect benefits, such as increased treatment efficacy or reduced healthcare costs.
- Time Horizon: Evaluating the impact of costs and benefits over a specific period, which may vary by therapeutic context.
- Risk and Uncertainty: Incorporating risk factors and uncertainties into the analysis, particularly in long-term cost and benefit projections.

Each sub-criterion addresses different facets of TDD procedures, including economic feasibility, regulatory compliance, and operational efficiency. The AHP framework compares sub-criteria pairwise to determine their relative importance.

- **Relative Weights and Priority Matrix**

In an AHP-based analysis, each criterion and sub-criterion could be compared using a pairwise comparison matrix [64]. This process assigns numerical weight based on the relative importance of each factor. While this book chapter does not provide empirical data or a fully executed AHP analysis, it is possible to apply the following steps in future studies:

1) Pairwise Comparisons: For example, decision-makers could be asked, "How important is Ec compared to GuComp?" and assign a value on a 1-to-9 scale to express the relative importance.

2) Consistency Check: The matrix would then be checked for consistency. If the consistency ratio (CR) exceeds a threshold (usually 0.1), adjustments would be made to the comparisons to ensure logical coherence.

3) Priority Weights: After calculating the normalized priority vectors, a priority matrix would be constructed, showing the relative weights of all criteria and sub-

criteria. This matrix would offer insights into which aspects of TDD should be prioritized based on the decision-makers' goals and the relative importance of each factor.

A full AHP analysis would offer a quantitative framework to assess complex decisions, such as optimizing drug delivery systems or balancing economic and procedural goals. Future studies could apply the methodology described to generate concrete insights into the trade-offs and decisions involved in TDD optimization.

9.6. In-Vivo and In-Vitro Evaluations in Biomedicine

9.6.1. The Risk of Human Subjects

9.6.1.1. Clinical Trials and Ethical Considerations

In-vivo evaluation methods in biomedicine refer to laboratory experiments conducted on living organisms, including animals and humans, to assess the safety and efficacy of drugs, therapies, or medical devices. These methods involve animal models, such as rodents, rabbits, or dogs, to simulate human conditions and evaluate how treatment might affect human patients. A fundamental ethical principle in these studies is to minimize risks to human subjects by first researching animal models, aligning with the principles of translational research. This stepwise progression ensures that treatment outcomes are thoroughly evaluated before moving to human trials, reducing the risk to human participants.

For example, as reported in [65; p. 1364], researchers used fluorescently labeled gold nanoparticles in mice for intravital imaging. This allowed observation of nanoparticle behavior within the liver, specifically Kupffer cells and sinusoids. By varying doses of Cy3-gold and Cy5-gold nanoparticles, the study demonstrated how different doses impacted nanoparticle localization, providing insights into how nanoparticles behave in vivo. This in-vivo method is invaluable for understanding drug biodistribution and potential efficacy before moving to human trials.

Several key factors, such as drug dosage, pharmacokinetics (PK), and the relationship between the disease and human metabolism, are critical to in-vivo evaluations. For instance, as noted in [66; p. 4], the intramuscular injection of calcium phosphate (CaP) nanoparticles in mice indicated enhanced immune responses, suggesting the potential of CaP nanoparticles as vaccine carriers.

Once preclinical animal model studies demonstrated safety and efficacy, clinical trials involving human subjects commenced. These trials are critical for ensuring treatment safety, efficacy, and regulatory compliance. For example, Jiang A.Y. *et al.* (2023) [67] highlighted the clinical success of lipid nanoparticle (LNP)-based mRNA vaccines, which required rigorous in-vivo and in-vitro studies. These studies, including experiments on mice and air-liquid interface (ALI) cultures, provided valuable data on dose responses and protein expression, facilitating the development of mRNA vaccines.

In Europe, TDD systems undergo comprehensive in-vivo evaluations using various methods. Techniques such as PET (positron emission tomography), fluorescence imaging, MRI (magnetic resonance imaging), tissue sampling, and microdialysis are employed to assess the behavior and efficacy of TDD systems. Each method offers unique insights into drug biodistribution, retention time, and local variations in drug levels, contributing to an improved understanding of the system's performance.

Examples include:

- PET: This imaging technique visualizes drug distribution and accumulation, providing insights into how TDD systems behave in real-time.
- Fluorescence Imaging: A non-invasive method that maps disease sites, drug biodistribution, and retention time.
- MRI: Used to visualize drug accumulation in specific organs, such as tumors, and assess the pharmacokinetic parameters of TDD systems.
- Tissue Sampling: Involves surgical removal of target tissues to analyze treatment efficacy, tissue response, and drug release kinetics.
- Microdialysis: An in-vivo sampling technique that measures local drug and metabolite levels, offering a granular view of drug behavior within specific tissues.

Ethical considerations are pivotal in in-vivo studies, ensuring that research adheres to guidelines that protect animal and human subjects while advancing biomedical knowledge. In 2022, global R&D spending in the pharmaceutical industry reached $244 billion, underscoring the industry's commitment to innovation. Regulatory bodies such as the U.S. Food and Drug Administration (FDA) oversee the

preclinical and clinical stages, ensuring that new drugs meet stringent safety and efficacy standards before reaching the market.

Pharmaceutical companies, driven by the need to innovate, invest heavily in R&D due to limited patent protections and the potential for sales erosion once generics enter the market. Patent expirations necessitate continued research into specialty drugs, which helps diversify product portfolios.

In recent years, significant shifts have occurred in pharmaceutical R&D, with companies increasingly outsourcing research to clinical research organizations (CROs) to reduce costs. Additionally, advancements in big data and predictive modeling, including real-world evidence (RWE), are transforming clinical research. These developments necessitate partnerships with technology companies to leverage data from various sources, including social media, to create safer and more effective drugs [68].

The growing reliance on predictive modeling and real-world data highlights the need for interdisciplinary collaboration in biomedicine [69]. These collaborations help pharmaceutical companies stay ahead of emerging challenges while fostering innovation in drug development.

9.7. Conclusion

The COVID-19 pandemic underscored the importance of global collaboration in the biotech and pharmaceutical sectors. As discussed in *Section 1*, partnerships across global supply chains and financial networks have proven essential for a post-pandemic recovery, with policies supporting health and safety, funding mechanisms, and regulatory frameworks driving economic decisions and industry investments across the EU.

Our research explored how nanocarriers interact within the body and traverse physiological barriers in targeted drug delivery (TDD). *Section 2* explored the structural features of various nanocarriers—such as liposomes and dendrimers—and their influence on systemic drug administration, including size, surface charge, and composition. These factors critically affect nanocarriers' behavior, highlighting the importance of optimizing their communication dynamics for effective drug delivery.

In *Section 3*, we examined the complexities introduced by the COVID-19 crisis in biotech labs focusing on drug delivery, showcasing the role of partnerships, ethical considerations, and marketing strategies in advancing TDD within the European biotech landscape. The scalability challenges discussed in *Section 4* highlighted the importance of AI in monitoring the development of nanocarriers and TDD systems. The integration of AI, especially in detecting trends in nanomedicine, exemplifies a forward-thinking approach in an industry where human and computational interactions are critical to success. However, we also emphasized the need for balance, ensuring that overreliance on AI does not overshadow human oversight and interdisciplinary collaboration. This sentiment was echoed in the discussion of *Eroom's Law* in *Sub-Section 4.2.4*, where we discussed how innovative drug delivery methods like TDD are part of the transition toward more efficient drug development models.

Section 5 highlighted the value of analytic hierarchy process (AHP) tools for evaluating the criteria driving TDD market expansion, particularly focusing on key factors such as economic considerations, regulatory compliance, and public awareness. This sophisticated analysis would not have been possible without employing machine learning (ML) and open-access tools, illustrating how modern digital tools enhance research and decision-making in biotech.

Lastly, *Section 6* addressed the importance of in-vivo and in-vitro evaluations in biomedicine, which remain indispensable despite the growing prominence of computational models. These evaluations play a vital role in refining the efficacy of nanocarriers and ensuring their safety. Integrating traditional bioanalytic strategies with modern technological advancements provides a comprehensive approach to understanding drug delivery systems, ensuring that TDD developments are innovative and ethically sound.

Our research contributes a balanced perspective by blending technical discussion with the broader implications of economics and patient behavior in the post-COVID-19 landscape. Integrating specialist language from medicine and healthcare with economic analysis allows a broader audience to appreciate the evolution of TDD methods. By considering TDD's technical and human elements, our work highlights the importance of empathy, collaboration, and interdisciplinary understanding in advancing healthcare innovation.

9.8. Contributions

Conceptualization: Romina Fucà; Literature review: Romina Fucà; Hypothesis development: Romina Fucà; Data collection and data sorting: Romina Fucà and Serena Cubico; Hypothesis evaluation: Romina Fucà; Discussion: Romina Fucà and João Leitão; reviewing: Romina Fucà, Serena Cubico, Giuseppe Favretto, Piermatteo Ardolino and João Leitão. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

We extend our heartfelt gratitude to Dr. Maria VLIORA and Professor Andreas FLOURIS for their organizational and editorial efforts in coordination with our Department at the University of Verona, Italy.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this chapter.

References

[1] International Monetary Fund (IMF). *World Economic Outlook. Managing Divergent Recoveries*. Washington, DC, April 2021, pp. 1-170.

[2] World Economic Forum. *Annual Report 2023-2024*. Geneva, Switzerland, 2023, pp. 1-70.

[3] European Central Bank (ECB) (2022). Speech by Luis de Guindos, Vice-President of the ECB, at the London School of Economics German Symposium. Frankfurt am Main, 10 February 2022.

[4] Luo C-M. The COVID-19 Crisis: The EU Recovery Fund and its Implications for European Integration - a Paradigm Shift. *European Review*. 2022; 30(3): 374-392. doi:10.1017/S106279872100003X

[5] World Economic Forum. *Future of Jobs Report 2023. Insight Report*. Geneva, Switzerland, May 2023, pp. 1-296.

[6] Jadhav, V.; Roy, A.; Kaur, K.; Kumar Rai, A.; and Rustagi, S. Recent advances in nanomaterial-based drug delivery systems, *Nano-Structures & Nano-*

Objects, Volume 37, 2024, 101103, ISSN 2352-507X. https://doi.org/10.1016/j.nanoso.2024.101103

[7] Byun, J.; Wu, Y.; Park, J. *et al.* RNA Nanomedicine: Delivery Strategies and Applications. *AAPS J* **25**, 95, 2023. https://doi.org/10.1208/s12248-023-00860-z

[8] Curran, M.A. Life Cycle Assessment: a review of the methodology and its application to sustainability. *Current Opinion in Chemical Engineering* 2013, 2:273-277. http://dx.doi.org/10.1016/j.coche.2013.02.002

[9] Dong, S.; Nessler, I.; Kopp, A.; Rubahamya, B.; and Thurber, G.M. Predictive Simulations in Preclinical Oncology to Guide the Translation of Biologics. *Front. Pharmacol.*, 03 March 2022, Sec. Experimental Pharmacology and Drug Discovery, Vol. 13, 2022, pp. 1-11. https://doi.org/10.3389/fphar.2022.836925

[10] Ashique, S.; Sandhu, N.K.; Chawla, V.; Chawla P.A. Targeted Drug Delivery: Trends and Perspectives. *Curr Drug Deliv.* 2021;*18*(10):1435-1455. doi: 10.2174/1567201818666210609161301. PMID: 34151759.

[11] Tewabe A.; Abate A.; Tamrie M.; Seyfu A.; Abdela Siraj E. Targeted Drug Delivery - From Magic Bullet to Nanomedicine: Principles, Challenges, and Future Perspectives. *J Multidiscip Healthc.* 2021 Jul 5; 14:1711-1724. doi: 10.2147/JMDH.S313968. PMID: 34267523; PMCID: PMC8275483.

[12] Choudhary, K.; DeCost, B.; Chen, C. *et al.* Recent advances and applications of deep learning methods in materials science. *npj Comput Mater* 2022, **8**, 59. https://doi.org/10.1038/s41524-022-00734-6

[13] Wilson, S.; Steele, S.; Adeli, K. Innovative technological advancements in laboratory medicine: Predicting the lab of the future. *Biotechnology & Biotechnological Equipment* 2022, *36*(S1), S9-S21, https://doi.org/10.1080/13102818.2021.2011413

[14] Shukla, R., Tripathi, T. (2021). Molecular Dynamics Simulation in Drug Discovery: Opportunities and Challenges. In: Singh, S.K. (eds) Innovations and Implementations of Computer Aided Drug Discovery Strategies in Rational Drug Design. Springer, Singapore. https://doi.org/10.1007/978-981-15-8936-2_12

[15] Greener, J.G.; Kandathil, S.M.; Moffat, L. *et al.* A guide to machine learning for biologists. *Nat Rev Mol Cell Biol* 2022, **23**, 40-55. https://doi.org/10.1038/s41580-021-00407-0

[16] Manzari M.T.; Shamay Y.; Kiguchi H.; Rosen N.; Scaltriti M.; Heller D.A. Targeted drug delivery strategies for precision medicines. *Nat Rev Mater.* 2021 Apr;*6*(4):351-370. doi: 10.1038/s41578-020-00269-6. Epub 2021 Feb 2. PMID: 34950512; PMCID: PMC8691416.

[17] Haleem, A.; Javaid, M.; Singh, R.P.; Suman, R. Telemedicine for healthcare: Capabilities, features, barriers, and applications. *Sens Int.* 2021; 2:100117. doi: 10.1016/j.sintl.2021.100117. Epub 2021 Jul 24. PMID: 34806053; PMCID: PMC8590973.

[18] Hossain S, Kabedev A, Parrow A, Bergström CAS, Larsson P. Molecular simulation as a computational pharmaceutics tool to predict drug solubility, solubilization processes and partitioning. *Eur J Pharm Biopharm.* 2019 Apr; 137:46-55. doi: 10.1016/j.ejpb.2019.02.007. Epub 2019 Feb 14. PMID: 30771454; PMCID: PMC6434319.

[19] Shoukat, R.; Khan, M.I. Carbon nanotubes: a review on properties, synthesis methods and applications in micro and nanotechnology. *Microsyst Technol* 2021, **27**, 4183-4192. https://doi.org/10.1007/s00542-021-05211-6

[20] Aqel, A.; Abou El-Nour, K.M.M.; Ammar, R.A.A.; Al-Warthan, A. Carbon nanotubes, science and technology part (I) structure, synthesis and characterization. *Arabian Journal of Chemistry* 2012 5(1); pp. 1-23, ISSN 1878-5352. https://doi.org/10.1016/j.arabjc.2010.08.022

[21] Anjum, S.; Ishaque, S.; Fatima, H.; Farooq, W.; Hano, C.; Abbasi, B.H.; Anjum, I. Emerging Applications of Nanotechnology in Healthcare Systems: Grand Challenges and Perspectives. *Pharmaceuticals* 2021 Jul 21;*14*(8):707. doi: 10.3390/ph14080707. PMID: 34451803; PMCID: PMC8401281.

[22] Döring, A.; Ushakova, E.; Rogach, A.L. Chiral carbon dots: synthesis, optical properties, and emerging applications. *Light Sci Appl* 2022, **11,** 75. https://doi.org/10.1038/s41377-022-00764-1

[23] Cheng, Z.; Li, M.; Dey, R.; Chen, Y. Nanomaterials for cancer therapy: current progress and perspectives. *J Hematol Oncol.* 2021 May 31;*14*(1):85. doi: 10.1186/s13045-021-01096-0. PMID: 34059100;

PMCID: PMC8165984.

[24] Salata O. Applications of nanoparticles in biology and medicine. *J Nanobiotechnology* 2004 Apr 30;*2*(1):3. doi: 10.1186/1477-3155-2-3. PMID: 15119954; PMCID: PMC419715.

[25] He, H.; Pham-Huy, L.A.; Dramou, P.; Xiao, D.; Zuo, P.; Pham-Huy, C. Carbon nanotubes: applications in pharmacy and medicine. *Biomed Res Int.* 2013; 2013:578290. doi: 10.1155/2013/578290. Epub 2013 Sep 30. PMID: 24195076; PMCID: PMC3806157.

[26] Ahmad, R.; Srivastava, S.; Ghosh, S.; Khare, S.K. Phytochemical delivery through nanocarriers: a review. *Colloids Surf B Biointerfaces.* 2021 Jan; 197:111389. doi: 10.1016/j.colsurfb.2020.111389. Epub 2020 Oct 5. PMID: 33075659.

[27] Lombardo, D.; Kiselev, M.A.; and Caccamo, M.T. Smart Nanoparticles for Drug Delivery Application: Development of Versatile Nanocarrier Platforms in Biotechnology and Nanomedicine. *Hindawi Journal of Nanomaterials* 2019, Article ID 3702518, pp. 1-26 https://doi.org/10.1155/2019/3702518. Retrieved from (lastly accessed February 21, 2023).

[28] Chamundeeswari, M.; Jeslin, J.; Verma, M.L. Nanocarriers for drug delivery applications. *Environ Chem Lett* 2019, **17**, 849-865. https://doi.org/10.1007/s10311-018-00841-1

[29] Murugaiyan, J.; Kumar, P. A.; Rao, G. S.; Iskandar, K.; Hawser, S. *et al.* Progress in Alternative Strategies to Combat Antimicrobial Resistance: Focus on Antibiotics. *Antibiotics* 2022, *11*(2), 200. https://doi.org/10.3390/antibiotics11020200

[30] Alavi, M.; Karimi, N.; and Safaci, M. Application of Various Types of Liposomes in Drug Delivery Systems. *Advanced Pharmaceutical Bulletin* 2017, *7*(1), 3-9. https://doi.org/10.15171/apb.2017.002

[31] McClements, D.J. Advances in nanoparticle and microparticle delivery systems for increasing the dispersibility, stability, and bioactivity of phytochemicals. Elsevier, 2018, pp. 1-37. Version of Record: https://www.sciencedirect.com/science/article/pii/S0734975018301368

[32] Mouhid, L.; Corzo-Martínez, M.; Torres, C. *et al.* Improving In Vivo Efficacy of Bioactive Molecules: An Overview of Potentially Antitumor Phytochemicals and Currently Available Lipid-Based Delivery Systems. *Hindawi Journal of Oncology* 2017, pp. 1-34. doi: https://doi.org/10.1155/2017/7351976

[33] Sultana, A.; Zare, M.; Thomas, V.; Sampath Kumar, T.S.; Ramakrishna, S. Nano-based drug delivery systems: Conventional drug delivery routes, recent developments and future prospects. *Medicine in Drug Discovery*, **15**, May 2022, 100134. https://doi.org/10.1016/j.medidd.2022.100134

[34] Mitchell, M.J.; Billingsley, M.M.; Haley, R.M. *et al.* Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov* 2021, **20**, 101-124. https://doi.org/10.1038/s41573-020-0090-8

[35] Bröring, S.; Laibach, N.; Wustmans, M. Innovation types in the bioeconomy. *Journal of Cleaner Production*, vol. 266, 2020, 121939, ISSN 0959-6526. https://doi.org/10.1016/j.jclepro.2020.121939

[36] Zhang, W.; Mehta, A.; Tong, Z.; Esser, L.; Voelcker, N.H., Development of Polymeric Nanoparticles for Blood-Brain Barrier Transfer—Strategies and Challenges. *Adv. Sci.* 2021, 8, 2003937. https://doi.org/10.1002/advs.202003937

[37] Sherje, A.P.; Jadhav, M.; Dravyakar, B.R.; Kadam, D. Dendrimers: A versatile nanocarrier for drug delivery and targeting. *International Journal of Pharmaceutics*, *548*(1), 2018, 707-720, ISSN 0378-5173. https://doi.org/10.1016/j.ijpharm.2018.07.030

[38] Hsu, J.C.; Tang, Z.; Eremina, O.E. *et al.* Nanomaterial-based contrast agents. *Nat Rev Methods Primers* **3**, 30 (2023). https://doi.org/10.1038/s43586-023-00211-4

[39] Adepu, S.; Ramakrishna, S. Controlled Drug Delivery Systems: Current Status and Future Directions. *Molecules* 2021, *26*(19), 5905. https://doi.org/10.3390/molecules26195905

[40] Stater, E.P.; Sonay, A.Y.; Hart, C. *et al.* The ancillary effects of nanoparticles and their implications for nanomedicine. *Nat. Nanotechnol.* **16**, 1180-1194. (2021). https://doi.org/10.1038/s41565-021-01017-9

[41] Vaishya, R.; Javaid, M.; Khan I.H.; Haleem A. Artificial Intelligence (AI)

applications for COVID-19 pandemic. *Diabetes Metab Syndr.* 2020, Jul-Aug; *14*(4):337-339. doi: 10.1016/j.dsx.2020.04.012. Epub 2020 Apr 14. PMID: 32305024; PMCID: PMC7195043.

[42] Naseem, M.; Akhund, R.; Arshad, H.; Ibrahim, M.T. Exploring the Potential of Artificial Intelligence and Machine Learning to Combat COVID-19 and Existing Opportunities for LMIC: A Scoping Review. *J Prim Care Community Health.* 2020 Jan-Dec; 11:2150132720963634. doi: 10.1177/2150132720963634. PMID: 32996368; PMCID: PMC7533955.

[43] Stahl, B.C., Antoniou, J., Bhalla, N. *et al.* A systematic review of artificial intelligence impact assessments. *Artif Intell Rev* **56**, 12799-12831 (2023). https://doi.org/10.1007/s10462-023-10420-8

[44] Secinaro, S., Calandra, D., Secinaro, A. *et al.* The role of artificial intelligence in healthcare: a structured literature review. *BMC Med Inform Decis Mak* **21**, 125 (2021). https://doi.org/10.1186/s12911-021-01488-9

[45] Wilson, S.; Steele, S.; Adeli, K. Innovative technological advancements in laboratory medicine: Predicting the lab of the future. *Biotechnology & Biotechnological Equipment* 2022, *36*(S1), S9-S21, https://doi.org/10.1080/13102818.2021.2011413

[46] Bae, Y.H.; Park, K. Advanced drug delivery 2020 and beyond: Perspective on the future. *Advanced Drug Delivery Reviews* 2020, **158**, pp. 4-16, doi: https://doi.org/10.1016/j.addr.2020.06.018

[47] Sun, J.; He, W.T.; Wang, L.; Lai, A.; Ji, X.; Zhai, X.; Li, G., Suchard, M.A.; Tian, J.; Zhou, J.; Veit, M.; Su, S. COVID-19: Epidemiology, Evolution, and Cross-Disciplinary Perspectives. *Trends in molecular medicine* 2020; *26*(5), 483-495. Retrieved from: https://doi.org/10.1016/j.molmed.2020.02.008 (lastly retrieved March 19, 2021).

[48] Alany R.G. COVID-19 pandemic: what can pharmaceutical formulation and drug delivery experts offer? *Pharm Dev Technol.* 2020 Jul;*25*(6):649. doi: 10.1080/10837450.2020.1764670. PMID: 32423342.

[49] Toussi, S.S.; Hammond, J.L.; Gerstenberger, B.S. *et al.* Therapeutics for COVID-19. Nat Microbiol 2023, **8**, 771-786. https://doi.org/10.1038/s41564-023-01356-4.

[50] Kim, M.-G.; Park, J.Y.; Shon, Y.; Kim, G.; Shim, G.; Oh, Y.-K. Nanotechnology and vaccine development. *Asian Journal of Pharmaceutical Sciences*, **9**, 2014, 227-235. http://dx.doi.org/10.1016/j.ajps.2014.06.002

[51] Mao, L.; Chen, Z.; Wang, Y.; Chen, C. Design and application of nanoparticles as vaccine adjuvants against human corona virus infection. *Journal of Inorganic Chemistry*, **219**, 2021, 111454. https://doi.org/10.1016/j.jinorgbio.2021.111454

[52] Jit, M.; Ananthakrishnan, A.; McKee, M.; Wouters, O.J.; Beutels, P.; Teerawattananon, Y. Multi-country collaboration in responding to global infectious disease threats: lessons for Europe from the COVID-19 pandemic. *The Lancet Regional Health-Europe*, **9**, 2021, 100221, https://doi.org/10.1016/j.lanepe.2021.100221

[53] Torabi, S.; Bahreini, F.; and Rezaei, N. Gallium Components-Based Drug Delivery: A Potential Treatment for COVID-19. *Infectious Disorders - Drug Targets* 2022, **22**, pp. 1-3, doi: 10.2174/1871526522666220127120617.

[54] Cavalcanti, I.D.L.; de Fátima Ramos Dos Santos Medeiros, S.M.; Dos Santos Macêdo, D.C.; Ferro Cavalcanti, I.M.; de Britto Lira Nogueira, M.C. Nanocarriers in the Delivery of Hydroxychloroquine to the Respiratory System: An Alternative to COVID-19. *Curr Drug Deliv.* 2021; *18*(5):583-595. doi: 10.2174/1567201817666200827110445. PMID: 32860358.

[55] European Federation of Pharmaceutical Industries and Associations (EFPIA). *The Pharmaceutical Industry in Figures. Key Data*, 2024, pp. 1-28. https://www.efpia.eu/media/2rxdkn43/the-pharmaceutical-industry-in-figures-2024.pdf

[56] Bereczki, Z.; Benczik, B.; Balogh, O.M.; Marton, S. *et al.* Mitigating off-target effects of small RNAs: conventional approaches, network theory and artificial intelligence. *Br J Pharmacol.* 2024 Sep 18. doi: 10.1111/bph.17302. Epub ahead of print. PMID: 39293936.

[57] Haripriyaa, M.; Suthindhiran, K. Pharmacokinetics of nanoparticles: current knowledge, future directions and its implications in drug delivery. *Futur J Pharm Sci* **9**, 113 (2023). https://doi.org/10.1186/s43094-023-00569-y

[58] Biswas, L.; Mahtab, A.; Verma, A.K. Chapter 7 - Pharmacokinetics and in vivo

evaluation of nanoparticles, Editor(s): Prashant Kesharwani, Kamalinder K. Singh, Nanoparticle Therapeutics, Academic Press, 2022, pp. 265-289, ISBN 9780128207574. https://doi.org/10.1016/B978-0-12-820757-4.00006-5

[59] Lambrinidis, G.; Tsantili-Kakoulidou, A. Multi-objective optimization methods in novel drug design. *Expert Opin Drug Discov.* 2021 Jun;16(6):647-658. doi: 10.1080/17460441.2021.1867095. Epub 2020 Dec 31. PMID: 33353441.

[60] Nicolotti, O.; Giangreco, I.; Introcaso, A.; Leonetti, F.; Stefanachi, A.; Carotti, A. Strategies of multi-objective optimization in drug discovery and development. *Expert Opin Drug Discov.* 2011 Sep;6(9):871-84. doi: 10.1517/17460441.2011.588696. Epub 2011 May 27. PMID: 22646211.

[61] Lopalco A.; Iacobazzi, R.M.; Lopedota, A.A.; Denora, N. Recent Advances in Nanodrug Delivery Systems Production, Efficacy, Safety, and Toxicity. *Methods Mol Biol.* 2025;2834:303-332. doi: 10.1007/978-1-0716-4003-6_15. PMID: 39312172.

[62] Alavi, S.E.; Alharthi, S.; Alavi, S.F.; Alavi, S.Z.; Zahra, G.E.; Raza, A.; Shahmabadi, H.E. Microfluidics for personalized drug delivery, *Drug Discovery Today*, Volume 29, Issue 4, 2024, 103936, ISSN 1359-6446. https://doi.org/10.1016/j.drudis.2024.103936

[63] Sanjay, S.T.; Zhou, W.; Dou, M.; Tavakoli, H.; Ma, L.; Xu, F.; Li, X.J. Recent advances of controlled drug delivery using microfluidic platforms, *Advanced Drug Delivery Reviews*, Volume 128, 2018, pp. 3-28, ISSN 0169-409X. https://doi.org/10.1016/j.addr.2017.09.013

[64] Saaty, R.W. The Analytic Hierarchy Process—What It Is and How It Is Used. *Math. Modelling* 1987, *9*(3-5), pp. 161-176. https://doi.org/10.1016/0270-0255(87)90473-8

[65] Ouyang, B.; Poon, W.; Zhang, YN. *et al.* The dose threshold for nanoparticle tumour delivery. *Nat. Mater.* **19**, 1362-1371 (2020). https://doi.org/10.1038/s41563-020-0755-z

[66] Mao, L.; Chen, Z.; Wang, Y.; Chen, C. Design and application of nanoparticles as vaccine adjuvants against human corona virus infection. *Journal of*

Inorganic Chemistry, **219**, 2021, 111454. https://doi.org/10.1016/j.jinorgbio.2021.111454

[67] Jiang, A.Y.; Witten, J.; Raji, I.O. *et al.* Combinatorial development of nebulized mRNA delivery formulations for the lungs. *Nat. Nanotechnol.* (2023). https://doi.org/10.1038/s41565-023-01548-3

[68] Romasanta, A.S., van der Sijde, P. & van Muijlwijk-Koezen, J. Innovation in pharmaceutical R&D: mapping the research landscape. *Scientometrics* **125**, 1801-1832 (2020). https://doi.org/10.1007/s11192-020-03707-y

[69] Chen, Z.; Liu, X.; Hogan, W.; Shenkman, E.; Bian, J. Applications of artificial intelligence in drug development using real-world data, *Drug Discovery Today*, Volume 26, Issue 5, 2021, pp. 1256-1264, ISSN 1359-6446. https://doi.org/10.1016/j.drudis.2020.12.013.

Chapter 10
Pioneering the Future of Drug Delivery

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Localized drug delivery has undergone a notable evolution, transitioning from mere controlled-release formulations in the mid-20th century to the contemporary frontier field of nanorobotic devices. Initially the focus was primarily on minimizing the systemic side effects of certain compounds and enhancing their therapeutic efficacy. However, with the advent of injectable nanorobots (often called nanobots) as carriers with the capability of precise localized drug delivery, an ocean of possibilities is opening up in terms of therapeutic approaches.

In particular, recent advancements in nanotechnology, materials science, and bioengineering have enabled the development of magnetically steerable nanorobots, a technology now positioned to revolutionize angioplastic surgery and localized drug delivery, in particular for after-stroke intervention and oncological therapies. These nanorobots, characterized by programmability, biocompatibility, and responsiveness, offer a versatile platform for navigating the complexities of vascular networks up to the smallest vases thanks to the magnetic guidance systems. This capability holds promise for delivering drugs to specific locations previously identified through a variety of diagnostic techniques.

With such premises, it does not surprise that market projections indicate substantial growth in the global nanomedicine market by 2030, with nanorobotic technologies in angioplastic surgery and drug delivery expected to contribute significantly to this field. Increased research investments, technological advancements, and the demand for personalized medicine are anticipated to drive the birth/expansion of this field.

In this book chapter we'll examine the history, characteristics, and future potential of magnetically steerable nanorobots as an innovative solution in drug

delivery, not only as tools to improve patients' clinical outcomes, but also from the point of view of prospective entrepreneurs willing to enter this budding market. With this perspective, the following paragraphs are covered be food for thought that will hopefully spark further research for the topics treated.

10.1. The Intersection of Nanotechnology and Medicine

Injectable nanorobots stand at the crossroads of various scientific disciplines, technological domains, and economic sectors, reflecting a convergence that holds considerable promise to advance healthcare.

Nanorobotics epitomize the synergy between physics, chemistry, materials engineering, biology and medicine. However, scientific cross-pollination is merely just the start of the challenge: the development and fabrication of such nanorobots involves intricate collaborations between all the parties involved, thus making necessary to have a multidirectional constant exchange of information among the players in academia with the ones from the industries supplying the necessary components to the actual nanobots manufacturers and their operators, that have novelty elements in their own right.

The integration of nanorobots into medical practice extends beyond the world of atoms: the software component needed to manage them, that is the algorithms for data analysis and decision making about guidance/delivery in real-time is an equally challenging innovation; this hardware-software integration is a further axis along which it's necessary to collaborate.

In the realm of economic activities, the development of injectable nanorobots has spurred investments and collaborations across academia and pharmaceutical companies, who are actively exploring the integration of nanorobots into drug delivery systems. Their aim is to enhance the efficacy and specificity of therapeutic interventions, even with well-known molecules already on the market. Venture capital firms are increasingly investing in startups working on healthcare-focused nanorobotics, acknowledging its potential for disruptive innovation. The economic ecosystem around nanorobots extends to manufacturing, with precision fabrication techniques and quality control processes playing a critical role in the scalability and commercial viability of these technologies.

As research in the field progresses, unexplored directions with high potential

emerge, particularly in the realm of oncological therapy. Oncology itself is a very multidisciplinary field, so the collaboration among practitioners from all the involved medical disciplines has been inevitably dense, unraveling the nuances of tumor microenvironments and complex interactions at the cellular and molecular levels. Additionally, elucidating the immunomodulatory potential of nanorobots in the context of cancer immunotherapy remains an area ripe for exploration.

10.2. A Blueprint for Success: Starting a Medical Technology Company

Embarking on the journey to establish a medical technology company requires meticulous planning, strategic vision, and a deep understanding of the industry's nuances. At its core, the process of starting a company involves several fundamental steps, common to any industry. Identifying a clear market need or gap in existing technologies is the first crucial step. This involves conducting comprehensive market research to understand the competitive landscape, potential customer base, and regulatory requirements. With a validated idea, the next step is to create a robust business plan that outlines the company's mission, vision, target market, revenue model, and growth strategy. Securing funding, whether through investors, grants, or loans, is often a difficult but necessary step, which enables turning the conceptual framework into a tangible reality. Assembling a talented and diverse team, capable of executing the company's vision, is equally critical. Availability of resources and team quality are almost always linked in a sort of gravitational lock, so we'd advise aspiring entrepreneurs to dedicate extreme attention to both. Legal considerations, such as choosing an appropriate business structure, securing intellectual property rights, and compliance with regulatory frameworks, form the backbone of a resilient foundation for the company.

In the MedTech industry, where innovation intersects with healthcare regulations, the time-to-market is typically very long due to the complexities of the R&D and approval processes; therefore, the resources needed are typically sizable. One doesn't improvise such a venture: starting a MedTech company demands an ex-ante heightened awareness of specific challenges. Regulatory approval processes, dictated by agencies such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA), necessitate a thorough understanding of the stringent requirements for medical devices and technologies. Compliance with

Good Manufacturing Practices (GMP) and other quality assurance standards is non-negotiable, emphasizing the importance of integrating robust quality control measures into the company's operations. Furthermore, even if it doesn't directly affect the products commercialized by the new company, it's relevant to be familiar with the complex landscape of healthcare reimbursement policies and other healthcare industry money-flow peculiarities to understand the behavior of some players. Collaborations or partnerships with established industry players, healthcare professionals and institutions can enhance credibility and facilitate market penetration.

Intellectual property considerations are paramount in the MedTech sector, where innovation is the lifeblood not only of success, but of survival itself. Securing patents for novel technologies or at the very least ensuring freedom-to-operate through IP scans is a necessity very early in the development process and a safeguard against potential legal challenges later. Additionally, fostering relationships with research institutions and universities can provide access to cutting-edge research, talent, and potential collaborative opportunities, whose value can go beyond the aim to avoid future litigations.

Financial planning in the MedTech industry requires a keen understanding of the unique cost structures associated with research, development, clinical trials, and regulatory compliance. Diligent financial management, along with a realistic assessment of the time required for product development and market entry, is essential for long-term sustainability.

In conclusion, starting a medical technology company is an intricate process that demands a comprehensive understanding of both general business principles and industry-specific challenges. In the MedTech sector, careful consideration of regulatory pathways, quality assurance standards, intellectual property protection, and financial planning is essential. By navigating these intricacies with foresight and diligence, aspiring entrepreneurs can lay a solid foundation for success in the dynamic and impactful field of medical technology.

10.3. Navigating the Landscape of Targeted Drug Delivery

10.3.1. Mapping Out the Targeted Drug Delivery Ecosystem

The targeted drug delivery ecosystem is a complex interplay of organizations

active in research & development, manufacturing, commercialization, regulations, and clinical implementation. In this paragraph, we'll try to dissect it following an idealized (somewhat chronological) order, in the hope of identifying all the categories of players and highlighting the recursive patterns.

Research is where it all starts; this phase typically happens at universities, research centers and private companies. Research can be split into basic research and applied research. The latter is aimed at translating the scientific discoveries of basic research into practical applications for targeted drug delivery, including formulation development, biomaterials engineering, and nanoparticle synthesis.

Once there's input from research, there's the phase of (product) development, which typically happens in private companies; sometimes it can be externalized. This phase can be split in formulation development (designing/optimizing drug formulations to enhance targeting, stability, and therapeutic efficacy); preclinical testing (conducting in vitro and in vivo studies to evaluate the safety, efficacy, and pharmacokinetics of targeted drug delivery systems); clinical trials (assessing the safety and efficacy of targeted drug delivery products in human subjects, including Phase I, Phase II, and Phase III trials).

If the trials go well, then the new targeted drug delivery solution goes on to the manufacturing phase. This phase can be split into: production of drug carriers (nanoparticles, liposomes, micelles, hydrogels, etc.); encapsulation (also known as drug loading - incorporating therapeutic agents into drug carriers); quality control (ensuring product quality and consistency). Each of these processes can be performed in-house or by a third party.

Then the product goes into the distribution phase. Here the relevant processes are packaging (picking the appropriate dosage forms, such as vials, syringes, or implants); distribution (logistic channels for transporting targeted drug delivery products from manufacturing facilities to healthcare providers, pharmacies, hospitals, etc.); storage/handling. These processes are typically entrusted to partners, but they may as well be internalized.

In order to commercialize the product, private companies need to establish market access strategies for targeted drug delivery products through; this includes mapping the reimbursement mechanisms or any other dynamics specific

to the medical solutions they aim to market. They'll have to promote the products to healthcare professionals, patients, and caregivers through customized marketing campaigns, sales representatives, and educational initiatives. These activities are typically done in-house, but they may as well be outsourced to agencies or communication partners.

Healthcare Providers are the ones who actually use targeted drug delivery products in clinical practice settings; this includes hospitals, clinics, etc. Patient education and support organizations operate around these activities; they may prove to be interesting stakeholders in the ecosystem.

Throughout the above, there could be various types of interactions with regulatory agencies, such as the FDA (U.S. Food and Drug Administration) or EMA (European Medicines Agency). These actors are clearly external to targeted drug delivery companies, who often have their own compliance teams or a provider of such services, to produce the required documentation, reporting, and adherence to quality standards throughout the product lifecycle.

In summary, each of the phases or processes mentioned above could in principle be done by a separate entity or internally. It's important to map out all the players in the ecosystem, in order to understand the causal relationships and the interactions among them or bilaterally between them and the company; a particular focus should be put on understanding the dependency relationships and the risks of something going offside.

10.3.2. Market Outlook: Existing and Potential Players

The targeted drug delivery market is currently characterized by a diverse array of stakeholders, including established pharmaceutical companies, research institutions, startups, and biotechnology firms. Large pharmaceutical companies often engage in strategic partnerships or acquisitions of innovative startups to integrate cutting-edge targeted drug delivery technologies into their portfolios. Startups, on the other hand, drive innovation by focusing on niche applications and novel molecules or delivery mechanisms. Research institutions contribute to the development of foundational knowledge and often collaborate with industry players to translate scientific discoveries into tangible products. As the market is in a state of constant ebullience, the potential for new entrants and interdisciplinary

collaborations remains high. The dynamic nature of the field offers opportunities for partnerships between technology developers, clinicians, and regulatory experts, fostering an ecosystem conducive to the advancement of targeted drug delivery solutions.

10.3.3. Types of Customers

Targeted drug delivery technologies cater to a diverse set of customers within the healthcare ecosystem. From the point of view of a drug delivery innovative company, established pharmaceutical companies are the primary customers, either as developers of proprietary technologies with which to integrate the innovative one or as consumers seeking innovative solutions to enhance the efficacy of their therapeutic compounds. Healthcare providers, including hospitals and clinics, are the end-users employing these technologies in clinical settings; some innovative companies may decide to directly pursue them as customers, although it's relatively infrequent. Additionally, academic and research institutions may serve as both customers and collaborators, contributing to the continual evolution of targeted drug delivery through foundational research and technology development. Collaborative efforts between these customer segments are instrumental in refining technologies, conducting clinical trials, and ensuring successful market adoption.

10.3.4. How to Estimate Market Size and Make Assumptions

Estimating the market size for targeted drug delivery involves a rigorous analysis of various factors, including the addressable patient population, the prevalence of diseases targeted by the technology, the potential market penetration of novel therapies, and the palatability thereof considering their cost or side effects. Comprehensive market research, incorporating data from competitor analysis and industry reports on top of the internally sourced data, provides insights into existing market dynamics. Assumptions are often made based on extrapolations from preclinical and early clinical data, understanding the regulatory landscape, and anticipating potential challenges and opportunities. Scenario analyses, considering variations in adoption rates, technological advancements, and competitive or legislative landscapes, aid in developing a nuanced understanding of the potential market size. These sensitivity analyses are often required by investors or corporate partners. Engaging with key opinion leaders, regulatory experts, and market analysts further refines these assumptions and gives them credibility, ensuring a

comprehensive and informed approach to estimating the market potential for a specific targeted drug delivery technology.

10.4. Unlocking the Potential: From Lab to Market

10.4.1. Regulatory Framework

Navigating the regulatory landscape is a critical aspect of transitioning from research to production. Regulatory agencies, such as the FDA and EMA, play a central role in evaluating the safety and efficacy of medical technologies. Timelines may range from several years to over a decade, depending on the complexity of the technology, the therapeutic area, and regulatory requirements. However, any agency has several ways to obtain accelerated approval; we leave to the reader an in-depth analysis of what they are. In any case, the process requires meticulous documentation, data analysis, and compliant submissions, which have to be taken care of by expert professionals.

During the research phase, regulatory considerations about biocompatibility, toxicology, and preclinical data generation are paramount. As the transition to production occurs, adherence to specific regulatory frameworks for medical devices becomes imperative. Understanding the nuances of these frameworks, including the classification of the nanorobots, submission requirements, and engagement with regulatory authorities, is essential for a seamless path from lab to market.

10.4.2. What Happens After Approval

Upon achieving regulatory approval, the focus shifts towards commercialization and market penetration. Scaling up manufacturing capabilities to meet market demand, establishing distribution channels, and engaging with healthcare providers for product adoption become key priorities. Companies must ensure that the labeling and advertising of their drug delivery method comply with FDA/EMA regulations.

Post-market surveillance becomes an ongoing obligation, involving continuous monitoring of product performance, addressing potential safety concerns, and staying abreast of technological advancements for product improvement.

Companies must report any adverse events associated with their drug delivery

method to the regulatory agencies. This includes serious adverse events, unexpected adverse events, and adverse events that result in death, hospitalization, or disability. Building long-term relationships with key stakeholders, including healthcare professionals and patients who could provide genuine feedback about these aspects, is integral for sustained success in the market.

10.4.3. Frequently Overlooked Aspects

Frequently, in the enthusiasm to move from research to production, certain issues and risks may be overlooked, potentially jeopardizing the success of the nanorobotic venture. One common oversight is the length and/or complexity of the regulatory pathways, which may extend the time to market. Another critical issue is the frequent underestimation of the difficulty of scaling medical production processes, leading to challenges in maintaining product consistency and meeting demand. These issues are sometimes compounded by product issues: the design of the product could prove to be prone to misuse by practitioners. In general, it's good practice to heavily test the product before release; this will not only let the bad design appear clearer, but it could be an opportunity to analyze the cases of misuse by unprepared personnel, and hence to create a reparative course of actions for each case of misuse. Finally, it may be a good idea to create processes to prevent or repair the solution in case there could be issues caused by inappropriate transportation/handling/maintenance.

To mitigate all these risks, a best practice is to include early engagement with regulatory agencies, establishing robust quality control measures, and conducting thorough feasibility studies for scaled production. Collaborations with experienced manufacturing partners and leveraging industry-specific expertise can help navigate challenges effectively.

10.4.4. Tough Decisions When Things Go Well

If the venture proves successful and the market responds favorably, tough decisions may emerge concerning expansion, strategic partnerships, or diversification. Assessing whether to maintain independence or explore acquisition offers from larger pharmaceutical companies becomes a strategic consideration. Additionally, decisions related to further research and development investments for product enhancements or the exploration of new therapeutic applications require careful

evaluation. Weighting growth opportunities with the core mission and values of the company becomes a fine balancing act during this phase of success.

10.5. Securing Funding: From Grants to Venture Capital

10.5.1. Intro on Funding

Setting up a venture always comes with a relevant need of resources and this is all the more so in the medtech industry: the research itself can already be lengthy and costly; then pre-clinical and clinical trials can become very expensive; producing/commercializing a product can be the most resource-intensive phase. The funding arch typically begins with early-stage research grants, progresses through non-dilutive funding sources and may (or may not) culminate in venture capital (VC) investments during later stages. The funding journey aligns with the maturation of the technology, from proof-of-concept in the research phase to product development, clinical trials, and eventual market entry.

10.5.2. Non-Dilutive Funding

Non-dilutive funding sources, such as grants or debt, could be pivotal during the early stages of any company, especially a medtech one, which might as well be a university or corporate spinoff. These funding mechanisms are called “non-dilutive” because there is no equity give-away: the entrepreneurs retain ownership and control over their venture. While it may seem appealing at first glance, there are also downsides to this category of funding: they’re often one-off transactions, with little hopes of a follow up; the funding entity has no “skin in the game” and it will provide little besides the resources; in the case of debt, there’s a repayment risk that comes with it. For the sake of this analysis, we will disregard debt instruments (often simply inaccessible to startups without history nor revenues) to focus on grants.

Grants may come in different forms:

- Public research grants: typically offered by government agencies, these grants support early-stage research and development initiatives of national/regional public interest. Sometimes the key to obtaining them may be “political”: the funded activity has to fit within an intervention theme by the funding agency.
- Small business innovation research: these are also public grants, but the focus

is more on creating jobs in higher added-value industries and/or increasing the competitiveness of small companies. It can be limited to recently formed companies, but not necessarily.

- Technology transfer grants: a form of public grants as well, where the focus is on the valorization/commercialization of technology that has been developed within publicly-funded programs; also in this case, the desired outcome is related to occupation and competitiveness.
- Foundation Grants: private philanthropic institutions, non-profit organizations, foundations, etc… may also fund certain healthcare and medical innovative activities, with a greater discretion than public programs. Here the focus is more about the funding organization intervention themes (for example, foundations dedicated to curing a specific disease) or just the decision-makers willingness to intervene on a specific topic.
- Corporate grants and competitions: some large corporations offer grants or host competitions to encourage innovation in the medtech sector. These opportunities may include funding, mentorship, and access to resources. Their interest is in fostering a certain ecosystem, where they may later on scout for new trends and technologies.
- Accelerator/incubator programs: more and more often there are accelerator/incubator programs that do not necessarily require an equity investment in exchange for access to their mentorship and resources; sometimes, small grants are included in the support measures for assisted companies. This happens only in programs that are sponsored by larger entities.
- Clinical trial grants: some organizations provide funding for research that directly involves patients, supporting clinical trials for certain products. Sometimes these types of grants are funded directly by wealthy patients willing to volunteer for experimental cures.

The list above is by no means exhaustive, but it should give the reader an overview of the variety of non-dilutive sources that could be available to a budding medtech entrepreneur.

Whatever the grant, a very important aspect to be analyzed carefully is the time of payment and the reporting requirements. Some grants may come with a

"reimbursement" logic: the company has to first spend the grant amount and only then claim it from the grant-issuing entity; this implies that the amount has to be financed in some other way. Moreover, the reporting requirements are often very specific, so after having spent the amount, the claim might be rejected or only partially refunded. If overlooked, these aspects can become existential risks to the new venture, so they deserve appropriate attention.

10.5.3. Dilutive Funding

If the non-dilutive options are unavailable or unfavorable for any reasons, aspiring medtech entrepreneurs should consider dilutive funding, when new investors enter in the company's cap table and therefore "dilute" existing shareholders, reducing their total percentage ownership. While a grant is basically just a donation (although often with strings attached) and a debt could be always repaid, "equity is forever": typically, there isn't any pre-agreed manner of parting ways between the new shareholders and the old ones.

This category of investment can take many forms:

- Convertible note: this is a "quasi-equity" type of funding, because the investor agrees to invest a certain amount as debt (an interest-carrying note), which can later be converted into equity, often at a discount, upon certain pre-set conditions. Here the advantages are for both counterparties. If things don't go too well, the investor is protected against losses: in case of bankruptcy, the unconverted debt will be senior to the equity, which means that when assets are liquidated, the proceeds are distributed first to creditors and then, if there is anything left, to the shareholders. If things go well, the investor can become a shareholder and enjoy the upside of holding equity. For the entrepreneur, convertible notes can be a way to access resources in a fairly quick and effective manner (there's no need to come up to a valuation or share price and the paperwork is often very light, there are even some industry standards such as the SAFE - simple agreement for future equity), without too much dilution: if the company is successful, the conversion of the note into equity typically happens at a discount to the next equity valuation, which is more favorable than having to sell equity early on.
- Priced round: this is a classic equity investing, where the entrepreneur and the investor agree on a valuation and new shares are issued (hence they "price" the

shares). Usually, investors will require special privileges that are not granted to holders of common stock to compensate for the risk of investing in unproven technologies. Some of the most common rights/privileges are: board seat representation, pro rata rights, anti-dilution protection, drag-along and tag-along rights, right of first refusal, liquidation preference. The list can be much longer; we advise the reader to thoroughly research them before engaging in negotiations of a "term sheet" (the document with all the terms and conditions of the investment) with investors.

- Mezzanine: this is another form of debt that ends up being quasi-equity. It is debt often issued by mature startups in the growth phase, to bridge the gap between priced funding rounds or other cash inflows, or for acquisitions, recapitalization, management/leveraged buyouts and other extraordinary operations. It's called "mezzanine" because this debt stands in between equity and senior debt in the liquidation process; to compensate for this risk, investors usually require a higher interest compared to other forms of debt, frequently in addition to the possibility of converting the debt into equity if it is not repaid.

Besides the category of investments, there can be different categories of investors, whose nature can shape the dynamics of the relationship with the company:

- FFF: the acronym stands for "family, friends and fools"; typically the very first backers of aspiring entrepreneurs. They typically offer relatively modest amounts - their own personal savings and are open to convertible notes or equity rounds at low valuations.
- Angel investors: these are high-net-worth individuals who invest their personal funds in early-stage startups, often providing mentorship and industry expertise in addition to capital. They favor priced rounds, but can be open to convertible notes.
- Venture capital (VC) funds: institutional investors who manage money for other institutions, called limited partners (pension funds, insurances, university endowments, family offices, etc.). They often have a theme and a stage focus. This type of investors almost always has a preference for priced rounds, typically at higher valuations than the former two categories and with a minimum percentage that they'd take.

- Family offices: professionally managed large fortunes used to be limited partners in VC funds, but more and more often are starting to do direct investments in startups, with similar dynamics of VC funds, but with a higher degree of freedom, since they manage their own resources.
- Corporations: the possibility of receiving investments by large companies is not to be overlooked, but it's noteworthy that they almost always have a direct interest (as a client, supplier or even competitor) that could change the decision-making process when important decisions have to be made.
- Corporate venture capital funds: the investment arms of established healthcare or technology corporations can invest in and collaborate with innovative medtech startups. They are often run independently, but the corporation interests are an inevitable part of the background anyways.
- Public venture capital funds: they're run similarly to private VC funds, taking stakes in the equity of early-stage companies, but besides the return on capital, they also try to maximize the returns for the local economy or national interests.
- Banks and other financial intermediaries: it's rare, but traditional financial institutions could become shareholders in certain new ventures, if it fits their interests. Usually, these investors are not the lead investor, but rather part of a syndicate.
- Private equity funds and other: these funds are not interested in early stage companies, but rather in mature ones: it's rare, but they could be considered for mezzanine financing operations.

When there's a professional investor involved, entrepreneurs will almost always be asked to have their shares subject to a vesting plan: they will "earn" their shares by staying at the company (usually vesting plans last for 48 months, but it can vary). This mechanism keeps investor and entrepreneur interests aligned and prevents behavioral issues among founders.

We'd suggest the reader to research further the aforementioned topics, as they are part of the basic knowledge that an entrepreneur must have in order to negotiate with investors.

10.5.4. Specific dynamics in MedTech funding

MedTech funding exhibits unique dynamics influenced by the complex and regulated nature of the industry. Investors in the MedTech sector often seek a balance between the potential for significant returns and the inherent risks associated with regulatory approval processes. Recent trends indicate a growing interest in early-stage MedTech investments, with a focus on technologies that address unmet medical needs and demonstrate clear clinical value. Additionally, strategic partnerships between MedTech startups and established corporations have become prevalent, providing startups not only with funding but also with access to industry expertise, distribution channels, and regulatory guidance. The rise of specialized MedTech venture funds and corporate venture arms signifies a recognition of the distinct challenges and opportunities within the MedTech sector. As the industry evolves, there is a notable trend towards value-based investing, emphasizing technologies that offer demonstrable improvements in patient outcomes and healthcare efficiency.

However, as of 2023, there has been a slight reduction in the number of rounds and amounts thereof, largely attributable to a decline in exits (both M&A and IPOs). So, albeit the trend is positive, aspiring entrepreneurs should be aware of a more competitive landscape to obtain funding and prepare accordingly.

10.6. Making a Plan

10.6.1. Business Model

The first planning activity to do is to create a solid business model, which is integral to the successful translation of research into a viable and sustainable venture. A starting point could be the so-called Business Model Canvas, for which templates can easily be found online. This tool helps the entrepreneur to think and map out all the variables and the way they influence each other. Aspiring entrepreneurs should remember that of all the value they can manage to create, they'll be able to capture only a fraction of it. The business model then should encapsulate the key elements that define how the company will create, deliver and capture value. An effective business model outlines the value proposition, target customer segments, channels of distribution, customer relationships, key resources, key activities, key partnerships, revenue streams and cost structure. The model should be adaptable

to the unique intricacies of the medtech industry, considering regulatory compliance, manufacturing precision, and the integration of nanorobotic technologies into the existing healthcare ecosystem. So, should the environmental or internal circumstances change, an entrepreneur will know how this change affects the business activity.

10.6.2. Value Proposition: What Are You Going to Sell to Whom Exactly?

Out of all the elements of a business model, the Value Proposition deserves particular attention. It should go beyond the technical attributes of the technology; it should rather emphasize the specific benefits it delivers to end-users and stakeholders. For medical professionals, the value proposition might focus on enhanced precision in drug delivery and surgical procedures, leading to improved patient outcomes. For healthcare institutions, the proposition could highlight potential cost savings and efficiency gains. Communicating the value proposition effectively requires an understanding of the unique needs and pain points of each customer segment, ranging from pharmaceutical companies and clinicians to healthcare administrators.

Some important aspects of focus could be:

- Market segmentation: understand the diverse needs of different customer segments and tailor product offerings accordingly to maximize sales effectiveness. Concretely: it doesn't make sense to push a pioneering solution to hospitals or professionals that are not ready; instead, try to have different solutions that could be adopted in an easier way by slower-moving organizations. Conversely, try to offer frontier-tech to pioneer customers as soon as possible.
- Timing: recognize that customer needs and preferences evolve over time, necessitating adaptive sales strategies to offer the right products at the right moment. Concretely: similarly to what mentioned in the former point, as an organization becomes more familiar or more ready, it's possible to pitch to them more complex solutions. Viceversa, when key professionals leave the customer, it might be appropriate to propose to scale-down the solution.
- Customer relationship: a strong customer relationship is not good just to repeat sales, but above all to get insights from the power users and so identify the correct time/manner to introduce or promote new solutions.

- Reactiveness: the ability to regularly monitor market conditions in general and customer preferences will hopefully ensure the alignment of the company's offer with evolving demands by existing customers.

Focusing on the above aspects should increase the chances of getting the value proposition right.

10.6.3. Cost Structure and Revenue Streams

Another paramount aspect of the business model, which is the bottom section of the business model canvas, is the structure of costs and revenues. It's key to spell out all the cost sources, including research and development expenses, manufacturing costs, regulatory compliance expenditures, and marketing and distribution expenses. A comprehensive understanding of the cost landscape is vital for establishing the minimum price at which it's possible to operate (we'll leave the in-depth analysis on pricing strategies to the reader).

Concurrently, identifying the possible revenue streams is equally key. Revenue sources may include the sale of nanorobotic products, licensing agreements, partnerships, or even service-oriented models. The diversification of revenue streams contributes to the resilience of the business model and has also an influence on pricing decisions.

10.6.4. Business and Financial Plan

A few noteworthy quotes regarding planning:

"Who fails to plan is planning to fail"
"No plans survive the impact with reality"

Seasoned entrepreneurs would find both quotes very agreeable, but how is it possible to reconcile them? Is it pointless to have a plan if it will inevitably be changed? Absolutely not. Planning is a necessity, even if when writing there's already the acute awareness that some aspects will need revision some time down the road. The business and/or financial plan serve as dynamic documents, evolving in tandem with the progression of the business, the technology and the shifting industry landscape.

A robust business plan serves as a roadmap, outlining the company's mission, vision, and strategic objectives. It encompasses the business model, market

analysis, competitive landscape, marketing and sales strategies, operational plan, risk assessment/management and financial aspects.

The financial plan could be treated separately if needed, with only a summary of it included in the business plan. This document delves into revenue projections, expense forecasts, capital requirements, and sensitivities of the most impacting variables to the business metrics.

For MedTech companies some areas of particular focus should be the timeline for regulatory approvals, clinical trial expenditures, and market entry costs.

10.7. The Power of Collaboration: Forging Partnerships

10.7.1. Pros and Cons of Partnerships in MedTech

Partnerships in the MedTech sector can be instrumental in accelerating the development and commercialization of innovative technology. The primary advantages are:

- Access to resources: the pooling of expertise, physical resources, and organizational capabilities, enabling a more comprehensive approach to address the complexities of research, development, and market entry. Sometimes it's not just a plus, but rather a necessity, because the startup may lack certain essential resources.
- Shared costs/risks: by partnering with another organization, the startup can share its financial burden and/or the risks associated with developing and launching a new product.
- Increased market reach: besides access to existing channels, partnerships can enhance credibility and validate the technology through association with reputable organizations.

However, navigating partnerships in MedTech requires careful consideration of potential challenges. Some frequent ones include:

- Loss of control: By partnering with another company, the startup may have to give up some control over its product or business. This impacts not only the decision-making process at critical crossroads, but also the way business is conducted: differences in organizational culture and the need for extra-clear communication (often through additional formalities) to ensure alignment of

goals can considerably slow down the speed at which the startup can move.

- Conflicts of interest: conflicts of interest can arise if the goals of the startup and its partner are not aligned. On those occasions, the bargaining power of the larger partner could be felt in an unpleasant way.
- Legal issues: if the partnership or alliance was not structured properly or if there are disagreements between the partners, there could be nasty legal issues that could endanger the development of the product or even the very existence of the junior partner. This can often relate to intellectual property, which is paramount for the startup.

10.7.2. Types of Partnerships

Partnerships in the MedTech industry come in various forms, each tailored to address specific needs and stages of technology development. For example, an emerging startup may consider a research collaboration with academic institutions or research centers that can provide access to cutting-edge knowledge, facilities, and talent; it may as well consider a strategic alliance or industry partnership with other pharmaceutical or medical device companies to speed up the development or improve the distribution of its products, or to get financial support, regulatory guidance, market access, etc. The possibilities are countless and so there's a great variety of the types of partnerships possible, summarized in the following categories:

- Joint Venture: two companies come together to form a third distinct legal business entity, by contributing resources, capabilities, and core competencies. This new entity is a "child company" in which the parent companies have a shared interest.
- Equity Strategic Alliance: this type of alliance is created when one company purchases a certain equity percentage of another company and therefore it typically also acquires some voting power. Besides the financial investment, there's often also a formal agreement to work together.
- Non-equity Strategic Alliance: in this alliance, companies work together to achieve a specific objective without making a financial investment in each other. The agreement can be relatively generic or very detailed, to the discretion of the involved parties.

Licensing agreements, allowing the use of intellectual property in exchange for royalties or other considerations, are also common in MedTech and can characterize all the aforementioned types of partnerships.

10.7.3. How to Structure a Partnership

Structuring a partnership in the MedTech sector requires careful consideration of the goals, expectations, and contributions of each party. This is true not only for the organizations, but also for the individuals involved: understanding their perspective is key to forge a partnership. Needless to say, some caution about the turnover of the involved people would be wise.

In general, clear communication and a shared vision are foundational elements. Establishing a Memorandum of Understanding (MOU) or Letter of Intent (LOI) at the outset can provide a framework for discussions and negotiations.

Later in the collaboration, it is crucial to define the roles, responsibilities, and contributions of each partner, including financial commitments, intellectual property arrangements, and milestones.

Addressing potential conflicts and establishing mechanisms for dispute resolution lowers the risk of actually incurring in those.

Additionally, having a well-defined exit strategy in case the partnership needs to be dissolved or evolves into a different form provides clarity and mitigates potential challenges.

10.8. Conclusions

Entrepreneurs looking to start a company in this field should be encouraged by the growing interest in localized drug delivery systems. The recent breakthrough technologies enabling on-demand controlled drug release can spark a revolution in medicine, and create numerous opportunities for entrepreneurs to make a significant impact in patient health and clinical outcomes. The growth of the ecosystem is making it easier to start a company, with the possibility of externalizing non-core activities and to receive support by pharmaceutical companies and health systems, complemented by more and more resources available from more and more interested specialized investors.

This is a challenging, yet fertile ground for innovation. The call to action is

straightforward: aspiring entrepreneurs should embrace this complexity with a pragmatic mindset. Engage regulators early, diversify funding sources strategically, and cultivate transparent collaborations. In a realm where breakthroughs are tempered by stringent regulatory processes and financial realities, success lies in a no-nonsense approach and an unwavering commitment to driving impactful change in healthcare.

Appendix: Resources for Aspiring Entrepreneurs

Media

Popular industry publishers: ACS, ScienceDirect, Drug Discovery and Development, PubMed.

The top 100 influencers discussing Pharma & Biotechnology online. Source: Onalytica.

Toolkits

Legal (incorporation, contracts, IP, investments, etc.)

- Orrick: Startups Forms Center
- NOLO: DIY Products
- Series Seed Financing Documents
- Techstars Docs

Miscellaneous

- OwnYourVenture - equity calculator
- Nubie - business plan
- Strategyzer - business canvas template
- F6S - free resources for startups

Incubators/accelerators (non-exhaustive list)

- 1 Kubator
- 50 Partners
- Accelerace
- Accelerating Asia
- ANGIE accelerator
- Base Launch
- Bayer G4A
- Bizion Group
- Capital Accelerate & Scale Tech Superstars
- Cites
- Cork BIC
- Creative Accelerator
- DayOne
- Digitalhealth London
- Eckert Life Science Accelerator
- EIT
- Fast Track Malmö
- Flying Health
- G2 Startups
- Greater Philadelphia Alliance for Capital and Technologies
- Hatch
- HCF Catalyst
- Health Hub Vienna
- Health venture lab
- Health2B
- HealthCare Lab
- HSEVEN
- i&i Prague
- I3P
- Impact Hub Milan
- Incentive Incubator
- Innopeaks
- InnovationRCA
- Invest Ottawa
- Iowa Startup Accelerator
- ITACA
- MAN Impact Accelerator
- MEDX Xelerator

- Nurture Ventures
- OJAS MedTech Accelerator
- Open accelerator
- Richi Entrepreneurs
- Rockstart Health
- Rubik Hub
- Sberbank-500
- Schweizer Kapital Global Impact Fund
- SELLALAB
- SINE
- Startup Bootcamp Digital Health
- Startup Leadership Program
- Startup Lighthouse MedTech DDW
- StartX
- Syddansk Innovation
- Terkko Health Hub
- Ubiz Accelerator
- Wilco
- WorldStartup

VC/CVC (non-exhaustive list)

- AbbVie Biotech Ventures Inc. (CVC)
- Abingworth
- Albion VC
- Alexandria Venture Investments (CVC)
- Almi
- Ananda
- Apex Ventures
- Archventure
- Ascension
- Astellas Venture Management (CVC)
- b2Ventures
- Balderton Capital
- BGV
- Biomed
- Black Pearls
- Boehringer Ingelheim Venture Fund (CVC)
- Eight Roads
- EQT Life Science
- Forbion
- Fprime Capital
- General Catalyst
- Giant
- GlaxoSmithKline (CVC)
- Heal Capital
- Health Cap
- Healthy Capital
- High Tech Gründerfonds
- Hoxton Ventures
- Index Ventures
- Industrifonden
- Inhealth
- Inkef
- Inovo
- Johnson & Johnson Innovation (CVC)
- Karista VC
- Kurma Partners
- Leaps by Bayer CVC
- Merck Global Health Innovation Fund (CVC)
- Meta Planet
- MPM Capital (CVC)
- Nina Capital

- Novartis Venture Fund (CVC)
- Novo Ventures (CVC)
- Octopus Ventures
- Panakes
- Pfizer Venture Investments (CVC)
- Qualcomm Ventures (CVC)
- Red Alpine
- Remind
- Roche Venture Fund (CVC)
- Sanofi-Genzyme BioVentures (CVC)
- Seed camp
- Seventure
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$9.00
ISBN 979-8-89507-901-0
50900>
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