Chapter 12
Limitations of Current Cancer Theranostics

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Introduction

Throughout the last decade, a tremendous breakthrough in nanotechnology has resulted in highly versatile nanomaterials worthy of recognizing, tracking, regulating, and curing disease progression (Caldorera-Moore et al., 2011). Nanocarriers have shown promising results in the therapy of cancer, which is the second leading cause of death worldwide. Nearly 7,556,956 deaths are caused by cancer in the year 2020. Owing to new studies reported in *The Lancet Oncology*, the worldwide cancer prevalence is expected to increase by greater than 75% by the year 2030.This surge is expected to become even greater in the developing countries, with the poorest countries witnessing a predicted rise of higher than 90%. The complex composition of cancerous tumors quite often makes it complicated to provide an accurate diagnosis and effective treatment. Interindividual tumor variability is due to the wide variability of the types of tumors, distinct genetic factors, and histogenesis (Bray et al., 2019). Conversely, traditional cancer treatment modalities, such as chemotherapy and radiotherapy, lack the individualized treatment approach as tumor characteristics vary from person to person (Guo et al., 2019; Peng et al., 2019; Thorat et al., 2019). Currently, the nanotheranostic approach has been widely applied in cancer treatment for early tracking and diagnosis.

The idea of theranostics usually includes combining medication, diagnostic tools, and image analysis methodologies into a single procedure for cancer care regimen. Integrating nanostructures (nanocarriers, imaging nanoagents) with theranostics on a single framework is referred to as "nanotheranostics." One of the goals of the nanotheranostic approach is to develop personalized and uniquely engineered

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chemotherapeutic agent-associated nanocarriers, which can both provide therapy and perform diagnosis according to the tumor variability of an individual (Tang et al., 2019a, 2019b; Yu et al., 2019; Liao et al., 2020; Boehnke et al., 2020). Leveraging the application of nanocarrier and anticancer agents makes nanotheranostics an attractive technique for cancer treatment. Nanotheranostics deliver and release chemotherapy agents in reaction to internal or external signals or stimuli to elicit a successful therapy (An et al., 2019). Simultaneously, monitoring and regulating drug release can allow cancer clinicians to supervise excess chemotherapy-induced adverse effects or insufficient dose (Ding et al., 2019).

Although nanotheranostics have numerous advantages, some challenges need to be tackled for successful delivery. The biggest obstacle in the preclinical characterization of nanotheranostics is the need for a thorough comprehension of nanoformulations. The consistency of the formulations under the varying environments that could affect their efficacy should be scrutinized at each point of the process of production. The application of multifunctional nanomedicine platforms is limited by high levels of production expenditure and difficulties in their development process. The main obstacle for their clinical therapy is the conflicting intervals and proportions of imaging and therapeutic agents used in these platforms. Although the primary aim of the imaging technique is to use the minimum quantity of imaging agent for a short period to achieve a high signal-to-noise ratio, for therapeutics, the maximum allowable dose (maximum tolerated dose) is required to induce a good potential cytotoxic effect (Svenson, 2013). Another drawback is the difficulty in successfully encapsulating both cytotoxic and image contrasting agents within a single nanocarrier. According to the principle, if the encapsulated sections do not modify the surface properties and dimension of the nanostructures, combined encapsulation is not necessary, given that these are administered simultaneously in a balanced proportion. However, as it is proven, the implanted substance can affect physicochemical processes; there is a consistent drawback of targeting strategy. The main aim of combining sophisticated guided delivery systems is to enhance the specificity of presently existing therapies. There is a reason for integrating multiple approaches within a single nanocomposite to address the shortcomings of each modality, leading to the development of multidisciplinary nanotheranostics.

Current Nanotheranostic Platforms for Cancer

Numerous nanotheranostic frameworks have become introduced over the last tenure. However, the most widely used nanotheranostics are gold nanoparticles, mesoporous silica nanoparticles, carbon nanotubes, liposome-based nanocomposites, and upconversion nanoparticles. These nanocomposites make it possible to imagine and track the path followed by the formulation, to provide insights on pharmacokinetics, intra-organic and intra-tumor utilization, and drug efficacy and safety profile. The stability of nanoparticles should be maintained inside the body. It must withstand intervention from the host's defense mechanism before it enters its key trigger

site and is ingested. The form and size of nanotheranostics used are relevant to their potency at a binding site (Penet et al., 2014).

Gold Nanoparticle (AUNPs)

AuNPs made with gold bases are a revolutionary device that displays special attributes for theragnostic delivery. They are biocompatible primed by chemical modification with hydrogen tetrachlorocuprate and are often in the form of balls, cubes, sticks, cages, and wires. The gold nanoparticles can be easily customized into different sizes and shapes or can be conjugated with the other surface. Researchers can take advantage of this flexibility to explore its potential in nanotheranostics, particularly in malignant tumors. Compared to other nanoparticles, gold nanoparticles have demonstrated an antineoplastic impact caused by oxidative emphasis on the cellular level. The powerful aspects of gold nanoparticles involve characteristic diagnostic properties, monodispersible ability, surface-to-volume ratio, less toxicity, and ability to connect the biomolecule, and packing of therapeutic agents is carried out by electrostatic activity as well as covalent conjugation (Norden et al., 2008; Fan et al., 2017). Wang and his work colleagues had already created a double stratified device for chemotherapeutic drug delivery. The LDH-Gd/Au nanoparticles demonstrated a strong non-anionic chemotherapeutic drug volume fraction of DOX (264 mg drug/g carrier, which shows a feature of pH-sensitive activation.

Magnetic Nanoparticle (MNP)

MNPs have proven to be effective in enhancing targeted cancer drug delivery with the aid of magnetic resonance imaging. Nanoparticles can be retained in tumor tissues in conjunction with an external force field. Due to this field, the magnetic center elevates the targeted delivery of nanoparticles. Iron oxide nanoparticles (IONPs) typically comprise of a magnetic center, e.g., magnetite/iron oxide and an external polymeric shell starch and dextran are used. They provide a valid approach of application in theranostics, owing to potential superparamagnetic effects, suitable biocompatibility, and its use as a contrast agent in MRIs. MNPs have proven promise as nanomedicines in enhancing drug delivery at cancer sites with the benefit of MRI tracking. IONPs are best known to be biocompatible since they degrade in the biological process and metabolize into the serum (Sonali et al., 2018). The key limitation of magnetic nanoparticles is the low solubility in water and accumulation within the cell. For example, Santra and his colleagues used poly(acrylic amide) (PAA) to embed lipophilic NIR dye and anticarcinogenic drug Taxol inside hydrophobic spaces, combining a double-fluorescence nanostructure with MRI imaging tracking of drug delivery in a theranostic.

Quantum Dots (QD)

QDs are nanosized inorganic multifunctional platforms for nano-therapy. Quantum dots have lately been identified as appealing diagnostic operators for therapeutic purposes, which are noticed to be better than traditional organic fluorophores. For example, cadmium and zinc sulfide-based QDs are the most common nanostructures in clinical diagnostics. It comprises Cd-Se (cadmium-selenium) center that is laminated with a heap of zinc sulfide (Zhang et al., 2017). For example, QDs-525 and QDs-585 are selective for HER2 (generally expressed in breast cancer) and type IV collagen (ECM) and have explored the function of HER2 in breast cancer cell lines.

Carbon Nanotubes (CNTs)

CNTs have a cylindrical tube resulting from stacks of graphene layers (allotropic forms). They have unique electronic and mechanical properties ideal for theranostic formulation. CNTs will boost cancer therapy, which offers a good substitute for therapeutic implementation and also provides potentially lethal heat for NIR irradiation. If the cells are taken up, they can also interfere with proteins and DNA to influence cell signaling or mechanism for other treatments.

Mesoporous Silica Nanoparticles (MSNPs)

Mesoporous silica nanoparticles are developing delivery systems and are widely studied in terms of their configurable size. A lot of diverse drugs like paclitaxel, camptothecin, doxorubicin, and methotrexate are incorporated into mesoporous nanoparticles. Bioresponsive MSN prevents early release and provides fluorescent images, whereas trifunctional MSN covers the benefit of target specificity (ATN0647N is used as a contrasting agent) and minimal damage to healthy cells.

Upconversion Nanoparticles (UCNPs)

Components are activated by the uptake of low power radiation at a higher wavelength, accompanied by the transmission of light with greater intensity. Such feature makes upconversion nanoparticles successful nanotheranostic applications (Fang & Wei 2016). A narrow and sharp UCNP emission band considerably enhances the productivity and responsiveness of upconversion nanotheranostics. Lanthanide containing upconversion nanoparticles is most widely used, as they are rapidly removed

from circulation (Auzel 2004). Upconversion nanoparticles are insoluble in water due to their hydrophobic nature, and they are also sometimes water-dispersible. The benefits of UCNPs are small emission levels, strong physical-chemical stability, broad stroke shift range, and reduced toxicity. UCNPs are also important substitutes to traditional fluorescent probes for clinical applications (Sonali et al., 2018).

Polymeric Nanoparticles (PNPs)

Polymeric nanoparticles have proven unique benefits due to their ability to trap antineoplastic agents and restrict their metabolization (Van Haute et al., 2018). The therapeutic value of various water-soluble or insoluble drug products has been shown to enhance bioavailability, solubility, and retention (Invernici et al., 2011). Such traditional natural polymers that are used for the development of nanoparticles involve chitosan, gelatine, polyanhydride, etc.

Polymeric Micelles (PMs)

The pH-sensitive polymeric micelle self-assembled from a biodegradable brush-type copolymer (PHF-g-(PCL-PEG)) showed a threefold increase of cumulative drug release at pH 5.0 than that of at pH 7.4. PEG, poly(acrylic acid), and dextran are also reported to create the micellar outer shell, which offers defense from drug clearance by suppressing opsonization process and reducing clearance by the RES uptake. The extent and design of the hydrophilic polymers had an impact on the dimension of the polymeric micelles and also expressed the particle aggregation pattern at the tumor site and subsequent internalization capacity of the tumor cells.

Solid Lipid Nanoparticles (SLNs)

Solid lipid nanocarriers are effective possible opportunities in anticancer therapy. It is synthesized by dispersing lipids and surfactant with aqueous media. This adds the perks of both lipidic formulation and polymeric nanoparticles and exhibits an enhanced degree of safety in the host's biological conditions. SLN can be loaded with various imaging agents and can also incorporate various contrasting agents. This nanostructure is accountable for the continued delivery of anticancer drugs and has effective penetrability throughout the outer cellular membrane and enhanced cytotoxicity. Table 12.1 highlights the advantages and disadvantages of current nanotheranostic platforms.

Limitations of Current Cancer Nanotheranostic Approach

Design and Development Limitations

The development of nanotheranostics has largely benefited from nanoscience advancements since many drug deliveries based on nanoparticle platforms represent a logical and basic choice for developing nanotheranostic systems (Cui & Wang, 2016). There are few nanotheranostic platforms that have been present for decades. However, frequently employed are traditional platforms like silica and gold, silver nanoparticles, liposomes, quantum dots, and composite nanoparticles.

The physicochemical property of nanoparticles like size, shape, surface functionalization, and charge decides their fate. Nanoparticles with small size (<20 nm) undergo rapid distribution but also get subjected to quick renal clearance. On the other hand, nanoparticles of larger size (>200 nm) undergo clearance by the mononuclear phagocytic system and accumulate in various organs like the spleen and liver. Also, the size distribution is a key factor to be taken into consideration while designing nanotheranostics. The normal size distribution for a wide variety of particles is <200 nm in size, to confer the full advantage of nanomedicines. The pore size of the endothelial junction in the tumor environment lacks lymphatic drainage, which further enhances the retention effect and permeability of nanoparticles. Nanoparticles have a unique characteristic that affects their application in imaging and functionalization. For example, certain sizes are strongly recommended and serve advantageous for targeting specific sites and exhibit their action particularly in anticancer treatment. The particular size supports their circulation time over standard anticancer therapeutics in vivo and enhances the absorption from the tumor blood vessels into tissues via tumor vasculature. Some methods of nano-fabrication utilize toxic raw material or generate toxic by-products. This phenomenon needs to be understood completely to be clear with the harmful effects of engineered nanoparticles as it largely depends on the species as well as the size and geometry of particles (Murty et al., 2013). After size, the surface property of nanoparticles plays an important role, which affects interaction and behavior with cells and protein. For example, in the case of siRNA, the diffusion across the plasma membrane is thwarted by anionic charge and large size, which prevents accumulation intracellularly (Gavrilov & Saltzman, 2012). Another example is of Myocet® and Doxil® is quite relative. Non-stealth or conventional liposomes have a higher affinity for the mononuclear phagocytic system, and they get quickly removed from circulation. PEGylated liposomes of doxorubicin have shown prolonged half-life, enhanced drug concentration, and better efficacy with limited side effects as compared to conventionally available doxorubicin formulation. Myocet®, the non-PEGylated liposomes of doxorubicin combined with cyclophosphamide for breast cancer, is superior to it. The component variations allow Myocet to exhibit reduced toxicity and no hand-foot syndrome.

Some important factors that are to be considered while working with nanoparticles in nanotheranostics are:

Table 12.1 Highlighting the advantages and limitations of current nanotheranostics platforms

Nanotheranostics platforms	Advantages	Limitations	References
Gold nanoparticles	These are easy to synthesize, and the Surface can be modified easily. The targeted delivery can be achieved with attachment of ligands	There are many toxicity concerns related to gold nanoparticles. The lack of standardized assay methods results into altered interpretation which limits their application	Arvizo et al. (2010) Cell et al. (2019)
Polymeric micelles	They provide high drug loading capacity especially for hydrophobic drugs. These are nontoxic platforms And can exhibit controlled release	They show variation in blood circulation time. The stability shows variation in some cases	Ahmad et al. (2014) Yokoyama (2014)
Polymeric nanoparticles	The method of preparation is easy. It provides targeted delivery and high therapeutic efficiency	They show cytotoxicity via accumulation inside organs. They exhibit limited capacity of targeting. Some of them demonstrate carcinogenicity and inflammation	Singh et al. (2017) Gopalasatheeskumar et al. (2017)
Mesoporous silica nanoparticles	They have large Pore size, great compatibility, and biodegradability. They make stable dispersion in aqueous environment	The reproducibility is complex, And they also require expensive processes for manufacturing	Vallet-Regí et al. (2018) Jafari et al. (2019)
Carbon nanotubes	They exhibit great Mechanical strength; they have high surface area and aspect ratio and exhibit excellent conductivity. They increase the capacity of molecular imaging by enhancing sensitivity and selectivity of detection	They demonstrate toxicity by rupturing cell membrane, show cytotoxicity, produce reactive oxidative species, and also show biochemical toxicity	Gholizadeh et al. (2016) Porwal et al. (2017) Shao et al. (2013)
Quantum dots	They show good stability. They have broad band spectrum and high Surface-to-volume ratio	They usually have large size (10–30 nm) and also exhibit blinking response They also induce cytotoxicity	Barroso (2011)

(continued)

Table 12.1 (continued)

Nanotheranostics platforms	Advantages	Limitations	References
Magnetic nanoparticles	Magnetic nanoparticles demonstrate another type of hyperthermia behavior, are superparamagnetic, and also demonstrate effective targeting	They demonstrate varied toxicity depending on sizes. Also MNPs can Induce a cytotoxic reaction upon internalization. Further issue is with their poor degradation and accumulation in organs	Mandal et al. (2017) Markides et al. (2012)
Upconversion nanoparticles	They exhibit high signal-to-noise ratio, and they have greater photo stability; they show minimal photo damage and demonstrate deep tissue penetration. They can also show light stimuli drug release	They exhibit low targeting efficiency and thus require more strategies to deliver therapeutics at a target site. Only a small fraction reaches to tumor site	del Rosal et al. (2019) Ang et al. (2011) Wu et al. (2015)
Lipid nanoparticles	They enhance bioavailability of poorly soluble drugs. They are biocompatible and show controlled release	The design of lipid nanoparticle is complicated and often shows instability	Shahi et al. (2015) Lee et al. (2012)

1. Thorough knowledge of the target cell type and biomarkers present at the target site.
2. Route of therapeutic administration and pathway that will be followed to reach the site of action.
3. In vivo stability of nanoparticles (they must show resistance to immune reaction).
4. The nanoparticle's size and shape play a vital role and it directly controls the efficacy.

Rod-shaped, disk-shaped, and worm-like, all differ in their drug loading capacity, absorption at the target site, circulation time, and uptake at the target site, for example, for cancer theranostics, the recommended shape is spherical.

A simple alteration in nanoparticle shape provides new labeling opportunities. Example nano-prisms show interaction with light differently as compared to spherical particles and subsequently appear differently colored. This variation provides the basis of multiplexed assays, wherein nanoparticle labels are made from the same material but depend on shape differences to generate unique optical signals (Emerich & Thanos, 2006).

5. The size range of nanoparticles may vary from 50 nm to 200 nm. In the intestine, this size range has been tested.

All these characteristics of nanoparticles were found beneficial in the field of personalized medicines in diagnosis even based on biomarker identification.

Based on the reports, the ideal size required for tumor targeting nanoparticles is in the range of 70–200 nm. From a technical standpoint, polymeric particles can hardly be made with a size smaller than 5 nm. To highlight the major role of size, the difficulties of getting precise measurements of size will be the first standpoint. However, out of all techniques, most cited ones are SEM and LS (Gaumet et al., 2008).

Another challenge in the development of nanotheranostic platforms is the successful manufacturing of nanotheranostics. Conventional formulation development does not involve the creation of a 3D system of nanoscale multi-components, and this leads to a series of challenges for scale-up of nanoparticles. Complete knowledge of multi-components with their interaction is the main requirement to define key characteristics of the formulation. Identifying important manufacturing conditions is essential to achieve the main function and attributes. Based on conditions, the procedure may result in a changed chemical structure of API with a substantial quantity of impurities. In the case of macromolecules specifically biologics, it may lead to altered conformation, cross-linking, denaturation, coagulation, and degradation. Ideally, the manufacturing process should be robust and should be streamlined so that it ensures easy scale-up for production. Nanoparticles which are to be administered by parenteral route need sterilization, where it will face problems related to particle size and composition; also they are known to get damaged by the method of sterilization. The sterilization method is not problematic when the structure is malleable or has flexibility (Desai, 2012). Another issue during the manufacturing of nanoparticles is the safety of the environment. The handling and dealing with dry matter of nanometer range requires specific caution as nanoparticles which are airborne distribute as aerosols. The deposition of these nanoparticles in the lung leads to pulmonary toxicity. During the preparation of the dosing solution, the aerosolization should be avoided to prevent unintended exposure. Nanoparticles which are created in liquid environments demonstrate low impact on the environment, presumably the same as standard manufactured liquid pharmaceutical formulations. The most challenging aspect of developing nanomedicines is a selection of the most suitable analytical method to characterize nanomedicines whether biologically, physically, or chemically from technical and regulatory perspectives. More innovative methods of testing are continuously being developed and used for the analyzing nanoparticles. However, these tests cannot differentiate between an active and inactive formulation effectively. The most critical feature for the intended function is the spatial distribution of these moieties. To determine these aspects, another series of tests are required. Also to validate the highly reproducible process of manufacturing, it is essential to have a well-established "structure-function" test. Some of the nanotherapeutics have complex complicated components (protein, nucleic acid), forming an integral part of nanotherapeutics, which might be sensitive to the condition of the manufacturing process and sometimes can undergo changes during manufacturing. These components are not necessarily the "active" moiety, but their presence might play a role in targeting biological pathways or specific cells or

distribution in the body. These ingredients can't be counted as inactive and should be characterized completely by an accurate analytical method. However, with the latest upcoming techniques, this limitation can be overcome (Neuberger et al., 2005).

Biopharmaceutical Limitations

It is important to achieve desired pharmacokinetic (PK) and pharmacological (PD) profiles for successful nanomedicines. However, few limitations are associated while applying the standard criteria of small therapeutic molecule PK to nanomedicines PK. The fact is well known that small changes in composition can affect the biodistribution largely. To characterize the behavior of the wide range of potential nanoparticles, the standard pharmacological strategies are not appropriate. Several factors like composition, physicochemical properties, and geometry influence the PK and biodistribution of therapeutic within nanoparticles compared to the conventional approach. The uniform effective method of designing nanomedicines to achieve optimized PK profile still doesn't exist. A unique approach was the attempt to prolong circulation using nanomedicines to take advantage of EPR effect. However, for required indication, this approach might not be appropriate every time and in a few cases might result in reduced efficacy and undesired exposure. In summary for nanomedicines, the standard PK might not be appropriate as plasma PK is not always representative of PK within tumor and site of disease, and hence it might fail to predict clinical activity. Contrary to this, it is more relevant to consider PK at the site of action since it shows a better correlation with therapeutic efficacy. This is mainly in the case of targeted nanoparticles. In conclusion, the development of medicines based on nanoparticles has numerous biopharmaceutical limitations. When a certain parameter gets altered, it results in changes in the PK profile of nanoparticles, and hence there is a need for different pharmacokinetic approaches for various diseases. Rather than using the standard approach for testing PK of plasma, it is more relevant to consider physicochemical properties and accumulation of active agents at the target site for evaluating the activity of nanomedicines and ensuring reproducibility. In the future, these approaches may also be effective for characterizing the bioequivalence of nanotechnology-based products (Desai, 2012).

Immunological Limitations

The different factors elicit an immune response. Nanoparticles sometimes themselves can be antigenic, where immunogenicity is influenced by size, charge, hydrophobicity, and surface characteristics. Sometimes nanoparticles get recognized as foreign bodies and are opsonized by plasma proteins, which activate complement pathways causing phagocytosis and clearance by macrophages. The complement

activation also causes undesired provoking consequences which include life-threatening allergy, hypersensitivity, and anaphylactic reactions along with activation immune response against the nanoparticles. Nanoparticles have also been reported to be associated with hematologic safety concerns like thrombogenicity and hemolysis. The antibodies against nanoparticles can induce immunogenic or non-immunogenic hemolysis. It has been demonstrated that a positive surface charge enhances the damage of erythrocytes and hemolytic potency. The nanoparticles whose surface is modified with hydrophobic-hydrophilic region act as a surfactant to disturb erythrocyte membrane. These toxicity limitations of nanoparticles impose significant limitations to assure the safety of medicines based on nanoparticles. A preferred safety profile would be required for careful adjustments of composition and key parameters during manufacturing. Minute differences in components or conformation arise, which could change the nanoparticle toxicities. Another challenge is the testing of nanoparticle toxicity. Listed in vitro assays may test the interaction between nanoparticle and immune system, which includes hemolysis assay, plasma coagulation, platelet aggregation, and phagocytosis. For predicting the immunological response, rodents are not very predictive, whereas rabbits show hypersensitivity to antigens. The preclinical study of toxicity specifically related to immunotoxicity cannot precisely predict the safety of nanomedicines. More likely in the case of nanomedicinal products, the immunological studies might need to be carried out in human trials (Desai, 2012).

Limitations Related to the Interaction of Nanotheranostics

To overcome various biologic barriers, nanomaterials must be engineered skillfully so that they can perform therapeutic action at a disease site. The biological effect of a complex interaction between nanomaterial and barriers is still not understood completely. Interaction between nanomaterial and various biological components in the cancer microenvironment will be discussed in this section. Table 12.2 describes the key factors influencing the interaction between biomolecules and nanotheranostics.

Interactions with Complement

The dual activation of the complement system by the surface of nanoparticles results into uncontrolled release of high pro-inflammatory mediators known as “anaphylatoxins” and opsonization by C3b or iC3b, which leads to the uptake of nanoparticles by phagocytic cells. However, pseudo-allergy related to activation of complement induced by nanocarriers is highly concerned. There is more preclinical and clinical research required to understand the implication of complement activation on the performance of nanocarriers (Anchordoquy et al., 2017).

Table 12.2 Addressing key factors influencing the interaction between nanotheranostics and biomolecules

Factor	Significance
Size	The small particle size promotes rapid interaction with the cell membrane and leads to greater accumulation
Shape	The shape of the nanoplatform influences the endocytosis process, biodistribution, elimination, and internalization
Surface modification	The surface chemistry influences the plasma protein binding, absorption, bypassing BBB, and colloidal behavior
Protein corona	The interaction between nanoparticles and protein results in the formation of protein corona which alters the physicochemical and biological identity of nanoplatforms

Interaction with Serum Protein

During the circulation of nanoparticles within the body, they get exposed to a complex system of fluid which contains biomolecules, blood, lymph, cytoplasm, etc. Protein and some other biomolecules like albumin, fibrinogen, and transferrin compete with each other for the binding site on the nanomaterial surface. This will result in alteration of the secondary structure, and it results into the formation of soft as well as hard protein corona. This formed corona on the surface of nanomaterials impacts the biological interaction of nanomaterials like biodistribution and cellular compartments. The physicochemical characteristics of nanomaterials will be influenced by the properties, formation, and composition of the protein corona. Nanomaterials with negative charge show enhanced uptake and improved lysosomal escape with no toxicity; they also exhibit particular interaction with a biological membrane of negative charge (Dorothy et al., 2021).

Interaction with Mononuclear Phagocytic System (MPS)

The main factor for reduced concentration of therapeutics at tumor site and less efficacy of therapy is MPS. The liver, lymph node, and spleen contain the majority of MPS. The nanomaterials with positive charge have a high affinity for macrophages as compared to the anionic and neutral nanomaterial. Upon attaching surface protein like opsonins, there are greater chances of them getting recognized by scavenger receptors of Kupffer cells. Around 95% of administered nanoparticles get cut off by MPS. However, strategies to avoid uptake by MPS have been established, such as modification of the surface with zwitterions, PEG, and dysopsonic proteins, which allows bypassing the phagocyte-mediated barrier and enhances blood circulation time and efficacy of theranostics.

Limitations Related to Tumor Targeting

The microenvironment of a tumor mainly consists of tumor cells, stromal cells, cells from the immune system, extracellular matrix, etc. The existing therapies have mainly failed due to the tumor microenvironment which limits the access of drugs to tumor cells. When nanomaterials enter through the blood vessel leakage, they will first interact with the microenvironment of the tumor, where it acts as a physical and biological barrier for drug delivery to solid tumors. The M2 type of macrophage, which is a different type, traps and degrades the nanomaterial delivered to the tumor. Also, the high pressure of tumor interstitial fluid forces the nanomaterial to go back to circulation, thus preventing them from reaching the target site. All these pathophysiological characteristics affect and delay the intratumoral delivery of nanotheranostics. In the case of metal and metal oxide NPs, there is another phenomenon that is called "dissolution" because of the large surface area and reactivity. Different approaches have been used to overcome these stromal barriers and increase intratumoral targeting (Jang et al., 2003). In the case of active targeting, the strategy is that the targeted NP demonstrates a reduction in tumor penetration than non-targeted NPs. Though macromolecules like polymers, antibodies, and nanoparticles predominantly accumulate in the tumor over healthy tissues, when they reach the target site, they exhibit reduced penetration because of the reduced rate of suppressed convective movement and diffusion in the tumor. When the targeted nanoparticle gets bound to the target followed by extravasation, their mobility in that tissue reduces, which enhances heterogeneity in the intratumoral distribution of NPS and results in recurrence of tumor and drug resistance. Li et al. had shown that targeted LPD nanoparticles did not enhance the tumor uptake compared to non-targeted PEGylated nanoparticles (Chen et al., 2012). In the case of passive targeting, there is enhanced permeation due to the tumor's defective vasculature, which causes ischemia and low perfusion in the tumor. This deficit perfusion decreases the delivery of blood-borne compounds to the microenvironment of the tumor. This dysfunctional lymphatic system is responsible for the retention and enhanced interstitial pressure, which counteracts the drug diffusion from the bloodstream to tumors (Shohdy & Alfaar, 2013). Another limitation is the size of nanoparticles. The size range optimal for tumor targeting is between 10 nm and 200 nm, and lesser than this get removed by the kidney, whereas larger size gets accumulated in extracellular space and hence fails to reach the target site. From patient to patient, the vascular fenestration varies for each tumor type even overtime during tumor treatment; hence, developing a size-specific targeting system will be challenging.

Limitation Related to the Safety of Nanotheranostics

The toxicity of nanoproducts depends upon their size, as it can have a great impact on safety. The nanoparticles of size less than 100 nm can be toxic as they can travel to other sites other than the targeted site, cross cell membranes and blood-brain barriers, and accumulate in the healthy cells of the body (Oberdörster et al., 2005). The human body does not have natural biological mechanisms for dealing with nanomaterials such as carbon, gold, silver, and titanium. Thus, they might harm the health and appropriate development of the nanoproducts necessary, which are suitable for safe delivery (Buzea et al., 2007). Nanotheranostics' impact on the body depends on their physicochemical properties as they have an impact on protein binding and cellular uptake (Vishwakarma et al., 2010). Exposure of particles through inhalation or penetration through damaged skin leads to translocation to the dermis and lymph nodes, causing uptake by dendritic cells and macrophages affecting the immune system (Köhler & Som, 2008). Various in vivo studies of titanium dioxide showed inflammatory reactions and cytotoxicity after UV irradiation (Gurr et al., 2005; Shukla et al., 2011), whereas iron nanoparticles showed interaction with proteins and DNA, which damages structure (Könczöl et al., 2011). Silicon dioxide interacts with cell membranes and causes a hemolytic effect (Barnes et al., 2008). Quantum dots also have major toxicity issues due to the release of free radicals and the use of materials such as selenium or cadmium. They can accumulate in adipose tissue and can impact the kidney and liver (Xu et al., 2008; Rzigalinski & Strobl, 2009). To avoid such toxicities, the regulatory agency should scrutinize the nanotheranostic development process. Traditional chemical toxicity testing methods can be useful as a primary approach for nanomaterial testing. The parameters for toxicity screening are physicochemical analysis, in vivo studies, and in vitro assays of nanotheranostics. The biological activity of the product depends upon its physicochemical properties. Hence, characterization of size, shape, surface charge, aggregation, and solubility should be performed at administration as well as conclusion time. Cellular and non-cellular assays should be conducted to determine the pharmacokinetic and pharmacodynamic behavior of the material on the body. Risk assessment of exposure as well as the hazard is done by exposure modelling and epidemiological studies. The regulatory agencies should come together to make decisions regarding the safety guidelines of the nanotheranostics (Nel, 2006). Environmental concerns are also associated with nanotheranostics, and studies suggested that they accidentally enter into the environment through the disposal of wastes, emissions from production sites, or natural sources. They can stay in the environment for a longer period, causing accumulation in the environment. Toxicological studies indicated that nanometals such as silver, zinc, and copper oxides are toxic to underwater organisms. Still, the proper data is not available, and many researchers are studying the environmental effects of nanotheranostics. Therefore, there is a need for stringent

regulatory processes for the safety of mankind as well as the environment (Gaur et al., 2020).

Pitfalls of Nanotheranostic Research

Clinical research is based on the interpretation of multiple experimental statistics. The foundation of these studies is the validity of conclusions from statistical analysis which are mainly based on the significance of statistical results. An example of famous research where difficulty was highlighted was by Lui et al. Florence highlighted the lacuna of the statistical significance of results observed by Lui et al. on carbon nanotube's fate in mice, in which accumulation in tumor did not go beyond 6% of the dose, and the study concluded that carbon nanotubes are efficient in tumor targeting. Moreover, few studies of nanoparticle targeting ignored the essence of satisfying all the criteria for a successful drug delivery system, declaring the success of a few target mechanisms that satisfied only the subset of criteria. The gold nanoshells were fabricated by Choi et al. by using monocytes isolated from human whole blood's buffy coat, and to examine their hypothesis, the researchers administered a breast cancer mouse model which was metastasized to CNS using macrophages laden with nanoparticles and tracked the location of the macrophages using another NP for moment microspheres labeled fluorescently. The results signify that macrophages were able to cross BBB and delivered the nanoparticles to near cells width away from closer metastatic cells, giving a paradigm to the delivery method of Trojan horse. It was considered as the "first successful disclosure of active delivery." Although controlled release criteria are not discussed, more research requires an understanding of how macrophages unload their cargo. Despite dynamic knowledge of tumor biology, the development of cancer-targeted nano-particulates is still moving at a slow pace. The oncology drugs have an attrition rate at the last stage of as high as 70% and 59% for Phase II and III trials, respectively. Many characters of cancer biology have been elucidated; however, there are only a few models for preclinical studies. The current studies focus less on the cancer cell and more on medicines. More research studies are required to detect the cancer cell's behavior, and many failures in the nanomedicine field come from this point (Shohdy & Alfaar, 2013).

Case Study

Challenges in the Development of nab-Paclitaxel

The development of nab-paclitaxel demonstrates the challenges in the manufacturing, formulation, and testing of nanoparticles with suitable physicochemical characteristics. Nab-paclitaxel is the first approved nanomedicine based on proteins that were subjected to extensive testing at a small-scale level. A wide range of manufacturing conditions was analyzed along with proteins of different sources; quality and purity were also investigated. Altered conditions often result in the suboptimal formulation; this is the challenge that can be overcome only by conducting trial and error. This hurdle for successful nab-paclitaxel scale-up was further demonstrated by failed attempts in the marketplace to copy the nab-paclitaxel formulation. Challenges in the development of therapeutics based on nanoparticle optimization batches were carried out to define the composition and components of nanoparticle and to develop a robust process which assures reproducibility and consistency for scale-up.

As an outcome, the nanoparticles of nab-paclitaxel have shown many key characteristics for an injectable nanoformulation. The size distribution in solution form was in a narrow range with a mean particle size of 130 nm measured using dynamic laser light scattering (Merisko-Liversidge et al., 1996). Cryo-TEM and TEM images revealed that the nanoparticles had a spherical shape with a size >200 nm. The surface of albumin has zeta-potential which falls in negative, which leads to steric stabilization, prevents aggregation, and provides good stability to suspension. The X-ray powder diffraction showed that in nanoparticles, paclitaxel is non-crystalline, which makes the drug bioavailable with no time lag required for paclitaxel dissolution as is well known for nanocrystals (Langer et al., 2003). Nab-paclitaxel consists of nanoparticles which have albumin-coated cross-linked therapeutics; the paclitaxel is bound to albumin noncovalently via hydrophobic interactions, thus allowing high bioavailability with quick distribution to tissues. In contrast with other nanoparticles based on albumin reported in literature, this involves the addition of glutaraldehyde or any other cross-linking agent during formation and also requires enzymatic metabolism of albumin for in vivo drug release (Lin et al., 1993). In conclusion, selection of main components, identification of key characteristics, and thorough knowledge of critical steps of manufacturing should be done carefully as they determine whether the formulation will have the desired or required PD, PK, and safety profiles to obtain the said therapeutic action. For testing in-process quality and controls, multiple orthogonal methods of analysis are required. Deviation from desired parameters and processes would result in a negative effect on the efficacy and safety of nanomedicines.

Regulatory Concerns of Cancer Nanotheranostics

Regulatory Evaluation of Cancer Nanotheranostics

Nanotheranostics is an emerging approach of nanotechnology, used for prevention, treatment, and diagnosis of diseases, which improves the quality of life. Today, more than 200 pharmaceutical companies are focusing their research work on developing nanotherapeutics and theranostics, with 38 nanomedicines on the market (Wagner et al., 2006). Among various diseases, cancer is one such area where theranostic research has been carried out prominently. Nanomedicines for biomedical applications have emerged recently, but the lack of established general guidelines for animal studies and analysis of these products has limited their scope for forwarding development in humans (Peer et al., 2007).

The regulatory process of cancer theranostics depends on various parameters such as composition of the product, therapeutic or diagnostic function, mechanism of action, imaging mode, drug delivery, and whether they are combination or companion products (Bardhan et al., 2011). As per the regulatory agencies, the characteristics of the nanomedicine evaluation primarily rely on the active pharmaceutical ingredient (API), which suggests that nanomaterial must be reviewed for the biologic specification along with those for new chemical entities (NCEs). The human use of these diverse and advanced nanoproducts largely depends on the characterization and evaluation of certain properties. Their characteristics can be easily changed by modification in raw materials as well as in manufacturing processes. Such small modifications can significantly affect biological and biodistribution patterns (Duncan & Gaspar, 2011). Along with these, researchers attach tracking and imaging molecules with nanomedicines; new sophisticated methods and assays need to be developed to significantly determine their physicochemical properties, drug release, protein binding, metabolism, and cellular uptake (Tinkle et al., 2014). Another barrier is the development and manufacturing process of this nanotherapeutics. Every process should be identified for a critical point during the scale-up process. The recent approach of "Quality by Design" to access the critical points during production helps to solve the problems in a systematic manner. This concept gave rise to the International Council for Harmonisation (ICH) guidelines Q8, Q9, and Q10 for pharmaceutical development. Another obstacle in the regulatory pathway is the data collection during the life cycle of products, including animal and clinical studies. Hence, regulatory authorities should draft regulations for the successful development and scale-up of nanoproducts (Sainz et al., 2015).

The US Food and Drug Administration

The FDA evaluates cancer nanotheranostics according to the regulations that apply to other existing drugs and devices with particular information about the nanomaterial with a route of administration, dose, and biological behavior in both efficacy

and safety studies. It ensures that the study design and clinical studies conducted are safe for human volunteers (Commissioner, 2019). Regulatory bodies and advisory boards responsible for the safety of human beings must carry out a risk-benefit ratio that measures possible harm both unknown and known. The FDA regulatory pathway depends on whether the theranostic is a drug, device, or a combination product (Clancy, 2014).

The FDA suggests early consultation of new and emerging drugs, biologics, or devices. Early-phase clinical trials can be conducted for a new product, but the FDA requires proof of efficacy. The investigator (sponsor) should submit preclinical data including first-in-human (FIH) studies, and research data should ensure the safety of participants in further trials (Kimmelman, 2007). Early trials for a new drug or device give a risk-benefit ratio and identify endpoints to study complicated factors such as dose, population characteristics, and delivery of a new intervention drug or device. Adverse effects accompanied by FIH trials are scrutinized by regulatory and ethics boards resulting in changes in study designs and trials (Kimmelman, 2012a).

Exploratory IND studies are implemented in Phase 1, involving less human exposure to new intervention drugs. This study predicts the pharmacokinetics and pharmacodynamics of new theranostics (2006). The exploratory IND includes population characteristics such as age, indications, contraindications, diagnostic approach and outcomes, and treatment parameters such as schedule, route of administration, and risk mitigation. This helps to determine unknown effects and helps to frame efficient study designs that can form the basis of further trials of theranostics with the main aim of providing safety to human subjects. The investigator should determine both known and unknown risks before and during these early studies. The risk analysis must include the severity and frequency of adverse effects of the theranostics. If the investigator fails to do this, it can cause a delay or halt in the study. The FDA suggests a device evaluation strategy "failure mode and effect analysis" (FMEA) for investigational device exemptions (IDEs) in early device trials. This assessment benefits to translate research to further clinical trials (Kimmelman, 2012b).

The Investigational New Drug (IND) application of any cancer theranostics should be submitted to the FDA before clinical trials. The application form 1571 is the guidance document for IND, which tells requirements that include investigational plan, study protocol, investigator's brochure, IRB information, facilities, manufacturing and chemistry data, pharmacological and toxicological information, and any existing INDs of human use (2020a). When a drug application is received by the Center for Drug Evaluation and Research (CDER), it is reviewed by the Office of Hematology and Oncology Products (OHOP). The sponsor has to wait 30 days before initiating clinical trials. The FDA, while evaluating the IND, requires all other additional information; the key goal is to ensure the well-being of the participants in the trial. The IND also needs information about theranostics, including function and composition, preclinical or prior human use, laboratory or animal study data, and manufacturing and clinical conditions to be treated by theranostic. On this basis, the FDA gives the IND approval (Center for Drug Evaluation and Research, 2019). The changes made to the drug or process such as change of the ingredients, equipment, and manufacturing facilities after approval by the FDA

should be notified in a stipulated time. The FDA informs the sponsor to file a New Drug Application (NDA), Abbreviated New Drug Application (ANDA), or Biologics License Application in case of potential changes that severely affect the characteristics of the product (Office of the Commissioner, 2020). Other types of INDs includes an investigator IND, which is submitted by a physician who conducts and initiates the trial, i.e., a research IND given by a physician to study a new drug or an approved drug for a new indication and an emergency IND that allows approving a new interventional drug in an urgent situation that has no time to follow the regulations and treatment IND submitted for a drug, showing promising results in clinical testing for life-threatening situations while the final clinical studies are conducted.

An approved cancer drug theranostics will need an application if it involves (Clancy, 2014):

1. Replacement of a new drug when the standard is favorable.
2. Supplementary chemotherapy when the patient has a low risk of occurrence, if the study will result in a change in labeling, or if standard therapy has good results.
3. Use of cytotoxic drugs in case of no standard treatment.
4. Animal studies should determine the safe schedule or starting dose, including:
   (a) New drug combinations indicating synergistic toxicity effect.
   (b) Change in the route of administration.
   (c) Change in dose.
   (d) Radiosensitizers or chemosensitizer drugs.

If Phase 1 trial shows safety in healthy volunteers, then the new investigational drug can be allowed to test in a larger population. Phase 2 and 3 trials are conducted to determine the safety, efficacy, as well as toxicity in diseased patients. If the results of these trials ensure efficacy outweighing the risk, then the sponsor can submit a New Drug Application (NDA) to the FDA. The NDA must include bioavailability data, analytical data, chemistry, manufacturing, and control (CMC) data for each and also toxicological data. It is submitted to the Center for Drug Evaluation and Research (CDER) for review (2020b). There are three types of NDA under Sect. 505:

505(b)(1): It includes the use of a drug which has not been approved previously**.**

505(b)2: It involves reports regarding changes in strength, dosage form, and route of administration or change of an active pharmaceutical ingredient in an approved combination product. This applies to nanoproducts where nanocarriers are used in an approved product (2020c).

505(j) (Generics): This application is for generic products which include that the product is the similar inactive ingredient, route of administration, strength, dosage form, label, quality, performance, and use in comparison to an approved drug called "Abbreviated New Drug Application" (ANDA), which does not require animal studies and human trials to determine safety and efficacy (2019a).

Biologics License Application (BLA) This is for biological components submitted to the Center for Biologics Evaluation and Research (CBER) or CDER for

review. It includes information the same as the NDA such as manufacturing, chemistry and control, and clinical and toxicity data of the biological product. It is marketed under the Public Health Service (PHS) Act (2019b).

In case of cancer, the sponsor can request the FDA for a faster process through programs such as breakthrough, fast track designation, prior review designation, and accelerated approval. The FDA reviews the application in a faster way but with a great degree of scrutiny, providing high chances of availability of a new product. During this process, the FDA conducts efficient and clear communication with an investigator during the development process of a drug (2020d).

Regulations of Combination Products and Companion Products

Combination products are composed of any drug with a device or biological product or combined drug, device, and a biological product. For approval of these products, the investigator should communicate with the Office of Combination Products (OCP) of the FDA. The investigator should have data on the product such as its use, mechanism of action, therapeutic activity, and targeted population before consulting OCP. A request for determination (RFD) of a maximum of 15 pages describing the product and IND and IDE status of physicochemical or pharmacological characteristics, mechanism of action, use, route, schedule, and manufacturing details of the product should be submitted. The OCP will give a decision within 60 days of receipt. The FDA requires separate applications for a component which has been approved, and labeling requires a change based on the new activity. The application must include all the information about the products with the approval status of a component (2019c).

Companion products are combined with drug/biological therapeutic agents and diagnostic devices/imaging modalities. According to the FDA, healthcare professionals must be able to rely on the results, if diagnostic device results are an important parameter in a treatment. The in vitro diagnostic companion products can have severe consequences if the product fails to be performed analytically or clinically. The investigator should consult the Center for Drug Evaluation and Research (CDER) and Center for Devices and Radiological Health (CDRH) for study designs and development of the companion products. Both products must be developed and reviewed together in the same clinical investigation (2020e).

The final step in the pathway of new drug/device/biological products from the laboratory to clinical trials is reviewed by the Institutional Review Board (IRB) or the Institutional Ethics Committee (IEC). The investigator should not start the clinical trials on human subjects unless the IRB reviews and approves. The IRB protects human subjects and can stop or delay the study. Hence, early consultation is required in the process. Ethics guidelines should be signed to avoid the risk involved in nanotheranostics trials. The informed consent should be taken from subjects before

initiating the trials. The study should outweigh the risk and provide benefits to the patients and society (Clancy, 2014).

European Medicine Agency

The European Medicine Agency (EMA) rules help in preparing the market approval application for drugs and devices. The methodology of the EU Member State and the Agency demonstrates the requirement for authorization, which includes safety, quality, and efficacy of the product. The EMA collaborates with a different organization to evaluate the risk-benefits at the early stage of development of theranostics (Tambe et al., 2019).

Nanomedicines composed of drugs or medical devices or both have to be evaluated for risk assessment. Any drug or device gets market approval only under the guidance of "clinical trial directives," which includes applying good manufacturing practices (GMP) and good clinical practices (GCP). The application for a clinical trial should include information on the investigational medicinal product (IMP) and the clinical trials. The information should be in the format of a common technical document and should contain all the information in the Investigational Medicinal Product Dossier (IMPD). It includes the manufacturing, production, and pharmacological and toxicological data of the IMP. The clinical trial has to be approved by the national competent authority as well as the local ethical committee. The clinical trial application with EudraCT number is important for every clinical trial generated by the European Union Drug Regulating Authorities Clinical Trials (EudraCT) database system. The application should include an investigator brochure (IB) and study protocol, including study designs, objective, human volunteer inclusion, exclusion criteria, and scientific background of the clinical trials. Along with this, an informed consent form is also required, stating that the patient is known of all the risks and consequences of the trial. Besides this, the standard operating procedures, investigator information, and relationship between the sponsor and the trial site should be provided for evaluation. The trials should be monitored efficiently to avoid unnecessary risk to the study participants and conducted according to the regulations of the state (Kolenc Peitl et al., 2019).

Marketing of Cancer Nanotheranostics

The market of nanotheranostics is prominently growing nowadays due to the FDA's guidelines. The FDA's Emerging Technology Program (ETP) encourages the development and production of novel pharmaceuticals, which includes early trials and constant feedback from regulatory authorities. This embraces the industries and academic researches to develop nanotheranostics.

The FDA's Nanotechnology Task Force is an initiative to collaborate with industries, hospitals, and academia to investigate the nanomaterials' influence on the

body and to develop innovative and effective drugs and devices. The FDA also started public-private partnerships (PPPs) to produce awareness among the people regarding the researches in the field of nanotheranostics (2018). It was also created to improve and help the public and private sectors in taking the research from the lab to the bedside. Besides this, it is necessary to perform pharmacoeconomic studies for new nanotheranostics to indicate the economic and social benefits in comparison with the existing products. Quality-adjusted life expectancy years (QALYs) and future consecutive hospitalization costs are indicators necessary in the development of new innovative products (Gaspar et al., 2014).

The Unwither Conference in 2009 quoted the development of nanomedicines such as nanofluidic devices for delivering therapies, functionalized nanoparticles, implants, or nanodevices with sensors to detect drug delivery and other motors or nanobots traveling through the circulatory system to cure diseases. The commercialization of nanotheranostics is increasing, and over 200 companies are investing in the development of nanoproducts. The Grand View Research statistics predicted that by the year 2025, the global market of nanomedicines will be 350.8 billion USD (Bawa, 2009).

The challenges faced by the stakeholders such as researchers, stockholders, and patients in marketing include improper definitions for nanotechnology, technological difficulties, the need for proper regulatory guidelines, and the necessity of financial aid. There is a need to overcome those challenges to bring more nanotheranostics from the lab to commercial scale to improve the health of mankind.

Table 12.3 gives the summary of all types of limitations and the factors influencing it.

Conclusion

Despite tremendous efforts, the morbidity related to cancer disease is still inescapable. The emerging nanotechnology has provided better opportunities to advance the design and manufacturing of novel nanotheranostics. Nanotechnology has transformed the treatment and diagnosis of cancer by enabling early tumor detection, which in turn is followed by effective delivery of therapeutic drugs. These nanoscale platforms have gradually travelled from benchtop to the bedside and have improved overall management of cancer. Nanotheranostics is an “act on-site” strategy that narrows the time required for the detection of cancer and treatment. The development of smart nanotheranostics which acts on the bioresponsive system has been evolved and offers promising outcomes with high efficiency and accuracy at the target site. Nanotheranostics is an upcoming efficient field which offers cost-effective quick detection and delivery of therapeutics to the targeted site with reduced side effects. However, there is an urgent need to address certain limitations of nanotheranostics for their successful clinical application. By considering these limitations and developing an effective strategy to overcome them, an efficient and successful smart nanotheranostic platform can be constructed. Based on this

Table 12.3 Summary of all limitations discussed above and factors influencing them

Type of limitations	Key factors
Design and development	Major aspects of design like size, shape, surface properties, and composition regulate overall performance and applicability of nanoplatforms
Biopharmaceutical limitations	Some of the nanotheranostic platforms demonstrate variation in PK and biodistribution, and sometimes it is difficult to attain optimal PK profile
Immunogenic reaction	The surface properties, charge, and size induce a lethal immunogenic reaction, which needs to be addressed
Interaction with biomolecules	The interaction with the cellular system, complement system, mononuclear phagocytic system, and proteins alters performance of nanotheranostics
Targeting related limitations	The presence of biological barriers, design of nanotheranostics, their physicochemical properties, and type of targeting all influence targeting
Safety concerns	The toxicity of nanotheranostics is largely influenced by their size; some of the nanometals (carbon, gold, iron, etc.) have shown harmful effects on health
Regulatory aspects	Based on various parameters such as composition of the product, therapeutic or diagnostic function, mechanism of action, imaging mode, drug delivery, and whether they are combination or companion products

concept, the efficiency of these platforms should be observed before and after their administration, as well as during the therapy and after collecting sufficient data of cytotoxicity, immunogenicity, cost-effectiveness, and genotoxicity; these nanotheranostic therapeutics can be used in routine as a crucial agent of predictive and personalized medicine.

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