# Recent Trends for Nanomedicine Safety

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**Abstract**

Nanomedicine has gained tremendous attention in medical professionals owing to its smart strategies of treatment and huge applications in healthcare. Nanomedicine is mainly used for drug delivery with special attention to drug targeting, their excellent properties of high drug loading, sustained drug release, surface tunability, surface modification possibilities, and unique surface properties. A major portion of nanomedicine has been occupied by nanocarrier systems. For diagnosis and therapeutic purposes, nanoparticles, nanoliposomes, dendrimers, and other nanoparticulate devices have been reported for treatment/diagnosis of diseases. Several nanomedicines with emerging therapeutic efficacy have been already commercially available and many more are in pipeline. Despite their impressive therapeutic benefits, some critical challenges are associated with their transition from laboratory to clinical usage. Toxicity caused by nanoformulated drug is the most adverse barrier for its broad range of application. In this chapter, we have reported different nanocarriers available for their diversified applications in the management of several diseases, with a special emphasis on recently published reports, clinical evidence, and, their toxicity and safety concerns.

**Keywords**

Nanotoxicity · Nanomedicine · Biosafety · Clinical trial · FDA-approved nanoformulations

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## 18.1 Introduction

Nanomedicine is a branch of medical science, which deals with an emerging field of knowledge, applications, and novel tools of nanotechnology for diagnostic and therapeutic smart approaches to manage human healthcare system. According to the European Science Foundation, nanomedicine is defined as nanosized tools used for diagnosis, prevention, and treatment of diseases with an increased understanding of the complex pathophysiology associated with it [1]. Nanocarrier system reigns over a vast area in nanomedicine. Hence, we mostly focus on nanocarriers in the current discussion. Due to the nanoscale size, enhanced surface area to volume ratio, and new surface properties such as super magnetism, surface charge, etc., the nanosize drug delivery systems are considered to achieve prolonged circulation time, greater stability, improved accumulation at the target site, and controlled release of drug in a sustained manner that enhances their therapeutic efficacy with reduced cytotoxicity to normal tissues. Nanocarriers have been reported for the treatment of cardiovascular diseases, different types of cancer, brain tumor, HIV/AIDS, gastrointestinal diseases, skin disorders, respiratory diseases, pulmonary fungal infections, neurological diseases, stroke, hypertension, leishmaniasis, visceral diseases, epilepsy, ocular diseases, and many more. For the last few decades, emerging research on nanomedicine evolves various biocompatible and biodegradable nanoscale materials which include nanoparticles, nanoliposomes, dendrimers, micelles, polymer-protein conjugates, albumin-drug conjugates, DNA-drug conjugates, antibody-drug conjugates, ligand-conjugated polymeric nanoparticles or nanoliposomes for targeting specific organs of interest, and so on. Nanoliposomal formulations have a great advantage for their ability to include both hydrophilic and hydrophobic drug types in the delivery system and to cross various physiological barriers. Designing of the nanomedicinal targeted anticancer drug delivery systems are of great scientific interest in the field of biomedical research. Many of these nanoparticulate medicines have been translated into clinical trials and some already been introduced commercially, reflecting the successful outcome of laboratory research on nanomedicinal formulations. Despite their well-versed medical applications and fast-developing future prospect, some unavoidable toxic outcomes often limit their extensive use. Evidence in animal studies suggested that certain nanomaterials can interact in cell constituents in vivo in a different manner than small molecules [2]. They can produce a wide range of alterations such as oxidative stress, induction of inflammatory responses, protein aggregation, mitochondrial perturbation, blood coagulation and cell death, induction of autophagy and apoptosis, complement activation, etc., as observed in experimental models. The ability of nanomaterials to cross several biomembranes (e.g., blood–brain barrier) may produce off-target effects and unpredictable dose-response profile. Assessment of environmental exposure to nanomaterials in humans, animals, and our ecosystem and their potential hazards, if any, their persistent use causing nanomaterial-related effect, their immediate effects, and long-term risk should be monitored minutely. Development of suitable devices, testing methods, and guidelines for assessment are in need and currently under the supervision of many

regulatory authorities throughout the world to ascertain the safety concerns. Different strategies are required to develop to avoid nanomedicine-mediated in vivo toxicity in humans and animals.

In this chapter, we have mainly provided recent reports on several nanomedicinal drug carriers used for treatment, diagnostic, and theranostic purposes. The toxicity profiles of many such nanomedicinal materials observed in various experimental models were also discussed to understand the problems associated with current research in the field and requirements to cross the hurdles of clinical trials. Nanomaterials under clinical investigations and their outcomes and safety concerns are also discussed.

## 18.2 Polymeric Drug Nanocarriers

Polymeric drug nanocarriers have been successfully used for cancer drug delivery since last few decades. Polymers can be natural, synthetic, or pseudosynthetic. In the design of commercial nanocarriers, size, surface morphology, and characteristics have been used to achieve successful delivery. The design of optimized formulation for effective delivery to target sites of action with minimum off-target effects remains a vital research objective. Hence, it is in an urgent requirement to develop new chemotherapy using polymer-based nanocarriers to enhance therapeutic efficacy. Polymer nanoparticles can be fabricated in a wide range of varieties and sizes from 10 to 999 nm. They can range in size from a single polymer chain used directly as a therapeutic or as a modifying agent for a drug or diagnostic agent to large aggregates within the nanoscale [1, 3, 4]. Polymer nanodrugs can be categorized as: (1) degradable polymer forms for controlled release applications and (2) polymer–drug conjugates that increase circulation time and drug half-life or improve biocompatibility/solubility [1].

Two of the top ten best-selling drugs in the U.S. in 2013 were polymeric drugs, Copaxone (glatiramer acetate injection), approved in 1996 for the treatment of relapsing-remitting multiple sclerosis, and Neulasta (pegfilgrastim), approved in 2002 for chemotherapy-induced neutropenia [1]. Polymer (NPs) can facilitate drug release for weeks without accumulating in the body. Therefore, polymeric NPs are considered promising carriers for numerous medications, including treatments for cancer, cardiovascular disease, diabetes, bone-healing therapies, and vaccinations [1, 3]. One of the most well-established polymers is polyethylene glycol (PEG). Plegridy, a pegylated interferon beta-1a formulation, has been approved in 2014 for the treatment of relapsing forms of multiple sclerosis, showed improved drug half-life and exposure. Another such pegylated form is Adynovate (antihemophilic factor [recombinant]), which was approved in 2015 for bleeding prophylaxis and hemophilia A [1, 5]. Rebinyn (coagulation factor IX [recombinant], glycopegylated), was approved in 2017 for treatment and control of bleeding episodes and perioperative bleeding management in patients with hemophilia B [6, 7]. Apart from pegylated polymers, biodegradable polymers are also of prime interest because they can be fully metabolized and removed from the body. Poly-(lactide-co-glycolic acid)

(PLGA), Polyhydroxyalkanoates (PHAs), and cyclodextrins (CDs) are the most commonly used polymer for core fabrication [8]. Polyvinyl alcohol (PVA), PEG, and monomethoxy poly-(ethylene glycol) (mPEG) have been applied in surface modification of polymer-based nanocarriers which gives nontoxic hydrophilic outer shells and outstanding blood biocompatibility. The United States Food and Drug Administration (USFDA) has approved biodegradable polymer such as PLGA and PLA for human use [9]. Poly lactic-co-glycolic acid (PLGA) is an especially intriguing example of a biodegradable polymer because relative proportions of polylactic acid (PLA) and polyglycolic acid can be used to tune finely the biodegradability of PLGA [3]. Zilretta is extended-release injectable suspension microspheres consisting of crystals of triamcinolone acetonide embedded in a PLGA copolymer matrix used for the treatment of osteoarthritis knee pain [6, 7]. In addition to increasing half-life, polymer conjugation can improve passive tumor targeting by increasing the size of a drug [10]. Abraxane® and Transdrug® are the clinically approved passively tumor-targeted nanoparticles in cancer therapy. Abraxane®, a solvent-free, albumin-bound nanoparticle of paclitaxel which is also known as nab-paclitaxel presently used in breast cancer and Transdrug® contains cytotoxic drug doxorubicin currently used to treat hepatocarcinoma clinically [11].

In a recent study, Nosrati et al (2019) developed a mono methoxy poly (ethylene glycol)-poly (ε-caprolactone) (mPEG-PCL) co-polymer based on novel methotrexate sodium (MTX) drug delivery carrier with the objective of enhancing the loading efficiency of the drug in nanocarrier as well as achievement of an effective control release rate of the drug. They showed that these polymersomes provided an ideal carrier for the delivery of MTX to breast cancer cells (MCF-7) [12]. In another research article, Zheng et al (2019) reported that PEGylated poly (lactic-co-glycolic acid) nanoparticles conjugated with LFC131 (a peptide inhibitor of CXCR4) co-delivery of sorafenib and metapristone via the CXCR4-targeted nanoparticles showed a synergistic therapy against hepatocellular carcinoma. Here, they showed enhanced cytotoxicity, colony inhibition, apoptosis, and caspase signaling pathways. Their results also suggested combinational treatment of chemotherapeutics enhanced circulation and target accumulation at tumor sites and consequently inhibited tumor growth in an animal model [13]. Many polymer-containing nanodrugs are being investigated in clinical trials and are discussed in the relevant section of this text. CRLX101 (camptothecin conjugated cyclodextrin-PEG formulation) is currently under clinical trial for lung cancer and solid tumors [3].

## 18.3 Liposomal Drug Nanocarriers

Liposomes were initially described in 1965 and its drug delivery properties were first proposed in the 1970s. It is a spherical vesicle composed of a self-assembling lipid bilayer membrane arranged around an empty core which carries and delivers both hydrophilic and hydrophobic molecules within the cores. This special characteristic, along with biocompatibility and biodegradability, makes liposomes more unique as a drug delivery carrier. In a structural point of view, liposomes can be divided into

unilamellar vesicles (UVs) and multilamellar vesicles (MLVs) on the basis of lipid bilayers. Each of UVs is composed of an aqueous core enclosed by a lipid bilayer, separating the inner aqueous core from the outside, and MLVs consist of various layers of lipid bilayers along with the aqueous core. Fabrications of liposomes are the first drug delivery system in the field of nanoscale to make the transformation from concept to clinical practice [14]. USFDA approved lipids used for the preparation of liposomal vesicles are 1, 2-distearoyl-sn-glycero-3-phospho-ethanolamine (DSPE), hydrogenated soybean phosphatidylcholine (HSPC), phosphatidylglycerol (eggPG), and 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC) [15]. Most of the conventional chemotherapeutic agents circulate nonspecifically in the body and have poor pharmacokinetic profiles and systemic toxicity associated with major side effects. Hence, the establishment of nanodrug delivery systems able to target the tumor site is becoming a genuine challenge [16]. Liposome-based nanomedicines showed the ability to circulate in the bloodstream for an extended time thus providing a longer treatment to affect and accumulate more drugs at the site of a tumor or infection [1, 3, 10]. Attachment of polyethylene glycol chains at liposomal surface proved a 4- to 16-fold enhancement in drug delivery during malignancies, in contrast to prior non-liposomal trials [17]. Liposomes can reach tumor site passively through the leaky vasculature surrounding the tumors by the increased permeability and retention effect although ligands modified at the surface of liposomes allow specific targeting by binding to the receptors overexpressed by cancer cells or angiogenic endothelial cells [16]. By using lipids of different fatty-acid-chain lengths, liposomes can be made temperature or pH sensitive, thereby controlling the release of their contents under specific environmental conditions [3, 10]. Drugs with low bioavailability or high toxicity have been successfully delivered by liposomes [1, 4]. Co-encapsulation of drugs in nanoformulations can also provide a novel means of drug delivery. More specifically, these formulations can deliver drugs sequentially and at specific molar ratios within the tumor microenvironment, allowing for maximal synergy that is not possible with conventional drug delivery methods [10].

Starting with the approval of Doxil in 1995, many nanodrugs incorporating liposomes have been approved, including antifungal agent, anticancer drug, and analgesic [1, 10, 18]. Myocet® is a remarkable example presently used to treat breast cancer clinically in combination with another chemotherapeutic agent (cyclophosphamide). Doxil is used to treat metastatic breast cancer and AIDS-related Kaposi's sarcoma. Other liposomal non-PEGylated systems have been approved such as DaunoXome® and Onco-TCS® for drugs daunorubicin and vincristine [15, 19]. Recently, in 2017, Vyxeos, a liposomal formulation of cytarabine and daunorubicin in a 5:1 fixed molar ratio, also got FDA approval for the treatment of acute myeloid leukemia (AML) [20, 21].

Guan et al (2019) reported doxorubicin-loaded 8-mer and 16-mer D-peptide ligand-modified liposome preparation and studied systemically enhanced immunocompatibility. The biodistribution and biosafety of two different peptide-modified liposomes were assessed in healthy BALB/C mice and antiglioblastoma effect was determined in nude mice bearing intracranial glioblastoma [22]. In

another research work, Awad et al (2019) showed that human serum albumin (HSA) modification of pegylated liposome remarkably increased their binding to the surface of the breast cancer cell line MCF-7 and MDA-MB-231, resulting in the increased uptake of the drug by cancer cells. Therefore, the HAS-coated liposomes coupled with ultrasound-mediated enhanced drug release indicate desirable prospective in breast cancer chemotherapy [23].

## 18.4 Nanocrystal Drug Nanocarriers

Nanocrystal is formed by an optically active core which emits tunable, narrow, symmetric, photochemically stable spectrum and is surrounded by a shell which makes nanocrystal less sensitive to photo-oxidation and medium changes [24]. These nanocrystal-based medicines are composed entirely of drug compound (s); therefore, the surface area of these drugs is increased and their dissolution speed and saturation solubility are also enhanced. Due to increased saturation solubility, they get absorbed through the gastrointestinal tract easily [1, 4]. Nanocrystal formulations improve the Pharmacokinetic/Pharmacodynamic (PK/PD) properties of poorly water soluble organic or inorganic drug by increasing their bioavailability and solubility [4, 25]. However, the mechanism behind their oral absorption and behavior after subcutaneous injection are not fully understood.

First FDA-approved (2000) organic nanocrystal medicine was Rapamune that contains bacteria-derived immunosuppressant sirolimus and acts to prevent organ rejection (particularly kidney) after transplantation. This formulation makes poorly soluble sirolimus into an extended release drug. This technique is also used in other types of formulation such as tablets, oral suspension, and intramuscular injections [1]. After the approval of Rapamune, several other nanocrystal medicines were marketed using the techniques like Tricor, Emend, etc., and provided the potential solution for solubility issue of many compounds [18]. In comparison to organic nanocrystal formulation, FDA approval of inorganic nanocrystal formulations are only limited to hydroxyapatite and calcium phosphate nanocrystal as a bone-graft substitutes [1].

## 18.5 Micelle Nanocarriers

Micelle nanoparticles contain a hydrophobic internal core for easy encapsulation of poorly aqueous soluble drugs but adequate polarity on its outer surface helps them to dissolve in aqueous solution [1]. These are self-assembling polymeric amphiphilic structures and may be customized for slow and controlled delivery of hydrophobic drugs as well as their structures can be finely tuned to get desired particle size, drug loading, and release characteristics [10]. A huge number of new chemical entities coming out of research laboratories suffers from low water solubility, making them slightly challenging to the manufacturer for administration and often causing delay in drug formulation and development. Additionally, so many poorly soluble drugs

have not achieved their potential on the market due to intolerable levels of toxicity from the drug or the excipients in the formulation. The most common hydrophilic block used to make the hydrophilic shell is the FDA-approved excipient poly (ethylene glycol) (PEG) or poly (ethylene oxide) (PEO) [26]. Research on nanocarriers composed of block copolymer micelles is a rapidly developing and exciting area of drug delivery. These systems are being studied for stabilization, solubilization, and delivery of the most challenging therapeutic agents. The distinctive architecture, small size, stability, and ability of block copolymer micelles to be modified for good compatibility with the drugs are best preferable properties for a drug delivery system [27]. To target abnormal cancer cells actively, special types of ligands are used to modify the micelle surface, namely aptamers, folic acid, carbohydrates, peptides, and antibodies. The core of the micelle can be functionalized to release the drug at the right concentration to the target site. The stimuli used in smart drug delivery systems based on micelles are changes in temperature, pH gradients, ultrasound, enzymes, and oxidation [28].

Optimized doxorubicin polymeric micelles (NK911, Nippon Kayaku, Co.) were the first clinically evaluated in 2001. NK911 micelles have been tested for metastatic pancreatic cancer in Phase II clinical trials, but the results have not been reported [29]. Paclical (orphan drug status in 2009 by the FDA) and Genexol-PM (approved in South Korea) are two examples of micellar formulation of paclitaxel for ovarian cancer and metastatic breast cancer and advanced lung cancer treatment, respectively, with significantly less toxicity [4, 10]. Genexol-PM consists of low-molecular-weight amphiphilic diblock copolymer, monomethoxy poly (ethylene glycol)-block-poly (D, L-lactide) (mPEG-PDLLA) and drug paclitaxel [30]. Micellar formulation of estradiol (Estrasorb) got FDA approval in 2003 and used for the treatment of menopause-related moderate-to-severe vasomotor symptoms. As it is administered transdermally, gastrointestinal side effects may be avoided [31]. Toxicity of Kolliphor-based paclitaxel drug can be reduced by micellar formulation. Nephrotoxicity of cisplatin drug also reduced due to micellar formulation [32]. Therefore, due to this broad applicability of micellar-based nanoformulations, we can expect many new products in the near future.

Recently, Seo et al (2015) revealed a co-delivery scheme based on the temperature-responsive micelle that can carry genes along with anticancer drugs [33]. Doerflinger et al (2019) developed a targeting aptamer ligand to functionalize polydiacetylene micelles [34].

## 18.6 Protein-Based Nanocarriers

In protein-based nanocarriers, proteins are used as a carrier, active therapeutic agents as well as for targeted delivery and reduction of toxicity [1]. In the last decade, albumin is mainly studied as a drug carrier and many albumin-based nanomedicines are at present in clinical trials. The advantages of these albumin-based nanomedicines are higher passive accumulation in the tumor site and also facilitating the cellular uptake of the drug by the albumin receptor [35]. Abraxane is an early

albumin-based nanoparticle conjugated with paclitaxel drug and got FDA approval in 2005. This formulation eliminates the use of toxic Kolliphor solvent required to make paclitaxel soluble as it causes immune reaction [1]. Therefore, an improvement in infusion time, drug efficacy, drug PK, and reduction in toxicity was observed during Abraxane use [36]. After the successful entry of Abraxane into the market, several other albumin-bound nanoparticles are in clinical trials for improving the efficacy of the drugs like docetaxel, rapamycin, heat shock protein inhibitor, etc. [31]. Nowadays, apart from unmodified proteins, engineered particle complexes are also being designed to enable active targeting and Ontak is an example of this engineered fusion targeting proteins with cytotoxic molecules. It is an interleukin (IL)-2 receptor antagonist and used to treat non-Hodgkin's peripheral T-cell lymphomas and helps to suppress overexpression of IL-2 receptor on T cells [1]. Ontak also showed a significant reduction in organ toxicity and thus may be an effective treatment for many hematological malignancies which are related to overexpression of IL-2 receptor [37].

## 18.7 Dendrimers

Dendrimers are well-defined, multivalent molecules with a nanometer size branching structure. There are three distinct components of dendrimer: core, branching dendrons, and surface-active groups. Conventional dendrimers face immune system clearance and lower uptake by cancer cells. Modification of the conventional dendrimer is the solution to these limitations. Chemical modification, linear polymer copolymerization, and hybridization with other nanocarriers are some of the choices for overcoming these limitations as reported so far [28]. To target the cancer cells, peptides, proteins, carbohydrates, aptamers, antibodies, etc., can change the surface of the dendritic structure. The surface of the dendritic structure can also be modified for different stimuli-responsive systems, such as light, heat, pH change, protein, and enzyme transformation [38, 39].

Most of the successful nanocarriers were synthesized using classical linear, random coil polymers, such as polyethylene glycol (PEG), poly (glutamic acid) (PGA), N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, poly (ethyleneimine) (PEI), and dextrin (a-1, 4 polyglucose). These polymers have produced conjugates and polyplexes that have now been used to develop formulations and that are in the clinical trial [40].

The cationic nature of PAMAM (polyamidoamine) makes it extremely beneficial for the delivery of genetic materials among other dendrimers. The effectiveness of delivery relies on PAMAM generation. In 1993, Haensler and Szoka were the first to report the delivery of PAMAM nucleic acid [41]. The tumor imaging dendritic contrast agent is also very promising [42].

## 18.8 Other Nanocarriers

Recently, the use of other different nanomaterials has been tried to build effective nanocarriers for drug delivery applications [43]. A large number of inorganic materials, such as metal, metal oxide, silica, carbon nanotubes, etc., can be used to create nanoformulations for therapeutic and imaging applications and metal and metal oxide are being explored intensely.

### 18.8.1 Carbon Nanotubes

Carbon nanotubes (CNTs) are cylindrical large molecules composed of a hexagonal structure of hybridized carbon atoms that may be composed of one sheet of graphene (single-walled CNTs) or by rolling up various sheets of graphene in a concentric manner (multi-walled CNTs) [44]. CNTs have some unique physicochemical and biological features and high surface modification capabilities that make them a successful drug delivery carrier. The distinctive shape of the nano-needle is particularly interesting, as it enables endocytosis to cross the cell membrane, while CNTs with size ranging from 50 to 100 nm are easy to be engulfed [45]. Any drugs can either be encapsulated in the internal space or be attached (covalent or noncovalent functionalization) to the surface of the CNTs.

The main issues with CNTs are their low water solubility, nonbiodegradable, and cytotoxicity. Although, CNT nanocarriers have the potential to be surface-functionalized (chemically or physically) which render them water-soluble, biocompatible, and nontoxic or less toxic. While PEGylation is used to improve solubility, prevent reticuloendothelial system (RES), and reduce toxicity, their surface functionalization with poly (N-isopropylacrylamide) polymer could be used to modify the CNTs for stimulus-responsive (temperature) nanocarriers. Castillo et al (2013) reported the formulation of a graphene electrode modified with peptide-conjugated nanotubes and folic acid for improving target specificity of human cervical cancer cells overexpressing folate receptors [46]. Lu et al (2019) developed anti-IGF1R antibody (IGF-1R Ab) coupled carbon nanotubes for photothermal therapy of orthotopic pancreatic cancer guided by optical imaging [47].

### 18.8.2 Metal and Metal Oxide Nanoformulations

Gold nanoparticle (AuNP) is the most distinctive inorganic material in nanotechnology with a wide range of biological and biomedical applications. It has been suggested for drug delivery and gene delivery applications as nontoxic carriers [48]. Passive targeting is well known to be accomplished by using AuNP as a carrier due to its better tumor cell accumulation (Enhanced Permeability and Retention) effect [49]. In fact, AuNP particular characteristics, such as its elevated surface to volume ratio, unique optical properties, simple synthesis, and flexible surface functionality, are committed to cancer therapy in the clinical sector [50]. In addition,

AuNP has optical properties that can be readily adjusted according to their form and structure to desirable wavelengths, enabling photothermal and imaging applications. Battogtokh et al (2019) reported glycol chitosan-coated near-infrared photosensitizer-encapsulated gold nanocages for glioblastoma in vitro and in vivo [51]. In another article, Liu et al (2019) reported that zwitterionic gadolinium (III) complex dendrimer entrapped AuNP showed target specificity to αvβ3 integrin expressing cells and enhanced CT/MRI imaging of lung cancer metastasis model in vivo [52]. Albertini et al (2019) developed RGD-link pentapeptide decorated AuNP for diagnosis and treatment of cancer. The peptide conjugation was selected for its ability to recognize the αvβ3 integrin receptor [53]. Most of the gold nanoparticles are at the in vivo stage (preclinical), and few have reached clinical trials.

In several studies, iron oxide nanoformulations have been considered for examining their use as contrast enhancement reagents for magnetic resonance imaging (MRI) [1, 4]. The iron nanoformulations for treating chronic kidney disease (CKD)-associated anemia are Venofer (iron sucrose injection), Ferrlecit (sodium ferric gluconate complex in sucrose injection), Infed (iron dextran injection), and Dexferrum (iron dextran injection). These formulations avoid toxicity and thus are administered rapidly in large doses, without increasing free iron levels in the blood [1]. Superparamagnetic iron oxide nanoparticles (SPIONs) have low toxicity, more half-life, and are biodegradable that respond strongly when exposed to a magnetic field; used both as targeted and nontargeted contrasting MRI agents to target specific tumors [3, 10]. Three FDA-approved SPION drug formulations are—Feraheme, Feridex, and GastroMARK. Feraheme is used to treat CKD and is also being deliberated as an imaging agent in clinical trials [32]. SPIONs also release energy in a magnetic field, permitting them to be used as promising hyperthermia agents against tumors in preclinical and early clinical studies. Nanotherm is one of such SPIONS to treat glioblastoma tumors; the subsequent injection in the tumor causes programmed and non-programmed cell death due to local thermal heating [1, 10].

Several metals, including silver, are known to be potent antimicrobials as they can easily penetrate bacterial cells and induce toxic effects. Cornell dots are inorganic silica nanoparticles that are being developed at Cornell University as a diagnostic and therapeutic tool in cancer treatment [1, 4, 10]. Although designed for lymph-node mapping in cancer patients, these nanoparticles have also been found to induce cancer cell death in vitro and reduce the size of tumors after multiple high-dose injections were administered to mice. They are composed of an internal silica core labeled with a near-infrared fluorescent dye, a targeting moiety, and an antifouling polymer layer. This design has created a nanoparticulate system that is more stable and 20 to 30 times brighter than a conventional solution of the constituent dye. Various FDA approved nanomedicines used in cancers and non-cancerous diseases have been given in Tables 18.1 and 18.2.

**Table 18.1** FDA-approved nanomedicines available for cancer treatment

| Drug | Nanomedicine | Manufacturer | Delivery system | Indications | Benefits over free drug | Route of administration | FDA approved Year |
|---|---|---|---|---|---|---|---|
| *Liposomal* | | | | | | | |
| Doxorubicin | Doxil | Janssen | PEGylated liposomes | HIV-associated Kaposi's sarcoma, ovarian cancer, metastatic breast cancer, multiple myeloma | Increased delivery to disease site, decreased systemic toxicity of free drug | i.v. | November 1995 |
| Daunorubicin | DaunoXome | Gilead Sciences | Liposomes | First-line treatment for patients with advanced HIV-associated Kaposi's sarcoma | 10 times higher accumulation in tumors than free drug. | i.v. | April 1996 |
| Daunorubicin and cytarabine | Vyxeos | Jazz pharmaceuticals | Liposomes | Acute myeloid leukemia (AML), AML with myelodysplasia-related changes | Increased efficacy through synergistic delivery of co-encapsulated agents | i.v. | August 2017 |
| Vincristine | Marqibo (OncoTCS) | Spectrum Pharmaceuticals | Liposomes | Adult patients with lymphoblastic leukemia | Increased delivery to tumor site, decreased systemic toxicity | i.v. | August 2012 |
| Irinotecam | Onivyde | Ipsen biopharmaceuticals | Liposomes | Metastatic pancreatic cancer | Increased delivery to tumor site, decreased systemic toxicity | i.v. | October 2015 |

(continued)

**Table 18.1** (continued)

| Drug | Nanomedicine | Manufacturer | Delivery system | Indications | Benefits over free drug | Route of administration | FDA approved Year |
|---|---|---|---|---|---|---|---|
| Verteporfin | Visudyne | Bausch and Lomb | Liposomes | Age-related macular degeneration, myopia, ocular histoplasmosis | Increased delivery to site of diseased vessels, photosensitive release | i.v. | April 2000 |
| Cytarabine | Depocyt | Sigma-tau | Liposomes | Lymphomatous meningitis | Increased delivery to tumor site, decreased systemic toxicity | i.v., i.t. | August 1999 |
| *Polymer* | | | | | | | |
| Pegademase bovine | Adagen | Leadiant biosciences | PEGylated adenosine deaminase enzyme | Severe combined immunodeficiency disease associated with adenosine deaminase deficiency | Longer circulation time, decreased immunogenicity | i.m | March 1990 |
| Pregasprase | Oncaspar | Baxalta U.S. | PEGylated L-asparaginase | Acute lymphoblastic leukemia | Greater protein stability | i.v., i.m. | July 2006 |
| Pegfilgrastim | Neulasta | Amgen | PEGylated granulocyte colony stimulating factor | Neutropenia associated with cancer chemotherapy | Greater protein stability | s.c. | January 2002 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Pegvisomant | Somavert | Pfizer | PEGylated human growth hormone receptor antagonist | Acromegaly | Greater protein stability | s.c. | March 2003 |
| Leuprolide acetate | Eligard | Tolmar | Polymeric nanoparticles | Prostate cancer | Longer circulation time, controlled payload delivery | s.c. | January 2002 |
| *Protein* | | | | | | | |
| Denileukindiftitox | Ontak | Eisaii Inc | Protein-drug conjugate | Cutaneous T-cell lymphoma | Targeted T-cell specificity, lysosomal escape | i.v. | February 1999 |
| Paclitaxel | Abraxane | Abraxis bio science, AstraZeneca | Protein-drug conjugate | Breast cancer, NSCLC, pancreatic cancer | Greater solubility, increased delivery to tumor | i.v. | January 2005 |
| Monoclonal human EGF receptor-2antibody and DM1 | Kadcyla | Genentech | Protein-drug conjugate | Metastatic breast cancer | Selectively deliver to EGF receptor-2-expressing cells | i.v. | February 2013 |
| *Nanocrystal* | | | | | | | |
| Sirolimus | Rapamune | Wyeth pharmaceuticals | Nanocrystals in tablets | Immunosuppressant | Greater bioavailability | Oral | August 2000 |

**Table 18.2** FDA-approved nanoparticles available for treatment of diseases other than cancer

| Drug | Name of nanoformulation | Manufacturer | Delivery System | Indications | Benefits over free drug | Route of administration | FDA approved Year |
|---|---|---|---|---|---|---|---|
| *Liposomal* | | | | | | | |
| Poractant alfa | Curosurf | Chiesi USA | Liposomes | Respiratory distress syndrome | Increased delivery with smaller volume, decreased toxicity | Intratracheal | October 1998 |
| Morphine | DepoDur | Pacira pharmaceuticals | Liposomes | Postoperative analgesia | Extended release | Epidural injection | May 2004 |
| Amphotericin B | Fungizone | Apothecon | Surfactant-based nanoformulation | Systemic fungal infections | Increase solubility | i.v. | March 1966 |
| | Abelcet | Sigma tau | Lipid-based (non-liposomal) | Invasive fungal infections | Decreased toxicity | i.v. | November 1995 |
| | Amphotec | Alko Pharma USA | Lipid-based (non-liposomal) | Fungal infections | Decrease infusion time | i.v. | November 1996 |
| | Ambisome | Gilead Sciences | Liposomes | Fungal and protozoal infection | Significantly lower nephrotoxicity, and infusion-related chills/ rigors | i.v. | August 1997 |
| Bupivacaine | Exparel | Pacira pharmaceuticals | Liposomes | Post-surgical analgesia | Sustained release, increase safety | Local/ Depofoam | November 2011 |

| *Polymer* | | | | | | | |
|---|---|---|---|---|---|---|---|
| Glatimer acetate | Copaxone | TEVA pharmaceuticals | Polymeric drugs | Multiple sclerosis | Controlled clearance | s.c. | January 1997 |
| Pegaptanib | Macugen | Bausch and Lomb | PEGylated anti-VEGF aptamer | Age-related macular degeneration | Greater aptamer stability | Intravitreal | December 2004 |
| Antihemophilic factor (recombinant) | Adynovate | Shire | PEGylated polymer protein conjugate | Hemophilia | Greater protein stability, longer half-life | i.v. | November 2015 |
| Certolizumab pegol | Cimzia | UCB | PEGylated humanized antiTNF-alpha antibody fragment | Rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis | Longer circulation time, greater stability in vivo | s.c | May 2009 |
| Pegloticase | Krystexxa | Horizon | PEGylated polymer protein conjugate | Chronic gout | Greater protein stability | i.v. | September 2010 |
| Epoetin beta | Mircera | Hoffman-LaRoche | PEGylated polymer protein conjugate | Anemia associated with CKD | Greater aptamer stability | i.v., s.c. | November 2007 |
| IFN alpha-2a | Pegasys | Genentech | PEGylated polymer protein conjugate | Hepatitis B, hepatitis C | Greater protein stability | s.c. | October 2002 |
| IFN alpha-2b | PegIntron | Merck | PEGylated polymer protein conjugate | Hepatitis C | Greater protein stability | s.c. | January 2001 |

(continued)

**Table 18.2** (continued)

| Drug | Name of nanoformulation | Manufacturer | Delivery System | Indications | Benefits over free drug | Route of administration | FDA approved Year |
|---|---|---|---|---|---|---|---|
| IFN beta-1a | Plegridy | Biogen | PEGylated polymer protein conjugate | Multiple sclerosis | Greater protein stability | s.c. | August 2014 |
| Coagulation factor IX (recombinant) | Rebinyn | Novo Nordisk | GlycoPEGylated | Hemophilia B | Longer half-life, greater drug levels between infusions | i.v. | June 2017 |
| Poly(allylamine hydrochloride) | Renagel and Renvela | Genzyme | Polymeric drug of sevelamer hydrochloride and Sevelamer carbonate | Chronic kidney disease (CKD) on dialysis | Longer circulation time and therapeutic delivery | Oral | October 1998 |
| Triamcinolone acetonide ER | Zilretta | Flexion therapeutics | Polymeric drug | Osteoarthritis knee pain | Extended release | Intra-articular | October 2017 |
| *Nanocrystal* | | | | | | | |
| Aprepitant | Emend | Elan, Merck | Nanocrystal | Antiemetic | Greater absorption and bioavailability | Oral | March 2003 |
| Megestrol acetate | MegaceES | Par pharmaceuticals | Nanocrystal | Anorexia, cachexia | Lower dosing | Oral | July 2005 |
| Fenofibrate | TriCor | Elan, Abbott | Nanocrystal | Anti-hyperlipidemic | Greater drug loading and bioavailability | Oral | May 2004 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Morphine sulfate | Avinza | Pfizer | Nanocomplex | Psychostimulant | Greater drug loading and bioavailability, extended release | Oral | March 2002 |
| Methylphenidate HCl | Ritalin LA | Novartis | Nanocomplex | Psychostimulant | Greater drug loading and bioavailability | Oral | June 2002 |
| Dexamethylphenidate HCl | Focalin | Novartis | Nanocomplex | Psychostimulant | Greater drug loading and bioavailability | Oral | May 2005 |
| Hydroxyapatite | EquivaBone | Zimmer Biomet | Nanocrystal | Bone substitute | Mimics bone structure | Bone graft | September 2009 |
| | NanOss | RTI surgical | Nanocrystal | Bone substitute | Mimics bone structure | Bone graft | 2005 |
| | Ostim | Heraeus Kulzer | Nanocrystal | Bone substitute | Mimics bone structure | Bone graft | 2004 |
| | OsSatura | IsoTis Orthobiologics | Nanocrystal | Bone substitute | Mimics bone structure | Bone graft | 2003 |
| Calcium phosphate | Vitoss | Stryker | Nanocrystal | Bone substitute | Mimics bone structure | Bone graft | 2003 |
| Paliperidone Palmitate | Invega Sustenna | Janssen | Nanocomplex | Schizophrenia, schizoaffective disorder | Slow release | Oral, i.m | December 2006 |
| Dantrolene sodium | Ryanodex | Eagle pharmaceuticals | Nanocomplex | Malignant hypothermia | More rapid rate of administration at higher doses | i.v. | July 2014 |

(continued)

**Table 18.2** (continued)

| Drug | Name of nanoformulation | Manufacturer | Delivery System | Indications | Benefits over free drug | Route of administration | FDA approved Year |
|---|---|---|---|---|---|---|---|
| Tizanidine HCl | Zanaflex | Acorda | Nanocomplex | Muscle relaxant | Greater drug loading and bioavailability | Oral | August 2002 |
| *Inorganic* | | | | | | | |
| Iron dextran | Infed | Actavis Pharma | Nanocomplex | Iron deficiency in CKD | Increased dose | i.v. | August 1995 |
| | Dexferrum | American regent | | Iron deficiency in CKD | Increased dose | | February 1996 |
| Iron oxide | Feraheme | AMAG pharmaceuticals | Metal oxide nanoparticles | Iron deficiency in CKD | Prolonged, steady release with less frequent dosing | i.v. | June 2009 |
| Sodium ferric gluconate | Ferrlecit | Sanofi-Aventis | Nanocomplex | Iron deficiency in CKD | Increased dose | i.v. | February 1999 |
| Iron sucrose | Venofer | American regent | Nanocomplex | Iron deficiency in CKD | Increased dose | i.v. | November 2000 |
| *Emulsion* | | | | | | | |
| Estradiol | Estrasorb | Novavax | Nanoemulsion | Vasomotor symptoms associated with menopause | Controlled delivery | Topical and transdermal | October 2003 |
| Difluprednate | Durezol | Sirion therapeutics | Nanoemulsion | Eye inflammation, uveitis | Sustained release, increase absorption | Ocular | June 2008 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cyclosporine A | Restasis | Allergan | Nanoemulsion | Dry eye syndrome | Sustained release, increase absorption | Ocular | October 2003 |
| *Imaging agents* | | | | | | | |
| Iron oxide | Feridex | AMAG pharmaceuticals | Metal oxide nanoparticles | Liver/spleen lesion magnetic resonance imaging | Vertical irritant effect | i.v. | February 1996 |
| Silicone-coated iron oxide nanoparticles | Lumirem | AMAG pharmaceuticals | Silicone-coated, superparamagnetic iron oxide | Imaging agent | Vertical irritant effect | Oral | May 1996 |

Source References: [7, 31, 54–59]

## 18.9 Toxicity Aspect of Nanomaterials

In the following section, we have described in brief the observed toxicity of nanomaterials used for medicinal purposes in an organ-specific/physiological system-dependent manner.

### 18.9.1 Neurotoxicity

Several nanomaterials such as polymeric nanoparticles, liposomes, inorganic metallic nanoparticles, carbon nanotubes, dendrimers, quantum dots, etc., have been applied for diagnosis and treatment of brain diseases. They can enter the brain by penetrating through the blood–brain barrier with the help of a series of transporters or receptors expressed on the endothelial cells of brain capillaries, or through adsorption-mediated transcytosis, or through the intranasal route; bypassing the blood–brain barrier [60]. Nanosized drug carriers, if permeating through the blood–brain barrier can interact with the hippocampal cells of the brain and can cause alteration of brain functions [61]. The common mechanisms of nanoformulation-induced neurotoxicity involves oxidative stress, induced cell apoptosis and autophagy, and immune response and inflammation resulting in activation-specific signaling pathways which can subsequently alter the neuronal structure or activity and also can alter the function of the blood–brain barrier [60]. Charge of nanoparticles has an impact on its permeability through the blood–brain barrier. For example, anionic wax nanoparticles were found to penetrate the blood–brain barrier better than its neutral or cationic counterparts [62]. Considering the role of surfactants in penetration of nanoparticles through the blood–brain barrier and toxicity of polymeric nanoparticles in the neuronal system, one study revealed that Polysorbate 80-modified chitosan nanoparticles after intravenous injection in rats causes a dose-dependent accumulation of the nanoparticles in the frontal cortex and cerebellum, with neuronal apoptosis, mild inflammation, increased oxidative stress, and loss of body weight [63]. Liposomes are considered to be superior over many other formulations in delivering drugs through the blood–brain barrier but empty liposomes had also found to possess toxic effects on the neuronal system. In a comparative study of the cisplatin containing liposomal formulation, free-drug and drug-free liposomes, it was found that cisplatin-loaded liposomes increased in vitro cytotoxicity against glioma cells and high tumor retention in glioma-bearing rats compared to the free cisplatin but the drug-free liposomes also induced minimal to severe neuroinflammation and necrosis in control rats. This study suggested the intrinsic toxicity of the liposomes alone [64]. Dendrimers of different generations with various surface groups were used to assess the neurotoxic effects on human neural progenitor cells. It was found that cationic dendrimers of higher generation altered mitochondrial activity, induced oxidative stress, apoptosis, and subsequent DNA damage; also can interfere with neuronal differentiation, and gene expression. Metal nanoparticles were also studied extensively to elucidate their neuronal toxicity [65]. Gold nanoparticles may accumulate in the brain where through transportation

via the BBB or olfactory nerve and can induce neurotoxic effects such as increased seizure activity, cognition effects, and astrogliosis [66]. The neurotoxicity of silver nanoparticles involves increased ROS generation, caspase activity, and cytokine release resulting in inflammation and cell death. Additionally, the release of silver ions from nanoparticles of silver or its oxides can directly trigger necrosis through the disruption of membrane integrity [67]. Iron oxide nanoparticles were also found to alter synaptic transmission and nerve conduction leading to several inflammatory responses [68]. $TiO_2$ nanoparticles were also reported to induce similar toxicities resulting in the alteration of synaptic plasticity and disrupted signaling pathways [69]. The intranasal administration of silica nanoparticles also leads to the accumulation of nanoparticles in the brain and subsequently to cognitive dysfunction and impairment, synaptic changes, and pathologies similar to neurodegeneration. Carbon nanotubes enter the brain through olfactory or systemic administration [70]. Studies have shown that the inhaled carbon nanotubes can accumulate in the olfactory bulb, causing activation of microglial cells and subsequent inflammatory responses. Multi-walled carbon nanotubes (MWCNT) were reported to induce higher neurotoxic effects than single-walled carbon nanotubes (SWCNT) [71]. Neurotoxicity associated with carbon nanotubes can also result in neurobehavioral changes such as anxiety and depression [60]. Oberdörster et al (2004) studied the effect of colloidal fullerenes on the neuronal system of largemouth bass and observed increased lipid peroxidation in brain and reduction of glutathione in gills after 48 h of dosing [72]. The neurotoxic effects of quantum dots are similar to the general neurodegenerative toxicity, including increased oxidative stress and cell function damage; and are dependent on their size, surface charge, concentration, surface coating, the nature and the solubility of the constituent materials [60].

### 18.9.2 Cardiotoxicity

Metal nanoparticles were most extensively studied to elucidate their toxicity to the heart. Using a zebrafish model, it was revealed that titanium nanoparticles can translocate between organs and can accumulate in the heart through crossing the blood-heart barrier. Thinning of cardiac muscles, tissue inflammation followed by cell necrosis and cardiac biochemical imbalance are the consequences of chronic exposure to $TiO_2$ nanoparticles. Molecular analysis revealed that $TiO_2$ nanoparticles can bind with lactate dehydrogenase (LDH) thereby increasing its activity along with the activity of some other enzymes such as AST (aspartate aminotransferase), CK (creatine kinase), HBDH (α-hydroxybutyrate dehydrogenase), which leads to myocardial injury [73]. The other reasons found to be the elevation of ROS (reactive oxygen species) level: mitochondrial swelling, increased activity of caspase-3, and augmentation of DNA peroxidation in cardiac muscle [74]. ZnO nanoparticles are mainly bioaccumulated by the interaction of Zn and sulfur-containing proteins. ZnO nanoparticles were found to be toxic in both in vitro and in vivo studies. Apart from heart, acute oral exposure to ZnO nanoparticles also targets liver, spleen, pancreas, and bone [75]. Long-term exposure of rats to ZnO inhalation was reported to cause

both cardiac damage and lung inflammation [76]. In order to find a correlation between cardiac and respiratory toxicity of ZnO nanoparticles, Bessemer et al (2015) conducted a study on freshwater fish model *Catostomus commersonii*. They concluded that myocardial damage was due to increased parasympathetic input in the heart, which is a consequence of gill neuroepithelial cell damage by ZnO [77]. While studied at the molecular level, increased level of troponin T, CPK-MB (creatine phosphokinase-MB), and myoglobin were found to be responsible for ZnO-induced myocardial damage [78]. Like $TiO_2$ and ZnO, Ag nanoparticles can also induce ROS and upregulation of cytokine activity, thus producing oxidative stress and inflammation. While searching for molecular marker behind Ag-induced myocardial injury, in a study on chicken, it was found that Ag nanoparticles downregulate FGF-2 (fibroblast growth factor-2, a modulator of cardiomyopathy) and upregulate VEGF-A (vascular endothelial growth factor-A, an angiogenesis modulator) [79, 80]. Inhaled carbon nanoparticles and carbon nanotubes can also cause cardiac damage through depletion of serum thiol content and an increase in lipid peroxidation products. Administration of multi-walled carbon nanotubes (MWCNT) through intratracheal instillation can worsen ischemia/reperfusion (I/R) injury [74]. Both intravenous and intratracheal administration of fullerene was found to cause myocardial infarction [81]. Exposure to silica nanoparticles can also create inflammatory responses in the cardiovascular system and is associated with an increased level of eotaxin-1, LDH (lactate dehydrogenase), and CKMB (creatine kinase MB) [82].

### 18.9.3 Pulmonary Toxicity

The main toxic effects of nanoparticles on the pulmonary system are inflammation, oxidative stress, and functional disturbances. Inorganic metal nanoparticles such as Co, $TiO_2$, $SiO_2$, Ni, and ZnO were found to induce lung epithelial damage, leading to inflammation and this effect is prominently higher in case of nanosized particles compared to their macrosized congeners. Carbon black nanoparticles can also generate similar inflammation and its effect is worse than ZnO nanoparticles. Release of interleukin-8 is responsible for such inflammatory conditions [61]. Heavy metal nanoparticles like cadmium, iridium, and gold showed variable toxicity depending on their solubility and reactivity to the tissues. Inhalation of insoluble iridium and gold nanoparticles did not induce pulmonary inflammation, while soluble cadmium nanoparticles at high doses were found to induce pulmonary injury via translocation from the lung to the liver [83]. Exposure to carbon nanoparticles are mainly through inhalation in occupational level, hence lung is the primary target for carbon nanoparticles. But inhaled carbon nanoparticles and carbon nanotubes can distribute in heart, liver, kidney, and brain and cause multiple dysfunctions such as necrosis of liver and kidney tissue, inflammation, depletion of serum antioxidants such as GSH (glutathione) and SOD (superoxide dismutase), and abnormalities of alveolar microvessels [84]. When instilled in the lung, SWCNTs (single-walled carbon nanotubes) can be phagocytosed by lung epithelial cells and

these result in both local and systemic inflammation [74]. A study about carbon black nanoparticle toxicity revealed that these nanoparticles can increase intracellular calcium by controlling cellular ion channels, resulting in impaired phagosome transport and cytoskeletal dysfunction [85]. Presence of airway inflammation, asthma, and obstructive pulmonary diseases can increase the retention of inhaled nanoparticles and this could worsen the situation. This hypothesis was also tested in animal models [86]. Smaller sized nanoparticles were found to be superior in the induction of inflammatory responses compared to larger nanoparticles or microparticles. Tumorigenesis associated with the inhalation of nanoparticles is invariably related to its size and the smaller congeners are often more severe than their larger analogs. This is supposed to be due to poor detection of smaller nanoparticles by macrophages thereby improper clearance, resulting in nanoparticle buildup, chronic inflammation, fibrosis, and eventually tumorigenesis [87].

### 18.9.4 Hemotoxicity

Hemotoxicity of nanoformulations is directly related with their circulation half-life, interaction with RBC and macrophages, ability to escape from hepatic reticuloendothelial system, and affinity for enzymes present in serum. The size of nanoparticles regulates their persistence in circulation in a variable way. Nanoparticles with 5–10 nm diameters are rapidly cleared from systemic circulation after systemic administration but those with 10–70 nm diameters can penetrate through blood capillary wall and distribute easily throughout the body. Nanoparticle with 70–200 nm diameters needs longer time to penetrate the blood capillary wall and also can persist in systemic circulation for a longer period. Hence, their toxicity also varies depending on their circulation half-life. Senior and Gregoriadis found that neutral liposome with a diameter of less than 100 nm had circulation half-life up to 20 h while the anionic liposomes had a half-life of less than 1 h [88]. The high surface to volume ratio nanoformulations provide a large exposure of surface molecules toward the circulation system, and this is one of the major reasons behind nanoformulation-related RBC damage. Another important factor is the surface charge which interacts with the cell membrane of RBC. Cationic polystyrene nanoparticles were found to cause hemolysis and blood clotting while such effects were absent in case of its anionic counterparts. Carbon nanotubes were found to cause platelet aggregation and in vivo thrombosis in experimental models while carbon fullerenes with almost similar diameter had not produced such effects. This can be explained with the difference of their shape which plays an important role in binding with platelets [61].

### 18.9.5 Hepatotoxicity and Nephrotoxicity

Polymeric nanoparticles used in drug delivery have variable effects on liver depending on their physicochemical properties such as functional groups present,

size, biodegradability, etc. For example, polyalkylcyanoacrylate nanoparticles were found to produce mild and reversible inflammatory condition to the liver in animal model due to their biodegradable nature and rapid clearance from systemic circulation. Polystyrene nanoparticles indeed, produce severe toxicity in the liver due to their nonbiodegradable nature and prolonged circulation [61]. Polyamidomamine dendrimers can induce lysosomal dysfunction in the liver, resulting in vacuolization of hepatocytes as observed in the experimental mice model [89]. Acrylic nanoparticles like cyanoacrylate and isobutylcyanoacrylate nanoparticles showed high accumulation in kidneys in experimental rats and this can cause renal injury and proteinuria [90]. Metal nanoparticles were studied extensively to elucidate their effects on hepatorenal system. Isoda et al (2017) reported size-dependent toxicity of Pt nanoparticles with a comparative study of 1 nm and 8 nm Pt nanoparticles and found that 1 nm Pt nanoparticle can cause acute hepato-renal injury in mice by increasing serum aminotransferases and blood urea nitrogen [91]. They also concluded that upregulation of interleukin-6 and interleukin-1β is responsible for such hepatic and renal injury, respectively. Similar findings were also reported by Yamagishi et al (2013) who found that administration of Pt nanoparticles less than 1 nm diameter to mice for several weeks produces urinary casts, tubular atrophy, and accumulates inflammatory cells [92]. Negatively charged superparamagnetic iron oxide nanoparticles were found to significantly damage actin cytoskeleton of kidney and brain cells in both in vitro and in vivo studies [74].

### 18.9.6 Genotoxicity

Nanoparticles when entered inside the cell can interact directly with the nucleus and transport into the nucleus through the formation of nuclear pore complexes (NPC). As the diameter of NPC is around 30 nm, nanoparticles with a diameter of 30 nm or less can cross nuclear envelope through NPC. Nanoparticles larger than 30 nm enter into the nucleus during mitotic division when the nuclear membrane dissembles. Inside the nucleus, nanoparticles can interact with DNA and affect replication and transcription of DNA. In a study, Li et al (2014) showed nanoparticles of 3–46 nm size have a high affinity for DNA and strongly inhibit replication of DNA [93]. Tsoli et al (2005) reported gold nanoparticle of 1.4 nm size interacts with major grooves of DNA in a unique manner which could account for its genotoxicity [94]. Not only size but also the charge of NPs can affect its transportation into the nucleus. In a study using THP-1 cells, Nabiev et al (2007) demonstrated that green quantum dots (2.1 nm) can enter the nucleus through NPC while the red ones (3.4 nm) cannot, and concluded such transportation is mediated by histone binding [95]. Apart from direct interaction with DNA, nanoparticles can interfere with DNA repair through the interaction of DNA repairing molecules in BER and NER pathways (base excision repair and nucleotide excision repair). Carbon nanotubes due to their similarities with cellular microtubules can interact or mimic mitotic spindle, resulting in loss or gain of chromosomes (known as aneugenic effect). Such aneugenic effects were also observed in CuO and gold nanoparticles [96, 97]. Transition metal nanoparticles

(like Fe, Ag, Cu, Mn, Ni NPs) can release free metal ions which can directly induce ROS generation through Fenton reaction and this accounts for a major metal nanoparticle-induced genotoxicity as a consequence of oxidative stress. This phenomenon is also known as inflammation-induced or secondary genotoxicity. International Agency for Research on Cancer (IARC) evaluated that amorphous silica is not carcinogenic to humans but the crystalline is carcinogenic. Several in vivo studies using amorphous $SiO_2$ have supported this with mild or no genotoxicity induction (DNA damage) [98]. For crystalline silica, the secondary inflammation-driven genotoxicity mechanism is recognized as an important mechanism for its carcinogenicity. In several in vitro studies, it was found that smaller $SiO_2$ nanoparticles induce more toxicity due to higher penetration and ultimate lysosomal overload. Although most of the in vivo studies reported $TiO_2$ anatase did not induce micronuclei formation in hepatic reticulocytes and leukocytes but some of the studies reported significant micronuclei formation in bone marrow cells and peripheral blood cells (erythrocytes), which is a hallmark of genotoxicity [98]. However, intratracheal or inhalation administration of $TiO_2$ nanoparticles to mice and rat has been reported to induce inflammation in the lung but not significant genotoxicity in both lung epithelial lung cells and erythrocytes [99]. Intraperitoneal administration of $TiO_2$ NPs has been found to accumulate titanium in liver, kidney, and bone marrow and to induce oxidative stress-mediated genotoxicity in those organs [100]. In the case of gold nanoparticles, high genotoxicity was found in the administration of larger nanoparticles compared to smaller ones. Both acute and chronic intraperitoneal administration of differently sized AuNPs (10 nm and 30 nm, citrate coated) in rats induced DNA damage in blood and liver cells as evaluated by comet assay [101]. As gold can cross the blood–brain barrier, while studying genotoxicity of AuNPs in rat it was found to cause DNA damage in the cerebral cortex. Toxicity of gold nanoparticles greatly depends on its surface chemistry and manufacturing methods. For example, AuNPs prepared in aqueous media did not produce cyto- or genotoxicity but those prepared from pure acetone solution produced remarkable genotoxicity as studied by Di Bucchianico et al (2015) [102]. They concluded the presence of amorphous carbon and enolate ions on the surface of acetone-derived gold nanoparticles were responsible for this effect. Genotoxicity of silver nanoparticles was reported to be mainly oxidation-induced and was prominent in case of larger nanoparticles (200 nm). Several in vitro studies revealed genotoxicity and mutagenicity of silver nanoparticles in vitro, and were confirmed by micronuclei formation, DNA double-strand break, and comet assay [98]. Intranasal administration of MWCNT was found not to induce genotoxicity in lung up to 90 days of treatment but produced pulmonary inflammation in animal models (rats) [103]. Even they (MWCNTs) have not induced DNA damage or micronuclei formation in peripheral blood leukocytes or bone marrow erythrocytes. On the other hand, SWCNT and carbon nanofibers even after single-dose administration can persist in the lung for a long period and can induce micronuclei formation, nuclear protrusions, and pulmonary fibrosis [84]. Catalán et al (2016) showed that the structure and dispersion of carbon nanotubes and their administration routes can give variable results. They showed straight-walled MWCNTs were able to induce DNA damage in mouse BAL (broncho-alveolar lavage) cells after inhalation but not after pharyngeal

aspiration, while both straight-walled and tangled MWCNT can induce DNA damage in lung cells (alveolar) irrespective of aforementioned routes of administration [104]. When functionalized by carboxylation, both SWCNT and MWCNT induced chromosomal aberration in bone marrow cells [98]. This suggests the presence of important interaction of COOH groups with chromosomes in vivo. Dendrimers can form complexes with DNA and this complexion can damage DNA through distortion or complete separation of double-stranded DNA. G4 and polyamidoamine dendrimers were reported to induce considerable genotoxicity [61].

### 18.9.7 Methods of Assessment of Toxicity of Nanomaterials

Nanotoxicity refers to the biological adverse effects caused by nanomaterials. Toxicity assessments of nanomaterials should follow a standardized set of rules to avoid confusion and misconduct in designing nanomaterials for biomedical applications [60]. Overall walkthrough of nanomaterial toxicity assessment involves (a) characterization of nanomaterials, (b) in vitro and in vivo studies, and (c) final clinical trials. Subsequently, we discussed these aforementioned analyses in detail.

*(a) Characterization of nanomaterials*: There are several essential physicochemical characteristics to be studied. They include but not limited to, particle shape and size, distribution, surface charge and reactivity, surface area, chemical composition, solubility and partition properties, aggregation tendency in relevant media, crystallinity, porosity, and sample purity. Chemical reactivity, surface chemistry, redox potential, and photocatalytic activity are some of the chemical analysis necessary to identify the chemical nature of nanomaterials. Nanomaterial characterization is generally achieved through spectroscopy, electron microscopy, X-ray diffraction, differential light scattering, magnetic resonance, mass spectrometry, chromatography, zeta potential measurement, thermal techniques, and circular dichroism [60]. Sterility of the formulations is generally assessed by endotoxin test through kinetic turbidity LAL assay [105]. Standardized guidelines should be implemented on the physicochemical characterization of nanomaterials to generate reproducible nanomaterials with desired physiochemical properties. The results obtained in this step should be relevant to the objective and end-point target of the study. Interaction of nanomaterials with body components, especially with proteins and receptors can change its surface characteristics. So, there is a possibility of discrepancies between cellular studies and theoretical predictions based on physicochemical properties and care should be taken to assess such evaluations. Furthermore, combination with biological macromolecules can promote intracellular uptake, reducing body clearance and can lead to chronic and degenerative changes.

*(b) In vitro studies*: In vitro toxicity assessments are crucial for investigating the mechanism of nanomaterial-induced toxicity on biological entities. Conventional in vitro models consist of different cell culturing systems, xenograft models, and studies on tissue sections. Choice of cultures and tissues depends on the organ of interest on which toxicity needs to be evaluated. In vitro toxicity assessments can be categorized into cell proliferation, apoptosis, necrosis, cell cycle, oxidative stress,

and DNA damage assays. The most common methods involved in in vitro experiments are fluorescence, chemiluminescence, analytical, and molecular marker-based detection systems; often accompanied by chromatographic separation techniques [60]. Cell proliferation analysis is the primary study to detect both the efficacy of cytotoxic agents and unwanted toxicity toward cells of 'not interest'. Cytotoxicity is generally assessed by MTT assay and LDH assay [106]. Cell death can occur through two different routes, namely apoptosis and necrosis. Apoptosis is a programmed cell death, characterized by changes in the nuclear morphology owing to chromatin fragmentation and condensation and identified by specific biomarkers (translocation of phosphatidylserine), occurrence of apoptotic bodies, and cell shrinkage. The main mechanisms of apoptosis involve caspase activation, mitochondrial swelling, release of cytochrome c, and DNA fragmentation. On the other hand, necrosis represents accidental cell death due to trauma, hypoxia, or pathogens; characterized by nuclear swelling, chromatin flocculation, loss of organelle function, membrane break, extracellular release of cytoplasmic content, etc.; and can be identified by microscopic studies. Another necessary assay involves the detection of oxidative stress as a response to the exposure of cells to nanomaterials. Major in vitro studies involve estimation of ROS (reactive oxygen species), activity of GSH (glutathione), and SOD (superoxide dismutase). In vitro studies in the evaluation of genotoxicity involves mutation testing in bacteria and mammalian cells, in vitro cytogenic effects and micronuclei analysis, micronuclei testing in erythrocytes, comet assay, and chromosomal aberration in bone marrow cells [98]. The major limitations of in vitro assay using cell culture involve the inability of the cell to mimic the native tissue microenvironment and reactivity of formulation ingredients with the assay components. However, the recent development of organ culture established in vitro studies more reliable in evaluation of actual phenomena happening in vivo on exposure to nanomaterials and also opened a new era of diverse biological experiments [60].

*(c) In vivo studies*: In vivo experiments are mandatory for investigation of nanomaterial toxicity and are superior over other methods as they allow assessment of physiological action for the whole organ and cannot be modeled in vitro. Several in vivo models and techniques have been developed to assess the organ distribution of nanomaterials. The most widely used invasive techniques are analysis of blood and tissue sampling after intravenous injection, microdialysis, quantitative autoradiography, and autopsy. The most popular noninvasive techniques are fluorescent and radio-imaging [60]. Assessment of organ-specific toxicity involves the measurement of various parameters specific to the organ system. For example, assessment nanomaterial-induced neurotoxicity primarily involves evaluation of behavioral changes regarding movement, learning, memory, motor coordination or reflexes, tremor, or paralysis; analysis of synthesis, release, and uptake of neurotransmitters; and histopathological observation of neuronal system [107]. Regarding cardiotoxicity assessment, the primary studies involve echocardiography, cardiac magnetic resonance imaging, Speckle-Tracking imaging, and analysis of several biomarkers such as troponin I, CKMB (creatine kinase-MB), LDH (lactate

dehydrogenase), myoglobin, myeloperoxidase, FGF-2 (fibroblast growth factor-2), NT-proBNP (N-terminal pro-B type natriuretic peptide), etc. [74].

## 18.10 Theranostic Applications of Nanomedicines

The term "Theranostic" was first coined by Furkhouser in 2002. It refers to "any material" with a dual ability of therapeutic and diagnostic potential. The application of nanotechnology in the field of therapeutics gives rise to the development of nanotheranostics which provide significant promise to develop much effective precision medicine by tuning the treatment depending on the molecular understanding of the disease and genetic makeup of the patients leading to protection of patients from adverse side effects. Thus, nanotheranostics help to monitor simultaneously the bioavailability of therapeutics and noninvasive evaluation of therapeutic efficacy in real time. Thus, nanotheranostics belong to a platform which provides integration between molecular therapy and molecular imaging. This integration offers myriads of promising characteristics such as early detection of disease, disease staging, therapy selection, planning and scheduling of treatment, identification of adverse effects at early stages of the treatment, and finally, planning of follow-up therapies. Plenty of researchers prefer to see the nanotheranostics as an integrated platform of nanomedicine and nanosensor due to the ability of nanosensor to identify significant numbers of biomarkers from a small sample volume and nanomedicine can extravasate from the blood vessel and deliver the therapeutic payloads predominantly into the target tissue by receptor-mediated active targeting. Plethoras of materials used for the development of nanomedicines are explored for the production of theranostic nanomedicines as described below.

### 18.10.1 Drug-Polymer Conjugate

Covalent interaction between drug and polymer depending on the functional group present in drug and polymer carrier resulted in the formation of drug–polymer conjugate. N-(2-hydroxypropyl) methacrylamide (HPMA) polymer is predominantly explored for the formation of drug-polymer conjugate because of its stability, nontoxicity, and biocompatibility for in vivo application. I-131 labeled HPMA-doxorubicin conjugate (HPMA-DOX conjugate) had been studied in Phase-1 clinical trial.

### 18.10.2 Polymers, Liposomes, Micelles, and Dendrimers

Nanocarriers made up any of these platforms have been widely explored to deliver the drug to the central nervous system (CNS) through the blood–brain barrier, neoplastic cells, and to remote organs such as lungs due to their biocompatibility, stability, cellular membrane-mimicking properties, and their ability to release the

drug in a sustainable manner. They can be converted into theranostic nanomedicine by dual loading of imaging modalities and therapeutic entities. Examples of imaging modalities include magnetic resonance imaging (MRI) contrast agents, radioactive agents for radionuclide imaging via positron emission tomography (PET) or single-photon emission computed tomography (SPECT), fluorescent agents for fluorescent imaging, and nano/microbubbles for ultrasound imaging. Each imaging modality has its own advantages and disadvantages and therefore their usage depends on their suitability for the maximum desired outcome.

### 18.10.3 Noble Metal Nanoparticles

Gold and silver at their nanodimension acquire optical properties known as surface plasmon response which occurs due to excitation and relaxation from the surface of nanoparticles and the surrounding solution. Optical property can be modulated by tailoring their size, shape, and surface properties. The use of surface plasmon response for cancer detection has limited to superficial sites due to their inability to penetrate deep even in the presence of near-infrared region where the absorbance of tissue is minimum. In contrary, noble metal nanoparticles provide promising outcome X-ray computed tomography (CT) imaging as they are highly dense in comparison to human soft tissues due to the presence of certain vital characteristics such as higher X-ray absorption coefficient, long circulation time in blood, and high surface area for easy attachment of targeting and therapeutic agents. They create high-contrast regions by dampening the amplitude of X-ray leading to much better, noninvasive real-time molecular imaging of solid tumors as compared to iodine, the commonly used CT contrast agent.

Heo et al (2012) synthesized gold nanoparticles (AuNPs) functionalized with PEG, biotin, and rhodamine B-linked beta-cyclodextrin with an objective to function as a theranostic system for the treatment of glioma. Among the two types of nanoformulations developed by them, AuNPs-5 showed a more promising result as it exhibited much better interaction with cancer cells as compared with normal cells. Further, the authors revealed that the developed theranostic system can simultaneously monitor pharmacokinetic profiles of loaded-drug and detection of cancer cells upon induction by laser light [108].

### 18.10.4 Quantum Dots (QDs)

Pioneer work by Brus and his coworkers at the Bell laboratories gave the birth of QDs in the year 1983. QDs are inorganic semiconductor nanocrystals which can serve as a versatile tool for molecular diagnostics and nanotherapeutics. The absorption and emission spectra of QD are predominantly dependent on size and thus optical spectrum can be finely adjusted by tailoring the size of the nanoparticulate core. Among the various types of QDs, cadmium selenide (CdSe)/zinc sulphide (ZnS)-based QDs are most popularly explored for diagnostic purpose and they

contain a core made up of CdSe which is overcoated with layers of ZnS. QDs offer long-term repetitive bright imaging as they are devoid of photobleaching, overcoming the disadvantages associated with the organic chromophore. One serious drawback associated with QDs is the toxicity of cadmium and their inability to penetrate to a deeper part of tissues leading to the detection of cancers at superficial sites such as skin cancer, esophageal cancer, etc.

Yang et al (2017) developed photostable and multifunctional carbon QDs (known as carbon dots) which was tailored with polyamine containing organosilane molecules for simultaneous cell imaging and anticancer drug delivery. The amine groups of polysilane allowed extremely high loading of doxorubicin (DOX), i.e. 62.8%. Further, the surface hydroxyl groups ensured its significantly good dispersibility in water and the fluorescence property enabled to dynamically trace the drug-release characteristics. Results of in vitro investigations revealed carbon dot-doxorubicin complex (CDs –DOX) was effectively internalized by MCF-7 cells and upon internalization, DOX detached from the complex and moved to the nucleus whereas CDs resided in cytoplasm. Findings of the in vivo investigations revealed that CDs-DOX complex showed much improved performance as compared to free DOX. Further, in vivo investigation revealed that CDs–DOX showed negligible systemic toxicity and was able to successfully illuminate fungal, bacterial, and mammalian cells, signifying it to function as a universal cell imaging reagent. Finally, they concluded their investigations might accelerate the development of carbon dots as a novel nanotheranostic for various biomedical applications [109].

### 18.10.5 Carbon nanotubes

Cylindrical shaped carbon nanotubes (CNTs) are considered as allotropes of carbon with hindered biodegradation and poor biocompatibility. They can be branched into different types such as fullerene, CNTs, graphene, and carbon dots. All of these varieties have characteristic electronic and mechanical properties which make them suitable for theranostic applications. Further, both single-walled and multi-walled carbon nanotubes designated as SWCNTs and MWCNTs, respectively, have a high surface area and internal volume which are quite sufficient to simultaneously load the therapeutics and imaging agents.

## 18.11 Nanomedicines in Clinical Trials

Meanwhile, several nanodrugs are available commercially and many more are at clinical trials. Subsequent test-approval by FDA generally leads to clinical trials which are normally done to govern safety and efficacy in humans. These trials can be classified into phase I (dosing, toxicity, and excretion in healthy subjects), phase II (safety and efficacy in subjects with the target illness), and phase III (randomized, placebo-controlled, multicenter trials). When these trials are accomplished, a new drug formulation can be filed with the FDA for approval [1]. The bulk of the

nanoformulations which are in clinical development are generally based on different types of drug distribution methods such as polymeric, micelles, liposomes, dendrimers, and inorganic nanoparticles [110]. Liposomal nanoformulations of doxorubicin currently being studied in clinical trials are HER2-targeted MM-302 (Merrimack Pharmaceuticals, Inc.) and thermosensitive Thermodox (Celsion Corp.). HER2 targeting is expected to improve efficacy compared to non-targeted liposomal doxorubicin; a phase I clinical trial is well tolerated and the phase II trial of this formulation is ongoing in patients with HER2-positive breast cancer [10]. Thermodox comprehends liposome-bound doxorubicin formulated with thermally sensitive lipids. The combination of this nanodrug with radiofrequency thermal ablation shows site-specific targeted combat in phase III trials in the treatment of hepatobiliary tumors [4, 10, 111]. CPX-351 is another liposomal formulation containing dual drugs, cytarabine and daunorubicin, recently passed phase II clinical trial with improved efficacy and reduced side effects to sensitive patients. Pegylated liposomal formulations of irinotecan IHL-305 and MM-398 were also well tolerated in phase I clinical trial with reduced side effects (such as neutropenia and diarrhea) compared to commercially approved formulation FOLFIRI. MM-398 also crossed the hurdles of phase II and phase III clinical trials and is awaiting FDA approval. A phase II clinical study was conducted using liposomal irinotecan sucrosofate for metastatic pancreatic cancer-affected patients whose success led to a global phase III trial (NAPOLI-1) [112]. But this study was also not devoid of common adverse effects of anticancer agents such as diarrhea, nausea, anorexia, vomiting, alopecia, neutropenia, and leucopenia. Hepatocyte-directed vesicular (HDV) insulin is a nanoformulation of liposomal insulin that provides prolonged delivery of the drug directly to the liver. An oral formulation of HDV insulin is also undergoing evaluation in phase II and III clinical trials [10]. Among the liposomal formulations that underwent clinical trials, many are terminated due to low treatment benefits (in spite of reduced side effects too); e.g. L-NDDP, SPI-77, lipoplatin, and Li-PlaCis; or due to success of other formulations, e.g. LEP-ETU and EndoTAG-1 which were left over because of the success of albumin-based and polymeric formulations of paclitaxel (e.g. NK015).

A protein-based nanoparticle RSV-F (Novavax) containing a respiratory syncytial virus (RSV) fusion protein has completed phase II trial and is being used in healthy women of childbearing age. The formulations of Pulmaquin (Aradigm Corp.) in combination with liposomal and aqueous-phase ciprofloxacin have completed company-sponsored phase II studies in cystic fibrosis (CF) or non-CF bronchiectasis patients. An important candidate SGT-53 (SynerGene Therapeutics) containing anti-transferrin antibody fragment for its binding to glycoprotein receptor on cancer cells have completed phase I and II trials to use in glioblastoma, solid tumors, and metastatic pancreatic cancer [1, 10]. Dendrimer-based nanodrug DTXSPL8783 has been investigated in phase I clinical trials among patients with progressive cancer.

Polymeric nanoformulation can potentially improve chemo-radiotherapy treatment through tumor-specific delivery of the drugs, which increases efficacy while decreasing toxicity in normal tissues. Nanoformulations such as SN-38 and

Genexol-PM have been completed both the phase I and phase II trials in treating triple-negative breast cancer and advanced lung cancer, respectively. Genexol-PM also showed fairly low toxicity and good overall response (40–60%). SP1049C is a polymeric formulation of doxorubicin, which completed phase II clinical trial and obtained the title of orphan drug for the treatment of advanced gastric cancer and currently undergoing a phase III clinical trial. Opaxio and CRLX101 are two promising examples. Opaxio, a polyglutamic acid-conjugated paclitaxel formulation is currently under clinical investigation of ovarian and fallopian tube cancer. But while used in NSCLC (non-small cell lung cancer), Opaxio did not improve survival compared to Taxol, and when used in combination with carboplatin, it worsened the situation causing grade III/IV hemotoxicity (neutropenia, leucopenia) and significant neurotoxicity. CRLX101, a drug–conjugate formulation of camptothecin and a cyclodextran-PEG polymer, is being studied in numerous phase I and II clinical trials in the treatment of lung cancers (SCLC and NSCLC), gynecological malignancies, and solid tumors. Clinical studies of CRLX101 in renal cell carcinoma and gastrointestinal cancers have been completed and have shown promising early clinical results [10, 32, 113]. Nanoparticle Lipoxal that contains the drug Oxaliplatin, has been used in Phase II trials for colorectal cancer and glioma [114]. Docetaxel having the nano preparation LE-DT has completed Phase I/II in cure of solid tumors and pancreatic cancers. Drugs like K105 and Paclical are in Phase III trials to cure gastric cancer and ovarian cancer, respectively [10, 114].

Antimicrobial agents have also been profusely used in nanodrugs trials. Polymer nanoparticles with antibacterial properties are also being investigated in the treatment of active infections. Quaternary ammonium polyethylenimine-based polymers are promising as they have a potent activity to disrupt a number of gram-positive and gram-negative bacteria membranes. Such activity make this polymeric nanoparticle particularly promising [10, 115]. Polymeric nano-form of doxycycline have demonstrated a more sustained release and improved efficacy in the treatment of chronic periodontitis. Two polymeric nanoformulations of antiretroviral agents are being investigated for HIV treatment. Efavirenz, a non-nucleoside reverse transcriptase inhibitor, and Lopinavir, a protease inhibitor, are commonly used in combination therapy against HIV. NANOefavirenz and NANOlopinavir are nanoformulations of these antiretroviral agents that have been developed with the aim of reducing total dosage while maintaining clinical efficacy, thereby improving patient tolerability and decreasing treatment costs [10, 116]. One antifungal agent amphotericin B which has been used as nanoformulation (MAT2203) in phase II trials among chronic candidiasis patients. Another antiviral/antibiotic compound, VivaGel (Starpharma), is now being used in patients having bacterial vaginosis (BV) in phase III clinical trials after being effective in phase II.

Inorganic nanoparticles are also an excellent choice for nanoimaging technology and nanomedicine. Superparamagnetic iron oxide nanoparticles (SPIONs) are used as promising hyperthermia agents in the treatment of solid tumors. One such formulation, MFL AS1 (aminosilane coated SPION) has passed phase I clinical trial with no systemic toxicity following intratumoral injection but skin irritation was observed in some patients due to high heat generation in local region (44 °C).

Aurimune (CytImmune) has been developed as recombinant human TNF which is attached to gold NPs using a PEG linker. During its Phase I trials, Aurimune was shown to be well tolerated in patients with advanced cancer. CYT-6091, another from CytImmune, was the first product in a clinical trial using gold nanoparticles for solid tumor patients [61]. AuroLase® is silica-AuNPs decorated with PEG and is approved by the FDA for a pilot test to treat solid tumors [117]. In February 2017, it has been applied for the treatment of patient's tumors of head and neck cancers. AuroLase was explored for the treatment of primary or metastatic lung cancer in another clinical trial (Phase I) [118]. More recently, in patients with recurrent multiform glioblastoma or gliosarcoma, Nu-0129 has been started the clinical trial using spherical nucleic acid [119]. But till date, the FDA has not yet approved any gold-based nanodrugs [1, 120]. Hafnium oxide is another promising nanoparticle suitable for intratumoral injection and a good candidate for radiation-based chemotherapy. NBTXR3 is a hafnium oxide nanoparticle which is undergoing several phase I clinical trials in patients with soft tissue sarcomas and head and neck cancers. A recent "first in human" trial demonstrated a favorable and safety profile when used as a tumor imaging agent, allowing investigation in additional trials with humans in the near future [1, 121].

For the last few years, several nucleic acid nanotherapies are under progress to address nucleic acid targets in the study of organ-specific diseases. These therapies are generally siRNA mediated. Several such formulations are DCR-MYC, ALN-RSV01, TKM-130803, and AGN211745 which were dismissed after phase I and II trials. Reasons of termination are not always related to safety issues (as in case of AGN211745) but some gave fatal output, e.g., TKM-130803 (9 out of 12 died in a study within 14 days). CALAA-01 and PRO-040201 are used for curing solid tumors, but terminated in a clinical trial due to only modest activity in vivo [10].

## 18.12 Regulatory Authorities for Monitoring Nanomedicines and Their Adverse Effects and Safety Concerns

In the U.S., USFDA is the main regulatory authority in the approval of foods, drugs and formulations, cosmetics, medical devices, and veterinary products. National Nanotechnology Initiative was programmed by USFDA in the objective of development and regulation of nanoscale products, development of new and world-class nanotechnology as well as academic and industrial progress of nanotechnology. Office of Science and Health Coordination (OSHC) under FDA regularly coordinates information delivered by major experts of different internal organizations under FDA [122]. Apart from USFDA, the Center for Drug Evaluation and Research, the Center for Biologics Evaluation and Research, and the Center for Devices and Radiological Health are some regulatory bodies who shared responsibilities of risks associated with nanotechnology and involved in assessment and regulation of nanoscale products.

Under the control of EU, REACH (Registration, Evaluation, Authorization, and restriction of Chemicals) is a policy for controlling chemicals and is a hoe for proper

evaluation and regulation of nanomaterials in European territory [123]. REACH provides a set of standard tests, testing procedures, and testing requirements which are practically feasible in assessing the safety of nanoscale materials. EMA (European Medicines Agency) has also taken initiatives for the development of nanotechnology-based medicinal products. In the UK, government organization DEFRA (Department of Environment, Food & Rural Affairs) is working in the issue of nanotechnology-related risks and published its report on the risk of engineered nanomaterials on human health and environment. European Nanosafety Cluster (NSC) is a forum for Framework Projects like FP6 and FP7, which are national projects in EU member states and seeks to maximize the synergies between the existing projects by addressing toxicology, ecotoxicology, and exposure and risk assessment, mechanisms of interaction, and standardization issues. The European Academies Science Advisory Council (EASAC) and the Joint Research Centre of the European Commission (JRC) recently published a report entitled "Impact of Engineered Nanomaterials on Health: Considerations for Benefit–Risk Assessment". The report pointed on the limitation of current knowledge and technologies and provides a guideline for further research; focusing on 'safety-by-design' principle for the successful implementation of the emerging nanotechnologies [124].

Airborne nanoparticles are the main occupational hazards in manufacturing units of engineered nanomaterials. UCLA (University of California, Los Angeles, CA) has developed new testing methods for measurement of airborne nanomaterials in manufacturing units, analysis of the exposure of workers to those materials, and its associated health risks; and also suggested guidelines for the safe manufacturing of engineered nanomaterials. Some nongovernment industries like QuantumSphere are also working on the regulation of occupational hazard in cooperation with government agencies such as NIOSH (National Institute for Occupational Safety and Health) in the USA [122]. The concern of adverse effects of nanomaterials on environment and ecosystem motivated many research organizations to conduct individual research on the toxicity of nanomaterials on the ecosystem. CBEN (Center for Biological and Environmental Nanotechnology), under the regulation of Rice University, is one of the leading organizations currently working on water-based ecosystem. The International Council on Nanotechnology was established in 2004 as an extension of CBEN and involved in the exploration of health and environmental risks of nanotechnology, data management and screening of knowledge gathered from nanotechnology-related publications, and also in increasing public awareness of nanotechnology. Environmental Protection Agency is a government organization in the USA sharing the responsibilities on the assessment of toxic effects on the environment [122]. It possesses the authority to regulate the manufacturing, usage, commercial distribution, and disposal of existing chemical substances as well as new chemical entities. Incorporation of engineered nanomaterials in food, medical, and pharmaceutical industry also comes under the scrutiny of this organization.

## 18.13 Conclusion

Thus, nanomedicines, their potential uses, and even their scientific and commercial aggression before human healthcare system in the near future cannot be ignored. However, their toxicity and safety concerns should not be jeopardized by the enormous possibilities of favorable sea-change in human healthcare.

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