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REVIEW

Nanomedicines: Recent Progress, Impact and Challenges in Applications

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The ultra-fine size of nanoparticles gives them a unique material characteristics, such as remarkably vast surface area and high mobility or diffusibility in free state. They can be classified as hard or soft, depending upon the composition. Nanoparticles have proven to be highly promising in medicine, giving rise to a new field of study, nanomedicine. This rapidly evolving and upcoming branch of medicine is the study of nanomaterials and nanotechnology used for diagnosis, treatment and prevention of diseases. The review presented here summarizes the applications of nanotechnology in the field of medicine *i.e.* nanomedicine, specifically in diagnostics and drug delivery systems. It broadly covers nanotherapeutics with special focus on cancer, nanoformulations for drug delivery in biological systems, nanoimaging for diagnostics of life threatening diseases like cancer, CVD, neurodegenerative diseases and nanotoxicity on human health and concerns for environment safety.

Keywords: Nanomedicine, Nanoparticles, Imaging, Diagnostics, Drug delivery systems, Cancer therapy, Nanoformulations.

INTRODUCTION

The nanoparticles are the substances occurring at a nanometer scale (1-100 nm) at least in one direction and having specific properties like uniformity, conductance and optical nature. They can be of 0D, 1D, 2D or 3D based on their overall shape and structure [1]. Although the field is hardly 5-6 decades old, but it is being realized that nanoscale particles had been used for centuries, *e.g.* the alternate sized silver and gold particles used in stained glass paintings in medieval churches. However, if we look at the history of nanotechnology, the idea was first proposed by American physicist Richard Feyman in 1959, in which he had described a process to manipulate and control molecules. Because of this reason, Feyman is considered as Father of Nanotechnology. More than a decade later, Prof. Norio Taniguchi of Tokyo Science University had given the term "Nanotechnology" to the precision machining of materials in atomic scale. But it was not fully understood until the development of scanning tunneling microscope in early eighties. Then, Alexei Ekimov from Russia in 1981 had first synthesized nanocrystalline, semiconducting quantum dots in a glass matrix and did pioneering studies of their electronic and optical properties [2]. By early 2000, number of nanomaterial based consumer products started coming in market, such as lightweight nanotechnology-enabled automobile bumpers that resist denting and scratching, golf balls that fly straighter, tennis rackets that are stiffer (therefore, the ball rebounds faster), nanosilver antibacterial socks, clear sunscreens, wrinkle- and stain-resistant clothing, deep-penetrating therapeutic cosmetics, scratch-resistant glass coatings, faster recharging batteries for cordless electric tools and improved displays for televisions, cell phones and digital cameras.

The application of nanotechnology has been revolutionary in improving technologies in different industries, *viz.* energy, security, environment, information technology, medicine pharmaceuticals, *etc*. An example of nanomaterial with improved properties is nanoscale films on eyewear lenses and screens of computer and camera to make them scratch resistant, antireflective, self-cleaning and resistant to UV or IR light. Similarly, nanotechnology has been implemented in electronics where transistors for computers are becoming smaller and smaller, from >100 nm to just 1 nm in size! And from quantum dots for high definition display screens or television to rollable, foldable, bendable electronics and countless other similar

products are possible now a days. In a number of environmental and energy efficiency areas, nanotechnology has played significant role. The nanostructured solar cells, quick-charging batteries, nanoporous filters for efficient and early water clean-up or erosion-resistant, antifouling, scratch-resistant, self-cleaning, fire-resistant nanocoating for paints have been successfully developed and revolutionized many such fields. Nanoparticles have been categorized in various ways, *viz.* according to their shape, size, morphology or chemical properties. Table-1 illustrates the various types of nanoparticles with their characteristic properties and major applications.

Nanomedicine: Many novel therapeutic and diagnostic approaches based on nanotechnology have been developed in recent years. Nanomedicine is the branch of nanotechnology, which refers to the field of science studying the application and implementation of nanotechnology for treatment, diagnosis and prevention of diseases. The major applications of nanotechnology in healthcare is being seen in (i) drug delivery and therapeutics, (ii) disease diagnostics and imaging, (iii) studying the molecular mechanisms and disease pathogenesis, (iv) to study *in vivo* efficacy of therapeutic agents and (v) nanoscale technologies to accelerate basic research. Fig. 1 is a schematic representation of the wide application of nanoparticles in diagnostics and drug delivery.

The nanomaterials which have been studied for all such purposes are nanoshells, liposomes, polymeric nanoparticles, micelles, dendrimers, nucleic acid nanoconstructs, viral nanoparticles, magnetic nanoparticles, quantum dots or silicon oxide nanoparticles [25]. Fig. 2 shows the percentage of types of nanodrugs for approved (1a) and investigational (1b) clinical use [26].

Nanotechnology for disease diagnostic and imaging: Magnetic resonance imaging (MRI), computed tomography (CT) and single photon emission tomography (PET) are the routine procedures for diagnostic imaging to evaluate and identify various pathologies inside the body. All such technologies greatly rely on contrasting agents which enhance the sensitivity of employed method. In this field, the nanotechnology

TABLE-1
TYPES OF NANOPARTICLES, THEIR CHARACTERISTICS AND MAJOR APPLICATIONS

Type of nanoparticle (NP)	Shape/structure/characteristics of NP	Applications	Ref.
Fullerenes	Globular or spheroidal with hollow cage made of 60 or more atoms	As filters, gas adsorbents, in energy remediation, support material for immobilizing various organic and inorganic catalysts	[3]
Carbon nanocomposites or carbon nanoparticles	Often spherical with 10-20 µm diameter and high crystalinity.	Catalytic, as electrode for electrochemical sensors, magnetic applications, biomedical applications	[4,5]
Carbon nanotubes	Most studied carbon nanomaterial, have unique atomic structure and extraordinary strength and flexibility	Wide applications in thermal conductivity, energy storage, fibres and fabrics, biomedical applications *etc*.	[4]
Carbon nanofibres	50-200 nm, similar to single walled carbon nanotubes	Polymer composites, electronics and drug delivery	[4]
Metal nanoparticles	Mostly synthesized in living organisms *e.g.* fungi, bacteria or plants	With unique properties like surface plasmon and optical properties	
Gold nanoparticles	Chemical reduction method, Burst Schifrin method (17 Harish)	In electrical wiring, nanotubes, fuel cell, catalyst, sun-screen lotion, therapeutic like anti-infective, anti-angiogenic, tumor diagnosis, rheumatoid arthritis drug, radio therapy *etc*.	[6,7]
Silver nanoparticles	Reduction by citrate anion or reduction by Gallic acid (21 Harish)	Wide application in photography, diagnostics, catalysis, biosensor, antimicrobial *etc*.	[8]
Platinum nanoparticles	Ionic or molecular platinum used as precursor	Electrocatalysts and catalytic converters, magnetic nanopowders, Polymer membranes, cancer therapy, Coatings, plastics, nanofibers and textiles.	[6]
Lead nanoparticles	Lead oxide, lead sulphide NPs	In magnetic resonance imaging, magnetic data storage	[6,9]
Ceramic nanoparticles	Inorganic solid in amorphous, polycrystalline, dense, porous or hollow forms	In photocatalysis, photodegradation of dyes, imaging applications	[10]
Organic or polymeric nanoparticles (PNPs)	Nanosphere or nanocapsular	Used in a variety of applications	[11]
Lipid nanoparticles	Sphere of 10-1000 nm with core made of lipid and matrix containing lipophilic molecules	Wide applications in drug delivery; as drug carrier; for RNA delivery in cancer therapy, as ultrasound contrast agent, anesthetic *etc.*	[12-14]
Protein nanoparticles	Animal origin: Albumin, gelatin, collagen, silk proteins, elastin. Plant origin: zein, gliadins, soy proteins and lectins	As drug delivery system in Cancer, HIV, malaria *etc*. Abraxane for breast cancer, non-small cell lung carcinoma and pancreatic cancer	[15,16]
Glycan based nanoparticles	Poly lactic-*co*-glycolic acid (PLGA)	Targeted drug delivery, for glycosylation of liposomes as lectin targets, vaccine delivery platform	[17,18]
Virus nanocarriers	Virus like Vaccinia virus; Virus like particles (VLPs)	As DNA, siRNA and protein loading particles, in biosensor	[19,20]
Synthetic polymer nanoparticles	Micelles made of Polyethylene glycol and polyaspartate (PEG-PAA)	Versatile drug delivery vehicle of toxic anticancer drugs	[21,22]
Drug conjugates	Covalent conjugation of active moelcules	Antibody-Drug conjugates for lymphoma, breast cancer. For enhanced tumor uptake	[23,24]

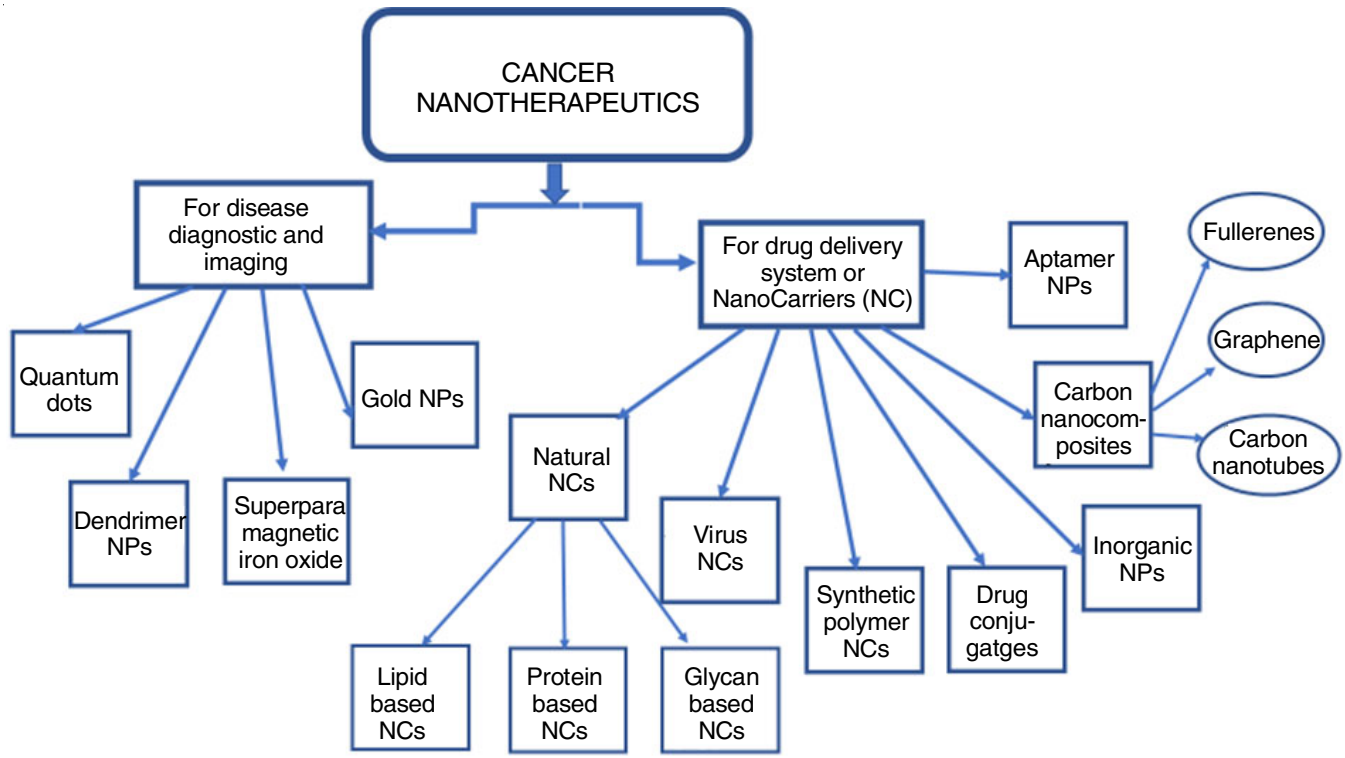
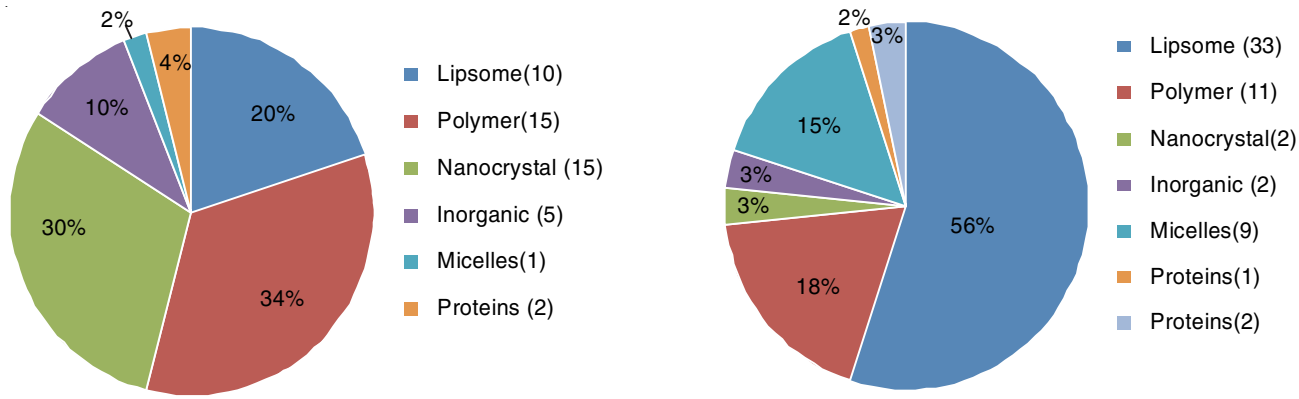


Fig. 1. Application of nanoparticles in diagnostics and drug delivery



(a) Types of nanodrugs being used in therapeutics (b) Types of nanodrugs undergoing clinical trials

Fig. 2. Percent use of nanoparticles in approved and investigational drugs (sourced from [Ref. 26])

is making significant impact. The rapidly expan-ding field of nanotechnology into imaging and detection of injured or diseased cells or tissue have given some very prom-ising examples which have gone beyond proof-of-concept stage. The nanoscale systems usually contain contrasting agents or radiopharmaceuticals for imaging. These nanoscale systems can be utilized for *in vivo* imaging by techniques, such as PET, MRI, fluorescence microscopy, CT and ultrasound (USG). Such techniques have enabled the detection of diseases in a non-invasive manner and has helped in planning of therapies and surgeries [27,28]. The review summarizes below the main classes of nanotechnology-based contrasting agents, being studied in all major imaging techniques.

Quantum dots for imaging: Quantum dots (QDs) are the nanocrystals of hundreds of atoms of crystalline solids. They have minute structures with dense electron cloud, known as quantum confinement, which creates unique optic and electronic properties in them. Quantum dots have a promising future in imaging due to their minute size. The sizes of QDs govern the various optical, electronic and chemical phenomena, making them more versatile. Quantum dots are used as injections which are excited using long wavelengths causing generation of fluorescence. The cost of such technology is lower than MRI but toxicity of these materials have restricted their use for *in vivo* procedures. Voura *et al.* [29] reported the potential of quantum dot nanocrystals in tracking metastatic tumor cells in lung tissue and the spectral images taken with the help of fluorescence emission scanning microscopy has identified five different populations of cells. In more recent developments, an electrode prepared by conjugating zinc sulphide capped cadmium selenite

quantum dots with procalcitonin (PCT) specific monoclonal antibody on indium-tin oxide (ITO) coated glass substrate, was used for quantification of PCT, the protein over expressed in urinary tract infections (UTIs). This quantum dot based immuno-electrode had sensitivity range of 1 ng/mL to 10 µg/mL [30]. Graphene quantum dots have enhanced the signal in time-of-flight secondary ion mass spectrometry (ToF-SIMS) analysis for single cell imaging [31]. The use of N-carbon quantum dots (N-CQDs) to detect pathogenic fungi *Candida albicans* is also reported, in which the synthesized N-CQDs were modified with amphotericin B on their surface. The method reduced the detection time and enabled the process for complex samples like beef sausage [32]. Quantum dots (QDs) have been used with phospholipid micelles and silicon nanospheres to increase solubility and reduce its accumulation in liver and bone [33].

Quantum dots have also been used as labels in immunoassays, immunohistochemical staining and cellular imaging. QDs of different sizes were coated with antibodies of cholera toxin, ricin, shiga-like toxin 1 and *Staphylococcal enterotoxin* B for simultaneous detection of these infections. As the various sizes of QDs give different emission colours, a sensitive multiplex immunoassay is developed with lowest detectable concentrations of 10 ng/mL (cholera toxin), 30 ng/mL (ricin), 300 ng/mL (shiga-like toxin 1) and 3 ng/mL (*Staphylococcal enterotoxin* B) [34]. Similar multiplex diagnostic system for simultaneous detection of hepatitis B virus, hepatitis C virus and HIV, in human serum samples has been developed by using antigen coated QDs in polystyrene beads and microfluidic chip [35]. It has been claimed to have 50 times greater sensitivity and turn-around-time of less than 1 h. QDs have also been studied for genomic analysis andnucleic acid detection. Single nucleotide polymorphisms (SNPs) of cytochrome p450 were detected by using a combination of nanocrystals of QD encoded latex beads having two emission coloursand different intensity levels [36].

Though having great potential for *in vitro* applications, the use of QDs for *in vivo* imaging has been restricted due to its highly toxic nature and also clearancemechanisms from the body is yet not clearly understood [37].

Dendrimer nanoparticles for imaging: Dendrimers are branched and globular macromolecules, containg three critical components, the core, peripheral layer consisting of different functional group and interior coat composed of multiple layers of building blocks. They can be synthesized by several methods using a one-step procedure by condensation and self-condensing vinyl polymerization conditions. Most of these methods did not have structure control, but in one such study the structurally controlled synthesis of these hyper branched polymers was explained [38]. The molecular weight, dispersity, number of branching points, branching density and end functional groups in the synthesized dendrimers were controlled. The properties of these nanoparticles can be modified in different ways, which may include various shapes, sizes, charge, surface properties, *etc*. The dendrimer nanoparticles have been studied in improving MRI contrasting agent. It has significantly reduced retention time and toxicity of large dendrimers or albumin MRI products. The particles will easily be excreted by kidneys as well. The dendritic polymers have been conjugated with MRI contrast agent En-DOTA-Gly4 or Gd-DOTA for *in vivo* MRi optic imaging in preclinical glioma animal model [39].

Recently, self-assembling supramolecular nanostructures have been synthesized from amphiphillic dendrimes. These dendrimer nanoparticles contained multiple positron emission tomography (PET) reporting units at the surface terminals. They self-assembled into uniform nanomicelles and accumulated in tumors for effective PET imaging with enhanced sensitivity and specificity of upto 14-fold increased PET signals than the conventional 2-fluorodeoxyglucose. The system was then studied *via* quantification of nanomicelles uptake in xenograft mouse models for human prostate carcinoma, human glioblastoma, human colorectal adenocarcinoma and human pancreatic adenocarcinoma. The system was found to be superior to conventional method. It was even more effective for detecting those tumors also which were otherwise undetectable. Complete safety level and excellent pharmacokinetics for PET imaging were additive advantages reported for the studied system [40].

(i) Superparamagnetic iron oxide nanoparticles: Superparamagnetic iron oxide nanoparticles can be synthesized from magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3), then encapsulated in polysaccharide or synthetic monomer. The pharmacological properties of these nanoparticles are governed by iron oxide core and hydrophilic coating both. Iron oxide particles can be conjugated with other proteins, enzymes, antibodies, nucleotides or drugs and thus can be directed towards a specific organ or tissue. Such enhancements can also be utilized for drug delivery, radiation therapy, MRI planning, tissue repair, *etc*. [41,42]. The application of ultra small super paramagnetic iron oxide (USPIO) nanoparticles in MRIs as contrasting agent is being done where blood brain barrier breakdown is seen for assessing neuroinflammation and neoplasm. They have also been found to improve visualization in dynamic MRI examination for tumor vasculature, relative cerebral blood volume measurements, tumour-associated inflammation, inflammatory immune mediated disorders, stroke and vascular malformations. A newer USPIO, ferumoxytol (30 nm size with coating of polyglucose sorbitol carboxymethyl ether), is being successfully used in imaging CNS neoplasms, CNS inflammations and cerebral malformations and for studying pathophysiology of multiple sclerosis and epilepsy [43]. Ferumoxytol has also been studied as a contrast agent for USPIO based MRI to quantify arterial wall inflammation, where its uptake is higher in atherosclerotic plaques than non-plaque wall segments [44,45]. USPIOs have also found significant application in molecular imaging for diagnosing *in vivo* cellular and molecular damage. This non-invasive and quantitative technique of molecular imaging have been studied for a number of purposes in medicine, such as early disease detection, accurate prognostic assessment, personalized treatment strategy monitoring efficacy of treatment, *etc*. [46]. The technique has also been used to study cellular interactions *in vivo*, using iron oxides of different particle size and surface properties like neutral or charged.

Another approach to improve imaging for CNS tumors is to enhance contrast, which is done by targeting/activating

inflammatory cells. It is reported that activated microglia are present in higher number within and around malignant brain tumors. The USPIOs are targeted for such cells which are higher along the periphery of lesion and in the surrounding brain tissue. Weinstein *et al.* [46] have demonstrated the significance of technique by imaging ferumoxtran-10 (USPIO of 15-30 nm with dextran as coating agent) from rats brain into cervical lymph nodes. The process can be used to study pathogenesis of multiple sclerosis and Alzheimer's disease. The application of USPIOs in detection of parenchymal ischemic injury has significantly reduced the minimum required as compared to standard MRI methods, which could be done only after 6-12 h after onset of symptoms [47]. The USPIOs have also been utilized for imaging carotid atherosclerosis. It is now understood that composition and stage of atherosclerotic plaque is an important marker for stroke risk assessment. The application of USPIO for enhanced detection of plaque inflammation can prove to be important screening tool for minimizing stroke incidences because majority of embolic infarcts in carotid stenosis may be present without any signs or symptoms. The use of iron oxide nanoparticles in multiple sclerosis and acute disseminated encephalomyelitis (ADEM) MRI imaging has been found to determine the amount and distribution of inflammatory lesions [48]. A nanoparticle made of superparamagnetic iron oxide loaded PEGylated polymer served as successful pigment for fluorescent imaging in glioblastoma and also the conjugated particle was used as paclitaxel drug carrier with minimal systemic toxicity [49]. The multiple uses of USPIOs have in detail been discussed in brain tumors, cerebral ischemia, carotid atherosclerosis, multiple sclerosis, brain injury and epilepsy. In a recent review, the various applications of iron oxide nanoparticles have been discussed, such as diagnostic application in liver and lymph node inflammation, MRI for vascular imaging; therapeutic applications in advanced cancer treatments, macrophage polarization, magnetic fluid hyperthermia and magnetic drug targeting and finally its application in theranostic purpose, where diagnosis and therapy are combined [50].

(ii) Gold nanoparticles in imaging: Gold nanoparticles (GNP) can be conveniently synthesized in organic and aqueous solvents, using reducing agents trisodium citrate or sodium borohydride thereby leading to nucleation of gold ions ($AuCl_4^-$), precisely controlling citrate concentration results in formation of uniform GNPs with sizes in range from few to hundreds of nanometres [51,52]. Both gold nanorods and nanospheres are extensively used to enhance the contrast between cancerous and normal tissues when imaged using X-ray computed tomography (CT). This property of contrast enhancements is due to higher X-ray attenuation of gold as compared to iodine at same molar concentrations [53]. Sun *et al.* [54] demonstrated the outstanding property of heparin-DOPA conjugated GNPs as liver-specific CT imaging agents and molecular imaging probe for examining the therapeutic effect of anticancer drugs against liver cancer by non-invasive ways. The collagen-conjugated gold nanoparticles that effectively target myocardial scar and provide adequate contrast for CT imaging, leading to superior capability to quantify transmural extent of myocardial scar have also been reported [55]. Myocardial scar was created in coronary artery and CT-imaging was done *in vivo*, GNPs coated with collagen-adhesion peptide (CNA35) provided uniform and prolonged opacification of myocardial vascular structure over 6 h. Another study by Meir *et al.* [56] showed real-time non-invasive quantitative and longitudinal tracking of tumor-specific T-cells labelled with 20 nm GNPs *in vitro*, the cells were then injected to melanoma-bearing mice, whole-body *in vivo* CT imaging enabled examination of the biodistribution, migration and prevalence of T-cells in vicinity of tumor. Currently, Hybrid nanoparticles such gadolinium coated GNPs [57], antibiofouling polymer-coated GNPs [58], PEG coated GNPs [59] are being designed as *in vivo* vascular contrast enhancement in CT imaging. They can also be modified in various ways by incorporating other imaging contrast agents such as rare earth metals and dyes [53].

Due to phenomenon of surface plasmon resonance, gold nanoparticles exhibit intense scattering and absorption peak which make them suitable contrast agents for reflectance-based optical imaging technique [60]. GNPs can easily be conjugated to antibodies or peptides through coordinate bonding or electrostatic charge interaction and tend to aggregate in cellular environment to produce even larger optical signal by increasing scattering cross section per particle, which can be exploited to provide good contrast to specific cellular biomarkers in cancer cells with high affinity and accuracy, thus, greatly assisting clinicians in cancer prognosis. Also, non-cytotoxicity, long-term stability, water solubility, inertness and biocompatibility of GNPs make them suitable for *in vivo* imaging applications. Several studies support the successful application of GNPs in optical probing. Rayavarpu *et al.* [61] synthesized gold nanorods with optical extinction bands in regime of 650 nm to 850 nm and bioconjugated these gold nanorods with HER81 antibodies, which in turn binds with high efficiency to cell membrane of SKBR3 breast carcinoma cells *via* HER2 receptors. Thereafter, bioconjugates on HER2 positive cell line was successfully confirmed by confocal microscopy, while Kah *et al.* [62] synthesized and conjugated GNPs to monoclonal anti-epidermal growth factor receptor (EGFR), when imaged under confocal microscopy the reflectance property of nasopharyngeal carcinoma CNE2 cells increased significantly as compared to normal human lung fibroblast (NHLF) confirming high expressions of EGFR in cancer cells. Similarly, Aaron *et al.* [63] synthesized 25 nm GNPs *via* sodium citrate reduction of gold tetrachloride and conjugated to anti-EGFR antibodies, reflectance images of cervical biopsies clearly demonstrated over expression and nanoscale spatial distribution of EGFR in cell membrane of neoplastic cells. Mallidi *et al.* [64] reported the efficacy of multi-wavelength photoacoustic imaging in detecting A431 keratinocyte cancer cells labelled with anti-EGFR GNPs using 3-D tissue model. GNPs have been successfully studied for CNS imaging and drug transport both [65]. Due to low toxicity, they pass and move through brain endothelium rapidly *via* brain parenchyma therefore GNPs can be detected in neurons and neuroglia within minutes of infusion.

Ando *et al.* [66] demonstrated dynamic surface-enhanced Raman spectroscopy imaging using gold nanoparticles inside

living cells that helps in tracking of particle motion and allows to detect intracellular molecules at 50 nm temporal resolution and 65 nm spatial resolution, thus, providing molecular maps for understanding dynamic biological functions such as organelle transport, membrane protein diffusion, lysosomal accumulation, nuclear entry and rearrangement of cellular cytoskeleton. Thus, gold nanoparticles provide versatile tool in imaging as a result of their superier chemical, physical and optical properties as compared to other nanomaterials such as quantom dots. Also, owing to their small size, they can easily be cleared through kidneys and did not accumulate in the liver or spleen confirming their safe use in *in vivo* studies [67].

Nanotechnology for drug delivery systems: For any drug to be successfully launched, it is important that it crosses all stages of drug discovery characteristics *i.e.* ADMET properties. However, most conventional drug forms like suspension or emulsion have limitations of low availability, instability, intolerance and such. Cancer therapy is one such field which has been highly affected by drug delivery limitations. The cancer treatment includes chemotherapy, radiation therapy and surgery, which not only destroy tumor cells but damages normal tissues also. The delivery of drugs due to poor solubility, poor distribution in tissues and combination of multiple drugs with distinct pharmacokinetic and pharmacodynamic properties, drug resistance at cellular or non-cellular levels, distribution and clearance of drugs are some of the limitations encountered in cancer therapeutics. Poor vascularization of tumors, reduced access of drugs to tumors, altered enzyme activity, altered apoptosis are other problem areas due to which tumors show resistance towards therapeutic agents. Lastly, the high toxicity of anticancer drugs on normal cells along with tumors, limits their use for the strong side effects. Thus, drug delivery systems needed such novel carriers which could overcome these problems.

Nanoparticles because of their tiny size, exhibit this unique advantage of enhanced bioavailability and site-specific drug targeting. The targeted approach of many toxic drugs protects other tissues and cells from deleterious side effects. More than thousand nanomedicine formulations for cancer therapy have been registered till date as per clinicaltrials.gov website. Food and drug administration (FDA) considers all products as nanoformulation, which would contain colloidal nanoparticles of size 1-100 nm. Major advantage of such nanoparticle based drugs are increased bioavailability, increased half-life, increased receptor specificity, enhanced target specificity, thereby reducing the drug quantity and reduced drug toxicity by specific delivery of drugs to target site and protection to non-target tissues [68]. They have been studied as drug carriers across blood brain barrier and cell membranes. Nanodrug formulations have been synthesized from liposomes, polymers, micelles, metal nanoparticles, nanocrystals, proteins and carbon nanotubes. Fig. 3 is a representation of various nanoparticle platforms being tried in therapeutics [23].

Kinetics for drug release from nanoparticles: The most important factors for a successful nanodrug development are efficient drug release and polymer degradation. The rate of drug release mainly depends on (i) drug diffusion through nanoparticle, (ii) desorption of drug, (iii) solubility of drug and (iv) degradation of nanoparticle [68]. In a nanodrug, an active agent is commonly encapsulated or conjugated with a nanoparticle, which alters its pharmacokinetic properties. The major strategies employed for nanodrug delivery are:

(A) Passive targeting: In passive targeting, the drugs are deposited in tumor micro-environment determined by specific characteristics inherent to tumor cells and not present in healthy cellular milieu. Passive targeting is based on enhanced permeability and retention (EPR) phenomenon, first described by Matsumura & Maeda [69,70]. The phenomenon depends on nanometer size range of nanoparticles and two important neoplastic characteristics, namely leaky vasculature and impaired lymphatic drainage [71]. First generation nanomedicine has been synthesized by modulating pharmacokinetic and biodistribution properties of the compound. Doxil® a doxorubicin-HCl liposome injection indicated for ovarian cancer, multiple myeloma, Kaposi's sarcoma and Abraxane® a albumin protein bound paclitaxel injectable suspension recommended for metastatic breast cancer and non-small cell lung cancer (NSCLC) are two significant examples of first generation nanodrugs based on passive targeting. An important consideration for the EPR phenomenon for nanodrug uptake in neoplastic tissue to be effective is the protection and escape from reticuloendothelial system and thereby increasing the half-life to be able to concentrate in tumor cells [71]. The EPR is also a macromolecular size dependent phenomenon. The molecules of lower molecular weight (< 40-50 kDa) are quickly eliminated from circulation by renal clearance. These molecules need to be present in circulation for more than 6 h to be able to concentrate in neoplastic tissues by EPR effect [72]. However, passive targeting does not prevent accumulation of nanocarriers in other fenestrated endothelial organs [23]. Though pharmacokinetics facilitates efficient localization of nanoparticles inside tumor interestitium, it has not been able to promote their uptake by cancer cells, which is more efficiently seen in active targeting of nanoparticles.

(B) Active targeting: Active targeting is achieved by attaching various ligands on the surface of nanoparticle-drug conjugate. The ligands attach to specific receptors present on target cells with high specificity and thus increase intracellular drug uptake and accumulation. These second generation nanomedicines have improved functionalities and increased efficacy to overcome the limitations of passive targeting. Various small and macromolecules have been used as ligands, such as folic acid, carbohydrates, proteins, antibodies, *etc*. Most important factor for consideration is that the ligand must be specific to binding with cancer cells with minimum binding with normal healthy cells and must not initiate unwarranted initiation of immune system. The specific ligands should be stable enough to avoid premature degradation or cleavage by reticuloendothelial system. The chemotherapeutic drugs which have been tried by active targeting are doxorubicin and paclitaxel with different ligand targets, *viz.* small peptides, hyaluronic acid, folic acid, antibodies, aptamers, polysaccharides, *etc.* [73]. For example, Yin *et al*. [74] have demonstrated higher cellular uptake, tumor accumulation and inhibition rate of paclitaxel micelle conjugated with hyaluronic acid, targeted towards

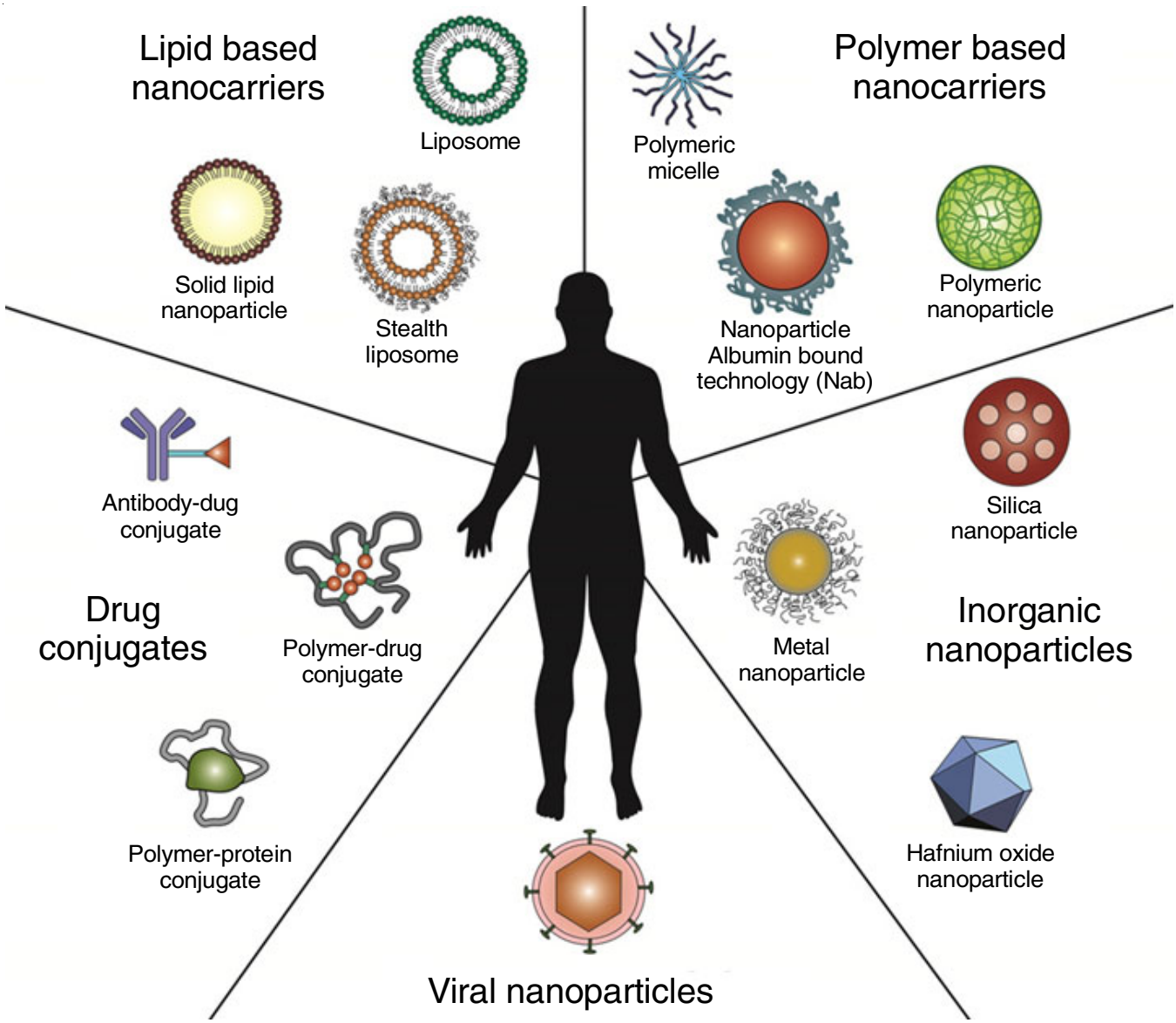


Fig. 3. Various nanoparticles being studied for therapeutics (reproduced with permission from [Ref. 23])

CD44 receptor in human breast adenocarcinoma cell lines *in vitro* and murine hepatic carcinoma cell lines xenograft *in vivo*. The several advantages of active targeting of nanodrugs are enhanced selectivity of cancer cells, enhanced drug accumulation and anticancer activity in tumor cells. However, the major disadvantage of this approach is the limited clinical use because specific receptors are expressed in only certain types of cancer. In some cases, the nanocarriers are not always internalized, but deposition of the small drug molecules in vicinity of target tumors is sufficient, *e.g.* small molecules of doxorubicin can cross cell membrane by passive diffusion. Curative properties of noninternalized antibody-drug conjugates have been successfully studied in experimental animals [75].

External stimuli-trigger release: It is the phenomenon where the release of drugs from nanocarriers takes place in response to physical, chemical or biological triggers generated by the neoplastic tissue. The extracellular and intracellular environments of cancerous and normal cells have many differences in their physico-chemical properties, surface electric charge, elasticity, *etc*., which have been useful in designing many stimulus responsive drug release nanoparticles [76]. These drug delivery systems are being tailored to be responsive towards pH variations, redox potential, enzymatic activation, thermal gradient, magnetic fields, light or even ultrasound as well as a combination of more than such stimulus. The approach has also been tried for sustained release from the carrier, only when the release is needed. Common example based on the concept can be insulin delivery vehicles that release insulin in response to high tissue glucose concentration. Similarly, pH of endosomes or lysosomes is different from that of blood or cytoplasm and this change can be used as an internal stimuli. Biggest advantage of drug release by external stimuli response is that it decreases drug side effect. pH responsive microparticles of phosphatidyl choline were synthesized with the drug ketoprofen. It was shown to release 50% of drug at pH 4.5, whereas 80% was released at pH 7.4 after 12 h [77]. A pH induced liposome

targeted towards tumor extracellular matrix (ECM) has shown rapid drug release in acidic conditions. These ECM targeting liposomes accumulated in tumors and showed efficient anticancer activity *in vivo* with lower hepatic and renal toxicity [78]. Enzyme sensitive release is also one of the many such strategies. Many biochemical molecules and enzymes are exposed differently in normal and cancer cells, like proteases, glucoronidases, lipases, oxidoreductases, *etc.* and this concept has been successfully employed for designing drug delivery system triggered by biocatalytic action [79,80]. Hyaluronidase, an enzyme rich in tumor microenvironment, has been used as a trigger to induce drug release in a magnetic iron oxide silica nanoparticle conjugated with anticancer drug doxorubicin and chlorambucil [81]. Also by decreasing tumor collagen intensity or decreasing internal fluid pressure by nomalizing tumor microvessels, the accumulation and uptake of nanoparticles could be enhanced in tumors [82]. External stimuli like local hyperthermia have been used to release drugs from nanocarriers, which are temperature responsive. Thermodox, a thermosensitive liposomes are used for intravascular release of doxorubicin after mild hyperthermia [83]. Similarly, ultraviolet and infrared light or ultrasound waves are some other external stimuli being tested [84].

Multifunctional nanomedicine: The nanoparticles are also being studied as multifunctional platforms where two or more different structures are combined for therapeutics. Thus, these multifunctional nanocarriers are another promising and latest methodology to combine more than one function in parallel like delivery of multi-targeted drugs or combination therapies, such as doxorubicin and DNA loaded nanoparticles [85]. In another such report, gold nanoparticles were combined with lipid formulations to deliver CRISPR-Cas9 (bacterial antiviral defense system) into tumor cells. The attachment of Tat peptide to gold nanoparticles enabled them to cross cell nucleus membrane. At the site, CRISPR-Cas9 plasmid released RNA targeting Plk-1 gene. The system was activated later by laser irradiation to disassemble lipid-nanogold vehicle to release CRISPR-Cas9 system to knock out the targeted gene leading to apoptosis and tumor growth inhibition *in vitro* as well as *in vivo*. This whole assembly was coated with lipids for better cellular uptake [86]. These gold particles heat upon irradiation, so they can act as thermotherapeutic agents themselves. Nanoparticles coloaded with doxorubicin and bortezomib both have shown better synergistic effect on ovarian cancer [87]. More emerging multifunctional nanocompounds are theronostics, where diagnostics and treatment are carried out with same nanoformulations. Being used as personalized medicine as per individual patient need is another important achievement of theronostics. Though not much clinical trials have been done, significant results are obtained in a number of preclinical studies, such as miRNA theragnostic with wide application in targeted delivery of personalized medicine in multiple myeloma patients [88]. Similarly, Ananta *et al.* [89] have shown concurrent silencing of oncomiRNAs followed by temozolomide treatment for glioblastoma, an aggressive brain malignancy with poor prognosis. They have reported significant reduction of viable cells and many fold increased cell cycle arrest at G2/M phase with the use of these PLGA nanoparticles, tailored for co-delivery of multiple siRNAs in an *in vitro* study. Such studies are also paving way for personalized medicine approach.

Nanoparticles as drug delivery systems: The major types of nanocompounds being used as drug carriers are fullerenes, viral vectors, drug conjugates, lipid based, *etc*. Many of these nanomedicines have cleared phase I clinical trial in solid tumors, whereas some have been approved for clinical care, majority for cancer therapy.The FDA approved nanodrugs available for clinical use are given in Table-2, which is reproduced from the detailed review on nanomedicines in clinics by Ventola [26].

TABLE-2
APPROVED NANODRUGS FROM FDA AND ARE AVAILABLE FOR CLINICAL USE [Ref. 26]

Trade name (Manufacturer)	Generic name	Indication (s)*	Benefit of nanoparticles**
Liposome nanoparticles			
Curosurf (Chiesi USA)	Poractant alfa	Respiratory distress syndrome	Increased delivery with smaller volume, decreased toxicity
Doxil (Janssen)	Doxorubicin HCl liposome injection	Karposi's sarcoma, ovarian cancer, multiple myeloma	Increased delivery to disease site, decreased systemic toxicity of free drug
Abelcet (Sigma-Tau)	Liposomal amphotericin B lipid complex	Fungal infections	Decreased toxicity
AmBIsome (Gilead Sciences)	Liposomal amphotericin B	Fungal/protozoal infections	Decreased nephrotoxicity
DepoDur (Pacira Pharmaceuticals)	Liposomal morphine sulphate	Postoperative analgesia	Extended release
DepoCyt (Sigma-Tau)	Liposomal cytarabine	Lymphomatous meningitis	Increased delivery to tumor site, decreased systemic toxicity
Marqibo (Spectrum Pharmaceuticals)	Liposomal vincristine	ALL	Increased delivery to tumor site, decreased systemic toxicity
Onivyde (Ipsen Biopharmaceuticals)	Liposomal irinotecan	Pancreatic cancer	Increased delivery to tumor site, decreased systemic toxicity
Visudyne (Bausch and Lomb)	Liposomal verteporfin	Wet AMD, ocular histoplasmosis, myopia	Increased delivery to site of diseased vessels, photosensitive release
Vyxeos (Jazz Pharmaceuticals)	Liposomal daunorubicin and cytarabine	AML, AML with myelodysplasia-related changes	Increased efficacy through synergistic delivery of co-encapsulated agents

Polymer nanoparticles			
Adagen (Leadiant Biosciences)	Pegademase bovine	SCID	Longer circulation time, decreased immunogenicity
Adynovate (Shire)	Antihemophilic factor (recombinant), pegylated	Hemophilia	Greater protein stability, longer half-life
Cimzia (UCB)	Certolizumab pegol	Crohn's disease, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis	Longer circulation time, greater stability *in vivo*
Copaxone (Teva)	Glatimer acetate	Multiple sclerosis	Controlled clearance
Eligard (Tolmar)	Leuprolide acetate and polymer	Prostate cancer	Longer circulation time, controlled payload delivery
Krystexxa (Horizon)	Pegloticase	Chronic gout	Greater protein stability
Macugen (Bausch and Lomb)	Pegaptinib	Neovascular AMD	Greater aptamer stability
Mircera (Vifor)	Methoxy polyethylene glycol-epoetin beta	Anemia associated with CKD	Greater aptamer stability
Neulasta (Amgen)	Pegfilgrastim	Chemotherapy-induced neutropenia	Greater protein stability
Oncaspar (Baxalta U.S.)	Pegaspargase	ALL	Greater protein stability
Pegasys (Genentech)	Pegylated IFN alpha-2a	Hepatitis B, hepatitis C	Greater protein stability
PegIntron (Merck)	Pegylated IFN alpha-2b	Hepatitis C	Greater protein stability
Plegridy (Biogen)	Pegylated IFN beta-1a	Multiple sclerosis	Greater protein stability
Rebinyn (Novo Nordisk) (available in 2018)	Coagulation factor IX (recombinant), glycopegylated	Hemophilia B	Longer half-life, greater drug levels between infusions
Renvela (Genzyme); and Renagel (Genzyme)	Sevelamer carbonate; and Sevelamer HCl	CKD	Longer circulation time and therapeutic delivery
Somavert (Pfizer)	Pegvisomant	Acromegaly	Greater protein stability
Zilretta (Flexion Therapeutics)	Triamcinolone acetonide ER injectable suspension	Osteoarthritis knee pain	Extended release
Micelle nanoparticles			
Estrasorb (Novavax)	Micellar estradiol	Vasomotor symptoms in menopause	Controlled delivery
Nanocrystal NPs			
Avinza (Pfizer)	Morphine sulfate	Psychostimulant	Greater drug loading and bioavailability, ER
EquivaBone (Zimmer Biomet)	Hydroxyapatite	Bone substitute	Mimics bone structure
Emend (Merck)	Aprepitant	Antiemetic	Greater absorption and bioavailability
Focalin (Novartis)	Dexamethylphenidate HCl	Psychostimulant	Greater drug loading and bioavailability
Invega Sustenna (Janssen)	Paliperidone palmitate	Schizophrenia, schizoaffective disorder	Slow release of injectable low-solubility drug
Megace ES (Par Pharmaceuticals)	Megestrol acetate	Antianorexic	Lower dosing
NanOss (RTI Surgical)	Hydroxyapatite	Bone substitute	Mimics bone structure
Ostim (Heraeus Kulzer)	Hydroxyapatite	Bone substitute	Mimics bone structure
OsSatura (IsoTis Orthobiologics)	Hydroxyapatite	Bone substitute	Mimics bone structure
Rapamune (Wyeth Pharmaceuticals)	Sirolimus	Immunosuppressant	Greater bioavailability
Ritalin LA (Novartis)	Methylphenidate HCl	Psychostimulant	Greater drug loading and bioavailability
Ryanodex (Eagle Pharmaceuticals)	Dantrolene sodium	Malignant hypothermia	More rapid rate of administration at higher doses
Tricor (AbbVie)	Fenofibrate	Hyperlipidemia	Greater bioavailability simplifies administration
Vitoss (Stryker)	Calcium phosphate	Bone substitute	Mimics bone structure
Zanaflex (Acorda)	Tizanidine HCl	Muscle relaxant	Greater drug loading and bioavailability
Inorganic nanoparticles			
Dexferrum (American Regent)	Iron dextran	Iron deficiency in CKD	Increased dose
Feraheme (AMAG Pharmaceuticals)	Ferumoxytol	Iron deficiency in CKD	Prolonged, steady release with less frequent dosing
Ferrlecit (Sanofi-Aventis)	Sodium ferric gluconate complex in sucrose injection	Iron deficiency in CKD	Increased dose
Infed (Actavis Pharma)	Iron dextran	Iron deficiency in CKD	Increased dose
Venofer (American Regent)	Iron sucrose	Iron deficiency in CKD	Increased dose
Protein NPs			
Abraxane (Celgene)	Albumin-bound paclitaxel	Breast cancer, NSCLC, pancreatic cancer	Greater solubility, increased delivery to tumor
Ontak (Eisai)	Denileukin diftitox	Cutaneous T-cell lymphoma	Targeted T-cell specificity, lysosomal escape

*Refer to complete prescribing information. **Compared with conventional formulations.
ALL = acute lymphoblastic leukemia; AMD = age-related macular degeneration; AML = acute myeloid leukemia; CKD = chronic kidney disease; ER = extended release; HCl = hydrochloride; IFN = interferon; NP = nanoparticle; NSCLC = non–small-cell lung cancer; SCID = severe combined immunodeficiency disease.

Natural compound nanocarriers: There are wide varieties of natural compounds for drug delivery, ranging from lipids, proteins to glycans. Some of them are discussed as:

(I) Lipid based nanocarriers: Lipids have been extensively used as nanocarriers due to their advantage of self-assembly [90]. Liposomes, micelles, nanoemulsions or nanosuspensions are some of the systems used for drug delivery to reduce drug toxicity. Of them, liposomes or phospholipids bilayers and micelles are the most commonly used lipids. They have been found to have much higher drug carrying capacity per molecule as compared to other forms. The lipids systems are well tolerated by body and formulations of water insoluble drugs require such systems for their stability in aqueous media. The protein based formulations are protected against enzyme degradation within body thus reducing required dose and related toxicity. Solid lipid nanoparticles, nanoemulsions and nanocapsules as well as liposomes have shown good results for oral administration of peptides and proteins [13]. These nanocarriers can be used to alter pharmacokinetic properties and biodistribution of drugs by specific targeting, thus better accumulation at target site and lower non-specific distribution to other parts of body. The surface modification of such lipid nanoparticles has been done to minimize rejection and opsonization or removal by phagocytes, thereby improving stealth and immunogenicity of such formulations [91]. Gan *et al.* [92] reviewed the lipid based nanocarriers for non-invasive drug delivery systems, including liposomes, cubosomes, niosomes, emulsions and commercially available lipid-nanodrugs have been described.

Liposomes are one of the most successful nanocarriers for drug delivery for differenct purposes and many FDA approved formulations have come in market. Doxil®, a liposomal doxorubicin is the first US-FDA approved nanocarrier [93]. Nal-IRI, nanoliposomal irinotican is an efficient, high loading nanomedicine with improved biodistribution and pharmacokinetics, with less systemic toxicity [94]. Recently, PEGylated irinotecan with fluorouracil/leucovorin, Onivyde MM-398 (Merrimack) has cleared phase III randomized clinical trial for treatment in advanced pancreatic cancer [95] and is an US-FDA approved nanomedicine for metastatic pancreatic cancer and other solid malignancies. Similarly, Myocet® (non-pegylated liposomal doxorubicin), DaunoXome® (non-pegylated liposomal daunorubicin), DepoCyt® (non-pegylated liposomal cytarabine), Marqibo® (vincristine sulfate liposomes), Mepact® (liposomal mifamurtide) are some of the other FDA approved lipid based nanomedicines for cancer treatment [26]. However most of these approved liposomal systems have similar designs *i.e.* non-targeted and encapsulating a single drug. Combinations of two drugs are also being tried. CPX-351 is a combination of two anticancer drugs (cytarabine and daunorubicin) having passed early clinical trials [96].

Some other FDA approved lipid based nanoformulations are definity, a perflutren lipid microspheres is approved by FDA in 2001 as ultrasound contrast agent. Diprivan, a lipid based nanoparticle anesthetic, AmBisome, a liposomal amphotericin B for *Aspergillus*, *Candida* or *Cryptococcus* secondary infections in immunocompromized hosts, liposomal verteporfin for macular degenaration are some other FDA approved nanomedicines [97]. There is a long list of intravenous nanoparticles for therapy and diagnostics, which are undergoing clinical trials for cancer therapeutics. ThermoDox, thermosensitive liposomal doxorubicin for breast, liver and other refractory solid tumors, Vyexos CPX-351, liposomal formulation of cytarabine for leukemias and lipocurc, liposomal curcumin for solid tumors are some representative examples [97]. Lipid nanocapsules as a *trans*-dermal drug delivery system for ibuprofen has been found to be more efficient than drug solution alone [98]. Immunoliposomes (ILs) are liposomes conjugated with antibody for selective targeting of antigen expressing cells and have been used for improving efficacy, reducing toxicity as well as in immunoassays, immunotherapy and imaging; however very limited clinical trials and no FDA approved drug has been reported yet [86].

The RNA interface (RNAi), like siRNA (small interfering RNA) and miRNA (micro RNA) are being explored extensively for therapeutics of various ailments, like malignancies, autoimmune disorders, neurological diseases [14]; but quick degradation and poor permeability are some limitations of these systems. Recently, liposomes, lipid nanoemulsions and other lipid nanocarriers have shown promising results as RNA delivery vehicle [99]. Different nanoencapsulation agents are being used for safe delivery of bioactive food ingredients and their protection from biodegradation in unfavourable environments, masking of odour or taste or lowering incompatabilities [100]. Similarly, targeted liposomes for siRNA delivery has resulted in enhanced drug uptake and reduced cytotoxicity in number of xenograft studies [101]. Other such examples are DCR-MYC, lipid nanoparticle knocking down a key oncoprotein MYC or Atu027, the liposomal siRNA formulation targeting to knock down PKN3, an important malignant cell growth gene. Apart from cancer therapeutics, some other important examples are ARB-001467, a liposomal siRNA which is designed to knock down three key hepatitis B gene [102]; a siRNA lipid nanoparticle (ND-L02-0201) being developed for treatment of hepatic fibrosis [103]; liposome formulation, CAL02, used as broad antitoxin therapy for bacterial pneumonia [104] and prednisolone liposomal formulation for acute inflammation [97]. Thermosensitive liposomes (TSL) is another example of target specific drug delivery system, wherein target tissue is exposed to localized hyperthermia by an image guided device and TSLs do precise drug delivery. Recent advancement in the field is intravascular trigger release in which the drug is released within seconds when TSLs pass through heated tissue region, thus enabling 20-30 times higher uptake of drugs [105].

(II) Protein and peptide based nanocarriers: A promising and versatile delivery vehicle is cell penetrating peptides, which is a non-viral transmembrane vector for transport of short regulatory oligonucleotides [15]. Protein based nanocarriers have attracted much attention due to their many advantages like low cytotoxicity, high drug binding capacity, increased uptake into targeted cells, having high nutritional value as well as being GRAS (generally regarded as safe). Also the ease to prepare them from many renewable sources and scale up in manufacture make them a promising candidate for drug and

gene delivery. Various functional groups available in a polypeptide give the flexibility for different three dimensional networks to be synthesized for providing protective matrix to molecules and increasing specific targets at the site of action. Thus, self-assembling peptide based nanogels are being used as drug delivery carrier in cancer and other therapeutic methods [106]. Protein based nanoparticles were initially based on blood serum proteins for better transport and dissolution of drugs in circulation. But until now proteins from animal as well as plant origins have been tried as nanocarriers, like gelatin, collagen, albumin, silk proteins and elastin of animal origin and zein, gliadins, soy proteins and lectins of plant derived proteins [107]. But albumin based nanoparticles are the most prominent in this category, because being non-toxic, non-immunogenic and biocompatible they give improved pharmacokinetics of drugs and increased passive accumulation in solid tumors [26]. It also has high binding capacity due to higher percentage of charged amino acids and thus presents many binding sites for drugs. Paclitaxel-albumin nanoparticle, Abraxane® is the only FDA approved chemotherapeutic nanodrug for breast cancer, non-small cell lung carcinoma and pancreatic cancer. Abraxane® is a NAB-paclitaxel with improved drug solubility but without much difference in overall survival. However, albumin bound rapamycin (ABI-009) for bladder cancer and pulmonary arterial hypertension and albumin-bound thiocolchicine analog (ABI-011) for solid tumors are two more protein based cancer nanodrugs undergoing clinical trials [97]. Another protein based nanodrug with respiratory syncytial virus (RSV) protein RSV-F (Novavax), developed to treat RSV in infants is undergoing phase II clinical trial in healthy women of child bearing age for this nanodrug's safety and immunogenicity of different formulations [26].

Though animal protein, gelatin, has been reported as nanocarriers of drugs for HIV, malaria, analgesics, *etc*. it has only been tried as paclitaxel loaded nanoparticle in cancer for longer retention and higher accumulation in tissues [108]. Nanomicelles of β-caesin containing chemotherapeutic drugs, mitoxantrone, vinblastine, docetaxel and irinotecan have been synthesized but not reached clinical trials yet. Silk sericin protein is used in formation of self-assembled micellar nanoparticle carrying hydrophilic FITC-inulin and hydrophobic paclitaxel drugs with promising results in cytotoxicity assay *in vitro* using breast cancer MCF-7 cells in comparison to free paclitaxel [109]. The hydrophobic plant proteins zein and gliadin have been found to have some advantage over hydrophilic animal proteins, by providing sustained drug release and being more cost effective. They also reduce risk of infections like mad cow disease (spongiform encephalitis) [110]. However, only paclitaxel loaded poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles against colon cancer cells have found some success in this regard. Lectins, a diverse class of carbohydrate binding proteins have been extensively studied for glycotargeting of cancer drugs and have shown significant autophagy in cancer cells [107]. Despite being synthesized in various combinations, protein nanoparticles have not given much satisfactory results due to their heterogeneity, batch wise variations in synthesis and also rapid solubilization due to their hydrophilic nature.

(III) Glycan based nanocarriers: Poly lactic-*co*-glycolic acid (PLGA) is one of the most studied biodegradable polymers of lactic acid and glycolic acid, which has got USFDA and European Medicine Agency approval for parenteral drug delivery system. Rationale behind using carbohydrates is that lectins are good and specific carbohydrate targets present on cell surface and thus targeting C-type lectin receptors (myeloid) present on antigen presenting cells, has been shown to be a useful strategy. Therefore, many studies have been reported glycosylation on liposome nanoparticles for cell specific targeting and better immune response [111]. Like, drug delivery to hepatocytes can be done by specifically targeting cell surface lectins, asialoglycoprotein receptors. The mannose and *N*-acetylglucosamine conjugated nanoparticles are few such tried examples [17]. Glycan based polymers have provided numerous benefits to drug delivery nanopolymers, like preferential targeting, extended residence time and enhanced recognition by cancer cells. Many pH and temperature sensitive glycan nanoparticles and enzyme cleavable polysaccharides for target delivery have been successfully synthesized and undergoing trials at various levels [112]. Modification with mannose on the surface of hydroxyethyl starch PEG nanocarriers have given high and specific affinity for dendritic cells with low protein adsorption from blood [113].

PLGA polymers as vaccine delivery platforms has added advantages of enhanced efficiency, efficient targeting of dendritic cells, better depot effect and enhanced immune response [18]. Number of *in vivo* and *in vitro* studies has been done to evaluate PLGA based anticancer nanodrugs [114]. PEGylated PLGA nanoparticles containing doxorubicin were found to have enhanced antitumoral efficacy than free drug. However, no approved glycan based nanodrug has yet come in market. Cyclodextrin nanoparticle based camptothecin drug for advanced solid tumors have cleared phase II clinical trials [115]. Also cyclodextrin nanocarriers with methotrexate for melanoma, lonidamine for prostate cancer, exemestone for breast cancer and vorinostat for lymphoma are some other examples [116]. The advantages of cyclodextrin encapsulated nanomedicines are increased drug loading capacity, enhanced drug circulation, reduced toxicity and targeted sustained release. The ADME properties of cyclodextrin nanodrugs have been described and reviewed. Polymer particle of cyclodextrin and PEG (CALAA-01) for siRNA delivery is reported to silence the expression of ribonucleotase reductase [117].

(IV) Virus nanocarriers: Viruses have emerged as an important nanocarrier platform due to their various advantages like uniform morphology, biocompatibility and ability to self-assemble to package viral nucleic acid. This has given advantage of tailoring viruses at genetic level to be used as reagents, catalysts, *etc*. Similarly, virus like particles (VLPs) has also been used as nanovectors and nanocarriers. DNA-loaded VLP for gene therapy, siRNA loaded VLP and protein conjugated VLP are some other examples of the vast applicability of these multiprotein structures lacking viral genome [19]. Plant viruses, bacteriophages and VLPs have also been utilized as nanoscaffolds for enzyme nanocarriers and enzyme kinetic studies [118]. Pox viruses, like Vaccinia virus replicate specifically in

cancer cells, which has been utilized in JX-594 pox virus to destroy tumor cells by activating EGFR-Ras-MAPK signaling [119]. In the phase III clinical trial (OPTiM) for stage III and IV melanoma patients, intralesional administration of oncolytic virus talimogene laherparepvec (T-Vec) has improved response and survival as compared to granulocyte-macrophage colony stimulating factor (GM-CSF) [120].

The major drawback of these nanomedicines is their biosafety and cytocompatibility concerns and that is the reason none of the several oncolytic viruses have been approved for cancer therapy, despite clearing clinical trials. Bäcker *et al.* [121] described a newer approach to electrochemical biosensor combining tobacco mosaic virus (TMV) nanotubes and coat protein as enzyme nanocarriers.

(V) Synthetic polymer nanocarriers: Synthetic polymer nanoparticles can be modified in various ways like molecular weight, biodegradability or hydrophobicity; thus providing an advantageous drug encapsulating molecule. The customization can make manipulation of these nanoparticles easier, facilitating and controlling drug release rate and amount [21]. Therefore, these chemically versatile synthetic polymers are quite promising nanocarrier tool in cancer therapeutics. Many synthetic polymers are undergoing clinical trials, some such examples are Docetaxel-PNP for solid tumors, Lipotecan for liver and renal cancer, nanotax and nanoxel polymer nanoparticles of paclitaxel for peritoneal neoplasma and advanced breast cancer respectively [122,123]. Micelle formulations of highly toxic anticancer drugs, like paclitaxel and docetaxel are undergoing clinical trials. Polyethylene glycol and polyaspartate (PEG-PAA) polymeric micelle, NK-105 is a synthetic nanocarriers for paclitaxel that has cleared phase II/III clinical trials for gastric and breast cancer [124]. Similarly, SP1049C, a doxorubicin polymeric micelle for advanced adenocarcinoma and NC-6004, a PEG-PAA polymer micelle (nanoplantin)for pancreatic cancer with less neuro and ototoxicity are undergoing clinical trial phase III. Docetaxel encapsulated PLGA-PEG nanoparticles, BIND-014, is the first targeted polymer nanoparticle undergoing clinical trials [122]. Cyclodextrin based nanoparticles (CRLX101) are undergoing clinical trials for highly toxic drug camptothecin delivery. The system has shown tumor reduction in 74% of patients [97]. A reduction responsive drug delivery nanocarriers is synthesized from a linear polyester with disulphide bonds, conjugated with PEG. The nanoparticle has shown faster payload release in response to intracellular reducing environment and thus generating superior anticancer activity towards PC-3 cells [125].

(VI) Drug conjugates: Drug conjugates form the most wide and successful nanomedicine group in cancer therapeutics. In contrast to any natural or synthetic nanocarriers where the drugs are usually encapsulated, here the active molecules are conjugated covalently to a target antibody or peptide; so the resultant conjugate formulation is a monomer or oligomer and thus have minimum effect on drug solubility or biodegradability [23]. The three approved antibody drug conjugate (ADC) in the market today are Adcetris®, Kadcyla® and Zevalin®. The drug conjugate Adcetris® (Seattle Genetics) contains brentuximab vedotintargeting CD30 in non-Hodgkin lymphoma (NHL) and was approved in 2011 [126]. Vedotin is much less toxic and more effective when combined with anti-CD30 antibody (brentuximab), which redirects the drug selectively to CD30 expressing cancer cells. Kadcyla® by Roche/ImmunoGen contains trastuzumab emtansine targeting HER-2 in breat cancer and got approval in 2013. Similarly, Zevalin® is also approved for NHL in 2002. Drug conjugates with polymer are also a vast group of nanodrugs and at least twenty different anticancer conjugates undergoing or completing different phases of clinical trials [23]. Also combination therapy, combining a synergistic ratio of two anticancer drugs, cytarabine and daunorubicin has shown advantage over single use [127]. However, two separate drugs may exhibit distinct pharmacokinetic profiles, thus combination therapies can be challenging in delivering exact molar ratio of two drugs.

N-(2-Hydroxypropyl)methacrylamide (HPMA) based copolymer is a promising carrier with enhanced tumor uptake and antitumor activity [24]. PK-1 is a HPMA polymer-doxorubicin conjugate, which have significantly low cardiotoxicity and aloepecia than free doxorubicin [128]; whereas PK-2 is a modified PK-1 with galactosamine residues making it the first drug conjugate for active targeting in hepatocellular carcinoma. Many ADC with better properties are expected to clear clinical trials and get approval in future.

(VII) Inorganic nanoparticles: Inorganic molecules have found wide applications in biomedical field because of their small size and unique shape. Nanoparticles using inorganic substances are being used in imaging, radiotherapy and drug delivery in cancers. Gold, silver and platinum metal nanoparticles are showing good scope as drug delivery system for cancer therapeutics [129]. To increase tumor targeting and enhanced permeability and retention (EPR) effect for breast cancer treatment, a precise gold nanoparticle system is developed by coadministering iRGD peptide and legumain. The modified gold nanoparticles showed higher penetration and accumulation in breast tumor tissues *in vivo* [130]. AuroLase is a gold nanoparticle undergoing clinical trial for site-selective phototherapy of primary or metastatic lung tumors [97]. Aurimune is another nanoformulation conjugating AuNPs with PEG for delivery of highly toxic tumor necrosis factor alpha (TNF-α). The drug has passed phase I trial with good results in patients with advanced cancer [131].

Silica nanoparticles are also widely studied in drug delivery, imaging applications and theronostic capabilities, because of their biocompatibility, ease of modification and degradation, flexible morphology, *etc*. Different types of silica nanoparticles are solid spheres, mesoporous hollow spheres, foam like nanoparticles, nanotubes, mesoporous red blood cell shaped nanoparticles, *etc*. [132]. They can be conjugated with other organic or inorganic components. Such integrated systems have shown wide applications like target drug delivery, radiation therapy, MRI, optical imaging, hyperthermia therapy, photodynamic therapy, immune therapy and in theronostics. However, they are yet to make a significant impact yet. Cornell-dots are the only silica based conjugated nanoparticles for melanoma and malignant brain tumor PET and optical dual-modality imaging, which is close to getting FDA approval [26].

The most studied metal nanoparticles are iron oxide nanoparticles, mainly for diagnostic purposes. Iron oxide and magnetite have been used since long as nontargeted contrast agents for MRI. A multifunctional silica nanosphere containing superparamagnetic iron oxide nanocrystals and anticancer drugs have been synthesized for simultaneous functions of enhanced drug delivery, fluorescent imaging and target delivery [133]. NanoTherm® (Magforce®) are superparamagnetic iron oxide nanoparticles in late clinical trial stages, to be used as hyperthermia agent in combination with radiotherapy for glioblastoma patients. NanoTherm® injected into tumor and heated up by magnetic field thereby either destroying the tumor cells or sensitizes them for radio or chemotherapy [134]. The nanodrug is being investigated for brain, pancreatic, prostate and esophageal cancers also and has got marketing approval in many European countries. Many different iron oxide nanoparticles were approved by European Union, but only three iron oxide nanoparticles, Feraheme®, Feridex® and GastroMARK™ have been approved by FDA; of which later two of them were withdrawn also. However, feraheme is still available for treatment of anemia associated with CKD and also being studied in clinical trials as imaging agent. Magnablate is also an iron based nanoparticle drug for thermal ablation in prostate cancer, which has entered clinical trial stage. Synthesized from hafnium metal oxide, NBTXR3 (developed by Nanobiotex) is a novel metallic nanoparticle which increases radiotherapy efficacy. It had reached phase II/III clinical trials for soft tissue sarcoma by 2016 [135].

(VIII) Carbon nanocomposites (fullerenes, graphene and carbon nanotubes): Many biomedical uses of fullerenes are described in a review by Bakry *et al*. [136]. Some examples are their antiviral and anti-HIV properties of anionic or cationic fullerenes [137] by inhibiting HIV protease, as biological antioxidants, where they have been studied for the protective effects against apoptosis [138], as cytoprotective agent against UVA radiation [139]. Fullerenes have shown great potential as antimicrobial agent as well, due to their ability to induce reactive oxygen species after photoexcitation [140]. Apart from these, fullerenes have also been extensively studied as a carrier for targeted delivery and controlled release of carried drug or gene. They belong to inorganic nanoparticle category due to their size of < 1 nm. Their core is essentially hydrophobic to which many functional groups can be added. The hydrophilic moieties are also added to make them polar and soluble in aqueous medium to which drugs or genes are carried across cell membranes. While carrying DNA, fullerenes form a protective layer around DNA, due to which they increase DNA lifetime in endosomes and finally incorporate with chromosomes [141]. In another study, a lipophilic slow release drug delivery system of C_{60}-paclitaxel conjugate had shown significant anticancer activity in cell culture. The toxicity studies in the cell cultures and *in vivo* studies have indicated low toxicity of the water soluble fullerenes [136].

Carbon nanotubes (CNT) are cylindrical structures which can be single walled or multi-walled, made up of carbon atoms in graphene sheets. These cylinders can be of a diameter of 0.7-1.5 nm for single walled and 2-50 nm for multi walled CNTs. They have a variety of intracellular effects like reactive oxygen species (ROS) generation, lipid peroxidation, oxidative stress, mitochondrial dysfunction and cell morphology changes [140]. Platelet aggregation was induced by CNTs, but not by C_{60}-fullerenes. Similarly, graphene are being studied as photothermal agents to kill bacteria and thus replace antibiotics. Several reports are available describing their role as drug delivery agent in different settings. In a recent study, graphene nanosheets were used as pH sensitive drug carrier for doxorubicin. The graphene sheets were exfoliated by poly(vinyl pyrrolidone) and their anticancer effects was also evaluated along with drug carrier capacity. It is reported that the cytotoxic impact is due to modulation of mitochondrial activity, elicitation of oxidative stress due to generation of ROS and initiation of caspase-3 activity, proliferation capability and formation of tight junctions in cancer cells. The study has been done *in vitro* in a number of cancer cell lines to elucidate the effects [142]. CNTs have also been used by modifying them in various ways by addition of different simple or complex functional groups, which may alter CNT behaviour *in vivo* and *in vitro*, like addition of polar groups will make CNT soluble whereas that of non-polar will make them insoluble. Variations in total charge, catalyst residue and length of nanotube are some important factors governing behaviour and use of CNTs in drug delivery systems.

Another way in which carbon nanoparticles can be used is by combing them with magnetic particles. Conjugates of ferromagnetic materials with activated carbon have been used in drug delivery systems and for magnetic separation of biomolecules. The ferromagnetic part has the magnetic property with carbon being utilized as drug adsorption matrix. But the process has not got much application due to its weak binding and its easy separation with each other while in circulation. The first report of magnetic particles for drug delivery is of colloidal magnetite coated with cross linked albumin, which have been used for encapsulating doxorubicin and used in sarcoma tumors implanted in rat tails [143]. In another report, particles with size between 0.5 and 5.0 nm were used to absorb and desorb doxorubicin, a potent chemotherapeutic with a narrow therapeutic index. Chemical analysis showed the particles to be composed primarily of carbon, iron and oxygen, with traces of phosphorous, hydrogen, nitrogen and sulfur and tracesof metals [4].

Thus all the carbon naocomposites, including fullerenes, carbon nanotubes and grapheme have been studied as carrier for a number of chemotherapeutic drugs, such as paclitaxel (taxol), docetaxel (DTX), doxorubicin, *etc*. [144]. In one such study, the conjugate of DTX and fullerene C_{60} (C_{60}/DTX) showed four times more bioavailability of DTX and 50% less clearance of the drug [145]. Thus the study, design and manipulation of carbon nanomaterials has found varied application in biomedical field, including drug delivery, imaging, detecting and treating different cancers. However, the toxicity, solubility and specificity are some of the characteristics which have not given very satisfactory results for these carbon nanostructures to be taken as a desirable material for an approved nanodrug.

(IX) Aptamer nanoparticles: Aptamers are short, single strands of RNA or DNA fragments that bind specifically to a target site like protein, carbohydrate, toxin, small molecules or living cells. Being extremely versatile, aptamers can be targeted towards different molecules with high selectivity and specificity. They are considered better than antibodies due to their high stability in extreme conditions, small size, easy modification, high flexibility and low immunogenicity [146]. Because of their specific properties, aptamers are studied for synthesizing aptamer nanomaterial complexes for application in diagnostics and therapeutics. The first FDA approved aptamer based drug was Mucugen, which is a vascular endothelial growth factor specific aptamer for treatment of age-related macular degeneration [147]. In another report, NOX-A12 Spiegelmer, an L-enatiomer RNA oligonucleotide, combined with tyrosine kinase inhibitor reduced leukemia burden in experimental animals as well as *in vitro* [148]. Few other such examples are ARC1779 aptamer conjugated to 20kDa PEG for purpura [149] and NOX-A12, combined with drugs bendamustine and rituximab for chronic lymphocytic leukemia and NOX-A12 in combination with bortezomib and dexamethasone in relapsed multiple myeloid leukemia [150].

Aptamers have also been studied for specific and selective drug carrier vehicles for targeted delivery. A drug-aptamer conjugate of doxorubicin with A10 aptamer recognized prostat specific membrane antigen (PMSA), an over-expressed protein in prostate cancer. The nanoconjugate was found to be highly useful because it could selectively deliver drug to both PMSA (+) and (-) prostate cancer cells [151]. Numerous aptamer based nanomaterials are reported for diagnosis and as biosensors. An aptamer based quantum dot modified probe for cocaine detection in samples or aptamer modified gold nanoparticles (AuNPs) for a sensitive and rapid detection of cancer cells are few such examples. Such aptamer-AuNP conjugates have been demonstrated for specific identification and diagnosis of other molecules or disease. The aptamer photosensitizer gold nanorods or multiple aptamer-modified gold nanostars have been developed for selective and effective drug delivery vehicle [147]. A theronostic complex of doxorubicin drug intercalated with aptamer and quantum dot complex has been developed successfully for simultaneous detection of target cells and specific drug delivery both [152]. The conjugation of aptamers on liposome surface has also given specific target directed drug delivery. A dextran-encapsulated PEG-liposome immobilized with sgs8 aptamer is one such example for selective killing of CEM-CCRF target cells. In the same way, aptamer-modified PEG-polylactic acid micelles are designed for endothelial cells in brain [153] as well as for increased efficiency of fluribiprofen, an Alzheimer's disease drug bound with aptamer nanomicelle. The superparamagnetic iron oxide nanoparticle (SPIONs) has found widespread application in imaging and MRI as described above. But these SPIONs may also be utilized in therapy by drug-aptamer immobilization on their surface. The SPIONs conjugated with doxorubicin and PSMA (+) and (-) aptamers have shown effective and selective release of drug to prostate cancer cells. Thus, the aptamer-nanomaterial conjugates have given promising results for target specific drug delivery, sensitive imaging and biosensor systems. However, short half-life and susceptibility to serum degradation are some of the limitations for their widespread usage in nanomedicine.

Challenges with nanomedicine: The adverse and toxic effects of nanoparticles have been studied, documented and reported. However, it is difficult to make a common or generalized listing of toxicological effects for all types of nanoparticles, due to diverse variety of nanomaterials. Nanoparticles are exposed to different body systems like blood, cytoplasm, extracellular matrix, cellular organelles, *etc*. where they may cause free radical generation and reported that oxidative stress due to which is responsible for inflammation of liver, lung or brain. The toxicity by nanoparticles at molecular, cellular and tissue level is very well documented [154]. Certain specific effects like mitochondrial damage, platelet aggregation, uptake through olfactory epithelium ad cardiovascular effects are seen only with nanoparticles and not with larger particles [140]. As the properties of nanoparticles change significantly from micron sized particles, thus a good understanding of their physico-chemical properties with regard to blood brain barrier (BBB) passage, distribution through systems and blood coagulation pathways is to important. Like the uptake of nanoparticles in brain take place by two specific mechanisms (i) uptake through blood brain barrier and (ii) *trans*-synaptic transport *via* olfactory epithelium inhalation. Though healthy BBB has defense system inhibiting the uptake of blood borne nanoparticles; however studies have shown toxic effects of cationic nanoparticles and high concentration of anionic nanoparticles [140]. Lung inflammation by carbon nanotubes is reported by Wolfram *et al.* [154]. Hepatotoxicity following intravenous administration of positively charged nanoparticles has been found. However, toxicity for certain nanoparticles is more serious than others, *e.g.* silver and iron oxide nanoparticles. It is also observed that gold nanoparticles of 1.4 nm diameter are toxic, whereas that of 15 nm were not. But, gold nanoparticles are usually toxic when used in high concentration and for a long period because of slow clearance and accumulation in blood and tissues [26].

Thus, it is important to understand the physico-chemical properties of nanoparticles for their safety and toxicological issues, *e.g.* the toxic effects of nanoparticles can be reduced by surface modifications or these properties can be harnessed to ablate diseased tissue in cancer therapy. So despite gaining lot of attention as potential drug carrier in therapeutics and in medical imaging, nanomedicines have many limitations and concerns. This can be understood by the example of withdrawal of feruglose and resovist nanodrugs, due to safety concerns, despite getting FDA approval [26]. Due to these reasons, the latest strategy being implemented is mandatory phase IV post marketing study after FDA approval to analyze and assess any potential risk associated with nanodrugs [135]. Another important aspect is analysis of pharmacoeconomic aspect to determine economic and social aspects related to nanoformulations as compared to standard treatment. In general, it has been beneficial to make nanoformulation of already approved conventional drug than using any new chemical entity. Thus, the sales of abraxane, first generation nanomedicine for various oncology is estimated to be more than US $ 900 million, making it still

one of the best-selling nanodrugs in the world [155], however the nanomedicine field is expected to grow to approximately US $200 billion by 2020 [26].

Conclusion

Nanomedicine has found wide applications in all fields of medicines, with most potential application of nanoparticles in cancer (Fig. 4), atherosclerosis, neurodegenerative diseases, *etc*. It has improved the field of imaging and detection as well as therapeutics of various cancers and also other diseases. Lot of understanding in response mechanisms of these nanoparticles towards pH, light, magnetic field, thermal gradients, ultrasound and enzymatic response, have helped nano scientists in developing 'smart' and site-specific drug delivery systems for stimulus responsive controlled release [76]. However, the ratio of approved nanomedicines with those undergoing clinical trials is very low. Less than 1% of nanoparticle encapsulated drugs actually reach target due to their filtration from blood by liver and spleen. Variety of nanoparticles has been studied as probes for detection and the technology is expected to reduce cost and time in disease diagnostics and therapy. A specific example is the incorporation of nanoparticle mimicking high density lipoprotein (HDL) and thus effectively shrinking plaque [156]. Nanomaterials have been studied to replace conventional materials for tissue and bone regeneration as well as in dental resins. Recently, graphene nanoribbons giving very promising results in regeneration and repair of spinal cord injuries, have caught scientists attention [157]. Nanotechnology in improving the field of vaccines is another frontier of this technology where non-injectable vaccines and universal vaccine for all influenza virus strains, are few such examples. So days are not far when will hear about human organs grown with the help of nanomaterials for organ transplantation.

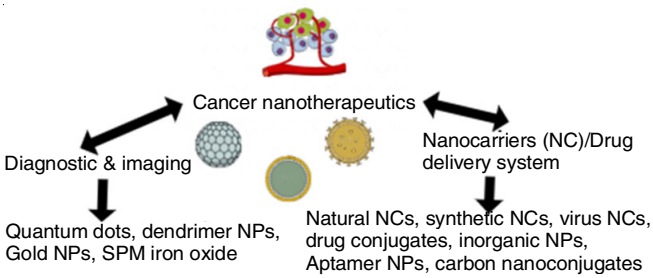


Fig. 4. Application of nanoparticles in cancer

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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