Nanotechnology in the Life Sciences

Muthupandian Saravanan
Hamed Barabadi *Editors*

Cancer Nanotheranostics

Volume 2

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Nanotechnology in the Life Sciences

Series Editor

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Nano and biotechnology are two of the 21st century's most promising technologies. Nanotechnology is demarcated as the design, development, and application of materials and devices whose least functional make up is on a nanometer scale (1 to 100 nm). Meanwhile, biotechnology deals with metabolic and other physiological developments of biological subjects including microorganisms. These microbial processes have opened up new opportunities to explore novel applications, for example, the biosynthesis of metal nanomaterials, with the implication that these two technologies (i.e., thus nanobiotechnology) can play a vital role in developing and executing many valuable tools in the study of life. Nanotechnology is very diverse, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale, to investigating whether we can directly control matters on/in the atomic scale level. This idea entails its application to diverse fields of science such as plant biology, organic chemistry, agriculture, the food industry, and more.

Nanobiotechnology offers a wide range of uses in medicine, agriculture, and the environment. Many diseases that do not have cures today may be cured by nanotechnology in the future. Use of nanotechnology in medical therapeutics needs adequate evaluation of its risk and safety factors. Scientists who are against the use of nanotechnology also agree that advancement in nanotechnology should continue because this field promises great benefits, but testing should be carried out to ensure its safety in people. It is possible that nanomedicine in the future will play a crucial role in the treatment of human and plant diseases, and also in the enhancement of normal human physiology and plant systems, respectively. If everything proceeds as expected, nanobiotechnology will, one day, become an inevitable part of our everyday life and will help save many lives.

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Preface

Nanotechnology is an interdisciplinary research field that integrates chemistry, engineering, biology, and medicine. Nanomaterials offer tremendous opportunities as well as challenges for researchers. Of course, cancer is one of the world's most common health problems, responsible for many deaths. Exploring efficient anticancer drugs could revolutionize treatment options and help manage cancer mortality. Nanomedicine plays a significant role in developing alternative and more effective treatment strategies for cancer theranostics. This book mainly focuses on the emerging trends using nanomaterials and nanocomposites as alternative anticancer materials. The book is divided into three main topic areas: how to overcome existing traditional approaches to combat cancer, applying multiple mechanisms to target the cancer cells, and how nanomaterials can be used as effective carriers. The contents highlight recent advances in interdisciplinary research on processing, morphology, structure, and properties of nanostructured materials and their applications to combat cancer.

Cancer Nanotheranostics is comprehensive in that it discusses all aspects of cancer nanotechnology. Because of the vast amount of information, it was decided to split this material into two volumes. In the first volume of *Cancer Nanotheranostics*, we discuss the role of different nanomaterials for cancer therapy, including lipid-based nanomaterials, protein- and peptide-based nanomaterials, polymer-based nanomaterials, metal-organic nanomaterials, porphyrin-based nanomaterials, metal-based nanomaterials, silica-based nanomaterials, exosome-based nanomaterials, and nano-antibodies. In the second volume, we discuss the nano-based diagnosis of cancer, nano-oncology for clinical applications, nano-immunotherapy, nano-based photothermal cancer therapy, nano-erythrosomes for cancer drug delivery, regulatory perspectives of nanomaterials, limitations of cancer nanotheranostics, the safety of nano-biomaterials for cancer nanotheranostics, multifunctional nanomaterials for targeting cancer nanotheranostics, and the role of artificial intelligence in cancer nanotheranostics.

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Nanotheranostics: Emerging Strategies for Early Diagnosis and Therapy of Cancer

Rekha Pachaiappan and Kovendhan Manavalan

Introduction

Global Scenario of Cancer

Worldwide, cancer was a great threat which produces physical and mental pain to the victim. After cardio-related problems, cancer holds the next place to cause death to the patients. According to the World Health Organization (WHO), cancer is the main cause of death and accounted for 7.6 million deaths in 2008. Key statistics report shows that the number of deaths due to cancer might reach 13 million by 2030. From low- and middle-income countries alone, ~10–11 million new cancer cases might be recorded by 2030. The WHO has initiated certain relevant strategies in order to reduce the economic burden and social stress caused by cancer. By supporting national cancer control programmes, it creates awareness, change in lifestyle, screening and vaccination which were done towards cancer. In this context, it's to be noted that early detection of cancer with appropriate treatment will improve the lifetime of cancer-affected people. Cancer types such as oral, breast, cervical and colorectal cancers are entirely curable if detected at an earlier stage followed by effective therapies (https://www.who.int/cancer/resources/keyfacts/en/). Current detection modalities of cancer include imaging methods like positron emission tomography, single photon emission computed tomography, magnetic resonance imaging, ultrasound imaging and hybrid of these techniques (Karpuz et al., 2018). In the treatment of cancer, physicians follow surgery, radiotherapy, chemotherapy,

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hormonal therapy, immunotherapy, gene therapy and a combination of these therapies (https://www.cancer.gov/about-cancer/treatment#:~:text=Some%20people%20with%20cancer%20will,targeted%20therapy%2C%20or%20hormone%20therapy). Majorly chemotherapy was considered to increase the survival rate of the patient by solo or when combined with surgery and radiotherapy (https://www.medicalnewstoday.com/articles/326031). In particular survival rate of breast cancer was improved to 91%, 84% and 80% at 5, 10 and 15 years after diagnosis (https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/breast-cancer-facts-and-figures/breast-cancer-facts-and-figures-2019-2020.pdf). However, practically many impediments exist in the diagnosis and treatment of cancer. Lack in significant cancer biomarkers, early diagnosis, efficient mass screening strategies and cancer awareness are the main difficulties in cancer diagnosis. In the case of the treatment process, the cancer site contains heterogeneous molecular structure, and the possibility of removing the entire cells from the tumour was difficult. Hence, some cells retain in the tumour as 'cancer stem cells' (CSCs), and targeting these CSCs is complex. Further, drug resistance characteristics of these CSCs make them resistant against anticancer drugs given during therapy period. Another fact is that CSCs are not producing appreciable cell division to allow chemotherapy agents to act on it. Further, lack of identification of genetic mutation is related to malignancy, deficiency in epigenetic profiling and demand of specific epigenetic drugs. Finally, during diagnosis of different cancer types, various difficulties are raised which increase the complexity in the treatment process (Chakraborty & Rahman, 2012).

Nanoscience and Nanotechnology in Cancer

In this scenario, the recent advancement in nanoscience and nanotechnology has introduced vast nanoparticles and nanocomposites in the field of oncology. These nanoparticles are smaller in size, exhibit higher surface-area-to-volume ratio, surface plasmon resonance, biocompatibility, higher stability, tunable optical property, multifunctionality, etc. Hence, find its key role in imaging, tumour targeting and drug delivery to proceed for cancer diagnosis and therapy. Integration of the words diagnostics and therapeutics has given out a new word 'theranostics' suggested by John Funkhouser in 2002 (https://www.ddw-online.com/theranostics-an-emerging-tool-in-drug-discovery-and-commercialisation-1024-200210/). Novel features of nanoparticles made it readily available for both treatment and diagnosis of cancer and evolved as 'nanotheranostic' (Gindy & Prud'homme, 2009). Nanotheranostic agents are expected to possess certain features to carry out efficient diagnosis and therapy. A nanotheranostic agent must be biocompatible and less or no toxic towards the target system. Further, it should be stable under different physiological conditions when injected or introduced into the target. Also, it should have the capability to overcome the physical hindrances on its way to enter into the tissue or organ to be diagnosed and treated. These nanotheranostic agents settle more on the tumour

site due to leaky blood networks and then selectively inhibit the growth of cancer cells by interacting with it effectively (Acharya & Sahoo, 2011; Mura & Couvreur, 2012). Many exotic nanotheranostic agents were synthesized to improve the diagnosis and treatment modalities of cancer. Nanotheranostic agents have the ability to conjugate with different anticancer drugs. A nanocarrier carries the anticancer drugs and releases it upon the target cell under suitable optical excitation. The anticancer drugs might be biomolecules (peptides, enzymes, etc.) or conventional chemical drugs. For these nanotheranostic agents with anticancer drugs, the circulation time found to be elevated due to inhibition towards regular renal excretion. Drug delivery was targeted to specific tumour sites avoiding more accumulation on normal tissues (Ho et al., 2017). Many researchers have emerged with the successful nanoplatform by employing both organic and inorganic nanotheranostic agents in the detection and treatment of cancer. Inorganic nanotheranostic agents were synthesized from noble metals gold and silver, silica, various metal oxide and sulphides (Eyvazzadeh et al., 2017; Barabadi et al., 2020; He et al., 2012; Roy et al., 2016; Zou et al., 2018). Organic nanotheranostic agents include polymers, liposomes, organic/inorganic hybrid nanotheranostic agents, dendrimers and micelles (Perumal et al., 2019; Prasad et al., 2020; Kumar et al., 2020). In this review, the most recent nanotheranostic agents prepared from various inorganic- and organic-based nanoparticles were categorized and discussed with regard to its potential in cancer diagnosis, and therapy was given in detail. This chapter will surely provide the researchers the summary of recent nanotheranostic agents and help them evolve with an idea to bring out new nanotheranostic agents.

Various Nanotheranostic Agents

Noble Metal-Based Nanotheranostic Agents

Noble metals gold and silver have their unique place in the cancer diagnosis and therapy. These metals have merits such as facile synthesis, biocompatibility, surface plasmon resonance, high surface-area-to-volume ratio, optical properties, flexible structure, multifunctionality, non-cytotoxicity, etc. For both in vivo and in vitro analyses, these nanoparticle-based nanotheranostic agents were employed in cancer imaging and therapy. Recent studies on gold- and silver-based nanotheranostic agents were elaborated below.

Gold-Based Nanotheranostic Agents

Gold nanoparticle in a colloid form was first synthesized by Michael Faraday in year 1857. Following this in the year 1908, Mie proposed that depending on the size of the nanoparticle, the colour of the nanoparticle/nanoparticle colloid varies. Due

to novel characteristic features of gold nanoparticle such as biocompatibility, high surface-area-to-volume ratio, simple synthesis process, desired size and shape, less toxicity during in vivo studies, surface plasmon resonance, etc. have made it to achieve its importance in the diagnosis and treatment of cancer. Subsequently, gold nanoparticles were used in cancer theranostic because of its functionalization towards the tumour target agent and specific interaction on the target cancer cells.

Gold nanoparticles possess better bioconjugation with cancer cells when introduced into the human system. Multifunctionalized gold nanoparticles with nanopopcorn shape (~28 nm) have shown the imperative surface-enhanced Raman signal for human prostate cancer cells (LNCaP). This surface-enhanced Raman signal was better for prostate cancer cells when compared with that of non-cancerous and breast cancer cell lines in the presence of rhodamine 6G dye. The particular sensitive detection of prostate cancer cells was obtained when gold nanoparticles were excited with the near-infrared irradiation (785 nm of diode laser). Subsequently, the exposure of laser light caused the irreversible damage of the cancer cell due to the generation of localized heating known as photo-thermal effect. Prostate-specific membrane antigen (PSMA) found to be more in prostate cancer cells than the normal cells producing alteration in diagnostic signals. So, the gold nanoparticles binded into the cancer cells upon excitation by near-infrared laser light have paved a way for the real-time early cancer diagnosis and destruction of cancer cells (Lu et al., 2010). Similarly, Nagy-Simon et al. (2018) have synthesized the gold nanoparticle loaded with IR-780 dye and coated with pluronic copolymer. This IR-780 dye was found to be extremely active at near-infrared regions and helps in cancer cell tracking. Preparation technique yields a reproducible nanoparticle which is found to be a stable system. Initially, nanoparticle preparation was validated for colon carcinoma cancer cells of murine sample (C26). Then, live cancer cell imaging was performed by employing surface-enhanced resonance Raman scattering (SERRS) microspectrometer. Photodynamic and photothermal therapies were employed to treat cancer tissues. It was observed that pluronic-fixed gold nanoparticles possess a cytotoxic effect under these therapies and showed an enhanced SERRS signal under NIR excitation. Further, the quantum yield of these gold nanoparticles is found to be like the photosensitizer present in organic solvent which produces singlet oxygen (Fig. 1.1).

Lin et al. (2017) have proposed the multifunctional unimolecular spherical micelle nanocarrier fabricated from β-cyclodextrin-{poly(lactide)-poly(2-(dimethylamino)ethyl methacrylate)-poly[oligo(2-ethyl-2-oxazoline)methacrylate]}$_{21}$ (β-CD-(PLA-PDMAEMA-PEtOxMA)), gold nanoparticle and doxorubicin (DOX). By increasing the concentration of doxorubicin, its penetration into the inner core of PLA was improved. In in vivo study xenografted HepG2 tumour was imaged using this gold nanoparticle-based nanohybrid detector which yielded more intensity than a regularly employed contrast agent omnipaque. In vitro and in vivo anticancer effects were performed with HePG2 liver cancer cells using this nanoparticle. Accumulation of nanoparticles in the tumour cells produced breakdown of the nucleus of the cancer cell leading to apoptosis. Thus, β-CD-(PLA-PDMAEMA-PEtOxMA, gold nanoparticle and DOX have combined to produce a better synergic

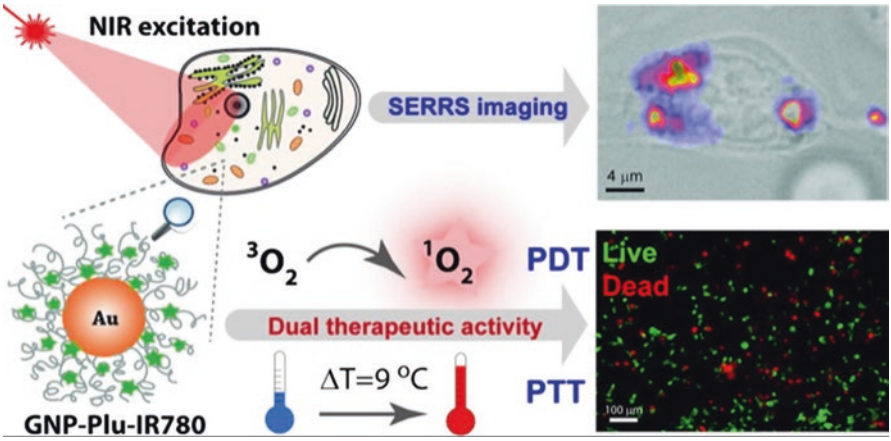

Fig. 1.1 Gold nanoparticle loaded with IR780 dye used as a nanotheranostic agents to perform photodynamic and photothermal therapies under near-infrared laser excitation for intracellular tracking by surface-enhanced resonance Raman scattering imaging. (Adopted from Nagy-Simon et al., 2018)

nanotheranostic effect in the detection of cancer cells using computed tomography imaging and delivery of drug both in vitro and in vivo studies based on pH conditions.

Miao and Tang (2019) have reported on the multipedal DNA walker strands formed on the base of gold nanoparticles used to identify the circulating tumour cells (CTC) available in the biofluids. CTC have transmembrane receptor protein which is binded with the integrated aptamer sequence. So, early detection was possible due to the accumulation of gold nanoparticles specifically on the surface of CTC. Here, they have utilized a reducing agent tris(2-carboxyethyl)phosphine to improve the electrochemical signal and to detect the CTC in blood samples at a rapid rate. Recently, Zhang et al. (2020) have designed a pH-based gold nanoparticle coupled with a zwitterionic surface (~15 nm) to produce an anticancer effect. From a phenomenon given by Otto Warburg, it was observed that the microenvironment of the tumour sites was more acidic in nature than the normal tissue sites known as 'Warburg effect' (Vander Heiden et al., 2009). The gold nanoparticles were launched more on the tumour target due to its acidic nature and accumulated into greater size of order (~180 nm). Photoacoustic signals were given out by the tumours upon near-infrared (808 nm) exposure producing appropriate imaging of the cancer spread. In addition to the detection of cancer cells, upon continuous light exposure, these gold-based nanotheranostic agents affect the tumour cells and destroy it with less side effects, known as photothermal therapy. Thus, simultaneously this pH-based gold nanoparticle with zwitterionic charge surfaces acts as a diagnostic and therapeutic agent. In depth research studies are needed to evaluate the toxicity, biodistribution and side effects of the gold nanoparticle to approve it as a good nanotheranostic agent.

Silver-Based Nanotheranostic Agents

Similar to gold nanoparticle, silver nanoparticle also possesses unique characteristics like biocompatibility, easy fabrication, high surface-area-to-volume ratio, cost effective, etc. Silver nanoparticles offer low toxicity, enhanced SERS signal, biodistribution and cellular uptake that made it readily available for the detection and therapy process, representing as an excellent nanotheranostic agent.

Triple-negative breast cancer cell lines (MDA-MB-231) were identified and treated using the nanocomposite made from silver nanoparticle combined with polymer chitosan and R-phycoerythrin protein. The binding status of the nanoparticle, polymer and protein was evaluated using flow cytometry analyses. Further, the activity of nanocomposite against the cancer cell was determined by evaluating the percentage of caspase protein and tumour suppressor gene with the release of reactive oxygen species. Nanocomposite produced was found to be highly stable providing real-time application both in diagnosis and therapy. This study suggested that silver-based chitosan-phycoerythrin was a potential nanotheranostic agent killing breast cancer cells but not affecting the normal cells (Thangam et al., 2015).

Sahoo et al. (2016) have demonstrated nanotheranostics technique in the diagnosis and therapy of HeLa and A549 cancer cell lines by employing silver nanoclusters conjugated with folic acid forming nanocomposite. Silver nanoclusters were found to exhibit high fluorescence upon optical excitation. These nanoclusters carry the prodrug paracetamol dimer (PD) to the target cell. Prodrug became active and toxic because of the emergence of reactive oxygen species from silver nanoparticles. Toxicity of PD induces apoptosis of cancer cells. Further, folic acid fixed in silver nanoparticles was employed to differentiate HeLa and A549 cell lines, depending on the overexpression of folic acid receptors and downregulation by those cell lines, respectively. Dual role of cancer diagnosis and therapy was successfully done with PD-silver nanocomposite.

Mishra and Kannan (2017) have developed the nanotheranostic agent by using metals (silver) merged with neodymium. These particles were found to possess fluorescence at near-infrared irradiation and magnetic characteristics when injected into the biosystem. In the presence of these silver-based nanoparticles, combinations of near-infrared imaging, magnetic resonance imaging and computed tomography were possible in the detection of cancer cells. Computed tomography has produced contrast image as the silver nanoparticle was very small, whereas neodymium possesses large X-ray attenuation coefficient. Photothermal therapy was done with the silver-neodymium nanoparticle functionalized with chitosan on its surface. This polymer with bimetallic nanoparticle used to carry the paclitaxel an anticancer drug and delivers it into the cancer cells. Loading of drug with the nanoparticle was confirmed using isothermal titration calorimetry method which shows seven drug molecules fixed on a single nanoparticle. On interaction of drugs on the cancer cells, it gets destroyed while the healthy cells are retained. Ghaemi et al. (2018) have successfully reported on the silver nanoparticle embedded on the semiconducting material zinc oxide nanoparticle to yield a novel nanocomposite. This nanocomposite was tested against the detection and treatment of breast cancer.

Since, the zinc oxide nanoparticles were active under ultraviolet excitation, it produces appreciable photoactivity under dark scenario. Reactive oxygen species were given out by the charge transfer that happened between silver and zinc oxide nanoparticles upon the suitable light excitation. Hence, the photocatalytic activity and reactive oxygen species generation support apoptosis of cancer cells. In detail, the p53 tumour suppressor gene, cytochrome complex and executioner caspase were activated to destroy the cancer cell. The mitochondrial pathway suggests that the ratio of BAX/BCl2 got increased which seems to produce cancer cell death in the presence of silver-zinc oxide nanocomposite. Further, the nanocomposite has been supported in imaging the cancer region by computed tomography and optical imaging techniques. Thus, this study had shown the capability of silver-based semiconductor nanocomposite as an agent in diagnosis and therapy of cancer.

Silica-Based Nanotheranostic Agents

Silica nanoparticles were considered for their novel properties such as biocompatibility, desired particle size and large pore volume for a given surface area. Hence, silica nanoparticles find their major role in the biomedical field for diagnosis and therapy by performing imaging and drug delivery of the target sample. Among porous silica nanoparticles, mesoporous silica was considered to be best in delivering the drug due to its small size ranging from 2 to 50 nm. Kresge et al. (1992) have reported on the fabrication of mesoporous ordered silica nanoparticle from aluminosilicate gel as a precursor and a suitable surfactant by employing liquid crystal method. The required size of mesoporous silica nanoparticle could be achieved by tuning the surfactant, experimental conditions and other chemicals. Vallet-Regi et al. (2001) have developed the disk-shaped mesoporous silica of various porous sizes using C12TAB and C16TAB as a surfactant to perform drug delivery. In this study, these silica nanoparticles were employed to carry the drug ibuprofen which released into the target, upon charging the nanoparticle.

Fan et al. (2017) have reported on mesoporous silica nanoparticles attached with the aggregation-induced emission luminogen and copper sulphate nanoparticles. Luminogen was used to induce fluorescence emission to produce imaging of the biological sample they encounter. Copper sulphate nanoparticles were used to produce a photothermal effect upon optical excitation (808 nm) along with chemotherapy to kill the cancer cells. Drug doxorubicin was introduced into the silica nanoparticles settled down in its pores. Doxorubicin was found to be released more from the pores under the near-infrared excitation and in an acidic environment created by the presence of cancer cells. In vitro cancer cell line study was done with HeLa and DLD-1 cells. Samples have exhibited the emission at the blue wavelength region for the given silicon-based nanocomposite. This study concludes that mesoporous silica nanocomposites have the potential in providing the anticancer effect along with chemotherapy and photothermal effect.

Similarly, another work on a novel multifunctional upconversion nanoparticles coupled with mesoporous silica nanoparticles using surface-protected hot water etching technique was proposed by Fan et al. (2014). Both diagnosis and treatment of cancer were carried out successfully. In the detection of cancer cells, dual-mode magnetic resonance and upconversion luminescent imaging techniques were used. Chemotherapy, radiotherapy and photodynamic therapy were combined as 'tri-modal therapy' under the exposure of near-infrared and X-ray radiations. In vitro study of upconversion-mesoporous silica nanoparticles have shown more destruction of HeLa cancer cell lines, together with drug Dtxl and radiotherapy. Both in vitro and in vivo studies have shown that cancer cells were treated and entirely destroyed. This study proves the potential of nanothernaostics approach using mesoporous silica nanoparticles with a synergetic effect of bimodal imaging and tri-modal therapies.

Jin et al. (2018) have designed a multifunctional nanocomplex out of mesoporous silica nanoparticle capped by gold nanoparticle linked with the amino acid L-cysteine and ferrous iron ions. This silica-based nanocomplex was found to be the best anticancer agent. It works on the requirement such as the acidic pH level which controls the drug release followed by chemo-photothermal therapies. Based on Fenton reaction, the disintegration of hydrogen peroxide takes place to release more hydroxyl radicals which enhance the treatment efficacy. The potential of the mesoporous silica with gold nanoparticle and ferrous iron was checked against the in vivo study of mice. It was observed that after 2 weeks the treatment of tumour with a nanocomplex inhibited the growth of tumour along with chemo-photothermal therapy (Fig. 1.2).

Another work utilized silicon nanorods fabricated using microwave synthesis joined with gold nanoparticles to explore its characteristic as a nanotheranostic agent. Thus, the prepared silicon-gold nanorods were injected into the sample and exposed to near-infrared rays. These nanostructures possessed high photothermal stability and photothermal conversion efficiency, yielding better diagnostic data by exploring photoacoustic and infrared thermal imaging techniques. This was possible due to more deposition of modified nanostructures on the targeting tumour with peptide ligands of ~8.74% ID g^{-1}. In vivo study on mice models have shown no toxicity and destroyed the tumour cells alone and also no recurrence of cancer cells even after 60 days of photothermal therapy. Silicon-gold nanorods have exhibited dual features adapting multimodal imaging along with simplified surface modification which concludes its potential towards the real nanotheranostic approach (Cui et al., 2019).

Dendrimer-Based Nanotheranostic Agents

Dendrimers are hyperbranched molecules which were discovered by Fritz Vogtle in 1978. Synthesis of dendrimer was successfully done by Donald Tomalia and co-workers in 1980 simultaneously by George R. Newkome independently. Dendrimers

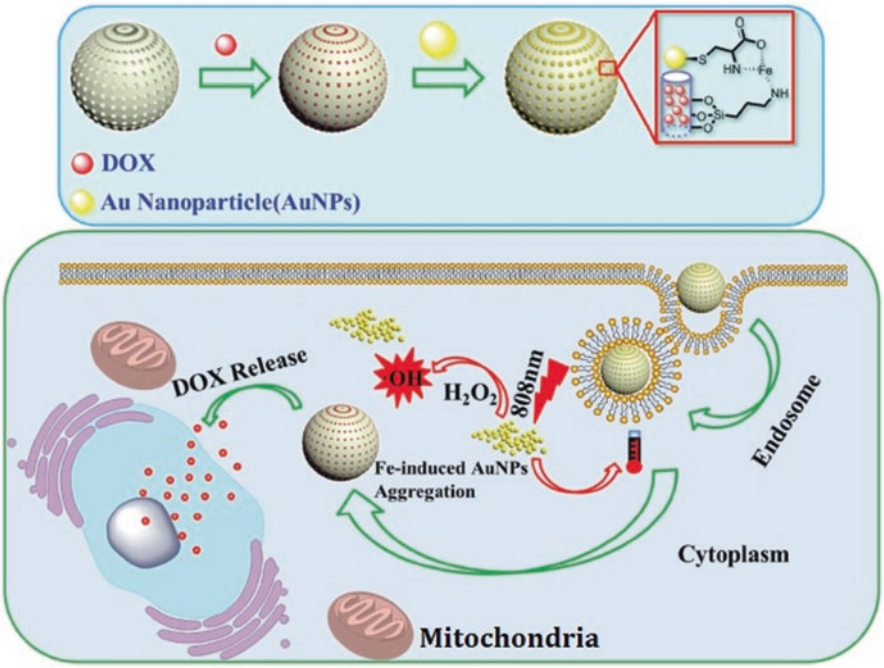

Fig. 1.2 Schematic representation of nanotheranostic agent prepared with the combination of mesoporous silica, iron and gold nanoparticles to provide drug delivery doxorubicin under near-infrared laser light irradiation produced reactive oxygen species to destroy cancer cells. Controlled mechanism occurred by forming and breaking of ligand coordination bonds between mesoporous silica, iron and gold nanoparticles. (Adopted from Jin et al., 2018)

mean 'trees' in Greek also known as 'cascade molecules'. Since it's a hyperbranched molecule, modifications in its structure include changes in its physicochemical property (Abbasi et al., 2014). The change in physicochemical characteristics made them readily available for cancer diagnostic imaging and anticancer therapies. Maciejewski was the first one to propose the dendrimer as molecular containers in 1982. Then the idea got extended to carry the anticancer drug to a specific target. Drug-conjugated dendrimer possesses several calibers like high solubility, less toxicity and tumour accumulation (Palmerston Mendes et al., 2017).

Chen et al. (2015) have designed the multifunctional dendrimer captured the gold nanoparticles combined with gadolinium (Gd) chelator and surface modification done by thiolated cyclo(Arg-Gly-Asp-Phe-Lys-(mpa)) (RGD) peptide (Gd−Au DENPs-RGD). Thus developed multifunctional dendrimer-based nanoparticles found to be stable at different pH ranging from 5 to 8 and temperature range 4–50 °C. The investigation has shown that with the obtained X-ray attenuation and T1 MR relaxometry (2.643 mM^{-1} s^{-1}) properties, the Gd−Au DENPs-RGD have been probed to carry out the dual-mode imaging using computed tomography and magnetic resonance imaging techniques. In vivo imaging of a xenograft small

tumour model overexpressing $\alpha_v\beta_3$ integrin via RGD-mediated targeting pathway was done, and the same could be employed for a variety of overexpressing $\alpha_v\beta_3$ integrin tumours. Similarly, Xu et al. (2017) have also proposed on the fabrication of multifunctional dendrimer entrapped with gold nanoparticles and followed by modification using arginine–glycine–aspartic peptide. Radionuclide $^{99m}T_c$ having a half-life period of 6 h was fixed with prepared nanoparticles for labelling purposes. These nanocomplexes have shown characteristics such as biocompatibility, radiostability and uniform size. Imaging of overexpressing $\alpha_v\beta_3$ integrin tumours was done with integrated dual-mode single photon emission computed tomography and computed tomography imaging methods. Both in vivo (subcutaneous tumour model) and in vitro analysis of cancer cells were performed in this dendrimer-based nanotheranostic study. In another work expressed by Nigam and Bahadur (2017), the peptide dendrimer is coupled with super paramagnetic iron oxide nanoparticle which acts as a stimuli-responsive carrier of drug to the target cancer sites. These nanoparticles were found to be better due to their unique properties like biocompatibility, stability and multifunctionality. Hence, this magnetic peptide dendrimer's drug transportation and releasing rate were challenging to a commonly used PAMAM dendrimer. These magnetic peptide dendrimers have successfully delivered the doxorubicin drug into the cancer cell supporting chemotherapy to produce magnetic hyperthermia in the cells to face the apoptosis and proved to be the best alternative to widely used common PAMAM dendrimers.

Another work proposed on the synthesis of ^{177}Lu dendrimer coupled with bombesin- folate and gold nanoparticle (^{177}Lu–DenAuNP–folate–bombesin) to locate internally T47D breast cancer cells and evaluated its characteristics as a nanotheranostic particle. This dendrimer-based nanocomplex had exhibited fluorescence emission after its exposure to near-infrared irradiation at 825 nm which leads to optical imaging of the cancer cell environment. Also, the nanocomplex possesses plasmonic properties, increasing the temperature of the cell from 39.1 °C to 46.8 °C. The rise in temperature causes a decrease in viability (~90%) of T47D cancer cells with an absorbed dose of (63.16 + 4.20 Gy). Thus these properties of ^{177}Lu–DenAuNP–folate–bombesin have paved a way for better optical detection, photothermal therapy and radiotherapy in the diagnosis and treatment of breast cancer (Mendoza-Nava et al., 2017).

Fan et al. (2019) have developed a nanotheranostic agent from pyridine-functionalized generation 5 poly dendrimer fixed to copper (II) (G5.NHAc-Pyr/Cu(II)) to detect tumour and tumour metastasis. By employing this dendrimer-based nanotheranostic agent, the detection and treatment of tumour were carried out with the combination of magnetic resonance imaging, radiotherapy and chemotherapy. In the presence of copper (II), the G5.NHAc-Pyr/Cu(II) nanocomplex was found to be stable, reducing the cancer cell proliferation and causing cell apoptosis. The detection with magnetic resonance imaging of xenografted tumour model and lung metastatic nodules was significant due to r_1 relaxivity of 0.7024 mM^{-1} s^{-1} under radiotherapy followed by improved chemotherapy of tumour models. They have reported that in order to treat a particular type of cancer the dendrimer complex with

copper (II) may be altered with targeting ligand to act as a better nanotheranostic agent. Recently the same group, Fan et al. (2020), has developed another strategy in the field of nanotheranostics. They have introduced the nanocomplex out of phosphorous dendrimer combined with copper (II) complex (1G3-Cu) which enables the ultrasound in the diagnosis and treatment of cancer. For magnetic resonance (MR) imaging at T1 weightage, the relaxivity r_1 was found to be 0.7024 mM^{-1} s^{-1}. Half maximal inhibitory concentration IC50 was found to be 1.24μM to produce complete inhibition against the pancreatic cell under investigation producing cell apoptosis. Along with apoptosis decrease in energy carrying intracellular adenosine triphosphate (ATP), upregulation of Bcl-2-associated x protein, tumour protein P53 and PTEN protein and downregulation of Bcl-2 protein take place. Due to the ultrasound effect, high permeability was introduced in the cell wall and cell membrane which attracts more 1G3-Cu nanocomplexes which improves MR imaging and chemotherapy. The complex may be modified and coupled with ultrasound-targeted microbubble destruction (UTMD) such that it could be adaptable for various types of cancer (Fig. 1.3).

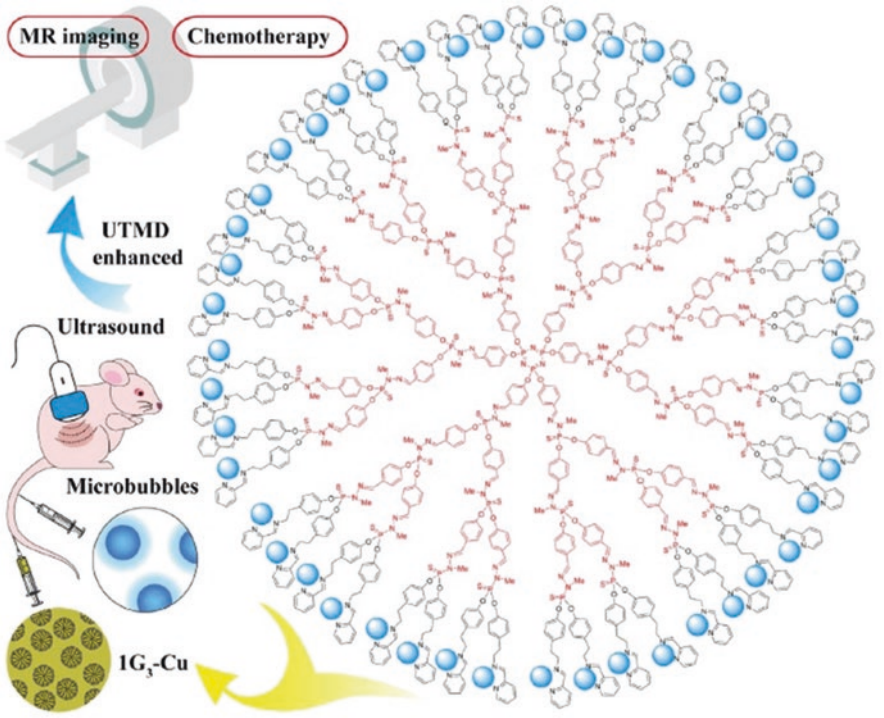

Fig. 1.3 Schematic representation of phosphorous dendrimer coupled with copper (II) complexes for ultrasound-targeted microbubble destruction to support magnetic resonance imaging and chemotherapy of tumour. Right picture represents the synthesized dendrimer-based nanotheranostic agent, and copper (II) ions are shown as blue balls. (Adopted from Fan et al., 2020)

Similarly, Inoue et al. (2019) have performed the ability of dendrimer (G2) as an inhibitor and its breaking effect on amyloidogenic transthyretin (ATTR) V30 M protein of β sheet structure. The ATTR triggers the formation of amyloid fibrils, misfolding in protein structures, by reducing the β sheet structure. Amyloid fibrils deposit on the tissue which requires a suitable therapy to inhibit its growth and a suitable treatment to remove it. Here, 3D-structured nanomaterials from polyamidoamine dendrimer (dendrimer) with cationic polymer have been injected into transgenic rats. The dendrimer (G2) has shown its potential in inhibiting and destroying those ATTR deposited on tissues of rats. The detailed mechanism of dendrimer (G2) can help implement it in the clinical setup for cancer nanotheranostic application.

Recently, Song et al. (2020) have reported on the preparation of LyP-1 peptide-modified ^{131}I-labelled dendrimer. This dendrimer as a nanotheranostic agent was employed to carry out the multitask on single photon emission computed tomography imaging, radionuclide and antimetastasis therapies in the detection and treatment of cancer. Also, a study on cytocompatibility was done for ^{131}I-dendrimer at a concentration of 0.1–10μM for the period of 24 h. It was noted that radiochemical purity and stability (>90%) were appreciable even at 16 h. Thus developed multifunctional platforms have improved the local hypoxia in the system leading to apoptosis of cancer cells. Another work by Chen et al. (2020) has fabricated multifunctional dendrimer entrapped the gold nanoparticles combined with Fluo-4. They have performed by entrapping 2-nm-sized gold nanoparticles into the poly(amidoamine) dendrimers modified with polyethylene glycol (hydroxyl terminated). Extra amine groups present on the dendrimers have undergone the acetylation process. Finally, the nanoprobe was emerged by the covalent bonding between the dendrimer and calcium ion in the presence of polyethylene glycol hydroxyl group. This nanoprobe has been employed in monitoring and tracking the T-cell due to its novel nature of cytocompatibility, high X-ray attenuation, water solubility and excellent T-cell labelling. Imaging was done as the nanoprobe possessed the fluorescence emissions upon irradiation. For this confocal fluorescence microscopy and computed tomography were employed to view the T-cells.

Polymeric-Based Nanotheranostic Agents

In nanotheranostic application, polymer nanoparticles are considered for its delivery of anticancer drugs. Drug delivery can be regulated by controlling the physicochemical properties like molecular weight, crystallinity, water solubility, etc. Both natural polymers and synthetic polymers were utilized for nanoparticles. in cancer theranostics, natural polymers like chitosan, albumin and collagen whereas in synthetic polymers polyethylene glycol, polylactic acid, poly glutamic acid are regularly used (Luk & Zhang, 2014).

Zhu et al. (2017) have engineered the semiconducting polymer which was active under exposure of near-infrared irradiation to carry out photodynamic therapy for advance and optimized treatment of cancer. The polymer acted as a NIR fluorescent

photodynamic therapy agent. Nanoceria coupled with polymer has played an important role in regulating the reactive oxygen species based upon the pH level and has no reaction over NIR exposure. In a murine mouse model, the nanoceria-doped polymer has shown decreased damage to normal cells, whereas it improved photodynamic response towards the cancer cells. Thus, this study was a better example of an organic polymer-based approach in therapy of cancer.

Jia et al. (2017) have demonstrated the photodynamic therapy with plasma membrane-activatable polymeric nanodrug assembled with protoporphyrin IX (photosensitizer), polyethylene glycol and glycol chitosan. Due to π–π, strong stacking interaction protoporphyrin present in the core shell of nanoparticles has exhibited fluorescence quenching effect. However, when this nanocomplex encounters the plasma membrane, disassembly of the protoporphyrin from the core shell occurred and fixed to the plasma membrane due to its high affinity towards it. Now, the nanocomplex deposits more on the tumour after intravenous injection and laser irradiation have produced enhanced fluorescence signals and also generated singlet oxygen. Further, this scenario of high fluorescence emission is guided to carry out photodynamic therapy.

Similarly, Shao et al. (2019) have explained the triple collaborative method utilizing auto-fluorescent polymer nanotheranostic agents in self-monitorization of cancer therapy. The biodistribution of this polymer nanoparticle was observed due to its self-fluorescence property leading to the best real-time fluorescence and photoacoustic imaging. Enhanced photocytotoxicity and neovascularization with xenograft mouse model by this polymer nanocomplex were due to its efficient photothermal conversion property. Through this work they have shown the capability of nanocomplex polymers in inhibiting the growth of the tumour concurrently on tumour cells and vasculature which occurred by RNA interference, anti-angiogenesis and photothermal therapy process. An et al. (2018) have carried out the synthesis of bovine serum albumin (BSA)-regulated organic polymer to perform as a nanotheranostic agent. In this process, BSA coated on the gallic acid–iron (III) of size ~ 3.5 nm was generated. This nanoparticle possessed good biocompatibility and excellent response at near-infrared excitation. Ultrasmall size of nanoparticle helps them filtrate through renal excretion, thus reducing the potential toxicity. Magnetic resonance (MR) imaging (T1 weighted) of tumour-bearing mice model was recorded prior and after the injection of these polymer nanoparticles. It was observed that MR images were different and exhibiting respective changes with injection of polymer nanoparticles. The real-time MR imaging of tumours have shown the guidance to carry out laser ablation in the destruction of solid tumours. This study has created trust towards the polymer-based BSA-coated nanoparticle that could serve as a nanotheranostic agent.

Yang et al. (2019) have developed platinum encapsulated by prodrug polyphenols and gadolinium ions with thermal-sensitive polymers. Two types of polymers were employed, polyethylene glycol–perylene diimide–poly(diisopropanol amino ethyl methacrylate) and 2-octyldodecyl–perylene diimide–poly(ethylene glycol), in yielding nanotheranostic platform. These polymer-based nanoparticles were also responsive to mild pH environments. Near-infrared radiation from laser exposure

has produced controlled drug release in the acidic microenvironment of the tumour. In vivo imaging with photoacoustic, magnetic resonance and positron emission tomography have shown the accumulation of polymer nanoparticles in the tumours. Subsequently with laser exposure of wavelength 671 nm have supported the combination of chemotherapy and photothermal therapy, taking these nanoparticles as a cancer nanotheranostic agent into the clinical environment.

Hu et al. (2019) have designed a gadolinium-chelated coupled polymer-based nanotheranostic agent. These nanomaterials have shown the admirable characteristics such as less biotoxicity and chemical and optical stability. In vivo imaging was carried out with this nanomaterial to analyse the antitumor effect under 24 h of administration in 4 T1 tumour-bearing syngeneic mice model. As expected the imaging of the tumour site got improved after the exposure of these nanomaterials into the cancer site. It's a semiconductor polymer with low band gap allowing NIR absorption for integration of photoacoustic imaging and second near-infrared fluorescence imaging (1000–1700). Magnetic resonance imaging (T1-weighted) guiding to perform photothermal therapy was due to the carboxyl group from polymer chelate with gadolinium ions. So, a unique organic nanotheranostic agent was evolved possessing good spatial resolution along with deep penetration to view and treat the tumours. Zarepour et al. (2019) have designed and fabricated a nanocomplex with three polymeric layers covering the iron oxide nanoparticles (43±1.5 nm). The three layers were polymeric β-cyclodextrin, polyacrylic acid combined with sulfadiazine and polyethylenimine. Sulfadizine was hydrophobic which appears at the first and third layers. Here, doxorubicin was employed as an anticancer drug which was studied for its characteristics under normal and acidic pH environment, on/off switching mechanism followed for drug release. Mainly the biocompatibility test has proved that the nanocapsule possessed its anticancer effect on cancer cells even at low concentration of 0.3μg mL^{-1}, whereas no effect towards the components of blood or immune system. These biocompatibility tests were carried out using various methods: MTT assay, coagulation assay, hemolysis, MTT and complement activation. Thus, they have successfully proposed the 'switchable' nanotheranostic capsule depending on pH environment, cancer nanotherapeutic agent with low concentration and magnetic property of iron nanoparticles which provides 160% drug loading capacity.

Recently, Men et al. (2020) have developed a nanostructure made up of polymer coupled with doxorubicin-iohexol suspended at hydrogel which was found to be thermosensitive. When these nanostructures exposed to the near-infrared irradiation have experienced controlled melting and drugs were released at this state. Also, hydrogel degradation takes place upon NIR excitation. This unique nanomaterial found to enhance the synergic effect of imaging methods like photoacoustic imaging, computed tomography imaging and fluorescence imaging in producing the detection of tumour. Notably, tumours were appreciably treated with chemotherapy combined with photothermal therapy. Thus, this work proves the novelty of polymer hydrogel as a nanotheranostic agent integrating synergic effects of imaging techniques and chemo- and photothermal therapies under controlled drug release.

Liposome-Based Nanotheranostic Agents

In 1964, British haematologist Alec D. Bangham was the first to propose on liposomes. The name derived from two Greek words *lipo* and *soma* means fat and body, respectively. The main constituents of liposomes are phospholipids and cholesterol. It's a spherical structure consisting of two layers of lipids with aqueous core. Hence, hydrophilic substances are attracted towards aqueous core, and hydrophobic materials are encapsulated. Liposomes are noted for its remarkable nature of biocompatibility, nanosize, biodegradability, less toxicity, nanocarriers, etc. (Tang et al., 2018). Liposomal-based anticancer drugs of different types have been synthesized to target cancer cells.

Feng et al. (2017) have proposed on the synthesis of multipurpose liposome from encapsulation of AQ4N (hydrophilic) and hexadecylamine (hydrophobic) combined with photosensitizer chlorin e6 (hCe6) which chelated using copper isotope (^{64}Cu) forming an effective imaging probe. In vivo trimodal imaging was carried out positron emission tomography (PET), fluorescence and photoacoustic imaging methods with the liposome nanocomplex. After the injection of AQ4N-hCe6-liposome into the tumour mouse model, a light-emitting diode of 660 nm was exposed at the tumour site. Tumour sites loaded with liposome nanostructure have triggered severe hypoxia followed by activation of the AQ4N drug due to photo-irradiation. Thus, occurrence of tumour hypoxia induces the cancer treatment by sequential photodynamic therapy along chemotherapy as a significant treatment. Due to biocompatibility, tumour hypoxic nature, overcoming of classical photodynamic therapy and real-time imaging of AQ4N-hCe6-liposome had made it available as a cancer nanomedicine.

Chauhan et al. (2017) have fabricated the gold nanorods supported by liposomal nanohybrid in the diagnosis and therapy of cancer under near-infrared light excitation. The study was carried out against the breast cancer cell lines MDA-MB-231 using the gold-liposome nanohybrid. Notable effects were shown by this novel nanohybrid. First, remarkable mechanical strength of the liposome improved with gold nanorods by supporting the inner and outer layer of it. Control over the release of drug doxorubicin into the cellular environment. In particular, premature drugs were not released upon photothermal excitation. Nanohybrids have shown appreciable anticancer response towards the employment of both chemotherapy and photothermal therapy instead of any single therapy. Further, this biocompatible gold-liposome nanohybrid has exhibited the best contrast agent to carry out imaging, especially yielding highly resolved computed tomographic images guiding cancer therapies. So, this study was promising in giving out the best nanotheranostic agent using liposome-based gold nanohybrid. This nanohybrid had shown several potential characteristics such as biocompatibility, contrast agent, stability and photoactive which find its application in the field of oncology.

Shen et al. (2017) have reported on the development of a nanodelivery system based on liposome-encapsulated ruthenium polypyridine coordination complex $[(Ru(phen)_2dppz](ClO_4)_2]$. Breast cancer cells MDA-MB-231 were treated with

ruthenium complex. After being irradiated, an active compound ruthenium polypyridine emits strong fluorescence signals. Ruthenium complex integrates with hydrophobic lipid bilayers and settles more in the cancer cells and tumours. Liposome-ruthenium got settled in the cancer cell causing severe DNA damage interrupting G2/M phase and leading to apoptosis of those cancer cells. Growth of triple-negative breast cancer tumours was well inhibited by liposome-ruthenium. Imaging of liposome-ruthenium incorporated in cancer cells was obtained by utilizing confocal microspectroscopy, intravital microscopy and in vivo preclinical imaging techniques. In particular, liposome-ruthenium nanocomplex does not cause any physiochemical changes to the major organs and emerged as an adaptable nanotheranostic agent.

Sun et al. (2018) have engineered the liposome-based nanotheranostic agent by gadolinium-doped mesoporous silica nanoparticles coupled with indocyanine green fixed with thermosensitive liposomes loaded with doxorubicin drug (DOX@GdMSNs-ICG-TSLs). Here gadolinium facilitates the T1-weighted magnetic resonance imaging, whereas indocyanine green supports the near-infrared fluorescence imaging and photoacoustic imaging. Thus, the trimodal imaging system was obtained successfully. Under near-infrared laser, excitation doxorubicin and indocyanine green have supported to carry out the chemotherapy and photothermal therapy guided by trimodal imaging. Thus, an excellent trimodal imaging with chemo- and photothermal therapies was done by utilizing DOX@GdMSNs-ICG-TSLs to provide significant antitumor results for both in vivo and in vitro analysis.

Yu et al. (2018) have proposed the development of multi-compartment membrane-derived liposomes (MCLs). This was obtained by reassembling the cancer cell membranes with polysorbate/tween 80. In order to carry out the imaging and drug delivery, generated multi-compartments were joined with ^{89}Zr (deferoxamine chelator) and loaded with tetrakis(4-carboxyphenyl) porphyrin, respectively. Positron emission tomography (PET) and photodynamic therapy (PTT) were employed for imaging and therapy processes. Radiochemical stability was found to be high in turn supporting long-term PTT in in vivo study against 4 T1 tumours. Then the liposome-derived radionuclide particles were excreted through action of phagocyte system, i.e. reticuloendothelial system through hepatobiliary excretion process. Merits of this study include no toxicity by MCLs, detailed imaging data of lymph nodes by ^{89}Zr-MCLs and flexibility to adapt for other types of cells. Hence, a novel nanotheranostic platform for imaging and treatment of cancer was given out with liposomes.

Prabhakar and Banerjee (2019) have emerged with a nanotheranostic platform from nanobubble liposome complexes working under ultrasound to create imaging and drug delivery in cancer scenarios. This was done by the combination of chemotherapy medicine paclitaxel entrapped by liposomes (entrap efficiency ~85.4 ± 4.39%) incorporated with nanobubble. Conjugation efficiency was found to be ~98.7 ± 0.14%. Finally, a nanobubble paclitaxel liposome of size ~528.7 ± 31.7 nm was received. Sonoporation of MiaPaCa-2 cancer cells was performed by using ultrasound and a prepared nanobubble system. Cellular permeability was enhanced because of the above-said combination resulting in 2.5-fold increase in the uptake

of liposomes from nanocomplex than individual liposomes. Several merits of nanobubble liposomes like echogenic stability, minimally invasive and more anticancer effect than commercial medicine ABRAXANE/PACLITAX. In order to find its place in clinics, some more deep preclinical studies need to be done.

Karpuz et al. (2020) have engineered the radiolabelled liposome of nanosize (150–180 nm) used for in vitro analysis of folate-target of non-small cell lung cancer (H1299 and A549). Liposome was covered by paclitaxel (PCX) and vinorelbine (VNB) containing encapsulation efficiency of 15% and 20%, respectively. After introduction of active- and passive-type targeted liposomes, the uptake rate was found to be more for H1299 cells than A549 cells. Also, higher rates of active targeted liposomes were uptaken by cells than the passive targeted ones. Significance of the anticancer effect was enhanced due to the inclusion of two drugs paclitaxel and vinorelbine instead of single-drug encapsulated liposomes. This in vitro study of co-drug-encapsulated biocompatible liposomes has exhibited nanotheranostic characteristics in the detection and treatment of non-small cell lung cancer through endocytosis initiated by folate receptor.

Recently, De Oliveira et al. (2020) have fabricated a novel nanotheranostic particle liposome, from 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine, pluronic F127 which was covalently modified with a fluorescence probe 5(6)-carboxyfluorescein to perform photodynamic therapy. Thus the obtained polymer was subjected to solid dispersion technique followed by sonication to achieve the final lipid-based polymer liposome. These liposomes had vesicles of size 100 nm which was sufficient to entrap the photosensitizer verteporfin. Time-resolved fluorescence microspectroscopy was employed to determine the structure using fluorescence lifetime of the liposome. The fluorescence data of liposomes are found to possess monomeric form with heterogeneous distribution of verteporfin and 5(6)-carboxyfluorescein in its vesicles. Verteporfin have settled down in the nucleus level, whereas 5(6)-carboxyfluorescein was found in the liposome membrane. The capability of liposome in the detection and photodynamic therapy was done against glioblastoma multiforme cell line T98G. Verteporfin concentration of 1.0μmol L^{-1} was sufficient to decrease 99% cell viability at blue LED excitation. This study had shown the efficient way of performing cancer therapy with photoactivated liposomes which were not toxic under the absence of light.

Micelle-Based Nanotheranostic Agents

Micelles are colloidal suspension formed by the aggregation of amphiphilic surfactant molecules. This term means tiny particle derived from the Latin word *mica* which means particle and then forms a new word tiny particle (elle diminutive) and coined during the nineteenth century. Mainly micelles due to their ultrasmall size, spherical shape with core, amphiphilic nature, etc. have been employed in drug delivery. Recent research works on micelles have shown the advancement in

synthesis of various micelles and its role for anticancer drug delivery and in imaging techniques (Oerlemans et al., 2010).

Yang et al. (2018) have reported on the development of nanostructures based on polymer conjugated with near-infrared dye. Nanostructures formed with polymer and near-infrared dye have the capability to self-assemble themselves in order to create micelles. The biodegradable polymer involved in construction of nanostructure was responsible to yield hydroxyl radical and hydrogen peroxide which amplifies the oxidative stress in the system. Due to excellent absorption in the near-infrared region, these micelle-based nanostructures facilitate dual imaging, i.e. fluorescence and photoacoustic imaging. In vivo study was carried out with these micelles on intravenous injection to tumour (~250 mm^3)-bearing mice model. More number of micelles was settled down at tumour because of enhanced permeation and retention effect. Now, upon NIR laser irradiation, a strong fluorescence signal was emitted by the tumour with micelle nanostructure. The emission occurred after 24 h of injection and lasts for more than 48 h. Tumours were destroyed upon excitation with near-infrared laser light by the combination of photothermal effect and photodynamic therapy. Hence, this study has shown the potential of micelles with NIR excitation in producing dual imaging and photo anticancer therapy as a good nanotheranostic agent (Fig. 1.4).

Similarly, Chen et al. (2017) have designed the micelles from copper sulphide nanoparticles to explore its capacity for imaging and cancer therapy. Copper sulphide nanoparticles have increased in temperature upon near-infrared radiation due to the functionalization of polymers poly(acrylamide-acrylonitrile) and polyethylene glycol. This property enables them to use photothermal therapy. Further, poly(acrylamide-acrylonitrile) has displayed drug encapsulation due to transition

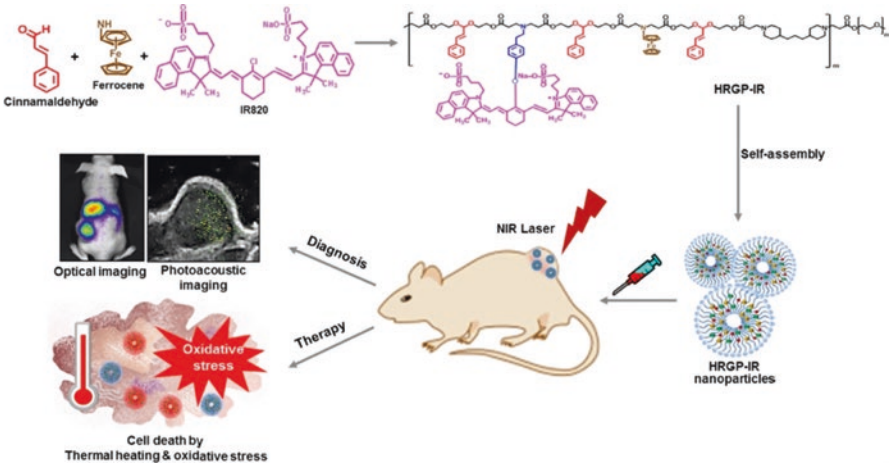

Fig. 1.4 Schematic diagram representing near-infrared dye conjugated hydroxyl radical-generating biodegradable polymer (HRGP-IR) micelles to carry out dual imaging fluorescence and photoacoustic imaging techniques followed by combinational anticancer therapy, with oxidative stress and thermal heating causing cancer cell apoptosis. (Adopted from Yang et al., 2018)

from hydrophobic to hydrophilic transition of micelles. This is due to the rise in the temperature of the polymer greater than the upper critical solution temperature upon excitation of the laser. This temperature arousal helps do controlled chemotherapy by NIR. Also, CuS-micelles coupled with aminoflavone-loaded GE11 peptides tested against triple-negative breast cancer model. Two-dimensional single-layered cells and three-dimensional multi-layered tumours were analysed with micelles. This nanotheranostic agent was found to be best in both imaging and therapies by NIR excitation. Here, photoacoustic imaging, chemotherapy and photothermal therapy have produced the synergic effect in the nanotheranostic of cancer.

Wang et al. (2019) have designed matrix metalloproteinase-2-controlled nanoscale micelles to provide cancer therapy by heterogeneic targeting. Nanoscale micelle system named as HEKM was introduced into the tumour environment. Tumour targeting effect was obtained when HEKM identifies and binds into the EGFR-HER2 complex (tumour heterogeneity marker). HEKM remains as nanorods under normal physiological condition. It changes its shape to sphere under tumour microenvironment due to the presence of matrix metalloproteinase-2. These nanospheres HEKM invade tissues of tumours leading to apoptosis through proapoptotic element integration. In vivo dual imaging was performed with magnetic resonance and near-infrared fluorescence imaging techniques along with fluorophores and gadolinium-loaded HEKM. Both in vivo and in vitro studies have shown that HEKM was suitable for heterogeneous tumour environments.

Yan et al. (2021) have engineered micelle-based nanostructure in liver cancer diagnosis and treatment. They have synthesized polymer (poly-ε-caprolactone cystamine carboxymethyl chitosan-glycyrrhetinic acid) which self-assembles to micelles. These lipid-based structures are uniform and deliver anticancer drugs – pheophorbide A and doxorubicin. Sensitiveness of micelles exists towards response characteristics of antioxidant glutathione. Lysosomes from liver carcinoma cells (HepG2) have made the release of pheophorbide A and doxorubicin drugs with a release rate of 92.1% and 86.3%, respectively. This co-delivery system of drugs improves the treatment of cancer. Tumour sites were accumulated with higher amounts of drugs by glycyrrhetinic acid and EPR effect. The capability of this nanoplatform was visualized by in vivo study on living tumour-bearing mice. Near-infrared radiation was employed to irradiate the tumour site on mice. Then, a synergetic effect was achieved by utilizing photothermal therapy, photodynamic therapy and chemotherapy to have the improved inhibition rate by anticancer drugs. This study has insisted upon the simultaneous imaging and treatment of cancer exploring the concept of co-delivery drug system.

Recently, Wei et al. (2020) have proposed on the production of hybrid organosilica micellar-based upconversion nanoparticle to carry out cancer therapies. This hybrid nanoparticle consists of upconversion nanoparticle core at centre. Then the core was followed by hydrophobic polystyrene middle layer and then outer organic shell doped with disulphide. These middle and outer layers were loaded with Ce6 and doxorubicin respectively. Thus, the prepared micelles have shown good stability. Near-infrared laser light was employed to excite the core of upconversion nanoparticles. Through the photodynamic effect, transfer of energy happened in

Ce6 to release cytotoxic 1O2. Based on cytotoxic and GSH response, the cell viability was recorded for SMMC-7721 and MCF-7/ADR cells. Release of loaded drug to inhibit tumour growth depends upon the higher amount of GSH present in tumour cells and disruption of disulphide-doped organic shells. In the case of normal cells, the negligible toxicity level was observed with these drugs. The values obtained clearly represents the potential of the synthesized upconversion nano-hybrid based on micelles loaded with Ce6 and DOX on tumour cells.

Other Nanotheranostic Agents

Apart from the above-discussed nanotheranostic agent, some other novel nanomaterials used in the diagnosis and therapy of cancer were represented in Table 1.1.

Conclusion and Future Direction

Organic, inorganic and hybrid nanotheranostic agents have shown significant results in cancer diagnosis and therapy due its discussed novelties. However, certain limitations are to be addressed in order to take it to successful real clinical practice. Both in vitro and in vivo studies have exhibited that the nanotheranostic effect depends upon the nanoparticle's size, shape and dosage injected. There might be a chance of affecting normal cells during imaging and therapy process. Also, if the nanocarriers used are insoluble, they might get accumulated on tissues. For example, gold nanoparticles have issues over non-biodegradability leading to accumulation in target tissues and toxicity (Lasagna-Reeves et al., 2010; Jain et al., 2014). In-depth understanding is required to evaluate the toxicity of nanoparticles and to fix the safe dose. Another issue with nanotheranostic agent speaks upon the stability of the nanoparticles. In lipid- and polymer-based nanotheranostic agent, stability issues were reported by several studies (Luk & Zhang, 2014). Due to hydrophilic property, high solubility of nanoparticle takes place which prevent prolong drug release to cause cancer cell apoptosis and in turn necrosis. After dissolving the nanoparticles, it was removed from the system circulation by regular clearance. Hence, concern to build up the nanotheranostic agent to improve solubility rates by opting to appropriate cross-linkers and binding moieties was given. Next is tumour targeting with the nanotheranostic agent. Tumour targeting efficiency of nanotheranostic agent is necessary to consider both active and passive tumour targeting mechanisms. Dynamics involved in active and passive process must be analysed to improve tumour targeting by a nanoparticle (Li et al., 2017). This could be achieved by conjugating the nanoparticle to suitable ligands so as to bind with the receptors released only from tumour target-enhancing cancer cell death. Also, it's necessary to consider cancer stem cells to produce complete cancer therapy. Successful cancer confrontation is possible when nanotheranostic agents are capable of doing early-stage diagnosis

Table 1.1 Novel nanotheranostic materials for the diagnosis and therapy of cancer

S. no.	Nanomaterial	Nanotheranostic agent	Excitation	Sample	Diagnosis contrast agent	Drug for therapy	Study result	References
1.	Zinc oxide (ZnO)	Upconverted nanoparticle mesoporous silica shell-ZnO (UPSC-mSiO_2-ZnO)	980 nm – NIR laser	HeLa cells (in vitro); Balb/c mouse (in vivo)	UPSC-mSiO_2-ZnO	Doxorubicin	Timodal imaging (CT, MRI and upconversion luminescence) with pH triggered, high therapeutic effect	Wang et al. (2015)
2.	Iron-platinum (FePt); graphene oxide (GO)	fcc-FePt/PEGlyated GO sheets	Fluorescence	MCF-7, HeLa and HepG2 cells (in vitro); Mouse (in vivo)	FePt/GO; dimercaptosuccinic acid (DMSA)	Iron ions	Dual imaging (MRI and CT); WST-1 assay done - significant toxicity on MCF-7, HeLa and HepG2 cells – ROS generation	Yue et al. (2017)
3.	Copper sulphide (CuS)	Gadolinium-CuS bovine serum albumin	980 nm NIR laser	SK-OV-3 cells (in vitro); SK-OV-3 tumour-bearing mice (in vivo)	Gd:CuS@BSA	Gd:CuS@ BSA+NIR	Dual imaging (MR and PA)-guided PTT; significant tumour target with no toxicity	Yang et al. (2016)
4.	Europium	Europium complexes – Grafted-oxidative dopamine (ECOD)	808 nm – NIR laser	4 T1 cells (in vitro); 4 T1 tumour-bearing mice (in vivo)	Calcein-AM and propidium iodide	ECOD+Laser	CT/PL imaging-guided PTT; high iodine of ECOD leads to strong X-ray attenuation; low toxicity	Zou et al. (2019)

(continued)

Table 1.1 (continued)

S. no.	Nanomaterial	Nanotheranostic agent	Excitation	Sample	Diagnosis contrast agent	Drug for therapy	Study result	References
5.	Zinc ion	Zinc ion-doped Prussian blue nanotheranostic agent (SPBZn)	808 nm – NIR laser	4 T1 cells (in vitro); 4 T1 tumour-bearing mice (in vivo)	SPBZn	SPBZn(10%) + Laser	Effective result with MRI and PTT-obtained Zn2+– doped ultrasmall SPBZn(10%) nanoprobe	Shou et al. (2020)
6.	Molybdenum disulphide	Molybdenum disulphide-polyethylenimine – hyaluronic acid	808 nm – NIR laser	MCF-7-ADR cells (in vitro); MCF-7-ADR tumour-bearing BALB/c mice (in vivo)	MoS2-PEI-HA + NIR	Doxorubicin	PET imaging to provide spatial-temporal controlled accurate therapy	Dong et al. (2018)
7.	Iron oxide	Superparamagnetic iron oxide-amphiphilic polymers-PEG combined with antibodyHuCC49ΔCH2 and fluorescent dye 5-FAM	–	Colon cancer cell line (LS174T)	Prussian blue	Doxorubicin	Cancer cell imaging and targeted drug delivery depending on pH	Zou et al. (2010)

guided by biomarkers at a molecular level and synergic effect of imaging techniques guided to perform therapeutic methods. Researchers are involved to achieve the superior nano-combater holding the above values to confront cancer.

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Chapter 2
Multifunctional Nanoparticles for Targeting Cancer Nanotheranostics

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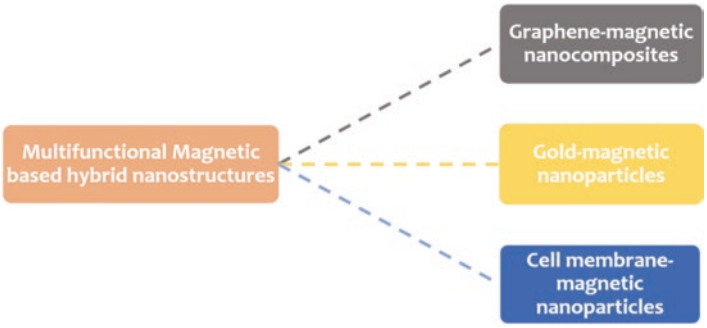

Introduction to Cancer and Nanomedicine

Cancer is a disease condition of abnormal cell growth that divides uncontrollably leading to infiltration and destroying other healthy cells (Ahmad et al., 2019). It is usually caused by many external or genetic factors affecting any part of the body, resulting in more than 100 types of cancer. Most importantly, it is the second most disease responsible for causing 9.6 million deaths worldwide by 2018 (Bray et al., 2018). And the World Health Organization (WHO) estimates 29.5 million (M) new cases and approximately 16.5 M deaths by 2040 globally. In Europe, more than 20% of deaths are caused by cancer every year (~1.7 M deaths/year), the estimated number of 5.2 M new cases, and 2.5 M deaths after cardiovascular diseases (Raza

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et al., 2019) is represented in Fig. 2.1. The conventional therapies possess a plethora of side effects and are incapable of circumventing the biophysical barriers posed by tumor microphysiology (Shahbazi et al., 2016). Also, more than 80% of patients reportedly show a poor response to conventional therapy, ultimately increasing their healthcare costs. So, there is an urge to develop novel treatments and therapies to treat cancer, which is currently being achieved by the field of revolutionizing nanomedicine.

Nanomedicine, an innovative and promising field, plays a vital role in the efficient treatment and diagnosis of cancer by overcoming the drawbacks of conventional anticancer therapy (van der Meel et al., 2019). Last three decades, scientists have experimented with and exploited a huge number of nano-based formulations specifically for cancer theranostics. This is made possible by employing various nanostructures as a site-specific targeting agent mainly affecting the cancer cells and sparing the healthy ones which made its prominent path in research and development. In specific, the versatility of multifunctional nanoparticles has gained much more interest in the field of cancer therapy which revolutionizes early-stage detection, treatment, and therapy becoming altogether a theranostic agent (Seeta Rama Raju et al., 2015).

This area of multifunctionality opened vast research on using various combinations of size, shape, surface functionalization, targeting molecule, drug attachment, linkers, etc. In most cases, the amalgamation of both organic and inorganic nanomaterials gave a distinctive property for its efficient use (Navya et al., 2019). These nanoparticles are more effective and specifically advantageous in various aspects. For instance, prolonged blood circulation time, improving stability and solubility, controlled and sustained release of drugs, decrease immune system activation, less cytotoxicity to various organs was investigated to have better knowledge and in-depth understanding of the system. This led to a major outbreak on biomedical purposes with their physicochemical properties leading to various clinical trials and practices. Based on the designing property of nanocarriers, it can be easily tuned to release the drugs by external manipulation such as heat and ultrasound also. Briefly,

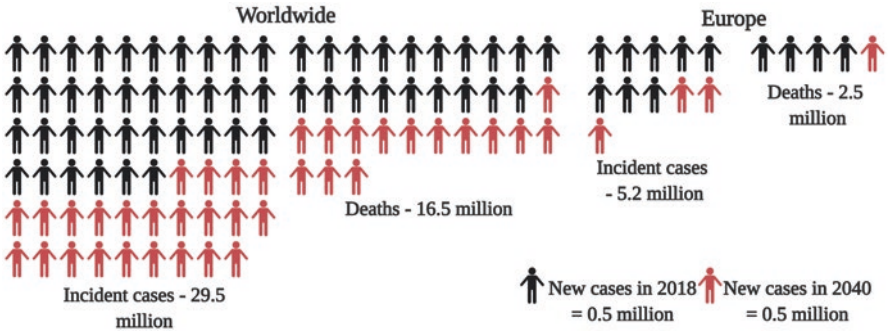

Fig. 2.1 Estimated number of incident cases and deaths from 2018 to 2040 worldwide and in Europe for all cancer types. (Created with BioRender.com)

the doped multimetallic, organometallic, and mesoporous nanostructure finds its way in the field of healthcare and biomedical applications. For example, in the case of magnetoplasmonic nanoparticles, it can function as a drug delivery, photodynamic, photothermal, hyperthermal, and contrast agent which is widely used from diagnosis to prognosis (Urries et al., 2014). This kind of nanoparticles is strategically designed to attain a diverse therapeutic property to increase the possibility of finding a cure and to improve the patients' health substantially.

The major impact of anticancer nanotherapies is usually superior to the conventional systems because of its site-specific targeting and biodistribution of drugs, thus minimizing the drug toxicity to healthy tissues. So far, extensive research gave a potential outcome in the nano-based therapeutic strategy such as chemo and radiation, gene, and immunotherapies. Thus, the multifunctional or a combinatorial therapeutic agent will lead to the future of cancer nanotheranostics and the possibility of personalized medicine approach which could have a great impact on a patient's health by largely minimizing the side effects of current life-disrupting therapies.

Multifunctional Magnetic-Based Hybrid Nanoparticles

Magnetic nanostructures are one of the widely used and studied in cancer therapeutics (drug delivery, contrast agents, and hyperthermia) (Knežević et al., 2019) because of their unique superparamagnetic properties, and it can be externally manipulated using a magnetic field to target and deliver the drugs in a site-specific region (Farzin et al., 2020). In some cases, magnetic nanoparticles (MNPs) have the inherent capability to differentiate the healthy and cancer cells based on their threshold electrical field which aids in inducing electroporation (Dehvari et al., 2018; Rodzinski et al., 2016). But it is mandatory for making it water-soluble to use it for biomedical applications which are usually done by post-surface functionalization process using surfactant molecules, polymers, etc. Thus, various materials were incorporated to have a synergistic effect for combinatorial therapy. So, here we have concentrated our spotlight on some of the successful and potential magnetic nanoparticle-based hybrids which include graphene, gold, and finally cell membrane-cloaking technology shown in Fig. 2.2. Some of the abovementioned hybrids are in the process of clinical translation which could have a major impact on the cancer patients in the future. Thus, we outline some of the major advancements and provide a current understanding for the readers.

Graphene-Magnetic Nanocomposites (GO-MNPs)

In the last few decades, the discovery of graphene received a wide interest in biomedical applications because of its inherent excellent properties like surface area, biocompatible, optical, electrical, mechanical, and thermal (Priyadarsini et al.,

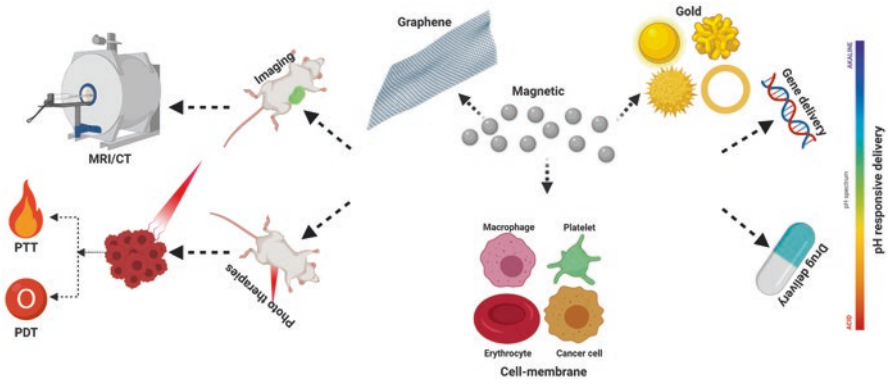

Fig. 2.2 Schematic representation of magnetic-based hybrid nanostructures and their applications in the cancer theranostics. (Created with BioRender.com)

2018; Banerjee, 2018). To be specific, 2D-based materials such as graphene, graphene oxide, and reduced graphene oxide along with various covalent and noncovalent surface functionalities, doping, and other derivatives are extensively studied in material science (Cheng et al., 2017). Also, the coupling of various metallic and organic nanostructures, such as gold, magnetic, upconversion, and polymeric structures, further enhances the functionality to be employed for cancer disease. Thus, the multifunctional behavior and the possibilities were explored for bioimaging and sensing, targeted gene and drug delivery, photothermal and dynamic therapy along with combination therapies, theranostics, and finally tissue engineering (Syama & Mohanan, 2019). Currently, GO's immense application in biomedicine has been exploited with the MNPs as a composite to apply in the nanotheranostics (Alegret et al., 2017).

Xie et al. designed a multifunctional layer-by-layer assembly of GO-MNP nanosheets loaded with doxorubicin for drug delivery and photothermal therapy (PTT) (Fig. 2.3). In most cases, GO-MNPs aggregate/precipitate under physiological conditions which prevent its efficiency. But this self-assembled structure was produced with the help of natural polyelectrolytes (chitosan and sodium alginate) to decrease the aggregation of particles and also to increase the stability in the biological medium which is highly suitable for biological applications. Interestingly, using both electrostatic interaction and via π-π stacking, the drug loading was maximum which is more than 137% w/w. This nanocomposite showed enhanced dispersion, pH-based drug delivery, and PTT effect. The drug delivery was maximum at acidic pH (pH 5) by decreasing the interaction between the composite and doxorubicin which is favorable for attacking the cancer tissues. The case of PTT at near-infrared (NIR) laser (808 nm, 1 W/cm^2) showed a concentration-dependent temperature increment to 51 and 53 °C at 100 μgmL^{-1} and 200 μgmL^{-1}, respectively. Thus, this novel self-assembled nanocomposite proves it to be a multifunctional and high-stable candidate for drug delivery and PTT (Xie et al., 2019).

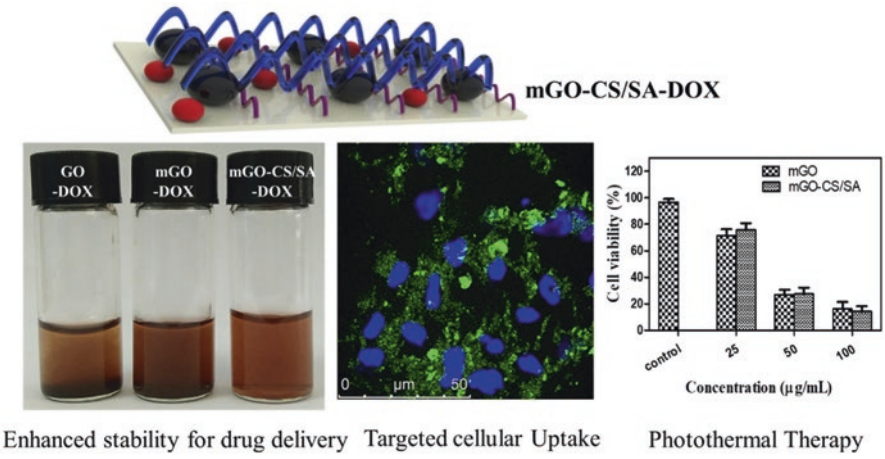

Fig. 2.3 Layer-by-layer assembly of GO-MNP synthesis, drug-loading strategy, stability, cellular internalization, and its applications as a PTT agent. (Reprinted with permission from Ref. (Xie et al., 2019) from Elsevier)

The superparamagnetic behavior of MNPs is widely employed for magnetic resonance imaging (MRI) (Zarepour et al., 2017; Stephen et al., 2011). There are very few studies where the researchers have developed and investigated GO-MNP nanocomposite to effectively employ it in MR-based imaging. So, Rodriguez et al. proposed a GO-MNP composite and exploited it for drug delivery, fluorescence, and MRI imaging. The authors synthesized multifunctional GO-MNPs labeled with doxorubicin as an anticancer drug for pH-based drug delivery system, exploit intrinsic fluorescence behavior of GO as a bioimaging agent and finally use the whole nanocomposite as a contrast marker in MRI. This system avoids the toxicity from the external fluorophores and increases the biocompatibility. From the results it is clearly shown that the drug loading of eight times less concentration than the usual amount resulted in ~62% killing of cancer cells. In case of in vivo MR imaging depicted, enhanced relaxivity values (~10.7) and optical pH sensing using GO fluorescence have been studied in vitro using different normal and cancer cell lines aided to track the doxorubicin internalization within 3 h of transfection with the signal difference of four- to fivefold representing the specificity of the nanosystem. Thus, this multimodal agent helps in drug delivery, tracking, and detection (Gonzalez-Rodriguez et al., 2019).

Last three decades, heavy metal gadolinium-based contrast agents for MRI have been widely used which have accumulated in most of the patient's tissue, especially in vital organs such as the brain, bone, and kidneys. The detrimental effects are seen with kidneys leading to renal toxicity (Rees et al., 2018). So now it is time to shed a spotlight on other non-toxic contrast agents. To address this issue, recently Luo et al. synthesized 5-nm-ultrasmall superparamagnetic MNPs which are functionalized with nano-GO and doxorubicin. This composite was employed for dual applications like pH-based chemotherapy and T1 MRI agent. The drug release was based

on the pH responsiveness which differentiates the tissues from healthy and cancerous. The MR imaging in vivo studies were evaluated which shows the MNPs greatly contribute to r1 rather than r2 value resulting in high-resolution T1-weighted imaging which is due to the size of the nanoparticles. Later using cell counting kit-8 assay, renal and hepatic function tests were carried out to determine the cytotoxicity of the nanocomposite showing more than 90% of cell viability. This proves that the developed composite has a promising application as a T1 contrast agent (Luo et al., 2019) depicted in Fig. 2.4.

Recently, a versatile chemo-photodynamic platform was developed and studied its efficiency by Vinothini et al. In this work, reduced GO was used and decorated with the MNPs subsequently with a cancer drug camptothecin and a photosensitizer 4-hydroxycoumarin for a combinational therapy. The drug release was evaluated based on the pH-sensitive system and the photodynamic therapy (PDT) using a 365-nm laser. The cytotoxicity of the nanocomposite was also determined using both healthy and cancerous cell lines. One of the major advantages of this design is to enhance the photo-irradiation behavior and stimuli-based drug release for effective synergistic effects on the cancer cells rather than the monotherapy. From the studies, it is determined that the synthesized nanocomposite is highly cytotoxic for its effective employment in both in vitro and in vivo studies. The drug release was maximum at the acidic pH which is based on the environment-based stimuli response. The PDT effect of 4-hydroxycoumarin in rats induced with breast cancer, under laser irradiation for a short period, showed an enhanced effect in vivo by generating reactive oxygen species (ROS), along with both nuclear and cellular damages, thus

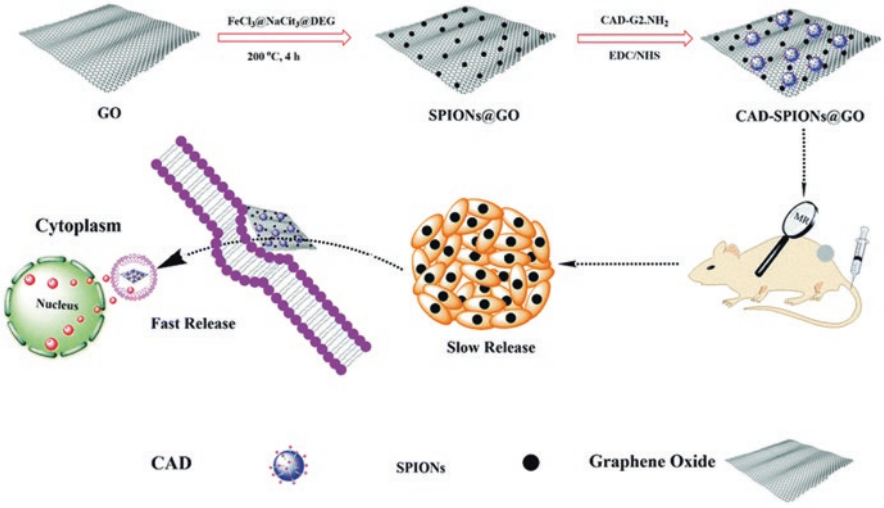

Fig. 2.4 Schematic illustrations of the multifunctional GO-MNPs with doxorubicin loading and testing its efficacy in breast cancer model for both tumor diagnosis and therapy. (Reproduced from Ref. (Luo et al., 2019) with permission from The Royal Society of Chemistry)

proving it to be an extraordinary and synergistic composite for dual therapies (Vinothini et al., 2020).

Most of the research is carried out on NPs based on its functionalization for cancer therapeutics with less interest in microsphere (MS) structure. In some studies, MSs were employed instead of NPs because of high porosity and surface area which can be highly suitable to maximize the loading of drugs, PDT agents, and other moieties. In the present investigation, the authors proposed an advanced approach to developing a chemo-photothermal nanocomposite system using MSs with hollow-reduced GO intended to use it for PTT near-infrared (NIR)-light-responsive system (Fig. 2.5). And interestingly this study employs a 3D-reduced GO-connected structure, where very few reports have been published on this kind of structure. The fabrication of this system involves various processes, where reduced GO synthesis initially using a spray dry method using silicon dioxide which is later etched to obtain GO hollow MSs. Then the MNPs and doxorubicin were decorated on the reduced GO MSs. The resulting sphere exhibited a very high surface area (~120 m^2g^{-1}) and pore volume (~1 cm^3g^{-1}), which is advantageous for doxorubicin loading (~18%). The on-off pattern of NIR triggered PTT-induced enhanced conversion efficiency. The combinatorial chemo-photothermal therapy showed higher cytotoxicity in vitro confirming an enhanced antitumor efficiency of the MS-based drug delivery system (Liang et al., 2020).

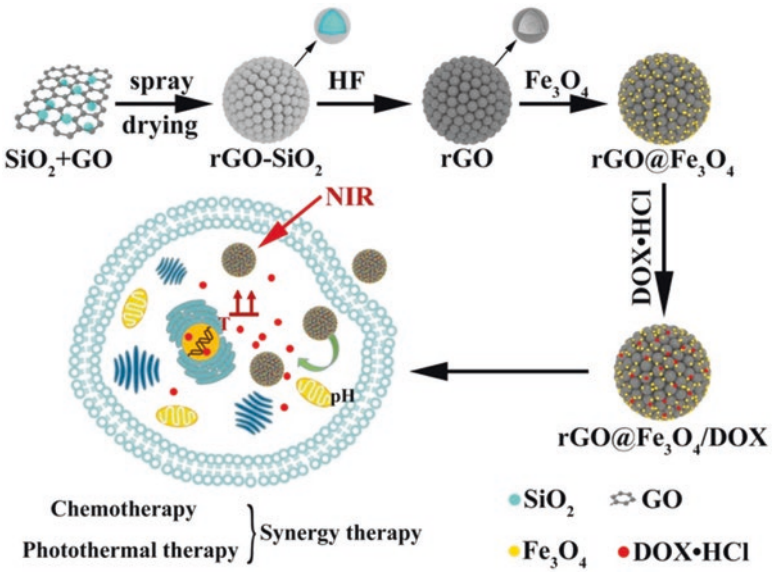

Fig. 2.5 Schematic illustration representing the design and synthesis of reduced GO-MNPs with doxorubicin decoration for combined photothermal and chemotherapy for tumor inhibition (Liang et al., 2020)

Gold-Magnetic Nanocomposites (Au-MNPs)

Noble metals have always created a niche in the field of biomedical applications (Yaqoob et al., 2020; Chugh et al., 2018), and it is the earliest known nanostructure to the field of nanotechnology. In specific, gold nanomaterials have always made their contribution and have also become an inevitable research topic in various sectors. Gold nanoparticles (GNPs) are widely celebrated and exploited for their tunability in shape, size, and surface chemistry, biologically non-reactive, and most importantly localized surface plasmon resonance (SPR) resulting in extraordinary optical, electronic, and thermal properties. Thus, novel synthesis and bioconjugation methods were employed in order to extend its suitability for in vivo diagnostics and therapeutics (Fan et al., 2020; Elahi et al., 2018). In the field of cancer theranostics, GNPs are constantly studied with the focus of using them as a drug delivery carrier, tumor imaging, photothermal, radiofrequency, and antiangiogenic therapies aiming to eradicate the disease (Peng et al., 2019; Sztandera et al., 2019). Thus, the researchers came out with a concept of a unique multimodal platform using the amalgamation of both MNPs and GNPs to synthesize majorly a core of MNPs and GNPs of various morphologies like satellite/shell/hybrid/star/rods or Janus-shaped nanostructures for treating, diagnosing, and preventing cancer. These magnetoplasmonic nanoparticles with proper design and biofunctionalization provided an innovative approach in site-specific targeting, diagnostic tools (MRI, computed tomography (CT), Raman, and photoacoustic), and therapies (PTT, PDT) (Manisekaran, 2018; Das et al., 2019). In this section, we chose some of the promising investigations and discussed them in detail.

Core-shell NPs are quite often employed for cancer theranostics (Mukherjee et al., 2020; Dhas et al., 2018). But the gold encapsulation is a tedious process to ensure complete coverage, and some researchers employ the iteration procedure to protect the MNPs from reactivity, oxidation, and other environmental factors (Wang et al., 2008). Our group has designed a core-shell NPs using an iterative-seeding method five times with co-functionalization of targeting agent-folic acid and anticancer drug-doxorubicin as a multifunctional nanovehicle for cancer theranostics. This process enhanced stability, cytocompatibility, and SPR compared to one-step procedure coating. The nanovehicle exhibited a first-order rate kinetics of drug profile at an endosomal pH, as an efficient T2 contrast agent in case of in vitro studies with the cancerous cell line and finally showed an increased temperature during the in vitro hyperthermia and chemohyperthermia which is capable enough to kill the cancer cells within a short span of time. This proves it to be a potential candidate as a versatile agent for cancer theranostics (Das et al., 2019).

Anisotropic gold nanostructures (nanostar, rods, plates, flowers, cubes, etc.) are one of the most fascinating discoveries in material science which has been widely exploited in the last two decades. These structures are synthesized using the seed/seedless method by employing various polymers or surfactants, reducing agents chemically, or green approach (Ortiz-Castillo et al., 2020). The impact of slight morphology change is clearly seen majorly in the SPR adsorption mostly shifting

from visible to NIR region which can be readily used for nano-bio-interface reactions (Stone et al., 2017). Some investigations used these structures along with MNPs to the next level of cancer theranostics (Singh et al., 2018).

Gold nanostars are efficiently used for surface-enhanced plasmon resonance (SERS)-based applications because of their hot-spot concept with signal enhancement in the case of diagnostics (Tian et al., 2016). But, Hung-Yu et al. designed a multifunctional platform using gold nanostar-coated MNPs with the surface functionalization of folic acid and employ three different versatile imaging agents (MR/CT/photoacoustic-PA) and for PTT of cancerous tissues schematically illustrated in Fig. 2.6. Interestingly, this nanostructure possessed enhanced stability, heme, and cytocompatibility. After evaluating and finding the excellent characteristics of the NPs, it was further used as an imaging marker both in vitro and in vivo. For example, it expressed a high r2 relaxivity with the value of 549.07 mM^{-1} s^{-1} because of the superparamagnetic behavior of MNPs. Then in the case of CT phantom studies and PA imaging, the nanostar concentration is directly proportional to the signal enhancement which is calculated using the CT value and PA signal intensity vs nanostar concentration, respectively, and finally with PTT (808-nm laser, 1 W/cm^2, 300 s) expressed a temperature increment based on the nanostar concentration reaching up to 63.3 °C at 20 mM. It is also seen that by increasing the laser power to 1.5 W/cm^2 the temperature raised to 68.5 °C. Thus, it proved its multifunctionality and extended its possibility for different cancer types with translational medicine applications (Hu et al., 2016).

Multifunctional MNP-based Janus nanoparticles are exceptionally important because of their inherent magnetic, chemical, optical, and electronic properties (Bradley et al., 2016). Chen et al. investigated magnetoplasmonic Janus NPs for trimodal imaging and therapy of cancer cells with good stability and biocompatibility (Fig. 2.7). The synthesized structure resulted in a strong SPR band (786 nm) in the NIR region which makes it highly suitable for PTT. The NIR laser irradiation on Janus NPs which is incubated with the cells showed an instant temperature increment of 42 °C which resulted in damage and irreversible death. And interestingly, the authors say more than 40% of photothermal conversion efficiency value compared to other methods. The in vitro trimodal imaging in this study is aimed at

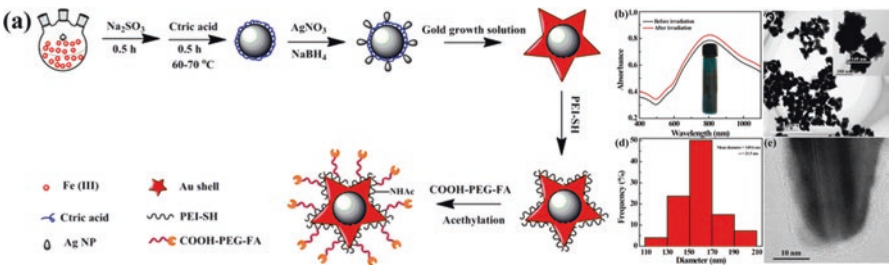

Fig. 2.6 Schematic illustration depicting the synthesis of versatile Au nanostar MNPs with folic acid (**a**) and their characterization representing the stability, morphology, and size distribution (b–e, **b**) (Hu et al., 2016)

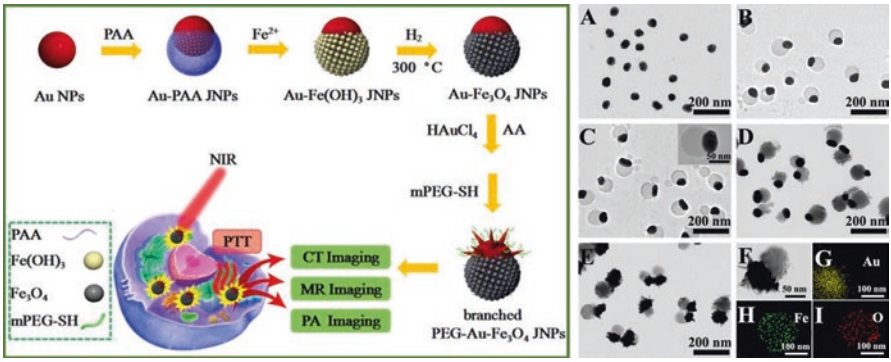

Fig. 2.7 Schematic illustration of the Au-MNP-branched Janus nanoparticles for trimodal imaging agent and for in vitro PTT (**a**). TEM images of individual nanostructure and final nanoparticles along with its elemental mapping (A-I, **b**). (Reproduced from Ref. (Chen et al., 2017) with permission from The Wiley)

enhancing diagnostics featuring high accuracy, practicability, and efficacy. GNP's inherent higher X-ray absorption coefficient was exploited to use it as a CT imaging agent, where the signal enhancement was purely dependent on gold concentration. From the evaluation, it is identified that only 20 mg/ml was enough to have a similar signal with that of 300 mg/ml of conventional iodine. The magnetic property of MNPs was used for MR imaging which gave rise to an r2 relaxivity value of 93.5 mm^{-1} s^{-1} than the usual core-shell NPs. The Janus NPs represented a high PA signal attenuation leading to enhanced PA imaging based on the concentration, thus proving it to be an exceptional nanoplatforms for cancer theranostics (Chen et al., 2017).

Innovative nanostructures are designed by researchers aiming to synthesize an "all-in-one" multifunctional platform in the field of cancer theranostics. One such example is developing a hybrid magnetoplasmonic NPs which is made up of octahedral-shaped MNPs with spherical GNPs which is shown in Fig. 2.8. This dual platform was functionalized with two different fluorescent dyes (Cy5 and Nile red) and an anticancer drug (doxorubicin) for effective tracking and cargo delivery simultaneously. The major advantage of the octahedral MNP's shape represents very high magnetization and becomes perfectly suitable for MRI contrast agents. The capability of hybrid NPs was tested both in vitro and in vivo effectively. In the case of in vitro drug delivery, the release profile was a little less when compared to the free drug due to the time taken for passive diffusion, internalization, with the cell membrane, and finally delivering the drugs at the endolysosomal pH effectively. So, the authors studied the internalization based on the enhanced permeation and retention (EPR) effect which was found to be 3% in a period of 24-h injection. The site-specific drug release was tracked using Cy5 dye which accumulated majorly in the tumorous tissue rather than the normal cells and proving its specificity. MR imaging in both in vitro and in vivo studies showed that they express high r2 relaxivity values

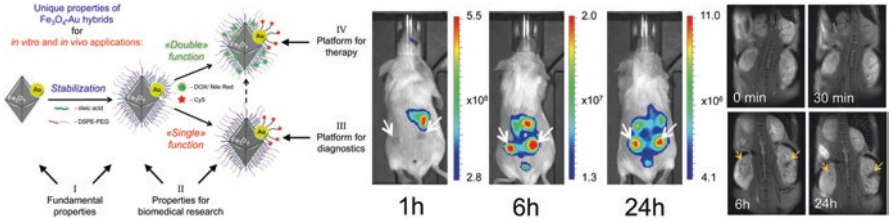

Fig. 2.8 Synthesis of Au-MNP's all-in-one platform as a theranostic agent (**a**), in vivo studies on NP accumulation in tumors (**b**), and photographs of T2-weighted MRI contrast agents (**c**) detected at various time intervals (Efremova et al., 2018)

which are due to its angular structure, high crystallinity, and magnetization effect (Efremova et al., 2018).

Cell-Membrane-Camouflaged Magnetic Nanoparticles (CmC-MNPs)

The nanomedicine sector is escalating tremendously and rapidly aiming to treat all life-threatening illnesses but still features a certain limitation of translation to clinical use efficiently. Thus, very few nanoformulations are successfully passed from clinical trials to the Food and Drug Administration (FDA) approval for patient use. Some of the important aspects are blood circulation time, toxicity, activation of an immune response, uptake by the reticuloendothelial system (RES), and not site-specific. To overcome these issues, many biocompatible polymer-based formulations are administered (multiple times) with major FDA-approved products for many diseases. Nonetheless, it is necessary to find an efficient system that ultimately leads to biomimicry for designing a nanosystem composed of both natural and artificial nanomaterials (Li et al., 2018b). This quest is responsible for the state-of-the-art technology of cell membrane coating/camouflaging nanocarriers reported in 2011 (Hu et al., 2011), and its gradual development in the exploitation of various cell membrane timelines is depicted in Fig. 2.9. The cell is one of the life fundamental units with various biological moieties responsible for complex functionalities whose membrane can be employed for effective biointerfacing (Fang et al., 2018).

This novel technology employs various cellular membranes such as erythrocytes, leukocytes, platelets, immune cells, cancer cells, stem cells, fibroblast, etc. which are obtained by hypotonic isolation, thawing, or ultrasonic disruption. Then by using either membrane extrusion, electroporation, or ultrasonic treatment, it is fused with the desired NPs and the membrane of interest-based on applications or targeting the disease, thus resulting in numerous filing of patents and clinical studies (Liu et al., 2019). Considering cancer therapy, CmCs are widely investigated with tremendous possibilities involving different cell membranes for drug and gene delivery, imaging, photo- and immunotherapies, etc. (Wu et al., 2019; Pereira-Silva

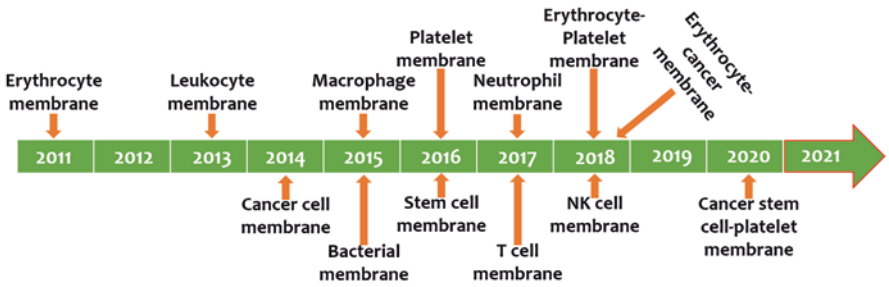

Fig. 2.9 Timeline of major advancements in the development of cell membrane-encapsulated NPs in a decade (Chen et al., 2020). (Adapted with permission from Ref. (Chen et al., 2020) from Elsevier)

et al., 2020) Being a versatile theranostic tool, MNPs with various cell membrane encapsulations along with a wide range of surface functionalization are widely employed for immunotherapies because of their inherent properties like real-time characterization, magnetic navigation, monitoring cellular responses, etc. (Cheng et al., 2020), and we present in brief some of the outstanding achievements on CmC-MNPs.

One of the most interesting and alternative ways of encapsulating NPs was by a cancer cell membrane which avoids the tedious and biofunctionalization process. This structure helps stability, biocompatibility, and readily cellular uptake. So, Li et al. designed a cationic superparamagnetic iron oxide nanoparticles (SPIONs) functionalized with photosensitizer and in turn encapsulated with human hepatocellular carcinoma line cells and evaluated (in vitro and in vivo) antitumor efficacy and dual-mode MRI/NIR fluorescence imaging (Fig. 2.10). The synthesized nanocarrier represented high stability, better imaging agent in MRI, and generated ROS under 670-nm irradiation with anticancer effect (Li et al., 2018a).

Most of the studies employ post/surface functionalization to convert the hydrophobic nanoparticles to hydrophilic for efficient coating of the extracted cellular membrane. So, one of the recent investigations deals with the oil-to-water microemulsion to transfer hydrophobic semiconductor-based MNPs using well-known and highly exploited red blood cell (RBC) membranes to employ NIR-mediated PTT and MRI agents as shown in Fig. 2.11. Surprisingly this study aims to develop a nanostructure to use in the NIR-II window instead of conventional NIR-I-based PTT and also to enhance molecular imaging through MRI. Thus, in the case of PTT, it largely minimizes the scattering of light and increases the tissue adsorption favorable for efficient therapy with no recurrence of cancer cells. Therefore, the authors camouflaged the SPION@$Cu_{2-x}S$ nanoparticles with RBC membrane because of their outstanding circulation time, accessibility, and most importantly low immunogenicity (cluster of differentiation-CD47 and CD59 surface markers). The synthesized nanocluster was evaluated for in vitro PTT using 1064-nm laser irradiation which represented a photothermal conversion efficiency of 69.6%, and in the case of MRI, it resulted to be an excellent T2-weighted image agent with an r2 value of

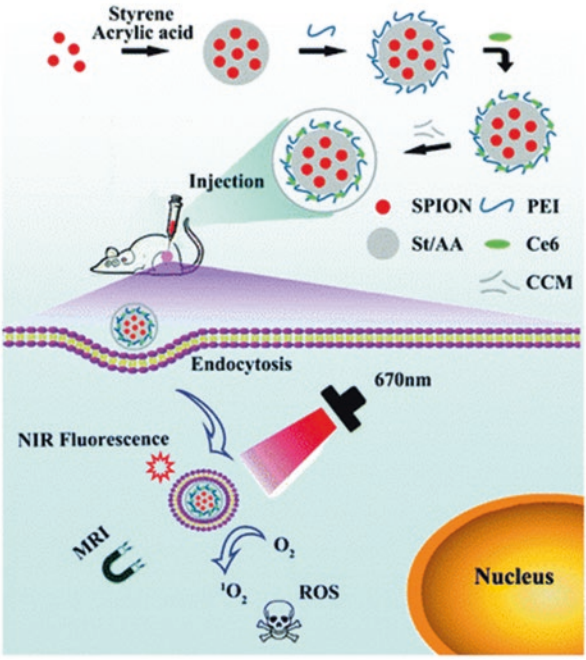

Fig. 2.10 Schematic images of the cancer cell membrane-coated NPs for dual-modal imaging and PDT at 670 nm. (Reproduced from Ref. (Li et al., 2018a) with permission from The Royal Society of Chemistry)

130 mM^{-1} s^{-1}. Later, in vivo studies were conducted in BALB/c nude mice carrying HeLa tumors; under laser irradiation, the temperature in the tissue escalated rapidly and reached to 54.1 °C (10 min) when compared with control groups. In a 7.0 T MRI scanner, NP-treated mice were exposed after 24 h post-injection, representing an enhanced region of interest (ROI) intensity in the presence of a magnetic field with high accumulation and relaxivity. This proves the possibility of next-generation CmC-based MNPs with futuristic applications (Lin et al., 2020).

Last few years, the field of cancer immunotherapy is gaining its pace to become a novel and new universal therapy for the treatment of various cancer types by stimulating the immune system to diagnose and assault the tumor cells (Chen & Mellman, 2013). Considering cancer immunotherapy, there is a wide range of studies but lacking its potentiality in clinical approval and success. This is due to some of the major blockades, such as dealing with the highly complex tumor microenvironment (TME) which is caused by the tumor-associated macrophages (TAMs) and immunosuppressive cells (Van Der Burg et al., 2016). Thus, many researchers are developing a novel approach using NPs to produce an immune response like vaccines to generate antigen-specific responses to increase the efficacy of immunotherapies. Recently, a kind of cell death process has been identified known as ferroptosis which can be induced by the glutathione peroxidase 4 (GPX4) resulting

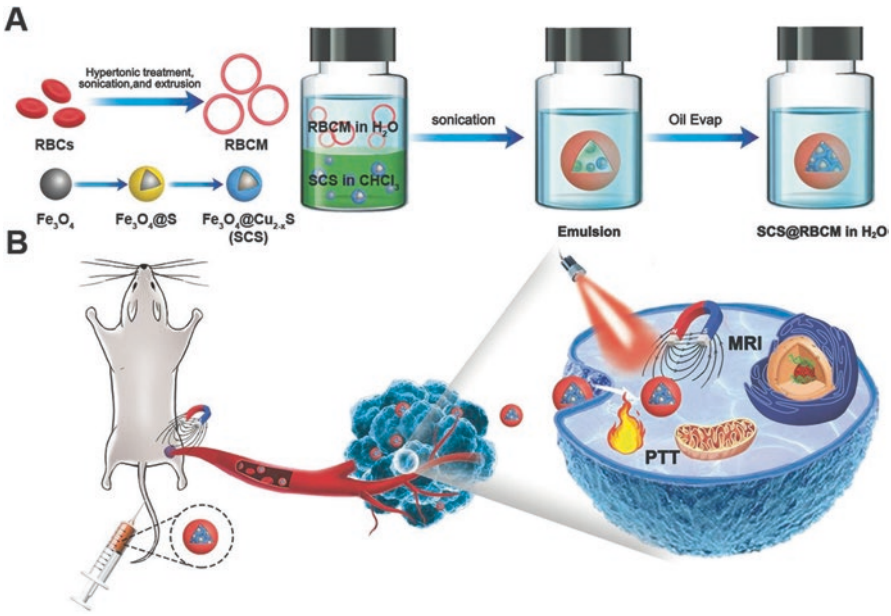

Fig. 2.11 Schematic illustration of the synthesis of RBC membrane-encapsulated NPs (**a**) employed as an MRI and NIR-II PTT agent (**b**). (Reproduced from Ref. (Lin et al., 2020) with permission from The Royal Society of Chemistry)

in the production and scavenging of ROS leading to lipid peroxidation in the cancer cells (Dixon et al., 2012). Based on this tactic, Jiang et al. designed a platelet membrane-camouflaged drug (sulfasalazine)-modified MNPs to employ it for in vivo ferroptosis-based immune checkpoint blockade therapy. The choice of platelet membrane is so specific because of its inherent advantage and increasing circulation time with less hepatic uptake.

The authors evaluated the cytotoxicity, cellular uptake, and production of lipid peroxide and finally assessed the ferroptosis both in vitro and in vivo to determine the efficacy of the designed biomimetic NPs. In general, the NPs are highly efficient not only in evasion from the immune system but also showed enhanced uptake by the metastasized tumor tissues. The in vivo study showed that the biomimetic NPs can enhance the process of ferroptosis by inhibiting certain pathways to increase the efficacy of blockade therapy, thereby decreasing the tumor growth, and the mechanism is depicted in Fig. 2.12. Thus, this novel system proved it to be clinically promising for combating cancer in a unique way rather than the common single therapies (Jiang et al., 2020).

In immunotherapy, TAMs play a vital role including tumor development, invasion, and migration of the cells, thereby inhibiting antitumor immune reaction. The TAMs are classified into two types, M1 and M2, cells for inhibiting and promoting tumor growth, respectively. But in most solid tumors, M2 dominates in tumor tissue development and accounts for the poor prognosis of patients (80%) (Bingle et al.,

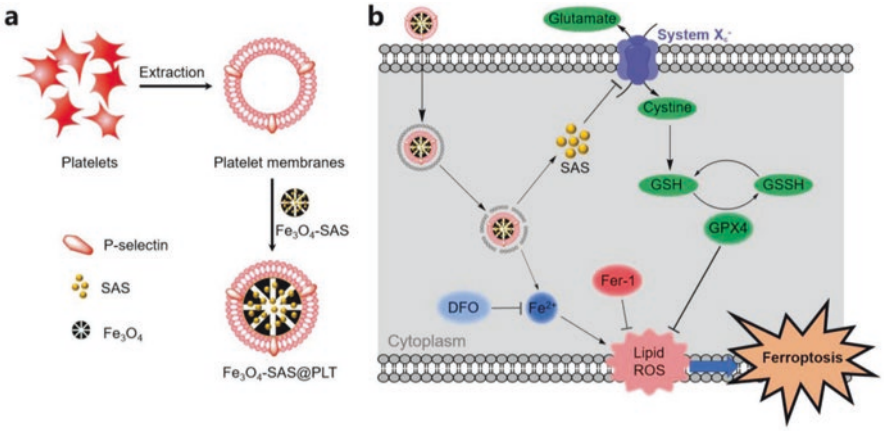

Fig. 2.12 Schematic images showing the synthesis of platelet membrane-camouflaged MNPs (**a**) and mechanism of cell death by ferroptosis in metastasis tumors (**b**). (Reproduced from Ref. (Jiang et al., 2020) with permission from The Wiley)

2002). Thus, the nanomedicine field aims to regulate M1 type using novel drug carriers making it effective for TAM-based therapy. In specific, the keen interest among researchers is to promote the TAM polarization to M1 type dominantly for effective immunotherapy.

In the recent study, a biomimetic nanocarrier was developed to target breast cancer using MNPs with certain other specific markers encapsulated using the macrophage membrane to target with high specificity and for polarizing TAMs. This nanocarrier was composed of multiple elements, such as polymeric PLGA coating of MNPs which is encapsulated with imiquimod (R837), and then the whole structure is in turn engulfed using lipopolysaccharide-treated macrophage membrane. In this design, MNPs and R837 are used for macrophage stimulation by interferon regulatory factor 5 and nuclear factor kappa-B pathway, respectively. These help in enhancing the polarization of macrophages synergistically. Thus the in vivo studies showed the uptake of nanocarrier helps mainly in the TAM polarization from M2 to M1 which was confirmed by analyzing various signaling pathways. From the evaluation, it is seen that the ratio of M1/M2 polarization effect was found to be 2.88 by the synergetic effect of MNPs and R837 effectively. Thus, this extraordinary nanosystem shows an effective and enhanced antitumor effect and opens a novel line of research by manipulating TME for future cancer immunotherapy (Liu et al., 2020) (Fig. 2.13).

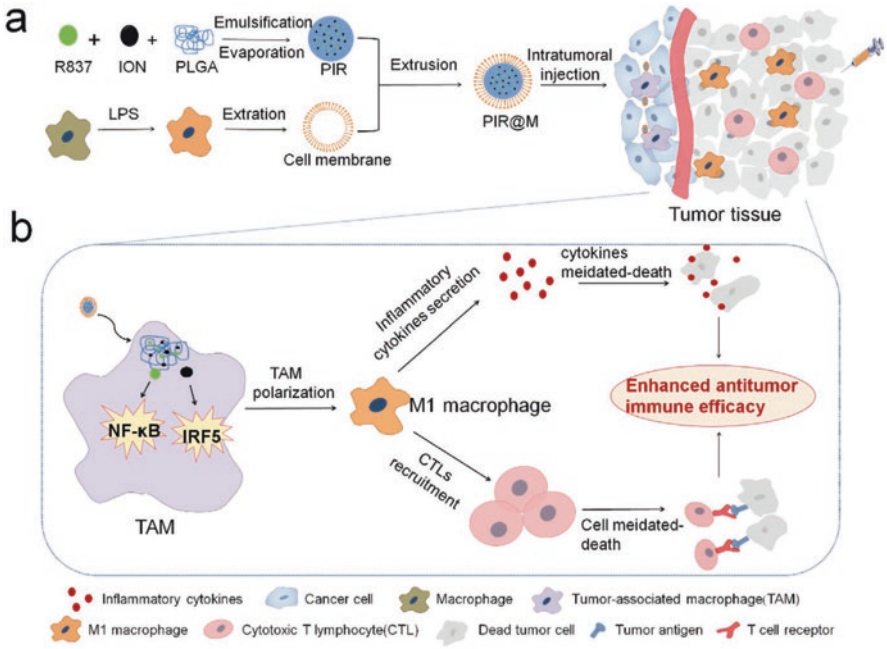

Fig. 2.13 Schematic illustrations of macrophage cell membrane-coated MNP nanocarriers (**a**) and the mechanism depicting the function of NPs responsible for the TAM polarization through various signaling pathways (**b**). (Reproduced from Ref. (Liu et al., 2020) with permission from The Wiley)

Prospects and Challenges

This chapter describes some of the major developments and potentials of magnetic-based hybrid nanostructures including their design, strategies, and multifunctionalities in a broad spectrum. The different aspects of nanomaterials are widely exploited based on the area of interest and field of applications to fabricate an individual nanocarrier. Nonetheless, the field of nanomedicine has expanded enormously but with various limitations to make it to clinical transformations or the market for human use. But currently, the scenario is changing majorly because of more out-of-the-box and biomimetic approaches. For example, the cell membrane technology gives an opportunity to use various cellular membranes, thereby helping in overcoming the major drawbacks of the conventional nanocarriers, considering all the abovementioned structures which provide huge flexibility and uniqueness in fine-tuning of their properties to increase the cargo-carrying efficiency. Thus, this kind of platform aids in specific targeting and attacking the cancerous cells instead of normal cells.

Hybrid-based nanostructures have enormous potential in cancer therapy but limited by a number of challenges preventing them from the clinical translation. First, the synthesis technique or production of these nanostructures is not completely

mature considering the nano-bio-interface and biomimetic behavior, because the process of scaling-up or manufacturing on an industrial scale needs more precision and reproducibility in a high manner rather than the lab-scale level. Next, the major point is resources in the case of cell membrane technology, which is from a biological source. Each time the extraction protocol depends on the infrastructure, and sophistication of the workplace seems to be a major deal to obtain high-quality and sterile membranes. Then most importantly for the gram scale-up method, the culture technique must expand to meet the need to have a sufficient membrane. And novel strategies should be developed to isolate autologous blood cells to avoid the immune response of the structures. Then comes the critical factor in maintaining the purity and storage of the extracted cellular membranes which must be carried out aseptically for extended use. In the case of both graphene- and gold-based structures, more in-depth studies are unavoidable to have a concentration-dependent dose-response, biocompatibility, shelf-life, immune responses, and large-scale production techniques.

Despite several challenges, these hybrid nanoparticles which result from the amalgamation of both metallic and biomimetic structures establish the most outstanding and advantageous strategies for various types of applications in cancer therapy, such as targeting drug delivery, contrast, and phototherapy agents, with magnetic field manipulation to the precise location for enhanced therapeutic efficacy. This confirms the promising characteristics of these nanostructures and their potential to be a boon in nanomedicine. But in the coming days, the researchers are held to resolve and tackle certain issues associated with nano-based drug delivery systems pharmaco-kinetics, bio-interphase, and safety, in the human body to increase and improve the clinical translation prospects. Therefore, with these great efforts and measures, an excellent, safe, magnetic-based hybrid nanoparticle will become a favorable multifunctional platform in cancer.

Conclusion

Hybrid magnetic nanoparticles have reached a tremendous advancement in the last decade with various contributions from different nanostructures starting from semiconductor to metal. These novel structures with a huge potential can aid in clinical translation due to their inherent optical, magnetic, and structural properties as a versatile theranostic platform. The magnetic nanoparticles have become an inevitable nanostructure in the cancer theranostic field, and the researcher has a wide variety of opportunities to play and deliver a power-packed outcome. Thus, this chapter summarized some of the developments which mainly involves in the anisotropic structures of graphene (layer by layer, sheet, encapsulation), gold (star, shell, rods, particles), and most importantly the upcoming cell membrane technology (cancer cell, erythrocyte, macrophage membrane, platelet membrane) with their extended possibilities. The advantage of these nanostructures is based on the synthesis technique with high tunability in sizes, shapes, surface properties, and

cloaking mainly to decrease the toxicity, nonspecific cellular internalization, enhance their targeting specificity/efficacy, finally increase blood circulation time, and minimize or avoid immune responses. Thus, a multifunctional or all-in-one hybrid structure can be responsible for future clinical transformations with a major impact on tumor imaging, drug targeting/delivery, and therapy in cancer patients. Future research and promising clinical trials are focused on overcoming the drawbacks and hurdles to develop innovative, novel safe, and efficient therapeutic/eradication methods soon.

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Chapter 3
Nano-oncology: Clinical Application for Cancer Therapy and Future Perspectives

Priya Singh and Sanjeeb Kumar Sahoo

Introduction

Cancer is a multifactorial disease involving dynamic changes in the genome due to defects in regulatory pathways that control normal cell homeostasis and proliferation. The consequence of such defects is uncontrolled proliferation of cells which invade normal tissues and organs and eventually metastasize to spread throughout the body (Hanahan & Weinberg, 2000). Despite significant understanding of mechanisms underlying the disease and technological and therapeutic advances, cancer is still the second main cause of death globally (https://www.who.int/news-room/fact-sheets/detail/cancer).

There are several factors which govern poor treatment outcomes. One of them is late detection of cancer, which significantly increases the mortality rate. The available diagnostic techniques include imaging (via X-ray, computed tomography (CT), endoscopy, magnetic resonance imaging (MRI), and ultrasound) and morphological analysis of cells or tissues. These imaging techniques can detect cancer at a stage when there are visible changes in the tissues, but by that time, several cancer cells have already proliferated and some have even metastasized. Moreover, these imaging techniques cannot distinguish between malignant and benign tumors. On the other hand, morphological analysis of tissues by histopathology or cytology cannot be used independently for early diagnosis of cancer (Zhang et al., 2019). Another reason for treatment failure is that conventional chemotherapeutics have nonspecific distribution and poor drug delivery to the target site which causes severe side effects and eventually leads to multidrug resistance (Wang et al., 2008). Further, the new class of anti-neoplastic drugs are antibodies, proteins, and nucleic acids which are unstable in vivo and undergo rapid degradation before reaching the target site (Mu & Yan, 2018). Another major reason for poor treatment outcomes is metastasis and

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relapse which are responsible for 90% of cancer-related deaths (Seyfried & Huysentruyt, 2013). Research evidence suggests that a small sub-population of cells called cancer stem cells (CSCs) are responsible for driving metastasis and relapse because of their inherent ability of self-renewability, differentiation, and chemoresistance. Available chemotherapeutic agents target only rapidly proliferating bulk cancer cells, thereby enriching slow-cycling CSCs in the tumor mass which then reseed new tumors (Singh et al., 2017; Mohapatra et al., 2020). Accordingly, there is a requirement for a more sensitive and accurate diagnostic method, specific targeting, efficient drug delivery, and new therapies targeting CSCs for reducing the morbidity and mortality associated with the disease.

Nanotechnology is a branch of science which amalgamates physics, chemistry, biology, and engineering for the development of a diverse array of nanoparticles which find application in varied fields including medical and healthcare applications (Ventola, 2012; Sahoo & Labhasetwar, 2003). These nanoparticles have at least one dimension in size range of 1 to 100 nm, which imparts them with unique physical, structural, and optical properties that enable them to interact with the biological system at a molecular level. The material composition of nanoparticles is such that they have the ability to self-assemble and can maintain stability and specificity (Wang et al., 2008). By virtue of these properties, nanotechnology can offer significantly advantageous applications which can address the unfulfilled clinical needs of oncology (Di Lorenzo et al., 2018).

In this chapter we have addressed first how nanotechnology can transform oncology; second, types and characteristics of different nanoparticles which can be used for diagnosis and treatment of cancer; and lastly, we have illustrated the current state of the art of nano-oncology with discussion on nanoformulations which are in the market or are undergoing clinical trials. In addition, it also sheds light on the challenges in clinical translation of nanomedicine and future perspectives.

Nano-oncology: Transforming Cancer Therapy

The application of nanotechnology for detection, diagnosis, and therapy of cancer is termed as nano-oncology. The two major goals for achieving better cancer treatment are to increase the targeting selectivity and enable the therapeutic or diagnostic agents to reach the target site overcoming the biological barriers (Ferrari, 2005). If nanotechnology is employed efficiently with established cancer research, it can serve as an excellent tool to accomplish the above goals. Nanoparticles are capable of selective targeting by employing passive or active targeting which prevents damage to healthy tissues and also helps the drug reach the target site with minimal loss in activity (Bamrungsap et al., 2012; Sahoo et al., 2007). In passive targeting, the nanoparticles take advantage of the unique pathophysiological properties of tumor vasculature such as enhanced permeability and retention (EPR) effect (Wang et al., 2008). In comparison to normal tissues, the vasculature of a tumor is leaky as it is composed of poorly aligned defective endothelial cells with gaps as large as

600–800 nm. Further, lack of smooth muscle layer and hyper production of vascular mediators like vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), bradykinin and others result in hyperpermeable vascular architecture which allows nanoparticles to extravasate and accumulate in tumor, and since the lymphatic drainage is poor, it allows for longer retention. This phenomenon is termed as EPR effect (Greish, 2010; Acharya & Sahoo, 2011). It is observed that when a drug is delivered through nanoparticles, there is approximately a tenfold or higher increase in drug accumulation in tumor as compared to its native form (Northfelt et al., 1996). However, there are several factors which affect the accumulation of drug by this phenomenon like biocompatibility, surface characteristics, size, and circulation half-life of nanoparticle and the degree of angiogenesis, size, type, and location of tumor (Maeda et al., 2013; Prabhakar et al., 2013). Because of this prevailing heterogeneity in EPR, an alternative strategy of active targeting was evolved. In active targeting nanoparticles are conjugated with targeting moiety that specifically binds a receptor or antigen which is exclusively present or overexpressed in cancer cell surface, thereby increasing the selectivity of drug for cancer cells. Thus, in totality it can be said that both the strategies increase the selectivity of the drug, but passive targeting increases the accumulation of nanoparticles in the tumor microenvironment, whereas active targeting facilitates the uptake of nanoparticles directly by cancer cells (Bazak et al., 2015).

Further, nanotechnology can not only bestow selective targeting but can also be used to increase the pharmaceutical properties of drugs like solubility, stability, and circulation half-life drugs. It can also enable sustained drug release, help in delivery of bio-macromolecular drugs like antibodies and genes, co-delivery of multiple therapeutic agents to overcome multiple drug resistance (MDR) (Parhi et al., 2012), and provide more sensitive detection, diagnosis, and imaging of cancer (Shi et al., 2017; Parveen et al., 2012). These will be discussed in detail in subsequent sections. Thus, it can be rightly said that nanotechnology-based therapeutics can significantly improve the treatment outcomes in oncology and have the potential to reshape or transform the present stature of cancer therapy.

Nanotechnology Toolbox for Cancer Therapy

Before proceeding to the application of nanotechnology in oncology, it is essential to have an overview of current nanotechnologies available at our disposal. Nanoparticles can be made from diverse materials like lipids, polymers, inorganic metals, ceramics, and others. Depending on the type of material used and the method employed, nanoparticles vary in size, shape, and properties (Wang et al., 2008). This section describes various types of nanoparticles available for cancer therapeutics (Fig. 3.1).

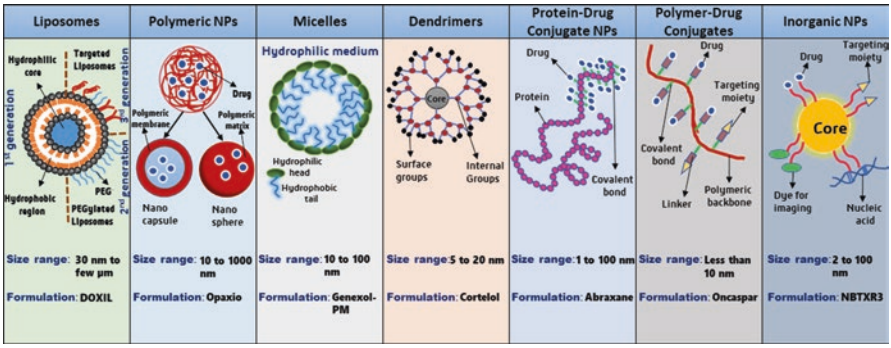

Fig. 3.1 Schematic representation of different types of nanoparticles used in oncology with information regarding size range and clinically approved formulation

Liposomes

Liposomes are spherical and self-assembling artificial vesicles composed of lipid bilayers surrounding an aqueous core. Because liposomes have lipid bilayer which is the core component of cell membrane structure, research interest in this nanoparticle began early, and eventually these became the first nanoparticles to be used in medicine (Min et al., 2015). Liposomes are used extensively for drug delivery due to their ease of preparation, biodegradability, and acceptable toxicity. They are formed impulsively when an amphiphilic liquid is added to aqueous solution, generating spheres of size 30 nm to several micrometers (Akbarzadeh et al., 2013). They can be employed to encapsulate both hydrophilic and hydrophobic drugs, where hydrophilic drug remains in aqueous core, whereas hydrophobic drug gets encapsulated between the lipid bilayers (Tran et al., 2017). The first generation of liposomes had an unmodified phospholipid surface, and their size was around 400 nm. Because of this large size, they were rapidly cleared by the mononuclear phagocytic system (MPS) which requires preliminary opsonization by the immune system; thus, the second generation of liposomes came into existence. These liposomes were called stealth liposomes and had a covering of hydrophilic carbohydrates or polymer, usually polyethylene glycol (PEG) on the surface. The process of coating the surface of liposomes with hydrophilic PEG chains is termed as PEGylation which helps repel the absorption of opsonin proteins on the surface, thereby blocking the opsonization process and significantly increasing the circulation half-life. The third generation of liposomes have increased selectivity and specificity as they incorporate targeting ligand on the surface (Reggio et al., 2011).

Polymeric Nanoparticles

Polymeric nanoparticles are solid carriers of the size range 10–1000 nm which are composed of natural or synthetic polymers. Natural polymers which are used include chitosan, gelatin, dextran, and alginate. The synthetic polymers provide an edge over natural polymers as they allow tunability of many key factors like biodegradability, molecular weight, and hydrophobicity. The synthetic polymers used include poly(lactic acid) (PLA), PEG, and poly(glycolic acid) (PGA) and their copolymers which have been extensively studied and approved by the Food and Drug Administration (FDA) owing to their biocompatibility, biodegradability, and safety profile (Tran et al., 2017; Reggio et al., 2011). Polymeric nanoparticles are extensively researched because of several properties such as ease of synthesis, water solubility, biocompatibility, biodegradability, non-immunogenicity, non-toxicity, and low cost (Bolhassani et al., 2014; Parveen & Sahoo, 2008). Broadly there are two strategies for preparation of polymeric nanoparticles which include top-down and bottom-up approach. In top-down approach, pre-formed polymers are dispersed to produce nanoparticles. The techniques used to prepare nanoparticles from top-down method solvent emulsification–evaporation, solvent emulsification–diffusion, dialysis, and nanoprecipitation. In a bottom-up approach, monomers are polymerized to form nanoparticles. Techniques used for this approach include emulsion polymerization, interfacial polycondensation, interfacial polymerization, and molecular inclusion (Rai et al., 2019). The anti-cancer drug can be entrapped, dissolved, encapsulated, adsorbed, or covalently linked to the polymeric backbone. Polymeric nanoparticles are used for controlled drug release as they have dense metrics with well-known degradation curves, which makes it easier to play with drug release as compared to other drug delivery systems (Tran et al., 2017).

Polymeric Micelles

Micelles are spherical nano-constructs with a core-shell structure and narrow size distribution of 10–100 nm. They are formed spontaneously by self-assembly of amphiphilic molecules in aqueous media to form closed lipid monolayers with hydrophobic core and polar surface (shell) to minimize the system energy. The hydrophobic core determines the drug loading capacity, drug release profile, and stability, which depends on the type of material used for forming the core. The hydrophilic shell determines the steric stability and protects the nanoparticle from sequestration by reticuloendothelial system (RES), thus increasing the circulation time of the nanoparticle. The surface can be modified by incorporating targeting moiety which can increase the selectivity of tumor targeting (Yousefpour Marzbali & Yari Khosroushahi, 2017). Critical micellar concentration (CMC) is the concentration of surfactant above which micelles are formed, and it is a characteristic feature of surfactants. Generally high molecular weight and hydrophobicity result in

low CMC which provides higher stability. Higher CMC means a higher concentration of surfactant will be required to prevent the micelle from dissociation, and this high level of surfactant can be toxic to cells. As micelles are subjected to dilution upon administration, it is advantageous to use low CMC surfactants (Lu et al., 2018). Polymeric micelles are capable of controlled release and can deliver two or more drugs or imaging agents simultaneously. The drug can be released by diffusion through polymeric matrix, erosion of biodegradable polymer, or polymer swelling (Oerlemans et al., 2010).

Dendrimers

Dendrimers are hyper-branched, spherical, and synthetic macromolecules with easily modifiable surfaces. Because of their easily adjustable shape and size, they are burgeoning as an important class of nanoparticles for drug delivery. Their architecture is composed of three main topological components: a central core, repetitive branching units, and multiple peripheral functional groups. The addition of concentric branched layers around the central core is referred to as generation of dendrimer, which is responsible for highly amplified, symmetrical, and globular structure. The size of dendrimer ranges from 1 to 15 nm. Chemotherapeutic drugs or oligonucleotides can be encapsulated or attached either to the internal core region through hydrophobic interactions, chemical linkage, or hydrogen bonds or bound to the surface through electrostatic adsorption or covalent bond formation (Palmerston Mendes et al., 2017). The dendrimers which are most widely researched and used for biomedical application include poly(amidoamine) (PAMAM) and poly(propylene imine) dendrimers. PAMAM dendrimers have numerous active amine groups on the surface which makes them highly water soluble. This property is harnessed for enhancing the solubility of hydrophobic drugs. In addition to this, the surface of PAMAM dendrimers can be engineered with numerous reactive groups which can enhance the targeting selectivity of the nanoparticle (Chauhan, 2018).

Protein-Drug Conjugate Nanoparticles

Protein-based nanoparticles are stable, biodegradable, metabolizable, and non-immunogenic with ease of modification of the surface and particle size. These can be used for controlled release of drugs by incorporating them in the microscopic structure of biodegradable polymers. These particles can covalently attach drugs and ligands (Verma et al., 2018; Parveen & Sahoo, 2006). Further, due to the very small size of protein nanoparticles, they can be transmitted through the cell by endocytosis. The proteins used in these nanoparticles include water-soluble proteins like bovine and human serum albumin; and water-insoluble proteins like gliadin and zein. They can be prepared by various chemical, physical, and self-assembly

methods. The chemical methods used include coacervation/desolvation and emulsion-based methods where shape and size of nanoparticle can be controlled. Physical methods include electrospray and nanospray techniques which are easy for industrial scale-up. Self-assembly methods generate nanoparticles with high stability and encapsulation efficiency, but the shape and size cannot be controlled (Hong et al., 2020).

Polymer-Drug Conjugates

Polymer-drug conjugates are also referred to as polymeric pro-drugs. They are composed of three main components, namely, solubilizing agent, targeting moiety, and the drug, and all these are covalently incorporated in the polymeric backbone (Alven et al., 2020). The application of biological molecules like active enzyme, proteins, peptides, and nucleic acid as drugs is well established in medicine. But the problem associated with this therapeutic technique is rapid clearance and enzymatic degradation on administration. Further, some of these proteins and peptides can induce immunogenic response, which in turn leads to rapid clearance. Most of these molecules have poor solubility, and they require solvents for administration, which themselves are toxic. Polymer-drug conjugates are able to address the above issues and improve the clinical outcomes of such drugs. Conjugation of polymers to drugs such as proteins and peptides provides several biological and pharmacological advantages. When hydrophilic polymers are used, they increase the solubility of proteins and peptides, thus eliminating the requirement of toxic solvents. Further, the conjugation increases the size of these small molecules which increases the circulation time by reducing the uptake of small molecules by RES and decreasing the renal clearance. This increase in size also reduces off-target effects and therefore reduces the systemic toxicity. Furthermore, they increase the stability of these molecules to enzymatic degradation and immune-mediated clearance (Min et al., 2015). The polymers which are used for formulation of this type of nanoparticles include PEG, N-(2-hydroxypropyl) methacrylamide (HPMA), and PLA.

Inorganic Nanoparticles

Magnetic Iron Oxide Nanoparticles

Iron oxide compounds in a nanoparticulate form possess superparamagnetic properties which are otherwise not found in them. Due to this they serve as excellent contrast agents in magnetic resonance imaging (MRI), where they can provide strong paramagnetic signals in the presence of an external magnetic field (A. Singh & Sahoo, 2014). These magnetic iron oxide nanoparticles consist of three principal components, namely, a magnetic core which is used as a carrier for both contrast

and therapeutic agents, a coating for stability, and a therapeutic agent. Additionally, it may contain a targeting moiety for tumor-specific targeting. These nanoparticles can be used for cancer diagnosis and treatment as they can serve as both contrast agents and a carrier for drugs or genes (Dilnawaz & Sahoo, 2015). Further, these nanoparticles can produce heat; thus they can be used for clinical hyperthermia. Current research on these nanoparticles focusses on loading both contrast and therapeutic agents in the same nanoparticle for developing theranostic agent which can be used for personalized medicine for real-time monitoring of therapeutic response (Martinkova et al., 2018).

Gold Nanoparticles

Gold nanoparticles (AuNPs) have excellent optical properties due to which they find application in ultrasensitive detection and imaging-based therapeutic techniques (P. Singh et al., 2018). Further, gold is a high Z-element. It has a high number of protons and neutrons in its nucleus, which can help enhance the effect of radiotherapy. On exposure of electromagnetic radiation to AuNPs, the electrons are excited which produces strong fields, resulting in production of localized heat on relaxation. This heat is sufficient enough to destroy the surrounding cancer cells. Because of this, AuNPs not only find application in radiotherapy but also in photothermal therapy (PTT) and photodynamic therapy (PDT) (Hainfeld et al., 2004). AuNPs can be easily conjugated with drugs, nucleic acids, and proteins; thus they can also be used for delivery of therapeutic agents (Daraee et al., 2016).

Hafnium Nanoparticles

Hafnium oxide is a chemically inert inorganic compound which has a high Z-number and, thus, has been used as a radiosensitizer. When these hafnium oxide nanoparticles are excited by radiation, the electron density increases, and thus absorption leads to production of localized heat on relaxation within the irradiated tissue. As these nanoparticles are chemically inert, they are well tolerated both locally and systemically; thus, they can enhance the therapeutic window of radiotherapy (Pottier et al., 2014).

Clinical Application of Nanotechnology in Oncology

Nanomedicine is a relatively new field in medicine. Despite this it has already contributed to the development of several products ranging from in vitro diagnostic agents, contrast agents for imaging, and therapeutics to medical devices. There are around 20 nanomedicine products in the market for cancer (Table 3.1). Apart from this, there are several clinical trials going on for already approved nanomedicine for

Table 3.1 List of nanoformulations clinically approved for oncology

S. no.	Trade name and company	Nanoparticle type/drug	Approved indication	Approved (year)	Route of administration	Number of studies on ClinicalTrials.gov	References
	Cancer therapy						
1.	Doxil (Janssen)	Liposomal doxorubicin (PEGylated)	Refractory ovarian cancer AIDS-associated Kaposi's sarcoma (secondary to chemotherapy) Multiple myeloma (secondary, in combination of bortezomib)	FDA (1995) EMA (1996)	Intravenous injection	635	(https://www.accessdata.fda.gov) (https://www.clinicaltrials.gov) (Anselmo & Mitragotri, 2019)
2.	DaunoXome (Galen)	Liposomal daunorubicin (non-PEGylated)	HIV-associated Kaposi's sarcoma	FDA (1996)	Intravenous injection	151	(https://www.accessdata.fda.gov; https://www.clinicaltrials.gov) (Anselmo & Mitragotri, 2019)
3.	Myocet (Teva UK)	Liposomal doxorubicin (non-PEGylated)	Metastatic breast cancer (primary, in combination with cyclophosphamide)	EMA (2000)	Intravenous injection	6	(https://www.ema.europa.eu; https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)

(continued)

Table 3.1 (continued)

S. no.	Trade name and company	Nanoparticle type/drug	Approved indication	Approved (year)	Route of administration	Number of studies on ClinicalTrials.gov	References
4..	Abraxane (Celgene)	Albumin particle-bound paclitaxel	NSCLC (primary, in combination with carboplatin when surgery and radiation are not the options) Metastatic breast cancer (secondary to chemotherapy) Metastatic pancreatic cancer (primary, in combination with gemcitabine)	FDA (2005) EMA (2008)	Intravenous injection	1315	(https://www.accessdata.fda.gov; https://www.clinicaltrials.gov) (Anselmo & Mitragotri, 2019)
5.	VYXEOS CPX-351 (Jazz Pharmaceuticals)	Liposomal formulation of cytarabine/daunorubicin (5:1 M ratio)	Acute myeloid leukemia (primary)	FDA (2017) EMA (2018)	Intravenous injection	28	(Anselmo & Mitragotri, 2019; https://www.accessdata.fda.gov; https://www.clinicaltrials.gov)
6.	Marqibo (Spectrum)	Liposomal vincristine (non-PEGylated)	Philadelphia chromosome-negative acute lymphoblastic leukemia (in second or greater relapse or whose disease has progressed following two or more anti-leukemia therapies)	FDA (2012)	Intravenous injection	345	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019; https://www.accessdata.fda.gov)

S. no.	Trade name and company	Nanoparticle type/drug	Approved indication	Approved (year)	Route of administration	Number of studies on ClinicalTrials.gov	References
7.	MEPACT (Millennium)	Liposomal mifamurtide (non-PEGylated)	Osteosarcoma (primary following surgery)	EMA (2009)	Intravenous injection	3	(https://www.ema.europa.eu; Anselmo & Mitragotri, 2019; https://www.clinicaltrials.gov)
8.	Onivyde MM-398 (Merrimack)	Liposomal irinotecan (PEGylated)	Metastatic pancreatic cancer (secondary, after disease progression following gemcitabine-based therapy)	FDA (2015)	Intravenous injection	33	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019; https://www.accessdata.fda.gov)
9.	DepoCyt (SkyPharma Inc.)	Liposomal cytarabine	Lymphomatous meningitismeningitis	FDA (2007)	Intrathecal injection	32	(https://www.accessdata.fda.gov; https://www.clinicaltrials.gov) (Thakor & Gambhir, 2013)
10.	Oncaspar (Enzon Pharmaceuticals)	Covalent conjugation of PEG with L-asparaginase	Acute lymphocytic leukaemia	FDA (1994)	Intramuscular or intravenous injection	79	(https://www.clinicaltrials.gov; https://www.accessdata.fda.gov; Dinndorf et al., 2007)

(continued)

Table 3.1 (continued)

S. no.	Trade name and company	Nanoparticle type/drug	Approved indication	Approved (year)	Route of administration	Number of studies on ClinicalTrials.gov	References
11.	Zinostatin stimalamer (launched by Yamanouchi later Astellas Pharma)	Conjugation product of zinostatin with SMANCS or SMA (polystyrene-co-maleic acid-half-butylate) copolymer	HCC	Approved in Japan in 1994	Intra-arterial injection	0	(Okusaka et al., 2012; Okusaka et al., 1998)
12.	Genexol PM (Samyang Biopharm)	Polymeric micelle of paclitaxel	Recurrent and metastatic breast cancer (primary) NSCLC (primary)	Approved in South Korea in 2006	Intravenous injection	1 NCT03008512	(Werner et al., 2013; https://www.clinicaltrials.gov)
13.	Eligard (Tolmar)	Polymeric matrix formulation of leuprolide acetate	Advanced prostate cancer	FDA (2002)	Subcutaneous injection	105	(https://www.clinicaltrials.gov; Weissig et al., 2014) (https://www.accessdata.fda.gov)
14.	Ontak (Eisai)	Recombinant fusion protein of fragment A of diphtheria toxin and subunit binding to interleukin-2 receptor	Persistent or recurrent cutaneous T-cell lymphoma whose malignant cells express CD25 component of the IL-2 receptor	FDA (initial approval 1994/ full approval in 2008)	Intravenous injection	0	(https://www.accessdata.fda.gov; Weissig et al., 2014)
15.	Opaxio (paclitaxel poliglumex) (Cell Therapeutics)	Polymeric nanoparticles with paclitaxel conjugated with polyglutamate	Glioblastoma	FDA (orphan drug status in 2012)	Intravenous injection	0	(Weissig et al., 2014)

S. no.	Trade name and company	Nanoparticle type/drug	Approved indication	Approved (year)	Route of administration	Number of studies on ClinicalTrials.gov	References
16.	Kadcyla (Roche)	Protein-drug conjugate of trastuzumab (Herceptin), connected via a stable linker to a microtubule assembly inhibitor (DM1)	Adjuvant treatment of patients with HER2-positive early breast cancer who have residual invasive disease after neoadjuvant taxane- and trastuzumab-based treatment	FDA (2019)	Intravenous injection	72	(https://www.fda.gov/drugs; Alphandéry et al., 2015) (https://www.clinicaltrials.gov)
17.	NanoTherm (MagForce)	Aminosilane-coated superparamagnetic iron (hyperthermia)	Glioblastoma	EMA (2013)	Intratumoral injection	0	(Martinelli et al., 2019; Weissig et al., 2014)
18.	Lipo-Dox (Taiwan Liposome Company)	Liposomal doxorubicin	Metastatic breast cancer (secondary), ovarian cancer (secondary), and AIDS-related Kaposi's sarcoma	Taiwan (1998)	Intravenous injection	0	(https://adisinsight.springer.com; Tran et al., 2017)
19.	NBTXR3	Hafnium oxide nanoparticles (radiosensitizer)	Locally advanced squamous cell carcinoma	FDA (2019)	Selective transcatheter arterial or intrahepatic lesion injection	9	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)
20.	DHP107	Paclitaxel lipid nanoparticles	Gastric cancer	Korea (2016)	Oral	1 NCT03326102	(https://www.clinicaltrials.gov; Salvioni et al., 2019)
	Cancer diagnosis (imaging)						
21.	Combidex (Ferumoxtran-10) (AMAG)	Dextran-coated iron oxide nanoparticles	Imaging of lymph node metastases	Netherlands (2013)	Intravenous injection	7	(https://www.clinicaltrials.gov; Fortuin et al., 2018)

exploring their role in other indications. Further, numerous non-approved nanomedicines are in clinical trials (Table 3.2), and around 18 new nanomedicines (for intravenous injection) have entered clinical trials since 2016. Out of these 18 nanomedicines, 17 are for cancer (Anselmo & Mitragotri, 2019). From this it can be inferred that hopes for nanotechnology to meet the unmet challenges of cancer are high. In the subsequent sections of this chapter, we will explore the clinical data available on nanomedicines which are both approved or are undergoing clinical trials for application in cancer (for nanoformulations in clinical trials, we have selected those trials which are recruiting, not yet recruiting, active, not recruiting studies).

Nanotechnology in Early Detection and Diagnosis

In the fight against cancer, early detection and diagnosis are without doubt one of the most efficient means for effective cancer treatment. For instance, patients with breast cancer when detected at a local stage show a 5-year survival rate of nearly 90% in comparison to merely 27% when detected at an advanced metastasis stage (Y. Zhang et al., 2019). Similar data is reported for lung cancer where the 5-year survival rate is 57% when diagnosed at stage I compared with only 3% when diagnosed at stage IV (Crosby et al., 2020). The available techniques lack sensitivity to detect cancer at an early stage. Nanoparticles by virtue of their small size have large surface-area-to-volume ratio in comparison to bulk materials which allows for surface functionalization with antibodies, small molecules, aptamers, peptides, and other functional groups. This functionalization can help detect cancer-specific biomarkers. Further, as it allows for incorporation of various binding ligands, multivalent effects can be achieved, thereby increasing the specificity and sensitivity of available detection and diagnostic techniques.

Nano-enabled In Vitro Diagnostic Devices

In vitro diagnostics are tests based on detection of various biological molecules found in blood, urine, saliva, and other tissues of the body. They are helpful in screening, diagnosis, and monitoring therapeutic response of a disease. These in vitro diagnostic devices are biosensors which include a biological recognition element (enzyme/antibody/nucleic acid/cell/aptamer) and a transducer which is used to convert biochemical reaction into a measurable signal which can be detected (Fig. 3.2). The transduction mechanisms may depend on electronic, magnetic, or light effects. In the case of cancer, there are certain biomarkers which are released in the body like secreted or cell surface proteins, tumor-shed exosomes, circulating tumor DNA, miRNA, and others which can be used for early detection of cancer or tumor recurrence. But the problem associated with biomarker detection is that the concentration of these is very low in body fluids, which makes it immensely challenging to detect. Further, heterogeneity in the abundance of biomarkers within

Table 3.2 List of unapproved nanoformulations undergoing clinical trials (recruiting, not yet recruiting, and active, not recruiting studies)

S. no.	Name/company	Type of nanoparticle	Drug	Investigated indication	Number of studies in ClinicalTrials.gov	References
	Nanotechnology in chemotherapy					
1.	Halaven E7389-LF (Eisai)	Liposome	Eribulin mesylate	Solid tumors	64	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)
2.	Promotil (LipoMedix Pharmaceuticals)	PEGylated liposome	Mitomycin C	Solid tumors	1 NCT03823989 (Ph 1): recruiting	(Anselmo & Mitragotri, 2019; https://www.clinicaltrials.gov)
3.	Mitoxantrone hydrochloride liposome (CSPC ZhongQi Pharmaceutical Technology)	Liposome	Mitoxantrone	Lymphoma, breast cancer, & and advanced HCC	4 NCT04509466 (Ph 2): not yet recruiting NCT03776279 (Ph 2): recruiting NCT04548700 (Ph 1): not yet recruiting NCT04331743 (Ph 1): not yet recruiting	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)

(continued)

Table 3.2 (continued)

S. no.	Name/company	Type of nanoparticle	Drug	Investigated indication	Number of studies in ClinicalTrials.gov	References
4.	Lipusu (Nanjing Luye Sike Pharmaceutical Co. Ltd.)	Liposome	Paclitaxel	Advanced squamous non-small cell lung cancer	1 NCT02996214 (Ph 4): active, not recruiting	(Anselmo & Mitragotri, 2019; https://www.clinicaltrials.gov)
5.	LiPlaCis (LiPlasome Pharma)	Liposome (specific degradation-controlled drug release by phospholipase A2 (PLA2)	Cisplatin	Phase 1: advanced or refractory solid tumors Phase 2 Part: Metastatic breast cancer, prostate cancer, and skin cancer	1 NCT01861496 (Ph 1/2): active, not recruiting	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)
6.	AZD2811 (AstraZeneca with BIND therapeutics)	Polymeric nanoparticles (PLA-PEG copolymer matrix with PEG blocks oriented toward particle surface, slow release)	AZD2811 (Aurora B kinase inhibitor)	Relapsed small-cell lung cancer subjects with c-MYC expression and acute myeloid leukemia	2 NCT04525391 (Ph 2): Recruiting NCT03217838 (Ph 1/2): Recruiting	(https://www.clinicaltrials.gov) (https://www.ddfevent.com)
7.	NC-6004 (Nanocarrier)	Polymeric micelle	Cisplatin	Head and neck cancer (secondary, after failed platinum regimen)	1 NCT03771820 (Ph 2): recruiting	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)

8.	Docetaxel-PM DOPNP201 (Samyang Biopharmaceuticals)	Micelle	Docetaxel	Esophageal carcinoma and advanced solid tumor	2 NCT03585673 (Ph 2): recruiting NCT04066335 recruiting	(https://www.clinicaltrials.gov)
9.	CriPec (Cristal Therapeutics)	Micelle	Docetaxel	Platinum-resistant ovarian cancer	1 NCT03742713 (Ph 2): recruiting	(https://www.clinicaltrials.gov)
10.	CRLX101 (Cerulean)	Polymeric nanoparticle (cyclodextrin-polyethylene glycol (CD-PEG) co-polymer)	Camptothecin	Progressive metastatic castration-resistant prostate cancer and relapsed/refractory small-cell lung cancer	2 NCT03531827 (Ph 2): recruiting NCT02769962 (Ph 1/2): recruiting	(https://www.clinicaltrials.gov; Young et al., 2011)
11.	ABI-009 (Aadi with Celgene)	Protein nanoparticles (albumin bound)	Rapamycin	Metastatic colorectal cancer, sarcoma, PEComa, and high-grade recurrent glioma	7	(https://www.clinicaltrials.gov)
12.	Liposomal Annamycin (Moleculin Biotech)	Liposome	Annamycin	Acute myeloid leukemia	2 NCT03388749 (Ph 1/2): recruiting NCT03315039 (Ph ½): active, not recruiting	(https://www.clinicaltrials.gov)

(continued)

Table 3.2 (continued)

S. no.	Name/company	Type of nanoparticle	Drug	Investigated indication	Number of studies in ClinicalTrials.gov	References
13.	TLD-1/Talidox (InnoMedica)	Liposome	Doxorubicin	Advanced solid tumors	1 NCT03387917 (Ph 1): recruiting	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)
14.	NC-6300 (Nanocarrier)	Micelle	Epirubicin	Advanced solid tumors or soft tissue sarcoma	1 NCT03168061 (Ph 1/2): recruiting	(Anselmo & Mitragotri, 2019; https://www.clinicaltrials.gov)
15.	Imx-110 (Immix Biopharma Australia)	Micelle	Stat-3/NF-kB/poly tyrosine kinase inhibitor and low-dose doxorubicin	Advanced solid tumors	1 NCT03382340 (Ph 1/2): recruiting	(https://www.clinicaltrials.gov)
	Nanotechnology in PDT					
16.	Visudyne (Bausch and Lomb)	Liposome	Verteporfin	Recurrent prostate cancer, Solid Pancreatic Tumors or Advanced Pancreatic Cancer & Recurrent High-Grade EGFR-Mutated Glioblastoma	3 NCT03067051 (Ph 1): Recruiting NCT03033225 (Ph 2): Recruiting NCT04590664 (Ph 1/2): Not yet recruiting	(https://www.clinicaltrials.gov)

17.	Foslip (Originator: QuantaNova, Developer: Biolitec)	Liposome	Meta-tetra(hydroxyphenyl)chlorin (mTHPC), also known as temoporfin or Foscan	Inoperable Bile Duct Cancers (PDT)	1 NCT03003065 (Ph 2): Recruiting	(Obaid et al., 2016; https://www.clinicaltrials.gov) (https://adisinsight.springer.com)
	Nanotechnology in PDT					
18.	ThermoDox (Celsion)	Liposome (thermosensitive release)	Doxorubicin	Pediatric Refractory Solid tumors & Breast cancer	2 NCT02536183 (Ph 1): Recruiting NCT03749850 (Ph 1): Recruiting	(https://www.clinicaltrials.gov)
19.	AuroShell (Nanospectra Biosciences)	Inorganic nanoparticles (PEG-coated silica gold nanoshells)	Gold nanoparticles	Prostate cancer	2 NCT04240639 Recruiting NCT02680535 (Active, not recruiting)	(https://www.clinicaltrials.gov) (Stern et al., 2016)

(continued)

Table 3.2 (continued)

S. no.	Name/company	Type of nanoparticle	Drug	Investigated indication	Number of studies in ClinicalTrials.gov	References
	Nanotechnology in radiotherapy					
20.	AGuIX nanoparticles	Inorganic nanoparticles	Polysiloxane Gadolinium	Advanced cervical cancer & brain metastases	3 NCT03308604 (Ph 1): Recruiting NCT03818386 (Ph 2): Recruiting NCT04094077 NCT03818386 (Ph 2): Recruiting	(https://www.clinicaltrials.gov)
	Nanotechnology in gene therapy					
21.	Oncoprex (Genprex)	Liposome	FUS1 (TUSC2) Tumor suppressor gene	Lung cancer	1 NCT01455389 (Ph 1/2): Active, not recruiting	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)
22.	siRNA-EphA2-DOPC (M.D. Anderson Cancer Center)	Liposome	siRNA for EphA2 knockdown	Solid tumors	1 NCT01591356 (Ph 1): Recruiting	(https://www.clinicaltrials.gov)

23.	BP1001 (Bio-Path Holdings)	Neutral liposomes	Growth factor-bound protein-2 (Grb-2) antisense oligonucleotide	Advanced or recurrent solid tumors	2 NCT04196257 (Ph 1): Not yet recruiting NCT02781883 (Ph 2): Recruiting	(https://www.clinicaltrials.gov) (Anselmo & Mitragotri, 2019)
24.	SGT-53 (SynGene Therapeutics)	Cationic liposome (targeting moiety: anti-transferrin receptor antibody)	Wild-type p53 sequence	Metastatic Pancreatic cancer, Recurrent or Progressive CNS Malignancies & PMID: 23609015	3 NCT02340117 (Ph 2): Recruiting NCT03554707 (Early Ph 1): Not yet recruiting NCT02354547 (Ph 1): Recruiting	(https://www.clinicaltrials.gov; Senzer et al., 2013)
25.	Lipo-Merit (BioNTech RNA Pharmaceuticals)	Liposomes	Four naked ribonucleic acid (RNA)-drug products (DPs) RBL001.1, RBL002.2, RBL003.1, and RBL004.1	Cancer vaccine for advanced melanoma	1 NCT02410733 (Ph 1): Active, not recruiting	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)

(continued)

Table 3.2 (continued)

S. no.	Name/company	Type of nanoparticle	Drug	Investigated indication	Number of studies in ClinicalTrials.gov	References
26.	MTL-CEBPA (Mina alpha)	Amphoteric liposomes (pH-triggered endosomal escape for intracellular delivery)	"Small"- or "short"-activating RNAs (saRNA) for inducing expression of transcription factor CCAAT/enhancer-binding protein alpha (CEBPα), a tumor suppressor and critical regulator of hepatocyte function	Advanced solid tumours	1 NCT04105335 (Ph 1): Recruiting	(https://www.clinicaltrials.gov; Setten et al., 2018)
	Nanotechnology in immunotherapy					
27.	m-RNA-4157 (ModernaTX, Inc.)	Lipid nanoparticle	mRNA targeting 20 tumor-associated antigens (TAAs) that are specifically expressed by the patient's cancer cells	Solid Tumors & High-Risk Melanoma (Personalized cancer vaccine)	2 NCT03313778 (Ph 1): Recruiting NCT03897881 (Ph 2): Recruiting	(https://www.clinicaltrials.gov; Kowalski et al., 2019)
28.	mRNA-2416 (ModernaTX, Inc.)	Lipid nanoparticle	mRNA encoding for the OX40L protein, which is a co-stimulatory membrane-bound protein that enhances the expansion, function, and survival of T cells to attack cancer cells	Solid tumor malignancies or lymphoma	1 NCT03323398 (Ph 1): Recruiting	(https://www.clinicaltrials.gov; Kowalski et al., 2019) (https://www.modernatx.com)
29.	Anti-EGFR-IL-dox Swiss group for clinical Cancer research	Immunoliposomes (anti-EGFR)	Doxorubicin	Advanced triple-negative EGFR positive breast cancer	1 NCT02833766 (Ph 2): Active, not recruiting	(https://www.clinicaltrials.gov)

30.	IVAC_W_bre1_ulD (BioNTech SE)	Liposomes	RNA (specificity for antigen expression on a patient's tumor) Personalized cancer immunotherapy	Triple-negative breast cancer	1 NCT02316457 (Ph 1): Active, not recruiting	(https://www.clinicaltrials.gov)
	Nanotechnology in in vivo imaging of cancer					
31.	Definity (Lantheus Medical Imaging)	Lipid microspheres	Perflutren (as ultrasound contrast agent)	HCC, breast cancer, ocular melanoma, head and neck cancer, and sarcome	9	(https://www.clinicaltrials.gov)
32.	Optison (GE Healthcare)	Protein nanoparticles (albumin stabilized)	Perflutren (as ultrasound contrast agent)	HCC	1 NCT03199274 (early Ph 1): Recruiting	(https://www.clinicaltrials.gov)
33.	SonoVue (Bracco imaging)	Lipid nanoparticle	Sulfur hexafluoride (ultrasound contrast agent)	Liver neoplasms, prostate, breast and pancreatic cancer	21	(https://www.clinicaltrials.gov; https://www.ema.europa.eu)
34.	Visudyne (Bausch and Lomb)	Liposome	HSP90 inhibitor-linked verteporfin	Solid tumor	1 NCT03906643 (Ph 1): Recruiting	(https://www.clinicaltrials.gov)
35.	Sonazoid (GE Healthcare)	Lipid nanoparticle	F-butane (ultrasound contrast agent)	HCC & breast cancer	8	(https://www.clinicaltrials.gov)
36.	Cornell Dots (C -Dots) (Memorial Sloan Kettering Cancer Center)	Silica nanoparticles (PEG and surface functionalized with cRGDY, tumor-targeting peptide)	Near-infrared fluorophore and ^{124}I	Image-guided intraoperative mapping of nodal metastases	1 NCT02106598 (Ph 1/2): Recruiting	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)

(continued)

Table 3.2 (continued)

S. no.	Name/company	Type of nanoparticle	Drug	Investigated indication	Number of studies in ClinicalTrials.gov	References
37.	ONM-100	Micelle	Indocyanine green	Intraoperative fluorescence imaging agent for the detection of cancer	1 NCT03735680 (Ph 2): Recruiting	(https://www.clinicaltrials.gov; Anselmo & Mitragotri, 2019)

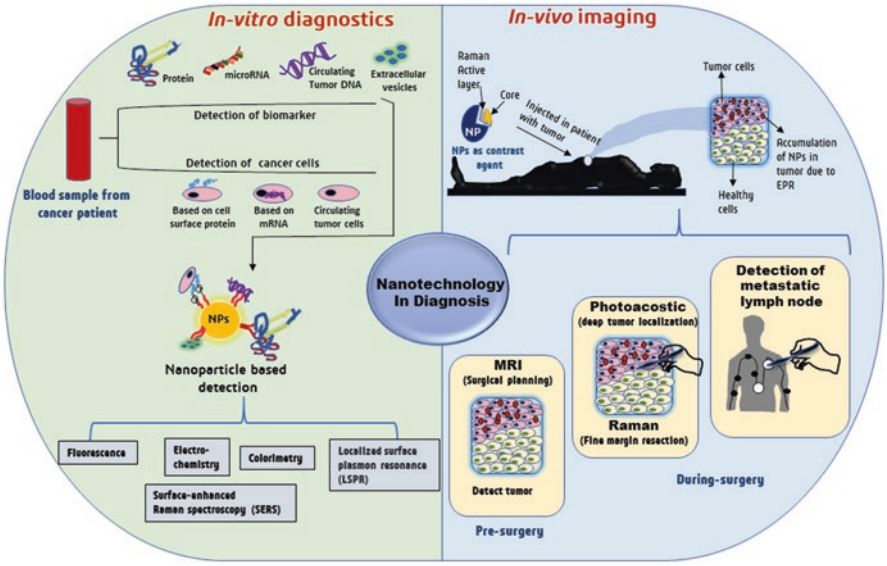

Fig. 3.2 Illustration of application of nanotechnology in in-vitro and in-vivo diagnosis of cancer. In in-vitro diagnosis, nanoparticles help to detect extracellular cancer biomarkers or cancer cells in samples collected from patients, which could help in early detection of cancer. In in-vivo imaging, nanotechnology not only helps to detect cancer, but can also aid in surgery by helping in delineating tumor margins, identifying residual cancer cells and locating metastatic lymph nodes

patients is another barrier in its translation. Nanotechnology-enabled in vitro diagnostic devices can address these problems as they offer high selectivity and sensitivity and detection of multiple targets simultaneously (Y. Zhang et al., 2019). However, presently there are no nano-enabled in vitro diagnostic devices for cancer in the market, but there are several nanoparticle-based medical screens and tests already in use for other indications like the use of AuNPs for home pregnancy tests (https://www.cancer.gov/nano/cancer-nanotechnology/detection-diagnosis#in-vitro). So, research is actively being conducted in this field, and the hopes for translation of this technique in cancer are high. One such research was conducted at Northwestern University where they designed a nanoparticle-based bio-barcode assay for ultrasensitive detection of free prostate-specific antigen (PSA) for identification of disease relapse after surgical treatment of prostate cancer. This technique utilizes magnetic nanoparticles (MMPs) functionalized with PSA monoclonal antibodies which bind to target proteins in the sample. These MMP-protein hybrids are then combined with AuNPs which are highly functionalized with DNA barcodes and polyclonal anti-PSA. Target protein-specific DNA barcodes are then released in solution and are detected by scanometric assay with sensitivities in femto-picomolar range (Nam et al., 2003). Another nanoparticle-based diagnostic magnetic resonance sensor platform operated by smartphone technology was developed by Ralph Weissleder at Massachusetts General Hospital for accurate diagnosis of malignant tumors from fine-needle aspirate biopsies. Weissleder's system utilizes

nanoparticle-based magnetic affinity ligands for detection of protein markers that are associated with cancer. This system exploits changes in the transverse relaxation signal of water molecules in a magnetic field as a sensing mechanism for magnetic nanoparticle-labeled analytes. They conducted experiments with this technology in a clinical setting using cells obtained through fine-needle aspiration of suspected lesions in 50 patients. They further validated the results with an independent cohort of 20 other patients. They reported a 96% accuracy in cancer diagnosis which was way ahead of clinical analyses by immunohistochemistry (Haun et al., 2011).

In Vivo Imaging

In vivo imaging platforms have provided immense opportunities for both clinical diagnosis and research by endowing the means to peer deeply within tissues in living objects. These imaging techniques create the image of internal structure and tissues. The visibility of these physiological structures can be greatly enhanced by the use of contrast agents. By employing nanotechnology, contrast images with high resolution can be obtained which is essential for precise and accurate diagnosis. Nanotechnology-based contrast agents can significantly enhance the detection of tumor in vivo by conventional scanning and imaging devices such as MRI, PET, and CT scans (Fig. 3.2). This is mainly because nanomaterials can deliver large imaging payloads, allow for multiplexing, and yield improved sensitivity. In certain imaging modalities, nanomaterials themselves are used as a source of image signal rather than as a delivery platform (Smith & Gambhir, 2017). One such example is Combidex-enhanced MRI (CEM) which is also known as magnetic resonance lymphography. Combidex (ferumoxtran-10) is a dextran-coated iron oxide nanoparticle of size range 20–50 nm which has been approved for clinical use in the Netherlands for imaging of lymph node metastases. Lymph node status in patients with prostate cancer is evaluated either by imaging or lymphadenectomy. Lymphadenectomy is an invasive method where extended pelvic lymph node dissection (e-PLND) is the standard procedure to detect metastatic lymph nodes in intermediate and high-risk prostate cancer patients. The procedure evaluates lymph nodes only in the dissection field and misses small and/or distant nodes. It is reported that in 60–80% of proven lymph node-positive patients, lymph nodes were present outside the e-PLND. Thus, the sensitivity of this technique is quite low. The other technique available for assessing is imaging which is non-invasive and is of two types, conventional and functional. Both the techniques are unable to detect small metastatic nodes, and as metastatic nodes in prostate cancer are small, the sensitivity of these techniques is reduced. Since the early 1990s, CEM has shown very promising results, but unfortunately it was withdrawn from the registration process in Europe by the manufacturer. But, Radboud University Medical Center (Radboudumc) obtained all rights and documents for Combidex in 2013, and with the help of an accredited pharmacy, they were able to manufacture the contrast agent with original specifications. In 2015, the rights were transferred to SPL Medical B.V. in Nijmegen, the Netherlands, which is presently the sole manufacturer for the contrast agent.

Combidex is administered intravenously with a slow-drip infusion 24–36 h prior to MRI imaging. The nanoparticles are taken up by macrophages, which accumulate in normal lymph nodes, whereas metastatic nodes do not uptake these nanoparticles. Therefore, in MRI images normal lymph nodes appear black, whereas metastatic nodes appear white. Thus, with CEM, there is a sharply defined distinction with normal and metastatic nodes (Fortuin et al., 2018). Another example of nanotechnology-based contrast agent is Definity which are lipid microspheres comprising echogenic microbubbles filled with octafluoropropane gas. It is developed by Lantheus Medical Imaging and was approved by the FDA in 2001 as an injectable cardiovascular ultrasound enhancement agent (https://www.accessdata.fda.gov). It is now in clinical trials for ultrasound enhancement for early-stage detection of liver, breast, ovary intraocular, and pancreatic cancer (https://www.clinicaltrials.gov). Optison is a GE healthcare-developed nanotechnology-based ultrasound contrast agent which was FDA approved in 1997 for echocardiography. Optison are microspheres of human serum albumin with perflutren for contrast enhancement in ultrasound imaging procedures (https://www.accessdata.fda.gov). They are presently in clinical trials for detection of sentinel lymph nodes which are the first targets of cancer cell and for screening of renal cell carcinoma recurrence. Apart from this they are in early phase I clinical trial for assessing whether perflutren protein type A microsphere contrast-based ultrasound can work in improving response to radioembolization therapy in patients with liver cancer (https://www.clinicaltrials.gov). SonoVue, a nanotechnology-based product developed by Bracco Imaging, is an ultrasound contrast agent consisting of phospholipid-stabilized microbubble containing sulfur hexafluoride. It was approved by the European Medicines Agency (EMA) in 2001 for echocardiography (https://www.ema.europa.eu). It is now in clinical trials for early detection of liver neoplasms, prostate, breast, and pancreatic cancer, which are difficult to detect in early stages (https://www.clinicaltrials.gov). Another nanotechnology-based product in clinical trials is Cornell Dots (C Dots) which are silica nanoparticles that deliver near-infrared fluorophore (optical imaging contrast agent) and radiolabeled iodine (^{124}I, PET contrast agent) in the same platform. They are coated with PEG and surface functionalized with cRGDY (cyclic arginine–glycine–aspartic acid) targeting peptide for tumor-specific targeting (Anselmo & Mitragotri, 2019). C Dots have been undergoing FDA Investigational New Drug (IND) clinical trials for metastatic melanoma since 2010 and have successfully made through initial clinical trials. These are intended to be used for real-time image-guided intraoperative mapping of nodal metastases where they would assist the surgeon in removing metastatic lymph nodes. They are also in clinical trials for other cancers like breast, brain, and colorectal cancer (https://www.clinicaltrials.gov).

Nanotechnology in Treatment and Therapy

Conventional cancer therapy includes chemotherapy, radiation therapy, and surgery. Nanotechnology finds application in these fields by improving the pharmacokinetics and targeting ability of chemotherapeutics, enhancing radiosensitization, and aiding surgery by better diagnostic imaging. Additionally, the ease of fabrication and engineering of nanoparticles is helping to develop novel therapies for cancer like gene therapy, immunotherapy, and photodynamic therapy (Misra et al., 2010) (Fig. 3.3). This section of chapter will illustrate the clinical status of nanotechnology in advancement of oncotherapy.

Nanocarriers for Delivering Chemotherapy

Conventional chemotherapeutics is the major treatment approach used either individually or in combination with other therapies for cancer. But its effects are significantly reduced due to several limitations which include poor aqueous solubility, lack of selectivity for cancer cells, short circulation time, systemic toxicity, and development of MDR (Chidambaram et al., 2011). Thus, the long-established use of nanotechnology in oncology has shown to improve the pharmacokinetics, targeting selectivity, and delivery of chemotherapeutics to the tumor tissues. This is achieved through either encapsulating or conjugating the drug to nanoparticles which act as delivery systems. And by virtue of their inherent characteristics, they are able to improve the overall therapeutic index of the drug and selectively deliver the therapeutics to tumor tissues either by passive or active targeting (Cho et al., 2008). This

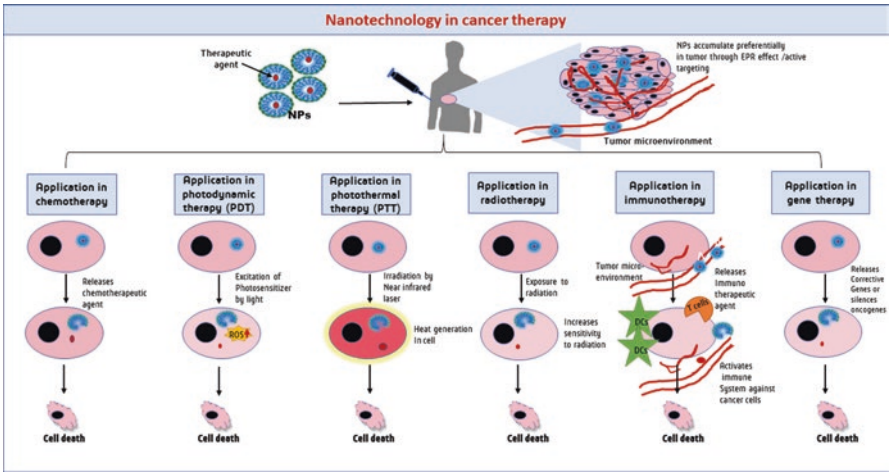

Fig. 3.3 Figurative representation of application of nanotechnology in varied therapies for oncology where nanoparticles helps in improving the pharmacokinetics of the therapeutic agent and selective targeting of tumor

has led to the development of several nanomedicines which have been approved and are extensively used for different types of cancer. The first nanomedicine which was approved for cancer is DOXIL which is a liposomal formulation of doxorubicin. Doxorubicin is one of the first chemotherapeutics for cancer and is used in various cancers like gastric, lung, ovarian, breast myeloma, lymphomas, leukemia, and sarcoma. But, one of the major side effects of this drug is severe cardiotoxicity which is dependent on dose and is cumulative (Rivankar, 2014). As liposomes offer several advantages as delivery vehicles like they can deliver both hydrophobic and hydrophilic drugs and can improve the pharmacokinetics and biodistribution of the encapsulated drug, scientists began hypothesizing that it could improve the therapeutic index of doxorubicin. The first generation of liposomal doxorubicin which entered in clinical trials was of the size range 300–500 nm and had doxorubicin encapsulated in lipid bilayer. Pharmacokinetics and biodistribution studies revealed that DOXIL, though administered at higher dose, provided lower peak levels of free doxorubicin. This was mainly because liposomes were rapidly cleared from circulation by reticuloendothelial system (RES) or MPS due to their large size. Thus, liposomes encountered a major problem in their translation. This led to the development of a second generation of liposomes which were coated with PEG on the surface to make "stealth liposomes" which were not easily detected by RES. This new formulation entered clinical trials between 1991 and 1994 in Israel with the aim of the study to understand the pharmacokinetics of DOXIL. The study revealed that DOXIL had remarkably higher area under curve (AUC) in comparison to native doxorubicin. Further, the volume of distribution was much smaller for DOXIL (4 L) in comparison to native doxorubicin (25 L), and the clearance of DOXIL was also slower as compared to native doxorubicin. Intriguingly, the drug concentration in malignant effusions was 4–16-fold higher for DOXIL as compared to free doxorubicin. This result was one of the first in a clinical setting to support the concept of EPR utilized by nanoparticles. Eventually the drug entered the clinical investigation phase and received the first FDA-accelerated approval for treatment of chemotherapy-refractory Kaposi's sarcoma associated with AIDS in 1995 (Min et al., 2015). Later it received FDA-accelerated approval for treatment-refractory ovarian cancer in 1999 and for relapsed and refractory multiple myeloma in 2007 as a combination treatment with bortezomib (https://www.accessdata.fda.gov). There are around 635 clinical trials going on DOXIL for various cancers (https://www.clinicaltrials.gov).

Another liposomal formulation approved for cancer is DaunoXome which is a non-PEGylated liposome formulation of daunorubicin. Daunorubicin is an anthracycline drug from which doxorubicin is obtained. DaunoXome, entered in clinical trials for AIDS-associated Kaposi's sarcoma and pharmacokinetic study, revealed that both AUC and alpha half-life of DaunoXome were significantly better as compared to native daunorubicin. Further, investigators found that in 22 patients who received DaunoXome, the partial response (PR) or clinical complete response (CR) rate was 55% (Gill et al., 1995). Further, randomized phase III trials were conducted to compare the effect of DaunoXome and doxorubicin, and it was found that overall response rate was almost the same (Gill et al., 1996). Based on these results, DaunoXome was granted accelerated approval by the FDA for Kaposi's sarcoma in

1996 (Kaposi's sarcoma: DaunoXome approved, 1996). Clinical trials were also conducted to compare DaunoXome and DOXIL, and it was found that DaunoXome was less potent in comparison to DOXIL. It has been evaluated for several cancers, but since it does not offer higher efficiency in comparison to other standard treatments, it has not been approved for other cancers (Min et al., 2015). There are around 151 clinical trials going on for DaunoXome for various types of cancer (https://www.clinicaltrials.gov). Arabinofuranosyl cytidine commonly known as cytarabine (ara-C) is an antimetabolite class of chemotherapeutic used for certain types of leukemia and lymphomas. It is one of the few drugs which is effective in treating meningeal leukemia. But the intrathecal administration of drugs is toxic and requires frequent dosing (Baker et al., 1991). As one of the key features of liposomes is long circulation time and slow drug release (Bozzuto & Molinari, 2015), investigators thought employing these as delivery vehicles can address the clinical issues of cytarabine. This led to the development of liposomal cytarabine called DepoCyt, which uses multivesicular liposomes as a depot for cytarabine. DepoCyt entered clinical trials in 1991, and phase I clinical trial revealed that DepoCyt had prolonged half-life in comparison to free cytarabine (Chamberlain et al., 1993). Based on the encouraging results of phase I trial, two randomized clinical trials were undertaken to compare DepoCyt with cytarabine (Glantz et al., 1999). In both trials the response rate was significantly higher for DepoCyt in comparison to cytarabine, which eventually led to its FDA-accelerated approval for lymphomatous meningitis in 1999. The formulation received full approval in 2007 (https://www.accessdata.fda.gov). There are around 32 clinical trials going on DepoCyt for various cancers (https://www.clinicaltrials.gov). Other liposomal formulations which have been approved for treatment of cancer include VYXEOS, Myocet, Marqibo, MEPACT, and Onivyde. VYXEOS is a first nanoformulation having dual drug loading to be approved by the FDA in 2017 and EMA in 2018 for treatment of acute myeloid leukemia. It is a liposomal formulation containing cytarabine and daunorubicin in the ratio (5:1 M ratio) (Anselmo & Mitragotri, 2019). It is currently undergoing 28 clinical trials for various indications in cancer (https://www.clinicaltrials.gov). Myocet is a non-PEGylated liposomal doxorubicin which was granted approval by the EMA in 2000 for sale in Europe and Canada as the first line of treatment for metastatic breast cancer in combination with cyclophosphamide (https://www.ema.europa.eu). There are around six clinical trials going on Myocet for various cancers (https://www.clinicaltrials.gov). Marqibo is a non-PEGylated liposomal vincristine which received approval for a rare condition in adult patients with Philadelphia chromosome-negative (Ph-) acute lymphoblastic leukemia (ALL) in second or greater relapse by the FDA in 2012. Presently, Marqibo is undergoing around 345 clinical trials for different indications in cancer (https://www.clinicaltrials.gov). Another non-PEGylated liposomal formulation is MEPACT which encapsulates mifamurtide as an immune modulator. The product received EMA approval for use in the European Union for treatment of high-grade non-metastatic osteosarcoma in 2009 (https://www.ema.europa.eu). It is presently undergoing three clinical trials for osteosarcoma (https://www.clinicaltrials.gov). Onivyde, a PEGylated liposomal formulation of irinotecan which is a topoisomerase I inhibitor, has been

approved by the FDA in 2015 for use in combination with fluorouracil and leucovorin for treatment of metastatic adenocarcinoma of the pancreas after disease progression following gemcitabine-based therapy (https://www.accessdata.fda.gov). Currently, it is undergoing 38 clinical trials for different indications of cancer (https://www.clinicaltrials.gov).

Apart from liposomes, protein nanoparticles-based chemotherapeutics are also in the market. One such successful formulation is Abraxane. It is an albumin-bound nanoformulation of paclitaxel which has been granted FDA approval in 2005 for treatment of metastatic breast cancer (secondary), for locally advanced or metastatic non-small cell lung cancer (NSCLC) (primary) in combination with carboplatin, and as first-line treatment for metastatic adenocarcinoma of the pancreas in combination with gemcitabine (https://www.accessdata.fda.gov). Paclitaxel has limited aqueous solubility, so initially it was prepared as a Cremophor−/ethanol-based preparation to improve its solubility and in vivo delivery. But this preparation had several side effects like acute hypersensitivity reaction leading to severe and at times fatal allergic responses, decreased drug clearance, and volume of distribution. Thus, the Cremophor EL delivery system decreased the therapeutic index of paclitaxel and increased the toxicity. This led to the search for a new delivery system for paclitaxel which eventually resulted in development of Abraxane that greatly enhanced the overall solubility and in vivo transport efficiency of the drug, without causing any toxicity (Min et al., 2015). Abraxane is presently undergoing around 1315 clinical trials for different types of cancer (https://www.clinicaltrials.gov). The type of nanoparticle-based formulation in the market for oncology is Oncaspar. It is a polymer-drug conjugate formed by covalent conjugation of PEG with native L-asparaginase which was approved by the FDA in 1994 for use in acute lymphocytic leukemia patients who developed hypersensitivity to the free form of asparaginase (Dinndorf et al., 2007). It is undergoing around 79 clinical trials for different indications of cancer (https://www.clinicaltrials.gov). Zinostatin stimalmer is another drug-polymer conjugate which was approved for use in Japan in 1994 for hepatocellular carcinoma (HCC). It is a conjugation product of neocarzinostatin (Zinostatin) with SMANCS or SMA (polystyrene-co-maleic acid-half-butylate) copolymer (Okusaka et al., 2012).

Polymeric micelle formulations are also clinically available for oncology application. One such formulation is Genexol PM which is composed of amphiphilic diblock copolymer, monomethoxy poly(ethylene glycol)-block-poly(D, L-lactide) (mPEG-PDLLA) and paclitaxel. It has been approved for use as a first-line therapy for recurrent and metastatic breast cancer and NSCLC in South Korea in 2006. The formulation shows reduced toxicity in comparison to Taxol (Werner et al., 2013). Currently it is undergoing phase II clinical trial to assess the activity and safety profile of weekly administered Genexol PM in patients with advanced HCC for whom sorafenib treatment has failed (https://www.clinicaltrials.gov). Apart from these there are other nanotechnology-based chemotherapeutics which are not approved but are under clinical investigation. The details of which are provided in Table 3.2.

Nanotechnology in Photodynamic Therapy (PDT)

Photodynamic therapy is an emerging treatment strategy which is based on the excitation of non-toxic photosensitizers (PS) by light of a specific wavelength that matches the absorption properties of PS, resulting in production of reactive oxygen species (ROS) that are toxic to the target cancerous tissues. The success of PDT depends on the selection of PS. A suitable PS should be selective to neoplastic cells, excited by far-red or near-infrared spectral regions, and capable of yielding long-lived triple excited stages. The generation of ROS depends on two distinct pathways which are interchangeable. In type 1 pathway, electron transfer to or from the triple excited state of PS leads to the production of oxygen-free radicals such as hydroxyl radicals, superoxide, and hydroperoxides. Type 2 pathway leads to the production of singlet oxygen where triple excited PS interacts to molecular oxygen which is triplet in its ground state leading to singlet excited to singlet ground-state relaxation (Mfouo Tynga & Abrahamse, 2018). For effective and safe PDT, it is quintessential that PS is delivered in therapeutic concentration to target cancerous tissues without being absorbed by non-target tissues. But there are two problems in realizing this outcome. One of it is that PS being highly hydrophobic in nature accumulates in aqueous environment which results in lower efficiency as their monomeric form is more photoactive, and the other is PS lacks specificity for tumor tissues. Both the issues can be effectively addressed by employing nanotechnology in PDT, where nanoparticles can deliver PS specifically to target cancer cells and at the same type prevent these hydrophobic agents from stacking up by preventing them from aqueous environment (Hamblin et al., 2015). The first nanotechnology-based PS formulation to get FDA approval is Visudyne® (verteporfin) which is a non-PEGylated liposomal formulation of PS benzoporphyrin derivative (BPD) consisting of egg phosphatidylglycerol, dimyristoyl phosphatidylcholine, ascorbyl palmitate, and butylated hydroxytoluene. It has been approved as a first-line treatment for age-related macular degeneration (AMD) in year 2000. The success of PDT using Visudyne® in AMD has led to its investigation in cancer. One such investigation was conducted in advanced pancreatic cancer, where a 690-nm laser light was delivered after 60–90 min of sensitization with Visudyne®, under computed tomography (CT) guidance for photochemical activation of Visudyne® within the tumor interstitium. It was observed that at 40 J, this therapy induced 12 mm of necrosis in the tumor without any adverse effects. Thus, it was concluded that verteporfin PDT in locally advanced pancreatic cancer is safe and effective. Though presently it is not approved for cancer therapy, several clinical trials are ongoing in different types of cancer (Obaid et al., 2016). It is undergoing four clinical trials for cancer (https://www.clinicaltrials.gov). Other nanotechnology-based products in context of PDT in clinical trials are Foslip and Fospeg, which are liposomal formulations of PS meta-tetra(hydroxyphenyl)chlorin (mTHPC), also known as temoporfin or Foscan. Foslip is a conventional liposomal formulation, whereas Fospeg is a PEGylated liposomal formulation. It has been observed that the PDT efficiency of both the formulations is higher in comparison to native PS, but in context of tumor selectivity, Fospeg is more efficient in comparison to Foslip (Obaid et al., 2016). Presently, Foslip is

undergoing one clinical trial for inoperable bile duct cancers. However, there are no ongoing clinical trials on Fospeg.

Nanotechnology in Photothermal Therapy (PTT)

PTT is another phototherapy used for cancer treatment apart from PDT where a photothermal (PT) agent is stimulated by both a near-infrared laser and vibrational energy to produce local heating through optical absorption for selective killing of cancer cells (Montaseri et al., 2020). Recently nanomaterials have garnered significant attention in this field as they can themselves act as photothermal transducers as they exhibit strong absorption in the near-infrared range (Doughty et al., 2019). This strategy of employing NP-mediated hyperthermia is advantageous as selective hyperthermia in tumor tissues can be achieved without toxicity to healthy tissues. In PTT, once intratumoral accumulation of plasmonic nanoparticles is achieved following administration, they are illuminated with near-infrared light which causes synchronized oscillations in electrons of the conduction band in nanoparticles which converts light energy into heat that kills cancer cells (Pinto & Pocard, 2018). Several nanomaterials with different structures have been explored for PTT like gold, tungsten, copper, carbon-based materials, iron oxide, and molybdenum (Fernandes et al., 2020). Of these plasmonic gold nanostructures and iron nanoparticles have made it to the clinical trials. Currently, PEG-coated silica gold nanoshells, called AuroShell®, are undergoing two clinical trials for anti-cancer PTT specifically termed AuroLase® therapy (https://www.clinicaltrials.gov). Early-phase clinical trials for evaluation of safety of AuroShell®-directed PTT in the treatment of prostate disease have found that the safety profile of AuroShell particles is excellent (Stern et al., 2016). Another NP-based PTT in clinical trials is Magnablate, which is an iron oxide nanoparticle that has completed phase 0 clinical trial for thermal ablation of prostate cancer (https://www.clinicaltrials.gov). PTT is also being evaluated for aiding in selective activation of nanotechnology-based therapeutics in tumors. One such clinical investigation has been conducted with ThermoDox® which is a PEGylated liposomal formulation of doxorubicin to thermally release the drug upon radiofrequency ablation (RFA). It has completed Phase III HEAT trial for HCC, and the results indicate that employing extended duration of RFA can be critical in improving the outcome. This finding has led to a new phase III trial, OPTIMA for HCC (Obaid et al., 2016).

Delivering or Synergizing Radiotherapy

Radiotherapy is one of the most effective treatment modalities in oncology with more than 60% of cancer patients receiving the therapy during the course of treatment. Since the discovery of ionizing radiation's capacity to cause cytotoxicity through DNA damage, they have been extensively used clinically to kill rapidly proliferating cancer cells as they are more susceptible to DNA damage. Radiotherapy

can be applied through both external beams (electrons, protons, or photons) and internal radioactive source depending on the type of tumor, its location, and size. Though a lot of progresses have been made in the field, still several challenges exist. These challenges include reducing the toxicity to healthy tissues and increasing the therapeutic efficiency in tumors which are resistant or exhibit low response to radiotherapy (Zhao et al., 2016). These unmet challenges can be addressed by employing nanotechnology, where nanoparticles can be used as a cargo for delivering radionucleotides or radiosensitizers in tumors, thereby enhancing the tumor selectivity and at the same time alleviating toxicity to normal tissues (Mi et al., 2016). There are several radioisotopes which are used in cancer therapy which include beta-emitters, like 188Re, 166Ho, 186Re, 89Sr, 32P, and 90Y, and alpha-emitters, like 211At, 225Ac, and 213Bi (Hamoudeh et al., 2008). But the major problem associated with radioisotopes is that they are rapidly eliminated from the body either through renal clearance or by MPS owing to their small size. Several preclinical studies have shown that encapsulating or conjugating these radioisotopes with nanocarriers can significantly enhance their biological half-life (L. Zhang et al., 2010). However, one liposomal formulation of Rhenium-188, 188Re-BMEDA-liposome, entered phase 1 clinical trials in 2014 for evaluating the maximum tolerance dose (MTD) and safety profile in patients with primary solid tumor in advanced or metastatic stage. But it has been terminated in 2020 due to some concerns regarding the accumulation of radioactivity in the liver and spleen (https://www.clinicaltrials.gov).

Interestingly, one nanotechnology-based radio-enhancing agent, NBTXR3, has been approved by the FDA in 2019 for the treatment of locally advanced squamous cell carcinoma. NBTXR3 is a spherical 50-nm size hafnium oxide nanoparticle which is functionalized with a negative surface charge. After its injection in the tumor, the tumor is irradiated with high-energy photons from external beams, which are absorbed by tissues leading to the generation of electrons that activate the hafnium oxide nanoparticles. These excited nanoparticles then emit high-energy electrons leading to the production of high levels of ROS which further causes double-stranded DNA damage, thereby enhancing the effect of radiotherapy (Anselmo & Mitragotri, 2019). Currently, NBTXR3 is undergoing nine clinical trials for different indications in cancer (https://www.clinicaltrials.gov). Apart from this, gadolinium-based AGuIX nanoparticles have recently entered in phase 1 clinical trial for evaluating the safety and tolerability of escalating doses of AGuIX-NP in combination with radiation and cisplatin in patients with locally advanced cervical cancer. There are two more clinical trials going on with AGuIX nanoparticles for the treatment of brain metastases (https://www.clinicaltrials.gov).

Chemo- and radiation therapies are concurrently required for the treatment of several cancers; this combination therapy is termed as chemoradiotherapy. But this combination therapy causes a significant increase in toxicity. For instance, Shimmyo et al. have reported that the chemoradiotherapy bears a mortality risk of 5% which is higher than both the individual therapies in lung cancer (Minami-Shimmyo et al., 2012). Thus, there is a need for novel approaches to reduce the toxicity associated with chemoradiotherapy. In this context, radiotherapy with nanotechnology-based

chemotherapeutics has been explored in several clinical trials. The first reported clinical trial of this sort used liposomal doxorubicin (Caelyx) with radiotherapy for the treatment of NSCLC and head and neck cancer. The results indicated 40% complete response and 87% partial response for patients with stage IIIb of NSCLC (Koukourakis et al., 1999). There are around 271 clinical trials going on with Abraxane and radiotherapy in different indications of cancer (https://www.clinicaltrials.gov).

Delivering Gene Therapy

Gene therapy is a novel treatment modality which has bloomed from the increasing knowledge of tumor cells and its surrounding microenvironment. It involves introduction of exogenous nucleic acids, such as genes, miRNAs, siRNAs, oligonucleotides, or gene segments to cancer cells or its microenvironment for silencing the oncogenes or restoring the expression of tumor suppressor genes or allowing synthesis of an exogenous protein. A detailed account on these mechanisms is beyond the scope of this chapter; however it has been very nicely outlined in a recent review (Roma-Rodrigues et al., 2020). There are two mechanisms of gene transfer into tumor cells, namely, ex vivo and in vivo. In the ex vivo approach, tumor cells from patients are collected, propagated, and genetically mutated which are then transferred back into the host. This approach however is not very suitable in the case of cancer because it requires proliferation of transfected cells, which is antagonistic to the main objective of cancer gene therapeutics that aims to inhibit the tumor progression by targeting the proliferative ability of cancer cells (Amer, 2014). The other approach is in vivo where therapeutic nucleic acids are introduced systematically or pre-systemically via oral, transdermal, ocular, or nasal delivery route depending on the type and location of tumor. But the problem with this approach is that nucleic acids are highly unstable in systemic circulation, sensitive to degradation by nucleases, and susceptible for phagocyte uptake and renal clearance (Chen et al., 2016). Thus, they require the use of vectors or carriers which can protect them from degradation and deliver them efficiently to the tumor. For this, initially, viral vectors were explored, but immunogenicity, poor selectivity, insertional mutagenesis, and poor delivery efficiency led to the search of other delivery systems (Roma-Rodrigues et al., 2020). Nanotechnology-based gene delivery systems have been found to increase the stability of genetic therapies as they protect these agents from degradation and also facilitate their endosomal escape which is a critical step for efficient nucleic acid therapeutics (Balazs & Godbey, 2011). The area of nano-based gene therapy in oncology is in its infancy, so presently no product is clinically available in the market, but in the last two decades, several products have entered in phase I/II clinical trials (Table 3.2). Adenovirus type 5 E1A gene has been found to have antitumor activity which is demonstrated through repression of HER-2/neu and induction of apoptosis. Preclinical studies in HER-2/neu-overexpressing breast and ovarian cancers in nude mice have shown that E1A gene therapy indeed caused regression of these tumors. Based on these results, cationic liposomal formulation

of E1A gene has been assessed for feasibility in patients with both HER-2/neu-overexpressing and low HER-2/neu-expressing breast and ovarian cancers in a phase I clinical trial. The results indicated that the therapy was well tolerated and most importantly the expression of E1A gene was observed in tumor cells suggesting successful gene therapy (Hortobagyi et al., 2001). Another phase I clinical trial has been conducted with liposomal nanodelivery complex (scL) consisting of anti-transferrin receptor (TfR), scFv, as the targeting molecule for the delivery of p53 tumor suppressor gene (SGT-53) in advanced solid tumors. It was observed that treatment showed minimal side effects with only one patient experiencing serious side effects of chest pain and tachycardia. Further, in the context of treatment response, it was found that the majority of patients demonstrated stable disease (Senzer et al., 2013). In another phase I trial, Atu027, which is a liposomal formulation containing siRNA that silences the expression of protein kinase N3 in vascular endothelium, was evaluated for safety in advanced solid tumors. The formulation was well tolerated with only low-grade toxicities and led to stabilization of disease in 41% of the patients after at least 8 weeks (Schultheis et al., 2014).

Nano-enabled Immunotherapy

Immunotherapy offers immense scope in transforming oncology by a number of approaches involving immune checkpoint blockade and cellular therapies. As of now, the observed effect of immunotherapy has been moderate for a subset of cancer like melanoma and microsatellite-unstable cancers with approximately 35% objective response rate, but response rate for most of the cancer is generally low. In order to enhance the impact of immunotherapy in oncology, a greater understanding of the tumor-host immune system is required. Further, there are several challenges which impede this therapy, for instance, dosing can be limited due to lack of specificity which elicits auto-immune-type pathologies. Also, it is a challenge to break the local immune tolerance in tumors without breaking the systemic immune tolerance. Recent advancements in the field of nanotechnology bestow opportunities to significantly improve the arena of cancer immunotherapy by providing with the system that can enable the release of payloads (immunostimulatory or immunomodulatory molecules) on particular cell types and at a specific anatomical location (Goldberg, 2019). Apart from this, several new nano-immuno-based approaches are being investigated for oncology like nanoparticle vaccines, nanoparticle-based capturing of tumor antigens following radiotherapy to develop personalized treatments, and employing nanotechnology to create immune depots in or near tumors for in situ vaccination. Not only this, nano-enabled devices are also being harnessed for molecular and functional analysis of single cells to investigate both tumor and immune cells in order to elucidate biomarkers and understand immune responses to therapy. However, all these are in a nascent stage of development and will gradually advance as our understanding of cancer immunotherapy will evolve (https://www.cancer.gov/nano/cancer-nanotechnology/treatment#immunotherapy). As of now there are only a few nano-based immunotherapies which have entered in clinical

trials for cancer. One of them is CYT-6091 (Aurimune) which is a colloidal gold-bound tumor necrosis factor (TNFα). TNFα is a potent anti-cancer molecule which causes apoptosis, cell lysis, and induction of pro-inflammatory pathways. But its exogenous administration causes severe side effects like hypotension, septic shock, etc. which are bottleneck in its clinical translation. This led to the search for a delivery system which could reduce the toxicity, and eventually nanotechnology was sought for; gold-bound TNFα NPs were developed which were able to improve the killing of tumors and at the same time reduced the toxicity in preclinical models. But the problem with this system was that it was rapidly cleared by macrophages in the RES. So, a new formulation was developed which used PEGylation to reduce the detection by RES where thiol-derivatized PEG was conjugated with recombinant TNFα on the gold NP surface. These nanoparticles were tested in several different preclinical studies, and it was observed that they improved the biodistribution and showed significant antitumor effects. The promising preclinical results subsequently led to its first clinical trial in advanced or metastatic solid tumor malignancies to evaluate the safety profile, where it was found that CYT-6091 is safe for use in humans. Cytimmune, the manufacturer of Aurimune is planning for phase II trials to further establish the clinical efficacy (Min et al., 2015). In another phase I clinical trial, a mutanome-engineered RNA immunotherapy (MERIT) is being investigated with liposomal IVAC_W_bre1_uID and IVAC_M_uID in triple-negative breast cancer as a novel concept of individualized cancer (IVAC) immunotherapy. The TNBC-MERIT trial uses two complementary strategies, the IVAC® WAREHOUSE (IVAC_W_bre1_uID) which targets shared tumor-associated antigens and the IVAC® MUTANOME (IVAC_M_uID) concept, which identifies tumor-specific mutations in single patient and targets multiple neo-antigens derived from mutated epitopes, thus resulting in two custom-made IVAC® investigational medicinal products (IMPs) (IVAC_W_bre1_uID and IVAC_M_uID) for individual patients (https://www.clinicaltrials.gov).

Challenges in Clinical Translation

The field of nanotechnology has progressed significantly in the past few decades with extensive research being undertaken which has resulted in reporting new nanoformulations and their novel applications on an almost daily basis. Thus, the academic progress has been overwhelming, but the clinical translation and industrial acceptance of nanomedicine have been quite grim. In this section we will discuss the challenges in the clinical developmental path of nanomedicine starting from issues related to product development followed by preclinical and clinical aspects. A careful consideration of end-point users and market requirements in the early phase of development may contribute to promote success of nanomedicine in clinics (Metselaar & Lammers, 2020; Singh et al., 2020).

Product Development

The chemistry (structural and physicochemical) of nanomedicine is far more complex in comparison to conventional formulations like capsules, pills, tablets, and injections which pose several challenges in their large-scale manufacturing like batch-to-batch variability, scalability problems, poor quality control, high cost of manufacturing, poor consistency and storage stability of the final product, and degradation of the encapsulated active pharmaceutical agent during manufacturing process. Further, the task becomes even more difficult with multi-functional nanoparticles, which have multiple targeting moieties, encapsulate more than one therapeutic agent, or have surface modifications with coating. This integration of multiple components in one nanocarrier increases steps in the production process which consequently leads to problems in large-scale good manufacturing (cGMP) production with added difficulty in evaluation of quality assurance and quality control (Hua et al., 2018). As per FDA guidelines, chemistry, manufacturing, and controls (CMC) information is mandatory for investigational new drugs (IND) in each phase of trials in order to ensure the identity, purity, potency, and quality of drug and its product (https://www.fda.gov/regulatory-information). Thus, it is essential that the manufactured nanomedicine is well characterized and is reproducible, so that it does not face rejections in initiation for clinical translation.

Bridging Gap Between Preclinical Efficacy and Clinical Outcome

The next major challenge is the translation of preclinical efficacy to clinical success. There are several examples of nanoformulations which have shown very promising results in animals but failed miserably when tested in patients. This may be because the efficacy of nanomedicines is essentially dependent on pharmacokinetics, tissue distribution, accumulation, penetration, and payload release at target site which are critically influenced by tumor vasculature, stroma, and macrophage infiltration at target site. And these tissue morphology aspects vary dramatically in animal model versus human patients, as well as among different patients (Metselaar & Lammers, 2020). Attempts should be made to use suitable animal models which are reflective of human disease. As compared to orthotopic sites, subcutaneous models are less reliable due to abnormal stromal and vascular biology. Further, the effect on immunological parameters should also be assessed during preclinical testing which would help in predicting the toxicity profile of nanomedicine. In addition to this, phase 0 studies should be done which would give information regarding pharmacokinetic profile and tumor localization potential of nanoformulations in humans. The advantage of this trial is that it is cheaper than phase I clinical trials and provides quicker feedback on clinical feasibility of the investigated nanomedicine (Gharpure et al.,

2015). But still we need better tools and technologies to bridge the gap between preclinical efficacy and clinical outcome.

Advancing Novel Preclinical Models to Predict EPR

Targeting ability is one of the major advantages of employing nanoparticles which helps in increasing the efficacy and at the same time reducing off-target effects (Attia et al., 2019). As most of the nanoparticles rely on the EPR effect for selective accumulation in tumors, it becomes imperative to develop techniques which could accurately predict EPR effects for assessing the accuracy of delivery (Gharpure et al., 2015). For this, we need to put efforts in understanding the inter- and intra-individual heterogeneity of the EPR effect along with establishing tools to image EPR-based targeting. In this context Theek et al. used contrast-enhanced functional ultrasound imaging to characterize EPR-mediated passive drug targeting of near-infrared fluorophore-labeled polymeric drug carrier (pHPMA-Dy750) in CT26 tumor-bearing mice and found that a higher number of nanoparticles accumulated in highly vascularized tumors. This study provided the concept of using simple ultrasound imaging of vasculature to predict EPR effects, which can be utilized in other preclinical and clinical models (Theek et al., 2014).

Toxicological Studies

Another major challenge in clinical translation of nanomedicine is addressing the gap between preclinical toxicological studies and safety in patients. Apart from the inherent toxicity profile of encapsulated payload (drug or diagnostic agent), nanomedicines can cause toxicity at three different levels. Firstly, the biodistribution profile of drugs significantly changes when delivered through nanoparticles, thus resulting in the uptake and release of drug at off-target sites, may result in local overexposure and toxicity. It is widely known that nanoparticles have high propensity for accumulation in lymphoid organs and kidney. Secondly, unexpected toxicity may occur from the use of components which are not yet declared safe in humans. Though safety profile of commonly used phospholipids and polymers in nanomedicine has been studied, the use of different synthetic components for surface functionalization, coatings, and deployment of targeting ligands in multi-functional nanomedicine can have a significant impact on toxicological profile of nanomedicines following in vivo administration. The toxicity issues at these two levels can be addressed by employing early preclinical pharmacokinetic and biodistribution studies. Further, off-target toxicity can be assessed by extensive histopathological study and using clinical chemistry protocols, taking void nanocarriers in different doses as key controls (Metselaar & Lammers, 2020; Hua et al., 2018). The third level where nanomedicine may show safety issues is at the immunological level which are

difficult to predict using small laboratory animals. Certain specific nanoparticle–blood interactions have been reported to elicit complement cascade activation which play a major role in immunological side effects. Thus, to address this issue, cell interaction and complement binding assays can be performed in vitro. Further, for preclinical toxicological studies, lager animals like pigs can be used (Szebeni et al., 2018).

Optimizing the Administration Route

The route of administration used during preclinical studies to evaluate the efficacy of nanomedicine should be the same as that expected to be administered in patients (Gharpure et al., 2015). This would help in reducing the response rate and therapeutic efficacy variability in preclinical and clinical studies to some extent. In certain preclinical studies, intratumoral injections are given to prove the efficacy of nanomedicine (Al-Ghananeem et al., 2009; Chattopadhyay et al., 2012); however, it is not always possible to give intratumoral injections in patients like in the case of metastatic disease. In these circumstances the same effects would not be obtained when it would be given by another route as the nanomedicine will have to incur several biological barriers before reaching the tumor. Thus, it is important to optimize the route of administration during preclinical studies.

A Look into the Future of Nano-oncology

Nanotechnology has already established its potential in oncology with several formulations already in the market for both diagnosis and therapeutics. But it still holds immense potential in revolutionizing the field by providing means of integrating novel technologies to further aid in diagnosis and therapy. The relatively new additions in the field of nano-oncology are photoacoustic tomography (PAT), theranostic nanomedicine, nano-based cancer stem cell therapies, and personalized medicine. PAT is an upcoming imaging modality which is based on acoustic detention of optical absorption from either internal chromophore like deoxy-hemoglobin and oxy-hemoglobin or external contrast agents like nanoparticles to generate images of high resolution in both the optical ballistic and diffusive regimes (Xia et al., 2014). The amalgamation of PAT with nanoparticles probes holds a huge potential in increasing the sensitivity as well as accuracy of detention of cancer in early stages (Conde et al., 2012). The other new technology in nanomedicine is theranostic which is the combination of diagnosis and therapy. Nanotechnology by virtue of its ability to carry multiple components in one nano-cargo holds the ability to deliver both the therapeutic and diagnostic agents simultaneously which would help in monitoring real-time therapeutic response, studying off-target effects, and developing personalized medicine (Lymperopoulos et al., 2017). Another very

recent advancement in the field of nano-oncology is the development of nano-based cancer stem cell therapies. Cancer stem cells are notorious cells in tumor mass which are responsible for chemoresistance, metastasis, and relapse that are the major hurdles in achieving complete cancer cure. Till today there is no therapy in the market which could target and kill these cells, but nanotechnology provides hope for specific targeting of CSCs. Nanoparticles can be engineered in such a way that they themselves can differentiate CSCs into non-CSC phenotype, which would then be more susceptible to available chemotherapeutics (Geng et al., 2020). Not only this, nanotechnology holds the potential of developing personalized medicine which could assess response or risk of disease in individual patients. It opens new avenues to design tools which can obtain and integrate biological information from biomarkers, cancer cells, and tumor environments from individual patients to design materials which can respond according to the received information (PMID: 30563441). The success of these new nano-fields can bring paradigm shift in clinical oncology.

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Chapter 4
Cancer-Targeted Nanotheranostics: Recent Advances and Future Perspectives

Hector Katifelis and Maria Gazouli

Introduction

Cancer represents a leading cause of morbidity and mortality at a global scale. Based on the WHO statistics, cancer is the cause of 9.6 million deaths in 2018, while approximately 17% of deaths is due to malignancies (World Health Organization, 2018). Even worse, new cases are expected to rise to 22 million by 2040 (Kohler et al., 2015). Thus, the need for the development of new methods for the efficient and personalized cancer management is of high priority.

During the past years, several potential applications of nanoparticles in the field of medicine have been studied thoroughly in critical areas that involve targeted drug delivery and medical imaging.

One of the most promising approaches is the combination of therapy and diagnosis (mostly refers to medical imaging) in a single platform (in the case of nanoparticles, it is referred to as a nanoplatform). These approaches resulted in the development of nanotheranostics which are expected to be important tools in the hands of clinicians. The need for such nanoplatforms is highlighted by the rapidly growing numbers of related publications (Viswanadh et al., 2018). Hopefully, these nanoplatforms will allow the monitoring of the disease (the extent of the affected tissues) and the visualization of the drug delivery kinetics, while the therapeutic efficacy will be increased.

In the current chapter, we present the most important concepts of nanotheranostics towards cancer management. In the first paragraphs, we discuss the most common forms of nanocarriers, and subsequently we examine their potential in therapeutic and imaging applications. Finally, the most novel nanoplatforms in

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clinical studies are described, while a discussion about translating nanotheranostics into clinical practice and their limitations is discussed at the end of the chapter.

Nanocarriers

Carriers (which due to their nanoscale size are referred to as nanocarriers) are a crucial element of every nanotheranostic system which aims to fuse therapeutic and imaging agents in a single platform (the terms nanoplatforms and nanocarriers are equivalent). Several types of nanomaterials have been studied for their potential uses in such platforms ranging from organic polymers to noble metal nanocarriers (Daglar et al., 2014; Fatima et al., 2020). Figure 4.1 shows some commonly used nanocarriers. In the following paragraphs, we will discuss the most promising carriers to date, liposomal, polymeric and inorganic nanocarriers.

Liposomal Nanocarriers

Phospholipids (and most importantly phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine) are the most aberrant components of any mammalian cellular membrane. Theoretically, any type of nanocarrier that would use these molecules would be expected to be highly biocompatible. Indeed, lipid nanoparticles of miscellaneous forms (such as micelles) have already been used in drug delivery systems. A typical example of the use of liposomal solutions in clinical practice is the use of doxorubicin (an anti-tumor agent) in the form of liposome injection (Access Data FDA, 2020). However, doxorubicin is not the sole drug that makes use of the unique opportunities that liposomal carriers offer. Several other drugs are

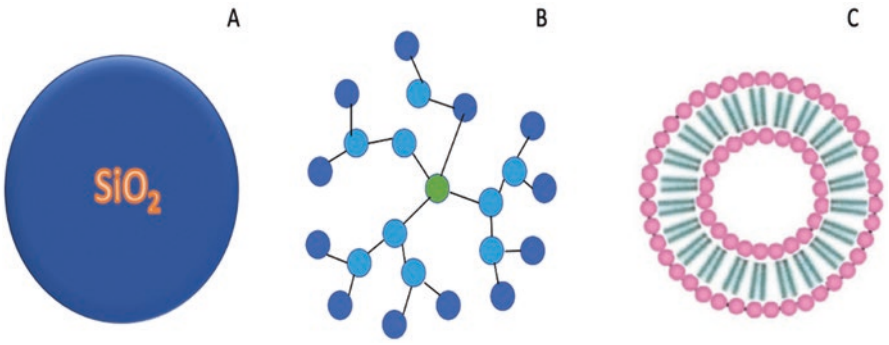

Fig. 4.1 Nanotheranostic platforms can use different types of nanoparticles that range from inorganic compounds such as SiO_2 (**a**) to organic compounds that include dendrimers (**b**) and liposomes (**c**). (Copyright Wiley-VCH GmbH. Reproduced with permission) (Ma et al., 2016)

already available for clinicians to prescribe, while the list of other liposomal-based drugs under clinical trials is constantly growing (Bulbake et al., 2017).

The liposomes used in these formulations can be of either natural origin or alternatively via chemical synthesis, while their size varies; some formulations are only a few nm in diameter, while others are more than an order of magnitude larger. When it comes to liposome formulation, a typical method is the rehydration of a lipid film followed by sonication (Luk et al., 2012). In order to form a theranostic nanocarrier, an additional step is required; an additional functional agent has to be loaded.

Due to the dual nature of a liposome (it contains a hydrophobic phase between the lipid layer and a hydrophilic aqueous core), they represent one of the most versatile "vehicles" for an agent to be attached. Whether hydrophilic or hydrophobic, many different agents in terms of chemistry and aqueous behaviour can be loaded. To date, many anti-tumor agents apart from doxorubicin have been successfully merged with nanocarriers in order to achieve a targeted release. Among these, cisplatin, one of the most efficient chemotherapeutic drugs and one of the most notorious for its side effects, has been added in liposomal formulations (Aldossary, 2019; Zahednezhad et al., 2020). However, the advantages of liposomes are not limited to their ability of loading both hydrophilic and hydrophobic agents. They also shield the loaded agents from the extracellular hostile environments that they face which results in prolonged circulation time and enhanced tumor accumulation which is boosted due to the enhanced permeability and retention effect (EPR Effect) (Golombek et al., 2018).

It should be noted that liposomes are not the only lipid-based nanocarriers available. Other formulations including oil-in-water emulsions that can encapsulate metal oxide nanoparticles (most notably iron oxides) and a dye (Cy7) that allows the monitoring of the effectiveness of a particular carrier are used in combination with the conventional MRI so as for medical imaging of increased clinical value to be obtained (Ma et al., 2016). Furthermore, solid nanolipids have been employed to deliver a combination of paclitaxel and a Bcl-2 targeting siRNA into cancer cells (Bae et al., 2013; Albuquerque et al., 2015).

Another advantage of using structures similar to these of a typical cell is immune clearance evasion. Membranes derived from red blood cells have been fused with polymer nanoparticles and have shown prolonged elimination half-life, a finding indicative of their possible anti-tumor potential (Hu et al., 2011).

Polymeric Nanocarriers

Polymeric nanoparticles represent a vast category of highly heterogeneous nanoparticles that include, among others, oligodendrimers, polymersomes and microbubbles. Their main purpose is to achieve a drug delivery technique with increased efficiency. A conjugation between a polymer and a chemotherapeutic drug (or any other type of drug) may augment the hydrodynamic size, decrease metabolic

clearance and at the same time provide a sufficient blood circulation half time. Combined, these parameters can improve the accumulation at tumor sites, while at the same time off-target effects could be reduced. The most commonly used polymers used in such conjugates are PEG and PLGA which have already met the approval criteria. The addition of an additional drug or imaging agent to increase the theranostic efficiency is currently a field of intense research (Ma et al., 2016).

Dendrimers represent a class of organic, intensely branched molecules with a dense exterior surface with several applications in medicine. When compared to standard polymers, these nanoparticles can form mono-dispersive nanostructures with abundant functional groups. Thus, theranostic agents can be bound via noncovalent bonds, while functional linkages permit responses triggered by different stimuli that can lead to the release of its cargo (in our case a chemotherapeutic drug).

Several dendrimer-based systems have been described in the field of cancer theranostics that include, among others, PPI (polypropylenimine) and PEG (polyethylene glycol) (Lo et al., 2013).

Shi et al. found that a dendrimer-based platform of generation 5-entrapped gold nanoparticle linked with a-tocopheryl succinate can increase CT imaging quality without reducing its therapeutic effects (Zhu et al., 2014).

Another type of polymers used in nanoplatforms is the amphiphilic block copolymers. These blocks contain a hydrophilic compartment (such as PEG) and a lipophilic compartment which triggers a controlled accumulation of these blocks due to hydrophobic interactions that occur inside an aqueous medium. Moreover, this block can be used to form structures of different shapes and sizes such as micelles and vesicles which are among the most useful structures that can serve the purposes of nanotheranostics. Such a structure has already been prepared by Liu et al. These structures are made from PEG-b-PKGA vesicles that have been loaded with doxorubicin and gadolinium that allow MR imaging while they show anti-cancer effects simultaneously (Liu et al., 2014b). A similar nanotheranostic approach was adopted by Chen et al. In this study, a chemotherapeutic compound and a fluorescent dye were engulfed in a polymeric micelle. This resulted in a nanotheranostic compound that allowed an image-guided anti-cancer treatment (Wan et al., 2014).

Despite the promising results of several self-assembled formulations, some major limitations are present. Most importantly, these structures may self-disassemble when the micelle concentration is not high enough. Another important issue is the maximum drug capacity that can be loaded in micelles. When approaches use both an imaging agent and a therapeutic one, the available space is limited. Thus, the anti-cancer drug load may be smaller to that of a micelle loaded only with an anti-cancer agent. A related issue is a possible mismatch between the fluorescent dye and the therapeutic agent that could disturb the desirable ratio among them, resulting in poor clinical outcomes.

Other possible candidates for theranostic applications are proteins. Their increased biocompatibility and their versatile nature that allows several modifications make them ideal molecules for such uses. Other advantages of proteins include their biodegradation and their non-immunogenic nature. By using a polypeptide that is composed of repetitions of a small peptide sequence, their assembly into

vesicles (or micelles) can be performed with increased precision. Zhu et al. (2015) used elastin-like polypeptides in order to build nanostructures (via self-assembly) that are stimuli-responsive. Interestingly, these formulations resulted in an almost full tumor regression.

Another example of the use of proteins is drug delivery systems based on albumin. Albumin is the most abundant protein in human plasma, and it is not surprising that it was one of the first proteins to be studied for such applications. An albumin-based system that aims to deliver paclitaxel (another chemotherapeutic agent) has already been approved by the FDA from 2005 (Mackay et al., 2009). Another abundant protein, apoferritin (which is ferritin without the Fe atoms) has also been used in nanoplatforms. Cutrin et al. (2013) encapsulated an MRI contrast agent and curcumin in apoferritin. The result was a nanocarrier which showed increased bioavailability, while the therapeutic effects of curcumin were preserved.

Practically, protein of any origin can be tested in nanocarrier formation. Indeed, nature has provided science several invaluable drugs and most notably antibiotics such as penicillin that allowed the treatment of the so-called white plague, tuberculosis (Barberis et al., 2017).

Thus, it is reasonable that several non-human proteins have been employed in nanocarrier formulations. For instance, gelatin has been widely used in nanocarriers. A 2019 study (Abdelrady et al., 2019) showed that gelatin nanocarriers were capable of delivering methotrexate during lung cancer therapy. The findings of this study were indicative of gelatin nanocarrier's potency; the IC50 of methotrexate was reduced to a fourth of that of methotrexate alone. A great advantage of gelatin is its abundant ionizable groups that allow the conjugation of several drugs or any other type of chemical modifications (Lohcharoenkal et al., 2014).

Another promising candidate is elastin. This protein is crucial for the connective tissue function as its name suggests it provides elasticity. A recent study (Dhandhukia et al., 2017) revealed that elastin-like nanocarriers were capable of suppressing tumor growth in a mouse model. This action was mediated by the encapsulation of rapamycin which resulted in superior tissue targeting.

Interestingly, researchers have moved even further from humans and animals, and plant proteins have also been tested recently. One of these is gliadin, a gluten protein that can be found in wheat and that has already been used in several pharmaceutical products (Arangoa et al., 2000). Its natural origin combined with its high biocompatibility and biodegradability has made it a potent tool in the field of nanophytotechnology. A recent study showed that anti-cancer drugs can be loaded into gliadin nanoparticles for the treatment of breast cancer (Gulfam et al., 2012), while its uses have been studied outside the field of oncology, even in the treatment of auto-immune diseases (Freitag et al., 2020).

Proteins found in milk may also be of use for such purposes. Two milk proteins b-lactoglobulin and casein have studied for their potential use as nanocarriers. The former has the characteristic of retaining its conformation even at acidic tissue environments while at the same time it can resist proteolytic processes (such as chymotryptic digestion). Its low cost combined with its abundance makes it a promising candidate for several drug delivery systems. A 2019 study (Bijari et al., 2019)

showed that irinotecan-loaded b-lactoglobulin nanoparticles had an increased effect on HT-29 cancer cells compared to the free drug.

Casein has also favourable physicochemical properties. It can withstand most processing treatments (heat and mechanical stress included), leaving its micelles intact. An in vivo study (Gao et al., 2019) showed prolonged survival times in mice that received casein nanoparticles loaded with 10-hydroxycamptothecin compared to the group that received 10-hydroxycamptothecin alone. At the same time, these nanocarriers managed to bypass the blood-brain barrier, which most of the time limits the therapeutic effect of conventional drugs. Thus, casein nanoparticles have a potential use for brain tumors and other brain pathologies.

Due to their abundance, soy proteins have been studied for several purposes in the field of medicine. Its balance between amino acids with different side chains (polar, non-polar and charged side chains) allows its conjugation with both hydrophilic and hydrophobic drugs. A 2019 study (Qian et al., 2019) employed phenylboronic acid in soy nanoparticles in order to make the tumor's environment. Indeed, these nanoparticles reduced the interstitial fluid pressure (which is increased in solid tumors due to blood vessel leakage and lymph vessel malformations). This finding is promising, since by reducing IFP, solid tumors could become sensitive to antitumor agents that otherwise could not penetrate the tumor cell (Heldin et al., 2004).

Inorganic Nanocarriers

Inorganic nanocarriers represent another wide category that includes several different nanomaterials with the potential use in theranostic nanosystems. Several metals including gold (Au) and platinum (Pt) as well as non-metals most notably silica (Si) have been tested the past decades to such uses (Lin et al., 2016).

Indeed, silica nanoparticles have been used in various forms that include, among others, mesoporous, solid and hollow nanoparticles. Additionally, the sol-gel preparation technique in SiO_2 nanoparticles allows the stabilization and the cross-linking between various therapeutic and diagnostic agents by forming a SiO_2 shell.

A 2020 study (Carniato et al., 2019) used a delivery system based on mesoporous Si that included rhodamine dyes, while the porous were impregnated with mitoxantrone (a chemotherapeutic agent). Interestingly, this nanotherapeutic system showed increased cytotoxicity on the MFC7 cells compared to the free drug, while medical imaging that was obtained (by using MRI scan) showed increased contrast enhancement when compared to untreated cells.

Another study (He et al., 2014) showed that mesoporous Si nanoparticles loaded with ruthenium polypyridyl complexes exhibit an increased cytotoxic effect on cancer cells via the induction of apoptotic pathways. The autofluorescence of the Ru complex served as an imaging agent making this formulation a promising theranostic nanocarrier.

Equally promising are the results of another type of non-metal nanocarriers, carbon-based platforms. These platforms are characterized by their high versatility

in terms of their possible formations. Fullerene nanoparticles (which are an allotrope of carbon and are composed of carbons linked with single and double bonds that form a mesh) are widely used in several biomedical applications (Lin & Lu, 2012). Their applications involve their use in cancer diagnostics (Sagman, 2002) and cancer treatment such as the targeting of cancer of melanoma. Their ability to bypass the BBB is also indicative of their potency as nanotheranostic carriers (Lin & Lu, 2012).

Carbon nanotubes are also valuable tools in cancer nanotheranostics. An easy way to visualize their shape is thinking of them as a graphene sheet that rolls up in many different ways forming the "nanotube". Their main classification refers to the number of layers that form the tube's walls. Thus, carbon nanotubes are categorized as either single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs) (Sanginario et al., 2017). CNTs can be loaded with anti-cancer agents which can be attached covalently or noncovalently. Noncovalent bond is important, since it has been suggested that any covalent modification of the therapeutic agent could decrease its anti-cancer potency. On the other hand, the weaker nature of noncovalent bond strength could decrease the attachment efficacy. Regarding the targeted drug delivery, a novel and promising approach includes the sealing of the nanotube's end with molecules that can be cleaved intracellularly. Thus, when the CNT has reached its destination (in this case the cancer cell), it can unload its cargo and selectively affects its target and no other tissue cells. Additionally, the CNT environment allows the attachment not only of drugs such as paclitaxel but also of small interfering RNA (Madani et al., 2011).

Graphene is another form of carbon that has been intensively studied the past few years. Its unique characteristics involve its particularly large surface area and the ease for cargoes to be loaded. Recently, Zhang et al. attached doxorubicin and Gd complexes to graphene oxide nanoparticles and showed their theranostic behaviour (Zhang et al., 2013).

Metallic nanoparticles represent perhaps the most important category of inorganic nanocarriers. Several different metals both noble and basic (and their alloys) have been tested with promising results. For instance, gold nanoparticles have been shown as potent contrast agents in X-ray scans and computed tomography (Mahan & Doiron, 2018). Similarly, Cu nanoparticles have been proved efficient for PET imaging applications (Lu et al., 2018). Recently, Han et al. (2019) showed that iron oxide nanoparticles (IONPs) can be used in dual modal imaging for the detection of breast cancer.

When compared to other nanoplatforms (regarding theranostic applications), metal nanoparticles have several important advantages. Firstly, their synthetic routes are well characterized, and practically metal nanoparticles can be formed in almost any desired shape and size (Abedini et al., 2016). Secondly, many metal NPs can serve as therapeutic agents on their own. For example, Ag NPs have been shown as promising anti-cancer agents in the literature (Raja et al., 2020). Thirdly, the versatile nature of metals allows their integration in structures of different metals that can have a synergistic effect. Lian et al. (2014) showed that IONPs engulfed in Au nanoshells can be used as MRI contrast agents and photothermal therapy (PTT). This

action was mediated due to a peak in the plasmonic resonance of the Au nano-shell in the near-infrared region.

The list of nanoparticles that can be used in theranostic nanocarriers is constantly growing in size as new materials are being tested. Semiconductor crystals, titanium dioxide nanoparticles (TiO_2 NPs) and metal organic frameworks (MOFs) have also been tested as promising candidates. MOFs are characterized by an excellent drug-loading capacity, bionic catalytic properties and satisfactory biocompatibility. Moreover, MOFs can be modified so as active targeting to be achieved via the use of ligands or the addition of antibodies (Cai et al., 2020).

All the aforementioned available materials for nanoplatforms make the whole process of selecting the most suitable nanocarrier a challenge. Moreover, among the myriad combinations between the different nanocarriers, therapeutic and diagnostic agents to make a selection of the most suitable make this challenge even greater. Important factors that will favour a nanocarrier over another include its maximum cargo loading capacity (in this case, a chemotherapeutic agent) and its release profile at tumour tissues. Additionally, the nanocarrier needs to be biocompatible; immune responses must not be triggered. The differences between the intensity of the EPR effect among different nanocarriers must be taken into serious consideration. The EPR effect refers to the selective accumulation of a substance at tumour tissue due to vessel malformation and poor lymphatic drainage (Patra et al., 2018). This effect represents a passive targeting approach that can result in a more efficient theranostic nanocarrier with less off-target effects. Another major limitation that may occur is possible alterations of physicochemical characteristics when switching from in vitro approaches to the bloodstream. Critical parameters include the nanoplatform's stability and its biological half-life. Finally, every nanocarrier with a potential of being translated into a clinical tool needs to be easily modified and to have an affordable production cost (Ma et al., 2016).

Anti-tumour Agents

Most conventional cancer therapies include chemotherapy, radiotherapy and surgery either alone or combined. However, each of them is tied with several side effects that severely affect a patient's life or worse; the possible side effects could exclude him from a potential therapy. Chemotherapy side effects may include thrombocytopenia and anaemia, cardiotoxicity, nausea and vomiting, among others (Oun et al., 2018). Radiation is associated too with several side effects. These include not only with the direct action of radiation (such as skin ulcers) but indirect effects as in the cases of head and neck cancer where it could lead to tooth decay and tooth loss because of the destruction of saliva glands (Mohan et al., 2019). Surgical approaches, apart from the risk that are associated with any surgical procedure, sometimes require patient rehabilitation and replacement of the lost tissues, since healthy tissue must also be removed in order to ensure no cancer cells were left behind (Benjamin, 2014).

Despite the serious side effects of any chemotherapy, its beneficial effects are considered greater, and this is the reason they are still used in clinical practice, since it increases the patient survival rates (Huang et al., 2017). The standard chemotherapy agent categorization includes different classes of chemotherapeutic drugs including alkylating agents (such as cisplatin), anti-metabolites (dehydrogenase inhibitors, nucleoside inhibitors, topoisomerase II inhibitors, kinase inhibitors) (Abotaleb et al., 2018), anti-tumor antibiotics (such as plicamycin) (Gao et al., 2020) and phytogenic anti-tumour agents. Figure 4.2 shows some common side effects of cisplatin as well as its chemical structure.

Unfortunately, despite the plethora of available cancer chemotherapy options, very few cancer nanomedicines have gained FDA approval. Indicative of the lack of such medicines is the publication of a review commenting exactly on the scarcity of nano-chemotherapeutic agents (Venditto & Szoka Jr, 2013). The first cancer nanodrug to be approved was Doxil (PEGylated liposomal doxorubicin) in 1995, followed by DaunoXome (liposomal daunorubicin). The past 5 years, five nanodrugs have been approved either in Europe or in the USA for cancer treatment: ONIVYDE (liposomal irinotecan), DHP107 (paclitaxel lipid nanoparticles), Vyxeos (liposomal daunorubicin combined with cytarabine), Apealea (a micellar form of paclitaxel) and Hensify (which is composed of hafnium oxide nanoparticles) (Salvioni et al., 2019).

A promising approach for incorporation with theranostic nanoplatforms is the use of prodrugs (precursor forms of drugs that are inactive). The use of prodrugs can be beneficial in terms of reducing the drug's toxicity other than the target tissues. For instance, Cao et al. used a cisplatin prodrug in a nanoparticle formulation (cationic lipid-assisted nanoparticles) in order to load greater amounts on the cancer cells and to counter tumour drug resistance (Cao et al., 2016).

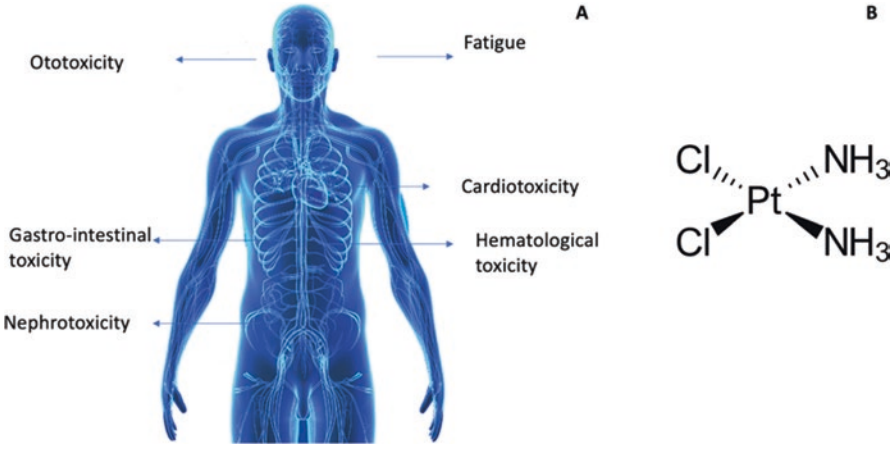

Fig. 4.2 Adverse effects of cisplatin (**a**) and the chemical structure of cisplatin (alkylating agent) (**b**)

A major goal of each delivery system is its ability to load and subsequently deliver a high drug amount at the desired target. However, an increased amount of drug may decrease its solubility in aqueous solvents. In order to avoid this issue, several nanosystems use platforms with a high surface-area-to-volume ratio or mesoporous formulations for use in theranostic approaches (Ma et al., 2016). Porous Si nanoparticles have been successfully integrated sorafenib (a kinase inhibitor) with a drug-loading percentage of approximately 28%, while the therapeutic agents can be released in a sustained fashion (Wang et al., 2015).

Another major barrier that cancer nanodrugs need to overcome is multidrug resistance (MDR) which is usually triggered by single-drug treatment protocols. A possible strategy to deal with this issue is the simultaneous administration of antitumor agents with P-glycoprotein inhibitors. Indeed, recent studies showed that this combination decreased cell viability compared to the use of the chemotherapeutic agent alone. Interestingly, the P-glycoprotein inhibitor when used alone did not provoke any decrease of cellular viability (Nanayakkara et al., 2018). Masking the charge of anti-tumour agents could also decrease MDR (Brigger et al., 2002).

A second commonly used approach is the simultaneous use of two different chemotherapeutic agents so as for synergistic effects to take action. However, this is no easy feat. Common issues that may occur during the combination of different drugs is the limited solubility of one or both drugs, limited permeation (which may result in a difference than the desired intracellular levels of both drugs) and even different drug stabilities (Jain & Thareja, 2019).

Nanoparticles in Medical Imaging

Imaging quality is a major characteristic of any theranostic nanosystem. Both preclinical and clinical trials involve computed tomography (CT), magnetic resonance imaging (MRI), ultrasounds (US) and positron emission tomography scan (PET scan) (Sanchez et al., 2013). All the aforementioned techniques are characterized by their excellent sensitivity and specificity making them reliable diagnostic methods that can be used during the initial diagnosis and during the monitoring of the disease (e.g. PET scan is a valuable technique to evaluate the effectiveness of a cancer treatment that involves surgical excision). Additionally, they can be used for the patient follow-up for the early detection of a possible metastatic site (Vensby et al., 2017).

Positron Emission Tomography

PET scan is an imaging technique commonly used during nuclear medicine applications. It is capable of providing 3D images that can be either static or dynamic (real-time imaging). Shortly, its principle of function includes the use of a nuclide that emits b+ radiation (positrons) which after a very short distance are annihilated via

the collision with an electron. Thus, two opposite photos occur and are subsequently detected by the imaging system. So far, the most commonly conventional PET radioisotopes include ^{11}C and ^{18}F (which can replace an H atom in a glucose molecule) (Ma et al., 2016; Vaquero & Kinahan, 2015). The complexation of the nuclide and the nanoparticle is an important aspect for the development of any radiotracer. The most common radiolabelling strategy involves the attachment of the radioactive metal to the nanosystem via chelators. Thus a "cold" nanoparticle is used, and the isotope is subsequently added, converting it into a "hot" nanoparticle. One of the most promising classes of radiolabelled theranostic nanoparticles, suitable for PET scan imaging, is silica nanoparticles. Their biocompatible nature and their well-defined chemistry make silica a promising candidate for the incorporation in PET scan imaging theranostic nanosystems. Ultrasmall silica NPs (with diameter of approximately 6 nm) have been approved by the US FDA while they have already been used for imaging in metastatic melanoma (Phillips et al., 2014; Goel et al., 2017).

Computed Tomography

CT imaging is an X-ray technique widely used in medicine that was developed more than half a century ago by Hounsefield and Cormack (Goodman, 2010). Currently, the clinically approved contrast agents that are used include small iodinated molecules and several barium (Ba) suspensions (Cormode et al., 2014). Unfortunately, these agents have been proved nephrotoxic in several cases, and thus renal function must always be checked. Thus, several patients may be excluded from the use of these agents and the increased imaging quality that this technique could offer (Andreucci et al., 2014). The fact that these agents are used in large doses due to their low X-ray absorption could also trigger hypersensitivity reactions (Ma et al., 2016). The basic principles of both PET scan and CT are shown in Fig. 4.3.

Nanotechnology research has focused on the development of several potential materials that could serve as CT contrast agents. Metals with high atomic numbers (Z greater than 50) are believed to be effective agents for CT imaging. A recent example is the research of Liu et al. who used PEGylated WO3-x nanoparticles for CT imaging applications merged with photothermal therapy. The formulation used showed no harmful effects upon normal tissue. On the contrary, tumour cells were ablated when exposed to near-infrared radiation (NIR) making this nanomaterial for nanotheranostic applications (Liu et al., 2014a).

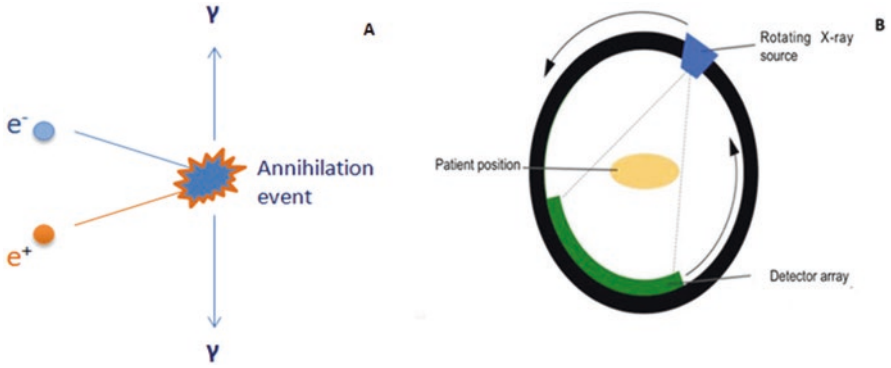

Fig. 4.3 The source of information during a PET scan derives from two opposite-direction photons that occur after an annihilation event between an electron and a positron (**a**). During a CT scan, the source of radiation is placed outside the patient, and the medical image is formed from the photons that reach the detector array (**b**). (Adapted from Open Access journal under the term of Creative Commons Creative Commons Attribution 4.0 License) (Cormode et al., 2014)

Nanotheranostics in Clinical Studies

The past 25 years, 50 different nanomedicines have received FDA approval and are currently used in clinical practice (Ventola, 2017). Most commonly approved formulations include polymeric, liposomal and nanocrystal nanodrugs, while drug delivery nanosystems based on NPs have been employed in approved nanodrugs including metal oxides and several other inorganic compounds (Ventola, 2017; Bobo et al., 2016). It is worth noting that a large percentage of the already approved nanodrugs are characterized by a reduced toxicity, while their efficacy is not heavily improved compared to standard formulations. Indeed, the main reason that several nanodrugs have failed clinical development is their inability to show a higher efficacy, since reduced toxicity can be already achieved by other drugs (conventional drugs and nanodrugs) (Caster et al., 2017). The basic characteristics of clinical trials (phase I and II) are shown in Fig. 4.4.

Nanoplatin (NC-6004, NanoCarrier Co., Ltd.) is a micellar formulation of cisplatin and is currently being investigated under phase 1 and phase 2 clinical trials either alone or combined with other chemotherapeutic agents such us gemcitabine. Another nanoformulation that is being tested is SN-38. Sn-38 is an active metabolite of a topoisomerase inhibitor (irinotecan). At least two phase 1 trials have been completed and a phase 2 trial in solid tumours (including breast cancer and non-small cell lung carcinoma, NSCLC). Additionally, Genexol PM (Samyang Biopharma) which is a micellar PEGylated formulation of paclitaxel is considered as an alternative to Kolliphor-based paclitaxel. This nanodrug has already been approved for use in patients with metastatic breast cancer in South Korea and currently is under phase 2 clinical trials in other countries.

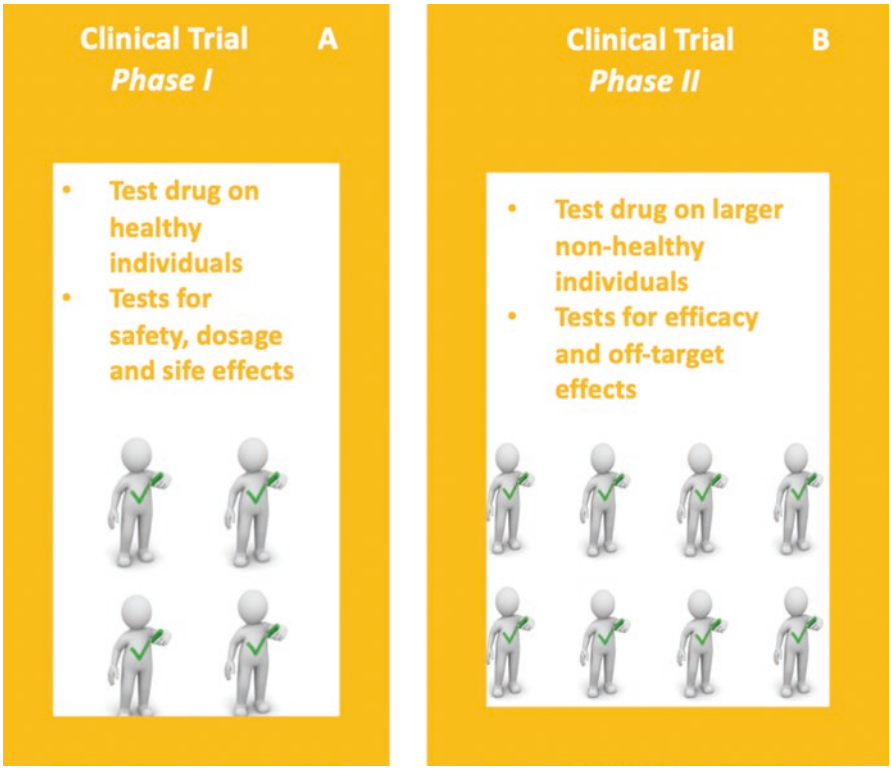

Fig. 4.4 The basic characteristics of phase I (**a**) and phase II (b) of a clinical trial

Cornell dots are capable of inducing cellular death and to reduce tumor size after several injections in treated mice. Their structure is based on an internal Si core which is labelled with a NIR dye, a targeting moiety and a polymer layer. This formulation results in a stable nanoparticle which is more than 20 times brighter than any conventional solution of the dye used. A human trial that involved five participants demonstrated a promising pharmacokinetic and safety profile when used as an imaging agent (Bobo et al., 2016).

Limitations and Future Perspectives

Although there are several research papers focusing on the benefits of several nanodrugs and nanoformulation for the treatment of cancer, there are several reasons that these agents fail to be translated into clinical practice. One of the most important limitations is the often-limited comprehension of interactions between biological components and the nanoparticle itself. Most importantly, the protein corona means that the nanoparticle's surface is covered with proteins which heavily alters its

stability, clearance and the possible immune response. The formulation of blood-like media is a promising effort that will allow the deeper understanding of this phenomenon. Regarding theranostic system, the control of the nanostructure's physicochemical properties is crucial. Usually, theranostic systems consist of an imaging and a therapeutic agent that work separately. Thus, accurate control is essential so as for the results of preclinical trials and in vitro research to be validated in clinical practice. An answer to that problem could be the development of smart theranostic systems. For instance, environmental stimuli that could include pH changes and enzymes at the target tissue could facilitate the accumulation or the activation of the nanoplatform. In order for this goal to be accomplished, the targeting agents and therapeutic agents could be designed so as to work synergistically and without negatively affecting the actions of one another.

Another important issue is the technical challenges that occur during productions. For example, in 2017, the production of DepoCyt was halted due to non-specified technical issues that affected its production (He et al., 2019; Pacira halts production of Depocyt, 2020).

Moreover, safety issues occur, despite the toxicity screening that each and every product under clinical trial has to face. Two examples are the MRX45 which failed at phase 1 since one out of five patients experienced serious adverse events from the immunity system and MM-3210 (2019) which also failed in phase 1 since it caused cumulative peripheral neuropathy (Mirna Therapeutics Halts Phase 1 Clinical Study of MRX34, 2020; Merrimack Discontinues Development of MM-310, 2020).

Another issue is the "controversial" EPR effect. While initially it was believed that the EPR effect was one of the greatest advantages of the use of nanoparticles that resulted in the tumor passive targeting (it has even been referred to as the golden principle), controversial statements are common in newer research papers. Such cases include the failure of the EPR effect in clinical studies or the presence of the EPR effect on mice but its absence on humans. An additional barrier is the poor pharmacokinetics that several nanoparticles show. The bloodstream levels of several nanoparticles draw rapidly due to the mononuclear phagocyte system. The speed of this process, which can range from minutes to hours, can affect the drug efficacy and can even lead to non-specific distribution of the nanoparticles to unwanted sites (Albanese et al., 2012). It should also be noted that despite the plethora of cancer animal models, their reliability at some cases is less than satisfactory. No known animal models can reproduce all the aspects of human disease (including cancer-driving mutations and the metastatic profile). This could be the reason that great differences occur in the therapeutic efficacy of a given drug between preclinical and clinical studies (Shi et al., 2017).

In summary, theranostic nanosystems are more than promising strategies that could bring precision medicine into clinical practice. There are several and important barriers in this field, but the intrinsic advantages of nanoparticles will sooner or later allow their extended use in clinical trials. For that purpose, the combined knowledge of researchers of different scientific backgrounds (chemistry and material science, biology and medicine) will ensure the know-how of building a

successful and at the same time smart nanosystem that its different parts (therapeutic and imaging agents) will work synergistically in order to provide maximum results.

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Chapter 5
Nanotheranostics: The Future Remedy of Neurological Disorders

Saba Sohail and Fakhar-Ud-Din

Introduction

Nanotheranostics and Neurological Disorders

Nanotheranostics

An emerging and promising area of medicine "theranostics" inculcates both diagnosis and therapy and treats difficult diseases effectively (Sumer & Gao, 2008). The existing treatment modalities are only for treating limited populations and only at the selective stage of disease, so that there is a firm need to develop a subject-specific approach that provides superior therapeutic regimen and improved prognosis (Xie et al., 2010). Nanotheranostics involves nanotechnology to treat different diseases effectively with improved diagnosis and therapy. It incorporates different types of nanocarriers, e.g. dendrimers, micelles, liposomes, niosomes, inorganic, polymer conjugates and metal nanocarriers (Din et al., 2017; Khatoon et al., 2017; Mir et al., 2017; Shahzad et al., 2020). The advantage of nanotheranostics is to treat disease at its early phase, so it acts as a new angled, advanced form and game changer class to treat various diseases at the level of cell or molecule with the help of nanotechnology and due to incorporated therapeutic and diagnostic moiety (Janib et al., 2010). Nanotheranostics has a great potential to cure deadly neurological disorders (WHO report, 2007). This chapter depicts a detail review on the new breakthroughs in advance nanotheranostics for treating neurodegenerative diseases.

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Nanotheranostics as Both Therapeutic and Diagnostic Moieties

Deadly diseases are cured in an effective manner if treated at an early phase. It is an area of great concern that all treatments that exists are not very much effective in diagnosing and treating brain disorders at an initial disease stage; therefore the game changer nanotheranostics can diagnose and treat disease effectively and help in individualizing neuronanomedicine. The usually used diagnostic agents in nanotheranostics are quantum dots, iron oxides, radionuclides, heavy metals and fluorescent dyes. Some therapeutic agents are organic drugs, proteins, hydrophobic agents, genetic materials and peptides (Janib et al., 2010; Xie et al., 2010; Ye & Chen, 2011). Nanocarriers are the main components of nanotheranostics besides imaging or diagnostic agents. Nanocarriers stimulate pharmacokinetics, increases distribution of incorporated therapeutic or diagnostic moiety at specific site (Wicki et al., 2015), increases efficiency of drug, reduces the toxicity due to reducing nonspecific biodistribution (Bhojani et al., 2010; Kawasaki & Player, 2005). These nanocarriers are functionalized with certain ligands or biomarkers for achieving specific treatment and diagnostic ability in the real time. In addition therapeutic entities like hydrophobic agents, oligonucleotides and peptide drugs showed improved stability when entrapped into the nanoparticles (Muthu et al., 2014).

The CNS and Blood-Brain Barrier: "The Opponent at the Doors"

The neurological diseases show a social, economic and medical problem of chief attention, because their incidence is increasing day by day along with increases in life expectancy (Hofman et al., 2011). The aetiology of neurodegenerative diseases is known, but still their performance is still a challenge in the therapeutic interventions in the neurological disorders, although potential treatment is provided by experimental studies for CNS disorders. The CNS is a complicated and vulnerable system that has evolved efficient defence systems against external substances. Ironically, the strength of these defensive mechanisms makes therapeutic interventions in the CNS more difficult. As a result, the battle against neurological illnesses is often fought "at the gates" of the brain. The three major barriers that exchange fluid between blood and brain parenchyma include blood-brain barrier (BBB), choroid plexus epithelium and arachnoid epithelium. The blood-brain barrier is the structural foundation of the functional system known as the neurovascular unit, which is produced by a monolayer of endothelial cells. Endothelial cells contain high concentration of p-glycoproteins. The p-glycoproteins that are ATP dependent hinder diffusion of the huge molecular weight hydrophobic molecules (approximately 400 Da) while actively passing small molecular weight drugs out of the brain (Schinkel, 1999). Thus BBB permeates hydrophobic small molecules while for transportation of large molecules follows receptor-mediated endocytosis or

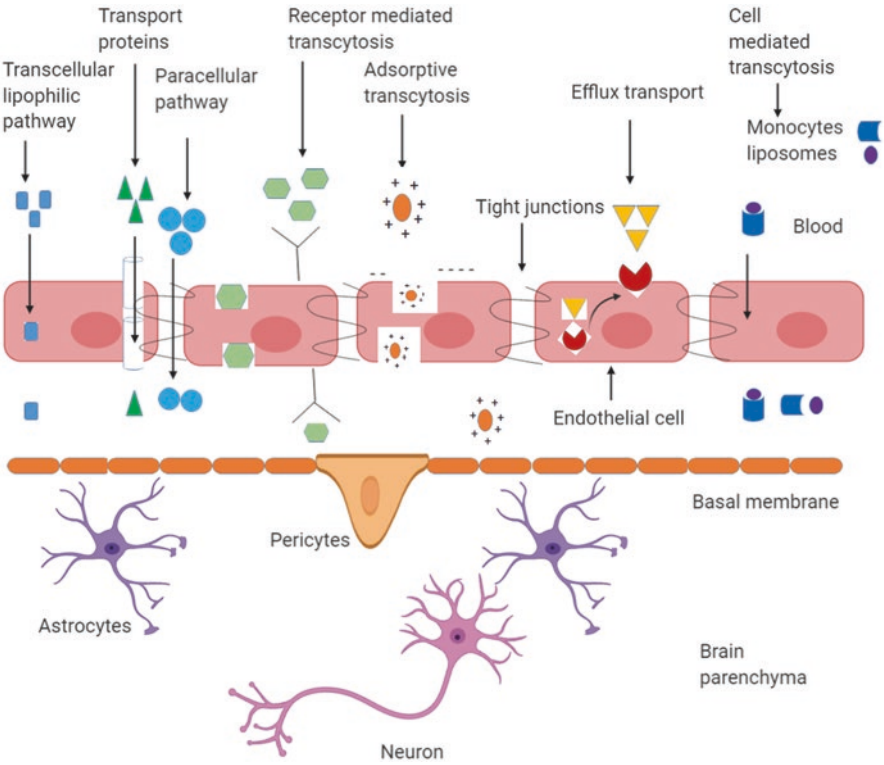

Fig. 5.1 Graphical representation of the numerous mechanisms of entrance of molecules and specific pathways that transverse the blood-brain barrier (BBB)

carrier-mediated systems. Figure 5.1 illustrates the schematic representation showing the mechanism of entrance of molecules and specific pathways that cross the BBB. Those molecules that do not transport through the above mechanisms follow some other strategies to penetrate through the parenchyma of the brain to further reach and target the site of action. One way to bypass BBB is by using intraparenchymal injection of the needed substance. For continuous drug infusion, another uses implantable system that further use osmotic pumps or enhanced convection devices. The surgical invasive and risky procedures can't be used when implantable tools traverse the crucial areas of the brain. These devices are also used for intraventricular or intrathecal administration of drugs that contain cerebrospinal fluid brain barrier that evades BBB. Intranasal administration is a new noninvasive method of bypassing the blood-brain barrier and allowing big charged medicinal molecules to enter the brain (Dhuria et al., 2010). Rather bypassing it disrupts the BBB temporarily and perforates the tight junctions of endothelial cells. The osmotic shock transiently opens the BBB by using mannitol, arabinose or hypertonic solutions that act on receptors of bradykinin (Borlongan & Emerich, 2003) and induce focalized perforation in small parts of the brain by using MRI-guided ultrasound with

microbubbles of contrast agents (Meairs & Alonso, 2007). The hydroelectric changes during BBB opening is a dangerous procedure and suppresses the mechanism that further regulate the entry of molecules in the brain (Diringer & Zazulia, 2004; Suzuki et al., 1988). There are also some suitable methods that penetrate the molecules across the brain parenchyma without altering the BBB, when the early-mentioned mechanisms are inaccessible or unsuitable. The use of chemical derivatives partially modifies drug structure to increase penetration of drug across BBB, while its activity is not affected such as by adding aliphatic chains to enhance lipophilicity (Vlieghe & Khrestchatisky, 2013). Another option is to use prodrugs that is not active in their native state but later active by undergoing some enzymatic or chemical changes in the parenchyma of the brain (Rautio et al., 2008). The most multifaceted approach is by using drug carriers "Trojan horses" that contain the therapeutic agent that can effectively cross the BBB. In this scenario, nanotechnology shows an effective role to combat these deadly neurological disorders.

Nanotechnology to Overcome BBB for Improved Drug Delivery

Nanocarriers involved in drug delivery system can increase the penetration across the brain by the use of influx transporters. In this section of the chapter, we highlighted the brain-targeting nanoparticle-based delivery strategies that include receptor-mediated transcytosis (RMT), adsorptive-mediated transcytosis (AMT), cell-mediated transport, carrier-mediated transport and BBB disruption-enhanced transport.

Carrier-Mediated Transcytosis (CMT)

The CMT is an important corridor to transport nutrients and hormones. Glutathione or glucose molecules attach to the carrier proteins and move from one side to another side of the membrane by concentration gradient after activating the conformational pathway in these carrier proteins. For the diagnosis and treatment of gliomas, the reported nutrient transporters such as GLUT1, large amino acid transporter 1 (LAT 1) and choline transporters show promising delivery for the nanoparticle-based drug delivery system (NDDS) (Du et al., 2014; Guo et al., 2011; Huang et al., 2013). Among all transporters, the most famous one GLUT1 is expressed in increased levels in brain capillary endothelial cell (BCEC) receptors (Uchida et al., 2011). Recently research group of scientists, Anraku et al., developed an approach that increased the BBB passage of glycosylated nanoparticles with the function of glycaemic-control (Anraku et al., 2017). The findings indicated that these glycosylated nanoparticles pass from BBB through these GLUT1 transporters, with its uptake that depends on the concentration of glucose in the blood. Mannose and

mannose analogues in addition to glucose can also target the GLUT1 transporter, and they have higher binding affinity as compared to glucose (Umezawa & Eto, 1988). In this scenario, another group of research scientists, Singh et al., designed p-aminophenyl-a-D-mannopyranoside (MAN)-modified solid lipid nanoparticles (SLNs) used for the brain transport of docetaxel (DTX) (Singh et al., 2015). Moreover, Ying et al. efficiently designed liposomes functionalized with (MAN) to transport the daunorubicin across BBB for the effective management of glioma (Ying et al., 2010).

Receptor-Mediated Transcytosis (RMT)

RMT transports big endogenous molecules that are significant for the functioning of the brain, whereas CMT transports small molecules into the brain. Several macromolecules or ligand-binding nanoparticles move across the BBB due to the presence of receptors on the luminal side of the BBB. Among all of these receptors, the most well-established ones are Tf receptor 1 (TfR1), glutathione receptor, LDL receptors (LDLRs), lactoferrin (Lf) receptor, scavenger receptor class B type 1 and insulin receptors (Banks, 2016; Chen & Liu, 2012). TfR1 receptor has been widely studied due to the promising role in the clinical translation (Choudhury et al., 2018). It increases the delivery of iron via the intracellular trade of iron-binding plasma glycoprotein tf in the brain (Moos & Morgan, 2000). Moreover, this one is overexpressed in blood-brain barrier and brain tumours (Prior et al., 1990). Many Tf-based nanoplatforms have been broadly used in brain distribution (Choudhury et al., 2018). So in this scenario, research group of scientists, Zhu et al., designed indocyanine green (ICG)-holo-Tf nanoplatforms for bimodal photoacoustic/fluorescence (PA/FL) imaging-guided photothermal therapy (PTT) designed for the management of orthotopic U87MG tumours (Zhu et al., 2017). Additional study developed conjugates that are modified Tf micelles encapsulated with cyclo-(Arg-Gly-Asp) (cRGD)-paclitaxel (PTX) that efficiently increased the mean survival time of orthotopic U87MG tumour mice from 34.8 to 42.8 days by paclitaxel-loaded micelle (Zhang et al., 2012). The most extensively considered receptors for delivery of the brain includes LDLRs, importantly peptides (Apo B and Apo E fragments and angiopep-2 (ANG) (Salvia et al., 2016). LDLRs includes a receptor's family such as (LDLR, LDLR-related protein 1 (LRP1), and LRP2) that further interfere the cholesterol-rich LDL endocytosis, such as apolipoproteins, cholesterol and tocopherol (Lanthier et al., 2002). Among all the aforementioned receptors, LRP-1 is the most important to deal with due to its increased expression on the BBB and many categories of tumour cells including malignant glioma cancer cells (Boyé et al., 2017). A 19-mer peptide (ANG) (TFFYGGSRGKRNNFKTEEY) shows powerful closeness to LRP-1. The two ANG-conjugated forms with drug that entered the clinical trials include ANG4043 (ANG-trastuzumab conjugate) and ANG1005 (ANG-PTX conjugate) for the management of brain metastasis from HER2+ breast cancer and repeated high-grade glioma, respectively. A group of researchers, Jiang

et al., developed ANG-conjugated polymersomes (ANG-PS) to deliver an effective natural protein toxin saporin (SAP) to the neurons (Jiang et al., 2018a).

Adsorptive-Mediated Transcytosis (AMT)

CMT and RMT depend on the specific receptors or transporters (Anraku et al., 2017; Cox et al., 2018; Jiang et al., 2018a, b), and AMT is used for transportation of large molecules and depends on electrostatic interactions among substrates that are positively charged and plasma membrane that are negatively charged. It has a non-specific binding as compared to RMT. Moreover, AMT shows high capacity and low-binding affinity to RMT. The transportation saturation capacity is for threefold high for AMT as compared to RMT (Hervé et al., 2008). AMT transports many peptides or proteins (CPPs and cationic proteins) through the BBB via AMT pathway (Lu et al., 2005; Salvia et al., 2016; Zou et al., 2013). A research collection of scientists, Lin et al., designed nanocarriers composed of CPP conjugated with albumin to transport the two hydrophobic drugs synergistically (fenretinide and PTX) for the treatment of brain tumour (Lin et al., 2016).

Cell-Mediated Transport

Generally, some side effects are reported for pathways such as CMT, RMT and AMT in the penetration across the BBB, and moreover no one of these receptors/transporters is used for the brain delivery exclusively. Protein and peptide use may be restricted due to its instability and immunogenicity. Recently cell-mediated transport has obtained an increasing acceptance. Mesenchymal stem cells (MSCs) (Clavreul et al., 2015; Huang et al., 2013; Li et al., 2011; Qiao et al., 2018), neural stem cells (NSCs) (Cheng et al., 2013; Gutova et al., 2013), macrophages (Li et al., 2017) and exosomes (Jia et al., 2018) are capable of transporting the therapeutic and diagnostic agent to malignant gliomas, due to its intrinsic tumour-homing capacity (Ali & Chen, 2015). MSC has the self-renewal property and monitors the cells of tumour. It is utilized for the distribution of many cargoes, such as replication-competent oncolytic adenovirus (CRAd), herpes simplex virus thymidine kinase (HSV-tk), IL-2, IL-18, IL-23, TRAIL, TNF-a, and TNF-b for therapy against oncolytic virus, immunotherapy and anti-glioma chemotherapy (Kosztowski et al., 2009). But it is difficult to encapsulate multifunctional modalities in these stem cells for the therapeutic and diagnostic purposes in various diseases. So to overcome these impediments, MSCs can be manipulated with multifaceted nanocarriers by cellular internalization or surface conjugation method. Authors reported that mesenchymal stem cells modified with multi-disciplinary FITC/ZW800/64Cu/Gd^{3+} entrapped mesoporous silica nanocarriers for the treatment of gliomas and multiplexed FL/PET/MR imaging (Huang et al., 2013). The findings indicated that these

NP-labelled MSCs have high-fold tumour accumulation compared to free NPs and provide targeted drug delivery in gliomas (Huang et al., 2013). Moreover, another research group of scientists, Kim et al., modified MSCs with mesoporous silica-coated hollow manganese oxide ($HMnO@mSiO_2$) nanocarriers by cellular internalization with the method of electroporation (Kim et al., 2011), and the findings indicated that these nanocarriers showed sustained release over 14 days in mice brain (Kim et al., 2011). In addition, another group of research scientists, Li et al., developed silica-based nanorattles that encapsulated doxorubicin (SN-DOX) with antibodies and precisely bind to CD73/CD90 proteins that are overexpressed on MSC membranes (Li et al., 2011). The results indicated that improved apoptosis and suppression of tumours grow due to extended and enhanced biodistribution of DOX in tumours by SN-DOX-labelled MSC nanoplatforms as compared to free DOX or SN-DOX (Li et al., 2011). Currently bi-functional luminescence nanocomposites (LPLNP-PTT/TRAIL) have been reported by Wu et al. to monitor MSCs and bring apoptosis in tumour cells by TRAIL gene therapy (Wu et al., 2017). These nanocomposites simultaneously showed persistent fluorescence in NIR region along with effective migration in glioblastoma cells in vitro and in vivo and resulted in effective reduced growth of glioblastoma (Wu et al., 2017). MSC nanoplatforms has a promising role in brain tumour and shows a great role in stem cell-based theranostics agents in clinical applications.

BBB Disruption-Enhanced Transport

A significant transport called BBB disruption is used for enhanced BBB penetration of nanoplatforms. Another promising strategy, focused ultrasound (FUS) with microbubbles (MBs), induces opening of the BBB and is used for the localized delivery of pharmaceutical agents (Fan et al., 2016). Recently a research group of scientists, Zheng et al., simultaneously designed a 50-nm hollow mesoporous organosilica nanoplatform (HMON) into the orthotopic brain tumour with exposure by FUS strategy (Wu et al., 2018). Other external stimuli other than FUS that includes hyperthermia effects or produces mechanical forces on brain capillary endothelial cells (BCECs) produce opening of the BBB for the brain delivery. A research group of scientists, Choi et al., used near-infrared (NIR) ultrasound pulsed laser to persuade leakage transiently in the blood vessel, but integrity cannot be effected, and these are used for the delivery of various nanomodalities into the healthy brain such as quantum dots (QDs), tetramethylrhodamine-conjugated magnetic oxide NP and FITC-dextran (Choi et al., 2011). The brain permeability can also be amplified by the use of various chemical modulators. A research group of scientists, Han et al., reported strategies like autocatalytic brain tumour targeting (ABTT) for increased delivery of nanocarriers in brain delivery (Han et al., 2016). In this scenario, less amount of ABTT-NPs via transcytosis or passive diffusion deliver to the brain, but after transversing BBB, the nanocarriers discharge the BBB modulators and open BBB transiently. It showed enhanced NP penetration and

ultimately increased brain accumulation. In addition, authors also reported ABTT-related brain priming approaches can also be used for the greater transfer of small-molecule drugs (PTX) and genes (such as plasmid-expressing tumour necrosis factor-related apoptosis-inducing ligand (pTRAIL) for the management of malignant brain cancer (Han et al., 2016).

Nanotheranostics: Developing Approaches for Early Diagnosis and Therapy of CNS Disorders

The treatment methods that are currently used to treat neurodegenerative diseases are radiotherapy, chemotherapy, surgery and immunotherapy; in addition some new treatments that are used to treat neurological disorders are gene therapy, stem cell therapy and hyperthermia therapy (Delhaye et al., 2012; Simonato et al., 2013; Thomas & Omuro, 2014; Yoo et al., 2013). However, for the efficient therapy, diagnosis at early stage is important so various imaging techniques such as single-photon emission tomography (SPECT), MRI, CT, X-rays and positron emission tomography (PET) will guide to decide timely and further reduce recurrent therapies and occurrence of disease (Nasrallah & Wolk, 2014; Pereira et al., 2014; Perrin et al., 2009; Small et al., 2006). There are still limited treatments that are successful despite of the fact that there are many progresses in the management of CNS diseases in the area of diagnostic modalities, due to certain drawbacks of current treatments that includes (1) time-consuming and low sensitivity in MRI (Ladd et al., 2018), (2) meagre permeability and increased breakup in brain tissues in fluorescence imaging (Louie, 2010), (3) along with drug there is inability to track disease progression and (4) toxic effects shown in normal brain tissues in case of PET and SPECT due to the absence of targeting ability in these imaging techniques that are radioactive based (Lu & Yuan, 2015). In addition, various conventional treatments such as chemotherapy, photodynamic therapy, radiotherapy, immunotherapy and surgery faced various challenges that includes (1) incapacity to pass through BBB, (2) insufficient uptake of drug in tissues of the brain, (3) nonspecific delivery and biodistribution, (4) meagre biocompatibility, (5) solubility is poor along with low half-life and less retention time and (6) irritating effects on healthy cells that are rapidly dividing. To control all these limitations and to attain efficient delivery through the brain, there is an unrelenting necessity to develop advanced methods that have high resolution, high sensitivity, deep penetration power and ability to track the development of the disease in the real time along with biosafety. In this scenario, ultra-small multimodal nanotheranostics plays a key role and shows a great potential for therapy as well as biomedical imaging of neurological diseases (Miao et al., 2017; Zhang et al., 2016c). In a chapter review of this section, we have made an effort to provide a glance of targeted nanotheranostic nanocarriers in various neurodegenerative diseases in the research field. We wish that this chapter review will be contributing in inspiring the collection of information of clinicians

and researchers on numerous nanotheranostics nanoplatforms that are under examination, as effective therapeutics against various neurodegenerative disorders. There are many types of targeted theranostic nanocarriers such as polymeric NPs (PNPs), lipid NPs, organic, metal-based NPs (Fig. 5.2) and some others that are being explored for the potential treatment of diagnosis and treatment of CNS disorders (Lim et al., 2013; Peng et al., 2015). The applications of theranostic nanocarriers are assembled and explained in Table 5.1.

Neurological Disorders and Specific Targeted Theranostic Nanocarriers in Neurological Diseases

The growing occurrence of neurodegenerative diseases stated that it is the most shared reason of global death and disability and in addition considered as irregular physical situation of nervous system. The leading causes of death are by various

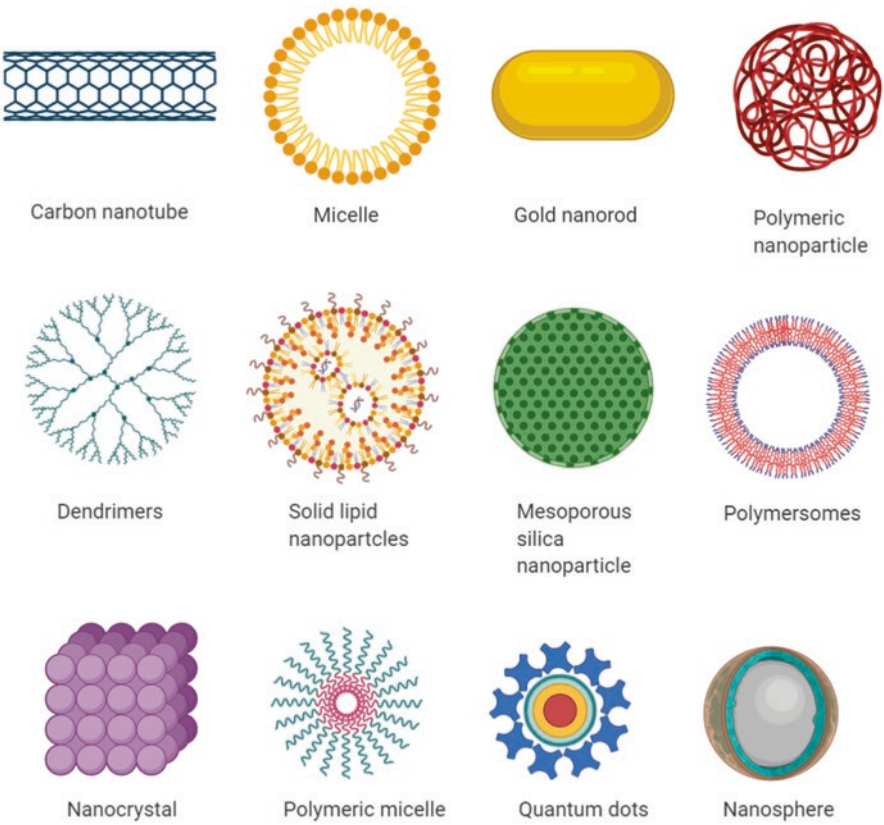

Fig. 5.2 Theranostic nanocarriers used in brain disorders

Table 5.1 Tabular representation of theranostic nanocarriers explored for the treatment of neurological diseases and preclinical studies in CNS disorders

S. no.	Theranostic NPs with hydrodynamic size (nm)	Ligand attached/ transport method used	Animal model/cell lines used	Imaging/ diagnostic moiety	Therapeutic moiety	Neurologic diseases	Result	References
1.	Magnetic nanocarriers (Magnevist®) (Gd-DTPA) with 239 ± 4.1 nm	IgG-antiamyloid antibody + chitosan + 125I	Human brain *microvascular endothelial cell* line *(hCMEC/D3)*	Magnevist® (MRI contrast agent)	CYC (cyclo-phosphamide)	AD	Used for contrast imaging of cerebrovascular amyloid with MRI, SPECT, reduced pro-inflammatory cytokine as contrast to cyclophosphamide	Agyare et al. (2014)
2.	Au nanoparticles (30 nm)	Carboxyl-conjugated AuNPs (negatively charged)	Neuroblastoma cells, BE(2)-C	AuNPs	–	AD	Disorganized Aβ fibrillation and break the preformed fibrils	Liao et al. (2012)
3.	Double-coated silica shells with layers of semiconducting polymer with ~ 15 nm	Cyclic RGD peptides	In vitro 4T1 human breast cancer epithelial cell line and in vivo includes the use of tumour-induced female mice	Fluorescence and photoacoustic brightness imaging	–	Brain cancer	Amplifying photoacoustic brightness for silica-coated SPNs as compared to SPNs, photothermal heating rate increased, moreover also provides increased fluorescence and PA imaging for polymeric nanocarriers by surface engineering method	Zhen et al. (2017)

4.	Liposomes with 109.98± 0.54 nm	Phosphatidylserine-targeting antibody	In vitro U87MG human glioma cell line, in vivo includes the use of tumour-induced nude mice	Iron oxide nanocarriers and near-infrared fluorescence (NIR) dye/NIR fluorescence imaging and magnetic resonance imaging (MRI)	–	Brain cancer	Nanoplatform of phosphatidylserine-targeting liposome (PS-L) increase targeted delivery across the brain of contrast agents by effectively penetrate through BBB	Zhang et al., (2016a)
5.	Gd micelles with 20.2 ± 2.8 nm	–	In vivo Wistar male rats	Gadolinium/ magnetic resonance imaging	–	Ischemic stroke	Diagnosis for evaluation of rtPA-haemorrhage risks	Shiraishi et al. (2017)
6.	Liposomes with ~140 nm	Endocytosis	Female nude mice	Quantum dots	Apomorphine	Parkinson's disease	Findings indicate that increased brain targeting, enhanced fluorescence intensity, approximately 2.4 folds increased in drug accumulation	Wen et al. (2012)
7.	Tocopheryl polyethylene glycol succinate (TPGS) liposomes with size below 200 nm	Transferrin	Rats used Charles Foster (CF)	Quantum dots (QDs)	Docetaxel	Brain cancer	Increased drug delivery, provides targeted therapy, approximately two folds more effectively than non-targeted liposomes	Sonali et al. (2016b)

(continued)

Table 5.1 (continued)

S. no.	Theranostic NPs with hydrodynamic size (nm)	Ligand attached/ transport method used	Animal model/cell lines used	Imaging/ diagnostic moiety	Therapeutic moiety	Neurologic diseases	Result	References
8.	Carboxymethyl dextran (CMC) – magnetoliposome with (50–100 nm)	Passive transport	Human neuroblastoma (SH-SY5Y) cells	Supramagnetic iron oxide nanoparticles (SPIONs)	Doxorubicin (DOX)	Brain tumour	Increased drug-loading capacity and enhanced stability, efficient T2 contrast agent, decreased toxicity as compared to doxorubicin	Guo et al. (2015)
9.	M40401 liposomes with 170 ± 50 nm	Porphyrins	Mice used	Mn(11) cation	Superoxide dismutase (SOD)	Cerebral ischemia	Used as T1 contrast agent for MRI imaging, increased neuroprotective outcome of mimetic SOD	Shazeeb et al. (2014)
10.	Stealth immunoliposome with ~100 nm	Anti-HSP72	Rats induced with ischemia	Rhodamine/ gadolinium	Citicoline	Cerebral ischemia	As targeted theranostic nanoplatform, provides targeted therapy	Agulla et al. (2013)

11.	RGD-TPGS liposome with 182.3 ± 7.5 nm	RGD/receptor-mediated endocytosis	Rats	Quantum dots	Docetaxel (DTX) and quantum dots (QDs)	Brain cancer	Provides sustained release, approximately 6.47 folds more competent than Docel, biocompatible liposomes for codelivery of DTX and QDs, enhanced efficiency of drug encapsulation by approximately 70%	Sonali et al. (2016a)
12.	pyE-lipid nanoparticle with approximately 30 nm	Apo E3/transcytosis	Orthotopic U87-GFP cell line, tumour-induced mice	Porphyrin	Porphyrin	Glioblastoma	Provided theranostic characteristics such as therapeutic agent as well as contrast agent, in vitro uptake by low-density lipoprotein receptor (LDLR7)-mediated endocytosis	Rajora et al. (2017)
13.	Liposome with 140–190 nm	Anti-CD20	Used athymic nude mice	SPIONs	Rituximab	Primary central nervous system lymphoma (PCNSL)	Enhanced storage capacity of nanoparticles and targeted drug delivery, theranostic nanoplatform for (PCNS), strong anti-lymphoma activity	Saesoo et al. (2018)

(continued)

Table 5.1 (continued)

S. no.	Theranostic NPs with hydrodynamic size (nm)	Ligand attached/ transport method used	Animal model/cell lines used	Imaging/ diagnostic moiety	Therapeutic moiety	Neurologic diseases	Result	References
14.	Polymeric nanoparticle with <100 nm	Endocytosis	Rat	Supermagnetic iron oxide	Temozolomide (TMZ)	Glioma (malignant)	Increased the survival rate, efficiently reduces the growth of glioma, used as MRI contrast agent, provided convection-enhanced drug delivery (CED)	Bernal et al. (2014)
15.	Iron oxide cores coated with chitosan with 76 nm	Chlorotoxin	Used of GBM6-bearing mice	SPION	O6-Benzylguanine (+oral temozolomide)	Brain tumour	Real-time monitoring by MRI, biodistribution in the tumour region significantly	Stephen et al. (2014)
16.	Polyfluorene-chitosan with 150 nm	Adsorptive transcytosis	Hybridoma cell line derived from endothelial cells (EAhy 926.1)	Polyfluorene	Polyfluorene	AD and presenile dementia	Inhibits amyloid conjugates, a novel polymer conjugate, sensing ability due to their distinctive optical properties	Chowdhury et al. (2018)

17.	Polyethylene glycol-poly lactic acid nanoparticles (PEG-PLA NPs) with approximately 100 nm	TGN and QSH peptides	Alzheimer's disease mice model	1,1-Dioctadecyl-3,3,3,3-tetramethylindotricarbo-cyanine iodide (DIR)	Coumarin-6	Alzheimer's disease (AD)	Provides specific delivery to amyloid plaque in mice brain, high distribution, and uptake in the brain	Zhang et al. (2014b)
18.	Iron oxide core with alginate coat with 139.6 nm	G23 peptide-/ receptor-mediated endocytosis	U87-Luc2 tumour-induced mice	Iron oxide	Doxorubicin	Brain tumour	Significant shrinkage of tumour after time period of 7 days, provided brain tumour therapy in a safe manner, used as MRI contrast agent both in in vitro and in vivo	Su et al. (2016)
19.	Polymeric nanoparticle with 125± 11.12 nm	F3 peptide	Rats with glioma	Iron oxide	Photofrin	Brain tumour	Increased survival rate significantly, provides targeted drug delivery, increased therapeutic efficiency	Bhojani et al. (2010)

(continued)

Table 5.1 (continued)

S. no.	Theranostic NPs with hydrodynamic size (nm)	Ligand attached/ transport method used	Animal model/cell lines used	Imaging/ diagnostic moiety	Therapeutic moiety	Neurologic diseases	Result	References
20.	Poly(2-hydroxyethyl methacrylate)-RA-poly (carboxybetaine) (PHEMA-RA-PCB-CPP) polymeric nanocarrier with approximately 100 nm	CPP/endocytosis	2×Tg (transgenic) – mice that have induced Alzheimer's disease	SPIONs	siSOX9	AD	Real-time checking and movement of neural stem cells (NSCs) by SPIONs, increased cellular uptake, improve neurological changes	Zhang et al. (2016b)
21.	PCB polymer with 150 nm	Endocytosis	2×Transgenic mice that have induced Alzheimer's disease	SPIONs	let-7b antisense oligonucleotide and simvastatin	AD	Provides controlled drug release with high loading capacity, memory deficit improved, traceable and self-assembled nanocarriers	Li et al. (2018b)
22.	SPIONs with 10 nm	Anti-Aβ monoclonal antibodies (aAβmAbs)	–	SPIONs	–	AD	Provides a temperature-sensitive and magnetic drug delivery system along with specific drug delivery	Dehvari and Lin (2012)

23	SPIONs with 15.0 ± 1.3 nm	Anti-Aβ monoclonal antibodies (aAβmAbs)	Used transgenic mice with (AD) disease	Fluorescent SPIONs	BAM10	AD	Inhibits amyloid beta (Aβ40) fibrillation by five folds, detects (Aβ40) by using fluorescence and MRI imaging	Skaat et al. (2013)
24.	Iron oxide magnetic nanocarriers with 13 nm	Penetration	APPswe/PS1dE9 transgenic mouse used	Iron oxide magnetic nanoparticles (MNPs)	Rutin	AD	Provides controlled release in a H_2O_2-responsive manner, inhibits oxidative stress, MRI imaging detects aggregated Aβ	Guo et al. (2015)
25.	Den-RGD-Reg peptide nanocarrier with 7.9 nm	c(RGDyK)	Used orthotopic U87-GFP tumour-induced mice	Near-infrared region (NIR) fluorophore IR640 B	Paclitaxel	Brain tumour	Increased rate of survival, glioma-targeted drug delivery, provides image-guided chemotherapy	Gao et al. (2018)

neurodegenerative diseases such as Alzheimer's disease (AD), epilepsy, Parkinson's disease (PD), ischemic stroke, multiple sclerosis, migraine, neuroinfections, malignant glioma and traumatic disorders. These disorders affect the central and peripheral nervous system. Approximately 50 million of people around the globe suffer from epilepsy according to the World Health Organization (WHO) report (WHO report, 2007). Approximately 47 million people suffer from dementia among that 36 million have (AD) (Realdon et al., 2016). Stroke is one of the foremost causes of death globally according to recent reports and findings. The prevalence rate of stroke was 42.4 million in 2015, while haemorrhagic stroke prevalence was about 18.7 million, and ischemic stroke was 24.9 million globally according to the report of the American Heart Association (Feigin et al., 2015). Among all types of cancers, brain cancer is one of the most invasive and fatal category of neurological disorders (Davis, 2016; McFaline & Lee, 2018). Gliomas are heterogeneous and consist of approximately 27% of brain tumour categories and 80% of malignant tumours. Glioblastoma is the most frequent glioma and comprises of 56% with poor consequences. These disorders affect patient lives and their families, cause huge socioeconomic burden affecting individuals and society multifariously. So here the game changer nanotheranostics save and raise the patient's quality of life that can't be treated with conventional treatment modalities successfully; also it presents a special boon for patients suffering from various brain disorders.

Alzheimer's Disease

Today millions of people affected due to Alzheimer's disease (AD) neurological disorder. It occurs due to amyloid plaque aggregation accumulation that is caused by Aβ protein aggregation and hyperphosphorylated tau protein aggregating due to intracellular neurofibrillary tangles (NFTs) (Faraji & Wipf, 2009; Gabathuler, 2010; Kaur et al., 2008). Moreover, the characteristics of disease include loss of verbal fluency and loss of temporal and spatial orientation. The theory that is accepted widely for the cause of AD is amyloid cascade hypothesis. The results show the buildup and accumulation of Aβ42, which promotes additional pathological effects such as the presence of NFTs, decreased the release of neurotransmitters along with disruption of the synaptic transmission and activation of astrocytes and microglia in the response of chronic inflammation that ultimately leads to dementia and causes neuronal loss. Researchers are working to develop suitable nanoplatforms that diagnose and treat CNS diseases (Begley, 2004; Kreuter et al., 2003). Stealth liposomes reported by Tanifum et al. for the encapsulation of Aβ targeted lipid conjugates for deposition of amyloid plaque in the preclinical model (Tanifum et al., 2012). The results indicated the efficient delivery across BBB and binding with deposited Aβ plaque. Zhang et al. designed polyethylene glycol-poly(lactic-co-glycolic acid) nanoparticles (PEG-PLGA NPs) modified with lectin that efficiently increased the delivery through the brain by intranasal administration (Zhang et al., 2014a). Combination of Solanum tuberosum lectin (STL) to nanocarriers with incorporated basic fibroblast growth factor (bFGF) increased the attachment with N-acetyl

glucosamine on the nasal membrane for increased brain distribution and resulting in even-size nanocarriers that has negative zeta potential. For tracing of brain delivery of 125 I-bFGF, use radioisotopic tracing method by intranasal administration for brain delivery in rats. These Nps (STL-bFGF-NP) was more effective than many other preparations for memory and spatial learning of AD rats. Zhang et al. designed and targeted bi-functional nanocarriers that consist of PEG-PLGA-NPs (Zhang et al., 2014b). The PEG-PLGA-NPs were surface conjugated with two peptides TGNC and QSH. TGNC targeted ligands at BBB, while QSH has an excellent closeness with Aβ42. These surface-engineered NPs provide increased and targeted brain delivery in amyloid plaque of AD models in mice. These PEG-PLGA-NPs formed toxic formation of oligomers by self-aggregation and showed avoidance of Aβ1–40 and Aβ1–42 peptides and moreover also showed enhanced fluorescence due to higher affinity with Aβ species. Hu et al. designed a nanotheranostic platform (Congo red/rutin-MNPs) for targeted drug delivery and in vivo imaging of Aβ plaques alongside with H_2O_2-controlled release of therapeutic agent rutin. The results indicated the stimuli-responsive distribution of rutin via Aβ-induced manufacture of H_2O_2 and identification of amyloid plaques by magnetic resonance imaging (MRI) (Hu et al., 2015).

Epilepsy

The main CNS disorder "epilepsy" shows disruption in nerve cell activity and further causes spontaneous and recurrent epileptic seizures in the brain. Epilepsy is classified into generalized and partial seizures. Both sides of hemispheres are affected in generalized seizures (De Tiège et al., 2007; Fisher & Ho, 2002), and they are classified further into absence, myoclonic, grand mal, atonic, clonic and tonic seizures, whereas in partial seizures, small areas of the brain are affected. Moreover, epilepsy is classified into symptomatic, presumed symptomatic (cryptogenic) and idiopathic. Scientists are focused and working to deliver antiepileptic drugs (AEDs) that target the brain and in turn reduce adverse effects and seizures. Various methods including IV injection and pills are used to deliver AEDs through the brain but with certain limitations associated with limited penetration through BBB. The best alternative is to encapsulate these AEDs into nanocarriers for effective free AED delivery in the brain. The reported causes of epilepsy are malformation, infection in the brain, head trauma, arteriovenous malformations, reduced oxygen supply, perinatal injuries and cerebrovascular diseases (Moody et al., 1974; Rutecki et al., 1985). Huang et al. (2009) designed a chip to deliver antiepileptic drug ethosuximide (ESM). The chip was made up of magnetic core-shell nanocarriers that encapsulated drug into the electrically conductive elastic polyethylene terephthalate (PET) substrate by using the technique of electrophoresis. The in vivo findings indicated reduced seizures and reduction in spike and wave discharge in the epileptic patients. The chip methodology is highly affective as related to conventional drug delivery owing to broader versatility, dosage improvement and easy surgery. Polymeric nanocarriers are efficient to deliver AEDs owing to high stability in the biological

fluids, and its preparation technique is easy (Couvreur & Vauthier, 2006). A research group of scientists prepared a nanoplatform composed of superficial covered poly(butylcyanoacrylate) with polysorbate 80 was designed as a noncompetitive N-methyl-d-aspartate (NMDA) receptor antagonist, and MRZ 2/576 (8-chloro4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-b] quinoline5-oxide choline salt) was encapsulated into NMDA. The polysorbate coating on these NPs attaches to apolipoprotein E and B (Friese et al., 2000), when exposed to blood, and in turn showed high brain delivery across BBB. Another group of researchers, Hsiao et al., developed injectable nanogel which has a property of thermo-gelling (Hsiao et al., 2012). In a Long Evans rat model, nanogels loaded with ESM were found to be effective in suppressing spike-wave discharges. MRI was used to observe noninvasively the gel clearance at the site of administration. Ying et al. prepared (Ying et al., 2014) electroresponsive hydrogel NPs (ERHNPs) that manipulated with brain-targeting ligand peptide angiopep-2 (ANG) that promoted the release of phenytoin and AED after triggered by external magnetic field. Fu et al. prepared (Fu et al., 2016) functionalized SPIONs targeted with anti-1L-1β monoclonal antibody (mAb) used for MRI diagnosis and treatment. The results showed increased particles in astrocytes and neurons in antiepileptic tissues along with efficiently provided therapeutic brain delivery.

Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative illness in which there is loss of neurons that produce dopamine (DA) that originates from substantia nigra pars compacta to the corpus striatum. Because DA is important in the transfer of electric impulses to normal physical motion, its absence causes bradykinesia, stiffness, and resting tremor. Levodopa is an effective drug that is used to treat PD, but it causes a long-term side effects (Singh et al., 2007). In PD patients there is development and deposition of Lewy bodies that are composed of protein alpha-synuclein proteins. Many routes (sublingual, rectal, pulmonary) are used, but they are not much effective, and there is still a challenge to deliver the localized and targeted drug delivery for the researchers. Moreover, if the drug is not completely distributed, that results further in dyskinesia and psychiatric problems. Advancements in nanotechnology help overcome the impediments that are placed in the management of PD. Yurek et al. (2009) prepared plasma-derived glial cell line-derived neurotrophic factor (pGDNF) DNA NPs and when injected into the striatum produced GDNF protein overexpression and in turn provided neurotrophic support to DA neurons. Wen et al. developed a modified nanocarriers with odorranalectin (OL) by adding OL-conjugated with polyethylene glycol-poly(lactic-co-glycolic acid) (OL-PEG-PLGA) nanoplatforms (Wen et al., 2011). The findings indicated that these nanocarriers increased the delivery of macromolecular drug urocortin-loaded NPs (UCN-NPs) in PD. Hu et al. (2011) designed lactoferrin NPs (LF NPs) and found very successful methodology for the treatment of PD.

Huntington's Disease

Huntington's disease (HD) is a neurological disease that affects mostly cortical, striatal and spiny neurons. The features of this disease show rapid involuntary actions, alterations in mood and oxidative injury that leads to death. Moreover, also neural loss is seen in HD due to dysfunction of mitochondria. In addition, changes seen in intracellular pathway that are accountable for the existence of neurons are also shown in the development of HD. The pathogenesis of HD involves dysfunction of mitochondria, but the exact causes for pathogenesis of HD remain unidentified (Morrison, 2010; Pringsheim et al., 2012; Ross & Tabrizi, 2011; Schon & Manfredi, 2003; The Huntington's Disease Collaborative Research Group, 1993; Wexler et al., 2004). There is a need of development of the method that can overcome the impairment in mitochondria for the successful treatment, and none other than nanotheranostics have the potential to treat these HD disorders. Sandhir et al. (2014) developed entrapped curcumin solid lipid nanoparticles (C-SLNs) to improved 3-nitropropionic acid-induced HD rats. The findings showed the increased Nrf2 mRNA expression along with increased nuclear and cytosolic fractions. Bhatt et al. (2015) developed rosmarinic acid (RA)-loaded SLNs, and findings indicated that RA-SLNs showed a promising tool for effective treatment against PD through administration by intranasal route.

Ischemic Stroke

Ischemic stroke is the foremost reason of death or disability, and here nanotheranostic nanocarriers show high efficiency for this disease treatment. For the detection of stroke, various biomarkers were measured that includes glial fibrillary acidic protein and vascular cell adhesion molecule that may have conjugated with nanocarriers followed by computed tomography or MRI scan (Jickling & Sharp, 2011). For acute ischemic stroke, improvement of fibrin targeted nanocarriers as a substitute to recombinant tissue plasminogen activator (r-TPA) and has a striking targeted strategy to decline illness and death in patients. Some other nanoparticles include quantum dots (QDs) which were successfully used to deliver TPA (Chen et al., 2012; Lin et al., 2013; Marsh et al., 2011). Dendrimers also displayed high efficacy to deliver heparin in rodent model of ischemic stroke, thus efficiently preventing deep vein thrombosis (Bai & Ahsan, 2009). Regarding carbon nanotube (CNT) applications in stroke, it exhibited the electrical neutrality in the injured tissues of neurons and thus increased functional recovery (Moon et al., 2012). Researchers studied anandamide CNTs showed enhanced cell viability and reduction of oxidative stress and thus have been proposed as an efficient nanoplatform with neuroprotective effects (Hassanzadeh et al., 2017). In this context NGF (nerve growth factor) played a crucial role in CNS disorders (AD, ischemic stroke) as NGF promotes the antioxidant protein expression and prevents neuronal insult and excitotoxic damage (Bianchi et al., 2012; Fantacci et al., 2013; Lad et al., 2003). Meanwhile efficiency of NGF has been affected negatively due to slow penetration in tissues and its short half-life

and also affected by environmental factors (Pfister et al., 2007). NGF-conjugated nanomaterials that target the brain have been developed in this regard (Kurakhmaeva et al., 2009). A research group of scientists designed amine-functionalized CNT-NGF that showed enhanced neuroprotective effects by suppressing cytotoxicity induced by ischemia (Allen & Bayraktutan, 2009; Hassanzadeh et al., 2017). In recent studies, polyphenolic compound showed great efficiency against ischemic stroke owing to their antioxidant, anti-inflammatory and neuroprotective effects (Hassanzadeh et al., 2015). However, its efficiency is inadequate owing to their small half-lives and poor penetration across BBB (Adam et al., 2002; Li et al., 2008). So in this scenario, numerous nanoformulations incorporated in these polyphenolic compounds were designed to improve the pharmaceutical and pharmacological properties of these compounds and in turn reduce ischemia-related damage of neurons (Ghosh et al., 2013; Kakkar et al., 2013).

Multiple Sclerosis

MS is a chronic neuroinflammatory disease marked by degeneration of neurons and immune cell invasion of the central nervous system, particularly lymphocytes and macrophages (Banks, 2016). The cause yet be unknown but linked to various environmental factors that include smoking, low levels of vitamin D, some genetic factors and Epstein-Barr virus (EBV) (Brown, 2016). Among the ages of 20 and 40 years, approximately 20 million people suffer globally from MS (Brown, 2016). MS is classified into relapse-remitting MS (RRMS), primary progressive MS (PPMS) and secondary progressive MS (SPMS) (Schmidt, 2016). The nanomaterial-based approach provides a promising role for the management of MS as shown in animal models of MS (Kannan et al., 2012; Menjoge et al., 2010). Specifically, yttrium and cerium oxides (Y_2O_3 and CeO_2) NPs showed mitigation of ROS in vitro by using hippocampal cells of neurons (Orive et al., 2009; Schubert et al., 2006). Furthermore, other authors designed poly(amidoamine) dendrimers that showed therapeutic localization in astrocytes and microglia cells of brain diseases, ultimately reduced neuroinflammation and improved motor function (Dai et al., 2010; Kanwar et al., 2012). Machtoub et al. injected ultra-small superparamagnetic iron oxide nanoparticles (*USPIO-NPs*) conjugated with anti-CD4 antibodies, and results showed successfully imaging of brain lymphocytes in amyotrophic *lateral sclerosis* (*ALS*) models of rat brain via MRI and surface-enhanced coherent anti-Stokes Raman scattering *microscopy* (*SECARS*) (Machtoub et al., 2010). Another group of research scientists designed teriflunomide-nanostructured lipid carriers (TFM-NLCs) in MS by intranasal route, and results indicated that TFM-NLCs produced remyelination in cuprizone-managed animals and further decreased the entry number in elevated plus maze (*EPM*) model (Gadhave & Kokare, 2019). Another group of scientists designed dimethyl fumarate (DMF) nanolipoidal nanocarriers for the treatment of MS. Results indicated that these DMF nanocarriers rejuvenated the myelin sheath, effective brain delivery along with reduced dosage frequency (Kumar et al., 2018).

Gliomas

A familiar category of tumour that originates from glia guides the developing neurons. Gliomas make up about 33% of all brain tumours and come in a variety of aggressiveness and differentiation grades. Symptoms depends upon the part of the brain damaged and moreover also depend on the degree of malignancy; they include headache, vomiting, nausea, vertigo, speech difficulties, motor alterations and in advanced phases of seizures a common demonstration too. Classification of glioma as reported by the World Health Organization (WHO) includes astrocytoma, glioblastoma, anaplastic astrocytoma, oligodendrogliomas, ependymomas and mixed gliomas (Wesseling & Capper, 2018). Among all glioblastoma multiforme (GBM) is the most ordinary malignant and comprises greater than 60% of primary astrocytomas (Rock et al., 2012). GBM is rarely common in children, but it is most common in adults as a high-grade primary brain tumour (Aldape et al., 2015). GBM is known as the lethal form of brain tumour and originating from glial cells. The survival rate is 14 months despite of many therapies and its combination (chemotherapy, radiation therapy), so there is a firm need to develop such strategies to eradicate the GBM in an effective way (Hanif et al., 2017; Koshy et al., 2012). Another recent approach used is immunotherapy, but still it cannot eradicate the tumours completely, and recurrence occurs due to intratumoral heterogeneity in patients. Several nanoformulations marked with specific ligands showed notably improvement in elimination and treatment of brain tumours (Dixit et al., 2015; Gao et al., 2012; Guo et al., 2011; Lee et al., 2017; Madhankumar et al., 2006; Mahmud et al., 2018). Another research group of scientists designed theranostic polyfunctional gold iron-oxide nanoparticles (polyGIONs) conjugated with surface therapeutic miRNA to mice in GBM. The results indicated trafficking and multimodality imaging in vivo along with concurrent systemically delivered temozolomide (TMZ) effectively in intranasal delivery (Uday et al., 2019). Another research group of scientists developed cetuximab (C225) entrapped core-shell Fe_3O_4 @Au magnetic nanoparticles (Fe_3O_4 @Au-C225 composite-targeted MNPs) against magneto-photothermal therapy against the cells of glioma. The results indicated that it showed a great potential to treat human glioma and used a potential of great value to medical use in the future (Lu et al., 2018). Wang et al. designed core/shell/shell NaYF4:Yb, Er@NaGdF4:Yb@NaNdF4:Yb nanoplates and searched their utilization in NIR-II FL imaging and photodynamic therapy (PDT) of glioblastoma. The results indicated that these NPs showed increase accumulation in brain tumours, showed enhanced tumoricidal activity, and resulted in complete removal of glioblastoma at the 25th day post treatment (Wang et al., 2017).

Current Developments in Nanotheranostics for Neurological Disorders

Nanotechnology in Neurosurgery

In near the future, nanosurgery will be the upcoming medical frontline in neurobiology that contributes to advancements in neurosurgery (Ebbesen & Jensen, 2006; Kohli & Elezzabi, 2009). Nanosurgery will remove the defects in cellular or subcellular levels and also involves nanoimaging. Synthetic nanomagnetic materials (such as cytobots and karyobots) will have magnificent properties and will regenerate the axons which damaged and halted the deleterious processes (such as haemorrhaging) by nanomanipulations (Freitas, 2005; Khawaja, 2011). Nano-neuromodulators and nonsurgical nanorepairs will be stimulating and monitoring the damaged neurons. In this context, in the last period, single-cell nanosurgery was the philosophy of neurosciences, but the recent advances of QDs for nanoimaging multifaceted nanocarriers for neuromodulations, and AFM cantilever for nanomanipulations became reality (Jeffries et al., 2007). Moreover, a research group of scientists implanted AFM tip as sharp needle's nanoscissors that can penetrate only 1 micrometre in cell wall. This was much gentle than the routine method that was tough to control in microcapillary procedures. AFM tip easily penetrates to the nucleus and returns back, and the cell wall returns to its normal shape (Obataya et al., 2005). It further rises in the new perspective for single-cell neurorepair like the coating tip with monoclonal antibodies (mAb) that interacts specifically to traffic of intracellular proteins and enables real-time monitoring of intracellular chemistry. Microrobotics and nanobodies, which use tiny magnetically driven spinning screws to swim along veins and deliver medications to infected tissues or even burrow into damaged brain cells, have gone from science fiction to reality (Hernot et al., 2012; Wang & Gao, 2012).

Challenges and Their Potential Solutions

BBB-crossing nanoplatforms provided excessive chances to transport imaging probe and therapeutic moieties for efficient treatment of brain disorders. In view of this, NanoTherm and Opaxia therapy approved by European Regulatory Agency seem promising. Although there are many promising strategies, they are still facing many limitations, and effort must be made to translate nanotheranostics from the bench to the bedside of the patients. The major issue facing is inconsistent metabolic destiny of nanoacarriers after the systemic administration. In incubation of biological fluids, nanocarriers from protein corona adsorb molecules on their surface that further alter their surface chemistry and charge (Walkey et al., 2012) and nullify their potential diagnostic and therapeutic performance. This issue can be solved by coating nanoparticles with PEG chains that reduced macrophage uptake

and opsonization and increases blood circulation. However, it is reported by other studies that further issues occurred due to immunogenicity of PEG moiety (Shukla et al., 2018). Recently, a research group of scientists, Cox et al., recognize that protein corona from blood to brain faces dramatic and dynamic molecular remodelling during transportation however stable beyond BBB (Cox et al., 2018). The authors designed mercapto-1-undecanesulfonate (MUS)-covered gold NP (All MUS NP) nanoplatforms and displayed their BBB passage capability by bio-TEM of hCMEC/D3 cells to visualize the apical to basolateral dynamic movement of these internalized nanocarriers (All MUS NPs). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) study of protein corona NPs in the Transwell system (Cox et al., 2018) indicates that nominated proteins were enhanced in blood side (apical compartment), whereas undetectable proteins were visualized in the basolateral compartment (brain side). These findings indicated that protein corona NP alters after interaction with BBB, so it is a significant aspect to deal when manipulating and analysing nanocarriers for diagnosis and treatment for neurological disorders. The second challenge is the limited brain delivery, despite of many targeted nanotheranostic nanocarriers to transverse BBB, targeting proteins or receptors in brain capillary endothelial cells (BCECs), but is still challenging due to some receptors shown in healthy brain tissues as well. Furthermore, BBB and blood-tumour barrier (BTB) further hamper the drug buildup and diffusion in brain delivery. In this scenario, highly selective and preferred targeting ligands are required for NP functionalization. New developments are conductive to enhancing brain delivery that includes integration of NPs with cell-penetrating peptide (CPP) and development of spherical-shaped NPs (rod like, worm like) (Lin et al., 2016; Zeng et al., 2016; Lee et al., 2017). For on-demand drug release in a spatiotemporally controlled manner, more emphasis should be made on TME-responsive nanosystems. For the transformation of these nanocarrier-based theranostics, agent safety remains a predominant factor that needs to be highly addressed. It is worth mentioning that most efficient nanocarriers assemble in major organs (liver, kidneys, spleen) before removal. Moreover, the aforementioned systemic toxicity and possible neurotoxicity caused by far-off targeted nanocarriers remain the legal concerns in healthy brain tissues. Several metal and metal oxide nanocarriers caused neurotoxicity through increased oxidative stress and induce cellular apoptosis (Su et al., 2018). Examples include TiO_2 and ZnO NPs in CNS which were found to result in cellular inhibition, imbalance in oxidative stress, DNA damage and ultimately neurodegeneration (Aijie et al., 2017). The promising answer to this matter is to conceal NPs with biocompatible polymers or cell membrane (Cai et al., 2016; Guo et al., 2017; Rao et al., 2016), or another one is to modify nanocarriers with surface-conjugated brain-targeting ligands that promote effective brain delivery as well as reduce the far-off targeted delivery in healthy brain tissues. Another solution to reduce neurotoxicity is to avoid premature drug release by developing TME-responsive nanoplatforms that release their cargoes in a responsive manner. Although some nanoformulations cause low acute and systemic toxicity, their side effects of long-term and clearance mechanism are not completely recognized. Furthermore, additional practical matters include extended stability, reproducibility and immunogenicity of these

nanoparticles that impede their clinical applications. Despite the current challenges of scientific and regulatory approvals for clinical translation, the novel development of multifunctional theranostic nanocarriers with high BBB-crossing capacity for brain disorders includes tumour selectivity, and it remains a great boon (Fig. 5.1). In addition, clinical studies using theranostic-based nanoplatforms, which have been done for diagnosis and treatment of CNS disorders, are concise and discussed in Table 5.2.

Table 5.2 Tabular representation of clinical stage of development of nanotheranostics systems for neurological disorders

S. no.	Theranostic NPs	Description	Brain disorders/ cancer type	References and/or identifier/clinical trial ID/phase
1.	Gold Nanocarriers	To evaluate the efficiency of NU-0129 (nucleic acids organized on the external of a round gold nanocarrier)	Glioblastoma	NCT03020017 Early Phase 1
2.	Ferumoxytol	Superparamagnetic iron oxide covered with polyglucose sorbitol carboxymethyl ether	Epilepsy	NCT02084303
3.	^{67}Cu-peptide conjugates	MTD study of ^{64}Cu-SARTATE	Neuroblastoma	NCT04023331 Phase 1 and 2
4.	Ferumoxytol	Composed of superparamagnetic iron oxide coated with polyglucose sorbitol carboxymethyl ether	Brain glioblastoma	NCT00660543 Yankeelov et al. (2014)
5.	Nanoliposomal CPT-11	Liposomal irinotecan	Recurrent high-grade gliomas	NCT00734682 and NCT02022644
6.	Ferumoxytol	Composed of superparamagnetic iron oxide covered with polyglucose sorbitol carboxymethyl ether	Brain neoplasm	NCT00659126 Yankeelov et al. (2014)
7.	SGT-53	Nanocomplex of liposomes incorporating a wild category p53 DNA arrangement, used in conjugation with temozolomide	Recurrent glioblastoma	NCT02340156
8.	Ferumoxtran-10	Ultra-small superparamagnetic iron oxides coated with dextran	Multiple sclerosis	Singh et al., (2010)
9.	Caelyx™	PEGylated liposomal DOX (PEG-Dox)	Brain tumour	NCT02766699
10.	Ferumoxides (Feridex)	Superparamagnetic iron oxide nanocarriers coated with dextran	Multiple sclerosis, amyotrophic lateral sclerosis	Tomitaka et al. (2019)
11.	Ferumoxytol	Superparamagnetic iron oxide covered with polyglucose sorbitol carboxymethyl ether	Migraine headache	NCT02549898

Future Prospects and Concluding Remarks

Despite the continuous improvements in the technical arena, brain-associated disorders remain a significant problem with high danger of mortality. Apart from the above-mentioned therapy and imaging techniques, we can glance in novel bioengineering devices such as 4D and two-photon imaging that can detect the movement of NPs at the BBB and brain tumours over a period of time (Scheibe et al., 2011; Tang et al., 2019). Furthermore, recent advances in genome editing, such as the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9 system (Li et al., 2018a), may bring new technologies for delivering genetic materials to the brain, particularly for the treatment of brain cancer. Moreover, nanoplatforms that can cross BBB can treat various advance stages of brain disorders such as stroke, PD, AD and epilepsy as mentioned above in the section of targeted theranostic nanocarriers for brain disorders (Saraiva et al., 2016). NPs modified with BBB or disease-targeting ligands are used to deliver cargoes to dopaminergic neurons for PD and deliver neuronal stem cells for the repair of neurons and targeting microglia for neuroinflammation (Khan et al., 2018). The issues like opsonization and toxicity must be addressed for clinical applications of neurological disorders. The use of nanoplatforms to treat brain disorders is yet in its initial phases. In recent years improvements in techniques for imaging and therapy of brain disorders enhanced our living understanding. Even though BBB is still a powerful challenge, nanocarrier-based delivery systems (ligand functionalization) show promising results preclinically. Moreover, some backup solutions to enhance nanoformulations into brain delivery include focused ultrasound (FUS)/chemical/biological-facilitated BBB distraction. In contrary to other therapeutic and imaging agents, nanoparticles have the ability to modify some adjustments in shape, size and ligand-targeting ability to control the impediments linked with blood-brain barrier and blood-tumour barrier (BTB). We expect that more efforts and continued developments in the area of nanomedicine will overcome multiple snags and will further improve patient outcomes and quality of life, and then we can shift innovation from laboratory to the bedside of the patient for individualized therapy.

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Chapter 6
Cancer Nanoimmunotherapy: Recent Advances and New Opportunities

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Introduction

Cancer is known as the very common deadly illness that affects human beings adversely worldwide. Mutations that occur in the DNA within cells cause cancer. DNA is packaged and folded into a greater number of individual genes, each of which contains a set of instructions informing the cell about the functions that all have to be performed by the cells and also how to grow and divide. If errors occur in the instructions, it could cause the cell to stop its normal function, and that leads a cell to become cancerous. To overcome problems like this, modern therapeutic paths are required because of the complication of cancer as a disease. The usual immunotherapy depends on in vivo immune balance controlled by unfavourable (tumour) and favourable (host) factors. But it is very hard to keep up such linear immune balance. When it comes to nanoimmunotherapy, nanocarriers could produce potential, stable, organized and targeted transmission of drugs for effective treatment and/or stimulating immune reactions. Pharmaceutical nanotechnology also known as cancer nanotechnology or nanomedicine has been giving an efficient

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quick fix to sort out the barriers of traditional immunotherapy (Li et al., 2014). The main advantage of cancer nanoimmunotherapy is that nanomedicines—therapeutics made up of transporter components usually lesser than 100 nm—had been made for widening the uptaking of chemotherapy substances by carcinoma and for reducing their off-target toxicity. Nanomedicines, such as NPs, gather within tumours via the improved permeation and retention effect, targeting the drug in tumour sites (Irvine & Dane, 2020). Nanoparticle (NP) delivery methods have been formulated to sort out many obstacles to the safe and efficient transfer of nucleic acid therapeutics to immune cells. NPs protect the therapeutic cargo, to evade nuclease degradation and to increase circulation half-life (Whitehead et al., 2014). Nanosystems formed to arrive at immune particles and cells might let the improvement of accesses that would utilize the patient's immune structure as a further precise tool to fight against cancer (Conniot et al., 2014). In recent days, cancer immunotherapy using NPs have been developed due to their effective role in cancer treatment. The following chapter brings to light an in-depth picture of nanoimmunotherapy that is paramount in cancer treatment. We would address the futuristic technology of artificial intelligence (AI).

Nanoimmunotherapy

Nanoimmunotherapy is mainly designed to develop nanotechnology to sort out the issues occurring in immunotherapy, and its focus is mainly about different types of nanocarrier development to deliver antigens to dendritic cells in a constant, limited and targeted way. We extend to emphasis mechanisms of NPs on tumour therapeutics. Cancer nanomedicine generally targets to advance the direct destroying of tumour cells by developing the delivery of chemotherapeutic drugs to tumours and metastases. Recently, nanomedicine formulations are utilized to increase the potentiality of anticancer immunity with clinically settled immunotherapeutics (Shi & Lammers, 2019). A promising solution to separate with the traditional drug advancement example and direct the delivery of immunotherapeutics is driving their action on target tissues (i.e. tumours and tumour-draining lymph nodes) or cell types, to control the time duration and location of immune modulation. To overcome this, nanomedicine-based proposals, i.e. the formulation of drugs in transporter materials that are lesser than ~100 nm, may increase both the defence and the therapeutic effectiveness of bountiful immunotherapies (Irvine & Dane, 2020). Nanoimmunotherapy is developed in nanotechnology to strengthen immunotherapy, which combines the advancement of nanocarriers to deliver antibodies on targeted tumour cells (passive immunotherapy) and of antigens to dendritic cells to induce immune reactions towards the disease (Li et al., 2013a, b).

Mechanisms of Nanoparticle Therapeutics

Nanoparticle therapeutics are customarily fragmented makeup of therapeutic bodies like nucleic acids, proteins, mini-molecule drugs, peptides and elements that gather with the therapeutic entities, such as lipids and polymers to form NPs. Those NPs could have increased anticancer properties correlated with the therapeutic bodies they contain (Davis et al., 2010). Targeted NPs have the following features that differentiate those NPs from other therapeutic approaches for cancer. (i) NPs can transport a huge payload of drug material and also save them from depravity. For instance, a 70-nm nanoparticle can have relatively 2000 short interfering RNA (siRNA) molecules (Bartlett & Davis, 2007). (ii) The NPs are adequately abundant to consist of various targeting ligands that would let on multivalent attaching to cell-surface receptors (Hong et al., 2007). (iii) NPs are big enough to shelter various kinds of drug molecules. Numerous therapeutic mediations can be applied together with a nanoparticle in a controlled way. (iv) The discharge kinetics of drug molecules from NPs could be modified to meet the mode of action. For instance, topoisomerase I inhibitors such as the camptothecin-based chemotherapeutic drugs are reversible binders of the enzyme. So, the mechanism of action for camptothecin-based drugs on the topoisomerase I enzyme recommends enhanced strength with extended exposure to the drug (Pommier, 2004). (iv) NPs can have the capability of bypass multidrug resistance processes that associate cell-surface protein pumps (e.g. glycoprotein P), as they go into cells through endocytosis. The physicochemical properties of the NPs could determine the stage of complexity for its function, whether it is organic or inorganic. For example, reacting nanoparticle's surface area with thiols could promptly functionalize gold NPs, whereas organic polymers need more plan of actions so that side chains 475 are reactive prior to the nanoparticle synthesis (Shi et al., 2009). Generally, it may control the mixing of these traits by nanoparticle construction that could reduce the adverse side effects of anticancer medicines while increasing potency, and results obtained by clinical studies are proposed that this potential is opening to be understood.

Nanomedicinebased drug formulations had formerly been generated for changing the pharmacokinetics and toxicity outlines of chemotherapy promoters and to develop their aggregation in cancer-affected cells. The capability of focusing on drugs could be within cancer cells or the tumours.

The tumour microenvironment (TME) is applicable of improving immunotherapy. Nevertheless, nanomaterials also let on new mechanisms of action for immunotherapy promoters, including the potentiality to show ligands to the immune cells, drive intracellular transfer of cell-impermeable mixture and restrict the drug-releasing time and/or activation. Mechanism of nanoparticles delivery has been described in Fig. 6.1.

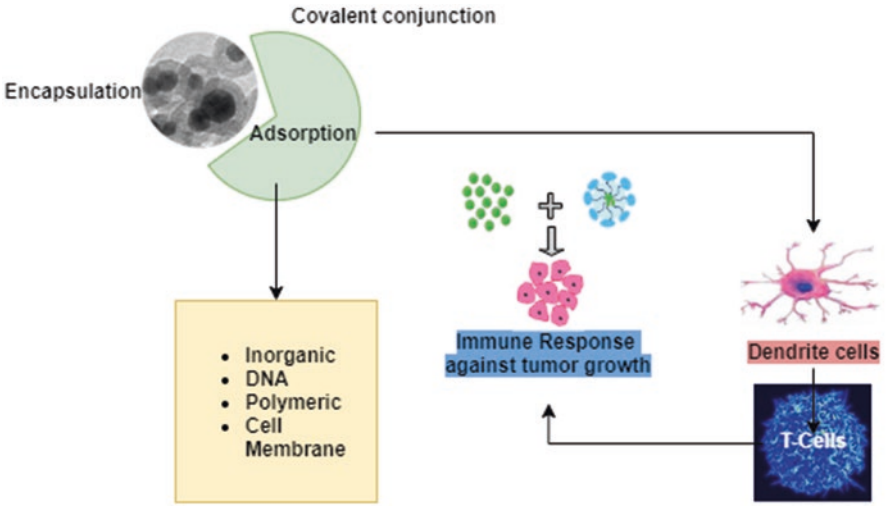

Fig. 6.1 Mechanism of nanoparticle delivery

Developing Immunogenic Tumour Cell Death

An anti-tumour immune response could be promoted by tumour cell death which is stated as immunogenic cell death (ICD). Nanomedicine formulations are a potential method to develop ICD since the cytotoxic agents could get targeted in tumour cells. Also, nanomaterials would be made to precisely interact with outer energy sources, letting on amplification of ICD provoked by therapies called radiotherapy and magnetic hyperthermia (Duan et al., 2019). Nanomedicines could be utilized as radio enhancers that straight away combine with ionizing radiation to improve ICD (Rancoule et al., 2016). By exchanging magnetic fields to induce paramagnetic iron oxide NPs within the tumour macro-environment, localized hyperthermia could be understood. Tumour ICD can be evoked by localized hyperthermia, whereas CD8+ T-cell-mediated immune response can also be promoted by the same in preclinical models of glioma, colon adenocarcinoma and melanoma (Toraya-Brown et al., 2016; Yanase et al., 1998). After getting positive results from these preclinical studies, it was clinically studied for the utilization of magnetic NPs to induce tumour hyperthermia. Since iron NPs studied comparatively very less or non-toxic and are acceptable to functionalize with targeting molecules, they have the potentiality to be successful. The experiment was done on encapsulation of oxaliplatin in the same nanoparticle-granted mixed ICD-promoting reactions like photodynamic therapy, chemotherapy and provoked regression of irradiated primary tumours and non-irradiated secondary tumours in mouse models of colorectal cancer (He et al., 2016).

Ligand Presentation to Immune Cells

The advancement of ligand-targeted NPs to remove solid tumours is expressed in advance to have a great impact on human wellness. The choice of a targeting ligand is mainly instructed by the receptors existing on the target cells. Clustering ligands on NPs contain a major profit of strengthening the affinity of receptor binding. This renowned phenomenon can increase ligand affinity by various orders of magnitude because of the simultaneous occupation of receptor-binding sites on the cell surface. Targeted NPs will drive the path to decrease the lethal side effects of general cancer treatments and to reduce the number of deaths related to cancer worldwide (Duskey & Rice, 2014). Ligands of NPs connect with cell-surface receptors which let on the gathering of high intracellular NP concentrations by receptor-mediated endocytosis. For example, cyclo-arginine-glycine-glutamic acid (cRGD) is a peptide that attaches to integrin receptors expressed on the surfaces of various kinds of tumours (Ahmad et al., 2019). Polymeric micelles for siRNA delivery consist of a cRGD ligand over the micellar surface that especially identifies tumour cells and increases their intracellular ability (Christie et al., 2012). PK2 (FCE28069), an HPMA-polymer-Gly-Phe-Leu-Gly-doxorubicin conjugate which has sugar galactosamine, was the pioneer nanoparticle targeted at the ligand to arrive at the clinic. The galactose-based ligand is used against the asialoglycoprotein receptor (ASGPR), and that reacts on hepatocytes. Hence, it is believed that its high expression is maintained on primary liver cancer cells (Seymour et al., 2002).

Nano-delivery Systems for Cancer Immunotherapy

Nanocarriers encompass multipurpose composition, tunable size and morphology, and surface functions. Immuno-nanomedicine is one of the advanced techniques that have been utilized in different ways in cancer therapy. Nanocarriers could be classified into inorganic NPs, polymeric NPs, lipid-based nanovesicles, DNA nanostructures, biomimetic and naturally derived particles based on their composition. Nanosystem-based identification of specific tumour neoantigens is a promising field (Wang et al., 2018a, b). Since nanomaterials have several significant characteristics such as high effectiveness for drug loading, low drug loss ratio and high stability of avoiding body clearance, they act as effective drug delivery carriers (Shi et al., 2011). Further, we discussed in detail about antigenic peptide delivery systems and monoclonal antibody (mAb) delivery systems. Nano-delivery using various systems has been shown in Fig. 6.2.

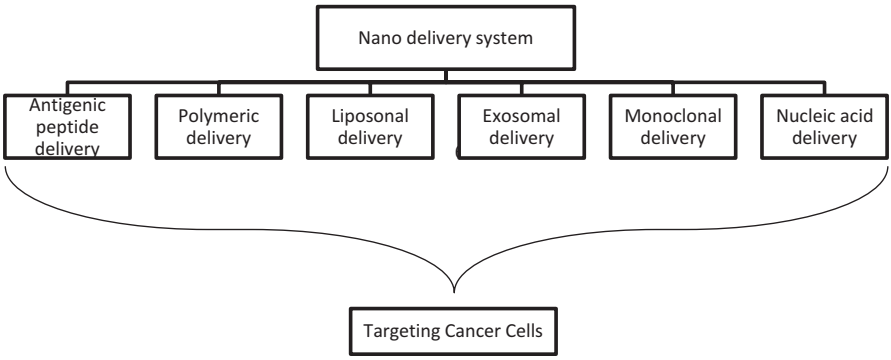

Fig. 6.2 Different types of nano-delivery system targeting cancer cells

Antigenic Peptide Delivery Systems

Although several nanostructures exist, peptide self-gathered nanostructures have gained more consideration for anticancer drug delivery and become a promising platform to treat cancer. The peptide has the potentiality of self-assembling into several various types of nanostructures like NPs, nanotubes, nanovesicles and nanofibers that form hydrogels (Yu et al., 2015). These hydrogels with injectable features could also be utilized directly to contact with the tumour sites for enhancing the potency of tumour treatment (Yishay-Safranchik et al., 2014). Gold nanorods inserted dipeptide microspheres and stacked with the anticancer agent, doxorubicin (DOX), have been chosen as a smart drug delivery stage for natural, steady and pulsatile drug release. Outcomes of the experiments revealed the ability to attain a sustained and on-demand DOX discharge from the microspheres by using the laser exposure timing (Erdogan et al., 2016). NPs' surface chemistry can be designed to target tumour-derived protein antigens. The NPs which are designed to capture the antigen further improve the exposure between the antigens to APC. Researchers showed the utilization of NPs that capture antigens (AC-NPs) increases the abscopal effect, a phenomenon by which local radiotherapy provokes a systematic response of immune cells and also the reversion of metastatic lesions. Radiation would generate pro-inflammatory proteins and enhance the liability of immune cells to tumour-specific antigens once the cancer cells are induced to death (Barker et al., 2015). A study showed the natural properties of antigen clubbing over NPs (adsorption versus encapsulation) and the surfactants (poly(vinyl alcohol) (PVA) or PF127) disturbed the DC activation, and it has been revealed that antigen-adsorbed NPs promote the MHC II on DCs in a highly expressed way, whereas antigen-encapsulated NPs promote the maximum expression of MHC I. It was concluded that antigen-encapsulated NPs promoted the response of antigen-specific T-cell (Zupančič et al., 2017). Delivering peptide antigens fixed to NPs provides various benefits. NPs are capable of protecting the peptides from enzyme peptidases, during prolonged transportation of peptide circulation and delivery. Virus-like distribution

in NPs helps them get identified and captured by antigen-presenting cells, driving to a huger gathering of antigens in lymphoid tissues. Antigens and immune adjuvants could be simultaneously co-delivered by NPs to prevent immune tolerance (Kuai et al., 2017).

Polymeric Systems

Delivery of antigens or antibodies towards either cancer cells or dendritic cells (active immunotherapy or passive immunotherapy) to provoke the immune system would happen efficiently by the polymeric NPs because of their composition, convenient particle size and particular intelligent characteristics (Li et al., 2013b). Poly(lactic-co-glycolic acid) (PLGA) is one of the most successfully used biodegradable polymers that was approved by the FDA. Various types of therapeutics had been encapsulated in PLGA NPs for their potential use in the field of the pharmaceutical industry. Tumour lysate, OVA and antigenic peptides are loaded in PLGA particles to evoke T-cell responses after intradermal injection (Cruz et al., 2014; Mueller et al., 2012; Zhang et al., 2011). A pH-responsive amphiphilic polymeric micelle has been fabricated by the group of researchers in order to dual delivery of OVA antigen and CpG adjuvant at the same time (Wilson et al., 2013). The responses of anti-tumours can rebuild after depleting MDSCs (myeloid-derived suppressor cells) with nanomaterials. MDSCs are valuable types of immunosuppressive cells, which have been found in several types of cancers such as gastrointestinal cancer, breast cancer, hepatocellular carcinoma and lung cancer (Parker et al., 2015). Poly(ethylene glycol)-poly(propylene sulphide) (PEG-PSS) polymer micelles are loaded with 6-thioguanine (MCTG) to deplete MDSCs in tumour-bearing mice and increase T-cell-mediated anti-tumour responses (Jeanbart et al., 2015). Recently, endosome-disrupting polymersomes have been utilized by Wilson and his colleagues for intracellular delivery of interferon gene stimulator (STING) agonist in which the natural formation does not overpass the cell membrane. Therapy with these polymersomes made better the anticancer immunity as well as the efficiency of checkpoint blockade therapy substantially (Shae et al., 2019). Rowan, Figdor and their fellow workers engineered synthetic APCs made on poly(isocyano peptide) altering with three to five anti-CD3 antibodies/150–200 nm of the polymer chain by which the expression of CD69 (early T-cell activation marker) has been induced and also the IFN-γ production has been promoted (Mandal et al., 2013). Gao and associates used NPs based on pH-sensitive PEG-polymethacrylate polymers for the efficient delivery of antigens to APCs in lymph nodes. NPs loaded with antigen evoked forceful vaccination than free antigens incorporated with conventional adjuvants (e.g. polyinosinic:polycytidylic acid (i.e. poly(I:C))), likely by stimulating the pathway of STING (Luo et al., 2017).

Liposomes

Nanocapsules, liposomes, micelles, nanoemulsions and solid lipid NPs are generally known lipid-based NPs that are administered by different directions such as oral, topical and parenteral (Dong & Mumper, 2010). A liposome is the most known NPs that are accepted medicine for cancer treatment (Qu et al., 2014). Since liposomes have the ability to raise the targeting and reduce the elimination and harmful adverse effects of chemotherapeutic agents, they are promising targets and delivery materials of the chemotherapy (Mandal et al., 2017). The study was described that doxorubicin (DOX) loaded with PEGylated egg phosphatidylcholine-cholesterol liposomes containing ~100 nm passively assembled in the tumour vessels of a multidrug-resistant breast cancer xenograft model, expressing a phenomenal antitumour effect, where the free DOX fails to deliver any detectable therapeutic reaction (Kibria et al., 2016). Mitoxantrone (MTO), anthracenedione relevant to anthracyclines, was encapsulated in PEGylated liposomes, and these MTO-encapsulated liposomes reduced the toxicity that let on the highest MTO dose administration of maximum MTO dose administration to treat breast carcinoma on mice (Pedrosa et al., 2015). A new nanocarrier of emulsion liposomes having perfluoropentane nanodroplet inside the aqueous interior of a dipalmitoylphosphatidylcholine liposome, along with the anticancer drug DOX, has been explained. Studies carried out in vitro resulted in liposomes showing an effective release of DOX over the application of less-intensity ultrasound at 20 kHz, 1.0 MHz and 3.0 MHz. This new drug delivery process ensures the effective delivery of DOX, and comparatively they are capable of minimizing the adverse effects of cardiotoxicity produced by DOX than old stealth liposomes (Lin et al., 2014). Liposome particles either with encapsulated cytokines (IL-15, IL-21) or drugs (glycogen synthase kinase-3 β inhibitor TWS119) were conjoined on the living T-cell surface through thiol-reactive maleimide head groups over the surface of lipid bilayer particles. These surface-coated NPs are not harmful to their carrier T-cells which have not interfered with intrinsic cell action or migration patterns. The function of these carrier cells extensively improved with the utilization of very few drug doses, and that was not effective enough while using alone old systemic routes. After crossing the endothelial barrier, 83% (± 3%) of their original NP cargo was still physically attached to the carrier CD8+ T-cells (Stephan et al., 2010).

Exosomes

Exosomes delivered as resourceful drug tools have gained attention because of their internal skill of shuttling proteins, lipids and genes among cells and their native affinity towards target cells. Salient properties of exosomes, such as the size of nanoscope, less immunogenicity, great biocompatibility, encapsulation of several cargoes and the strength to defeat biological blockades, differentiate them from

other nanocarriers (Zhang et al., 2019). Exosomes show an effective drug delivery because of their satisfactory biodistribution, biocompatibility and low immunogenicity. Exosomes contain better permeability and could pass the utmost biological membranes. Inspired by natural exosomes, researchers developed exosomes mimicking nanocarriers for siRNA delivery. A good yield of nano-sized vesicles, called exosome-mimics (EMs), by extruding non-tumourigenic epithelial MCF-10A cells through filters with various pore sizes was obtained (Yang et al., 2016). After encapsulated in exosome-based nanocarriers, protein/peptide drugs can obtain improved pharmacokinetic properties, increased bioavailability and the potentiality to reach and penetrate targeting tissues (Sterzenbach et al., 2017). Apart from native exosomes, exosomes using particular ligands could be made and designed in vitro to spot tumour cells effectively. For example, αv integrin-specific iRGD peptide provides exosomes which were utilized to supply doxorubicin and strengthened the anti-tumour efficacy in αv integrin positive breast cancer cells in vivo compared to free drug group (Tian et al., 2014). Different therapeutic cargoes such as anti-cancer drugs and cancer gene suppressors could be packed with exosomes in order to destroy cancer cells efficiently. Notably, exosomes give their therapeutic cargoes straight away to the cellular compartment with the capability of mediating cell-to-cell communication (Li et al., 2018; Turturici et al., 2014). Doxorubicin (DOX), paclitaxel (PTX), celastrol and curcumin are chemotherapeutic drugs that have been found to encapsulate into exosomes. Diverse explorations have proved that the drug-loaded exosomes are capable of increasing the effectiveness of chemotherapy (Hadla et al., 2016; Aqil et al., 2016). Exosomes have the feature of showing improved stability of blood which allows them to move far inside the body under both physiological and pathological conditions. Additionally, exosomes are having a hydrophilic core, which makes them as host water-soluble drugs (Jiang & Gao, 2017). Because of the advancements on tumour treatment, exosomes are used in cancer diagnosis, immunotherapy and drug delivery vehicles (Li et al., 2018).

Monoclonal Antibody (mAb) Delivery Systems

Cancer cell-specific treatment became possible with the improvement of a technique to develop monoclonal antibodies (mAbs) in year 1975 (Köhler & Milstein, 1975). Nanomaterials facilitated the supply of bioactive monoclonal antibodies (mAbs) and drugs to tumours which encompass the great advantage as they are well-known to improve permeability and retention (EPR) effect as well. The combination of leaky tumour vasculature and poor tumour drainage through the lymphatics provides a better advantage for nanoconjugates (Torchilin, 2005; Hofheinz et al., 2005). For the first time in year 1982, Levy and fellow workers used mAbs to cure human malignancy (Miller et al., 1982). It has not been done until 1986, and then the US Food and Drug Administration (FDA) approved the first monoclonal antibody [Orthoclone OKT3). The FDA approved the first humanized monoclonal antibody in 1997 against CD25 to treat multiple sclerosis in adults. Russia approved

Table 6.1 Types of nanoparticle delivery in cancer immunotherapy

Nanoparticle delivery	Targeted cancer type	References
Liposome	Advanced colorectal cancer	ClinicalTrials.gov Identifier: NCT00361842
	Hepatocellular carcinoma	NCT02112656
	Metastatic breast cancer	MM-398 (Inman, 2015)
Colloid gold NPs	Late-stage cancers	NCT00356980
Polymeric micelle	Breast cancer and non-small cell lung cancer	Smith (2013)
	Lymphoblastic leukaemia	Cerqueira et al. (2015)
Monoclonal antibody: CD20	CD20-positive B-cell non-Hodgkin's lymphoma	Asadujjaman et al. (2020)
Monoclonal antibody: EGFR	Metastatic colorectal and head and neck carcinoma	
Dendrimers	Leukaemia	Tekade et al. (2009)
	Glioblastoma	Kaneshiro and Lu (2009)
Lipid NP-siRNA against PLK1	Advanced hepatocellular carcinoma	NCT01808638
Lipid NP-siRNA against KSP	Solid tumours	NCT00882180 and NCT01158079

the first cancer vaccine Oncophage in 2008. Sipuleucel-T (Provenge) was approved by the FDA in 2010 as therapeutic cancer to treat prostate cancer (Waldmann, 2003; Parish, 2003). Schneck's team examined the synergy between PLGA-based antigen-presenting cells and anti-PD1 monoclonal antibody (mAb). This particular combination promoted the higher-level secretion of IFN-γ in vitro and delayed tumour growth in vivo with long survival (Kosmides et al., 2017). In many cases, anti-CD28 mAbs are alone, not able to work, and their usage has to be followed by antigen-dependent T-cell receptor (TCR)-interfered signals to activate T-cells. 4-1BB which is also called as CD137 is possible to identify on T-cells, natural killer cells, DCs, mast cells and even sometimes endothelial cells of metastatic tumours (Vinay & Kwon, 2012). Application of anti-4-1BB in this receptor induces signalling pathways that drive to the strengthened expression of anti-apoptotic genes. As like 4-1BB, OX40 is another type of the TNF receptor superfamily, and anti-OX40 mAbs are efficient to stimulate CD4+ and CD8+ T-cells (Aspeslagh et al., 2016). Adjuvants, cytokines, and mAbs all perform as immunotherapeutic agents that would benefit from the improved transport given by nano-delivery. Table 6.1 shows the different types of nanoparticle delivery in cancer immunotherapy.

Nucleic Acid-Based Delivery Systems

The extreme need for a vector that is able to perform efficiently in transporting and supplying nucleic acid (NA) therapeutics towards the target cells has prompted intense research. NA delivery methods could be of endogenous (viral vectors) or

exogenous (natural and synthetic delivery materials) origin (Yin et al., 2014; Xiao et al., 2019). Nanomaterials play important roles in the delivery system of siRNA, and nanomaterial-mediated siRNA delivery in cancer immunotherapy is one of the major directions for future clinical cancer therapy. siRNA is known as a double-stranded RNA that contains the length of 19–21 nucleotides and has been broadly checked for potential cancer therapy in animal models. Nano-sized non-viral carriers like liposomes, polyethyleneimine (PEI), polypeptides, chitosan, inorganic NPs, etc. have been promoted as promising vehicles in the process of nucleic acid delivery (Mei et al. 2019). RNAi consists of post-transcriptional gene silencing mediated by endogenously produced mini (19–25 base pairs) oligoribonucleotides with the potency of degrading a target RNA specifically and selectively, thus repressing translation of an encoded protein (Whitehead et al., 2009). NA therapeutics have been considered as effective applicants for cancer treatment, including immunotherapy (Opalinska & Gewirtz, 2002). NA therapeutics are a broad category of DNA or RNA; they are plasmids, mRNA, ASO, siRNA, miRNA, small-activating RNA (saRNA), aptamers, gene-editing gRNA as well as immunomodulatory DNA/RNA. NA therapeutics are multifunctionalities ranging from gene expression alteration (up- or downregulating) to immune response modulation (Pastor et al., 2018; Kleinman et al., 2008; Ishikawa & Barber, 2008). siRNA is responsible for gene regulation, whereas ASO is responsible for regulating gene expression after transcription and silence-targeted genes further regulating intracellular signalling pathway which plays a role in cancer progression (Dahlman et al., 2014). NA immune stimulants such as unmethylated cytosine-guanine deoxynucleotides (CpG), poly I:C, 5′-triphosphate RNA as well as di-cyclic nucleotides that active stimulator interferon genes (STING) stimulate anticancer immune activation (Barber, 2015; Vollmer & Krieg, 2009; Kyi et al., 2018). mRNA therapeutics and plasmid DNA (pDNA) can be made to express proteins or peptides of interest, such as antigens or cancer immunotherapeutic proteins. Genome editing-related nucleic acids such as gRNA are currently started using to edit target genes accurately which could modulate gene expression for cancer immunotherapy (Gilboa et al., 2015; Yin et al., 2017). The ability of mRNA to express essentially any proteins and peptides for a longer duration on nuclear localization for gene expression makes mRNA therapeutics of tremendous potential for versatile applications, including cancer immunotherapy (Sahin et al., 2014). mRNA can now be manufactured in vitro at large scales at a low cost. Particularly, mRNA can be reproducibly synthesized by in vitro transcription (IVT) using DNA templates, a T7, a T3 or an Sp6 phage RNA polymerase (Pardi et al., 2018). These technology developments have altogether provided RNA therapeutics as a very powerful platform for cancer immunotherapy by multipurpose approaches like ex vivo mRNA transfer for therapeutic adoptive cell engineering and using cancer-specific antigen-encoding mRNA as tumour therapeutic vaccines (Sahin et al., 2014). siRNA is a dsRNA that consists of 21–23 nucleotides. siRNA guides RNA-induced silencing complexes to bind to the specific sequence of mRNA and subsequently degrades it. Given that some genes are highly expressed in many diseases including cancer, siRNA can be used as a therapeutic agent to silence them (Agrawal et al., 2003). Guillermo et al. showed that hMCP1 siRNA-DOPC

NPs suppress tumour growth and decrease the infiltration of CD68+ and F4/80+ macrophage cells in tumour samples obtained from mice models that are under day-to-day restraint stress (Armaiz-Pena et al., 2015). Arvizo et al. delivered MICU1 siRNA/positively charged AuNPs to human ovarian cancer cell lines (OVCAR5, OV167 and OV202). The decreased expression of *Bcl-2* simultaneously raises in the range of cytosolic $[Ca2]_{cyto}$ leading to the activation of the mitochondrial pathway of apoptosis. An experiment shows MICU1 as a novel regulator, which prevents apoptosis in tumour cells (Arvizo et al., 2013). EZH2 is a histone-lysine N-methyltransferase enzyme and is functional in some cell processes. It tends to be increased in some tumour cells. EZH2 suppresses the expression of vasohibin-1 with antiangiogenic properties. Gharpure et al. established that siRNA coated with CS NPs, along with docetaxel against EZH2, reduces angiogenesis and tumour mass in HeyA8 and SKOV-3ip1 orthotopic mouse models (Gharpure et al., 2014). In a study, Lingegowda et al. used a siRNA targeting the platinum resistance genes ATP7A and ATP7B in ovarian carcinoma. For in vivo delivery, they utilized neutral nanoliposome DOPC with incorporated siRNA to decrease the expression of ATP7B in 48 h. Tumour shrinkage, cancer cell apoptosis and proliferation reductions have been reported (Mangala et al., 2009). Hatakeyama et al. delivered CTGF (a key factor in hypothermia resistance) siRNA-DOPC nanoliposome to xenograft HTRSKOV-3 and HeyA8 mice. And then PEG-CuS NPs were intravenously injected. Due to CTGF underexpression and hyperthermia, tumour burden was decreased in the HeyA8 model. Besides, local hyperthermia and CTGF silencing led to decreased metastasis rate and tumour burden in HTR SKOV-3 tumours (Hatakeyama et al., 2016). Clinical trials involving lipoplexes containing RNA oligonucleotides are at the starting blocks within the Mutanome Engineered Nanomaterials 2016, 6, 131 15 of 22RNA Immuno-Therapy (MERIT) project, an initiative that has got research financial support from the European Union, coordinated by BioNTech AG. CLs to shape RNA lipoplexes, namely, MERIT-Lipo (ClinicalTrials.gov Identifier: NCT02410733), has been selected for the clinical trial entitled "Evaluation the safety and tolerability of i.v. administration of a cancer vaccine in patients with advanced melanoma (Lipo-MERIT)". The cationic liposomes of the Lipo-MERIT vaccine entail four naked ribonucleic acid (RNA)-drug products (DPs) such as RBL001.1, RBL002.2, RBL003.1 and RBL004.1. These are the ability to induce antigen-specific CD8+ and CD4+ T-cell responses in contradiction of designated malignant melanoma-associated antigens. The corresponding investigation under the clinical phase I trial (Campani et al., 2016). Anti-tumour immune responses are elevated by adjuvants. NA-based adjuvants include CpG-oligodeoxynucleotide (CpG-ODN) (Wang et al., 2016, 2018a, b; Kadiyala et al., 2019), polyinosinic:polycytidylic acid (poly I:C) (Yang et al., 2016; Zhang et al., 2019) and cyclic guanosine monophosphate-adenosine monophosphate (cGMP). These can induce pattern recognition receptors (PRR) and consequently activate the immune response (Shae et al., 2019; Cheng et al., 2018; Wilson et al., 2018).

Obstacles and Future Perspective

NPs have been revealing a huge promise in cancer immunotherapy. However, it should be noted that the obstacles of this technology meet the stability of nano-dimensions at the target site under different physiological circumstances, protein corona formation and accumulation. Therefore, it should be discussed the fundamental to incorporate systematic investigations on nanomaterials with biological systems, to finalize the nano-formulation in the nano-treatment. Further, we would give a glimpse of futuristic technology like artificial intelligence in cancer nanoimmunotherapy. Nanotechnology with its special features can multiply cancer immunotherapy considering the present barriers and technical complications. The growth of nanotechnology, more specifically NPs, grants a novel paradigm for cancer immunotherapy. For instance, we could say that few researchers have obtained PD-1-expressing cellular NPs utilizing genetically designed cells to give immunological molecules of smaller sizes. New concepts for personalized immunotherapy may attain by these strategies. Although the targeting capability of NPs is confined by the controllability, they can play an exclusive role in targeted delivery for cancer immunotherapy. The main obstacle in all types of therapy depends on the time, dose and patient-specific at any point of treatment. To sort out these issues, the development towards nanomedicine-mediated co-delivery of multiple treatments has created the potentiality of collaborating artificial intelligence (AI) along with nanomedicine for maintaining the optimization of combinatorial nanotherapy. AI-facilitated paths that essentially account for things like drug targeting, ratiometric delivery and other features are activated by nanotechnology-mediated delivery, and also a dynamic patient reaction to treatment would need exceptional levels of actionability during drug administration. This necessity shows the chances for the field of artificial intelligence (AI) (Zarrinpar et al., 2016). Nanomaterials encompass an extraordinary function in targeted delivery to provide effective cancer immunotherapy, though their targeting capacity is narrowed by the controllability of nanomaterials. Hence, the use of nanomaterials relies on the advancement of analysis and characterization techniques, as well as the constant updating of clinical data.

Conclusions

The advent of nanotechnological development in cancer immunotherapy exhibited the prominent results to overcome the obstacles of transferring water-soluble drugs in hydrophobic lipid particles, improving the target-specific activity and delivering rapid phagocytosis by immune cells. Futuristic technology of artificial intelligence would pave the improvement in the selection and advance the process in nanoimmune cancer therapy. To date, the significance of nanomaterials on cancer patients and clinical transformations is insufficient. Despite the clinical evolution of nanomaterials which still has a lot of objections, challenges and questions, the

improvement of nanotechnology, clinical research and the form and fabrication of nanomaterials will largely support the evolution of safe and powerful cancer immunotherapeutics. The interfusion and prolongation of nanotechnology, as well as the cancer immunotherapy, will move ahead in the future world.

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Chapter 7
Recent Advances in Lipid-Based Nanoformulations for Breast Cancer Theranostics

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Introduction

Breast cancer is one of the world's leading causes of cancer deaths in women. According to the World Health Organization (WHO), it is expected that the deaths due to breast cancer can reach 17.5 million by year 2050 (Ferlay et al., 2010). Several theories are proposed on the origin of breast cancer. There are several theories proposed to explain its origin. 'Misplacement somatic stem cell' theory hypothesizes that breast cancer originates from misplacement of mammary somatic stem cells (Wang et al., 2013). 'Linear hierarchy model' postulates that breast cancer arises from the deregulation of self-renewal in mammary stem cells (Dontu et al., 2003; Wicha et al., 2003). According to this theory, tumour-initiating breast cancer stem cells (BCSCs) originate from acquired mutations of normal stem cells (NSCs). It was also reported that BCSCs develop by epithelial-to-mesenchymal transition (EMT) in response to chemotherapeutic agents and environmental toxicants (Owens & Naylor, 2013; Pindiprolu et al., 2018b). The conventional therapeutic modalities for breast cancer include radiation therapy, chemotherapy with anticancer agents (tamoxifen, doxorubicin (DOX), paclitaxel (PTX), docetaxel, cisplatin, etc.) and in severe cases surgery (Zhao et al., 2016; Swaminathan et al., 2013; Sai Kiran Pindiprolu et al., 2020). These treatment modalities, however, are associated with

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significant limitations like chemo-/radioresistance, off-target effects, incomplete eradication, induction of EMT, and tumour relapse (Vinogradov & Wei, 2012).

In recent years, the emergence of nanosized (up to 1000 nm) formulations or nanomedicines improved the therapeutic outcomes in cancer (Singh & Lillard Jr, 2009). These nanoformulations can easily permeate via the leaky vasculature of the tumour tissues and lead to passive drug targeting. This is called enhanced permeability and retention (EPR) effect (Torchilin, 2011). Tumour-targeted nanoformulations enhance the cellular internalization of therapeutic or diagnostic agents by the process of endocytosis (Siddhartha et al., 2018). The surface of these nanoformulations can be altered/modified by using ligands binding to the cancer cell-specific receptors to enhance the targeting efficiency further. Different research groups are working on developing nanoformulations for improving the therapeutic efficacy of anticancer agents. These include carbon nanotubes (Miyako et al., 2008), solid lipid nanoparticles (SLNs) (Pindiprolu et al., 2018a), lipid drug conjugates (LDCs), nanostructured lipid carriers (NLCs) (Chintamaneni et al., 2017), polymeric nanoparticles (Muntimadugu et al., 2016), lipidic micelles, etc. (You et al., 2015). A compelling body of evidence suggests accurate and informative visualization of tumours and anticancer agents within the tumours can play an essential role in designing effective treatment plans. To achieve this, researchers focused on formulating combined diagnostic and therapeutic (theranostic) modalities in a single nanocarrier (Pindiprolu et al., 2018c). Nanotheranostics allow real-time monitoring of drug distribution, on-demand release, response and efficacy, in a non-invasive and real-time manner. Theranostic modalities, therefore, overcome the limitations associated with conventional chemotherapeutic agents (Liu et al., 2018). Nanotheranostics are multicomponent nanosized formulations, made of organic or inorganic materials, capable of theranostic actions, such as gene/drug delivery (Sola et al., 2020), light-assisted therapy (photodynamic therapy (PDT) (Pais-Silva et al., 2017) and photothermal therapy (PTT)) and imaging modalities (magnetic resonance imaging (MRI), photoacoustic imaging (PAI), positron emission tomography (PET) and single-photon emission computed tomography (SPECT)) (Naczynski et al., 2014) (Figs. 7.1 and Table 7.1). Nanotheranostics offer advantages such as enhanced accumulation in tumours, controlled drug release and multiple imaging modalities for optimized therapeutic outcomes. In recent years, various nanoformulations are being developed and tested in clinical trials for theranostic applications in cancer (Table 7.2). Among different nanotheranostic modalities, lipid-based nanoformulations offer several advantages for cancer theranostics. They include biocompatibility, improved cellular uptake, controlled drug release, etc. (Pindiprolu et al., 2019). In the present chapter, we discuss the recent advancements in the lipid-based nanoformulations (liposomes, SLNs, NLCs, LDCs and lipid micelles) for theranostics applications in breast cancer.

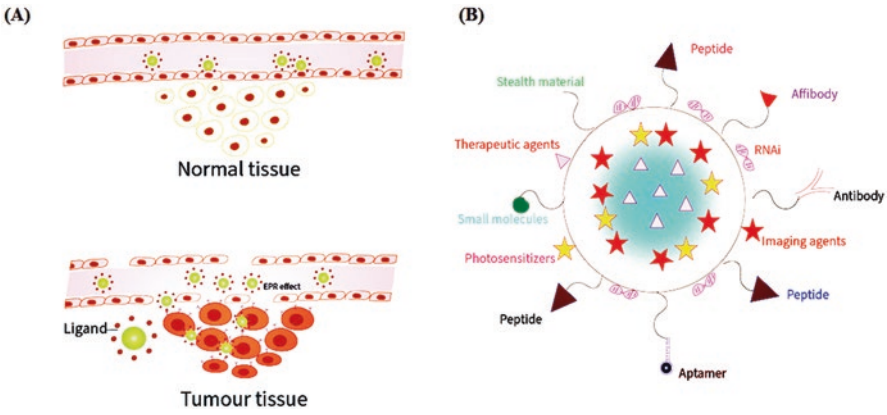

Fig. 7.1 (**a**) EPR effect and (**b**) design of nanotheranostic modality

Table 7.1 Primary therapeutic and diagnostic modalities in cancer theranostics

	Technique	Advantages	Disadvantages
Therapeutic modalities	Photothermal therapy	Efficient heat production Do not require oxygen to interact with the target cell Less harmful to healthy tissues	Unable to penetrate deeper tissues Costly
	Photodynamic therapy	High sensitivity Targeted therapy Non-invasive	Unable to penetrate deeper tissues Ineffective against metastasized tumours
Diagnostic (imaging) modalities	Fluorescence Imaging	High sensitivity Multi-colour imaging	Limited clinical translation Low depth penetration
	MRI	Clinical translation High resolution and soft tissue contrast	High cost Long imaging time
	PET	Clinical translation High sensitivity with unlimited penetration	High cost
	SPECT	Clinical translation Unlimited penetration	Limited spatial resolution

Nanomedicine for Cancer Theranostics

Design and Targeting Principles

The passive targeting strategy utilizes the unique tumour microenvironment and the physicochemical properties of nanoparticles. Nanoparticles with average size of 100–200 nm, after *i.v.* administration, get accumulated in the tumour tissues

Table 7.2 Nanoformulations in clinical trials for cancer theranostics

S. no.	Nanoformulation	Cancer	Clinical trial ID/ phase
1.	Carbon nanoparticles	Colorectal tumour	NCT03350945
2.	Gold nanoparticles	Glioblastoma	NCT03020017/ Phase 1
3.	hafnium oxide (HfO2) nanoparticle	Prostate cancer	NCT02805894/ Phase 1
4.	Liposomes	Breast cancer	NCT03409198/ Phase 2B
5.	Polymeric nanoparticles	Acute myeloid leukaemia	NCT03217838/ Phase 1
6.	Polysiloxane Gd-nanoparticles	Advanced cervical cancer	NCT03308604/ Phase 1
7.	Protein-based nanoparticles	Solid tumours	NCT02495896/ Phase 1
8.	Silica nanoparticles	Colon, head and neck, breast cancer	NCT02106598/ Phase 2
9.	Polymeric nanoparticles	Colon cancer	NCT03774680/ Phase 1
		Solid tumour	NCT03712423/ Phase 1
		Prostate cancer	NCT03531827/ Phase 2
10.	Liposomes	Solid tumours	NCT02271516/ Phase 1
11.	Liposomes	Solid tumours	NCT02191878/ Phase 3
12.	Micelles	Head and neck squamous cell carcinoma	NCT02639858/ Phase 2
13.	Protein-based nanoparticles	Bladder cancer	NCT02009332/ Phase 1
		CTCL	NCT00211198/ Phase 4
14.	67Cu-peptide conjugates	Neuroblastoma	NCT04023331/ Phase 2
15.	CCK2 receptor targeting 111	Thyroid carcinoma	NCT03246659/ Phase 1
16.	Gold nanoparticles	Lung cancer	NCT01679470
17.	Superparamagnetic iron oxide nanoparticles (SPIONs)	Head and neck cancer	NCT01895829/ Phase 1
18.	Lipid-based nanoparticles	Liver tumours	NCT02181075/ Phase1
19.	Polymeric nanoparticles	Squamous cell, NSCLC	NCT02283320/ Phase 2
20.	Protein-based nanoparticles	Solid tumours	NCT02975882/ Phase 1
21.	PSMA conjugates	Prostate cancer	NCT03392428/ Phase 2

passively, due to the presence of leaky vasculature and abnormal tissue architecture. Further, the absence of lymphatic drainage in these tumour tissues retains nanoparticles and results in EPR effect (Ward & Thompson, 2012; Torchilin, 2011; Maeda et al., 2000). Thus, passive targeting by nanoparticles reduces the off-target effects of encapsulated anticancer agents and improves their anticancer effects. However, a compelling body of evidence suggests that sufficient cellular internalization and targeting cannot be achieved with a passive targeting strategy. Hence, in recent years scientists have focused their research towards active targeted delivery by modifying the surface of nanoparticles with tumour-targeting ligands (antibodies, peptides, proteins, aptamers, nucleic acids, proteins, polysaccharides, small molecules). These ligands recognize and can bind to complementary receptors or molecules, which are generally overexpressed on tumour cells. Thus, an active targeting approach improves the cell specificity, cellular uptake and anticancer efficiency of encapsulated anticancer drugs (Zhang et al., 2012; Torchilin, 2010).

Localized Imaging of Tumours

Specific and early imaging (diagnosis) of tumours is a major challenge in oncology. The limitations associated with small molecular imaging agents are rapid clearance, non-specific biodistribution, low uptake by the tumour tissues, nephrotoxicity, poor biocompatibility and low image resolution. In recent years, materials such as Br, Gd, Pb, U, Dy, Cu, Yb, AU, Bi, Lu and organic dyes were, therefore, extensively owing to their high radiocontrast imaging ability (Singh et al., 2011; Nasongkla et al., 2006; Yang et al., 2015; Shaik et al., 2020). However, their low cellular uptake and toxicities due to off-target actions are the significant limitations of these contrast agents. In this context, nanocarriers offer several benefits by overcoming the limitations associated with these contrast agents, enabling effective bio-imaging and diagnosis of cancer (Li et al., 2014; Chen et al., 2017).

Light-Assisted Cancer Therapy

PDT is one of the promising approaches in cancer diagnosis and treatment, which utilizes photosensitizers (PSs) for the eradication of tumours. In PDT, tumours are destroyed by the toxic oxygen species, ROS, produced by these PS upon exposure to light. However, off-target effects of PSs, oxidative stress in healthy tissues and low cellular uptake are the PDT limitations. Further, the clinically approved PSs like porphyrin and its derivatives in the visible region limit penetration of light, which reduces their PDT efficiency (Moret et al., 2013; Edmonds et al., 2012).

PTT is another attractive approach for light- and heat-mediated non-invasive and selective ablation of tumours. Moreover, greater depths of penetration of NIR light, high photo conversion efficiency and lower uptake by healthy tissues make PTT an

efficient approach to deal with tumours. However, off-target effects of ROS and low cellular uptake of photothermal agents are the limitations of PTT (Yoon et al., 2017; Li et al., 2017). Nanomedicine overcomes the setbacks associated with PDT/PTT and improves the therapeutic outcomes in cancer. In recent years various nanoformulations are being developed for efficient PDT/PTT in addition to image-guided anticancer therapy (Xie et al., 2020).

Lipid-Based Nanoformulations for Breast Cancer Theranostics

Lipid-based nanoformulations are one of the most significantly investigated in breast cancer theranostics. They have resulted in several advantages owing to their biodegradability and biocompatibility. Further, their formulation can be personalized for various applications. Herein, we discuss recent advances in lipid-based nanoformulations for breast cancer theranostics (Fig. 7.2).

Liposomes

Liposomes are spherical bilayers of lipids, which are formed by the controlled self-assembly, and are having hydrophilic interior moiety and hydrophobic exterior moiety (Joo et al., 2013; Díaz & Vivas-Mejia, 2013). These are the first-generation lipidic nanoformulations, explored for intracellular delivery of chemotherapeutic agents (Yingchoncharoen et al., 2016). They can be classified as multilamellar vesicles (0.5–10 μm) with multiple lipid bilayers based on the particle size and a number of lipid layers, giant unilamellar vesicles (1000 nm), large unilamellar vesicles (100 nm) and small unilamellar vesicles (20–100 nm) (Yingchoncharoen et al., 2016). The charge on the surface of the liposomes determines their cellular uptake and in vivo circulation capabilities (Su et al., 2012). Liposomes are classified as anionic, cationic and neutral based on surface charge. Neutral and anionic liposomes adsorb fewer serum proteins and have longer circulation half-life than cationic nanoparticles (Allen, 1994). However, cationic nanoparticles have been shown to have greater cell uptake (Sood et al., 2013). Modified liposomes such as transfersomes are made of phospholipids and an edge activator, generally an extended single-chain surfactant (Honeywell-Nguyen & Bouwstra, 2005). Ethosomes are another class of modified liposomes comprising phospholipids and ethanol. Phosphatidylcholine, DSPE, DOPE, etc. are commonly used lipids in the preparation of liposomes (Godin & Touitou, 2003). Controlled release, effective intracellular delivery of chemotherapeutics and ease of preparation are the advantages of liposomes. However, drug leakage during storage is the limitation of liposomes (Yingchoncharoen et al., 2016).

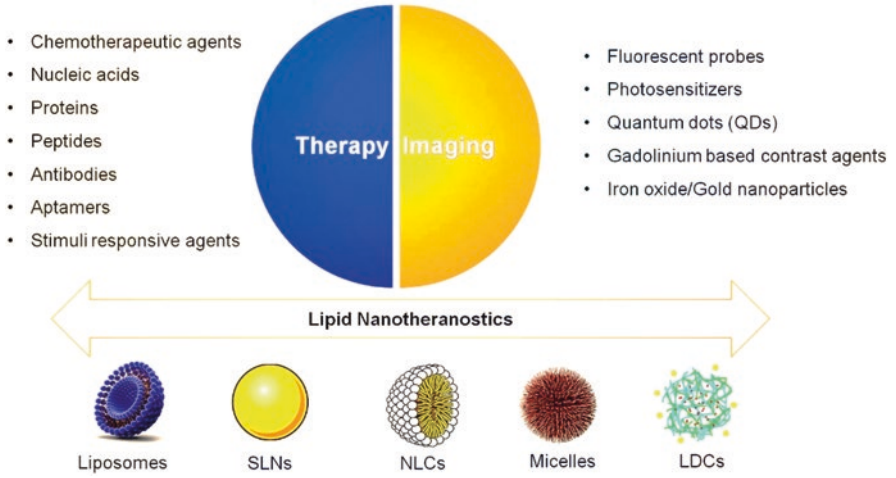

Fig. 7.2 Lipid-based nanoformulations for breast cancer theranostics

Preparation of Liposomes

Thin Film Hydration

In this method, liposomes can be prepared by dispersing lipids or their mixtures in a suitable organic solvent. The mixture of lipids and the organic solvent is subjected to rotary flash evaporation under reduced pressure, to form a thin film of dry lipid. The formed dry lipid film will be hydrated by adding aqueous buffer and by maintaining temperatures beyond phase transition temperature of the lipids employed. While maintaining the conditions, the mixture is agitated until the entire lipid film gets dispersed (preferably for 1 hour), resulting in formation of liposomes (Singh et al., 2019, 2020). The formed liposomes are further size reduced by sonication or by extrusion through polycarbonate filters, to get liposomes of desired size ranges. Even though the method is easy and is widely employed for the formulation of liposomes, it suffers from potential limitations of low drug encapsulation efficiencies (Yingchoncharoen et al., 2016).

Reverse-Phase Evaporation

This technique involves the preparation of a water-in-oil emulsion, with lipids, buffers and excess of organic phase by sonication or mechanical process. Followed by which the organic solvent is completely removed under vacuum, resulting in the formation of oligolamellar and large unilamellar vesicles. This technique is useful in the formation of inverted micelles (Vemuri & Rhodes, 1995; Laouini et al., 2012).

Detergent Removal Method

In this method, lipids are solubilized in a detergent to form mixed micelles. Then detergent will be removed either by dialysis, chromatography or adsorption (Torchilin et al., 2003; Vemuri & Rhodes, 1995).

Heating Method

This method doesn't involve any organic solvent and is one of the most uncomplicated and fast processes in a liposome preparation. The lipid components are hydrated at temperatures above phase transition temperatures of lipid for about 1 hour. by mechanical stirring (generally at about 120 °C), in glycerol (3% v/v). As they are prepared by a direct heating method, further sterilization is not required. This enables ease of formulation and scale-up (Patil & Jadhav, 2014).

Spray Drying

This method involves dispersing lipids into organic solvents, followed by sonication and spray drying. The obtained amorphous product can be hydrated using aqueous media. This results in the formation of lipid vesicles with high encapsulation efficiencies. The vesicular size of the final product depends on the volume of the aqueous phase used for hydrating the spray-dried product (Laouini et al., 2012).

Freeze-Drying

This method involves dispersing lipids in tertbutyl alcohol/solvent system at appropriate ratios. The above mixture is then sterilized and is processed for freeze-drying at controlled temperature and pressure conditions. This freeze-dried product, when dispersed in aqueous media, results in the formation of homogenous liposomal suspension (Patil & Jadhav, 2014). Particle size instability during freeze-drying, high production cost and varying EE are the limitations associated with this method (Ranade & Cannon, 2011).

Supercritical Reverse-Phase Evaporation

In this method, lipid components are dissolved in pressurized carbon dioxide with ethanol. Following which, the mixture is subjected to quick depressurization, along with simultaneous mixing, resulting in rapid precipitation of lipids into the aqueous phase, thereby forming liposomes (Frederiksen et al., 1997).

Applications of Liposomes in Breast Cancer Theranostics

A variety of liposomal such as Doxil® and Marqibo® have already been clinically approved for the treatment of breast cancer (Barenholz, 2012). Liposomes have also been studied as carriers for a variety of imaging agents including ^{14}C and ^{64}Cu isotopes, quantum dots (QDs), gadolinium contrast agents, fluorescent probes and superparamagnetic iron oxide nanoparticles (SPIONs). Liposomes are thus emerging as theranostic tools, with a wide range of anticancer applications (Al-Jamal & Kostarelos, 2011). Encapsulating MRI contrast agents into liposomal carriers together with anticancer agents is an effective approach for developing advanced nanotheranostics with non-invasive MRI. Mikhaylov et al. prepared ferrimagnetic iron oxide (FMIO) nanoparticles and cathepsin-encapsulated long-circulating liposomes for simultaneous imaging and killing of cancer cells. This liposomal formulation resulted in an improved MRI imaging with greater uptake by the tumour cells and adjacent stroma. This liposomal nanoformulation is a non-invasive, real-time tool for breast cancer (Mikhaylov et al., 2011). In another study, the authors reported the beneficial effects of liposomes encapsulated with hypoxia-activated prodrug (AQ4N) and hCe6 for breast cancer theranostics. After chelation with ^{64}Cu, these liposomes displayed properties suitable for PET. Further, it was reported that combining PET, fluorescence and PAI with PDT enhanced the anticancer theranostics by sequential PDT and hypoxia-activated therapy. Overall, this liposomal theranostic tool is a promising candidate for breast cancer theranostics (Feng et al., 2017). Rizzitelli et al. have developed a long-circulating liposomal nanoformulation encapsulated with gadoteridol and doxorubicin. After systemic administration, the nanoformulation resulted in localized drug release by ultrasound. This approach significantly improved the therapeutic efficacy in a breast tumour model (Rizzitelli et al., 2015). Folate receptor (FR) is an important cell surface receptor reported to be overexpressed on the surface of breast cancer cells due to their abnormal metabolism. FR is an essential target for tumour-specific delivery and imaging of therapeutic agents (Meier et al., 2010). In a recent study, Liu et al. have formulated multifunctional Gd-DTPA-ONB (GDO) lipid by introducing the Gd-DTPA contrast agent moiety and nitro-benzyl ester lipid for enhanced breast cancer theranostics (Fig. 7.3). In another study, doxorubicin-loaded, FR-targeted long-circulating liposomes containing polymers enhance theranostic efficacy in breast cancer (Gabizon et al., 2003; Moret et al., 2013). This nanotheranostic platform was found to be safe for treatment of breast cancer. In another study, He et al. have developed luteinizing hormone-releasing hormone (LHRH) receptor-targeted liposomes by conjugating gonadorelin (LHRH ligand) to the liposomal nanocarriers containing magnetic iron oxide nanoparticles (MIONs) and anticancer drug, mitoxantrone. This nanotheranostic formulation effectively controlled tumour progression, in addition to real-time and non-invasive tumour visualization (He et al., 2014). In another study, Lozano and coworkers reported the theranostics propensity of long-circulating liposomes co-encapsulated with ICG and doxorubicin, functionalized with a monoclonal antibody (hCTM01). The authors demonstrated non-invasive theranostics by

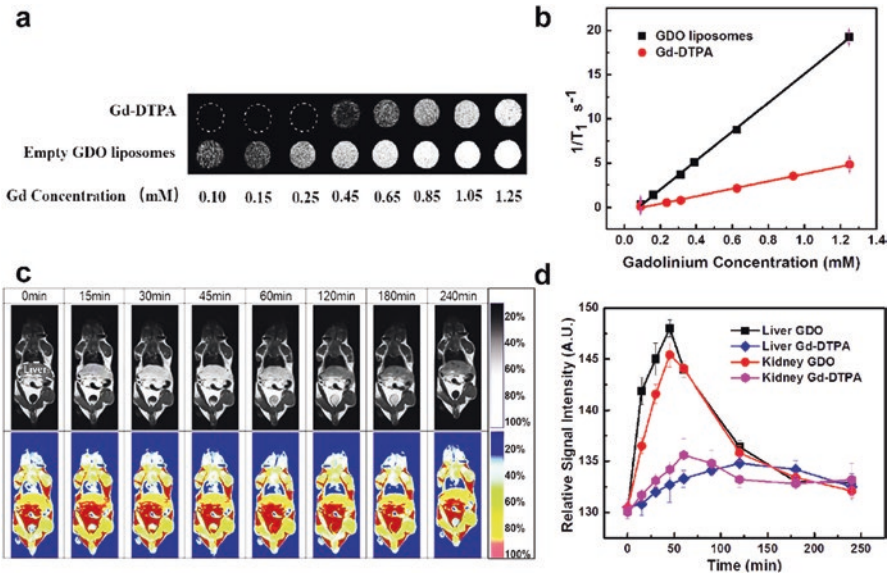

Fig. 7.3 GDO liposomes as an MRI contrast agent, in comparison with commercially available Gd-DTPA. (**a**) T_1-weighted MR images of different Gd-equal concentrations of Gd-DTPA and GDO. (**b**) A plot of $1/T_1$ against Gd concentration. (**c**) T_1-weighted MR images of mice injected with GDO liposomes. (**d**) Comparison of signal intensities of Gd-DTPA and GDO in the liver and kidney of mice (Liu et al., 2019). (Reproduced with permission, Copyright Elsevier 2019)

multispectral optoacoustic tomography (MSOT) and anticancer activity of liposomal nanotheranostics in breast cancer model (Gubbins, 2016).

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype with higher tumour relapse and metastasis (Dietze et al., 2015). Dai et al. developed integrin α3 receptor-targeted liposomes loaded with DOX and a NIR probe (DiD) and evaluated for efficacy in the TNBC model. In this study, the authors reported the beneficial effects of liposomes for significant regression and real-time monitoring of tumours (Dai et al., 2014). In another study, the authors prepared liposomes loading DOX, gemcitabine, or cisplatin, as well as the corresponding DNA barcode for probing the tumour sensitivity towards chemotherapeutic agents. After systemic administration, the liposomes can target tumour cells, confirmed by the fluorescent monitoring of the diagnostic imaging agent fluorescent dye indocyanine green (ICG). The DNA barcode established the superior therapeutic efficacy of gemcitabine compared to other tested drugs. PDT is an effective treatment modality, owing to its selectivity, residual systemic toxicity and non-invasive nature (Pais-Silva et al., 2017). In an in vivo proof-of-concept study, the authors reported the beneficial effects of long-circulating thermosensitive liposomes for PDT by incorporating ICG in liposomes. Liposomal tumour accumulation and excellent tolerability, with almost nearly complete tumour eradication, was observed from NIR imaging (Yoon et al., 2017). Zhao et al. developed long-circulating

thermal-responsive liposomes for chemo-PDT. The liposomes containing doxorubicin and ICG were designed, and their efficacy was evaluated in vivo in a rodent model. The developed liposomal formulation allowed temperature-controlled release of DOX along with real-time imaging of biodistribution.

Further, significant tumour regression with minimal off-target effects was observed in this study. It was reported that Ru-derived complexes had marked anticancer and imaging propensity without affecting healthy cells (Luk et al., 2012). Shen et al. have fabricated long-circulating liposomes containing Ru polypyridine complex for simultaneous imaging and induction of apoptosis in TNBC cells. This biocompatible, liposomal nanoformulation was found to be effective in selective induction of apoptosis in TNBC cells (Shen et al., 2017).

Lipid Nanoformulations with a Solid Matrix

Lipid nanoformulations with the solid matrix are interesting vectors for tumour-targeted drug delivery. These include:

(i) *SLNs*

SLNs or lipospheres are often referred to as second-generation liposomes. They are nanoparticles with 50–1000 nm size range. SLNs are made of fatty acids and also contain surfactants (Weber et al., 2014; Talluri et al., 2017). Easy preparation, stability and controlled drug release are some of the advantages of SLNs. Triglycerides, phospholipids, lipid acids (e.g. stearic acid, palmitic acid), glyceride mixtures or waxes and cholesterol are common lipids in the preparation of SLN formulations. Tween 80, lecithin and sodium glycolate are commonly used lipids for SLN preparation (Feng & Mumper, 2013). Compatibility for intravenous (i.v.) administration, controlled drug delivery and better cellular internalization are the characteristics of SLNs for useful applications in tumour-targeted drug delivery. However, drug expulsion and low drug-loading capacity are the limitations of SLNs (Muchow et al., 2008). SLNs have a wide range of theranostic applications in breast cancer (Weber et al., 2014).

(ii) *NLCs*

NLCs consist of a mixture of solid and liquid lipids with distinct nanostructures (Weber et al., 2014; Muchow et al., 2008; Pindiprolu et al., 2020). The lipid matrix of NLCs varies from crystal to amorphous structure. Compared to SLNs, NLCs have higher drug loading and better release profiles. However, rapid clearance is the disadvantage of NLCs (Shao et al., 2015; Patlolla et al., 2010; Beloqui et al., 2016). Soya bean oil, oleic acid, vitamin E/α-tocopherol, corn oil, etc. are the liquid lipids used in the preparation of NLCs. Stearic acid, carnauba wax, cetyl palmitate, glyceryl monocaprate, etc. are the solid lipids used in the preparation of NLCs. Tween 80, lecithin, sodium dodecyl sulphate, etc. are the emulsifiers used in the preparation of NLCs (Beloqui et al., 2016). NLCs are gaining interest in their theranostic

applications due to their high versatility. Further, high drug-loading capacity and improved stability at room temperature are the advantages of NLCs over SLNs for their applications in cancer theranostics (Beloqui et al., 2016).

(iii) *LDCs*

LDCs or lipidic prodrugs are prepared by the formation of salts with fatty acids or by covalent bonds, dipole moments, ion interactions, electrostatic interactions or hydrogen bonds either to the lipid directly or with the help of a functional spacer group (Hussain et al., 2015; Müller & Olbrich, 2004; Muchow et al., 2008; Müller & Olbrich, 1999). Further, surface modification of LDCs with suitable ligands provides active targeted drug delivery. LDCs are similar to SLNs and NLCs in terms of preparation methods and lipid components. The physicochemical challenges of drugs/photosensitizer dyes can be overcome with LDCs (Müller & Olbrich, 2004; Olbrich et al., 2002).

Preparation of Lipid Nanoformulations with the Solid Matrix

Homogenization

In this method, lipid nanoparticles are formed by dispersing the hot lipid phase (up to 90 °C) into an aqueous phase containing surfactants. The pre-emulsion is homogenized at 90 °C. Finally, the obtained o/w emulsion is cooled down to room temperature to solidify the nanoparticles (Weber et al., 2014; Muchow et al., 2008).

Emulsification Solvent Evaporation

In this method, the dissolved lipids in the organic solvent will be added to the aqueous phase with continuous stirring at 70–80 °C. Then the organic phase will be removed by continuous stirring. The obtained nanoemulsion will be then cooled to 5 °C to form lipid nanoparticles (Talluri et al., 2017; Pindiprolu et al., 2019).

Microemulsion Method

In this method, the oil and aqueous phases are heated up to the same temperature. Then the hot aqueous phase will be added to the oil phase under stirring. The hot o/w emulsion is dispersed in cold water at a 1:50 ratio to obtain solidified lipid nanoparticles (Siddhartha et al., 2018).

Ultrasonic Solvent Emulsification Method

In this method, lipid nanoparticles are formed by dissolving lipid phase in the organic phase (dichloromethane) and heated up to 50 °C. After partial evaporation of the organic solvent, the aqueous phase containing surfactants will be added. The obtained emulsion is sonicated for an appropriate time and finally cooled (Muchow et al., 2008).

Applications in Breast Cancer Theranostics

SLNs

IR-780 iodide is a NIR dye with higher fluorescence intensity and strong optical absorbance at 700–900 nm. Following laser irradiation, IR-780 can kill cancer cells in a non-invasive manner. However, hydrophobicity off-target effects are the challenges for the clinical use of IR-780 (Pais-Silva et al., 2017). Kuang et al. reported the theranostic efficacy of tumour-targeted IR-780 iodide dye-loaded SLNs to monitor PTT. These theranostic SLNs were biocompatible, stable and selectively accumulated at the tumour site (Peira et al., 2003). In another study, Kallinen et al. have reported the beneficial effects of thermally hydrocarbonized porous silicon (THCPSi)-SLN nanocomposite for passive targeting and imaging of breast cancer. The authors encapsulated THCPSi nanoparticles within SLNs to form THCPSi-SLN nanocomposite (THCPSi-SLNC) and radiolabelled with 18F (a PET-compliant dye) for imaging. The efficacy of developed THCPSi-SLNC was evaluated in an orthotopic breast cancer model. The authors reported that encapsulation of THCPSi in SLN improved the accumulation of nanoformulation at the tumour site at a 7-week point of time (Kallinen et al., 2014). SLNs are promising carriers for PSs/dyes for enhancing their phototherapeutic efficacy. Meso-(tetrahydroxyphenyl) chlorin (mTHPC) is one of the most potent photoactive compounds for clinical use. However, its effective intracellular delivery is challenging. It was reported that mTHPC-encapsulated SLNs enhanced the PDT efficacy in MCF-7 cells. (Navarro et al., 2014).

Brezaniova et al. have prepared stable thermoresponsive SLNs of temoporfin (T-SLNP). The prepared SLNs have a particle size below 50 nm. The efficacy of SLNs was evaluated in vitro using 4T1 and MDA-MB-231 breast cancer cells. Besides, the efficacy was evaluated in tumour-bearing *Nu*/*Nu* mice (Brezaniova et al., 2016) (Fig. 7.4). In another study, the authors developed trastuzumab-functionalized SLNs loaded with quantum dots and rapamycin for HER-2-targeted breast cancer theranostics. The results showed enhanced therapeutic efficacy of targeted SLNs over native drugs and unconjugated SLNs in the SKBR3 cells. Further, the authors demonstrated enhanced inhibition of the mTOR signalling pathway and enhanced apoptosis. The bio-imaging propensity of SLNs was studied in both monolayer and 3D tumour spheroid models (Parhi & Sahoo, 2015).

NLCs

Huipeng Li et al. have recently reported the beneficial effects of NLCs for targeted delivery of IR-780 and PTT of breast cancer cells. In this study, the author's developed CXCR4 receptor-targeted NLCs with AMD3100 (CXCR4 antagonist) and IR-780 (IR-780-AMD-NLCs). CXCR4-mediated tumour targeting and IR-780-induced PTT resulted in the prevention of metastasis. The encapsulated IR-780 displays better PTT than naïve IR-780 when exposed to NIR irradiation. These findings suggest CXCR4-targeted IR-780 delivery system holds a potential for PTT-induced anticancer effects and preventing metastasis (Li et al., 2017) (Fig. 7.5). In another study, Zhang et al. developed folic acid (FA)-modified NLCs loaded with PTX and chlorin e6 (Ce6). The NLCs have long circulation and tumour targeting. This system also enhanced the solubility and intracellular uptake of PTX and Ce6. Besides, after laser irradiation, this nanoformulation produced sufficient local ROS and induced cytotoxicity in breast cancer cells. Overall, these findings demonstrated that PDT combined with chemotherapy might have more significant tumour regression and theranostic effects (Zhang et al., 2019).

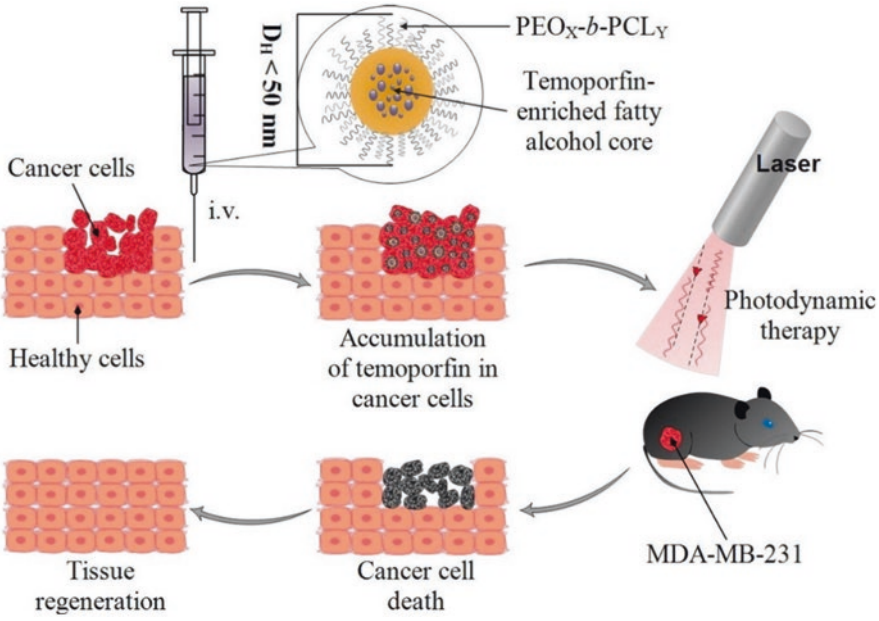

Fig. 7.4 PS temoporfin (T-SLNP) for PDT of breast cancer. (Reproduced with permission, Copyright Elsevier 2016) (Brezaniova et al., 2016)

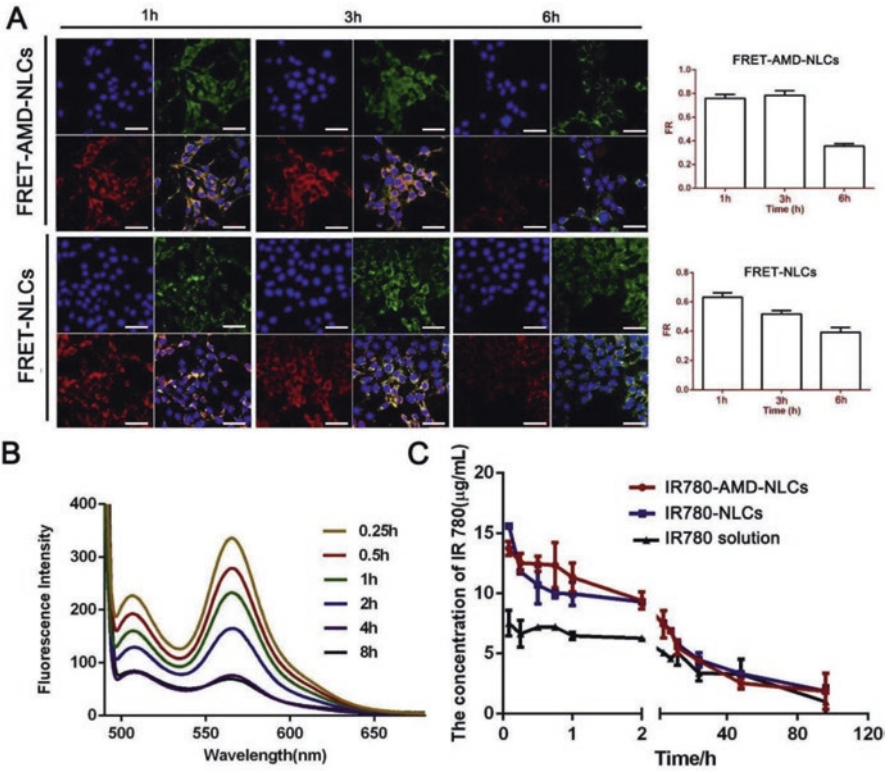

Fig. 7.5 (**a**) Confocal images of 4T1-luc cells incubated with FRET-AMD-NLCs. (**b**) The FRET signal change of FRET-AMD-NLCs in plasma. (**c**) Plasma concentration time curves of IR-780-AMD-NLCs after intravenous injection of various IR-780 formulations. (Reproduced with permission, Copyright Elsevier 2017) (Li et al., 2017)

LDCs

Muddineti et al. have recently synthesized PEGylated lipid and Ce6 conjugate nanoformulation for enhanced cancer imaging and NIR light-induced PDT. In another study, Lee et al. have developed fatty acid-conjugated PS nanoformulation for tumour targeting and PDT (Muddineti et al., 2020). In this work, the authors prepared PS LDC formulation comprising oleic acid (OA), pullulan (polymer) and Ce6 (OA-Pullulan-Ce6, OPuC). In this study, the imaging and anticancer propensities of OPuC were observed in colon, breast, and lung cancer cell lines. OPuC was reported to exhibit 3.27-fold greater cell uptake than non-OA-conjugated polymer (Pullulan-Ce6, PuC). Upon NIR irradiation, OPuC could generate singlet oxygen and results in cellular apoptosis and necrosis. OA-conjugated polymeric PS is, therefore, a potential tumour targeting and PDT agent for effective theranostics of breast cancer (Lee & Na, 2020). Also, in recent years, the researchers are focused on surface modification of nanoparticles with lipids to enhance their cellular uptake,

to achieve multifunctionality and to improve biocompatibility. For instance, Junjing Yin et al. have prepared PEGylated phospholipid membrane-coated catalase nanoparticles for targeting tumour hypoxia and enhanced chemo-PDT. The hydrophobic photodynamic agent of DiD and cytotoxic soravtansine were inserted in the phospholipid membrane of 1,2-distearoyl-snglycero-3-phosphoethanolamine-N-[methoxy(PEG)-2000] (DSPE-PEG). This lipid membrane was then surface modified on catalase nanoparticles to develop the nanocatalase system of DiD and soravtansine (Cat@PDS). Cat@PDS nanoformulation penetrated deep tumour sites and resulted in combinational chemo-PDT of breast cancer (Yin et al., 2020). In another study, Tang et al. have developed lipid-coated calcium phosphate nanoparticles (LCP NPs) for tumour-targeted combined PTT and gene therapy of TNBC. In this work, lipid-coated calcium phosphate nanoparticles (LCP NPs) were devised, with the out-layer lipids being readily PEGylated. The surface was further functionalized with a bispecific antibody (BsAb) of the EGFR receptor for targeted delivery. Cell death (CD) siRNA and ICG were co-loaded into LCP NPs for efficient NIR imaging and anticancer effects (Fig. 7.4). LCP-BsAb NPs are, therefore, promising theranostic platforms for breast cancer (Tang et al., 2019).

Lipid Micelles

Lipid micelles are colloidal lipidic amphiphilic systems (Torchilin, 2005). Unlike liposomes (lipid bilayer structure), they form a monolayer with the lipophilic tails forming the inner core and hydrophilic heads. They may be either spherical rods or ellipsoidal structures (Arleth et al., 2005). Lipid micelles can effectively deliver hydrophobic drugs, which are localized in the inner core of the micellar structure (Mu et al., 2005). Lipid micelles have the potential for effective intracellular delivery of drugs and genes to cancer cells. However, they have limited drug-loading capacity due to their low hydrophobic volume (Feng & Mumper, 2013).

Preparation of Lipid Micelles

Lipid micelles can be prepared by dispersing lipids in an organic solvent. The solution will then be injected into an excessive aqueous media to form lipid nanoparticles. Ether/ethanol is used as an organic solvent to form micelles and is diluted to a point below CMC, causing dissolved lipids to self-assemble in the aqueous phase and form small particles (under 100 nm) (Deamer & Bangham, 1976).

Applications of Lipid Micelles in Breast Cancer Theranostics

Lipid micelles are promising theranostic nanoformulations with the advantage of small size (5–100 nm), encapsulation of hydrophobic drugs/contrast agents, long circulation and greater cellular uptake for theranostic applications (Arleth et al., 2005). Tymish Y et al. have developed a phospholipid micellar nanoformulation for MRI-guided cancer therapy. Magnetoplasmonic nanoplatform combining gold nanorods (GNR) and FeO nanoparticles within phospholipid-based polymeric nanomicelles (PGRFe) was prepared. An external magnetic field guides the fabricated nanotheranostic micelles. The application of an external field enhances photoacoustic signal and also results in enhanced cell uptake. Upon laser irradiation, formulation efficiently generates plasmonic nanobubbles in cancer cells causing cell destruction. The combined plasmonic and magnetic functionalities of these nanoplatforms enable efficient anticancer theranostics (Ohulchanskyy et al., 2013). In another study, Kang et al. have developed EGFR-targeted lipid micelles co-encapsulating QDs and PTX for breast cancer theranostics. In this work, the authors co-encapsulated QD and PTX in lipid micelles for imaging and eradication of breast cancer cells. Further, to achieve an active target, the surface of lipid micelle nanoformulation was modified with EGFR aptamer or antibody. The developed micellar nanoformulation specifically accumulated in EGFR-positive MDA-MB 453 breast cancer cells and for imaging and enhanced tumour regression (Kang et al., 2018). Lipid micellar nanoformulations are, therefore, promising nanocarriers for theranostic applications in breast cancer (Fig. 7.6).

Future Prospects

Nanotheranostics is the fastest-growing field in life sciences. The biocompatibility, biodegradability, structural simplicity and extended functionality make lipid-based nanoformulations as promising tools for theranostic applications in cancer (Yang et al., 2015; Pindiprolu et al., 2018c; Paliwal et al., 2014). However, successful clinical translation of these nanoformulations for theranostic application in breast cancer is still challenging. The primary concerns with lipid-based nanotheranostics include (i) limited preclinical and clinical efficacy and safety data; (ii) complete eradication of tumours and precise imaging of tumours are still challenging to achieve; (iii) biodegradation and clearance mechanisms need to be elucidated; and (iv) the interactions between tumour microenvironment and nanotheranostics are yet to be elucidated (Chen et al., 2017; Kievit & Zhang, 2011). To overcome the above challenges to improve the theranostic and clinical translation potential of lipid-based nanoformulations in breast cancer, the following strategies can be adopted.

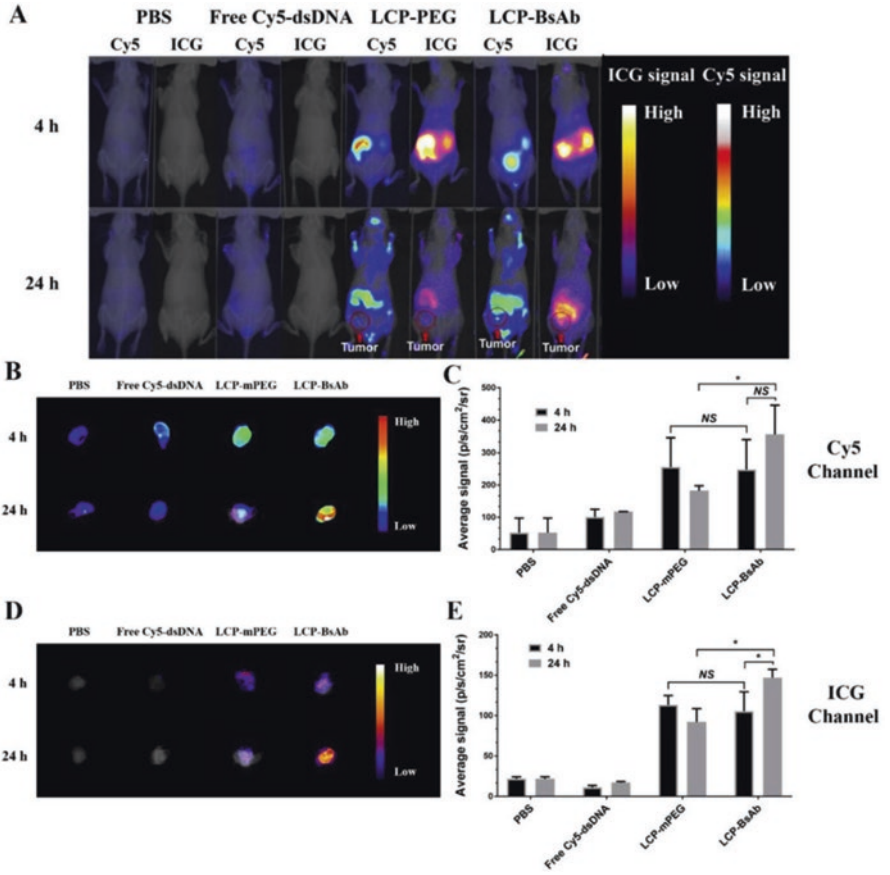

Fig. 7.6 NIR images of Cy5-dsDNA and ICG dual-labelled LCPs NP in human, murine tumour xenograft models (**a**). Nude mice-bearing MDA-MB-468 human tumour xenografts. Tumours are indicated with circles and arrows. Ex vivo images of tumour tissues collected from sacrificed mice at excitation wavelength for Cy5 (**b**) and ICG (**d**). Semi-quantitative analysis of fluorescent signals of tumour tissues for Cy5 (**c**) and ICG (**e**). (Reproduced with permission, Copyright Elsevier 2017) (Tang et al., 2019)

Multifunctional Nanotheranostics

Tumour imaging with different modalities, monitoring of drug/gene delivery, stimuli-sensitive release of payload and enhanced image-guided treatment or surgical removal of tumours can be achieved by incorporation of multiple components in a single formulation (Li et al., 2014; He et al., 2014). However, multifunctions and diverse components in a single nanotheranostic formulation make the system complicated and pose significant difficulties for scale-up and clinical translation. Researchers should, therefore, also focus on scale-up challenges for the effective design of multifunctional lipid-based nanotheranostics (Paliwal et al., 2014).

Combinatorial Chemo-/Gene Therapy and Phototherapy

Chemotherapy, phototherapy and radiation therapy are stand-alone treatments so far for a variety of cancers. However, these alone are not sufficient for the complete eradication of tumours. A combination of these stand-alone strategies could be more beneficial for significant tumour regression. In recent years, researchers, therefore, focused on combinatorial strategies such as a combination of chemotherapeutic agents and gene therapy with PSs to achieve complete tumour eradication (Abeylath et al., 2011; Amreddy et al., 2018).

Targeted Theranostics Towards BCSCs

A compelling body of evidence suggests that the presence of tumours initiating BCSCs even after chemotherapy or radiotherapy results in tumour relapse. Elimination of BCSCs along with bulk tumour cells results in a radical cure for breast cancer. It is, therefore, necessary to develop lipid-based nanoformulations to target self-renewal pathways for imaging and elimination of BCSCs (Pindiprolu, Krishnamurthy, & Chintamaneni, 2018b; Pindiprolu & Pindiprolu, 2019).

Stable Drug Loading and Prolonging Circulation Time

Stable drug loading within a nanoformulation is necessary for enhanced bioavailability and the improved pharmacokinetic relationship between drug and delivery systems. Covalent conjugation, active loading or strong complexation between the drug and the vehicle result in stable drug loading. Stable incorporation methods are needed to establish effective delivery and theranostics of PSs in breast cancer. PEG surface modification or other hydrophilic modifications is a recognized approach for prolonging circulation time, minimizing non-specific binding and reducing clearance by immune cells. Extending circulation time may to some extent mitigate the effect of their poor extravasation into tissues (Knop et al., 2010; Maeda et al., 2000).

Regulatory Aspects of Lipid-Based Nanoformulations

Biocompatible and inert lipids without significant toxicity are needed to be employed for effective clinical applications of lipidic nanoformulations. The use of lipids/excipients listed as generally regarded as safe (GRAS) by US FDA needs to be utilized for preparing lipid nanoformulations (Burdock & Carabin, 2004). Besides, an

inactive ingredient guide (IIG) also provides the safest concentrations of excipients in nanoformulations (FDA, 2009). By considering the data from GRAS and IIG, the formulation scientist can select the excipients for developing a new lipidic nanoformulation. Further, quality and safety issues associated with these formulations in preclinical and clinical studies are essential from the regulator's point of view as the lipids are used to undergo a chemical modification in the structure during conjugation, which may alter their properties (Namiki et al., 2011). Further, immunological reactions and toxicity profile of lipid-based nanoformulations need to be well studied for successful theranostic applications of lipid-based nanoformulations.

Conclusion

With the advent of nanomedicine, there is a paradigm shift in the imaging and treatment of cancer. In recent years, researchers focused on developing nanotheranostics for simultaneous tumour imaging and therapy. Nanotheranostics offer advantages such as enhanced tumour accumulation, controlled drug release and multiple imaging modalities for optimized therapeutic outcomes. A compelling body of evidence suggests that nanotheranostic have promising efficacy and negligible side effects. Further, nanotheranostics is a clinical 'weapon' for targeted cancer therapy. Among various nanotheranostics, lipid-based nanoformulations offer multiple advantages for imaging and therapy of breast cancer. They include biocompatibility, improved cell uptake and reduced off-target effects. However, nanotheranostics are struggling for clinical translation due to their multifunctionality and complexity. Multidisciplinary collaborations and knowledge interchange between academia, biomedical scientists and medical specialists are, therefore, needed to address the various translational challenges of nanotheranostics.

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Chapter 8
Nanoparticle for Photoresponsive Minimal-Invasive Cancer Therapy

Shazid Md. Sharker

Introduction

Cancer treatment is one of the most challenging problems confronting the worldwide healthcare system. The most common types are breast, lung, liver, stomach, and brain cancer, which are about ten million new cases every year. The cancer therapies used against this devastating disease are chemotherapeutic drugs, radiation, and surgical interventions. The choice of therapy depends on location, staging, and types of cancer (Sharker, 2019). Each of these therapies has some advantages and disadvantages because these cytotoxic treatments often kill healthy tissues and can cause resistance in cancer cells.

Another effective strategy that is currently gaining huge attention toward the researchers is the light-induced minimal-invasive cancer phototherapy, which includes photodynamic therapy (PDT) and photothermal therapy (PTT) (Gürbüz et al., 2020). In cancer phototherapy, the applied low energetic light has no interaction with the tissue area, and light can trigger externally from the body. The use of light to activate DDS for chemotherapy is yet another attempt to reduce the side effects and toxic effects of cancer chemotherapy. Moreover, combination treatments are a widely accepted strategy in cancer therapy, where surgical intervention, radiation, and chemotherapy are administered concurrently to cancer patients. It would therefore be desirable to develop photo-based therapies that may provide benefit from chemo- or radiotherapy with minimal adverse health effects (Huang & El-Sayed, 2011). The minimal-invasive therapy like chemotherapy, radiation, and phototherapy may gain popularity to cancer patients as they allow at least hospitalization, rehabilitation, and side effects.

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The use of light as a therapy dates back 3000 years. During that time, traditional Egyptians, Chinese, and Indians medicine used light to treat rickets, vitiligo, psoriasis, and other skin diseases. In modern medicine, Niels Ryberg Finsen received the Nobel Prize in 1903 for his treatments of cutaneous tuberculosis and smallpox pustules with ultraviolet (UV) and red light. This is the inauguration of a systematic study and understanding of the photochemical and photophysical processes of diseases and its treatment. As a result, the direct use of light has been used in vitamin-D deficiency, neonatal jaundice, manic depression, and other diseases (Tong & Kohane, 2012).

Light is a form of energy known as electromagnetic radiation (EMR). All types of light can travel at the same velocity with straight lines; however, they vary owing to the different sizes of wavelengths. For example, humans can visualize the spectral region of 400–600 nm of wavelength. Ultraviolet (UV) light has a shorter wavelength (200–400 nm), while infrared (IR) rays have a longer wavelength (600 nm–few μm size). Light consists of photons, and the wavelength of light is inversely proportional to energy ($E = h\nu$) (Tong & Kohane, 2012).

Based on the nature of medium in which it is propagating, light can be absorbed, transmitted, scattered, or reflected. The biological organic molecules, hemoglobin, and tissue heterogeneity have scattered and absorbed most UV and visible light, whereas the longer (650–900 nm) wavelength near-infrared (NIR) light can reach up to 10 cm depth. In this context, the maximum tissue-permeable NIR light and light-responsive NPs can be used for minimal-invasive optical imaging and phototherapy.

Minimal-Invasive Cancer Therapy

Moreover, the absorbed light can induce specific photochemical or photophysical reactions in the presence of light-responsive agents (Fig. 8.1). Those useful properties can be used for therapeutic or diagnostic purposes or both. The photochemical reaction included photo-cleavage or photo-switching, whereas the photophysical interaction included generation of cytotoxic singlet oxygen ($^1O_2$) or conversion of light into hyperthermia. Photoresponsive targeted cytotoxic $^1O_2$ therapy, known as photodynamic therapy (PDT), and photothermal heat-mediated therapy, known as photothermal therapy (PTT), are the main two branches of minimal-invasive cancer phototherapy (Gürbüz et al., 2020). At the same time, the emission of light at different wavelengths from light-responsive agents can induce bioimaging and diagnosis. Systemic study and understanding of photochemistry and nanotechnology in one platform has contributed to the clinical translation of promising NPs to address minimal-invasive PDT and PTT.

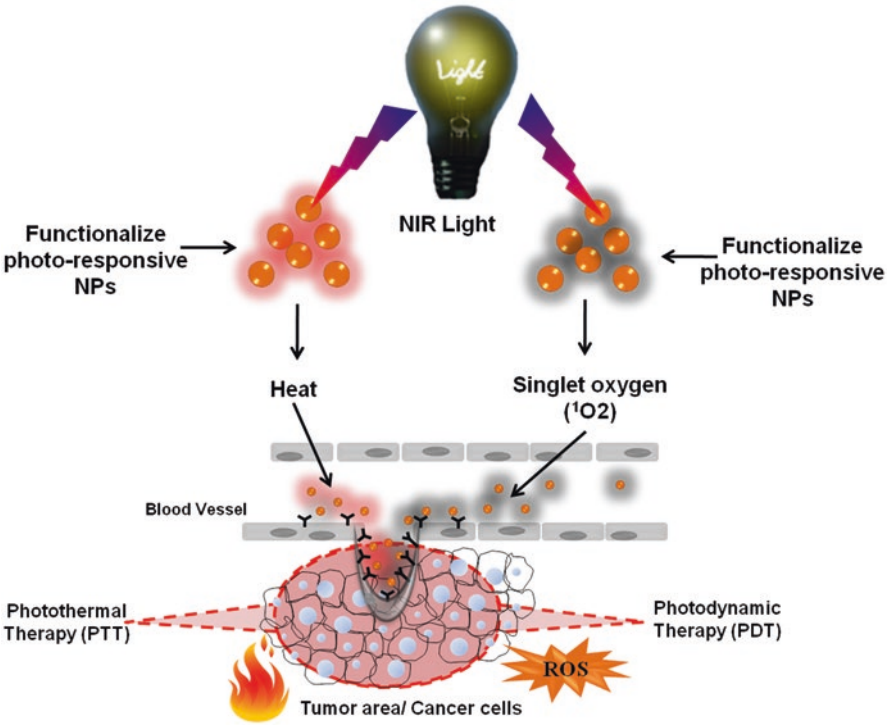

Fig. 8.1 Light-responsive nanoparticles (NPs) for the applications of PDT, PTT, and chemotherapy and theranostics

Photodynamic Therapy (PDT)

Photodynamic therapy (PDT) needed photosensitizer (PS) agents, which are excited in the presence of molecular oxygen (O_2). The subsequent photochemical reactions have generated reactive oxygen species (ROS) and transform the molecular oxygen (O_2) to cytotoxic singlet oxygen $^1O_2$ species. The reactive and singlet oxygen species followed two pathways: direct necrosis and apoptosis and indirect microvascular damage and antitumor immune responses (Hou et al., 2018).

The first PDT-based clinical results were in a dermatological site for the treatment of skin cancer. The key challenges involved in this PDT are tissue thickness, dose of PS, and continuity of treatments. Moreover, traditional PS are facing difficulty in water solubility, stability, and untargeted activity with the diseased tissue. Additionally, typical PDT agents utilize short-wavelength (UV and visible) light for activation, which cannot penetrate deep inside the tissue. PS agents having targeted molecule-modified nanocarriers and upconversion (UC) nanomaterials which can convert long-wavelength excitation light to short-wavelength have the potential merits to improve curative effect and reduce side effects (Hou et al., 2018).

Moreover, several nanocarrier-based PDT agents also showed promising fluorescence imaging capability, which can empower imaging-guided therapy and diagnosis, following optimum cancer therapy. Currently, NIR-responsive organic fluorescence dye including ICG, IR825, and IR780 and inorganic and hybrid nanomaterials like gold nanoparticles, carbon nanotubes, and graphene oxide have performed improve and efficacious PDT (Kim et al., 2016a).

Photothermal Therapy (PTT)

Photothermal therapy (PTT) has utilized a photosensitizer (PS) which can convert light energy into heat to kill cancer cells (Hou et al., 2018). The tissue-penetrating near-infrared (NIR) light and tumor-targeted photothermal agents are the main two factors controlling the efficiency of PTT. The ligand-based active targeting and the nano-fabrication-based passive targeting strategy can be used to define the control delivery of PTT agents. Moreover, imaging modules, like fluorescence imaging-guided PTT, may have a precise tumor-killing effect. Typically, the temperature above 42 °C exerts antitumor effects by damaging the tumor cell membrane, destroying the cytoskeleton, and inhibiting DNA synthesis. The duration and extent of PTT can be optimized through controlling NIR laser power or the concentration of photothermal agents. However, increasing laser power is associated with biosafety issues, and photothermal agents might not provide complete biocompatibility (Hou et al., 2018).

Moreover, heat shock proteins (HSPs) may overexpress during PTT, which allow repair of protein damage and cell apoptosis. The tumor cells can get protection, and the resulting heat resistance slows down the effects of PTT. The use of antagonizing HSPs, such as HSP70 and HSP90, during treatment could overcome the failure of PTT. Additionally, PTT-based hyperthermia can activate immune response by secreting cytokines and upregulating the expression of HSPs. The innate immunity and the adaptive immune system activated by tumor-associated antigens and hyperthermia therapy can kill residual or metastatic tumors (Ali et al., 2016).

Combined Phototherapy and Chemotherapy

Chemotherapies are typically cytotoxic small molecular drugs that can kill fast proliferating cells. It is the first choice of cancer treatment, and chemotherapy can increase the life span of cancer patients when it is possible to cure cancer (Gürbüz et al., 2020). The main side effect of cytotoxic chemotherapeutic drugs is that it also affects normal healthy tissue like digestive tracts and the bone marrow. The therapeutic success depends on the extent of cytotoxicity between normal cells and cancer cells. Therefore, the choice of drug, the dosage, and their biodistribution determine the effectiveness of chemotherapy.

Moreover, because most chemotherapeutic drugs are poorly aqueous-soluble, they can only be administered one at a time, and their pharmacokinetic profiles allow them to be rapidly excreted before tumor accumulation. Those limitations can be overcome by developing nanocarrier systems for commonly used chemotherapeutic drugs including doxorubicin (DOX), docetaxel (DTX), cisplatin (CPT), and paclitaxel (PTX). In this context, the long blood circulation time of nanocarriers is required for sufficient accumulation at the tumor site. However, most nanocarriers are usually taken by the liver and spleen during blood circulation, and long-term deposition becomes a toxic effect for a particular organ. As a result, there are still challenges in preventing drug release during circulation and controlling supply at the tumor site (Gürbüz et al., 2020). Combinational therapies including radiotherapy with chemotherapy and PTT and PDT with chemotherapy can beat this challenge by corporately fighting cancer.

Photoresponsive Nanoparticles (NPs)

The incident light which is the basis of photoresponsive NPs can cause photon injury. It depends on power density, spot size, irradiation time, and wavelength of incident light. The most common photon injury is photothermal damage where rising temperature damages surrounding tissue through protein denaturation, detritions of molecular tertiary structure, and subsequent fluidization of membranes. Based on the power of photothermal heat, the cell may undergo apoptosis (55–58 °C), apoptosis and necrosis (60–68 °C), or direct cell death (72 °C or higher) (Tong & Kohane, 2012). The increased and controllable photothermal heat in a target tumor is used in PTT. The incident light can also interact with endogenous chromospheres, like heme proteins and flavoproteins for the generation of free radicals. The free radical is another form of photochemical damage in the living system. Typically, long-term exposure to high energetic (short-wavelength UV) light is responsible for such photochemical injury.

The American National Standards Institute (ANSI) recommended maximum permissible exposures (MPEs) for different energetic light. The MPEs depend on light duration, pulse of light, and wavelength of light (Tong & Kohane, 2012). For the 10 W cm^{-2} (watt per square centimeter) power sources in a 700-nm continuous wave, it is no longer than 1 sec. In case of longer wavelengths, higher power fluxes are permissible. Typically, NIR-responsive NPs have been triggered by a 0.1–10 W cm^{-2} power source with a continuous pulse laser in most cancer therapies.

Light-Responsive Plasmonic NPs for PDT, PTT, and Diagnosis

Photoresponsive NPs have been synthesized to perform cancer therapy and are classified into inorganic (metallic), organic (polymeric, liposome), and hybrid systems. The nanosized metallic nanoparticles are photoactive and have a distinct photophysical property. Metallic NPs possess free electrons which are oscillating on its surface. The incident light with similar frequency can interact with an oscillating electron, and the collective coherent oscillation of metal exhibits absorption of resonance light. However, based on size and shape, the metallic NPs can also scatter or couple incident light. The distinct photophysical properties of metallic NPs are known as localized surface plasmon resonance (LSPR) (Huang & El-Sayed, 2011).

Light absorption in metallic NPs is due to the loss of photon energy by inelastic (lost or increased) processes, whereas light scattering results in electronic oscillation of photon energy in NPs. Interestingly, the scattering phenomenon has contributed to the emission of photons with the same frequency as the incident light. The photophysical light absorption and scattering properties of metallic NPs can be explained by Mie theory. The presence of LSPR effect in most of the metallic NPs can increase both light absorption and scattering efficiencies. For example, inorganic gold NPs have 1000 times the absorption or scattering properties of any existing organic molecule. This makes them well-suited as photoresponsive agents (Fig. 8.2) (Huang & El-Sayed, 2011; Sharker et al., 2015a).

The unique LSPR of metallic NPs depends on the particle size, shape, structure, electron charge density, and the surrounding medium on the particle surface. For example, spherical gold (AuNPs), silver (AgNPs), and copper (CuNPs) nanoparticles have a strong light absorption band in visible areas, whereas other metallic NPs exhibit broad and weak absorption bands in the UV area. Furthermore, modified hollow structures or core-shell NPs can show a red shift (longer wavelength) when compared to unmodified NPs (Huang & El-Sayed, 2011). The shape of NPs, such as rods, triangles, or branch structure, can also red shift the wavelength, known as anisotropic effects.

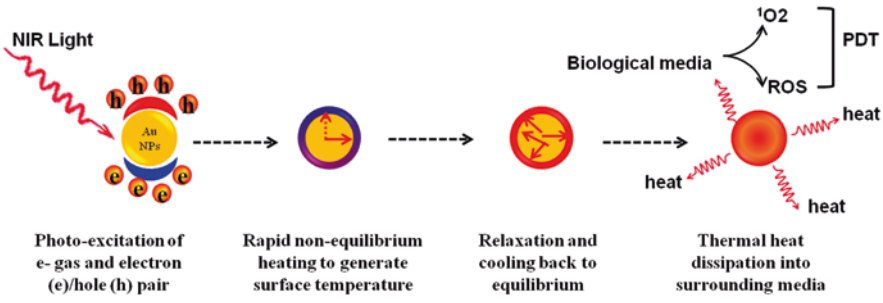

Fig. 8.2 Light-responsive plasmonic NPs and photochemical conversion for the applications of PDT, PTT, and chemotherapy and diagnosis

Among the different noble metallic NPs, the gold nanoparticle (AuNPs) has been explored more in cancer therapy. In cancer therapy, the LSPR frequency of AuNPs can be shifted to the NIR area by changing the structure and shape (Huang & El-Sayed, 2011). This is particularly important because the NIR light can penetrate significantly in biological tissue. For example, in core-shell NPs, the silica core at 100–200 nm and a shell of gold at 5–20 nm demonstrated coupling between the inner and outer shell surfaces for red-shifting of the absorption band. It has been found that decreasing thickness of the core-shell ratio can control predictable shift from visible to NIR absorption area. As a result, the absorption band changes from 700 nm to 1000 nm, owing to the decreasing shell thickness from 20 nm to 5 nm. The metallic LSPR frequency has decreased near-exponentially with the decreasing shell thickness-to-core radius ratio. The rational nano-chemical synthesis and nanotechnology approach allow us to develop NPs according to our needs.

The surface of metallic NPs allows conversion of absorbed light energy into heat. This includes a series of photophysical processes where the absorbed light energy oscillates the surface electron of the metal. The simultaneous electron-electron and electron-phonon relaxation as a result of oscillating electrons has generated substantial hot electron temperature in this metallic lattice. At the end of this translation, the lattices cool again through phonon-phonon relaxation, but the heat is dissipated from the particles into the surrounding medium (Huang & El-Sayed, 2011). Such light-to-heat conversion and heat propagation can be strategically used to raise the local temperature of the local environment. For sufficient amounts, heat from these hot NPs can change the function of the cells and even destroy them. For example, in photothermal therapy (PTT), cancer cells can be hyperthermally destroyed by targeted delivery and irradiation of metallic NPs.

Moreover, photoactivated metallic NPs can generate cytotoxic singlet oxygen ($^1O_2$) that is used in another cancer therapy called photodynamic therapy (PDT). With respect to LSPR of metallic NPs, it is assumed that a low-energy state of some NPs can transfer energy from irradiated absorbed light to molecular oxygen (O_2). Though molecular oxygen is present in living cells, target delivery and generation of singlet oxygen can be used to destroy cancer cells.

In addition to photoresponse property, metallic NPs like AuNPs have the potential to be used in cancer diagnosis. It can be used as a contrast agent or luminescence NPs for bioimaging exploration. Furthermore, the photothermal property of AuNPs opens up the possibility of developing a heat-responsive drug delivery system (DDS). For example, temperature-responsive polymer-coated AuNPs and loaded drugs can release in response to rising heat. In liposome DDS, photothermal heat has shown microbubble generation, leading to breaking of the liposome and drug release (You et al., 2012). It can be accomplished upon light irradiation to trigger the release of drug for the maintenance of dose-response curve. Even though the biocompatibility and biodistribution of metallic NPs remain contradictory, light-trigger DDS is a promising branch for anticancer drug and gene delivery.

Light-Responsive Polymeric NPs for PDT, PTT, and Theranostics

Photoresponsive organic NPs have shown promising outcomes for cancer phototherapy and diagnosis. Such organic NPs like liposome, polymeric micelles, polymersomes, and dendrimers possess favorable stability, biocompatibility, and biodegradability to satisfy clinical translation of diagnostic and therapeutic demand (Fig. 8.3). Moreover, organic NPs can be easily modified by a simple chemical route, which can load versatile drugs and deliver them to the targeted disease site without degradation.

The unique NIR-responsive organic NPs can efficiently harvest light for emission, allowing for versatile optical imaging while also acting as a PDT and PTT agent. Nonetheless, there are still some challenges, and further research is needed to address them for their future clinical translation. For example, some NPs are low photo-stable, do not intrinsically absorb light, and have a poor light-to-heat conversion efficiency (PTT), a low level of singlet oxygen generation (PDT), and clearance issues. To address this issue, novel conjugated polymer dyes, biodegradable biomaterials with attached cancer ligands, and antibodies must be developed for photoresponsive organic NPs. Moreover, the injection of a photo-cleavable or photo-switching moiety during NP synthesis can precisely overcome the above shortcoming. The photo-cleavable group can either disrupt or deform the NP structure where irradiate light can be absorbed the most to perform photoresponsive activity. Advance formulation strategy could control NP size less than 5 nm, which can solve clearance issues through urinary excretion. Thus, the interface of biology

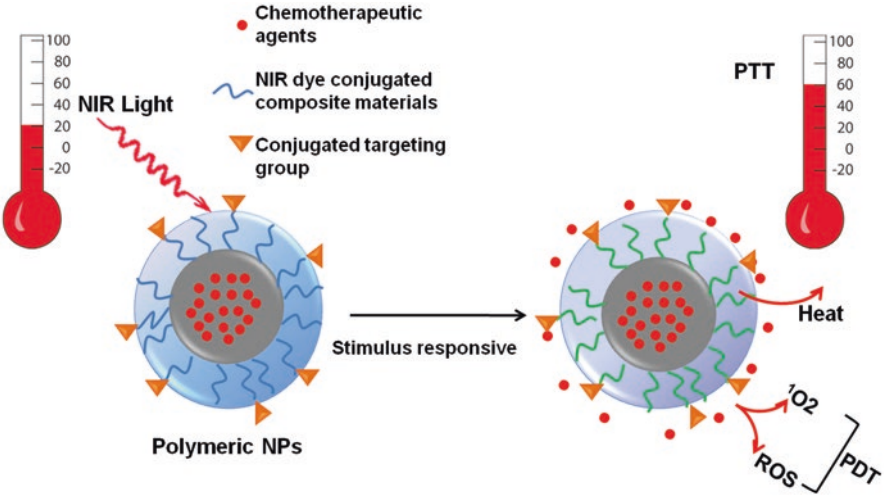

Fig. 8.3 Light-responsive polymeric NPs and functional modification for the application of PDT, PTT, and chemotherapy and theranostics

and chemistry in nanotechnology should be tailored for the application of NIR-responsive organic NPs clinically.

In photoresponsive NPs, the photochemistry and photoactivity of conjugated or loaded dye slightly differ when compared with free state. The free dye molecule absorbs light energy and releases fluorescence emission, whereas in nanosystems, the conversion of heat or singlet oxygen has been noticed in different organic photoresponsive NPs.

Previous studies showed that heat-sensitive liposomes loaded IR780 dye in its lipid layer and DOX loaded in the aqueous cavity can perform photoresponsive chemotherapy release. The mechanism was that the NIR laser excited IR780 to produce heat that will disrupt liposome structure, finally allowing DOX to be released from the liposome. In one example, organic NPs made by photocaging groups were shown to have an automatically degraded cleavage moiety, allowing loaded dyes to be released. Furthermore, organic NPs made with methoxy polyethylene glycol (mPEG) and poly(lactide-co-glycolide) (PLGA) conjugated to porphyrin have shown a micelle-like structure capable of loading both hydrophilic and hydrophobic drugs (Denkova et al., 2018). The release of those drugs could be controlled thermally by irradiation of NIR laser. The NIR light-irradiated organic liposome composed of pyropheophorbide has shown promising light to conversion efficiency for the use of PTT. Such systems can simultaneously deliver DOX during thermal therapy. The study and use of dual functional organic NPs have recently gain considerable interest, which might be due to PTT potentially improving the efficacy of chemotherapy.

Organic NPs photoactivated with photosensitizers can not only serve as phototherapeutic agents but also act as optical nanoagents for bioimaging. The NIR-absorbing organic nanoparticles or loaded MR (magnetic resonance), US (ultrasound), and CT (computed tomography) contrast within organic NPs endow them with single-modal or multimodal imaging-guided cancer therapy. Previous biological activity studies performed on photosensitizer organic NPs revealed that they should be water-soluble and tumor-targetable, have a high yield of heat or singlet oxygen generation, and be rapidly eliminated from healthy tissue.

Light-Responsive CDs for PDT, PTT, and Theranostics

Carbon dots (CDs) offer the development of theranostic (therapeutic and diagnostic) nanoparticles for combined cancer imaging and therapy. The fascinating photoluminescence (PL) property of CDs can be used in different biomedical fields accurately, like cancer diagnosis and therapy. Typically, the CDs show a full absorption spectrum in the ultraviolet (UV) region, owing to the extensive π-conjugated electrons in an sp2 atomic framework. It attributed π-π* transition for aromatic C=C bonds and n-π* transition for the C=O bonds or other connected groups. Additionally, the CDs with different size, composition, structure, or surface passivation effect can change its light absorption spectrum (Kim et al., 2016a; Gupta et al., 2014).

However, the emission spectrum of CDs shows excitation-dependent behavior ranging from UV to visible or near-infrared (NIR) region. Therefore, it is highly promising for multicolor bioimaging applications. Although the mechanism of PL emission is still a controversial issue of CDs, it is an important tool for biomedical use. For this, there are two proposed mechanisms: the quantum confinement effect (QCE) mechanism based on the band gap of the conjugated π-electron and the edge effect mechanism based on the surface defect states of both sp3- and sp2-hybridized carbons. This result has increased the localization of electrons on CDs and contributes to multicolor fluorescence emissions. Furthermore, the inherent photostability and introduction of surface passivation properties could amplify the fluorescent properties and empower the biological application in different ways. In the case of surface passivation (functionalization), the increased densities of the π-electron have facilitated the radiative combination and the quantum confinement of electron-hole (e/h) pair that improves fluorescent properties of raw CDs (Sharker et al., 2015b) (Fig. 8.4).

CDs or FCNs (fluorescent carbon nanoparticles) are considered an excellent luminescence probe for bioimaging due to their unique optical properties, size tuning capacity, surface functionalization capacity, and less photo-blinking and photobleaching characteristics (Sharker et al., 2015b). Though the surface functionalization of CDs is a complicated engineering method, the resultant fabricated and functionalized CDs are capable of exhibiting multiple functions. They can work as a remarkable drug carrier system and a gene delivering aid. Still, they can also be modified as an excellent phototherapeutic agent and sensor molecule for various therapeutic and diagnostic purposes, possibly due to their maximum drug-loading capacity (Choi et al., 2014). Numerous in vitro and in vivo studies have shown low or non-toxic nature of CDs compared to other fluorescent nanomaterials. Moreover, there is the ease of administration of CDs via different routes, such as oral, nasal, and parenteral, which make them better and more convenient forms of drug delivery systems to deliver therapeutic substances.

The ability to generate detectable acoustic waves in response to excitation waves is another attractive property of CDs, which can work as contrast agents in

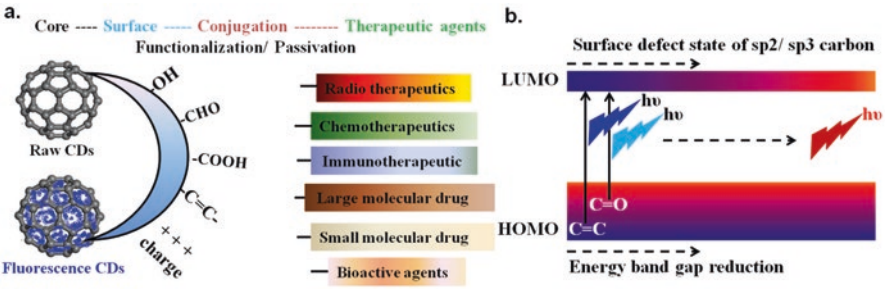

Fig. 8.4 (**a**) The photoresponsive carbon dot (CDs) NPs and the potential applications for PDT, PTT, and chemotherapy and diagnosis, (**b**) the light-responsive fluorescence and energy conversion process of CDs

photoacoustic imaging. An essential feature of photoacoustic imaging is that it offers an excellent spatial resolution to monitor the smallest areas, like axillary lymph nodes in breast cancer. Moreover, light-triggered photosensitizing properties of CDs alone or, in combination with photosensitizing agents, can generate reactive oxygen species (ROS) in tumor cells. This is another branch of cancer therapy, commonly known as near-infrared (NIR) light-irradiated photodynamic therapy (PDT). For example, PEG-functionalized CDs with photosensitizer chlorin (Ce6), protoporphyrin, or zinc phthalocyanine (ZnPc) were generated ROS under the excitation of CDs for targeted PDT (Choi et al., 2014).

Like PDT, the NIR-responsive photothermal therapies (PTT) can hyperthermally kill the targeted cancer cells. Different carbon-based NPs have this ability to absorb NIR light in the electromagnetic spectrum and consequently convert into reasonable heat to thermally destroy the malignant cells. For example, the carbonized polydopamine (pDa) have performed NIR-responsive photothermal conversion and multicolor fluorescence emission in different excitation wavelengths (Kim et al., 2016a). Moreover, CD-based hybrid systems can simultaneously perform PDT, PTT, and pH or NIR-responsive drug release. Such a multifunctional smart delivery system enables synergistic cancer therapy. For example, PEG chitosan-integrated CD nanogels have shown pH and NIR light dual-responsive drug release and PTT against tumor cells. In another attempt, carbonized fluorescence hyaluronic acid (HA-FCN)-conjugated boronic acid (BA) with β-cyclodextrin showed multi-responsive paclitaxel (PTX) DDS (Sharker et al., 2015c). This kind of DDS exploits acidic pH-dependent and remote external NIR-responsive on-demand cooperative controlling strategy. Such development of cooperative stimulus-responsive DDS having bioimaging potentiality is a promising method for chemotherapeutic release that can be adjusted according to physiological needs (Sharker et al., 2015b).

Light-Responsive CNTs for PDT, PTT, and Theranostics

The carbon nanotubes (CNTs) are one-dimensional (1D) needle-like structures which have generated significant interest in cancer therapy and diagnosis. Structurally, the CNTs are sp2-hybridized cylindrical tube-like shape carbon-based nanomaterials. The CNTs are prepared by rolling single- or multi-layered sp2 carbon, known as single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs) (Liang & Han, 2006). The unique CNTs belong to a vast surface area that can load small molecular as well as large molecular drugs and diagnostic probes.

The CNTs are soluble in a wide range of solvents and can be covalently functionalized on their side walls and the edge of tubes. The functionalization of CNTs is a first step to load the therapeutic agents. Moreover, conjugation of a diagnostic probe would provide added advantages for bioimaging, detection, and monitoring of the treatment process. The potential applications of CNTs can lead to the development of several new drug delivery systems that are still poorly explored.

The CNTs, specifically SWNTs, have intense NIR absorption bands due to the presence of intact sp2-hybridized carbon sheets resembling graphene. It has been found that the NIR light absorption band has decreased with an increased level of chemical conjugation. However, the chemical functionalization offers greater water solubility and biocompatibility. The mechanism behind the NIR responsiveness is that the energy levels of one-dimensional (1D) SWNTs have split due to quantum confinement effects. The 1 eV (electron volt) energy band gaps between the SWNTs allowed exciton wavelength-dependent fluorescence emission in the NIR area (Barone et al., 2005). The characteristic Stokes shift (energy band gaps) in this NIR area provided lower autofluorescence of biological tissues during bioimaging and diagnosis.

The incident light absorbed by NIR-responsive SWNTs can act as a photochemical catalyst in surrounding molecules. The photochemical reactions organized by SWNTs with molecular oxygen (O2) are able to generate cytotoxic singlet oxygen ($^1O_2$). The photodynamic conversion of cytotoxic $^1O_2$ can play a key role in cancer PDT. Although several photoresponsive NPs have already shown promising PDT, the SWNTs can be an interesting one due to NIR responses. For example, the noncovalent conjugation of SWCNTs with pyrenyl-functionalized distyryl-BODIPY has shown PDT agent in response to 660 nm light. In another study, photosensitizer 5-aminolevulinic acid-loaded polyamidoamine dendrimer-modified MWCNTs exhibited photodynamic destruction of tumor when they were excited to 632 nm light. Furthermore, the zinc monocarboxyphenoxy phthalocyanine (ZnMCPPc) with spermine- and uridine-loaded SWCNTs have shown high level of triplet and ^{1}O2 yield for the efficient PDT against melanoma A375 cells (Gupta et al., 2019).

Concurrent diagnosis and therapy is the key feature of CNTs in cancer theragnostics. The CNTs allowed broad electromagnetic absorbance spectrum in the NIR windows that can be a unique feature for the development of next-generation photothermal agents (Singh & Torti, 2013). This is because the NIR-responsive CNTs can efficiently convert the absorbed light into heat for the photothermal ablation of tumors. The absorbed NIR light allows CNTs to migrate excited states, and when it does, it releases absorbed energy in the form of vibrational energy, which is converted into heat energy to exhibit photothermal therapy (PTT). Moreover, it has been observed that the broad absorption window of CNTs is more advantageous than that of plasmonic metallic NPs, whose absorption band changes with size and shape of particles.

The photothermal studies of CNTs are well-known established methods in cancer therapy. However, the exciting result has been found in NIR-induced treatment of vascular inflammation, remote control gene expression, and implantable bioelectronic devices operated by laser irradiation from outside the body (Singh & Torti, 2013). Moreover, combining PTT and PDT can be a more efficient cancer treatment than only PDT or PTT. For example, Ru(II) complex SWCNTs (Ru-SWCNTs) produce $^1O_2$ through the photothermal effect of this complex, which exhibits enhanced anticancer efficacy. It is not surprising that new engineering strategies and unique functionalization schemes of CNTs have tremendous potential for human biological use.

Light-Responsive Graphene Oxides for PDT, PTT, and Theranostics

Graphene and its derivative graphene oxide (GO) have shown the most promising nanomaterials for cancer diagnosis and therapy. Structurally, graphene is two-dimensional (2D) and sp2-hybridized, and GO consists of both sp2- and sp3-hybridized planar carbon sheets. The planar carbon sheets bonded together to form a π-conjugated hexagonal pattern like a honeycomb. The basal plane of GO consists of a network of sp2- and sp3-hybridized carbons bearing hydroxyl (-OH) and epoxide (-O-) groups, whereas the edges are furnished by carboxyl (-COOH) and carbonyl (-CO-) functional groups (Lin et al., 2014; Kim et al., 2015).

The source of carbon-based GO is mainly graphene, which is available everywhere and can be prepared at a low cost. An essential feature of GO on its basal plane is pi-pi stacking/interaction, which can attach various molecules to its surface. Additionally, the carboxyl (-COOH), carbonyl (-CO-), and hydroxyl (-OH) of graphene oxide allow the conjugation of different therapeutic agents. The attached and conjugated GO nanocomplexes have the potential to improve the aqueous solubility of different anticancer therapies, a major problem facing most chemotherapeutic agents. The experimental results GO nanocomplexes promise to deliver a broad range of therapeutic agents in upcoming advanced drug delivery systems. In recent years, the analyses of GO nanocomplexes have led us to believe that it has the potential to solve cancer diagnosis and therapy at an early stage (Kim et al., 2015).

The GO can conjugate with organic photosensitizer through π-π stacking, hydrophobic interactions, and electrostatic interaction (Karimi et al., 2017; Sharker et al., 2016). The versatile interaction allows for increased loading efficiency and highly efficient PDT. Moreover, PDT can achieve the cancer target with the simultaneous conjugation of a targeting ligand. The photodynamic activities are caused by lipid peroxidation, depolarization of mitochondrial, increased caspase-3 activity, and finally apoptosis and death of target cancer cells.

The novel features of photosensitizer-loaded GO make them well-suited for cancer therapy. For example, chlorine e6 (Ce6), zinc phthalocyanine (ZnPc), and 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-α (HPPH) molecules loaded with PEGylated GO have shown high-rated singlet oxygen production ability sufficient for PDT (Kim et al., 2016b). Even though their fluorescence emission intensity has decreased due to the quenching properties of graphene oxide, their cellular uptake and permeability are very promising.

The GO sheets have broad UV to NIR absorption windows, which can be a potential candidate for hyperthermia cancer therapy (PTT). The presence of characteristic band gaps and edges/defects of GO has contributed reasonable photoluminescent properties and produces intensive heat at the time of laser irradiation. In PTT, the heat production efficiency depends on light absorbance ability. The light absorption efficiency of GO can be increased significantly when it is reduced. The reduced graphene oxide (rGO) can be developed through chemical and physical approaches including heat and light exposure. Moreover, polyethylene glycol (PEG)

and hyaluronic acid (HA)-modified biocompatible GO and fluorescent dyes such as Cy7, Hilyte647, or rhodamine B-loaded GO have shown bioimaging-guided PTT when they were applied into xenograft mice (Kim et al., 2016b).

GO can attach hydrophobic aromatic compounds like anticancer drugs by π-π stacking and hydrophobic interaction. The strategic design of GO-drug complex with a targeting agent can serve as a controlled drug release system. The nanosized GO carrier system belongs to a vast surface area and showed better accumulation in tumor microenvironments (Sharker et al., 2015d). Moreover, PDT and PTT can be anchored with this carrier system to achieve multimodal cancer therapy.

Previous studies showed that doxorubicin (DOX) can be released from the PEG-GO-DOX complex in response to glutathione (GSH) and NIR hyperthermia treatment. The NIR-induced photothermal heat has disrupted the endosome, which allows GO-DOX to escape into the cytoplasmic matrix. In other studies, the PEGylated GO with branched polyethyleneimine (BPEI) has performed combined DOX delivery and PTT. The interesting layer-by-layer methods were used for GO-poly (allylamine-hydrochloride)(PAH) nanocomplexes and pH-responsive drug delivery and PTT simultaneously. The photothermal chemotherapy may reduce chemotherapy resistance, which has prompted extensive study in this area. For example, the protein-functionalized reduced graphene oxide (rGO) nanosheet showed stimuli-responsive controlled DDS. The multifunctional GO-IONP-PEG-DOX complex was developed by superparamagnetic graphene oxide-iron oxide with loaded DOX, which showed NIR-responsive PTT, magnetically targeted DOX delivery, and magnetic resonance (MR) imaging of tumor (Kim et al., 2016b).

Conclusions

A collective effort from nanotechnology has required the development of cancer phototherapeutics to overcome the hurdle of translating photoresponsive NPs. The preliminary works of photoresponsive NPs are very promising because of small sizes, functionalization potentiality, and the ability to introduce multiple therapeutic agents on its surface. Moreover, the photoluminescence properties of photoresponsive NPs play an additional advantage for the bioimaging and diagnosis of tumors. The combined therapeutic with diagnostic functionality is known as theranostics, which holds the main potential of photoresponsive NPs to address the challenges of cancer therapy. It can be a paradigm shift in the way that we traditionally treat cancer. In photoresponsive NPs, cancer therapeutic is still in the midst of development; however, it has the technical capability to develop a brand-new DDS that can bring new hope for diagnosing, treating, and preventing cancer in the near future.

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Chapter 9
Biologically Synthesized Plant-Derived Nanomedicines and Their In vitro-- In vivo Toxicity Studies in Various Cancer Therapeutics: Regulatory Perspectives

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Introduction

Cancer remains a big health issue all over the world and is the second largest cause of mortality. As per the World Health Organization report, 9.6 million deaths were reported in 2018, and more than 9.8 million deaths at the end of 2020 were expected (https://www.who.int/health-topics/cancer#tab=tab_1). There are several therapies for the management of different forms of cancer. The most effective treatment used to treat certain forms of cancer is chemotherapy in conjunction with cytotoxic agents (Santhosh et al., 2020a; Santhosh & Chandrasekar, 2020). However, these

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therapeutic approaches are correlated with serious adverse effects, particularly multidrug resistance (Huang et al., 2017; Housman et al., 2014; Wang et al., 2019). There are several adverse side effects from chemotherapy alone or in conjunction with cytotoxic drug treatment or radiation therapy (Suganya et al., 2020; Santhosh et al., 2019; Wang et al., 2018). The National Cancer Institute (USA) has promoted studies into the possible antitumor activities of plant extracts focused on these adverse side effects (Menon et al., 2018). The discovery of anticancer medications is believed to be an important step forward for natural compounds derived from medicinal plants. Since the 1960s, examination of active compounds isolated from herbal plants has been of great interest for different biological activities, such as anticancer behavior. Many herbal plants have shown resistance against many metabolic disorders and cancers (Vetrivel et al., 2019; Santhosh et al., 2020b; Yuan et al., 2016; Santhosh et al., 2020c). Methods, such as nanotechnology, in numerous fields attracted and influenced major streams including drug delivery, chip laboratory, delivery vehicle, diagnostics, barcoding, cosmetics, paints, solar cells, batteries, chromatography, and so on (Saifi et al., 2018). In connection to that, the medicinal plant-derived nanoparticles have been a particular interest among researchers because of their minimal size. Nanotechnology is widely recognized below the size limit of 100 nm (Santhosh et al., 2015a; Rajakumar et al., 2017; Santhosh et al., 2015b).

There are no definite limits on the size spectrum; however, particles within the submicron range can be found in the literature. In contrast, humans are more exposed to a variety of nanosized materials, and modern growing nanotechnology fields become another potential risk to the lives of humans (Bahadar et al., 2016). Due to their smaller size, nanoparticles enter into all parts of the body by bypassing biological barriers in almost all major organs. Recently, researchers have proven with in vitro and in vivo studies that the nanoparticles act as a gas during inhalation and reach the liver, spleen, brain, heart, etc. while taken orally as well as inhaled, disrupting the normal cell biochemical environment (Sarwar et al., 2020; Li et al., 2020a).

The normal difficulties related with existing malignancy medicines are confinement of the treatment to tumor destinations, drug obstruction by tumors, and short medication course times (Thakkar et al., 2020). Additionally, toxicity-related anticancer drugs prompt significant side effects, for example, heart-related problems and decreased white blood cells (Park et al., 2020). Immune systems are further activated so as to clear nanoparticles from the human body since the approximate half-life of nanoparticles is about 700 days in the lungs, which ultimately affect the respiratory system. In order to overcome the issues associated with toxicity in nanoparticle usage to humans, distinctive intuition intending to add to safe utilization of nanoparticles is urgently required (Salieri et al., 2020). Latest nanotechnology-based cancer therapy drug delivery technologies are both on the market and in clinical trial investigations (Barkat et al., 2020; Slika & Patra, 2020). Moreover, nanocarriers have benefits in drug delivery due to the large surface size, good solubility, higher entrapment efficiency, lower degradation, low toxicity, etc. It leads to enhanced bioavailability and therapeutic effectiveness. Various nanocarriers, such

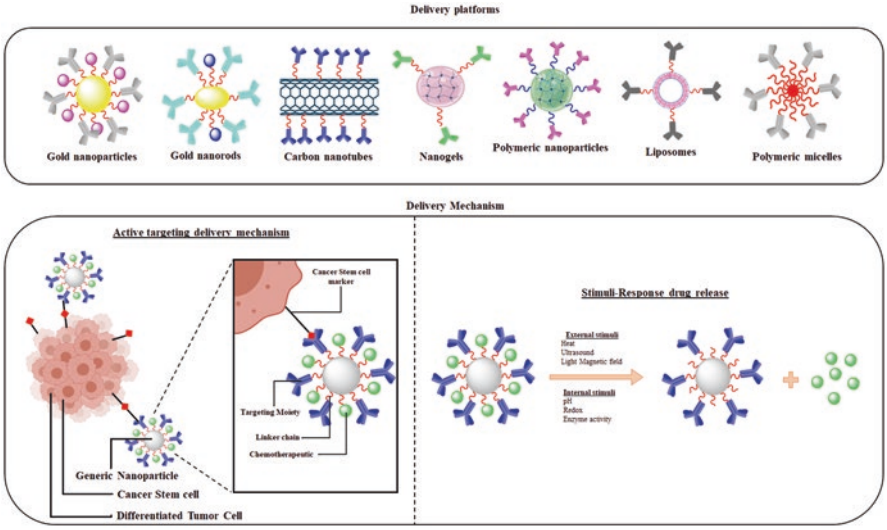
Table 9.1 Risks related to nanoparticles toward humans

Nanoscale materials	Chances of threat toward the human body
Carbon, silver, and gold nanoparticles	Toxicity to the respiratory tract and liver
Carbon nanoparticles	Pulmonary toxicity, inflammation of the lungs, development of excess fibrous connective tissue in organs, and cytotoxicity
Cadmium-based nanomaterials	Toxic liver disease, pulmonary toxicity, cell and DNA smash-up. Impaired development and cytotoxicity
Copper and copper oxide nanoparticles	Damaging the immune system
Titanium dioxide nanoparticles	Damages genetic material within a cell that triggers mutations that can lead to cancer; damage to the brain or peripheral nervous system
Zinc oxide nanoparticles	Severe toxicity to the liver
Quantum dots	Decreases sperm count and quality, embryo toxicity, and teratogenicity
Nanometal-organic framework materials	Toxicity of reproductive and respiratory organs, immunotoxicity, neurotoxicity, carcinogenicity

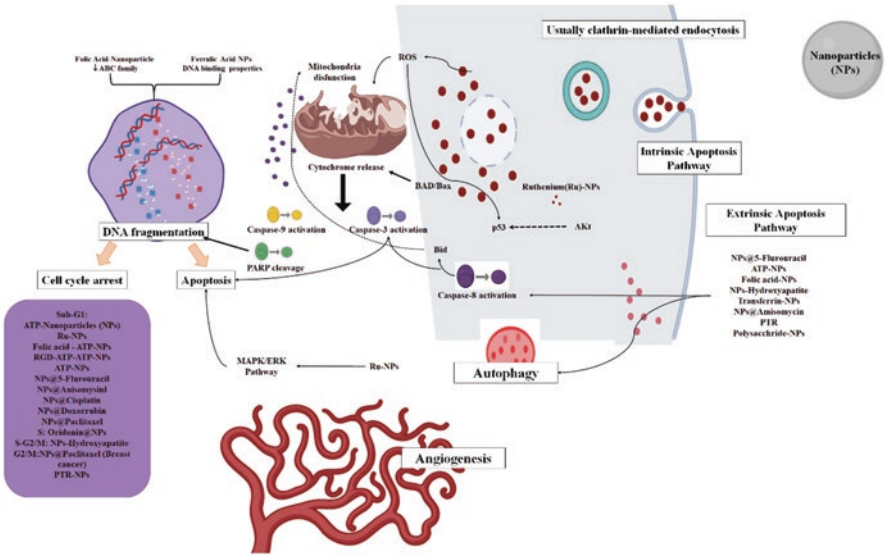
as liposomes, nanoparticles, SLNs, carbon nanotubes, nanosuspension, and nanoemulsion, are the thrust research area in herbal drug delivery of cytotoxic drugs (Mir et al., 2020).

Plants are an important source of novel compounds with anticancer activity, high therapeutic activity, and fewer side effects compared to synthetic drugs, such as paclitaxel (PTX), vincristine, vinblastine, irinotecan, topotecan, etoposide, teniposide, saffron derivatives, curcumin, etc. There are studies on numerous herbal anticancer medicines, such as curcumin liposomal formulation and PTX nanomicelle among others. Carbon nanotubes (CNTs) are carbon-based materials composed of graphite sheets rolled in the form of a tube, creating either single-walled or multiwalled CNTs. Focused on those developments, some of the US FDA-approved anticancer nanomedicines are now in clinical use, such as paclitaxel (Abraxane) and doxorubicin (Doxil). The metal nanoparticles such as Ag, Au, Ce, Zn, and Ti have many biomedical applications. Moreover, carbon-based nanoparticles play a prominent role in a variety of biomedical and industrial applications. Twofold specific characteristics of nanoparticles such as ultrafine scale, high surface area, surface charge, and adsorption make them an enormous tool for different applications (Montané et al., 2020; Lichota & Gwozdzinski, 2018; Jogi et al., 2018; Salatin et al., 2015).

The goal of nanomedicine, nanotechnology, and nanotoxicology is to improve human health (Saifi et al., 2018). Larger amounts of manufacturing the natural and anthropogenic nanoparticles predispose the atmosphere to overexposure. Nanoparticles endanger plants, animals, humans, and other environmental biosystems. Total overall studies suggest that these nanoparticles have a strong environmental effect which threatens the health of terrestrial and aquatic ecosystems (Karn et al., 2009). Toxicological evaluation of nanomaterials is crucial. We have very few toxicological data on the scope of nanotechnology research about safety of



Scheme 9.1 Nanoparticle-mediated targeted drug delivery to cancer stem cells



Scheme 9.2 Plant-based nanoparticles through cancer mechanism

nanomaterials. The well-being parts of nanoparticles have brought worries up in businesses, research networks, and administrative bodies.

There are no such outlined rules for toxicological assessment of nanomaterials. In this section, we have centered around therapeutic plants and biologically synthesized nanoparticles from herbal plants with possible anticancer exercises and

nanotoxicological issues, as well as diverse in vitro and in vivo toxicological investigations completed with the absolute most generally utilized nanoparticles. Risks related to nanoparticles toward the human body are listed in Table 9.1. Further, Scheme 9.1 and 9.2 depict the nanoparticle-mediated targeted drug delivery to malignant stem cells and cancer mechanism via plant-based nanoparticles, respectively.

Bioanalytical Approach-Estimation of Toxicity of Nanomaterials

Different techniques are accessible for the toxicity assessment of nanoparticles on organisms. The methods for toxicity assessment can be classified as in vitro, in vivo, and in silico modeling.

In vitro Assessment on Toxicity of Nanomaterials

One of the essential techniques is the measurement of in vitro nanoparticle toxicity. Lower cost, quicker, and minimum ethical concerns are included in the focus points. Necrosis assay, oxidative stress assay, proliferation assay, apoptosis assay, and DNA damage assays can be categorized through assessments (Fig. 9.1).

The MTT assay is often referred to as a colorimetric assay used to assess cell metabolic activity. (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a tetrazolium salt (MTT) that is most frequently used to test nanoparticles for in vitro toxicity. Rahmani et al. reported superparamagnetic iron oxide nanoparticles evaluated their cellular toxicities by MTT assay (Rahmani et al., 2020). Mohan et al. (2020) reported that the IC_{50} value of myricetin gold nanoparticles was found to be 13μg/mL against the MCF-7 cell line. It showed the depolarization of mitochondrial membrane potential and production of reactive oxygen species. Carneiro & El-Deiry (2020) have reviewed the apoptosis pathways and other signaling pathways that interact in cancer therapy. Yang et al. demonstrated that lemon-derived extracellular vesicles (LDEVs) can induce apoptosis of gastric cancer cells. They have used a flow cytometer to perform an apoptosis experiment and found that the LDEVs induced substantial apoptosis in three cell lines of gastric cancer (Yang et al., 2020). Evidence suggests that the oxidative stress in cell culture systems can cause apoptosis and DNA damage (Ryter et al., 2007). Biochemical markers such as malondialdehyde (MDA) and glutathione (GSH) have been investigated for oxidative impact (Akhtar et al., 2010). The toxicity of different nanomaterials was studied in vitro on both regular and malignant cell lines. Single-walled carbon nanotubes (SWCNTs), carbon nanotubes (MWCNTs), and metal nanoparticles such as Ag, Au, Ti, and Si are two of the major nanoparticles studied.

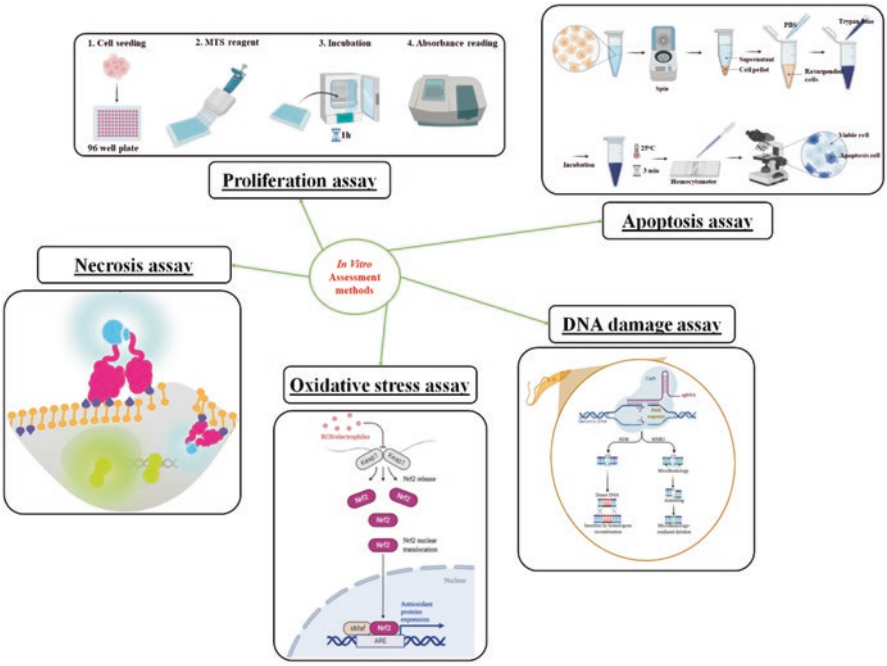


Fig. 9.1 In vitro assessment methods

In vivo Toxicity Assessment Methods

Usually, in vivo toxicity testing was conducted on animal models, such as mice and rats. Biodistribution, clearance, hematology, serum chemistry, and histopathology are assessment tools for in vivo toxicity (Fig. 9.2).

Nanoparticles are identified by radiolabels, and clearance can be carried out by analyzing the excretion rate and metabolism of nanoparticles at different times of exposure. Some other approaches for in vivo toxicity evaluation are serum chemistry changes and cell types. Nanoparticles exposed cell, tissue, and organ histopathology (Table 9.2).

Physicochemical Parameters for Toxicity Assessment

In materials that interact with biological systems, particle size and surface area play a prominent role. Apparently, reducing the size of the materials leads to an exponential increase in the area of the surface relative to the thickness, making the surface of the nanomaterials more reactive to itself and its neighboring environment. How

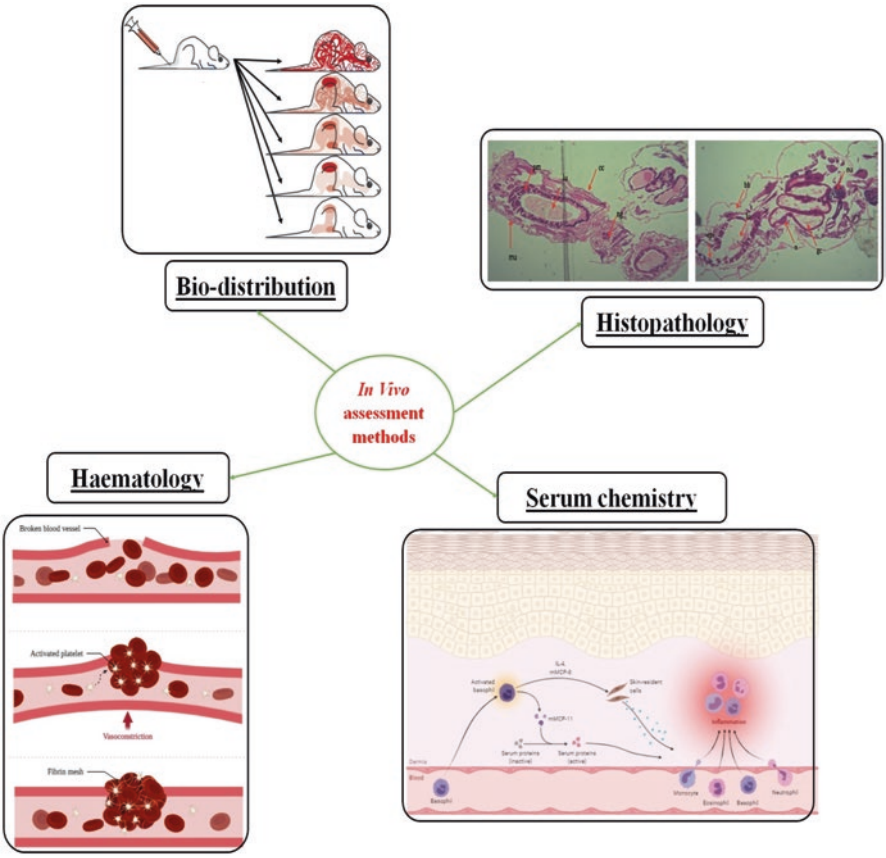


Fig. 9.2 In vivo assessment methods

the device reacts, distributes, and removes the materials was determined by the size and surface area (Powers et al., 2007).

Size, Surface Area, and Surface Electrostatic Status

Cytotoxicity was caused by nanomaterials resulting from the interaction between the surface and cellular components of the nanomaterials. If the diameter goes down, the surface area of the particle steadily increases. Thus, while particles have the same structure, they may have significantly different levels of cytotoxicity depending on both particle size and surface reactivity. In addition, particle size induces substantial differences in the in vivo delivery and distribution process of drugs. Chemical properties and size-dependent cytotoxicity are important in determining the cytotoxicity of nanomaterials in this sense, but the amount of size-dependent cytotoxicity is also important. In vitro cytotoxicity of nanoparticles of

Table 9.2 Methods of estimating the toxicity of nanomaterials

Evaluations	Methods	Advantages	Disadvantages
In vitro evaluation using dye exclusion method	Trypan blue, eosin, Congo red,	Simple, cheap, and a good indicator of membrane integrity and dead cells	Counting errors, poor dispersion of cells, inaccurate dilution of cells
Dye exclusion	Erythrosine B assays	Versatility and biosafety	Time-consuming and labor-intensive
Colorimetric assays	MTT assay	Simple to use, safe, and highly reproducible	It forms purple needle-shaped crystals in the cells and insoluble in water
	MTS assay	In vitro cytotoxicity assay, rapid, economic, and precise	This assay has an incubation time of 1–3 hours
	XTT assay	High sensitivity and precision	The efficiency of the XTT assay depends on the reductive ability of viable mitochondrial dehydrogenase cells
	WST-1 assay	It is easy to use, is safe, has a high reproducibility	The normal WST-1 incubation time is 2 hours
	WST-8 assay	WST-8 is not cell-permeable	Changes in intracellular metabolic activity that have no direct impact on overall cell viability influence the reduction of assay substrates
	LDH assay	Reliability, speed, and simple evaluation	The key drawback of this assay is that the intrinsic activity of serum and some other compounds is LDH
	SRB assay	Simple, fast, and sensitive	For high assay results, cellular clumps/aggregates should be avoided
	NRU assay	Speed and rapid evaluation are some of the benefits of this test	–
	Crystal violet assay	Quick and versatile assay	Insensitive to modifications in the metabolic activity of cells
Fluorometric assays	alamarBlue assay	Inexpensive and more sensitive than tetrazolium assays	Test compound fluorescent interference and the frequently ignored; there can be clear toxic effects on the cells
	CFDA-AM assay	Nontoxic to cells	There is scope for fluorescent interference from test compounds

(continued)

Table 9.2 (continued)

Evaluations	Methods	Advantages	Disadvantages
Luminometric assays	ATP assay	Most sensitive	Limited by reproducibility
	Real-time viability assay	Measurement of cell viability/cytotoxicity	Maximum incubation time
In vivo evaluations	Zebrafish	Low cost relative to mouse husbandry; in vivo imaging	Expensive to maintain and also more difficult to modify genetically Several duplicate genes
	Mouse, rat, and guinea pig	Reduce the human trials	Both have a nonethical nature and a lengthier period of evaluation
In silico evaluations	Computational simulation	Relation between nanotoxicity and physicochemical properties. The developed data set is focused on the accurate findings of experimental toxicity obtained through in vitro and in vivo studies	In silico findings cannot be relayed entirely because the findings in vitro and in vivo can differ from the results in silico

various sizes using different cell types, culture conditions, and exposure times was examined by various researchers (Perumal et al., 2020). He et al. developed the rhodamine B-labeled carboxylate chitosan-grafted size-dependent absorption polymeric nanoparticles with different particle sizes and similar zeta potentials (−35 mV) to deliver the protein drugs (He et al., 2012). Many toxicological studies have shown that, compared to larger particles of the same material, small nanoparticles <100 nm from plant source have potential effects on larvicidal activity (Cittrarasu et al., 2019). The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction was reported (Hoshyar et al., 2016). The variables relating to the biodistribution and clearance of nanoparticles were demonstrated by Wei et al. (2018). The zebrafish model was documented as a predictive screening model to test the macrophage clearance of in vivo liposomes (Sieber et al., 2019). The in vivo assessment of safety, biodistribution, and pharmacokinetics of laser-synthesized gold nanoparticles has been demonstrated by Bailly et al. (2019). The quantum dot size and surface-grafted peptide density on cellular absorption and cytotoxicity were assessed by Maksoudian et al. (2020). Similarly, previous studies have reported that the size-dependent cellular uptake and localization profiles of silver nanoparticles, cell type-based and size-dependent cellular uptake, cytotoxicity, and in vivo distribution of gold nanoparticles were demonstrated (Wu et al., 2019; Sun et al., 2017; Xia et al., 2019).

Morphology

Morphology is a critical issue and a significant problem in nanotoxicology. Nanoscale fibers (e.g., carbon nanotubes), like other well-established inhalable fibers (e.g., asbestos), are considered to pose a serious risk of pulmonary toxicity. In addition, prolonged exposure can cause numerous cancers (Wright et al., 2020). It is difficult to determine whether single nanotubes or an array of such tubes have a certain toxic effect. Few studies have found carbon nanotubes to be more dangerous than other ultrafine black carbon or silica powders. Lung lesions above the legal allowable exposure level have been found by many staff exposed to single-walled carbon nanotubes (SWCNTs) (Bustamante, n.d.; Das et al., 2018). Interestingly, CNTs have been shown to cause targeted death of kidney cells through impaired growth of cells caused by adhesive reduction of cells. Human penetration of fullerene (also called buckyball) has resulted in significant lung injury (Tripathi et al., 2015).

Status of Agglomeration

Agglomeration may be a potent stimulator of human inflammatory lung injury, regardless of any physical and chemical properties of nanoparticles, such as chemical compounds (Gupta & Xie, 2018). It has been assumed that exposure to higher levels of such chemicals contributes to serious chronic diseases such as fibrosis and cancer (Shin et al., 2015). In a living organism, it remains to be identified which characteristics cause any toxicological effects.

Toxicity of Carbon and Graphene-Based Nanomaterials

Toxicity of Carbon Nanomaterials

In biomedical areas, including drug delivery, biomedical imaging, biosensors, tissue engineering, and cancer treatment, carbon nanomaterials are widely used. Nevertheless, numerous studies on the toxicity of CNTs have been carried out, still suffering from their toxic effect on biological systems (Mohanta et al., 2019). Several studies have stated that the CNT toxicity is caused by different factors. The influence of metal impurities in the CNTs may significantly affect toxicity (Yuan et al., 2019; Pumera & Miyahara, 2009). The length-dependent retention of CNTs in the pleural space of mice initiates sustained inflammation, and progressive fibrosis was reported by Murphy et al. (2011). The CNTs and its properties in relation to pulmonary toxicology have been studied (Donaldson et al., 2006). They analyzed the invaluable factors in recognizing important toxicity factors, such as scale, shape, purity, and functionality, which can attenuate CNT toxicity (Madani et al., 2013).

Moreover, Nam et al. suggested that solubilizing agent sodium dodecyl sulfate played a significant role in toxicity of CNTs and solubilized SWCNTs exert genotoxic effect in renal epithelial cells. Nanosized carbon nanoparticle C-60 for optimized delivery of berberine into leukemia (CCRF-CEM) cells has been investigated by Grebinyk et al. (2019); C60-berberine nanocomplexes increased particle size steadily from 110 to 152 nm. In a range of concentrations from 5 to 50μM, free berberine exhibited dose- and time-dependent toxicity against CCRF-CEM cells. The IC_{50} value of berberine was found to decrease by 3.2, 4.8, and 6.3 times in the C60-Ber nanocomplexes at 1:2, 1:1, and 2:1 molar ratios, respectively (Table 9.3).

Due to its distinctive properties for medicinal use, CNTs have drawn considerable interest. Via surface functional groups, drugs and biological molecules may bind to the CNTs or can be loaded within the tubes. Reports suggest that up to five million drug molecules can load CNTs with a diameter of 80 nm. The CNTs are well-investigated for the delivery of anticancer herbal medicines such as PTX, quercetin, ginsenoside, oridonin, analogues to camptothecin, derivatives of gallic acid, vinblastine, and betulinic acid (Table 9.4).

CNTs are novel carriers which are used for drug delivery, especially for anticancer herbal drugs. The structure of CNTs is such that it enables loading of drugs inside the tube and also allows surface coating of the drug on the tubes. Conjugation of targeting moieties or functionalization through polymers enables drug attachment and higher loading efficiency. Many in vitro reports are available about CNT-based herbal drugs. However, more focus and investigations about in vivo and clinical studies are still required for CNT-based herbal drugs to establish this novel architect as promising delivery vehicles.

Toxicity of Polymeric Nanomaterials

Chitosans

Facchi et al. successfully obtained the physical cross-linked sodium tripolyphosphate (TPP) nanoparticles based on N, N-dimethyl chitosan (DMC) and N, N, N-trimethyl chitosan (TMC), using water/benzyl alcohol emulsion. Curcumin (CUR) was loaded into nanoparticles and simulated intestinal fluid and in simulated gastric fluid was tested in controlled release experiments. Cytotoxicity studies showed that, compared to unloaded TMC/TPP particles, only loaded TMC/TPP particles containing CUR were marginally cytotoxic to human cervical tumor cells (SiHa cells). Alternatively, loaded nanoparticles (TMC/TPP/CUR and DMC/TPP/CUR) were more biocompatible than unloaded NPs (TMC/TPP and DMC/TPP/CUR), in particular, with safe VERO cells (Facchi et al., 2016). Dhanavel et al. have recently reported that the chitosan-reduced graphene oxide (CS/rGO) nanocomposite loaded with 5-fluorouracil (5-FU) and CUR) was prepared using a simple chemical method. With the addition of 5-FU + CUR-loaded CS/rGO nanocomposite, synergistic cytotoxicity was observed, demonstrating the system's efficacy in

Table 9.3 Toxicity of carbon and graphene-based nanomaterials

Source	Formulation	Size (nm)	Cell line	Assays	Cancer type	References
Catharanthus roseus	Carbon quantum dot-hydrothermal carbonization technique	5	MCF-7	MTT assay, cytotoxicity, and bioimaging	Breast cancer	Arumugham et al. (2020)
Doxorubicin	Carboxymethyl cellulose/graphene quantum dot nanocomposite hydrogel films filled with doxorubicin	4–6	K562	MTT assay	Blood cancer	Javanbakht & Namazi (2018)
Tea leaves	Cadmium sulfide (CdS) quantum dots (QDs) using waste matured tea leaves	2.5–4	MCF-7 cells, IC_{50}–30μg/mL	MTT assay, Western blot analysis	Breast cancer	Shivaji et al. (2019)
Gynostemma pentaphyllum	Core-shell nanocarriers with ZnO quantum dot-conjugated Au nanoparticle	12.2	Hela	In vitro cytotoxicity	Cervical cancer	Chen et al. (2013)
Angelica species	Copper oxide quantum dots (CuO QDs) on chitosan	5–10	The human osteoblast cells	MTT assay		Hu et al. (2019)
(C-dots/PEI/Au), (C-dots/PEI/Ag) dots/polyethyleneimine/silver dots/polyethyleneimine/gold	Microwave irradiation method	6.5 ± 2	Vero cell line	No sign of toxicity. Very low conc. 200 mg/mL; biocompatibility assay, MTT assay		Emam et al. (2017)

Hydroxyl-modified graphene quantum dots	Purchased	5.6 ± 1.1 nm	H1299 and A549	50μg/mL CCK-8 assay analysis of cell viability, cell cycle distribution analysis, immunoblotting and immunofluorescence analysis	Human lung carcinoma cell lines	Tian et al. (2016)
Single-walled carbon nanotube-paclitaxel-folic acid conjugate	Chemical methods		MKN45 cell line	High toxicity for the SWCNTPTX-FA sample compared to PTX (50 ml) after 48 h. MTT assay	Gastric cancer	Tavakolifard et al. (2016)
TAT-CS conjugates, MWCNTs-TC and MWCNTs-C	Chemical method		MD-MBA-231, L929, human umbilical vein endothelial cells	Cell growth of CuO QDs/ CS-Ang by concentration-dependent cytotoxicity assessment	Anticancer	Dong et al. (2015)
Quantum dots encapsulated with curcumin	Solvent displacement technique	~100 nm	HCT-116, MCF-7, HEK-293	Dose-dependent cytotoxic effect on both HCT-116 and MCF-7 cells. MTT assay, clonogenic assay	Colon cancer, breast cancer	Khan et al. (2020)

Table 9.4 The CNT-based carrier for anticancer drug delivery

Biological source	Formulations	Size (nm)	Cell line	Assay	Cancer type	Ref
Paclitaxel	Conjugation with polyethylene-imine, folic acid -MWCNTs	20	HeLa, HUVEC cells	Confocal microscopy assay, MTT assay	Cervical cancer	Tian et al. (2011)
Quercetin	Conjugation with poly-methacrylic acid. MWCNTs	NA	HeLa	Cell viability test	Cervical cancer	Cirillo et al. (2013)
Ginsenoside	MWCNTs	NA	MCF-7 PANC-1	MTT assay, double-label immunocytochemical staining	Breast cancer/ pancreas cancer	Lahiani et al. (2016)
Oridonin	Conjugation with carboxylic acid-MWCNTs	0.30	HepG-2 cell. IC_{50}–7.29 μg/mL	Cytotoxicity testing, CCK-8 assays	Liver cancer	Wang & Li (2016)
Camptothecin	Conjugation with diamino trimethylene glycol-MWCNTs	–	MKN-28 H22 tumor cells-mouse	Cytotoxicity	Gastric cancer	Wu et al. (2009)
Betulinic acid	Conjugation with COOH -MWCNTs	–	A549, HepG2 IC_{50}–2.7 and 11.0μg/mL,	Cytotoxicity assay	Lung cancer, liver cancer	Tan et al. (2014a)
Paclitaxel	Conjugation with PEGylation-SWCNTs	20.6	4T1 murine breast cancer	Cell toxicity assay	Breast cancer	Liu et al. (2008)
Betulinic acid	Conjugation with COOH- SWCNTs Conjugation with PEG Tween-80 Tween-20 chitosan-SWCNTs	–	3T3, A549, HepG2	Cytotoxicity evaluation	Lung cancer, liver cancer	Tan et al. (2014b), Tan et al. (2016)

the vincristine-loaded folic acid chitosan-conjugated nanoparticles.
Kumar et al. (2018) have reported that MTT assays on NCI-H460 cells evaluated chitosan-based biodegradable nanoparticles has been stated by Gupta et al. (2015). The enhanced apoptotic and anticancer potential of paclitaxel-loaded (2020). The enhanced apoptotic and anticancer potential of paclitaxel-loaded chetgeq nanocomposites with an IC20 of 53.8μg\mL were observed (Dhanaraj et al., inhibiting the growth of HT-29 colon cancer cells. Better cytotoxicity for dual-drug-

Poly(lactic-co-glycolic acid)

Prabhuraj et al. (2020a) using the nanoprecipitation process encapsulated curcumin and niclosamide in poly(lactic-co-glycolide) (PLGA) nanoparticles in the presence of poly(vinyl alcohol). Dual-drug-loaded PLGA nanoparticles had a higher anticancer effect on MDA-MB-231 breast cancer cells compared to a bare two-drug mixture in DMSO with an IC50 value of 13 (μM). In addition, in the treatment of triple-negative breast cancer, PEGylated PLGA nanoparticles are used to deliver curcumin to MDA-MB-231 and L929 cells (Prabhuraj et al., 2020b). Sufi et al. have reported the drug retention of curcumin and indole-curcumin analogs (ICA)-laden polysorbate 80-stabilized PLGA nanoparticles against colon cancer cell line SW480. On the SW480 cell line, IC_{50} of free CUR and ICA were found to be 25–27μM and 15–17μM for 24 hours, respectively (Sufi et al., 2020). Kumari et al. (2020) have developed that the CUR-loaded PLGA nanoparticles showed significant cytotoxicity toward HepG2 cells, whereas they were found to be cytocompatible toward HEK293 cells. Durymanov et al. (2020) developed the intravenous silibinin-loaded PLGA-based nanoparticles to reduce drug-induced hepatotoxicity.

Poly(amidoamine) Dendrimers

Ghaffari et al. reported that trapping CUR in a polyamidoamine (PAMAM) dendrimer improved its solubility and bioavailability, and a polyplex was formed to build PAMAM-Cur/Bcl-2 siRNA nanoparticles by grafting Bcl-2 siRNA to the amine surface groups. Higher cytotoxicity than PAMAM-CUR and free CUR were seen (Ghaffari et al., 2020). Bhatt et al. have synthesized octa-arginine (R) and vitamin-E succinate (VES), conjugated to the PEGylated generation 4 polyamidoamine dendrimer (D) to form delivery carrier (RVES-PD). The efficacy of RVES-PD-paclitaxel antitumor in vivo was determined using tumor-bearing B16F10 mice. The cytotoxicity study showed that in the paclitaxel range of concentrations of 0–50μg/mL, RVES-PD-paclitaxel showed high cytotoxicity of paclitaxel compared to nontargeted VES-PD- paclitaxel and free paclitaxel (Bhatt et al., 2020).

In vivo Toxicity of Polymeric Nanomaterials

Chitosans

Zafar et al. demonstrated enhanced chemotherapeutic efficacy of chitosan-grafted lipid nano-capsules beside resistant human breast cancer cells with co-delivery of docetaxel and thymoquinone. They found that antiangiogenic activity in vivo was assessed using chick embryo chorioallantoic membrane (CAM) assay, which showed a superior antiangiogenic effect (Zafar et al., 2020). In order to increase water solubility and bioavailability, CUR was encapsulated by liposomes

(CUR-Lip) that were further coated with thiolated chitosan (CSSH) to form liposomal hydrogels (CSSH/CUR-Lip gel). The in vivo experiment demonstrated that the CSSH/CUR-Lip gel prevents the BC from recurring after tumor resection and repairs the injured cells in the CSSH/CUR-Lip gel group (Li et al., 2020b).

Poly(lactic-co-glycolic acid)

Gracia et al. developed PLGA curcumin and tested it in various subcutaneous models of prostate cancer xenograft in nude mice (PC3, 22rv1, and DU145 PCa cell lines). Their findings contrasted with those of immunohistochemical study (Trichromic, Ki67 and TUNEL stainings) assessed by tumor progression of the commercial preparation of curcumin (Gracia et al., 2019). Malathi et al. (2020) developed the nanopatterned PLGA films using cast molding technique based on polydimethylsiloxane (PDMS). In vivo analysis in the skin papilloma cancer Swiss albino mice model was used to find the therapeutic efficacy of 1000 succinate (TPGS) combinations with curcumin, and tocopherol poly(ethylene glycol) was explored. Godara et al. (2020) prepared and characterized the lipid-PLGA hybrid paclitaxel nanoparticles using a single-phase nanoprecipitation process. In vivo experiments found that when compared to nanoparticles without a lipid coat, lipid-coated human serum albumin-based nanoparticles showed extended circulation period.

Poly(amidoamine) Dendrimers

Montazerabadi et al. (2019) developed third-generation dendrimers by using methoxy-PEGylated poly(amidoamine) along with curcumin and iron oxide nanoparticles. Chen et al. (2004) reported that more cytotoxic and hemolytic than anionic or PEGylated dendrimers were found to be cationic dendrimers. Patel et al. (2016) suggested that sialic acid, glucosamine, and concanavalin A can be used as ligands to incorporate poly(propyleneimine) dendrimers to improve anticancer drug delivery to the brain for improved therapeutic outcome. Anti-ovarian cancer activity of poly(propyleneimine) antibody conjugates containing encapsulated paclitaxel was reported in vivo by Jain et al. (2015). Rompicharla et al. have demonstrated the effective targeting of paclitaxel in cancer using biotin-functionalized PEGylated poly(amidoamine) dendrimer conjugation (Rompicharla et al., 2019).

Toxicity of Metallic Nanoparticles

In vitro Toxicity Studies of Gold Nanoparticles

Gold as a nanomaterial has been extensively researched in recent years due to its use in various biomedical applications. In general, gold in bulk form is known to be an inert material, but the literature is not consistent in the case of nanoparticles, and some studies highlight the significance of nanometer size in the biological effects observed. In the documented in vitro experiments, the most common approach was to evaluate cytotoxicity after exposure to gold nanoparticles of different sizes and, at times, most frequently between 3 and 24 hours, using the MTT assay (Dykman & Khlebtsov, 2011). Several studies have reported biocompatibility of the gold nanoparticles (Sulaiman et al., 2020) (Table 9.5).

Toxicity Effects of TiO_2 Nanoparticles

In vitro Toxicity Studies of TiO_2

A broad variety of experiments using different cell models were performed to test the in vitro toxicity of TiO2 nanoparticles. Several studies examine toxic effects but at very high doses as well (Kose et al., 2020). Ekstrand-Hammarström et al. (2012) compared the toxicity of various TiO2 nanoparticles in normal human bronchial epithelial (NHBE) cells and epithelial cell lines (A549 and BEAS-2B). Carbon black and titanium dioxide nanoparticles (15 nm) have been shown to cause apoptotic cell death in bronchial epithelial cells, according to Hussain et al. (2010). Several experiments have stated that TiO_2 nanoparticles can cause damage to DNA using, e.g., the comet assay, but results from some experiments are negative or indicate only effects when UV light is present (Karlsson, 2010). TiO_2 nanoparticles can cause inflammation, damage to the respiratory tract, fibrosis, and lung tumors and can be carcinogenic to humans. While cancer is a disease involving mutation, several studies on the genotoxicity of TiO_2 nanoparticles have been conducted (Chen et al., 2014).

In vivo Toxicity Studies of TiO_2

Oberdörster et al. (Riediker et al., 2019) explored that the chances of primary particle size could influence the fate of particles after they are dumped and cause health issues. Pulmonary and oral toxicity studies have explored the influence of surface modifications and particle size through in vivo (Warheit & Brown, 2019). In summary, DNA disruption and cytotoxic effects have been seen in vitro studies of TiO_2

Table 9.5 Biosynthesized nanoparticles mediated from natural sources on toxicity analysis

Biological source and family	Metal	Shape and size	Cell line	IC_{50}	Tests	Ref
Vitis vinifera (Vitaceae)	Gold	Spherical (20–45)	HBL- 100	NA	Induction of apoptosis	Amarnath et al. (2011)
Couroupita guianensis (Lecythidaceae)	Gold	Spherical, triangular, tetragonal, pentagonal with irregular (7–48)	HL-60	5.14μM	MTT assay, DNA fragmentation, apoptosis by DAPI staining, and comet assay for DNA damage	Geetha et al. (2013)
Dysosma pleiantha (Berberidaceae)	Gold	Spherical (127)	HT1080	200μM	Cell viability using trypan blue exclusion assay, actin and (4,6-diamidino-2-phenylindole dihydrochloride) DAPI staining, transwell migration assay, Rac1-GTP assay	Karuppaiya et al. (2013)
Cajanus cajan (Fabaceae)	Gold	Spherical (29)	HepG2	6μg/mL	MTT, annexin-V/PI double-staining assay, cell cycle, comet assay, and flow cytometric analysis for apoptosis	Ashokkumar et al. (2014)
Punica granatum (Punicaceae)	Gold	Spherical (5–2)	Hela	62.5μg/mL	MTT assay	Lokina et al. (2014)
Corallina officinalis (Corallinaceae)	Gold	Spherical (14.57 ± 1)	MCF-7	NA	Cytotoxic activity, DNA fragmentation assay	El-Kassas & El-Sheekh (2014)
Podophyllum hexandrum (Berberidaceae)	Gold	Spherical (5–35)	Hela	20μg/mL	Cell viability, apoptosis	Jeyaraj et al. (2014)

Acalypha indica Linn (Euphorbiaceae)	Gold	Spherical (20–30)	MDAMB-231	NA	MTT assay, acridine orange/ethidium bromide dual staining, Caspase-3 assay, DNA fragmentation assay	Krishnaraj et al. (2014)
Cassia auriculata (Caesalpiniaceae)	Gold	Spherical (21)	A549	10μg/mL	Cytotoxicity, DNA fragmentation	Parveen & Rao (2015)
Illicium verum (Illiciaceae)	Gold	Hexagon (20–50)	A549	NA	Cell viability and cytotoxicity, oxidative stress, Caspase-3–7 levels	Sathishkumar et al. (2015)
Antigonon leptopus (Polygonaceae)	Gold	Spherical (22)	MCF-7	(GI50 = 257.8μg/mL)	Cytotoxic activity using MTT assay, DPPH free radical scavenging assay	Balasubramani et al. (2015)
Plumbago zeylanica (Plumbaginaceae)	Gold	Spherical (16)	DAL cell line	NA	MTT assay, DNA binding studies	Velammal et al. (2016)
Nigella sativa (Ranunculaceae)	Gold	Spherical (15–28)	A549	28.37μg/mL	MTT assay	Manju et al. (2016)
Genipa americana (Rubiaceae)	Gold	Spherical (15–40)	A549	NA	Cancer cell proliferation, MTT assay	Kumar et al. (2016)
Hibiscus sabdariffa (Malvaceae)	Gold	Spherical (10–60)	U87	1.5 ng/mL	MTT assay, analysis of cellular DNA degradation, degradation of glyceraldehyde-3-phosphate dehydrogenase	Mishra et al. (2016)
Rhus chinensis (Anacardiaceae)	Gold	Oval and spherical (20–40)	Hep3B MG63 MKN 28	150μg/mL	Antiproliferative assay, immunofluorescence staining	Patil et al. (2017)

(continued)

Table 9.5 (continued)

Biological source and family	Metal	Shape and size	Cell line	IC_{50}	Tests	Ref
Commelina nudiflora (Commelinaceae)	Gold	Spherical, triangular (24–150)	HCT116	200μg/mL	MTT, flow cytometry, and reverse transcription polymerase chain reaction	Kuppusamy et al. (2016)
Abutilon indicum (Malvaceae)	Gold	Spherical (1–20)	HT29	24 hours/210μg/mL 48 hours/180μg/mL	MTT assay, acridine orange/ethidium bromide (AO/EB) staining, annexinV-Cy3 staining, DAPI staining	Mata et al. (2016)
Tribulus terrestris (*Zygophyllaceae*)	Gold	Triangular, spherical (7)	AGS	NA	Cell viability assay by annexin-V/propidium iodide (PI) staining	Gopinath et al. (2019)
Sesuvium portulacastrum L. (Aizoaceae)	Gold	Spherical (37)	A549	14μg/mL	Cytotoxic assay, acridine orange/ethidium bromide (AO/EB) double-staining assay, DCFH-DA staining, rhodamine staining, DAPI staining	Ramalingam et al. (2016)
Musa paradisiaca (Musaceae)	Gold	Spherical (8)	MCF-7	24 hours <8μg/mL 48 hours/8μg/mL	Cell viability assay, cell cycle analysis and comet assay, apoptosis detection by dual-staining assay	Suganya et al. (2016)
			MDAMB-231	24 hours <2μg/mL 48 hours/2μg/mL		
Phoenix dactylifera (Arecaceae)	Gold	Spherical (95)	MCF-7	4.76μg/mL	Cytotoxicity, apoptosis, immunoassay	Banu et al. (2018)
Alternanthera bettzickiana (Amaranthaceae)	Gold	Spherical, aggregated (80–120)	A549	NA	MTT assay, DNA fragmentation study, protein assay, nuclear staining, Western blotting	Nagalingam et al. (2018)

Solidago canadensis (Asteraceae)	Gold	Spherical, triangular, and rod-like shape (238)	H4IIE-*luc* rat hepatoma cells. HuTu-80 cells	NA	Cytotoxicity assay	Botha et al. (2019)
Dragon fruit	Gold	Spherical, oval, and triangular (10–20)	MCF-7/ MDA-MB-231	NA	Cytotoxicity study	Divakaran et al. (2019)
Gelidium pusillum (Gelidiaceae)	Gold	Spherical to pseudo-spherical-shaped (12)	(MDA-MB-231)	43.09 ± 1.6μg/mL	MTT assay, apoptotic staining	Jeyarani et al. (2020)
Commiphora wightii (Burseraceae)	Gold	Spherical, triangular, and hexagonal (27.91)	MCF-7	66.11μg/mL	MTT assay	Uzma et al. (2020)
Annona muricata (Annonaceae)	Gold	Spherical (15)	Hep2	10.94μg/mL	Cytotoxicity	Kamala & Iyer (2020)
Ecklonia cava (Lessoniaceae)	Silver	Spherical (15–30)	HeLa	59μg/mL	Cytotoxicity assay, annexin V-FITC/propidium iodide (PI) staining	Venkatesan et al. (2016)
Citrullus colocynthis (L.) (Cucurbitaceae)	Silver	Spherical (31)	HEp-2 cell	3.42μg/mL	MTT assay, caspase −3 assay, lactate dehydrogenase leakage assay, and DNA fragmentation assay	Satyavani et al. (2011)
Artemisia princeps (Asteraceae)	Silver	Spherical (20)	A549	30μg/mL	DNA fragmentation, cell viability of L132 and A549 cells	Gurunathan et al. (2015)

(continued)

Table 9.5 (continued)

Biological source and family	Metal	Shape and size	Cell line	IC_{50}	Tests	Ref
Trapa natans (Lythraceae)	Silver	Spherical (30–90)	A431	64.2μg/mL	MTT assay	Saber et al. (2018)
Nepeta deflersiana (Lamiaceae)	Silver	Spherical (33)	HeLa cells	5μg/mL	MTT assay, cytotoxicity by neutral red uptake (NRU) assay, apoptosis/necrosis assessment using annexin V-PE and 7-AAD	Al-Sheddi et al. (2018)
Punica granatum (Punicaceae)	Silver	Spherical (30)	A549	54.5μg/mL	MTT assay, apoptotic assays, Caspase assay	Padinjarathil et al. (2018)
Abutilon indicum (Malvaceae)	Silver	Spherical (5–25)	COLO205	4μg/mL	MTT assay	Mata et al. (2015)
Gracilaria edulis (Gracilariaceae)	Silver	Spherical (55–99)	PC3	39.60μg/mL	MTT assay	Priyadharshini et al. (2014)
Phyllanthus emblica (Phyllanthaceae)	Silver	Spherical and cubic (188)	HEp-2 cell	20μg/mL	Cytotoxicity assay	Rosarin et al. (2013)
Cyperus conglomeratus (Cyperaceae)	Silver	Spherical (70–100)	MCF-7	5μg/mL	MTT Assay, V-FITC-PI double-staining kit, and real-time PCR for apoptotic gene	Al-Nuairi et al. (2020)
Phaseolus vulgaris (Fabaceae)	Copper oxide	Spherical (26.6)	HeLa	NA	Sulforhodamine-B assay	Nagajyothi et al. (2017)
Zingiber officinale (Zingiberaceae)/ *Allium sativum* (Amaryllidaceae)	Copper	Spherical (22.70 ± 5.67)	HeLa and HepG2	NA	MTT assay	Yaqub et al. (2020)

Trichoderma asperellum (Hypocreaceae)	Copper	Spherical (110)	A 549	24.7μg/mL	Cell viability, DAPI and Rh123 staining apoptosis, Western blot analysis	Saravanakumar et al. (2019)
Ficus religiosa (Moraceae)	Copper oxide	Spherical (577)	A 549	200μg/mL	MTT assay, apoptosis	Sankar et al. (2014)
Syzygium alternifolium (Wt.) (Myrtaceae)	Copper oxide	Spherical (17.2)	MDA-MB-231	NA	MTT assay	Yugandhar et al. (2017)
Olea europaea (Oleaceae)	Copper and copper oxide	Spherical (20–50)	SKOV-3	2.27μg/mL	Cell viability assay, clonogenicity assay, cytotoxicity using crystal violet dye, mitochondrial membrane potential assay	Sulaiman et al. (2018)
			AMJ-13	1.47μg/mL		
Azadirachta indica (Meliaceae), *Hibiscus rosa-sinensis* (Malvaceae), *Murraya koenigii* (Rutaceae), *Moringa oleifera* (Moringaceae), and *Tamarindus indica* (Fabaceae)	Copper and copper oxide	Spherical (12)	MCF-7	25.55 ± 1.96, 22.45 ± 1.32,25.32 ± 1.82, 26.71 ± 1.24,19.77 ± 0.98	MTT assay, apoptosis by Hoechst 33258 staining assay	Rehana et al. (2017)
			HeLa	26.73 ± 1.67, 21.63 ± 1.44,23.22 ± 1.36, 30.08 ± 1.84, 20.32 ± 1.16		
			Hep-2	28.59 ± 2.08, 22.59 ± 1.96, 25.59 ± 1.61, 29.58 ± 1.34, 21.66 ± 1.22		
			A549	26.03 ± 1.84, 20.15 ± 1.22, 25.05 ± 1.31, 34.37 ± 1.81, 18.11 ± 0.93		
			NHDF	>100		
Nigella sativa L.	Pt	Spherical (1–6)	MDA-MB-231	36.86μg/mL	Cytotoxicity study	Aygun et al. (2020)
			HeLa	19.83μg/mL		

(continued)

Table 9.5 (continued)

Biological source and family	Metal	Shape and size	Cell line	IC_{50}	Tests	Ref
Punica granatum	Pt	Spherical (20–100)	MCF-7	17.84μg/mL	Cell viability test, propidium iodide staining, comet assay	Şahin et al. (2018)
Padina gymnospora	Pt	Truncated octahedral, tetrahedral, and spherical (25)	*Artemia salina* nauplii	(LC_{50}) 100 ± 4 mg/mL	Anti-crustacean assay	Ramkumar et al. (2017)
Mentha piperita	Pt	Spherical (54.3)	HCT 116	20μg/mL	Cytotoxicity assay	Yang et al. (2017)

nanoparticles but only at very high doses. Inflammatory and genotoxic effects are shown in animal studies, indicating that inhalation should be avoided, while exposure to the skin (e.g., TiO_2 with low photocatalytic activity) is unlikely to pose much risk (Table 9.6).

Regulation Perspectives of Nanomaterial Toxicity

Nanotechnology has emerged over the previous years, and currently, its revolution has been utilized in multiple sectors through an integrated approach. Moreover, increases in the number of products containing nanomaterials and nanotechnology-based applications have become widely available. The pharmaceutical field isn't an exception in this revolution, where nanotechnology is tremendously involved in the drug development process with the capability of providing a new and innovative medical solution to fulfill the current existence gap in healthcare (Bleeker et al., 2013; Perez de la, 2014).

Definition and Regulation Concerns for the Nanomaterial's Characterizations

The use of nanotechnology applications in medicine is referred to as nanomedicine, which is defined as the use of nanomaterials for prevention, diagnosis, treatment, and monitoring of diseases. However, still, there is a controversy regarding the uniform and standard definition of nanomaterials among the different international regulatory agencies. Therefore, several efforts took place to find a more relevant descriptive definition of nanomaterials, since nanomaterials have very small particle sizes with novel physicochemical properties which are different from the bulk chemical equivalent materials (Tinkle et al., 2014). Also, it is necessary to establish a definition that explicitly describes the presence of nanomaterials. Indeed, a definition to distinguish whether the material is considered as nanomaterial or not was created by the European Commission (EC); however, the EC definition was not further categorized as the present nanomaterial hazardous or safe. The US FDA has considered nanomaterials as any materials within the range of nanoscale or certain materials that pose related dimension-dependent characteristics. On the other hand, it recognizes nanotechnology products as products that consist of or are manufactured using nanomaterials (Bleeker et al., 2013; Guidance, 2011). As per the EC, nanomaterials refer to a natural, adjuvant, or manufactured material containing particles, either in an unbound state or as an aggregate with a size range of 1–100 nm for ≥50% of the particles according to the number size distribution. Materials whose surface area by volume is more than 60 m^2/cm^3 as well as material with one or more external dimension structure below 1 nm, like single-walled carbon nanotubes and

Table 9.6 Toxicity studies of TiO_2 nanoparticles mediated from plant sources

Biological source	Formulations	Shape and size	Cell line	IC_{50}	Assays	Reference
Aloe vera	Green hydrothermal synthesized Ag@ TiO_2 nanoparticles	Rod-shaped and small spherical. 38 and 57 nm	A549	115μg/mL	MTT cell viability assay, acridine orange/ethidium bromide (AO/EtBr) staining, dichlorodihydrofluorescein diacetate (DCFH-DA) assay	Hariharan et al. (2020)
Withania somnifera, *Eclipta prostrata*, *Glycyrrhiza glabra*	Biomodified TiO_2 nanoparticles -hydrothermal method	Rod and spherical. 7.5 nm, 9.5 nm, 12.5 nm, 11.5 nm	KB oral cancer cell line	NA	MTT assay	Maheswari et al. (2020)
Ledebouria revoluta	Bioengineered TiO_2 nanoparticles-green synthesis	Tetragonal structure and spherical. 47 nm	A549	53.65μg/mL. LC_{50} (18.960 mg/mL)	MTT assay	Aswini et al. (2020)
Rheum emodi	TiO_2 nanoparticles	Crystalline 45 nm	HepG2	400μg/mL	MTT assay	Sharma et al. (2018)

fullerenes, should be recognized as nanomaterials (Commission Recommendation, 2011).

Overall, with respect to the former definition of nanomaterials, there are three fundamental components to determine the presence of nanomaterials:

I. *Size:* It's one of the important aspects to be taken into consideration while determining the presence of nanomaterials. Though 1–100 nm is the conventional size range of nanomaterials, however, there is no clear cutoff line to set this limit. Additionally, the maximum size that should be used to consider the material as nanomaterial is a random value, because the biological and physicochemical properties of the material do not change randomly at 100 nm; therefore, with respect to this context, it is assumed that other properties also should be considered.
II. *Particle size distribution (PSD):* The PSD reflects the material size variation, and it's one of the largely used parameters for distinguishing the nanomaterials from other bulk chemical equivalent materials since nanomaterials are usually exhibiting the polydisperse properties.
III. *Surface area:* Determining the surface area by volume is additionally strengthening the legislation of the nanomaterial's regulation. The defined limit of surface area by volume for considering the material as nanomaterials is more than 60 m^2/cm^3; however, the PSD shall prevail, even though the material surface area by volume is lower than 60 m^2/cm^3 (Bleeker et al., 2013; Boverhof et al., 2015; Lövestam et al., 2010).

The Regulation Challenge for the Nanomaterials with Respect to the Pharmaceutical Context

The novel physicochemical properties of nanomaterials provided great opportunities in the majority of the drug development process. However, some of the concerns regarding their safety aspects were raised, predominantly, the nano-formulation safety concern, which can result in alteration in the pharmacokinetic parameters including the absorption, distribution metabolism, and elimination. Moreover, the ability to cross biological barriers more easily, their toxic characteristics, and their prolonged presence in the human body and environment are some examples of concerns over the involvement of nanomaterials in pharmaceutical files (Bleeker et al., 2013; Perez de la, 2014; Tinkle et al., 2014; Guidance, 2011). These nano-formulation alterations and other safety concerns are posing a significant challenge in pharmaceutical development and manufacturing processes, primarily in the identification of the critical parameters and technologies for analyzing and evaluating the safety aspects of nanomedicine (Hodge et al., 2010; Gaspar et al., 2014; Sainz et al., 2015). Quality by Design (QbD), in conjunction with process analytical technology (PAT), is one of the important systems recognized for the systematic regulation and control of nanomaterials involved in pharmaceutical drug development

processes. Quality by Design work is mainly based on the identification of quality attribute (QA), which is known as the biological, physical, and chemical, or any other relevant properties of nanomaterials (U.S Food and Drug Administration, 2004; European Medicines Agency, 2017). It is important to note that these nanomaterials properties may be altered during and/or after the manufacturing processes; such modifications are considered as QA and should be controlled with a certain range of quality (Verma et al., 2009; Riley & Li, 2011; Bastogne, 2017). PAT is playing a major role in evaluating and controlling the QA during the manufacturing process by designing, analyzing, and controlling the attributed performance and quality of raw materials and intermediated and finished products, which eventually lead to ensuring the safety of any high-quality nanomedicine product.

Nanotoxicology and Biocompatibility

Appropriate compatibility between the biological environment and the administered drug is one of the essential requirements in the design of a safe and effective drug delivery system. Biocompatibility refers to the capability of a material to produce an appropriate effect on the targeted organ without triggering undesired response (Williams, 2003; Keck & Müller, 2013). Therefore, ensuring appropriate biocompatibility during the nanomedicine manufacturing process through certain biocompatibility testing program at the preclinical level is crucial for safe and effective nanomedicine; on the other hand, if biocompatibility is not granted nanomaterials, then it's inevitable that toxicological concern arises (Hussain et al., 2015). The biocompatibility assessment at the preclinical testing level for the nanomaterials involved in vivo studies complemented by certain in vitro assay to prove safety. Despite the efforts made from different pharmaceutical regulatory agencies, academia, and other governmental bodies to develop guidelines and protocols for short- and long-term nanotoxicological assessments, still, nanomaterials are handled as conventional chemical materials. However, several initiatives from the FDA, OECD, and EC scientific projects emphasize on the regulatory aspects concerning the safety of nanomaterials. Identifying various physicochemical properties of nanomaterials and correlating them with the effect on the organs are important for a better understanding of how certainly nanomaterials interact with the living system (Juillerat-Jeanneret et al., 2015).

Surface physicochemical properties, shape, and size of nanomaterials play a major role in the pharmacokinetics of nanomaterials, particularly the distribution of nanoparticles in the body. For example, the large nanoparticles were found distributed in the liver and blood, whereas nanoparticles with 10 nm size were detected in the heart, kidney, blood, liver, spleen, thymus, and testis (De Jong et al., 2008; Adabi et al., 2017). Note that the serum proteins available in the systemic blood circulation influence the cellular uptake of the nanoparticles; therefore, considering an assessment of protein profile through in vivo studies is required crucially for

ensuring safe and effective nanoparticle concentration index at the cellular level (Keck et al., 2013).

The clearance of nanomaterials is relying upon the surface and size of nanoparticles, for example, large size nanoparticles more than 200 nm are uptaken by the liver, spleen, and bone marrow through the mononuclear phagocytic system, whereas small-sized nanoparticles below 20–30 nm are rapidly excreted through the renal system (Moghimi et al., 2001). However, still, studies are required to certainly explain the absorption, biodistribution, degradation, and elimination of nanoparticles in the living systems.

In vitro Assessment Methods of Nanotoxicology

The in vitro assay approach was one of the important methods for evaluating the toxic effects of nanoparticles, which includes the studies of cytotoxicity potency of nanoparticles through providing detailed information about the biological interaction between the cells and nanoparticles including the inflammatory responses, cell viability, and stress (Kroll et al., 2009). The major merits of in vitro assay include less time consumption, not expensive, and minimum ethical concerns. However, the main drawbacks of in vitro experiments are lacking the ability to reproduce the entire complex and interrelated mechanism at the cell, tissues, and membranes of the human body; moreover, in vitro assay also cannot predict the compensatory response of the human body when it's imposed with a toxic effect. Nevertheless, the possible interaction between the reagents used in the in vitro assay and the nanoparticles can lead to significant changes in the physicochemical properties of the nanoparticles and consequently influence the adsorption, biodistribution, dissolution, and PH of the nanoparticles (Kroll et al., 2009; Fadeel et al., 2013). Therefore, many concerns have been raised about the integrity and harmony of nanotoxicological data produced by the in vitro assay. Novel approaches for consistent evaluation of nanoparticle cytotoxicity are, hence, demanded. In this regard, new models like silicon nanotoxicology have emerged, which has combined the power of information technology (computational tools) and biostatistics tools to provide more accurate and various possible nanotoxicological pathways (Warheit, 2008; Raunio, 2011).

In vivo Assessment Methods of Nanotoxicology

The in vivo experimental approach involves evaluating the nanotoxicity in the animal models. Mainly, the in vivo studies evaluate the pharmacokinetic and pharmacodynamic parameters of nanoparticles through assessing the biodistribution, cytotoxicity, metabolism, and excretion of rats or mice imposed with nanoparticles. In the biodistribution studies, the determination of various allocation pathways of the nanoparticles to the organ cell and tissue is carried out through radiolabel

technique, which detects the presence or absence of the nanoparticles in living or dead animals (Kim et al., 2001). Another method for in vivo assessment is evaluating metabolism, excretion, and changes in serum chemistry profile at various time points after nanoparticle exposure (Li et al., 2001; Baker et al., 2008). The cytotoxicity level in cells imposed by nanoparticles can be also determined by histopathology of organ cells and tissues using an advanced technique such as microfluids and micro-electrochemistry (Zhu et al., 2008; Ewing et al., 1983).

General Evaluation Methods of Nanotoxicology

Several evaluation methods exist to find out the impact of nanoparticles toxic effects on the organism of living systems, including:

Colony Forming Efficiency (CFE) Assay or Plate Count Method

The biological toxic effect of the nanomaterials can be evaluated by counting the number of the cells prior and post-exposure of treatment with nanoparticles. It determines the capability of the surviving cells to produce colonies, and the counting process takes place using a plate reader, microscope, or naked-eye approach. The cells are diluted first prior to counting the cell numbers before and after exposure to the nanoparticle treatment, and the results are presented as a relative ratio. Reduction in the number of formed colonies reveals cytotoxic effects, whereas a decrease in colony size indicates cytostatic effects. The CFE assay method for counting the formed colonies may sometimes underestimate the actual number of living cells since bacteria can replicate in clumps or chains. Indeed, when using the microscopic approach, the number may be overestimated, because it takes into account both living and dying cells. Therefore, there is a controversial concern when determining the nanoparticles' biological effects through the microscopic approach. However, the antibacterial activity was evaluated using the plate count method for copper nanoparticles, zinc oxide nanoparticles, and silver nanoparticles (Kumar et al., 2017; Mohamed et al., 2017; Lv et al., 2018; Orou et al., 2018).

Optical Density (OD)

The safety and efficacy of antibacterial consist of nanomaterials can also be evaluated using ultraviolet-visible spectroscopy at 600 nm OD. The amount of scattered light from the cell determine the antibacterial activity of the nanoparticles. A reference solution consisting of a mixture of sterilized water plus bacterial solution has to be compared to the sample solution at OD600 carried out under similar incubation conditions to determine the bacterial growth rate under the exposure to nanoparticles. Various nanoparticles, such as metal complexes, Ag nanoparticles, and

BaTiO3, and their effects on the bacterial growth rate of *E. coli* were studied using the OD technique (Qiao et al., 2019; Alshareef et al., 2017; Shah et al., 2018; Liu et al., 2018).

Adenosine Triphosphate (ATP) Cell Viability Assay

The living cells gain their energy in the form of ATP; therefore, measuring the ATP generated by bacteria incubated with nanoparticle solution helps in evaluating nanotoxicology. Almost all of the ATP is constant in the cell, which by default adds the advantage of more accuracy in terms of quantitative measurements of ATP amount changes, which reflect the estimation of the present living bacteria in the sample. The measurement of ATP is considered by the generated light from the conversion of D-luciferin to oxyluciferin via luciferase. This generated light is quantified by using luminometer and expressed as relative light unit (RLU)/mol of ATP, which has a linear relationship with the cell viability; hence, it is used for evaluating the nanotoxicology impact (Kumar et al., 2017). There are several other testing techniques used for evaluating the cytotoxicity of nanoparticles, such as the omics method, fluorescence microscope, solid-state nuclear magnetic resonance studies, oxidative stress, apoptosis, proliferation, and necrosis assays (Soares et al., 2018; Lin et al., 2011; Greish et al., 2012; Perovic, n.d.).

Conclusion and Future Perspectives

Using bionanomaterials as nanoparticles in the anticancer drug delivery mechanism continues to be a significant field of future nano-pharmaceutical studies. It is clear that bionanomaterials will bring many developments in drug delivery and will continue to draw interest from this field's researchers. Extensive study has been carried out concerning nanosized drug carriers based on natural and synthetic polymers. The use of biomaterial nanoparticles as drug carriers has been expanded to help in the delivery of antibiotics, anticancer medications, vaccines, and genomes. Not only do biomaterials with special characteristics such as biocompatibility, biodegradability, and nontoxicity have a vast range of applications, but they are also capable of being modified and functionalized to meet those criteria. These particles can be modified to have unique properties to assist them in successful drug delivery and release of drugs, such as improved bioavailability, active targeting of the site of administration by receptor recognition, and reduced recognition by the immune system. Several studies have been performed in the case of tumor targeting and anticancer drug delivery via nanocarriers from biomaterials. Development of large-scale manufacturing processes to prepare large quantities of nanoparticles is needed. These manufacturing methods ought to be cost-effective and time-efficient and allow the control of particle size and surface properties, directly influencing drug release. Despite developments made in research on drug carriers with polymeric

nanoparticles, in vivo testing is required to help resolve the challenges that have been faced in in vitro research and to expand the findings and knowledge that have been collected so far. It should really be noted that while these drug delivery systems show positive reports in in vitro studies, they may fail in vivo trials. In vivo and clinical trial research can help to much better understand and address the mechanisms for drug targeting and drug uptake. In conclusion, despite the major advances shown in recent findings, a lot of problems and obstacles must still be overcome and answered.

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Chapter 10
Nanoerythrosome-Biohybrid Microswimmers for Cancer Theranostics Cargo Delivery

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Introduction

Cancer causes about ten million deaths in 2018, and globally it is the second leading cause of cardiovascular disease. Malignant tumor was invigorated by the alteration of precancerous lesion which develops cancer as multiplex processes. Cancerous cells multiply and grow to other organs in the body, called metastases. For the survival of patients and to reduce the medication cost, it requires earlier observation of cancer and treatment (WHO, 2018). In spite of the notable developments in cancer treatment, particular side effects of chemotherapy and radiotherapy have yet to be discovered (Misra et al., 2010). Based on this matter, scientists put enormous results to evolve new nanomedication at the molecular level to treat cancer (Wu et al., 2015a; Zhang et al., 2019). Compared to free drugs, nanomedicines show high delivery efficiency, efficient retention time, lesser side effects, and prolonged circulation time (Gandhali, 2016). So, this encourages research related to nanoparticles, which would help to discover abnormalities or as carriers to transport drugs to the desired cell or as therapeutic means (Bharali et al., 2009). The concentration of

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enhanced drugs found in cells with traces of cancer was functionalized by targeting ligands, enclosing multidrug molecules and detouring ancestral drug resistance. This helps to encapsulate both the medicinal and diagnostic agents as a unified particle (Cao et al., 2017).

The distribution of nanoparticles, identification and isolation of toxic content, initiation of clearance by the renal system, and maintenance of the mononuclear phagocytic system (MPS) are biological barriers still faced by cancer nanomedicines. The phagocytic cell mediator system gets rid of nanoparticles in the liver and also ejects the hydrodynamic diameter of nanoparticles less than 5.5 nm, which is observed within the kidney (Liu et al., 2013a). Studies disclose about 0.7% nanoparticle is injected and delivered to solid tumors (Wilhelm et al., 2016). Encapsulation of various bioactive compounds (enzymes, peptides, toxins, genetic materials), ample cells in the human body, and long blood circulation up to 120 days (40–50% of blood level) used to deliver drugs were observed as the major properties of RBC. The enhancement of cancer cell membrane coating drug delivery efficacy requires surface refunctionalization of erythrosome-based nanoparticles with target moieties (Xu et al., 2018). The nanoerythrosome drug loading method requires ultimate care in handling and is also based on its type of medium. The hypotonic solution-mediated lysis (hemolysis) was used to remove the cytoplasmic content observed in the cells. This should be performed in a controlled way to load the required therapeutic agents exactly into erythrocytes and hemoglobin (Lieber & Steck, 1989). Seamless nanosized capsules should be formed in plasma membranes without disruption during the hemolysis process. One erythrosome can be fragmented into 4000–5000 nanoerythrosome, all depending on cell recovery after stripping the cell off its organelles.

Different techniques like sonication, extrusion, and electric pulses are handed down for the preparation of nanoerythrosomes from hemoglobin-free erythrocyte ghosts (Schwoch & Passow, 1973). Uniform nanoerythrosomes with minimal cell loss are achieved by extrusion sizing method, which is the most efficient technique. The firmness and shelf-life of nanoerythrosomes formulation were supported by flexibility and fluidity, processing temperature, sizing technique, and combination of lysis medium of nanoerythrosomes. The usage of encapsulation and contrast agents was performed by biomimetic properties. The small molecular weight drugs conjugated with surface nanoerythrosomes were discovered, for example, daunorubicin and pyrimethamine (Villa et al., 2016).

Insight on Microswimmers Used for Anticancer Drug Cargo

The vital locomotion and cargo delivery to a particular site were attained by live mobile microorganisms as biohybrid microswimmers showed much growing interest in the past decades (Alapan et al., 2019). Cell-constructed biohybrid microswimmers have inherent abilities of sensing and motility, extract suitable response to unnatural and environmental changes, and engage in the passage of cargo to isolated

locations within the human body. This helps to confine proactive transportation of medicinal bioagents which could also be used for performing diagnostics and isolation (Nguyen et al., 2016). Ghost cell bodies and beads coupled with bacteria were commonly observed (Park et al., 2017). Due to some drawbacks, these microorganisms show case risk to generate infectious agents, which leads to tremendous growth of bacteria in the biological environment and probable resistance toward antibiotics. Development of biohybrid systems has enlarged when compared to slow-growing motile microorganisms and shows greater need for biocompatibility (Ceylan et al., 2017). *C. reinhardtii* microalga was used to design and identify biohybrid microswimmers due to its active delivery of therapeutics.

Chlamydomonas reinhardtii (single-cell microalga) is designed as a biomodel microorganism along with a polymer-nanoparticle complex as an artificial component to extend the manufacturing efficiency up to 90%. The nanoparticle is coated on the cell wall of microalga by chitosan, the biopolymer. The accommodation of biomedical delivery particles was engineered because the smooth external layer does not damage the practicable and phototactic potential of microalgae. Moreover, thin-coated chemotherapeutic doxorubicin conjugated with nanoparticles is embedded by a photocleavage linker on request to cargo of drugs to cancerous cells, showing evidence for therapeutic confirmation (Erkoc et al., 2019). For future medicinal active cargo delivery jobs, the high-throughput plan can cover the way for the next-generation microswimmer swarms. Well-defined techniques to construct RBCm that combine different covalent and non-covalent conjugation methodologies are used. This technique also includes methods to monitor proper lipid insertion and implement biotin-avidin bridge; few other metrics such as EDC/NHS coupling, ligand receptor conjugation, and antibody conjugation are also considered. In addition to the above techniques, passive absorption (hitchhiking) is also considered to measure it for theranostic purposes. The implementation of the abovementioned different techniques and their intercommunication is depicted in Fig. 10.1.

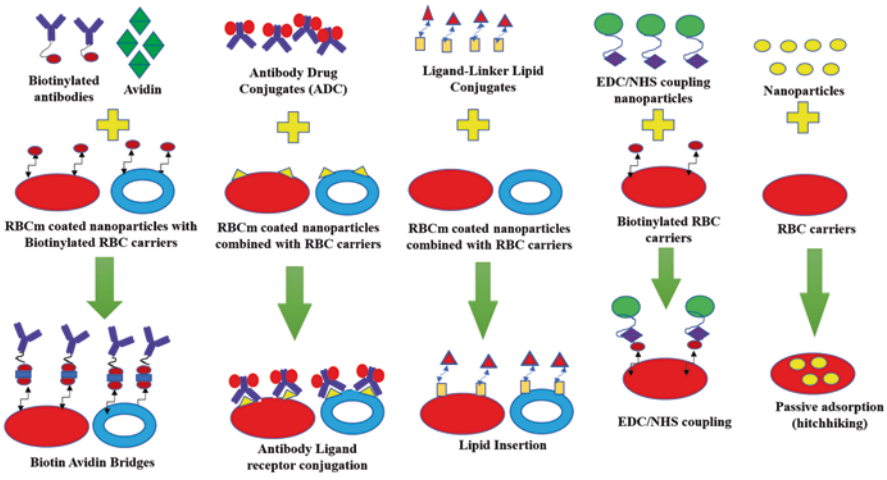

Fig. 10.1 Various patterns of operationalized erythrocyte construct for nanodrugs

Nanocarriers that work toward drug delivery both actively and passively such as polyethylene glycol (PEG) and polyethylene oxide (PEO) are copolymers adsorbed on the surface of nanoparticles with hydrophilic surfactants reduced by opsonization. In order to enhance the properties, the nanoparticle shows a high tailorability, and it can be easily used by ligands and other functional molecules. As a consequence, they constitute favorable and adaptable tools in cancer therapy and drug delivery (Stevanovic & Uskokovic, 2009). In vitro, the therapeutic payloads along with liposomes were coupled with magnetic iron oxide-producing magnetotactic bacteria (Taherkhani et al., 2014). In vivo, drug-loaded liposomes are delivered to the mouse tumor site using magnetic fields, which are guided by these modified bacteria (Felfoul et al., 2016) (Table 10.1). The combination of streptavidin genetically with altered *E. coli* substrain with nanoerythrosomes (biotin) was realized and fabricated with nanoerythrosome-functionalized biohybrid microswimmers. Recent advances in artificial biology made it possible to use bioengineered biohybrid microswimmers that do not have any inorganic constituents for delivering the biological cargo materials. The release of the constructed payload on the bacterial lysis was triggered to generate a therapeutic payload like alpha-hemolysin E. This is achieved by using genetically engineered bacteria, *S. typhimurium*. This is controlled and monitored such that it generates an activator/repressor to synchronize and control the cyclic continuous delivery process where this lysis event was overcome by these small numbers of bacteria (Din et al., 2016).

Table 10.1 Comparison of cargo delivery of biohybrid microswimmers with physical and chemical methods

Physiological function	Resource		
	Chemical method	Physical method	Biohybrid microswimmers
Pattern	20 μm Janus nanoparticle with MgTiO2 10 μm of Janus nanoparticle with CaCO3	300 μm of magnetic microrods 300 m of polymeric griper	1.2 μm bacteria combined with *S. typhimurium* 1 μm streptavidin-polystyrene nanoparticle with *L. monocytogenes*
Position of the stereotype (in vivo)	Stomach of mouse Liver of mouse	Rabbit and bile duct of pig	Colon of mouse and intraperitoneal cavity of mouse
Purpose	Cargo huge content of microswimmers Bleeding stops	Navigation through intraocular Biopsy (tissue)	Production of pore-forming toxin (α-hemolysin E) against tumor cells Gene expression using fluorescence imaging, delivering payload, and monitoring

Characterization and Design of a Biohybrid Microswimmer for Cancer Nanotheranostics

On the cell membrane of bacteria, the biotin acceptor peptides encode, and it is induced by isopropyl-beta-D-thiogalactopyranoside containing pOS233 plasmid in genetically engineered bacteria through autotransporter antigen 43 (Schauer et al., 2018). Green fluorescence protein expression was enhanced by using L-arabinose induced by bacterial species carrying another plasmid, which could be utilized to perform fluorescence detection in any bacteria. The induction of IPTG and L-arabinose is used to determine the growth characteristics of the bacterial cell and to investigate the growth rate of bacteria by observing a single colony upon infusion of plasmid to identify particular genes. Also, as the metabolic burden increased, these IPTG and L-arabinose growth rates decreased; further experiments were carried out to get better bacterial growth characteristics. To reach the OD value of 0.6 at OD 600, these genetically engineered bacterial cultures were incubated for 12–18 hours and then are pursued with IPTG. Biotin molecules are coated upon the cell membrane of bacteria; this was done after proper plasmid DNA genetic sequencing. Then, nanoerythrosome conjugation methods were performed after further incubation of streptavidin particles for 1 hour to modify the bacterial membrane. Anti-TER 19 antibodies combined with biotin (modified nanoerythrosomes) were realized for 1 hour for bacterial actuators in noninvasive conjugation with delivery carriers. The expression level of TER 119 proteins was high on mature erythrocyte membranes and is denoted as lineage markers (Kina et al., 2000).

The characterization of SEM analysis shows the perfect conjugation of genetically engineered bacteria-modified streptavidin with nanoerythrosomes. Fluorescence microscopy images demonstrate the fabrication of powered biohybrid microswimmers carried with nanoerythrosomes. The fabrication of nanoerythrosome was done in three steps. First by using a Percoll gradient assay, the RBCs were removed from murine blood; using centrifugation, the middle layer was taken, which is visualized as a dark red ring in the middle layer. Second, the dispersion of the cytoplasmic content was done by emptying RBCs using hypotonic-isotonic processes, which led in the formation of 100 nm pores on the cell membrane of RBCs (Delcea et al., 2012). In the last step, the cell extruder method is used as this emptied RBCs slightly moved through the polycarbonate membrane at 1 micrometer pore size (Fig. 10.2). The size and uniformity of surface-coated nanoerythrosomes were finalized by membrane pore size and extrusion speed (Ong et al., 2016). DLS analysis revealed polydisperse population with a maximum peak at 350 nm ranging from100 nm to 1 μm diameter, and these inhabitants with vesicle sizes smaller than the extracted pore size were noticed (Hu et al., 2013). Flow cytometry investigation shows more than 90% of the population are double-positive for TER 119 and CD47 proteins on the membrane, and fluorescence signals as green and red were given to differentiate both TER 119 and CD47 proteins, and staining procedures were performed for identification process. In addition, it is also observed that 2D motility characterization done on the free swimming bacteria that are genetically engineered and biohybrid microswimmers sowed the mean speed of 19.91 ± 9.37 μm/s and 14.06 ± 6.71 μm/s (Kim et al., 2009).

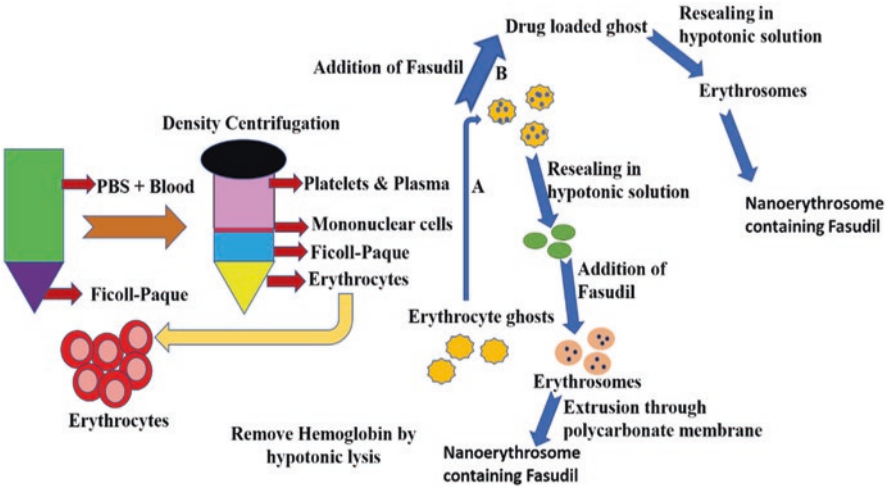

Fig. 10.2 Diagrammatic representation of hypotonic lysis method

Functionalization of Micro-/Nanoswimmers with Different Bioreceptors Toward Targeting Tumor Cells

Lipid Insertion

The presence of biological molecules on RBCm performed by natural carriers like RBC- non-disruptive functionalization process performs better when compared to chemical analysis methods. In lipid insertion process done by direct mixing or placing the alkyl chain or lipid part inserted into lipid bilayer at 37 °C room temperature, the unwanted section or debris of lipid conjugates were filtered by centrifugation (Fang et al., 2013). Ligand-linker-lipid conjugates insert surface proteins without any disturbances along with therapeutic molecules. It also facilitates RBCm to be made functional by performing and enhancing targeting processes and imaging processes (Hymel and Peterson 2012). The most cell surface modification was performed by ligand-linker-lipid couples through detectable poly(ethylene glycol) PEG-lipid conjugates (Zalipsky et al., 1999). While analyzing the various tumor types, the folate receptors are overexpressed, and DSPE-PEG-folate acts as an active cargo agent in cancer drug delivery. Although some side effects have been observed, folate-modified RBCm-cloaked nanoparticles express similar physicochemical properties (Rao et al., 2017). This large delivering ligand with MW of approximately 9000 Da conjugated to a smaller lipid anchor with MW of approximately 748 Da shows an excellent conjugation with RBCm (Fang et al., 2013).

Biotin-Avidin Bridges

Lipid insertion process and chemical alteration are used by biotin-avidin bridges, which utilize indirect surface engineering of RBCm. Biotinylated RBCm method-derived vesicle shows less damage than chemical modification, e.g., NHS-biotin or Sulfo-NHS-biotin. Four subunits show similarity in tetrameric avidin, which strongly binds the biotin. A rapid formation and stable bonding of non-covalent interaction between proteins and ligands shows about Kd = 10–15 mol/L value, which shows the strongest bond (Hofmann & Kiso, 1976). One hour at room temperature, this avidin/streptavidin was incubated and rinsed using PBS to destrain any unreacted traces of biotin (Sun et al., 2017). To select glioma and transport chemotherapeutic drugs, the tumor-targeting peptide c(RGDyK) is fixed with RBC-coated drug nanocrystals by lipid insertion method through biotin-avidin bridges (Chai et al., 2019).

EDC/NHS Coupling

An amine reactive O-acylisourea intermediate was formed by a carboxyl group merged with primary amines, which is mediated by 1-ethyl-3-3-dimethylaminopropyl--carbodiimide cross-linking substance that reacts with amide bond, giving the final product isourea, and unwanted and excessive things are removed by gel filtration process (Conde et al., 2014). Sulfo-NHS (N-hydroxysulfoxuccinimide) is used for active ester stabilization; while doing this, the hydrolysis reaction was optimized, and nanoparticle aggregation was prevented, and the pH and ratio of NHS/EDC controlled. UCNP nanoparticle-coated RBCs are utilized for fluorescence imaging during tumor surgery (Shen et al., 2009).

Antibody/Ligand-Receptor Conjugation

In order to evade the difficulties faced in ex vivo alteration and transfusion, antibody/ligand receptor process is combined with circulating RBCs in vivo with therapeutic agents (Villa et al., 2015). By intravascular injection of antibody-drug conjugates, it chokes significant binding to the blood stream of circulating RBCs. Band 3, complement receptor 1 cr-1, and glycophorin A (GPA) were used as cargo receptors for ADC, which shows approximately 1,000,000 per single RBC. The encapsulation of co-polymer-based nanoparticles on various GPA ligands like ERY1 is done through fluorescent BSA with RBCm and protamine-based CPP without damaging RBCm. Ligands in complexing nanoparticles with RBCm showed efficient in vitro study by protamine-based CPP (Sahoo et al., 2017).

Passive Adsorption (Hitchhiking)

Passive adsorption utilizes electrostatic interaction, hydrogen bonds, and van der Waals forces, which were nanoparticle-functionalized on the surface of RBC carriers. Theranostic-based nanoparticles are transported on the surface of RBC, referred to as RBC hitchhiking, which prolongs the circulation. Chambers and Mitragotri in 2004 initiated *Hemobartonella* mammalian pathogen and first developed this strategy at a diameter of 0.2–2 μm (Chambers & Mitragotri, 2004). Compared to large particles, 220 nm and 450 nm particles show the largest circulation times of >7 hours, and polystyrene nanoparticles are removed from RBCs due to cell-cell contact and shear forces and are cleared in the spleen. The surface of doxorubicin (DOX)-loaded RBCs showed positively charged chitosan-coated nanoparticles (CTSs). This suppresses pulmonary metastatic melanoma by reducing metastatic nodes by threefold (Liang et al., 2019).

System Integration and Propelled Navigation of Microswimmers to Promote Cancer Cell Targeting

The independent movement was authorized by motile methods such as flagellar, crawling, ciliary movement, and contractile deportment by propulsion of bacteria, sperm cells, microalgae, and macrophages. The controllable biohybrid microswimmers are considered as a major important and steerable movement because the absence of an external factor makes the movement of a particular microorganism very random. There are two methods for controlling the motion: one is intrinsically controlled by microorganisms which are thrusted chemotaxis, surrounded by chemical energy (Tu et al., 2017), and another method by extrinsically controlled by synthetic chambers which are connected to the living organisms or sound wave-based control (Behzadi et al., 2017). The hydrogen peroxide (H_2O_2) level is increased to generate oxidative stress by cancerous cells. These micro-artificial swimmers utilize the hydrogen peroxide as a good energy source for propulsion. Catalysts such as platinum are layered in the inner side of motors, which are used to convert chemical energy into mechanical energy and for catalytic decomposition of hydrogen peroxides. Unidirectional force is utilized for propulsion where oxygen bubbles come out through one opening and the center of motors are tapered as hollow texture observed in motors. The navigation of drugs to target cells is caused by magnetic fields and is controlled by embedding Fe3O4 nanoparticles into the human body system, whereas gold nanoparticles are coated for drug release, which focused on external IR triggers. DOX uptake of HeLa cells was determined by Janus motors for maximum motility and high surface area. In order to increase the biocompatibility, one end was molded with immobilized catalase, and hollow polyelectrolyte multilayer capsules were created (Ma et al., 2015).

Propelled Ultrasound Nanoswimmers Used for Identification of miRNA in Intact Tumor Cells

MicroRNAs (miRNAs) are encoded in plant, animal, and viral genomes with more than 25 nucleotides, ascetic, and biomolecules compiling RNAs. Hematopoiesis, cell difference and multiplication process, and amplitude of mRNA expression were regulated by this process (Dong et al., 2013). Abnormal manifestation of miRNA results in certain diseases ranging from cancer to diabetes. Ryoo et al. (2013), used miRNAs as a biomarker for the diagnosis of diseases and also for therapeutic purposes, considered as important in clinical purposes. Different methods like electrochemical, Northern blotting, real-time quantitative PCR, and electrochemiluminescence of photoelectric cell take longer time to detect the expression of miRNA (Yoo et al., 2014). To overcome this difficulty, the novel nanomotors were identified for rapid intracellular miRNA detection. The intracellular hybridization process was accelerated into cells, which were internalized by ultrasound propelled nanowires supported by graphene oxide with motor-based miRNA used to detect fluorescence biosensing and quenching of nucleic acids. Single-cell step processes such as miRNA intracellular biosensing nanomotor plan revealed in tumor cells were also suggested by Avila et al. (2015). Also, the efficient penetrating power in intact tumor cells was possessed by gold nanowire covered with graphene oxide. The procedures followed in this method are fluorescence shift ON-OFF intracellular method, intrinsic cell nanomotor process, and dye-coated ssDNA-quenched probe attached to cargo miRNA from displaced surface GO quenching.

Steered Segregation of Circulating Cancer Cells for Perception

Circulating cancer cells (CCC) were first observed in patients having cancer cells in their blood that had been transmuted (Ashworth 1869). The cancerous genotype mechanism evolved during the growth of tumor cells, and enormous information was provided by CCCs. The forecast message of tumor cells and treatment were done, and earlier detection of noninvasive cancer cells was efficiently observed in CCC patient's blood samples. Technological provocation was satisfied by detecting 1 ml of blood, which contains more than eight million leukocytes and three million erythrocytes, showing greater importance of CCCs (Yap et al., 2010). The enhanced approach of CCCs is presently examined as a current research area.

Various methods to separate CCCs were developed using physical effects such as moving properties, density, size, charge, and unique cell-type properties, including melanocyte grains in melanoma cells. During the circulation of blood, flow cytometry is considered as an efficient tool for distinguishing CCC from other cells by

immune separation processes (Yu et al., 2011). Quick research advances for the detection of CCC were surveyed by micro-/nanomotors. The energetic charging, transportation, and managed declaration of magnetic nanoparticles were authorized by magnetic segments that are inserted into nanowire motors. This review includes disassembled motors, location targeted by contacting assemblage of micromotors, and early identification of micromotors in human B lymphocyte. Isolation of CCC and micromotor development were studied by these initial works (Balasubramanian et al., 2011). Miniature motors that are technologically performed and huge cellular targeted transport in biological liquids are done with high viscosity and ionic strength (Gao et al., 2018).

Enhanced Intracellular Cancerous Cargo Delivery By Powered Cell Membrane Penetration

During the drug delivery process, there are tremendous problems in handling tumor cells (Behzadi et al., 2017). Hydrophilic nature, increased molecular weight, and negative charges cause restriction in penetration through plasma membranes (Au et al., 2016). Endosomal entrapment of drug bioactivity inside the cytoplasm leads to endocytosis of cell opening, which regulates nanoparticle formulations. The drug is delivered within the cell by introducing a constriction lesser than the cell diameter and by squeezing through microfluidic methods (Szeto et al., 2015). In the cell membrane, the pores are created through an array of bubbles introduced by micro-cavitation, which explodes pulsing laser formed via electroporation laser associated with microfluidic method (Wu et al., 2015b). Straight cytosolic approach was achieved by piercing cell membrane to provide liquid pipelines in cells created by nano-designed nano-straws (VanDersarl and Xu, 2012).

Helical nanomotor was designed for cargo delivery, which is a mechanical-based principle exploited to load lipoplex gene delivery with more force for propulsion to deliver through cell membrane (Qiu et al., 2015). Without the use of transfection materials, the initial delivery of nanomotors to transport DNA and miRNA inside the cells was attempted. Cells propelled externally using ultrasound mechanics by cordial upper-layer chemistry and forceable transport for quick internalization by nanomotors. Cellular incision was generated for surgeon to microdaggers developed by nanomotors to enter the cell membrane powered using unique abilities. Microdaggers are microneedles raised from Dracaena .sp, coated with Ti-Fe layers of alloy to accept the exterior magnetic field. These microdaggers are cultured with HeLa cells and stabbed into the cell membrane during magnetic orientation, which leads to cell death and acts as anticancer drug with increased tumor cell damage by releasing camptothecin.

Insight on Cancer Drug Delivery Erythrocyte-Based Nanomedicine

The significance of using erythrocyte-based nanomedicines includes good biocompatibility, a long mode of circulation, the ability to target a specific location for drug delivery with a nanosized structured erythrocyte membrane-coated nanoparticle, and nanocarriers that are designed as biomimetic cargo delivery methods with lesser immunogenicity to individual patients due to the use of their own erythrocyte. Specialized model drug carriers, such as red blood cells and macrophages, are considered as excellent biodegradable, easy to handle, and biocompatible (Villa et al., 2016). The cargo transport efficiency enhanced by maturation of RBCs from erythroid cell lineage, which lacks nucleus and cargo encapsulation space, is reduced, which gives huge deformability (Ji et al., 2011). The natural cargo carriers like biohybrid microswimmers derived from bacteria are considered as excellent carriers for encapsulation to specific regions. For energetic cargo transfer, red blood cells/erythrocyte bacterial microswimmers act as auto-cargo carriers for drug delivery (Alapan et al., 2018). Biotin-avidin irrevocable complex includes motile *E. coli* MG 1655 biologically engineered with SPIONS (superparamagnetic iron oxide nanoparticles) loaded with doxorubicin as an anticancer drug, which was constructed with RBCs as multifunctional activity for biohybrid microswimmers. RBC microswimmers have lots of functional behavior, such as acceptable delivery deformed by bacteria with force generation; noninvasive, simple construction; magnetic property; and NIR-activated hyperthermia switch termination (Park et al., 2017). Biohybrid microswimmer crafts the scheme for multimode targeted cargo drug delivery.

Construction of Nanoerythrosome Employed By Cell Extrusion Method

Manufacture of vital drug delivery from microbial/biomaterials in the human body has more significance in the medical treatment of cancerous cells (Buss et al., 2020). For specific medical application, bioactuators occupied in biohybrid microswimmers. Magnetotactic bacteria have an intrinsic magnetic navigating ability and excellent motility, but their withstanding capacity in the human body is reduced to less than an hour for short interventions. *E. coli* is slower compared to other microorganisms and has been optimized to withstand the situation and operate in human body conditions to perform medical functions (Song et al., 2018). The main critical factor is size, which is considered as an effective drug carrier and also affects the navigation in limited area and propulsion activation. The union of ultrasound and extrusion method helps to attain the propulsion of RBC motor, acting as an alternate switchable guide for fabrication of nanoerythrosomes (Fig. 10.3). The biohybrid nanosized microswimmers show quick propulsion and locomotion in complex

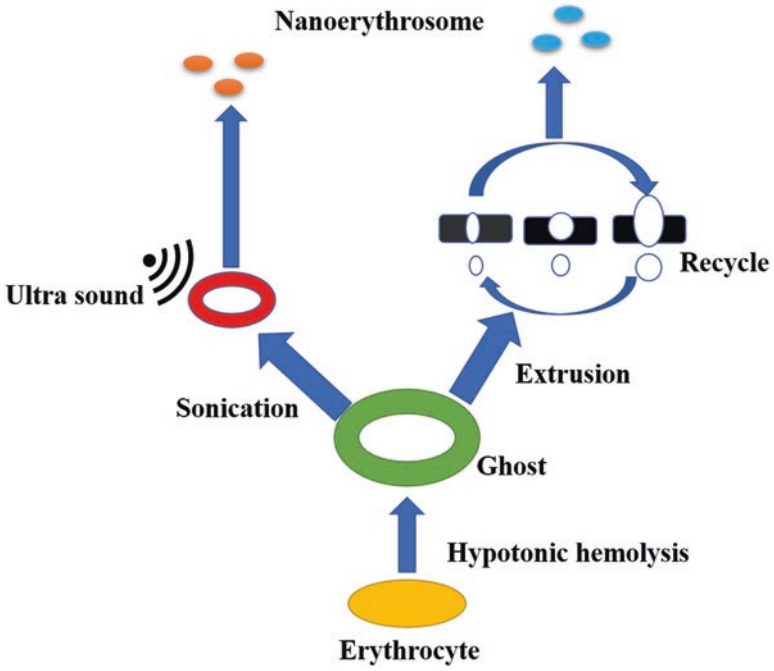

Fig. 10.3 Construction of nanoerythrosomes by sonication and cell extrusion methods

biotic environments. The cargo carriers are designed as biohybrid microswimmers assimilated with nanoliposomes and nanosized particles (SeungBeum et al., 2019). The unsought immune cells that need to be avoided are challenged to natural nanoerythrosomes, which were designed as nanocarriers containing membrane proteins (Wilchek et al., 2006).

Morphological and Physiochemical Characterization of Nanoerythrocytes

The recent development in the fields of human and veterinary medicine is nanoerythrocytes. A drug could be delivered by circulating for a long period or directly to the target specific sites like lymph node and other organs like the spleen or liver by using natural cells (circulating erythrocytes) or RBC carriers. Nanoerythrocytes have been successfully used for drug delivery, such as cancer therapeutic drugs like adriamycin, asparaginase, and methotrexate. The usage of drug-loaded erythrocytes for drug delivery is still in a preclinical phase. Resealed erythrocytes are also used in various applications like delivery of antiviral agents and removal of toxic agents

and RES iron overload (Banerjee and Singh 2013). It is also used in biopharmaceuticals, along with peptides and proteins that are therapeutically significant, vaccines, antigen, and drug delivery on targeted sites based on their nucleic acid. A good drug carrier should possess certain ideal characteristic features like the size and shape and physicochemical properties that could recognize the desired target site, and, after the release of the drug at the targeted site, should be biocompatible with minimum toxic side effect. It should maintain its stability during storage and should carry a broad spectrum of drugs and drug delivery in a controlled manner (Bhise et al., 2010). Latha et al. (2012), studied about erythrocytes used in patients with appropriate size and shape, which will not trigger immunological response and are biocompatible with minimum leakage before the target site is reached. To use RBCs as a carrier system, they have to be encapsulated with drugs, enzymes, and peptides; this is done by various methods; currently, it is done mainly based on the osmosis-based methods in which hypoosmotic dialysis is widely used. For effective disease management with controlled site-specific safe drug delivery system of various drugs at passive and active target sites, encapsulated erythrocytes have become a revolutionized effective delivery system (Nangare et al., 2016).

Mobility Assessment of Nanoerythrosome-Functionalized Biohybrid Microswimmers

A biohybrid microswimmer is an integrated synthetic cargo carrier that has been recently shown to be a promising invasive theranostic application. These biohybrid microswimmers are fabricated with various microorganisms, including bacteria and spermatozoids, which have autonomous control that can navigate through narrow gap and with controlled environmental stimuli with advanced medical functionalities that accumulate to necrotic regions of tumor environments (Nicole et al., 2020). The steer ability of biohybrid microswimmers with long-range applied external fields, with a broad range of medical active cargo delivery application such as acoustic or magnetic fields (Wu et al., 2014; Alapan et al., 2018), and fundamental taxis behaviors of biological actuators regarding numerous environmental stimuli, like chemoattractants, pH, and oxygen, make it a promising candidate. A biohybrid microswimmer that is genetically engineered bacterium studded with nanoerythrosomes can be loaded with molecular cargo and can be injected into the body and push itself through thick environments and tissue cells to dispense drugs at a tumor site. To get where it needs to go, the microswimmer could home in on a signal of some kind. A chemical signal could allow microswimmer dispatchers to take advantage, however passively, of a bacterium's natural sensing capabilities. Alternatively, magnetic or sound signals could allow for a more active, hands-on approach, in which microswimmer movements could be subjected to remote control. Nicole et al., (2020), reported fabricated biohybrid bacterial microswimmers by combining a genetically modified *E. coli* MG1655 substrain with natural cells, which are small

vesicular structures made from red blood cells. The new NEs were found to have a homogeneous size distribution, good stability, and increased shelf-life storage. Wang et al. (2019), engineered core-shelled structure palladium/gold and magnetite (Fe_3O_4) nanoparticles and doxorubicin-based spirulina microswimmers. It has the ability to transport and release the drug at the targeted site by inducing a hyperthermic effect in the cell population, thereby killing the cancer cells and acting as an effective system in cancer treatment. Another example of biocompatible microswimmer employed for drug delivery came from Darmawan et al. (2020), who studied a new biocompatible microswimmer for drug delivery with a self-folding helical magnetic structure, which on ultrasonic stimulation could rapidly release the preloaded drug doxorubicin. Similarly, Chen et al. (2020), employed magnetic and pH-sensitive double-layer microswimmers for in vitro sustained drug release, with two different designs: thumbtack-like and frisbee-like. The microswimmers contained magnetite nanoparticles, as well as doxorubicin, embedded first in a chitosan matrix and then into a calcium alginate hydrogel. Lee et al. (2020), designed needle-type microswimmers which were coated with nickel and titania (TiO_2) and subsequently loaded with the chemotherapeutic agent paclitaxe; this unique features facilitated piercing and a scaffold body for increased surface area through the target microtissue. The needle-type feature enables the microswimmers to spike all the way through the target cell population for fixation and reduces cancer cell viability. The release of chemotherapeutic agents by biohybrid microswimmers was reported by Akolpoglu et al. (2020).

Surface Functionalization of Erythrocyte-Based Nanomedicine for Improved Drug Delivery in Cancer Nanotherapy

Various surface moieties present in erythrocytes are involved in complex biological mechanisms when nanoparticles (NPs) are used as carriers loaded with drugs and directed toward a specific target site. NPs are commonly prepared by bottom-up approach of surface functionalization for site-specific targeted drug delivery. Surface functionalization is done by various chemical and nonchemical interactions by incorporating functional moieties such as antibodies, enzymes, ligands, and other functional target molecules onto the surface of drug delivery carriers. Through enhanced permeability and retention (EPR) effects, more than 80% of NP systems are available to treat cancer, but only a few tumors have been reported to attain NP accumulation through EPR effects. Zhang et al. (2013), stated that they used RBC membrane to cover NPs in order to neutralize the bacterial exotoxin and drug delivery for anticancer and antibacterial treatment (Gao et al., 2015). In one study, DOX was encapsulated into lactic-co-glycolic acid nanoparticles and camouflaged by red blood corpuscles membrane to increase the half-life. Through this approach, half-life of 40 h was achieved (Chai et al., 2019). In one more similar study, RBC

membranes masked with magnetic O-carboxymethyl-chitosan nanoparticles were used to encapsulate DOX and paclitaxel (PTX) to increase tumor inhibition (Fu et al., 2015). Red blood cell membrane (RBCM)-camouflaged NPs are excellent biomimetic nanoparticles, which can be used in biomedical applications. It has also involved a great deal of attention and opened up new doors for surface modification of nanoparticles for cancer therapy. Cell membrane-based NPs drug carriers possess multifunctional abilities and are shown to be a better system compared to synthetic NP-based drug carriers. Cell membrane-coated NP (CMCNPs) have been constantly observed for their ability to mimic cell surface functionality effectively, which can aid in minimizing the immune responses of artificial NPs in vivo and enhance the capability to merge both natural and artificial elements (Chai et al., 2019).

Shape-Changing Nano- and Micromotors for Cancer Therapy

In order to overcome challenges in cancer treatment and diagnostics, recently nanotechnology and nanomaterials have paved new ways in nanomedicine revolution, in which a variety of nanomaterials are used for fabrication such as quantum dots (QDs), upconverting nanomaterials (UCNPs), hydrogel sheets, magnetic nanobeads (MNs), and gold nanoparticles (AuNPs). These nanoparticles have the capacity to release the drug at a reasonable time and space at the targeted site. Other than the abovementioned nanomaterials, self-propelled micro-/nanomotors have gained more attention in the field of cancer diagnostics and treatment. These nanomaterials are biocompatible and have enabled precise diagnostics and therapy of cancers. Metal-organic framework-based biomedical microrobots were recently reported (Wang et al., 2019). These helical microswimmers were fabricated by nickel or titanium for magnetic actuation and subsequently with the zeolitic imidazolate framework-8. Using this principle, biodegradable MOFBOTS were developed and employed in vitro for doxorubicin delivery (Terzopoulou et al., 2020). A different type of biodegradable microswimmer, microrockets propelled by gastric acid, was developed by Zhou et al. (2019), which consisted of a microtube made up of polyaspartic acid surrounded by a zinc core covered with a thin iron layer, which helps drug transport to the stomach using an external magnet, and the acidic pH in the stomach triggers the bubble propulsion of the microrockets to the galvanic corrosion of the zinc core; this was demonstrated in vivo in mouse stomach using doxorubicin. Liu et al. (2020), developed similar microswimmers (organic-inorganic Janus) made up of a magnesium core partially surrounded by a mixture of poly(lactic-co-glycolic acid) and doxorubicin. Magnesium core and water undergo a catalytic reaction that produces a hydrogen bubble that helps in propelling the microswimmers. Alapan et al. (2020), reported a magnetic gold/nickel-coated silica Janus microspheres, which are employed for targeted doxorubicin delivery. Gao et al. (2012), designed nanomotors which are self-propelled and provide the carriers with continuous driving power to help them transport across biological tissues for

cancer cells. These self-propulsion abilities of nanomotors are more advantageous and efficient drug delivery systems with better therapeutically effects with less toxicity (Medina-Sánchez et al., 2016). Apart from other microswimmers, Hortelão et al. (2017), designed enzyme-powered nanomotors for enhancing anticancer drug delivery. Villa et al. (2018), reported superparamagnetic microrobots for cell manipulation and anticancer drug delivery, which are navigated by an external magnetic field and deliver bioactive molecules. Zhang et al. (2019), proposed a jellyfish-like micromotor for the detection of target DNA. The concave surface of the micromotor was assembled with sandwich DNA hybridization. The detection of DNA can be realized, which is simple, cheap, and fast. Yan et al. (2017), used spirulina microalgae to fabricate biohybrid magnetic robots, which allowed in vivo fluorescence imaging and remote diagnostic sensing; it is controlled and navigated in hard to reach cavities of the human body, making them promising miniaturized robotic tools. Nanomotors have been broadly used for cancer nanomedicine, as evidenced by breakthroughs now made in this area.

Evaluation of Stability Profiles of Erythrosomes and In vitro Release Studies

RBCs are engineered and developed rapidly in surface engineering to target diseases. These engineered RBCs can improve the pharmacokinetics and modulate immune response, which helps in targeting certain diseases. The natural cells can be used as releasing systems both as internal and external loading systems (Zhai et al., 2017). Various methods can be used in ex vivo surface engineering of RBCs (Xu et al., 2018). Recently, nanocarriers act as building substance to leverage engineered RBCs as drug carrier, the intracellular parts are removed by hypotonic lysis and by either sonication, microfluidic electroporation, or extrusion; the nanoparticles are coated to the membrane vesicle (Yang et al., 2017). These surface-engineered RBCs can circulate the complex proteins for a prolonged duration in the blood flow, and it is biocompatible to the mother cells (Wang et al., 2019). A biodegradable and biocompatible simple synthesized method of poly(lactic-co-glycolic acid) nanoparticles has been reported in cancer cargo delivery (Liu et al., 2013b).

RBC carriers are attached on either the alkyl chain or the lipid section of molecule; this is yet another approach in functional RBC carriers known as RBC carrier lipid insertion that requires direct mixing (stirring) for a few minutes to hours based on the necessity at normal temperature. RBC carrier lipid insertion facilitates more functionality toward precise outlining, targeting, and biological molecules without harming the proteins (Fang et al., 2013). The indirect surface engineering of RBCm can be done by biotin-avidin bridges, which can be done by modifying the holding element on the RBC either through inducing lipids or by performing chemical change. Zhu et al. (2018), modified folic acid and magnetic nanoparticles in the surface of erythrocyte tumor targeting, which facilitate the filtration of the

circulating tumor cells. Villa et al. (2018), used another method of antibody/ligand-receptor combination, in which the biological agents are made to circulate RBCs in vivo; this provides a relatively safe method to avoid complications associated with ex vivo modification and transfusion. The most recent method in which nanoparticles can be functionalized on the surface of RBC carriers via passive adsorption to deliver theranostic-based nanoparticles can prolong their circulation (Zelepukin et al., 2019). The latest advancement of surface functionalization performed in erythrocyte-dependent nanomedicine has confirmed the potential change-over of this as the next major step toward personalized tumor treatment.

Optimization of Drug Dosage in Nanoerythrosomes (NERs)

At an optimal dose, the nano-based cargo delivery focuses the drug to the target site. The medicinal agents having properties like polymer matrices and dispersed, encapsulated vesicles which are covalently bound are nanosized particle nanodrug cargo (Jadhav et al., 2015). Nano-based drug delivery systems improve the therapeutic property of the drug and comparatively have few negative reactions in the body. Drugs are loaded in NERs by extrusion, sonication, and electrical breakdown method. In extrusion method, with the means of the membrane filter, the erythrocyte ghost combination is ejected. Using a microscope, the obtained erythrocyte ghost mixture pigmented with uranyl acetate was visualized. The above processes are usually performed in a thermostatically restricted device at room temperature. In general, the size of the vesicles seems to be independent of the temperature but decreases considerably with an increase in pressure (Abhilash et al., 2013). In sonication method, electrical signal is converted into physical vibration. The transducer was induced by a signal created by this device. By creating a mechanical vibration, the transducer further transmits to electric signals. The sonicator boosts up the vibration molecules and interacts through the probe (Nandgude and Bhisa 2013). In the electrical breakdown method, erythrocyte ghosts are converted into vesicles. The NERs are also referred to as lipo-proteosomes due to its analogy with liposomes. Due to the huge volume surface ratio, NERs are also considered as buoyant vesicles. The breakdown by electrical method in liquid is the formation of bubbles and superheating by electricity. The breakdown process in liquids seems to be more complicated because pressure is applied on the liquid by the electric field's strength by hydrodynamic effects (Ierardi et al., 2012).

Nano-/Microswimmers: Toward Clinical Translation

In accordance with the treatment of disease and preclinical trials, it is very much advanced by nanotechnology-dependent methods. Scientific research is essential for generating a step forward in medical advances. Moreover, the translation of

clinical practices is not attained by initial scientific research. Thus, the outcome of maximum clinical trials reveals that the majority of the nanotechniques are less effective than the current standards of care. Hence, it is necessary to recognize the positivity and negativity and maintain the significance in mind for further processing and implementation.

The perspective of the present scenario nanorobotics is dependent on their translational shifting. The design of nanorobots with smartness is focused nowadays due to their environmental sensing applications. Even though a lot of research has been performed, few issues like environmental manipulation, micro-/nanorobot retrieval, and toxicology are still required to be labelled. Nevertheless, substantial improvement has been undergone in the field of micro-/nanorobotics technology. In order to apply the nanowires in clinical practices, it is indispensable to regulate the reproducibility of various aspects related to micro-/nanorobotics research.

Biohybrid Microswimmers as Cargo Delivery Agents

In recent times, biohybrid microswimmers have been reported to be promising toward invasive theranostic applications. Erkoc et al. (2019) have reported that biohybrid microswimmers are made of unified biological controllers and artificial cargo carriers. Synthetic cargo carriers with magnetotaxis (Alapan et al., 2018), chemoattractants (Zhuang & Sitti, 2016), and pH and oxygen (Zhuang et al., 2015) make biohybrid microswimmers a potential aspirant for a wide range of therapeutic applications (Alapan et al., 2019).

Lucratively nanoerythrosomes are employed to enhance the human system circulation by passive cargo carriers (Hu et al., 2012). Gupta et al. (2014) reported that by intravenous, subcutaneous route and intraperitoneal administration, the treatment of lymph node, spleen, and liver diseases can be treated through nanoerythrosomes (Gupta et al., 2014). For example, decreased detection of immune cell drug-packed particles was observed in intravenous mice injection when attached to RBC membrane (Wibroe et al., 2017). The transportation of nanocarriers to specific locations of organs is uplifted by conjugating with RBCs and also changing the bioaccumulation process of nanocarriers (Brenner et al., 2018).

RBCs were also used in the production of biohybrid microswimmers powered by motile bacteria to meet cargo transport requirements in medicine (Alapan et al., 2018). RBCs coupled with drugs were set in motion by bacteria and guided via magnetic areas.

Production of active cargo delivery systems using bacterial cells presents a promising perspective to modernize drug delivery and cancer treatment (Brenner et al., 2018). One of the essential parameters in the cargo carrier selection is its size because the size has a direct effect on the impulsion performance. Therefore, for speedy motion in biotic surroundings, the nanosized carriers in biohybrid microswimmers were used and designed with more advanced technology (Samira et al., 2014).

Future Perspectives

The development in the field of microswimmers has achieved considerable advances. Various types of microswimmers have been announced with therapeutic applications at in vitro conditions. However, several issues and challenges need to be considered in order to apply the microswimmers for clinical applications. The benefits of microswimmers in in vivo conditions for treatment of several diseases are far away in doubt. Further research should be focused on various aspects of preclinical studies and the methods of clinical translation. Making use of microswimmers in in vivo is a creditable goal; in vivo therapeutic appeal must be accessible in a much-tapered timeframe.

Customized microswimmers can be extensively used in diagnosing a low quantity of selected constituents by "probing" the specimen. For diagnostic applications, the intriguing solution was given by improved imaging offered by microswimmers. Microswimmers are required to fulfill the requirements from scientific and regulatory bodies. It is also indispensable to consider the ethical, economic, and social inferences to employ medical nanorobotics. These implications are likely to be on par with those of the considerable biological revolutions. Thus, microswimmers need to sustain the considerable challenges in the upcoming future. There are more challenges highlighted despite the rapid development of microswimmers. We also believe that the enormous curiosity and rational implication in the development of microswimmers are likely to throw in appropriate solutions to transferral challenges.

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Chapter 11
Role of Artificial Intelligence in Cancer Nanotheranostics

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Introduction

Artificial intelligence stands for the intellect of man-made machines which became possible through computer-based algorithms. The integration of various branches of science such as computer science, robotics, engineering, medicine, physiology, linguistics, and psychology resulted in the development of this novel branch of science. The era of artificial intelligence came into glare of publicity by continuous research across the world, and currently it is used as a common name for machine-based algorithms that are being used in various fields. AI can make rapid and accurate decisions similar to the human brain to overcome various hitches faced in diagnosis and therapy of several diseases (Lo et al., 2017).

Even though the algorithms of artificial intelligence were designed to overcome the challenges of industrial applications like maintenance of aseptic conditions, labeling of products, and packaging, now it is widely utilized to predict the severity of diseases, to make decisions related to dose optimization, and to manage surgical procedures especially in clinical oncology (Shiraishi & Moore, 2016; McIntosh et al., 2017). The prevalence and death rate caused by cancer is continuously increasing owing to lifestyle changeover and usage of harmful chemicals for

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agricultural and industrial practices. The recurrent cases can be minimized with the help of modern technologies such as tumor adjuvant therapy, robotic surgery, and laparoscopic surgery (Simmons et al., 2017).

The two major difficulties in cancer therapy are delayed diagnosis and its recurrence. Besides that, the precise prognosis and perfect assumptions are not possible in conventional methods. The diagnosis at early stage is very challenging for some types of cancers due to asymptomatic nature and cannot be predicted through scans and other techniques like mammograms. Therefore, there was a boom for the development of several predictive methods for cancer based on statistical tools and modern diagnostic techniques with more accuracy. However, the accurate prediction and diagnosis through artificial intelligence was a big hurdle for scientists and was getting resolved day by day. The algorithms based on AI are playing a vital role in the prognosis of cancer at an early stage. Furthermore, it is providing insights of cancer vulnerability, relapse, and predictions of survival. The literature survey reveals that the magnitude of AI has developed tremendously using the available huge data of clinical oncology (Obermeyer & Emanuel, 2016).

Artificial intelligence is a field of research that correlates mathematical and statistical approaches to build up algorithms for processing large sets of clinical data. Ultimately, this results in accurate conclusions derived by machines without human observation. The branches of AI are deep learning, machine learning, and artificial neural networks. All these subdomains of AI utilize algorithms to identify, analyze, and categorize the data for precise conclusions (Wang & Summers, 2012). Ultimately, the highly advanced computer-based algorithms like gradient boosting, support vector machines, and random forest methods would outpace the classical methods and statistical approaches (Valdes et al., 2016; Valdes et al., 2018).

AI had been adopted in the field of radiology for decision-making in such a way that even it may replace the role of human prophecy in the near future. Furthermore, AI will reveal the facts concealed in the huge clinical data that were needed for decision-making

(Dilsizian & Siegel, 2014; Kolker et al., 2016). Through these data and images from radiology, appropriate diagnosis and treatment of tumors will become possible (Sherbet et al., 2018; Houssami et al., 2019). Hence, the newly rooted AI technology will pave the way for concrete applied research involving clinical oncology and robotics (O'Leary, 2013; Iafrate, 2018). This chapter focuses on the various aspects of artificial intelligence such as delineation of algorithms, development of nanosensors and nanoarrays for computational screening, clinical decision-making, artificial neural networks for synthesis and formulation of nanoparticles, dose determination, and successful cancer treatment by means of nanotheranostics.

Application of AI in Medical Imaging

Artificial intelligence (AI) involves the use of computerized algorithms to analyze data. AI has been used clinically in diagnostic imaging and detection and quantification of an extensive array of clinical conditions. Computer-aided diagnostics have been reported to show excellent precision, sensitivity, and specificity for the detection of abnormalities, with the prospective to improve health.

Machine learning (ML), a part of AI (Samuel, 1959), includes approaches that allow computers to learn from data without being explicitly programmed (Lee et al., 2017). Among the various techniques of machine learning, deep learning (DL) has emerged as a promising technique. DL methods belong to representation learning methods with multiple levels of representation, which process raw data for the detection of clinical conditions (LeCun et al., 2015). At present, many AI imaging studies estimate diagnosis of clinical conditions by calculating sensitivity and specificity (Liu et al., 2019). AI detects minor image alterations, new diagnosis of advanced disease, disease requiring treatment, and conditions likely to affect long-term survival. The occurrence of symptoms, need for disease-modifying therapy, and mortality strongly affect the quality of AI-based investigations (Park et al., 2019).

AI imaging is widely employed in cancer detection and characterization. High-power quantitative analysis of image alterations is used to predict the probability of malignancy and anticipated tumor kinetics. Let us consider an example of prostate cancer. Prostate cancer has the most prevalent neoplasm in men and lacks an effective screening approach. In the past 5 years, multiparametric MRI was revealed to increase the detection of clinically relevant prostate cancer, but variability remains a major obstacle (Stabile et al., 2020). Deep learning algorithms have been reported to enhance the assessment of MRI features such as texture, volume, and shape.

Due to accuracy in results of AI imaging, physicians have an ability to diagnose advanced prostate cancer while decreasing biopsies in low-probability cases (Yoo et al., 2019). The approach for adrenal incidentalomas could also benefit from AI-based imaging analysis. Adrenal nodules are the most frequently encountered incidental radiographic finding and can reflect malignant or benign conditions (Oren et al., 2020).

Computational Analysis of Multiplex Nanosensors for Differentiating Wild Type and Cancerous Gene

A new trend of employing multiplex nanosensors for differentiating wild types and cancerous genes has attained phenomenal development. These nanosensors are modeled to analyze and quantify the specific chemicals based upon the detectable signals. Some of the commonly used nanosensors are carbon nanowires and nanotubes, gold nanoparticles, and quantum dots. The major significance of these nanosensors includes early diagnosis, continuous monitoring of disease progression, and

specific treatment plan for control of cancerous growth (Swierczewska et al., 2012). The analysis of multiplex nanosensors through computational methods requires specialized methods for constructing clusters to classify the huge amount of data collections. Eventually, this analysis will reveal the importance of these nanosensors over other conventional sensors (Scott et al., 2006).

The nanoarray technique was used to recognize genetic profile and expression pattern of subtypes of NSCLC which was earlier based on microarray (Koivunen et al., 2008; Ohashi et al., 2012). These studies about genes and its subtypes involved in lung cancer are useful to plan a treatment method targeting toward the specific site. The mutations in the genes responsible for EGFR and ALKR are significantly affecting NSCLC (non-small cell lung cancer), and the management options based on molecular approaches are recommended (Lawrence et al., 2013). This new approach focuses on the correlation between molecular profile and pathological symptoms to uproot the molecular mechanism and biological phenomenon behind the origin and development of cancer. Ultimately, this results in the appropriate screening and suitable site-directed therapy. Two different researches on KRAS (Tam et al., 2006) and EGFR (Ninomiya et al., 2009) revealed that there is a close relationship between pathological symptoms and mutations in tumor-related genes. The molecular abnormalities at several loci of oncogenes as well as tumor suppressor genes related to NSCLC had been studied (Djuric et al., 2017).

The integration of machine learning algorithms and nanosensors is much useful to find out the subtypes of cancer, growth pattern, and metastasis (Li et al., 2014). The genetic profiling of cancerous genes through microarray technique is helpful in the identification of gene subtypes of NSCLC. This is also useful to design machine learning algorithms to analyze genomic pathways. The identification of adenocarcinoma is mainly based on the microarray data and widely applied to recognize the probable NSCLC biomarkers (Kikuchi et al., 2003). The major pitfall in this approach is the multidimensional analysis of data obtained from imaging and genome profiling, which is a new area of clinical oncology and needs more concrete research. The analysis of genomic profile data results in the identification of transcriptional and translational events of cancerous genes. The images obtained from radiology give details about the pathological changes, cancer development, and spread to the surrounding tissues. The comparison of genomic and imaging data of specific tumors will be more useful to predict the consequences faced from the onset of cancerous growth to its recovery. On the other hand, designing a model to handle such a huge clinical data is a great concern (Wang et al., 2019). But, the advanced sequencing methods along with machine learning algorithms and accurate nanosensors will help the oncologists to distinguish the subtypes of NSCLC on the basis of genome analysis (Podolsky et al., 2016).

There are several in vitro methods developed judiciously to recognize biomarkers such as DNA, RNA, protein, and metabolites in liquid samples (saliva, sweat, urine, and blood) and breath by combining a ligand that can detect the target biomarkers through suitable nanomaterials (Kosaka et al., 2014). Quantum dot nanosensor with a DNA strand conjugated with streptavidin is capable of emitting fluorescence after binding with the mutated gene. It is widely used to differentiate

the wild and cancerous KRAS with point mutation at a specific locus (Zhang et al., 2005). These types of sensors can be restructured to identify several other mutant genes and will become the appropriate diagnostic tools to monitor cancer patients through further improvisation. Medically authorized diagnostic tools are already available to isolate malignant cells from blood by magnetic beads incorporated with antibodies for EpCAM (epithelial cell adhesion molecule) (Green et al., 2016). The highly sensitive nanosensors will be more useful for the earlier identification of biomarkers even at femtomolar concentrations during the first stage of cancer development (Fig. 11.1) (Acimovic et al., 2014).

Nanosensors, which can act as olfactory systems and are capable of quantifying the volatile organic compounds (VOC), are available for complex analysis of several metabolites in gaseous state (Wilson & Baietto, 2009). The major advantage of these electronic nose nanosensors is that they do not rely on the antibodies but it can specifically react with volatile organic compounds and produces several patterns in the nanosensor array. This facilitates the application of nanosensor arrays for wide targets such as lung, breast, prostate, and colorectal cancers. The gold nanoparticles with specific ligands are commonly employed nanosensors that can differentiate normal people and cancer patients by analyzing the breath samples (Peng et al., 2010). Several models of electronic nose sensors were developed to detect VOC from the exhalation samples of suspected patients at an early stage (Phillips et al., 1999; Altomare et al., 2013).

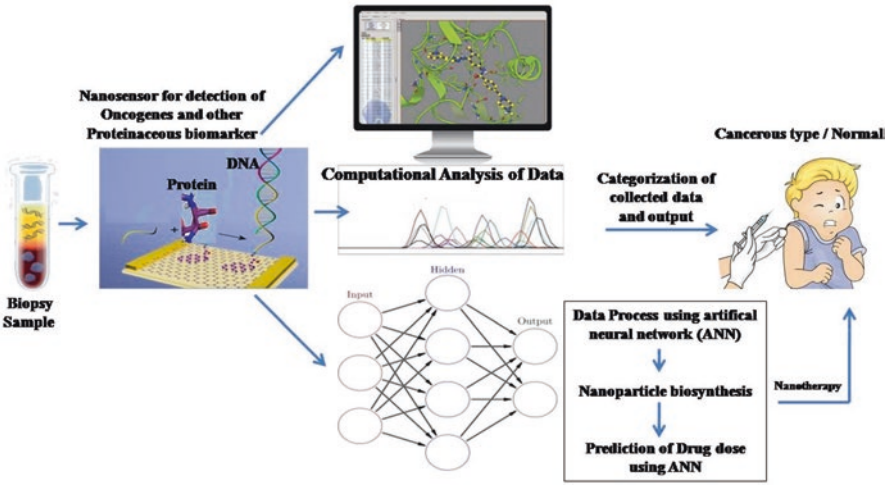

Fig. 11.1 Integration of nanosensors and artificial neural networks along with computational analysis using AI methods supports earlier prediction of cancerous biomarkers, suitable drug designing, and dose determination

Computational Analysis in Nanopore Sequencing Using Artificial Neural Networks

Precision medicine is a personalized treatment plan designed for treating cancer patients based on their unique molecular profile. Generation of molecular profile for each patient involves compilation of disease-related biomarkers, which are generated by applying various omics approaches, as molecular signature (Chen et al., 2012). Advent of nanopore sequencing and real-time sequencing of single molecules enhanced the accuracy and speed of omics data generation as they permit direct sequencing of template DNA (Korlach et al., 2010). Single-molecule real-time sequencing provides epigenomic information such as identification of lesions and DNA methylations, which could be utilized as biomarkers for detecting malignancy and other types of cancer (Flusberg et al., 2010). Nanopore sequencing technology is based on detection of change in ionic current during translocation of single-stranded DNA (under a specific voltage) across a lipid membrane (Deamer and Branton, 2002). But, the dispersion of applied electric voltage to adjacent nucleotides near the nanopore affects the detection efficiency and remains a major challenge in the field of nanopore sequencing (Lindsay, 2016). In order to overcome this pitfall, artificial intelligence involving neural network algorithms is widely used in nanopore sequencing to translate voltage signals into nucleotide sequence.

Neural networks consist of several layers of interconnected nodes, which are trained by using known nucleotide sequences, and the alteration in the electrical signals imposed by each nucleotide was recorded to obtain sequencing results with high accuracy (Goodwin et al., 2015). Quantum sequencing, which is an advanced form of nanopore sequencing, involves nanoscale electrodes to measure the changes in electron tunneling when the nucleotide gets translocated through the nanopore (Huang et al., 2010). Machine learning algorithms, like support vector machine algorithms, are being exploited for the interpretation of such electrical signals.

Combination of feature selection with machine learning algorithms can be utilized for detection of disease-specific molecular signatures. Features, such as genes, that are specific to a particular type of cancer can serve as a biomarker in cancer detection based on machine learning algorithms (Ren et al., 2013). Efforts are being taken to combine various omics data, such as epigenomic, proteomic, genomic, transcriptomic, microbiomic, and metabolomic data, to create disease profiles for each patient with high accuracy (Kim et al., 2016).

Role of Artificial Neural Networks in Nanoparticle Biosynthesis

The nanoparticle biosynthesis has reached maximum progress due to increasing awareness to produce nanoparticles by ecofriendly green synthesis. Especially, extracellular biosynthesis of gold nanoparticles using phytochemical extracts and

microbes like actinomycetes, fungi, and bacteria had been widely studied (Shameli et al., 2012). The accurate models are needed to study a chemical reaction on the basis of data obtained from experimental research. ANNs (artificial neural networks) are used as powerful tools for the calculation of nanoparticle dimension and percentage yield in different branches of medical as well as engineering fields. The major advantages of artificial neural network include precise prediction without any previous familiarity, its ability to deal with multiple datasets, and detection of both positive and negative correlations (Chandwani et al., 2015).

The nanoparticles were synthesized using varying concentrations of chitosan solution with pH 6.2 and sodium triphosphate to determine the mass ratio for maximum yield of desirable nanoparticles. The characterization of nanoparticles was carried over by the comparative analysis of particle size, zeta potential, and yield. These parameters were also analyzed through artificial neural networks to determine the optimum conditions, and it was found that sodium triphosphate concentration is positively influencing the particle size yield (%). The maximum yield was recorded as 91.5% with 227 nm particle size and + 24.13 zeta potential, and a stable spherical structure was also observed. The analysis of nanoparticles through FTIR spectroscopy and DSC revealed that there are positive interactions between chitosan and sodium triphosphate. The role of artificial neural networks on nanoparticle size and yield determination was useful to formulate the nanoparticle with desired characteristics and high percentage of yield (Rania et al., 2016).

ANN was used to determine the size and polydispersity of nanoparticles synthesized using polymers. A mathematical method was developed using artificial neural networks to analyze the characteristics and yield of biopharmaceutical polymer-based nanoparticles. In addition to that, the effects of polymeric properties such as viscosity, surface activity, and hydrophobicity on the particle size were determined. This research study revealed the prediction of the properties of nanoparticles through in silico method within a least time period than the already existing conventional methods (Youshia et al., 2017).

In another work, gold nanoparticles were biosynthesized using $AuCl_4^-$ (tetrachloroaurate) and water-based extract of leaf samples obtained from *Camellia sinensis* L. The plant extract played the role of a reducing agent as well as a stabilizing agent. These nanoparticles were characterized using TEM, XCRD, and UV-visible spectroscopy. The cost-effective strategies based on ANN algorithms were used to assess the properties of biosynthesized gold nanoparticles. This ANN method was optimized using a biogeography method and chaotic map, and these improvisations lead to less mean squared error (0.0134) and maximum R^2 value (0.9822) when compared to all other ANN-based algorithms. The accurate prediction of gold nanoparticle size became possible through this optimization protocol. The measurement through ANN was based on the process parameters, such as quantity of leaf extract and $AuCl_4^-$, reaction temperature, agitation speed, and time, and it was comparatively analyzed with the results of transmission electron microscopic images. The IBBO (4–3-1 architecture) algorithm forms the basis of this artificial neural network, and it is more advanced than the other existing algorithms. The experimental results revealed that the agitation speed, time of reaction, temperature,

and volume of tetrachloroaurate are positively correlated with the essential characteristics of the nanoparticles. On the other hand, the volume of leaf extract was negatively correlated with the particle size of gold nanoparticles. The particles obtained ranged between 7 and 36 nm under various process conditions. This model was found to be a highly useful method to calculate the size of biosynthesized gold nanoparticles (Shabanzadeh et al., 2019).

Similarly, the computational ANN method was used to optimize and evaluate the size of silver nanoparticles. In this method, four input data, such as the concentration of bentonite, starch, silver nitrate, and gallic acid, were compared with the output (particle size). The production of silver nanoparticles is based on a green biogenic reduction method. The LM algorithm was used to develop ANN. The architecture of the ANN was 4–10-1(4 input nodes, 10 neurons, 1 output layer) and named as MLP (multilayer perceptron). ANN results were compared with the experimental outcomes and revealed that the method was well suitable for in silico analysis of silver nanoparticles. The statistical analysis revealed less MSE and high R^2 value. The predominant factor that affects the nanoparticle size and yield was determined as the concentration of silver nitrate. This study proved that the application of ANN based on LM algorithm (4–10-1) is an economically feasible alternative method to predict the results of nanoparticle biosynthesis (Anupama et al., 2018).

Optimizing Drug Combinations Using AI-Based Tools

The synergistic effects of drug combinations can be obtained by optimization of a set of drugs, which will be useful to improvise the positive results of cancer treatment. There is a great difficulty faced by the researchers in the selection of drug combinations, dosage, and treatment intervals without toxicity. Due to the complex metabolic machineries existing in the living system, some toxic effects may come across during treatment by chance. Though nanomedicines enhance the therapeutic efficiency dramatically, they also have the same problems in the optimization process. Therefore, the combined application of nanomedicine and artificial intelligence will be more useful for the effective management of cancer (Ho et al., 2019).

Several research works to find out the drug combinations for various cancer therapies were carried out in the past few decades to reveal the possibilities behind these obstacles. The combinations of manifold drugs against the renal adenocarcinoma cell line 786–O were studied by Weiss et al. (2015). This type of carcinoma is mostly resistant to radiotherapy and chemotherapy, and the specific drug combinations with targeted release will be useful to overcome this resistance. They found that the optimum concentration of broad spectrum anticancer drugs such as erlotinib, AZD4547, axitinib, and dasatinib minimized the negative effects drastically with their synergistic effect.

An AI-based method named as CURATE.AI was programmed and introduced by Pantuck et al. (2018). This is based on the topographic analysis of the cell surface, and correlation is made with probable physical changes of target cells. This

technique was earlier employed for the treatment of TB patients under immunosuppression for liver transplantation. Later, it was applied to control prostate cancer using enzalutamide and ZEN-3694. This technique was useful to adjust the dosage according to the specific response of the patients under observation. Thus, the CURATE.AI-based therapy for prostate cancer diminished the specific antigen that induces malignancy and arrested the disease spread.

The feedback system control method was introduced by Wang et al. (2018) to optimize drug dosage for cancer treatment by nanotheranostics. This AI-based method was used to determine the optimum concentration and drug-dose relationship with higher cytotoxicity. The drugs optimized were unmodified paclitaxel, nanodiamond-bleomycin, nanodiamond-mitoxantrone, and nanodiamond-doxorubicin. The different amalgamations were tested on various breast cancer cell lines. Their study revealed that AI-based drug dosage and combinations gave better results than the normal conventional methods.

Utilization of Machine Learning Algorithms in Nanotheranostic Formulation to Predict Encapsulation Efficiency

Computational models are being employed efficiently to hasten the development of novel drug delivery systems. Liposomes are considered to be an effective vehicle for delivering drugs. Particularly, nanosized liposomes are of great importance as they are highly stable and allow controlled drug release. Moreover, they can pass through the membrane passively and are efficient in delivering drugs to the target (Barenholz, 2003). Computational modeling could be applied to predict the efficiency of liposome-mediated drug delivery systems. Quantitative structure-property relationship (QSPR) models have been generated using in silico tools to predict the suitability of drugs for remote loading (high intra-liposomal concentration) with 90% accuracy. This high loading efficiency is determined by the structure of the drug molecule and experimental conditions (Cern et al., 2014). Moreover, drug-to-lipid ratio also determines the high loading capacity, as this ratio suggests whether the effective dose of the drug could be achieved by using liposomal drug delivery system. Several imaging techniques and computational models have been designed to monitor the biodistribution and QSPR of liposomes. Artificial intelligence could be exploited as an imaging agent, in which the encapsulation efficiency of the drug can be predicted by loading the drug onto the nanoparticle and by employing machine learning algorithms (Hathout & Metwally, 2016).

Prediction of Personalized Drug Potency Using Computational Tools

Computational tools play a key role in predicting the potential of a drug for each patient individually and enhance their recovery rate. But the inadequacy in algorithm and data availability makes drug response prediction a difficult one. Recent advancement in designing computational models increases the accuracy of prediction, and these machine learning techniques are of great use to the clinicians for prescribing personalized medicines. For instance, computational tools for analyzing single-cell profiles help to sort out the most effective combination of drugs in treating cancer (George et al., 2020).

Neural networks, such as graph convolutional networks, have been designed on the basis of biological information related to cancer (Hamilton et al., 2017). In the field of pharmacogenomics, the relationship between various features, like mutation and gene expression pattern, are incorporated by using conditional scaling technique. In order to understand the complete biological background and to achieve high accuracy in drug screening, integration of multiple models and algorithms becomes mandatory.

Automation in designing and testing neural networks is of prime importance in case of predicting the drug response (Zoph & Le, 2016). Molecular simulation involves designing of drugs as well as the target receptor molecules to elucidate the mechanism of binding and action potential (Xiaoqian et al., 2020).

Biomarker system has been developed to determine binding of drugs and its response. Development of efficient diagnostic kits requires a large set of experimental data and computational tools for analyzing it. Integration of statistical and machine learning tools also gives insights of drug resistance in preclinical (Dhandapani & Goldman, 2017) and clinical levels (Perez-Gracia et al., 2017). Development of drug and elucidation of its response using computational tools and neural networks minimizes the time and expenses, which in turn facilitate the availability of drugs for treating cancer (Xiaoqian et al., 2020).

Relating Drug Dosage, Biodistribution Profiles, and Therapeutic Efficacy of Nanoparticles

AI tools can correlate the condition of the patient and a treatment option to predetermine the effect of a particular drug and its dosage. One such model named pharmacogenetic predictor was used to determine the effect of doxorubicin, fluorouracil, cyclophosphamide, and paclitaxel in breast cancer patients. The genetic profile data obtained from 82 breast cancer patients treated with these drug combinations revealed that the treatment given was effective in 92% of the study population. These AI-based predictors will be much useful to determine the patient-specific dose determination (Hess et al., 2006).

The nanomaterial produced using bismuth has been designed as a nanotheranostic tool to get a quality treatment measure for cancer based on the enhancement of image quality. These nanoparticles should be excreted out of the body through the renal pathway if they didn't reach the target tissue. Based on their biodistribution profile, clearance after usage, and other therapeutic efficacies, bismuth-based nanomaterials had been widely studied in vivo. The bismuth-based nanomaterials showed less cytotoxic effects, prolonged biodistribution profile, and appropriate renal clearance pathways. Which were proved through in vitro and in vivo studies. The application of these bismuth-based nanomaterials as a nanotheranostic drug after a clinical approval will result in an effective nanotherapy (Badrigilan et al., 2020).

The nanomedicines are variable chemical structures that should be treated with suitable agents to modify its surface characteristics to escape from phagocytosis (Cho et al., 2008). Besides this, several other factors also severely affect the transport of drugs toward the target tissues. These factors can be avoided from binding with the nanomedicines and nanocarriers by using suitable co-polymers such as glycerate-based chitosan nanoparticles. The glycerate will form stabilized micelles to protect the drug through the intestinal tract and circulation. These co-polymers can be optimized by using AI models (Tian et al., 2015). Therefore, the nanomedicine delivery to the target tissue is mainly influenced by physicochemical and surface characteristics of the nanoparticles. To overcome these biological barriers, the surface-modified carriers are being used in a tissue-specific drug delivery (Fortuna et al., 2011).

Rationalization of Nanomedicine Interaction with Membrane Receptors

Drug-loaded nanoparticles are generally coated with specific ligands like membrane-bound receptor ligands, antibodies, etc. for the selective binding of nanomedicine to the target tissues. But these drug-loaded nanoparticles with specific ligands cannot ensure proper release of the drug in the target site and its therapeutic efficiency. Hence, rationalization of the nanoparticle has to be performed during the designing stage of nanomedicine in order to enhance the success rate of nanotherapy. Computational modeling paved the way to study the interaction between nanoparticles with vascular endothelium, cell membrane, etc. based on the binding energy of the nanomedicine with the target tissues (Fig. 11.2).

Metropolis Monte Carlo algorithm is widely used for rationalization of the nanoparticles, wherein the computational tools based on system simulation will be used to determine the influence of antibody coverage ratio of each nanoparticle on its binding energy to the system. The results obtained using computational analysis were in congruent with the experimental analysis based on in vivo models and cell culture techniques (Liu et al., 2010).

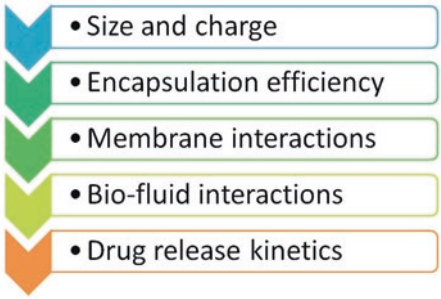

Fig. 11.2 Role of computational tools on nanoparticle design

Computational models are essential to predict the capability of nanomedicine to cross the barriers before reaching the target cells. For example, Shityakov et al. (2017) applied various computational models for predicting the efficiency of nanoparticles in crossing blood-brain barriers as well as the potential toxicity of nanoparticles. These models could be useful for enhancing the efficiency of nanoparticle formulations targeting the brain. But the construction of these models is highly complex and involves large computational capacity, as they require a wide knowledge on physical and biological mechanisms that govern the permeation of nanoparticles.

Machine learning algorithm has been designed to envisage the permeability of nanoparticles against the blood-brain barrier and to determine its side effects and chemical properties (Gao et al., 2017). Designing the machine learning algorithm involves a huge set of experimental data to infer the correlation among the variables for predicting the model. Machine learning models should be selected appropriately to achieve high accuracy. Data types and mathematical relationships among the variables are essential for designing a computational model. For instance, DNA sequences in text format were divided into short oligonucleotides and then converted into two-dimensional image forms in order to create a neural network for predicting the structure of DNA chromatin. This type of data conversion from text to image could be useful for designing computational models for image processing with high degree of accuracy (Yin et al., 2018).

Contribution of Artificial Neural Networks in Survival Prediction of Cancer

Artificial neural networks (ANNs) are widely used in diagnosis of cancer (Cichetti, 1992). ANNs have been effectively employed for pattern recognition and survival prediction of several diseases (Dybowski et al., 1996). The crucial goal of cancer prediction and prognosis are distinct from the goals of cancer identification and analysis. The benefit of a neural network is the capability of the model to capture nonlinearities and complex interactions among factors (Burke, 1996). Trained on a

number of predictive factors, neural networks have been employed to progress the precision of endurance calculation for patients with lung and colorectal cancer (Burke et al., 1997).

Accurate evaluation of survival and prediction duration is the most significant part of a clinical decision-making process in patients with cancer (Chen et al., 2009). The following are the three predictive foci which are to be considered in cancer patients: the calculation of cancer vulnerability, the prediction of cancer reappearance, and the survival prediction of cancer. In the first case, one is trying to predict the possibility of emergence of cancer prior to the incidence of the disease. In the second case, one is trying to predict the probability of reemergence of cancer after the obvious resolution of the disease. In the third case, one is trying to predict the life anticipation, survivability, tumor drug sensitivity, and succession. In the latter two situations, the success of the analytical prediction is apparently reliant on the quality of the diagnosis. However, a disease prognosis can be identified after the diagnosis (Hagerty et al., 2005).

The prediction of diagnosis includes a huge range of decisions about the different aspects of cancer treatment (Van Vliet et al., 2012). The gene expression in normal tissue and diseased tissue will bring an insight and understanding of the cancer pathology (Shimada et al., 2009). Checking gene expression patterns for attributes coupled with the clinical performance is very significant, because these patterns examine prognosis and lead to the alternative approach to recognize the molecular and physiological mechanism.

Previous research reports have given an insight on the influence of analytical methods than histological and clinical data in survival prediction. Recently in the artificial intelligence field, increasing clinical decision support systems based on machine learning methods to evaluate gene expression data have facilitated the medical diagnosis. Studies have revealed the higher precision of machine learning algorithms than regression models in cancer survival prediction (Spechler, 2013). Gene expression data have the possibility to avoid errors caused by fatigue and annoyance of oncology experts in the evaluation of cancer survival. Analyzing such data with machine learning techniques leads to the development of clinical decision support systems for the accurate estimation of survival time and hence provides appropriate treatments to patients.

Predicting Potential Toxicity of Nanoparticles Using Computational Analysis

Nanotheranostics aimed at enhancing the efficiency of the drug to the target system and reducing the side effects. But the toxicological nature and biocompatibility of some nanoparticles create a major hindrance in the clinical translation of nanoparticles. Machine learning models for determining toxicological properties of nanoparticles are limited due to ethical issues, time consumption, and high cost.

Chen et al. (2016) classified nanoparticles on the basis of its ecotoxicity using machine learning models. The toxicity data from multiple sources were utilized to develop species-specific models as well as for predicting toxicity for different species using a single model. Ecotoxicology of iron oxide nanoparticles on kidney cells was predicted by using neural network models. The kidney cell viability was predicted based on the surface charge, concentration, incubation time, and particle size of nanoparticles (Hataminia et al., 2019).

Challenges in Clinical Implementation and Future Prospects

Machine learning algorithms have improved the accuracy of cancer detection, treatment, and posttreatment monitoring of patients. Artificial intelligence in integration with traditional imaging techniques such as X-rays, CT, and MRI scans paved the way for early prognosis of cancer and its treatment. It could be useful in the interpretation of some rare pathological data, to which most of the clinicians remain unaware.

Secondly, precision medicine contributes to a major revolution in the treatment of cancer. It also provides newer insights on the distribution of nanomedicine and their therapeutic potential. Unsupervised learning methods aimed at clustering patients into different groups based on their unique feature for developing medical regime. For instance, Franconi and Campesi (2014) found that the gender of the patient is an important feature which affects the therapeutic efficiency, pharmacokinetic properties, and adverse effects of the administered drug, and hence, it has to be considered for developing treatment plans. Similar results were observed by Serpooshan et al. (2018), in which a patient's gender influences the uptake of nanoparticles by amniotic stem cells.

Moreover, utilization of easy-to-use and portable nanosensors can help in the advancement of a patient's follow-up procedure. Development of electronic skin nanosensors allows continuous monitoring of patients via their saliva, sweat, and blood profile analysis (Jin et al., 2016). Gao et al. (2010) designed a sensor for monitoring sweat electrolytes and connected the sensor to a mobile app using Bluetooth. This kind of smart, user-friendly sensor simplifies the follow-up procedure of patients and reduces the work burden of clinicians.

Although artificial intelligence plays a critical role in cancer nanotheranostics, various hurdles and challenges have to be addressed before its clinical translation. Curation and annotation of medical data remains a major obstacle in the construction of artificial neural networks as they require professional expertise.

The most important challenge in clinical translation of AI includes lack of ground-truth data for validation. To evaluate the reliability of AI, the results have to be compared with human experts. But the paucity of the patient's ground clinical data makes the training and validation of AI a difficult one. In spite of various ethical issues, medical data should be made accessible to improve the collaborative research and to develop AI-based computational algorithms. Recently, the National

Institutes of Health supported AI scientists by sharing CT scan and chest X-ray data from their repository (Wang et al., 2017). Moreover, increase in availability of genomics data from various patients enables AI to unravel the unique relationship among patient subgroups, which helps in developing better treatment plans (Kalinin et al., 2018).

In addition to the abovementioned hindrance, there is a lack of transparency among different companies regarding AI techniques. But for the validation of AI algorithms, reproducibility of experimental data is mandatory. Hence, transparency in the techniques along with a well-defined research protocol may help in further improvement and implementation of AI techniques (Hutson, 2018).

Clinicians and patients have to adopt this computational revolution in healthcare instead of experience-based medicines. Doctors have to update their knowledge on AI techniques for using the tools efficiently and for providing effective treatment to the patients (Mesko et al., 2017). Implementation of AI tools in the pathology field helps not only in the diagnosis but also for exploring the ground truth behind them (Zomnir et al., 2018). On the other hand, there are lots of probabilities for the patients to get misinterpreted clinical data. Hence, clinicians should make the patients aware about the risks and benefits of AI (Mesko et al., 2017).

Achieving accuracy in computational techniques remains a major challenge as it requires large datasets for training the ML algorithm. Data collected from a large group of heterogeneous individuals is necessary for developing the models. Experts from various disciplines, such as medicine, nanoparticles, and computer science, should be involved to devise, optimize, and validate the computational tools for increasing the clinical relevance. Further complications in the clinical approval of precision medicine, its production cost, and toxicity analysis have to be addressed.

Successful implementation of precision nanomedicine in cancer treatment will enhance the therapeutic efficacy of the administered drug and prevent the development of drug resistance. The role of computational tools and AI is inevitable in cancer diagnosis as well as in designing, rationalization, and implementation of nanomedicines for treating cancer. But the challenges posed by this digital healthcare transformation should be addressed properly to ensure the safety of the patients.

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Chapter 12
Limitations of Current Cancer Theranostics

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Introduction

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

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

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

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

Current Nanotheranostic Platforms for Cancer

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

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

Gold Nanoparticle (AUNPs)

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

Magnetic Nanoparticle (MNP)

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

Quantum Dots (QD)

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

Carbon Nanotubes (CNTs)

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

Mesoporous Silica Nanoparticles (MSNPs)

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

Upconversion Nanoparticles (UCNPs)

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

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

Polymeric Nanoparticles (PNPs)

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

Polymeric Micelles (PMs)

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

Solid Lipid Nanoparticles (SLNs)

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

Limitations of Current Cancer Nanotheranostic Approach

Design and Development Limitations

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

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

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

Table 12.1 Highlighting the advantages and limitations of current nanotheranostics platforms

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

(continued)

Table 12.1 (continued)

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

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

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

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

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

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

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

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

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

Biopharmaceutical Limitations

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

Immunological Limitations

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

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

Limitations Related to the Interaction of Nanotheranostics

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

Interactions with Complement

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

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

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

Interaction with Serum Protein

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

Interaction with Mononuclear Phagocytic System (MPS)

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

Limitations Related to Tumor Targeting

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

Limitation Related to the Safety of Nanotheranostics

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

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

Pitfalls of Nanotheranostic Research

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

Case Study

Challenges in the Development of nab-Paclitaxel

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

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

Regulatory Concerns of Cancer Nanotheranostics

Regulatory Evaluation of Cancer Nanotheranostics

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

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

The US Food and Drug Administration

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Regulations of Combination Products and Companion Products

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

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

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

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

European Medicine Agency

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

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

Marketing of Cancer Nanotheranostics

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

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

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

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

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

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

Conclusion

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

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

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

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

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Chapter 13
Safety of Nanobiomaterials for Cancer Nanotheranostics

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Introduction

Cancer is the second leading cause of human death worldwide, with approximately 7.5 million deaths reported till date in 2020 (Cancer Statistics – Worldometer, n.d.. Globally, about one in six deaths is due to cancer (https://www.who.int/westernpacific/health-topics/cancer). As per the WHO, there might be approximately around 13 million deaths from cancer in 2030 (WHO | Key statistics, 2020). Cancer is one of the fatal diseases for which scientists have been battling for decades (Anand et al., 2020). The traditional therapy of cancer involves chemotherapy, radiotherapy, and surgery (Shukla et al., 2019). Chemotherapy kills both the normal and the healthy cells, also attributing to multidrug resistance (Brannon-peppas & Blanchette, 2004). Radiotherapy is appropriate for localized cancer, but it also lacks specificity, leading to toxicity and damage to neighboring cells (Lungu et al., 2019).

Cancer theranostics have combined action of diagnosis as well as a therapeutic effect (Gobbo et al., 2015). Nanotheranostics is a rapidly evolving area for tracking the delivery and release of drugs and therapeutic evaluation at the same time and efficacy by a single nanoscale carrier. Nanoparticles are modified to integrate different bioconjugated moieties for accurate detection and therapy (Gobbo et al., 2015; Indoria et al., 2020). The use of nanobiomaterials overcomes the drawback associated with the traditional methods. These materials are nontoxic, biocompatible, and biodegradable and allow controlled and sustained release of anticancer drugs. The major attractions include ease of size and charge manipulation, decrease in adverse effects, site specificity, and flexibility of route of administration (Pandurangan et al., 2016). Nanobiomaterials should interact with cancer cells without disturbing

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normal biological functions. In cancer therapy, they are useful in molecular imaging, early detection of cancer, bioinformatics, and targeting of cancer cells (Mody et al., 2016). These materials exhibit different properties in bulk form compared to when they are nanosized. The safety of the nanobiomaterials lies in the difference in this property (Tekade et al., 2018). This chapter addresses the thin line that exists between safety and toxicity of nanobiomaterials and will cover various nanobiomaterials used in cancer theranostics, their safety, evaluation parameters, and regulatory perspectives.

Nanobiomaterials Used in Cancer Theranostics

There are various nanobiomaterials used for cancer theranostics. For better understanding, they have been categorized into metal-based nanobiomaterials, polymeric nanobiomaterials, carbon-based nanobiomaterials, nanomaterials derived from natural origin, and protein-based nanomaterials. The metal-based nanomaterials include gold nanoparticles, silver nanoparticles, iron oxide nanoparticles, silica, selenium, titanium dioxide, and zinc oxide-based nanobiomaterials (Mody et al., 2016). Applications like bioimaging, phototherapy, gene delivery, drug delivery, and use as a biosensor are possible using the optical, surface, chemical, and electrical properties of gold nanoparticles. Gold nanoparticles can absorb near- infrared light and transfer light energy to localized surface plasmon resonance for bioimaging or as a photothermal agent in cancer theranostics (Jiang et al., 2012). The whole-body scan is possible using this optical property. Since epidermal growth factors express themselves in several cancers, one can conjugate gold nanoparticles with endothelial growth factor receptor

antibodies. Using these conjugates for imaging is possible with the help of a scanning confocal microscope (Guo et al., 2017). The oxidative stress induction and cytotoxic effects of gold nanoparticles make it a potential candidate for cancer therapy (Saravanan et al., 2019). Gold nanoparticles can serve as a suitable marker for increasing intratumor localization of anticancer drugs (Rejinold et al., 2015). The inherent optical property of silver nanoparticles makes silver interact with the wavelength of light (400nm) (Seeta et al., 2019). Compared to other nanobiomaterials, silver nanoparticles show better light absorption and resolution (Mihail et al., 2016). Silver alone or its amalgam with other metals can be used for medical imaging (Seeta et al., 2019). The nanosized silver nanoparticles can enter the tumor efficiently by passive or active targeting. They affect the mitochondria-producing reactive oxygen species, leading to oxidative stress and apoptosis of cancer cells. At 435 nm, it shows maximum cytotoxicity and anticancer activity due to activation of the apoptotic gene (Aydin et al., 2019). The capacity of iron oxide nanoparticles to bind with antibodies, chemotherapeutic agents, and nucleic acid also makes it a suitable candidate for cancer theranostics (Santhosh & Ulrih, 2013). Iron oxide nanoparticles (IONPs) are suitable as MRI contrast agents. These nanoparticles can bind covalently or noncovalently with the overexpressed cancer biomarker (Zhu et al.,

2016). They can also navigate through tumor margin, metastasis, and inflammatory areas and can find the location of angiogenesis, making it possible to visualize and predict the stage of cancer. IONP labeling of cells can help to determine the spread of immune-competent cells in the tumor (Singh & Sahoo, 2013) and for biosensing. The study by *Liu et al*. depicted that they are sensitive and precise in detecting biomarkers at higher spatial and temporal resolution. Using the phenomenon of magnetic fluid hyperthermia, one can apply external heat to IONPs to target the cancer cells. It will convert magnetic energy to heat energy and will selectively kill the cancer cells (Liu et al., 2013). Research has increased in the development of mesoporous silica nanoparticles (MSNs) due to their unique properties like high surface area, flexibility in size, and ability to modify functional groups. Imaging and drug delivery are feasible using these properties. A study by *Bobo et al.* showed a synergistic effect of using a photosensitizer, porphyrin, and a drug, camptothecin, in lecithin-targeted mesoporous silica nanoparticles (Gary-Bobo et al., 2011). *Cheng et al.* developed polydopamine poly(ethylene)glycol-folic acid-modified MSNs for delivering doxorubicin for the treatment of cervical cancer. This novel system has higher antitumor activity in vivo and is a promising carrier (Cheng et al., 2017). Selenium is one of the extensively used chemotherapeutic agents. It is due to the ability to regulate cell cycle, inhibit migration of tumorcells, stimulate apoptosis, and invade them in vitro. Selenium can enhance anticancer effect of photodynamic therapy, modulate growth-stimulating hormone systems, and decrease selenium-binding protein expression for personalized therapy for people suffering from hepatocellular carcinoma (Sanmartin et al., 2012). The investigation of difference in disulfide, thioether, diselenide, carbon, and selenoether bonds depicts that selenoether and diselenide bonds produce more reactive oxygen species (ROS) and enhance cytotoxicity of paclitaxel-citronella prodrug conjugate (Sun et al., 2019). Titanium dioxide can stop the growth of tumors and bring improvement in cancer therapy. Nanoformulations developed from titanium oxide affect the proliferation of cells by blocking cell cycle. It exhibits an increased cytotoxicity effect and potential dose-dependent effect on cell proliferation and leads to cell death. It also leads to decrease in ATP level, inhibition of apoptosis, and necrotic cell death (Raja et al., 2020). Sonodynamic therapy involves the use of titanium dioxide and zinc oxide. Using a conjugation of antibodies with titanium oxide and zinc oxide helps in targeting specific receptors. It also decreases the adverse effects associated with daunorubicin and doxorubicin (Çeşmeli & BirayAvci, 2019). A study by *Bai et al.* depicted that the use of 20 nm-sized zinc oxide nanoparticles induced considerable cytotoxicity in human ovarian cancer cells by induction of ROS, which affects cells by apoptosis, autophagy, and mitochondrial malfunction. Photodynamic therapy using zinc oxide produces ROS, leading to significant cytotoxicity in cancer cells, thereby increasing the selectivity and decreasing the adverse effects. It gets localized in tumor cells, and by focusing light on that region, selective and specific therapeutic action is achievable (Ancona et al., 2018).

Polymeric nanobiomaterials include poly(lactide-co-glycolic acid), polycaprolactone, chitosan, polylactide acid, polyethylene glycol-poly(lactic acid-co-glycolic acid) (PEG-PLGA), etc. PEGylated form of PLGA nanoparticles (NPs) increases

the therapeutic index of paclitaxel (Prabhu et al., 2015). The hyaluronic acid-based paclitaxel nano-lipid carrier gives a high therapeutic index and site specificity (Shukla et al., 2019). Chemotherapeutic drugs like paclitaxel, doxorubicin, docetaxel, camptothecin, and 5- fluorouracil are delivered using chitosan as a carrier. Conjugating chitosan with quantum dots, gadolinium, supermagnetic iron oxides, etc. is useful for imaging (Fernandez-Fernandez et al., 2011). PLGA has been used to encapsulate vincristine sulfate, dexamethasone, paclitaxel, doxorubicin, and cisplatin. It has completed its journey from benchside to bedside (Fernandez-Fernandez et al., 2011). Doxorubicin chitosan-polyalkyl cyanoacrylate nanoparticles show an increased therapeutic effect on cancer cells expressing folate receptors (Kumar et al., 2019). Polycaprolactone (PCL) can enhance the oral efficacy of the cytotoxic drugs – doxorubicin and paclitaxel. A PEGylated derivative of PCL was used as a nanoparticulate implant to be administered postsurgery. Polycaprolactone-hydrazine linkage releases the drug in acidic media with significant toxicity on cancer cells (Kumar et al., 2019). The cisplatin PEGylated polyglutamic acid micelles exhibited longer circulation time and accumulation in Lewis lung carcinoma cells. The treatment gave promising tumor regression, 20 times higher accumulation, and no weight loss in animal models (Prabhu et al., 2015).

Carbon nanomaterials consist of carbon nanotubes, carbon nanohorns, and fullerenes. Its graphene and carbon cage-like structure allows functional modification, imparts stability, and increases its drug-carrying capacity. Its unique property makes it flexible for incorporating both hydrophilic and hydrophobic drugs. Fullerenes being smaller in size can easily penetrate intracellularly (Yamashita et al., 2012). Drugs like paclitaxel and doxorubicin conjugated with fullerenes are efficient cancer theranostic. It is used as self-labeled probes designed for imaging, tracking drug delivery, mitigating adverse effects associated with chemotherapeutic drugs, and increasing selectivity toward cancer cells. The functional derivatives are capable of downregulating various angiogenic factors and suppress metastasis (Chen et al., 2012). Carbon nanotubes have the potential to penetrate the cell membrane and diffuse through lipid bilayer without killing normal cells. As per reports, it can selectively kill cancer cells using NIR light heating effect (Veerapandian et al., 2009), showing its utility in the field of thermal ablation and imaging. Gadolinium atoms are inserted inside carbon nanotubes for MRI and modified drug delivery (Mody et al., 2016). Carbon nanotubes take advantage of folic acid overexpression in cancer cells and get selectively bound to the surface of folic acid (Eskandari et al., 2014).

The natural origin-based nanobiomaterials include starch nanoparticles, alginate nanoparticles, pullulan nanoparticles, heparin-based nanoparticles, and silk fibroin (Mody et al., 2016). In the past decade, scientists have been attracted to natural polysaccharide like starch as a carrier. Due to the virtue of several hydroxyl groups, it can easily attach hydrophobic side chains and facilitate substitution, which enables the dissolution of the final product. It is biocompatible, biodegradable, nontoxic, non-immunogenic, renewable, economic, and biocompatible with drugs. Docetaxel is a potent chemotherapeutic drug. To tackle the low aqueous solubility, researchers

have encapsulated this drug in starch nanoparticles (Dandekar et al., 2012). In the experiment conducted by Li H *et al.*, the group

observed synergism in suppressing human lung cancer cells by simultaneously delivering doxorubicin and siRNA via folate-biotin-quaternized starch nanoparticles. The nanoparticles exhibited high cytotoxicity and inhibited proliferation in A549 cells and had specificity and potential in treating lung cancer (Li et al., 2019). Alginate is sensitive to acidic environment of the tumor vasculature. The polymer is being envisaged for its application in the design of smart cancer theranostics. Alginate-based magnetic nanogel by *Peng N et al.* caused rapid endocytosis and release of doxorubicin in the presence of a magnetic field. Also, it exhibited a desirable effect as an MRI and contrast agent (Peng et al., 2018). *Pei M et al.* discovered that in the acidic microenvironment of cancer, real-time noninvasive locating of cancer cells was possible (Pei et al., 2017). Pullulan nanoparticles have shown to prolong blood circulation time along with better stability and active tumor targeting. Pullulan-based doxorubicin nanoparticles developed by *Li H et al.* showed low cardiotoxicity. The group concluded that the developed pullulan-based doxorubicin nanoparticles not only improved the therapeutic efficacy but also eradicated chemoresistance and exhibited synergism effect compared to single-drug therapy (Li et al., 2015). *Hua et al.* loaded adriamycin-O-urocanyl pullulan nanoparticles to overcome drug resistance in cancer cells. There was higher cellular uptake of adriamycin due to avoidance of export by P-glycoprotein. This helped in reversing drug resistance in cancer cells (Guo et al., 2014). According to studies, heparin is a good anti-metastatic agent. It is due to heparinase, which inhibits metastasis or binding to growth factors or binding to platelets to expose circulating tumor cells to natural killer cells. The study performed by *Sun H et al.* displayed that doxorubicin-heparin had anti-metastatic activity and synergism (Sun et al., 2018). Yang and coworkers observed that heparin could overcome problems like low solubility, low selectivity, and improper release of drugs (Yang et al., 2017). Silk fibroin is obtained from silkworm. The biocompatibility, biodegradability, mechanical strength, and flexibility make it a suitable candidate for sustained release of drug. In a study performed by Montalban *et al.*, curcumin silk fibroin nanoparticles showed cytotoxicity in two different cell lines (Hep3B cells and Kelly cells) without decreasing viability in normal cells (Montalban et al., 2018). Since most of the anticancer drugs have poor aqueous solubility, a carrier like silk fibroin can increase bioavailability. Application of doxorubicin-silk fibroin film in residual tumor bed, after removal of tumor, has shown to prevent regrowth of tumor (Jastrzebska et al., 2015). Cisplatin-loaded silk fibroin nanoparticles could internalize in the A549 cell line and could exhibit significant inhibition (Qu et al., 2014).

Albumin is protein-based commonly used as nanobiomaterial. Abraxane is the marketed formulation of albumin-based paclitaxel formulation used in the treatment of breast cancer. It has high penetration and selective anticancer activity with minimum harm to normal cells. Being flexible, it exhibits the EPR effect (Shukla et al., 2019). In a study performed on albumin nanoparticles, it was observed that there was an increase in cytotoxicity when tested in MCF-7 and A549 cells along with prolonged distribution in tumor, leading to slower tumor growth and increase in

mice survival (Pandurangan et al., 2016). Concentration-dependent cytotoxicity is seen in MCF-7 cells by paclitaxel albumin nanoparticles. It gives optimal therapeutic efficacy with minimal side effects (Lomis et al., 2016). The combination of nano-albumin-bound paclitaxel along with gemcitabine for treating pancreatic cancer showed good antitumor activity and doubled the rate of survival. Nanoparticles also exhibited synergistic effect due to modulation of cytidine deaminase (Fanciullino et al., 2013).

Safety Aspects

Safety of Nanobiomaterials

It is of utmost importance for a formulation to be safe when administered to a patient. It is necessary to understand a thin line between safety and toxicity of these nanobiomaterials. Safety aspects of these nanobiomaterials are described in the following text.

The bulk form of gold is inert, biocompatible, and nontoxic; however, with size reduction to prepare nanoparticles, safety is compromised (Fratoddi et al., 2015). The cytotoxicity of gold nanoparticles depends on the shape and concentration. A study by *Steckiewicz et al.* illustrated that star-shaped gold nanoparticles were the most cytotoxic, whereas spherical forms were the least cytotoxic. The larger the size, the lesser is the cytotoxicity (Steckiewicz et al., 2019). There is no significant toxicity of tumor necrosis factor observed with colloidal gold delivery (Powell et al., 2010). The toxicity also depends on the surface charge. Serious and five times severe toxicity is observed in cationic compared to anionic nanoparticles. It is due to the interaction between negatively charged cell membranes and positively charged cationic, which leads to internalization and disruption of membranes (Jiang et al., 2012). Silver nanoparticles are safe and effective for cancer treatment. There is a thin line between the safety and adverse effects of silver nanoparticles, which can be governed by monitoring the physicochemical properties (Mihail et al., 2016). The size of the silver nanoparticles influences the cell viability, reactive oxygen species generation, and lactate dehydrogenase action. *Akter et al*. demonstrated that the generation of reactive oxygen species in the macrophage cell lines was more by nanoparticles of size 15 nm and less by nanoparticles of size 55 nm (Akter et al., 2018). As per findings, toxicity was due to the changes in biological media. It disrupted the mitochondrial respiratory chain, thereby increasing the ROS level. It also interfered with the production of ATP, causing DNA damage. It had also inhibited cell proliferation by activating signaling pathways (Singh et al., 2017). Safety is maintained if we ensure the controlled release of silver ions. An in vivo study using zebrafish described size-dependent toxicity, with 100% mortality by the end of 120 hours. Toxicity can be decreased by sulfidation of silver nanoparticles, thereby reducing the release of silver ions. Another approach was to coat the nanoparticles

with organic (citrates, proteins, polymers, etc.) or inorganic (carbonate, chloride, sulfide, etc.) capping agents. This coating stabilizes the nanoparticles, manages the surface chemistry, gives a proper shape, and reduces the amount of silver ions (Akter et al., 2018). In our body, iron oxide nanoparticles convert into elemental iron species for hemoglobin production. IONPs are safe up to a concentration of 200 μg/ml. A high dose of IONPs leads to ROS generation, affecting the normal functioning of the cell and cell apoptosis (Thomas et al., 2013). ROS generated in higher amounts leads to cell damage, DNA disruption, alteration in gene transcription, and protein alteration. The surface charge should be neutral. The cationic surface charge may lead to hemolysis and aggregation of platelets (Liu et al., 2013). Coating the surface will mask the oxidative sites, rendering the nanoparticles less reactive. For coating, various organic polymers like chitosan, poly(ethylene)glycol (PEG), dextran, and organic surfactants like sodium oleate, dodecyl amine, and inorganic metals can be used (Singh & Sahoo, 2013; Zhu et al., 2016). IONPs of 10–100 nm have promising pharmacokinetics (Zhu et al., 2016). Santhosh and Ulrich's study concluded that PEG-coated IONPs showed no cytotoxicity, and cytotoxicity was due to uncoated IONPs (Santhosh & Ulrih, 2013).While evaluating dextran-coated IONPs, there was a decrease in proliferation; and cell death was observed. It was due to the breaking of the dextran coat that exposes cells to iron oxide aggregates (Singh et al., 2010). These nanoparticles may get detached from the surface of tumor due to cell division or leakage, so it is advisable to study the clearance pathway in the initial phase to develop efficient targeted systems (Liu et al., 2013). The fluorescent mesoporous nanoparticles were compatible at therapeutic doses, and it reduces the associated toxicity of chemotherapeutic agents along with excellent tumor suppression (Lu et al., 2010). Selenium in large quantity leads to toxicity. Some chemical forms have reported genotoxicity, but there is insufficient reported data to claim it a carcinogen. At times, selenosis occurs; hence, long-term use is not recommended. The toxic level leads to hair loss, damage, or removal of nails and skin lesions and affects the nervous system (Brozmanová et al., 2010).

Titanium dioxide exhibits noticeable toxicity like genotoxicity and cytotoxicity in humans. The large surface area and redox activity also contribute to the toxicity. The intraperitoneal injection of titanium dioxide in mice results in acute toxicity like tremor, lethargy, loss of appetite, and passive behavior. It showed more toxic effect on the kidney compared to the liver (Chen et al., 2009; Jinyuan et al., 2009). Zinc oxide can dissolve in the extracellular region, which increases the intracellular level of zinc oxide, leading to toxicity. Toxicity can also be due to uptake of zinc oxide by cells, followed by its dissolution. The systemic exposure of zinc oxide leads to neurological effects (Pandurangan & Kim, 2015).

In a study on polymeric nanobiomaterials by *Jesus S. et al.*, the group concluded that oral toxicity associated with chitosan nanoparticles is ruled out, confirming compatibility of nanoparticles with the blood components. Dose-dependent toxicity is seen on intravenous (iv) injection. In various studies, there is a proportionate increase in ROS associated with chitosan nanoparticles. The generation of ROS is less in the nonlethal concentration (1%) of chitosan. Further, it is reported that

PLGA NPs did not exhibit toxicity on oral or intravenous (iv) administration. Only one study reports the toxicity of daunorubicin-PEG-PLL-PLGA nanoparticles (Jesus et al., 2019). The toxicity evaluation of cationic and anionic polyamidoamine in zebrafish demonstrated that cationic form caused cardiovascular dysfunction and decreased survival rate, whereas anionic form did not show such toxicity (Jia et al., 2019). As per findings, bovine serum albumin decreased cytotoxicity associated with PLGA (Razavi & Khandan, 2017). Literature also reports that there is toxicity associated with polycaprolactone (PCL) on intravenous (iv) and intraperitoneal (ip) administration (Garcia et al., 2014). Administering paclitaxel-tamoxifen in polyethylene oxide-polycaprolactone via the iv route had a significant anticancer activity with minimal toxicity (Prabhu et al., 2015).

The macrophage cells are inefficient in completely engulfing the long fibers of carbon nanotubes. It leads to the production of ROS and inflammatory response. Contents of metal impurity in carbon nanotubes (CNTs) also determine the carcinogenicity. Apart from this, particle length and width also have an impact on safety. CNTs having a large diameter or tangled ones are comparatively less toxic. The fabrication should be such that it is biocompatible, biodegradable, and water-soluble; otherwise, it will lead to chronic toxicity (Yamashita et al., 2012). If CNTs are present as aggregates, it becomes difficult for macrophages to recognize them, leading to potential systemic toxicity. Doping the surface can increase or decrease toxicity. The acid-oxidized CNTs induce more toxicity, whereas nitrogen doping decreases toxicity (Narei et al., 2018). Injecting multi-walled CNTs in zebrafish leads to long-term reproductive toxicity and a higher death rate. It may be due to metal catalyst residues that is not removed during purification (Jia et al., 2019). Using purification techniques like sonication in different media, treatment with hydrochloric acid, ion-exchange chromatography, etc. can tackle this problem (Eatemadi et al., 2014).

The blank starch nanoparticles are safe, and there is no significant effect on cell viability, even at a dose of 2 mg/ml (Dandekar et al., 2012). *Yu et al.* performed chemo-photothermal therapy to eradicate tumors using hydroxyethyl starch-based nanoparticle systems. It was biodegradable and biocompatible and had efficient and safe in vivo performance (Yu et al., 2019). *Zhao K et al.* also found hydroxyethyl starch nanoparticles safe compared to the free doxorubicin. The conjugate had lower organ toxicity; hence, long-term administration is possible (Zhao et al., 2017). Saralkar P and Dash A prepared curcumin-resveratrol alginate nanoparticles for evaluating the effect on prostate cancer cell line DU145. The blank nanoparticles were found to be safe as they did not cause hemolysis and showed cytotoxic effect on cancer cells (Saralkar & Dash, 2017). Alginate is biocompatible, biodegradable, nontoxic, and hemocompatible (Bhunchu & Rojsitthisak, 2014). On investigating pullulan, it did not show any alteration in liver and kidney tissues. Even the orally administered dose did not show significant signs of toxicity. There was no change in clinical findings even after 14 days of repeat toxicity study (Raychudhuri et al., 2020). The folate-conjugated pullulan acetate nanoparticles for cervical cancer did not exhibit mortality in the control as well as the experimental group. There is a minor change in vital organs, along with inflammation in experimental groups

(Tang et al., 2015). The long-term use of heparin leads to thrombocytopenia, osteoporosis, and bleeding in women. Also, it triggers the immune system, forms abnormal clots, and leads to myocardial infarction, stroke, and ischemia. Therefore, monitoring of patients is required (Hwang & Lee, 2016).The doxorubicin-silk fibroin hydrogel is safe and efficacious compared to iv doxorubicin in the treatment of breast cancer. It is efficient in reducing tumor growth and metastasis (Jastrzebska et al., 2015).

Albumin selectively accumulates in the tumor as the tumor cells require it to meet their increasing need of amino acids and energy (Li et al., 2020). Albumin is biodegradable, biocompatible, and non-immunogenic and has specificity for glycoprotein 60 receptor present in cancer cells. It allows the delivery of various anticancer drugs without inducing an immune response (Lomis et al., 2016). Drugs bound to albumin are likely taken up by cancer cells compared to normal cells rendering it safe. Usually, cationic polymer-based nanoparticles exhibit incompatibility and cause hemolysis and cytotoxicity. But, the bovine serum albumin nanoparticles do not damage RBC, cell line, or endothelial cells in vitro (Taguchi et al., 2013).

Importance of Dose of Nanobiomaterials

It is the dose that decides whether the outcome will exhibit a therapeutic effect or toxicity. It is necessary to determine a practically feasible dose from pharmacokinetic and pharmacodynamic studies. It is important to observe the effects of a high dose of nanobiomaterial as well as the toxicity due to long-term exposure. Also, determine appropriate dosage form, route of administration, dosing frequency, and exposure time, and establish safety protocols. There is a rare possibility of developing a neurodegenerative disorder, asthma, etc. due to exposure to high dose of nanomaterial. It necessitates the dose calculation of nanomaterial along with active pharmaceutical ingredients (Tekade et al., 2018). To explain the importance, we would like to quote certain examples. Using 10/20/50 nm-ranged gold nanoparticles can lead to liver damage. However, a single dose of gold nanoparticles did not lead to liver toxicity. Based on the observations of the same study, it was proposed that administering gold nanoparticles dose of 2.5 mg/kg after every 48 hours for 21 days did not cause any liver or brain toxicity (Pastoris Muller et al., 2017). In another example, the biotin-modified pullulan nanoparticle did not show apparent acute toxicity up to 200 mg/kg (Tang et al., 2015). The intraperitoneal injection of 1.5 g/kg of mesoporous silica nanoparticles leads to distress or death in mouse. It is attributed to high doses and size of nanoparticle (Lu et al., 2010). The use of selenium in low dose helps in cancer prevention, reduces inflammation, and regulates blood pressure, but intake of 300–700 μg/day leads to toxicity (Sanmartin et al., 2012).

Safe-by-Design Strategy for Developing Safer Nanotherapeutics

On seeing the adverse effects associated with nanomaterials, there is a need for efforts to minimize the risk during the development stage. Scientists have proposed a safe-by-design (SbD) strategy for developing safer nanotherapeutics (Yan et al., 2019). It is an innovative approach that stands on the pillars of safe materials, safe production, and safe use for maximizing safety while maintaining the efficacy of the final product (Schmutz et al., 2020).

The word "design" in safety-by-design does focus not only on the properties of nanomaterials that we modify but also on the entire process, the materials, as well as the final product. The implementation starts with defining the workflow of the project along with a schedule of evaluating the collected data of nanomaterial property, defining prerequisites, characterizing process, and product safety profile (Kraegeloh et al., 2018).

The product developed should be safe and meet all the regulatory requirements. Being a new concept, it is not a part of the ICH, FDA, or EMA guidelines (Schmutz et al., 2020). It follows REACH and OECD guidelines. REACH stands for Registration, Evaluation, Authorization, and Restriction of chemical substances. It emphasizes risk management by following three principles: evaluating the effect, assessing the exposure, and characterizing the risk. Effect evaluation involves the collection of data that affect toxicity like size, distribution of size, shape, surface area, aggregation, stability, surface property, and reactivity. As per reports, smaller particle size showed more toxicity due to greater uptake by cells. The positive charge exhibited more toxicity due to an increase in interaction with negative charge present on the biological membrane. Also, high ionic dissolution and rod-shaped nanomaterials caused damage to the cells. Exposure assessment identifies all the likely sources of exposure in the manufacturing process. Finally, risk characterization involves adapting a strategy for testing and managing the risk (Zielińska et al., 2020). The Organisation for Economic Co-operation and Development (OECD) has published a guideline on the quantitative structure-activity relationship for the environment, health, and safety (Schmutz et al., 2020). The OECD is used to develop nano-QSAR for developing a relationship between physicochemical properties of nanobiomaterials and observed desirable and undesirable effects. However, this technique requires more quantitative data of structure and chemical properties to develop a robust technique to co-relate the structure with the response (Yan et al., 2019).

Various strategies under safe-by-design can establish safety in products. It involves coating, doping, grafting, loading, optimizing size/shape, managing surface charge, reducing persistence, reducing interaction, and passivating defect site (Torres Andón & Fadeel, 2014; Yahaya & Zain, 2017; Yan et al., 2019; Reijnders, 2020). Coating involves encapsulating toxic material inside a biocompatible carrier to decrease the potential side effects (Yan et al., 2019). Coating the inorganic nanomaterial with polymers or silica decreases undesirable contact with biologics, while coating the rare-earth oxide with phosphate reduces the impact of damage caused

by phosphonates due to phosphate stripping. The coating of carbon nanotubes with poloxamer reduced lung fibrosis (Reijnders, 2020). The coated gold nanoboxes were capable of developing personalized nanosystems for treating lung cancer (Movia et al., 2014). Doping involves the addition of a small amount of foreign atoms to modify the electrical, optical, or magnetic properties. It leads to change in energy near the surface, causing charge separation which will interfere ROS generation and oxidative stress (Yan et al., 2019). As per literature, doping the copper oxide nanoparticles with 1–10% iron reduced cytotoxicity, rendering it safe to the environment (Naatz et al., 2017). Doping nano-silica with iron and titanium minimizes inhalation hazards (Reijnders, 2020). Grafting is the covalent attachment of targeting ligands to nanomaterials. There are two modes of grafting – "grafting-to" and "grafting-from." Grafting-to approach attaches reactive species to functionalized surface of nanomaterial, whereas grafting-from involves embedding nanomaterial inside a matrix (Yan et al., 2019). Grafting the carbon with small organic molecules reduced cytotoxicity. Loading is like grafting, but here it involves noncovalent bond formation. It helped in improving drug delivery and imaging (Reijnders, 2020). Next comes the optimization of properties. The cellular uptake and its distribution in the tissues depend upon the size of nanomaterial. The higher the uptake by the cells, the higher is the toxicity observed (Torres Andón & Fadeel, 2014). Studies have reported that the size of nanomaterials used in cancer should be in the range of 2–200 nm for having suitable half-life and accumulation in tumor via EPR effect (Yan et al., 2019). The small particle size in the range of 1–100 nm had a hazardous profile compared to bulk or large particle size formulation (Dekkers et al., 2020). The shape of nanomaterial will also determine the toxicity. The macrophages can efficiently engulf ellipsoidal-shaped particles compared to spherical nanoparticles. Nanoformulations that are in the form of needle or multi-walled nanotubes resist uptake of macrophages and cause damage to cellular membranes (Torres Andón & Fadeel, 2014). The shape of nanomaterial influences the stability, surface adsorption, transport, and absorption in the body (Zielińska et al., 2020). The surface charge of the nanocarrier is also responsible for the interaction with biological membranes. If the nanocarriers have net positive charge, it interacts with the negative charge of the cell surface. It increases the rate of internalization and associated toxicity of positively charged nanocarriers compared to negatively charged nanocarriers (Torres Andón & Fadeel, 2014). All the above discussed points were strategies to promote safety; in the following sections, we will discuss what parameters can be controlled or reduced to enhance safety of nanobiomaterials. Avoiding or reducing the use of toxic elements eventually decreases toxicity (Yahaya & Zain, 2017). Attempts to modify the oxidative state to alleviate reactivity of nanocarriers are required. One can reduce the release of toxic material from the matrix by optimizing Van-der Waals, coordination and ionic and covalent bonding present between matrix and nanoparticle with the aid of stabilizer or compatibilizers. There is a need to reduce the persistence of such materials or develop strategies to control their end life (Yahaya & Zain, 2017). Sometimes, the presence of defects like steps, kinks, corners, and edges have atoms possessing weak bonds that can lead to change in electronic structure and reactivity. Passivation of such defects

during the development stage inhibits such toxic reactions without hampering desired activity of nanomaterials. It is possible by simple coating, for example, zinc oxide, and iron oxide nanoparticles can be coated with silica shells to shield the reactive site on the surface. Such coatings impart stability and biocompatibility, thereby maintaining functionality (Yan et al., 2019).

Safe-by-design (SbD) also helps in risk assessment, addressing the uncertainty, indeterminacy, and responsibility toward the design. This uncertainty is managed by identifying the risk and defining the consequences if the risk occurs without ignoring the facts. Sometimes, the risk is due to scenario uncertainty; to resolve this, apply a safety factor that makes it several times safer than expectations. Another approach is substituting all the dangerous parameters with less dangerous ones. However, one must understand that it is not possible to control all the hazards, but addressing only known parameters develops more uncertainty. Ruling out the indeterminacy will make the design more adaptable. The operators should have adequate expertise and skills to improve safety. There should be constant self-improvement within workers to remove indeterminacy and exposure to unknown hazards. Lastly, there should be a sense of responsibility, to not only safeguard themselves but also protect the end user (Van De Poel & Robaey, 2017).

"NaNoREG" and "Prosafe" are European projects for guiding the industry for SbD of manufactured nanomaterial. It is a combination of the innovative management process, risk assessment, environment, health, and safety assessment with regulatory affairs and data management. It consists of four elements: innovative projects, safety dossier, safety profile, and SbD protocols. The objective is to transfer precautionary measures to practical actions. It involves the use of all the precautionary measures to eradicate uncertainty and associated risks that may hinder the product's entry to the market (Kraegeloh et al., 2018). NaNoREG has introduced an innovative approach for effective communication between regulators, researchers, decision-makers, and industry. It has developed SbD, on the stage-gate model, where the entire project is divided into various stages from the proposal of idea to its entry in the market and contains a gate between every stage and where decisions regarding cancellation, modification, or its entry into the next stage are taken (Micheletti et al., 2017). Another project named GoNanoBioMatSbD was developed from the SbD approach to deal with polymeric nanocarriers. It involves design of the material, evaluation, human health and environment risk, manufacturing and control, storage, and transport. The initial stage includes all the set of questions such as type of drug, its application, dose, and design of nanocarrier, which is an extensive literature search. It is followed by screening the model for toxicity with the aid of QSAR modelling. It is necessary to evaluate human risk at the initial stage with the help of literature search and toxicity modeling. The material design stage compares all the nanobiomaterials and attempts to maintain a proper balance between safety, efficacy, and budget. Then, it characterizes the polymer properties for optimizing the batch. It is tested for all types of toxicity, like immunotoxicity, carcinogenicity, and mutagenicity, and the endpoints are noted. All the environment risks

are listed, and after comparing all the nanobiomaterials, at least one nanobiomaterial is selected by the end of this stage. It is followed by manufacturing and control steps. It is also necessary to apply the good manufacturing practices (GMPs) and define critical quality attributes (CQAs) and critical process parameters (CPPs), scale-up, storage, and transportation measures (Schmutz et al., 2020).

Gold Standard for Safety Assessment

The use of zebrafish is considered a gold standard for safety assessment (Jia et al., 2019). The evaluation is done on zebrafish from the environment, health, and safety perspective of nanomaterial, which helps in risk assessment and framing guidelines on safety, precautionary measures, control, and strategy development to improve characteristics of nanobiomaterial and decrease the associated toxicity (Chakraborty et al., 2016). Zebrafish is gaining importance due to similarities with humans. Compared to rodents' models, it has more sensitivity toward toxins, and it develops a toxicity mechanism quickly. It is preferred due to small size, ease of handling, and less requirement of chemicals to determine toxicity (Kim et al., 2019). It is widely used in bioimaging to characterize the toxicity profile of nanomaterials. An experiment depicted that silver nanoparticles in the size range of 30–72 nm were able to diffuse in zebrafish embryos, leading to potential toxicity. To evaluate the cytotoxicity of gold nanoparticles, use the zebrafish model. The 20-day exposure of 16 and 55 μg/g dry weight of gold nanoparticles caused change in oxidative stress, neurotransmission, and mitochondrial metabolism. The evaluation of cytotoxicity of carbon nanotube was assessed using this model, which showed bioaccumulation of 16 L/kg wet weight of fish and biochemical alterations (Chakraborty et al., 2016). There was a disturbance in the behavior and development of zebrafish exposed to cadmium tellurium quantum dots. Titanium dioxide leads to neurotoxicity when it is in the form of nanoparticles compared to bulk titanium dioxide. The metal oxides interfered with hatching of zebrafish. Thus, to assess the safety of nanobiomaterials, the zebrafish response is evaluated (Haque & Ward, 2018).

Toxicology Study

The most common toxicological studies of nanomaterials involve analysis of physical and chemical parameters and in vitro, in silico, and in vivo evaluations. In vivo and in vitro toxicological studies are mostly carried out in animal models or cell models (Pandey & Mishra, 2019).

In Vitro and in Vivo Toxicology Study in Vitro Assessment of Nanomaterial Toxicity

The in vitro toxicology studies of nanobiomaterial involve the test for cytotoxicity, genotoxicity, apoptosis, and markers of oxidative stress. The benefits of in vitro study include reduced animal testing, faster analysis, and lower costs, and it is currently required to produce and confirm in vitro assays to determine nanomaterial toxicity. For evaluating the cytotoxicity of biomaterials, use multiple assays like cell membrane integrity, functionalization assay, and cell proliferation assay (Pandey & Mishra, 2019; Stone et al., 2009).

Table 13.1 gives a brief description of all assays for in vitro toxicology study.

In Vivo Toxicology

In vitro characterization used to estimate the nanotoxicity of nanomaterials is not sufficient to ensure complete human safety (Tekade et al., 2018). In vivo toxicology study is also commonly conducted on animal models like mice and rats. Zebrafish (Danio rerio) is also a popular model and has several distinct benefits in toxicological testing over its mammalian counterparts (Jia et al., 2019). The biodistribution, clearance, hematology, serum chemistry, and histopathology are among the evaluation techniques for in vitro toxicity. Biodistribution studies investigate the path of localization nanoparticles to the tissue or organ. Nanoparticles are detected in the killed or live animals through radiolabels. One can perform the clearance studies of nanoparticles to analyze the excretion and metabolism of nanoparticles at different intervals after exposure (Kumar & Sharma, 2017). The examination of alteration in serum chemistry and cell type following exposure of nanoparticles is another technique for in vivo toxicity evaluation. Studies have been conducted to evaluate the histopathology of the cell, tissue, or organ after exposure to determine the toxicity effect induced by nanoparticles (Lei et al., 2008). Histopathology examination determines nanoparticle accumulation in tissues such as the lungs, eyes, brain, liver, kidneys, heart, and spleen (Baker et al., 2008).

Biocompatibility Study

Biocompatibility is related to the capacity of a biomaterial to carry out its specific medical therapy role without having any unintended effect on the patient or effect of the patient on the therapy (WEBSTER et al., 2013). It determines the incompatibility with the biological system. The compatibility with blood is an important attribute to claim safety. Nanomaterial incompatibility with blood can result in protein complexes and complement activation of the system by forming a clot. Different

Table 13.1 In vitro toxicology studies to determine toxicity of nanomaterials and evaluate them for cytotoxicity, genotoxicity, apoptosis, and cell viability

Assay	Details	Merits	Demerits	Reference
Trypan blue dye exclusion assay	It helps in determining the cell viability. It involves assessment based on cell membrane integrity. The dead cells uptake the dye, whereas living cells do not uptake the dye	This method is easy to apply, economical, and widely used	This method cannot distinguish between apoptosis and necrosis. It has low sensitivity	(Strober & Diseases, 2019; Adan & Baran, 2016)
Lactate dehydrogenase (LDH) assay	It is used to assay the cellular cytotoxicity. It is a colorimetric technique which measures LDH enzymes released from dead cells. The released LDH is determined by a coupled enzymatic action which gives red-color formazan	This method is simple and reliable facilitates faster evaluation	It determines only the last apoptosis/ necrosis stage. It interferes with the culture media. The use of this method is limited to the compounds with low-serum or serum-free compounds	(Katriina Lappalainen et al., 1994), (Adan & Baran, 2016; Aslantürk & Aslantürk, 2018)
MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay	It determines cytotoxicity and cell variability by estimating activity of mitochondrial enzymes. The principle involves reduction of MTT to water-soluble insoluble purple formazan by mitochondrial NADH enzymes	It is easy and safe to use. It has high reproducibility. It is widely used and is superior to exclusion dye methods	There is significant well- to-well error is observed in this method. Formazan is insoluble in water. It is difficult to remove cell culture media	(Stone et al., 2009) (Langdon, 2003; Aslantürk & Aslantürk, 2018)

(continued)

Table 13.1 (continued)

Assay	Details	Merits	Demerits	Reference
XTT (sodium 3,3′ – [1(phenylamino)-carbonyl]-3,4-tetrazolinium]-3(4-mdthoxy-6-nitro) benzene sulfonic acid hydrate) assay	This method helps in determining cellular viability by estimating the activity of mitochondrial enzymes. These enzymes reduce XTT to water-soluble orange formazan	This method is more sensitive and easier compared to MTT	The outcome of this method depends upon the reductive capacity of viable cells with mitochondrial dehydrogenase activity	(Aslantürk & Aslantürk, 2018; Kuhn et al., 2003)
MTS (3-(4,5-dimethyl thiazol-2-yl)-5- (3-carboxymethoxyphenyl)-2-(4-sulphonphenyl)-2H-tetrazolium, inner salt) assay	This method determines cytotoxicity and cell viability. Here, the mitochondrial enzymes reduce the tetrazolium salt MTS to water-soluble formazan compound	This method has high precision. The identification of outcome is faster. This method is also cheap	The absorbance is influenced by incubation time, cell number, and cell type	(Malich et al., 1997), (Aslantürk & Aslantürk, 2018)
WST (water-soluble tetrazolium) assay	This method identifies the number of viable cells. The principle involves the reduction of tetrazolium salt WST to water-soluble formazan by mitochondrial enzymes in presences of electron acceptor like mPMS (1- methoxy-5-methyl-phenazinium methyl sulfate)	This technique has high reproducibility. It is safe and easy to use	The reflection of effect of addition of WTS-1, on testing at different endpoints is unclear	(Aslantürk & Aslantürk, 2018), (Adan & Baran, 2016)

(continued)

Table 13.1 (continued)

Assay	Details	Merits	Demerits	Reference
H3 Thymidine Uptake	This method helps in determining DNA synthesis and cell proliferation	There are no significant advantages associated with this method	This method has harmful effects. It is time-consuming and difficult to handle	(Madhavan, 2007), (Adan & Baran, 2016)
BrdU (bromodeoxyuridine) assay	This method helps in determining DNA synthesis and cell proliferation. The DNA synthesis can be determined by measuring the incorporation of BrdU in S-phase using a monoclonal antibody or BrdU ELISA	This method requires less time and equipment. It is suitable for simultaneous technique	BrdU is a potential carcinogen	(Leif et al., 2004; Adan & Baran, 2016)
Ki-67 antigen	This method helps in determining the DNA synthesis and cell proliferation. It gives information about the growth of tumor cell and the effect of drug on it	This method can determine all the phases of cell cycle and mitosis	This method is not suitable for formalin-fixed paraffin sections	(Scholzen & Gerdes, 2000),(Singhal et al., 2001), (Romar et al., 2016; Adan & Baran, 2016)

(continued)

Table 13.1 (continued)

Assay	Details	Merits	Demerits	Reference
ATP (adenosine triphosphate) assay	It determines cell viability and cytotoxic effects by estimating the ATP levels. The enzymes luciferase and luciferin react with cellular ATP producing light that can be measured using luminometer. The luminescence light is directly proportional to viability of cells	This method has very good sensitivity and is widely applicable. It has a short protocol to be followed.	This method cannot differentiate between cytotoxic and cytostatic cells	(Crouch et al., 1993; Mueller et al., 2004; Adan & Baran, 2016)
Ames test	This method determines genotoxicity. It uses *Salmonella Typhimurium* which has mutation in gene encoding histidine enzyme. Therefore, subjecting to toxic insult leads to reverse mutation This reverse mutation helps in identification of mutagen/ carcinogen	This method is approved by regulatory bodies. It is simple and gives faster results	This method is less suitable for bacterial agents. Sometimes, the prokaryotic models might reflect eukaryotic model	(Dusinska et al., 2012) (Arne Biesiekierski et al., 2018)

(continued)

Table 13.1 (continued)

Assay	Details	Merits	Demerits	Reference
Annexin-V assay	The method helps in the identification and quantification of apoptotic cells. Annexin-V binds to phosphatidyl serine on the surface of apoptotic cells. The flow cytometer allows identification of both apoptotic and necrotic cells	This method can identify the type of cell undergoing apoptosis. It can also determine the early phase of apoptosis	In this method, the intensity andthe patterndiffer depending on efficacy of injection protocol. This method is expensive sometimes	(Kumar & Sharma, 2017) (Michiko et al., 2002)
TUNEL (terminal transferase dUTP labelling) assay	The method helps in the identification and quantification of apoptotic cells. The assessment is possible due to the selective binding to the fragmented end of DNA strand	This method can detect the concentrated DNA fragments. The commercial kits of this test are available	The method sometimes gives false positive results. Therefore, there is a need of additional assay to confirm the results. It is time consuming and expensive	(Shmuel, 1992) (Arne Biesiekierski et al., 2018) (Michiko et al., 2002)
Comet assay	It helps in the detection of DNA damage and repair The principle is based on separation of DNA fragment using gel electrophoresis. The relative intensity of tail of comet to its head accounts for the DNA damage	This technique has high sensitivity. It requires small number of cells per cycle	This method does not detect aneugenic effect and epigenetic effect of DNA. It cannot detect DNA fragments from apoptosis and necrosis	(Sligo et al., 2018) (Costa & Paulo Teixeira, 2014)

nanomaterials have been documented to induce a hemolytic impact through different mechanisms, such as oxidative damage to the membrane, changes in osmotic stability, enzymatic modifications, and alterations in the physical properties of blood (Pandey & Mishra, 2019). Different assays, such as bleeding time, clotting time, prothrombin time, thrombin time, and activated partial thromboplastin time, are useful for analyzing the influence of nanomaterial on extrinsic and intrinsic pathways of blood (Tekade et al., 2018).

Risk Assessment of Nanomaterials

Risk assessment involves identifying the potential of risk, usually by giving a score or ranking. The main aim of risk assessment is to provide details that will be useful in evaluating substitutes (Hegde et al., 2015). To choose any nanomaterial, the human health risk assessment must be correlated with exposure to hazard assessment (Jesus et al., 2019). Exposure assessment is an estimation of the concentrations or doses that the human population experiences through the environment or environmental compartments (Hegde et al., 2015). Human exposure to nanomaterials is possible via numerous routes at various phases of nanomaterial synthesis (Sligo et al., 2018).The different exposure routes include respiratory, oral, ocular, dermal, and parenteral route (injectable and implantable). We will further discuss the most prominent exposure routes and characteristics of NMs (Sharma et al., 2016). The respiratory system is the most popular route of exposure for ENM in the occupational environment. The particle size in the respiratory system has a significant effect on their distribution and lung aggregation (Kreyling et al., 2009; Pietroiusti et al., 2018). In the alveolar area, particulate size around 20 nm has the largest percentage of rate of deposition, and a size less than 55 nm will reach the alveoli more successfully compared to particle size 200 nm or larger. Nanomaterials with a positive charge show higher interaction with the negative charge of mucus, thus avoiding fast mucociliary clearance (Jesus et al., 2019). The skin is the largest organ in the human body and hence has a potential role in dermal exposure to ENMs. Estimates of potential dermal exposure to generate ENMs have been recorded in the workplace. However, there is no convincing evidence for the entry of ENM into systemic circulation by intact or even injured or inflamed skin. However, dermal penetration may lead to nanoparticles penetrating the skin's superficial layers, the dermis, causing a local inflammatory reaction (Gulson et al., 2010). For customers, the gastrointestinal path is theoretically important. However, at least in contrast to the pulmonary path, oral exposure was lower in staff. It is notable that a large proportion of nanoparticles inhaled are cleared into the oral cavity by the mucociliary escalator cells and then ingested into the gastrointestinal tract (Pietroiusti et al., 2018). The absorption depends on several variables, such as the

form of nanoparticles, and essential physicochemical characteristics: particle size, dispersibility, and charge (Patricia, 2015). The particles having a diameter of about <50 nm and <500 nm cross epithelial barriers through paracellular and endocytosis. The nanomaterial having a positive charge has more affinity toward the intestinal mucus; hence, retention is more, and absorption is less. Neutrally charged nanomaterials diffuse more efficiently through the mucus layers s(Jesus et al., 2019). Hazard assessment is an evaluation of the nature and severity of biological effects (typically in toxicology studies). The hazard assessment concept is the same for nanomaterials as for other substances (Kuempel et al., 2012). Hazard evaluation of nanomaterial is done by using various toxicology tests and assays. Determination of hazard by using experimental testing helps to identify the properties of a chemical or substance and its potential that leads to harmful health effects of human, terrestrial, or aquatic organisms (Hegde et al., 2015).

Risk Management of Nanomaterials

Risk management for nanomaterials is assumed as naturalistic (Murashov, 2015). Risk management pays attention mostly to choosing and implementing the most appropriate risk monitoring step. It is widely seen that conventional structure and devices for risk management systems do not cover all problems related to developing, handling, and use of engineering nanomaterial. Hence, it is required to develop a new approach to become more responsive to the nano-specific problem (Marchant et al., 2008). There are a variety of technical documents and recommendations released by international organizations and standard-setting bodies that advise on risk management problems and control measures associated with ENMs. According to the risk management strategy, all possible hazards and exposures are determined, tested, and evaluated (Oksel, 2017). The most effective hazard control strategy is based on (1) limiting, substituting, and modifying the nanomaterials, (2) the engineering process to minimize or eliminate exposure to the nanomaterials, (3) implementing administrative controls that limit the quantity or duration of exposure to the nanomaterials, and (4) providing for use of PPE (NIOSH, 2012). The Control of Substances Hazardous to Health (COSHH) regulation, which requires employers to properly control the occupational exposure to all chemicals used in the workplace concentrates on preventing or reducing exposure to hazardous substances by controlling equipment, procedures, and worker behavior, demonstrating the clear importance given to management controls (e.g., supervision and training to reduce exposure; Oksel, 2017).

Regulatory Aspects

Legal Requirements

Cancer theranostics is one of the emerging field of cancer treatment and has multiple functions, such as diagnostic and therapeutic functions, targeted and regulated release of medicinal agents, and effectiveness of therapy (Svenson, 2013). Regulatory approval of pharmaceutical drug products for human use, particularly those that are biological products in which a nanomaterial in the finished products is present, needs extensive toxicology and safety studies. The same is applicable for any newly developed cancer theranostics. This can be a challenging job, as size, shape, composition, surface properties, loading of drug, dosage, route of administration, biodistribution, and pharmacokinetics are all variables that can influence the toxicity profiles (Cole et al., 2011).

Nanotechnology is used in a wide variety of products governed by the FDA, such as human drugs and biologics. Products containing nanobiomaterials have quality characteristics which differ from those products that do not contain nanobiomaterials and therefore require analysis. The guidance document and review processes provided by the FDA addresses issues such as public health impact, safety, effectiveness, or the regulatory status of pharmaceutical products containing nanomaterials on case-by-case basis; and the guidance provided should be used as supplementary with other documents ('Guidance for Industry Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology', 2011; FDA/CDER/"Yeaton, 2017).

It includes characterization of the nanomaterial, understanding of the expected use and application of the nanomaterial, and how nanomaterial attributes contribute to product quality, safety, and efficacy; it is also an effective structure for assessing potential risks associated with nanomaterial-containing drug products. All drugs including finished drug products and drugs that are subject to OTC monograph regulations should be manufactured under current good manufacturing practice (cGMPs) as mentioned in the following sections: 501(a)(2)(B) of the Food, Drug, and Cosmetic Act (FD&C Act); 21 CFR parts 210, 211, and 212; and the regulations in 21 CFR parts 600–680. Building a knowledge base to better understand potential threats to product safety, identification, strength, consistency, and purity characteristics during the manufacture of nanomaterial-containing drug products is important for robust control strategies and successful process validation protocols to be put in place (FDA/CDER/"Yeaton, 2017; Guidance for Industry Considering Whether an FDA- Regulated Product Involves the Application of Nanotechnology, 2011).

The current FDA guidance on determining new excipient safety is applicable when a standard excipient is intentionally transformed into a nanoscale material. An appropriate safety assessment is required when existing safety data does not completely demonstrate the safety of nanomaterials with regard to exposure period,

exposure level, and route of administration. In case, a typical excipient has been deliberately modified to be a nanomaterial or inserted into a nanomaterial; it is important to research the effect on safety and exposure of the materials (Guidance for Industry Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology, 2011).

For Nonclinical Studies

All current guidelines from the International Council for Harmonisation (ICH) on nonclinical safety studies of the drug product as well as the components are generally applicable to nanomaterial-containing drug products. In terms of safety, newly developed drug products containing nanomaterials should be carefully analyzed:

(a) absorption, distribution, metabolism, and excretion (ADME) – Nanomaterial drug products including excipients as drug carriers' biological fate and possible safety impacts in addition to active ingredient are required to be studied. Radiolabelled or fluorescence of nanomaterial will help in biodistribution studies of materials.
(b) Risk assessment for routes of administration – While evaluating safety of a drug product containing nanomaterials, the following route-specific issues should be addressed and may require special evaluation in addition to the nonclinical studies usually performed in support of drug product production.
(c) Testing of representative nanomaterial – Before conducting toxicity studies, the nanomaterial to which the human is exposed should be known; and different factors, media, and in vitro and in vivo solvents that affect the aggregation and surface properties of the drug should be understood. Adequate validated method of analysis should be employed to examine the test articles used in nonclinical studies. In general, such nonclinical evaluations, normally carried out to support the manufacture of any drug product, will be sufficient to evaluate nanomaterial-containing drug products when the clinical content is tested in nonclinical studies.
(d) Bridging toxicology (a drug product not containing nanomaterials to a drug product containing nanomaterials) – When an existing approved drug product is changed to nanomaterial ADME and a bridging toxicology analysis may be adequate and necessary to allow reliance on prior nonclinical expertise, provided that other regulatory requirements are met. Consideration should be given to the effect of the transition on the drug ADME and the possible effects of the transition on toxicity (FDA/CDER/"Yeaton, 2017; Guidance for Industry Considering Whether an FDA- Regulated Product Involves the Application of Nanotechnology, 2011).

For Clinical Studies

Nanomaterial-containing drug products should be manufactured according to all policies and guidelines that apply to the NDA, ANDA, IND, BLA, clinical efficacy, and safety studies (Narang et al., 2018).

505(b)(2) Submissions – For the NDA (New Drug Application) submitted under section 505(b)(1) and approved by 505(c) section (Guidance for Industry Considering Whether an FDA- Regulated Product Involves the Application of Nanotechnology, 2011)

505(j) Submissions – Approval for generic product referencing a nanomaterial drug product can be applied by submitting an ANDA under section 505(j)of FD&C Act (Guidance for Industry Considering Whether an FDA- Regulated Product Involves the Application of Nanotechnology, 2011).

351(k) Submissions – For the development of a biological reference product containing nanomaterials, existing guidelines on biosimilars should be followed. As part of product development, the contribution of the nanomaterial to product potency, safety, and purity should be assessed. Sponsors are encouraged to approach the FDA early on to develop nanomaterial-containing biosimilars (Guidance for Industry Considering Whether an FDA- Regulated Product Involves the Application of Nanotechnology, 2011).

Bioanalytical Methods – After the administration of products containing nanomaterials, the clinically important elements, i.e., the parent drug and major active metabolites, should be calculated in the required biological matrices. It is recommended to use verified, relevant, and highly sensitive methods for examination of free and nanomaterial-associated drugs (Guidance for Industry Considering Whether an FDA- Regulated Product Involves the Application of Nanotechnology, 2011).

In vitro Tests – The following parameters such as biocompatibility, plasma protein binding, stability, in vitro clearance, and metabolism should be carried out with human biomaterials (Guidance for Industry Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology, 2011).

Immunogenicity – Applications for general guidelines for risk reduction associated with adverse immune responses shall address the FDA Guidance on the Evaluation of Industry Immunogenicity for Therapeutic Protein Products and the ICH Guideline S8 Immunotoxicity Research for Human Pharmaceuticals on sample approaches. Assessments of the probability of immunogenicity of biological products having a nanomaterial nonbiological component should consider the adjuvant properties of the component. Accordingly, the biological products that contain a nanomaterial component may have different immunogenic properties, which are important to be assessed (Guidance for Industry Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology, 2011).

Environmental Impact Considerations

The National Environmental Policy Act (NEPA) requires federal agencies to determine the environmental effects and to ensure that environmental analysis is made known to the concerned and affected public. Applicants must submit an environmental assessment (EA) or claim categorical exclusion [21 CFR 25.15(a)]. The information registered in EA will be reviewed by the CDER (Center for Drug Evaluation and Research) or CBER(Center for Biologics Evaluation and Research) to decide if it is reliable, or the proposed action may have a substantial effect on the quality of the human environment. They encourage industries to notify them in early development phase of their plan to either demand a categorical exclusion or apply for an EA in order to assist them in decision-making and late-cycle information requests (Guidance for Industry Considering Whether an FDA- Regulated Product Involves the Application of Nanotechnology, 2011; Narang et al., 2018).

Global Regulatory Strategy

Organisation for Economic Cooperation and Development (OECD)

Within its chemical safety framework, the Organisation for Economic Cooperation and Development (OECD) initiated a strategic initiative in 2006 which provides a global forum to discuss and support responsible development of nanomaterials processed, in particular their safety assessment and risk assessment. They established Working Party on Manufactured Nanomaterials (WPMN). It facilitates global collaboration on aspects of the human health and environmental protection of manufactured nanomaterials and focuses on the production of suitable methodologies and techniques to ensure the safe use of nanotechnology (Rauscher et al., 2017).

They are divided into six groups, namely, environment, health, and safety research strategy on manufactured nanomaterials, development of research database nanomaterials, safety testing of a set or representative manufactured, cooperation on voluntary schemes and prevention, manufactured nanomaterial test guidelines, and cooperation on risk assessments and exposure measurement (Park & Yeo, 2016).

Data obtained in accordance with the guidelines are covered by the Mutual Acceptance of Data (MAD) agreement of the OECD for the assessment of chemicals. MAD is a critical component for international harmonization of chemical safety approaches by regulatory acceptance of these test guidelines. Therefore, MAD also includes data on nanomaterials collected following OECD test guidelines that are specific to nanomaterials (Rauscher et al., 2017).

International Organization for Standardization (ISO)

ISO was established in June 2005 and is composed of 33 member countries and 15 observing countries. The ISO/TC 229 conducted ISO standardization of nanotechnologies based on four working groups (WGs): terminology and nomenclature (WG 1), measurement and characterization (WG 2), health safety and environmental aspects of nanotechnologies (WG 3), and material specifications (WG 4). It plays a significant role in developing basic framework for global nanotechnologies for risk assessment, risk control, and standardization (Park & Yeo, 2016).

Emerging Green Nanomaterial Approach for Cancer Theranostics

Researchers are moving toward safer and environment friendly approaches, which are not only safe for the environment but also for health. Many advances had been made in research toward a greener approach, where natural plant extracts, sources, and microorganisms are used for the synthesis of biogenic nanoparticles. The manufacturing of nanoparticles can be carried out using three methods: physical, chemical, and biological (green) methods. The conventional methods, physical and chemical methods, have certain limitations such as high energy consumption, low production rate, use of toxic and hazardous chemicals, instability, huge production cost, and environmental and health hazards (Si et al., 2020). The alternative methods are biological methods based on green nanotechnology (Saravanan et al., 2019). The advantages of green nanotechnology over conventional are low cost production, less energy consumption, use of renewable sources, resolving sustainability problems of climate change, reduction in use of toxic chemicals, simplicity of handling, and biodegradable yet recyclable products (Barry, 2019; Si et al., 2020). In the last few years, metal nanoparticles such as gold, silver, platinum, palladium, selenium, zinc, and copper have proven to be of great interest in the field of cancer theranostics.

Plants Used as a Natural Source for Green Approach

The phytochemicals extracted from the plant source are useful in combating cancer. Phytochemicals present in the medicinal plants had shown cytotoxic effects against cancer cells. Though phytonanotechnology has high potential in synthesizing biogenic nanoparticles, the exact mechanism of the phytosynthesis is yet to be understood (Saravanan et al., 2019). Plant parts such as roots, leaves, and barks are

collected, washed, and cut into small pieces for extraction under sterile conditions. The extract is purified by filtration and centrifugation. Extract, metal salt, and water are incubated for the growth of nanoparticles. The natural compounds used are starch, glucose, chitosan, sucrose, and calcium alginate as reducing and/or capping agents. Nontoxic, biodegradable polymers such as polyethylene glycol(PEG) and carboxymethyl cellulose(CMC). Nanoparticles synthesized are generally spherical in shape (Noruzi, 2015). Reduction and agitation methods, choice of suitable types of protecting agents, and concentration; synthesizing conditions such as pH, concentration of reductive biomass, temperature, and time; and use of alternative energy sources such as ultrasound and UV light are some of the factors responsible for the sizes and shapes of nanoparticles (Barry, 2019).

Microorganisms Used as a Natural Source for Green Approach

Various microorganisms such as yeast, fungi, bacteria, and algae are studied and used for synthesis of biogenic nanoparticles. One of the drawbacks of using microorganisms is that they require long incubation period for reduction, whereas the plant-based synthesis is quick. The use of microorganisms also has biosafety issues, where they are resolved in green synthesis using plant extracts (Ovais et al., 2016).

There are two approaches of synthesis, i.e., intracellular and extracellular. The advantage of extracellular process over intracellular process is that it is devoid of downstream processing steps. Downstream processing steps in an intracellular process are the recovery steps. It includes sonication, centrifugation, and washing steps for purification of nanoparticles. There are some important factors that play a crucial role in synthesis. Some important factors play a crucial role in synthesis, including metal-resistant agents, proteins, peptides, enzymes, reducing cofactors, and organic materials that play a role of a reducing agent (Soni et al., 2019).

For nanoparticles synthesis, the widely used bacterial species include *Actinobacteria* sp., *Escherichia coli*, *Klebsiella pneumonia*, *Lactobacillus* spp., *Bacillus cereus*, *Corynebacterium* sp., and *Pseudomonas* sp. (Soni et al., 2019).

In recent years, the studies have confirmed the biocompatibility and effectiveness of green nanoparticles and can be used as theranostic agent for cancer. The anticancer potential of phytosynthesized metallic NPs has grown over the past decade, with relatively little research on their genotoxicity, pharmacokinetics, pharmacodynamics, and safety profiles alone or in combination with others. The studies conducted on in vivo models elicit potential value of green nanoparticles, and future research would allow us to conclude more on the anticancer activity of green nanoparticles (Saravanan et al., 2019).

Conclusion and Future Aspects

The use of nanobiomaterial in cancer theranostic is an innovative approach toward cancer diagnosis and therapeutics. This dual-purpose technique aims at interacting with the cells such that there is no interaction with the normal cells, thereby promoting selectivity and sensitivity toward cancer cells and reducing the duration of cancer treatment. There are various nanobiomaterials used in cancer cells, briefly divided as metal-based, polymer-based, derived from natural origin, carbon-based, and protein-based nanobiomaterials. These nanobiomaterials show promising results in cancer therapy if used properly. Scientists need to optimize the physicochemical properties and the dose of these nanomaterials to achieve maximum safety and minimal toxicity. Though toxicity cannot be eradicated, efforts can be made to make the formulation safe for end users. Researchers have adopted the use of the "safe-by-design" strategy that not only focuses on the physicochemical properties of these materials but also considers the entire process, material, and final product. The REACH guideline emphasizes evaluating the effect, assessing the exposure, and characterizing the risk, whereas the OECD guidelines suggest using QSAR modelling to establish a relationship between physicochemical properties and observed effects. Various techniques under SbD include coating, grafting, loading, doping, optimizing size/shape/charge, reducing persistence, and passivating defects. NaNoREG has developed SbD on the stage-gate model, whereas GoNanoBioMatSbD was developed for dealing with polymeric NBM. Both were concerned about the product right from the initial stage to its entry into the market.

The in vitro evaluation of toxicology involves cell membrane integrity assay, trypan blue dye exclusion assay, lactate dehydrogenase assay, metabolic activity assay, MTS assay, MTT assay, XTT assay, cell proliferation assay, and assay for genotoxicity. In vivo evaluation involves various animal models, but zebrafish is preferable due to its peculiar characteristics. Biocompatibility studies are performed to evaluate if any incompatibility exists with the biological system. The various routes by which exposure to nanobiomaterials occurs involve pulmonary, dermal, oral, ocular, and parenteral root. Optimizing the size, shape, and surface charge minimizes the exposure and associated exposure. As per the Control of Substances Hazardous to Health (COSHH) regulation, the employers should properly control the occupational exposure to all chemicals used in the workplace. They should concentrate on the prevention and/or reduction of exposure to hazardous substances by controlling worker behavior, equipment, and procedures.

As per the FDA, characterization of the nanomaterial, understanding of the expected use and application of the nanomaterial, and how nanomaterial characteristics contribute to product safety, efficacy, and quality are an effective structure for assessing potential risks associated with nanomaterial-containing drug products. The current FDA guidance on determining new excipient safety applies where a typical excipient is intentionally transformed into a nanomaterial. An appropriate safety assessment should be ensured when existing safety data regarding exposure level, duration of exposure, and route of administration do not completely

demonstrate the safety of the nanomaterials. New products containing nanobiomaterials should be tested for ADME and risk considerations for the route of administration, testing nanobiomaterial for in vivo, in vitro, vehicle, media, and surface properties, thereby bridging regulations of conventional dosage forms and nanoformulations. They should follow clinical trials similar to the recommendations for the IND, NDA, ANDA, and BLA. The FDA also requires applicants to submit an environmental assessment or some similar document. The OECD facilitates international collaboration on aspects of the human health and environmental protection of processed nanomaterials and focuses on the production of suitable methods and techniques to ensure the safe use of nanotechnology. ISO also plays a significant role in developing a basic framework for global nanotechnologies for risk assessment, risk control, and standardization.

Advances in research are being achieved by moving toward the use of green technology. The advantages of green nanotechnology over conventional are low cost production, less energy consumption, use of renewable sources, resolving sustainability problems of climate change, reduction in the use of toxic chemicals, simplicity of handling, and biodegradable yet recyclable products. The in vivo studies of green nanoparticles elicit its potential for developing safe nanotherapeutics in the future.

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