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Receptor-Targeted Surface-Engineered Nanomaterials for Breast Cancer Imaging and Theranostic Applications

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ABSTRACT: Breast cancer is one of the most frequently diagnosed cancers in women and the major cause of worldwide cancer-related deaths among women. Various treatment strategies including conventional chemotherapy, immunotherapy, gene therapy, gene silencing and deliberately engineered nanomaterials for receptor mediated targeted delivery of anticancer drugs, antibodies, and small-molecule inhibitors, etc are being investigated by scientists to combat breast cancer. Smartly designed/fabricated nanomaterials are being explored to target breast cancer through enhanced permeation and retention effect; and also, being conjugated with suitable ligand for receptor-mediated endocytosis to target breast cancer for diagnostic, and theranostic applications. These receptor-targeted nanomedicines have shown efficacy to target specific tumor tissue/cells abstaining the healthy tissues/cells from cytotoxic effect of anticancer drug molecules. In the last few decades, theranostic nanomedicines have gained much attention among other nanoparticle systems due to their unique ability to deliver chemotherapeutic as well as diagnostic agents, simultaneously. Theranostic nanomaterials are emerging as novel paradigm with ability for concurrent delivery of imaging (with contrasting agents), targeting (with biomarkers), and anticancer therapeutics with one delivery system (as cancer theranostics) and can transpire as promising strategy to overcome various hurdles for effective management of breast cancer including its most aggressive form, triple-negative breast cancer.

KEY WORDS: breast cancer, receptor, ligand, active targeting, imaging, nanoparticles

ABBREVIATIONS: BRBP1, brain metastatic breast cancer cell-(231-BR)-binding peptide; **DOX**, doxorubicin; **EGFR**, epidermal growth factor receptor; **EPR**, enhanced permeability and retention; **FA**, folic acid; **FR**, folate receptor; **FSiNP**, fluorescent silica nanoparticles; **HA**, hyaluronic acid; **HER2**, human epidermal growth factor receptor 2; **ICG**, indocyanine green; **IONPs**, iron oxide nanoparticles; **LHRH**, luteinizing hormone-releasing hormone; **MDR**, multidrug resistance; **MMC**, mouse mammary carcinoma; **MRI**, magnetic resonance imaging; **NIR**, near-infrared; **NPs**, nanoparticles; **PAMAM**, poly(amidoamine); **PA**, photoacoustic; **PT**, photothermal;

PAI, photoacoustic imaging; **PAT**, photoacoustic tomography; **PEG**, polyethylene glycol; **PLA**, polylactic acid; **PLGA**, poly (D, L-lactide-co-glycolide); **QDs**, quantum dots; **RGD**, arginine-glycine-aspartic acid; **scFv**, single-chain fragment variable; **SPIONs**, superparamagnetic iron oxide nanoparticles; **Tf**, transferrin; **TfR**, transferrin receptors; **TNBC**, triple-negative breast cancer; **TPGS**, D-α-tocopheryl polyethylene glycol 1000 succinate; **VEGF**, vascular endothelial growth factor; **VEGFR**, vascular endothelial growth factor receptor

I. INTRODUCTION

Breast cancer is one of the most common cancers in females, with an annual incidence of about 2.3 million in 2020. It has been associated with the largest number of cancer-related deaths in women and caused approximately 685,000 deaths, accounting for 15% of all cancer-related mortality in females. It has been recently declared as the world's most prevalent cancer with a count of 7.8 million cases in the last five years by the WHO in March 2020.[1] While the incidence rates of breast cancer are on the rise in almost every region, the rates are relatively higher in the developed world.[2] Recurrence is a major concern in the treatment of breast cancer and patients with metastatic breast cancer show a mean five-year survival rate of 27%.[3] The prognosis of breast cancer is dependent upon the extent of expression of three receptors namely human epidermal growth factor receptor-2 (HER2/Neu/ErbB2), estrogen and progesterone receptors.[4] Heterogeneity in their molecular expression in different solid tumor tissue makes it difficult to diagnose and treat breast cancer. Triple-negative breast cancers (TNBC) which do not express any of these targets, i.e., estrogen, progesterone, or HER2 receptors are the most challenging to treat as these show a poor response to both HER2 targeted and hormonal therapies.[5,6] Breast cancer therapy has advanced over time from monotherapy to a combinational approach. However, the development of multidrug resistance (MDR) has also emerged as a huge challenge in the treatment of breast cancer. MDR to breast cancer occurs through various complex mechanisms mediated by cellular and non-cellular pathways.[7,8]

Various advanced approaches such as nanotechnology, precision medicine, and three-dimensional (3D) printing have recently emerged as an important area of interdisciplinary research that has the potential to offer solutions for a variety of unresolved disease issues.[9–15]

Further, the advent of novel metallic, lipids, and polymeric nanoparticles (NPs), as well as consequent smart design and development of targeting ligand-based NPs have shown the utility of nanotechnology in the last two decades in the treatment of cancer as well as other diseases including infectious diseases, cardiovascular and brain disorders, etc.[16–20] The main objective of nanocarrier-mediated anticancer drug delivery are, to enhance the drug concentration in the cancerous tissue through active and passive targeting, containment of drug during transit in the body, reducing its distribution in the normal tissues through improvement in its pharmacokinetic profile, facilitate intravenous administration by improving drug solubility, provide better stability and prevent degradation, improve bioavailability and patient compliance, and to enhance cellular uptake/intracellular delivery of anticancer agent.[21–25]

Nanotechnology-based carriers have been widely explored to reduce the dose of anticancer therapeutics by preventing its off-target accumulation using active and passive

targeting approaches. Engineered NPs can also be developed to vitalize the conventional cancer therapeutic modalities.[26,27] Multifunctional NPs have the unique strength that they can carry multiple payloads including therapeutic and diagnostic agents or their combination (i.e., theranostic) for the treatment as well as to evaluate the progress of therapy,[28] as depicted in Fig. 1. This parallel multifunctional property opens a new horizon of opportunities in cancer diagnosis and therapy that have been conceptualized recently.

Breast cancer is one of the major thrust areas for which various nanotechnological therapeutic approaches are being explored and some of the NPs have shown promising results and even reached the clinical stage for therapy of resistant breast cancer cases. For example, several liposomal formulations of doxorubicin (DOX) have been designed and developed to improve its therapeutic index without altering its efficacy.[29] Thus, nanotechnology-based drug targeting approaches can be further explored to resolve long-standing pitfalls in the treatment of breast cancer for a better prognosis of the therapy.

II. OVERVIEW AND SIGNIFICANCE OF DRUG TARGETING IN BREAST CANCER

Targeted nanocarriers have special features that make them suitable for targeted delivery of anticancer drugs by facilitating the accumulation of drugs via leaky vasculature in the tumor. If the nanocarrier is decorated with a targeting ligand, it can enter the tumor cells by endocytosis in an efficient manner.[30] Intracellularly targeted nanocarriers also protect the drug particles from substrate-specific efflux pumps and prevent their expulsion.[31] The drug targeting approach is classified into two major categories, i.e., passive and active targeting approach (Fig. 2).

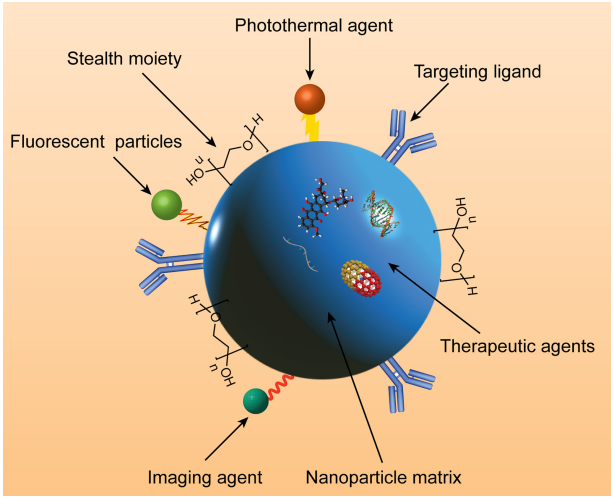

FIG. 1: Diagram of cancer-targeted theranostic nanomedicine

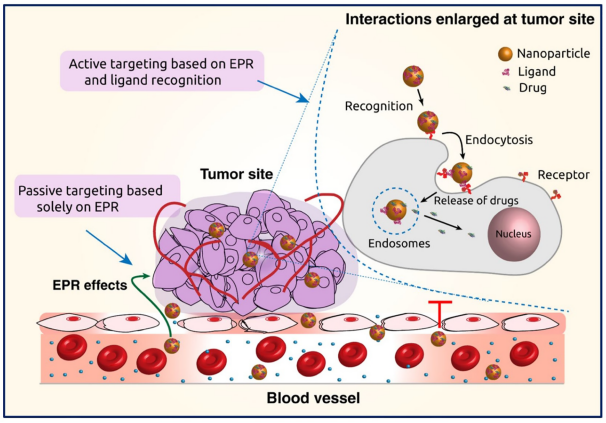

FIG. 2: Diagram of passive and active targeting to tumor site depicting passive targeting of NPs via EPR effect where NPs can pass through leaky vasculature of tumor blood vessels and accumulates in tumor tissue, while in active targeting ligand conjugated NPs bind to receptors present on tumor cells and enter tumor cell via receptor-mediated endocytosis. Figure reproduced from Navya et al.[26] under a Creative Commons license.

Tumor vasculature has a very high number of proliferating endothelial cells and an aberrant basement membrane. This leads to an increased vascular permeability and the presentation of open fenestrations and inflammatory processes on the vascular endothelium. Further, tumor tissue also develops intermittent hypoxic areas.[32,33] The mentioned abnormal structural alterations provide an opportunity that is exploited by circulating nanocarrier systems having a size range of 20–200 nm to extravasate and accumulate in the tumor microenvironment. Further, due to the absence of lymphatic drainage in the tumor, the accumulated nanocarriers are detained in tumor tissues leading to their prolonged retention at the target tissue, i.e., tumor. This phenomenon is called the "enhanced permeability and retention (EPR) effect."[8,34–36] Thus, the abnormal vascular structure is crucial for the EPR effect and drug targeting at the tissue level in cancer. The phenomenon of the EPR effect has been characterized in almost all types of cancers. The EPR effect applies to and is utilized by almost all noncarriers and it is regarded as the gold standard in designing cancer targeted drug delivery systems.[37–39] Various factors affect the EPR effect in solid tumors, including: (i) unique and abnormal tumor vasculature, (ii) active angiogenesis and high vascular density, (iii) impaired lymphatic clearance, and (iv) extensive production of vascular mediators that facilitate extravasation including bradykinin, nitric oxide, carbon monoxide, heme oxygenase-1, vascular endothelial growth factor (VEGF), prostaglandins and inflammatory cytokines, collagenase (matrix metalloproteinase), angiotensin-converting enzyme inhibitors, etc.[40–43] The factors affecting optimal EPR effect are schematically presented in Fig. 3.

The optimal EPR effect is obtained in the case of carriers that can evade the immune system and can remain in circulation for a relatively longer duration. It can lead to many fold increase in accumulation of the anticancer drug in tumors

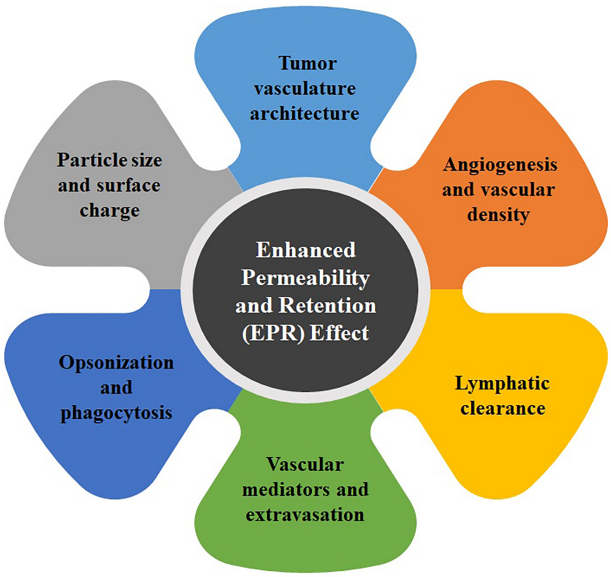

FIG. 3: Schematic representation of factors affecting EPR effect

compared with normal tissues.[44] To achieve this fate a noncarrier needs to have three ideal properties; firstly, its size should be in the range of 10–100 nm. In any case, the size for extravasation should not exceed 400 nm. If the size is below 10 nm, it will be filtered out by kidneys and if is above 100 nm, there is a probability of it being trapped by the liver. Secondly, the carrier should have a neutral or anionic charge to prevent its elimination by kidneys. Thirdly, it should bypass the trapping, opsonization, and phagocytosis by the reticuloendothelial system.[45] Opsonization refers to an immune process, which utilizes proteins called opsonins, for tagging the foreign nanocarriers which can be recognized and eliminated by phagocytes. Major opsonins include immunoglubulins and complement components (C3, C4, and C5). Opsonization is often unfavorable in active-targeting approaches as the adhered opsonins masks the targeting ligands, resulting in a marked reduction in specificity. The effective interaction between the opsonins and nanocarriers depends on the size, surface charges and composition of the nanocarriers. Generally, hydrophobic and charged particles tend to be opsonized more easily in the bloodstream.[46,47] Different properties of NPs, including size, surface properties, composition, etc which affect their *in vitro* and *in vivo* performance including targeting ability in the treatment of cancer are shown in Fig. 4.

The degree of tumor vascularization and angiogenesis may limit the passive targeting efficiency of NPs and therefore, the extravasation will vary depending upon anatomical location and type of tumor. Sometimes, solid tumors exhibit high fluid pressure in the interstitium which affects the uptake and distribution of nanocarriers.[39,48] Thus, a combination of high interstitial fluid pressure and poor lymphatic drainage can be

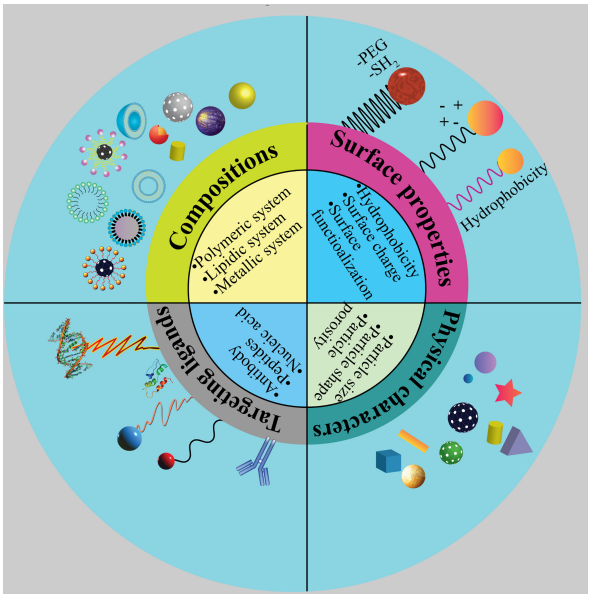

FIG. 4: Tunable physicochemical characteristics of cancer-targeted nanomedicine influencing *in vitro/in vivo* performance of loaded therapeutics/imaging agents to advance the therapy outcome in breast cancer

correlated as the relation between EPR effect and size, i.e., there is more retention of larger and long-circulating carriers than the smaller molecules.[49,50]

Active targeting involves the attachment of a ligand to the nanocarrier which can bind to the receptors overexpressed by tumor cells or vasculature but not or less expressed by normal cells of the body.[51,52] Such targeted receptors should have a homogenous distribution in the targeted tumor tissue. These targeting ligands could be non-antibody peptides, antibody fragments, monoclonal antibodies, or endogenous ligands such as folate, estrone, etc. Tumor penetration is affected by the binding affinity of the targeting ligand to the binding site and higher affinity binding is observed in tumor vasculature due to easy accessibility to the cells owing to the dynamic environment of systemic circulation.[37,53–55] The understanding of the difference in tumor microenvironment compared with that of normal tissue has been utilized by researchers in designing better anticancer therapies. Here, the differential expression of targeted receptors in normal and cancerous cells has particular significance in designing tumor-targeted nanocarriers for delivery of anticancer drugs in breast cancer.[56–58] The mechanism of receptor-mediated targeting is depicted in Fig. 5.

III. RECEPTOR-MEDIATED TARGETING IN BREAST CANCER IMAGING

Imaging agents are crucial in the management of cancer attributed to their critical role in screening and diagnosis of cancer which helps in therapy monitoring and planning

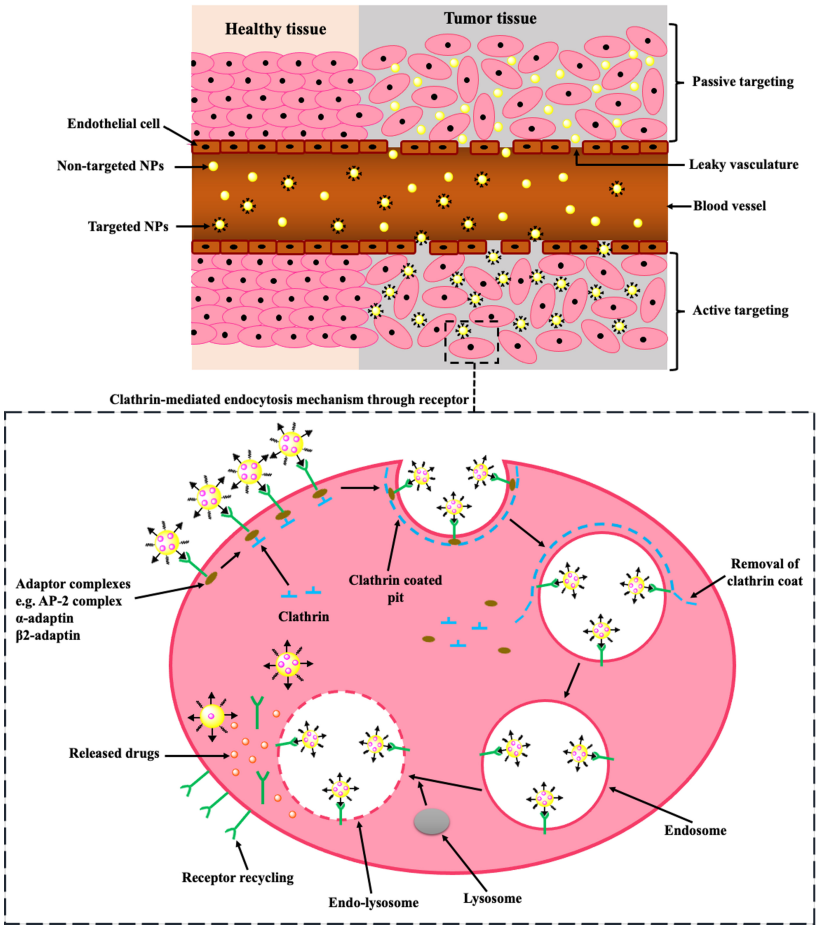

FIG. 5: Schematic representation of the mechanism of receptor-mediated targeted delivery

of current and future treatment strategies.[59,60] In breast cancer, the degree of hormone-receptor expression is directly related to the beneficial effects offered by hormonal therapy. For example, in a patient with overexpression of HER2 receptors, there are more binding sites available for the monoclonal antibody (trastuzumab) to work.[61,62] Although immune-histochemistry has been the conventional method used to determine the degree of hormone-receptor expression, its use in quantifying receptor expression is not widespread.[63] In addition, an attempt to detect different types of receptor proteins at once on a single tumor specimen requires several steps.[61,64] Receptor-targeted nanomedicines, on the other hand, can quantify and profile several biomarkers more accurately and sensitively, thus offering clear advantages over immune-histochemistry.[65,66]

Nanomedicines are being engineered as imaging and contrast agents for breast imaging techniques including magnetic resonance imaging (MRI),[67–70] fluorescence imaging,[71–74] ultrasound imaging,[75–78] and photoacoustic tomography (PAT).[79–82] In addition,

nanoparticulate systems that can serve as multimodal imaging contrast agents are also being developed and investigated.[83–85] Various receptors which are overexpressed in breast cancer are presented diagrammatically in Fig. 6.

A. MRI

MRI, a radiological technique, is widely used to analyze tissues. In the case of MRI, firstly, hydrogen atoms present in tissues are excited by the application of the magnetic field of appropriate resonance frequency, then these excited hydrogen atoms return to their equilibrium state while emitting a radiofrequency signal which is used to form the image. The contrast difference among different tissues is attributed to the different rates of relaxation of hydrogen atoms of different tissues. MRI offers high spatial resolution without any ionizing radiation. Superparamagnetic iron oxide nanoparticles (SPIONs) are the majorly used MRI contrast agents among various paramagnetic and superparamagnetic metals.[86–89]

Various nanocarriers apart from SPIONs has also been designed and investigated by scientists for targeted imaging of breast tumors. Luteinizing hormone-releasing hormone (LHRH) templated ferrosoferric oxide (Fe_3O_4) NPs has been investigated as MRI agent for targeted diagnosis and treatment of breast tumors. The designed LHRH-Fe_3O_4 NPs showed promising results as a contrast agent to be used in the T2 field as observed in MRI experiments performed in female Balb/c mice.[79] G3 decorated ankyrin repeat protein and fluorescein-5-maleimide multifunctional SPIONs (G3-5MF-SPIONs) have been designed by Li and coworkers for MRI of breast tumors. The G3-5MF-SPIONs showed selective binding (even in the presence of trastuzumab), long retention time, significant accumulation, and biodistribution in SK-BR-3 tumor-bearing female Balb/c

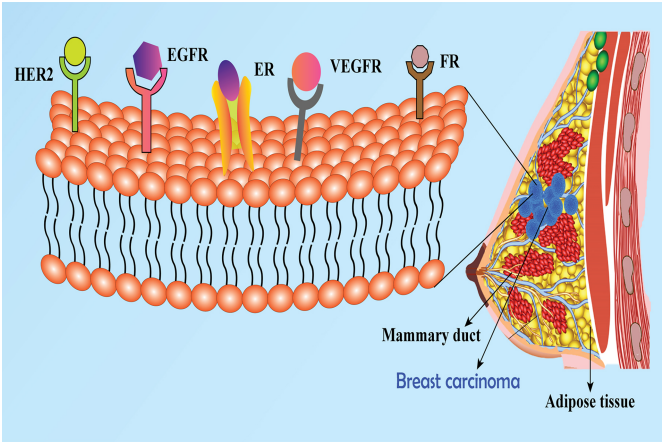

FIG. 6: Diagrammatic illustration of commonly overexpressed receptors in breast cancer. HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor (HER1); ER, estrogen receptor; VEGFR, vascular endothelial growth factor receptor; FR, folate receptor.

nude mice. Additionally, G3-5MF-SPIONs showed the selectivity to image HER2-positive breast tumors with remarkable T2 signal reduction after 24 h. These findings supported the promising credential of targeted nanomedicine in imaging breast cancer by MRI.[90]

Ding et al. developed anti-HER2 single-chain antibody-conjugated iron oxide nanoparticles (IONPs) as MRI agents. The NPs showed higher affinity toward HER2 overexpressing cells (NCI-N87), compared with HER2 lower expressing cells (SUIT2) in *in vitro* cell lines studies. Further, these results were supported by *in vivo* studies with NCI-N87 and SUIT2 tumor-bearing mice where a significantly higher reduction in MRI signals was observed with NCI-N87 tumors (19.3%) than SUIT2 tumors (6.2%) attributed to overexpression of HER2 receptors on NCI-N87 cells.[91] In another study, Lee et al. observed that hyaluronic acid (HA)-modified manganese ferrite ($MnFe_2O_4$) nanocrystals enhanced the detection ability of MRI in CD44 expressing breast carcinoma cells (MDA-MB-23 and MCF-7).[92] Lim et al. also observed that hyaluronan-modified magnetic nanoclusters benefit in MRI technique for the detection of CD44-overexpressed breast cancer cells in MCF-7 and MDA-MB-231 cell lines as well as in MDA-MB-231 bearing female Balb/c nude mice. It was found that the developed nanoparticulate system exhibited superior ability for targeted diagnosis of CD44-overexpressed breast cancer with excellent sensitivity for MRI.[68]

Meier et al. developed folic acid (FA) conjugated ultra-small SPIONs which showed specific retention in imaging in various breast carcinoma cell lines including MDA-MB-468, ZR-75-1, MDA-MB-435, MCF-7, SK-BR-3, and MDA-MB-231 as well as *in vivo* imaging in folate receptors (FR) overexpressing cancer xenograft mice model. The retention of FR-specific SPIONs led to a significant negative enhancement of FR expressed breast tumors on delayed magnetic resonance images at 24 hours post injection.[67] Similarly, Meng et al. found that LHRH-conjugated SPIONs exhibited much greater efficiency than that of unconjugated SPIONs by MRI technique in the detection of LHRH overexpressed human breast cancer (Hs 578T) as well as breast cancer xenografts model in addition to metastases in the lungs of athymic nude mice.[93] From the research discussed above, it is clear that nanoconjugates systems investigated by scientists for MRI of breast cancer have shown promising results and are encouraging enough for scientists to further explore possible clinical applications of these nanomedicine-based MRI contrast agents for detection of breast cancer to achieve better prognosis in cancer management.

B. Fluorescence Imaging

Fluorescence spectroscopy is an important technique being used for years for imaging in various fields related to biological and medical sciences such as biochemistry, biotechnology, molecular biology, pharmaceutical technology, nanotechnology, and nanomedicine, etc. Fluorescence spectroscopy can provide molecular specificity as many biomolecules such as proteins, lipids, amino acids, drug molecules such as DOX can inherently fluoresce on excitation with UV-visible light and hence can be used as

fluorescent probes to label target molecules in fluorescent imaging.[94,95] In this spectroscopy, the image is formed by photons emitted either from biomolecules having inherent fluorescence ability or from external fluorescent active probes.[96,97] It offers an inexpensive imaging technique with suitable spatial resolution and is similarly sensitive to radioisotopes generally used in single-photon emission computed tomography (SPECT) and positron emission tomography (PET).[98–100] Limited tissue penetration with significantly high noise, interference from water molecules, light absorption by proteins, tissue scattering, and autofluorescence are the few limitations of fluorescence microscopy. Some of these challenges such as tissue scattering and autofluorescence have been reduced to some extent with tissue penetration to many centimeters by use of near-infrared (NIR) light during *in vivo* imaging.[101–103]

Gold nanoclusters encapsulating protein are being explored for bioimaging and biosensing applications in cancer due to their biocompatibility, relatively safe profile, and significant fluorescing ability. Scientists have designed mannose conjugated bovine serum albumin (BSA) encapsulated gold (Man-BSA-Au) nanoclusters for detection of concanavalin A and imaging of breast tumor cells using fluorescence imaging technique (Fig. 7A). Man-BSA-Au nanoclusters showed bright red fluorescence after incubation with mannose receptor-expressing MDA-MB-231 cells for 1 h attributed to targeting of nanoclusters to cancer cells mediated by mannose receptors, whereas cells incubated with phosphate buffer and BSA encapsulated gold (BSA-Au) nanoclusters showed no noticeable fluorescence (Fig. 7B).[73]

Scientists have fabricated platinum nanocluster bio-nanoprobes with red emission using polyamidoamine (PAMAM) dendrimer combined with Protein A (adapter), which exhibited small size, low cytotoxicity and exceptional photostability for fluorescence imaging of HER2 in SK-BR-3 cells. These bio-nanoprobes exhibit minimal nonspecific binding that allows highly sensitive optical imaging with visualization of deep anatomy and increase in tissue penetration due to long-wavelength emission.[74] Yamaguchi et al. observed that technetium-99m (^{99m}Tc) and indocyanine green (ICG) labeled anti-HER2 antibody decorated PAMAM-coated silica NPs showed higher imaging efficiency with stronger NIR fluorescent signals for HER2 positive SK-BR-3 cells than that in HER2 negative MDA-MB-231 cells. The *in vivo* imaging results with SK-BR-3 and MDA-MB-231 xenograft tumor mice model were also found in accordance with the *in vitro* results.[104]

Quantum dots (QDs) have been exploited by scientists for spectrum analysis and immunofluorescence detection of epidermal growth factor receptors (EGFR). The outcomes of this study showed the importance of EGFR in the prognosis of lymph node-positive and HER2-positive invasive breast cancer via immunofluorescent detection.[63] Li et al. developed FA-functionalized two-photon absorbing 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine **(**DSPE)- polyethylene glycol (PEG) NPs with aggregation-induced emission for specific targeting and imaging in MCF-7 cancer cells using a two-photon fluorescence microscope.[105] Wu et al. described the targeted imaging potential of arginine-glycine-aspartic acid (RGD) peptide-doped fluorescent silica NPs (FSiNPs) in athymic nude mice bearing the MDA-MB-231 tumor on intravenous injection. It

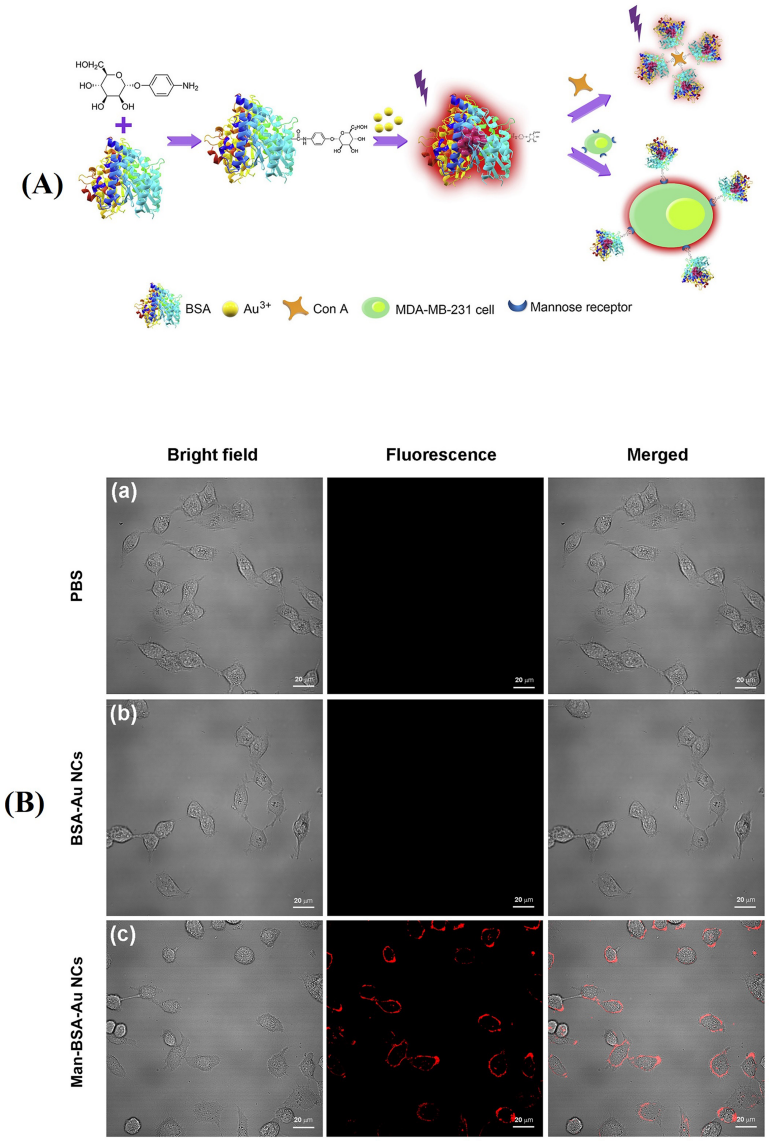

FIG. 7: (A) Schematic presentation of preparation of Man-BSA-Au nanoclusters for fluorescence imaging of human breast cancer cells. (B) Confocal fluorescence images of MDA-MB-231 cells after incubation with (a) PBS, (b) BSA-Au nanoclusters, and (c) Man-BSA-Au nanoclusters for 1 h (excitation wavelength: 488 nm; scale bar: 20 μm) (reprinted from Sha et al.[73] with permission from Elsevier, copyright 2020).

exhibited a high and specific $\alpha_v\beta_3$ integrin expression level in the MDA-MB-231 tumor due to the specific targeting potential of RGD peptide-doped FSiNPs and tumor fluorescence reached maximum intensity after 1 h of injection.[72]

Different nanomaterials including lipidic NPs, polymeric NPs, metallic NPs, QDs, and dendrimers, etc have been engineered smartly by researchers for fluorescent imaging of breast cancer with promising results in preclinical studies in animals.

C. Ultrasound Imaging

Ultrasound imaging is widely used as a tomography technique for the clinical diagnosis of breast cancer. It can show minute internal details of organs and tissues attributed to the use of sound waves of a shorter wavelength having frequencies of 2 MHz or greater. Transmitting and receiving transducers are set to capture ultrasound wave interactions.[106] Ultrasound imaging is commonly used to diagnose various diseases via imaging different internal organs, muscles, and tendons, and gives information related to the structure, size, or presence of any abrasion, injury, or lesions in these areas. High resolution with low cost, non-invasive procedure, and precise targetability of ultrasound energy deposition are some of the advantages of ultrasound imaging.[86,107]

Xu et al. developed dual-targeted gold nanoshell poly (D,L-lactide-co-glycolide) (PLGA) nanocapsules decorated with vascular endothelial growth factor receptor (VEGFR)-2 and p53 antibodies for specific ultrasound molecular imaging of breast cancer, which showed excellent biocompatibility. The results of *in vivo* studies in the MCF-7 orthotopic mice model indicated efficient binding to cells overexpressing VEGFR2 or p53 protein with no signs of organ damage.[77] Polylactic acid (PLA) NPs have been designed and evaluated for comparison of targeted ultrasound imaging of HER2-overexpressing and HER2-negative breast cancer cells by scientists. In results, HER2-overexpressing cells showed more staining after incubation with NPs/antibody conjugates, whereas minimal staining was seen in HER2-negative cells, indicating direct receptor binding of targeted NPs. The average gray matter of HER2-positive cells was significantly higher in NP-treated cells compared with the control shown in high-resolution ultrasound images.[76]

In another study, Sakamoto et al. carried out the molecular analysis of breast cancer using herceptin conjugated IONPs-based ultrasound imaging agents. The ultrasound imaging results supported the ability of herceptin decorated IONPs to differentiate between HER2/neu positive SK-BR-3 cells as compared with the non-tagged NPs.[75] These studies validated that conjugated NPs may have promising applications in receptor-targeted non-invasive imaging of breast cancer.

D. Photoacoustic Imaging/Tomography

Photoacoustic imaging (PAI) or photoacoustic tomography (PAT) is an emerging non-ionizing imaging tool combining high optical contrast and high ultrasound resolution.[108] PAT makes use of pulsed laser light to generate light-induced acoustic signals.[109] PAT with finite element reconstruction has been used to image small nanometer-sized particles containing objects with high resolution.[110] Photoacoustic provides *in vivo* morphological and functional information regarding the tumors within the surrounding tissue.

Photoacoustic (PA) with the use of targeted contrast agents is also capable of performing *in vivo* molecular imaging, thus enabling further molecular and cellular characterization of cancer.[59,111,112]

In vivo PAI studies using VEGF targeted, anti-VEGFR antibody-conjugated iron platinum alloy NPs showed that with these NPs the PAI depth could be extended to more than 5 cm in chicken breast tissues with the use of a laser energy density within the safe limit. Further, it was observed that NPs were removed from the site of the tumor and metabolized mainly by the liver supporting the safe use of this nanomedicine in early detection and theragnosis of breast cancer with whole-body PAI.[82] Balasundaram et al. demonstrated that folate decorated NPs showed ~ 4-fold enhancement in the PA signal in the MCF-7 breast cancer xenograft model compared with untargeted NPs, thus providing a better delineation from the surrounding tissue.[113] Furthermore, Kanazaki et al. developed a single-chain fragment variable (scFv) anti-HER2 moiety conjugated IONPs for PAI studies. Their results demonstrated that anti-HER2 scFv-conjugated IONPs showed high affinity and specific binding to HER2-expressing N87 tumor-bearing female Balb/c-nude mice and SUIT2 cells compared with untargeted NPs.[114]

Scientists have investigated breast cancer imaging techniques by combining high-resolution NIR induced PAT using NIR dye-labeled with amino-terminal fragments of urokinase plasminogen activator receptor-targeted magnetic IONPs in orthotopic mouse mammary tumor model (mouse bearing mammary carcinoma cell line 4T1). Their results showed 4–10 fold amplification in PA signals in the tumor with receptor-targeted NPs as compared with non-targeted ones.[115] Wang et al., 2014 designed FR-targeted ICG-loaded PLGA (ICG-PLGA) lipid NPs. It exhibited high targeting efficiency for FR and remarkable optical absorption in NIR wavelengths resulting in excellent PA signals in *in vitro* as well as *in vivo* studies in MCF-7 bearing female Balb/c nude mice over non-targeted ICG-PLGA lipid NPs.[116] Furthermore, a strategically designed study using HA decorated gold NPs for PAI showed that PA/photothermal (PT) signals from targeted NPs in MDA-MB-231 cancer cells were 10- to 50-fold higher than untargeted NPs, and the rate of flashing PA signals significantly increased in Balb/c nude mice model.[79]

The use of receptor-targeted nanomedicines such as antibody-conjugated NPs, folate, HA conjugated nanomaterials, etc have shown that the use of these ligand conjugated nanomedicines targeted toward a particular receptor overexpressed on tumor cells can promisingly improve *in vivo* imaging efficiency of PAI along with may provide further details regarding the cellular and molecular characterization of tumor.

E. Multimodal Imaging

The intrinsic merits and demerits of each imaging technique are significant and hardly overcome by the improvement of imaging instrumentation alone. Multimodal imaging includes the combined usage of various imaging tools such as MRI/fluorescence imaging and it has gathered significant attention to advance the currently used techniques for diagnosis of breast cancer. It is a powerful technique that provides more consistent and accurate recognition of disease sites.[117,118]

Du et al., 2020 developed brain metastatic breast cancer cell-231-BR-binding peptide (BRBP1)-functionalized ultra-small IONPs containing DiR fluorescent dye for targeted MR/NIR fluorescent dual-modal detection of breast cancer brain metastasis. Their results showed that the NPs exhibited a size of 10 nm with core-shell structure, high relaxivity values, and efficient photon emission *in vitro*. Moreover, the NPs presented a T2 contrast imaging effect and NIR fluorescence signal enhancement. The MR/NIR fluorescence signal of BRBP1-modified NPs in tumor tissue was significantly enhanced as compared with the control. The results showed that BRBP1-IONPs-mPEG NPs loaded with DiR can be employed as an attractive targeting strategy for the detection of breast cancer brain metastasis.[85] Similarly, Han et al. developed CD44 monoclonal antibodies coupled magnetic-fluorescent iron oxide carbon hybrid nanomaterials which demonstrated strong excitation wavelength-dependent fluorescence in the blue-red region with a quantum yield of 58.4%, and they displayed higher stability and T2 relaxivity than simple IONPs. Moreover, there was significant preferential uptake of the conjugates by 4T1 breast cancer cells as seen in biological transmission electron microscope imaging. In addition, the developed CD44-hybrid nanomaterials were able to distinguish 4T1 cells from normal cells by virtue of its high binding affinity and specificity of the CD44 antibodies to the CD44 receptors on the surface of cancer cells.[119]

Herceptin-decorated paclitaxel-loaded ultrasmall superparamagnetic iron oxide nanobubbles exhibited significantly enhanced cytotoxic effects against HER2-overexpressing SK-BR-3 breast carcinoma cells and significantly lower cytotoxicity against HER2-negative MDA-MB-231 breast carcinoma cells. In addition, the developed magnetic nanobubbles showed ability to enhance ultrasound, magnetic resonance, and photoacoustics trimodal imaging.[120] Hyaluronan-modified SPIONs were designed for multimodal imaging of breast cancer which showed significantly enhanced specific cellular uptake of HA-SPIONs in HA receptor-overexpressing MDA-MB-231 cells which was confirmed by fluorescence imaging. In addition, HA-SPIONs demonstrated significant negative contrast enhancement on T2-weighted MR images of HA receptor-overexpressing MDA-MB-231 breast tumor-bearing balb/c nude mice compared with untargeted SPIONs.[121] Kievit et al. developed herceptin decorated fluorescent dye loaded multifunctional SPIONs that exhibited a 4-fold improvement in cellular uptake and specificity to target the neu/HER2-overexpressing mouse mammary carcinoma cells. It showed significant contrast enhancement in magnetic resonance images of breast tumors.[83]

These studies demonstrated that nanomedicine may advance the diagnosis of various breast cancers by utilizing receptor-mediated targeting of imaging agents. The potential of various receptor-targeted nanomedicines explored by different researchers/investigators for the detection or imaging of breast cancer are summarized in Table 1.

IV. RECEPTOR-MEDIATED THERANOSTICS IN BREAST CANCER TREATMENT

NPs can be designed with some inherent properties to offer unique imaging and surface functionalization ability. Also, NPs have optimum size, high surface area to volume

TABLE 1: Receptor-targeted nanomedicines for detection/imaging of breast cancer

Imaging technique	Types of nanomedicine	Ligand	Receptor targeted	Breast cancer model	Outcome	Ref.
Magnetic resonance imaging	Fe_3O_4 NPs	LHRH	LHRH	Human MCF-7 breast cancer cells	LHRH-Fe_3O_4 effectively accumulated in the tumor region due to magnetic targeting. It showed a good negative enhancement effect that significantly reduced the intensity of T2 weighted images, allowing it to be used as a contrast agent	70
	Fe_3O_4-Au NPs	Thiolated AS1411 aptamer	Urokinase-type plasminogen activator (uPA)	4T1, mouse mammary carcinoma, and HFFF-PI6, human foreskin fibroblast cell lines	–Developed nanoprobe produced strongly darkened T2-weighted MR images with 4T1 cells, whereas brightened images with HFFF-PI6 cells –Specific targeting of 4T1 cells overexpressing nucleolin on the cell surface –Nanoprobe binds specifically to breast cancer cells and has very low accumulation in normal cells.	69
	$MnFe_2O_4$ nanocrystals	HA	HA receptor	*In vitro*/ MDA-MB-231 and MCF-7 cells	Excellent MR imaging sensitivity to diagnose CD44-overexpressing cancer cells	92
	Magnetic nanoclusters	Hyaluronan	HA receptor	*In vitro*/ MDA-MB-231 and MCF-7 breast carcinoma cells *In vivo*/Balb/c-Slc nude mice bearing MDA-MB-231 cancer cells	Nanoclusters showed excellent ability for targeted detection of CD44-positive breast carcinoma cells by showing excellent sensitivity as MR imaging agents	122

TABLE 1: (*continued*)

Imaging technique	Types of nanomedicine	Ligand	Receptor targeted	Breast cancer model	Outcome	Ref.
	SPIONs	FA	FA receptor	*In vitro*/ MDA-MB-231 *In vivo*/breast cancer xenograft in female athymic Harlan rats	–Specific retention in FR-positive MDA-MB-231 cell line and in an FR-positive human breast carcinoma xenograft model *in vivo* –Significant negative enhancement of FR-positive breast tumors on delayed MR images at 24 hours post injection	67
	SPIONs	LHRH	LHRH receptor	*In vitro*- Hs 578T cells *In vivo*-breast cancer xenografts and lung metastases of athymic nude mice	–LHRH conjugated SPIONs exhibited significantly higher uptake in the cells than that of the unconjugated SPIONs in both *in vitro* and *in vivo* studies –The enhanced uptake of intracellularly accumulated LHRH conjugated SPIONs provided T2 contrast enhancement leading to improved spatial resolution in MRI by classical T2 imaging	93
Fluorescence imaging	Red-emitting Platinum nanoclusters	Protein A and anti-HER2 antibody	HER2	SK-BR-3 breast cancer cells	Platinum bio-nanoprobe binds to HER2-overexpressing SK-BR-3 cells with a higher affinity and selectivity	74
	Mannose functionalized BSA Gold Nanoclusters	Mannose	Concanavalin A on Mannose receptors	Human breast cancer MDA-MB-231 cells, HUVEC cells	Increased sensitivity and selectivity of Man-BSA-Au nanoclusters for determination of Concanavalin A on mannose receptor	73
	99mTc-radiolabeled nanosilica system	Trastuzumab	HER2	Female Balb/c nude mice SK-BR-3 breast cancer cells	–Radiolabeled system exhibited an enhanced active targeting to the tumor, compared with EPR-based passive diffusion –Significantly enhanced accumulation of SiNPs within the HER2 overexpressing SK-BR-3 cells	123

TABLE 1: (*continued*)

	DSPE-PEG NPs	FA	FA receptor	*In vitro*/MCF-7 breast cancer cells	Two-photon fluorescence images obtained from the fluorescence signal from cytoplasm after incubation with developed NPs clearly distinguished the MCF-7 breast cancer cells from others	105
	FSiNPs	RGD peptide	Integrin receptor	*In vitro*/ MDA-MB-231 cell *Ex vivo*/athymic nude mice bearing the MDA-MB-231 tumors	RGD peptide-conjugated FSiNPs were able to image the specific $\alpha_v\beta_3$ integrin expression level in the MDA-MB-231 cells due to its special receptor-based targeting effects	72
Ultrasound imaging	Gold-Nanoshells PLGA Magnetic Hybrid NPs	Anti-HER2 antibodies	HER2	Human breast cancer SK-BR-3 cells	Conjugation with anti-HER2 antibodies led to specific and sensitive binding with HER2 overexpressing SK-BR-3 breast cancer cells. Excellent contrast enhancement *in vitro*	78
	Gold nano-shelled PLGA nanocapsules	Anti-VEGFR2 and anti-p53 antibodies (DNCs)	Vascular endothelial growth factor receptor type 2	HUVECs, 4T1, MCF-7, and MDA-MB-231 breast cancer cells MCF-7 cell bearing Female Balb/c nude mice	–DNCs exhibited high target specificities *in vitro* in VEGFR2- and p53-overexpressing cells compared with control cells –Ultrasound imaging studies using novel nano-sized, dual-targeted PLGA-Au nanocapsules ultrasound contrast agents allowed highly precise and reliable detection of breast cancer in MCF-7 orthotopic mice model	77
	Anti-EGFR antibody-conjugated gold nanorods	Anti-HER2 antibody	EGFR	EGFR-negative MCF-7 cells EGFR-positive MDA-MB-231 cells Mouse model of human TNBC	Ultrasound-guided PAI using anti-EGFR decorated gold nanorods showed a very high sensitivity for the selective visualization of EGFR-positive primary breast tumors as well as Lymph Nodes micrometastases	124

TABLE 1: (*continued*)

Imaging technique	Types of nanomedicine	Ligand	Receptor targeted	Breast cancer model	Outcome	Ref.
	PLA-NPs	Anti-HER2 antibody	HER2 receptor	*In vitro*/SK-BR-3 and MDA-MB-231 cell line	In high-resolution ultrasound B-mode images, the average gray scale of the HER2-positive cells (SK-BR-3 cell) was consistently and significantly higher	76
	IONPs	Herceptin	HER2 receptor	*In vitro*/SK-BR-3 human breast cancer cells	Ultrasound imaging exhibited excellent sensitivity and specificity in the detection of HER2 overexpressed breast cancer cells	75
Photoacoustic (PA) imaging	Iron-platinum NPs	anti-VEGFR	VEGF	4T1 breast cancer cells	Accumulation in the tumor region, Targeted delivery of NPs by EPR effect	82
	Irradiated nanodiamonds	Anti-HER2 peptide	HER2	4T1.2 breast cancer cells Female Balb/c mice	–PA images demonstrated that nanodiamonds significantly accumulated in breast tumors and traced the entire tumor in less than 10 hours –HER2 conjugation significantly enhanced the imaging of HER2-positive tumors –The conjugation of PEGylated nanodiamonds with anti-HER2 peptide led to enhanced internalization of INDs by HER2 positive tumor cells (4T1.2-neu) and longer residence time in the region of HER2 positive tumor	81
	ICG conjugated PLGA lipid NPs	FA	FR	*In vitro*/MCF-7 cells *In vivo*/Female Balb/c nude mice bearing human breast cancer (MCF-7) cells	Developed NPs showed excellent optical properties with reduced toxicity, enhanced tumor-targeting capability, prolonged circulation time, and significantly improved PA contrast for preclinical imaging applications	116

TABLE 1: (*continued*)

	Gold nanorods	Bombesin (BBN)	Gastrin releasing peptide (GRP) receptor	Breast tumor-bearing Balb/c mice T47D cells	*In vitro* studies showed a very selective binding of PEG-BBN gold nanorods towards breast cancer cells. Biodistribution studies showed that after the injection of PEG-BBN gold nanorods conjugate (high uptake) and PEG gold nanorods conjugate (low uptake) approved the targeting ability of PEG-BBN gold nanorods conjugate to breast cancer cells. PEG-BBN gold nanorods is a reliable vector for targeting GRP receptors over-expressed in various cancers	125
	IONPs	NIR830	Urokinase plasminogen activator receptor	*In vivo*/Balb/c mice bearing Mouse mammary tumor 4T1 cells	*In vivo* studies in mice showed 4- and 10-fold enhancement in PA signals of IONPs compared with non-targeted IONP or control Balb/c mice	115
	Gold carbon nanotubes	CD44 receptor-specific antibody	CD44 receptor	*In vitro*/ MDA-MB-231 cells *In vivo*/nude mice	–PA/PT signals from targeted MDA-MB-231 cells were 10 to 50-fold higher than those from unbound nanotubes distributed randomly in suspension between cells at relatively high laser fluences of 300–500 mJ/cm^2 –After subsequent injections of CD44-decorated gold carbon nanotubes, the rate of flashing PA signals increased to 46.7 ± 6.8 cells/min in nude mice	79

TABLE 1: (*continued*)

Imaging technique	Types of nanomedicine	Ligand	Receptor targeted	Breast cancer model	Outcome	Ref.
Multimodal imaging	Ultra-small IONPs	BRBP1 peptide	EGFR receptor	Mice bearing 231-BR xenografts	–Ultra-small IONPs offered a T2 contrast imaging effect and enhanced NIR fluorescent signal –The MR/NIR fluorescence imaging signal of BRBP1-modified NPs in tumor tissue was significantly enhanced as compared with control NPs, which shows the targeting ability of BRBP1 peptide	85
	Natural magnetic NPs with Gold nanorods	Folate	FA receptor	*In vitro* MDA-MB-231 cell line Mouse model injected with breast cancer cells	–Magnetotactic bacteria, natural magnetic NPs, and natural magnetic NPs-gold nanorods can be used for scanning as well as in PA and PT imaging –Significant enhancement in PA signals from natural magnetic NPs and natural magnetic NPs-gold nanorods both *in vitro* and *in vivo* –Natural magnetic NPs-gold nanorods were able to provide for real-time counting of labeled cells, magnetic trapping of labeled cells (e.g., CTCs), and killing of diseased circulating cells	84
	SPIONs	Herceptin	HER2 receptor	*In vitro*/ MDA-MB-231 cells *In vivo*/transgenic breast cancer mice	–SPIONs showed up to 4-fold improved cellular uptake and specific ability to target neu/HER2 overexpressing mouse mammary carcinoma (MMC) cells *in vitro* and *in vivo* –Significant enhancement in the contrast of SPIONs MR images in primary breast tumors	83

ratio, higher loading capacity for contrast agents, targeting ligands, and therapeutic molecules.[126] Furthermore, many NPs have inherent imaging properties, which can further be functionalized to become nanotheranostics. Theranostics refers to the integrated applications of diagnostic/ imaging agents and therapeutic moiety in one system. Theranostic nanomedicines or nanotheranostics are multifunctional nanosystems designed for precise and personalized disease management.[127] They consist of macromolecular materials/ polymers in which imaging and therapeutic agents can be absorbed, adsorbed, encapsulated, entrapped, or conjugated for simultaneous imaging and therapy at the cellular and molecular level.[128] Ideal theranostic nanomedicines should have the following properties: (i) safe for administration in humans, (ii) shows rapid and selective accumulation at the target site, (iii) recognizes biochemical and morphologic characteristics of a disease, (iv) able to deliver sufficient drug on-demand without damaging healthy organs, and (v) rapidly cleared from the body within hours or be biodegraded into nontoxic byproducts.[129]

Generally, theranostic nanomedicines can be fabricated in several ways: (i) therapeutic agents (e.g., anticancer drugs and photosensitizers) may be adsorbed, conjugated, entrapped, or loaded, to existing NPs having intrinsic imaging abilities such as QDs, IONPs, and gold nanocages; (ii) attaching the imaging agents, such as fluorescent dyes, optical or magnetic NPs, and various radioisotopes, to existing therapeutic NPs; (iii) encapsulating both diagnosing and therapeutic agents together in different nanocarriers such as polymeric NPs, porous silica NPs and ferritin nanocages; and (iv) some unique NPs (e.g., porphysomes, ^{64}Cu-CuS, and gold nanoshells or cages) with dual inherent imaging and therapeutic properties can also be engineered for theranostic applications.[130,131]

The surface of these nanocarriers can be modified with PEG and various targeting ligands to prolong the blood circulation half-life and to provide the active tumor-targeting capability. Targeting ligands can be exploited to identify and selectively bind to cell surface receptors overexpressed on tumor cells or surfaces. Various targeting ligands including, antibodies, small peptides, aptamers, lectins, some proteins, or protein fragments can be used depending on the physicochemical properties of theranostic nanocarrier or tumor of interest. In the last few decades, substantial efforts have been made by researchers to design and evaluate different receptor-targeted nanomedicines for simultaneous diagnosis and treatment of breast cancer with certain promising results; however, some challenges have also been faced by scientists while designing targeted theranostic nanomedicines. The following subsection of the article discusses different receptor-targeted nanomedicines for theranostic applications in the breast cancer treatment particularly in the invasive variants such as TNBC and deals with the challenges encountered while designing and developing these theranostic nanomedicines.

A. HER2 Targeted Theranostics

HER2 receptors are overexpressed on breast cancer and have been explored by scientists for targeted theranostic applications. Mechanistic approaches for exploring HER2 receptors for targeted breast cancer theranostics are shown diagrammatically in Fig. 8. Recently, Khaniabadi et al. investigated the theranostic potential of superparamagnetic Fe_3O_4 NPs

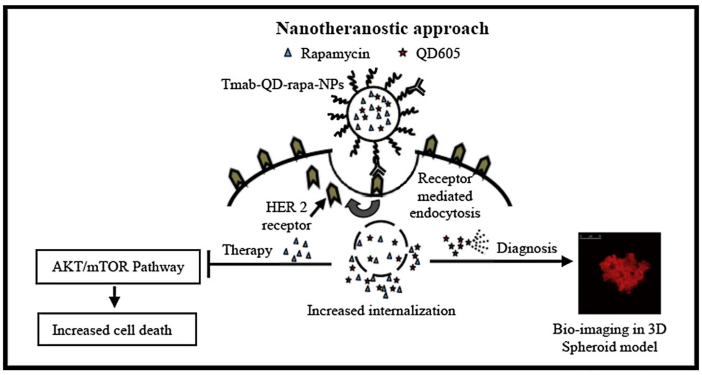

FIG. 8: Diagrammatic representation of theranostic applications achieved with HER2 receptor targeting, where QD: Quantum dots; Tmab-QD-rapa-NPs: Trastuzumab-conjugated rapamycin and QDs-loaded theranostic NPs (reprinted from Parhi and Sahoo[134] with permission from Elsevier, copyright 2015)

decorated with protoporphyrin and trastuzumab for imaging and treatment of EGFR2-overexpressing breast cancer. The results showed no significant cytotoxicity after incubating the MCF-7 breast cancer cells under various Fe concentrations of NPs and theranostic agents, whereas *in vitro* photothermal ablation of targeted NPs showed a reduction of 74% in MCF-7 cells after 10 min at the highest Fe concentration.[132] In another study, Choi et al. developed IONPs and DOX-loaded multifunctional nanocarriers with an ability of integrated cancer-targeting via a HER2 monoclonal antibody for controlled delivery of antineoplastic drug (DOX) as well as imaging agent (IONPs) for MRI and NIR fluorescence imaging. The results of *in vitro* studies demonstrated up to 5-fold higher cellular uptake and significantly higher cytotoxicity to HER2 overexpressing SK-BR-3 cells than HER2 negative MCF-7 cells, signifying HER2 receptor-based cancer targeting. In *in vivo* tumor xenograft model, antibody-targeted nanocarriers exhibited significantly higher uptake in the cancerous cells and the size of the tumor significantly reduced than the non-targeted ones.[133]

Parhi and Sahoo developed trastuzumab-conjugated rapamycin, and QDs-loaded theranostic NPs which exhibited significantly higher uptake in SK-BR-3 cells and MDA-MB-231 cells compared with untargeted NPs. Targeted NPs showed ~ 3 and ~ 1.5-fold higher toxicity to SK-BR-3 cells and MDA-MB-231 cells, respectively, compared with untargeted NPs. The cellular uptake studies were performed using coumarin-6 dye and the results of cell uptake studies showed that confocal images indicated a higher fluorescence intensity in tumor spheroids treated with targeted NPs than that treated with untargeted NPs (Fig. 9). Further, the therapeutic benefits of targeted NPs were explored at the molecular level and the results showed augmented downregulation of mTOR signaling pathway thereby inducing further cell death.[134]

Kievit et al. developed multifunctional SPIONs for diagnosis and treatment of metastatic breast cancer and evaluated in a FVB/N transgenic mouse model. They fabricated fluorescent dye-loaded NPs using a copolymer of chitosan and PEG for optical detection

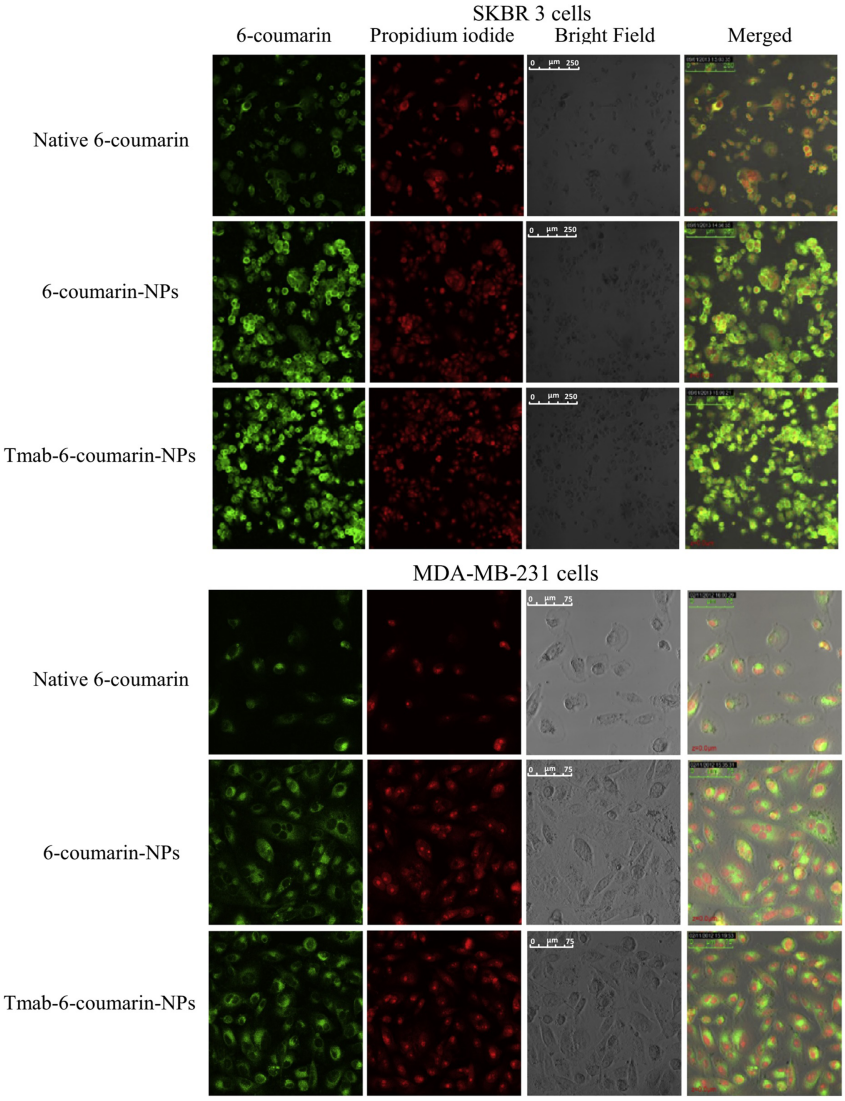

FIG. 9: Qualitative intracellular uptake of coumarin-6, coumarin-6-NPs, and Tmab-coumarin-6-NPs (50 ng/mL) in SKBR 3 cells and MDA-MB-231 cells by confocal microscopy after 2 hrs of incubation (reprinted from Parhi and Sahoo[134] with permission from Elsevier, copyright 2015)

and labeled them with trastuzumab against the HER2 receptor. The results of cellular uptake studies showed that trastuzumab-doped NPs exhibited a 4-fold higher cellular uptake and could specifically target HER2 overexpressing mouse breast cancer cells. Also, the dye-coated NPs provided significant enhancement in contrast of MR images of primary breast tumors that were detected with MRI. Moreover, these NPs were able

to detect micrometastases by recognizing and tagging spontaneous micrometastases in the lungs, livers, and bone marrow of these mice.[83]

In another study, Day et al. developed NIR resonant anti-HER2 antibody conjugated gold-gold sulfide NPs as theranostic agents for management of breast cancer via multiphoton microscopy and higher intensity photoablation. The study demonstrated that anti-HER2 antibody-doped NPs could efficiently bind to SK-BR-3 breast cancer cells. Antibody conjugated NPs emitted luminescence upon excitation with a pulsed laser resulted from a two-photon absorption process. At 1 mW laser power, Anti-HER2 antibody conjugated gold-gold sulfide NPs could specifically visualize SK-BR-3 cells and cell death was induced upon increasing laser power to 50 mW followed by membrane blebbing.[135] Similarly, Yang et al. developed anti-HER2 antibody conjugated DOX-loaded magnetic PLGA NPs for imaging and treatment of breast cancer. The *in vitro* studies of anti-HER2 antibody conjugated DOX NPs using HER2/neu-overexpressing fibroblast NIH3T6.7 cells and HER2-negative SK-BR-3 and MDA-MB-231 cell lines demonstrated 87.4 times higher fluorescence intensity than that of other cells. The multimodal NPs demonstrated remarkable cancer cell affinity. Also, the NPs followed a sustained release of loaded anticancer drugs for 3 weeks.[136]

HER2 receptors and HER2 targeting antibody, trastuzumab have been investigated for targeted theranostic applications in cancer and have shown efficacy to be explored as promising theranostic nanomedicines for treatment and imaging of breast cancer.

B. Folate-Targeted Theranostics

FR are highly overexpressed on different types of cancer cells including breast cancer and have been exploited by scientists for targeted delivery of antineoplastic drugs, contrast agents as well as theranostic applications in cancer. Soleymani et al. fabricated folate-targeted IONPs which showed a significant reduction in the T2-weighted MR signal intensity of breast tumors indicating the accumulation and retention of NPs in the tumor tissue. Moreover, a significant reduction in tumor progression was seen in the mice.[137] In another study, Alibolandi et al. developed folate-decorated QDs and DOX-loaded theranostic polymersomes for the imaging and treatment of breast cancer. The results of fluorescence microscopic and cytotoxicity studies showed that the folate-conjugated DOX-QD NPs displayed significantly higher cellular uptake and cytotoxicity in 4T1 and MCF-7 cells compared with untargeted NPs and pure drug solution. Fluorescence imaging studies in Balb/c mice bearing 4T1 breast adenocarcinoma demonstrated that the targeted NPs showed significant accumulation at the tumor site after 6 h post intravenous injection. Also, acute toxicity studies showed no evidence of long-term harmful histopathological and physiological changes in the treated animals. Moreover, the targeted NPs demonstrated much better therapeutic efficacy *in vivo* compared with untargeted NPs and free drugs.[138]

FA and cisplatin prodrug conjugated gold nanoclusters showed that FA-conjugation led to a significant increase in the cellular uptake and cytotoxicity of cisplatin gold nanoclusters in murine 4T1 breast cancer cells. Fluorescence imaging studies in 4T1

tumor-bearing nude mouse showed that FA-cisplatin gold nanoclusters selectively accumulated in the 4T1 tumor cells and produced intense fluorescence signals due to the tumor-targeting effect of FA. In addition, FA-cisplatin gold nanoclusters showed significant inhibition in the growth and lung metastasis of the orthotopically implanted 4T1 breast tumors.[139] In another study, Heidari Majd et al. developed tamoxifen-loaded FA-armed PEGylated $MnFe_2O_4$ NPs for imaging and treatment of the FR-positive breast cancer cells. Fluorescence imaging and flow cytometry analyses revealed substantial interaction of developed NPs with FR-overexpressing MCF-7 breast cancer cells. Cytotoxicity studies demonstrated significant inhibition in the growth of MCF-7 cells treated with developed NPs.[140] Muthu et al. also developed FR-targeted D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) coated theranostic liposomes containing docetaxel and QDs for diagnosis and therapy of breast cancer. Confocal laser microscopy study showed that folate-targeted liposomes exhibited a significantly higher red fluorescence intensity and higher cell uptake in MCF-7 cells compared with untargeted liposomes. Also, they observed a reduction in IC_{50} values of docetaxel-loaded TPGS coated targeted and non-targeted liposomes by 97.58% and 83.64% compared with Taxotere® after 24 h incubation with MCF-7 cells.[141]

FA targeting has shown promising outcomes in the treatment of breast cancer and could be utilized for its potential theranostic applications in cancer.

C. CD44-Targeted Theranostics

CD44 receptors are overexpressed in cancer cells including breast cancer. HA is a principal ligand for CD44 receptors and scientists have explored HA conjugated nanomedicines to achieve targeted delivery of therapeutic, diagnostic and theranostic agents. Scientists have investigated HA-decorated, red emission cationic BSA protected gold nanoclusters loaded with ICG and paclitaxel for chemo-photothermal therapy and nitric oxide to modulate the tumor microenvironment and enhance the delivery of drug to the cancer cells. The developed nanoclusters presented size-reducible ability in presence of hyaluronidase and significantly higher accumulation in breast cancer cells with the homogenous intra-tumor distribution. Furthermore, their study showed a suppression of 95.3% in *in situ* tumor growth and 88.4% inhibition of lung metastasis growth.[142]

Similarly, Wang et al. developed pH-responsive HA prodrug micelles with aggregation-induced emission properties by a chemical graft of biocompatible phosphorylcholine, DOX, and aggregation-induced emission fluorogen tetraphenylene to the HA backbone. The intracellular distribution of HA was observed by fluorescence microscopy. Flow cytometry and fluorescent microscopy indicated that HA prodrug micelles were effectively internalized by MDA-MB-231 carcinoma cells due to HA-mediated endocytosis. Also, the cytotoxicity results indicated that the prodrug micelles showed significant inhibition in the proliferation of cancerous cells. HA backbone could greatly enhance the cellular internalization ability, furthermore, they observed a significant inhibition in the proliferation of cancer cells and higher cytotoxicity in MDA-MB-231 cells.[143] Receptor-targeted theranostic nanomedicines based on CD44 receptor overexpression

on cancer cells have shown the ability for simultaneous targeted delivery of antineoplastic drugs and imaging agents to cancer cells.

D. EGFR-Targeted Theranostics

EGFR receptors are overexpressed on breast cancer cells and scientists have explored anti-EGFR antibodies and aptamers for targeted theranostic applications in breast cancer. Kim et al. designed aptamer-loaded lipid-based nanocarriers containing QDs and siRNAs for imaging and treatment of TNBC. The hydrophobic QDs were incorporated in the lipid bilayer of liposomes, and then liposomes containing QDs were complexed with therapeutic siRNAs and functionalized with anti-EGFR aptamer for targeting TNBC. The results of fluorescence imaging studies showed enhanced delivery of developed nanocarriers to EGFR overexpressing cancerous cells and thus, more effective gene silencing and enhanced tumor imaging compared with non-targeted nanocarriers. Moreover, combinatorial therapy with Bcl-2 and PKC-ι siRNAs loaded into the anti-EGFR QD lipid nanocarriers in tumor-bearing female Balb/c nude mice caused remarkable inhibition in tumor growth and metastasis.[144]

In another study, Wang et al. developed anti-EGFR nanobody-decorated QDs based theranostic micelles loaded with an antineoplastic drug, aminoflavone for TNBC therapy. The results of *in vitro* studies in EGFR overexpressing MDA-MB-468 cells showed that anti-EGFR nanobody 7D12 conjugation resulted in an enhanced cellular uptake and cytotoxicity of the QD-based micelles. Furthermore, there was a higher concentration of targeted micelles in tumors as compared with non-targeted ones, leading to more effective tumor regression in an orthotopic TNBC xenograft mouse model. Also, they did not observe any systemic toxicity with the treatments.[145] From these studies, it could be concluded that EGFR targeted nanomedicine could be explored promisingly for theranostic applications in breast cancer.

E. Transferrin-Targeted Theranostics

Transferrin (Tf) functionalized nanomedicines have significantly been explored for targeted delivery of anticancer therapeutic agents. Scientists are also exploring Tf for targeted theranostic applications in breast cancer. Tf-functionalized DOX-loaded luminescent blue copper (Tf-Cu) nanoclusters have been fabricated by Goswami et al. for breast cancer theranostic applications. The results of Förster resonance energy transfer within the DOX-loaded Tf-Cu nanoclusters showed striking red luminescence, wherein the blue luminescence of Tf-Cu nanoclusters (donor) was quenched due to absorption by DOX (acceptor). The blue luminescence of Tf-Cu nanoclusters was restored in the cytoplasm of cancer cells upon Tf mediated internalization. Finally, the gradual release of DOX from the nanoclusters led to the generation of red luminescence inside the nucleus. Moreover, a confocal microscopy image of HeLa cells revealed successful internalization of Tf-Cu nanoclusters in the cells. The results of *in vivo* studies showed superior targeting efficiency of DOX-loaded Tf-Cu nanoclusters on transferrin receptor

(TfR)–positive HeLa and MCF-7 cells compared with TfR-negative HEK-293 and 3T3-L1 cells. Furthermore, the Combination index showed synergistic interaction of Tf-Cu nanocluster and DOX upon treatment with DOX-loaded Tf-Cu nanoclusters. *In vivo* assessment of the nanoclusters on TfR overexpressing Dalton's lymphoma ascites-bearing mice model showed significant inhibition of tumor growth rendering prolonged survival of the mice.[146]

In another study, Muthu et al. designed docetaxel and ultra-bright gold clusters loaded Tf-tagged TPGS based theranostic micelles. Their results indicated that the micelles showed significantly higher cytotoxicity and cellular uptake along with the targeted co-delivery, and enhanced theranostic activity compared with the non-targeted micelles. Also, they observed a reduction in IC_{50} values of docetaxel-loaded TPGS based non-targeted and targeted micelles by 15.31- and 71.73-fold as compared with that of Taxotere® after 24 h incubation with MDA-MB-231-luc breast cancer cells, respectively. Thus, they proposed that the developed micelles could be an attractive system for the co-delivery of poorly soluble anticancer drugs and imaging agents to TfR overexpressing carcinomas.[147] Tf-conjugated micelles, nanoclusters and other nanomedicines have shown promising potential for targeted delivery of antineoplastic drugs and simultaneous imaging of TfR overexpressing carcinoma.

The potential of receptor-targeted theranostic nanomedicines explored for breast cancer is summarized in Table 2. Systematic research on ligand conjugated nanomedicines for receptor-mediated targeting of breast cancer for theranostic applications may result in a promising outcome and will surely aid in the clinical management of breast cancers including its most invasive variant TNBC.

V. CHALLENGES IN DEVELOPMENT OF BREAST CANCER THERANOSTICS AND FUTURE PERSPECTIVES

Major challenges in developing theranostic nanomedicines include (i) developing simple, controllable, and reproducible methods for synthesis of nanomedicines; (ii) insufficient batch-to-batch reproducibility, low yield, and variable physicochemical characteristics; (iii) understanding the *in vivo* biochemical mechanisms through which nanomedicines function (i.e., how nanomedicines target certain tissues and factors that affect the release of drugs from nanocarriers); (iv) understanding the biodistribution of NPs *in vivo*; (v) identifying the transformation and metabolic pathways; (vi) understanding the chronic toxicity of nanotheranostics *in vivo*; (vii) stringent regulatory and safety guidelines for timely and effective translation of theranostics to market. Finally, to bring nanomedicine to the clinic for the effective diagnosis and treatment of human diseases, we need multidisciplinary knowledge and techniques from pharmaceutical scientists, nanochemists, nanophysics, nanotoxicologists, clinical doctors, and so forth to construct safe and effective nanotheranostics.[154,155]

The high heterogeneity of breast cancers poses a big challenge for its successful prognosis and treatment, which could be resolved by selectively targeting the theranostic agents to cancer cells. Although, development of an effective theranostic nanomedicine

TABLE 2: Receptor-targeted theranostic nanomedicines for breast cancer treatment

Receptor targeted	Targeting ligand	Type of nanomedicines	Type of imaging	Imaging agent	Therapeutic agent	Breast cancer model	Outcome	Ref.
HER2	Trastuzumab	SPIONs	MRI	Protoporphyrin	IONPs	MCF-7 breast cancer cell line	*In vitro* photothermal ablation of IONP-protoporphyrin-trastuzumab revealed a 74% reduction in MCF-7 cells after 10 min exposure at the highest Fe concentration	132
	Anti-HER2 antibodies	Gold nanoshell PLGA hybrid nanocapsules	Ultrasound/ MRI	Perfluorooctyl bromide	DOX and SPIONs	HER2-positive SK-BR-3 cells and HER2-negative MDA-MB-231 cells	–Significant photothermal cytotoxicity Significantly enhanced anti-tumor effect	148
	Herceptin	Graphene QDs	Fluorescence	Graphene QDs	DOX	HER2-negative MCF-7 breast cancer cells, HER2-positive BT-474 breast cancer cells	Significant accumulation of nanocarriers in the cancer cells, Internalization into the cells via endocytosis	149
	Trastuzumab	Lipid NPs	Fluorescence	QDs	Rapamycin	SK-BR-3 cells, MDA-MB-231 cells	significantly higher cellular uptake, higher fluorescence intensity in tumor spheroid, ~ 3 and ~ 1.5-fold higher toxicity to SK-BR-3 cells and MDA-MB-231 cells, respectively	134

TABLE 2: (*continued*)

	Herceptin	IONPs	MRI and Fluorescence	IONPs	DOX	*In vitro*/ SK-BR-3 and MCF-7 breast cancer cells *In vivo*/ Athymic nude mice bearing SK-BR-3 cancer cells	–5 fold higher cellular uptake –Significantly enhanced tumor regression *in vitro* and *in vivo*	133
	Herceptin	Mesoporous silica NPs	Ultrasound	Mesoporous silica NPs	Mesoporous silica NPs	*In vitro*/ SK-BR-3 and MDA-MB-231 breast cancer cells	–Significantly higher cellular uptake –Significant enhancement in ultrasound image contrast and enhanced breast tumor-specific toxicity	150
	Trastuzumab	SPIONs	MRI	Fluorescent dye	SPIONs	*In vivo*/MMC cells *In vivo*/FVB/N transgenic mouse model	–4-fold higher cellular uptake –Significant contrast enhancement in MR images of primary breast tumors *in vitro* and *in vivo*	83
	Herceptin	Magnetic PLGA NPs	MRI	Magnetic nanocrystals	DOX	*In vitro*/ NIH3T6.7, SK-BR-3, and MDA-MB-231 cancer cells	–87.4 times greater fluorescent intensity –Significantly higher cancer cell affinity –Significantly higher cellular uptake –Excellent tumor growth retardation both *in vitro* and *in vivo*	136

TABLE 2: (*continued*)

Receptor targeted	Targeting ligand	Type of nanomedicines	Type of imaging	Imaging agent	Therapeutic agent	Breast cancer model	Outcome	Ref.
Folic acid	FA	Fe_3O_4 NPs	MRI	IONPs	IONPs	MC4-L2 cells, Female Balb/c mice	–The NPs were significantly accumulated and retained within the tumor tissues. –MRI experiments showed a significant decrease in T2-weighted MR signal intensity of breast tumors indicating the accumulation and retention of NPs in the tumor tissue *in vivo*. –Reduction in tumor progression	137
	FA	Nanocomposite	Fluorescence	Graphene oxide manganese-doped zinc sulfide QDs	DOX	Breast cancer cell line MDA-MB-231 (FA receptor positive) and NIH-3T3 cell line (FA receptor negative)	–FA functionalization improves the selectivity for discriminating positive FR cancer cells. –Significant reduction in the toxicity of the nanocomposite	151

TABLE 2: (*continued*)

	FA	IONPs	MRI	IONPs	DOX	Breast cancer cell lines, i.e., MCF-7, BT549, and MD-MBA-231 Female Balb/c nude mice	–FA conjugated NPs showed the strongest cytotoxicity against breast cancer cells, compared with non-targeted NPs, and caused cellular apoptosis. –Suppression in *in vivo* tumor growth in mice, No signs of toxicity against healthy organs	152
	FA	PEGylated magnetic NPs	MRI	Magnetic NPs	Tamoxifen	*In vitro*/MCF-7 breast cancer cell line	–Significantly enhanced cellular uptake –Significant inhibition in growth of MCF-7 cells	140
	FA	TPGS micelles	Fluorescence	QDs	Docetaxel	MCF-7 breast cancer cells	–Significantly higher red fluorescence intensity and higher cellular uptake –Significantly improved cytotoxicity *in vitro*	141
CD44	HA	Cationic BSA protected gold nanoclusters	PAI	ICG	Paclitaxel	4T1, A549, and RAW 246.7 Cells Female Balb/c mice	–Significantly enhanced cellular uptake –Suppression in *in situ* tumor growth by 95.3%	142

TABLE 2: (*continued*)

Receptor targeted	Targeting ligand	Type of nanomedicines	Type of imaging	Imaging agent	Therapeutic agent	Breast cancer model	Outcome	Ref.
	HA	HA prodrug micelles	Fluorescence	Aggregation-induced emission fluorogen tetraphenylene	DOX	CD44-overexpressing MDA-MB-231 cell line CD44 negative NIH3T3 cell line	–Flow cytometry and fluorescent microscopy indicated that HA prodrug micelles were effectively internalized by MDA-MB-231 cells due to HA-mediated endocytosis –Significant inhibition of the proliferation of cancer cells –Significantly enhanced cytotoxicity against MDA-MB-231 cells	143
EGFR	Anti-EGFR aptamer	Lipid nanocarriers	Fluorescence	QDs	siRNAs	EGFR positive MDA-MB-231 and EGFR negative MDA-MB-453 cell lines, Tumor-bearing female Balb/c nude mice	–Enhanced delivery to target cancer cells –Significant reduction in target gene expression –Inhibition of tumor growth and metastasis	144

TABLE 2: (*continued*)

	Anti-EGFR Nanobody	Micelles	Fluorescence	QDs	Aminoflavone	EGFR overexpressing MDA-MB-468 TNBC cells MDA-MB-468 breast cancer xenograft mouse model	–Nb-conjugated micelles accumulated in tumors at higher concentrations, leading to more effective tumor regression in the mouse model –No systemic toxicity observed –Enhanced cellular uptake and increased cytotoxicity	145
Transferrin	Tf	Nanoclusters	Fluorescence	luminescent blue copper	DOX	TfR positive HeLa, TfR positive MCF-7 cells, TfR partially positive HEK-293 and TfR negative Mouse embryo fibroblast cell lines	–Tf-CuNC-DOX-NPs showed excellent targeting efficiency on TfR overexpressed cells (HeLa and MCF-7) as compared with the less TfR expressed cells (HEK-293 and 3T3-L1) –Confocal microscopy images of HeLa cells revealed successful internalization of Tf-Cu nanoclusters in the cells	146

TABLE 2: (*continued*)

Receptor targeted	Targeting ligand	Type of nanomedicines	Type of imaging	Imaging agent	Therapeutic agent	Breast cancer model	Outcome	Ref.
	Tf	TPGS micelles	Fluorescence	Ultra-bright gold nanoclusters	Docetaxel	*In vitro*/MDA-MB-231-luc and NIH-3T3 fibroblast cells *In vivo*/female CB-17 mice bearing MDA-MB-231-luc cells induced tumor	Significantly higher cellular uptake and theranostic effect 4.6-fold reduction in IC_{50} value compared with non-targeted micelles	147
	Tf	PEGylated gold NPs	PAI	Fluorescein isothiocyanate	Gold NPs	*In vitro*/Hs578T cancer cells	–6-fold higher cellular uptake in breast cancer cells –Reduced in laser power effective for therapy from 1600 W/cm^2 to 7 W/cm^2, which is more than two orders of magnitude lower	153

with targeting propensity toward breast cancer cells may encounter many challenges, yet it will be beneficial to develop these nanomedicines as the conventional techniques used for diagnosis and management of breast cancer are not efficient enough to image the breast tumors completely with minute detailing. Further, targeted theranostic medicines, which can be sorted out using novel techniques and strategies, give a unique opportunity to maximize the accumulation of anticancer therapeutics to cancer cells which could result in an improved therapeutic index with a significant reduction in toxicity which is one of the major hurdles in the treatment of cancer along with the development of resistance to chemotherapeutic agents.

Cancer nanotechnology has advanced over the last two decades to reach a stage where the novel approach of "cancer theranostics" has been conceptualized and developed for the diagnosis and therapy of breast cancer. It provides a unique combination of drug and a diagnostic probe having controlled release and receptor-mediated targeting potential. Theranostics offers an effective and patient-friendly alternative. Various polymeric, lipid-based and inorganic NPs have been considered for this approach. They have shown therapeutically significant and noteworthy results in various investigations. However, the majority of theranostic applications in breast cancer have only been demonstrated at the pre-clinical level. In addition, due to nanoscale sizing, complex synthetic pathways can alter the physicochemical properties and consequently become a source of toxicity at the cellular or subcellular level. Various investigations related to the same have established the potential of nanomaterials for biochemical and physiological alterations. Therefore, a correlation needs to be established between their efficacy and safety. The study of *in vitro*/*in vivo* correlation has also become a challenge due to the non-availability of suitable tools. Thus, there is an unmet need to match the changing paradigm of breast cancer therapy using receptor-mediated nanomedicine with advanced bio-pharmaceutical and toxicity assessment tools.

VI. CONCLUSION

Breast cancer is emerging as one of the most frequently diagnosed cancer in women in developed and developing countries. Management of breast cancer is a difficult task if not diagnosed at an early stage. Further, the most aggressive form of breast cancer, i.e., TNBS is very difficult to manage due to metastasis which is further worsened by poor prognosis. Receptor-targeted nanomedicines are being explored to augment the selectivity of drug delivery to cancer cells. With the help of suitable ligand and engineering technology, promising nanomedicines may be designed to target the therapeutic, imaging agents, or both simultaneously to cancer cells. The result of ongoing studies and studies done in the last few decades are strongly supporting the candidature of receptor-targeted nanomedicines for targeted delivery of therapeutic and diagnostic moieties to breast cancer with an improved therapeutic index.

Further nanomedicine-based approaches are also being explored for theranostic applications in breast cancer with promising results which have been obtained in research studies conducted in the last decade. These nanomedicines based theranostic approaches

have revealed an ability to deliver the therapeutic agents to breast cancer, selectively, to enhance the efficacy of cancer therapy with simultaneous imaging of cancer microenvironment for better prognosis and management of breast cancer. So, from the result of these investigations, it could be expected that continuous and coherent investigation in this direction may help in developing promising therapeutic approaches based on nanomedicines for targeted delivery of diagnostic agents, therapeutic moieties, and theranostic modalities to breast cancer, which may get approval for clinical use.

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REFERENCES

1. World Health Organization. Cancer. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer.
2. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer (Dove Med Press). 2019;11:151–64.
3. Cancer.Net. Breast cancer - metastatic: Statistics. Available from: https://www.cancer.net/cancer-types/breast-cancer-metastatic/statistics.
4. Francis I, Altemaimi R, Al-Ayadhy B, Alath P, Jaragh M, Mothafar F, Kapila K. Hormone receptors and human epidermal growth factor (HER2) expression in fine-needle aspirates from metastatic breast carcinoma – Role in patient management. J Cytol. 2019;36(2):94–100.
5. Yao H, He G, Yan S, Chen C, Song L, Rosol TJ, Deng X. Triple-negative breast cancer: Is there a treatment on the horizon? Oncotarget. 2017;8(1):1913–24.
6. Khan MA, Jain VK, Rizwanullah M, Ahmad J, Jain K. PI3K/AKT/mTOR pathway inhibitors in triple-negative breast cancer: A review on drug discovery and future challenges. Drug Discov Today. 2019;24(11):2181–91.
7. Ji X, Lu Y, Tian H, Meng X, Wei M, Cho WC. Chemoresistance mechanisms of breast cancer and their countermeasures. Biomed Pharmacother. 2019;114:108800.
8. Ahmad J, Akhter S, Greig NH, Amjad Kamal M, Midoux P, Pichon C. Engineered nanoparticles against mdr in cancer: The state of the art and its prospective. Curr Pharm Des. 2016;22(28):4360–73.
9. Ahmad J, Kohli K, Mir SR, Amin S. Lipid based nanocarriers for oral delivery of cancer chemotherapeutics: An insight in the intestinal lymphatic transport. Drug Deliv Lett. 2013;3(1):38–46.
10. Ahmad J, Amin S, Rahman M, Rub R, Singhal M, Ahmad M, Rahman Z, Addo R, Ahmad F, Mushtaq G, Amjad Kamal M, Akhter S. Solid matrix based lipidic nanoparticles in oral cancer chemotherapy: Applications and pharmacokinetics. Curr Drug Metab. 2015;16(8):633–44.
11. Ahmad J, Akhter S, Rizwanullah M, Amin S, Rahman M, Ahmad MZ, Rizvi MA, Amjad Kamal M, Ahmad FJ. Nanotechnology-based inhalation treatments for lung cancer: State of the art. Nanotechnol Sci Appl. 2015;8:55–66.
12. Afsana, Jain V, Haider N, Jain K. 3D printing in personalized drug delivery. Curr Pharm Des. 2019;24(42):5062–71.

13. Pardhi VP, Verma T, Flora SJS, Chandasana H, Shukla R. Nanocrystals: An overview of fabrication, characterization and therapeutic applications in drug delivery. Curr Pharm Des. 2019;24(43): 5129–46.
14. Joshi K, Chandra A, Jain K, Talegaonkar S. Nanocrystalization: An emerging technology to enhance the bioavailability of poorly soluble drugs. Pharm Nanotechnol. 2019;7(4):259–78.
15. Jain K, Shukla R, Yadav A, Ujjwal RR, Flora SJS. 3D printing in development of nanomedicines. Nanomaterials. 2021;11(2):1–24.
16. Jain K, Jain NK. Dendrimers as nanobiopolymers in cancer chemotherapy. In: Nanobiomedicine. Houston, TX: Studium Press; 2015. p. 289–306.
17. Rizwanullah Md, Ahmad J, Amin S. Nanostructured lipid carriers: A novel platform for chemotherapeutics. Curr Drug Deliv. 201;13(1):4–26.
18. Akhter MdH, Rizwanullah Md, Ahmad J, Ahsan MJ, Mujtaba MdA, Amin S. Nanocarriers in advanced drug targeting: Setting novel paradigm in cancer therapeutics. Artif Cells Nanomed Biotechnol. 2018;46(5):873–84.
19. Jain V, Jain K. Molecular targets and pathways for the treatment of visceral leishmaniasis. Drug Discov Today. 2018;23(1):161–70.
20. Jain K, Mehra NK, Jain NK. Potentials and emerging trends in nanopharmacology. Curr Opin Pharmacol. 2014;15(1):97–106.
21. Soni N, Jain K, Gupta U, Jain NK. Controlled delivery of gemcitabine hydrochloride using mannosylated poly(propyleneimine) dendrimers. J Nanoparticle Res. 2015;17(11):1–17.
22. Pardhi V, Chavan RB, Thipparaboina R, Thatikonda S, Naidu VGM, Shastri NR. Preparation, characterization, and cytotoxicity studies of niclosamide loaded mesoporous drug delivery systems. Int J Pharm. 2017;528(1-2):202–14.
23. Rizwanullah M, Amin S, Mir SR, Fakhri KU, Rizvi MMA. Phytochemical based nanomedicines against cancer: Current status and future prospects. J Drug Target. 2018;26(9):731–52.
24. Jain K. Nanohybrids of dendrimers and carbon nanotubes: A benefaction or forfeit in drug delivery? Nanosci Nanotechnol. 2018;9(1):21–9.
25. Dahiya S, Dahiya R, Hernández E. Nanocarriers for anticancer drug targeting: Recent trends and challenges. Crit Rev Ther Drug Carrier Syst. 2021;38(6):49–103.
26. Navya PN, Kaphle A, Srinivas SP, Bhargava SK, Rotello VM, Daima HK. Current trends and challenges in cancer management and therapy using designer nanomaterials. Nano Converg. 2019;6(2):23.
27. Jain K, Jain NK. Surface engineered dendrimers as antiangiogenic agent and carrier for anticancer drug: Dual attack on cancer. J Nanosci Nanotechnol. 2014;14(7):5075–87.
28. Rahman M, Akhter S, Ahmad MZ, Ahmad J, Addo RT, Ahmad FJ, Pichon C. Emerging advances in cancer nanotheranostics with graphene nanocomposites: Opportunities and challenges. Nanomedicine. 2015;10(15):2405–22.
29. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: An updated review. Pharmaceutics. 2017;9(2):12.
30. Din FU, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S, Zeb A. Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. Int J Nanomed. 2017;12:7291–309.
31. Kirtane AR, Kalscheuer SM, Panyam J. Exploiting nanotechnology to overcome tumor drug resistance: Challenges and opportunities. Advanced Drug Delivery Reviews. 2013;65(13-14):1731–47.
32. Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. Adv Drug Deliv Rev. 2013;65(1):71–9.
33. Schaaf MB, Garg AD, Agostinis P. Defining the role of the tumor vasculature in antitumor immunity and immunotherapy article. Cell Death Dis. 2018;9(2):1–14.
34. Fang J, Nakamura H, Maeda H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv Drug Deliv Rev. 2011;63(3):136–51.

35. Khawar IA, Kim JH, Kuh HJ. Improving drug delivery to solid tumors: Priming the tumor microenvironment. J Control Release. 2015;201:78–89.
36. Shi Y, van der Meel R, Chen X, Lammers T. The EPR effect and beyond: Strategies to improve tumor targeting and cancer nanomedicine treatment efficacy. Theranostics. 2020;10(17):7921–4.
37. Danhier F, Feron O, Préat V. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release. 2010;148(2):135–46.
38. Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC. Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev. 2014;66:2–25.
39. Shi J, Kantoff PW, Wooster R, Farokhzad OC. Cancer nanomedicine: Progress, challenges and opportunities. Nat Rev Cancer. 2017;17(1):20–37.
40. Fang J, Islam W, Maeda H. Exploiting the dynamics of the EPR effect and strategies to improve the therapeutic effects of nanomedicines by using EPR effect enhancers. Adv Drug Deliv Rev. 2020;157:142–60.
41. Subhan MA, Yalamarty SSK, Filipczak N, Parveen F, Torchilin VP. Recent advances in tumor targeting via epr effect for cancer treatment. J Pers Med. 2021;11(6).
42. Islam R, Maeda H, Fang J. Factors affecting the dynamics and heterogeneity of the EPR effect: Pathophysiological and pathoanatomic features, drug formulations and physicochemical factors. Expert Opinion on Drug Delivery. 2021:1–14.
43. Halin C, Neri D. Antibody-based targeting of angiogenesis. Crit Rev Ther Drug Carrier Syst. 2001;18(3):299–339.
44. Golombek SK, May JN, Theek B, Appold L, Drude N, Kiessling F, Lammers T. Tumor targeting via EPR: Strategies to enhance patient responses. Adv Drug Deliv Rev. 2018;130:17–38.
45. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, Diaz-Torres LS, Grillo R, Swamy MK, Sharma S, Habtemariam S, Shin H. Nano based drug delivery systems: Recent developments and future prospects. J Nanobiotechnol. 2018;16(1):71.
46. Fam SY, Chee CF, Yong CY, Ho KL, Mariatulqabtiah AR, Tan WS. Stealth coating of Nanoparticles in drug-delivery systems. Nanomaterials. 2020;10(4):1–18.
47. Hillaireau H, Couvreur P. Nanocarriers' entry into the cell: Relevance to drug delivery. Cell Mol Life Sci. 2009;66(17):2873–96.
48. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. Eur J Pharm Biopharm. 2015;93:52–79.
49. Xie J, Yang Z, Zhou C, Zhu J, Lee RJ, Teng L. Nanotechnology for the delivery of phytochemicals in cancer therapy. Biotechnology Advances. 2016;34(4):343–53.
50. Attia MF, Anton N, Wallyn J, Omran Z, Vandamme TF. An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. J Pharm Pharmacol. 2019;71(8):1185–98.
51. Zhong Y, Meng F, Deng C, Zhong Z. Ligand-directed active tumor-targeting polymeric nanoparticles for cancer chemotherapy. Biomacromolecules. 2014;15(6):1955–69.
52. Yoo J, Park C, Yi G, Lee D, Koo H. Active targeting strategies using biological ligands for nanoparticle drug delivery systems. Cancers. 2019;11(5).
53. Bazak R, Houri M, el Achy S, Kamel S, Refaat T. Cancer active targeting by nanoparticles: A comprehensive review of literature. J Cancer Res Clin Oncol. 2015;141(5):769–84.
54. He K, Zeng S, Qian L. Recent progress in the molecular imaging of therapeutic monoclonal antibodies. J Pharm Anal. 2020;10(5):397–413.
55. Vyas SP, Singh A, Sihorkar V. Ligand-receptor-mediated drug delivery: An emerging paradigm in cellular drug targeting. Crit Rev Ther Drug Carrier Syst. 2001;18(1):1–76.
56. Danhier F. To exploit the tumor microenvironment: Since the EPR effect fails in the clinic, what is the future of nanomedicine? J Control Release. 2016;244(Pt A):108–21.
57. Roma-Rodrigues C, Mendes R, Baptista PV, Fernandes AR. Targeting tumor microenvironment for cancer therapy. Int J Mol Sci. 2019;20(4).

58. Ashfaq UA, Riaz M, Yasmeen E, Yousaf M. Recent advances in nanoparticle-based targeted drug-delivery systems against cancer and role of tumor microenvironment. Crit Rev Ther Drug Carrier Syst. 2017;34(4):317–53.
59. Mallidi S, Luke GP, Emelianov S. Photoacoustic imaging in cancer detection, diagnosis, and treatment guidance. Trends Biotechnol. 2011;29(5):213–21.
60. Lima ZS, Ebadi MR, Amjad G, Younesi L. Application of imaging technologies in breast cancer detection: A review article. Open Access Maced J Med Sci. 2019;7(5):838–48.
61. Yezhelyev MV, Gao X, Xing Y, Al-Hajj A, Nie S, O'Regan RM. Emerging use of nanoparticles in diagnosis and treatment of breast cancer. Lancet Oncol. 2006;7(8):657–67.
62. Wang J, Xu B. Targeted therapeutic options and future perspectives for HER2-positive breast cancer. Signal Transduct Target Ther. 2019;4(1):1–22.
63. Li Y, Yang X-Q, Chen C, Peng C-W, Hou J-X, Liu S-P, Qi C-B, Gong Y-P, Zhu X-B, Pang D-W. Quantum dot-based quantitative immunofluorescence detection and spectrum analysis of epidermal growth factor receptor in breast cancer tissue arrays. Int J Nanomed. 2011;6:2265.
64. Wu L, Qu X. Cancer biomarker detection: Recent achievements and challenges. Chem Soc Rev. 2015;44(10):2963–97.
65. Chen C, Peng J, Xia HS, Yang GF, Wu QS, Chen LD, Zeng LB, Zhang ZL, Pang DW, Li Y. Quantum dots-based immunofluorescence technology for the quantitative determination of HER2 expression in breast cancer. Biomaterials. 2009;30(15):2912–8.
66. Pelaz B, Alexiou C, Alvarez-Puebla RA, Alves F, Andrews AM, Ashraf S, Balogh LP, Ballerini L, Bestetti A, Brendel C, Bosi S, Carril M, Chan WCW, Chen C, Chen X, Chen X, Cheng Z, Cui D, Du J, Dullin C, Escudero A, Feliu N, Gao M, George M, Gogotsi Y, Grünweller A, Gu Z, Halas NJ, Hampp N, Hartmann RK, Hersam MC, Hunziker P, Jian J, Jiang X, Jungebluth P, Kadhiresan P, Kataoka K, Khademhosseini A, Kopeček J, Kotov NA, Krug HF, Lee DS, Lehr CM, Leong KW, Liang X, Lim ML, Liz-Marzán LM, Ma X, Macchiarini P, Meng H, Möhwald H, Mulvaney P, Nel AE, Nie S, Nordlander P, Okano T, Oliveira J, Park TH, Penner RM, Prato M, Puntes V, Rotello VM, Samarakoon A, Schaak RE, Shen Y, Sjöqvist S, Skirtach AG, Soliman MG, Stevens MM, Sung HW, Tang BZ, Tietze R, Udugama BN, VanEpps JS, Weil T, Weiss PS, Willner I, Wu Y, Yang L, Yue Z, Zhang Q, Zhang Q, Zhang XE, Zhao Y, Zhou X, Parak WJ. Diverse applications of nanomedicine. ACS Nano. 2017;11(3):2313–81.
67. Meier R, Henning TD, Boddington S, Tavri S, Arora S, Piontek G, Rudelius M, Corot C, Daldrup-Link HE. Breast cancers: MR imaging of folate-receptor expression with the folate-specific nanoparticle P1133. Radiology. 2010;255(2):527–35.
68. Lim EK, Kim HO, Jang E, Park J, Lee K, Suh JS, Huh YM, Haam S. Hyaluronan-modified magnetic nanoclusters for detection of CD44-overexpressing breast cancer by MR imaging. Biomaterials. 2011;32(31):7941–50.
69. Keshtkar M, Shahbazi-Gahrouei D, Khoshfetrat S, Mehrgardi M, Aghaei M. Aptamer-conjugated magnetic nanoparticles as targeted magnetic resonance imaging contrast agent for breast cancer. J Med Signals Sens. 2016;6(4):243–7.
70. Nian D, Shi P, Sun J, Ren L, Hao X, Han J. Application of luteinizing hormone-releasing hormone–ferrosoferric oxide nanoparticles in targeted imaging of breast tumors. J Int Med Res. 2019;47(4): 1749–57.
71. Yang J, Lee CH, Park J, Seo S, Lim EK, Song YJ, Suh JS, Yoon HG, Huh YM, Haam S. Antibody conjugated magnetic PLGA nanoparticles for diagnosis and treatment of breast cancer. J Mater Chem. 2007;17(26):2695–9.
72. Wu P, He X, Wang K, Tan W, Ma D, Yang W, He C. Imaging breast cancer cells and tissues using peptide-labeled fluorescent silica nanoparticles. J Nanosci Nanotechnol. 2008;
73. Sha Q, Guan R, Su H, Zhang L, Liu BF, Hu Z, Liu X. Carbohydrate-protein template synthesized high mannose loading gold nanoclusters: A powerful fluorescence probe for sensitive Concanavalin A detection and specific breast cancer cell imaging. Talanta. 2020;218:121130.

74. Tanaka S, Wadati H, Sato K, Yasuda H, Niioka H. Red-fluorescent Pt nanoclusters for detecting and imaging HER2 in breast cancer cells. ACS Omega. 2020;5(37):23718–23.
75. Sakamoto JH, Smith BR, Xie B, Rokhlin SI, Lee SC, Ferrari M. The molecular analysis of breast cancer utilizing targeted nanoparticle based ultrasound contrast agents. Technol Cancer Res Treat. 2005;4(6):627–36.
76. Liu J, Li J, Rosol TJ, Pan X, Voorhees JL. Biodegradable nanoparticles for targeted ultrasound imaging of breast cancer cells in vitro. Phys Med Biol. 2007;52(16):4739–47.
77. Xu L, Du J, Wan CF, Zhang Y, Xie SW, Li HL, Yang H, Li F. Ultrasound molecular imaging of breast cancer in MCF-7 orthotopic mice using gold nanoshelled poly(lactic-co-glycolic acid) nanocapsules: A novel dual-targeted ultrasound contrast agent. Int J Nanomed. 2018;13:1791–807.
78. Dong Q, Yang H, Wan C, Zheng D, Zhou Z, Xie S, Xu L, Du J, Li F. HER2-functionalized gold-nanoshelled magnetic hybrid nanoparticles: A theranostic agent for dual-modal imaging and photothermal therapy of breast cancer. Nanoscale Res Lett. 2019;14(1):235.
79. Galanzha EI, Kim JW, Zharov VP. Nanotechnology-based molecular photoacoustic and photothermal flow cytometry platform for in-vivo detection and killing of circulating cancer stem cells. J Biophotonics. 2009;2(12):725–35.
80. Xi L, Grobmyer SR, Zhou G, Qian W, Yang L, Jiang H. Molecular photoacoustic tomography of breast cancer using receptor targeted magnetic iron oxide nanoparticles as contrast agents. J Biophotonics. 2014;7(6):401–9.
81. Zhang T, Cui H, Fang CY, Cheng K, Yang X, Chang HC, Forrest ML. Targeted nanodiamonds as phenotype-specific photoacoustic contrast agents for breast cancer. Nanomedicine. 2015;10(4): 573–87.
82. Liu Y, Wu PC, Guo S, Chou PT, Deng C, Chou SW, Yuan Z, Liu TM. Low-toxicity FePt nanoparticles for the targeted and enhanced diagnosis of breast tumors using few centimeters deep whole-body photoacoustic imaging. Photoacoustics. 2020;19:100179.
83. Kievit FM, Stephen ZR, Veiseh O, Arami H, Wang T, Lai VP, Park JO, Ellenbogen RG, Disis ML, Zhang M. Targeting of primary breast cancers and metastases in a transgenic mouse model using rationally designed multifunctional SPIONs. ACS Nano. 2012;6(3):2591–601.
84. Nima ZA, Watanabe F, Jamshidi-Parsian A, Sarimollaoglu M, Nedosekin DA, Han M, Watts JA, Biris AS, Zharov VP, Galanzha EI. Bioinspired magnetic nanoparticles as multimodal photoacoustic, photothermal and photomechanical contrast agents. Sci Rep. 2019;9(1):1–12.
85. Du J, Zhang Y, Jin Z, Wu H, Cang J, Shen Y, Miao F, Zhang A, Zhang Y, Zhang J, Teng G. Targeted NIRF/MR dual-mode imaging of breast cancer brain metastasis using BRBP1-functionalized ultrasmall iron oxide nanoparticles. Mater Sci Engin C Mater Biol Appl. 2020;116:111188.
86. Luk BT, Zhang L. Current advances in polymer-based nanotheranostics for cancer treatment and diagnosis. ACS Appl Mater Interfaces. 2014;6(24):21859–73.
87. Grover VPB, Tognarelli JM, Crossey MME, Cox IJ, Taylor-Robinson SD, McPhail MJW. Magnetic resonance imaging: Principles and techniques: Lessons for clinicians. J Clin Exp Hepatol. 2015;5(3): 246–55.
88. Estelrich J, Sánchez-Martín MJ, Busquets MA. Nanoparticles in magnetic resonance imaging: From simple to dual contrast agents. Int J Nanomed. 2015;10:1727–41.
89. Fern I, Muñoz-Hernando M, Ruiz-Cabello J. Iron oxide nanoparticles: An alternative for positive contrast in magnetic resonance imaging. Inorganics. 2020;1–22.
90. Li DL, Tan JE, Tian Y, Huang S, Sun PH, Wang M, Han YJ, Li HS, Wu HB, Zhang XM, Xu YK, Wang QS. Multifunctional superparamagnetic nanoparticles conjugated with fluorescein-labeled designed ankyrin repeat protein as an efficient HER2-targeted probe in breast cancer. Biomaterials. 2017;147:86–98.
91. Ding N, Sano K, Kanazaki K, Ohashi M, Deguchi J, Kanada Y, Ono M, Saji H. In vivo HER2-targeted magnetic resonance tumor imaging using iron oxide nanoparticles conjugated with anti-HER2 fragment antibody. Mol Imaging Biol. 2016;18(6):870–6.

92. Lee T, Lim EK, Lee J, Kang B, Choi J, Park HS, Suh JS, Huh YM, Haam S. Efficient CD44-targeted magnetic resonance imaging (MRI) of breast cancer cells using hyaluronic acid (HA)-modified MnFe2O4 nanocrystals. Nanoscale Res Lett. 2013;8(1):1–9.
93. Meng J, Fan J, Galiana G, Branca RT, Clasen PL, Ma S, Zhou J, Leuschner C, Kumar C, Hormes J, Otiti T, Beye A, Harmer M, Kiely C, Warren W, Haataja M, Soboyejo W. LHRH-functionalized superparamagnetic iron oxide nanoparticles for breast cancer targeting and contrast enhancement in MRI. Mater Sci Engin C. 2009;29(4):1467–79.
94. Cubeddu R, Comelli D, D'Andrea C, Taroni P, Valentini G. Time-resolved fluorescence imaging in biology and medicine. J Phys D Appl Phys. 2002;35(9):R61.
95. Sarkar I, Mishra AK. Fluorophore tagged bio-molecules and their applications: A brief review. Appl Spectrosc Rev. 2018;53(7):552–601.
96. Sarder P, Yazdanfar S, Akers WJ, Tang R, Sudlow GP, Egbulefu C, Achilefu S. All-near-infrared multiphoton microscopy interrogates intact tissues at deeper imaging depths than conventional single- and two-photon near-infrared excitation microscopes. J Biomed Opt. 2013;18(10):106012.
97. Sajedi S, Sabet H, Choi HS. Intraoperative biophotonic imaging systems for image-guided interventions. Nanophotonics. 2018;8(1):99–116.
98. Basu S, Alavi A. SPECT-CT and PET-CT in oncology – An overview. Curr Med Imaging Rev. 2011;7(3):202–9.
99. Chopra A, Shan L, Eckelman WC, Leung K, Menkens AE. Important parameters to consider for the characterization of PET and SPECT imaging probes. Nucl Med Biol. 2011;38(8):1079–84.
100. Miletich RS. Positron emission tomography and single-photon emission computed tomography in neurology. Continuum (Minneap Minn). 2016;22:1636–54.
101. Frangioni JV. In vivo near-infrared fluorescence imaging. Curr Opin Chem Biol. 2003;7(5):626–34.
102. Ntziachristos V, Bremer C, Weissleder R. Fluorescence imaging with near-infrared light: New technological advances that enable in vivo molecular imaging. Eur Radiol. 2003;13(1):195–208.
103. Janib SM, Moses AS, MacKay JA. Imaging and drug delivery using theranostic nanoparticles. Adv Drug Deliv Rev. 2010;62(11):1052–63.
104. Yamaguchi H, Tsuchimochi M, Hayama K, Kawase T, Tsubokawa N. Dual-labeled near-infrared/99mTc imaging probes using PAMAM-coated silica nanoparticles for the imaging of HER2-expressing cancer cells. Int J Mol Sci. 2016;17(7).
105. Li K, Jiang Y, Ding D, Zhang X, Liu Y, Hua J, Feng S, Liu B. Folic acid-functionalized two-photon absorbing nanoparticles for targeted MCF-7 cancer cell imaging. Chem Commun. 2011;47(26): 7323–5.
106. Christensen-Jeffries K, Couture O, Dayton PA, Eldar YC, Hynynen K, Kiessling F, O'Reilly M, Pinton GF, Schmitz G, Tang MX, Tanter M, Sloun R. Super-resolution ultrasound imaging. Ultrasound Med Biol. 2020;46(4):865–91.
107. Guo R, Lu G, Qin B, Fei B. Ultrasound imaging technologies for breast cancer detection and management: A review. Ultrasound Med Biol. 2018;44(1):37–70.
108. Hoelen CGA, de Mul FFM, Pongers R, Dekker A. Three-dimensional photoacoustic imaging of blood vessels in tissue. Opt Lett. 1998; 3(8):648.
109. Copland JA, Eghtedari M, Popov VL, Kotov N, Mamedova N, Motamedi M, Oraevsky A. Bioconjugated gold nanoparticles as a molecular based contrast agent: Implications for imaging of deep tumors using optoacoustic tomography. Mol Imaging Biol. 2004;6(5):341–9.
110. Yuan Z, Jiang H. Quantitative photoacoustic tomography. Philos Trans A Math Phys Eng Sci. 2009;367(1900):3043–54.
111. Manohar S, Dantuma M. Current and future trends in photoacoustic breast imaging. Photoacoustics. 2019;16:100134.
112. Steinberg I, Huland DM, Vermesh O, Frostig HE, Tummers WS, Gambhir SS. Photoacoustic clinical imaging. Photoacoustics. 2019;14:77–98.
113. Balasundaram G, Ho CJH, Li K, Driessen W, Dinish US, Wong CL, Ntziachristos V, Liu B, Olivo M.

Molecular photoacoustic imaging of breast cancer using an actively targeted conjugated polymer. Int J Nanomed. 2015;10:387–97.
114. Kanazaki K, Sano K, Makino A, Shimizu Y, Yamauchi F, Ogawa S, Ding N, Yano T, Temma T, Ono M, Saji H. Development of anti-HER2 fragment antibody conjugated to iron oxide nanoparticles for in vivo HER2-targeted photoacoustic tumor imaging. Nanomedicine. 2015;11(8):2051–60.
115. Xi L, Grobmyer SR, Zhou G, Qian W, Yang L, Jiang H. Molecular photoacoustic tomography of breast cancer using receptor targeted magnetic iron oxide nanoparticles as contrast agents. J Biophotonics. 2014;7(6):401–9.
116. Wang H, Liu C, Gong X, Hu D, Lin R, Sheng Z, Zheng C, Yan M, Chen J, Cai L, Song L. In vivo photoacoustic molecular imaging of breast carcinoma with folate receptor-targeted indocyanine green nanoprobes. Nanoscale. 2014;6(23):14270–9.
117. Lee DE, Koo H, Sun IC, Ryu JH, Kim K, Kwon IC. Multifunctional nanoparticles for multimodal imaging and theragnosis. Chem Soc Rev. 2012;41(7):2656–72.
118. Forte S, Dellas S, Stieltjes B, Bongartz B. Multimodal ultrasound tomography for breast imaging: A prospective study of clinical feasibility. Eur Radiol Exp. 2017;1(1):27.
119. Han C, Zhang A, Kong Y, Yu N, Xie T, Dou B, Li K, Wang Y, Li J, Xu K. Multifunctional iron oxide-carbon hybrid nanoparticles for targeted fluorescent/MR dual-modal imaging and detection of breast cancer cells. Anal Chim Acta. 2019;1067:115–28.
120. Song W, Luo Y, Zhao Y, Liu X, Zhao J, Luo J, Zhang Q, Ran H, Wang Z, Guo D. Magnetic nanobubbles with potential for targeted drug delivery and trimodal imaging in breast cancer: An in vitro study. Nanomedicine. 2017;12(9):991–1009.
121. Yang RM, Fu CP, Fang JZ, Xu XD, Wei XH, Tang WJ, Jiang XQ, Zhang LM. Hyaluronan-modified superparamagnetic iron oxide nanoparticles for bimodal breast cancer imaging and photothermal therapy. Int J Nanomed. 2017;12:197–206.
122. Lim EK, Kim HO, Jang E, Park J, Lee K, Suh JS, Huh YM, Haam S. Hyaluronan-modified magnetic nanoclusters for detection of CD44-overexpressing breast cancer by MR imaging. Biomaterials. 2011;32(31):7941–50.
123. Rainone P, Riva B, Belloli S, Sudati F, Ripamonti M, Verderio P, Colombo M, Colzani B, Gilardi MC, Moresco RM, Prosperi D. Development of 99mtc-radiolabeled nanosilica for targeted detection of HER2-positive breast cancer. Int J Nanomed. 2017;12:3447–61.
124. Zhang M, Kim HS, Jin T, Yi A, Moon WK. Ultrasound-guided photoacoustic imaging for the selective detection of EGFR-expressing breast cancer and lymph node metastases. Biomed Opt Exp. 2016;7(5):1920.
125. Heidari Z, Sariri R, Salouti M. Gold nanorods-bombesin conjugate as a potential targeted imaging agent for detection of breast cancer. J Photochem Photobiol B Biol. 2014;130:40–6.
126. Stephen BJ, Suchanti S, Mishra R, Singh A. Cancer nanotechnology in medicine: A promising approach for cancer detection and diagnosis. Crit Rev Ther Drug Carrier Syst. 2020;37(4):375–405.
127. Chen H, Zhang W, Zhu G, Xie J, Chen X. Rethinking cancer nanotheranostics. Nat Rev Mater. 2017;2:17024.
128. Muthu MS, Feng SS. Theranostic liposomes for cancer diagnosis and treatment: Current development and pre-clinical success. Exp Opin Drug Deliv. 2013;10(2):151–5.
129. Jokerst JV, Gambhir SS. Molecular imaging with theranostic nanoparticles. Acc Chem Res. 201;44(10):1050–60.
130. Chen F, Ehlerding EB, Cai W. Theranostic nanoparticles. J Nucl Med. 2014;55(12):1919–22.
131. Chen F, Cai W. Tumor vasculature targeting: A generally applicable approach for functionalized nanomaterials. Small. 2014;10(10):1887–93.
132. Khaniabadi PM, Shahbazi-Gahrouei D, Aziz AA, Dheyab MA, Khaniabadi BM, Mehrdel B, Jameel MS. Trastuzumab conjugated porphyrin-superparamagnetic iron oxide nanoparticle: A potential PTT-MRI bimodal agent for herceptin positive breast cancer. Photodiagnosis Photodyn Ther. 2020;31:101896.

133. Choi W il, Lee JH, Kim JY, Heo SU, Jeong YY, Kim YH, Tae G. Targeted antitumor efficacy and imaging via multifunctional nano-carrier conjugated with anti-HER2 trastuzumab. Nanomedicine. 2015;11(2):359–68.
134. Parhi P, Sahoo SK. Trastuzumab guided nanotheranostics: A lipid based multifunctional nanoformulation for targeted drug delivery and imaging in breast cancer therapy. J Colloid Interface Sci. 2015;451:198–211.
135. Day ES, Bickford LR, Slater JH, Riggall NS, Drezek RA, West JL. Antibody-conjugated gold-gold sulfide nanoparticles as multifunctional agents for imaging and therapy of breast cancer. Int J Nanomed. 2010;5(1):445–54.
136. Yang J, Lee CH, Park J, Seo S, Lim EK, Song YJ, Suh JS, Yoon HG, Huh YM, Haam S. Antibody conjugated magnetic PLGA nanoparticles for diagnosis and treatment of breast cancer. J Mater Chem. 2007;17(26):2695–9.
137. Soleymani M, Khalighfard S, Khodayari S, Khodayari H, Kalhori MR, Hadjighassem MR, Shaterabadi Z, Alizadeh M. Effects of multiple injections on the efficacy and cytotoxicity of folate-targeted magnetite nanoparticles as theranostic agents for MRI detection and magnetic hyperthermia therapy of tumor cells. Sci Rep. 2020;10(1):1–14.
138. Alibolandi M, Abnous K, Sadeghi F, Hosseinkhani H, Ramezani M, Hadizadeh F. Folate receptor-targeted multimodal polymersomes for delivery of quantum dots and doxorubicin to breast adenocarcinoma: In vitro and in vivo evaluation. Int J Pharm. 2016;500(1-2):162–78.
139. Zhou F, Feng B, Yu H, Wang D, Wang T, Liu J, Meng Q, Wang S, Zhang P, Zhang Z, Li Y. Cisplatin prodrug-conjugated gold nanocluster for fluorescence imaging and targeted therapy of the breast cancer. Theranostics. 2016;6(5):679–87.
140. Heidari Majd M, Asgari D, Barar J, Valizadeh H, Kafil V, Abadpour A, Moumivand E, Mojarrad JS, Rashidi MR, Coukos G, Omidi Y. Tamoxifen loaded folic acid armed PEGylated magnetic nanoparticles for targeted imaging and therapy of cancer. Colloids Surf B Biointerfaces. 2013;106:117–25.
141. Muthu MS, Kulkarni SA, Raju A, Feng SS. Theranostic liposomes of TPGS coating for targeted co-delivery of docetaxel and quantum dots. Biomaterials. 2012;33(12):3494–501.
142. Liu R, Xiao W, Hu C, Xie R, Gao H. Theranostic size-reducible and no donor conjugated gold nanocluster fabricated hyaluronic acid nanoparticle with optimal size for combinational treatment of breast cancer and lung metastasis. J Control Release. 2018;278:127–39.
143. Wang L, Zhang H, Qin A, Jin Q, Tang BZ, Ji J. Theranostic hyaluronic acid prodrug micelles with aggregation-induced emission characteristics for targeted drug delivery. Sci China Chem. 2016;59(12):1609–15.
144. Kim MW, Jeong HY, Kang SJ, Jeong IH, Choi MJ, You YM, Im CS, Song IH, Lee TS, Lee JS, Lee A, Park YS. Anti-EGF receptor aptamer-guided co-delivery of anti-cancer siRNAs and quantum dots for theranostics of triple-negative breast cancer. Theranostics. 2019;9(3):837–52.
145. Wang Y, Wang Y, Chen G, Li Y, Xu W, Gong S. Quantum-dot-based theranostic micelles conjugated with an Anti-EGFR nanobody for triple-negative breast cancer therapy. ACS Appl Mater Interfaces. 2017;9(36):30297–305.
146. Goswami U, Dutta A, Raza A, Kandimalla R, Kalita S, Ghosh SS, Chattopadhyay A. Transferrin-copper nanocluster-doxorubicin nanoparticles as targeted theranostic cancer nanodrug. ACS Appl Mater Interfaces. 2018;10(4):3282–94.
147. Muthu MS, Kutty RV, Luo Z, Xie J, Feng SS. Theranostic vitamin E TPGS micelles of transferrin conjugation for targeted co-delivery of docetaxel and ultra bright gold nanoclusters. Biomaterials. 2015; 39:234–48.
148. Dong Q, Wan C, Yang H, Zheng D, Xu L, Zhou Z, Xie S, Du J, Li F. Targeted gold nanoshelled hybrid nanocapsules encapsulating doxorubicin for bimodal imaging and near-infrared triggered synergistic therapy of HER2-positve breast cancer. J Biomater Appl. 2020;35(3):430–45.
149. Ko NR, Nafiujjaman M, Lee JS, Lim HN, Lee YK, Kwon IK. Graphene quantum dot-based theranostic agents for active targeting of breast cancer. RSC Adv. 2017;7(19):11420–7.

150. Milgroom A, Intrator M, Madhavan K, Mazzaro L, Shandas R, Liu B, Park D. Mesoporous silica nanoparticles as a breast-cancer targeting ultrasound contrast agent. Colloids Surf B Biointerfaces. 2014;116:652–7.
151. Diaz-Diestra D, Thapa B, Badillo-Diaz D, Beltran-Huarac J, Morell G, Weiner BR. Graphene oxide/ZnS:Mn nanocomposite functionalized with folic acid as a nontoxic and effective theranostic platform for breast cancer treatment. Nanomaterials. 2018;8(7):1–18.
152. Pan C, Liu Y, Zhou M, Wang W, Shi M, Xing M, Liao W. Theranostic pH-sensitive nanoparticles for highly efficient targeted delivery of doxorubicin for breast tumor treatment. Int J Nanomed. 2018;13:1119–37.
153. Li JL, Wang L, Liu XY, Zhang ZP, Guo HC, Liu WM, Tang SH. In vitro cancer cell imaging and therapy using transferrin-conjugated gold nanoparticles. Cancer Lett. 2009;274(2):319–26.
154. Ma X, Zhao Y, Liang XJ. Theranostic nanoparticles engineered for clinic and pharmaceutics. Acc Chem Res. 2011;44(10):1114–22.
155. Singh D, Dilnawaz F, Sahoo SK. Challenges of moving theranostic nanomedicine into the clinic. Nanomedicine. 2020;15(2):111–4.