Targeted approaches for HER2 breast cancer therapy: News from nanomedicine?

Serena Mazzucchelli, Marta Truffi, Luisa Fiandra, Luca Sorrentino, Fabio Corsi

Serena Mazzucchelli, Luisa Fiandra, "L. Sacco" University Hospital, 20157 Milan, Italy
Marta Truffi, Fabio Corsi, Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, 20157 Milan, Italy
Luca Sorrentino, Fabio Corsi, Surgery Division, "Luigi Sacco" Hospital, 20157 Milan, Italy
Author contributions: All authors contributed to this paper.
Correspondence to: Fabio Corsi, MD, Professor, Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, via G. B. Grassi, 74, 20157 Milan, Italy. fabio.corsi@unimi.it
Telephone: +39-2-39043449 Fax: +39-2-50319846
Received: July 28, 2014   Revised: August 29, 2014
Accepted: September 23, 2014
Published online: December 9, 2014

Abstract

About 30% of human breast cancers are human epidermal growth factor receptor 2 (HER2)[+]. This particular biological portrait is characterized by the overexpression of HER2 receptor with the subsequent deregulation of downstream pathways, which control cellular survival and proliferation. The most effective treatment for HER2^{+} cancer is represented by therapy with HER2-targeted agents. Anti-HER2 therapy dramatically improves clinical outcomes, although it shows some limitations in achieving a proper treatment. These drawbacks of HER2-targeted therapy may be overcome with the development of HER2-targeted drug delivery nanodevices. These nanoparticles possess an internal three-dimensional compartimentalization, which allows to combine the specific target recognition with their capability to act as a drug reservoir for the selective delivery of chemotherapics to tumor sites. Moreover, nanoparticles useful in photothermal ablation or in photodynamic therapy have been functionalized in order to match specificity in tumor cell recognition and suitable chemical properties. Here, we summarize the state of the art concerning the HER2^{+} breast cancer and anti-HER2 therapy, in particular deepening the contribution of the nanomedicine. Description of preclinical studies performed with HER2-targeted nanoparticles for HER2^{+} breast cancer therapy will be preceded by an overview on HER2-targeting molecules and nano-conjugation strategies. Further investigation will be necessary to introduce these nano-drugs in clinical practice; however promising results encourage an upcoming translation of this research for the next future.

Core tip: About 30% of human breast cancers are characterized by the overexpression of human epidermal growth factor receptor 2 (HER2) receptor, which determines the deregulation of cell survival and proliferation pathways. The HER2-targeted therapy is the most effective treatment, despite some related limitations, which could be bypassed with the development of nanoparticles for HER2-targeted drug delivery, photothermal ablation or photodynamic therapy. Here, we describe HER2^{+} breast cancer features and anti-HER2 therapy, and focus on the contribution of nanomedicine in this context, by reporting HER2-targeted nanoparticles under preclinical investigations. Promising results suggest upcoming clinical application of these nanocompounds in the next future.

INTRODUCTION

The erythroblastic leukemia viral oncogene homolog (ErbB) family of receptors and associated pathways main-
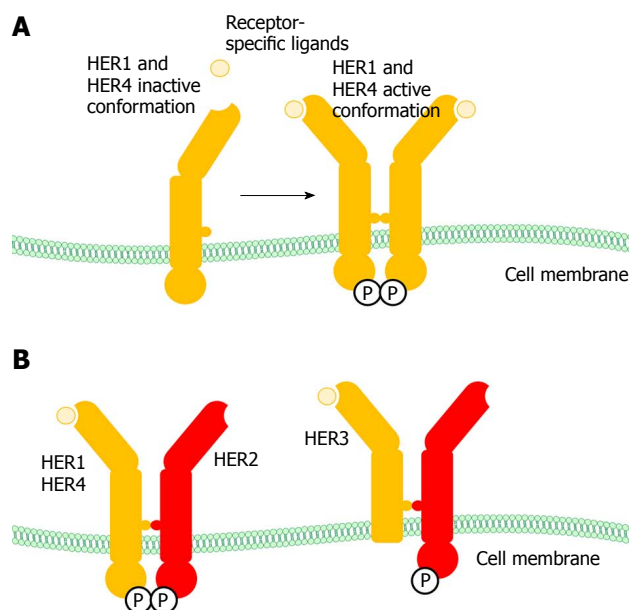
Figure 1 Characteristic features of ErbB receptors family. A: Schematic representation of HER1 and HER4 conformational change upon ligand interaction; B: Schematic representation of HER2 heterodimers. HER: Human epidermal growth factor receptor 2; ErbB: erythroblastic leukemia viral oncogene homolog.

ly regulate cell survival and proliferation. They include many actors, which cross-talk with each other and which may have overlapping functions. The typical redundancy of robust physiological processes, including the ErbB pathways, is employed by normal cells to guarantee their survival. However, it may also be highly dangerous during the early stages of tumor development, since it contributes to increase the proliferative potential of cancer cells[1]. Actually, the main goal in clinical practice is to selectively kill the tumor cell before it can acquire the capability of metastasizing and to reduce the onset of severe side effects related to chemotherapics. Based on this rationale, several nanotechnological devices have been developed to target delivery of chemotherapeutic agents toward cancer cells in order to minimize their toxic effects on healthy tissues while increasing the antitumor efficacy[2]. Although the number of nanoparticle strategies developed for drug delivery is increasing rapidly, they can be classified into two major groups: (1) particles containing organic molecules; and (2) particles that use inorganic elements, usually metals, as a core.

In this review, we focus on the ErbB receptor human epidermal growth factor receptor 2 (HER2) with the aim to summarize the state of the art of HER2+ breast cancer and related targeted therapy. In particular, we wish to explore the contribution of nanotechnology in the development of HER2-targeted nanoparticles for therapeutic purpose.

HER2 AND THE ErbB FAMILY OF PROTEINS

HER2 is a cell membrane-bound tyrosine kinase receptor that is overexpressed in 20%-30% of breast cancer

in humans. It belongs to the ErbB family of proteins, consisting of four different membrane receptors: epidermal growth factor receptor 1 (EGFR, HER1, ErbB1), 2 (HER2, ErbB2), 3 (HER3, ErbB3) and 4 (HER4, ErbB4)[3]. Each receptor includes an extracellular domain recognized and bound by the ligand, an α-helical transmembrane portion and an intracellular tyrosine-kinase domain[4]. Within the ErbB family there are also 13 polypeptide ligands that share the conserved epidermal growth factor (EGF) domain. The EGF family of polypeptides specifically binds the ErbB receptor and generally include three classes of proteins. The first one contains several EGFR ligands such as EGF, transforming growth factor (TGF)-α, amphiregulin and Epigen. The second group is constituted of β-cellulin, heparin binding EGF and epiregulin, which display dual specificity toward EGFR and HER4. The last group contains neuregulins (NRGs), which are divided into two subclasses depending on recognition of HER3 and HER4 (NRG-1 and NRG-2) or HER4 only (NRG-3 and NRG-4)[5]. Generally, ErbB receptors take on an inactive conformation. The binding of the physiological ligand determines and stabilizes a conformational change that makes the dimerization domain within the extracellular portion accessible to other receptors of the family (Figure 1A). The receptor dimerization is essential for ErbB function and for activating the downstream cascade of signal transduction. Dimerization can take place between two different ErbB receptors (heterodimerization) or between two identical ErbB molecules (homodimerization). The receptor dimerization causes transactivation of the tyrosine-kinase domain by phosphorylation, so that each receptor activates its partner[4]. In the ErbB family, only HER3 and HER2 are non-autonomous: the first one does not have intrinsic kinase activity since it is unable to bind adenosine triphosphate (ATP), whereas HER2 is an orphan receptor, since it lacks a physiological ligand[3,4].

PHYSIOLOGICAL MECHANISM OF ACTION: FROM THE RECEPTOR TO THE PATHWAYS

Since it is an orphan receptor, HER2 is always in a constitutively active conformation, which exposes the dimerization domain to other receptors of the ErbB family. Therefore, HER2 cannot homodimerize and needs to be activated by heterodimerization with ligand-activated HER1, HER3 or HER4 (Figure 1B)[3]. After dimerization, the cross-phosphorylation of dimer partner creates docking sites for the engagement of downstream signaling actors. Depending on the type of ligand and the type of ErbB receptor recruited by HER2, different adaptor proteins are engaged and different pathways are activated[4]. Two key pathways are activated by HER2: the mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase (PI3K)/Akt pathway, which promote proliferation and cell survival, respectively. The activation of the MAPK pathway is due to
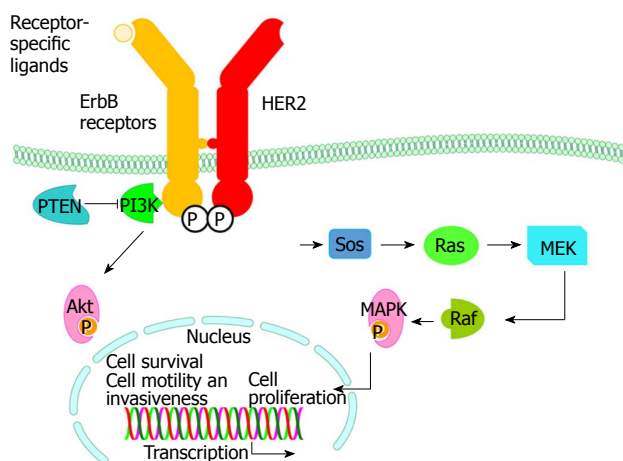
Figure 2 Schematic representation of HER2 physiological pathways. Ras: Rat sarcoma protein; Sos: Son of sevenless; PI3K: Phosphoinositide 3-kinase; MAPK: Mitogen-activated protein kinase; HER: Human epidermal growth factor receptor; ErbB: Erythroblastic leukemia viral oncogene homolog.

the recruitment and activation of the rat sarcoma protein (Ras) by the transducer molecule son of sevenless, which is in turn activated by growth-factor-receptor-bound-2 previously activated by Src-Homology-2 containing (Shc). Shc is activated upon interaction with the phosphorylated tyrosine residues within the HER2 intracellular domain. Activation of Ras kinase triggers the activation of the MAPK signaling cascade, which includes the phosphorylation of Raf, MEK and MAPK. Upon phosphorylation by MEK, MAPK translocates into the nucleus, where it regulates the transcription of genes involved in angiogenesis, proliferation and cell cycle control (Figure 2).

Differently, the PI3K pathway is activated through interaction of the phosphorylated serine or threonine residues of the receptor with the PI3K or with one of its transducer proteins, such the ubiquitin ligase Cbl. The activation of PI3K leads to the conversion of Phosphatidyl inositol 2 (PI) into PI3, with subsequent activation by phosphorylation of the Akt kinase. Once phosphorylated, Akt interacts with several transcription factors involved in cell cycle control, suppression of apoptosis and cell survival, such as mTOR, p27, nuclear factor-κB, glycogen synthase kinase-3β, and modulates their activation/inhibition[4,6]. The formation of PI3 is antagonized by the phosphatase PTEN, which acts reverting PI3 in PI2. Interestingly, HER2 is also able to translocate into the nucleus, where it interacts with the cyclooxygenase-2 promoter and directly activates the transcription of specific HER2-dependent genes (Figure 2)[7].

MOLECULAR FEATURES AND PATHOGENESIS OF HER2^{+} BREAST CANCERS

HER2[+] breast cancer is characterized by HER2 overexpression due to Her2 gene amplification or aneuploidy in more than 90% of cases[8]. In addition to gene amplifica-

tion and aneuploidy, HER2 overexpression may derive from transcriptional deregulation involving *cis*-acting enhancer elements near Her2 promoter or overexpression of transcription factors that bind this region[9]. As a result of HER2 overexpression, many intracellular signaling proteins and physiological pathways are activated[1]. Moreover, the negative regulatory loops usually active in normal cells are impaired, further contributing to pathology onset[10]. Frequently, in HER2^{+} breast cancer the deregulation of the PI3K/Akt pathway takes place. Indeed, the PI3K activity is maintained high by the preferred interaction of HER2 with HER3. HER3 has impaired kinase activity and is unable to form homodimers but it contains six docking sites for the PI3K interaction that makes it the major PI3K activating receptor of the ErbB family. HER3 is the preferred partner of HER2 and the HER3/HER2 dimer functions as an oncogenic unit[5]. The activation of PI3K leads to the phosphorylation and subsequent activation of Akt, which determines, among others, many important downstream effects in the oncogenic process, such as the downregulation of cyclin D1 and p27, which increase tumor cell proliferation and survival[11]. Another typical outcome of HER2 overexpression is the hyperactivation of the MAPK pathway that results in the transcription of genes that drive cell proliferation and migration, thus conferring to tumor cells poor differentiation, invasiveness and metastatic behavior[1,11].

Generally, HER2 overexpression is also combined with increased angiogenesis, since HER2 is able to modulate the balance between pro- and anti-angiogenic factors. In particular, high HER2 expression has been related to high levels of the pro-angiogenic molecules VEGF, IL-8 and angiopoietin-2[11].

It has to be noted that HER2 extracellular portion is subjected to metalloproteinase cleavage, which generates a kinase-active p95 fragment. At present, it is unknown whether this activated fragment undergoes nuclear translocation and regulates HER2-dependent genes expression[12]. Moreover, decreased levels of phosphatase expression (*e.g.*, PTEN), increased expression of ErbB receptor partners and/or their ligands[12,13], cross-talk with other tyrosine-kinases (*e.g.*, IGF-IR) are alternative mechanisms leading to HER2 hyperactivation even in absence of HER2 overexpression[1,5,8,14].

CLINICAL FEATURES OF HER2^{+} BREAST CANCER

As widely stated in literature, breast cancer is a heterogeneous disease and includes various subsets with distinct biological portraits. HER2^{+} breast cancer is characterized by a poor clinical outcome when anti-HER2 therapy is not administered. Notoriously, HER2 overexpression is related to lower hormonal receptor (HR) positivity, higher index of mitosis, and frequent p53 mutations. Clinical implications of these features include shorter metastasis-free and overall survival[15]. A retrospective
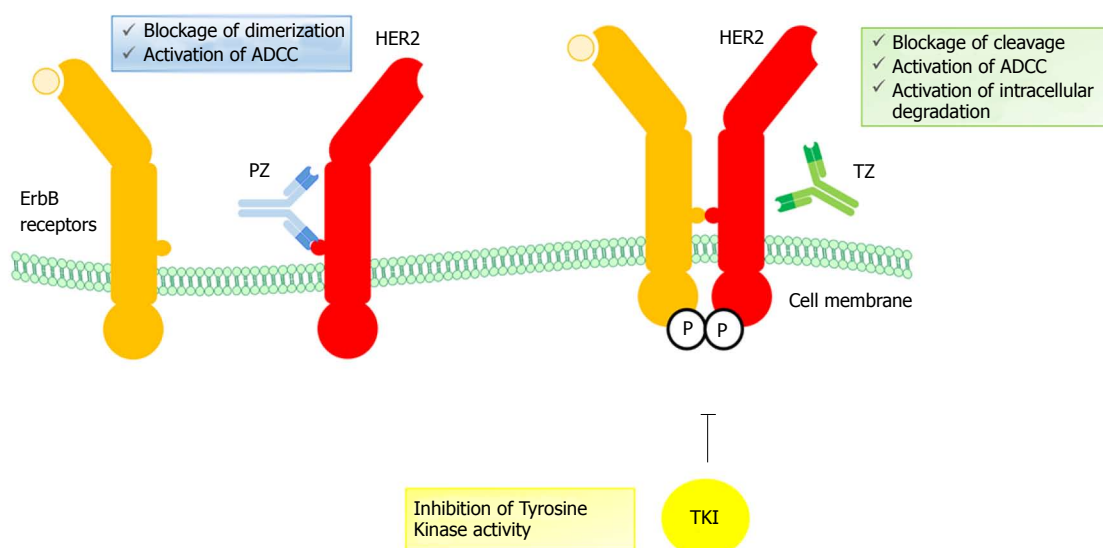
Figure 3 Schematic representation of mechanism of action of HER2-targeted drugs. PZ recognizes an epitope within the HER2 dimerization domain, thus preventing interaction with other activated ErbB receptors. Moreover PZ recruits natural killer cells, which mediate ADCC. TKI act on HER2 tyrosine kinase activity, by blocking intracellular signaling. TZ binds the juxtamembrane portion of HER2, thus preventing receptor cleavage and stimulating ADCC response and receptor degradation after endocytosis of the HER2-TZ complex. PZ: Pertuzumab; TZ: Trastuzumab; TKI: Tyrosine kinase inhibitors; ADCC: Antibody dependent cellular cytotoxicity; HER: Human epidermal growth factor receptor; ErbB: erythroblastic leukemia viral oncogene homolog.

study on 892 breast cancer patients showed a significantly higher frequency of distant metastases for HER2^{+} tumors, together with a lower 9-year disease free survival and a lower 7-year overall survival compared to the other subgroups[16]. However, the clinical behavior of HER2^{+} cancers may also depend on HR status[17]. A recent prospective cohort study was conducted on 3394 HER2^{+} breast tumors: among these, HR[-] cancers more likely presented higher T stage (T3 to T4 in 17% *vs* 10%, $P < 0.001$), nodal involvement (52% *vs* 45%, $P < 0.001$), and higher histologic grade (81% *vs* 60%, $P < 0.001$)[18]. Interestingly, HER2^{+}/HR[-] cancers were associated with more frequent brain recurrences (OR = 1.75, $P = 0.033$), and less frequent bone metastases as a first distant recurrence (OR = 0.53, $P = 0.005$), thus indicating a more aggressive disease. Therefore, HER2^{+} cancers may be divided into two distinct clinical entities based on HR status, although further studies are needed.

MANAGEMENT OF HER2^{+} BREAST CANCER

Among the different therapeutic strategies employed for HER2^{+} breast cancer, Trastuzumab (TZ) is the most widely used agent. TZ is a humanized monoclonal antibody developed starting from the murine antibody 4D5 and constituted of two antigen-binding sites that recognize the juxtamembrane portion of HER2 receptor. It functions by blocking the downstream signaling activity of HER2, thus causing cell cycle arrest and reduced angiogenesis[4]. TZ inhibits the PI3K survival pathway by increasing PTEN membrane localization and activity, with resulting inhibition of proliferation[14]. The inhibition of PI3K signaling may also result from HER2

internalization and degradation upon TZ interaction. However, it is still under debate whether HER2 may effectively be downregulated by TZ or not[19,20]. Moreover, there is evidence that TZ-HER2 interaction activates the immunological response mediated by the antibody-dependent cellular cytotoxicity (ADCC), through recruitment of natural killer (NK) cells. NK cells express on their surface the Fcγ receptor IIIa, which recognizes and binds the Fc domain of TZ[1,4,12]. In addition, the interaction of TZ with HER2 prevents the proteolytic cleavage of HER2 extracellular domain, its serum-release and the production of the truncated p95 kinase active fragment by masking the cleavage site to metalloproteinase (Figure 3)[12,13]. At present, it is still unknown if TZ can act directly on HER2 intracellular partners[14,21]; however TZ likely inhibits signaling downstream HER2-HER1 heterodimers[4]. Finally, TZ treatment blocks the cell cycle in the G_1 phase, leading to reduced proliferation. This event is coupled to reduced expression of proteins involved in the sequestration of the cyclin-dependent kinase inhibitor p27KIP1, including cyclin D1. This results in increased expression of p27KIP1 protein, which causes cell cycle arrest in S phase[1,12].

The significant improvement in overall survival and disease free survival achieved with TZ in HER2^{+} breast cancer may be considered a paradigm of the importance of targeted therapy in clinical practice, since TZ-based chemotherapy regimens have changed the clinical course of the disease. A phase Ⅱ randomized clinical trial on HER2^{+} metastatic breast cancers showed that the addition of TZ resulted in a significantly improved overall response rate (61% *vs* 34%, $P = 0.0002$), and overall survival (31.2 mo *vs* 22.7 mo, $P = 0.0325$)[22]. In a recent Cochrane systematic review on a total of 1497 patients in which TZ was administered in combination with chemotherapy,

the efficacy of TZ was confirmed, with an improved overall survival (HR = 0.82, P = 0.004), progression-free survival (HR = 0.61, P < 0.00001), and overall response rate (RR = 1.58, P < 0.00001), although an increased risk in congestive heart failure was evident[23]. Because of the great efficacy as a first-line and adjuvant treatment, TZ has been successfully introduced also in the neoadjuvant setting. Interestingly, the first phase III randomized trial on neoadjuvant TZ was prematurely stopped due to the evident superiority of TZ-based chemotherapy[24]. Other more extensive trials, such as GeparQuattro and NOAH, demonstrated similar results, with a substantial improvement of pathologic complete response rates and 5-year event-free survival in the TZ arm[25,26].

Besides TZ, other antibodies against HER2 are currently under investigation. In particular, Pertuzumab (PZ) is a humanized monoclonal antibody that recognizes an epitope within the HER2 dimerization domain. It is able to inhibit heregulin-induced activation of HER2 phosphorylation and cell growth. Differently from TZ, PZ blocks the heterodimerization of HER2 with HER3, which is extremely relevant in tumorigenesis. However, PZ is not able to prevent the formation of EGFR-HER2 dimers, thus limiting its therapeutic efficacy[27,28]. As observed for TZ, PZ efficacy is also mediated by the recruitment of the ADCC system (Figure 3). Because of its capability to inhibit HER2 dimerization with HER3, PZ has been approved by FDA for clinical use in association with TZ, thus helping to overcome resistance to anti-HER2 treatment. This approval has been obtained on the basis of a phase III Clinical Evaluation of PZ and TZ (CLEOPATRA) trial, in which placebo plus TZ plus docetaxel was compared to PZ plus TZ plus docetaxel for first-line treatment of 808 HER2^{+} metastatic breast cancer[4]. Median progression-free survival was significantly higher in PZ group (18.5 mo *vs* 12.4 mo), and preliminary analysis showed also a favorable trend about overall survival. Currently other studies are investigating the role of PZ in HER2^{+} breast cancer in progression under TZ treatment, confirming a synergistic role between the two antibodies[29].

In the last ten years, an antibody-drug conjugate, named T-DM1 (Genentech), has been developed, and it is constituted by a TZ molecule conjugated with the anti-microtubule agent DM1. TZ-DM1 recognizes HER2, is internalized and release DM-1 into the cytoplasm of HER2^{+} cells[30]. In February 2013, T-DM1 was approved by FDA for treatment of metastatic HER2^{+} breast cancer previously treated with TZ and taxanes. The efficacy of T-DM1 in this setting has been assessed in comparison with Lapatinib on 991 patients, with an overall survival of 30.9 mo *vs* 25.1 mo (P < 0.001): in particular, T-DM1 was associated with a higher objective response rate (43.6% *vs* 30.8%, P < 0.001) and a lower toxicity profile[31].

Another class of biological drugs for HER2-targeted therapy is represented by tyrosine-kinase inhibitors (TKI). They are small molecules that bind the ATP binding site of ErbB receptors, and prevent the activation of both PI3K and MAPK signaling pathways, thus increasing apoptosis and reducing proliferation (Figure 3)[4]. Among them, the most clinically advanced is Lapatinib, a dual inhibitor of HER2 and EGFR[32]. Lapatinib has the advantage to act also on p95 activated fragment of HER2, which strongly correlates with poor prognosis[33,34]. Lapatinib has gained a great interest for breast cancer treatment mainly for two reasons: its orally available formulation and its efficacy in the treatment of TZ-resistant metastatic HER2-positive breast cancer, with a reduction in risk of death by 26%[35]. Moreover, Lapatinib has been recently studied in the neoadjuvant setting in association with TZ (NeoALTTO Trial), with a pathologic complete response of 51.3% *vs* 29.5% with TZ alone[36]. Other TKIs, such as HKI-272, ARRY-334543 and BIBW-2992, are under clinical investigation for breast cancer[4].

Finally, some inhibitors of the Heat Shock Protein 90 (Hsp90) have been developed for breast cancer therapy. Indeed, Hsp90 has a role in controlling the stability of nascent and mature forms of HER2. Inhibition of its activity results in HER2 ubiquitination and subsequent proteasomal degradation, thus blocking HER2 downstream signaling pathway[3]. A phase II trial has been conducted on 31 patients with HER2^{+} breast cancer in progression after TZ treatment, subsequently treated with the Hsp90 inhibitor tanespimycin: the objective response rate was 22% with a progression-free survival of 6 mo, therefore demonstrating the efficacy of the drug against this subset of breast cancer[37]. However, tanespimycin has been suspended for further clinical studies, and other novel Hsp90 inhibitors are currently studied.

ONSET OF RESISTANCE

Frequently, HER2^{+} cancers develop resistance to HER2-targeted therapies[38]. In particular, the development of resistance toward the widely used TZ has been extensively examined. Generally, resistance to TZ occurs because of three different mechanisms: (1) epitope masking; (2) upregulation of HER2 signaling; and (3) alterations of the immune response[39]. As regards to epitope masking, two candidates have been identified: Mucin 4 (MUC4) and the CD44/hyaluronan polymer complex. MUC4 is an O-glycosylated membrane-associated protein, which is upregulated in TZ-resistant JIMT-1 cells. Binding of TZ to HER2 was reduced in JIMT-1, while it was restored after knockdown of MUC4[40]. A similar result was observed with the CD44/hyaluronan polymer complex, where knockdown of CD44 or chemical inhibition of hyaluronan synthesis restored TZ-HER2 recognition in JIMT-1 cells. In both cases, the TZ-resistance is probably due to the steric hindrance of the complex that prevents TZ binding and internalization, without altering HER2 signaling[41]. Upregulation of HER2-signaling is another mechanism found to bypass TZ hurdle. It results from the overexpression of some ErbB family members and the subsequent increase in heterodimer formation. Indeed, in presence of an excess of ErbB ligands the

resulting heterodimers drive cells towards proliferation and inhibition of apoptosis, thus interfering with TZ action[42]. However, the HER2/HER1 complex may also undergo antibody-induced internalization, ubiquitination, and proteolysis, that disable its transforming activity[1]. Moreover, up to 30% of HER2+ breast cancers express p95, an amino-terminal truncated form of HER2. Since p95 is a constitutively active kinase lacking the TZ binding site, it is able to confer TZ resistance[43]. In this situation, treatment with PZ or with one of TKIs may replace responsiveness to anti-HER2 therapy[44]. Another mechanism to bypass the TZ-mediated blockade of HER2 signaling is the activation of downstream effectors by alternative routes, *e.g., via* the insulin-like growth factor 1 receptor (IGF-1R) or c-Met, often overexpressed in TZ-resistant cells, and able to hyperactivate the PI3K/Akt pathway. Treatments with inhibitors of IGF-1R or c-Met may restore TZ sensitivity[8,45]. Decreased expression of the Akt inhibitor PTEN is another crucial factor in TZ resistance. TZ upregulates the microRNA miRNA-21, a physiological inhibitor of PTEN phosphatase[46]. The reduction of PTEN expression maintains Akt active, and diminishes TZ efficacy[14]. Moreover, the hyperactivity of the PI3K pathway causes epigenetic changes, which result in the inhibition of FoxO, the transcription of antiapoptotic genes[47] and the downregulation of p27KIP1[44]. Finally, the alteration of the immune response may cause TZ resistance in tumor cells. It is well known that TZ treatment induces ATCC, which triggers tumor cell death[48]. It exists a FcγRIIIa polymorphism, which makes it less effective at inducing ATCC. This mechanism of resistance is common to both TZ and PZ treatments[49].

Besides these three main mechanisms, other minor ones are involved in TZ resistance and have to be taken into account. These concern the discovery of HER2 mutants with modulated receptor activities, and subsequent more aggressive tumor phenotype[16], and the increased activation of Notch receptors upon TZ or Lapatinib treatment, which contributes to the development of resistance[50,51].

To overcome the previously described mechanisms of resistance to TZ a new agent against HER2+ cancers, called Neratinib, is being investigated for clinical use. Neratinib is a pan-HER irreversible TKI, also available for oral administration, and ErbB2 mutations were found to be sensitive to Neratinib in some preclinical studies[52]. In a phase Ⅱ trial on patients with or without previous treatment with TZ, Neratinib was administered daily at 240 mg dosage. The 16-wk progression-free survival rates was 59% for patients with prior TZ and 78% for the other group of patients and the most common adverse event was diarrhea[53]. Interestingly, Neratinib was recently administered in combination with weekly paclitaxel and TZ in a phase I trial on metastatic HER2+ positive cancers previously treated with TZ, Lapatinib or T-DM1, with an objective response in 38% of patients and a median time to progression of 3.7 mo, therefore suggesting that dual anti-HER blockade with Neratinib and TZ may be more effective than single-agent inhibition[54]. Therefore Neratinib represents a promising tool for HER2+ TZ-resistant breast tumors, and a phase Ⅲ trial comparing Neratinib plus Capecitabine and Lapatinib plus Capecitabine in metastatic HER2+ breast cancer is ongoing[55].

THERAPEUTIC IMPROVEMENT FROM NANOTECHNOLOGY

Over the last thirty years, we all have witnessed the great development of nanotechnologies. In particular, nanomedicine has shown promising scenarios for clinical practice with the development of more effective, less toxic and smart therapeutics[56]. The novel field of nano-oncology was created and several nanodevices for tumors treatment have been developed in order to overcome limitations of conventional therapies. Indeed, chemotherapy lacks of selectivity toward tumor cells and therefore it is highly toxic toward healthy tissues. It has limited accessibility to the tumor tissues, and requires high doses to be efficient[2]. Moreover, conventional chemotherapics are usually unable to cross biological barriers, thus bearing limited efficacy at several metastatic sites[57]. Nanoparticles possess physical and chemical properties suitable for molecular and cellular interactions, partially due to their high surface-to-volume ratio. Moreover, their capability to form internal 3D nanostructures gives them the appropriate flexibility to be exploited as drug delivery devices able to overcome biological barriers, and to transport hydrophobic and poorly water-soluble drugs. Nanoparticles can be designed to have a large therapeutic payload and to be applied in combinatorial therapy since they can accommodate multiple drugs. Moreover, nanoparticles protect embedded drugs, thus allowing in certain cases to overcome drug resistance, which is crucial for effectiveness of cancer treatment. Finally, nanoparticle surface can be engineered with antibodies, peptides or other biologically active molecules in order to achieve a selective targeting of tumor malignancies[58].

In the next paragraphs, we will overview the ligands and the conjugation employed for the development of HER2-targeted nanoparticles, and we will report some nanotechnological approaches for the targeted-therapy of HER2+ breast cancer.

HER2-TARGETED LIGANDS FOR NANOPARTICLES BIOENGINEERING

An active targeting strategy relies on the coupling of a targeting moiety to the surface of nanoparticles, thus providing specific binding to cancer biomarkers overexpressed at the target site. Such a targeting mechanism increases specific recognition of tumor cells and internalization of the nanocomplex through receptor-mediated endocytosis[59-61]. The influence of a targeting molecule on the pharmacokinetics, biodistribution and tumor accumulation of nanoparticles depends on several
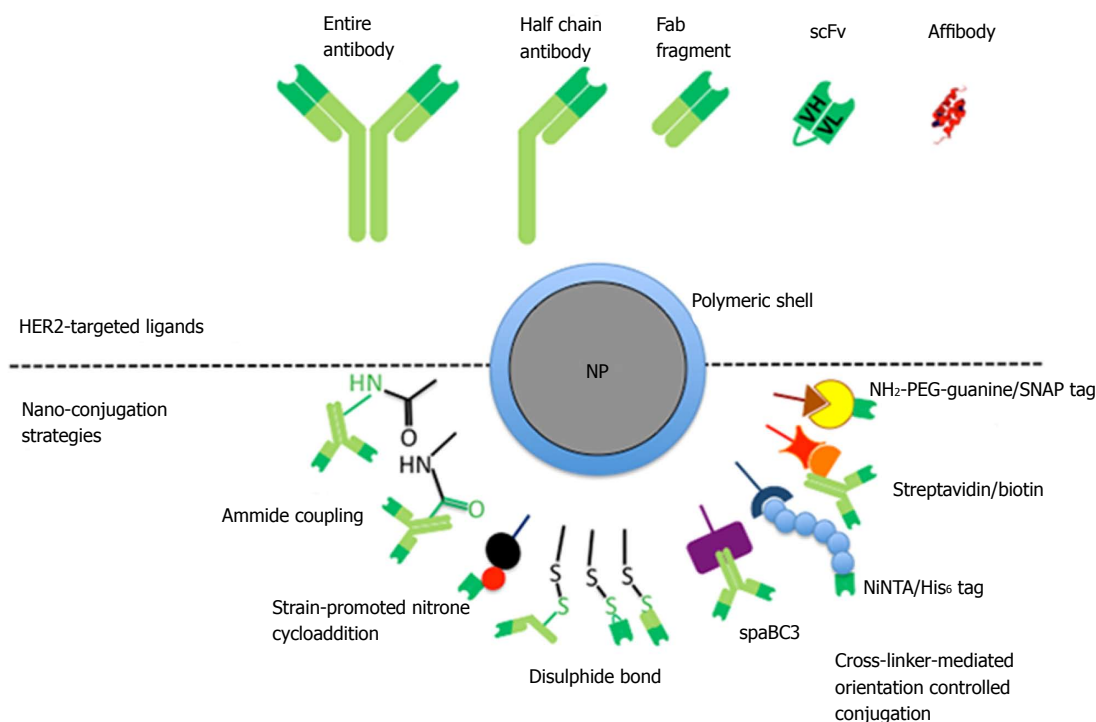
Figure 4 Schematic representation of HER2-targeting ligands and conjugation strategies employed for NPs functionalization. HER: Human epidermal growth factor receptor; scFv: Single-chain fragment variable antibodies.

factors, including the nature of the ligand, its density on the surface of the nanoparticle and its activity[62]. Targeting moieties exploited for nanoparticle functionalization include peptides, proteins, oligonucleotides, aptamers, carbohydrates, lipids, and other biologically active molecules. Among them monoclonal antibodies and antibody-derived ligands, are widely used (Figure 4). Indeed, antibody-based therapy has received wide attention because of its stability to selectively target tumor cells through receptor-specific interactions[63-65]. The selective tumor targeting capability of antibodies can be exploited in nanotechnology by covalently coupling antibodies directed against HER2 to the surface of colloidal nanoparticles, thus achieving higher cellular uptake and improved antitumor efficacy of nanoformulated drugs. In the context of HER2-targeted nano-therapy the most studied example of nanoparticle-conjugated ligand is TZ, a humanized monoclonal antibody already used as single agent after chemotherapy or in combination with chemotherapy in HER2-overexpressing metastatic breast cancer treatment. TZ has been used as a targeting moiety to be conjugated onto the surface of different nanoparticles, including quantum dots, magnetic and gold nanoparticles, in order to achieve selective recognition of HER2^{+} tumor cells for both imaging and therapeutic applications, with promising results obtained in preclinical studies on HER2^{+} breast cancer-bearing animal models. Different kinds of TZ-functionalized nanoparticles have been extensively reported[1,66]. However, conjugating entire antibodies onto nanoparticles may lead to increased immunogenicity of the resulting nano-compound and reduced circulation time and tissue penetration[64]. Recombinant

antibodies with small size have been developed in order to overcome such problems. Nano-conjugation of the half-chain of the monoclonal antibody TZ dramatically improves the intracellular trafficking and the long-term stability of the nano-compound in both *in vitro* and *in vivo* settings[67]. Anti-HER2 Fab fragment of the monoclonal antibody TZ has also been shown to enhance tumor cell uptake resulted from HER2-mediated internalization of HER2-targeted liposomes[68,69]. Innovative and intriguing ligands, which have provided promising results, are single-chain fragment variable antibodies (scFv), variable V_H and V_L regions of antibodies connected through a synthetic loop[63]. Anti-HER2 scFv immobilized onto the surface of magnetic nanoparticles has proved to be highly effective in selectively targeting HER2^{+}-breast cancer cells, and has shown faster cellular interaction and incorporation of nanoparticles when compared to entire TZ ligand[67]. A number of nanoparticles have also been functionalized with HER2 affibody molecules, small proteins mimicking the active portion of the Fab region of TZ[70,71]. Nanoparticle-affibody conjugates have shown highly specific targeting and efficiency toward HER2^{+}-breast cancer, thus representing another promising class of targeting ligands with simple, robust, and precise structure and high affinity.

Besides antibodies and antibody-derived ligands, other active molecules directed toward HER2 have been proposed as interesting ligands for nano-formulation. In particular, Lapatinib is a dual inhibitor of the tyrosine kinase receptors EGFR and HER2 used to treat advanced breast cancers, and its poor water solubility has been overcome by conjugation with lipoprotein-like nanopar-

ticles (LTNPs). Such nano-compounds could be taken up by breast tumor cells by endosomes through clathrin-dependent pinocytosis and macropinocytosis, with subsequent escape from endosomes to the cytoplasm. Within tumor cells, LTNPs induce a significant cell arrest at G_0/G_1 phase compared with equal concentrations of classical lapatinib. They also could passively accumulate into the tumor *in vivo via* the enhanced permeability and retention effect where they induce elevate anti-tumor activity[72,73].

NANO-CONJUGATION OF HER2-TARGETED LIGANDS

When designing nano-devices for targeted treatment, a crucial issue concerns the optimization of functionalization strategies to achieve an efficient and specific targeting. The structural features of a nano-compound may affect its biological functions; hence many efforts have been focused on development of new strategies for nanoparticle surface bioengineering (Figure 4). In particular, fine control of positioning, spatial orientation and conservation of the activity of targeting biomolecules have reveled essential for the generation of nano-compounds with well-defined and reproducible properties[74,75]. Reliable conjugation strategies include physical adsorption and formation of covalent chemical connections, often through coupling with appropriate crosslinkers[75,76]. Physical adsorption is usually related to protein ligands destabilization. Moreover, ligand orientation, number of immobilized molecules and bond stability are completely out of control. Instead the covalent coupling between the ligand and the nanoparticle gives some advantages in terms of stability of the ligand conjugation and versatility of the conjugation strategy. Indeed, chemical properties of nanoparticles have sometimes to be modulated with different functionalities depending on the functional groups found on the targeting ligands. Frequently superficial amino and carboxylic groups on the surface of nanoparticles are employed for amide coupling, thus obtaining covalent binding between the ligand and the biocompatible polymers coating the nanoparticles surface. Cysteine residues have also been found as preferred conjugation sites on proteins in general, and further exploited for HER2-targeted ligands bioconjugation. Such cysteines, either naturally present in the polypeptide sequence or introduced at specific positions by site-directed mutagenesis in case of recombinant ligands, can be activated with reducing agents and used to form disulfide bonds with properly modified nanoparticles surface[77,78]. Traditionally, poly ethylene glycol (PEG) or poly ethylene oxide molecules are used to coat nanoparticles surface in order to reduce eventual aspecific interactions of the nanoparticle with the cells and function as spacer. Anti-HER2 antibodies have been conjugated to PEGylated nanoparticles, by covalent attachment to superficial amino and carboxylic groups[79-81]. Polyvynil-pyrrolidone (PVP) and poly-D,L-lactic-co-glycolic acid (PLGA) are other clinically safe polymers used

to coat nanoparticles, which can interact with a variety of agents[82]. Vivek *et al*[83] have developed TZ-conjugated PVP-PLGA nanoparticles for targeted delivery of drugs to HER2-overexpressing breast cancer cells.

Optimization of nanoparticle functionalization has lead to the development of smart conjugation techniques, which allow fine-tuning of the orientation of the targeting biomolecules, in order to maintain and/or further exploit the targeting capability and the therapeutic efficacy of HER2-directed ligands[75,84]. In several cases both TZ and the nanoparticle surface have been modified with heterobifunctional linkers, such as N-succinimidyl-3-(2-pyridyldithio)propionate, succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate and succinimidyl iodoacetate, commercially available and widely used crosslinkers for bioconjugation. They allow to determine the exact number of reactive amines on the nanoparticle surface, thus widely contributing in controlling ligand density onto the surface of the resulting nano-compound[7,84-86]. A classical approach, although not applicable in medicine, is based on the strength of streptavidin-biotin complex. Basically biothinylated TZ reacts with streptavidin-modified nanoparticles, thus generating HER2-targeted nano-compounds[87]. Another smart conjugation strategy consists in taking advantage of spaBC3, a monodomain variant of protein A, a natural peptide linker endowed with high affinity for IgGs. It has been used to bind iron oxide and gold nanoparticles for tight TZ immobilization through the Fc fragment, thus achieving an optimal presentation of the target-directed Fab fragments and keeping full binding capacity of the bound antibody[88]. In several cases the use of a protein biolinker is suited for a controlled site-specific conjugation of HER2-targeted ligands and it also contribute in stabilizing nanoparticle while producing.

Recombinant ligands offer an extremely desirable versatility in terms of nanoparticle conjugation, since their polypeptide sequence can be easily genetically engineered leading to generation of useful functionalities for nanoparticles conjugation. Anti HER2 scFv were modified inserting a His-tag in N-terminal position leading to conjugation of NiNTA functionalized nanoparticles. Otherwise, the mutation of a serine residue with a cysteine within the V_H-V_L linker region or the insertion of a N-terminal serine have been probed to nanoparticle conjugation through disulphide bridges formation nanoparticles or nitrone cycloaddition[78]. These different chemical immobilization strategies of anti-HER2 scFv have been developed and tested, thus leading to multiple scFv specific and uniform orientations on the surface of nanoparticles and demonstrated subsequent effect on the targeting efficiency of the nano-compound. Another recently explored bioconjugation approach exploits the genetic fusion between the scFv module and a small enzyme (*i.e.*, SNAP tag), which works as "capture" unit. Nanoparticles have been functionalized with a suicide inhibitor of the enzyme, allowing covalent, irreversible and specific immobilization of the scFv on the nanoparticle

Table 1 Nanoparticles for breast cancer therapy

Nanoparticle	Delivered active molecule	Activity on HER2 + breast cancer cells or tumors
TZ-polymeric NPs[90]	DOX	nuclear drug delivery and apoptotic effect (*in vitro* study)
TZ-PLGA-PEG NPs[91]	DTX	HER2 specific targeting, cellular internalization and cytotoxic activity (*in vitro* study)
anti-HER2 affibody-PLA-PEG-Mal NPs[70]	Pxtl	HER2 specific targeting, cellular internalization and cytotoxic activity (*in vitro* study)
rhuMAbHER2 (Fab')-PLGA NPs[92]	PE38KDEL	HER2 specific targeting, cellular internalization and cytotoxic activity (*in vitro* study); anti-tumor activity (*in vivo* study)
scFv-PEG-PLA NPs[96]	*siPlk1*	cellular internalization, Plk1 silencing, apoptotic effect (*in vitro* study); *siPlk1* accumulation in tumors and anti-tumor activity (*in vivo* study)
Herceptin-PLA-TPGS+TPGS-COOH NPs[97]	DTX/IOs	cellular internalization and cytotoxic activity (*in vitro* study)
anti HER2-PEG–gold NPs[98]	phthalocyanine	HER2 specific cytotoxic activity (*in vitro* study)

TZ: Trastuzumab; DOX: Doxorubicin; DTX: Docetaxel; PLGA: Poly-D,L-lactic-co-glycolic acid; PEG: Poly ethylene glycol; IOs: Iron oxides; HER2: Human epidermal growth factor receptor 2.

surface. In this case the immobilized molecules are fully active since the bioconjugation reaction takes place in mild conditions without affecting scFv stability[89].

HER2-TARGETED NANOPARTICLES FOR BREAST CANCER THERAPY

In breast cancer therapy, many studies have been devoted to the development of HER2-targeted nanodevices as delivery system for chemotherapics (anthracyclines and taxanes) or other molecules exerting an anti-tumor effect (Table 1). Most of them are mainly focused on the development and characterization of bioengineered NPs for HER2^{+} breast cancer cells targeting, and have demonstrated their cytotoxic effect *in vitro*. In 2009, Shi and collaborators developed an amphiphilic copolymeric NP, where surface furan groups were used to bind, by a simple diels-alder coupling chemistry, both an anti-HER2 antibody and the chemotherapeutic doxorubicin (DOX)[90]. The DOX-conjugated immuno-NPs were able to efficiently deliver DOX into the cytoplasm, and then into the nucleus of HER2^{+} breast cancer cells, where DOX exerts its function. This intracellular DOX accumulation was significantly higher than that measured when DOX was delivered by non-functionalized NPs. These results demonstrated, for the first time, the great prospective of a surface-conjugation strategy for the development of nanoformulated DOX, which proved to be more efficient than the conventional encapsulation for the nuclear delivery of this drug. The enhanced HER2-mediated intracellular uptake of DOX also resulted in increased apoptosis of HER2^{+} breast cancer cells, when compared to non-functionalized DOX-NPs.

In 2011, Koopaei *et al*[91] developed a copolymeric immuno-nanocarrier for the active delivery of docetaxel (DTX) to human breast cancer cells. DTX was encapsulated in PLGA-PEG nanoparticles functionalized with TZ. A fast and over time sustained release of DTX from NPs was first observed *in vitro*, together with a specific interaction of DTX-PLGA-TZ with HER2^{+} breast cancer cells. Cytotoxicity of HER2-targeted DTX-PLGA was compared with that of free DTX and non-specifically targeted nanoformulates. The greatest cytotoxic effect was obtained with the immune NPs as results of their specific interaction with HER2 receptors on cancer cell surface.

Another taxane commonly used in clinical practice, the paclitaxel (Ptxl), has been nanoformulated to be actively delivered to breast cancer cells. An interesting study of Alexis and colleagues[70] addressed the numerous drawbacks of the antibody-based approach for an efficient drug delivery to tumors, mainly related to the large hydrodynamic size of the ligand. Here, it was sponsored the use of an anti-HER2 affibody, which shows several advantages in comparison to the entire monoclonal antibody: (1) smaller size (15 kDa *vs* 150 kDa); (2) considerable distance between the functional end group and the conjugation site; and (3) high *in vitro* and *in vivo* stability. Copolymeric NPs conjugated to the anti-HER2 affibody (NPs-Affb) efficiently bound HER2^{+} cancer cells and were internalized. The cytotoxic effect of Ptxl encapsulated into targeted NP-Affb was then evaluated in comparison to that of nude NPs, non-targeted NPs (Pxtl), NPs-Affb and free Pxtl. A significant decrease of cells viability was observed both with free Pxtl and non-targeted NPs (Pxtl) after 2 h, but a further significant decrease in cell viability was obtained with NP-Affb (Pxtl).

Despite the conspicuous literature about the *in vitro* therapeutic potential of nanostructured chemotherapics, only few researchers have assessed the efficacy of biofunctionalized nanodevices *in vivo*. In 2009, Gao and collaborators decided to encapsulate the anti-cancer *Pseudomonas* exotoxin A (PE)-based immunotoxin into PLGA nanoparticles[92]. In particular, PE38KDEL, a 38 kDa mutant form of PE, was loaded into PLGA nanoparticles targeting HER2 (PE-NP-HER), where the anti-HER2 portion was represented by a Fab' fragment of a humanized anti-HER2 monoclonal antibody (rhuMAbHER2). Once assessed that the integrity and the potent activity of PE38KDEL were maintained after encapsulation in PLGA particles, *in vitro* interaction of PE-NP-HER with HER2^{+} breast cancer was compared to that obtained with

HER2-negative cells and the cytotoxic effect on the two cell types was also evaluated. PE-NP-HER were exclusively internalized by HER2$^+$ cells and a strong cytotoxicity occurred specifically in these cells. *In vivo* toxicity studies were performed upon intravenous injection of PE-NP-HER, PE-NP, PE-HER and PE38KDEL in mice. A 3-fold lower LD$_{50}$ (mg/Kg) and no influence on hepatic functionality were observed for PLGA-loaded PE, compared to non-encapsulated toxins. A dose-dependent inhibition of tumor growth was observed in mice injected both with PE-HER and PE-NP-HER, even though a 2-fold higher dose of the PE-HER was necessary to obtain the same effects of the nanoformulated immunotoxin.

A recent nanotechological approach proposes the employment of NPs for the delivery of small interfering RNA (siRNA)[93-95]. In the current year, it has been developed a nanocarrier for the delivery of siRNA targeting the gene encoding polo-like kinase 1 (Plk1)[96]. The si*Plk1* was encapsulated in a PEG-PLA shell functionalized with the anti-HER2 scFv (ScFv$_{Her2}$-NP$_{siPlk1}$), to exert an active targeting of HER2$^+$ breast cancer. ScFv$_{Her2}$-NP$_{siPlk1}$ were efficiently internalized by cancer cells and promoted Plk1 silencing, inducing tumor cell apoptosis. Nanocomplex-mediated accumulation of si*Plk1* in HER2$^+$ breast tumors was also observed *in vivo*, in parallel with a dose-dependent anti-tumor efficacy: ScFv$_{Her2}$-NP$_{siPlk1}$ significantly increased the inhibition of tumor growth, when compared to non-targeted NP$_{siPlk1}$, and allowed to reduce the active dose of injected siRNA.

Nanotechnology has found a great application also in thermal therapy where gold or magnetic NPs have proved to be very useful in triggering ablation of cancer cells. In 2012, Mi and colleagues identified a multimodal strategy for breast cancer treatment, where the chemotherapy DTX was formulated with a PLA-tocopheryl-PEG-succinate and carboxyl group-terminated TPGS (TPGS-COOH) copolymer, containing iron oxides (IOs) for hyperthermia therapy[97]. TPGS-COOH molecules were conjugated with TZ for HER2 targeting. The *in vitro* therapeutic efficiency of these multimodal NPs was tested on HER2$^+$ breast cancer cells. A stronger cytotoxic activity was observed on cells incubated with TZ-IO-NPs under the exposure to an alternating current field, or with TZ-DTX-NPs, in comparison to the corresponding non-targeted NPs.

In photodynamic therapy of cancer, irradiation with visible and/or near-infrared light induces the activation of photosensitiser drugs, able to generate reactive oxygen species and trigger apoptotic or necrotic response of target cells, thus leading to cell death. Stuchinskaya and collaborators developed a PEG-gold NP conjugated to the phthalocyanine and functionalized with an anti-HER2 antibody on PEG chains. Upon red laser irradiation a strong cytotoxic effect of these NPs was observed on HER2$^+$ cells and not on HER2-negative cells[98].

CONCLUSION

About 30% of breast cancers are associated with HER2 receptor overexpression, which strongly correlates with a poor prognosis. Indeed, HER2 regulates several highly redundant pathways involved in cellular survival and proliferation, which are deregulated in HER2$^+$ cancer. At present, conventional therapy with biological drugs, such as Trastuzumab, Pertuzumab or Lapatinib, has provided satisfactory results, although still shows some limitations in achieving a proper treatment. In this context, the development of HER2-targeted nanoparticles exploited as drug delivery systems may overcome these drawbacks. Specific HER2 ligands have been conjugated on the surface of nanoparticles, thus providing a specific recognition of HER2$^+$ cancer cells. Their specific target recognition is combined with the nanoparticles capability to act as a drug reservoir for a selective delivery to tumor sites. In addition, therapeutic efficiency can be reached also by combining targeting molecules with nanoparticle useful for photothermal ablation. In this review we have extensively analysed HER2$^+$ breast cancer features and related targeted therapy, particularly underlining the precious contribution that nanomedicine may provide. Moreover, we have described various molecules used to target HER2 and related nano-conjugation strategies, and provided a detailed overview of preclinical studies performed with HER2-targeted nanoparticles developed for cancer therapy. Further investigations and synergic collaborations between nanotechnologists and physicians will hopefully allow to achieve the introduction of these nano-drugs in clinical.

REFERENCES

1   **Colombo M**, Corsi F, Foschi D, Mazzantini E, Mazzucchelli S, Morasso C, Occhipinti E, Polito L, Prosperi D, Ronchi S, Verderio P. HER2 targeting as a two-sided strategy for breast cancer diagnosis and treatment: Outlook and recent implications in nanomedical approaches. *Pharmacol Res* 2010; **62**: 150-165 [PMID: 20117211 DOI: 10.1016/j.phrs.2010.01.013]

2   **Shapira A**, Livney YD, Broxterman HJ, Assaraf YG. Nanomedicine for targeted cancer therapy: towards the overcoming of drug resistance. *Drug Resist Updat* 2011; **14**: 150-163 [PMID: 21330184 DOI: 10.1016/j.drup.2011.01.003]

3   **Citri A**, Yarden Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol* 2006; **7**: 505-516 [PMID: 16829981 DOI: 10.1038/nrm1962]

4   **Baselga J**, Swain SM. Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. *Nat Rev Cancer* 2009; **9**: 463-475 [PMID: 19536107 DOI: 10.1038/nrc2656]

5   **Hynes NE**, MacDonald G. ErbB receptors and signaling pathways in cancer. *Curr Opin Cell Biol* 2009; **21**: 177-184 [PMID: 19208461 DOI: 10.1016/j.ceb.2008.12.010]

6   **Kau TR**, Way JC, Silver PA. Nuclear transport and cancer: from mechanism to intervention. *Nat Rev Cancer* 2004; **4**: 106-117 [PMID: 14732865 DOI: 10.1038/nrc1274]

7   **Chen J**, Saeki F, Wiley BJ, Cang H, Cobb MJ, Li ZY, Au L, Zhang H, Kimmey MB, Li X, Xia Y. Gold nanocages: bioconjugation and their potential use as optical imaging contrast agents. *Nano Lett* 2005; **5**: 473-477 [PMID: 15755097 DOI: 10.1021/nl047950t]

8   **Jin Q**, Esteva FJ. Cross-talk between the ErbB/HER family and the type I insulin-like growth factor receptor signaling pathway in breast cancer. *J Mammary Gland Biol Neoplasia* 2008; **13**: 485-498 [PMID: 19034632 DOI: 10.1007/s10911-008-9107-3]

9   **Freudenberg JA**, Wang Q, Katsumata M, Drebin J, Nagato-

mo I, Greene MI. The role of HER2 in early breast cancer metastasis and the origins of resistance to HER2-targeted therapies. *Exp Mol Pathol* 2009; **87**: 1-11 [PMID: 19450579 DOI: 10.1016/j.yexmp.2009.05.001]

10   **Amit I**, Wides R, Yarden Y. Evolvable signaling networks of receptor tyrosine kinases: relevance of robustness to malignancy and to cancer therapy. *Mol Syst Biol* 2007; **3**: 151 [PMID: 18059446 DOI: 10.1038/msb4100195]

11   **Dean-Colomb W**, Esteva FJ. Her2-positive breast cancer: herceptin and beyond. *Eur J Cancer* 2008; **44**: 2806-2812 [PMID: 19022660 DOI: 10.1016/j.ejca.2008.09.013]

12   **Suzuki E**, Toi M. Improving the efficacy of trastuzumab in breast cancer. *Cancer Sci* 2007; **98**: 767-771 [PMID: 17428260 DOI: 10.1111/j.1349-7006.2007.00455.x]

13   **Molina MA**, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. *Cancer Res* 2001; **61**: 4744-4749 [PMID: 11406546]

14   **Nagata Y**, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 2004; **6**: 117-127 [PMID: 15324695 DOI: 10.1016/j.ccr.2004.06.022]

15   **Ferrero-Poüs M**, Hacène K, Bouchet C, Le Doussal V, Tubiana-Hulin M, Spyratos F. Relationship between c-erbB-2 and other tumor characteristics in breast cancer prognosis. *Clin Cancer Res* 2000; **6**: 4745-4754 [PMID: 11156229]

16   **Wang SE**, Narasanna A, Perez-Torres M, Xiang B, Wu FY, Yang S, Carpenter G, Gazdar AF, Muthuswamy SK, Arteaga CL. HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. *Cancer Cell* 2006; **10**: 25-38 [PMID: 16843263 DOI: 10.1016/j.ccr.2006.05.023]

17   **Lee HJ**, Park IA, Park SY, Seo AN, Lim B, Chai Y, Song IH, Kim NE, Kim JY, Yu JH, Ahn JH, Gong G. Two histopathologically different diseases: hormone receptor-positive and hormone receptor-negative tumors in HER2-positive breast cancer. *Breast Cancer Res Treat* 2014; **145**: 615-623 [PMID: 24820412 DOI: 10.1007/s10549-014-2983-x]

18   **Vaz-Luis I**, Ottesen RA, Hughes ME, Marcom PK, Moy B, Rugo HS, Theriault RL, Wilson J, Niland JC, Weeks JC, Lin NU. Impact of hormone receptor status on patterns of recurrence and clinical outcomes among patients with human epidermal growth factor-2-positive breast cancer in the National Comprehensive Cancer Network: a prospective cohort study. *Breast Cancer Res* 2012; **14**: R129 [PMID: 23025714 DOI: 10.1186/bcr3324]

19   **Valabrega G**, Montemurro F, Sarotto I, Petrelli A, Rubini P, Tacchetti C, Aglietta M, Comoglio PM, Giordano S. TGFalpha expression impairs Trastuzumab-induced HER2 downregulation. *Oncogene* 2005; **24**: 3002-3010 [PMID: 15735715 DOI: 10.1038/sj.onc.1208478]

20   **Austin CD**, De Mazière AM, Pisacane PI, van Dijk SM, Eigenbrot C, Sliwkowski MX, Klumperman J, Scheller RH. Endocytosis and sorting of ErbB2 and the site of action of cancer therapeutics trastuzumab and geldanamycin. *Mol Biol Cell* 2004; **15**: 5268-5282 [PMID: 15385631 DOI: 10.1091/mbc.E04-07-0591]

21   **Delord JP**, Allal C, Canal M, Mery E, Rochaix P, Hennebelle I, Pradines A, Chatelut E, Bugat R, Guichard S, Canal P. Selective inhibition of HER2 inhibits AKT signal transduction and prolongs disease-free survival in a micrometastasis model of ovarian carcinoma. *Ann Oncol* 2005; **16**: 1889-1897 [PMID: 16219625 DOI: 10.1093/annonc/mdi405]

22   **Marty M**, Cognetti F, Maraninchi D, Snyder R, Mauriac L, Tubiana-Hulin M, Chan S, Grimes D, Antón A, Lluch A, Kennedy J, O'Byrne K, Conte P, Green M, Ward C, Mayne K, Extra JM. Randomized phase II trial of the efficacy and

safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol* 2005; **23**: 4265-4274 [PMID: 15911866 DOI: 10.1200/JCO.2005.04.173]

23   **Balduzzi S**, Mantarro S, Guarneri V, Tagliabue L, Pistotti V, Moja L, D'Amico R. Trastuzumab-containing regimens for metastatic breast cancer. *Cochrane Database Syst Rev* 2014; **6**: CD006242 [PMID: 24919460 DOI: 10.1002/14651858.CD006242.pub2]

24   **Buzdar AU**, Ibrahim NK, Francis D, Booser DJ, Thomas ES, Theriault RL, Pusztai L, Green MC, Arun BK, Giordano SH, Cristofanilli M, Frye DK, Smith TL, Hunt KK, Singletary SE, Sahin AA, Ewer MS, Buchholz TA, Berry D, Hortobagyi GN. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. *J Clin Oncol* 2005; **23**: 3676-3685 [PMID: 15738535 DOI: 10.1200/JCO.2005.07.032]

25   **Untch M**, Rezai M, Loibl S, Fasching PA, Huober J, Tesch H, Bauerfeind I, Hilfrich J, Eidtmann H, Gerber B, Hanusch C, Kühn T, du Bois A, Blohmer JU, Thomssen C, Dan Costa S, Jackisch C, Kaufmann M, Mehta K, von Minckwitz G. Neoadjuvant treatment with trastuzumab in HER2-positive breast cancer: results from the GeparQuattro study. *J Clin Oncol* 2010; **28**: 2024-2031 [PMID: 20308670 DOI: 10.1200/JCO.2009.23.8451]

26   **Gianni L**, Eiermann W, Semiglazov V, Lluch A, Tjulandin S, Zambetti M, Moliterni A, Vazquez F, Byakhov MJ, Lichinitser M, Climent MA, Ciruelos E, Ojeda B, Mansutti M, Bozhok A, Magazzù D, Heinzmann D, Steinseifer J, Valagussa P, Baselga J. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort. *Lancet Oncol* 2014; **15**: 640-647 [PMID: 24657003 DOI: 10.1016/S1470-2045(14)70080-4]

27   **Cai CQ**, Peng Y, Buckley MT, Wei J, Chen F, Liebes L, Gerald WL, Pincus MR, Osman I, Lee P. Epidermal growth factor receptor activation in prostate cancer by three novel missense mutations. *Oncogene* 2008; **27**: 3201-3210 [PMID: 18193092 DOI: 10.1038/sj.onc.1210983]

28   **Gaborit N**, Larbouret C, Vallaghe J, Peyrusson F, Bascoul-Mollevi C, Crapez E, Azria D, Chardès T, Poul MA, Mathis G, Bazin H, Pèlegrin A. Time-resolved fluorescence resonance energy transfer (TR-FRET) to analyze the disruption of EGFR/HER2 dimers: a new method to evaluate the efficiency of targeted therapy using monoclonal antibodies. *J Biol Chem* 2011; **286**: 11337-11345 [PMID: 21282108 DOI: 10.1200/JCO.2011.37.4207]

29   **Cortés J**, Fumoleau P, Bianchi GV, Petrella TM, Gelmon K, Pivot X, Verma S, Albanell J, Conte P, Lluch A, Salvagni S, Servent V, Gianni L, Scaltriti M, Ross GA, Dixon J, Szado T, Baselga J. Pertuzumab monotherapy after trastuzumab-based treatment and subsequent reintroduction of trastuzumab: activity and tolerability in patients with advanced human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol* 2012; **30**: 1594-1600 [PMID: 22393084]

30   **Lewis Phillips GD**, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, Blättler WA, Lambert JM, Chari RV, Lutz RJ, Wong WL, Jacobson FS, Koeppen H, Schwall RH, Kenkare-Mitra SR, Spencer SD, Sliwkowski MX. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res* 2008; **68**: 9280-9290 [PMID: 19010901 DOI: 10.1158/0008-5472.CAN-08-1776]

31   **Verma S**, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, Pegram M, Oh DY, Diéras V, Guardino E, Fang L, Lu MW, Olsen S, Blackwell K. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med* 2012; **367**:

1783-1791 [PMID: 23020162 DOI: 10.1056/NEJMoa1209124]

32   **Spector NL**, Xia W, Burris H, Hurwitz H, Dees EC, Dowlati A, O'Neil B, Overmoyer B, Marcom PK, Blackwell KL, Smith DA, Koch KM, Stead A, Mangum S, Ellis MJ, Liu L, Man AK, Bremer TM, Harris J, Bacus S. Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. *J Clin Oncol* 2005; **23**: 2502-2512 [PMID: 15684311 DOI: 10.1200/JCO.2005.12.157]

33   **Pedersen K**, Angelini PD, Laos S, Bach-Faig A, Cunningham MP, Ferrer-Ramón C, Luque-García A, García-Castillo J, Parra-Palau JL, Scaltriti M, Ramón y Cajal S, Baselga J, Arribas J. A naturally occurring HER2 carboxy-terminal fragment promotes mammary tumor growth and metastasis. *Mol Cell Biol* 2009; **29**: 3319-3331 [PMID: 19364815 DOI: 10.1128/MCB.01803-08]

34   **Scaltriti M**, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J, Di Cosimo S, Matias-Guiu X, Ramon y Cajal S, Arribas J, Baselga J. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst* 2007; **99**: 628-638 [PMID: 17440164 DOI: 10.1093/jnci/djk134]

35   **Blackwell KL**, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, Ellis C, Casey M, Vukelja S, Bischoff J, Baselga J, O'Shaughnessy J. Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. *J Clin Oncol* 2010; **28**: 1124-1130 [PMID: 20124187 DOI: 10.1200/JCO.2008.21.4437]

36   **Baselga J**, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, Gómez H, Dinh P, Fauria K, Van Dooren V, Aktan G, Goldhirsch A, Chang TW, Horváth Z, Coccia-Portugal M, Domont J, Tseng LM, Kunz G, Sohn JH, Semiglazov V, Lerzo G, Palacova M, Probachai V, Pusztai L, Untch M, Gelber RD, Piccart-Gebhart M. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet* 2012; **379**: 633-640 [PMID: 22257673 DOI: 10.1016/S0140-6736(11)61847-3]

37   **Modi S**, Stopeck A, Linden H, Solit D, Chandarlapaty S, Rosen N, D'Andrea G, Dickler M, Moynahan ME, Sugarman S, Ma W, Patil S, Norton L, Hannah AL, Hudis C. HSP90 inhibition is effective in breast cancer: a phase II trial of tanespimycin (17-AAG) plus trastuzumab in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. *Clin Cancer Res* 2011; **17**: 5132-5139 [PMID: 21558407 DOI: 10.1158/1078-0432.CCR-11-0072]

38   **Zhang H**, Berezov A, Wang Q, Zhang G, Drebin J, Murali R, Greene MI. ErbB receptors: from oncogenes to targeted cancer therapies. *J Clin Invest* 2007; **117**: 2051-2058 [PMID: 17671639 DOI: 10.1172/JCI32278]

39   **Martin HL**, Smith L, Tomlinson DC. Multidrug-resistant breast cancer: current perspectives. *Breast Cancer* (Dove Med Press) 2014; **6**: 1-13 [PMID: 24648765 DOI: 10.2147/BCTT.S37638]

40   **Nagy P**, Friedländer E, Tanner M, Kapanen AI, Carraway KL, Isola J, Jovin TM. Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. *Cancer Res* 2005; **65**: 473-482 [PMID: 15695389]

41   **Pályi-Krekk Z**, Barok M, Isola J, Tammi M, Szöllosi J, Nagy P. Hyaluronan-induced masking of ErbB2 and CD44-enhanced trastuzumab internalisation in trastuzumab resistant breast cancer. *Eur J Cancer* 2007; **43**: 2423-2433 [PMID: 17911008 DOI: 10.1016/j.ejca.2007.08.018]

42   **Diermeier S**, Horváth G, Knuechel-Clarke R, Hofstaedter F, Szöllosi J, Brockhoff G. Epidermal growth factor receptor coexpression modulates susceptibility to Herceptin in HER2/neu overexpressing breast cancer cells via specific erbB-

receptor interaction and activation. *Exp Cell Res* 2005; **304**: 604-619 [PMID: 15748904 DOI: 10.1016/j.yexcr.2004.12.008]

43   **Gajria D**, Chandarlapaty S. HER2-amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. *Expert Rev Anticancer Ther* 2011; **11**: 263-275 [PMID: 21342044 DOI: 10.1586/era.10.226]

44   **Nahta R**, Yuan LX, Zhang B, Kobayashi R, Esteva FJ. Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. *Cancer Res* 2005; **65**: 11118-11128 [PMID: 16322262 DOI: 10.1158/0008-5472.CAN-04-3841]

45   **Shattuck DL**, Miller JK, Carraway KL, Sweeney C. Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. *Cancer Res* 2008; **68**: 1471-1477 [PMID: 18316611 DOI: 10.1158/0008-5472.CAN-07-5962]

46   **Gong C**, Yao Y, Wang Y, Liu B, Wu W, Chen J, Su F, Yao H, Song E. Up-regulation of miR-21 mediates resistance to trastuzumab therapy for breast cancer. *J Biol Chem* 2011; **286**: 19127-19137 [PMID: 21471222 DOI: 10.1074/jbc.M110.216887]

47   **Chakrabarty A**, Bhola NE, Sutton C, Ghosh R, Kuba MG, Dave B, Chang JC, Arteaga CL. Trastuzumab-resistant cells rely on a HER2-PI3K-FoxO-survivin axis and are sensitive to PI3K inhibitors. *Cancer Res* 2013; **73**: 1190-1200 [PMID: 23204226 DOI: 10.1158/0008-5472.CAN-12-2440]

48   **Clynes RA**, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med* 2000; **6**: 443-446 [PMID: 10742152 DOI: 10.1038/74704]

49   **Musolino A**, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, Laccabue D, Zerbini A, Camisa R, Bisagni G, Neri TM, Ardizzoni A. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *J Clin Oncol* 2008; **26**: 1789-1796 [PMID: 18347005 DOI: 10.1200/JCO.2007.14.8957]

50   **Osipo C**, Patel P, Rizzo P, Clementz AG, Hao L, Golde TE, Miele L. ErbB-2 inhibition activates Notch-1 and sensitizes breast cancer cells to a gamma-secretase inhibitor. *Oncogene* 2008; **27**: 5019-5032 [PMID: 18469855 DOI: 10.1038/onc.2008.149]

51   **Politi K**, Feirt N, Kitajewski J. Notch in mammary gland development and breast cancer. *Semin Cancer Biol* 2004; **14**: 341-347 [PMID: 15288259 DOI: 10.1016/j.semcancer.2004.04.013]

52   **Greulich H**, Kaplan B, Mertins P, Chen TH, Tanaka KE, Yun CH, Zhang X, Lee SH, Cho J, Ambrogio L, Liao R, Imielinski M, Banerji S, Berger AH, Lawrence MS, Zhang J, Pho NH, Walker SR, Winckler W, Getz G, Frank D, Hahn WC, Eck MJ, Mani DR, Jaffe JD, Carr SA, Wong KK, Meyerson M. Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of ERBB2. *Proc Natl Acad Sci USA* 2012; **109**: 14476-14481 [PMID: 22908275 DOI: 10.1073/pnas.1203201109]

53   **Burstein HJ**, Sun Y, Dirix LY, Jiang Z, Paridaens R, Tan AR, Awada A, Ranade A, Jiao S, Schwartz G, Abbas R, Powell C, Turnbull K, Vermette J, Zacharchuk C, Badwe R. Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. *J Clin Oncol* 2010; **28**: 1301-1307 [PMID: 20142587 DOI: 10.1200/JCO.2009.25.8707]

54   **Jankowitz RC**, Abraham J, Tan AR, Limentani SA, Tierno MB, Adamson LM, Buyse M, Wolmark N, Jacobs SA. Safety and efficacy of neratinib in combination with weekly paclitaxel and trastuzumab in women with metastatic HER2-positive breast cancer: an NSABP Foundation Research Program phase I study. *Cancer Chemother Pharmacol* 2013; **72**: 1205-1212 [PMID: 24077916]

55   **Mohamed A**, Krajewski K, Cakar B, Ma CX. Targeted therapy for breast cancer. *Am J Pathol* 2013; **183**: 1096-1112 [PMID: 23988612 DOI: 10.1016/j.ajpath.2013.07.005]

56   **Schroeder A**, Heller DA, Winslow MM, Dahlman JE, Pratt

GW, Langer R, Jacks T, Anderson DG. Treating metastatic cancer with nanotechnology. *Nat Rev Cancer* 2012; **12**: 39-50 [PMID: 22193407 DOI: 10.1038/nrc3180]

57   **Esteva FJ**, Valero V, Pusztai L, Boehnke-Michaud L, Buzdar AU, Hortobagyi GN. Chemotherapy of metastatic breast cancer: what to expect in 2001 and beyond. *Oncologist* 2001; **6**: 133-146 [PMID: 11306725]

58   **Desai N**. Challenges in development of nanoparticle-based therapeutics. *AAPS J* 2012; **14**: 282-295 [PMID: 22407288 DOI: 10.1208/s12248-012-9339-4]

59   **Lammers T**, Kiessling F, Hennink WE, Storm G. Nanotheranostics and image-guided drug delivery: current concepts and future directions. *Mol Pharm* 2010; **7**: 1899-1912 [PMID: 20822168 DOI: 10.1021/mp100228v]

60   **Torchilin VP**. Passive and active drug targeting: drug delivery to tumors as an example. *Handb Exp Pharmacol* 2010; **(197)**: 3-53 [PMID: 20217525 DOI: 10.1007/978-3-642-00477-3_1]

61   **Yu MK**, Park J, Jon S. Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. *Theranostics* 2012; **2**: 3-44 [PMID: 22272217 DOI: 10.7150/thno.3463]

62   **Allen TM**. Ligand-targeted therapeutics in anticancer therapy. *Nat Rev Cancer* 2002; **2**: 750-763 [PMID: 12360278 DOI: 10.1038/nrc903]

63   **Holliger P**, Hudson PJ. Engineered antibody fragments and the rise of single domains. *Nat Biotechnol* 2005; **23**: 1126-1136 [PMID: 16151406 DOI: 10.1038/nbt1142]

64   **Sanz L**, Cuesta AM, Compte M, Alvarez-Vallina L. Antibody engineering: facing new challenges in cancer therapy. *Acta Pharmacol Sin* 2005; **26**: 641-648 [PMID: 15916728 DOI: 10.1111/j.1745-7254.2005.00135.x]

65   **Slamon DJ**, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; **344**: 783-792 [PMID: 11248153 DOI: 10.1056/NEJM200103153441101]

66   **Corsi F**, Fiandra L, De Palma C, Colombo M, Mazzucchelli S, Verderio P, Allevi R, Tosoni A, Nebuloni M, Clementi E, Prosperi D. HER2 expression in breast cancer cells is down-regulated upon active targeting by antibody-engineered multifunctional nanoparticles in mice. *ACS Nano* 2011; **5**: 6383-6393 [PMID: 21790185 DOI: 10.1021/nn201570n]

67   **Fiandra L**, Mazzucchelli S, De Palma C, Colombo M, Allevi R, Sommaruga S, Clementi E, Bellini M, Prosperi D, Corsi F. Assessing the in vivo targeting efficiency of multifunctional nanoconstructs bearing antibody-derived ligands. *ACS Nano* 2013; **7**: 6092-6102 [PMID: 23758591 DOI: 10.1021/nn4018922]

68   **Hoang B**, Ekdawi SN, Reilly RM, Allen C. Active targeting of block copolymer micelles with trastuzumab Fab fragments and nuclear localization signal leads to increased tumor uptake and nuclear localization in HER2-overexpressing xenografts. *Mol Pharm* 2013; **10**: 4229-4241 [PMID: 24066900 DOI: 10.1021/mp400315p]

69   **Kirpotin DB**, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, Marks JD, Benz CC, Park JW. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. *Cancer Res* 2006; **66**: 6732-6740 [PMID: 16818648 DOI: 10.1158/0008-5472.CAN-05-4199]

70   **Alexis F**, Basto P, Levy-Nissenbaum E, Radovic-Moreno AF, Zhang L, Pridgen E, Wang AZ, Marein SL, Westerhof K, Molnar LK, Farokhzad OC. HER-2-targeted nanoparticle-affibody bioconjugates for cancer therapy. *ChemMedChem* 2008; **3**: 1839-1843 [PMID: 19012296 DOI: 10.1002/cmdc.200800122]

71   **Gao J**, Chen K, Miao Z, Ren G, Chen X, Gambhir SS, Cheng Z. Affibody-based nanoprobes for HER2-expressing cell and tumor imaging. *Biomaterials* 2011; **32**: 2141-2148 [PMID: 21147502 DOI: 10.1016/j.biomaterials.2010.11.053]

72   **Gao H**, Cao S, Chen C, Cao S, Yang Z, Pang Z, Xi Z, Pan S, Zhang Q, Jiang X. Incorporation of lapatinib into lipoprotein-like nanoparticles with enhanced water solubility and anti-tumor effect in breast cancer. *Nanomedicine* (Lond) 2013; **8**: 1429-1442 [PMID: 23451915 DOI: 10.2217/nnm.12.180]

73   **Zhang L**, Zhang S, Ruan SB, Zhang QY, He Q, Gao HL. Lapatinib-incorporated lipoprotein-like nanoparticles: preparation and a proposed breast cancer-targeting mechanism. *Acta Pharmacol Sin* 2014; **35**: 846-852 [PMID: 24902791 DOI: 10.1038/aps.2014.26]

74   **Algar WR**, Prasuhn DE, Stewart MH, Jennings TL, Blanco-Canosa JB, Dawson PE, Medintz IL. The controlled display of biomolecules on nanoparticles: a challenge suited to bioorthogonal chemistry. *Bioconjug Chem* 2011; **22**: 825-858 [PMID: 21585205 DOI: 10.1021/bc200065z]

75   **Avvakumova S**, Colombo M, Tortora P, Prosperi D. Biotechnological approaches toward nanoparticle biofunctionalization. *Trends Biotechnol* 2014; **32**: 11-20 [PMID: 24182737 DOI: 10.1016/j.tibtech.2013.09.006]

76   **Anand G**, Sharma S, Dutta AK, Kumar SK, Belfort G. Conformational transitions of adsorbed proteins on surfaces of varying polarity. *Langmuir* 2010; **26**: 10803-10811 [PMID: 20433160 DOI: 10.1021/la1006132]

77   **Yoon TJ**, Yu KN, Kim E, Kim JS, Kim BG, Yun SH, Sohn BH, Cho MH, Lee JK, Park SB. Specific targeting, cell sorting, and bioimaging with smart magnetic silica core-shell nanomaterials. *Small* 2006; **2**: 209-215 [PMID: 17193022 DOI: 10.1002/smll.200500360]

78   **Mazzucchelli S**, Sommaruga S, O'Donnel M, Galeffi P, Tortora P, Prosperi D, Colombo M. Dependence of nanoparticle-cell recognition efficiency on the surface orientation of scFv targeting ligands. *Biomaterial Sciences* 2013; **1**: 728-735 [DOI: 10.1039/c3bm60068h]

79   **Han H**, Davis ME. Single-antibody, targeted nanoparticle delivery of camptothecin. *Mol Pharm* 2013; **10**: 2558-2567 [PMID: 23676007 DOI: 10.1021/mp300702x]

80   **Kouchakzadeh H**, Shojaosadati SA, Tahmasebi F, Shokri F. Optimization of an anti-HER2 monoclonal antibody targeted delivery system using PEGylated human serum albumin nanoparticles. *Int J Pharm* 2013; **447**: 62-69 [PMID: 23454849 DOI: 10.1016/j.ijpharm.2013.02.043]

81   **Zhao J**, Feng SS. Effects of PEG tethering chain length of vitamin E TPGS with a Herceptin-functionalized nanoparticle formulation for targeted delivery of anticancer drugs. *Biomaterials* 2014; **35**: 3340-3347 [PMID: 24461325 DOI: 10.1016/j.biomaterials.2014.01.003]

82   **Duncan R**. The dawning era of polymer therapeutics. *Nat Rev Drug Discov* 2003; **2**: 347-360 [PMID: 12750738 DOI: 10.1038/nrd1088]

83   **Vivek R**, Thangam R, NipunBabu V, Rejeeth C, Sivasubramanian S, Gunasekaran P, Muthuchelian K, Kannan S. Multifunctional HER2-antibody conjugated polymeric nanocarrier-based drug delivery system for multi-drug-resistant breast cancer therapy. *ACS Appl Mater Interfaces* 2014; **6**: 6469-6480 [PMID: 24780315 DOI: 10.1021/am406012g]

84   **Occhipinti E**, Verderio P, Natalello A, Galbiati E, Colombo M, Mazzucchelli S, Salvadè A, Tortora P, Doglia SM, Prosperi D. Investigating the structural biofunctionality of antibodies conjugated to magnetic nanoparticles. *Nanoscale* 2011; **3**: 387-390 [PMID: 20877896 DOI: 10.1039/c0nr00436g]

85   **Chen H**, Wang L, Yu Q, Qian W, Tiwari D, Yi H, Wang AY, Huang J, Yang L, Mao H. Anti-HER2 antibody and ScFvEGFR-conjugated antifouling magnetic iron oxide nanoparticles for targeting and magnetic resonance imaging of breast cancer. *Int J Nanomedicine* 2013; **8**: 3781-3794 [PMID: 24124366 DOI: 10.2147/IJN.S49069]

86   **Shukla R**, Thomas TP, Peters JL, Desai AM, Kukowska-Latallo J, Patri AK, Kotlyar A, Baker JR. HER2 specific tumor targeting with dendrimer conjugated anti-HER2 mAb. *Bioconjug Chem* 2006; **17**: 1109-1115 [PMID: 16984117 DOI:

10.1021/bc050348p]

87   **Artemov D**, Mori N, Okollie B, Bhujwalla ZM. MR molecular imaging of the Her-2/neu receptor in breast cancer cells using targeted iron oxide nanoparticles. *Magn Reson Med* 2003; **49**: 403-408 [PMID: 12594741 DOI: 10.1002/mrm.10406]

88   **Colombo M**, Mazzucchelli S, Collico V, Avvakumova S, Pandolfi L, Corsi F, Porta F, Prosperi D. Protein-assisted one-pot synthesis and biofunctionalization of spherical gold nanoparticles for selective targeting of cancer cells. *Angew Chem Int Ed Engl* 2012; **51**: 9272-9275 [PMID: 22833476 DOI: 10.1002/anie.201204699]

89   **Colombo M**, Mazzucchelli S, Montenegro JM, Galbiati E, Corsi F, Parak WJ, Prosperi D. Protein oriented ligation on nanoparticles exploiting O6-alkylguanine-DNA transferase (SNAP) genetically encoded fusion. *Small* 2012; **8**: 1492-1497 [PMID: 22431243 DOI: 10.1002/smll.201102284]

90   **Shi M,** Ho K, Keating A, Shoichet MS. Doxorubicin-conjugated immuno-nanoparticles for intracellular anticancer drug delivery. *Adv Funct Mater* 2009; **19**: 1-8 [DOI: 10.1002/adfm.200801271]

91   **Koopaei MN**, Dinarvand R, Amini M, Rabbani H, Emami S, Ostad SN, Atyabi F. Docetaxel immunonanocarriers as targeted delivery systems for HER 2-positive tumor cells: preparation, characterization, and cytotoxicity studies. *Int J Nanomedicine* 2011; **6**: 1903-1912 [PMID: 21931485 DOI: 10.2147/IJN.S23211]

92   **Gao J**, Kou G, Wang H, Chen H, Li B, Lu Y, Zhang D, Wang S, Hou S, Qian W, Dai J, Zhao J, Zhong Y, Guo Y. PE38KDEL-loaded anti-HER2 nanoparticles inhibit breast tumor progression with reduced toxicity and immunogenicity. *Breast Cancer Res Treat* 2009; **115**: 29-41 [PMID: 18481173 DOI:

10.1007/s10549-008-0043-0]

93   **Piao L**, Li H, Teng L, Yung BC, Sugimoto Y, Brueggemeier RW, Lee RJ. Human serum albumin-coated lipid nanoparticles for delivery of siRNA to breast cancer. *Nanomedicine* 2013; **9**: 122-129 [PMID: 22542825 DOI: 10.1016/j.nano.2012.03.008]

94   **Meng H**, Mai WX, Zhang H, Xue M, Xia T, Lin S, Wang X, Zhao Y, Ji Z, Zink JI, Nel AE. Codelivery of an optimal drug/siRNA combination using mesoporous silica nanoparticles to overcome drug resistance in breast cancer in vitro and in vivo. *ACS Nano* 2013; **7**: 994-1005 [PMID: 23289892 DOI: 10.1021/nn3044066]

95   **Deng ZJ**, Morton SW, Ben-Akiva E, Dreaden EC, Shopsowitz KE, Hammond PT. Layer-by-layer nanoparticles for systemic codelivery of an anticancer drug and siRNA for potential triple-negative breast cancer treatment. *ACS Nano* 2013; **7**: 9571-9584 [PMID: 24144228 DOI: 10.1021/nn4047925]

96   **Dou S**, Yang X-Z, Xiong M-H, Sun C-Y, Yao Y-D, Zhu Y-H, Wang J. ScFv-Decorated PEG-PLA-Based Nanoparticles for Enhanced siRNA Delivery to Her2(+) Breast Cancer. *Adv Healthc Mater* 2014; **3**: 1792-803 [PMID: 24947820 DOI: 10.1002/adhm.201400037]

97   **Mi Y**, Liu X, Zhao J, Ding J, Feng SS. Multimodality treatment of cancer with herceptin conjugated, thermomagnetic iron oxides and docetaxel loaded nanoparticles of biodegradable polymers. *Biomaterials* 2012; **33**: 7519-7529 [PMID: 22809649 DOI: 10.1016/j.biomaterials.2012.06.100]

98   **Stuchinskaya T**, Moreno M, Cook MJ, Edwards DR, Russell DA. Targeted photodynamic therapy of breast cancer cells using antibody-phthalocyanine-gold nanoparticle conjugates. *Photochem Photobiol Sci* 2011; **10**: 822-831 [PMID: 21455532 DOI: 10.1039/c1pp05014a]

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com