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Targeted therapies in breast cancer: Where are we now?

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ABSTRACT

Over the past several years significant advances have been made in our understanding of a growing number of critical pathways involved in breast cancer. These advances have led to the development of novel therapies that are being collectively known as molecularly targeted in order to highlight their specificity and their interference with key molecular events responsible for the malignant phenotype.

Examples of approved targeted agents in breast cancer include agents directed against the human epidermal growth factor receptor 2 (HER2) such as trastuzumab and lapatinib and the anti-VEGF bevacizumab. In addition, there are classes of therapies under evaluation including novel anti-HER2 therapies, agents against other tyrosine kinases including Src and Insulin-Like Growth Factor Receptor agents interfering with critically important signalling pathways such as the PI3K/Akt/mTOR inhibitors and agents that promote apoptosis such as Parp inhibitors and others.

The challenges that are being brought by these novel therapies are different from those being faced with conventional chemotherapy. They include the selection of appropriate dose and schedule, safety issues, selection of the patient population most likely to benefit and early readouts of clinical benefit. We will present these novel therapies and will analyse for each target the developmental status of some of the agents as well as target-specific challenges.

1. Introduction

In recent years, improvements in the understanding of the altered molecular events leading to breast cancer have led to the identification of new molecular targets and the development of targeted therapy. One of the earliest validations to this approach has been observed with the anti-HER2 monoclonal antibody (MAb) trastuzumab in patients with human epidermal growth factor receptor 2 (HER2)-overexpressing tumours and with the anti-angiogenesis MAb bevacizumab, respectively.[1,2] In the case of patients with HER2 amplified breast tumours, a group of tumours with poor prognosis, trastuzumab has markedly improved the survival of this subgroup of patients and has markedly changed the outcome of HER2 positive breast cancer.

Other potential hallmarks of malignancy that represent a new opportunity for therapeutic targeting include evasion of apoptosis, lack of senescence, invasion and metastasis and genomic elasticity.[3] Therefore, new compounds are being developed that may interfere with these hallmarks and that may prove to be effective in monotherapy or in combination with cytotoxic therapy or other targeted therapies. This review highlights targeted agents that are furthest along in their clinical development as novel breast cancer therapeutics.

The development of these agents will require a new set of skills. First, these agents, unlike chemotherapy, will only

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work in the subset of tumours that show dependency on the target that the therapy is being directed to. This is again well exemplified by the anti-HER2 agent trastuzumab that is only active against tumour with high level of expression and/or amplification of the HER2 gene. Taking into account that HER2 is overexpressed in only 25% of breast tumours and that the single agent activity of trastuzumab is modest, if trastuzumab would have been developed in an unselected patient population, its antitumour activity would have been missed due to a dilutional effect brought in by the non-HER2-overexpressing population. This principle probably applies to the majority of classes of agents under study. The implication of this principle is that patient selection strategies will be of paramount importance in the development of these agents. Second, in early clinical studies with these agents in addition to establish their safety and optimal doses and schedules, it may prove to be instrumental to also check for the presence of the target in the studied tumours and to seek for indications of target engagement with the study agent. A debate, heated at times, is underway among the proposers of careful analysis of markers of target engagement – also known as pharmacodynamic studies (reviewed in [4]) – and those who are supporters of a more classical drug development process based on maximally tolerated doses. This review will not solve this discussion topic, but suffice to say that the need to determine target engagement is currently being taken into account with the majority of clinical trials with novel agents that are moved into the clinic. Third, the therapy end-points with these agents also need to be revisited. Some of these agents are not expected to result in tumour shrinkage (or response), and therefore we cannot propose a unified definition of clinical benefit as it has been done with chemotherapy in the past. Fourth, some of these agents will have limited activity by themselves and yet have the capacity to markedly enhance the antitumour activity of conventional agents like chemotherapy or even other biological agents. This later point is well exemplified by the anti-angiogenesis MAb bevacizumab that has no activity as a single agent and yet is clinically active when combined with chemotherapy. Lastly, with the increase in the number of available lines of therapy in breast cancer, there is a danger that novel agents will be tested in a heavily pre-treated patient population. While there is little debate that for early clinical studies patients with advanced disease that have received multiple lines of therapy may be appropriate study participants, there is also a growing concern that patients with advanced disease may not be the ideal population to detect the antitumour activity of novel agents since their tumours may have become highly resistant to any type of therapy. In addition, the drive to identify new biomarkers of target engagement and sensitivity with these novel agents is also promoting the search for new clinical study designs in a minimally pre-treated population. Studies of novel agents in the neo-adjuvant setting in breast cancer are therefore being incorporated with targeted therapeutics.

2. HER2 receptor inhibitors

The epidermal growth factor receptor (EGFR) family is composed of cell surface tyrosine kinase receptors that are involved in the regulation of cellular proliferation and survival of epithelial cells. The EGFR family includes four receptors: EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4. These receptors have an extracellular domain (ECD), a transmembrane region and an intracellular domain with tyrosine kinase activity. Except for HER2, binding of receptor-specific ligands to the ectodomain of EGFR, HER3 and HER4 results in the formation of homodimeric and heterodimeric kinase-active complexes to which HER2 is recruited as a preferred partner.[5] HER2 is unable to directly interact with ErbB ligands but can potently enhance signalling by HER2-containing heterodimers, and/or increase the binding affinity of receptor ligands to EGFR and HER3/4. HER2 receptor activation leads to the phosphorylation of the intracellular catalytic domains, and ultimately activation of signal transduction pathways that promote proliferation and survival, including the phosphatidylinositol 3′-kinase (PI3K)/Akt/mTOR, the Erk1/2 mitogen-activated protein kinase (MAPK) and the Jak/Stat pathway.[6]

HER2 overexpression drives malignant transformation of mammary epithelial cells in experimental systems.[7] In patients, HER2 is overexpressed and/or amplified in one-fourth of breast tumours and confers a more aggressive clinical course and a worse survival.[8,9] The outcome of these highly aggressive tumours has markedly improved with the development of anti-HER2 therapies. Trastuzumab (Herceptin®) is a recombinant humanised monoclonal antibody that binds with high affinity to the extracellular juxtamembrane domain of HER2 and inhibits the proliferation of human tumour cells that overexpress HER2 *in vitro* and *in vivo*.[10–12] In patients with HER2 amplified tumours, trastuzumab has single agent activity and improves survival in the first-line setting when combined with chemotherapy in patients with advanced disease.[1,13] Recently, a number of well-powered clinical trials have demonstrated that administration of trastuzumab in the adjuvant setting, in combination and/or sequentially after chemotherapy, results in an improvement in disease-free survival as well as overall survival.[14–17]

Another strategy to target HER2 is with low molecular weight tyrosine kinase inhibitors. Lapatinib (Tykerb®) is a dual EGFR and HER2 inhibitor that has been studied extensively in multiple clinical settings (Table 1). Lapatinib increases survival in patients with advanced HER2-overexpressing breast cancer when given in combination with the chemotherapeutic agent capecitabine compared to capecitabine alone in patients that had previously failed anthracyclines and taxanes.[18] In this pivotal trial that leads to lapatinib's regulatory approval, it was also observed that fewer patients in the lapatinib group developed central nervous system (CNS) metastases. Although the difference was not statistically significant, this clinical observation together with lapatinib's low molecular weight and capacity to cross the blood-brain barrier has led to a clinical trial to study the role of lapatinib in the treatment of brain metastasis in patients with HER2-overexpressing breast cancer.[19,20] Lapatinib is also active in patients with newly diagnosed inflammatory breast cancer, both alone and when used in combination with paclitaxel.[21] Currently, the role of lapatinib in early disease is being explored both in the first line and in the (neo) adjuvant settings. In this regard, the ALTTO study is comparing the adjuvant administration of trastuzumab and lapatinib given alone or in combination in a study population of 8000 patients after

Table 1 – Lapatinib: main results.

	Setting		(*n*) Patients	Phase	Schedule	Outcomes
Treatment with LAPATINIB						
Alone	Heavily pre-treated	MBC	140	II	1500 mg/day	ORR = 4.3%
						16 week PFS = 13%
	1–2 Prior therapy		78			ORR = 7.7%
						16 week PFS = 22%
	First-line treatment		60	II	150 mg/day; 500 mg b.i.d.	ORR = 28%
						12 week PFS = 40%
	Heavily pre-treated	CNS met	107	II	750 b.i.d.	ORR = 6%
						24 week PFS = 22%
+Trastuzumab	Heavily pre-treated	MBC	54	I/II	750–1500 mg/day	ORR = 22%
+Capecitabine	Prior anthracyclines/taxanes and trastuzumab	MBC or LABC	324	III	750 mg/day	TTP 8.4 versus 4.4 months, $p < .001$
						ORR = 22% versus 14%, NS
+Paclitaxel	Up-front	IBC	30	II	1500 mg/day	ORR = 77%
						pCR = 17%

MBC = metastatic breast cancer; CNS = central nervous system; IBC = inflammatory breast cancer.

or concomitantly with approved chemotherapy regimens. The Neo-ALTTO study exemplifies the potential of the neoadjuvant approach in incorporating a biological only therapeutic window and complex biomarker analyses (Fig. 1).

Other anti-HER tyrosine kinase inhibitors in clinical development include HKI-272 and BIBW 2992, two irreversible inhibitors that covalently bind to the intracellular kinase domain. HKI-272 has shown activity against HER2 patients in the initial phase I study[22]; similarly, treatment with BIBW 2992 has resulted in early evidence of clinical benefit.[23]

Other classes of novel anti-HER2 agents include antibodies that interfere with HER2 dimerisation and inhibitors of the chaperone HSP90 that accelerate HER2 degradation. HSP90 inhibitors are being discussed in another section of this review. Pertuzumab is a recombinant, humanised MAb that targets an epitope within the HER2 dimerisation domain.[24] Once bound, pertuzumab inhibits ligand-activated HER dimerisation with HER2 and thereby inhibiting activation of intracellular signalling.[25] A phase II study of pertuzumab was conducted in patients that had failed up to three lines of chemotherapy and trastuzumab, and whose disease had progressed during trastuzumab therapy as the last regimen for metastatic disease.[26] Study treatment was to commence within 9 weeks of the last dose of trastuzumab given as previous therapy and trastuzumab was continued together with pertuzumab. In the initial report, pertuzumab had an overall response rate of 18% and an overall clinical benefit rate (patients with either a response or disease stabilisation) of 36%. These highly encouraging results have led to the initiation of a large, phase III registration study in the first-line setting.

3. Inhibitors of the PI3K/Akt/mTOR pathway

The PI3K/Akt pathway plays a central role in diverse cellular functions including proliferation, growth, survival and metabolism. In addition to their physiological role, several isoforms of the PI3K family are implicated in disease. In particular, members of class 1A PI3Ks, which are heterodimers comprising a p85 regulatory and a p110 catalytic subunit, are often mutated in human cancer.[27–32] As a result of receptor tyrosine kinase activation and phosphorylation, PI3K interacts with the intracellular domain of the receptors, either directly or indirectly via adaptor molecules such as IRS1, thus removing the inhibitory effect of p85 and leading to an active kinase. The GTPase Ras can also directly activate PI3-kinase. The catalytic subunit next converts PIP2 to PIP3, itself being responsible for facilitating the phosphorylation of Akt (also known as PKB) at Thr308 by PDK1. Conversely, PI3K is negatively regulated at the level of PIP3 by phospholipid phosphatases, such as phosphatase and tensin homologue PTEN.[33] A second phosphorylation event at Ser473 by the mammalian target of rapamycin (mTOR)-rictor complex is required for maximal Akt activity.[34,35] Akt is the central effector of the pathway. It is able to execute its myriad cellular operations via a host of effectors and promotes protein synthesis and cell growth by alleviating TSC1/2 suppression of mTOR, allowing the latter to act as part of the mTOR-raptor complex on 4EBP1 and ribosomal protein S6 kinases (S6K).[36] Akt reduces cell cycle inhibitors p27 and p21, and promotes cell cycle proteins c-Myc and cyclin D1, resulting in enhanced cellular pro-

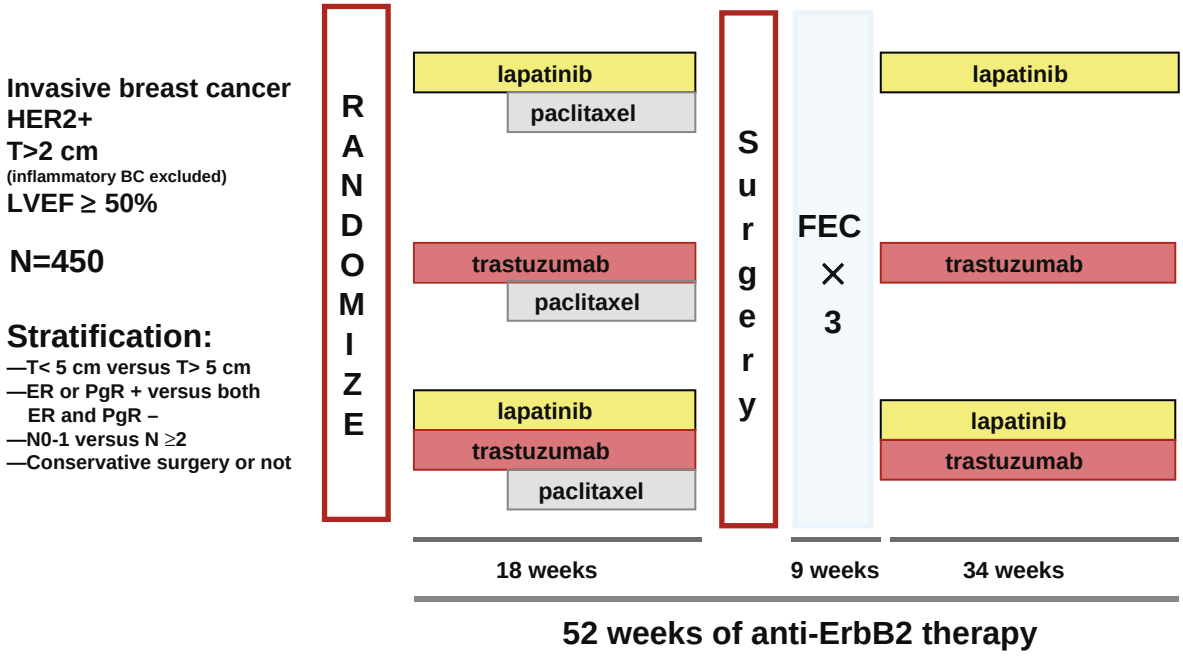

Fig. 1 – Study design of the Neo-ALTTO study.

liferation. Its influence extends to a host of pro- and anti-apoptotic proteins, such as the Bcl-2 family member Bad, limiting programmed cell death and boosting cellular survival.

There is growing evidence that uncontrolled activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway, achieved via numerous genetic and epigenetic alterations, contributes to the development and progression of human cancers, including breast cancer.[37] These genetic alterations include PTEN deletions[38,39] and 'hot-spots' mutations of the PI3K gene,[27] and they have been shown to have transforming capacity *in vitro* and *in vivo*.[40,41] Further, they may result in resistance to upstream anti-receptor agents. For example, trastuzumab depends on intact PTEN for its action in HER2-overexpressing breast cell lines, and PTEN loss predicts for trastuzumab resistance.[42]

Therefore, this pathway is an attractive target for novel anticancer agents. Clinical trials are currently underway with mTOR, PI3K and Akt inhibitors.

mTOR inhibitors are those further ahead in development within this class of agents. Rapamycin derivatives such as everolimus, temsirolimus and deforolimus are potent inhibitors of mTOR and do not share the problems of poor solubility and chemical stability of rapamycin. In unselected patients with breast cancer these agents have modest antitumour activity in the range of around 10%.[43] There is therefore a need to identify the subset of patients that may benefit from it, and PI3K/Akt/mTOR-dependency genetic signatures are being developed. In this direction, it has been recently observed that a majority of locally advanced and inflammatory breast cancers overexpress the translation regulatory protein 4E-BP1 and the initiation factor eIF4G, both of them mTOR downstream targets. In animal models and cell studies, the overexpression of 4E-BP1 and eIF4G has been shown to orchestrate a hypoxia-activated increase of tumour angiogenesis and growth at the level of selective mRNA translation. While additional studies are planned to further dissect this interaction, it does seem reasonable to explore the benefits of mTOR inhibitors in the treatment of locally advanced breast cancer.[44]

A potential explanation for the limited activity of mTOR inhibitors in breast cancer and other tumour types may be related to a 'collateral effect' of mTOR blockade. mTOR inhibition blocks the natural negative feed-back on IGF-1R signalling impinging on PI3K.[45,46] The result is an increase in PI3K and Akt activations which could potentially counteract the inhibition of mTOR. Dual inhibition of both IGF-1 signalling, with either MAbs against the receptor or tyrosine kinase inhibitors, and mTOR results in superior anti-proliferative effect over each single strategy. In the clinic there is indirect evidence that this approach may be fruitful as well. In patients with refractory neuroendocrine tumours although octreotide has limited activity, it has been shown to inhibit IGF-1R signalling. The combination of everolimus and octreotide has resulted in an impressive activity with four patients achieving a partial response and 19 patients having stable disease of 32 treated patients.[47]

Based on the cross-talk between the oestrogen receptor and the PI3K/Akt/mTOR pathways, mTOR inhibitors clinical trials have explored the combination of rapamycin analogues and aromatase inhibitors. A randomised phase II study compared oral temsirolimus combined with letrozole to letrozole alone in 104 patients with hormonal receptor positive metastatic breast cancer.[48] However, due to the toxicity of the high-dose schedule that resulted in dose delays/reductions or discontinuations, the protocol was amended to low-dose schedules. After the amendment, early data from 92 patients suggested that PFS could be longer for the combination arms than for the letrozole alone, and, therefore, a large phase III trial was initiated. However, this study was terminated before

accrual was completed lack of efficacy for the combination. The unexpected result raised the issue that phase I studies evaluating the appropriate dose of signal transduction inhibitors should aim to identify the biologically effective dose using pharmacodynamic end-points rather than focusing on the maximum tolerated dose. Following this premise, a phase I study of everolimus as a single agent recommended a dose of 10 mg daily for further phase II–III development based on the toxicity profile and the molecular pharmacodynamic findings.[49] In a more recent phase II double blind randomised study of everolimus in combination with letrozole versus placebo and letrozole in the neo-adjuvant setting, the combination arm proved to be superior over letrozole alone with a higher statistically significant response rate (68% versus 59%).[50] This study incorporated carefully conducted pre and on-study tumour biopsies, and pharmacodynamic studies demonstrated a near doubling of response rate by decreases in the cell cycle indicator Ki67 in the everolimus treated group. This is potentially important since Ki67 drops in the neo-adjuvant setting has been recently demonstrated to correlate to long-term outcome.[51] The safety profile of the combination was acceptable and in line with the known side-effects of rapamycin analogues (stomatitis, rash, thrombocytopaenia, asthaenia and medically manageable hypercholesterolemia). As this study included pre- and post-treatment tissue analyses, results of gene profiling analysis are impatiently awaited.

In terms of PI3K inhibitors, a new generation of compounds is emerging, overcoming earlier problems of poor selectivity, unfavourable pharmacokinetic profiles and unacceptable toxicity. A number of these agents have entered early phase clinical trials, such as the NVP-BEZ235, a synthetic low molecular weight compound belonging to the class of imidazoquinolines that potently and reversely inhibits class 1 PI3K catalytic activity by competing at its ATP-binding site.[52]

4. Src-family tyrosine kinases inhibitors

The v-Src (Rous sarcoma virus) tyrosine kinase was the first oncogenic gene discovered.[53] The corresponding cellular gene, c-Src, is a non-receptor signalling kinase that functions as a hub of a vast array of signal transduction pathways that influence cellular proliferation, differentiation, motility and survival.[54]

Several mechanisms lead to increased Src activity in tumours. Src is downstream in signalling from a number of growth factor receptors including PDGF receptor (PDGFR), epidermal growth factor receptor (EGFR), insulin-like growth factor-1 receptor (IGF-1R) and hepatocyte growth factor/scatter factor receptor.[55] In many tumour types, including breast cancer, overexpression of these receptors, their ligands or both is frequent.[56] Finally, overexpression of other Src binding partners, including focal adhesion kinase, also activate Src.[57–60]

Recent studies suggest an association between Src tyrosine kinase and the development, progression and metastasis of breast cancer.[61,62] Transgenic mice with polyoma middle T antigen under the control of mouse mammary tumour virus promoter were found to develop highly metastatic mammary tumours with increased Src activity.[63] Moreover, when these mice were cross-bred with Src-deficient mice, the resulting chimeric mice no longer developed mammary tumours.[63] In addition, it is described that mice overexpressing the HER2 oncogene develop highly metastatic mammary tumours with elevated Src activity.[61] Taken together, these results strongly indicate that the Src may play an important role in the development and progression of breast cancer.[64] Further supporting the role of Src in breast cancer, it has been demonstrated that Src activity is profoundly increased in human breast cancer tissues compared with benign breast tumours or adjacent normal breast tissues[62,65], and that elevated c-Src tyrosine kinase activity is correlated with early systemic relapse.[66]

Currently, small-molecule Src inhibitors are in early phases of clinical development either as single agents, in combination with cytotoxic agents, biological therapies or in combination with hormonal treatment. Originally, dasatinib (BMS-354825) was selected as a synthetic small-molecule inhibitor of Src-family kinases; then, it was found to inhibit at least four other protein tyrosine kinases: bcr-abl, c-Kit, EphA2, PDGF-beta. Dasatinib was recently approved for the treatment of patients with imatinib-refractory chronic myelogenous leukaemia and Philadelphia chromosome-positive acute lymphoid leukaemia and is currently being studied in clinical trials for the treatment of solid tumours, including breast cancer. Preclinical evidence suggests that dasatinib could be effective in breast cancer cell lines of basal-like subtype.[67] These findings provide scientific rationale for the clinical development of dasatinib in the treatment of patients with 'triple-negative' breast cancer[67], a tumour subtype that is categorised as being aggressive and lacking effective targeted treatments, such as endocrine therapies and anti-HER2 strategies.

More recently, AZD-0530 a highly selective, dual-specific, orally available small-molecule inhibitor of Src kinase and Bcr-Abl has entered clinical trials.[68] In healthy volunteers, AZD-0530 has shown only mild adverse events. A phase I clinical study indicates for the first time in cancer patients that AZD-0530 is able to decrease the levels of bone resorption markers in serum and urine, suggesting a potential effect on osteoclasts.[69,70] More importantly, the use of pharmacodynamic end-points such as activation of the Src downstream targets, paxillin and focal adhesion kinase, which are mediators of cell motility and invasion, have allowed the identification of a dose that is biologically relevant and have finally demonstrated that the recommended maximum tolerated dose should be taken for further development.[70] Phase II studies with AZD-0530 in breast cancer are currently ongoing.

5. PARP inhibitors

Poly(ADP-ribose) polymerase 1 (PARP-1) is the initial and best characterised member of a family of enzymes largely associated with the maintenance of genomic stability. Activation of PARP-1 is part of the immediate cellular response to DNA strand breaks, converting them into an intracellular signal via poly(ADP-ribosylation) of nuclear proteins.[71,72] This results in a highly negatively charged target, which in turn leads to the unwinding and repair of the damaged DNA through the

base excision repair pathway. In addition, PARP-1 is also known to bind dsDNA breaks (DSB) preventing accidental recombination of homologous DNA.[73] Upon binding DNA breaks, the catalytic activity of PARP-1 is stimulated >500-fold.[74] Enhanced PARP-1 expression or activity has been also observed in a number of different tumour cell lines and could provide a greater level of resistance to both endogenous genotoxic stress and to DNA damage-inducing therapeutic agents.

PARP inhibitors have been developed to investigate the role of PARP-1 in cell biology and to overcome DNA repair-mediated resistance of cancer cells to genotoxic agents.[75] These novel PARP inhibitors have been shown to enhance the antitumour activity of DNA-methylating agents, such as temozolomide, topoisomerase poisons and ionising radiation in preclinical studies,[76] and to restore sensitivity of resistant tumours to methylating agents or topoisomerase I inhibitors, agents presently used for the treatment of breast cancer. Studies of PARP expression in various tumour types identified that breast cancers with negative oestrogen receptor, progesterone receptor and HER2 expression were much more likely to overexpress PARP.[77] Additionally, it has been recently shown that BRCA1 and BRCA2 dysfunction sensitises cells to the inhibition of PARP enzymatic activity, resulting in chromosomal instability, cell cycle arrest and subsequent apoptosis. This seems to be because the inhibition of PARP leads to the persistence of DNA lesions normally repaired by homologous recombination.[77]

Initial phase I studies with the PARP inhibitor AZD2281 (KU-0059436) have shown that this agent is safe, in patients with advanced breast cancer or ovarian cancer associated with an inherited mutation in one of the cancer genes, BRCA1 or BRCA2.[78] Data from 21 patients with hereditary BRCA-associated ovarian cancer demonstrated a response rate of 43%. First responses were observed at 100 mg bid on an intermittent schedule (14 days' dosing in a 21-day cycle) and subsequently at 200 mg bid and 400 mg bid continuous dosing. The study is still ongoing. There are now a number of phase II studies underway to further define its activity in patients with hereditary breast and ovarian cancer. Based on the strong rationale to combine cisplatin with PARP inhibitors

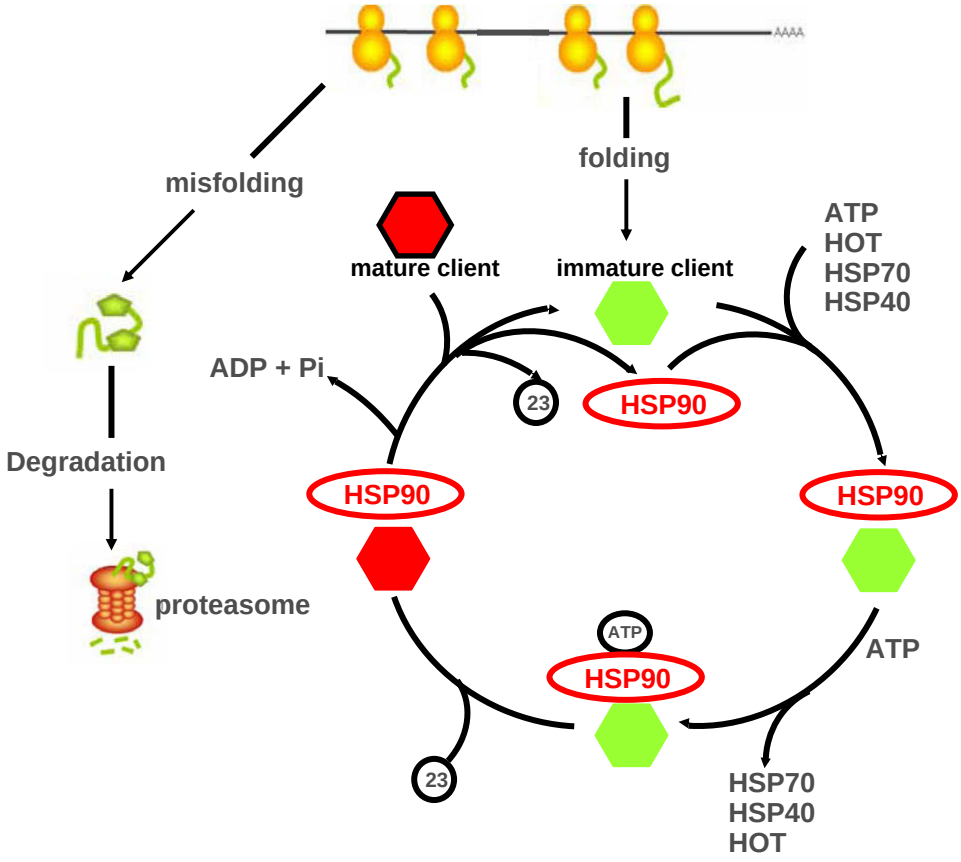

Fig. 2 – HSP90-mediated repair of proteins. The chaperone activity of HSP90 is dependent on its transient N-terminal dimerisation, which stimulates the intrinsic ATPase activity.[90] This process is controlled by an orchestrated set of interactions with a range of accessory proteins referred to as chaperones. Initially, client protein interacts with the HOT-HSP70-HSP40 complex. Then, HSP90 exchanges ADP for ATP, undergoes conformational changes, which includes the transient dimerisation of the N-terminal domains. This leads to the dissociation of HSP70/HSP40/HOP allowing the ATP-dependent association of other co-chaperones p23 to form the mature complex. It is while the HSP90 chaperone is in the mature state that it can clamp onto proteins, such as the steroid hormone receptor and the Akt, for folding, transport and aggregation. Inhibition of ATP binding to HSP90 prevents the formation of the mature complex, and results in the proteasome-dependent degradation of associated client protein.

and the emerging data in triple-negative breast cancer showing high level of activity with cisplatin, we will be starting briefly a neo-adjuvant study in patients with triple-negative breast cancer of cisplatin given in combination with AZD2281.

6. HSP90 inhibitors

Heat shock protein 90 (HSP90) is a molecular chaperone required for the stability and function of several conditionally activated and/or expressed signalling proteins.[79] Many of these client proteins such as Akt, HER2, Bcr-Abl, c-Kit, EGFR and PDGFR-α are oncoproteins and important cell-signalling proteins.[80,81] As signal transducers and molecular switches, these client proteins are inherently unstable. HSP90 keeps unstable signalling proteins poised for activation until they are stabilised by conformational changes associated with the formation of signal transduction complexes. As such, it is a single molecular target that is a central integrator of multiple pathways important to cancer. Activation of HSP90-dependent client proteins proceeds through an ordered sequence of events linked to the ATPase activity of HSP90 and involves a variety of co-chaperone complexes (Fig. 2).

HER2 is among the most sensitive client proteins of HSP90, demonstrating degradation within 2 h of HSP90 inhibition in cell culture experiments.[82] Geldanamycin analogues (17-allylamino-17-demethoxygeldanamycin [17-AAG] and 17-dimethylaminoethylamino-17-demethoxygeldanamycin [17-DMAG]) have demonstrated potent inhibition of HSP90 function in HER2-overexpressing cell lines, demonstrating significant antitumour activity in both cell culture and animal studies.[82,83] In addition to significant inhibition of full length HER2, HSP90 inhibitors also lead to significant decreases of the p95, the truncated form of HER2.[84]

In the clinic, initial studies with the HSP90 inhibitor tanespimycin (KOS-953)[85] and the second-generation HSP90 inhibitor, alvespimycin (KOS-1022)[86] have demonstrated safety and antitumour activity and tolerability in combination with trastuzumab in patients with trastuzumab-refractory HER2-positive metastatic breast cancer patients (Table 2). It will be important to determine whether HSP90 inhibitors will have activity as single agents. In order to address this question, we will start shortly a phase II study with IPI-504, a novel HSP90 inhibitor that has already shown to be safe and active in refractory advanced melanoma and in gastrointestinal stromal tumours.[87]

7. Targeted therapies in breast cancer: the way forward

We have reviewed some of the most promising new targeted agents in breast cancer. This list, however, is far from complete. Additional classes of agents in clinical development in breast cancer include inhibitors of angiogenesis, anti-IGF-1R MAbs and TKI's, other tyrosine kinase inhibitors and also enhancers of apoptosis. We all hope that this list will keep enlarging.

As mentioned in the introduction, the clinical development of these agents will require a new set of skills and a greater degree of complexity when compared to conventionally developed chemotherapeutic agents. It will be required to include appropriate subpopulation of patients, identification of target engagement by the study agent, incorporation

Table 2 – Results with anti-HSP90 agents.

	Phase	Setting (*n* patients)	Regimen		Main outcomes
Modi S, 2007	II	Refractory[a] HER2 breast cancer[26]	Tanespimycin (KOS-953) 450 mg/m^2 i.v. +trastuzumab, weekly	ORR = 25% SD > or = 4 months: 4/25	Grade 3 toxicities-all reversible: – 1 patient with fatigue and headache – 1 patient with transaminitis – 1 patient with unsteady gait and euphoric mood
Miller K, 2007	I	Heavily pre-treated[b] HER2 MBC[18]	Alvespimycin (KOS-1022) 60–100 mg/m^2 i.v. +trastuzumab, weekly	ORR = 1/18 SD ⩾ 12 weeks = 3/18	DLT at the highest dose: – 1 patient with hypoxia and ↓ LVEF – 1 patient with grade 3 transaminitis
Demetri GD, 2007	I	Refractory GIST[21]	IPI-504 90–400 mg/ m^2 i.v, days 1, 4, 8, 11; every 3 weeks	ORR = 66% (by PET) 33% of patients received ⩾ 5 cycles	Grade 3 toxicities: – 1 patient with nausea – 1 patient with transaminitis – 1 patient with ↑ lipase
Ongoing	I	Recurrent or MBC	mycograb + docetaxel 75 mg/m^2, every 3 weeks		
	II	Refractory HER2 MBC	IPI-504 400 mg/m^2 i.v, days 1, 4, 8, 11, every 3 weeks		
	I/II	Solid tumour	docetaxel + IPI-504		

a Second and third line therapy.
b Median number of prior regimens 6.

of novel end-points of clinical benefit such as tumour and surrogate-based biomarkers, and treatment of patients that have not been extensively pre-treated in the past.

Breast cancer is not a single disease entity but rather the summation of different subclasses that have different clinical outcome[88] and that may be respond differentially to therapy. New agents are increasingly being developed only in those subtypes that are more likely to benefit. For example, we have already mentioned that PARP inhibitors and platinum salts are being developed in basal type breast cancer. In addition, some agents will be developed across subtypes based on a specific gene response signature. This could be exemplified by PI3K signatures in the development of agents interfering with this pathway.

The need for longitudinal monitoring of tumours will be important in order to assess target engagement, biomarkers of response and tumour burden. Circulating tumour cells (CTCs) represent a potential alternative to invasive biopsies as a source of tumour tissue for the detection, characterisation and monitoring of tumour cells and are being incorporated in clinical trials. Novel technologies are allowing for the isolation of viable CTCs that could be used to monitor response to therapy.[89]

Finally, we will need to study these agents in less heavily pre-treated population of patients. In order to achieve this goal in addition to being able to obtain early readouts of clinical benefit, these agents are increasingly being developed in the neo-adjuvant, presurgical setting. The neo-adjuvant approach allows for the therapy of patients not previously treated, to obtain early indications of clinical response and to incorporate sequential tumour sampling for biomarker studies. It does even permit the incorporation of a 'window' therapy period with the biological agent alone as is in the ongoing Neo-ALTTO study in patients with HER2-positive disease (Fig. 1).

Conflict of interest statement

None declared.

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