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REVIEW

The Pharmacology of Trastuzumab and Its Use in Breast Cancer

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Abstract: Trastuzumab is a therapeutic humanised monoclonal antibody targeting the Her2 receptor tyrosine kinase. Her2 is over-expressed, due to gene amplification, in approximately 30% of breast cancers and confers a worse prognosis. Trastuzumab has been used clinically for over a decade and is now used routinely in the treatment of Her2-positive breast cancer in the metastatic and adjuvant setting. Its mechanisms of action and resistance, as well as its pharmacokinetics and efficacy in different clinical scenarios have been explored and increasingly clarified during its pre-marketing and post-marketing development. Nonetheless, numerous scientific and clinical issues remain not fully elucidated. This review discusses the pharmacodynamic and pharmacokinetic properties of trastuzumab and its current use in the treatment of breast cancer.

Keywords: trastuzumab, Her2, breast cancer

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Introduction

Trastuzumab is a humanised monoclonal antibody (mAB) used therapeutically in the treatment of Epidermal Growth Factor Receptor 2 (ErbB2, Her2, Neu) positive breast cancer. The efficacious use of antibodies in the treatment of cancer has been a goal of biologists for over 100 years.[1] That it has recently come to fruition is due to numerous technical advances over the last 40 years, not least the development of monoclonal antibody technology. In the 1970s Kholer and Milstein developed a process whereby the fusion of antigen-exposed mouse spleen cells with mouse myeloma cells, followed by clonal selection, generated unlimited supplies of highly specific and clonally-derived antibodies.[2] They immediately recognised and commented on the therapeutic potential of this technology. However, substantial barriers to the clinical exploitation of mABs remained, not least immunogenicity of murine antibodies in humans.[3] Subsequent developments, using the nascent recombinant DNA technology of the 1970s and 80s, saw the splicing of human and rodent antibody sequences[4,5] to the point where hypervariable regions of human light and heavy immunoglobulin (Ig) chains could be substituted with antigen specific hypervariable regions of rodent origin prior to transfection into melanoma cell lines and clonal selection.[6] At the time of writing there are 31 FDA-approved monoclonal antibodies, 12 of which are used in oncology, either therapeutically or for imaging.[7]

The period between the end of the last century and the beginning of the current one has seen great improvements in the management of breast cancer, to the extent that an increased incidence in Europe has been accompanied by a decrease in mortality.[8] The decrease in mortality is attributable to the introduction of screening programs in a number of nations and improvements in therapy resulting from improved understanding of the molecular etiology of the disease. However, breast cancer is the most commonly occurring cancer in women, remains the second most common cause of death from cancer overall and is the most common cause of death from cancer in women aged between 20 and 59.[9] It is a heterogeneous disease and elements of this heterogeneity have been exploited to develop therapeutic options. For example ~70% of breast cancers are estrogen and/or progesterone receptor positive. Many of these tumours are dependent on estradiol for survival and proliferation, and therefore susceptible to inhibition of proliferation by small molecule estrogen signalling antagonists.[10] Her2 is over-expressed, due to gene amplification, in approximately 25% to 30% of breast cancers and is associated, in the absence of targeted therapy, with a poorer prognosis than Her2-negative tumours.[11] A 185 kDa receptor tyrosine kinase (RTK), Her2 in common with other RTKs has an extracellular domain, a transmembrane domain and a cytoplasmic kinase domain.[12,13] In contrast to other RTKs no extracellular ligand for Her2 has been demonstrated. Despite the lack of identified ligand, Her2 is still capable of mediating intracellular proliferative and pro-survival signalling via heterodimerisation with other members of the ErbB family of receptors and is believed to be the preferred binding partner of EGFR (Her1), Her3 and Her4.[14,15] This may be of particular importance for the facilitation of ligand driven Her3 signalling, as Her3 has limited intrinsic tyrosine kinase activity and requires the activity supplied by the heterodimeric partner.[16,17] Of the potential combinations of ErbB dimers the Her2/Her3 heterodimer is believed to be the main oncogenic dimer.[18] It has also been hypothesised that ligand-independent homodimerisation is sufficient to drive proliferative signalling.[19] This review will discuss the metabolism and pharmacology of trastuzumab and the use of trastuzumab in the clinic.

Metabolism

The study of the catabolism of therapeutic antibodies presents a challenge to pharmacologists. Generically the degradative metabolism of small molecule drugs can be categorised as a process of enzymatic transformation of a compound to more hydrophilic product (Phase I), more amenable to direct excretion or conjugation with an endogenous molecule (Phase II) prior to excretion.[20] The metabolism of small molecule drugs predominantly, but not exclusively, occurs in the liver and excretion generally occurs via urine and faeces. Therapeutic proteins generally, and therapeutic antibodies specifically, are not typically substrates for the characterised enzymes of drug metabolism. While the mechanism of trastuzumab metabolism has not been extensively studied, it is assumed that the pathway of degradation accurately reflects that of endogenous IgG antibodies, the endpoint of catabolism being the

release of the constituent amino acids by intracellular proteolytic degradation. The mechanisms that influence the metabolism of antibodies can be classified into three broad categories (Fig. 1).

FcγR

The FcγRs—may have a role in the clearance of antibodies,[21] but they also serve as the bridge between the Ab:Ag interaction and the effector mechanisms of the Ab-mediated immune response. By analogy, the FcγR molecules are essential for some of the proposed mechanisms of action of trastuzumab and will be discussed in the context of pharmacodynamics.[22]

FcRn

In the mid 1960s two apparently disparate observations of the behaviour of IgG *in vivo* were elegantly synthesised to generate a readily testable hypothesis.[23] These observations were:

- That ingested maternal IgG was transmitted across the intestinal epithelium of neonatal mice by pinocytosis and that this transmission was saturable, with functional antibody re-entering the circulation or being degraded in the epithelium.
- That the rate of IgG catabolism increased in proportion to concentration.

Brambell and colleagues postulated that both phenomena could readily be explained by the presence of a receptor that protected IgG from proteolysis in lysosomes. This receptor, termed FcRn for neonatal Fc receptor, was initially identified in the intestine of neonatal rats[24] and subsequently in the syncytiotrophoblasts of humans[25] and is the receptor that mediates the transfer of IgG from maternal to infant circulation in rats and humans respectively. Structurally the FcRn is a membrane bound heterodimer comprised of an alpha chain, which is a member of the MHC class I family, and a β2 microglobulin chain (β2M).[24] That the FcRn is also the IgG protective receptor postulated in the Brambell hypothesis has been demonstrated definitively, at least in mice, in gene knockout experiments. Mice deficient in either β2M or the FcRn alpha chain have both a lower concentration of endogenous IgG and a more rapid clearance of radio-labelled IgG.[26–30] In humans, FcRn is expressed in placenta, heart, lung, liver, kidney, pancreas, breast, endothelial cells and dendritic cells.[25,31–33] The Fc portion of antibodies interacts with the FcRn receptor at pH6, but not at physiological pH[25] and it is likely that plasma IgG is taken into endothelial cells by fluid phase endocytosis rather than receptor-mediated transfer. Once in the early endosome the FcRn bound IgG is protected from the lysosomal proteolysis and may potentially be transferred into the tissue or returned to the circulation. While the former scenario has not been disproved the latter has been demonstrated in a Tie 2 Cre/Lox mouse model engineered to have no expression of the FcRn alpha chain in endothelial or haematopoietic cells.[30] Phenotypically this mouse model resembles both the FcRn and β2M knock-out mice with a lower concentration of endogenous IgG and increased rate of clearance of human IgG, indicating that the expression of FcRn in these tissues alone

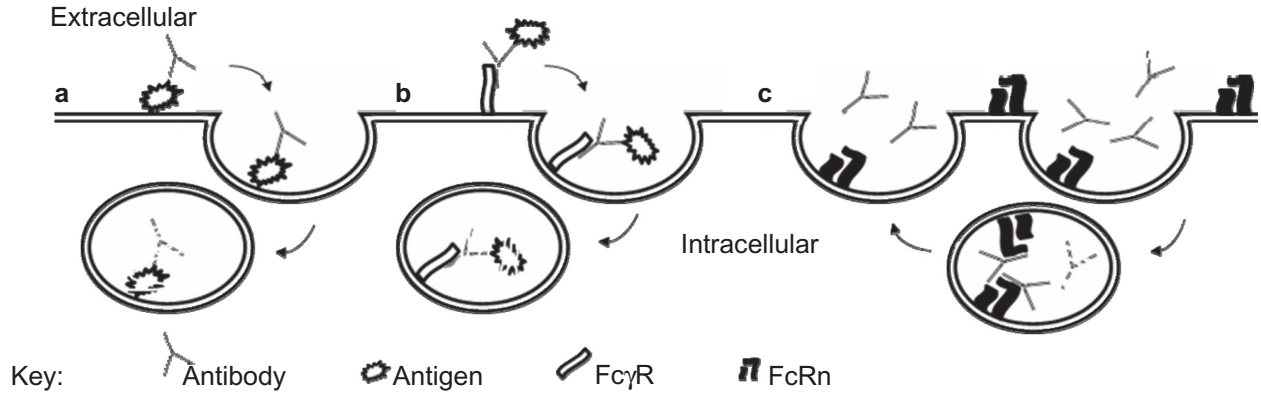

Figure 1. Receptor mediation of IgG catabolism. **a**) Antigen mediated endocytosis. Antigen on cell membrane is recognised by the antibody and the complex is then amenable to endocytosis and subsequent proteolysis in the lysosome. **b**) FcR mediated endocytosis of Ab:Ag complex. Fc Receptors on the surface of phagocytic cells interact with the antibody Fc region and may trigger endocytosis. **c**) FcRn mediated protection of IgG. Following fluid phase endocytosis and intra vesicular acidification IgG in the vesicle can interact with the FcRn receptor and is protected from proteolysis and available for recycling back to the interstitial compartment.

is sufficient to maintain the stability of IgG in blood.[30] Whether FcRn functions predominantly as a stabiliser rather than a transporter in all other adult tissues is not known. It is hypothesised that expression of FcRn in the proximal tubular cells of the kidney contributes to re-absorption of IgG from the urine[31] and it has been observed that IgG concentrations in the bile are very low despite the presence of FcRn expression on hepatocytes,[34] both phenomena that will result in reduced plasma IgG clearance. While FcRn function increases the stability of IgG and is the saturable molecule that results in nonlinear clearance of IgG at high concentrations, it is not envisaged that endothelial FcRn expression will influence the blood concentration of trastuzumab, which at maximum measured concentrations is ~5% of the IgG serum concentration, and at steady state is ~0.5%.[35,36] However, it is of interest that FcRn is expressed in normal breast and breast tumour tissue,[33] and may protect local concentrations of drug at the intended target. While FcRn should not influence the blood concentration of trastuzumab at therapeutic concentrations, under physiological conditions it is worth noting that high-dose corticosteroids do result in increased catabolism of IgG in both mice and humans[37,38] and it has been demonstrated that corticosteroids lead to a decrease in FcRn expression.[39]

Antigen-mediated catabolism

The interactions of antibody with antigen can influence the catabolism of antibodies, both by receptor-mediated endocytosis and the endocytosis of immune complexes following interaction of the antibody with soluble Ag.[21,40] This process will be saturable and may contribute to a non linear aspect of the pharmacokinetics of trastuzumab at low concentrations.[41,42] However, in contrast to the relationship between FcRn and IgG, the phenomenon of generic Ab:Ag-mediated catabolism cannot be extrapolated to the influence of membrane or soluble Her2 on the catabolism of trastuzumab. While the interaction between trastuzumab and the Her2 antigen may influence catabolism, it is also essential for the pharmacodynamics of trastuzumab and will be discussed below.

Pharmacokinetics

Trastuzumab is currently administered clinically as a 90 minute IV infusion of a 4 mg/kg loading dose followed by 2 mg/kg every week, or by a three weekly regimen of 8 mg/kg as a loading dose followed by 6 mg/kg every three weeks. A population PK analysis of data from 476 patients extracted from previous studies, the majority of whom were treated with the weekly regimen, revealed a two compartment linear model as the most appropriate fit of the data. The model predicted a steady-state trough concentration of 66 µg/ml with clearance of 0.225 l/d, volume of distribution (Vd) of 2.95 l and a terminal half-life (t½) of 28.5 days.[43] This compares with a t½ of 23 days for endogenous IgG and the Vd corresponds to plasma volume. High levels of circulating Her2 extracellular domain (ECD), more than 4 sites of metastases and high body weight were all associated with a lower predicted plasma concentration. While these covariates were statistically significant the magnitude of the effect was small compared to the overall variability of exposure and they are unlikely to be of clinical or predictive benefit.[43] The estimated steady-state trough concentration is comparable to that seen in the three weekly schedule.[36,44,45]

The population PK analysis was in contrast to earlier observations. Although the three initial phase one studies of trastuzumab have not been published *in extenso*, the available data show that half-life tended to increase with increasing dose, from approximately 1 day in the lowest dose group (10 mg) to 2 weeks in the highest dose group (500 mg).[46] Early phase II studies with a PK element reported a mean half life of between 1.1 and 11 days, with a high level of ECD being associated with a shorter half life and lower trough concentration.[41,47,48] The first published study using the weekly weight-adjusted regimen[48] reported PK parameters that, other than the mean t½ value of 6.5 days, were consistent with the subsequent population PK model[43] with Vd of 2.7 litres and a mean trough concentration at steady state of approximately 70 µg/ml. The first dose escalation study of trastuzumab to be published included concentration-time curves over a three week period from patients administered 1, 2, 4 or 8 mg/kg. The dose concentration-time curve at the highest dose appeared to show biphasic elimination with a fast initial decrease in trastuzumab concentration over 4 days followed by a slower decrease over the subsequent 11 days. Mean estimates of half life were short compared to those predicted from the two compartment linear model developed by Bruno et al,

ranging from 2.7 days following 1 mg/kg to 10.4 days following administration of the 8 mg/kg dose. A similar non-linear mechanism was seen, with clearance which decreased with increasing dose, suggesting that the pharmacokinetics of trastuzumab were non-linear and saturable at clinically relevant doses.[42] However, when plotted against dose the clearance and t½ values appeared to plateau at 4 mg/kg, indicating trastuzumab pharmacokinetics are linear and non-saturated at higher doses, suggesting that two distinct mechanisms of distribution or elimination influence clearance.

The initial assumption that trastuzumab pharmacokinetics were non-linear and the observation that accumulation can occur for up to 32 weeks before steady-state is reached, led to the hypothesis that a longer duration dosing schedule, more convenient for the patient, could be administered without lowering exposure to the drug. The three-weekly schedule results in a higher peak and lower trough concentration than seen with the weekly schedule, but overall exposure to drug and efficacy are comparable.[36,44] The effectiveness of the three-weekly schedule is more likely to be due to the t½ of 28.5 days than non-linear PK.

There are no apparent pharmacokinetic drug interactions between trastuzumab and cyclophosphamide, cisplatin, gemcitibine, paclitaxel or lapatinib.[43,44,46,49–51] With regard to the potential interaction with other monoclonal antibodies, a phase I trial of trastuzumab in combination with the anti-Vascular Endothelial Growth Factor (VEGF) monoclonal antibody bevacizumab reported that the pharmacokinetics of either agent were unaltered by co-administration.[52]

Mechanism of Action

The mechanism by which trastuzumab exerts an anti tumour activity is complex and likely to be multifactorial. The question of the clinical relevance of proposed mechanisms of action is also complicated by differences in the response of tumour cells to trastuzumab *in vivo* and *in vitro*. For example, while trastuzumab-induced apoptosis has been observed in clinical tissue and xenografts,[53,54] it is not commonly observed in tissue culture models. All proposed mechanisms are predicated on the interaction of the complementary determining region (CDR) of the antibody with the antigen, and the development of trastuzumab as an antibody specifically targeting Her2 can be viewed as a success. However, the complexity of trastuzumab's mechanism of action lies in the downstream consequences of the Ab:Ag interaction, and can be categorised as those that are immunologically mediated and those that are antagonistic of signalling pathways.

Immunological mechanisms

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immunological process whereby the cytolytic activity of a subset of lymphocytes, natural killer (NK) cells, is targeted to the destruction of host tissues by target-specific antibodies (Fig. 2). The process is

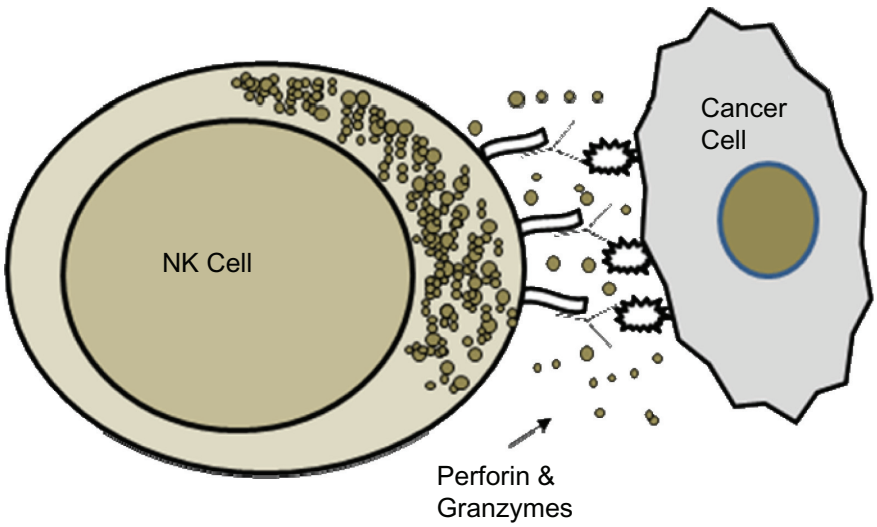

Figure 2. ADCC mechanism: Antibodies of the IgG isotype (Trastuzumab) bind the antigens (Her2) on the surface of target cell. Fc gamma Receptors (FcγR) on the NK cell recognizes the Fc region of the bound antibody on the target cell. Cross-linking of the FcγR on the NK cells promotes the release of Perforin and Granzymes, which induce apoptosis in the target cell.

mediated by the interaction of the antibody CDR with the antigen on the target cell surface and the interaction of the Fc portion of the antibody with the FcγRIII receptor on the surface of the NK cell. The formation of this interaction then triggers the release of cytotoxic effector proteins from the NK cell and the death of the target cell.[55] The role of ADCC in the efficacy of trastuzumab was first suggested *in vitro* as part of the initial characterisation of the drug[56] and has been subsequently demonstrated in murine models, *ex vivo* and in clinical samples. For example, trastuzumab prevents the growth of Her2-positive BT474M1 explants in wildtype nude mice, but this inhibition is abrogated in FcγR –/– animals of the same background.[57] Administration of trastuzumab enhances *ex vivo* ADCC activity in the neoadjuvant[58,59] and metastatic setting[60] and the induction of ADCC is associated with improved response. An increase in tumour-associated NK cells has also been observed in Her2-positive primary breast tumours in trastuzumab treated patients compared to controls not treated with trastuzumab.[22] The FcγRIIIa gene contains a single nucleotide polymorphism that results in a substitution of a valine for a phenylalanine at residue 158. There are suggestions that breast cancer patients who are homozygous for the valine genotype have increased ADCC activity and prolonged progression free survival when treated with trastuzumab.[59,61]

The clinical relevance of other antibody mediated immune effector mechanisms is uncertain. Low affinity anti-Her2 CD8+ T cells have been identified and can be clonally expanded *ex-vivo*. There is *in vitro* evidence that trastuzumab enhances the cytolytic activity of these cells, via internalisation of Her2 and increased expression of Her2 epitopes in the context of Class I MHC, but the clinical significance of this is unknown.[62] It has also been demonstrated that while trastuzumab can recruit complement to the Her2-positive cell surface this has no effect on cell death.[63]

Cell signalling mechanisms

The growth of Her2-positive cells is inhibited *in vitro* by trastuzumab and this growth inhibition is concomitant with inhibition of the pro-survival and proliferative PI3K/AKT and MAPK signalling pathways.[64–66] Of these two pathways, *in vitro* data suggest that the PI3 K/AKT axis is the most important. It has been demonstrated that introduction of constitutive AKT activity by transfection is sufficient to abrogate trastuzumab-mediated growth inhibition,[67] cell-cycle arrest and differential gene expression.[68] There is also evidence that functional PTEN, a phosphatase that antagonises PI3 K signalling, is essential for trastuzumab-mediated growth inhibition.[69,70] The lack of trastuzumab-induced apoptosis in cell line models is surprising in light of the observed inhibition of PI3 K signalling. In contrast, increased levels of apoptosis are observed in clinical tumour samples from patients treated with trastuzumab, but paradoxically this increase is seen without an increase in AKT phosphorylation in the same samples.[53] Another study has revealed an association between poor survival outcome in patients treated with trastuzumab and a potential high PI3 K activity phenotype, characterised by low PTEN expression or PI3 K mutation conferring constitutive activation.[70]

Anti-angiogenesis

Angiogenesis is the formation of new blood vasculature in response to low oxygen tension or angiogenic signalling pathways. It is considered a hallmark of cancer and is necessary for the growth of solid tumours beyond the size dictated by the diffusion of oxygen through the tissue.[71] Both low oxygen and the PI3 K/AKT signalling pathway can mediate an increase in the activity of the Hypoxia Inducible Factor-1 transcription factor and the subsequent induction and down-regulation of pro and anti angiogenic factors respectively.[72,73] These include VEGF which is a potent inducer of endothelial proliferation, and the anti angiogenic factor thrombospondin-1. Her2 expression has been shown to be correlated with VEGF expression in breast tumours.[74] This association appears to be mediated via the PI3 K/AKT signalling pathway[74,75] and induction of VEGF can be abrogated by trastuzumab *in vitro*.[76] Treatment with trastuzumab of mice bearing established tumours from xenografts of Her2-positive cells results in normalisation and decreased density of tumour vasculature.[77]

Inhibition of Her2 shedding

Her2 is cleaved by a metalloproteinase, ADAM10, to yield the released extra-cellular domain found in

plasma and a 95 kDa fragment that is the membrane-bound internal kinase domain.[78,79] In the pre-trastuzumab era expression of the p95 fragment was associated with poor prognosis.[80] Trastuzumab inhibits the cleavage of Her2 in SKBR3 and BT-474 cells *in vitro*.[81]

Early Clinical Studies

The early clinical development of trastuzumab, unlike the vast majority of anti-cancer agents, has been characterized by the exclusive focus on a particular subset of patients, i.e. the Her2-positive breast cancer patients. Since the first three phase I trials, patients have been pre-selected on the basis of Her2 expression in tumours. In these initial studies the authors failed to reach a maximum tolerated dose (dose range from 10 to 500 mg IV weekly) and none of the four reported serious adverse events was considered to be related to trastuzumab treatment.[46] Four subsequent phase II trials evaluated the activity of weekly administration, both as monotherapy and in combination. In particular, a first study of trastuzumab given as single-agent at a flat dose of 100 mg weekly with a loading dose of 250 mg showed an overall response rate (ORR) of 11.6% in 46 patients with pre-treated (median of 2 prior regimens) metastatic tumours expressing Her2 in more than 25% of cells (more than 50% of cells in 85% of cases).[36] A second combination study of trastuzumab (same schedule as above) and cisplatin (75 mg/m^2 every 28 days) showed an interesting ORR of 24% in a series of 37 heavily pre-treated patients. In this trial tumours were required to have evidence of over-expression (2+ or 3+) of Her2 as determined by immuno-histochemistry.[41] A third, much larger, multicentre trial evaluated the activity of a single-agent, weight-based weekly regimen (loading dose 4 mg/kg followed by 2 mg/kg) in 222 pre-treated patients. The ORR assessed by an independent response evaluation committee was 15% in the intent-to-treat population and the most clinically significant adverse event was cardiac dysfunction, occurring in 4.7% of cases.[48] Finally, a randomized trial conducted in previously untreated patients demonstrated the equivalence in terms of activity and safety of two different weekly schedules (2 mg/kg or 4 mg/kg). Most interestingly, a retrospective correlative analysis of Her2 gene amplification by fluorescent *in situ* hybridization (FISH) showed that objective remissions (26%) were almost entirely confined to FISH-amplified cases: ORR was in fact 34% in 79 FISH-positive cases and only 7% in 29 FISH-negative patients.[82] Taken together, the results of these initial clinical studies prompted the manufacturer and the researchers to broaden their evaluation within controlled settings.

Efficacy

Metastatic setting

In 2001 a pivotal randomized phase III trial provided evidence of trastuzumab efficacy in combination with chemotherapy in patients with previously untreated, Her2-positive, metastatic breast cancer.[83] Patients received chemotherapy (doxorubicin/cyclophosphamide or paclitaxel) either alone or in combination with the antibody. The addition of weekly trastuzumab conferred a clinically-significant increase in time to disease progression (4.6 vs. 7.4 months), ORR (50% vs. 32%), duration of response (9.1 vs. 6.1 months) and median survival (25.1 vs. 20.3 months). A second randomized trial of docetaxel alone or with trastuzumab achieved similar results.[84] Concomitantly with these large controlled trials two important phase II studies showed that a three-weekly schedule (8 mg/kg loading dose followed by 6 mg/kg), in combination with paclitaxel or as monotherapy, was able to achieve the same results in terms of response rates and plasma trough levels as the standard weekly regimen.[36,44] Beside the taxanes, other chemotherapeutic agents, such as vinorelbine, capecitabine and carboplatin have been tested in combination with trastuzumab in various non-randomized and randomized trials. The combination with vinorelbine, in particular, has been compared in a small randomized phase II trial with a weekly regimen of trastuzumab plus a taxane (either paclitaxel or docetaxel) and the results of this comparison revealed equivalence between the two arms in terms of efficacy and safety.[85]

A recent review of a large mono-institutional cohort of patients has shown that, despite the well-documented negative prognostic influence of Her2 over-expression, Her2-positive metastatic breast cancer patients treated with trastuzumab can achieve a better overall outcome than Her2-negative patients.[86]

Adjuvant setting

When used as adjuvant treatment for early stage Her2-positive breast cancers, trastuzumab has been consistently demonstrated to reduce the risk of recurrence by 30 to 50%. Table 1 shows the major findings of four large and two smaller randomized trials in terms of efficacy and toxicity, along with some notable differences with regard to various aspects, including the size of patient population, duration of treatment, type of associated adjuvant chemotherapy, trastuzumab schedule and timing of administration.

In particular, the large HERA trial[87–89] adopted a rather pragmatic approach, encompassing a wide range of adjuvant chemotherapy regimens in its inclusion criteria. This feature makes the study highly representative of clinical practice and confers a wide applicability to the results. Trastuzumab every three weeks was administered for one year after the completion of chemotherapy in this setting and a third randomization arm (data not released yet) evaluated a two-year treatment duration. The NSABP B-31 and the NCCTG-N9831 trials[90,91] reflect the popularity of the AC → P schedule (Doxorubicin/Cyclophosphamide followed by Paclitaxel). They both focused on the concomitant administration of paclitaxel and weekly trastuzumab, which had been shown to be synergistic, but the NCCTG-N9831 researchers also considered a comparison with a sequential arm. The BCIRG 006 study[92,93] aimed to specifically evaluate the efficacy of trastuzumab in association with an anthracycline-free regimen (Docetaxel/Carboplatin) or with a more standard regimen (Doxorubicin/Cyclophosphamide followed by Docetaxel).

As can be seen from a cross-comparison of the efficacy results reported in Table 1, the concomitant administration of trastuzumab (NSABP B-31/NCCTG-N9831 joint analysis and BCIRG 006) with chemotherapy seems to confer a slightly larger benefit in terms of efficacy, compared with the sequential administration, although these data could be biased by different durations of follow-up and different rates of treatment cross-over among the studies. On the other hand, incidence of cardiotoxicity appears slightly higher in the trials of concomitant trastuzumab.

Table 1. Reported trastuzumab adjuvant trials.

Study	Most recent update	Pts no.	Stage	Design	Trastuzumab administration	Pts with LVEF drop (%)
HERA	Gianni et al,[89]	5102	Node-pos and node-neg (tumour size > 1 cm)	Ch	–	0.8*
				Ch → H	3W; total 1 year	3.7*
				Ch → H	3W; total 2 years	NR
NSABP B-31	Perez et al,[91]	2006	Node-pos	AC → P	–	NR
				AC → PH	W; total 1 year	NR
NCCTG N9831	Perez et al,[91]	3505	Node-pos or high risk Node-neg	AC → P	–	NR
				AC → PH	W; total 1 year	
				AC → P →H	W; total 1 year	NR
BCIRG 006	Slamon et al,[93]	3222	Node-pos or high risk Node-neg	AC → T	–	11**
				AC → TH	W with T, then 3W; total 1 year	19**
				TCaH	W with TCa, then 3W; total 1 year	9**
FinHER	Joensuu et al,[95]	232	Node-pos or high risk Node-neg	T-FEC or V-FEC	–	10.5***
				TH-FEC or VH-FEC	W with T or V; total 9 weeks	6.8***
FNCLCC-PACS 04	Spielmann et al,[96]	528	Node-pos	FEC or ET	–	2.6****
				FEC or ET → H	3W; total 1 year	11.1****

The small FinHer trial[94,95] provided interesting findings on the potential efficacy of weekly trastuzumab administered with chemotherapy (vinorelbine or docetaxel) for a very limited period (9 weeks).

It is noteworthy that a recent medium-sized study of adjuvant trastuzumab failed to demonstrate a clear advantage in disease-free survival and overall survival.[96] The authors identified a potential explanation for this result in the rather high rate of treatment discontinuation, mainly due to cardiac toxicity.

Neo-adjuvant setting

Many non-randomized studies have demonstrated the extreme activity of trastuzumab in association with chemotherapy in the pre-operative setting.[97–99] A very promising randomized phase II trial confirmed the efficacy of this approach,[100] achieving an unprecedented rate of pathological Complete Remissions (pCR) (66.7% for chemotherapy plus trastuzumab versus 25% for chemotherapy alone) in a limited series of patients with operable primary breast cancer. Recently, a large randomized phase III trial has evaluated the combination of trastuzumab with a poly-chemotherapy regimen in the setting of locally-advanced breast cancer, achieving a benefit in terms of pCR rate and a substantial prolongation in event-free survival, with an acceptable cardiac safety.[101] In this study, which included non-inflammatory T4, inflammatory and N2 or ipsilateral tumours, trastuzumab was administered both pre-operatively (in association with doxorubicin, paclitaxel and cyclophosphamide/methotrexate/fluorouracil) and post-operatively, to complete one year of treatment. Importantly, 19% of patients in the control arm crossed over to adjuvant trastuzumab after the results of the major randomized trials became available. Despite the exciting results in terms of pCRs many clinical questions, including choice of chemotherapeutic drugs, combination with anthracyclines and duration of treatment, remain unsolved and use of pre-operative trastuzumab in clinical practice is still not widely established.

Grade III–IV cardiotoxicity + deaths (%)	Cardiac deaths (no.)	Median follow-up (months)		DFS (HR)	P	OS (HR)	P	Cross-over (%)
0.1	1			ref	–	ref	–	
0.8	0		48	0.76	<0.0001	0.85	0.1087	52
NR	NR			NR	–	NR	–	
0.9	1	JOINT ANALYSIS AC → P		ref	–	ref	–	
3.8	0							
0.3	1		35					21
2.5	0	AC → PH		0.48	<0.00001	0.65	0.0007	
0.7	0			ref	–	ref	–	
2	0		65	0.64	<0.001	0.63	<0.001	2.1
0.4	0			0.75	0.04	0.77	0.038	
1.7	0			ref	–	ref	–	
0.9	0		62	0.65 (DDFS)	0.12	0.55	0.094	0
0.4	0			ref	–	ref	–	
1.5	0		47	0.86	0.41	1.27	NR	0

Abbreviations: Ch, any chemotherapy; A, adriamycin; C, cyclophosphamide; H, herceptin; P, paclitaxel; T, docetaxel; Ca, carboplatin; F, fluorouracil; E, epirubicin; V, vinorelbine; W, weekly; 3W, 3-weekly; NR, not reported; LVEF, left ventricle ejection fraction; DFS, Disease Free Survival; HR, Hazard Ratio; OS, Overall Survival; DDFS, Distant Disease-Free Survival; Cross-Over, Proportion of patients in the observation arm who crossed-over to the trastuzumab arm after 1st release of efficacy data.
Note: *significant LVEF drop, **LVEF drop > 10%, ***LVEF drop > 20%, ****LVEF < 45% and/or 45%–49% + ≥15% decrease.

Predictors of efficacy

Trastuzumab was originally developed and is routinely used only in Her2 over-expressing and/or amplified breast tumours and the implementation of the recent American Society of Clinical Oncology/College of American Pathologists guidelines on Her2 testing is contributing to improve patient selection for this targeted treatment.[102] A retrospective analysis of Slamon's pivotal phase III trial of trastuzumab in advanced patients confirmed that the benefit was limited to patients with Her2-amplified tumours.[83,103] A recent exploratory analysis from the NSABP group suggested that the advantage of adjuvant trastuzumab may not be limited to Her2-positive cases,[104] but it must be highlighted that these results emerge from a central re-testing of samples that were considered Her2-positive in peripheral laboratories. Many randomized trials have been retrospectively evaluated for the potential role of estrogen receptor (ER) and progesterone receptor (PR) expression in trastuzumab efficacy. All reports consistently show that the benefit derived from use of trastuzumab is similar in hormone-receptor positive and in hormone-receptor negative patients, in advanced,[105] adjuvant[87,90] and neo-adjuvant setting.[101,106] Likewise, the co-expression of EGFR and Her2, evaluated in a limited series of advanced patients, does not seem to predict any additional effect.[107] Other biologic markers potentially implicated in response to therapy will be discussed in the context of trastuzumab resistance.

Safety

Trastuzumab is generally well tolerated and the most frequent acute adverse event is a hypersensitivity-like infusion reaction.[108] However, the occurrence of cardiac dysfunction can be a major concern in a minority of patients and definitive trastuzumab discontinuation following cardiotoxicity might favour recurrence or progression of the disease.

The potential mechanisms of this significant side effect have been studied recently. There is abundant laboratory evidence that Her2 has an important role in cardiomyocyte development and function. Her2 in fact functions as a co-receptor for Her3 and Her4 and their peptide ligands, the neuregulins, all of which are expressed in cardiac tissue. According to one of the most accepted models, the inhibition of Her2 signalling leads to mitochondrial dysfunction and ATP depletion and, in turn, to reduced contractility of the cardiomyocyte.[109,110]

Clinically, trastuzumab-related cardiotoxicity can span a range of various clinical situations, from asymptomatic variations in the heart contractility (measured as Left Ventricular Ejection Fraction—LVEF) to severe and sometimes fatal cardiac failure, and has some peculiar features such as the absence of ultrastructural changes in the heart muscle and a general tendency to reversibility.

In the pivotal registration trial,[83] congestive heart failure (CHF) was reported in an unexpectedly high proportion of patient: its incidence in the paclitaxel plus trastuzumab and the anthracycline plus trastuzumab arms was respectively 13% and 27%. In the four large adjuvant trials,[89,91,93,111] where cardiac eligibility criteria were stringent and trastuzumab was interrupted or discontinued in response to the development of cardiac dysfunction, the incidence of grade III-IV CHF ranged from 0.4 to 3.8% (Table 1). Exhaustive analyses of clinical data in both the advanced and early settings have shown that older age, pre-existing cardiac diseases or risk factors and use of anthracyclines can all contribute to an increased likelihood of developing trastuzumab-related cardiotoxicity.[112]

In clinical practice, a widely accepted approach to minimize this potentially life-threatening side-effect includes a baseline assessment of LVEF followed by regular on-treatment monitoring, possible interruption/discontinuation of trastuzumab and the introduction of appropriate cardiac medication (e.g. Angiotensin-Converting Enzyme Inhibitors).[113]

Controversial Clinical Issues

Monotherapy or combination

Trastuzumab has been shown to be effective both as a single agent and in combination with chemotherapy. In order to define the best treatment strategy, two similar randomized studies have recently compared a 'sequential' therapy with the combination of trastuzumab plus docetaxel as first-line treatment in two series of patients with advanced breast cancer.[114,115] In the first trial the sequential arm consisted of trastuzumab three-weekly, followed by docetaxel alone (100 mg/m^2) at progression, whereas in the latter trastuzumab monotherapy was given weekly and docetaxel (60 mg/m^2) was introduced at progression without suspending the

antibody. It should be noted that in the first paper the vast majority of patients had visceral metastases and in the second trial patients with only bony disease were excluded from the trial. In terms of safety, no substantial difference emerged between the sequence and the combination, whereas ORR and progression-free survival (PFS) were substantially higher in the combination arm compared to the monotherapy stage of the sequential arm in both studies. The subsequent introduction of docetaxel did not seem to completely fill the gap in terms of efficacy, since the hazard ratio for overall survival (OS) was 2.72 ($P = 0.04$) in favour of the combination in Inoue's work[115] and median OS were 20.2 months in the sequential arm and 30.5 months for the combination in Bontenbal's trial,[114] although this difference did not achieve statistical significance. Taken together, these results seem to favour the combination regimen, at least in this subset of advanced patients with mostly visceral disease.

Combination with hormone treatments

A recent study has shown that the addition of trastuzumab to anastrozole in the treatment of metastatic patients with both Her2-positive and Hormone Receptor-positive tumours confers a significant benefit in terms of ORR (6.8% vs. 20.3%) and PFS (2.4 months vs. 4.8 months), without an evident impact on safety.[116] As a result, the European Medicines Agency approved the use of the combination of trastuzumab with an aromatase inhibitor in this setting. Although many oncologists argue that a traditional combination of trastuzumab and chemotherapy would better control this subset of tumours, the hormonal/trastuzumab approach could be useful where chemotherapy is not feasible or in patients with minimal bony metastatic disease, in order to delay the introduction of traditional cytotoxic drugs.

Combination with anthracyclines

The combination of trastuzumab with doxorubicin was initially found to cause an unacceptable rate of heart failure[83] and was never introduced in clinical practice, although its synergism was proved in '*in vitro*' and clinical studies. In order to overcome this drawback, many authors have evaluated the use of alternative, less cardiotoxic anthracyclines. In particular, liposomal doxorubicin, both in the pegylated and in the non-pegylated form, has been shown to be active and relatively safe in association with trastuzumab in several phase II studies.[117–119] On the other hand, conflicting results have been reported with regard to cardiac safety of trastuzumab plus epirubicin.[120,121] Although promising early evaluations have been reported, the simultaneous use of anthracyclines and trastuzumab is currently limited to clinical trials.

Trastuzumab and CNS metastases

Her2-positive breast cancer patients have a higher risk of developing metastases to the central nervous system (CNS) and treatment with trastuzumab does not seem to alter this risk.[122,123] Trastuzumab has been shown to penetrate the blood-brain barrier (BBB) to a very limited extent, with cerebrospinal fluid (CSF) concentrations approximately two orders of magnitude lower than serum concentration, despite a possibly impaired BBB.[124,125] Nonetheless, several retrospective studies have shown that trastuzumab is able to prolong survival in patients who develop CNS metastases,[126,127] compared with control groups of Her-2 negative patients and Her2-positive patients who did not receive trastuzumab.[123] Whole brain radiation treatment (WBRT) is generally considered to be the standard of care for this subset of patients and is able to improve the CSF:serum trastuzumab concentration ratio by approximately five-fold.[124] Since different agents can achieve objective remissions in the CNS, both alone and in combination with WBRT (i.e. temozolomide, capecitabine, lapatinib, topotecan),[128] many oncologists believe that a reasonable strategy could be to continue trastuzumab in order to control metastases outside the brain and to administer active therapies to control CNS disease. New approaches in order to deliver trastuzumab directly to the CNS are under investigation.

Use in Imaging

Trastuzumab has been evaluated as an imaging tool in the context of nuclear medicine techniques. In particular, the development of indium-111 (^{111}In)—labelled trastuzumab and its use in Single Photon Emission Computed Tomography (SPECT) have shown potential value for clinical staging of Her2-positive breast cancers in terms of identification of new tumour lesions, but when the myocardial distribution of the drug was evaluated, the authors

where not able to demonstrate any predicting ability with regard to trastuzumab-related cardiotoxicity.[129] In order to overcome the limited spatial resolution of ^{111}In-DTPA-trastuzumab SPECT imaging, trastuzumab has been successfully radiolabelled with Zirconium-89 (^{89}Zr) and its uptake has been detected by Positron Emission Tomography (PET), achieving a higher spatial resolution and a better signal-to-noise ratio.[130]

In another approach the Fragment antigen-binding F(ab')2 of trastuzumab was labelled with Gallium-68 (^{68}Ga), and detected with PET.[131] Both the whole antibodies and the antibody fragments, which are characterized by a faster clearance, have allowed non-invasive imaging of the pharmacodynamics of anti-cancer agents as part of pre-clinical studies in animal models[132,133] but they are not currently used in clinical practice.

Resistance

Only 15 to 30% of Her2-positive metastatic breast cancers respond to trastuzumab administered as a single agent, whereas the response rate when used in combination with taxanes is approximately 60 to 70%. This suggests that a proportion of patients are primarily resistant to trastuzumab. Moreover, virtually all patients with advanced disease treated with trastuzumab will have recurrent disease, indicating an acquired resistance. Furthermore, in the adjuvant setting there are a significant proportion of patients who suffer from recurrent disease despite having received trastuzumab.

These clinical observations have prompted several researchers to examine in depth the biological mechanisms of trastuzumab resistance.[134]

Two Her2 isoforms, the first lacking the extracellular domain (p95HER2) and the second lacking a small portion of the juxta-membrane extracellular domain (HER2Δ16), were described to harbour enhanced transforming activity[135–137] and recent *in vitro* and clinical works show that these variants are involved in trastuzumab resistance.[138,139] Increased signalling from members of the ErbB family of receptors and from Insulin-like Growth Factor Type 1 Receptor (IGF-1R) has been observed by Motoyama et al[66] and Natha et al[140] in the context of trastuzumab-resistant cells and these initial data have been confirmed by the recent demonstration of a heterotrimeric (Her2/Her3/IGF-1R) complex which would disrupt effective binding of trastuzumab to Her2 and activate unique down-stream signalling.[141] Activation of the PI3K pathway has been shown to be important in trastuzumab downstream effects and can be regulated by Phosphatase and Tensin homolog (PTEN). *In vitro* and clinical studies suggest that activation of this pathway and loss of PTEN function may contribute to trastuzumab unresponsiveness.[142] The membrane-associated glycoprotein MUC4, which may hinder trastuzumab binding to its epitope on Her2, has also been proposed as an additional marker of resistance.[143] Some of these pathways and mechanisms are currently being evaluated as potential therapeutic targets in pre-clinical and clinical studies.[144]

A large trial compared the use of capecitabine monotherapy with the combination of capecitabine and lapatinib, a Her2 and EGFR associated tyrosine kinase-inhibiting small molecule, in trastuzumab-resistant patients. With this combination the authors obtained an advantage in terms of ORR and PFS[145] and lapatinib is now approved for the treatment of Her2-positive breast cancer.

Use of trastuzumab beyond progression of disease

Since limited second-line options are currently available in the clinic for patients who experience disease progression after first-line trastuzumab-containing therapies, many oncologists have been using trastuzumab following disease progression for several years, mostly in combination with various chemotherapeutic agents. This choice was based on trastuzumab's known chemo-sensitizing property and supported by data from a number of retrospective series.[146–148] Recently, a randomized study has assessed the efficacy of continuation of trastuzumab in association with capecitabine[149] and has confirmed the clinical benefit of this option, at least in terms of PFS. A second controlled study, which assessed the efficacy of continued trastuzumab in association with lapatinib versus lapatinib alone, demonstrated an advantage for the combined anti-Her2 therapy.[150]

These observations, along with several *in vitro* experiments,[151] seem to suggest that in patients who lose clinical responsiveness, the anti-tumour effect of trastuzumab is in part retained and can be exploited in the context of different combination regimens.

Place in Therapy

Trastuzumab has a widely-established role in the routine treatment of early and advanced Her2-positive breast cancer patients. In the adjuvant setting, trastuzumab is commonly administered for a total duration of one year, with variations regarding the timing and schedule of administration (Table 1). In metastatic patients, a combination therapy with chemotherapeutic agents is usually preferred: in particular, taxanes are the most extensively-studied partner drugs in this context. However, combination with different agents, including aromatase inhibitors, is feasible and effective. The pharmacodynamic and pharmacokinetic equivalence of the weekly and 3-weekly regimen allows clinicians to choose the most convenient schedule, taking into account patients' preference, associated chemotherapy regimen and hospital facilities. Furthermore, the safety and tolerability of this treatment allow a prolonged treatment in the vast majority of cases. Besides the introduction of lapatinib in trastuzumab-resistant patients, the development of new drugs and combinations which can potentially refine the treatment of Her2-positive cancers is a wide and exciting field of investigation.

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