Current Perspective – Trastuzumab

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Over the last decade, the increased understanding of the molecular basis of cancer has resulted in the development of a wide range of effective targeted therapies which has expanded the armamentarium of active drugs available to the oncologist. Trastuzumab is the first of this new generation to be successfully applied in breast cancer and it now plays an important part in the management of metastatic and early breast cancer. The introduction of Trastuzumab has been heralded as a successful example of modern "bench to bedside" development – from experimental models to translational experiments to clinical trials in the metastatic then adjuvant setting. This process has been made possible by worldwide collaboration between laboratory scientists, the pharmaceutical industry and clinical trialists.

The HER-2/neu (HER2) oncogene, also called c-erbB2, was discovered in the 1980's and is a member of the erbB-like oncogene family. It is related to, but distinct from, the epidermal growth factor receptor (EGFR) and shares a role in the regulation of cell proliferation. HER2 is over-expressed in 15-30% of breast cancers and carries an adverse prognosis.(1, 2) HER2 cell surface protein over-expression is usually caused by amplification of the HER2 gene. (3, 4) HER2 positive breast cancers represent one end of a spectrum of increasingly defined breast cancer subtypes, characterised by a high risk of recurrence and metastasis and reduced overall survival.

Trastuzumab is a recombinant humanised monoclonal antibody (IgG) directed against the HER2 extracellular domain. It is made with the hyper-variable antigen binding regions of a potent murine anti-HER2 monoclonal antibody grafted into a human IgG. Its exact mechanism of action remains unclear and appears to differ in vivo compared to in vitro. Whether or not Trastuzumab induces HER2 receptor down-regulation remains a matter of controversy. It may act by blocking HER2 receptor cleavage, inhibiting intracellular signalling pathways or even by anti-angiogenesis effects. (5) It is clear that its action is not

only cytostatic but also cytotoxic and this may be in part due to recruitment of the immune system by antibody dependent cell mediated cytotoxicity.(6) In addition to its direct mechanism of action, a theoretical synergy with chemotherapy may be important.

Standardised selection of the correct population who benefit from Trastuzumab therapy is vital and has been the subject of ongoing development. Guidelines are now well established at an international level that define HER2 over-expression (HER2 "positivity") and thus predict a high chance of sensitivity to Trastuzumab.(7) Algorithms use a mixture of immunohistochemistry (IHC) to measure the level of HER2 protein at the cell surface, and in-situ hybridisation (FISH) to look for gene amplification. FISH is widely held as the gold standard not just because there is a good correlation between DNA copy number and protein levels, but also because routine fixation processes mean that protein levels as measured by IHC may not always be an accurate indicator of the level in vivo in the patient.

Trastuzumab in metastatic breast cancer

As a single agent Trastuzumab can produce response rates up to 35% in selected metastatic breast cancer patients. (8, 9) In vitro demonstration of additive or synergistic activity with a number of active chemotherapy drugs led to early clinical development in combination with chemotherapy. (10) Landmark phase II and phase III trials reported response rates of 50-84% using Trastuzumab in combination with standard chemotherapy (paclitaxel, docetaxel or doxorubicin, and cyclophosphamide combinations) and demonstrated improvement in time to progression, duration of response and survival compared with the same chemotherapy alone as therapy for metastatic breast cancer over-expressing HER2.(11-13) Although combinations with a taxane or anthracycline were efficacious, anthracycline containing regimens produced unexpected and limiting cardiotoxicity.

Trastuzumab after progression?

Trastuzumab has clearly revolutionised treatment for HER2 positive patients, however, half of the patients still have non-responding tumours and disease progression occurs within 1 year in the majority of cases. It remains uncertain whether further Trastuzumab either in combination with further chemotherapy or as a single agent is worthwhile. It is also unknown whether retreatment on relapse is useful for patients who were treated with adjuvant Trastuzumab. Current guidelines do not recommend further treatment options after progression on Trastuzumab. (14) There is, however, emerging evidence suggesting efficacy with further anti-HER2 therapy and many clinicians will continue Trastuzumab after progression. Continuing Trastuzumab after cancer progression is undoubtedly an attractive option for patients and their treating oncologist due to low toxicity and lack of established alternatives. The implications for those funding healthcare are however significant, with the costs of trastuzumab and its administration at around Euro 40,000 (£32,000) for 1 year of treatment.(15)

Initially the practise of continuing Trastuzumab after progression was based on preclinical studies that suggested Trastuzumab can slow down tumour growth in the presence of disease progression. Clinical evidence that does exist is largely derived from retrospective studies and includes an extension trial to the pivotal phase III trial testing Trastuzumab in combination with first line palliative chemotherapy. They suggest a reasonable safety and cardiotoxicity profile with continued treatment(16-18) and responses are at least as good when combined with second line chemotherapy compared to historical controls.(19-21) A prospective observational database has been set up by Genentech with the hope of adding to this information. (22)

More convincing data comes from the randomised trial GBG26/TBP which set out to compare capecitabine with or without trastuzumab in patients who had progressed or relapsed after any prior trastuzumab treatment. This was closed early on the recommendation of the IDMC after recruiting 156 patients. The final analysis recently reported statistically significant advantages for the combination arm with increased response rates and mean TTP of 5.6 vs 8.2 months (p=0.034). This is the strongest evidence to date supporting the role for continuing trastuzumab therapy in this situation. (23)

The small molecule Lapatinib acts by inhibiting the receptor tyrosine kinase activity of HER2 and also the EGFR (ErbB1) receptor. A pivotal trial randomised between Capecitabine monotherapy and Capecitabine in combination with Lapatinib in patients who had been previously treated with Trastuzumab. Recently published interim results reported a statistically significant improvement in the primary endpoint of time to progression (HR 0.57 (95% confidence intervals 0.43 – 0.770, p=0.00013, medians of 4.3 months versus 6.2 months respectively). This demonstrated efficacy has led to approval for use in the US, EU and Switzerland. (24, 25)

Both of these studies support ongoing anti-HER2 therapy in combination with Capecitabine in patients with progressive cancer after Trastuzumab. The level of benefit though does not appear as great as that seen when Trastuzumab was combined with anthracyclines or taxanes, and therefore there does remain some uncertainty as to the true level of benefit from continued HER2 blockade. Importantly, they only provide evidence for patients pre-treated with anthracyclines, taxanes and trastuzumab. There remains no definitive evidence guiding the use of anti-HER2 therapy in other patient groups. In particular there is no proof

that continuing Trastuzumab as a single agent is efficacious in this situation. There remains much uncertainty which clearly still needs to be addressed by good quality adequately powered trials. Prospective randomized trials called THOR (Italian) and PANDORA (multinational) are attempting to address this need – comparing second line chemotherapy with or without continued Trastuzumab – but these types of studies have always been difficult to recruit to, due in part to the expectation that continued anti-HER2 therapy is of benefit.

Ultimately we need to further develop our understanding of the mechanisms by which tumours develop resistance to Trastuzumab if they are to be overcome. Proposed mechanisms are numerous and varied but include increased cell signalling (PTEN loss, increased AKT activity), alternative cell signalling mediated by EGFR family pathways (TGF-a over-expression, neuregulin over-expression) and alternative cell signalling mediated by different pathways (VEGF over-expression, IGF1R over-expression).(5) In particular, when considering the potential benefit of an intracellular signalling blockade with small molecules such as Lapatinib, is the possible relevance of the truncated HER2 receptor, p95, which results in constitutive activation and no extra-cellular target to which an antibody such as Trastuzumab can bind. (26) In addition to Lapatinib, other agents at an earlier stage of development are also showing promise. Pertuzumab is a monoclonal antibody similar to Trastuzumab which targets a different region of the HER2 receptor. It has demonstrated activity in early phase trials when added to Trastuzumab for patients with disease progressing on Trastuzumab therapy, which incidentally provides further evidence of retained tumour sensitivity to anti-HER2 therapy even though resistance to Trastuzumab has developed.(27) Gefitinib, a small molecule tyrosine kinase inhibitor (TKI) acting against ErbB1 has also been tested, but has failed to demonstrate a significant clinical effect when

used in combination with Trastuzumab.(28) Another agent KOS-953 (17-AAG) inhibits the activity of heat shock protein 90 resulting in degradation and reduced expression of the HER2 receptor and has also produced responses in an early report from a phase II study.(29)

Recent enthusiasm has also focused on the association between vascular endothelial growth factor receptor (VEGFR) and HER2 expression in breast tumours.(30) Evidence suggests that VEGFR expression is linked to HER2 signalling and over-expression of HER2 results in induction of VEGFR.(31) Hence Trastuzumab is currently under evaluation in combination with Bevacizumab, an antibody directed against VEGF. An impressive response rate of 46% was seen in a phase II trial testing this combination,(32) although concerns exist surrounding the potential combined cardiotoxicity of these two drugs. Pazopanib is a small molecule multi-targeted TKI which also inhibits VEGF and has recently demonstrated tolerability and activity when tested in combination with Lapatinib in a phase II trial. (33)

It is now well recognised that HER2 positive breast cancer has a high rate of CNS involvement, and cerebral metastasis as the site of progression after Trastuzumab is unexpectedly common.(34-37) The particular challenge facing us now is how best to control this disease, particularly when presenting in the setting of responding extra-cranial metastasis.(38) Trastuzumab does not appear to efficiently cross the blood brain barrier, and so it is unclear if the current practise of local CNS therapy and continued Trastuzumab is optimal. Although early data suggests that Lapatinib may play a role in controlling CNS disease(39), it is not known if switching to this agent would be of any advantage.

Adjuvant Trastuzumab

The clear benefit of using Trastuzumab in patients with advanced HER2 positive breast cancer quickly led to the initiation of a series of large trials testing the hypothesis that the use of Trastuzumab in HER2 over-expressing early breast cancer could improve disease-free survival. Four large and two smaller trials have all now reported, and all but one has shown a benefit with the use of Trastuzumab in addition to adjuvant chemotherapy in both disease free survival and overall survival (table 1). In fact Trastuzumab is the first monoclonal antibody to produce a survival advantage when used as adjuvant therapy. Altogether these trials include more than 13,000 patients and provide a firm evidence base supporting its use in this setting. The only trial not to show a clear benefit was the smaller PACS04 trial, (40) where the patients randomised to Trastuzumab, as in the FINHER trial (vide infra) were a subset of patients in a larger trial whose primary objective was a chemotherapy question. Thus, although there is little doubt for the role of adjuvant Trastuzumab, the differing designs of these trials leave many questions as to the optimum timing and duration of its use.

Three North American studies (table 1) – the North Central Cancer Treatment Group (NCCTG) Intergroup N9831, the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 and the Breast Cancer International Research Group (BCIRG) 006 trials based their design on data from trials in metastatic breast cancer using a taxane-Trastuzumab combination. Using a standard sequence of anthracycline followed by taxane, they added Trastuzumab to the taxane, continuing to a year in total. The similar designs of N9831 and B-31 led to the joint analysis of their combined data. (41-43) The HERceptin Adjuvant (HERA) trial recruited from most of the rest of the world. This tested the addition of one or two years of trastuzumab given as a single agent after completion of standard chemotherapy and radiotherapy. (44)

Table 1. Large adjuvant trastuzumab trials

	N9831 / B-31 (42)	BCIRG 006 (45)	HERA (46)
	AC → Paclitaxel AC → Paclitaxel Trastuzumab (H) x	A → Docetaxel A → Docetaxel Trastuzumab x 1 Docetaxel Carboplatin (TC) Trastuzumab x 1	Chemo → Observation Chemo → Trastuzumab x 1 Chemo → Trastuzumab x 2
Chemotherapy doses	AC = doxirubicin 60mg/m^2 cyclophosphamide 600mg/m^2 B-31: paclitaxel 175mg/m^2 3 wkly x4 or 80mg/m^2 wkly x12 N9831: paclitaxel 80mg/m^2 wkly x12	AC = doxirubicin 60mg/m^2 cyclophosphamide 600mg/m^2 TC = docetaxel (T) 75mg/m^2 carboplatin AUC 6	Chemo = standard chemotherapy from a permitted list
Trastuzumab doses	4mg/kg loading dose 2mg/kg weekly	4mg/kg loading dose 2mg/kg weekly	8 mg/kg loading dose 6 mg/kg 3 weekly
Patient No.	3,969	3,222	3,401 (no trastuzumab and 1 year arms)
Median follow up	2.9 years	3 years	2 years
Disease Free Survival	73% vs. 86% at 4 years (HR 0.49 [95 % confidence interval (CI) 0.41 to 0.58])	AC-T 77% at 4 years AC-TH 83% at 4 years (HR 0.61 [95% CI 0.48-0.76]) TCH 82% at 4 years (HR 0.67 [95% CI 0.54-0.83])	74% vs. 81% at 3 years (HR 0·64 [95% CI 0·54–0·76])
Overall Survival*	89% vs. 93% at 4 years (HR 0.63 [95% CI 0.49 to 0.81])	AC-T 86% at 4 years AC-TH 92% at 4 years (HR 0.59 [95% CI 0.42-0.85]) TCH 91% at 4 years (HR 0.66 [95% CI 0.47-0.93])	92% vs. 90% at 3 years (HR 0·66 [95% CI 0·47–0·91])

Early reporting of trials led to initial concerns over data immaturity – perhaps no survival advantage would be seen? (47) In fact the combined analysis of the two US trials N9831 and NSABP B-31 reported a 33% improvement in survival after a median follow-up of two years. Analysis of the HERA trial also at a median 2 years in 2006 saw a similar 34% improvement in overall survival. (46)

The duration of treatment with Trastuzumab varies hugely between trials from 9 weeks to 2 years. One year of treatment, starting either with the taxane component of chemotherapy, or after chemotherapy, has been widely adopted as standard treatment across the world. This treatment duration remains arbitrary at present but will be addressed in due course with the maturation of data from the third arm of the HERA trial. This has not yet been released by the IDMC, but will allow us to compare 2 years to 1 year of Trastuzumab after chemotherapy.

Researchers in Finland have added a new dimension to the debate with the publication of the FinHER trial. 231 women with HER2 positive tumours were identified within a larger adjuvant chemotherapy study. They were randomised to treatment with or without Trastuzumab immediately after surgery concomitantly with the non-anthracycline part of their chemotherapy. 3-year recurrence-free survival was better in those who received Trastuzumab (89 percent vs. 78 percent; P=0.01) with a halving of the risk of recurrence achieved with only nine weeks Trastuzumab. It resulted in women with HER2 positive breast cancer having the same prognosis as those with HER2 negative breast cancer in the same study. (48)

The FINHER data suggest a shorter duration could be just as effective as the longer durations tested in other trials. It is perhaps no surprise that in both the UK and France, it is government money that supports two similar trials with a planned joint analysis to address this question. In the UK the Health Technology Assessment have funded a trial called Persephone which tests a shorter duration of Trastuzumab, comparing 1 year to 6 months of treatment after chemotherapy. Detailed health economic data collection within Persephone will add a wealth of information to the ongoing debate surrounding the cost of new expensive adjuvant drug treatments. The PHARE trial in France, sponsored by Institut National de Cancer, follows a very similar design, and having opened earlier has already recruited over 1,800 patients at 12 months which represents over half of its targeted accrual. In addition, another trial from Finland, SOLD, is comparing the 9 week schedule used in FINHER with a full year's post-op trastuzumab as used in the HERA trial. This trial is similar in concept to a small randomised phase II study from ECOG, E2198.(49) Whilst the trial was designed to address a safety, not an efficacy question, it included a very similar number of HER2 +ve patients as FinHER, and the 5-year outcome data from this study suggests little additional benefit from the maintenance Trastuzumab after completion of adjuvant chemotherapy plus Trastuzumab.

An equally important, and perhaps more challenging, question addresses the optimal timing of Trastuzumab in relation to chemotherapy. Preclinical experiments suggest that concurrent administration is necessary to produce cytotoxicity, whereas sequential administration may be, at best, cytostatic.(50) Administration with the taxane portion of accepted anthracycline-taxane sequences is possible and efficacious, but trials have produced conflicting results between this and consecutive treatment. The N9831 trial had a third arm where Trastuzumab was given for one year, starting after chemotherapy. In the early analysis of

N9831 this arm appeared much less effective, in contrast to the HERA trial where it almost halves the rate of recurrence. It is interesting to note that in the HERA trial, the 26% of patients who received taxane-based chemotherapy had an apparently lower benefit from the subsequent year's Trastuzumab. However, neither of these data have been updated, so one cannot be sure that it is more effective to commence the Trastuzumab with the taxane, and this has the added disadvantage of starting the treatment only 3 weeks after a dose of anthracyclines. Indirect comparisons of the cardiac toxicity data between the two approaches suggests a lower rate of cardiac toxicity when the Trastuzumab is given further away from the last anthracycline dose.

The main barrier to concurrent administration of Trastuzumab and chemotherapy is the high risk of cardiotoxicity with an anthracycline-Trastuzumab combination.(12) The novel schedule of platinum-taxane-Trastuzumab suggested strong synergy in pre-clinical studies and with efficacy being confirmed in metastatic breast cancer patients. (51). Therefore the BCIRG006 included a third non-anthracycline containing arm aiming to reduce cardiac toxicity and build on preclinical data. This proved to be almost as effective and definitely less cardiotoxic compared to the anthracycline containing arms. Many argue that this is the most pragmatic treatment strategy in light of the absence of data confirming long term cardiac safety with the use of both an anthracycline and a taxane in the adjuvant treatment schedule. There is, however, convincing evidence suggesting that patients with HER2 positive tumours derive particular benefit from anthracyclines compared to patients with HER2 negative tumours.(52-54) Although this benefit may only be seen then HER2 is co-amplified with topoisomerase II,(55) this is hard to ignore. One potential strategy for minimising cardiotoxicity with anthracycline-Trastuzumab combinations may by the use of

liposomal doxorubicin and this topic has been recently reviewed in detail by Rayson et al. (56)

Future Directions

Perhaps a next step required to fully integrate Trastuzumab into the curative treatment algorithm for breast cancer patients is to fully evaluate its role as neo-adjuvant therapy and attempts are now being made to extend the benefit seen with adjuvant Trastuzumab as neo-adjuvant treatment. The MD Anderson centre conducted a randomised trial where patients either received Trastuzumab or not, concurrently with neo-adjuvant chemotherapy. The study was stopped prematurely after only 42 patients had been enrolled, because a significantly increased rate of pathological complete response was seen (43% vs 23%; p=0.002).(57) An interim analysis of the NOAH trial has recently been presented. This larger study demonstrated that pathologically complete response was doubled when Trastuzumab was added to neo-adjuvant chemotherapy for HER2-positive breast cancer (43% versus 22%, P=0.002, n = 228) thus paralleling the adjuvant benefit. (58) Both of these studies included concurrent use of Trastuzumab with the anthracycline epirubicin, and reported acceptable cardiac toxicity.

Regarding the role of Trastuzumab in the 45-50% patients(44) with HER2 positive tumours co-expressing HER2 and the oestrogen receptor (ER), pre-clinical evidence supports an interaction between their dependant signalling pathways.(59) This is important because of the relative resistance of HER2 positive tumours to endocrine therapy. The TANDEM trial looking at this patient population demonstrated improved PFS with Anastrozole plus Trastuzumab over Anastrozole alone (median 4.8 vs 2.4 months, p=0.0016).(60) Given the

rapid disease progression in these patients, the question is raised as to whether these patients proceed directly to chemotherapy in combination with Trastuzumab. (61)

Conclusion

A robust strategy for determining optimal treatment strategies for HER2 positive breast cancer as a unique disease is essential. Laboratory and clinical data point towards HER2 blockade being a critical drug treatment for most patients with HER2 positive disease. The limited clinical trial data to date do suggest, in a manner not dissimilar to what is seen with sequential endocrine agents and ER-positive breast cancer, that the development of resistance to one anti-HER2 agent, Trastuzumab, does not preclude benefit from further anti-HER2 blockade, but the optimum strategy and true level of benefit remain unclear. In the adjuvant setting, Lapatinib is the next in line for testing in HER2 positive early breast cancer. The ALLTO study - a very large multinational trial designed to assess Lapatinib and Trastuzumab, each alone, concurrently and sequentially - will confirm or refute the value of this approach. Ensuring maximum patient benefit by optimising the treatment of this disease with effective anti-HER2 blockade as the backbone of patient's therapy should remain high on the list of current research priorities.

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