"Advances in Molecular Classification and Targeted Therapy for Breast Cancer"

Debasish (Debu) Tripathy, M.D.

INTERNAL MEDICINE GRAND ROUNDS
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER AT DALLAS

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This is to acknowledge that Dr. Tripathy has disclosed (any) financial interests or other relationships with commercial concerns related directly to this program. Dr. Tripathy will be discussing off-label uses in his/her presentation. Dr. Tripathy receives research support from Genentech, Inc. and occasionally serves as a consultant for purposes of trial design. One of the topic covered will be the off-label use of adjuvant trastuzumab as the results of multi-center cooperative group trials in this area are summarized.

Part I - Molecular Heterogeneity of Breast Cancer

Introduction

Considerable heterogeneity exists among breast cancer cases, and even within specific biological or clinical subsets, there are significant variations that affect cell behavior and associated clinical outcomes. As newer techniques become available to measure the expression of a very high number of proteins, and to assess multi-gene expression and copy number variations, it is clear that distinct subsets of patients with definable clinical features and be identified. This holds the promise to not only provide more accurate prognosis and to individualize therapy, but also to discover new targets that can be addressed pharmacologically to tailor therapy and reverse resistance to convention anti-cancer drugs. Already, there are established molecular patterns of hormonal therapy responsiveness and expression of oncogenes, such as HER2 (human epithelial receptor 2).

The HER2 receptor, as other receptors, initiates and modulates a complex pathway that is actually better described as a multiply branched network with many negative and positive feedback loops (Figure 1). The end results of the HER2 signal are manifold, and include proliferation, survival, the elaboration of proteolytic and angiogenic molecules, alterations in cytoskeletal and motility functions, and responses to hormonal and cytotoxic therapies. The nature of the signaling output depends not only on initiation via the HER2 receptor, but also on tumor-specific gene and protein abnormalities in the HER2 network as well as germline polymorphisms in components of the system. It stands to reason that the "wiring" of individual HER2-positive cancer cases is different and that functional subclasses can be identified that are clinically relevant. The development of HER2 targeted therapy has heightened the importance of understanding the functional variability of HER2.

Two general strategies have been used to characterize HER2-positive breast cancer. One has been to systematically assess individual components of the HER2 pathway and the other to use multi-parametric analysis at the genomic, gene expression and protein levels to identify individual genes and proteins or patterns and profiles that correlate with prognosis. Both these approaches have also been used to study outcome as well as to understand predictive factors to trastuzumab, kinase inhibitors, chemotherapy and hormonal therapy.

Heterogeneity in HER2 pathway components and prognosis

Individual genes and proteins within the HER2 network including HER2 itself, src, ras, raf, mek, erk, map kinase, akt, PTEN, and PI3 kinase are being studied in preclinical models and patient tumor samples. Also, proteins involved in cell cycle control, apoptosis and estrogen receptor (ER) function are being assessed since these pathways "crosstalk" with HER2 signals.

Inherited polymorphisms in the HER2 gene may affect receptor function and tumor behavior, although as is typically the case with inherited mutations or polymorphisms (defined as more frequent variants of a gene that do not radically alter function), the principle phenotype of greatest concern is the heightened risk of cancer. A polymorphisms at the HER2 transmembrane domain (Val[655]Ile) is present at an allelic frequency of about 20%, and in one case-control study, homozygosity for that allele was associated with an increased risk of breast cancer (Xie 2000). While this polymorphism leads to truncated phosphorylated forms of the receptor in vitro, its effect on clinical behavior is unknown (Tommasi 2004).

The HER2 receptor is phosphorylated at specific residues upon activation, and assays using phospho-HER2- specific antibodies have been performed on tumor tissue have shown that it correlates with higher HER2 levels and worse outcome (Thor 2000). Additionally, phosphoryation of HER2 at serine 1113 has been shown to be associated with HER1 expression (possibly due to heterodimerization and cross-phosphorylation) as well as a worse outcome in HER2-positive breast cancer (Ouyang 2001). These data suggest that early receptor events of auto- and trans- phosphorylation mediated by ligands (heregulin/neuregulin) and trastuzumab or kinase inhibitors can be characterized and might be useful in decision-making.

Downstream signaling proteins and their activation (phosphorylation) status have been examined in small series of patients. In one report, signal transducer and activator of transcription-5 (Stat 5) was progressively lost in the transition of normal breast tissue to cancer and independently associated with a favorable outcome in early stage breast cancer (Nevalainen 2004). Another series showed several members of the Akt/mammalian target of rapamycin (mTOR) pathway, pAkt, mTOR, and 4E-BP1 transcription factor all to be independently associated HER2 expression and a poorer disease-free survival (Zhou 2004). HER2-positive cases had significantly worse survival with cytoplasmic compared to nuclear or absent distribution of p21 (Xia 2004). These finding suggest that the status of other signaling proteins either due to alternate or intersecting pathways, or to additional genetic or epigenetic alterations of these proteins, can functionally subclassify HER2-positive breast cancer.

Heterogeneity in HER2 pathway components and response to therapy

HER2 expression has also been found to be associated with sensitivity to doxorubicin and resistance to CMF and tamoxifen therapy in some but not all retrospective analyses (reviewed in Ross 2003). A few studies have examined the heterogeneity of HER2 breast cancer in relationship to response to nonHER2-targeted therapies. Ones study that showed relative resistance to CMF in HER2-positive cases implied that expression of the p53 inducible cell cycle inhibitor p21, but not p34/Cdc2 (cyclin-dependent kinase) phosphorylation, may play a role in ErbB2-mediated CMF resistance (Yang 2003). Topoisomerase IIα is a target of doxorubicin and the gene is on the same chromosome 17q21 amplicon as HER2, but not always co-amplified with HER2. Sensitivity to doxorubicin in one study was confined to cases where topoisomerase IIα was co-amplified with HER2 (Jarvinen 2001).

Markers of response and resistance to trastuzumab

Molecular classifications of HER2-positive breast cancer that identify trastuzumab-sensitive and resistant patients are important given the side effects and costs of trastuzumab, particularly as this agent undergoes testing in early stage disease. Very few randomized trials of trastuzumab have been conducted, and there is limited availability of tissue available for correlative studies. Small retrospective series have shown a few markers that correlate with response to trastuzumab alone or more often, in combination with chemotherapy, but none has been reproduced extensively. To date, only HER2 expression itself has emerged as a reliable predictive marker to response to trastuzumab (Vogel 2002). This is mainly due to the fact that immunohistochemical assays are subject to observer variability and degradation of antigen in tissue blocks leading to both false negative and false positive results. Fluorescence in situ hybridization, which measures gene copy number, appears to be more accurate in predicting response, but inter-observer variability still exists. (Mass 2001, Perez 2004). Also, the degree of HER2 gene amplification normalized to chromosome 17 centromere appears to produce incrementally higher responses to single agent trastuzumab (Press 2003). In one report, monosomy of chromosome 17 was associated with a lower response to trastuzumab and chemotherapy (Risio 2005).

Of increasing interest are mutations within the HER2 receptor based on the fact that mutations in the ATP-binding pocket of the kinase domain of the HER1 receptor (EGFR) have been found to strongly correlate with response to HER1 kinase inhibitors (Lynch 2004, Paez 2004). To date, mutations in this region of HER2 appear to be exceedingly rare in breast cancer and correlations to trastuzumab response are only now being systematically analyzed. However, in lung cancer, one report found mutations in the HER2 kinase domain in 4% of 120 cases, raising the possibility that HER2 targeted therapy could be effective in that small subset of patients (Stephens 2004).

Downstream signaling components were examined using a tissue microarray approach that allows a large number of immunohistochemical studies on a tissue set of patients treated with trastuzumab plus chemotherapy. In this analysis, overexpression of HER1, neuregulin (HER2 ligand), TGFα (one of several HER1 ligands), and interestingly, low levels of insulin-like growth factor receptor-1 (IGFR-1, another receptor signaling pathway) and elevated downstream phosphorylated ribosomal S6 predicted response (Smith 2004). Recent studies, especially in the neo-adjuvant setting, where tissue pre and post therapy is more easily accessible, have included correlative analyses of markers and in relation to responses to therapy. Chang and colleagues showed that resistance to pre-operative trastuzumab was associated with higher baseline proliferation index (Ki-67) and nuclear pAkt (Chang 2003). Another series showed that absence of PTEN, a phosphatase that inhibits Akt (and its associated cell survival effects) was associated with resistance to trastuzumab plus paclitaxel, and this report was accompanied by elegant preclinical data showing the role of PTEN in trastuzumab response (Nagata 2004).

Gene expression and proteomic patterns in HER2-positive breast cancer

Gene expression profiles of human breast cancers reveal distinct clusters that interestingly correlate with biological classifications that are already known and used in clinical decision-making, namely, estrogen and HER2 receptor expression (Sorlie 2003). Additionally, within the HER2 cluster, there is a separation between ER-positive and negative cases. The expression signature of HER2-positive tumors tends to be associated with other markers of aggressiveness including other genes on the 17q21 amplicon, GATA4, angiogenesis and proteolysis genes, and estrogen receptor negativity (Bertucci 2004). In one study, expression profiling of HER2-positive cases revealed 3 subsets – one containing ER responsive genes, one lacking these genes, and one with a predominance of cell/cell and cell/stroma interaction genes, but no difference in outcome between these groups (Helland 2004). Proteomic approaches using mass spectroscopy may also define patterns at the protein level that correspond to HER-targeted therapy, and preliminary success has been demonstrated in animal xenografts in response to trastuzumab and a HER1 kinase inhibitor (Reyzer 2004).

Considerable heterogeneity exists among HER2-positive breast cancers. Certain subgroups are associated functional and clinical differences that might be used not only to more appropriately tailor HER2-targeted therapy, but also to better identify markers of resistance that could be addressed pharmacologically to create more effective combination regimens. Much more information on single genes and proteins as well as predictive profiles will soon be available as tissue from larger prospective series are examined with high throughput techniques.

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Part 2 - Targeted Therapy for Breast Cancer – The HER2 Example

The *neu* oncogene was initially identified over 20 years ago in rat carcinogen-induced neural tumors. The human homologue of this gene, HER2/*neu* (HER2), encodes a transmembrane tyrosine kinase receptor, which belongs to a family of receptors that are involved in numerous functions including embryological development and cell growth. Amplification of the HER2 gene and overexpression of the protein product was found to present in 20-30 percent of primary breast tumors and accompanied by a worse outcome, suggesting that this may represent a potential therapeutic target. Monoclonal antibodies to HER2 were shown to inhibit HER2-expressing breast cancer cells and to act synergistically with certain chemotherapies such as taxanes and platinum agents. A humanized anti-HER2 antibody, trastuzumab, has now been tested in small and larger clinical trials and demonstrated activity as shown in Tables 1 and 2.

Thus, this biologically targeted therapy is one of the few that have been shown to improve survival in metastatic breast cancer. However, cardiomyopathy, which is felt to be due to modulation of HER2-mediated signaling in myocytes has been observed, especially in combination with anthracyclines. Otherwise, therapy is generally well tolerated. Trastuzumab is currently indicated in combination with paclitaxel for first line therapy of HER2-positive metastatic breast cancer and as a single agent for refractory disease. Clinical trials have shown promising results with trastuzumab in combinations with other agents such as weekly paclitaxel, docetaxel and vinorelbine. A more recent randomized trial comparing docetaxel alone to docetaxel plus trastuzumab showed a significant improvement in response, time to progression and survival with trastuzumab, even in patients who according to protocol were allowed to cross over to receive trastuzumab upon progression to docetaxel along. Based on preclinical synergy with platinum agents, pilot studies have shown activity of taxane, platinum and trastuzumab combinations with a recent randomized trial showing an improvement in response and time to disease progression with the addition of carboplatinum to paclitaxel and trastuzumab. Other agents and hormonal combinations are also being studied.

Table 1. Phase II Trials Trastuzumab for Breast cancer

Therapy	**Prior Chemotherapy for Advanced Disease**	**N**	**Response Rate**	**Median Response Duration**	**Median Time to Disease Progression**
Trastuzumab	Any	46	11%	6.6 mo	5.1 mo
Trastuzumab plus Cisplatin	1 or 2 prior regimens	39	24%	5.3 mo	Not reported
Trastuzumab[a]	1 or 2 prior regimens	222	15%	9.1 mo	3.0 mo
Trastuzumab[b]	None	114	26%	16.6 mo	3.5 mo

a Trastuzumab was given as a loading dose of 4 mg/kg followed by 2 mg/kg intravenously every week

b Patients were randomized to 4 mg/kg followed by 2 mg/kg intravenously every week vs 8 mg/kg followed by 4 mg/kg every week

Table 2. Randomized Trial of Chemotherapy vs. Chemotherapy plus Trastuzumab

Treatment	N	Median Time to Disease Progression	p value	Median Survival	p value
Chemotherapy	234	4.6 mo	0.001	20.3 mo	0.046
Chemotherapy + Trastuzumab	235	7.4 mo		25.1 mo	
AC	138	6.1 mo	0.001	21.4 mo	0.16
AC + Trastuzumab	143	7.8 mo		26.8 mo	
Paclitaxel	96	3.0 mo	0.001	18.4 mo	0.17
Paclitaxel + Trastuzumab	92	6.9 mo		22.1 mo	

Treatment	N	Response Rate	p value	Median Response Duration	p value
Chemotherapy	234	32%	0.001	6.1 mo	0.001
Chemotherapy + Trastuzumab	235	50%		9.1 mo	
AC	138	42%	0.02	6.7 mo	0.005
AC + Trastuzumab	143	56%		9.1 mo	
Paclitaxel	96	17%	0.001	4.5 mo	0.01
Paclitaxel + Trastuzumab	92	41%		10.5 mo	

AC= Anthracycline (doxorubicin or epirubicin) plus cyclophosphamide

One study has examined the pharmacokinetics and safety of trastuzumab given every three weeks using a loading dose of 8 mg/kg followed by 6 mg/kg every 3 weeks. Trough levels were within the target range of 20 μg/ml were achieved and no unexpected toxicity was seen. The use of trastuzumab beyond disease progression remains unclear. Several retrospective analyses have demonstrated efficacy with several trastuzumab-containing regimens after progression on another trastuzumab-based therapy, but the independent contribution of trastuzumab cannot be determined from these studies. No new safety signals have arisen, and cardiotoxicity tends to occur early upon exposure and not after cumulative exposure based on the limited data so far. Table 4 summarizes published reports of trastuzumab beyond progression.

Table 4. Studies of Trastuzumab beyond Progression

Study	Line of Trastuzumab Therapy	N	RR (%)	Clinical Benefit (%)	MTTP (Mo)
Tripathy et al.	Second line	93	11	22	6.7
Gelmon et al.	Second line	65	32	58	6.0-7.8
Fountzilas et al.	Second line	80	24	52	5.2
	Third line	49	14	38	3.5

N = Number of patients; RR = Response Rate; Clinical Benefit=RR+ stable disease for > 6 mo; MTTP = Median Time to Disease Progression; MDR = Median Duration of Response; Surv = Median Survival; Mo = Months; ND = Not done

The optimal way to determine HER2/neu tumor status and likelihood of response to trastuzumab remains somewhat controversial. Immunohistochemical staining may be falsely negative due to tissue fixation but can also be falsely positive. Direct analysis of gene amplification using fluorescence in situ hybridization (FISH) may allow for a more accurate assessment of HER2 status and for better patient selection. Additionally, many patients with truly HER2-positive tumors do not respond to trastuzumab for reasons that are not known, and there is active research ongoing to identify protein and genetic markers that will better predict responsiveness to therapy.

Four major large multi-center trials have recently been completed for patient with early stage breast cancer in both Europe and the United States. In general, eligible patients had HER2-positive tumors (3+ by immunohistochemical staining or positive by FISH) and positive axillary nodes. Two trials tested doxorubicin and cyclophosphamide (AC) followed by paclitaxel given with or without trastuzumab and another trial is also tested trastuzumab with a platinum agent and docetaxel. A worldwide study (HERA) randomized patients following any chemotherapy to observation or 1 or 2 years of trastuzumab. Interim pooled analysis of the American Intergroup and NSABP B-31 trials as well as the European HERA study have all showed reductions in all recurrences by about one half, as well as reductions in distant recurrences. The pooled American trial also demonstrated a reduction in mortality.

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FIGURE 1 – The HER2 Signaling Network