FUTURE ONCOLOGY

TECHNOLOGY, PRODUCTS, MARKETS AND SERVICE OPPORTUNITIES

A NEW MEDICINE PUBLICATION AUGUST 31, 2008 VOLUME 9, NUMBER 7/8

DRUG DEVELOPMENT IN ONCOLOGY

THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) PATHWAY IN CANCER

PART V — CLINICAL TRIALS OF COMBINATIONS OF APROVED ERBB-PATHWAY INHIBITORS WITH NOVEL AGENTS

DRUG DEVELOPMENT IN ONCOLOGY

THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) PATHWAY IN CANCER

PART V — CLINICAL TRIALS OF COMBINATIONS OF APROVED ERBB-PATHWAY INHIBITORS WITH NOVEL AGENTS

Despite the incredible effort invested in the development of targeted therapeutics addressing the ErbB pathway, first generation drugs only provide incremental benefits to patients. Therefore, the quest for improved approaches to target this pathway is proceeding in numerous fronts, such as:

- Better understanding of the role of the ErbB pathway in malignancy
- Identification of other contributors to the role of ErbB in cancer, in terms of upstream and/or downstream effectors
- Improving targeting approaches by the use of novel delivery systems
- Resolving any differences between monoclonal antibody (MAb)-based agents and small molecule receptor tyrosine kinase (RTK) inhibitors in terms of therapeutic effectiveness
- Overcoming intrinsic and acquired resistance of cancer stem/progenitor cells to ErbB pathway inhibitors
- Evaluating combinations of targeted drugs to significantly enhance their effectiveness
- Evaluating biomarkers associated with toxicity and response
- Identifying patient populations most likely to benefit from ErbB inhibitors using *in vitro* approaches to profile molecular markers expressed in tumors

EXPLORING AND EXPLOITING THE COMPLEXITIES OF *IN VIVO* SIGNALING IN THE MODULATION OF THE ERBB PATHWAY

As preclinical and clinical research in applying molecularly targeted agents in oncology expands and intensifies, it is becoming clearer and clearer that this approach to cancer treatment is far more complex than ever imagined. Targeted approaches face similar obstacles as did standard cytotoxic therapies, namely marginal effectiveness in advanced disease, resistance issues, and toxicities. Unlike standard cytotoxics, however, targeted therapeutics also present another serious dilemma for their developers.

Although in certain cases they appear to be highly effective, selecting out the patients who may benefit in such occasions leaves significantly fewer candidates for the treatment, negatively impacting the financial incentive of the company developing the drug. The effect of this emerging challenge in the development of targeted therapeutics may be mitigated by the employment of combination strategies using several targeted drugs, either concurrently or sequentially, to address different members of a pathway, including mutant forms, thus removing 'escape' routes and combating resistance.

To date, approved ErbB-pathway inhibitors have been evaluated with other approved drugs, mostly cytotoxics in the hope that the anticipated small gains in effectiveness would expand the clinical utility of the targeted agent leading to approvals in new indications or stimulating off-label use. Over 1,200 trials have been initiated worldwide with these agents and about 50% of these trials are still ongoing. The amount of data to be generated by these trials is staggering and the interpretation, comparison and assessment of the results a major undertaking, particularly when small improvements either in effectiveness or tolerability separate the results.

Molecular Targets/Pathways

Previous FUTURE ONCOLOGY issues described the ErbB pathway in great detail. Here, molecular moieties and pathways are described impacting the ErbB pathway tangentially, conferring positive effects by enhancing the sensitivity of cancer cells to ErbB inhibitors or negative ones by giving rise to resistance against such inhibitors.

Resistance mechanisms associated with ErbB inhibitors are not fully understood but one theory is that in many instances 'escape routes' are activated in tumors allowing them to bypass blockade of the EGFr/Ras/mitogen-activated protein kinase pathway (MAPK) signaling axis.

In order to define genes conferring resistance or susceptibility to ErbB inhibitors, investigators at Fox Chase Cancer Center (Philadelphia, PA) conducted an extensive *in silico* search of publicly available data sources for genes in the EGFr signaling network, including *Drosophila* and *C. elegans* evolutionary conserved pathway orthologs, and genes differentially expressed upon EGFr modulation. A 638-gene siRNA library (two siRNA per gene) was constructed and the effects of these siRNA on the viability of the human epidermoid carcinoma cell line A431 were studied in the presence of vehicle and IC_{30} to IC_{40} concentrations of erlotinib, panitumumab, U0126, and camptothecin. Using this functional genomics strategy, a specific set of genes conferring resistance to EGFr inhibitors as well as non-specific apoptosis resistance genes within the ErbB pathway were identified, which may be used to generate profiles of resistance or sensitivity, both in multiple cancer cell lines and in clinical samples, and optimize anti-EGFr cancer therapy (Astsaturov IA, AACR08, Abs. 914).

Targets/pathways implicated in the positive or negative effects of ErbB inhibition in cancer are many, varied and their contribution has not been fully elucidated. FUTURE ONCOLOGY Volume 8 issue #11/12 described many of the targets/pathways associated with the ErbB pathway. Here, additional or newly identified contributors to ErbB signaling are described.

A disintegrin and metalloprotease (ADAM) family (ADAM 9, ADAM 10, ADAM 17) members are referred to as sheddases because they cleave membrane proteins at the cell surface shedding soluble ectodomains (ECD). These zinc-dependent metalloproteases cleave ErbB ligands by ECD shedding that is essential for their functional activation; it allows ligands such as growth factors to be shed from their attachment to a receptor and go on to stimulate another. Sheddases such as ADAM enzymes 9, 10 and 17, promote growth through all four ErbB pathways. Therefore, inhibition of ligand shedding and receptor cleavage may improve clinical outcomes when used in combination with ErbB-based approaches.

Sheddase inhibition blocks two different pro-oncogenic mechanisms, generation of active EGFr ligands and a constitutively active HEr2 kinase. For instance, ADAM 10 is responsible for HEr2 shedding, enhancing the activity of the HEr2 receptor. In HEr2-overexpressing cells, the ECD is frequently cleaved, rendering the remaining transmembrane portion of HEr2 (p95) constitutively active. The presence of both serum ECD and cellular p95 protein have been linked to poor clinical outcome and reduced effectiveness of some treatments, suggesting that signaling via p95 is clinically relevant and may represent an attractive target for therapeutic intervention.

Sheddase inhibitors synergize with clinically relevant cancer therapeutics and show no overt or compounding toxicities, including fibroplasia, the dose-limiting toxicity (DLT) associated with broad-spectrum matrix metalloprotease (MMP) inhibitors (Fridman JS, etal, AACR-NCI, EORTC07, Abs. C198). ADAM metalloproteases block HEr2 ECD cleavage further potentiating the antitumor effects of trastuzumab that also inhibits ECD shedding. Also, sheddases can activate HEr3 through generation of the HEr3 ligand, heregulin. As this pathway is involved in resistance to current EGFr-targeted therapies, decreasing the activity of this pathway with sheddase inhibitors may be beneficial.

Sheddase inhibition may be effective in blocking EGFr activation upstream of the receptor providing an alternative route to direct receptor inhibition. Investigators at the Lawrence Berkeley National Laboratory (Berkeley, CA) used 3-dimensional culture models of breast cancer cell lines representative of the basal phenotype, to examine the regulation of EGFr-dependent signaling in this disease. Expression of ADAM17 and the EGFr ligand, transforming growth factor (TGF)-α, are positively correlated in human breast cancer of the the basal subtype, and are predictive of poor prognosis. ADAM17 was identified as the key reg-

ulator of EGFr ligand shedding in these cell lines. Both small molecule and siRNA inhibition of ADAM 17 blocked EGFr signaling by preventing ligand mobilization. Furthermore, because breast cancer cell response to EGFr inhibitors such as gefitinib is strongly dependent on the levels of free EGFr ligands in the extracellular milieu, impeding ligand bioavailability using ADAM 17 inhibitors may sensitize tumors to these EGFr inhibitors (Kenny PA and Bissell MJ, AACR-NCI-EORTC07, Abs. C253).

Incyte (Wilmington, DE) has an active development program in sheddase inhibitors. The lead agent, INCB7839, is being evaluated in phase II clinical trials in combination with trastuzumab (see below). INCB003619, another agent identified by Incyte is a potent, selective, small molecule inhibitor of ADAM that also blocks HEr2 ECD shedding, and enhances the antitumor activities of trastuzumab in HEr2-overexpressing human breast cancer cell lines.

INCB003619 dramatically enhances the antiproliferative activity of suboptimal doses of trastuzumab in HEr2-overexpressing/shedding breast cancer cell lines, accompanied by reduced extracellular signal-regulated kinase (ERK) and Akt phosphorylation. Furthermore, this potentiating effect is detected only with active inhibitors of ADAM 10, and not with inhibitors that lack ADAM 10 activity, but still possess inhibitory activities against other ADAM or MMP targets. In addition, INCB003619, in combination with trastuzumab, augments the proapoptotic and antiproliferative effects of paclitaxel. Consistent with these *in vitro* data, INCB003619 reduces serum ECD levels and enhances the antitumor effect of trastuzumab in a xenograft tumor model derived from the HEr2-overexpressing BT-474 breast cancer cell line. Therefore, it appears that blocking HEr2 cleavage with selective ADAM inhibitors may represent a novel therapeutic approach for the treatment of patients with HEr2-overexpressing breast cancer (Liu X, etal, AACR-NCI-EORTC07, Abs. B207).

Investigators at Queen's University (Belfast, UK) studied the mechanism by which EGFr is activated following chemotherapy treatment in colorectal cancer (CRC) and non-small cell lung cancer (nsclc) cells. GM6001 (Ilomastat), a broad spectrum inhibitor of MMP abrogated chemotherapy-activated EGFr phosphorylation in CRC and nsclc cells, indicating that EGFr activation was mediated by metalloproteases. ADAM 17 was the principal ADAM involved in chemotherapy-induced EGFr activation. ADAM 17 also regulated TGF-α shedding following chemotherapy treatment; TGF-α was the main ligand involved in chemotherapy-induced activation. In addition, silencing of ADAM 17 or TGF-α sensitized CRC and nsclc cells to chemotherapy-mediated apoptosis.

The importance of ligand shedding for chemotherapy-induced EGFr activation was further demonstrated by using cetuximab. Co-treatment with cetuximab attenuated chemotherapy-induced activation of EGFr and sensitized CRC and nsclc cells to chemotherapy. According to these findings, EGFr activity following chemotherapy is attributable to ADAM 17-mediated shedding of TGF-α, suggesting that specific metalloproteases in combination with chemotherapy may be effective in the treatment of CRC and nsclc (Kyula JN, etal, AACR-NCI-EORTC07, Abs B117).

Akt/ Protein kinase B (PKB) is a cytosolic protein that promotes cell survival by phosphorylative inactivation of targets in apoptotic pathways. Akt is downstream of ErbB-pathway members and its activity is, therefore, dependent on the status of several other effectors further upstream as described in this section.

BIM, a BH3-only Bcl-2 family protein, interacts by forming heterodimers with other members of the Bcl-2 protein family, including a number of antiapoptotic Bcl-2 proteins, and acts as an apoptosis activator. Upon activation, BIM can antagonize all the pro-survival Bcl-2 proteins, leading to apoptosis.

BIM mediates EGFr tyrosine kinase inhibitor (TKI)-induced apoptosis in nsclc and is linked to resistance conferred by EGFr mutation T790M and the novel L747S mutation. In nsclc cell lines that are either sensitive or resistant to reversible TKI, gefitinib-induced apoptosis was linked to upregulation of BIM, which was observed only in gefitinib-sensitive lung cancer cells with EGFr mutations. To determine if BIM was also involved in secondary resistance conferred by acquired EGFr mutations, investigators analyzed the common T790M mutation that accounts for half the tumors with acquired resistance to gefitinib, and a novel acquired EGFr mutation in exon 19, a leucine to serine substitution at position 747 (L747S) in *cis* to the activating L858R mutation identified in a patient with nsclc with a 40-month response to gefitinib prior to progression.

Significant attenuation of apoptosis occurred in constructs containing L858R-L747S and L858R-T790M upon gefitinib exposure, with the later being the most resistant mutation. Gefitinib-induced upregulation of BIM and apoptosis were inhibited by the presence of T790M, and to a lesser extent by L747S. In the cell line NCI-H1975, which harbors the L858R-T790M mutation, upregulation of BIM and apoptosis were only seen after exposure to CL-387,785, a specific and irreversible anilinoquinazoline EGFr inhibitor, and not gefitinib. Knockdown of BIM by siRNA significantly attenuated apoptosis induced by TKI in EGFr-mutant cell lines HCC827 that carries an exon 19 deletion and is highly sensitive to gefitinib, and in NCI-H1975. These results not only describe a novel secondary gefitinib-resistant mutation, but also provide insights into a key effector of TKI-induced apoptosis and suggest that induction of BIM may have a role in the treatment of TKI-resistant nsclc (Costa DB, etal, AACR07, Abs. LB-61).

CXCL12 and CXCr4 are members of a group of cytokines generally known as chemokines. Chemokines and their receptors promote migration and homing of cells to target tissues. Cancer cells use a chemokine receptor

axis for metastasis formation at secondary sites. CXCL12 and CXCr4-induced HEr2 transactivation contributes to enhanced invasive signals and metastatic growth in the bone microenvironment and helps explain the role of CXCr4 in promoting bone metastasis (Chinni SR, etal, AACR07, Abs. 2030). Also, CXCr4 has been implicated in increased metastatic potential in breast and colon cancer and as a scaffolding protein required for optimal HEr2 function. CXCr4 is upregulated in breast cancer cells expressing HEr2, resulting in a higher proliferative rate and changes in signal transduction/cell-cycle pathways. Therefore, antagonists of CXCL12/CXCr4 may prevent or reverse trastuzumab resistance.

Investigators at the University of Texas Southwestern Medical Center (Dallas, TX) describe a novel specific alteration in a cell line model of acquired trastuzumab resistance, the targeting of which reverses resistance. A trastuzumab-resistant subclone (BT-474HR) of the HEr2-positive breast cancer line BT-474 was developed that exhibited a 3-fold more rapid growth rate in the absence of trastuzumab. Following trastuzumab exposure, G0/G1 arrest was observed in sensitive compared to resistant cells (84% versus 68%), with fewer cells in S-phase (3% versus 14%). There were fewer changes in gene expression in the trastuzumab-resistant subclone compared to sensitive subclone. Insulin-like growth factor (IGF)-1 and cyclin-dependent kinase 2 (CDK2) were upregulated but p27 was not induced in BT-474HR cells treated with trastuzumab, and no difference was seen in phosphatase and tensin homolog (PTEN) expression. Differentially expressed genes included CXCr4, cystatin A (CSTA) a cysteine protease inhibitor, and mitotic checkpoint regulators. Gene-specific knockdown of CXCr4 led to re-sensitization of BT-474HR to trastuzumab, with a >60% reduction in cell number in both resistant and sensitive cells (Mukhopadhyay P, etal, AACR07, Abs. 2338).

Cyclin-dependent kinases (CDK) comprise a family of serine/threonine kinases that regulate cell-cycle progression in proliferating eukaryotic cells. At least 9 CDK have been identified to date. Abnormal CDK control, and loss of cell-cycle checkpoint function, may be attributed to cyclin overexpression, loss of endogenous CDK inhibitor function, and/or CDK substrate alterations.

Simultaneous inhibition of CDK and ErbB-pathway inhibitors may represent a novel combination for the treatment of malignancies overexpressing ErbB. Overstimulation of these receptors results in the increased production of cyclin D1, a signaling molecule that, by activating CDK, plays a central role in the ability of cancer cells to evade destruction and become resistant to the effects of such anticancer drugs as erlotinib or trastuzumab. This CDK-mediated proliferation prevents the natural process that either repairs or eliminates cells containing genetic alterations that may potentially become malignant. In a study conducted by Cyclacel Pharmaceuticals (Berkeley Heights, NJ), a synergistic activity was observed between seliciclib, a CDK2 inhibitor, and several EGFr inhibitors, including erlotinib, in models of nsclc, and trastuzumab in models of breast cancer (Fleming I, etal, Clin Cancer Res, 1 Jul 2008;14:4326-4335).

Confirmation of the synergy between seliciclib and erlotinib was observed in mouse xenograft models of nsclc. In these studies, the combination of seliciclib and erlotinib resulted in a 93% tumor growth inhibition, a result that was statistically significant when compared to tumor growth inhibition for erlotinib or seliciclib alone. Immunohistochemistry (IHC) analysis confirmed a dramatic reduction in cyclin D1 production in the xenografts treated with the combination of seliciclib and erlotinib compared with xenografts from mice treated with either agent alone (Frame S, etal, AACR07, Abs. 4003).

Cyclooxygenase-2 (COX-2) is the rate-limiting enzyme in prostaglandin synthesis. Overexpression of COX-2 has been linked to carcinogenesis and tumor progression in many human malignancies. COX-2 plays a key role in tumor-dependent angiogenesis and growth by activating the ERK/MAPK pathway. COX-2 is overexpressed in breast neoplasms, is associated with other markers of poor prognosis, and is significantly associated with worse survival independent of known prognostic factors (Zerkowski MP, etal, Cancer Invest, Feb 2007;25(1):19-26).

Fibroblast growth factor receptor 1 (FGFr1) is a member of the FGFr family comprising 4 transmembrane receptors with intrinsic PTK activity. FGF and the high affinity FGFr represent a complex cellular growth and survival system. Inappropriate activation of FGFr may be involved in tumor angiogenesis.

The consequences of FGF/FGFr-mediated signal inhibition alone or in combination with agents blocking EGFr was investigated in human nsclc cells. Inhibitors of FGF-mediated pathways prevented FGF-induced phosphorylation of ERK/MAPK and, in the majority of nsclc cell lines proliferation was inhibited and massive cell death, representing mainly apoptosis, was induced after treatment for a few days with FGFr inhibitors. In 3 nsclc cell lines (VL-8, A549 and A427) tested, subcutaneous tumor formation in SCID mice was completely inhibited (VL-8) or significantly reduced (A549 and A427) by the presence of a dominant-negative FGFr1 IIIc-GFP (dnFGFr1) fusion protein introduced by adenoviral transduction.

Comparable to dnFGRr1, small molecule FGFr inhibitors also blocked FGF-induced phosphorylation of ERK/MAPK and S6 ribosomal protein and, in more than 50% of the investigated cell lines, exhibited distinct anti-migratory as well as cytostatic and in some cases also cytotoxic effects. In addition, simultaneous blockage of FGFr and EGFr signals with small molecule inhibitors and the respective dominant-negative receptor constructs, generally exerted synergistic antiproliferative effects and further reduced MAPK and PI3K pathway activities. Therefore, targeting the FGF/FGFr system should be considered as new tool for multimodality treatment of nsclc, especially in combination with EGFr inhibitors (Hendrik F, etal, AACR08, Abs. 141).

Growth factor receptor binding protein 2 (Grb2) is a ubiquitously expressed adapter protein with two Src homology 3 (SH3) domains and a central Src homology 2 (SH2) domain. Binding of EGFr to EGF induces phosphorylation of the tyrosine residues in the cytoplasmic domain activating Grb2, which transmits the external EGF signals into the cytoplasm. Although Grb2 has no enzymatic activity, its SH2 domain recognizes the phosphotyrosine residues of activated EGFr, and its SH3 domains bind to Sos, a Ras GTP exchange factor. In this manner, Grb2 links activated EGFr to downstream signaling molecules to induce gene expression and morphologic changes in cells. In addition to activating Ras, Grb2 may also play a role in other signaling pathways in mammalian cells (Morimatsu M, etal, PNAS USA, 13 Nov 2007;104(46):18013-8).

Several small synthetic binding antagonists of Grb2 have been developed that potently disrupt functions mediated by its interaction with cognate proteins (Giubellino A, etal, Cancer Res, 1 Jul 2007;67(13):6012-6).

Heat shock protein 90 (Hsp90) is an important molecular chaperone that regulates the folding and stability of key signaling molecules (client proteins) involved in cell growth and survival. Hsp90 is an attractive oncology drug target because many of its client proteins are key mediators in pathways disrupted in cancer. Hsp90 is specifically activated in tumor cells where it controls multiple oncogenic proteins such as EGFr, HEr2, and Raf, and their downstream signaling molecules such as Akt, and ERK that are critical to the proliferation and survival of tumors. Hsp90 interacts with CDK4 and CDK6, and stabilizes mutated proteins such as v-Src, as well as fusion proteins resulting from chromosomal translocations such as Bcr-Abl.

Inhibition of Hsp90 leads to degradation of the client proteins, loss of signaling, and inhibition of cell growth. Examples of cancer-associated Hsp90 client proteins include c-Kit in gastrointestinal stromal tumors (GIST), EGFr in lung cancer, and Bcr-Abl in chronic myelogenous leukemia (CML). Numerous Hsp90 inhibitors have been investigated in preclinical and clinical trials. At least 12 such agents have been investigated in clinical trials with at least 11 currently in active clinical development for the treatment of solid tumors or hematologic malignancies.

Hsp90 inhibition may also sensitize drug-resistant cell lines expressing EGFr. Investigators at the University of Michigan Medical School (Ann Arbor, MI) discovered that EGFr is activated on exposure to cisplatin and gemcitabine in head and neck cancer cell lines, leading to ubiquitin-mediated degradation of the receptor itself, causing cellular toxicity and radiation sensitization. To test the hypothesis that the degradation of the receptor is a critical event in this setting, several head and neck cancer cell lines were screened that were either sensitive or resistant to cisplatin. Because EGFr was not degraded in cisplatin-resistant cell lines, it was hypothesized that promoting EGFr degradation, would sensitize these cells to cisplatin-mediated radiosensitization. For this purpose, EGFr was targeted using Hsp90 inhibition in cisplatin-sensitive and cisplatin-resistant head and neck cancer cell lines treated with cisplatin, geldanamycin or a combination of the two.

Results indicate that Hsp90 is activated by cisplatin only in cisplatin-sensitive cells; EGFr and Hsp90 interact and can be co-immunoprecipitated; and geldanamycin, an inhibitor of Hsp90, accelerates the degradation of EGFr in cisplatin-resistant cells, leading to both cellular toxicity and significant radiosensitization. These findings demonstrate that EGFr degradation after chemotherapy depends on Hsp90. Furthermore, they suggest that the new generation of geldanamycin analogs that are entering the clinic may potentiate cisplatin-mediated cytotoxicity and radiosensitization via EGFr degradation (Ahsan A, etal, AACR08, Abs. 410).

Histone deacetylases (HDAC) are enzymes that catalyze the removal of acetyl groups from the amino-terminal lysine residues of core nucleosomal histones. Deacetylation of histones by HDAC inactivates tumor suppressor genes leading to neoplastic transformation. The balance between transcriptional activity and gene silencing is often disturbed in tumors and, therefore, inhibition of HDAC may activate tumor suppressor genes, restoring normal growth control. HDAC inhibition may be antiproliferative, and induce differentiation and/or apoptosis. HDAC inhibitors exert their antitumor effects through acetylation of both histone and non-histone proteins, leading to modulation of gene expression, inhibition of cell-cycle progression and promotion of differentiation.

The effect of HDAC inhibitors on EGFr expression in colon cancer cell lines was investigated by researchers at Norris Comprehensive Cancer Center (Los Angeles, CA) to ascertain the value of combining inhibitors of both of these markers in the treatment of CRC. In human ovarian and lung cancer cell lines, HDAC inhibitors significantly downregulate EGFr protein expression. The effects of two HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA) and LBH589, were investigated on EGFr mRNA and protein expression levels. SAHA and LBH589 alone induced growth inhibition and suppressed EGFr mRNA and protein expression in a dose-dependent manner in a panel of colon cancer cell lines with varying levels of EGFr expression. In addition, analysis of EGFr-activated pathways following HDAC inhibition indicated decreased activation of the downstream targets MAPK and Akt. Also, the combination of cetuximab and HDAC inhibition resulted in synergistic inhibition of cell proliferation. Therefore, there is a scientific rationale for the clinical utility of the combination of HDAC inhibition with EGFr-targeted therapies for the treatment of metastatic CRC (LaBonte MJ, etal, AACR07, Abs. 683).

An alternative in combining different targeted drugs against relevant effectors is the use of multitargeted agents with intrinsic activity against the same targets. A case in point is a novel multitargeted small molecule drug against

EGFr, HEr2, and HDAC, developed by Curis (Cambridge, MA), that entered a phase I clinical trial (protocol ID: CUDC-101-101; NCT00728793) in August 2008. In animal studies, daily dosing of CUDC-101 for two weeks induced tumor regression (32.2%) or tumor stasis in nude mouse xenografts of the human HCC cell lines HepG2 and Hep3B2, whereas very limited antitumor effects were noted with SAHA and erlotinib. CUDC-101 displays potent inhibition of both HDAC activity and EGFr phosphorylation. No toxicity has been observed during the 2-week efficacy studies (Bao R, etal, AACR08, Abs. 737).

Insulin-like growth factor 1 receptor (IGF1r), a tyrosine kinase strongly overexpressed in many human tumors, is essential for transformation of cells *in vitro* by various oncogenes. IGF1r plays an important role in tumor-cell growth and survival and protects tumor cells from apoptosis induced by a variety of anticancer agents. Therefore, inhibition of the IGF1r tyrosine kinase activity may sensitize cancer cells to cytotoxic treatments. The ability of the IGF axis to influence chemosensitivity may be attributed not only to its function in regulating apoptosis, but also its direct involvement in the cellular response to DNA damage. There is also evidence that supports combining IGF1r inhibitors with other biologic treatments, including ErbB-pathway inhibitors. IGF1r:EGFr complexes have been detected in human breast and lung cancer cells and clinical samples of breast cancer, and similar complexes have been observed in lung tumors resistant to EGFr inhibitors. Indeed, the IGF axis is increasingly recognized as a candidate mediator of resistance to inhibition of RTK including EGFr and HEr2, and of downstream effectors including mTOR. Proof that co-targeting of the IGF1r and EGFr and IGF1r and HEr2 is synergistic is currently being sought in the clinical setting.

Investigators correlated the frequency of IGF1r expression with both clinical features of patients previously treated for advanced nsclc and molecular markers in the EGFr pathways in patients treated with gefitinib. Patients from an expanded access trial with >1 week of gefitinib treatment were included in the analysis. Tissue from 83 patients was evaluated for IGF1r expression by IHC; IGF1r positivity was defined as presence of any staining. Also, samples from 81 patients were evaluated for EGFr protein expression by IHC, and EGFr gene copy number and chromosome 7 copy number by fluorescence *in situ* hybridization (FISH). Among 78 patients with both IGF1r expression and FISH analysis available, 48 (61.5%) had adenocarcinoma, 12 (15.4%) were never smokers, and performance status (PS) was 0 in 71 (91.0%) patients.

The response rate (CR+PR) to gefitinib was 15.4%. Median progression-free survival (PFS) was 3.0 months and median overall survival (OS) was 7.3 months. The IGF1r score was positive in 71.08% of tissue samples, and was marginally ($p<0.1$) associated with smoking. In this study IGF1r expression by IHC was relatively frequent in nsclc, but did not correlate with clinical parameters. No significant association was found between IGF1r score and age, gender, histology, or PS. There was a significant association between IGF1r positivity and EGFr IHC ($p=0.002$), chromosome 7 copy number ($p=0.03$), and EGFr gene amplification ($p=0.01$) suggesting that combining an IGFr inhibitor with an EGFr TKI may improve outcomes in selected patients with nsclc (Batus M, etal, ASCO08, Abs. 22080).

Integrin α2 (ITGA2) is a type I membrane protein belonging to the integrin α chain family. The integrins constitute a family of heterodimeric integral membrane glycoproteins composed of two non-covalently associated subunits, a distinct α chain and a common β chain. Integrins are cell-surface receptors for extracellular matrix (ECM) components involved in cell adhesion and in a variety of processes including embryogenesis, hemostasis, tissue repair, immune response, and tumor-cell metastasis.

According to researchers at Kurume University (Fukuoka, Japan), integrin α2β1 physically associates with EGFr and functions in serum-independent activation of EGFr at intercellular adhesion sites. In lysates of A431 human epithelial carcinoma cells, immunoprecipitation with anti-integrin α2 MAb or anti-integrin β1 MAb resulted in preferential coprecipitation of EGFr, while anti-EGFr MAb coprecipitated integrin α2β1. The association of integrin α2β1 with EGFr was confirmed by chemical crosslinking and by double immunofluorescence staining. The association was not affected by EGF-induced EGFr stimulation. EGFr, localized at cell-cell contact sites, was phosphorylated even in serum-depleted conditions, while EGFr localized to other sites was totally dephosphorylated. Blocking integrin α2 abrogated EGFr phosphorylation at intercellular contact sites under serum-depleted conditions. Also, EGFr phosphorylation in serum-depleted conditions was not observed in suspended cells, or was largely abrogated in sparse cells, indicating that cell-cell adhesion is required for EGFr phosphorylation (Yu X, etal, J Cell Science, 2000; 113(12):2139-47).

E7820, an aromatic sulfonamide derivative under development by Eisai (Tokyo, Japan), modulates the expression of integrin α2 in human umbilical vein endothelial cells (HUVEC) leading to the inhibition of angiogenesis. E7820 inhibits tumor growth in a variety of human tumor xenograft models, including colon, breast, pancreatic and kidney cancer. E7820 has also been evaluated preclinically, in combination with erlotinib, in the treatment of nsclc. Nude mice bearing human nsclc A549 cells were treated with either single agent E7820, or erlotinib, or a combination of the two. The antitumor activity of the combination of E7820 and erlotinib at well tolerated doses was superior to either agent alone. The combination decreased tumor vascularity and increased apoptosis of tumor-associated endothelial cells within tumors as compared with each agent alone. These *in vitro* data suggests that investigation of this combination may be warranted in patients with nsclc (Ito K, etal, AACR08, Abs. 4018).

Kras is a proto-oncogene belonging to the ras family of genes. Kras plays an important role in cell growth regulation and oncogenesis. Recently, it has been confirmed that patients with metastatic CRC and Kras mutations do not benefit from treatment with EGFr inhibitors. As a result, assays are available to preselect patients for treatment with EGFr inhibitors, another indication of the trend towards personalized medicine.

A retrospective analysis of efficacy data from a multicenter (n=201), international, randomized, open label, controlled, phase III clinical trial (protocol ID: EMR 62202-013, NCT00154102), dubbed CRYSTAL, was conducted to assess the impact of tumor Kras mutation status on PFS and response rate in chemotherapy-naïve patients with metastatic CRC treated with FOLFIRI with or without cetuximab. The population with available tissue for Kras analysis was representative of the overall intent-to-treat (ITT) population (n=1198).

Among 540 patients with evaluable tumor samples, Kras mutation was detected in 192 (35.6%), while wild type (wt) Kras was detected in 348 (54.4%). In patients with wt Kras (n=348), cetuximab had a statistically significant effect on PFS (p=0.017; HR=0.68), with PFS of 9.9 months and 8.7 months in patients treated with FOLFIRI and cetuximab and in those with FOLFIRI alone, respectively. Response rate (CR+PR) was 43.2% in the FOLFIRI group (n=176) and 59.3% in the FOLFIRI and cetuximab group (n=172; p=0.0025). In patients with Kras mutation (n=192), treatment with cetuximab had no statistically significant benefit (p=0.75, HR=1.07), with a PFS of 7.6 months and 8.1 months in the FOLFIRI plus cetuximab and the FOLFIRI groups, respectively. Therefore, patients with Kras mutant tumors do not benefit from the combination of FOLFIRI and cetuximab. The Grade 3/4 AE profile was similar in the wt Kras and mutant populations (Van Cutsem E, ASCO08, Abs. 2).

Mammalian target of rapamycin (mTOR) is a member of the phosphoinositide 3-kinase (PI3K)-related kinase (PIKK) family and a central modulator of cell growth, proliferation and survival by influencing protein synthesis and transcription. mTOR plays a critical role in transducing proliferative signals mediated through the PI3K/Akt signaling pathway, principally by activating downstream protein kinases that are required for both ribosomal biosynthesis and translation of key proteins required for the progression of G1 to S phase. Targeting mTOR inhibits signals required for cell-cycle progression, cell growth, and proliferation.

Clinical evaluation of combinations of mTOR inhibitors and ErbB-pathway inhibitors is far more advanced than any other clinical program with ErbB-pathway inhibitors and other drugs addressing novel targets. Most of these trials involve the drug everolimus (Afinitor; Novartis), an mTOR inhibitor that is approved for a non-cancer indication and is in late stage development in cancer (see below).

To determine the optimal schedule of combining everolimus and gefitinib and to address whether the blockade of EGFr by gefitinib prevents everolimus-induced p-Akt, cancer cells (BT-474, MDA-MB-468 and DU-145) were incubated with gefitinib and/or everolimus at various doses, or sequentially exposed to gefitinib and everolimus, and vice versa. Simultaneous treatment of BT-474 and DU-145 cells with gefitinib and everolimus inhibited cell growth more than each drug alone and the effect was additive. Sequential treatment with gefitinib followed by everolimus produced synergistic effects in both cell types. The effect of the combination in MDA-MB-468 cells, which are relatively resistant to gefitinib, was synergistic when the drugs were added simultaneously, and additive when gefitinib was followed by everolimus. Sequential exposure to everolimus followed by gefitinib produced antagonistic effects in all cell lines. Gefitinib, at its IC_{50} dose, completely prevented everolimus-induced p-Akt and also significantly enhanced everolimus-mediated decrease in p-4E-BP1 and p-p70S6K. Therefore, antitumor interaction between everolimus and gefitinib is sequence dependent. Accordingly, the optimal therapeutic schedule of the combination may be gefitinib administered during or before everolimus. These data also support everolimus as a promising candidate for use in combination with gefitinib either in gefitinib-sensitive or resistant tumors (Di Cosimo S, etal, ASCO04, Abs.3074).

The synergistic effect of a EGFr TKI and an mTOR inhibitor was investigated in a phase I clinical trial conducted at the Cedars-Sinai Medical Center (Los Angeles, CA) with gefitinib and sirolimus in adult patients (n=21) with recurrent glioblastoma multiforme (GBM) previously treated with surgery, radiotherapy, or chemotherapy with or without immunotherapy (vaccine). Primary endpoints were safety and toxicity of this combination. Secondary endpoints include TTP, OS, and quality of life (QoL). Patients were administered oral sirolimus at 2 mg/day, adjusted to 4-12 ng/ml, and oral gefitinib (500 mg/day), which was escalated to 1000 mg/day in patients treated with dexamethasone or enzyme-inducing anti-epileptic drugs (EIAED). Among 21 patients (EIAED=12) enrolled in this trial,18 were evaluable. Most common toxicities were diarrhea, rash and mucositis, and 3 cases of Grade 3/4 nonhematologic toxicities including rash, renal failure, hypotension, dyspnea, coagulopathy, and elevated LFT. Wound infection unrelated to treatment developed in one patient. There were 2 (11%) minor responses and disease stabilized in 6 (33%) patients, for a clinical benefit of 44%. Median TTP was 20 weeks, and MST was 37.6 weeks. Oral daily co-administration of gefitinib and sirolimus is safe and tolerable in this heavily pretreated patient population, with modest antitumor activity (Phuphanich S, etal, ASCO08, Abs. 2088).

Met/hepatocyte growth factor receptor (HGFr), a proto-oncogene, is a transmembrane RTK for hepatocyte growth factor/scatter factor (HGF/SF) that plays an impor-

tant regulatory role in cell motility, morphogenesis and proliferation. Met regulates a complex array of cellular events involved in invasive growth that are essential for normal development and wound repair, but are frequently co-opted by tumors to promote their own growth, motility, and invasion. Met receptor levels are governed in part by Cbl-mediated ubiquitination and degradation; uncoupling of Met from Cbl-mediated ubiquitination promotes its transforming activity. Tumor cells with Met gene amplification are highly dependent on Met tyrosine kinase signaling for proliferation and survival making them particularly sensitive to Met inhibition.

Simultaneous inhibition of Met and ErbB members that activate common downstream signaling pathways, such as the Ras/Raf/MEK/MAPK and PI3K/Akt, is being investigated as a means of overcoming resistance to TKI like gefitinib or erlotinib in nsclc (Blazek ER, etal, ASCO08, Abs. 22223). Also, gefitinib, in combination with AZD6244, a MEK 1/2 inhibitor, potently suppresses the proliferation and survival of gastric cancer cells by blocking Ras/MAPK and PI3K/Akt signaling. Both EGFr and Ras/Raf/MEK/MAPK are important drivers of gastric cancer cell proliferation, and simultaneous inhibition of these pathways could be an effective strategy for the treatment of gastric cancer (Yoon Y-K, etal, AACR08, Abs. 4853).

Stimulation of EGFr or HEr3 mediates resistance to Met inhibition in Met-amplified gastric cancer cells coexpressing EGFr and HEr3, thus blocking the antiproliferative effects of Met inhibition. Combined inhibition of Met, EGFr and HEr3 signaling sensitizes these cells to Met inhibition, making it a promising approach for Met-amplified tumors coexpressing EGFr and/or HEr3 (Bachleitner-Hofmann T, etal, ASCO08, Abs. 4975).

MicroRNA-7 is one of numerous microRNAs (miRNA) that are short non-coding RNA molecules playing regulatory roles in animals and plants by repressing translation or cleaving RNA transcripts. MicroRNAs inhibit expression of numerous targets, usually through binding to the 3'-UTR. The specific modulation of several miRNA has been recently associated with some forms of human cancer, suggesting that these short molecules may represent a new class of genes involved in oncogenesis.

MicroRNA-7 is a putative tumor suppressor in glioblastoma multiforme (GBM) inhibiting both the EGFr and the Akt pathways. Transfection with microRNA-7 potently suppressed EGFr expression through its 3'-UTR, and independently inhibited the Akt pathway through targeting of upstream regulators of Akt. Expression of microRNA-7 was downregulated in GBM relative to normal brain, with a mechanism involving impaired processing. Importantly, transfection with microRNA-7 decreased the viability and invasiveness of established and primary GBM lines. Therefore, microRNA-7 as a regulator of major cancer pathways and may have a therapeutic potential in GBM (Kefas B, etal, AACR08, Abs. 5021).

PC cell-derived growth factor (PCDGF/GP88), an 88-kDa glycoprotein growth factor overexpressed in 80% of cases of invasive ductal carcinoma of the breast, stimulates proliferation and confers trastuzumab resistance to HEr2-overexpressing breast cancer cells. Scientists at A&G Pharmaceutical (Columbia, MD) sought to determine whether increased levels of PCDGF/GP88 confer trastuzumab resistance in ErbB2-overexpressing MCF-7 and SKBR3 breast cancer cell lines. Exogenous introduction of PCDGF/GP88 induced phosphorylation of HEr2 in a dose and time-dependent manner in these cells. In addition, overexpression of PCDGF/GP88 conferred trastuzumab resistance in ErbB2-overexpressing cells. Furthermore, overexpression of PCDGF/GP88 in HEr2-overexpressing cells provided a growth advantage over ErbB2-overexpressing cells without increased levels of PCDGF/GP88. Lastly, PCDGF/GP88 induced the phosphorylation of MAPK in a time-dependent manner in ErbB2-overexpressing cells, and pretreatment with trastuzumab did not attenuate the phosphorylation levels of MAPK induced by PCDGF/GP88. These data suggest that PCDGF/GP88 confers trastuzumab resistance in HEr2-overexpressing cells. Thus, increase in PCDGF/GP88 levels may indicate trastuzumab unresponsiveness in breast cancer (Kim WE, and Serrero G, Cancer Res, 15 Jul 2006;12(14 Pt 1):4192-9).

Periostin, a mesenchyme-specific gene product, is a contributor to epithelial-mesenchymal transition (EMT) and metastatic potential. During the tumor metastasis process, epithelium-derived tumor cells undergo EMT, a fibroblast-like transformation, which contributes to aggressive tumor behavior. Investigators at the University of Massachusetts Amherst (Springfield, MA) identified periostin as a contributor to EMT and metastatic potential. Transduction of periostin into 293T cells induces cell invasive activity through EMT. Stable expression of a periostin transgene in tumorigenic but nonmetastatic 293T cells caused cells to undergo fibroblast-like transformation accompanied by increased expressions of vimentin, EGFr, and matrix metalloproteinase-9 (MMP9). Migration, invasion, and adhesion increased in cells expressing ectopic periostin by 2 to 9-fold. Invasive characteristics required signaling through integrin $\alpha v\beta 5$ and EGFr. In addition, periostin-engineered 293T cells formed metastases in immunodeficient mice. Therefore, periostin plays an active role in EMT and metastasis that requires cross talk between integrin and EGFr signaling pathways (Yan W, etal, J Biol Chem, 14 Jul 2006;281(28):19700-8).

Phosphatidylinositide 3-kinases (PI3K) comprise a family of enzymes that phosphorylate the 3'-hydroxyl of phosphatidylinositol (PtdIns). They constitute a family of evolutionarily conserved lipid kinases that regulate a vast array of fundamental cellular responses, including growth, proliferation, transformation, differentiation and protection from apoptosis, survival, motility, invasion, and angiogenesis.

Investigators from the University of California San Francisco (UCSF) studied EGFr-driven glioma differing in PTEN status, treated with erlotinib and PI-103, a dual inhibitor of PI3K catalytic α polypeptide (PIK3C2A) and mTOR, developed by PIramed Pharmaceuticals that was acquired by Roche in May 2008. Erlotinib blocked proliferation only in wt PTEN cells expressing EGFr. In glioma with mutated PTEN, PI-103 greatly enhanced the antiproliferative activity of erlotinib. Furthermore, in wt PTEN glioma combining PI103 and erlotinib was superior to either monotherapy or to therapy combining erlotinib with either rapamycin or PIK90 that inhibits PI3Kα. Findings of this study offer a mechanistic rationale for targeting EGFr, PI3Kα, and mTOR in the treatment of EGFr-driven, PTEN-mutant glioma (Fan QW, etal, Cancer Res, 1 Sept 2007;67(17):7960-5).

Platelet-derived growth factor receptor (PDGFr) is a class III RTK that, upon PDGF-induced receptor dimerization, mediates the mitogenic effects of PDGF by binding and phosphorylating a variety of intracellular signaling proteins.

Because there is an approved drug, Sunitinib malate (Sutent; Pfizer), an oral, multitargeted inhibitor of VEGFr and PDGFr TKI with efficacy in patients with metastatic renal cell carcinoma (RCC), it has been possible to investigate the combination of PDGFr inhibitors with EGFr inhibitors to determine the effect of simultaneous inhibition of PDGFr and EGFr TKI in the clinical setting. In addition to trials in RCC, a randomized phase III clinical trial is evaluating the combination of erlotinib and sunitinib versus erlotinib and placebo in metastatic, platinum-refractory nsclc; and a phase II clinical trial is evaluating sunitinib, in combination with trastuzumab in metastatic HEr2-overexpressing breast cancer. However, sunitinib's multitarget mechanism of action, particularly its inhibition of VEGFr, limits its relevance as a measure of the effectiveness of PDGFr inhibition in combination with ErbB-pathway inhibitors.

A phase I/II clinical trial (protocol ID: A6181038; NCT00113529) was conducted with sunitinib, in combination with gefitinib, in chemotherapy-naïve patients with metastatic RCC refractory to one immunotherapy regimen. According to the protocol, patients were treated with PO sunitinib (37.5 mg) at the MTD determined in the phase I portion of this trial, once daily in 6-week cycles (4 weeks on treatment, 2 weeks off) plus gefitinib (250 mg/day). The primary endpoint of the phase II trial was objective response rate (ORR) by RECIST.

A total of 35 patients were treated at the MTD from phase II (n=31) and phase I (n=4); 24 (69%) of these patients had been previously treated with immunotherapy. An exploratory analysis of VEGF pathway biomarkers was performed in phase II. The ORR was 34%, and disease stabilized in 11 (31%) patients. Median response duration was 18 months, median PFS was 11 months, median OS had not been reached, and the 1-year survival probability was 82%. The most common Grade 3 treatment-related AE included diarrhea (14%) and gastrointestinal (GI) hemorrhage (6%); 3 patients were withdrawn from the trial because of treatment-related AE (ejection fraction decline, cardiac arrhythmia, and asymptomatic ventricular tachycardia). Increases in plasma VEGF-A levels and decreases in plasma VEGF-C and soluble VEGFr2 and VEGFr3 levels were observed in patterns generally consistent with those for sunitinib monotherapy. In a preliminary analysis, no statistically significant correlations between these protein levels and efficacy were noted. Results from this trial demonstrate the feasibility of this combination, with similar efficacy and tolerability to that observed with sunitinib monotherapy, along with similar patterns of biomarker modulation (Redman BG, etal, ASCO08, Abs. 6014).

Investigators at M. D. Anderson Cancer Center (Houston, TX) conducted a study to determine whether coexpression of EGFr and PDGFr, often observed in urothelial carcinoma, is redundant or if cross talk exists between them in regulating biologic functions. The expression levels of EGFr, and PDGFr were determined in 10 urothelial carcinoma cell lines. The UM-UC5 cell line, which expresses EGFr but not PDGFr-β and is highly sensitive to therapy with cetuximab, was stably transfected with a PDGFr construct to assess the effects of treatment with cetuximab alone and in combination with a PDGFr inhibitor. Based on cell proliferation assays using single and combination therapy in cell lines which coexpress both RTK, two response patterns were identified, one in which EGFr and PDGFr blockade was additive (UMUC3 and UMUC14) and one in which there was no additive affect (UM-UC13 and KU7).

In similar experiments performed on UM-UC5 and its PDGFr-expressing derivates, the cetuximab IC_{50} for cellular growth inhibition shifted from <20 nM in UM-UC5 to >100 nM in UM-UC5/PDGFr-expressing clones. Furthermore, significant apoptosis was observed in the cetuximab-sensitive parental cells compared to none in the PDGFr transfectants. PDGFr expressing clones were 3 times more invasive than the parental cells, although this effect was blocked by either PDGFr or EGFr inhibition. Dual inhibition of the receptors did not have an additive effect as was the case in the UM-UC13 and KU7 cell lines, which naturally co-express the two receptors and are resistant to cetuximab treatment. PDGFr-β appears to have a role in increasing cell proliferation, invasion and survival of human urothelial carcinoma. Based on this model, receptor expression alone does not predict the need for combination targeted therapy of RTK and suggests other factors may affect cross talk between the EGFr and PDGFr. However, PDGFr should be considered as a therapeutic target in urothelial carcinoma because its expression results in tumor cell proliferation, invasion and survival (Brown G, etal, AACR06, Abs. 1646).

Proteasome, present in all cells, plays an important role in degrading endogenous proteins such as transcrip-

tion factors, cyclins that must be destroyed to prepare for the next step in the cell cycle, proteins encoded by viruses and other intracellular parasites, and proteins that are folded incorrectly because of translation errors or encoded by faulty genes. Proteolysis is an irreversible way of limiting protein activity. Unlike other forms of regulation, such as phosphorylation and acetylation, proteolysis by the 26S proteasome represents a final and definitive means of protein removal.

The role of the proteasome in cancer centers on its indirect effects on nuclear factor κB (NFκB), a dimer encoded by two genes that is relatively abundant and controls the expression of numerous genes. In its normal state, this factor resides in the cytoplasm as an inactive cytoplasmic complex bound to inhibitory proteins of the NFκB inhibitor (IκB) family. This inactive complex is activated by a variety of stimuli; release of NFκB facilitates its translocation to the nucleus, where it promotes cell survival by initiating the transcription of genes encoding stress-response enzymes, cell-adhesion molecules, proinflammatory cytokines, and antiapoptotic proteins (Panwalkar A, etal, Cancer, 15 Apr 2004;100(8):1578-89).

In response to proinflammatory cytokines, IkB is phosphorylated, ubiquitinylated, and degraded, freeing NFκB to enter the nucleus and promote the production of interleukin (IL)-1 and IL-6, and survival factors, including inhibitors of apoptosis. One proteasome inhibitor, bortezomib (Velcade; Millennium Pharmaceuticals), a peptide boronate, prevents the proteasome in degrading IκB thus preventing NFκB from stimulating the production of these effectors. Bortezomib has been approved globally for the treatment of multiple myeloma and, as a commercialized drug, represents an attractive partner in combination trials with ErbB-pathway inhibitors. Trials are ongoing evaluating bortezomib in combination with cetuximab, erlotinib and trastuzumab.

A preclinical study performed by investigators at the Montefiore Medical Center (Bronx, NY), examined the schedule-dependent interaction between erlotinib and bortezomib in 4 cell lines, 2 sensitive to erlotinib (H-322, H-358) and 2 resistant (A-549, H-1299). In the H-358 cell line, the combination was more active than either single agent, but the effect was not additive. Bortezomib produced a G2/M arrest followed by a time-dependent enhancement of apoptosis. Erlotinib produced a G1 arrest that was most pronounced in sensitive cells. In erlotinib-sensitive cells, G1 arrest was followed by an enhancement of apoptosis. The combination of erlotinib and bortezomib produced an accumulation of cells at both G1 and G2/M, but the apoptosis enhancement was not additive.

The two agents were then administered 24 hours apart using two different schedules. Exposing the cells to bortezomib before erlotinib treatment, resulted in a similar effect as concomitant treatment with no significant apoptosis enhancement. Exposing the cells to erlotinib before bortezomib treatment resulted in G1 arrest and prevented bortezomib-induced G2/M arrest. The effect was observed in both erlotinib sensitive and resistant cells, and was followed by increased cell survival and decreased apoptosis. The results indicate that bortezomib has a narrower range of activity compared to erlotinib in human nsclc cell lines, the combination of bortezomib and erlotinib is more effective than either single agent in H-358 cell lines, but the effect is not significantly additive and that a schedule-dependent antagonistic effect is observed when cells are treated with erlotinib first in both erlotinib-sensitive and resistant cells (Piperdi B, etal, AACR04, Abs. 4010).

A preclinical study was performed to examine bortezomib and trastuzumab in HEr2 overexpressing breast cancer cell lines MDA-MB-361 [HEr2+/estrogen receptor (Er)+], MDA-MB-453 (HEr2+/Er-), HEr-2-transfected-MCF-7 (HEr2+++/Er+), and MCF-7 cells (HEr2-/Er+) as controls. A significant synergistic effect between both drugs was demonstrated *in vitro*, which was stronger in HEr2+++ than in HEr2++ cells. Response to bortezomib and trastuzumab correlated to HEr2 status and was independent of Er status and cell-growth rate. The combination of bortezomib and trastuzumab was observed to be synergistic *in vitro*, especially in cells with high levels of HEr2 (Laes J, etal, SABCS03, Abs. 369).

Toll-like receptors (TLr) comprise a family of at least 10 members, with each member involved in recognizing a variety of microorganism-derived molecular structures. TLr ligands include cell wall components, proteins, nucleic acids, and synthetic chemical compounds, all of which can activate dendritic cells (DC) as immune adjuvants (Kaisho T and Akira S, Curr Mol Med, Dec 2003;3(8):759-71). TLr are potent activators of the innate immune system and generate signals leading to the initiation of an adaptive immune response that can be used for therapeutic purposes.

TLr9, a member of the TLr family, plays a fundamental role in pathogen recognition and activation of innate immunity. Immunostimulation with TLr9 agonists also interferes with cancer cell proliferation and angiogenesis by mechanisms still incompletely understood.

An agonist of another TLr, TLr4, E6060, under evaluation by Eisai, enhances the efficacy of trastuzumab in a human HEr2-transfected syngeneic tumor in a human HEr2 transgenic mouse model (Shangzi Wang, etal, AACR08, Abs. LB-227).

The combination of a TLr agonist, in this case IMOxine (IMO-2055), an agonist of TLR9 under development by Idera Pharmaceuticals, in collaboration with Merck KGaA, was investigated alone and in combination with erlotinib, and bevacizumab, in the H358 nsclc xenograft model. Peritumoral injections of IMOxine, 3 times-a-week for 7 weeks, resulted in 53.6% growth inhibition on week 7. Intragastric administration of erlotinib once a week or intraperitoneal administration of bevacizumab twice a week for 7 weeks resulted in 47.6% and 46.4% tumor growth inhibition, respectively. Co-treatment with IMOxine

and erlotinib or bevacizumab led to 63.0% and 76.1% tumor growth inhibition, respectively, whereas the triple combination of IMOxine, erlotinib and bevacizumab led to 88.8% tumor growth inhibition. MST of mice in the single agent IMOxine, erlotinib, or bevacizumab groups was prolonged to 25, 7, or 22 days, respectively. Co-treatment with IMOxine and erlotinib or bevacizumab prolonged survival to 36 and 40 days, respectively, whereas triple combination resulted in complete regression of tumors in 43% of mice, and extended MST for >121 days. Therefore, IMOxine exerts potent antitumor activity and enhances antitumor effects of erlotinib and bevacizumab in mice (Wang D, etal, AACR08, Abs. 2078).

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), also referred to as Apo2 ligand (Apo2L), is expressed as a type II membrane protein and signals apoptosis via the death domain-containing receptors TRAIL-r1 (Dr4) and TRAIL-r2 (Dr5). Soluble recombinant derivatives of TRAIL (sTRAIL) or agonistic MAb to either functional TRAIL-r1 or TRAIL-r2 are considered as novel tumor therapeutics because of their selective apoptosis inducing activity in a variety of human tumors but not in normal cells.

Resistance to TRAIL therapy is frequently encountered necessitating combination treatments with sensitizing agents. Standard chemotherapeutics in combination with TRAIL agonists enhance TRAIL sensitivity. Also, various approaches using targeted inhibitors that either neutralize apoptotic blockades or suppress prosurvival signals, including EGFr signaling, may provide a means of overcoming TRAIL resistance (Kruyt FA, Cancer Lett, 8 May 2008;263(1):14-25).

In a study of the effects of gefitinib on apoptosis in a panel of human bladder cancer cell lines, conducted at M. D. Anderson Cancer Center, the drug failed to promote apoptosis induced by conventional chemotherapeutic agents such as gemcitabine and paclitaxel, but interacted with recombinant human TRAIL to induce high levels of apoptosis in gefitinib-responsive but not unresponsive cell lines. Molecular mechanisms downregulated active Akt and X-linked inhibitor of apoptosis protein (XIAP) expression and were mimicked by chemical inhibitors of the PI3K/Akt pathway but not of the MAPK/ERK kinase (MEK) pathway. Furthermore, direct small interfering RNA-mediated knockdown of Akt resulted in downregulation of XIAP and TRAIL sensitization, and knockdown of XIAP itself was sufficient to reverse TRAIL resistance. Therefore, EGFr pathway activation limits TRAIL-induced apoptosis via an Akt and XIAP-dependent mechanism in EGFr-dependent human bladder cancer cells, justifying further evaluation of the combination in relevant preclinical *in vivo* models (Shrader M, etal, Cancer Res, 15 Feb 2007;67(4):1430-5).

In another study, conducted at Okayama University, in Japan, the antitumor effects of gefitinib alone or in combination with TRAIL were investigated in human esophageal squamous cell cancer lines. Although all cells expressed EGFr at the protein level, the effect of gefitinib on cell growth did not correlate with the level of EGFr expression and phosphorylation of EGFr. Gefitinib caused a dose-dependent growth arrest at the G0/G1 phase associated with increased p27 expression. Gefitinib, in combination with TRAIL, was tested in TE8 cells that are resistant to TRAIL, to see if this combination induced apoptosis via the inhibition of EGFr signaling by gefitinib. Gefitinib inhibited the phosphorylation of Akt, and enhanced TRAIL-induced apoptosis via activation of caspase-3 and caspase-9, and inactivation of Bcl-xL. These findings indicate that gefitinib has anticancer properties against human esophageal cancer cells and augments the anticancer activity of TRAIL, even in TRAIL-resistant tumors (Teraishi F, etal, FEBS Lett, 1 Aug 2005;579(19):4069-75).

Vascular endothelial growth factors (VEGF) and their receptors represent the most actively pursued targets in oncology because of the marketplace success of the VEGF inhibitor bevacizumab (Avastin; Genentech). Also, the approved status of bevacizumab has encouraged its combination with approved ErbB-pathway inhibitors in numerous clinical trials, including over 10 phase III clinical trials. Although it is too early for definitive results from most of these late stage trials, results from earlier trials were somewhat promising.

Because bevacizumab's mode of action differs from VEGFr inhibitors as it involves blockade of the VEGF itself, clinical investigation is also being pursued in combining novel VEGFr inhibitors with ErbB-pathway inhibitors. The *in vivo* and *in vitro* antitumor activity of one such inhibitor, Zactima (ZD6474), under development by AstraZeneca was investigated in combination with cetuximab in human cancer cell lines with a functional EGFr autocrine pathway. *In vitro*, this combination resulted in synergistic growth inhibition in all cancer cell lines tested. *In vivo*, in nude mice bearing established human colon carcinoma (GEO) or lung adenocarcinoma (A549) cancer xenografts, treatment with ZD6474 or cetuximab delayed tumor growth by 21-28 days compared with untreated controls.

The combination of these two drugs resulted in a tumor growth delay of 120-140 days compared with controls, significantly greater than with either single agent therapy ($p<0.001$). Following combination treatment, in 3/10 A549 and 4/10 GEO xenograft-bearing mice there was no histologic evidence of tumor at the end of the experiment. Cooperative inhibition of cancer cell proliferation was observed as well as an almost complete suppression of tumor angiogenesis. The double blockade of EGFr in combination with inhibition of VEGFr2 signaling appears to be a viable treatment option (Morelli MP, etal, ASCO06, Abs. 13170).

WW domain-containing oxidoreductase (Wwox) is a tumor suppressor that is altered in many human malignancies, including breast cancer. Wwox interacts with the ErbB4 receptor, reduces nuclear translocation of the

cleaved intracellular domain of ErbB4, and inhibits its transactivation function mediated through Yes-associated protein.

In a series of 556 breast cancer samples, tested by investigators at Ohio State University (Columbus, OH), Wwox expression was absent in 36%. Also, loss of Wwox expression was associated with unfavorable outcome (p=0.02). Membranous location of ErbB4 was associated with favorable survival compared with cancer cases that lacked such ErbB4 expression (p=0.02). Wwox expression was strongly associated with membranous ErbB4 localization (p=0.0003) and with overall ErbB4 expression (p=0.0002). Coexpression of membranous ErbB4 and Wwox was associated with favorable outcome compared with cases with membranous ErbB4 and no Wwox immunoreactivity (p=0.002). *In vitro*, Wwox is associated with the two ErbB4 isoforms, JM-a CYT-1 and JM-a CYT-2, expressed in breast cancer. Moreover, expression of Wwox both *in vitro* and *in vivo* led to accumulation of total full length membrane-associated ErbB4. These results suggest that expression of Wwox is associated with ErbB4 expression and that their coexpression has prognostic significance in breast cancer (Aqeilan RI, etal, Cancer Res, 1 Oct 2007;67(19):9330-6).

CLINICAL TRIALS OF COMBINATIONS OF APPROVED ERBB-PATHWAY INHIBITORS WITH NOVEL AGENTS

This review only describes in some detail clinical trials involving non-commercialized novel targeted agents in combination with approved ErbB-pathway inhibitors, namely cetuximab (Erbitux; ImClone Systems), panitumumab (Vectibix; Amgen), nimotuzumab, erlotinib (Tarceva; OSI Pharmaceuticals), gefitinib (Iressa; AstraZeneca), trastuzumab (Herceptin; Genentech), and lapatinib (Tykerb; GlaxoSmithKline). In one case, the mTOR inhibitor everolimus (Afinitor; Novartis), an approved drug for a non-cancer indication has been considered a novel targeted agent because it has not as yet been approved for the treatment of cancer.

The novel drugs, targeting a variety of effectors (Exhibit 1), are being combined with approved ErbB-pathway inhibitors based on their mechanism of action and preclinical findings of possible synergism *in vivo*. These agents (Exhibit 2) represent a cross section of targeted drugs in clinical trials.

The worldwide market for ErbB-pathway inhibitors was $6,780.7 million in 2007 (Exhibit 3), and stood at $4,072.6 million in the first half of 2008 (Exhibit 4).

Although domestic revenues represent the largest single market, sales outside the USA accounted for nearly 61% of this sector's global sales in 2007, and 64.8% in the first half of 2008. Serving the global markets carries its own challenges in terms of treatment costs and reimbursement regulations and may also necessitate different patient selection procedures because of ethnic or racial variations in relevant biomarkers.

It is still too early to determine which combination of ErbB-pathway inhibitors with novel targeted agents would produce significant improvement in their effectiveness. Most of the trials are at an early stage and results are not expected until completion of at least phase II trials. Also, considerable research is still necessary to delineate the role of the various pathways in cancer, primarily in *in vivo* animal models. Numerous issues remain unresolved, from basic science to clinical trial design and patient selection.

Over 1,240 clinical trials were initiated with ErbB inhibitors globally, with more than half of these currently ongoing. The majority of these are combination trials with other approved drugs, including targeted drugs and standard cytotoxics. Results from these trials will have major implications in clinical practice and influence the development of novel agents.

ERBITUX COMBINATIONS

Cetuximab is under investigation in combination with numerous approved and novel agents. The market for this drug has expanded rapidly (Exhibits 3 and 4) but its future growth depends on broadening its utility within approved indications and demonstrating effectiveness in new cancer indications as monotherapy but most likely in combination with other approved anticancer agents.

At least 240 clinical trials have been initiated with cetuximab worldwide, mostly in combination with other approved anticancer agents, with 165 currently ongoing.

One of the challenges associated with the use of cetuximab, as is the case for all the drugs in this group, is selection of patients who may benefit from the drug. However, although identification of markers linked to drug performance may prove advantageous to patients and health care reimbursement systems, it would probably have a negative effect on the commercial prospects of a drug. Incorporation of the status of one marker, Kras, that predicts response to cetuximab in the CRC indication, has nearly halved the number of candidates for this treatment.

Solid Tumors

Everolimus was evaluated in a phase I clinical trial in combination with cetuximab in patients with advanced solid tumors, to assess safety, PK, and pharmacodynamic endpoints of mTOR inhibition and tumor vascular permeability. Patients with EGFr-expressing advanced solid tumors were randomized to a 3-week run-in of single agent everolimus or cetuximab, every week, followed by weekly everolimus plus cetuximab. Standard dosing of IV cetuximab was used and PO everolimus was dosed at 30 mg to 70 mg. In addition to plasma analysis for everolimus PK, 18FDG-PET and dynamic contrast-enhanced (DCE) MRI was performed before and during combination therapy, to assess any early changes in tumor metabolic activity and vascular permeability. Phosphorylation of p70S6K, a biomarker for everolimus activity, was measured in peripheral blood mononuclear cells (PBMC).

Exhibit 1
Targets of Agents Being Evaluated in Combination with ErbB-pathway Inhibitors

Developer ❑ Affiliate(s)	Generic Name ❑ Brand Name ❑ Other	Target	Most Advanced Clinical Trial Phase*
National Cancer Institute (NCI)	Alvocidib, flavopiridol ❑ NSC-649890, L86-8275, HMR-1275, HMR1275	Cyclin-dependent kinase (CDK1)	Phase II
Amgen	AMG-655, AMG 655	Tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor 2 (TRAIL-r2, TRAILr2), death receptor 5 (Dr5)	Phase I/II
Genentech	Apomab	TRAILr2	Phase II
Daiichi Sankyo ❑ Tragara Pharmaceuticals	Apricoxib ❑ TP2001, TG01, R-109339, CS-706	Cyclooxygenase-2 (COX-2)	Phase II
ArQule ❑ Kyowa Hakko Kogyo	ARQ-650RP, ARQ 650RP, ARQ 197, ARQ197	Met [hepatocyte growth factor receptor (HGFr)/c-Met]	Phase II
Bristol-Myers Squibb	Brivanib alalinate ❑ BMS-582664	Vascular endothelial growth factor (VEGF) receptor 2 (VEGFr2, VEGFr-2)/(FLK1, Flk-1)/KDR VEGFr3, VEGFr-3/(FLT4, Flt-4)/PCL Fibroblast growth factor 2 (FGF-2, FGFB, FGF2, BFGF)	Phase II
AstraZeneca	Cediranib ❑ Recentin ❑ AZD2171, AZD 2171	VEGFr Kit	Phase III
Chroma Therapeutics ❑ Cancer Research Technology, U Virginia	CHR-2797, CHR2797	Aminopeptidase M1	Phase II
Biogen Idec ❑ Memorial Sloan-Kettering Cancer Center, Duke U	CNF2024, BIIB021	Heat-shock protein 90 (Hsp90)	Phase II
Pfizer Global Research and Development	CP-751871, CP-751,871	Insulin-like growth factor 1 receptor (IGF1r, IGF-1r, IGFIr, IGF-Ir)	Phase III
Ariad Pharmaceuticals	Deforolimus ❑ AP23573, MK-8669	Mammalian target of rapamycin (mTOR)	Phase III
Eisai	E7820, NSC 719239	Integrin α2	Phase II
Eli Lilly	Enzastaurin ❑ LY317615	Protein kinase C (PKC) β (PKC-B) Phosphatidylinositol 3`kinase (PI3K) Akt (protein kinase B, PKB)	Phase III
Novartis	Everolimus ❑ Afinitor ❑ RAD001, RAD001C	mTOR	Registered in a non cancer indication
Pierre Fabre Medicament ❑ Merck	F50035, MK-0646, MK0646	IGF1r	Phase II/III
Merck KGaA ❑ Idera Pharmaceuticals, Isis Pharmaceuticals	IMOxine ❑ HYB2055, IMO-2055	Toll-like receptor 9 (TLr9), CD289	Phase II
ImClone Systems ❑ Dyax	IMC-A12, NSC-742460	IGF1r	Phase II
Incyte	INCB7839, INCB007839	Epidermal growth factor receptor (EGFr) family (HEr) A disintegrin and metalloproteinase domain 17 (ADAM 17), TACE	Phase II

— continued on next page

Dynavax Technologies ❑ U California San Diego	ISS-1018, 1018 ISS	TLr9, CD289	Phase II
Schering-Plough	Lonafarnib ❑ Sarasar ❑ SCH 66336, SCH66336	C-H-Ras	Phase II
Amgen ❑ Takeda Pharmaceutical Company	Motesanib diphosphate ❑ AMG-706	VEGFr1, Flt-1 VEGFr2 VEGFr3 Platelet-derived growth factor receptor β (PDGFrB, PDGFr) Kit Ret proto-oncogene (Ret), glial cell-line derived neurotrophic factor receptor (GDNFr)	Phase II
SuperGen ❑ U Arizona	MP470, MP-470, HPK-56, MP470.HCl	Kit Met Ret Rad51 PDGFr Axl	Phase I
Syndax Pharmaceuticals ❑ Bayer Schering Pharma	MS-275, MS275, SNDX-275	Histone deacetylase (HDAC)	Phase II
Wyeth	Neratinib ❑ HKI-272	HEr2 EGFr	Phase II
GlaxoSmithKline	Pazopanib HCl ❑ Armala ❑ GW786034, 786034	VEGFr1 VEGFr2 VEGFr3	Phase III
AEterna Zentaris ❑ Keryx Biopharmaceuticals	Perifosine ❑ KRX-0401, D-21266	Protein kinase C (PKC) Akt (protein kinase B, PKB)	Phase II
Genentech ❑ Roche	Pertuzumab ❑ Omnitarg ❑ R1273, 2C4, rhuMAb-2C4	HEr2	Phase II
Pfizer ❑ Ludwig Institute for Cancer Research, U Iowa,	PF-3512676, CPG 7909, PF-3,512,676	TLr9, CD289	Phase III
Genentech ❑ Amgen, U Pittsburgh	PRO1762, TRAIL/Apo2L, Apo2L/TRAIL, AMG 951, rhApo2L	TRAILr-2 TRAIL receptor 1 (TRAILr1), death receptor 4 (Dr4)	Phase II
Cyclacel Pharmaceuticals ❑ Institute of Cancer Research (ICR)	Seliciclib ❑ CYC200, CYC202, r-roscovitine	CDK2/cyclin E complex	Phase II
Kosan Biosciences	Tanespimycin ❑ KOS-953	Hsp90 HEr2	Phase III
Janssen Pharmaceutica ❑ Kyowa Hakko Kogyo, Ortho Biotech Products	Tipifarnib ❑ Zarnestra ❑ R115777	Ras	Phase III
AstraZeneca	Vandetanib ❑ Zactima ❑ ZD6474, AZD6474, AZD-6474	VEGFr2 EGFr Ret	Phase III
Novartis Pharmaceuticals ❑ Bayer Schering Pharma	Vatalanib ❑ PTK-787, PTK787 (PTK/ZK, ZK-222584, ZK224584, CGP-79787)	VEGFr2 Kit PDGFr	Phase III
Biogen Idec ❑ Eli Lilly, U California San Diego, PDL BioPharma	Volociximab ❑ Eos 200-4, M200	α5β1 (α-5-β-1, α5β-1, a5b1, AAB1) integrin	Phase II
Exelixis ❑ GlaxoSmithKline	XL184, EXEL-7184, NSC 718781	VEGFr2 Met	Phase III

**The trial phase is the most advanced for this agent in any setting.*

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), August 2008

A total of 12 patients in 3 cohorts were treated at 30 mg to 50 mg of everolimus. Observed toxicities were all Grade 1/2, including mucositis (2/12), rash (8/12), fatigue (6/12), anorexia (4/12), nausea (5/12), and vomiting (5/12). Disease stabilized in 2 patients with parotid and ovarian cancer, for 4+ months as best response; serum CA-125 also decreased in the patient with ovarian cancer. PET findings in patients with stable disease confirm decreased tumor metabolic activity. At these doses, neither drug appears to increase the toxicity of the other. Preliminary PET findings suggest biologic activity of the combination (Avadhani AN, etal, ASCO07, Abs. 14075).

Colorectal Cancer and Other Gastrointestinal Malignancies

Colorectal cancer (CRC) is a key indication for cetuximab. Numerous trials have been completed or are ongoing investigating this drug in every imaginable setting in all stages of CRC, mostly in combination with approved anticancer agents but also to some degree in combination with targeted novel agents.

1018 ISS, under development by Dynavax Technologies (Berkeley, CA), is an immunostimulator consisting of a short synthetic DNA sequence that triggers potent and specific biologic responses in multiple parts of the immune system, enhancing its ability to fight disease and control chronic inflammation.

A multicenter (n=3), open label, dose-escalation, phase I clinical trial (protocol ID: DV2-ONC-01; NCT00403052) was initiated in November 2006, with 1018 ISS in combination with irinotecan and cetuximab, in patients with metastatic CRC previously treated with a fluoropyrimidine, oxaliplatin or irinotecan regimen with or without bevacizumab. The objectives of this trial are to establish a safe, tolerable, and active dose of 1018 ISS, administered SC, and determine tumor response, TTP, and OS. The safety and tolerability of 1018 ISS is evaluated by periodic laboratory assessments, physical examinations, and compilation of AE. Patients assigned to one of the dose levels of 1018 ISS are treated with two 4-week cycles of 1018 ISS plus irinotecan every other week and with weekly cetuximab. Irinotecan and cetuximab are continued thereafter until disease progression or unacceptable toxicity.

In this trial, conducted at Premiere Oncology (Santa Monica, CA), under PI Lee Rosen, MD; Lombardi Comprehensive Cancer Center at Georgetown University (Washington, DC), under PI Jimmy Hwang, MD; and at the Center for Cancer and Blood Disorders (Bethesda, MD), under PI Ralph Boccia, MD, 3 cohorts of heavily pretreated patients were administered 0.2 (n=5), 0.5 (n=4), or 1.0 (n=5) mg/kg of 1018 ISS SC on weeks 2, 3, 4, 6, 7, and 8. Doses were escalated after treatment of the third patient in each cohort reached week 4. Irinotecan (180 mg/m^2) every other week, and standard dose cetuximab every week were dosed simultaneously. Patients were followed for AE, and biomarker and clinical response.

As expected because of irinotecan, GI toxicities such as nausea and diarrhea were observed, which may have been slightly increased at the highest dose of 1018 ISS; a Grade 3 diarrhea was observed in 1 patient at the 1.0 mg dose, but otherwise AE were <Grade 2. 1018 ISS did not impact irinotecan-induced myelosuppressive effects or cetuximab-induced acneform rash. MTD of 1018 ISS was not reached. The biomarker of drug activity, i.e. measurement of interferon(IFN)-α-induced genes in PBMC 24 hours post 1018 ISS dosing, was considered positive if a 3-fold increase was observed in gene copies in 2/3 genes tested in a patient. This biomarker was positive in 2/5 patients at 0.2 mg/kg, 3/4 patients at 0.5 mg/kg, and 1/2 patients at 1.0 mg/kg. Also, the amplitude of gene response (geometric mean of the fold amplification) was 6.1 ± 9.3 at 0.2, 33.1 ± 22.8 at 0.5, and 1.4 ± 1.2 at 1.0 mg/kg.

Among 12 evaluable patients, disease progressed in 7 at a median of 70 days (range=21-258). Disease stabilized in the remaining 5 for a median of 120 days (range=56-180); 2 patients were too early to be assessed. Results from this trial indicate that 1018 ISS may be administered safely over a range of doses with standard doses of irinotecan and cetuximab. The appropriate dose of 1018 ISS among those tested appears to be 0.5 mg/kg, based upon biomarker assessment. These results are encouraging as more than half the patients enrolled in this trial had been previously treated with either or both irinotecan and cetuximab (Hwang JJ, etal, ASCOGI08, Abs. 317).

Apo2L/TRAIL, a dual proapoptotic receptor agonist directly activating both proapoptotic death receptors Dr4 and Dr5, under development by Genentech (South San Francisco, CA), in collaboration with Amgen (Thousand Oaks, CA), is being investigated in an ongoing multicenter (n=3), open label, dose-escalation phase Ib clinical trial (protocol ID: APO3583g; NCT00671372), initiated in the USA, in July 2006. According to the protocol, multiple doses of IV Apo2L/TRAIL are administered in combination with IV irinotecan and cetuximab in patients with previously treated metastatic CRC, refractory to 5-FU-based chemotherapy. The trial's primary objective is to determine the safety and tolerability of this combination. Approximately 27 patients are to enroll in this trial, being conducted at Duke University Medical Center (Durham, NC), Sarah Cannon Cancer Center (Nashville, TN), and Northwest Medical Specialties (Tacoma, WA).

Apomab, under development by Genentech, is a fully human, optimized IgG1/3 MAb that induces tumor-cell apoptosis by targeting TRAIL-r2 (Dr5).

A multicenter (n=3), open label, dose-escalation, phase Ib clinical trial (protocol ID: APM4187g; NCT00497497) was initiated in October 2007, in the USA, with Apomab in combination with cetuximab and irinotecan in patients with metastatic CRC following, or intolerant to, treatment with 5-FU, oxaliplatin, and bevacizumab-based therapy. The trial's primary objective is incidence and nature of DLT. Secondary objectives include incidence, nature, and

severity of AE; change in vital signs; incidence and severity of infusion reactions; change in clinical laboratory results; incidence of anti-Apomab antibodies; rate and duration of objective response; PFS; changes in serum biologic tumor markers; and predictors of response to Apomab in archival tumor tissue and sera. The trial is to enroll 15 patients. Participating institutions include California Pacific Medical (San Francisco, CA), University of Colorado Health Sciences Center (Aurora, CO), and M. D. Anderson Cancer Center (Houston, TX).

Brivanib (BMS-582664), under development by Bristol-Myers Squibb, is a dual inhibitor of VEGFr2 and fibroblast growth factor receptor 1 (FGFr1). Several trials have been completed or are ongoing evaluating brivanib in combination with standard dose cetuximab.

Brivanib is being investigated in a multicenter (n=11), randomized, double blind, phase I/II clinical trial (protocol ID: CA182-025; NCT00594984), initiated in May 2008, in the USA, Argentina, and Republic of Korea, in combination with cetuximab and irinotecan, in patients with advanced, metastatic CRC. Primary outcomes are safety, tolerability, and PK and markers of exploratory coagulation pathways. Secondary outcomes are tumor progression and PFS. Patients in arm 1 are treated with the standard cetuximab regimen, IV irinotecan (350 mg/m^2) every 3 weeks, and daily PO brivanib escalated from 200 mg, to 400 mg, 600 mg, and 800 mg, until disease progression. Treatment in arm 2 consists of cetuximab and irinotecan as in arm 1, plus placebo. The trial is to enroll approximately 114 patients.

An international, multicenter (n=6), dose-escalation phase I clinical trial (protocol ID: CA182-003; NCT00207051) was initiated in September 2005, in the USA, Canada and Europe (Netherlands), to determine the safety, PK and pharmacodynamics of brivanib, in combination with full dose cetuximab, in patients with advanced CRC previously treated with no more than two regimens for metastatic CRC. Expected total enrollment is 30 patients. Among participating centers is the University of California San Francisco (protocol ID: 5.05453), Karmanos Cancer Center, under PI Pat LoRusso, MD, and VU Medical Center (Amsterdam, Netherlands), under PI C.J. van Groeningen, MD. This trial was closed to recruitment in 2007.

A multicenter (n=8), nonrandomized, open label, dose-escalation, phase I clinical trial (protocol ID: CA182-003; NCT00207051) was initiated in January 2006, in the USA, Netherlands, and Canada, to assess the safety, PK, and pharmacodynamics of brivanib, combined with full dose cetuximab, in patients with advanced refractory GI malignancies. Primary outcomes are safety, DLT assessed during the dose-escalation portion of the protocol, and MTD. Secondary outcomes are duration of response and TTP during treatment. Treatment consists of daily brivanib (800 mg) PO and standard weekly cetuximab dosing, for up to 48 weeks. Participating centers include USC/Norris Comprehensive Cancer Center, Georgetown University Lombardi Comprehensive Cancer Center, H. Lee Moffitt Cancer Center, University of Miami Miller School of Medicine, and Karmanos Cancer Institute. Approximately 50 patients are to enroll in this trial. This trial was reported closed as of February 2008.

A multicenter, randomized, double blind, placebo-controlled, phase III clinical trial (protocol ID: CAN-NCIC-CO20; CAN-NCIC-CA182009; NCT00640471) of brivanib in combination with cetuximab versus placebo in combination with cetuximab, was initiated in May 2008, by the National Cancer Institute of Canada, under Study Chair Lillian L. Siu, MD, at Princess Margaret Hospital (Toronto, Canada), in patients previously treated with combination chemotherapy (e.g., 5-FU, capecitabine, raltitrexed, or tegafur-uracil) in the adjuvant setting and/or for metastatic CRC. According to the protocol, patients are randomized to 1 of 2 arms. In arm 1, patients are treated with oral brivanib once daily and cetuximab infused over 60-120 minutes once weekly. In arm 2, patients are treated with oral placebo once daily and IV cetuximab as in arm 1. In both arms, treatment continues in the absence of disease progression or unacceptable toxicity. Tumor tissue and blood samples are analyzed for biomarker levels (collagen IV, FGF-2, and epiregulin, amphiregulin and Kras mutations status) and correlated with response. After completion of treatment, patients are followed at 4 weeks and every 8 weeks thereafter. The trial's primary objective is OS. Secondary objectives are PFS, objective response rate, duration of response, QoL, economic evaluation, safety profile, and molecular markers. Approximately 750 patients are to enroll in this trial, expected to be completed in December 2010.

E7820, under development by Eisai, is an inhibitor of integrin α2 (ITGA2) being investigated in a phase II clinical trial (protocol ID: E7820-A001-204; NCT00309179) initiated in August 2006, at the University of Miami, under PI Caio Max S. Rocha Lima, MD, and the University of Southern California, in combination with cetuximab in treating patients with advanced CRC. The trial's objectives are to determine the safety and efficacy of E7820 in this setting and identify any biomarkers relating to the drug's activity. Primary outcome measures are objective tumor response (CR or PR) and disease control (CR, PR or SD) rates based on RECIST and modified WHO criteria. Secondary outcome measures include PK and pharmacodynamics. Approximately 99 patients are to enroll in this trial.

This phase II clinical trial is based on the finding that the activity of E7820 and cetuximab is additive in CRC xenograft models. Cross talk between integrin α2 and EGFr pathways provided an additional rationale for this combination.

This phase II trial was preceded by a phase I trial of E7820 in combination with cetuximab, in patients with advanced solid tumors to establish the phase II dose and the

safety and tolerability of this combination. In the phase I clinical trial, PO E7820 was administered daily, starting on day 1 of each cycle. Standard dosing of cetuximab was started on day 8 of the first cycle and continued weekly. Secondary objectives included the evaluation of multiple pharmacodynamic markers, and serial FDG-PET imaging.

A total of 17 patients were treated in 3 cohorts at E7820 doses of 40, 70, and 100 mg/day, which is the MTD of E7820 as a single agent. A total of 46 cycles were administered (range=1-6). A single DLT, Grade 3 transaminitis, was observed at 70 mg/day, but none occurred at 100 mg. Grade 3 toxicities were acneiform rash (n=2), pruritus (n=1), and elevated AST (n=1). Grade 1/2 toxicities included rash, pruritus, anorexia and fatigue. E7820 attained maximal concentration by ~4 hours with a half life (t1/2) of 3.6 to 4.9 hours. Cmax and AUC increased dose dependently up to 100 mg. At the 100 mg dose level, integrin $\alpha 2$ levels in individual patients decreased over the first month to 72% of baseline values. VEGF levels increased at end of cycle 1 to 163 ± 103% in patients treated with E7820 at 100 mg/day. There were no significant changes in platelet function assays. There was 1 unconfirmed PR in a patient with tonsilar cancer, and disease stabilized in 5 patients (CRC=3, gastroesophageal cancer=1 and pancreatic cancer=1). These results confirm that E7820 (100 mg/day) may be safely administered with standard doses of cetuximab (El-Khoueiry AB, etal, ASCO08, Abs. 3568).

Enzastaurin, under development by Eli Lilly, is a potent, selective inhibitor of PKC-β, with antiangiogenic activity. The drug is in late stages of development for a variety of cancer indications.

A multicenter (n=19), randomized, open label, uncontrolled, phase II clinical trial (protocol ID: 10538; H6Q-MC-S018; NCT00437268), was initiated in the USA, in March 2007, to evaluate the safety and efficacy of irinotecan plus cetuximab with or without enzastaurin in patients with recurrent CRC. The trial's primary objective is to determine PFS at 6 months. Another objective is to determine PFS survival time. IV irinotecan (300 mg/m^2) is administered on day 1 every 21 days, and the standard IV cetuximab regimen is administered on day 1, 8 and 15 of a 21-day cycle until progressive disease. PO enzastaurin at a 1125 mg loading dose and 500 mg thereafter, is administered daily in 21-day cycles until progressive disease. The trial, to enroll about 115 patients, was closed as of August 2008.

Everolimus is being investigated in a randomized, open label, phase I/II clinical trial, initiated in August 2007, in the USA, at Indiana University Simon Cancer Center (Indianapolis, IN), under PI Gabriela Chiorean, MD, and Northern Indiana Cancer Research Consortium (South Bend, IN), under PI Jose Bufill, MD, to evaluate the safety and efficacy of a second line regimen consisting of irinotecan and cetuximab with or without everolimus in patients with metastatic CRC. According to the protocol, during phase I, patients are administered IV cetuximab on days 1, 8, and 15, IV irinotecan (1225 mg/m^2) on days 1 and 8, and PO everolimus daily at a dose determined at the time of registration. During phase II, patients are randomized based on UGT1A1 *28 7/7 genotype or prior irinotecan exposure. In arm 1, patients are administered cetuximab and irinotecan as above. At the time of progressive disease, patients from arm 1 crossover to be treated with cetuximab and irinotecan as in arm 1, and everolimus at MTD. In arm 2, patients are administered cetuximab, irinotecan, and everolimus at MTD. Arm 2 treatment is discontinued in the case of progressive disease.

A multicenter (n=10), open label, phase I clinical trial (protocol ID: CRAD001C2242; NCT00478634) was initiated in April 2007, in the USA, to investigate the combination of everolimus, cetuximab and irinotecan as second line treatment after failure of FOLFOX (or XELOX) plus bevacizumab (if administered as part of local standard practice) in patients with metastatic CRC. Patients are excluded if homozygous for the UGT1A1*28 allele as determined by sequencing, or were previously treated with irinotecan-based therapy or an mTOR inhibitor. The trial's primary objective is to determine DLT. Secondary objectives are to assess the drugs' PK, and PFS, OS, and the objective response rate. Approximately 80 patients are to be accrued in this trial.

IMC-A12, under development by ImClone Systems (NY, NY), is a human IgG1 MAb that blocks ligand-dependent signaling in tumor cell lines by inhibiting IGF1r.

A multicenter (n=4), randomized, open label, phase II clinical trial (protocol ID: CP13-0605; NCT00503685) was initiated in June 2007, in the USA, to evaluate the efficacy of IMC-A12 as a single agent or in combination with cetuximab in patients with metastatic CRC that progressed on at least one prior anti-EGFr therapy. According to protocol, patients in arm 1 are treated with single agent IMC-A12 (10 mg/kg) IV over 1 hour, every 2 weeks, until progressive disease, unacceptable toxicity, or withdrawal consent. In arm 2, patients are treated with cetuximab (500 mg/m^2) IV over 2 hours, followed by a 1-hour observation period, then IMC-A12 (10 mg/kg) administered IV over 1 hour; both treatments are administered every 2 weeks. PK and pharmacodynamic assessments are performed using samples from the first 10 patients enrolled in each arm at Memorial Sloan-Kettering Cancer Center (20 patients total). The trial's primary objective is to assess objective response rate (ORR) by RECIST re-evaluated every 6 weeks. Participating centers include University of California Los Angeles (UCLA) Medical Center, under PI J. Randolph Hecht, MD; Yale Comprehensive Cancer Center, under PI M. Wasif Saif, MD, Memorial Sloan-Kettering Cancer Center, under PI Leonard B. Saltz, MD; and Roswell Park Cancer Institute, under PI Marwan Fakih, MD. The trial is expected to enroll 75 patients.

Exhibit 2
Combination Trials of Approved ErbB-pathway Inhibitors with Novel Agents

Developer ❑ Affiliates	Generic Name ❑ Brand Name ❑ Other	Description ❑ Administration Route	Development Status ❑ Indications
Combination Trials of Cetuximab and Novel Agents in Development			
AstraZeneca	Vandetanib ❑ Zactima ❑ ZD6474, AZD6474, AZD-6474	Potent vascular endothelial growth factor receptor 2 (VEGFr2) and epidermal growth factor receptor (EGFr) tyrosine kinase inhibitor ❑ PO	Phase I (begin 2/07, ongoing 5/08)➢USA ❑ refractory metastatic colorectal cancer
Biothera ❑ Collaborative Group, U Louisville	Imprime PGG, Imprime WPG	Binds to neutrophils, allowing them to 'see' cancer as if it were a yeast or fungal pathogen and trigger tumor cell killing ❑ injection, PO	Phase Ib/IIa (begin 10/07)➢ Philippines ❑ advanced colorectal cancer
Bristol-Myers Squibb (BMS)	Brivanib alalinate ❑ BMS-582664	Dual inhibitor of VEGFr2 and fibroblast growth factor receptor 1 (FGFr1) kinases ❑ PO	Phase I/II (begin 5/08)➢ USA, Argentina, Republic of Korea ❑ metastatic, refractory colorectal cancer, second or third line; phase I (begin 1/06, closed 2/08) ➢USA, Canada, Netherlands ❑ advanced or metastatic gastrointestinal (GI) cancer
Dynavax Technologies ❑ U California San Diego	ISS-1018, 1018 ISS	Short synthetic DNA sequence that triggers potent and specific biological responses in multiple parts of the immune system, enhancing its ability to fight disease and control chronic inflammation ❑ SC	Phase I (begin 11/06, closed 10/07)➢USA ❑ colorectal cancer, metastatic, refractory
Eisai	E7820, NSC 719239	Aromatic sulfonamide derivative that, by inhibiting integrinα2, exerts antiangiogenic activity blocking endothelial cell proliferation and tube formation ❑ PO	Phase II (begin 8/06, ongoing 6/08)➢USA ❑ inoperable or metastatic colorectal cancer
Eli Lilly	Enzastaurin ❑ LY317615	Potent, selective inhibitor of PKC β with antiangiogenic activity ❑ PO	Phase II (begin 3/07, ongoing 5/08)➢USA ❑ advanced or metastatic, recurrent colorectal cancer
Genentech ❑ Roche	Pertuzumab	Recombinant human monoclonal antibody (MAb), a HEr dimerization inhibitor (HDI), that binds to HEr2 and blocks the interaction of HEr2 and other HEr family members (EGFr, HEr3, and HEr4), designed to target tumors with normal, rather than overexpressed levels of HEr2 ❑ IV	Phase I/II (begin 11/06, suspended 6/08)➢USA ❑ locally advanced or metastatic, refractory colorectal cancer
Genentech ❑ Amgen, U Pittsburgh	PRO1762, TRAIL/Apo2L, Apo2L/TRAIL, AMG 951, rhApo2L	Soluble recombinant human Apo2L/TRAIL (rhApo2L) dual proapoptotic receptor agonist directly activating both proapoptotic death receptors (Dr4 and Dr5), selectively inducing apoptosis in a variety of cancer cells, while sparing most normal cells ❑ IV, intralesional	Phase Ib (begin 7/06, ongoing 7/08)➢USA ❑ metastatic, refractory colorectal cancer

— continued on next page

Genentech	Apomab	Fully human, optimized IgG1/3 MAb that induces tumor cell apoptosis through Dr5 ❑ IV	Phase Ib (begin 10/07, ongoing 7/08)➢USA ❑ metastatic refractory colorectal cancer
GlaxoSmithKline	Pazopanib HCl ❑ Armala ❑ GW786034, 786034	Indazolylpyrimidine inhibitor of VEGFr2 ❑ PO	Phase I (begin 10/07, ongoing 6/08)➢France ❑ relapsed or refractory, metastatic colorectal cancer, second line
ImClone Systems ❑ Dyax	IMC-A12, NSC-742460	Fully human IgG1 MAb that inhibits ligand-dependent signaling in tumor cell lines by inhibiting IGF1r ❑ IV	Phase II (begin 6/07, ongoing 7/08)➢USA ❑ metastatic colorectal cancer; phase II (begin 1/08, ongoing 7/08)➢ USA ❑ metastatic, recurrent or refractory head and neck cancer
Merck KGaA ❑ Idera Pharmaceuticals, Isis Pharmaceuticals	IMOxine ❑ HYB2055, IMO-2055	Second generation immunomodulating oligonucleotide (IMO) agonist of Toll-like receptor 9 (TLr9) containing a CpR dinucleotide motif ❑ SC, intranasal	Phase Ib (begin 9/08) ➢USA ❑ metastatic, refractory colorectal cancer
Novartis	Everolimus ❑ Afinitor ❑ RAD001, RAD001C	An ester of the macrocytic immunosuppressive agent sirolimus (rapamycin), it is an inhibitor of mammalian target of rapamycin (mTOR) kinase ❑ PO	Phase I (begin 4/07, ongoing 8/08)➢USA ❑ metastatic, refractory colorectal cancer; phase I/II (begin 8/07, ongoing 8/08)➢USA ❑ metastatic, progressive colorectal cancer, second line
Pierre Fabre Medicament ❑ Merck	F50035, MK-0646, MK0646	Recombinant humanized MAb targeting IGF-1r ❑ IV	Phase II/III (begin 11/07, ongoing 6/08)➢Europe (Germany, Spain, UK) ❑ metastatic colorectal cancer
Combination Trials of Erlotinib and Novel Agents in Development			
ArQule ❑ Kyowa Hakko Kogyo	ARQ-650RP, ARQ 650RP, ARQ 197, ARQ197	Orally administered small molecule c-Met inhibitor, the lead compound of a series of proprietary compounds generated through ArQule's Cancer Survival Protein (CSP) modulation program ❑ PO	Phase I (begin 2/08) ➢USA ❑ advanced solid tumors; phase I/II (begin 3/08)➢USA ❑ advanced non-small cell lung cancer (nsclc)
Biogen Idec ❑ Eli Lilly, U California San Diego, PDL BioPharma, Ophthotech	Volociximab ❑ Eos 200-4, M200, anti-α5β1 ❑ integrin	Chimeric MAb against α5β1 integrin (AAB1), a member of the integrin family of proteins involved in angiogenesis ❑ IV, PO	Phase II (begin 9/05, closed 2/07)➢USA ❑ advanced, metastatic or recurrent nsclc
Chroma Therapeutics ❑ Cancer Research Technology, U Virginia	CHR-2797, CHR2797	Novel, synthetic, orally active metalloenzyme inhibitor with pleiotropic activity against a range of human malignancies, based on inhibition of intracellular aminopeptidases ❑ PO	Phase I/II (begin 8/07, terminated 3/08)➢USA ❑ advanced (Stage IIIb), metastatic (Stage IV), or recurrent nsclc
Cyclacel Pharmaceuticals ❑ Institute of Cancer Research (ICR)	Seliciclib ❑ CYC200, CYC202, r-roscovitine	Small molecule inhibitor of the cyclin-dependent kinase 2 (CDK2)/ cyclin E complex leading to cell cycle changes and subsequent apoptosis in cancer cells ❑ PO	Phase I (begin 5/08)➢ Europe (Spain) ❑ advanced or metastatic nsclc
Daiichi Sankyo ❑ Tragara Pharmaceuticals	Apricoxib ❑ TP2001, TG01, R-109339, CS-706	Selective cyclooxygenase (COX-2) inhibitor being developed as an anticancer agent ❑ PO	Phase II (begin 4/08) ➢USA ❑ metastatic, refractory nsclc; phase II (begin 7/08)➢USA ❑ locally advanced or metastatic, refractory pancreatic cancer

— continued on next page

Eli Lilly	Enzastaurin ❑ LY317615	See above	Phase I/II (begin 5/07, ongoing 6/08)➢USA ❑ locally advanced (Stage IIIb) or metastatic (Stage IV), refractory nsclc
Exelixis ❑ GlaxoSmithKline	XL184, EXEL-7184	Small molecule potent inhibitor of Met and VEGFr2, preventing tumor growth, and inducing regression of various types of large tumors in xenograft models ❑ IV, PO	Phase I/II (begin 12/07, ongoing 4/08)➢USA ❑ advanced (Stage IIIb) or metastatic (Stage IV), refractory nsclc
Genentech ❑ Roche	Pertuzumab ❑ Omnitarg ❑ R1273, 2C4, rhuMAb-2C4	See above	Phase I (begin 8/06, closed 7/08)➢Europe (Belgium, Spain, UK) ❑ locally advanced or metastatic, refractory nsclc
GlaxoSmithKline	Pazopanib HCl ❑ Armala ❑ GW786034, 786034	See above	Phase I (begin 11/07, ongoing 6/08)➢USA ❑ advanced, refractory solid tumors
Janssen Pharmaceutica ❑ Kyowa Hakko Kogyo, Ortho Biotech Products	Tipifarnib ❑ Zarnestra ❑ R115777	An imidazole farnesyl transferase inhibitor (FTI) targeting activated p21 ras ❑ PO	Phase I (begin 5/04, suspended 11/04, closed 6/07)➢USA ❑ advanced solid tumors
Merck KGaA ❑ Idera Pharmaceuticals, Isis Pharmaceuticals	IMOxine ❑ HYB2055, IMO-2055	See above	Phase Ib (begin 9/07, ongoing 6/08)➢USA ❑ advanced or metastatic, refractory nsclc
Novacea ❑ Oregon Health & Science U	Asentar ❑ DN-101	Novel oral formulation of high dose calcitriol, a naturally occurring hormone, and the biologically active form of vitamin D, with potent anticancer effects ❑ PO	Phase II (begin 9/07, terminated 11/07)➢USA ❑ advanced pancreatic cancer, first line
Novartis	Everolimus ❑ Afinitor ❑ RAD001, RAD001C	See above	Phase I/II (begin 12/05, ongoing 8/08)➢USA ❑ metastatic breast cancer, third line; phase I/II (begin 12/05, ongoing 1/07)➢USA ❑ metastatic breast cancer; phase I (begin 3/05, closed 8/07)➢USA ❑ advanced solid tumors; phase I/II (begin 6/05, ongoing 8/08)➢USA, Canada, and Europe (France) ❑ advanced nsclc, first line; phase II (begin 3/08)➢USA ❑ inoperable or metastatic, refractory pancreatic cancer
Pfizer	CP-751871, CP-751,871	Fully human IgG2 antibody with high affinity for IGF1r ❑ IV	Phase III (begin 5/08)➢USA advanced refractory nsclc of non adenocarcinoma histology
Pfizer ❑ Ludwig Institute for Cancer Research, U Iowa	Formerly ProMune ❑ PF-3512676, CPG 7909, PF-3,512,676	Synthetic CPG-containing DNA mimic, a toll-like receptor 9 (TLr9) agonist, capable of stimulating cellular and humoral immune responses ❑ IV, intradermal (ID), intratumoral, SC	Phase II (begin 8/06, ongoing 6/08)➢USA ❑ advanced or metastatic, refractory nsclc, second line
Pierre Fabre Medicament ❑ Merck	F50035, MK-0646, MK0646	See above	Phase I/IIa (begin 3/08) ➢Europe (Spain) ❑ locally advanced (Stage IIIb) or metastatic (Stage IV), recurrent nsclc

— continued on next page

SuperGen ❑ U Arizona	MP470, MP-470, HPK-56, MP470.HCl	Small molecule inhibitor of the platelet-derived growth factor (PDGF)/c-Kit receptor family of tyrosine kinase receptors, and Rad51 ❑ PO	Phase I (begin 12/07, ongoing 3/08)➢USA ❑ advanced solid tumors
Syndax Pharmaceuticals ❑ Bayer Schering Pharma	MS-275, MS275, SNDX-275	Benzamide derivative, HDAC1 and HDAC3 inhibitor with potent and unique cytotoxicity and anticancer activity ❑ PO	Phase I/II (begin 12/07, ongoing 6/08)➢USA ❑ locally advanced (Stage IIIb) or metastatic (Stage IV), refractory nsclc
Combination Trials of Gefitinib and Novel Agents in Development			
AstraZeneca	Cediranib ❑ Recentin ❑ AZD2171, AZD 2171	Novel, orally active inhibitor of VEGFr and c-Kit tyrosine kinases ❑ PO	Phase I (begin 9/05, ongoing 2/06)➢Europe (Netherlands) ❑ advanced, refractory, solid tumors; phase I (begin 10/05, closed 3/08)➢USA, Europe (Spain) ❑ previously untreated or recurrent, head and neck cancer, or metastatic or recurrent nsclc
Combination Trials of Panitumumab and Novel Agents in Development			
Amgen ❑ Takeda Pharmaceutical Company	Motesanib diphosphate ❑ AMG-706	Potent, oral, small molecule angiogenesis inhibitor that selectively targets multiple kinases, including all VEGF, and PDGF receptors, and Kit and Ret ❑ PO	Phase I (begin 1/05, terminated 8/07)➢USA ❑ advanced solid tumors
Amgen	AMG-655, AMG 655	Fully human agonistic IgG1 MAb targeting TRAILr2 that induces apoptosis via caspase activation ❑ IV	Phase I/II (begin 1/08, ongoing 6/08)➢USA, Belgium ❑ metastatic colorectal cancer
Combination Trials of Trastuzumab and Novel Agents in Development			
AEterna Zentaris ❑ Keryx Biopharmaceuticals	Perifosine ❑ KRX-0401, D-21266	Novel heterocyclic alkylphospholipid (alkylphosphocholine derivative) analog of hexadecylphosphocholine (miltefosine), oral Akt inhibitor that activates the MAP kinase (MEK/ERK) pathway leading to the activation of p21 independently of p53 ❑ PO	Phase II (begin, ongoing 5/05)➢USA (combination) ❑ metastatic, refractory, breast cancer, overexpressing HEr2
Ariad Pharmaceuticals ❑ Merck	Deforolimus ❑ AP23573, MK-8669	Phosphorus-containing sirolimus analog that blocks cancer cell growth and proliferation by mTOR inhibition ❑ PO, IV	Phase II (begin 7/08) USA, Europe ❑ HEr2-overexpressing, refractory or relapsed breast cancer
Biogen Idec ❑ Memorial Sloan-Kettering Cancer Center, Duke U	CNF2024, BIIB021	Oral, fully synthetic, non-ansamycin-derived inhibitor of heat shock protein 90 (Hsp90) ❑ PO	Phase I (begin 11/07, ongoing 3/08)➢USA ❑ advanced, refractory breast cancer, second line
Genentech ❑ Roche	Pertuzumab ❑ Omnitarg ❑ R1273, 2C4, rhuMAb-2C4	See above	Phase II (completed 5/05) Europe (UK, Spain, Finland, Italy, Belgium); phase II (begin 12/05, closed 10/07)➢USA ❑ locally advanced or metastatic, refractory breast cancer, second line; phase III (begin 12/07, ongoing 6/08)➢USA ❑ locally or metastatic, HEr2 expressing, breast cancer, first line; phase II (begin 12/07, ongoing

— continued on next page

			6/08) Australia, Brazil, Canada, Europe (Austria, Italy, Poland, Spain, Sweden, Switzerland, Turkey, UK), Israel, Korea, Mexico, Peru, Russia, Taiwan, and Thailand ❑ locally advanced, inflammatory, or early stage HEr2-positive breast cancer
Incyte	INCB7839, INCB007839	Novel orally available inhibitor of the enzymatic activity of sheddases ADAM 10/ADAM 17 that play a role in controlling the growth and spread of certain malignancies that are regulated by members of the HEr family of RTK ❑ PO	Phase II (ongoing 6/08) ➢USA ❑ advanced, HEr2-expressing breast cancer
Janssen Pharmaceutica ❑ Kyowa Hakko Kogyo, Ortho Biotech Products	Tipifarnib ❑ Zarnestra ❑ R115777	See above	Phase II (begin 1/03, closed 3/04)➢USA ❑ metastatic, refractory breast cancer
Kosan Biosciences (Bristol-Myers Squibb)	Tanespimycin ❑ KOS-953	Novel formulation of 17-allylamino-17-demethoxy-geldanamycin (17-AAG) ❑ IV	Phase I (ongoing 4/06)➢USA phase I/II (ongoing 2/07); phase II (begin 12/05, ongoing 2/07)➢USA ❑ metastatic, refractory breast cancer
National Cancer Institute (NCI)	Alvocidib, flavopiridol ❑ NSC-649890, L86-8275, HMR-1275, HMR1275	Semisynthetic analog of rohitukine isolated from the bark of the Indian tree Dysoxylum binectariferum that is a potent inhibitor of CDK1, arresting cell-cycle progression in either G1 or G2 ❑ continuous IV (CIV)	Phase I (begin 1/02, completed 6/05)➢USA ❑ HEr2-positive metastatic breast cancer
Novartis Pharmaceuticals ❑ Bayer Schering Pharma	Vatalanib ❑ PTK-787, PTK787 (PTK/ZK, ZK-222584, ZK224584, CGP-79787)	Vatalanib is an orally available angiogenesis inhibitor that targets VEGFr2, Kit, PDGFr ❑ PO	Phase I/II (begin 1/05, terminated 9/06)➢USA ❑ locally recurrent or metastatic, HEr2-overexpressing breast cancer, first line
Pfizer ❑ Ludwig Institute for Cancer Research, U Iowa, Coley Pharmaceutical Group	Formerly ProMune ❑ PF-3512676, CPG 7909, PF-3,512,676	See above	Phase I/II (begin 10/01, completed 10/03)➢USA; phase I/II (begin 7/02, completed 4/04)➢USA ❑ refractory metastatic breast cancer
Schering-Plough	Lonafarnib ❑ Sarasar ❑ SCH 66336, SCH66336	Orally bioavailable nonpeptide tricyclic farnesyltransferase (FTase) inhibitor (FTI) in the pyridobenzocycloheptene class ❑ PO	Phase I (begin 8/03, ongoing 6/08)➢Europe ❑ advanced (Stage IIIb/c) or metastatic, HEr2-overexpressing breast cancer
Wyeth	Neratinib ❑ HKI-272	Orally active, irreversible dual inhibitor of HEr2 and inhibits EGFr kinase ❑ PO	Phase II (begin 3/07, closed 3/08)➢USA, China, Europe (Spain, Switzerland), South Africa ❑ advanced (Stage IIIb/c) or metastatic (Stage IV) breast cancer overexpressing HEr2
Combination Trials of Lapatinib and Novel Agents in Development			
Daiichi Sankyo ❑ Tragara Pharmaceuticals	Apricoxib ❑ TP2001, TG01, R-109339, CS-706	See above	Phase II (begin 4/08)➢USA ❑ refractory, HEr2-overexpressing breast cancer

— continued on next page

GlaxoSmithKline	Pazopanib HCl ❑ Armala ❑ GW786034, 786034	See above	Phase I (begin 7/07, ongoing 4/08)➢Japan; phase I (begin 10/04, closed 06)➢USA, Europe (Netherlands) ❑ advanced solid tumors; phase II (begin 6/06, ongoing 6/08) USA, Canada, Europe (France, Hungary, Poland, Russia, UK), India, Israel, Korea, Mexico, Pakistan, Peru, Singapore, Thailand ❑ advanced or metastatic breast cancer, HEr2-overexpressing, first line; phase III (begin 12/07) USA, Australia, Brazil, Canada, Chile, China, Egypt, Europe (Belgium, Czech Republic, France, Germany, Greece, Italy, Romania, Russia, Spain, UK), Hong Kong, Israel, Korea, Morocco, Pakistan, Peru, Philippines, Singapore, Taiwan, Thailand, Tunisia, Turkey ❑ HEr2-overexpressing, refractory or relapsed inflammatory breast cancer; phase II (begin 11/06, ongoing 5/08)➢USA, Europe, Canada, India, Thailand, Argentina, Peru, Mexico ❑ metastatic or recurrent cervical cancer; phase I (begin 8/06, ongoing 4/08)➢USA, UK ❑ recurrent malignant glioma

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), August 2008

Treatment with IMC-A12 also enhances the efficacy of cetuximab and gemcitabine therapy in human pancreatic carcinoma xenografts. The efficacy IMC-A12 in combination with cetuximab and gemcitabine was investigated in mice bearing established BxPC-3, L3.7pL, or MiaPaCa-2 pancreatic xenografts, treated with IMC-A12, cetuximab, gemcitabine, cetuximab plus gemcitabine, or IMC-A12 plus cetuximab and gemcitabine. The efficacy of IMC-A12 was significant (T/C%=41% and 37%, respectively) in the BxPC-3 and MiaPaCa-2 cell lines but minimal (T/C%=91%) in the L3.7pL model. The efficacy of cetuximab plus gemcitabine was significant in all three models, with a T/C% of 17%, 23%, and 46% for BxPC-3, L3.7pL, and MiaPaCa-2, respectively. Adding IMC-A12 to cetuximab plus gemcitabine therapy significantly increased ($p< 0.02$) the treatment's antitumor effects. The 3-agent combination was more active (T/C%=9%, 11%, and 16% in BxPC-3, L3.7pL, and MiaPaCa-2, respectively) than either monotherapy or the cetuximab plus gemcitabine combination. Therefore, this study supports adding IMC-A12 therapy to cetuximab plus gemcitabine to increase antitumor effects in pancreatic cancer (Prewett M, etal, AACR07, Abs. 652).

IMOxine (IMO-2055), a second generation immunomodulating oligonucleotide (IMO) agonist of TLr9, is to be investigated in a nonrandomized, open label, dose-escalation, phase Ib clinical trial (protocol ID: IMO-2055-210; NCT00719199), in combination with cetuximab and irinotecan, in patients with metastatic CRC refractory to first line chemotherapy, to be initiated in September 2008 at Vanderbilt-Ingram Cancer Center (Nashville, TN), under PI Mace Rothenberg, MD. According to the protocol, SC IMOxine, and a standard IV cetuximab regimen in combination with IV irinotecan (300 mg/m^2) are administered weekly in 3-week cycles. There are two distinct parts in this trial. Part 1 is a dose-escalation trial of IMOxine, administered in 4 dose groups, to establish the recommended phase II dose to be administered to a total of 12 patients for confirmation of this dose. Part 2 further explores the tolerability, PK and pharmacodynamics of this combination. Estimated enrollment is 50 patients, and estimated completion date is March 2010.

Imprime PGG, under development by Biothera (Eagan, MN), is an injectable immunomodulator that primes neutrophils, monocytes, and macrophages without

activating inflammatory cytokines. Imprime PGG binds with specific receptors on neutrophils and primes them for heightened immune activity. Imprime PGG-bound neutrophils attack and kill cancer cells in the presence of complement and antibodies, in effect, engaging the innate immune system against cancer.

In October 2007, Biothera initiated a multicenter, nonrandomized, dose escalation, open label, parallel assignment, phase Ib/IIa clinical trial (protocol ID: BT-CL-PGG-CRC0713; NCT00545545) with Imprime PGG and cetuximab in patients with metastatic, recurrent, or progressive CRC not previously treated with cetuximab or irinotecan. Primary endpoints are safety and MTD of Imprime PGG when used in combination with cetuximab and concomitant irinotecan in patients previously treated with a 5-fluorouracil-containing regimen or capecitabine (Xeloda; Roche). Secondary endpoints include PK, tumor response rates, TTP, duration of response, and duration of SD. Total enrollment is 18 patients in 3 cohorts administered Imprime PGG weekly at a dose of 2.0, 4.0, or 6.0 mg/kg, in combination with cetuximab and irinotecan. The trial is being conducted in the Philippines under PI Gerardo Cornelio, MD, at Philippine General Hospital (Manila, Philippines), and at the Medical City Hospital (Makati City, Philippines). Biothera plans to follow this trial with a double blind, placebo-controlled, phase IIb trial in a larger patient population.

MK-0646, under development by Merck, in collaboration with Pierre Fabre Medicament, is a recombinant humanized MAb targeting IGF1r. A multicenter (n=3), randomized, double blind, placebo-controlled, phase II/III clinical trial (protocol ID: 2007_529; MK0646-004; NCT00614393), was initiated in November 2007, in Europe (Germany, Spain, and UK), to evaluate the efficacy of MK-0646 in combination with cetuximab and irinotecan in patients with metastatic CRC refractory to both irinotecan and oxaliplatin-containing regimens. The trial's primary objectives are to determine OS and PFS. Secondary objectives are to assess efficacy. According to the protocol, all patients are treated with irinotecan and cetuximab. The irinotecan dose is the same as the dose of the most recent therapy administered to the patient, i.e 125 mg/m^2 once every week for 4 weeks followed by 2 weeks of rest, 180 mg/m^2 once every two weeks, or 350 mg/m^2 once every 3 weeks. Cetuximab is administered at the standard regimen. Patients are then assigned to one of 3 treatment arms at 3 different doses of MK-0646 (7.5 mg/kg, 10 mg/kg or 15 mg/kg). In arm 1 patients are administered MK-0646 (10 mg/kg) by IV infusion over 60 minutes once weekly. In arm 2, patients are administered IV MK-0646 (15 mg/kg) over 60 minutes for their first treatment once every other week. During subsequent infusions, MK-0646 (7.5 mg/kg) is administered over 60 minutes. Placebo is infused over 60 minutes when MK-0646 infusions are not scheduled. In arm 3, patients are administered placebo over 60 minutes once weekly. In all arms, treatment continues until disease progression or unacceptable toxicity. The trial is to enroll about 1,112 patients.

Pazopanib, under development by GlaxoSmithKline, is an inhibitor of VEGFr1, VEGFr2, and VEGFr3 in late stage of development for a variety of malignancies.

A multicenter (n=2), nonrandomized, open label, phase I clinical trial (protocol ID: VEG108925; NCT00540943) was initiated in October 2007, in France, to determine the MTD of pazopanib in combination with irinotecan and cetuximab as second line therapy in patients with relapsed or refractory metastatic CRC. Primary outcomes are safety and tolerability. Secondary outcomes are various PK parameters, ORR, SD at 4 months, and TTP. Approximately 45 patients are to enroll in this trial.

Pertuzumab, under development by Genentech, is a recombinant human MAb HEr dimerization inhibitor (HDI), that binds to HEr2 and blocks the interaction between HEr2 and other HEr family members (EGFr, HEr3, and HEr4). It is designed to target tumors with normal, rather than overexpressed levels of HEr2 protein.

A phase I/II clinical trial (protocol ID: DFCI-07070; NCT00551421) that was initiated in November 2006, to evaluate the safety and efficacy of pertuzumab in combination with cetuximab and irinotecan in patients with previously treated, locally advanced or metastatic CRC, has been suspended. The trial's primary objectives were to determine the recommended phase II dose and objective tumor response rate.

Vandetanib, a potent VEGFr2 and EGFr inhibitor under development by AstraZeneca, is in late stage of development in many types of malignancies, including CRC.

A phase I clinical trial (protocol ID: NCT00436072) with vandetanib, cetuximab and irinotecan was initiated in February 2007 in patients with refractory metastatic CRC. Primary objectives are to determine the tolerability and MTD of the combination. Secondary objectives are to determine response rate, PFS and OS. The main purpose of the trial is to find the highest dose of vandetanib that is safe with this combination. Initially vandetanib is to be combined with cetuximab alone. Dose escalation of vandetanib and cetuximab continues until the highest possible dose is established. Dose-escalation then proceeds with irinotecan to determine the safety of all three drugs combined. Some trial participants are to be treated with vandetanib monotherapy for 2 weeks prior to starting cetuximab and/or irinotecan treatment. Each cycle of treatment is 8 weeks long. All participants start taking vandetanib orally, on day one and continue taking it at home thereafter. Cetuximab and irinotecan are administered IV; cetuximab once weekly, and irinotecan on day 15 of cycle one, then every other week. Patients without progressive disease may continue on the trial as long as they tolerate

the drug. A total of 46 patients are to enroll in this trial. The trial is being conducted at Dana-Farber Cancer Institute, under PI Jeffrey Meyerhardt, MD, MPH; Massachusetts General Hospital, under PI Andrew Zhu, MD; and Beth Israel Deaconess Medical Center, under PI Sanjay Jain, MD.

Head and Neck Cancer

Head and neck cancer is also a key indication of cetuximab.

IMC-A12 is being investigated in a multicenter (n=3), randomized, open label phase II clinical trial (protocol ID: CP13-0706; NCT00617734), initiated in January 2008 in the USA, either as monotherapy or in combination with cetuximab, in patients with recurrent or metastatic squamous cell carcinoma of the head and neck (oropharynx, hypopharynx, or larynx) that progressed on previous platinum-based chemotherapy with or without radiotherapy. According to the protocol, patients in arm 1 are treated with a IMC-A12 (10 mg/kg) over 1 hour every 2 weeks. In arm 2, patients are treated with IMC-A12 (10 mg/kg) followed in an hour by cetuximab (500 mg/m^2), administered IV over 2 hours. A cycle is defined as 4 weeks of therapy. Patients continue treatment until progressive disease or unacceptable toxicity. The trial's primary objective is PFS. Secondary objectives include 6-month PFS rate, OS rate, duration of response, safety and tolerability and AE profile. An estimated 90 patients are to enroll in this trial to be completed in June 2009. Participating institutions include M. D. Anderson Cancer Center (Orlando, FL), under PI Jennifer Tseng, MD; University of Chicago, under PI Ezra Cohen, MD; and Thoracic Head and Neck Medical Oncology (Houston, TX), under PI Bonnie Glisson, MD.

VECTIBIX COMBINATIONS

Compared to other EGFr inhibitors, panitumumab, a late starter, is being currently evaluated in fewer clinical trials than cetuximab with which it shares the same target and a similar mechanism of action. It appears that panitumumab and cetuximab bind to surface exposed amino acids in domain III of EGFr and inhibit all known EGFr ligands, resulting in inhibition of receptor activation (Freeman D, etal, ASCOGI08, Abs. 392).

Colorectal Cancer

CRC is a key indication of panitumumab. Regarding combinations with novel targeted agents, currently, the drug is mostly evaluated with novel agents under development by Amgen, the drug's supplier.

AMG-655, under development by Amgen, is a fully human MAb agonist of TRAILr2. A multicenter (n=3), open label, phase I/II clinical trial (protocol ID: 20060332; NCT00630786) was initiated in January 2008, in the USA and Europe (Belgium), to determine the safety and efficacy of AMG 655 in combination with panitumumab in patients with metastatic CRC. Primary outcomes are tolerability in phase I and ORR in phase II. Treatment in phase I consists of 3 dose levels of AMG 655 (10 mg/kg, 3 mg/kg, or 1 mg/kg) to be evaluated in a de-escalation manner in combination with panitumumab (6 mg/kg). In phase II, AMG 655 is administered at a tolerable dose level determined in phase I, in combination with panitumumab. Approximately 65 patients are to enroll in this trial.

AMG-706, also under development by Amgen, is a multitargeted oral inhibitor of VEGFr, PDGFr, Kit and Ret, in phase II clinical development in numerous indications. A multicenter (n=9), open label, dose-finding, phase Ib clinical trial (protocol ID: UCLA-0410089-01; AMGEN-20040205; NCT00101894; NCT00107328) was initiated in December 2004, in the USA and Australia, to evaluate the safety of AMG-706, panitumumab and FOLFIRI (irinotecan/folinic acid/5-FU) or FOLFOX (oxaliplatin/5-FU/leucovorin) in the treatment of patients with metastatic CRC refractory to one prior chemotherapy for advanced disease (second line) and no prior oral VEGFr multikinase inhibitor or anti-EGFr therapy. Participating sites include the Jonsson Comprehensive Cancer Center at UCLA, West Clinic (Memphis, TN), Duke University Medical Center (Durham, NC), Cancer Center of the Carolinas (Greenville, SC), Ashford Cancer Center, in Australia, Prince of Wales Hospital (Randwick, Australia), Austin Hospital (Heidelberg, Australia), Sarah Cannon Cancer Center (Nashville, TN), among others. Objectives are to establish safety, PK, and MTD of AMG-706 with this regimen.

In this trial, treatment consisted of a standard FOLFIRI or FOLFOX regimen plus IV panitumumab (6 mg/kg) on day 1 of each 2-week cycle, and escalating doses of AMG-706 (50, 75, 125 mg daily or 75 mg twice daily) from day 3 of cycle 1. As of November 2006, 45 patients, 64% having been previously treated with chemotherapy, were enrolled and treated with at least one dose of AMG-706 (FOLFIRI=33 and FOLFOX=12). Treatment-related AE occurred in 10% of patients. PK of AMG-706 at 50 mg daily with FOLFOX and 50-125 mg daily with FOLFIRI were comparable to data from monotherapy trials at the same dose levels. AMG-706 did not significantly alter the PK profiles of irinotecan or its metabolites. Among 32 evaluable patients, overall tumor response (CR+PR) was observed in 16 (50%) (FOLFIRI/FOLFOX=11/5) patients; CR was observed in 1 patient (FOLFOX), PR in 15 (11/4), SD in 13 (10/3), and progressive disease in 3 (1/2) patients (Schwartzberg LS, etal, ASCO07, Abs. 4081).

Non-small Cell Lung Cancer

AMG-706 was also investigated in a multicenter open label, dose-finding, multicenter (n=3) phase Ib clinical trial (protocol ID: UCLA-0408078-01, AMGEN-20040153, NCT00094835; NCT00107224) with or without carboplatin/paclitaxel or panitumumab in patients (n=70) with advanced (Stage IIIb) or metastatic (Stage IV) nsclc, initiated in the USA, in November 2004, to determine the safe-

ty, PK, and response rate for these regimens. This trial was closed as of December 2007.

According to this trial's protocol, patients with no prior chemotherapy for nsclc (segment A and C) or one previous regimen for nsclc (segment B) were treated with PO AMG-706 either once daily (50 mg or 125 mg) or twice daily (75 mg), in combination with paclitaxel (200 mg/m^2) and carboplatin (AUC=6 mg/mL/min) once every 3 weeks (segment A); with panitumumab (9.0 mg/kg) every 3 weeks (segment B); or with the carboplatin/paclitaxel and panitumumab regimen (segment C). AMG-706 was dosed continuously in 21-day cycles (days 3-21 in cycle 1 and days 1-21 in cycle 2 and beyond). Patients were sequentially enrolled into escalating AMG-706 dose cohorts. As of September 2005, 22 patients had been enrolled (segment A=10, segment B=12) into AMG-706 dose cohorts of 50 mg (segment A=6, segment B=7) and 125 mg (segment A=4, segment B=5) once daily. In the 125 mg once daily cohort in segment B, there was one case of Grade 5 pneumonia. Treatment-related AE occurring in >5% of patients include fatigue (segment A=3); hypertension (segment A=4; segment B=2) and other Grade 3 events (segment A=6; segment B=4). AMG-706 PK profiles were similar when administered with carboplatin/paclitaxel either 30 minutes or 48 hours apart. AMG-706 at 50 mg once daily had no effect on the PK of paclitaxel and could be combined safely with carboplatin/paclitaxel or panitumumab in patients with advanced nsclc (Blumenschein GR, etal, ASCO06, Abs. 7119).

A multicenter (n=3), open label, dose-finding, phase Ib clinical trial (protocol ID: 20040206, NCT00101907) of AMG-706, in combination with panitumumab, gemcitabine, and cisplatin, was initiated in January 2005, in the USA, in patients with advanced solid tumors (mostly nsclc) previously treated with only one chemotherapy regimen. The trial's objectives are to determine DLT, safety and tolerability, PK, MTD and response rate. The trial was to enroll 80 patients, but was terminated before completion at the recommendation of reviewers.

According to an interim report from this trial, as of November 2006, 36 patients (nsclc=19, pancreatic cancer=4, other10, unknown primary=3) had enrolled in the trial with 42% having been treated with prior chemotherapy. Patients were treated with IV panitumumab (9 mg/kg) on day 1 of each 3-week cycle, plus IV gemcitabine (1250 mg/m^2) on days 1 and 8, and IV cisplatin (75 mg/m^2) on day 1, and escalating doses of PO AMG-706 (50, 75, 100, 125 mg daily, or 75 mg twice daily) administered continuously from day 1 of cycle 1. Grade 5 pulmonary embolism at a daily dose of 50 mg was the sole DLT. Treatment-related AE included nausea and vomiting, fatigue, hypertension, and anorexia; thromboembolic events occurred in 39% of patients treated with AMG compared to 25% treated with the other drugs without AMG-706. There was a Grade 1 case of cholecystitis, and a Grade 3 case of gallbladder pain. AMG-706 PK at the 125 mg daily dose was comparable to that from monotherapy trials at the same dose level. Based on 29 patients evaluable for response, there was 1 (3%) CR in a patient with breast cancer, 9 (31%) PR in patients with nsclc (n=6), pancreatic cancer (n=2), and unknown primary (n=1), and disease stabilized in 17 (59%) patients and progressed in 1 (3%). AMG-706 was tolerable in this regimen, which had little effect on its PK (Crawford J, etal, ASCO07, Abs. 14057).

TARCEVA COMBINATIONS

Erlotinib is being investigated in a very broad clinical program in combination with cytotoxics and/or approved targeted agents. Over 343 clinical trials, mostly in combination with approved targeted anticancer drugs or cytotoxics have been initiated globally with erlotinib, with 220 currently ongoing.

The global market for Tarceva has grown rapidly reaching $886 million in 2007, up 36.3% from 2006 levels, and even faster in the first half of 2008, up 46.6% at $599.8 million (Exhibits 3 and 4).

Solid Tumors

ARQ 197, under development by ArQule (Woburn, MA), is a c-Met inhibitor that in preclinical studies demonstrated antitumor activity against several types of xenografted human tumors in mice.

A multicenter (n=3), nonrandomized, open label, phase I clinical trial (protocol ID: ARQ 197-111; NCT00612703), was initiated in the USA, in February 2008, to evaluate the safety and efficacy of ARQ 197 in combination with erlotinib in treating patients with advanced solid tumors. The trial's primary objectives are to determine safety, tolerability, and recommended phase II dose. Secondary objectives are to determine the PK profile and to assess preliminary antitumor activity. The trial is to enroll about 40 patients.

MP470, under development by SuperGen (Dublin, CA), was specifically developed to bind to mutant forms of the c-Kit receptor. While a potent inhibitor of wt c-Kit, because of its unique binding mode, MP470 is even more effective against clinically relevant mutants, including V560G, D816V, N822K and K642E. Because the activity of MP470 is increased in cells with mutant receptors, it may work in cases where other inhibitors fail. Also, because MP470 is active against mutated forms of these receptors it has high selectivity for cancer cells, thus potentially reducing side effects and toxicity.

A multicenter, open label, dose-escalation, phase I clinical trial (protocol ID: SUPERGEN-SGI-0470-02; NCT00602875) of MP470 and standard chemotherapy was initiated in the USA, in December 2007, in patients with advanced solid tumors appropriate for treatment with carboplatin/paclitaxel, carboplatin/etoposide, topotecan hydrochloride, docetaxel, or erlotinib. Chris H. Takimoto, MD, of South Texas Accelerated Research Therapeutics (San Antonio, TX) is Study Chair. According to the proto-

Exhibit 3
Global Sales of ErbB-pathway Targeted Agents in 2006/2007

		USA Sales ($ million)			ROW Sales ($ million)			Total Sales ($ million)		
Developer	**Drug Designation**	**2007**	**2006**	**Change (%)**	**2007**	**2006**	**Change (%)**	**2007**	**2006**	**Change (%)**
Genentech ❑ Roche	Herceptin ❑ Trastuzumab	1,287.0	1,234.0	4.3	2,750.0	1,900.0	44.7	4,037.0	3,134.0	28.8
ImClone Systems ❑ Merck KGaA, Bristol-Myers Squibb	Erbitux ❑ Cetuximab	691.0	646.0	6.9	656.7	454.0	44.6	1,347.7	1,100.0	25.5
OSI Pharmaceuticals ❑ Genentech, Roche	Tarceva ❑ Erlotinib	417.0	402.0	3.7	469.0	248.0	89.1	886.0	650.0	36.3
AstraZeneca	Iressa ❑ Gefitinib	9.0	8.3	8.4	229.0	228.7	NA	238.0	237.0	0.4
Amgen	Vectibix[1] ❑ Panitumumab	170.0	39.0	NA				170.0	39.0	NA
GlaxoSmithKline	Tykerb[2] ❑ Lapatinib	72.0	NA	NA	30.0	NA	NA	102.0	0.0	NA
Total		2,646.0	2,329.3	13.6	4,134.7	2,830.7	46.0	6,780.7	5,160.0	31.4

[1] *Approved in the USA in September 2006 and the European Union in December 2007*
[2] *Approved in the USA in March 2007*
Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), August 2008

col, the trial is evaluating 5 regimens. In regimen 1, patients are treated with paclitaxel IV over 3 hours and carboplatin IV over 1 hour on day 1, and oral MP470 once daily on days 2-21 of course 1 and on days 1-21 of all subsequent courses. In regimen 2, patients are treated with carboplatin IV over 1 hour on day 1 and etoposide IV over 2 hours on days 1-3, and MP470 as in regimen 1. In regimen 3, patients are treated with topotecan IV over 30 minutes on days 1-5, and MP470 as in regimen 1. In regimen 4, patients are treated with docetaxel IV over 1 hour on day 1, and MP470 as in regimen 1. In regimen 5, patients are treated with oral erlotinib once daily on days 1-21, and oral MP470 once daily on days 16-21 of course 1 and on days 1-21 of all subsequent courses. In all arms, treatment repeats every 21 days for up to 8 courses in the absence of disease progression or unacceptable toxicity.

Primary outcome measures include safety as assessed by patient-reported and investigator-observed AE, clinical laboratory tests (i.e., hematology, metabolic profile, and urinalysis), ECG, echocardiograms or MUGA scans, and physical examination; response to treatment as assessed by RECIST; and effects of MP470 on the PK of standard of care agents as assessed by concentrations in plasma before and after dosing with MP470. Also, Rad51 expression in skin punch biopsies is assessed as a pharmacodynamic marker. After completion of treatment, patients are followed for 30 days. Estimated enrollment is 105 patients.

Pazopanib has been entered in a 2-center, nonrandomized, open label, phase I clinical trial (protocol ID: VEG109607; NCT00619424), initiated in November 2007, to evaluate the drug's safety in combination with either erlotinib or pemetrexed (Alimta; Lilly) in patients with advanced solid tumors refractory to standard therapy or for which there is no standard therapy. Primary outcome is MTD. Secondary outcomes are antitumor activity, PK (AUC, Cmax, tmax and t1/2), response rate (CR, PR, SD), and levels of circulating cytokine and angiogenic factors biomarkers such as IL-2, IL-10, VEGF, and sVEGFr2. Cohorts of 3 patients in each arm are administered escalating doses of pazopanib and erlotinib or pazopanib and pemetrexed. Subsequently, 6 to 12 additional patients in each arm are to be enrolled at the MTD. Approximately 55 patients are to enroll in this trial.

Tipifarnib, a farnesyltransferase inhibitor (FTI) targeting activated Ras, originally under development by Janssen Pharmaceutica (Beerse, Belgium), is currently being investigated in several combination trials by the National Cancer Institute (NCI) in both solid tumors and hematologic malignancies.

Exhibit 4
Global Sales of ErbB-pathway Targeted Agents in the First Half of 2008

		USA Sales ($ millions)			ROW Sales ($ millions)			Total Sales ($ millions)		
Developer	**Drug Designation**	**2007**	**2008**	**Change (%)**	**2007**	**2008**	**Change (%)**	**2007**	**2008**	**Change (%)**
Genentech ❑ Roche	Herceptin ❑ Trastuzumab	640.0	677.0	5.8	1,300.3	1,682.6	29.4	1,940.3	2,359.6	21.6
ImClone Systems ❑ Merck KGaA, Bristol-Myers Squibb	Erbitux ❑ Cetuximab*	322.0	382.6	18.8	303.3	458.1	51.0	625.3	840.6	34.4
OSI Pharmaceuticals ❑ Genentech, Roche	Tarceva ❑ Erlotinib	204.0	230.0	12.7	205.0	329.8	60.8	409.0	599.8	46.6
AstraZeneca	Iressa ❑ Gefitinib	5.0	3.0	-40.0	108.0	122.0	13.0	113.0	125.0	10.6
Amgen	Vectibix ❑ Panitumumab	96.0	57.0	-40.6	NA	9.0	NA	96.0	66.0	-31.2
GlaxoSmithKline	Tykerb ❑ Lapatinib	26.0	42.0	61.5	6.0	40.0	>100	32.0	81.6	>100
Total		1,293.0	1,391.6	7.6	1,922.6	2,641.5	37.4	3,215.6	4,072.6	26.7

Includes sales to Canada

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), August 2008

A dose-escalation, phase I clinical trial (protocol ID: MAYO-MC0212, NCI-6014, NCT00085553) of erlotinib and tipifarnib in patients with advanced solid tumors, was initiated in May 2004 at the Mayo Clinic (Rochester, MN), under PI Alex Adjei MD, PhD, to determine MTD, toxicity, and tumor response. Patients are administered PO erlotinib once daily on days 1-28, and PO tipifarnib twice daily on days 1-21. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Cohorts of 3-6 patients are administered escalating doses of erlotinib and tipifarnib until the MTD is determined; up to 20 additional patients are then treated at the MDT. Patients are administered tipifarnib as above, and erlotinib once daily on days 1-28 (days 8-28 of course 1 only). Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Patients are followed at 3 months. A total of 18-50 patients (20 treated at MTD) are to be accrued for this trial. The trial was closed as of May 2008.

Among 12 patients treated through 4 dose levels, including 75 mg erlotinib with 200 mg tipifarnib, 100 mg erlotinib with 200 mg tipifarnib, 100 mg erlotinib with 300 mg tipifarnib, and 150 mg erlotinib with 300 mg tipifarnib, 9 treated at dose levels 1 to 3 have been administered 20 cycles of treatment and are evaluable. Treatment was also initiated in 3 patients at 150 mg erlotinib with 300 mg tipifarnib (the single agent MTD), who are not yet evaluable for toxicity. There were no DLT. Observed >/=Grade 2 treatment-related toxicities in all cycles of treatment included rash (Grade 2=15%), diarrhea (Grade 2=5%, Grade 3=5%), fatigue (Grade 2=10%), and abnormal LFT (Grade 2=10%). There were no objective responses. Disease stabilized in 4 patients for at least 2 months. Additional patients will be enrolled at the recommended phase II dose, which is anticipated to be 150 mg erlotinib with 300 mg tipifarnib, for further evaluation of dose and AE relationship, PK, and markers of farnesyltransferase and EGFr inhibition (Ma C, etal, ASCO05, Abs. 3000).

Non-small Cell Lung Cancer

Non-small cell lung cancer is a key target indication for erlotinib. Over 168 clinical trials have been initiated with the drug in nsclc, with 121 currently ongoing.

Apricoxib is an orally available selective cyclooxygenase 2 (COX-2) inhibitor. The drug was developed by Daiichi Sankyo that holds rights for the agent in Japan and other Asian markets, and has been licensed to Tragara Pharmaceuticals (San Diego, CA) in the rest of the world.

A multicenter (n=35), randomized, double blind, placebo-controlled phase II clinical trial (protocol ID: TP2001-201; APRiCOT-L; NCT00652340) was initiated in April 2008, in the USA, to evaluate the safety and efficacy

of apricoxib, in combination with erlotinib, in patients with refractory metastatic nsclc. This trial is comparing the antitumor efficacy of apricoxib and erlotinib with placebo and erlotinib to test the hypothesis that downregulation of COX-2 and EGFr in tumors with upregulated COX-2 expression will have a clinical benefit compared with erlotinib alone. According to the protocol, in arm 1, patients are treated with apricoxib (100 mg), 4 times daily, and erlotinib, and with erlotinib and placebo in arm 2. Primary outcome measures are to determine the antitumor activity of the combination of apricoxib and erlotinib compared with placebo and erlotinib as measured by TTP. Secondary outcome measures are PFS, OS, and safety and tolerability. Estimated enrollment is 115 patients. Estimated completion date is June 2009.

ARQ 197 may be effective in combination with erlotinib in nsclc because development of resistance in nsclc to therapy with EGFr inhibitors may be linked to an increase in c-Met signaling. In addition to published scientific literature supporting the role of c-Met in the onset of resistance to EGFr therapy, preclinical efficacy studies in nsclc cells, conducted by ArQule, indicate a synergy between ARQ 197 and erlotinib in halting cancer cell proliferation.

In March 2008, patient dosing was initiated in a phase I/II clinical trial of ARQ 197, administered in combination with erlotinib, in patients with advanced nsclc. The phase I trial is designed to determine the safety, tolerability and a recommended phase II dose of ARQ 197 when administered in combination with erlotinib in this patient population, and also evaluate the PK profile and assess the preliminary antitumor activity of this combination therapy. Upon completion of phase I, a multicenter, randomized, placebo-controlled, phase II trial is to be initiated to compare ARQ 197 plus erlotinib against placebo plus erlotinib.

CHR-2797, under development by Chroma Therapeutics (Abington, UK), is a novel, synthetic, orally active metalloenzyme inhibitor with pleiotropic activity against a range of human malignancies, based on inhibition of intracellular M1 aminopeptidase.

A multicenter (n=7), open label, multiple dose, phase I/II clinical trial (protocol ID: CHR-2797-005; NCT00522938) was initiated in the USA, in August 2007, to evaluate the safety and efficacy of CHR-2797, co-administered with erlotinib, in patients with histologically or pathologically confirmed Stage IIIb (with pleural effusion), metastatic (Stage IV), or recurrent nsclc. This trial involved 2 distinct phases. In phase I, the primary objectives are to determine safety, tolerability, MTD, and PK. In phase II, the primary objective is to determine the objective tumor response rate, as well as the safety, tolerability, and the trough levels of CHR-2797 and erlotinib after co-administration for 28 days. This trial was terminated as of March 2008 because of poor accrual.

CP-751,871, under development by Pfizer, is a fully human IgG2 antibody with high affinity for IGF1r.

A multicenter (n=11), randomized, open label, phase III clinical trial (protocol ID: A4021018; NCT00673049), within the ADVIGO (ADVancing IGF-Ir in Oncology) program in lung cancer, was initiated in May 2008, in the USA, to evaluate the efficacy of erlotinib alone or in combination with CP-751,871 in patients with advanced refractory nsclc of non-adenocarcinoma histology. Primary outcome is OS. Secondary outcomes are presence of circulating tumor cells (CTC) expressing IGF1r, PFS, safety, tolerability, efficacy, PK, and occurrence of anti-drug antibody in response to CP-751,871. Patients are assigned to 1 of 2 treatment arms. Treatment in arm 1 consists of IV CP-751,871 (20 mg/kg) on days 1 and 2 in cycle 1, and PO erlotinib (150 mg/day), every 3 weeks. Treatment in arm 2 consists of PO erlotinib (150 mg/day) at least 1 hour before or 2 hours after the ingestion of food. Approximately 600 patients are to enroll in this trial.

Enzastaurin was entered into a multicenter (n=11), phase I/II clinical trial (protocol ID: 11183; H6Q-MC-S030; NCT00452413), initiated in May 2007, in the USA, to evaluate the drug in combination with erlotinib in patients with refractory solid tumors and advanced (Stage IIIb with malignant pleural effusion) or metastatic (Stage IV) nsclc refractory to 1 or 2 prior regimens. The primary outcome measure of the phase I portion of the trial is to determine the MTD of this combination and of the phase II portion to establish PFS. Secondary objectives in phase I include safety and AE profile, and PK interactions between enzastaurin and erlotinib. Secondary objectives in phase II include OS, tumor response, duration of response and safety. According to the protocol, in phase I, enzastaurin doses are escalated to establish a phase II dose for the combination to be used with erlotinib (150 mg), daily, for 28-day cycles. Estimated enrollment is 58 patients. Estimated completion date is July 2009.

IMOxine is being evaluated in a multicenter (n=7), phase Ib clinical trial (protocol ID: 2055-200; NCT00633529) in combination with erlotinib and bevacizumab, in patients with advanced or metastatic nsclc refractory to first line chemotherapy. The trial, initiated in the USA in September 2007, is designed to assess the safety of the IMOxine, erlotinib, and bevacizumab combination and to determine the recommended dose of IMOxine for use in the subsequent phase II trial. A total of 3 dose regimens of weekly SC IMOxine will be tested. Target enrollment is 40 patients. The trial is being conducted in the USA, at Vanderbilt University Medical Center, under PI David Johnson, MD. Following analysis of the phase Ib results, a 4-arm, randomized, placebo-controlled, phase II clinical trial is planned to evaluate erlotinib alone, IMOxine and erlotinib, erlotinib and bevacizumab, and IMOxine, erlotinib, and bevacizumab.

The combination being evaluated in this trial is based on a preclinical evaluation of IMOxine alone and in combination with erlotinib, and bevacizumab in an H358 nsclc xenograft model. The combination of IMOxine and erlotinib or bevacizumab led to 63.0% and 76.1% tumor growth inhibition, respectively, whereas triple combination of IMOxine, erlotinib and bevacizumab led to 88.8% tumor growth inhibition. The combination of IMOxine and erlotinib or bevacizumab prolonged survival to 36 and 40 days, respectively, whereas triple combination resulted in complete regression of tumors in 43% of mice, and extended median survival for >121 days. These results demonstrate that IMOxine exerts potent antitumor activity and enhances antitumor effects of erlotinib and bevacizumab in mice (Wang D, etal, AACR08, Abs. 2078).

MK-0646, is being evaluated in a nonrandomized, open label, dose comparison, phase I/IIa clinical trial (protocol ID: 2007_605, MK0646-007; NCT00654420), initiated in March 2008, in Europe (Spain), in combination with erlotinib, in patients with locally advanced or metastatic, recurrent, Stage IIIb/IV nsclc that relapsed after chemotherapy or chemoradiotherapy. The trial's primary objectives are to determine safety, tolerability, and PFS. Secondary objectives are to determine the response rate and OS. According to the protocol, the phase I part of the trial is a safety assessment of erlotinib in combination with MK0646. Erlotinib (150 mg) is administrated PO daily, and MK0646 (5 mg/kg) is administered IV weekly; the dose is subsequently escalated to 10 mg/kg weekly to determine DLT. If the 5 mg/kg dose is not tolerated, then phase I will be terminated and phase II will not be initiated. This trial is to enroll about 68 patients.

Pertuzumab was entered in a multicenter (n=3), open label phase I clinical trial (protocol ID: WO20024), initiated in August 2006, in Europe (Belgium, Spain, UK), in combination with erlotinib, to assess the safety and tolerability, PK, and preliminary activity and determine the MTD of this regimen in patients with locally advanced or metastatic nsclc after failure of at least one previous chemotherapy regimen. Tumor specimens for biomarker evaluation potentially associated with response were available from all patients.

According to the protocol, patients were recruited in 2 cohorts. The first cohort was treated with IV pertuzumab at a loading dose of 840 mg and a 420 mg maintenance dose every 3 weeks plus daily PO erlotinib at 100 mg. If MTD was not reached, a second cohort was to be recruited to be treated with pertuzumab as the first cohort plus daily erlotinib (150 mg). Based on experience with the first cohort, the protocol was modified to exclude rash as a DLT (rash was manageable and responded to interruption or reduction of the dose of erlotinib), and a second cohort (n=9) was recruited. In the first cohort tolerability was good, the common AE being diarrhea in 3 (50%) patients, which was generally mild and self limiting, and rash, which was reported by all 6 (100%) patients. Rash was severe (Grade 3) in 3 patients, but responded to either dose reduction of erlotinib to 50 mg daily or to withdrawal of treatment. In the second cohort, the combination was well tolerated with no DLT reported to date. The dose schedule used in the second cohort appears suitable for phase II evaluation (Felip E, etal, ASCO08, Abs. 19134).

PF-3512676, under development by Pfizer, is a synthetic cytidine-phosphate-guanosine (CpG)-containing DNA mimic that is a TLR9 agonist, capable of stimulating cellular and humoral immune responses.

A multicenter (n=39), randomized, open label, phase II clinical trial (protocol ID: A8501006; NCT00321815) was initiated in the USA, in August 2006, to investigate the safety and efficacy of erlotinib with or without PF-3512676 in patients with advanced EGFr-positive nsclc after failure of at least 1 prior chemotherapy regimen. Primary outcome is PFS. Secondary outcomes are TTP, ORR, duration of response, OS, biomarkers of immune activation, and safety. Approximately 130 patients are to enroll in this trial.

Seliciclib, under development by Cyclacel (Berkeley Heights, NJ) is a small molecule inhibitor of the CDK2/cyclin E complex leading to cell cycle changes and subsequent apoptosis in cancer cells. An investigator-sponsored phase I clinical trial was initiated in May 2008, at Vall d'Hebron University Hospital (Barcelona, Spain), under PI Emiliano Calvo, MD, to investigate seliciclib in combination with erlotinib in patients with advanced nsclc.

SNDX-275/MS-275, under development by Syndax Pharmaceuticals (Waltham, MA), is an oral HDAC1 and HDAC3 inhibitor with potent and unique cytotoxicity and anticancer activity.

A multicenter (n=4), randomized, phase I/II clinical trial (protocol ID: SNDX-275-0401; NCT00602030) was initiated in December 2007, at two Rocky Mountain Cancer Centers (Denver and Lakewood, CO), under PI Samir Witta, MD, at Texas Oncology-Sammons Cancer Center (Dallas, TX), under PI Kartik Konduri, MD, and at Dayton Oncology (Kettering, Ohio), under PI Robert Raju, MD, to evaluate the safety and efficacy of SNDX-275 in combination with erlotinib in patients with advanced (Stage IIIb) or metastatic (Stage IV) nsclc refractory to at least one but no more than two prior chemotherapy regimens. Primary outcome is PFS at 4 months. Secondary outcomes are PFS at 6 months, safety, tolerability, and PK. The lead-in, open label, phase I portion is to identify a safe dose of SNDX-275 in combination with erlotinib for further evaluation.

Patients are randomized to 1 of 3 treatment arms. Patients in arm 1 are treated with SNDX-275 tablets (5 mg in cohort 1 and 10 mg in cohort 2) on days 1 and 15 of a 28-day cycle for a maximum of 6 cycles, plus daily erlotinib (150 mg). In arm 2, SNDX-275 (5 mg or 10 mg as determined by the lead-in findings) is administered on days

1 and 15 of a 28-day cycle for a maximum of 6 cycles until progression or unacceptable toxicity, plus daily erlotinib (150 mg). In arm 3, erlotinib is administered as in arm 1 and 2 plus a matched placebo. Patients in arm 3 with progressive disease may cross over to be treated with SNDX-275 and erlotinib for up to 6 28-day treatment cycles. Approximately 107 patients are to enroll in this trial.

Volociximab, under development by Biogen Idec (Cambridge, MA), in collaboration with PDL BioPharma (Redwood City, CA), is a chimeric MAb against $\alpha5\beta1$ integrin (AAB1), a member of the integrin family of proteins known to be involved in angiogenesis.

An open label, multicenter phase II clinical trial (protocol ID: UCLA-0504066-01; PDL-M200-1206; NCT00278187) was initiated in January 2006, at the Jonsson Comprehensive Cancer Center at UCLA, under PI Robert A. Figlin, MD, to evaluate the response rate in patients with locally advanced (Stage IIIb) or metastatic (Stage IV) nsclc treated with volociximab and erlotinib. Secondary objectives are determination of TTP and duration of response, PK, and safety and tolerability. According to the protocol, patients are treated with volociximab IV over 30 minutes once every 2 weeks and with oral erlotinib daily for 52 weeks in the absence of unacceptable toxicity or disease progression. After completion of trial treatment, patients are followed at 3 and 6 months. A total of 40 patients will be accrued for this trial. This trial was reported closed as of February 2007.

XL184, under development by Exelixis (South San Francisco, CA), is a small molecule inhibitor of Met and VEGFr2 RTK that prevented tumor growth, and induced regression of various types of large tumors in xenograft models.

A randomized, open label, phase I/II clinical trial (protocol ID: XL184-202; NCT00596648) was initiated in December 2007, in the USA, to evaluate the safety, tolerability, and highest safe dose of XL184 with or without erlotinib in approximately 86 patients with nsclc (Stage IIIb or Stage IV in phase I only or Stage IIIa with pleural effusion, Stage IIIb or Stage IV in phase II only). Primary outcomes in phase I are safety, tolerability, MTD, pharmacodynamics, and PK. Treatment in phase I consists of escalating daily doses of PO XL184 and erlotinib (150 mg). Primary outcomes in phase II are ORR of XL184 with or without erlotinib in patients with progressive disease after responding to erlotinib, and pharmacodynamics and PK of XL184 as a single agent and in combination with erlotinib. Secondary outcomes are PFS, duration of response, and OS, assessed every 8 weeks. Treatment in phase II consists of XL184 (dose determined from phase I portion of the trial) and erlotinib (150 mg) daily or XL184 daily, administered as a single agent at the dose determined from phase I.

Pancreatic Cancer

Pancreatic cancer is an important indication for erlotinib. The drug is being evaluated in over 33 ongoing trials in this indication, mostly in combination with other approved agents.

Apricoxib was entered in a multicenter, randomized, placebo-controlled phase II clinical trial (protocol ID: TP2001-203; APRiCOT-P; NCT00709826), initiated in July 2008, in the USA, to evaluate the safety and efficacy of the combination of apricoxib, gemcitabine and erlotinib compared with placebo, gemcitabine and erlotinib in patients with refractory locally advanced or metastatic pancreatic cancer. According to the protocol, apricoxib (100 mg), 4 times daily, is added to gemcitabine and erlotinib in arm 1 and is replaced by placebo in arm 2. Primary outcome measures are to determine the antitumor activity and TTP of the combination in the treatment of patients with advanced pancreatic cancer. Secondary outcome measures are PFS, OS, and safety and tolerability. Estimated enrollment is 80 patients. Estimated primary completion date is February 2009.

Asentar (DN-101) is a novel oral formulation of high dose calcitriol with potent anticancer effects. The drug was under development by Novacea (South San Francisco, CA), in collaboration with Schering-Plough, when in November 2007, Novacea ended the phase III ASCENT-2 clinical trial (protocol ID: 011-007; NCT00273338) of Asentar for the treatment of patients with androgen-independent prostate cancer, because of an imbalance of deaths between the two treatment arms, as observed by the Data Safety Monitoring Board (DSMB) for the trial. Subsequently, in April 2008, Schering-Plough terminated the collaboration agreement with Novacea relating to the development of Asentar and Novacea, about to merge with Transcept Pharmaceuticals (Pt. Richmond, CA), terminated development of the agent.

A randomized, placebo-controlled, multicenter phase II clinical trial (protocol ID: 011-017; NCT00536770) of Asentar in patients with advanced pancreatic adenocarcinoma, was initiated in September 2007, at Vanderbilt-Ingram Cancer Center (Nashville, TN), under PI Mace L. Rothenberg, MD. Schering-Plough sponsored the trial and Novacea was overseeing its execution. This phase II clinical trial, to enroll approximately 132 chemotherapy-naïve patients, was evaluating the effect of weekly Asentar, combined with weekly gemcitabine plus or minus daily erlotinib in the first line treatment of inoperable, locally advanced or metastatic pancreatic adenorcarcinoma. The primary endpoint of the trial is to assess the 6-month survival rate, with secondary endpoints of objective response rate, PFS, OS, and safety and tolerability. Enrollment in this trial was suspended in November 2007, as per the DSMB.

Everolimus was entered in a phase II clinical trial (protocol ID: 2007-0666; NCT00640978) in combination with erlotinib, initiated in March 2008, in the USA, at M. D. Anderson Cancer Center, under PI Milind Javle, MD, in patients with previously treated inoperable or metastatic pancreatic cancer. There is no limit to the number of prior regimens. The trial's primary objective is to determine if this treatment slows the growth of advanced pancreatic cancer. A secondary objective is determination of the safety of this combination. According to the protocol, patients are treated with PO erlotinib (150 mg), daily, for 28 days, and PO everolimus (30 mg) weekly for 4 weeks. Estimated enrollment is 40 patients.

Breast Cancer

Breast cancer is a secondary indication for erlotinib with approximately 13 combination trials currently ongoing. One area of emphasis is triple negative breast cancer.

Everolimus was entered in a nonrandomized, open label, uncontrolled, phase I/II clinical trial, initiated in December 2005, at Vanderbilt University Medical Center, under PI Ingrid A. Mayer, MD, in patients with metastatic (Stage IV) breast cancer. Patients must have been treated with an anthracycline in the adjuvant setting or failed anthracycline treatment in the metastatic setting (total cumulative dose of lifetime exposure of doxorubicin not >360 mg/m^2 or epirubicin not >640 mg/m^2). Patients must have also failed previous taxane (paclitaxel or docetaxel) therapy, in the adjuvant setting with metastatic relapse within 12 months of therapy; progressed on taxane therapy in the metastatic setting; or discontinued taxane therapy in the metastatic setting secondary to lack of resolution of a Grade 2 or higher toxicity. Use of trastuzumab in the first line treatment of metastatic breast cancer is required for patients with HEr2/neu overexpressing tumors. The trial's primary objectives are to determine the safety, antitumor activity, and rate of clinical benefit. Other objectives are to determine TTP, baseline PTEN, pAkt, pP70S6K 1, and pEGFr in primary tumors. An estimated 55 patients will enroll in this trial.

IRESSA COMBINATIONS

Gefinitib is being evaluated in a limited degree with either other approved or novel agents; approximately 38 trials are currently ongoing. Almost all the drug's sales (Exhibits 3 and 4) come from overseas, primarily from Asian countries.

Solid Tumors

Cediranib (AZD2171), under development by AstraZeneca, is a novel, orally active inhibitor of VEGFr and c-Kit tyrosine kinases.

An open label, phase I clinical trial (protocol ID: D8480C00004; NCT00502060) was initiated in the Netherlands, under PI G Giaccone, MD, Vrije Universiteit Medical Centre, in Amsterdam, to assess the safety, tolerability and PK of ascending multiple oral doses of AZD2171 when co-administered with fixed multiple oral doses of gefitinib (250 mg or 500 mg) once daily, in patients with advanced, refractory solid tumors. The trial's objectives are to determine the safety, tolerability, PK, and efficacy of this regimen.

This trial was conducted in four parts. PO AZD2171 (20, 25, 30 or 45 mg) daily and gefitinib 250 mg (part A1) or 500 mg (part B1) were administered until DLT. The potential PK interaction of AZD2171 (30 mg) with gefitinib (250 mg) was studied in an expanded cohort (part A2). Part B2 followed the design of part A2, with a gefitinib dose of 500 mg. A total of 83 patients were treated with AZD2171 plus gefitinib (parts A1 and A2, n=31; parts B1 and B2, n=52). Primary diagnoses included RCC=15, CRC=15, lung cancer=11, and melanoma=11.

Most frequently reported AE were diarrhea (93%), anorexia (67%), fatigue (65%), and hypertension (57%). In part A1, DLT at the AZD2171 30 mg dose was Grade 3 hypertension (n=1) and at the 45 mg dose, Grade 3 QTc prolongation (n=1) and Grade 3 hypertension (n=2). In part B1, DLT at the AZD2171 20 mg dose was Grade 3 hand-foot syndrome, anorexia, and nausea (n=1); at the 37.5 mg dose, DLT were Grade 3 hand-foot syndrome and anorexia (n=1), Grade 3 hand-foot syndrome (n=1), and Grade 3 hypertension (n=1).

AZD2171 (30 mg) steady-state PK data were not different when the drug is administered alone or in combination with gefitinib (250 or 500 mg) while gefitinib steady-state plasma PK data in combination with AZD2171 were similar to those observed when administered as monotherapy. AXD2171 was generally well tolerated with manageable side effects at doses up to and including 30 mg/day plus gefitinib 250 mg, and 37.5 mg/day plus gefitinib 500 mg. Hypertension was manageable with standard treatment. There was no sign of steady-state PK interactions between AZD2171 and gefitinib.

Confirmed PR were noted in 6 patients (RCC=4; mesothelioma=1; osteosarcoma=1). Disease stabilized in 31 patients, in a wide range of cancer types including CRC (n=9), RCC (n=7), and nsclc (n=4). Changes in mean arterial pressure were observed in patients treated with AZD2171 and gefitinib (Van Cruijsen H, etal, ASCO06, Abs. 3017).

At an updated interim analysis there were 6 PR among 16 (38%) patients with RCC. Median duration of response was 6 months (3/6 responses were ongoing at data cut off). Disease stabilized in 7/16 (44%) patients and a confirmed 10%-30% reduction in maximum tumor diameter was observed 4/7 patients (van Herpen C, etal, ASCO07, Abs. 3560). This trial has been completed as of July 2007.

Non-small Cell Lung Cancer

Non-small cell lung cancer is the key indication of gefinitib, accounting for most of its revenue worldwide.

Cediranib (AZD2171) was entered into a 2-center, nonrandomized, open label, placebo controlled, phase I clinical trial (protocol ID: D8480C00015; NCT00243347), initiated in December 2005, in the USA and Europe (Spain), to evaluate its efficacy in treating patients with previously untreated or recurrent, inoperable, Stage IIIb/IV nsclc, or patients with metastatic or recurrent head and neck cancer. The trial, to enroll about 26 patients, was reported closed as of March 2008.

HERCEPTIN COMBINATIONS

Herceptin is the leader among ErbB-pathway inhibitors in terms of global sales that were $3,134 million in 2007, up 28.8% from 2006 levels (Exhibit 3). Two-digit revenue growth continued in the first half of 2008, with global sales reaching $2,359.6 million (Exhibit 4).

To date, the drug has been entered in over 273 clinical trials, with about 137 currently ongoing, with the majority evaluating trastuzumab in combinations with other approved drugs in patients with various types of breast cancer.

Breast Cancer

Trastuzumab is in the envious position to have been shown effective in delaying recurrence of HEr2-positive breast cancer, in both the adjuvant and metastatic setting, and it is therefore routinely employed in almost all clinical trials recruiting patients with HEr2-overexpressing tumors.

Alvocidib (flavopiridol), a semisynthetic analog of rohitukine isolated from the bark of the Indian tree *Dysoxylum binectariferum*, is a potent CDK1 inhibitor, arresting cell-cycle progression in either G1 or G2. The drug is being investigated in numerous clinical trials sponsored by the NCI.

The combination of trastuzumab and flavopiridol in treating patients with HEr2-positive metastatic breast cancer was investigated in a multicenter phase I clinical trial (protocol ID: DFCI-01177; NCI-5867; NCT00039455) that was initiated in January 2002, in Boston, at Beth Israel Deaconess Medical Center, Dana-Farber Cancer Institute, and Massachusetts General Hospital Cancer Center. Lyndsay Harris, MD, at Dana-Farber was Trial Chair. IV trastuzumab was administered over 30-90 minutes on days 1, 8, and 15, followed by IV flavopiridol continuously over 24 hours on days 1 and 8. Courses were repeated every 21 days in the absence of disease progression or unacceptable toxicity. Cohorts of 3-6 patients were treated with escalating doses of flavopiridol until MTD is determined. Once the MTD is determined, 10 additional patients were to be treated with flavopiridol at the MTD and trastuzumab on the once weekly schedule to assess the true toxicity and a second cohort of 10 patients were to be treated with flavopiridol at the MTD and trastuzumab once every 21 days to assess the tolerability of this schedule. Approximately 50 patients were to enroll in this trial that was completed as of June 2005.

CNF2024, under development by Biogen Idec, is a fully synthetic, orally available non-ansamycin derived inhibitor of Hsp90.

A multicenter (n=3), open label, dose-escalation, phase I clinical trial (protocol ID: 120BC101; NCT00412412) was initiated in November 2007, to evaluate CNF2024 as monotherapy (arm A), or in combination with trastuzumab (arm B), in patients with refractory, HEr2-positive advanced breast cancer. Trial objectives are to determine safety, PK, and pharmacodynamics of these regimens. This trial, being conducted in the USA, is to enroll 70 patients.

Deforolimus (AP23573, MK-8669), under development by Ariad Pharmaceuticals (Cambridge, MA), in collaboration with Merck, is a phosphorus-containing sirolimus analog that blocks cancer cell growth and proliferation by inhibiting mTOR.

A nonrandomized, open label, phase II clinical trial (protocol ID: AP23573-08-207; NCT00736970), was initiated in July 2008, to evaluate the safety and efficacy of deforolimus in treating patients with HEr2-positive, trastuzumab-refractory, metastatic breast cancer. The trial's primary objective is to determine ORR. According to the protocol, patients are administered PO deforolimus (40 mg) once daily for 5 consecutive days each week, followed by 2 days without deforolimus. Patients are also administered a single IV infusion of trastuzumab every week with an initial dose of 4 mg/kg over 90 minutes, then 2 mg/kg over 30 minutes. The trial, to enroll about 37 patients at about 15 medical centers in the USA and Europe, is expected to be completed by the second half of 2009.

INCB7839, under development by Incyte Pharmaceuticals, is a potent, orally bioavailable sheddase inhibitor active against ADAM 10 and 17. The agent was effective in preclinical as monotherapy and in combination with EGFr inhibitors and chemotherapy.

INCB7839 was well tolerated in a completed phase Ib clinical trial in which disease stabilized for 8-16 weeks in 3/4 patients with HEr2-positive breast cancer. By day 15, circulating HEr2 ECD levels were reduced by 60%-80% and were maintained at this level throughout disease stabilization in these 3 patients with elevated circulating HEr2 ECD [Infante J, etal, San Antonio Breast Cancer Symposium (SABCS) 2007, Abs. 6064].

A phase II clinical trial was initiated in the fourth quarter of 2007 to determine the effectiveness of INCB7839 when used in combination with trastuzumab in patients with advanced, HEr2-expressing breast cancer.

Lonafarnib, under development by Schering-Plough, is a non-peptide tricyclic farnesyltransferase inhibitor (FTI) with antiangiogenic properties.

A multicenter, nonrandomized, open label, dose escalation, phase I clinical trial (protocol ID: SPRI-P01900; EORTC-16023-10051; NCT00068757) of lonafarnib, trastuzumab, and paclitaxel in patients with HEr2-overex-

pressing Stage IIIb/c, or Stage IV breast cancer was initiated in August 2003, at the Institut Curie (Paris, France), Institut Jules Bordet (Brussels, Belgium), and the Netherlands Cancer Institute, in Amsterdam, under PI Jan Schellens, MD, PhD. This trial was designed to determine this regimen's MTD, recommended phase II dose, toxicity, PK, and response.

In course 1, patients are administered a loading dose of trastuzumab IV over 90 minutes on day 1, and over 30 minutes on days 8 and 15. Patients are also administered paclitaxel IV over 3 hours on day 1. In course 2, patients are administered trastuzumab IV over 30 minutes on days 1, 8, and 15, and paclitaxel IV over 3 hours on day 2. Patients are also administered oral lonafarnib twice daily, on days 3-21. In course 3 and all subsequent courses, patients are administered oral lonafarnib twice daily, on days 1-21, trastuzumab IV over 30 minutes on days 1, 8, and 15, and paclitaxel IV over 3 hours on day 1. Courses repeat every 21 days in the absence of disease progression or unacceptable toxicity. Cohorts of 3-6 patients are administered escalating doses of lonafarnib until MTD is determined. Patients are followed every 8 weeks until disease progression. Estimated enrollment is 36 patients.

Neratinib (HKI-272), under development by Wyeth, is an orally available, irreversible inhibitor of HEr2. This agent also inhibits EGFr kinase and blocks the proliferation of EGFr-dependent cells.

A multicenter (n=18), nonrandomized, open label, phase I/II clinical trial (protocol ID: 3144A1-202; NCT00398567), was initiated in March 2007, to evaluate the safety and efficacy of neratinib in combination with trastuzumab in patients with refractory, advanced or metastatic breast cancer overexpressing HEr2. The trial's primary objectives are to assess the drug's safety, tolerability, and efficacy, and to determine MTD. Secondary objective is to determine PFS. According to protocol, 3 to 6 patients are enrolled in each dose group. AE and DLT are assessed from the first dose of neratinib through day 21. An additional 30 patients will be enrolled at MTD, and followed for approximately 1 year to determine PFS. The trial, to enroll about 50 patients, is being conducted in the USA, China, Europe (Spain and Switzerland), and South Africa. This trial was reported closed as of March 2008.

Perifosine, under development by AEterna Zentaris (Quebec City, Canada), in collaboration with Keryx Biopharmaceuticals (NY, NY), is an oral Akt inhibitor that activates the MAP kinase (MEK/ERK) pathway leading to the activation of p21 independently of p53.

A randomized phase II clinical trial (protocol ID: 042005-017), of 3 doses of perifosine in combination with trastuzumab is being conducted at Parkland Memorial Hospital (Dallas, TX) under PI Debasish Tripathy, MD, in patients with metastatic HEr2-positive breast cancer, refractory to at least one prior trastuzumab-containing regimen for the treatment of metastatic disease. The trial's objectives are to identify the best dose level of perifosine administered in combination with trastuzumab, and evaluate the safety and tolerability and determine the effectiveness of this regimen.

Pertuzumab has been entered in a phase II clinical trial (protocol ID: NCI-06-C-0035; NCI-P6660; NCT00263224; NCT00301899), initiated in December 2005, to evaluate its efficacy and safety in combination with trastuzumab in patients with HEr2-overexpressing, locally advanced (Stage III), metastatic (Stage IV) or recurrent breast cancer refractory to trastuzumab-based therapy. According to the protocol an IV dose of trastuzumab is administered on day 1. Patients who have not been treated with trastuzumab in over 1 month, are treated with a loading dose of 8 mg/kg. Those having been treated with trastuzumab within 1 month, are administered an IV maintenance dose of 6 mg/kg. A loading dose of pertuzumab (840 mg) is administered IV on day 2. A maintenance dose of trastuzumab (6 mg/kg) and pertuzumab (420 mg) is administered on day 22. Within 3 days before treatment on day 22, a repeat cardiology evaluation is performed in all patients, as well as tumor biopsies in those patients who underwent biopsy before day 1. Both agents are administered every 3 weeks from then on until disease progression or unacceptable toxicity.

Patients are formally evaluated for cardiotoxicity using quantitative echocardiography before every cycle or at any point if there is clinical indication. Measurable disease is re-assessed every 6 weeks. Exploratory correlative studies investigating downstream signaling markers of HEr2 are conducted on both tissue and blood samples. Primary objectives are to determine ORR and the safety and tolerability of this regimen in these patients. Secondary objectives are to determine TTP, PFS, duration of response, and the percentage of patients free from disease progression at 3, 6, and 12 months, and correlate pre-treatment HEr2 phosphorylation and phosphorylation of downstream markers of signaling pathways with pertuzumab sensitivity and/or trastuzumab resistance. Janice M. Walshe, MD, at the NCI Medical Oncology Branch is Study Chair. Total expected enrollment is 37 patients. This trial has been closed to enrollment as of October 2007.

A multicenter (n=57), randomized, double blind, placebo-controlled, phase III clinical trial (protocol ID: TOC4129g; WO20698; NCT00567190) was initiated in December 2007, in the USA, to evaluate the safety and efficacy of pertuzumab in combination with trastuzumab, and docetaxel versus placebo in combination with trastuzumab and docetaxel, in treating patients with previously untreated HEr2-positive, metastatic breast cancer. The trial's primary objective is PFS. Secondary objectives are to determine OS, TTP, objective response, cardiac function, AE and SAE, and laboratory abnormalities. The trial is to enroll about 800 patients.

A multicenter (n=80), randomized, open label, phase II clinical trial (protocol ID: WO20697; NCT00545688), was

initiated in December 2007, in Australia, Brazil, Canada, Europe (Austria, Italy, Poland, Spain, Sweden, Switzerland, Turkey, and UK), Israel, Korea, Mexico, Peru, Russia, Taiwan, and Thailand, to evaluate the safety and efficacy of pertuzumab versus pertuzumab in combination with docetaxel, in treating patients with locally advanced, inflammatory or early stage HEr2-positive breast cancer. The trial's primary objective is to establish the pathologic CR rate. Secondary objectives are to assess disease-free interval, PFS, breast-conserving surgery rate, AE, laboratory parameters, and LVEF.

According to the protocol, patients are randomized to one of 4 treatment arms before surgery, and treated with 4 cycles of trastuzumab plus docetaxel (arm 1), or trastuzumab, docetaxel and pertuzumab (arm 2), or trastuzumab and pertuzumab (arm 3), or pertuzumab and docetaxel (arm 4). Pertuzumab is administered IV at a loading dose of 840 mg, then at 420 mg 3 times weekly. Trastuzumab is administered IV at a loading dose of 8 mg/kg, and then at 6 mg/kg 3 times weekly, and docetaxel is escalated from 75 mg/m^2 to 100 mg/m^2 3 times weekly. During the entire pre- and post-surgery period all patients are administered adequate chemotherapy as per standard of care, as well as surgery and/or radiotherapy as required. The trial is to enroll about 400 patients.

Tanespimycin (KOS-953), under development by Bristol-Myers Squibb (BMS), has demonstrated encouraging, potent antitumor activity and a high level of tolerability in HEr2-positive metastatic breast cancer. This drug was originally developed by Kosan Biosciences, which was aquired by BMS in July 2008.

A phase II clinical trial was initiated in December 2005, at Memorial Sloan-Kettering Cancer Center and the Arizona Cancer Center (Tucson, AX), to evaluate KOS-953 in combination with trastuzumab in patients with HEr2-positive metastatic breast cancer that progressed following treatment with trastuzumab in either the adjuvant or metastatic setting. In this trial, patients are treated with weekly doses of KOS-953 following infusion of trastuzumab. The trial's primary objective is to assess tumor response based on RECIST.

In December 2006, preliminary data from this trial were reported at the San Antonio Breast Cancer Symposium (SABCS). According to the protocol, patients were administered IV tanespimycin (450 mg/m^2) with a standard dose of trastuzumab. A total of 12 patients were enrolled who had been treated with one prior trastuzumab-containing regimen; many patients had been heavily pretreated with additional cytotoxic chemotherapy (median n=3) without trastuzumab, and 4 patients had been administered prior hormonal therapy. Grade 3 toxicities, seen in 3 patients (headache=2, fatigue=1), were manageable and reversible. Grade 1/2 toxicities included dizziness (n=4), fatigue (n=3), headache (n=2), dry eyes (n=2), nausea (n=1), and diarrhea (n=1). In updated results, a 55% clinical benefit was observed in patients with trastuzumab-refractory disease, including 5 PR in 20 evaluable patients. This combination therapy shows signs of activity in patients with HEr2-positive metastatic breast cancer previously treated with trastuzumab, and toxicity is manageable (Modi S, etal, SABCS06, Abs. 1102).

In February 2008, Kosan withdrew its Hsp90 inhibitor alvespimycin from development in order to commit resources to the development of tanespimycin for the treatment of breast cancer, based on several factors, including clinical experience to date, strength of intellectual property protection, and risk and time to commercialization.

In an ongoing phase I clinical trial, patients with refractory, HEr2+ metastatic breast cancer, and those with refractory ovarian cancer (HEr2 status unknown) with progressing disease on standard chemotherapy, were treated with escalating doses of alvespimycin in combination with trastuzumab. A total of 25 heavily pretreated patients [median prior cytotoxic regimens=6.5 (range=1-15)] with HEr2+ breast cancer and ovarian cancer were treated with the drug at 60 (n=9), 80 (n=10), and 100 mg/m^2 (n=6). DLT, observed at 100 mg/m^2 in a single patient, was hypoxia and lowered LVEF. DLT at 80 mg/m^2 included 2 patients with Grade 3 reversible keratitis who were retreated upon resolution. The cohort at the 80 mg dose level was expanded because of Grade 2 arthralgia, myalgia, and fatigue at the 100 mg/m^2 dose level. At the 80 mg dose level drug-related toxicity included diarrhea (61%), fatigue (48%), arthralgia (44%), headache (39%), nausea (35%), myalgia (26%), blurry vision (26%), extremity pain (26%) and dry eye (22%); drug-related Grade 3+ toxicity (other than DLT) included 1 episode of fatigue and diarrhea. According to PK t1/2 was 19.1 hours, and the clearance rate was 16.9 L/hour with no change upon weekly dosing.

There was 1 CR with significant improvement in dyspnea in a patient with HEr2+ breast cancer that had metastasized to the lung. This patient had been treated with 13 prior regimens. Also, a 10% reduction in tumor mass with change consistent with tumor necrosis was noted in a patient who had been treated with 11 prior regimens. Disease stabilized in 5 additional patients with HEr2+ breast cancer for 4, 5, 6+, 11+, and 12 months, respectively. A near CR in ascites and pleural effusion was observed in a patient with ovarian cancer (16+ months on trial) at the end of cycle 2. This patient had been previously treated with 13 regimens In this trial, the combination of trastuzumab and alvespimycin was active in HEr2+ metastatic breast cancer (Modi S, etal, ASCOBC07, Abs. 165). In updated results from 27 heavily pretreated patients (HEr2+ breast cancer=24, and ovarian cancer=3), clinical benefit was observed in 8/19 (42%) evaluable patients with breast cancer, including a PR involving a 52% decrease in hepatic lesions in a patient who had been treat-

ed with 5 prior regimens. This trial was expanded to add weekly dosing with paclitaxel in 3 to 4-week cycles.

Tipifarnib was entered in an open label, phase II clinical trial (protocol ID: SACI-IDD-01-44, NCI-5330, UTHSC-IDD-01-44, NCT00054470), initiated in January 2003, at the San Antonio Cancer Institute, under PI Garry Schwartz, MD, in combination with trastuzumab in patients with metastatic breast cancer previously treated with trastuzumab. Trial objectives are to determine the antitumor activity, safety, and tolerability of this regimen. Patients are administered oral tipifarnib twice daily on days 1-21, and trastuzumab IV over 30-90 minutes on day 1. Courses repeat every 4 weeks in the absence of disease progression or unacceptable toxicity. Patients are followed every 2 months. A total of 18-40 patients were to be accrued for this trial, which was closed in early 2004.

Vatalanib, under development by Novartis, is an orally available angiogenesis inhibitor that targets VEGFr2, c-kit, and PDGFr.

A multicenter (n=8), nonrandomized, open label, uncontrolled, phase I/II clinical trial (protocol ID: HOG BRE04-80; NCT00216047) was initiated in January 2005, by the Hoosier Oncology Group (HOG), under Study Chair Kathy Miller, MD, of the Walther Cancer Institute and Indiana University Cancer Center (Indianapolis, IN), to investigate PTK787, combined with trastuzumab in treating patients with newly diagnosed, HEr2-overexpressing, locally recurrent or metastatic breast cancer. The primary objective of the phase I portion was to assess the safety and tolerability of this combination. The primary objective of the phase II portion was assessment of response rate. Secondary objectives in phase II were assessment of safety and tolerability, TTP and clinical benefit of this combination. Treatment consisted of PTK787 daily plus IV trastuzumab (4 mg/kg) during week 1, followed by 2 mg/kg weekly with disease evaluation every other cycle. Treatment continued until disease progression or unacceptable toxicity. Expected total enrollment was 37 patients. This trial was terminated in September 2006, because of low patient enrollment and serious toxicities.

TYKERB COMBINATIONS

Lapatinib, a selective dual inhibitor of EGFr and HEr2, is a latecomer in a sector dominated by trastuzumab. Drug revenues have been growing rapidly, albeit from a small base (Exhibits 3 and 4).

The drug has been entered in over 129 trials with 78 ongoing, mostly in combination with other approved targeted drugs and cytotoxics. Regarding combinations with novel agents, lapatinib is being primarily investigated with pazopanib, also under development by GlaxoSmithKline.

Apricoxib and Lapatinib

A multicenter (n=25), randomized, double blind, placebo-controlled, phase II clinical trial (protocol ID: TP2001-202; NCT00657137), dubbed APRiCOT-B (Apricoxib in Combination Oncology Treatment-Breast), was initiated in April 2008, in the USA, to determine the efficacy and safety of apricoxib in combination with lapatinib and capecitabine in the treatment of patients with HEr2-overexpressing breast cancer refractory to trastuzumab, an anthracycline, and a taxane. According to the protocol patients are treated with apricoxib (100 mg), 4 times daily, in combination with lapatinib and capecitabine.

This trial will compare the antitumor efficacy of apricoxib, lapatinib and capecitabine with lapatinib, capecitabine, and placebo, as measured by TTP, and evaluate urinary PGE-M measurements or baseline COX-2 expression in tumor tissue by IHC as a surrogate selection criterion for patients who will benefit from future treatment with apricoxib. Secondary outcome measures include PFS, and safety and tolerability. Estimated enrollment is 120 patients. Estimated completion date is June 2009.

Pazopanib and Lapatinib

A multicenter (n=2), nonrandomized, open label, phase I clinical trial (protocol ID: 109693; NCT00516672) was initiated in July 2007, in Japan, to determine the safety, tolerability, and PK of pazopanib alone and in combination with lapatinib in treating patients with advanced solid tumors. Approximately 36 patients are to enroll in this trial.

A nonrandomized, open label, uncontrolled, phase I clinical trial (protocol ID: VEG10006; NCT00158782) was initiated in October 2004 in patients (n=65) with advanced solid tumors to determine safety, tolerability, PK, and clinical activity of pazopanib administered concurrently with lapatinib. This trial was closed in 2006. Dose escalation occurred in cohorts of 3-6 patients based on DLT. In an interim report involving 33 patients treated with lapatinib/pazopanib doses of 750/250 (n=4), 750/500 (n=6), 1000/250 (n=3), 1000/400 (n=2), 1000/500 (n=4), 1250/250 (n=6), 1250/400 (n=5) and 1500/200 (n=3) mg once daily, most frequent AE were diarrhea (Grade 1=10, Grade 2=2, Grade 3=3); fatigue (Grade 1=7, Grade 2=5; Grade 4=1); nausea (Grade 1=9, Grade 2=2), anorexia (Grade 1=8, Grade 2=3), vomiting (Grade 1=9), hair depigmentation (n=7), rash (Grade 1=6; Grade 2=1) and abdominal cramps (Grade 1=3, Grade 2=2, Grade 3=1).

Prolonged SD lasting >16 weeks (median=21.5 weeks) was observed in 10 patients (RCC=3; CRC=3; GIST=1; mesothelioma=1; adenocarcinoma of gastroesophageal junction=1; aggressive fibromatosis=1). Tumor shrinkage <30% (i.e., SD by RECIST) was observed in 3 patients (RCC=2; giant cell tumor of the bone=1). Concurrent administration of pazopanib and lapatinib was generally well tolerated. Co-administration of lapatinib may alter the PK of pazopanib (Dejonge M, etal, ASCO06, Abs. 3088).

A multicenter (n=86), randomized, open label, phase II clinical trial (protocol ID: VEG20007; NCT00347919) comparing the efficacy and safety of pazopanib in combination with lapatinib to lapatinib alone as first line therapy

in patients with advanced or metastatic breast cancer with HEr2 FISH-positive tumors, was initiated in June 2006, in the USA, Canada, Europe (France, Hungary, Poland, Russia, UK), India, Israel, Korea, Mexico, Pakistan, Peru, Singapore, and Thailand. Primary outcome is rate of disease progression at 12 weeks, and secondary outcome is RR at 12 weeks, OS, time to response, duration of response, safety, and tolerability. A total of 140 patients are to enroll in this trial.

A multicenter, randomized, phase III clinical trial (protocol ID: VEG108838; NCT00558103), was initiated in November 2007, in the USA, Australia, Brazil, Canada, Chile, China, Egypt, Europe (Belgium, Czech Republic, France, Germany, Greece, Italy, Romania, Russia, Spain, UK), Hong Kong, Israel, Korea, Morocco, Pakistan, Peru, Philippines, Singapore, Taiwan, Thailand, Tunisia, and Turkey, to compare the combination of pazopanib and lapatinib with lapatinib monotherapy in patients with HEr2-overexpressing inflammatory breast cancer that progressed or relapsed following treatment, which must have included a chemotherapy regimen and trastuzumab, if available. HEr2 overexpression, based on local results is either 3+ overexpression by IHC or HEr2 gene amplification by FISH or CISH. Archived tumor tissue must be provided for all patients for HEr2 FISH testing by the central laboratory. Central testing is for analysis only; patients will remain on the trial based on local HEr2 expression results. The primary outcome measure is PFS. Secondary outcome measures include overall response rate (ORR), OS, and safety and tolerability. An estimated 320 patients are to enroll in this trial scheduled to be completed in May 2010.

A multicenter (n=77), randomized, open label, phase II clinical trial (protocol ID: VEG105281; NCT00430781) was initiated in November 2006, in the USA, Europe, Canada, India, Thailand, Argentina, Peru, and Mexico, to compare the efficacy and safety of pazopanib combined with lapatinib to lapatinib alone or pazopanib alone in patients with recurrent or persistent, metastatic cervical cancer (Stage IVb). Primary outcome is PFS measured every 6 weeks. Secondary outcomes are OS; CR, PR or SD at 6 months; time to response; duration of response; and safety and tolerability. Approximately 180 patients are to enroll in this trial.

A multicenter (n=11), nonrandomized, open label, phase I clinical trial (protocol ID: VEG102857; NCT00350727) was initiated in August 2006, in the USA and the UK, to assess the safety and tolerability of pazopanib and lapatinib administered in combination with enzyme-inducing anticonvulsants in patients (n=105) with recurrent malignant glioma (Grade III or IV). Primary outcome measures are AE, and changes in vital signs and laboratory values. Secondary outcomes are PK and plasma concentrations of the circulating biomarkers VEGF, sVEGFr1, and sVEGFr2.

Editor's note:

The information presented in this issue was obtained for NEW MEDICINE's Oncology KnowledgeBASE (nm|**OK**), a comprehensive subscription-based resource, residing at http://www.newmedinc.com. Although this article presents a detailed review on current developments within its topic, it is a snapshot in time. In the oncology sector, advances or changes happen constantly at breakneck speed. nm|**OK** tracks the information presented here on a daily basis, offering the user the best of two worlds, i.e. a thorough analysis of important issues in oncology, and a continuous updating of all the pieces that make up the topic of this article.

nm|**OK** is a multi-tiered data source covering every aspect of drug development in oncology and related areas. This knowledgebase profiles over 4,000 drugs (2,000 in active development) and technologies in great detail, in an easy to navigate, intuitive format. It is not simply a compilation of data, but a market research and product development environment designed to provide the user with a continuum of fully descriptive actionable information about developers, pipelines, technologies, mechanisms, targets, preclinical and clinical development, revenues (where applicable) and potential markets, etc.

Another feature of nm|**OK** is a comprehensive presentation of *in vitro* testing approaches in the oncology sector from general diagnostics for specific malignancies, to prognostic, pharmacogenomic, and theragnostic tests to aid in patient/treatment selection, disease monitoring, clinical trial design, and drug development. This information is presented by malignancy, agent, developer, and for individual trials. The *in vitro* testing market is a rapidly expanding sector in the oncology field with over 350 participating companies worldwide offering a variety of products or services.

INDEX OF COMPANIES & INSTITUTIONS

— continued on next page

INDEX OF COMPANIES & INSTITUTIONS

FUTURE ONCOLOGY

PUBLISHED BY NEW MEDICINE, INC.

PUBLISHER AND EDITOR: **Katie Siafaca**
ASSOCIATE EDITOR: **Sudha Khurana, PhD**
CONTRIBUTING EDITOR: **Timothy Tankosic, MD**
ASSISTANT EDITOR: **Vyvy Phan**
ASSISTANT EDITOR: **Brad R. Natsume**
TECHNICAL CONSULTANT: **Feisal Yamani, MD**
DESIGN & PRODUCTION: **Jill Burch**

NEW MEDICINE, INC. MAILING ADDRESS:

P.O. Box 909 ■ Lake Forest, CA 92609
Tel: 949. 830. 0448 ■ Fax: 949. 830. 0887

e-mail: info@newmedinc.com
www:http://www.newmedinc.com
www:http://www.oncologyknowledgebase.com
www:http://www.nmok.net

SUBSCRIPTION INFORMATION:

- FUTURE ONCOLOGY (ISSN 1082-331X) is published as 12 issues (several double issues) per subscription period, with a free index listing companies/institutions and subjects covered.
- A one-volume subscription to the electronic version is US $840 and to a hard copy version, sent first class to U.S. addresses, is US $1040, and sent air mail to foreign addresses, US $1,100.
- Volumes V7 and V8 are $1,400 (U.S.) and $1,460 (outside the U.S.).
- Additional subscriptions sent to the same location are $390 each.
- Payment must accompany your order; checks must be drawn on a U.S. bank. (A purchase order number is acceptable; however, the subscription will not begin until payment is received.) Make checks payable to NEW MEDICINE. Payment may also be made by AMERICAN EXPRESS, VISA, or MASTERCARD, wire transfer or direct deposit; please call 949. 830. 0448 for details.