multiple myeloma, breast, renal, and liver tumor cell lines as indicated by combination indices below 0.7. In the colon cancer cell line SW620 cell cycle analysis revealed G2M arrest as mechanisms of action for Perifosine, whereas two representative antimetabolites, i.e. 5-Fluorouracil and 6-Thioguanine, induced S-phase arrest as expected. In combination, synergistic effects were observed in terms of apoptosis, e.g. caspase activation.
In summary, these results demonstrate potent synergistic activity of Perifosine with various antimetabolites in human colon, multiple myeloma, breast, renal, and liver tumor cell lines. Synergism seems to be based on combining G2M arrest by Perifosine and S-phase arrest by the antimetabolite resulting in synergistic induction of cellular apoptosis. Further experiments addressing Perifosine's mechanism of action in combination with antimetabolites are ongoing. Currently, Perifosine is in a phase III clinical trial in combination with Capecitabine in patients with refractory advanced colorectal cancer.

204 POSTER

Effects of EGFR inhibition with tyrosine kinase inhibitors on invasive properties of EGFR mutant and wild type lung cancer cells

I.A. Umelo[1], O. De Wever[2], E. Teugels[1], M. Bracke[2], J. De Grève[1]. *[1]Universitair Ziekenhuis Brussel, Laboratory of Molecular Oncology and Department of Medical Oncology, Brussel, Belgium; [2]Universitair Ziekenhuis Ghent, Laboratory of Experimental Cancer Research and Department of Radiotherapy, Ghent, Belgium*

Background: The epidermal growth factor receptor (EGFR) pathway is known to be involved in the invasive and metastatic process. Furthermore, it is also known that activating mutations of EGFR confer increased sensitivity to small molecule tyrosine kinase inhibitors (TKI's) in the treatment of non-small cell lung cancer (NSCLC). The effects of various TKI's on lung cancer cell proliferation and survival have been previously investigated. However, the effects on invasion (and metastasis) have been studied less. Our present study investigates the effects of EGFR TKI's on the invasion of lung cancer cells with a wild-type or a mutant EGFR gene in an *in vitro* invasion assay.
Materials and Methods: The model used is based on the preparation of native collagen type I, the main interstitial matrix component of solid tumors. Three NSCLC cell lines − NCI-H358 (EGFR-WT), NCI-H1650 (EGFR-ΔE746-A750) and NCI-H1975 (EGFR-L858R/T790M) − were evaluated with several inhibitors of EGFR (erlotinib, lapatinib, BIBW2992, and cetuximab) for the effects on their invasive properties. Invasion- induced changes in cellular structure and F-actin organization were analyzed with phase contrast and confocal microscopy techniques. Invasive index, and factor shape were measured via image processing.
Results: Qualitative and quantitative analysis show that while lapatinib and cetuximab have a moderate effect on the attenuation of epidermal growth factor (EGF) stimulated invasion of mutant NCI-H1650, erlotinib and BIBW 2992 significantly abrogate cellular invasion ($P < 0.0001$). Similarly, BIBW 2992 abrogates invasion in the T790M mutant NCI-H1975 cell line ($P < 0.01$), whereas no effects are observed with any of the first-generation inhibitors. Interestingly, erlotinib significantly promoted EGF stimulated invasion of wild-type NCI-H358 ($P < 0.001$), while BIBW2992 did not.
Conclusions: These findings show that, as assessed in the pre-clinical *in vitro* collagen type I assay, erlotinib has differential effects on the invasive phenotype depending on the genomic status of the EGFR gene, promoting invasion in wild type cells. Our study also supports the use of BIBW 2992 as a therapeutic option in tumors bearing the EGFR-T790M resistance-conferring mutation.

205 POSTER

Distinct inhibitory properties of MEK inhibitors on pathway feedback translate into differential potency in BRAF and RAS mutant cancer cells

M. Belvin[1], L. Berry[1], J. Chan[1], K. Hoeflich[1], B. Liu[1], S. Malek[2], M. Merchant[1], C. Orr[1], K. Song[1], G. Hatzivassiliou[1]. *[1]Genentech Inc., Cancer Signaling/Trans Onc, South San Francisco, USA; [2]Genentech Inc., Biochemical Pharmacology, South San Francisco, USA*

Background: The RAS/RAF/MEK pathway is active in over 30% of human tumors, often due to mutation in BRAF or RAS family members. Several MEK inhibitors, aimed at treating tumors with RAS/RAF pathway alterations, are in various stages of clinical development. Despite their similarities, MEK inhibitors from distinct chemical series differ in their ability to modulate and inhibit signaling in BRAF and KRAS mutant cell lines and tumors. Here we explore the biochemical nature of this differential potency.
Results: GDC-0973 is a potent, selective, allosteric MEK1/2 inhibitor that is currently being tested in early stage clinical trials. G-573 is an allosteric MEK1/2 inhibitor from a distinct structural class with similar biochemical potency and selectivity as GDC-0973. While GDC-0973 and G-573 have similar cellular potencies in $BRAF^{V600E}$ mutant cells, G-573 displays up to 10 fold higher potency in KRAS mutant cell lines, and shows greater efficacy in vivo in KRAS mutant xenograft tumors. In vivo, GDC-0973 shows stronger maximal efficacy than G-573 in $BRAF^{V600E}$ mutant xenograft models whereas G-573 shows stronger efficacy than GDC-0973 in KRAS mutant models. To investigate the basis for the different activities of these two MEK inhibitors, we analyzed their effects on components of the RAF/MEK/ERK pathway in $BRAF^{V600E}$ vs. RAS mutant cells. We found that GDC-0973, but not G-573, increases levels of phosphorylated MEK (pMEK) and displays a potency shift in blocking pERK in KRAS vs, $BRAF^{V600E}$ mutant cells. This pMEK increase is mediated by RAF family members which are activated due to the release of negative feedback in the MAPK pathway. Although G-573 leads to a similar negative feedback release and RAF activation, it blocks MEK phosphorylation by activated RAF and is more effective at blocking downstream ERK activation in KRAS mutant cells. This effect translates into distinct cellular potencies for the two inhibitors in RAS mutant models, where the negative feedback is present, but not in $BRAF^{V600E}$ models, where it is absent.
Conclusions: These findings provide an explanation for the potency differences of MEK inhibitors in RAS vs. $BRAF^{V600E}$ mutant xenograft tumors and support a model in which potency in RAS mutant tumors correlates with the ability of MEK inhibitors to effectively block MEK activation by RAF. As a consequence, different classes of MEK inhibitors may show distinct efficacy profiles in the clinic.

206 POSTER

The cis/trans effect of the T790M drug resistant mutation in non-small cell lung cancer

I. Umelo[1], G. Chen[1], E. Teugels[1], J. De Grève[1]. *[1]Universitair Ziekenhuis Brussel, Laboratory of Molecular Oncology and Department of Medical Oncology, Brussel, Belgium*

Background: Activating mutations of the epidermal growth factor receptor (EGFR) in non-small cell lung cancer (NSCLC) confer increased sensitivity to small molecule tyrosine kinase inhibitors (TKIs). However, despite initial response, tumors often develop the resistance conferring T790M mutation. Although resistance mechanisms of T790M have been studied, the impact of T790M arising in either *cis* or *trans* to the activated allele remains to be established. The aim of our study was to compare the effects of EGFR primary activating mutations associated with TKI sensitivity to the TKI insensitive EGFR-T790M arising in *cis* or *trans*.
Materials and Methods: The model used is based on the interleukin-3 (IL-3) dependent Ba/F3 system. We transformed the Ba/F3 cells to an epidermal growth factor (EGF) dependent system by the exogenous introduction of wild-type and mutant forms of the EGFR gene through stable transfection (wild-type EGFR, mutant EGFR and *cis* constructs) and stable co-transfection (*trans* configurations). We assessed the functionality of our constructs with two EGFR tyrosine kinase inhibitors, erlotinib and a novel irreversible inhibitor, BIBW 2992 through ^{3}[H]thymidine incorporation, MTS, Annexin V/7-AAD and western blot analysis.
Results: Our results show that T790M arising in *trans* to a primary activating EGFR mutation exhibits increased activation of AKT, ERK1/2 and STAT5 when compared to its *cis* counterpart. We also found that BIBW 2992 overcomes resistance in all erlotinib resistant T790M conformations by decreasing proliferation, increasing apoptosis and promoting G_1 cell cycle arrest.
Conclusions: The T790M mutation activates the EGFR signal transduction pathway more effectively in the *trans* than in the *cis* conformation relative to primary activating mutations. The covalent EGFR/HER2 inhibitor BIBW 2992 has activity in both conformations.

207 POSTER

A novel selective MET inhibitor combined with erlotinib overcomes erlotinib facilitated resistance in patient derived NSCLC xenografts in vivo

M. Kissel[1], T. Müller[2], I. Fichtner[3], H. Hess-Stumpp[1]. *[1]Global Drug Discovery Bayer Schering Pharma Wuppertal Berlin Germany, GDD, Wuppertal, Germany; [2]Global Drug Discovery Bayer Schering Pharma Wuppertal Berlin Germany, Statistics Department, Berlin, Germany; [3]EPO, GmbH, Berlin, Germany*

Most advanced non-small-cell lung cancers (NSCLCs) and especially the fraction with activating epidermal growth factor receptor (EGFR) mutations initially respond to the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. However, most tumors develop acquired resistance to EGFR TKIs via secondary resistance mutations. The amplification of the MET oncogene is present in 20% of TKI-resistant tumors, and in half of the cases