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ACTB-1003 – a unique oral pan FGFR and PI3K pathway inhibitor with divergent modes of activity

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Introduction: ACTB-1003 is an oral kinase inhibitor targeting cancer mutations via FGFR inhibition, angiogenesis through inhibition of VEGFR2 and Tie-2, and induces apoptosis potentially by targeting RSK and p70S6K potentially induces apoptosis. The multi-activity of ACTB-1003 translates to efficacy with dose-dependent tumor growth inhibition in a variety of *in vivo* tumor models including endometrial, bladder and gastric cancers.
Results: ACTB-1003 inhibits FGFR1 (IC_{50} = 6 nM), VEGFR2 (2 nM), Tie-2 (4 nM), RSK (IC_{50} = 5 nM) and p70S6K (32 nM)). ACTB-1003 is highly active in mechanistic and proliferation assays using cell lines with FGFR genetic alterations. This translates to *in vivo* activity with dose-dependent tumor growth inhibition in OPM2 human multiple myeloma and the murine leukemia TEL-FGFR1 Ba/F3 model. OPM2 cells harbors the FGFR3 t(4:14) translocation, FGFR3 K650E mutation and PTEN deletion while the Ba/F3-TEL-FGFR1cells are driven by FGFR1 over-expression. The multiple modes of action of ACTB-1003 are demonstrated through cell-based and *in vivo* mechanism of action studies. Inhibition of RSK and p70S6K pathway is correlated with the induction of apoptosis (PARP) in H460 NSCLC *in vivo* tumor model. Additionally, ACTB-1003 is shown to inhibit tumor angiogenesis evident by the inhibition of CD31 staining in these tumor sections. Thus, ACTB-1003 has a unique activity profile inducing both the apoptotic and inhibiting angiogenic pathways. ACTB-1003 is combinable with 5-FU or paclitaxel without diminishing the activity or increasing the toxicity of these chemotherapy agents in the HCT-116 colon tumor xenograft model.
Conclusion: ACTB-1003 is an effective anti-cancer agent with a unique activity profile that inhibits tumor cell growth. This creates the potential for additive or synergistic antitumor efficacy to be achieved not only within each pathway but across multiple pathways with one drug and may overcome mechanisms of resistance. ACTB-1003 is projected to enter Phase 1 clinical testing for the treatment human solid cancer in 2010.

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GDC-0941 PI3K inhibitor activity in preclinical lung cancer models

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Background: Non-small cell lung cancer (NSCLC) is a heterogeneous disease with multiple genetic defects and activated signaling pathways. The activation of the PI3K pathway has been implicated in NSCLC via PTEN loss, PIK3CA mutation and amplification, and upstream growth factor signaling such as through EGFR, cMet, and K-ras mutation. Additionally, the PI3K pathway has been implicated in cancer cell survival upon challenge with chemotherapeutics.
Methods: The oral, potent, selective PI3K inhibitor GDC-0941 was assessed in vitro and in xenograft models of NSCLC. GDC-0941 cellular potency was determined in a panel of NSCLC lines using celltiterglo assay, and markers correlating with response were investigated. For a subset of lines combinations of GDC-0941 with chemotherapeutics were also pursued in vitro and in NSCLC xenograft models.
Results: GDC-0941 PI3K inhibitor showed a spectrum of potency in a broad panel of NSCLC cell lines. The spectrum of activity indicates that significant potency can be obtained regardless of K-Ras mutation status. The potential correlation of potency with putative predictive biomarkers such as PTEN loss, LKB1, FBXW7, PIK3CA mutations and PIK3CA amplification will also be discussed. In vivo, GDC-0941 displayed dose-dependent tumor growth inhibition in several NSCLC xenograft models, including mutant K-Ras xenograft models. In vitro, the combination of GDC-0941 with cisplatin was synergistic in some cell lines, using the Chou and Talalay method of combination index. In vivo combination efficacy greater than either agent alone was observed when GDC-0941 is administered daily and cisplatin weekly.
Conclusions: These findings indicate that the GDC-0941 PI3K inhibitor has promising activity in preclinical models of NSCLC.

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Growth response of human colorectal tumour cell lines to treatment with BIBW2992, an irreversible EGFR/HER1 and HER-2 tyrosine kinase inhibitor

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Currently the only EGFR inhibitors approved for treatment of patients with metastatic colorectal cancer are the anti-EGFR monoclonal antibodies (mAbs) cetuximab and panitumumab. While EGFR inhibitors improve survival in cancer patients, the duration of response is often limited. In addition, there has been no clear association between the expression of EGFR in colorectal cancer and response to EGFR inhibitors. Previously we examined the growth response of a panel of human colorectal tumour cell lines to treatment with our anti-EGFR mAb ICR62 and/or genfitinib, a reversible EGFR TKI, and found that most colorectal tumour cell lines were relatively resistant to treatment with both inhibitors. This study aimed to investigate the affect of BIBW2992, an irreversible EGFR/HER1 and HER-2 TKI, on the growth *in vitro* of a panel of human colorectal tumour cell lines (DiFi, Colo2, Colo13, HCT116, CCL-221, CCL-225, CCL228, CCL244), using the SRB colorimetric assay. We also investigated whether there was any association between the expression levels of the EGFR family members and response to treatment with BIBW2992 and ICR62. Of the 8 colorectal tumour cell lines examined, DiFi were the most sensitive to treatment with BIBW2992 and complete inhibition was achieved at concentrations above 198 nM (IC_{50} = 45 nM). In contrast, mAb ICR62 induced complete growth inhibition of DiFi cells at concentrations above 6.25 nM (IC_{50} = 4.33 nM). The growth of CCL244 and other colorectal tumour cell lines were also inhibited completely by BIBW2992 but at concentrations above 1.5 μM and 3.1 μM respectively with an IC_{50} which ranged from 318 nM (CCL-244) to 1.62 μM (HCT116). FACS analysis showed the mean fluorescence intensities (MFIs) for EGFR expression ranged from 4.5 (CCL244) to 513 (DiFi) and for HER-2 expression ranged from 17 (HCT116) to 64 (CCL-221). Interestingly, all colorectal tumour cell lines were found to be HER-4 negative while expressing very low levels of HER-3 with MFI values ranging from 9 (HCT116) to 25 (CCL-225). Our results suggest an association between the expression of EGFR or HER-2 and response to treatment with BIBW2992 and underline the need to better understand the molecular markers determining sensitivity. Further studies with BIBW2992 as single agent or in combination with standard or targeted therapies in colorectal cancer are warranted.

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Reduced expression of HER3 with a specific RNA antagonist is associated with antitumor effects in preclinical models of cancer

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The cytoplasmic tail of HER3 can be trans-phosphorylated by other HER family members and is a critical link to the PI3K/AKT axis. Since HER3 plays a role in HER2-mediated tumor growth, and hyperactivation of HER3 can mediate resistance to antitumor agents that target HER1 (EGFR) or HER2, agents that inhibit HER3 may have broad utility. However, unlike other HER family members, HER3 does not have kinase activity, and therefore is not amenable to inhibition with tyrosine kinase inhibitors. Beyond this, antibodies that neutralize HER3 may have limited effects if the cytoplasmic tail of HER3 remains activated. To overcome these limitations, we have developed HER3 antisense molecules to down regulate HER3 protein expression. In particular, we have used locked nucleic acid-based oligonucleotides (LNA-ON) since these third generation molecules are highly potent, are resistant to nuclease degradation, and have a proven track record of activity in animal models, including non-human primates, for the control of cholesterol and hepatitis C infection (Elmen et al., 2008. Nature. 452: 896; Lanford et al., 2010. Science. 327:198). EZN-3920, a LNA-ON that has complementarity to HER3, was identified based on *in vitro* inhibition of HER3 mRNA, protein expression and tumor cell proliferation. The 16-mer oligonucleotide ablated HER driven signaling and potently inhibited the HER3 signaling pathway in various tumor cells. In particular, EZN-3920 inhibited the growth of a variety of cell lines *in vitro*, including a lung tumor cell line (HCC827) that was selected for resistance to gefitinib and an ovarian cell (SKBR3) that overexpresses HER2. *In vivo*, systemic administration of EZN-3920, prepared in saline, resulted in specific down-modulation of HER3 mRNA and protein expression, as well as blockade in PI3K/AKT signaling pathways in both HCC827 and BT474 xenograft models. In established Polyomavirus middle T transgenic mammary

tumors transplanted into syngeneic FVB mice, perturbation of HER2/HER3 signaling pathway with EZN-3920 resulted in tumor growth inhibition, reduced expression of HER3, and induction of apoptosis as assessed by immunohistochemical methods. It is concluded that EZN-3920 provides a novel strategy to effectively down modulate HER3-mediated addiction to growth factors. Furthermore, down regulation of HER3 may provide a novel strategy to overcome resistance to HER1 and HER2 targeted therapies.

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Utility of microRNA analysis for understanding treatment mechanism of action: Necitumumab +/− gemcitabine/cisplatin in NSCLC models

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The contribution of mRNA regulation through effects on microRNA (miRNA), to the anti-tumor effects of therapy needs further study. Here, we utilized SABiosciences PCR arrays to identify oppositely regulated miRNA and predicted mRNA targets in A549 and NCI-H1650 non-small cell lung cancer (NSCLC) xenograft tumors growing in nu/nu athymic mice. Necitumumab, a recombinant human IgG1 targeting EGFR, alone or in combination with cisplatin+gemcitabine (cis/gem), inhibited the growth of these tumors. In the A549 model, 34% of 125 human mRNAs that were >2-fold up or down-regulated by necitumumab, cis/gem, or necitumumab+cis/gem ($p < 0.05$ by t-test versus saline control, $n = 3$), were predicted to be targets of at least one of the 16 tumor miRNA affected oppositely by the same respective treatment, indicating potential regulation of mRNA by miRNA in these tumors. In the NCI-H1650 model, cis/gem treatment did not affect the expression levels of the miRNAs evaluated, however 40% of 52 human mRNAs that were >2-fold up or down-regulated by necitumumab or necitumumab+cis/gem treatment were predicted to be targets of at least one of the 5 miRNA affected oppositely by the same respective treatment, again supporting the potential involvement of miRNA in the regulation of mRNA in tumors. The potential value of miRNA analysis for understanding mechanism of action *in vivo* was further highlighted by the observed necitumumab induced increase in mir-15b (18.3 fold, $p = 0.037$), let-7g (6.5 fold, $p = 0.03$) and miR-150 (2.3 fold, $p = 0.026$), that may impact tumor cell apoptosis and cell proliferation through modulation of BCL2 (-46.5 fold, $p = 0.002$), Cyclin D1 (−11.1 fold, $p = 0.0009$) and TP53 (-133.8 fold, $p = 0.0003$) mRNA in NCI-H1650 tumors. In the A549 model necitumumab increased the level of miR-148a (3.44 fold, $p = 0.0034$), miR-148b (2.54 fold $p = 0.006$), and miR-29 (2.1 fold, $p = 0.02$), that may target mRNA for the methyltransferase DNMT3b (−4.5 fold, $p = 0.02$) to restore normal patterns of methylation-silenced tumor suppressor genes such as CDKN2A (66.7 fold, $p = 0.004$), RARA (8.7 fold, $p = 0.006$), RARB (9 fold, $p = 0.007$) and RARG (6.67 fold, $p = 0.001$), contributing towards the treatment induced reduction in tumor growth. To summarize, utilizing necitumumab +/− gem/cis efficacy in NSCLC subcutaneous xenograft tumor models, we demonstrate that miRNA analysis can be an important tool in understanding the mechanism of action underlying antitumor effects of therapy.

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Specific MET inhibition using SU11274 impairs cholangiocarcinoma cells proliferation, motility and invasion

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Background: Cholangiocarcinomas (CCA) are highly malignant tumors of unmet medical needs often displaying aberrant MET signaling. MET activation either by HGF stimulation, MET over-expression or mutations induces cell proliferation, invasion, and angiogenesis, offering opportunities for investigating the potential of novel MET inhibitors such as SU11274.
Materials and Methods: SU11274, a pyrrole indolinone, specifically inhibits overexpressed and oncogenic MET activation at nanomolar concentrations. Antiproliferative effects of SU11274 were evaluated in human CCA cell lines (Mz-chA1, Mz-chA2 and SK-ch) using MTT assay. Baseline and phosphorylated (p-) protein levels were assessed by Western blot analysis. mRNA expressions used qRT-PCR. Mobility was investigated by wound-healing and matrigel invasion assays. Cell cycle distribution was studied by FACS analysis.
Results: MET-protein and -mRNA expression was detectable in our three CCA cell lines and cells were found responding to HGF stimulation as detected by activation of p-MET$^{Tyr1234/35}$ in all cell lines. MET activation by HGF was associated with increase of p-GAB1, p-ERK1/2, and p-AKT^{ser473} in Mz-chA2 cells. SU11274 displayed antiproliferative effects at concentrations ranging 2−5 μM after 48−72h exposures in our three cell lines without HGF stimulation. Cell cycle analysis of CCA cells exposed to 5μM SU11274 for 72h demonstrated accumulation of cells in G2/M phase. In HGF-stimulated Mz-chA2 and SK-ch cells, SU11274 blocked p-MET and p-GAB1 at MET-specific concentrations ranging 0.5−2 μM that were also shown yielding antiproliferative effects. At these concentrations, SU11274 inhibited HGF-induced downstream MET signaling by reducing p-AKT^{473} and p-ERK1/2. No significant effect of SU11274 on E-cadherin and vimentin expression was observed. SU11274 (5μM) decreased spontaneous HGF-independent cell motility of Mz-chA2 cells and their invasion in matrigel.
Conclusion: Inhibition of p-MET by SU11274 inhibited HGF-dependent MET signaling that resulted in inhibition of cell proliferation, motility and invasion in CCA cells. CCA may be an interesting tumor type to evaluate novel MET inhibitors either as single agents and/or in combination with other targeted therapies.

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Pharmacological characterization of NMS-P506, a novel second generation HSP90 inhibitor

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Background: the molecular chaperone heat shock protein 90 (HSP90) is essential for the conformational maturation and stability of a variety of key proteins, including kinases, implicated in cancer development and progression.
Prototype geldanamycin derivatives are the most advanced compounds, but their liabilities may ultimately limit clinical applications and justify the development of new non ansamycin drugs. In this perspective, the identification of inhibitors capable of long lasting pharmacodynamic modulation of client proteins in tumour tissues might prove important in order to improve the therapeutic window in the clinics.
Results: here we describe the *in vitro* and *in vivo* characterisation of NMS-P506, representative of a novel class of fully synthetic non-ansamycin HSP90 inhibitors. NMS-P506 binds HSP90α with an affinity of 65 pM, and has no significant activity against a broad panel of kinases, as well as other relevant ATPases.
When tested against a panel of tumor cell lines of various tissue origins, NMS-P506 showed widespread antiproliferative activity, with an average IC_{50} of 95 nM. When characterized by Biacore analysis the compound showed a very slow rate of dissociation from HSP90, with a K_{off} of $8.09E^{-5}$ 1/sec, which translates in cells in a long lasting degradation of HSP90-dependent oncoproteins and up regulation of HSP70.
In mice, NMS-P506 had a favourable pharmacokinetic profile with a $t_{1/2}$ of 6 hours in plasma. Selective retention was observed in tumours, with an extended half-life of 139 hours, and relevant concentrations of the compound were found in the brain.
In vivo, NMS-P506 showed an excellent anti tumor efficacy in the B-RAF V600E driven A375 melanoma model, as well as in the A2780 ovarian cancer model, resulting in tumor shrinkage after weekly intravenous administrations with a good tolerability profile. This *in vivo* activity was associated with apoptosis induction and prolonged degradation of HSP90 client proteins.
In conclusion, we report the characterization of NMS-P506, a new second generation Hsp90 inhibitor, capable of long lasting pharmacodynamic modulation of client proteins in cells and in xenograft tumors. NMS-P506 has a very potent *in vitro* and *in vivo* activity, with a good PK profile and selective retention in tumors, which makes it a candidate for further development. Moreover, the brain penetration makes this compound potentially attractive also for brain tumors or metastases.

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Therapeutic targeting of the pro-survival transcription factor CREB sensitizes glioblastomas to temozolomide-based therapy

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Background: Glioblastoma multiforme (GBM) is the most common, lethal primary brain tumor in adults. Following debulking surgery, adding temozolomide (TMZ) with, and after, radiation therapy has become the standard of care for newly diagnosed GBM, yielding 15 months median overall survival versus 12 months with radiation alone. Despite this advance, more effective therapeutic options are needed, particularly