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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