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Foretinib (GSK1363089) inhibits growth of gastric cancer cell lines through blockade of inter-receptor tyrosine kinases networks

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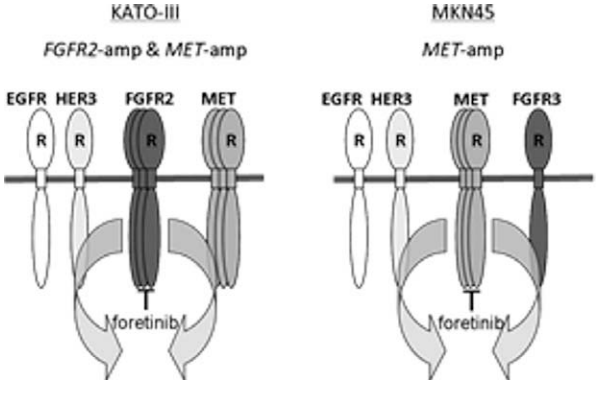
Background: Very few molecularly-targeted agents have been applied to the treatment for gastric cancer. Foretinib (GSK1363089) is an oral multikinase inhibitor targeting MET, RON, AXL, and vascular endothelial growth factor receptors (VEGFRs). The purposes of this study were to determine mechanisms of action of foretinib and to explore possible biomarker to predict sensitivity to the agent in gastric cancer.
Materials and Methods: We evaluated effects of foretinib on cell growth and cell signaling using a panel of gastric cancer cell lines; KATO-III, MKN-1, MKN-7, MKN-45, and MKN-74. All cell lines have been evaluated for copy number of *MET* and fibroblast growth factor receptor 2 (*FGFR2*) genes, and only MKN-45 and KATO-III cell lines are known to have *MET*- and *MET*- and *FGFR2*-amplifications, respectively.
Results: Only MKN-45 and KATO-III were highly sensitive to foretinib (IC_{50}; MKN-45 vs. KATO-III vs. the others, 7 vs. 30 vs. 800 nM<). In MKN-45, 1 μM of foretinib and 1 μM of PHA665752, another MET inhibitor, inhibited phosphorylation of MET and downstream signaling molecules including Akt as expected. However, in KATO-III, inhibition of phosphorylation of MET with PHA665752 did not coincide with the inhibition of phosphorylation of Akt. Instead, 1 μM of foretinib and 1 μM of PD173074, a selective inhibitor of FGFRs, inhibited phosphorylation of FGFR2 and Akt, suggesting that foretinib works through FGFR2 in KATO-III. While activity of foretinib against FGFR2 has not been reported before, we confirmed it in another *FGFR2*-amplified gastric cancer cell line, OCUM-2M. With phospho-receptor tyrosine kinase (RTK) array, we found that in MKN-45 1 μM of foretinib inhibited phosphorylation of epidermal growth factor receptor (EGFR), HER3 and FGFR3 via MET inhibition (figure). Similarly in KATO-III, 1 μM of foretinib inhibited phosphorylation of EGFR, HER3 and MET via FGFR2 inhibition (figure). To explore the role of HER3 and FGFR3 in MKN-45, we knocked down them with SiRNA and found that phosphorylation of Akt and cell growth was partially inhibited.
Conclusions: Foretinib appears effective against cell lines with *MET*- or *FGFR2*-amplified gastric cancer cell lines. Foretinib showed inhibitory effects on MET and FGFR2, and blocked the inter-RTKs signaling driven by *MET* or *FGFR2* gene-amplification in gastric cancer cells.



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Targeting focal adhesion kinase (FAK) in primary human schwannoma

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Neurofibromatosis type 2 (NF2), a dominantly inherited disease, is caused by loss of tumour suppressor protein Merlin. Loss of merlin also causes a variety of spontaneous tumours. NF2 patients normally suffer from multiple non-cancerous nervous system tumours i.e. schwannoma, meningioma and ependymoma. As these tumours are benign, chemotherapy is not effective. Tumour localisation and multiplicity make surgery and radiosurgery very challenging. Thus, a new therapeutic approach is urgently required. Focal adhesion kinase (FAK) is a tyrosine kinase localised in the cytoplasm and focal adhesions acting as a mediator of various signalling pathways. Our previous results revealed that FAK is strongly overexpressed and basally activated in human schwannoma, and autophosphorylated FAK (Y397) colocalises with active ERK1/2 at the focal adhesions in schwannoma.
By using an unique *in vitro* model comprising primary human schwannoma tumour cells and healthy Schwann cells, we tested the hypothesis that FAK is a key regulator of elevated focal adhesion and proliferation in human schwannoma. To target FAK, a small inhibitor PF573228 (Pfizer) and lentivirus containing shRNA against FAK had been applied in this study. We demonstrate that FAK is activated upon stimulation of the overexpressed IGF1−/IGF-IR and integrins/IGFBP-1 leading to the increased proliferation and adhesion. In IGF-IR mediated signalling, FAK recruits ERK1/2 and JNK pathways to regulate proliferation and adhesion. Nuclear localisation of FAK in schwannoma also suggested its potential contribution to proliferation or survival independent of growth factor receptors. Based on these data we suggest that FAK represents a good therapeutic target for the treatment of human schwannoma.

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Endothelin A receptor antagonism with zibotentan (ZD4054) augments androgen ablation-induced inhibition of prostate tumor cell growth

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Background: Endothelin-1 A and B receptors (ET-1 ET_A and ET_B) have emerged as important contributors to the development of castration-resistant prostate cancer (CRPC). Plasma ET-1 and tumor ET_A levels being elevated while ET_B expression is lost, sets the stage for progression to advanced CRPC. The current study focuses on the molecular response of the ET axis to androgen deprivation and effects on ET_A antagonism in the hormone-deprived prostate cancer (PCa) environment.
Materials and Methods: *In vitro,* PCa cell viability was assessed in hormone-depleted conditions with and without specific ET_A antagonist (zibotentan, ZD4054) treatment. *In vivo*, mice bearing established LNCaP xenograft tumors were either castrated or left intact, treated with zibotentan or vehicle, at the time of castration (castrate-immediate [CI]) or 7 days post-castration (castrate-delayed [CD]) and then analyzed for tumor proliferation, vascularization and serum prostate-specific antigen (PSA).
Results: Zibotentan significantly reduced cell viability in androgen-sensitive PCa cells during androgen deprivation. Castration decreased tumor growth rate compared with intact animals and combining castration with zibotentan led to additional reductions in tumor growth, the largest of which was in the CD group (15% lower, $P < 0.05$ vs castrate controls). This latter group also had 60% lower tumor weight vs castrate controls ($P < 0.05$). Time before removal from the study due to maximum allowable tumor volume (survival) was longest in the CI and CD groups, followed by the castrate controls. The intact-vehicle group had no animals remaining in the study by day 42, whereas the intact-zibotentan group still had 20% of animals surviving. Significant reductions in serum PSA were observed in zibotentan-treated castrate mice vs castrate controls (>30%) or intact mice (>100%) and a decrease in proliferation (Ki67) was observed in zibotentan-treated castrate mice vs castrate controls or intact mice.
Conclusion: Zibotentan reduced PCa cell viability and tumor growth in androgen deprived conditions and may provide additional tumor growth inhibition in CRPC through blockade of a cell survival/growth pathway active during hormone depletion.

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Targeting mTOR: a potential therapeutic approach for chondrosarcoma treatment

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Chondrosarcomas are the second most frequent primary malignant type of bone tumor for which no effective systemic treatment is available. If surgical resection remains the most reliable means of cure, this procedure does not restrain the high rates of local recurrence and life threat of chondrosarcoma. Thus, there is a need to develop innovative systemic therapies for the treatment of chondrosarcoma.
The aim of the present study was to determine the effects of Everolimus® (RAD001) on chondrosarcoma tumor progression. RAD001 was tested in vivo (i.p. 1 mg/kg, twice a week) alone or in combination with adriamycine

(i.p. 1 mg/kg, twice a week) in a rat chondrosarcoma model. The therapeutic efficiency of RAD001 and of the combination RAD001/adryamicin was evaluated using chondrosarcoma volume evolution (MRI), tumor necrosis percentage, tumor MVD quantification analysis between the treated and control groups.
We showed that in comparison to adriamycin, RAD001 significantly inhibited tumor growth progression (tumor doubling time of 33 days and 7 days respectively for RAD001 treated-tumors and control ($p < 0.01$)) and that targeting mTOR along with chemotherapy treatment did not exhibited additive antitumor effects in vivo when compared with RAD001 as single agent (adriamycin, RAD001 and RAD001+Adriamycin induced respectively tumor inhibition rate of: 43%; 74% ($p < 0.01$); 52% ($p < 0.05$)(. No histological differences or treatment-induced necrosis could be observed between the different groups. RAD001 inhibited the phosphorylation of mTOR and S6 and did not alter the activation of Akt.
Taken together, our preclinical data indicate that RAD001 alone has a beneficial effect *in vivo* on chondrosarcoma tumor progression and may be effective as single agent in treating chondrosarcoma patients.

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Novel small-molecule inhibitors of Interleukin-6 (IL-6) signalling

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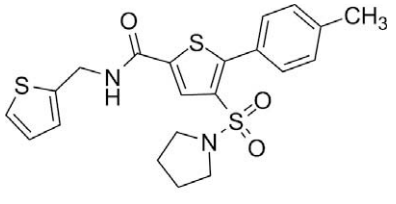
Interleukin-6 (IL-6) is a pro-inflammatory cytokine that plays a key role in the pathophysiology of several cancers. It binds to the IL-6 receptor (IL-6R), inducing dimerisation of the gp130 complex, and initiating signalling through the STAT3 pathway. For example, its over-expression is implicated in the pathology of multiple myeloma, renal cell, prostate, cervical and breast carcinomas, and the discovery of IL-6 inhibitory agents could lead to new classes of anti-cancer drugs.
As part of a drug discovery programme aimed at identifying small-molecule inhibitors of this pathway, a library of arylsulphonamidyl thiophene amides has been prepared through an efficient 4-step synthetic route. Library members were evaluated in a standard MTS assay to evaluate cell viability in STAT3-dependent MDA-MB-231 breast cancer cells, in which STAT3 signalling is IL-6 stimulated. In parallel, the compounds were also evaluated in STAT3-null A4 cells as a control. These experiments identified the "hit" arylsulphonamidyl thiophene amide RH06 (Figure 1) which had apparent selective inhibitory activity at the low micromolar level, with the ability to reduce the viable cells in the RH06-treated MDA-MB-231 line by ~40% compared to control. Furthermore, the high percentage of viable cells remaining after carrying out a trypan blue exclusion assay in the same cell lines indicated that RH06 is cytostatic rather that cytotoxic. Next, a luciferase reporter assay was used to evaluate the selectivity of RH06 towards the STAT3 promoter in HeLa cells using a SV40 promoter as control. The STAT3 promoter-luciferase cells were treated with Oncostatin M as an IL-6 mimic to activate STAT3 signalling *via* the IL-6/gp130 receptor. In these experiments RH06 showed demonstrated selective inhibition of STAT3 transcriptional activity with an EC_{50} in the 1 μM range. Finally, Western Blot studies of the effect on RH06 on STAT3 protein levels in MBA-MD-231, Hela and CT26 cells showed that it inhibits STAT3 phosphorylation which may account for its overall mechanism of action at the cellular level.



RH06

Figure 1: Structure of RH06

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The dual PI3K/mTOR blocker NVP-BEZ235 sensitizes cancer cells against irreversible ErbB inhibitors

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Epidermal growth factor (EGF) receptor-related antigens (EGFR, ErbB1−4, HER1−4) represent emerging drug targets in oncology. However, resistance against ErbB-targeting drugs occurs frequently in cancer patients. Drug-resistant cells may exhibit drug-refractory phosphatidylinositol 3-kinase (PI3K) or mitogen-activated protein kinase (MAPK) signaling, but the relative impact and contribution of these two downstream pathways to drug resistance are still controversially discussed. We examined the effects of the two very potent, irreversibly binding ErbB receptor tyrosine kinase inhibitors (RTKIs) pelitinib (EKB-569) and canertinib (CI-1033) on PI3K- and MAPK activity in ErbB RTKI-sensitive and ErbB RTKI-resistant breast and ovarian cancer cells. Western blot analysis revealed that ErbB phosphorylation was abrogated by the inhibitors in both drug-sensitive and drug-resistant cells, whereas AKT- and GSK3b phosphorylation were drug-dependently downregulated only in drug-sensitive cells. ErbB RTKI sensitivity did not correlate with expression of wildtype PTEN or PIK3CA, nor was it associated with drug-dependent silencing of ERK1,2 in the breast and ovarian cancer cell lines examined. Moreover, exogenous AKT, but not MEK, significantly induced drug resistance. Our data demonstrate that blocking AKT phosphorylation is essential and sufficient, whereas abrogation of ERK phosphorylation is not required for ErbB RTKI anticancer efficacy. AKT phosphorylation may thus be a useful biomarker of ErbB RTKI sensitivity in breast and ovarian cancer cells. Supported by 'Medical Scientific Fund of the Mayor of the City of Vienna' (#08037) and 'Initiative Krebsforschung' Medical Univ. Vienna.

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Discovery and characterization of PI3Kbeta isoform-selective inhibitors

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Background: Abnormal PI3K pathway activation plays a major role in cancer, as a result of either RTK activation and/or somatic mutations of major components of the pathway, including activating point mutations and amplification of the *PIK3CA* gene, as well as loss of negative regulatory proteins such as PTEN. Most of the ATP-competitive PI3K inhibitors currently in clinical development inhibit all class I PI3K isoforms: however, several recent reports support the development of isoform-specific inhibitors. In particular, while PI3Kα specific inhibitors are predicted to inhibit growth of tumors with PIK3CA mutations, PTEN-deficient tumors have been shown to depend on PI3Kβ. In addition, isoform specific PI3K inhibitors may exhibit better safety profiles compared to pan-selective PI3K inhibitors, and thus be more suitable to combine with other targeted or cytotoxic therapies.
Methods: ATP-competitive inhibitors with selectivity for PI3Kβ were identified via high-throughput screening and optimized using a structure-based design approach. Biochemical activities against the different PI3K isoforms were measured using a HTRF assay. Compound effects on AKT phosphorylation were measured in different cell lines using western blotting or Meso Scale Discovery multi-array techniques.
Results: PI3Kβ-selective inhibitors with biochemical IC50 values below 100 nM and good selectivity over other PI3K isoforms and a diverse panel of protein and lipid kinases were identified. Cellular assays demonstrate that PI3Kβ compounds potently inhibit phosphorylation of AKT (cellular IC50s <300 nM) in *PTEN*-deficient tumor cell lines. Consistent with its biochemical selectivity profile, the most PI3Kβ-selective inhibitors were inactive on AKT phosphorylation in PI3Kα-activated tumor cells. *In vivo* pharmacodynamic analyses following oral administration of these isoform-selective inhibitors to mice bearing xenografted tumors demonstrated dose-dependent inhibition of phosphorylation of PI3K downstream effectors at well-tolerated doses.
Conclusions: Novel PI3Kβ-selective small molecular mass inhibitors were identified and characterized *in vitro* and *in vivo* for PI3K pathway modulation in the context of different activating genetic abnormalities. Cellular selectivity for *PTEN*-deficient tumor cells versus PI3Kα-activated tumor cells was demonstrated. These preclinical results support the development of isoform-selective PI3Kβ inhibitors for the treatment of cancer patients harboring tumors with *PTEN*-deficient specific genotypes.