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Synergistic antitumor activity of the combination of the multi-targeted tyrosine kinase inhibitor sorafenib and of EGFR inhibitors in human colon and lung cancer cell lines

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Purpose: Tumor cell survival, growth, invasion and metastasis depend on efficient tumor cell proliferation and on tumor-induced angiogenesis. Simultaneous targeting of both these pathways could provide a better anticancer therapeutic strategy. Pre-clinical studies have shown that the combination of EGFR inhibitors, such as cetuximab, a chimeric human-mouse anti-EGFR monoclonal antibody, or erlotinib, a small molecule EGFR-selective tyrosine kinase inhibitor, and of anti-angiogenic drugs results in the potentiation of anti-tumor activity. In this study, we have evaluated the efficacy of the combination of sorafenib, a multi-targeted tyrosine kinase inhibitor and cetuximab or erlotinib, which are currently used for the treatment of metastatic colorectal cancer and non small cell lung cancer (NSCLC), respectively.
Methods: The antiproliferative effects of sorafenib in combination with gefitinib or cetuximab against a panel of human lung (A549, GLC-82, Calu3, H460) and colon (GEO, HCT-15, HCT-116, HT-29, SW480) cancer cells with a functional EGFR autocrine pathway, were determined after a concurrent 5 days exposure using a soft agar anchorage-independent growth assay. Combination effects were analyzed using the isobolographic model according to the Chou and Talalay method. Cell cycle distribution and apoptosis were quantitated by flow cytometry.
Results: Treatment with sorafenib, erlotinib or cetuximab caused a dose-dependent inhibition of soft agar growth in all the nine human cancer cell lines tested. A dose-dependent synergistic effect in growth inhibition and in apoptosis was observed by the combined treatment with sorafenib and erlotinib or with sorafenib and cetuximab in all cancer cell lines. Sorafenib induced cell cycle arrest in G1, while cetuximab and erlotinib did not induce significant changes in cell cycle distribution as compared to control untreated cells. The combined treatment with sorafenib and each EGFR inhibitor induced a significant increase in the G1 phase of the cell cycle.
Conclusion: The combination of sorafenib with erlotinib or of sorafenib with cetuximab resulted in a strong antiproliferative and pro-apoptotic activity providing a rationale for the development of multi-targeted anticancer strategies.

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Genetic polymorphisms in xenobiotic-metabolizing enzymes and their association with colorectal cancer

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Background: Carcinogenesis is a complex process associated with genetic and lifestyle factors. One of the most common forms of cancer is colorectal cancer (CRC). CRC affects approximately 5% of worldwide population. More than 75% of CRC cases represent sporadic forms. Susceptibility to nonhereditary CRC is significantly influenced by polymorphisms and mutations in low-penetrance genes. Genetic polymorphisms in xenobiotic-metabolizing enzymes may result in variations in detoxification capacity and thus influence the levels of carcinogenic compounds and subsequently the risk of cancer. Therefore, we aimed to study associations of polymorphisms in genes coding biotransformation enzymes with CRC. Based on frequency in Czech population and functional effects we selected polymorphisms in CYP1B1, EPHX1, GSTM1, GSTT1, GSTP1, NQO1, SOD2 and MPO.
Materials and Methods: Through the PCR RFLP and DNA sequencing analysis we followed their prevalence in groups of 500 CRC patients and 500 controls.
Results: Statistical analysis showed: (1) The lack of association of particular polymorphisms with CRC risk in unselected population. (2) Female carriers of variant genotype in NQO1 were at significantly higher risk of CRC in comparison with those carrying normal genotype. There was no association of this polymorphism with CRC risk in males, but previously we reported its role in breast cancer in Czech and Austrian populations. (3) Age played no role as confounding factor.
Conclusions: First study of this kind on Czech population showed that polymorphisms in xenobiotic-metabolizing enzymes may present risk factors in CRC. Further study should be focused at searching for differences in exposure between genders and assessment of importance of polymorphism combinations. Identified risk modifying factors may be used for formulation of preventive and therapeutic strategies.
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Pharmacokinetic characterization of BI 2536 – a novel Plk1 inhibitor – in advanced cancer patients

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Background: BI 2536 is a novel highly potent and specific inhibitor of the serine-threonine Polo-like kinase 1 (Plk1), a key regulator of cell cycle progression. The pharmacokinetics of BI 2536 after intravenous administration was investigated in the first treatment cycle of three different dosing schedules in advanced cancer patients (Schedule 1: 60 minutes infusion on day 1; Schedule 2: 60 minutes infusion on days 1 and 8; Schedule 3: 60 minutes infusion on days 1, 2 and 3).
Methods: Sequential cohorts of 3–6 patients with advanced or metastatic solid tumours received infusions of BI 2536 following a toxicity guided dose escalation design. Dose of BI 2536 within the different schedules were: Schedule 1: 25–250 mg (39 patients); Schedule 2: 25–200 mg (42 patients); Schedule 3: 50−70 mg (20 patients). Blood samples to determine the drug plasma concentration were taken at different time points in the first treatment cycle before, during and after the infusion.
Results: BI 2536 showed no relevant deviation from dose proportionality within the maximum plasma concentration or exposure (AUC) over the dose range tested (25–250 mg). BI 2536 revealed a multi-compartmental pharmacokinetic behaviour. The plasma concentration showed a fast decline after the end of infusion indicating a very fast disposition phase, most likely representing distribution into tissue. The plasma concentration decreased within 30 minutes after end of infusion to about 1/3 of the concentration at the end of infusion. BI 2536 is a high clearance drug with only minor contribution of urinary excretion of parent compound to the total clearance. No accumulation was observed within the Schedule 2 between days 1 and 8, whereas a slight accumulation was observed in the Schedule 3 between days 1 and 3. Neutropenia as a mechanism-related toxicity indicates target inhibition in vivo. Therefore the exposure after the first infusion (Schedules 1 and 2) was correlated to the decrease in neutrophil count on day 8 (assumed nadir). In addition influence of age, weight, gender and body surface area on the exposure was investigated. For none of the covariates a relevant influence on the exposure in the range tested was observed.
Conclusion: BI 2536 showed dose proportional behaviour in the dose range 25–250 mg. No accumulation after once weekly and slight accumulation after once daily dosing was observed. BI 2536 showed a high clearance and a high distribution. There was a clear correlation between drug exposure and the degree of neutropenia induced by BI 2536. No relevant influence of the covariates tested on the pharmacokinetics was observed.

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Biological and clinical significance of fatty acid synthase overexpression in cancer

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Background: The enzyme fatty acid synthase (FAS) has been implicated in the development and progression of several human cancers and is an attractive target for cancer therapy. FAS expression is low in most normal tissues, but is elevated in prostate cancer subsets with poor prognosis. While FAS expression is androgen responsive, it persists or is reactivated in tumors after androgen ablation, and is high in 83% of lethal tumors examined at autopsy.
Materials and Methods: To investigate FAS upregulation in cancer as a therapeutic target we tested FAS antimebolite therapy on the transgenic adenocarcinoma of mouse prostate (TRAMP) model using C-75 and Orlistat. FAS expression was evaluated by immunohistochemistry of TRAMP tissues, including primary and metastatic lesions in mice of varying ages with and without treatment, FAS enzyme activity was determined by 14C-acetate incorporation into lipids as a measure of pathway activity

and effect of antimetabolite treatment was assessed by urogenital and prostate weights, tumor grade and proliferation, and apoptotic markers. To study possible roles for FAS in prostate cancer, we examined the genetic alterations, which are associated with FAS protein expression and activity knockdown. We conducted a gene microarray analysis of prostate cancer cells with FAS knockdown by FAS gene specific siRNA in comparison to control treated cells.
Results: The anti-tumor efficacy of FAS inhibitors c75 and Orlistat was dose dependent and demonstrated a strong correlation to inhibition of akt phosphorylation and FAS pathway activity, reduced prostate and urogenital weights and decreased tumor grade compared to vehicle treated mice. Additional anti-tumor mechanistic studies demonstrated inhibition of tumor cell proliferation and induction of apoptosis. Our gene array data revealed that numerous genes are altered in expression including many proliferation and apoptotic genes with FAS knockdown that play significant roles in many pathways including cell growth, development, and cell signaling. These data suggest the upregulation of FAS expression plays a key role in tumorigenesis and provide insight into dysregulation of this gene in cancer.
Conclusions: These results indicate that the antitumor activity of FAS inhibitors may be mediated by direct effects on tumor cell growth or survival mechanisms.

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Cell death pathways as therapeutic targets for cancer

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Proper execution of cell death plays an essential role in tumor suppression. Apoptosis is a form of regulated cell death. Defects in this process are a hallmark of cancer and contribute to chemotherapy resistance. Our laboratory recently determined that the proapoptotic protein Bim determines tumor responsiveness to taxanes and that Bim inactivation by MAPK-mediated phosphorylation and degradation in proteasomes confers chemotherapy resistance. The same studies also revealed a mechanism by which addition of a proteasome inhibitor reactivates apoptosis and abrogates MAPK pathway-dependent resistance to taxanes enabling tumor regression. These preclinical studies are now being translated to a clinical trial of taxane and bortezomib combinatorial treatment for solid tumors with activated MAPK pathway, and set an example of rationally designed tumor genotype-specific chemotherapy.
An alternative to reactivation of apoptosis is to divert apoptosis-resistant tumor cells to an alternate pathway of cell death such as type II programmed cell death (autophagy). Beclin1 is a key regulator of autophagy and defective autophagy plays a role in mammary oncogenesis since beclin1 haploinsufficiency is common in human breast carcinomas. We have developed a novel mouse mammary epithelial model for studying the mechanisms regulating breast tumorigenesis and are applying this model to determine the role of autophagy in breast cancer progression and treatment responsiveness. Primary mouse mammary epithelial cells (MMECs) were isolated from beclin1 +/− and beclin1 +/+ mice, immortalized (iMMECs) by inactivation of the retinoblastoma and p53 pathways, and their response to metabolic stress, capacity for 3D-morphogenesis, and tumorigenicity were compared. Allelic loss of beclin1 in iMMECs increased susceptibility of iMMECs to metabolic stress, indicating that autophagy is indeed a survival, and not a cell death, mechanism in mammary epithelial cells. Furthermore, beclin1 +/− iMMECs were more tumorigenic than beclin1 +/+ iMMECs after orthotopic injection demonstrating that beclin1 haploinsufficiency promotes mammary tumorigenesis. Thus, autophagy may function as a tumor suppression mechanism by mitigating metabolic stress, thereby preventing the accumulation of damaged tumor cells that can promote tumor progression. These findings also suggest that autophagy inhibitors may be a means to drive apoptosis resistant tumor cells to cell death in response to metabolic stress.

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Pharmacodynamics (pd) of xl880, a novel spectrum selective kinase inhibitor (SSKI), administered orally to patients (pts) with advanced solid tumors (AST)

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Background: XL880 is a sub-nM inhibitor of the hepatocyte growth factor receptor (Met) and vascular endothelial growth factor (VEGF) family receptor tyrosine kinase (RTK), with low in vitro nM inhibition of PDGFRβ, KIT, FLT3, Tie-2 and Ron. It is the 1st orally bioavailable small molecule Met inhibitor to enter the clinic. An ongoing phase I study of XL880 in pts w/ASTs showed that XL880 is well tolerated up to 3.6 mg/kg, with definition of the maximum tolerated dose ongoing. Two dose limiting toxicities have been observed (1 grade 3 lipase and 1 grade 3 transaminase). Manageable hypertension and edema have been seen in pts treated at the highest doses for prolonged times. Two pts w/spontaneous papillary renal cell carcinoma (SPRC) have a PR (1 unconfirmed), 2 pts w/carcinoid and melanoma have had MRs and a pt w/medullary thyroid cancer showed tumor reduction by physical exam and decreased cortisol levels while receiving XL880.
Methods: Blood samples were collected from all pts. Tumor and normal (surrogate) tissues from selected pts were collected at baseline and following administration of XL880. Plasma samples were analyzed for ligands and soluble receptors via ELISA. Selected blood samples and tumor biopsies, including diagnostic (archival) paraffin embedded tumor sections, were analyzed for mutation of Met at known mutation hotspots. Tumor, skin, and hair follicles were processed for extensive IHC analyses.
Results: Staining of Met, phospho-Met (pMet), RON, pRON, pERK, and pAKT was detected in normal tissue, skin, and tumor tissue from a pt with melanoma who experienced a MR. Administration of XL880 decreased tumor staining of pMet, pRON, pERK, and pAKT, but staining for Met and RON was unchanged. Decreased tumor cell proliferation (Ki67 staining) and increased tumor cell apoptosis (TUNEL) were also observed. No hotspot mutations were observed in SPRC pts who exhibited PRs. However, when compared to adjacent normal renal tissue, staining for Met, pMet, RON, pRON and Ki67 was elevated in untreated tumor tissue. Additional pt tumors and tumor vasculature are under analysis.
Conclusions: In pts with solid tumors, administration of XL880 is associated with decreased activation of Met and RON, decreased activity of associated signaling pathways (AKT and ERK), decreased tumor cell proliferation, and increased tumor cell death. These data from clinical human samples are consistent with preclinical data demonstrating that XL880 exhibits potent anti-tumor activity by targeting MET. The responsiveness of SPRC pts to XL880 was not associated to mutational activation of Met. In the absence of clinical evidence of target inhibition with this novel SSKI, the tumor staining confirms activity against Met at doses at or below VEGF receptor and PDGFRβ inhibition.

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A phase I dose-escalation study of the safety and pharmacokinetics of a XL184, a VEGFR and Met kinase inhibitor, administered orally to subjects with advanced malignancies

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Background: XL184 is an orally available small molecule inhibitor of multiple receptor tyrosine kinases involved in tumor cell growth and angiogenesis. The primary targets of XL184 are Met, VEGFR2/KDR, and additional targets include KIT, FLT3, and Tie-2. The purpose of this study is to define the maximum tolerated dose (MTD) and pharmacokinetics (PK) of XL184. In addition, exploratory pharmacodynamic (PD) assays are being evaluated using blood plasma samples.
Methods: Patients (pts) with advanced solid malignancies are enrolled in successive cohorts to receive XL184 orally as a single dose on day 1 with pharmacokinetic (PK) sampling, followed on day 4 by 5 consecutive daily doses with additional PK sampling and observation until day 21. In subsequent cycles, pts receive daily dosing for 5 days every 14 days. Tumor response is assessed every 8 weeks by RECIST criteria. PD blood samples were collected from all pts and plasma samples will be analyzed for ligands and soluble receptors via ELISA.
Results: To date, a total of 12 pts (carcinoid [3], mesothelioma [1], gastric [1], pancreatic cancer [1], breast cancer [1], parotid carcinoma [1], cholangiocarcinoma [1], T-cell lymphoma [1], angiosarcoma [1] and gastro/esophageal carcinoma [1]) have been treated across 3 dose levels: 0.08, 0.16, and 0.32 mg/kg. Currently, the maximum tolerated dose is not yet defined and dose escalation continues. Of 12 treated pts, 3 have had stable disease greater than 3 months (ongoing stability at 7, 6 and 4 months), including one patient with carcinoid carcinoma with liver metastases who has had approximately 20% reduction in tumor size (treated at 0.08 mg/kg). There have been no drug-related AEs or SAEs to date. Preliminary PK analysis (0.08–0.32 mg/kg) indicates that systemic drug exposure (area under the plasma concentration-time curve; AUC) and peak plasma levels (Cmax) tend to increase with increasing XL184 dose. Average Cmax values were 34.2±20.7, 70.0±51.6, and 189.3±49.6 ng/mL following the fifth dose at 0.08, 0.16 and 0.32 mg/kg, respectively. The terminal half-life was approximately 90 hours after 5 days of dosing, with levels as high as