to EGFR-KIs because of reduced cell death. AEE788 (50 mg/Kg twice a week for 8 weeks) administered to orthotopic TIC-xenografts did not significantly affect the survival. Experiments are ongoing to see whether AEE788 pretreatment reduces tumorigenicity of TIC-cells *in vivo*.
Conclusions: (1) These established cell lines and xenografts represent valuable models for both basic and preclinical research on ependymoma, for which the availability of tumor models is extremely limited. (2) Human ependymoma TICs are sensitive to EGFR-KIs *in vitro,* but not *in vivo*, prompting preclinical evaluation of combination treatment strategies.
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215 POSTER

Anti-tumoral effects of the multi-targeted kinase inhibitor AEE788 in BRAF mutated colorectal cancer cells

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Background: Advanced colorectal cancer patients with tumours harboring a mutation in the KRAS or BRAF genes do not derive benefit from the administration of epidermal growth factor receptor (EGFR)-directed monoclonal antibodies, such as cetuximab or panitumumab. Therefore, other targeted therapies are needed. AEE788 is a novel synthesized oral small-molecule multitargeted kinase inhibitor with potent inhibitory activity against both EGFR and vascular endothelial growth factor receptor (VEGFR). The aim of this study was to determine the efficacy of AEE788 to inhibit cell proliferation in colorectal cancer cells with different RAS/BRAF mutational status, and to explore the involved mechanisms.
Materials and Methods: The human colorectal cancer cell lines SW48 (KRAS/BRAF non-mutated), Caco-2 (BRAF V600E) and HCT-116 (KRAS G13D) were treated with different doses of AEE788, in the presence or the absence of EGF or VEGF. Cell proliferation was measured using an XTT assay. Apoptosis was determined using both cell death detection ELISA and annexin flow cytometry assays. The expression and phosphorylation levels of EGFR, VEGFR, Akt and Erk1/2, and COX-2 expression were determined by western-blot using the corresponding specific antibodies.
Results: In all the three cell lines AEE788 effectively inhibited the phosphorylation of EGFR induced by EGF. In addition, AEE788 was capable to reduce the EGF-driven cell proliferation of SW48 and Caco-2 cells, but not of HCT-116 cells. Significantly, AEE788 reduced the VEGF-dependent cell proliferation of Caco-2 cells, that efficiently expresses cyclooxygenase-2 (COX-2), but not of SW48 or HCT-116 cells with low or undetectable expression of this enzyme, respectively. The antiproliferative effects of AEE788 in Caco-2 cells were associated to reduced activation of the EGFR/VEGFR downstream kinases Akt and ERK1/2 and enhanced apoptosis.
Conclusions: AEE788 exerts anti-proliferative and apoptotic effects in BRAF mutated colorectal cancer cells, by inhibiting both EGF- and VEGF-dependent intracellular signaling. Our results support that AEE788 may be effective in the management of colorectal cancer in a non-mutated KRAS setting, independently of BRAF mutational status.

216 POSTER

Preclinical characterization of EMD 1214063 − a selective c-Met kinase inhibitor in clinical phase 1

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The relevance of the oncogenic receptor tyrosine kinase c-Met for tumor progression, metastasis and aggressiveness has been convincingly demonstrated in preclinical and early clinical settings. c-Met can be activated by different mechanisms such as HGF binding and dimerization, over-expression, gene amplification or activating mutations. Several compounds with different selectivity profiles inhibiting c-Met are currently under preclinical/clinical investigation and might emerge as valuable cancer therapeutics in the future.
After the optimization of a hit structure identified during a high throughput screening, the highly selective c-Met kinase inhibitor EMD 1214063 was identified as clinical candidate for further development and is currently being investigated in a phase 1 clinical trial. This compound inhibits enzymatic and cellular c-Met kinase activity with IC_{50} values in the low nanomolar range. The pyridazinone EMD 1214063 displayed an impressive kinase selectivity of at least 300 fold when tested in vitro against a panel of more than 280 kinases at a concentration of 1 μM. The mechanism of action of our clinical candidate, including inhibition of phospho-c-Met, down-regulation of cyclin D1 and up-regulation of p27 in a dose and time dependant manner, has been shown in PK/PD experiments *in vivo*. This compound also demonstrated excellent anti-tumor activity *in vivo* in a variety of xenograft models, e.g. the gastric cancer cell line Hs746T, the lung cancer cell line EBC-1 or the glioblastoma cell line U87MG, either as single agent or in combination. Depending on the sensitivity of the particular model, complete regression and tumor free survival was observed with doses as low as 6 mg/kg/d administered per os. The overall profile of EMD1214063 including the chemical structure, structure–activity relationships, in vitro potency, selectivity profile, pharmacokinetic and *in vivo* data will be discussed.

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Specific TGF-beta receptor-I inhibition using LY364947 impairs signaling, motility, and invasion in parental and multikinase inhibitor-resistant hepatocarcinoma cells

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Background: Hepatocarcinomas (HCC) are highly malignant tumors of unmet medical needs. LY364947, a selective ATP-mimetic inhibitor, specifically inhibits TGF-β receptor (TβR)-I activation at nanomolar concentrations. TβR-I activation induces angiogenesis, cell invasion, and epithelial-to-mesenchymal transition (EMT), offering opportunities for investigating the potential of novel TβR-I inhibitors such as LY364947 in HCC.
Materials and Methods: We investigated the antiproliferative effects of LY364947 in a panel of human HCC and other gastrointestinal cancer cells by MTT assay, baseline and phosphorylated (p-) protein levels by western blot analysis, mRNA expressions by qRT-PCR, motility by wound-healing assay, and invasion by matrigel assay.
Results: LY364947 was tested in SK-HEP1 cells and the derived-counterparts SK-HEP-1R cells selected by stepwise exposure to the multikinase inhibitor sunitinib (cross-resistant to sorafenib). Protein- and mRNA-expressions of TGF-β1, TGF-β2, and TβR-I were detectable in SK-HEP1 and SK-HEP1-R cells, a low expression of mRNA TβR-II (with no protein) signal being observed in these cells. Exogenous stimulation of SK-HEP1 and SK-HEP1-R cells with TGF-β yielded the downstream activations of p-Smad2 and p-Smad3 as well as p-ERK1/2, p-AKT^{ser473}, and p-S6 in SK-HEP1 cells. In TGF-β-stimulated SK-HEP1 and SK-HEP1-R cells, LY364947 inhibit p-Smad3 at μmolar concentrations. LY364947 also inhibits TGF-β-induced downstream p-AKT^{473} and p-ERK1/2 signaling in SK-HEP1 cells. LY364947 displays moderate antiproliferative effects at concentrations up to 20 μM after 72 h exposure in our cell lines without exogenous TGF-β stimulation. Using 5 and 10μM LY364947, a decrease in spontaneous TGF-β-independent cell motility was observed in SK-HEP1 and SK-HEP-1R cells in wound-healing assay. Using 10 μM, LY364947 also decreases TGF-β-independent invasion in both SK-HEP1 and SK-HEP1-R in matrigel assay.
Conclusion: Inhibition of TGF-β/TβR-I activation using LY364947 inhibits TGF-β-dependent cell signaling and reduces cell motility and invasion in parental and multikinase-resistant HCC cells. HCC appears as an interesting tumor model to evaluate and antimetastatic potential of novel TGF-β inhibitors, either as single agents and/or in combination with other anticancer drugs.

218 POSTER

Down-modulation of the androgen receptor (AR) with EZN-4176 inhibits the growth of prostate tumor and potentiates the inhibitory effect of MDV-3100, a novel anti-androgen

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Background: While androgen-deprivation therapies are effective initially for the treatment of prostate cancer (PC), the recurrence of castration-resistant prostate cancer (CRPC) frequently occurs. In such cases, the AR still plays a critical role since new agents that deplete testosterone (Abiraterone; Reid et al., 2010. J. Clin Oncol. *28*: 1447−9) or block ligand binding (MDV-3100; Scher et al., 2010. Lancet. *375*: 1437−46), and are in Phase III evaluation, remain effective. These findings suggest that down-modulation of AR expression may provide an alternative strategy for treating CRPC. Here we describe a novel locked nucleic acid (LNA)-based antisense oligonucleotide (ONs), designated EZN-4176, that down-modulates the AR and inhibits prostate tumor growth in vitro and in vivo.

Methods: The mRNA, growth, luciferase activity, protein, and prostate specific antigen (PSA) were evaluated by qRT-PCR, MTT, SteadyGlo, western blot analysis, and ELISA assay, respectively. The effect of EZN-4176 on AR transcriptional activity was evaluated in LNCaP (androgen-dependent) and C4−2b (castration-resistant) cells. In vivo, therapeutic efficacy was evaluated in the androgen-dependent AR-positive CWR22 tumors.
Results: *In vitro*, EZN-4176 specifically inhibited the growth of both LNCaP and C4−2b cells. This effect correlated with down-modulation of AR (mRNA and protein), as well as AR transactivation. Interestingly, we found that the combination with anti-androgens (MDV-3100 or bicalutamide) showed much improved inhibitory effect in growth assays. *In vivo*, EZN-4176 demonstrated tumor inhibition (comparable to bicalutamide or MDV-3100) in CWR22 tumor xenografts, which was accompanied with down-modulation of mRNA of AR, PSA, and TMPRSS2 as well as protein level of AR. More importantly, EZN-4176 dramatically potentiated the tumor inhibitory effect of MDV3100. In C4−2b tumor xenografts, EZN-4176 potently and specifically down-modulated AR-luciferase reporter activity, confirming the uptake of EZN-4176 and down-modulation of AR in the tumors. Further examinations of the effect on a panel of LNA-ONs that specifically target AR splice variants, which also may play a role in resistance, could enhance the repertoire of antisense molecules to treat CRPC.
Conclusions: Our data suggest that EZN-4176 alone or in combination with MDV-3100 offers a new strategy to treat CRPC. These preclinical data support initiation of phase I studies in patients with prostate cancer.

219 POSTER

PI3K delta: Discovery of potent and selective inhibitors for treating hematopoietic malignancies

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Background: The phosphoinositide-3-kinase (PI3K) pathway is one of the most frequently activated pathways in human cancer. The PI3K isoform PI3Kδ is expressed primarily in leukocytes, and has important roles in immune cell function and development. PI3Kδ and its downstream target Akt have been reported to be frequently activated in leukemic blasts from patients with B cell malignancies and acute myeloid leukemia (AML). Constitutive activation of the PI3K/Akt pathway in malignant leukocytes results in sustained proliferation and survival of tumor cells. Therefore, the development of selective inhibitors of PI3Kδ with high therapeutic index offers a new approach for treating hematological malignancies.
Methods: Small molecule inhibitors of PI3Kδ were identified by high-throughput screening and optimized through medicinal chemistry techniques. Biochemical and cell based assays were used to measure compound potency and selectivity, and Akt pathway activation was measured by Western blot assay. Pharmacokinetic studies were conducted in mice, rats, dogs and cynomolgus monkeys.
Results: We identified potent inhibitors of PI3Kδ (biochemical IC50s <10 nM). The compounds are ATP-competitive with >100-fold selectivity over other PI3K Class I isoforms (PI3Kα, PI3Kβ, PI3Kγ) and a diverse panel of protein kinases. In cellular assays using the Raji cell line (lymphoblast-like derived cells from Burkitt's lymphoma), the compounds inhibit phosphorylation of targets downstream of PI3Kδ including Akt at threonine-308 and serine-473 (IC50s <100 nM) and PRAS40 at threonine-246 (IC50 < 300 nM). Additional cellular profiling has identified several lymphoma and leukemia cell lines that are preferentially sensitive to PI3Kδ inhibition. The compounds also inhibit the anti-IgM stimulated release of TNFα in vitro (IC50 < 30 nM) and IgE triggered mast cell degranulation in vivo (EC50 <1 mg/kg). Pharmacokinetic studies across multiple species show the compounds to have high oral bioavailability (60–100%) and are well tolerated at plasma exposures >500-fold over the cell-based IC50s.
Conclusions: Selective and potent inhibitors of PI3Kδ were identified that are highly active in cells and inhibit PI3K pathway signaling. A subset of lymphoma and leukemia cell lines with high sensitivity to the compounds was identified. The compounds have good in vivo exposure and PD activity and are being used to explore the in vivo anti-tumor effects of PI3Kδ inhibition.

220 POSTER

Discovery of selective inhibitors of fibroblast growth factor receptor (FGFR): Antitumor activity in cellular and xenograft tumor models with FGFR activation

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Background: Deregulated FGF signaling promotes oncogenesis in several tumor types including gastric, bladder, endometrial, breast and multiple myeloma. Tumor genomic analyses of these cancers has identified amplifications, translocations, and activating mutations in FGFR1, FGFR2, and FGFR3. Inhibitors selective for the FGFR family provide an opportunity to target diverse cancer subtypes driven by FGFR activation while avoiding potential complications from VEGFR/PDGFR inhibition and anti-angiogenic therapy.
Methods: Small molecule inhibitors of FGFR family kinases were identified by high-throughput screening and lead optimization using in vitro enzymatic assays. FGFR2 activation was measured in cultured cells and in xenograft tumors using phosphorylation of FGFR and downstream signaling proteins FRS2, ERK, and AKT. Cell viability was assessed by measuring cellular ATP levels. Xenograft tumors were grown in nude mice and compounds dosed by oral gavage.
Results: FGFR inhibitors were identified with potent biochemical activity against FGFR1, FGFR2, and FGFR3 (IC_{50} 10–100 nM). X-ray crystallographic studies with FGFR2 demonstrated that the compounds bind in the ATP binding pocket. Cell viability assays were used to identify FGFR-dependent tumor cell lines. Most of these lines have genetic alterations in FGFR family members such as the colon adenocarcinoma line NCI-H716 which contains an amplification of FGFR2. Treatment of NCI-H716 cells with FGFR inhibitors blocks phosphorylation of FGFR2 and the downstream proteins FRS2, ERK, and AKT (IC_{50} 10–100 nM) as well as a broader phosphotyrosine signaling network that includes the HER family kinases. In vivo pharmacodynamic studies with orally bioavailable compounds demonstrated target inhibition in NCI-H716 xenograft tumors as assessed by a reduction in pFGFR2. Efficacy studies in the NCI-H716 xenograft model showed up to 70% tumor regression at well tolerated doses. Distinct chemical subseries were identified that selectively inhibit FGFR2 vs. other family members or that inhibit FGFR2 with mutations at the gatekeeper residue V584, a common source of resistance to kinase inhibitor therapy.
Conclusions: In FGFR-driven tumor models, FGFR selective inhibitors block receptor activation and downstream signaling, reduce cell viability in vitro, and inhibit the growth of xenograft tumors. These results support advancement of FGFR selective inhibitors for the treatment of select cancer subtypes identified by tumor genomics.

Monoclonal antibodies and targeted toxins/nuclides/agents

221 POSTER DISCUSSION

89Zr-bevacizumab PET imaging in renal cell carcinoma patients: feasibility of tumor VEGF quantification

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Background: Renal cell carcinomas (RCCs) are characterized by high VEGF production resulting in excessive angiogenesis. Systemic VEGF levels are only partially tumor derived and do not predict response to angiogenesis inhibitors. We developed a novel imaging technique for non-invasive quantification of VEGF levels in the tumor and its microenvironment with the PET tracer ^{89}Zr-bevacizumab. We evaluate the feasibility of VEGF imaging before and during treatment in patients with RCC (NCT00831857). Here we report results of baseline scans.
Material and Methods: patients with RCC who start treatment with either sunitinib or bevacizumab plus interferon undergo VEGF-PET imaging at baseline and at 2 and 6 weeks after start. 37 MBq ^{89}Zr-bevacizumab (5 mg protein dose) is injected IV 4 days before each scan. PET scans are fused with baseline CT scans. Mean Relative Uptake Value (RUV), defined as the mean uptake in a region of interest devided by mean uptake in the whole body, is calculated for normal organs and for up to 10 tumor lesions per patient.
Results: in this ongoing study 11 patients underwent a baseline scan. Distribution in normal tissues showed high uptake in the heart (reflecting blood pool: RUV 4.6, range 3.7−5.6) and liver (RUV 5.6, range 4.5−7.5), intermediate uptake in lungs (RUV 1.2, range 0.7−1.7) and low uptake in the brain (RUV 0.21, range 0.12–0.33). In all patients tumor lesions (range 1 to >10) were visualized with VEGF-PET. A total of 64 lesions were quantified, resulting in a mean RUV of 7.1 (range 1.3–20.9). Between patients, mean tumor RUV varied from 2.3 to 13.2. Within individual patients, tumor RUV in different lesions varied with a factor 1.2 to 8.2.