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.