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.

Conclusions: ^{89}Zr-bevacizumab PET visualizes tumor lesions in RCC patients. Tumor quantification shows large differences within and between patients, whereas differences in normal tissue uptake between patients are small. With its high tumor-to-background ratio in target lesions this technique is expected to perform well in serial VEGF quantification during treatment with angiogenesis inhibitors.

222 POSTER

A first-in-human, phase I trial of the anti-DLL4 antibody (OMP-21M18) targeting cancer stem cells (CSC) in patients with advanced solid tumors

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Background: DLL4 is a ligand that activates the Notch pathway which is important for CSC survival. OMP-21M18 is a humanized, anti-DLL4 antibody that has been shown to inhibit tumor growth and decrease CSC frequency in minimally passaged human xenograft models, using an in vivo tumorigenicity limiting dilution assay. Inhibition of DLL4 has also been shown to cause dysfunctional sprouting of new vessels resulting in an antiangiogenic effect.
Methods: Patients with advanced solid tumors were enrolled in escalating dose cohorts and received OMP-21M18 weekly or every other week. The primary study objective was to determine the safety profile of OMP-21M18. Other objectives included: immunogenicity, pharmacokinetics, antitumor activity and biomarkers of Notch signaling and CSCs in blood, hair follicles and tumor cells.
Results: As of June 4, 2010, 33 patients were treated and 27 were evaluable for safety. Patients were treated with 0.5, 1, 2.5, or 5 mg/kg weekly or 2.5, 5 or 10 mg/kg every other week. The median age was 60 years and the patients had received a median of 4 prior chemotherapy regimens. The maximum tolerated dose (MTD) was not reached. Adverse events were predominantly grade 1 and unrelated to study drug. Adverse events reported as related to study drug and occurring in at least 3 patients included: hypertension (12), asthenia/fatigue (7), nausea (5), headache (3), and abdominal pain (3). Six of the 12 subjects who developed hypertension requiring oral antihypertensive treatment, had a diagnosis of hypertension at study entry. GI bleeding was observed in 4 patients and isolated BNP elevations were also observed. The half-life of OMP-21M18 was 12 days and administration of OMP-21M18 was associated with little immunogenicity. OMP-21M18 was shown to alter Notch signaling in blood and hair follicle cells. A waterfall plot of the % change in RECIST tumor measurements/patient suggests antitumor activity, in this heavily pretreated patient group, at doses of 5 mg/kg weekly and 10 mg/kg every other week and an unconfirmed partial response was observed in a patient with pancreatic cancer.
Conclusion: OMP-21M18 was generally well tolerated with asymptomatic hypertension being the most common drug related toxicity. The MTD was not reached at 10 mg/kg every other week. Encouraging early evidence of biologic and clinical activity has been observed. Enrollment is ongoing and updated results will be presented.

223 POSTER

Trastuzumab-DM1: mechanisms of action and mechanisms of resistance

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Overexpression of the HER2 receptor tyrosine kinase (RTK) occurs in approximately 20−25% of human breast cancer. Patients with elevated HER2 levels show more rapid disease progression and worse survival than breast cancer patients with HER2-negative disease. Trastuzumab (Herceptin®) is a humanized antibody developed specifically to treat HER2-positive breast cancer and is used in both the adjuvant and metastatic setting. Although trastuzumab is efficacious for many patients, a number of patients will have disease progression through trastuzumab therapy. We have recently developed an antibody–drug conjugate comprised of trastuzumab covalently linked through a stable linker to the microtubule inhibitory agent DM1, as an alternative treatment for patients whose cancers overexpress HER2. Trastuzumab-MCC-DM1 (T-DM1) is currently undergoing clinical testing in metastatic HER2-positive breast cancer patients. We performed cell culture studies to compare the potency of T-DM1 to chemotherapeutic agents and to determine T-DM1 mechanisms of action (MOA). Viability and clonogenic assays on HER2-amplified breast cancer cells show that T-DM1 is more potent than taxane or vinca alkaloid agents. Treatment with T-DM1 results in G2/M cell cycle arrest, as measured in cell cycle experiments or by Western analysis of the G2/M markers cyclin B1 and phospho-histone H3. Subsequently, G2/M-arrested cells undergo apoptosis as indicated by PARP cleavage and loss of XIAP expression. Like trastuzumab, T-DM1 suppresses signaling through the PI3 kinase pathway. However, T-DM1 inhibits PI3 kinase signaling in trastuzumab-insensitive cells; this activity was shown to be mediated by the DM1 component of T-DM1. Anti-proliferative activity of T-DM1 was also compared to T-DM1 components (trastuzumab, DM1, SMCC linker) and identified catabolites (Lys-MCC-DM1 and MCC-DM1). Only T-DM1 and DM1 potently inhibit growth of HER2-overexpressing breast cancer cells. Mechanisms of resistance to T-DM1 were investigated in 3 cell lines developed to have acquired T-DM1 resistance. One T-DM1-resistant line shows upregulation of multi-drug resistance (MDR) transporters. Increased expression of EGFR and other RTKs, as well as several erbB ligands was also observed. Mutations in beta 1-tubulin were not detected. These studies demonstrate the potent anti-tumor activity of T-DM1 compared to conventional chemotherapeutic agents, show multiple MOA for T-DM1, and shed light on potential mechanisms of resistance.

224 POSTER

Her3 as an emerging target for lung tumor initiating cells

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Background: Lung cancer is the leading cause of cancer mortality in the world. Current therapy is relatively ineffective and survival rate is approximately 15%. The human ErbB (or HER) receptor family plays multiple roles in normal cell functions as well as in tumor development and maintenance of lung cancer. Mutational activation and/or gene-amplification of ErbB family members is observed in several human malignancies. Given this key role in oncogenic signalling, several agents, antibodies and small molecules designed to target EGF/HER receptors, have been developed clinically. These include the antibodies cetuximab, panitumumab (EGFR), trastuzumab and pertuzumab (HER2) and the small molecule tyrosine kinase inhibitors (TKIs) gefitinib, erlotinib (targeting EGFR) and lapatinib (targeting EGFR/HER2). However, clinical efficacy for all these agents has been less pronounced than predicted based on preclinical models and responses are generally transient leading to the emergence of drug resistance. Intra-tumoral heterogeneity possibly underlies resistance of lung cancers to current therapies. Malignant pleural effusions (MPE) represent an opportunity to culture a wide variety of cancer cells from a single individual and possibly predict the response to a given therapy.
Materials and Methods: HER3 represents a crucial player in signal transduction and acquired resistance to TKIs, and a potential new drug target. In fact, HER3 has been shown to influence maintenance and survival of tumor cells through activation of the PI3K/AKT pathway. Since HER3 is devoid of kinase activity, it cannot be targeted by small molecule inhibitors. In contrast, antibodies are a viable strategy for pharmacological intervention. We have recently generated and started to characterize a set of five novel hybridomas directed against different epitopes of HER3. These antibodies significantly affected ligand-dependent signal transduction, viability and maintenance of cancer cells of different origins *in vitro*.
Results: In this study, we have characterized MPE primary cultures for ErbB receptors expression, phosphorylation and expression of the relevant ligands. In particular, HER3 was expressed at high levels and thus could be considered a relevant target for cancer stem-like cells. Importantly, anti-HER3 antibodies were capable of blocking signal transduction, cell proliferation and spheroid-forming activity.
Conclusions: We are currently establishing predictive *in vitro* and *in vivo* systems to test our anti-HER3 antibodies for their capability to block tumorigenicity of MPEs and revert acquired resistance to other antibodies and TKIs. Our results may have a significant impact on current standard therapies for lung cancer.