that is implicated in the PI3K/Akt pathway. Therefore, mTor inhibition is believed to contribute positively to the pathway shutdown. We present a detailed characterization of ETP-187, an advanced analog of this chemical series and potent dual inhibitor of PI3K alpha (IC_{50} 0.12 nM) and mTOR (1.5 nM), discussing its isoform and mutant profile and its selectivity against other kinases. In U2OS cells, ETP-187 blocks PI3K/Akt pathway signaling effectively as shown by inhibition of Akt phosphorylation at Ser473 (EC_{50} 1.1 nM) and of other downstream biomarkers. In addition, *in vivo* PK data will be presented.

53 POSTER

Down regulation of beta-catenin by a locked nucleic acid oligonucleotide antagonist inhibits tumor growth in experimental models of human cancer

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β-catenin is a transcriptional regulator that is critical in the development of numerous human cancers. The protein can be activated by many mechanisms including the loss of APC (colon cancer) or mutation of β-catenin itself (many human cancers e.g. hepatocellular carcinoma). Either event leads to stabilization of β-catenin. The protein can then translocate into the nucleus thereby activating pro-proliferative and survival signals within the cancer cells. It has been exceedingly difficult to identify specific small molecule inhibitors that target only protein–protein/DNA interactions involving β-catenin; this provides an ideal opportunity for the use of antisense-based therapy. Herein, we describe a locked nucleic acid (LNA) oligonucleotide (ON) antagonist of β-catenin, EZN-3892, with potent in vitro and in vivo anti-tumor properties. EZN-3892 targets the 3′ UTR of the β-catenin mRNA and results in specific down regulation of β-catenin mRNA and protein associated with inhibition of cell proliferation and death in human cell lines. LNA-ONs possess exceptionally high binding affinity for mRNA and high resistance to nuclease degradation. Therefore, we have been able to identify numerous human cell lines that take up the β-catenin LNA–ONs (without transfection) associated with marked target down modulation and growth inhibition. Colo-205 (colon) and NCI-H1581 (lung) are two such cell lines that are sensitive to EZN-3892 (EC_{50} = 80 nM and 2.5 mM, respectively). Consistent with this, mice bearing Colo-205 and NCI-H1581 tumor xenografts administered 50 mg/kg of EZN-3892 prepared in saline and given Q3Dx6 IV, show a 71 and 76% tumor growth inhibition, respectively ($p < 0.005$). In addition, we have used $APC^{+/-}$ mice to explore the utility of EZN-3892, since polyps within these animals have sustained activity of β-catenin. Administration of EZN-3892 at 60 mg/kg, given Q3Dx2 to the polyp-bearing mice results in a 50% reduction in β-catenin mRNA in polyp tissue ($p < 0.0001$) as well as several β-catenin target genes (Myc, Survivin and Cyclin D1, $p < 0.0001$) while non-related mRNAs are not altered. Taken together, EZN-3892-mediated β-catenin target down regulation associated with growth inhibition occurs in relevant tissues without a delivery vehicle and thus holds much promise for the control of cancer in patients where β-catenin plays a critical role in tumor progression.

54 POSTER

Biological characterization of ETP-45299, a selective small molecule inhibitor of PIM1, in human tumor cell lines

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The Pim family of serine/threonine kinases, particularly Pim1, has been shown to be misregulated in a variety of human malignancies; hence inhibitors of the PIM kinases are of therapeutic interest. In order to evaluate the therapeutic potential of targeting the Pim kinases a biochemical screen was performed to identify low molecular weight inhibitors of Pim 1. This screen identified the imidazo (1,2-b) pyridazines as potent, but nonselective inhibitors of Pim1. Chemical optimization of the imidazo (1,2-b) pyridazines lead to the discovery of ETP-45299 a potent and selective inhibitor of Pim1. ETP-45299 has a Ki of 30 nM for Pim1 and Ki's of 1,049 and 81 nM for Pim2 and Pim3, respectively. Unlike other imidazo (1,2-b) pyridazines, ETP-45299 was 25 times more selective towards Pim1 than Flt-3. The compound had no significant inhibitory activity against an additional 21 kinases that were tested. ETP-45299 inhibited the phosphorylation of BAD and 4EBP1 in a dose dependent manner and induced cell cycle arrest in MV4:11 tumors cells. ETP-45299 suppressed the proliferation of several non solid and solid human tumor cell lines. It also suppressed the migration MDA-MB-231 breast cancer cells through matrigel indicating a potential role for Pim inhibitors in metastatic disease. Dual inhibition of PI3K and Pim signaling was tested by combining the selective PI3K inhibitor GDC-0941 with ETP-45299. The combination of the two inhibitors was strongly synergistic in the MV4:11 cells indicating that dual inhibition of Pim and PI3K signaling could be efficacious in AML.

55 POSTER

The TORC1/TORC2 inhibitor, palomid 529 (P529), reduces tumor growth and sensitizes to chemotherapy and radiotherapy aggressive hormone refractory prostate cancer cells both in vitro and in vivo

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Background: Several studies have suggested that the AKT-mediated survival-signaling pathway is an attractive target for cancer therapy: (i) AKT pathway is relatively inactive in resting cells and amplification of the AKT gene occurs in some tumors; (ii) loss of the tumor suppressor gene PTEN (phosphatase and tensin homolog deleted on chromosome 10), present in about 30% of prostate primary tumors and in more than 50% of aggressive castrated resistant prostate cancers, constitutively activates AKT. AKT is indeed activated at the cancer invasion front stimulating local invasion and neoangiogenesis as well as decreasing sensitivity to chemotherapeutics and radiotherapy. A novel PI3K/Akt/mTOR inhibitor, Palomid 529 (P529), which inhibits the TORC1 and TORC2 complexes shows both inhibition of Akt signaling and mTOR signaling as well as inhibits tumor cell proliferation.
Aim and methods: We analyzed the in vitro effects of P529 on a panel of eight prostatic cancer cell lines having or not basal activation of Akt as well as the in vivo effects on aggressive castrated resistant PC3 (high basal Akt activity) and 22rv1 (low basal Akt activity) cell lines xenografted in nude mice.
Results: P529 inhibited cell proliferation with IC50 values ranging between 5 and 30 mM calculating at 48 hr of treatment. These values seems to be scarcely related to basal Akt activity and also cells possessing low Akt levels are sensitive to P529. However, the re-expression of PTEN in PTEN negative PC3 cell line reduced significantly the effects of P529 as well as the siRNA for PTEN sensitizes PTEN positive DU145 and 22rv1 cells to P529. However, we observed that the effects of P529 treatment were more marked when this drug were added to culture in clonogenic assays suggesting that at longer time prostate cancer cells are able to increase Akt activity in an autocrine manner for example secreting EGFR/Her2 ligands and exogenous addition of EGF (50 ng/ml) was able, indeed, to increase P529 efficacy. In this report we showed that the inhibition of Akt pathway by P529 (Palomid) enhanced the sensitivity to docetaxel and cisplatin of both PTEN positive and negative prostate cancer cells in vitro and in vivo. We demonstrated also that this during was able to reduce cell proliferation and to induce cells death increasing the activity of death receptors TRAILR-5 and Fas and downmodulating the expression of cellular-FLICE-inhibitory protein (c-FLIP), Bcl2 and survivin.
Conclusions: Therefore, these combinatorial treatments might open a promising therapeutic approach for the elimination of hormone-refractory prostate cancers, which are largely resistant to conventional therapies.

56 POSTER

A novel interplay between Ret oncoprotein and Fap-1 controls CD95-mediated apoptosis in medullary thyroid cancer

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Medullary thyroid cancer (MTC) represents the major cause of death in type 2 multiple endocrine (MEN2) syndromes. The most aggressive form, MEN2B, is caused in more than 95% of cases by the germline mutation M918T of the Ret receptor tyrosine kinase (Ret-MEN2B). The same mutation is present in one-third of sporadic MTCs where it has been associated with poor prognosis. Ret-MEN2B is a potent activator of cell survival pathways. However, the emerging proto-Ret function as dependence receptor suggests that Ret oncoproteins might also evade the receptor pro-apoptotic activity in the absence of ligand by directly impacting the apoptosis machinery. Preclinical studies have shown the antitumor potential of targeting Ret kinase with small molecule inhibitors. The present study is aimed at dissecting mechanisms of regulation of survival/apoptosis pathways by Ret oncoproteins to identify new targets exploitable in therapeutic drug combinations.
We found that in the human MEN2B-type MTC cell line MZ-CRC-1, inhibition of Ret activation and signaling by the tyrosine kinase inhibitor

RPI-1 was associated with Ret downregulation and apoptosis characterized by early caspase-8 activation and relocalization of CD95 death receptor into lipid rafts. This event was allowed by the downregulation of Fap-1, an inhibitor of CD95 trafficking to the cell membrane. Ret was found associated with both Fap-1 and procaspase-8 which were tyrosine dephosphorylated upon drug treatment. We propose a model in which tyrosine dephosphorylation of procaspase-8 induces its local activation initiating Ret proteolytic processing and destabilizing Fap-1. The following caspase-dependent degradation of Fap-1 may thus allow releasing the negative constraint on CD95. Accordingly, we found that drug-induced cell growth inhibition and apoptosis were enhanced in the presence of the CD95 agonist antibody CH11. Moreover, exogenous expression of the RET-M918T mutant in HEK293 cells upregulated Fap-1 and analysis of MTC specimens showed high levels of Fap-1 in RET-MEN2B type as compared to RET-wild type MTCs.
Overall, these findings reveal a functional interplay between Ret-MEN2B proteins and the extrinsic apoptosis pathway mediated by Fap-1 and caspase-8. The ability of Ret-MEN2B to exert a control on CD95 cell surface expression may contribute to MTC malignant phenotype and provide a rational basis for novel treatment strategies combining Ret inhibitors and CD95 agonists.

57 POSTER

Glioblastoma multiforme is characterized by high incidence of PDGFRalpha expression and susceptibility to the PDGFRalpha-specific antibody MEDI-575 in mouse tumor models

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Background: The platelet-derived growth factor receptor alpha (PDGFRα) acts not only as a receptor tyrosine kinase in tumor cells but also as a mediator of stromal support for cancer growth and survival. In glioblastoma multiforme (GBM), PDGFRα is activated in an autocrine fashion which is reminiscent of PDGFRα functions during development of mesenchymal-derived tissues. Since GBM is a malignant tumor derived from the mesenchyme, we hypothesized that blockade of the tumoral PDGFRα signaling cascade would result in anti-tumor activity.
Material and Methods: Human tumor microarrays with primary and recurrent GBM were stained for PDGFRα. Human GBM cell lines were grown as subcutaneous xenografts in nude mice and treated with MEDI-575, a fully human IgG2 monoclonal antibody that selectively targets PDGFRα without blocking PDGFRβ. Efficacy of MEDI-575 with temozolomide (TMZ) was measured by tumor growth inhibition (dTGI) and by delay in tumor regrowth after cessation of single and combination treatments.
Results: Tumor microarray analysis demonstrated PDGFRα expression in 41% of primary (n = 59) and 38% of recurrent (n = 53) GBM. The human GBM cell lines U118-MG, SNB-19 and U251-MG displayed high PDGFRα protein levels. MEDI-575 at ~2 nM inhibited ligand-induced phosphorylation of PDGFRα in all three GBM lines and PDGF-AA ligand was readily detected in tissue culture medium from SNB-19 cells. Mice with U118-MG or SNB-19 GBM xenograft tumors were dosed with MEDI-575 at 1 mg/kg (2×/wk) which resulted in anti-tumor efficacy of 118% or 78% dTGI, respectively. In U251-MG xenografts, 3 mg/kg (2×/wk) of MEDI-575 produced 71% dTGI. Efficacy in the U118-MG and U251-MG tumor xenografts was achieved at an exposure level of MEDI-575 in mouse serum of 34 ug/ml and 73 ug/ml of MEDI-575. Furthermore, combinations of 10 mg/kg of MEDI-575 with the GBM standard of care drug TMZ at 1 mg/kg or 15 mg/kg did not result in weight loss in mice. Both combination regimens delayed the regrowth of U251-MG xenograft tumors after stopping treatment when compared to treatment with MEDI-575 or TMZ alone.
Conclusions: High incidence of PDGFRα expression in primary and recurrent GBM together with high efficacy of MEDI-575 in GBM xenografts supports further testing of MEDI-575 with or without chemotherapy and radiation in preclinical and clinical settings to develop innovative medicines for GBM.

58 POSTER

Development and characterization of novel orally available Hypoxia-inducible factor (HIF) signaling inhibitors as dual-mechanism cancer therapeutics

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The HIF signaling pathway is crucial, in particular for solid tumors, to circumvent the constraints of low oxygen supply (hypoxia) to induce angiogenesis and maintain proliferation. The oxygen regulated subunit of the transcription factor hypoxia-inducible factor 1 (HIF1), HIF1alpha, is a key factor in tumor growth, and its expression has been correlated with poor patient prognosis in a number of settings.
Here we here present in vitro and in vivo data for a novel series of orally available small-molecule HIF signaling modulators that show nanomolar inhibition of the HIF signaling pathway, in addition to potent anti-proliferative activity against a large number of cell lines derived from solid and blood tumors (EC50 range: 1–100 nM). Phenotypically, the compounds elicit an initial G2/M arrest, followed by the induction of caspase-3/7 and the onset of apoptosis.
The in vitro results also translate into in vivo xenograft models. The lead compounds from the series demonstrate efficacy in such tumor models in mice, with dose-dependent tumor growth inhibition of >60% after oral dosing (breast, renal, and multiple myeloma models). Structural optimization has allowed us to improve the PK and physicochemical profile of the compounds, with a lead candidate having entered formal pre-clinical development. The objective is to commence Phase I clinical studies in Multiple Myeloma and other indications in the second half of 2011.
In conclusion, we believe that the development towards clinical Proof-of-Concept of this novel class of Dual-Mechanism Inhibitors (DMIs) impairing HIF signaling and cellular proliferation presents a promising new treatment option for cancer patients.

59 POSTER

Dual targeting of mTOR and HSP90 for therapy of pancreato-biliary carcinomas

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Background: Although mTOR has been identified as a therapeutic target in pancreato-biliary cancers, inhibitors to mTOR may lead to an undesired feed-back-loop activation via the TORC2 complex that *per se* exerts oncogenic activity, thus counteracting the antineoplastic potential. Interestingly, heat shock protein 90 (Hsp90) inhibitors harbor the potential to impair a broad range of oncogenic signaling molecules, including AKT, ERK and IGF-IR. Since activation of mTOR and IGF-IR signaling cascades represent driving oncogenic forces in pancreato-biliary carcinomas, we focused on elucidating the molecular effects of a dual Hsp90/mTOR inhibition in cholangiocarcinoma and pancreatic cancer cell lines, using 17DMAG, one novel synthetic Hsp90 inhibitor, and the mTOR inhibitor RAD001.
Materials/Methods: The effects of RAD001 and HSP90 were investigated in human cholangiocarcinoma cells (EGI-1, TFK1) and pancreatic cancer cells (L3.6pl), both K-ras mutated. Constitutive and IGF-I-induced signal transduction pathways were investigated by Western blotting. Cell proliferation was determined via an *in vitro* colorimetric BrdU-assay and incubation with RAD001 and/or Hsp90 inhibitors.
Results: Although effective inhibiting p-mTOR(Ser2448) and p-S6 (Ser240/244), the mTOR inhibitor RAD001 induced a positive feedback activation of p-AKT(Ser473), p-AKT(Thr308) and p-p44/42-MAPK(Thr 202/Tyr 204) in cancer cell lines. By adding the HSP90 inhibitor, this feedback was completely abrogated. However, inhibition of Hsp90 induced Hsp27 in all cancer cell lines except EGI-1. Inhibition of proliferation was achieved for up to 20% in L3.6pl and EGI-1 cells with RAD001. The HSP90 inhibitor dose-dependently reduced proliferation up to 25% in EGI-1 cells and up to 75% in L3.6pl cells. This effect was further increased in L3.6pl cells by adding RAD001, whereas no significant additive effect was observed in EGI-1 cells. However, the Hsp27 inducible cholangio cancer cell line TFK1 responded well to Hsp90 inhibition, suggesting Hsp27 as a potential marker for responsiveness. Moreover, IGF-I-induced signaling pathways were effectively blocked by HSP 90/mTOR inhibition.
Conclusion: Dual-targeting of mTOR/Hsp90 appears valuable for treating pancreato-biliary cancers through synergistic effects. However, despite achieving a robust signaling inhibition by mTOR/Hsp90 blockade, some cholangiocarcinomas are more susceptible towards Hsp90 inhibition, than a combinational therapy. Hsp90 serves as an interesting target for molecular therapy of pancreato-biliary carcinomas.