(SPC) from RHuT or RRT vaccinated mice were mixed with neu+ tumor cell lines and injected subcutaneously into the mammary fat pad of wt mice, a significant difference in tumor incidence was observed, being SPC from RHuT vaccinated mice the most effective. Immunohistochemical analysis revealed a clear differences in the type of the immune infiltrate in the tumor site. Mechanisms responsible for this kind of phenomena are under investigation.

Wednesday, 17 November 2010

Poster Sessions

Late breaking posters

4LB LATE BREAKING POSTER

A first synthesis of [18F]-lapatinib: a new agent for positron emission tomographic studies of kinase receptors

For full abstract, see p. 4.

5LB LATE BREAKING POSTER

Anti-tumor activity of MPC-9528, GMX1778 and APO866: Nampt inhibitors of three different structural classes

For full abstract, see p. 4.

6LB LATE BREAKING POSTER

The Nampt inhibitor MPC-9528 and the PARP inhibitor olaparib synergize in killing a BRCA-deficient cancer cell line

For full abstract, see p. 5.

Molecular-targeted therapies – preclinical

50 POSTER

A phase I study of the safety, tolerability and pharmacokinetics of pazopanib (P) in combination with gemcitabine (G) for advanced solid tumors

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Background: P (Votrient®), an oral angiogenesis inhibitor targeting VEGFR-1, -2, and -3, PDGFR-α and -β, and c-Kit, is FDA approved for advanced RCC. The combination of P + G is a novel regimen that may have broad clinical applicability.
Methods: Eligible pts had progressed on standard therapy, had adequate organ function, and received no prior anti-VEGF(R) therapy. A 3 + 3 dose escalation design was followed by an expansion phase. G was administered as a 30-min IV infusion on days 1 and 8 of each 21-day cycle at escalating dose levels (DL) of 1000 or 1250 mg/m^2. P was administered orally once-daily at escalating DLs of 400 or 800 mg. In the dose escalation phase, sparse sampling was performed to estimate peak and trough concentrations of P (C1D1: pre-dose and 3.5 h; C1D8 and C2D1 at pre-dose), and of G and its metabolite dFdU (C1D1 and C1D8 at pre-dose and end of infusion). In the cohort expansion phase, serial PK sampling was performed to characterize full PK profiles of G and dFdU with G given alone (C1D1) and in combination with P (C2D1). 24-hour P PK was determined on C2D1.
Results: 22 pts were enrolled; common tumor types were melanoma n = 8, NSCLC n = 4; CRC n = 4. The most frequent drug-related AEs (as a % of all pts) were fatigue 68%, neutropenia 59%, nausea 55%, anorexia 50%, and thrombocytopenia 41%. Most common Gr 3/4 AEs (as a % of all pts) were neutropenia 45% and thrombocytopenia 18%. Gr 3 (without Gr 4 observed) AEs were ALT increase 18%, and 9 % each for: lymphopenia, fatigue, diarrhea, abdominal pain, hyperbilirubenia. A non-neutropenic pt in DL 0 with sarcoma metastatic to lungs died of pneumonia at Day 105. Mean DIs of P and G for each DL are listed in Table. DL 2 was expanded to 13 pts to further assess tolerability. PK analysis indicates that systemic exposures of P, G, and dFdU in both dose escalation and cohort expansion phases appeared consistent with historical data. Comparison of G and dFdU PK parameter ratios (C2D1/C1D1) in expansion phase indicates P has no effect on the PK of G or dFdU. Best responses were: 1 PR (melanoma), 14 SD, 4 PD, 3 UNK. Durable disease control was observed with various tumor types; cholangiocarcinoma (cycle 17), melanoma (cycle 14), CRC (cycle 12).
Conclusions: P + G appears clinically active and tolerable for extended periods. Although an MTD was not reached, based upon analysis of tolerability as a function of DI, P 800 mg daily + G at 1000 mg/m^2 on days 1 and 8 every 21 days will be tested in phase II studies.
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Dose level (n)	P (mg) daily	G (mg/m^2 d1, 8 q21 d cycle	Mean (%) P/G dose intensity (DI)	No. of subjects/ type of DLT
0 (n = 6)	400	1000	92.4/79.7	1/Gr 4 thrombocytopenia
1 (n = 3)	800	1000	81.7/83.1	0/
2 (n = 13)	800	1250	94.9/86.1	1/Gr 3 fatigue

51 POSTER

Deacetylation and inactivation of peroxiredoxin by SIRT2 increases sensitivity of breast cancer cells to oxidative stress

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SIRT2, the mammalian ortholog of yeast *Hst2,* is a predominantly cytosolic and nuclear member of the class III, NAD+ dependent, histone deacetylase family. In the cytosol, SIRT2 co-localizes with microtubules and deacetylates α-tubulin at the lysine-40, indicating a role in proper cytokinesis. Here, we determined the other biologically important SIRT2 targets and their functions. In HEK293 cells, knockdown of SIRT2 followed by differential in-gel electrophoresis of the cytosolic extract and mass spectrometric analysis identified several significantly hyper-acetylated proteins, including peroxiredoxin 1. Peroxiredoxins (PXDs) are ubiquitous family of evolutionarily conserved, thiol-dependent peroxidases, which catalyze the reduction of hydrogen peroxide (H2O2). SIRT2 co-immunoprecipitated with PXD1, and ectopic over-expression of SIRT2 deacetylated PXD1 in HEK 293 cells. While hyperacetylation activates, deacetylation is known to inhibit the antioxidant activity of PXD1. Ectopic over-expression of SIRT2, but not the catalytically dead SIRT2 mutant, markedly increased intracellular reactive oxygen species (ROS) levels and increased ROS-induced DNA damage (determined by comet assay), as well as increased the sensitivity of the human breast cancer MCF7 and MDA MB231 cells to oxidative stress induced by H2O2. SIRT2 over-expression also significantly increased the sensitivity to ROS-inducing agents such as arsenic trioxide and menadione. Ectopic over-expression of SIRT2 induced FOXO3a and BIM levels, which was associated with increased caspase-3 activity and apoptosis of breast cancer cells. Additionally, wild type zebrafish embryos 48 hours post-fertilization were treated with 1.0 mM of splice-blocking morpholino (MO) targeting the exon 6 of the SIRT2 pre-mRNA versus the mismatch-control MO and/or 3.0 mM of H2O2. Exposure of zebrafish embryos to control MO and H2O2 increased ROS and produced cardiac edema and abnormal body curvature. In contrast, knockdown of SIRT2 in the zebrafish embryos decreased H2O2-induced ROS levels and abrogated ROS mediated cardiac edema and abnormal body curvature. These in vitro and in vivo findings demonstrate that SIRT2 regulates PXD1 acetylation and sensitivity to oxidative stress. These results also highlight the possibility that increased SIRT2 levels and activity can be therapeutically exploited for augmenting antitumor effects of agents that induce cancer cell death by increasing intracellular ROS levels.

52 POSTER

Discovery of novel fused thiadiazoles as potent inhibitors of phosphoinositide-3-kinase (PI3K) and/or the mammalian target of rapamycin (mTOR)

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Several inhibitors of phosphoinositide-3-kinase (PI3K) are currently being evaluated in early clinical studies for their ability to block PI3K/Akt pathway signaling that is found to be activated in many tumors. Here, we disclose a novel series of PI3K inhibitors based on fused thiadiazole bicycles and present their synthesis, structure–activity and structure–property relationships. We identified analogs with potent PI3K activity in the low nanomolar range and discuss their cross-reactivity with Flt3 as well as their cellular activity.
The mammalian target of rapamycin (mTOR), a class IV PI3K protein kinase, is - like PI3K itself - an important regulator of cell growth

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