

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 α (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.

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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.

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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 K_i of 30 nM for Pim1 and K_i '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.

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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 IC_{50} 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.

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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