(64.2±7.9 nM, n = 3) and FLCN-wt UOK257 cells (634.3±147.9 nM, n = 4). Differential ability to induce caspase 3/7 activity by mithramycin was also detected in a dose dependent manner. Clonogenic survival studies showed mithramycin to be ~10 fold more cytotoxic to FLCN-null than FLCN-wt UOK257 cells (200 nM). Following mithramycin exposure, UOK257-FLCN-null cells were mainly arrested and blocked in S and G2M phases of the cell cycle and low dose of rapamycin (1 nM) potentiated mithramycin sensitivity (1.5 fold in G2M population and 2 fold in G2M period time, 2 × GI50, 48 hrs).

Conclusions: These results provide a basis for further evaluation of mithramycin as a molecularly-targeted therapy for RCC associated with BHD.

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Discovery of BMS-817378: a novel prodrug of the dual Met/VEGFR-2 inhibitor BMS-794833

R. Borzilleri[1], J. Fargnoli[2]. [1]*Bristol-Myers Squibb, Oncology Chemistry, Princeton New Jersey, USA;* [2]*Bristol-Myers Squibb, Oncology Biology, Princeton New Jersey, USA*

Met receptor tyrosine kinase (RTK) is expressed predominantly on epithelial and endothelial cells, and serves as the only known high-affinity receptor for the mesenchyme-derived ligand, hepatocyte growth factor (HGF). Met activation can occur via ligand binding, receptor overexpression, and/or activating mutations. Subsequent signal transduction leads to complex biological responses, such as cellular proliferation, motility, migration, invasion, survival, morphogenesis and angiogenesis. Dysregulated Met-HGF signaling promotes tumor formation, growth, proliferation and metastasis, and thus, has been implicated in a wide array of human malignancies. Several agents that modulate the Met-HGF signaling axis have progressed to various stages of clinical development, including the potent and selective Met "superfamily" kinase inhibitor BMS-777607. In an effort to identify a second development candidate with a broader spectrum of antitumor activity relative to BMS-777607, compounds within the 2-aminopyridine series were screened against additional kinase targets, particularly those that play a role in tumor angiogenesis. The potent ATP-competitive Met/VEGFR-2 kinase inhibitor BMS-794833 was identified, which demonstrates enhanced activity versus both Met-dependent and Met-insensitive tumor lines. BMS-794833 also inhibits Ron (Met family), Axl (phylogenetically related Axl/Tyro3/Mer subfamily) and Flt-3 with IC_{50} values <3 nM. The compound was selective versus a panel of >200 additional RTKs, non-RTKs and serine/threonine kinases based on biochemical or Ambit binding assays. In cell culture, BMS-794833 inhibited the proliferation of human tumor cell lines containing constitutively activated Met receptor (GTL-16 gastric carcinoma). Tumor cell lines whose growth is stimulated by HGF (U87 glioblastoma) were also effectively inhibited by BMS-794833. *In vivo*, BMS-794833 demonstrated dose-dependent tumor growth inhibition following oral administration in the GTL-16 and L2987 lung carcinoma (Met-insensitive) xenograft models. Despite the impressive antitumor activity, BMS-794833 showed dissolution rate-limited absorption from solid dosage forms. The phosphooxymethyl prodrug, BMS-817378 was found to effectively liberate BMS-794833 in various *in vitro* and *in vivo* systems. On the basis of its desirable pharmacological profile, acceptable *in vitro* ADME and safety characteristics, and favorable pharmacokinetic properties in multiple species, BMS-817378 was selected for clinical development. The preclinical profile of BMS-794833 and the prodrug strategy leading to BMS-817378 will be presented.

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In vivo anti-tumor structure–activity relationships of Judemycin C and E, small molecule modulators of the spliceosome

T.R. Webb[1], C. Lagisetti[1], A. Pourpak[2], S.W. Morris[3], P.M. Potter[1]. [1]*St. Jude Children's Research Hospital, Chemical Biology and Therapeutics, Memphis TN, USA;* [2]*St. Jude Children's Research Hospital, Pathology, Memphis TN, USA;* [3]*St. Jude Children's Research Hospital, Pathology and Oncology, Memphis TN, USA*

Background: The spliceosome is an emerging target for cancer therapy that remains to be significantly exploited. We have recently reported the concise synthesis of Judemycin C, C1 and E (Figure 1), spliceosome modulators that show *in vivo* anti-tumor activity without notable toxicity. We are currently developing new analogs in this series that modulate splicing activity with the goal of yet further improving their anti-tumor efficacy.

Materials and Methods: Splice variants for many genes have been identified in mammalian cells, and in some instances the presence of these variant transcripts in tumors correlates with poorer clinical outcome (e.g. *MDM2*). By RT-PCR, we observed the modulation of *MDM2* splicing following exposure of the Rh18 rhabdomyosarcoma cell line to 0.1, 1, or 10 μM Judemycin C1 for up to 24 hours.

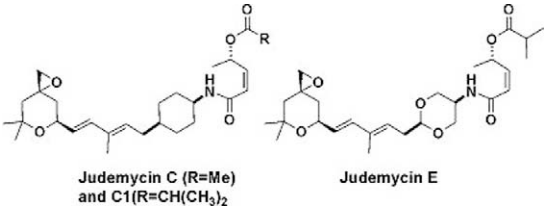

Figure 1. The structures of the lead compounds used in this study.

These compounds have also been tested in XTT cytotoxicity assays against a number of cancer cell lines and several sensitive lines have been identified, that possess IC_{50}s in the 80–200 nM range to these small molecules. Sensitive cancer cell lines (e.g. the JeKo-1 mantle cell lymphoma line) have been selected for *in vivo* xenograft anti-tumor efficacy studies in a NOD/SCID murine model. For our JeKo-1 animal studies, mice were treated with saline, vehicle, Judemycin E, or the proteasome inhibitor drug bortezomib for 5 consecutive days with 2 days off, for a total of 4 weeks. We will present new details on these extended dosing studies with Judemycin E.

Results: Our spliceosome modulatory compounds Judemycin C1 and E are cytotoxic against several cancer cell lines with IC_{50}s in the nanomolar range. In addition, we have observed modulation of *MDM2* splicing following treatment with the Judemycin compounds, resulting in shorter, alternatively spliced transcripts in drug-treated cells as compared to control samples. We have also demonstrated superior growth inhibition of the JeKo-1 mantle cell lymphoma xenograft *in vivo* as compared to the clinically approved mantle cell lymphoma drug bortezomib. Taken together, these new results are consistent with the hypothesis that Judemycin C1 and E alter splicing of *MDM2* (and other genes to be discussed in the presentation), presumably by interacting with the SF3b subunit of the spliceosome, resulting in the formation of aberrant mRNAs. We will also report on the structure–activity relationships of unpublished new Judemycin analog compounds prepared in our laboratory.

Conclusions: Judemycin C1 and E are examples of a new promising class of anti-cancer compounds. We have found that modulation of the alternative splicing of *MDM2* is a useful and sensitive marker for monitoring the splicing-modulatory effects of these compounds *in vivo*. This method is reproducible, requires small numbers of cells, and potentially could be used to document drug exposure and activity *in vivo* for a variety of tumor types. Studies to assess the latter possibility are currently underway.

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AEZS-129, an orally active PI3K inhibitor in preclinical development

I. Seipelt[1], E. Guenther[2], S. Baasner[1], L. Blumenstein[1], G. Mueller[3], J. Fensterle[1], J. Engel[4], M. Teifel[1], M. Gerlach[3]. [1]*Aeterna Zentaris GmbH, Preclinical Development, Frankfurt am Main, Germany;* [2]*Aeterna Zentaris GmbH, Alliance Management, Frankfurt am Main, Germany;* [3]*Aeterna Zentaris GmbH, Medicinal Chemistry, Frankfurt am Main, Germany;* [4]*Aeterna Zentaris GmbH, Management Board, Frankfurt am Main, Germany*

The phosphatidylinositol 3-kinase (PI3K)/Akt pathway plays a critical role in the regulation of cell growth, proliferation and survival in cancer. In many tumor types, the PI3K/Akt pathway is frequently activated through either the amplification/mutation of PIK3CA or the loss of the tumor suppressor phosphatase PTEN. Inhibition of the PI3K pathway therefore represents a high therapeutic value for patients with breast, colon, ovary, liver and other tumors.

AEZS-129 inhibits PI3Ka with an IC_{50} value of 28 nM with high specificity and proved to be a potent inhibitor of Akt phosphorylation in cellular assays. Mode-of-action studies showed that AEZS-129 acts as an ATP competitive compound. The *in-vitro* anti-proliferative activity against different human tumor cell lines (MDA-MB 468, U87, Hct116, PC-3, A549 and others) was determined, with EC_{50} values in the nanomolar range. AEZS-129 demonstrates favorable properties in early *in-vitro* ADMET screening including Caco-2 permeability, microsomal stability, plasma stability and testing against cardiac ion-channels.

Oral dosing of AEZS-129 results in high plasma levels in *in-vivo* pharmacokinetic experiments. Oral treatment with AEZS-129 is well tolerated and leads to significant tumor growth inhibition in multiple mouse xenograft cancer models, including colon (Hct116), lung (A549), prostate (PC-3) and endometrium (Hec1B) at 45 mg/kg repeated daily administration.

Based on these data, AEZS-129 was selected as preclinical development candidate.