196 POSTER

PK/PD models using a selective Ephrin B4 inhibitor JI-101 alone and in combination with other targeted agents and chemotherapy: Results of preclinical and ex-vivo studies

S. Sharma[1], R. Mullangi[2], M. Wade[1], S. Gurav[3], S. Sajja[3], D. Shankar[3], N. Srinivas[4], R. Govindarajan[5], L. Miller[6], M. Velleca[7]. [1]*Huntsman Cancer Institute − University of Utah, Center for Investigational Therapeutics, Salt Lake City UT, USA;* [2]*Jubilant Innovation, Dmpk, Bangalore, India;* [3]*Jubilant Biosys, Oncology, Bangalore, India;* [4]*Vanthys Pharmaceuticals, COO, Bangalore, India;* [5]*Jubilant Innovation, CMO, Bangalore, India;* [6]*Expert Medical Consultants, Oncology, Tuxeo Park NY, USA;* [7]*CGI Pharmaceuticals, SVP&Founder, Branston CT, USA*

Background: Combining drugs with different molecular mechanisms optimizes anticancer activity. JI-101, a highly selective angiogenesis inhibitor, has unique EphB4 activity and VEGFR2 and PDGFRb inhibitory activity. JI-101 has excellent preclinical profiles and is well tolerated in phase I trials. We present combination results for JI-101 and mTOR inhibitor Everolimus (E), chemotherapy agents, and other targeted agents (imatinib, EGFR inhibitors, other anti-angiogenesis inhibitors), as well as additional information on the results of JI-101 and its EphB4 activity in additional preclinical and ex-vivo modeling.
Materials and Methods: *In vitro* methods: Cell lines (chronic myelogenous leukemia, K-Ras mutant colon cancer, ovarian cancer) are treated with JI-101 as single agent or combined with chemotherapy agents (oxaliplatin, gemcitabine, 5-fluorouracil), E, or other targeted agents (imatinib, EGFR inhibitors, other anti-angiogenesis agents). Readouts presented include cell proliferation (MTS), apoptosis and ephrin B4 phosphorylation. *In vivo* methods: 6 groups of female nude athymic mice bearing HT-29 xenografts received vehicle, E 2.5 mpk, JI-101 25 mpk, JI-101 50 mpk, JI-101 25 mpk + E 2.5 mpk and JI-101 50 mpk + E 2.5 mpk for 4 weeks, given as q3d and qd regimen. Drug-drug interaction potential between JI-101 and E was studied in pooled human liver microsomes (PHLM).
Results: JI-101 was additive/synergistic with a variety of chemotherapy and targeted agents *in vitro*. *In vivo*, % tumor growth inhibition was 43, 45, 64, 65, and 73 for E 2.5 mpk, JI-101 25 mpk, JI-101 50 mpk, JI-101 25 mpk + E 2.5 mpk and JI-101 50 mpk + E 2.5 mpk, respectively. The PHLM study showed that % metabolized of JI-101 and E was decreased by 23% when they were co-spiked in PHLM.
Conclusion: Broad data on combination potential of JI-101 with various agents including targeted agents/therapies and mTOR inhibitor is presented. *In vivo*, combination potential of JI-101 and E is addressed, as well as ephrin B4 phosphorylation to build a PK/PD model.

197 POSTER

AEZS-132, a new orally bioavailable PI3K/Erk inhibitor with antitumor effects

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

The Ras/Raf/Mek/Erk and the PI3K-Akt signaling pathways promote cellular proliferation and survival. In many tumors molecular events lead to parallel activation of both pathways. Feed-back loops and cross-talk between the pathways further limit the effectiveness of single targeted approaches. Therefore it is likely that dual or multiple-targeted approaches may result in improved clinical responses.
Here we show the major *in-vitro* and *in-vivo* characteristics of AEZS-132. Our multi-parameter optimization program for kinase inhibitor selectivity, cellular efficacy, physico-chemical and *in-vitro* ADMET properties has led to the identification of a small molecular compound with an uniquely advantageous dual kinase inhibition profile. AEZS-132 acts as an ATP competitive compound and inhibits Erk and PI3K in the nanomolar range while exerting high selectivity against other serine threonine and tyrosine kinases. The anti-tumor efficacy of the dual kinase inhibitor was evaluated in diverse human tumor cell lines like Hct116, A549, MDA-MB 468 and PC-3. Physicochemical and *in-vitro* ADMET and safety parameters have been widely assessed.
AEZS-132 showed prolonged plasma exposure in *in-vivo* pharmacokinetic experiments and significant anti-tumor efficacy in mouse xenograft models including colon (Hct116) and endometrium (Hec1B) at repeated daily oral doses of 30 mg/kg.
Due to its dual PI3K and Erk inhibition, a broad anti-tumor activity is expected in tumors with over-activation of the Ras/Raf/Mek/Erk and the PI3K-Akt signaling pathways.

198 POSTER

Assessing the role of phosphoinositide 3-kinase (PI3K) in head and neck cancers

W. Shen[1], M. El Dinali[1], J. Braegelmann[1], J. Zoergiebel[1], A. Kundu[1], E. El-Hashani[1], R. Salgia[1], T. Seiwert[1]. [1]*University of Chicago, Hematology/Oncology, Chicago IL, USA*

Background: The phosphoinositide 3-kinase (PI3K) − AKT pathway is a key signal pathway for cell survival that is commonly activated in human cancers. Here we investigated the role of the PI3K as novel therapeutic target in head and neck cancers.
Material and Methods: We evaluated the status of PTEN and AKT in 13 HNSCC cell lines by immunoblotting. In order to evaluate mutations we sequenced two subunits of the PI3K protein, the p110-alpha catalytic subunit (PIK3CA) and the p-85 alpha regulatory subunit (PIK3R1) for 11 cell lines and 20 tumor tissue samples, Effects of PI3K inhibition (LY294002), dual PI3K/mTOR inhibition (NVP-BEZ235), and mTORC1 inhibition (Rad001) on viability in 12 cell lines were determined using Resazurin viability assays. We further evaluated apoptotic effects of NVP-BEZ235 using a Caspase 3/7 Assay or Annexin V-Propidium Iodide Assay. Moreover, we studied synergistic effects between NVP-BEZ235 and the MET-inhibitor SU11274/the EGFR-inhibitor Erlotinib.
Results: The PI3K-AKT pathway was activated in the majority of HNC cell lines. PTEN expression was variable and absent in 2 cell lines. Sequencing revealed 2 hotspot mutations in the helical and kinase domains of the catalytic subunit (PIK3CA). No mutations in PIK3R1 were identified. Resazurin viability assay showed many cell lines to be sensitive to BEZ235, including but not limited to cell lines with PIK3CA hot spot mutations (20−60 nM) to PI3K. On the other hand mTORC1 inhibitors were less effective (about 15 uM). Caspase 3/7 Assays revealed apoptotic effects of NVP-BEZ235. The Annexin V-Propidium Iodide Assay stated that NVP-BEZ235 is a potent cytotoxic inhibitor in several HNC cell lines (10−40% increase of apoptotic population).
Combined inhibition with MET/EGFR inhibitors and PI3K inhibitor showed modest synergy, but results were not consistent in all lines.
Conclusions: PI3K appears to be an attractive novel target for Head and Neck cancers including but not limited to those with PIK3CA mutations. NVP-BEZ235 is a promising inhibitor that is able to induce apoptosis. Further follow-up studies are indicated.

199 POSTER

Tumor colonizing bacteria − potential tumor therapy with *Salmonella enterica* serovar Typhimurium

K. Kochrübe[1], S. Weiss[1]. [1]*Helmholtz Center for Infection Research, Molecular Immunology, Braunschweig, Germany*

Systemic administration of facultative anaerobe bacteria is a promising application in anti-cancer therapy. Some bacteria have the capacity to accumulate in solid tumors and exhibit anti-tumor activity. *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) is one of those bacteria. Systemic administration in tumor-bearing mice revealed a high accumulation in tumors compared to organs like spleen and liver, where the number of bacteria is lower. Although the accumulation of bacteria in the tumor induces tumor shrinkage, the bacterial infection still leads to a decline in the health situation of mice, resulting in a high death rate 2−4 weeks after infection. This is probably due to the long-term burden of *Salmonella infection*.
Consequently, possibilities to improve consecutive symptoms and decrease number of bacteria after successful tumor therapy have been investigated. Antibiotic treatment of mice after Salmonella infection with different classes of antibiotics has been tested showing promising results. After Ciprofloxacin treatment a high number of bacteria is eliminated within 5 days after treatment. Subsequently, mice completely recover from infection and the death rate declines dramatically.
Additionally, the therapeutic potential of LPS alone and UV-killed bacteria has been assessed to avoid longterm bacterial infection. Interestingly, both exhibit a high tumor regression potential. After systemic administration a fast reduction of tumor growth, comparable to the reduction after administration of alive SL7207, can be observed. Nevertheless, depending on the amount of LPS or UV-killed bacteria the mice recover completely or, in the case of low doses, don't get sick at all.
Increasing knowledge about the molecular mechanism behind tumor regression by alive bacteria or bacterial components can ameliorate a possible application in tumor therapy.