Title

THER-06. PROTEASOME INHIBITION IN PRIMARY MEDULLOBLASTOMA CELL CULTURE AND PATIENT-DERIVED XENOGRAFT MODELS: A POTENTIAL THERAPEUTIC IMPLICATION

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compared to controls. Our findings show for the first time OECs tropism to pediatric glioma tumors and their potential for brain cancer gene therapy using intranasal delivery. OECs have the advantage over other typical stem cells in that they can be easily obtained from olfactory bulb, allowing autologous transplantation and overcoming ethical issues.

THER-05. CONTINUOUS AND BOLUS INTRAVENTRICULAR TOPOTECAN PROLONG SURVIVAL IN A MOUSE MODEL OF LEPTOMENINGEAL MEDULLOBLASTOMA

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Leptomeningeal metastasis remains a difficult clinical challenge. Some success has been achieved by direct administration of therapeutics into the cerebrospinal fluid (CSF) circumventing limitations imposed by the blood brain barrier. Here we investigated continuous infusion *versus* bolus injection of therapy into the CSF in a preclinical model of human Group 3 medulloblastoma, the molecular subgroup with the highest incidence of leptomeningeal disease. Initial tests of selected Group 3 human medulloblastoma cell lines in culture showed that D283 Med and D425 Med were resistant to cytosine arabinoside and methotrexate. D283 Med cells were also resistant to topotecan, whereas 1 µM topotecan killed over 99% of D425 Med cells. We therefore introduced D425 Med cells, modified to express firefly luciferase, into the CSF of immunodeficient mice. Mice were then treated with topotecan or saline in five groups: continuous intraventricular (IVT) topotecan *via* osmotic pump (5.28 µg/day), daily bolus IVT topotecan injections with a similar daily dose (6 µg/day), systemic intraperitoneal injections of a higher daily dose of topotecan (15 µg/day), daily IVT pumped saline and daily intraperitoneal injections of saline. Bioluminescence analyses revealed that both IVT topotecan treatments effectively slowed leptomeningeal tumor growth in the brains. Histological analysis showed that they were associated with localized brain necrosis, possibly due to backtracking of topotecan around the catheter. In the spines, bolus IVT topotecan showed a trend towards slower tumor growth compared to continuous (pump) IVT topotecan, as measured by bioluminescence. Both continuous and bolus topotecan IVT showed longer survival compared to other groups. Thus, both direct IVT topotecan CSF delivery methods produced better anti-medulloblastoma effect compared to systemic therapy at the dosages used here.

THER-06. PROTEASOME INHIBITION IN PRIMARY MEDULLOBLASTOMA CELL CULTURE AND PATIENT-DERIVED XENOGRAFT MODELS: A POTENTIAL THERAPEUTIC IMPLICATION

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Recent research has shown a role of proteasome ubiquitination in medulloblastoma tumorigenesis and proteasome inhibition as a possible therapeutic target. In medulloblastoma primary cell culture and in a *Patched1* haploinsufficiency medulloblastoma mice model, bortezomib, a proteasome inhibitor, has been shown to limit tumor growth via induction of apoptosis and inhibition of cell proliferation. However, its clinical utility is limited by its inability to cross the blood brain barrier and association with peripheral neuropathy. Marizomib, a more lipophilic proteasome inhibitor, has been shown to cross the blood brain barrier in preclinical studies and is being used in Phase III studies for adult glioblastoma in combination with radiation and temozolomide. Here, we evaluated dose dependent cell killing of marizomib in two group 3, myc-amplified medulloblastoma cell cultures. IC50's were determined and were consistent with and slightly lower than that seen in adult glioblastoma cell cultures. We suggest marizomib warrants further investigation as a potential therapeutic target for recurrent medulloblastoma. Future studies will include determination of IC50's for marizomib in cell lines for all medulloblastoma molecular subgroups and primary cell cultures as well as in vitro testing with patient-derived xenograft models.

THER-07. A PHASE 0 PHARMACODYNAMIC AND PHARMACOKINETIC STUDY OF EVEROLIMUS IN VESTIBULAR SCHWANNOMA (VS) AND MENINGIOMA PATIENTS

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BACKGROUND: Inhibition of mTORC1 signaling has been shown to diminish growth of NF2 deficient tumors in preclinical studies, and clinical data suggest that everolimus, an orally administered mTORC1 inhibitor, may slow tumor progression in a subset of adult and pediatric NF2 patients with VS. To assess the pharmacokinetics, pharmacodynamics and potential mechanisms of treatment resistance, we performed a pre-surgical ("phase 0") clinical trial of everolimus in patients undergoing surgery for VS or meningiomas. METHODS: Eligible patients with meningioma or VS requiring tumor resection received everolimus 10 mg daily for 10 days immediately prior to surgery. Everolimus blood levels were determined immediately prior to and after surgery. Tumor samples were collected intraoperatively. RESULTS: Ten patients completed protocol therapy, including 5 patients with NF2-related meningioma, 3 patients with sporadic meningioma, and 2 patients with NF2-related VS. Median pre- and post-operative plasma levels of everolimus were found to be in a high therapeutic range (17.4 ng/ml and 9.4 ng/ml, respectively). Median tumor tissue drug concentration determined by mass spectrometry was 24.3 ng/g (range 9.2–169.2), and median tumor tissue to post-operative plasma drug concentration ratio was 0.39. We observed only partial inhibition of phospho-S6 in the treated tumors, indicating incomplete target inhibition compared to matched control tissues from untreated patients ($p = 0.005$). Consistent with prior observations that inhibition of mTORC1 may lead to MAPK pathway activation through a PI3K-dependent feedback loop, we observed a statistically significant increase of phospho-ERK ($p < 0.03$) versus untreated controls. CONCLUSIONS: In patients with meningioma or VS, treatment with everolimus leads to incomplete inhibition of mTORC1 signaling and upregulation phospho-ERK. These data may explain the limited anti-tumor effect of everolimus observed in clinical studies for NF2 patients and identify upregulation of phospho-ERK as a likely resistance mechanism that could be addressed with combination therapies.

THER-08. RAPLINK-1 COOPERATES WITH INHIBITORS OF BCL-2/BCL-XL TO INDUCE APOPTOSIS IN GLIOBLASTOMA

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BACKGROUND: Glioblastoma (GBM) has dysregulated signaling of the mTOR pathway. Rapalink-1, a bivalent inhibitor created by linking rapamycin and sapanisertib, can bind to both drug binding pockets, potently inhibits mTORC1 *in vitro* and *in vivo*. Although Rapalink-1 represents a novel path towards mTOR inhibition, cellular responses in glioblastoma are mostly cytostatic. B cell lymphoma 2 (Bcl-2) family members play a central role in programmed cell death, orchestrating pro- and anti- apoptotic signals. Pro-survival Bcl-2 family member Mcl-1 is overexpressed in GBM. We hypothesize that blockade of Bcl-2 family members in combination with RapaLink-1 will drive apoptosis in glioblastoma. METHODS: We screened a panel of prototype and clinical Bcl-2 family inhibitors, namely ABT-737, ABT-199 (Venetoclax), ABT-263(Navitoclax), and GX15-070(Obatoclax) in combination with Rapalink-1, against pediatric (SF188) and adult (LN229, SF767, U373) and GBM cell lines. We assayed cell viability, annexin V FITC flow cytometry and western blot. RESULTS: Rapalink-1 in combination with Bcl-2 family inhibitors inhibited cell viability significantly more than single drug. ABT-199, a selective inhibitor of Bcl-2, did not induce apoptosis when combined with Rapalink-1. GX15-070, a pan pro-survival Bcl-2 family inhibitor and inducer of autophagy actually blocked apoptosis when combined with RapaLink-1. ABT-737 and its clinical derivative ABT-263, both of which inhibit Bcl-2, Bcl-xl and Bcl-w, synergized with Rapalink-1 to induce apoptosis in multiple GBM cell lines, evidenced by PARP cleavage and annexin V staining. We found that Rapalink-1 potently downregulated expression of Mcl-1 and induced pro-survival members Bcl-2 and Bcl-xl, suggesting that blockade of mTORC1, Bcl-2 and Bcl-xl, in combination cooperate in driving apoptosis. These results present a preclinical strategy to drive tumor cells towards mTOR inhibition-induced cytotoxicity, offering a translatable strategy for treatment of pediatric GBM.

THER-09. EVALUATION OF PROTEIN KINASE INHIBITORS WITH PLK4 CROSS-OVER POTENTIAL IN PEDIATRIC EMBRYONAL BRAIN TUMORS

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