TRTH-33. COMPARATIVE PLASMA AND CEREBROSPINAL FLUID PHARMACOKINETICS OF BRAF AND MEK INHIBITORS IN A NONHUMAN PRIMATE MODEL
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PURPOSE: BRAF and MEK mutations are present in several pediatric CNS tumors, particularly pilocytic astrocytomas, gangliogliomas and pleomorphic xanthoastrocytomas. Several BRAF inhibitors are under clinical investigation, alone and in combination with MEK inhibitors. To guide the clinical development of BRAF and MEK inhibitors, we evaluated CNS penetration of dabrafenib, selumetinib, vemurafenib, and trametinib (using CSF as a surrogate) in a nonhuman primate model predictive of pharmacokinetics (PK) in pediatric populations. METHODS: This study was approved by the NCI Animal Care and Use Committee. Agents were administered orally to rhesus macaques (n=4), with human equivalent dosing as follows: dabrafenib (161 mg/m²) and selumetinib (50 mg/m²). Planned studies include vemurafenib (516 mg/m²) and trametinib (1 mg/m²). Serum, paired plasma and CSF samples were collected from 0–24 hr for dabrafenib and 0–48 hr for selumetinib. Dabrafenib was quantified using a validated ultra HPLC-MS/MS method (lower limit of quantitation (LLOQ) in plasma and CSF = 1 and 0.1 ng/mL, respectively). Selumetinib was quantified by Covance Bioanalytical Services (LLOQ = 0.5 ng/mL in plasma and CSF). PK parameters were calculated using noncompartmental methods. RESULTS: In plasma, mean half-life and dose-normalized AUC₀₋₂₄ for dabrafenib were 3.2 (±1.2) hr and 73.9 (±34.0) hr*ng/mL/mg, respectively, and for selumetinib, 10.8 (±2.5) hr and 122 (±17.8) hr*ng/mL/mg, respectively. In CSF, 5.0 (±1.7) hr and 0.43 (±0.21) hr*ng/mL/mg, respectively. CSF levels of selumetinib were detectable in one animal, with an AUC₀₋₂₄ of 0.39 hr*ng/mL/mg. CSF penetration of dabrafenib and selumetinib were poor (0.37 (±0.18) % and 0.4%, respectively). CONCLUSIONS: Quantifiable concentrations of dabrafenib were found in plasma and CSF for 24 hr after administration, but, as with selumetinib, CSF penetration was low. Due to inter-animal variability, an additional animal will be studied. Alternate delivery methods (intranasal and intrathecal) may be useful to evaluate in efforts to increase CNS exposure.

TRTH-34. NOVEL ORAL PRODRUGS OF 6-DIAZO-5-OXO NORLEUCINE IMPROVE BRAIN PENETRATION AND DEMONSTRATE EFFICACY AGAINST MYC-DRIVEN ORTHOTOPIC MEDULLOBLASTOMA XENOGRAFTS
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Increased MYC levels can alter cellular metabolism, creating a reliance on glutamine. Glutamine PET and MRI spectroscopy demonstrate that aggressive brain malignancies have increased uptake of glutamine and increased glutamate relative to normal brain, suggesting that agents targeting glutamine metabolism may be active in brain tumors. The most aggressive subgroup of medulloblastoma tumors are driven by high expression of MYC, so we hypothesized that these tumors would have altered glutamine metabolism and be sensitive to 6-diazo-5-oxo norleucine (DON), a glutamine analog. Western blotting revealed that expressing MYC in human cerebellar-derived neural stem and progenitor cells (CB NSC) induced the expression of glutaminase (GLS), the enzyme that converts glutamine to glutamate—a critical step in glutamine metabolism. Human neural stem cells transformed with SV40 do not express MYC and thus do not express GLS. MYC-expressing patient-derived medulloblastoma cell lines (D425MED and D283MED) also express GLS. We treated our MYC-transformed CB NSC and medulloblastoma cell lines with DON and observed an increase in apoptosis of up to 450% as determined by cleaved caspase-3 immunofluorescence (p=0.001). DON treatment did not significantly increase apoptosis in SV40-immortalized CB NSC (p=0.65) and had no effect on normal, untransformed human NSC. DON is not orally bioavailable, so we decided to develop DON prodrugs designed for improved oral bioavailability and brain penetration. In non-human primates, our DON prodrug exhibited superior 10-fold enhanced CSF/plasma ratio versus DON. In orthotopic xenograft models, our novel DON prodrugs increased the median survival of mice bearing D425-MED MYC-driven medulloblastoma tumors by 60 percent (22 days for vehicle treated mice compared to 35 days for prodrug treated mice, p<0.01 by log-rank test). DON prodrugs can be engineered to improve oral bioavailability and brain penetration, and these drugs have efficacy in orthotopic xenograft models of aggressive MYC-driven medulloblastoma tumors.