also detected. These observations were markedly enhanced in tumor-bearing animals following cranial irradiation. Therapeutic inhibition with LLL12B could mitigate these effects, indicating a dependency on STAT3. Within the tumor compartment, granulocytic myeloid cells that successfully infiltrated following treatment demonstrated a pro-inflammatory phenotype denoted by interferon-gamma expression. Improved survival was also observed following combination treatment with LLL12B and radiation or immune checkpoint blockade. CONCLUSIONS: These findings advocate a critically important role for STAT3 in regulating granulocytic myeloid cell mobilization and trafficking to GBM tumors. It further illustrates the plasticity of these cells within these tumors, which may be useful in designing successful immunotherapeutic strategies.

EXTH-09. TUMOR PHARMACOKINETICS, PHARMACODYNAMICS AND RADIATION SENSITIZATION IN PATIENT-DERIVED XENOGRAFT MODELS OF GLIOBLASTOMA TREATED WITH THE AURORA KINASE A INHIBITOR LY3295668

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Cell-cycle deregulation is at the crux of all malignancies, including glioblastoma (GBM). Aurora Kinase A (AURKA) plays a central role in G2/M transition and faithful chromosome segregation. In this study, we evaluated the pharmacokinetics, pharmacodynamics, and radiation sensitization properties of LY3295668, a highly specific AURKA inhibitor, in orthotopic patient-derived xenograft (PDX) models of GBM. Mice with intracranial tumors were randomized to 50 mg/kg LY3295668 PO BID x 4 days vs. placebo. LY3295668 levels in plasma and contrast-enhancing tumor tissue were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). Unbound fractions were determined by equilibrium dialysis. Immunohistochemistry was performed to assess levels of pAURKA (T288), phospho-Histone H3 (pHH3), and cleaved caspase 3 (CC3). For survival studies, mice with intracranial tumors were randomized to four cohorts – vehicle, radiotherapy, LY3295668 monotherapy, and LY3295668 plus radiotherapy. The median unbound concentration of LY3295668 was 270.88 nmol/L and 22.33 nmol/kg in plasma and tumor tissue, respectively – significantly higher than the biochemical IC_{50} of LY3295668 for AURKA inhibition (0.8 nM). A decrease in pHH3(+) cells (0.8% vs. 6.4%, p=0.036) indicated drug-induced mitotic arrest and was accompanied by an increase in CC3(+) cells (6.4% vs. 8.0%, p=0.67). Combination of LY3295668 with radiotherapy prolonged survival compared to either therapy alone in orthotopic GBM PDX models. LY3295668 is well tolerated, achieves pharmacologically-relevant unbound concentrations in GBM PDX models, and is associated with significant target modulation. Preclinical combination of LY3295668 with radiation therapy leads to synergistic effects and supports future clinical study of this multimodal strategy in glioblastoma patients.

EXTH-10. EXPLORATION OF A NOVEL TOXIN-INCORPORATING CAR T CELL: HOW DOES CHLOROTOXIN RECOGNIZE GLIOBLASTOMA CELLS?

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Chlorotoxin, a peptide toxin component of scorpion venom, binds selectively to glioblastoma and other neuroepithelial tumors, with minimal binding to non-malignant cells. We have recently developed a CAR T cell incorporating chlorotoxin (CLTX) as its target recognition domain, and CLTX-CAR T cells are now in clinical trial for recurrent glioblastoma (NCT04214392). We determined in preclinical studies that surface MMP-2 was required for CLTX-CAR T cell killing. However, the precise composition and structure of the cell surface complex recognized by CLTX-CAR T cells remains an unresolved question of increasing importance. Previous investigations have variously proposed matrix metalloprotease-2 (MMP-2), ClC-3 chloride channels, regulators of MMP-2, annexin A2, and neuropilin-1, as receptors, or components of receptors, for CLTX. To approach this question, we have visualized bound Cy5.5-conjugated CLTX peptide (CLTX.Cy5.5) or biotin-conjugated CLTX peptide (CLTX.biotin) on tumor cells in fixed sections of patient resections, on tumor cells in organotypic cultures of patient resections, and on cells of cultured patient-derived glioblastoma tumor lines. At tissue- and cell-level spatial resolution, we saw good correlation of CLTX binding with MMP-2 expression in patient tumor samples and on cultured GBM cells, and between CLTX binding and tumor cell death (cleaved caspase-3) in organotypic GBM cultures. However, at subcellular resolution, surface binding of CLTX was related to, but not precisely overlapping, with MMP-2 or other putative receptors. Rather, on fixed PBT003, PBT030 and PBT106 cells in monolayer cultures, MMP-2 staining was clustered, with diffuse loosely associated CLTX.biotin staining. Diffuse distribution of CLTX.Cy5.5 was also seen in fixed xenograft sections of PBT003-4, PBT1206, PBT030-2, PBT051 and PBT138 tumor cells, and not obviously associated with more discrete staining for IL13Rα2 and EGFR. Ongoing experiments are further examining association of CLTX with other putative components of CLTX receptor complexes, and their redistribution during binding by CLTX and CLTX-CAR T cells.

EXTH-11. COLD ATMOSPHERIC PLASMA SELECTIVELY INDUCES APOPTOSIS AND FERROPTOSIS THROUGH REACTIVE OXYGEN SPECIES IN HIGH-GRADE GLIOMA

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INTRODUCTION: Cold atmospheric plasma (CAP) selectively induces reactive oxygen and nitrogen species (ROS/RNS) in many types of cancerous cells. ROS-mediated lipid peroxidation is thought to induce ferroptosis, apoptosis, and autophagy. We hypothesize that ferroptosis and apoptosis are key mechanisms of CAP-mediated cytotoxicity in high-grade glioma (HGG). METHODS: B16, U87, GL261, EPD-210FHTC and human astrocyte NHA hTERT cells were treated with CAP for 10, 30, 60, 90, and 180 seconds. Proliferation and propidium iodide (PI)/annexin V flow cytometry assays were employed to quantify cytotoxicity, cell cycle phases and apoptosis. Mitochondrial superoxide concentration was measured using MitoSOX Red. Cells were pre-treated with ferroptosis inhibitors Ferrostatin-1 and Deferoxamine (DFO) in rescue assays. RESULTS: Survival of GL261 and U87 cells after 90 seconds of CAP treatment was 3.7% and 7%, respectively, compared to 62% in NHA cells. A CAP dose-dependent increase in mitochondrial superoxide concentration was observed in GL261 and NHA (R^2=0.88 and 0.99, respectively). A shift of EPD and NHA cells into G_0 phase was noted after 180 seconds of treatment, compared to baseline (55.4% versus 1.2%, 100% vs. 27.5% respectively). Early apoptosis was more prominent in NHA cells (79% of dead cells), and late apoptosis in EPD cells after 60 seconds of treatment (86% of dead cells). DFO pre-treatment significantly reduced CAP cytotoxicity in GL261 (93% vs. 58% after 10 seconds) and U87 cells (85% vs. 13% after 60 seconds). DFO pre-treatment had no effect on NHA response to CAP. CONCLUSION: CAP treatment induces dose-dependent increases in ROS and apoptosis in HGG lines tested more significantly than in NHA cells. CAP induces G1-phase cell cycle arrest in treated HGG cells and G0 arrest in non-cancerous cells. CAP-mediated cytotoxicity was significantly mitigated with DFO pre-treatment in HGG cells, suggesting that ferroptosis plays a critical role in the mechanism of CAP treatment in HGG.

EXTH-12. INHIBITION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND PLATELET-DERIVED GROWTH FACTOR RECEPTOR-ALPHA EXERTS SYNERGISTIC EFFICACY IN GLIOBLASTOMA

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Epidermal growth factor receptor (*EGFR*) alterations, including amplification and activating mutations, occur in more than half of GBM cases. EGFR is located on Chr. 7, and Chr. 7 gain is one of the earliest events precipitating gliomagenesis. EGFR inhibitors, monoclonal antibodies, vaccines, and CAR-T cells have failed in GBM due to intrinsic heterogeneity and receptor tyrosine kinase (RTK) bypass pathways that mediate therapeutic resistance. New targeted therapeutic approaches to leverage synergistic combinations are desperately needed to improve GBM prognosis. Using the TCGA and other GBM databases, we previously demonstrated that *PDGFRA* amplification in patients with *EGFR*-amplified GBM carries significantly worse survival. *EGFR* and *PDGFRA* co-expression occur in more than one-third of GBM patients. The PDGFRA ligand PDGFA is also located on Chr. 7, and its expression is significantly increased with Chr. 7 gain and *EGFR* copy number increase. Therefore, Chr. 7 gain inherently leads to co-activation of both EGFR and PDGFRA signaling. We used patient-derived glioblastoma cells with Chr. 7 gain to test combined inhibition of EGFR and PDGFRA *in vitro*. We found that combined inhibition of both EGFR and PDGFRA using FDA-approved EGFR-targeted agents (Erlotinib, Gefitinib, Dacomitinib, Neratinib, and Osimertinib) and Crenolanib, respectively, leads to synergistic cytotoxicity *in vitro*. Inhibition of either EGFR or PDGFRA led to receptor cross-activation, and EGF and PDGF-AA-induced RTK activation was blocked by Neratinib and Crenolanib. Immunoprecipitation experiments and proximity ligation assays demonstrated that combined inhibition prevents EGFR and PDGFRA heterodimerization and pathways of therapeutic resistance. This combined inhibition led to decreased activation of downstream signaling pathways, including PI3K and MAPK. We show that

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