EXTH-45. DELTA-24-RGDOX ACTIVATION OF THE IDO-KYN-AHR CASCADE IN GLIOBLASTOMA: OLD TARGETS FOR A NEW THERAPY

Teresa Nguyen[1], Dong Ho Shin[1], Hong Jiang[1], Derek Wainwright[2], Sagar Sohoni[1], Sumit Gupta[1], Ashley Ossimetha[1], Frederick Lang[1], Marta M Alonso[2], Juan Fueyo[1], and Candelaria Gomez-Manzano[1]; [1]MD Anderson Cancer Center, Houston, TX, USA, [2]Northwestern University, Chicago, IL, USA, [3]Clinica Universidad de Navarra, Pamplona, Spain

Immune enhancement of virotherapy by reshaping the tumor immune landscape may improve its success rates. IDO, an IFNγ inducible tryptophan catabolizing enzyme, is upregulated in glioblastoma, correlating with poor prognoses. IDO-mediated tryptophan depletion in the tumor-microenvironment decreases proliferation and induces apoptosis of surrounding effector T-cells. Kynurenine, a metabolite of tryptophan, induces T-cell differentiation into immunosuppressive Tregs. Excess kynurenine elicits AhR-mediated lymphocyte dysfunction and immunosuppression. The immune stimulating effect of oncolytic-virus, Delta-24-RGDOX, triggers IFNγ production contributing to a positive IDO-Kynurenine-AhR feedback loop. We hypothesized that combining Delta-24-RGDOX with IDO inhibitors will synergize to effectively treat glioblastoma. We characterized IDO and AhR in Delta-24-RGDOX infected cancers using immunofluorescence, qRT-PCR, and flow cytometry and found increased expression of both proteins *in vitro* and *in vivo*. We also observed induction of AhR in CD4+ and CD8+ T-cells by Delta-24-RGDOX *in vivo*. Delta-24-RGDOX also increased activity of AhR in cancer cells as indicated by an AhR responsive elements transcription assay. We used a murine glioblastoma model to test the efficacy of combining Delta-24-RGDOX with IDO inhibitor, 1MT/indoximod; the combination produced 30% more long-term survivors compared Delta-24-RGDOX alone (P=0.03), which we showed, through lymphocytic depletion studies, was dependent on CD4+ T-cell activation. We observed 100% survival in the re-challenged long-term glioblastoma survivors, indicating the establishment of immune memory by the combination. Functional studies showed significant increases in anti-tumor activity of splenocytes from combination-treated mice compared to Delta-24-RGDOX-treated mice (P=0.009). Flow cytometry studies revealed that combination-treated mice yielded the highest levels of chronically activated T-cells and lowest levels of Tregs and myeloid derived suppressor cells compared to Delta-24-RGDOX single treatment (P≤0.05). This microenvironment remodeling correlated with complete tumor elimination. Altogether, Delta-24-RGDOX activates the IDO-Kyn-AhR cascade in gliomas, identifying new targets, which when inhibited have the potential to enhance the anti-glioma effect of oncolytic-viruses by reversing tumor immunosuppression.

EXTH-46. ARTIFICIAL INTELLIGENCE-BASED IDENTIFICATION OF COMBINED VANDETANIB AND EVEROLIMUS IN THE TREATMENT OF ACVR1-MUTANT DIFFUSE INTRINSIC PONTINE GLIOMA

Diana Carvalho[1], Peter Richardson[2], Nagore Gene Olaciregui[3], Reda Stankunaite[4], Cinzia Emilia Lavarino[3], Valeria Molinari[1], Elizabeth Corley[4], Ruth Ruddle[1], Adam Donovan[1], Akos Pal[1], Florence I Raynaud[1], John P Overington[2], Anne Phelan[2], Dave Sheppard[2], Andrew Mackinnon[4], Michael Hubank[4], Ofelia Cruz[3], Andres Morales La Madrid[5], Sabine Mueller[6], Angel Montero Carcaboso[5], Fernando Carceller[4], and Chris Jones[7]; [1]Institute of Cancer Research, London, United Kingdom, [2]Benevolent AI, London, United Kingdom, [3]Hospital Sant Joan de Déu, Barcelona, Spain, [4]Royal Marsden Hospital NHS Trust, London, United Kingdom, [5]Hospital Sant Joan de Déu, Barcelona, United Kingdom, [6]University of California, San Francisco, San Francisco, CA, USA, [7]The Institute of Cancer Research, London, United Kingdom

Somatic mutations in *ACVR1*, encoding the serine/threonine kinase ALK2 receptor, are found in a quarter of children with the currently incurable brain tumour diffuse intrinsic pontine glioma (DIPG). Treatment of *ACVR1*-mutant DIPG patient-derived models with multiple inhibitor chemotypes leads to a reduction in cell viability *in vitro* and extended survival in orthotopic xenografts *in vivo*, though there are currently no specific ACVR1 inhibitors licensed for DIPG. Using an Artificial Intelligence-based platform to search for approved compounds which could be used to treat ACVR1-mutant DIPG, the combination of vandetanib and everolimus was identified as a possible therapeutic approach. Vandetanib, an approved inhibitor of VEGFR/RET/EGFR, was found to target ACVR1 (Kd=150nM) and reduce DIPG cell viability *in vitro*, but has been trialed in DIPG patients with limited success, in part due to an inability to cross the blood-brain-barrier. In addition to mTOR, everolimus inhibits both ABCG2 (BCRP) and ABCB1 (P-gp) transporter, and was synergistic in DIPG cells when combined with vandetanib *in vitro*. This combination is well-tolerated *in vivo*, and significantly extended survival and reduced tumour burden in an orthotopic *ACVR1*-mutant patient-derived DIPG xenograft model. Based on these preclinical data, three patients with *ACVR1*-mutant DIPG were treated with vandetanib and everolimus. These cases may inform on the dosing and the toxicity profile of this combination for future clinical studies. This bench-to-bedside approach represents a rapidly translatable therapeutic strategy in children with *ACVR1* mutant DIPG.

EXTH-47. PRECISION IMMUNOTHERAPY VACCINES FOR GLIOBLASTOMA USING CANCER IMMUNOGENOMICS AND SELECTIVE GENE ENRICHMENT STRATEGY

Vrunda Trivedi[1], Changlin Yang[2], Oleg Yegorov[1], Kyle Dyson[1], and Duane Mitchell[2]; [1]University of Florida, Gainesville, FL, USA, [2]University of Florida, Department of Neurosurgery, Gainesville, FL, USA

BACKGROUND: Development of successful immunotherapy targeting antigens in Glioblastoma (GBM) remains a challenge owing to the heterogeneous nature of GBM and the low mutation burden. It is therefore important to identify multiple tumor antigens that can be targeted simultaneously. Our goal is to develop a personalized RNA vaccination approach that employs the use of cancer immunogenomics in identifying tumor immunogenic epitopes and target enrichment strategies that allow for targeting only the tumor specific antigens. APPROACH: RNAseq and WES was performed for murine GBM tumors KR158 and GL261. Using our *O*pen *R*eading Frame *A*ntigen *N*etwork (O.R.A.N.) algorithm, we identified neoantigens and tumor-associated antigens (TAAs) which includes cancer testis and developmental antigens that are aberrantly over-expressed in the KR158 and GL261 tumors. The genes identified as harboring the target epitopes were subjected to a gene enrichment strategy which included hybridization, capture and amplification of our identified genes from a pool of total tumor cDNA. The precision RNA libraries generated from the enriched cDNA samples were then validated for enrichment of target genes using RNAseq. RESULTS: We predicted 12 neoantigens and 15 TAAs as immunogenic targets for the KR158 tumor and 192 neoantigens and 37 TAAs for GL261. Pre-capture and amplification, the 27 KR158 tumor genes constituted only 1.14% of the total tumor cDNA pool, however, we were able to enrich the selected genes to up to 95% of the cDNA pool. The IVT RNA library generated from the enriched cDNA templates also consisted of a 90–95% majority of the predicted tumor-specific genes and only 5–10% of the genes were background. CONCLUSION: We have demonstrated the ability to generate tumor antigen specific personalized RNA libraries for murine GBM tumors. RNA vaccines targeting specifically and simultaneously numerous tumor antigens, will pave way for the future of immunotherapy vaccines without the induction of intolerable autoimmunity.

EXTH-48. BMP4 BINDING PEPTIDE AMPHIPHILE NANOFIBERS FOR THE TREATMENT OF PEDIATRIC HIGH-GRADE GLIOMAS

Cara Smith[1], Sonali Nayak[1], Ashorne Mahenthiran[1], Yufen Wang[1], Timmy Fyrner[1], Mark McClendon[1], Barbara Mania-Farnell[2], John Kessler[1], Tadanori Tomita[1], Charles James[3], Samuel Stupp[1], and Guifa XI[1]; [1]Northwestern University, Chicago, IL, USA, [2]Purdue University Northwest, Hammond, IN, USA, [3]Northwestern Feinberg School of Medicine, Chicago, IL, USA

Pediatric high-grade glioma (pHGG) is among the most formidable cancers occurring in childhood. Bone morphogenetic protein 4 (BMP4) reduces the number of glioma stem-like cells and induces apoptosis. Treating tumors with exogenous BMP4 could prove effective in treating gliomas. However, a short half-life limits its clinical application. Glycosylated peptide amphiphile (GlycoPA), with a design inspired by heparin's natural ability to bind growth factors including BMP4 through non-covalent interactions, was previously characterized and found to form high-aspect ratio supramolecular nanofibers that present growth-factor binding sulfated monosaccharides on their surface. These supramolecular nanofibers could carry excessive amounts of growth factor and markedly enhance their biological function. In this study, we verified that GlycoPA is able to bind BMP4 and dramatically increase its half-life with an ELISA assay. We also show that GlycoPA-BMP4, in comparison to free BMP4, significantly decreases pHGG cells' proliferation *in vitro*. Initial *in vivo* intracranial distribution experimental results showed that GlycoPA has a superior normal brain distribution in comparison to a control PA (E2 PA) that has the same base structure as GlycoPA except with no glycosylated group. Preliminary results show that GlycoPA-BMP4 markedly decreases pediatric glioma tumor growth in comparison to free BMP4. Our combined *in vitro* and *in vivo* results demonstrate PA supramolecular nanofibers as an innovative and promising BMP4 delivery platform for clinical application in the treatment of brain tumors. Our future directions will investigate the therapeutic efficacy of GlycoPA-BMP4 in pediatric HGG through testing large numbers of animals and introducing first-line clinical chemotherapy drugs in combination and in various consequence.