were resistant (mean AAC < 0.33) to 4 and 7-day treatments of abemaciclib. One CSC from a newly diagnosed GBM bearing CDK4, MYC and EGFR amplifications was sensitive to both inhibitors (mean AAC = 0.5 abemaciclib and ribociclib), while CSC from the matched recurrent tumor presenting the same driver genomic alterations was significantly more resistant (mean AAC = 0.2 for abemaciclib and ribociclib) ($p < 0.05$, t-test). Additionally, we exposed a sensitive cell line to conditioned media from a resistant cohort, resulting in significantly reduced proliferation and increased resistance to CDK4/6i ($p< 0.05$, Dunn). These findings underscore the importance of a utilizing a robust molecular profiling approach in evaluating which patients will benefit from CDK4/6i therapy.

EXTH-37. TARGETING EPIGENETIC VULNERABILITIES IDENTIFIED FROM A CRISPR SCREEN IN H3.3K27M DIPG

Eshini Panditharatna[1], Neekesh Dharia[1], Deyao Li[1], Alexander Beck[2], McKenzie Shaw[1], Li Jiang[3], Maria Trissal[1], Ilon Liu[1], Caleb Lareau[4], Jamie Anastas[5], Michael Quezada[6], Olivia Hack[1], Hafsa Mire[1], William Jerome[1], Guillaume Kugener[4], David Root[4], Francisca Vazquez[4], Lingling Dai[1], Tingjian Wang[1], Nathan Mathewson[1], Yang Shi[5], Kimberly Stegmaier[1], Michelle Monje[7], Todd Golub[5], Jun Qi[1], and Mariella Filbin[8]; [1]Dana-Farber Cancer Institute, Boston, MA, USA, [2]Ludwig-Maximilians-University of Munich, Munich, Germany, [3]Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, MA, USA, [4]Broad Institute, Boston, MA, USA, [5]Boston Children's Hospital, Boston, MA, USA, [6]Stanford University, Stanford, CA, USA, [7]Stanford University School of Medicine, Palo Alto, CA, USA, [8]Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, MA, USA

Children diagnosed with diffuse intrinsic pontine glioma (DIPG), a type of high grade glioma in the brainstem, currently have a dismal 5-year overall survival of only 2%. The majority of DIPG patients harbor a K27M mutation in histone 3.3 encoding genes (H3.3K27M). To understand if the aberrant epigenetic landscape induced by H3.3K27M provides an opportunity for novel targeted therapies, we conducted the first CRISPR/Cas9 screen using a focused library of 1,350 epigenetic regulatory and cancer related genes in six H3.3K27M DIPG patient-derived primary neurosphere cell lines. We identified gene dependencies in chromatin regulators, polycomb repressive complexes 1 and 2 (PRC1 and PRC2), histone demethylases, acetyltransferases and deacetylators as novel tumor cell dependencies in DIPG. We hypothesized that targeting dysregulated functions of chromatin regulators by genetically deleting and chemically targeting these epigenetically induced vulnerabilities, we could ameliorate, or even reverse the downstream oncogenic effects of the aberrant epigenetic landscape of DIPG. In our secondary CRISPR nanoscreen, we first used six single guide RNAs (sgRNA) to knockout each gene using CRISPR/Cas9 ribonucleoprotein nucleofections, followed by use of three best sgRNAs combined with homology directed repair templates. Compared to lentiviral delivery, nucleofection is a rapid method, with reduced off-target toxicity, suitable for single gene knockouts in DIPG neurospheres. Secondary CRISPR validations confirmed dependencies in *BMI1, CBX4, KDM1A, EZH2, EED, SUZ12, HDAC2,* and *EP300*. Next, we conducted a chemical screen using 20 inhibitors and degraders to target the aberrant activity of HDAC, KDM1A, P300/CBP, PRC1 and PRC2. We identified eight chemical compounds that were effective in H3.3K27M DIPG neurosphere cell lines at low drug concentrations. Among these, an inhibitor and degrader targeting P300/CBP activity indicates a novel strategy of epigenetic therapy in DIPG. Through our combinatorial testing, we will identify a synergistic combination of epigenetic therapy for treating children diagnosed with H3.3K27M DIPG.

EXTH-38. ORTHOGONAL IN VIVO MODELS WERE THE SOLE PRECLINICAL PREDICTOR OF CLINICAL EFFICACY IN PHASE 1 TRIALS OF TARGETED THERAPIES FOR GLIOBLASTOMA: RESULTS OF A SYSTEMATIC REVIEW

Ashray Gunjur[1], Adithya Balasubramanian[1], Umbreen Hafeez[1], Siddharth Menon[2], Lawrence Cher[1], Sagun Parakh[1], and Hui Gan[3]; [1]Austin Health, Melbourne, Australia, [2]Olivia Newton-John Cancer Research Institute, Melbourne, Australia, [3]Olivia Newton-John Cancer Research Institute, Heidelberg, Victoria, Australia

INTRODUCTION: No drug has improved survival in recurrent glioblastoma despite encouraging activity preclinically. We undertook a systematic review of matched preclinical and Phase 1 trials (P1Ts) of targeted agents to investigate potential preclinical predictors of clinical efficacy. METHODS: We identified all adult glioblastoma monotherapy P1Ts of targeted agents & preceding preclinical data published between 2006–2019 via structured searches of EMBASE/MEDLINE/PUBMED. For preclinical studies, data regarding *in vitro* models, *in vivo* models (species, implantation site, cell-line type) and efficacy (growth inhibition, regression rate, survival) were extracted. For P1T, response rate (RR) data were collected as absolute (%) and categorical (RR< 5% vs. RR≥ 5%) variables. Associations were compared by chi-square/Fisher's exact test, Kruskal-Wallis or Mann-Whitney U testing as appropriate with 2-sided p-values. RESULTS: We found 28 P1Ts with median RR 2.9% (range 0.0–33.3%) and mOS 8.0mo (range 4.6–13.0mo). Seven (25%) had 'minimal' published pre-clinical data (5 missing entirely; 2 *in vitro* only); 12 (43%) utilised one cell line *in vivo* ('single model' group); and 9 (32%) used 2+ biologically distinct *in vivo* models ('orthogonal' group). There was strong reliance on U87-based cell lines (14/21 (71%)) in the latter groups. None of the variables tested were associated with RR except for use of 'orthogonal models'. Compared to the 'orthogonal' group, the P1T RR rate was lower in 'single' and 'minimal' groups (6.8% vs 1.2%, p= 0.043 and 6.8% vs 0.0%, p= 0.026 respectively). The frequency of P1T with a RR > 5% was also higher in the 'orthogonal' compared to the same two groups (78% vs 20%, p= 0.042 and 78% vs 17%, p= 0.041). CONCLUSION: The availability of good quality pre-clinical data, especially the use of orthogonal models *in vivo,* was significantly associated with P1T response rates and warrants further investigation as a minimal threshold of evidence in future drug development.

EXTH-39. HEXON SWAPPING MITIGATES ANTI-VIRAL IMMUNE RESPONSE DURING BRAIN TUMOR VIROTHERAPY

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

Cancer virotherapy is a paradigm-shifting treatment modality based on the capabilities of virus-mediated oncolysis to elicit an anti-tumor immune response. Phase 1 and 2 clinical trials have demonstrated the safety and efficacy of our oncolytic adenovirus DNX-2401 (Delta-24-RGD) for patients with recurrent malignant gliomas. While a subset of the patients showed significant benefits, our goal is to improve the response rate. Clearance of the therapeutic virus by dominant anti-viral immune responses may contribute to the observed limits of the virotherapy. Adenovirus serotype 5 that provides the backbone of most oncolytic adenoviruses is highly prevalent in the human population and neutralizing antibodies against the capsid protein hexon may inhibit viral infection and replication. In this study, we showed using immunofluorescence that in mice bearing orthotopic syngeneic glioblastoma GSC005 treated with Delta-24-RGD, IgG antibodies crossed the disrupted blood-brain barrier and infiltrated the brain tumor parenchyma to colocalize with the viral hexon, suggesting that the systemic immune response may eradicate the virus within the infected tumor. To overcome this obstacle, we generated a chimeric virus called Delta-24-RGD-H43m with hexon hypervariable regions replaced with those from a lesser prevalent serotype 43 to avoid recognition by antibodies generated against serotype 5. The molecular swapping of the hexon did not significantly interfere with virion assembly nor attenuate its anti-glioma effect. Thus, the two viruses showed comparable efficacy in vitro (P= 0.568) and in vivo for animals without prior virus exposures (P= 0.228). Importantly, Delta-24-RGD-H43m evaded neutralizing antibodies generated against Delta-24-RGD and maintained its oncolytic ability (P< 0.0001). We conclude that hexon swapping strategies may improve virotherapy by alleviating the dominant immune response against the virus. Despite limited understanding of the interaction between oncolytic viruses and the host immune system, further research on strategies to circumvent virus-specific immune responses should aid the development of enhanced, glioma-targeted virotherapies.

EXTH-40. THERAPEUTICALLY TARGETING DE NOVO PURINE BIOSYNTHESIS IN DIFFUSE INTRINSIC PONTINE GLIOMA

Ian Mersich[1], and Biplab Dasgupta[2]; [1]University of Cincinnati and Cincinnati Children's Hospital, Cincinnati, OH, USA, [2]Cincinnati Children's Hospital, Cincinnati, OH, USA

Diffuse intrinsic pontine glioma (DIPG) is an incurable brainstem malignancy in children with median survival less than 1 year and 5-year overall survival only 2 percent. Little progress has been made in treating this deadly disease due to its inoperable location and treatments aimed at targets defined in adult gliomas. Despite recent advances in genetic characterization of DIPGs there are still no targeted therapies that significantly improve overall survival. We recently generated a metabolic profile for DIPG elucidating an upregulation in purine metabolism, specifically in de novo purine biosynthesis (DNPB). Normally nucleotide salvage maintains cellular purine levels by recycling degraded bases, however DNPB is needed when purine levels are depleted. Purine metabolism provides the basic components of nucleotides needed for tumor proliferation and thus considered a high-priority target in cancer treatment. DNPB is a sequential ten step enzymatic process resulting in the production of inosine monophosphate. The last step in DNPB is carried out by the bifunctional enzyme ATIC which is upregulated in DIPG cell lines, and in patient tumors. Our preliminary data demonstrates DIPG cell lines are sensitive to pharmacological inhibition and genetic ablation of multiple enzymes in the DNPB pathway. Strikingly, cell viability could be rescued by purine supplementation when inhibiting this pathway