HGG-41. CHARACTERIZATION OF THE IMMUNE RESPONSE FOLLOWING VARIOUS RADIOTHERAPY TREATMENTS IN GLIOBLASTOMA

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Malignant gliomas represent 6.5% of all childhood brain neoplasms with a 5-years survival rate of less than 20%. Current standard of care for these tumors include radiotherapy; recent data in solid tumors indicate that adequate radiation protocols may synergize with immunotherapy strategies for better outcomes. Nonetheless, a great discrepancy between preclinical studies and clinical trials outcomes persists, the basis of which is not fully understood. One hypothesis may be due to different radiation protocols used. We used the GL261 syngeneic mouse model of glioma to test this hypothesis and characterize the immune response to radiotherapy, with either a single dose of 10Gy, a dose often used in preclinical models, or a fractionated treatment of 2Gy for five consecutive days (2Gyx5), as fractioned radiotherapy is most often used in patients. The immune content of the brain and the blood was assessed by flow cytometry in un-irradiated (control), 10Gyx1 and 2Gyx5 treated mice for three weeks after radiation. In the brain, both radiation regimens drastically reduced the number of $CD45^+$ cells for the first two weeks after treatment. When compared to controls, 10Gyx1 but not 2Gyx5 treated mice showed a significant increase in tumor infiltrating lymphocytes ($CD3^+$) starting from the second week following treatment. This effect persisted until three weeks post treatment. The 10Gyx1 dose was better tolerated by the resident microglia ($CD45^{low}CD11b^+$) when compared to the 2Gyx5 treatment. Our data describe the dynamics through which the immune microenvironment responds to two radiation regimens over time. Our results show that 10Gyx1 is the most effective regimen to impede tumor growth and to induce lymphocyte infiltration once the system recovers from the treatment. Our work suggests that, in the GL261 model, the fractionated radiation treatment we tested may be less optimal in priming glioma cells to the immune system.

HGG-42. PEDIATRIC H3K27M MUTANT DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) SHOWS ROBUST RESPONSE TO IMIPRIDONE BASED COMBINATION THERAPY

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ONC201 is a first-in-class small molecule imipridone therapy, which is known to selectively induce apoptosis of cancer cells independent of p53. This novel chemotherapeutic, as well as its analogs ONC206 and ONC212, has been shown to have potent preclinical efficacy against H3K27M mutant diffuse intrinsic pontine glioma (DIPG). We sought to identify synergy between imipridones and other FDA-approved chemotherapeutics. Seven patient-derived DIPG cell lines, six H3.3K27M mutant (SU-DIPG-IV, SU-DIPG-13, SU-DIPG-25, SUDIPG-27, SU-DIPG-29, SF8628), and one H3.1K27M mutant (SU-DIPG-36) were grown in culture and exposed to first and second generation imipridones, both as monotherapies and in combination with histone de-acetylase inhibitors [HDACi], Marizomib, Etoposide, and Temozolomide. A dose dependent response was demonstrated across all cell lines, with increased potency of ONC206 and ONC212 as compared to ONC201, with half maximal inhibitory concentration (IC_{50}) of 0.11 μM, 0.03 μM, and 1.46 μM respectively. Strong synergy is demonstrated between ONC201 and Panobinostat with best combination index (CI) of 0.01. ONC201 similarly shows strong synergy with Romidepsin with best CI of 0.02, and Marizomib with best CI of 0.18. Combination of ONC201 and Etoposide or Entinostat shows some synergy, with best CI of 0.53 and 0.71 respectively. When combined with Temozolomide, some synergy is evident, however, there is overall poor efficacy, with lack of cell death even at the highest doses of Temozolomide. Second generation imipridones show a similar pattern of strong synergy with Panobinostat, Romidepsin, and Marizomib. Immunoblotting showed evidence of apoptosis, as measured by the induction of PARP cleavage, with a combination of imipridones and Panobinostat, as well as induction of integrated stress response with a combination of imipridones and Romidepsin. These results are indicative of promising synergy between imipridones and Panobinostat, Romidepsin, or Marizomib against H3K27M mutant DIPG, combinations which should be considered for future clinical trials.

HGG-43. INTERROGATING THE ROLE OF PEA3 ONCOGENIC TRANSCRIPTION FACTORS IN PEDIATRIC HIGH-GRADE K27M GLIOMAS

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Pediatric high-grade gliomas (HGGs) are an aggressive form of pediatric brain tumors which pose a grim five-year survival with little advancement in therapeutic efficacy, often requiring a multimodal therapeutic combination of chemotherapy, resection, and radiation. We have previously shown that proper function of ETS transcription factors is necessary for gliomagenesis in Ras/MAPK-driven pediatric gliomas. It is our hypothesis that ETS transcription factors are necessary for tumor initiation in HGGs by promoting the necessary glial cell fates in glioma. Further, we hypothesize that functional inhibition of ETS proteins following tumor formation will improve survival and outcome in HGG. Functional inhibition of ETS proteins using a competitive dominant-negative mutant was shown to completely rescue neural stem cell depletion, tumor formation and tumor-free survival in two rodent models of HGGs. Mechanistically, we show evidence that Pea3 factors may induce glial-cell fate by promoting Olig2 expression and activation of glial transcriptional programs. Indeed, transcriptomic analysis of ETS-perturbed HGG tumors revealed that Sox9 and Olig2 transcription factor networks were dependent on proper ETS function. Further, we show evidence that Etv5 can directly interact with promoter regions of glial fate master regulators in human primary glioma cell lines. To empirically determine the effect of Pea3 proteins on tumorigenesis, we have created a novel methodology for inducible gain- and loss-of-function genetic interrogation of these factors in vivo. Our survival results and combined single-cell RNA-sequencing of individual groups show that inhibition of the Pea3 family leads to a marked increase in survival in K27M glioma by regulating key features of glioblasts. All in all, our group provides evidence that the ETS family of transcription factors is necessary for glial specification of tumor cells and induce pro-glial transcriptional programs by activating OPC- and astrocyte-specific genes in K27M-driven tumors.

HGG-44. REVEALING VULNERABILITIES IN DIPG THROUGH ONC201

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Emerging evidence from clinical and preclinical studies suggests that the imipridone ONC201 is well tolerated and may have some clinical impact in discrete diffuse intrinsic pontine glioma patients (DIPG). A primary goal of our work is to determine if DIPG are uniquely sensitive to ONC201 and if so, whether ONC201 itself can be used as a tool to illuminate novel vulnerabilities in DIPG. To accomplish this, we are utilizing a combination of patient-derived cell lines as well as mouse xenografts that dovetail with a variety of molecular, epigenetic and metabolomic tools. A central finding from our work is that ONC201 primarily activates the mitochondrial protease, ClpP in DIPG patient-derived cell lines, an effect consistent with recently described ONC201 mechanism of action in other tumors. We further demonstrate that activation of ClpP by ONC201 leads to a host of downstream effects in DIPG model systems including distinctive effects on the metabolome leading to direct alterations in the unique epigenetic signature of DIPG. By directly manipulating these metabolic and epigenetic factors we provide prospective mechanistic insight into how ONC201 as well as ClpP activity impacts DIPG growth and tumorigenicity. These preclinical research findings shed light on potential therapeutic vulnerabilities in DIPG as well as ways that these strategies may be combined to enhance their potential.

IMMUNOLOGY/IMMUNOTHERAPY

IMMU-01. THE ONCOLYTIC VIRUS DELTA-24-RGD IN COMBINATION WITH AN AGONISTIC CD40 MAB INDUCES A DURABLE AND SYNERGISTIC ANTI-TUMOR IMMUNE EFFECT IN DIPG PRECLINICAL MODELS

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With a 2-year survival less than 20%, Diffuse Intrinsic Pontine Glioma (DIPG) is the principal cause of pediatric death. Despite recent advances in the current treatments, the outcome for children with DIPGs remains dismal. Since the approval of T-VEC for melanoma by the FDA, oncolytic adenoviruses have emerged as a promising therapeutic strategy for brain tumors. Thus, our group launched the first world clinical trial phase I with the oncolytic adenovirus Delta-24-RGD (DNX-2401 in the clinic) for newly diagnosed DIPG (NCT03178032), which has shown safety and feasibility. Despite DNX-2401 increases the recruitment of T cells into the tumor, they

usually become inactive due to the suppressive tumor microenvironment evidencing the urgent need to improve this strategy focusing on the generation of effective long-term immune responses. Therefore, we decided to combine the Delta-24-RGD with the targeting of the costimulatory molecule CD40 in two unique orthotopic immunocompetent mouse models of DIPG. The activation of the CD40 receptor, which is expressed by antigen presenting cells (APC) such as microglia, macrophages and dendritic cells, is known to increase antigen presentation and enable T-cell priming and activation. Here, we have observed that in addition to Delta-24-RGD anti-tumor effects, the stimulation of CD40 on the tumor APCs results in a remodeling of the tumor immune compartment with a more efficient T-cell tumor infiltration. Of importance, there is an increase of the survival of mice treated with the combination as compared to single treatments or non-treated mice. In addition, the combination therapy induced a complete regression of tumors in 25% of treated mice indicating the development of long-term anti-tumor immunity. We believe that these results provide a translational breakthrough in the treatment of these lethal tumors and open the door for a future innovative clinical trial.

IMMU-02. CH14.18 IN THE TREATMENT OF HIGH-RISK NEUROBLASTOMA: A META-ANALYSIS

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High-risk neuroblastoma (HRNB) is a rare malignancy that mainly affects young children. Long-term survival remains low despite aggressive, multimodal treatment regimens. The aim is to assess the effect of Ch14.18, a novel anti-GD2 antibody, on survival in HRNB. In this meta-analysis, Pubmed/MEDLINE, EMBASE, Cochrane CENTRAL and Clinicaltrials.gov bibliographic databases were searched from inception to 1st July 2018. Prospective or retrospective interventional clinical trials with at least two arms or observational cohort or case-control studies were eligible. Participants had to have HRNB. Ch14.18 was the intervention of choice. Any control, comparator treatments were accepted. Studies were appraised for inclusion by two independent reviewers. Data was extracted from published reports. Primary outcomes assessed were Event-Free Survival (EFS) and Overall Survival (OS) and presented as Weighted Mean EFS (%) ± Standard Error (SE) and Weighted Mean OS (%) ± Standard Error (SE), respectively. Combined effect size was elicited for EFS±SE and OS±SE. The search yielded 367 results of which 7 studies conducted on 1727 patients were eligible for inclusion. The weighted mean EFS±SE for Ch14.18 regimens (n=779) and control regimens (n=787) were 55.6±2.0% and 41.2±1.7%, respectively. The weighted mean OS±SE for Ch14.18 regimens (n=430) and control regimens (n=348) were 63.4±2.3% and 53.6±2.7%, respectively. Results of the meta analysis yielded statistical power >94%. The combined effect size of Ch14.18 regimens for EFS±SE was 0.2907±0.05 [95% CI: 0.19–0.39, p<0.001] and the combined effect size for OS±SE was 0.26±0.07 [95% CI: 0.11- 0.4, p<0.001]. Significant superior survival outcomes were achieved with the use of Ch14.18 in the treatment of HRNB. These findings support using Ch14.18 as an adjunct in maintenance therapy in high-risk neuroblastoma. This meta analysis is the first of its kind. Survival outcomes are to be interpreted with caution due to confounding bias.

IMMU-03. CHARACTERIZING THE IMMUNE MICROENVIRONMENT OF PEDIATRIC BRAIN TUMORS

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Therapy for pediatric central nervous system (CNS) malignancies can be toxic, and outcomes are suboptimal. Immunotherapy holds promise as a therapeutic avenue, but one of the challenges in its application is the poorly understood microenvironment of pediatric CNS tumors. The Children's Brain Tumor Network released the Pediatric Brain Tumor Atlas, containing the expression profile of nearly 700 primary CNS tumors. To study the immune microenvironment, a classification from The Cancer Genome Atlas project is applied. High-grade lesions are predominantly lymphocyte deplete (C4, 80%) or immunologically quiet (C5, 7.6%). Low-grade lesions are more mixed with 43% C4, 26% C5, and a higher proportion of inflammatory subtype (C3, 28%). For survival parameters, immune subtype and tumor grade are associated. Using a multivariate cox regression model, the hazard ratio is 2.2 (0.86 – 5.4, p = 0.102) and 3.6 (1.2 – 10.9, p = 0.02) for C4 and C5, respectively. Deconvolution of immune cell gene signatures provides insight into the phenotype of lymphocyte infiltrate, which averages 8.6% (IQR 5.4% – 9.8%) across all samples. For high-grade samples, greater than median expression of T cell-, monocyte-, macrophage-, and B cell-gene signatures are each associated with decreased survival (p < 0.05). Microglia gene signatures have decreased relative expression in high-grade samples compared to low-grade samples (p < 0.001). It is hypothesized that the expression of inhibitory immunomodulators contributes to a pro-tumorigenic microenvironment and represent potential therapeutic targets. In the absence of normal samples in the data set, differential gene expression experiments between disease states can reveal upregulated immunomodulators. Focusing on diffuse midline glioma, immunologic pathways are downregulated. Furthermore, 9 inhibitory immunomodulators, including *KDM1A, EZH2, CD276,* and *VTCN1*, are significantly expressed relative to midline low-grade glioma with equivalent immune subtype. Overall, our analysis contributes to the understanding of the immune microenvironment and mechanisms of immune escape for pediatric CNS malignancies.

IMMU-04. FIRST-IN-CHILDREN PHASE 1B STUDY USING THE IDO PATHWAY INHIBITOR INDOXIMOD IN COMBINATION WITH RADIATION AND CHEMOTHERAPY FOR CHILDREN WITH NEWLY DIAGNOSED DIPG (NCT02502708, NLG2105)

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Background: Diffuse intrinsic pontine glioma (DIPG) is a uniformly fatal brain tumor with no available cure. Indoximod blocks the IDO (indoleamine 2,3-dioxygenase) pathway, thereby reversing IDO-mediated immune suppression in the tumor microenvironment. Methods: Patients aged 3 to 21 years with treatment-naive DIPG were eligible for this phase 1b dose-confirmation study of indoximod. The treatment regimen comprised continuous oral indoximod (38.4 mg/kg/day divided twice daily) with conformal photon radiation (54 Gy in 30 fractions), followed by cycles of indoximod with temozolomide (200 mg/m2/day, days 1–5 in 28-day cycles). Results: Thirteen patients (median age 9 years, range 5 to 20 years) with DIPG were treated. Median OS was 14.5 months (follow-up ranged 4.8 to 29.3 months), 12-month OS was 61.5% (8/13), and 18-month OS was 30.8% (4/13), with 1 patient remaining in follow-up at the data cutoff. This compared favorably to expected median OS of approximately 10.8 months, 12-month OS of 45.3%, and 18-month OS of 16.2% taken from published historical data from the Pediatric Brain Tumor Consortium. Two patients showed near-complete responses lasting until relapsing after 7.6 months and 13.3 months of study therapy, respectively. Many patients had increased circulating non-classical monocytes (nc-Monos, CD16+, CD14neg, CD33+, HLA-DR+) within the first 3 treatment cycles, and elevation of this early pharmacodynamic marker was predictive of subsequent OS. Patients with nc-Monos >10% (n=7) had median OS of 19 months, whereas patients with nc-Monos below 10% (n=5) had median OS of 7 months (p=0.0047). No patients stopped therapy for toxicity. The most common indoximod-attributed adverse events were thrombocytopenia, neutropenia, nausea, vomiting, dizziness, and fatigue. Conclusions: Adding indoximod immunotherapy to conventional radiation and chemotherapy for front-line treatment of pediatric patients with DIPG was well-tolerated. Improved outcomes were observed in patients having evidence of pharmacodynamic response. A follow-on phase 2 study is in progress (NCT04049669).

IMMU-05. IMMUNOTHERAPY WITH RESIQUIMOD REPOLARIZES TUMOR-ASSOCIATED MYELOID CELLS AND IMPROVES EVENT-FREE SURVIVAL IN A TRANSGENIC MOUSE MODEL OF SONIC-HEDGEHOG (SHH) MEDULLOBLASTOMA

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Resiquimod is a synthetic small molecule agonist of Toll-like receptors 7 and 8 (TLR-7/8) that modulates innate immune cells. We found TLR-7/8 are expressed in medulloblastoma exclusively by tumor-associated myeloid cells