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 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 10 Gy, a dose often used in preclinical models, or a fractionated treatment of 2 Gy for five consecutive days (2Gx5), as fractionated 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), 10Gx1 and 2Gx5 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, 10Gx1 but not 2Gx5-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 10Gx1 dose was better tolerated by the syngeneic glioma (CD45+CD11b+) when compared to the 2Gx5 treatment. Our data describe the dynamics through which the immune microenvironment responds to two radiation regimens over time. Our results show that 10Gx1 is the more effective treatment to impede tumor growth and to induce immune 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 IMPRIBIDONE BASED COMBINATION THERAPY
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ONC201 is a first-in-class small molecule imipridone therapy, which is less toxic and as efficacious as TMZ in inducing apoptotic cell death of primary pediatric HGGs and is approved for upfront TMZ-refractory pediatric H3K27M mutant diffuse intrinsic pontine glioma (DIPG). We sought to identify synergy between imipridones and other FDA-approved chemotherapeutics. Most treatment. The 10Gx1 dose was better tolerated by the syngeneic glioma (CD45+CD11b+) when compared to the 2Gx5 treatment. Our data describe the dynamics through which the immune microenvironment responds to two radiation regimens over time. Our results show that 10Gx1 is the more effective treatment to impede tumor growth and to induce immune 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.

ONC201 and 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. Most treatment. The 10Gx1 dose was better tolerated by the syngeneic glioma (CD45+CD11b+) when compared to the 2Gx5 treatment. Our data describe the dynamics through which the immune microenvironment responds to two radiation regimens over time. Our results show that 10Gx1 is the more effective treatment to impede tumor growth and to induce immune 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-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 tumor with a poor 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 Razek et al. It is our hypothesis that transcription factors are necessary for tumor initiation in HGGs by promoting the necessary glial cell fates in glioma. Furthermore, 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 gli-cell fate by promoting Olig2 expression and activation of the Olig2 transcriptional reporter. Indeed, transcriptomic analysis of ETS-primed HGG tumors revealed that Sox9 and Olig2 transcription factor networks were dependent on proper ETS function. Further, we show evidence that Ets3 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 gliompecification of tumor cells and induce pro-gli-proliferative dorgrams 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 ORNO2 and its chemical analogs ONC206 and ONC212 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 pro-apoptotic pathway. Our in vitro findings shed light on potential therapeutic vulnerabilities in DIPG as well as ways that these strategies may be combined to enhance their potential.