lationships in pediatric brain tumor patients. METHODS: Forty-nine patients (ages 7-18y.o.) with any brain tumor diagnosis underwent resting state functional Magnetic Resonance Imaging (rsfMRI) during regularly scheduled clinical visits. All patients were tested with the NIH Toolbox Cognition Battery. One-hundred thirty-nine age- and sex-matched typically developing children were used as controls. All data were processed to minimize artifactual sources of variance. Functional brain networks were created for each patient via rsfMRI data from 300 regions of interest that sample the whole brain. Multilinear models were implemented to examine brain-behavior relationships, while accounting for demographic and clinical factors. RESULTS: Functional network organization was significantly altered in patients compared to controls (p<0.001). Network organization was more affected in patients who received whole-brain radiation therapy than those who did not (t=2.52, p<0.015). Patients demonstrated significant impairments in multiple domains of cognitive performance, e.g. attention (p<0.0001). Weak relationships were found between cognitive performance and network organization, none of which survived multiple comparison correction. CONCLUSIONS: Brain network architecture is significantly altered in pediatric brain tumor patients. Whole-brain radiation was related to the largest changes. Most network and cognitive changes were significant with large effect sizes, yet brain-behavior relationships were weak. Our results suggest that systems-level changes in brain organization may provide insight into long-term changes in brain function in pediatric brain tumor patients.

IMG-15. RADIOMIC PROFILING OF PEDIATRIC LOW-GRADE GLIOMA IMPROVES RISK STRATIFICATION BEYOND CLINICAL MEASURES

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PURPOSE: Treatment response is heterogeneous among patients with pediatric low-grade glioma (pLGG), the most frequent childhood brain tumor. Upfront prediction of progression-free survival (PFS) may facilitate more personalized treatment planning and improve outcomes for the pLGG patients. In this work, we explored the additive value of radiomics to clinical measures for prediction of PFS in pLGGs. We further sought associations between the derived risk groups and underlying alterations in key genomic and transcriptomic variables. METHODS: Quantitative radiomic features were extracted from pre-operative multi-parametric MRI scans (T1, T1-post, T2, T2-FLAIR) of 96 patients with newly diagnosed pLGG (median age, 8.59, range, 0.35-18.87 years; median PFS, 25.23, range, 3.03-124.83 months). Multivariate Cox proportional hazard's (Cox-PH) regression models were fitted using 5-fold cross-validation on a training cohort of 68 subjects and tested on 28 patients. Three models were generated using (1) only clinical variables (age, sex, and extent of tumor resection), (2) radiomic features, and (3) clinical and radiomic variables. The dimensionality of radiomic features in Cox-PH models was reduced by applying Elastic Net regularization penalty to identify a subset of variables that are most predictive of PFS. The patients were then stratified into three groups of high, medium, and low-risk based on model predictions. RESULTS: Cox-PH modeling resulted in a concordance index (c-index) of 0.55 for clinical data, 0.65 for radiomics, and 0.73 for a combination of clinical and radiomic variables, highlighting the additive value of radiomics to the readily available clinical information in prediction of PFS. Radiogenomic assessments revealed significant differences in expression of BRAF, NF1, TSC1, ALK (p<0.01), and RB1 (p<0.05) genes in the high-risk group, compared to the medium and low-risk groups. CONCLUSION: Our results demonstrate the value of integrating radiomics with clinical measures to improve risk assessment of patients with pLGG through improved pretreatment prediction of PFS.

IMG-16. NON-INVASIVE METABOLIC IMAGING OF RESPONSE TO THERAPY IN DIFFUSE MIDLINE GLIOMAS

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Diffuse midline gliomas (DMGs) are universally lethal pediatric tumors that are defined by the presence of histone H3K27 alterations. The infiltrative nature and anatomical location of these tumors prohibit surgical resection. Radiotherapy, which is standard of care, does not significantly enhance long-term outcome. Novel therapies are sorely needed for DMG patients. Imipridone drugs ONC201 and ONC206 have demonstrated anti-tumor activity in preclinical cancer models, including DMGs and have shown promise in pilot studies in DMG patients. Successful clinical deployment of imipridones requires the identification of companion imaging biomarkers that report on response to therapy. Magnetic resonance spectroscopy (MRS) is a safe, non-radioactive, non-invasive method of imaging metabolism *in vivo*. ^{1}H-MRS assesses steady-state metabolite levels and is used in clinics. ^{2}H-MRS following administration of ^{2}H-labeled substrates is a novel, clinically translatable method of imaging metabolic pathway activity. Our results indicate that treatment of SF7761 DMG cells with ONC206 causes a significant reduction in ^{1}H-MRS-detectable lactate, glutamate, glutathione and phosphocholine, pointing to inhibition of glycolysis, oxidative phosphorylation, redox and phosphatidylcholine biosynthesis respectively. Examination of [6,6'-^{2}H]-glucose metabolism using ^{2}H-MRS indicates that lactate production from [6,6'-^{2}H]-glucose is significantly reduced in ONC206-treated SF7761 cells relative to controls. We then investigated the effect of ONC206 on mice bearing orthotopic SF8628 DMG tumors. At day 7 following the treatment onset, at a timepoint when no change in tumor volume can be observed by anatomical imaging, *in vivo* ^{1}H-MRS-detectable lactate and total choline are reduced relative to day 0. Collectively, our studies indicate that imipridones induce alterations in DMG metabolism that can be leveraged for non-invasive ^{1}H- and ^{2}H-MRS-based imaging of response to therapy. By providing clinicians with an early readout of treatment response prior to anatomical changes, our biomarkers will enable early assessment of treatment response and, thereby, clinical translation of these promising therapeutics.

IMG-17. ADVANCED MRI ON THE CELLULAR AND VASCULAR PHENOTYPE OF MOUSE EPENDYMOMA MODELS AND CHEMO-RADIATION TREATMENT RESPONSE

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Ependymoma (EPN) is an aggressive pediatric brain tumor, for which the benefits of chemotherapy in pediatric patients have not been defined. EPN treated with surgery and radiation recur in 23-66% of patients. Our group has previously established aggressive behaviors of EPN, including high tumor cellularity, cytological anaplasia, high mitotic index, tumor necrosis, and the presence of inflammatory cells such as M2-type myeloid cells. Here we report on an advanced 9.4 Tesla MRI protocol for characterizing the cellular and vascular phenotype and treatment response to chemo-radiation therapy (CRT) in an orthotopic mouse model of patient derived xenografts (PDX) of pediatric EPN . Female severely immune deficient (SCID) mice were used for intracranial inoculation of disaggregated tumors from pediatric EPN patients (n=22). High-resolution T2w-MRI was able to detect cerebellar microlesions as small as 0.2 mm diameter; the median tumor volumes at the baseline were 21±12 mm3. Using diffusion-weighted based cell-size imaging, iron-oxide based vessel-size imaging and quantitative T2-maps, the EPN-specific phenotype was characterized by an increased cell size (S=14 microns), increased vessel density index (Q=0.54), and low ADC values (0.63x10-3). Once the intracranial tumors reached at least 5 mm3, animals were treated with CRT (10 Gy radiation plus 30 mg/kg 5-fluorouracil, n=6). CRT resulted in a tumor shrinkage, tumor necrosis with decreased cell sizes and increased ADC values, and a dramatic vascular-inflammatory response (decreased Q and DT2 values with the injection of iron oxide nanoparticles as macrophage-specific contrast). In summary, orthotopically implanted PDX EPN in mice closely mimic histological features, anatomical location and radiological features of the primary tumors. A significant decrease in vessel size density and an increase in inflammatory cells were seen as soon as 2 days after CRT. The late response (2 weeks post CRT) is characterized by decreased cellularity, cell size, and tumor volumes.

IMMUNOTHERAPY

IMMU-01. COMBINING CD28 AND 4-1BB COSTIMULATION *IN TRANS* ENHANCES THE ANTI-GLIOMA EFFICACY AND PERSISTENCE OF B7-H3 CAR T CELLS IN IMMUNE-COMPETENT BRAIN TUMOR MODELS

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We and others have demonstrated that B7-H3 CAR T-cells have potent antitumor responses in xenograft models for brain tumors; however, these models do not recapitulate the immunosuppressive tumor microenvironment (TME) in patients with high-grade glioma. To evaluate the safety and efficacy of antigen-specific CAR T-cells, we adapted the immune-

competent GL261 glioma model which recapitulates human disease and host immune barriers. We generated a library of B7-H3 CARs with different transmembrane (CD8, CD28), costimulatory (CD28, 4-1BB), and activation (ζ, mutζ) domains. We then compared their cytolytic activity, expansion, and anti-tumor activity. Results show that B7-H3 CARs with CD28 transmembrane and costimulatory domains have superior efficacy compared to CARs with CD8 and 4-1BB domains. Additionally, CARs with mutated ζ activation domain have better overall persistence. However, providing costimulation signals through CD28 or 4-1BB alone does not induce superior anti-glioma efficacy of B7-H3 CAR T-cells *in vivo*. Thus, we next investigated whether incorporating 4-1BB signaling into CD28-based CARs using *in trans* design enhances the therapeutic efficacy of B7-H3 CAR T-cells. We found that in repeat stimulation assays, surface expression of 4-1BBL enhanced expansion of B7H3 CAR T-cells at least 300-folds more than T-cells with CD28 or 4-1BB costimulatory domains alone. Additionally, 4-1BBL expression significantly enhanced the sequential killing capacity compared to CD28- or 41BB-based B7-H3 CAR T-cells. High dimensional flow cytometry analysis of GL261 tumors post CAR T-cell injection revealed unique immune clusters including dendritic cells and lymphoid predominant populations in mice treated with 4-1BBL expressing CARs. Thus, expression of 4-1BBL on CD28-based CARs reshaped the TME and enhanced persistence and anti-glioma efficacy of B7-H3 CAR T-cells. Studies examining transcriptional and epigenetic programs, and TME/CAR T-cell interactions are in progress. Results will define pathways that dictate CAR T-cell performance and will identify unique mechanisms for further improvements utilizing other members of TNF-superfamily.

IMMU-02. TARGETED INNOVATIVE ANTIBODY FRAGMENT-BASED IMMUNOTHERAPY FOR MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common malignant pediatric brain tumor accounting for ~20 % of childhood brain tumors. One third of all MB are characterized by constitutive activation of the Sonic Hedgehog (SHH)-signaling pathway. This tumor type shows overexpression of the epidermal growth-factor receptor (EGFR), which we detected in SHH-MB patient samples, transgenic SHH MB mouse models, and MB-cell lines. In contrast, non-neoplastic cells only express EGFR at low levels. Intensive radio-/chemotherapy often leaves the young patients with significant long-term burdens including problems in brain development and cognitive deficits. Thus, there is an urgent need to develop new targeted therapies that can prevent tumor recurrence without affecting healthy cells. We selected EGFR as a potential therapy target using EGFR-specific antibody fragments (scFvs) as part of immunoconjugates, namely bispecific T-cell engagers (BiTEs) and immunotoxins (ITs). Both, the EGFR-specific BiTEs and the ITs showed specific binding and cytotoxic activity in MB cells. Effector- and target-cell specificity was demonstrated via flow cytometry for the BiTEs. BiTEs and ITs selectively killed MB-tumor cells and showed pro-apoptotic effects without unspecific effects. Furthermore, preliminary results from an innovative hiPSC-based in vitro-BBB-model suggest, that the ITs are able to cross the BBB. Finally, by having a functional cloning- and expression system for the BiTEs and ITs available, target-scFvs can be easily exchanged by novel antigens or peptides to obtain additional targeted immunotherapies. Together, these results pave the way for preclinical in vivo experiments and future clinical trials in patients with SHH MB.

IMMU-03. SYNERGY BETWEEN TMZ AND INDIVIDUALIZED MULTIMODAL IMMUNOTHERAPY TO IMPROVE OVERALL SURVIVAL OF IDH1 WILD-TYPE MGMT PROMOTER-UNMETHYLATED GBM PATIENTS

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The prognosis of IDH1 wild-type MGMT promoter-unmethylated GBM patients remains poor. Addition of Temozolomide (TMZ) to first-line local treatment shifted the median overall survival (OS) from 11.8 to 12.6 months. We retrospectively analysed the value of individualized multimodal immunotherapy (IMI) to improve OS in these patients. All adults meeting the criteria and treated 06/2015-06/2021 were selected. Thirty-two patients (12f, 20m) had a median age of 47y (range 18-69) and a KPI of 70 (50-100). Extent of resection was complete (11), <complete (12) or not documented (9). Seven patients were treated with surgery/radio(chemo)therapy and subsequent IMI (Group-1); 25 patients were treated with radiochemotherapy followed by maintenance TMZ plus IMI during and after TMZ (Group-2). Age, KPI and extent of resection were not different amongst both groups. The median OS of group-1 patients was 11m (2y OS: 0%). Surprisingly the median OS of group-2 patients was 22m with 2y OS of 36% (CI95%: 16-57), which was significantly (Log-rank: p = 0.0001) different from group-1. The data suggest that addition of IMI after local therapy on its own has no relevant effect on OS in these GBM patients, similar to maintenance TMZ. However, the combination of both TMZ + IMI significantly improved OS. This finding might also have implications in the search for novel combined treatment approaches for children with malignant glioma.

IMMU-04. TRANSCRIPTIONAL ANALYSIS REVEALS DISTINCT MICROENVIRONMENTAL SUBGROUPS ACROSS PEDIATRIC NERVOUS SYSTEM TUMORS

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INTRODUCTION: Recent clinical trials of immune checkpoint inhibitors indicated 5-11% response rate in pediatric patients depending on cancer type and expression of target proteins. Currently, a systematic analysis characterizing the immune microenvironment of childhood tumors is lacking. The main objective of this study is to uncover the features of immune microenvironment in pediatric nervous system tumors (pedNST). METHODS: We compiled transcriptomes of 925 tumors from three initiatives, Therapeutically Applicable Research To Generate Effective Treatments (TARGET, n = 149), International Cancer Genome Consortium (ICGC, n = 195) and Children Brain Tumor Tissue Network (CBTN, n = 581). We analyzed the performance of immune deconvolution tools and used publicly available datasets to define immune genesets. We conducted a consensus analysis to assign genes to cell-types and identify immunological groups. RESULTS: We found wide variability in immune infiltration across and within cancer types ranging from cold tumors such as medulloblastoma (2.7% infiltrate) to infiltrated entities such as neurofibroma (22.6%). Consensus clustering revealed four distinct immune clusters. The pediatric inflamed group (10%) included MYCN non-amplified neuroblastoma and ATRT. The myeloid-predominant group (30%) showed decreased infiltration of lymphoid cells but enrichment of myeloid cell genesets. The pediatric-cold group (42%) harbored no enrichment of immune genesets and included 72% of ependymomas and 65% of medulloblastomas. The immune excluded group (18%) showed depletion of immune cell-types and included sonic-hedgehog medulloblastoma. 71% of pedNST belonged to the lymphocyte depleted or immunologically quiet clusters, indicating the cold immune microenvironment in pedNST compared to adult cancers. CONCLUSION: We report characteristics of the immune microenvironment in pedNST. We found an overall cold microenvironment with low lymphocyte infiltration in this population compared to common adult cancers. We identified ~10% of tumors harboring a relatively inflamed microenvironment. Our data uncover characteristics of immune infiltration in pediatric tumors with potential implications to guide therapy.

IMMU-05. INTEGRATIVE TRANSCRIPTOMIC ANALYSIS OF PILOCYTIC ASTROCYTOMAS REVEALS CNS REGION-ASSOCIATED CHARACTERISTICS

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