Next, we expressed either WT zebrafish or human TP53 in tp53-/- animals along with kRASG12D and both genes suppressed tumor initiation and growth. We co-expressed TP53C176F (found in two ERMS patients) and TP53P153del (identified in a patient with osteosarcoma in our clinic) in zebrafish ERMS, and find that the TP53C176F allele significantly suppressed tumor initiation with effects predominantly on enhanced apoptosis. However, the TP53P153del allele initiated tumors at similar frequency compared to tp53-/- animals but increased the initiation of tumors in the head musculature. DISCUSSION/SIGNIFICANCE OF FINDINGS: Different TP53 alleles identified in patient tumors have very different effects on tumorigenesis in vivo and can respond differently to potentially therapeutic compounds. Thus, the type of precision modeling demonstrated here promises to help further define patient-specific TP53 biology and improve clinical strategies in the future.

92418

Molecular imaging of the tumor microenvironment to predict response to combination treatment with immunotherapy in triple negative breast cancer

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ABSTRACT IMPACT: Insights from this project will provide clinical guidance in treatment of immunotherapy in triple negative breast cancer and identify early imaging biomarkers of treatment response. OBJECTIVES/GOALS: Significant research that addresses monitoring and predicting patient response of triple negative breast cancer (TNBC) to immunotherapy is needed. Using positron emission tomography (PET) imaging to probe the tumor microenvironment (hypoxia, T-cell activation), we aim to predict early response to immunotherapy for in mouse models of TNBC tumors. METHODS/STUDY POPULATION: Female Balb/c mice with 4T1-luciferase mammary carcinoma cell tumors were administered paclitaxel (PTX; 10 mg/kg), anti-PD1 (200 μg), both, or vehicle (saline) intraperitoneally. Treatment was given on days 0, 2, and 5 for cohort 1 (n=16) who underwent granzyme B specific (GZP) PET imaging (T-cell activation) and days 0, 2, 5, and 8 for cohort 2 (n=12) who underwent [18F]-fluoromisonidazole (FMISO)-PET imaging (hypoxia). Bioluminescence (BLI) imaging and caliper measurements were performed to track tumor size changes at multiple timepoints and tumors were collected for histological validation on day 20. Mean standard uptake value (SUVmean) was calculated as percent of day 0, and statistical analyses were performed with unpaired t-tests and Wilcoxon-rank sum tests. RESULTS/ANTICIPATED RESULTS: Non-responders to treatment had a significantly higher tumor volume compared to responders starting on day 6 ($p<0.05$). Although no significant differences in BLI between control and single-agent therapies were found, BLI data revealed that treatment with combination PTX and anti-PD1 significantly decreased viability signal between days 3 and 6 ($p=0.04$). SUVmean from GZP-PET was over 250% higher in responders compared to non-responders by day 6 ($p=0.03$). SUVmean from FMISO-PET was 80% less in responders compared to nonresponders, indicating less tumor hypoxia ($p=0.04$). DISCUSSION/SIGNIFICANCE OF FINDINGS: Non-invasive PET imaging of the tumor microenvironment can provide data on T cell activation and hypoxic response predicting response to combination immunotherapy and chemotherapy. Utilizing advanced imaging to understand biologically distinct features of the TNBC tumor microenvironment can aid in personalizing anti-cancer therapies.

Team Science

84357

A TL1 Team Approach to Integrating Mathematical and Biological Models to Target Myeloid-Derived Immune Cells in Glioblastoma

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ABSTRACT IMPACT: Predicting therapeutic responses in GBM. OBJECTIVES/GOALS: The goal of this team approach is to integrate mathematical models of glioblastoma (GBM) infiltrating myeloid cells that contribute to the immunosuppressive phenotype in glioma with experimental data to predict therapeutic responses to combined chemokine receptor and immune checkpoint blockade. METHODS/STUDY POPULATION: Orthotopic murine KR158-luc gliomas were established in fluorescent reporter CCR2WT/RFP CX3CR1WT/GFP mice. Subsequently, an anti-CD31 injection was administered to label the vasculature. Fluorescent imaging and quantification of anti-CD3 stained sections were performed on a range of tumor sizes to acquire vasculature, tumor, T cell, and myeloid cell densities. In parallel, a system of ordinary differential equations was formulated based on biological assumptions to evaluate the change over time of tumor cells, T cells, and infiltrating myeloid cells. The model was then refined and validated by experimental results. RESULTS/ANTICIPATED RESULTS: Fluorescent imaging and quantification revealed a correlation between tumor size and abundance of (CX3CR1+, CCR2-) and (CX3CR1+, CCR2+) myeloid cell populations in the tumor microenvironment. The density of these cell populations and vasculature remained constant as the tumors increased in size. Computer simulations of the mathematical model will predict tumor, myeloid, and T cell dynamics. These simulations will be particularly useful to uncover information regarding myeloid cell dynamics, such as cell entry time into the tumor microenvironment. Parameter sensitivity analysis of the model will inform us of the biological processes driving these tumor-immune cell dynamics. DISCUSSION/SIGNIFICANCE OF FINDINGS: GBM is a challenge as current intervention are ineffective. This study improves the understanding of glioma infiltrating myeloid cells and their impact on tumor progression. The data will serve as a basis for quantitatively predicting therapeutic responses of a novel combination treatment.

Translational Science, Policy, & Health Outcomes Science

11791

Gray matter volume differences in bilingual compared to monolingual children

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ABSTRACT IMPACT: This study examines gray matter volume differences resulting from the bilingual experience in children and

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adults allowing us to better understand the brains of over half of the world's population that speaks more than one language. OBJECTIVES/GOALS: Literature is mixed regarding a bilingual advantage in executive control (EC). While it has been shown that young adult bilinguals have greater gray matter volume (GMV) than monolinguals in EC regions, there is behavioral evidence that suggests such difference would be more pronounced in children. METHODS/STUDY POPULATION: Using SPM12 to test this hypothesis, we used a whole-brain t-test to compare GMV in 35 English-speaking monolingual and 20 Spanish-English early (learned both languages before 6 years old) bilingual children. Next, we submitted both groups of children to an ANOVA with 42 English speaking monolingual and 26 Spanish-English bilingual adults to test for an interaction of Language Experience by Age Group at the level of the whole brain. RESULTS/ANTICIPATED RESULTS: e between-group comparison of bilingual and monolingual children, revealed more GMV in bilingual compared to monolingual children in regions associated with EC (right middle and inferior frontal gyri, superior parietal lobule, and precuneus). Our second analysis, an ANOVA comparing bilingual and monolingual children and adults, revealed an interaction in which bilingual>monolingual GMV in children was greater than any bilingual>monolingual GMV (or bilingual=monolingual GMV) in the adult groups in the right superior parietal lobule (BA1). No regions indicated that bilingual>monolingual GMV was more pronounced in adults. DISCUSSION/SIGNIFICANCE OF FINDINGS: These results provide further evidence for GMV differences in early bilinguals in regions associated with EC and indicate that more GMV differences exist between bilingual and monolingual children than adults.

16886

Moyamoya-Like Vasculopathies Observed In a Novel Mouse Surgical Model

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ABSTRACT IMPACT: Development of our animal model of moyamoya will provide a meaningful assessment of therapeutic efficacy of interventions applicable to the clinical setting. OBJECTIVES/GOALS: Moyamoya is a cerebrovascular condition with progressive stenosis of the internal carotid arteries (ICA) and formation of abnormal vascular collaterals at the base of the brain, all of which result in ischemic and hemorrhagic strokes. We aim to develop a needed animal model of this condition in order to develop new therapeutics. METHODS/STUDY POPULATION: Male and female C57Bl/6J mice (4 months old) underwent surgery for the unilateral placement of a microcoil (0.16 mm ID) onto the proximal ICA or sham control. After 30 and 60 days (N = 6-8/time point), the brain blood vessels were examined for changes in diameter, number of anastomoses, and development of new collaterals using DiI stain. Brain tissue was examined for micro-hemorrhages using Prussian blue stain, and cross-sections of blood vessels were examined for intimal thickening using H&E and smooth muscle actin. Expression of vascular endothelial growth factor (VEGF), which is associated with angiogenesis and moyamoya syndrome, was quantified by qPCR. Blood samples were also analyzed for inflammatory biomarkers using ELISA. RESULTS/ANTICIPATED RESULTS: Within 30 days, the distal ICA and anterior cerebral artery (ACA) had significantly decreased diameters at the Circle of Willis, with an initial decrease in the number of cortical anastomoses. Histology demonstrated smaller lumen diameter and alterations to in the various layers of the blood vessels, indicating intimal thickening and stenosis of the affected blood vessels. There was also a significant increase in the number of intracranial micro-bleeds, suggesting a compromised vascular integrity. This may be due, in part, to a significant upregulation in VEGF gene expression within the striatum, a region of hemorrhagic occurrence in moyamoya patients. DISCUSSION/SIGNIFICANCE OF FINDINGS: We report the development of an animal model with vasculopathies that mimic those observed in patients with moyamoya syndrome. With further characterization, this animal model will have a positive impact as a meaningful assessment of therapeutic efficacy of interventions applicable to the clinical setting.

38227

Specific and highly potent human monoclonal antibodies against SARS-CoV-2

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ABSTRACT IMPACT: We devised a new method to produce highly potent SARS-CoV2-specific that can be used to treat severely ill patients with Covid-19. OBJECTIVES/GOALS: Neutralizing antibodies against SARS-CoV-2 are thought to offer the most immediate and effective treatment for those severely afflicted by Covid-19. We devised an approach for rapid and efficient generation of human monoclonal antibodies with neutralizing activity against SARS-CoV-2. METHODS/STUDY POPULATION: SARS-CoV-2 S1 spike protein-specific memory B cells were isolated from 12 subjects recovering from infection with that virus. Paired end single index sequencing was performed using up to 10,000 antigen-specific B cells per subject. Antigen-specific B cell clones were identified by unique diversity and joining gene V(D)J rearrangements and the CDR3 regions. VH and VL regions were cloned and the products expressed in 293T/17 cells to generate spike-specific human monoclonal antibodies. RESULTS/ANTICIPATED RESULTS: Forty-three human monoclonal antibodies were produced. Every monoclonal antibody so generated neutralized viruses pseudotyped with Spike protein of the Wuhan-1 strain. Eighteen monoclonal antibodies neutralized pseudotyped viruses with half-maximal inhibitory concentration (IC50s) between 1 pg/mL and 1 ng/mL (6.7 x 10E-15 M to 6.7 x 10E-12 M), exceeding by 10-100-fold the potency of previously reported anti-SARS-CoV-2-neutralizing monoclonal antibodies. Eight monoclonal antibodies neutralized viruses pseudotyped with mutant spike proteins previously identified in clinical isolates, including receptor binding domain mutants and the C-terminal D614G mutant with IC50<6.7 x10E-12M. DISCUSSION/SIGNIFICANCE OF FINDINGS: We show that SARS-CoV-2 evokes high affinity B cell responses. Some B cells produce antibodies that are broadly neutralizing; others produce strain-specific antibodies. However, antigenic variants that would potentially escape control by immunity or vaccination were nonetheless identified.

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