scRNA-seq on these samples. Following unbiased clustering, we observed and characterized five distinct fetal NK cell subsets in the umbilical cord blood and four fetal NK cell subsets in the corresponding umbilical cord tissue. Our findings revealed that HCMV+ fetal NK cells primarily consisted of mature NK cell subsets, while HCMV- fetal NK cells constituted the majority of the immature subsets. Importantly, we identified a unique subset of NKG2CHi fetal NK cells that were significantly elevated in the HCMV+ fetuses. Finally, we defined a group of transcription factors involved in the formation of antiviral fetal NK. DISCUSSION/SIGNIFICANCE: Here, we demonstrate that HCMV infection can induce the formation of distinct NK cell subsets and drive their unique transcriptional profiles. These findings have the potential to guide the development of an innovative NK cell immunotherapy that could help prevent fetuses from developing symptomatic cCMV.

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BiP knockdown decreases antibody production in malignant and non-malignant plasma cells

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OBJECTIVES/GOALS: Numerous diseases, including AL amyloidosis, are due to expression of aberrant antibodies. Significant effort has gone into plasma cell toxic therapies with varying degrees of success, but no therapies preventing antibody synthesis have been developed. The goal of this study is to assess BiP targeting to prevent antibody secretion in plasma cells. METHODS/STUDY POPULATION: Using 4 multiple myeloma cell lines (KMS11, RPMI8226, ANBL-6, U266), we knocked down BiP expression with RnaseH dependent siRNA or subA toxin, a bacterial toxin that specifically cleaves BiP, and measured changes in unfolded protein and intracellular light chains by flow cytometry during drug induced ER stress created by the intracellular calcium depleting agent thapsigargin. BiP is the master regulator of the unfolded protein response (UPR), an ER stress pathway important for protein folding. BiP is also an ER resident protein folding chaperone important for proper antibody folding. We hypothesized that BiP downregulation will lead to decreased folded antibody in the cell, increased unfolded antibody and constitutive activation of the UPR. RESULTS/ANTICIPATED RESULTS: 1 to 4 hours after treatment with thapsigargin plus siRNA against BiP, levels of BiP are significantly decreased. The levels of intracellular light chains decrease, and the level of unfolded protein within the cells increases dramatically. Interestingly, in alignment with the UPR literature, 24 hours post treatment, these levels have normalized again in surviving cells. SubA treatment increased BiP expression by 4 hours, contrary to our hypothesis, and minimally increased unfolded proteins and minimally decreased intracellular light chains. We expect that further functional testing of antibody secretion by ELIspot assays will show decreased secretion of antibody with BiP siRNA treatment. Combination therapies with other UPR stressing agents may act synergistically to affect antibody production. DISCUSSION/SIGNIFICANCE: BiP knockdown reduces antibodies and boosts unfolded proteins. SubA toxin ineffectiveness likely stems from increased BiP due to feedback loops. Combining anti-BiP treatments with UPR stressing drugs like bortezomib may halt antibody synthesis and induce cell death. These findings support BiP as a viable drug target for antibody-related diseases.

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Unitary neural correlates of self-control in pediatric transdiagnostic psychopathology*†

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OBJECTIVES/GOALS: Childhood psychopathology is a worsening public health crisis leading to negative life outcomes, including self-harm and suicide. Difficulty in self-control as early as 3 years old predicts psychopathology, but the mediating mechanisms of brain function are unknown. Here, we tested one mechanism: functional connectivity (FC) integration. METHODS/STUDY POPULATION: We studied a sample of 204 children [53 F/149 M/2 NC; mean age (SD)=11 years (1.7)] with diverse self-control difficulties (e.g., attention deficit disorder [n=80]; autism spectrum disorders [n=91]). We extracted a general factor of psychopathology ("p-factor") from the parent-reported Child Behavior Checklist. For participants with high quality fMRI data on 3 self-control tasks (n=79), testing flexibility, working memory, and inhibition, we calculated FC connectomes reflecting a general self-control state, and applied connectome predictive modeling (CPM) to reveal connections predicting overall task impairment. We then measured individual variance in cross-network integration of regions with the most predictive connections and tested for association with p-factor in a multiple linear regression. RESULTS/ANTICIPATED RESULTS: We repeated CPM 1,000 times with 10-fold cross validation to generate a distribution of accuracies for predicted vs. observed task impairment scores (mean r=0.25, permutation p=0.02). Connections selected a maximum of 10,000 times (10 folds * 1,000 repetitions) were strongly predictive of task impairment (r=-0.5, p<0.001), highlighting connectivity of canonical executive networks as well as the default mode network. Regions (n=22) with the top 5% most selected connections were in lateral parietal and frontal cortices and implicated motor control. Between-network integration, operationalized with the graph theory metric participation coefficient, of one of these regions in left posterior superior frontal gyrus significantly predicted p-factor ($R2=0.26$, $F(22,56) = 0.87$; $B = -0.49$, $p<0.05$). DISCUSSION/SIGNIFICANCE: A portion of dorsolateral prefrontal cortex, associated with executive control, explained individual variance in p-factor. We plan to test alternative predictive models. Identification of such a neuro behavioral mechanism underlying psychopathology may lead to novel intervention targets.

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Beyond Antibiotics: Monensin and its Derivatives as Promising Anti-Breast Cancer Agents

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OBJECTIVES/GOALS: Although the approval of immune checkpoint inhibitors (ICIs) revolutionized the treatment of metastatic

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breast cancer (BC), yet about 30% remain unresponsive. Since the potency of ICIs depends on the efficient presentation of tumor-specific antigens by cancer cells, compounds which increase such presentation could increase efficacy of ICIs. METHODS/STUDY POPULATION: A library of the ester and urethane derivatives of polyether ionophore antibiotic, monensin (MON) has been synthesized. MTT cell viability assays were performed on the panel of human and mouse BC cell lines, and non-cancerous breast epithelial cells to determine IC50 values of MON and its derivatives. Selectivity Indexes were calculated to identify the most selective compounds towards cancer versus non-cancer cells. Major Histocompatibility Complex (MHC) class I and II presentation and Programmed death-ligand 1 (PD-L1) expression have been determined using flow cytometry. Proteins involved in apoptosis, autophagy and immunogenic cell death were identified through immunoblotting. At least three biological replicates have been performed for each experiment. RESULTS/ANTICIPATED RESULTS: MON and several of its derivatives shown activity in nanomolar range against MDA-MB-231 human BC cell line. MON and its most potent derivatives significantly increased MHC class I and II presentation and downregulated the expression of PD-L1 in BC cell lines. DISCUSSION/SIGNIFICANCE: Present findings will lead to the development of new therapeutic approaches that can serve as single agents or be used in combination with existing ICIs for the treatment of metastatic BC. By pushing the boundaries of our understanding and developing new therapies, this research can make an impact in improving outcomes for patients with metastatic BC.

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The Analysis of N-glycans and Collagen to Predict Prostate Adenocarcinoma Outcome*

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OBJECTIVES/GOALS: Distinguishing indolent from aggressive prostate cancer and early identification of men at risk of developing aggressive, metastatic disease is of great importance. We aim to explore the relationship between N-glycan and collagen composition in prostate tumor tissue and the long-term outcome of the disease. METHODS/STUDY POPULATION: Matrix assisted laser desorption/ionization mass spectrometry can be utilized to characterize N-glycan profiles in formalin fixed paraffin embedded tissues. Collagen may also be characterized using ECM-targeted collagenase MALDI imaging. These approaches were used to analyze prostatectomy samples with different clinical outcomes. Tissue microarrays containing tissues from 75 non-progressors (no evidence of disease; NED) and 50 metastatic cases (MET) were examined. From a combined list of 90 N-glycans and 500 collagenase peptides, the average AUC intensity value for each glycan and collagen peptide was extracted and assessed as a predictor of metastatic progression. Ancestral informative markers were analyzed and polygenic hazard risk scores were generated for samples as well. RESULTS/ANTICIPATED RESULTS: Three N-glycans and three collagen peptides were found to discriminate between NED and MET cases with statistical significance. The best performing N-glycan was Hex6HexNAc6Fuc1 with an AUC of 0.77 ($p<0.001$). While the best performing collagen peptide was COL1A2 with an AUC of C 0.77 ($p<0.001$). DISCUSSION/SIGNIFICANCE: Both a collagen peptide and N-glycan were discovered as promising biomarkers to predict metastasis. Future validation studies are needed to confirm biomarker potential and to determine if the addition of these biomarkers can strengthen current genomic classifier's ability to predict metastatic prostate cancer.

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Pathogenicity of a CCDC6-RET Fusion in Malignant Peripheral Nerve Sheath Tumor (MPNST)

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OBJECTIVES/GOALS: RETgene fusions in sarcoma are rare and their impact on pathogenicity is unknown. Malignant peripheral nerve sheath tumors (MPNST) are a deadly, genomically heterogenous soft tissue sarcoma rarely harboring targetable aberrations. We present a case of a CCDC6-RET fusion MPNST sensitive to RET-inhibitor therapy in a xenograft model. METHODS/STUDY POPULATION: Lung tumor tissue was obtained per an approved collection protocol from a 21yo male patient with a spontaneous MPNST harboring an inactivating mutation in NF-1 and a CCDC6-RET gene fusion detected by a commercially available sequencing panel (Signatera). To confirm pathogenicity of the RET fusion, fresh tumor tissue was engrafted into immunocompromised NSG mice in the anterior and posterior flanks, harvested at ~10 weeks, and re-transplanted into bilateral flanks. When tumor diameters reached 0.5-1cm (~4 weeks), mice were randomized into 3 groups (n=6/group) and treated with either vehicle (V) (PBS), the RET-specific inhibitor selpercatinib (S) (20mg/kg twice daily), or the multi-kinase inhibitor cabozantinib (C) (30mg/kg daily) by oral gavage. Mice were monitored weekly for weight and tumor size. RESULTS/ANTICIPATED RESULTS: 92% (33/36) of implanted tumors were evaluable for treatment response. Pre-treatment tumor volumes (mm3) across all three groups were similar (mean/Std Dev – V: 230/111, S: 271/132, C: 230/123). At day 7, tumor growth was significantly inhibited by S and C versus V (ANOVA $p < 0.001$, post-hoc Tukey's V vs S $p= 0.0178$, V vs C $p< 0.0001$, S vs C $p= 0.0005$). V-treated tumors increased in volume by 60% while S reduced tumor volume by ~80% and C reduced tumor volume by ~20%. S and C treatments were tolerated well. and S improved survival with 100% of mice alive at day 63 vs 0% in V and C groups. 6 of the 12 implanted tumors treated with S, 50% increased in size after ~6-weeks following a >90% initial tumor reduction in tumor volume. Follow-on molecular studies in S-resistant tumors are ongoing. DISCUSSION/SIGNIFICANCE: Targetable genomic changes in MPNST, especially in RET, are infrequent and often considered stochastic. Our findings suggest that precision medicine approaches pairing genomic sequencing and in vivotesting of target gene pathogenicity may guide treatment planning and novel discovery for rare, difficult to treat sarcomas.

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Prototyping a mobile phone application for Chimeric Antigen Receptor (CAR) T-cell therapy patient monitoring and data collection post-discharge

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OBJECTIVES/GOALS: Research objectives include prototyping a mobile phone application that allows physicians to monitor CD19-directed CAR T-cell therapy patients remotely after discharge. This app will also enable standardized data collection across different

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