the structures of distant bacterial homologs. DISCUSSION/SIGNIFICANCE OF IMPACT: We are using these experimentally verified structures and functional data to answer questions about the mechanism of ferroportin iron transport, structural dynamics and the significance of mutations in ferroportin seen in different populations, especially the Q248H mutation found in Africans and black Americans with moderate to high prevalence.

4369 Reprogramming of vascular smooth muscle cells to multipotent progenitor cells contributes to progression of atherosclerosis
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OBJECTIVES/GOALS: Our lab previously identified a population of vascular smooth muscle (SMC)-derived progenitor cells (AdvSca1-SM) which expand robustly in response to disease and can differentiate into multiple cell types. We now aim to define the role of these AdvSca1-SM cells in atherosclerotic plaque progression.

METHODS/STUDY POPULATION: Goal one uses SMC lineage tracing mice and a model of atherosclerosis to track reprogramming of SMCs to AdvSca1-SM cells in the setting of disease. Arteries are analyzed using flow cytometry and immunofluorescence to quantify changes in number of mature SMCs and AdvSca1-SM cells. Goal two uses AdvSca1-SM lineage tracing mice with high cholesterol-induced atherosclerosis and plaque neovascularization. Arteries are analyzed using flow cytometry and immunofluorescence to quantify changes in number of mature SMCs and AdvSca1-SM cells. Goal two uses AdvSca1-SM lineage tracing mice with high cholesterol-induced athero

RESULTS/ANTICIPATED RESULTS: Flow cytometry from SMC lineage tracing mice revealed a 7- to 13-fold expansion of AdvSca1-SM cells in atherosclerotic plaque progression.

4307 Role of Pre-pregnancy Uterine Natural Killer Cells in Human Embryo Implantation
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OBJECTIVES/GOALS: Human placentation requires complex coordination between maternal and fetal cell types but remains incompletely understood. We hypothesize that uterine natural killer (uNK) cells, an immune cell type that increases in abundance during the implantation window, is essential for appropriate implantation and placentation.

METHODS/STUDY POPULATION: We plan to examine stromal cell (SC) decidualization, spiral artery remodeling, and EVT invasion, processes vital for early pregnancy establishment, in the presence or absence of secretory phase uNK cells. Fetal extravillous trophoblasts (EVTs) will be isolated from first trimester pregnancy tissue; maternal SCs, endothelial cells (ECs) and uNK cells will be obtained from secretory phase uterine tissue. SCs will be placed in monoculture and coculture with uNK cells and prolactin will be measured to evaluate decidualization. To study EVT invasion, we will utilize our novel “implantation-on-a-chip” device to determine how addition of uNK cells affects EVT migration through a collagen-matrigel matrix. In this system, we will also examine spiral artery remodeling with or without uNK cells via TUNEL staining.

RESULTS/ANTICIPATED RESULTS: We anticipate that uNK cell addition to SCs will lead to a significant increase in SC prolactin levels, suggesting a role of uNK cells in endometrial decidualization. In vitro, we expect the addition of uNK cells will increase EC apoptosis and promote EVT invasion.

DISCUSSION/SIGNIFICANCE OF IMPACT: Although decidual NK cells are known to participate in placentation, the role of pre-pregnancy uNK cells is unknown. uNK cell involvement in processes important for the earliest stages of pregnancy would provide a potential marker for abnormal placentation and offer avenues for intervention to decrease placenta-associated perinatal morbidity.

4121 The beneficial, anti-fibrotic effects of chemokine receptor 2 and 5 antagonists on fat-exposed mouse primary hepaticstellate cells (pHSCs)
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OBJECTIVES/GOALS: Non-alcoholic steatohepatitis (NASH) is a leading cause of cirrhosis in the world for which no anti-fibrotic therapies exist. We hypothesized that BMS-22 and maraviroc (MVC), chemokine receptor 2 (CCR2) and 5 (CCRS) antagonists, respectively, would diminish the fibrogenic activity of ‘fat-exposed’ murine pHSCs. METHODS/STUDY POPULATION: pHSCs were isolated from livers of 6 week old male mice following 4 weeks on a NASH-inducing choline-deficient high fat diet (CDADFHD, "fat-exposed") or standard diet (SD) and passaged in vitro. Early passage (6-12) pHSCs were plate-adhered and TGF-b-treated (10ng/mL) to maximally activate their pro-fibrogenic genes, collagen 1α1.
Immune Therapeutics, Inc. and MVC, respectively, can selectively dampen the pro-fibrogenic pHSCs are urgently needed. CCR2 and CCR5 antagonists, BMS-22 and TGF-b activated CDAHFD pHSCs had a 2-fold greater, dose-dependent attenuation of their pro-fibrogenic activity in response to BMS-CCR2-22 and MVC, when compared with plate- and TGF-b-activated SD pHSCs, as measured by reductions in collagen 1α1 (Col1A1) and α-smooth muscle actin (ACTA2) gene expression. TIMP1 gene expression was unaffected by drug treatment for 48 hours. Cell viability was not affected up to doses of 30ng/mL of each drug. pHSCs also demonstrated a dose-dependent increase in CCR2, CCR5 and MMP-9 gene expression in response to surface receptor antagonism. Migration assays comparing CDAHFD and SD pHSCs in response to escalating doses of MVC and BMS-22 are ongoing and expected to demonstrate a significantly decreased migratory capacity of CDAHFD pHSCs than SD pHSCs in response to therapy, reflecting the increased susceptibility of the "fat-exposed" pHSCs to anti-fibrotic therapy than normal pHSCs. DISCUSSION/SIGNIFICANCE OF IMPACT: Anti-fibrotic drugs that damp pro-fibrogenic activities of "fat-exposed" pHSCs are urgently needed. CCR2 and CCR5 antagonists, BMS-22 and MVC, respectively, can selectively dampen the pro-fibrogenic response of fat-exposed pHSCs, and must be considered for future trials in human NASH. CONFLICT OF INTEREST DESCRIPTION: Dr. Jill Smith has a patent licensing agreement with Immune Therapeutics, Inc.

The role of creatine in developmental myelination and remyelination

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OBJECTIVES/GOALS: Oligodendrocytes (OL) are glial cells of the central nervous system (CNS) responsible for the energy demanding task of generating myelin sheaths during development and remyelination after demyelinating injury. One metabolite shown to significantly increase ATP production in OL is the nitorgenous organic acid, creatine. Creatine plays an essential role in ATP buffering within tissues with highly fluctuating energy demands such as brain and muscle. Interestingly, mature OL, which are the cells capable of myelin production, are the main cells in the CNS expressing the rate-limiting enzyme for creatine synthesis, guanidinoacetate methyltransferase (Gamt). Patients with mutations in Gamt display intelectual disabilities, impaired myelination and seizures. Therefore, we hypothesize that creatine may be essential for developmental myelination and improve remyelination.

METHODS/STUDY POPULATION: To investigate these hypotheses, we developed a new transgenic mouse model with LoxP sites flanking exons 2-6 of the Gamt gene where excision leads to expression of a green fluorescent tag allowing us to track the cells normally expressing Gamt.

RESULTS/ANTICIPATED RESULTS: In this mouse model, we show a 95% (±0.47%, n = 3) co-localization of Gamt within mature OL during postnatal (P) day P14. Next, we show that knocking out Gamt leads to a significant reduction in OL in the major CNS white matter tract, the corpus callosum, at P14 and P21 (P14: 0.007, n = 3; P21: 0.04, n = 3). Here, we also investigate whether dietary creatine can enhance remyelination in the cuprizone model of toxic demyelination. DISCUSSION/SIGNIFICANCE OF IMPACT: These studies highlight the important role creatine plays in developmental myelination and investigate whether creatine can provide a therapeutic value during a CNS demyelinating insult.

The Utilization of Polyethylene Glycol Fusion to Improve Facial Reanimation

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OBJECTIVES/GOALS: This study’s goal is to determine whether intraoperative treatment of facial nerves with polyethylene glycol (PEG) fusion technology improves facial paralysis outcomes. Improved facial nerve regeneration in facial paralysis patients would lead to improved recovery time and effectiveness. METHODS/STUDY POPULATION: 30 rats were utilized; 15 underwent facial nerve regeneration without PEG fusion, and 15 with PEG fusion. Facial paralysis was initiated on the left by transection of the buccal and marginal mandibular branches of facial nerve. The buccal branch was repaired though microsuture technique. Neurorrhaphy sites of rats in the PEG group were exposed to calcium free saline, methylene blue, and polyethylene glycol. Nerve continuity was assessed post-operative in 5 animals in each group through electron microscopy. Functionality was assessed in the other 10 per group by EMG and whisker analysis after surgery, and weekly for 8 weeks. At 8 weeks, nerves and distal muscles were histologically analyzed. RESULTS/ANTICIPATED RESULTS: PEG fusion technology immediately restored axonal continuity following surgery, demonstrated by electron microscopy. Electrophysiology was also similarly restored across the site immediately, determined through intraoperative nerve stimulation, in the PEG fusion group. The nonintervention group showed dramatically reduced functional recovery than the PEG fusion group following surgery, shown by lower whisking activity and poor electrophysiology outcomes. Furthermore, the PEG fusion group showed statistically significant higher fascicle counts, myelination diameter, axonal diameter, and distal muscle fibers histologically. DISCUSSION/SIGNIFICANCE OF IMPACT: This study demonstrates that polyethylene fusion technology may improve facial reanimation outcomes. PEG is already a FDA-approved drug, and thus the pathway to translational clinical application of this work may thus be streamlined, bringing new options to patients with facial paralysis.

Utilization of swept source optical coherence tomography to optimize characterization of cystoid macular edema in preterm infants

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OBJECTIVES/GOALS: The goal of this study is to evaluate and optimize the characterization of cystoid macular edema (CME) using an investigational swept source (SS)-OCT system. Our knowledge of...