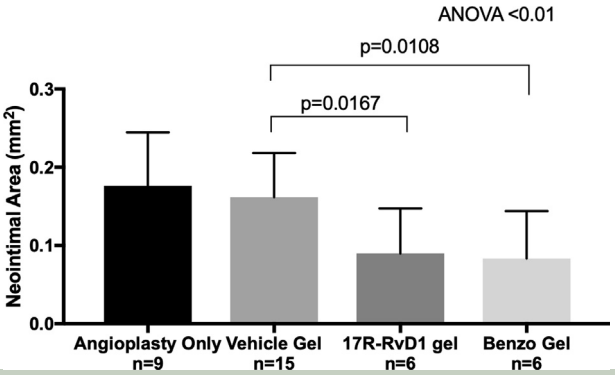

Fig 2.

Author Disclosures: E. C. Werlin: Nothing to disclose; **A. Kim: Employment,** UCSF Department of Surgery; **Research Grant,** NIH R25, NHLBI-VITA; **H. Kagaya:** Nothing to disclose; **M. Chen:** Nothing to disclose; **B. Wu:** Nothing to disclose; **G. Mottola:** Nothing to disclose; **M. R. Spite:** Nothing to disclose; **B. Sansbury:** Nothing to disclose; **M. S. Conte: Research Grant,** Metagenics Inc; **Stock Shareholder,** VasaRx.

Programmed Death Ligand-1 Regulates T Cells and M2 Macrophages to Control Wall Thickening During Arteriovenous Fistula Maturation

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Background: Vascular remodeling during arteriovenous fistula (AVF) maturation is characterized by infiltration of T cells and macrophages. We have previously shown that M2 macrophages play an important role in AVF maturation to reduce inflammation and promote wall thickening. Although T cells can activate macrophages, little is known about T-cell regulation of macrophage function during AVF maturation. Programed death ligand 1 (PD-L1) induces regulatory T cells (Treg) to suppress other T cells and is expressed in endothelial cells. We hypothesized that PD-L1 induces Treg accumulation in the AVF to promote AVF maturation.

Methods: We used the mouse aortocaval AVF model. Intraperitoneal injection of PD-L1 antibody (3 times per week) was used to inhibit PD-L1; control was matched IgG2 isotype antibody. Helper T-cells (Th)-1, Th2, Treg, macrophage accumulation in the AVF was assessed by immunofluorescence with their specific markers. Vascular wall thickening was assessed by elastin van Gieson stain.

Results: Inhibition of PD-L1 significantly increased accumulation of Th1 (15.9 cells/hpf vs 8.6 cells/hpf; $P < .05$) and Th2 (18.0 cells/hpf vs 9.7 cells/hpf; $P < .05$), but decreased Treg (3.8 cells/hpf vs 11.2 cells/hpf; $P < .05$) cells compared with control. PD-L1 significantly inhibited accumulation of TGM2$^+$ (M2) macrophages (5.8 cells/hpf vs 15.7 cells/hpf; $P < .05$) and CD206$^+$ (M2) macrophages (2.6 cells/hpf vs 13.2 cells/hpf; $P < .05$), but not iNOS$^+$ (M1) macrophages (8.8 cells/hpf vs 10.0 cells/hpf; $P = .23$) or tumor necrosis factor-α^+ (M1) macrophages (9.8 cells/hpf vs 12.3 cells/hpf; $P = .29$). There was less wall thickening in AVF treated with PD-L1 antibody compared with control (8.2 μm vs 17.55 μm; $P < .01$) as well as reduced AVF maturation ($P = .03$; n = 16-17; Fig).

Conclusions: Inhibition of PD-L1 is associated with reduced vascular wall thickening as well as less Treg and M2 macrophage accumulation in the maturing AVF. These results suggest that PD-L1 induces Treg cells to promote M2 macrophage accumulation during AVF maturation.

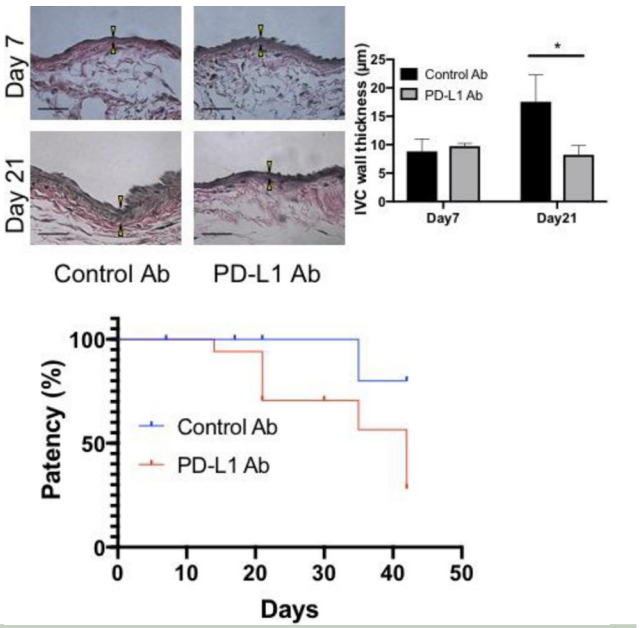

Fig.

Author Disclosures: Y. Matsubara: Nothing to disclose; **R. Taniguchi:** Nothing to disclose; **B. Yatsula:** Nothing to disclose; **A. Dardik:** Nothing to disclose; **L. Gonzalez:** Nothing to disclose; **J. Liu:** Nothing to disclose; **A. Fereydooni:** Nothing to disclose; **J. Langford:** Nothing to disclose; **S. Lee:** Nothing to disclose; **J. Gorecka:** Nothing to disclose; **M. Gao:** Nothing to disclose; **X. Gao:** Nothing to disclose.

TRPC6 Depletion Results in Loss of Myocardin and Phenotypic Modulation in Vascular Smooth Muscle Cells

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Objective: The contractile phenotype of vascular smooth muscle cells (VSMC) is regulated by expression of the master transcription factor, myocardin. Loss of myocardin is observed during VSMC phenotypic switching and within the hyperplastic neointima after vascular intervention. We have shown that depletion of the nonvoltage gated calcium-permeable channel, canonical transient receptor potential 6 (TRPC6) channel, promotes VSMC phenotypic modulation and more severe carotid stenosis following wire injury. The goal of this study was to determine if TRPC6 regulates VSMC phenotype by altering expression of myocardin.

Methods: Myocardin expression was assessed in primary aortic wild-type (WT) and TRPC6$^{-/-}$ VSMC as well as common carotid artery (CCA) explants from WT and TRPC6$^{-/-}$ mice. Acute TRPC6 knockdown was performed in immortalized mouse VSMC (MOVAS cells) using small interfering RNA. Functional alteration of VSMC was assessed in vitro using a wound healing assay.

Results: Myocardin mRNA levels were significantly reduced in primary aortic TRPC6$^{-/-}$ VSMC compared with primary aortic WT VSMC (mean 5.53×10^7 fold reduction; n = 4; $P < .001$). Myocardin protein was also reduced in TRPC6$^{-/-}$ primary aortic VSMC compared with WT, although not to the same degree (mean, 2.03-fold reduction; n = 3; $P = .001$). Myocardin protein was significantly decreased in whole tissue explants of TRPC6$^{-/-}$ CCA versus WT CCA (55.5% reduction; n = 4 mice per genotype; $P = .007$), demonstrating that significant differences in myocardin expression were present in vivo. In MOVAS cells, acute small interfering RNA-mediated TRPC6 knockdown induced a 44.7% decrease in myocardin protein (n = 3; $P = .044$). Wound healing assays showed a significant increase in the number of migrating TRPC6$^{-/-}$ VSMC compared with WT

VSMC in response to growth factor stimulation, supporting our previous findings that TRPC6$^{-/-}$ VSMC are modulated from a contractile to a proliferative phenotype.

Conclusions: TRPC6 depletion is associated with decreased myocardin and the emergence of pathogenic VSMC behavior. TRPC6-dependent signaling may, therefore, be a therapeutic target to promote myocardin expression and stabilize the VSMC contractile phenotype after arterial injury.

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Periadventitial Delivery of Simvastatin from Microparticles Attenuates Arteriovenous Fistula Outflow Vein Neointimal Hyperplasia

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Background: Venous neointimal hyperplasia (VNH) is vexing problem to maintain arteriovenous fistula (AVF) patency in end-stage renal disease patients. The available drug delivery systems to prevent VNH formation are limited. VNH is characterized with increased expression of transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor-A (VEGF-A), and monocyte chemoattractant protein-1 (MCP-1). We sought to determine whether periadventitial delivery of microparticles coated with simvastatin (MP-SIM) could prevent VNH formation via inhibition of gene expression of TGF-β1, VEGF-A, and MCP-1 in a murine AVF model with chronic kidney disease.

Methods: At day −28, 8-week-old C57BL/6J male mice were randomly grouped into control group (MP alone) or MP-SIM group and nephrectomy was used to induce chronic kidney disease. At day 0, an AVF was created. A volume of 20 μL of phosphate-buffered saline with 16.6 mg/mL of either MP or MP-SIM was applied to the periadventitia of the proximal AVF outflow vein at the time of AVF creation. Fistula patency was assessed weekly using Doppler ultrasound examination. Mice were humanely killed at day 3 and 28 for gene expression and immunohistochemistry staining respectively.

Results: At day 3, the gene expression of TGF-β1, VEGF-A, and MCP-1 was significantly decreased in MP-SIM group. At day 28, there was a significant increase in the peak systolic velocity and decrease in the average neointimal area and cell density in MP-SIM group. At day 28, as assessed using immunohistochemistry staining, there was a significant increase in apoptosis and a decrease in the smooth muscle cell, fibroblasts, macrophages, fibrosis, and cellular proliferation in MP-SIM group.

Conclusions: Our study indicates that periadventitial delivery of MP-SIM attenuates VNH at 4 weeks after AVF creation. Further studies using a porcine animal model to confirm these findings are recommended. The potential clinical applicability of controlled release simvastatin to decrease expression of TGF-β1, VEGF-A, and MCP-1 while increasing apoptosis and decreasing cellular proliferation is encouraging.

Author Disclosures: C. Zhao: Nothing to disclose; **S. T. Zuckerman: Ownership Interest,** Affinity Therapeutics; **C. Cai:** Nothing to disclose; **M. L. Simeon:** Nothing to disclose; **A. Singh:** Nothing to disclose; **S. Kilari:** Nothing to disclose; **J. N. Korley: Stock Shareholder,** Affinity Therapeutics; **S. Misra:** Nothing to disclose.

ABSTRACT SESSION II: ATHEROSCLEROSIS AND THE ROLE OF THE IMMUNE SYSTEM

Absence of Cpla2 in Lrp1 Smooth Muscle Cell-Deficient Mice Promotes Severe Aortic Atherosclerotic Disease

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Smooth muscle cell targeted deficiency of low-density lipoprotein (LDL) receptor-related protein 1 (LRP1) in a mouse model (smLRP1$^{-/-}$) results in accelerated aortic atherosclerosis through activation of cytoplasmic phospholipase A2 (cPLA2), leading to reduced ABCA1 expression in vascular smooth muscle cells, and increased intracellular cholesterol accumulation. We therefore hypothesized that deficiency of cPLA2 would impede atherogenesis in the smLRP1$^{-/-}$ mouse model.

Methods: Adult male smLRP1$^{-/-}$;cPLA2$^{-/-}$;LDLR$^{-/-}$ (triple knockout) mice were placed on a high cholesterol diet for 16 weeks and compared with age- and diet-matched sibling control smLRP1$^{+/+}$;cPLA2$^{-/-}$;LDLR$^{-/-}$ mice. Histologic analysis was performed using en face whole aorta Oil red O (ORO) staining, as well as a cross-sectional analysis of the aortic root with ORO, Picro Sirius red, Alizarin red, and immunofluorescence. Immunoblot protein analysis was performed using lysed whole aortas. Data are presented as mean ± standard error of the mean. Statistical analysis was performed using one- and two-way analysis of variance with Tukey's correction.

Results: En face ORO analysis revealed increased lipid accumulation in triple knockout mice as compared with controls (60 ± 3% vs 13 ± 2%; $P <$ .001) (Fig). Uniquely, triple knockout mice develop extensive necrotic cores and thin fibrous caps in atherosclerotic lesions in the aortic root (Fig). ABCA1 is paradoxically increased both in whole aorta lysate as well as in immunofluorescence staining of the aortic root.

Conclusions: Deficiency of cPLA2 in the smLRP1$^{-/-}$ mouse model rescued ABCA1 expression, but unexpectedly increased lipid accumulation within the plaque and generated more vulnerable plaque. Future studies will underpin the mechanisms that guide severe disease development in our triple knockout mice via regulation of ABCA1.

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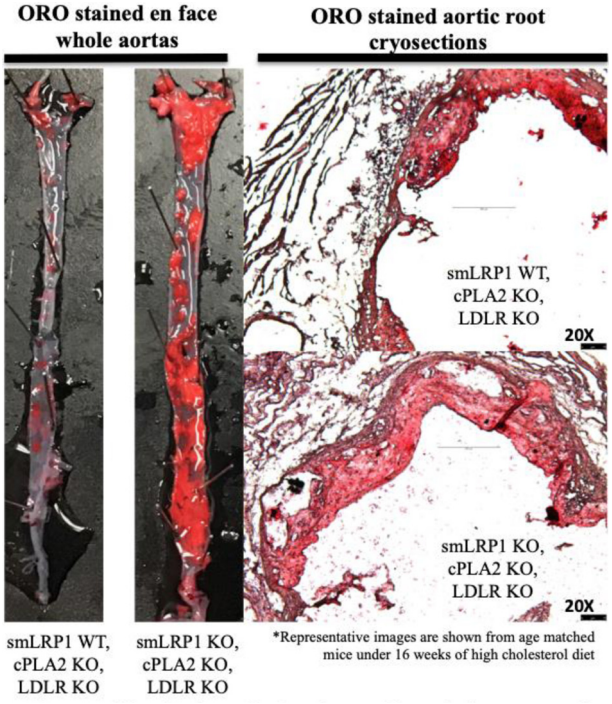

Fig. Histologic analysis using en face whole aorta and aortic root cryosection Oil red O (*ORO*) staining for atherosclerotic lesions.

Author Disclosures: R. I. Mota Alvidrez: Nothing to disclose; **S. Tsai:** Nothing to disclose; **J. Herz:** Nothing to disclose.

Serum Circulating Fatty Acid Synthase as a Diagnostic Biomarker for Chronic Limb-Threatening Ischemia

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Objectives: Circulating fatty acid synthase (cFAS), a de novo lipogenesis enzyme, is elevated in the serum of patients with diabetes mellitus (DM)