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

Chenglei Zhao,[1] Sean T. Zuckerman,[2] Chuanqi Cai,[1] Michael L. Simeon,[1] Avishek Singh,[1] Sreenivasulu Kilari,[1] Julius N. Korley,[2] Sanjay Misra[1]. [1]Mayo Clinic, Rochester, Minn; [2]Affinity Therapeutics, LLC, Cleveland, Ohio

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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

Roberto Ivan Mota Alvidrez, Shirling Tsai, Joachim Herz. University of Texas Southwestern Medical Center, Dallas, Tex

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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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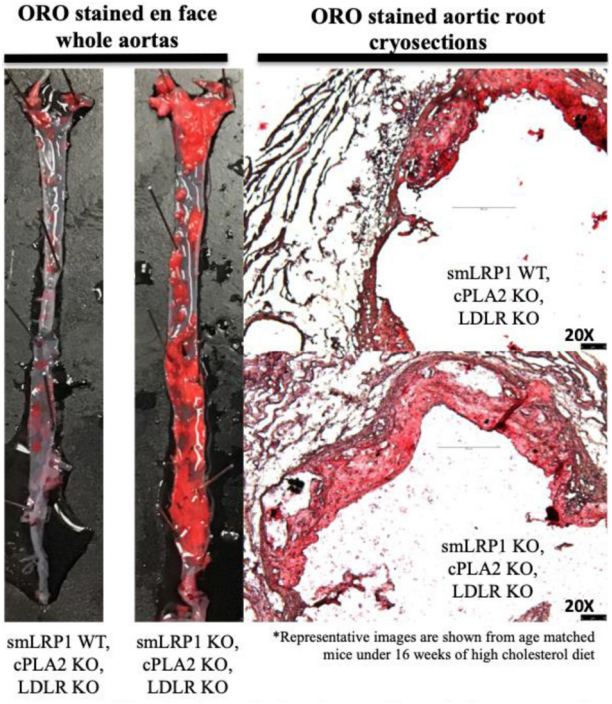

Fig. Histologic analysis using en face whole aorta and aortic root cryosection Oil red O (*ORO*) staining for atherosclerotic lesions.

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

Shirli Tay, Amanda Penrose, Gayan S. De Silva, Yan Yan, Clay F. Semenkovich, Mohamed A. Zayed. Washington University School of Medicine, St. Louis, Mo

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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)

and carotid artery stenosis. However, it is unknown whether cFAS is similarly elevated in patients with DM and advanced peripheral artery disease. This study aims to evaluate whether cFAS content and enzyme activity are biomarkers for clinical severity in patients with DM and chronic limb-threatening ischemia (CLTI).

Methods: Serum samples were prospectively collected from patients undergoing arterial revascularization procedures and maintained in an institutional review board-approved institutional biobank. The cFAS content and enzyme activity were evaluated using colorimetric ELISA assays. Multivariable logistic regression was used to evaluate DM and CLTI outcomes while adjusting for patient clinical characteristics. Hosmer-Lemeshow tests and C-index assessed goodness of fit and classification accuracy. All tests were two sided and a *P* value of less than .05 was considered significant.

Results: A total of 86 patients underwent cFAS analysis (67 content; 63 activity). Mean age was 65.0 ± 8.5 years with 67.4% male, 46.5% had DM, and 47.7% had CLTI. Bivariable analyses demonstrated association of CLTI with cFAS content ($P < .01$), DM ($P = .01$), and insulin use ($P = .01$); DM was associated with body mass index ($P = .001$), CKD ($P = .001$), and current smokers ($P < .05$). On multivariable analysis, CLTI was associated with cFAS content (odds ratio [OR] 1.16; 95% confidence interval [CI], 1.02-1.32; $P = .02$) and closely with DM (OR, 2.72; 95% CI, 0.94-7.89; $P = .066$), whereas DM was associated with body mass index (OR, 1.13; 95% CI, 1.05-1.23; $P < .01$) and CKD (OR, 4.86; 95% CI, 1.72-13.73; $P < .01$). No interactions were observed to be significant. Both models showed good fit ($P > .15$) and classification (Fig).

Conclusions: Serum cFAS content is associated with an increased risk of CLTI but not DM. Each unit increase in cFAS content increases the odds of CLTI by 16%. Future analysis with a larger sample and statistical bootstrapping will determine whether cFAS content is a clinically relevant biomarker of CLTI severity in patients with advanced atherosclerosis.

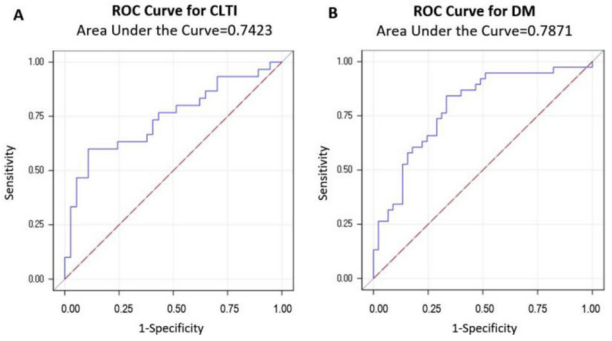

Fig. C-index and area under the receiver operating characteristic curve (AUROC) for crotocal limb-threatinging ischemia (*CLTI*) and diabetes mellitus (DM) multivariable logistic regression models. **A**, CLTI model with cFAS content and DM, AUROC = 0.7423 (95% conifdence interval, 0.6184-0.8663). **B**, DM model with BMI and chronic kidney disease (CKD); AUROC, 0.7871 (95% confidence limit, 0.8869).

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ZEB2 Regulates Activation and Exhaustion Programming of CD8 T Cells in Atherosclerosis

Dawn Fernandez,[1] Nicolas F. Fernandez,[1] Adeeb Rahman,[1] Christopher Hill,[1] Roza Shamailova,[2] Seunghee Kim-Schulze,[1] J. Mocco,[1] Peter Faries,[1] Miriam Merad,[1] Chiara Giannarelli[1]. [1]Mount Sinai, New York, NY; [2]Icahn School of Medicine, New York, NY

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Background: T cells are among the most prevalent immune cells found in human atherosclerotic lesions, yet their role remains obscure. In previous single-cell immunophenotyping studies we found that CD8$^+$ T cells of carotid atherosclerotic plaques display a spectrum of functionally heterogeneous states that vary based on differentiation, activation, and exhaustion. Furthermore, CD8$^+$ T-cell profiles varied between patients without (asymptomatic) and with (symptomatic) recent cardiovascular events (ie, transient ischemic attack and stroke), suggesting that T cells might contribute to adverse outcomes. The transcriptional regulator *ZEB2* has dual roles in T-cell differentiation and cardiovascular disease, as genome-wide association studies have reported *ZEB2* polymorphisms as independent risk alleles for coronary artery disease and myocardial infarction.

Methods: Our independent analysis identified *ZEB2* as a key driver of CD8$^+$ T-cell alterations in atherosclerotic lesions.

Results: We found that *ZEB2* was differentially regulated between patient types, with asymptomatic patients expressing higher *ZEB2* and *GZMB* levels compared with symptomatic (Fig, *A*). *ZEB2^{high}* CD8$^+$ T cells upregulated genes involved in cytotoxic functions, and conversely *ZEB2^{low}* CD8$^+$ T cells upregulated *PD-1* signaling in T-cell exhaustion (Fig, *B*). To probe the mechanistic implications of *ZEB2*, we performed in vitro chronic stimulation assays of human primary CD8$^+$ T cells using depleted of *ZEB2* using CRISPR/CAS9. *ZEB2* knockout cells had reduced cytotoxic function and had a dampened activation state upon acute stimulation.

Conclusions: Persistent stimulation-induced exhaustion in these cells showed that *ZEB2* knockout increased PD-1 expression levels, a protein marker that is critical for T-cell exhaustion (Fig, *C*). Collectively, these experiments suggest that *ZEB2* may contribute to the regulation of T-cell activation and exhaustion states, which is different between clinical phenotypes.

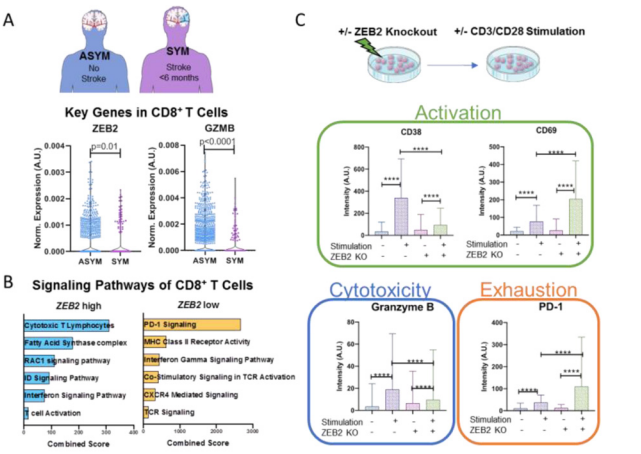

Fig. ZEB2 is a key driver of CD8* T cells in atherosclerosis. **A**, Expression of key genes from CD8* T cells stratified by patient type. Statistics by Welch's *t*-test. **B**, Signaling pathways upregulated in ZEB2^{high} and ZEB2^{low}CD8 T cells from scRnaseq data. **C**, ZEB2 knockout by CRISPR/CAS9 in human PBMCs and evaluated for expression of expression of activation (CD38, CD69), cytotoxicity (granzyme B), and exhaustion (PD-1) markers.

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The Role of Mast Cells in Atherosclerotic Plaque Calcification

Nikolaos-Taxiarchis Skenteris,[1] Malin Kronqvist,[1] Mariette Lengquist,[1] Ulf Hedin,[2] Erik L. Biessen,[3] Ljubica Matic[2]. [1]Karolinska Inst, Solna, Sweden; [2]Karolinska Inst, Stockholm, Sweden; [3]Maastricht University Medical Center, Maastricht, The Netherlands

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Background: Vascular calcification is a key feature of atherosclerosis and has been associated with major adverse cardiovascular events.