images/dish). Mb were treated with the PLK1-inhibitor BI2536 (10 nmol/L; 1 hour) to assess checkpoint responsiveness. Analysis of variance with post hoc analysis confirmed statistical significance ($\alpha = 0.05$).

Results: γH2AX expression was elevated in all PAD groups compared with PAD$^-$ Mb (n = 3-4/PAD group). While PLK1 expression was similar across groups, CDC2 was lower in all ischemic PAD groups relative to perfused PAD Mb. BI2536 attenuated CDC2 expression in perfused PAD Mb ($P = .006$), whereas Mb in ischemic PAD remained unresponsive. AIM2 expression was significantly higher in ischemic Mb compared with perfused and PAD$^-$ Mb, correlating with high γH2AX and low CDC2. Results are summarized in the Figure.

Conclusions: Here, we show that in PAD Mb, AIM2 correlates with DNA damage, attenuated DNA damage checkpoint protein expression, and diminished CDC2 responsiveness to BI2536. Thus, AIM2 may promote pyroptosis in the setting of defective DNA damage mechanisms that would otherwise prompt apoptosis. Whether these pathways are reversed by revascularization is a topic of further study.

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22-VIRC-487-AHA-VD

Modifiable Mesenchymal Stem Cell Defects in Veterans with Diabetes Mellitus

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Background: Diabetes mellitus (DM) affects 34.2 million Americans. DM impairs the body's reparative machinery leading to early onset chronic illness (peripheral arterial disease [PAD], neuropathy, nephropathy, and retinopathy, the latter being hallmarks of microvascular disease). Patients with DM and PAD have increased risk of major amputation. Mesenchymal stem cells (MSCs) are reparative cells found in all tissues providing paracrine and trophic support for new tissue. This study's objective was to identify intracellular and epigenetic mechanisms of how DM impairs MSC function and test if these defects are modifiable with culture rejuvenation.

Methods: MSCs obtained from bone marrow of 13 consecutive male veterans undergoing lower limb major amputation were cultured in 10% fetal bovine serum or 5% human platelet lysate for culture rejuvenation. Groups were DM and PAD (n = 8) and PAD with no DM (n = 5). Intracellular signaling was analyzed with multiplexed enzyme-linked immunosorbent assay. Epigenetic differences were identified by ATAC-sequencing.

Results: DM and PAD MSCs had modifiable AKT signaling defects with platelet lysate (Fig 1) and a discrete DNA profile identified in their introns and intergenic regions (Fig 2). MSCs from PAD alone had increased transcription factor binding at Wnt and cGMP-PKG pathways ($P = .04$). DM and PAD MSCs had increased binding at MAPK ($P = .01$) and Rap1 ($P = .01$) pathways.

Conclusions: DM is a complex disease disrupting reparative mechanisms and can lead to major complications. MSC dysfunction in DM may have common mechanisms throughout the body. We have identified potentially druggable pathways that may provide therapeutic solutions to relieve chronic illness for endogenous MSCs and to expand MSC donor pool for regenerative medicine strategies.

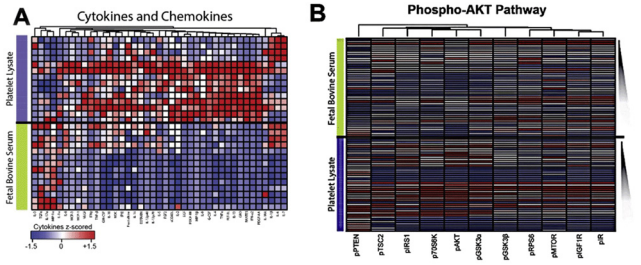

Figure 1. Modifiable Pathways in Diabetic MSCs P. A) Overall, cytokines expression is increased after PL treatment in this panel of 32 cytokines and chemokines. Each row represents a sample, and all columns are z-scored for expression. **B)** Z-scored expression of 11 phosphoprotein in PI3K/AKT at 0, 15, and 30 minutes after fetal bovine serum (FBS) or PL incubation. At 30 minutes, AKT, PTEN and P70SK phosphorylation is significantly increased in PL group compared to FBS.

Fig 1.

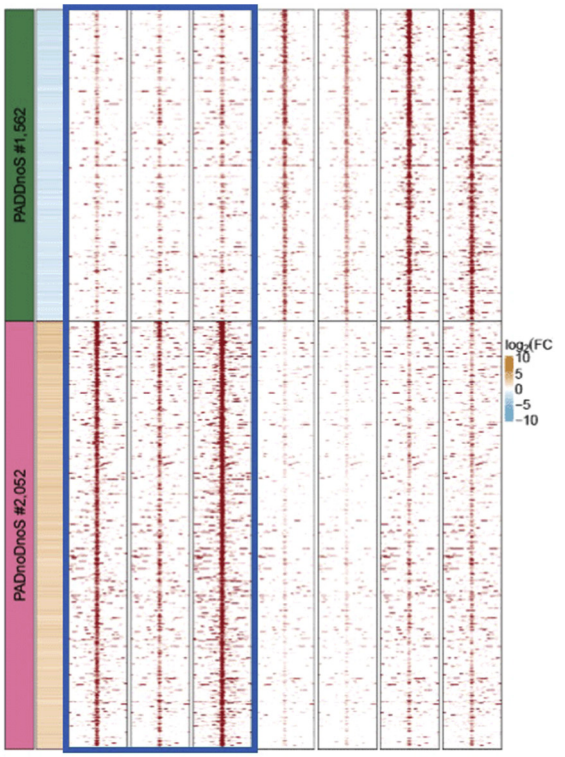

Figure 2. Influence of diabetes mellitus on DNA accessibility.
Comparison of chromatin accessibility determined by ATAC-seq of MSCs from diabetic patients with PAD or PAD alone, which are outlined in the blue box. Differential chromatin accessibility between two conditions was defined as a change of log2(Fold change) >1 and FDR < 0.05. MSCs from diabetic patients with PAD demonstrated 1,562 new gained chromatin accessible sites and 2,052 lost sites, suggesting a dramatic alteration in the binding of transcription factors.

Fig 2.

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22-VIRC-491-AHA-VD

Phosphodiesterase 10A Regulates Medial Artery Calcification

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Background: Vascular calcification results from deposition of calcium hydroxyapatite crystals in the vessel wall. It is highly prevalent in patients with chronic kidney disease (CKD), diabetes, and peripheral artery disease. In lower extremity arteries, elevated calcification levels are associated with an increased risk of ischemic events including amputation. The cyclic nucleotides cyclic adenosine monophosphate and cyclic guanosine monophosphate, controlled by distinct cyclic nucleotide

phosphodiesterase (PDE) isozymes, play important regulatory roles in a variety of human diseases.

Methods: Using a quantitative polymerase chain reaction PDE array, we found that PDE10A was the most highly induced isoform in a rodent model of arterial calcification. PDE10A was also markedly increased in calcifying vascular smooth muscle cells (VSMCs) in vitro, calcified arteries from rodents with CKD, and calcified human tibial arteries.

Results: Knockdown or inhibition of PDE10A markedly attenuated high phosphate-induced VSMC osteogenic transformation and calcification in both rat and human VSMCs. Importantly, deficiency of PDE10A significantly decreased arterial calcification in both ex vivo aortic ring and in vivo vitamin D_3 medial calcification models. Several laboratories, including our own, have previously demonstrated that matrix metallopeptidases (MMPs) are involved in vascular calcification. Using a loss-of-function strategy and bioinformatics analysis, we found that MMP-3 expression was regulated by PDE10A in calcifying VSMCs. Our further mechanistic studies showed that PDE10A could regulate vascular calcification by controlling p38 MAPK signaling and MMP-3 activity through cyclic guanosine monophosphate/PKG signaling.

Conclusions: These findings suggest that PDE10A plays a critical role in the development of medial artery calcification, and that targeting it may provide a novel therapeutic strategy to reduce medial calcification in patients with peripheral artery disease and CKD with the ultimate goal of preventing major limb amputation.

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Role of Cellular Communication Network Factor 2 and Male Sex on Flow-mediated Arterial Remodeling and Atherosclerotic Plaque Formation

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Background: Cellular communication network factor 2 (CCN2) or connective tissue growth factor is upregulated in response to disturbed flow and arterial stiffening. Carotid artery disease and peripheral arterial disease (PAD) are associated with disturbed flow and arterial stiffening. We have identified a key role for CCN2 in stiffened arterial remodeling and flow-mediated focal atherosclerotic plaque formation in translational models of carotid and PAD and in patient carotid plaques and PAD arteries. The objective of this project was to determine the modifiability of CCN2 pathways in pathologic arterial remodeling and atherosclerosis.

Methods: To test our hypothesis that CCN2 was an important mediator of pathology arterial remodeling in vascular disease, we used murine models of stiffened arteries (fibulin-5 knockout [KO]) in atherogenic conditions (PCSK9 + HFD) and clinical arterial tissue from patients with PAD and carotid artery disease.

Results: Disturbed flow increases EC expression of CCN2 3.5 fold in wild-type carotid arteries. This is doubled again in Fibulin 5 KO carotid arteries. These KO carotid arteries are approximately three times stiffer than littermate control arteries, which is a similar ratio of PAD compared with healthy control arteries (Figure, *A*). Under disturbed flow and atherogenic conditions KO arteries had more elastin breaks ($P = .04$), greater plaque area ($P = .007$) and more lipid deposition ($P = .02$), and Glagov's outward remodeling ($P = .02$) (Fibure, *B, C*). This was most pronounced in male mice. CCN2 staining is significantly increased in PAD arteries and carotid endarterectomy plaques ($P < .05$) (Figure, *D, E*). To evaluate the modifiability of CCN2, we used $CCN2^{ECKO}$ animals. We found that male (but not female) $CCN2^{ECKO}$ animals had significantly less atherosclerotic plaque burden ($P = .001$) and similar elastin breaks/lipid deposition compared with littermate controls (Figure, *F, G*).

Conclusions: CCN2 may play an important and modifiable role in flow-mediated pathologic arterial remodeling and focal atherosclerotic plaque burden.

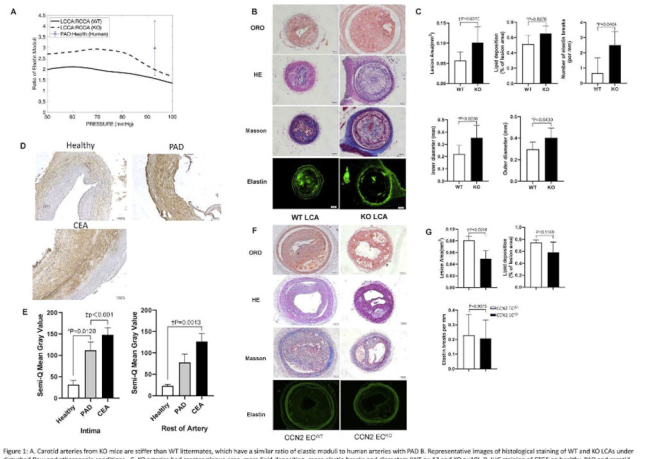

Figure 1: A. Carotid arteries from KO mice are stiffer than WT littermates, which have a similar ratio of elastic moduli to human arteries with PAD B. Representative images of histological staining of WT and KO LCAs under disturbed flow and atherogenic conditions. C. KO arteries had greater plaque area, more lipid deposition, more elastin breaks and diameters (WT n= 12 and KO n=10). D. IHC staining of CTGF on healthy, PAD and carotid endarterectomy plaques. E. CCN2 expression is significantly increased in PAD arteries and carotid endarterectomy plaques. Mean + SD; one-way ANOVA test; Healthy n=5; PAD n=5; CEA n=6. F. Representative images of histological staining of LCAs from vascular endothelial cells specific knockout of CCN2 and littermate control under disturbed flow and atherogenic conditions. G. CCN2ECKO animals had less atherosclerotic plaque burden and similar elastin breaks/lipid deposition compared to littermate controls (CCN2 ECWT n=5; CCN2 ECKO n=5). Mean + SD; unpaired t-test with significance set at P<0.05. *P<0.05; †<.01; ‡<.001 in B and F.

Fig.

Author Disclosures: L. P. Brewster: Expert Witness, two law firms; **F. Li:** Nothing to disclose; **V. Omojola:** Nothing to disclose; **S. Kumar:** Nothing to disclose; **R. L. Gleason:** Nothing to disclose; **H. Jo:** Ownership Interest, FloKines Pharma

22-VIRC-452-AHA-VD

Association of Influenza Pneumonia with Abdominal Aortic Aneurysm Disease

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Objectives: The renin-angiotensin system contributes to the pathogenesis of abdominal aortic aneurysms (AAA). The pulmonary system regulates circulating angiotensin II and 1-7 via angiotensin-converting enzyme and angiotensin-converting enzyme 2 activity. Pulmonary diseases, including chronic obstructive pulmonary disease and sleep apnea are associated with increased AAA risk. This study examined whether a similar association exists with influenza pneumonia (IP).

Methods: Using a cohort discovery tool with institutional review board approval for informed consent waived, electronic health records from Stanford health system were retrospectively queried to identify patients aged 50 years or more with a history of IP or noninfluenza pneumonia (NIP). After controlling for gender, race, and cigarette smoking, AAA prevalence and odds ratios were calculated for patients with IP or NIP as a function of age and diabetes status.

Results: We identified 935 and 6145 AAA patients in the IP (45,110) and NIP (1,993,760) cohorts, respectively. IP was associated with increased AAA prevalence regardless of sex, age, smoking, diabetic status or racial classification (excepting Native Americans) (Table I). After controlling for White race and male sex, AAA prevalence remained higher in IP smoking patients in most age groups regardless of diabetic status. OR for IP-associated AAA risk ranged from 1.48 to 2.31 (Table II). In White male nonsmokers, AAA prevalence was also higher in IP than NIP patients, particularly in nondiabetics (odds ratio, 10.10-228.86) (Table III).

Conclusions: This study suggests that influenza pneumonia is positively associated with AAA disease prevalence in older patients. Additional study is needed to determine whether this association is causal and, ultimately, whether AAA screening and surveillance protocols should be adjusted accordingly.

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