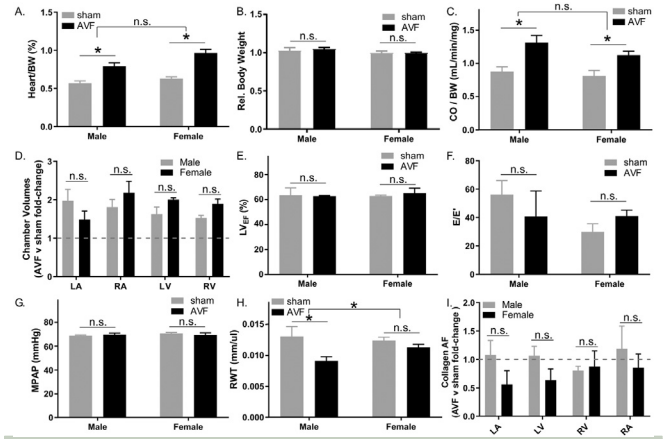


**Fig 1.**

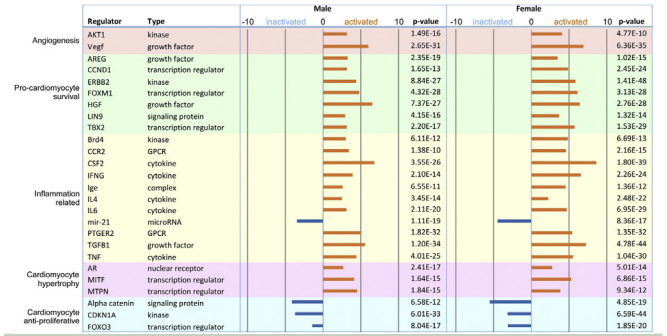


**Fig 2.**

**Author Disclosures: A. Dardik:** Nothing to disclose; **L. A. Gonzalez:** Nothing to disclose; **N. Guerrera:** Nothing to disclose; **J. Langford:** Nothing to disclose; **S. Lee:** Nothing to disclose; **A. Sinusas:** Nothing to disclose; **R. Taniguchi:** Nothing to disclose; **S. Thorn:** Nothing to disclose

## RI08.

## Delivery of Thrombospondin-2 Small Interfering RNA for Suppression of Intimal Hyperplasia

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**Objectives:** Treatments for peripheral arterial disease, which affects over 8.5 million individuals in the United States, include angioplasty/stenting and graft bypass. However, these are limited by the pervasive development of intimal hyperplasia, which leads to in-stent restenosis and reduced graft patency. We propose a novel technique to suppress the progression of intimal hyperplasia using bioengineered hydrogels (ClickGels) for localized small interfering RNA (siRNA) delivery and suppression of thrombospondin-2 (TSP2), an upregulated gene of intimal hyperplasia, in an in vivo rat carotid angioplasty model.

**Methods:** The rat carotid angioplasty model is an established in vivo animal model for intimal hyperplasia. Vessel injury was induced by Fogarty catheter angioplasty of the left common carotid artery in 32 rats, which were equally divided into three control arms (control injury, ClickGel, control siRNA delivery) and one experimental arm (ClickGel delivering TSP2-suppressing siRNA). At postoperative day 21, bilateral common carotid arteries were harvested for evaluation of intimal hyperplasia via intima-to-media ratio calculation, polymerase chain reaction quantification of TSP2 messenger RNA expression, and immunohistochemical analysis of CD31, CD68, and ki-67 expression.

**Results:** Masson's trichrome stain demonstrates an injured left carotid (Fig, *A*) with significant intimal hyperplasia as compared with an uninjured right carotid (Fig, *B*) of the same rat. Intima-to-media ratio is significantly increased (Fig, *C*) in the control injury arm compared with the TSP2 siRNA experimental arm (2.02 ± 0.55 vs 1.24 ± 0.57; $P = .01$). Quantitative real-time polymerase chain reaction demonstrate successful TSP2 messenger RNA expression knockdown (Fig, *D*) in the TSP2 siRNA experimental arm compared with the control injury arm (0.081 ± 0.01 vs 6.32 ± 5.47; $P = .063$).

**Conclusions:** Preliminary in vivo experiments suggest the promising ability of ClickGel delivery of TSP2 siRNA to successfully suppress TSP2 expression and limit the development of intimal hyperplasia in a rat carotid angioplasty model. Further translational research with larger animal models are warranted for continued optimization of this delivery system and eventual aim of clinical trials.

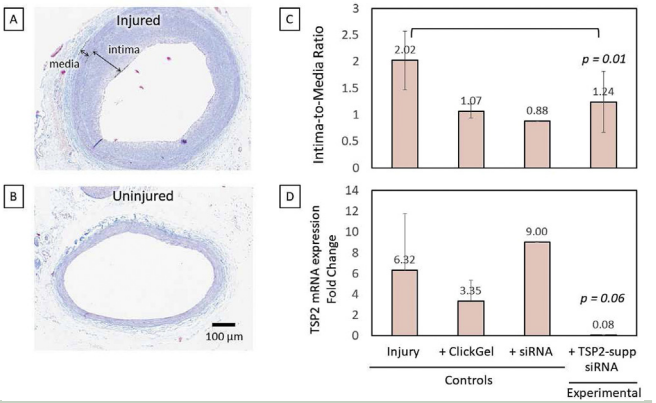


**Fig.**

**Author Disclosures: M. Contreras:** Nothing to disclose; **J. R. Li:** Nothing to disclose; **P. Liang:** Nothing to disclose; **F. LoGerfo:** Nothing to disclose; **D. Mooney:** Nothing to disclose; **K. Piper:** Nothing to disclose; **L. Pradhan-Nabzdyk:** Nothing to disclose; **A. Stafford:** Nothing to disclose; **M. Zhu:** Nothing to disclose

## RI09.

## Investigating the Role of Receptor-interacting Protein Kinase 3 in Venous Thrombosis

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**Objectives:** Venous thromboembolism is a disease that encompasses both deep vein thrombosis (DVT) and pulmonary embolism. Recent investigations have shown that receptor interacting protein kinase 3 (RIPK3), a protein known for its role in the programmed form of cell death necroptosis, may play a role in thrombosis. Specifically, RIPK3 has been shown to promote platelet activation in arterial thrombosis and mixed lineage kinase domain like pseudokinase (MLKL), a protein downstream of RIPK3 in the necroptosis pathway, has been shown to promote neutrophil extracellular trap formation in DVT. This investigation sought to comprehensively investigate the role of RIPK3 in DVT.

**Methods:** The inferior vena cava ligation/stasis model of DVT was used in C57BL/6J, littermate wild type ($Ripk3^{+/+}$), and littermate RIPK3-deficient ($Ripk3^{-/-}$) mice. RIPK3 levels were determined by western blotting and immunostaining.

**Results:** $Ripk3^{-/-}$ mice formed smaller DVTs compared with Ripk3+/+ mice (Fig 1). C57BL/6J mice showed significant increases in thrombus weight from 6 to 24 hours and 24 to 48 hours. RIPK3 progressively