**INTRODUCTION to SB-030**

SB-030 is a novel molecule that binds to the sub-endothelial collagen matrix exposed during vascular intervention or surgical procedures.

It is composed of collagen-binding peptides covalently attached to a heparin backbone.

The collagen-binding peptides are derived from a platelet receptor to collagen and thus directly compete for platelet-collagen binding sites.

The heparin backbone has multiple functions, both providing a hydrophilic barrier and binding growth factors to modulate cellular function.

Unlike heparin, SB-030 lacks anti-coagulation activity.

**PURPOSE**

- Severe vessel occlusion in the lower extremities is treated surgically by peripheral vein graft procedures. However, ~40% peripheral vein grafts fail within the first year.
- Negative remodeling in the vessel walls of the grafts leading to intimal hyperplasia and stenosis is recognized as an important factor for graft failure.
- Vascular smooth muscle cells (SMCs) and fibroblasts present in the vessel walls are key drivers of the negative remodeling process.
- Interestingly, heparin inhibits in vitro proliferation of SMCs and fibroblasts in vein explant cultures, which correlates with good graft outcome in patients.

Therefore, in this study, we evaluated the effect of SB-030 on SMC and fibroblast proliferation in vitro, and also evaluated the effect of ex vivo vein treatment with SB-030 on the outgrowth of fibroblast-like cells from vein walls.

**METHODS**

1. In vitro assessment of SMC and fibroblast proliferation in presence of SB-030.
   - Human coronary artery smooth muscle cells or human lung fibroblasts were seeded and allowed to attach onto fibrillar collagen coated 96-well plates.
   - Cells were treated simultaneously with SB-030 and PDGF-BB, a fibrogenic growth factor.
   - Proliferation was assayed at 48 hours post treatment by staining with Cyquant nucleic acid dye (Thermo Fisher) and reading fluorescence on a plate reader.

2. In vitro assessment of endothelial cell proliferation in presence of SB-030.
   - Human umbilical vein endothelial cells (HUVECs) were seeded and allowed to attach onto fibrillar collagen coated 96-well plates.
   - Once attached, the cells were treated with varying concentrations of SB-030 and proliferation was assayed at 48hrs as above.

3. In vitro assessment of SB-030 binding to PDGF-BB by solid-phase ELISA.
   - Recombinant PDGF-BB protein (R&D Systems) was coated on 96-well plates and subsequently incubated with varying concentrations of biotinylated SB-030.
   - Unbound SB-030 was washed, and plates were incubated with HRP-streptavidin detection reagent. Bound HRP was detected colorimetrically by measuring absorbance at 450nm.

**RESULTS**

1. SB-030 inhibits in vitro proliferation of SMCs and fibroblast.
   - SB-030 at 0.5 mg/mL and 1 mg/mL significantly inhibits PDGF-BB induced proliferation of SMCs and fibroblasts in vitro (p<0.0001) to a greater extent than heparin in this assay.
   - SB-030 also significantly inhibits PDGF-BB independent proliferation of SMCs and fibroblast in vitro (p<0.0001) in this assay.

2. SB-030 does not inhibit in vitro proliferation of endothelial cells.
   - SB-030 does not inhibit the proliferation of HUVECs in this assay.

   - SB-030 and heparin bind to PDGF-BB recombinant protein.

**CONCLUSION**

- SB-030 is a novel matrix regulating molecule that inhibits proliferation of vascular smooth muscle cells and fibroblasts—two key fibrotic cell types involved in the negative remodeling of vein grafts. At the same time, SB-030 does not have inhibitory effects on endothelial cell proliferation.
- SB-030 binds growth factors such as PDGF-BB, which are present in the inflammatory environment during negative vessel remodeling. Binding to PDGF-BB may be one of the important mechanisms by which SB-030 inhibits proliferation of SMCs and fibroblasts.
- A single ex vivo treatment of veins by soaking in SB-030 inhibits outgrowth of fibroblast-like cells from vein walls in vitro. Thus, we propose that soaking veins in SB-030 prior to grafting will have a beneficial anti-fibrotic effect with potential to reduce negative remodeling and stenosis in peripheral vein grafts.
- A clinical trial, SHIELD (NCT00258293), is underway to evaluate SB-030 in peripheral artery disease.

**DECLARATION OF INTEREST**

- This study was sponsored by Symic Bio. All authors are employees and shareholders of Symic Bio.

**ACKNOWLEDGEMENTS**

- The authors thank Andrew Wicolely of Symic Bio for staining and imaging α-SMA in vein explant cultures, and Alyson Matsuo of Sutter Institute of Medical Research for harvesting fresh porcine veins for ex vivo explant studies.