SOCS3 is limited by its short biological half-life. Therefore, mutation of all 9 Lys residues that are potential sites of ubiquitination to Arg should produce a mutated SOCS3 resistant to ubiquitin-mediated proteasomal degradation ("Lys-less" SOCS3). We hypothesise that a stabilised "Lys-less" SOCS3 may have greater therapeutic potential versus wild type (WT) SOCS3 in limiting JAK/STAT mediated processes responsible for neointimal hyperplasia and vein graft failure in type 2 diabetes mellitus (T2DM).

Methods Smooth muscle cells (SMCs) and endothelial cell (ECs) isolated from human saphenous vein (HSV) were transduced with recombinant lentiviruses, MOI = 3.6 (WT), 22.2 (Lys-less SOCS3) and 5.6 (GFP) tu/cell. Successful transduction was confirmed by immunofluorescence and immunoblotting. Ubiquitylation was tested by immunoprecipitation and immunoblotting and half-life was determined by immunoblotting following incubation with protein synthesis inhibitor emetine. HSVSMC proliferation (cell counting and CyQuant proliferation assay) and migration (Boyden chamber assay) were assessed in transduced HSVSMCs. Finally, the effect of WT and Lys-less SOCS3 gene delivery on IL-6 and PDGF-BB signalling in HSVSMCs was assessed by phosphorylation of STAT3 (Y705) and ERK1/2 (Thr202/Tyr204) by immunoblotting.

Results Lentiviral transduction of WT and Lys-less SOCS3 in HSVSMCs and ECs was highly efficient after 48hrs with 97±0.9% (n=4) and was sustained for at least 2 weeks. Lys-less SOCS3 was resistant to ubiquitylation in HSVECs in contrast to WT (n=3). Lys-less SOCS3 was also more stable (t1/2 = 2±4 h) than WT (t1/2=2±4 h) (n=6, p<0.001). Concomitant with a significant reduction in proliferative response to sIL-6R/IL-6 in HSVSMCs treated with WT and Lys-less SOCS3 was a selective inhibition of sIL-6R/IL-6-mediated STAT3 activation by 74±6% and 80±6% respectively (n=5, p<0.001 versus sIL-6R/IL-6 alone) but not ERK1/2. Time course experiments indicated that PDGF-BB-induced STAT3 and not ERK1/2 activation was blocked by WT SOCS3 in HSVSMCs by 59±4% at 5 minutes and 38±1% at 15 minutes (n=3 p<0.05 versus PDGF-BB/GFP). WT and Lys-less SOCS3 did not affect proliferative responses to 20% foetal bovine serum as well as PDGF-BB-induced migration.

Conclusions WT and Lys-less SOCS3 can be successfully transduced into HSVSMCs and ECs with high efficiency using recombinant lentiviruses. Lys-less SOCS3 is more stable than WT yet functionally equivalent in inhibiting HSVSMC proliferation. WT SOCS3 was also capable of inhibiting both IL-6 and PDGF-BB signalling. These results provide evidence for the possible therapeutic targeting of SOCS3 to limit SMC dysfunction responsible for graft failure.

Conflict of interest None