Selective Targeting of Matrix-Associated TGFβ1 is an Attractive Approach for Anti-Fibrotic Therapy

Rohan Manohar, **Justin W. Jackson**, George Coricor, Adam Fogel, Abhishek Datta, Christopher T. Brueckner, Kailyn Looby, Christopher Chapron, Mohammed Qatanani, Gregory J. Carven, Thomas Schurpf

Introduction

- TGFβ inhibition is a promising approach for anti-fibrotic therapy. However, non-selective inhibition of all 3 TGFβ isoforms is associated with safety findings[1, 2, 3]
- Scholar Rock, Inc previously identified an isoform selective TGFβ1 inhibitory antibody with an improved preclinical safety profile compared to non-selective TGFβ inhibition and is associated with on-target immune cell activation[4]. This profile is advantageous for therapeutics in immuno-oncology, but may be less desirable for fibrosis
- Here, we describe the development of a context-dependent isoform selective antibody, LTBP-49247 that inhibits matrix-associated TGFβ1 complexed with LTBP1 and LTBP3 and does not bind TGFβ1 presented by immune cells via GARP or LRRC33. This antibody, LTBP-49247, shows an equivalent reduction in TGFβ signaling (pSMAD2) and histological fibrosis as a context-independent TGFβ1 antibody in preclinical models of renal fibrosis

Figure 1: Latent TGFβ1 is covalently bound to presenting molecules that are found in distinct cellular milieus

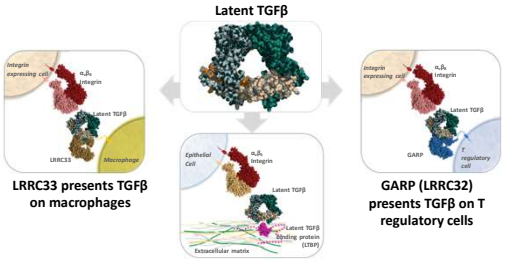
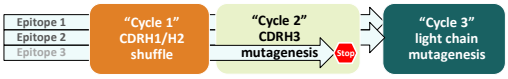


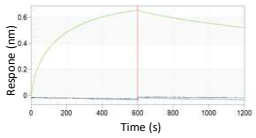
Figure 2. Three cycles of antibody engineering were performed to identify high affinity antibodies to LTBP-TGFβ1



- 3 low-affinity (KD~100 nM) antibodies against TGFβ1 that target non-crossblocking epitopes were selected for affinity maturation
- A yeast display campaign utilizing multiple rounds of positive selections with LTBP-TGFβ1 and negative selections with GARP-TGFβ1 and LTBP presenting molecule alone were performed

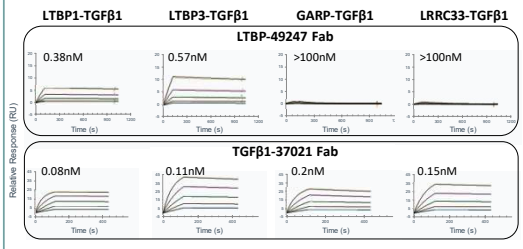
Figure 3: LTBP-49247 is TGFβ1 isoform selective

- LTBP-49247 binds to proTGFβ1 C4S but not to mature growth factors TGFβ1, TGFβ2, or TGFβ3 in an Octet binding assay (shown at right)
- LTBP-49247 binds to LTBP1-TGFβ1 but not LTBP1-TGFβ2 or LTBP1-TGFβ3 (not shown)



- A context-independent antibody TGFβ1-37021 similarly binds only to latent TGFβ1 (not shown)

Figure 4: LTBP-49247 only binds to LTBP-TGFβ1, while TGFβ1-37021 binds to TGFβ1 complexed with all 4 presenting molecules



- LTBP-49247 and TGFβ1-37021 exhibit high affinity to human LTBP-TGFβ1 in a Biacore binding assay (shown at top). The context-independent antibody TGFβ1-37021 also exhibits high affinity to human GARP- and LRCC33-TGFβ1
- Both antibodies are cross-reactive to cyno, mouse, and rat (not shown)

Figure 5: LTBP-49247 is a Potent Inhibitor of LTBP-TGFβ1 activation

- LN229 cells that endogenously express LTBPs and $\alpha_v\beta_8$ integrin that activates TGFβ1, are transiently transfected with human proTGFβ1
- 24 hours after transfection, LN229 cells are treated with test antibodies and co-cultured with a mink lung epithelial cell TGFβ reporter line (Mv1Lu-CAGA12)

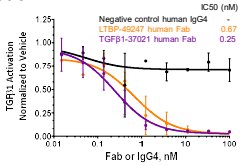
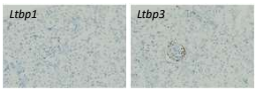


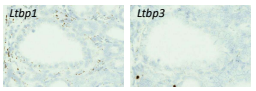
Figure 6: Ltbp expression in kidneys from preclinical models

Col4a3 KO Mice



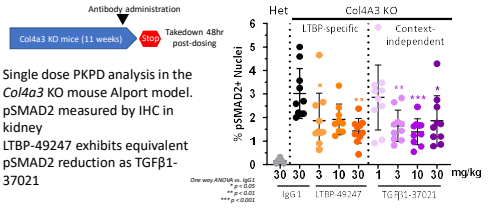
- Low levels of *Ltbp1*, *Lrrc32* and *Lrrc33* transcripts
- *Ltbp3* observed in the fibrotic regions, glomerulus and Bowman's capsule

Adenine fed Rats



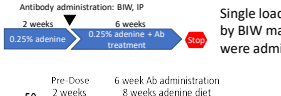
- *Ltbp1* and *Ltbp3* identified in the fibrotic regions of the cortex. *Ltbp1* also found proximal to dilated tubular epithelial cell basement membrane
- *Lrrc32* and *Lrrc33* also detected

Figure 7: LTBP-49247 reduces pSMAD2 to a similar extent as TGFβ1-37021 in Mouse Alport Kidneys

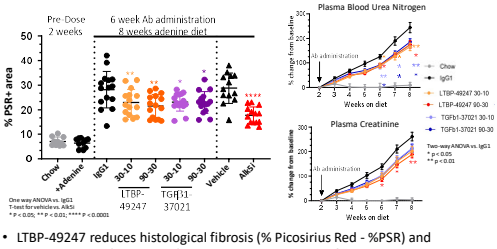


- Single dose PKPD analysis in the *Col4a3* KO mouse Alport model. pSMAD2 measured by IHC in kidney
- LTBP-49247 exhibits equivalent pSMAD2 reduction as TGFβ1-37021

Figure 8: LTBP-49247 exhibits similar antifibrotic efficacy as TGFβ1-37021 in Adenine fed Rat Kidneys



Single loading doses (90 or 30mg/kg) followed by BIW maintenance doses (30 or 10mg/kg) were administered to adenine fed rats



- LTBP-49247 reduces histological fibrosis (% Picosirius Red - %PSR) and hydroxyproline (not shown) to a similar extent as TGFβ1-37021. Reduction in fibrotic gene expression was observed as well (not shown)
- Both antibodies also reduce blood urea nitrogen and creatinine that are circulating markers of kidney damage

Conclusions

- We have identified a highly selective antagonist of matrix-associated LTBP-TGFβ1 . LTBP-49247 exhibits high affinity to LTBP1- and LTBP3-complexed TGFβ1, >100x selectivity vs. GARP- and LRRC33-TGFβ1 and is potent in a TGFβ1 activation assay
- LTBP-49247 exhibited a similar reduction of pSMAD2 and histological fibrosis *in vivo*, in the *Col4a3* KO model of Alport syndrome and an adenine-fed rat model of CKD, respectively. A longer-term multiple dose study is being run in *Col4a3* KO mice to determine if inhibition of matrix-associated LTBP-TGFβ1 is sufficient to reduce fibrosis
- Data in preclinical models of fibrosis along with a dose-ranging 13-week non-GLP safety study suggests that LTBP-49247 may offer a safety profile better suited to treating chronic fibrotic indications in which immune cell activation is not desirable

References

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