Discovery of an isoform-specific inhibitor of TGFβ1 activation using antibody display technology

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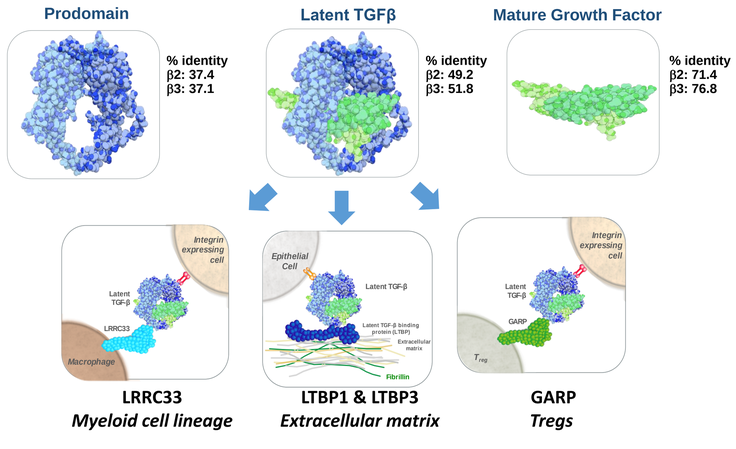
Abstract

TGFβ1 dysregulation has been implicated in the pathogenesis of a variety of diseases including fibrosis, cancer and immune dysregulation, but its tractability as a drug target has proven challenging due to toxicities and lack of selectivity over closely related family members. The TGFβ1 pro-protein is proteolytically processed into a small latent complex (SLC) consisting of the mature growth factor and the latency-associated protein, which acts as an inhibitor of receptor binding. The SLC is further assembled into a Large Latent Complex (LLC) via disulfide linkage with either matrix-associated Latent TGFβ Binding Protein (LTBP) or cell type-specific transmembrane proteins GARP and LRRC33. LLCs tether latent TGFβ1 to cell surfaces or the matrix until appropriate activation events, such as integrin binding, induce release of the active growth factor from the complex. The LLC provides an opportunity to develop antibodies that can bind to the complex with exquisite selectivity and modulate TGFβ1 activation in a tissue or disease-targeted manner with a potentially improved safety profile. We have successfully generated recombinant TGFβ1 LLC complexes and employed both phage and yeast display technologies for antibody discovery. By targeting the prodomain of TGFβ1, which shares very low sequence identity to prodomains of TGFβ2 and TGFβ3, we identified SR-AB1, a fully human monoclonal antibody that binds proTGFβ1 with exquisite selectivity. In addition, SR-AB1 is capable of binding and inhibiting the integrin mediated activation of all TGFβ1 LLC tested *in vitro*. We have also shown SR-AB1 inhibits endogenous TGFβ1 in a number of primary cells *in vitro*, including dermal myofibroblasts and Treg activity *in vitro*. In addition, we tested the *in vivo* efficacy of TGFβ1 inhibition via this novel mechanism in multiple preclinical models of tissue fibrosis. We find that SR-AB1 suppresses the induction of profibrotic genes, p-SMAD signaling and tissue fibrosis to levels similar to those achieved in pan-TGFβ antibody-treated animals. Taken together, our data show that inhibition of latent TGFβ1 activation is efficacious in preclinical mouse models of fibrosis and has a potentially improved safety profile as compared to pan-TGFβ inhibition.

Introduction



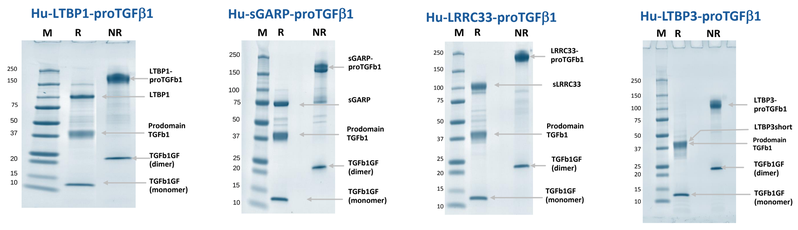
- TGFβ (TGFβ1, TGFβ2, and TGFβ3) is expressed as a pro-protein that is proteolytically cleaved intracellularly into an N-terminal prodomain and a C-terminal growth factor.
- The covalent association of the growth factor (GF) with the prodomain prevents GF from receptor binding and signaling.
- proTGFβ1 is covalently associated with presenting molecules through disulfide bonds.
- Presenting molecules provide an anchor for integrins to exert traction force on latent TGFβ1, releasing the active growth factor.
- To date, four TGFβ1-presenting molecules have been identified:
  - Latent TGFβ Binding Proteins 1 & 3 (LTBP1 and LTBP3) - fibrillin-like proteins that link latent TGFβ1 to the ECM.
  - Glycoprotein-A Repetitions Predominant (GARP) & Leucine-Rich Repeat-Containing Protein 33 (LRRC33) - transmembrane proteins that present latent TGFβ1 on the surface of activated regulatory T cells (Tregs) and myeloid cells, respectively.
- Dysregulated TGFβ1 activation has been associated with a number of pathologies, including fibrotic diseases
- Chronic TGFβ1 activation drives myofibroblast transdifferentiation and overexpression of extracellular matrix proteins.

Therapeutic approaches and challenges

- Multiple therapeutic approaches have been tried including antibodies or soluble ligand traps that bind and block the TGFβ growth factors, or small molecular inhibitors of the TGFβ receptor kinase ALK5.
- pan-TGFβ inhibition was found to cause dose-limiting heart valvulopathies, leading to concerns about toxicity of this therapeutic approach.
- Therapeutic approaches targeting TGFβ1 specifically has been challenging because of the high homology between the three TGFβ growth factor isoforms

Results

Fig. 1: Generation of recombinant TGFβ1 Large Latent Complexes to enable antibody discovery & characterization



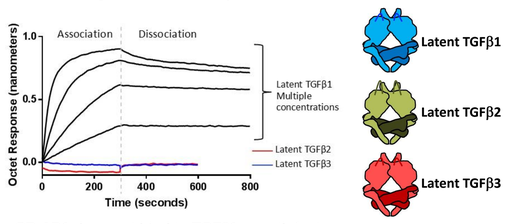
- ~40 distinct proteins TGFβ proteins expressed to support antibody discovery & characterization
- TGFβ1 Large latent complexes, small latent complexes & presenting molecules of multiple species have been expressed and purified from HEK293 cells

Antibody display campaign to identify highly selective binders

Proprietary positive and negative selection strategies utilized to identify highly selective mAbs	**Multi-arm yeast display campaign** Synthetic library based on human repertoire Realized display diversity >10^{10} Fully human Fab & IgG based

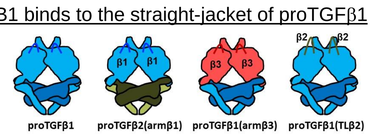
Fig. 2: SR-AB1 selectively binds prodomain of latent TGFβ1

A. ForteBio octet measurement of SR-AB1 binding to latent TGFβ isoforms



- **SR-AB1 does not bind to TGFβ1 growth factor**

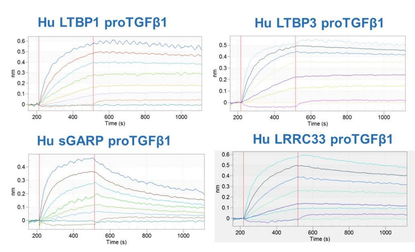
B. SR-AB1 binds to the straight-jacket of proTGFβ1



Octet binding to SR-AB1	+++	-	+	+++

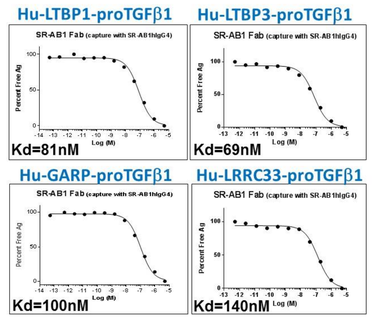
Fig. 3: SR-AB1 binds to all TGFβ1 Large Latent Complexes (LLC) and has good developability properties

A. ForteBio octet measurement of SR-AB1 binding affinity to TGFβ1 LLC



- **SR-AB1 does not bind presenting molecules alone**

B. Equilibrium affinity measurement of SR-AB1 Fab affinity to TGFβ1 LLC using MSD-SET assay



- A range of conc. Of SR-AB1 Fab is incubated in solution with 25pM (for LTBP1- & LRRC33-proTGFβ1) or 50pM (for LTBP3- & GARP-proTGFβ1) of biotinylated antigen for 20-22hr
- Free antigen is captured with 20nM of SR-AB1hIgG coated on a MSD plate.
- Captured biotinylated antigen was detected using labeled SA antibody

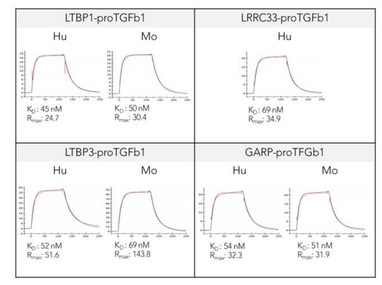
Table 1: SR-AB1IgG affinity against TGFβ1 LLC

TGFβ1 Large Latent Complex	KD (nM)
Hu LTBP1 proTGFβ1	0.40
Mu LTBP1 proTGFβ1	0.50
Cy LTBP1 proTGFβ1	0.64
Ra LTBP1 proTGFβ1	0.71
Hu LTBP3 proTGFβ1	0.14
Mu LTBP3 proTGFβ1	0.58
Cy LTBP3 proTGFβ1	0.22
Hu Garp proTGFβ1	2.62
Mu Garp proTGFβ1	2.50
Cy Garp proTGFβ1	1.55
Hu LRRC33 proTGFβ1	1.08
Mu LRRC33 proTGFβ1	0.65

- SR-AB1hIgG4 (25nM) was immobilized AHC sensor tip
- TGFβ1 complexes were presented from 200 nM (rat and cyno antigens) or 50 nM (hu- and mu- antigens) in kinetics buffer. 6 concentrations were tested with a series of 2 fold dilutions.
- Global KD was determined from all traces with a calculated dissociation rate and a response greater than 0.1 nM

C. SR-AB1 Fab binding affinity to TGFβ1 LLC using Biacore

b-antigen on SA Chip, Fab in solution (100 nM)



Antigen RU Capture Levels:	
Hu LTBP1-proTGFb1	238
Mo LTBP1-proTGFb1	341
Hu LTBP3-proTGFb1	326
Mo LTBP3-proTGFb1	1136
Hu GARP-proTFGb1	398
Mo GARP-proTFGb1	406
Hu LRRC33-proTGFb1	500

D. SR-AB1 shows good developability properties

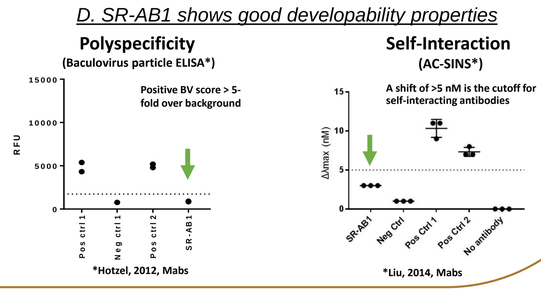
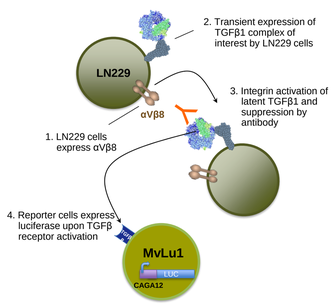
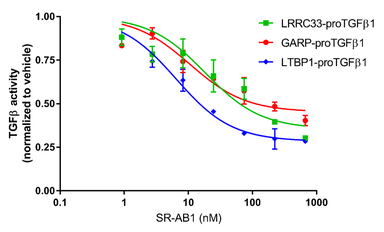


Fig 4: SR-AB1 is an inhibitor of latent TGFβ1 activation irrespective of presenting molecule

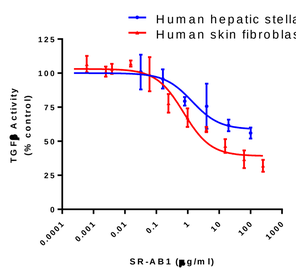
A. *Schematic of LN229 assay*



B. SR-AB1 inhibits activation of TGFβ1 irrespective of presenting molecule



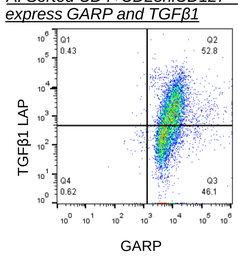
C. SR-AB1 inhibits activation of TGFβ1 endogenously presented by human cells



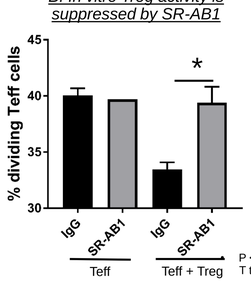
- Inhibitory activity of SR-AB1 was tested on overexpressed TGFβ1 complexes (Fig. 4A)
  - LN229 glioblastoma cells express αVβ8
  - Transfected LN229 cells express latent LTBP1-proTGFβ1, GARP-proTGFβ1, or LRRC33-proTGFβ1
  - Latent TGFβ1 complexes activated through αVβ8
  - TGFβ1 activity detected by reporter cells
- SR-AB1 inhibits all latent TGFβ1 complexes tested (Fig. 4B)
  - SR-AB1 is a context-independent inhibitor of TGFβ1 activation
- SR-AB1 inhibits endogenously presented latent TGFβ1 by human hepatic stellate cells and skin fibroblasts (Fig. 4C)
  - Hepatic stellate cells or fibroblasts were co-cultured with SW480 cells over expressing αVβ6 integrin which activated the latent TGFβ1
  - TGFβ1 activity is measured using a luciferase reporter cell

Fig. 5 : SR-AB1 blocks regulatory T cell (Treg) function *in vitro*

A. Sorted CD4+CD25hiCD127- Tregs express GARP and TGFβ1

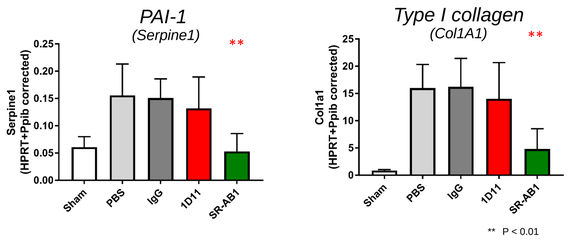


B. In vitro Treg activity is suppressed by SR-AB1



- SR-AB1 suppresses Treg function *in vitro*
  - Activated human Tregs express GARP and TGFβ1 prodomain (LAP) on their surface (Fig. 5A)
  - Division of activated human effector T cells (Teff) is suppressed by autologous Tregs (Fig. 5B)
  - SR-AB1 inhibits suppression by Tregs, but not division of Teff cells alone (Fig. 5B)

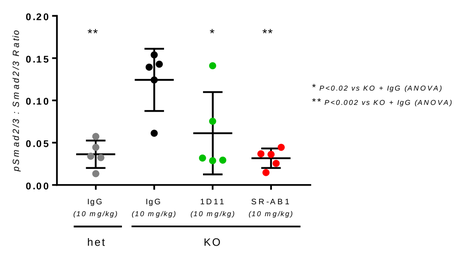
Fig. 6 : SR-AB1 suppresses profibrotic gene expression in UUO model of kidney fibrosis



- SR-AB1 (30 mg/kg) inhibits activation of latent TGFβ1 in the kidney
  - Male CD-1 mice underwent sham or unilateral ureteral occlusion (UUO) surgery
  - Injured kidneys were collected 5 days post surgery and mRNA levels analyzed by multiplexed Quantigene RNA assays
  - Dosing of mice with SR-AB1 suppressed expression of TGFβ-responsive and profibrotic genes
- Specific inhibition of TGFβ1 was at least as efficacious as pan-TGFβ inhibition with 1D11

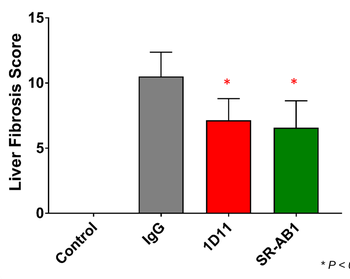
Fig. 7: SR-AB1 inhibits TGFβ1 activation in progressive kidney fibrosis model

Alport Syndrome model (COL4A3 KO)



- 6 weeks old F1 pups from 129+/- X B6+/- cross were dosed with antibody @5mg/kg 2X week
- Mice were sacrificed at 6wks following start of dosing
- Kidney tissues were used to measure pSmad2/3:Smad2/3 ratios using ELISA assay

Fig. 8: TGFβ1 inhibition with SR-AB1 ameliorates CCl4-induced liver fibrosis



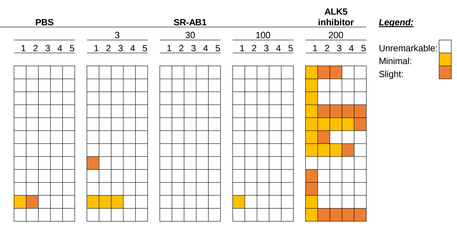
- Treatment of mice with SR-AB1 (30 mg/kg) reduced fibrosis, confirming suppressive activity of SR-AB1 in liver.
  - Liver fibrosis was induced in BALB/c mice with CCl4.
  - Therapeutic dosing with antibodies was initiated after two weeks and continued for four weeks.
  - Liver pathology was assessed by histology on picrosirius red-stained liver slices, and extent of fibrosis was scored.
- Similar to kidney, specific inhibition of TGFβ1 isoform was as effective as pan-TGFβ inhibition with 1D11

Conclusions

- Successfully generated purified TGFβ1 large latent complexes which have enabled the discovery of a TGFβ1 specific antibody SR-AB1 using yeast display technology. Isoform specificity achieved by targeting the prodomain of TGFβ1.
- SR-AB1 binds all proTGFβ1 large latent complexes and is a potent inhibitor of integrin mediated activation of latent TGFβ1 both *in vitro* and *in vivo*.
- Inhibition of TGFβ1 with SR-AB1 in preclinical mouse models of kidney and liver fibrosis is at least as effective as pan-TGFβ inhibition.
- Specific inhibition of TGFβ1 avoids cardiac toxicity such as valvulopathies associated with pan-TGFβ inhibition.

Fig. 9: No cardiac toxicity observed with SR-AB1 up to 100 mg/kg in 4-week rat study

Cardiac pathology (microscopic findings)



- TGFβ1-specific inhibitor SR-AB1 tested at three dose levels in 4-week rat toxicology study
- No treatment-related cardiac toxicity of SR-AB1 up to 100 mg/kg per week, the highest dose tested
- No toxicology findings with SR-AB1 in comprehensive list of tissues and organs
- Dosing of ALK5 inhibitor for 5 days recapitulated published cardiac valve toxicity of pan-TGFβ inhibition
- Efforts underway to determine minimum efficacious dose of SR-AB1 *in vivo*