A novel, highly specific TGFβ1 inhibiting antibody demonstrates antifibrotic activity without cardiotoxicity

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Abstract

Background: Transforming growth factor-β1 (TGFβ1) has diverse biological functions, including regulation of immune response and tissue homeostasis. TGFβ1 activation has been associated with diseases including kidney fibrosis, where chronic activation is a key driver. Because of high homology between the TGFβ1 growth factor and its close relatives TGFβ2 and TGFβ3, truly TGFβ1-specific inhibitors have remained elusive. Pan-TGFβ inhibition, on the other hand, can cause dose-limiting heart valvulopathies, leading to concerns with long-term dosing. TGFβs are expressed as pro-proteins that are proteolytically cleaved into a C-terminal growth factor and an N-terminal prodomain that remains noncovalently associated with the growth factor, preventing receptor binding. This latent TGFβ complex resides on cells or in the extracellular matrix until it is activated by integrins, freeing the growth factor and allowing receptor binding.

Methods: To identify TGFβ1-specific antibodies, we targeted the prodomain, which shares much lower homology to TGFβ2 and TGFβ3 than the growth factor.

Results: We identified SR-AB1, a monoclonal antibody that binds latent TGFβ1 with no detectable binding to latent TGFβ2 or TGFβ3. SR-AB1 blocks latent TGFβ1 activation by αVβ6 or αVβ8 integrins, providing specificity unachieved by biologics that target the TGFβ1 growth factor/receptor interaction. SR-AB1 further inhibits latent TGFβ1 complexed with all four known TGFβ-presenting molecules, allowing targeting of TGFβ1 in multiple tissues. SR-AB1 blocks activation of endogenous TGFβ1 in a number of primary cells, including dermal myofibroblasts and hepatic stellate cells. Critically, while pan-TGFβ inhibitors show evidence of valvulopathy or other cardiotoxicity, SR-AB1 is free of such toxicities in 1 and 4 week rat studies. We further tested the *in vivo* efficacy of TGFβ1 inhibition via this novel mechanism in models of kidney and liver fibrosis, showing that SR-AB1 suppresses fibrosis to levels similar to those achieved by pan-TGFβ inhibition.

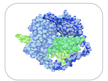
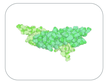
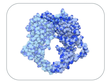
Conclusions: Our data show that isoform-specific inhibition of latent TGFβ1 is efficacious in a preclinical fibrosis model and has a superior safety profile compared to pan-TGFβ inhibition.

Background

TGFβ structure

- The **three TGFβ isoforms**, TGFβ1, TGFβ2, and TGFβ3, are expressed as pro-proteins that are cleaved before secretion into an N-terminal prodomain and a C-terminal growth factor.
- The growth factor remains noncovalently associated with the prodomain, forming a **latent complex** that cannot bind receptor to induce signaling (see Panel A, below)
  - Growth factor release from prodomain is catalyzed by αV Integrins
- The three TGFβ growth factors share a high degree of sequence identity across the three isoforms (Panel A, below), which allows them to signal through the same receptor complex. TGFβ prodomains, however, are much less conserved.

A

Latent TGFβ1	Mature TGFβ1 Growth Factor	TGFβ1 Prodomain
Prodomain prevents receptor binding	*Binds TGFβ receptor*	*Growth factor release catalyzed by Integrins*
		
Secreted form, resides on cell surface & ECM	*Domain identity to:* ***TGFβ2****: 71.4%* ***TGFβ3****: 76.8%*	*Domain identity to:* ***TGFβ2****: 37.4%* ***TGFβ3****: 37.1%*

B **Clinical-stage Antibodies Targeting Mature TGFβ1 are not Isoform Specific**

	TGFβ1	TGFβ2	TGFβ3
CTR-AB1	< 1 pM	No binding	15.2 nM
CTR-AB2	< 1 pM	23.4 nM	5.6 nM
CTR-AB3	< 1 pM	< 1 pM	18.7 nM
CTR-AB4	< 1 pM	< 1 pM	< 1 pM
CTR-AB5	< 1 pM	1.7 nM	< 1 pM
1D11	< 1 pM	2.8 nM	< 1 pM

Biological functions of TGFβ

- As demonstrated by the distinct phenotypes of TGFβ knockout mice, the three TGFβ isoforms have non-redundant biological functions.
- 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 TGFβ inhibition

- Approaches include antibodies or soluble ligand traps that bind and block the TGFβ growth factors and small molecular inhibitors of the downstream TGFβ receptor kinase ALK5.
- pan-TGFβ inhibition has been reported to cause dose-limiting heart valvulopathies, leading to concerns about toxicity of this therapeutic approach *(Anderton et al., Tox. Path. 2011; Stauber et al., J. Nonclin. Tox., 2014).*
  - Genetic and expression data suggest role for TGFβ2 and/or β3 isoforms in cardiotoxicity *(Doetschman et al., Cell Tissue Res., 2012; Bertoli-Avella et al., J. Am. Coll. Cardiol., 2015)*
- Specifically targeting the TGFβ1 isoform has been challenging because of the high homology between the three TGFβ growth factors (see Panel B, above).

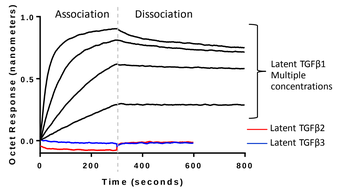
Hypothesis

We hypothesized that inhibitors targeting the much less conserved TGFβ1 prodomain could inhibit growth factor release. This approach would achieve TGFβ1 isoform specificity, potentially providing a superior safety profile compared to pan-TGFβ inhibition.

Results

Figure 1: SR-AB1 specifically binds prodomain of latent TGFβ1

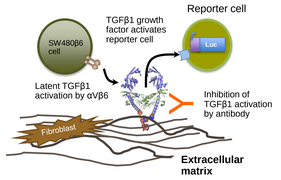
Bio-Layer Interferometry (Octet)



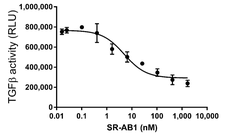
- Human monoclonal antibody SR-AB1 discovered by yeast display
- Specifically binds latent TGFβ1, without detectable binding to latent TGFβ2 or TGFβ3 (Fig. 1)
  - No binding of TGFβ1 growth factor
  - Antibody epitope maps to straitjacket of TGFβ1 prodomain
  - Cross-reacts with mouse, rat, & cynomolgus monkey TGFβ1

Figure 2: SR-AB1 inhibits activation of human fibroblast-expressed latent TGFβ1

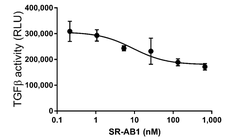
A *Schematic of fibroblast co-culture assays*



B *SR-AB1 inhibits TGFβ in human dermal fibroblasts*



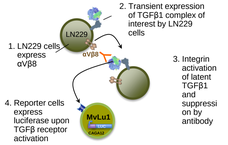
C *SR-AB1 inhibits TGFβ1 in human hepatic stellate cells*



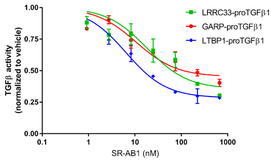
- Co-culture of human fibroblasts with αVβ6-expressing cells activates endogenous fibroblast TGFβ (Fig. 2A)
- SR-AB1 blocks αVβ6-dependent activation of latent TGFβ1 produced by dermal fibroblasts or activated hepatic stellate cells (Fig. 2B,C)

Figure 3: SR-AB1-mediated latent TGFβ1 inhibition is not dependent on the presenting molecule

A *Schematic of LN229 assay*



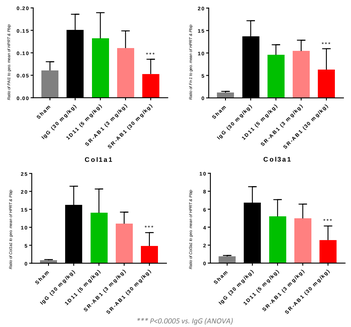
B *SR-AB1 inhibits activation of TGFβ1 irrespective of presenting molecule*



- Latent TGFβ1 is held in extracellular space by presenting molecules on the cell surface (GARP, LRRC33) and in ECM (LTBP1)
- SR-AB1 inhibition tested against specific TGFβ1/presenting molecule complexes (Fig. 3A)
  - Transfected LN229 cells express latent LTBP1-proTGFβ1, GARP-proTGFβ1, or LRRC33-proTGFβ1
  - Latent TGFβ1 complexes activated through αVβ8 (endogenously expressed in LN229 cells)
  - TGFβ1 activity detected by reporter cells
- SR-AB1 inhibits all latent TGFβ1 complexes tested (Fig. 3B)
  - SR-AB1 is a context-independent inhibitor of TGFβ1 activation

Figure 4: SR-AB1 suppresses profibrotic gene expression in a mouse UUO model of kidney fibrosis

Ab dosing started 1 day prior to UUO, Kidneys harvested 5 days after surgery

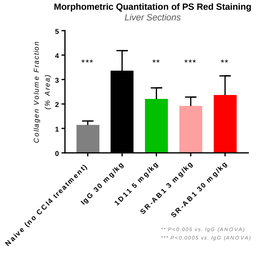


*** P<0.0005 vs. IgG (ANOVA)

- Gene expression measured by Quantigene branched DNA assay
- SR-AB1 suppressed expression of TGFβ-responsive and profibrotic genes
- Specific inhibition of TGFβ1 is sufficient to block UUO-induced fibrosis

Figure 5: SR-AB1 inhibits fibrosis in a mouse carbon tetrachloride (CCl_4) induced model of liver fibrosis

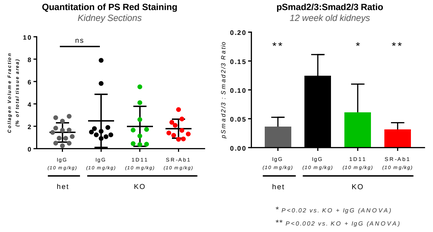
- *Mice dosed 2x weekly (i.p.) w/ 1mL/kg CCl_4 for two weeks prior to antibody dosing*
- *Antibodies dosed 1x weekly (i.p) at indicated doses for four weeks (CCl_4 dosing ongoing during this period)*
- *Livers harvested at end of study (6 weeks)*



- Liver fibrosis measured by morphometric quantitation of Picrosirius Red (PS Red) stained sections
- SR-AB1 ameliorated liver fibrosis at doses as low as 3 mg/kg
- Specific block of proTGFβ1 shows equivalent efficacy to pan-TGFβ inhibition by 1D11

Figure 6: TGFβ1 inhibition completely blocks disease-related Smad2/3 phosphorylation in Col4a3$^{-/-}$ kidneys

- *F1 mice generated from 129/SvJ Col4a3$^{+/-}$ x C57/Bl6 Col4a3$^{+/-}$ crosses*
- *Antibodies dosed 10 mg/kg weekly (5mg/kg BiW, i.p) starting at 6 weeks old*
- *Kidneys harvested from 12 week old mice*

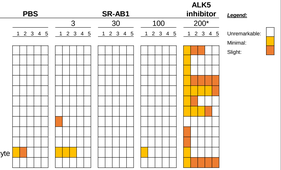


- Limited fibrosis (PS Red quantitation) in Col4a3$^{-/-}$ mice suggests early/mid-stage disease
  - Consistent with moderately elevated BUN (data not shown)
- Elevated pSmad2/3:Smad2/3 ratio in KO+IgG group indicates increased TGFβ signaling
  - TGFβ signaling measured by pSmad2/3- and Smad2/3-specifc ELISAs
- Reduced Smad2/3 phosphorylation in 1D11- and SR-Ab1-treated KO mice
  - Complete inhibition by SR-Ab1 suggests that TGFβ signaling in kidney fibrosis is due to TGFβ1 isoform

Figure 7: No cardiac toxicity observed in rats treated with up to 100 mg/kg SR-AB1 (4-week study)

- *Sprague-Dawley rats treated weekly for 4 weeks with indicated doses of antibody*
  - *ALK5 inhibitor (small molecule) dosed daily for 5 days*

Cardiac pathology (microscopic findings)



- TGFβ1-specific inhibitor SR-AB1 tested at three dosing levels in 4-week rat toxicology study
- No treatment-related cardiac toxicity of SR-AB1 up to 100 mg/kg per week
- No toxicology findings with SR-AB1 in comprehensive list of tissues and organs (data not shown)
- Dosing of ALK5 inhibitor for 5 days recapitulated published cardiac valve toxicity of pan-TGFβ inhibition *(Anderton et al., Tox. Path. 2011; Stauber et al., J. Nonclin. Tox., 2014)*

Summary

- By targeting the TGFβ1 prodomain, **we identified highly specific TGFβ1 antibodies** with no cross-reactivity to TGFβ2 or TGFβ3 (Figure 1).
- One of these antibodies, **SR-AB1, blocks αVβ6 Integrin-mediated activation of endogenously expressed TGFβ1** in primary cultured human dermal fibroblasts and in human hepatic stellate cells (Figure 2).
- Because TGFβ1 is presented on the cell surface or in the ECM by several presenting molecules, we tested the ability of SR-AB1 to inhibit TGFβ1 in the context of each of these presenting molecules. We show that **SR-AB1 can inhibit αVβ8 Integrin-mediated activation of TGFβ1 presented by LTBP1, GARP, or LRRC33** (Figure 3).
- **SR-AB1 inhibits fibrotic markers in kidney** (Figure 4) **and liver** (Figure 5) at doses as low as 3 mg/kg.
- Weekly 10 mg/kg dosing of **SR-AB1 completely blocks disease-associated Smad2/3 phosphorylation** in Col4a3$^{-/-}$ kidneys (Figure 6)
- In a 4 week toxicology study in rats with up to 100 mg/kg/wk **SR-AB1 showed no test article-related toxicities** including cardiac toxicity previously associated with pan-TGFβ inhibition (Figure 7).

Conclusions

- SR-AB1 specifically binds the TGFβ1 prodomain and inhibits αVβ6- or αVβ8-Integrin mediated activation of TGFβ1
- TGFβ1 inhibition is sufficient to reduce fibrosis in several preclinical models and completely blocks disease associated Smad2/3 signaling. Together, these findings point to TGFβ1 as the critical pathogenic isoform in fibrosis
- In a 4 week toxicity study, TGFβ1 inhibition exhibits a superior safety profile compared to pan-TGFβ inhibition