Defeating primary checkpoint resistance: SRK-181 is a first-in-class, fully human antibody that renders resistant tumors sensitive to anti-PD-1 (Abstract 4090)

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Introduction

Despite the profound advances in cancer immunotherapy, primary resistance to checkpoint blockade therapy (CBT) remains a major unmet need for patients; a majority of patients' cancers still fail to respond to PD-(L)1 inhibition. Retrospective analysis of urothelial cancer and melanoma tumors has recently implicated TGFβ activation as a potential driver of primary resistance, very likely via multiple mechanisms including exclusion of cytotoxic T cells from the tumor as well as their expansion within the tumor microenvironment (immune exclusion). These observations and subsequent preclinical validation have pointed to TGFβ pathway inhibition as a promising avenue for overcoming primary resistance to CBT. However, therapeutic targeting of the TGFβ pathway has been hindered by dose-limiting preclinical cardiotoxicities, most likely due to inhibition of signaling from one or more TGFβ isoforms.

Upon secretion, TGFβ growth factor is held in a latent complex with its non-covalently associated prodomain. TGFβ activation is triggered by extracellular events that release the growth factor from this latent complex. We previously demonstrated that antibody-based isoform-specific inhibition of TGFβ activation can be achieved by targeting a specific latent TGFβ complex and preventing release of one isoform (e.g., TGFβ1) while avoiding other isoform complexes (e.g. TGFβ2 and TGFβ3), thus creating a potential avenue to avoid the toxicities observed with less selective TGFβ pathway inhibition.

Hypothesis

Delineation and selective targeting of the TGFβ isoform(s) that is most relevant in the tumor microenvironment may enable a therapeutically tractable approach to overcoming primary resistance to CBT.

Figure 1: TGFβ1 is the predominant isoform in many human tumors

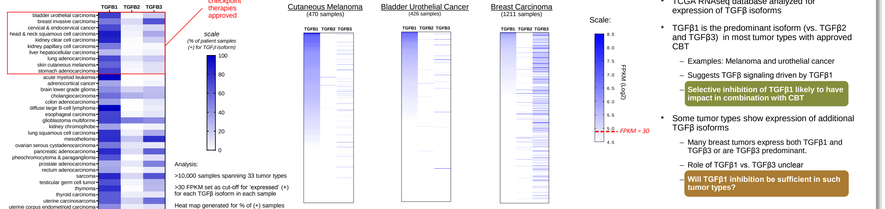
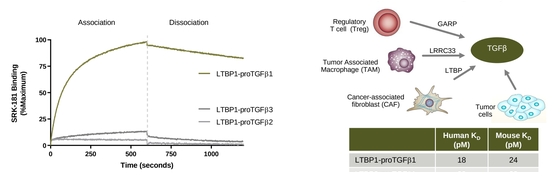
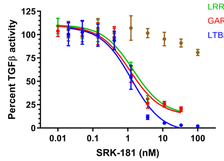


Figure 2: SRK-181 is a fully human, isoform-specific anti-latent TGFβ1 antibody that binds with high affinity and potently inhibits TGFβ1 activation in vitro

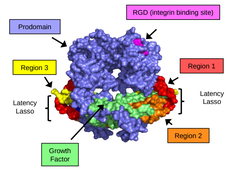


	Human K_D (pM)	Mouse K_D (pM)
LTBP1-proTGFβ1	18	24
LTBP3-proTGFβ1	28	22
GARP-proTGFβ1	16	21
LRRC33-proTGFβ1	63	48



- SRK-181 is specific for latent TGFβ1 as determined by Octet (Fig. 2A)
  - Minimal or no binding to latent TGFβ2 and TGFβ3 complexes
  - No binding to active TGFβ1 growth factor
- SRK-181 binds with high affinity to all large latent TGFβ1 complexes, as measured by equilibrium titration (Fig. 2B)
  - SRK-181 therefore targets latent TGFβ1 from multiple sources, including fibroblasts, myeloid cells, and regulatory T cells
  - Subnanomolar affinity of SRK-181 for rat and cynomolgus TGFβ1 complexes confirmed
- SRK-181 inhibits activation of latent TGFβ1 by integrins (Fig. 2C) and proteases (not shown) in cell-based assays
  - SRK-181 inhibits activation of all large latent TGFβ1 complexes but not of latent TGFβ3 complex

Figure 3: SRK-181 binds to the latency lasso region of latent TGFβ1



- SRK-181 Fab binding protects three regions on latent TGFβ1 from hydrogen/deuterium exchange (H/DX; Fig. 3A)
  - Region 1 is on TGFβ1 prodomain
  - Regions 2 and 3 are on TGFβ1 growth factor
- Protected regions suggest allosteric mode of inhibition of integrin-dependent activation and competitive inhibition of proteolytic activation (not shown)
- Sequence diversity of protected regions across three TGFβ isoforms is consistent with isoform specificity of SRK-181 (Fig. 3B)

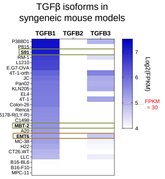
Figure 4: Selection of murine syngeneic tumor models that best reflect human primary resistance to CBT

Phenotype of Human Tumors

- Subset responsive to PD-(L)1 axis blockade
- Immune exclusion
- Evidence of TGFB1 expression and TGFβ signaling

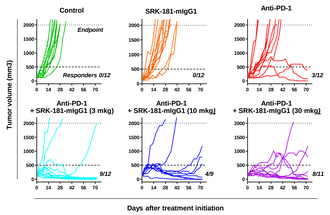
Criteria for Selection of Mouse Tumor Model

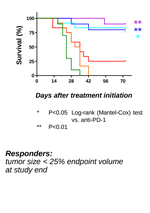
- Partial to no response to PD-(L)1 inhibition
- Immune-excluded tumors
- TGFB1 expression and evidence of TGFβ signaling



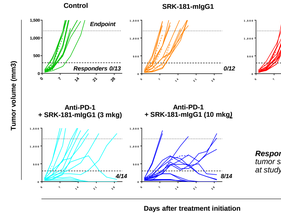
- Commonly used syngeneic mouse models used for I/O do not recapitulate TGFβ1 bias or anti-PD-1 resistance
- **S91** melanoma and **MBT-2** urothelial cancer models selected to test TGFβ1-specific inhibition with SRK-181 in TGFβ1-predominated tumor
- **EMT6** breast cancer model chosen for testing TGFβ1-specific inhibition with SRK-181 in TGFβ1/3-expressing tumor

Figure 5: SRK-181 sensitizes TGFβ1-predominated tumors to anti-PD-1

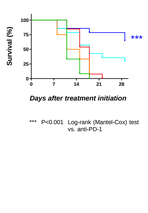




Responders:
tumor size < 25% endpoint volume at study end

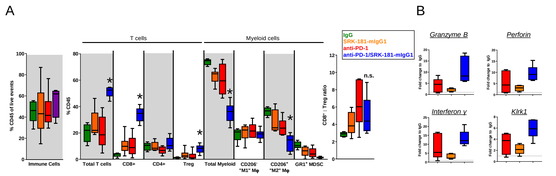


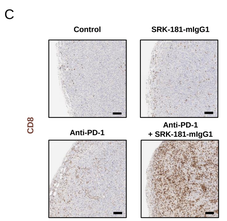
Responders:
tumor size < 25% endpoint volume at study end

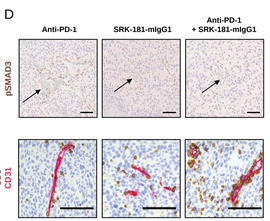


- Treatments of mice bearing s.c. S91 and MBT-2 tumors initiated when tumor volume reached 125-196 mm^3 and 30-80 mm^3, respectively. Anti-PD-1 was dosed at 10 mkg twice weekly. SRK181-mIgG1 was dosed weekly as indicated.
- Treatment with anti-PD-1 or SRK-181-mIgG1 alone had little to no effect on tumor growth.
- In combination with anti-PD-1, blockade of TGFβ1 activation with SRK-181-mIgG1 with doses as low as 3 mg/kg per week resulted in synergistic tumor growth delay as manifested by either complete responses, tumor regressions, or tumor control (< 25% tumor volume endpoint; Fig. 5A,C).
- Combination treatment leads to significant survival benefit in both models over anti-PD-1 alone (*, P<0.05; **, P<0.01; ***, P<0.001 Log-Rank test; Fig. 5B,D). In the MBT-2 study, the 10 mg/kg combination group did not reach median survival. In the S91 study, the median survival was not reached with any combination group.

Figure 6: Anti-PD-1/SRK-181 combination enables infiltration and expansion of CD8⁺ T cells in MBT-2 tumors while reducing immunosuppressive myeloid cells

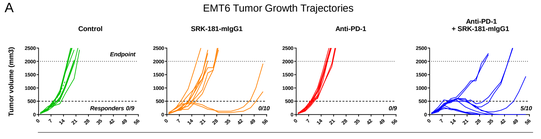


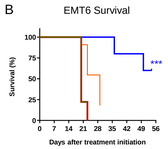


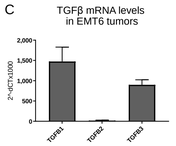


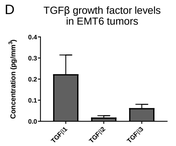
- Immune contexture analyzed at day 10 post-treatment. Anti-PD-1 was dosed at 10 mkg twice weekly. SRK181-mIgG1 was dosed weekly at 10 mkg.
- While the overall tumor-infiltrating immune cell fraction remains constant across treatment groups, anti-PD-1/SRK-181-mIgG1 causes (Fig. 6A):
  - Significant increase in intratumoral CD8⁺ T cells (* P<0.05, two-sided T test vs. anti-PD-1 group).
  - Significant increase in Tregs (* P<0.05), however, the CD8⁺:Treg ratio is unchanged (n.s., not significant vs anti-PD-1)
  - Significant reduction of myeloid cells compared to any other group, driven by a reduction of immunosuppressive M2 macrophage and myeloid-derived suppressor cell (MDSC) populations (* P<0.05, two-sided T test vs. anti-PD-1 group)
- Quantitative PCR analysis of whole tumor lysates confirms robust increase in CD8 effector genes (Fig. 6B). Dotted line, IgG control.
- Similarly, anti-PD-1/SRK-181-mIgG1 induces a marked increase in frequency of CD8⁺ T cells within the tumor mass, overcoming immune exclusion (Fig. 6C). Bar, 100 μm.
- PhosphoSMAD3 was found to be enriched near vascular endothelium within anti-PD-1-treated tumors. Treatment with SRK-181-mIgG1 abrogates this signal (Fig. 6D, top). Arrow points to intratumoral vasculature. Bar, 100 μm.
- Anti-PD-1-treated animals show some infiltrating CD8⁺ T cells closely associated with tumor vasculature (CD31 staining). Combination treatment supports further T cell infiltration. Proximity of CD8⁺ T cells to vascular endothelium suggests that T cells may infiltrate the tumor from the intratumoral vasculature (Fig. 6D, bottom).

Figure 7: Inhibiting TGFβ1 alone in a TGFβ1/3-expressing tumor is sufficient to sensitize to anti-PD-1 immunotherapy





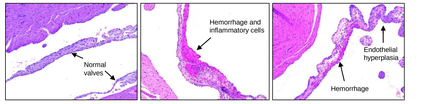




- EMT6 tumors were implanted subcutaneously. Treatment began when EMT6 tumors reached 30-80mm^3. Anti-PD-1 was dosed at 10 mkg twice weekly. SRK-181-mIgG1 was dosed once weekly at 10 mkg.
- Treatment with anti-PD-1 or SRK-181-mIgG1 alone had little effect on EMT6 tumor growth.
- In combination with anti-PD-1, blockade of TGFβ1 activation with SRK-181-mIgG1 resulted in synergistic antitumor efficacy, as evidenced by either complete responders or tumor growth delay (Fig 7A). Responders, tumor size < 25% endpoint volume at study end.
- Combination treatment leads to significant survival benefit over anti-PD-1 alone (***, P<0.001 Log Rank test; Fig. 7B). The median survival was not reached with the combination group.
- Blockade of the TGFβ1 isoform is sufficient to sensitize EMT6 tumors to anti-PD-1, despite the presence of TGFβ3 in EMT6 tumor lysates, as measured by qPCR (Fig. 7C, normalized to HPRT) or total TGFβ growth factor ELISAs (after acid activation, normalized to tumor volume; Fig. 7D).

Figure 8: TGFβ1 isoform specificity of SRK-181 results in improved preclinical toxicity profile vs. non-selective TGFβ pathway inhibitor

A Valvulopathies confirmed with **pan-TGFβ inhibition control reagents** in one week tolerability study



B Improved preclinical toxicity profile of **SRK-181**

Microscopic observations in heart	Control vehicle iv, qwk x 4	LY2109761 300 mg/kg po, qd x 8	PanTGFβAb 30 mg/kg iv, 1 dose	SRK-181 10 mg/kg iv, qwk x 4	SRK-181 30 mg/kg iv, qwk x 4	SRK-181 100 mg/kg iv, qwk x 4
Valvulopathy						
Atrium - Mixed cell infiltrate						
Myocardium - Degeneration/necrosis						
Myocardium - Hemorrhage						
Myocardium - Mixed cell infiltrate, base						
Coronary artery - Necrosis with inflammation						
Cardiomyocyte - Necrosis/inflammatory cell infiltrate						

Legend: Unremarkable, Minimal, Slight, Moderate

- Repeat dose pilot toxicology study in adult female Sprague Dawley rats
- Animals dosed with LY2109761 (ALK5 kinase inhibitor) or pan-TGFβ antibody (binds TGFβ1, TGFβ2, and TGFβ3 with high affinity) were sacrificed on day 8 (Fig. 8A), animals dosed with SRK-181 on day 29 for histopathology analysis (Fig. 8B).
- Exposure as assessed by SRK-181 serum concentration reached 2,300 μg/ml following 4 weekly doses of 100 mg/kg.
- No SRK-181-related adverse effects were noted up to 100 mg/kg.
- Importantly, no cardiotoxicities (valvulopathy) were noted with SRK-181 compared to LY2109761 and pan-TGFβ Ab.
- In conclusion, the no observed adverse effect level (NOAEL) for SRK-181 was the highest dose evaluated (i.e. 100 mg/kg), suggesting that the maximally tolerated dose (MTD) is >100 mg/kg.

Conclusions

- TGFβ1 is the predominant TGFβ isoform expressed in many human tumors and is the likely driver of TGFβ pathway signaling that contributes to immune exclusion, which renders a large fraction of tumors resistant to CBT.
- Some tumor types have considerable levels of TGFβ3, the contribution of which to immune exclusion and CBT resistance has been unclear.
- SRK-181 is a fully human antibody that binds latent TGFβ1 with high selectivity and subnanomolar affinity, and potently inhibits multiple mechanisms of activation of this growth factor.
- Importantly, SRK-181 binds and inhibits the activation of all large latent TGFβ1 complexes and therefore targets TGFβ1 from fibroblasts (LTBP), myeloid cells (LRRC33), and regulatory T cells (GARP).
- In murine syngeneic tumor models that reflect human primary resistance to CBT, treatment with SRK-181-mIgG1 renders tumors sensitive to anti-PD-1 therapy. SRK-181-mIgG1/anti-PD-1 combination treatment leads to:
  - an increase in effector T cells and an enrichment of CD8⁺ T cells around the tumor vasculature
  - a decrease in intratumoral immunosuppressive myeloid cells
  - pronounced tumor regression or tumor control, as well as a significant survival benefit
- Inhibition of TGFβ1 isoform with SRK-181-mIgG1 was sufficient to sensitize tumors to anti-PD-1, even in presence of intratumoral TGFβ3. These results are consistent with the hypothesis that TGFβ1 is the isoform that drives TGFβ signaling, immune exclusion, and primary resistance to CBT.
- Importantly, isoform-specific inhibition of TGFβ1 activation by SRK-181 is not sufficient to trigger valvulopathies in a 4 week rat toxicology study and results in an improved preclinical toxicity profile versus non-selective TGFβ pathway inhibition.
- In summary, the rationale for targeting TGFβ1 in CBT-resistant tumors is derived from analysis of clinically derived human tumors and associated responses. These results have led to the selection of SRK-181 as a clinical development product candidate for the treatment of tumors resistant to checkpoint blockade therapies, such as anti-PD-1 antibodies.

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