Defeating checkpoint resistance: Highly specific inhibition of latent TGFβ1 activation renders resistant solid tumors vulnerable to PD-1 blockade

Thomas Schürpf[1], Constance J Martin[1], Christopher Littlefield[1], Christopher Chapron[1], Stefan Wawersik[1], Ashish Kalra[1], Kevin Dagbay[1], Allison Simpson[1], Francis Danehy[1], Christopher Boston[1], Anastasia Nikiforov[1], Susan Lin[1], Justin Jackson[1], Pichai Raman[2], Elizabeth Rainbolt[3], Laurie Comfort[3], David Harris[3], Madelyn Cecil-Taylor[3], Lorne Celentano[3], Danielle Meadows[3], Gregory J Carven[1], Alan Buckler[1], Allan Capili[1], Abhishek Datta[1]

1) Scholar Rock Inc., Cambridge MA 2) Independent Consultant, Bryn Mawr, PA 3) Charles River Discovery Services, Morrisville NC

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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 multiple TGFβ isoforms.

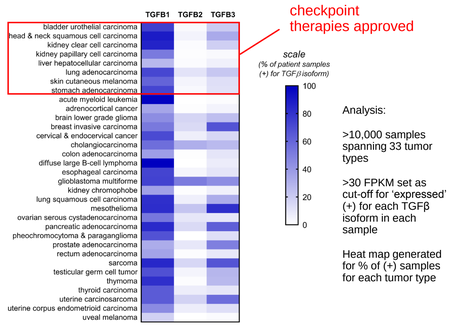
Upon secretion, TGFβ growth factor is held in a latent complex with its non-covalently associated prodomain. TGFβ activation is triggered by extracellular events that release the growth factor from this latent complex. We previously demonstrated that antibody-based isoform-specific inhibition of TGFβ 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

A: TGFβ isoform expression by tumor type



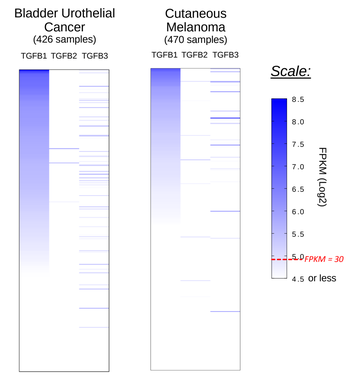
Analysis:

>10,000 samples spanning 33 tumor types

>30 FPKM set as cut-off for 'expressed' (+) for each TGFβ isoform in each sample

Heat map generated for % of (+) samples for each tumor type

B: TGFβ isoform expression in individual tumors

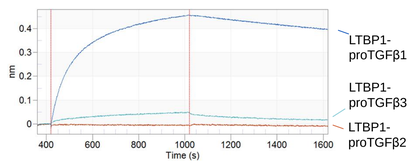


Human tumor data:

- For many tumor types, expression data suggest that TGFβ signaling is mainly driven by TGFβ1
- TGFβ1 is most prevalent (vs. TGFβ2 and TGFβ3) in cancers where checkpoint therapies are approved
- TGFβ1 is the predominant isoform in majority of urothelial cancer and melanoma tumor samples

Selective inhibition of TGFβ1 activity likely to have greatest impact in combination with checkpoint therapies

Figure 2: SRTβ1-Ab3 is a fully human, isoform-specific anti-latent TGFβ1 antibody with high affinity

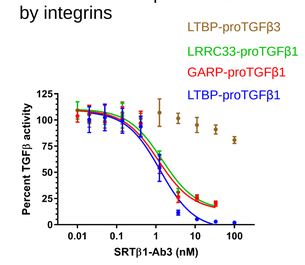


	Human K_D (pM)	Mouse K_D (pM)
LTBP1-proTGFβ1	18	24
LTBP3-proTGFβ1	28	22
GARP-proTGFβ1	16	21
LRRC33-proTGFβ1	63	48

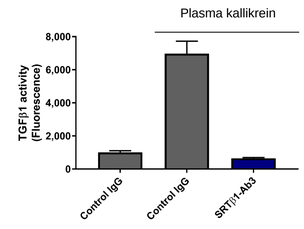
- SRTβ1-Ab3 is specific for latent TGFβ1
  - No meaningful binding to latent TGFβ2 and TGFβ3 complexes
  - No binding to active TGFβ1 growth factor
- SRTβ1-Ab3 binds with high affinity to all large latent TGFβ1 complexes
- Subnanomolar affinity of SRTβ1-Ab3 for rat and cynomolgus proteins confirmed

Figure 3: SRTβ1-Ab3 potently inhibits activation of latent TGFβ1

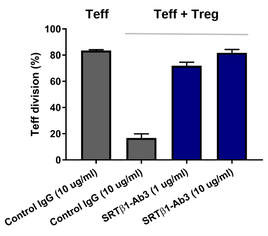
A Inhibition of TGFβ1 activation by integrins



B Inhibition of TGFβ1 activation by proteolysis



C Inhibition of human Tregs



- SRTβ1-Ab3 inhibits activation of latent TGFβ1 by both integrins and proteases (Fig. 3A,B)
  - SRTβ1-Ab3 inhibits activation of all large latent TGFβ1 complexes but not of latent TGFβ3 complex (Fig. 3A)
  - SRTβ1-Ab3 inhibits activation of latent TGFβ1 by plasma kallikrein (Fig. 3B)
- SRTβ1-Ab3 inhibits human Treg suppression of autologous CD4+ T cells in a co-culture assay (Fig. 3C)

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

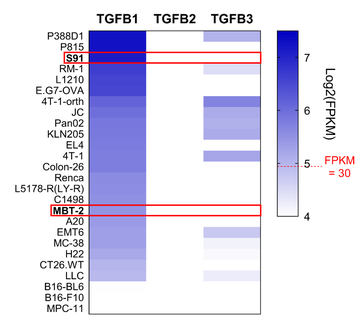
Phenotype of Human Patients in anti-PD-(L)1 Approved Therapies

- High TGFB1 over TGFB2/3 expression
- Subset responsive to PD-1 axis blockade
- Evidence for TGFβ signaling
- Immune exclusion

Criteria for Selection of Mouse Tumor Model

- High TGFB1 over TGFB2/3
- Partial to no response to PD-(L)1 inhibition
- Active TGFβ signaling
- Immune-excluded tumors

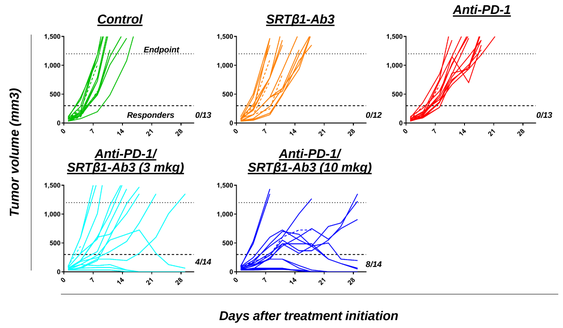
TGFβ isoforms in syngeneic mouse models (RNAseq)



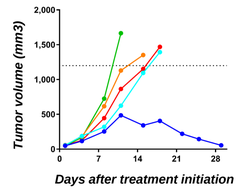
- Many tumor models are not truly refractory to CBT
- Many models do not exhibit an immune excluded phenotype
- Commonly used syngeneic mouse models used for I/O (MC38, EMT6, 4T1) do not recapitulate TGFβ1 bias observed in many human tumors
- **MBT-2** urothelial cancer and **CloudmanS91** melanoma models chosen for testing TGFβ1-specific inhibition using SRTβ1-Ab3 in combination with anti-PD-1 antibody

Figure 5: Synergistic effects of SRTβ1-Ab3 combination with anti-PD-1 on tumor growth in CBT-resistant tumors

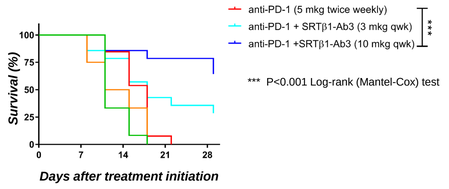
A MBT-2: Tumor Growth Trajectories



B MBT-2: Median Tumor Volume

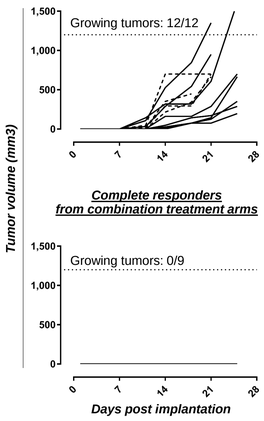


C MBT-2: Survival

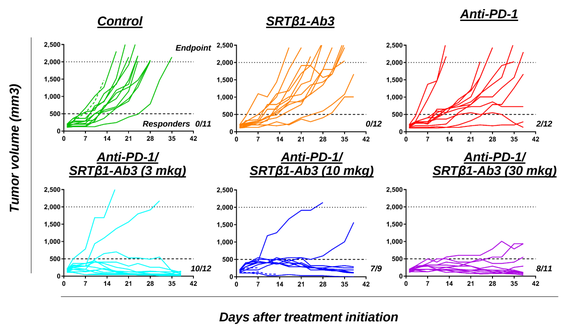


*** P<0.001 Log-rank (Mantel-Cox) test

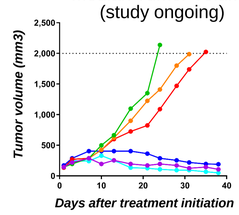
D MBT-2: Re-Challenge



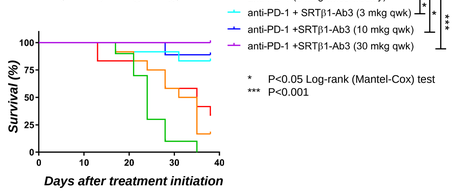
E S91: Tumor Growth Trajectories (study ongoing)



F S91: Median Tumor Volume (study ongoing)



G S91: Survival (study ongoing)

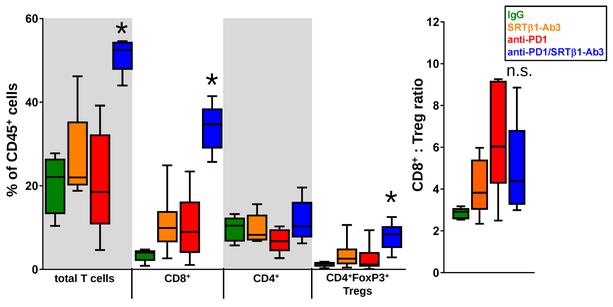


* P<0.05 Log-rank (Mantel-Cox) test
*** P<0.001

- Treatments of mice bearing s.c. MBT-2 and S91 tumors initiated when tumor volume reached 30-80 mm^3 and 125-196 mm^3, respectively.
- Treatment with anti-PD-1 (twice weekly) or SRTβ1-Ab3 (qwk) alone had little to no effect on tumor growth.
- In combination with anti-PD-1, blockade of TGFβ1 activation with SRTβ1-Ab3 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,B,E,F).
- Combination treatment leads to significant survival benefit in both models (Fig. 5C,G). 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 yet reached with any combination group (study ongoing).
- Verification of acquired T cell memory in complete responder mice: In contrast to age-matched naïve mice, complete responders from anti-PD-1/SRTβ1-Ab3 treatment arms rejected re-challenge with MBT-2 cells after a washout period of 7 weeks (Fig. 5D)

Figure 6: SRTβ1-Ab3 in combination with anti-PD-1 overcomes immune exclusion by enabling infiltration and expansion of CD8+ T cells in tumors

A



- Anti-PD-1/SRTβ1-Ab3 induces significant increase in intratumoral CD8+ T (* P<0.05, two-sided T test vs. anti-PD-1 group; Fig. 6A).
  - No changes in %CD45+ cells of total live cells observed across treatment groups (not shown)
- Anti-PD-1/SRTβ1-Ab3 causes significant increase in Tregs (* P<0.05), however, the CD8+:Treg ratio is unchanged (n.s., not significant vs anti-PD-1; Fig. 6A).
- Similarly, anti-PD-1/SRTβ1-Ab3 induces a marked increase in frequency of CD8+ T cells within the tumor mass, overcoming immune exclusion (Fig. 6B).

B

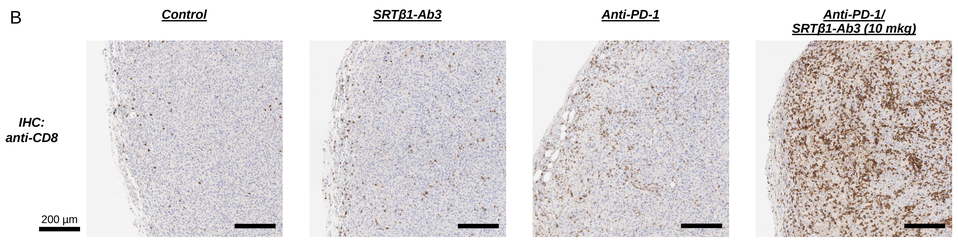
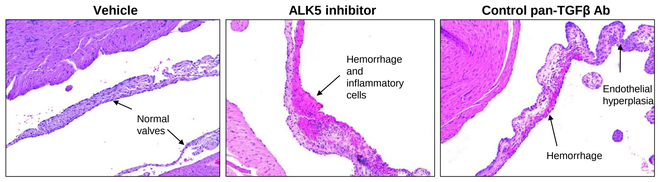


Figure 7: TGFβ1 isoform specificity of SRTβ1-Ab3 results in improved preclinical toxicity profile

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



B: Improved preclinical toxicity profile of **SRTβ1-Ab3**

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	SRTβ1-Ab3 10 mg/kg iv, qwk x 4	SRTβ1-Ab3 30 mg/kg iv, qwk x 4	SRTβ1-Ab3 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. 7A), animals dosed with SRTβ1-Ab3 on day 29 for histopathology analysis (Fig. 7B).
- Exposure as assessed by SRTβ1-Ab3 serum concentration reached 2,300 µg/ml following 4 weekly doses of 100 mg/kg.
- No SRTβ1-Ab3-related adverse effects were noted up to 100 mg/kg.
- Importantly, no cardiotoxicities (valvulopathy) were noted with SRTβ1-Ab3 compared to LY2109761 and pan-TGFβ Ab.
- In conclusion, the no observed adverse effect level (NOAEL) for SRTβ1-Ab3 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, particularly those for which CBT is approved. It is the likely driver of TGFβ pathway signaling that contributes to immune exclusion, which renders a large fraction of tumors resistant to CBT.
- SRTβ1-Ab3, a fully human antibody that binds latent TGFβ1 with high selectivity and subnanomolar affinity, potently inhibits multiple mechanisms of activation of this growth factor.
- In murine syngeneic tumor models that best reflect human primary resistance to CBT, including the predominance of TGFβ1, treatment with SRTβ1-Ab3 renders tumors vulnerable to anti-PD-1 therapy. SRTβ1-Ab3/anti-PD-1 combination treatment leads to effector T cell infiltration and expansion, resulting in pronounced tumor regression or tumor control, durable immunological memory, as well as a significant survival benefit.
- Importantly, isoform-specific inhibition of TGFβ1 activation by SRTβ1-Ab3 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. Collectively, our results point to a potential therapeutic avenue for overcoming primary resistance by selectively targeting TGFβ1, the likely driver of this pathway in many human tumors.

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