Biomarker Strategy for the Latent TGFβ1 Inhibitor SRK-181 Phase 1 Clinical Trial, DRAGON

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Disclaimer

- SRK-181 is an investigational drug candidate being evaluated in a Phase 1 clinical trial.
- SRK-181 has not been approved by the U.S. Food and Drug Administration or any other health authority for any indication.
- The safety and efficacy of SRK-181 in human subjects will not be discussed today.

SRK-181 overview

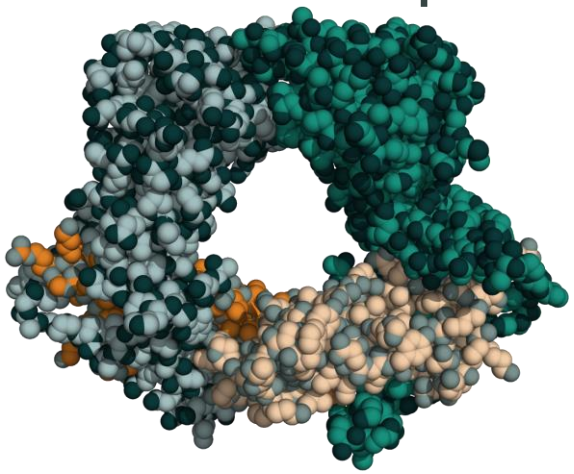
- Fully human monoclonal antibody[1]
- SRK-181 binds latent TGFβ1 complexes with picomolar affinities
  - Binds all TGFβ1 large latent complexes
  - Cross-reacts with mouse, rat, cyno
  - Minimal or no binding to latent TGFβ2 and TGFβ3 isoforms or to mature TGFβ growth factors
- Potent and selective inhibitor of latent TGFβ1 activation
  - Inhibits latent TGFβ1 activation triggered by integrins or proteolytic cleavage
- TGFβ1 Isoform specificity of SRK-181 leads to improved safety profile in preclinical studies[2]

1. Martin CJ, et al. Sci Transl Med 2020;12(536)
2. Welsh BT, et al. Int J Toxicol. 2021;40(4):322-336

Pro-domain Targeting: Isoform Specificity

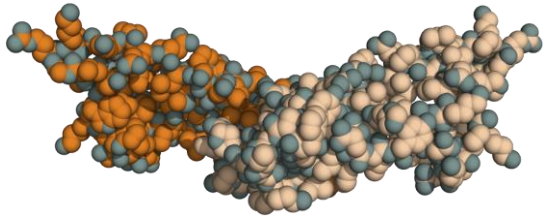
Targeting Latent TGFβs Creates Multiple "Handles" For Selectivity[1]

Latent TGFβ



- Proprotein is cleaved before secretion
- Prodomain & growth factor remain noncovalently bound
- Receptor binding requires growth factor release

Mature Growth Factor



Percent Identity

TGFβ1	TGFβ2	TGFβ3	
	71.4	76.8	TGFβ1
		79.5	TGFβ2
			TGFβ3

Prodomain

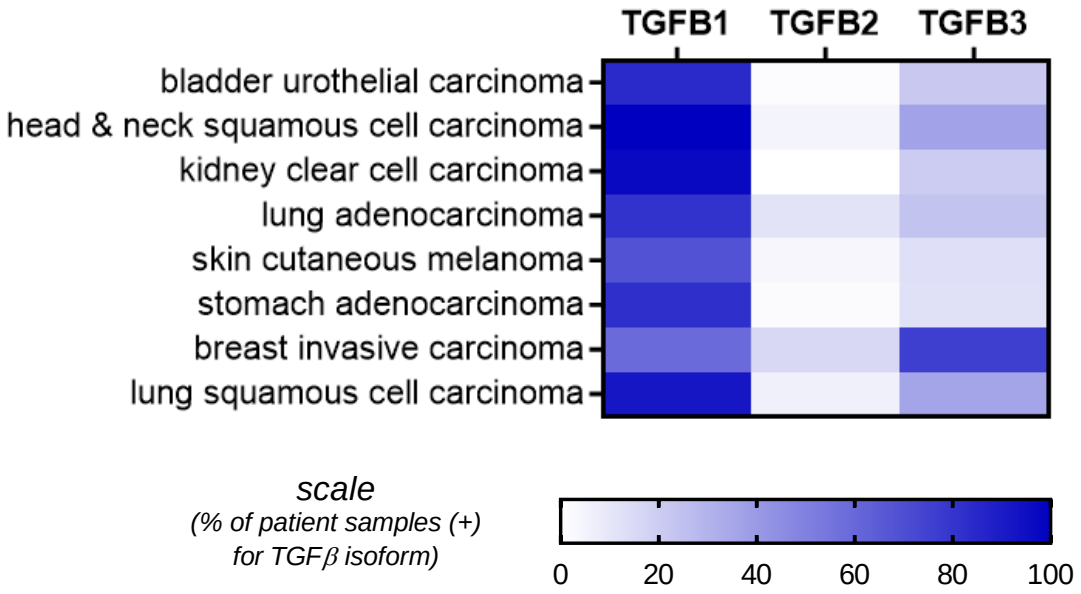
Percent Identity

TGFβ1	TGFβ2	TGFβ3	
	37.4	37.1	TGFβ1
		48.7	TGFβ2
			TGFβ3

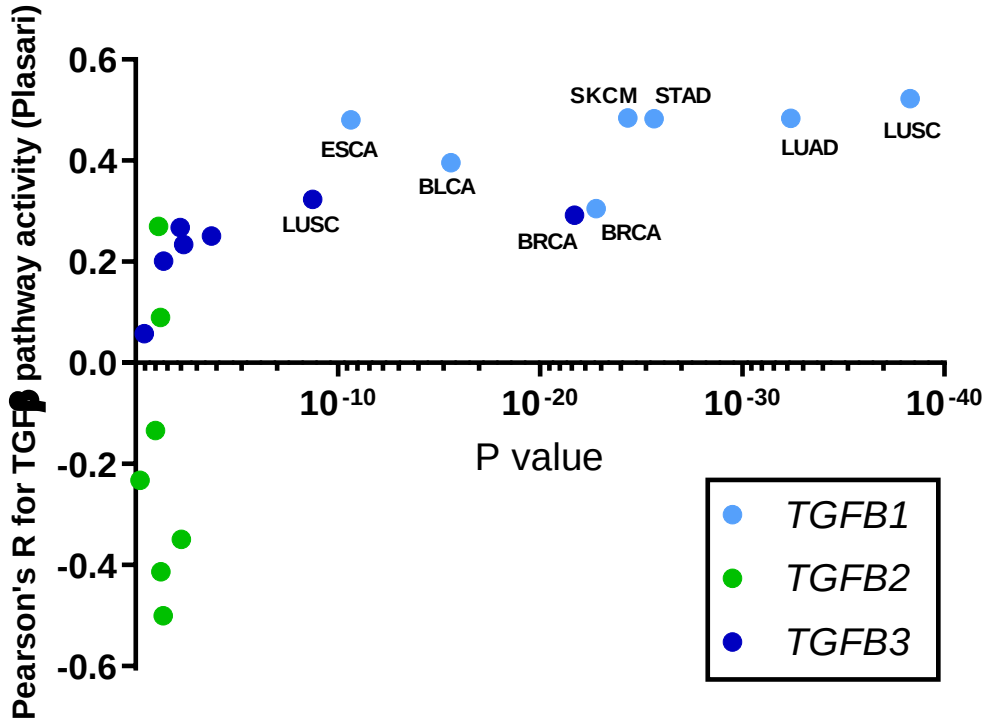
1. Martin CJ, et al. Sci Transl Med 2020;12(536)

TGFβ1 implicated as the most relevant TGFβ isoform in Human Tumors

TGFβ1 is most prevalent isoform in most human cancers[1].



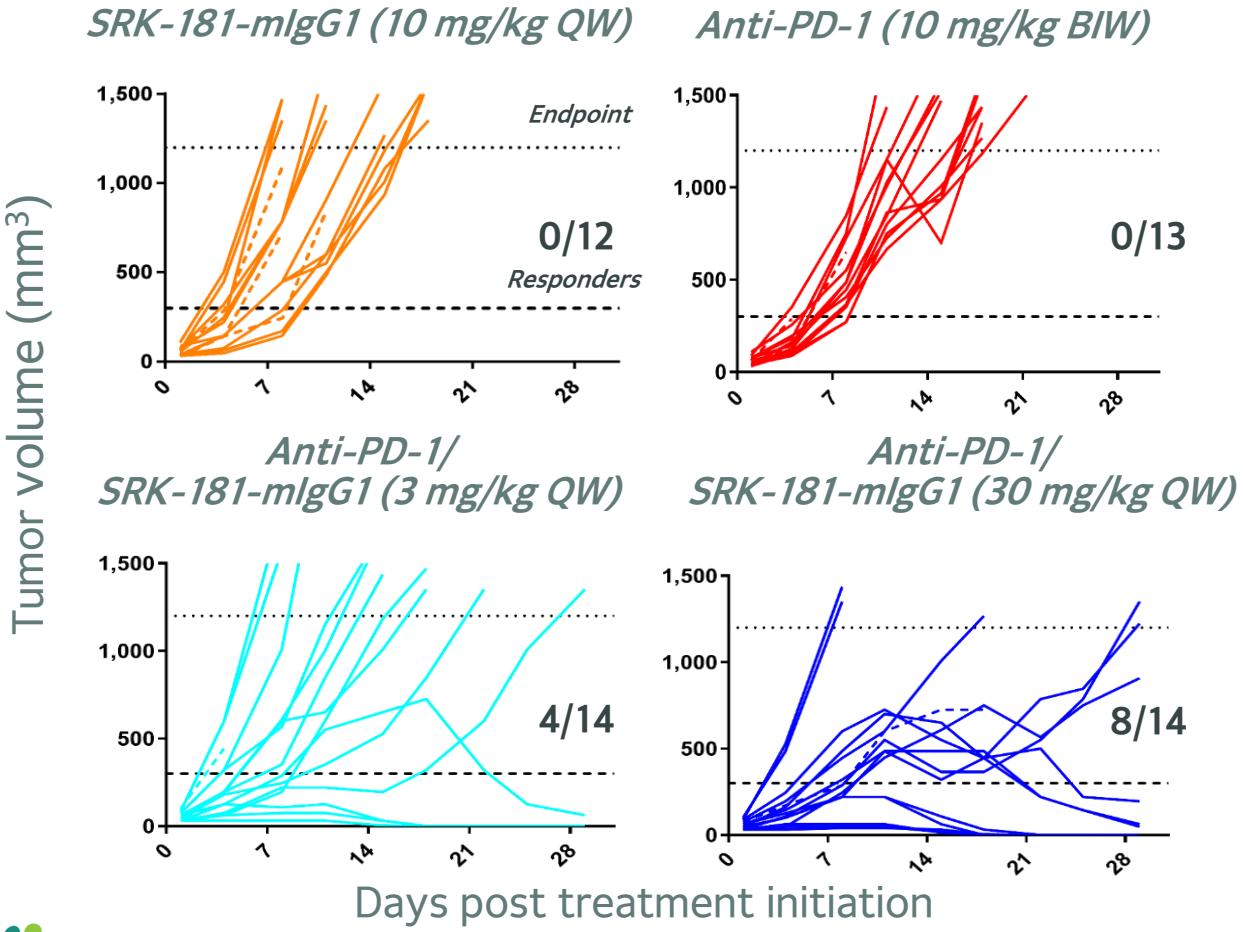
TGFβ1 expression correlates with TGFβ pathway activity in tumors[1].

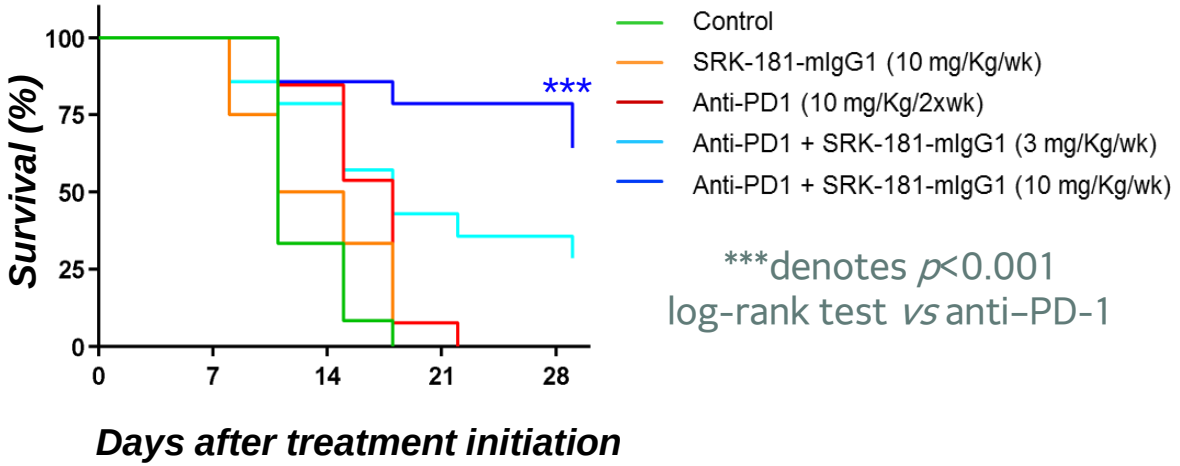


1. Martin CJ, et al. Sci Transl Med 2020;12(536)

TGFβ1 Blockade with SRK-181-mIgG1 Rendered Preclinical Tumor Models Susceptible to Anti-PD-1 Therapy

MBT-2 urothelial cancer model: combination treatment led to tumor regression and survival benefit[1]





***denotes $p<0.001$ log-rank test *vs* anti-PD-1

Similar results demonstrated in Cloudman S91 melanoma model and EMT-6 breast cancer model[1]

Model selection criteria: immune exclusion, minimal or no response to anti-PD-(L)1, evidence for TGFβ signaling

1. Martin CJ, et al. Sci Transl Med 2020;12(536)

DRAGON Phase 1 Clinical Trial

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DRAGON trial (NCT04291079) is a multicenter, open-label, phase 1, first-in-human (FIH), dose-escalation, and dose expansion study to evaluate the safety, tolerability, PK, PD and efficacy of SRK-181 alone, or in combination with anti-PD-(L)1

Part A

Part A1:

- SRK-181 as a single agent
- Modified 3+3 dose escalation
- Assess SRK-181 dose range of 80-3000 mg

Part A2:

- SRK-181 with approved anti-PD-(L)1
- 3+3 dose escalation

→

Part B

- SRK-181 in combo with approved anti-PD-(L)1 therapy
- 5 cohorts – each will enroll up to 40 patients
- Target indications include:
  - NSCLC
  - Urothelial carcinoma
  - Melanoma
  - Renal cell carcinoma
  - Other solid tumor types

Highlights from the DRAGON Part A data presented at SITC 2021

https://investors.scholarrock.com/news-releases/news-release-details/scholar-rock-presents-data-part-dragon-phase-1-trial-evaluating

SRK-181 biomarker strategy to support DRAGON Ph 1 study: 2 tier prioritization

Tier 1: Focuses on evaluation of biomarkers relevant to the MOA of SRK-181

1 **Immunophenotyping**
Assessment of immune landscape

- Assess ability of SRK-181 to overcome tumor immune exclusion
- Characterize tumor immune contexture

2 **TGFβ pathway evaluation**
Assessment of signaling pathway

- Assess the ability of SRK-181 to modulate TGFβ pathway
- Identify the prevalence of TGFβ signaling components within the tumors

Tier 2: Expands to orthogonal complementary biomarkers and approaches

3 **Multiplex immune biomarkers**

4 **Additional blood-based and predictive biomarkers**

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Preclinical data provides scientific rationale to support CD8 as a biomarker for SRK-181

Immunophenotyping
Assessment of immune landscape

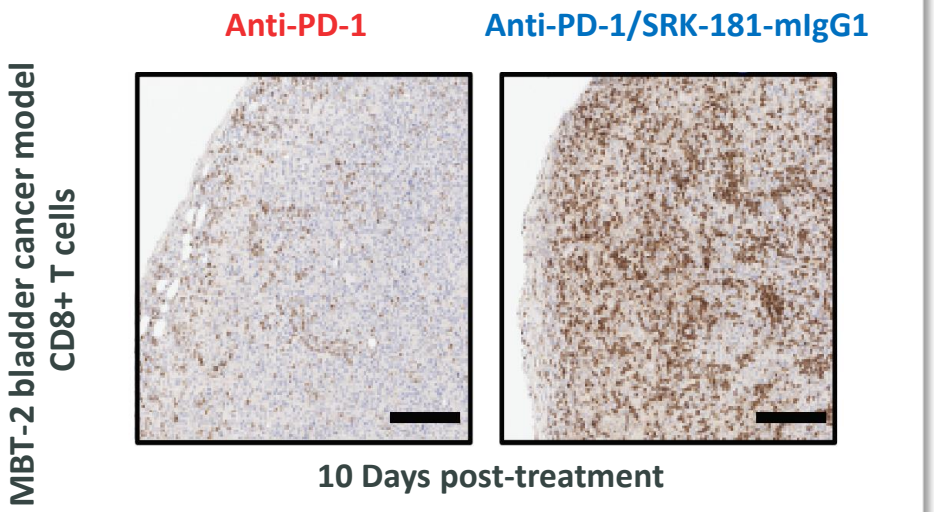
- CD8+ T cells plays a central role in cancer immunity[1,3]
- In preclinical tumor models, SRK-181 and α-PD1 combination leads to the influx of CD8+ T cells
  - *Significant increase of effector T cells correlated with efficacy in MBT-2 model, $p<0.05$*

Hypothesis: treatment-induced increase of tumoral CD8+ T cells correlates to anti-tumor immune response

SRK-181 treatment increased tumoral CD8+ T cells thereby supporting CD8 as a biomarker

Immune contexture analysis at day 10 post-treatment in MBT-2 model[1]

Anti-PD-1 (10 mg/kg BIW); SRK-181-mIgG1 (10 mg/kg QW)



Anti-PD-1/SRK-181-mIgG1 induces a marked increase in frequency of CD8+ T cells within the tumor mass (right). Bar, 100 µm.

1. Martin CJ, et al. Sci Transl Med 2020;12(536)
3. Mariathasan S, Turley SJ, Nickles D, et al. Nature. 2018;554:544-548

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Establishment of CD8 IHC digital pathology to enable identification of tumor immunophenotypes

IHC pilot study was performed utilizing commercially available human cancer samples

- Performed digital pathology analysis to characterize the tumor immune phenotypes
  - Compartmental analysis: quantify CD8⁺ T cells within tumor, tumor margin and stromal compartments
  - Tumor nest analysis: Quantified CD8⁺ T cells in tumor and tumor margin of each tumor nest
- Established the CD8⁺ cell baseline signals for bladder cancer and melanoma

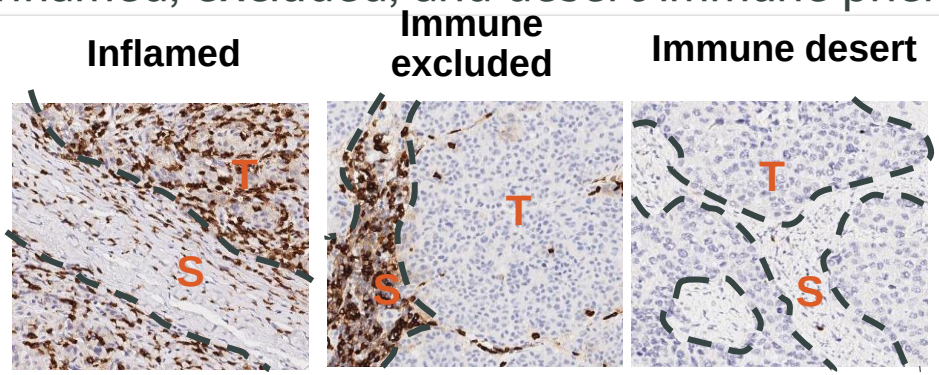
Future directions: multiplex IHC to evaluate T cell subtypes and enzymatic activity

Intratumor CD8 is used to characterize the tumor immune phenotypes

Categorization reviewed and confirmed by pathologists (Flagship Bio)

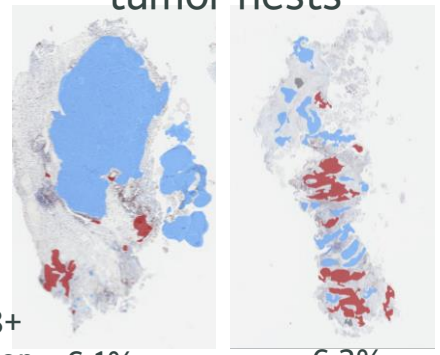
** %CD8⁺ cells across compartments are utilized to classify immune phenotypes. ≥5% CD8⁺ cells in tumor compartment are classified as inflamed, <5% CD8⁺ cells in tumor and ≥ 5% CD8⁺ cells at the margin are classified as immune excluded, and <5% CD8⁺ cells in all compartments are classified as immune desert*

CD8 compartmental analysis tumors to classify tumors into inflamed, excluded, and desert immune phenotypes

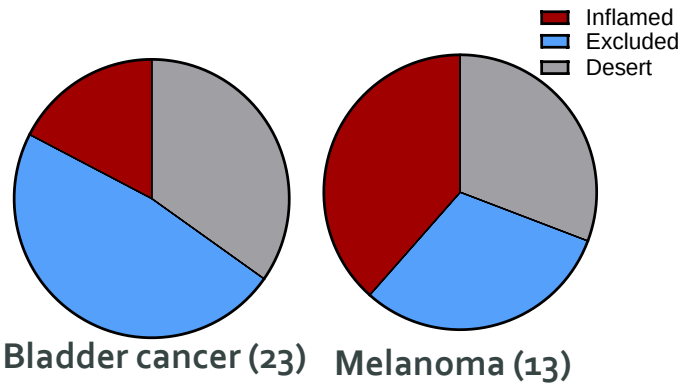


Dotted line represents margin between tumor and stroma compartments in the tumor (T) and stroma (S) compartments

Analysis of individual tumor nests



Dominant immune phenotypes*



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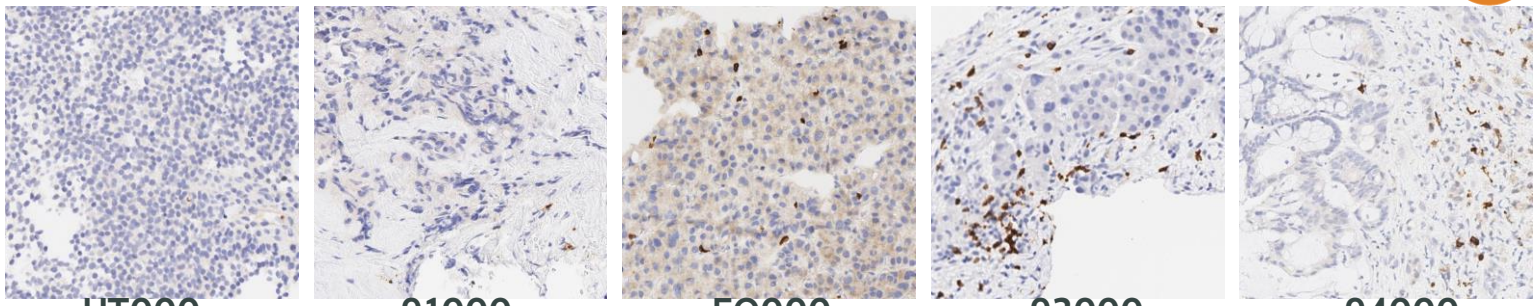
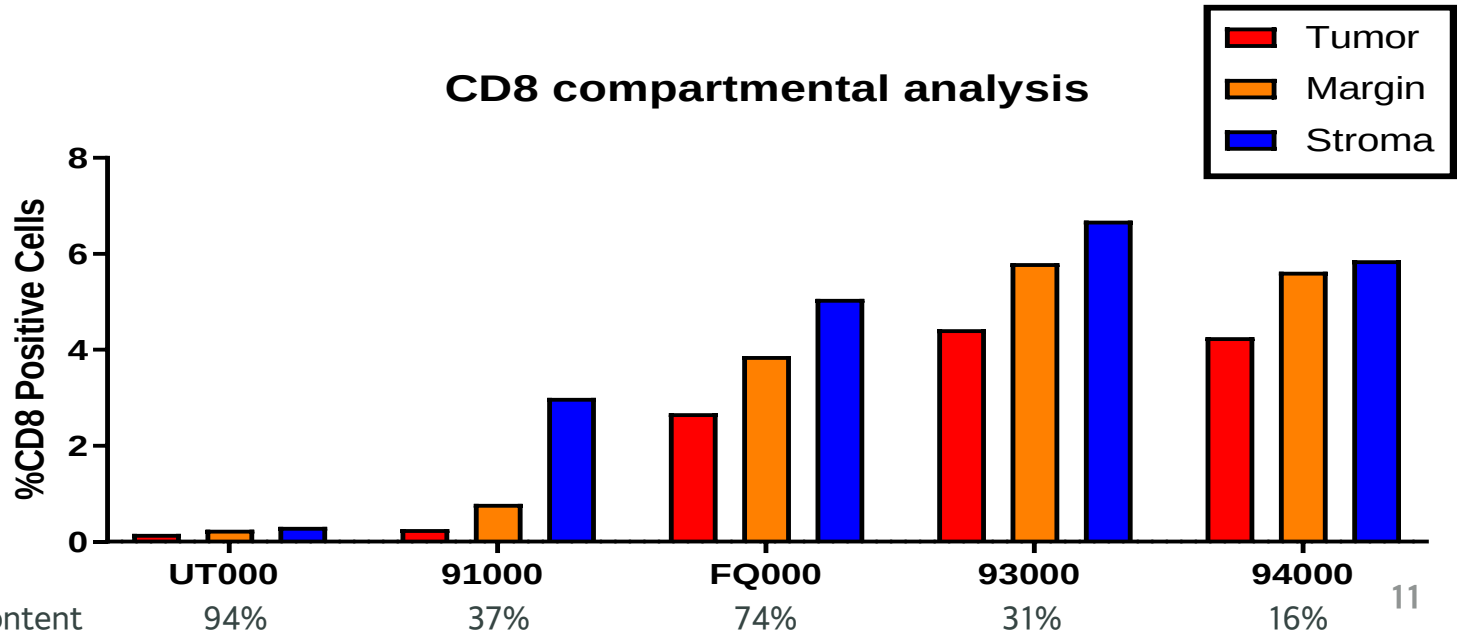
Compartmental analysis revealed differential localization of CD8 positive cells in clinical samples

CD8 IHC compartmental analysis revealed 2 classes of samples

1. Samples that displayed immune desert phenotype
   - ➢ Limited CD8+ cells was detected in all compartments
   - ➢ UT000, 91000
2. Samples that displayed mixed immune phenotypes
   - ➢ Heterogeneous distribution of CD8+ cells predominantly in the stroma compartment
   - ➢ e.g. 93000, 94000
   - ➢ Tumor Nest Quantification may provide additional insights



UT000 (Ovarian) | 91000 (Prostate) | FQ000 (Hepatocellular) | 93000 (Endometrial) | 94000 (Colorectal)



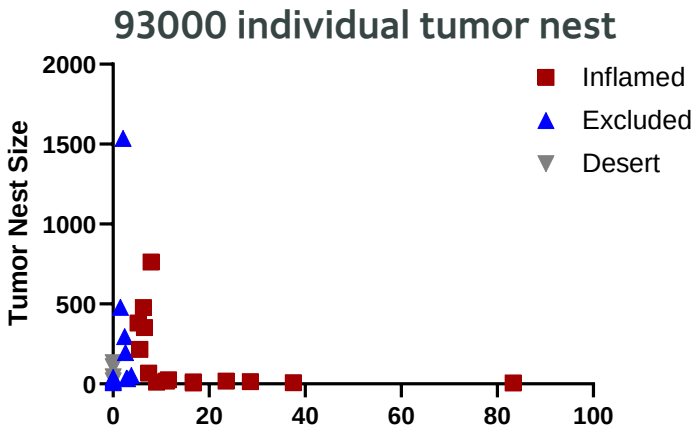
CD8 tumor nest analysis further refined the immune landscape of heterogeneous tumor samples

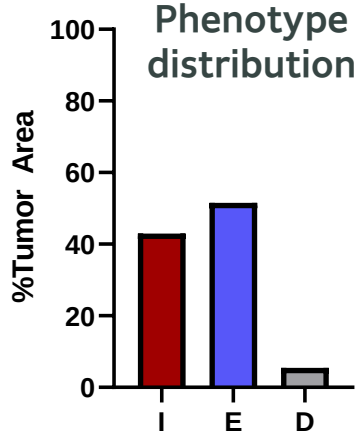
Perform analysis of individual tumor nests

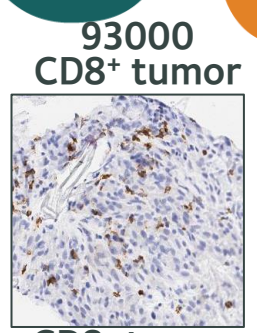
- Characterized the distribution of immune phenotypes of tumor nests relative to nest size

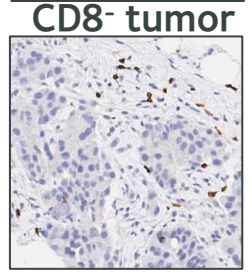
Revealed distribution of immune phenotypes distinct from compartmental analysis

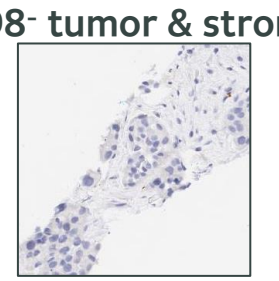
- 93000 and 94000 shown comparable CD8 positivity by compartmental analysis
- 93000 displayed a mix of inflamed and excluded tumor nests
- 94000 shown strong evidence of immune exclusion

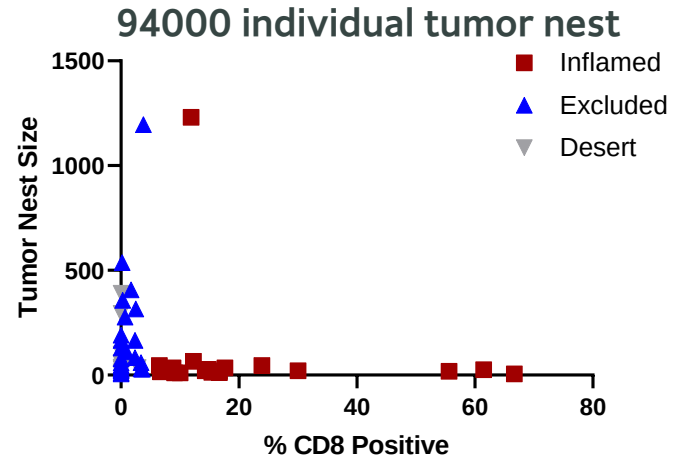


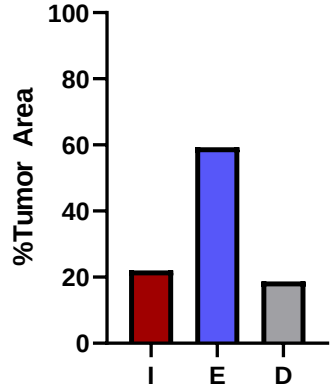












CD8 tumor nest analysis improves the definition of heterogeneous immune excluded tumors

Preclinical data provided scientific rationale to evaluate myeloid derived suppressor cells (MDSC) as SRK-181 biomarkers

Multiplex immune biomarkers

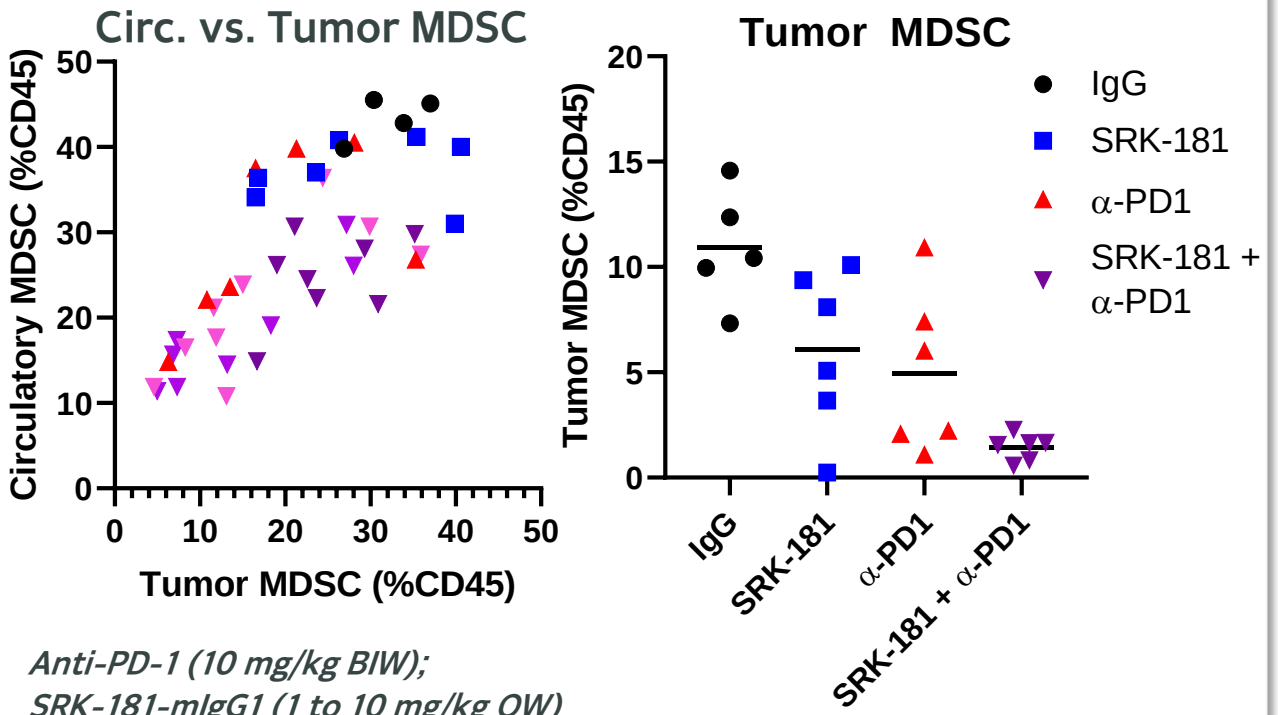
MDSCs have immune suppressive functions[5]

- Heterogeneous population of myeloid cells
- Two key subtypes of MDSCs were most studied :
  - ➢ Granulocytic MDSC (gMDSC)
  - ➢ Monocytic MDSC (mMDSC)
- Play a critical role in tumor development

SRK-181 alone or in combination with anti-PD1 reduced level of MDSC[1]

SRK-181 and anti-PD1 combination treatment reduced MDSC level, thereby supporting MDSC as a biomarker for SRK-181

Tumor MDSC were decreased at day 10 following SRK-181 and α-PD1 treatment in MBT-2 model[1]



Anti-PD-1 (10 mg/kg BIW);
SRK-181-mIgG1 (1 to 10 mg/kg QW)

1. Martin CJ, et al. Sci Transl Med 2020;12(536)
5. Elliott et al. Frontiers in Immunology. 2017;Vol. 8: Article 86
6. https://investors.scholarrock.com/investors-media/events-presentations

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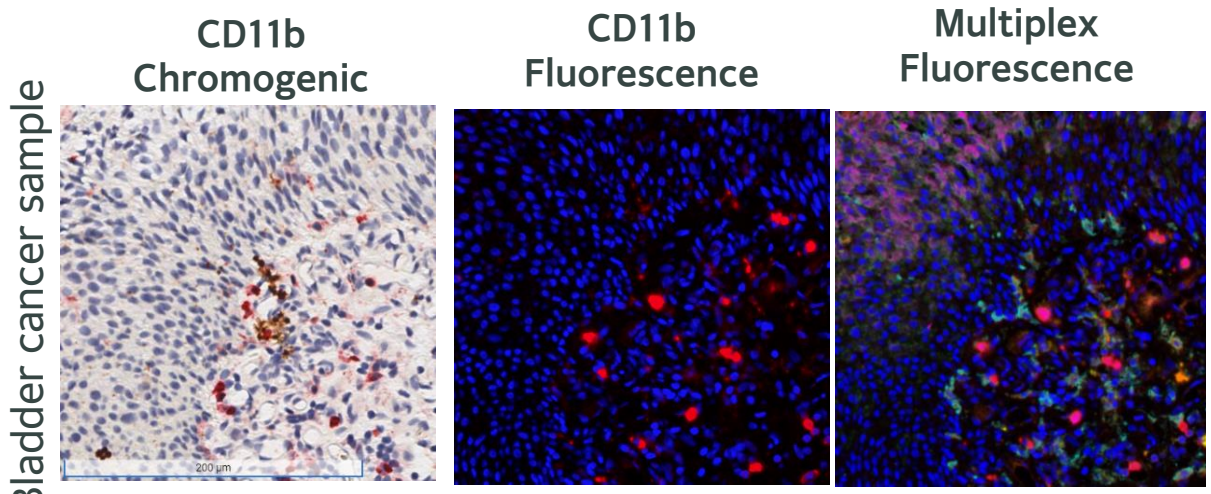
Selection of cell surface markers and antibody optimization for tumor MDSC assay development

- The prevalence of tumor MDSC in clinical studies is highly variable depending on markers utilized[5]
- Multiple markers were selected and evaluated to distinguish MDSC subtypes from other monocytes
  - Proposed markers include CD11b, CD33, CD66b, CD14, cD15 and HLA-DR
  - Chromogenic assay was performed for each Ab to define IF dynamic range
  - *Order of Ab staining was optimized for most robust signals (data not shown)*

Chromogenic Validation e.g. CD11b



Matching localization of CD11b staining between chromogenic monoplex assay and fluorescence assay

Defined IF dynamic range (exposure values):

- Confirmed minimal contribution of auto-fluorescent or bleed through artifacts
- Established gating strategy for image analysis

5. Elliott et al. Frontiers in Immunology. 2017;Vol. 8: Article 86

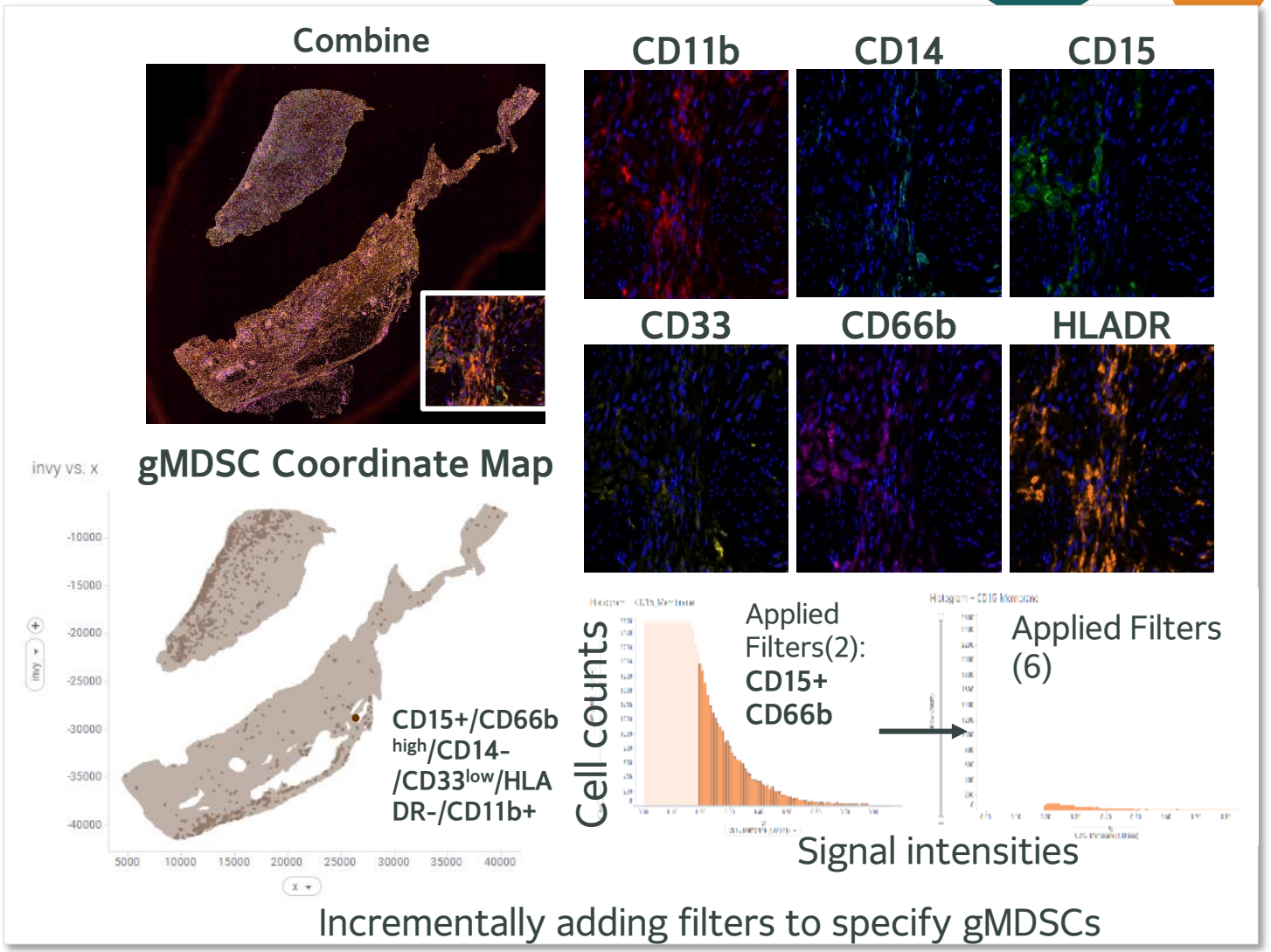
Established the signal intensity filter for each cell surface marker to enable identification of tumor MDSC

Signaling intensity for each marker was assessed and Intensity filters (cutoff) were used to identify MDSC

- Binary intensity selection: + vs. -
  e.g. distinguish CD14+ mMDSC from CD15+ gMDSC
- Binned categorically: intensity range
  e.g. Distinguish HLA-DR$^{low-neg}$ mMDSC from HLA-DR^{neg} gMDSC
- Signal Intensity filters were applied sequentially

Multiplex IHC distinguish putative tumor MDSC from other monocytes by defining cut-off for each marker

Categorization reviewed and confirmed by pathologists



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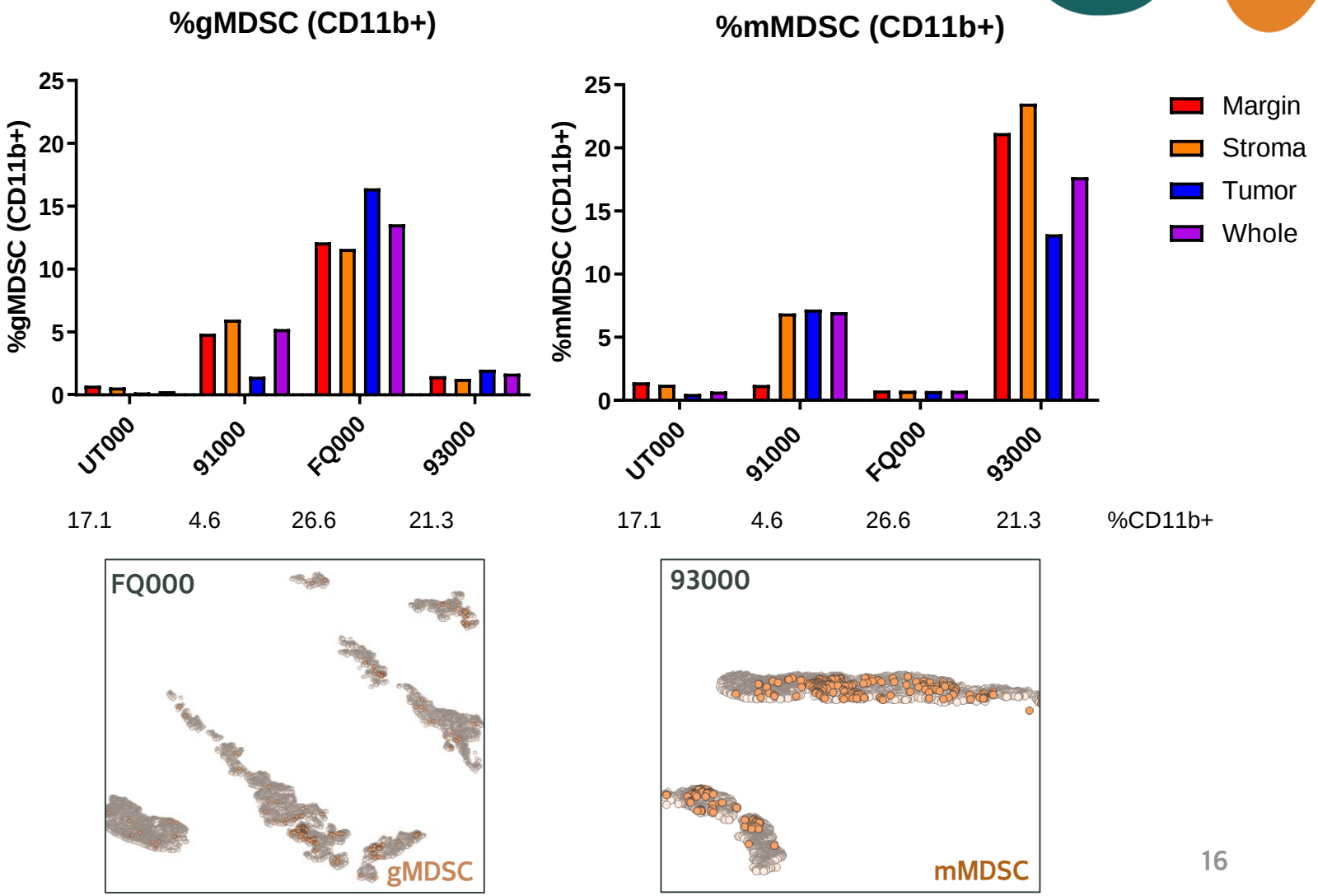
Multiplex IHC identified variable level of putative tumor MDSC in clinical samples

- Both putative tumor gMDSC and mMDSC were identified by the multiplex IHC assay
- Co-ordinate map further enables visualization of the location of putative MDSCs within the tumor
- The relative prevalence of gMDSC and mMDSC varies across samples
- No correlation of MDSC to CD8 has been observed in the limited number of samples



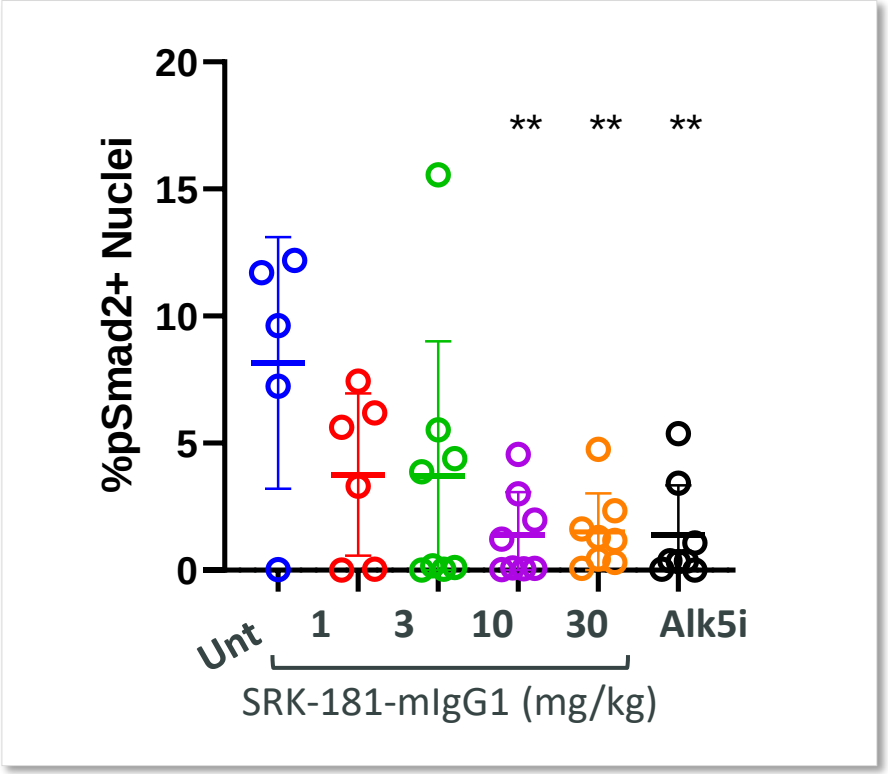
Preclinical data provided scientific rationale to support tumor P-Smad2 as a biomarker for SRK-181

TGFβ pathway evaluation
Assessment of signaling pathway

- Phospho-Smad2 (P-Smad2) is a key signaling mediator of TGFβ pathway[4]
  - Phosphorylation of Smad2 and Smad3 leads to heteromeric complex formation that translocates into the nucleus to regulate target gene expression
- Inhibition of TGFβ1 by SRK-181 leads to reduced pSmad2 in MBT2 bladder cancer model

SRK181 treatment reduced tumor P-Smad2 signal, thereby supporting P-Smad2 as a PD biomarker

MBT-2 Tumor P-Smad2 was analyzed at day 10 post-treatment of SRK-181-mIgG1 dosed weekly



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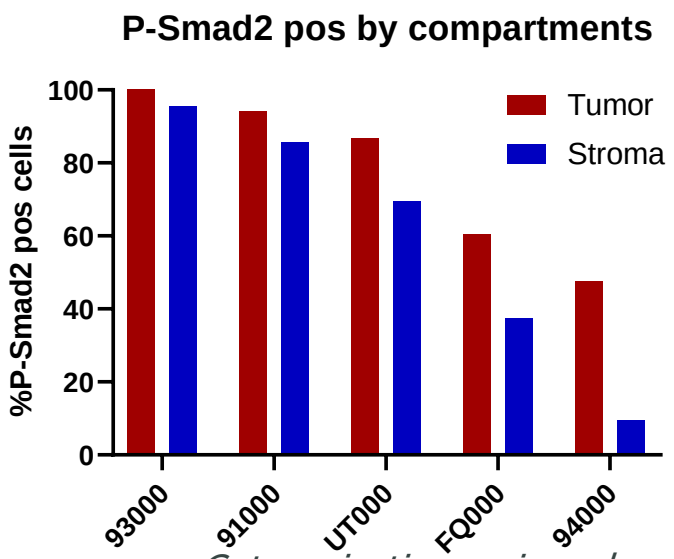
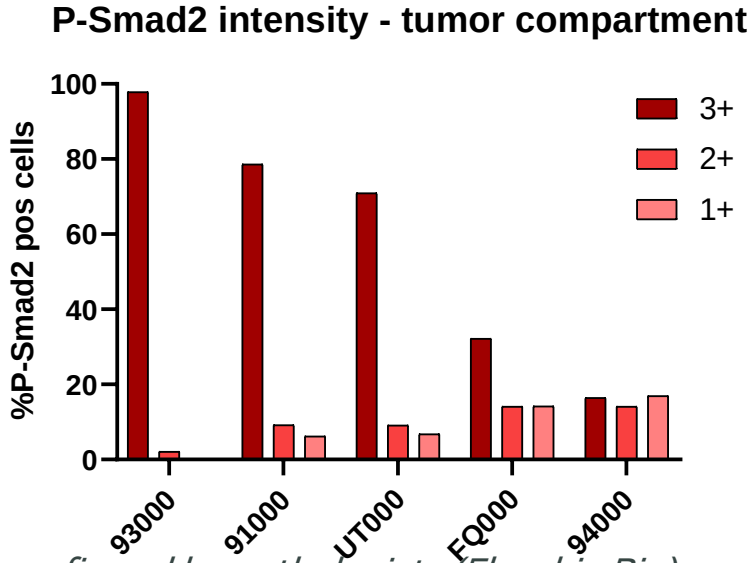
4. Liu S, et al. Signal Transduct Target Ther 2021;6(1):8

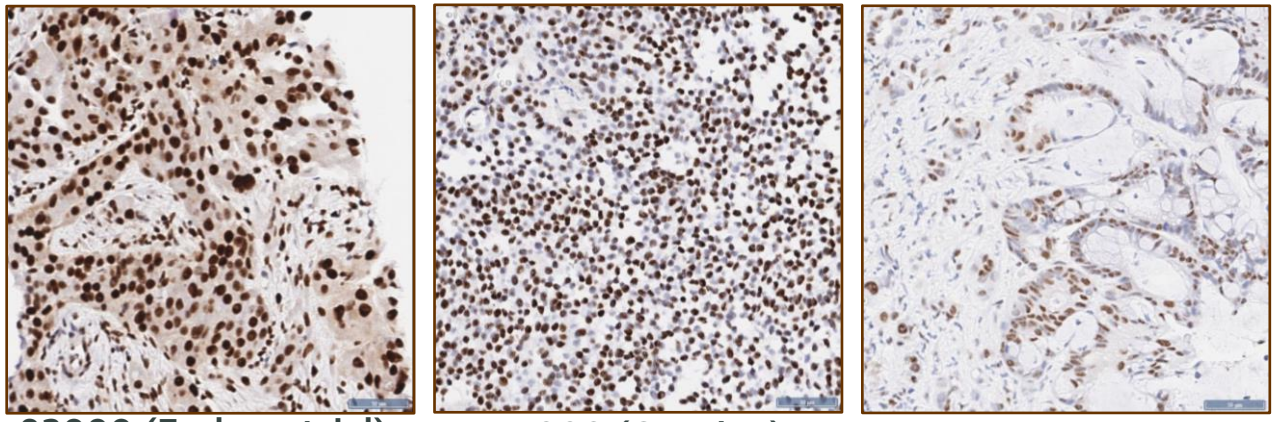
P-Smad2 assay performs within anticipated dynamic range for clinical samples from DRAGON Part A

- P-Smad2 IHC assay was developed using commercial human samples
  - Total Smad2 IHC was used as an orthogonal validation method
- A dynamic range of P-Smad2 signaling intensity was observed
  - High P-Smad2 samples showed comparable signals across compartments and predominant 3+ intensity e.g. 93000
  - Heterogeneous (UT000) or moderate P-Smad2 intensity (94000) samples displayed different readouts
- Compartmental and intensity analyses enable more exhaustive assessment of P-Smad2





Categorization reviewed and confirmed by pathologists (Flagship Bio)



93000 (Endometrial) UT000 (Ovarian) 94000 (CRC)

SRK-181 dose-dependent increase in a preclinical study demonstrates the utility of circulatory TGFβ1 as a target engagement biomarker

Additional blood-based biomarkers

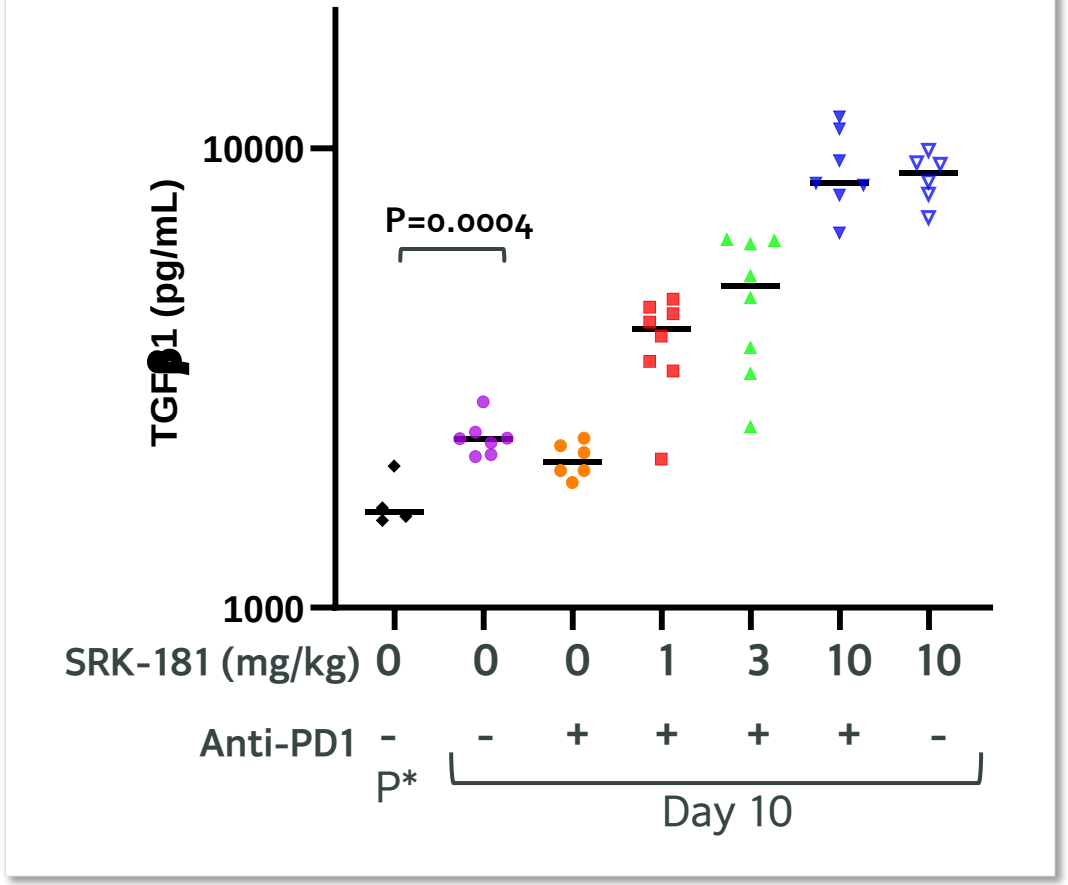
Measurement of circulatory TGFβ1 may provide direct evidence of SRK-181 binding to therapeutic target

Pharmacology study demonstrated SRK-181 dose-dependent increased of circulatory TGFβ1 in MBT-2 mouse tumor model

- Measurement of total TGFβ1 in circulation post-acidification (predominantly latent-TGFβ1)
- Circulatory TGFβ1 maybe elevated in tumor-bearing mice

Circulatory TGFβ1 may serve as a target engagement biomarker

Circulatory TGFβ1 was analyzed at day 10 post-tx; SRK-181-mIgG1 dosed weekly at 1, 3 and 10 mg/kg



P pre-implant*

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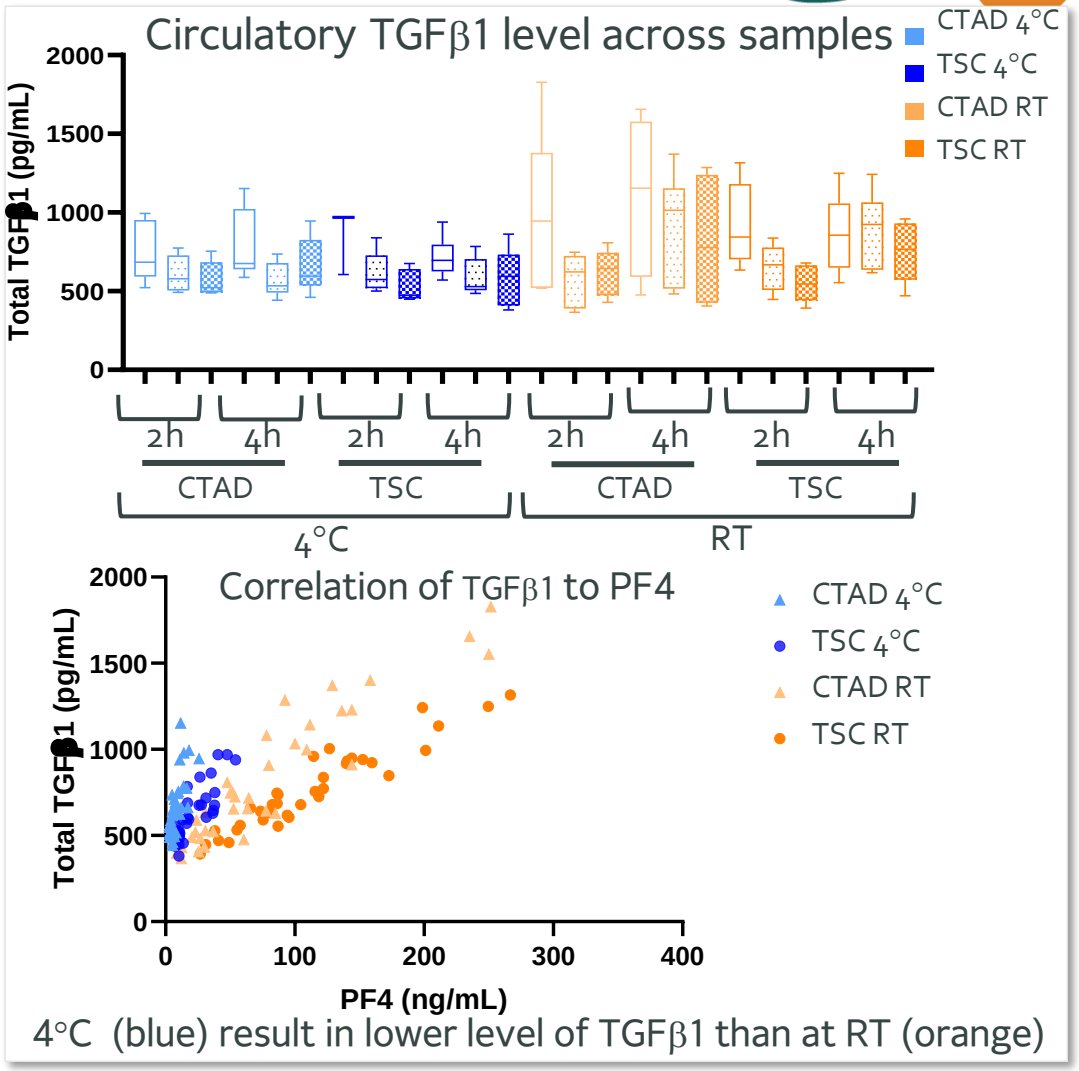
Strategy to mitigate platelet activation to minimize SRK-181 independent effect on circulatory TGFβ1

- Platelets enriched with TGFβ could be released if activated e.g. during clinical sample processing
  - ➢ May result in elevated TGFβ1 level independent of SRK-181 treatment
- A pilot study was performed to identify sample processing method to minimize platelet activation
  - ➢ Multiple platelet poor plasma (PPP) processing methods were evaluated using HV samples
  - ➢ Multiple factors affect platelet activation including temperature, anti-coagulant tube and centrifugation
  - ➢ Platelet factor 4 (PF4) is included to identify samples with significant platelet activation
- High level of PF4 correlated to high TGFβ1 level
  - ➢ Suggest used PF4 level to identify platelet activated samples



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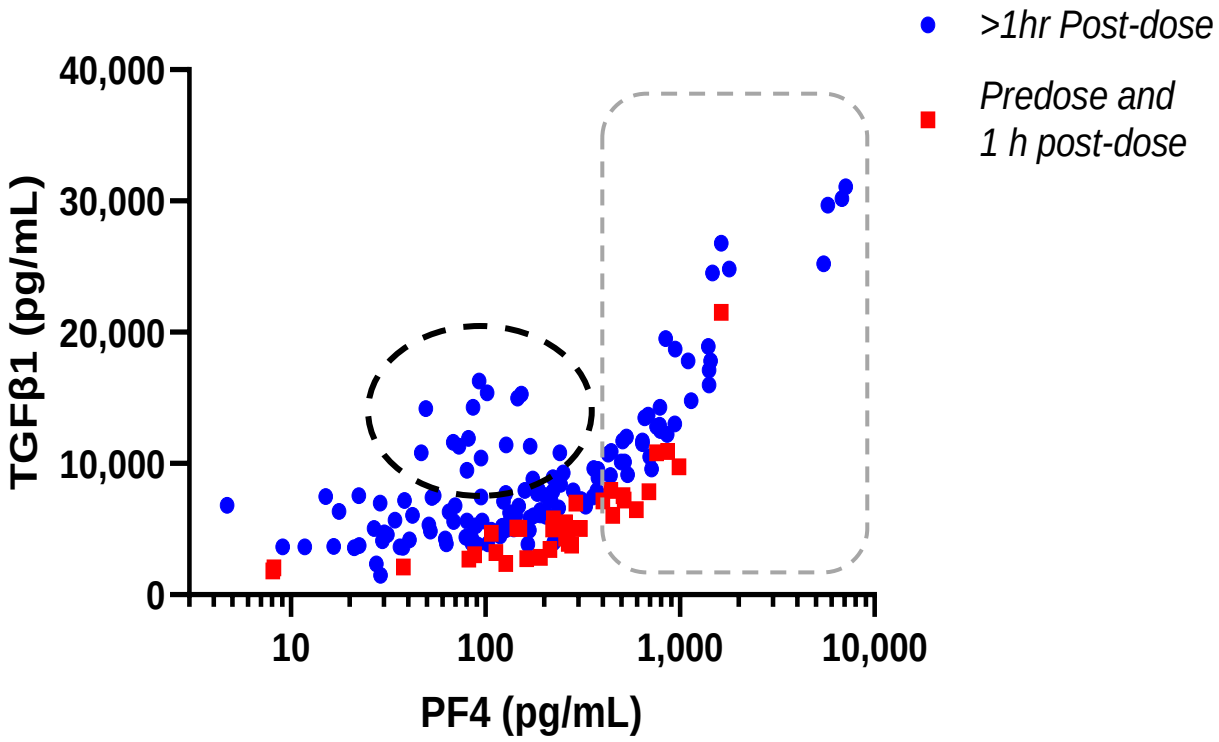
Preliminary analysis demonstrates SRK-181 may modulate circulatory TGFb1 in the DRAGON study

Preliminary data support PF4 readout may identify platelet-activated samples

- Samples with high PF4 demonstrate a strong correlation between PF4 and TGFβ levels (dotted square)

SRK-181 treatment increased the level of circulatory TGFβ1 when platelets were minimally activated (dotted circle)

- Caveats: very limited data set
- Target composition, saturability and tumor dependency will be assessed



Additional analysis will be presented at SITC 2022

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Next wave of immuno-oncology biomarkers

Robust biomarker data will increase probability of success for IO programs

1. Focus on the mechanism of action of the therapeutics supported by robust preclinical data
2. Include comprehensive image analysis of tumor biomarkers to evaluate the tumor immune landscape
   - Account for sample heterogeneity by assessing individual tumor nests within a sample
   - Measure spectrum of signaling intensity of individual antibody in multiplex assays to enable selection of specific immune cell types
3. Complement tumor biomarkers with paired circulatory biomarker data
   - Assessment of both tumor and circulatory MDSC in parallel
4. Enrich for pathway-centric biomarkers for target engagement and modulation of downstream signaling to achieve mechanistic POC
5. Aspiration: identify putative biomarkers that correlate with anti-tumor response

Acknowledgements

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Others

DRAGON

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Lu Gan
Heather Klodzinski
Ann Marie Kennedy
Connie Martin
Thomas Schurpf
All past and current team members

Flagship Biosciences collaborators
Roberto Gianani
Chuck Caldwell
Clara Troccoli
Caroline Chandler
Image Analysis team

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