Development of a Comprehensive 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 that is currently 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 efficacy and safety of SRK-181 in human subjects will not be discussed today.

SRK-181 overview

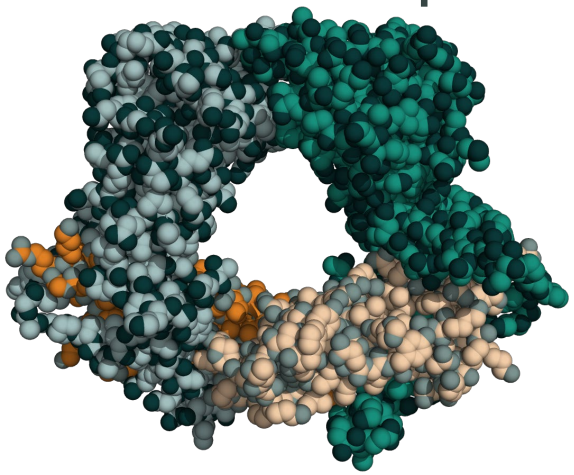
- Fully human monoclonal antibody[1]
- SRK-181 binds latent TGFβ1 with picomolar affinity
  - Binds all TGFβ1 large latent complexes
  - Cross-reacts with mouse, rat, cyno
  - Minimal or no binding to latent TGFβ2 and TGFβ3 isoforms or to active TGFβ growth factors
- Potent and selective inhibitor of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

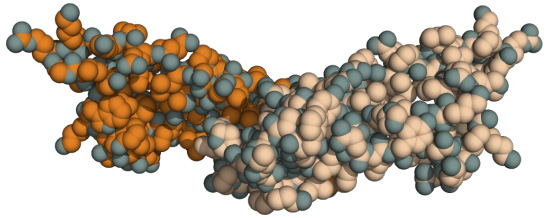
Targeting Latent TGFβs Creates Multiple "Handles" For Selectivity[1]

Latent TGFβ



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

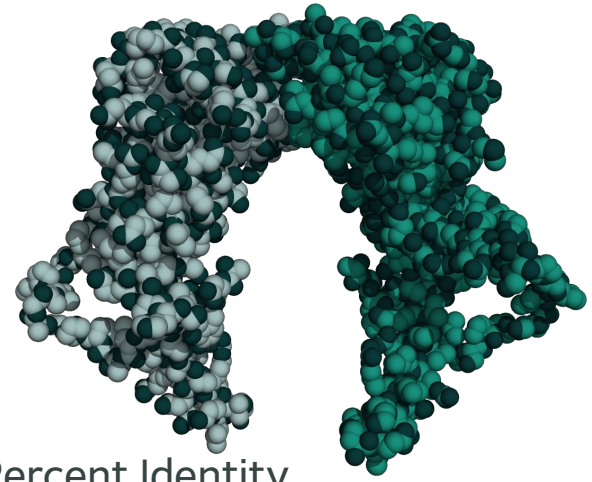
Mature Growth Factor



Percent Identity

TGFβ1	TGFβ2	TGFβ3	
	71.4	76.8	TGFβ1
		79.5	TGFβ2
			TGFβ3

Prodomain



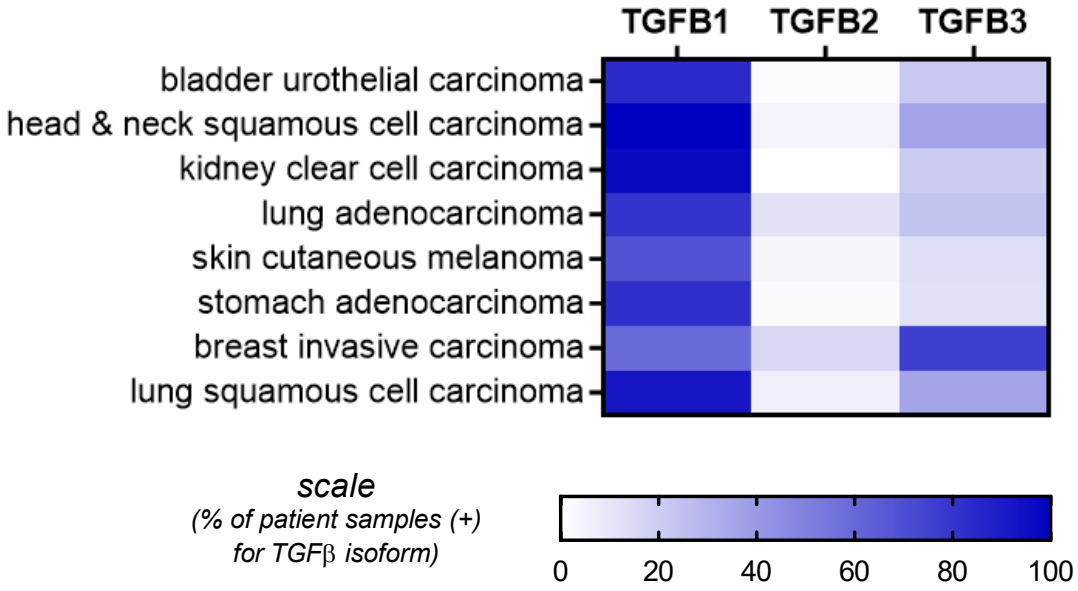
Percent Identity

TGFβ1	TGFβ2	TGFβ3	
	37.4	37.1	TGFβ1
		48.7	TGFβ2
			TGFβ3

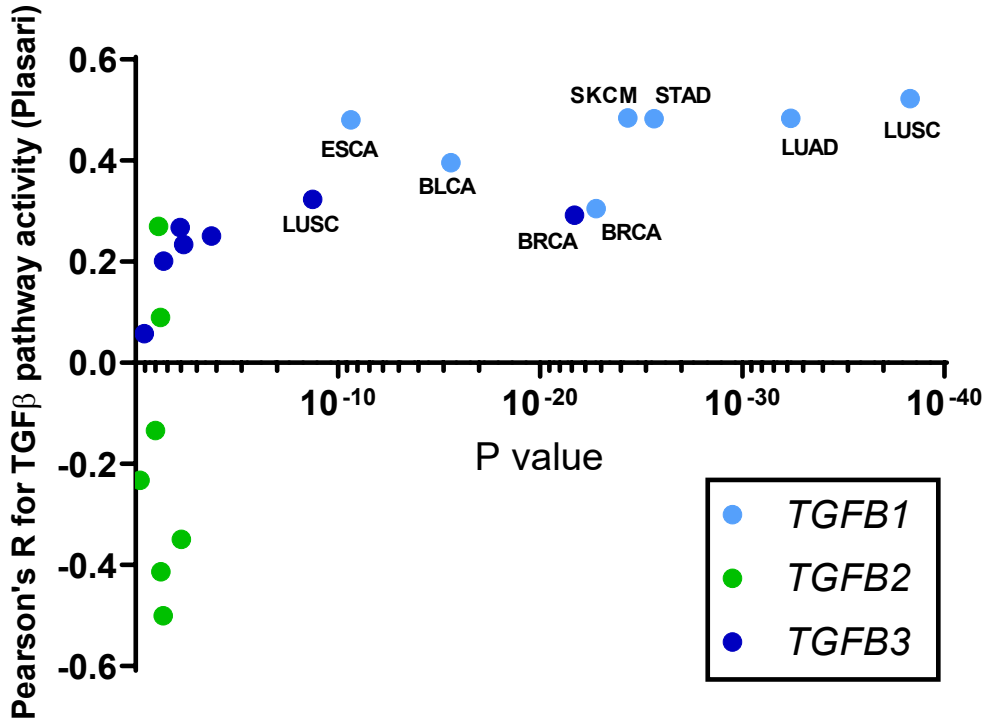
1. Martin CJ, et al. Sci Transl Med 2020;12(536)

TGFβ1 implicated as the most critical TGFβ isoform in Human Tumors

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



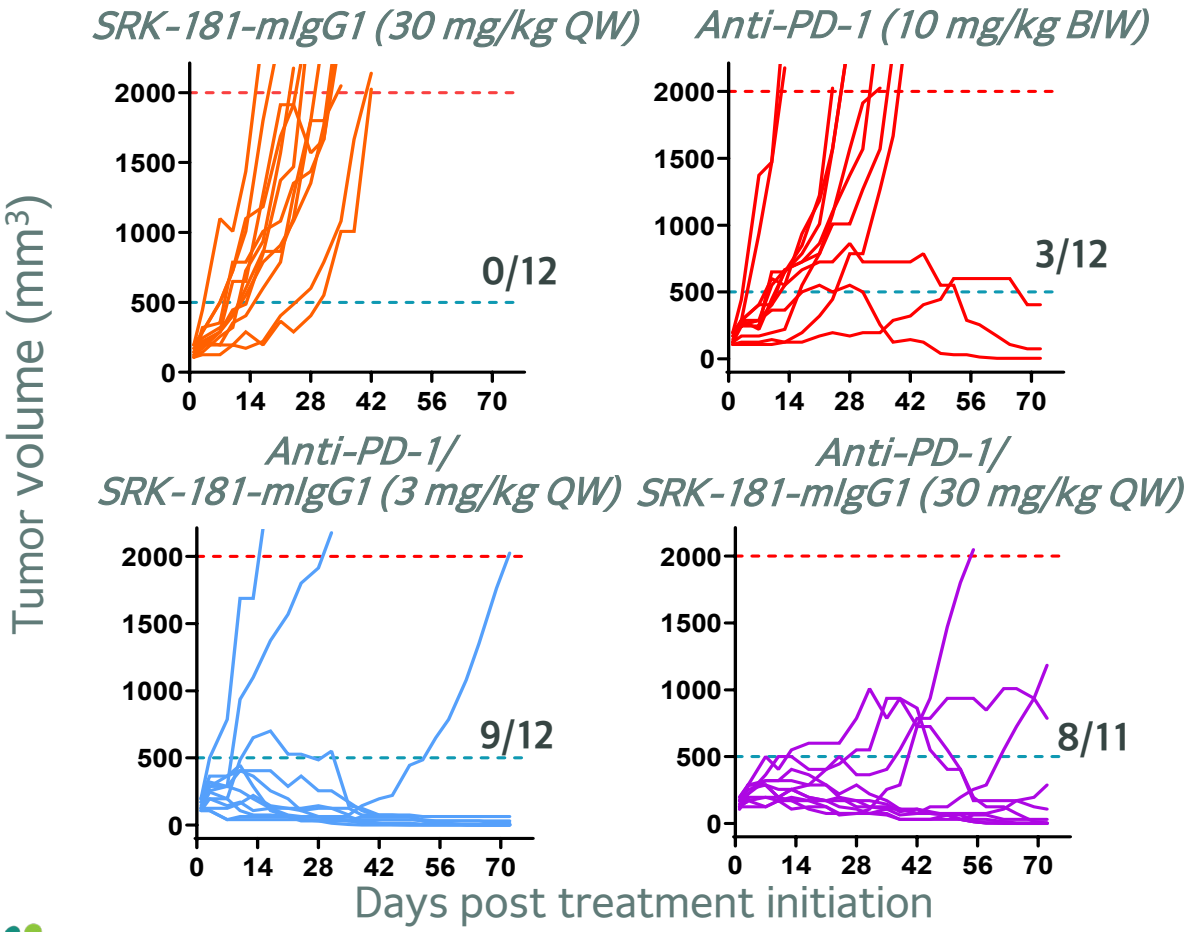
TGFβ1 expression correlates with TGFβ pathway activity in tumors[1].

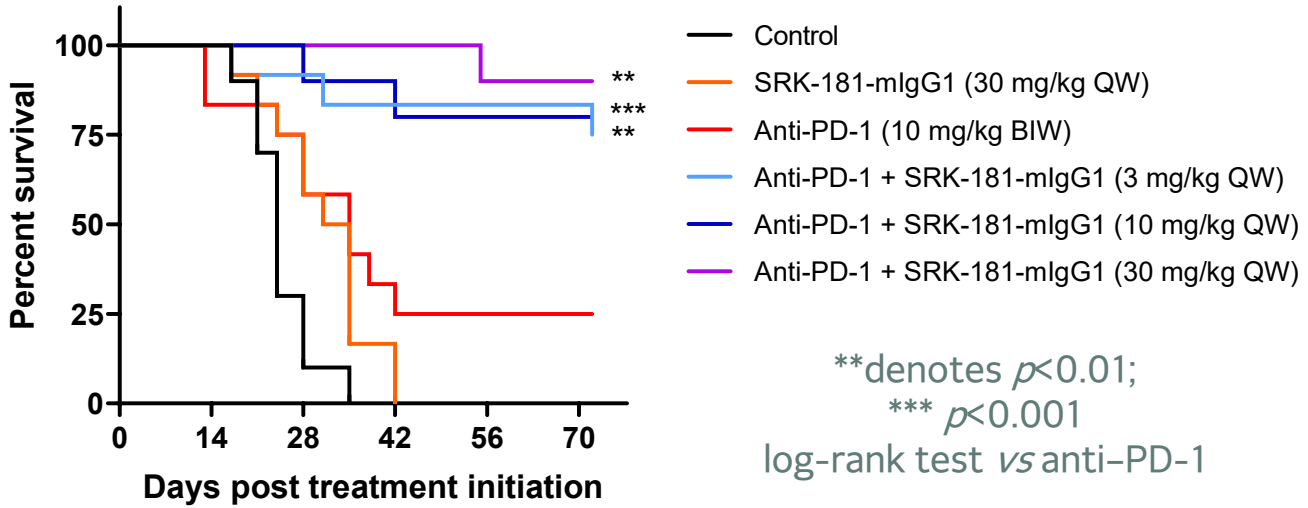


1. Martin CJ, et al. Sci Transl Med 2020;12(536)

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

Cloudman S91 melanoma model: Combination treatment led to tumor regression and survival benefit[1]





**denotes $p<0.01$;
*** $p<0.001$
log-rank test *vs* anti–PD-1

Similar results demonstrated in MBT-2 urothelial cancer model and EMT-6 breast cancer model[1]

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

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DRAGON Phase 1 Clinical study

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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 expected to 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 I 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

e.g. Tumor CD8

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

e.g. Tumor P-Smad2

Tier 2: Expand to orthogonal complementary biomarkers and approaches

3 **Multiplex immune biomarkers**

e.g. Tumor MDSC

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

e.g. Circulatory MDSC

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

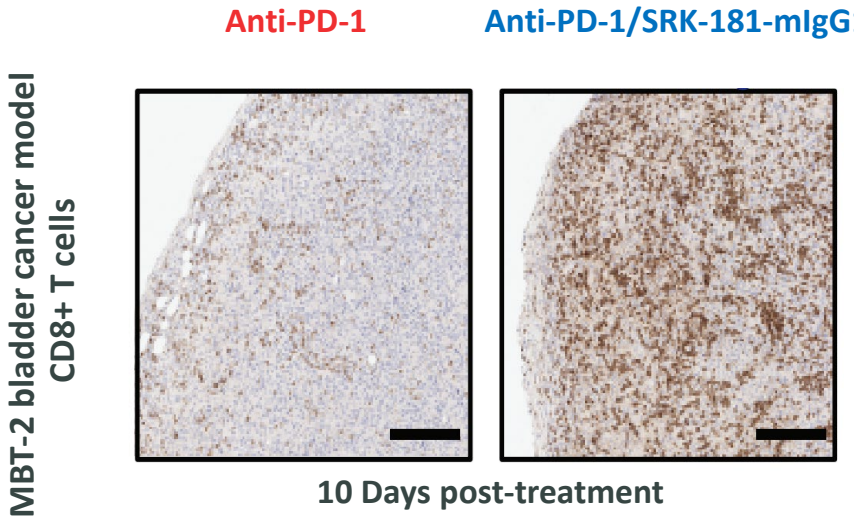
Immunophenotyping
Assessment of immune landscape

- 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/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

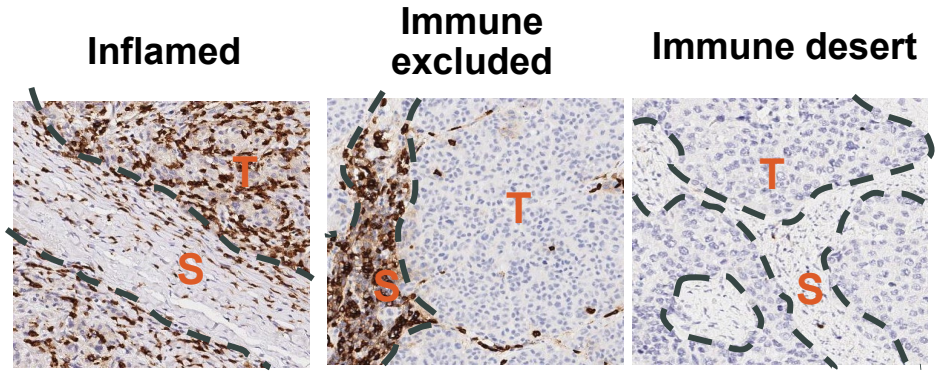
- Performed digital pathology analysis to characterize the tumor immune phenotypes
  - ➢ Quantify CD8⁺ T cells within tumor, tumor margin and stromal compartments
- Established the CD8⁺ cell baseline signals for bladder cancer and melanoma (DRAGON indications)

Intratumor CD8 is used to characterize the tumor immune phenotypes

** %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*

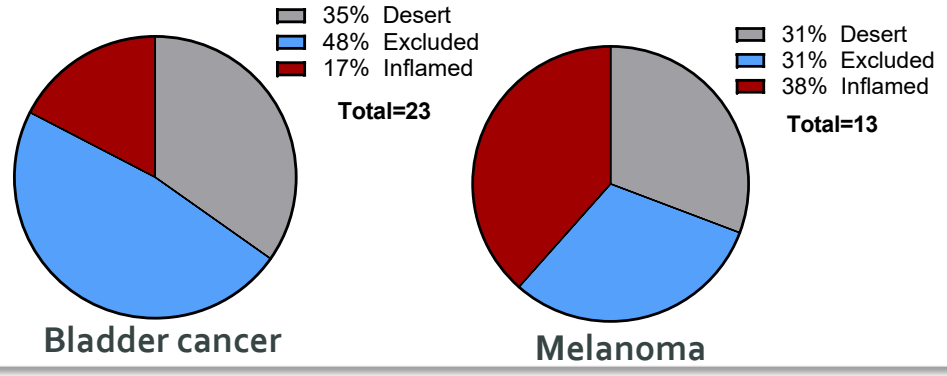
Categorization reviewed and confirmed by pathologists (Flagship Bio)

Classification of 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

Dominant immune phenotypes*



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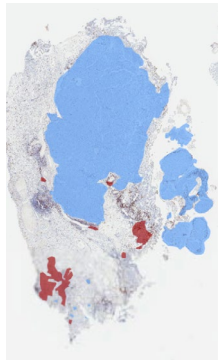
Evaluating CD8 of individual tumor nests improves the definition of heterogeneous immune excluded tumors

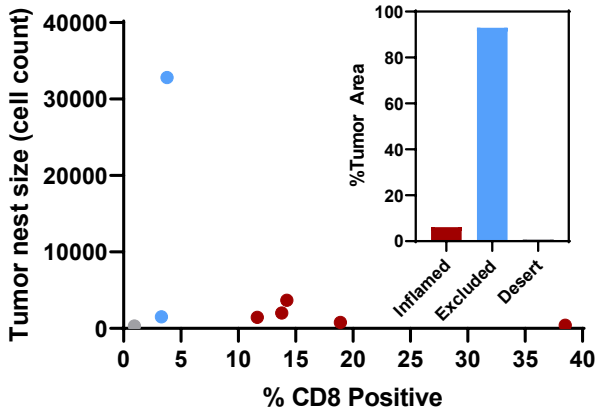
Perform analysis of individual tumor nests

- Quantified CD8⁺ T cells in tumor and tumor margin of each tumor nest
- Characterized the distribution of immune phenotypes of tumor nests relative to nest size
- Enabled more exhaustive assessment of the tumor immune microenvironment

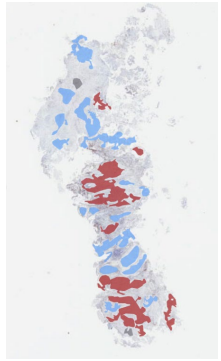
Intratumor CD8 is being evaluated in the DRAGON study

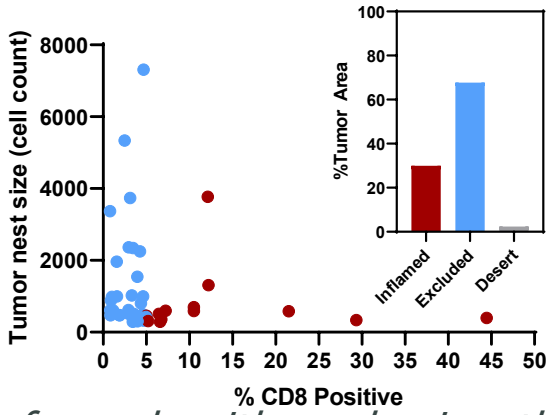
Refinement of tumor nest analysis to evaluate the distribution of immune phenotypes





Avg. %CD8⁺ cells in tumor = 6.1%





Avg. %CD8⁺ cells in tumor = 6.3%

Examples of sample with predominantly immune excluded phenotype tumor nests (blue)

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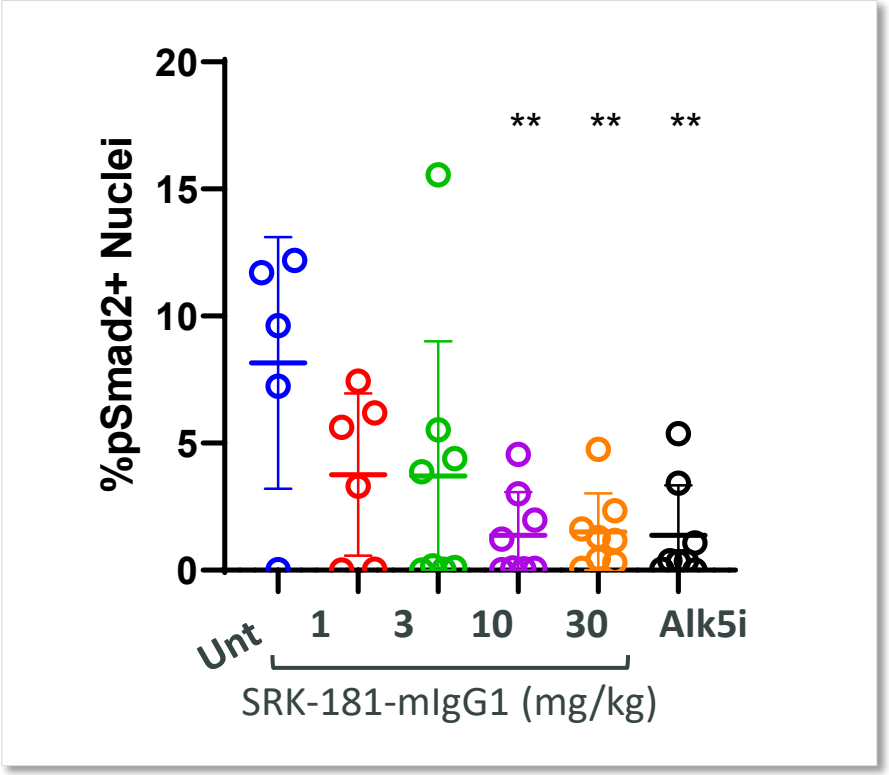
Preclinical data provided scientific rationale to support tumor P-Smad2 as a biomarker for SRK-181

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 translocate 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

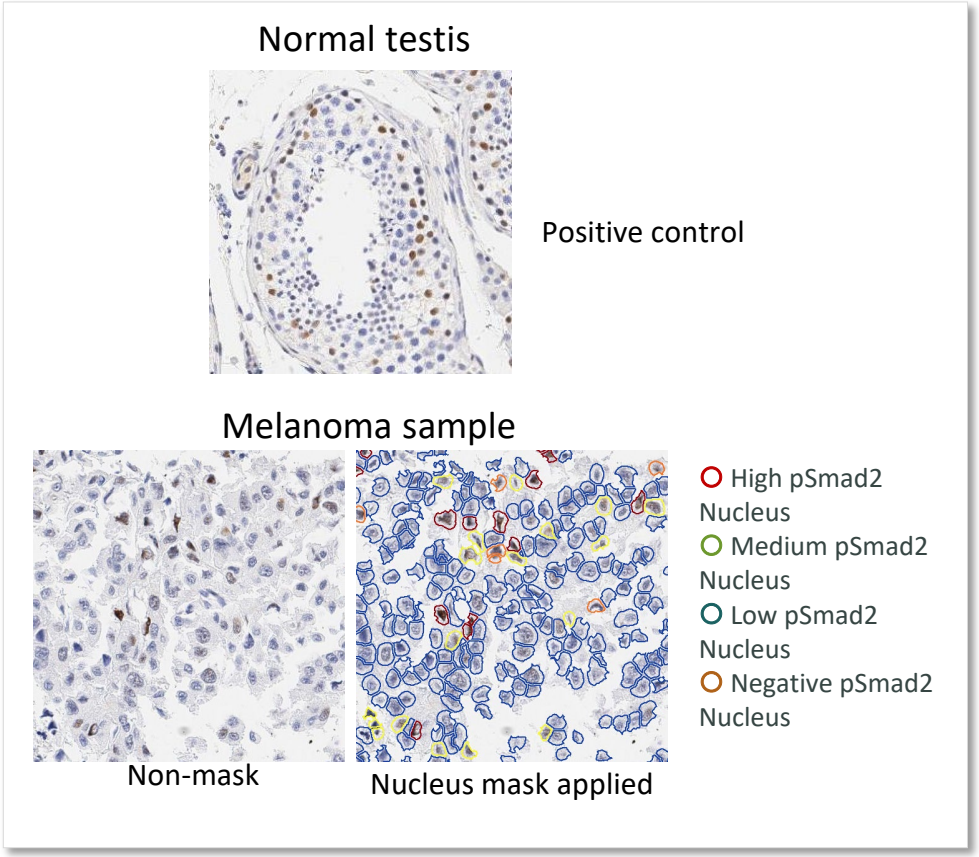
Establishment of P-Smad2 IHC assay to assess TGFβ signaling in clinic

P-Smad2 IHC assay was developed using commercial normal and cancer samples

- Positive control testis shows a consistent and robust staining pattern of P-Smad2 IHC
- An example melanoma sample stained for P-Smad2 identified a range of P-Smad2 nucleus staining intensity from high (red), medium (orange), low (yellow), to negative (blue) using digital image analysis
- Total Smad2 IHC is used as an orthogonal method for validation and demonstrates comparable staining as phospho-Smad2 (data not shown)

Level of tumor P-Smad2 is being evaluated in the DRAGON study

P-Smad2 IHC assay development



Categorization reviewed and confirmed by pathologists (Flagship Bio)

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

Multiplex immune biomarkers

Myeloid derived suppressor cells have immune suppressive functions[5]

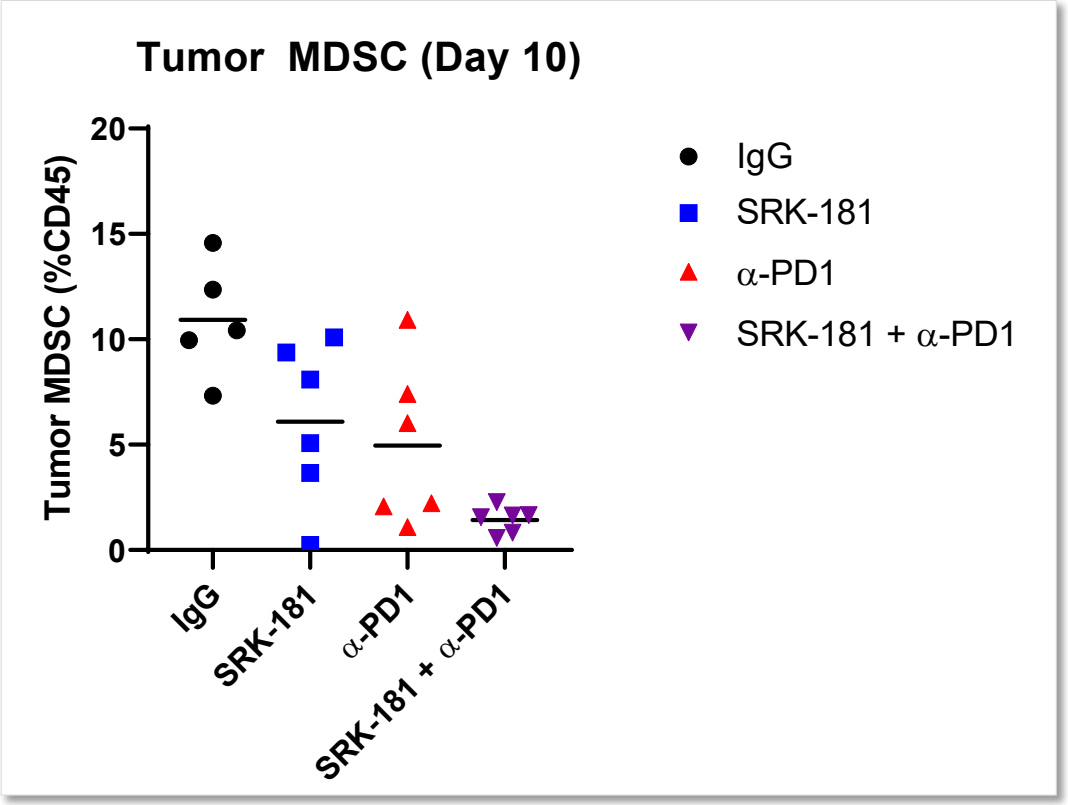
- They are a heterogeneous population of myeloid cells
- Two key subtypes of MDSC were most studied:
  - Granulocytic MDSC (gMDSC)
  - Monocytic MDSC (mMDSC)
- They play a critical role in tumor development

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

- Similar results were observed for circulatory MDSC[6] (data not shown)

SRK-181 and anti-PD1 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]



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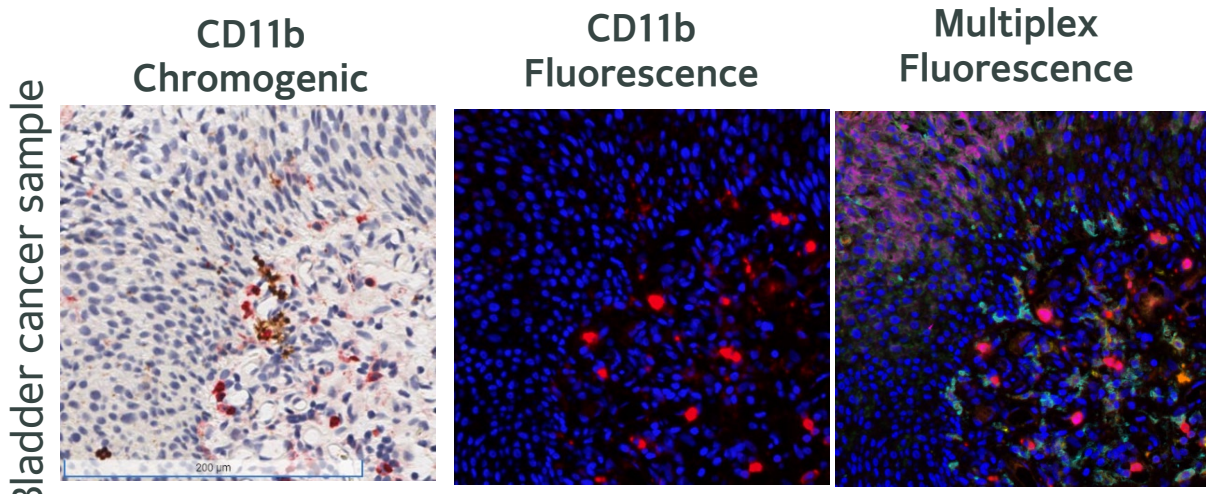
Selection of cell surface markers and antibody optimization for tumor MDSC assay development

- The prevalence of tumor MDSC in clinical studies are highly variable depending on markers utilized[5]
- Multiple markers were selected and developed to distinguish MDSC subtypes from other monocytes
  - Proposed markers are 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):

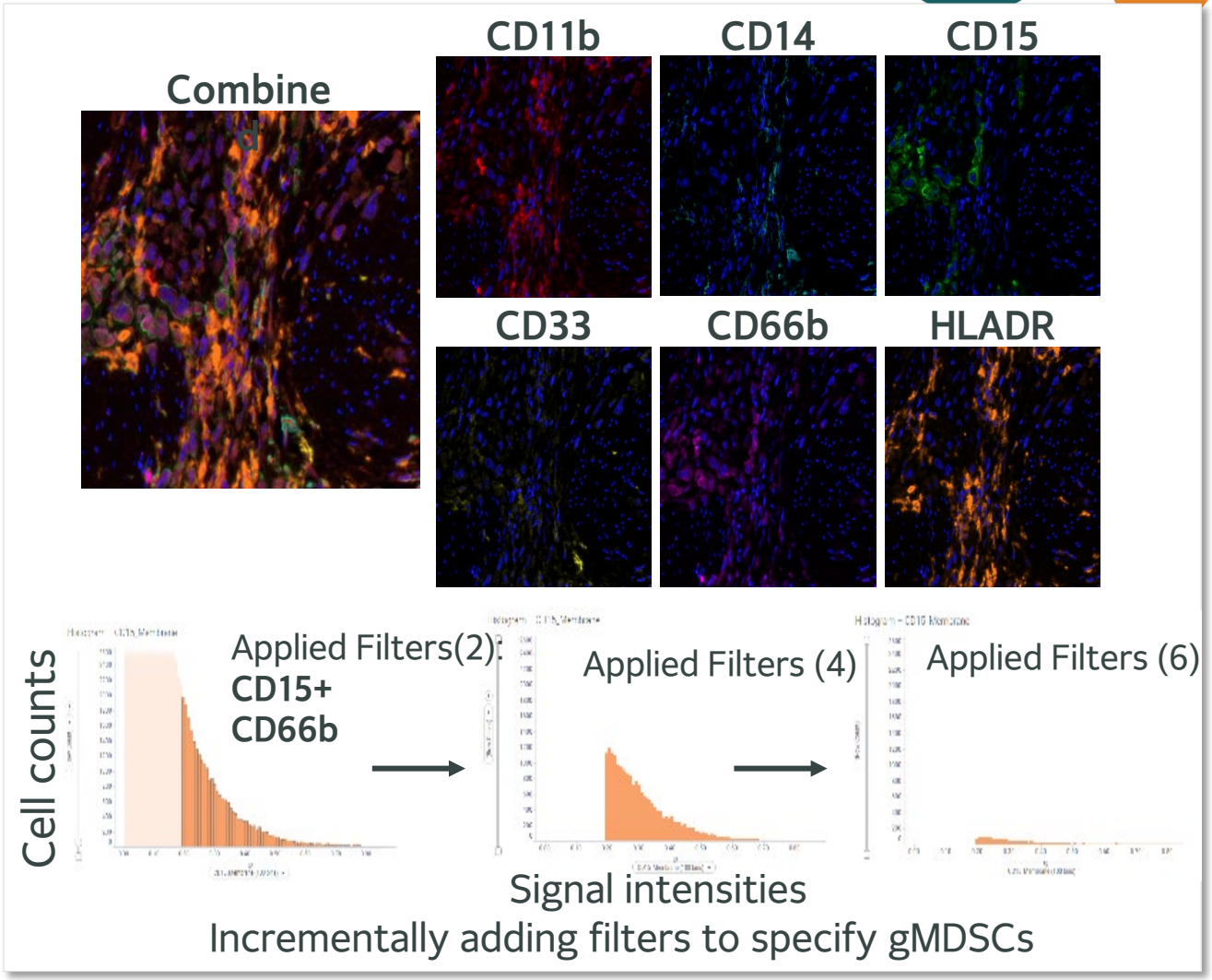
- Confirmed minimal contribution of auto-fluorescent or bleed through artifacts
- Established gating strategy for image analysis

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
- 2 types of signal Intensity filters (or cutoff) were used to identify MDSC
  - ➢ Binary intensity selection i.e. pos. vs. neg.
    e.g. distinguishing CD14+ mMDSC from CD15+ gMDSC
  - ➢ Binned categorically i.e. define a range of signal intensities
    e.g. Distinguish HLA-DR $^{low-neg}$ mMDSC from HLA-DR ^{neg} gMDSC
  - ➢ Signal Intensity filters were applied sequentially

Categorization reviewed and confirmed by pathologists



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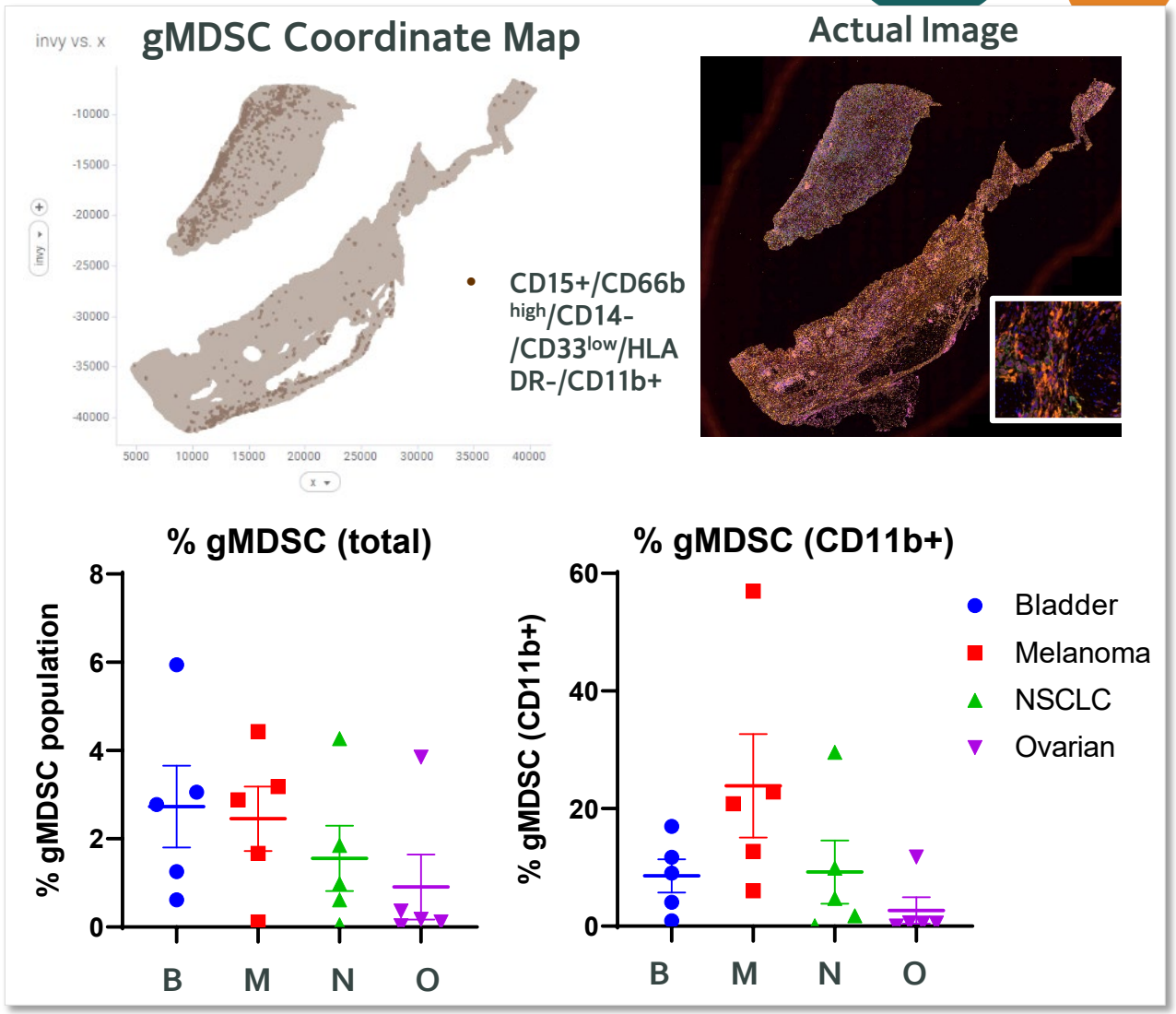
Prevalence of tumor MDSC in selected indications

Current multiplex IHC study distinguished putative tumor MDSC from other monocytes by defining cut-off for each marker

- Performed analysis in 4 indications to establish signal dynamic ranges
- Confirmed signal consistency between samples and across indications
- Observed lower level of gMDSC in ovarian cancer compared to other indications
- Low prevalence of mMDSC was identified (data not shown)

Both tumoral and circulatory MDSC are being evaluated in the DRAGON study



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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 the 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. Aspiration: identify putative biomarkers that correlate with anti-tumor response

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Flagship BIOSCIENCES