Development of a Comprehensive Biomarker Strategy to Support the Latent TGFβ1 Inhibitor SRK-181 Phase 1 Clinical Trial, DRAGON

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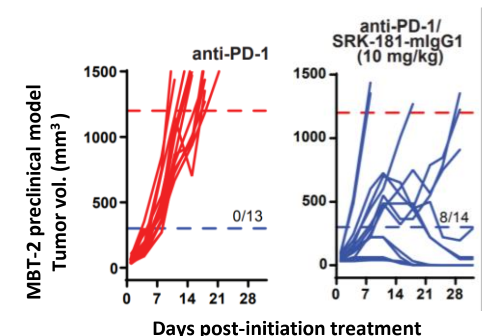
Introduction

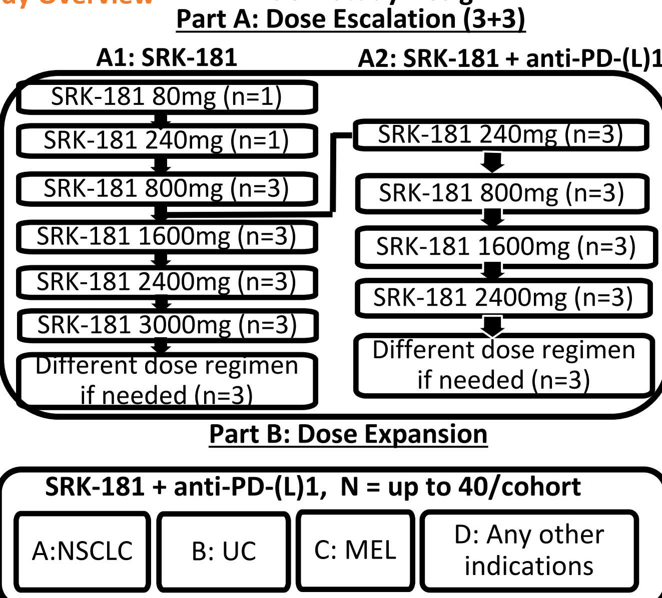
- Nearly 80% of patients do not respond to Checkpoint Inhibitor (CPI) therapies[1]
- Human data implicate TGFβ1 as a key driver of immune exclusion and primary resistance to CPIs[2,3]
- SRK-181 is a fully human monoclonal antibody that potently and selectively inhibits latent TGFβ1 activation, in pre-clinical studies[2]
- SRK-181 combination treatment with anti-PD-1 overcomes CPI resistance leading to anti-tumor effects in preclinical models including MBT-2 (Fig 1)
- The DRAGON trial (NCT04291079) is a multi-center, 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[4]
- A comprehensive biomarker strategy will support the ongoing DRAGON clinical trial and further explore the mechanism of action of SRK-181

Figure 1.
SRK-181 Sensitizes Tumors to Anti-PD-1 in Preclinical Models[2]



- Mice bearing s.c. MBT-2 tumors treated with anti-PD-1 at 10 mg/kg 2X weekly alone (—) or in combination with SRK181-mIgG1* 1X weekly at 10 mg/kg (—)
- Anti-PD-1 alone has no effect while SRK-181-mIgG1* and anti-PD-1 combination results in either complete responses, tumor regressions, or control

Study Overview



Biomarker Strategy includes Evaluation of Immune Landscape and TGFβ Pathway

1. Immunophenotyping Assessment of the tumor immune landscape
- Predictive → identify hot, cold or immune excluded tumors
- PD → ability of SRK-181 to convert 'excluded' tumors to 'hot'

e.g. CD8+ T cells

2. TGFβ signaling pathway Assessment of TGFβ1 signaling pathway
- Predictive → identify target/pathway prevalence to predict response
- PD → ability of SRK-181 to modulate TGFβ pathway activation

e.g. Circulatory TGFβ1, P-Smad2

Assay Development to Enable Implementation of SRK-181 Biomarker Strategy

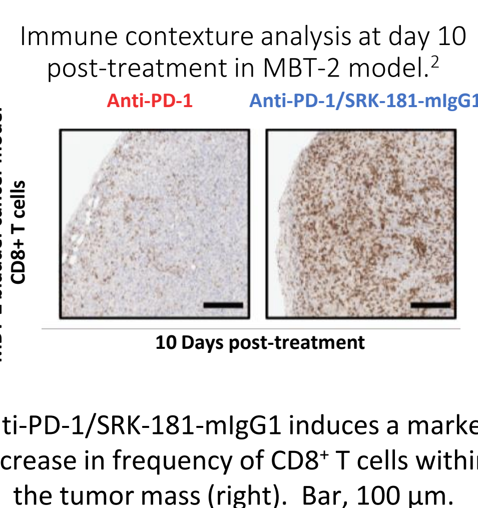
Development and further improvement of the biomarker assays would be the key to potentially unlock important clinical data including:

- ➢Refinement of image analysis for CD8 IHC assay (Figs. 2-4)
- ➢Development of P-Smad2 IHC assay (Figs. 5-6)
- ➢Improvement of platelet poor plasma processing method for assessment of circulatory TGFβ-1 (Fig.7)

Figure 2.
Refinement of Image Analysis for CD8 IHC Assay: CD8 is a Proposed PD Biomarker For DRAGON[2]

- CD8⁺ T cells plays a central role in cancer immunity[2,3]
- In preclinical tumor models, SRK-181 and α-PD1 combination leads to the influx of CD8+ T cells that correlates to treatment response (Fig 2)
- Intratumor CD8⁺ T cells evaluated in the DRAGON study
- IHC utilized to assess:
  - ➢ Modulation of CD8⁺ T cells between pre- and post-treatment biopsy samples
  - ➢ Digital pathology analysis performed to quantify CD8⁺ T cells across tumor, tumor Margin and stromal compartments
- Distribution of CD8⁺ T cells across the compartments may define the tumor immune landscape (Fig. 3A)

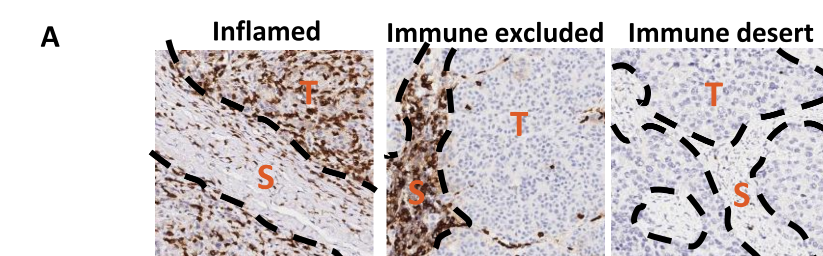
Immune contexture analysis at day 10 post-treatment in MBT-2 model.[2]



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

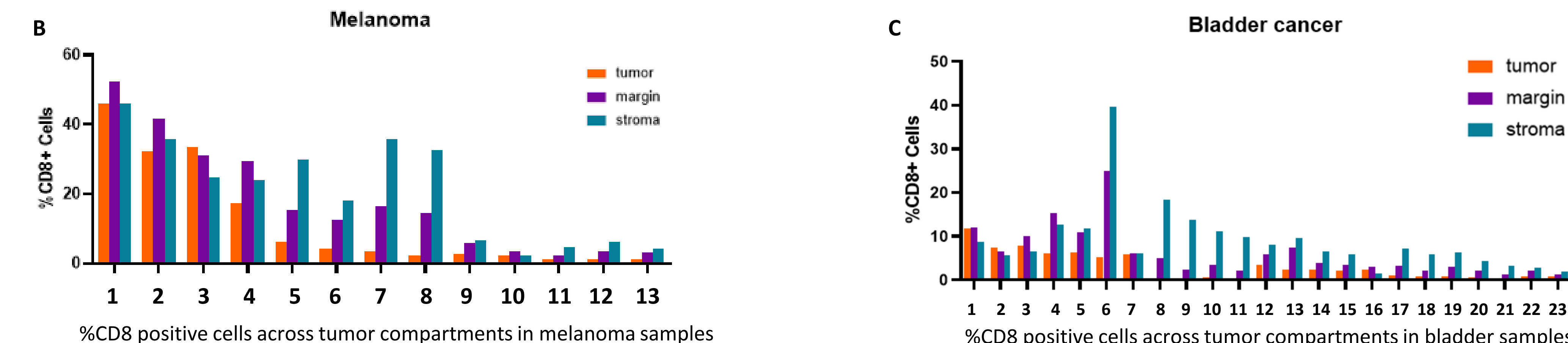
Figure 3.
Refinement of Image Analysis for CD8 IHC Assay: Digital Pathology Analysis Can Guide Tumor Immunophenotyping and Classification into Inflamed, Excluded and Desert Tumors

- IHC pilot study is performed utilizing commercially available human cancer samples[5]
- The study serves to validate the performance of the CD8 IHC assay, establish the CD8⁺ cell baseline signals for selected DRAGON indications, including bladder cancer and melanoma, and formulate a comprehensive image analysis plan



Representative images of cancer immune phenotypes (3A), inflamed (left), immune excluded (middle), immune desert (right). Dotted line represents margin between tumor and stroma compartments in tumor (T) and stroma (S) compartments. %CD8⁺ cells across compartments are utilized to classify immune phenotypes.[6] ≥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.

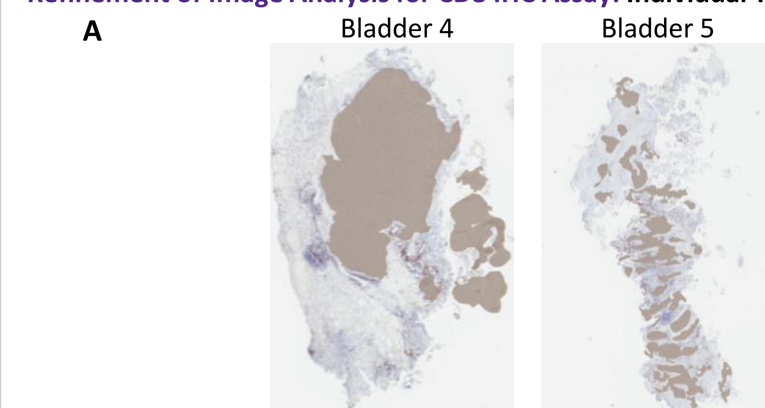
%CD8⁺ cells in tumor, tumor margin and stromal compartments quantified across melanoma (3B) and bladder cancer samples (3C).



%CD8 positive cells across tumor compartments in melanoma samples

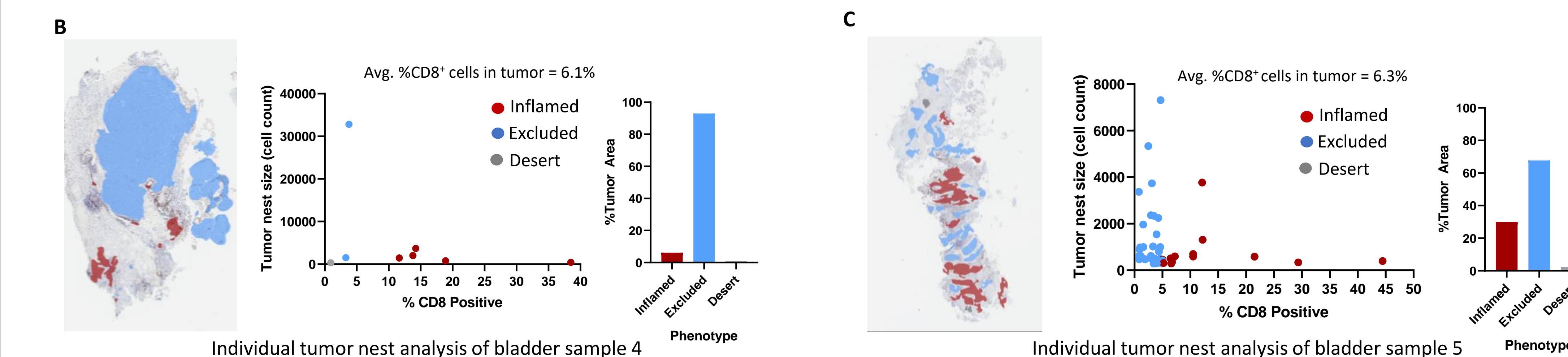
%CD8 positive cells across tumor compartments in bladder samples

Figure 4.
Refinement of Image Analysis for CD8 IHC Assay: Individual Tumor Nest Analysis Leads to Identification of Tumors with Mixed Immune Phenotypes



Tumor samples often consist of multiple tumor nests that are variable in number and size (4A, masked area). A tumor sample may have a single large immune excluded tumor nest (4B, blue) or multiple small tumor nests (4C, blue). In both examples, several small but highly inflamed tumor nests are present (4B and 4C, red).

Scatter plots show the analysis of each individual tumor nest including nest size, % CD8⁺ cells and immune phenotype. The highly inflamed tumor nests skewed overall %CD8⁺ cells of the samples. Bar graphs show combined tumor nest analysis and the distribution of immune phenotypes for each sample rather than classifying the sample into a single immune phenotype.



Individual tumor nest analysis of bladder sample 4

Individual tumor nest analysis of bladder sample 5

Summary

To implement the biomarker strategy, select biomarker assays were developed and improved including:
- Refinement of CD8 IHC to enable capture of sample heterogeneity and better assessment of the PD effects of SRK-181 and CPI combination
- Development of P-Smad2 IHC assay to detect modulation of TGFβ signaling by SRK-181
- Improvment of blood sample collection and PPP processing method to enable assessment of the PD effect of SRK-181 on circulatory TGFβ1

Establishment of these biomarker assays is part of a broader biomarker strategy that is poised for analysis of samples from the DRAGON clinical trial by the second half of 2021

Combination of CD8 and tumor gene signature analysis will be explored as potential predictive biomarkers for SRK-181 in the future

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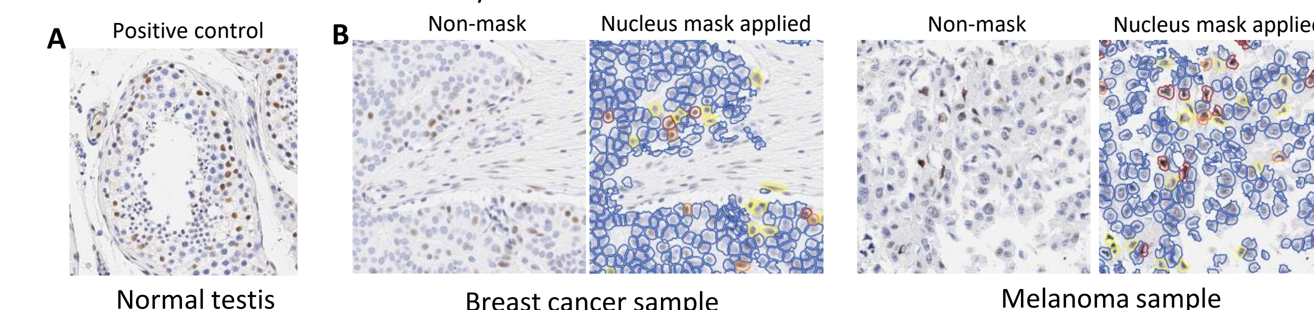
Figure 5.
Development of P-Smad2 IHC Assay: Exploring Tumor Tissue P-Smad2 Levels as Potential SRK-181 PD Marker

- Phospho-Smad2 (P-Smad2) is a key signaling mediator of TGFβ pathway.[7]
- TGFβ downstream signaling is activated through phosphorylation of Smad2 and 3, leading to heteromeric complex formation that translocates into the nucleus to regulate target gene expression (Fig 6).[7]
- The inhibition of TGFβ pathway leads to a decrease of P-Smad2.[8]
- Level of P-Smad2 is being evaluated in the DRAGON study
- P-Smad2 IHC assay is developed using commercially available normal and cancer samples

Establishment of P-Smad2 IHC assay



Testis is a positive control for P-Smad2 IHC assay that shows a consistent and robust staining pattern (5A). Digital image analysis is identified by a range of P-Smad2 nucleus staining intensity (5B) in breast cancer (left) and melanoma samples (right) ranging from P-Smad2 high (red), medium (orange), low (yellow) to negative (blue). Total Smad2 IHC is used as an orthogonal method for validation and demonstrates comparable staining between total Smad2 and phospho-Smad2 across samples (data not shown).

Figure 6.
TGFβ signaling pathway[7]

Figure 7.
Improvement of Platelet Poor Plasma Processing Method for Assessment of Circulatory TGFβ1: Circulatory TGFβ1 as a Potential Target Engagement BM for SRK-181

- A Preclinical study demonstrated SRK-181 treatment increased circulatory TGFβ1 and would serve as a target-engagement biomarker for SRK-181 (Fig. 7)[5]
- Platelets are enriched with TGFβ that may be released if activated during clinical sample processing leading to alteration of base level of TGFβ1 independent of SRK-181 treatment[9]
- To overcome the potential increase of circulatory TGFβ1 by platelet activation, a pilot study was performed using healthy volunteer blood samples to improve the collection and processing method for obtaining platelet poor plasma (PPP) (Fig. 8)
- Platelet factor 4 (PF4) is included to identify samples with significant platelet activation

Circulatory TGFβ1 was analyzed at day 10 post-treatment of SRK-181-mIgG1 dosed weekly at 10 mg/kg

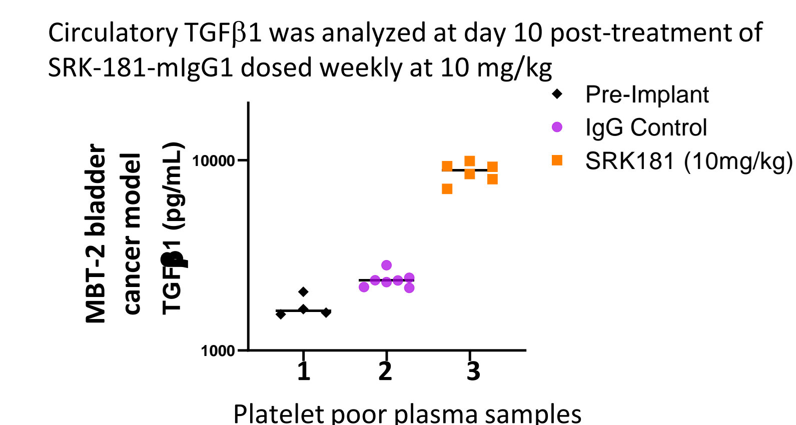
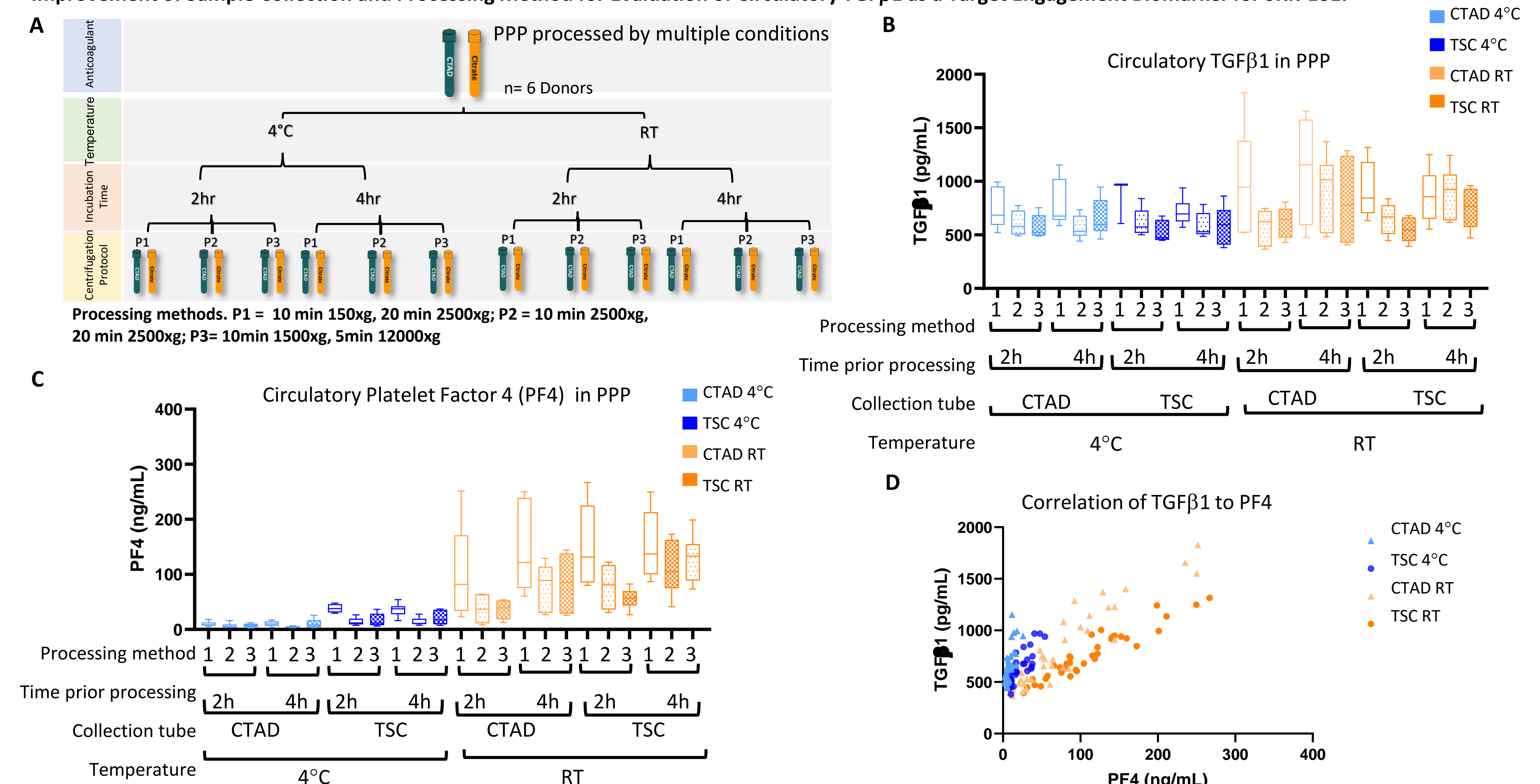


Figure 8.
Improvement of Sample Collection and Processing Method for Evaluation of Circulatory TGFβ1 as a Target Engagement Biomarker for SRK-181.



Blood samples collected from 6 healthy donors to generate platelet poor plasma (PPP) at 4°C or room temperature (RT), in either CTAD or sodium citrate (TSC) collection tubes, by different processing times and methods (8A).[5] Circulatory TGFβ1 evaluated using a Quantikine assay (R&D System). The concentration range of TGFβ1 is between 500 to >1500 pg/ml (8B) and platelet factor 4 (PF4) level minimal to 250 ng/ml (8C). Significantly higher levels of TGFβ1 and PF4 detected in samples processed at RT (orange) compared to 4°C (blue). Correlation of PF4 and TGFβ1 observed in these RT samples (8D) support PF4 may be used to identify samples with platelet activation leading to increased levels of TGFβ1. The lowest level of PF4 observed in samples collected in CTAD tubes and processed by method 2 (P2) thus implicated to be the optimal condition for processing PPP.

Disclaimer: SRK-181 is an investigational drug candidate that is currently being evaluated in a Phase 1 clinical trial. The safety and efficacy of SRK-181 have not been established. 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 have not been established.

* SRK181-mIgG1 , murine SRK181 monoclonal IgG1 antibody; BM, biomarker; CD8, cluster of differentiation 8; CTAD, citrate-theophylline, adenosine, dipyridamole; IgG, immunoglobulin; IHC, immunohistochemistry; MBT-2, mouse bladder tumor line-2; MEL, melanoma; mg/kg, milligram/killigram; NSCLC, non-small cell lung cancer; pg/ml, picogram/milliliter; SMAD9, mother against decapentaplegic homolog 9; TβR, TGFβ receptor; TSC, trisodium citrate; UC, urothelial carcinoma; PK, pharmacokinetics; PD, pharmacodynamics; S.C., sub-cutaneous; TGFβ1, transforming growth factor beta-1; SRK, ScholarRock; PD-1, programmed cell death protein-1

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