Tracking Multiplicity: In vitro and Clinical Pharmacokinetic Assays for Multi-target Therapeutics

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Introduction

- SAB-142 is a fully human, multi-specific, targeted, Anti-Thymocyte Globulin (hATG) for delaying onset and progression of type 1 diabetes (T1D).
- SAB-142 presents distinct challenges in developing a high sensitivity pharmacokinetic (PK) assay for monitoring SAB-142 exposure in clinical trials.
- The similar CD marker binding profiles of SAB-142 and rabbit ATG were determined by using Bw mouse cells lines engineered to overexpress human CD marker proteins.
- The diverse repertoire of multi-specific T-cell receptor binding led to the development of a novel PK assay utilizing pooled donor PBMCs to distinguish SAB-142 from endogenous antibodies.
- This PK Assay required high sensitivity due to the low dose levels of SAB-142 administered during the trial.
- The active components of SAB-142 bind to circulating lymphocytes as part of its mechanism of action – the remaining SAB-142 in participant serum was measured with this PK assay.
- This novel, high sensitivity PK assay was developed and validated for the quantitative measurement of the SAB-142 in participant serum samples from SAB-142-101 trial.

Methods

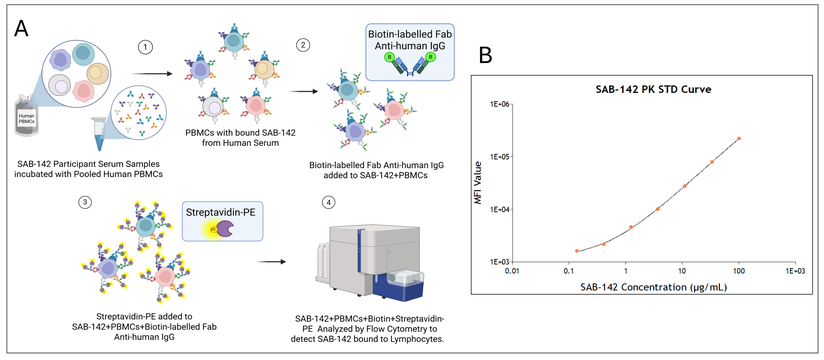

Figure 1A. Schematic diagram for the methodology of a multi-specific, lymphocyte targeted, flow cytometry-based PK assay quantifying SAB-142 in human serum. **1B**. Standard curve between SAB-142 concentration and fluorescence intensity of flow cytometry in human serum.

- Pooled human PBMCs ($5x10^5$ cells) were incubated with clinical trial serum samples for 1 hr at RT.
- Cells were washed, incubated with biotin $F(ab')_2$ goat anti-human IgG-Fc (30 mins at RT), washed, and finally incubated with streptavidin-PE (15 mins at RT)
- Fluorescently-labeled PBMCs were gated for lymphocytes and analyzed via flow cytometry. SAB-142 concentration is determined by interpolating Median Fluorescence Intensity (MFI) against the standard.
- Assay was validated for accuracy, precision, selectivity and range.

Results: Validation

Table 1. Accuracy in human serum samples spiked with SAB-142 at five QC concentrations

Sample[1]	Nominal (µg/mL)	Mean[2] (µg/mL)	Mean Recovery[3] (%)	Passed/Failed[4]
ULOQ	80.0	85.9	107%	Passed
HQC	15.0	14.8	99%	Passed
MQC	5.00	5.29	106%	Passed
LQC	1.00	1.03	103%	Passed
LLOQ	0.25	0.19	76%	Passed

[1]ULOQ = Upper Limit of Quantitation; HQC = High Quality Control; MQC = Medium Quality Control; LQC = Lower Quality Control; LLOQ = Lower Limit of Quantitation
[2]Average of Sample ran in triplicate (3 wells/replicate)
[3](Measured concentration/theoretical concentration)*100
[4]Acceptance Criteria: HQC, MQC & LQC = %Recovery between 80-120%; ULOQ & LLOQ = %Recovery between 70-130%

Table 2. Overall precision in human serum samples spiked with SAB-142 at five QC concentrations

Sample[1]	Nominal (µg/mL)	Mean[2] (µg/mL)	CV%[3]	Bias[4] (%)	Total Error[5] (%)	Passed/Failed[6]
ULOQ	80.0	82.4	5.8%	3.0%	8.7%	Passed
HQC	15.0	14.7	5.9%	2.0%	7.9%	Passed
MQC	5.00	5.18	12.8%	3.6%	16.4%	Passed
LQC	1.00	1.05	5.4%	5.0%	10.4%	Passed
LLOQ	0.25	0.26	21.6%	2.4%	24.0%	Passed

[1]ULOQ= Upper Limit of Quantitation; HQC = High Quality Control; MQC = Medium Quality Control; LQC = Lower Quality Control; LLOQ = Lower Limit of Quantitation
[2]Average across all precision runs
[3]Coefficient of Variation; (Standard Deviation/Mean)*100; Average across all precision runs
[4](Mean measured concentration-nominal concentration)/nominal concentration
[5]Sum of the absolute bias and CV%
[6]Acceptance Criteria: CV%: HQC, MQC, LQC ≤20%; ULOQ & LLOQ ≤ 30%; Total Error: HQC, MQC, LQC ≤30%; ULOQ & LLOQ ≤ 40%

Table 3. Selectivity in Type 1 Diabetes serum spiked with SAB-142 at HCQ and LLOQ concentrations

	Type 1 Diabetes									
Sample[1]	1	2	3	4	5	6	Mean (µg/mL)	Mean Recovery[2] (%)	CV%[3]	Passed/ Failed[4]
HQC	14.7	16.3	13.1	15.1	16.3	12.6	14.7	98%	11%	Passed
LLOQ	0.32	0.20	0.26	0.32	0.27	0.20	0.26	104%	24%	Passed

[1] HQC = High Quality Control; Lower Limit of Quantitation (0.25 µg/mL); Individual samples ran in triplicate
[2](Actual concentration/theoretical concentration)*100
[3]Coefficient of Variation; (Standard Deviation/Mean)*100
[4]Accpetance criteria: HQC %Recovery = 80-120% and CV% ≤25%; LLOQ %Recovery = 70-130% and CV% ≤30%

CD Marker Binding Profiles

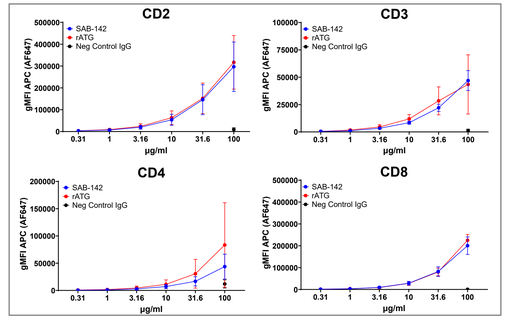

Figure 3. Directly labeled SAB-142, rATG or negative control IgG was incubated with Bw cells expressing the indicated T cell surface protein. The cells were analyzed by flow cytometry and data shown is the geometric mean fluorescent intensity with the background from the parental Bw cells subtracted. Error bars show SEM, N=6.

Results: SAB-142 Phase 1 PK Profile

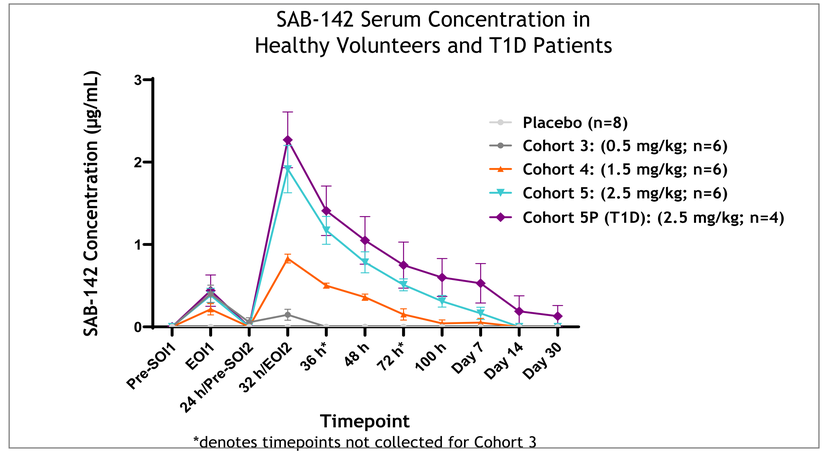

Figure 4. SAB-142 concentration (mean ± SEM) over time in serum from healthy volunteers and T1D patients following a single IV infusion of SAB-142 in Cohorts 3, 4, 5, 5P from Phase 1 study, SAB-142-101.

Conclusion

- CD Marker binding profiles demonstrated that SAB-142 targets numerous T cell receptors, similar to rATG.
- The novel PK assay developed was validated for accuracy, precision, selectivity, and range and exhibited high enough sensitivity to detect free unbound SAB-142 at very low circulating levels down to 0.25 µg/mL.
- The utility of this assay was successfully demonstrated where SAB-142 had a dose-proportional PK profile measured in the SAB-142-101 Phase 1 Study.
- No major differences were observed in systemic exposure to SAB-142 between Healthy Volunteers and T1D patients dosed at 2.5 mg/kg.