# Development of a Robust, Simplified Method to Measure Receptor Occupancy in Peripheral Blood from Patients Treated With a Novel Anti-PD-1 Agent, AB122

**Authors:** Ashok D, Piovesan D, Zhao X, Singh H, Walters MJ, Young S, Seitz L

Arcus Biosciences, Inc., 3928 Point Eden Way, Hayward, CA 94545, USA

## Introduction

Exhausted T-cells express high levels of immune checkpoint proteins, including programmed cell death-1 (PD-1) receptor. Preclinical and clinical data support the role of PD-1 and its ligand, programmed cell death ligand 1 (PD-L1), in promoting tumor evasion by curtailing immune responses. In a Phase 1 clinical trial of the anti-PD-1 monoclonal antibody AB122, we determined receptor occupancy (RO) in peripheral blood T-cells using a directly conjugated competitive antibody method and the previously established method using biotinylated anti-human IgG4.

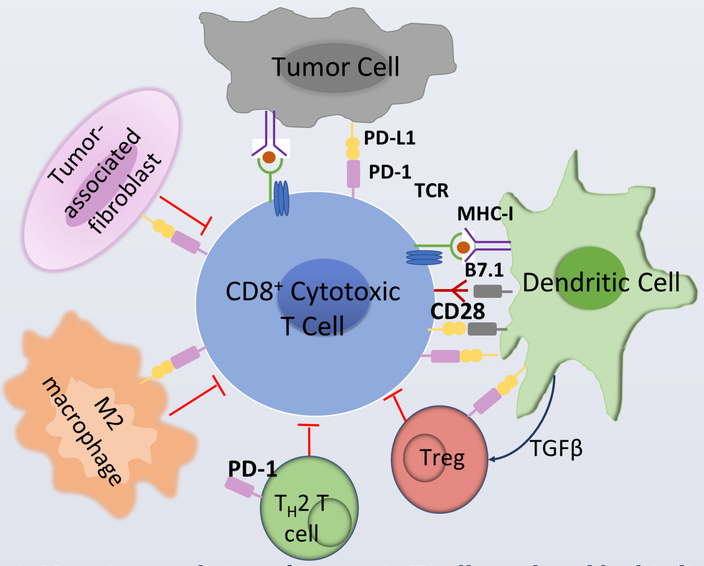


Figure 1. Negative regulation of cytotoxic T-cells mediated by binding of the receptor PD-1 to its ligand PD-L1 on multiple cell types.

## Methods

RO was evaluated using 2 methods: (1) saturation binding (using a biotinylated anti-hIgG4 for the detection of AB122, by the previously published method[1]) and (2) direct competition (via commercially available anti PD-1 antibody) that is competitive with AB122. Both methods of RO were optimized to identify proliferating cells by determining intra-cellular Ki-67$^+$ cells within lymphocyte sub-populations.

## Results

### Robust Multi-Color Flow Cytometry Assay for Determination of PD-1 RO in PBMCs and WB

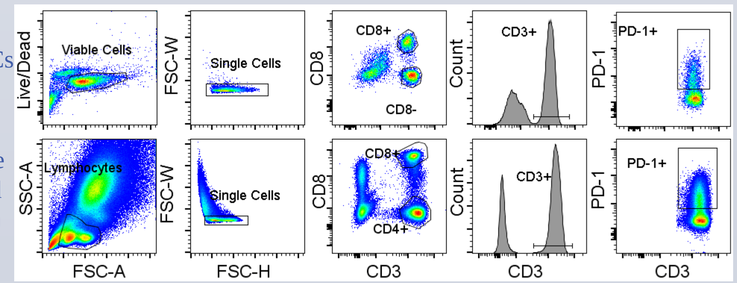


Figure 2. A multi-color flow cytometry panel was developed to identify PD-1 expression on total CD3$^+$ lymphocytes as well as CD3$^+$CD8$^+$ (cytotoxic T-cells) and CD3$^+$CD8$^-$ (surrogate markers for CD4$^+$ helper T-cells) using both methods of RO determination on PBMCs and WB.

### Comparable PD-1 Expression in Fresh and Freeze/Thaw PBMCs

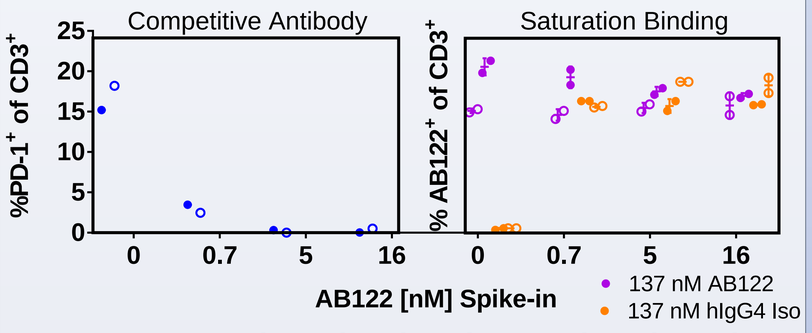


Figure 3: AB122 spike-in dose-dependent signal is observed in freshly isolated (closed symbols) and freeze/thaw PBMCs (open symbols) of healthy volunteers and shows comparable PD-1 detection. Data is representative of three independent runs.

### AB122 Dose-Dependent Competition is Observed in Whole Blood

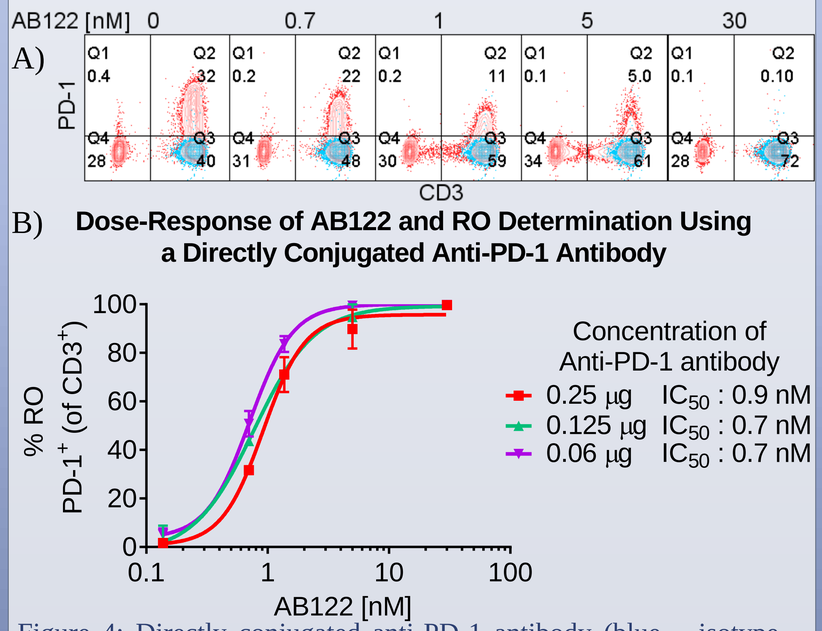


Figure 4: Directly conjugated anti-PD-1 antibody (blue – isotype, red – staining) was used to determine RO in whole blood in the presence of a dose-titration of AB122 (A). A four-parameter global fit curve (B) of a dose-response to AB122 by 2.5, 1.25 and 0.625 µg of anti-PD-1 antibody provides an $IC_{50}$ for AB122 of ~1 nM *in-vitro*.

### 10 nM AB122 Completely Blocks PD-1 in Human Whole Blood *In-vitro*

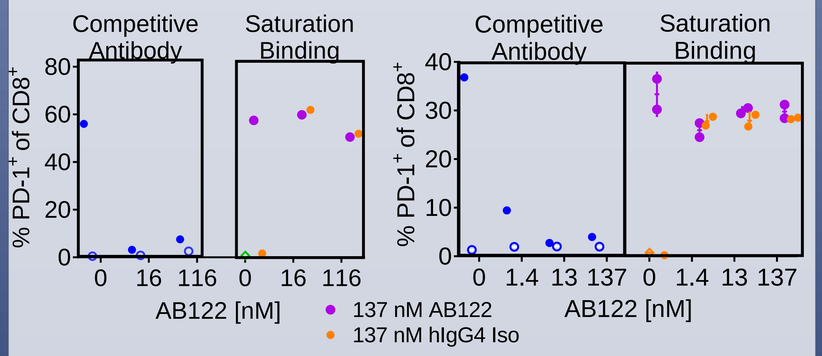


Figure 5: AB122 spike-in into healthy volunteer whole blood, donors A (left) and B (right) and their respective expression of PD-1 on CD8$^+$ cells using both methods of RO determination displays complete RO with ~10 nM of AB122. Open symbols (isotype).

### AB122 Exhibits Complete Target Coverage in Dose-Escalation Cohorts (80/240 mg, Q2W)

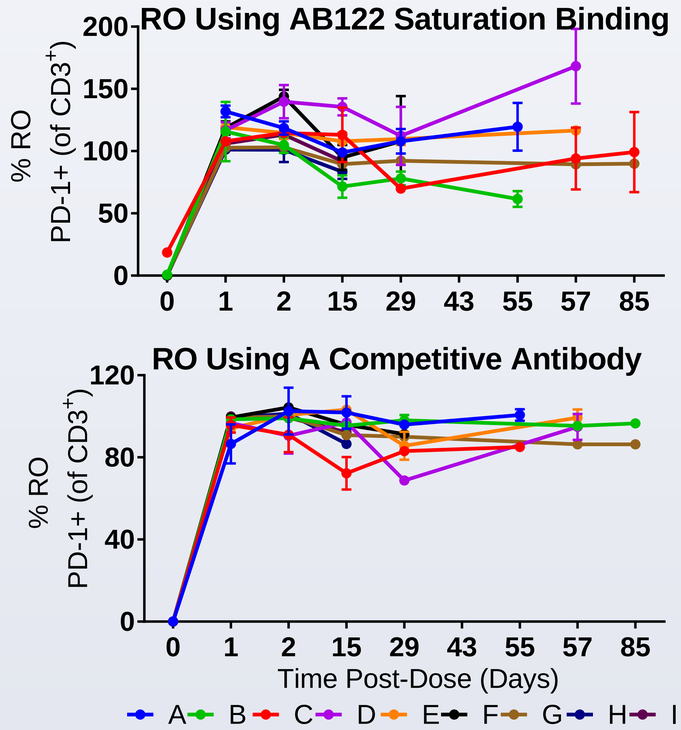


Figure 6: RO determined using the AB122 saturation binding and competitive antibody method in AB122 dosed subjects in the dose-escalation phase. Subjects A-C (80 mg Q2W), subjects D-I (240 mg Q2W). Please refer to poster # P673 at this meeting for additional clinical trial information.

### Both Methods of RO Determination are Compatible with Ki-67 Staining

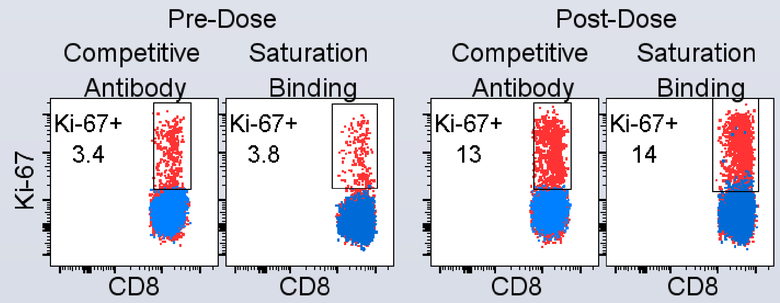


Figure 7: Representative plot of CD8$^+$Ki-67$^+$ PBMCs (blue-isotype, red-staining) from AB122 dosed patient at baseline (left) and post-dose (right) by both assay protocols of RO determination display comparable levels of Ki-67$^+$ cells.

## Conclusions

- AB122 exhibits excellent PD-1 target inhibition.
- Both methods of RO determination produce consistent results and are compatible with intra-cellular Ki-67 staining.

## References

[1]Brahmer JR, Drake CG, Wollner I, *et al*. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28(19):3167-75.