**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.

**Methods**

RO was evaluated using 2 methods: (1) saturation binding (using a biotinylated anti-hlgG4 for the detection of AB122, by the previously published method) 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 intracellular 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 IC50 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**