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Receptor Occupancy and Neo-Epitope Specific T-cell Repertoires in Patients with Solid Tissue Tumors Following Anti-PD-1 Therapy

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Introduction

Antigen-experienced 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 cancer immunotherapy. AB122 is an anti-PD-1 monoclonal antibody that is being evaluated in a dose-escalation monotherapy study as well as in combination with AB928, a potent small molecule inhibitor of the adenosine 2 receptor (A_2R). In both studies, PD-1 receptor occupancy (RO) on T cells was determined. A small but significant number of these T cells can recognize neo-epitopes (neoE) arising from patient specific mutations.

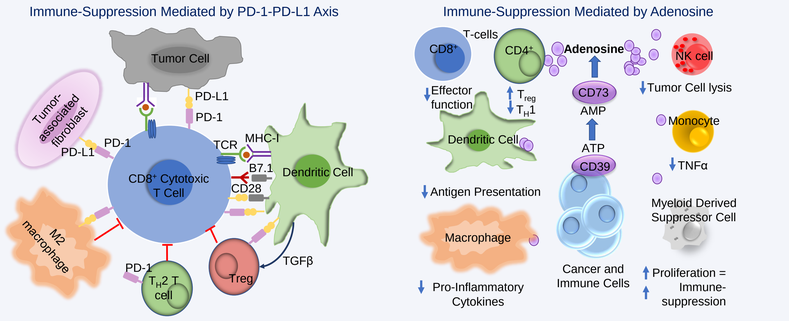


Figure 1. Negative regulation of cytotoxic T-cells and other immune cells mediated by two pathways; the PD-1 – PD-L1 axis and the adenosine pathway provide the rationale for combination therapy with anti-PD-1 and A_2R inhibitors.

Materials and Methods

Determination of PD-1 Receptor Occupancy (RO): Total $CD3^+$ lymphocytes, $CD3^+CD8^+$ cytotoxic T-cells and $CD3^+CD8^-$ (CD4 T cells) were identified by surface staining using flow cytometry. Within the T-cell subsets, PD-1 receptor occupancy was determined from isolated, cryopreserved peripheral blood mononuclear cells (PBMCs) or using whole blood (WB).The two methods were: 1) saturation binding using a biotinylated anti-hIgG4 for the detection of AB122 (previously published method) and 2) direct competition using a commercially available anti PD-1 antibody (Figure 2).

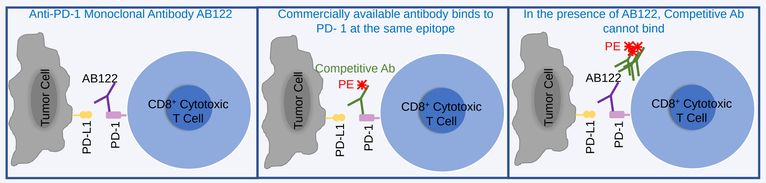


Figure 2. Determination of PD-1 receptor occupancy in cancer patients following administration of AB122 at defined intervals using a commercially available competitive antibody.

Evaluation of Proliferation: T-cell proliferation in cancer patients following dosing with AB122 was determined as a function of the frequency of $Ki\text{-}67^+$ cells. Intra-cellular staining was performed on both isolated PBMCs as well as whole blood post-RBC lysis and fixation.

Patient-Specific NeoE-Targeted CD8 T Cells

Prediction of neoEpitope (neoE) candidates from individual patient's tumor sequence → Generation & assembly of bar-coded neoE-HLA 'snare' candidates → Capture of neoE-specific CD8 T cells from patient blood that bind specifically to neoE-HLA snares → Reading of barcodes to verify NeoE targets → Quantification, sequencing & phenotypic characterization of patient-specific neoE-specific T cells

Results – PD-1 Receptor Occupancy

Complete PD-1 Receptor Occupancy Observed Following AB122 Dosing in the Monotherapy Trial

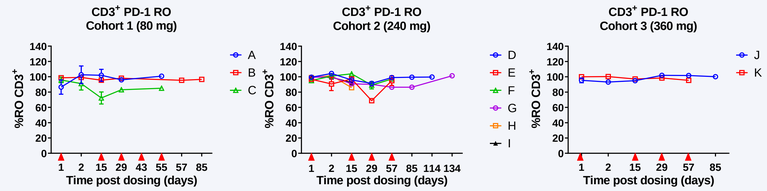


Figure 3. PD-1 receptor occupancy determined on PBMCs using the competitive antibody method in AB122-dosed subjects in the dose-escalation phase. Three dosing regimens were evaluated: Subjects A-C (80 mg Q2W), subjects D-I (240 mg Q2W) and subjects J and K (360 mg, Q2W). Comparable PD-1 receptor occupancy data were obtained using the previously published saturation binding method on total $CD3^+$ lymphocytes and the individual T-cell subsets (data not shown). Red arrows indicate AB122 dosing.

Complete PD-1 Receptor Occupancy Observed in Combination Studies with AB928

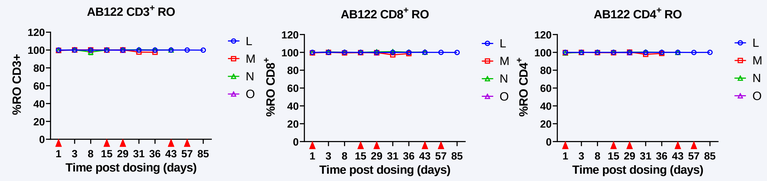


Figure 4. Comparable PD-1 receptor occupancy on T-cell subsets in whole blood was observed in an ongoing AB928 dose-escalation + AB122 combination trial. All subjects received 240 mg Q2W of AB122. Subjects also received once daily dosing of AB928: Subjects L and M (75 mg AB928) and subjects N and O (150 mg AB928).

Frequency of Ki-67+ Cells Varies Among Subjects When Comparing Baseline to 15 Days Post-AB122 Administration

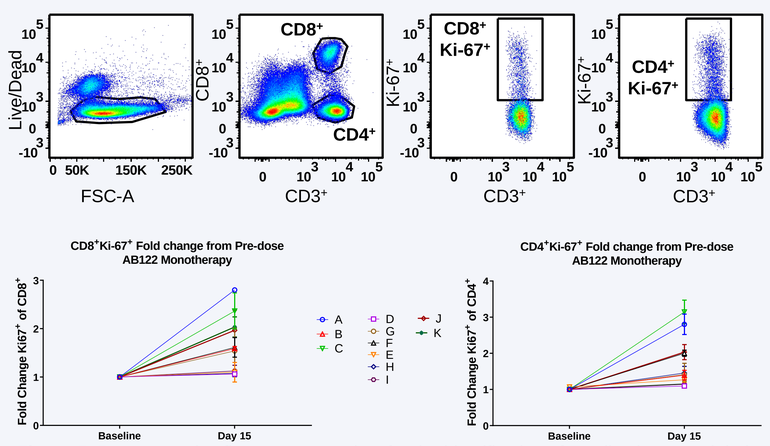
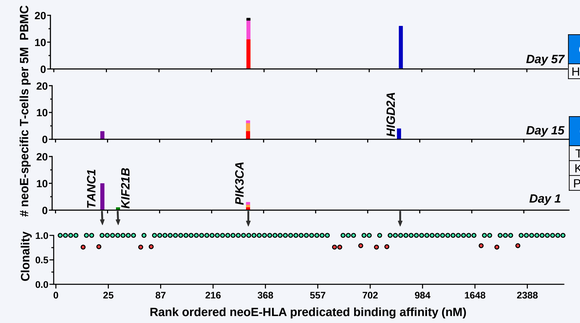


Figure 5. Representative flow cytometry plots for the identification of T-cell subsets and their respective frequencies of $Ki\text{-}67^+$ cells in AB122-dosed patients (top panel). Frequency of $Ki\text{-}67^+$ cells within the $CD8^+$ and $CD4^+$ (identified as $CD3^+CD8^-$) lymphocyte subsets was determined on all subjects shown at baseline (day 1) and 15 days post-AB122 administration. Data are represented as a fold change in the frequency of cells between the two timepoints (bottom plot). Subjects A-C (80 mg Q2W), subjects D-I (240 mg Q2W) and subjects J and K (360 mg, Q2W).

Results – NeoEpitope (NeoE)-Specific T Cells

NeoE-Specific T Cells Expand in Response to AB122, as Characterized With ImPACT™ Isolation Technology



Gene	HLA	neoE (wild type AA)
HIGD2A	B35:01	LATAAAI**(L)**TYG

Gene	HLA	neoE (wild type AA)
TANC1	C05:01	STDSPS**(C)**STL
KIF21B	A11:01	ARSVSSI**M(V)**R
PIK3CA	A11:01	R**A(D)**IDKIYVR

Figure 6. Evolution of NeoE-specific T-cell response to AB122 (anti-PD1) treatment, showing drug-dependent target-specific expansion, as captured from patient blood using PACT Pharma's imPACT™ isolation technology. Tumor-exclusive NeoE-HLA candidates are displayed by highest to lowest predicted binding affinity (bottom plot, x-axis) as well as the clonality/prevalence of tumor cells expressing the mutated neoantigen (y-axis, bottom plot). Green circles represent clonal mutations; red circles indicate sub-clonal neoantigen mutations. Identity/quantification of T cells that are verified by capture from peripheral blood to recognize different NeoE-HLA candidates is shown (top three plots): TANC1: Tetratricopeptide repeat, ankyrin repeat and coiled-coil domain-containing protein 1, KIF21B: Kinesin Family Member 21B, PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, HIGD2A: HIG1 Hypoxia Inducible Domain Family Member 2A. Please refer to abstract #4058 for additional data on neoE-specific T cells from peripheral blood or tumors from patients with different cancers using the imPACT™ technology.

Phenotypic Characterization of NeoE-Specific T cells in Peripheral Blood Using imPACT™

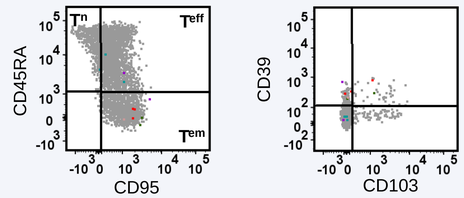


Figure 7: Representative flow cytometry plots characterizing individually captured NeoE-specific T cells (colored dots in plots correspond to imPACT-captured T cells in Fig 6). Evidence that neoE-specific T cells from peripheral blood are antigen-experienced (effector (T^{eff}), effector memory (T^{em}); left plot) and those T cells that potentially also trafficked to the tumor (CD39 / CD103, right plot).

Conclusions

- **AB122 exhibited complete PD-1 receptor occupancy on peripheral T cells at all dose levels of the monotherapy dose-escalation study with a dosing regimen of Q2W (n=11).**
- **Complete PD-1 receptor occupancy with AB122 was not impacted in a dose-escalation study of AB122 in combination with AB928 (Adenosine receptor antagonist).**
- **Patient-specific baseline levels and subsequent modulation post-AB122 of $Ki\text{-}67^+$ cells was observed in all cohorts of the AB122 monotherapy trial.**
- **In collaboration with PACT Pharma, an expansion of patient-specific NeoE T cells was observed following AB122 administration.**
- **Longitudinal immune monitoring of neoE-specific T cells from blood by imPACT™ analysis holds potential to establish when and how patients respond to treatment with immunotherapy agents.**

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