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Abstract#291

Validation of a Whole Blood and Proteomic Stabilized Blood Assay to Monitor the Engagement and Modulation of CD6 on T cells by Itolizumab as a Clinical Pharmacodynamic Biomarker in Autoimmune Diseases

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INTRODUCTION

Itolizumab is a novel first-in-class monoclonal antibody that selectively targets the co-stimulatory molecule CD6, a receptor that is highly expressed on CD4 and CD8 T cells and plays an important role in activation and migration. Monitoring target engagement and changes in receptor levels is critically important to interpreting clinical data. To evaluate the pharmacodynamic properties of itolizumab treatment on T cells in patients including those with graft versus host disease (GvHD), systemic lupus erythematosus (SLE), lupus nephritis and uncontrolled asthma, Precision for Medicine has developed and validated a 10-color flow cytometry assay to assess the engagement and modulation of cell-surface CD6 in both fresh whole blood (WB) samples and fixed whole blood samples using SMART™ Tube's proteomic stabilizer.

PURPOSE

Measuring cell-based receptor engagement and fate in patients on immuno-modulatory therapies is very challenging. This assay was designed and validated to be both sensitive and selective in the quantification of CD6 receptor occupancy and modulation to facilitate the determination of an optimal therapeutic dose in autoimmune and inflammatory diseases. The validation parameters assessed included intra-assay, inter-assay, inter-operator precision and post-staining stability.

METHOD

Step 1. Whole Blood Treatment with Itolizumab
- Whole blood from 3 healthy donors were used to validate the whole blood (WB) and fixed whole blood (Fixed WB) assays using multiple concentrations of Itolizumab (EQ001) based on expected PK/PD pharmacodynamics.
- In fixed WB samples, the EQ001 drug treatment was in whole blood then the samples were fixed and stabilized using SMART™ Tube's proteomic stabilizer, stored at -80°C for subsequent batched flow testing.

Step 2. Detection of Itolizumab bound CD6
- The red blood cells were lysed, washed and cells were stained with anti-human IgG1 antibody to detect drug bound CD6.

Step 3. Detection of Total Surface CD6
- To detect Total CD6 on the cell surface, the cells were stained with an anti-human distal receptor 3 CD6 antibody, this is a non-competing CD6 antibody to EQ001.

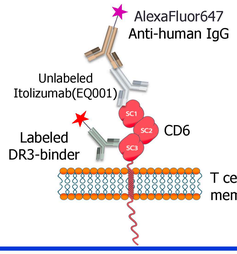
Step 4. Cell Surface Marker Staining
- Cells were stained with an antibody cocktail containing cell surface markers for identification of T cell subsets.
- Fresh WB Panel: CD3, CD4, CD8, CD25, CD127, CD45RO, CCR7
- Fixed WB Panel: CD3, CD4, CD8, CD25, CD127, CD45RO, CD45RA, Lin-(CD14,CD19,CD33, CD66b).

Step 5. Cells were acquired on a BD FACSCanto™-10 Color Cytometer

Step 6. Data analysis of CD6 Receptor Occupancy (RO)
- Data was analyzed with TreeStar's FlowJo™ software. CD6 %RO was calculated by determining frequency of CD6 bound to receptor as a ratio of Total CD6 on the cell surface.

RECEPTOR OCCUPANCY- DETECTION OF CD6

Figure 1. Receptor Occupancy of CD6: Drug (EQ001) bound CD6 receptors are detected with an anti-human IgG AlexaFluor647 antibody and Total Surface CD6 is detected with a fluorescently labeled non-competing CD6 antibody to the distal receptor 3 (DR3) of CD6.



RESULTS

Figure 2. Frequency of CD6 (%CD6 EQ001+) in CD4 T cell: Data representative of the 3 healthy donors tested. Mean of triplicates plotted with SD. In Fixed WB, 50ug/mL %CD6 EQ001+ detected was ≤ 83%. in Fresh WB , 50ug/mL %CD6 EQ001+ detected was ≤ 96% in CD4+ T-cells. **Total Surface CD6** in CD4 T cells was 98.9±0.03% in Fixed WB and 98.8±0.4% in Fresh WB. Different donors used for Fixed WB and Fresh WB.

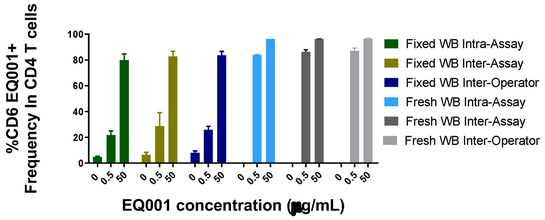


Figure 3. Frequency of CD6 (%CD6 EQ001+) in CD8 T cells: Data representative of the 3 healthy donors tested. Mean of triplicates plotted with SD. In Fixed WB, 50ug/mL %CD6 EQ001+ detected was ≤ 50%. In Fresh WB , 50ug/mL %CD6 EQ001+ detected was ≤ 75% in CD8+ T-cells. **Total Surface CD6** in CD8 T cells was 94.7±0.1% in Fixed WB and 89.2±0.2 % in Fresh WB. Different donors used for Fixed WB and Fresh WB.

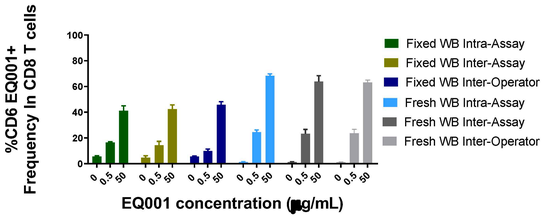


Figure 4A. Gating Strategy Proteomic Fixed Whole Blood CD6 RO Assay: CD4 & CD8 T cells and T-Memory cell subsets in fixed whole blood assay. CCR7 not included in this flow panel.

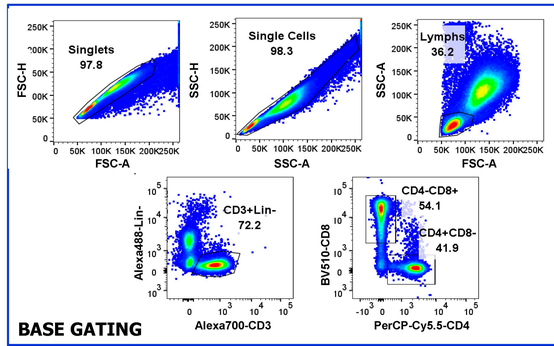
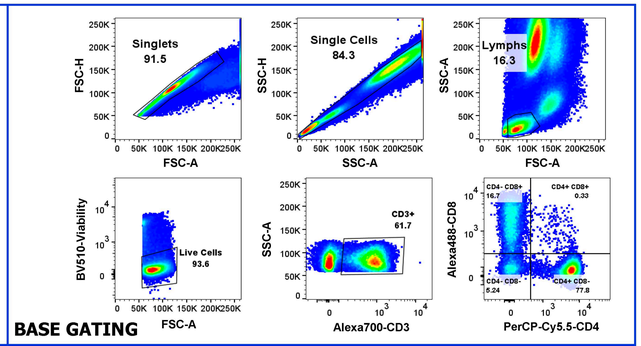
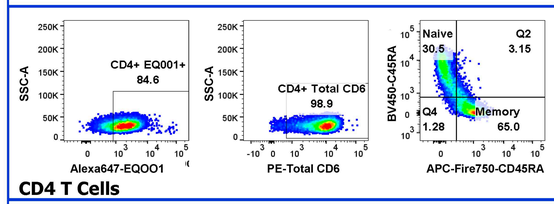
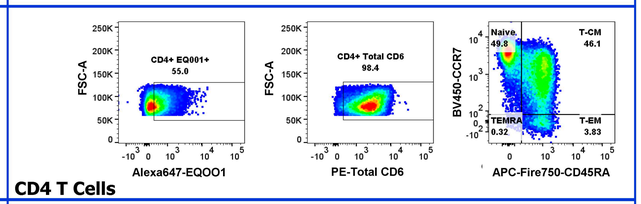
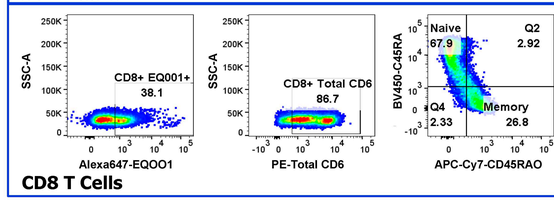


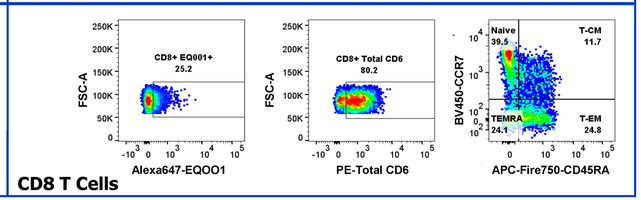
Figure 4B. Gating Strategy Whole Blood CD6 RO Assay: CD4 & CD8 T cells, T-Memory cell subsets in fresh whole blood.











RESULTS

Figure 5. CD4 T cell EQ001 Receptor Occupancy (%RO) Validation Results: Data representative of the 3 healthy donors tested. Mean of triplicates plotted with %CV for each parameter. At 50ug/mL %RO detected was ≤ 84% in Fixed WB CD4 T-cells and ≤ 87% in Fresh WB CD4 T-cells. Different donors used for Fixed WB and Fresh WB.

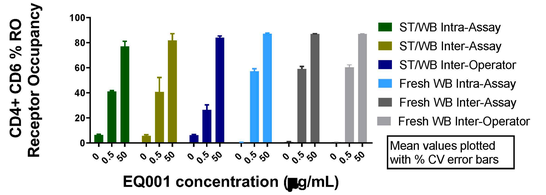
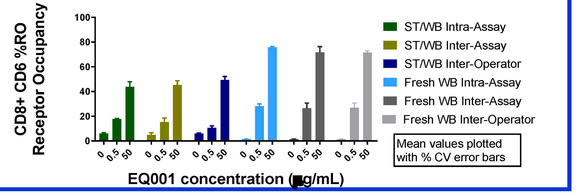


Figure 6. CD8 T cell EQ001 Receptor Occupancy (%RO) Validation Results: Data representative of the 3 healthy donors tested. Mean of triplicates plotted with %CV for each parameter. At 50ug/mL %RO detected was ≤ 49% in Fixed WB CD4 T-cells and ≤ 76% in Fresh WB CD4 T-cells. Different donors used for Fixed WB and Fresh WB.



CONCLUSION

Measuring cell-based receptor engagement and fate in patients on immuno-modulatory therapies is very challenging. This assay was designed and validated to be both sensitive and selective in the quantification of CD6 receptor occupancy and modulation to facilitate the determination of an optimal therapeutic dose in autoimmune and inflammatory diseases.

The whole blood and proteomic stabilized fixed whole blood receptor occupancy assays can be used to assess CD6 modulation and target engagement as a pharmacodynamic marker of Itolizumab on T cells in patients with graft versus host disease (GvHD)), systemic lupus erythematosus (SLE), lupus nephritis and uncontrolled asthma.

ACKNOWLEDGEMENTS

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