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An Assay to Monitor the Engagement and Modulation of CD6 on T cells as a Clinical Biomarker of Treatment with Itolizumab

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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), Precision for Medicine has developed and validated a 10-color flow cytometry assay to assess the engagement and modulation of cell-surface CD6.

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 assay using 5 concentrations of Itolizumab (EQ001) based on expected PK/PD pharmacodynamics.

Step 2. Detection of Itolizumab bound CD6
- 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.

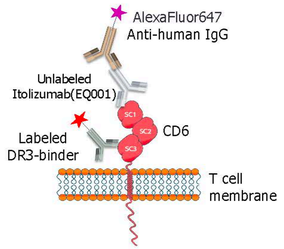
Step 4. Cell Surface Marker Staining
- Cells were stained with an antibody cocktail containing cell surface markers for identification of T cell subsets (T-Memory and T-Regulatory) CD3, CD4, CD8, CD25, CD127, CD45RO, CCR7. The red blood cells were lysed. The sample was washed and fixed for sample acquisition.

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

Summary of CD6 RO Assay Validation
The CD6 RO flow assay was validated in whole blood with three healthy donors assessing 5 concentrations of Itolizumab (EQ001) and no drug. Precision assessed the validation parameters of intra-assay, inter-assay, inter-operator and post-staining stability with pre-set assay validation criteria defined for each parameter being assessed.

Figure 2. Gating Strategy: CD4 & CD8 T cells, T-Memory and CD4 T-Regulatory cell subsets in whole blood

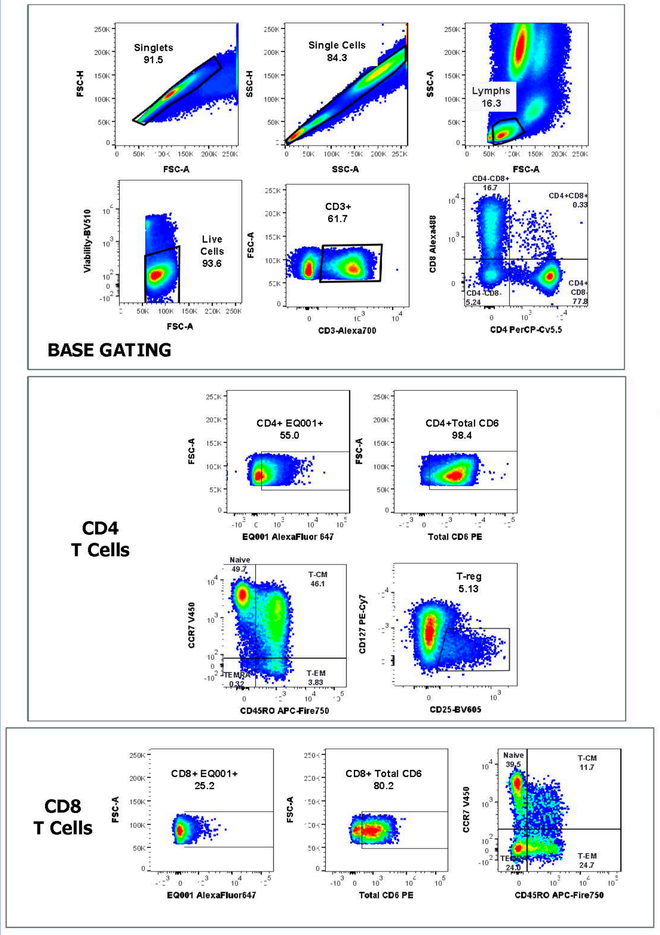


Figure 3. Frequency of CD6 (%CD6 EQ001+) in CD4+ T cell: Data representative of the 3 healthy donors tested. Mean of triplicates plotted with SD. At 50ug/mL %CD6 EQ001+ detected was ≤ 96% in CD4+ T-cells, with ≤ 1% background observed.
Total Surface CD6 frequency was 98.8±0.4% in CD4 T cells.

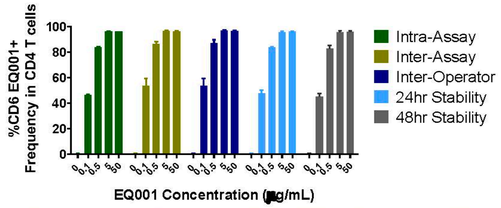


Figure 4. Geometric Mean Fluorescence CD6 EQ001+ (MFI) in CD4+ T cell: Data representative of the 3 healthy donors tested. Mean of triplicates plotted with SD. At 50ug/mL CD6 EQ001+ MFI detected was ≤ 1700 in CD4+ T-cells, with ≤ 400 background observed.
Total Surface CD6 MFI was 2738±77 in CD4 T cells.

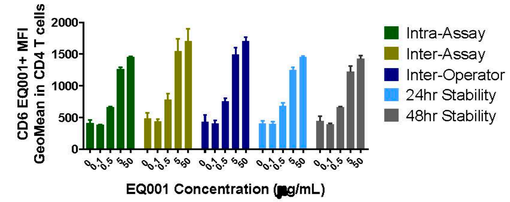


Table 1. %CD6 Receptor Occupancy in CD4 T cells Validation Results: These results represent the Mean and %CV of CD4+ EQ001 receptor occupancy (%RO) for the parameters tested in three healthy whole blood donors. The CD6 %RO results for CD8 T cells, T-Memory, and CD4 T-Regulatory cells passed assay validation criteria (*data not shown*).

	EQ001 Conc. (µg/mL)	Intra-Assay		Inter-Assay		Inter-Operator		24hr Stability		48hr Stability	
		Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
Donor 1	0.10	46.45	1.40	50.86	11.63	57.49	0.22	49.16	7.78	43.80	8.57
	0.50	84.24	0.30	85.08	2.98	89.00	1.72	84.12	0.20	81.82	4.18
	5.00	96.45	0.30	97.07	0.57	97.34	0.17	95.76	1.02	95.31	1.70
	50.00	96.62	0.10	97.51	1.41	97.20	0.56	96.55	0.10	95.99	0.92
Donor 2	0.10	1.87	9.10	2.51	22.57	2.82	6.98	1.90	1.56	1.59	25.39
	0.50	14.77	9.40	16.89	11.35	17.95	4.35	15.79	9.11	15.15	3.50
	5.00	81.85	0.50	84.35	2.67	85.38	0.67	84.18	3.93	81.22	1.10
	50.00	85.88	0.30	88.55	2.69	89.52	0.32	87.97	3.36	85.50	0.63
Donor 3	0.10	15.92	16.70	15.65	6.14	16.89	3.64	17.61	13.53	17.76	14.62
	0.50	57.30	3.30	59.08	3.25	60.30	3.45	57.14	0.39	57.47	0.42
	5.00	86.52	1.10	86.49	0.33	86.30	0.18	87.24	1.17	87.70	1.90
	50.00	87.22	0.70	86.92	0.35	87.03	0.19	86.21	1.67	86.82	0.66

- For each donor, no drug (0ug/mL) was tested to determine the background of CD6 RO in CD4 T cells, the CD6 %RO was <5% for each parameter.
- Specifically the CD6 %RO in CD4 T cells with no drug was ≤ 0.53% for intra-assay, ≤ 2.35% for inter-assay, ≤ 0.62% for inter-operator, ≤ 0.65% for 24hr post staining stability, and ≤ 0.55% for 48hr post staining stability.

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 ≤ 97% in CD4+ T-cells.

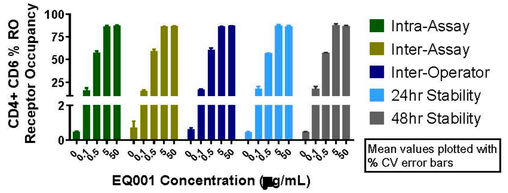
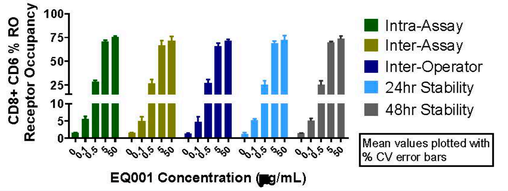


Figure 6. CD8+ T cell EQ001 Receptor Occupancy (%RO) Validation Results: Data representative of the 3 healthy donors tested. Mean of triplicates plotted with %CV for each parameter. At 50ug/mL %RO detected was ≤ 85% in CD8+ T-cells.



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.

This whole blood receptor occupancy assay 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).

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REFERENCES

Stewart, J. et.al., 2016 Cytometry Part B (Clinical Cytometry) 90B:110–116.
Liang, M. et. al., 2106 Cytometry Part B (Clinical Cytometry) Mar; 90(2): 117–127.
Nair, P. et. al., 2010 Clin Exp Immunol. 2010 Oct; 162(1): 116–130.
Fox, D. et. al., 2018 Cellular & Molecular Immunology volume 15, 1001–1002.

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