PRECISION for medicine®

CASE STUDY

Developing and Validating Clinical Receptor Occupancy Pharmacodynamic Biomarker Assays

Background

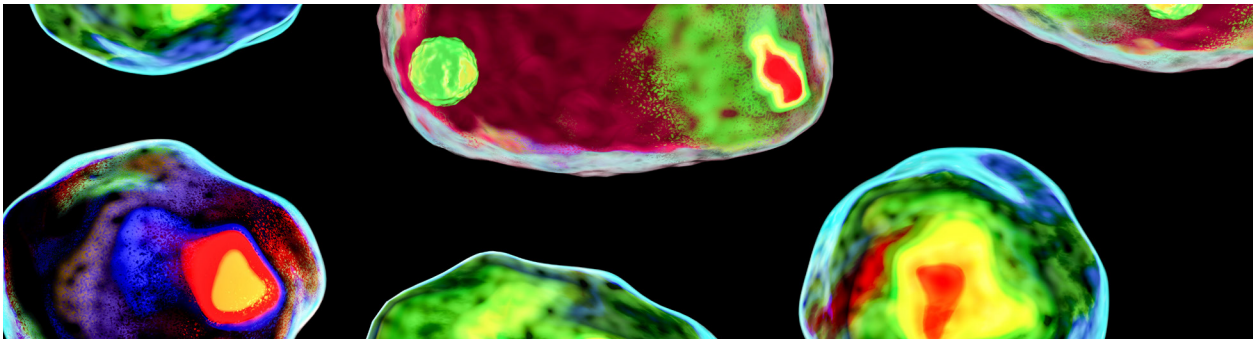
Receptor occupancy (RO) assays are a critical pharmacodynamic (PD) marker for determining target engagement and establishing pharmacokinetic (PK)/PD modeling for first-in-human studies. Often, RO should be tested using fresh whole blood samples to accurately determine the occupancy as isolation and cryopreservation of cells can cause the drug to disengage from the receptor. Additional challenges are that the receptor may be downregulated, internalized, or the target cells may undergo trafficking away from the periphery back to the tissues. Thus, when developing an RO assay, it is important to clarify the stability of the receptor and understand the biology. In general, RO assays need to be customized to the drug of interest, and it is critical to define the best approaches for designing and developing these assays.

Receptor occupancy assays need to be customized to the drug of interest for determining target engagement and establishing PK/PD modeling, and it is critical to define the best approaches for designing and developing these assays.

In this case study, the sponsor needed to monitor target engagement and changes in receptor levels for PK/PD modeling and interpreting patient data while they were on treatment with the drug. This drug is an immuno-modulatory therapy for the treatment of multiple autoimmune and inflammatory diseases.

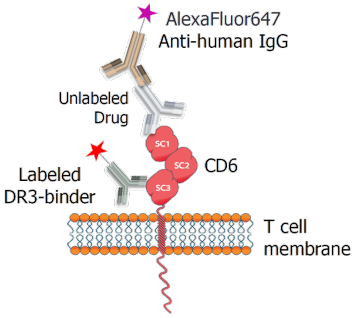
Case Study

To evaluate the PD properties of this specific drug therapy on T cells in patients and determine an optimal therapeutic dose, the sponsor needed an assay to quantify CD6 receptor occupancy and modulation. Ideally, the assay would allow for batched flow testing and to measure receptor occupancy in a stabilized whole blood sample.



Implementation

Figure 1. **Receptor Occupancy of CD6**



Precision for Medicine 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 (fixed WB) samples.

First, whole blood from healthy donors was used to validate the WB and fixed WB assays using multiple concentrations of the drug based on expected pharmacokinetics/pharmacodynamics. For fixed WB samples, whole blood was treated with the drug, and then the samples were fixed and stabilized using SMART™ Tube's proteomic stabilizer and stored at -80°C for subsequent batched flow testing. After red blood cell lysis and washing, cells were stained with:

- Anti-human IgG1 antibody to detect the drug-bound CD6
- Anti-human distal receptor CD6 antibody, a non-competing CD6 antibody to the drug, to detect total CD6 on the cell surface
- An antibody cocktail containing cell surface markers for identification of T cell subsets

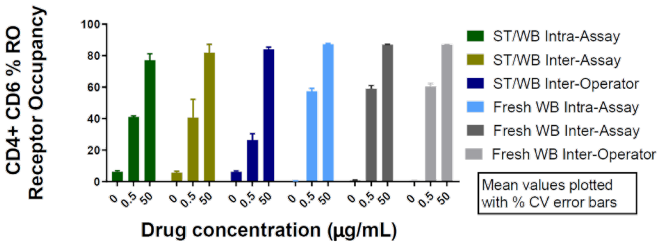
Cells were acquired on a Becton Dickinson FACSCanto™-10-Color Cytometer. CD6 percent receptor occupancy (percentage RO) was calculated by determining the frequency of CD6 bound to the receptor as a ratio of total cell-surface CD6.

Results

Validation parameters assessed included intra-assay, inter-assay, and inter-operator precision, and post-staining stability using multiple concentrations of drug. At a drug dose of 50 ug/mL, the CD6 percentage RO detected was*:

- ≤87% in WB CD4 T cells and ≤76% in WB CD8 T cells
- ≤84% in fixed WB CD4 T cells and ≤49% in WB CD8 T cells

Figure 2. **CD4 T cell CD6 Receptor Occupancy Validation**



Different donors were used for fresh WB and fixed WB samples.

These WB and proteomic-stabilized fixed WB receptor assays have been validated to be both sensitive and selective in assessing CD6 target engagement and modulation as a pharmacodynamic marker of the drug on T cells in patients with autoimmune and inflammatory diseases, such as graft-versus-host disease and systemic lupus erythematosus.

For more information on designing and validating custom flow cytometry assays, and to learn more about Precision for Medicine's full suite of immune monitoring solutions that can help accelerate your trial, please visit **precisionformedicine.com**.

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