Assessment of Receptor Occupancy by Flow Cytometry: A Powerful Tool in Therapeutic Discovery

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Introduction

Flow cytometry can be a powerful tool in therapeutic discovery. One of the areas proven to benefit from this technology is the assessment of receptor occupancy. Receptor occupancy assays describe the qualitative and/or quantitative assessment of binding of a therapeutic to its target receptor. Flow cytometry is an excellent tool for the investigation of monoclonal-based therapeutics, immunomodulators, and small molecules due to the ability to interrogate therapeutic binding, as well as free-receptor simultaneously on multiple cellular targets. Assessment of receptor occupancy by flow cytometry can take several approaches.

Detection of Free Receptor Expression

Free receptor expression, which defines those receptors not occupied by therapeutic, can be assessed by detection with either an antibody that competitively binds the therapeutic's target epitope or by therapeutic that has been fluorescently labeled. These assays are especially useful in the assessment of monoclonal therapeutics but are equally valuable for any targeted therapy where either a competitive antibody or the therapeutic itself is available and readily conjugated to the fluorochrome of choice.

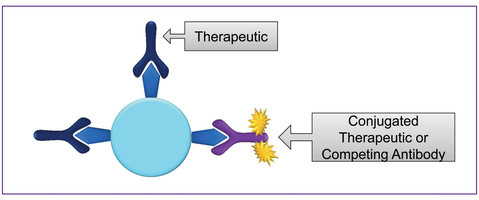

Figure 1. Simple depiction of basic free receptor assay utilizing conjugated drug or competing antibody

Detection of Total Receptor Expression

Total receptor expression, representing the total receptor available for therapeutic binding, can be assessed using a non-competitive antibody that binds to a different epitope from that targeted by the therapeutic or competitive antibody used in free receptor assessment. This type of assay can be valuable in assessing the degree of saturation at any given time point during therapeutic dosing by providing an assessment of the total receptor levels relative to that bound by the therapeutic. It may also assist in assessing if receptor modulation occurs in response to treatment by monitoring total receptor expression over time versus free site occupancy.

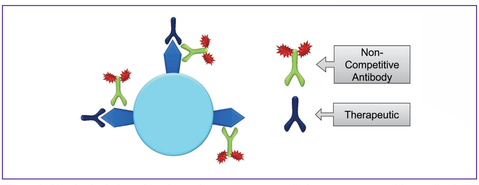

Figure 2. Simple depiction of basic total receptor assay utilizing a non-competitive antibody

Detection of Bound Receptor Expression

Bound receptor expression allows for the assessment of therapeutic that is actively bound to its target epitope. This is performed by using a fluorescently-labeled antibody directed to the therapeutic itself and ideally to the non-binding site region of the therapeutic to avoid competition of therapeutic away from its receptor. These types of receptor occupancy assays are often applied when monoclonal antibodies to the receptor are not readily available or conjugation of the therapeutic itself may compromise binding to its receptor target.

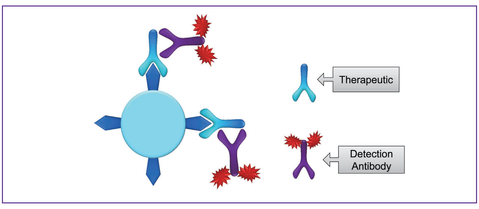

Figure 3. Simple depiction of basic bound receptor assay utilizing an anti-therapeutic antibody

Receptor Modulation

Receptor modulation assays, when properly designed, can allow for the assessment of the functional effect therapeutic binding may have on receptor expression. An example of this type of assessment may involve the monitoring of inhibition of receptor internalization in response to external modulation challenge as a result of therapeutic-receptor interaction. The degree of this modulation inhibition may be directly proportional to the amount of therapeutic-receptor binding present at the time of assessment.

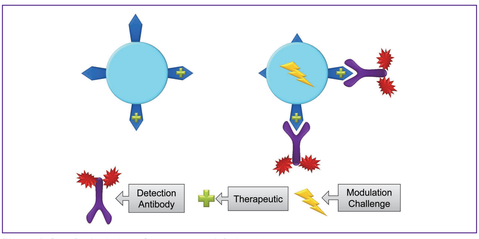

Figure 4. Simple depiction of receptor modulation assay

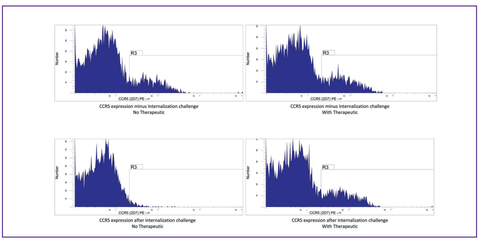

Figure 5. Example of an assay monitoring inhibition of CCR5 internalization by external challenge in the presence or absence of a CCR5 directed therapeutic.

Receptor Occupancy Assessment

There are many ways to assess receptor occupancy using the variety of assays described above. In its simplest form the data can be evaluated by monitoring the quantitative fluorescence expression of the total and/or free receptor antibodies. If designed properly this can be performed within the same tube to allow for a more direct comparison on the cells of interest and can be visualized as shown below.

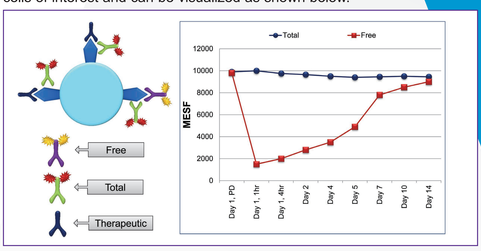

Figure 6. Assessment of the quantitative MESF expression of total and free receptors

Likewise, assessment of free versus bound receptors can also be performed simultaneously using quantitative expression as shown below.

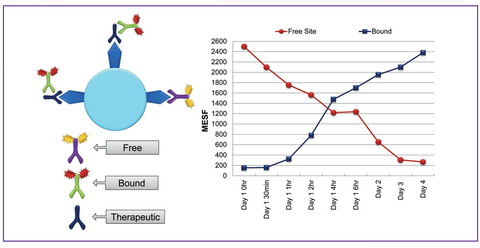

Figure 7. Assessment of the quantitative MESF expression of total versus bound receptors

Calculation of receptor occupancy can be performed using various combinations of either percentage positive results or quantitative expression data such as MESF. Receptor occupancy may be calculated and expressed in terms of percent saturation by using the ratio of free versus total receptors measured within the assay.

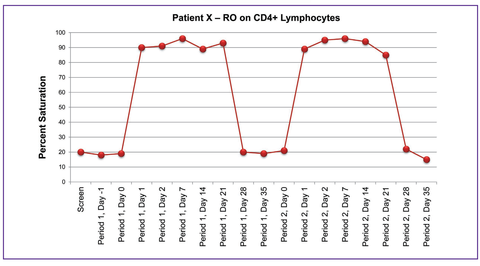

Figure 8. Example of receptor occupancy expressed as percent saturation on a two armed dosing study

Considerations for Receptor Occupancy Assay Development and Performance

Several factors must be considered when developing receptor occupancy assays.

- Choice of appropriate antibody clones.
- Choice of appropriate fluorochrome depending on assay requirements (qualitative versus quantitative assessment).
- Rigorous reagent QC to ensure saturation of target reagents while maximizing signal-to-noise.
- Consistency of lot-to-lot reagents for longitudinal quantitative assays.
- Rigorous instrument QC to ensure consistent data longitudinally.
- Stability of receptor post-collection and impact on assay design.
- Consideration of appropriate assay reportables (% positivity vs. fluorescence intensity).

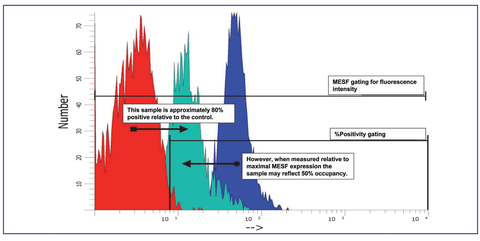

Figure 9. The above displays an example of an assay where percentage positive expression does not accurately reflect the level of receptor occupancy. In this example the cells of interest retain 80-90% positivity relative to the fluorescence control; however, measurement of fluorescence intensity demonstrates approximately 50% occupancy.

Summary

These assays, when properly designed and implemented, can serve as powerful tools in the pharmacodynamic assessment of therapeutic binding. The data generated from these assays can be used as companion data in the pharmacokinetic assessment of therapeutic activity during the course of treatment. Alternatively, these assays may provide independent assessment of therapeutic activity well beyond that seen in traditional pharmacokinetic monitoring.

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