# Use of Receptor Occupancy Assays on cells from Peripheral Blood as a Surrogate Model of the Tumor Microenvironment

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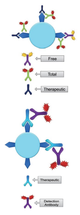
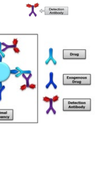
# 4041

## Introduction

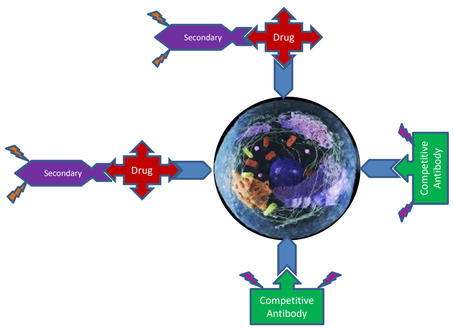
In the development of monoclonal antibodies for the treatment of solid tumors, monitoring receptor occupancy (RO) on peripheral blood lymphocytes can help illustrate potential therapeutic activity occurring in the tumor environment as an assessment of pharmacodynamics (PD). There is increased interest in using these PD assessments on cells from the periphery to assess the effectiveness and efficacy of investigational therapies. In this area of therapeutic development, much focus has been placed on checkpoint inhibitors proteins/receptors such as PD-1, PD-L1, TIM3, CTLA-4, TIGIT, and multiple others. These immune checkpoint inhibitors are suitable targets since they can be upregulated and/or modulated on exhausted T cells in cancer patients. Assessing the expression of these markers in the tumor itself would be an invasive process and provide little information to correlate with pharmacokinetic results throughout the course of a clinical trial to determine optimal dose selection. However, assessment of the receptor expression and occupancy in combination with pharmacokinetics can lead to a better understanding of drug levels required to achieve optimal therapeutic performance. Monitoring the expression and binding of these molecules by drug can determine specific treatment based on the ability of the patient's own immune system to act, while providing information to identify therapeutic responses against cancer. This poster will present an example of one such RO assessment in its development and implementation.

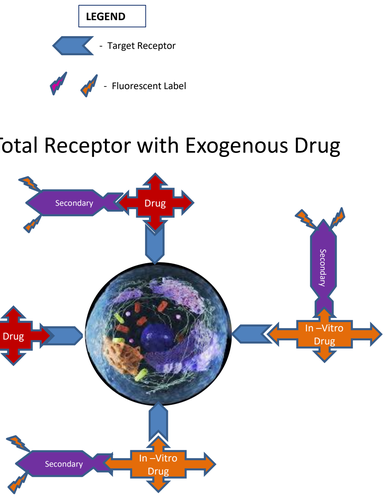
## Mechanisms to Measure Receptor Occupancy

Receptor Occupancy (RO) can be measured in a few different ways, each with its own intricacies that require proper planning and design.

| | | | |
|---|---|---|---|
| **Free Receptor** |  | An indirect determination of free receptors that are not occupied by therapeutic/drug, which is detected with conjugated antibody | Uses Competitive fluorescently labeled antibody or conjugated therapeutic/drug to identify unoccupied receptors |
| **Bound Receptor** |  | A direct determination of receptors that are occupied by therapeutic/drug, which is detected using an anti-drug binding secondary reagent | Uses fluorescently labeled anti-drug secondary antibody to identify occupied receptors |
| **Total Receptor** |  | A measurement of total receptors available on cells of interest using a detection antibody that is either not competitive for binding site with therapeutic/drug | Uses Non-Competitive fluorescently labeled antibody to identify total target receptors -- OR-- Total receptors are identified using in-vitro saturation with drug to fully occupy all possible receptor and detect with fluorescently labeled anti-drug secondary antibody |

### Free Receptor and Bound Receptor




### Total Receptor with Exogenous Drug




## Assay Development and Validation

The example chosen for this presentation utilizes the three main phases of receptor occupancy: free receptor using competitive antibody, bound receptor detecting drug bound to surface antigen of interest, and total receptor using in-vitro saturation with drug to fully occupy all available receptors to detect total available receptors of interest. Finding commercially available non-competitive reagents to assess total receptor is becoming increasingly difficult to identify with higher affinity therapeutics, particularly with checkpoint inhibitors due to their nature. As such, using exogenous drug/in-vitro saturation with detection using anti-drug secondary reagent is becoming the mechanism of choice for most assessments. This too, however, can be problematic in certain scenarios. As the use of a secondary reagent specific to the drug is needed, trials involving combination therapies with more than one treatment can wreak havoc on receptor occupancy determination, as the secondary antibody can bind to both therapeutics, thereby making it impossible to determine which (or both) receptors are being labeled. In these instances, free receptor may be the only option for determination of receptor occupancy (RO) as a pharmacodynamic assessment of drug performance.
The example followed through assay development and validation into the clinical setting is one of the checkpoint inhibitors noted in the introduction. It has been anonymized, as the results of this trial are still in progress.

**Chart 1 Development Data**

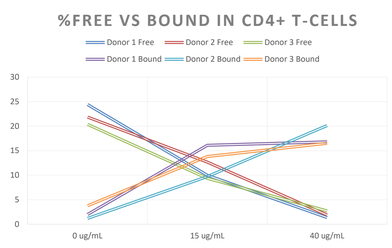



**Chart 1:** The flow results shown in Chart 1 show free receptor inhibition and detection of bound drug with donor blood samples treated with varying amounts of therapeutic. The results show a distinct correlation of detection of free receptor with the incremental in-vitro drug spiking to mimic patient treatment at different levels as well as correlation with detection of bound drug with incremental in-vitro spiking. This confirms that the detection of both is real and also helps to identify the mid-point of approximate 50% occupancy at approximately 15ug/mL in donor blood with this example.

**Chart 2 Development Data**

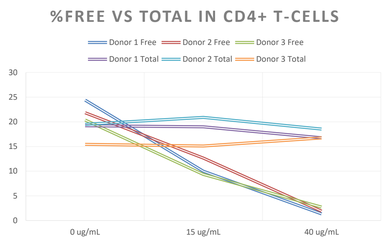
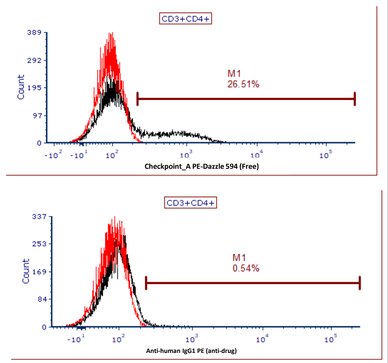


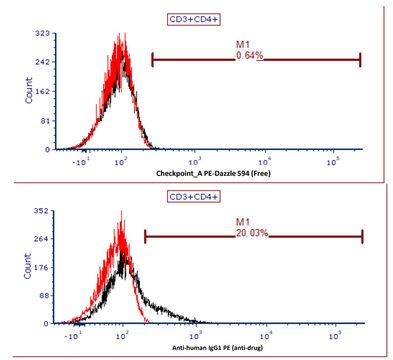

**Chart 2:** The flow results shown in Chart 2 show the ability to detect total receptor with donor blood samples treated with various amounts of therapeutic to mimic patient treatment with additional in-vitro saturation prior to assessment to ensure all receptor sites are occupied for total receptor assessment. These data are also plotted against free receptor data to observe approximate total inhibition at 40ug/mL in the example. It is also noted that prior to treatment (0ug/mL), a slightly higher percentage of free receptors were identified vs. total receptors. This is not atypical in receptor occupancy development and implementation as different antibodies and different methods were used to enumerate the receptor expression.

## Example Histograms

**Expression of Competitive (Free Site) antibody and Anti-human IgG1 (anti-drug) detecting antibody prior to therapeutic administration (no drug).**




**Expression of Competitive (Free Site) antibody and Anti-human IgG1 (anti-drug) detecting antibody after in-vitro saturation with exogenous drug.**




## Trial Data / Patient Results

**Chart 3: Patient Data : Free site vs. Total**

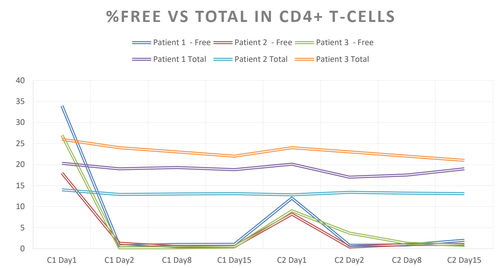



**Chart 3** The flow results shown in Chart 3 display the results of patient dosing through 2 treatment cycles with a recovery period between treatment cycles for %Free vs. %Total. Three example patients were selected for display with full data sets to view. The data shows that within the first day, prior to sampling at Day 2 of each cycle, that the drug dosing had achieved full or near full saturation and held mostly through the cycle of treatment. During this time, the total receptor monitored was also relatively stable over time. In the recovery period between cycles, the availability of free receptors was noted in the patients, indicating that the drug had cleared, at least partially prior to beginning cycle 2 treatment. This data also indicates that some target cells treated in cycle 1 may still be present prior to cycle 2 treatment, as the availability of free receptors had not recovered to near total receptor values.

**Chart 4: Patient Data : Free site vs. Bound receptor**

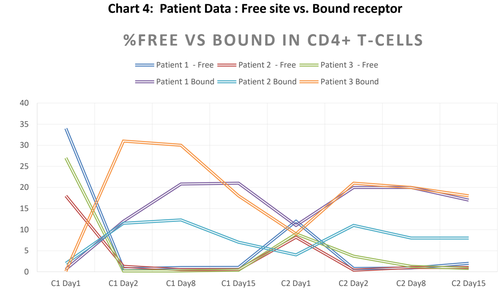



**Chart 4** The flow results shown in Chart 4 display the results of patient dosing through 2 treatment cycles with a recovery period between treatment cycles for %Free vs. %Bound. Three example patients were selected for display with full data sets to view. The data shows that within the first day, prior to sampling at Day 2 of each cycle, that the drug dosing had achieved full or near full saturation and held mostly through the cycle of treatment for free sites, however the detection of bound was a bit more erratic and variable. The data from this comparison confirms that in the recovery period between cycles, some target cells treated in cycle 1 were still be present prior to cycle 2 treatment, as the availability of free receptors had not recovered to near total receptor values and also that the percent of bound cells had not fully reduced to zero.

## Conclusions

When receptor occupancy assays are properly designed and implemented, they can serve as a powerful tool in the assessment of novel therapeutics. Receptor occupancy provides a valid observation of the pharmacodynamic assessments within target populations of interest. In the context of the tumor micro-environment, assessment of specific occupancy is not possible, however, observing changes in target cells or similar populations in the periphery can serve as a suitable alternative. These data, in combination with pharmacokinetic assessments, can prove as a valuable information during the development of novel therapeutics when evaluating patient outcome, current or future dosing regimens, and efficacy of the therapeutic.

Immune checkpoints consist of inhibitory and stimulatory pathways that help with immune response. In cancer, immune checkpoint pathways are often activated to inhibit the anti-tumor immune response. Immune checkpoint therapies act by blocking or stimulating these pathways to increase the body's anti-tumor effect. The example data displayed from an ongoing clinical trial displays the information that can be gained from receptor occupancy analyses. The example therapeutic targets a representative checkpoint inhibitor protein/receptor that is being evaluated as a targeted therapy with solid tumors. By attacking the upregulated checkpoint molecules present on exhausted T-cells (and other populations) in cancer patients, the treatment is intended to be more specific and less toxic to other populations than traditional chemotherapy. When these checkpoints are blocked, the T-cells can target and kill cancer cells more efficiently and prevent these T-cells from attacking other cells in the body. Specifically, these therapies are intended for the tumor micro-environment, but can be measured in the periphery using receptor occupancy as a snapshot of the effectiveness of therapy and to assist in determining optimal dose and timing.

## References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