A Platform Approach to Assess Lack of Monoclonal Antibody Effector Function Using an iQue® Screener PLUS

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Introduction

Sartorius has developed two platform approaches to assess lack of Antibody-dependent cellular cytotoxicity (ADCC) or Antibody-dependent cellular phagocytosis (ADCP) MoAs of monoclonal antibodies using the iQue® Screener PLUS. The iQue® Screener PLUS achieves a faster assay throughput than traditional flow cytometers by sampling only microliters from each well, and delivering an on-the-fly-defined flow of samples to the detectors. This technology transforms a low throughput, time-consuming flow cytometry approach into a high throughput process.

An increasing number of therapeutic antibodies are designed not to induce Fc effector functions such as ADCC and ADCP. One such molecule is nivolumab that targets the immune checkpoint protein PD-1. It is required during the development of these molecules to demonstrate that IgG MoAs do not occur. The key to a well designed lack of effector function assay is the identification of a suitable positive control molecule. The PC demonstrates that each individual assay run is capable of inducing the MoA under assessment. The data presented here was obtained from development studies performed at Sartorius Glasgow site.

1. Experimental Approach

Two methods have been developed, one to measure a lack of ADCC activity and another to measure a lack of ADCP activity. Figure 1 shows an overview of the ADCC and ADCP process. PBMC preparations are used as effector cells in the ADCC format and CD4+ monocytes isolated from whole blood are used as effector cells in the ADCP assay format.

1.1. ADCC Assay Format

ADCC assay specific death of these cells is used as the assay readout. For the ADCP assay CD14+ effector cells that have phagocytosed target cells will become positive for the cell encoder and therefore encoder+ CD14+ cells are considered to have performed ADCP.

Figure 1: Schematic diagram of ADCC (left) and ADCP (right) MoA functions of therapeutic molecules.

Figure 2 demonstrates the workflow offered by Sartorius of which a key step is the identification of a target cell line. We have a wide range of mouse cell lines, the capability to generate GMP cell banks if required and the technical expertise to isolate specific cell types such as T cells from fresh whole blood using on-site donor pools. This capacity combined with our house-platform live cell binding assay approach places us in the perfect position to work with our clients to identify a suitable target cell line (Figure 2).

1.2. ADCP Assay Format

The format is suitable for both biosimilar testing and new biological entities testing. We were able to identify a human IgG molecule with a constitutively expressed antigen to use as a positive control in the ADCP assay format. We have confirmed binding of this molecule to a range of cell lines including primary activated T cells (Figure 4).

For molecules such as nivolumab, their target antigens (PD-1 in this case) are not always expressed on target cells. We isolated T cells from whole blood and then activated them with CD3/CD28 dynabeads. The activated T cells express PD-1 and were used as target cells to develop lack of effector function assays for both ADCC and ADCP. The developed assay includes a binding readout to confirm target antigen expression in each assay run.

2. Gating Strategy

Gating strategies for the ADCC and ADCP assay formats are shown below. Experimental design includes fluorescence minus one (FMO) controls to enable accurate setting of key gates in the assay (Figure 3).

2.1. ADCC Gating

Figure 3 demonstrates the gating strategy for ADCC activity (Left) and ADCP activity (Right). Gating strategy for the ADCC assay format is shown in Figure 5.

2.2. ADCP Gating

Figure 5 demonstrates the gating strategy for ADCC assay format. For molecules such as nivolumab, their target antigens (PD-1 in this case) are not always expressed on target cells. We isolated T cells from whole blood and then activated them with CD3/CD28 dynabeads. The activated T cells express PD-1 and were used as target cells to develop lack of effector function assays for both ADCC and ADCP. The developed assay includes a binding readout to confirm target antigen expression in each assay run.

3. Identifying a Assay Positive Control

We are able to identify a human IgG molecule with a constitutively expressed antigen to use as a positive control in the assay format. We have confirmed binding of this molecule to a range of cell lines including primary activated T cells (Figure 4). Using activated T cells as target cells, we successfully developed assay formats for both ADCC and ADCP with the positive control molecule producing an assay response while nivolumab fails to elic it an assay response [as expected; representative assay data is shown in Section 5].

4. Results – Example of Assay Data

The ADCC and ADCP assay formats assess a 5 point dilution series of the therapeutic molecule which robustly tests if a PC response occurs in each assay to clearly demonstrate the assay can induce the MoA in question. A high, medium and low concentration of the test material is assessed to show a lack of MoA activity. Reproductions are reported alongside the background target and effector cell alone assay responses as well as the maximal and minimal PC response. Binding of a reference standard molecule at the high concentration confirms the test antigen expression; an important assay control when target cells are required to be stimulated to induce antigen expression. Example ADCC assay output is presented in Table 1 and ADCP output in Table 2. Data is taken from internal development studies for these methods.

5. Conclusions

• We have developed two off the shelf assay to assess lack of effector function MoA of anti PD-1 molecules and anti PD-1/2/3/4 molecules for both ADCC and ADCP
• Primary cells are used as both effector and target cells
• Cells are activated to induce antigen expression
• Allowing readout is included to assess target antigen expression in each run
• The off-the-shelf assay can be used to test any molecules that bind activated T cells and are not expected to induce ADCC or ADCP; for example natalizumab
• Our ADCC assay uses primary monocytes and is a measure of ADCC MoA function
• We have identified a human IgG positive control molecule that binds to a wide range of cell lines and can induce both ADCC and ADCP MoA responses
• The binding readout can be applied to any target cell line that our PC will bind, representing a true platform approach to the lack of effector function assay formats
• The format is suitable for both biosimilar testing and new biological entities testing

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Table 1: Example data taken from internally developed ADCC assay - nivolumab assay. In the assay target cells are activated T cells, effector cells are PBMCs and all TMs are nivolumab.

Table 2: Example data taken from internally developed ADCC assay – nivolumab assay. In the assay target cells are activated T cells, effector cells minus plus isolated from activated whole blood and all TM are nivolumab.