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Simplifying Progress

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

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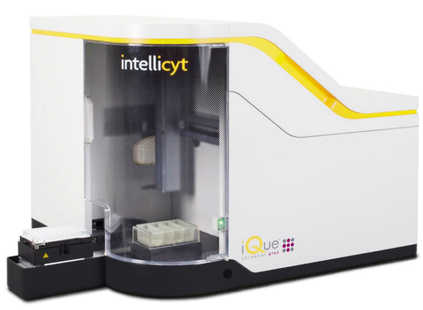
Introduction

Sartorius have developed two platform approaches to asses a 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 air-gap-delimited 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 ADDC and ADCP process. PBMC preparations are used as effector cells in the ADCC format and CD14+ monocytes isolated from whole blood are used as effector cells in the ADCP assay format.

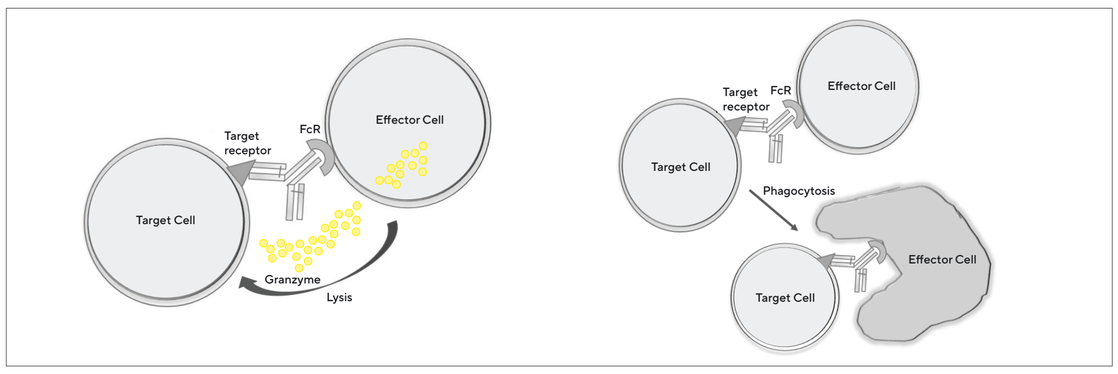


Figure 1: Schematic diagram of ADCC (left) and ADCP (right) MoA functions of therapeutic molecules.

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 in house 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 our on-site donor pools. This capacity combined with our in house platform live cell binding assay approach places us in the perfect positon to work with our clients to identify a suitable target cell line (Figure 2).

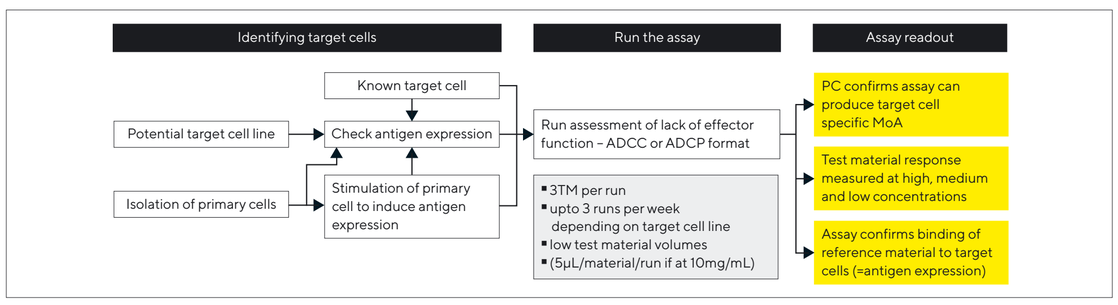


Figure 2: Schematic diagram of the work flow for a lack of effector function assay.

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 CD3/28 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).

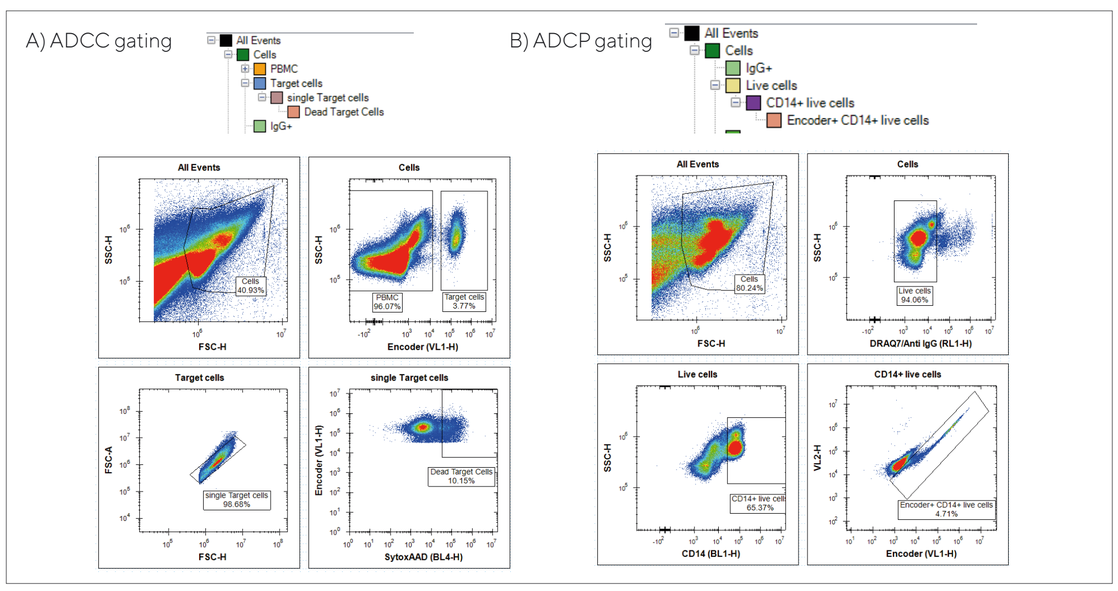


Figure 3: A) Gating strategy for lack of ADCC effector function assay format. B) Gating strategy for lack of ADCP effector function assay format

In both assays, target cells are encoded with a dye that enables specific identification of the cells within each well. For the 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, therefore encoder+ CD14+ cells are considered to have performed ADCP.

3. Identifying a Assay Positive Control

We were 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).

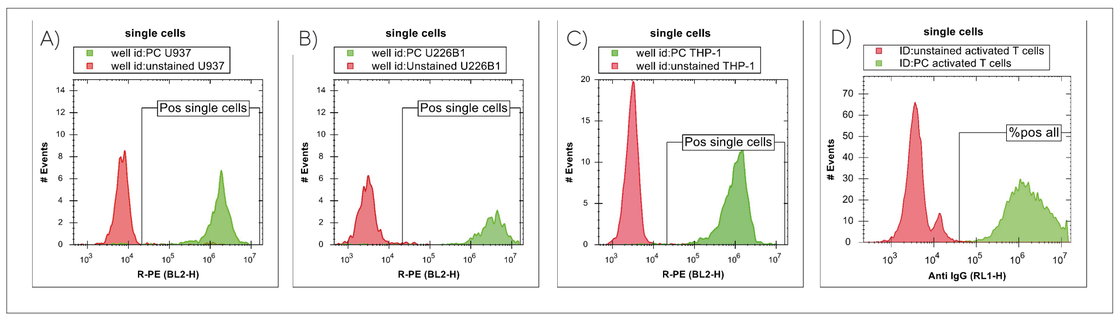


Figure 4: Binding of a positive control (PC) human IgG molecule was assessed in U937 cells (A), U226B1 cells (B), THP-1 cells (C) and primary activated T cells (D). Binding was confirmed across all cells tested as shown by a shift to the right in the histograms vs unstained cells.

Using activated T cells as target cells, we successfully developed assay formats for both ADCC and ADCP with the PC molecule producing an assay response while nivolumab fails to elicit an assay response (as expected), representative assay data is shown in Section 5.

4. Results – Example of Assay Data

The ADCC and ADCP assays formats asses a 5 point dilution series of the PC 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 materials are assessed to show a lack of MoA activity. Reponses are reported alongside the background target and effector cell alone assay response as well as the max and min PC response. Binding of a reference standard molecule at the high concertation confirms target 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.

Material position	Mean % target cell death			T&E % death	Lowest PC mean % response	Largest PC mean % response	RS binding (>25% positive)	Number of PC wells >3 s.d away from T&E response
	Low conc.	Medium conc.	High conc.					
RS	11.5	9.4	8.1					
A	8.9	9.2	8.5					
B	10.5	9.3	9.1	8.7	18.4	25.6	Pass	Pass
A	9.7	9.2	9.4					

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

Material position	Mean % ADCP			T&E % death	Lowest PC mean % response	Largest PC mean % response	RS binding (>25% positive)	Number of PC wells >3 s.d away from T&E response
	Low conc.	Medium conc.	High conc.					
RS	4.23	4.80	4.16					
A	4.50	4.11	4.09					
B	4.46	4.10	4.09	4.55	5.51	11.29	Pass	Pass
A	4.76	4.45	4.53					

Table 2: Example data taken from internally developed lack of ADCP effector function – nivolumab assay. In the assay target cells are activated T cells, effector cells monocytes isolated from whole blood RS and all TMs are nivolumab.

5. Conclusions

- We have developed two off the shelf assays to assess lack of effector function MoA of anti PD-1 molecules and anti PD-1 biosimilar molecules for both ADCC and ADCP
  - Primary cells are used as both effector and target cells
  - T cells are activated to induce antigen expression
  - A binding 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 either ADCC or ADCP, for example natalizumab.
- Our ADCP assay uses primary monocytes and is a measure of ADCP 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 experimental approach 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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