1. Introduction

Antibody-dependent cellular phagocytosis (ADCP) is an important mechanism of action of therapeutic antibodies that is effective at killing tumor cells as well as in antibody-dependent cell-mediated cytotoxicity (ADCC). Antibody drug development is beginning to address and realize the potential of the mechanism of action more effectively. Additionally, cell development is enabling the potential of an ADC response for vaccine effectiveness.

However, even more so than classic ADCs, bispecific ADCAs are tedious and tightly variable. They are FACS-based rather than plate-based, and run on isolation of primary cells, typically murine, that then need to be differentiated into diagnostic populations. Furthermore, the drug development is dependent on the stability of target cell lines. The stability of target cell lines is critical as antibody and target cell development could be improved.

Macrophage effector cells and other myeloid-derived phagocytes mediating ADCP are more dependent on Fcγ activating receptors beyond the NK cell expressing and ADCC-promoting FcγRIIa. The FcγRIIIa receptor has been reported to have the dominant contribution in ADCP by macrophages.

We describe here our development and evaluation of a human FcγRIIIa (CD16) specific reporter bioassay for quantifying antibody bioactivity in ADCP pathway activation. The FcγRIIIa in vivo is a key activator of ADCP. The assay was used to identify engineered macrophage effector cells that express the receptor and mediate FcγRIIIa-dependent macrophage activation of reporter expression only when receptors are bound by target cell-bound antibodies.

We confirmed surface expression of the FcγRIIIa (CD16) variant in our effector cells via FACS and passive stability of the cell line for cell bank development. We developed and optimized the FcγRIIIa reporter bioassay using the effector cell in three-plate format (immunization/immunPAL culture). We used a range of dose–response curves consistency. Rituximab, a chimeric Igg1 antibody drug with lower ADCP activity than ADCP, was used in much of the bioassay development. Using a series of reporter cell variants, we determined that at all human tissue culture levels. We further demonstrated that the assay can quantify the bioactivity of other antibodies such as anti-EGFR panitumumab (an IgG2) anti-HER-3 trastuzumab and on anti-GE2 antibody via the receptor. In a bioassay analysis, the assay was accurate, precise and demonstrated good linearity and range.

2. Generation of engineered FcγRlla/NFAT-RE-luc2 effector cells

Reporters for engineered FcγRlla/ADCP effector bioassay

Jacket cells were transformed to engage in the FcγRlla luc2 expression plasmid.

Jacket cells have the immunochemical signaling pathway elements for MACP expression of action in the bioassay.

Cells were prepared in thaw and-use assay-ready format.

3. Protocol and advantages of the bioassay

Plate surface antigen expression

Target cell - FcγRlla effector cells in thaw and-use format

Response induction (24 H)

Measure luminance

Simple, low variability, homogeneous, convenient bioassay

6. FcγRlla (H131 variant) bioassay shows appropriate IgG isotype bioactivities

Using an anti-CD20 human IgG1 isotype series, the assay demonstrates expected EC50 ranking based on published SPR binding affinities for FcγRIIa.

7. Bioassay can quantify bioactivity in ADCP pathway activation of a variety of drug and research antibodies

A. Rituximab and research-grade ‘equivalent’ ADCP Control Ab, anti-CD20

B. Trastuzumab

C. Pamitumumab

D. Anti-GD2

8. Potency qualification using ADCP Control Ab, Anti-CD20

Qualification results:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Accuracy</th>
<th>Expected Relative Potency</th>
<th>Recovery (%)</th>
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<tbody>
<tr>
<td>Linearity (%)</td>
<td>99.1</td>
<td>100.7</td>
<td>105</td>
</tr>
<tr>
<td>Interplate precision (%)</td>
<td>1.71</td>
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</tbody>
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Repeatability (%CV)

158.1, 111.2, 243

9. Bioassay demonstrates stability indicating properties using heat stressed rituximab

Rituximab was heat stressed at 63°C for 1-3 days and compared to control unheated sample. Results obtained following 6 hour induction using frozen thaw-and-use FcγRlla (H131) effector and Raji target cells.

10. ADCP reporter bioassay is robust

Biologic robustness was tested using thaw-and-use FcγRlla (H131) effector cells, Raji target cells, and ADCP Control Ab, Anti-CD20. The bioassay is robust for E50 within an induction time of 2-3 hours and in the range of 15K-35K Raji target cells/well. Effector cells were at 35K/well.

Summary

1. We have developed an FcγRlla-specific ADCP reporter assay that quantifies ADCP MOA pathway activation for the predominant Fcγ receptor mediating macrophage ADCP.

2. The luminescent 96-well plate assay is simple, homogeneous, convenient, and fast. It should provide a much less variable and tedious method of assessing ADCP pathway MOA than FACS analysis using monocore-derived macrophages differentiated in culture.

3. It is able to quantify ADCP pathway activation for a variety of research and drug antibodies and demonstrates appropriate human IgG isotype relative activities.

4. The bioassay is specific, robust and stability-indicating. In a qualification study it demonstrated excellent linearity, recovery and intra- and intermediate assay precision, indicating potential application for plate-based lot release testing.

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