Measurement of Fc-mediated ADCC and CDC of anti-TNFα and anti-VEGF Therapeutic Antibodies using Reporter-based Bioassays and Engineered TNFα+ and VEGF+ Target Cells

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1. Introduction

Fc-mediated effector functions are critical to the efficacy and safety of therapeutic antibodies. Measurement of Fc-mediated antibody-mediated cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) during antibody drug discovery and development is not only important for antibodies that harness ADCC and/or CDC as their primary mechanism of action (e.g. rituximab, trastuzumab), but also for antibodies designed to target and block soluble ligands such as TNFα and VEGF.

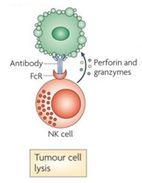
We previously reported the development of a cell-based reporter bioassay platform which has been used to measure ADCC and ADCP mediated through FcγRI, FcγRIIa and FcγRIIIa. These reporter bioassays exhibit the specificity, accuracy, precision, and robustness necessary for qualification according to ICH guidelines and have been used extensively to characterize and measure the potency of antibody-based biologics drugs that target cell surface immune receptors. In the current study, we sought to evaluate Fc-mediated ADCC and CDC activities of therapeutic antibodies designed to target and block soluble ligands including TNFα and VEGF.

To measure ADCC activity of anti-TNFα and anti-VEGF blocking antibodies, we developed engineered target cells that express either membrane-bound TNFα or VEGF. When used as target cells with reporter-based effector cells expressing a relevant FcγR, ADCC activity of adalimumab (anti-TNFα) and bevacizumab (anti-VEGF) was detected in a specific and dose-dependent manner. Similarly, when used in a luminescence-based CDC assay, the engineered target cells elicited an appropriate FcγR-mediated response. The assay signals demonstrated IgG isotype specificity as IgG4 variants showed minimal activity in both ADCC and CDC assays. Our results demonstrate that the combined use of cell-based reporter bioassays with target cells engineered to express membrane-bound soluble ligands can provide a simple, specific, and quantitative platform to measure Fc-mediated effector functions of therapeutic antibodies targeting soluble ligands.

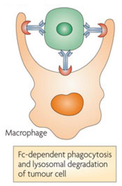
2. MOA-based ADCC & ADCP Reporter Bioassays

Surrogate Measure of In Vivo Biology

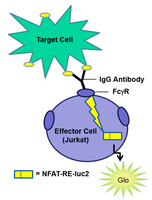
FcγR-mediated ADCC



FcγR-mediated ADCP



Fc Effector Reporter Bioassay

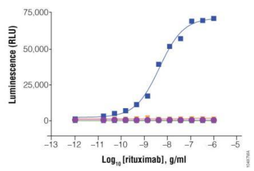


FcγR Reporter Bioassays are Specific

Variations of the ADCC Reporter Bioassay (FcγRIIIa-V158) were used to measure the Fc effector function of either Rituximab (anti-CD20) or Trastuzumab (anti-Her2) to demonstrate assay specificity.

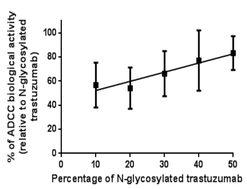
Assay signal is dependent on:

- Target cells expressing Ab-targeted antigen
- Effector cells expressing FcγR
- Antigen-specific IgG antibody
- **Similar results were obtained using human FcγRIIIa, FcγRIIa, FcγRI and mouse FcγRIII and FcγRIV bioassays.**

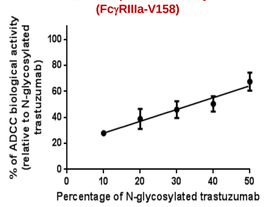


3. ADCC Reporter Bioassay Correlates with Primary Cell-based ADCC Cytotoxicity Assay

ADCC Cytotoxicity Bioassay (PBMCs from VV allele blood donors)



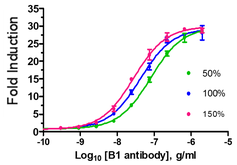
ADCC Reporter Bioassay (FcγRIIIa-V158)



Antibody (trastuzumab) potency was measured using a primary cell-based ADCC assay (**left panel**) and the ADCC Reporter Bioassay (FcγRIIIa-V158, **right panel**). The surrogate ADCC Reporter Bioassay shows similar potency ranking but much less variability (as indicated by the smaller error bars).

4. FcγR Reporter Bioassays Measure Antibody Relative Potency and Stability

Potency Determination



The **relative potency** of anti-CD20 Ab preparations were measured using the ADCC Reporter Bioassay (FcγRIIIa-V158).

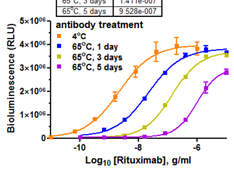
Excellent accuracy, precision, and linearity in the range of 50-150% relative potency (per ICH guidelines) was demonstrated (shown here and data not shown).

Stability Indication

Rituximab

CD20+ WIL2-S Target Cells

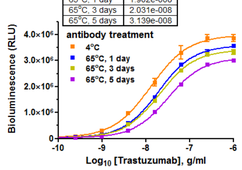
	EC50
4°C	2.133e-009
65°C, 1 day	2.148e-008
65°C, 3 days	1.411e-007
65°C, 5 days	9.528e-007



Trastuzumab

HER2+ SKBR3 Target Cells

	EC50
4°C	1.254e-008
65°C, 1 day	1.902e-008
65°C, 3 days	2.031e-008
65°C, 5 days	3.139e-008



The ADCC Reporter Bioassay (FcγRIIIa-V158) was used in a stability study of Rituximab and Trastuzumab following heat denaturation at 65°C for the indicated number of days.

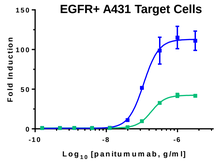
Similar results were obtained using human FcγRIIIa, FcγRIIa, FcγRI and mouse FcγRIII and FcγRIV bioassays.

5. Application of FcγRIIa & FcγRI ADCP Bioassays to Characterize Antibody MOA and Glycosylation

FcγRIIa-mediated MOA

Panitumumab (IgG2)

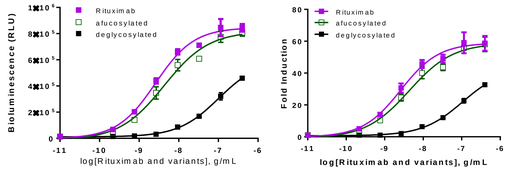
EGFR+ A431 Target Cells



FcγRIIa-H ADCP Reporter Bioassay

FcγRIIa-R ADCP Reporter Bioassay

Antibody Deglycosylation Measured by FcγRI

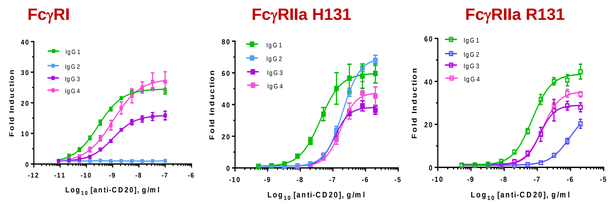


CD20+ Raji Target Cells were assayed with Rituximab preparations that were either untreated, afucosylated or deglycosylated, as indicated. Deglycosylated antibody showed a significant decrease in bioluminescence (left panel) and fold induction (right panel), as expected.

6. FcγR bioassays show appropriate IgG isotype Specificity

Test anti-CD20 antibody, rituximab IgG variants

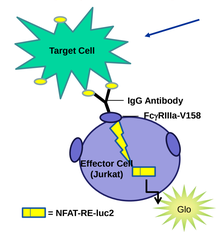
1. Human IgG1
2. Human IgG2
3. Human IgG3
4. Human IgG4



EC_{50} (g/mL)	FcgRI	FcgRIIa H131	FcgRIIa R131	FcgRIIIa F158	FcgRIIIa V158
IgG1	$2.8x10^{-10}$	$4.15x10^{-8}$	$7.03x10^{-8}$	$3.34x10^{-8}$	$4.50x10^{-9}$
IgG2	NB	$1.98x10^{-7}$	$>9.60x10^{-7}$	$3.55x10^{-7}$	$4.87x10^{-7}$
IgG3	$1.0x10^{-9}$	$1.14x10^{-7}$	$1.18x10^{-7}$	NB	NB
IgG4	~ $1.0x10^{-9}$	$1.71x10^{-7}$	$1.45x10^{-7}$	NB	NB

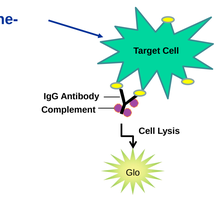
7. Anti-TNFα & Anti-VEGF ADCC & CDC Assays using Engineered Target Cells

ADCC Reporter Bioassay (FcγRIIIa-V158)



1. Target cell-bound antibody binds to FcγRIIIa-V158
2. FcγRIIIa-V158 signals through NFAT pathway in ADCC Effector Cells
3. Luciferase activity measured using Bio-Glo reagent

CDC Assay



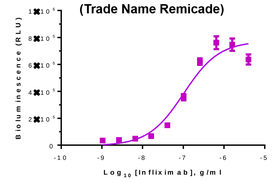
1. Complement binds to target cell-bound antibody
2. Activation of the complement cascade results in cell lysis
3. Cell viability or cytotoxicity of target cells is measured using **CellTiter-Glo (loss-of-signal)** or **CytoTox-Glo (gain-of-signal)**

Measure anti-TNFα mAb-induced ADCC & CDC Activity

ADCC Reporter Bioassay

Infliximab

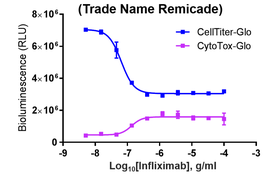
(Trade Name Remicade)



CDC Assay (CytoTox-Glo vs CellTiter Glo)

Infliximab

(Trade Name Remicade)

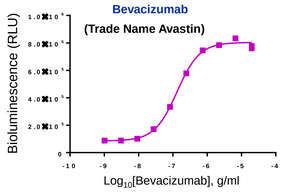


Measure anti-VEGF mAb-induced ADCC & CDC Activity

ADCC Reporter Bioassay

Bevacizumab

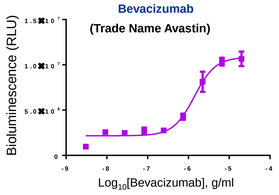
(Trade Name Avastin)



CDC Assay (CytoTox-Glo)

Bevacizumab

(Trade Name Avastin)



8. Conclusions

Functional cell-based assays for the measurement of ADCC and CDC are critical in the development of therapeutic antibodies. Here we show application of FcγR reporter bioassays, engineered target cells, cell viability and cytotoxicity assays to elucidate and characterize the Mechanism of Action (MOA) of antibody biologics for their Fc Effector Function.

Biologically relevant measurement of antibody Fc effector function

- ADCC reporter bioassays with specific FcγR-induced expression of NFAT-RE driven luciferase
- Specific Ab-mediated target cell killing via CDC
- Appropriate IgG isotype specificity in ADCC and CDC assays

Consistent and reliable measure of antibody activity

- Demonstrated precision, accuracy, reproducibility, robustness

Easy-to-implement

- Multiple product formats meet diverse workflows; commercial kits include necessary reagents
- Rapid protocols for standard 96-well plate format

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