Novel reporter-based bioassays for evaluating FcyR-dependent functions of therapeutic antibodies

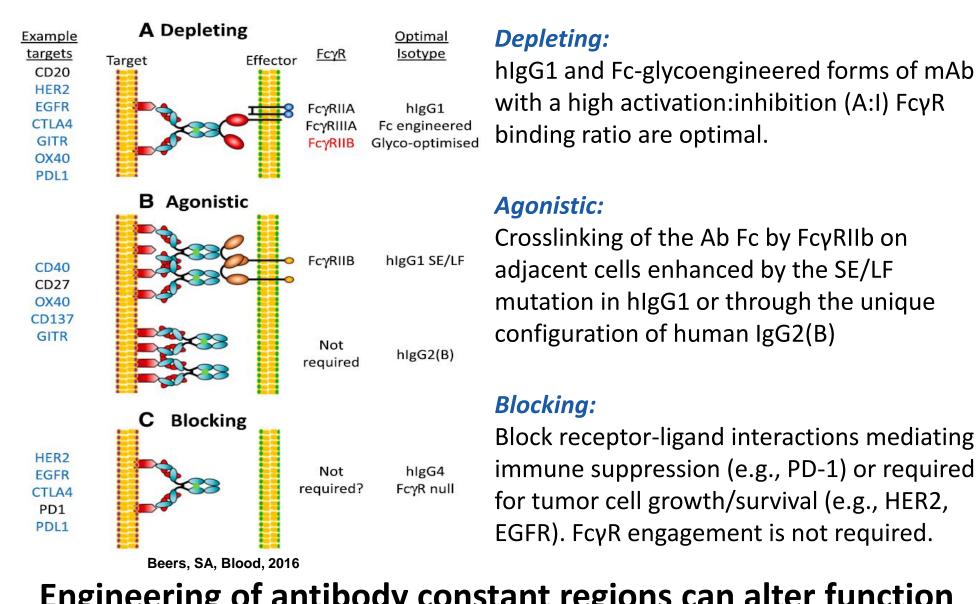
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Frank Fan, Mei Cong, and Jey Cheng

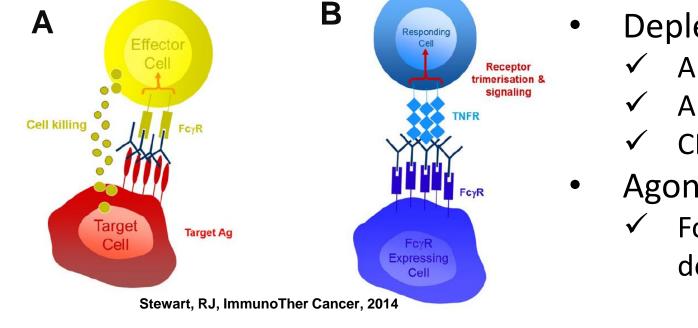
Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711



1. Antibody constant regions dictate functional activity



Engineering of antibody constant regions can alter function Comprehensive suite of bioassays to measure Ab Fc function



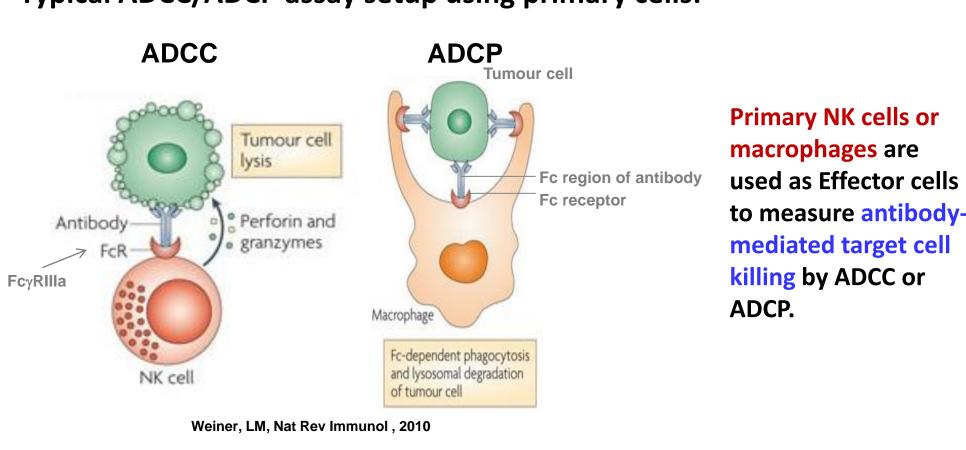
Depleting Abs **ADCC**

ADCP CDC Agonist Abs Fc crosslinkingdependence

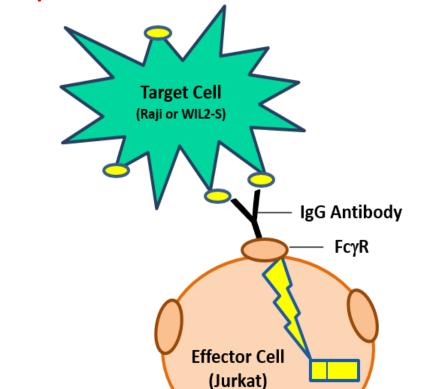
(A) Ab bound to High level of target antigen clusters FcγRs driving ADCC or ADCP (B) Low level of target receptor is clustered by Ab bound to FcγRs driving activating signals to receptor expressing cells

2. ADCC and ADCP bioassay designs

Typical ADCC/ADCP assay setup using primary cells:



VS.

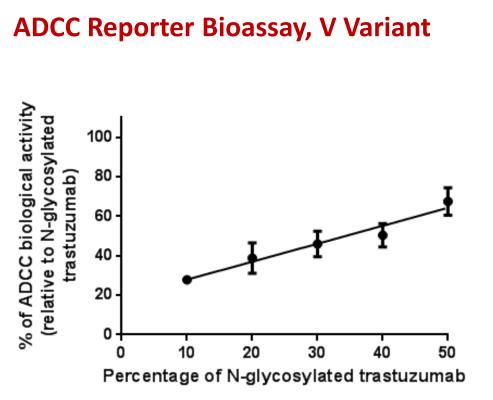


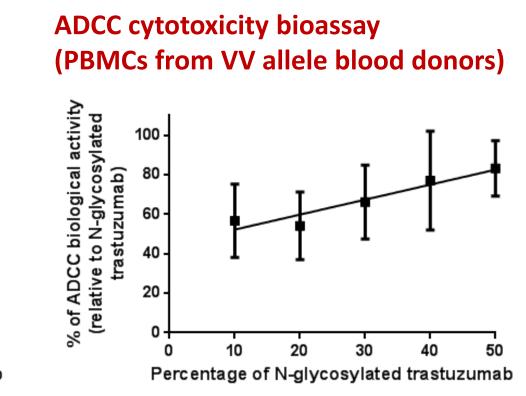
FcyR Reporter Bioassay:

Engineered reporter cell lines are used as Effector cells to measure antibody-mediated Fc receptor activation.

- **Human:** FcyRI FcyRIIa (H or R) FcyRIIb FcyRIIIa (V or F)
- Mouse: FcyRIII
- FcyRIV = NFAT-RE-luc2

3. Reporter-based ADCC bioassay correlates with primary cell killing assay



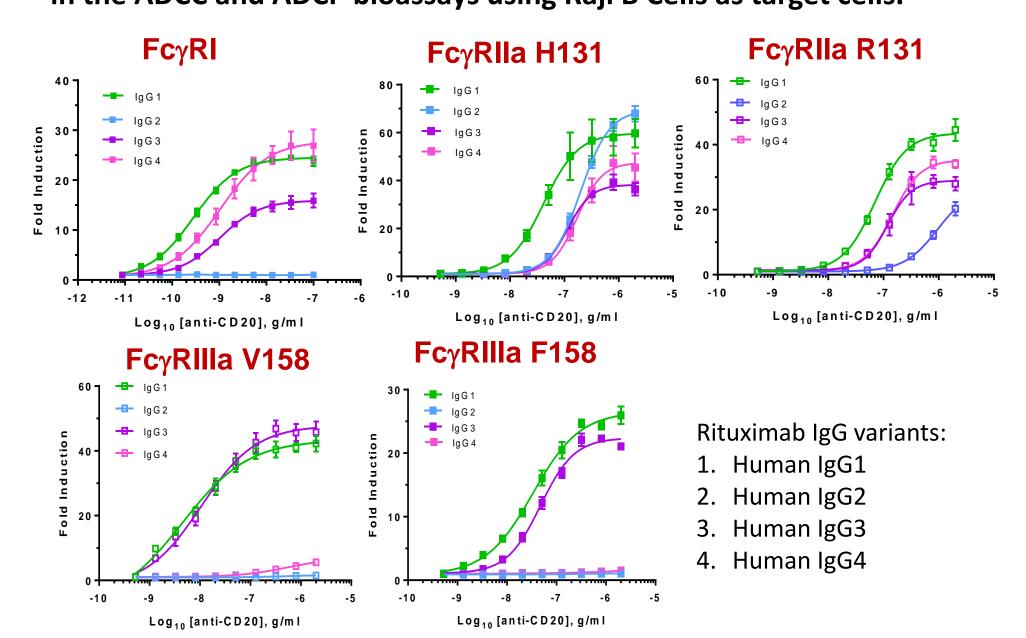


- A series of trastuzumab glycosylation blend mixtures were prepared by blending PNGase F-treated (deglycosylated) and untreated trastuzumab (N-glycosylated).
- The antibody samples were tested with SK-BR-3 target cells, using untreated antibody as 100% reference.
- Assay responses were correlated between reporter-based and cytotoxicity ADCC bioassays
- Reporter-based ADCC bioassay exhibited much lower variability

Cheng-ZJ et al., *J of Immunol Methods (*2014)

4. Bioassays demonstrate isotype selectivity for human FcyRs

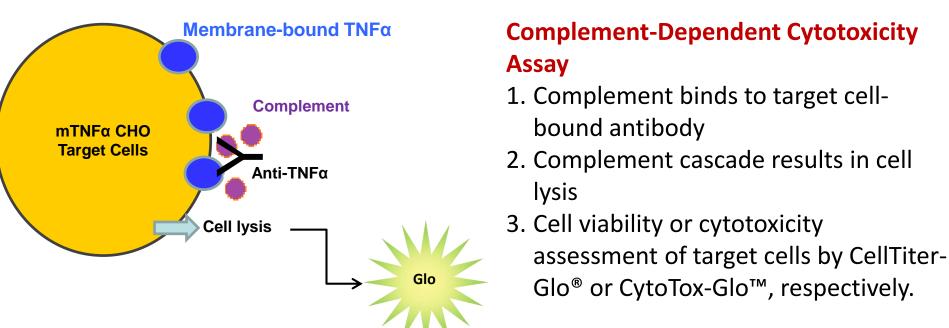
Research grade anti-CD20 Abs with different IgG isotypes were tested in the ADCC and ADCP bioassays using Raji B Cells as target cells.



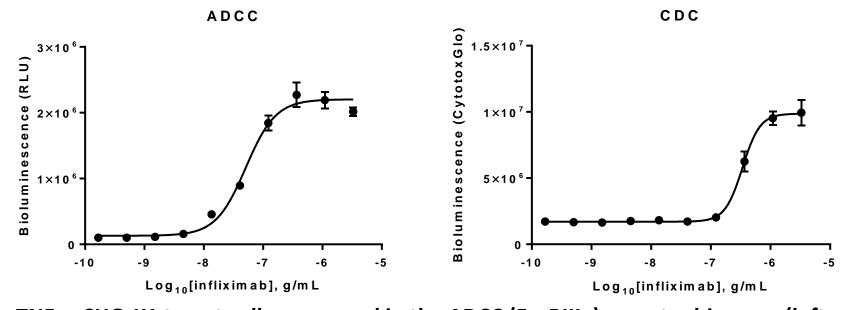
Summary table of EC₅₀ of each Ab isotype in the ADCC and ADCP bioassays

EC ₅₀ (g/mL)	FcγRI	FcγRIIa H131	FcγRIIa R131	FcγRIIIa F158	FcγRIIIa V158
lgG1	2.8x10 ⁻¹⁰	4.15x10 ⁻⁸	7.03x10 ⁻⁸	3.34x10 ⁻⁸	4.50x10 ⁻⁹
lgG2	NB	1.98x10 ⁻⁷	>9.60x10 ⁻⁷	3.55x10 ⁻⁷	4.87x10 ⁻⁷
lgG3	1.0x10 ⁻⁹	1.14x10 ⁻⁷	1.18x10 ⁻⁷	NB	NB
lgG4	~ 1.0x10 ⁻⁹	1.71x10 ⁻⁷	1.45x10 ⁻⁷	NB	NB

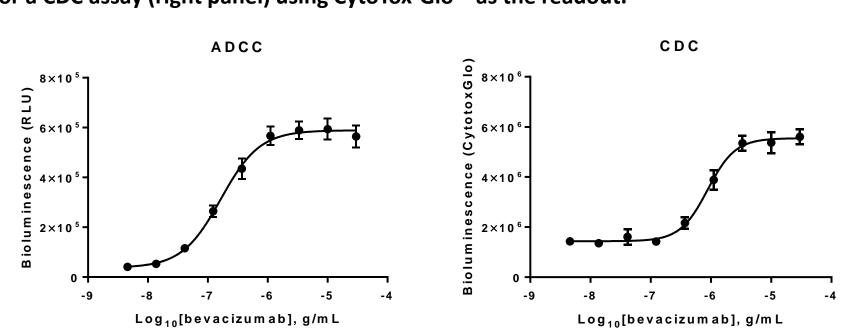
5. Cytotoxicity assay reagents and engineered target cells enable CDC assays



Engineered target cells can be used for ADCC and CDC assays

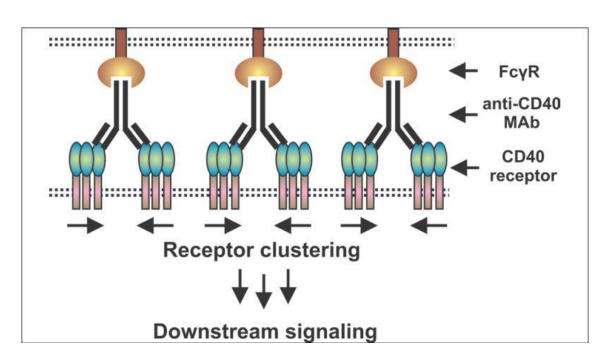


mTNF-α CHO-K1 target cells were used in the ADCC (FcγRIIIa) reporter bioassay (left panel) or a CDC assay (right panel) using CytoTox-Glo™ as the readout.



mVEGF CHO-K1 target cells were used in the ADCC (FcγRIIIa) reporter bioassay (left panel) or a CDC assay (right panel) using CytoTox-Glo™ as the readout.

6. Fc crosslinking by FcγRs can enhance agonist antibody functions



Crosslinking anti-TNFRSF agonist antibodies (e.g. anti-CD40, anti-OX40) via engagement of FcγR, particularly FcγRIIb, enhances receptor clustering and downstream signaling.

Fc₇R-dependent

Antibody 2

Fc_γR-independent

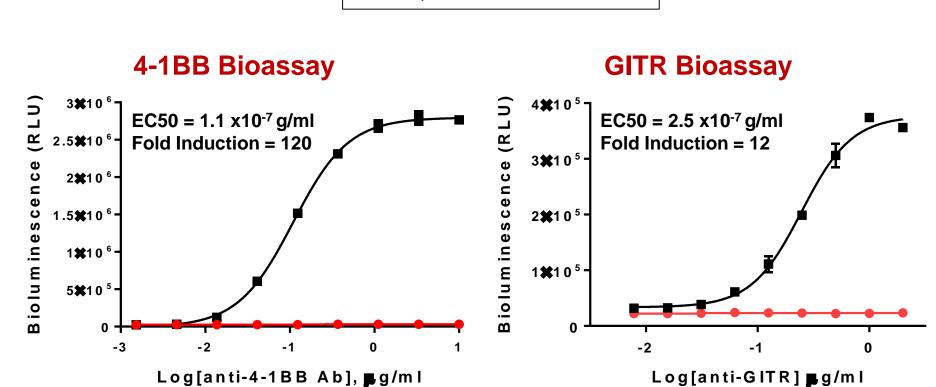
FcyRIIb CHO-K1 Cells Antibody ' **Co-stimulatory receptor Effector Cells**

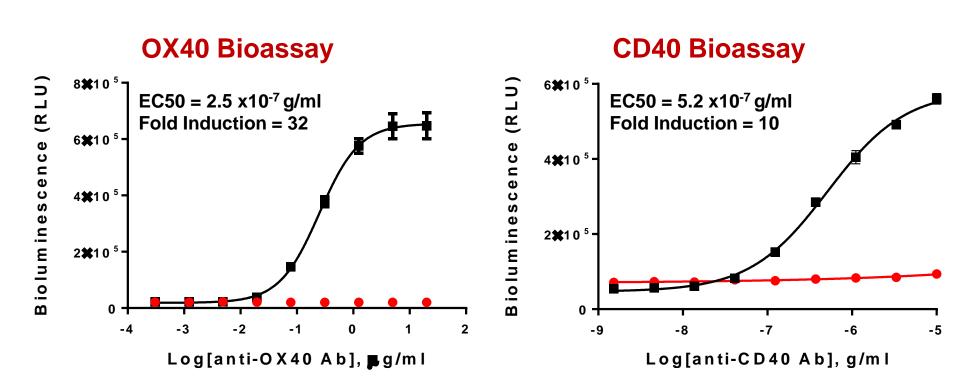
Co-stimulatory receptor Effector Cells

FcyRIIb CHO-K1 Cells can be used to test the FcyR-dependence of agonist Abs.

7. FcyRIIb CHO-K1 cells demonstrate Fc crosslinking dependence of agonist Abs

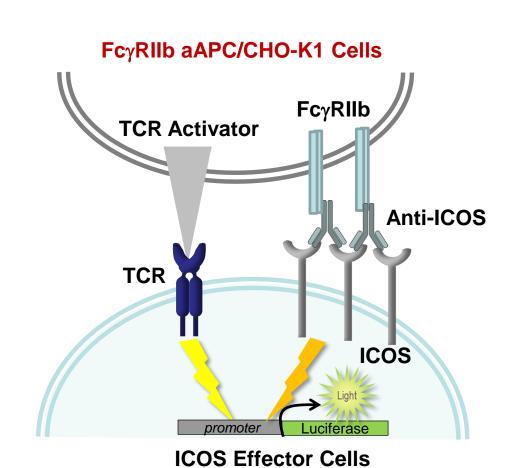






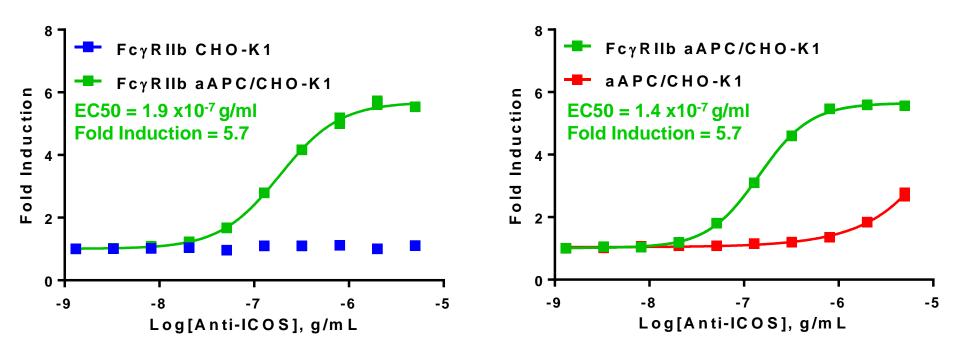
Functional analysis of research grade TNFRSF agonist antibodies in the context of FcyR crosslinking. 4-1BB, GITR, OX40, or CD40 reporter cells were activated with their respective agonist antibodies in the presence or absence of FcyRIIb CHO-K1 Cells. Fc crosslinking by FcyRIIb CHO-K1 cells enabled agonist activity of these antibodies.

8. FcyRllb aAPC/CHO-K1 cells are used with agonist Abs that require TCR co-stimulation



- 1. The TCR Activator protein binds to and activates the T cell receptor (TCR) complex
- Agonist anti-ICOS Ab Fc regions are crosslinked by FcyRIIb on the aAPC/CHO-K1 Cell. The F(Ab) regions of the ICOS Ab bind ICOS on the Effector Cell to induce activation.
- 3. A luciferase reporter integrates signals from both the TCR and ICOS.

Agonist anti-ICOS Ab requires both TCR activation and FcR crosslinking



The ICOS activation bioassay was performed using a research-grade anti-ICOS agonist Ab in the presence of FcyRIIb-expressing CHO-K1 cells (FcyRIIb CHO-K1), TCR Activator protein-expressing CHO-K1 cells (aAPC/CHO-K1) or CHO-K1 cells that co-express the TCR Activator protein and FcyRIIb (FcyRIIb aAPC/CHO-K1).

9. Conclusions

We have developed a platform of cell-based bioassays for characterizing the Fc function of therapeutic antibodies.

- A comprehensive suite of FcyR-specific bioassays have been developed for determining ADCC and ADCP activity of Ab
- The bioassays are homogeneous, easy to use, sensitive, and robust
- Reagents and Target cells to enable CDC assays
- FcγRIIb CHO-K1 cells enable easy determination of agonist antibody dependence on FcyR crosslinking of Fc regions
- Integration of a TCR Activator into FcγRIIb CHO-K1 cells enables analysis of co-stimulatory targets that require TCR activation

Together, these bioassay tools enable the comprehensive characterization of antibody Fc effector function