Development of a bioluminescent cell-based bioassay to measure Fc receptor functionality in antibody-dependent cell-mediated cytotoxicity

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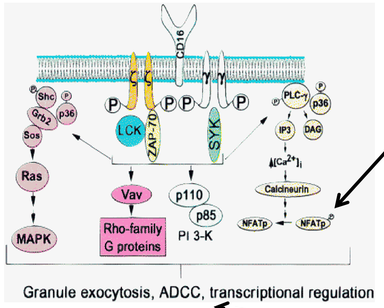
1. Abstract

Primary peripheral blood mononuclear cells (PBMCs) are routinely used in traditional bioassays to quantify antibody drug potency in antibody-dependent cellular cytotoxicity (ADCC). These bioassays are labor intensive and have high inherent assay variability. Here, we report the development of a bioluminescent cell-based bioassay which measures activation of effector cells via cross-linking of FcγRIIIA with target cell-bound antibodies. For this, Jurkat T-cell stable cell lines that stably express NFAT-luciferase reporter and human FcγRIIIA were generated to replace primary PBMCs as effector cells in ADCC bioassay. Effector cells were also developed in a frozen, thaw-and-use format to minimize assay variability due to cell culture and handling. The resultant bioassay using this format demonstrated good assay precision and accuracy in bioassay qualification. Bioassay using the engineered effector cells is robust, specific and is able to quantify the potencies of rituximab and trastuzumab, two monoclonal antibody drugs for cancer. When used to measure effects of Fc glycosylation on effector functions of therapeutic antibodies, a linear correlation was observed between relative antibody activity and the extent of Fc glycosylation. Thus, the bioluminescent cell-based reporter bioassay provides a simple and robust approach to measure potency of therapeutic antibodies in ADCC with high precision.

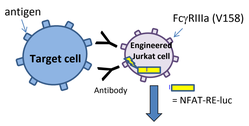
2. Introduction

FcγRIII A initiated signaling events



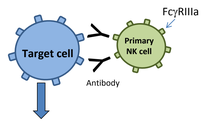
Leibson-PJ, *Immunity* 1997

New reporter-based bioassay



Read out: NFAT-RE reporter activation from effector cells

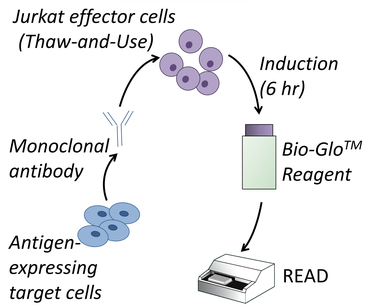
ADCC cytotoxicity bioassay



Read out: target cell cytotoxicity

3. Reporter bioassay method and assay format

Assay Format



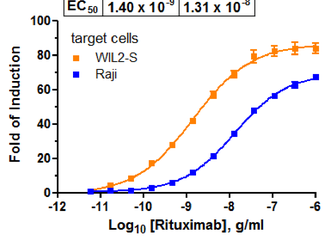
Assay Procedure

Plate target cells into assay plates
↓
Add biologic antibody to target cells
↓
Add FcγRIIIa/NFAT-luciferase-expressing Jurkat effector cells into assay plates
↓
Induce for 6 hours
↓
Add Promega Bio-Glo™ detection reagent, measure luciferase activity

4. Measuring Potency for Biologic Drugs Rituximab (anti-CD20) and Trastuzumab (anti-Her2)

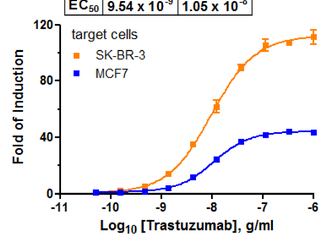
A. $CD20^+$ B cell lines (suspension) as target cells

	WIL2-S	Raji
EC_{50}	1.40 x 10^{-9}	1.31 x 10^{-8}



B. $Her2^+$ breast cancer cell lines (adherent) as target cells

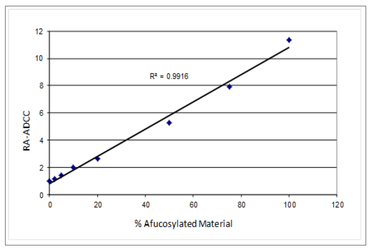
	SK-BR-3	MCF7
EC_{50}	9.54 x 10^{-9}	1.05 x 10^{-8}



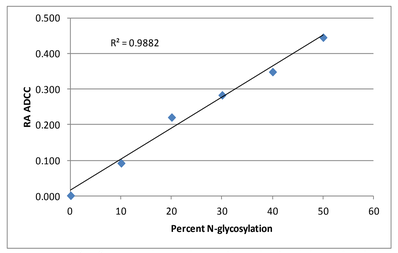
Quantifying the potencies of Rituximab (trade name: Rituxan, anti-CD20 chimeric monoclonal antibody drug) or Trastuzumab (trade name: Herceptin, anti-Her2 humanized monoclonal antibody drug) with appropriate target cell systems.

5. Fc effector functionality in new ADCC reporter bioassay correlates with antibody afucosylation and N-glycosylation

A. Relative ADCC reporter activity *vs* percentage of antibody afucosylation



B. Relative ADCC reporter activity *vs* percentage of Rituximab N-glycosylation

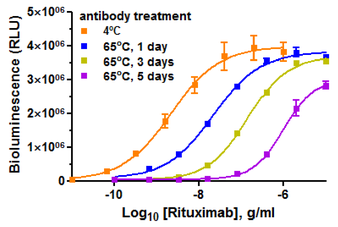


A. Linear correlation shown between percentage of afucosylated antibody in blended antibody samples and relative luciferase reporter activity in ADCC reporter assay
B. Linear correlation shown between percentage of N-glycosylated antibody in blended Rituximab samples and relative reporter activity in ADCC reporter assay

6. Stability indicating properties of new reporter bioassay for monoclonal antibody biologic drugs

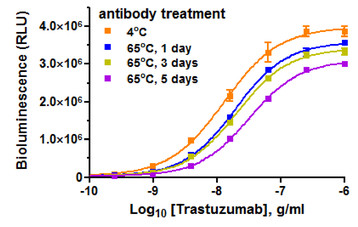
A. Potencies of heat-treated Rituximab

	4°C	65°C, 1 day	65°C, 3 days	65°C, 5 days
EC_{50}	2.13 x 10^{-9}	2.15 x 10^{-8}	1.41 x 10^{-7}	9.53 x 10^{-7}



B. Potencies of heat-treated Trastuzumab

	4°C	65°C, 1 day	65°C, 3 days	65°C, 5 days
EC_{50}	1.28 x 10^{-8}	1.90 x 10^{-8}	2.03 x 10^{-8}	3.14 x 10^{-8}



Quantifying the potencies of heat-treated Rituximab (A) or Trastuzumab (B) samples using ADCC reporter bioassay.

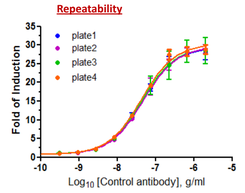
7. Bioassay Qualification: Repeatability, Precision, Accuracy and Linearity

Design:
- Two analysts
- Three days
- Four plates per day
- ✓100% vs 50%
- ✓100% vs 75%
- ✓100% vs 125%
- ✓100% vs 150%

Representative plate layout

	1	2	3	4	5	6	7	8	9	10	11	12	Plate1
A													
B		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		100%
C		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		50%
D		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		100%
E		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		50%
F		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		100%
G		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		50%
H													

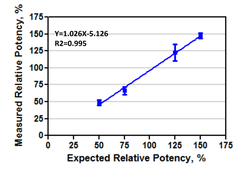
Repeatability



Precision = average of RSD (%) = 7.3%
Accuracy = average of Recovery (%) = 95.8%

	Antibody Sample	Measured Potency (%)	Mean Potency (%)	SD %	Recovery (%)	RSD (%)
day 1	50%	48.5	48.9	3.9	97.7	7.9
day 2		45.2				
day 3		52.9				
day 1	75%	63.1	66.4	5.9	88.5	8.9
day 2		62.9				
day 3		73.2				
day 1	125%	112.1	123.0	12.3	98.4	10.0
day 2		136.3				
day 3		120.5				
day 1	150%	148.8	147.6	3.6	98.4	2.4
day 2		150.4				
day 3		143.6				

Linearity



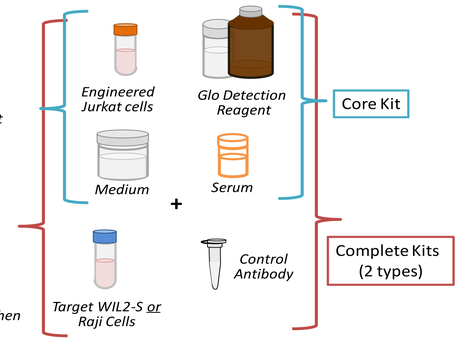
8. ADCC Bioassay kit configurations

Core Kit
- Engineered Jurkat Effector cells (NFAT-RE-luc2 / FcγRIIIa); *frozen, thaw-and-use*
- Bio-Glo™ Luciferase Assay System
- RPMI Medium
- Low IgG Serum

Use with your own Abs and target cells

Complete Kits (2 types)
- *Above PLUS:*
- Target WIL2-S cells; *frozen, thaw-and-use* or Target Raji cells; *frozen thaw-and-use*
- Control Ab (CD20)

Use as an assay run control, or when WIL2-S or Raji target cells are suitable or your target is CD20



9. Summary

- **We developed a bioluminescent cell-based reporter bioassay which can be used to quantify biologic potency of therapeutic monoclonal antibodies based on Fc effector functionality as a result of target cell-bound antibody activation of FcγIIIA receptor on engineered effector cells.**
- **Replacement of primary NK cells with the engineered Jurkat effector cells eliminates the high bioassay variability typically seen in assays using NK cells from blood donors.**
- **The bioassay was used to assess the impact of glycan afucosylation and N-glycosylation of antibodies on their Fc effector functionality. Linear correlations obtained between the extent of antibody afucosylation or N-glycosylation and relative antibody bioactivity show that this assay, like traditional ADCC bioassays, is able to appropriately quantify Fc effector functionality of monoclonal antibodies.**
- **Use of thaw-and-use effector and target cells improves assay precision due to tight control over consistency of cell preparations for the assay.**
- **The assay was qualified as a bioassay for therapeutic monoclonal antibodies.**