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Alternate Approaches Addressing Variability in ADCC Assay

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October 28, 2014

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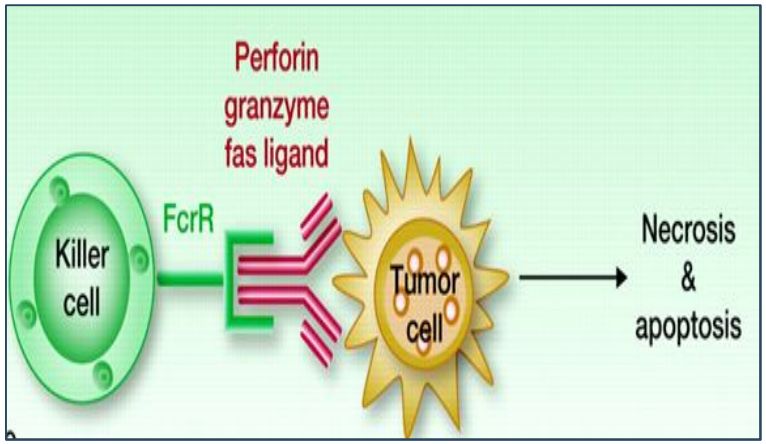
Introduction

- Monoclonal antibodies are gaining prominence as therapeutic agents to treat a wide spectrum of diseases.
- Several of these therapeutics antibodies mediate their action through antibody dependent cellular cytotoxicity (ADCC).
- ADCC is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies



Mechanism of action of ADCC Assay
***Source**: Matthay KK et al. Clin Cancer Res 2012;18:2740-2753*

Introduction

- Although *in vitro* ADCC mimics the mode of action of these antibodies in the clinic, ADCC is typically not used as a lot release assay.

- ADCC assay requires peripheral blood mononuclear cells (PBMCs) or purified Natural Killer (NK) cells expressing FcγRIIIa receptor isolated from pre-screened healthy human donors as effector cells.

- Traditional cell lysis based ADCC assays using PBMCs or NK cell lines can be challenging to develop and implement for routine testing.

Challenges with Conventional ADCC assay

- Currently, the only available *in vitro* ADCC assay involves acquiring PBMCs or purified Natural Killer (NK) cells expressing FcγRIIIa receptor as effector cells.

- These effector cells are isolated from pre-screened healthy human donors, a process that can be highly inconsistent.

- The extreme variability observed in the assay due to the varying response exhibited by the effector cells isolated from different donors.

Challenges with Conventional ADCC assay

- In addition, such an assay requires a ready supply of genotyped donors that are homozygous for each of two major CD16 alleles that confer significantly different binding affinities for the IgG Fc domain.

- ADCC is not only plagued with variability but use of PBMCs also raises ethical issues with human donors, cost associated with procuring blood samples, logistic issues of transporting the blood from clinic to lab, temperature control during transport, etc.

- Hence, different binding assays and other surrogate assays in lieu of traditional functional bioassays are being more routinely introduced.

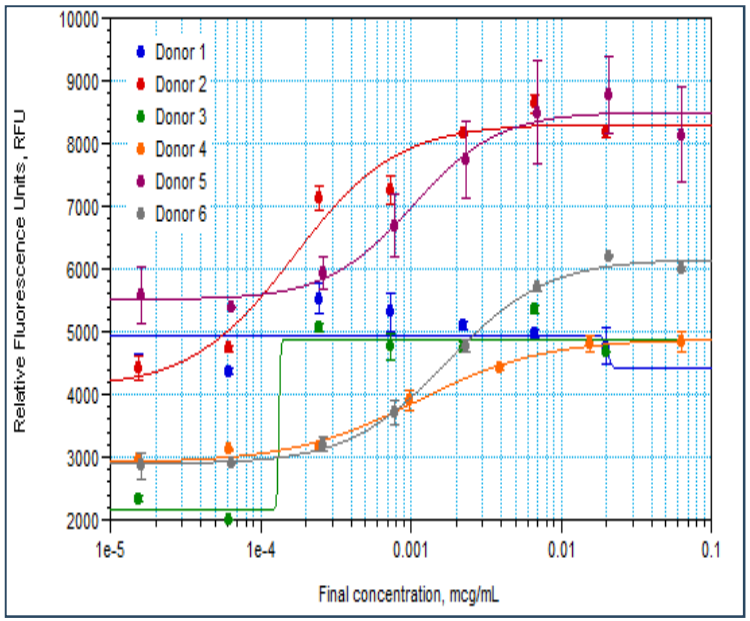
- In order to address these issues, our lab assessed several alternative approaches for performing ADCC assay

Improved Conventional ADCC Assay

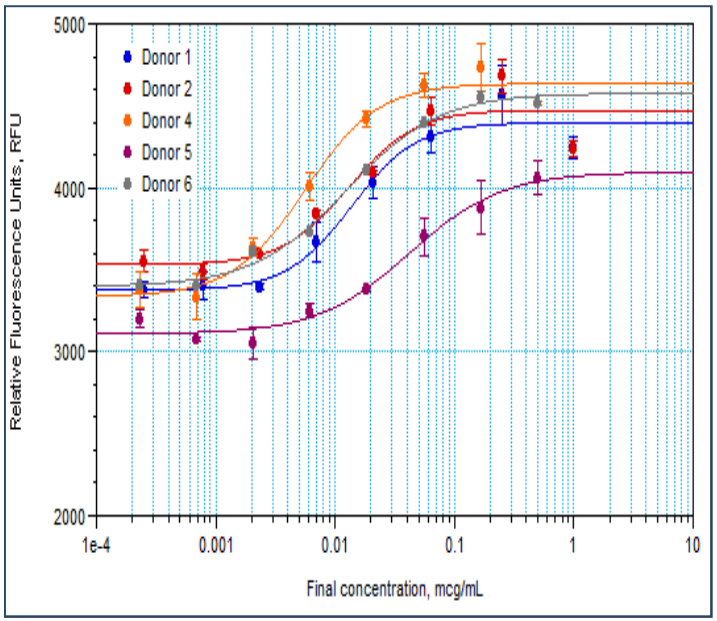
Fresh *Vs* Cultured PBMCs: Methodology is same as conventional ADCC assay except that the PBMCs cultured in presence of low IgG serum and IL-2 used as effector cells

Dose Response Curves of different donors PBMC – (a) with fresh PBMC and (b) with ex-vivo cultured PBMC

(a)



(b)

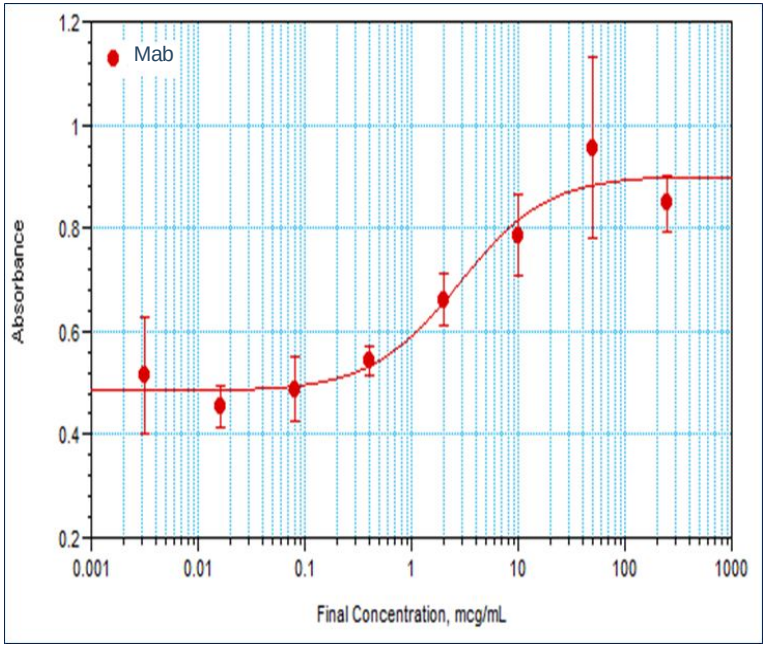


Dose response obtained with cultured PBMCs (Fig. b) exhibited less variability with improved overall response and defined asymptotes with consistent slope when compared to fresh PBMCs (Fig. a). Use of cultured PBMCs also improved the response in proportion to the dose as compared with fresh PBMCs, for e. g.: Donor 1.

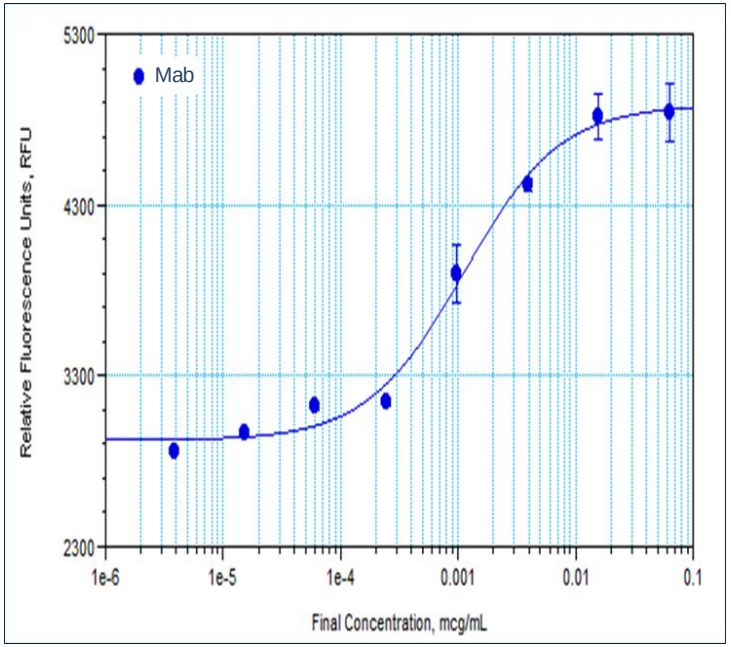
Choice of quantification reagent: Homogeneous and non-homogeneous assay formats

Representative Dose Response Curves of conventional ADCC Assay with two different quantification reagents

(a)



(b)

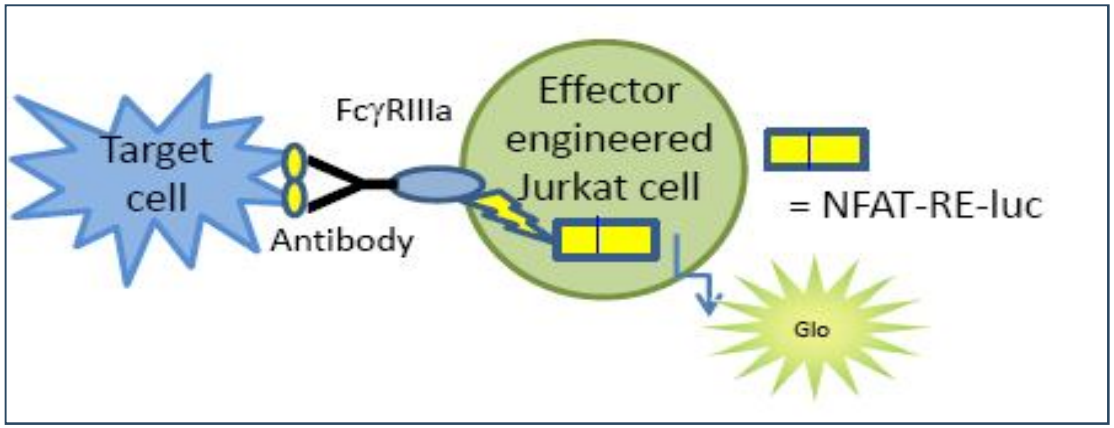


The variation in the replicates across the dose response curve was found to be high (5 - 22%) with non-homogeneous assay format (Fig. a), whereas with homogeneous format (Fig. b), the variation in the replicates was significantly low (0.2 – 4.4%).

Surrogate assay – Reporter based bioassay

- The approach for reporter based ADCC bioassay is similar to conventional ADCC assay except that the effector cells are engineered to express FcγRIIIa (V158) and NFAT-RE-luc2 luciferase, which is used in place of typical PBMCs/purified NK cells.

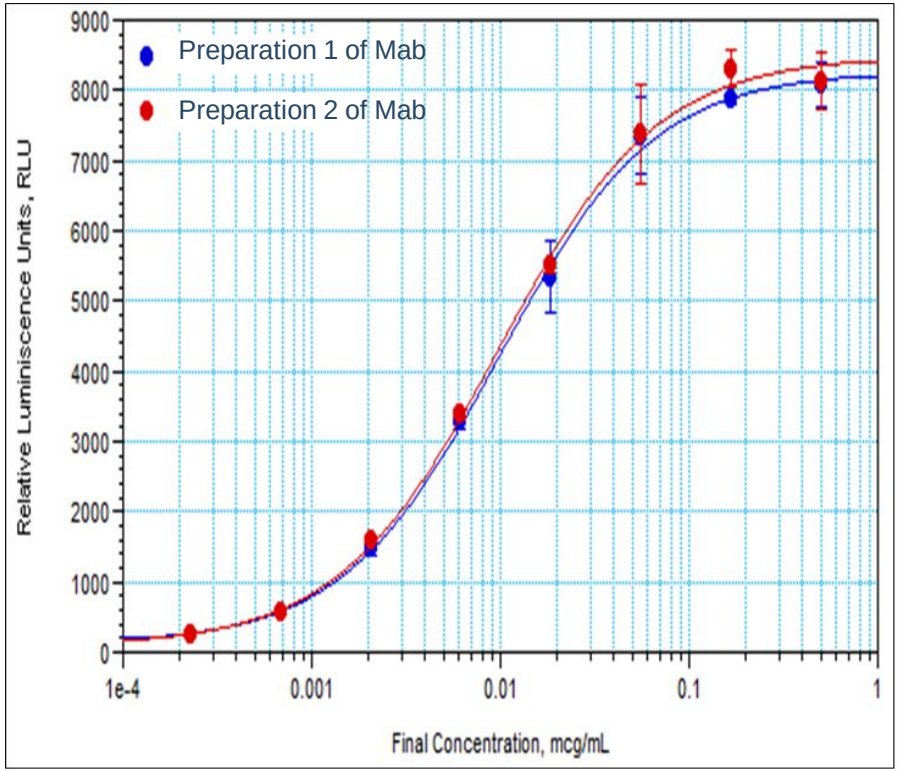
- Antibody cross-linking of target and effector cells results in transactivation of the response element and expression of luciferase which is detected by adding the Luciferase detection reagent and luminescence is measured immediately



Mechanism of action of Bioluminescent Reporter-based ADCC bioassay
ADCC Reporter Bioassay Principle, Promega

Surrogate assay – Reporter based bioassay

Representative Dose Response Curve with Engineered cell line



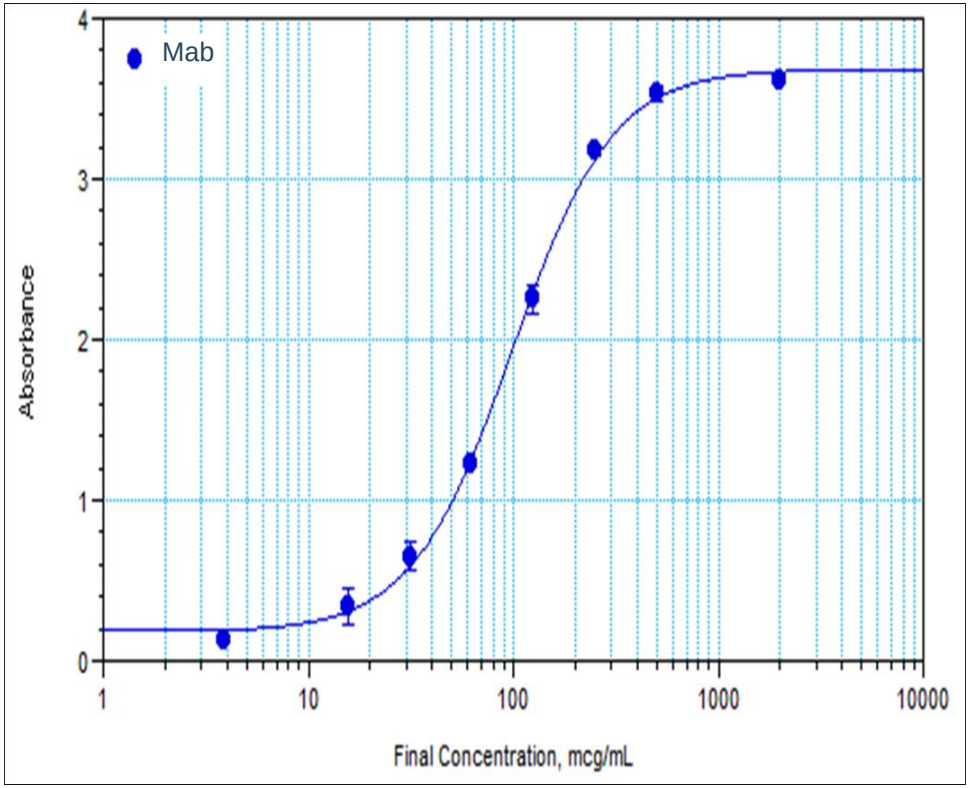
The dose response obtained with engineered cells was found to be consistent across days/analysts with well defined asymptotes, low variation in replicates, consistent slope and broader working window (fold response) when compared to conventional ADCC assay using cultured PBMCs.

Surrogate assay – Binding Assay

- An in-house developed method was used.
- The FcγRIIIa receptor was coated on the maxi sorp 96 well plates and various concentrations of the antibody were added.
- A secondary antibody and TMB substrate were used for detection of antibody bound to the FcγRIIIa receptor.
- The result obtained with the binding assay demonstrates low variability, consistent slope and better fold response.

Surrogate assay – Binding Assay

Binding Assay - Representative Dose Response Curve



The dose response obtained with binding assay was found to be consistent across days/analysts with well defined asymptotes, low variation in replicates, consistent slope and broader working window (fold response) when compared to conventional ADCC assay using cultured PBMCs.

Summary & Conclusions

Advantages and Disadvantages of various approaches for ADCC Assay

Method	Advantages	Disadvantages
Conventional ADCC Assay with fresh PBMC	Reflects the in vivo mode of action of the drug	▪ High background ▪ High variation due to donor variability ▪ Ethical and logistic issues ▪ Practical issues ▪ Laborious
Conventional ADCC Assay with cultured PBMC		
Reporter based bioassay	Highly precise, robust, less laborious and cost effective	Surrogate assay
FcγRIIIa binding assay	Highly precise, robust, less laborious and cost effective	Surrogate assay

Data obtained with cultured PBMCs exhibited less variability with improved dose response, defined asymptotes and consistent slope. Whereas data obtained with engineered cells and binding assay not only addressed the variability but also exhibited good precision and broader working window with low background and have the potential to become a robust lot release assay.

Summary & Conclusions

Comparison of assay parameters for conventional and non-conventional ADCC assays

Method	Dose-Response	Fold Response	Slope	Regression
Conventional ADCC	Highly Variable with ill defined asymptotes and occasionally the response is not observed with few donors.	≤ 2	Highly Variable (≥ 100% RSD)	Variable
ADDC with cultured PBMC's	Variable but always obtained defined asymptotes	≤ 3	Variable (≤ 45% RSD)	≥ 0.90
Reporter based bioassay	Very significant with well-defined asymptotes and consistent across days/analysts	≥ 15	Precise (≤ 15% RSD)	≥ 0.98
FcγRIIIa binding assay	Very significant with well defined asymptotes and consistent across days/analysts	≥ 20	Highly precise (≤ 10% RSD)	≥ 0.98

Note: Data presented is trend observed from ≥ 5 assays

Acknowledgements

- Dr. Ranjan Chakrabarti
- Dr. Disha Dadke
- Shubrata Khedkar
- T. Praveen Kumar

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Questions

Thank You