A novel method for quantification of ADCC activity based on the use of engineered effector cells and a series of matching target cells

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INTRODUCTION

Therapeutic antibodies are increasingly used to treat different types of cancer, including breast cancer, the second most commonly diagnosed cancer worldwide. The activity of a number of therapeutic antibodies is mediated at least in part by antibody dependent cell-mediated cytotoxicity (ADCC), including trastuzumab, an anti-HER2 antibody commonly used for treating HER2 positive breast cancer patients.

Here we present a method for quantification of ADCC activity based on the use of novel engineered effector cell line expressing the low affinity Fc receptor, FcgRIIIa (CD16), that responds to ligation of the Fc region of an antibody bound to the specific antigen expressed on target cells by activation of a NFAT responsive reporter gene. In addition, novel target cells have been developed that express a constant high level of the specific antigen, HER2, as well as the homologous control target cells which allows differences in ADCC activity to be determined with precision and a high degree of specificity.

ADCC REPORTER GENE CONCEPT

Antibody-dependent cell-mediated cytotoxicity (ADCC) is the lysis (killing) of an antibody-coated **target cell** by a cytotoxic **effector cell** through a nonphagocytic process, characterized by the release of the content of cytotoxic granules or by the expression of cell death-inducing molecules. ADCC is triggered through interaction of target-bound antibodies with certain Fc receptors (FcRs) present on the effector cell surface that bind the Fc region of the antibodies (Fig. 1).

Clinical bioassay characterization of an ADCC assay for quantification of anti-HER2 activity drug activity

When the *iLite* assay ready cells are developed, they are always validated for their functionality. Furthermore, we qualify and characterize the assay for different applications like potency testing of biologicals or immunogenicity assessment of therapeutics which includes different studies to ensure the assay functionality and suitability (recommended in several guidelines).

Here, we have tested the *iLite* ADCC HER2 assay for the most critical parameters included in the clinical bioassay characterization: Serum tolerance, cut-point determination, NAb assay sensitivity using different antibodies and drug tolerance evaluation at high NAb level.

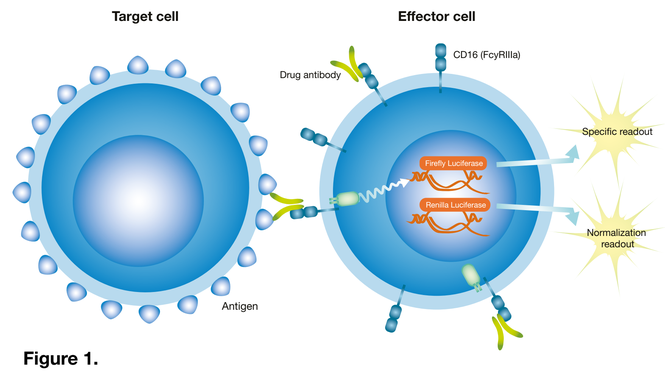
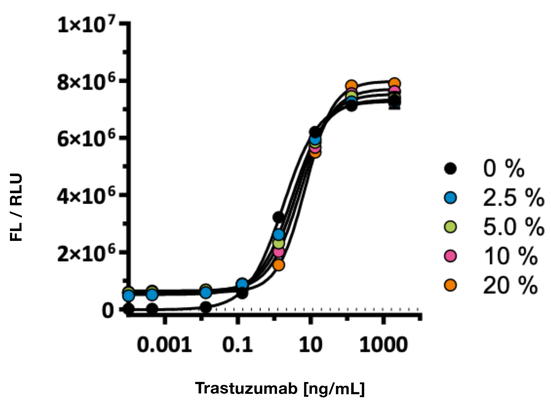


Figure 1.

In the ***iLite*® ADCC cell line** the effector cells serves as the "killing cell" but when activated, meaning when the antigen on the targets cell surface binds to the drug/antibody and in turn the Fc receptor on the target cells, the reporter gene construct is activated and firefly luciferase is produced.

SERUM TOLERANCE

The presence of patient's serum in a sample can effect the performance of the assay and the ability to detect either drug or anti-drug antibodies.



Human serum	0%	2.5 %	5.0 %	10%	20%
Best-fit values					
HILLSLOPE	0.89	0.95	0.99	1.02	1.14
EC50	1.77	3.12	4.15	5.42	7.25
FL 133 ng/ml/FL 0.00044 ng/ml	522	14.3	12.3	11.9	13.4
Goodness of Fit					
R^2	0.9993	0.9996	0.9995	0.9996	0.9998

Figure 2. Analysis of Trastuzumab dose-response curve (0-1000 ng/mL) in presence of different concentrations of human serum pool from healthy donors (0 – 20%). The *iLite* ADCC HER2 assay tolerates up to 20% serum in the assay. An increase in the EC50 of the dose-response curve can be observed at higher serum level while the induction of the reporter gene remains stable at higher serum level.

CUT-POINT

To help decide the presence or absence of a biomarker, a cut-off point for 'normal' or 'abnormal' is chosen. The sensitivity and specificity of a test vary according to the level that is chosen as the cut-off point (CP).

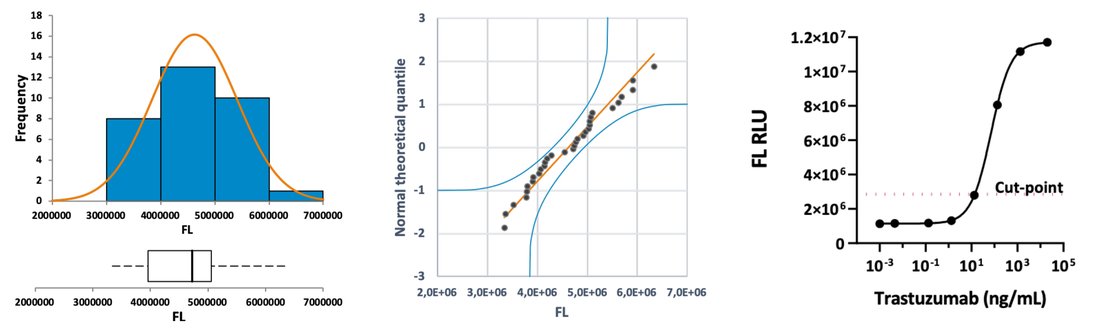


Figure 3. Determination of the ADCC HER2 assay cut-point by analysis of 16 serum samples from healthy donors spiked with 25 ng/mL Trastuzumab. All samples were analyzed in a 1:10 dilution in two replicates. The analysis was performed at two independent occasions by two different operators. Results shown are examples from one experiment. The analysis included the ADCC HER2 dose-response curve in three independent titrations in two replicates as well as negative controls. The cut-point was calculated at 9.5 ng/mL MRD Trastuzumab using normal distribution and Shapiro-Wilk test (Analyse-it V5.11) for the CP calculation.

NAb ASSAY SENSITIVITY

Threshold values for the distinction of positive and negative results in immunogenicity assays is key, due to risk-based strategy and a cut point value/NAb assay sensitivity is to be determined.

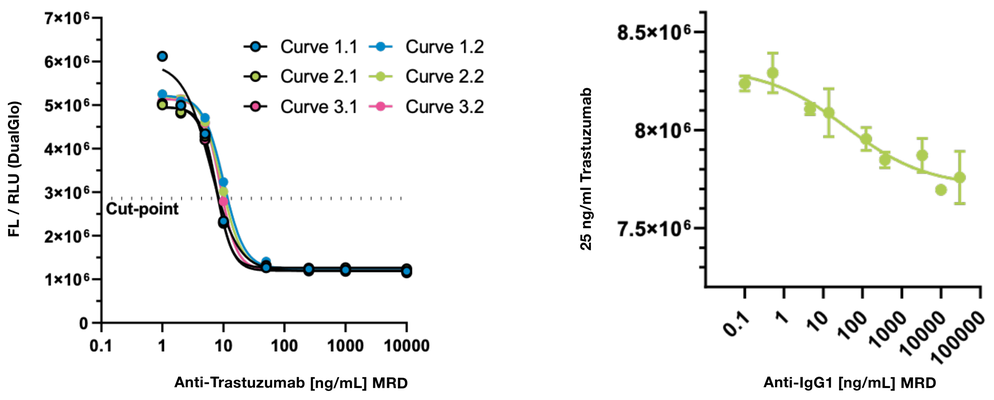


Figure 4. Evaluation of ADCC HER2 Nab assay application using a specific anti-Trastuzumab antibody and an anti-IgG1 Fc antibody. Serum pool prepared from healthy donors was spiked with 25 ng/mL Trastuzumab (MRD) and different concentrations of anti-Trastuzumab antibody directed against the HER2 binding site (0-10000 ng/mL) or an anti-IgG1 antibody (0-30000 ng/mL), pre-incubated for 30min at 37°C and subsequently analyzed as a 1:10 dilution in the *iLite* ADCC HER2 assay. The analysis with the anti-Trastuzumab antibody included three titration curves with two replicates each and was performed two times by two independent operators. The titration of the anti-IgG1 antibody was performed only once.

DRUG TOLERANCE – ANTI-TRASTUZUMAB

Drug tolerance describes the sensitivity of your anti-drug antibody assay in the presence of soluble drug. Tolerance is determined by assessing assay sensitivity in the presence of increasing amounts of added soluble drug.

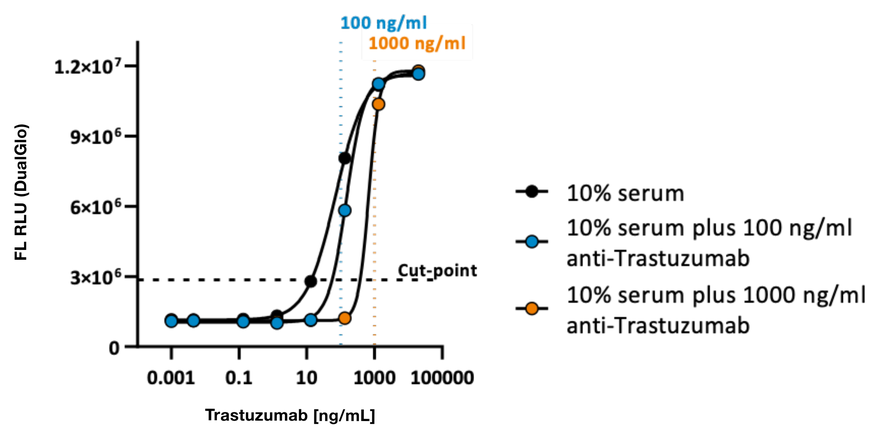


Figure 5. Evaluation of the drug tolerance in the ADCC HER2 assay. A serum pool prepared from healthy donors was spiked with increasing concentrations of Trastuzumab up to 20 µg/ml MRD and high level of anti-Trastuzumab antibody (0, 100 and 1000 ng/mL), pre-incubated at 37°C for 30 min and subsequently analyzed as 1:10 dilution in two replicates using the *iLite* ADCC HER2 assay.

CONCLUSION

Our engineered target cells and the homologous control cells allows drugs working trough an ADCC activity to be assessed with precision. Here, we have focused on the clinical bioassay parameters and tested the *iLite* ADCC HER2 assay for the most critical parameters including:

- Serum tolerance
- Cut-point determination
- NAb assay sensitivity (anti-Trastuzumab, anti-IgG1 Fc)
- Drug tolerance at high level of anti-Trastuzumab

The data presented suggest the following:

- The *iLite* ADCC HER2 assay tolerates serum up to 20%. An increase in the EC50 of the dose-response curve can be observed while the induction of the reporter gene remains stable.
- The cut-point analysis shows a normal distribution of healthy donors although the detected cut-point is quite high at 9.5 ng/mL. This is most likely due to the limited number of specimen included in the cut-point analysis.
- The results of the NAb assay evaluation indicate that the antibody specificity as well as, affinity and avidity have an impact on the NAb assay sensitivity. The anti-Trastuzumab showed a 5-fold reduction of the FL signal while the anti-IgG1 signal reduction was detected <2.
- The drug tolerance analysis indicated that Trastuzumab can be measured in serum samples (1:10 diluted) at a range between 133 – 1300 ng/mL in presence of 100 ng/mL anti-Trastuzumab.

Based on these preliminary results the *iLite* ADCC HER2 assay could be used for clinical applications to analyze patient sample and determine the presence of neutralizing antibodies in clinical samples.