Quantification of the ADCC activity of therapeutic antibodies and it´s tolerance to human serum

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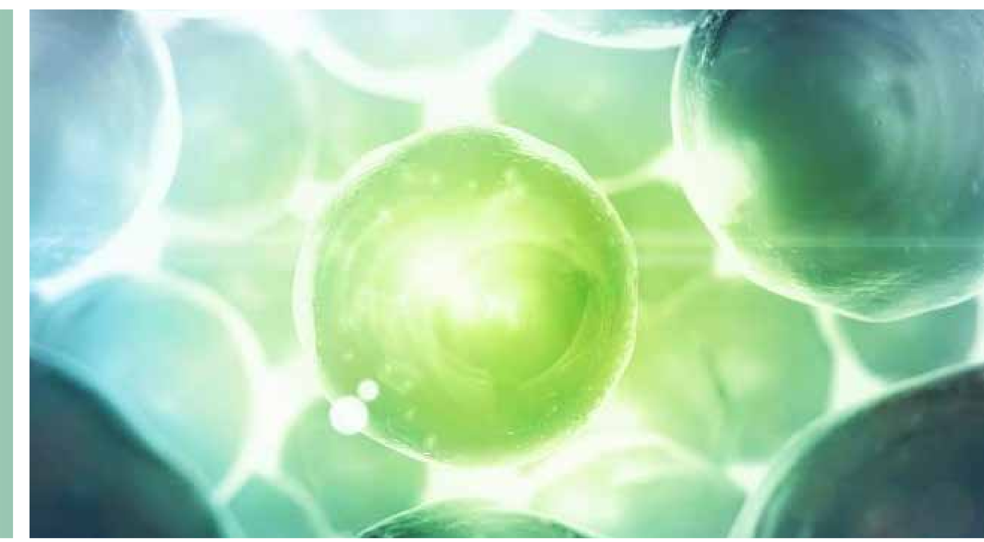


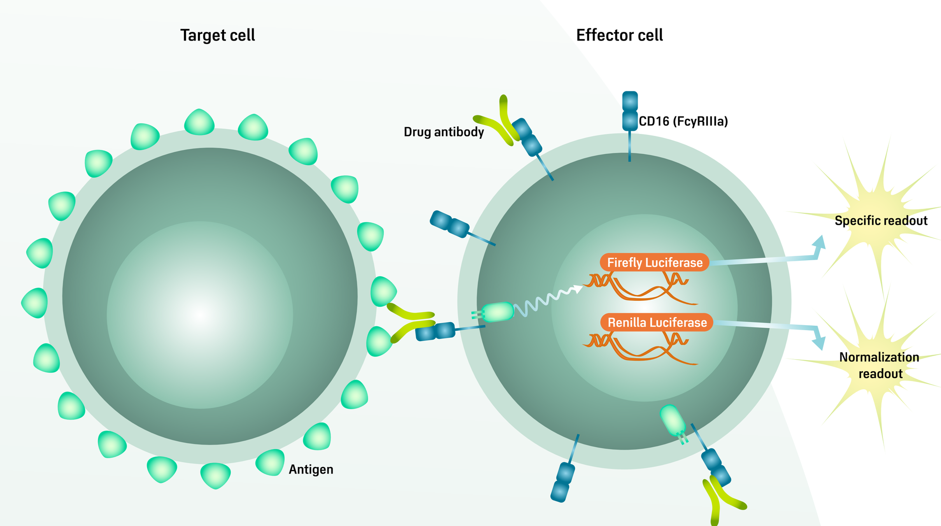
Introduction

The activity of numerous therapeutic antibodies is mediated in part by antibody-dependent cell-mediated cytotoxicity (ADCC). Traditional methods for quantifying ADCC activity are labor intensive and have a high level of inherent variability due to the use of primary human NK-cells from different donors as the effector cells. These limitations can be overcome in part by the use of an engineered effector cell line expressing the low affinity Fc receptor, FcgRIIIa (CD16), that responds to ligation of the Fc moiety of an antibody bound to the specific antigen expressed on target cells by activation of a NFAT responsive reporter gene. There is a need, however, for an ADCC assay with improved sensitivity, specificity, and tolerance to the presence of human serum.

Here we present a method for quantification of ADCC activity based on the use of novel engineered effector cells carrying a reporter gene. In addition, novel target cells have been developed that express a constant high level of the specific antigen 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 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.



In the *iLite* ADCC Cell Line the Effector cells serves as the "killing cell" but when activated, hence the target antigen binds to the drug/antibody and in turn the FC receptor on the target cells the reporter gene construct is activated and firefly luciferase produced.

Figure 1. Schematic illustration of the *iLite* Reporter gene ADCC Effector cell.

Establishment of an Engineered Effector Cell Line and target Cells

Briefly - Jurkat cells were co-transfected with a chimeric promoter containing binding sites for the principal transcription factors (NFAT, NFkB, AP1, CREB, and STAT) that mediate signaling from the FcgRIIIa receptor, driving transcription of the firefly luciferase (FL) reporter-gene from a minimal SV40 promoter (Figure 1), an expression vector for FcRgIIIa (v variant), and the NL reporter gene, under the control of a constitutive promoter, that allows drug-induced FL activity to be normalized with respect to the constitutive expression of NL (Figure 2), rendering assay results independent of variations in cell number, serum matrix effects, or lysis of the effector cells by the target cells.

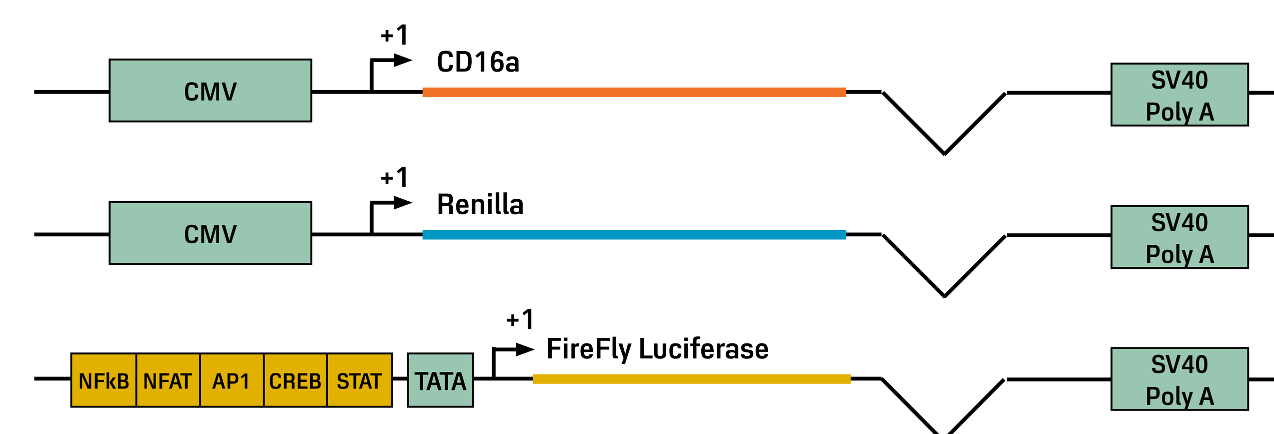


Figure 2.
Genetic constructs

Results

Quantification of ADCC activity of Trastuzumab together with HER2 (+) and (-) target cells

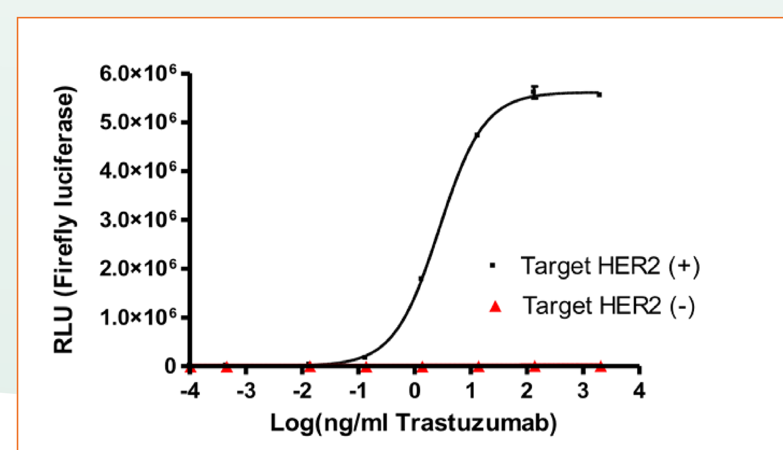


Figure 3.

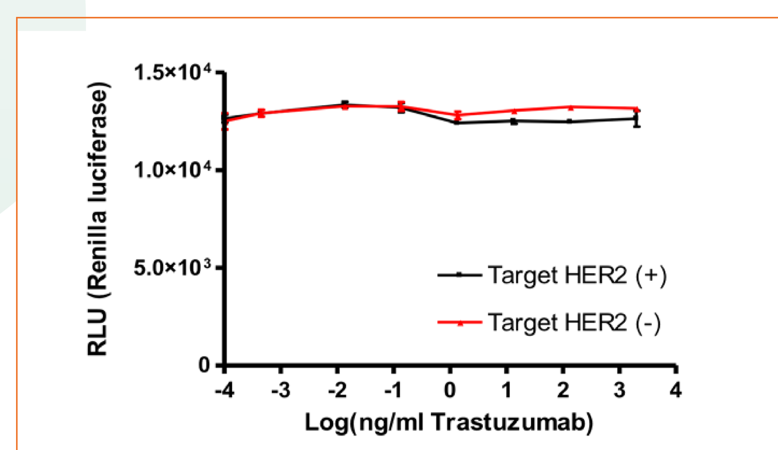


Figure 4.

iLite ADCC effector cells were assayed together with *iLite* target cells HER2 positive and negative and Trastuzumab. The results clearly state that the engineered positive target cells gives a clear dose-response curve, whereas the negative target minus cells gives, as expected, no response (Figure 3).

This can be compared to the Renilla readout from the normalization gene where both the positive and negative target cells give a very similar readout, due to the constitous promotor region controlling the renilla luciferase translation (Figure 4).

Quantification of activity of Infliximab together with mTNF(+) and (-) target cells

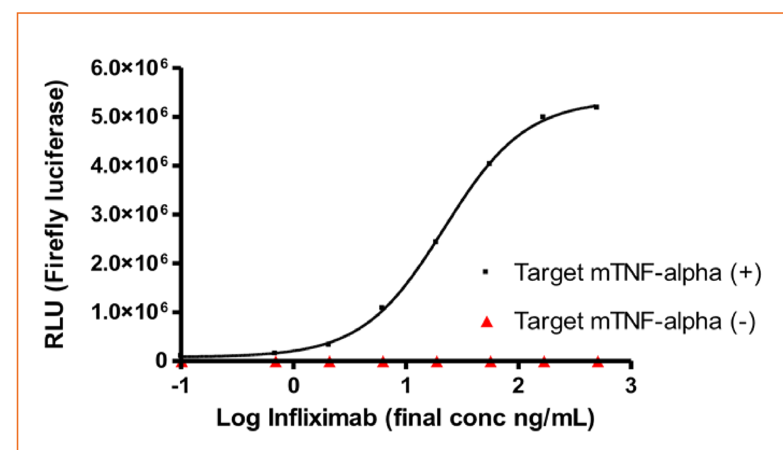


Figure 5.

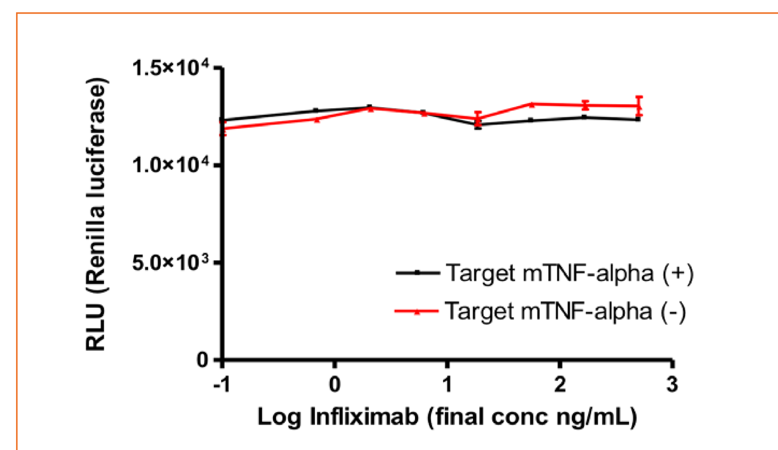


Figure 6.

In accordance with the results from HER2, *iLite* ADCC effector cells were assayed together with *iLite* target cells mTNF positive and negative and Infliximab. The results follow the expected set up where a positive target cells gives a clear dose-response curve, and whereas the negative target minus cells gives, as expected, no response (Figure 5).

The Renilla readout from the normalization gene where both the positive and negative target cells gives a dose independent readout that is similar for both the target (+) and (-) cells thus proving its independence from the stimulatory agent/drug and its usefulness as an internal control and/or normalization tool (Figure 6).

Quantification of the ADCC Activity of Trastuzumab and Infliximab in the presence of Normal Human Serum

Target HER2 - effect of human serum

The response of effector cells & HER2++ target cells upon activation with drug (trastuzumab) was visible in the presence of human serum (Figure 7) rendering a shrinking of the available dynamic range.

A dynamic range of approximately 557-fold and an EC50 of 2.4ng/ml was obtained for the effector cells & HER2 target cells in the absence of human serum, compared to a 12-fold and EC50 of 6,1 with a large presence of human serum (10 % final concentration), Figure 7.

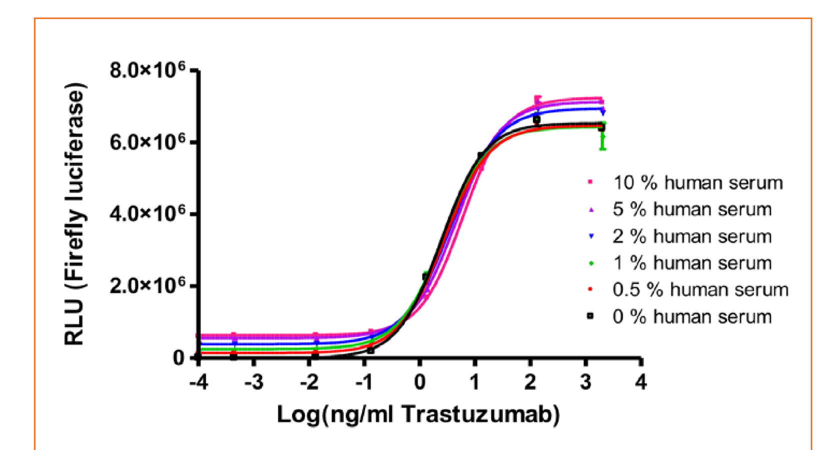


Figure 7.

Parameter	10%	5%	2%	1%	0.5%	0%
R2 (log(agonist) vs response - variable slope)	0,9993	0,9989	0,9994	0,9960	0,9994	0,9989
EC50	6,1	4,6	3,7	2,6	2,8	2,4
Fold induction 133 ng/ml/FL 0 ng/ml (Firefly luciferase)	12	14	19	28	49	557
Ratio Target HER2 (+)/Target HER2 (-) (133 ng/ml)	27	32	44	53	84	326
2000 ng/ml (RLU FL)	7111000	6967500	6832000	6180500	6351000	6403500
0 ng/ml (RLU FL)	606150	519200	363700	238250	132450	11905
Hillslope	1,1	1,1	1,0	1,1	1,1	1,1

Target mTNF-alpha - effect of human serum

The response of effector cells & mTNF+ target cells upon activation with drug (Infliximab) was visible in the presence of human serum (Figure 8) rendering a shrinking of the available dynamic range.

A dynamic range of approximately 50-fold and an EC50 of 21ng/ml was obtained for the effector cells & mTNF+ target cells in the absence of human serum, compared to a 2,3-fold and EC50 of 71 with a large presence of human serum (10 % final concentration), Figure 8.

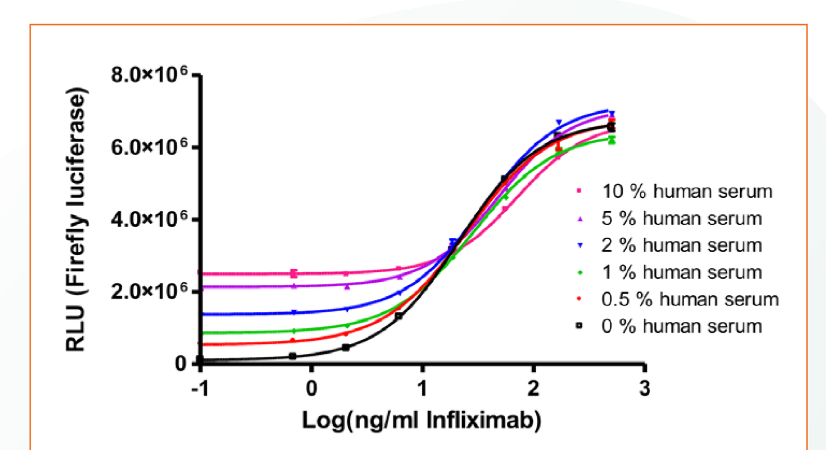


Figure 8.

Parameter	10%	5%	2%	1%	0.5%	0%
R2 (log(agonist) vs response - variable slope)	0,9991	0,9996	0,9981	0,9994	0,9992	0,9996
EC50	71	48	34	29	25	21
Fold induction 167 ng/ml/FL 0 ng/ml (Firefly luciferase)	2,3	2,9	4,9	6,9	11	50
Ratio Target mTNFa (+)/Target mTNFa (-) (167 ng/ml)	25	27	39	43	69	514
500 ng/ml (RLU FL)	6465000	6923500	6942000	6206000	6646000	6563500
0 ng/ml (RLU FL)	2528000	2129500	1356000	855250	556800	127050
Hillslope	1,4	1,3	1,3	1,2	1,2	1,2

Conclusion

The *iLite*® effector cell line JE5.35 provides a highly sensitive, precise, and specific means of quantifying ADCC activity. Potentially, JE5.35 cells can be used to quantify the ADCC activity of any biopharmaceutical carrying a Fc moiety, whether a monoclonal antibody or a fusion protein. The availability of both frozen ready-to-use effector cells and target cells, in addition to providing a convenient and cost-effective means of quantifying the ADCC activity of therapeutic antibodies, also provide the basis for the establishment of highly precise and reproducible assays with a low degree of vial-to-vial and lot-to-lot variation. The *iLite*® effector cell line and specific target cells and the homologous control cells can be used for both a potency assay for use in a CMC environment or for the quantification of ADCC activity or the anti-Fc humoral response in pre-clinical or clinical studies. In the later context the improved tolerance to the presence of human serum and presence of the Nano-Luc luciferase normalization gene provides a means for compensating for serum matrix effects or killing of the effector cells by the target cells observed at high concentrations of antibody or in the presence of certain clinical samples.

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