Quantification of the ADCC Activity of Trastuzumab

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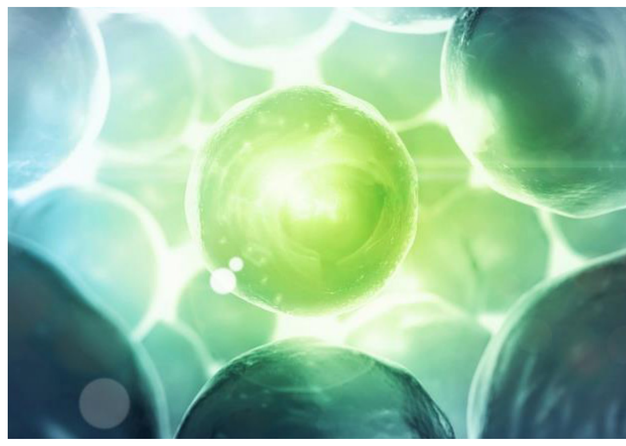

Introduction

The activity of a number of 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. This is due in part to the use of primary human NK-cells from different donors as the effector cells and the use of a complex endpoint, that is difficult to standardize, namely cytotoxicity. These limitations can be overcome in part by the use of an engineered effector cell line expressing the low affinity Fc receptor, FcγRIIIA (CD16a), that responds to ligation of the Fc moiety of an antibody bound to a specific antigen expressed on target cells by activation of a NF-AT responsive reporter gene. There is a need, however, for an ADCC assay with improved sensitivity, specificity and tolerance to the presence of human serum that integrates the principal pathways involved in ITAM signal transduction.

Methods

A novel recombinant effector cell line has been developed based on the human T-cell line Jurkat engineered to over express the low affinity Fc receptor, FcγRIIIA (CD16a), and the Firefly Luciferase (FL) reporter gene under the control of a chimeric promoter which incorporates recognition sequences for the principal transcription factors that regulate FcγRIIIA activation (Figure 1). The effector cell line has also been transfected with the coding region of the Nano Luciferase (NL) gene under the control of a constitutive TK promoter, thus allowing FL expression to be normalized with respect to the constitutive expression of NL activity rendering the assay independent of variations in cell number, serum matrix effects, or lysis of the effector cells by the target cells. The use of this novel effector cell line confers improved sensitivity, an improved dynamic range, and improved tolerance to human serum, relative to engineered effector cell lines that express a NF-AT regulated reporter-gene, when used in an ADCC assay together with engineered target cells. The target cells have been engineered to over-express a constant high level of the specific antigen recognized by the therapeutic antibody, and homologous control cells have been developed in which the gene encoding the specific drug target has been invalidated by CrisPR/Cas9 genomic editing.

Results

I. Establishment of an Engineered Target Cell Line Expressing High Constant Levels of ERBB2 at the Cell Surface

The gene encoding ERBB2 was invalidated in HEK293 cells (ATCC® CRL-1573) using CrisPR-Cas9 genome editing. Briefly a guide RNA sequence was designed, synthetized, and cloned into the nuclease vector in order to guide the Cas9 double stranded DNA endonuclease to a specific site within exon 6 of the ERBB2 gene in order to isolate ERBB2$^{-/-}$ HEK293 cells. HEK293$^{-/-}$ cells were then transfected with an ERBB2 expression vector and positive clones were enriched using fluoresce activated cell sorting and a FITC labelled anti-ERBB2 monoclonal antibody. Stable clones were isolated and characterized for ADCC activity in the presence of the *iLite*® target cells and Herceptin.

The response of *iLite*® effector cells & ERBB2^{++} HEK293 target cells (expressed as fold induction relative to the control sample without Herceptin), was found to be significantly greater than that of the NF-AT effector cells & wild type SK-BR-3 target cells (**Figure 1**). The response of *iLite*® effector cells & wild type SK-BR-3 target cells, was less than that obtained with the ERBB2 HEK293^{++} target cells but was nevertheless significantly greater than that observed with the NF-AT effector cells and wild type SK-RB-3 target cells (**Figure 2**). The response of *iLite*® effector cells and ERBB2$^{-/-}$ target cells, at the same E:T ratio as that used for the ERBB2^{++} target cells, to the presence of increasing concentrations of Herceptin did not differ from that of the control sample without Herceptin (data not shown).

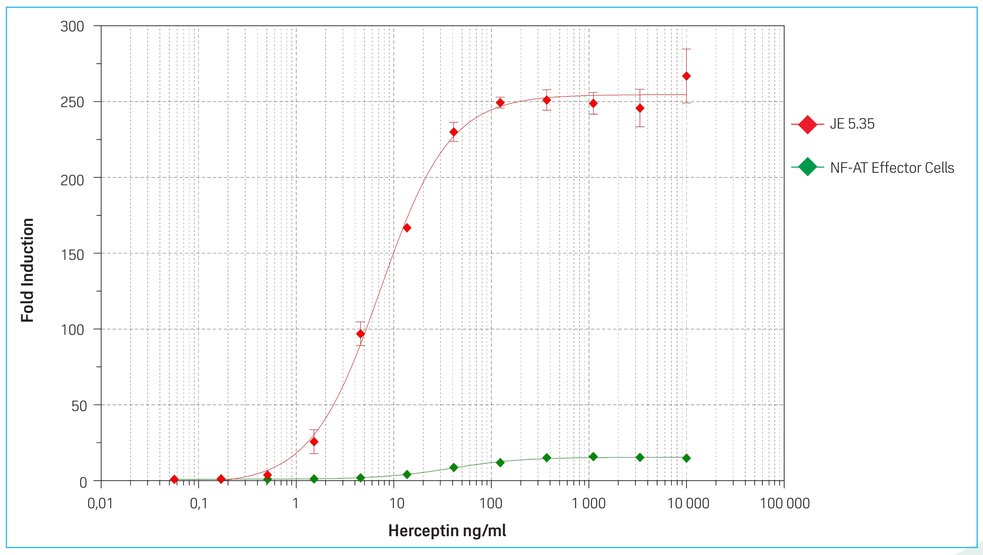

Figure 1. Quantification of the ADCC Activity of Herceptin using *iLite*® J5.35 Effector cells & ERBB2^{++} HEK293 Target Cells: vs NF-AT effector cells and ERBB2^{++} HEK293 Target Cells

Best-fit values	*iLite*® JE35.5	NF-AT
Top	254,6	15,55
Bottom	-2,466	1,005
LogIC50	0,8647	1,592
HillSlope	1,226	1,206
IC50	7,324	39,09
Fold Induction	257,1	14,55

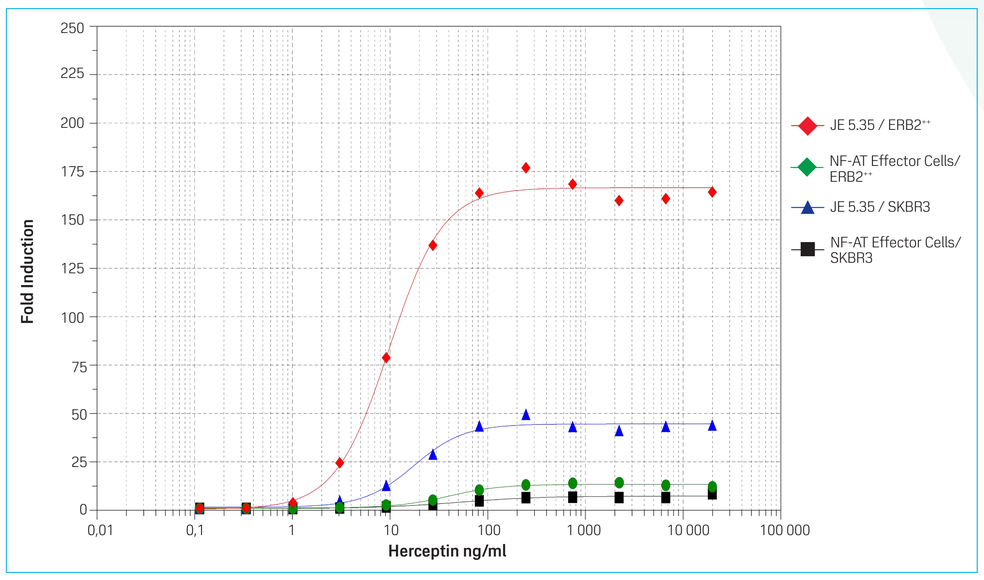

Figure 2. Quantification of the ADCC Activity of Herceptin using *iLite*® J5.35 Effector Cells & ERBB2^{++} HEK293 cells or SK-BR-3 Target Cells: vs NF-AT effector cells and ERBB2^{++} HEK293 target cells or SK-BR-3 Target Cells.

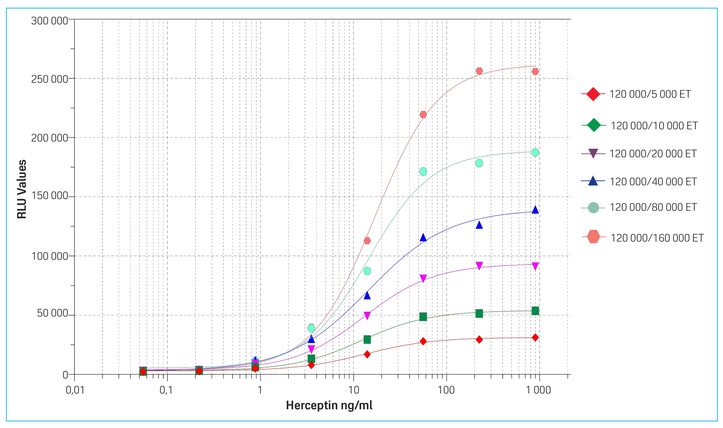

Figure 3A. Quantification of the ADCC Activity of Herceptin: Determination of the Optimal E:T ratio for Frozen Ready-to-Use *iLite*® JE5.35 Effector Cells and ERBB2^{++} HEK293 Target Cells (RLU Values, 6 hours)

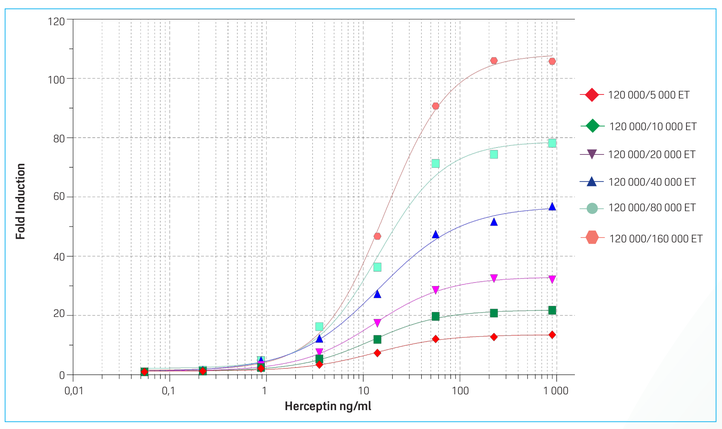

Figure 3B. Quantification of the ADCC Activity of Herceptin: Determination of the Optimal E:T ratio for Frozen Ready-to-Use *iLite*® JE5.35 Effector Cells and ERBB2^{++} HEK293 Target Cells (Fold Induction, 6 hours)

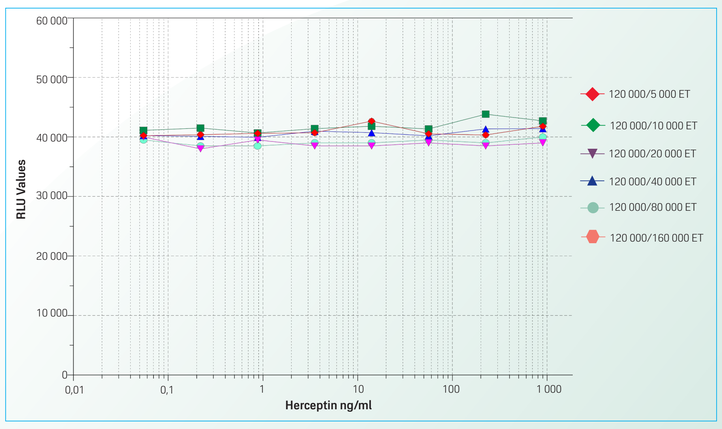

Figure 3C. Quantification of the ADCC Activity of Herceptin: Determination of the Optimal E:T ratio of Frozen Ready-to-Use Cells for *iLite*® JE5.35 Effector Cells and ERBB2 HEK293 Target Cells (Nano-Luc Expression, 6 hours)

Vials of *iLite*® effector cells and vials of ERBB2^{++} and HEK293 target cells were frozen separately using standard techniques. Upon thawing, effector cells and target cells were mixed at increasing E:T ratios and incubated for 6 hours in the presence of increasing concentrations of Herceptin. An E:T ratio of 4:1 was found to be optimal after 6 hours incubation of the effector and target cells (**Figures 3A & 3B**). The level of Nano-Luc expression did not increase as a function of the E:T ratio (**Figure 3C**) and can thus be used as a normalization gene.

In addition to providing a convenient and cost-effective means of quantifying the ADCC activity of therapeutic antibodies frozen ready-to-use effector and target cells 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.

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.