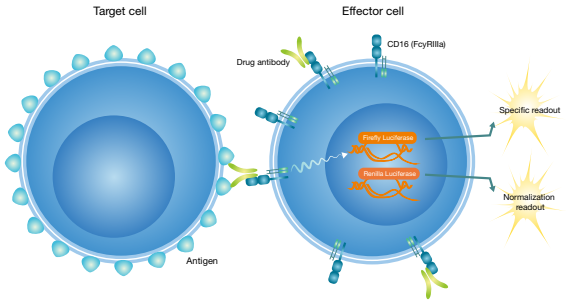
iLite® ADCC HER2
ASSAY READY CELLS

***iLite*® ADCC anti-HER2 Assay Ready Cells allow you to measure the ADCC activity of your anti-HER2 antibody in a simple, rapid and sensitive manner.**

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.

The *iLite* ADCC Target HER2 (+) Assay Ready Cells are human cells engineered to overexpress HER2 and optimized to give high sensitivity and specificity when used together with *iLite* ADCC Effector (V) Assay Ready Cells. This cell-based assay is unique in its setup – it does not require any culturing, and can be run within one workday. Since unspecific activation of ADCC can be a confounding factor when performing ADCC assays, we have also developed *iLite* ADCC Target HER2 (-) Assay Ready Cells which are depleted of HER2 expression, to be used as an internal control. The control cells can also be used for adjusting background levels when analyzing serum samples, thereby effectively removing any issues with varying background between samples.

iLite ADCC Effector (V) Assay Ready Cells, specifically designed for use with *iLite* ADCC Target Assay Ready Cells, are human cells which express constant, high levels of FcγRIIIa (CD16) which signals to a Firefly Luciferase (FL) reporter gene. The cells are engineered to have a high tolerance for serum, and include a second reporter gene, Renilla Luciferase (RL), which allows for normalization of cell counts, serum matrix effects or lysis of the effector cells by the target cells.



The iLite anti-HER2 ADCC Activity Set can be used for the quantification of ADCC activity of anti-HER2 antibodies

- Unparalleled sensitivity
- Normalization readout available
- High precision, due to Assay Ready Target cells with constant high HER2 expression.
- Assay Ready Target (-) cells for use as internal control and background adjustment

***iLite*® ADCC HER2 Assay Ready Cells**	
Product code	BM5001 *iLite*® ADCC Effector (V) Assay Ready Cells BM5011 *iLite*® ADCC Target HER2 (+) Assay Ready Cells BM5016 *iLite*® ADCC Target HER2 (-) Assay Ready Cells BM5090 *iLite*® anti-HER2 ADCC Activity Set
Host Cell	For BM5001: Human T lymphocyte cell line, Jurkat (ATCC #TIB-152) Fo BM5011, BM5016: Human embryonic kidney cell line, HEK293 (ATCC# CRL-1573)
Format	Assay Ready Cells
Application	The *iLite*® ADCC Effector (V) Assay Ready Cells can be used together with matched *iLite*® ADCC Target HER2 (+) and *iLite*® ADCC Target HER2 (-) Assay Ready Cells for the quantification ADCC activity. • Quantification of anti-HER2 ADCC activity (E-256-GB)
Assay time	4 hours (incubation)
Detection system	Luminescence
Availability	Research Use Only (RUO)*

**These products are intended for professional research use only. The data and results originating from using the products, should not be used either in diagnostic procedures or in human therapeutic applications.*

In accepting delivery of iLite® Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third party, and recipient is only to use them directly in assays. The iLite® cell-based products are covered by patents which are the property of Svar Life Science AB and any attempt to reproduce the delivered iLite® Assay Ready Cells would constitute an infringement.

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Quantification of the ADCC Activity of Trastuzumab

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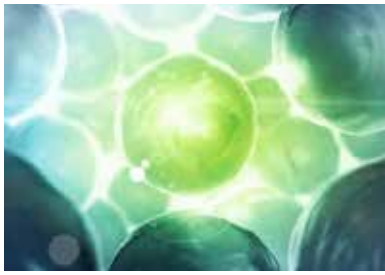

Introduction

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).

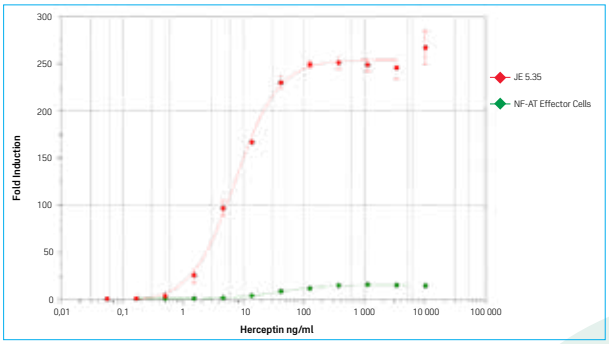

Figure 1. Quantification of the ADCC Activity of Herceptin using *iLite*® J5.35 Effector cells & ERBB2^{++} HEK293 Target Cells: vs NF-AT effector cells and ERBB2^{++} HEK293 Target Cells

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

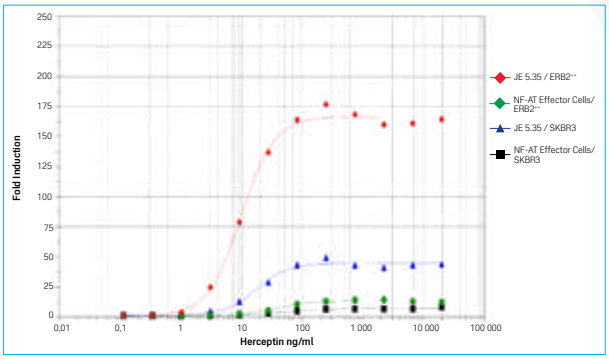

Figure 2. Quantification of the ADCC Activity of Herceptin using *iLite*® J5.35 Effector Cells & ERBB2^{++} HEK293 cells or SK-BR-3 Target Cells: vs NF-AT effector cells and ERBB2^{++} HEK293 target cells or SK-BR-3 Target Cells.

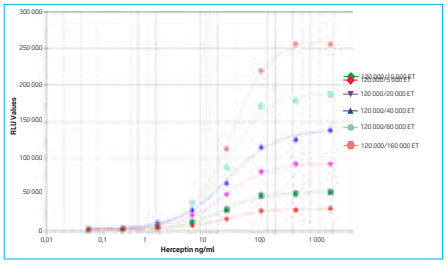

Figure 3A. Quantification of the ADCC Activity of Herceptin: Determination of the Optimal E:T ratio for Frozen Ready-to-Use *iLite*® JE5.35 Effector Cells and ERBB2^{++} HEK293 Target Cells (RLU Values, 6 hours)

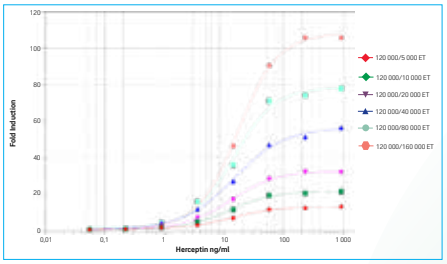

Figure 3B. Quantification of the ADCC Activity of Herceptin: Determination of the Optimal E:T ratio for Frozen Ready-to-Use *iLite*® JE5.35 Effector Cells and ERBB2^{++} HEK293 Target Cells (Fold Induction, 6 hours)

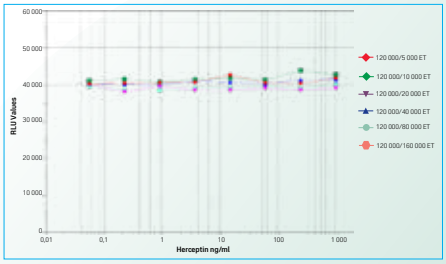

Figure 3C. Quantification of the ADCC Activity of Herceptin: Determination of the Optimal E:T ratio of Frozen Ready-to-Use Cells for *iLite*® JE5.35 Effector Cells and ERBB2 HEK293 Target Cells (Nano-Luc Expression, 6 hours)

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.