Evaluation of *iLite*® ADCC Effector cells using different CD20 target cells

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INTRODUCTION

The clinical activity of numerous monoclonal antibodies is to some extent mediated by antibody-dependent cell-mediated cytotoxicity (ADCC). Traditional methods for quantification of ADCC activity are labour intensive and show a high level of inherent variability due to the use of primary human NK cells from different donors as effector cells. The use of an engineered effector cell line has shown a significantly lower variation between assays. However, there is a need for a controlled set of target cells to allow differences in ADCC activity to be determined with precision and a high degree of specificity.

Here, we present a comparative case study using a novel ADCC effector cell line expressing the firefly luciferase reporter gene under control of the V-variant of the FcγIIIa receptor, to compare a novel target cell line overexpressing a constant high level of a specific antigen and a homologous wild type target cell line.

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.

In the *iLite*® ADCC Cell Line the effector cells serve as the "killing cell" and when activated, the antigen on the targets cell surface binds to the drug/ antibody and the Fc domain of the drug binds to the Fc-receptor on the effector cells initiating the transcription of the reporter gene construct and firefly luciferase is produced (Fig. 1).

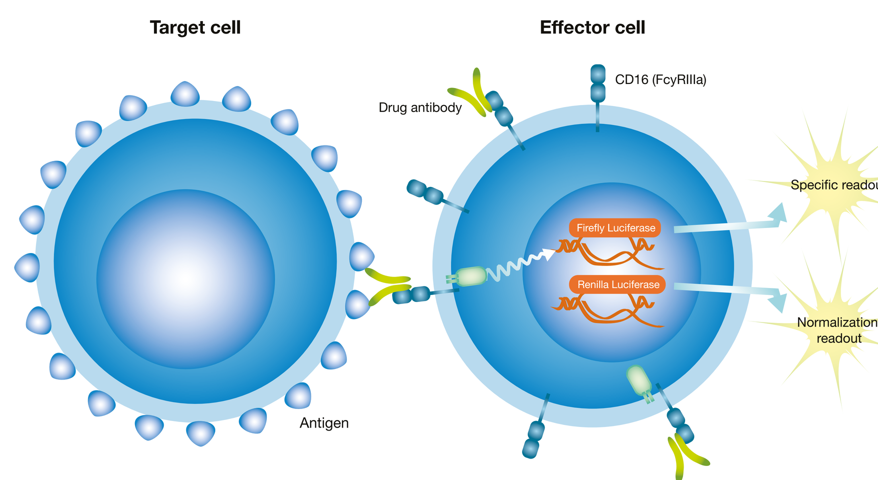

Figure 1. Schematic illustration of the *iLite* Reporter gene ADCC Effector cell.

ESTABLISHMENT OF AN ENGINEERED EFFECTOR CELL LINE AND TARGET CELL LINE

For the effector cells - 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 signalling from the FcgRIIIa receptor, initiating transcription of the firefly luciferase (FL) reporter-gene from a minimal SV40 promoter, an expression vector for FcRgIIIa (V variant), and the Renilla luciferase (RL) 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 RL.

For the 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 a homologous control cell line has been developed in which the gene encoding the specific drug target has been invalidated by CrisPR/ Cas9 editing.

> "Data shows that ADCC activity can be thoroughly assessed with an engineered target, just as well, and in some cases better, as with a traditional wild type target cell."

CONCLUSION

A series of novel target cells has been developed that expresses a constant high level of the specific antigen, recognizable by therapeutic antibodies, under the control of a strong constitutive promoter as well as the homologous control target cells in which the gene encoding the specific antigen has been inactivated by CRISPR/Cas 9 genome editing.

These engineered target cells and the homologous control cells allow differences in ADCC activity to be determined with precision. The compared assays cover a range from 50% to 150% nominal activity with good match between nominal and measured concentration. Further both target cells give a dynamic range of approx. 50% and an EC50 of approx. 3,7 ng/mL. The assays are stability indicating, specific and with a low in-assay variation of plate homogeneity. The data show that ADCC activity can be thoroughly assessed with an engineered target, just as well, and in some cases better, as with a traditional wild type target cell.

The availability of target cells specific for several of the most widely used therapeutic antibodies known to exhibit ADCC activity provides:

- a precise means of comparing the ADCC activity of biosimilars with that of the innovator product
- a comparison of the ADCC activity of variants of novel therapeutic antibodies that target the same antigen

Furthermore, availability of target cells in which the specific drug target has been invalidated provides the ideal control target cell for determining the specificity of an ADCC assay.

RESULTS

QUANTIFICATION OF ADCC ACTIVITY OF RITUXIMAB

A comparison of Ramos and *iLite* CD20 target cells

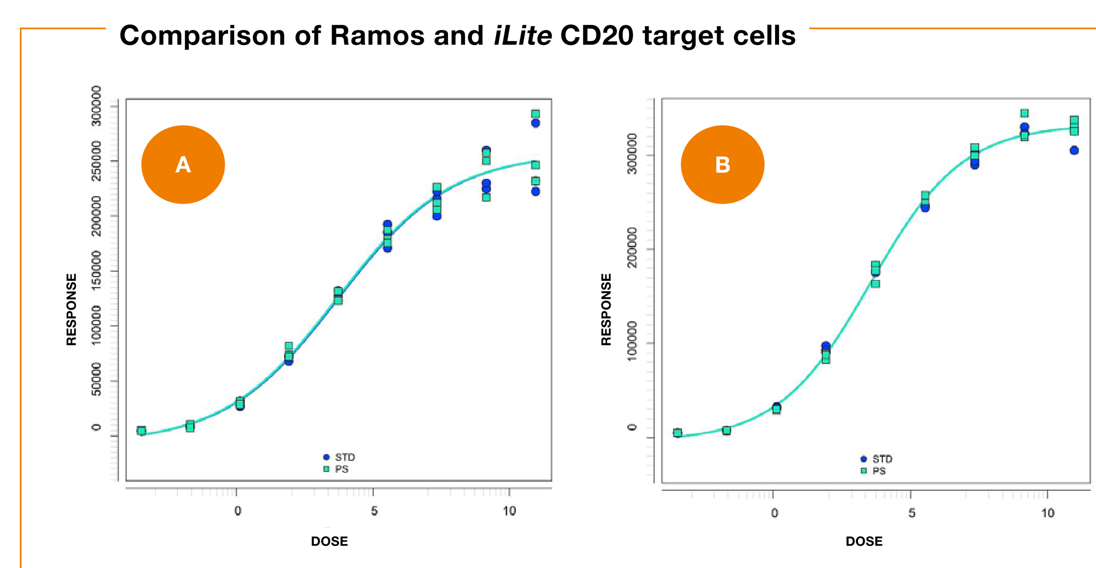

Figure 2.

iLite effector V cells were co-cultured with Ramos target cells (**A**) in presence of increasing concentrations of MabThera, or *iLite* CD20 target cells (**B**) in presence of increasing concentrations of MabThera. Subsequently, cells were lyzed, luciferase substrate BioGlo (Promega) added to the wells and the luminescence signal was read. Results were analyzed using PLA (Stegmann) software.

EVALUATION OF LINEARITY USING RITUXIMAB

Ramos and *iLite* CD20 target cells

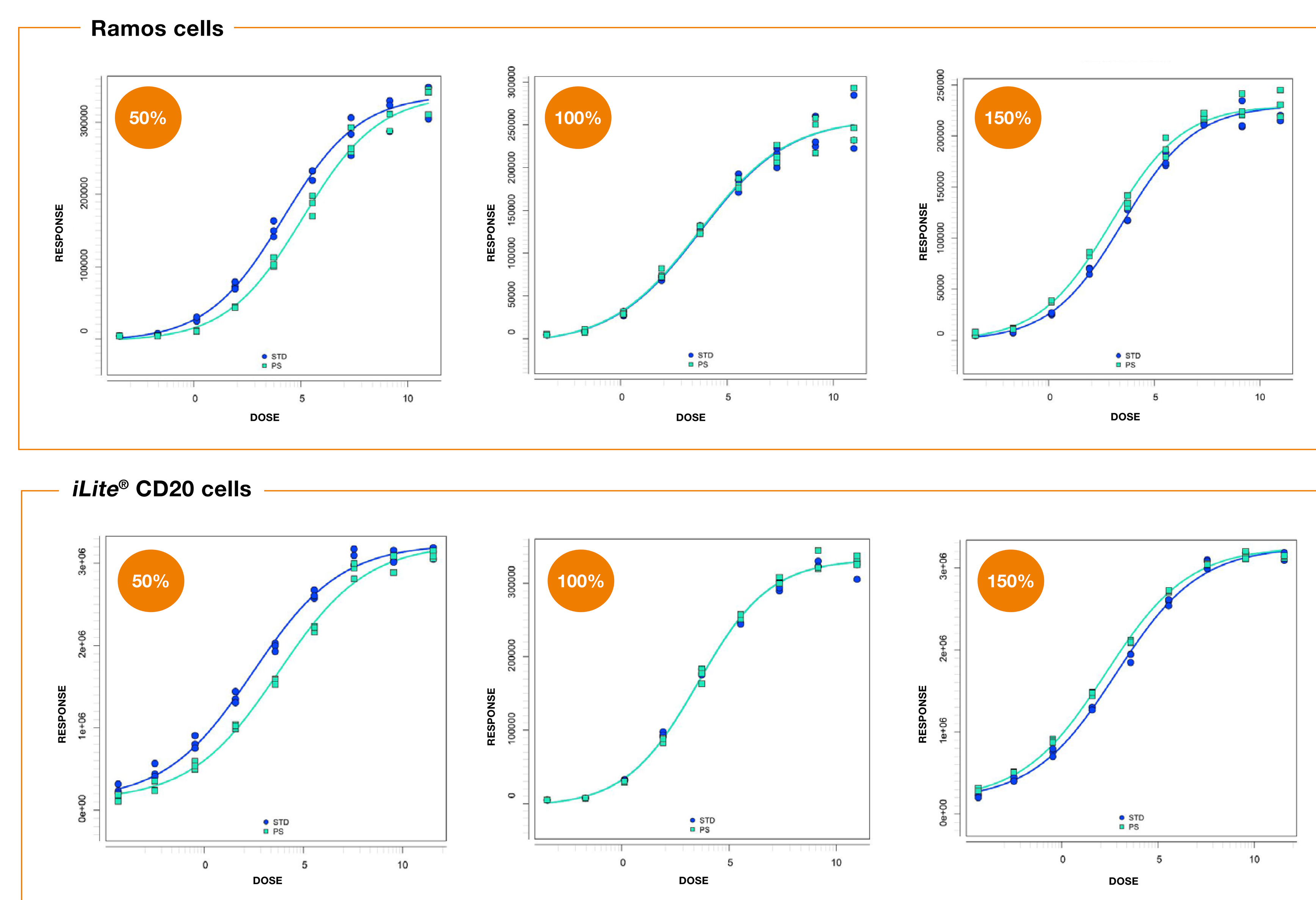

Figure 3.

iLite effector cells were co-culured either with Ramos target cells or *iLite* CD20 target cells with an E:T ratio of 3:1. Results were analyzed using PLA (Stegmann) software.

EVALUATION OF STABILITY INDICATING PROPERTIES

using Ramos cells and *iLite* CD20 target cells

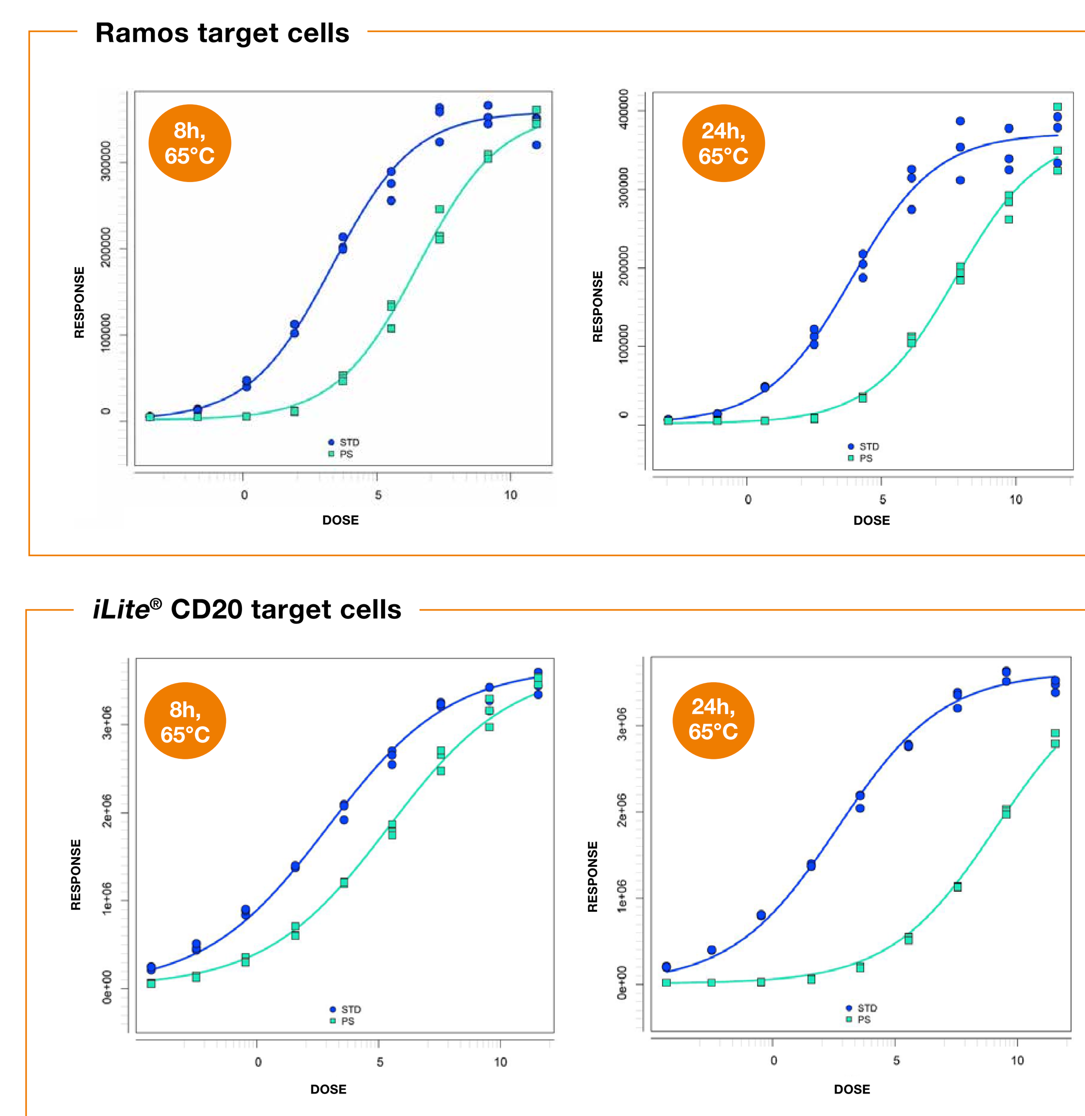

Figure 4a. Ramos target cells

iLite effector cells co-cultured with Ramos target cells; E:T ratio 3:1 and increasing concentrations of MabThera. MabThera was heat stressed at 65°C for 8h and 24h. Results were analyzed using PLA (Stegmann) software.

Figure 4b. *iLite* target CD20 cells

iLite effector cells co-cultured with *iLite* target CD20 cells; E:T ratio 3:1 and increasing concentrations of MabThera. MabThera was heat stressed at 65°C for 8h and 24h. Results were analyzed using PLA (Stegmann) software.

EVALUATION OF ASSAY SPECIFICITY USING A NON-RELEVANT MAB

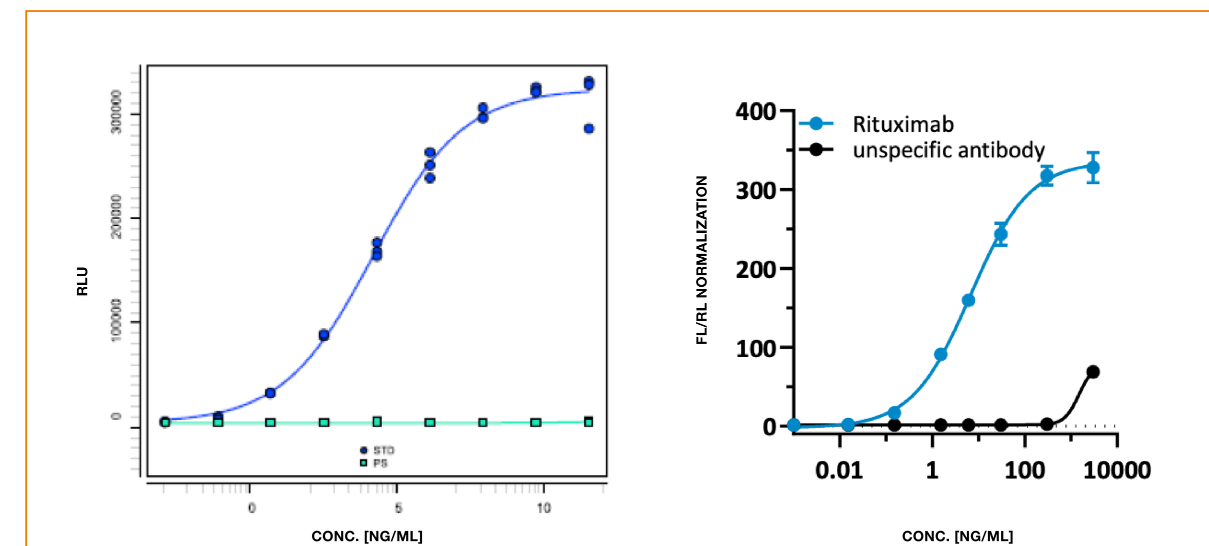

Figure 5.

iLite effector cells were co-cultured with either Ramos target cells or *iLite* CD20 target cells with an E:T ratio of 3:1 in presence of increasing concentrations of MabThera or an unspecific antibody at the same concentration range. Results were analyzed using PLA (Stegmann) for Ramos cells and a sigmoidal 4p curve fit, for *iLite* CD20 target cells.

PLATE HOMOGENEITY

using Ramos wild type target cells and *iLite* CD20 target cells

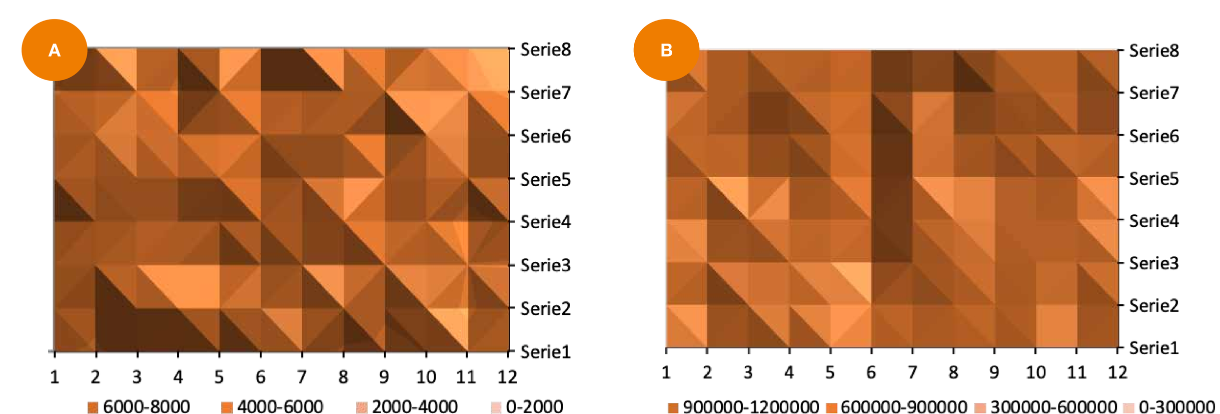

Figure 6.

A *iLite* effector cells were co-cultured with Ramos target cells; E:T ratio 3:1 and one concentration of MabThera.

B *iLite* effector cells were co-cultured with *iLite* CD20 target cells; E:T ratio 3:1, and one concentration of MabThera.