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, FcγRIIA (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 FcγRIIA 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, FcγRIIA (CD16a), and the Firefly Luciferase (FL) reporter gene under control of a chimeric promoter which incorporates recognition sequences for the principal transcription factors that regulate FcγRIIA activation (Figure 1). The effector cell line has also been transfected with the coding region of the Nano Luciferase (NL) gene under control of a constitutive TK promoter, thus allowing FL expression to be normalized with respect to this 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 enhanced 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 invalidatio in HEK293 cells (ATCC® CRL-1573) using CRISPR/Cas9 genome editing. Briefly a guide RNA sequence was designed, synthesized, 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 fluorescing activated cell sorting and a FITC labeled 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 1A). 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-BR-3 target cells (Figure 1B). 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 1A. Quantification of the ADCC Activity of Herceptin in iLite®-Effector Cells and ERBB2®-HEK293 Target Cells (RLU Values; 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 an 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 1A & 1B). The level of Nano-Luc expression did not increase as a function of the E:T ratio (Figure 1C) 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 the 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 inter- and intra-lot variation.

Conclusion

The iLite© effector cell line J5.35 provides a highly sensitive, precise, and specific means of quantifying ADCC activity. Potentially, J5.35 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 provides the basis for the establishment of highly precise and reproducible assays with a low degree of inter- and intra-lot variation.