High-level Production of Antibody Derivatives and Alternative Antibody Isotypes Using Scalable Cell Engineering

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Abstract

Antibody derivatives, such as bispecifics and Fc fragments, as well as alternative, non-IgG7 antibody isotypes represent promising classes of cancer immunotherapy. Their expression, however, can be challenging, complicating preclinical evaluation which can require significant amounts of recombinant protein. In this poster we highlight the production of milligram to gram quantities of quality bispecifics and other novel antibody derivatives and isotypes in cells relevant to bioproduction, including multiple CHO cell lines, using MaxCyte’s scalable cell engineering technology. We present data showing the high quality of transiently expressed antibody derivatives and their functional characterization. This capacity for rapid, large-scale production of high quality, functional antibody derivatives via transient expression is key to enabling development of novel biotherapeutics with improved efficacy, ADME profiles and manufacturability.

Strong Expression of Her2 x CD3 & CD19 x CD3 Functional BiTEs

Figure 1: Expression of Functional Bispecific T-cell Engaging (BiTE) Molecules in CHO Cells. 3d1 CHO-S cells were electroporated with a plasmid encoding a Her2 x CD3 or CD19 x CD3 tandem scFv. A) Conditioned media samples from electroporated-CHO-S cultures were equilibrated in fHabs binding buffer, and proteins enriched using NTA agarose. After dialysis against PBS, proteins were assayed by capillary electrophoreses. B) BiTE binding to SKBR-3 cells (Breast Cancer, Her2+CD3+) and Raji cells (Burkitt Lymphoma, her2+CD19+). C) Tumor cells were co-cultured with non-stimulated T-cells for 20 hours in the presence of [Her2]+ or CD3) or CD19 x CD3 BiTE molecules and cell lysis measured by a chromium release assay. Data Courtesy of Dr. Matthias Peppa, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University.

High Expression of Tribody Targeting Her2 x CD16

Figure 2: NK-mediated Lysis of Her2+ Tumor Cells Augmented by Tribodies. CHO-S cells were co-transfected with plasmids encoding a Her2 x CD16 tribody. SKBR-3 cells (derived from human breast cancer) were incubated with (Her2) x (CD16) tribody or with CD19 x CD3 BiTE molecules. FACs analysis showed binding of the tribody to Her2 antigens on SKBR-3 cells, whereas binding was not observed using a negative control CD19 x CD3 bispecific molecule (data not shown). SKBR-3 cells were co-cultured with mononuclear cells (MNC) at a 5:1 effector to target ratio in the presence of [Her2]+ cell tribody and cell lysis measured in a 4 hour chromium release assay. Data Courtesy of Dr. Matthias Peppa, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University.

Improved CHO-S Production of Bispecific Diabody

Figure 3: Production of Quality Diabodies. CHO-S cells were transfected with a bistatic expression plasmid encoding the components of a bispecific diabody via electroporation using the MaxCyte STX on the customer’s previous lipid-based transfection reagent. Concentrations of purified diabody were measured using ELISA. Diabody titers were more than 20-fold higher using MaxCyte electroporation. Analysis of purified proteins showed that 94% of the MaxCyte-produced protein was in a monomeric form.

Expression of Functional CD20-specific IgA2

Figure 4: Strong Fc Fusion Protein Production From CHO GS +/- Stable Pool. A CHO GS +/- cell line was created from parental CHO-K1 line. Either parental CHO-K1 or CHO GS +/- cells were electroporated with a construct expressing an Fc fusion protein using MaxCyte electroporation. Stable pools were maintained for 17 days. Titers produced by CHO GS +/- stable pools exceeded 1.5 g/L while parental cell line pools produced <25 mg/L. Data courtesy of LabelPharma.

Summary

• Flow Electroporation Technology is universal in nature and can produce a variety of high quality protein types including BiTEs, tribodies, FC fusion proteins, and alternative antibody isotypes such as IgA2 and IgG4.
• Ability to express alternative antibody-like molecules or non-IgG7 isotypes allows for fine-tuning of therapeutic effectors functions.
• High titer production using a variety of CHO cell lines allow maximum flexibility and early alignment with manufacturing cell line.
• Large-scale MaxCyte electroporation transfects up to 2e10 cells without the need for neopitrogenation allowing for purification of mg to gram quantities of antibodies from a single transfection.

High Titer IgG4 Antibody Production Using ExpelCHO Cell Line

IgG4 Titters Improved 4x4 by Post Transfection Seed Density Optimization

Figure 5: Increased Neutrophil-mediated Tumor Lysis via CD20-specific IgA2. 3d1 CHO-S cells were electroporated using large-scale MaxCyte electroporation with DNA encoding a CD20-specific IgA2. Purified IgA2 was incubated with CD02-bearing lymphoma cells and binding assessed. CD20-specific IgA2 control antibody and non-specific CD20-IGA2, as well as CD20-IGA2 expressed using MaxCyte electroporation were used in a chromium release cytotoxicity assay. Data Courtesy of Dr. Matthias Peppa, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University.

Figure 6: Higher ExpelCHO IgG4 Titters Using Flow Electroporation Technology. ExpelCHO cells were transfected with a human IgG4 expression plasmid using MaxCyte electroporation. Transfected cells were seeded at 4e6 cells/mL, 4e5 cells/mL, or 2e5 cells/mL, using ExpelCHO medium and feed. Viable cell density and antibody titers were determined at Days 3, 5, 7 and 10 post transfection.