Antibody derivatives, such as bispecific antibodies, represent a promising class of cancer immunotherapy either alone or as a component of combination therapies. For the preclinical evaluation of structure-function relationships and effector mechanisms triggered by different antibody formats, significant amounts of recombinant proteins are required. Efficient transient transfection systems are a prerequisite for comparison of various potential candidate molecules in parallel. In this poster we highlight the production of milligram to gram quantities of quality bi-specifics and other novel antibody derivatives in cells relevant to bioproduction, including multiple CHO cell lines, using MaxCyte’s scalable, cGMP-compliant cell engineering technology. We present data showing the high quality of transiently expressed antibody derivatives and their functionality through tumor cell-specific cytotoxicity, tumor cell binding and anti-inflammatory activity. This rapid, large-scale production of high quality, functional antibody derivatives via transient expression demonstrates the ability of MaxCyte’s enabling technology to delay stable cell line generation, thus reducing time and resource expenditures, until after toxicology studies while maintaining the integrity of candidate development.

Figure 1: Expression of functional bispecific T cell engaging (BITE) molecules in CHO cells. A). Conditioned media samples from CHO-B cells transiently expressing CD19 x CD3 or Her2 x CD3 bispecific molecules were equilibrated in buffer binding buffer, and proteins were enriched using NTA agarose. After dialysis against PBS, proteins were assayed by capillary electrophoresis. B). BITE binding to SKBR3 cells (Breast Cancer, Her2+/CD16+) and Raji cells (Burkitt Lymphoma, Neutrophilic) was evaluated. C). Tumor cells were co-enriched with non-stimulated T-cells for 20 hours in the presence of [Her2+, x CD3] or [CD19 + x CD3] BITE molecules and cell lysis measured by a chromium release assay. Data Courtesy of Dr. Matthias Peipp, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University.

High Expression of Tridoby Targeting Her2 x CD16
Antigen-specific Binding & Redirected Lysis by NK Cells

Figure 2: Binding & lysis of affinity enriched tribodies to tumour cells. A). Following co-transfection of CHO-S cells with plasmids encoding a Her2 x CD16 tribody, proteins were enriched from conditioned media samples using CH1-specific agarose beads. Proteins were assayed by capillary electrophoresis under reducing and non-reducing conditions. Arrows indicate bands of the expected sizes for single chains and intact tribody. B). SKBR3 cells (derived from human breast cancer) were incubated with (Her2α, x CD16) tribody or with CD19 x CD3 BITE molecules. FACS analysis showed binding of the tribody to Her2α antigens on SKBR3 cells, whereas binding was not observed using the negative control CD19 x CD3 bispecific molecules. C). SKBR3 cells were co-enriched with mononuclear cells (MNAC) in the presence of [Her2α, x CD16] tribody and cell lysis measured by a chromium release assay. Data Courtesy of Dr. Matthias Peipp, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University.

Expression of FC Protein in CHOZn Cells
Functional Stimulation of T Cells

Figure 3: Purified Fc fusion protein has anti-inflammatory activity. A). CHOZn2 cells were transfected with an expression plasmid encoding an Fc fusion protein containing a recombinant form of the naturally occurring human protein Alpha-1 Antitrypsin. Clarified media samples were loaded onto a 1ml Protein A Flow HiTrap® column (GE) at room temperature. Gel analysis of purified proteins expressed by transiently transfected cells showed quality and size attributes consistent with those of a reference protein and that produced in a customer lab. B). Two different donor T cells were challenged with an inflammatory substance either in the presence or absence of various quantities of the purified Fc fusion protein. Transiently produced Fc fusion protein had the expected anti-inflammatory activity as measured by an overall decrease in IL10 secretion. Data Courtesy of MilliporeSigma.

Expression of Functional CD20-specific IgA2
IgA increased Recruitment of Myeloid Effector Cells

Figure 4: Increased Neutrophil-mediated Tumor Lysis via CD20-specific IgA2. A). CHO-CHO-S cells were electroporated using large-scale MaxCyte electroporation on the MaxCyte STX with DNA encoding a CD20-specific IgA2. Transfection efficiency was assessed using GFP transfection (B). IgA2 was incubated with CD20-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 cytotoxicity assay. Data Courtesy of Dr. Matthias Peipp, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University.

Summary
- The MaxCyte delivery platform can produce high titers of antibodies and antibody-like molecules using a variety of CHO cell lines, including CHO-S and CHOZn2 cells, providing researchers the ability to use their CHO cell line of choice.
- Flow Electroporation Technology is universal in nature and can produce a variety of high quality protein types including bi-specifics, tribodies, Fc fusion proteins, IgA and alternative isotypes such as IgA2.
- Large-scale MaxCyte electroporation transfects up to 2x10^10 cells without the need for recaptimization allowing for purification of mg to gram quantities of antibodies from a single transfection.
- A variety of transiently produced protein types exhibited functional activity including tumor binding, tumor lysis and anti-inflammatory activity.
- Ability to express alternative antibody-like molecules or non-IgG isotypes allows for fine-tuning of therapeutic effector functions.

MaxCyte Transient Transfection Platform
The MaxCyte STX® and MaxCyte VLX® Transient Transfection Systems use fully scalable flow electroporation for rapid, highly efficient transfection.
- High efficiency & high cell viability
- Broad cell compatibility
- True scalability requiring no re-optimization
- Closed, computer-controlled instruments
- cGMP-compliant & CE-marked
- Master file with US FDA & Health Canada