

Production & Functional Characterization of Bispecifics and Other Novel Antibody Derivatives Using Scalable, Regulatory-compliant Cell Engineering



James Brady, Weili Wang, Rama Shivakumar, Pachai Natarajan, Krista Steger. MaxCyte, Gaithersburg, MD, USA

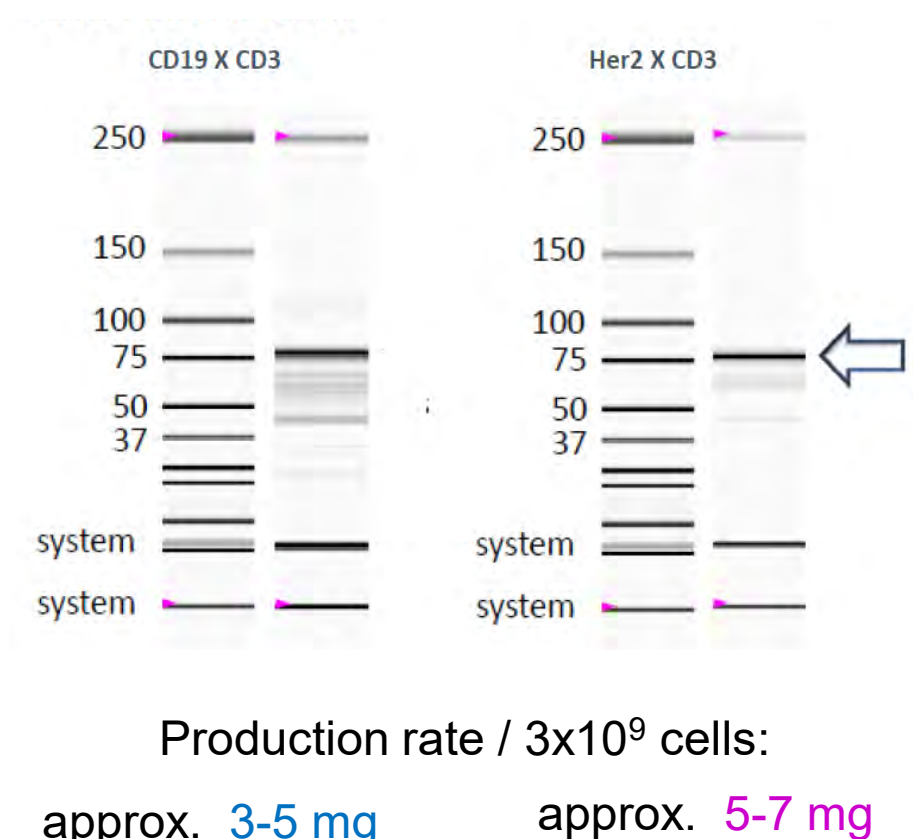
Abstract

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 risk and resource expenditures, until after toxicology studies while maintaining the integrity of candidate development.

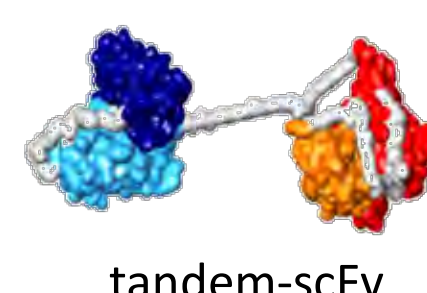
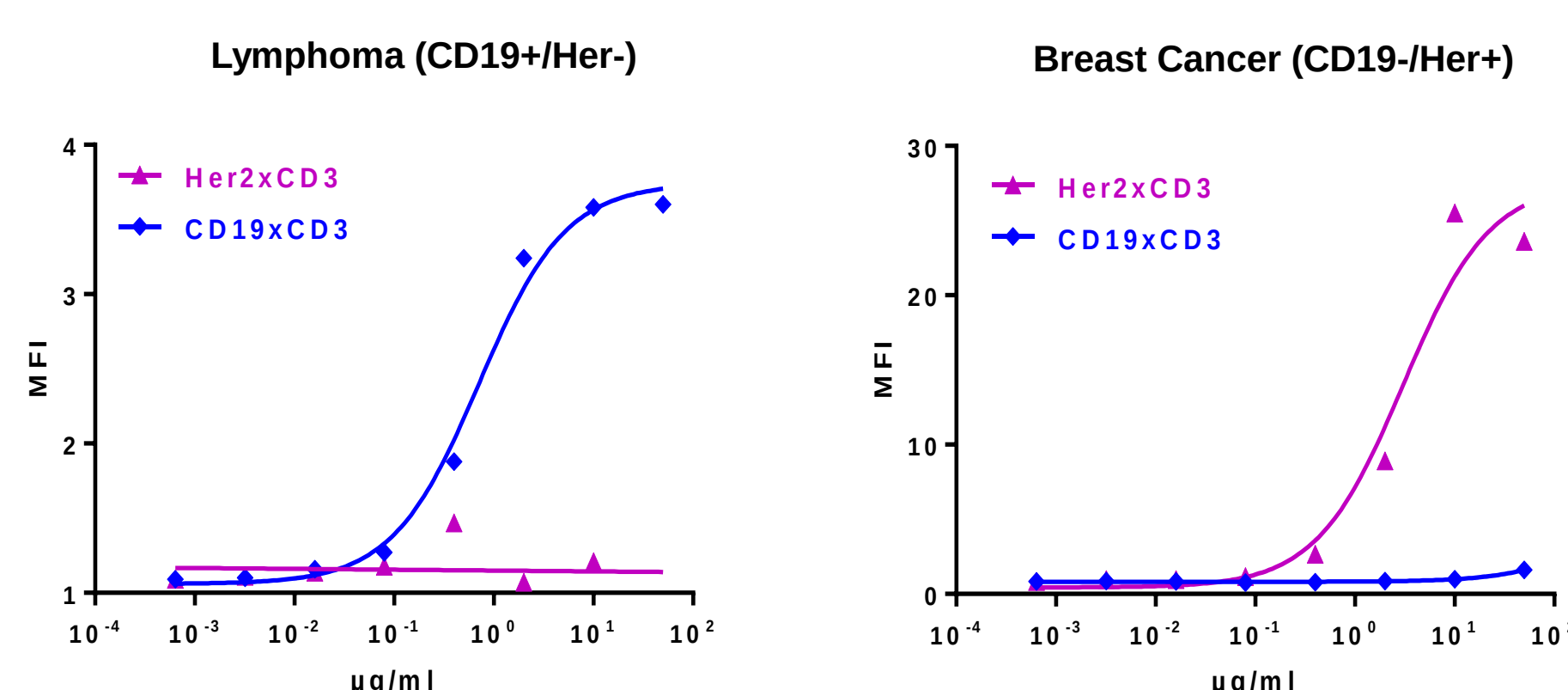
Strong Expression of Her2xCD3 & CD19xCD3 Functional BiTEs

Antigen-specific Binding & Redirected Lysis by Non-stimulated $\alpha\beta$ T Cells

A. Affinity Purification



B. Antigen-specific BiTE Binding to Tumor Cells



tandem-scFv

C. Specific Killing of Tumor Cells by Non-stimulated $\alpha\beta$ T Cells Mediated by BiTE Molecules

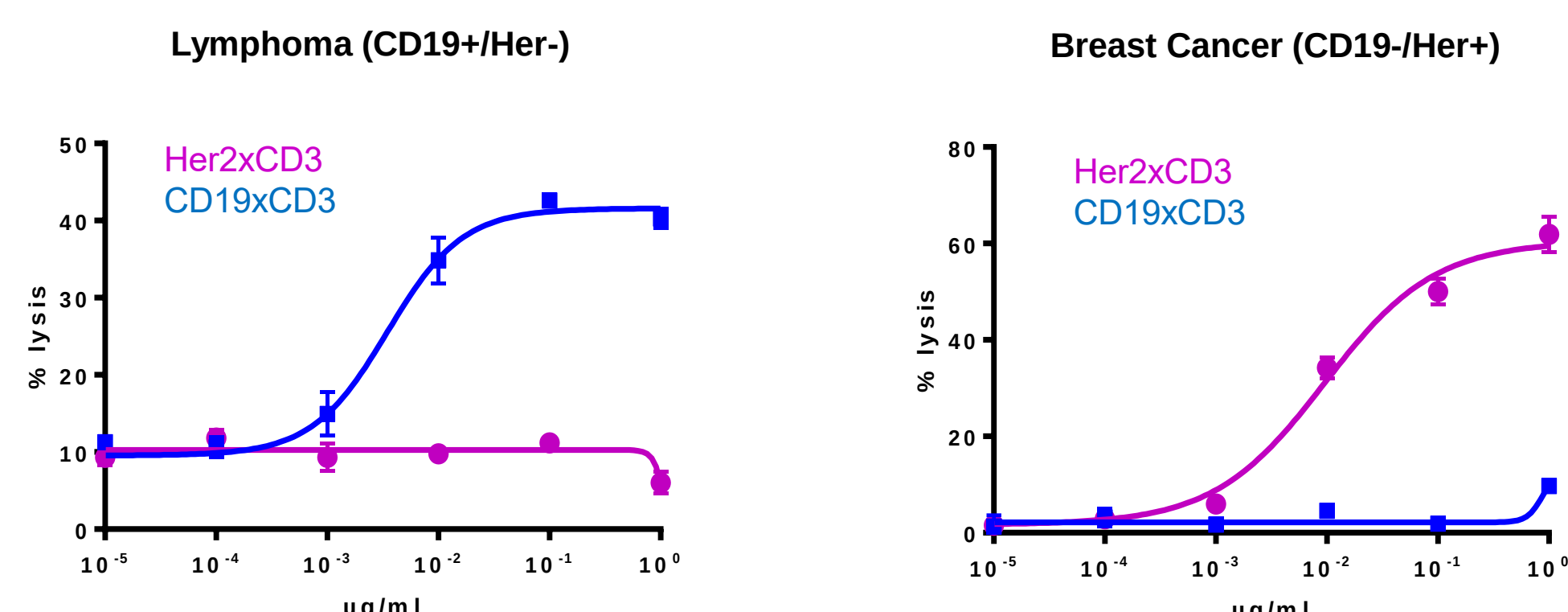
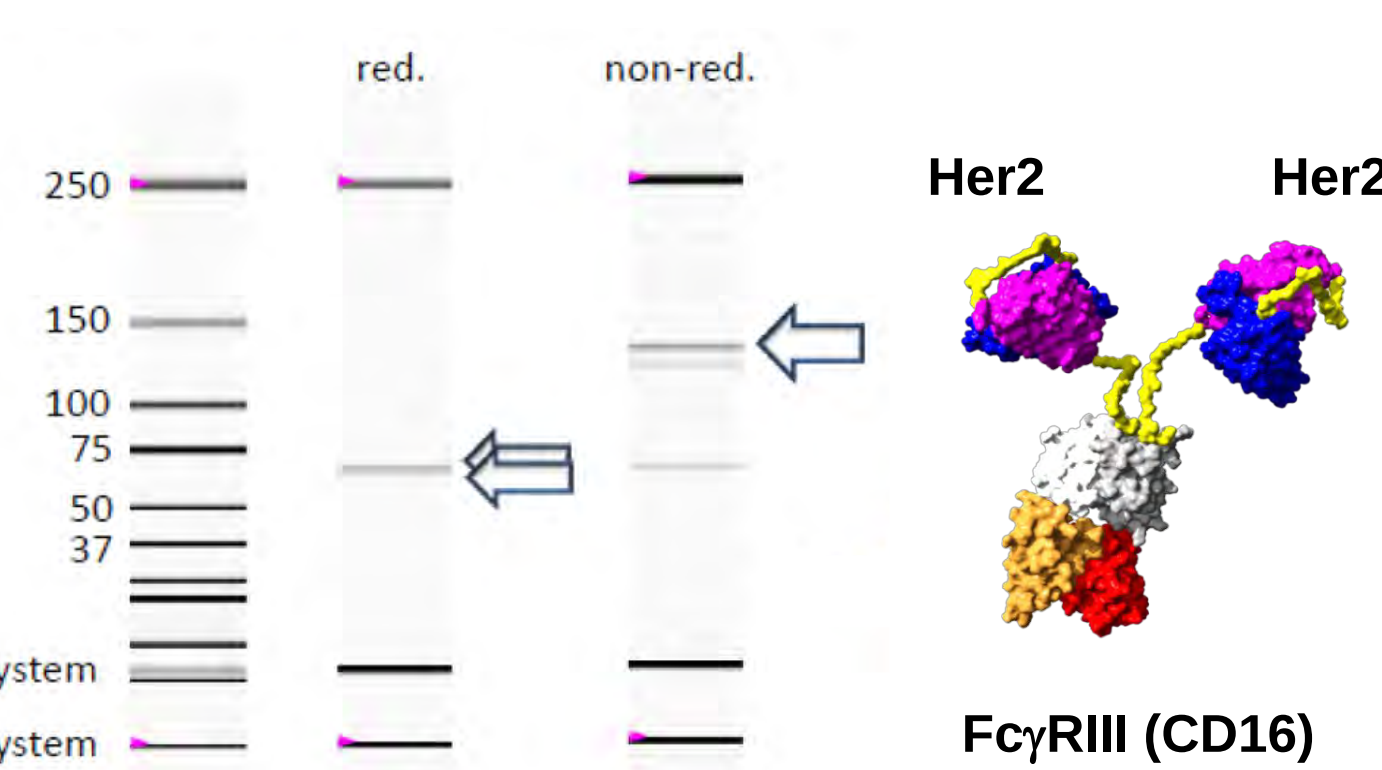


Figure 1: Expression of functional bispecific T cell engaging (BiTE) molecules in CHO cells. A). Conditioned media samples from CHO-S cultures transiently expressing CD19 x CD3 or Her2 x CD3 bispecific molecules were equilibrated in 6xHis binding buffer, and proteins were enriched using Ni-NTA agarose. After dialysis against PBS, proteins were assayed by capillary electrophoresis. B). BiTE binding to SKBR-3 cells (Breast Cancer, Her2+/CD19-) 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)₂ 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.

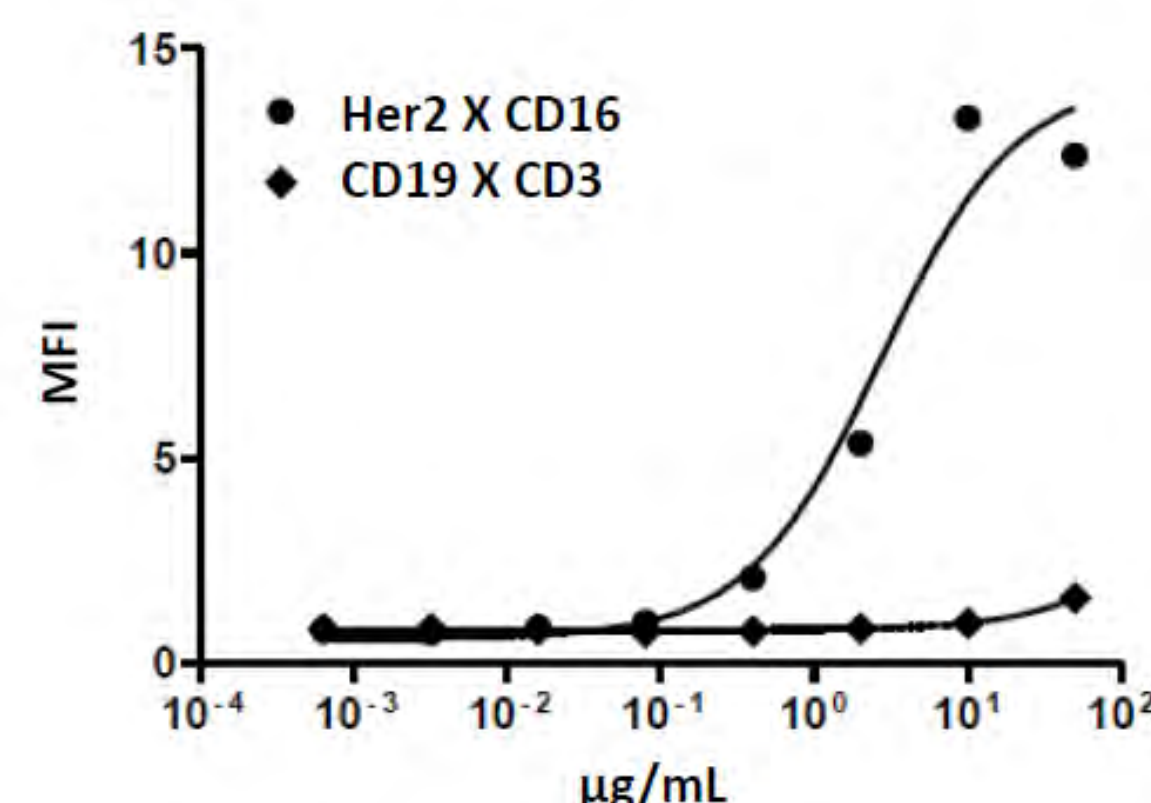
High Expression of Tribody Targeting Her2 x CD16

Antigen-specific Binding & Redirected Lysis by NK Cells

A. Affinity Purification



B. Specific Binding of Enriched Tribodies to Tumor Cells



C. NK Cell-Mediated Redirected Lysis

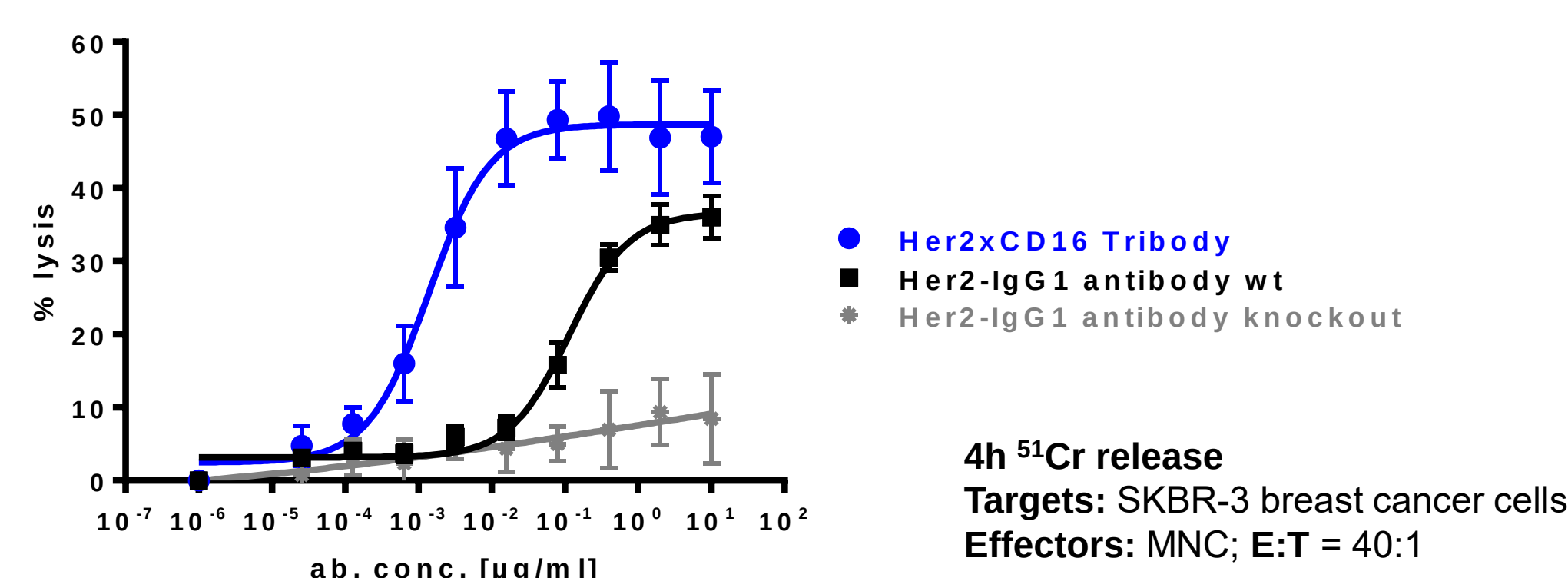
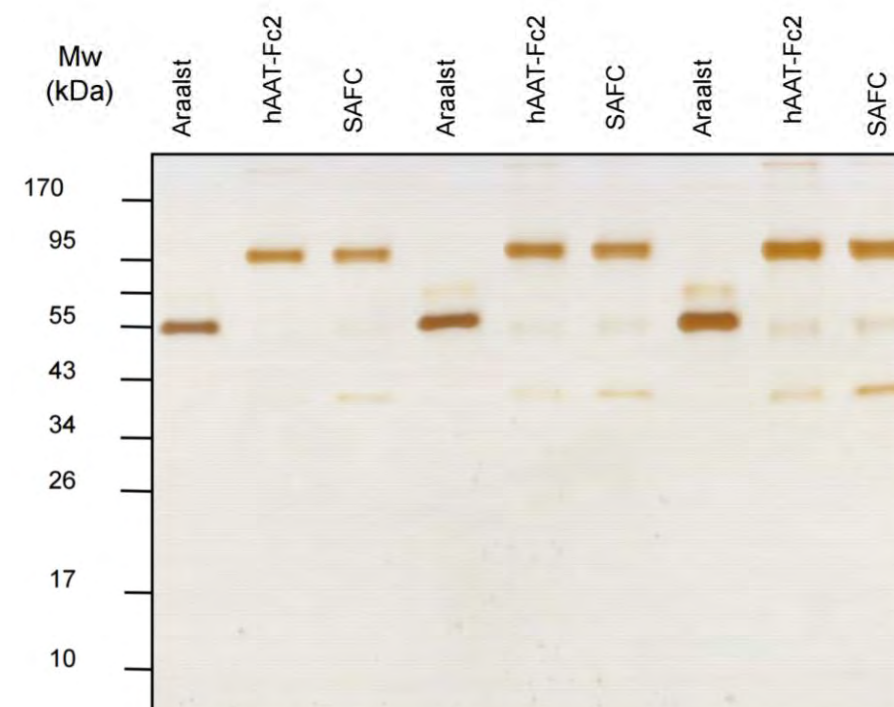


Figure 2: Binding & lysis of affinity enriched tribodies to tumor cells. A). Following co-transfection of CHO-S cells with plasmids encoding a Her2 x CD16 tribody, proteins were enriched from conditioned media samples using CH1-specific agarose beads. Proteins were assayed by capillary electrophoresis under reducing and non-reducing conditions. Arrows indicate bands of the expected sizes for single chains and intact tribody. B). SKBR-3 cells (derived from human breast cancer) were incubated with [(Her2)₂ 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 the negative control CD19 x CD3 bispecific molecules. C). SKBR-3 cells were co-cultured with mononuclear cells (MNC) in the presence of [(Her2)₂ 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 Fusion Protein in CHOZN® Cells

Functional Stimulation of T Cells

A. Fc Fusion Protein Gel Analysis



B. Stimulation of Donor Cell Measured by IL1β Secretion

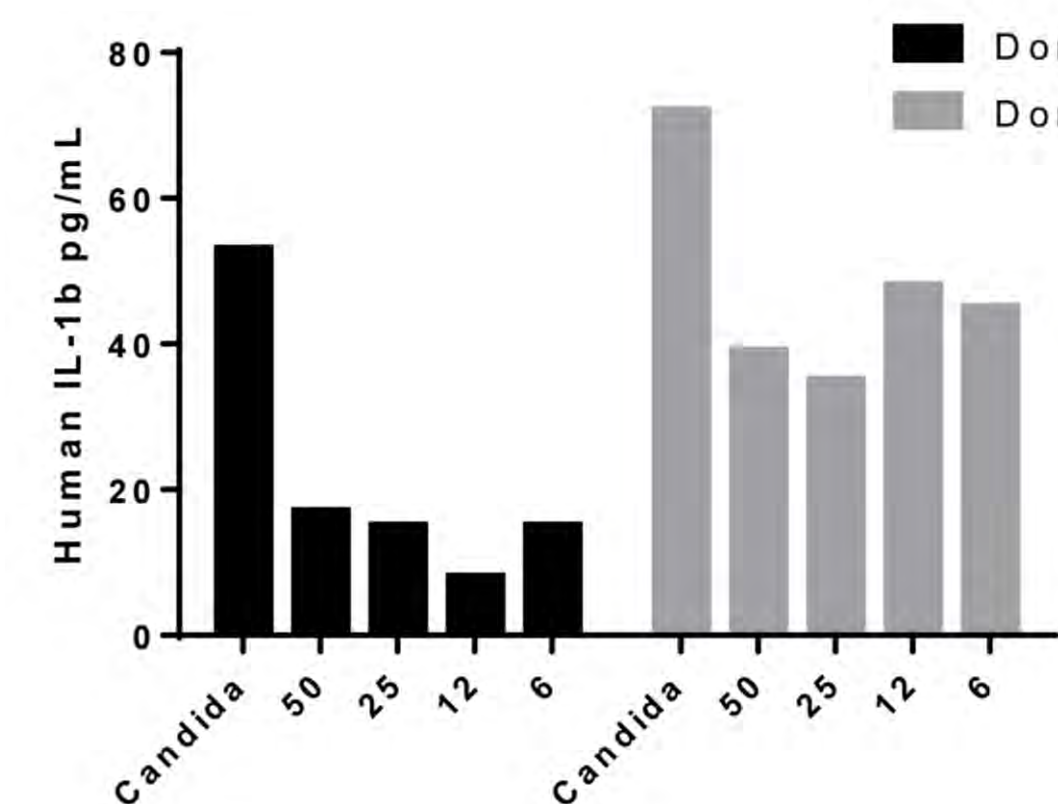
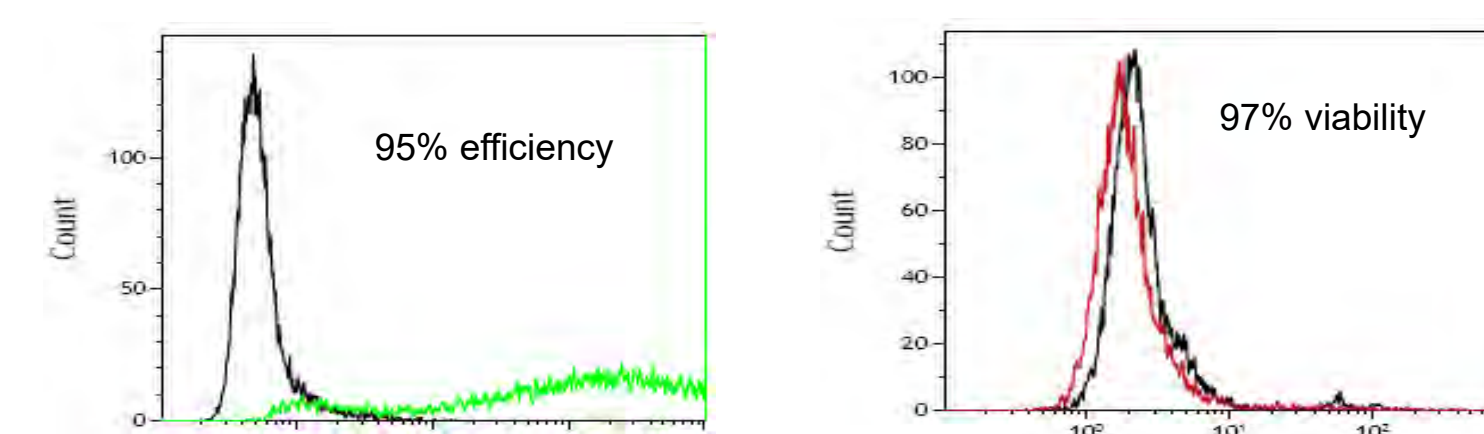


Figure 3: Purified Fc fusion protein has anti-inflammatory activity. A). CHOZN® cells were transfected with an expression plasmid encoding an Fc fusion protein containing a recombinant form of the naturally occurring human protein Alpha-1 Antitrypsin. Clarified media samples were loaded onto a 1ml rProtein A Fastflow 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 IL1b secretion. Data Courtesy of MilliporeSigma.

Expression of Functional CD20-specific IgA2

IgA: Increased Recruitment of Myeloid Effector Cells

A. Large-scale MaxCyte Electroporation of CHO-S cells



B. Antigen-specific Binding & Tumor Cell Lysis

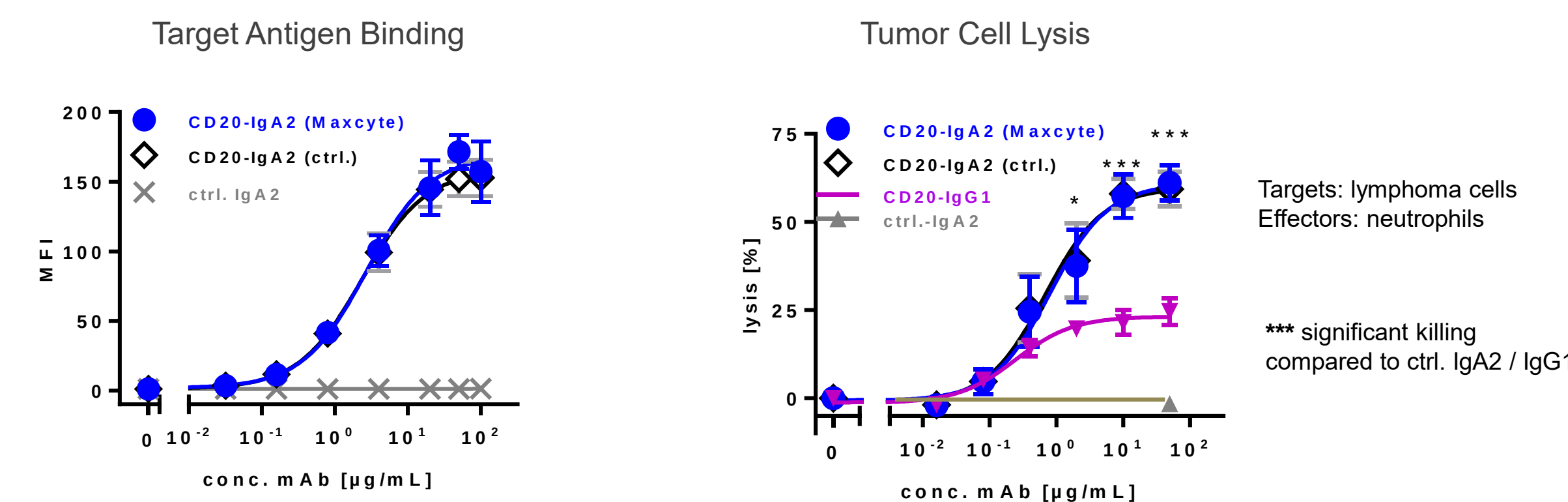


Figure 4: Increased Neutrophil-mediated Tumor Lysis via CD20-specific IgA2. A). 3e9 CHO-S cells were electroporated using large-scale MaxCyte electroporation on the MaxCyte STX with DNA encoding a CD20-specific IgA2. Transfection efficiency was assessed using GFP transfection. B). IgA2 was incubated 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 CHOZN® 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, IgG and alternative isotypes such as IgA2.
- Large-scale MaxCyte electroporation transfects up to 2e10 cells without the need for reoptimization 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 STX®
5E5 Cells in Seconds
Up to 2E10 Cells in <30 min



MaxCyte VLX®
Up to 2E11 Cells in <30 min

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