Reducing Timelines for Production and Characterization of Bispecifics and Other Novel Scaffolds.

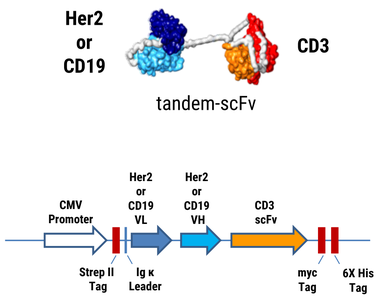
Joseph Abad, Weili Wang, Rama Shivakumar, Pachai Natarajan, Krista Steger, and James Brady. MaxCyte, Gaithersburg, MD, USA

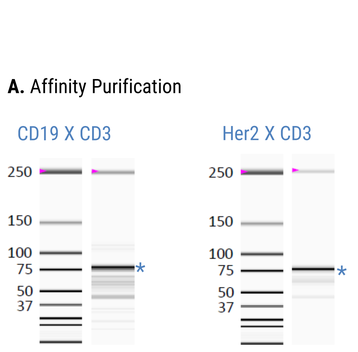
Abstract

Antibody derivatives, such as bispecifics and Fc fragments, as well as novel scaffolds, represent promising classes of biotherapeutics offering the ability to fine-tune specificity, half-lives, and anti-tumor effector functions. Their expression, however, can be challenging, complicating preclinical evaluation which can require significant amounts of recombinant protein. This poster highlights high titer, scalable production of functional bispecifics, tribodies and other scaffolds, such as membrane receptor targeting KnotBodies™, in cells relevant to bioproduction, including multiple CHO cell lines. Data are presented showing the quality of transiently expressed proteins and their functional characterization. This capacity for rapid, large-scale production of difficult-to-express proteins via transient expression is key to enabling rapid development and translation to the clinic of novel biotherapeutics with improved efficacy, ADME profiles and manufacturability. .

Case Study #1 - Strong Expression of Her2 x CD3 & CD19 x CD3 Functional BiTE & Tribody Targeting Her2 x CD16

Antigen-specific Binding & Redirected Lysis by Non-stimulated αβ T Cells





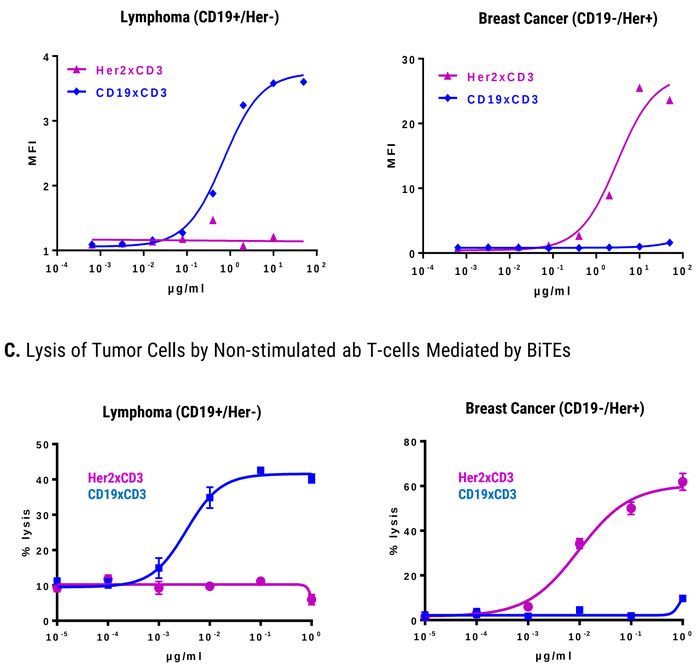


Figure 1: Expression of functional Bispecific T-cell Engaging (BiTE) molecules in CHO-S cells. 3e9 CHO-S cells were electroporated with a plasmid encoding a Her2 x CD3 or CD19 x CD3 tandem scFv. *A).* Conditioned media samples from electroporated CHO-S cultures were equilibrated in 6xHis binding buffer, and proteins 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.*

Augmentation of Antigen-specific, NK-cell-mediated Cytotoxicity

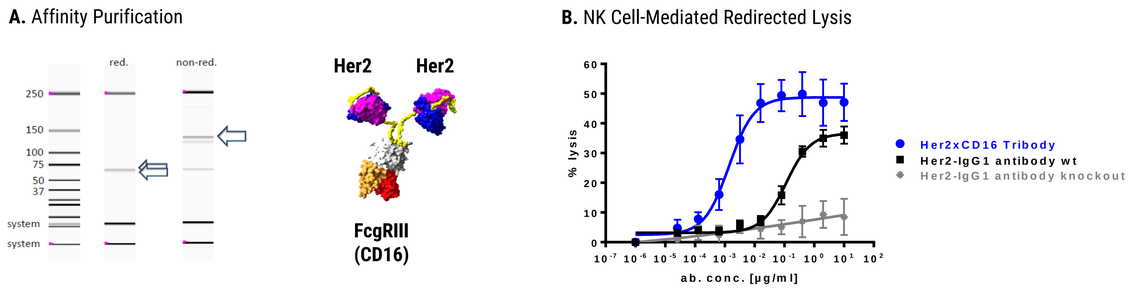
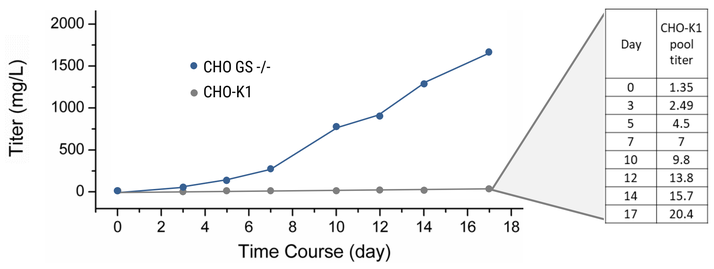


Figure 2: Binding & lysis of affinity enriched tribodies to tumor cells. *A).* Following co-transfection of CHO-S cells with plasmids encoding a Her2 x CD16 tribody, proteins were enriched from conditioned media samples using CH1-specific agarose beads. Proteins were assayed by capillary electrophoresis under reducing and non-reducing conditions. Arrows indicate bands of the expected sizes for single chains and intact tribody. *B).* SKBR-3 cells 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.*

Case Study #2 - >1.5 g/L Fc Fusion Stable Pool Production

Custom CHO GS -/- Cell Line Stable Pool Outperforms Parental CHO-K1 Cell Line



Day	CHO-K1 pool titer
0	1.35
3	2.49
5	4.5
7	7
10	9.8
12	13.8
14	15.7
17	20.4

Figure 3: 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 LakePharma.*

Case Study #3 - Improved CHO-S Production of Bispecific Diabody

Higher Quality and Titer Using MaxCyte Electroporation Compared to Lipid Reagents

Transfection Method	Expression (purified protein)	%HMW	%Monomer
MaxCyte STX	**173 .0 mg/L**	5.6	94.3
Lipid Reagent	7.3 mg/L	7.2	92.8

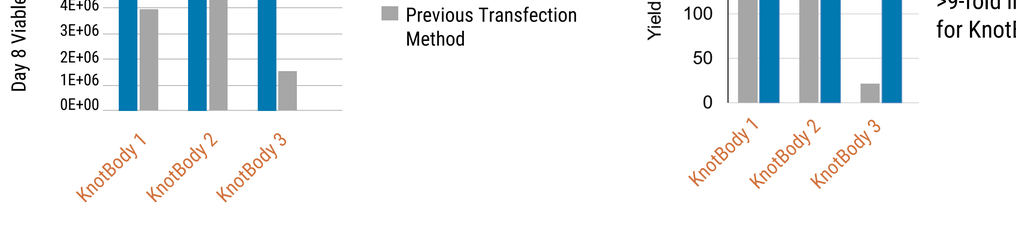


Figure 4: Production of Quality Diabodies. CHO-S cells were transfected with a bicistronic expression plasmid encoding the components of a bispecific diabody via electroporation using the MaxCyte STX or the customer's previous lipid-based transfection reagent. Concentrations of purified diabody were 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.

Case Study # 4 – Ion Channel Targeting KnotBodies™: Characterization, Activity Optimization & Stable Cell Line Generation

KnotBodies Combine the Benefits of Knottin Ion Channel Blocking with Long Half Life of Antibodies

Knottins

- Natural blockers of ion channels
- 30-40 amino acids
- Often lack specificity
- Short *in vivo* half life
- Difficult to engineer

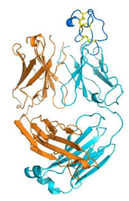
Antibodies

- Large, modular and provide specificity
- Long half life
- Amenable to engineering via *in vitro* selection technologies

KnotBodies™

- Insert knottins into peripheral antibody CDR loops
- Engineer other CDR loops for improved potency & selectivity

IONTAS



Improved Transient KnotBody Expression Expands Early Candidate Pools

Figure 5: High transfection efficiency & cell viability provide for increased transient KnotBody production. Bicistronic expression vectors encoding three different KnotBodies were transfected into ExpiCHO cells using either lipid-based transfection or MaxCyte's Flow Electroporation technology. Lipid transfected cells were cultured in ExpiCHO medium pre and post transfection using supplements and culture conditions recommended by the manufacturer. MaxCyte transfected cells were cultured in ExpiCHO medium prior to transfection, and cultured post transfection with lower cost commercial media and feed supplements with feed on day 1, 3, 6 and 8. Transfected cells were cultured for 9 days. *Data courtesy of Iontas.*

Summary

- High transfection efficiency and cell viability of Flow Electroporation Technology enable production of high quality, difficult-to-express protein formats including BiTEs, tribodies, Fc fusion proteins, KnotBodies™, and non-IgG1 antibody isotypes.
- Expression of novel antibody-like molecules or non-IgG isotypes allows for fine-tuning of therapeutic effector functions for improved efficacy.
- High titer production using a variety of CHO cell lines, including CHO-S, CHO-K1, CHOZN®, and genetically engineered CHO cell lines, allows maximum flexibility and early alignment with manufacturing cell line.
- Sophisticated, scalable cell engineering, including nuclease-mediated library construction, that support enabling applications such as mammalian display for rapid optimization of biotherapeutic candidates.
- Scalability of the ExPERT Platform provides for accelerated timelines from early discovery using high-level transient expression through pre-clinical and clinical development via high-producing stable pool and stable cell line generation.

Corresponding Author: James Brady; jamesb@maxcyte.com MaxCyte, Inc., Tel: (301) 944-1700, info@maxcyte.com, *www.maxcyte.com*