



B-Xpander[™]

Unleash the Power of B Cell Expansion for Clinical Research & Immunotherapies

Interest in B cells has recently increased in immunotherapies using Engineered B cells or Tumor-Infiltrating Lymphocytes (TILs). However, current methods for B cell expansion are inefficient, time consuming and can lead to insufficient B cell numbers and persistent T cell exhaustion. Our B-Xpander[™] protein improves current protocols for B cell stimulation by mimicking the natural interaction between CD40L and CD40 molecules on B cells.

B-Xpander[™] is a potent enhanced Multimeric human CD40L cytokine, for B cell activation, B cell expansion (proliferation) and cell therapy applications. In-house production using a proprietary protocol and a characterized and certified CHO cell line allows the supply of the most active Multimeric human CD40L proteins in GMP-like format.

B-Xpander[™] – B Cell Expansion (Proliferation)

B-Xpander[™] increases proliferation of human primary CD19⁺ B cells as efficiently as other grades of Multimeric human CD40L. Unlike most recombinant CD40L proteins on the market, our B-Xpander[™] and Multimeric CD40L proteins do not need an enhancer (antibody) to multimerize and to be active.

METHOD: Activity of B-Xpander[™] and 2 different research grades of Multimeric hCD40L is measured by proliferation of human primary CD19⁺ B cells. Cells (5 x 10⁴/well) are grown in RPMI medium, glutamine, 10% FBS, IL-4 (human) (10ng/ml), IL-21 (human) (10ng/ml) and different concentrations of B-Xpander[™] CD40L (human) (rec.) (Animal-Free) (#AG-40B-0010AF), CD40L (human) (multimeric) (rec.) (CSG; Certified Serum Grade) (#AG-40B-0010CSG), CD40L (human) (multimeric) (rec.) (#AG-40B-0010), or a control protein. Cell proliferation is quantified after 4 days using PMS/MTS (CellTiter 96[®] Aqueous One Solution Cell Proliferation Assay, Promega). Cells expanded by 10-fold after 4 days to reach 5 x 10⁵/well.

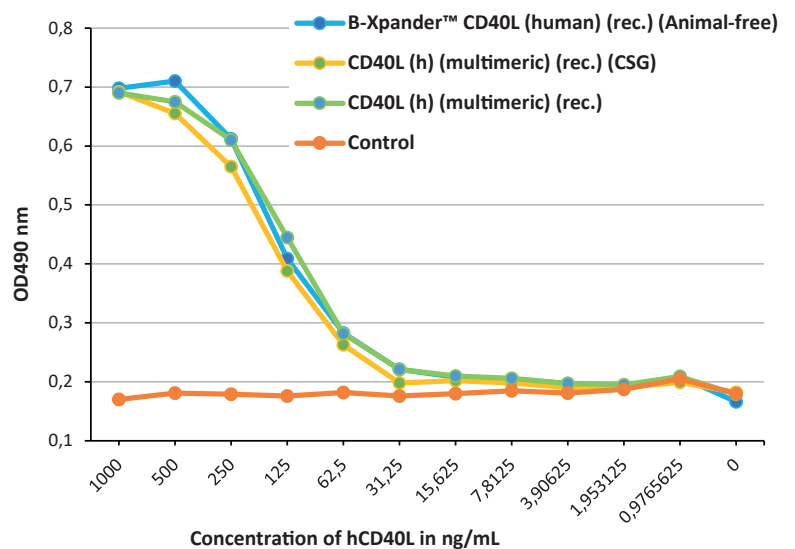


FIGURE: Measurement of B cell proliferation.

B-Xpander™ – B Cell Activation

- The multimeric structure of B-Xpander™ mimics the biological activity of membrane-bound human CD40L.
- B-Xpander™ is the most potent fusion protein alternative to activate human CD40.
- Using B-Xpander™, no enhancer (antibody) is required for multimerization and maximal activity.
- B-Xpander™ is an efficient B cell activation factor.

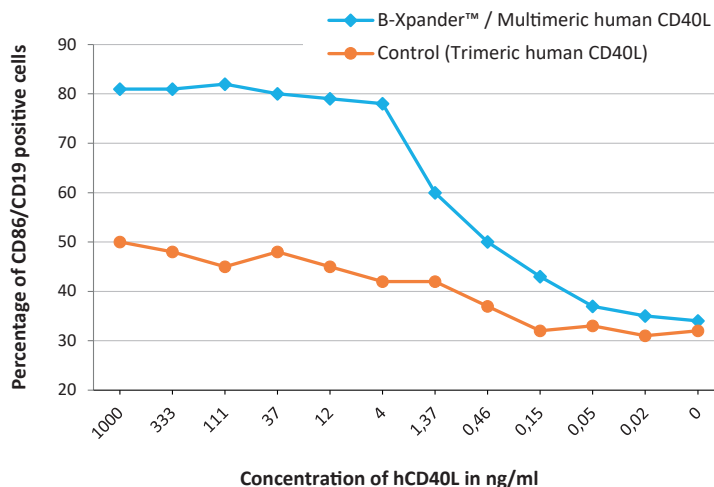


FIGURE: B-Xpander™ (Prod. No. AG-40B-0010AF) induces B cell activation.

METHOD: PBL cells were incubated in 96-well plates (2×10^5 cells/well in 100 μ l RPMI supplemented with 10% FCS) for 24 hours at 37°C with the indicated concentration of B-Xpander™ CD40L (human) (rec.) (Animal Free) (#AG-40B-0010AF), CD40L (human) (multimeric) (rec.) (#AG-40B-0010) or a trimeric human CD40L as a control. Cells were washed with PBS and stained with 2 μ l each CD86-PE and CD19-FITC in 50 μ l FACS buffer (PBS, 5% fetal calf serum, 0.02% azide) for 20 min. at 4°C in the dark. After two washes in FACS buffer, samples were then analyzed by flow cytometry.

B-Xpander™ Quality Features:

- **Production in characterized and certified CHO cell line (for GMP-grade manufacturing)**
- **High Bioactivity tested by ELISA/Cell-based Assays**
- **Verified Purity & Homogeneity by SEC**
- **Low Endotoxin Level**
- **Batch-to-Batch Consistency**
- **Animal-component free Production**
- **No cross-linking Reagents necessary**

B-Xpander™ CHO Cell Line:

The production of the B-Xpander™ uses a characterized and certified proprietary CHO cell line which has been tested on Master Cell Bank Biosafety and GMP compliance:

Biosafety and GMP Compliance:

- **Cell Growth, Sterility**
- **Mycoplasma (Compendial Method)**
- **Cell Line Identity DNA Barcoding**
- **Cell Profile Examination by TEM**
- **Extended S+L Assay (Test for Infectious Murine Xenotropic Retrovirus)**
- **Transcriptomic Analysis by Next Generation Sequencing (NGS)**

B-Xpander™ – The Multimeric human CD40L Structure

- B-Xpander™ / Multimeric human CD40L is a high activity fusion protein construct in which the human CD40L extracellular domain (aa 116-261) is artificially linked via the collagen domain of ACRP30.
- The ACRP30*headless* multimerization domain sequence from mouse Adiponectin allows the multimerization (10-12 x trimers) of the construct.
- This construct very effectively simulates the natural membrane-assisted aggregation of human CD40L *in vivo*.
- Multimeric Ligands developed by **AdipoGen Life Sciences** are ideal for *in vitro* & *ex vivo* applications.

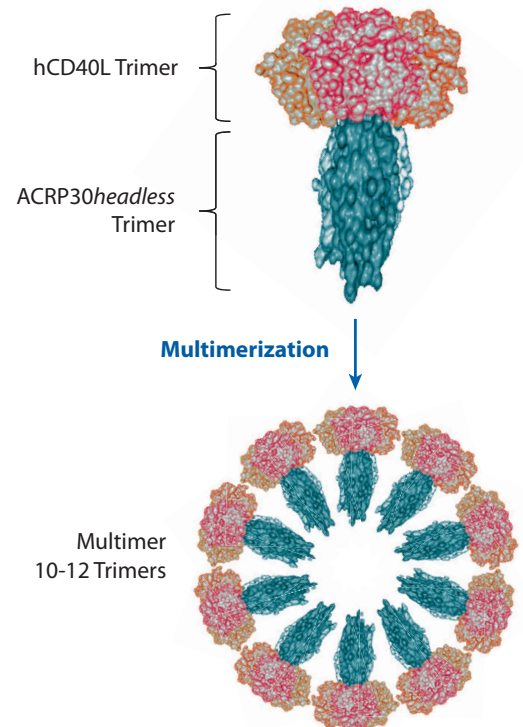


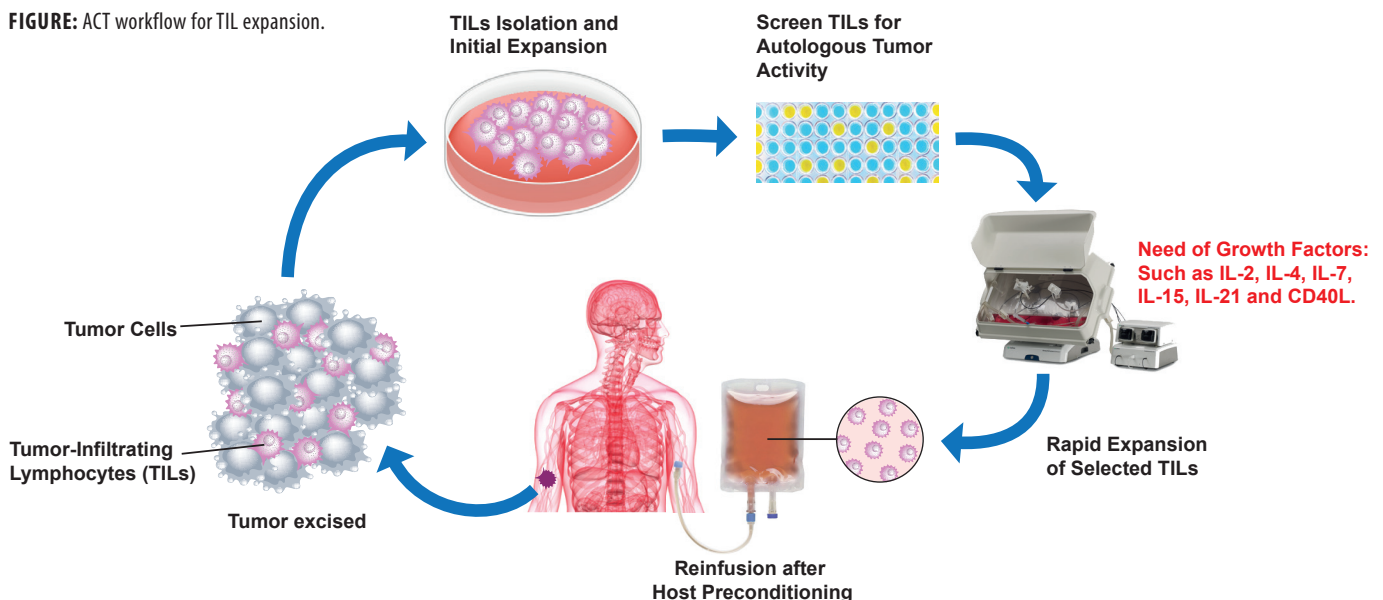
FIGURE: Structure of the B-Xpander™ or Multimeric human CD40L.

Adoptive Cell Therapy (ACT)

During ACT, T cells are isolated from a patient's blood or tumor tissue, expanded and activated *ex vivo*, and then re-infused back into the patient to target and eliminate cancer cells. T cells can be engineered to express chimeric antigen receptors (CARs) or T cell receptors (TCRs) that recognize tumor-specific antigens, or they can be expanded to enrich for tumor-infiltrating lymphocytes (TILs) that have natural tumor-specific reactivity. The patient's tumor cells can then interact with the enlarged TIL cells to screen effector TIL cells that can kill tumor cells.

Recently, it has been observed that B cells are a new key player in the Adoptive Cell Therapy. Higher presence of B cells infiltrated with TILs are associated with better TIL expansion and activation. B cells stimulated and amplified with B-Xpander™ and loaded with tumor-specific antigen improve tumor-specific TILs.

FIGURE: ACT workflow for TIL expansion.



B-Xpander™ – The Power of B Cell Expansion in Adoptive Cell Therapy

Embrace the future of immunotherapy research with Multimeric human CD40L-powered B cell expansion. Backed by rigorous scientific research and development, B-Xpander™, our cutting-edge Multimeric human CD40L-based B cell expansion tool, represents a major improvement in immunotherapy. During Adoptive Cell Transfer (ACT) immunotherapy, activation and expansion of B cells present with T cells in tumor-infiltrating lymphocytes (TILs) by B-Xpander™ leads to increased proliferation and greater abilities of T cells to fight cancer cells (Arnaud, et al. 2022).

LIT: Sensitive identification of neoantigens and cognate TCRs in human solid tumors: M. Arnaud, et al.; Nat. Biotechnol. 40, 656 (2022)

B-Xpander™ and Multimeric human CD40L Grades for your experiments:

Product Name	PID	Specifications / Applications
B-Xpander™		
B-Xpander™ CD40L (human) (rec.) (Animal Free)	AG-40B-0010AF	<ul style="list-style-type: none"> • Produced using animal component-free medium in characterized and certified CHO cells • GMP-like production • <i>Ex vivo</i> B Cell Expansion for Cell Therapy
B-Xpander™ CD40L (human) (rec.) (GMP-grade)	Custom Please Inquire	<ul style="list-style-type: none"> • GMP-Production outsourced to CRO • <i>Ex vivo</i> B Cell Expansion for Cell Therapy
Other Research Grades		
CD40L (human) (multimeric) (rec.)	AG-40B-0010	<ul style="list-style-type: none"> • Produced using standard serum/medium in CHO cells • <i>Ex vivo</i> B Cell Expansion for Research Use
CD40L (human) (multimeric) (rec.) (Certified Serum Grade)	AG-40B-0010CSG	<ul style="list-style-type: none"> • Produced using certified serum/medium in CHO cells for traceability • <i>Ex vivo</i> B Cell Expansion for Cell Therapy and Research Use

www.bxpander.com