Tools for BCMA Research

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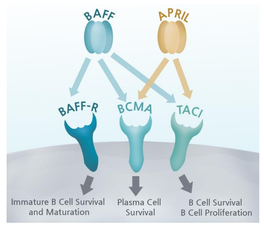
Introduction

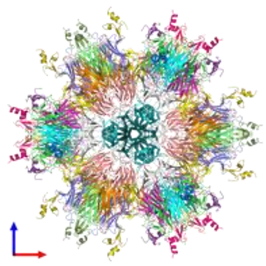
BCMA (B Cell Maturation Antigen), also known as TNFRSF17 (Tumor Necrosis Factor Receptor Super Family member 17), is a cell surface receptor preferentially expressed in mature B lymphocytes. It is activated by two ligands, APRIL and BAFF, which induce proliferation and participate in the regulation of B cell development and survival. BCMA plays a role in leukemia, lymphoma, and multiple myeloma. For example, upregulation of BCMA or APRIL is frequently observed and correlates with disease burden and worse prognosis in multiple myeloma. It has been shown that activation of BCMA by APRIL promotes tumor growth, chemoresistance and immunosuppression in the bone marrow microenvironment. Therefore, BCMA represents an attractive therapeutic target.



BCMA is activated by the TNF (ligand) superfamily, APRIL and BAFF, leading to NF-κB and MAPK8/JNK activation.

Several therapeutic approaches aim at engaging the BCMA pathway, two of which have been approved for the treatment of multiple myeloma, with many more undergoing clinical trials.

- ADC (Antibody-Drug Conjugate), including FDA-approved Belantamab mafodotin-blmf (BLENREP, Glaxosmithkline) composed of a humanized anti-BCMA monoclonal antibody conjugated to cytotoxic payload MMAF by a protease-resistant linker
- CAR-T cell therapy, including FDA-approved Idecabtagene vicleucel (Bluebird Bio)
- Anti-BCMA and anti-APRIL monoclonal antibodies
- BiTEs (Bi-specific Antibodies)

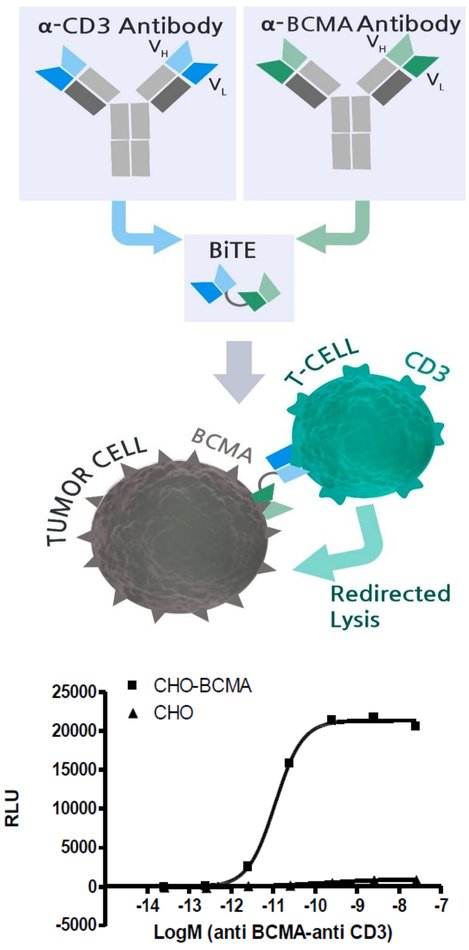


2.6 Å crystal structure of human soluble sTALL-1 and BCMA extracellular domain; PDB: 1OQD Liu *et al.* Nature (2003) 423: 49-56.

BiTE and TriTE Antibodies

Pharmaceutical biologics known as BiTEs (Bi-specific T cell Engagers) are multifunctional antibodies that bind to a cell surface tumor-specific antigen from one end, and from the other end to a T cell-specific molecule such as CD3, a T cell activator. By physically linking T cells and tumor cells, a BiTE engages T cell-mediated cytotoxicity toward the tumor cells.

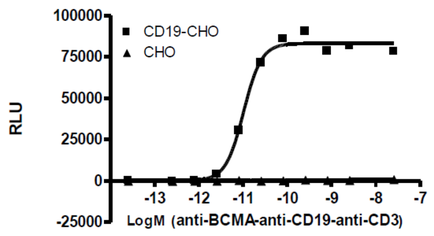
Our **anti-BCMA/anti-CD3** antibody is a recombinant human BiTE tested for specific activity against BCMA. This bispecific antibody binds simultaneously to BCMA on cancer cells and to CD3 on T cells, thus bringing the cells in close proximity. Binding of the antibody to CD3 activates the T cells and induces direct cytotoxicity against BCMA-expressing tumor cells.



Activation of NFAT Reporter Jurkat cells was measured using increasing amounts of anti-BCMA/anti-CD3 bispecific antibody in the presence of BCMA-CHO or CHO cells. EC50= 11.2 pM

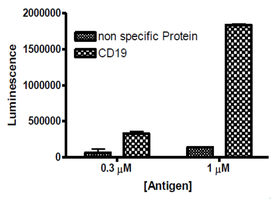
Antibodies	BPS #
Anti-BCMA Antibody (Single-Chain Variable Fragment)	100173
Anti-BCMA-Anti-CD3 Bispecific Antibody	100689
Anti-BCMA-Anti-CD19-Anti-CD3-His Trispecific Antibody	100761

TriTEs (Trispecific T cell Engagers) are engineered to bind three targets at the same time. The **anti-BCMA/anti-CD19/anti-CD3** antibody binds to tumor cell BCMA and CD19 (a B-cell marker that is expressed in many leukemia and lymphoma cells), and to T cell CD3. The multi-functionality of this trispecific antibody allows it to bind to BCMA and CD19 on the tumor cell and CD3 on T cells simultaneously. The binding event targets the tumor while providing co-stimulatory signals that promote T cell expansion and cytotoxicity against BCMA+ and CD19+ cancer cells.



Activation of CD3-expressing NFAT Reporter Jurkat cells was determined using increasing amounts of trispecific antibody in the presence of CD19-CHO or CHO cells. EC50= 10.3 pM

The trispecific antibody coated to an ELISA plate was incubated with CD19-biotin or with an unrelated biotinylated protein, followed by streptavidin-HRP and chemiluminescent detection.

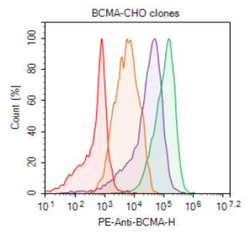


Services

BPS offers customized services for generating BiTE or TriTE constructs, producing specific antibodies, measuring the affinity of antibody binding to an antigen using ELISA-based assay or interferometry (Gator™, Probe Life), and assessing T cell activation using cell-based reporter assays. Over 200 cell lines expressing tumor antigens (cancer cells, CHO or HEK293 cells) are available, such as **CD19-CHO** cell line and several reporter cell lines. These include **Firefly Luciferase RPMI8226 Cells** as well as **NFAT and NF-κB reporter cell lines**, which are useful for the evaluation of BiTE or TriTE constructs in cell-based assays.

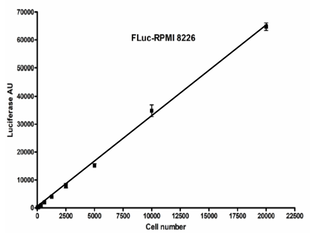
BCMA-expressing Cells

BCMA-CHO Cell Lines: Expression of BCMA was validated by flow cytometry using PE-conjugated anti-BCMA antibody (Biolegend #357504). High, medium, and low expression shown in green, purple and brown, respectively. Parental CHO cells: red.



Cell lines	BPS #
BCMA-CHO, high/low expression	79500
BCMA-Firefly Luciferase CHO	79724
BCMA-Gaussia Luciferase CHO	79830
BCMA-CD20-Firefly Luciferase CHO	78185
Parental Firefly Luciferase CHO	79725
RPMI8226-Firefly Luciferase	79834

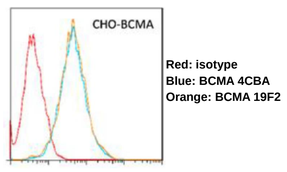
RPMI8226 Firefly Luciferase Cell Line: Human B cells originally isolated from a myeloma patient. These cells constitutively express BCMA and offer a **physiologically relevant platform** to evaluate anti-BCMA immunotherapies such as Chimeric Antigen Receptor (CAR) cells.
Luciferase activity is directly proportional to the number of cells.



Applications

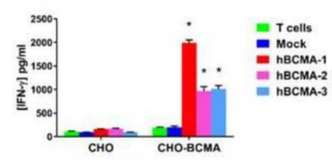
- Co-culture assays with immune cells targeting BCMA, including measurement of cytokine induction or cytotoxicity.
- *In vivo* quantification using animal imaging instruments: cell proliferation or survival studies. Measure the efficacy of BCMA-targeting therapeutics in mice.

BCMA-overexpressing CHO cells were used to validate a new monoclonal antibody for CAR-T cell therapy: Berahovich R. *et al*. CAR-T Cells Based on Novel BCMA Monoclonal Antibody Block Multiple Myeloma Cell Growth. *Cancers* (2018). *doi: 10.3390/cancers10090323. Figures used without modification under license CC BY 4.0*

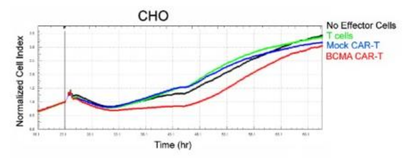


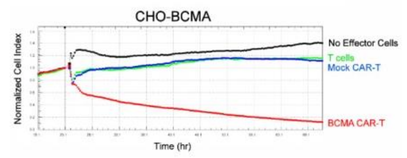
Red: isotype
Blue: BCMA 4CBA
Orange: BCMA 19F2

mAb 4C8A binds BCMA: mAb 4C8A, mAb 9F2, and mouse IgG1 isotype control were incubated with BCMA-CHO cells and detected by flow cytometry using PE-conjugated anti-mouse IgG.



IFN-γ production was quantified by ELISA in a real-time cytotoxicity assay.

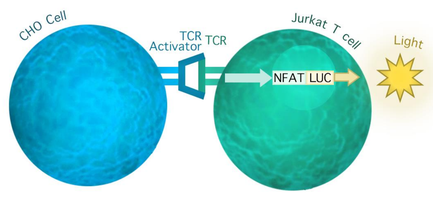




Anti-BCMA CAR-T cells killed CHO-BCMA cells in a real-time cytotoxicity assay. Anti-BCMA CAR-T cells, mock CAR-T cells and non-transduced T cells were added to monolayers of CHO cells and CHO-BCMA cells. The impedance, proportional to cell number of the monolayers, was monitored over time.

TCR-based Tools

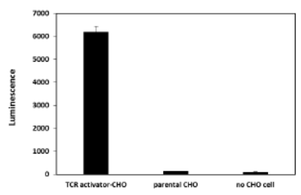
TCR Activator Cells



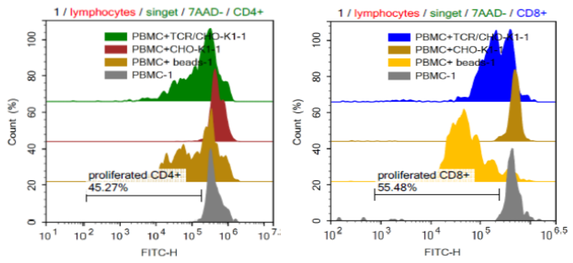
TCR Products
TCR CRISPR/Cas9 Lentivirus (integrating, non-integrating) and TCR Activator Lentivirus
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line
TCR Activator Recombinant Cell Lines; alone or with checkpoint regulators (PD-L1, PD-L2, CD155, HVEM)
TCR Activator Expression Kit (TCR and PD-L1 or PD-L2), mouse or human

Applications

- Activate T cells *in vitro.*
- Control for TCR activator/PD-L1, PD-L2, CD155 or HVEM.



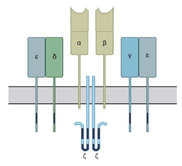
TCR activator CHO or parental CHO cells were co-cultured with NFAT Reporter-Jurkat cells. ONE-Step™ Luciferase reagent (#60690) was added to measure luciferase activity activated by TCR. Presence of the TCR activator cells induced luciferase activity in Jurkat cells.



TCR activator-CHO cells promote T cell proliferation: PBMCs were stained with CellTrace™ CFSE and co-cultured with TCR activator or parental CHO cells**, or** were activated with anti-CD3/CD28 beads for 72 hours. (Left) CD4+proliferation; (Right) CD8+ proliferation.

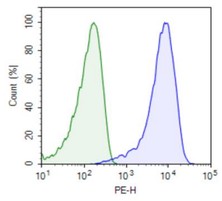
TCR Knockout Jurkat cells

CRISPR/Cas9 genome editing was used to remove TRAC (T-Cell Receptor α Constant) and TRBC1 (T-Cell Receptor β Constant) domains of TCRα/β chains from Jurkat cells constitutively expressing the firefly luciferase gene under the control of NFAT response elements. Loss of expression was confirmed by genomic sequencing and flow cytometry.



Applications

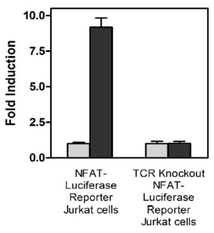
- Use as control in the generation of CAR-T cells.
- Determine CAR-T-specific cell killing.



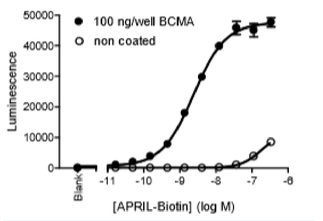
TCRα/β expression in TCR Knockout NFAT-Luciferase Reporter Jurkat cells. Flow cytometry analysis was performed using a PE-labeled anti-TCRα/β antibody. Parental cells are shown in blue, TCR Knockout cells in green.

Functional validation of TCR-KO cells: Parental or TCR-Knockout NFAT-luciferase Jurkat cells were incubated with or without 10 µg/ml soluble anti-CD3 for 5 hours at 37°C to stimulate TCR. ONE-Step Luciferase reagent (#60690) was added to measure luciferase activity resulting from TCR activation.

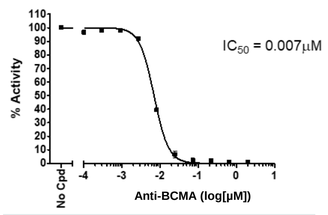
TCR was activated in the parental cells but not in the TCR-knockout cells.



BCMA-based Assays

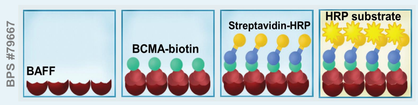


Binding of APRIL to BCMA was titrated by incubating increasing amounts of biotinylated APRIL to BCMA coated onto plates. Control consisted of non-coated wells.



Inhibition of BCMA:APRIL binding was measured by pre-incubating increasing concentrations of anti-BCMA Antibody prior to the addition of APRIL-biotin.

BAFF:BCMA Inhibitor Screening Assay Kit



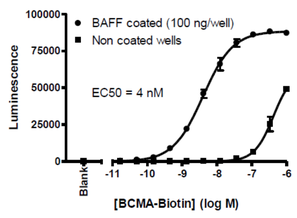
Assay Principle: BAFF is coated on a 96-well plate, incubated with BCMA-biotin, then with streptavidin-HRP. The HRP substrate is added last to produce chemiluminescence.

Alternatively, the BCMA:APRIL Inhibitor Screening Assay kit (#79722) uses unlabeled BCMA, which is coated onto the plate. APRIL-biotin is added to BCMA before incubation with streptavidin-HRP, followed by addition of the substrate and detection of the chemiluminescence.

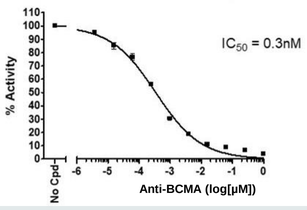
Both kits benefit from the high sensitivity of detection of biotin-labeled proteins by streptavidin-HRP.

Applications

- Screen inhibitors of the interaction between BCMA and its ligands.
- Validate antibody blocking activity/epitope
- Titrate blocking antibodies and inhibitors.



Binding of BCMA and BAFF was titrated by incubating increasing amounts of biotinylated BCMA to BAFF coated onto plates. Control consisted of non-coated wells.



Inhibition of BCMA:BAFF binding was measured by pre-incubating increasing concentrations of anti-BCMA Antibody (scFv) with BCMA-biotin, prior to addition to BAFF-coated plates.

Conclusion

Designing new therapeutic strategies requires the generation of appropriate tools, which can use considerable time and resources. BPS Bioscience has generated a varied portfolio of validated immuno-oncology tools to support drug development efforts as well as basic research projects, allowing the scientific community to focus on critical questions. These tools include anti-BCMA antibodies for BCMA detection, BiTEs and TriTEs, BCMA cell lines with or without reporter genes, biochemical assays, and BCMA lentivirus. Thus, BPS supports researchers at all phases of drug discovery to accelerate the development of new treatments for human diseases.