Enabling Research Tools for αβ and γδ T Cell-Based Immunotherapy

M. Samoudi, Z. Warburg, K. Skuster, S. Green, J. Bilakovics, Y. Zhang, H. Zhu, M. Kinbara
BPS Bioscience, San Diego, CA 92121

BACKGROUND

Significant progress has been made in T cell-based immunotherapy to fight cancer in recent decades, through advances such as naturally occurring or genetically engineered T cells to target cancer antigens and using multi-specific molecules that recruit immune cells to tumors, such as BiTE™ (bispecific T cell engager).

T cell lymphocytes are composed of two sub-populations, αβ T cells and γδ T cells, which are distinguished by the expression of either an αβ TCR (T Cell Receptor) or a γδ TCR, respectively. While they share some structural similarities, these populations differ in important aspects. αβ T cells are the predominant subset of T cells in the blood circulation and recognize antigens presented by MHC (Major Histocompatibility Complex) molecules. γδ T cells are less abundant and recognize antigens independently of MHC presentation. Both αβ T cells and γδ T cells contribute to cell cytotoxicity through distinct mechanisms to target and eliminate infected or abnormal cells. However, transplanted γδ T cells have a lower risk of causing GvHD (Graft-versus-Host Disease) and induce cytotoxicity against a wide range of tumor types.

Here, we demonstrate the successful isolation, activation, and expansion of αβ T cells and γδ T cells. Functional validations by two different BPS Bioscience's cytotoxicity assays (flow cytometry and luciferase-based assays) showed that T cells can effectively kill cancer cells *in vitro*. Furthermore, we demonstrate BPS Bioscience's T Cell Engagers can activate T cells to kill tumor cells in our model *in vitro* assays, while expanded γδ T cells kill target cells without needing antibodies.

PAN T CELL ISOLATION, ACTIVATION, AND EXPANSION

Fast, simple, and efficient isolation of pan-T cells from normal human PBMCs (Peripheral Blood Mononuclear Cells) was performed using specific antibody-conjugated magnetic beads. The isolated cells were then activated and expanded for analysis and functional validation.

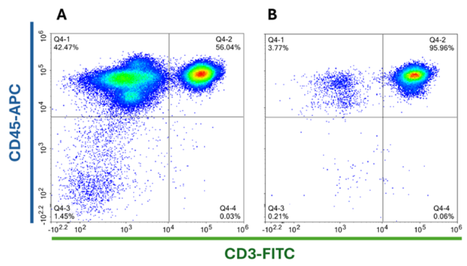

Figure 1: Analysis of human T cells isolated from PBMCs.

Comparison of PBMCs and untouched T cells isolated from PBMC using BPS Bioscience Human T Cell Isolation Kit (BPS Bioscience #82288). Flow cytometry analysis was performed before and after T cell isolation using an APC-labeled CD45 antibody (BioLegend #304011) and a FITC-labeled CD3 antibody (BioLegend #300406). ***A)*** *represents the starting PBMC cells.* ***B)*** *represents the population remaining after negative magnetic selection. Each plot was gated on FSC-A/SSC-A (to remove debris from analysis) and FSC-H/FSC-A (singlet discrimination).*

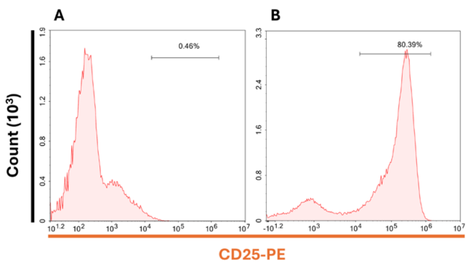

Figure 2: T cell activation assessment in isolated human T cells.

Isolated human T cells were activated with Human T Cell Activation Reagent (BPS Bioscience #82283) and cultured in complete T Cell Expansion Media (TCellM™, BPS Bioscience #78753) supplemented with 200 IU/ml of human interleukin-2 recombinant protein (BPS Bioscience #90184). T cells activation was measured by flow cytometry using a PE-labeled anti-human CD25 antibody (BioLegend #302606) on ***A)*** *day 0 and* ***B)*** *day 3. The y-axis indicates the cells count, while the x-axis represents PE intensity.*

T CELL ENGAGERS

T cell engagers definition

Multi-specific T cell engagers are a promising emerging therapeutic modality designed to recruit immune cells and activate their cytotoxicity against tumor cells. Research and development in this field continues to advance, contributing to the ongoing evolution of personalized and precise cancer treatment strategies. Defined, recombinant bispecific engagers can be used as control when designing and optimizing new co-culture assays, and as internal standard when comparing new candidates.

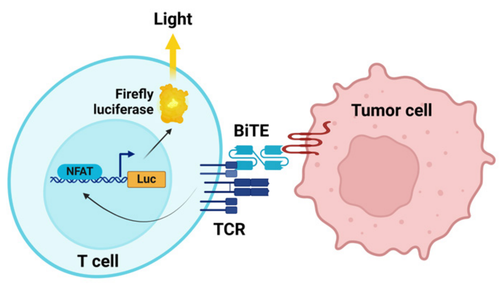

Figure 3: Illustration of Bispecific T cell engagers (BiTEs) mechanism of action.

T cell activation assays using NFAT reporter Jurkat cell lines and various T cell engagers

Various BPS Bioscience's target cell lines expressing an antigen of interest were co-cultured with NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #60621) in the presence of increasing concentrations of bispecific T cell engager molecules. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690).

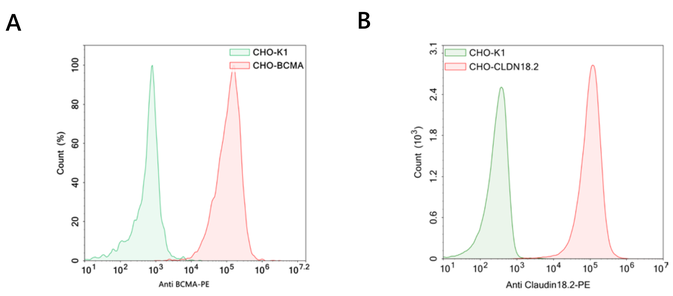

Figure 4: target cell lines expressing antigen of interest.

Cell surface expression of ***A)*** *BCMA in BCMA CHO Cell Line (BPS Bioscience #79500) and* ***B)*** *Claudin18.2 in Claudin18.2 CHO Cell Line (BPS Bioscience #78533-H) confirmed by flow cytometry.*

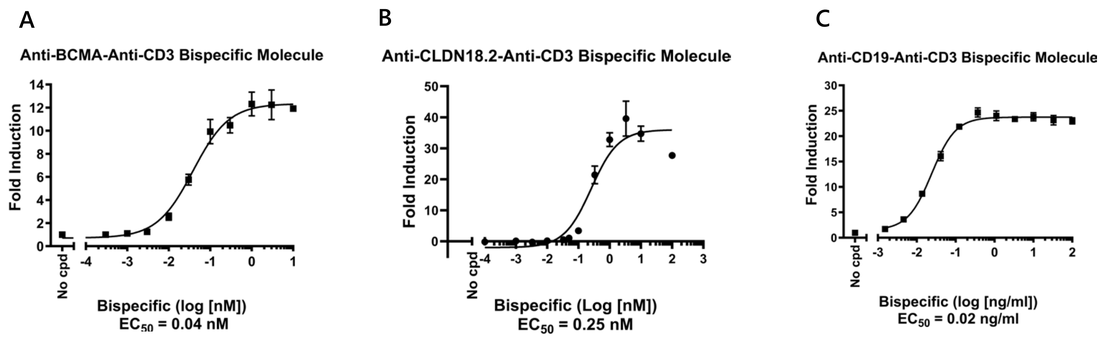

Figure 5: Dose-response curve of BPS Bioscience's T cell engagers in NFAT Reporter Jurkat cells co-cultured with different target cell lines.

A) *Anti-BCMA-Anti-CD3 bispecific antibody (BPS Bioscience #100689) was used with BCMA CHO Cell Line (BPS Bioscience #79500).* ***B)*** *Anti-Claudin18.2-Anti-CD3 bispecific antibody (BPS Bioscience #101541) was used with Claudin18.2 CHO cell line (BPS Bioscience #78533-H) or control CHO-K1 cells.* ***C)*** *Anti-CD19-Anti-CD3 bispecific molecule (BPS Bioscience #100441) was used with Raji cells, which are CD19-positive.*

CYTOTOXICITY ASSAYS

Flow cytometry-based cytotoxicity assay

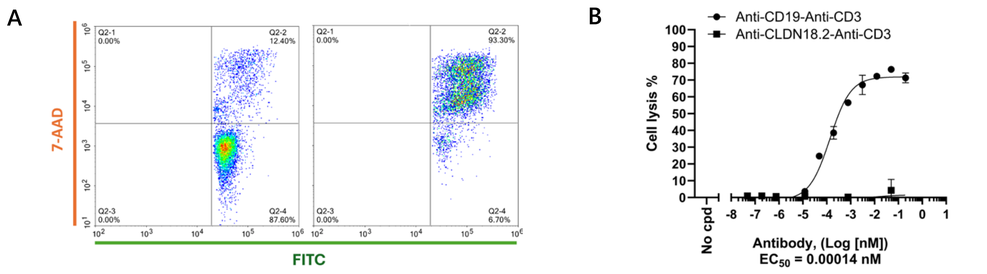

Figure 6: Flow cytometry assessment of in vitro cytotoxicity using PBMC Cytotoxicity Bioassay Kit, CFSE, 7-AAD (BPS Bioscience #82173).

A) *CFSE-labeled NALM6 cells were incubated with PBMCs at 10:1 (Effector:Target) cell ratio without (left) or with (right) 0.2 nM Anti-CD19-Anti-CD3 Bispecific Molecule (BPS Bioscience #100441) for 24 hours, stained with 7-AAD for 15 minutes and analyzed by flow cytometry. The CFSE⁺/7-AAD⁺ population corresponds to the dying target cells (Q2-2 of the density plots).* ***B)*** *PBMCs and CFSE-labeled NALM-6 cells were combined at a 10:1 ratio. The cells were incubated with a dilution series of Anti-CD19-Anti-CD3 Bispecific Molecule (BPS Bioscience #100441) or the control Anti-CLDN18.2-Anti-CD3 Bispecific Antibody (BPS Bioscience #101541) for 24 hours in a humidified 37°C incubator with 5% CO_2. Cells were stained with 7-AAD and analyzed by flow cytometry. The percentage of dying target cells (CFSE⁺/7-AAD⁺ population) in each well was calculated. The raw cell death percentage data were fitted to a sigmoidal three-parameter curve using GraphPad Prism® software.*

Luciferase-based cytotoxicity assay

A) Luciferase NALM6 cells (suspension)

B) Luciferase BCMA CHO cells (adherent)

Figure 7: Assessment of in vitro cytotoxicity using BPS Bioscience Luciferase-Based Assay.

PBMCs (BPS Bioscience #79059) were combined at a 10:1 (effector:target) ratio with ***A)*** *Luciferase NALM6 (BPS Bioscience #78494) or* ***B)*** *BCMA Luciferase CHO Cells (BPS Bioscience #79724) and incubated with a dilution series of Anti-CD19-Anti-CD3 Bispecific Molecule (BPS Bioscience #100441) or Anti-BCMA-Anti-CD3 Bispecific Molecule (BPS Bioscience #100689) for 24 hours. Luciferase activity was measured with ONE-Step™ Luciferase reagent (BPS Bioscience #60690). The raw luminescence data were fitted to a sigmoidal three-parameter curve using GraphPad Prism® software for EC_{50} determination.*

THE EMERGING ROLES OF γδ T CELLS IN CANCER IMMUNOTHERAPY

Antitumor activity of γδ T cells

As cancer therapies, γδ T cells are especially attractive because they directly recognize molecular "indicators of stress" in tumor cells, against which they exert potent cytolytic activity, rather than a single specific antigen on a tissue type. This results in little or no toxicity of γδ T cells towards healthy cells that may express the same antigens as the cancer cells. γδ T cells sense dysfunctional cells by recognizing tumor-associated metabolic byproducts such as butyrophilins (BTNs) on tumor cells or proteins like MHC class I-related chain A or B (respectively MICA or MICB) upregulated on stressed cells. Engagement of these ligands by their receptors on γδ T cells activates direct killing mechanisms via granzyme B and perforin rapidly without prior exposure to pathogen- or tumor-associated antigens. Vγ9Vδ2 T cells are the predominant subpopulation of γδ T cells in human peripheral blood.

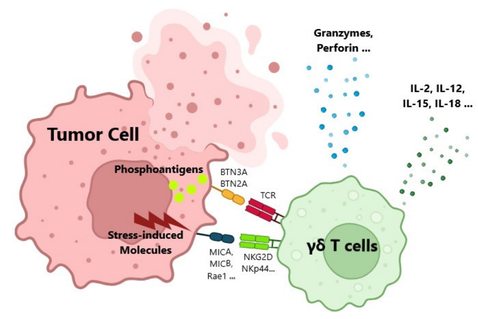

Figure 8: Illustration of γδ T cell anti-tumor activity mechanism of action.

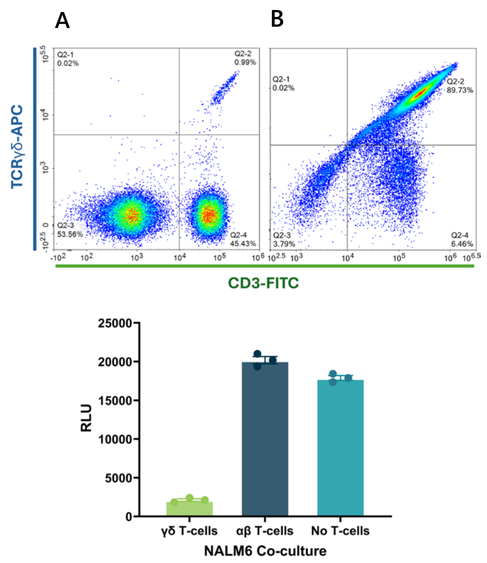

Figure 9: Flow cytometry analysis of human primary γδ T cell expansion using human γδ T cells expansion kit (BPS Bioscience #82551).

PBMCs (BPS Bioscience #79059) were stimulated with the activation reagent from the kit and human interleukin-2 recombinant protein (BPS Bioscience #90184), and expanded for 12 days in complete T Cell Expansion Medium (TCellM™ supplemented with 1000 IU/ml of IL-2). αβ T cells and B cells were depleted on day 6 using immunomagnetic cell separation by Negative Selection. Expansion fold change was calculated >1000. The purity of the expanded Vγ9Vδ2 T cells was determined by flow cytometry using an APC-labeled anti-human TCR Vγ9 Antibody (BioLegend #331310) and FITC-labeled anti-human CD3 Antibody (BioLegend #300406). Representative flow cytometry plots show the percentage of Vγ9Vδ2 T cells (CD3⁺ TCR Vγ9⁺) and αβ T cells (CD3⁺ TCR Vγ9⁻) in expanded cells at ***A)*** *day 0 and* ***B)*** *day 12.*

Figure 10: Luciferase-based cytotoxicity assay of expanded γδ T cells using Luciferase NALM6 cells as the target cells.

Expanded γδ T cells and Firefly Luciferase NALM6 cells (BPS Bioscience #78494) were combined at a 10:1 ratio in a 96-well white, clear bottom plate. The cells were incubated in a humidified 37°C incubator with 5% CO_2 for 24 hours. Luciferase NALM6 cells were also co-cultured with activated αβ T cell and without T cells as negative controls. Luciferase activity was measured with ONE-Step™ Luciferase reagent (BPS Bioscience #60690).

CONCLUSION

T cell-based cancer immunotherapy leverages the antitumor activity of T cells to treat certain blood cancers. In this modality, T cells are redirected against tumor cells by identifying and targeting cancer-specific or overexpressed self-antigens. αβ T cells, the predominant T cell subset in blood circulation, recognize antigens presented by MHC molecules, while γδ T cells are less abundant and can directly recognize and kill cancer cells independently of MHC presentation, which makes them a highly promising effector cell for cancer immunotherapy. This presentation highlighted the tools and products developed at BPS Bioscience to accelerate research in T cell-based therapeutics. We demonstrated how BPS Bioscience's expanded γδ T cells could effectively recognize and kill cancer cells in an *in vitro* assay. The presentation further described and validated different cytotoxicity assay kits developed at BPS Bioscience for being used in functional T cell-based assays. We also illustrated some of the BPS Bioscience's bispecific T cell engagers that could successfully activate our NFAT-Jurkat *in vitro* cell model which can facilitate the development of new cancer immunotherapies.

References

1) Morath A, and Schamel WW, 2020, *Journal of Leukocyte Biology* 107(6): 1045-55.

2) Fu M, *et al.*, 2019, *Front. Immunol* 10.1396.

Illustrations in Figure 3 and Figure 8 created with BioRender.com