#235

Novel biparatopic TIM3 antibody effectively blocks multiple inherent ligands and activates anti-tumor immunity

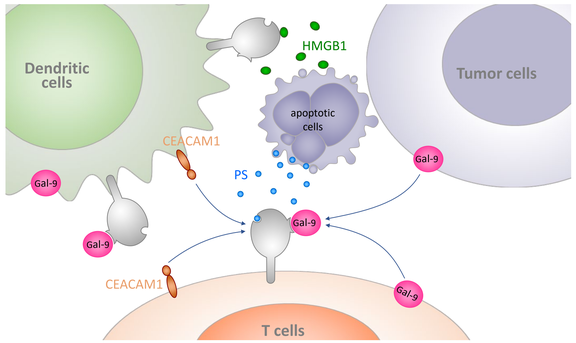
Yuji Mishima*, Kanto Nakajima, Mamoru Shiraishi, Haruka Matsumura, Takahiko Aramaki, Noriko Matsumoto, and Norihiro Nakamura

BrightPath Biotherapeutics Co., Ltd., Kawasaki Research Laboratory, Kawasaki, Japan

*Email contact:
mishima_y@brightpathbio.com

Background

T cell immunoglobulin and mucin domain-containing protein 3 (**TIM3**) is a part of modules expressed on dysfunctional or exhausted T cells as well as dendritic cells. TIM3 has been reported to have multiple ligands including galectin-9 (**Gal-9**), phosphatidylserine (**PS**), CEACAM-1 and HMGB1, which bind to different regions on the extracellular domain of TIM3. Most of the TIM3 antibodies developed to date are intended to inhibit PS that binds to the pocket in TIM3 immunoglobulin V domain. Gal-9 binds to carbohydrate motifs on the opposite side of PS-binding site in immunoglobulin V domain and thereby induces cell death in TIM3$^+$ T cells. We report herein novel antibodies that block TIM3 binding to multiple ligands including these two important ligands simultaneously.



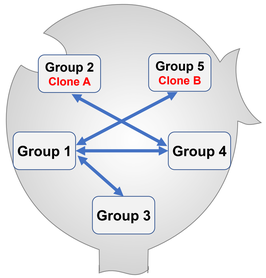
Methods

Anti-TIM3 antibodies were generated by immunizing mice with a purified recombinant human TIM3 protein and TIM3-expressing mammalian cell line. Phage display libraries were constructed using cDNAs of splenocytes and lymph node cells of the immunized mice, then subjected to the biopanning using recombinant TIM3 proteins.
After analyzing specificities and affinities to the TIM3 protein, scFvs obtained were classified by epitope bin and inhibitory effects on TIM3 binding to the multiple ligands. The scFvs were converted to scFv-Fc to generate biparatopic antibodies (BpAbs).

Results

Configuration of biparatopic TIM3 antibody

(A) Epitope binning of TIM3 antibodies

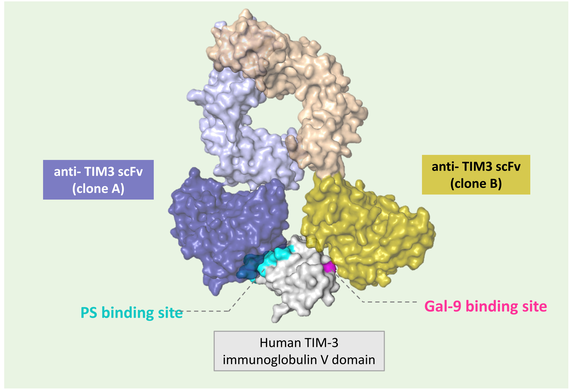


(B) Affinity of biparatopic antibody to recombinant TIM3

Homodimeric Ab (scFv-Fc)	ka (1/Ms)	kd (1/s)	KD (M)
Clone A	4.63E+05	6.82E-03	1.47E-08
Clone B	7.58E+04	2.43E-02	3.20E-07
Biparatopic Ab (scFv-Fc)	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**
BpAb A-B (clone A & B)	4.18E+05	1.16E-04	2.78E-10

(A) **Epitope classification of TIM3 antibodies**. The antibody groups connected by arrows do not compete with each other in binding to TIM3.
(B) The biparatopic antibody has increased binding affinity to recombinant TIM3 molecule with a significant reduction in the dissociation rate constants as compared to the original homodimeric antibodies.

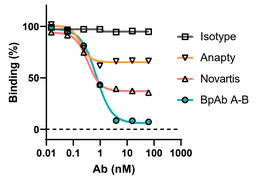
Biparatopic antibody strongly blocks the interaction of TIM3 to both galectin-9 and PS



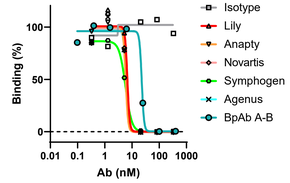
Expected binding form of biparatopic antibody; The epitope of scFv of TIM3 antibody clone A (shown in blue) was confirmed by analyzing crystal structure of scFv-TIM-3 complex. The structure of scFv of clone B (shown in yellow) was predicted from the primary amino acid sequence in silico.

Biparatopic antibody strongly blocks Galectin-9 binding to TIM3 molecules

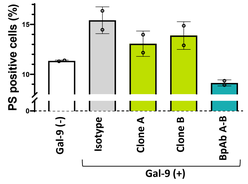
(A) Gal-9 binding inhibition



(B) PS binding inhibition



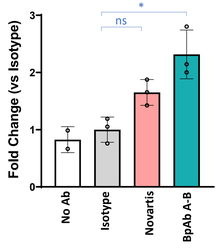
(C) Gal-9 induced apoptosis inhibition



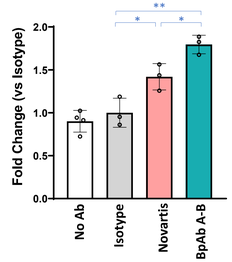
(A) The biparatopic antibody inhibited the binding between human TIM3 and human Gal-9 more potently than the pre-developed TIM3 antibodies.
(B) The biparatopic antibody completely blocked binding between PS and TIM3 at the similar concentration as the previously developed antibodies.
(C) The biparatopic TIM3 antibody completely inhibited exogenous Gal-9-induced apoptosis to primary CD4+T cells. Data are shown as mean of two independent donors.

Biparatopic TIM3 antibody increases Interferon-γ producing CD8-positive T cells

IFNγ+ / CD8+ T cells



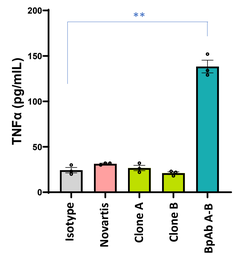
CD8+ T cells



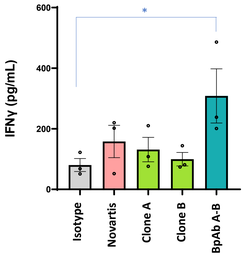
SEB stimulation assay : Effect of the TIM3 biparotopic antibody on antigen stimulation by SEB was evaluated. In the presence of the biparatopic TIM3 antibody, the total CD8+ T cells number was significantly increased compared to isotype control (1.80-fold increased). In addition, IFNγ-positive CD8+ T cell number was further increased up to 2.32-fold compared to isotype control. (* p<0.05, ** P<0.01)

Biparatopic TIM3 antibody promotes cytokine production from antigen-stimulated T cells

TNFα production



INFγ production



Cytokine production of antigen stimulated PBMCs: PBMCs were stimulated with CMV antigen peptides in the presence of test antibodies. The amount of TNFα and IFNγ produced in the culture supernatant was quantified. Only the biparatopic antibody significantly enhanced cytokine production of both TNFα and IFNγ. (* p<0.05, ** P<0.01)

Conclusion

- This study demonstrates the successful development of a novel biparatopic antibody against human TIM3.
- The biparatopic antibody has a higher binding affinity to human TIM3 and completely blocked both Gal-9 and PS ligand binding to TIM3 molecules.
- The biparatopic antibody increased the number of IFNγ producing CD8+T cells by antigen stimulation and promoted cytokine production of TNFα and IFNγ.
- The biparatopic antibody shows the advantages over conventional TIM3 antibodies and potentially manipulated for the development of human monoclonal antibodies for therapeutic treatment of cancer.

Disclosure

Mishima Y.: Employee of BrightPath Biotherapeutics

BrightPath Biotherapeutics