Bispecific antibodies against TIM-3 and CD39 induce anti-tumor efficacy and immune response by blocking multiple suppressive mechanisms.

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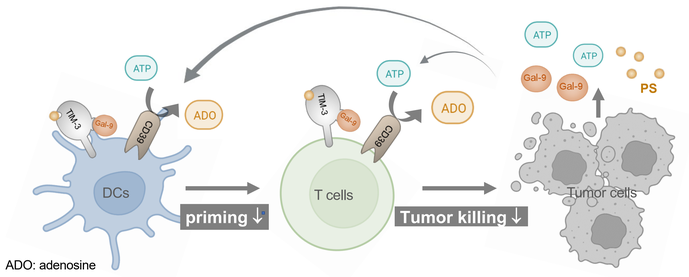
Background

Multiple immuno-suppressive mechanisms in tumor have been suggested to attenuate anti-tumor immunity. Here we developed bispecific antibodies against TIM-3 and CD39, negative regulators of anti-tumor immunity, in order to overcome the immune-suppression in tumor microenvironment.

BP1210 is a humanized TIM-3 biparatopic antibody (BpAb) that blocks the ligand binding of TIM-3 including phosphatidylserine and galectin-9. Monoclonal TIM-3 antibodies in clinical trials inhibit only some, but not all ligands. Thus, BP1210 potentially improves the efficacy of TIM-3 blockade.

BP1212 is a humanized bispecific antibody (BsAb) against TIM-3 and CD39. The expression of TIM-3 and CD39 are simultaneously induced in exhausted T cells and DCs and suppress the anti-tumor activity of T cells and DCs. By inhibiting CD39 in combination with TIM-3 blockade, BP1212 synergically enhances the anti-tumor immunity.

BP1210 and BP1212 that block TIM-3- and adenosine-signaling, provide new and effective therapeutic approaches.



ADO: adenosine

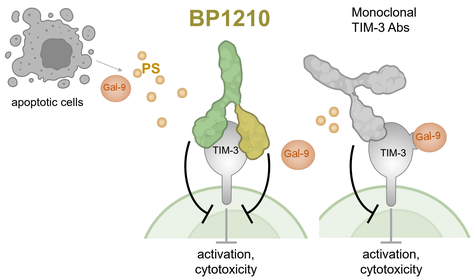
Conclusion

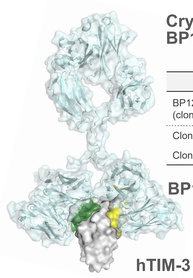
- **BP1210** completely inhibits the ligand-binding of both galectin-9 and phosphatidylserine. BP1210 enhances the antigen-dependent activation of T cells, and anti-tumor activity with higher potency than the reference antibody.
- **BP1212** binds and blocks CD39 and TIM-3 simultaneously, resulting in synergistic effects of TIM-3 and CD39 inhibition on antigen-stimulated CD8⁺ T cells.
- **BP1210 and BP1212** enhance T cell immunity by ameliorating the immuno-suppressive tumor microenvironment and provide the advantages over conventional TIM-3 and CD39 antibodies in cancer immune therapy.

Disclosure

Authors are employees of BrightPath Biotherapeutics

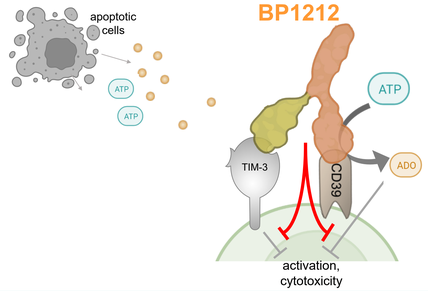
BP1210, a humanized biparatopic TIM-3 antibody





	Ka (1/Ms)	Kd (1/s)	KD (M)
BP1210 (clone A & B)	3.03E+05	2.23E-04	7.34E-10
CloneA (dimer)	2.34E+05	1.44E-03	6.13E-09
CloneB (dimer)	8.87E+04	1.41E-02	1.59E-07

BP1212, a humanized bispecific antibody for TIM-3 and CD39

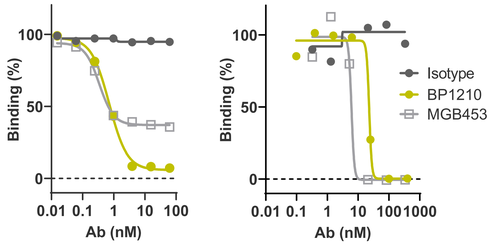


	hCD39⁺ cells	hTIM-3⁺ cells	hCD39⁺ hTIM-3⁺ cells
	KD (M)	KD (M)	KD (M)
BP1212 (TIM-3 x CD39)	17.56E-9	5.67E-9	8.31E-9
anti-TIM3 (dimer)	N.D.	1.97E-9	2.75E-9
anti-CD39 (dimer)	7.04E-9	N.D.	16.84E-9

N.D. not detectable

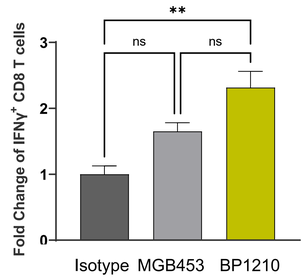
Inhibition of the ligand-binding

(A) inhibition of Gal-9 binding (B) Inhibition of PS binding



(A) Inhibition of Gal-9 binding to TIM-3 by the antibodies was quantified by ELISA.
(B) Inhibition of PS binding to human recombinant TIM-3 by the antibodies was analyzed by FACS.

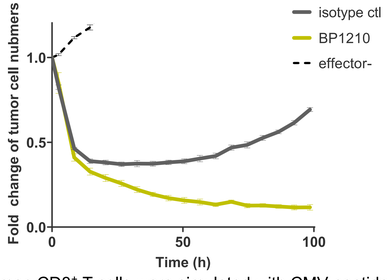
BP1210 Enhances IFNγ-producing T cells



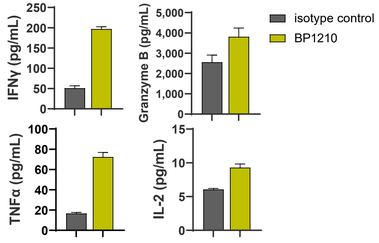
Human PBMCs were stimulated with SEB (Staph enterotoxin B) for 6 days in the presence of antibodies, then the IFNg⁺ CD8⁺ T cells were quantified by FACS. (** p<0.01, One-way ANOVA and Tukey's).

Enhanced cytotoxic activity of CTLs

(A) Growth rate of tumor cells



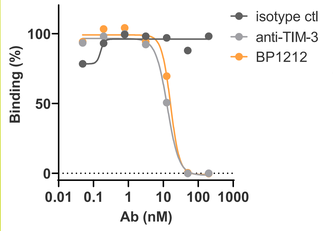
(B) Cytokine production



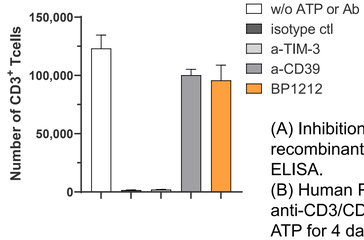
Human CD8⁺ T cells were simulated with CMV-peptide, then co-cultured with colon cancer cell line, SW620 expressing CMV pp65 antigen and RFP. HLA-types of PBMCs and SW620 are A02. (A) The number of RFP⁺ tumor cells were quantified under imaging analyzer. (B) Cytokines were quantified at 48hrs.

Inhibition of TIM-3 and CD39 functions

(A) Inhibition of PS binding

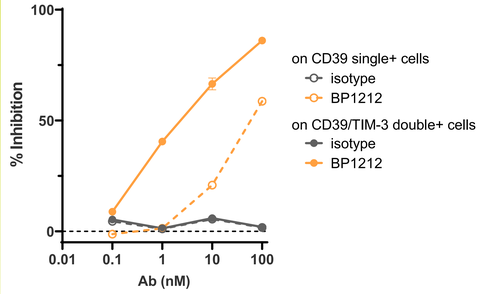


(B) Proliferation of activated-T cells exposed to ATP



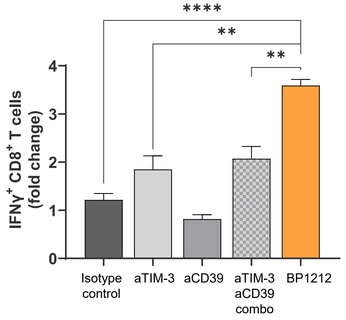
(A) Inhibition of PS binding to human recombinant TIM-3 was estimated using ELISA.
(B) Human PBMCs were stimulated with anti-CD3/CD28 beads in the presence of ATP for 4 days, then analyzed by FACS.

Enhanced inhibition of CD39 by BP1212



CD39 or CD39/TIM3 co-expressing cells were incubated with ATP in the presence of antibodies. The enzyme activity of CD39 was analyzed by quantifying the remaining ATP in the supernatant.

BP1212 Enhances the expansion of IFNγ-producing T cells



PBMCs were stimulated with SEB in the presence of antibodies for 6 days, then IFNγ⁺ CD8⁺ T cells were quantified by FACS. **p<0.01, ****p<0.001