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A novel therapeutic antibody against CD73 that ameliorates the tumor microenvironment and improves the efficacy of cancer immunotherapy

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Introduction

CD73, also known as ecto-5'-nucleotidase (NT5E), is a GPI-anchored cell surface enzyme expressed on certain types of immune cells and tumor cells. This enzyme catalyzes the hydrolytic conversion of extracellular adenosine monophosphate (AMP) to adenosine (Ado) that suppresses tumor-infiltrating immune cells via the adenosine receptors, allowing tumors to escape the immune system. Here, we introduce a novel anti-human CD73 antibody called BP1200 that strongly blocks the generation of Ado by CD73 and thereby reverses immune suppression in tumors.

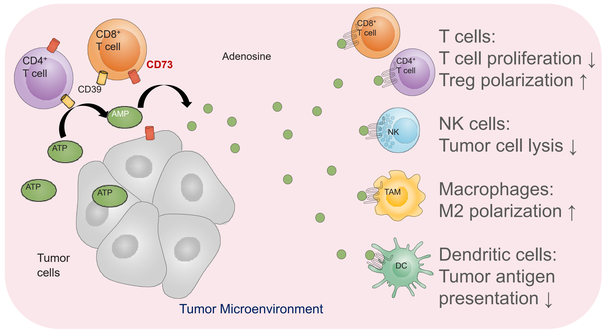
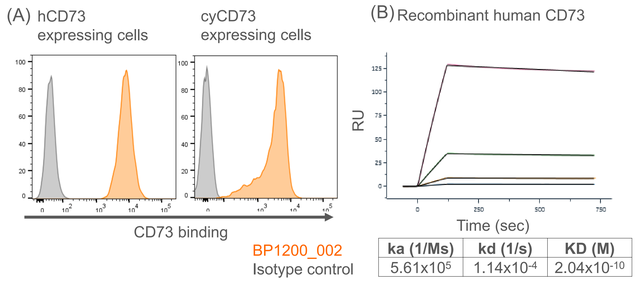


Figure 1. Ado generated by CD39 and CD73 suppresses immune cells, such as T cells, NK cells, macrophages and dendritic cells in TME.

Result

BP1200 binds human and cynomolgus CD73 with high affinity.



ka (1/Ms)	kd (1/s)	KD (M)
5.61×10^{5}	1.14×10^{-4}	2.04×10^{-10}

Figure 2. Binding of BP1200_002 to human and cynomolgus CD73 expressed on tumor or 293 cells(A) and the kinetics to a recombinant human CD73 (B).

BP1200 inhibits the activities of CD73 on the cell membrane and soluble CD73 without hook effect.

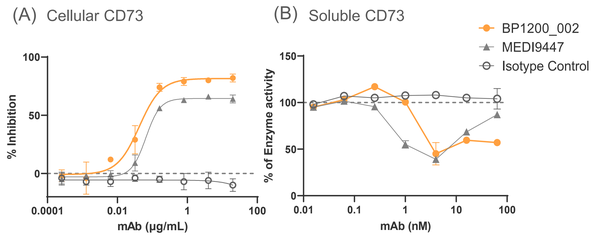
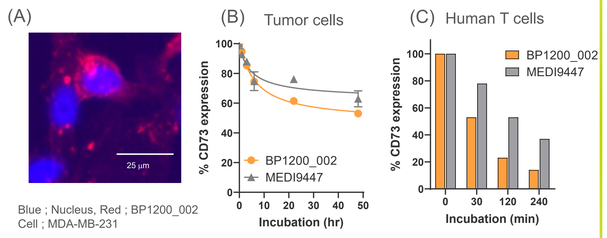


Figure 3. The CD73-expressing MDA-MB-231 cells (A) or recombinant human CD73 protein(B) were incubated with AMP in the presence of antibodies. The enzyme activity was analyzed by quantifying the remaining AMP in the supernatant. MEDI9447 is in-house produced analogues.

BP1200 internalizes and depletes cell surface CD73.



Blue ; Nucleus, Red ; BP1200_002
Cell ; MDA-MB-231

Figure 4. Internalization of CD73 was observed using microscopy (A). BP1200_002 downregulates CD73 on the cell surface of MDA-MB-231(B) and human T cells (C).

BP1200 enhances the proliferation of T cells in the presence of ATP.

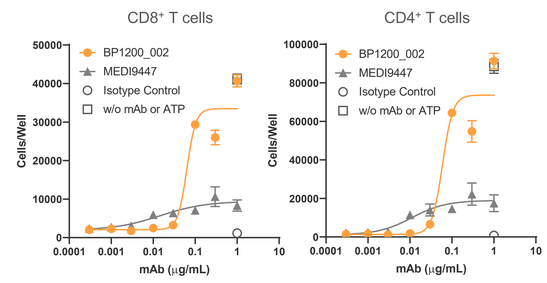


Figure 5. Human PBMC were stimulated with anti-CD3/CD28 beads in the presence of ATP. After 96h culture, the number of T cells were determined using flow cytometry.

BP1200 augments the cytotoxic activity of T cells.

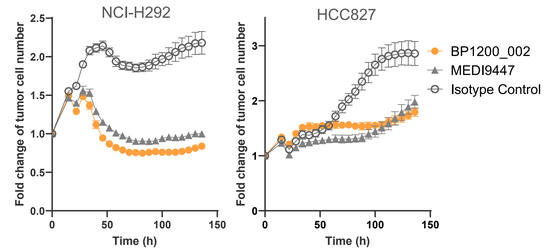


Figure 6. Human PBMC were co-cultured with tumor cells in the presence of ATP and antibodies. The tumor cells were quantified using imaging analyzer. Both NCI-H292 and HCC827 are human lung cancer cell lines expressing CD73 on the cell surface. Data are presented as means ±SEM.

The combination therapy of BP1200 with ICB leads tumor regression with increasing the number of TILs.

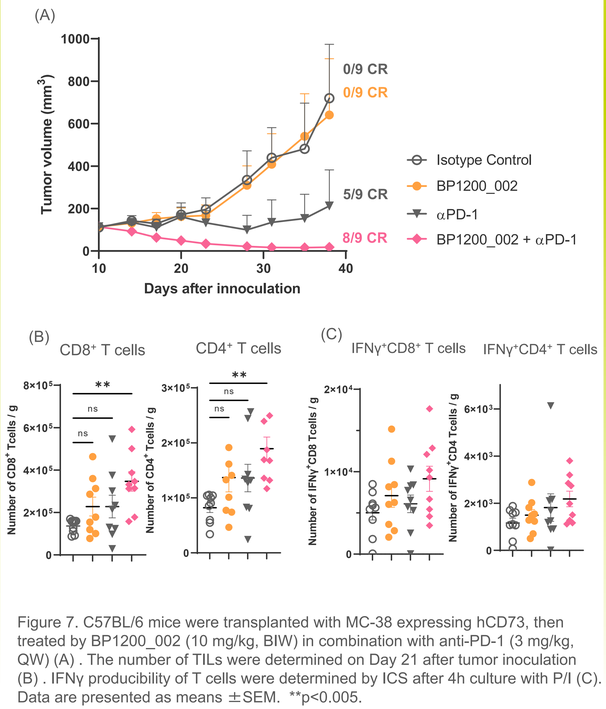


Figure 7. C57BL/6 mice were transplanted with MC-38 expressing hCD73, then treated by BP1200_002 (10 mg/kg, BIW) in combination with anti-PD-1 (3 mg/kg, QW) (A) . The number of TILs were determined on Day 21 after tumor inoculation (B) . IFNγ producibility of T cells were determined by ICS after 4h culture with P/I (C). Data are presented as means ±SEM. **p<0.005.

The combination therapy of BP1200 with ICB induces tumor-specific immunity in rechallenge model.

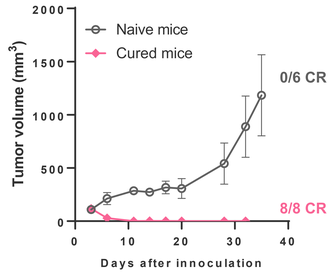


Figure 8. Cured mice treated with combination therapy of BP1200-002 and anti-PD1 were rechallenged with the MC-38 expressing hCD73 cells. Naïve mice were used as a control. Data are presented as means ±SEM.

Pharmacokinetics and Toxicokinetics of BP1200

Table 1. Pharmacokinetics of single intraperitoneal dose of BP1200 in female C57BL/6 mice

Dose	C_{max}	$AUC_{0\to\infty}$	Cl	V_{ss}	$t_{1/2}$	$MRT_{0\to\infty}$
mg/kg	μg/mL	hr×mg/mL	mL/hr/kg	mL/kg	hr	hr
10	91±15	24±2	0.41±0.03	119±12	201±27	290±39

Table 2.Toxicokinetics of single or multiple intravenous dose of BP1200 in female cynomolgus monkeys

Dose	Route	Day	C_{max}	$AUC_{0\to\infty}$	CL	V_{ss}	$t_{1/2}$	$MRT_{0\to\infty}$
mg/kg			μg/mL	μg・hr/mL	mL/hr/kg	mL/kg	hr	hr
5	iv. q1w	1	149	6900	0.7	52.8	51.2	73.9
5	iv, q1w	22	122	4600	2.6	173.3	42.9	61.9
25	iv, q1w	1	598	22200	1.1	68.4	41.9	60.4
25	iv, q1w	22	808	35700	0.7	57.6	57.7	83.2

Cmax, Maximum observed concentration; $AUC_{0\to\infty}$, Area under the concentration-time curve from 0 to infinity; CL, Total systemic clearance; V_{ss}, Volume of distribution at steady state; $t_{1/2}$, Terminal half life; $MRT_{0\to\infty}$, Mean residence time from 0 to infinity

Conclusion

- BP1200_002 is a humanized anti-CD73 antibody that attenuates the activity of CD73 as a non-competitive inhibitor without hook effect.
- BP1200_002 enhances the proliferation, cytotoxicity, and cytokine production of T cells under the TME condition.
- The combination of BP1200_002 and immune checkpoint antibody significantly suppressed tumor growth and lead long term immunotherapeutic efficacy.
- BP1200_002 showed good PK/TK profiles without remarkable organ toxicity in mice and monkeys.
- The combination of BP1200 and immune-checkpoint inhibitors for cancer treatment will be promising therapeutic option in clinical practice.

Disclosure

N. Matsumoto: Employee of BrightPath Biotherapeutics

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