JAB-X1800: a potent immunostimulatory antibody-drug conjugate (iADC) targeting CD73

Abstract #2923

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Background

- STING is an important immuno-oncological target that stimulates the production of type I interferon and downstream antitumor target genes. Clinically, the systemic administration of STING agonist may be challenged by toxicity due to broad biodistribution. Therefore, targeting STING activation by immunostimulatory antibody-drug conjugate (iADC) which conjugates STING agonist to an antibody targeting tumor cells is warranted.
- We have developed a highly potent STING agonist JAB-27670 with favorable solubility as an iADC payload.
- CD73 is an important immune checkpoint in adenosine pathway, highly expressed in many types of cancers and undergoes internalization after binding to antibody. We have developed a CD73 antibody JAB-BX102 which is in clinical development (NCT05174585).
- We have developed JAB-X1800, a first-in-class CD73-STING iADC by conjugating our own STING agonist to CD73 antibody. JAB-X1800 exhibits stability, target-specific internalization, potent antitumor activity and good tolerability in pre-clinical study.

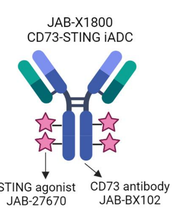


Figure 1. Schematic structure of JAB-X1800.

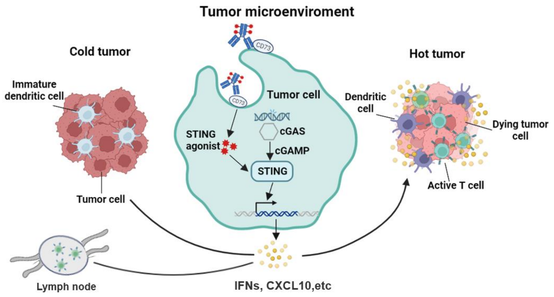


Figure 2. Schematic representation of the cGAS-STING signaling pathway in cancer.

Jacobio developed a highly potent STING agonist as an iADC payload

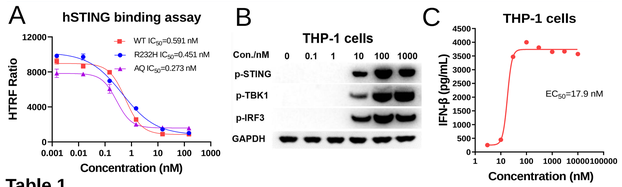
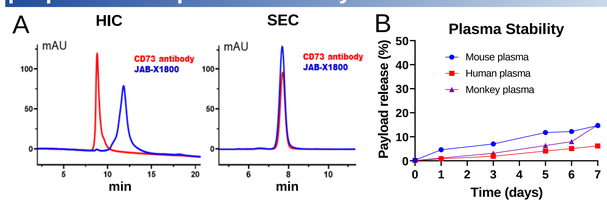


Table 1

STING variant	HEK293 Reporter assay	
	EC_{50}/nM (STING agonist)	EC_{50}/nM (2',3'-cGAMP)
WT	0.990	2636
R232H	1.73	3497
R293Q	1.77	4661
AQ (G230A-R293Q)	2.97	2989
HAQ (R71H-G230A-R293Q)	6.90	3156

Figure 3. Potency of the STING agonist.
A. Human STING binding assay shows high affinity of STING agonist on WT STING or variants.
B. THP1 cells were treated with STING agonist for 5 hours and the levels of p-STING, p-TBK1 and p-IRF3 were examined by WB.
C. THP-1 cells were treated with STING agonist for 5 hours and the level of IFN-β was examined by HTRF.
Table 1. HEK293 cells engineered with a secreted luciferase reporter for STING activity were incubated with STING agonist for 24 hour and the luciferase activity was examined to indicate STING activation.

JAB-X1800 demonstrates good physicochemical properties and plasma stability



Attribute	Value
Conc. of CD73 antibody	>10 mg/mL
Conc. of unconjugated CD73 antibody	<1 mg/mL
DAR (TOF)	~4
HMW	~1%

Figure 4. Physicochemical properties and plasma stability of JAB-X1800.
A. HIC and SEC analysis of JAB-X1800 compared to unconjugated CD73 antibody.
B. Plasma stability of JAB-X1800 in human, monkey and mouse plasma was assessed by release of STING agonist as free payload.

CD73-dependent internalization of JAB-X1800 in cancer cells, but not in peripheral B and T cells

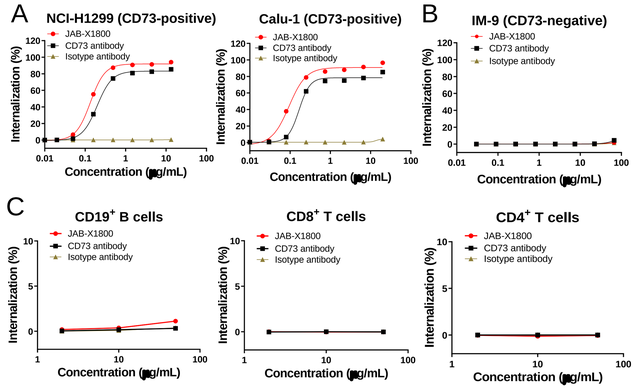


Figure 5. Internalization of JAB-X1800.
Internalization was assessed in CD73-positive cancer cells (NCI-H1299 and Calu-1) (**A**), CD73-negative cancer cells (IM-9) (**B**), and human B cells and T cells (**C**). Cells were treated with a series of concentrations of fluorescence-labeled compounds for 6 hours (**A**), 17 hours (**B**), or 16 hours (**C**), and the intracellular fluorescence was detected by FACS. Percentage of cells with fluorescence was calculated to evaluate internalization.
There is significant CD73-dependent internalization of JAB-X1800 on cancer cells. There is no detectable internalization of JAB-X1800 on B cells and T cells, which have minimal to modest expression of CD73, suggesting the activation of STING pathway in peripheral B and T cells by JAB-X1800 may be spared to minimize systemic toxicity.
CD73 antibody is the unconjugated anti-human CD73 antibody used as positive control of internalization assay, and isotype antibody was used as negative control.

JAB-X1800 induces anti-tumor cytokines and leads to potent killing of cancer cells

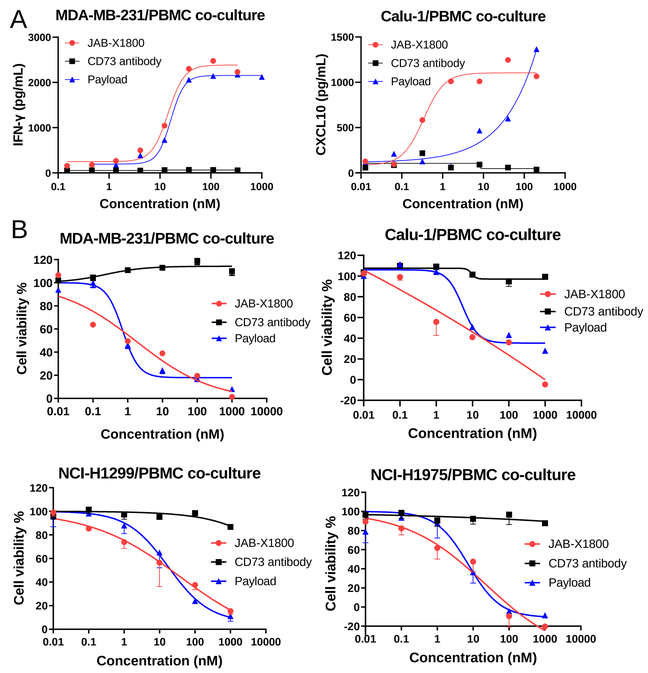


Figure 6. *In vitro* antitumor activities of JAB-X1800.
A. Release of IFN-γ and CXCL10 were assessed in co-culture of CD73-positive MDA-MB-231 and Calu-1 cancer cells with PBMCs. Co-culture was treated with a series of concentrations of JAB-X1800 for 24 hours and the cytokines in supernatant were evaluated by ELISA.
B. Cell viability was assessed in co-culture of CD73-positive cancer cells (MDA-MB-231, Calu-1, NCI-H1299 or NCI-H1975) with PBMCs. Co-culture was treated with a series of concentrations of JAB-X1800 for 72 hours and then cell viability was evaluated by CTG assay.
Payload is the free STING agonist used as positive control of co-culture assay, and CD73 antibody is the unconjugated anti-human CD73 antibody used as negative control.

JAB-X1800 exhibits potent anti-tumor activity with immune memory and good tolerability *in vivo*

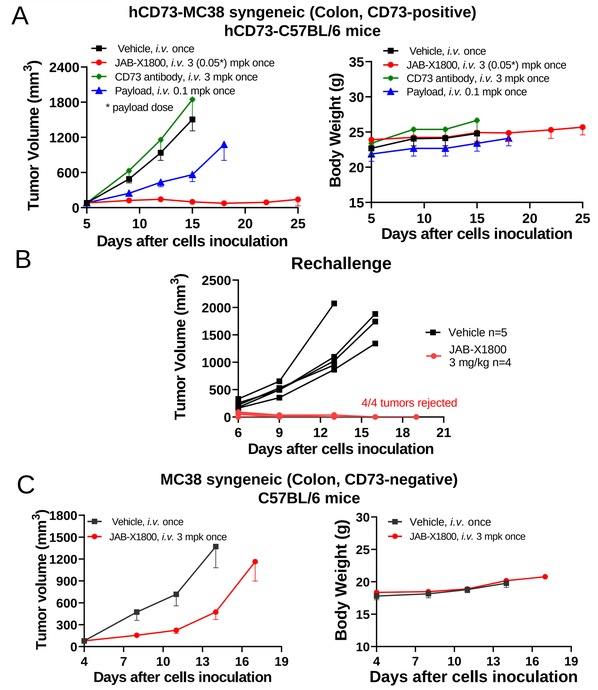


Figure 7. *In vivo* antitumor activities and tolerability of JAB-X1800.
A. Antitumor activity of JAB-X1800 in MC38 colon syngeneic model which expresses human CD73 transgene (hCD73-MC38).
B. Surviving mice treated with vehicle or JAB-X1800 for the original tumor implantation (A) were rechallenged with hCD73-MC38 tumor cells, showing inhibition of rechallenged tumor growth in JAB-X1800-treated mice.
C. Antitumor activity of JAB-X1800 in CD73-negative MC38 colon syngeneic model which has no expression of human CD73.
D. Antitumor activity of JAB-X1800 in CD73-positive breast cancer xenograft model (MDA-MB-231).
E. Antitumor activity of JAB-X1800 in CD73-positive non-small cell lung cancer xenograft model (NCI-H1975). Body weights are shown to demonstrate animal tolerability. 4-8 mice/group.
Payload is the free STING agonist and CD73 antibody is the unconjugated anti-human CD73 antibody. Isotype ADC is the STING agonist conjugated with isotype antibody.

References

1) Proc Natl Acad Sci U S A. 2022 Dec 6;119(49):e2214278119.
2) Curr Opin Pharmacol. 2020 Aug;53:66-76.
3) Cancers (Basel). 2021 May 30;13(11):2695.
4) Clin Cancer Res. 2022 Feb 15;28(4):677-688.
5) Nature. 2008 Oct 2;455(7213):674-8.

JAB-X1800 induces higher level of CXCL10 in tumor, and has limited effect in serum IL-6 concentrations

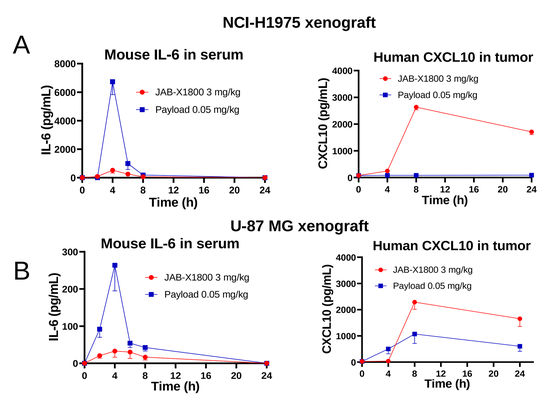


Figure 8. JAB-X1800 induces high levels of CXCL10 in tumor with minimal IL-6 induction in serum.
NCI-H1975 (CD73 positive) xenograft in BALB/c nude mice (**A**) and U-87 MG (CD73 positive) xenograft in CB17 SCID mice (**B**) were treated with JAB-X1800 3 mg/kg (equivalent 0.05 mg/kg payload) or payload 0.05 mg/kg, and the tumor tissue and plasma samples were collected at indicated time points to assess the levels of CXCL10 and IL-6 by ELISA. 3-4 mice/group. Intratumoral level of CXCL10 is used to indicate antitumor activity, and expression of IL-6 in serum is used to indicate risk of cytokine storm.

Synergy of JAB-X1800 in combination with anti-PD-1

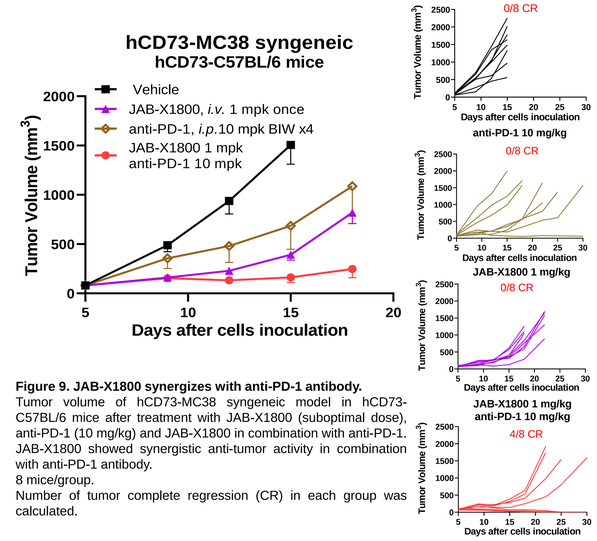


Figure 9. JAB-X1800 synergizes with anti-PD-1 antibody.
Tumor volume of hCD73-MC38 syngeneic model in hCD73-C57BL/6 mice after treatment with JAB-X1800 (suboptimal dose), anti-PD-1 (10 mg/kg) and JAB-X1800 in combination with anti-PD-1. JAB-X1800 showed synergistic anti-tumor activity in combination with anti-PD-1 antibody.
8 mice/group.
Number of tumor complete regression (CR) in each group was calculated.

Conclusions

- JAB-X1800 is a first-in-class iADC by conjugating potent STING agonist to CD73 mAb (CD73-STING iADC).
- JAB-X1800 has favorable plasma stability.
- JAB-X1800 shows target-specific internalization in CD73-positive cancer cells and induces anti-tumor cytokines, leading to potent killing of cancer cells in tumor cells and PBMC co-cultures.
- JAB-X1800 exhibits CD73-dependent and potent anti-tumor activity with a single dose administration in multiple mouse models.
- JAB-X1800 induces higher CXCL10 protein expression in tumor, and limited IL-6 concentrations in serum, suggesting an enhanced antitumor activity with low risk of cytokine storm.
- JAB-X1800 in combination with anti-PD-1 has synergistic effect.

Acknowledgment

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