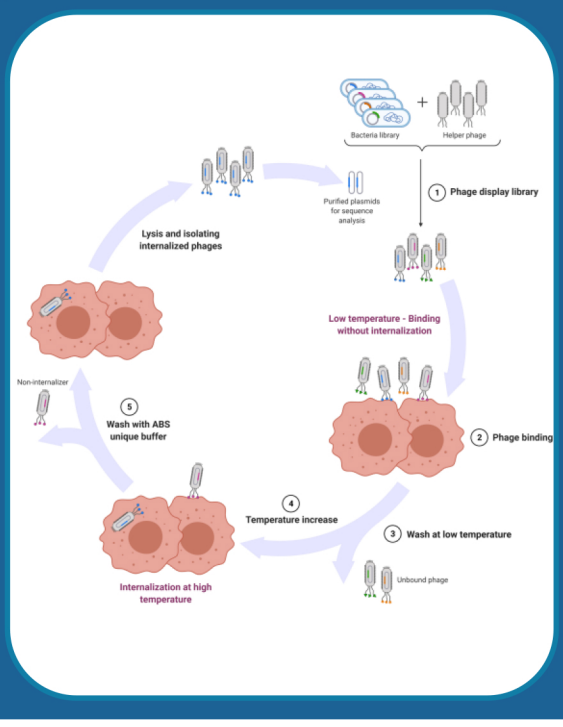
ABS-INT-001 Internalizing Ab-ADC for Cancer Therapeutics

Background

- Growth factor X is a well-characterized target that is involved in multiple proliferation-related signal transduction pathways
- Growth factor X expressed in multiple cancer types and therefore is an ideal target cancer therapies



Project Rationale

The need for antibody targets with internalizing capacity can greatly aid the development of ADC therapeutics for oncology

Internalizing Ab Platform

Ab Studio's unique internalizing antibody platform with computer-aided design allows for the discovery of true antibody internalizers. Combined with our patented screening strategy for characterization allows for rapid discovery.

Project Highlights

- Results:

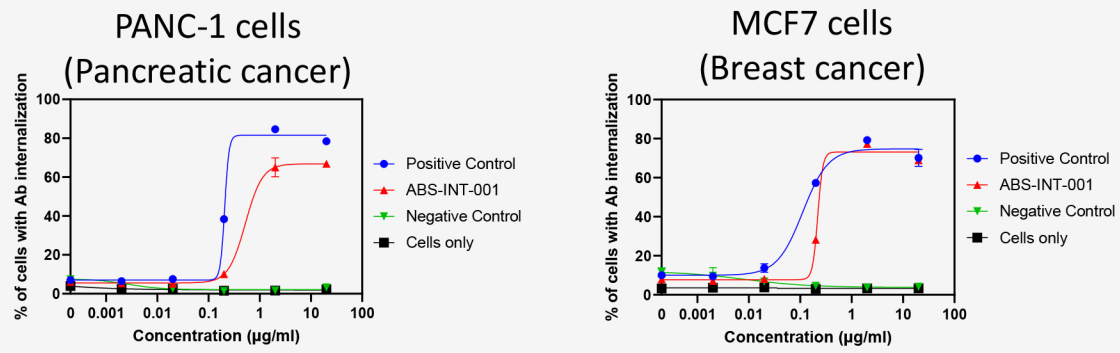
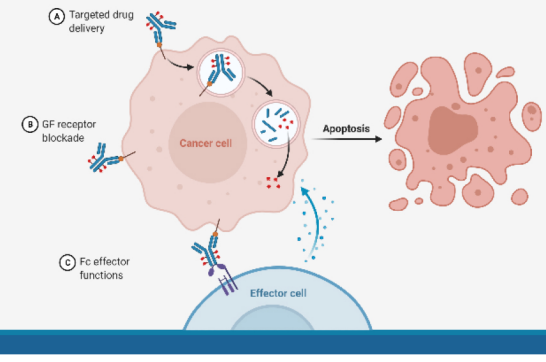


Figure 1. Using our screening method, ABS-INT-001 is internalized in both pancreatic and breast cancer cell lines, which is comparable to a positive control antibody

- Potential application: ADC development



Development of a novel bispecific antibody targeting PD-L1 and CD55 for cancer therapy

Amit Kumar Chaudhary[1]*, Jiadong Shi[3]*, Wenyan Cai[2,3], Bo Wang[3], Mohd Saif Zaman[1], Cai Huang [1], Jun Lin[2], Steven Z. Kan[2], Joe Zhou[2], Jianbo Dong[3] and Yue Liu[1,3]

1 Ab Therapeutics Inc., a JHBP company, 3541 Investment Blvd., Suite 2, Hayward, CA 94545

2 Genor Biopharma Co. Ltd., a JHBP company, 1690 Zhangheng Road, Building 3, Pudong New District, Shanghai, P.R.C.

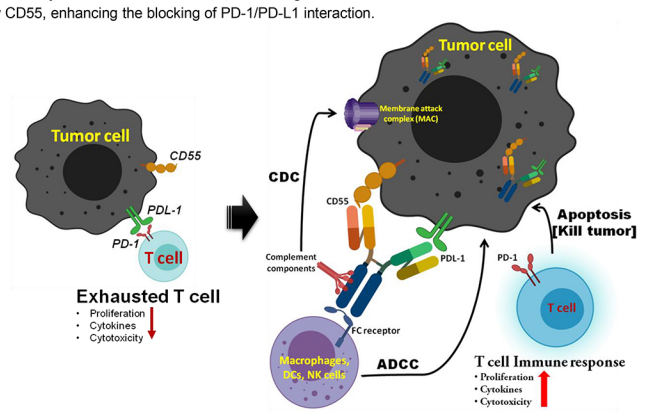
3 Ab Studio Inc., 3541 Investment Blvd., Suite 3, Hayward, CA 94545

*These authors contributed equally to this work

ABSTRACT

Immune checkpoint interaction helps tumors resist immunity-induced apoptosis. Blocking PD-1/PD-L1 leads to remarkable clinical responses; however, a large portion of patients develop acquired resistance after initial therapy. Many tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs) have been identified. TSAs are restricted to cancer cells only but TAAs are elevated on tumor cells and minimally expressed on healthy cells. Therefore, co-targeting TAAs/TSAs would show benefits over monotherapy.

CD55 is a TAA that regulates complement activation pathway and expressed on cells exposed to the complement system to protect against complement attack. Cancer cells escape this attack by raising CD55 expression. Additionally, CD55 acts as a virus receptor for internalization. Based on the roles of CD55, we designed PD-L1/CD55 bispecific antibody (GB262) with weaker CD55 binding arm to rule out the possibility of lethality to healthy cells but maintain its CD55 binding and internalization ability specifically to cancer cells. Moreover, co-binding to PD-L1 and CD55 leads to co-internalization of PD-L1 by CD55, enhancing the blocking of PD-1/PD-L1 interaction.



Overall MOA of GB262 including T cell activation, ADCC, CDC

Internalization of GB262 in target cells degrade intracellular PD-L1

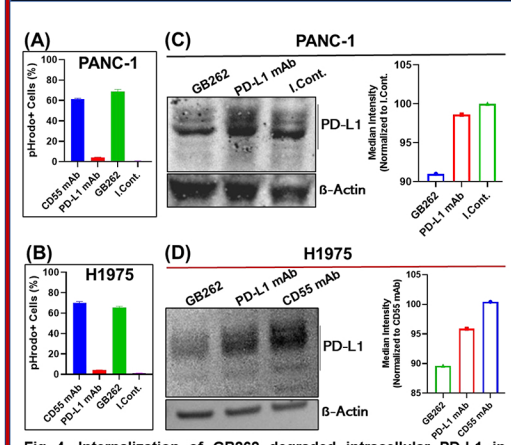


Fig 4. Internalization of GB262 degraded intracellular PD-L1 in target cells. **(A-B) Internalization:** Target cells were treated with 10 ug/mL respective antibodies followed by 10 ug/mL of pHrodo labelled-GAH secondary antibody. Internalization was analyzed by flow cytometry after 24 hr. **(C-D) Western blot:** Target cells were incubated with 10 ug/mL and 50 ug/mL respective antibodies for 72 hr and 48 hr. Intracellular PD-L1 was analyzed by western blot. Median intensity was quantified by iBright Analysis Software.

GB262 maintained ADCC with additional CDC MOA

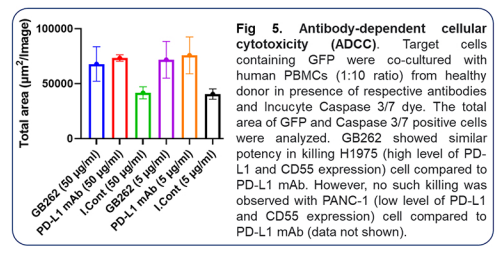


Fig 5. Antibody-dependent cellular cytotoxicity (ADCC). Target cells containing GFP were co-cultured with human PBMCs (1:10 ratio) from healthy donor in presence of respective antibodies and Incucyte Caspase 3/7 dye. The total area of GFP and Caspase 3/7 positive cells were analyzed. GB262 showed similar potency in killing H1975 (high level of PD-L1 and CD55 expression) cell compared to PD-L1 mAb. However, no such killing was observed with PANC-1 (low level of PD-L1 and CD55 expression) cell compared to PD-L1 mAb (data not shown).

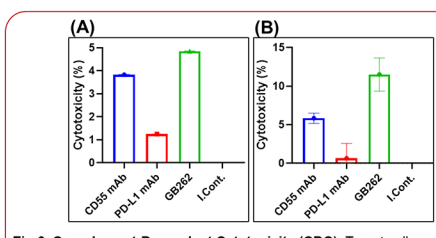


Fig 6. Complement-Dependent Cytotoxicity (CDC). Target cells were treated with 5-10% of human complement serum and test antibodies followed by analyzing cytotoxicity. **(A)** PANC-1 (50ug/mL) LDH assay. **(B)** MIA PaCa-2 (10ug/mL) Calcein is released in the media from dying MIA PaCa-2 and detected. GB262 was potent in inducing CDC compared to PD-L1 mAb.

In Vivo Study: B-NDG mouse model (Naked antibodies) (B-NDG mouse is not applicable for CDC MOA)

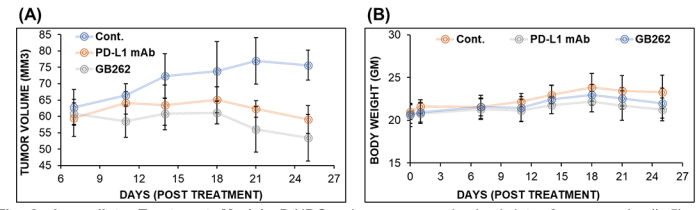


Fig 8. Immediate Treatment Model. B-NDG mice were randomized into 3 groups (n # 5) and subcutaneously injected on day 0 with a mixture of PANC-1 ($2.0x10^6$ cells/mouse) and human PBMCs ($8.0x10^6$ cells/mouse). The animals were immediately IP injected with either without (DPBS) or with PD-L1 mAb or GB262 at 1.5 mg/kg. Three additional doses of antibodies were given weekly. TV **(A)** and BW **(B)** of mice in the study were measured.

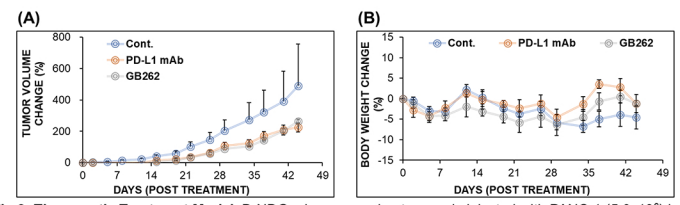


Fig 9. Therapeutic Treatment Model. B-NDG mice were subcutaneously injected with PANC-1 ($5.0x10^6$) in the right flank. Tumor-bearing animals were randomly enrolled (n # 5) into study groups when mean tumor size reaches approximately ~100 mm^3. The day treatment initiated was represented as Day 0. Treatment was done by IV injection with the combination of human PBMCs ($8x10^6$ cells/mouse) and 10 mg/kg antibodies. Five additional doses of antibodies were given weekly. TV **(A)** and BW **(B)** of mice in the study were measured.

Designing of Imbalanced GB262 (High PD-L1 and Low CD55 binding)

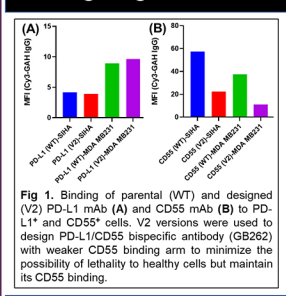
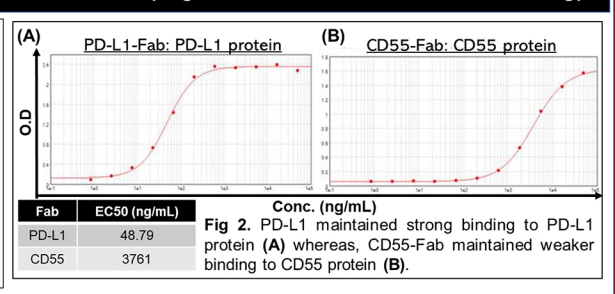


Fig 1. Binding of parental (WT) and designed (V2) PD-L1 mAb **(A)** and CD55 mAb **(B)** to PD-L1⁺ and CD55⁺ cells. V2 versions were used to design PD-L1/CD55 bispecific antibody (GB262) with weaker CD55 binding arm to minimize the possibility of lethality to healthy cells but maintain its CD55 binding.



Fab	EC50 (ng/mL)
PD-L1	48.79
CD55	3761

Fig 2. PD-L1 maintained strong binding to PD-L1 protein **(A)** whereas, CD55-Fab maintained weaker binding to CD55 protein **(B)**.

GB262 maintained T cell activation property like PD-L1 mAb

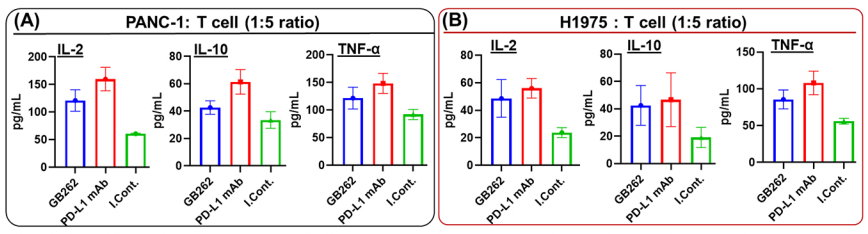


Fig 3. Cytokine release after co-culturing target cells with preactivated human T cell with Immunocult human CD3/CD28 T cell activator [T cell: Target cell ratio (5:1)]. T cell was activated for 72 hr and co-cultured with target cells in presence of antibodies for 72 hr. The release of cytokines were analyzed by ELISA using culture supernatant.

ADC Internalization and Therapeutic Efficacy

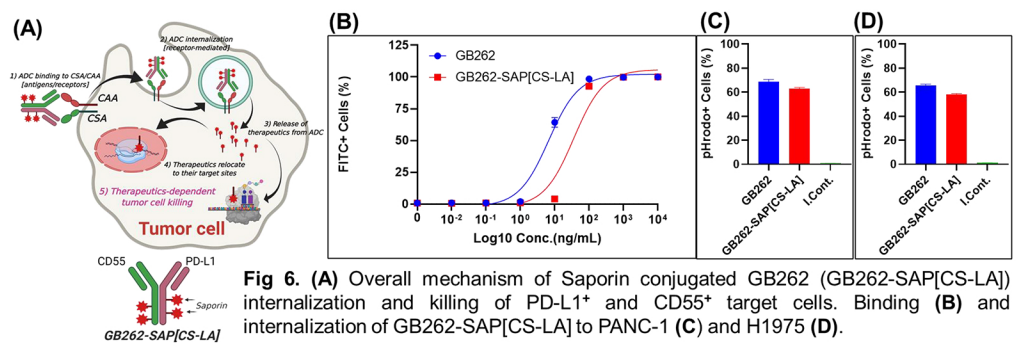
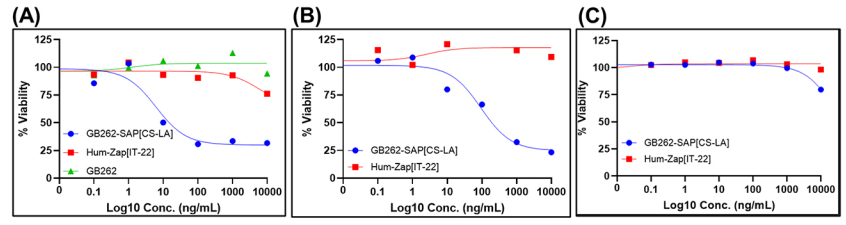


Fig 6. (A) Overall mechanism of Saporin conjugated GB262 (GB262-SAP[CS-LA]) internalization and killing of PD-L1⁺ and CD55⁺ target cells. Binding **(B)** and internalization of GB262-SAP[CS-LA] to PANC-1 **(C)** and H1975 **(D)**.



Cell lines	IC50-GB262-SAP[CS-LA] (ng/mL)
H1975	6.163
PANC-1	93.98
CHO-K1+CD55	29191

Fig 7. Antibody-Drug Conjugate (ADC)-mediated target cell killing. H1975 **(A)**, PANC-1 **(B)** and CD55⁺ CHO-K1 **(C)** cells were incubated with respective antibodies and % viability analyzed after 72 hr using crystal violet staining of live adherent cells.

In Vivo Study: B-NDG mouse model (ADC)

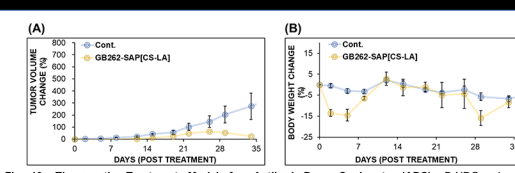


Fig 10. Therapeutic Treatment Model for Antibody-Drug Conjugate (ADC). B-NDG mice were subcutaneously injected with PANC-1-1 ($5.0x10^6$) in the right flank. Tumor-bearing animals were randomly enrolled (n # 5) into study groups when mean tumor size reaches approximately ~100 mm^3. The day treatment initiated was represented as Day 0. Treatment was done by IV injection with the combination of human PBMCs ($8x10^6$ cells/mouse) and 1 mg/kg antibody. Four additional doses of antibody was given weekly. TV **(A)** and BW **(B)** of mice in the study were measured.

Results and Conclusion

- GB262 maintained high PD-L1 and low CD55 binding. It also maintained T-cell activation and cancer killing (ADCC and CDC). GB262 internalization leads to intracellular PD-L1 degradation. GB262 showed similar regression of tumor growth in B-NDG mice compared to PD-L1 mAb. We believe due to the lack of human complement serum in B-NDG mouse, we did not observe additional CDC benefits by GB262 in vivo.
- GB262-saporin GB262-SAP[CS-LA] induced cancer killing in vitro and in vivo.
- GB262 is the first bispecific antibody that not only releases cancer repression on T-cell activation, but also releases cancer repression on CDC. GB262 has potential to serve as a novel therapy for many cancers.

Permanent Abstract Number

LB067