Using single B platform to develop a CAR T-cell therapeutic solution for a GPCR target

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Abstract

To expedite antibody-based drug development, DIMA Biotech developed more than 5000 on-shelf lead antibody molecules on over 300 druggable targets with its DimAb® single B platform. In this presentation, we will elaborate DimAb® platform and showcase how we developed anti-GPRC5D CAR T-cell therapy constructs from leads to IIT clinical trial stage with our collaborator. The preliminary data from ongoing clinical trial exhibits astonishing clinical efficacy on RRMM patients.

Experimental approach

DiMPro™ membrane protein purification platform

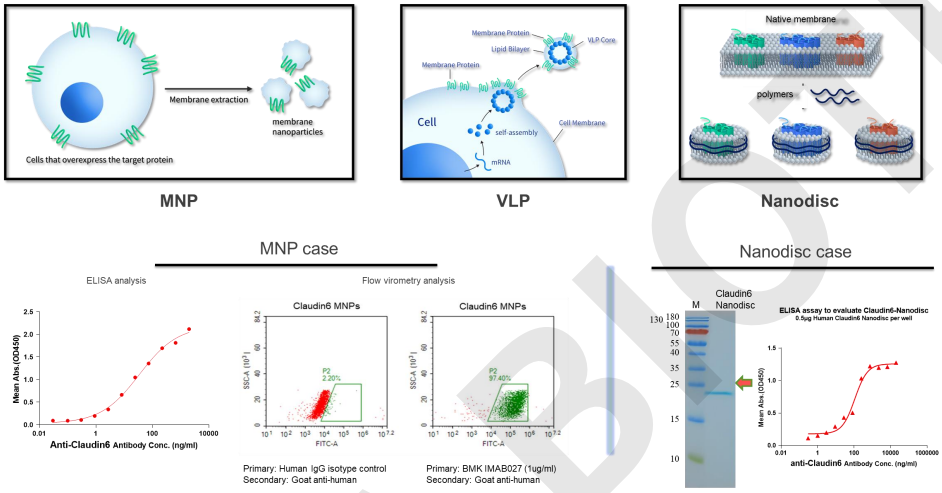


Figure 1. DiMPro™ multi-pass transmembrane protein production and purification platform. DIMA biotech has established a plethora of technology platforms to produce functional membrane proteins as immunogen for therapeutic antibody development.

DimAb® single B cell platform

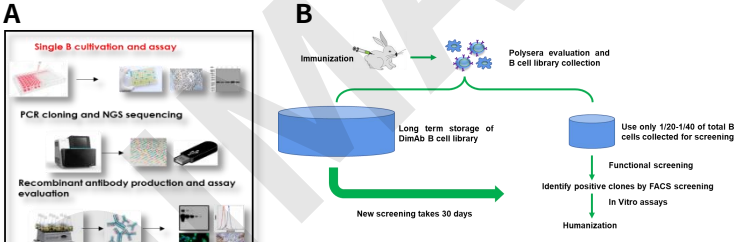
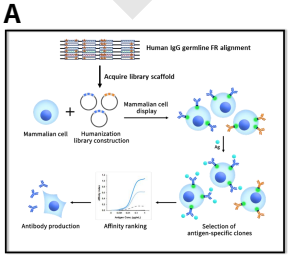


Figure 2. (A) **DimAb® single B cell workflow.** DimAb® single B platform takes advantages of unique B cell culture method, which allows fast and precise isolation of positive antibody sequences. (B) **DimAb® B cell library**. The unused B cells from immunized animals can be stored in liquid nitrogen for long term. More lead antibody molecules can be selected by initiating a new round of screening and cloning, which takes only 30 days.

DiLibrary™ antibody humanization platform

A



B

Analyte	Ka(1/Ms)	Kd(1/s)	KD (nM)	Rmax (RU)
Rabbit BCMA	4.3884E+4	1.1750E-5	0.267	65.3
Hu-BCMA1	3.1023E+4	4.3530E-6	0.140	105.2
Hu-BCMA2	2.8604E+4	4.5480E-6	0.159	78.9
Hu-BCMA3	6.4527E+4	1.0250E-6	0.015	81.3
Hu-BCMA4	3.3508+4	1.9060E-6	0.056	92.1

Analyte	Ka(1/Ms)	Kd(1/s)	KD (nM)	Rmax (RU)
Rabbit CEACAM5	6.3002E+4	2.3270E-5	0.369	88.9
Hu-CEACAM5-01	5.5353E+4	1.4308E-5	0.258	82.1
Hu-CEACAM5-02	5.4997E+4	1.0730E-6	0.019	78.9

Figure 3. (A) The workflow of DiLibrary™ antibody humanization. CDRs from parental antibody are first grafted onto a FR library constructed from a number of selected human IgG germline sequences. The final humanized antibody library will be displayed on HEK293 cell surface, and the best binders can be selected via FACS sorting with target protein. **(B) Examples of two humanization experiments.** The selected humanized antibodies show improved affinities (pM) comparing to the parental antibody.

Results

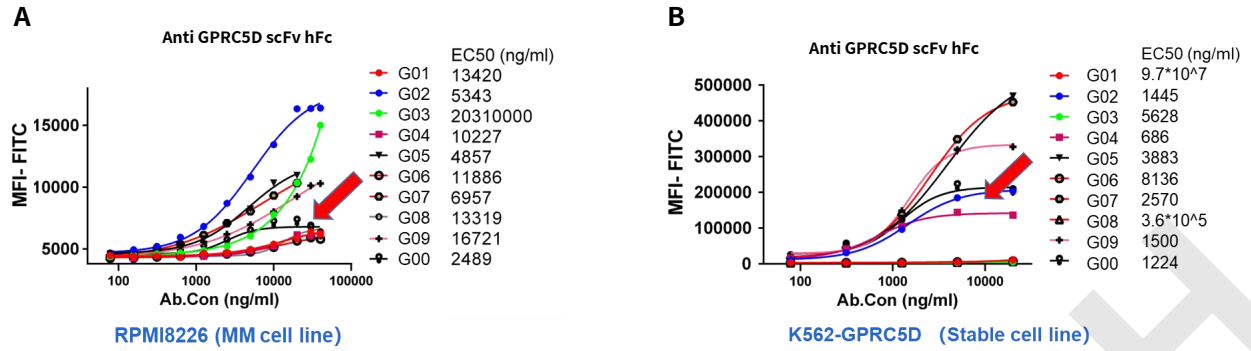


Figure 4. Affinity ranking of 9 humanized anti-GPRC5D DimAbs through flow assay. Comparing to BMK antibody (G00) pointed by red arrow, derived from a CAR construct currently in phase I clinical trial, 4 out of 9 DimAbs exhibit stronger binding affinity on both RPMI8226 or K562-GPRC5D stable cell line.

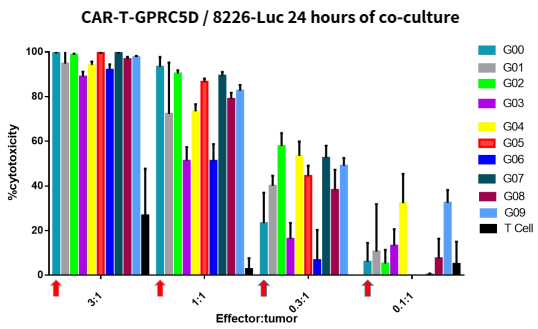


Figure 5. *In Vitro* tumor cell cytotoxicity assay. The experimental data showed that several of CARs constructed from DimAbs exhibit stronger cytotoxic capacity than bench-mark CAR (G00), pointed by red arrows, especially under low Effector:Tumor ratio.

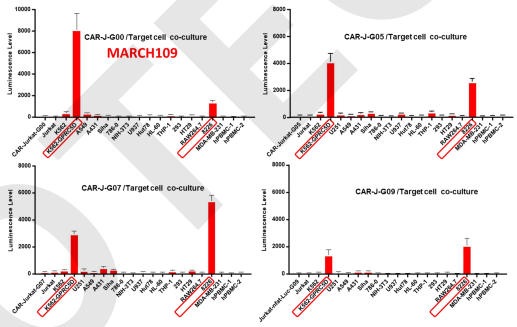
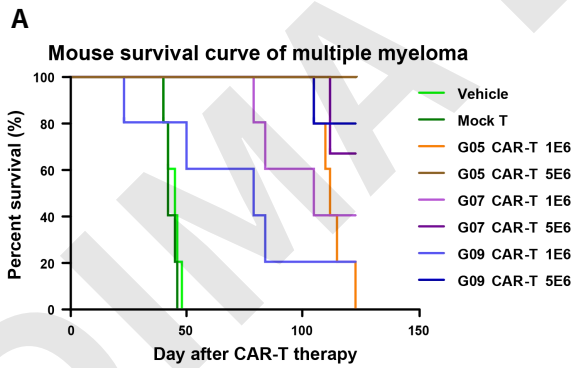


Figure 6. The selected CAR constructs exhibit high specificity in activation upon contact of target cells. We examined the specificity in activation of G00, G05, G07, and G09 CAR constructs through a CAR-Jurkat activation assay, and all CAR constructs show exclusive target cell specificity.



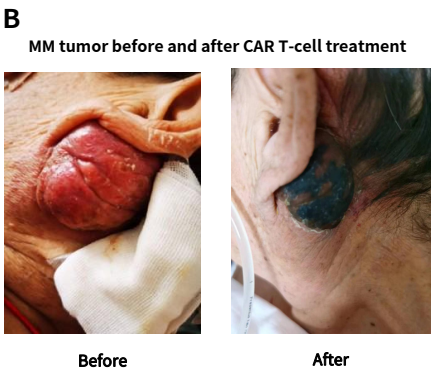


Figure 7. Animal survival data and early IIT clinical trial data. A. Multiple myeloma (MM) model mice infused with two dosage of CAR T-cells exhibit elongated survival time comparing with control groups. **B.** A RRMM patient relapsed from BCMA CAR T-cell therapy was treated with our anti-GPRC5D CAR T-cells. A metastatic tumor of large size behind patient ear nearly disappeared in 10 days after CAR T-cells infusion. (Currently our collaborators are recruiting more patients.)

Conclusion

DIMA Biotechnology Ltd is a biotech company dedicated on hit to lead stage therapeutic antibody discovery. Currently we have obtained more than 5000 IgG molecules on over 300 targets. In this anti-GPRC5D CAR T-cell project, we showcased how we developed 3 lead stage DimAbs and then chose one to push into IIT clinical trial. For this project, our collaborator is planning to recruit 18 patients for IIT and the clinical data will be reported separately.