Therapeutic mAb discovery for multi-pass transmembrane protein targets

DIMA BIOTECH
Recombinant mAbs and proteins

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Abstract

Many important druggable targets are multi-pass transmembrane proteins, including GPCRs, ion-channels and Claudins. Due to the extreme difficulties in obtaining purified functional proteins, so far only very few of these targets got FDA approved antibody drugs. To develop therapeutic lead mAbs on these targets, DIMA Biotech has systematically optimized every step of antibody discovery process to tackle these challenging targets. In this presentation, we will exhibit how we integrate DIMA's Nanodisc, Single B and Mammalian display technology platforms to speed up the mAb discovery. Here we showcase an example of using DIMA's technology platform to develop an anti-GPRC5D CAR T-cell therapy from protein purification to IIT clinical trial.

Experimental approach

Multi-pass transmembrane protein purification strategies

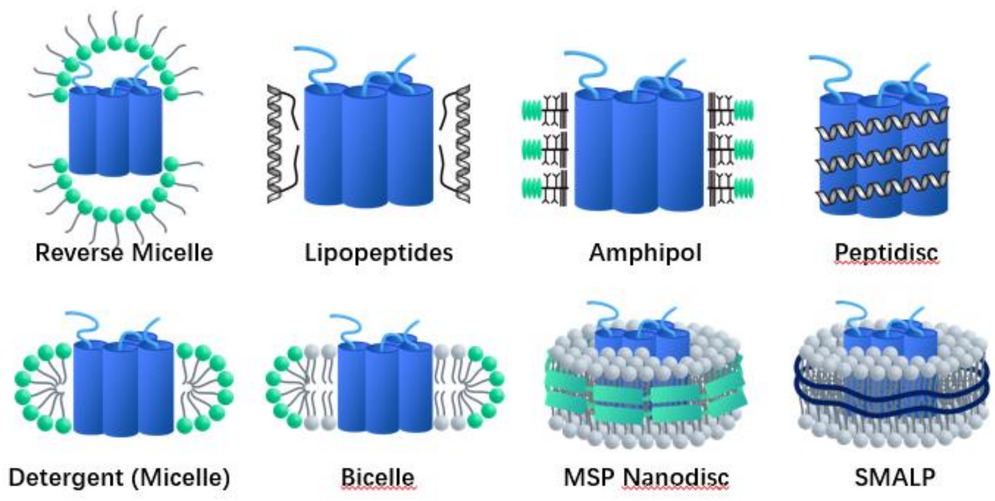


Figure 1. Different extraction and purification technologies for multi-pass transmembrane proteins.

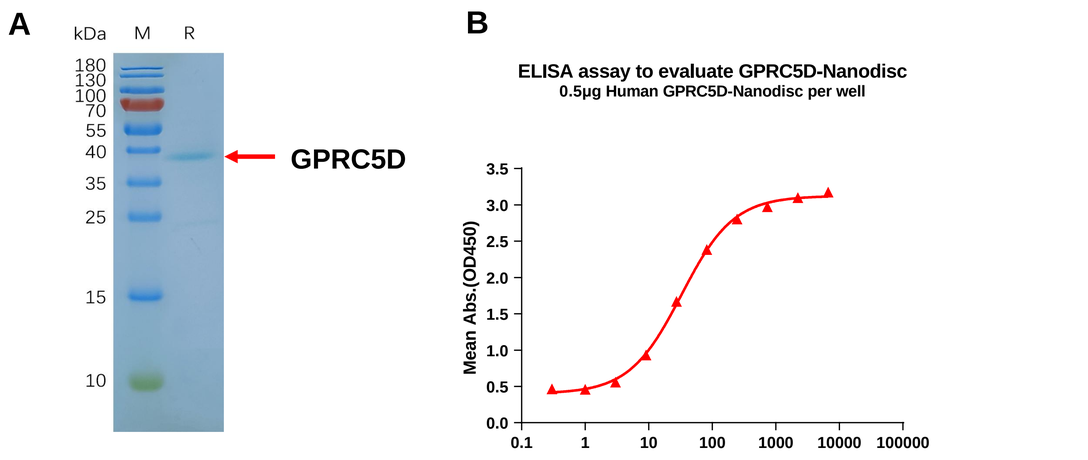


Figure 2. A. SDS-PAGE analysis on purified GPRC5D in Nanodisc format; **B.** GPRC5D-Nanodisc reacts with serial diluted anti-GPRC5D monoclonal antibody (Cat.DME100090).The EC50 is 32.86ng/ml.

Single B cell platform

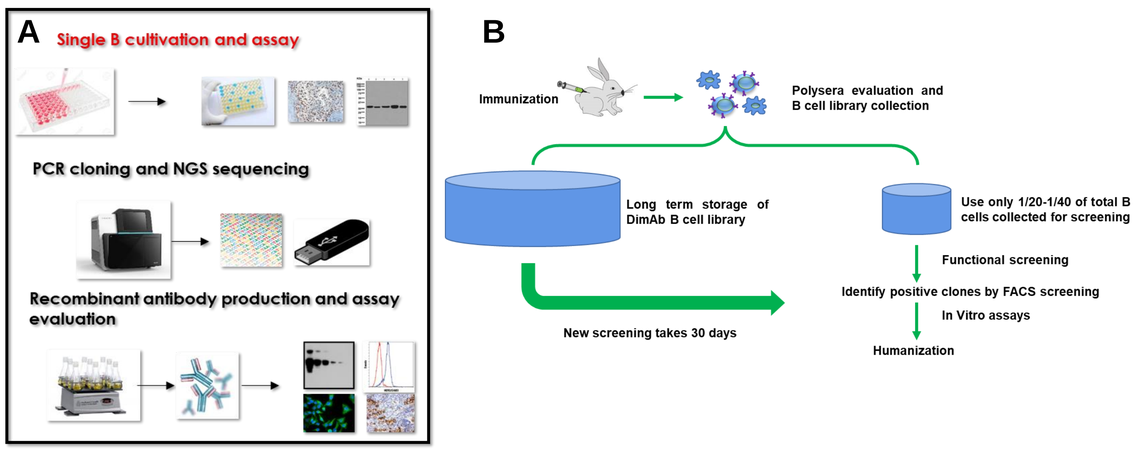
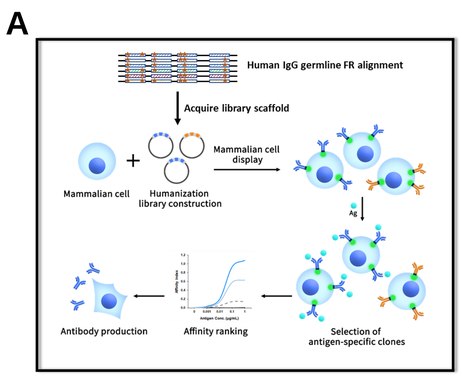


Figure 3. (A) **Dima single B cell workflow.** DIMA takes advantages of unique B cell culture method, which allows fast and precise isolation of positive antibody sequences. (B) As rabbits are larger in size than mice, only 1/40-1/20 total B cells are used for screening and the rest of B cells are frozen and ready for quick new-round screening that takes only 30 days to acquire positive clones.

Results

Humanization platform



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 4. Dima humanization workflow. Dima biotech has developed a mammalian cell-based humanization library. (A) The library is generated by FR shuffling to reach the better conformation of CDR, and thus to screen higher affinity humanized antibodies with good biological properties that fit down-stream drug development. (B) Cases of successful antibody humanization.

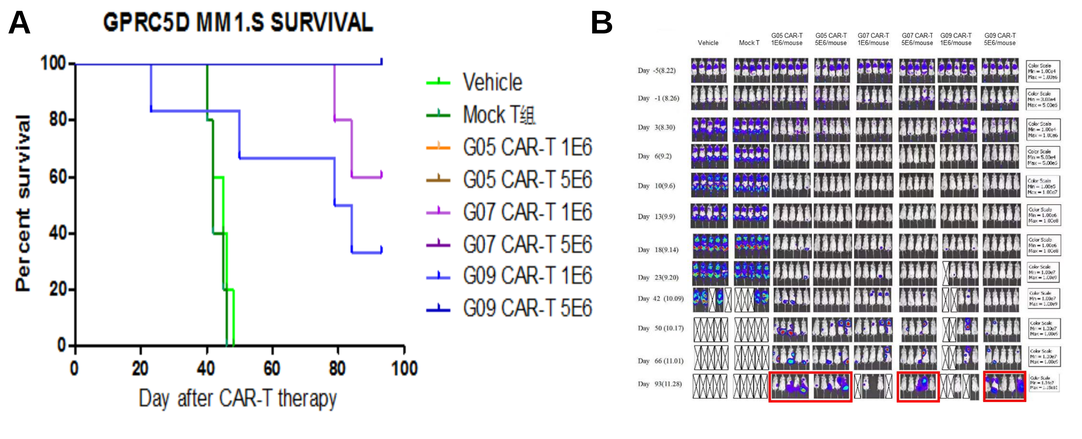


Figure 5. Survival of model mice with MM. G05, G07, and G09 CAR-T cells were injected to MM model mice. All CAR-T cells show effect of elongate mouse survival time after injection, proving the function of Dima GPRC5D CAR-T cells *in vivo*.

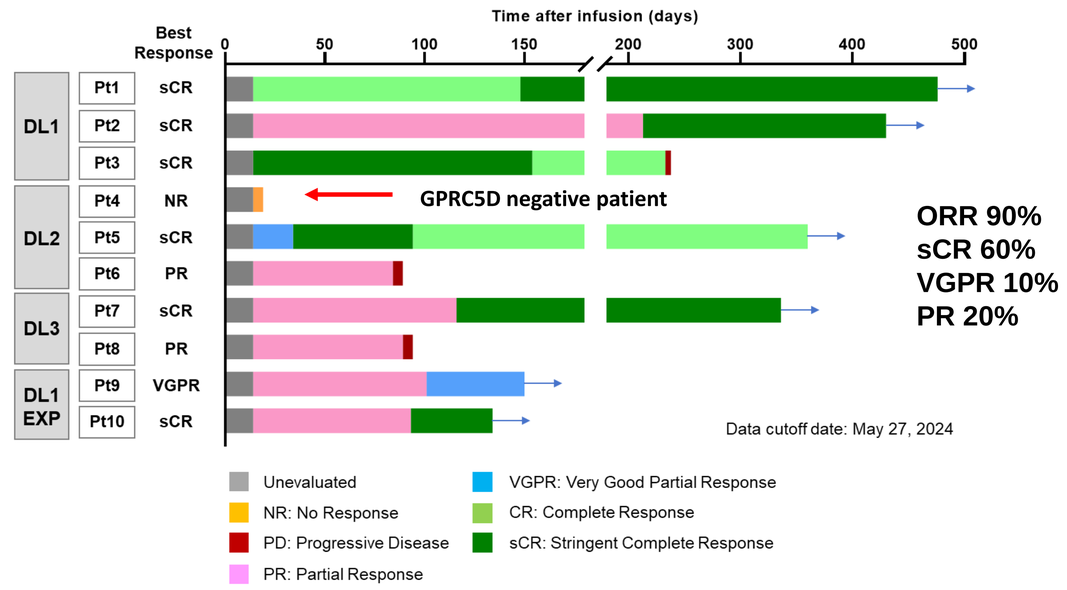


Figure 6. The IIT clinical data of anti-GPRC5D CAR T-cell therapy with RRMM patients.

Conclusion

One of the key challenges to develop therapeutic lead mAbs for multi-pass transmembrane targets is to obtain enough purified functional proteins. DIMA's synthetic nanodisc technology platform provides a perfect solution to extract and purify aqueous soluble multi-pass membrane proteins in high homogeneity. By applying DIMA's different technology platforms, we have successfully developed a group of good lead mAbs on GPRC5D, which is a seven transmembrane GPCR target. After functional evaluation, we identified molecules suitable for different therapeutic modalities, such as ADC, BsAb and CAR T-cell therapy. Through collaboration with our partners, we got very good clinical data on RRMM patient treatment.