The application of Nanodisc technology platform to develop therapeutic mAbs 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 poster presentation, we will exhibit how we integrate DIMA's Nanodisc technologies, including Syndisc™, PeptiNanodisc™, Single B enrichment and DiLibrary™ Mammalian display technology platforms to develop mAbs against a challenge multi-pass transmembrane target, CLDN18.2.

Experimental approach and data

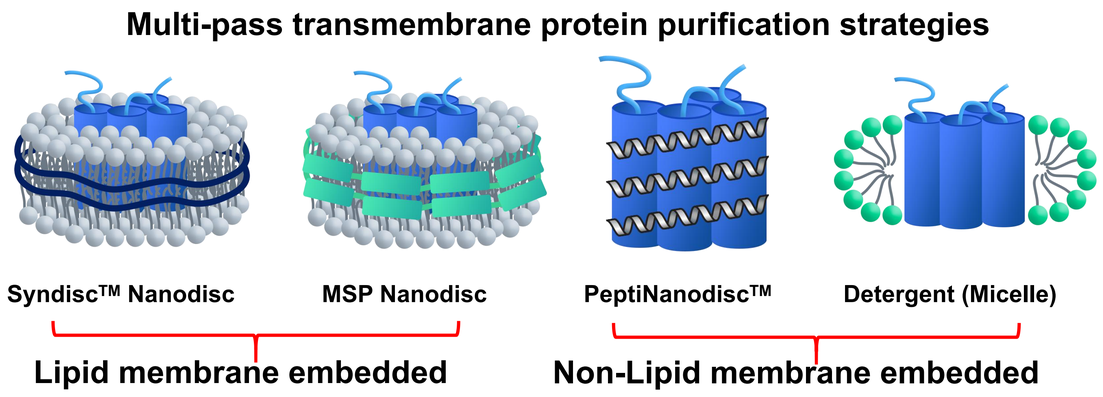

Figure 1. Different extraction and purification technologies for multi-pass transmembrane proteins.

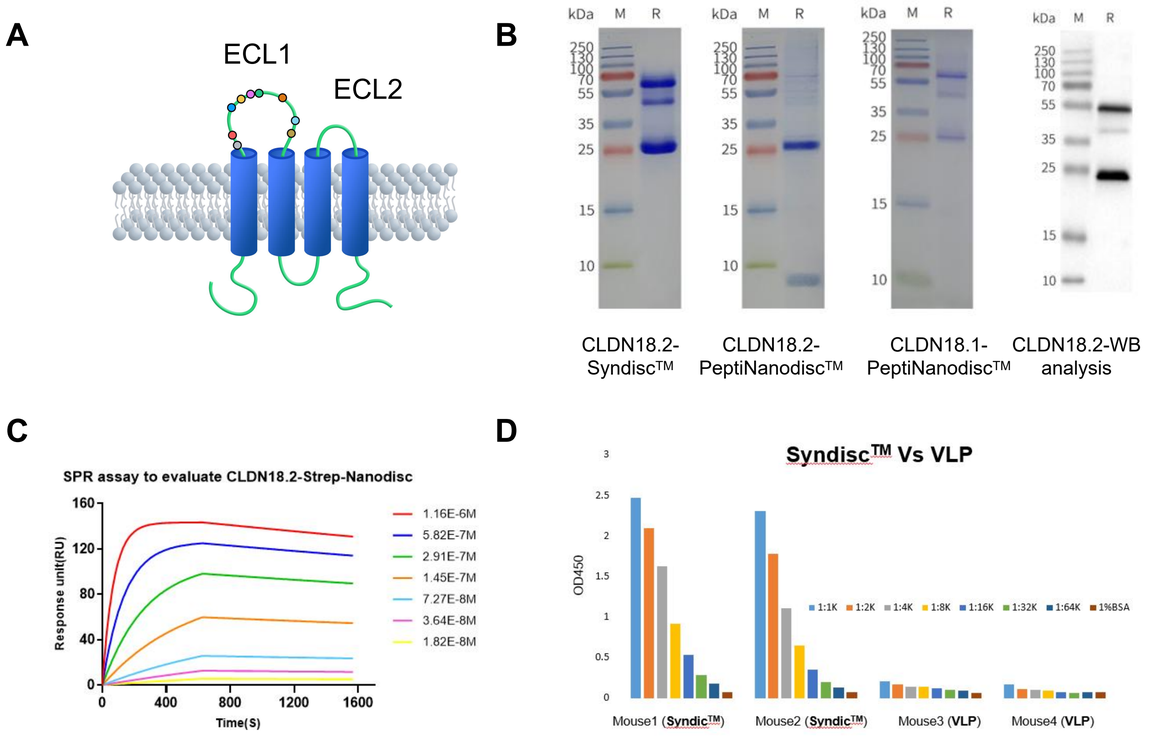

Figure 2. A) The structural topology of CLDN18.2/18.1. The therapeutic mAbs for CLDN18.2 should target exclusively on ECL1. **B)** The protein purity analyses of one round affinity-purified CLDN18.2/18.1 through different preparation approach. **C)** SPR analysis of the binding affinity between purified CLDN18.2-Nanodisc and Zolbetuximab (affinity constant at 8.09 nM). **D)** DIMA's SyndiscTM can stimulate much stronger immune response than VLP.

DIMA Single B cloning platform

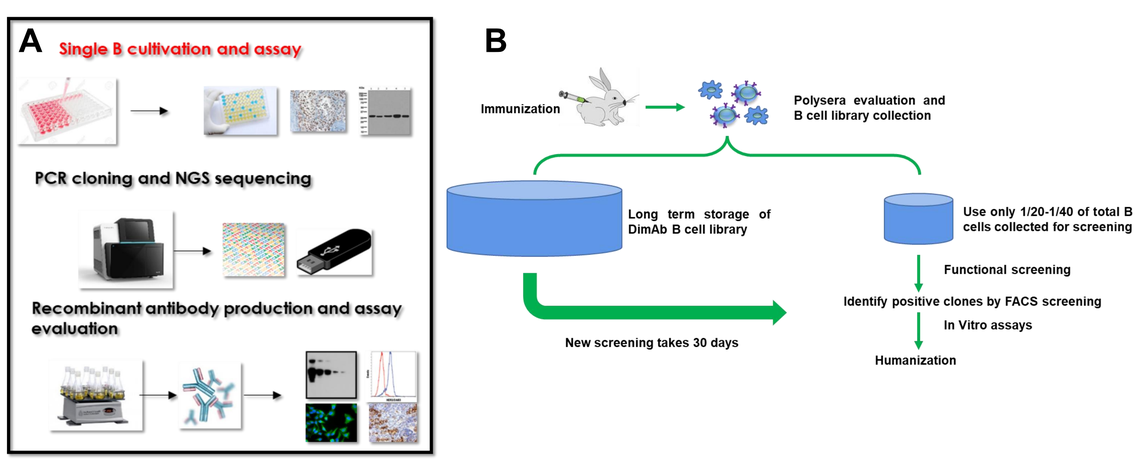

Figure 3. A) Dima single B cloning workflow. DIMA takes advantages of unique B cell culture method, which allows fast and precise isolation of positive antibody sequences. **B)** As rabbits are larger in size than mice, only 1/40-1/20 total B cells are used for screening and the rest of B cells are frozen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

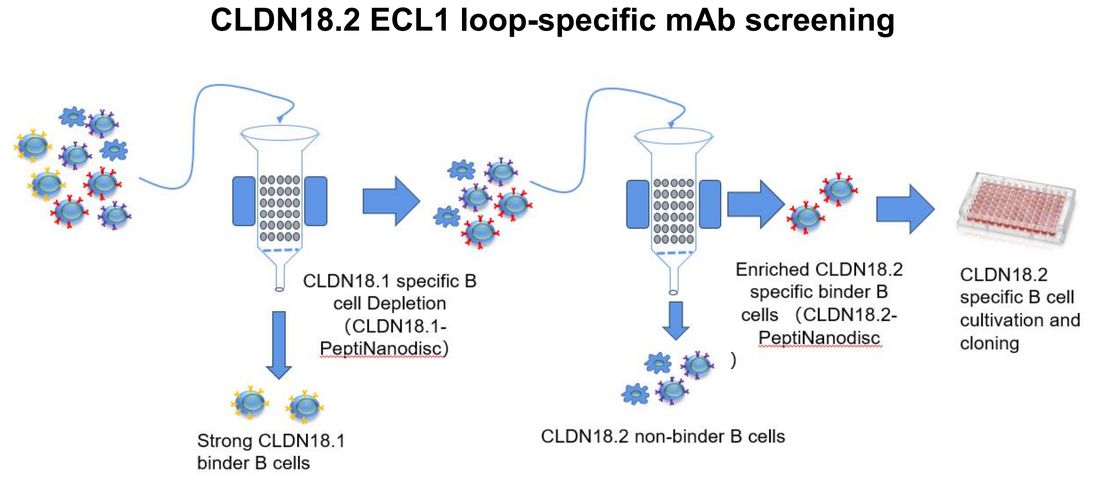

Figure 4. The flow chart of CLDN18.2 specific mAb cloning process. An alternative PeptiNanodisc depletion and enrichment processes were used to clone CLDN18.2 mAbs that exclusively target on ECL1 of CLDN18.2.

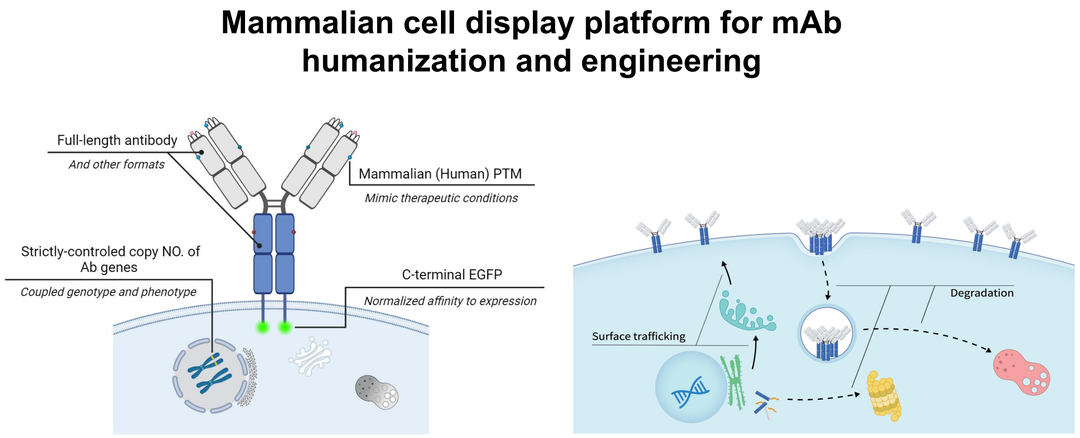

Figure 5. DiLibrary™ Mammalian cell display technology platform.

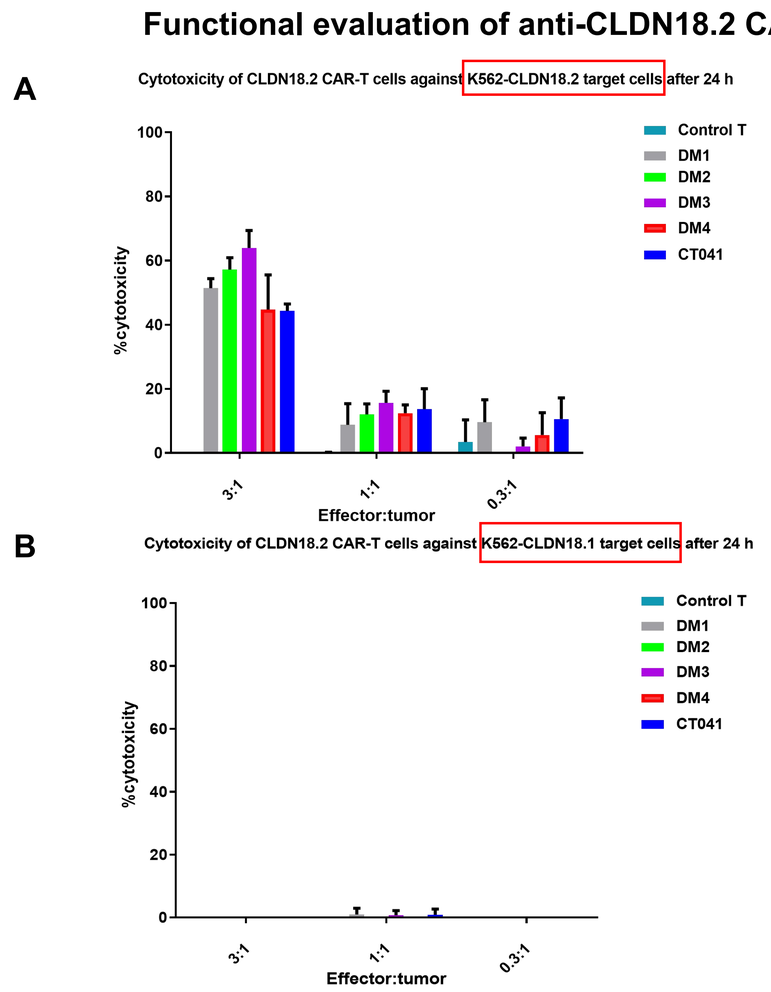

Figure 6. Four CLDN18.2 clones were humanized and constructed as CARs. All 4 clones exhibit strong ECL1 specific target cell killing efficacy. They show comparable efficacy as clinical stage BMK (CT041). (**A**. K562-CLDN18.2 overexpression cells. **B**. K562-CLDN18.1 overexpression cells.)

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 Syndisc™, PeptiNanodisc™ technology platforms provide 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 CLDN18.2, which is a four transmembrane protein. After functional evaluation, we identified molecules suitable for CAR T-cell therapy.