CARP: A High-Throughput Platform for Rapid VHH Discovery of Potent CAR-T Therapy

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Introduction

Conventional methods for CAR-T antibody discovery and screening presents significant bottlenecks, including the challenges in identifying high-quality antibodies with diverse epitope and broad affinity coverage. These methods also carry a high risk of overlooking functionally superior candidates or mistakenly identifying unsuitable leads for CAR development.

In this study, we develop **CARP** (**C**AR-T **A**I-powered **R**apid high-throughput screening **P**latform) as an efficient antibody discovery process to identify optimal CAR-VHHs with strong functionality and target antigen specificity. CARP was applied for CART therapy targeting Cadherin 17 (CDH17). After immunizing alpacas and llamas, we utilized the **VHHMAb®** platform to capture the full repertoire of binders through multiple proprietary methods. An AI-powered platform was then used to analyze hundreds of VHH hits for lineage determination (epitope coverage), humanized variant generation, and immunogenicity prediction. Over 400 pooled VHH CAR-T clones were screened using tumor cell line co-culture assays, designed to simulate long-term CAR-T/tumor cell interactions and assess target-specific expansion.

CARP identified top-ranked CAR-T clones with strong CDH17-dependent expansion capacity through Next-Generation Sequencing (NGS) and proliferation ranking. The platform enables high-throughput functional screening of binding hits, allowing for the selection of optimal VHHs that mediate robust, target-dependent CAR-T responses.

Methods

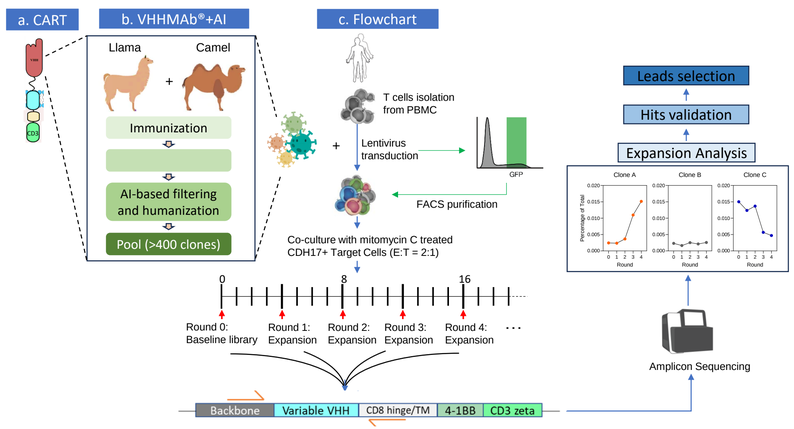

Figure 1. CARP high throughput screening workflow: It begins hits discovery by using **VHHMAb®** platform, followed by AI-powered VHH library construction that encompasses antibodies with diverse epitopes and low immunogenicity. This is followed by pooling CAR-T clones, co-culturing with target cells, performing NGS of amplicons from the amplified CAR-T clones, and concluding with lead validation.

Results

I. Pooled CART Preparation and Serial Proliferation with Target Cells

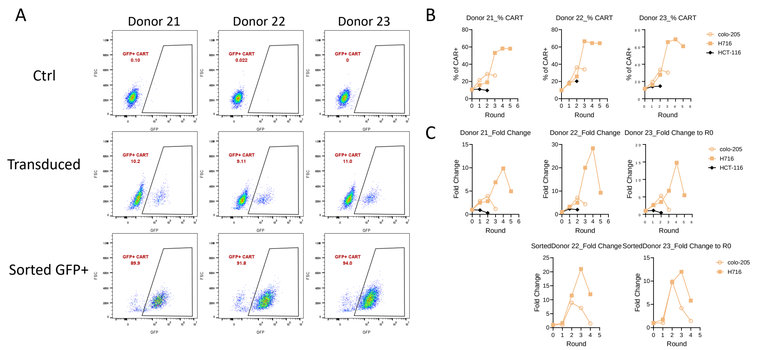

Figure 2. The preparation of pooled CAR-T and serial proliferation by co-culturing with target+ cell lines. After CAR-T preparation (A), the percentage (B) and fold change (C) of CAR-T proliferation were assessed after co-culturing with H716 (CDH17 high), Colo205 (CDH17 medium), and HCT-116 (CDH17 negative) cells.

II. Hit Proliferation Ranking by NGS and Slope Analysis

A

Effective CART cells	Grouping	Target cell
Donor 21	Group1	H716
	Group2	COLO205
Donor 22	Group3	H716
	Group4	COLO205
Donor 23	Group5	H716
	Group6	COLO205
Sorted Donor 22	Group7	H716
	Group8	COLO205
Sorted Donor 23	Group9	H716
	Group10	COLO205

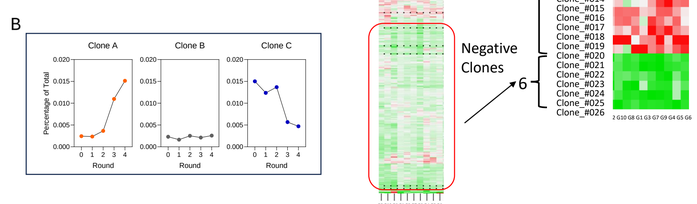

Figure 3. NGS and slope analysis for VHH hit ranking. (A) Grouping information. (B) The proliferation curve shows the clone percentage of each round based on NGS, with the slope of the curve calculated through fitting. Three examples are shown to demonstrate different types of slopes. (C) Hierarchical clustering analysis for lead selection. (D) Twenty positive and six negative clones were selected for validation.

Results

III. Lead Validation through individual Co-culturing with Target Cells

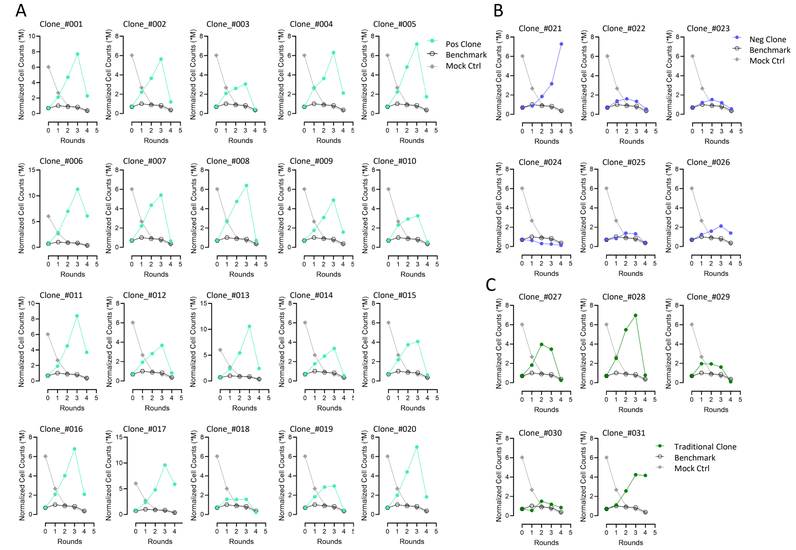

Figure 4. Leads validation to confirm CARP accuracy in lead selection. Selected CART leads were co-culturing with H716 cells. (A) In the group of positive clones, **19 out of 20** demonstrated strong CAR-T expansion. (B) In the group of negative clones, **5 out of 6** showed no or minimal CART expansion. (C) For comparison, conventional CAR-T leads (selected based on good binding affinity to proteins and cells) were tested, and only **2 out of 5** exhibited strong CAR-T expansion..

Conclusions

1. CARP provides a fast, powerful, and efficient screening method for identifying VHHs that demonstrate superior target-dependent CAR-T expansion.
2. Among the positive clones, 19 out of 20 exhibited superior functional expansion and robustness. In contrast, 5 out of 6 negative clones failed to proliferate, confirming CARP's accuracy in lead selection.
3. CARP identified VHHs that exhibited specific target domain enrichment and enhanced CAR-T expansion, outperforming clinical benchmarks.

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