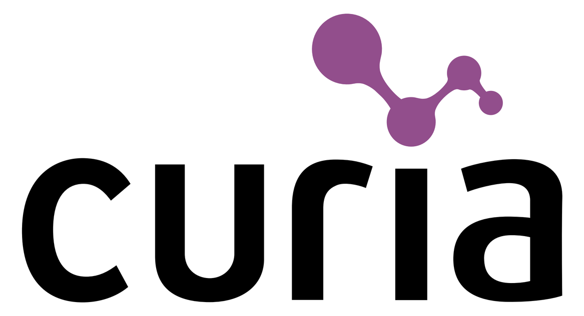
Curia's Hybridoma-based Antibody Discovery to Support CAR T Development



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ABSTRACT

Chimeric antigen receptor T cell (CAR T cell) therapy is effective in treating blood cancers and holds promise for treating solid tumors. The FDA has recently approved two types of CD19-targeting CAR T cells, tisagenlecleucel (Kymriah™, Novartis) in leukemia (August 2017) and lymphoma (May 2018) and axicabtagene ciloleucel (Yescarta™, Kite Pharma) in lymphoma (October 2017). Nearly 1,000 CAR T clinical trials are ongoing worldwide (Fig. 1). For the next generation of CARs, novel tumor antigen-binding mAbs are paramount. Here we describe the discovery of diverse, potent, fully human mAbs for two CAR T tumor targets.

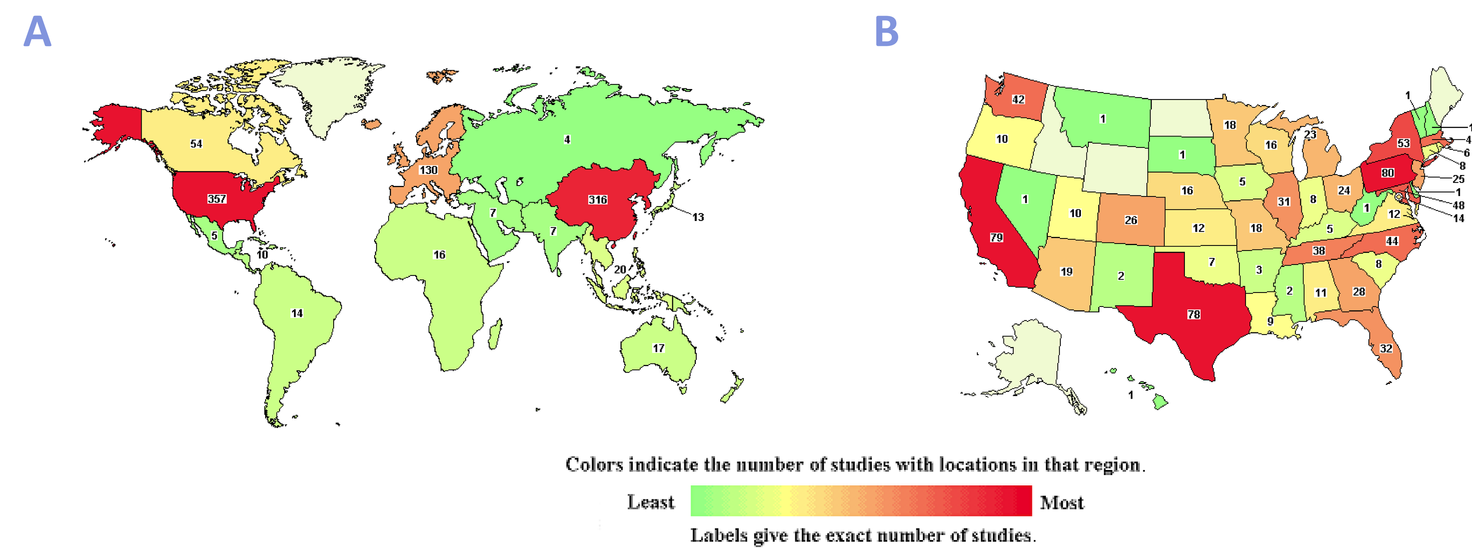


Figure 1. CAR T clinical trials. Geographic distribution of CAR T clinical trials worldwide (A) and specifically within the United States (B), as of October 2019, based on data retrieved from clinicaltrials.gov.

BACKGROUND

CARs are synthetic chimeric proteins introduced into patient T cells that commandeer antigenic specificity and redirect T cells to recognize tumor antigens and kill tumor cells. CARs typically comprise a single chain variable fragment (scFv) ectodomain originally derived from a tumor antigen-binding mAb; a transmembrane domain; a CD3ζ signaling domain, and usually one or two costimulatory domain(s) (Fig. 2). For a given CAR, it is difficult to predict *a priori* the optimal scFv tumor cell binding potency, affinity or target epitope specificity within a tumor antigen that will drive maximal CAR T efficacy. It is known that a wide range of scFv affinities (K_D 1 nM – 1 μM) can support effective anti-tumor CAR T-dependent killing. The position of the scFv target epitope within the tumor antigen can impact killing activity as well (e.g. a membrane-proximal epitope was superior to a membrane-distal epitope for a CD22-targeted CAR T). It may be advantageous to select scFvs from mAbs that do not trigger receptor internalization. It is also known that scFvs based on fully human variable domain sequences are less antigenic than mouse-derived antibodies and thus less susceptible to efficacy-killing anti-CAR responses *in vivo*. To maximize success and mitigate risk in CAR T development, a diverse selection of fully human target-binding mAbs is desirable.

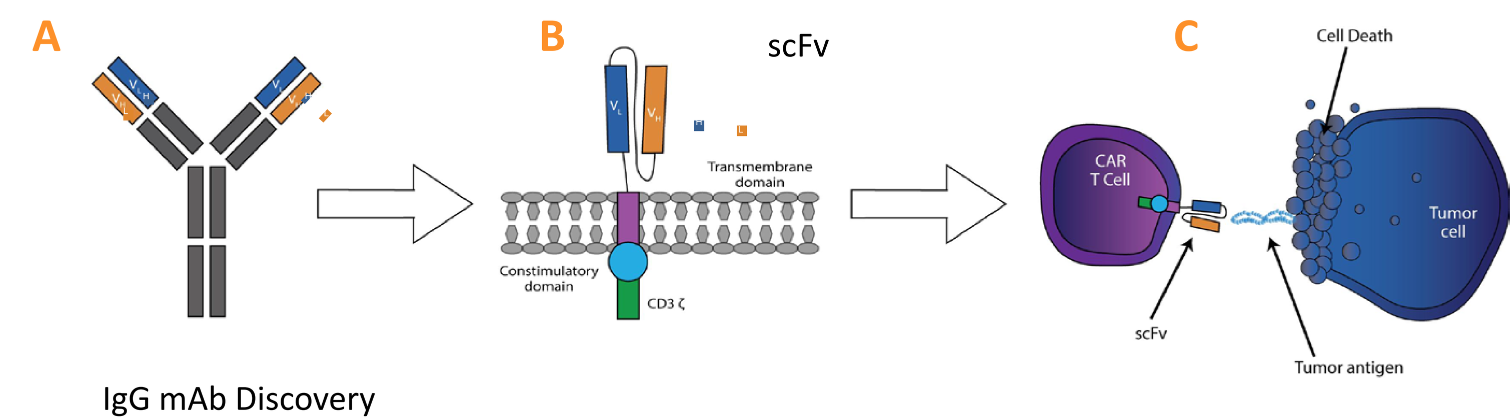


Figure 2. CAR Design and Tumor Cell Killing. (A) Tumor antigen-specific IgG monoclonal antibodies are identified by hybridoma-based antibody discovery. Immunizing transgenic mice that express fully human V_H and V_L domains reduces potential efficacy-limiting CAR antigenicity. (B) The V_H and V_L sequences are reformatted as a single chain fragment variable (scFv) domain, which comprises the CAR ectodomain. A transmembrane domain and intracellular signaling domain (e.g. costimulatory and CD3ζ sequences) complete the CAR. (C) Patient-derived T cells are typically transduced with a virus encoding the CAR construct, and these CAR T cells are expanded *in vitro* and infused into the patient where they specifically target and kill tumor cells.

RESULTS

TARGET A

Figure 3. In-life plasma titer checks over time identify mice with maximum titer for antibody recovery. Six differentimmu nization approaches were attempted (4 mice per cohort) using protein, DNA, and cellular immunogens. Mice immunized with Target A protein ECD (Cohort 1) or Target A protein ECD limited to the membrane-proximal domain (Cohort 4) using a HT-HOCK™ protocol (High Titer Hock injections) showed the best plasma titers. Plasma titers improved from D17 to D31/D38 in all mice in cohorts 1 (M1-4) and 4 (M5-8).

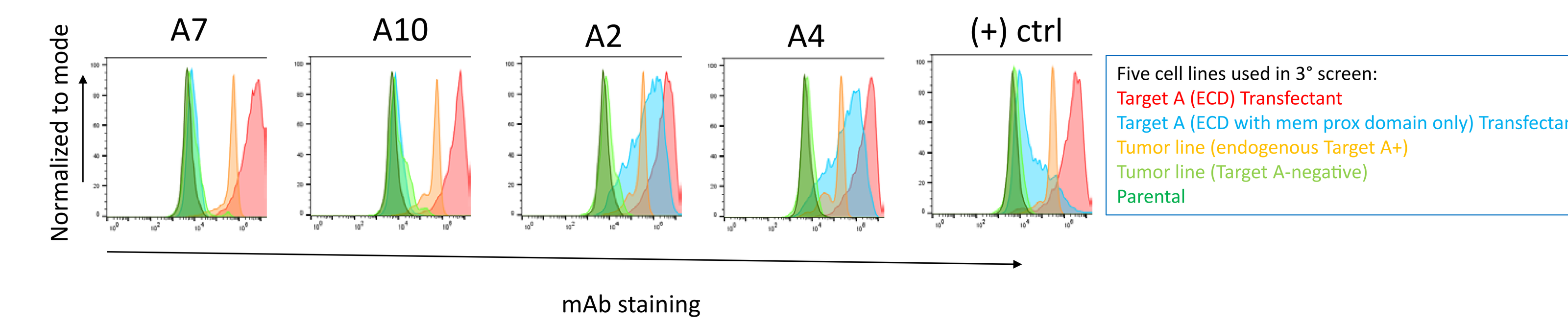
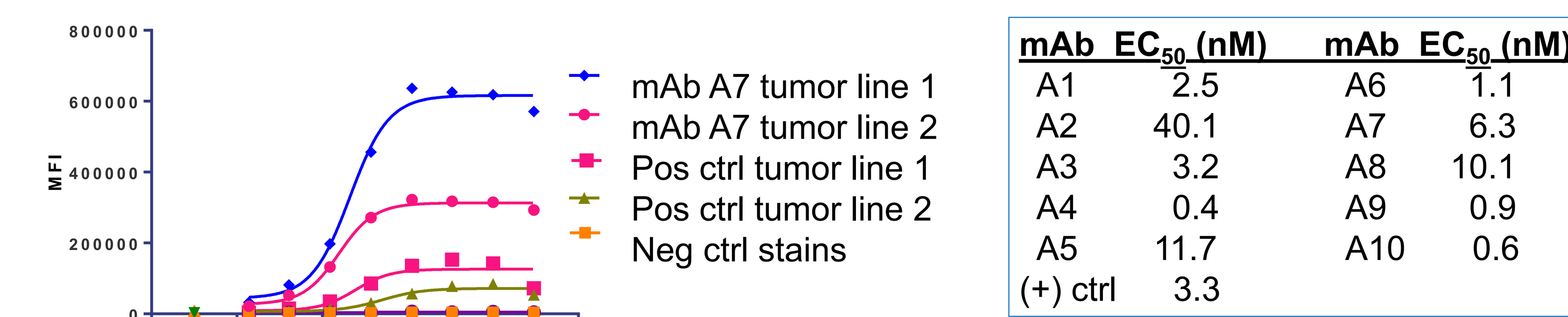


Figure 4. FACS tertiary screen confirms domain-specific Target A cell binding. 96 hits were identified in the primary ELISA screen. 33 hits confirmed in the 3° cell binding screen by FACS. 4 representative mAbs are shown. A7 and A10 originated from Cohort 1; A2 and A4 originated from Cohort 4. Antibodies arising from Cohort 4 stained cell lines expressing the membrane proximal domain only, as expected (*blue* staining).



mAb	EC_{50} (nM)	mAb	EC_{50} (nM)
A1	2.5	A6	1.1
A2	40.1	A7	6.3
A3	3.2	A8	10.1
A4	0.4	A9	0.9
A5	11.7	A10	0.6
(+) ctrl	3.3		

Figure 5. Tumor cell binding potency. FACS potency (EC_{50}) analysis of purified mAbs against tumor lines that express endogenous target. mAbs with a range of cell binding potencies (0.4 - 40.1 nM) were discovered, many exceeding the potency ofthe comparator a ntibody. Dose response curves are shown for mAb A7 and a (+) control mAb against 2 tumor lines (*left*), and EC_{50} values are shown for 10 lead candidates (*right*).

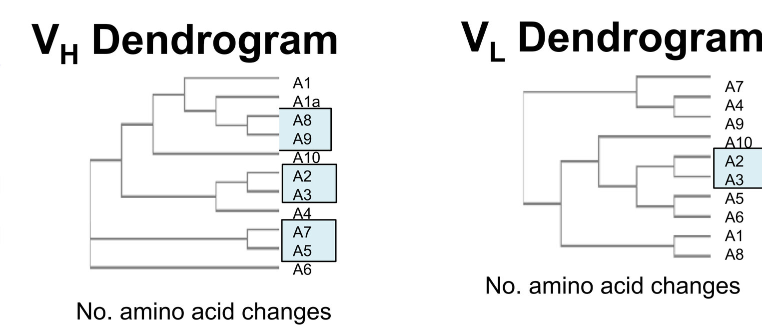


Figure 6. V_H and VL sequence diversity among Target A binders. Clustal alignment and dendrogram structures show relatedness of V_H and V_L sequences. mAb siblings based on V_H homology are indicated by shading.

TARGET B

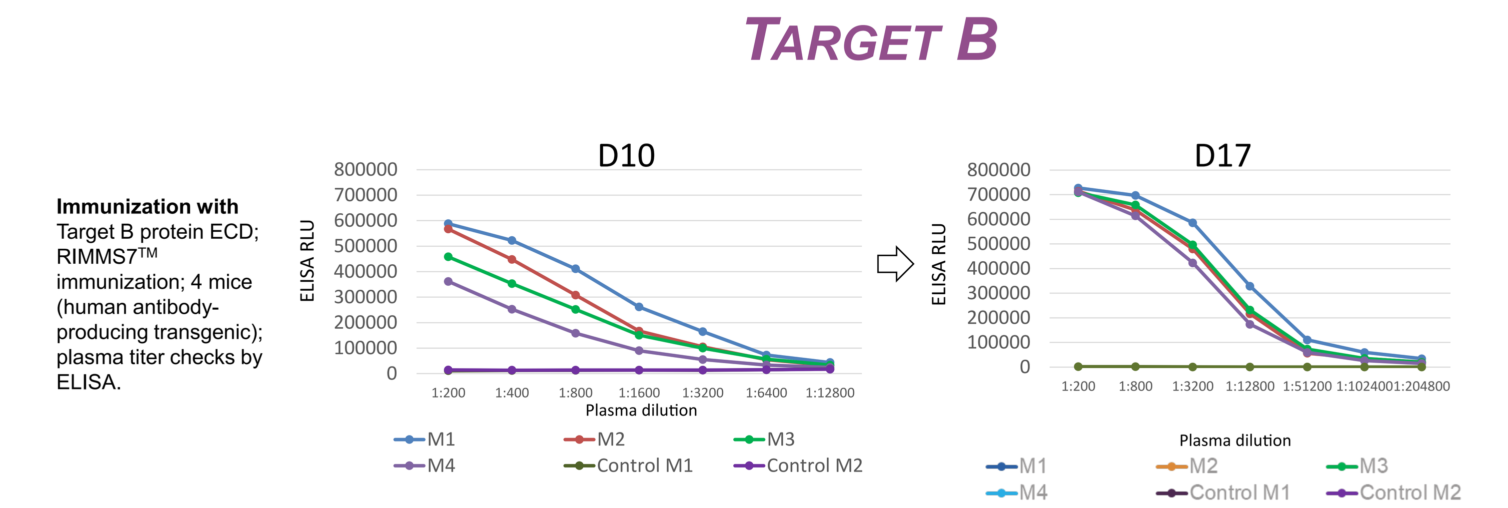


Figure 7. In-life plasma titer checks over time identify mice with maximum titer for antibody recovery. Mice were immunized using a RIMMS7™ protocol (Rapid Immunization Multiple Sites 7 (7 sites)). Plasma titers improved from D10 to D17 in all mice.

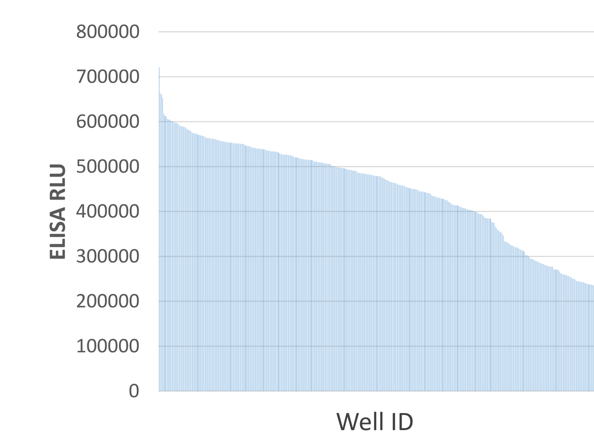


Figure 8. ELISA primary screen identified 384 hits with RLU >225,000. Ten (10) 384-well microplates were screened by ELISA against Target B protein. 104 hits confirmed in the secondary screen to bind Target B-positive cells by FACS.

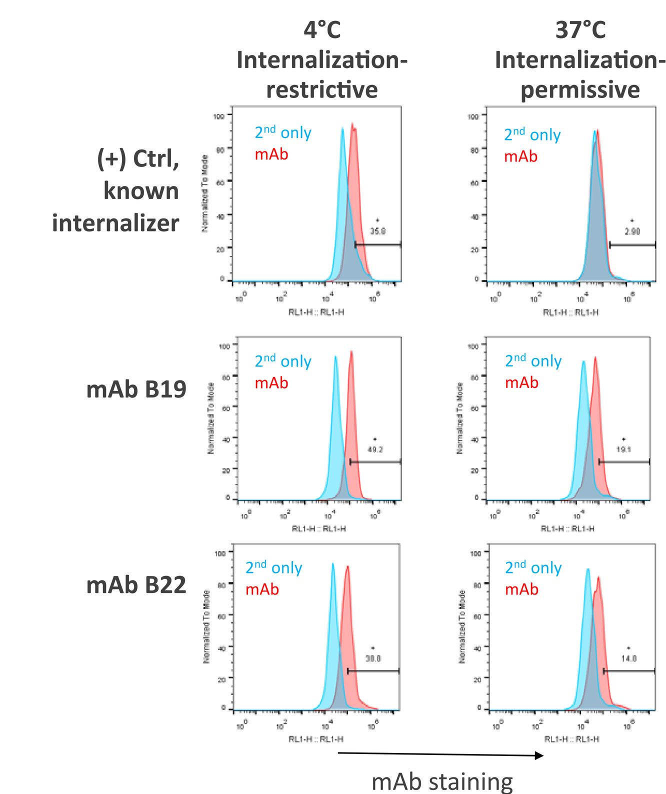


Figure 9. Identification of desired non-internalizing mAbs. Target B+ cell lines were incubated with discovered mAbs or positive control (10 µg/mL) at 4 °C, washed, and then incubated at internalization-restrictive (4 °C) or permissive (37 °C) temperatures for 1 h. Secondary antibody was then added to assess levels of surface staining by FACS. Surface staining with the (+) ctrl mAb, a known internalizer, was lostfol lowing 37 °C incubation, whereas surface staining with discovered mAbs B19 and B22 was retained.

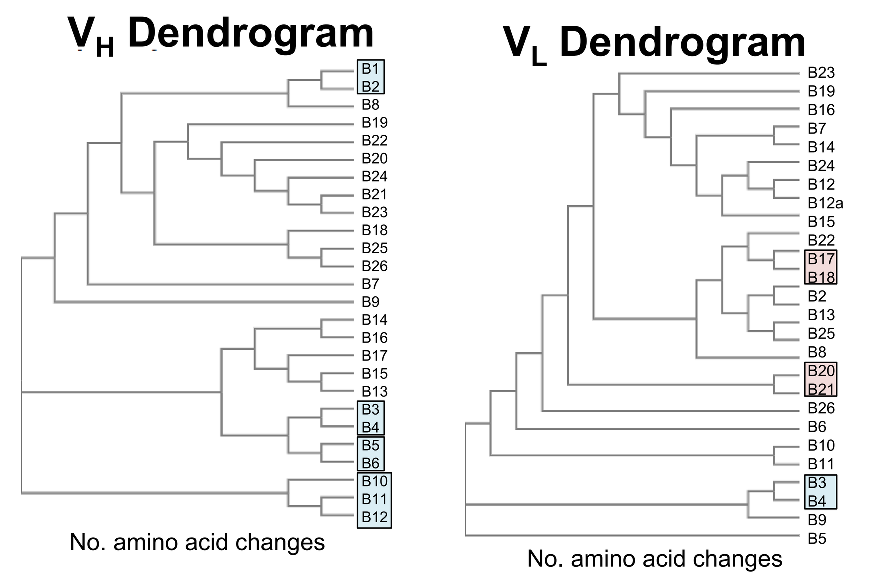


Figure 10. V_H and V_L Sequence Diversity among Target B Binders. Clustal alignment and dendrogram structures show relatedness of V_H and V_L sequences. mAb siblings based on V_H homology are indicated by blue shading; light chain siblings indicated by red shading.

CONCLUSIONS

- For Target A, we discovered many unique and diverse domain-specific leads. These IgGs are currently being reformatted as ectodomain scFvs for CAR development programs.
- For Target B, we discovered many unique diverse non-internalizing leads. These IgGs are currently being reformatted as ectodomain scFvs for CAR development programs.
- Given the large number of worldwide CAR T cell clinical trials (906), many of which are in states within or near Curia sites (CA, TX, MA) (Fig. 1), Curia is well-positioned to provide mAb discovery and CAR T cell development resources to help create new and effective cancer treatments.

Curia Provides Integrated Solutions for Biologics Development

San Carlos, CA	Belmont, CA	Hayward, CA	Worcester, MA	Hopkinton, MA
Antibody Discovery	Protein Engineering	Development + Manufacturing	Vector Engineering	Development + Manufacturing
Multiple discovery platforms available to support CAR T development			• AAV, lentivirus engineering and production • CAR T and stable cell line generation	

REFERENCES

[1]Watanabe et. al "Expanding the Therapeutic Window for CAR T Cell Therapy in Solid Tumors: The Knowns and Unknowns of CAR T Cell Biology", *Front Immunol Oct.* 2018.

Dotti et. al "Design and Development of Therapies using Chimeric Antigen Receptor-Expressing T cells", *Immunol Rev* Jan. 2014.

ClinicalTrials.gov