LB210

RenNano® mice: a heavy-chain-only antibody platform for the generation of nanobody therapeutics

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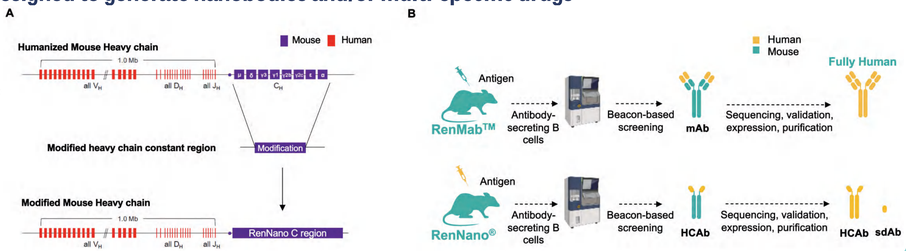
INTRODUCTION

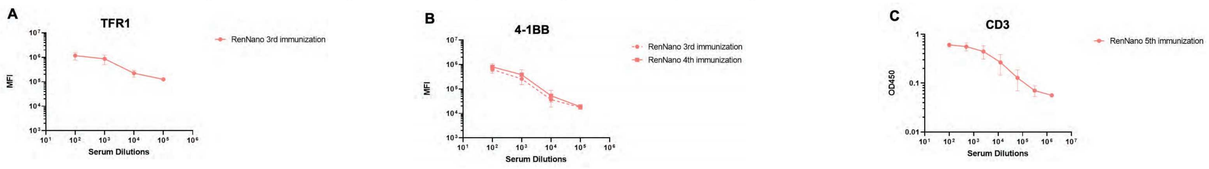
Monoclonal antibodies have been used to successfully treat various diseases, including tumors, autoimmune diseases, and infectious diseases. Traditional antibodies are comprised of a tetramer of two heavy chains and two light chains, totaling 150 kDa in molecular weight. However, the large size of antibodies can limit the therapeutic application; in particular, the penetration of tumors and the blood brain barrier (BBB) is not always feasible. In contrast to traditional antibodies, heavy chain-only antibodies (HCAbs) are significantly smaller (~75 kDa), as they contain only two heavy chains. Since the heavy chain variable domain of HCAbs (i.e., VHH or single domain antibody, sdAb, or nanobody) is solely responsible for antigen recognition, nanobodies can function independently as a therapeutic molecule, which may be advantageous for penetrating tumors or the BBB. Previously, we generated a fully human antibody mouse platform, RenMab™, in which the murine heavy chain and kappa light chain variable domains were replaced by the full human heavy chain and kappa light chain V(D)J loci in situ. Here, we have further modified the RenMab™ model to generate a fully human heavy-chain-only antibody mouse model, termed RenNano®. The modified heavy chain constant regions of RenNano® mice allow them to spontaneously produce HCAbs. Flow cytometry and biolayer interferometry confirmed that RenNano®-derived HCAbs can bind antigens without light chains. Despite this reliance on the heavy chain only variable regions for antigen specificity, RenNano® mice can generate antigen-specific antibodies with high affinity (10^{-8}-10^{-9} KD) upon immunization with various antigens. In addition, many RenNano®-derived HCAbs exhibited a longer CDR3 length, which could promote the recognition of difficult-to-reach epitopes. Furthermore, RenNano®-derived HCAbs have favorable diversity, and excellent developability properties such as a higher degree of hydrophilicity. Anti-4-1BB HCAbs can also activate 4-1BB-NF-κB signaling in a dose-dependent manner, as demonstrated in reporter assays. In summary, the full human heavy-chain-only antibody mice, RenNano®, can produce human HCAbs with high affinity and good efficacy. Thus, RenNano® is a powerful platform to discover HCAb/nanobodies for various therapeutic applications.

The RenNano® platform is designed to generate nanobodies and/or multi-specific drugs

Generation of the RenNano® mouse and its applications for sdAb and multispecific antibody discovery. A. Using chromosome engineering, the entire variable region of the mouse heavy chain was replaced by its human counterpart *in situ* (to generate RenMab™ fully human antibody mice). To generate RenNano® mice, further modifications were made to the RenMab™ constant region to produce humanized heavy-chain-only antibodies (HCAb). **B.** RenNano® mice produce HCAbs upon immunization by various antigens. The variable region of HCAbs can be developed into drugs alone as SdAbs, or two HCAbs/SdAbs can be easily assembled to construct multispecific antibody drugs.



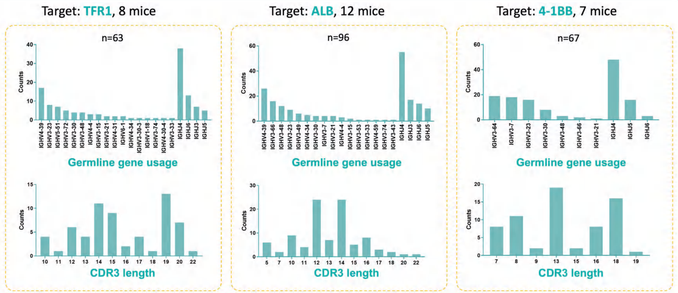
RenNano® mice generate strong immune responses against multiple antigens



RenNano® immune responses against TFR1, 4-1BB and CD3. A and B. Sera from RenNano mice immunized with TFR1 or 4-1BB were diluted and incubated with antigen-expressing CHO cells. Alexa Fluor 647-conjugated anti-mIgG secondary antibody was used to label the HCAb bound to the surface of the CHO cells, and mean fluorescence intensity (MFI) was measured using flow cytometry to indicate the antigen-specific antibody titer. **C.** ELISA was used to evaluate the antigen-specific antibody titer in RenNano mice immunized against CD3. Serum from immunized RenNano mice were incubated with the antigen-coated plates, and detected by HRP-conjugated anti-mIgG. OD450 measurements were used to determine the antigen-specific antibody titer.

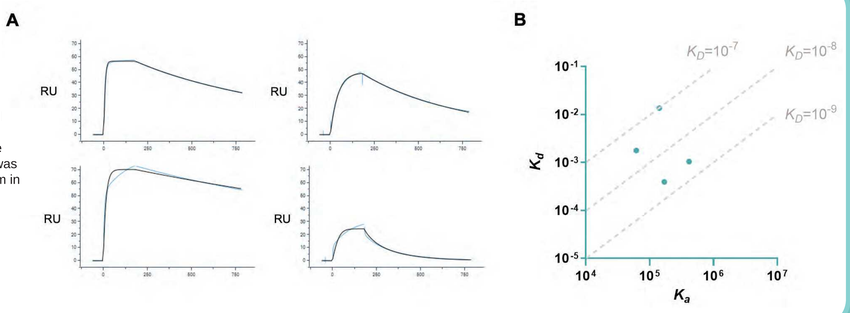
Sequence diversity of antigen-specific HCAbs derived from RenNano® Mice

HCAbs derived from RenNano mice are diverse and many contain longer CDR3 regions. Sequences of HCAbs with antigen specificity were analyzed by their heavy chain germline usage and CDR3 length. Analysis indicates broad IGHV germline diversity, indicating normal heavy chain recombination. For CDR3 length, most CDR3 lengths observed were longer than 12 AA, with some longer than 17 AA, which is commonly seen in HCAbs.



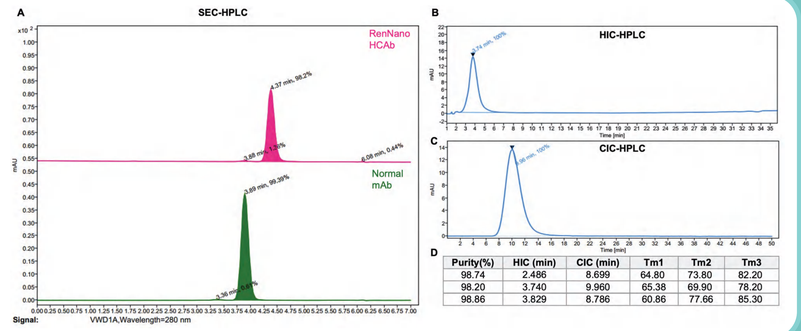
High affinity of HCAbs derived from RenNano® mice

RenNano HCAbs bind antigen with high affinity. A. 4 purified RenNano® anti-h4-BB HCAbs were immobilized on a protein A conjugated sensor chip as the ligand, then binding of the analyte (antigen h4-1BB protein) was measured under flow. Response was recorded as resonance units (RU) and displayed as a sensogram in real time. **B.** The kinetic binding constants were presented in a scatter plot. The K_D of 2 HCAbs were lower than the 10^{-8} level.



Favorable developability of HCAbs derived from RenNano® mice

Favorable developability of HCAbs derived from RenNano® mice. A. RenNano HCAbs show high purity after one step purification, as measured by SEC-HPLC. Due to the absence of light chains and CH1 domain, the molecular size of HCAb is lower than normal antibodies; therefore, HCAb showed longer retention time in the SEC-HPLC. **B-C.** Short retention time (RT) in HIC-HPLC and CIC-HPLC indicates superb hydrophilicity and specificity of RenNano HCAbs. **D.** Reasonable thermostability was confirmed by differential scanning fluorimetry (DSF). Summary chart of developability of three RenNano HcAbs.



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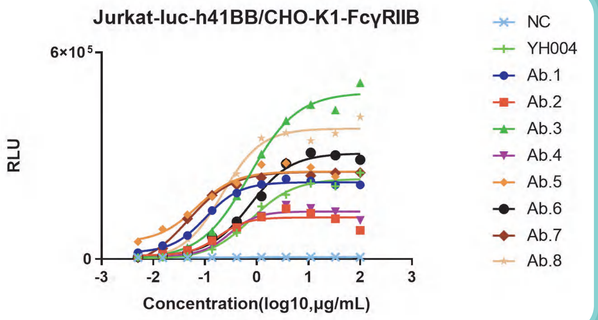
Purity(%)	HIC (min)	CIC (min)	Tm1	Tm2	Tm3
98.74	2.486	8.699	64.80	73.80	82.20
98.20	3.740	9.960	65.38	69.90	78.20
98.86	3.829	8.786	60.86	77.66	85.30

In vitro function of RenNano® anti-4-1BB HCAbs

Anti-4-1BB HCAbs activate 4-1BB-NF-κB signaling in a dose-dependent manner. PBS(NC), YH004 (an anti-4-1BB mAb as positive control) or RenNano HCAb Ab.1-Ab.8 were incubated with 4-1BB reporter Jurkat cells simultaneously co-cultured with FcγRIIB-expressing CHO-K1 cells. The activation of NF-kB signaling triggers luciferase expression in the reporter cells, which was quantified by luminescence after substrate addition.



SUMMARY

- RenNano® mice contain all human V,D, and J genes *in situ* with a modified murine constant region designed to generate HCAbs *in vivo*.
- RenNano® mice mount immune responses in response to multiple antigens.
- RenNano®-derived HCAbs exhibit diversity in germline gene usage and CDR3 length, and demonstrate high affinity and favorable developability characteristics.
- RenNano®-derived HCAbs are functional *in vitro*.
- RenNano® is a robust and powerful platform to discover HCAb/nanobodies for various therapeutic applications.

RenNano®

LB211

Discovery of RenNano®-derived human heavy-chain-only antibodies that cross the blood-brain barrier

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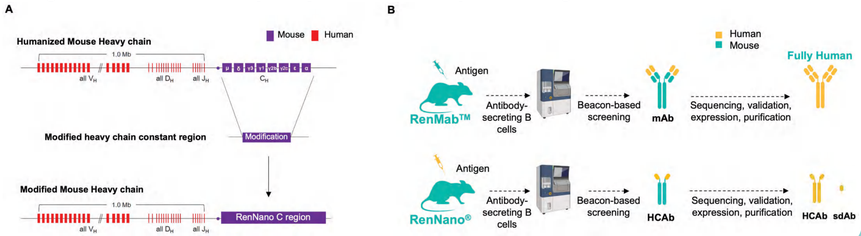
INTRODUCTION

The utility of conventional antibodies for neurological conditions is limited by the blood-brain barrier (BBB). Several strategies to address this issue have been reported, including receptor-mediated transcytosis (RMT) of antibodies using transferrin receptors. We hypothesize that this strategy could be further improved by the use of single-domain antibodies (sdAbs), which are significantly smaller, and therefore could be used to more efficiently transport drugs of interest across the BBB.

To this end, we developed anti-transferrin receptor 1 (TFR1) HCAbs utilizing our full human heavy-chain-only antibody mice (RenNano®). We immunized RenNano® mice with recombinant TFR1 proteins, isolated the B cells from spleen and lymph nodes, and performed single B cell antibody screening using the Beacon® Optofluidic system. Most of the antibodies tested were cross-reactive to human and monkey TFR1. The affinity of these HCAbs can reach 10^{-8}~10^{-9} (K_D). Of the 7 HCAbs we tested, 6 were internalized into the human brain microvascular endothelial cell line, hCMEC/D3.

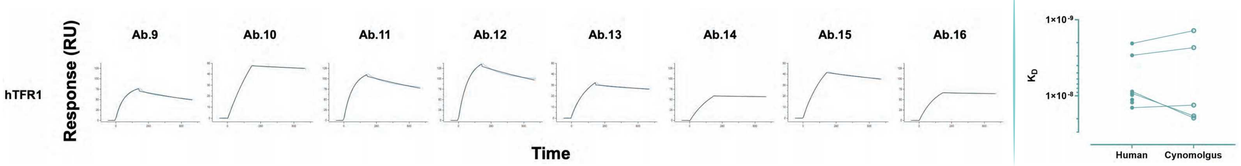
To assess brain penetration of these antibodies *in vivo*, mice expressing human TFR1 (hTFR1 mice) received a tail vein injection with either isotype control, Pabinafusp Alfa (a BBB-penetrating anti-TFR1 antibody conjugate) analog as positive control, or RenNano derived HCAbs. After 0.5, 6, 24, 72 h of exposure, mice brains were dissected for the quantification of hIgG and immunofluorescence. The level of anti-TFR1 HCAbs in the brain parenchyma was significantly higher than isotype controls and JR-141 analog. In brain sections, HCAbs can be clearly observed in the parenchyma, and were colocalized with mTUJ1 cells (neurons). These results demonstrate that HCAbs developed from RenNano® mice are able to penetrate the BBB. Taken together, these data highlight the tremendous potential for HCAbs and its variable domain sdAbs for transporting cargo across the BBB.

The RenNano® platform is designed to generate nanobodies (sdAb) and/or multi-specific drugs



Generation of the RenNano® mouse and its applications for single-domain antibody (sdAb) and multispecific antibody discovery. A. Using chromosome engineering, the entire variable region of the mouse heavy chain was replaced by its human counterpart in situ (to generate RenMab™ fully human antibody mice). To generate RenNano® mice, further modifications were made to the RenMab™ constant region to produce humanized heavy-chain-only antibodies (HCAb). **B.** RenNano® mice produce HCAbs upon immunization by various antigens. The variable region of HCAbs can be developed into drugs alone as SdAbs, or two HCAbs/SdAbs can be easily assembled to construct multispecific antibody drugs.

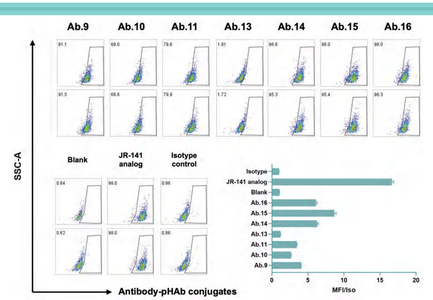
High affinity of TFR1 HCAbs screened from RenNano® mice



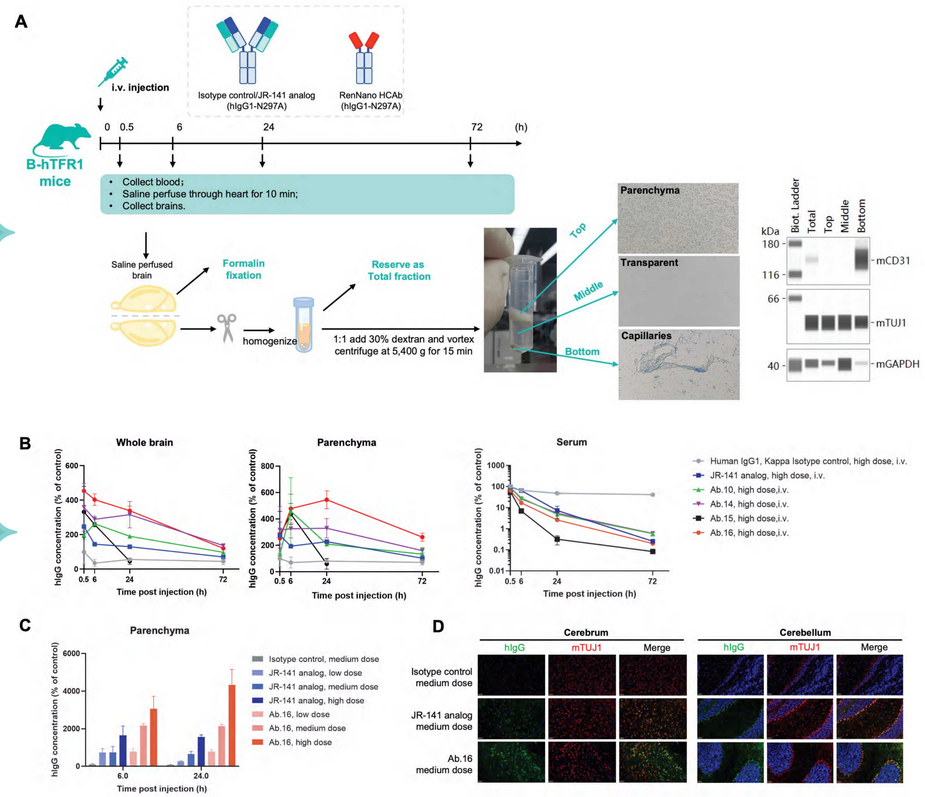
RenNano®-derived HCAbs (screened from 4 mice) exhibited high affinity and cross-species reactivity to TFR1. 5/8 of the purified antibodies tested demonstrate affinity in the nm range as assessed by SPR. 5/8 were cross-reactive to cynomolgus TFR1.

In vitro internalization of HCAbs from RenNano® mice

Anti-TFR1 HCAbs can be efficiently internalized by hCMEC/D3 cells. Mechanistically, anti-TFR1 crosses the BBB via transcytosis initiated by TFR1-expressing cells in the BBB. Internalization of the antibodies is the primary step. The isotype control, JR-141 analog (positive control) or RenNano HCAbs were conjugated to pHAb Amine- and Thiol-Reactive Dyes. pHAb dye-conjugated antibodies were then incubated with hCMEC/D3, a human brain microvascular cell line which expresses TFR1. Upon receptor-mediated internalization, antibody-pHAb conjugates traffic through the endosome and lysosomal system. At low pH, the antibody-pHAb conjugates fluoresce, which was detected by flow cytometry.



Penetration of anti-TFR1 HCAbs across the blood-brain barrier

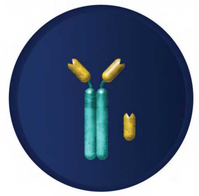


Anti-TFR1 HCAbs have the capacity to cross the BBB. A. Humanized B-hTFR1 mice were injected through tail veins with isotype control, JR-141 analog or RenNano-derived anti-TFR1 HCAbs (hIgG1). At 0.5, 6, 24 or 72 hours after exposure, serum was collected. At the endpoint, anesthetized mice were perfused by a saline injection through the left ventricle for 10 mins. The right hemisphere of the brain was subjected to immunofluorescence, and the left was homogenized and subfractionated (via dextran addition and centrifugation) to detect hIgG concentration. The homogenate was separated into three layers. The top layer consisted of neurons; the middle layer was clear and cells were rarely observed; the bottom pellet consisted of fibrous structures which could contain blood vessels. Protein was extracted from each fraction and immunoblots were performed to detect mCD31 (endothelial cell specific marker), mTUJ1 (neuron specific marker) and mGAPDH abundance in each fraction, which demonstrated the successful separation of capillaries and parenchyma. **B.** hIgG concentration in total fraction (whole brain), top fraction (parenchyma) and serum was detected by electrochemiluminescence (Meso Scale Discovery). For each plot, the Y axis was molar concentration and standardized relative to isotype control at 0.5 h. **C.** hIgG concentration in parenchyma exhibited a dose-dependent trend. For most conditions, Ab.16 concentration was significantly higher than JR-141 analog. Low, medium and high does respectively referred to 1,3,10 mg/kg for HCAbs, and the same molar dose for isotype control or JR-141 analog. **D.** Immunofluorescence for hIgG and mTUJ1 in the brain sections from mice injected with isotype control, Ab.16 or JR-141 analog 24 h prior to analysis. Both JR-141 analog and Ab.16 labeling can be observed in the neurons.

SUMMARY

- TFR1-targeting HCAbs developed from RenNano® mice have high affinity to human TFR1, and have functional capabilities, including internalization capacity.
- hTFR1-targeting HCAbs are able to penetrate the BBB efficiently, as evidenced by fractionation studies and histological analyses.
- Together, these data highlight the tremendous potential for HCAbs and its variable domain sdAbs for transporting cargo across the BBB. Due to their smaller size and simpler structure, sdAbs could ultimately provide therapeutic benefit for neurodegenerative diseases, and offer promising potential for tumor penetration.

RenNano®



2752

Targeting Intracellular Tumor Antigens Using Fully Human TCR Mimic Antibodies Derived From HLA Transgenic RenMice™

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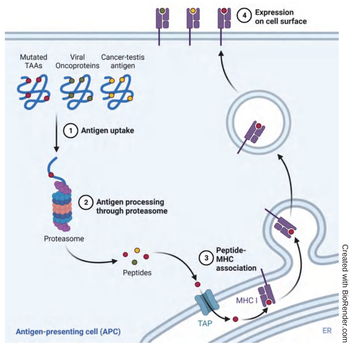
ABSTRACT

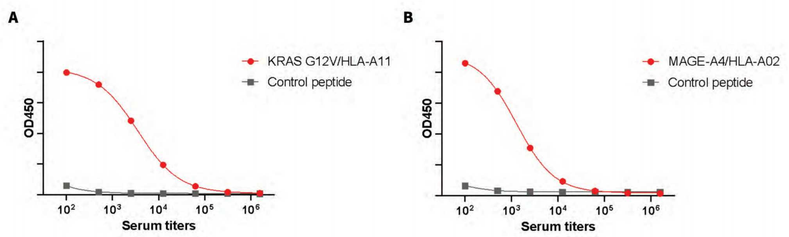
Therapeutic antibodies have ushered in a new age of cancer immunotherapy. Historically, these therapies have targeted a limited subset of soluble and cell surface tumor-associated antigens (TAAs). T cell receptors (TCRs) on cytotoxic CD8+ T cells recognize peptide antigens bound to major histocompatibility class I (MHC-I) proteins, called HLA-A/B/C in humans. By this pathway, antigen is regularly sampled from the intracellular peptidome, processed, and presented to cytotoxic T cells. Expanding TCR-based recognition of soluble, intracellular TAAs presented on the surface of malignant cells by this mechanism is a propitious therapeutic strategy. Here we describe a novel platform for generating T cell receptor mimic (TCRm) antibodies using our humanized immunoglobulin (RenMab™) mice engineered to express HLA. TCRm antibodies have the same binding properties as endogenous TCRs and recognize processed, HLA-bound peptides including intracellular tumor associated antigens, viral oncoproteins, and cancer-testis antigen (CTA). TCRm antibodies bind peptide-HLA with high specificity and up to nanomolar affinity. Our optimized immunization protocols and high-throughput screening methods allow for one-step TCRm antibody generation. TCRm antibodies can also be used to assemble bispecific T cell engaging antibodies (BiTEs) to enhance tumor targeting of cytotoxic T cells. Biocytogen's TCRm antibodies are a flexible and powerful tool for cancer immunotherapy. By enabling TCR-mediated recognition of an unrestricted repertoire of cancer neoantigens, TCRm antibodies may find broad clinical application.

Strategy to target intracellular tumor-associated antigens

- **An abundance of potential tumor-associated antigens (TAAs) exist inside tumor cells, including mutated proteins, viral oncoproteins, and cancer-testis antigen (CTA).**
- **Peptide/MHC-I complexes (pMHC), derived from intracellular antigens, are presented on the cell membrane for recognition by T cells.**
- **Can we discover a novel approach to target intracellular TAAs/pMHC to expand cancer therapeutic possibilities, in addition to recognition by endogenous T Cell Receptors (TCR)?**

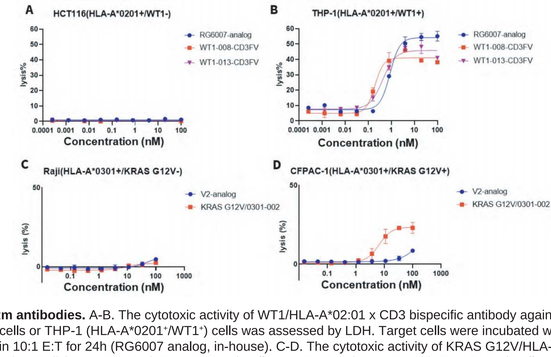


HLA-humanized RenMab mice generate specific immune responses to pHLA



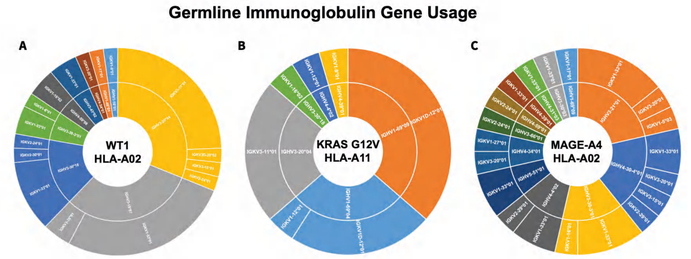
Sera from immunized HLA/RenMab mice were assessed by ELISA. A. Serum titration of KRAS G12V/HLA-A11. B. Serum titration of MAGE-A4/HLA-A02.

TCRm antibodies promote tumor cell killing in an antigen-specific manner



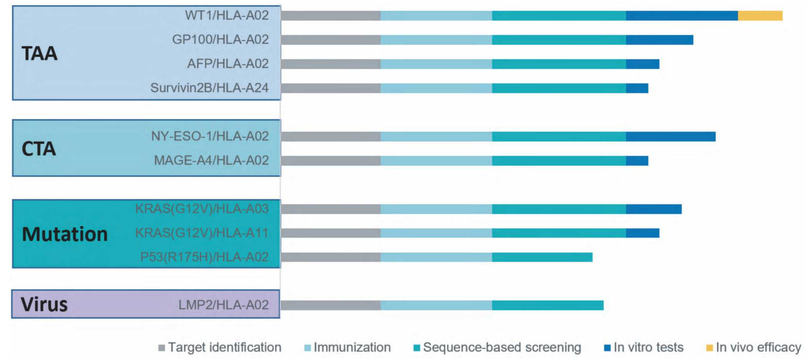
Cytotoxicity of TCRm antibodies. A-B. The cytotoxic activity of WT1/HLA-A*02:01 x CD3 bispecific antibody against HCT116 (HLA-A*0201^{+}/WT1^{neg}) cells or THP-1 (HLA-A*0201^{+}/WT1^{+}) cells was assessed by LDH. Target cells were incubated with antibody and human CD3^{+} T cells in 10:1 E:T for 24h (RG6007 analog, in-house). C-D. The cytotoxic activity of KRAS G12V/HLA-A*03:01 x CD3 bispecific antibody against Raji (HLA-A*0301^{+} /KRAS G12V^{neg}) cells or CFPAC-1 (LA-A*0301^{+} /KRAS G12V^{+}) cells was assessed by LDH. Target cells were incubated with antibody and human CD3^{+} T cells in 10:1 E:T for 72h (V2 analog, in-house).

Biocytogen's TCRm platform enables genetic diversity of antibody sequences



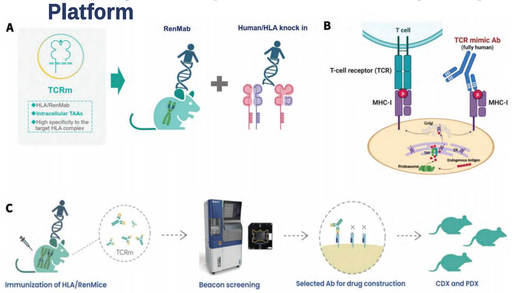
A variety of fully human antibody sequences can be generated from HLA/RenMab mice. Heavy chain and light chain germline usage of A. WT1/HLA-A02(tumor-associated antigen); B. KRAS G12V/HLA-A11 (mutation antigen); C. and MAGE-A4/HLA-A02 (cancer-testis antigen).

Pipeline of Biocytogen's TCRm discovery



Biocytogen's TCRm discovery progress. Each potential peptide will generate an antibody library through immunization.

Biocytogen's Fully Human TCR-mimic (TCRm) Antibody Discovery Platform

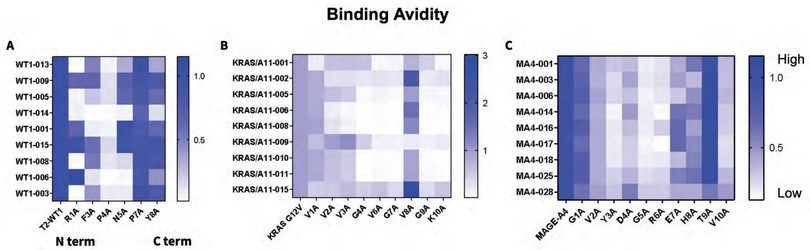


TCRm Platform Overview. A. Knock-in of Human HLA on RenMab™ background (fully human antibody mouse) results in antibodies with fully human variable domain sequences that can recognize pHLA. B. Mechanism of TCR-mimic antibody recognition of the pHLA complex. C. Biocytogen's fully human TCRm antibody discovery workflow. Following a proprietary immunization procedure to generate TCRm antibodies *in vivo*, tissues from the mice are subjected to Beacon® on-chip screening for fast, high-throughput discovery. Binders are selected for further *in vitro* and *in vivo* screening.

Advantages

- Does not rely on *in vitro* phage or yeast display
- Does not require human donors
- *in vivo* selection of high affinity binders
- Less cross-reactivity
- Naturally paired heavy and light chains

Diverse recognition sites ensure high specificity of antibody discovery



Binding avidity of WT1, KRAS G12V and MAGE-A4 alanine substituted peptides, as determined by flow cytometry MFI relative to isotype. WT1, KRAS G12V and MAGE-A4 peptide are used as control, and the positions are arranged from N-terminus (left) to C-terminus (right). Multiple WT1 antibodies bind to the N-terminal half of the peptide (Fig. A), KRAS G12V antibodies bind strongly to the C-terminal half of the peptide (Fig. B), while the MAGE-A4 antibodies show specific reactivity toward middle fraction of the peptide (Fig. C).

SUMMARY

- Biocytogen's TCR-mimic (TCRm) antibody platform can be deployed to generate antibodies in vivo to target pHLA complexes for mutated proteins, viral oncoproteins, and cancer-testis antigen (CTA)
- Germline gene usage and avidity assessments demonstrate the diversity of TCRm antibodies that can be generated using the platform
- When assembled via a T Cell engager strategy, TCRm sequences discovered using our platform demonstrate the ability to kill tumor cells in an antigen-specific manner

5320

The RenMice™ HiTS (Hyperimmune Target Specific) Platform Facilitates Identification of Novel Therapeutic Antibodies for Challenging Targets

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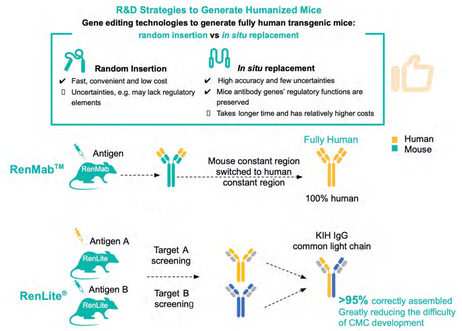
ABSTRACT

In recent years, an increasing number of therapeutic antibodies have shown to be effective for the treatment of cancer and other diseases. However, limitations in the traditional discovery process, including immune tolerance of highly homologous genes, challenges with antibody sequence humanization, clone selection, and model selection for drug efficacy and safety evaluation, often hinder the process of identifying new therapeutic antibodies. The RenMice™ HiTS (Hyperimmune Target Specific) Platform is a library of chromosome engineered mice with fully human immunoglobulin variable domains replacing the mouse loci, each with a specific drug target gene knocked out. These mice are designed to establish robust immune responses and generate antibodies that bind to more epitopes of the target protein, including conserved domains. The platform is ideal for challenging targets, such as proteins with high homology across species, or multi-pass transmembrane proteins (e.g. GPCRs/ion channels). Here, we show that the platform can be used to generate antibodies that cross-react with multiple species, like human, monkey, dog, and mouse targets, by immunizing with both human and mouse or dog antigen. We provide examples for newer campaigns, including species cross-reactivity and internalization of novel antibodies targeting NECTIN-4, and high-throughput in vivo efficacy screening of novel anti-PD-1 antibodies in wild-type mice. In the future, we will evaluate the preliminary toxicity of these cross-reactive antibodies in preclinical animal models. Thus, selection of the best antibody candidate based on in vivo efficacy and safety allows for a streamlined and successful preclinical phase. In conclusion, the RenMice™ HiTS platform facilitates the generation of developable antibodies that recognize novel epitopes and challenging targets.

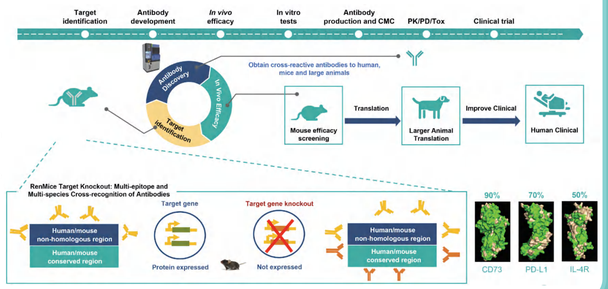
Introduction to RenMice™

Overview of RenMab and RenLite mice generation. Both models were modified to contain human antibody variable domains using in situ replacement, which has several advantages over random insertion. RenMab mice contain the full human variable domain, while RenLite mice generate antibodies with a common human kappa light chain. Once antibodies are generated in the mice, the murine constant region is swapped, yielding fully human antibodies.

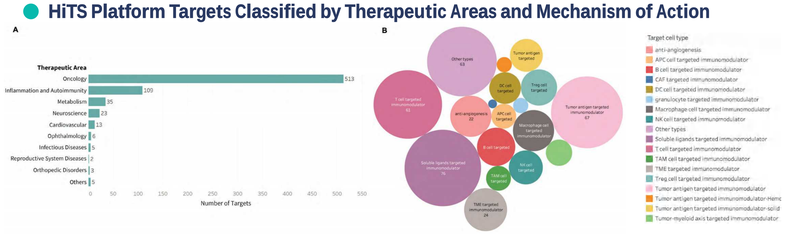


Introduction to RenMice™ HiTS Platform

Overview of the RenMice HiTS (Hyperimmune Target Specific) Platform. The platform utilizes specialized strains of RenMice modified to lack the target gene of interest, thereby resulting in a more robust immune response and cross-recognition of both human and mouse antigens. The platform is ideal for antigens with high levels of homology between mouse and human. After the cross-reactive antibodies are generated and selected, they can be screened for efficacy in mice and larger animals.

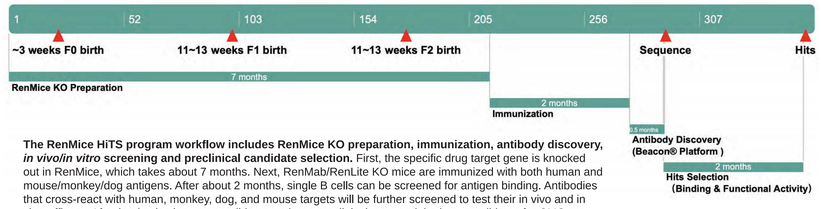


HiTS Platform Targets Classified by Therapeutic Areas and Mechanism of Action



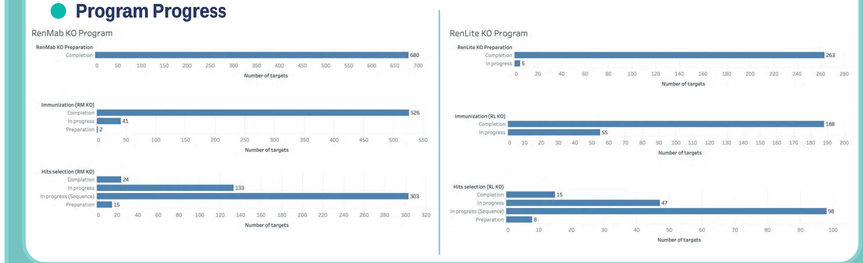
Therapeutic areas and mechanisms targeted by the RenMice HiTS Platform. For a full list of targets, visit RenMab.com/ko-library.

RenMice™ HiTS Program Workflow



The RenMice HiTS program workflow includes RenMice KO preparation, immunization, antibody discovery, *in vivo/in vitro* screening and preclinical candidate selection. First, the specific drug target gene is knocked out in RenMice, which takes about 7 months. Next, RenMab/RenLite KO mice are immunized with both human and mouse/monkey/dog antigens. After about 2 months, single B cells can be screened for antigen binding. Antibodies that cross-react with human, monkey, dog, and mouse targets will be further screened to test their in vivo and in vitro efficacy. After lead selection, top candidates undergo preclinical tests and the best candidates for CMC are selected.

Program Progress



Example 1 –Anti-Nectin-4 Campaign Using RenMice™ HiTS Platform

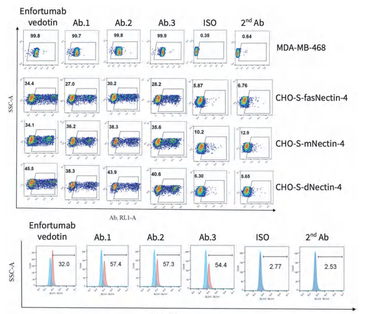
20 Nectin-4 knock-out RenMab™ Mice were immunized with human and mouse antigen

735 CD138+ plasma cells were isolated using Beacon® Platform

121 clones were identified with species cross-reactivity (human/monkey)

27 clones were selected according to affinity and internalization tests

Further evaluation (Antibody purification/in vitro test/PK/PD/TOX/Developability)



Nectin-4 knock-out RenMab mice were immunized with human and mouse antigens. We successfully obtained 20+ clones with better internalization activity than Enfortumab vedotin and can cross react with human/monkey Nectin-4. Antibody discovery using Nectin-4 knock-out RenLite® mice is also under development. 300+ clones with species cross-reactivity will be used for further research.

Example 2 –Anti-4-1BB Campaign Using RenLite® HiTS Platform

16 4-1BB Knock-out RenLite Mice were immunized with both human and mouse antigen

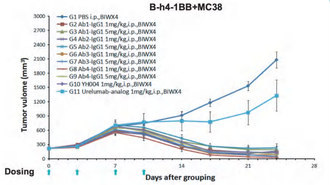
384 CD138+ plasma cells were isolated using Beacon® Platform

72 clones were identified with species cross-reactivity (human/monkey)

14 antibodies show comparable affinity with h4-1BB and cyno4-1BB

Top 4 clones were selected according to in vivo efficacy result

Further evaluation (PK/PD/TOX/Developability)



Sample (purified mAb)	Analyte	ka (1/Ms)	kd (1/s)	KD (M)
Ab1	h4-1BB-His	2E+05	2E-03	8E-09
	cyno4-1BB-His	3E+04	1E-03	4E-08
	m4-1BB-His	1E+05	7E-04	4E-09
	dog4-1BB-His	6E+04	3E-03	5E-08
Ab2	h4-1BB-His	4E+05	3E-03	6E-09
	cyno4-1BB-His	1E+05	1E-03	1E-08
	m4-1BB-His	1E+05	3E-04	2E-09
Ab3	h4-1BB-His	1E+05	4E-03	2E-08
	cyno4-1BB-His	5E+04	3E-03	6E-08
Ab4	h4-1BB-His	2E+05	5E-03	2E-08
	cyno4-1BB-His	2E+05	3E-03	1E-08

4-1BB knock-out RenLite® Mice were immunized with human and mouse antigen. In vivo efficacy was tested in humanized B-h4-1BB mice inoculated with MC38 cells. PBS or antibodies were dosed at day 0, 3, 7, 10 and the tumor volume was calculated twice every week. YH004 (developed by Biocytogen) and Urelumab were used as positive controls. We successfully obtained TOP4 clones which exhibit better efficacy than Urelumab and show comparable affinity with h4-1BB and cyno4-1BB.

Example 3 - Anti-PD-1 Campaign Using RenMab™ HiTS Platform

20 PD-1 knock-out RenMab Mice were immunized with both mouse and human antigen

778 CD138+ plasma cells were isolated using Beacon® Platform

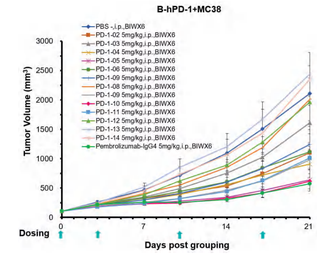
230 clones were identified with species cross-reactivity (human/monkey)

114 clones were selected according to in vitro blocking assay result

47 clones were selected according to affinity test

20 clones were selected for in vivo efficacy test

Further evaluation (in vitro test/PK/PD/TOX/Developability)



Clones cross-reactive with both human and monkey PD-1 were tested in humanized B-hPD-1 mice inoculated with MC38 cells. PBS or antibodies were dosed at day 0, 3, 10, 17 and the tumor volume was calculated twice every week. Pembrolizumab was used as a positive control. Several PD-1 clones exhibit efficacy comparable with pembrolizumab. Some clones cross react with human/mouse/monkey/dog PD-1.

Example 4 –Anti-CD40 Campaign Using RenMice™ Platform

30 RenMab™/RenLite® mice were immunized with both human and dog antigen

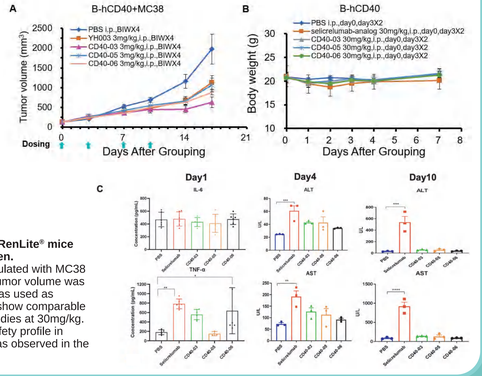
649 CD138+ plasma cells were isolated using Beacon® Platform

155 clones were identified with species cross-reactivity of human/monkey and 81 clones cross react with human/dog

60 clones were selected according to cell-based function assay

32 clones were selected according to in vivo efficacy result

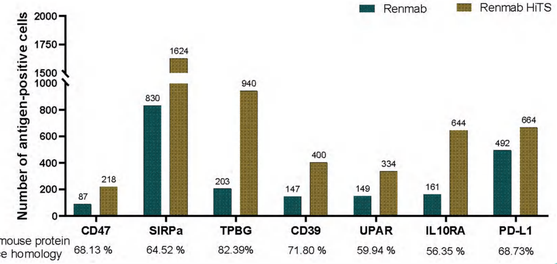
Further evaluation (PK/PD/TOX/Developability)



Knock-out of CD40 affects humoral immunity, so RenMab™/RenLite® mice were used for immunization with both human and dog antigen.
(A) In vivo efficacy was tested in humanized B-hCD40 mice inoculated with MC38 cells. PBS or antibodies were dosed at day 0, 3, 7, 10 and the tumor volume was calculated twice every week. YH003 developed by Biocytogen was used as positive control. We successfully obtained several clones which show comparable efficacy with YH003. **(B-C)** Toxicity assessments of CD40 antibodies at 30mg/kg. Selicrelumab was used as control. CD40 clones show a good safety profile in CD40 humanized mice. No significant transaminase elevation was observed in the CD40 mAb treatment groups compared with the PBS group.

The RenMab™ HiTS Platform Facilitates Antibody Discovery for Highly Homologous Targets

RenMab and RenMab HiTS mice (Hyperimmune Target-Specific Renmab KO mice, yellow) were immunized with antigens for the targets CD47, SIRPa, TPBG, CD39, UPAR, IL10RA and PD-L1, which have varying degrees of homology between mouse and human. Results indicate that RenMab KO mice generated more antibody-secreting B cells when the target has high homology between mouse and human.



SUMMARY

In conclusion, the RenMice™ HiTS (Hyperimmune Target Specific) Platform enables accelerated identification of fully human antibodies with increased diversity of antibody paratopes and species cross-reactivity, using fully human antibody mice engineered to lack the target antigen. The RenMice™ HiTS Program is focusing on identifying more first-in-class drug targets across a range of therapeutic areas.