

Antibody Engineering & Therapeutics Virtual 2020: Post-event Report

Antibody Engineering
& Therapeutics
US

Antibody Engineering
& Therapeutics **US**
VIRTUAL

December 14-16, 2020

A 3D molecular model of an antibody structure, rendered in shades of blue and green, set against a blue background with a bokeh effect. The antibody is shown in a Y-shaped configuration, with the central stem and two arms. The surface of the antibody is highly textured and irregular, representing the complex protein structure. The lighting is soft, highlighting the contours and depth of the model.

Introduction & Contents

Welcome,

The **Antibody Engineering & Therapeutics** virtual event welcomed over 800 attendees over the course of the 3-day program in mid-December. Featuring a slate of keynote presentations built into the live agenda, and an on-demand library of over 100 pre-recorded speaker presentations, it was packed with content.

A highlight of the event was the collection of 45-minute panel discussions which brought every speaker from the meeting into the live agenda for active discussions and audience Q&A.

Keynote presentations included Pamela Bjorkman of Caltech on [The Structural Basis of Neutralization by Antibodies Against Viral Fusion Proteins](#), Paul Carter of Genentech on [Engineering Bispecific Antibodies as Therapeutics](#) and Andrew Ward of Scripps on [Characterizing Polyclonal Antibody Responses Using Single Particle Electron Microscopy](#).

[Two live award presentations](#) were featured during the event including a student/post-doc best poster award and the inaugural Jim Huston science talent award presentation. A stirring tribute session honoring Jim Huston, the founder of the Antibody Society and long-time organizer of the conference (he passed away in 2020), featured live tributes from some of the most famous antibody scientists in the field.

We also introduced several new features, including a speed networking session and small group hot topic discussion sessions.

We look forward to welcoming everyone back to Antibody Engineering & Therapeutics in San Diego in 2021.

Stay safe and well,

Michael Keenan, Conference Producer, TIDES Europe



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Biology and bioinformatics consultant Silvia Hnatova looks back on the top presentation highlights across the week, including:

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- Keynote: Engineering Bispecific Antibodies as Therapeutics: Utilizing Intrinsic Heavy/Light Chain Pairing Preferences and Mitigating High Viscosity
- Keynote: Characterizing Polyclonal Antibody Responses Using Single Particle Electron Microscopy
- Isolation of Potent SARS-CoV-2 Neutralizing Antibodies and Protection from Disease in a Small Animal Model
- Biophysical Properties of Human B-Cell Derived Antibodies
- Engineered Antibody Platforms for Receptor Agonism

2. Panel Discussion: Bispecific T Cell Engagers

Silvia Hnatova revisits this popular discussion which explored the current challenges of T cell engager antibodies as cancer therapeutics.

3. Advancing Therapeutic Antibody Discovery Using RenMab/RenLite/RenMab KO Immunoglobulin Humanized Mouse Platforms

In this on-demand session, Dr. Li Hui presents a powerful tool for advancing current bispecific or multispecific antibody discovery.

4. Panel Discussion: Antibody Affinity Maturation and Developability Improvement for Lead Optimization

Silvia Hnatova highlights the key themes of one of the conference's hottest live discussions on antibody affinity maturation and developability improvements

5. Poster: RenMab Mouse: A Leading Platform for Fully Human Antibody Generation

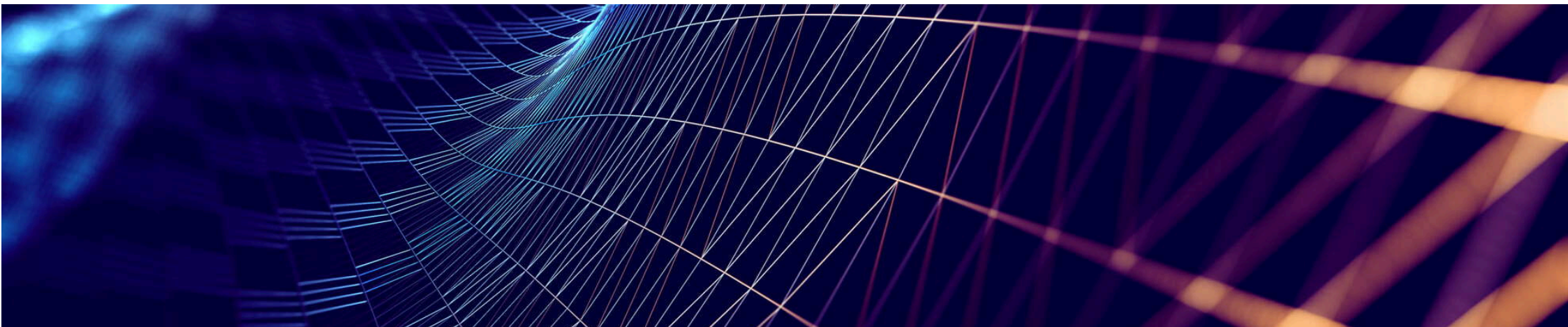
This poster and presentation explains the development and features of Biocytogen's fully human antibody mouse.

6. The Antibody Society Student/PostDoc Poster Competition Winners

Take a look at the full presentations and posters by innovative winners Felix Goerdeler and Nicolas Bery.

7. Novel TNFR2 humanized mouse model for in vivo validation of human TNFR2 antibody targeting hTNF /hTNFR2 signaling pathways

In this poster presentation, Biocytogen presents a useful preclinical model for in vivo efficacy of human TNFR2 therapeutics.



Key Themes of the Week

Executive Summary

Executive Summary: Key Themes of the Week

The conference was largely focused on COVID-19 antibodies, elaborating on the talks from the Antibody Engineering & Therapeutics Virtual Event held in summer 2020. The focus of this virtual event was on the structure of neutralization antibodies and relevance for vaccine

COVID-19 neutralization antibodies

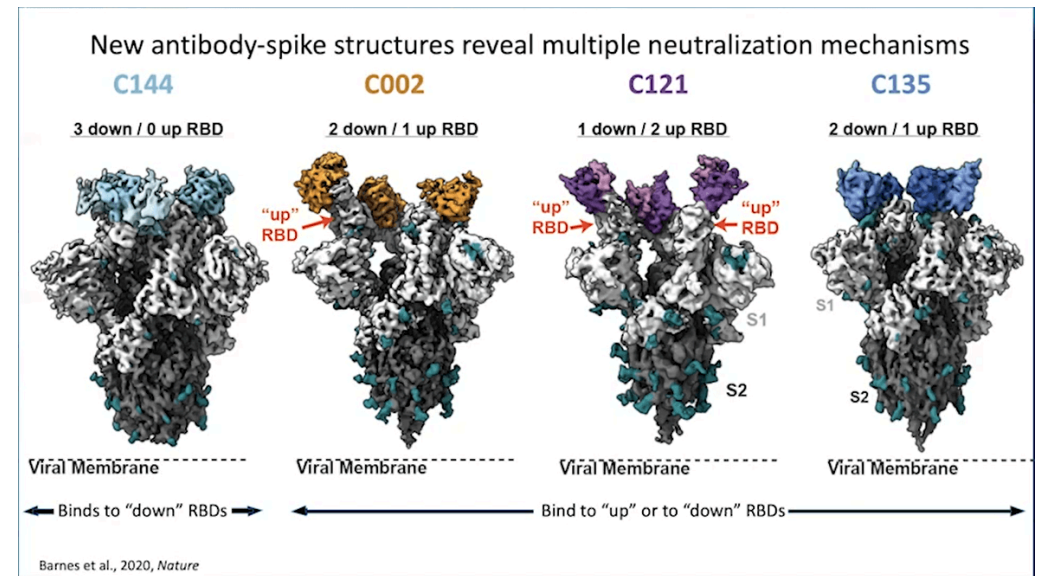
Prof Pamela Bjorkman from Caltech opened the topic with a keynote presentation on The Structural Basis of Neutralization by Antibodies Against Viral Fusion Proteins. She highlighted that the main strategy to prevent COVID-19 infection is via stopping COVID-19 entry into human cells, which would prevent the subsequent multiplication of the virus. As

COVID-19 infects human cells by binding virus' spike protein to the ACE2 receptor in human cells, neutralizing antibodies are best suited to stop this binding.

Prof Bjorkman, in collaboration with the Rockefeller University, found that patients who responded to COVID-19 infection (convalescent donors) produced monoclonal

antibodies. Using electron microscopy, the consortium asked whether they could determine the structural correlates of antibody-mediated neutralization of coronavirus infection. Prof

Bjorkman was also interested in whether there are multiple epitopes targeted in recovered individuals or a single predominant epitope. To examine this, Prof Bjorkman's group developed a new



technique called negative stain polyclonal epitope mapping (published by Bianchi et al., 2018; Nogal et al., 2020). Using this technique, they were able to determine the binding of single predominant epitopes in polyclonal antibodies from patients named COV21 and COV57.

Prof Bjorkman presented her research using single-particle cryo-

EM to highlight the structural correlates of neutralization for potent monoclonal antibodies. COVID-19 spike protein structure includes receptor binding domains (RBDs) that can be in two positions. To bind to ACE2, it needs to be in an “up” position. Neutralizing antibodies block the ACE2 binding site and only bind to “up” RBDs. All neutralizing antibodies identified by Prof Bjorkman were found to bind

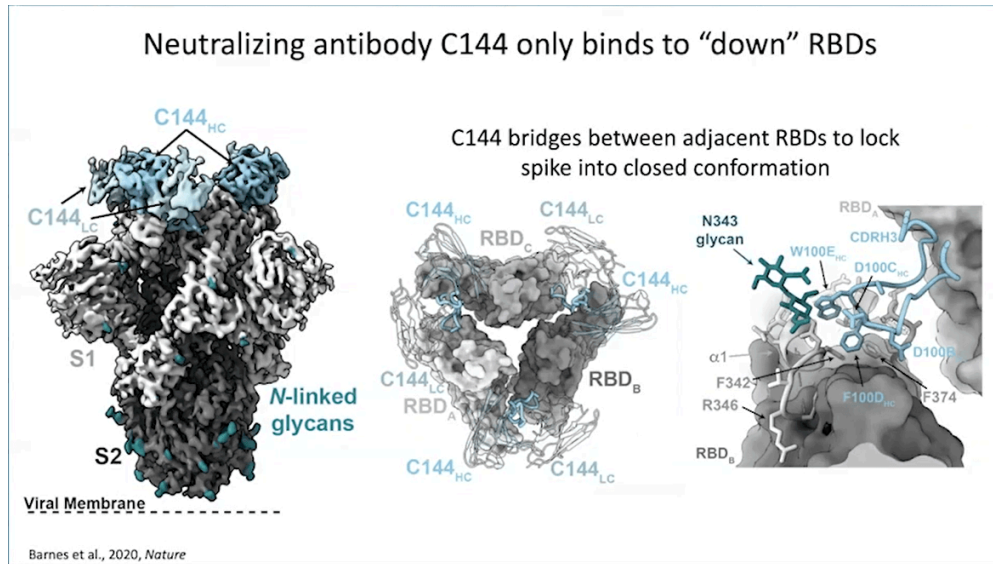
to C105 epitope, supported by previous work from other laboratories. Prof Bjorkman’s group summarized the neutralization mechanisms based on antibody-spike structures in a paper authored by Barnes et al. (2020).

Prof Bjorkman explained in detail the neutralization mechanism of one antibody named C144, that only binds to “down” RBDs. She revealed that C144 bridges between adjacent RBDs, locking the spike into closed conformation, highlighting the conformational changes in play.

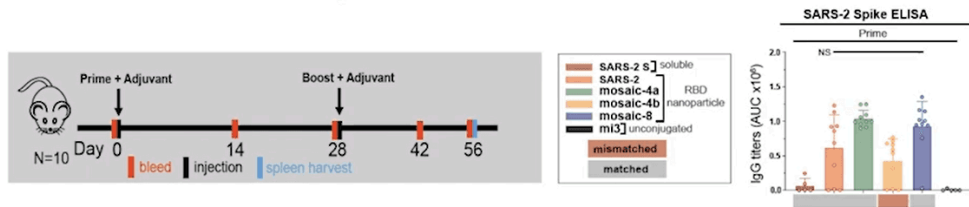
The structures of neutralizing antibodies were grouped into most abundant classes to lay the ground for vaccine and therapeutic strategies. Prof Bjorkman highlighted that the prediction between competition/no competition among neutralizing

antibodies is complicated. To illustrate her point, she presented an exception to the classification her group developed. BG1-22, a VH3053/VH3-66 antibody with a long CDRH3 binds to the class 1 RBD epitope.

In the second part of her talk, Prof Bjorkman pointed out that even after developing a COVID-19 vaccine, we are at risk of zoonotic transmission from coronaviruses from other hosts. She presented her work on a pan-coronavirus vaccine. Using a previously published system, immunogens can be attached to nanoparticles (Brune et al., 2016; Escolano/Gristic et al., 2019). Prof Bjorkman combined the SpyTag particle system with COVID-19 antigens to make four types of RBD-nanoparticles: homotypic SARS-2 and three mosaics (combining SARS-2 particles with other



Sera from RBD-nanoparticle-immunized mice bound to S trimer



Cohen et al., 2020, bioRxiv

particles). For this, they used RBDs from 8 sarbecovirus spike proteins for making nanoparticles, including RBDs from viruses with spillover potential. They immunized and boosted mice with RBD-nanoparticles of different types. SARS-2 spike ELISA assay results showed that antibodies raised against RBD monomers can bind to RBDs on S trimer.

Prof Bjorkman expressed her surprise that homotypic and mosaic sera led to the same degree of response in immunized mice. She said that “multimerization of RBDs on nanoparticles enhances immunogenicity compared with soluble antigen”. Mosaic sera bound and neutralized bat strains better than homotypic sera, but “neutralization of matched and

mismatched strains was only observed after priming”.

Prof Bjorkman pointed out at that protection against a mismatched strain can be induced via mosaic nanoparticles, implying neutralization. Prof Bjorkman’s group then repeated the ELISA’s using antibodies from plasma of people who overcame SARS-CoV-2, that did not bind well to coronaviruses of other types. She concluded that cross-neutralization response SARS-CoV-2 seems unlikely, by saying that “Co-display of SARS-2 RBD with other RBDs shows no disadvantages compared with homotypic SARS-2 nanoparticles for eliciting neutralizing Abs against SARS-Cov-2”. In summary, having had COVID-19 wouldn’t protect against the next pandemic.

Prof Bjorkman concluded the talk

by highlighting that mosaic-RBD nanoparticles are promising vaccine candidates for SARS-COV-2 and potential future emerging zoonotic sarbecoviruses. In her opinion, a mosaic nanoparticle strategy would be the best approach to protect against SARS-COV-2 and potentially emerging sarbecoviruses.

Prof Dennis Burton from Scripps Research Institute elaborated upon the results presented in previous virtual events four months ago in his presentation on Isolation of Potent SARS-CoV-2 Neutralizing Antibodies and Protection from Disease in a Small Animal Model. He first presented the collaborative effort to identify neutralizing antibodies from COVID-19 patients, published in Science (Rogers et al., 2020). In summary, the consortium looked into immune responses to SARS-CoV-2 spike protein and

compared it to the original SARS-CoV-1. The plasma from 17 donors was isolated soon after symptom onset, followed by neutralization assays in vitro using 2000 mAbs isolated from 8 donors. 33 mAbs from 3 donors showed notable neutralization. Prof Burton highlighted that vaccines can do much better than natural infection,

by targeted design.

Prof Burton's group took the potent antibodies forward to describe the binding mechanism to SARS-CoV-2. Binding to 3 major epitopes on RBD (RBD-A, RBD-B, RBD-C) and 3 epitopes that are non-RBD (D proteins) was described. Prof Burton explained that the

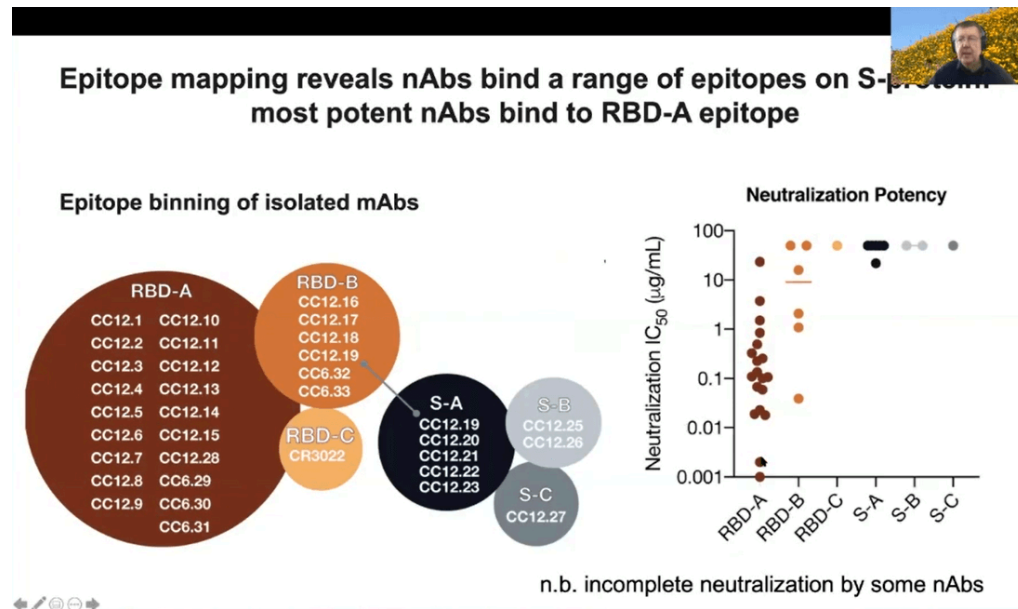
antibodies against RBD proteins proved to be most potent, although some antibodies showed incomplete neutralization. He focused the rest of his talk on the antibodies binding to RBD proteins.

RBD-A-ACE2 binding specific antibodies were most prevalent and most potent. Prof Dennis Burton explained this by competition between neutralizing antibodies to RBD-A epitope with the ACE2 receptor (Yuan et al., 2020): the most potent antibodies showed most competition with the ACE2 receptor. Prof Burton's consortium concluded that the antibodies are very potent with very few somatic mutations, leading to a concept of 'super-antibodies' that occur in a minority of infected individuals as a minor part of the antibody response. RBD-B seemed to be a better target for cross-reactive antibodies, despite

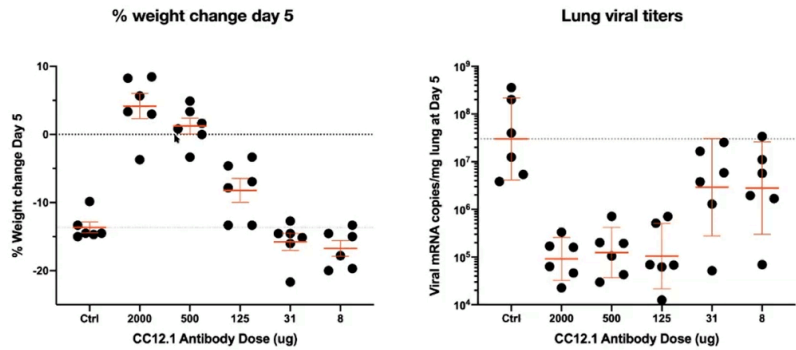
displaying a lower potency. Non-RBD S-protein nAbs demonstrated both weak and incomplete neutralization.

Prof Burton's groups further examined the potential of the potent antibodies from in vitro experiments to validate these in vivo using a Syrian hamster model. The antibodies selected were against RBD-A epitope and non-RBD epitopes. The potent neutralizing antibody CC12.1 against RBD epitope protects hamsters from COVID-19 disease, both weight loss and viral titers in the lungs. As expected, the CC12.23 antibody against S protein does not protect hamsters from COVID-19.

Prof Burton concluded his talk with very interesting results of antibody performance against different COVID-19 variants, including the recently emerged variant from



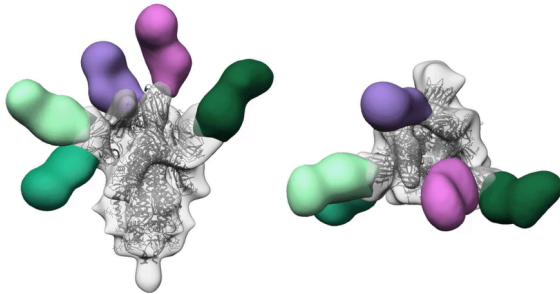
CC12.1 protects hamsters from COVID-19 disease



Immune responses to SARS CoV-2 spike vaccine candidates

Side View

Top View



- Polyclonal Fabs reveal at least 3 different NTD specificities and 2 different RBD specificities.
- Polyclonal Fabs resemble known neutralizing antibodies isolated from convalescent patients.
- Little to no detection of S2 antibody responses.

Europe. Selected most potent antibodies, including the antibody CC12.1 against RBD epitope, identified in convalescent plasma displayed protection against most COVID-19 variants. However, some COVID-19 variants escaped protection from monoclonal antibodies. Prof Burton closed his talk listing the SARS-CoV-2 nAbs that were licensed to Merck KGaA and Serum Institute of India to undergo clinical trials. He emphasised the need of providing the antibodies to low and middle-income countries.

Prof Andrew Ward from Scripps Research Institute dived deeper into COVID-19 antibodies and their structure with his keynote presentation on Characterizing Polyclonal Antibody Responses Using Single-Particle Electron Microscopy. He started by introducing a technique developed

in his lab: electron microscopy polyclonal epitope mapping (EMPEM; Bianchi et al., 2018). By combining traditional serology with EMPEM, Prof Ward's group can "visualize diverse polyclonal antibody responses to subunit vaccines and rapidly map epitopes, accelerating vaccine design process". Prof Ward presented his comprehensive work on HIV epitopes and he closed his talk by presenting his research on antibodies in sera of COVID-19 patients. He concluded that polyclonal Fabs resemble known neutralizing antibodies isolated from COVID-19 convalescent patients.

Antibody engineering strategies and biophysical properties

In parallel to the focus on COVID-19, the virtual event covered a range of talks that discussed antibody engineering strategies and biophysical properties of antibodies.

Dr Paul Carter from Genentech delivered a fascinating keynote presentation on Engineering Bispecific Antibodies as Therapeutics: Utilizing Intrinsic Heavy/Light Chain Pairing Preferences and Mitigating High Viscosity. Bispecific antibodies present an exciting development in the therapeutics field: there are 2 FDA approved antibodies (blinatumomab and emicizumab) and 186 in clinical development.


Dr Carter pointed out that bispecific antibodies have several advantages compared to traditional antibodies: their design exploits the modular architecture of antibodies, aiming to match the desired mechanism of action and clinical application, and they have long serum half-life.

Although the current manufacturing process for bispecific antibodies is very successful and robust, it is resource-intensive, expensive and inconvenient for manufacturing.

Using current methods, light and heavy chains are expressed separately in two separate cells and then assembled in vitro into

bispecific IgGs. Genentech improved and simplified the manufacturing process: bispecific IgGs were re-designed for 1-cell production. Dr Carter presented work on v11 IgG as an example of this design (Dillon et al., 2017). He showed that 1-cell and 2-cell bispecifics have similar in vitro potency and pharmacokinetics. Most significantly, 1-cell production of bispecific IgGs at large scale is successful. One such bispecific IgG has reached the clinic.

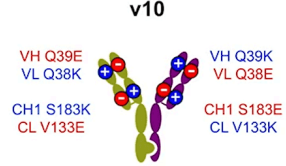
Building upon his work on 1-cell bispecific IgGs, Dr Carter presented results of cognate light chain/heavy chain (LC/HC) pairing preference. To evaluate the LC/HC preference, his group quantified bispecific IgG yield with orbitrap LC-M after transient expression of IgG pairs in a single cell (Joshi et al., 2019). It was found that cognate LC/HC pairing preference is a common occurrence and can be strongly influenced by residues in CDR L3 and CDR H3. Such knowledge may



Efficient 1-Cell Production of Bispecific IgG – Summary

- Demonstrated for many different antibody pairs tested
- 1-cell and 2-cell bispecific IgG have similar properties
- Successful expression and purification of bispecific IgG demonstrated from stable CHO cell lines for several programs
- Used for one bispecific IgG that has reached the clinic

v10




VH Q39E
VL Q38K
CH1 S183K
CL V133E

VH Q39K
VL Q38E
CH1 S183E
CL V133K

Dillon et al., mAbs, 2017

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Cognate LC/HC Pairing Preference is a Common Occurrence which Depends Upon Constituent Antibody Pairs

- Random LC/HC pairing is rare
- Cognate LC/HC pairing preference is common

mAb-2	mAb-1					
	IL-13	MET	EGFR	CD3	IL-4	HER2
IL-13	NA	87.6	97.0	75.2	70.3	66.6
MET	86.6	NA	72.3	66.7	53.1	59.9
EGFR	86.3	72.4	NA	23.9	45.4	22.0
CD3	75.5	54.8	32.5	NA	25.0	22.7
IL-4	68.7	58.0	44.1	26.9	NA	22.6
HER2	64.6	65.4	21.6	24.1	25.0	NA
DR5	90.4	95.1	53.3	53.4	53.8	34.7
FluB	87.7	69.5	52.3	32.0	60.8	72.7
RSPQ3	84.7	58.6	82.1	40.6	26.0	22.0
Factor D 25D7 v1	83.6	73.1	69.3	83.1	35.6	68.7
RSPQ2	83.5	51.1	78.5	38.7	22.3	71.3
IL-1β	74.2	63.5	80.4	77.8	63.7	65.9
GFRα1	73.9	40.6	77.5	79.6	33.5	68.0
PDGF-C	61.2	71.0	54.6	56.0	34.2	24.3
CD33	49.8	58.8	49.6	36.4	56.6	51.5
α5β1	45.9	62.2	31.0	41.4	48.4	72.6
IL-33	45.6	21.4	30.9	20.4	42.4	46.6
SIRPα	41.7	31.0	22.6	60.6	47.9	31.8
Factor D 20D12 v1	23.5	29.8	58.0	36.0	22.6	69.6
LGR5	21.7	56.2	53.8	23.6	22.8	22.1

BsIgG₁ yield (%)

BsIgG ₁ yield (%)	LC/HC pairing preference
< 30%	Random
> 30-65%	Moderate
> 65%	Strong

Joshi et al., mAbs, 2019

be advantageous to reduce risks occasionally encountered with engineered proteins and the number of Fab mutations needed for efficient production of bispecific IgG where there is strong intrinsic pairing preference.

In the second part of his talk, Dr Carter focused on the viscosity of bispecific antibodies. Due to their clinical potential, it is necessary to consider the delivery method. Currently, subcutaneous delivery

forms one-third of all delivery methods. Because it is highly convenient, it improves patient compliance with the drug. The cons of some antibodies are their high viscosity, limiting delivery to small injection volumes and increasing injection site pain.

Viscosity properties of bispecific antibodies are unknown. Although subcutaneous delivery and low viscosity would be highly desirable, Genentech found that bispecific

antibodies have an unusually high viscosity. Dr Carter suggested several strategies to resolve the high viscosity including the addition of excipients to antibody formulation (NaCl or Arginine-HCl), co-formulation with hyaluronidase or re-engineering of the antibody.

Genentech found that the introduction of specific mutations into a bispecific antibody reduces viscosity to its parent IgG. Dr Carter illustrated his point with the same strategy applied to monospecific antibodies, using anti-GCGR IgG antibody as an example. He demonstrated that mutations of single aromatic residues can help to reduce the viscosity of both monospecific and bispecific antibodies.

Related to Genentech's fascinating results on the engineering of bispecific antibodies, **Dr Greg**

Lazar (Genentech) delivered a presentation on Engineered Antibody Platforms for Receptor Agonism. There are several mechanisms on how to improve the agonist potential of antibodies.

Dr Lazar illustrated his point with an example: TNFRSF antibodies are typically poor agonists on their own and none of the current TNFRSF agonist antibodies has yet passed clinical trials to become therapeutics. The emphasis of the talk was on Fc-mediated cross-linking and our inability to control this.

Dr Lazar proposed several engineering design principles that could help to overcome this challenge, exploring receptor signalling without relying on Fc receptor engagement. He concluded the talk by highlighting that FcγR expression is variable and

not under our clinical control. Instead, engineering of the antibody may enable better control over the biological and pharmacological profile of the agonists.

Dr Laura Walker from Adimab delivered a unique presentation on Biophysical Properties of Human B-cell derived Antibodies. In her publication (Jain et al, 2020), she divided the biophysical properties of 400 human B cell-derived mAbs into five main groups: stability, hydrophobicity, long-term aggregation propensity, ELISA plate binding and cross interaction propensity.

The focus of her research was to address whether B cell subsets have different biophysical properties. She found a few correlations between the biological and biophysical properties of

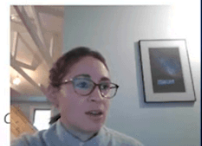
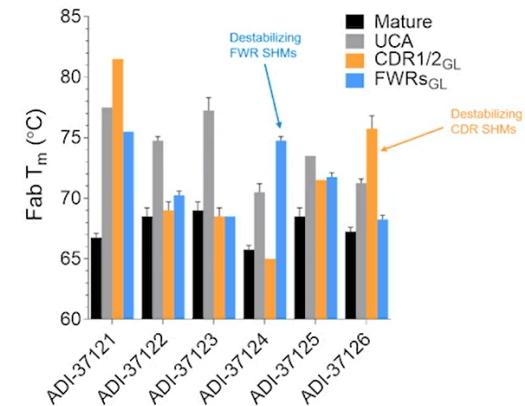
antibodies:

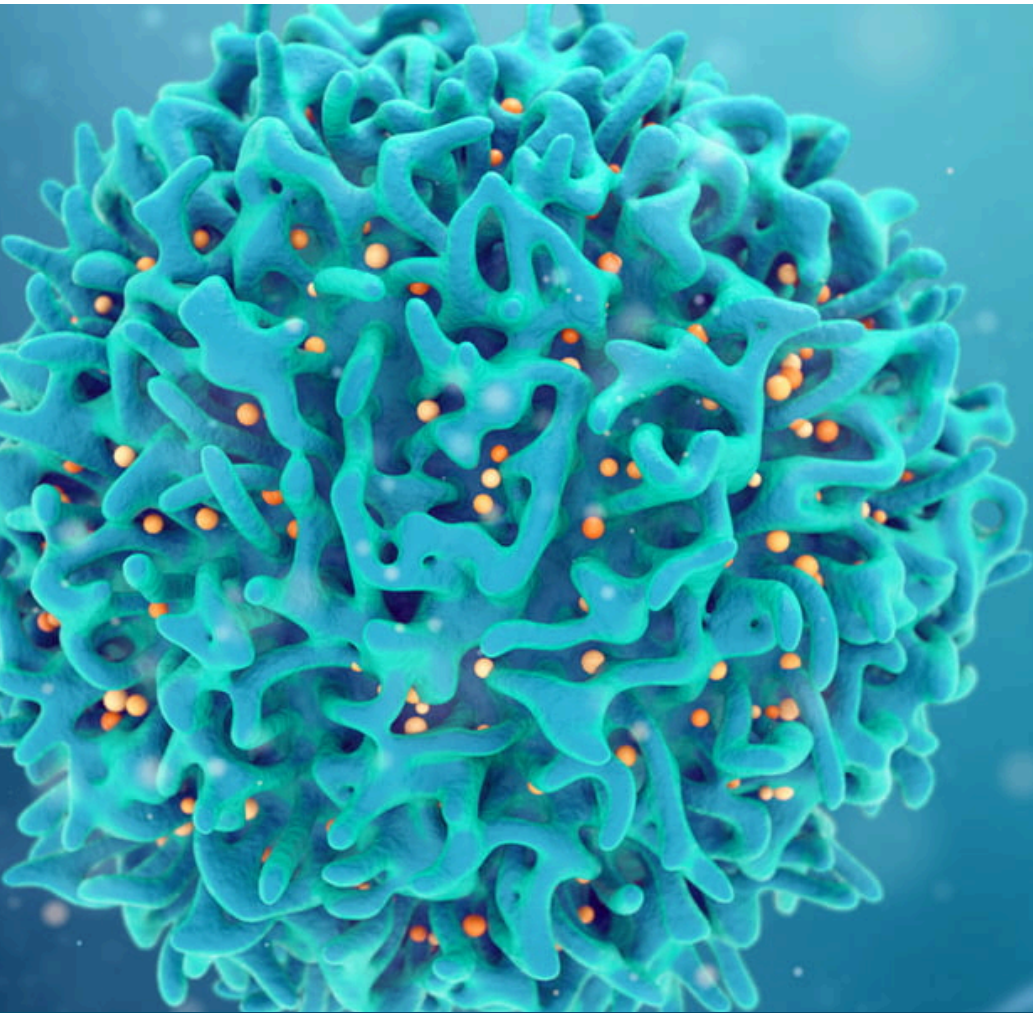
- higher polyreactivity score correlates with faster mAb clearance in humans
- with increasing levels of somatic hypermutation, the antibody polyreactivity decreases
- long CDRH3 lengths and high isoelectric points were associated with elevated polyreactivity
- VH1-69 germline gene usage and long CDRH3 lengths were associated with high hydrophobicity.

Dr Walker closed the talk with an interesting observation that naïve B cell-derived mAbs show higher thermostability compared to memory B cell-derived mAbs. Somatic mutations led to decreased thermostability and the location of destabilizing mutations was found to be antibody-dependent. In general, Kappa LCs

and VH4 germline antibodies were found to be more thermostable than other antibodies. Dr Walker concluded that human B cell-derived antibodies show favourable polyreactivity, hydrophobicity and thermal stability properties. Her work laid important ground on principles to control polyreactivity, hydrophobicity and thermal stability of antibodies.

The location of destabilizing mutations is antibody dependent





Bispecific T Cell Engagers

Panel Discussion Summary

Panel Discussion Summary: Bispecific T Cell Engagers

The discussion about bispecific T Cell Engagers built upon the talks from the virtual event, focusing on the current challenges of T cell engager antibodies as cancer therapeutics. Silvia Hnatova details the key points raised in the discussion, featuring:

- **Dr Katherine Harris**, Senior Director of Discovery at TenoBio
- **Dr Alison Crawford**, Senior Staff Scientist, Oncology and Angiogenesis at Regeneron Pharmaceuticals
- **Prof Paul Parren**, EVP, Head of R&D and Professor at Lava Therapeutics and Leiden University
- **Dr Teemu Junttila**, Principal Scientist, Translational Oncology at Genentech
- **Dr Hans van der Vliet**, Chief Scientific Officer at Lava Therapeutics BV
- **Dr Brian Avanzino**, Scientist III, Discovery at TeneoBio

Dr Katherine Harris opened the discussion highlighting that currently, there is only one therapeutic T cell engager bispecific antibody approved (blinatumomab targeting CD19 and CD3). However, this drug has serious side

effects including cytotoxicity. Dr Harris underlined the importance of an improved safety profile in developing next-generation bispecific T cell engagers. She then opened the forum for discussion on cytokine release syndrome (CRS).

Dr Alison Crawford agreed that toxicity and efficacy in solid tumours are major challenges for bispecific T cell engagers. She pointed out that our knowledge about the mechanism CRS is incomplete and that current clinical trials are hindered by initial CRS response. Dr Crawford highlighted that it is not yet understood why step-up dosing of bispecific antibodies works and for how long.

Prof Paul Parren, Dr Crawford and **Dr Teemu Junttila** built upon this by discussing the detection of different cytokines during CRS. Dr Junttila suggested that perhaps de-sensitisation could be taking place and CRS could be a result of the quickly escalating cytokine response in patients. He pointed out that further understanding of CRS would be needed for clinical use.

Prof Parren asked **Dr Hans van der Vliet** whether reduced toxicity could be achieved by reducing affinity to targets. Dr van der Vliet explained that there are T cell subsets involved in CRS. He said that T cell subsets could be triggered instantaneously, and have the “potential to have a different effect on the cytokine spike”. Dr van der Vliet pointed out that the size of the cell population makes a difference and different T cell subsets produce different cytokines – allowing specific targetting. He hypothesised that this could improve the safety profile.

There was a further discussion about specific cytokine targetting and liver toxicity, supported by data from Dr Crawford’s lab. **Dr Brian Avanzino** asked if such specific cytokine targetting could drive selection pressure for tumour antigen loss. Dr Crawford confirmed that antigen-negative relapse following CRS remains the main problem with anti-CD3 bispecifics.

Prof Parren answered a question from the audience about whether “any T cell targets

beyond CD3 to engage T cells would be possible in TME without systemic effects”. Dr van der Vliet elaborated on this question highlighting gamma-delta T cells that have a limited cytokine profile. He explained that the cell subset targetting would not have to be limited to T cells, giving examples of NK cells, in addition to pointing to his research on gamma-delta cells and their cytokine release. Dr van der Vliet concluded that there is not sufficient clinical data to answer the question of cell subset targetting at this time.

Dr Crawford suggested that mitigating systemic response to prevent CRS could prove more useful than targetting of T cell subsets, due to the need for engaging as many T cells as possible in big tumours. Dr Junttila agreed that efficacy of bispecifics in solid tumours is a more pressing issue than the toxicity, adding that we can ‘find a creative way of using the antibodies’, but they need to work.

Prof Parren switched gears, asking Dr van der Vliet if we could treat tumours that have high levels of gamma-delta T cells or we could

recruit those cells to the tumour. Prof Vliet added that there is evidence to suggest that the number of infiltrating gamma-delta T cells in different tumours differs.

He said that in his research, he did not see a lower limit for gamma-delta T cell numbers that would be needed to trigger a response, unless none present. He said we might want to implement a lower limit for gamma-delta T cell numbers later, once more is known about this cell subpopulation.

Dr Crawford elaborated on her statement that 'the larger the tumour, the more difficult to treat'. She explained that this is dependent on the size of the tumour vs T cell ratio. In her preclinical mouse models, she saw that a much bigger number of T cells is needed when tumours are too big in size (above 200mm) and they reach exponential growth, worsening the prognosis.

Dr Avanzino and Dr Crawford discussed targeting of different cytokines, especially those secreted at different times. Dr Harris added

that on-target/off-tumour toxicity is an issue and asked the panel about strategies to mitigate the toxicity. Dr Crawford suggested that affinity and checkpoints would be important to be taken into account to decrease toxicity in solid tumour targets.


There was a brief discussion about the relevance of animal models when studying bispecific antibodies. Dr Crawford talked about the xenogenic models she uses in her lab and humanized mouse models.

Humanized mouse models are very useful for studying potentially clinically relevant targets, she said, agreeing with Dr Junttila that mouse immune response is not always reflective of a

human immune response. It was agreed that questions should be selected carefully to enable the best use of animal models.

The last question was asked by Dr Avanzino to Dr Crawford's whether mouse models are reflective of potential toxicity in humans. Dr Crawford highlighted the importance of 'choosing the right question' and the right model for addressing toxicity.

She emphasised the need for 'the target expressed in the same place at the same level'. The discussion was concluded by everyone agreeing that despite unresolved challenges, it was an exciting time for bispecific antibodies and rapid advancement on the horizon.



"The discussion was concluded by everyone agreeing that despite unresolved challenges, it was an exciting time for bispecific antibodies and rapid advancement on the horizon."

Advancing Therapeutic Antibody Discovery Using RenMab / RenLite / RenMab KO Immunoglobulin Humanized Mouse Platforms

with Dr. Li Hui, Scientific Director at Biocytogen



Advancing Therapeutic Antibody Discovery Using RenMab/RenLite/RenMab KO Immunoglobulin Humanized Mouse Platforms

To human or to humanize? This is a great question for therapeutic antibody discovery.

Human antibodies that are generated from immunoglobulin transgenic mice have been proved to be successful due to the in vivo natural selection, affinity maturation of antibody secreting B cells.

To meet the expanding needs of the therapeutic antibody market, Biocytogen established first and second

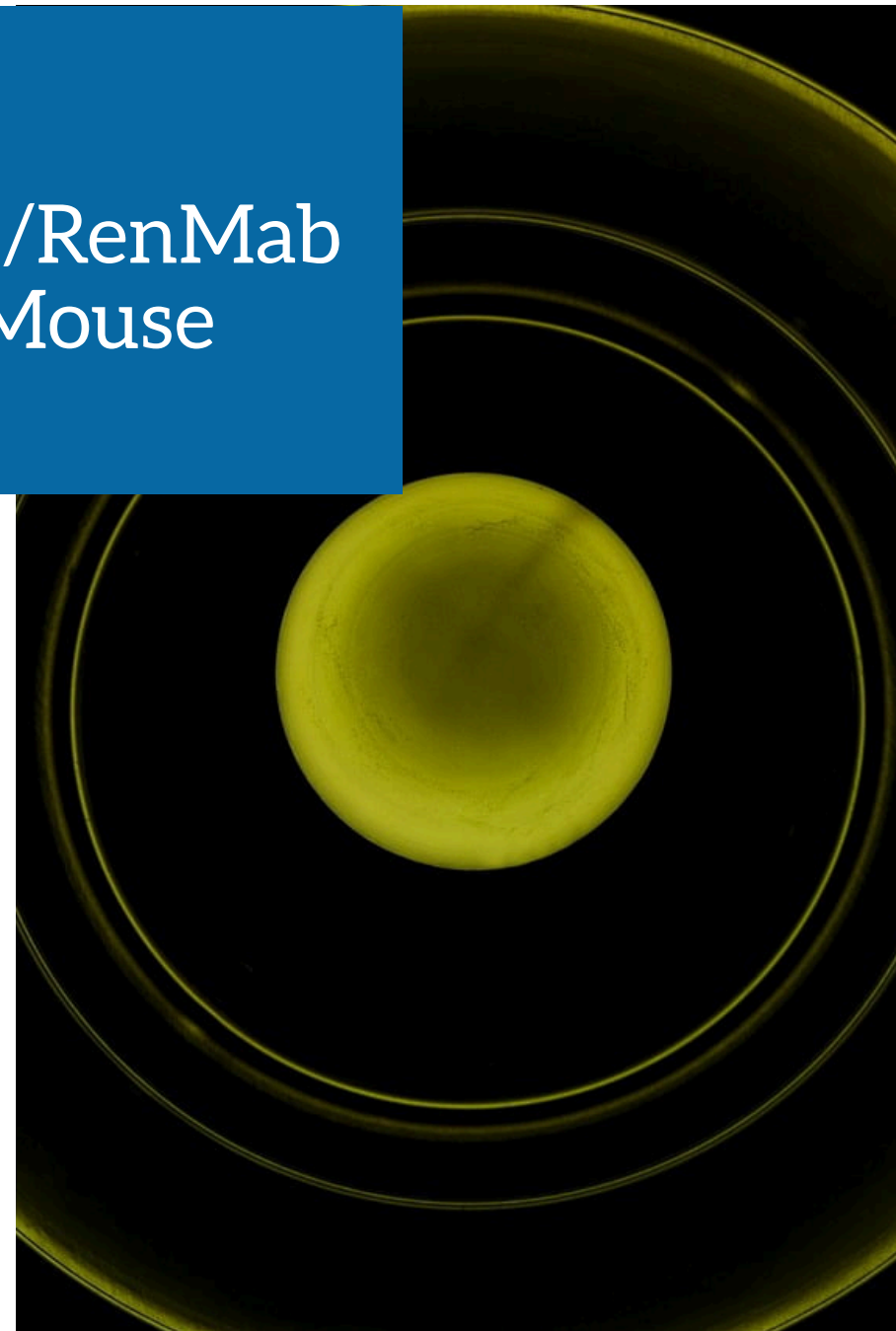
generation of immunoglobulin humanized mouse platforms: RenMab, RenMab KO and RenLite.

RenMab mice carry full human heavy chain and kappa light chain repertoire and has been proved to be a robust human antibody generating engine.

Neutralizing antibodies

against SARS-COV-2 were generated from RenMab mice and showed strong neutralizing function as well as high epitope diversity.

RenLite immunoglobulin humanized mouse is designed to overcome the common challenges of bispecific antibody discovery: immunogenicity and chain mispairing, at the



same time, provide a full human heavy chain repertoire diversity.

RenLite mice show robust immune response comparable to wild type mice and RenLite mice derived antibodies have broad binding affinity up to subnanomolar range.

RenLite antibodies also showed strong in vivo efficacy against tumor growth in xenograft mouse models.

Biocytogen's RenMab KO mice library contains a list of mice each with a specific target gene knocked out.






RenMab KO mice conquers challenging targets such as high homologous protein, GPCR and Ion channels.

RenMab KO mice also generates species cross reactive antibody candidates of which preclinical studies do not rely on surrogate antibodies.


In summary, RenMab/ RenLite/RenMab KO mouse platforms are powerful tool for advancing current bispecific or multispecific antibody discovery.

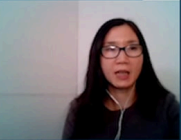
Click on the preview below to watch the full presentation

Biocytogen History

				
Founded in 2008 to provide gene targeting services	The Beijing headquarter started to operate, primarily providing gene editing services	Large animal facility in operations	Lab & vivarium capabilities were added in the Wakefield site, providing preclinical services	Expanded animal production capabilities will be available in 2020

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December 14-16, 2020

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Antibody Affinity Maturation and Developability Improvement for Lead Optimization

Panel Discussion Summary



Panel Discussion Summary: Antibody Affinity Maturation and Developability Improvement for Lead Optimization



One of the most popular live discussions across the week explored antibody affinity maturation and developability improvements. Silvia Hnatova revisits the top themes from the session, featuring:

- **Dr Stephen Parmley**, Vice President, Molecular Biology & Protein Science at AnaptysBio (Chair)
- **Dr Laura Walker**, Director at Adimab, LLC
- **Dr Andre Frenzel**, Founder and CSO at Yumab
- **Dr Danielle Dicara**, Principal Scientific Researcher, Antibody Engineering at Genentech
- **Dr Alon Wellner**, Postdoctoral Researcher at UC Irvine

Dr Stephen Parmley chaired a very practical session on antibody affinity maturation and developability improvements. He opened the session by highlighting the importance of improving antibody libraries free of liabilities.

The first discussion focused on the sequence of steps needed to correct antibody liabilities.

Dr Parmley asked the panel “if an antibody needs to be further optimized and has liabilities, would you initiate diversification for maturation before or after fixing the antibodies”? There were different opinions from panel members, depending on the models they employ, ultimately concluding that the answer would be dependent on the specific antibody.

Dr Laura Walker said that she would first try to identify where the liability comes from. **Dr Andre Frenzel** listed several approaches available for liability correction, including bioinformatic and biomedical approaches.

Dr Danielle Dicara added that the liable residues do not always have to be removed if they do not impact folding, although this may be dependent on the circumstances. **Dr Alon Wellner** added that in his system they cannot control mutations as the combinations are random and that liabilities can also emerge during affinity maturation.

Dr Parmley concluded that if the liability remains to be fixed at the end of the process, it

may impact the affinity maturation that may be context-dependent. He prefers to remove liabilities as early as possible in the process, which usually takes about two weeks using his platform.

Dr Wellner asked the panel whether a liability residue is always a liability or whether it is context-dependent. Dr Walker responded that this needs to be tested depending on a specific antibody, for example, if it does not impact binding it may not be a liability. Dr Parmley and Dr Frenzel agreed that some of the liabilities arise from manufacturing and may not be found in vivo.

Dr Parmley addressed a question to Dr Walker about liability in germline residues and whether they can go away. She responded that from her practice, the developability profiles of antibodies from B cells is broadly good, apart from naive B cells (see her talk).

She pointed out that thermostability may decline as an effect of mutations, that may accumulate during affinity maturation. Dr

Parmley pointed out that ultimately, the main liability will be the specificity of the antibodies.

Related to Dr Wellner's talk, Dr Dicara asked about T_m threshold as a function of somatic mutations. Dr Walker explained that below a certain point, B cells antigen uptake is compromised.

There was a discussion about how and why thermostability would be affected and how this could relate to gene expression in B cells.

The panel answered an interesting question from the audience about whether there is a list of sequence liabilities that is well-known. Dr Parmley and Dr Dicara explained that although some are well-described, some can be identified through stress-testing.

Next, the panel discussed possible dead ends during antibody optimization. Dr Wellner replied to Dr Parmley's concerns about mutation rates in his model being too high. He said that based on his model, the mutation rate is not yet as high as he would like it to be,

leading to dead ends. However, his only dead end so far was that he was unable to mature the antibody any further.

Dr Dicara voiced her concerns about stopping mutations in a continuous mutation system to identify and characterize them. Dr Wellner responded that there are points where he can isolate plasmids from specific clones, admitting that there may be some noise present.

The panel answered specific questions from the audience.

There was a discussion about improvements in antibody affinity by using insertions and deletions. Dr Parmley stated that although this is infrequent in his practice, the main improvement he has seen was in germline antibodies pulled from naive cells. He said that he may not have a complete picture because he only sees 'what was selected for', saying that insertions are mainly seen in CDRs (duplications of e.g. 8 residues).

Dr Frenzel answered a question from the

audience about whether he has seen 'epitope walking' with affinity maturation. In Dr Frenzel's experience, epitope drift may not be fully avoided. Dr Walker said that in her experience she only saw this once, that a true shift would happen.

There was an interesting question from the audience about how immunogenicity could be avoided, that was debated by the panel. Dr Parmley answered that there are a number of ways to reduce this, mainly good biophysical properties of the antibody and avoidance of T cell epitopes with in silico tools.

It was agreed that immunogenicity may not be spotted until the antibody is tried in humans. This was followed by a technical discussion on salt conditions during antibody optimization.

Dr Walker answered a question from the audience asking her opinion on the most successful methods of affinity maturation. Her opinion was that pre-made libraries targetting multiple residues at the same time would be the best method. Dr Wellner added that in his system the most mutations are achieved in CDR1 and CDR2 regions.

Dr Parmley pointed out that COVID-19 convalescent antibodies do not have much activity in H3 regions, suggesting affinity maturation could be possible in these regions.

Dr Parmely highlighted that posttranslational modifications are of main concern in clinical development, and tryptophan residues are the main focus in affinity maturation. Dr Parmley asked Dr Walker about developability properties of in vitro vs in vivo antibodies. Dr

Parmley cautioned that right filters are crucial when using antibodies from different kinds of display, e.g. phage.

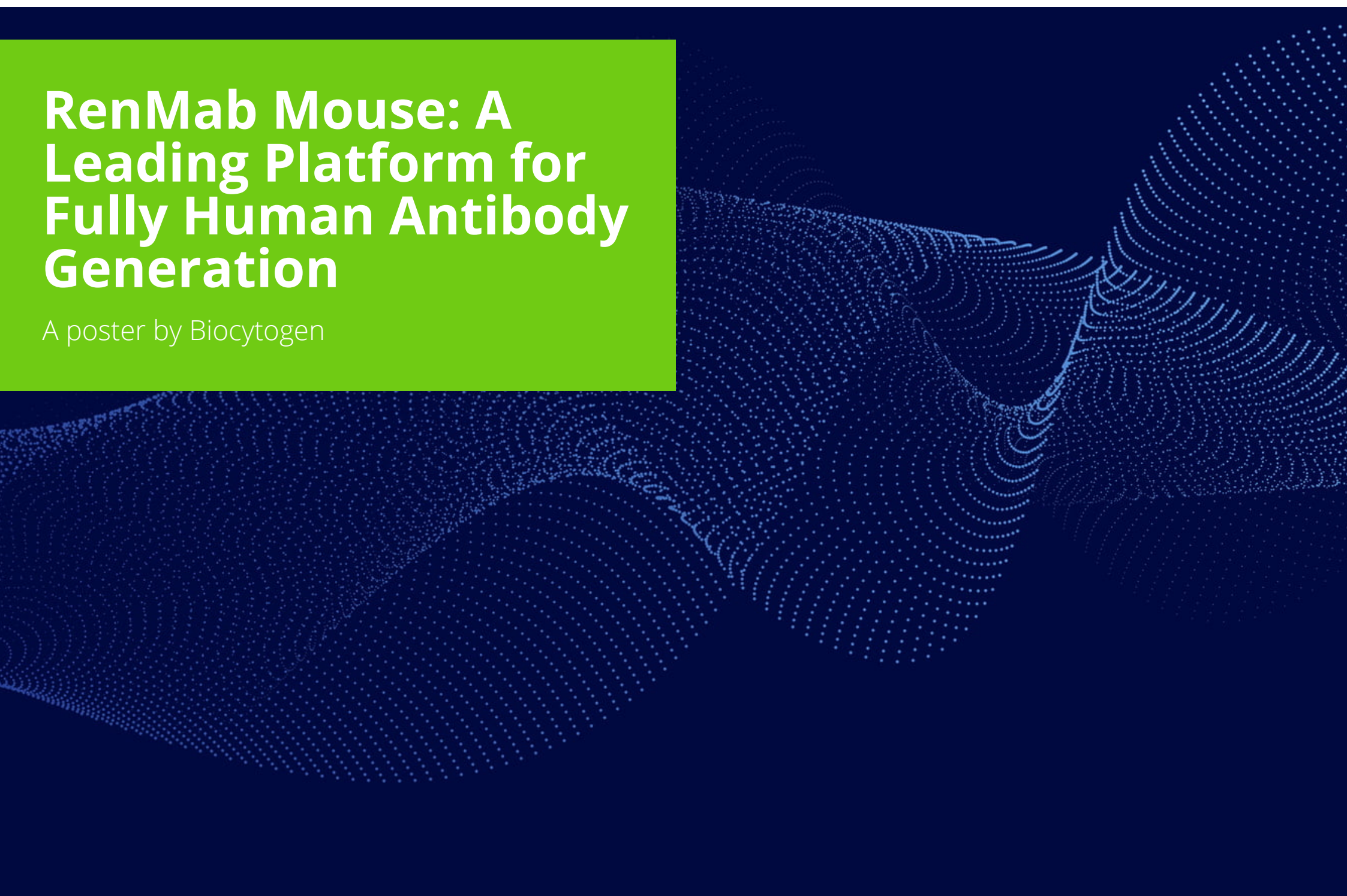
The discussion was closed with an interesting question from the audience on "how closely do current antibodies follow developability rules?", questioning the current practices and how successful they are in the antibody selection process.

Dr Walker pointed out that phage antibodies currently in the clinic did not follow developability rules employed now. She suggested that it would be "interesting to repeat the same analysis in 20 years", to see how successful current developability practices will prove to be.



RenMab Mouse: A Leading Platform for Fully Human Antibody Generation

A poster by Biocytogen



RenMab Mouse: A Leading Platform for Fully Human Antibody Generation

Chaoshe Guo, Benny Yang, Huizhen Zhao, Yabo Zhang, Lili Liu, Hui Lu, Shuwen Huang, Yuelei Shen, Biocytogen

With the development of immuno-oncology, therapeutic antibodies have been proven to be extraordinarily effective for cancer treatment.

Conventional human antibody discovery process can be divided into steps including target selection, target validation, screening preparation, hits generation, leads selection, lead optimization, and clinical candidate selection.

To accelerate antibody

development process, Biocytogen has developed RenMab™ Mouse, a fully human antibody mouse whose entire variable regions were replaced by human Ig heavy chain and κ light chain through Biocytogen's unique Mb-scale chromosome engineering technology.

RenMab™ Mouse provides a remarkably efficient therapeutic antibody discovery platform for fully human antibody generation, characterization, and rapid in vivo efficacy screening.



RenMab Mouse: A Leading Platform for Fully Human Antibody Generation

Chaoshe Guo¹, Benny Yang¹, Huizhen Zhao¹, Yabo Zhang¹, Lili Liu², Hui Lu², Shuwen Huang², Yuelei Shen¹
¹Biocytogen Boston Corp., Wakefield, MA, USA ; ²Beijing Biocytogen Co., Ltd., Beijing, China

Abstract

With the development of immuno-oncology, therapeutic antibodies have been proven to be extraordinarily effective for cancer treatment. Conventional human antibody discovery process can be divided into stages including target selection and validation, screening preparation, hits generation, leads selection and optimization, and clinical candidate selection. To accelerate antibody development process, Biocytogen has developed RenMab™ Mouse, a fully human antibody mouse whose entire mouse variable regions were replaced by human immunoglobulin heavy chain and κ light chain through Biocytogen's unique Mb-scale chromosome engineering technology. RenMab™ Mouse provides an efficient therapeutic antibody discovery platform for fully human antibody hit generation and characterization. We have characterized RenMab™ Mouse with the following features.

1. The entire mouse variable regions were replaced with complete human genome DNA in situ for complete human & mouse regulatory elements. The gene regulation of RenMab™ mouse is highly consistent with that of human.
2. Mouse constant region remains to ensure proper B cell development and maturation. Immune system of RenMab™ Mouse has been proven to be almost identical to that of wild type mouse. RenMab™ Mouse showed normal antibody immune responses to antigens.
3. RenMab™ Mouse generates a highly diverse repertoire of fully human antibody variable regions through V(D)J recombination. This capability can lead to promising hits for downstream leads and candidate selection in human therapeutic antibody discovery programs.



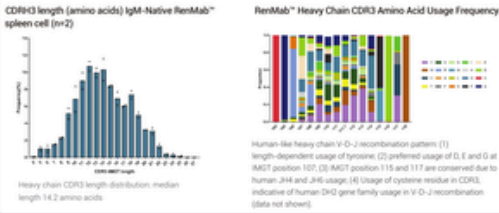
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ABSTRACT

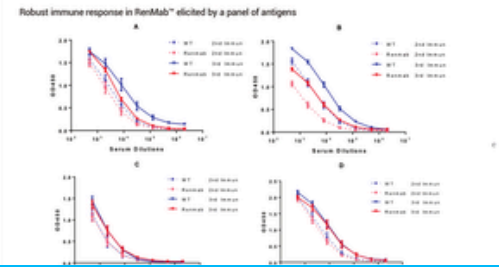
With the development of immuno-oncology, therapeutic antibodies have been proven to be extraordinarily effective for cancer treatment. Conventional human antibody discovery process can be divided into stages including target selection and validation, screening preparation, hits generation, leads selection and optimization, and clinical candidate selection. To accelerate antibody development process, Biocytogen has developed RenMab™ Mouse, a fully human antibody mouse whose entire mouse variable regions were replaced by human immunoglobulin heavy chain and a light chain through Biocytogen's unique Mb-scale chromosome engineering technology. RenMab™ Mouse provides an efficient therapeutic antibody discovery platform for fully human antibody hit generation and characterization. We have characterized RenMab™ Mouse with the following features.

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III. RenMab™ Mouse produces human-like antibody repertoire as shown by CDR3 analysis

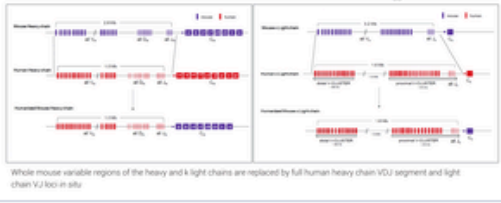


IV. RenMab™ Mouse demonstrates highly similar immune responses compared to WT mouse

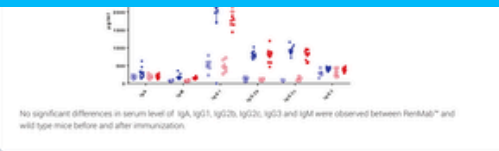
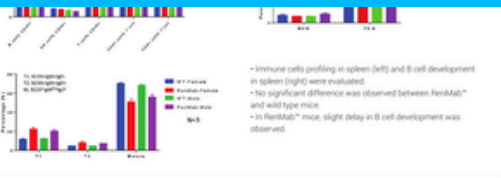


RESULTS AND DISCUSSIONS

Schematic of Humanization in The RenMab™ Mouse



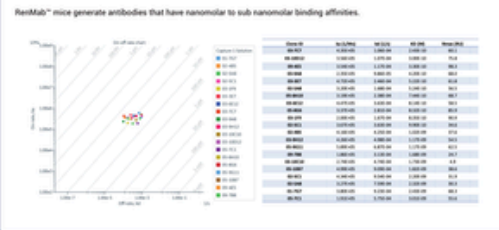
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B. Full human VDJ expression in the variable domains of heavy chain and kappa light chain genes



V. RenMab™ Mice produce high affinity antibodies



CONCLUSIONS

In conclusion, RenMab™ Mouse platform provides:

- Fully human antibody repertoire diversity of heavy chain and kappa light chain.
- Conservation of regulatory elements of both human (within VDJ loci) and mouse (outside VDJ loci).
- Normal B cell development and immune cell profile.
- Robust immune response comparable to wild type mouse.
- Antibody affinity of nanomolar to sub nanomolar range.
- RenMab™ 4G mouse for challenging targets such as proteins with high homology between human and mouse or GPCR/ion channels.
- Recent addition of RenLife™ mice with humanized heavy chain and fixed human light chain, suitable for bispecific antibody discovery.

Information available at www.renmab.com or send inquiry to info@biocytogen.com.

Combined with existing inventory of single, double or triple target humanized mouse models and a world leading proprietary gene editing platform, Biocytogen is dedicated to providing full support on every stage of your antibody discovery journey.

We have characterized RenMab™ Mouse with the following features.

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fully human antibody variable regions through V(D)J recombination. This capability can lead to improved hit rates in antibody drug discovery.

In conclusion, RenMab™ Mouse, a fully human antibody mouse developed by Biocytogen's chromosome engineering technology, has been proven to exhibit normal immune cell profile and rapid immune response.

Combined with existing inventory of single, double or triple target humanized mouse models and a world-leading proprietary gene editing platform, Biocytogen is dedicated to providing a one-stop solution for antibody discovery and development from target validation to IND application.

Find out more at biocytogen.com or renmab.com.



**The Antibody Society
Student/PostDoc
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Fighting protozoan parasites using carbohydrate-binding nanobodies

Felix Goerdeler, Phd Student at Max Planck Institute of Colloids & Interfaces

Find out more at mpikg.mpg.de.

Protozoan parasites such as Plasmodium, Leishmania or Toxoplasma are responsible for some of the most severe health problems worldwide.

The intracellular forms of these parasites carry a dense cover of carbohydrate chains attached to a lipid moiety called glycosylphosphatidylinositols (GPIs).

Whereas mammalian GPIs usually anchor proteins to the cell surface, the majority of parasitic GPIs is protein-free. Previous work revealed that these GPIs are highly immunogenic and can affect the severity of symptoms in the host.

A well-characterized example are Plasmodium GPIs which act as inflammatory toxins in

malaria.

Nanobodies are the smallest antigen-binding fragments from heavy-chain-only antibodies exclusively found in camelids (camel, llama, alpaca).

Their single-domain nature allows straightforward expression in bacterial systems and their small size (approx. 15 kDa) enables

Fighting protozoan parasites using carbohydrate-binding nanobodies



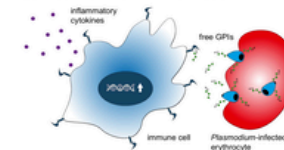
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BACKGROUND

Plasmodium and other protozoan parasites are coated with highly immunogenic and proinflammatory glycosylphosphatidylinositols (GPIs) [1].



GOALS

- develop and characterize a glycan-binding nanobody against Plasmodium GPIs
- examine the GPIs of various protozoan parasites with the nanobody
- functionalize the nanobody for diagnostic and therapeutic application

Camelids possess heavy-chain-only antibodies with ultra-small antigen-binding domains, called nanobodies [2].



METHODS

Glycan arrays

Isolation of cDNA and purification of glycan antibodies

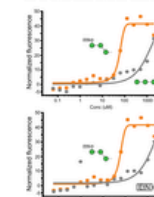
In silico identification and nanobody expression

Validation

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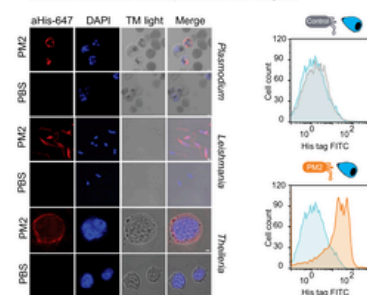
RESULTS

Nanobody PM2 binds to synthetic GPIs with micromolar affinity.



Structure	K _d (nM)
PM2 (Kunitz)	62 ± 12
PM2 (Kunitz + His tag)	>1000
PM2 (Kunitz + His tag + His tag)	>1000

PM2 recognizes Plasmodium, Leishmania and Theileria parasites but does not interact with Babesia, Besnoitia and Neospora.

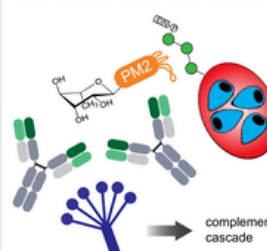


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OUTLOOK

Nanobody functionalization with immunogenic glycans will recruit endogenous antibodies and induce complement-dependent cellular cytotoxicity.



REFERENCES

- [1] Boullis et al. (2005) CTM: 297:145-85
- [2] Muyldermans (2013) Annu Rev Biochem; 82:775-97

them to reach less accessible antigens, e.g. intracellular targets, while maintaining high affinity and stability.

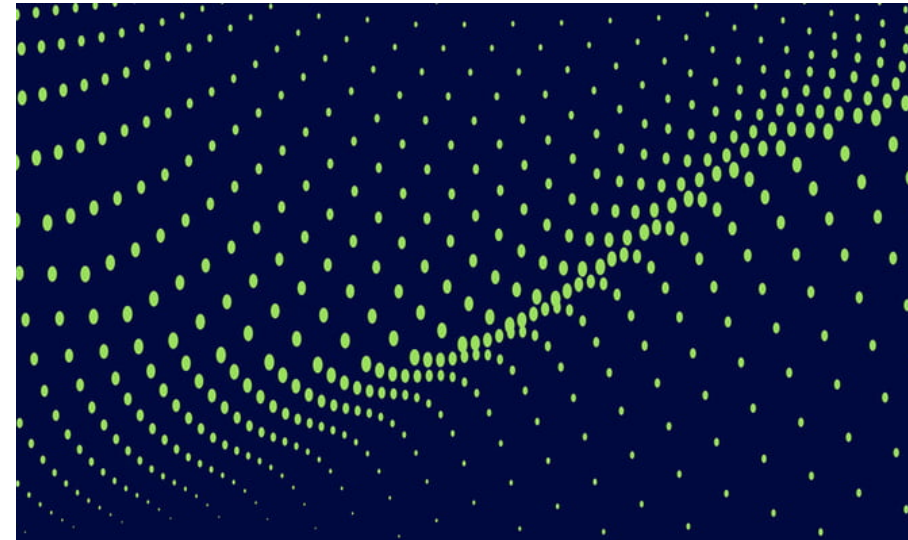
Furthermore, nanobodies can be easily functionalized to give them additional properties, such as multivalency or multispecificity, or to couple them to effector molecules, such as drugs or fluorescence labels. Due to the high structural complexity of glycans, hardly any glycan-targeting nanobodies were described so far.

Here, we show the successful development of glycan-targeting nanobodies against parasitic GPIs. Affinity measurements with

synthetic GPIs confirmed that nanobody binding is glycan-dependent. Furthermore, we found that our nanobody recognizes native glycans on the surface of Plasmodium falciparum, Leishmania major/mexicana and Theileria annulata parasites but not Babesia, Besnoitia or Neospora which renders it a useful diagnostic tool.

Next, we sought to functionalize the nanobody for therapeutic applications. Foreign and immunogenic glycan epitopes such as rhamnose or aGal, are present on the food that we consume. They initiate the development of glycan-specific naturally-circulating antibodies. We aim to exploit the presence of such

antibodies to amplify an anti-parasitic response using synthetic glycan-coupled nanobodies. The functionalized nanobodies will recruit naturally-circulating antibodies and subsequent anti-parasitic complement response.



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ACKNOWLEDGEMENTS

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Patricia Priegue
Katrin Sellrie

Parasite Infections GRK 2048

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Freie Universität Berlin

MAX PLANCK INSTITUTE OF COLLOIDS AND INTERFACES

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Sultan, the alpaca



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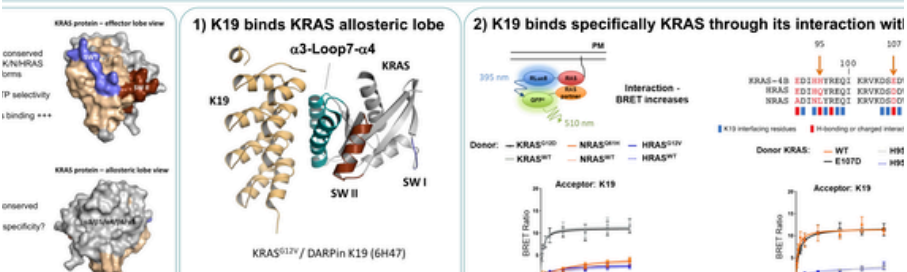
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Discovery of a potent KRAS macromolecule degrader specifically targeting tumours with mutant KRAS

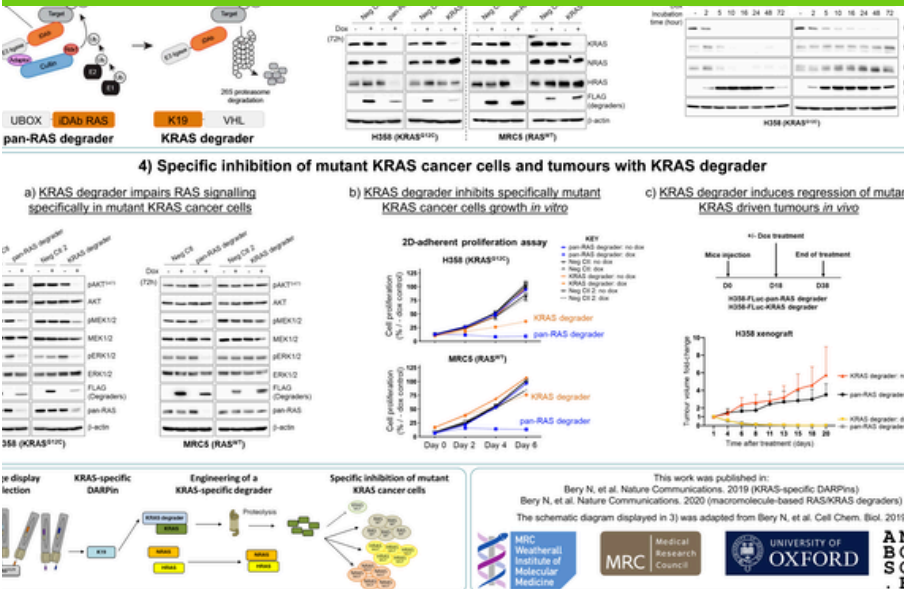
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KRAS-associated KRAS mutations are the most prevalent in the three RAS-family isoforms and involve many different amino-acids. Therefore, most of the small molecule inhibitors that interfere with mutant KRAS protein are potentially important for wide-ranging tumour therapy. We describe here (1) the selection and characterisation of a KRAS-specific antibody mimetic (Designed Ankyrin Repeat Protein, DARPIN K19) and (2) the engineering of this DARPIN into RAS degraders by fusing it to macromolecules fused to specific E3 ligases. The KRAS-specific DARPIN K19 fused to the VHL E3 ligase is compared to a pan-RAS intracellular antibody (iDab RAS) fused to the UBOX domain of the CHIP E3 ligase. We demonstrate that while the KRAS-specific DARPIN K19 degrader induces proteolysis of both mutant and wild type KRAS, it only inhibits proliferation of cancer cells expressing mutant KRAS *in vitro* and *in vivo*. Pan-RAS degradation, however, impairs proliferation irrespective of the RAS mutation. These data show that specific KRAS degradation is an important strategy to affect tumours expressing any of the range of KRAS mutations.



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Discovery of a potent KRAS macromolecule degrader specifically targeting tumours with mutant KRAS

Nicolas Bery, Postdoctoral Scientist at Cancer Research Centre of Toulouse (CRCT)

Find out more at crct-insERM.fr.

Tumour-associated KRAS mutations are the most prevalent in the three RAS-family isoforms and involve many different amino-acids.

Therefore, molecules able to interfere with mutant KRAS protein are potentially important for wide-ranging tumour therapy.

We describe here (1) the selection and characterisation of KRAS-specific antibody mimetics (Designed Ankyrin Repeat Proteins, DARPins) and (2) the engineering of these DARPins into RAS degraders based on protein macromolecules fused to specific E3 ligases.

The KRAS-specific DARPIN K19 fused to the VHL E3 ligase is compared to a pan-RAS intracellular single domain antibody (iDab) fused to the UBOX domain of the CHIP E3 ligase.

We demonstrate that while the KRAS-specific DARPIN degrader induces specific

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Pan-RAS protein degradation, however, affects proliferation irrespective of the RAS mutation.

These data show that specific KRAS degradation is an important therapeutic strategy to affect tumours expressing any of the range of KRAS mutations.

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Discovery of a potent KRAS macromolecule degrader specifically targeting tumours with mutant KRAS

Dr. Nicolas Bery

Antibody Engineering & Therapeutics, December 14-16, 2020

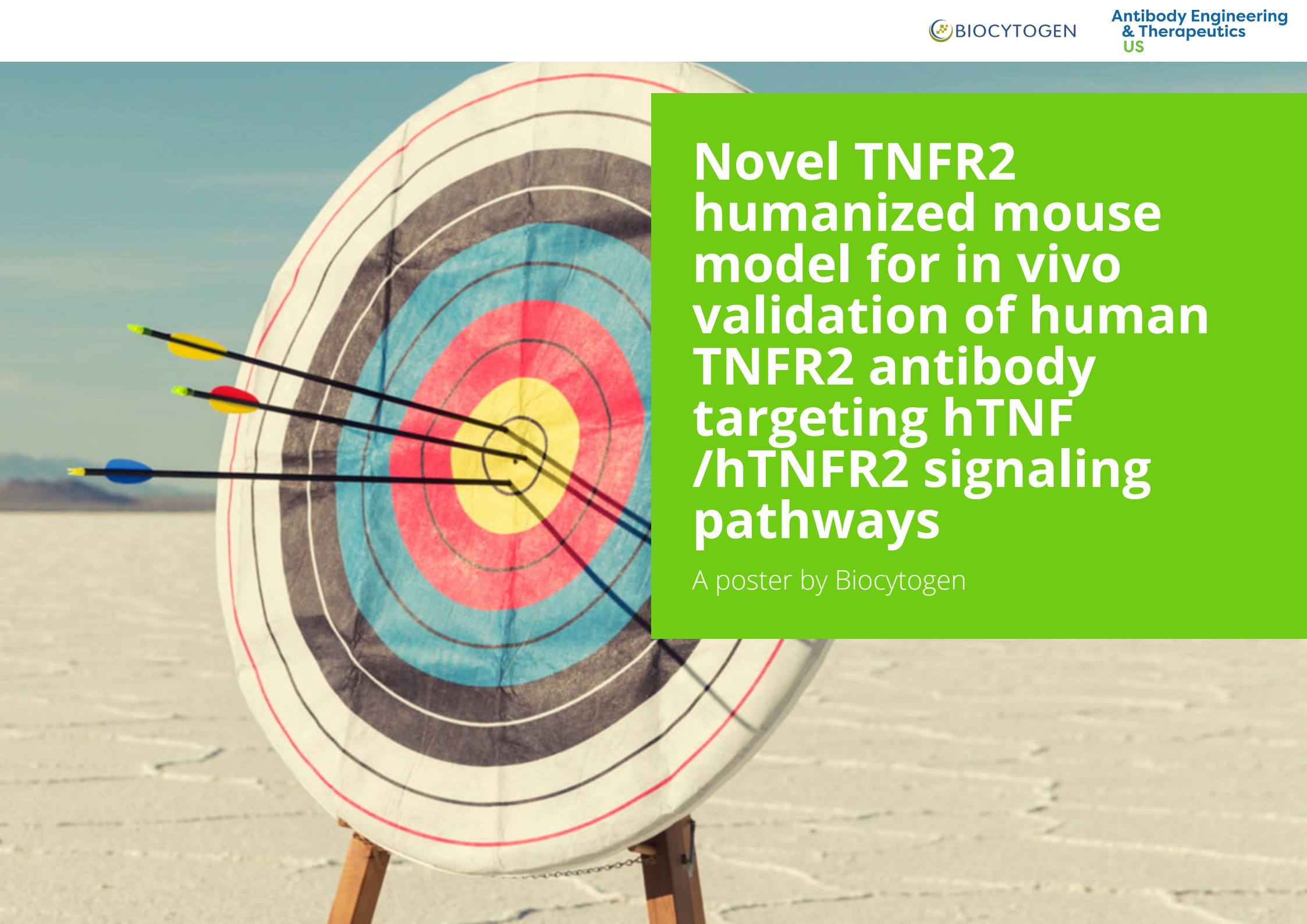
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Novel TNFR2 humanized mouse model for in vivo validation of human TNFR2 antibody targeting hTNF /hTNFR2 signaling pathways

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Novel TNFR2 humanized mouse model for in vivo validation of human TNFR2 antibody targeting hTNF /hTNFR2 signaling pathways



Yanan Guo, Yanan Li, Xiaofei Zhou, Youhong Su, Qingcong Lin., Biocytogen

Novel TNFR2 humanized mouse model for in vivo validation of human TNFR2 antibody targeting hTNF /hTNFR2 signaling pathways

Yanan Guo¹, Yanan Li¹, Xiaofei Zhou², Youhong Su¹, Qingcong Lin¹.
¹Biocytogen, Wakefield, MA; ²Biocytogen, Beijing

Abstract

In recent years, immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment. Tumor necrosis factor receptor 2 (TNFR2), also known as tumor necrosis factor receptor superfamily member 1B (TNFRSF1B), is a transmembrane receptor that plays an essential role in immune modulation and tissue regeneration (Chen et al., 2008). TNFR2 is mainly expressed on the surface of immune cells in particular CD8 and CD4 T cells, including regulatory T cells in inflammatory and tumor-microenvironment (Wu et al., 2013, Azizi et al., 2018). TNFR2 promotes the proliferation of Tregs through nuclear factor kappa B (NF- κ B) signaling (Rodriguez et al., 2011). TNFR2 also provided early costimulatory signals during CD4 and CD8 T cell activation (Kim et al., 2006). Both antagonistic and agonistic anti-TNFR2 antibodies have been developed to modify T cell functions and shown to inhibit tumor growth (Torrey et al., 2019, Tam et al., 2019). Therefore, TNFR2 has become an enthusiastically pursued therapeutic target for both immuno-oncology and autoimmune disease field.

To investigate the role of TNFR2, Biocytogen generated TNFR2 humanized mouse for both *in vitro* function validation of signaling pathway and *in vivo* efficacy evaluation of TNFR2 antibodies. In this model, the exons 2~6 of mouse *Tnfrsf1b* gene which encode the extracellular domain were replaced by human *TNFRSF1B* counterparts. Human extracellular domain of TNFR2 was detectable on the Tregs in spleen and the anti-human TNFR2 antibodies associated well to the splenocytes of the TNFR2 humanized mice. Basal leukocyte subpopulations of TNFR2 humanized mice were comparable to those of wild-type mice, including T/B cells, NK cells, DC, granulocytes and monocytes/macrophages. Anti-human TNFR2 antibodies bound with CD3+ T cells and inhibited tumor growth in TNFR2 humanized mice. Taken together, TNFR2 humanized mouse is a valuable tool for *in vivo* efficacy assessment of therapeutics that target human TNFR2.

In recent years, immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment.

Tumor necrosis factor receptor 2(TNFR2), also known as tumor necrosis factor receptor superfamily member 1B (TNFRSF1B), is a transmembrane receptor that plays an essential role in immune modulation and tissue regeneration (Chen et al., 2008).

TNFR2 is mainly expressed on immune cells specifically on the surface of potent regulatory T cells (Tregs) (Wu et al., 2008) and promote the proliferation of Tregs through nuclear factor kappa B (NF- κ B) (Chen et al., 2013; Rodríguez et al., 2011).

Anti-TNFR2 antibodies have been developed to inhibit NF- κ B driven growth and have revived excitement for the use of anti-



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TNFR2 antibodies in the clinic.

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comparable to those of wild-type mice, including T/B cells, NK cells, DC, granulocytes and monocytes/macrophages.

Anti-human TNFR2 antibodies bound well with CD3+ T cells and inhibited tumor growth in TNFR2 humanized mice.

Taken together, TNFR2 humanized mice is a useful tool for in vivo efficacy evaluation of therapeutics that target human.

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ABSTRACT

In recent years, immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment. Tumor necrosis factor receptor 2 (TNFR2), also known as tumor necrosis factor receptor superfamily member 18 (TNFRSF18), is a transmembrane receptor that plays an essential role in immune modulation and tissue regeneration (Chen et al., 2006). TNFR2 is mainly expressed on the surface of immune cells in particular CD8 and CD4 T cells, including regulatory T cells in inflammatory and tumor-microenvironment (Wu et al., 2013; Azou et al., 2016). TNFR2 promotes the proliferation of Tregs through nuclear factor kappa B (NF- κ B) signaling (Rodriguez et al., 2011). TNFR2 also provided early costimulatory signals during CD4 and CD8 T cell activation (Kim et al., 2006). Both antagonistic and agonistic anti-TNFR2 antibodies have been developed to modify T cell functions and shown to inhibit tumor growth (Tansey et al., 2019; Tam et al., 2019). Therefore, TNFR2 has become an enthusiastically pursued therapeutic target for both immuno-oncology and autoimmune disease field.

To investigate the role of TNFR2, Biocytogen generated TNFR2 humanized mouse for both in vitro function validation of signaling pathway and in vivo efficacy evaluation of TNFR2 antibodies. In this model, the exons 2-6 of mouse *Tnfrsf1b* gene which encode the extracellular domain were replaced by human TNFRSF1B counterparts. Human extracellular domain of TNFR2 was detectable on the Tregs in spleen and the anti-human TNFR2 antibodies associated well to the splenocytes of the TNFR2 humanized mice. Basal leukocyte subpopulations of TNFR2 humanized mice were comparable to those of wild-type mice, including T/B cells, NK cells, DC, granulocytes and monocytes/macrophages. Anti-human TNFR2 antibodies bound well with CD3+ T cells and inhibited tumor growth in TNFR2 humanized mice. Taken together, TNFR2 humanized mouse is a valuable tool for in vivo efficacy assessment of therapeutics that target human TNFR2.

RESULTS AND DISCUSSIONS

Fig 1. Humanized TNFR2 mouse model generation and mRNA expression analysis

(A) Targeting strategy. (B) RT-PCR analysis of hTNFR2 expression. Mouse *Tnfrsf1b* mRNA was detectable only in splenocytes of wild-type (+/+), but not in +/+ mice. Human TNFRSF18 mRNA was detectable only in +/+ mice. (C) RT-qPCR analysis of hTNFR2 expression. The expression of hTNFR2 in B-hTNFR2 (+/+), was similar to that of mTNFR2 in the C57BL/6 (+/+), mice at mRNA level. Values were expressed as mean \pm SEM.

Fig 2. Protein expression analysis in B-hTNFR2 mice

Strain specific TNFR2 expression in homozygous B-hTNFR2 mice. Splenocytes were collected from WT and homozygous B-hTNFR2 (+/+), mice stimulated with anti-CD3 in vivo and analyzed by flow cytometry with species-specific anti-TNFR2 antibody Human TNFR2. Human TNFR2 was exclusively detectable in T cells (A) and Tregs (B) of homozygous B-hTNFR2 but not in WT mice.

Fig 3. Analysis of spleen leukocytes cell subpopulations in B-hTNFR2 mice

Splenocytes were isolated from female C57BL/6 and B-hTNFR2 mice (n=3, 6-week-old). Flow cytometry analysis of the splenocytes was performed to assess leukocyte subpopulations. (A) Single live cells were gated for CD45 population and used for further analysis of leukocyte subpopulations as indicated in the representative FACS plots. (B) Results of FACS analysis. Percentages of T, B, NK, Myocyte, DC and macrophage cells in homozygous B-hTNFR2 mice were similar to those in the C57BL/6 mice, indicating that introduction of hTNFR2 extracellular domain

Fig 4. Analysis of spleen T cell subpopulations in B-hTNFR2 mice

Splenocytes were isolated from female C57BL/6 and B-hTNFR2 mice (n=3, 6-week-old). Flow cytometry analysis of the splenocytes was performed to assess T cell subpopulations. (A) Representative FACS plots. Single live CD45+ cells were gated for CD3 T cell population and used for further analysis as indicated. (B) Results of FACS analysis. Percent of CD8, CD4, and Treg cells in homozygous B-hTNFR2 mice were like those in the C57BL/6 mice, demonstrating that introduction of hTNFR2 extracellular domain in place of its mouse counterpart does not change the overall development, differentiation or distribution of these T cell subtypes in spleen. Values were expressed as mean \pm SEM.

Fig 5. Antibody binding assay in B-hTNFR2 mice

Anti-human TNFR2 antibodies inhibited MC38 tumor growth in B-hTNFR2 mice. Murine colon cancer MC38 cells (5E5) were subcutaneously implanted into homozygous B-hTNFR2 mice (female, 9-10-week-old, n=8). Mice were grouped when tumor volume reached approximately 100 mm³, at which time they were treated with anti-human TNFR2 antibodies with doses and schedules indicated in the panels. Anti-human TNFR2 antibodies were efficacious in controlling tumor growth in B-hTNFR2 mice. (B) Body weight changes during treatment. Values were expressed as mean \pm SEM.

Fig 6. In vivo efficacy of anti-human TNFR2 antibodies

(A) Anti-human TNFR2 antibodies inhibited MC38 tumor growth in B-hTNFR2 mice. Murine colon cancer MC38 cells (5E5) were subcutaneously implanted into homozygous B-hTNFR2 mice (female, 9-10-week-old, n=8). Mice were grouped when tumor volume reached approximately 100 mm³, at which time they were treated with anti-human TNFR2 antibodies with doses and schedules indicated in the panels. Anti-human TNFR2 antibodies were efficacious in controlling tumor growth in B-hTNFR2 mice. (B) Body weight changes during treatment. Values were expressed as mean \pm SEM.

CONCLUSIONS

1. Humanized TNFR2 mouse model (B-hTNFR2) expresses TNFR2 protein that bears human TNFR2 extracellular domain with the mouse transmembrane and cytoplasmic domains.
2. B-hTNFR2 showed normal subpopulations of immune cells.
3. Anti-human TNFR2 antibodies showed robust anti-tumor efficacy in MC38/B-hTNFR2 mice.
4. The B-hTNFR2 mice are a powerful preclinical model for in vivo efficacy evaluation of human TNFR2 therapeutics.

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