

# Discovery and development of a transferrin receptor antibody panel for efficient blood-brain barrier delivery as part of a multispecific antibody

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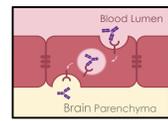


## Background – Developing blood-brain barrier transporters

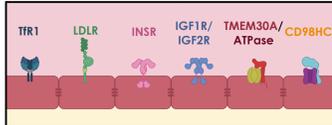


- The blood-brain barrier (BBB) presents a significant obstacle to the utility of conventional antibodies in the treatment of neurological conditions.
- One promising delivery approach utilizes receptor-mediated transcytosis (RMT) where the therapeutic cargo is targeted to a receptor present in the brain endothelia via a secondary binding domain which can facilitate transport across the BBB.
- Multiple receptors have been identified that facilitate transport across the BBB. Transferrin receptor 1 (TfR1) has been a key focus for these approaches due to its well-studied transport properties and clinical validation.
- Multispecific antibodies that target both the brain-specific therapeutic target and a transport receptor, like TfR1, are increasingly prevalent in the field.
- Therefore, we have developed a diverse panel of antibodies that target TfR1 and can be paired with many therapeutic modalities to cross the BBB.
- The TfR antibody panel developed here provides a novel toolset to aid in the development of complex multispecific antibodies across neurological indications.

### Receptor-Mediated Transcytosis (RMT)



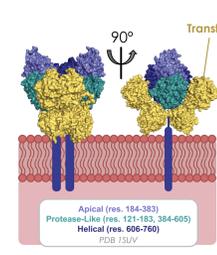
### RMT Receptors



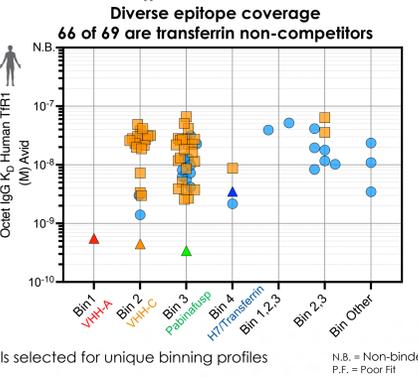
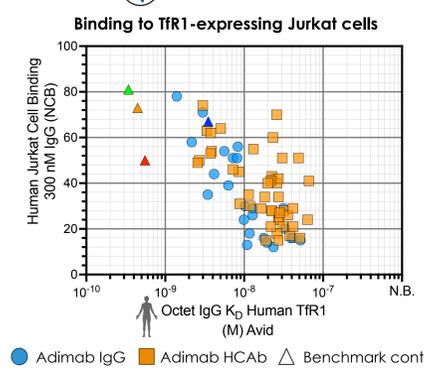
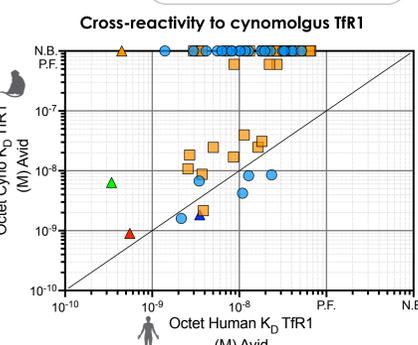
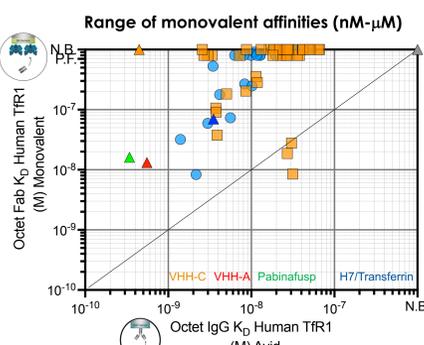
## Discovery of TfR1 antibodies

### Desired TfR1 antibody profiles

- CROSS-REACTIVITY**
  - Human TfR1: 10-100 nM (monovalent) ( $K_{on}$ :  $10^2 - 10^3$ )
  - Cyno TfR1: 94% sequence identity Required
  - Mouse TfR1: 76% sequence identity Desired
- THERAPEUTIC MECHANISM**
  - Engage with receptor to potentiate RMT

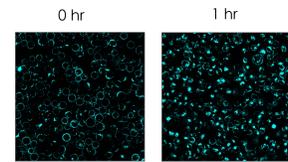


- DESIRED OTHER FEATURES**
  - Suitable for multiple formats: Fc-Fab, Fc-scFv, Fc-sdAb
  - Does not interfere with Transferrin (Tf) binding

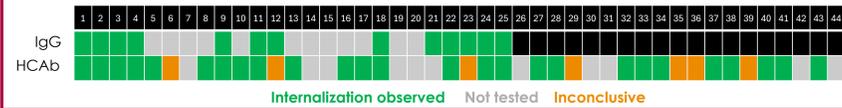


## Robust internalization observed for discovered antibodies

- Internalization assessed for primary discovery panel using human Jurkat cells expressing human TfR1
- Fluorescently conjugated antibodies were allowed to bind cells for 30 min; internalization was assessed following 1 hour at 37°C
- Internalization was confirmed by the appearance of cytoplasmic puncta on confocal microscopy



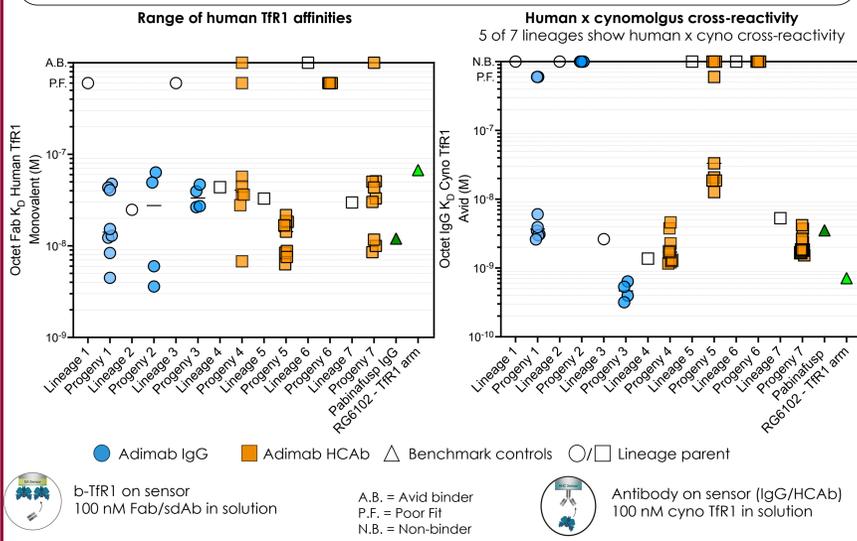
### Internalization Summary for full panel



## Lead clones were optimized for a range of affinities

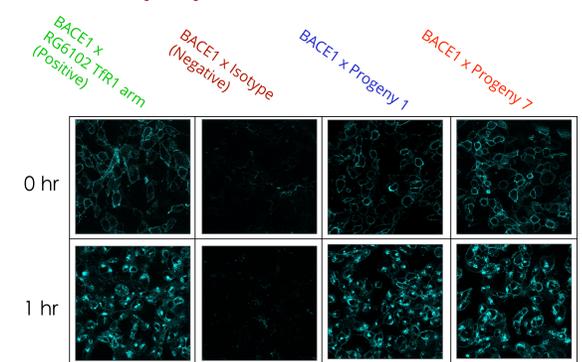
### Optimization goals

- 7 nominees selected from primary discovery - 3 IgGs and 4 HCAbs
- Human affinity optimization (range of monovalent hTfR1 affinities)
- Improved cyno cross-reactivity
- Maintain good developability characteristics



## Multispecific antibodies are rapidly internalized

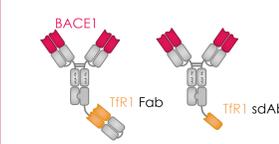
- Internalization assessed for 2+1 multispecific antibodies using hCMEC/D3 cell line
- Fluorescently conjugated antibodies were incubated with cells at 100 nM
- Internalization was assessed using confocal microscopy following 1 hour at 37°C
- Bright cytoplasmic staining was observed for all multispecific antibodies containing a TfR1 targeting arm



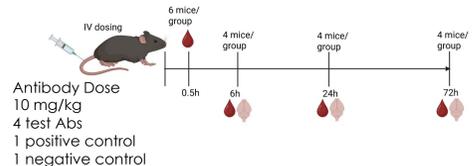
## Multispecific antibodies cross the BBB as well as positive control

### Study Design

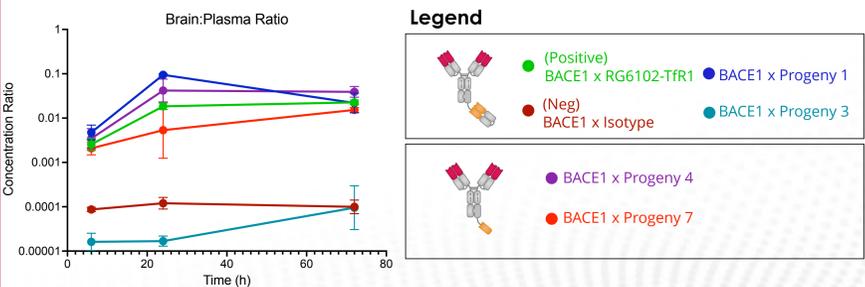
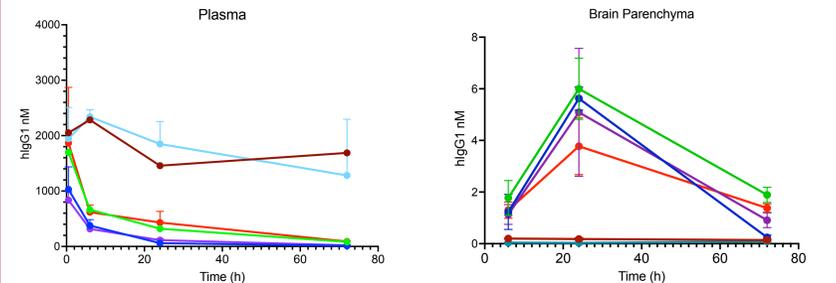
Bispecific Antibodies



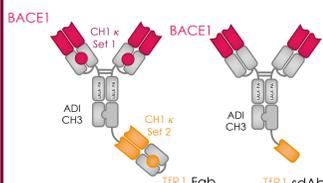
C57BL/6-Tfrc<sup>(tm1Tfrc)</sup>/Bgen (B-hTfR1)



### Three TfR1 antibodies show comparable brain accumulation to positive TfR1 control



## Efficient generation of multispecific BBB antibodies



### Input TfR1 binding arm data

C-Term / Fc Binding Arm	Hu Affinity (K <sub>d</sub> ) Avid	Hu Affinity (K <sub>d</sub> ) Mono	Cy Affinity (K <sub>d</sub> ) Avid	Hu TfR1 Jurkat Cell Binding (NCB) Fab/sdAb
RG6102 TfR1 arm (+ control)	0.7 nM	67 nM	0.7 nM	47
Isotype (- control)	N.B.	N.B.	N.B.	0
<b>Fab</b>				
Progeny 1	P.F.	12 nM	3.1 nM	103
Progeny 3	0.2 nM	27 nM	0.6 nM	51
<b>sdAb</b>				
Progeny 4	0.6 nM	28 nM	1.2 nM	37
Progeny 7	0.7 nM	33 nM	2.4 nM	47

- Multispecifics generated using anti BACE1 binding arms paired with a single TfR1 binding arm (Fab or sdAb)
- Correct HC-LC pairing was maintained by using Adimab's proprietary HC-LC pairing sets (set 1 and set 2)
- HC-HC pairing was maintained using Adimab's CH3 pairing sets
- All constructs were generated with LALA-PA silencing mutations in the CH2 domain

1. Barlow et al., *MAbs* 2025 Dec; 17:1, 2479531 (10.1080/19420862.2025.2479531)

## Conclusions

- We have generated a panel of 69 antibodies (VHH and HCAb) that target human TfR1 with a range of monovalent binding affinities to TfR1
- 66 of 69 antibodies in the panel do not compete with transferrin binding
- Binning against a panel of benchmark antibodies showed diverse epitope coverage
- 7 lineages (IgG & HCAb) have been optimized to improve human x cynomolgus TfR1 cross-reactivity and to provide a range of affinities
- Four of the optimized antibodies have been converted into 2+1 multispecific antibodies using Adimab's heterodimerization technologies
- These multispecific antibodies showed rapid internalization in a brain endothelial cell line (hCMEC/D3)
- Finally, using a humanized mouse TfR1 model, these antibodies showed improved accumulation in the brain compared to a negative control, and the accumulation was comparable to the clinically-validated TfR1 binding arm benchmark included in the study



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