



Novel TFRC humanized mouse model for drug delivery to the brain

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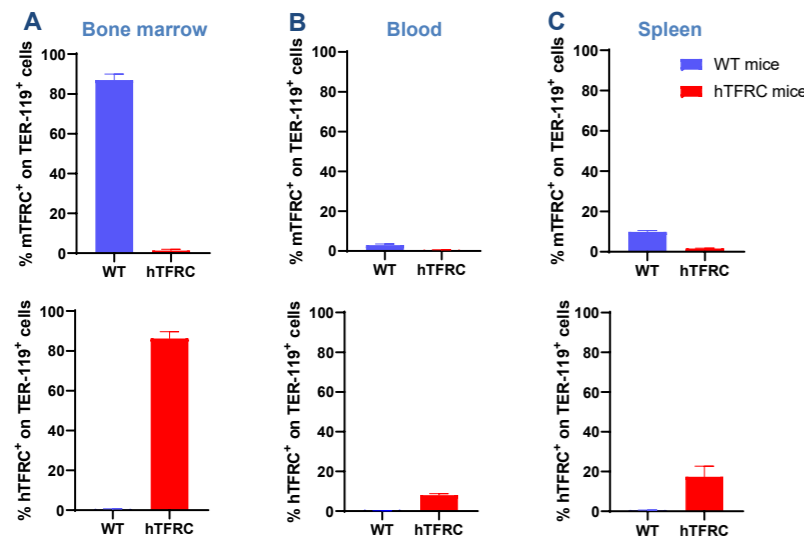


Background: Gliomas represent the most common neoplasm in central nervous system (CNS) (Gritsch et al., 2022). Treatment with immunotherapy has been challenged by the limited delivery of therapeutic agents through the brain blood barrier (BBB).

Transferrin receptor (TFRC) is abundantly expressed along the BBB, conjugation of therapeutic agents with molecule binding the TFRC represent an alternative strategy to efficiently deliver drugs into the CNS.

Here we show a novel humanized model of TFRC developed to enable assessment of compounds targeting human TFRC (hTFRC) and aiming to deliver therapeutic agents through the BBB.

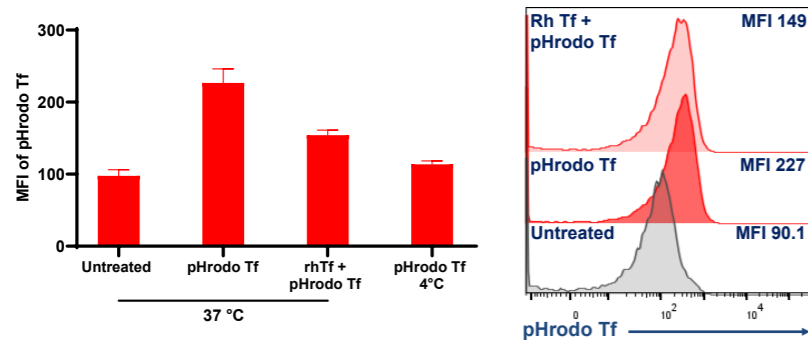
1. Human TFRC (hTFRC) expression on red blood cells



Expression of TFRC (anti-mouse TFRC clone C2, anti-human TFRC clone OKT-9) on TER-119⁺ cells from freshly isolated bone marrow cells (A), blood (B) and splenocytes (C), gated on single viable cells.

⇒ Expression of hTFRC mirrors expression of mTFRC

2. Human transferrin internalization in cells from hTFRC mice

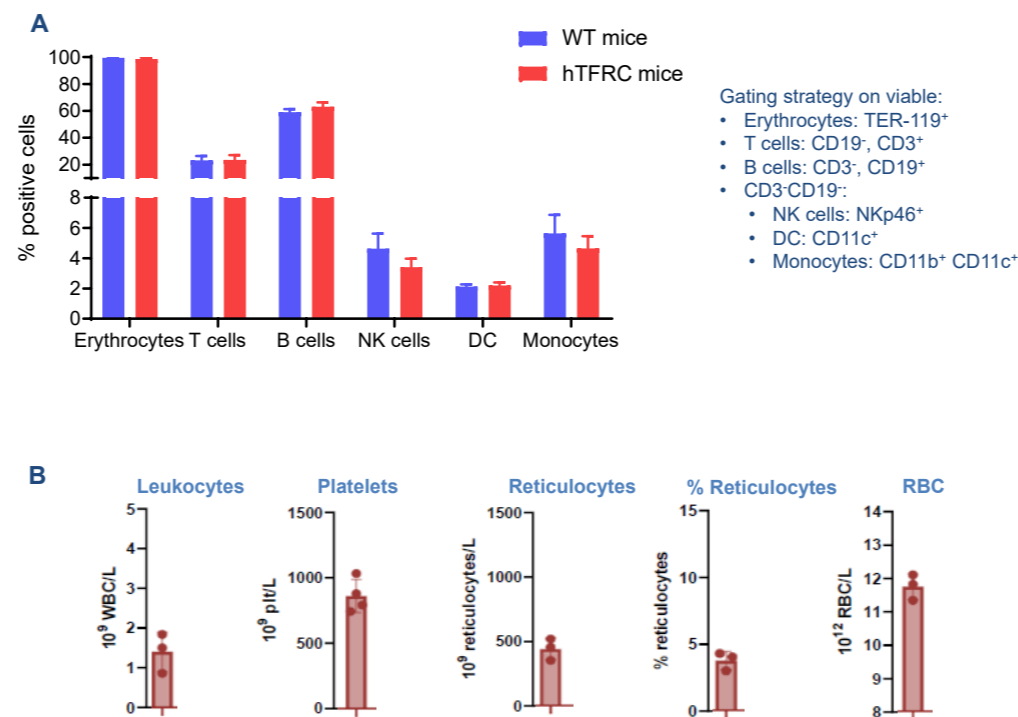


Splenocytes of hTFRC mice were incubated with human conjugate pHrodo red transferrin for 2 hours at 37°C. Internalization of human transferrin was confirmed by the increase of the MFI values of pHrodo Tf.

Specific activity of hTFRC: pre-incubation of splenocytes with pHrodo competitor, rh-transferrin, reduced the level of pHrodo internalization.

⇒ Human transferrin is efficiently internalized
⇒ TFRC is likely functional

3. Immune cell distribution and hematology parameters in blood of hTFRC mice



Frequency of distribution of immune cell populations in whole blood from WT and hTFRC mice was analyzed by flow cytometry (A); Hematology parameters in whole blood from hTFRC mouse model (B)

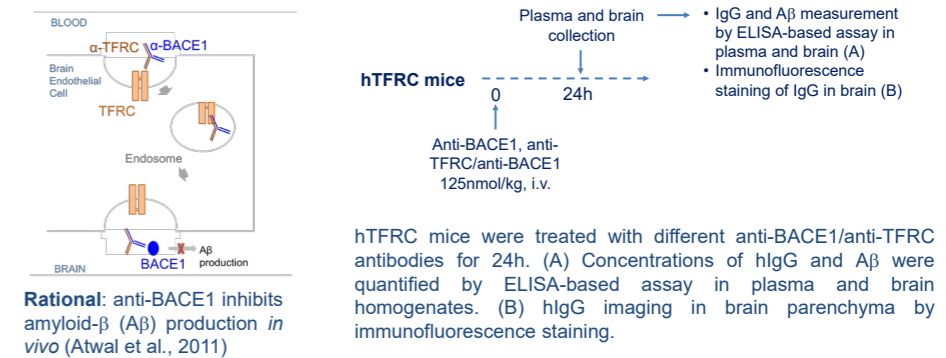
⇒ hTFRC model presents physiological distribution of immune cells and hematology parameters

| Model upgrade | Objective |
|-----------------|--|
| hTFRC/hSA/hFcRn | Better translatability of PK study of therapeutics with enhanced half-life through binding to hSA or hFcRn |
| hTFRC/hIgG1 | Tolerability to human IgG1 therapeutic antibodies |
| hTFRC/hFcγR | Better biodistribution of therapeutics to the brain by enabling drug shuttling through the BBB |

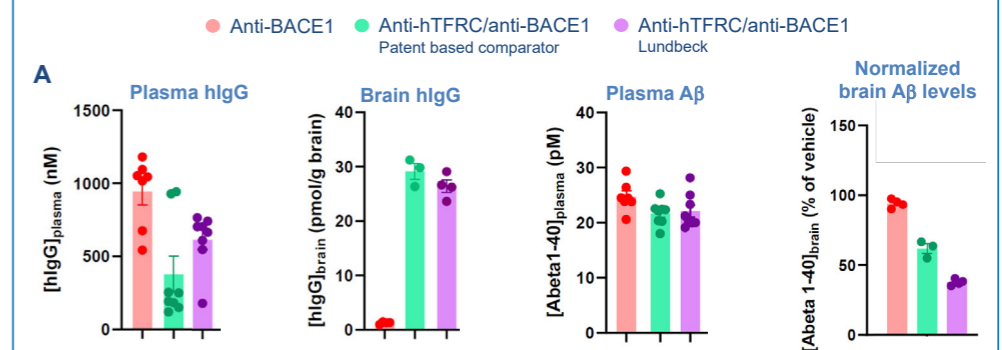
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4. Drug shuttling through the BBB in hTFRC mice

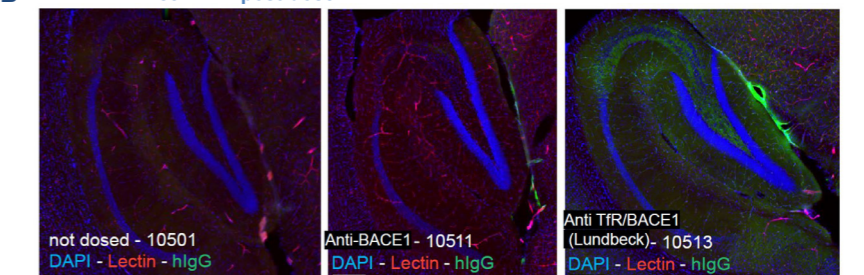


Rational: anti-BACE1 inhibits amyloid-β (Aβ) production *in vivo* (Atwal et al., 2011)



⇒ Combination of anti-hTFRC/anti-BACE1, but not anti-BACE1 alone, enables the biodistribution of IgG in the brain and the reduction of Amyloid-β.
⇒ Targeting human TFRC improves shuttling to the brain of the anti-BACE1 antibody

B hTFRC mice – 24h post-dose



⇒ Human IgG is detected in the brain of hTFRC mice upon administration of anti-hTFRC/anti-BACE1, but not anti-BACE1 alone
⇒ hTFRC is functional in hTFRC mice

Conclusion:

- Human TFRC mouse model is an efficient tool to assess the efficacy of therapeutic treatments targeting the CNS. Combination of existing therapies with anti-hTFRC allows overcoming the limitation of drug delivery through the BBB.
- The hTFRC model was intercrossed with the well-established hSA/FcRn humanized model (Viuff et al., 2016), to enable translatable PK and biodistribution of compounds targeting TFRC.