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Novel FcγR humanized mouse models enabling efficacy and PK/PD of therapeutic antibodies

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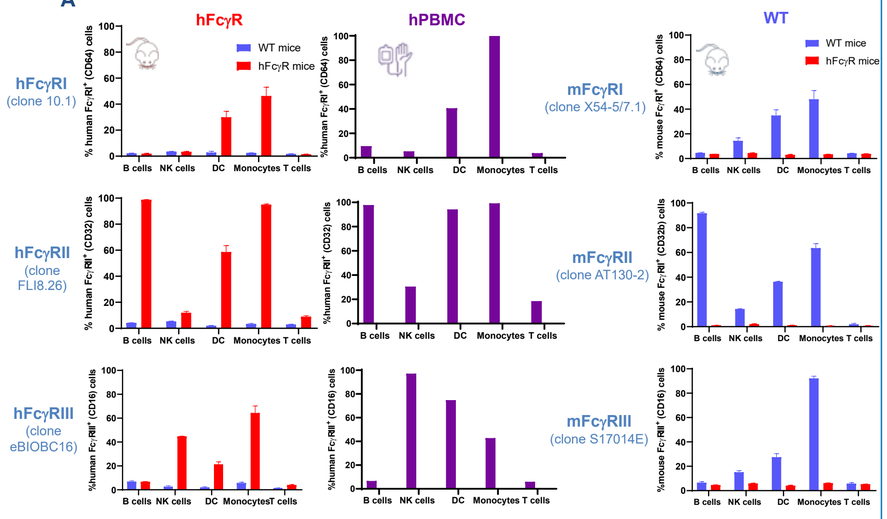
AACR 2024 Abstract #5341

Background: Therapeutic antibodies have revolutionized treatment of cancer. Enhanced activity of therapeutic IgG can be achieved by the modulation of Fc binding to Fcγ receptors (FcγR) which will modulate the Fc-effector function triggered upon crosslinking target effector cells by the therapeutic antibodies.

We report here a novel mouse model expressing humanized FcRI, FcγRIIA, FcγRIIB, FcγRIIIA and FcγRIIIB, instead of the mouse versions. This model was intercrossed with the previously described hSA/hFcRn mouse model (Viuff et al., 2016), which has shown to improve translatability of PK assessment of therapeutics with extended half-life via FcRn recycling and/or hSA binding.

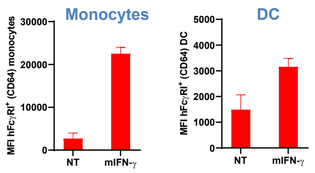
1. Expression of FcγRs

A



⇒ **hFcγR expression pattern in hFcγR mice is consistent with the expression in human PBMC**

B

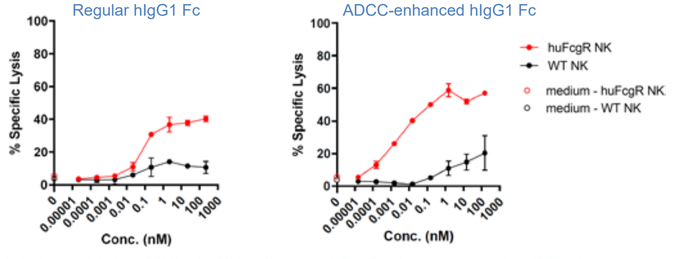


(A) FcγRI/II/III expression in freshly isolated splenocytes gated on $CD3^-CD19^+$ (B cells), $CD3^-CD19^-CD56^+$ (NK cells), $CD3^-CD19^-CD56^-CD11c^+CD14^-$ (Dendritic cells), $CD3^-CD19^-CD56^-CD14^+$ (Monocytes) and $CD3^+CD19^-$ (T cells)
(B) Expression of FcγRI on splenic monocytes and DC with or without 24-hours stimulation with IFN-γ (100 ng/mL)

⇒ **Expression of hFcγRI is increased upon treatment with IFN-γ on monocytes and DC as described in human cells (Bournazos et al., Microbiol Spectr., 2016) – Regulation of FcγRI expression is kept**

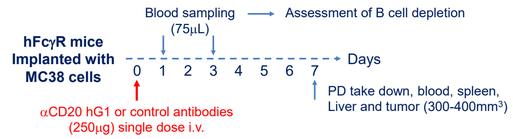
2. Functionality of hFcγR
2.1. *Ex vivo* ADCC by NK cells

- NK cells isolated from the spleen of WT or hFcγR mice loaded with B16F-10-CD20 cells loaded with ^{51}Cr (ratio 10:1) in the presence of Rituximab or Obinutuzumab
- Antibody-dependent cell cytotoxicity (ADCC) was measured by gamma radiation count in the supernatant

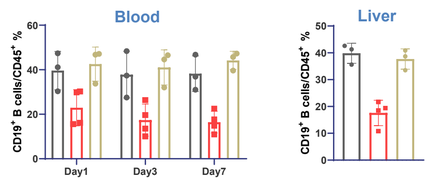


⇒ **Rituximab induces higher ADCC in NK cells from hFcγR mice compared to WT mice**
⇒ **Obinutuzumab, featuring an engineered Fc portion to enhance binding to FcγRIII (Mössner et al., Blood, 2010), induces higher lysis than Rituximab (regular Fc) in NK cells from hFcγR mice**

2.2. *In vivo* B cells depletion in hFcγR mice



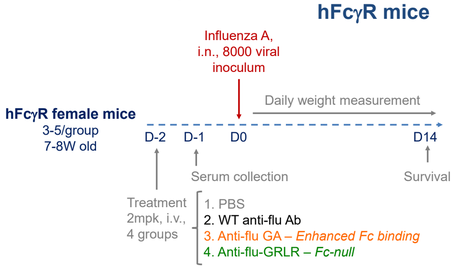
Assessment of B cells depletion by flow cytometry after treatment by anti-CD20 hG1 or control antibodies in hFcγR mice bearing MC-38 tumor
Note: No CD20 activity expected in MC38 cells – tumor model used to enable assessment of FcγR into the TME



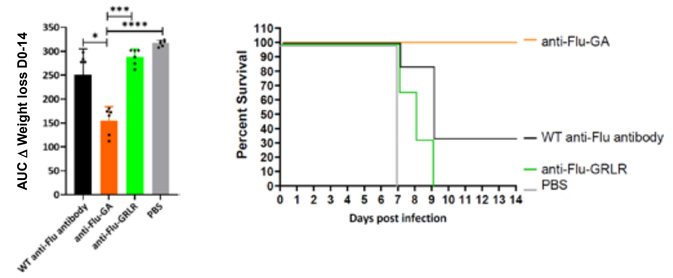
Frequency of total B cells in $CD45^+$ cells in blood and liver after treatment

⇒ **Anti-CD20 depletes approximately 50% of B cells in blood and liver from hFcγR mice**
⇒ **B cell depletion is dependent on Fc effector function (no activity in Fc-null anti-CD20 human IgG1)**

2.3. Enhanced protected effect of Fc-enhance-prophylaxic antibody in hFcγR mice



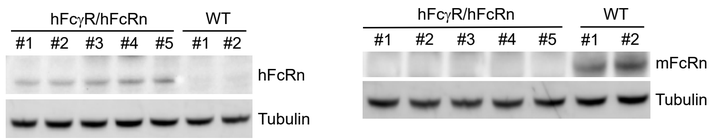
Assessment of antibodies efficacy in Influenza A virus-infected hFcγR mice through weight measurement and survival



⇒ **Anti-Flu mAb increases survival of Influenza A-infected hFcγR mice**
⇒ **Anti-Flu antibody with enhanced Fc binding improves protective activity and outperforms the WT antibody**
⇒ **No protective activity shown by Fc-null version of anti-Flu mAb**

3. Model upgrades

- hFcγR was intercrossed with the hFcRn mouse model to enable more trnslatable PK/PD assessment
- Expression of mouse or human FcRn in lung from hFcγR/hFcRn mice was determined by Western Blot analysis



⇒ **hFcRn is expressed in lung from hFcγR/hFcRn mice while mFcRn is expressed in lung from WT mice**
⇒ **Preliminary results show an enhanced PK translatability *(data not shown)***

Model upgrade	Rational
hFcγR/hSA/hFcRn	Better translatability of PK study of therapeutics with enhanced half-life through binding to hSA or hFcRn
hFcγR/hIgG1	Tolerability to human IgG1 therapeutic antibodies
hFcγR/hSA/hFcRn/Rag1$^{-/-}$	Assessment of translational PK/PD in immunodeficient model for engraftment with human cells
hFcγR/hTFRC	Better biodistribution of therapeutics to the brain through TFRC-mediated drug shuttling
hFcγR/Pan hCD3	Assessment of anti-CD3's efficacy
hFcγR/ immune checkpoint humanized models from genOway's catalog	Flexibility of therapeutics assessment: bispecific or combotherapies targeting immune checkpoints

Conclusion: Humanized FcγR model represents a useful tool for testing efficacy of Fc-engineered antibodies in several therapeutic domains: autoimmunity, immuno-oncology and viral infection.

References:
- Viuff D, Antunes F, Evans L, et al., Generation of a double transgenic humanized neonatal Fc receptor (FcRn)/albumin mouse to study the pharmacokinetics of albumin-linked drugs. J Control Release. 2016,10.1016
- Bournazos S, Wang TT, Ravetch JV. The Role and Function of Fcγ Receptors on Myeloid Cells. Microbiol Spectr. 2016 Dec;4(6):10.1128/microbiolspec.MCHD-0045-2016, 10.1128
- Mössner E, Brünker P, Moser S, et al., Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. Blood. 2010 Jun 3;115(22):4393-402, 10.1182

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