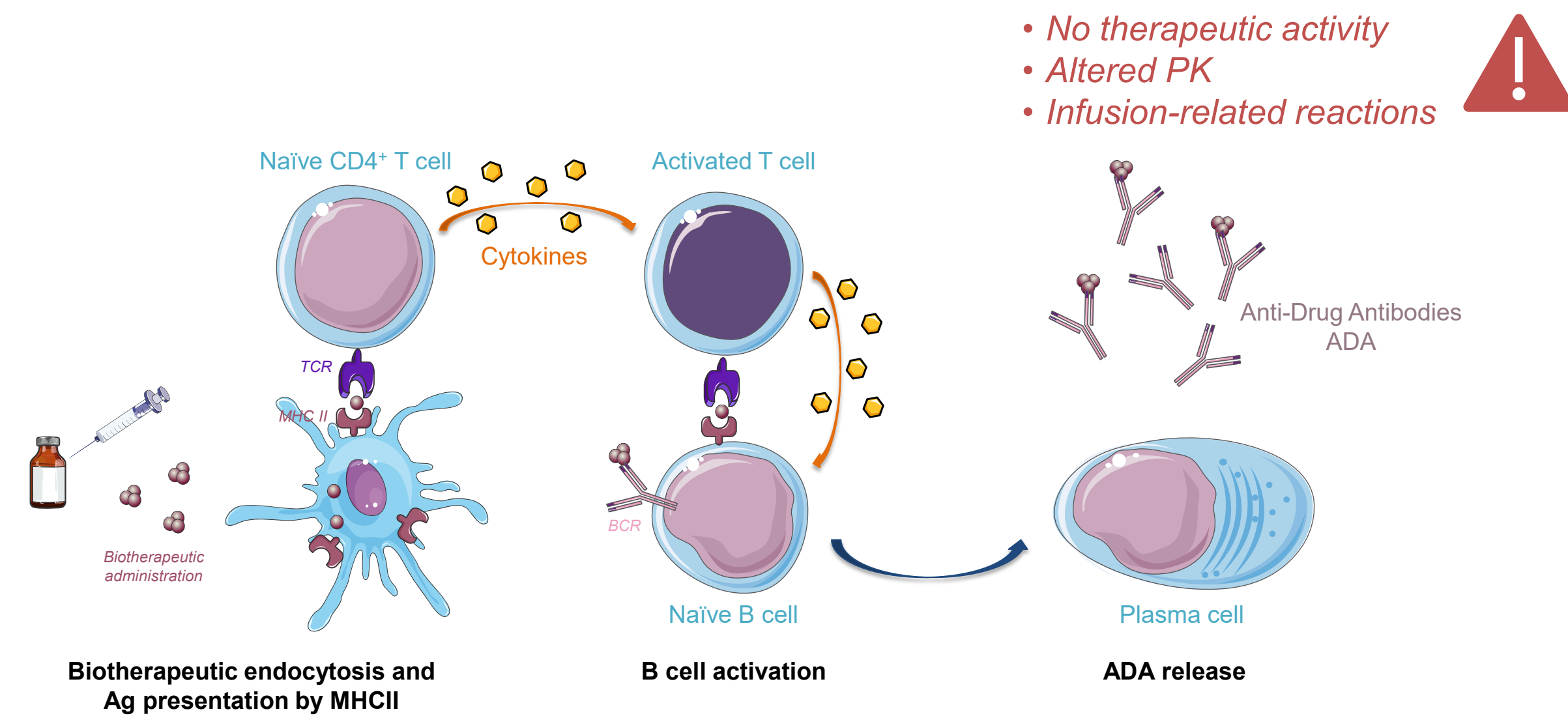


Background: Mouse models expressing human targets offer strong translational relevance for evaluating human antibody-based therapies in IO. However, when treated with human antibodies, these models frequently develop anti-drug antibodies (ADA), which can compromise the interpretation of preclinical data. ADA formation may alter pharmacokinetics (PK), reduce therapeutic efficacy, and introduce immune-related artifacts that do not reflect human responses. This immune recognition limits the duration and reliability of treatment studies, especially for repeated dosing or long-term efficacy assessments. Therefore, careful selection and engineering of preclinical models that minimize ADA formation are essential to accurately predict clinical performance and support the development of safe and effective biologics. We generated a model expressing humanized IgG1, the most used isotype in clinical development, which was designed to induce tolerance to human IgG1-based therapies. Herein, we describe the investigation of the tolerance induced by the expression of hlgG1 in a mouse model humanized for serum albumin, FcRn, which was developed to investigate PK profile of antibodies in a context of tolerance to hlgG1.

1. Anti-Drug Antibody (ADA): mechanism and consequences



- No therapeutic activity
- Altered PK
- Infusion-related reactions

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Adapted from Noel et al., Trends in Pharmacological Sciences, 2023

⇒ ADA leads to biased or lack of therapeutic activity, altered PK and potential infusion-related reactions

3. IgG1 humanization in other syngeneic models to induce tolerance to human IgG1-based therapies – Models upgrades

genO-Pan hCD3/hlgG1

- Assessment of T cell engager's efficacy while avoiding the ADA induced by hlgG1

Reference on genO-Pan hCD3 model:
• Gaspar et al., JITC, 2023
• Natoli et al., JITC, 2024
• Perico et al., Front. Immunol., 2024

genO-hFcγR/hlgG1

- Assessment of Fc-mediated therapies while avoiding ADA induced by hlgG1

Reference on genO-hFcγR model:
• Manasson et al., Arthritis Rheumatol., 2024
• Van Damme et al., Arthritis Rheumatol., 2025
• Stefanutti et al., Microbiol Spectr., 2025
• Van Damme et al., Sci. Immunol., 2026

genO-hTFRC/hlgG1

- Better biodistribution of therapeutics through epithelial layers by TFRC-mediated drug shuttling while avoiding ADA induce by hlgG1

Reference on genO-hTFRC model:
• Sônego et al., Cancer Res., 2024

Conclusion: Overall, these findings underscore the relevance of IgG1-humanized preclinical models for accurately predicting clinical performance by avoiding the development of ADA.



- References:**
(1): Viuff et al., Journal of Controlled Release, 2016
(2): Mandrup et al., Commun Biol., 2021
(3): Vantourout et al., Bioconjug Chem., 2021
(4): Archer et al., Clin Cancer Res., 2024
(5): Anthi et al., Nat Commun., 2025

2. genO-hSA/hFcRn model expressing humanized IgG1 to investigate PK profile and induce tolerance to human IgG1-based therapies

a. genO-hSA/hFcRn model expressing hlgG1

FcRn humanization:

- hFcRn knock-in at the mouse *FcRn* locus
- Mouse FcRn expression is invalidated
- Human FcRn driven by the mouse promoter
- Unmodified mouse β 2-m

Albumin humanization by knock-in:

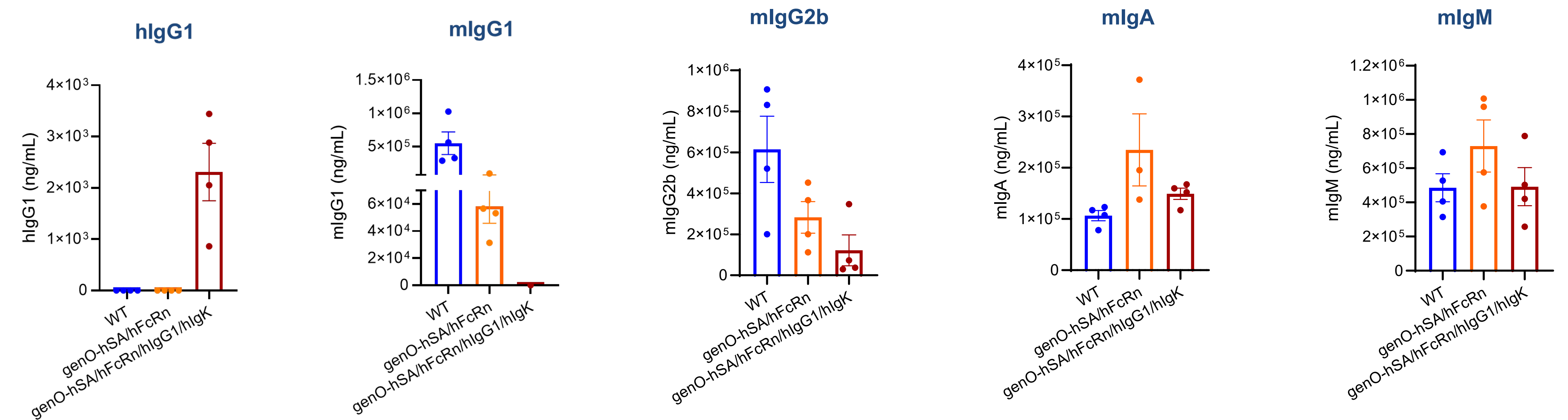
- hSA knock-in at the mouse *SA* locus
- Mouse SA is invalidated
- Signal peptide kept mouse to favor hSA processing in mouse cells
- Human SA driven by the mouse promoter

IgG1 humanized mice by knock-in:

- hlgG1 models, developed onto genO-hSA/hFcRn mice:
- Knock-in at the mouse *IgG* locus to humanize heavy chain
 - Only the constant domain of the IgG1 heavy chain is humanized
 - IgG1 variable domains are mouse
 - Physiological hlgG1 regulation: under mouse immunoglobulin locus regulation
 - Knock-in at the mouse *Igk* to humanize light chain

⇒ Model expresses hSA and hFcRn and has been successfully used to assess PK of compounds aiming at extended half-life through binding to hFcRn and hSA (1, 2, 3, 4, 5)

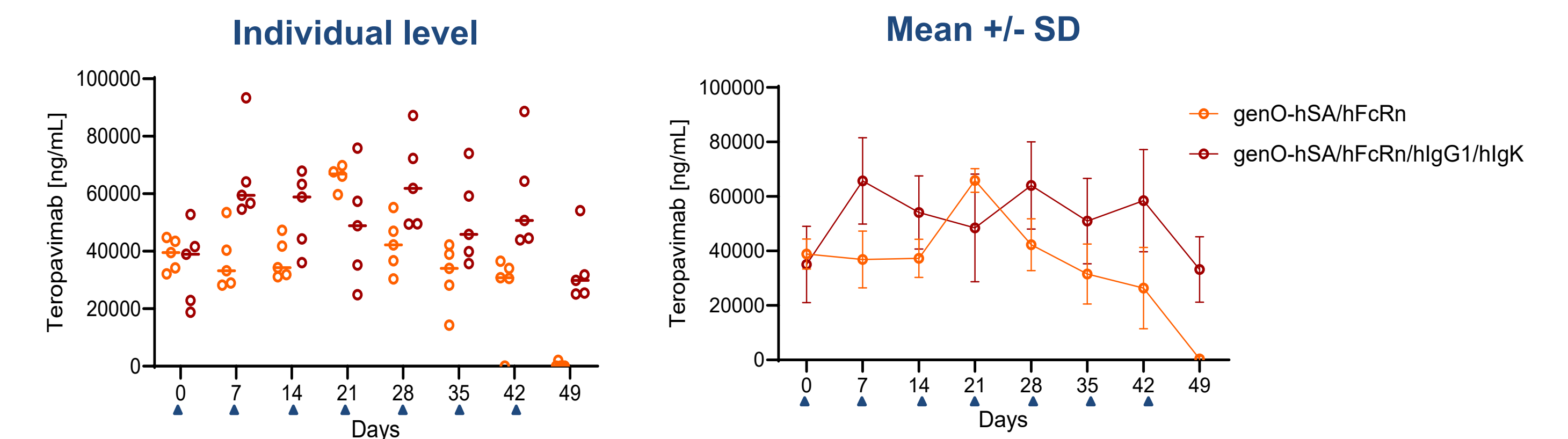
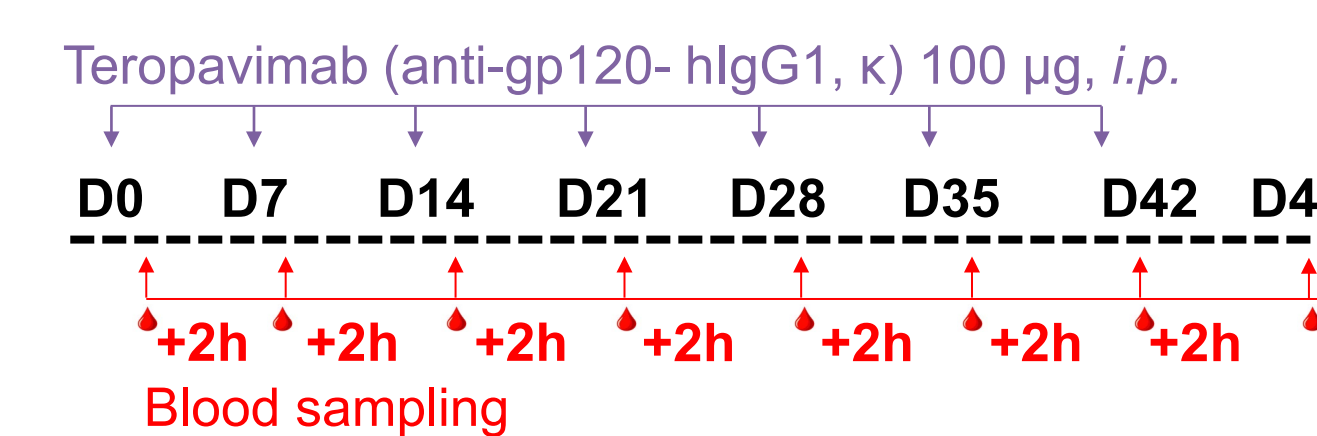
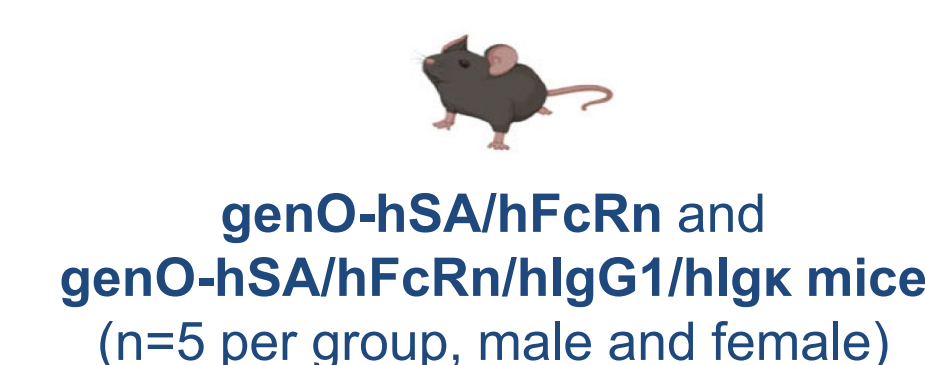
b. Immunoglobulins levels at steady state in IgG1 humanized model



⇒ As expected, hlgG1 is secreted in mouse humanized for IgG1, whereas mlgG1 is not detected
⇒ Ig class switch is not altered as mlgG2b, mlgA and mlgM are detected at similar levels in WT and hlgG1-expressing mice

c. hlgG1 expression is associated with sustained circulating levels of therapeutic antibody (hlgG1)

Mice received a weekly injection of Teropavimab (anti-gp120- hlgG1, κ , 3BNC117) for 7 weeks. Blood was collected 2 hours after each injection for quantification of circulating Teropavimab (ELISA).



⇒ Expression of hlgG1 is associated with maintenance of Teropavimab's circulating levels, suggesting better tolerance to multiples cycles of Teropavimab compared to genO-hSA/hFcRn mice

⇒ One week after the last injection (D49), Teropavimab is not detected in genO-hSA/hFcRn mice, whereas it is detected in 100% IgG1 humanized mice