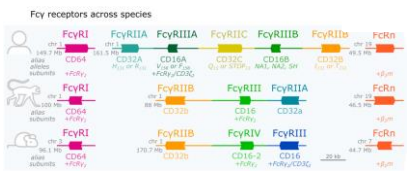
Cross-species cellular mapping and humanization of Fcγ receptors to advance antibody modeling

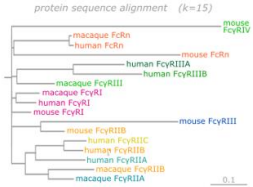
Karel F.A. Van Damme[1,2,#,*], **Dorine Sichien[1,2,3,#]**, Katrien Van der Borght[1,2,4,5#], Justine Van Moorleghem[1,2], Sofie Van Gassen[6,7], Elisabeth De Leeuw[1,2], Victor Bosteels[1,2,8], Pieter De Bleser[5,7], Manon Vanheerswynghels[1,2], Sofie De Prijck[1,2], Anna Bujko[5,9], Clint De Nolf[2,8,10], Martijn J. Schuijs[1,2,11], Bram Van Den Eeckhout[3], Ilse Rogiers[3], Robin Browaeys[6,12], Katrien Quintelier[6,7,13], Jinke D'Hont[14,15], Tino Hochepied[14,15], Patricia Isnard Petit[16], Séverine Augier[17], Rachel Courtois[17], Stijn Verwaerde[1,2], Stijn Vanhee[1,2,18], Charlotte L. Scott[5,9], Sophie Janssens[2,8], Yvan Saeys[6,7], Niels Vandamme[5,19], Antonio P. Baptista[1,2], Ariane Morel[17], Eric Vivier[17,20,21], Fabiane Sônego[16], Kader Thiam[16], Sofie Voet[3], Bianca Balbino[3], Bart N. Lambrecht[1,2,13,22,*]

[1]Laboratory of Mucosal Immunology, VIB-UGent Center for Inflammation Research, Ghent University, Belgium
[2]Department of Internal Medicine and Pediatrics, Faculty of Medicine and Health Sciences, Ghent University, Belgium
[3]argenx, Belgium
[4]VIB Flow Core, VIB Center for Inflammation Research, Belgium
[5]Department of Biomedical Molecular Biology, Faculty of Sciences, Ghent University, Belgium
[6]Department of Applied Mathematics, Computer Science and Statistics, Ghent University, Belgium
[7]Data Mining and Modeling for Biomedicine Group, VIB-UGent Center for Inflammation Research, Ghent University, Belgium
[8]Laboratory for ER Stress and Inflammation, VIB-UGent Center for Inflammation Research, Ghent University, Belgium
[9]Laboratory of Myeloid Cell Biology in Tissue Damage and Inflammation, VIB-UGent Center for Inflammation Research, Ghent University, Belgium
[10]Barriers in Inflammation, VIB-UGent Center for Inflammation Research, Ghent University, Belgium
[11]Cancer Research Institute Ghent, Ghent University, Belgium
[12]Bioinformatics Expertise Unit, VIB Center for Inflammation Research, Belgium
[13]Department of Pulmonary Medicine, Erasmus MC, The Netherlands
[14]Transgenic Core Facility, VIB Center for Inflammation Research, Belgium
[15]Transgenic Core Facility, Department of Biomedical Molecular Biology, Ghent University, Belgium
[16]genOway, France
[17]Innate Pharma, France
[18]Upper Airways Research Laboratory, Department of Head and Skin, Faculty of Medicine and Health, Ghent University, Belgium
[19]VIB Single Cell Core, Belgium
[20]Aix Marseille Université, Centre National de la Recherche Scientifique, INSERM, Centre d'Immunologie de Marseille-Luminy, France
[21]Assistance Publique-Hôpitaux de Marseille, Hôpital de la Timone, Marseille Immunopôle, France
[22]Department of Respiratory Medicine, Ghent University Hospital, Belgium
[#]Contributed equally
[*]Corresponding authors. Email: karel.vandamme@ugent.be; bart.lambrecht@ugent.be

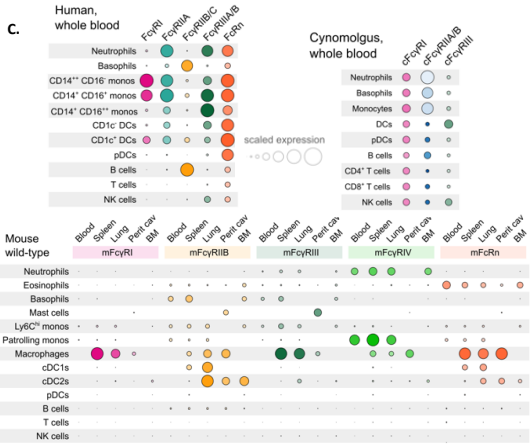
Substantial differences between human, mouse and macaque FcγRs and comprehensive mapping of human FcγR expression



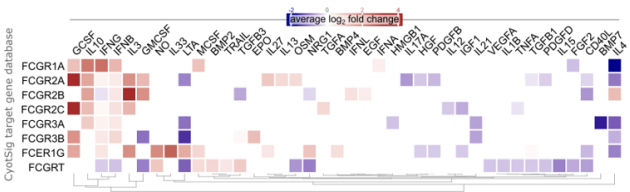
Genomic organization of the Fc-γ-receptor locus in human, cynomolgus macaque and mouse (UCSC Genome Browser). Similar colors indicate genes predicted to be orthologs and arrows indicate gene orientation.



Distance-based phylogenetic tree of human, cynomolgus macaque and mouse FcγR protein sequences.

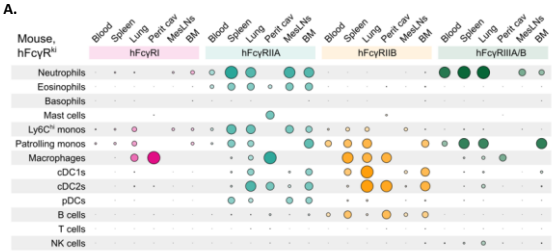


Protein expression profiles of FcγRs in healthy subjects and WT C57BL/6J mice determined by flow cytometry. Protein expression profile of FcγRs in cynomolgus monkeys was defined using CyTOF. The size and color of each data point represents the average MFI across at least 3 biological replicates, after correction for the negative control and scaling per marker.

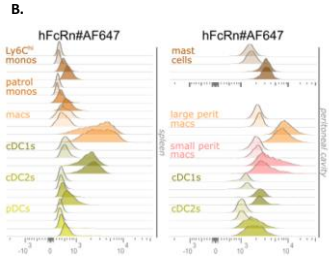


Cytokines which impact human FcγR transcript expression (based on CytoSig database).

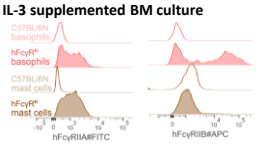
A novel mouse line with knock-in humanized FcγRs (hFcγR^{ki}) faithfully recapitulates human FcγR expression



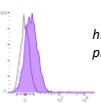
Expression profile of knock-in humanized FcγRs in hFcγR^{ki} mice determined by flow cytometry. The size and color of each data point represents the average MFI across at least 3 biological replicates after correction for the negative control and scaling per marker.



Intracellular hFcRn levels in spleen and peritoneal lavage of hFcγR^{ki} (express murine FcRn, light colors) or hFcγR/hFcRn^{ki} mice (dark colors).

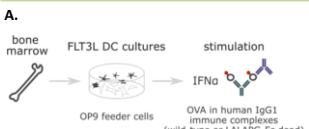


Expression of hFcγRIIA or hFcγRIIB on in vitro generated basophils or mast cells.

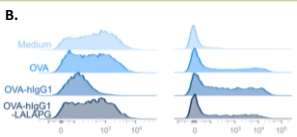


hFcγRIIA expression on platelets of hFcγR^{ki}mice.

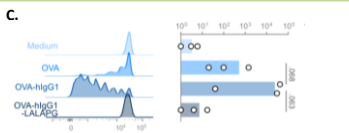
Functional validation of knock-in humanized FcγRs



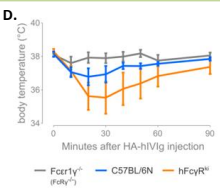
Schematic overview of BM-derived dendritic cell assay.



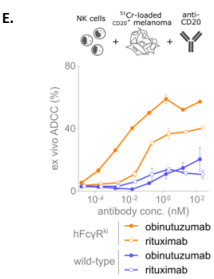
Expression of humanized FcγRI and uptake of OVA-AF647 (either free or in immune complex) by in vitro BM-derived hFcγR^{ki} cDC2s after stimulation with IFN-α.



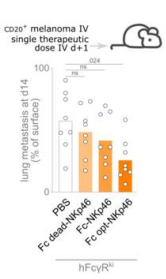
In vitro BM-derived cDC2s were stimulated with IFN-α and OVA (either free or in immune complex) and cocultured with CTV-labeled OVA-specific CD4+ T cells. 4 days later, T cell proliferation was assessed.



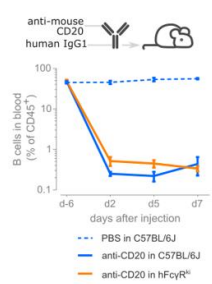
Body temperature of FcεR1γ$^{-/-}$, WT or hFcγR^{ki} mice after injection with 0.5mg human heat-aggregated human IVIg.



Ex vivo ADCC by NK cells isolated from hFcγR^{ki} or WT spleens, stimulated with WT (rituximab) or afucosylated (obinutuzumab) human IgG1 anti-CD20. ADCC was assessed as the % of maximal ^{51}chromium release from tumor cells.



hFcγR^{ki} mice injected IV with CD20$^+$ B16.F10 melanoma cells and multifunctional NK cell engagers 1 day later. Metastasis formation was determined as % of lung affected by metastasis at day 14.



% of B cells in peripheral blood following a single IV administration of a human anti-mouse CD20 IgG1 Ab.

Overall summary

We present a cross-species FcγR atlas and a humanized mouse model with refined humanized FcγR expression and antibody pharmacokinetics which will improve the preclinical assessment of antibody-based therapeutics.

PO-1497-EN-2611 **Rev A**