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Efficacy and safety assessment of CCR8-targeting agents in preclinical humanized models

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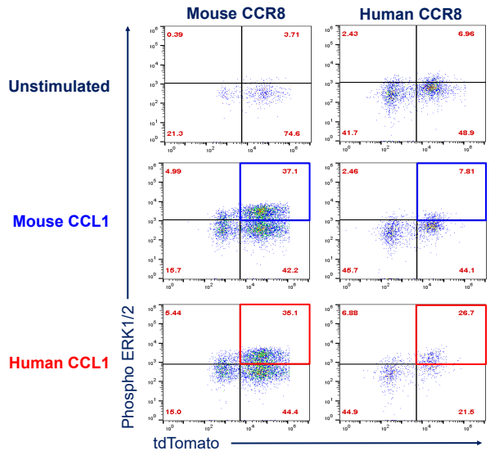
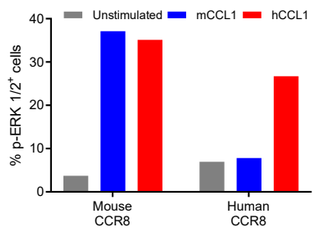
Abstract #443

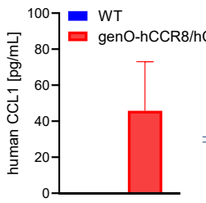
Background: CCR8 is a potential therapeutic target to treat cancer due to its role in the immunosuppression induced by regulatory T cells (Treg). The development of a physiologically relevant model to assess CCR8-targeting therapies depends, among others, on the maintenance of a proper interaction of the receptor and ligands. CCR8 has 2 ligands: CCL1 and CCL8. Mouse CCL8 induces specific calcium flux in human CCR8-transfected mouse cells(1), suggesting that humanization of CCL8 is not mandatory. However, *in vitro* studies showed that murine CCL1 was unable to interact with human CCR8. Lack of functional CCR8/CCL1 axis could have an impact on the suppressive activity of Treg cells, thus requiring the humanization of both CCR8 and CCL1. Here we describe two humanized models, genO-hCCR8/hCCL1 and genO-BRGSF-HIS(2,3) suitable for the assessment of efficacy and safety of CCR8-targeting compounds.

1. Mouse CCL1 does not induce signaling through human CCR8, requiring the humanization of both CCR8 and CCL1 to maintain functional CCR8/CCL1 axis





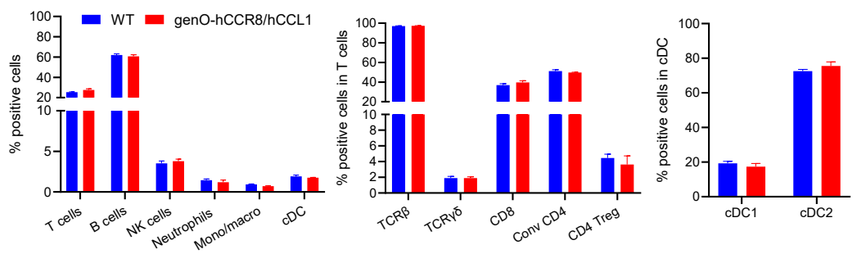
CCR8/tdTomato-transfected NIH-3T3 cells were stimulated with recombinant human or mouse CCL1 (100ng/mL). ERK phosphorylation was measured by flow cytometry.



⇒ **Human CCL1 is detected in genO-hCCR8/hCCL1 mice**

Quantification of human CCL1 in the plasma by ELISA (diluted to 1/2000)

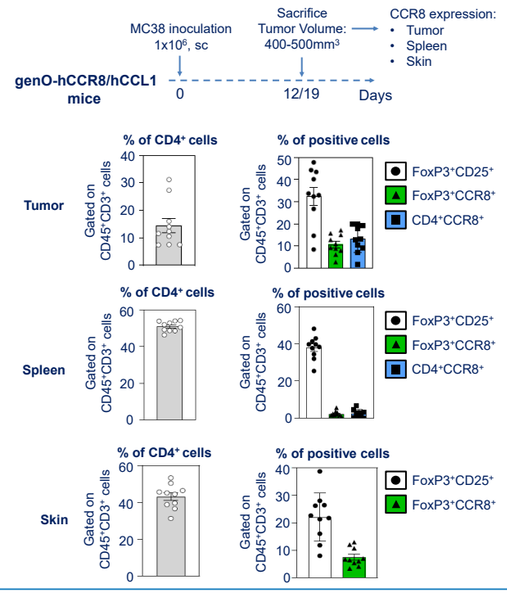
2. Humanization of both CCR8 and CCL1 does not alter immune cell distribution in genO-hCCR8/hCCL1 compared to WT mice



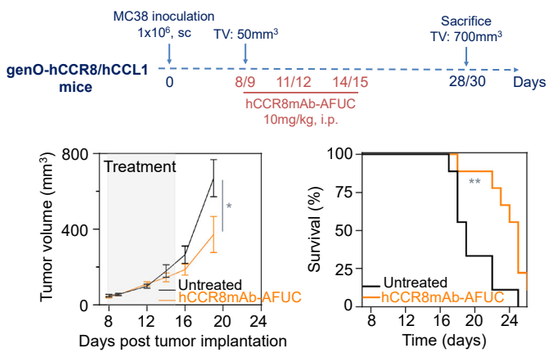
⇒ **Similar results of immune profiling in blood and bone marrow – *data not shown***

Freshly isolated splenocytes, gated on live CD3⁺ CD19⁻ (T cells) and CD3⁻ CD19⁺ (B cells), in CD3⁻CD19⁻cells: NKp46⁺ (NK cells), CD317⁺ (pDC), Ly-6G⁺ (Neutrophils), Ly-6G⁻ CD11c⁺ MHC-II⁺ (cDC) and Ly-6G⁻ Ly-6C⁺ CD11b⁺ (Monocytes/macrophages). In cDC: CD8⁺ CD11b⁻ (cDC1) and CD8⁻ CD11b⁺ (cDC2). T cell subsets gated on CD3⁺ TCRβ⁺ TCRγδ⁻: CD4⁻ CD8⁺ (CD8), CD4⁺CD25⁻ FoxP3⁻(convCD4) and CD4⁺CD25⁺FoxP3⁺ (Treg).

3. CCR8 is expressed on tumor infiltrating Treg cells but not in the spleen



4. Blocking of human CCR8 efficiently inhibits tumor growth



⇒ **CCR8-targeting therapies can be assessed in genO-hCCR8/hCCL1 model**

⇒ **However, CCR8 expression pattern differs between human and mouse, as, in humans CCR8 is expressed on both infiltrating and peripheral Tregs**

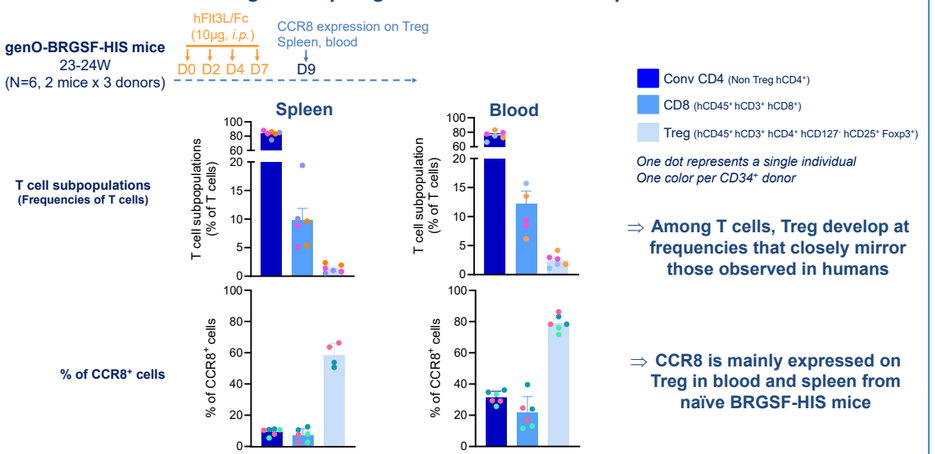
⇒ **Safety assessment requires a model with a human-like expression of CCR8**

5. genO-BRGSF-HIS model for safety assessment of CCR8/CCL1-targeting therapies

a. genO-BRGSF-HIS model features

BRGSF (BALB/c Rag2$^{-/-}$, IL2Rγ$^{-/-}$, SIRPα^{NOD}, Flt3$^{-/-}$):
- Highly immunodeficient with reduced murine myeloid cells
- Normal lifespan (no anemia, no weight loss, normal fur texture & integrity, normal activity & posture)
- Upon CD34⁺ cells injection:
  - Lymphoid and Myeloid compartment development

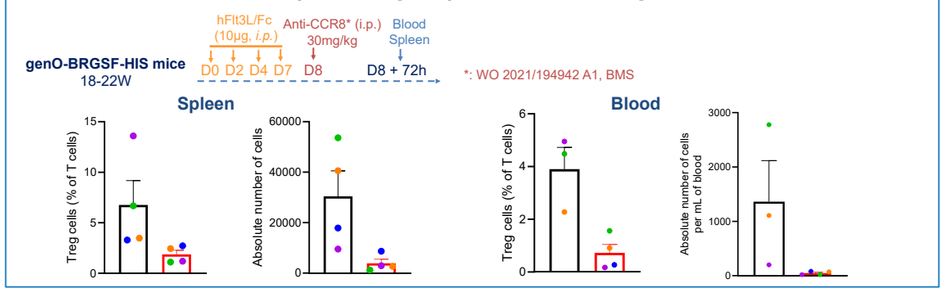
b. Treg develop in genO-BRGSF-HIS and express CCR8



⇒ **Among T cells, Treg develop at frequencies that closely mirror those observed in humans**

⇒ **CCR8 is mainly expressed on Treg in blood and spleen from naïve BRGSF-HIS mice**

c. Anti-CCR8 depletes Treg in spleen and blood of genO-BRGSF-HIS



Conclusion: Altogether, data suggest that genO-hCCR8/hCCL1 humanized model is a valuable tool for CCR8-targeting agents efficacy assessment, enabling the investigation of immunomodulatory mechanisms at the tumor microenvironment. Nevertheless, this model has limitations to assess safety of CCR8-targeting compounds as the pattern of expression differs between human and mouse. The human-like expression pattern of CCR8 in naïve Treg from genO-BRGSF-HIS model suggests it could be a more suitable model for safety and PD assessment.

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genO-hCCR8/hCCL1

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References:
(1): Islam *et al.*, Nat Immunol., 2011
(2): Martin *et al.*, Front Immunol., 2024
(3): Martin *et al.*, Front. Immunol., 2025