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genO-hCCR8/hCCL1 humanized model for efficacy assessment of CCR8/CCL1-targeting therapies

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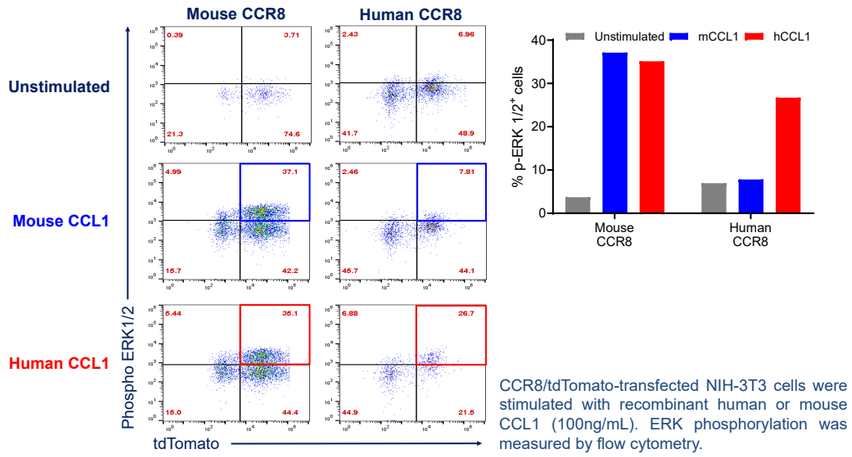
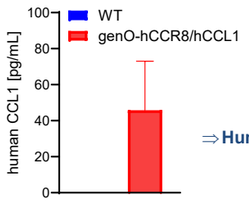
Background: Modulation of tumor microenvironment has shown to be a promising approach to treat cancer. Among diverse mechanisms responsible for tumor immunosuppression, those mediated by regulatory T cells (Treg) infiltrating in tumor are well identified and known to reduce the efficacy of anti-tumor therapies. CCR8 has been identified as a potential therapeutic target due to its role in the immunosuppression induced by Treg. CCL1 is the major CCR8 ligand and CCL1/CCR8 interaction induces STAT3-dependent up-regulation of FoxP3, CD39, IL-10 and Granzyme B, leading to the enhancement of the suppressive activity of Treg cells. A major challenge in the development of new therapeutic approaches relies on the choice of the right preclinical model, which could mimic the complexity of interactions among different cell types and closely predict the effects of therapeutics in humans. The current therapeutic approaches directed toward the CCR8/CCL1 axis target the receptor CCR8, requiring its humanization for assessment of therapeutics displaying species-specific activity. The development of a physiologically relevant model depends, among others, on the maintenance of a proper interaction of the receptor and ligand. Here we describe two humanized models, genO-hCCR8/hCCL1 and genO-BRGSF-HIS 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



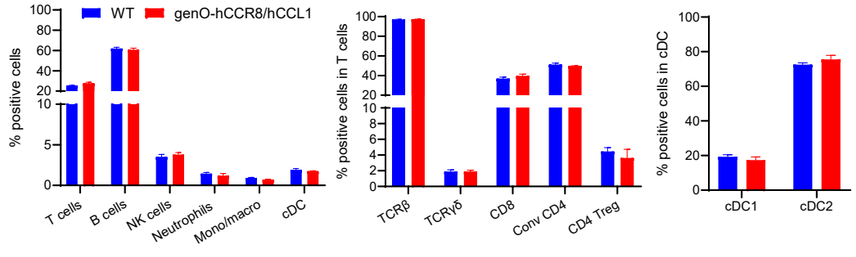
CCR8/tdTomato-transfected NIH-3T3 cells were stimulated with recombinant human or mouse CCL1 (100ng/mL). ERK phosphorylation was measured by flow cytometry.



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

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

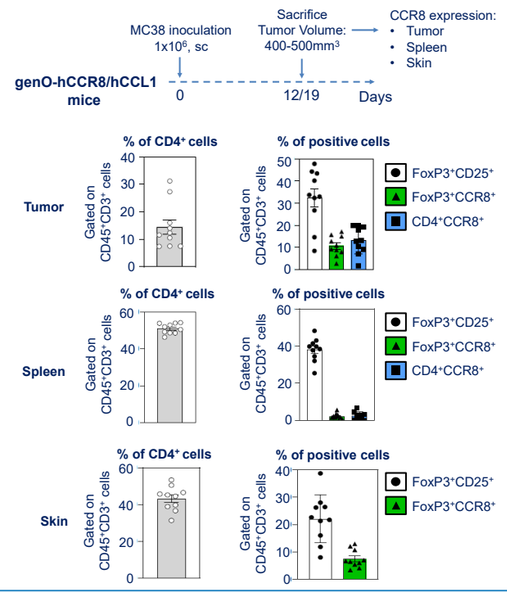
2. Humanization of both CCR8 and CCL1 does not alter immune cell distribution in genO-hCCR8/hCCL1 compared to WT mice



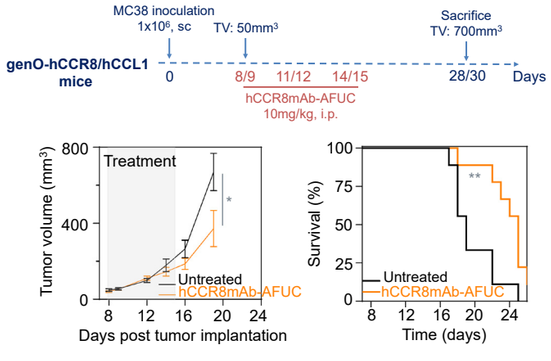
⇒ **Similar results of immune profiling in blood and bone marrow – *data not shown***

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

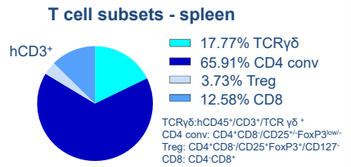


⇒ **CCR8-targeting therapies can be assessed in genO-hCCR8/hCCL1 model**
⇒ **However, CCR8 expression pattern differs between human and mouse, as, in humans CCR8 is expressed on both infiltrating and peripheral Tregs**
⇒ **Safety assessment requires a model with a human-like expression of CCR8**

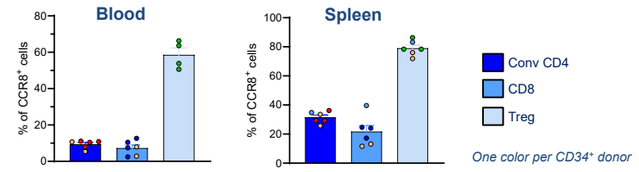
5. genO-BRGSF-HIS model for safety assessment of CCR8/CCL1-targeting therapies

a. genO-BRGSF-HIS model features

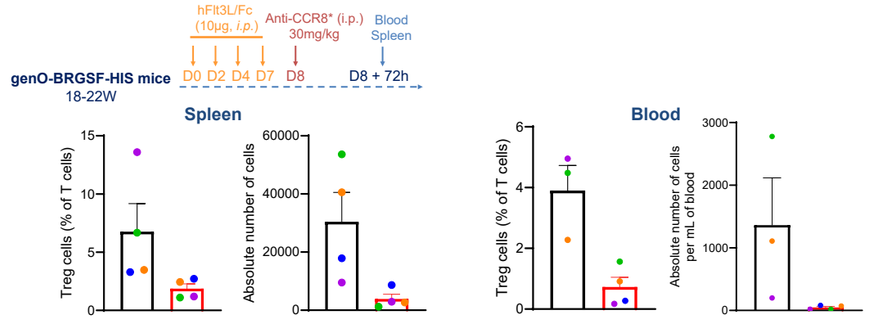
BRGSF (BALB/c Rag2$^{-/-}$, IL2Rγ$^{-/-}$, SIRPα^{NOD}, Flt3$^{-/-}$):
- Highly immunodeficient with reduced murine myeloid cells
- Normal lifespan (no anemia, no weight loss, normal fur texture & integrity, normal activity & posture)
- Upon CD34⁺ cells injection:
  - Lymphoid and Myeloid compartment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



b. In genO-BRGSF-HIS, CCR8 is expressed by naïve Treg in blood and spleen as in humans



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



*: WO 2021/194942 A1, BMS

Conclusion: Altogether, data suggest that genO-hCCR8/hCCL1 humanized model is a valuable tool for the efficacy assessment of CCR8-targeting drugs, enabling the investigation of immunomodulatory mechanisms at the tumor microenvironment. This is possible because the mouse immune system is functional and its interplay with mouse stroma and tumor cells enables the assessment of a finely regulated 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 makes it a more suitable model for safety and PD assessment.

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