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Functional human myeloid cells in BRGSF-HIS humanized mice enable myeloid-directed therapy assessment

Florence RENART-DEPONTIEU[1], Gaelle MARTIN[1], Valéry MOINE[2], Coline BURNET-MERLIN[2], Florent CREUSAT[1], Alexis GONON[1], Perrine MARTIN-JEANTET[1], Amélie REZZA[1], Fabiane SÔNEGO[1], Lise NOUVEAU[2], Edward H. VAN DER HORST[3],Yacine CHERIFI[1], Kader THIAM[1]

[1]genOway, Lyon, 69007, France; [2]Light Chain Bioscience, Plan-Les-Ouates Genève, Switzerland; [3]Sensei Biotherapeutics Inc., Boston, MA 02210, USA

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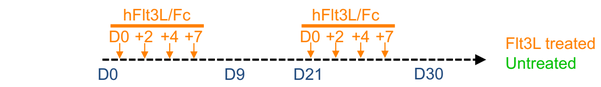
Background: Translational preclinical assessment of myeloid-targeting therapies is considered challenging due to the reduced availability of humanized mouse models expressing both lymphoid and myeloid compartments. Most of the humanized models harboring a myeloid compartment overexpress human cytokines, leading to body weight loss, anemia and a short life span. Recent reports described the BRGSF-HIS as an alternative model displaying all major human hematopoietic cell subsets, such as B, T, natural killer (NK), dendritic cells (DCs), plasmacytoid cells (pDCs) and monocytes/macrophages. Human myeloid cells were developed in the BRGSF-HIS mice without side effects and human immune system engraftment is stable for over a year (Labarthe et al., 2019), favoring long-term studies with agents requiring a wide therapeutic window.

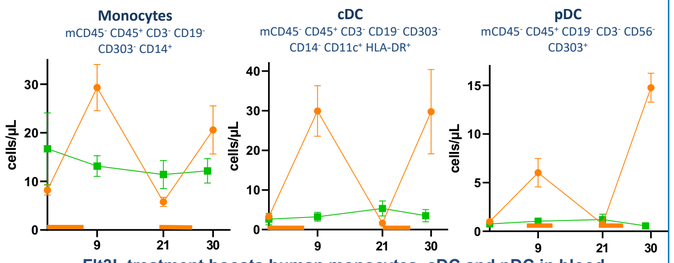
1. Model features

- BRGSF (Balb/C Rag2$^{-/-}$ IL2R$\gamma^{-/-}$, SIRPα^{NOD} and Flt3$^{-/-}$):
  - Highly immunodeficient with reduced murine myeloid cells
  - Normal life span (no anemia, no weight loss, normal fur texture & integrity, normal activity & posture)
- Upon hCD34$^+$ cell injection:
  - Lymphoid and myeloid compartment development
  - Boost by hFlt3L enhances human myeloid cell development and accumulation

2. Flt3L injections boost human myeloid cells in blood

In vivo Flt3L boosting: Mice were injected (i.p.) every 2 to 3 days with 10µg recombinant human Flt3 Ligand Fc



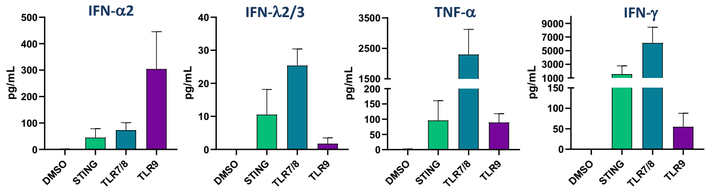


- Flt3L treatment boosts human monocytes, cDC and pDC in blood
- Boost is transient, and myeloid compartment can be reboosted

3. Functional STING and TLR pathways in myeloid cells from Flt3L-boosted BRGSF-HIS mice

Ex vivo
- Spleen collected 1 day after Flt3L treatment
- Quantification of secreted cytokines after *ex vivo* STING or TLR stimulation for 24h

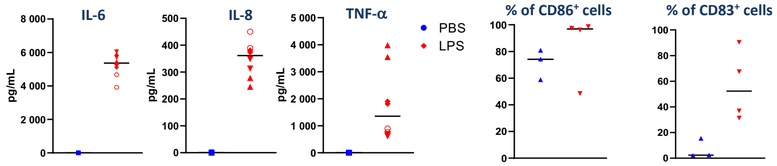
STING agonist: 2'3' cGAMP 50µg/mL
TLR7/8 agonist: Resiquimod (R848) 10µg/mL
TLR9 agonist: CpG ODN2216 10 µM



Quantification of secreted cytokines 4h after LPS injection

Spleen collected 1 day after LPS injection

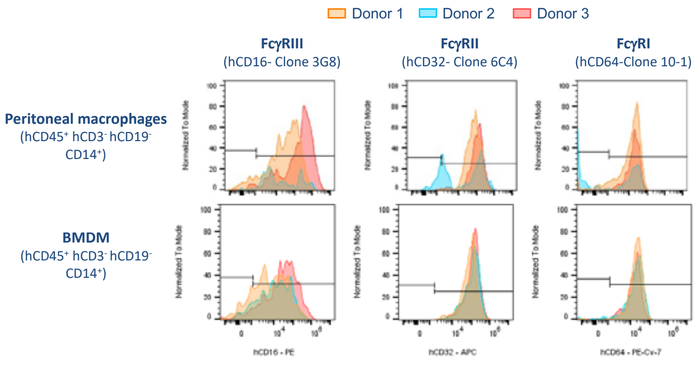
In vivo
- LPS injection (2mg/kg) 1 day after Flt3L treatment



- *Ex vivo* STING and TLR stimulation induces cytokine secretion
- TLR4 stimulation efficiently activates cDCs *in vivo*

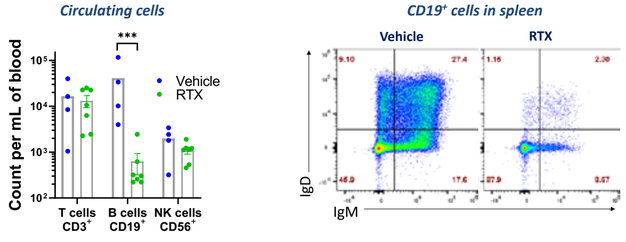
4. FcγR-mediated function in Flt3L-boosted BRGSF-HIS mice

- Analysis of FcγR expression after Flt3L boost, on:
  - Thioglycolate-elicited peritoneal macrophages were incubated for 3 days with human M-CSF
  - Bone marrow-derived macrophages



- FcγRI, II and III are expressed on peritoneal macrophages and BMDM

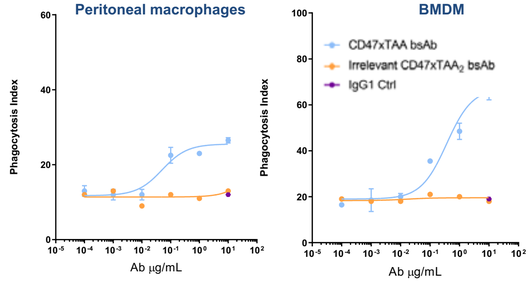
- Rituximab (RTX): anti-CD20 mAb (IgG1) which binds via FcγR (mostly FcγRIIa and FcγRIIIa) and triggers CDC-, ADCC- and ADCP-dependent mechanisms (Teige et al., Front. Immunol., 2019)
- B cell depletion was assessed in Flt3L-boosted BRGSF-HIS mice 48h post injection with RTX 30mg/kg (i.v)



- RTX induces circulating mature B cell depletion, suggesting an efficient FcγR-mediated monocyte and NK cell cytotoxicity

5. *Ex vivo* ADCP induction using CD47-TAA BsAb

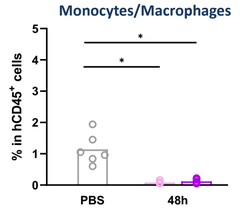
- BRGSF-HIS mice were boosted with Flt3L, and thioglycolate-elicited peritoneal macrophages and bone marrow-derived macrophages were incubated with CD47-TAA BsAb and TAA-expressing MKN45 cells for 2.5h
- Phagocytic index determined by fluorescence *(average number of target cells engulfed by 100 macrophages)*

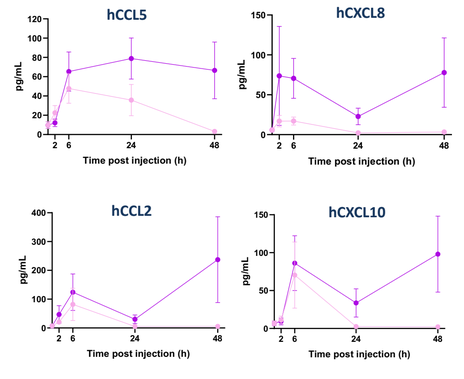


- Human macrophages phagocyte target cells when incubated with anti-CD47-TAA bsAb

6. Cytokine release induction and myeloid cells depletion upon myeloid-targeting therapeutic antibody treatment

- Quantification of cytokine serum levels upon anti-VISTA antibody (JNJ) injection, 1 day after Flt3L treatment
- Analysis of percentage of activated monocytes/macrophages and cDCs from spleen

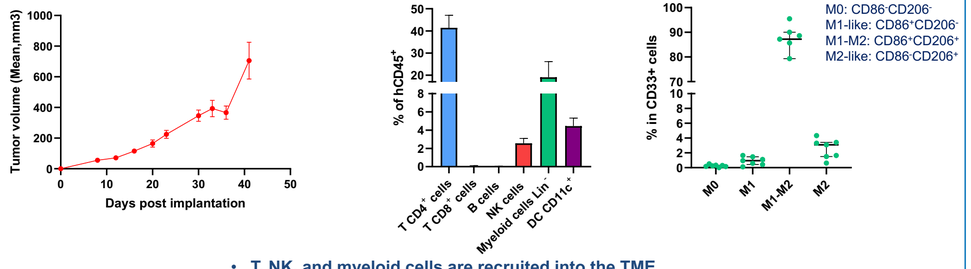




- Anti-VISTA treatment induces monocyte/macrophage and cDC depletion in 48h
- Anti-VISTA therapeutic antibody treatment preferentially induces secretion of monocyte/macrophage human chemokines

7. Recruitment of human myeloid cells in tumor microenvironment

- MDA-MB-231 cell injection (5.10^6 cells, s.c.) 1 day after Flt3L treatment
- Measurement of tumor volume and TME analysis at endpoint by flow cytometry



- T, NK, and myeloid cells are recruited into the TME
- Enrichment of transitional M1-M2 polarized cells (M2-like cells > M1) among myeloid cells

Conclusion: BRGSF-HIS mice develop functional human myeloid cells without side effects and have a wide therapeutic window, enabling the assessment of effector mechanisms such as ADCP and ADCC, triggered by myeloid-targeted therapies.

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