

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

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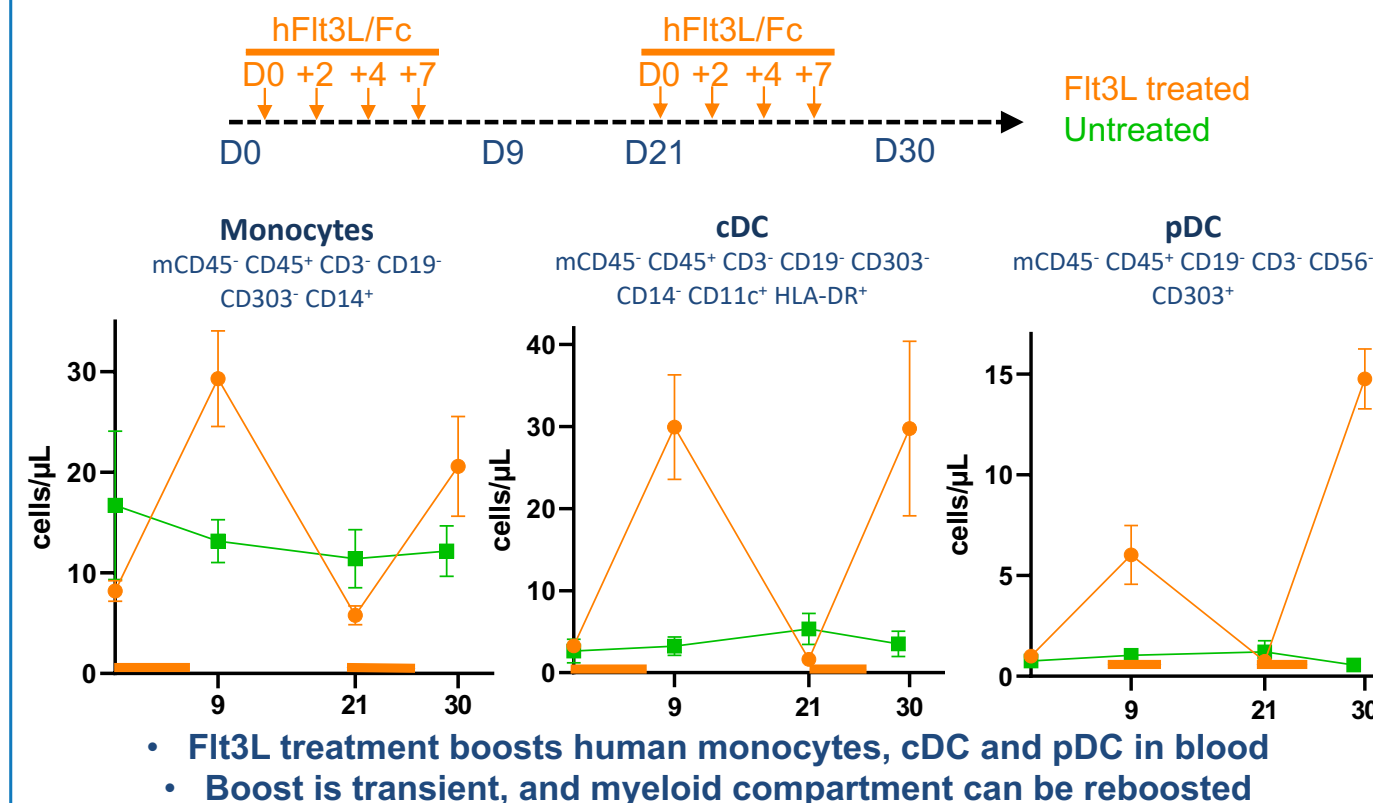
Background: Translational preclinical assessment of myeloid-targeting therapies is considered challenging due to the reduced availability of humanized mouse models expressing both lymphoid and myeloid compartments. Most of the humanized models harboring a myeloid compartment overexpress human cytokines, 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^{-/-} IL2Rγ^{-/-}, 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

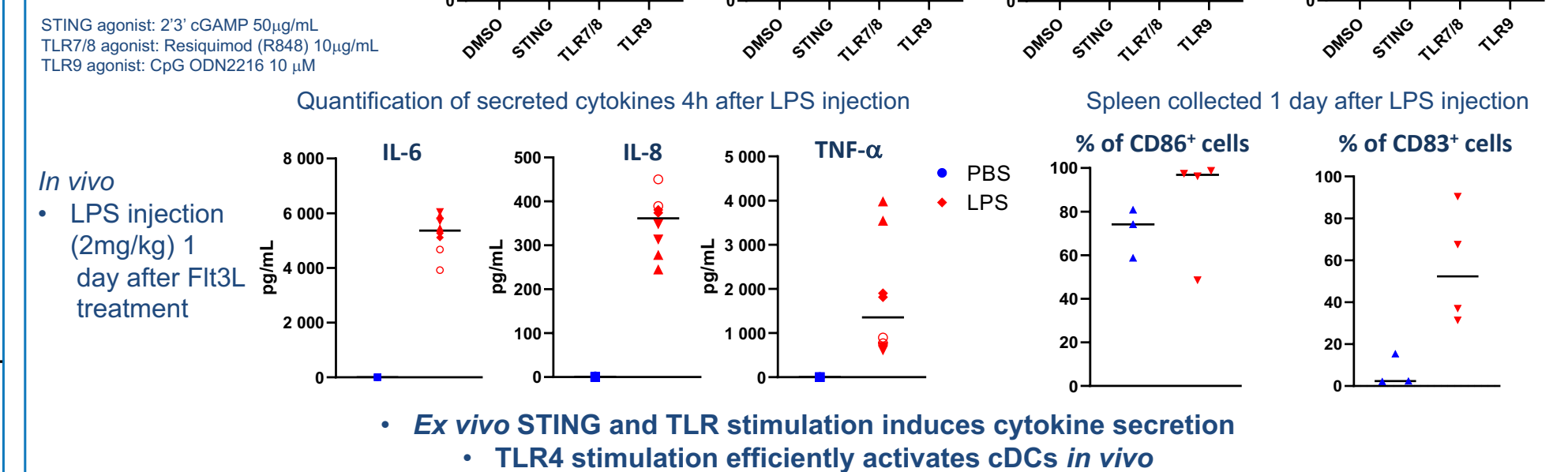
In vivo Flt3L boosting: Mice were injected (i.p.) every 2 to 3 days with 10μg recombinant human Flt3L Fc



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

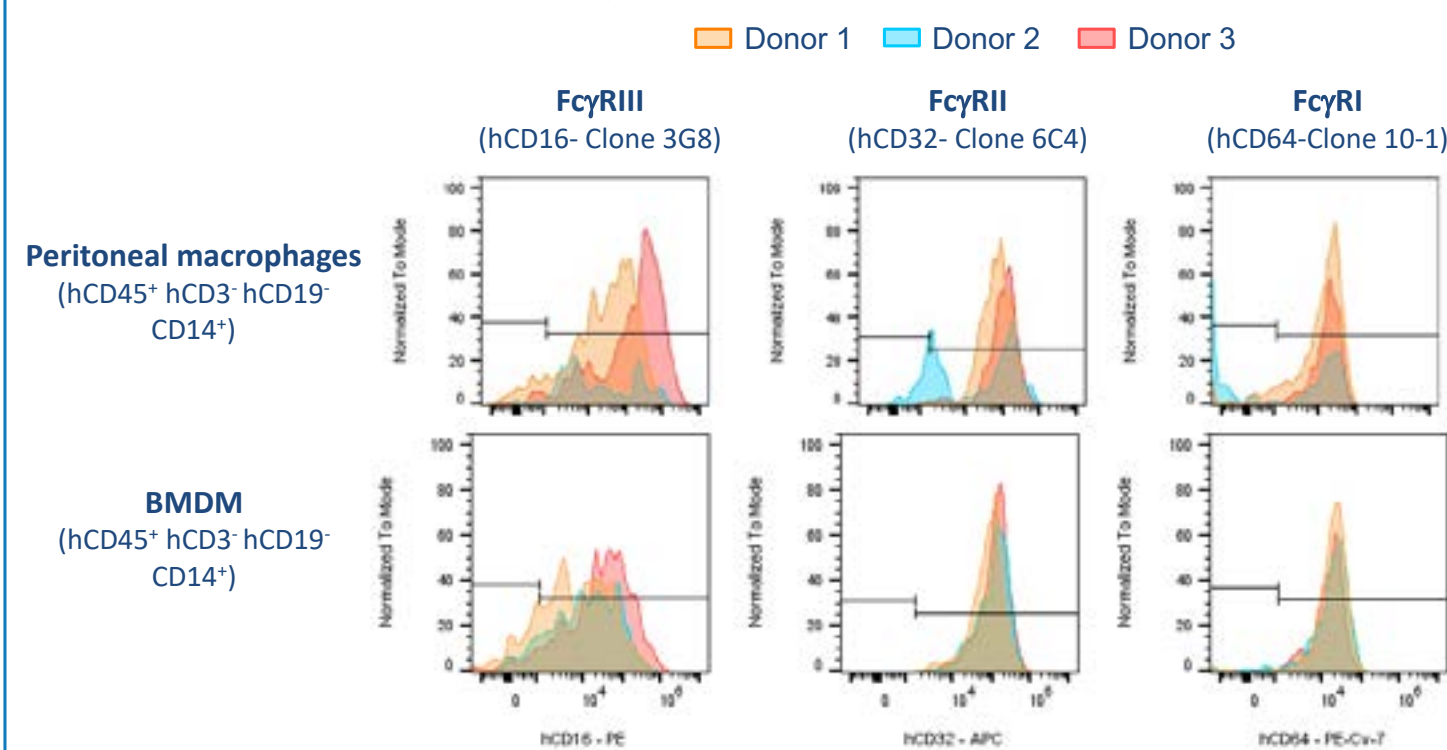
Ex vivo

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



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

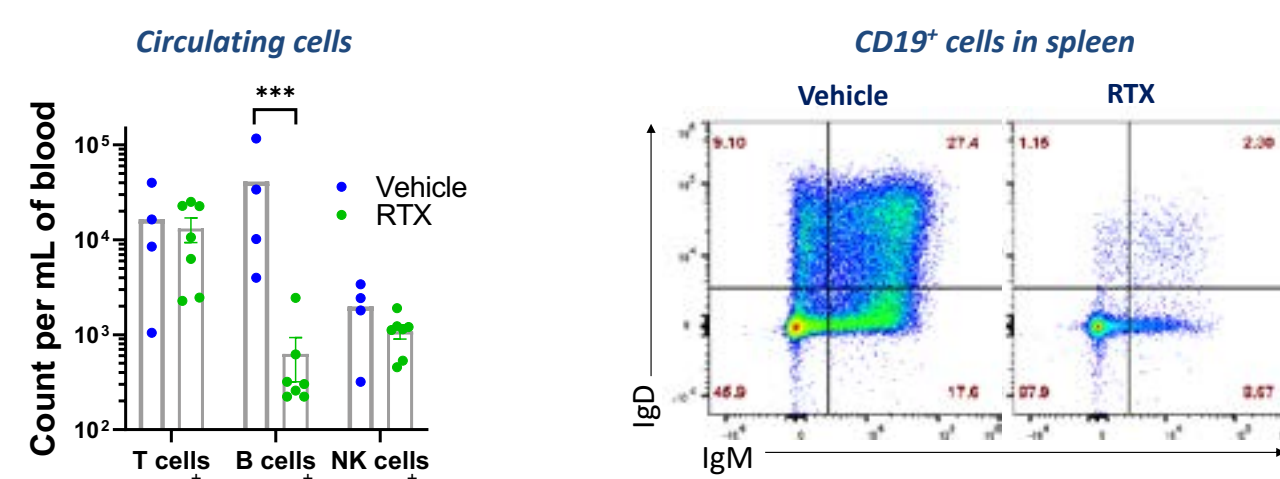
- Analysis of FcγR expression after Flt3L boost, on:
 - Thioglycolate-elicited peritoneal macrophages were incubated for 3 days with human M-CSF
 - Bone marrow-derived macrophages



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

- Rituximab (RTX): anti-CD20 mAb (IgG1) which binds via FcγR (mostly FcγRIIIa and FcγRIIIa) and triggers CDC-, ADCC- and ADPC-dependent mechanisms (Teige et al., Front. Immunol., 2019)

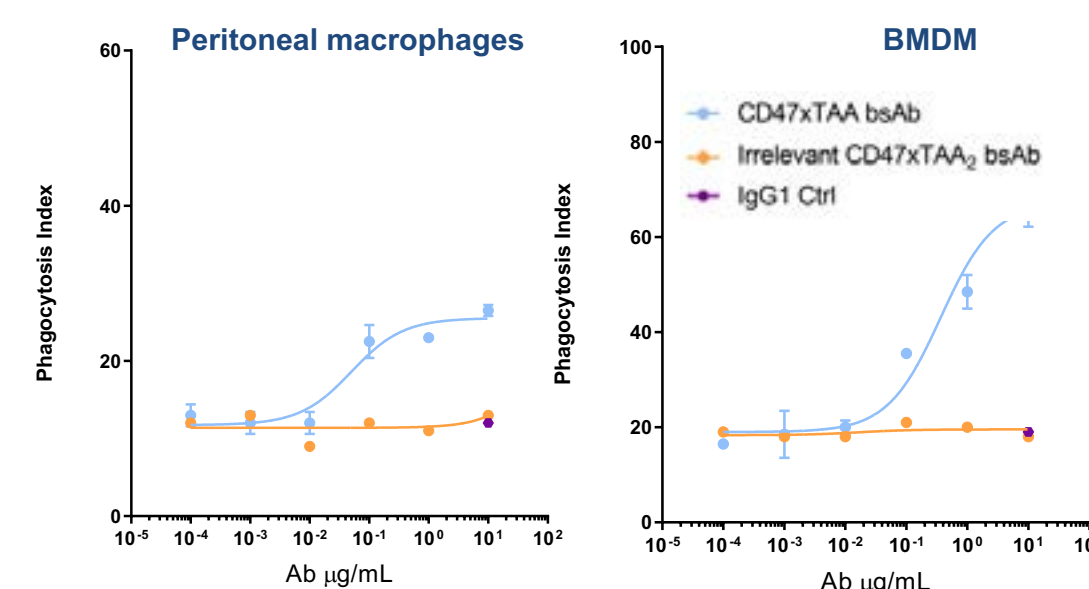
- B cell depletion was assessed in Flt3L-boosted BRGSF-HIS mice 48h post injection with RTX 30mg/kg (i.v.)



- 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

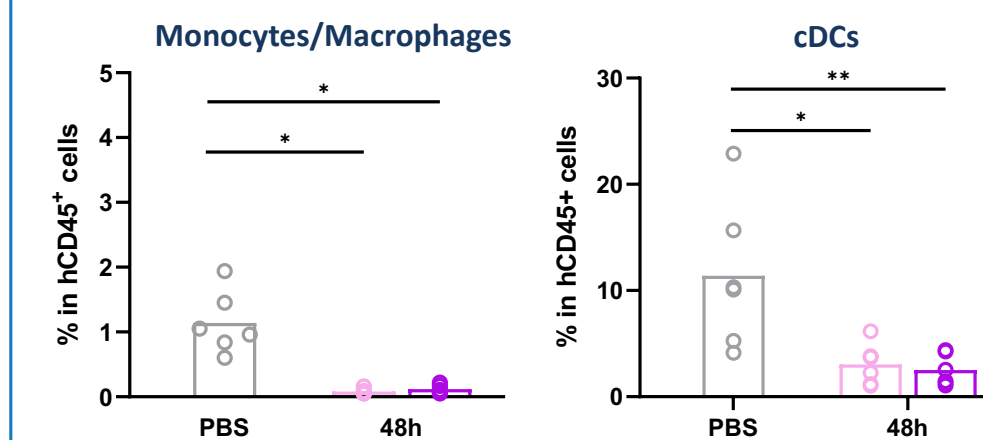
- BRGSF-HIS mice were boosted with Flt3L, and thioglycolate-elicited peritoneal macrophages and bone marrow-derived macrophages were incubated with CD47-TAA BsAb and TAA-expressing MKN45 cells for 2.5h
- Phagocytic index determined by fluorescence (average number of target cells engulfed by 100 macrophages)



- 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

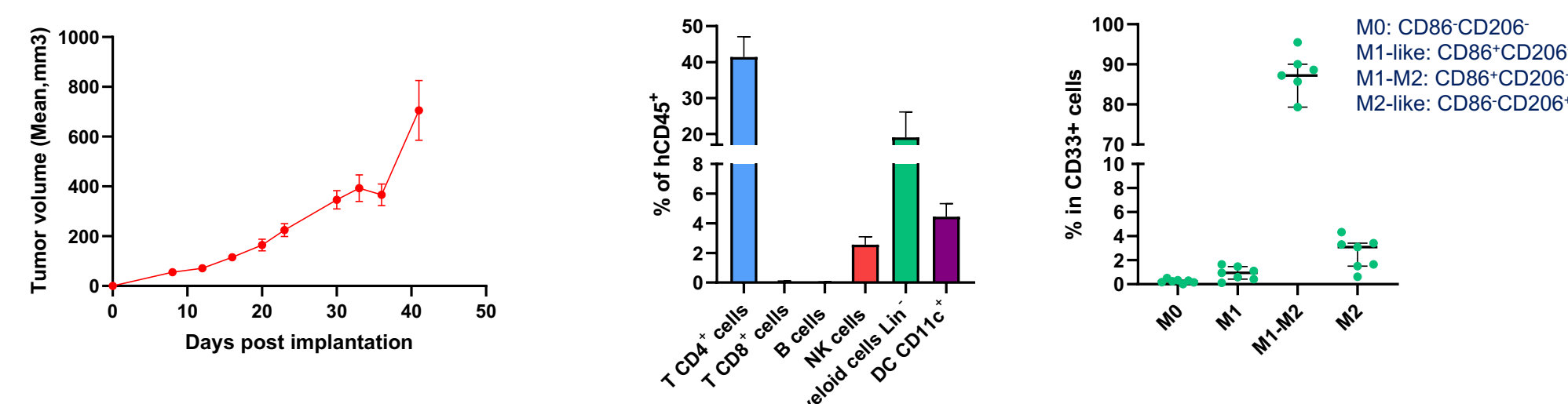
- Quantification of cytokine serum levels upon anti-VISTA antibody (JNJ) injection, 1 day after Flt3L treatment
- Analysis of percentage of activated monocytes/macrophages and cDCs from spleen



- 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 (5.10⁶ 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.