



ULTIMATE PREDICTABILITY

$\gamma\delta$ T-cells are functional and recruited into the TME in genO-BRGSF-HIS mice

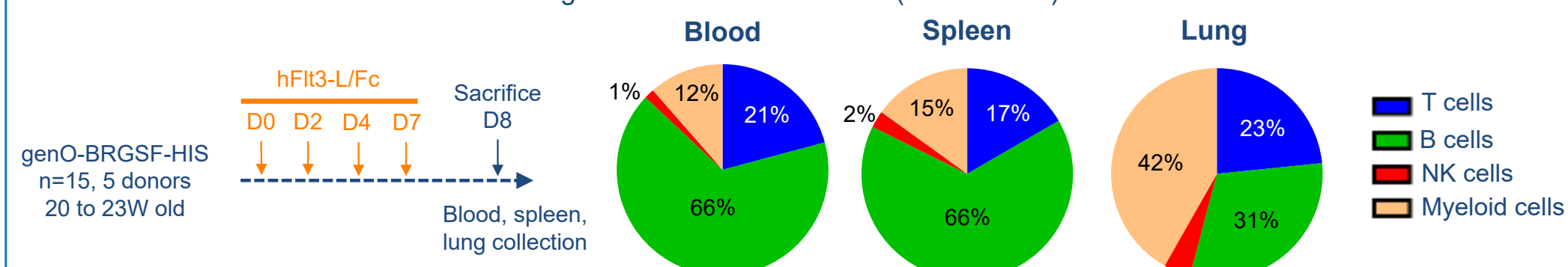
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Abstract #835

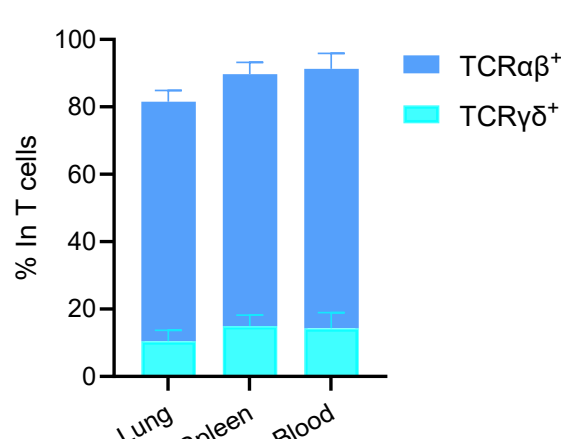
Background: Development of immunotherapies has been a major landmark in the field of oncology, leading to numerous effective treatments. From the first use of an immunomodulatory agent in clinical studies to modern immunotherapies, these were mainly focused on modulating adaptive immune response. However, only a fraction of patients can respond to these treatments, and the complexity of the tumor microenvironment requires other players to be targeted. $\gamma\delta$ T cells are unconventional T cells, as they recognize antigens mostly in a MHC-unrestricted fashion. They show a high diversity of effector functions, from cytotoxicity to mediator production and wound healing. Their preactivated state allows a quick immune response, and their role in tumor development, both in beneficial or deleterious manner, was demonstrated in numerous types of cancer. Current therapeutic approaches involving $\gamma\delta$ T cells include adoptive cell transfer, *in vivo* stimulation and combined therapies. While preliminary results are promising, investigation of such therapies in preclinical models is challenging, because $\gamma\delta$ T cells are not developed at satisfactory levels in most of the humanized mouse models. Here we describe the presence and functionality of $\gamma\delta$ T cells in genO-BRGSF (BALB/c Rag2^{-/-} IL2R γ ^{-/-}, SIRP α ^{NOD} and Flt3^{-/-}), a highly immunodeficient mouse featuring reduced murine myeloid cells. genO-BRGSF mice reconstituted with human cord blood CD34⁺ cells (genO-BRGSF-HIS) develop functional lymphoid and myeloid compartments. This engraftment is stable over a year⁽¹⁾ and mice do not develop GvHD.

1. $\gamma\delta$ T cells developed in genO-BRGSF-HIS mice

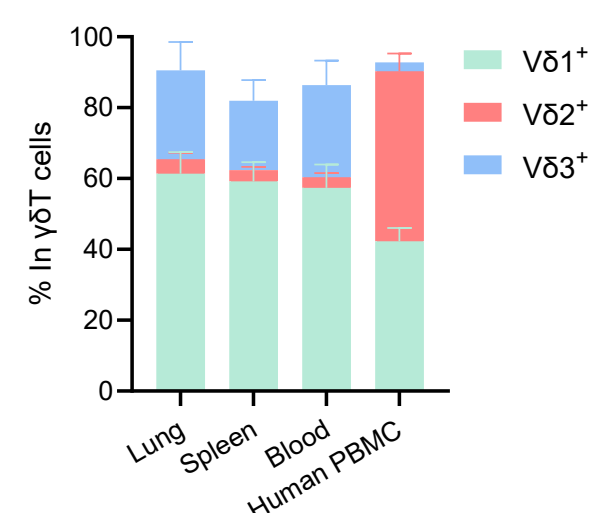
- Human immune cell reconstitution in genO-BRGSF-HIS animals (% in hCD45)



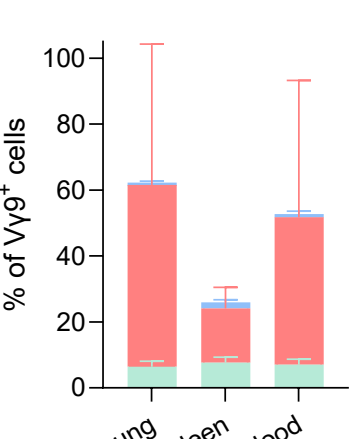
- T cells subpopulations (CD3⁺ CD56⁻)



- Characterization of human $\gamma\delta$ T cells



- V γ 9 expression on human $\gamma\delta$ T cells

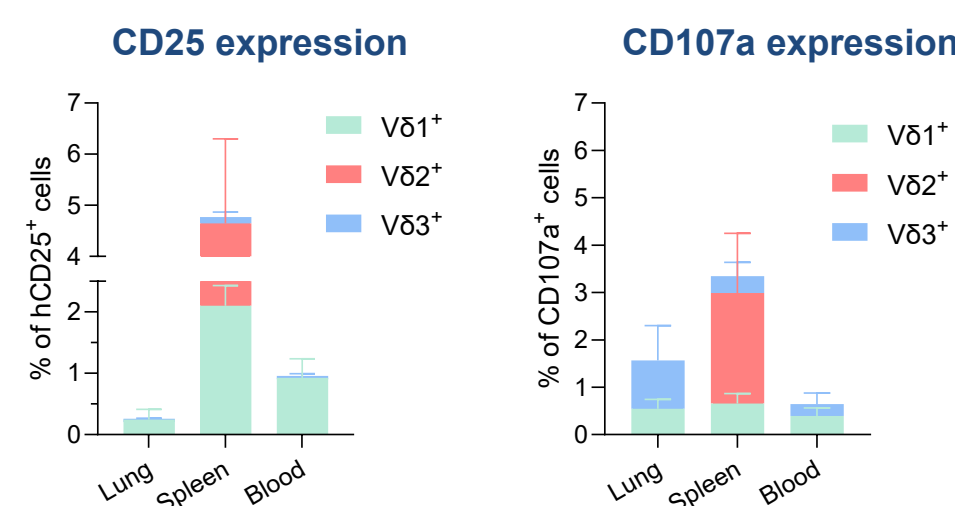


⇒ Ratio of TCRαβ vs TCRγδ is approximately 80:20 in blood, spleen and lung from genO-BRGSF-HIS mice, as reported in humans

⇒ $\gamma\delta$ cells develop in genO-BRGSF-HIS and express Vδ1, Vδ2 and Vδ3, although their ratios are different from human PBMC

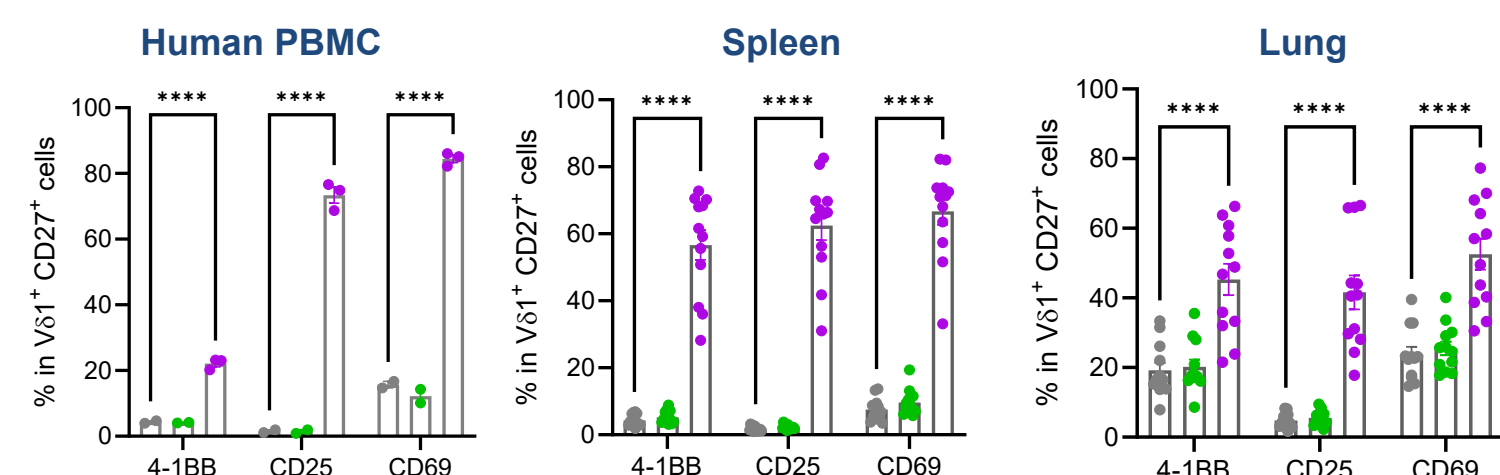
⇒ V γ 9 is preferentially paired with Vδ2+ cells, as reported in human PBMC
⇒ V γ 9 is also expressed in ~5% of Vδ1+ cells and very low to no expression is observed in Vδ3+ cells, as described in human physiology

- Human $\gamma\delta$ T cell activation phenotype



⇒ Low / no CD25 and CD107a expression in $\gamma\delta$ T-cell subpopulations, as would be expected in naive mice

2. Ex vivo $\gamma\delta$ T-cell activation in genO-BRGSF-HIS mice

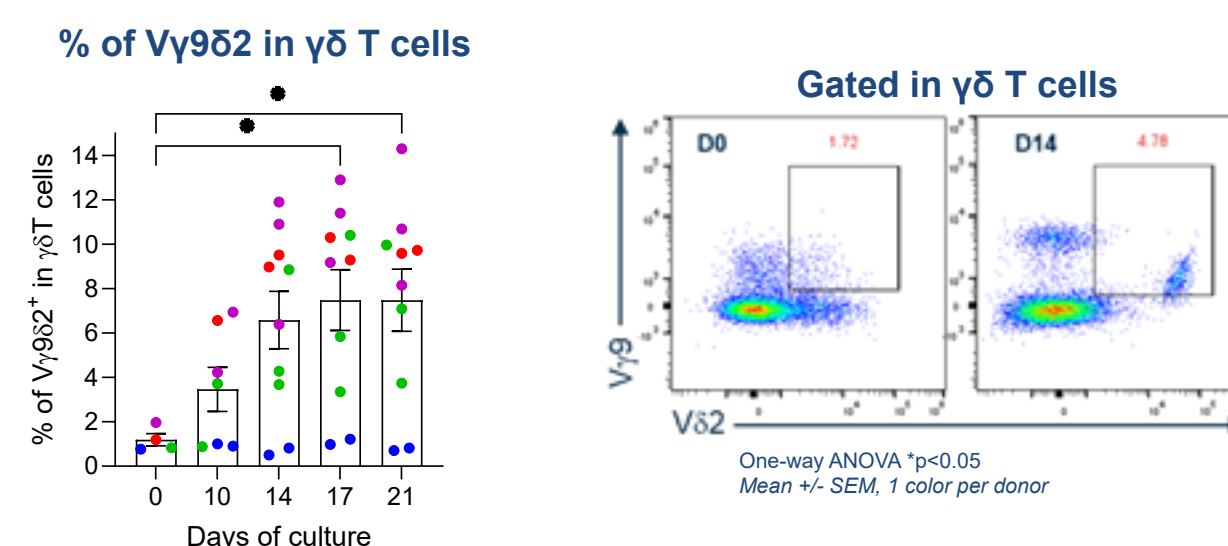


⇒ Compound B targeting Vδ1 cells shows similar activation profile on targeting Vδ1 cells developed in genO-BRGSF-HIS mice and human PBMC from a healthy donor

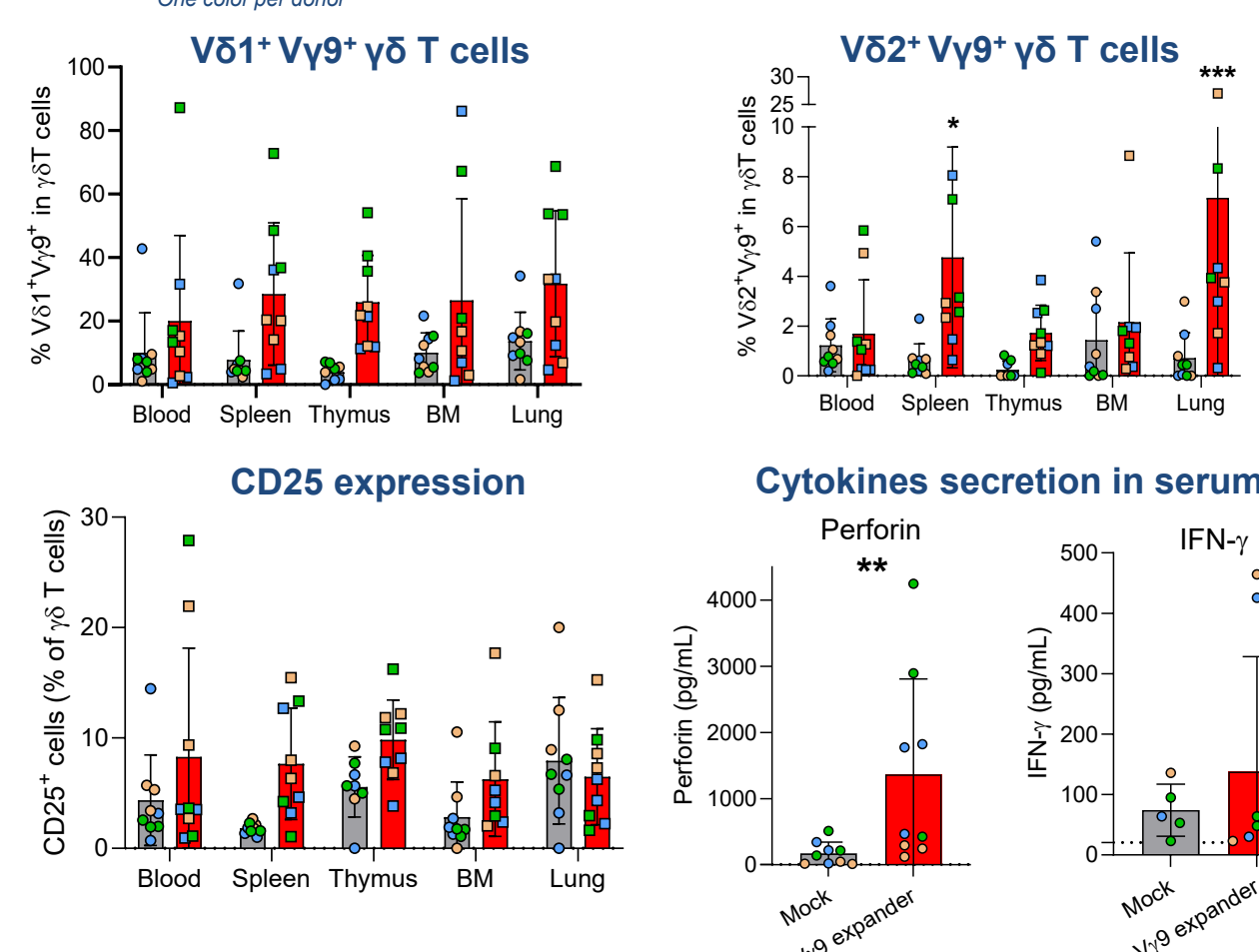
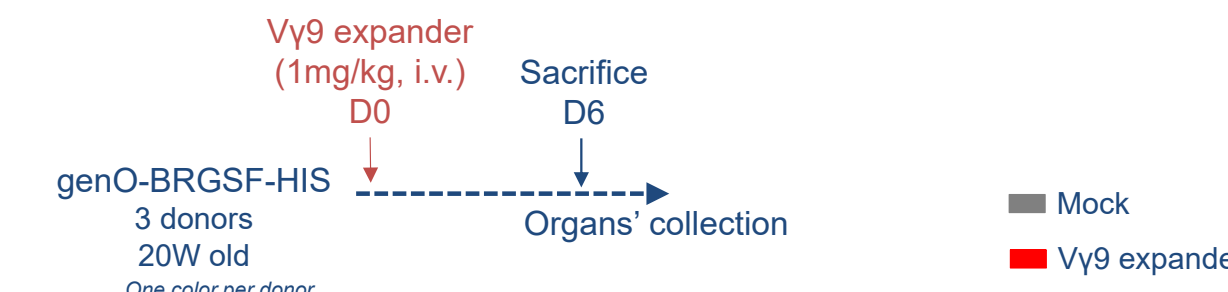
3. Expansion of $\gamma\delta$ T cells in genO-BRGSF-HIS mice

3.1 *In vitro* expansion of $\gamma\delta$ T V γ 9⁺ cells

hCD45 enriched splenocytes (4x10⁶) from genO-BRGSF-HIS were cultured in presence of Zoledronate, IL-2 and IL-15.



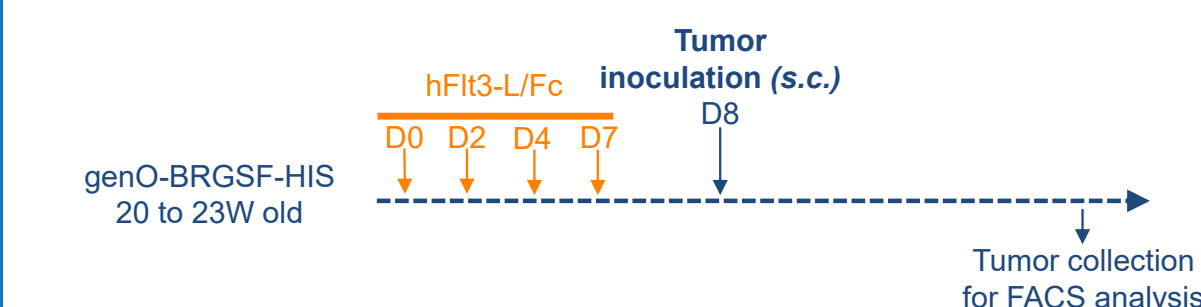
3.2 *In vivo* expansion of $\gamma\delta$ T V γ 9⁺ cells



⇒ V γ 9⁺ cells developed in genO-BRGSF-HIS mice
⇒ "Expander" activates V γ 9 proliferation *in vitro* and in all tested organs and increases their frequencies

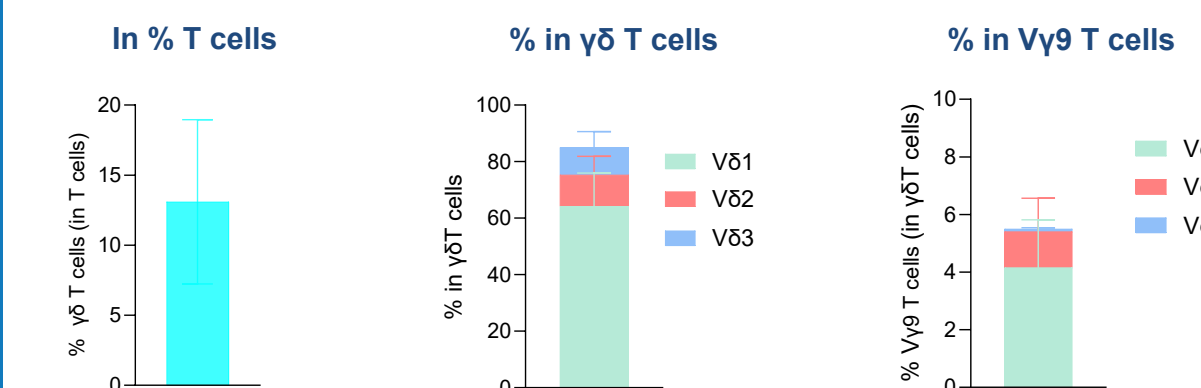
4. $\gamma\delta$ T cells in MDA-MB-231-bearing genO-BRGSF-HIS mice

4.1. Experimental workplan for the assessment $\gamma\delta$ T cells recruitment into the TME of tumor-bearing genO-BRGSF-HIS mice



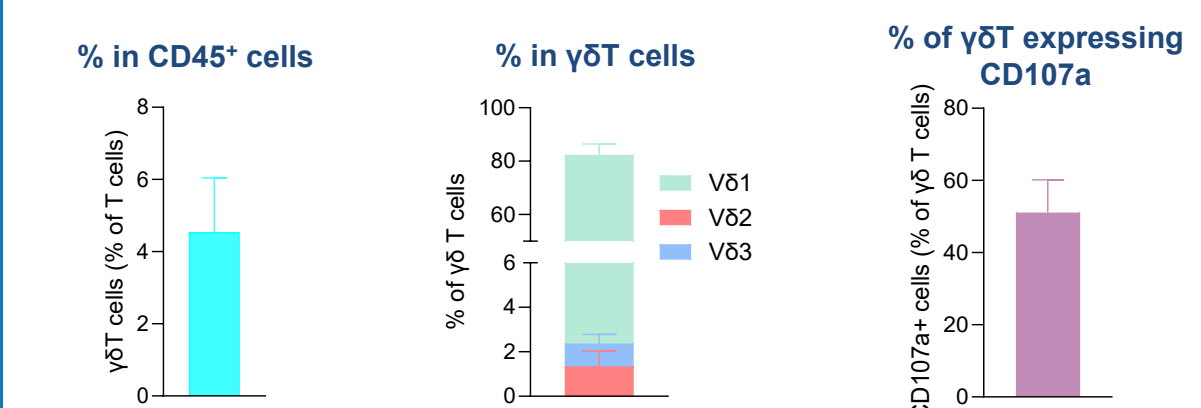
4.2. TME of DAUDI

genO-BRGSF-HIS mice were injected s.c. with the B lymphoblast DAUDI cell line (2x10⁶ cells). TME was analyzed when tumor volume = 1 000 mm³



4.3. TME of MDA-MB-231

genO-BRGSF-HIS mice were injected with the triple-negative breast cancer cell line MDA-MB-231 (1x10⁷ cells). TME was analyzed when TV > 800 mm³



⇒ $\gamma\delta$ T cells are recruited into the TME of MDA-MB-231 and DAUDI and express Vδ1, Vδ2 and Vδ3
⇒ Therapies designed to expand and activate $\gamma\delta$ T cells will be assessed in genO-BRGSF-HIS tumor-bearing mice

Conclusion: The development of functional $\gamma\delta$ T cells in genO-BRGSF-HIS mice brings a new perspective to the assessment of therapies targeting this cell population in humanized mouse models.

References:
(1) Labarthe et al., J Leukoc Biol. 2020

