γδ T-cells are functional and recruited into the TME in genO-BRGSF-HIS mice

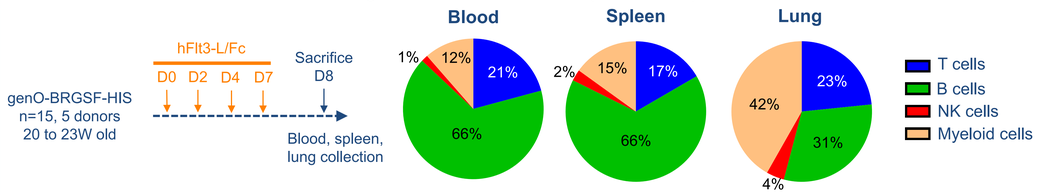
Florent CREUSAT, Siham HEDIR, Alexis GONON, Amélie MARGUIER, Camille LE GOUALLEC, Fabiane SÔNEGO, Yacine CHERIFI, Gaelle MARTIN, Kader THIAM
genOway, Lyon, France

SITC 2025
Abstract #835

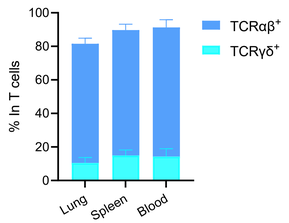
Background: Development of immunotherapies has been a major landmark in the field of oncology, leading to numerous effective treatments. From the first use of an immunomodulatory agent in clinical studies to modern immunotherapies, these were mainly focused on modulating adaptive immune response. However, only a fraction of patients can respond to these treatments, and the complexity of the tumor microenvironment requires other players to be targeted. γδ 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 beneficious or deleterious manner, was demonstrated in numerous types of cancer. Current therapeutic approaches involving γδ 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 γδ T cells are not developed at satisfactory levels in most of the humanized mouse models. Here we describe the presence and functionality of γδ T cells in genO-BRGSF (BALB/c Rag2$^{-/-}$ IL2Rγ$^{-/-}$, SIRPα^{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. γδ T cells developed in genO-BRGSF-HIS mice

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

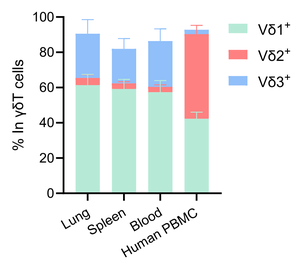


- T cells subpopulations (CD3^{+} CD56^{-})



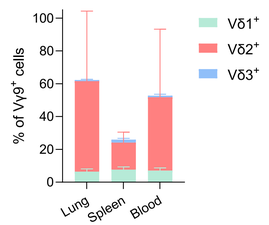
⇒ **Ratio of TCRαβ *vs* TCRγδ is approximately 80:20 in blood, spleen and lung from genO-BRGSF-HIS mice, as reported in humans**

- Characterization of human γδ T cells



⇒ **γδ cells develop in genO-BRGSF-HIS and express Vδ1, Vδ2 and Vδ3, although their ratios are different from human PBMC**

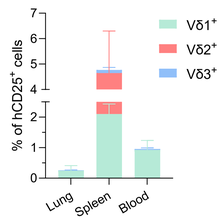
- Vγ9 expression on human γδ T cells



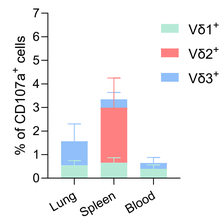
⇒ **Vγ9 is preferentially paired with Vδ2+ cells, as reported in human PBMC**
⇒ **Vγ9 is also expressed in ~5% of Vδ1^{+} cells and very low to no expression is observed in Vδ3^{+} cells, as described in human physiology**

- Human γδ T cell activation phenotype

CD25 expression

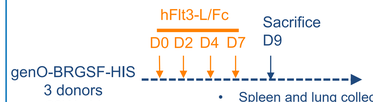


CD107a expression



⇒ **Low / no CD25 and CD107a expression in γδ T-cell subpopulations, as would be expected in naïve mice**

2. *Ex vivo* γδ T-cell activation in genO-BRGSF-HIS mice

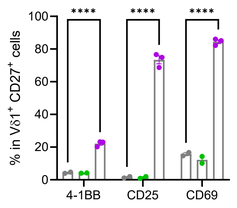


- Spleen and lung collection
- *Ex vivo* activation with Vδ1 targeting compounds for 24h

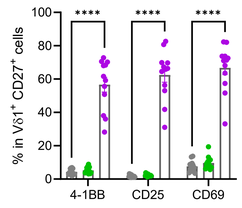
Unstimulated
Compound A
Compound B

Statistics: 2-way Anova
***** p<0.0001; ***p<0.0005; **p<0.005*

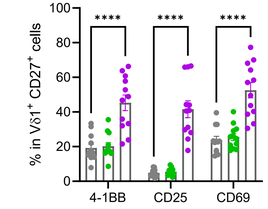
Human PBMC



Spleen



Lung



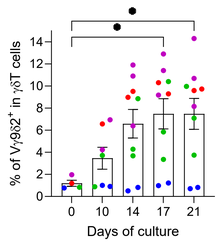
⇒ **Compound B targeting Vδ1 cells shows similar activation profile on targeting Vδ1 cells developed in genO-BRGSF-HIS mice and human PBMC from a healthy donor**

3. Expansion of γδ T cells in genO-BRGSF-HIS mice

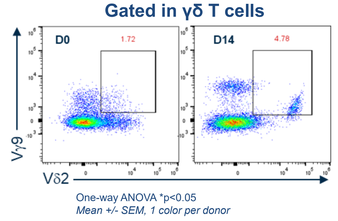
3.1 *In vitro* expansion of γδ T Vγ9^{+} cells

hCD45 enriched splenocytes (4x10^{6}) from genO-BRGSF-HIS were cultured in presence of Zolendronate, IL-2 and IL-15.

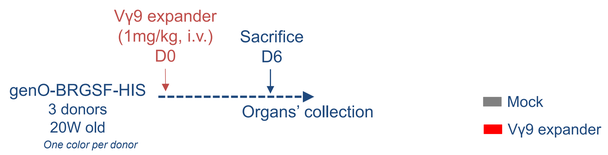
% of Vγ9δ2 in γδ T cells



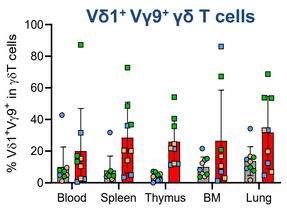
Gated in γδ T cells



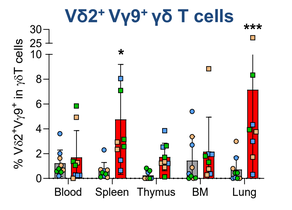
3.2 *In vivo* expansion of γδ T Vγ9^{+} cells



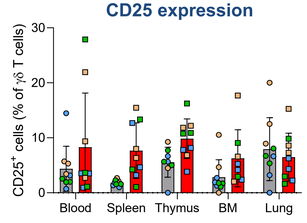
Vδ1^{+} Vγ9^{+} γδ T cells



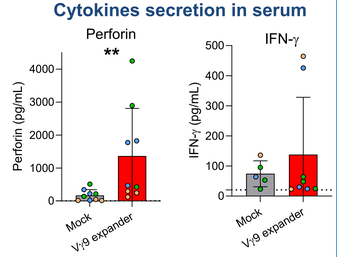
Vδ2^{+} Vγ9^{+} γδ T cells



CD25 expression



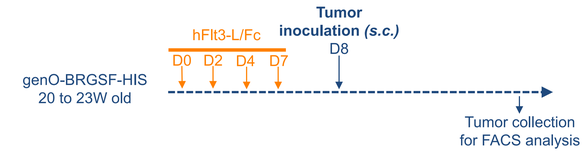
Cytokines secretion in serum



⇒ **Vγ9^{+} cells developed in genO-BRGSF-HIS mice**
⇒ **"Expander" activates Vγ9 proliferation *in vitro* and in all tested organs and increases their frequencies**

4. γδ T cells in MDA-MB-231-bearing genO-BRGSF-HIS mice

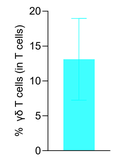
4.1. Experimental workplan for the assessment γδ T cells recruitment into the TME of tumor-bearing genO-BRGSF-HIS mice



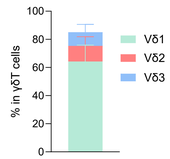
4.2. TME of DAUDI

genO-BRGSF-HIS mice were injected *s.c.* with the B lymphoblast DAUDI cell line (2x10^{6} cells). TME was analyzed when tumor volume = 1 000 mm^{3}

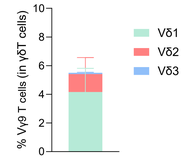
In % T cells



% in γδ T cells



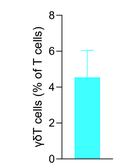
% in Vγ9 T cells



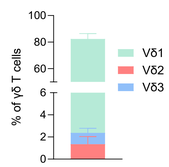
4.3. TME of MDA-MB-231

genO-BRGSF-HIS mice were injected with the triple-negative breast cancer cell line MDA-MB-231 (1x10^{7} cells). TME was analyzed when TV > 800 mm^{3}

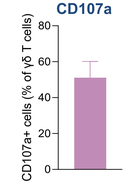
% in CD45^{+} cells



% in γδT cells



% of γδT expressing CD107a



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

Conclusion: The development of functional γδ 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