

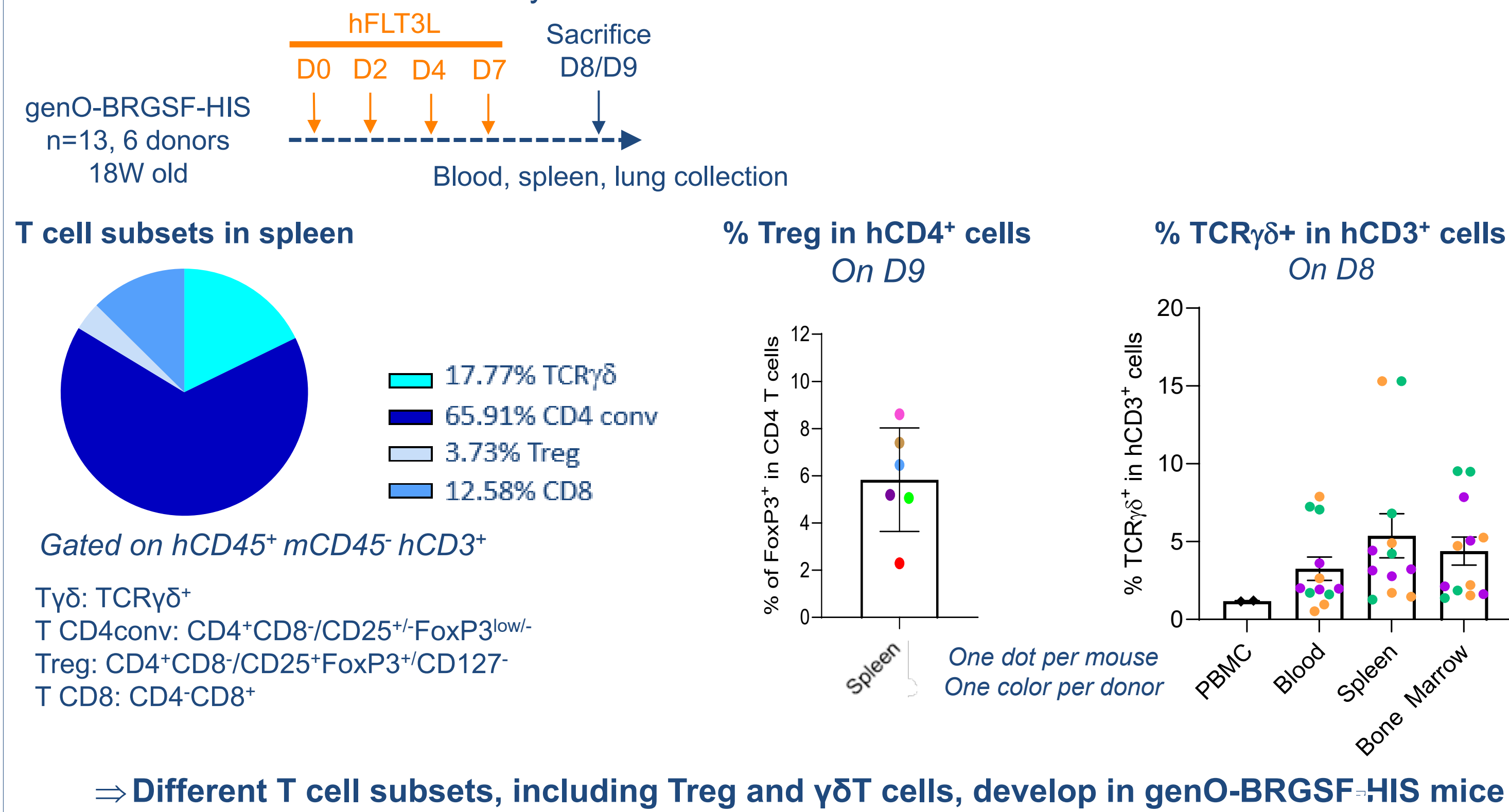
ULTIMATE PREDICTABILITY

Background: Therapies targeting regulatory T cells (Tregs) have emerged as a promising strategy to overcome immune suppression and enhance anti-tumor immunity. Tregs maintain immune tolerance under physiological conditions, but within the tumor microenvironment they promote immune evasion and resistance to immunotherapy. Selective depletion or functional modulation of Tregs can restore effector T-cell activity and improve responses to checkpoint inhibitors and other immunotherapies. However, the development of Treg-targeting approaches requires preclinical models that accurately recapitulate human Treg biology, including phenotype, activation status, and distribution in peripheral tissues and tumors. Conventional mouse models often fail to reflect these human-specific features, limiting their predictive value for safety and efficacy. Humanized models that support the development and long-term engraftment of functional Tregs and mimic human immune-tumor interactions are therefore essential to bridge the translational gap and guide clinical development of next-generation immunotherapies.

1. T cell subsets developed in genO-BRGSF mice engrafted with human hematopoietic stem cells (genO-BRGSF-HIS mice)

BRGSF (BALB/c Rag2^{-/-}, IL2Rγ^{-/-}, SIRPα^{NOD}, Flt3^{-/-}):

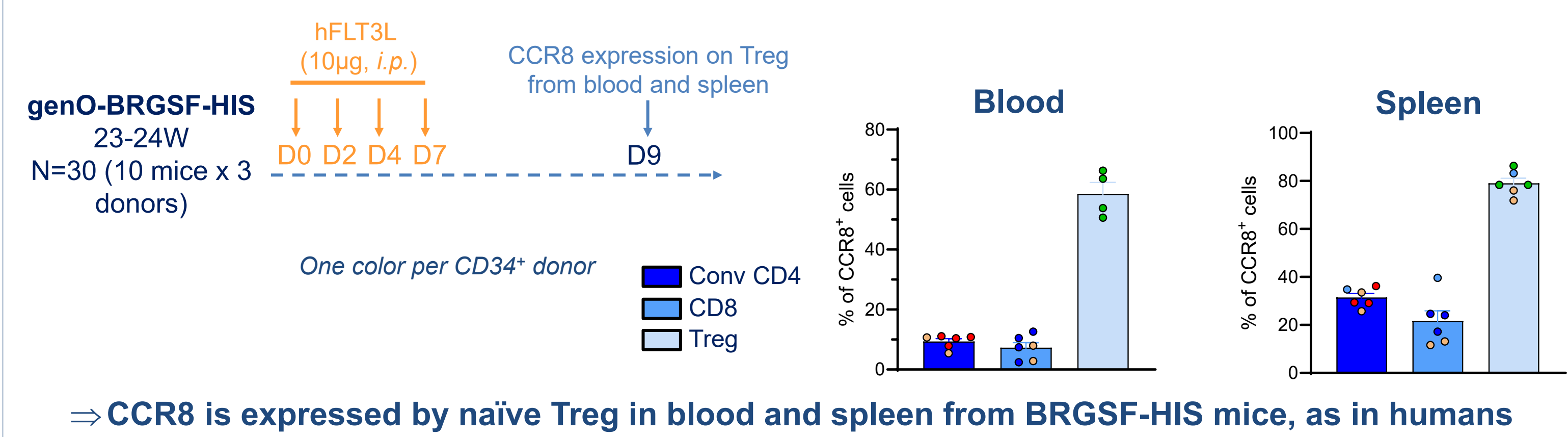
- Highly immunodeficient with reduced murine myeloid cells
- Normal lifespan (no anemia, no weight loss, normal fur texture & integrity, normal activity & posture)
- Upon CD34⁺ cells injection:
 - Lymphoid and Myeloid compartment development (1)
 - Stable reconstitution over a year (2)



2. Targeting Treg in naïve genO-BRGSF-HIS mice

CCR8 is expressed on Treg and depletion of CCR8⁺Treg is currently being tested as an approach to induce anti-tumor response. CCR8 expression pattern differs between human and mouse:

- In mice, CCR8 is only expressed on Tregs infiltrating the TME
- In humans, it is expressed on both tumor infiltrating and peripheral Tregs

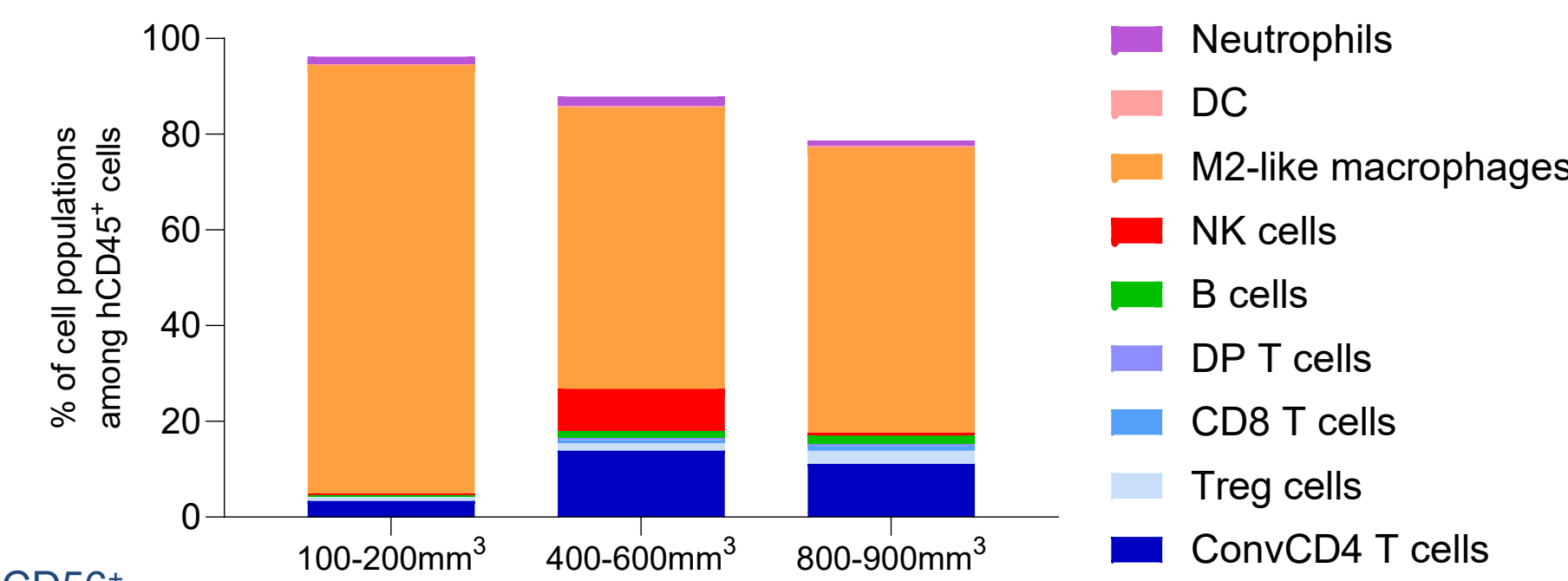


3. Treg infiltration in the tumor microenvironment (TME) of Cell Derived Xenografts (CDX) engrafted in genO-BRGSF-HIS

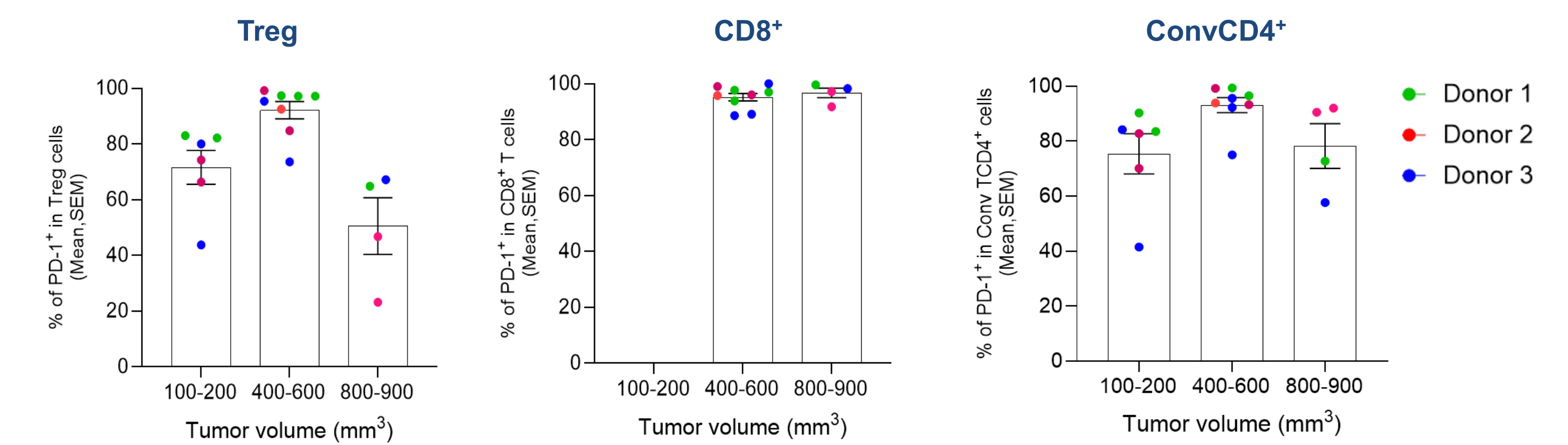
a. MDA-MB-231 (triple negative breast cancer tumor model): a highly myeloid infiltrated tumor model

genO-BRGSF-HIS mice were boosted with FLT3L and inoculated (5x10⁶ cells) with the triple negative breast cancer cell line MDA-MB-231. TME was analyzed over time.

TME human immune infiltrate and composition overtime



PD-1 expression in the TME overtime

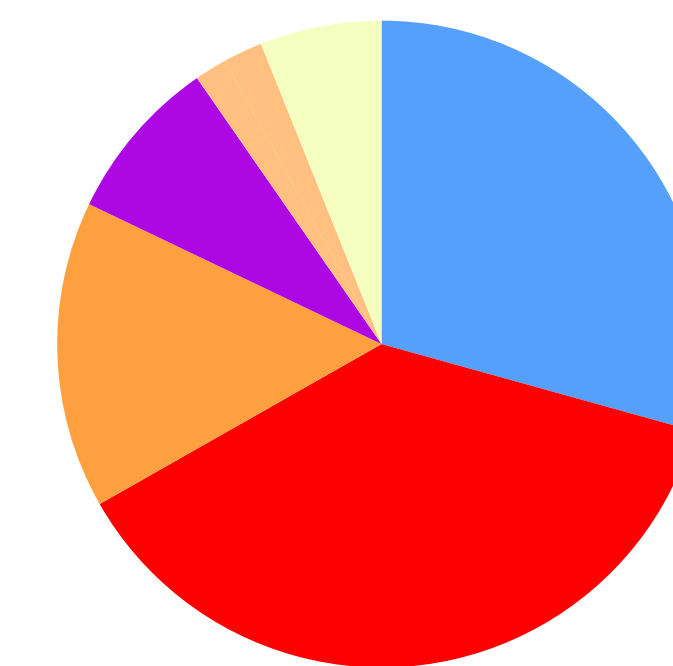


b. A549 (Non-Small Cell Lung Cancer NSCLC tumor model): a model infiltrated with T cells

genO-BRGSF-HIS mice were inoculated (5x10⁶ cells) with the human lung adenocarcinoma A549 cell line and TME was analyzed.

TME human immune infiltrate when TV=800mm³

- T cells (30%)
- NK cells (38%)
- Monocytes (15%)
- Neutrophils (8%)
- cDC (3%)
- pDC (6%)



Focus on Treg cells subsets

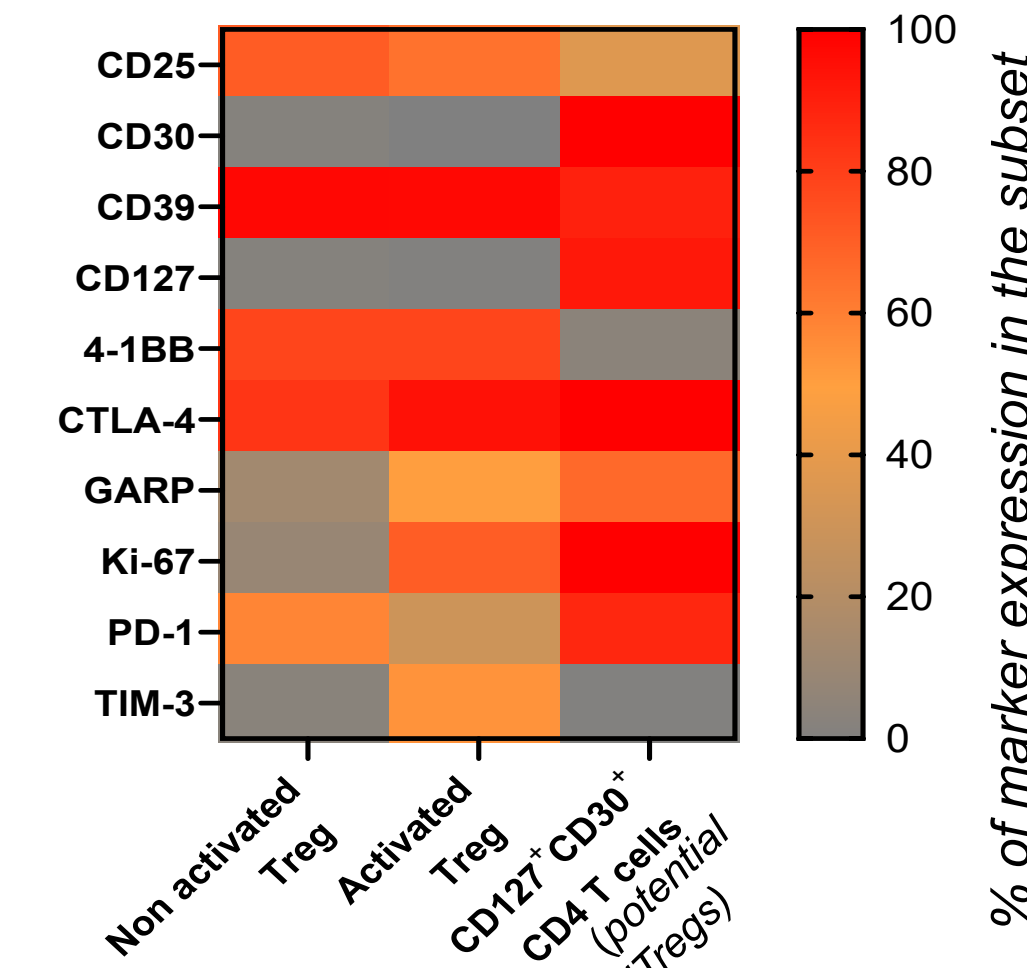
T cells: CD3⁺ CD19⁻ CD56⁻
NK cells: CD3⁺ CD19⁻ CD303⁻ CD56⁺
Monocytes: CD303⁺ CD14⁺
Neutrophils: CD303⁻ CD14⁺ CD16⁺
cDC1: CD33⁺ Lin⁻ CD303⁻ CD14⁻ CD16⁻ HLA-DR⁺ CLEC9A⁺ CD141⁺
cDC2: CD33⁺ Lin⁻ CD303⁻ CD14⁻ CD16⁻ HLA-DR⁺ CLEC10⁺ CD11C⁺
pDC: CD123⁺ CD303⁺

⇒ **TME of A549 engrafted mice is mainly composed of T cells (30%) and NK cells (38%)**

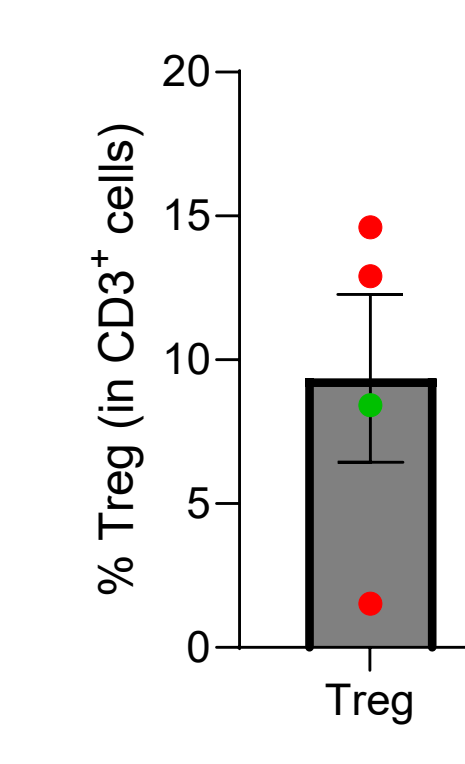
⇒ **Treg subsets develop in A549-bearing genO-BRGSF-HIS**

⇒ **2 subsets corresponding to activated and non-activated cells based on the expression of GARP, Ki-67 and TIM-3 markers**

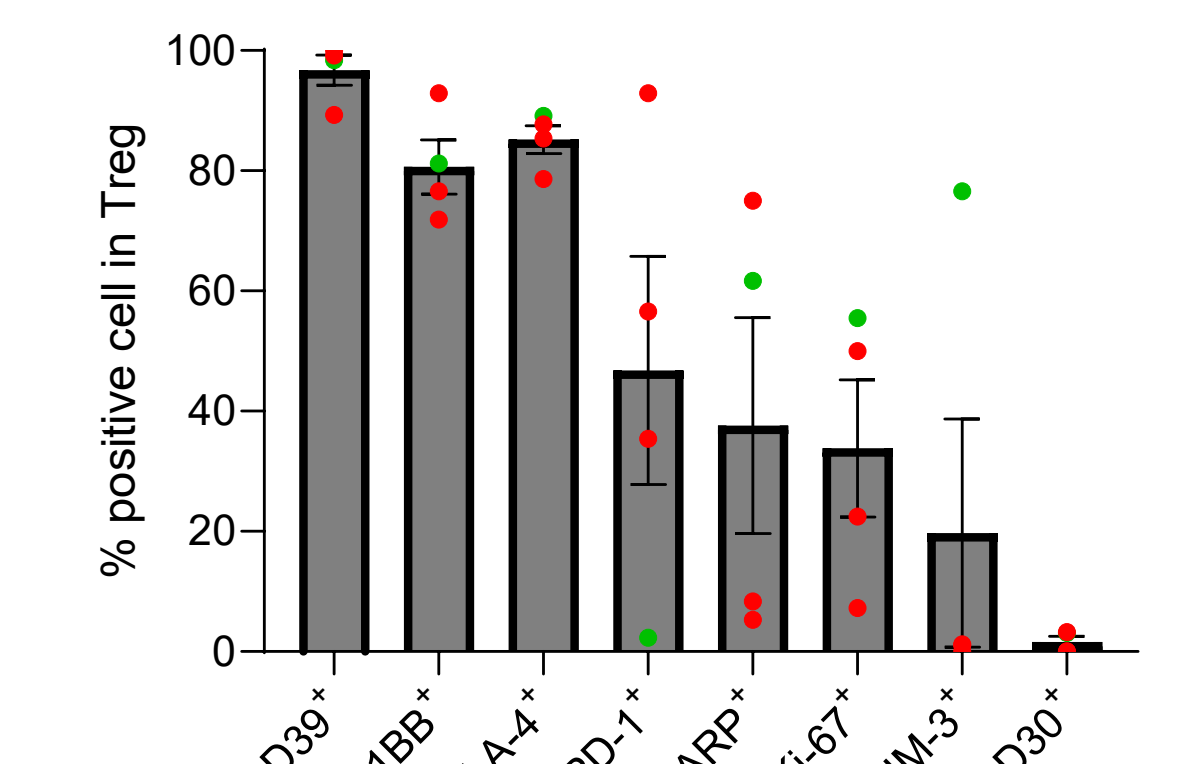
⇒ **A third subset, expressing CD127, CD30, CD39 and high levels of all activation markers is observed and could correspond to induced Tregs (iTregs) that contributes to tumor tolerance through their anti-inflammatory phenotype (3)**



CD127- Tregs in T cells

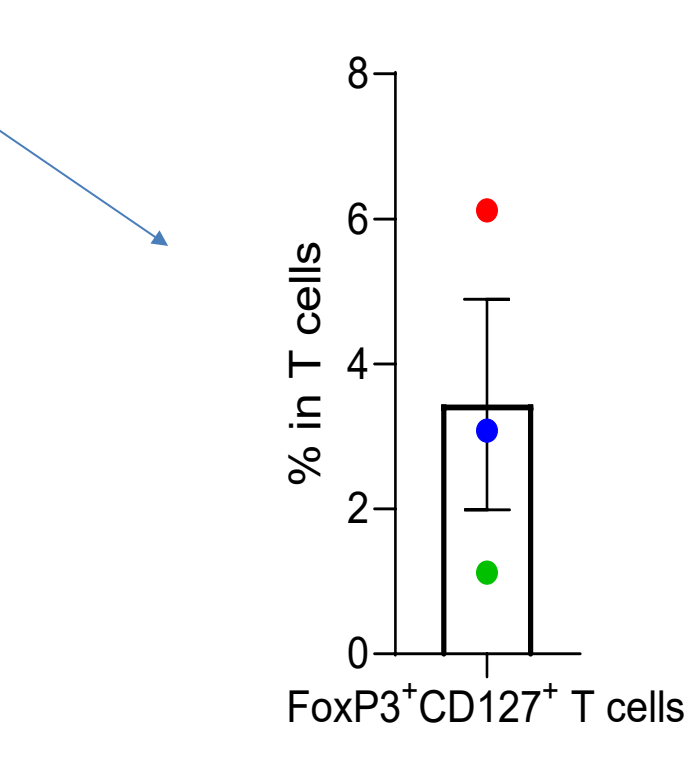


Activation markers

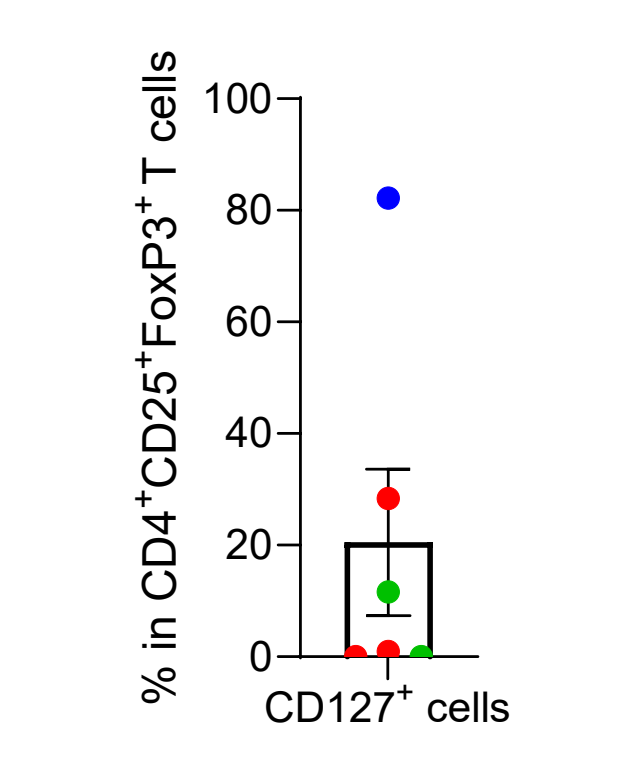


⇒ **Treg proportion at this time point represents 10% of CD3⁺ cells and expresses activation markers, but CD30**

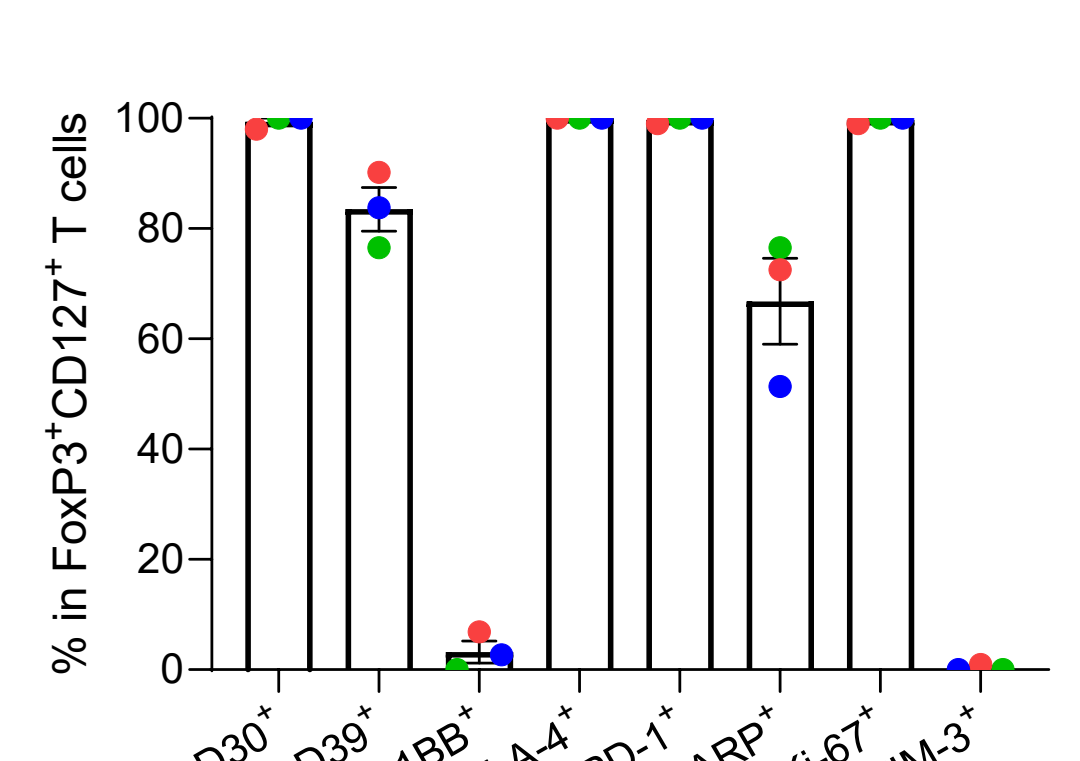
CD127+ Tregs in T cells



CD127+ Tregs in Tregs



Activation markers



Conclusion: Overall, these findings support the use of genO-BRGSF-HIS mice as a valuable tool to investigating Treg biology and evaluating human-specific therapeutic strategies in oncology.



References:
(1): Martin *et al.*, *Frontiers in Immunol.*, 2025
(2): Labarthe *et al.*, *J. Leukoc. Biol.*, 2019
(3): Curiel *et al.*, *Curr. Opin. Immunol.*, 2008