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Featuring the genO-BRGSF-HIS (scan or click)

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genO-BRGSF-HIS mice: A humanized mouse model for assessment of Treg-targeting therapies

Gaëlle H. Martin, Florent Creusat, Siham Hedir, Amélie Marguier, Camille Le Gouallec, Fabiane Sônego, Kader Thiam
genOway, Lyon, 69007, France

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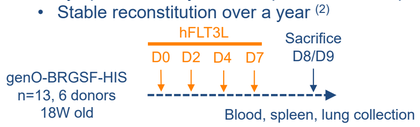
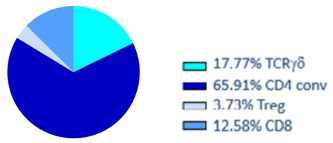
Background: Therapies targeting regulatory T cells (Tregs) have emerged as a promising strategy to overcome immune suppression and enhance anti-tumor immunity. Tregs maintain immune tolerance under physiological conditions, but within the tumor microenvironment they promote immune evasion and resistance 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)



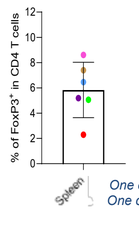
T cell subsets in spleen



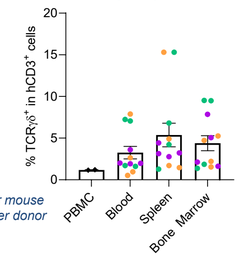
Gated on hCD45$^+$ mCD45$^-$ hCD3$^+$

Tγδ: TCRγδ$^+$
T CD4conv: CD4$^+$CD8$^-$/CD25$^{+/-}$FoxP3$^{low/-}$
Treg: CD4$^+$CD8$^-$/CD25$^+$FoxP3^{+}/CD127$^-$
T CD8: CD4$^-$CD8$^+$

% Treg in hCD4$^+$ cells *On D9*



% TCRγδ+ in hCD3$^+$ cells *On D8*

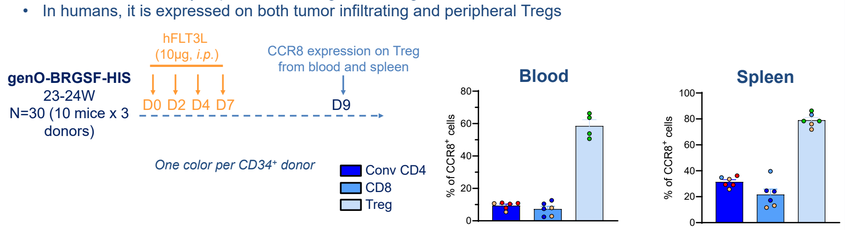


One dot per mouse
One color per donor

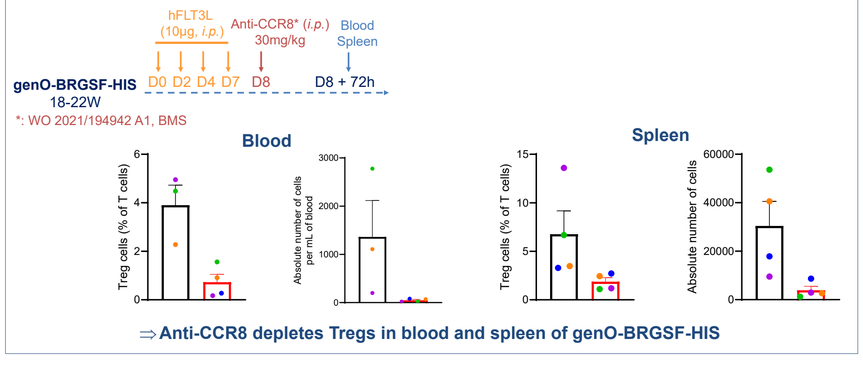
⇒ **Different T cell subsets, including Treg and γδT cells, develop in genO-BRGSF HIS mice**

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



⇒ **CCR8 is expressed by naïve Treg in blood and spleen from BRGSF-HIS mice, as in humans**



⇒ **Anti-CCR8 depletes Tregs in blood and spleen of genO-BRGSF-HIS**

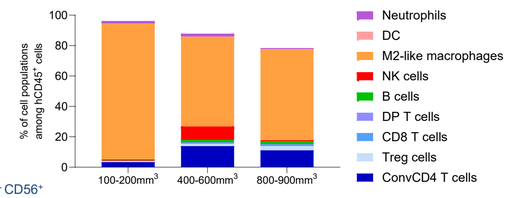
3. Treg infiltration in the tumor microenvironment (TME) of Cell Derived Xenografts (CDX) engrafted in genO-BRGSF-HIS

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

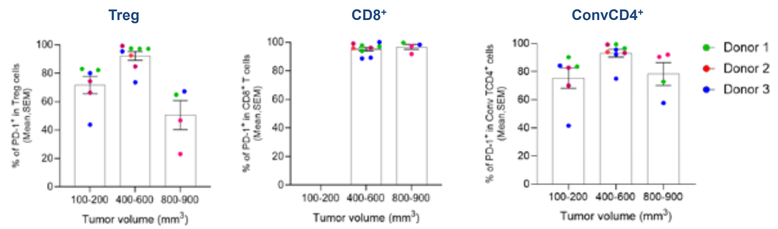
genO-BRGSF-HIS mice were boosted with FLT3L and inoculated (5x10^6 cells) with the triple negative breast cancer cell line MDA-MB-231. TME was analyzed over time.

TME human immune infiltrate and composition overtime

B cells: CD3$^-$ CD19$^+$ CD56$^-$
T cells: CD3$^+$, CD19$^-$ CD56$^-$
Conv CD4$^+$:CD4$^+$, Foxp3$^-$
Treg cells: CD4$^+$, Foxp3$^+$
Cytotoxic cells: CD8$^+$, CD4$^-$
NK cells: CD3$^-$ CD19$^-$ CD303$^-$ CD56$^+$
M2-like macrophages: CD14$^+$ CD206$^+$CD163$^+$CD80$^+$CD86$^+$
Neutrophils: CD14$^-$ CD33$^+$ CD16$^+$



PD-1 expression in the TME overtime

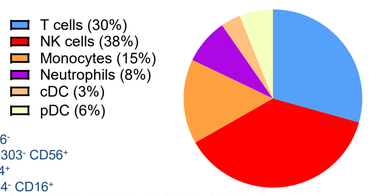


⇒ **Treg infiltration is tumor burden dependent**
⇒ **Treg are activated and express PD-1, suggesting that Treg cells respond to environmental stimuli, as well as other T cells subsets**

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

genO-BRGSF-HIS mice were inoculated (5x10^6 cells) with the human lung adenocarcinoma A549 cell line and TME was analyzed.

TME human immune infiltrate when TV=800mm^3



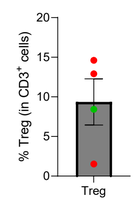
T cells: CD3$^+$ CD19$^-$ CD56$^-$
NK cells: CD3$^-$ CD19$^-$ CD303$^-$ CD56$^+$
Monocytes: CD303$^-$ CD14$^+$
Neutrophils: CD303$^-$ CD14$^-$ CD16$^+$
cDC1: CD33$^+$ Lin$^-$ CD303$^-$ CD14$^-$ CD16$^-$ HLA-DR$^+$ CLEC9A$^+$ CD141$^+$
cDC2: CD33$^+$ Lin$^-$ CD303$^-$ CD14$^-$ CD16$^-$ HLA-DR$^+$ CLEC10$^+$ CD1C$^+$
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**

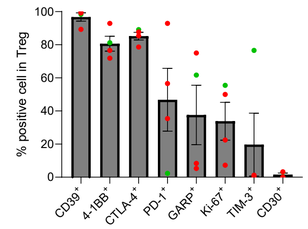
Focus on Treg cells subsets

*Gated on CD4$^+$ CD3$^+$ FoxP3$^+$ **CD127$^-$***

CD127$^-$ Tregs in T cells



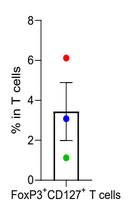
Activation markers



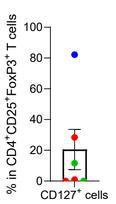
⇒ **Treg proportion at this time point represents 10% of CD3$^+$ cells and expresses activation markers, but CD30**

*Gated on CD4$^+$ CD3$^+$ FoxP3$^+$ **CD127$^+$***

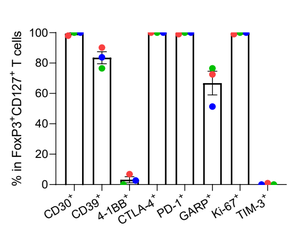
CD127$^+$ Tregs in T cells



CD127$^+$ Tregs in Tregs

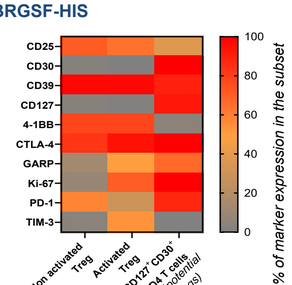


Activation markers



⇒ **A population of CD4$^+$FoxP3$^+$CD127$^+$ T cells is observed, and express CD30, CD39, CTLA-4, PD-1, GARP and Ki-67 but failed to express 4-1BB and TIM-3**

⇒ **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)**



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

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