



ULTIMATE PREDICTABILITY

# Human immune cell composition of TME depends on tumor cell line-derived xenograft subtype and tumor burden in genO-BRGSF-HIS mice

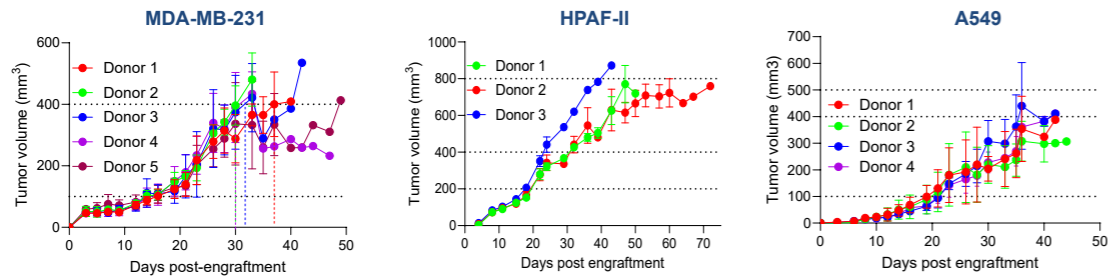
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Abstract #0841

**Background:** The relevance of preclinical models has vastly improved with mice bearing a human immune system, especially in the context of immunotherapy. genO-BRGSF (BALB/c Rag2<sup>-/-</sup>, IL2Ry<sup>-/-</sup>, SIRPα<sup>NOD</sup> and Flt3<sup>-/-</sup>) is a highly immunodeficient mouse featuring reduced murine myeloid cells. genO-BRGSF mice reconstituted with human cord blood CD34<sup>+</sup> cells (genO-BRGSF-HIS) develop functional lymphoid and myeloid compartments. This engraftment is stable for over a year<sup>(1)</sup> and mice do not develop GvHD. Additionally, the myeloid compartment can be transiently boosted with exogenous human Flt3L injections. In contrast to other models that overexpress human cytokines to develop human myeloid cells, Flt3L-treated genO-BRGSF-HIS mice do not show side effects. genO-BRGSF-HIS mice are permissive to mouse and human cancer cell line engraftment, hence representing a valuable preclinical model to study cancer development and evaluate novel therapeutics.

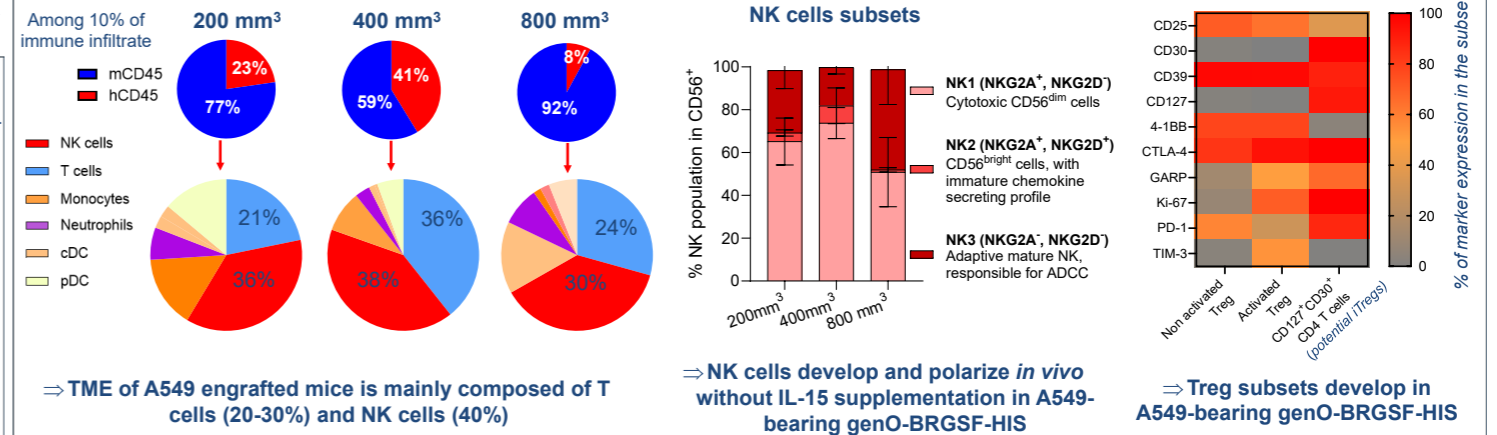
## 1. Permissiveness of genO-BRGSF-HIS mice to cell line xenograft engraftment

genO-BRGSF-HIS mice were boosted with Flt3L and inoculated with MDA-MB-231 or HPAF-II cells, or not boosted with Flt3L and inoculated with A549 cells. Tumor growth was analyzed.



## 2. TME of A549 is highly infiltrated with NK cells

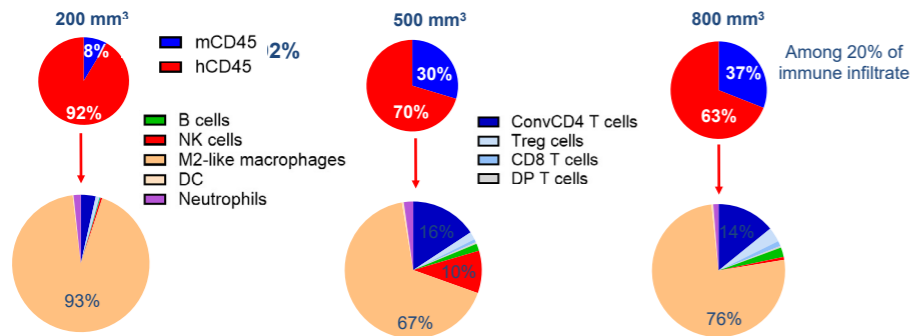
genO-BRGSF-HIS mice were inoculated (5x10<sup>6</sup> cells) with the human lung adenocarcinoma A549 cell line and TME was analyzed over time.



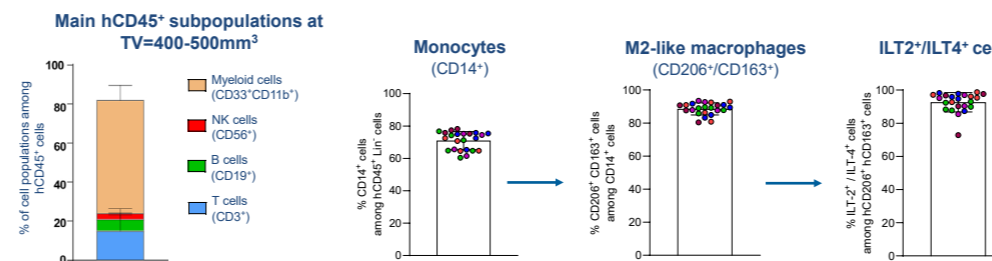
## 3. TME of MDA-MB-231 is highly infiltrated with myeloid cells

genO-BRGSF-HIS mice were boosted with Flt3L and inoculated (5x10<sup>6</sup> cells) with the triple-negative breast cancer cell line MDA-MB-231 and TME was analyzed over time (among the 20% immune cell infiltrate)

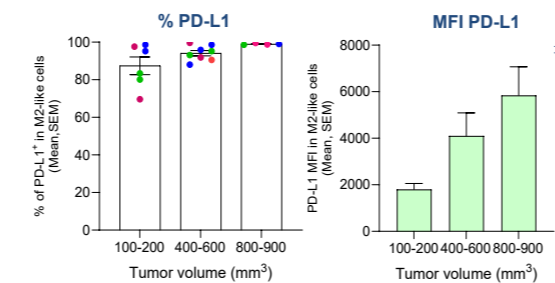
### 3.1. TME composition of MDA-MB-231 is shaped by tumor burden



### 3.2. Myeloid cell activation status in TME of MDA-MB-231



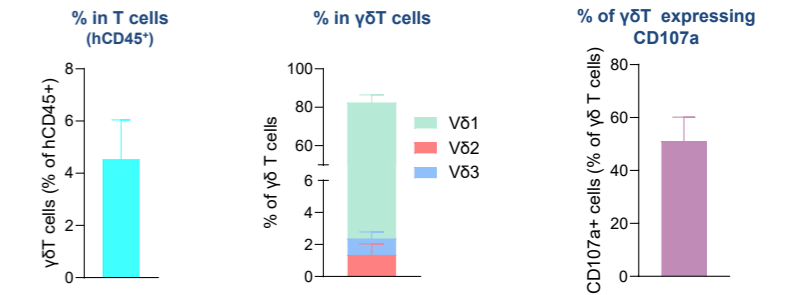
### PD-L1 expression on M2-like macrophages in the TME



⇒ TME of MDA-MB-231-bearing genO-BRGSF-HIS mice is enriched in myeloid cells, mostly CD206<sup>+</sup>/CD163<sup>+</sup> M2-like macrophages and they express both ILT2 and ILT4  
⇒ PD-L1 MFI increases with tumor burden, indicating myeloid cells activation

### 3.2. γδT cell recruitment in TME of MDA-MB-231

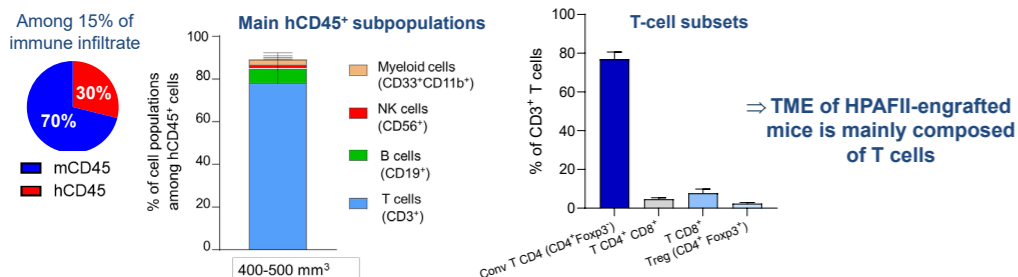
genO-BRGSF-HIS mice were injected with the triple-negative breast cancer cell line MDA-MB-231 (1x10<sup>7</sup> cells). TME was analyzed when TV > 800mm<sup>3</sup>.



⇒ γδT cells are recruited into the TME MDA-MB-231  
⇒ Therapies designed to expand and activate γδT cells will be assessed in genO-BRGSF-HIS tumor-bearing mice

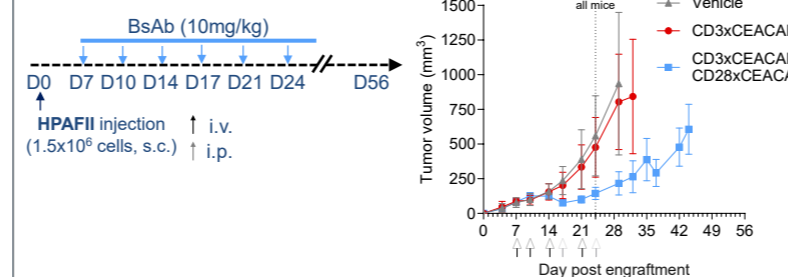
## 5. TME of HPAF-II is highly infiltrated with T cells

genO-BRGSF-HIS mice were boosted with Flt3L and inoculated (1.5x10<sup>6</sup> cells) with the human pancreatic adenocarcinoma HPAF-II cell line and TME was analyzed at TV= 400-500mm<sup>3</sup>



## 6. Response to T-Cell Engagers

genO-BRGSF-HIS mice were injected with the human pancreatic adenocarcinoma HPAFII and treated 6 times from D7 to D24 with bispecific antibodies (CD3xCEACAM5 and CD3xCEACAM5 + CD28xCEACAM5 at 10mg/kg)



**Conclusion:** The genO-BRGSF-HIS mouse model is a valuable tool to investigate immune cell infiltration in the TME, enabling a translatable assessment of mechanism of action of immunotherapies.

**References:**  
<sup>(1)</sup> Labarthe *et al.*, J. Leukoc. Biol., 2019  
<sup>(2)</sup> Majocchi *et al.*, Cancer Immunol. Res., 2024