



Tumor microenvironment composition is shaped by tumor cell line-derived xenograft subtype and tumor burden in BRGSF-HIS mice

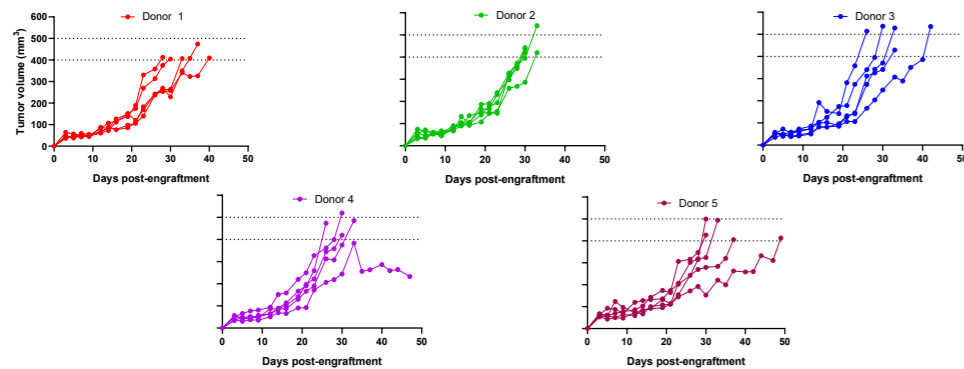
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Abstract #937

Background: The relevance of preclinical models has vastly improved with mice bearing a human immune system, especially in the context of immunotherapy. BRGSF (BALB/c Rag2^{-/-}, IL2Rγ^{-/-}, SIRPα^{NOD} and Flt3^{-/-}) is a highly immunodeficient mouse featuring reduced murine myeloid cells. BRGSF mice reconstituted with human cord blood CD34⁺ cells (BRGSF-HIS) develop functional lymphoid and myeloid compartments. This engraftment is stable over a year⁽¹⁾ and mice do not develop GvHD. Additionally, the myeloid compartment can be transiently boosted with exogenous human Flt3L injections. Contrary to other models which overexpress human cytokines to develop human myeloid cells, Flt3L-treated BRGSF-HIS mice do not show side effects. BRGSF-HIS mice are permissive to mouse and human cancer cell line engraftment.

1. Tumor growth is CD34⁺ donor-independent in MDA-MB-231-engrafted BRGSF-HIS mice

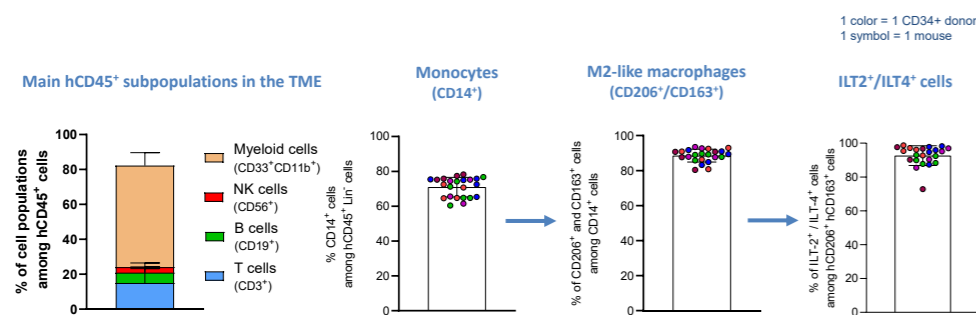
BRGSF-HIS mice were boosted with Flt3L and inoculated (5x10⁶ cells) with the triple negative breast cancer (TNBC) cell line **MDA-MB-231** and TME was analyzed when tumor volume reached ~400-500mm³



⇒ 100% tumor uptake
⇒ Tumor growth is CD34⁺ donor-independent

2. Immune cells recruitment into the TME of MDA-MB-231 engrafted BRGSF-HIS mice

BRGSF-HIS mice were boosted with Flt3L and inoculated (5x10⁶ cells) with the TNBC cell line **MDA-MB-231** and TME was analyzed when tumor volume reached ~400-500mm³



⇒ As seen in TNBC patients⁽²⁾, TME of MDA-MB-231-bearing BRGSF-HIS mice is enriched in myeloid cells, mostly CD206⁺/CD163⁺ M2-like macrophages
⇒ These M2-like macrophages express both ILT2 and ILT4

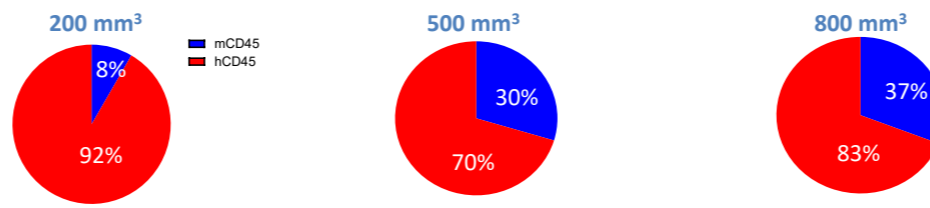
References:

- (1) Labarthe L, Henriquez S, Lambotte O, Di Santo JP, Le Grand R, Pflumio F, Arcangeli ML, Legrand N, Bourgeois C. Frontline Science: Exhaustion and senescence marker profiles on human T cells in BRGSF-A2 humanized mice resemble those in human samples. *J Leukoc Biol.* 2020; 10:1002
- (2) Zheng H, Sidharth S, Parida S, Wu X, Sharma D. Tumor Microenvironment: Key Players in Triple Negative Breast Cancer Immunomodulation. *Cancers.* 2021; 13(13):3357.

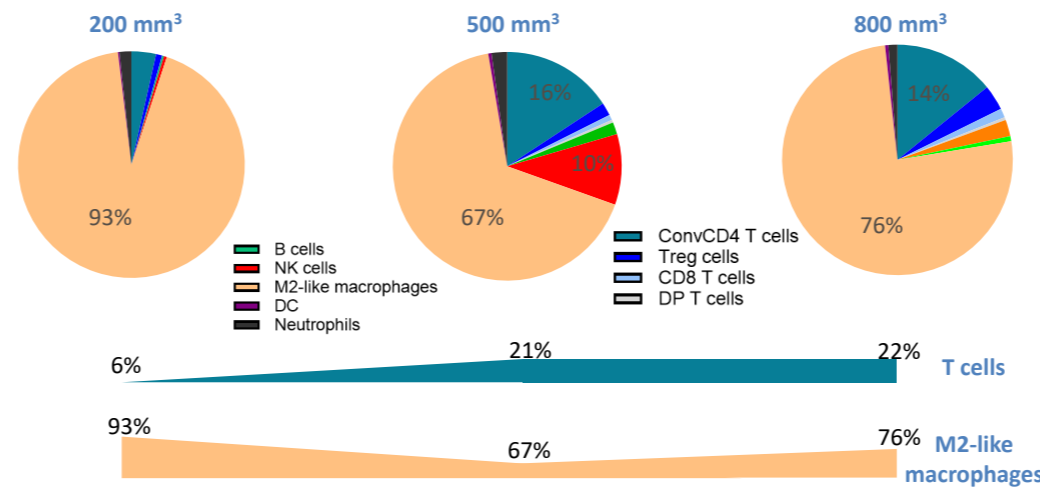
3. TME composition is tumor-burden dependent

BRGSF-HIS mice were boosted with Flt3L and inoculated (10x10⁶ cells) the TNBC cell line **MDA-MB-231** and TME was analyzed when tumor volume reached 100-200, 400-600 and 800mm³

Human and murine CD45⁺ cell recruitment in the TME



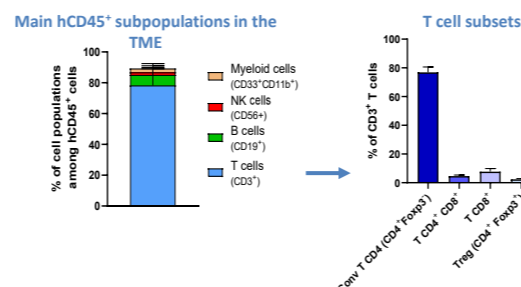
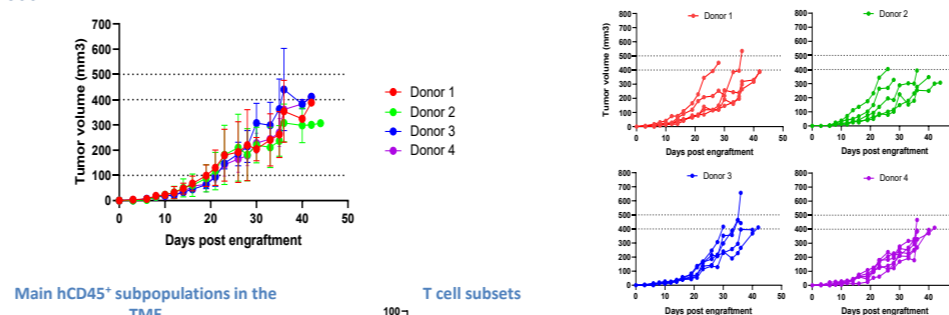
Human immune cell recruitment in the TME



⇒ TME composition evolves with tumor burden
⇒ Human cell types recruited shifts over time: myeloid compartment recruitment reduces in large tumors while T cell recruitment increases

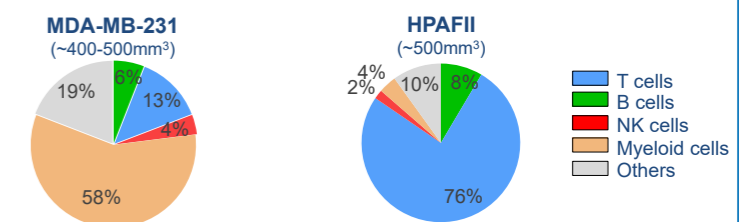
4. TME composition is tumor type dependent TME characterization of HPAFII engrafted BRGSF-HIS mice

BRGSF-HIS mice were boosted with Flt3L and inoculated (1.5x10⁶ cells) with the human pancreatic adenocarcinoma **HPAFII**. Tumor growth was monitored and TME was analyzed when tumor volume reached ~500mm³



⇒ 100% tumor uptake
⇒ Tumor growth is CD34⁺ donor-independent
⇒ TME of HPAFII engrafted mice is mainly composed of T cells

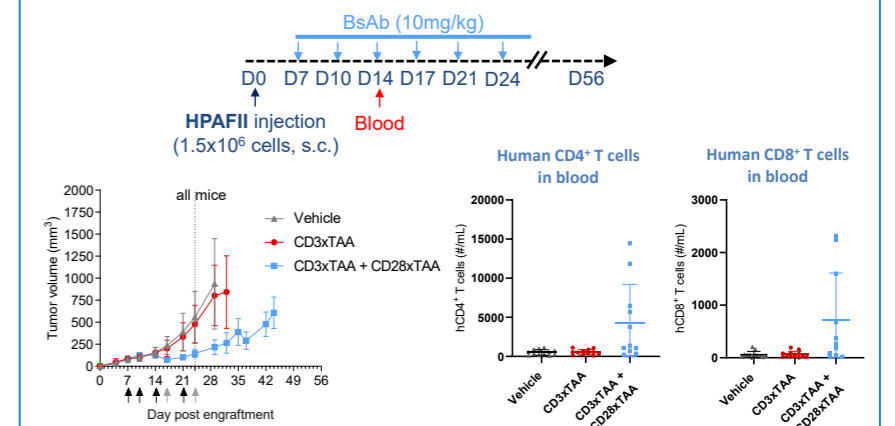
5. Comparison of global TME composition



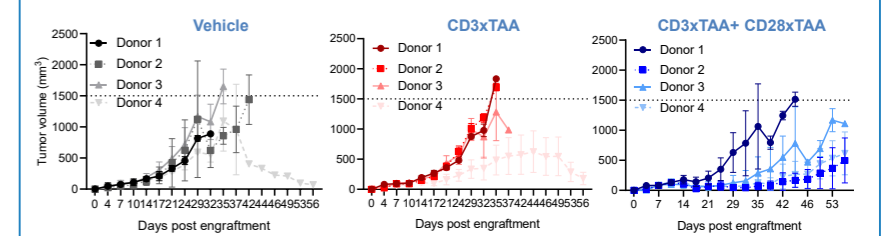
⇒ TME composition varies according to engrafted cell line and tumor burden

6. Response to immunotherapy is donor-dependent – Example of TCE

BRGSF-HIS mice were injected with the human pancreatic adenocarcinoma **HPAFII** (1.5x10⁶ cells) and treated 6 times from D7 to D24 with bispecific antibodies (CD3xTAA + CD28xTAA at 10mg/kg)



⇒ Treatment with combotherapy CD3xTAA + CD28xTAA efficiently reduces tumor growth *in vivo*
⇒ Combotherapy induces systemic immunomodulation in blood as CD4 and CD8 T cell numbers are increased



⇒ Response to treatment is CD34⁺ donor-dependent (“weak” and “good” responders)

Conclusion: 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.

