POWERED BY genOway's PRECLINICAL MODELS

Tumor cell line-derived xenograft subtype shapes tumor microenvironment composition in BRGSF-HIS mice

Perrine MARTIN-JEANTET[1] , Siham HEDIR[1], Gaelle MARTIN[1], Fabiane SÔNEGO[1], Julie GORRY[1], Yacine CHERIFI[1], Kader THIAM[1]
[1]genOway, Lyon, 69007, France

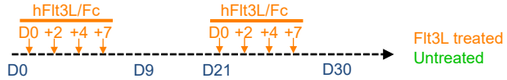
AACR 2024
Abstract #1530

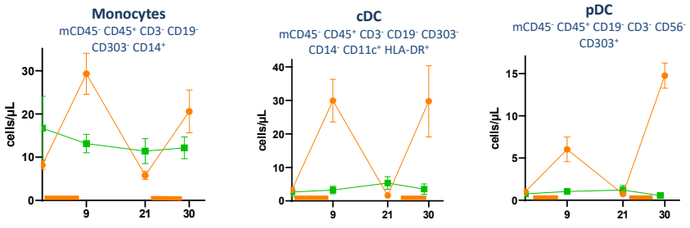
Background: The relevance of preclinical models has vastly improved with mice bearing a human immune system, especially in the context of immunotherapy. BRGSF (BALB/c Rag2$^{-/-}$ IL2R$\gamma^{-/-}$, SIRPα^{NOD} and Flt3$^{-/-}$) is a highly immunodeficient mouse featuring reduced murine myeloid cells. 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, myeloid compartment can be transiently boosted with exogenous human Flt3L injections. In 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 lines engraftment and represent a valuable preclinical model to study cancer development and evaluate novel therapeutics.

1. Boosting of human myeloid cells in blood

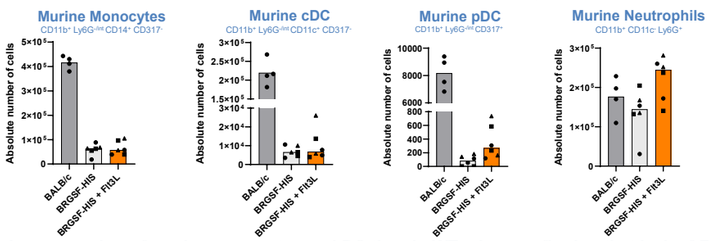
In vivo Flt3L boosting: Mice were injected (i.p.) every 2 to 3 day with 10µg recombinant human Flt3 Ligand Fc





⇒ Flt3L treatment boosts human Monocytes, cDC and pDC in blood
⇒ Boost is transient, peaks 2 days after treatment
⇒ Myeloid compartment can be reboosted

2. Remaining murine myeloid cells in BRGSF-HIS mice

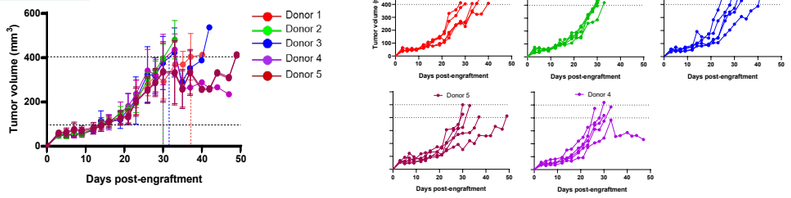


⇒ Lower number of murine monocytes and DC than in WT mice, confirming that lack of Flk2 impairs the development of myeloid lineage
⇒ Murine neutrophils are not reduced in BRGSF-HIS mice, suggesting that their differentiation pathway is independent of Flt3(2)

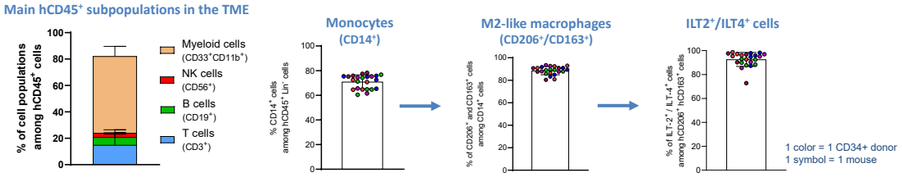
3. TME characterization of MDA-MB-231 engrafted BRGSF-HIS mice

- BRGSF-HIS mice were boosted with Flt3L and inoculated (5x10^6 cells) with the triple negative cancer cell line **MDA-MB-231** and TME was analyzed when tumor volume reached ~ 400-500mm^3

Early time point (~400-500mm^3)



Main hCD45$^+$ subpopulations in the TME

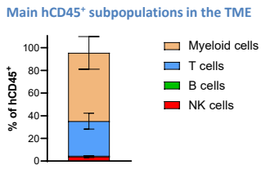


⇒ **100% tumor uptake**
⇒ **Tumor growth is CD34$^+$ donor-independent**
⇒ **TME of MDA-MB-231 bearing BRGSF-HIS mice is enriched in myeloid cells, mostly CD206$^+$/CD163$^+$ M2-like macrophages**
⇒ **These macrophages express both ILT2 and ILT4**

- BRGSF-HIS mice were boosted with Flt3L and inoculated (5x10^6 cells) with the triple negative cancer cell line **MDA-MB-231** and TME was analyzed when tumor volume reached ≥ 1000mm^3

Later time point (Tumor volume ≥ 1000mm^3)

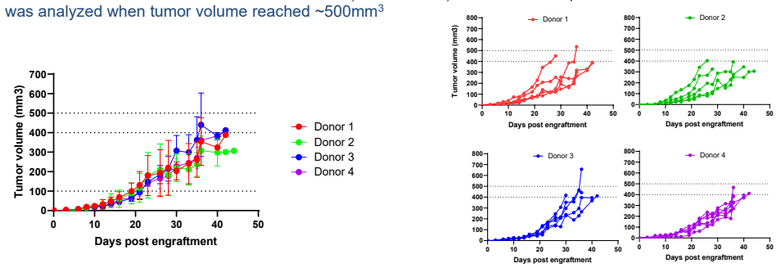
Main hCD45$^+$ subpopulations in the TME



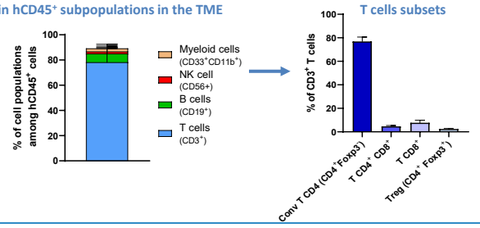
⇒ **TME composition evolves over time, with increased T cell frequency at later stage, depicting interaction between tumors cells and immune system**

4. TME characterization of HPAFII engrafted BRGSF-HIS mice

- BRGSF-HIS mice were boosted with Flt3L and inoculated (1.5x10^6 cells) with the human pancreatic adenocarcinoma **HPAFII** and TME was analyzed when tumor volume reached ~500mm^3

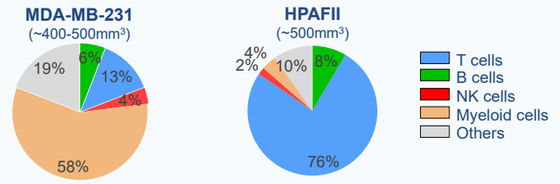


Main hCD45$^+$ subpopulations in the TME



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

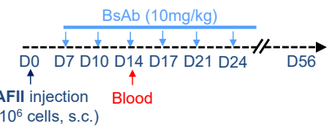
5. Comparison of global TME composition

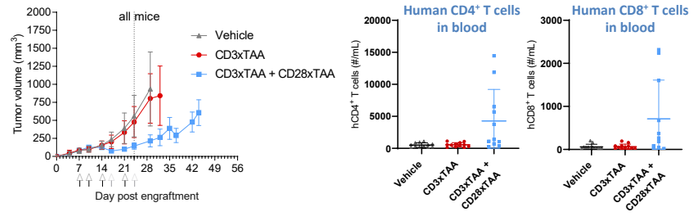


- ***In vivo*** **growth of human cell line-derived xenograft (CDX) is CD34$^+$ donor-independent and the TME composition varies according to cell line used and tumor burden**

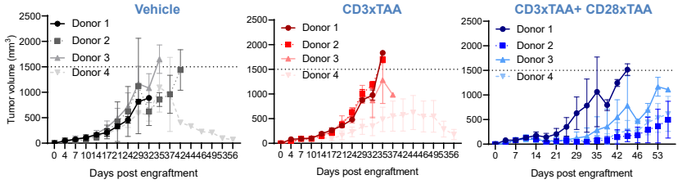
6. Donor variability in response to T cell engagers

- BRGSF-HIS mice were injected with the human pancreatic adenocarcinoma **HPAFII** (1.5x10^6 cells) and treated 6 times from D7 to D24 with bispecific antibodies (CD3xTAA and 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 cells numbers are increased**



⇒ **Response to treatment is CD34$^+$ donor-dependent ("weak" and "good" responders)**

Conclusion: This heterogeneity of response mimics what is observed in clinic and enables further investigations of treatment MoA (mode of action) and immune escape mechanisms.

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) https://www.rndsystems.com/pathways/hematopoietic-stem-cell-differentiation-pathways-lineage-specific-markers

www.genOway.com