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BRGSF-HIS and CD3ε epitope humanized mice: Translatable preclinical mouse models for assessment of T-cell engagers-induced CRS

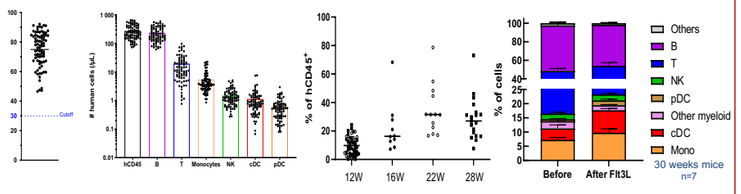
Perrine Martin-Jeantet[1]; Florence Renart-Depontieu[1]; Ludovic Bourre[2]; Gaëlle Martin[1]; Angela Pappalardo[1]; Dean O. Campbell[2]; Astrid Doerner[2]; Yacine Cherifi[1]; Fabiane Sônego[1]; Kader Thiam[1]
[1]genOway, Lyon, 69007, France; [2]Crown Bioscience, 16550 West Bernardo Drive, Building 5, Suite 525, San Diego, CA, USA

Background: T cell engagers show high efficacy in B cells malignances. High risk of immune-related adverse events, including cytokine release syndrome (CRS), is reported in patients due to on-target off-site effects of T cell engagers. Thus, reliable and translational mouse models are required to predict potential safety issues and investigate their rescue. Here we describe two preclinical models mimicking to some extent CRS induction upon activation with anti-CD3 antibodies.

BRGSF-HIS mice

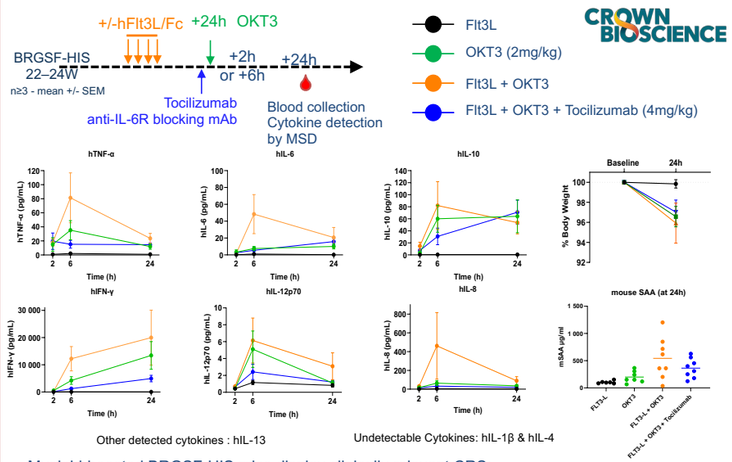
Model features

- BRGSF (Balb/C Rag2$^{-/-}$ IL2Rγ$^{-/-}$, SIRPα^{NOD} and Flt3$^{-/-}$):
  - Highly immunodeficient with reduced murine myeloid cells
  - Normal life span (no anemia, no weight loss, normal fur texture & integrity, normal activity & posture)
- Upon hCD34^{+} cells injection:
  - Lymphoid and Myeloid compartment development
  - Boost by hFlt3L enhances human myeloid cell development and accumulation



- Humanization level in blood (12 weeks/QC)
- Increase over time of T cells proportions
- Flt3L treatment boosts human myeloid cells proportions

OKT3-induced CRS is enhanced by myeloid compartment and can be mitigated by Tocilizumab

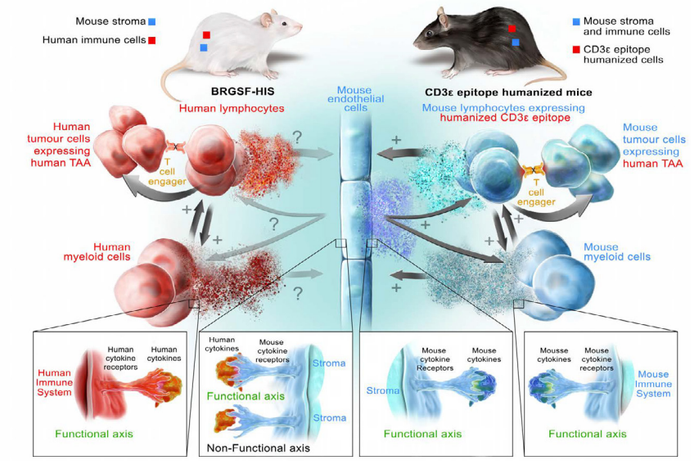


Other detected cytokines : hIL-13

Undetectable Cytokines: hIL-1β & hIL-4

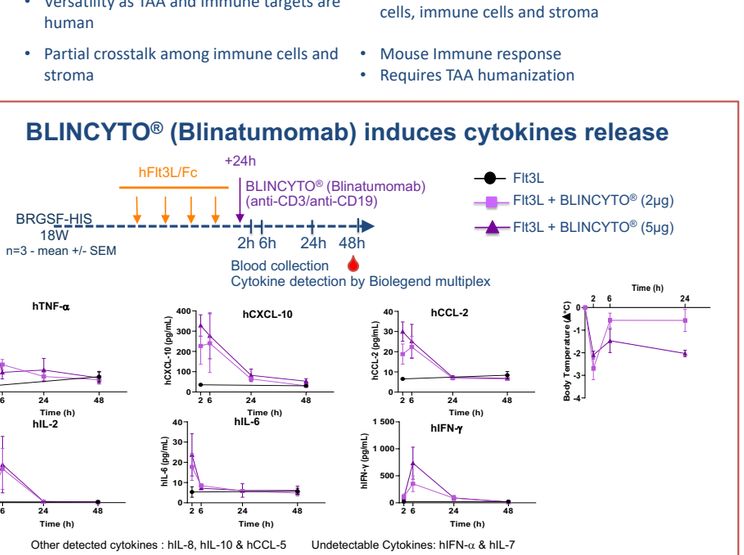
- Myeloid-boosted BRGSF-HIS mice display clinically relevant CRS:
  - T cells depletion both in blood and spleen (data not shown) and body weight drop are observed upon OKT3 injection
  - Flt3L treatment increases cytokine and mouse Serum Amyloid A (SAA) levels induced by OKT3
  - Tocilizumab (anti-IL-6R blocking mAb) mitigates cytokine secretion, mSAA & temperature (not shown) drops

Crosstalk between immune system & non-immune cells



	BRGSF-HIS	hCD3 KI
Pros	• Human Immune response • Versatility as TAA and immune targets are human	• Fully functional crosstalk among tumor cells, immune cells and stroma
Cons	• Partial crosstalk among immune cells and stroma	• Mouse Immune response • Requires TAA humanization

BLINCYTO® (Blinatumomab) induces cytokines release



Other detected cytokines : hIL-8, hIL-10 & hCCL-5

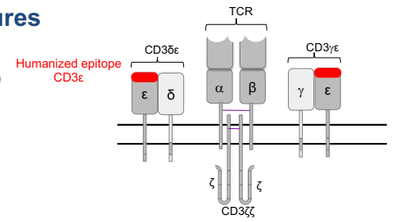
Undetectable Cytokines: hIFN-α & hIL-7

- BLINCYTO® induces cytokine release and body temperature drop in a dose-dependent manner

CD3ε epitope humanized mice

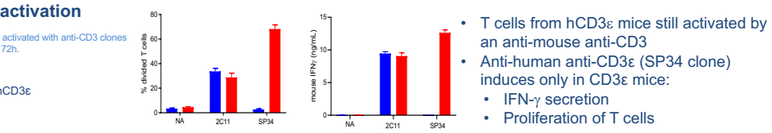
Model features

- CD3ε N-terminal epitope knock-in mice (hCD3ε mice) expressing the humanized epitope of anti-CD3 clone SP34:
  - Preserves the overall mouse CD3 complex functionality
  - Preserves the homeostasis of the immune system



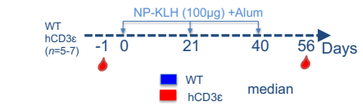
CD3ε humanization does not alter T cells functions

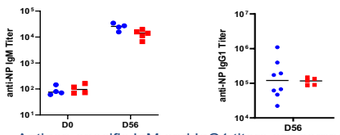
Ex vivo T cell activation



- T cells from hCD3ε mice still activated by an anti-mouse anti-CD3
- Anti-human anti-CD3ε (SP34 clone) induces only in CD3ε mice:
  - IFN-γ secretion
  - Proliferation of T cells

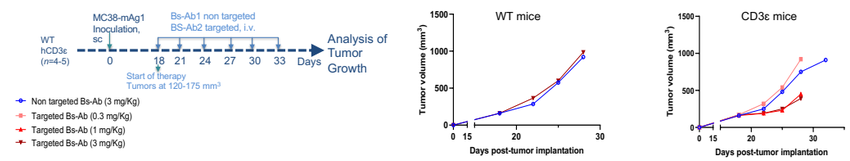
T-B cells cooperation





- Antigen-specific IgM and IgG1 titers are comparable in hCD3ε and WT mice

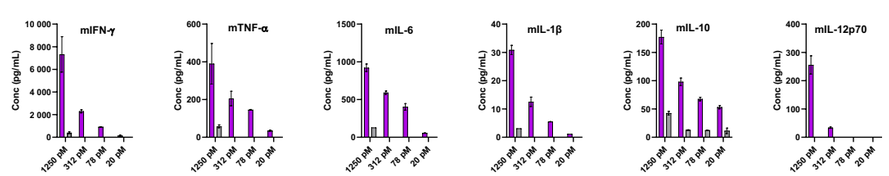
T cell engager-induced anti-tumor response



- Tumor regression induced by an anti-CD3ε bispecific antibody (ScFv) in hCD3ε mice only

Bispecific Ab T-cell engager induces cytokine production

Ex vivo co-culture of Hepa1-6 + splenocytes for 24h in presence of:
BsAb1: anti-CD3 derived from SP34; TAA expressed on Hepa1-6 cell
BsAb2: negative control - anti-CD3 derived from SP34; TAA not expressed on Hepa1-6 cell



mean +/- SEM

- Mouse cytokines production is concentration-dependent
- A 2nd BsAb targeting CD3ε and an irrelevant TAA does not induce cytokine release

Conclusion: BRGSF-HIS and hCD3ε models are complementary to assess T cell engager-induced CRS in addition to efficacy.

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