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SITC 2022

Syngeneic CD3 humanized and CD34⁺-reconstituted BRGSF mice as validated tool to assess immune-related adverse events of T cell engagers

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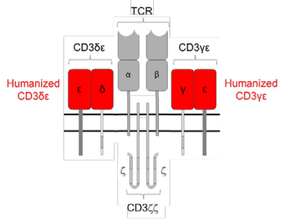
Background: T cell engagers show high efficacy in B cells malignances. Immune-related adverse events (IrAE), including cytokine release syndrome (CRS), is reported in patients due to on-target off-site effects of T cell engagers. Translational and predictive assays to assess IrAE of T cell engagers are key to avoid pitfalls in clinical trials. Syngeneic CD3 humanized mouse models enable the assessment of human-target antibodies in a fully immunocompetent mice, displaying a functional crosstalk among stroma, tumor, and immune cells. The main limitation of such models is that the read-out is focused on mouse biology. Alternatively, immunodeficient mouse models reconstituted with human CD34⁺ cells enable the assessment of human biology in a context of functional interaction of human immune and tumor cells, with a partial interaction with mouse stroma cells. Here we show the assessment of anti-CD3-induced CRS in a syngeneic CD3 humanized model as well as in BRGSF (Balb/C *Rag2*$^{-/-}$, *IL2Rγ*$^{-/-}$, *SIRPα*^{NOD} and *Flt3*$^{-/-}$) mice reconstituted with CD34⁺ cells.

Pan CD3 humanized mice

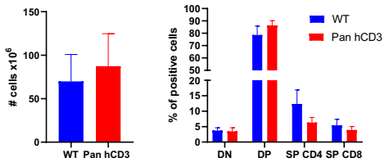
A versatile syngeneic model for T cell engagers assessment

Mice expressing human CD3γ, CD3δ , CD3ε:
- Preserve the overall CD3 complex functionality
- Preserve the homeostasis of the immune system



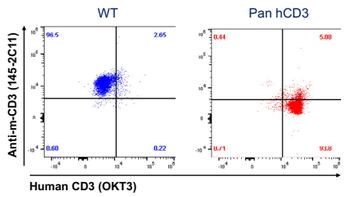
CD3 humanization does not alter T cells functions

Cell distribution in the thymus



- Pan CD3 humanized model show similar distribution of thymocytes along the maturation process

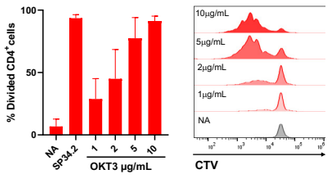
CD3 expression on CD4 T cells



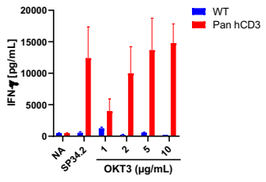
- Human CD3 is specifically expressed on Pan hCD3 CD4 T cells – *Similar results obtained for CD8 T cells (data not shown)*

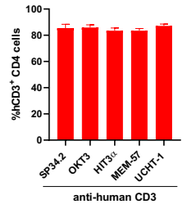
Ex vivo T cell activation

T cell proliferation



IFN-γ production



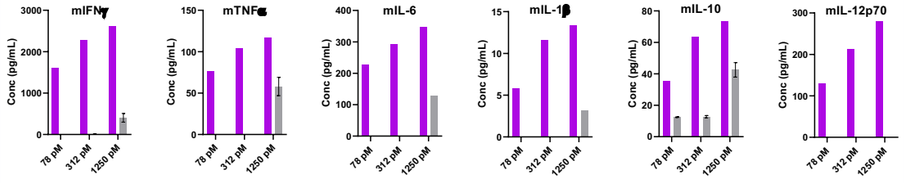


- Anti-human CD3 clones induce T cell proliferation and IFN-γ secretion from Pan hCD3
- Pan hCD3 CD4 T cells specifically bind all tested anti-human CD3 clones – *Similar results obtained for CD8 T cells (data not shown)*

Bispecific Ab T cell engager induces cytokine production

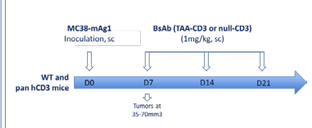
Ex vivo co-culture of Hepa1-6 + splenocytes for 24h in presence of:
BsAb1: anti-CD3 derived from SP34; TAA expressed on Hepa1-6 cell
BsAb2: negative control - anti-CD3 derived from SP34; TAA not expressed on Hepa1-6 cell

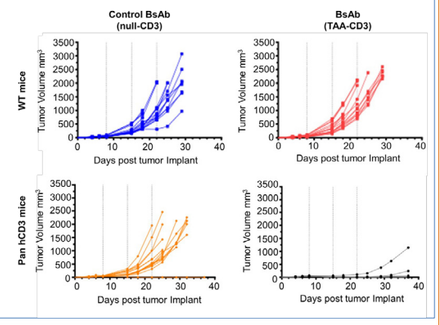
mean +/- SEM



- Anti-CD3 x TAA BsAb induces mouse cytokines production in a concentration-dependent manner

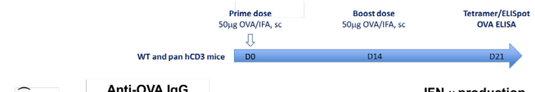
T cell engager-induced anti-tumor response

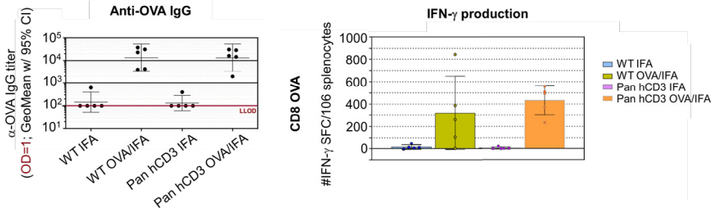




- Anti-CD3 x TAA BsAb specifically induces tumor growth inhibition in Pan hCD3 mice

Functional T-B cells cooperation in Pan hCD3 mice

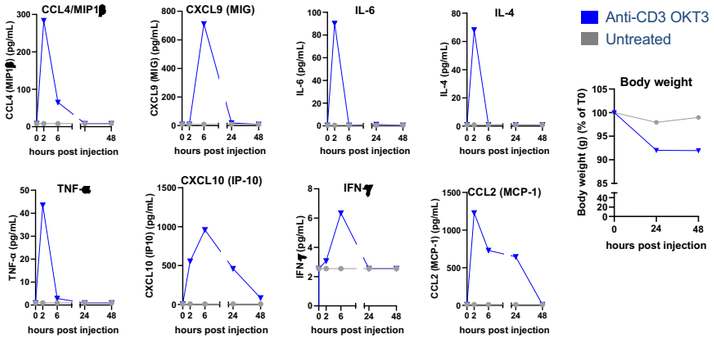




- Antigen-induced specific response is similar in Pan hCD3 and WT mice

OKT3-induced *in vivo* Cytokine Release Syndrome

Pan hCD3 mice were injected i.v. with anti-human CD3 antibody (OKT3 250µg/kg).
Cytokine release and body weight were measured at different time points after injection.



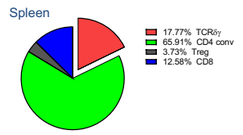
- OKT3 induces cytokine release and body weight drop, suggesting that Pan hCD3 mice can recapitulate CRS observed in humans

BRGSF-HIS mice

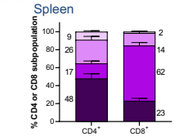
Snapshot on human immune cell subset

- BRGSF highly immunodeficient with reduced murine myeloid cells
- Normal lifespan and stable reconstitution overtime

T cell subsets

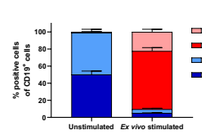


T CD4conv: CD4⁺CD8⁻/CD25⁺FoxP3$^{low/-}$; **T CD4reg**: CD4⁺CD8⁻/CD25⁺FoxP3^{hi}CD127⁻; **T CD8**: CD4⁻CD8⁺; **TαβδγTCRγδ**: hCD45⁺mCD45⁻/CD3⁺/ TCRγδ⁻ ; **Tγδ**:hCD45⁺mCD45⁻/CD3⁺/TCRγδ⁺



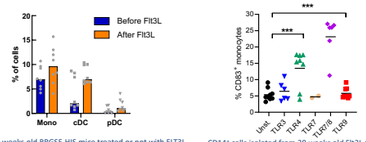
CM (Central Memory): CD45RA⁻ CCR7⁺; **EM** (Effector Memory): CD45RA⁻ CCR7⁻ ; **EMRA** (Effector Memory Re-expressing CD45RA): CD45RA⁺ CCR7⁻ ; **Naive**: CD45RA⁺ CCR7⁺

B cell subsets



hCD19⁺ isolated splenocytes activated for 6 days with αCD40, CpG ODN, IL-2, IL-10, IL-21

Myeloid cells

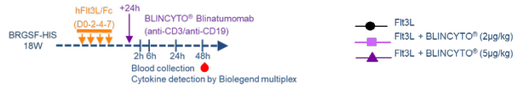


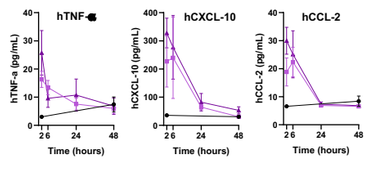
30 weeks old BRGSF-HIS mice treated or not with FLT3L (10µg, i.p.) every other 2-3 days for one week

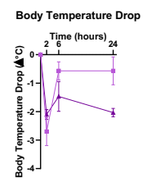
CD14⁺ cells isolated from 38 weeks old Flt3L-treated BRGSF-HIS, activated with TLR3 ligand: Poly(I:C) - 10µg/mL; TLR4 ligand: LPS – 0.1µg/mL; TLR7 ligand: R837/imiquimod - 100µg/mL; TLR7/8 ligand: R848/Resiquimod - 250µg/mL; TLR9 ligand: CpG class A : ODN 2216 - 250µg/mL

- BRGSF-HIS mice display lymphoid and myeloid compartments
- Myeloid compartment can by boosted by Flt3L and has been shown to be functional

BLINCYTO® (Blinatumomab) induces cytokines release







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

Conclusion:

- Pan CD3 humanized model shows a functional immune response and enables assessment of efficacy and CRS induced by T cell engagers
- BRGSF-HIS mice display functional human lymphoid and myeloid compartments, enabling translatable response of T cell engagers-induced CRS
- Pan CD3 and BRGSF-HIS are complementary models to evaluate toxicity induced by T cell engagers

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