



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

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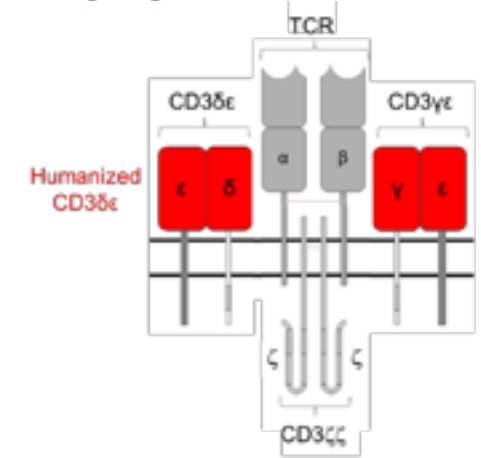
**Background:** T cell engagers show high efficacy in B cells malignances. Immune-related adverse events (IrAE), including cytokine release syndrome (CRS), is reported in patients due to on-target off-site effects of T cell engagers. Translational and predictive assays to assess IrAE of T cell engagers 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<sup>+</sup> 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*<sup>-/-</sup>, *IL2R*<sup>γ</sup><sup>-/-</sup>, *SIRPα*<sup>NOD</sup> and *Flt3*<sup>-/-</sup>) mice reconstituted with CD34<sup>+</sup> cells.

### Pan CD3 humanized mice

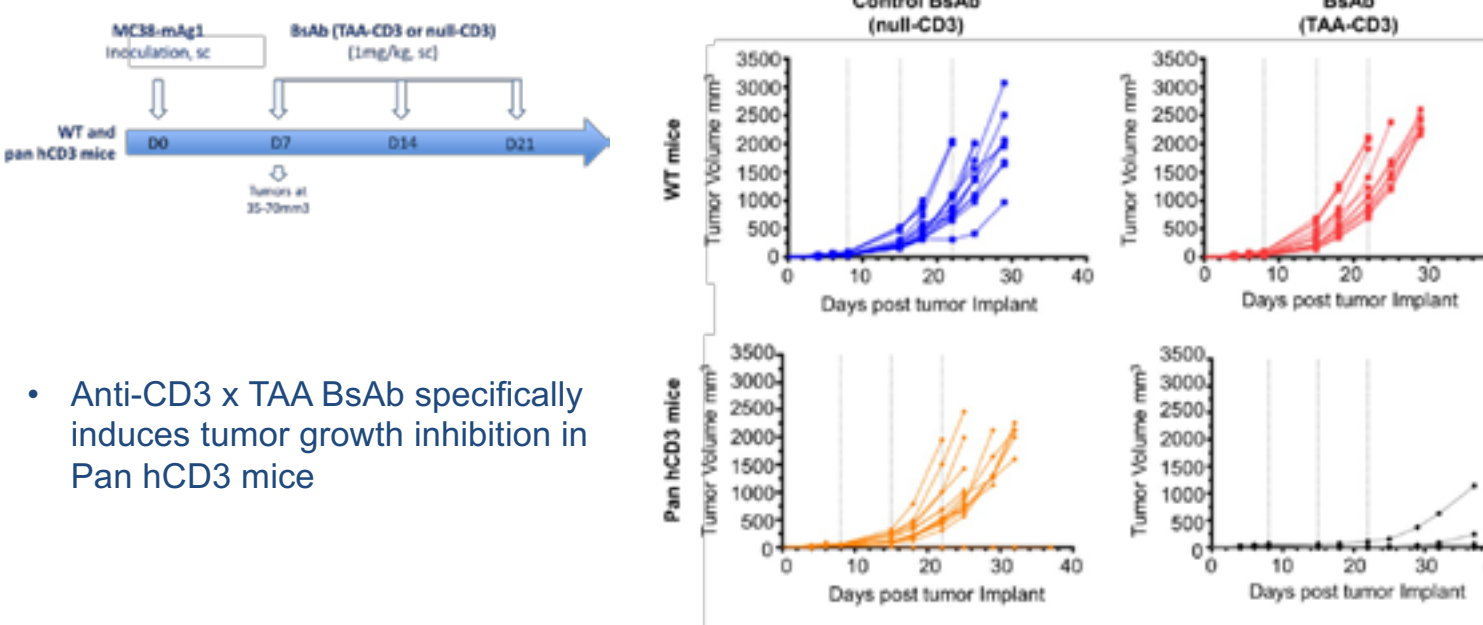
#### A versatile syngeneic model for T cell engagers assessment

Mice expressing human CD3 $\gamma$ , CD3 $\delta$ , CD3 $\epsilon$ :

- Preserve the overall CD3 complex functionality
- Preserve the homeostasis of the immune system



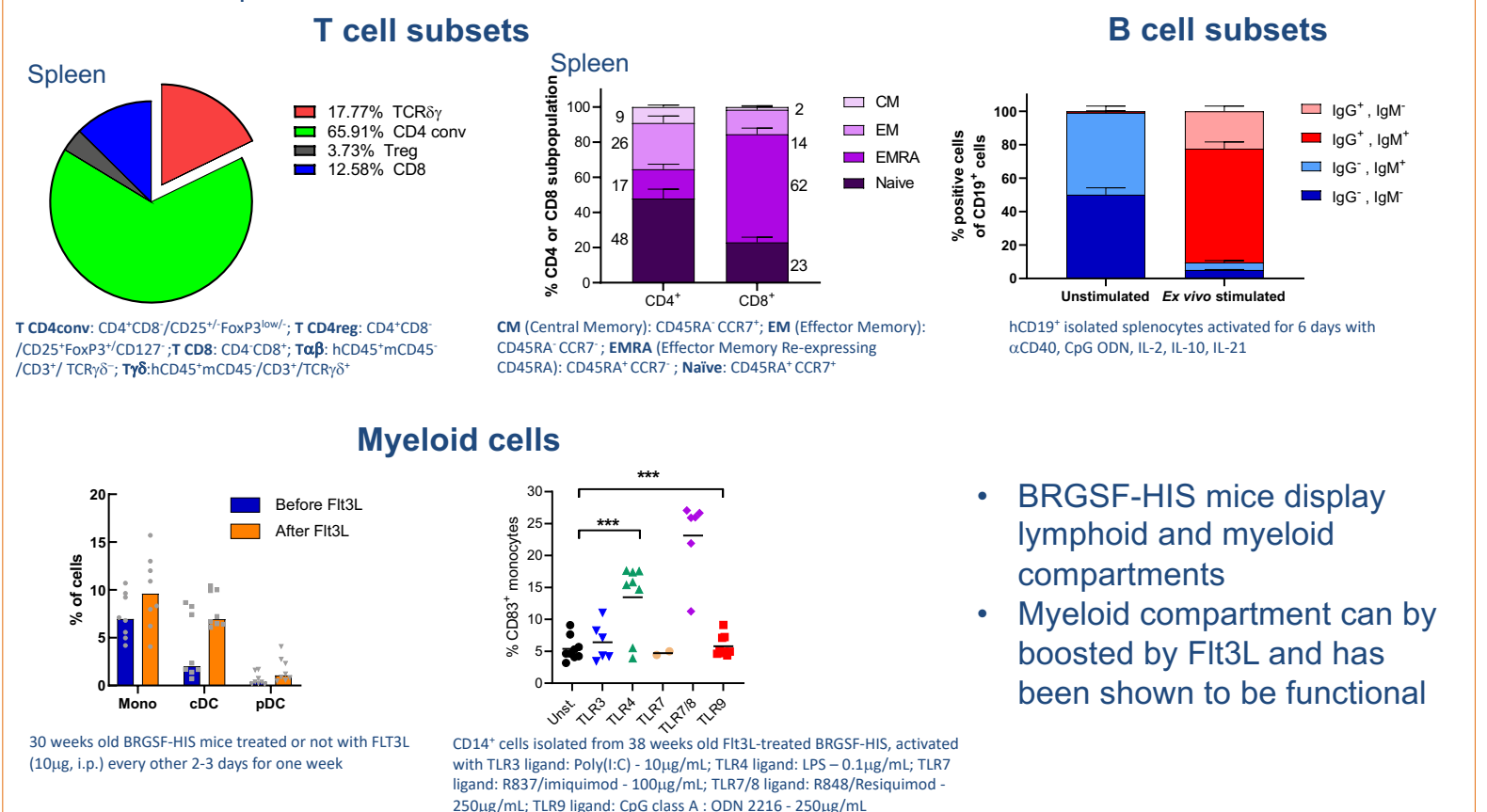
#### T cell engager-induced anti-tumor response



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

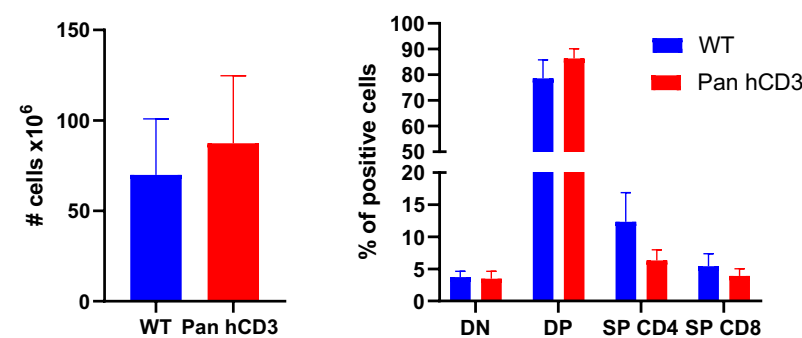
#### Snapshot on human immune cell subset

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

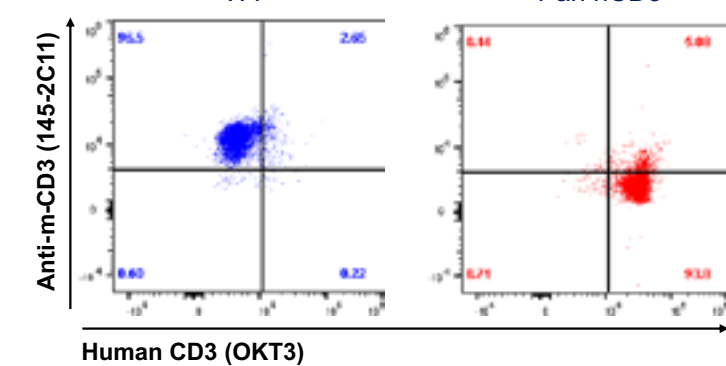


#### CD3 humanization does not alter T cells functions

##### Cell distribution in the thymus



##### CD3 expression on CD4 T cells

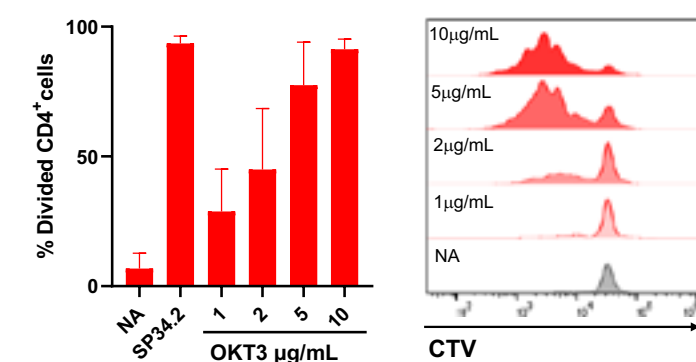


- Pan CD3 humanized model show similar distribution of thymocytes along the maturation process

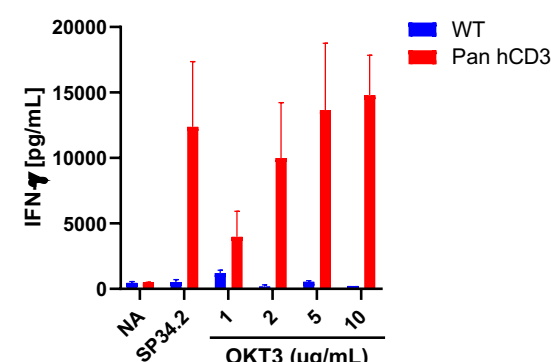
- Human CD3 is specifically expressed on Pan hCD3 CD4 T cells – Similar results obtained for CD8 T cells (data not shown)

##### Ex vivo T cell activation

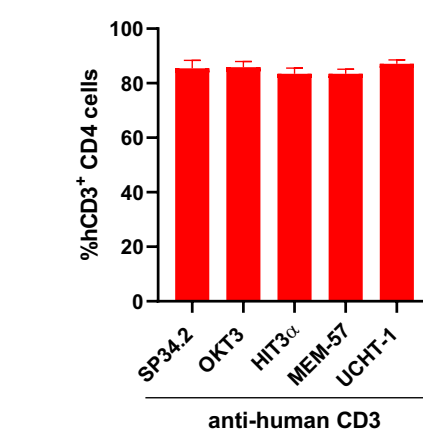
##### T cell proliferation



##### IFN-γ production

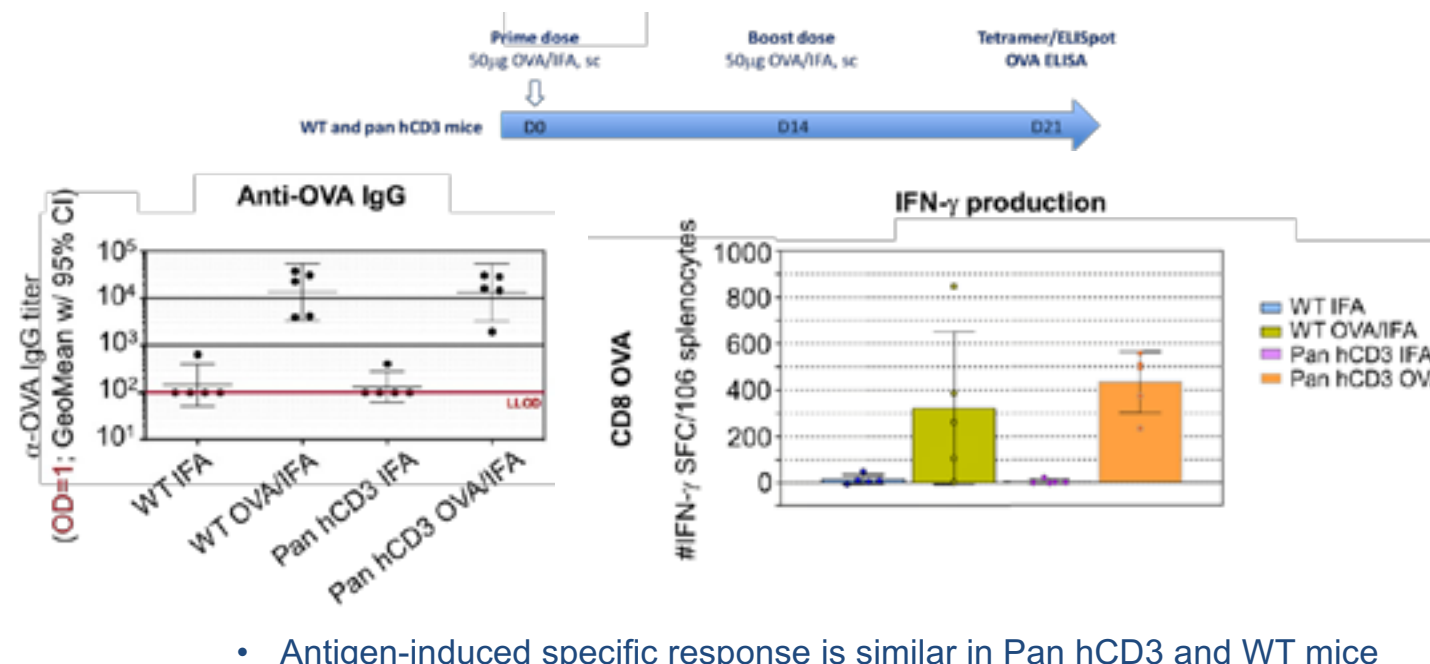


- Anti-human CD3 clones induce T cell proliferation and IFN-γ secretion from Pan hCD3



- Pan hCD3 CD4 T cells specifically bind all tested anti-human CD3 clones – Similar results obtained for CD8 T cells (data not shown)

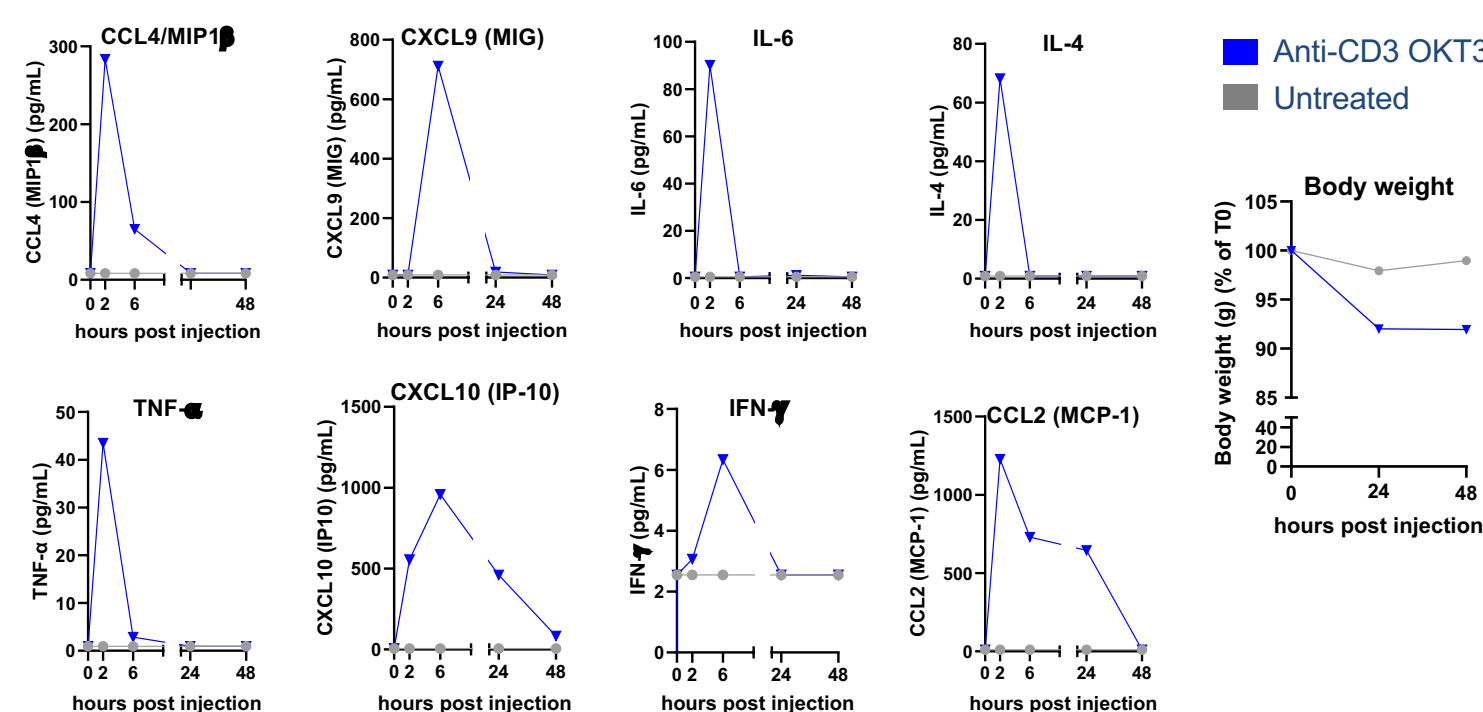
#### Functional T-B cells cooperation in Pan hCD3 mice



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

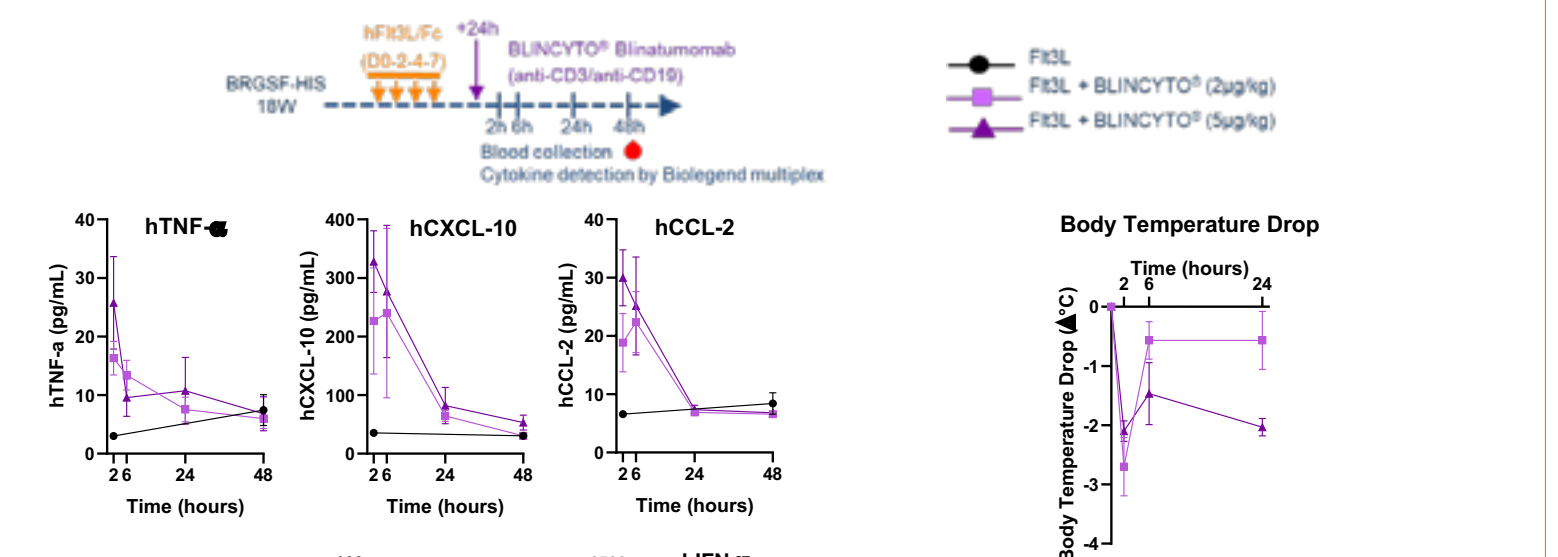
#### OKT3-induced *in vivo* Cytokine Release Syndrome

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

#### BLINCYTO® (Blinatumomab) induces cytokines release



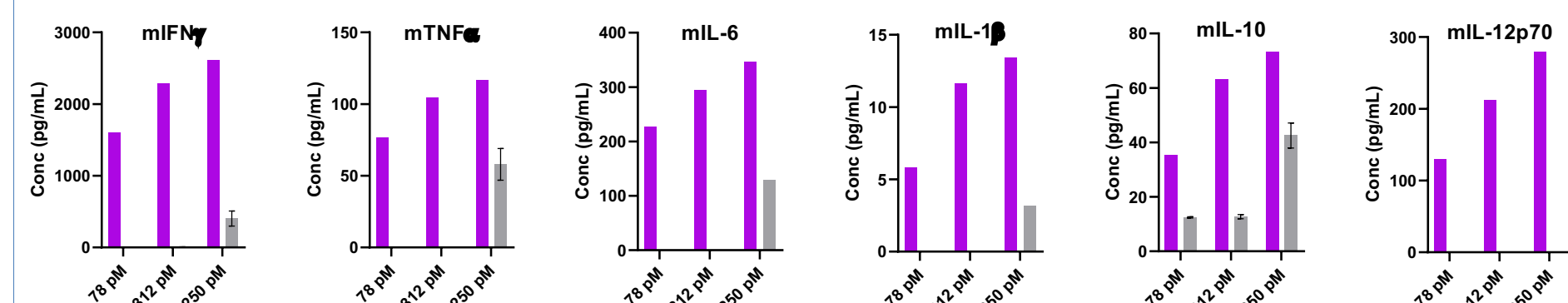
- BLINCYTO® induces cytokine release and body temperature drop in a dose dependent manner

#### 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

#### 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



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