Alternate Methods for Immunogenicity Assessment of Biosimilar Drug Products

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BsUFA III Regulatory Science Pilot Program: Progress Update – January 22, 2025

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Immunogenicity Assessment

- Immunogenicity is the ability of a substance to induce immune responses
- Reference products are evaluated for the overall level and effect(s) of immunogenicity
- Biosimilar immunogenicity assessment ensures the biosimilar is not significantly different from the reference product

BsUFA Research Goals

- Evaluate/develop alternatives to human clinical trials for evaluation of immunogenicity
- *In vitro* immunogenicity assays
  - Literature review and biosimilar application mining
- *In vivo* immunogenicity assessment
  - Can a humanized mouse produce immunogenicity to biological drug products?

Biosimilar Application Mining

- Determine if sponsors are submitting results from *in vitro* assays with their applications
- If they are:
  - What assay types are submitted?
  - Do the assays, as submitted, have interpretable data?
  - How do submitted assays compare to what is published in the literature?
  - Are the results consistent with clinical trial results?

Data Mining Results

- A total of 64 biosimilar applications were reviewed for 12 total reference products
- A wide range of assays were submitted including proliferation, DC:T-cell assay, ELISpot, mixed lymphocyte reaction (MLR), and cytokine release assays
- Many different cell types/cell lines used
- Some included adequate methodology to interpret data; some had no methods listed making interpretation difficult
- Wide range of assay parameters/protocols; in general, there was no consistency in how assays were run, number of donors used, inclusion of donor HLA-typing, and assay endpoints between sponsors
- Many more assays in published literature as compared to number included in 351k applications

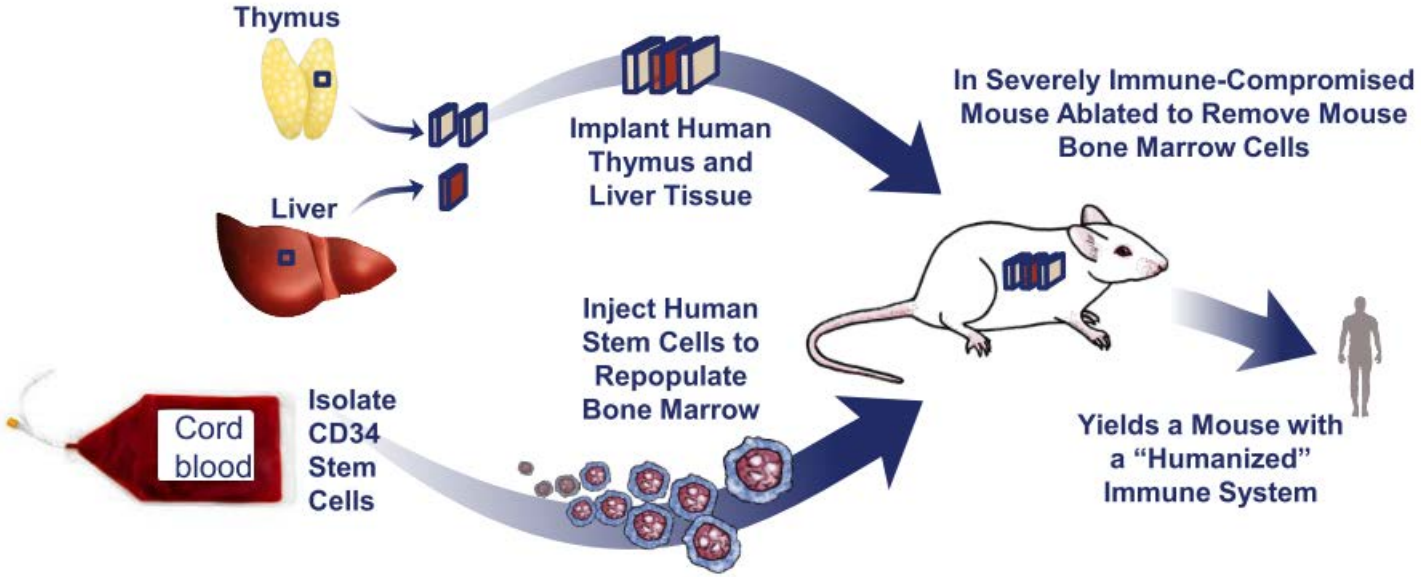
Data Mining Summary

- In vitro immunogenicity assessment is being conducted by sponsors
- Not always included in applications
- Great variability in assays used and methods
- At present, difficult to interpret and draw meaningful conclusions related to clinical immunogenicity

In vivo studies

- Current biosimilar guidance indicates animal studies are not required
- In part, due to lack of usefulness for most animal models because human biologics would be seen as ‘foreign’ by the host species
- Goal was to determine if mice with a human immune system could demonstrate immunogenicity to biological drug products

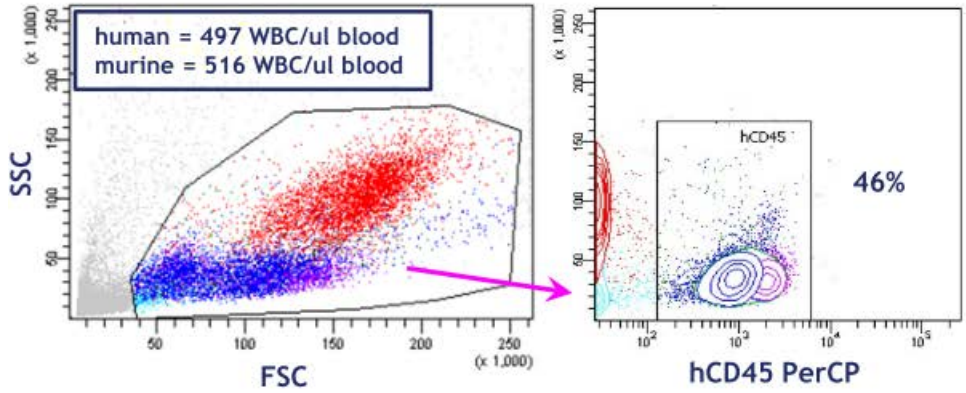
Bone Marrow-Liver-Thymus (BLT) Immune Humanized Mice

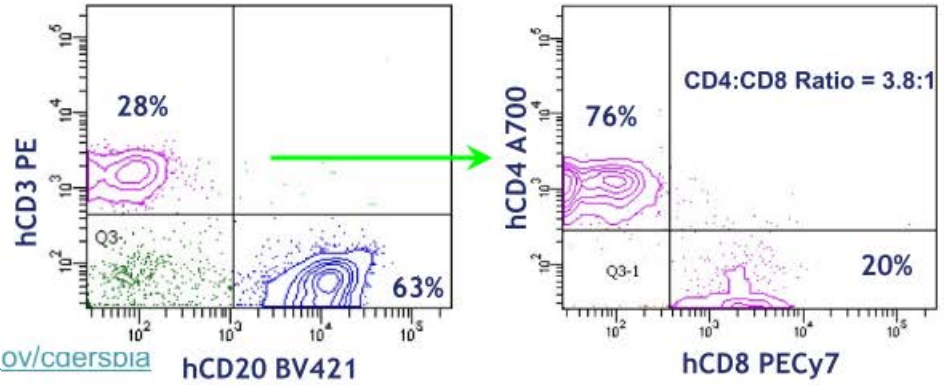


- Available human cell types: T cells, B cells, Monocytes, NK cells, Tregs, pDC, mDC
- Presence of matching human thymus and hematopoietic stem cells allows T:B cell interaction

Humanization of Blood and Thymus

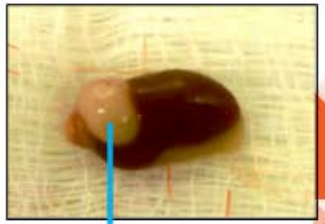
PBMC



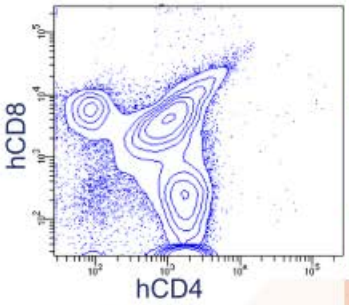


CD45: pan-WBC
CD3: All T-cells
CD20: Mature B-cells
CD4: helper T-cells
CD8: cytotoxic T-cells

Thymic organoid



Typical range of human CD4:CD8 ratio = 1.0 – 4.0



Study Design

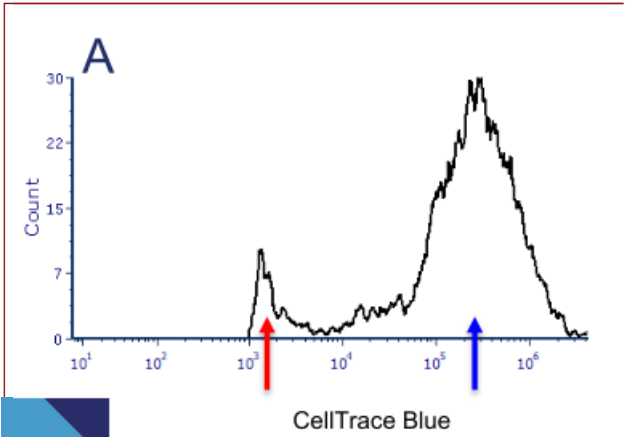
- BLT- or CD34-humanized mice were treated with either saline, KLH, infliximab, interferon-β, or a combination of two biologics
  - Study duration = 9 weeks
  - At study end peripheral lymph node and spleen were collected and processed to obtain cells
  - Lineage phenotype and functional assays were performed with freshly isolated cells

T-cell Function Assays

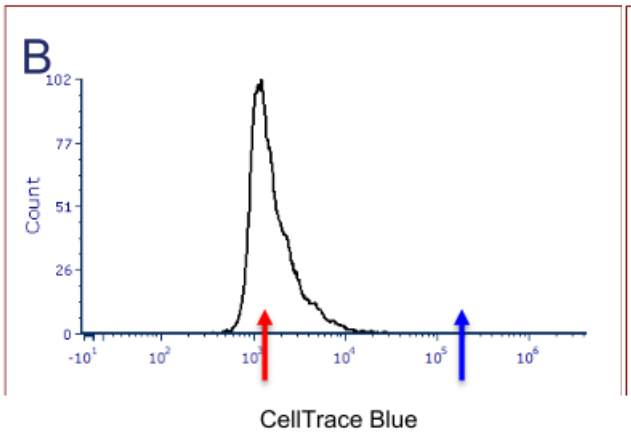
- Proliferation: cells are stained with a nuclear dye such as CFSE or CellTrace dyes, then restimulated in vitro for approximately 72 hours
  - Loss of dye indicates cell division, i.e. stimulation
- Intracellular cytokines: cells are stimulated *ex vivo* with antigens they were exposed to *in vivo,* with monensin (or brefeldin) added after one hour of culture; total culture is 5-6 hours
  - Cells are washed and stained for surface receptors, then fixed and permeabilized, and stained for intracellular proteins

Proliferation of LN cells

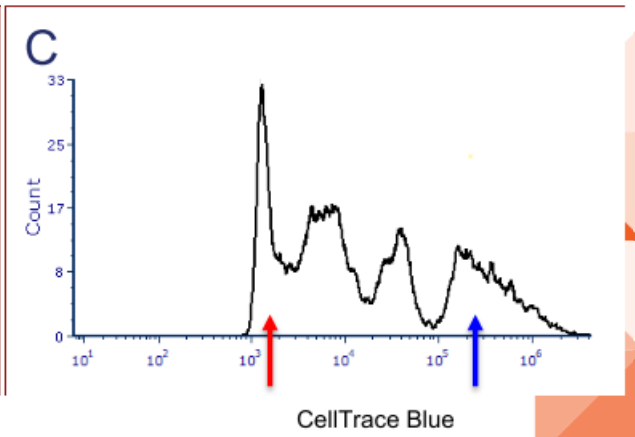
Stained, no stimulation



ConA stimulation



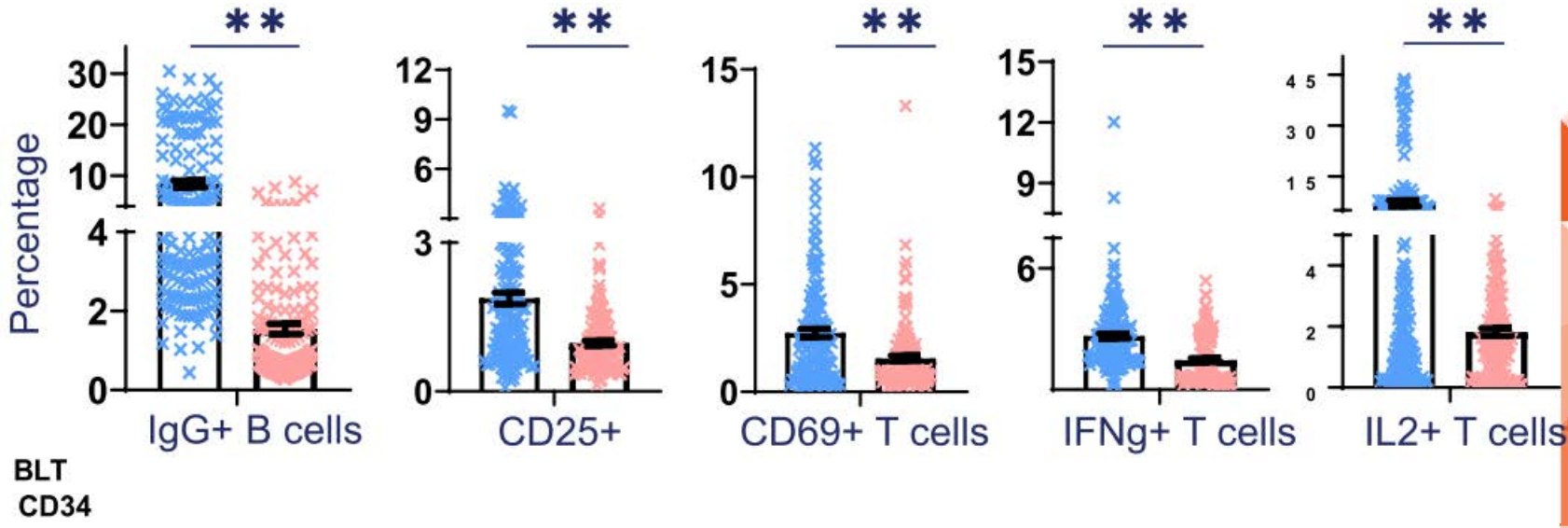
Infliximab stimulation



- Red arrows indicate cells that have divided; blue arrows indicate cells that have not divided
- Mitogen stimulation shows all cells dividing in 72 hours; no stimulation shows very few have divided
- Stimulation with the biologic infliximab shows significant division of cells in 72 hours

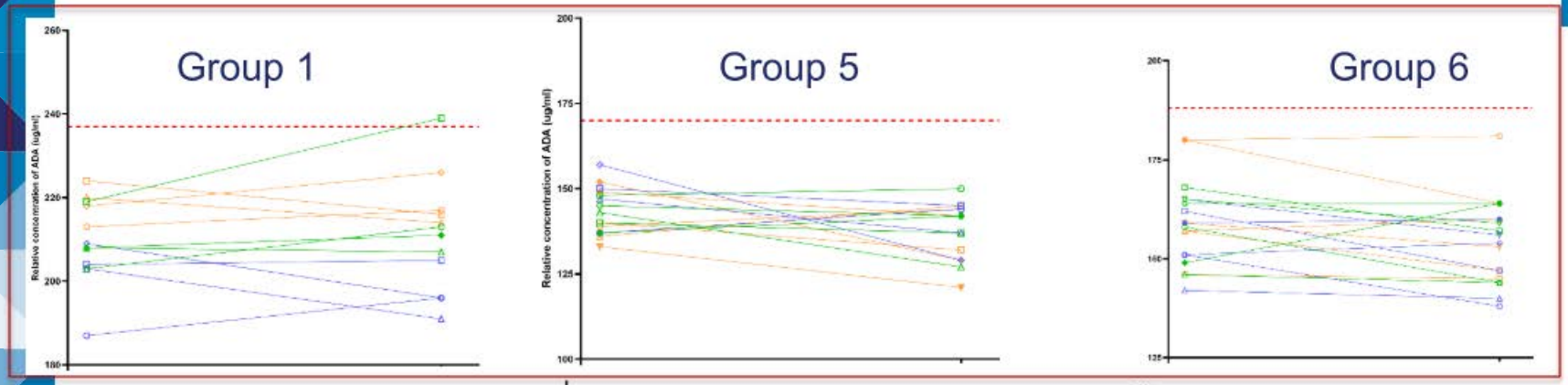
➢ Lymph node cells are capable of functionally responding to stimulation *ex vivo*

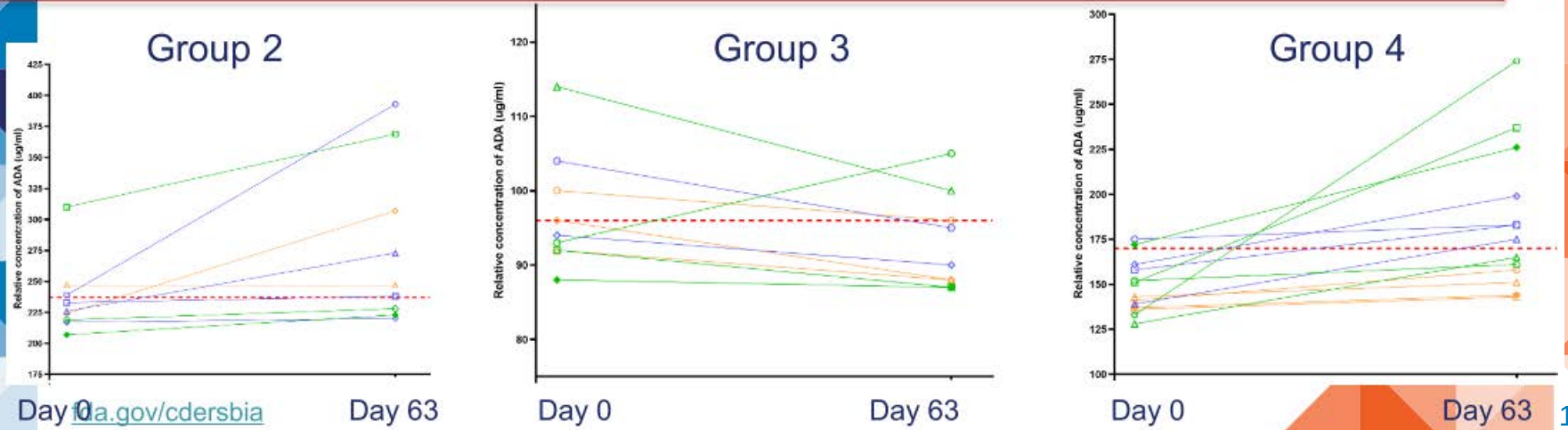
Comparative LN Activation



- Significant increases in all activation markers present for BLT versus CD34 mice

Anti-drug antibodies to IFNb





Summary

- Humanized mouse model makes a difference
- Those produced with human thymus can make measurable, functional immune responses
- BLT-humanized mice can make ADAs to biological drug products
- Model has potential to inform immunogenicity

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Questions?

Please submit your questions

If you have questions after the webinar, please contact me directly:

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Closing Thought

- Consider what *in vitro* immunogenicity assessments your organization conducts
- When submitting them in an application, please include detail of methodology used
- If your organization conducts *in vitro* assays, but do not currently submit; please consider submitting them


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