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Alternate Methods for Immunogenicity Assessment of Biosimilar Drug Products

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This presentation reflects the views of the presenter and should not be construed to represent those of the FDA.

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

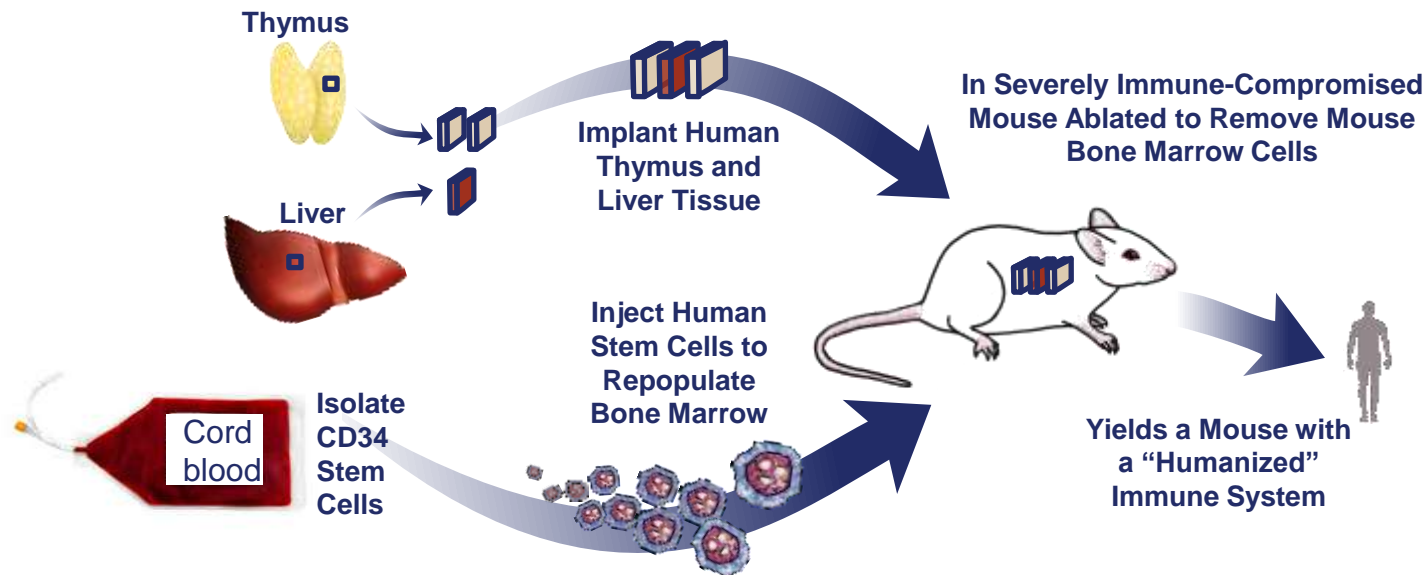


- In vitro immunogenicity assessment is being conducted by sponsors
- Not always included in applications
- Great variability in assays used and methods
- At present, difficult to interpret and draw meaningful conclusions related to clinical immunogenicity

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

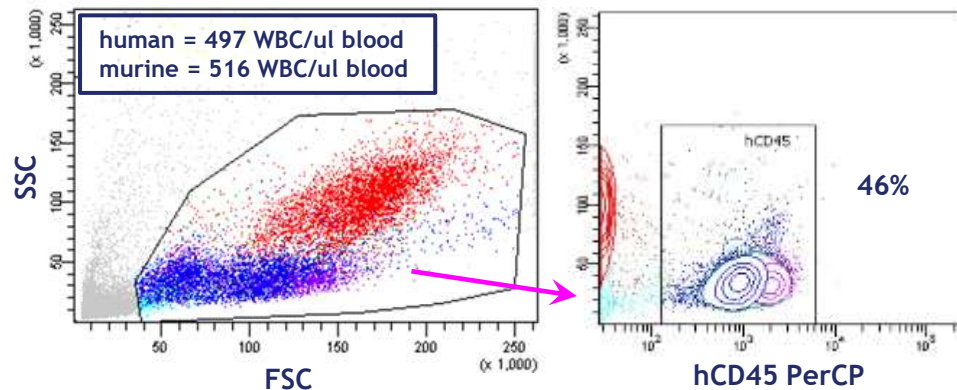


- Available human cell types: T cells, B cells, Monocytes, NK cells, Tregs, pDC, mDC
- Presence of matching human thymus and hematopoietic stem cells allows T:B cell interaction

Humanization of Blood and Thymus

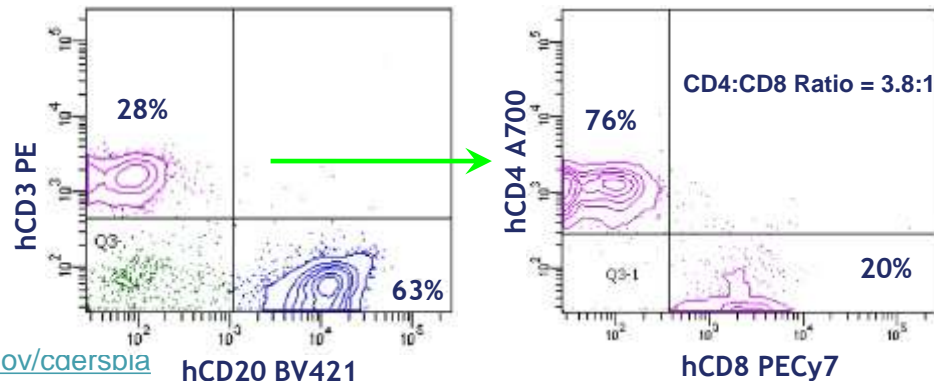


PBMC

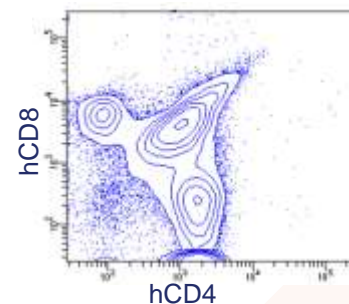


CD45: pan-WBC
CD3: All T-cells
CD20: Mature B-cells
CD4: helper T-cells
CD8: cytotoxic T-cells

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

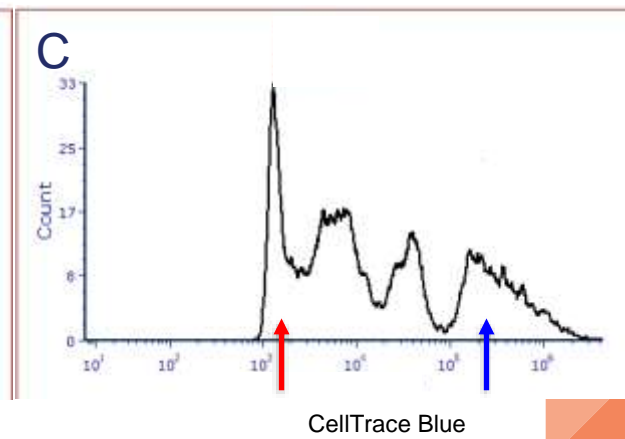
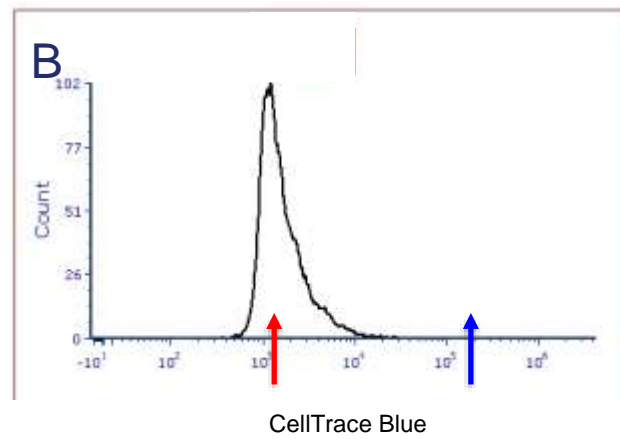
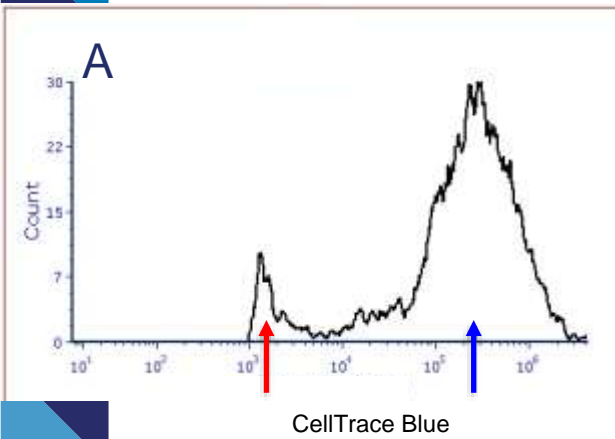
Proliferation of LN cells



Stained, no stimulation

ConA stimulation

Infliximab stimulation

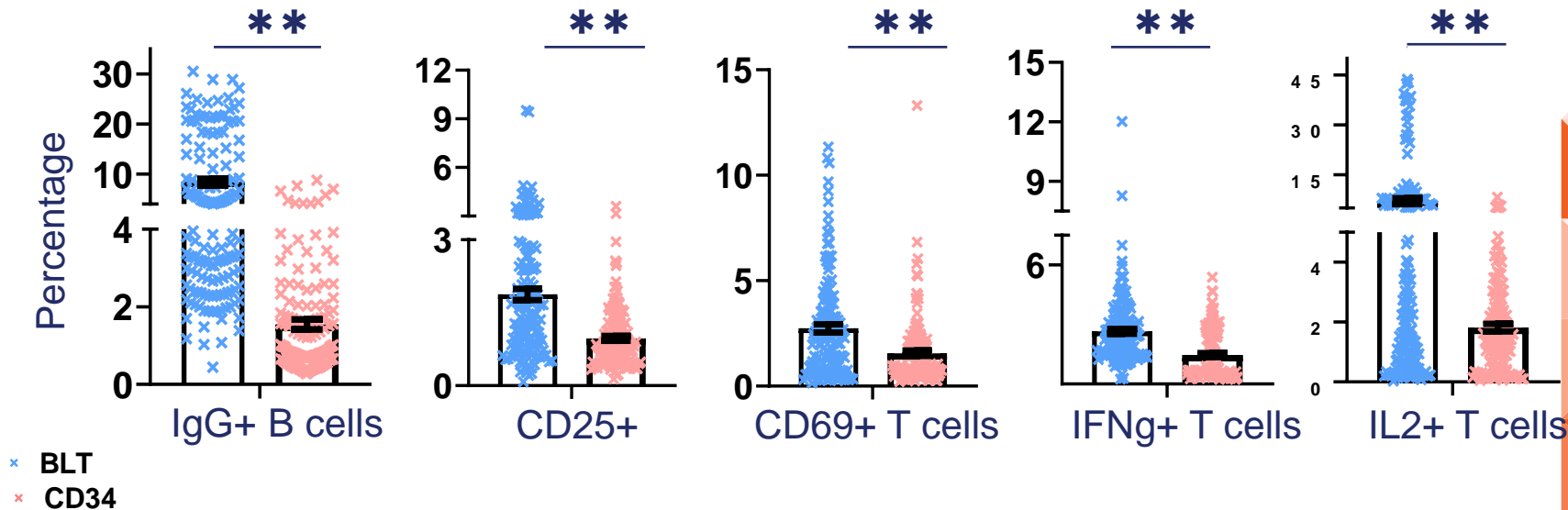


- Red arrows indicate cells that have divided; blue arrows indicate cells that have not divided
- Mitogen stimulation shows all cells dividing in 72 hours; no stimulation shows very few have divided
- Stimulation with the biologic infliximab shows significant division of cells in 72 hours

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

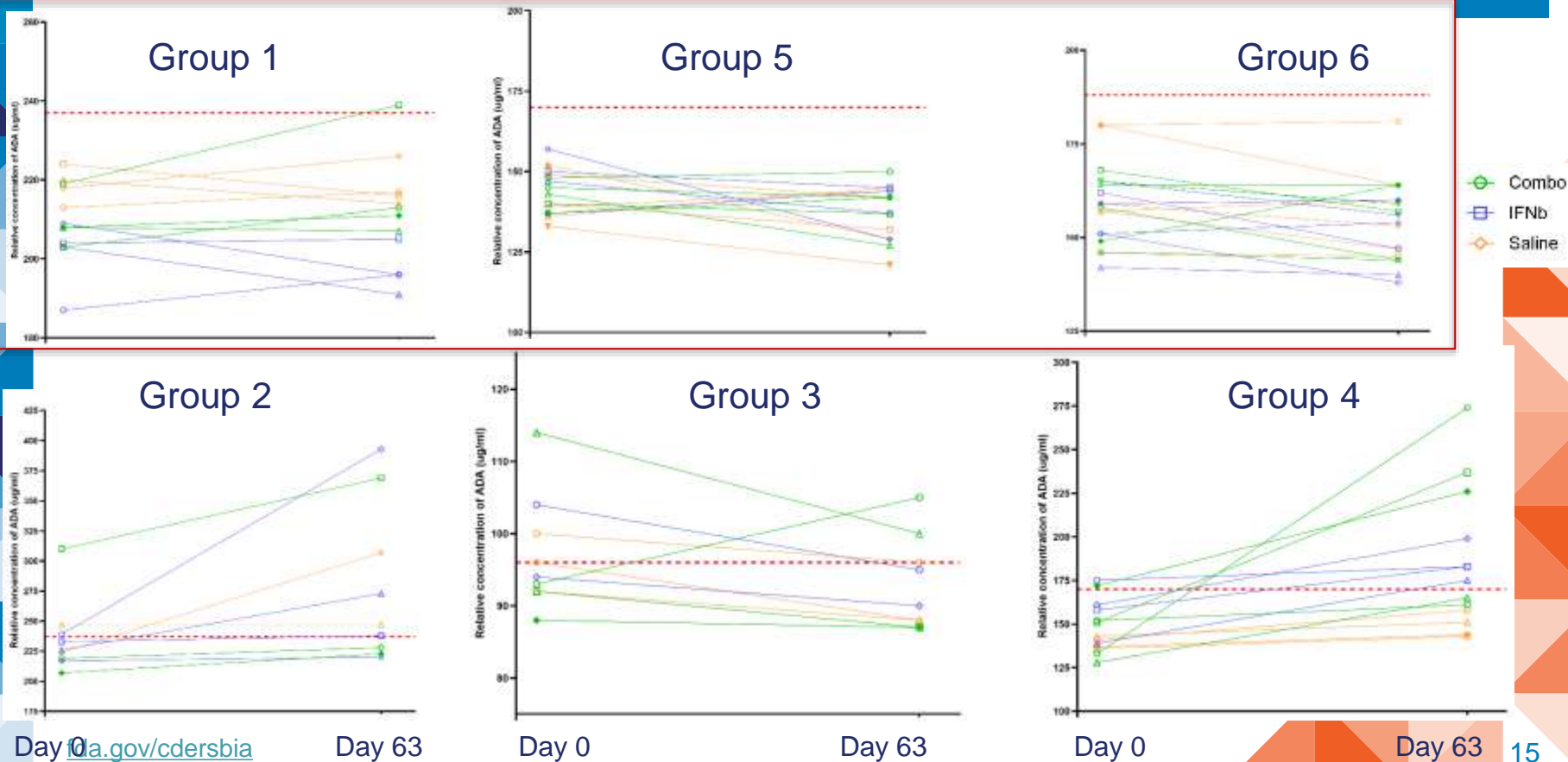
[fda.gov/cdersbia](https://www.fda.gov/cdersbia)

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

