

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

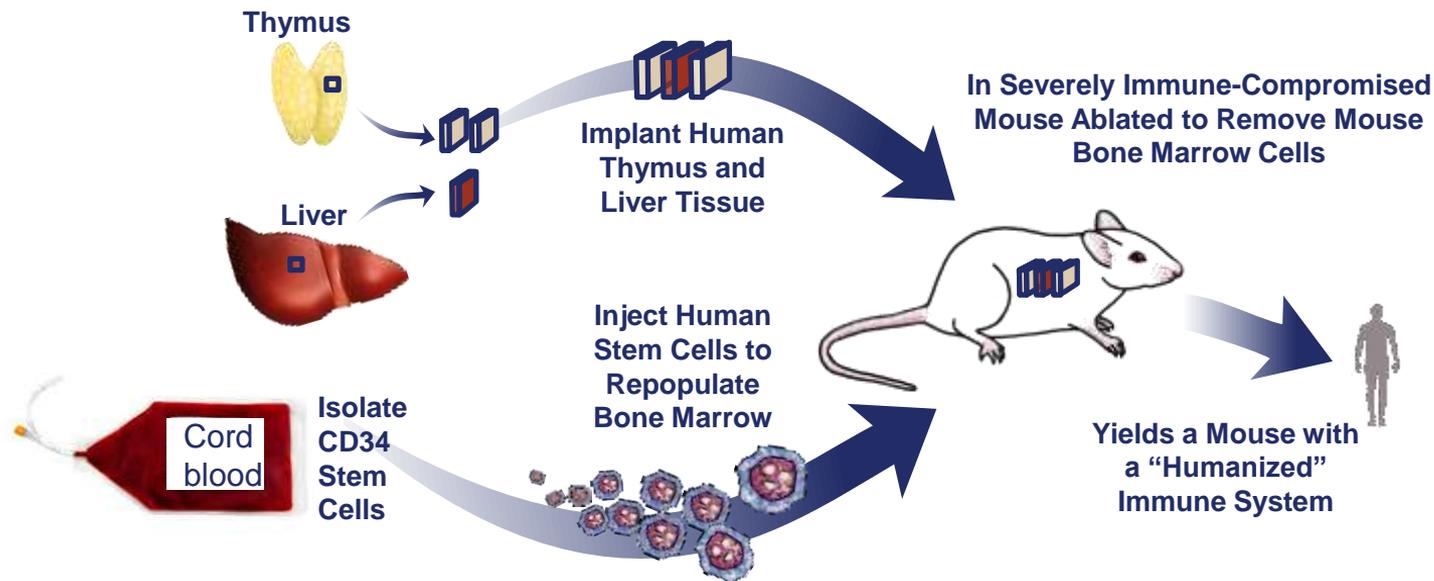
Data Mining Summary

- 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

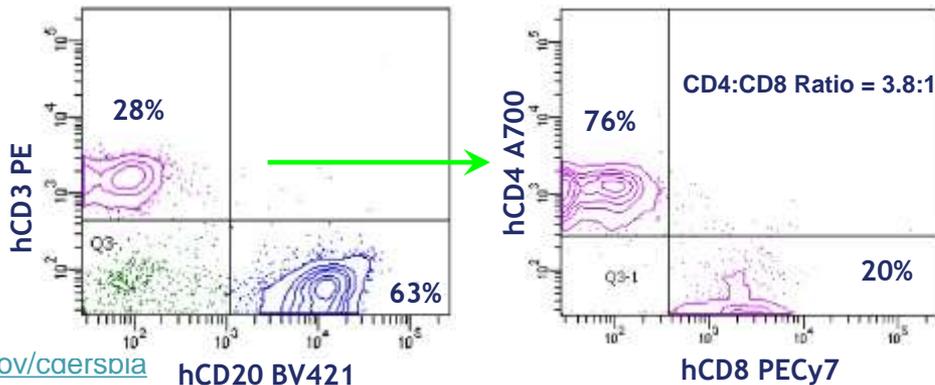
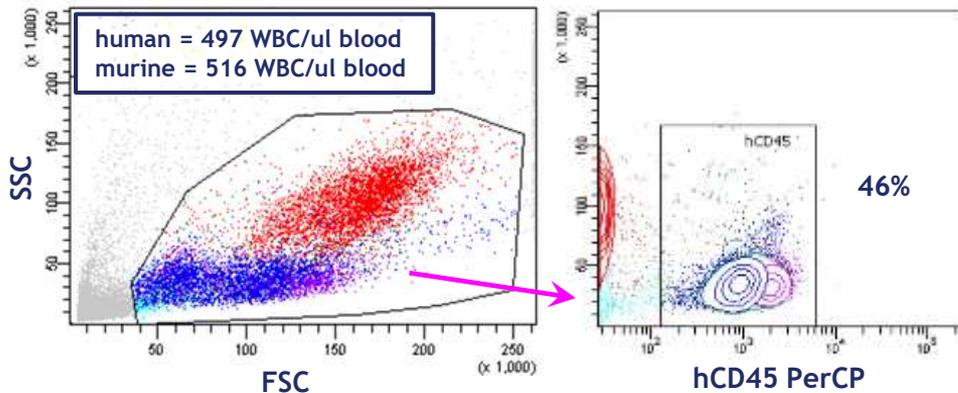


- Available human cell types: T cells, B cells, Monocytes, NK cells, Tregs, pDC, mDC
- Presence of matching human thymus and hematopoietic stem cells allows T:B cell interaction

Humanization of Blood and Thymus



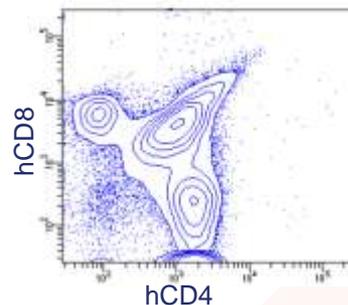
PBMC



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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

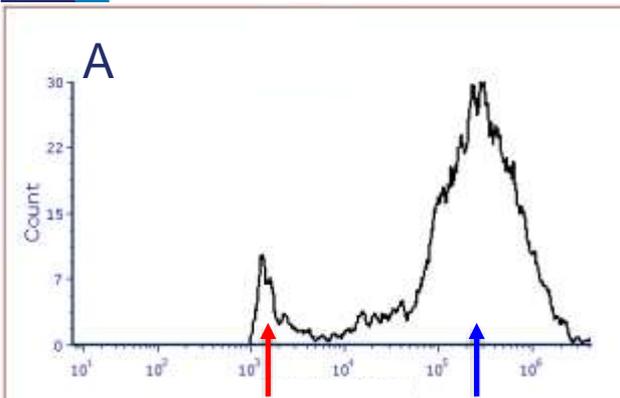
Proliferation of LN cells



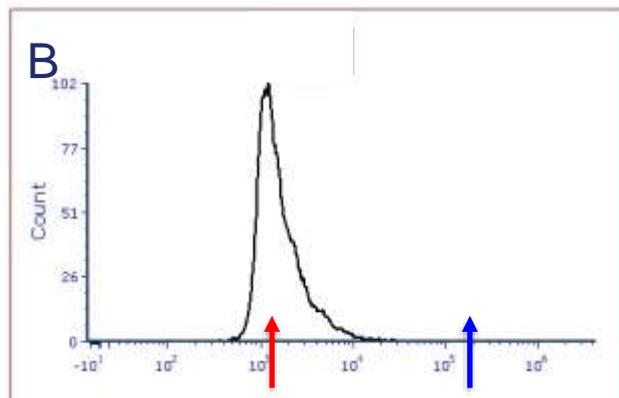
Stained, no stimulation

ConA stimulation

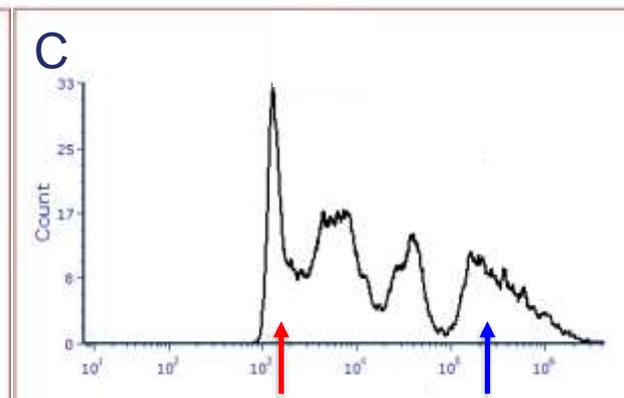
Infliximab stimulation



CellTrace Blue



CellTrace Blue



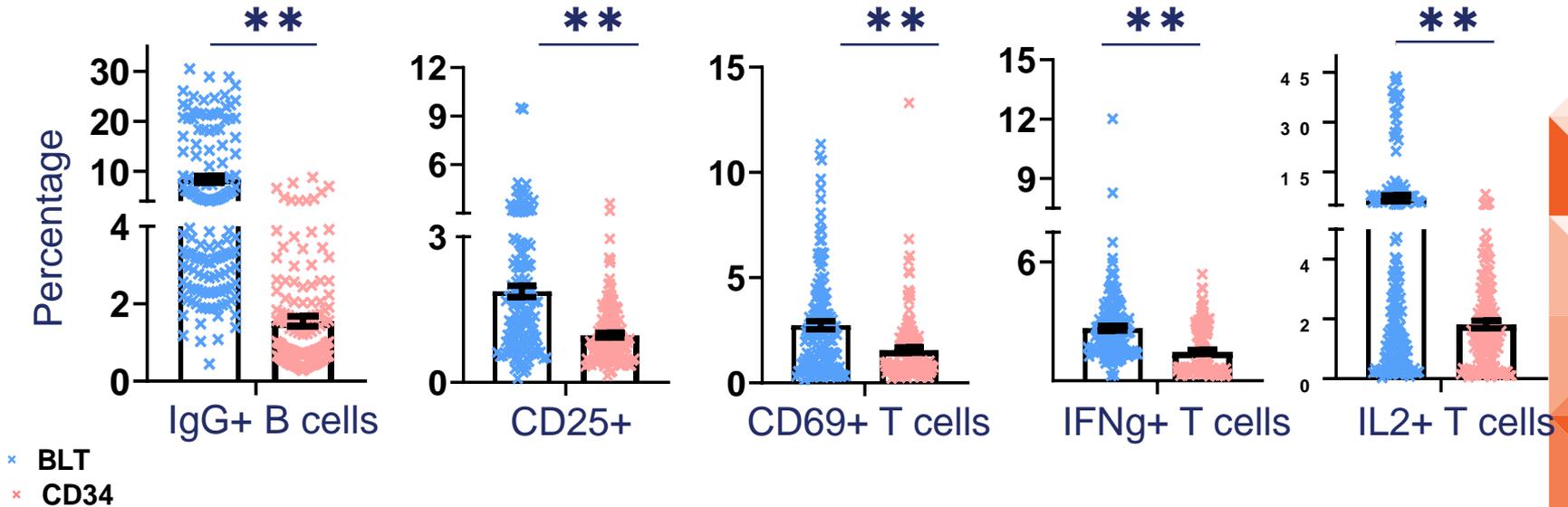
CellTrace Blue

- 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*

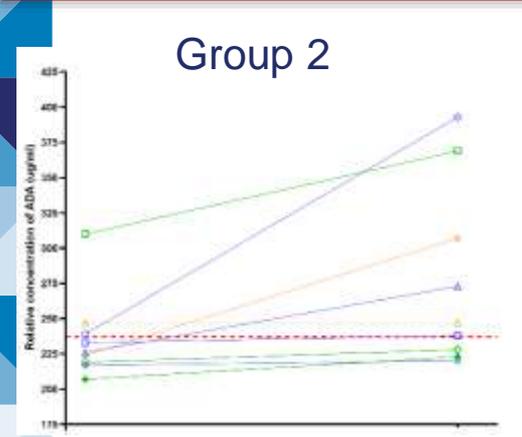
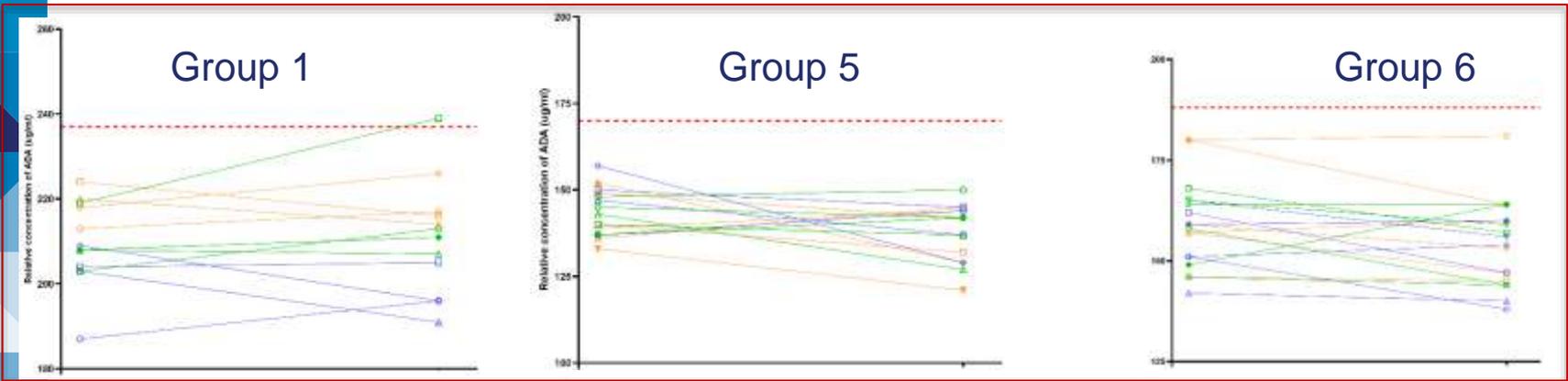
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Comparative LN Activation

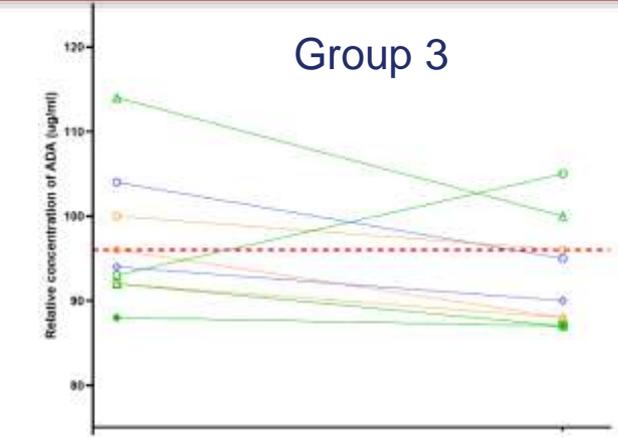


➤ Significant increases in all activation markers present for BLT versus CD34 mice

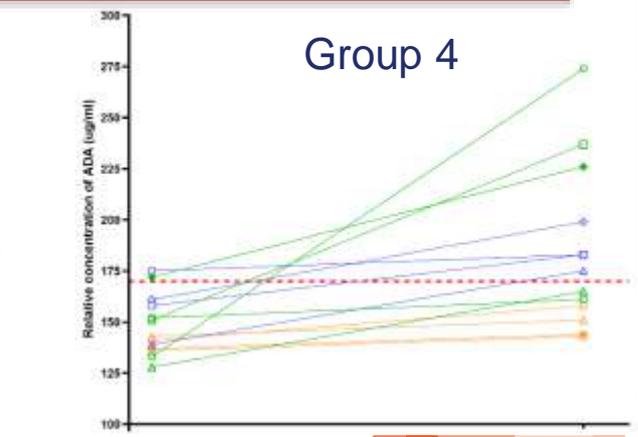
Anti-drug antibodies to IFN β



Day 0 www.fda.gov/cdersbia Day 63



Day 0 Day 63



Day 0 Day 63

- Combo
- IFN β
- Saline

Summary



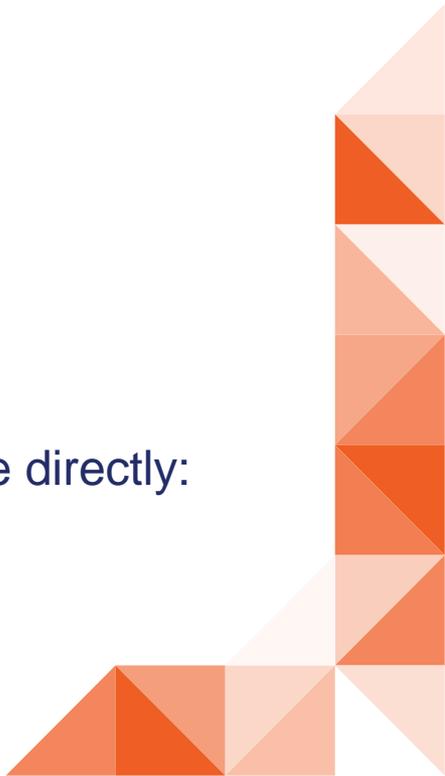
- Humanized mouse model makes a difference
- Those produced with human thymus can make measurable, functional immune responses
- BLT-humanized mice can make ADAs to biological drug products
- Model has potential to inform immunogenicity

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

