European Immunogenicity Platform

Immunogenicity of Biopharmaceuticals

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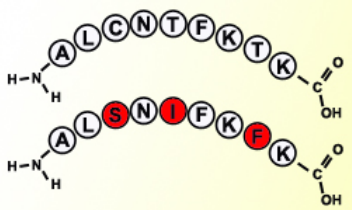
Factors Affecting the Immune Response

Modified from W. Jiskoot



Sequence Variation

Formulation

UNKNOWN

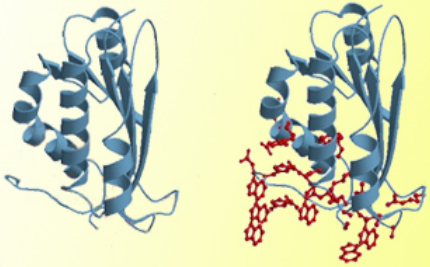
?

Application route

Patient Features

Length of Treatment

Product modification



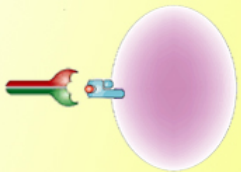
IMMUNOGENICITY

Nature of Disease

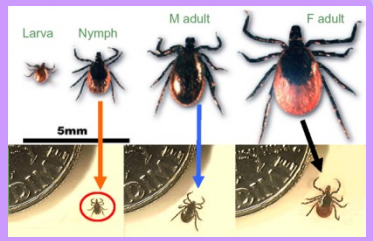
Contaminants and Impurities

Dose

Biological Activity



Concomitant Medications



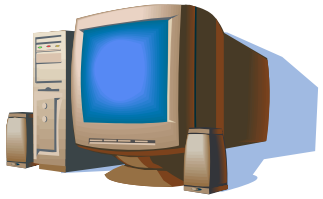
PRODUCT RELATED

Environmental

TREATMENT RELATED

Current Immunogenicity Prediction

In silico



Predicts the presence of a CD4 T cell epitope

In vitro

Provides evidence (proliferation/cytokine production) that the cognate epitope specific CD4 T cells are present

Neither

Predicts if there is an orthologous B cell epitope or anything about the myriad of other factors that impact immunogenicity

Current Immunogenicity Prediction

In vivo veritas

However

- **Animals recognize human sequences as “foreign” thus current models are not predictive of the human immune response**
- **However, animal studies may have comparative utility**
- **Only clinical studies accurately reflect immunogenicity**
- **New models?**

Something Closer to the Truth…?

The Humanized Mouse Model

Adapted from A. Rosenberg

Humanized Mouse Models

A. Transgenic Models
   1. Conventional
   2. Knock-In
   3. BAC/YAC
B. Hematopoietic Systems
   1. Hu-PBL-SCID
   2. Hu-HSC-SCID
   3. Hu-BLT-SCID
C. Limitations and Potential Solutions

Humanized Mouse Models

Transgenic Systems (1)

A transgenic (tg) animals' immune system is <u>tolerant</u> to the human transgene product

However:

- **Responses are influenced by the mouse strain**
- **Immune recognition and responses are murine, not human**
- **Not predictive, but a useful tool for testing product attributes**

- **Three Basic Variations of the Transgenic System**
  - **Conventional**
  - **Knock-In**
  - **BAC/YAC**

Humanized Mouse Models

Transgenic Systems (2)

Conventional Transgenics

- Murine homolog usually present
- Gene expression is controlled by tg P/E
- may be one or many copies of the gene
- the same gene copy number may ≠ same amount of protein
  - this may impact the level of tolerance

Knock-In Transgenics

- Murine homolog deleted/inactivated
- May be homozygous or hemizygous
- Gene expression is controlled by P/E of murine homolog
- Usually reflects mu gene expression

Humanized Mouse Models

Transgenic Systems (3)

BAC/YAC Transgenic

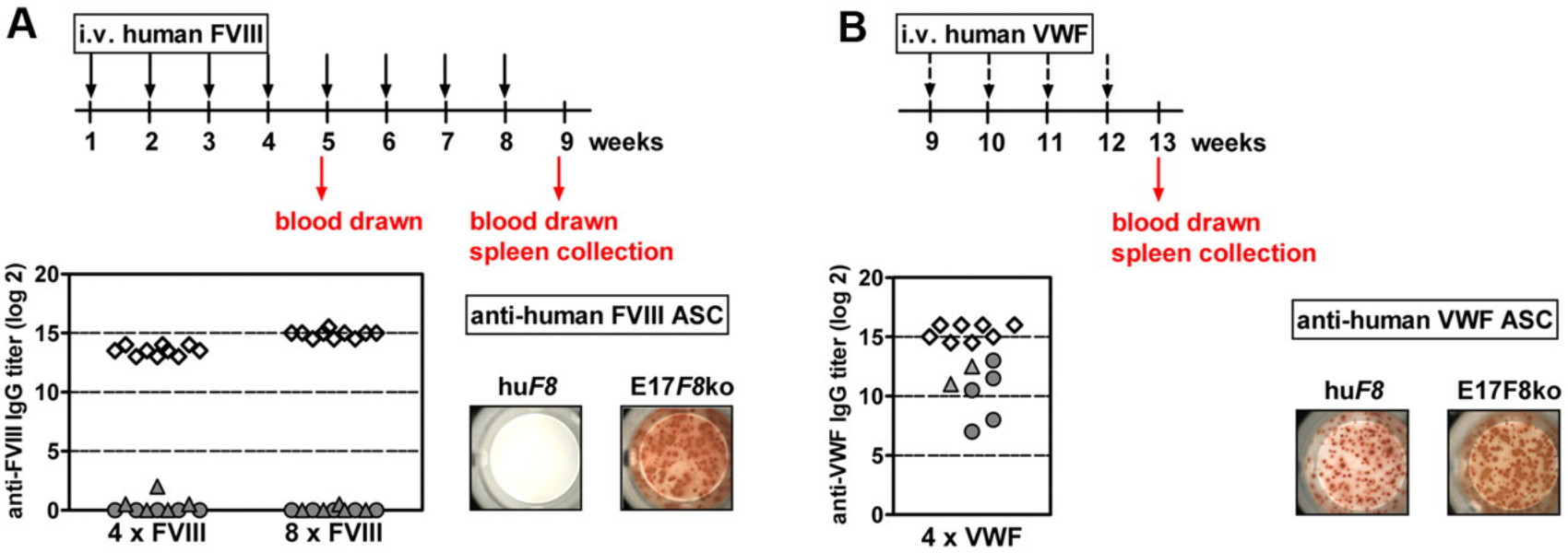
Instead of a single gene, a large chromosomal segment(s) is transferred

- **Murine homolog usually present (may also be deleted)**
- **Usually gene expression is controlled by native human P/E**
- **Usually has one copy of the gene**
- **More likely to reflect human pattern of gene expression**

Humanized Mouse Models

FVIII Transgenic Mouse

exhibit Ag specific tolerance

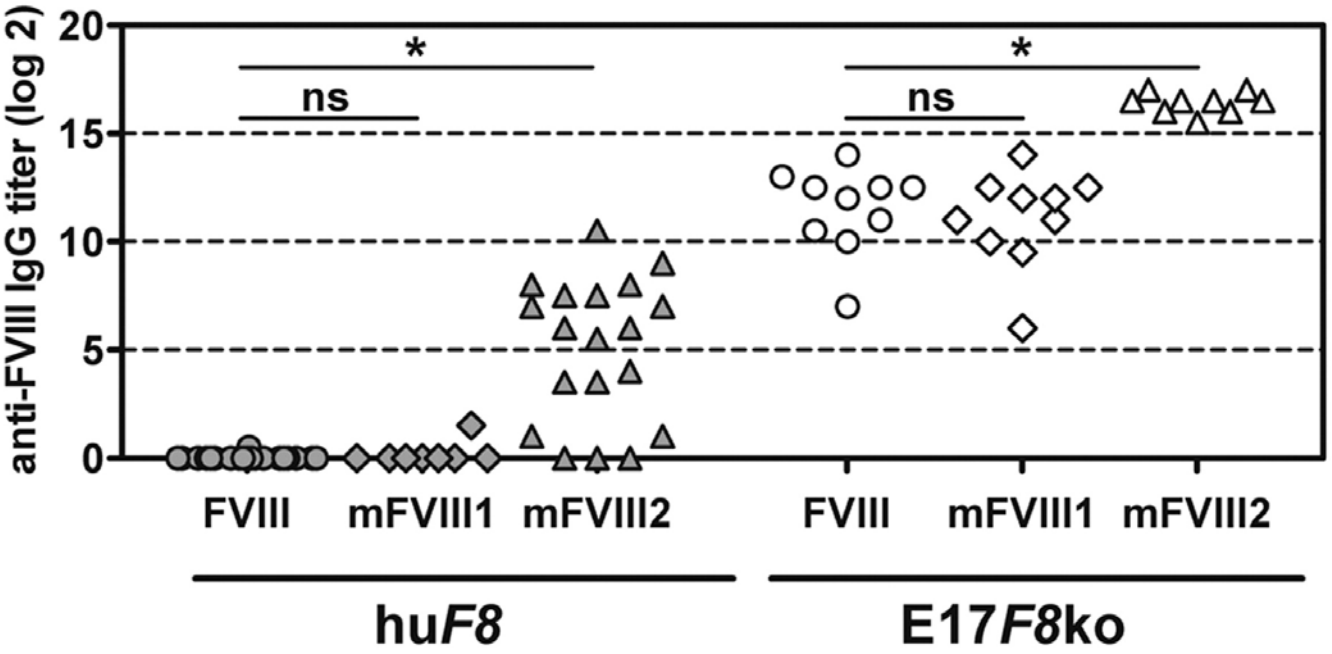


Pauline M. van Helden et al. Blood 2011;118:3698-3707

Humanized Mouse Models

FVIII Transgenic Mouse

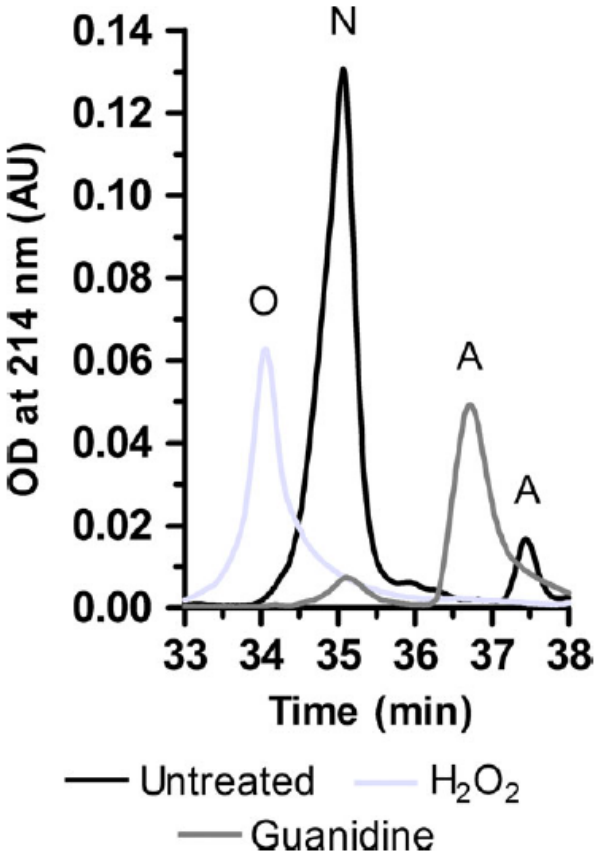
modified FVIII breaks tolerance

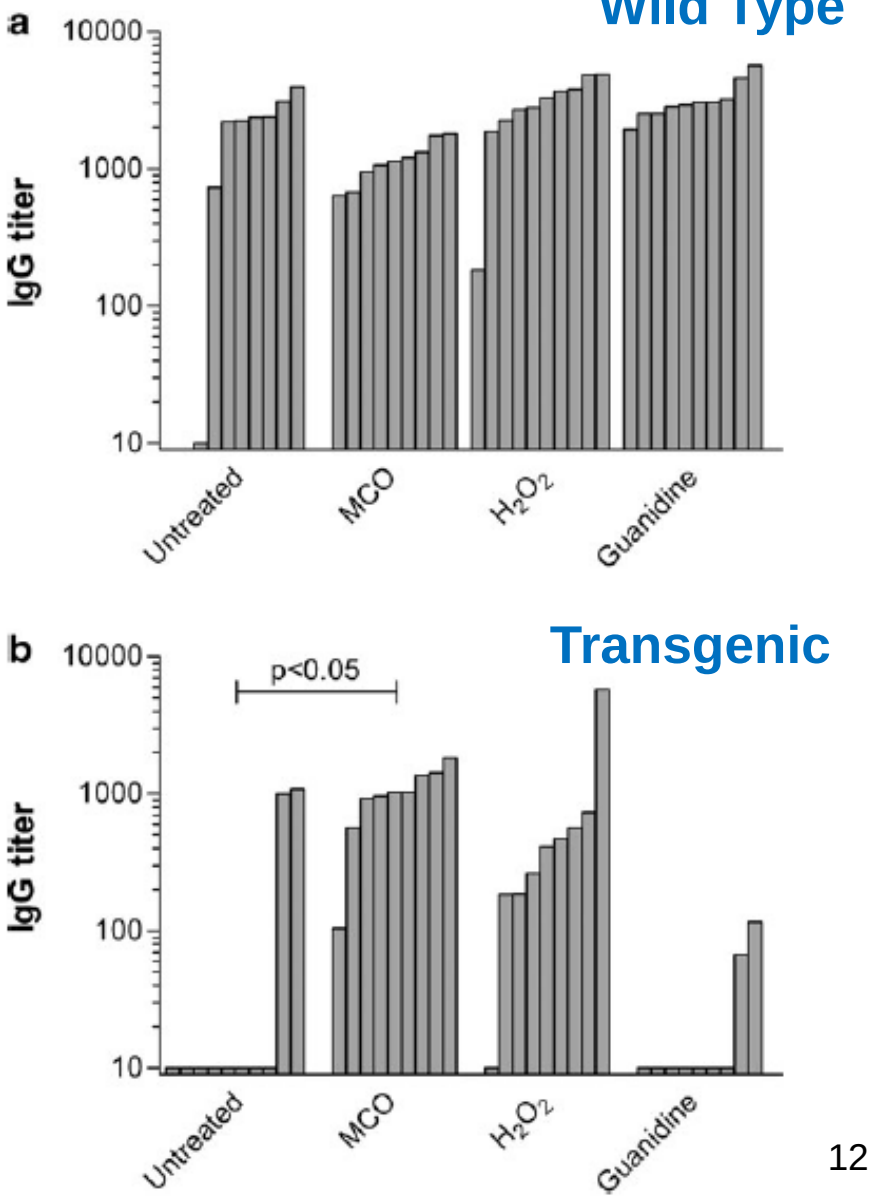


Pauline M. van Helden et al. Blood 2011;118:3698-3707

Advancing Humanized Mouse Models for Preclinical Testing

Stressed Protein Breaks Tolerance in IFN-β tg Mice



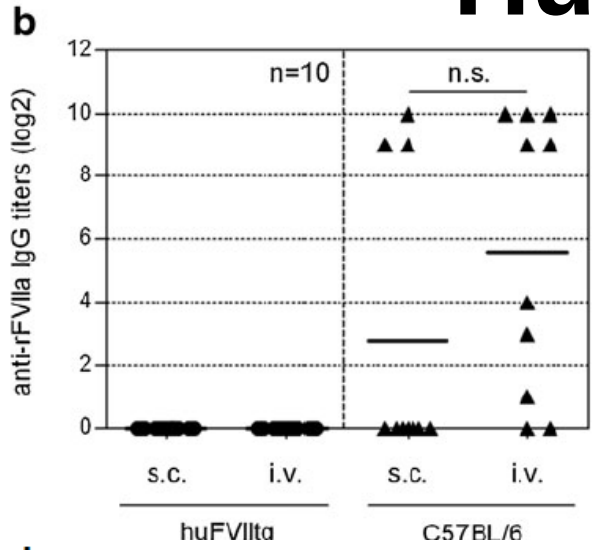


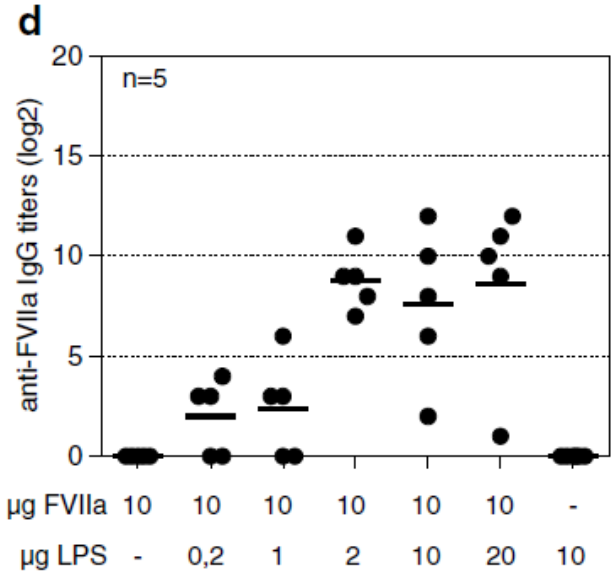
M. van Beers et al. Pharm Res (2011) 28:2393–2402

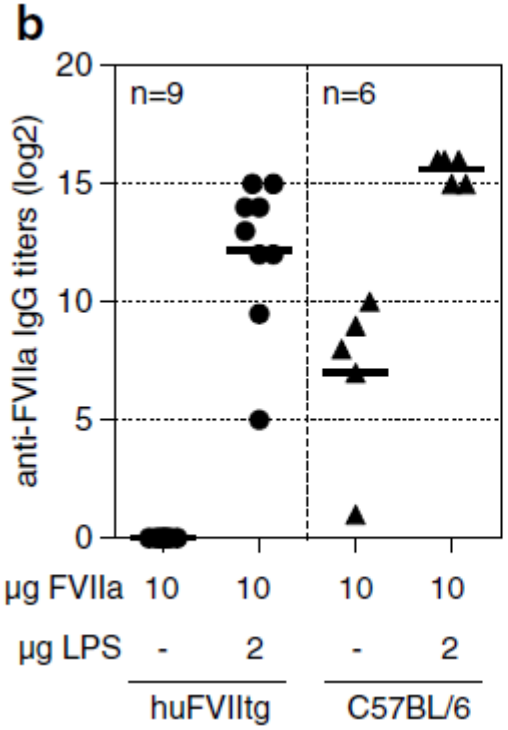
Humanized Mouse Models

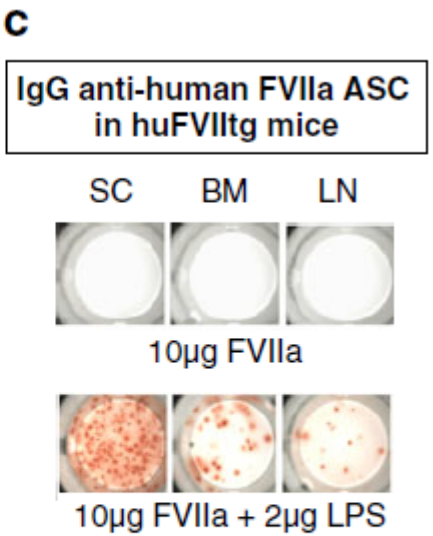
FVIIa Transgenic Mouse

PAMPs Break Tolerance









Christine Lenk et al. Pharm Res (2013) 30:2855–2867

Humanized Mouse Models
Hematopoietic Systems

- A mouse reconstituted with human hematopoietic cells (and tissues)
- Confers ability to make human-specific immune responses to pharmaceuticals and/or infectious agents
- Cannot be made genetically (e.g. knock-in)

Humanized Mouse Models

Hematopoietic Systems

Mice with a Human Immune System

Requirements:

- Immune deficient mouse that will not reject implanted/transplanted tissues and/or cells
- Human cells and/or tissues, e.g.
  - Hematopoietic stem cells (HSC)
    - Fetal liver
    - Cord blood
    - Peripheral blood
    - Bone Marrow
  - Fetal thymus/liver

Human Hematopoietic Mouse Models

- **Hu-PBL-SCID: *scid* mice injected with human peripheral blood mononuclear cells (PBMC)**
- **Hu-HSC-SCID: *scid* mice that have been sublethally irradiated and injected with CD34+ hematopoietic stem cells (HSC)**
- **Hu-BLT-SCID: Hu-HSC-SCID mice that have also been engrafted with autologous human thymus**

•Adapted from LD Shultz, et.al., 2007. *Nat. Rev. Immunol.*7:118

Human Hematopoietic Mouse Models

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Human Hematopoietic Mouse Models

Hu-HSC-SCID

- Hu T cell development in the mouse thymus
  - Immune recognition is mouse (H2), not human, and suboptimal
  - No GVHD (predicted)
  - However, hu T cells would recognize non-hematopoietic human proteins as “foreign”
  - Capable of H2 restricted effector responses (e.g cytokines) that are human

Human Hematopoietic Mouse Models

- Hu-PBL-SCID: *scid* mice injected with human peripheral blood mononuclear cells (PBMC)
- Hu-HSC-SCID: *scid* mice that have been sublethally irradiated and injected with CD34+ hematopoietic stem cells (HSC)
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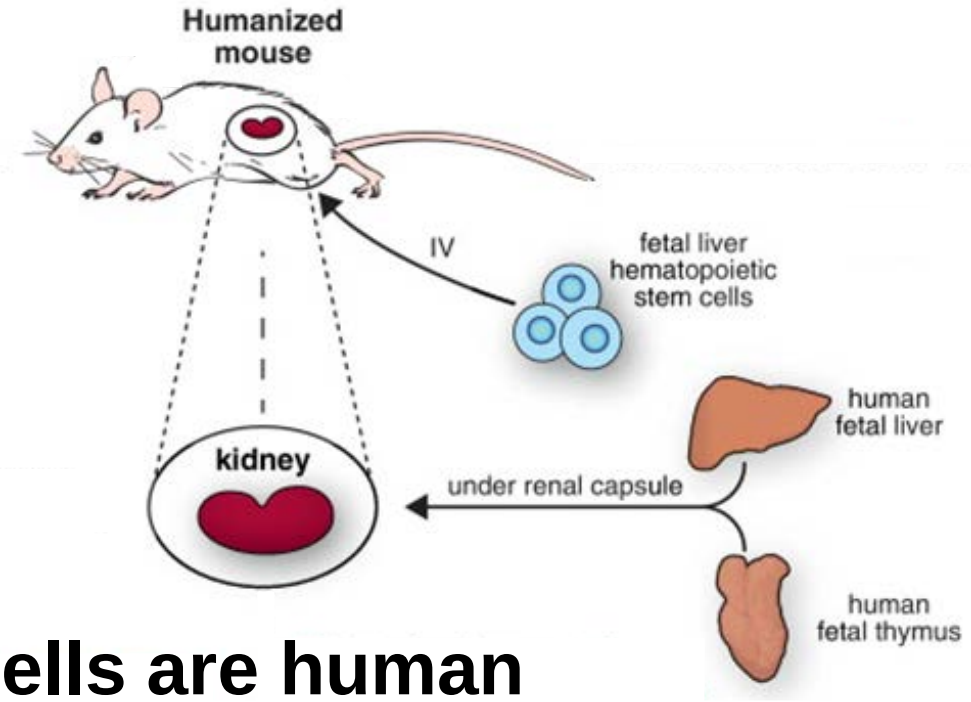
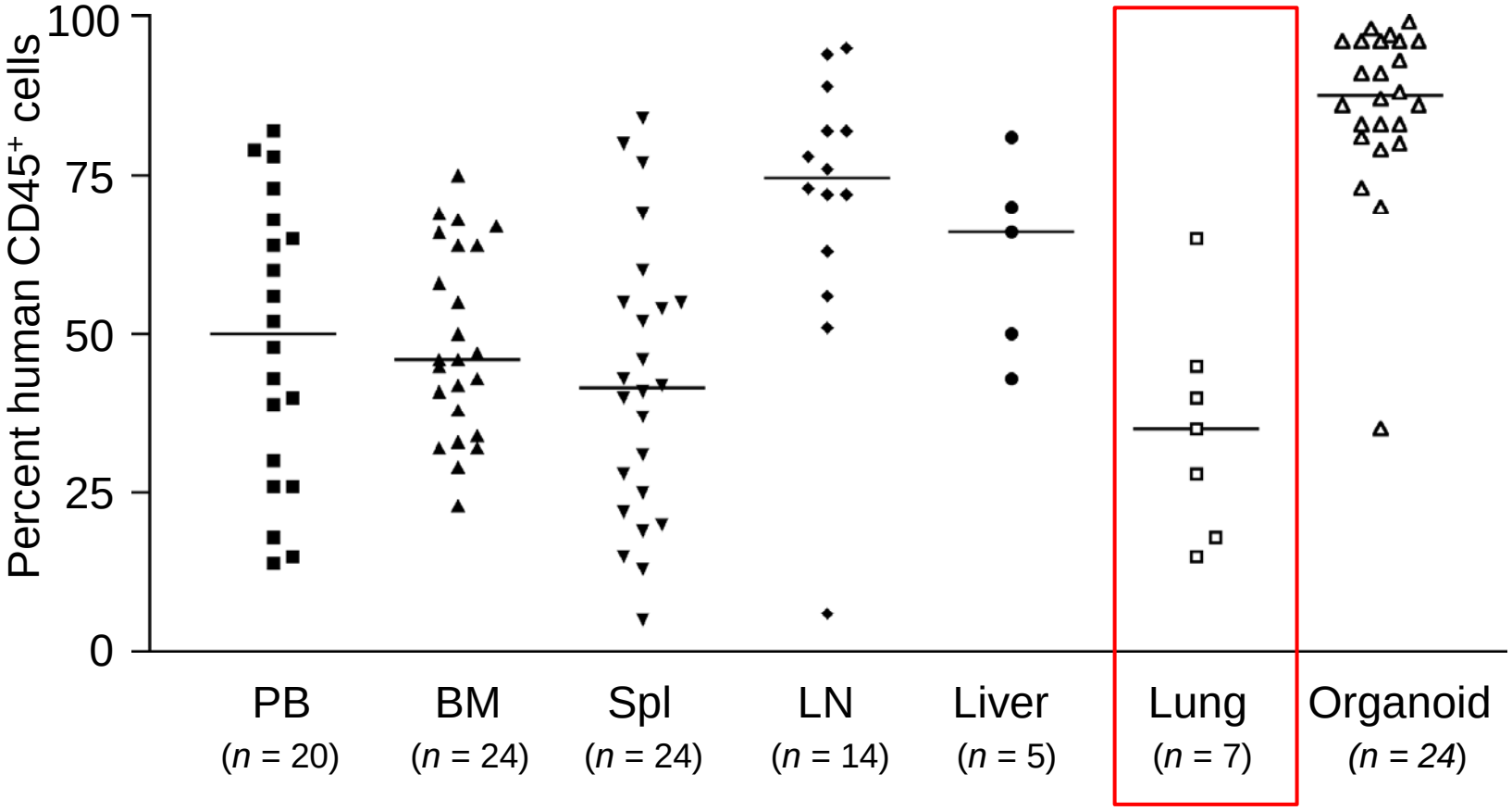
Humanized Mouse Models: BLT



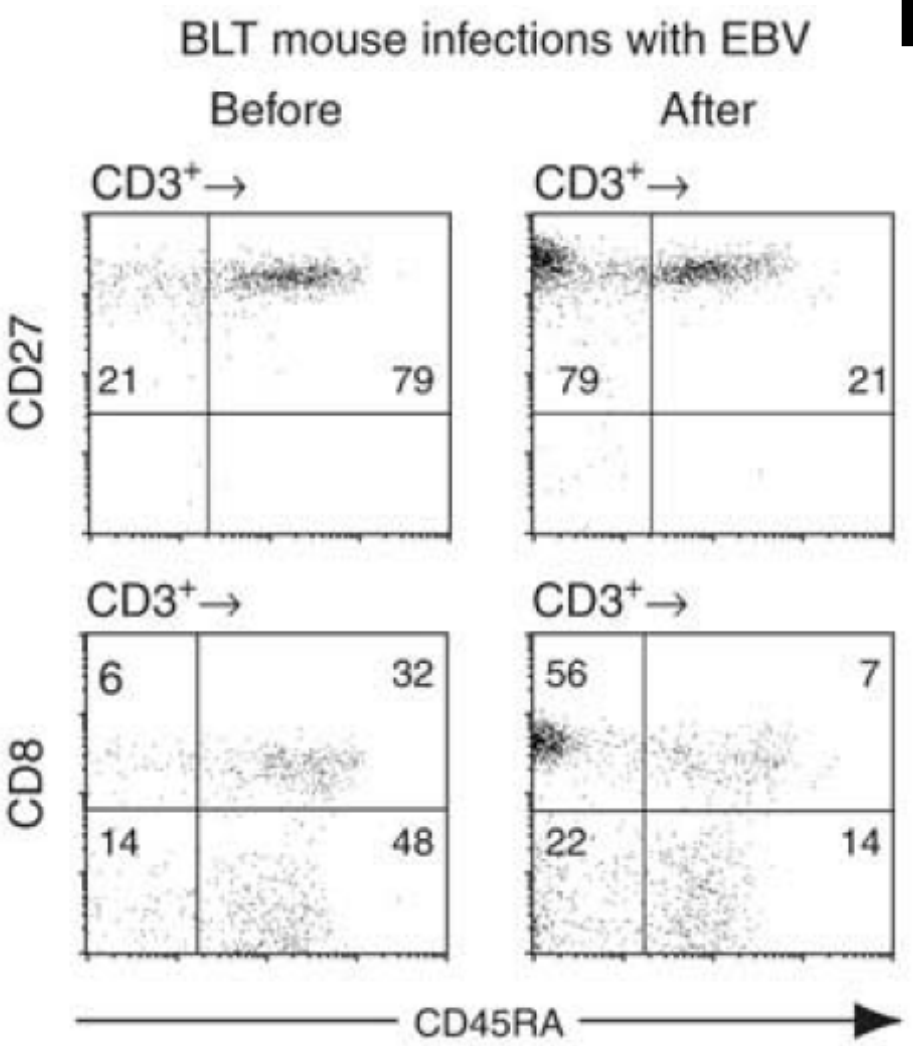
- Immune cells are human
- Unlike Hu-HSC-SCID
  - Immune recognition and responses are human
  - Immune system is tolerant to most human proteins

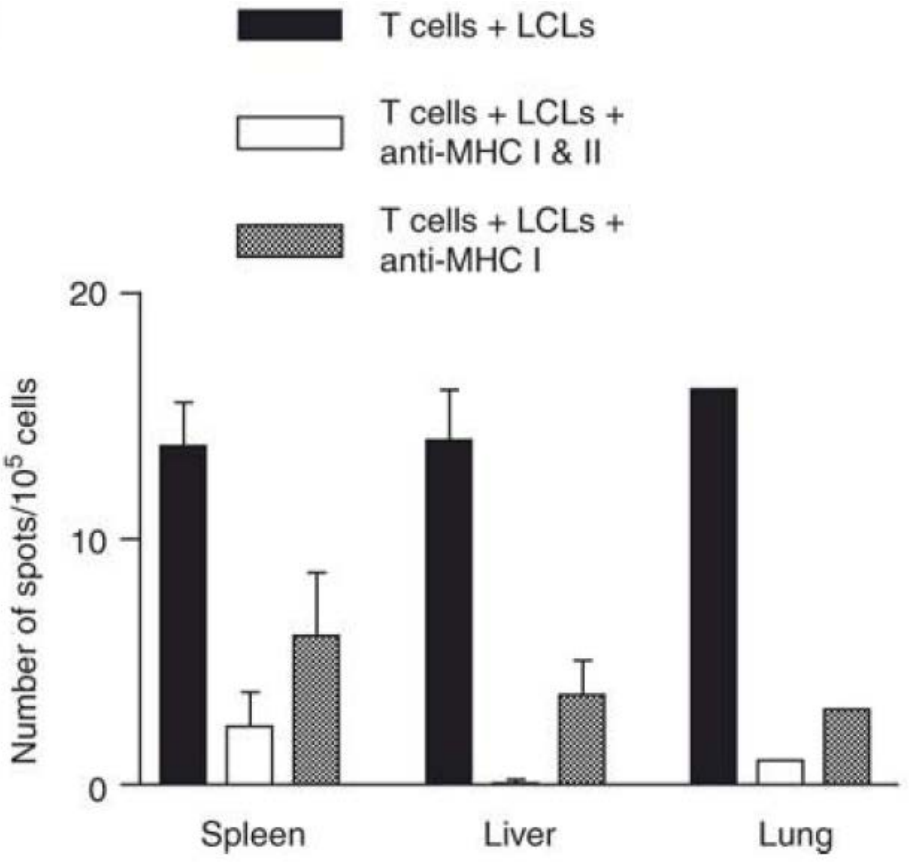
Systemic Human Reconstitution in BLT Mice



Melkus, et al. Nature Medicine. 2006

HLA Restricted EBV Specific T cell Responses in BLT Mice





Melkus et al. Nat. Med. 12, 1316-1322 (2006)

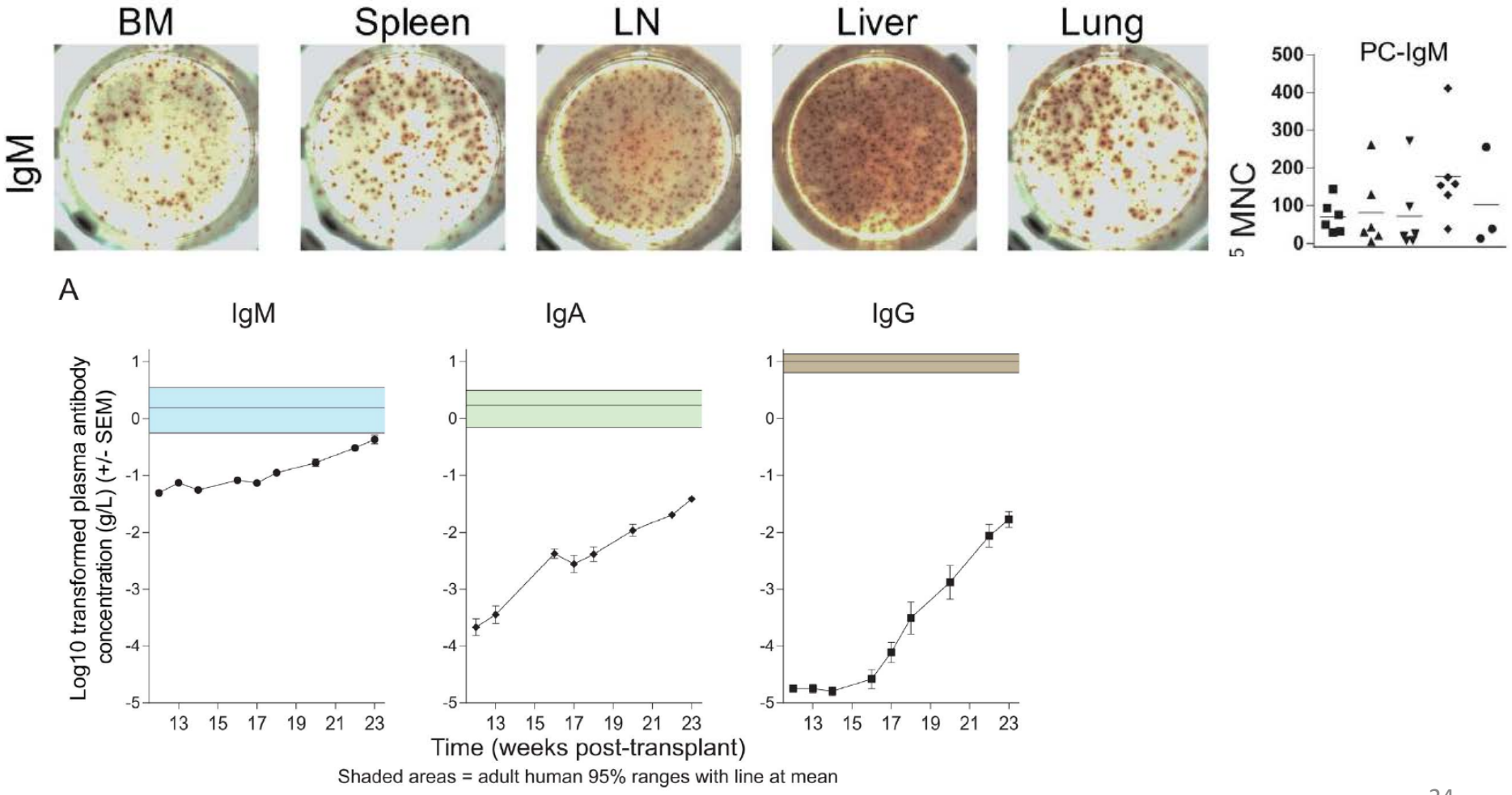
Do BLT Mice Mimic the Clinical Immunogenicity Seen with IFNβ?

Numerous clinical studies have shown IFNβ-1b products to be more immunogenic than IFNβ-1a products

IFNβ type	IFNβ-1a	IFNβ-1b
Production Method	CHO cells	*E. coli*
Glycosolation	Yes	No
Amino Acid Sequence	Same	Different
Aggregation	Low	High
Dosing	1X/wk	3X/wk

Advancing Humanized Mouse Models for Preclinical Testing

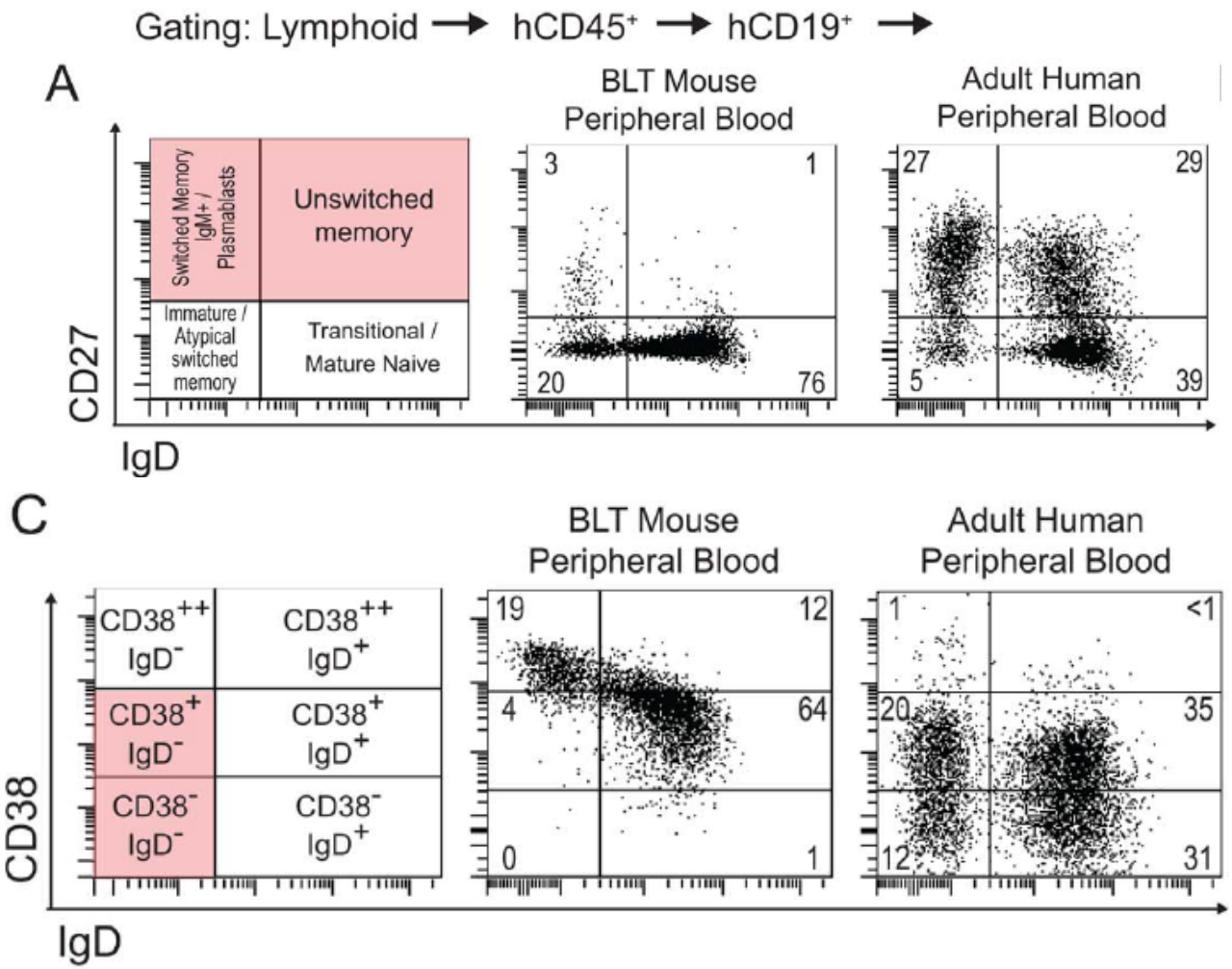
Limited Ig Class Switching



Martinez-Torres et al. 2014 PloS One 9(10): e108663

Advancing Humanized Mouse Models for Preclinical Testing

Limited B cell Maturation



Martinez-Torres et al. 2014 PloS One 9(10): e108663

Overcoming the Limitations of Hu-mu Models

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•Adapted from LD Shultz, et.al., 2007. *Nat. Rev. Immunol.*7:118

Challenges & Potential Solutions

2. GVHD in PBMC and BLT models
   a. H2 cl I and cl II ko
   b. C57BL/6 CD47 ko BLT
3. Lack of HLA molecules for T cell education and/or peripheral effector functions
   a. HLA tg-matched/unmatched HSC?
4. **Low levels of Ig and class switched Ig**
   a. **Cytokines e.g. NSG-Tg(BLyS)?**
   b. **BM stromal elements?**
   c. **Environmental Factors e.g. microbiota?**

Challenges & Potential Solutions

1. High Level Barrier facility; Labor Intensive Surgery and limited donor tissues
2. GVHD in PBMC and BLT models
   a. H2 cl I and cl II ko
   b. C57BL/6 CD47 ko BLT
3. **Lack of HLA molecules for T cell education and/or peripheral effector functions**
   a. **Tsplt of HLA matched target tissue**
   b. **HLA tg-matched/unmatched HSC?**

The BLT Mouse Team

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