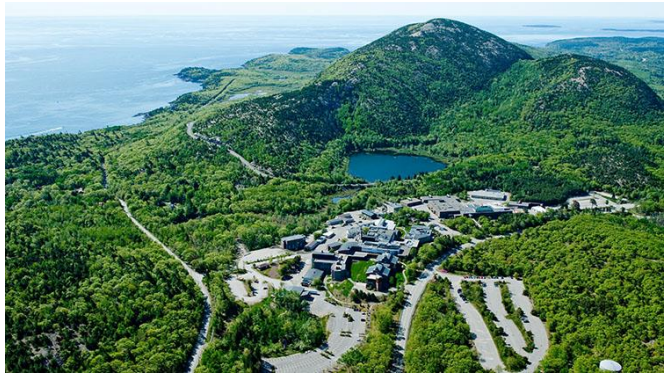
Next Generation Humanized Mouse Models for Human Infectious Diseases

Les Pensieres
Fondation Merieux Conference Center
Veyrier-du-Lac-France

April 27, 2017

Lenny Shultz
The Jackson Laboratory

For Distribution



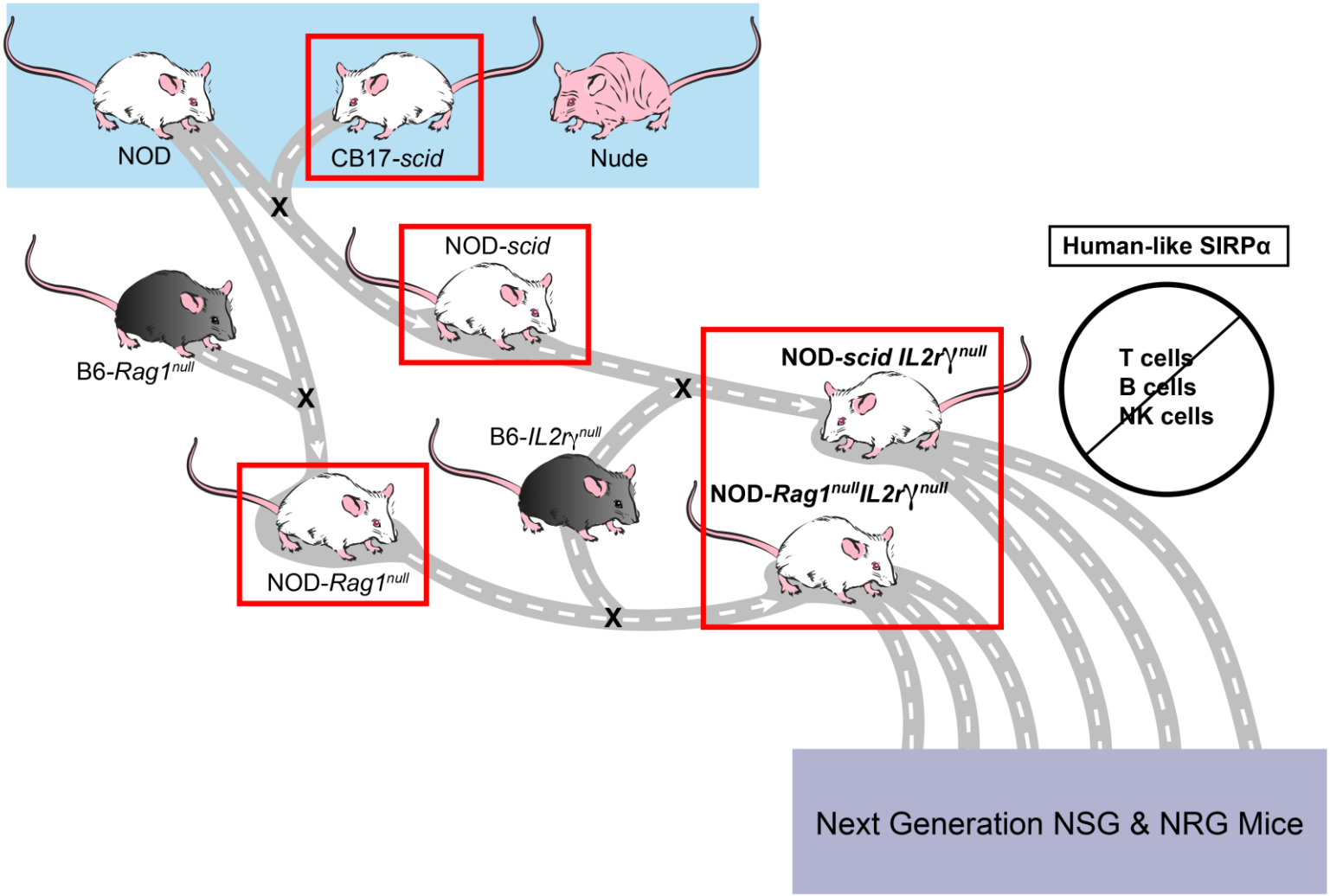
The Jackson Laboratory
Leading the search for tomorrow's cures

Mice are not Humans

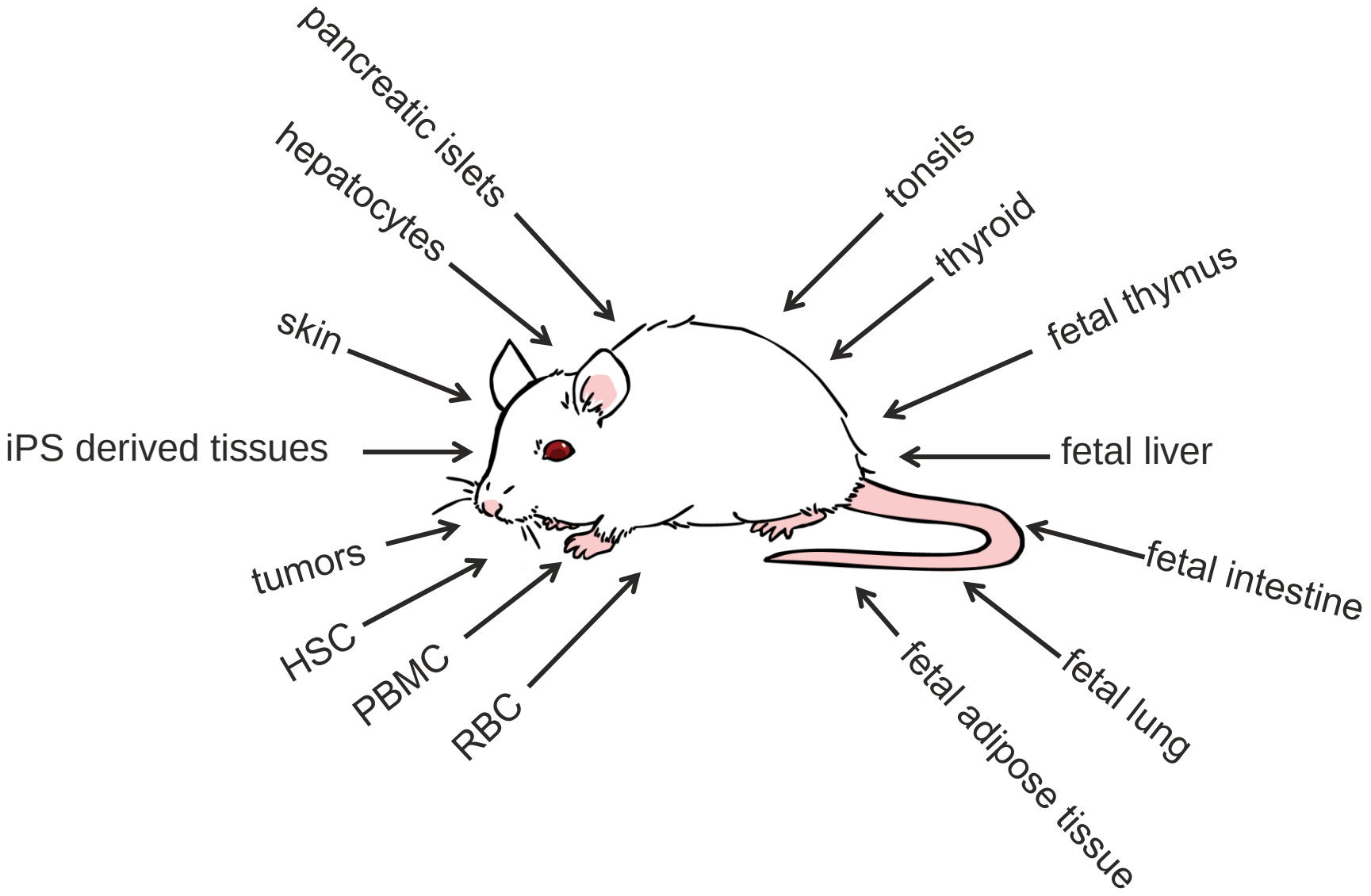
Humanized mice are immunodeficient mice that have been engrafted with functional human cells or tissues and support clinically relevant *in vivo* studies of human cells, tissues, and immune systems without putting patients at risk.

Outcomes predicted by murine studies are not always representative of outcomes in humans

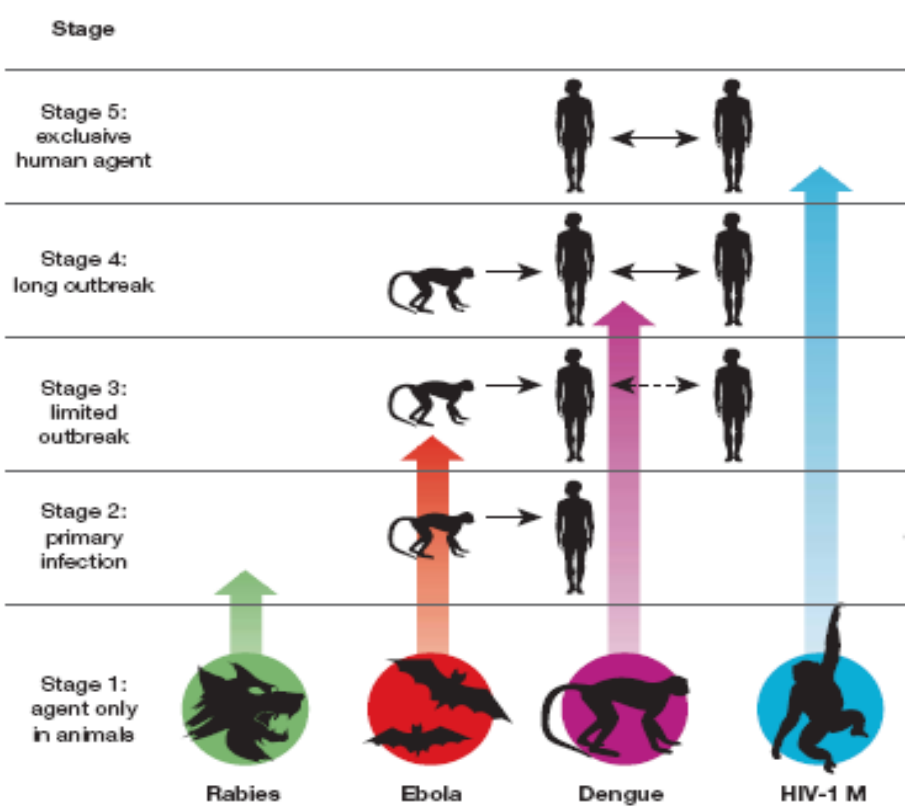
The Road to Humanized Mice



Engraftment of NSG Mice With Human Cells and Tissues



Human Infectious Agents Studied in Humanized Mice



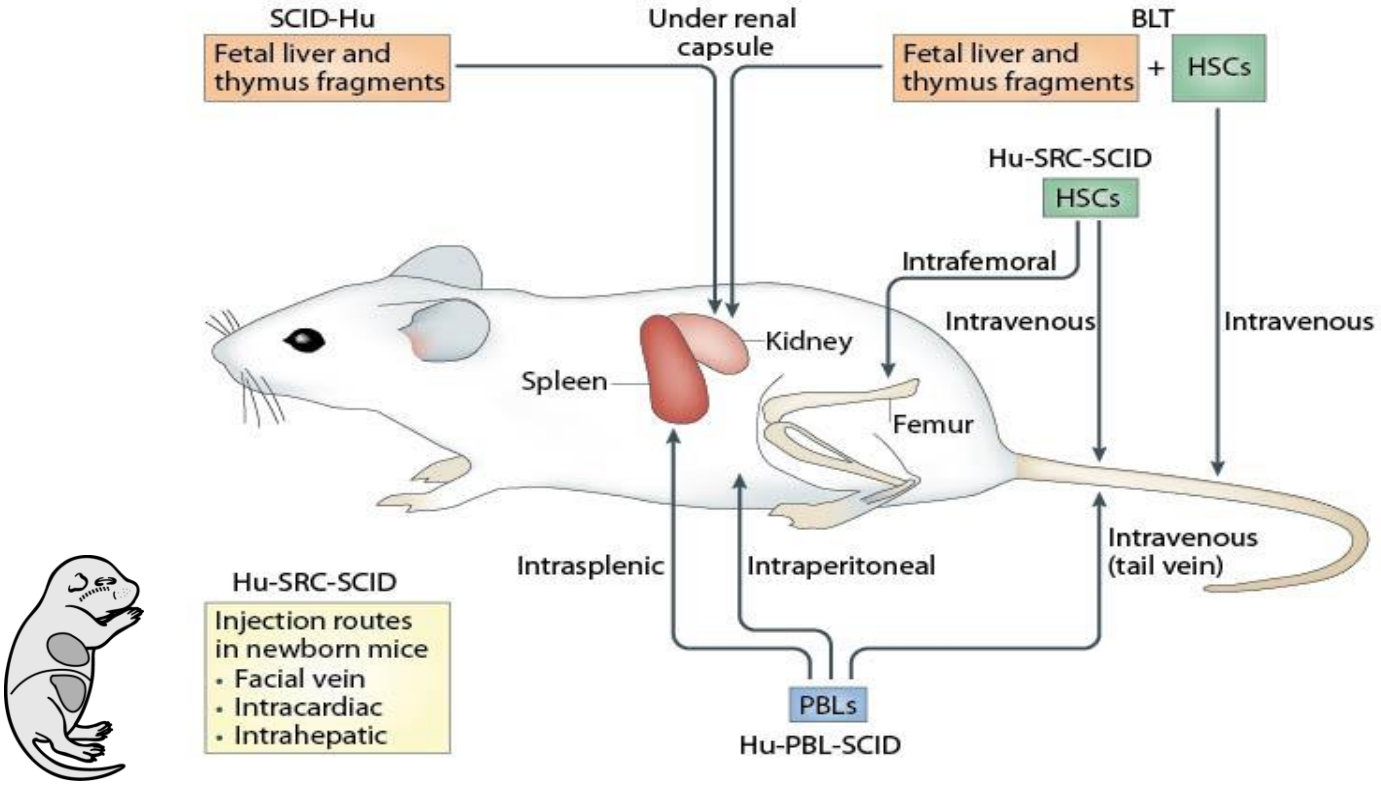
HIV
Human T cell leukemia virus
Ebola
Dengue virus
Epstein-Barr virus
Influenza virus
Hepatitis virus B and C
Human cytomegalovirus
Herpes simplex virus type 2
Human Herpes virus 6 and 8
Varicella-zoster virus
Nipah virus
Hantavirus
Plasmodium falciparum
Chlamydia trachomatosis
***Salmonella enterica* serovar typhii**
Mycobacterium tuberculosis
Emerging infectious diseases

N Wolfe et al (2007) Nature 447:279
M Brehm et al (2013) Curr Opin Immol 25:428
M Brehm et al (i2014) J Immunol Methods 410:3
Walsh NC et al (2017) Annu Rev Pathol 12:187

Strain Platforms for Humanized Mice

NSG	**NOD-*scid IL2rγ^{null}***
NRG	**NOD-*Rag1^{null} IL2rγ^{null}***
NOG	**NOD-*scid IL2rγ^{Trunc}***
BRG	**C;129-*Rag2^{null} IL2rγ^{null}***
MISTRG	**C;129S4-*Rag2^{null} Csf1$^{tm1(CSF1)Flv}$ Csf2/Il3$^{tm1.1(CSF2,IL3)Flv}$ Thpo$^{tm1.1(TPO)Flv}$ Il2rg^{null}* Tg(SIRPA)1Flv/J**
BRGS	**BALB/c-*Rag2^{null} IL2rγ^{null}Sirpa ^{NOD}***
H2^{d}RG	**Stock-H2^{d}-*Rag2^{null} IL2rγ^{null}***
C57BL/6	***Rag2^{null} CD47^{null} IL2rγ^{null}***

Engraftment of NSG Mice With Human Hematopoietic Cells and Tissues



Limitations of Humanized Mouse Models

Engraftment with PBMC

mature T cells > xenogeneic GVHD

Engraftment with HSC

T cell education in context of mouse MHC (H2) antigens

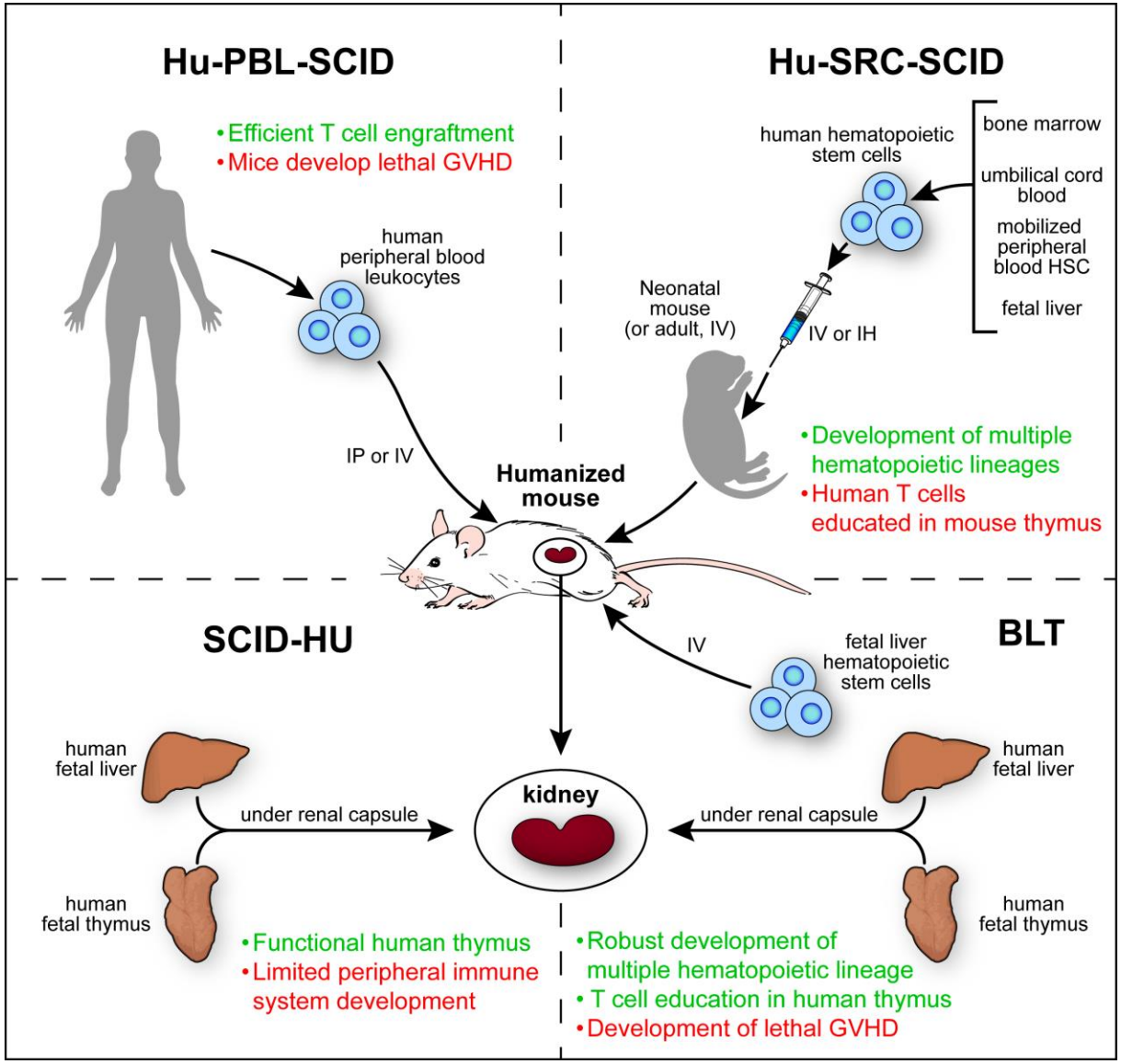
Lack of human cytokines impairs HSC growth & differentiation

Engraftment with fetal cells & tissues

Ongoing ethical controversy

Development of a lethal wasting disease

Hematolymphoid Engraftment Methods



Human Immune System Models

- **Hu-PBL-SCID mice:** SCID mice engrafted with human peripheral blood mononuclear cells (PBMC)

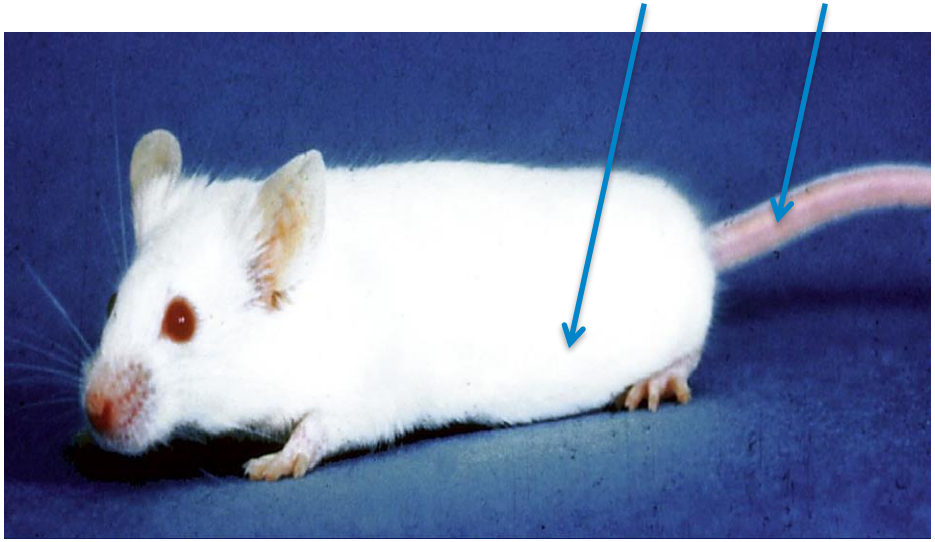
- **Hu-SRC-SCID mice:** SCID mice engrafted with human hematopoietic stem cells (HSC) scid repopulating cells (SRC) = $CD34^+$ cells

- **THY/LIV (BLT) mice:** SCID mice engrafted under the renal capsule with human fetal thymus/liver and injected IV with autologous $CD34^+$ human fetal liver cells

L Shultz, et al (2007) *Nat Rev Immunol* 7:118

Engraftment of NSG Mice with Human PBMC (Hu-PBL-SCID)

i.v. or i.p. injection of human PBMC



Human T cell function can be analyzed for 4-6 week prior to development of lethal xenogeneic GVH

Schleifman EB et al (2013) Mol Ther Nucleic Acids 2:e135
Kumar P et al (2008) Cell 134:577

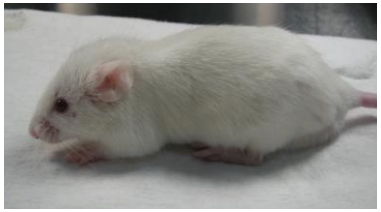
Xenogeneic GVHD Mediated By Human PBMC

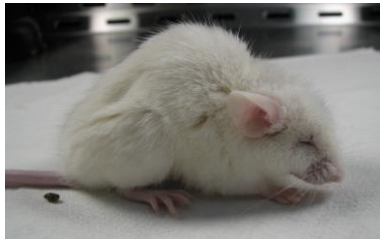
Intravenous Injection of 20 x 10^6 Human PBMC

NSG Mice

+ or - radiation

Follow for engraftment and disease





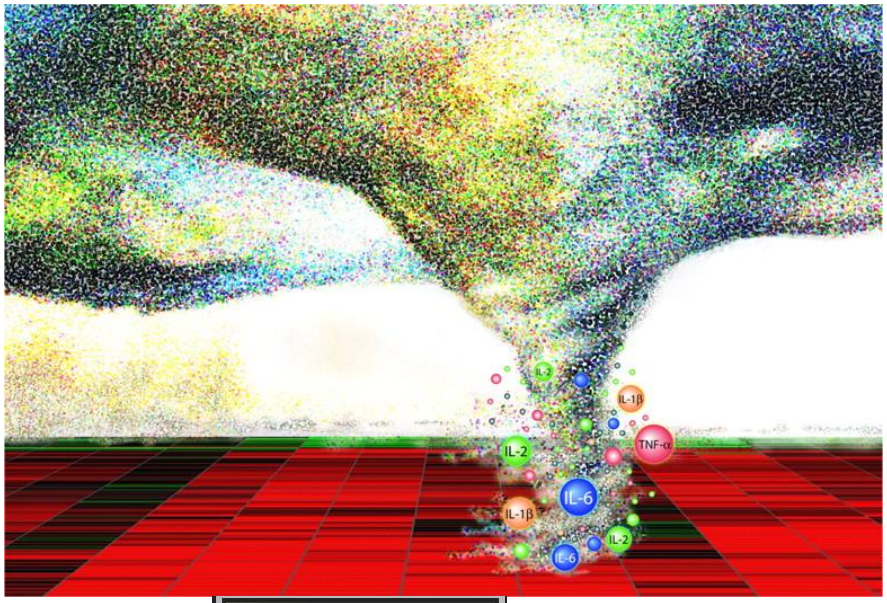
-hair loss/erythema
-hunched posture
-weight loss
-death

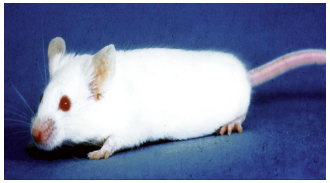
In the Eye of A Cytokine Storm

On March 13, 2006, six young healthy volunteers received TGN1412 anti-Hu CD28 superagonistic mAb in a phase 1 trial. Within 12-16 hours, the volunteers became critically ill (Activation of effector memory T cells)

TGN1412 was validated as safe in animal models. A 500X higher level of TGN1412 had no adverse events in cynomologous monkeys (Effector memory T cells of cynomolgus monkeys lack CD28).

Treatment of PBMC-engrafted NRG-HLA-DQ8 mice with TGN1412 induces a cytokine release syndrome and reproduces some of the effects seen in humans.





Humanized mouse

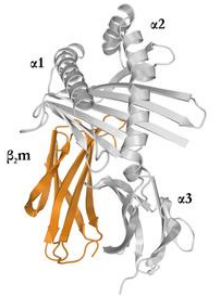
Cytokine storm **Unaffected** **Storm warnings**

G. Suntharalingam et al (2006) NEJM 355:1018
Tisoncik JR. 2012. Microbiol. Mol. Biol Rev.76: 16
JLBrady et al (2014) Clin Trans Immunol 3, e29; doi:10.1038/cti.2014.28
S Weibmuller et al (2016) PLOS ONE | DOI:10.1371/journal.pone.0149093 March 9, 2016

Reduced Xenogeneic GVHD in NSG Mice lacking Murine MHC Class I and II Molecules

Mouse MHC class I knockouts

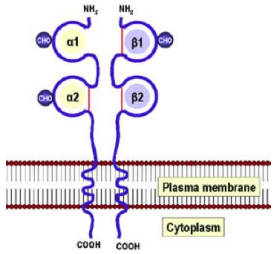
NSG *(β2M)*^{null}
NSG *(KD)*^{null}



M King et al (2009)
Clin Exp Immunol 157:104

Mouse MHC class II knockouts

NSG *(I-A)*^{null}
NSG *(I-A/I-E)*^{null}



L Covassin et al (2011)
Clin Exp Immunol 166:269

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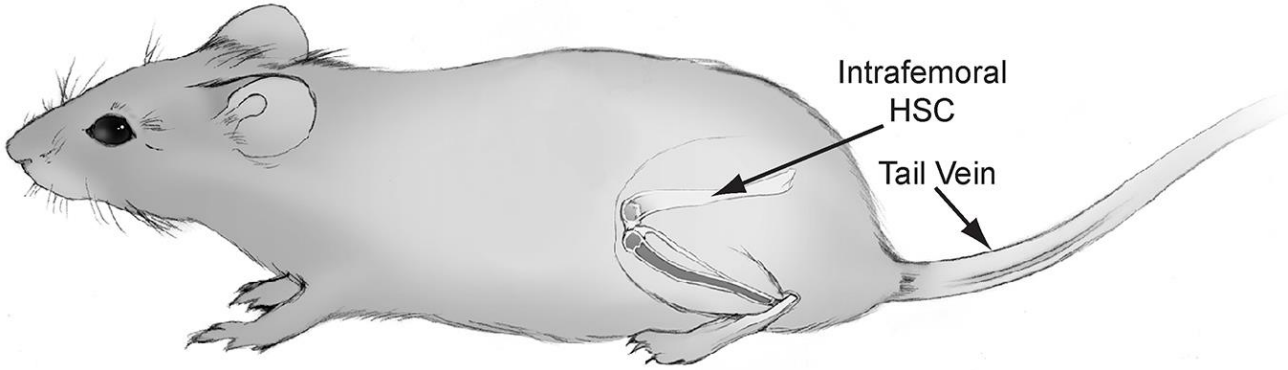
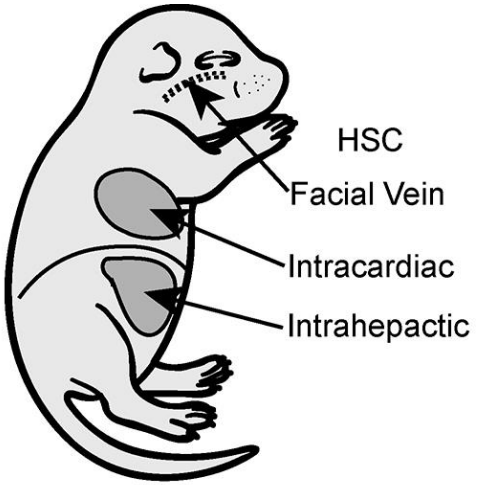
LD Shultz, et al (2007) *Nat Rev Immunol* 7:118

Engraftment of NSG Mice with Human Hematopoietic Stem Cells (Hu-SRC-SCID)

CD34+ cells from UCB, fetal liver, BM, mobilized stem cells



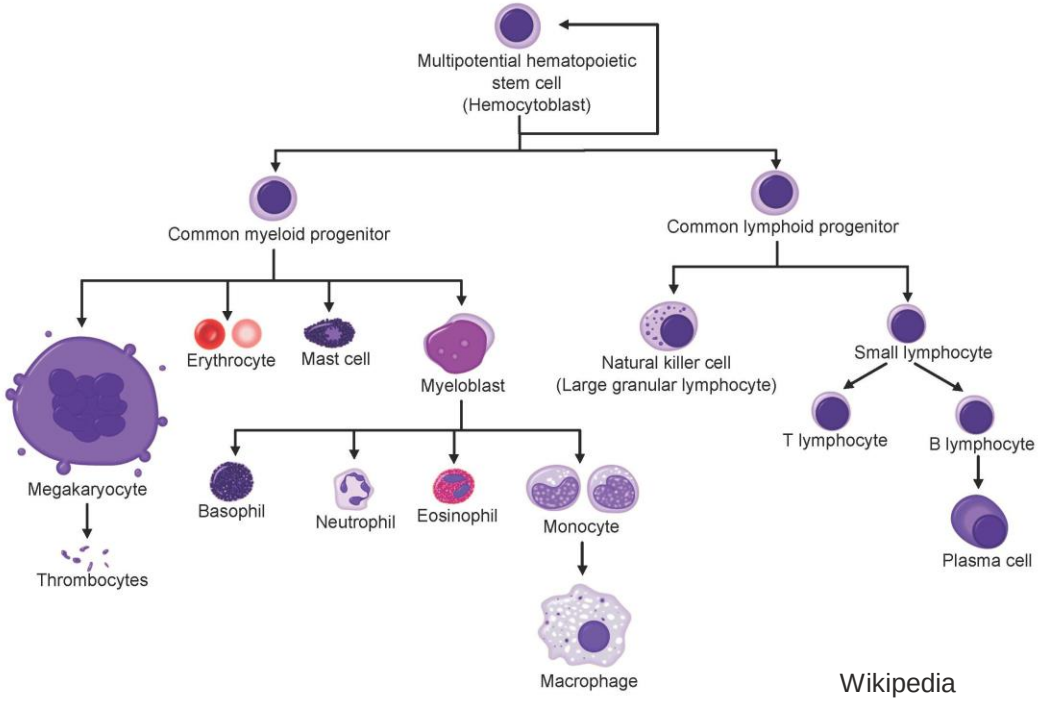


X-Ray dose 250cGy 100cGy

LD Shultz, et al (2007) *Nat. Rev. Immunol.* 7:118
T Pearson et al (2008) Current Protocols Immunol. 15:21

Human HSC-engrafted NSG Mice Develop All Hematopoietic Cell Lineages

- T cells
- B cells
- NK cells
- Dendritic cells
- Macrophages
- Granulocytes
- Red blood cells
- Platelets



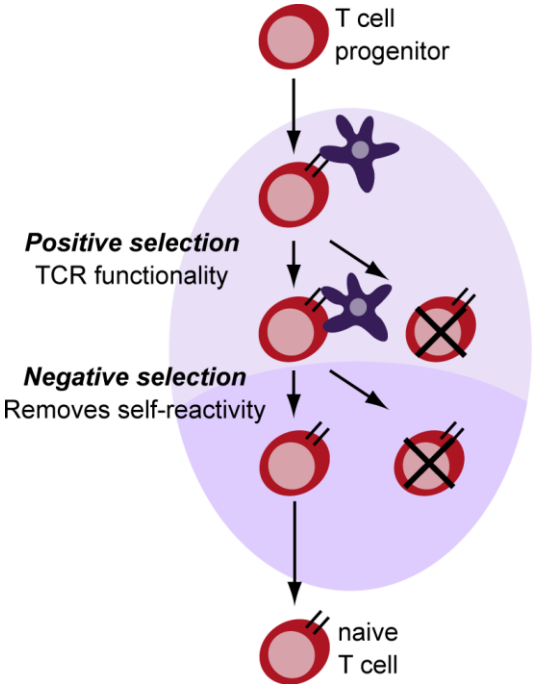
Wikipedia

Optimal engraftment protocol

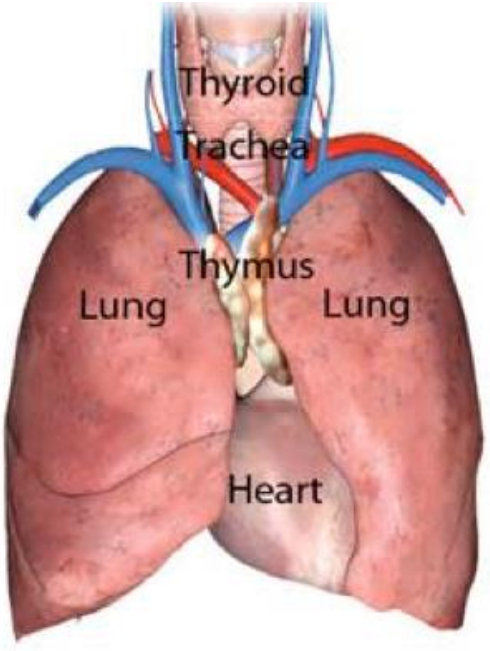
T Pearson et al (2008) *Curr Protocols in Immunology Supplement 81, Unit 21.1-21.21*

T Cell Education Occurs in the Thymus in the Context of MHC Molecules

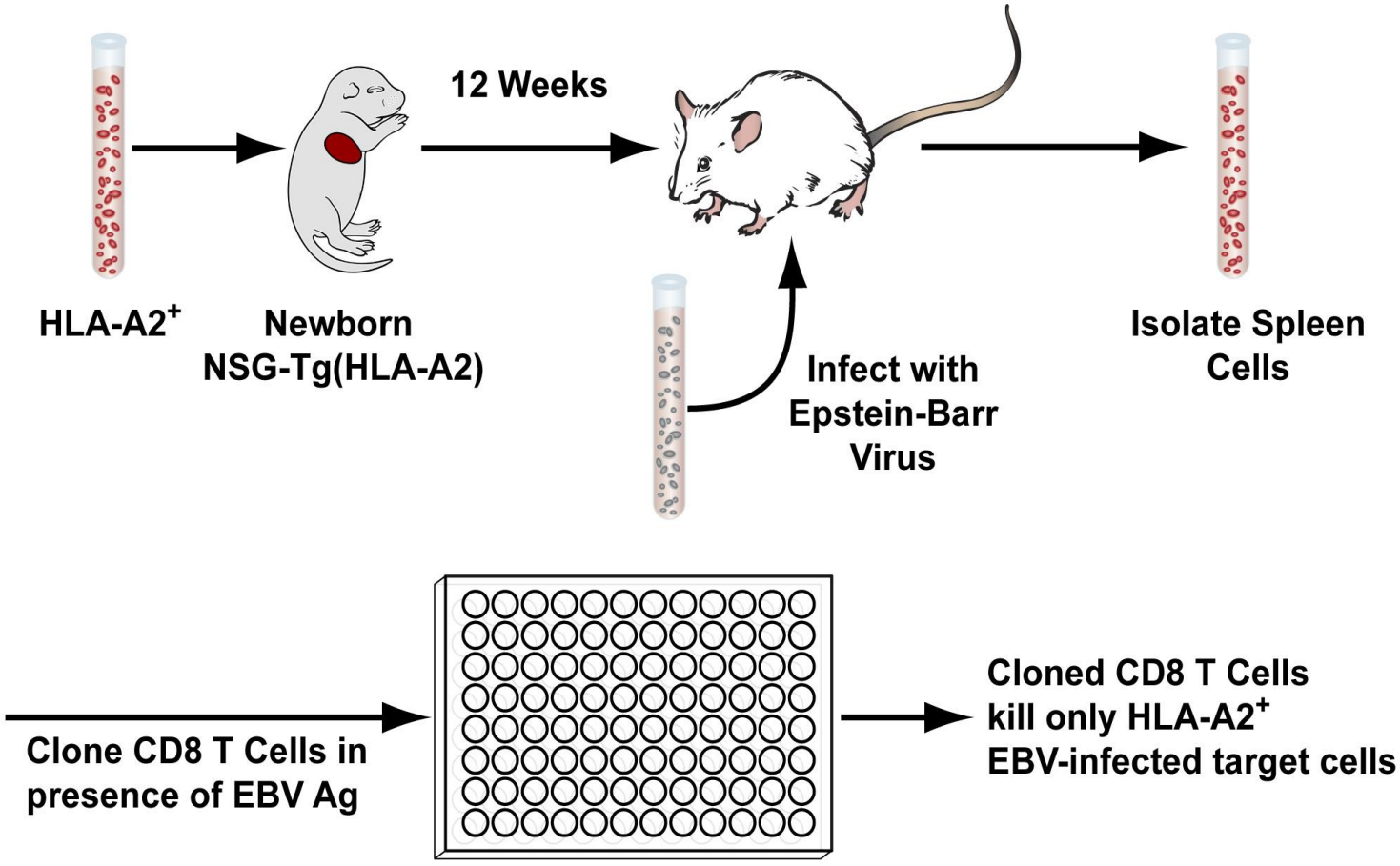
Mouse MHC – H2 Complex



Human MHC - HLA complex

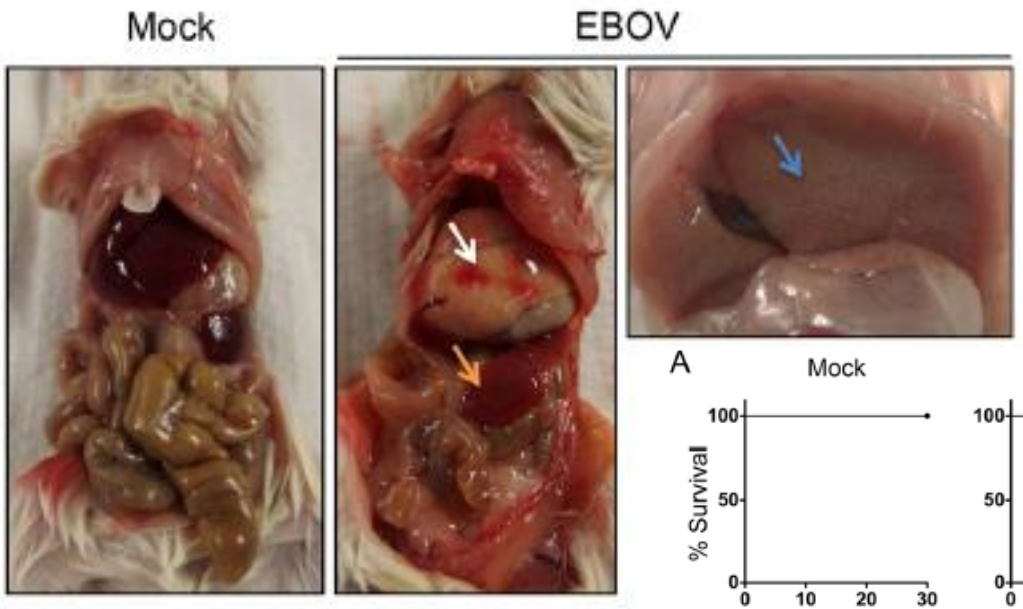


NSG-HLA-A2 Transgenic Mice Engrafted With HLA-A2+ HSCs Develop HLA-A2 Restricted Human Cytotoxic T Cells

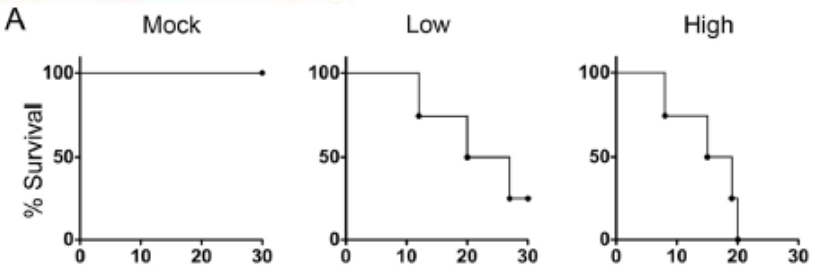


L Shultz et al (2010) PNAS 107:13022
S Jaiswal et al (2009) PLOS One e7251

Ebola Virus Infection of Human Myeloid Cells Following HSC Engraftment of NSG Tg(HLA-A2) Mice Results in Pathological Changes and High Lethality



Mice injected with Human EBOV developed liver hemorrhage and steatosis



A Ludtke et al (2015) J Virol 89:4700

A Humanized Mouse Model for Typhoid Fever

20 million new cases of Typhoid per year

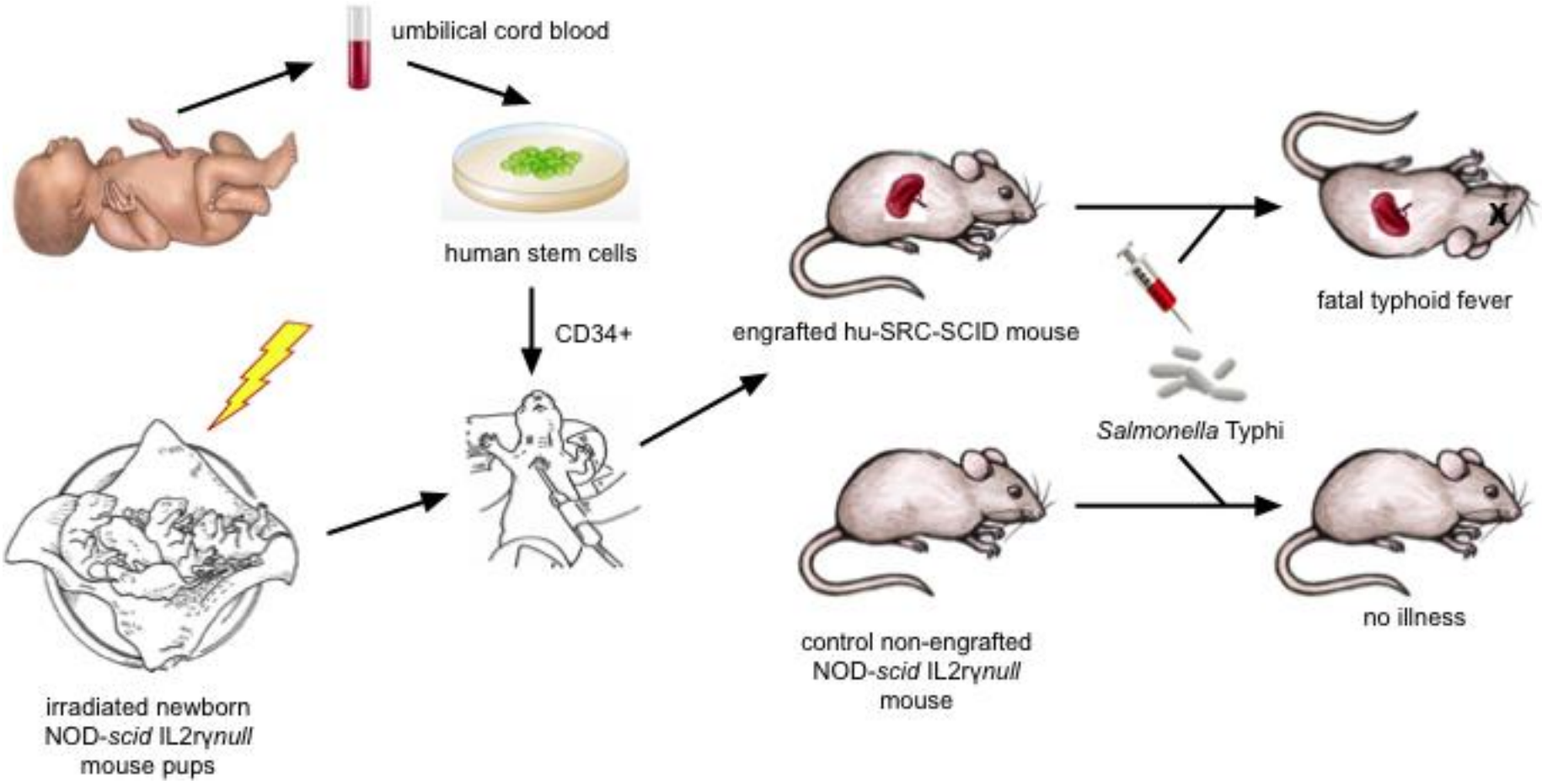
400 yearly cases in United States

200,000 deaths worldwide

Caused by infection with *S. typhi*

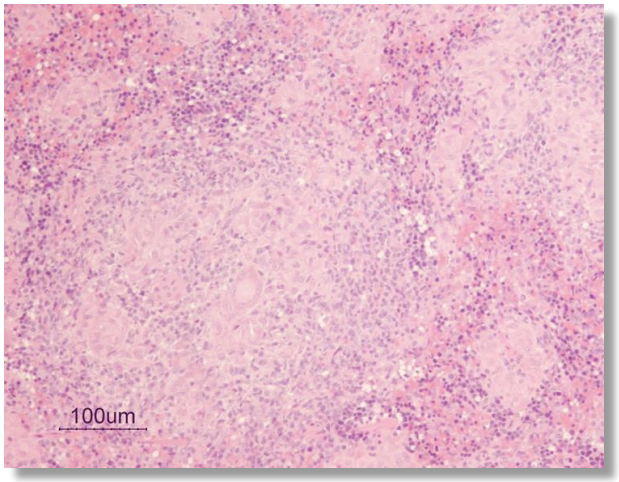
Humans are only known reservoir

A Humanized Mouse Model for Typhoid Fever

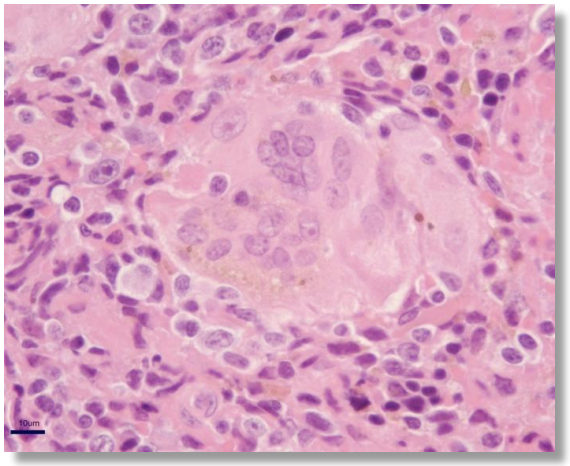


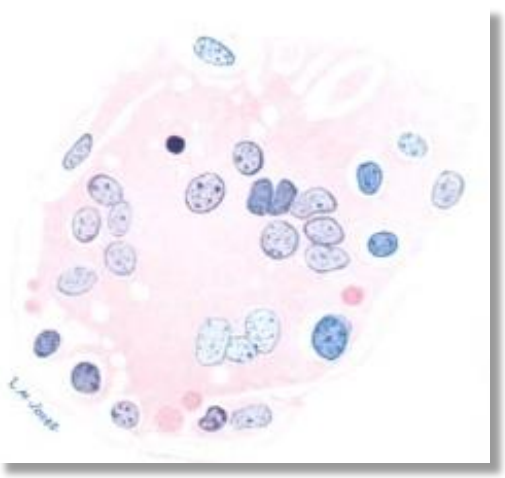
SJ Libby et al (2010). PNAS 107:15589

Splenic Granulomas in Humanized hu-SRC-SCID Mice Infected with *S.* Typhi



Granulomatous inflammation





Multinucleate Giant Cells

"In 1861 Billroth and Grohe, working independently, described certain very large multinucleated cells occurring in the mesenteric lymph nodes in typhoid fever. . . The multinucleated lymphoid cells of the early observers are for the most part the phagocytic cells."

-- Mallory, "A Histological Study of Typhoid Fever," *J Exp Med*, 1898

SJ Libby et al (2010). PNAS 107:15589

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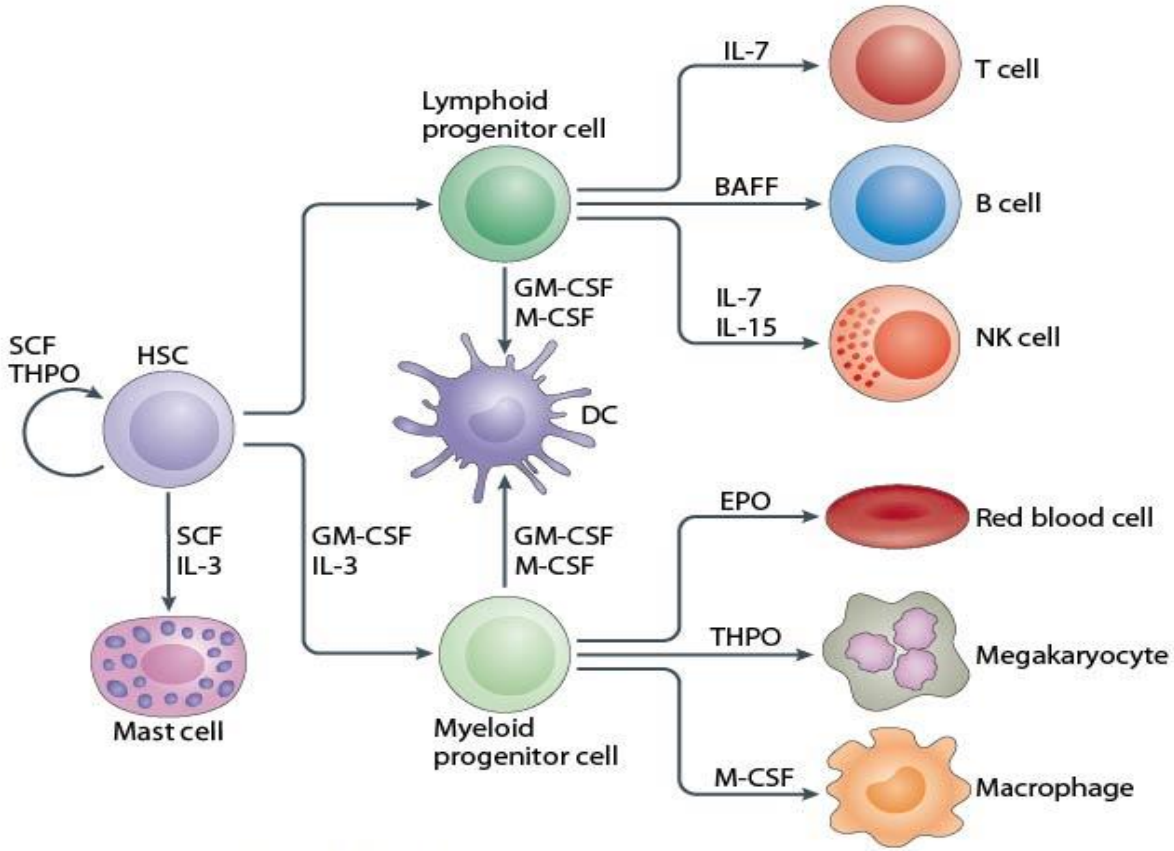
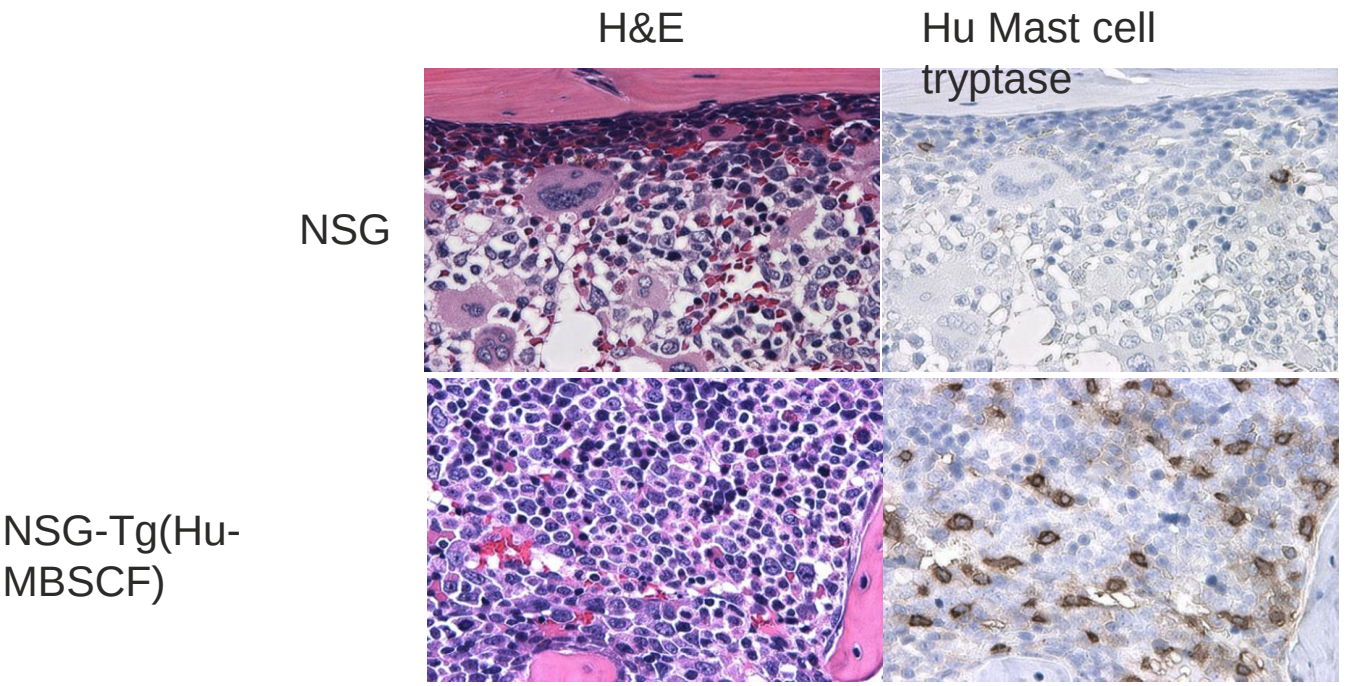
Human Cytokines Expressed Transgenically in Immunodeficient Mouse Strains



LD Shultz et al (2012) Nat Rev Immunol 12:786

Enhanced Human Mast Cell Development in NSG Mice Transgenically Expressing Human Membrane-Bound Stem Cell Factor

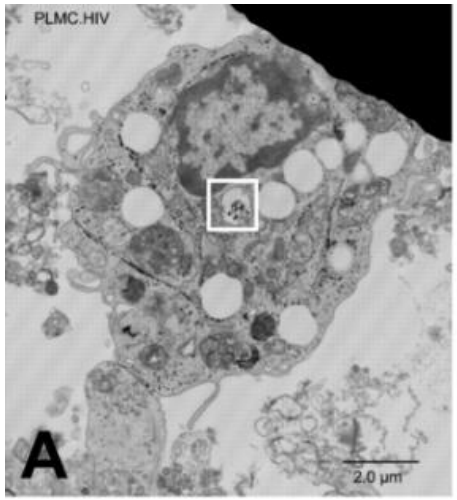


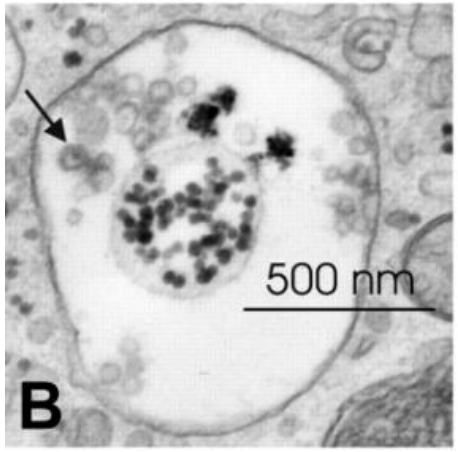
Bone marrow sections of mice irradiated at birth with 150 cGy and injected IV with 5 x10^4 human CD34+ CD38- HSC. Mice were examined 35 weeks post-engraftment

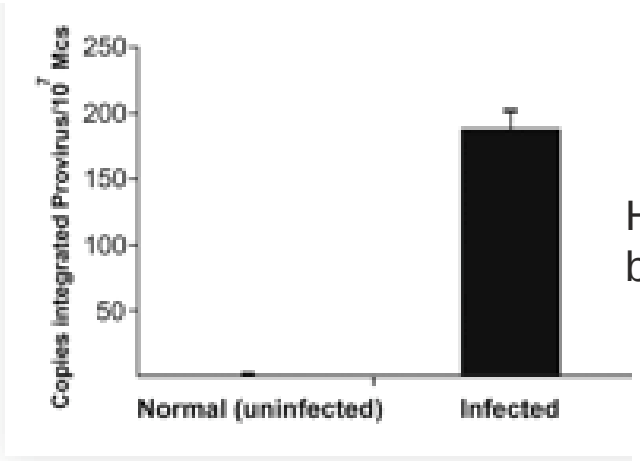
S Takagi et al (2012) Blood 119:276

Model human systemic anaphylaxis **PJ Bryce et al (2016) J Allergy Clin Immunol**

Human Placental Tissue Mast Cells Harbor Infectious HIV Virions







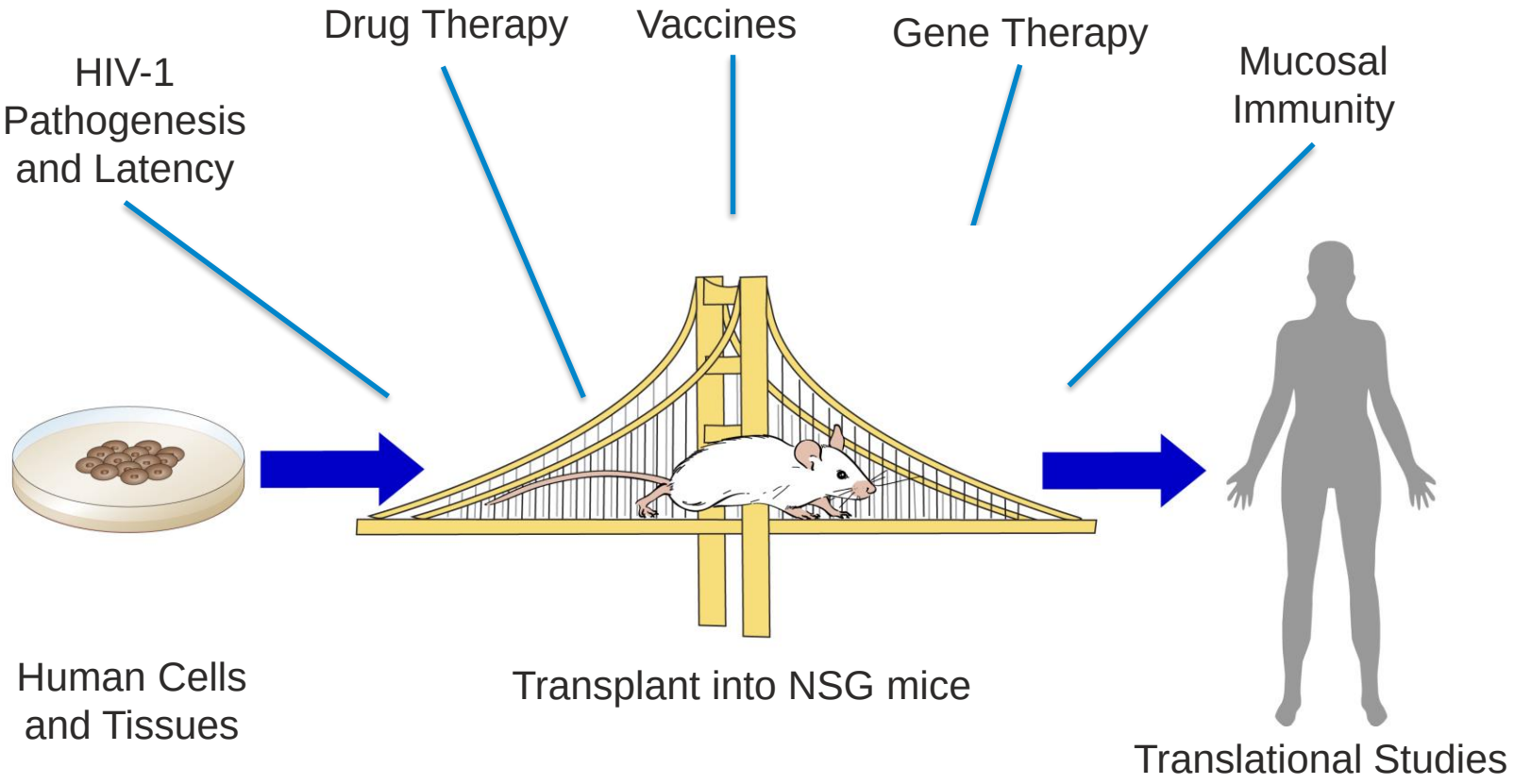
HIV-p24^{+}provirus released by cultured mast cells

HIV virions with typical nucleocapsid core particles located within cytoplasmic vesicular bodies

Immature mast cells are infected. Mature mast cells may serve as a site for latent HIV infection

JB Sundsrom et al (2007) Blood 109:5293

HUMANIZED MICE FOR HIV RESEARCH



Improvements and Limitations of Humanized Mouse Models for HIV Research: NIH/NIAID "Meet the Experts" 2015 Workshop Summary

R Akkina et al (2016) AIDS Research Human Retroviruses 32:109

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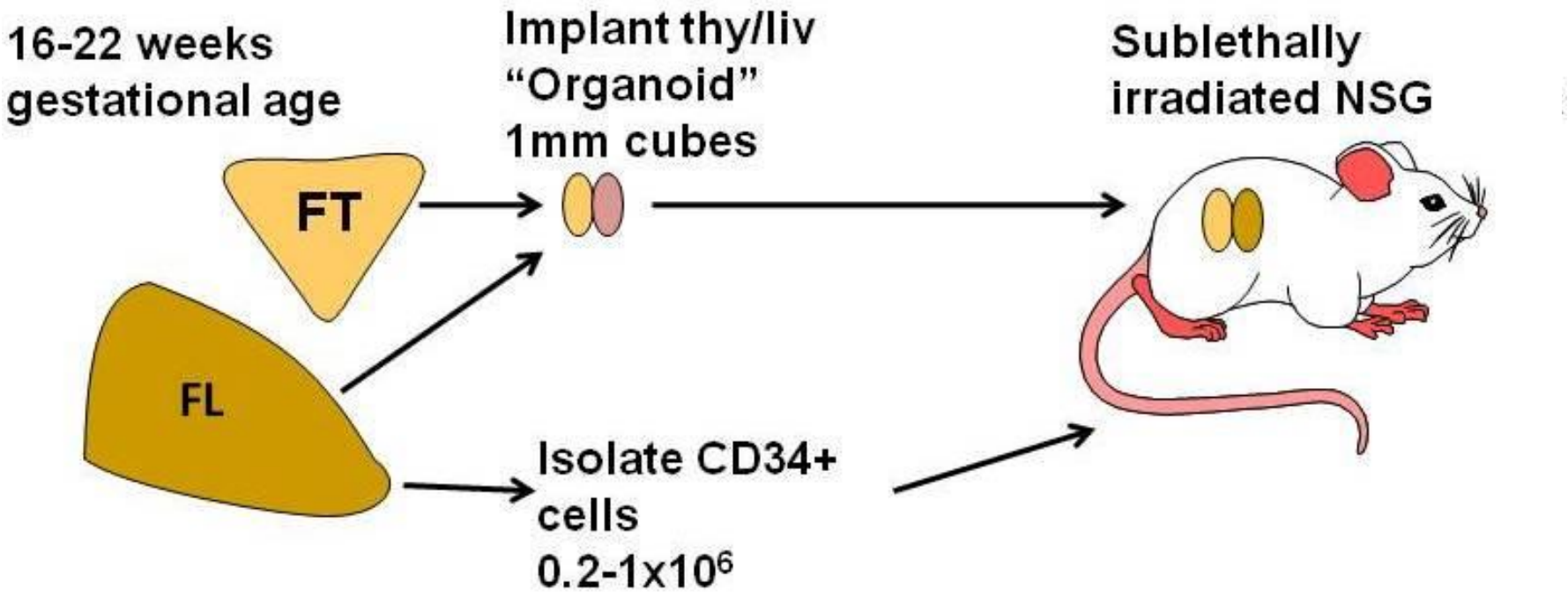
LD Shultz, et al (2007) *Nat. Rev. Immunol.* 7:118

Engraftment with Human Fetal Liver and Thymus (BLT Model)

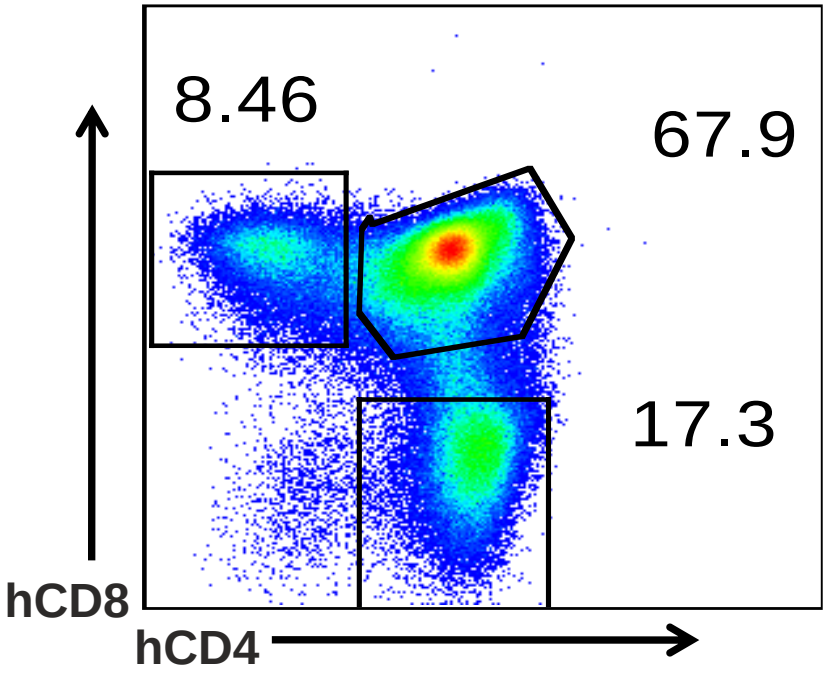


L Covassin et al (2013) Clin Exp Immunol 174:372
K Aryee et al (2014) Methods Mol Biol 1185:267

Thymus Development in BLT NSG Mice at 16 Weeks Post-Implant

Thymic Organoid

Thymocyte subsets



Scientific and Medical Advances Enabled by Fetal Tissue Research

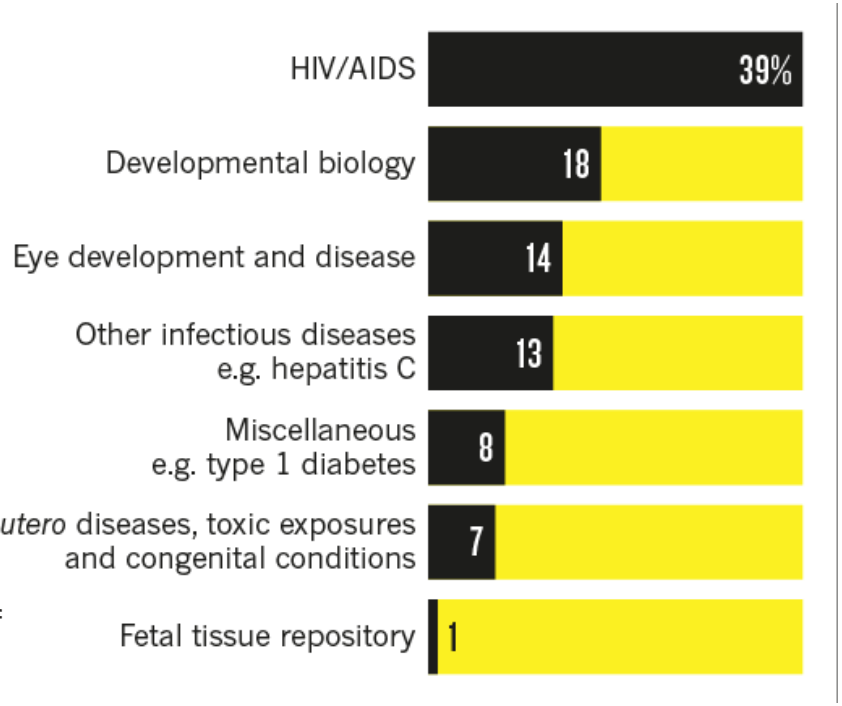
Human fetal fibroblast cell lines (WI-38 and MRC-5) have been used to support production of more than 5 billion doses of vaccines for measles and other infectious diseases

Vaccine research and development
Rubella
Zoster (chicken pox)
Hepatitis A
Polio

Targeting Fetal Tissue Researchers

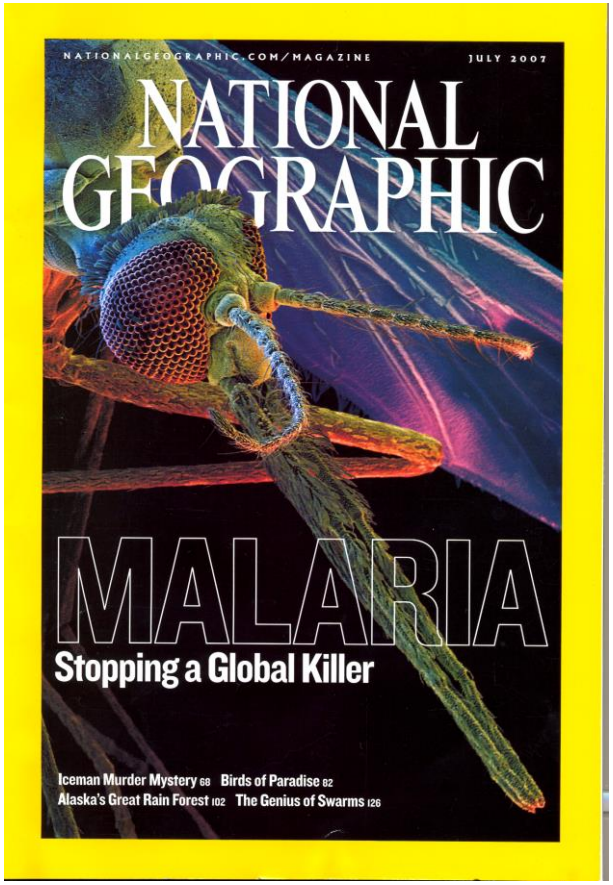
Congressional inquiry seeks the names and identities of academics, including graduate students, prompting criticism from scholars who say the probe is more about intimidation than information.

NIH funded fetal tissue research (164 projects)



M Wadman (2015) Nature 528:7581

Humanized Mice in Malaria Research



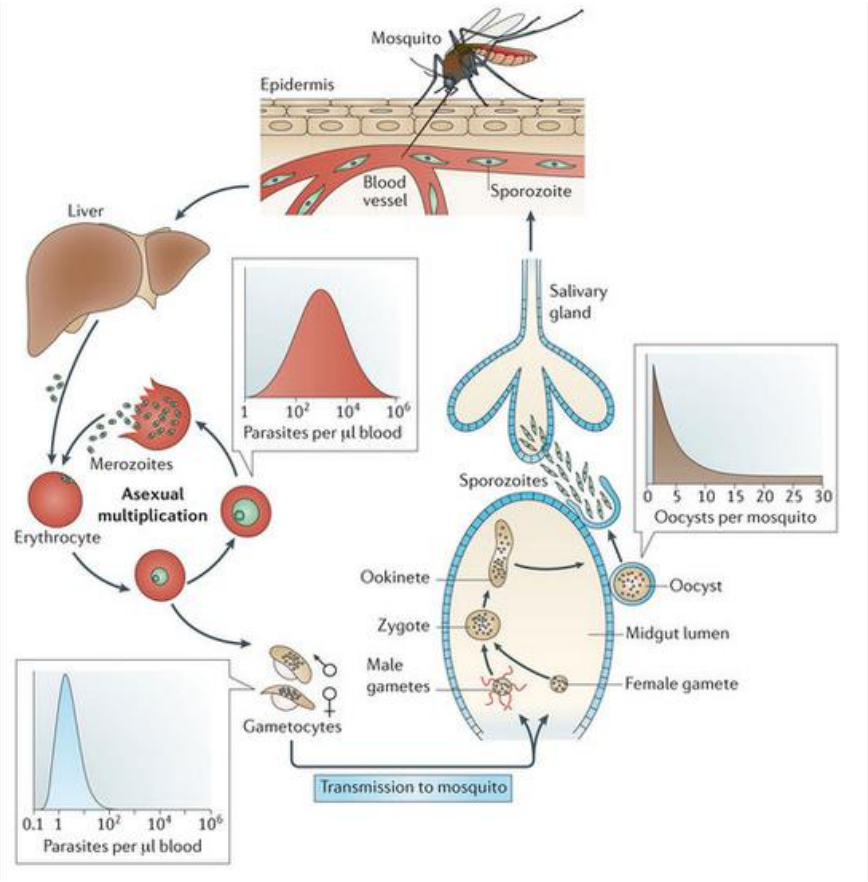
300-500 million cases per year worldwide

> 2 billion people at risk; > 1 million deaths

Plasmodium falciparum causes 95% of the mortality

GSK Diseases of the Developing World facility in Tres Contos, Spain is using *P. falciparum* parasitized human RBC engrafted NSG mice as a model for high throughput drug screening of anti-malarial agents

Plasmodium falciparum Life Cycle



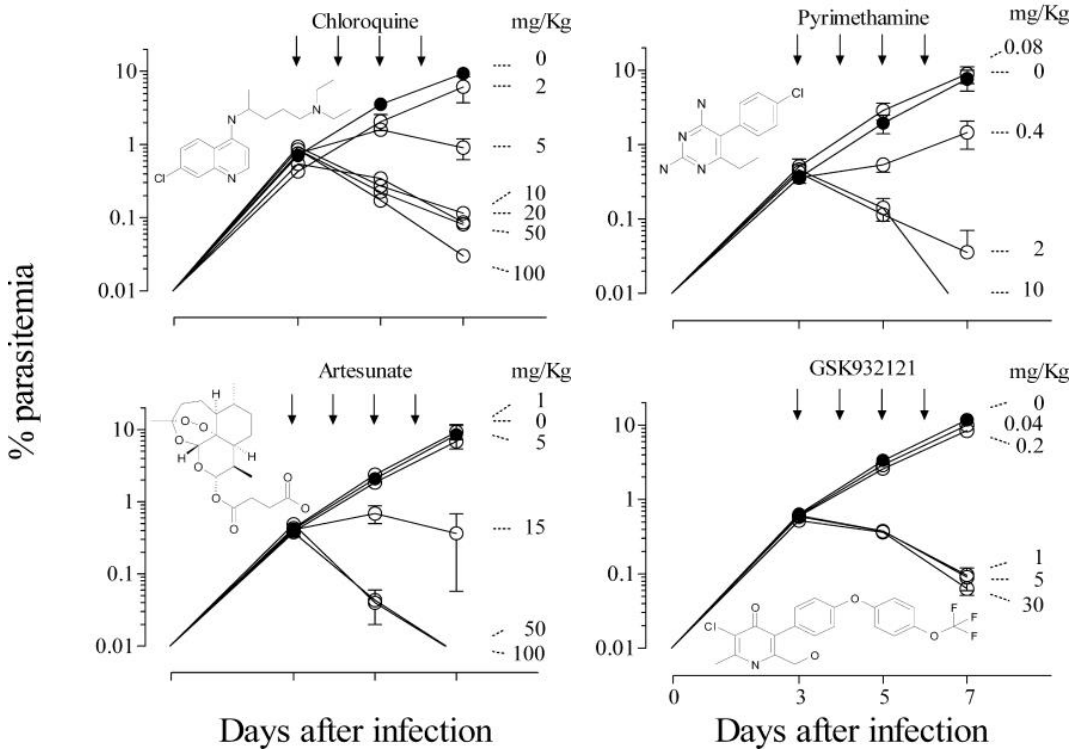
Female Anopheles mosquito injects sporozoites into bloodstream

Sporozoites travel to liver and invade hepatocytes

Merozoites are released into the bloodstream

Bousema T (2014) Nat Rev Microbiol 12:833

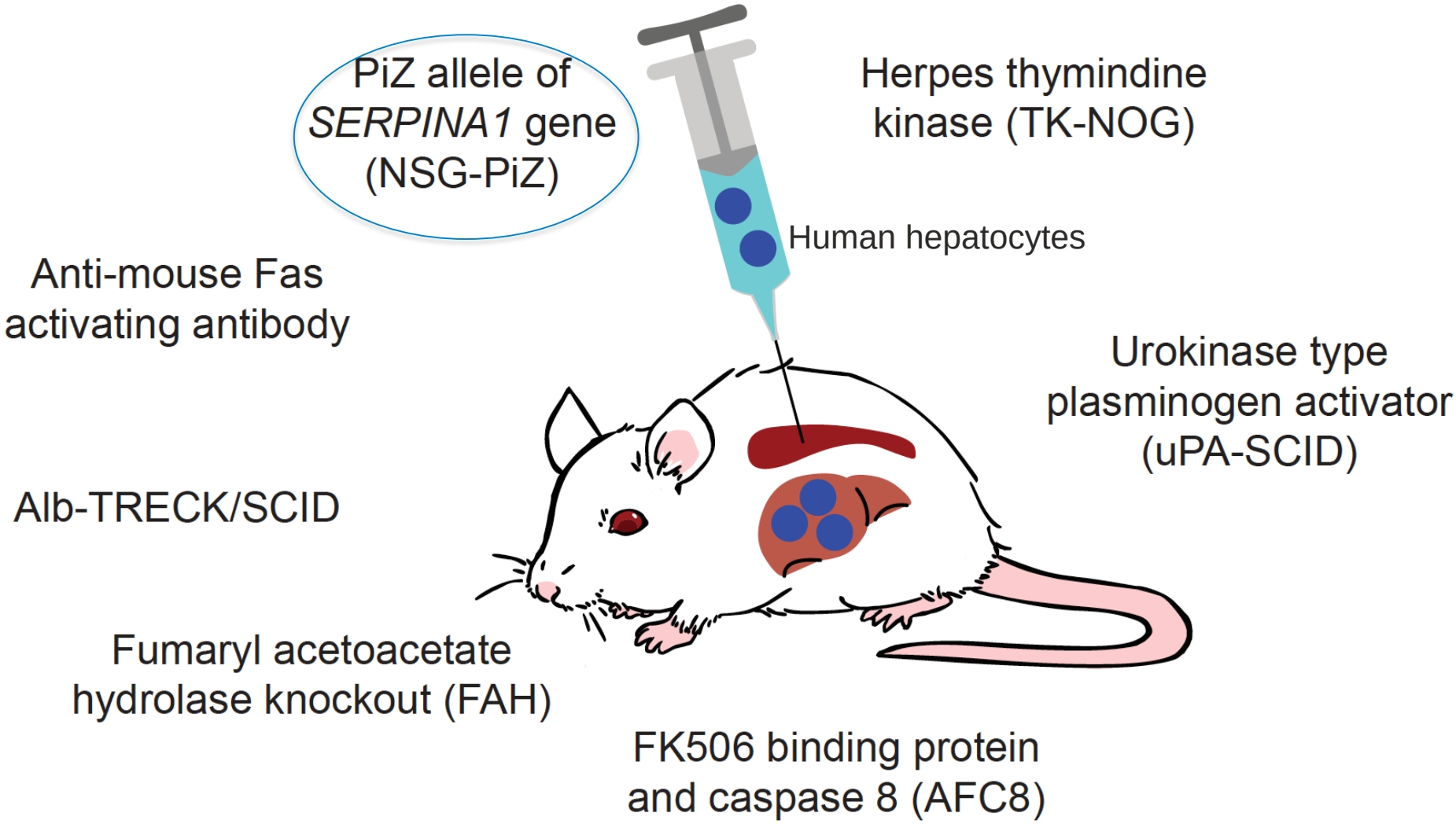
Anti-malarial Drugs are Effective in NSG RBC-Engrafted Mice



Therapeutic efficacy of anti-malarial drugs. Arrows indicate the day of treatment in a 4-day standard test. Values are levels of parasitemia in peripheral blood in three mice per group.

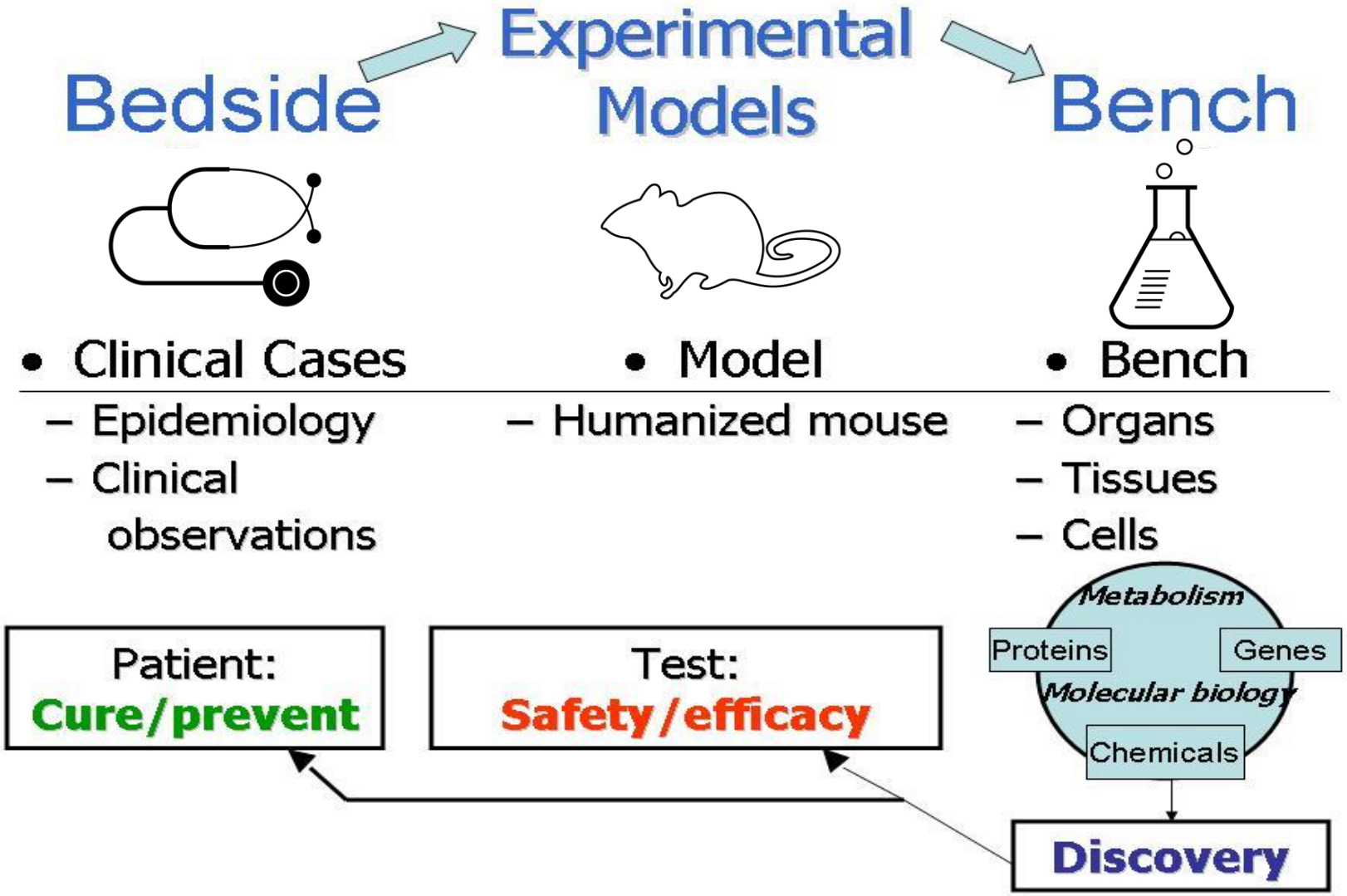
Jimenez-Diaz et al (2008) Antimicrob Agents Chemother 53(4533)

Humanized Mouse Models for Hepatocyte Engraftment



N. Walsh et al (2017) Annu Rev Pathol 12:187

Humanized Mice in Translational Research



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