Accelerating Global Health Protection: The Human-Immune-System DRAGA Mouse as a Preclinical Platform for Infectious Disease Countermeasures

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DISCLAIMERS

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The Need for Better Models: Limitations of

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Hum **odel**

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Review Altern Lab Anim. 2023 Mar;51(2):102-135. doi: 10.1177/02611929231157756.
Epub 2023 Mar 7.

Poor Translatability of Biomedical Research Using Animals – A Narrative Review

Lindsay J Marshall [1], Jarrod Bailey [2], Manuela Cassotta [3], Kathrin Herrmann [4], Francesca Pistollato [5]

Affiliations

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Free article

Abstract

The failure rate for the translation of drugs from animal testing to human treatments remains at over 92%, where it has been for the past few decades. The majority of these failures are due to unexpected toxicity - that is, safety issues revealed in human trials that were not apparent in animal tests - or lack of efficacy. However, the use of more innovative tools, such as organs-on-chips, in the preclinical pipeline for drug testing, has revealed that these tools are more able to predict unexpected safety events prior to clinical trials and so can be used for this, as well as for efficacy testing. Here, we review several disease areas, and consider how the use of animal models has failed to offer effective new treatments. We also make some suggestions as to how the more human-relevant new approach methodologies might be applied to address this.

(Photo courtesy of NIH)

(Photo courtesy of NIH)

HUMAN-IMMUNE-SYSTEM MICE

- Conventional animal models often fail to accurately replicate human immune responses.
- Numerous vaccine candidates demonstrating strong efficacy in animals failed to replicate those protective effects in human clinical trials

Human-Immune-System humanized mice as "surrogate *in-vivo* human" model

animal models

(Photo courtesy of NIH)

Human-immune system DRAGA mice

(Photo courtesy of NIH)

Humans

(Photo courtesy of NIH)

DRAGA Mice
(HLA-A2.HLA-DR4.Rag1KO.IL2RgcKO.NOD)

- Super-Immunodeficient mouse strain generated at NMRC
  - Able to engraft human hematopoietic stem cells from cord blood
- Express transgenically HLA class I and class II molecules
  - Needed for development of functional human T cells
  - Needed to support human B cell Ig class switching and antibody production

- Reconstitute a functional human-immune-system, human epi/endothelial cells and hepatocytes from infused human hematopoietic stem cells from umbilical cord blood

- Sustain infection with human pathogens (including but not limited to *Plasmodium falciparum/vivax,* HIV, scrub typhus, influenza A, dengue, SARS-CoV-2, Hantaan virus, and Zika virus)

- Elicit protective human cellular and antibody responses upon vaccination

- A "*surrogate in vivo human model*" for testing safety, immunogenicity, and efficacy of human vaccines/immunotherapeutics to protect the warfighter and the public

OUTLINE

<u>PART I</u>: Generation of Human-Immune-System humanized DRAGA mice

<u>PART II</u>: DRAGA as a "surrogate" *in vivo* human model for human infectious diseases

<u>PART III</u>: DRAGA mice as a rapid platform for generation of *therapeutic human monoclonal antibodies*

DRAGA MICE GENOTYPE

Genotype	Outcome	Purpose
NOD	Permissive strain	Favor human HSC engraftment
Rag1 KO	No mouse T and B cells	To prevent the rejection of human HSC cells
IL-2 receptor γ chain KO	No mouse NK cell activity	
HLA-DR4 transgenic	Human HLA class-II and I expression of the mouse thymus	Development of human CD4 T cells
HLA-A2 transgenic		Development of human CD8 T cells

- *Danner et al. PLoS One 6(5):e19826 (2011)*
- *Majji et al. Sci Rep 6:28093 (2016)*

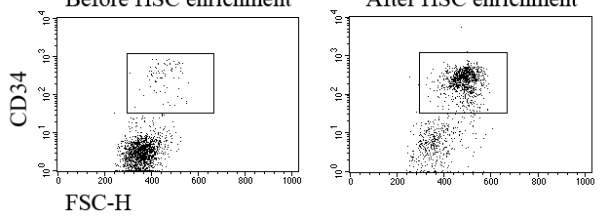
HUMANIZATION PROCEDURE

HLA-matched hHSCs from umbilical cord blood

(Photo courtesy of charlestonmoms)

T-cell depletion
1-2x10^5 hCD34$^+$/mouse

Human HSC enrichment



350 rads

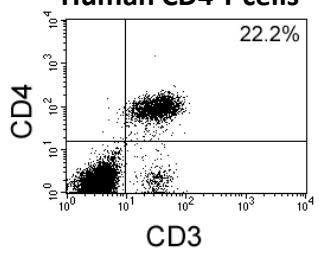
1.

2.

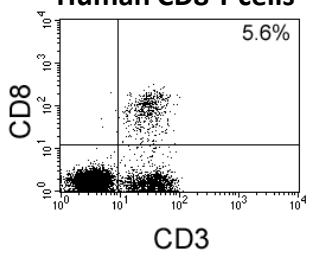
3-4 months

Human-Immune-System

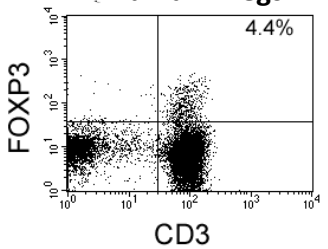
Human CD4 T cells



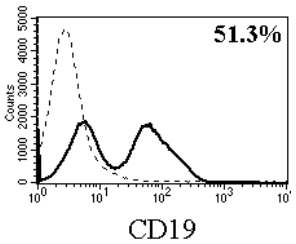
Human CD8 T cells



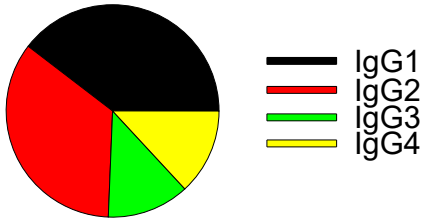
Human Tregs



Human B cells



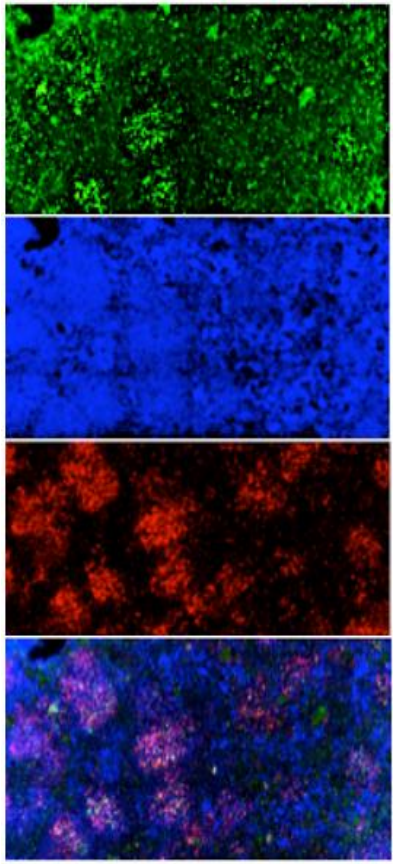
Serum levels of human IgGs



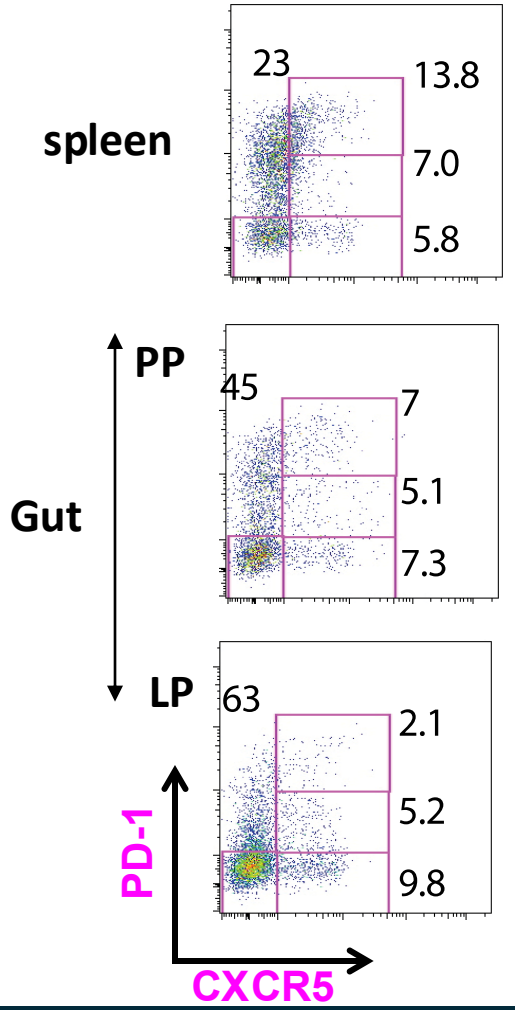
- **Human monocyte/macrophages, human dendritic cells, human neutrophils, human microglial cells, and human Kupffer cells**

Human T Follicular Helper Cells and Peyer's patches in gut

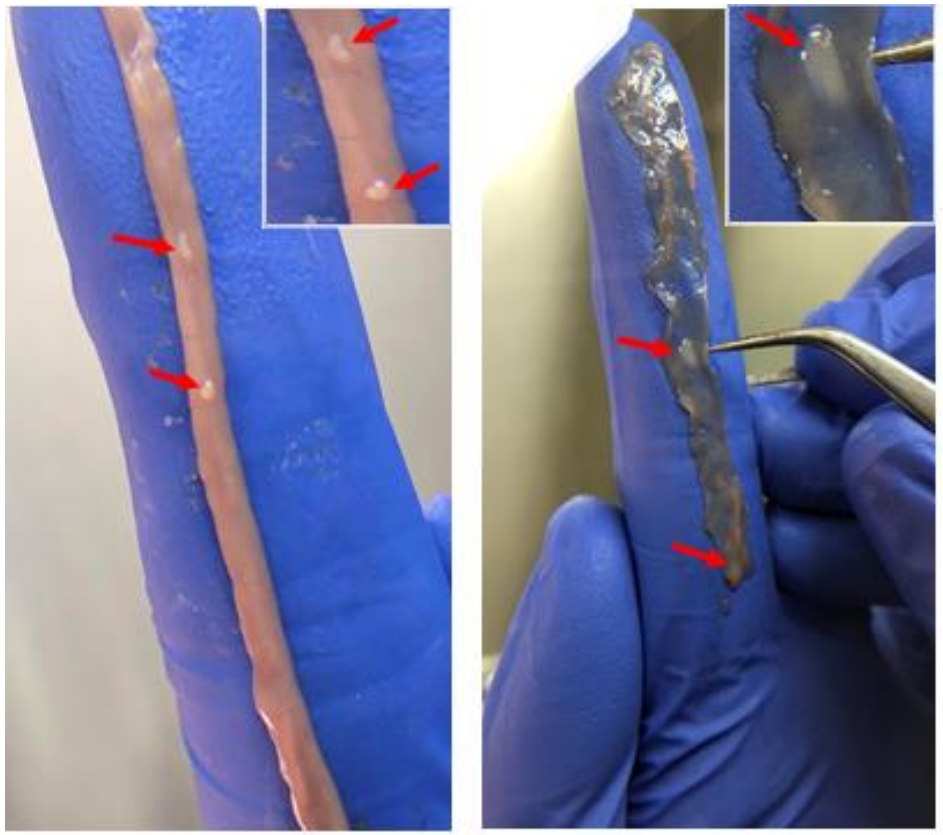
Human TFH cells



Gated human CD4 T cells



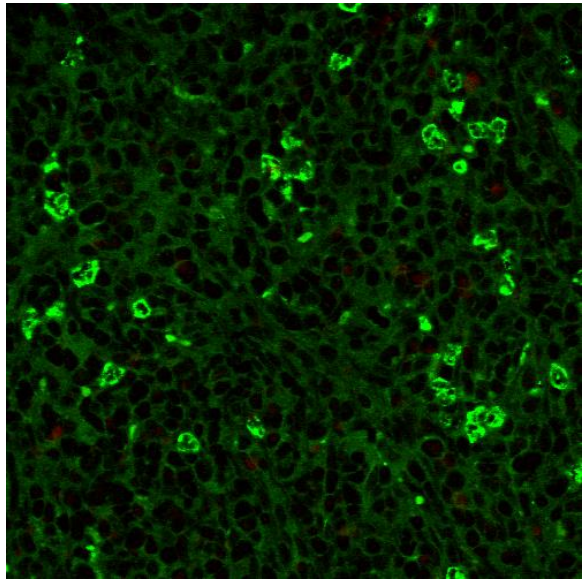
Peyer's Patches



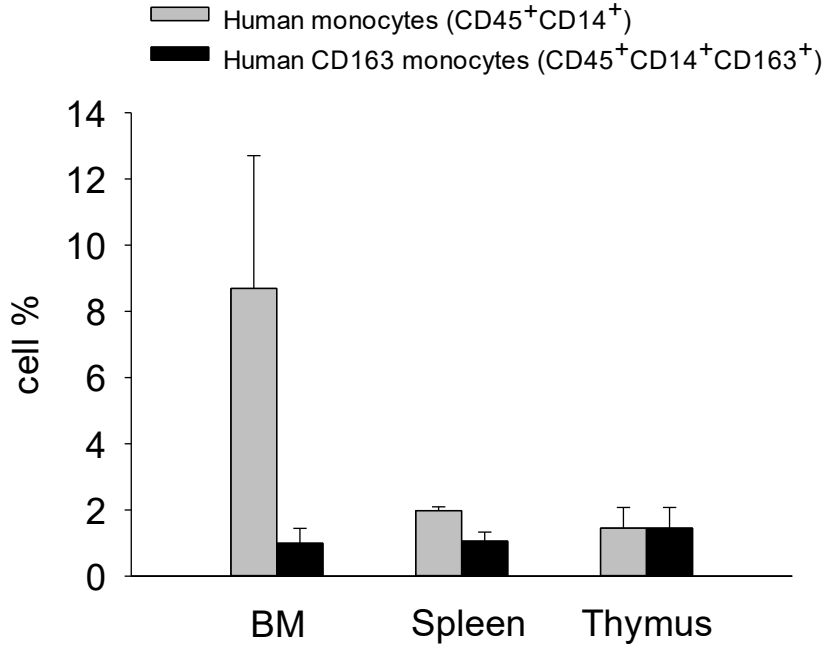
Sci Rep 5:10443 (2015); J Immunol Methods 454:40, 2018

HUMAN MONOCYTES/MACROPHAGES

Mesenteric Lymph node

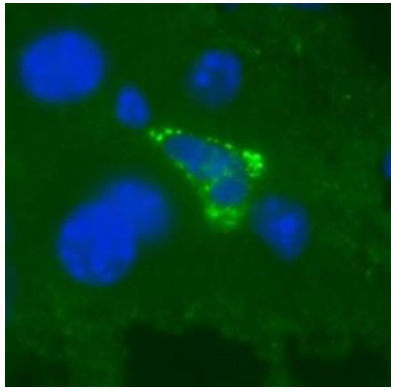


CD68



Human Kupffer cells

(*liver-resident macrophages*)



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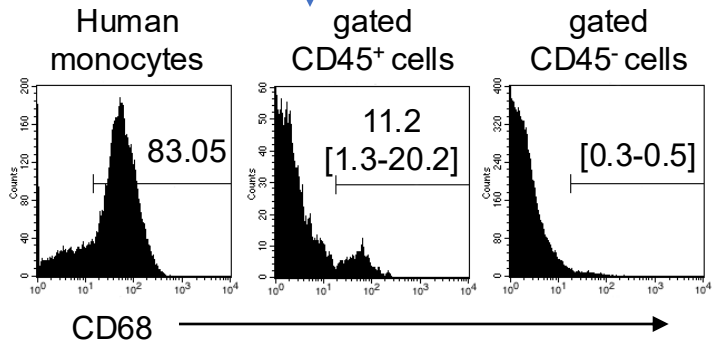
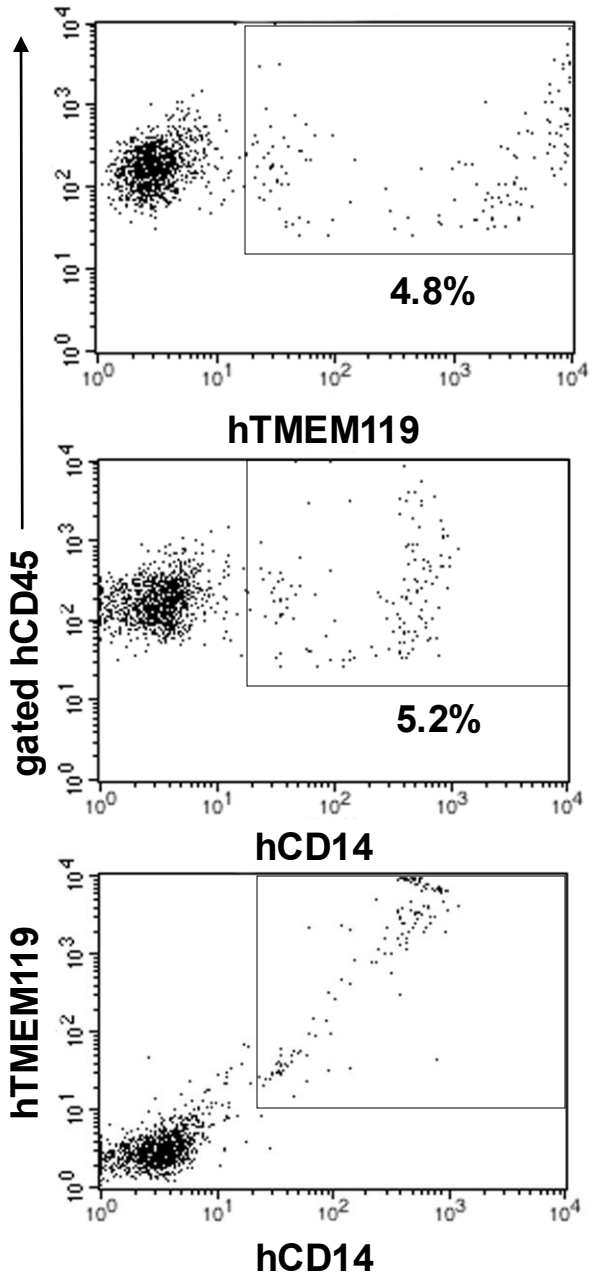
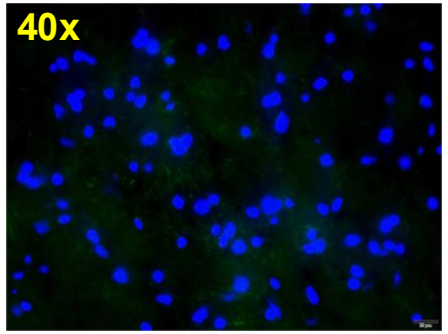


- *Malar J. 13: 386 (2014)*

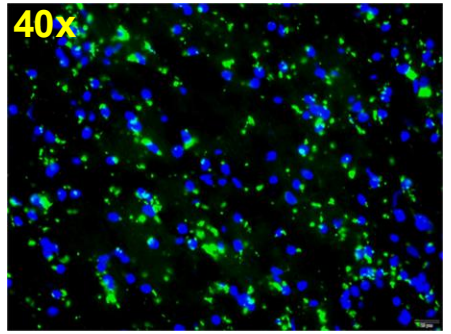
HUMAN MICROGLIA



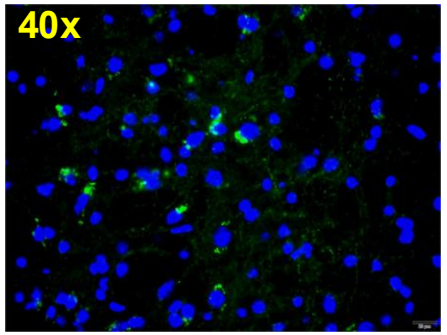
hTMEM119 (green) + DAPI (blue)



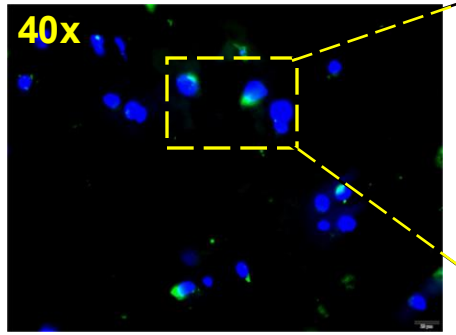
Control DRAGA brain



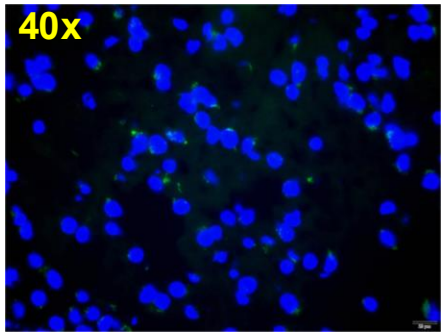
Human brain



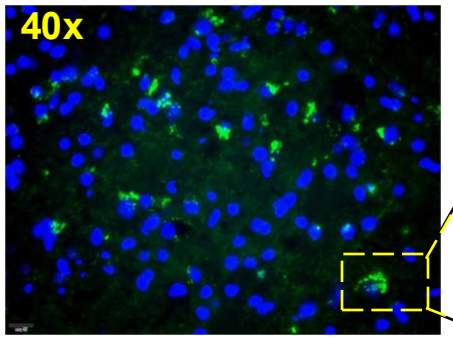
HIS-DRAGA brain#1



Human brain

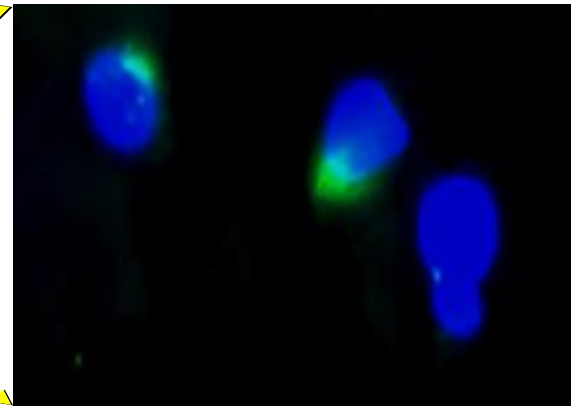


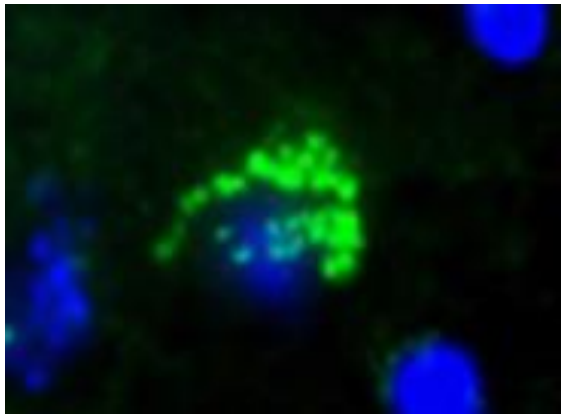
HIS-DRAGA brain#2



HIS-DRAGA brain#3

Front Cell Infect Microbiol 14:1367566, 2024



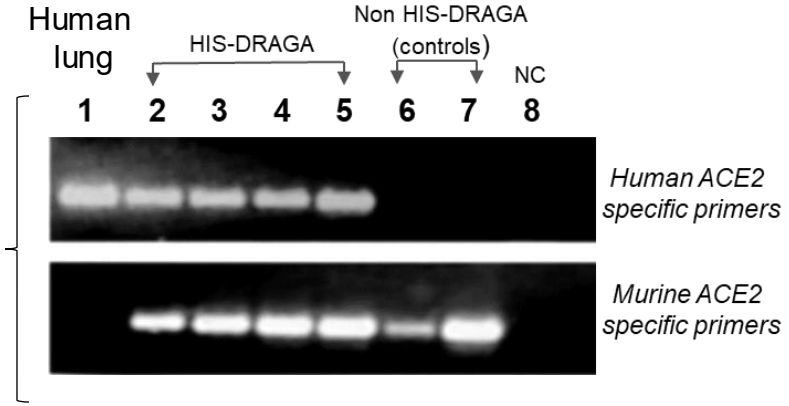


HUMAN LUNG CELLS EXPRESSING ACE2

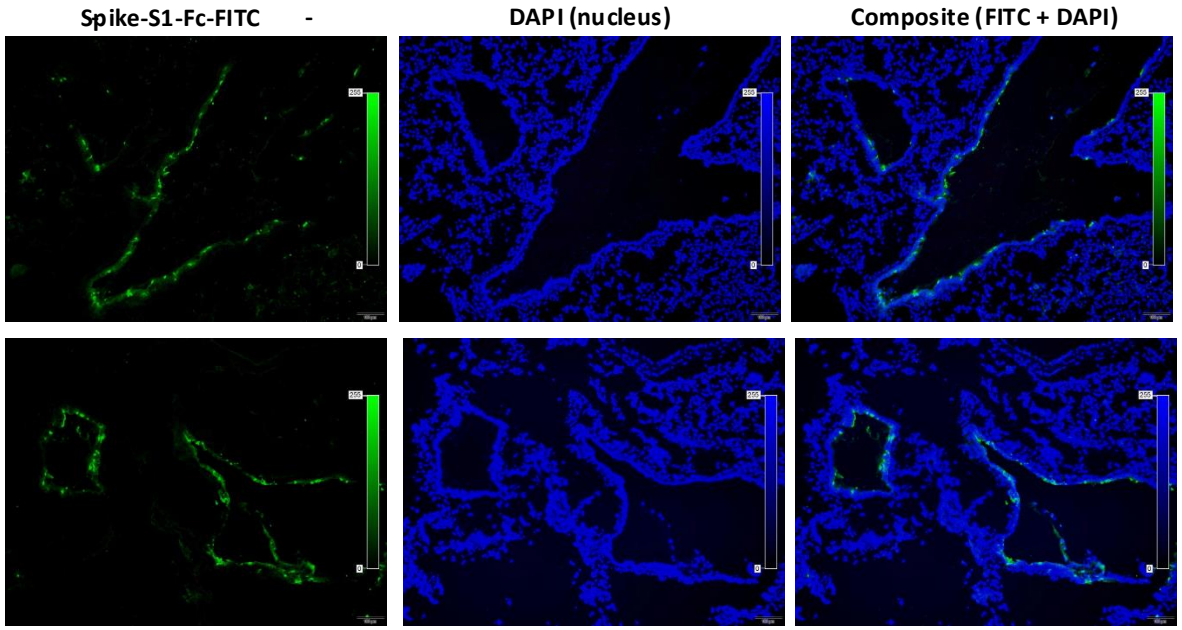
Lungs

(Photo courtesy of Pixabay)

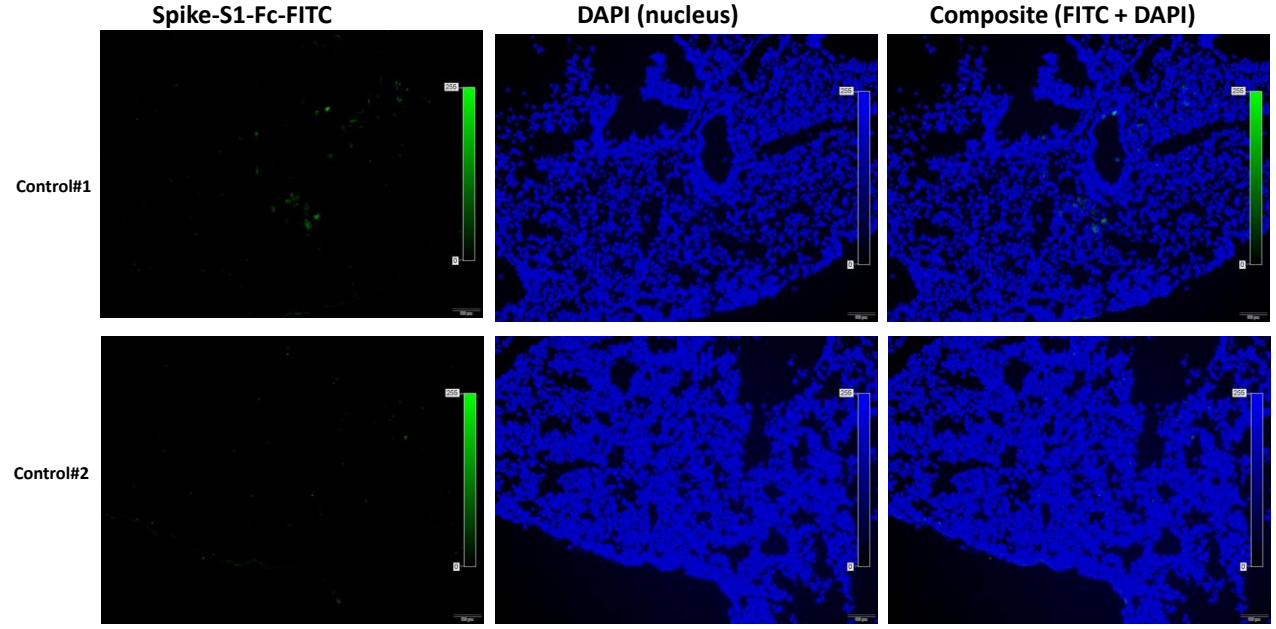
RT-PCR



Lung sections from Humanized DRAGA mice

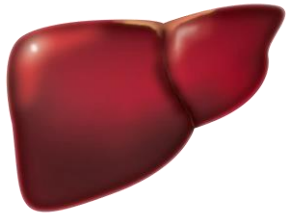


Lung sections from CONTROL (non- HIS) DRAGA mice

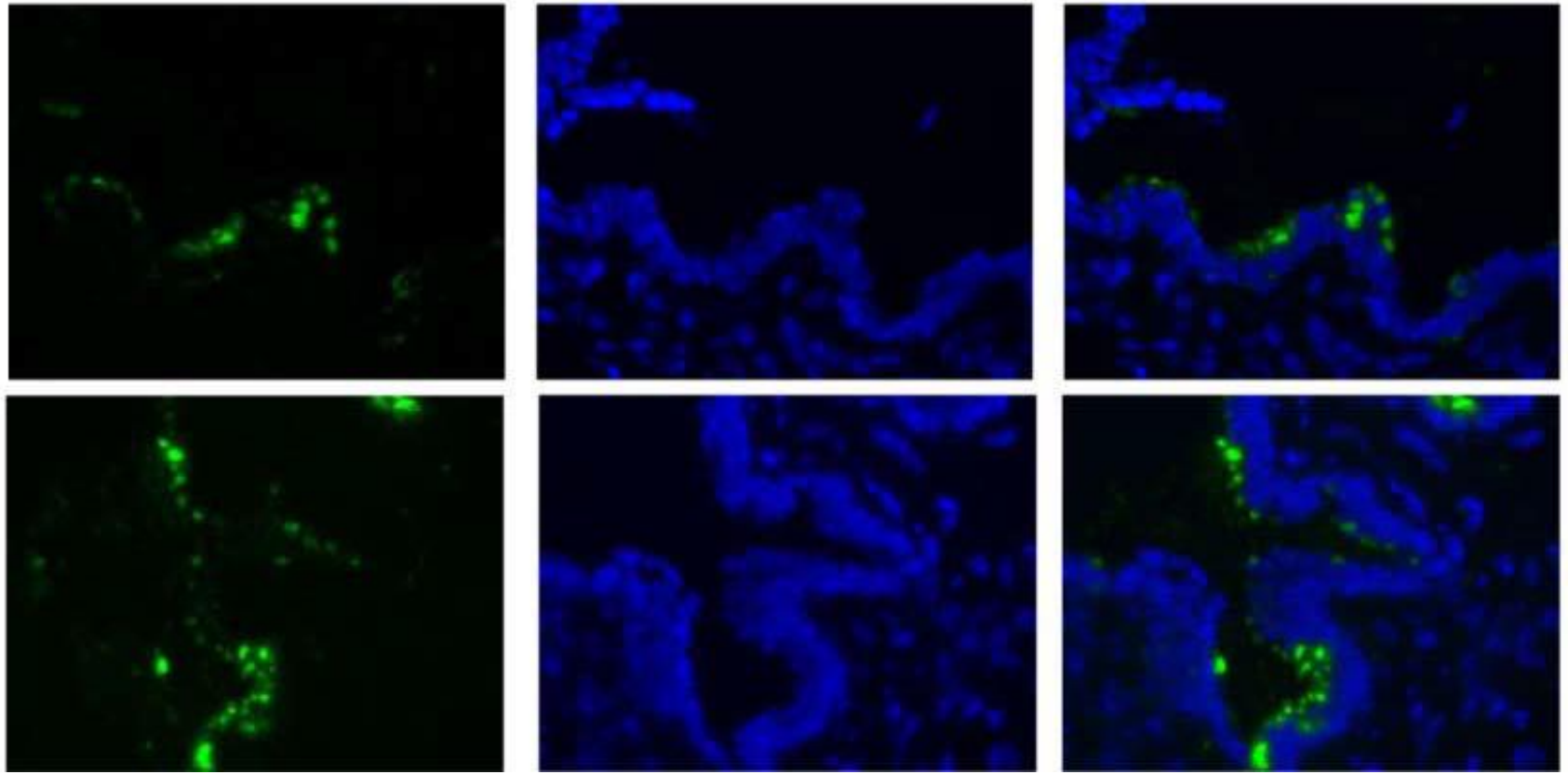


HUMAN ENDOTHELIAL CELLS

Liver



(Photo courtesy of Pixabay)



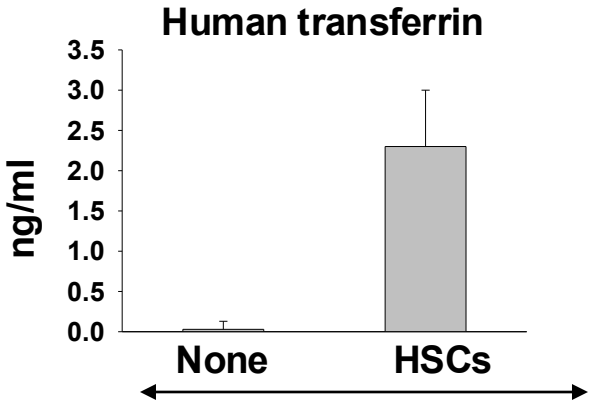
hCD36 (green) + DAPI (blue)

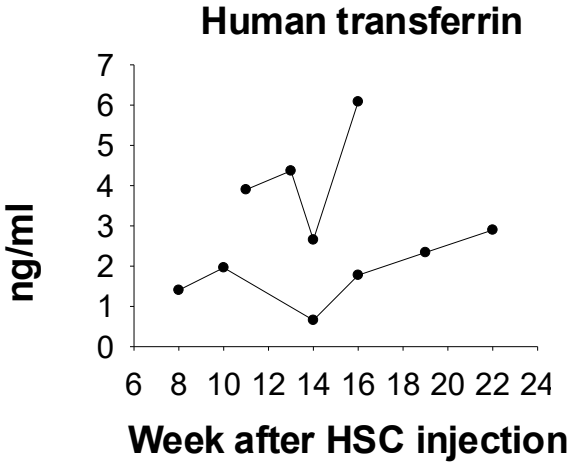
Malar J. 13: 386 (2014).

HUMAN HEPATOCYTES

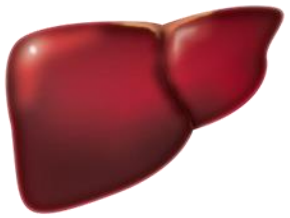
Serum

(Photo courtesy of NIH)

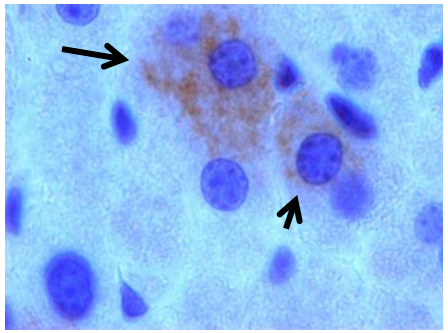




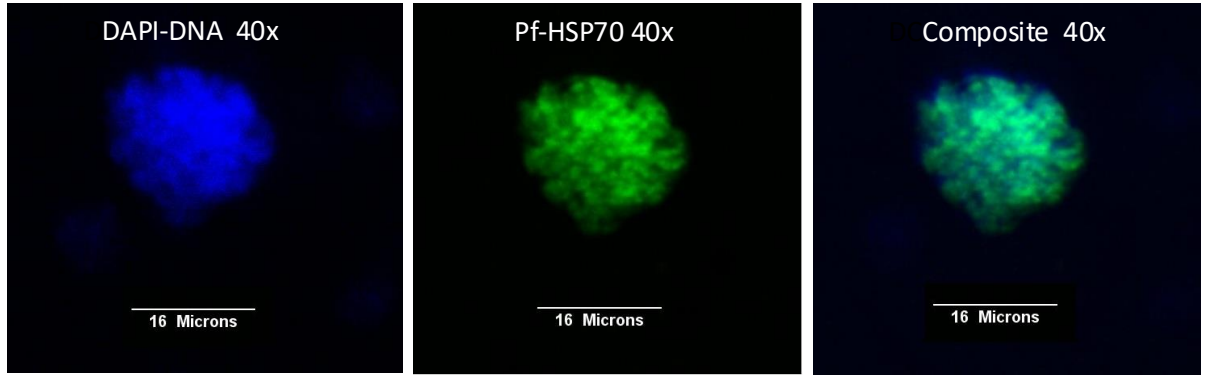
Liver



(Photo courtesy of Pixabay)



Human albumin



Malar J. 13: 386 (2014).

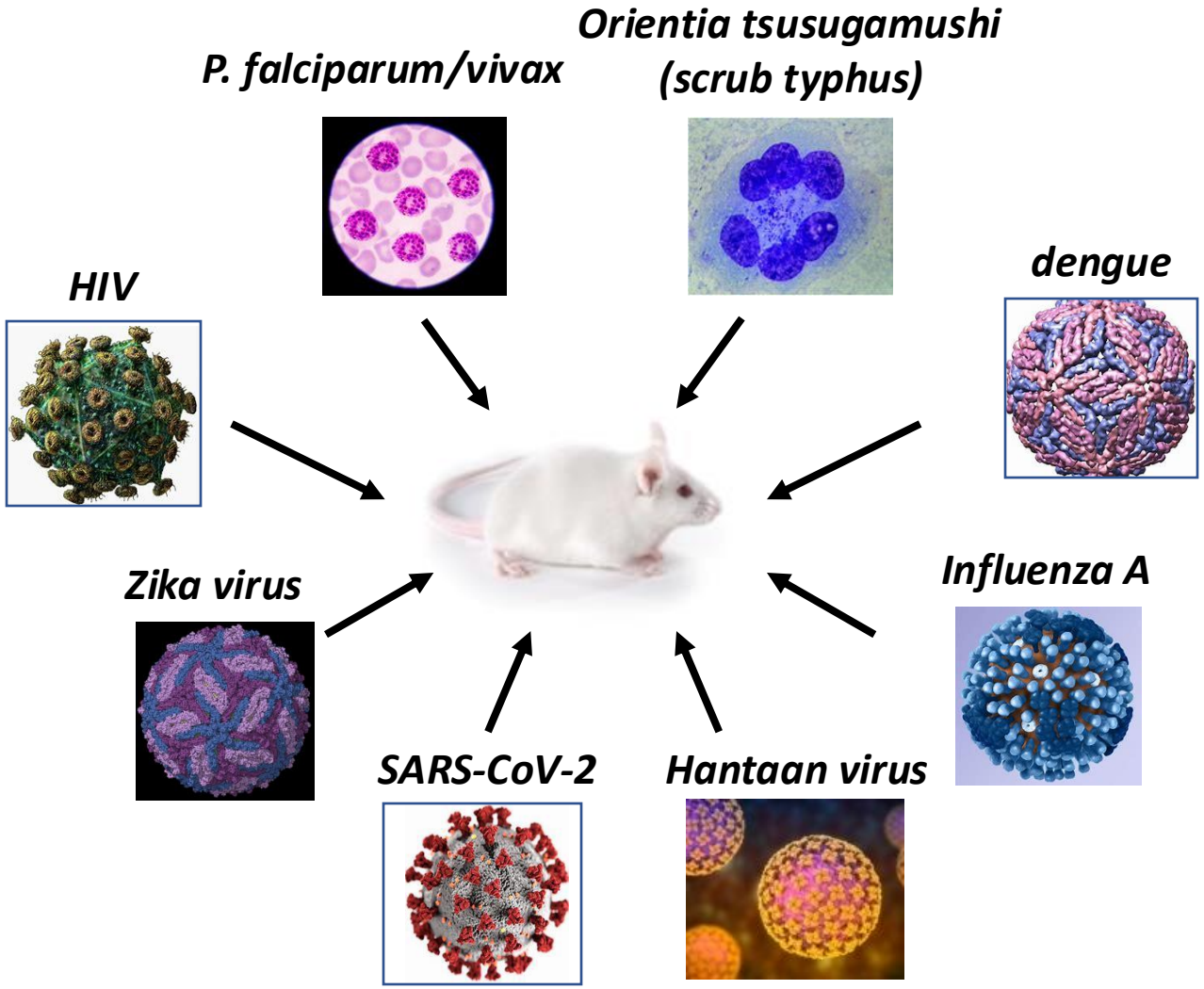
OUTLINE

PART I: Generation of Human-Immune-System humanized DRAGA mice

PART II: DRAGA as a "surrogate" *in vivo* human model for human infectious diseases

PART III: DRAGA mice as a rapid platform for generation of *therapeutic human monoclonal antibodies*

INFECTION WITH HUMAN PATHOGENS



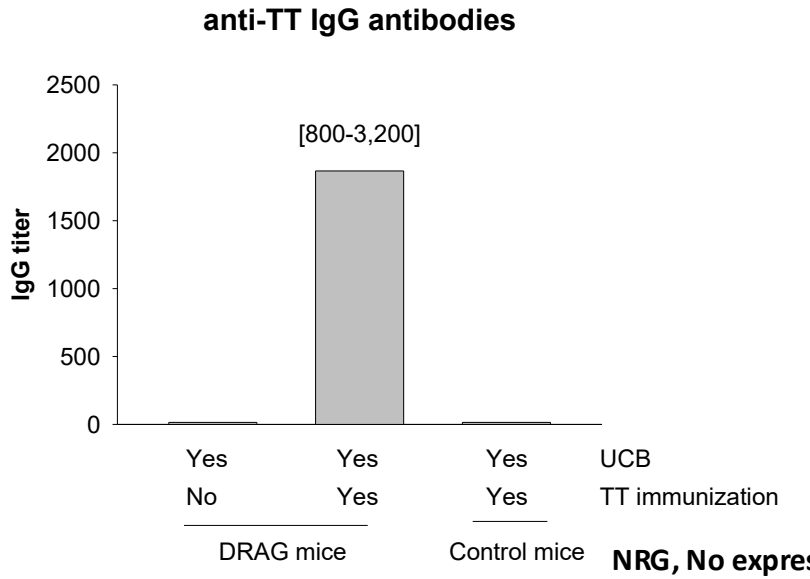
(Photos courtesy of NIH)

- *Malar J. 13: 386 (2014).*
- *Sci Rep 5:10443 (2015).*
- *AIDS Res Hum Retroviruses. 32:109-19 (2016)*
- *Sci Rep 6:28093 (2016)*
- *Front Immunol. 8:1405 (2017)*
- *Ebiomedicine doi:10.1016/j.ebiom (2017)*
- *Hum Vaccin Immunother doi:10.1080/21645515 (2018)*
- *J Immunol Methods* 454:40 (2018)
- *Mal J.* 17:114 (2018)
- *Front Immunol. 9:816 (2018)*
- *Hum Vacc Immunother.* Doi:10.1080/21645515 (2020)
- *Front Immunol 13:1047277 (2022)*
- *Hum Vacc Immunother 14:1367566 (2024)*
- *J Immunol. 214(11):3017-3027 (2025).*

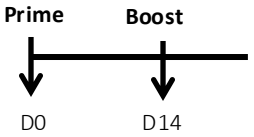
HUMAN ANTIBODY RESPONSES

(Photo courtesy of IndiaMART)

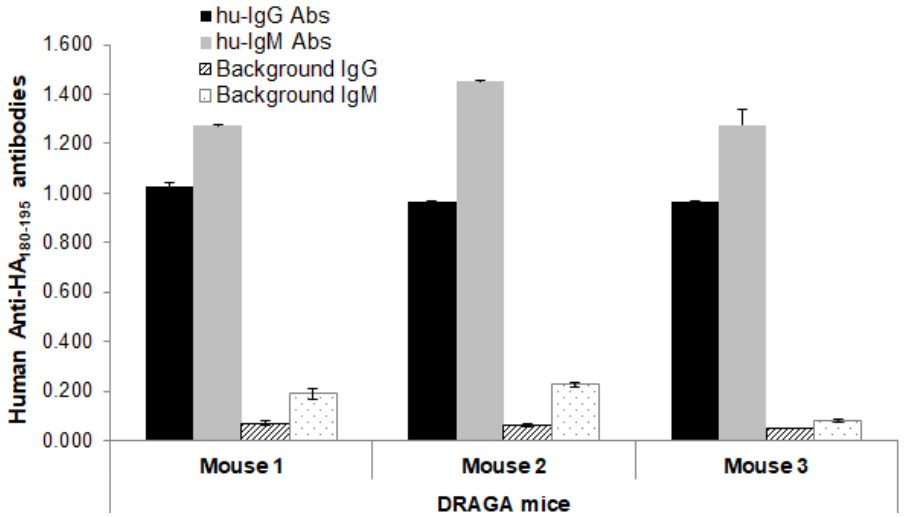
14 days p.i.



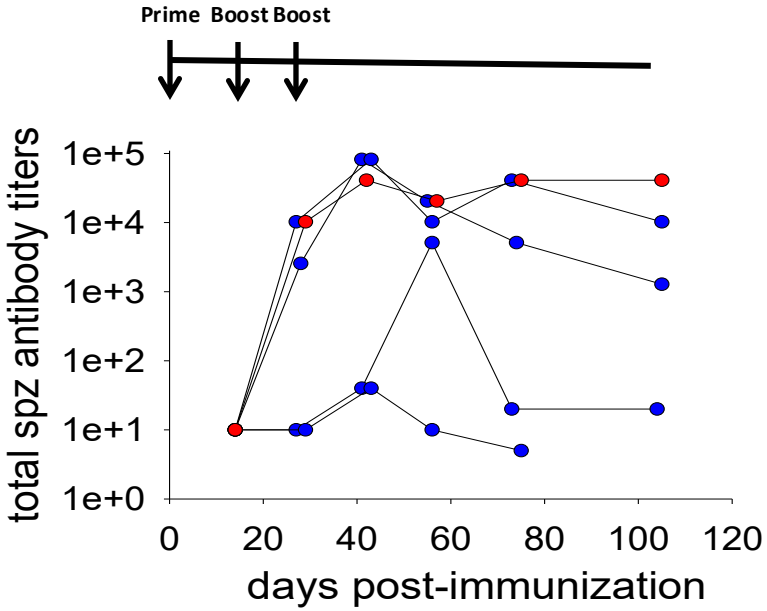
KLH-HA



NRG, No expressing HLA molecules



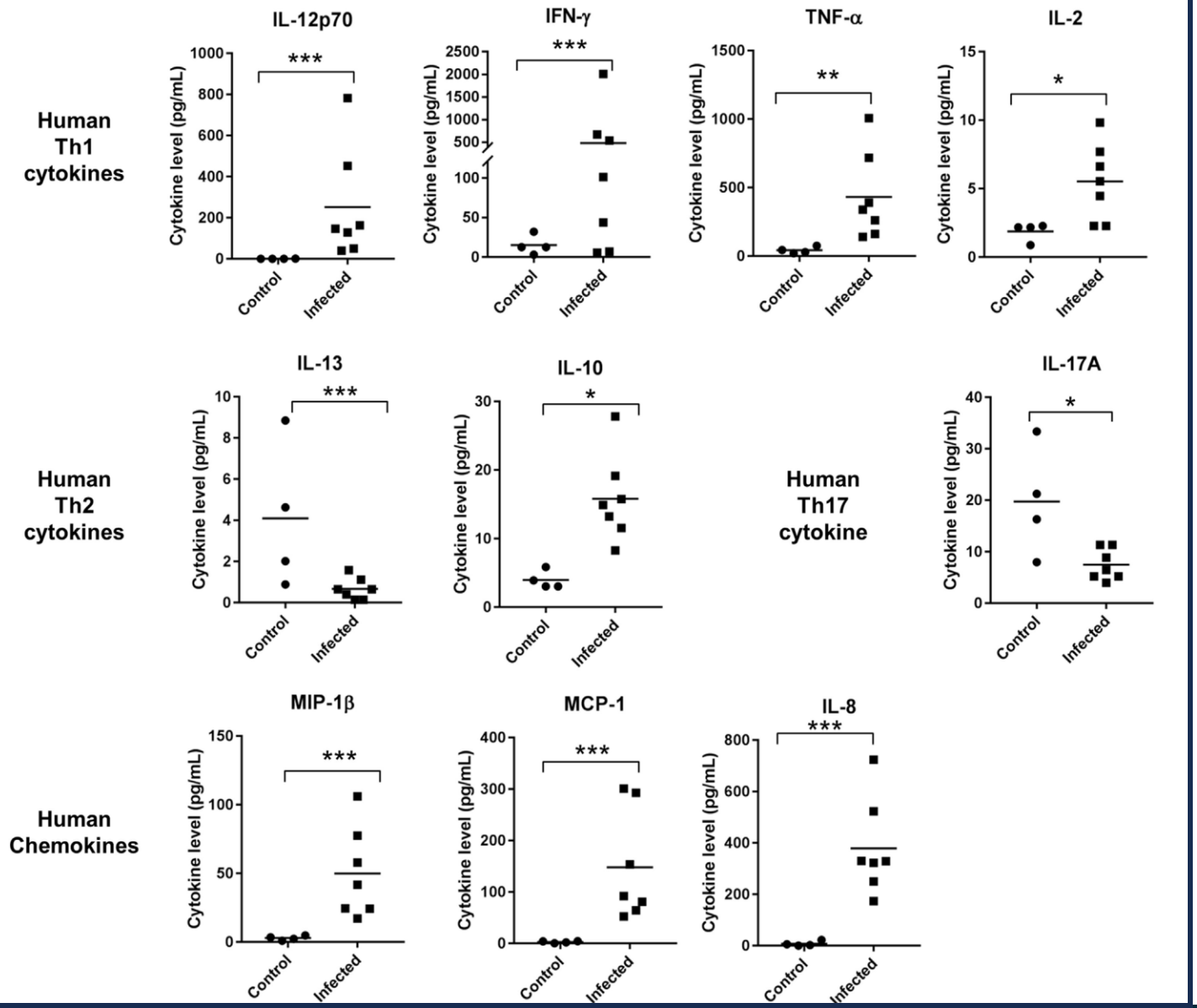
Live Pfspz under chloroquine prophylaxis



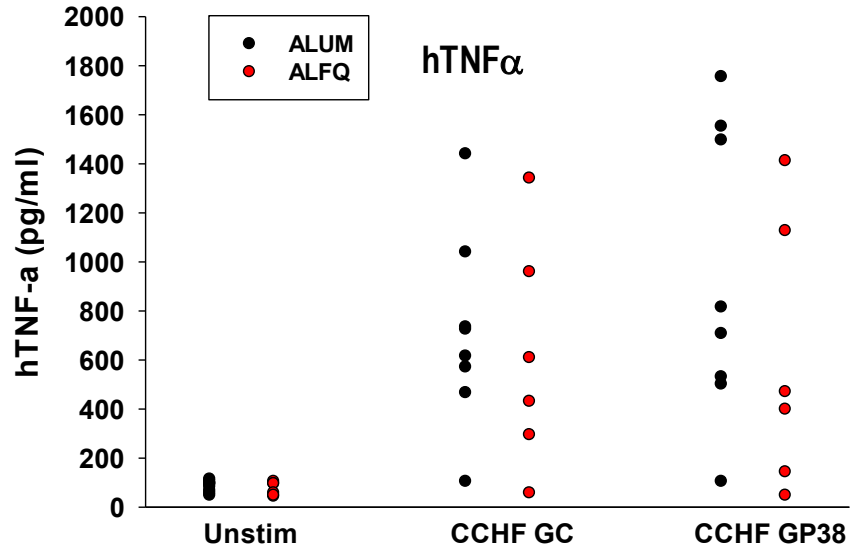
Hum Vacci Immunother doi:10.1080/21645515 (2018)
Hum Vacc Immunother. Doi:10.1080/21645515 (2020)
Mal J. 17:114 (2018)

HUMAN CELLULAR RESPONSES

***INFECTION**: Orienta tsusugamushi (scrub typhus)*
Serum cytokines



***IMMUNIZATION**: Crimean-Congo Hemorrhagic fever DNA vaccine (GP) + GP protein adjuvanted in Alum or ALFQ*



DRAGA Mice for Infectious Diseases and Therapeutic Testing

Human immune system

Infection with
human pathogens

Testing safety, immunogenicity and protective efficacy of human vaccine/immunotherapeutics

Protection against pathogen challenge

Majji et al. Malar J (2018) 17:114
https://doi.org/10.1186/s12936-018-2264-y

Malaria Journal

RESEARCH Open Access

Humanized DRAGA mice immunized with *Plasmodium falciparum* sporozoites and chloroquine elicit protective pre-erythrocytic immunity

Sai Majji[1†], Wathsala Wijayalath[1†], Soumya Shashikumar[1], Teodor D. Brumeanu[2] and Sofia Casares[1,2*]

EBioMedicine 25 (2017) 87–94

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

A DNA Vaccine Protects Human Immune Cells against Zika Virus Infection in Humanized Mice

Guohua Yi *[,1], Xuequn Xu [1], Sojan Abraham [2], Sean Petersen, Hua Guo, Nora Ortega, Premlata Shankar, N. Manjunath *

Center of Emphasis in Infectious Diseases, Department of Biomedical Sciences, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, TX, United States

The Journal of Immunology, 2025, **214**, 3017–3027
https://doi.org/10.1093/jimmun/vkaf185
Advance access publication August 13, 2025
Research Article

American Association of Immunologists

OXFORD

Humanized DRAGA mice are a valuable model to study novel immunotherapies for HIV-1

Pongthorn Pumtang-On [1], Liliana K. Thron [1,2], Negin Goodarzi[1], Brianna C. Davey[1], Emily N. Sevcik[1], Natalie Coleman-Fuller[3], Ahmad F. Karim[4], Vaiva Vezys[5], Mangala Rao[6], Branden S. Moriarity[7], Mary S. Pampusch[1], Aaron K. Rendahl[1], Sofia A. Casares[4], and Pamela J. Skinner[1,3,*]

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[2]Graduate Program in Molecular Pharmacology and Therapeutics, University of Minnesota, Minneapolis, MN, United States
[3]MarPam Pharma, LLC, St Paul, MN, United States
[4]Agile Vaccines and Therapeutic, Infectious Diseases Directorate, Naval Medical Research Command, Silver Spring, MD, United States
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Molecular Therapy
Advances
Original Article

NK cell immunotherapy after analytic treatment interruption is associated with HIV viral control

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OUTLINE

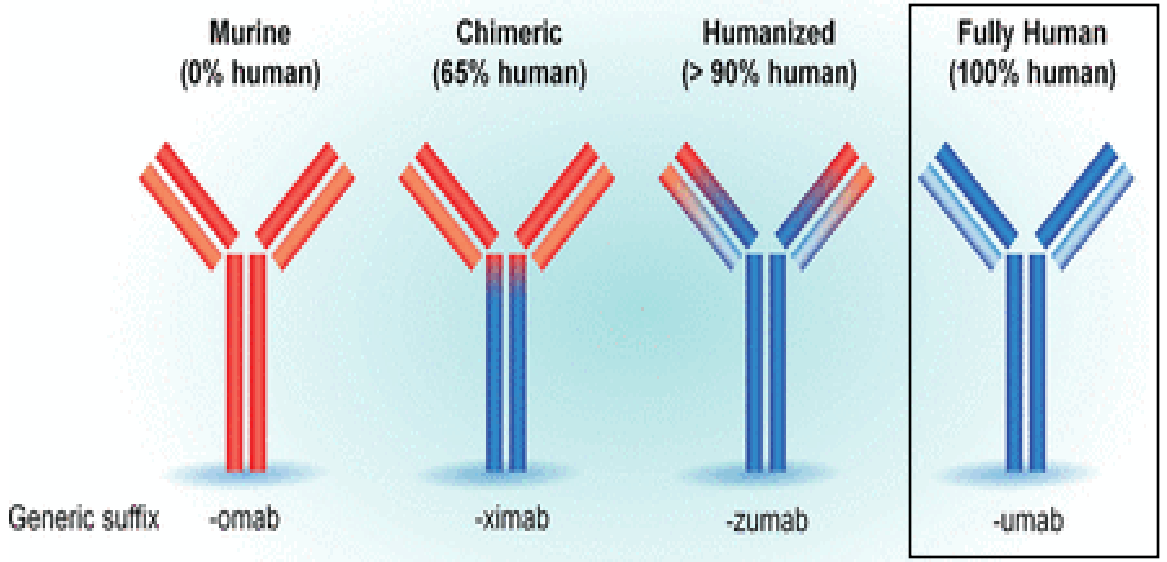
PART I: Generation of Human-Immune-System humanized DRAGA mice

PART II: DRAGA as a "surrogate" *in vivo* human model for human infectious diseases

PART III: DRAGA mice as a rapid platform for generation of *therapeutic human monoclonal antibodies*

FDA-APPROVED mAbs

Mouse origin		Human origin: Source
Mouse mAbs	Genetically-engineered Mouse mAbs	• Blood • Phage/yeast display • DRAGA mice



- ✓ Minimal immunogenicity
- ✓ Multiple treatments possible (high-level efficacy)
- ✓ Improved serum half-live

DRAGA MICE AS A RAPID PLATFORM FOR HU-MAB GENERATION

Human Immune System

1. Vaccination (*non-pharmaceutical grade*)
2. Experimental Infection with Human Pathogens

3. Recover human B cells from spleen/bone marrow

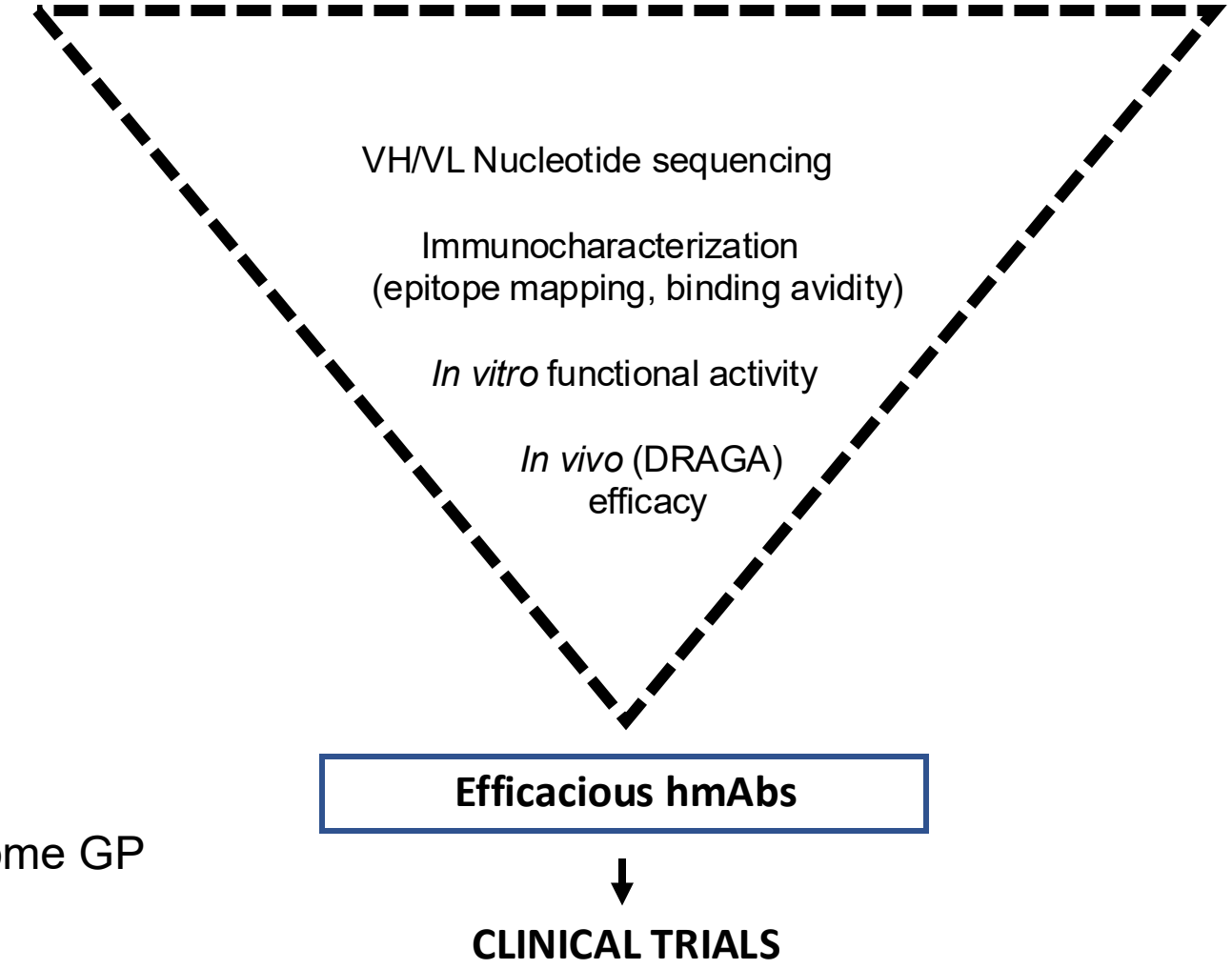
4. Generation of human mAbs

(Photos courtesy of kauveryhospital)

Hu-MABs GENERATED FROM DRAGA MICE

- ✓ *P. falciparum CSP*
- ✓ *P. vivax CSP*
- ✓ HA cross-reactive (influenza A)
- ✓ *Orientia Tsusugamushi* major protein
- ✓ Ebola GP
- ✓ SARS-CoV-2 spike protein
- ✓ Hantavirus GP
- ✓ Crimean-Congo Hemorrhagic fever GP
- ✓ Severe Fever with Thrombocytopenia syndrome GP

Strategy for hmAb down-selection



SUMMARY: DRAGA MICE

- ✓ Reconstitute human cells by infusion of human hematopoietic stem cells from umbilical cord blood
- ✓ Establishes a robust, highly translational *pre-clinical model* for the rigorous evaluation of human vaccine candidate safety, immunogenicity, and protective efficacy.
- ✓ Serves as an accelerated, high-throughput mechanism for the rapid *in vivo* generation of therapeutic human monoclonal antibodies.

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