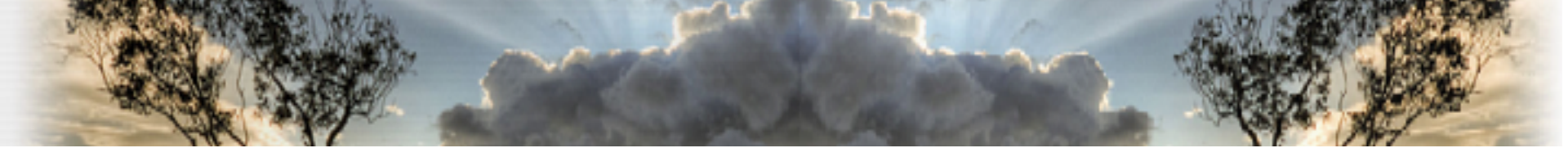


EBF

The Omicron Workshop

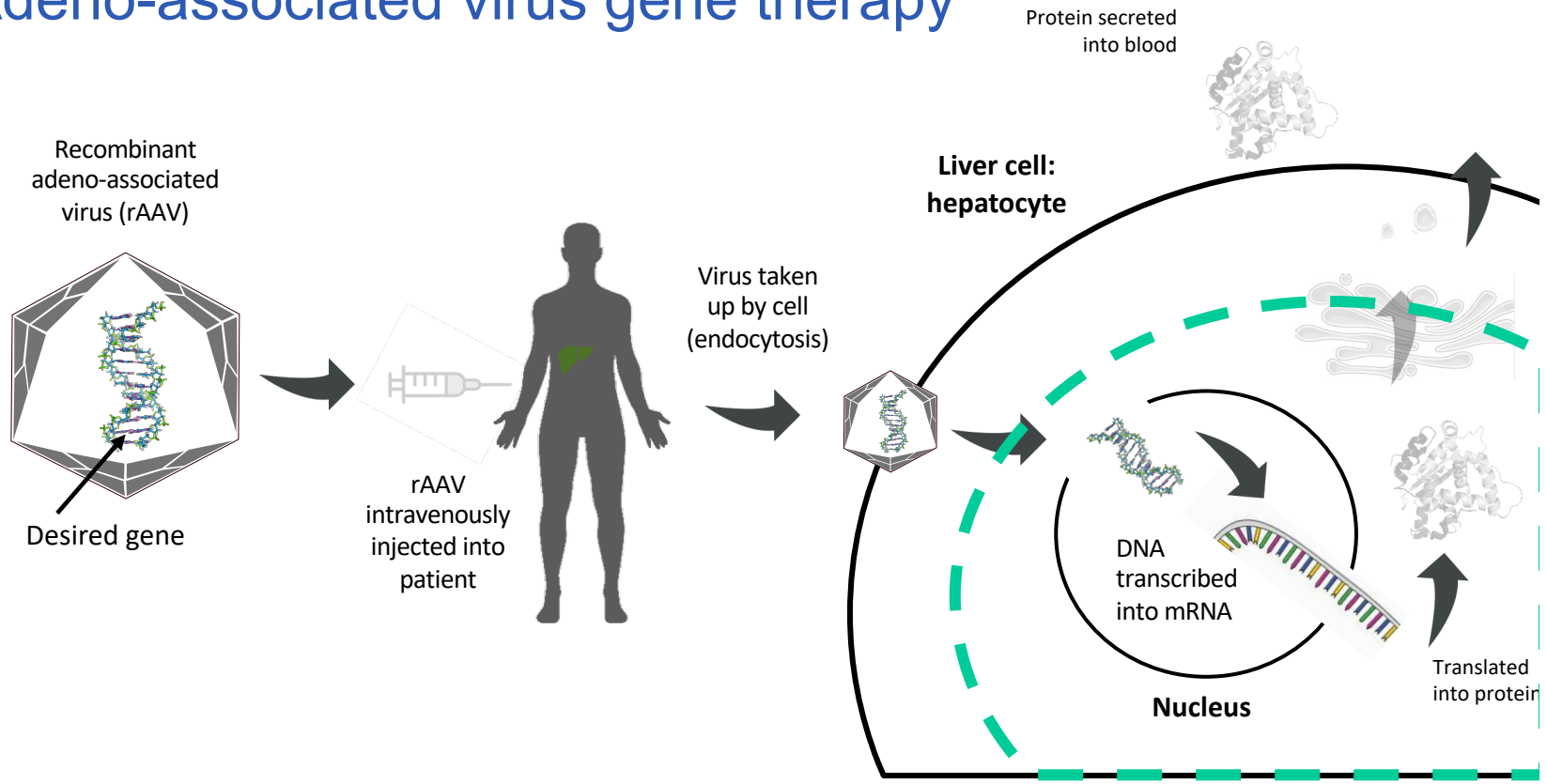
Points to Consider on Cut Points

Overview of regulatory expectations for cut points for pre-existing Ab

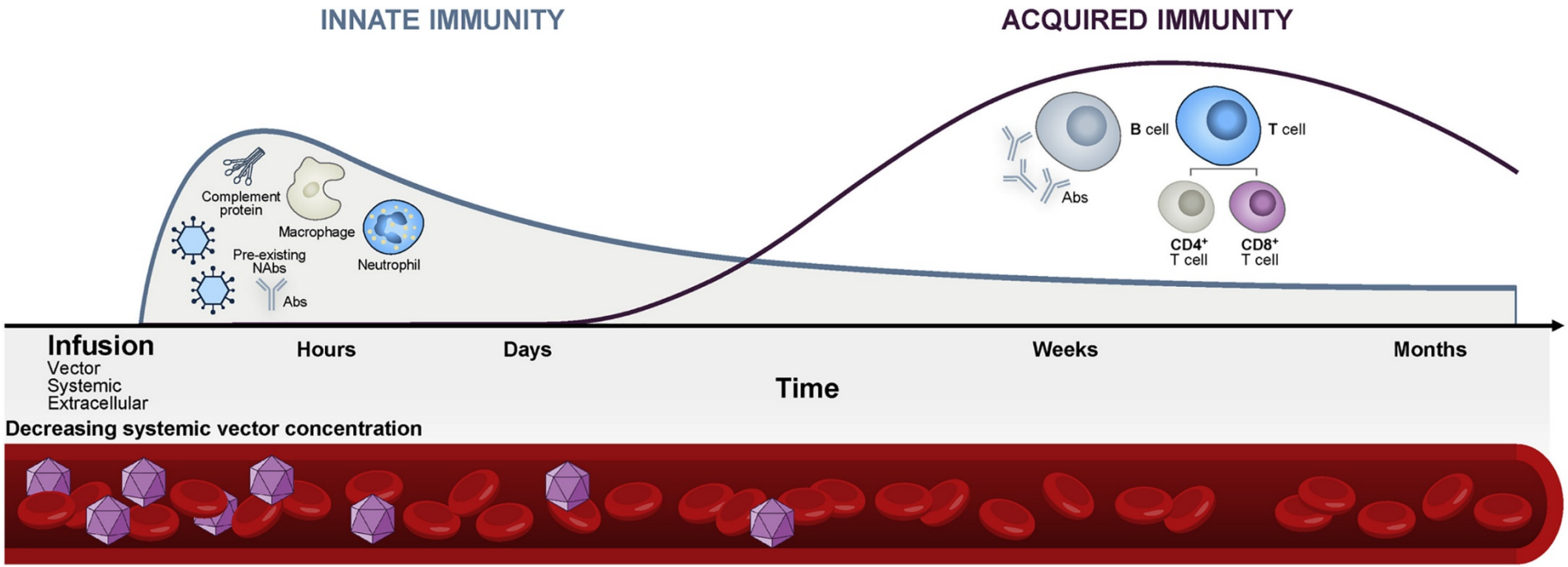
Johannes Stanta, on behalf of the EBF

28-29 April 2022 – in Cyberspace

Adeno-associated virus gene therapy



Golgi apparatus image adapted from https://qsstudy.com/biology/describe-golgi-apparatus. Protein image adapted from https://foodscience-techn.blogspot.com/2014/07/structure-of-protein.html. mRNA, messenger RNA; rAAV, recombinant adeno-associated virus



From Mendell et al., 2022, Mol Ther Methods Clin Dev. 2022 Jun 9; 25: 74–83.

AAV capsids can trigger a strong immune response

BioA challenges: anti-capsid antibodies

- Anti-capsid antibodies to AAV capsids are common in the general population
- NAbs linked to reduced efficacy
- Linked to patient selection
- Companion diagnostic

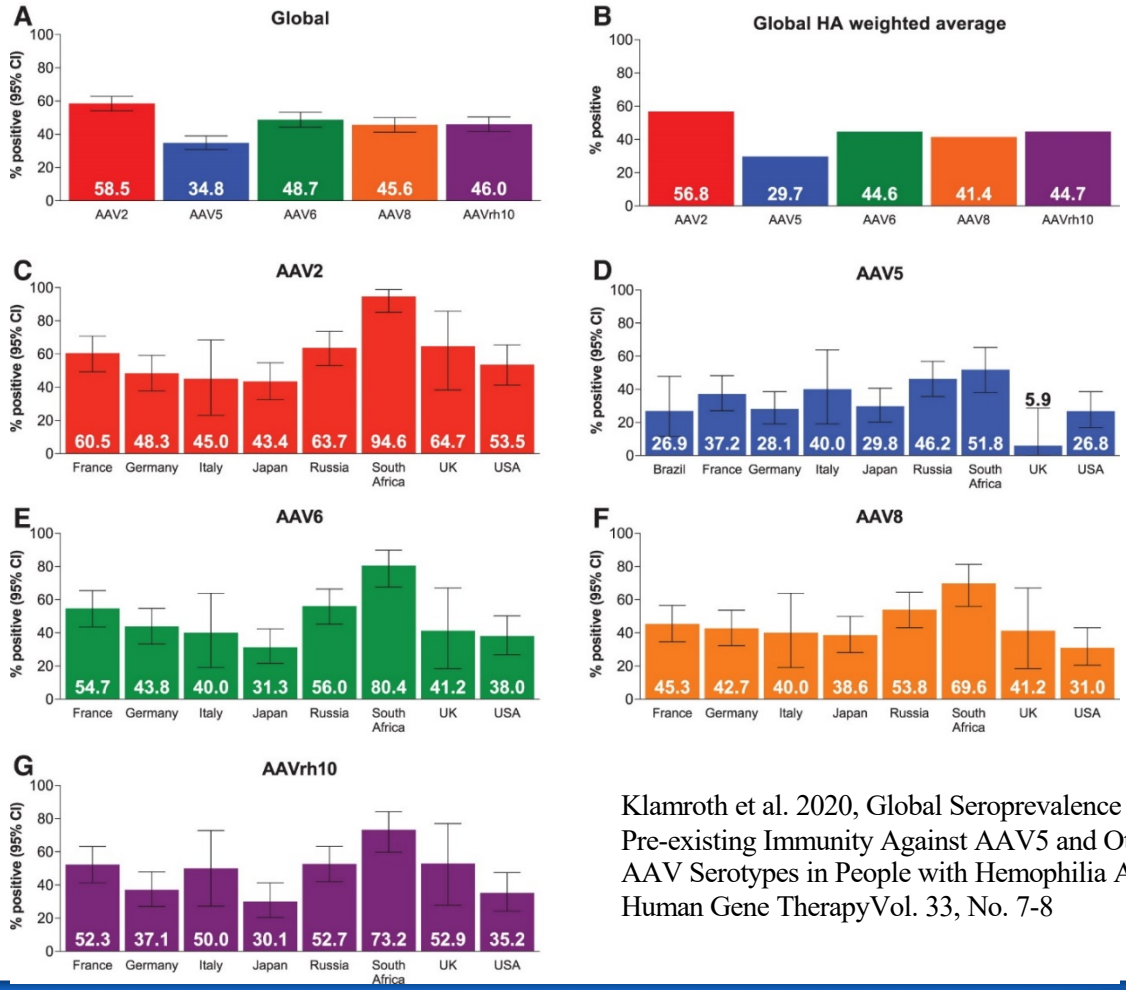
What is the seroprevalence of pre-existing immunity?

Open camera or QR reader and scan code to access this article and other resources online.

Global Seroprevalence of Pre-existing Immunity Against AAV5 and Other AAV Serotypes in People with Hemophilia A

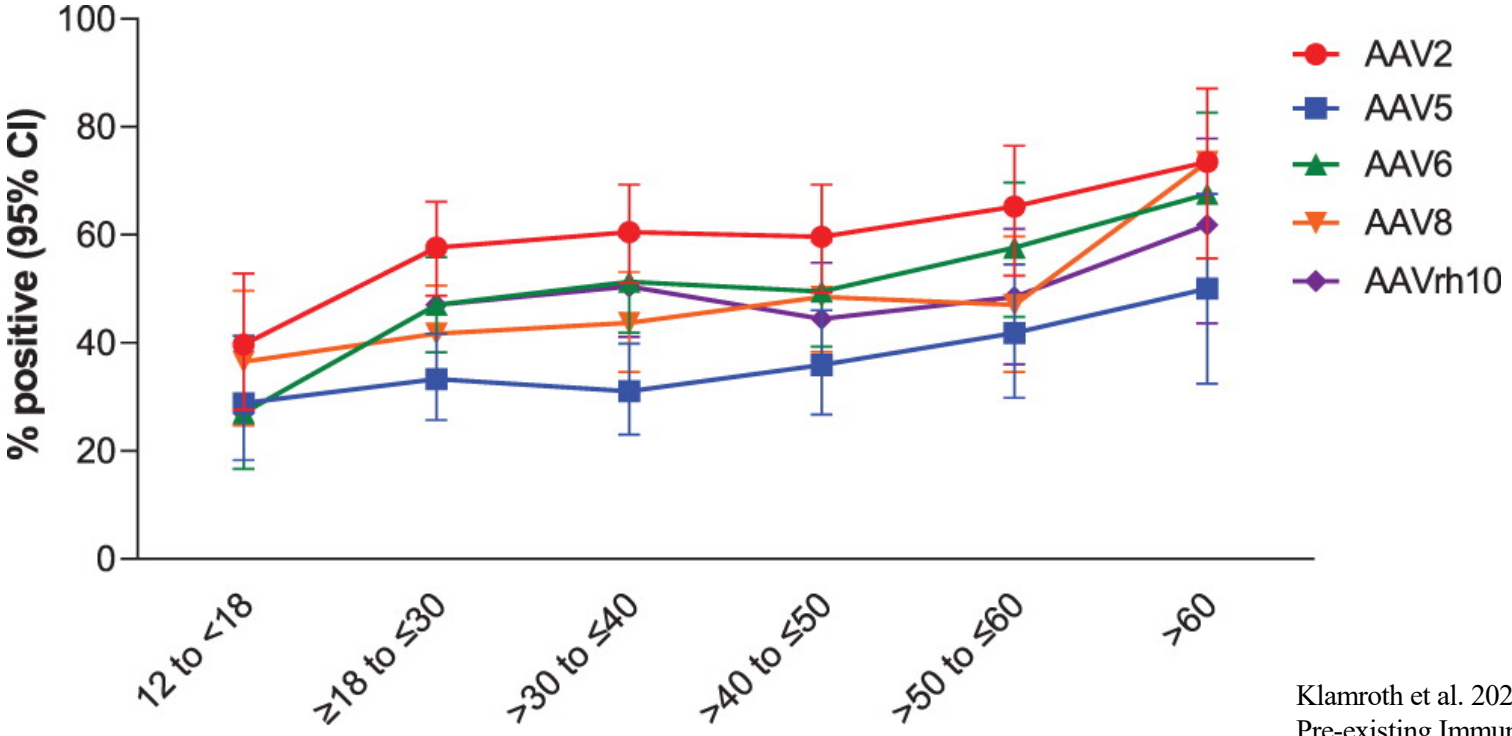
Robert Klamroth,[1,*] Gregory Hayes,[2] Tatiana Andreeva,[3] Keith Gregg,[2] Takashi Suzuki,[4] Ismail Haroon Mitha,[5] Brandon Hardesty,[6] Midori Shima,[7] Toni Pollock,[8] Patricia Slev,[8] Johannes Oldenburg,[9] Margareth C. Ozelo,[10] Natalie Stieltjes,[11] Sabine-Marie Castet,[12] Johnny Mahlangu,[13] Flora Peyvandi,[14,15] Rashid Kazmi,[16] Jean-François Schved,[17] Andrew D. Leavitt,[18] Michael Callaghan,[19] Brigitte Pan-Petesch,[20] Doris V. Quon,[21] Jayson Andrews,[2] Alex Trinh,[2] Mingjin Li,[2] and Wing Yen Wong[2]

Seropositivity to different AAVs and geographical regions



Klamroth et al. 2020, Global Seroprevalence of Pre-existing Immunity Against AAV5 and Other AAV Serotypes in People with Hemophilia A, Human Gene TherapyVol. 33, No. 7-8

AAV Seropositivity by age group



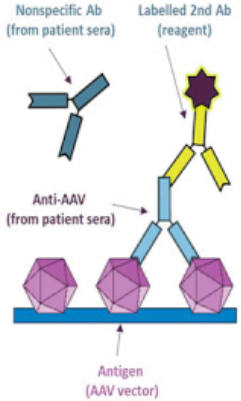
Klamroth et al. 2020, Global Seroprevalence of Pre-existing Immunity Against AAV5 and Other AAV Serotypes in People with Hemophilia A, Human Gene TherapyVol. 33, No. 7-8

TOTAL ANTIBODY ASSAY (ELISA BINDING ASSAY)

PREEXISTING INNATE IMMUNITY TO AAV CAN LEAD TO:

REDUCED EFFICACY

- Neutralization
- Opsonization



Detects **all** antibodies that bind to AAV antigen

- ✓ Neutralizing, opsonizing and complement-activating Ab
- ✓ Non-neutralizing Ab

NEUTRALIZING ANTIBODY ASSAY (CELL-BASED)

SAFETY CONCERNS

- Inflammation
- Cascade effector function (thrombocytopenia, aHUS, immune complex deposition)

Detects **only** antibodies that interfere with transduction

- ✓ Neutralizing Ab
- × Non-neutralizing Ab

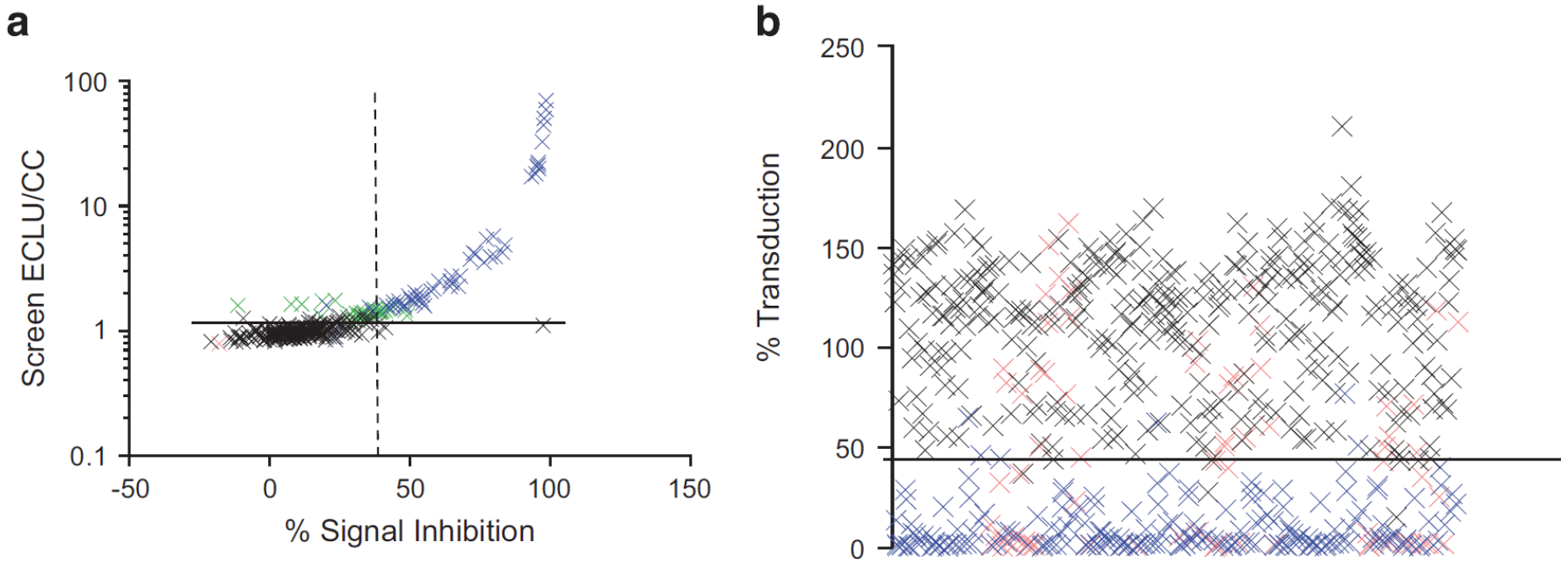
Mendell et al. Mol Ther Methods Clin Dev. 2022 Jun 9; 25: 74–83.

How to choose individuals for setting the cut-point?

Standard ADA guideline doesn't apply

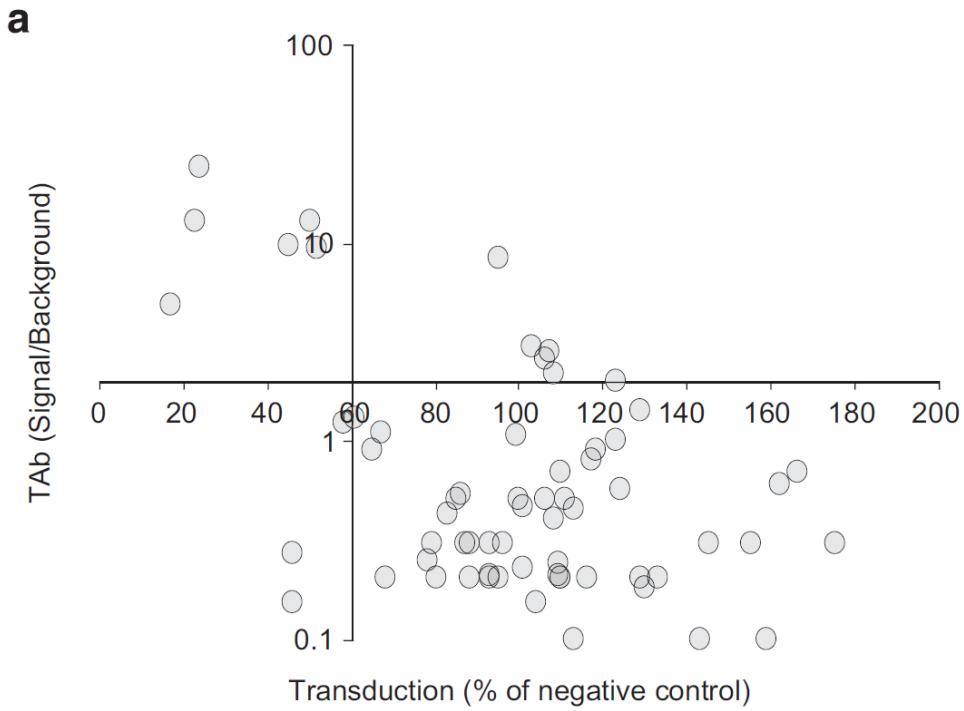
- Systematic removal of positive individuals (biological outliers)
- Screen/conf many individuals (300+) to find enough true negatives.
- Use pseudo negative population (depletion)

Challenges in setting the cut-point

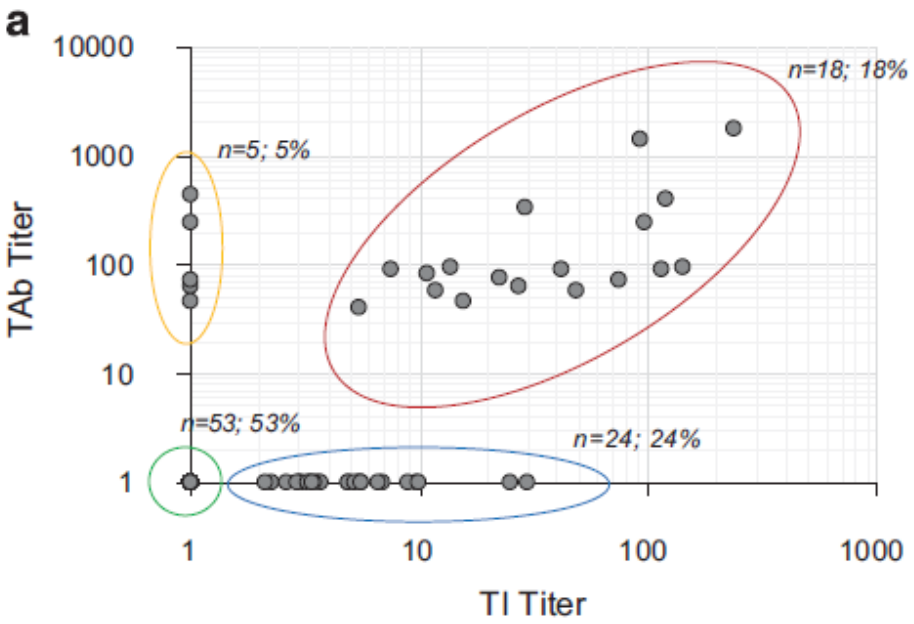


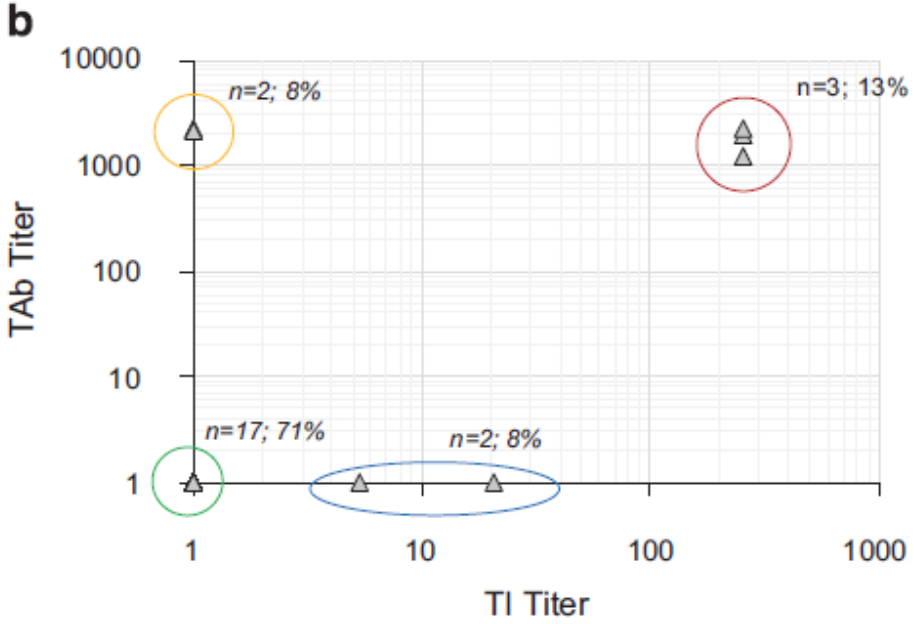
L Falese et al, 2017, Screening strategy for AAV immunity

Comparison of Tab and nAb results in NHP



L Falese et al, 2017, Screening strategy for AAV immunity





c

Healthy

TAb+ TI- (5%)	5	18	TAb+ TI+ (18%)
TAb- TI- (53%)	53	24	TAb- TI+ (24%)

Hemophilia A

TAb+ TI- (8%)	2	3	TAb+ TI+ (13%)
TAb- TI- (71%)	17	2	TAb- TI+ (8%)

All Data

TAb+ TI- (6%)	7	21	TAb+ TI+ (17%)
TAb- TI- (56%)	70	26	TAb- TI+ (21%)

L Falese et al, 2017, Screening strategy for AAV immunity

Use in clinical trials

Assay formats	Total = 30	Cut off
tAb (LBA)	5	>1:50 – 400
nAb (cell based)	18	≥1:5 – 1:40 (mainly not disclosed)
tAb and nAb	1	Not disclosed
None	3	-

Adapted from Mendell et al., 2022, Mol Ther Methods Clin Dev. 2022 Jun 9; 25: 74–83.

Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products, FDA, June 2015

- Allogeneic CT products, GT vectors, and proteins that might be produced by CGT products have the potential to elicit immune responses (immunogenicity). The induction of an immune response may be the desired effect of some products, such as therapeutic vaccines. For other CGT products, immunogenicity may be a risk. For example, pre-existing antibodies, or antibodies that develop after administration of the product, could reduce or extinguish a beneficial effect, cause an adverse reaction (e.g., an autoimmune syndrome), or influence safety or efficacy if there are any subsequent administrations.

Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products, FDA, June 2015

- There are additional considerations for selecting the subject population for certain product types. For certain gene therapies, pre-existing antibodies to either the vector or the transgene product may influence the safety or effectiveness of the product; therefore, the study might exclude subjects with such antibodies.
- In those cases where a special test, such as an antigen or antibody assay, could be critical to the safety or potential effectiveness of the product, the test might be regarded as a companion diagnostic product. If the specific use of the test is also investigational, then the Center for Devices and Radiological Health may need to evaluate the risk of that use. For additional information regarding companion diagnostics, please see the guidance document entitled "In Vitro Companion Diagnostic Devices – Guidance for Industry and Food and Drug Administration Staff" dated August 2014, http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf

Human Gene Therapy for Rare Diseases Guidance for Industry, FDA, Jan 2020

- Study population
  - Pre-existing antibodies to any component of the GT product may pose a potential risk to patient safety and limit its therapeutic potential.
  - Sponsors may choose to exclude patients with pre-existing antibodies to the GT product. In such cases, the sponsor should strongly consider contemporaneous development of a companion diagnostic to detect antibodies to the GT product. If an in vitro companion diagnostic is needed to appropriately select patients for study (and later, once the GT product is approved, for treatment), then submission of the marketing application for the companion diagnostic and submission of the biologics license application for the GT product should be coordinated to support contemporaneous marketing authorizations.

Human Gene Therapy for Hemophilia, Jan 2020

- Study Population
  - Pre-existing antibodies to the GT product may block delivery of the coagulation factor gene to its target (e.g., liver cells), limiting its therapeutic potential. Therefore, sponsors may choose to exclude patients with pre-existing antibodies to the GT product. In such cases, the sponsor should strongly consider contemporaneous development of a companion diagnostic to detect antibodies to the GT product. (Ref: CDx Guidance)
  - If an in vitro companion diagnostic is needed to appropriately select patients for study (and later, once the GT product is approved, for treatment), then submission of the marketing application for the companion diagnostic and submission of the BLA for the GT product should be coordinated to support contemporaneous marketing authorizations. In addition, the clinical development plan should include studies to assess the effect of such pre-existing antibodies on the safety and efficacy of the product.

Human Gene Therapy for Retinal Disorders, FDA, 2020

- No mention of pre-existing Abs.

Regulatory requirements for Clinical Trial inclusion/exclusion and CDx co-development

- **United States**
  - Assays are subject of an Investigational Device Exemption (IDE) application
  - For Phase 2b/3 clinical study the final candidate IVD assay is submitted to FDA through a modular Premarket Approval (PMA) application as a companion diagnostic (CDx)
- **Europe and UK**
  - From 26 May 2022 compliance with EU Regulations for in vitro diagnostic medical devices (IVDR). A technical file needs to be prepared in conformance with IVDR and submitted for approval by a country's Notified Body, such as the MHRA (UK), BfArM (De), EFECTIS (France) to name a few.

Summary

- Bioanalytical Immunogenicity guidance can't be applied word for word
- IVD regulations (CLSI) need to be followed, if used as exclusion criterion
- Several options for cut-point setting have been published and can be used as starting point based on the therapy and capsid
- Scientific justification based on therapy and capsid
- Changing the cut-point is not straight forward – use same method and cut-point across programs and diseases
- Age related cut-point should be avoided
- Clinical investigation between efficacy and cut-off/titer very difficult
  - Availability of Drug Product
  - Availability of patients and patient numbers

Acknowledgements

EBF Cell and Gene Therapy Team

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- Samuel Pine
- Munday James
- Philippe Ancian

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