
Total versus Neutralizing antibody assays against Adeno-associated virus, what is the best?
Vivek Nayak on behalf of Sabrina Lory
Inspired by patients.
Driven by science.

Agenda

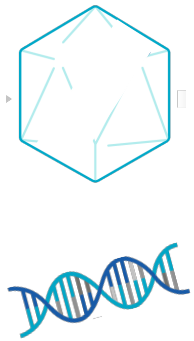
1. Gene therapy
2. Pre-existing antibodies.
3. Assay formats (TAb & NAb)
4. Assay results
5. Correlation of assay results
6. Impact of sero-positivity
7. Conclusion

AAV-based Gene Therapy

Gene Therapy is the therapeutic delivery of DNA into a patients cells as a drug to treat disease (→*in vivo* therapy)

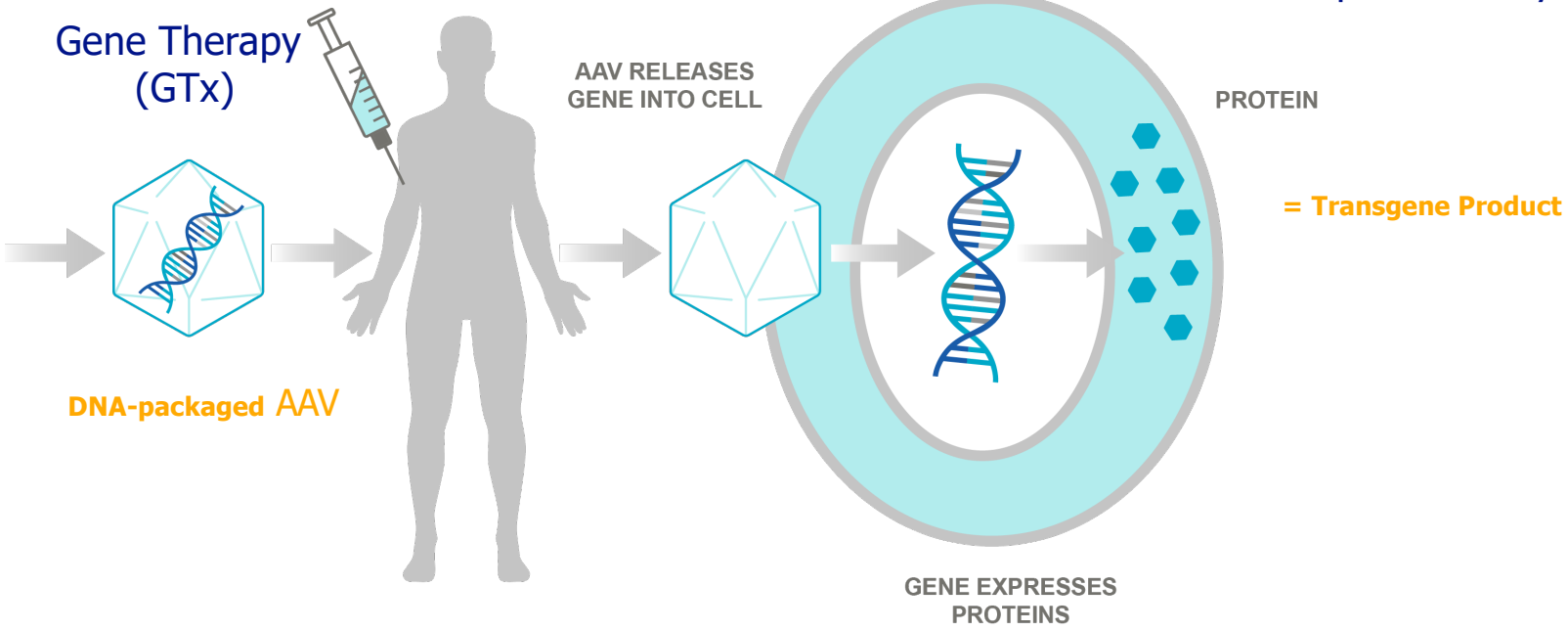
Delivery system

Adeno-associated viral vector (AAV)



Gene to deliver

DNA Construct
Transgene, promoter & stop codon

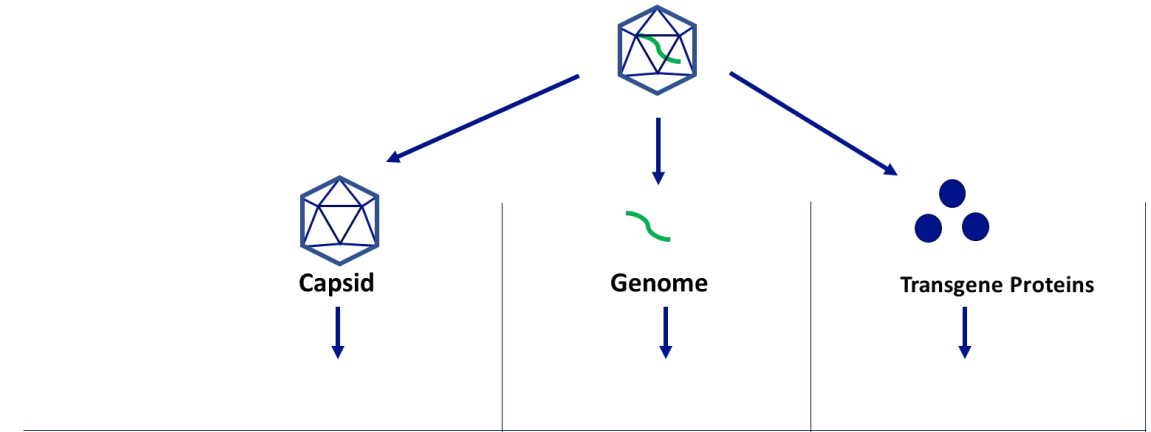


In-vivo produced Therapeutic Entity

= Transgene Product

Source: Adrenas Therapeutics Bridge Bio Adrenas Tx

Immunogenicity Considerations for Gene Therapy

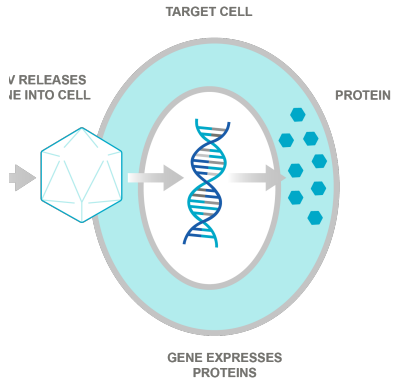


Innate	x	x	
Humoral	x		x
Cellular	x		x

The antibody responses against AAV can include both total antibodies (TAb) and neutralizing antibodies (NAb).

Impact of Pre-existing antibodies on safety and efficacy

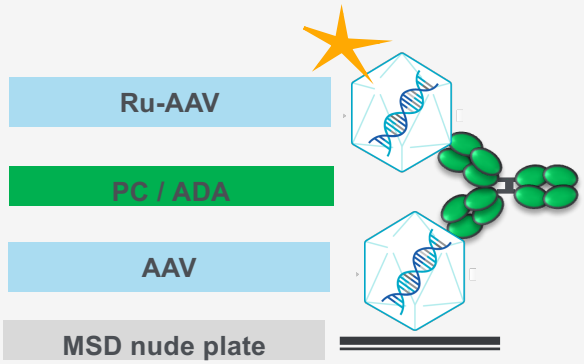
- Pre-existing ADA is known against AAV-based gene therapy
- The presence of Pre-existing AAV antibodies can impact Transduction efficiency and can trigger immunotoxicities.
- During preclinical development of AAV-based therapies, the impact of pre-existing antibodies against AAVs on the transduction efficiency and the level of transgene product should be evaluated.
- Grouping animals prior to start of the study based on their AAV antibody status, i.e negative or positive could be an approach



Animal Group	Seropositivity	AAV based GT Dose
1	**Seropositive**	Dose 1
2		Dose 2
3		Dose 3
4	**Seronegative**	Dose 1
5		Dose 2
6		Dose 3

TAb assay

Format and Characteristics



Assay format: Sequential bridging assay

- Capture Overnight
- Sample (MRD: 1/10)

Matrix: Cyno serum

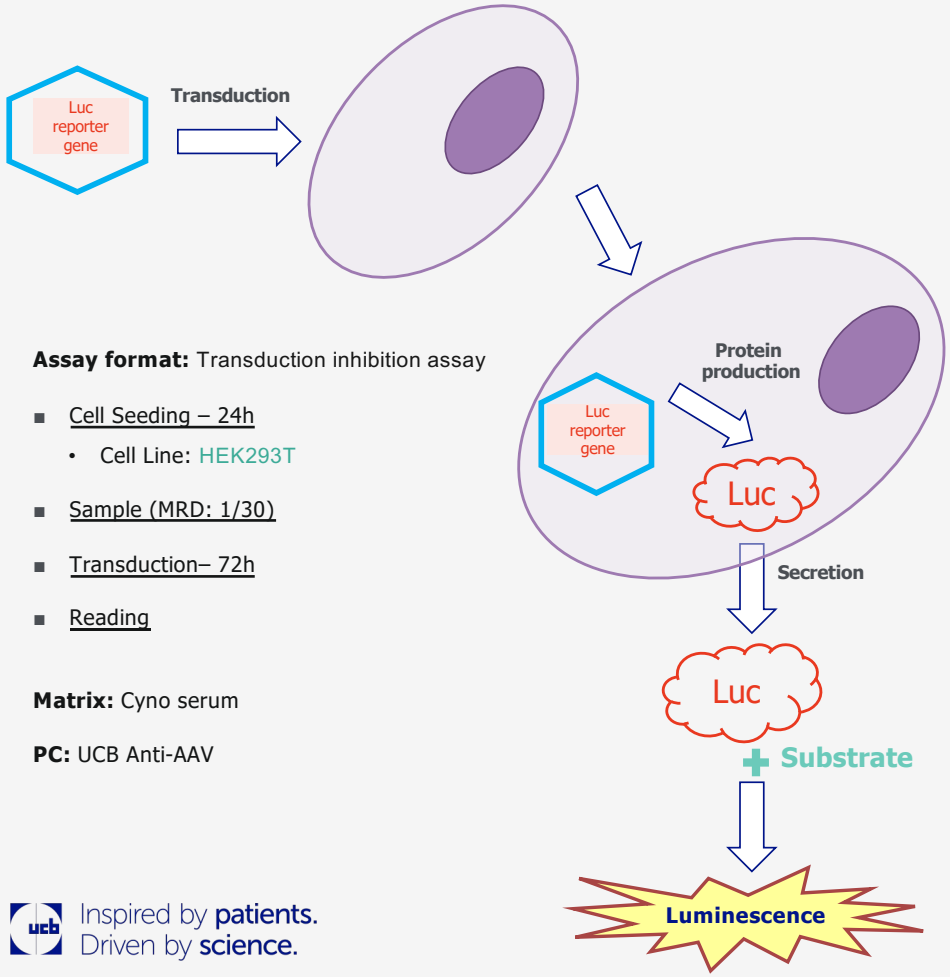
PC: Commercial Anti-AAV

Assay Qualification

PARAMETERS	Screening assay	Titration assay
Cut-Point	SCP factor (1% FPR) =1,16	TCP factor (6SD) =1,43
Selectivity	> 80% ind scored positive at LoPC level	/
Inter-assay precision	< 20%	MSR=2,07
Intra-assay precision	< 20%	/
Acceptance Limits	- Upper limits for NC - Upper and Lower limits for LoPC & HiPC	- Upper and Lower limits for Titer Control

NAb assay (Transduction inhibition assay)

Format and Characteristics



Assay format: Transduction inhibition assay

- Cell Seeding – 24h
  - Cell Line: HEK293T
- Sample (MRD: 1/30)
- Transduction– 72h
- Reading

Matrix: Cyno serum

PC: UCB Anti-AAV

Assay Qualification

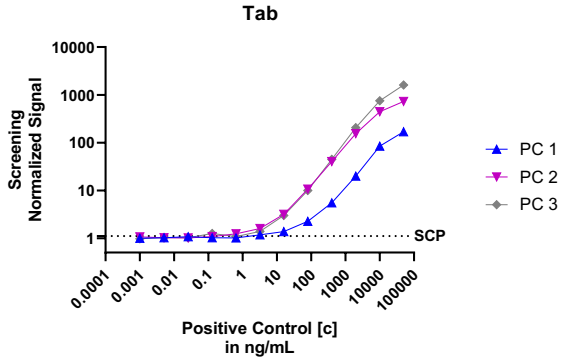
PARAMETERS	Screening assay	Titration assay
Cut-Point	SCP factor (1% FPR) =0,646	TCP factor = SCP factor
Selectivity	> 80% ind scored positive at LoPC level	/
Inter-assay precision	< 25%	MSR=2,11
Intra-assay precision	< 25%	/
Acceptance Limits	- Upper and Lower limits for NC, LoPC & HiPC	- Upper and Lower limits for Titer Control

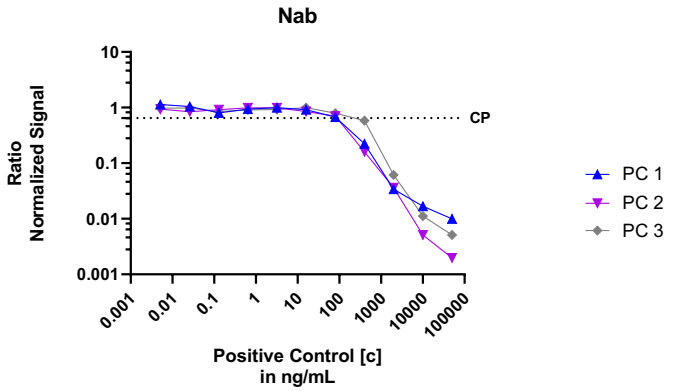
Sensitivity assessment

Comparison TAb versus NAb assay

- 3 PC tested (Monoclonal antibodies against AAV capsid)
- Same preparation tested in both assays

Interpolated Sensitivity (ng/mL)	PC 1	PC 2	PC 3
Tab	2,20	0,23	0,88
Nab	94,22	109,63	293,33

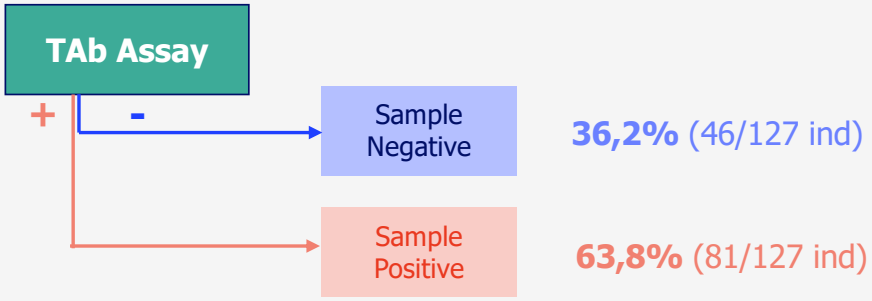




Tab assay more sensitive than Nab assay

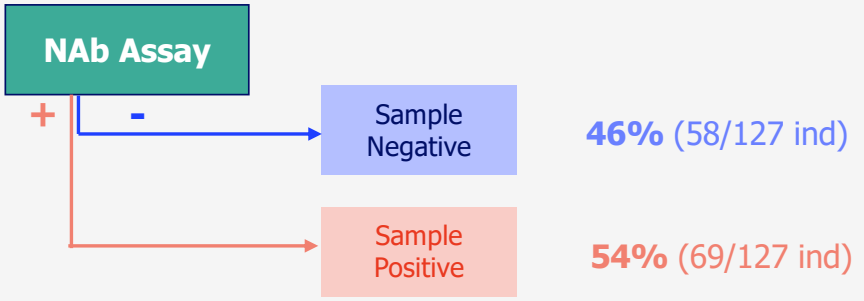
Prevalence of Pre-existing Antibodies against capsid

Results from TAb and NAb assays



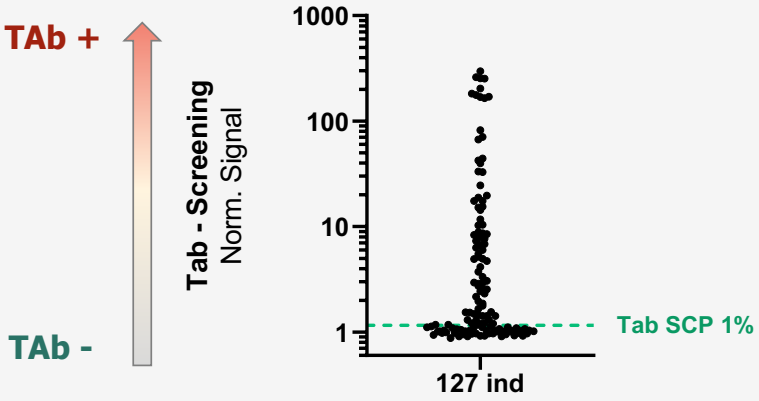
36,2% (46/127 ind)

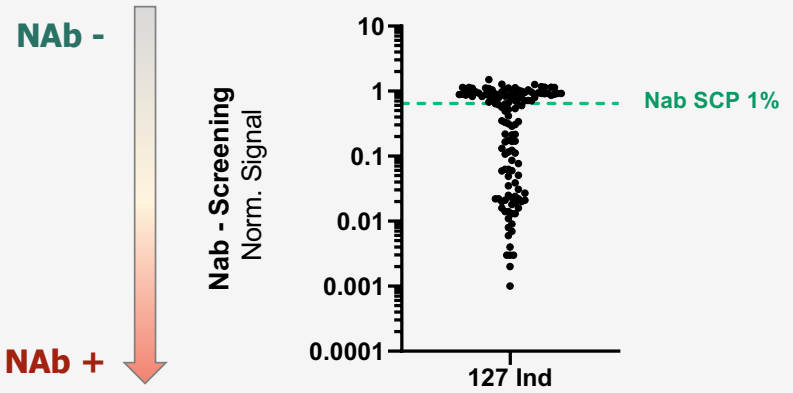
63,8% (81/127 ind)



46% (58/127 ind)

54% (69/127 ind)





Inspired by patients.
Driven by science.

Despite the difference of sensitivity observed between both assays, the prevalence is not so different

Correlation between Tab & Nab

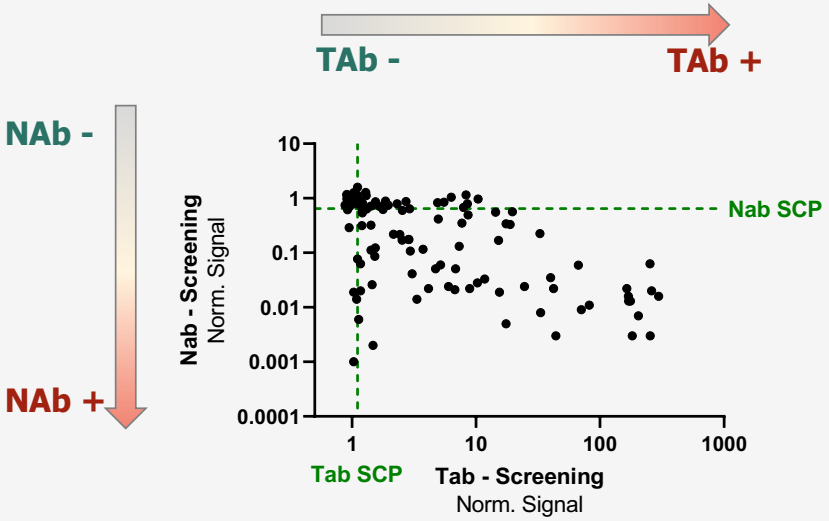
At screening status level

- 127 cynomolgus individual samples assessed in both assays

TAb - NAb - **30,7%** 41/127	**TAb + NAb -** **15%** 17/127
TAb - NAb + **5,5%** 7/127	**TAb + NAb +** **48,8%** 62/127

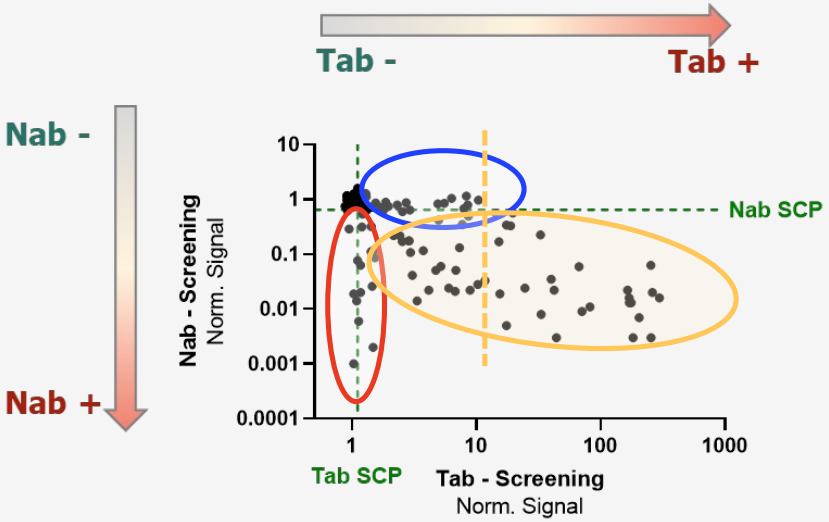
Good correlation between TAb and NAb status

- 79,5 % of ind are concordant between TAb & NAb
  - 30,7% of samples considered TAb & NAb Negative
  - 48,8% of samples considered TAb & NAb Positive
- 20,5 % of values are discordant between Tab and Nab



Correlation between TAb & NAb

At screening value level



48,8% of samples are TAb+ and NAb +

Correlation in term of signal not so clear

- Low TAb signal could correspond to a low NAb or a strong NAb signal
- Normalized signals >10 in TAb are corresponding to NAb + samples

20% of samples are not matching

5,5 % of samples are NAb + and TAb –
- Despite higher sensitivity in Tab assay -

Potential explanations:
- Samples containing antibodies directed against other proteins that are important for AAV transduction or AAV receptor
- Other non-antibodies based neutralizing factors
- Real Tab + samples not detected due to epitope masking during labeling campaing

15 % of samples are TAb + and NAb -

Potential explanations:
- Non neutralizing anti-AAVs antibodies
- Neutralizing antibodies not detectable in the Nab assay as the assay is less sensitive

Summary of TAb & NAb assays characteristics

	TAb	NAb = Transduction inhibition assay
Sensitivity	+++	+
Specificity	Total antibody against Capsid Neutralizing activity from NAb partially detected	Neutralizing activity - Antibodies against capsid - Non Antibody related neutralizing factors - Other antibodies which inhibits the transduction of AAV
Cost	€ €	€ € €
Ease of use	LBA platform – Straight forward	Cell based - required skilled person
Turn around	⏱	⏱ ⏱ ⏱

Different options for animal grouping

For animal grouping, in view of evaluating impact of seropositivity on transduction efficiency and safety :

- Confidence in Seronegative population "Truly Negative"
- High antibody level for Seropositive population
- Range of titer for Seropositive population to understand potential threshold for impact

→ 3 potential options

	Seronegative	Seropositive	Comments
Option 1	Negative samples in TAb	Highly positive samples in TAb	❑ In Seronegative group, animals being NAb+ may be present ❑ Highly positive animals expected to be NAb+ (potential range from low to high) → **Potential effects of NAb positivity may be present in the 'defined negative group'; and potentially misinterpreted as not related to Seropositivity**
Option 2	Negative samples in NAb	Highly positive samples in NAb	❑ In Seronegative group, animals being Tab+ at lower level may be present (Non neutralizing or due to difference of assay sensitivity). However, animal exhibiting a neutralizing activity are excluded ❑ In Seropositive group, animals presenting a high level of Nab+ may be Tab- → **Potential effects of Low Tab positivity may be present in the 'defined negative group'; and potentially misinterpreted as not related to seropositivity** → **No confidence that seropositivity effect is capsid driven. Results might be difficult to interpret.**
Option 3	Negative samples in TAb & NAb	Highly positive samples in TAb & NAb	❑ Seronegative group can be considered « truly negative» ❑ Seropositive group can be considered as « truly positive » with high level of Tab+ (Total antibodies against capsid) and with important neutralizing activity → **This approach is the more conservative approach and might be reflective of the worst case scenario to study impact of seropositivity** → **However, cost and timeline delivery are affected**

As a conclusion…

Bioanalytical Strategy

Context of Use

Assay Characteristics

Acknowledgement

Data Comparison and strategy discussion

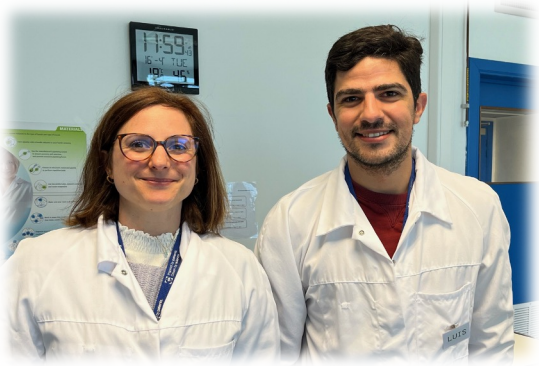
- Sabrina Lory
- Veerle Snoeck

Generation of TAb data

- Pierre-Jean Couble
- Patricia Guerreiro

Generation of NAb data

- Juliette Lamy
- Luís Aires Farinha de Castro e Almeida
- Patricia Guerreiro



Questions?