Immunogenicity Testing of Multi-Specific Nanobodies®

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European Immunogenicity Platform
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Ablynx
A SANOFI COMPANY

Acknowledgement to Contributors

First things first!

- **BAI Current and Former Scientists:**
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- **Ablynx**
  - Nanobody Research Platform
  - Clinical Sciences Operations
- **Sanofi**
  - Therapeutic Area Scientists
  - Drug Metabolism & Pharmacokinetics
  - Preclinical Sciences
  - Translational Medicine & Early Development

Ablynx Bioanalysis & Immunogenicity Team

Presentation Overview

- **Multi-specific Nanobodies®**
- **Binding ADA Methods**
- **ADA Characterization Methods**
- **Case Study 1 – Non-clinical binding ADA & Characterization**
- **Case Study 2 – Clinical characterization assays**

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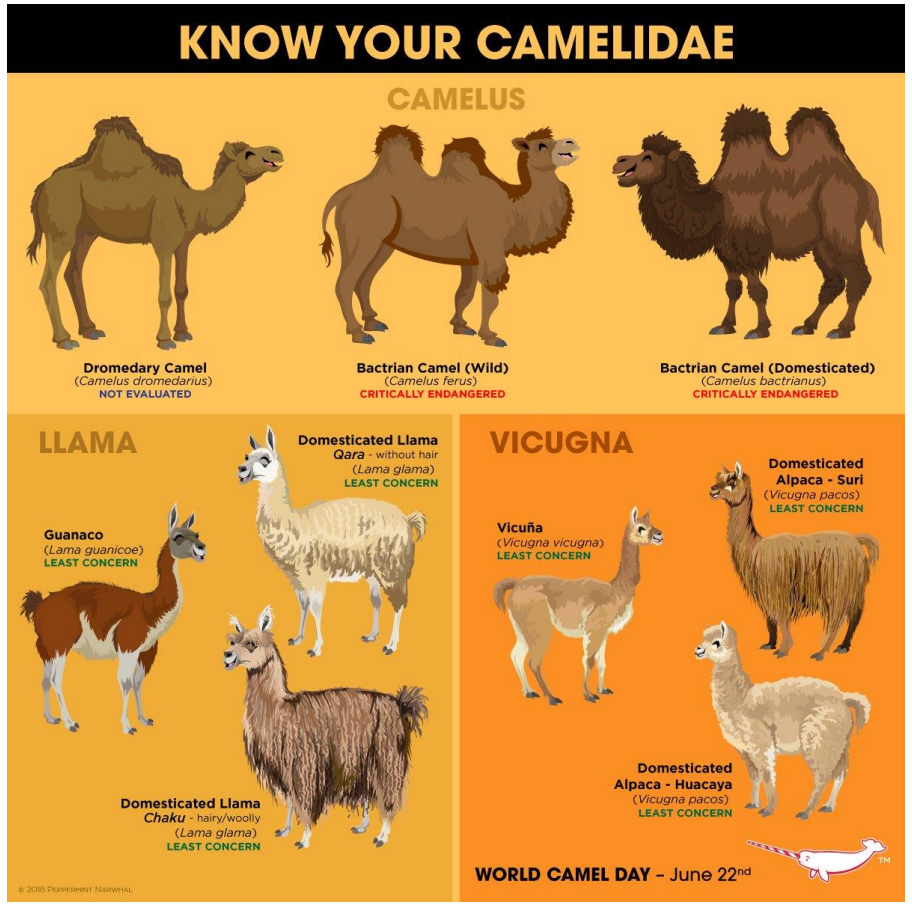
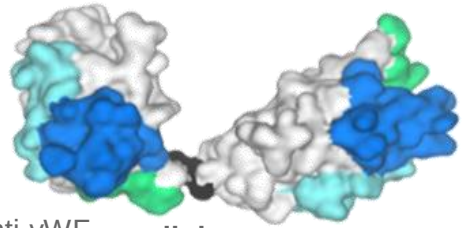


What is a Nanobody®?

Antibody-based biotherapeutic from Ablynx

Bivalent Nanobody® (28kD) for the treatment of aTTP

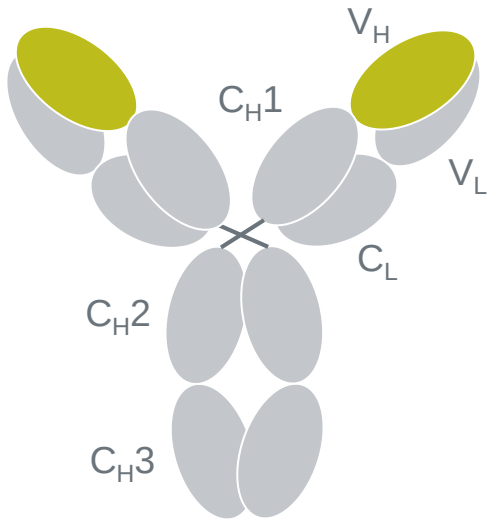
Cablivi®
caplacizumab



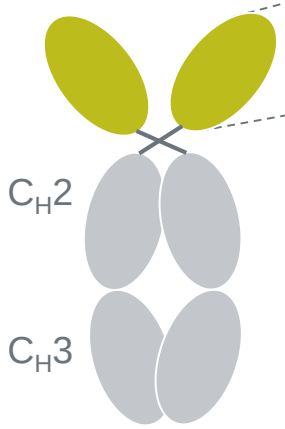
anti-vWF Nanobody®

linker

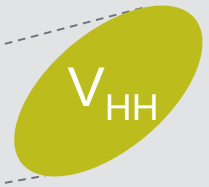
anti-vWF Nanobody®



Conventional antibodies



Heavy chain only antibodies



12-15kDa

Ablynx' Nanobody®

- small and robust
- easily linked together
- sequence homology comparable to humanized/human mAbs
- nano- to picomolar affinities
- able to bind and block challenging targets
- multiple administration routes
- manufactured in microbial cells

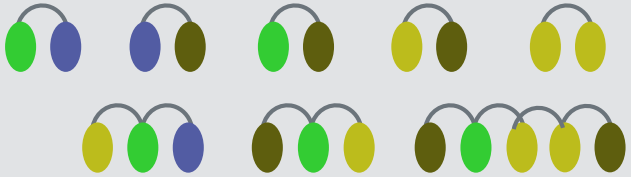
aTTP = acquired thrombotic thrombocytopenic purpura

Nanobody® advantages

Full-cycle R&D design and production platform

Mix and match

Multi-specific/multivalent Nanobodies® that address multiple targets in a single drug molecule

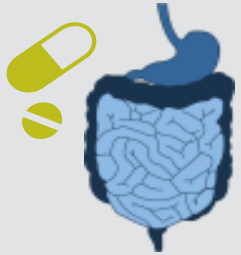


Multiple delivery routes

Injection

Inhalation

Ocular

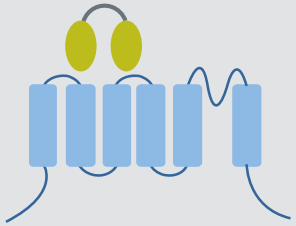


Oral-to-topical

Manufacturing

High-yield, high-concentration, low-viscosity, microbial production

Able to bind and block challenging targets



Nanobodies® against ion channels and GPCRs

Customised half-life extension

Hours/days/weeks

Albumin-binding Nanobody®

Fc

Presented at 11th EIP Symposium – 19 Feb 2020

Modularity of Next-Generation Nanobodies®

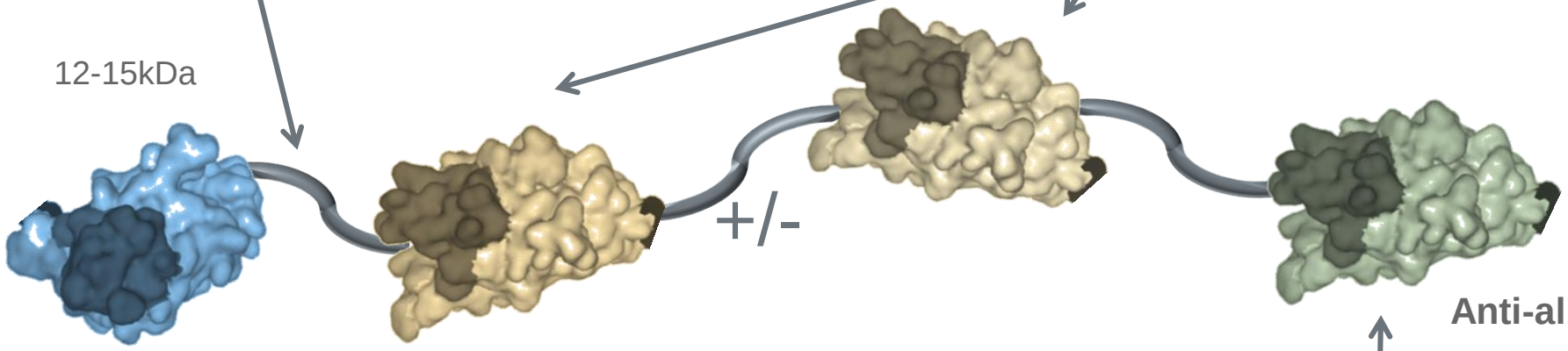
Mix & match components to build a custom therapeutic

Bi-paratopic:
binding multiple identical or different epitopes on same target

Custom linker

+/-

12-15kDa



+/-

+/-

Payload

Anti-albumin

anti-Target 1

anti-Target 2

Multi-specifics:
Individual binding arms with tailored affinity

Long serum half-life
Extend from hours to ~20 days

Conjugates
toxins, inhibitors, modulators, etc.

Next-generation Ablynx Nanobodies® include site-specific mutations designed to reduce most pre-existing antibody binding

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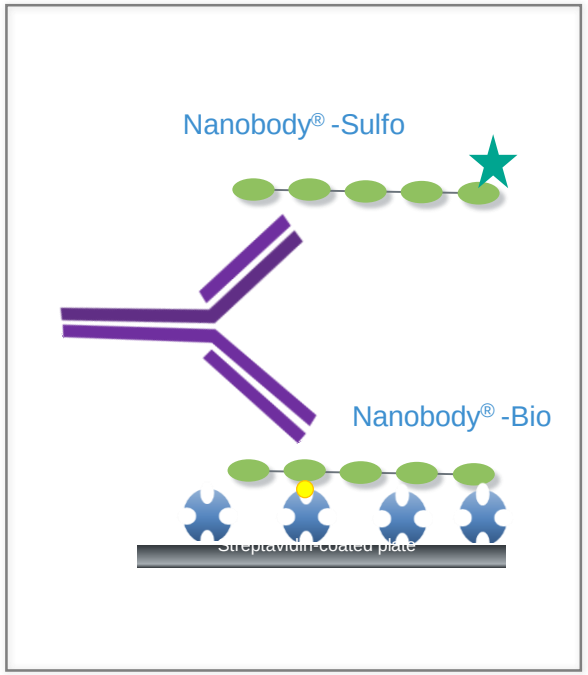
ADA Method Menu

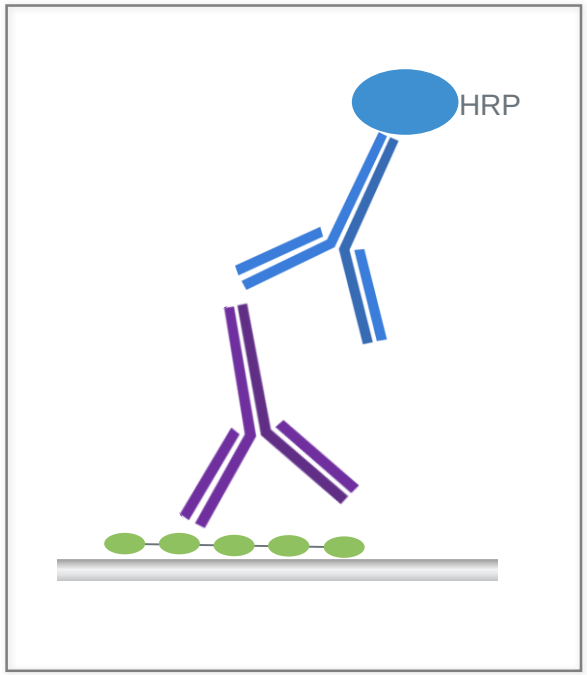
Fit-for-purpose selection of method

- Often have parallel early development tracks for empiric selection of method
- Pre-treatment and assay competitor options, e.g. BEAD, AD, Protein G, SPE, etc.
- Background (S/N), drug and target tolerance, as well as lab suitability considered

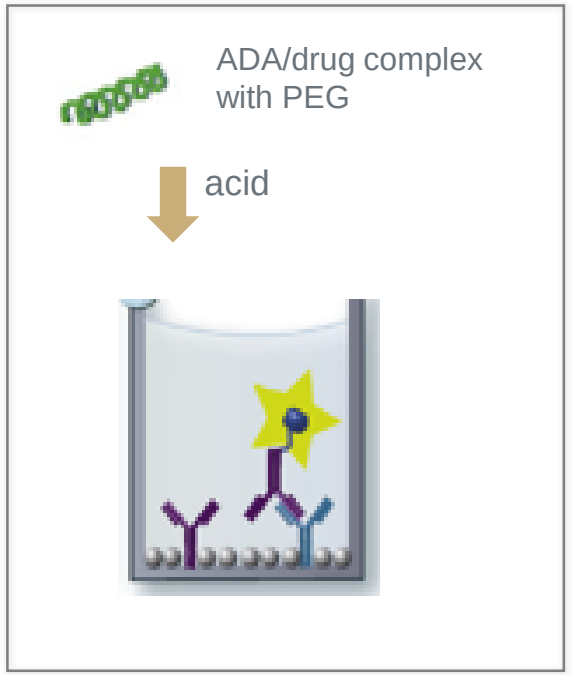
Bridging ECL



Direct ELISA



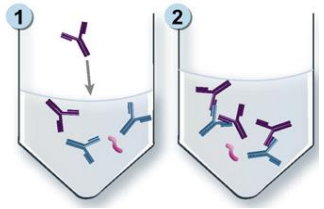
PandA



PandA Method Development

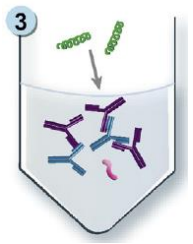
Thoughtful multi-parameter evaluations required

Step 1: Saturation of ADA with excess drug (complex formation)

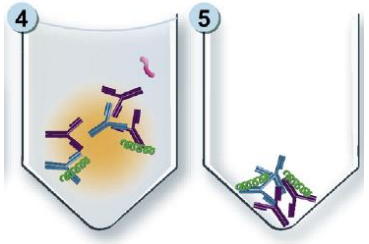


Add sample + assay buffer + drug

Step 2: Precipitation of complexes

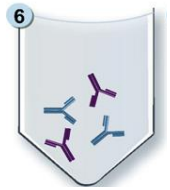


Add PEG



Spin down and collect supernatants
Add PEG and wash

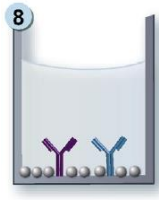
Step 3: Acid dissociation and surface coating



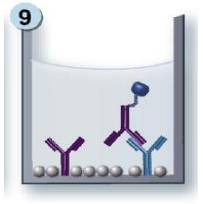
Add acid to reconstitute the pellet

Coat on plate

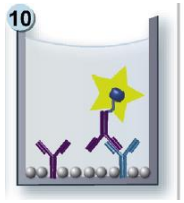
Step 4: Specific detection



Block plate

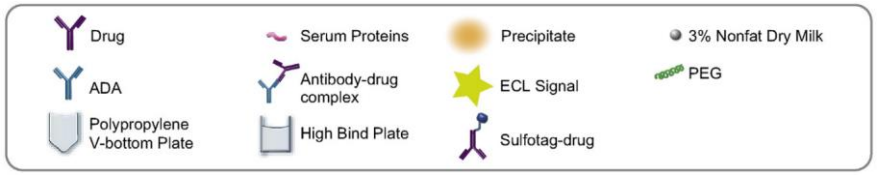


Add detector



Wash and read plate

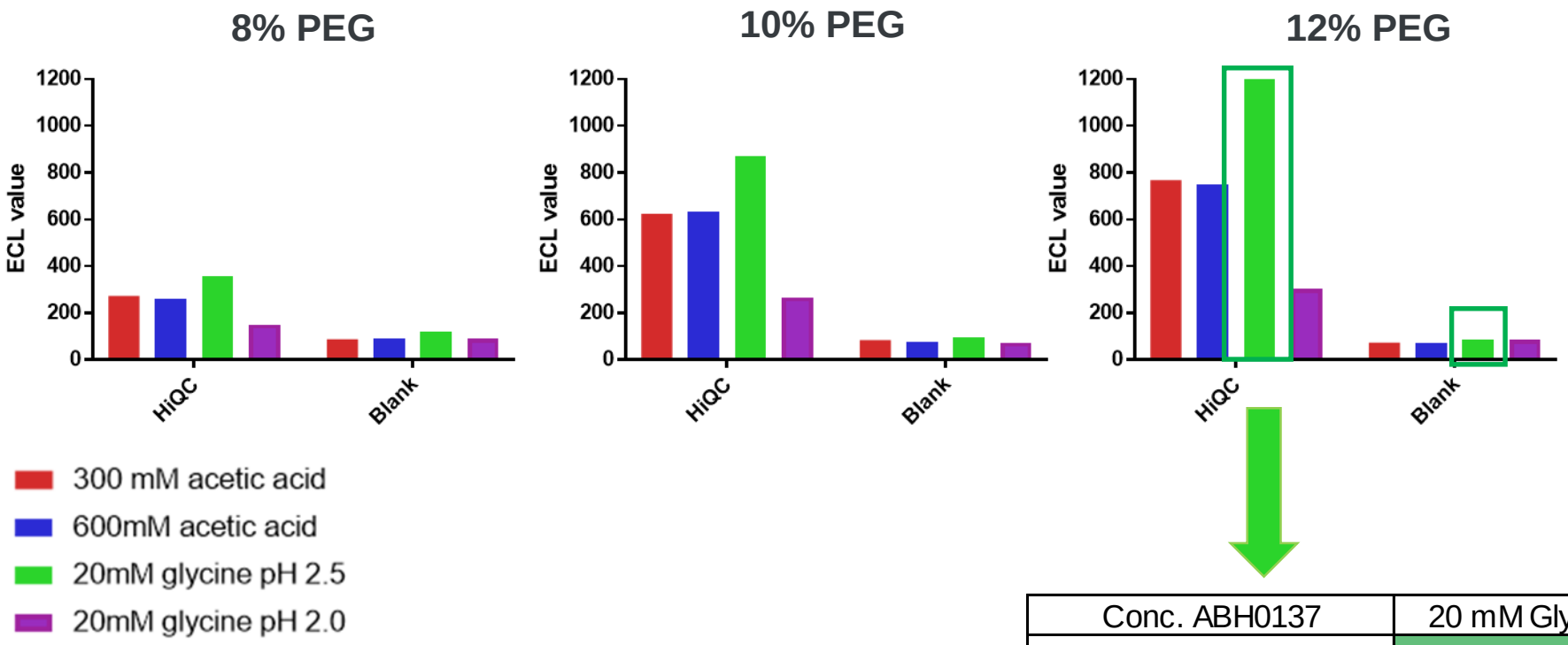
- Highly drug and target tolerant format
- Complex procedure with many variables
  - PEG concentration
  - acidic buffer composition
  - immune complex co-factors
  - other matrix effects
  - MRD
  - [coating], [detector], etc.
- Implementation and transfer challenges
- Robust method with careful development and optimization



Zoghbi et al, JIM 2015

PandA Method Development

Example parameter evaluation: PEG and acid conditions



- Increasing PEG concentration increased S/N
- Acidic conditions differentiate assay performance by type and concentration/pH

Conc. ABH0137	20 mM Glycine pH 2.5
10000.0 ng/mL	1197
500.0 ng/mL	139
100.0 ng/mL	92
0.0 ng/mL	84

Characterization Method Menu

Options for assessing impact & translational immunogenicity

- **Domain specificity**
  - Immunodepletion (confirmatory assay) with protein subunits
  - Individual Nanobody® building blocks usually available in sufficient quantity
- **Isotype analysis**
  - ADA sub- and isotype analysis for functional impact and immunological insights
  - SPR, LCMS or other platforms
- **NAb Epitope Characterization Assay (“NECA”)**
  - ‘Null-variant’ compounds = identical to drug with scrambled CDRs
  - Immunodepletion with null-variants can correlate with NAb responses
- **Functional NAb Assay: cell-based or competitive LBA**
  - CLBA for antagonistic soluble factors

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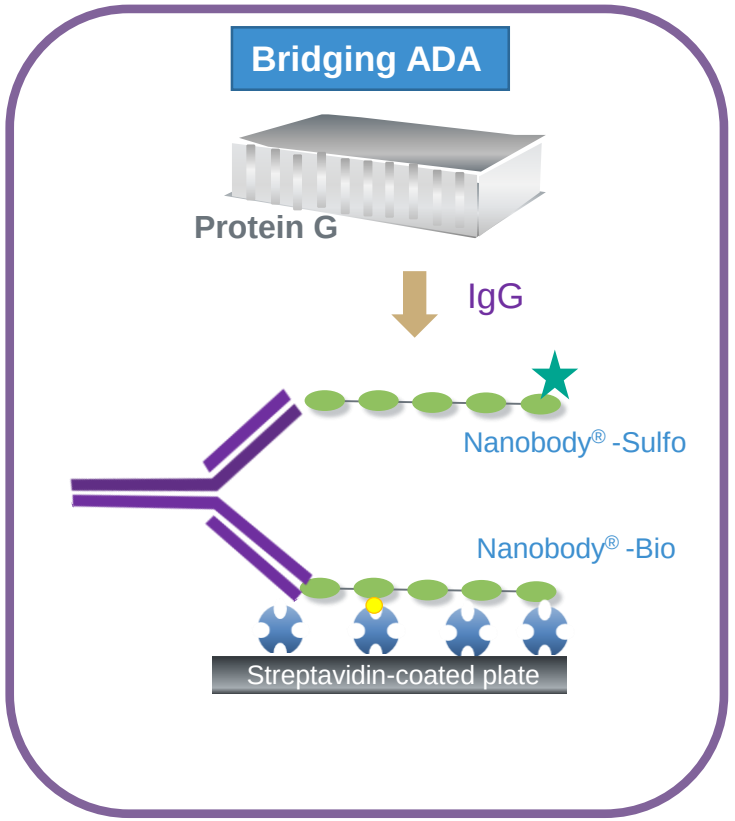
Case Study 1 – Non-clinical ADA analysis

ADA Method Description

- Pentavalent bi-specific Nanobody® tested in NHP PK/PD study
- Bridging ADA assay with sample pre-enrichment by protein G
- Optimized for sensitivity, drug (1-4 mg/mL) & target tolerance (up to 1 µg/mL)



	Screening				Confirmation				
PC	ECL duplicate 1	ECL duplicate 2	Average	%CV	ECL duplicate 1	ECL duplicate 2	Average	%CV	%inhibition
10000,00 ng/mL	120596	125158	122877	2,6%	80	76	78	3,6%	99,94
2500,00 ng/mL	17839	18046	17943	0,8%	61	58	60	3,6%	99,67
625,00 ng/mL	4553	3924	4239	10,5%	61	60	61	1,2%	98,57
156,25 ng/mL	1118	1139	1129	1,3%	61	60	61	1,2%	94,64
39,06 ng/mL	374	366	370	1,5%	64	63	64	1,1%	82,84
9,77 ng/mL	179	176	178	1,2%	78	72	75	5,7%	57,75
2,44 ng/mL	102	97	100	3,6%	59	62	61	3,5%	39,20
		SCP = Blank*1.3	**86**						

Interpolated sensitivity: <5 ng/mL

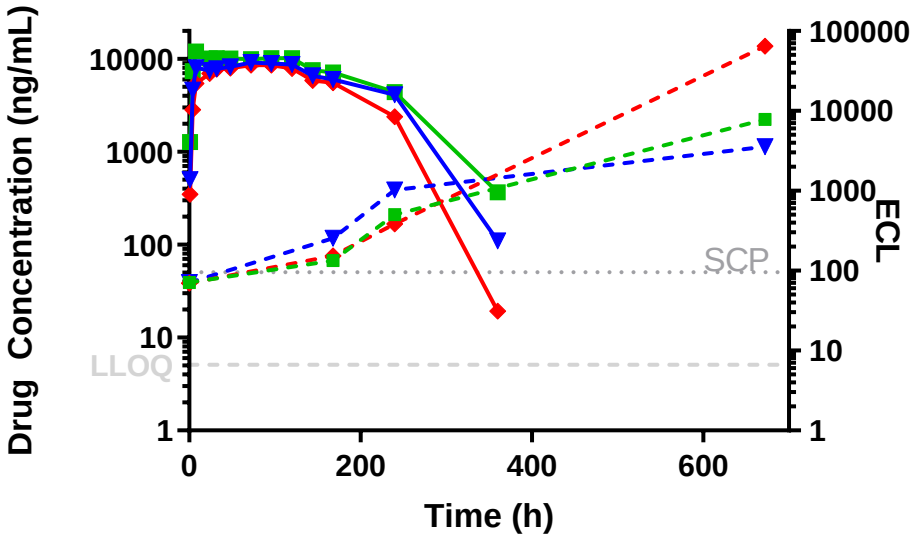
[PC]	S/N
10000,00 ng/mL	1861,8
2500,00 ng/mL	271,9
625,00 ng/mL	64,2
156,25 ng/mL	17,1
39,06 ng/mL	5,6
9,77 ng/mL	2,7
2,44 ng/mL	1,5

Case Study 1 – Non-clinical ADA Analysis

Binding data and early-stage characterization method

PK

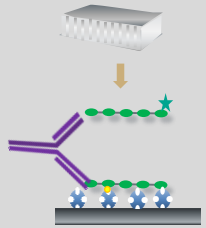
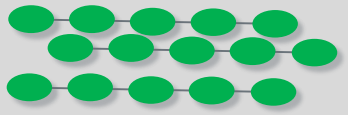
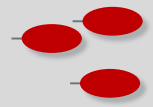
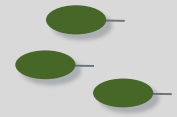
ADA

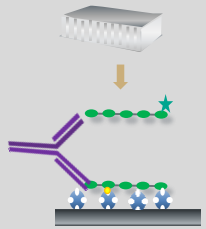
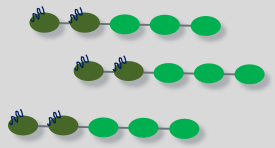
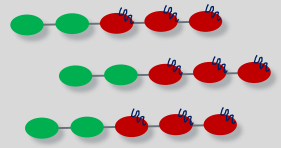


ADA response in primates to humanized Nanobody® correlated with drop in drug exposure.

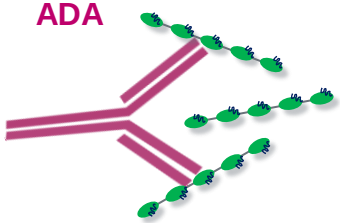
*Nanobody® structure for illustrative purposes only and not representative of actual compound

NECA, neutralizing antibody epitope characterization assay; CDR, complementarity-determining region; SCP, screening cutpoint, LLOQ, lower limit of quantitation;

	Domain-specificity assay (Confirmatory)		
Screening	**Full-length Nanobody**		
Bridging ADA + ProtG 	Screening + 	Screening + 	Screening + 

	Bi-specific NECA* (Confirmatory)	
Screening	**Monospecific null variant for Target 1**	**Monospecific null variant for Target 2**
Bridging ADA + ProtG 	Screening + 	Screening + 

Non-CDR-Binding ADA

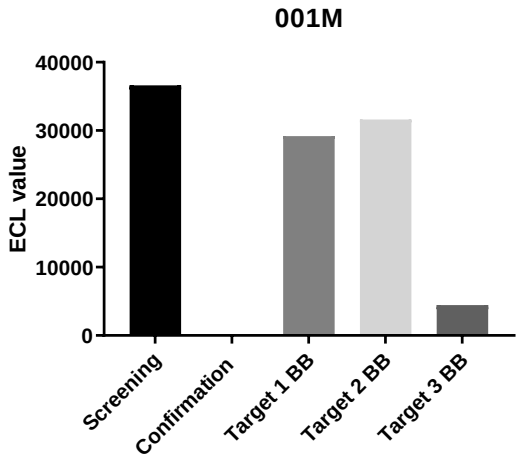


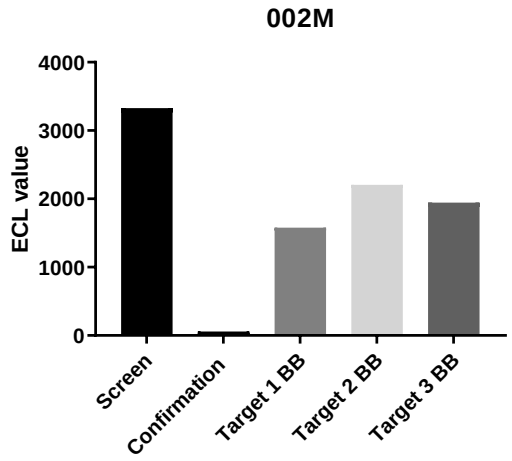
Null variant Nanobody®

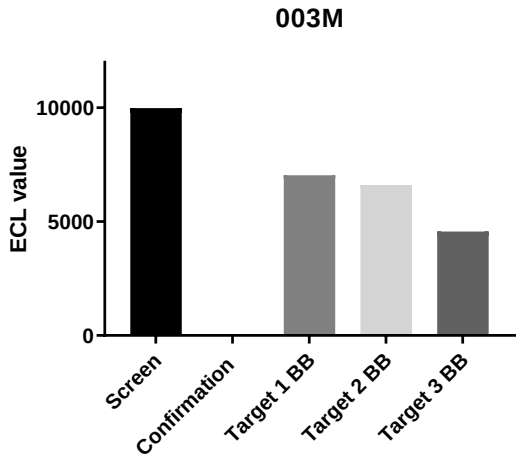
Case Study 1 – Non-clinical ADA Analysis

Early-stage immunogenicity characterization

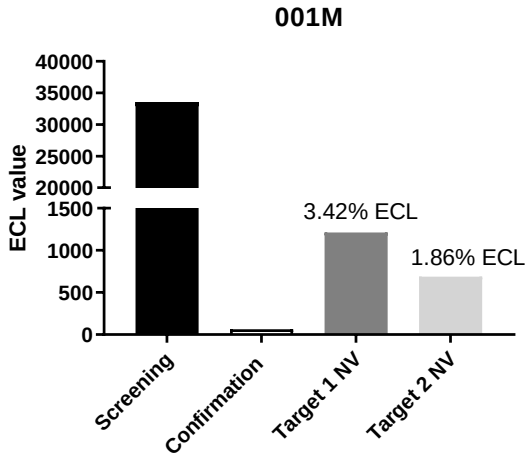
Domain-specificity assay (monovalent building blocks)

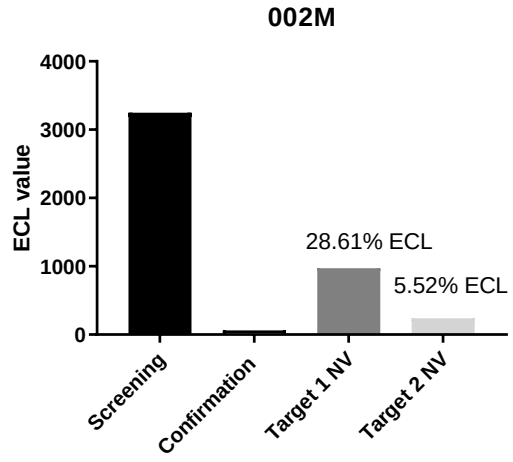


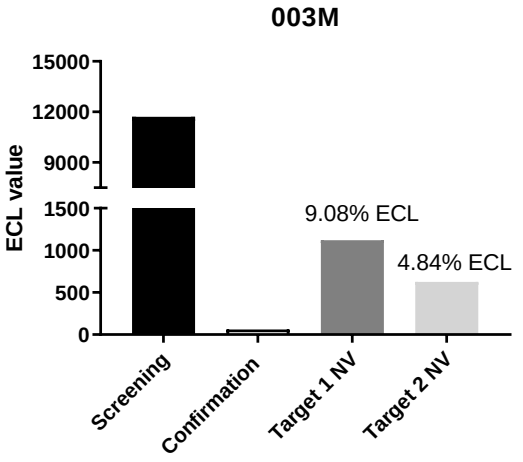




Bi-specific NECA method (pentavalent, mono-specific null-variants)







% ECL = proportion of ADA screening signal attributed to CDR-binding in a given sample's response

BB, building block; NV, null variant

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Case Study 2 – Clinical ADA Testing

Caplacizumab HERCULES Study – Ph3 Pivotal

Double-blind, randomized, parallel group, multicenter placebo-controlled	**Patients with aTTP; n= 144 with 1:1 active:placebo**	**i.v. + s.c. 10 mg per dosing**	1x i.v. prior to first PE + s.c after each PE session + daily s.c. for 30 days after last PE

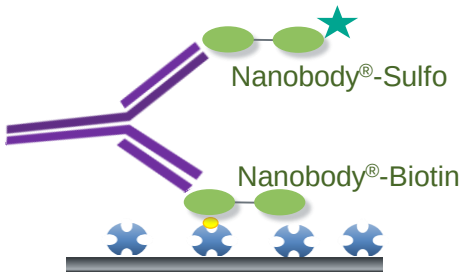
- Complex situation for immunogenicity interpretation
  - Standard of care for aTTP indication includes plasma exchange
  - Pre-existing ADA (pre-Ab) prevalence 4-63%, depending on population
  - Rare disease clinical impact and epitope characterizations for agencies
- Four immunogenicity assays implemented to monitor and characterize
  - Binding ADA: tiered bridging method for binding antibodies
  - Modified ADA (mADA): detects ADA directed to C-terminal region
  - NECA: ADA directed to the CDR of the Nanobody®
  - NAb Assay: functional ELISA based on vWF (target) interactions

Presented at 11th EIP Symposium – 19 Feb 2020

Case Study 2 – Clinical ADA Testing

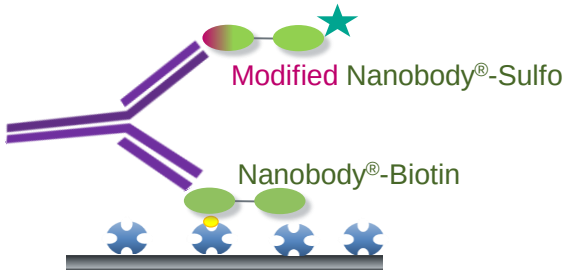
HERCULES assay formats

ADA



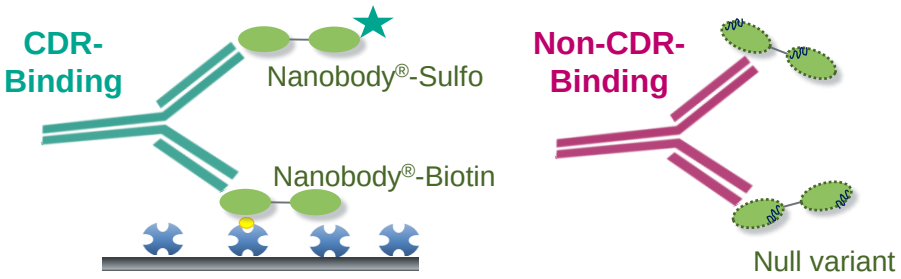
Sensitivity <25ng/mL

mADA



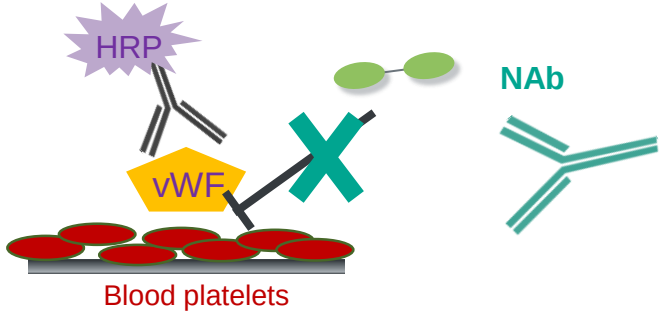
Sensitivity <25ng/mL

NECA



Sensitivity 28.8 ng/mL

NAb



Sensitivity: 91.7 ng/mL

Case Study 2 – Clinical ADA Testing

Assay results comparison

	Caplacizumab	Placebo
HERCULES Immunogenicity*	N=97 (%)	N=73 (%)
Treatment-emergent binding ADA	3 (3.1)	1 (1.4)
CDR-binding ADA, detected with NECA	4 (4.1)	1 (1.4)
NAb, detected with functional assay	2 (2.1)	0 (0.0)
Pre-Ab (sampling after first PE)	55 (56.7)	46 (63.0)

° incidence on the overall study period: double blind and open label period

- Plasma exchange (PE) complicates immunogenicity data interpretation; all patients received one PE prior to randomization.
  - i) pre-Ab can be transferred from donor plasma to the subject and/or
  - ii) treatment emergent ADA (TE ADA) might be diluted during PE.
- mADA assay used to define TE-ADA that did not recognize the C-terminal binding region at any given time point
- NECA allowed more sensitive and drug tolerant read-out in conjunction with NAb assay for confirmation of functional neutralization
- No impact of TE ADA or pre-Ab on clinical efficacy (time to platelet count response) or safety

Next-Generation Nanobodies®

Latest for multi-functional, sequence-optimized compounds

Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Single Ascending Doses of the Anti-ADAMTS-5 Nanobody®, M6495, in Healthy Male Subjects: A Phase I, Placebo-Controlled, First-in-Human Study

Guehring et al, 2019 ACR/ARP Annual Meeting

Recent clinical data presented with a next-gen Nanobody®

- SAD in healthy male volunteers; 6 x cohorts of n=9 (2:1 active:placebo)
- Well-tolerated with TEAEs in line with protein therapeutic and similar between treatment and placebo groups
- Positive PK/PD results – exposure increased with slightly greater-than-dose proportional manner; target marker reduced in dose-proportional manner.
- Low ADA incidence, 1/53 (1.9%) pre-existing ADA reactivity
  - Overall 6/53 (11.3%, 1 in each of 4 active dose groups, 2 in placebo)
  - No impact on PK/PD, consistent with other clinical programs.

Summary & Conclusions

Multiple options for multi-specifics

- Multi-specific, multi-domain Nanobodies® present unique challenges and opportunities to immunogenicity assay developers.
  - Modular format allows precise targeting of domain specificities.
  - Protein tool production is built into the discovery process, enabling more choices for assay developers.
  - Next-generation Nanobodies® have increased complexity and expected lower pre-Ab reactivity
- Drug and target tolerance remain challenging parameters, but many choices are available.
  - Nanobodies® offer their own unique characteristics that must be addressed with optimizations of critical reagent choices and buffer compositions.
  - Multiple targets in multiple species must be addressed.
- Tool creation allows many choices for early non-clinical immunogenicity characterizations.
  - Parallel tracks for assay development
  - Mix-and-match confirmatory set-ups
- Additional assay data can help inform clinical scientists and regulators on clinical impact and safety in complex treatment situations.
  - Clinical experience supports the need for additional methods in certain disease settings
  - Techniques developed for measuring relevant TE-ADA in the face of pre-existing antibody
  - NECA and NAb assays provide sensitivity & direct functionality when used together.
  - In agreement with regulators, NECA could be used as a stand-alone NAb assay.

Related Publications

Further reading and information

- **2019 ACR/ARP Annual Meeting :**
  - *Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Single Ascending Doses of the Anti-ADAMTS-5 Nanobody®, M6495, in Healthy Male Subjects: A Phase I, Placebo-Controlled, First-in-Human Study*, H. Guehring, T. Balchen, K. Goteti, et al.

- **2020 EBF YSS:**

POSTPONED

A novel approach for immunogenicity cut-point determination in the presence of pre-existing antibodies, G. Daelman, O. Van de Vyver, E. Pattyn and A. Coddens

POSTPONED

The Rise of Multi-specific Nanobodies® in Pharmacokinetic Assays, V. Allemon, K. van Lysebetten, S. Poelmans, T. Antoine and S. Pine

- **NECA Paper:**
  - *An innovative method for characterizing neutralizing antibodies against antibody-derived therapeutics*, A. Coddens, V. Snoeck, L. Bontinck, M.A. Buyse, S. Pine (in press).

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  - Drug Metabolism & Pharmacokinetics
  - Preclinical Sciences
  - Translational Medicine & Early Development

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Thank you for your attention and to the 2020 EIP Organizers!