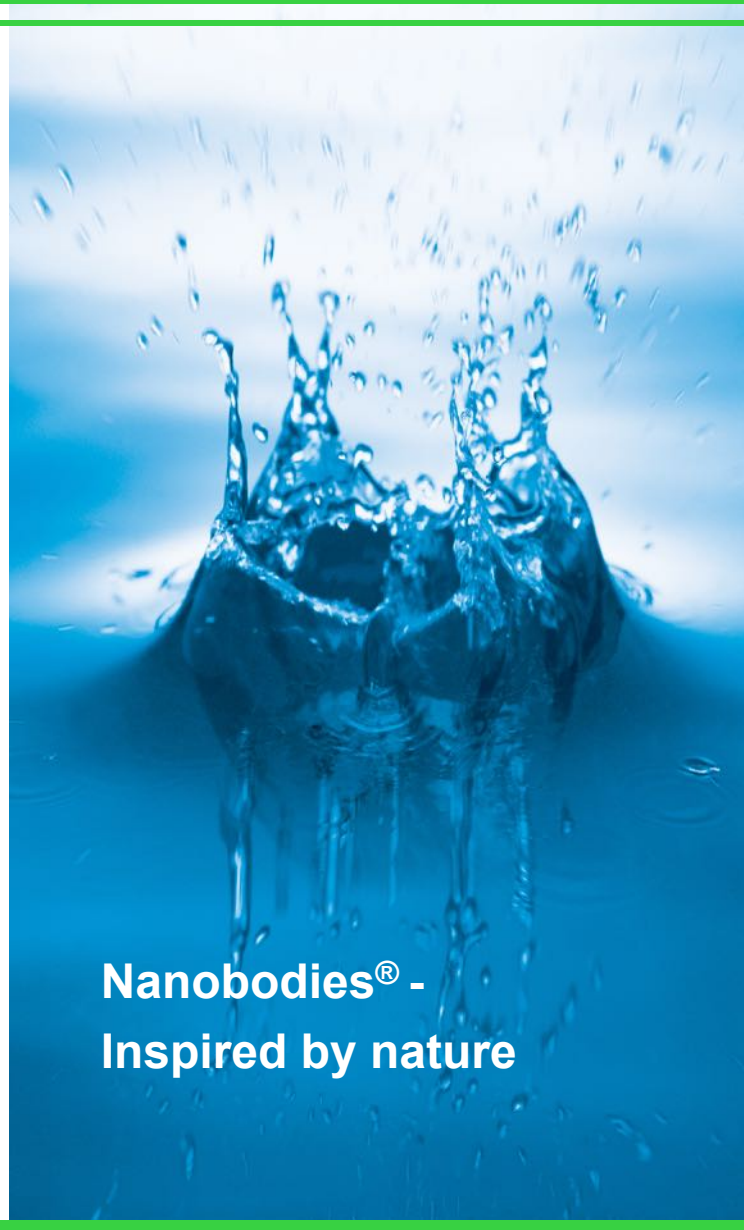
Current Experience in Immunogenicity Assessment of next Generation Biologics- Nanobodies®

European Immunogenicity Symposium

Veerle Snoeck

Ablynx NV



Outline

- Introduction
- Nanobodies - low immunogenicity by design
- Nonclinical experience
- Clinical experience
- Conclusion

Outline

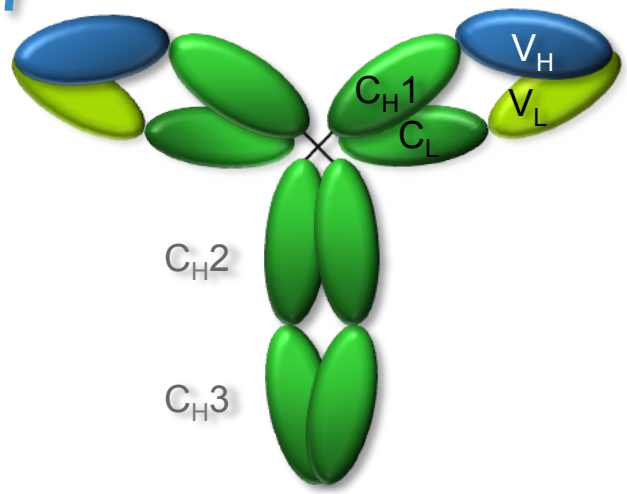
- Introduction
- Nanobodies - low immunogenicity by design
- Nonclinical experience
- Clinical experience
- Conclusion

Ablynx – company overview

- Drug discovery and development company based in Ghent, Belgium
- A pioneer in next generation biologics - Nanobodies®
- Worldwide exclusive rights to commercialise Nanobody products in human healthcare
- ~25 programmes in the R&D pipeline
- Two products achieved clinical proof-of-concepts in RA
- 5 Nanobody products in the clinic - 2 Phase II & 3 Phase I
- Exclusive rights to >500 patent applications and granted patents
- Partnerships with Boehringer Ingelheim, Merck Serono, Novartis and Merck & Co
- >250 employees

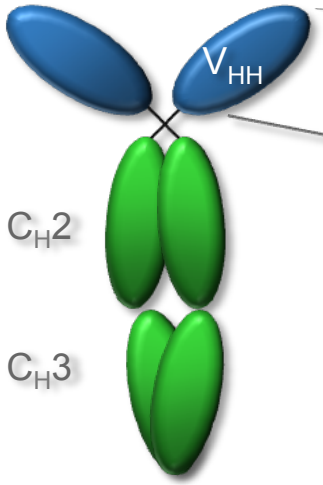
Ablynx's Nanobodies – proven single variable domain approach

Camelidae family has both forms



Conventional antibody

- Heavy and light chains
- Both chains required for antigen binding and stability
- Large size and relatively low formatting flexibility
- Administered through injection



Heavy-chain antibody

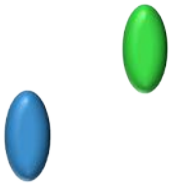
- Only heavy chains
- Full antigen binding capacity and very stable

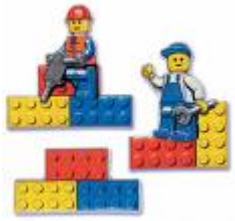
Ablynx's Nanobody®

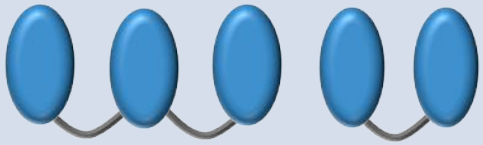
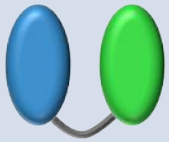
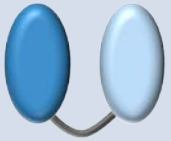
- Small (1/10 size of a mAb)
- Flexible formatting
- Highly potent, robust and stable
- Broad target applicability
- Multiple administration routes
- Ease of manufacture
- Speed of discovery

Powerful Nanobody formatting features: multivalent, biparatopic and multispecific formats

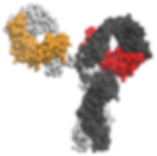
- Formatting = Linking together of two or more Nanobody building blocks which:





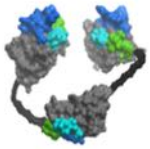
Multivalent	Biparatopic	Multispecific
		
bind to same epitope on single target	bind to different epitopes on single target	bind to different targets

Immunogenicity assessment of novel biologics



Monoclonal antibodies

- 28 approved therapeutic mAbs
- >30 years of clinical experience



Antibody fragments

- Fab (PEGylated)
- scFv
- dAbs
- Nanobodies
- ...

- First approvals
- Limited clinical experience

Fragments of other origin

- DARPINS
- Anticalins
- Affibodies
- ...

- First clinical experience

Immunogenicity of Nanobodies

- >20 non-clinical studies
- 9 clinical studies in 5 different programmes
- Low observed immunogenicity in non-clinical development
  - incidence between 0% and 37% in safety pharmacology/toxicology studies
  - generally not impeding the interpretation of PK/PD and safety studies
  - low incidence of clearing ADA
- Low observed immunogenicity in clinical development
  - incidence of ADA mainly transient
  - incidence up to 3% of neutralizing ADAs
  - generally no influence on safety and efficacy

Platform so far has shown very benign immunogenicity profile

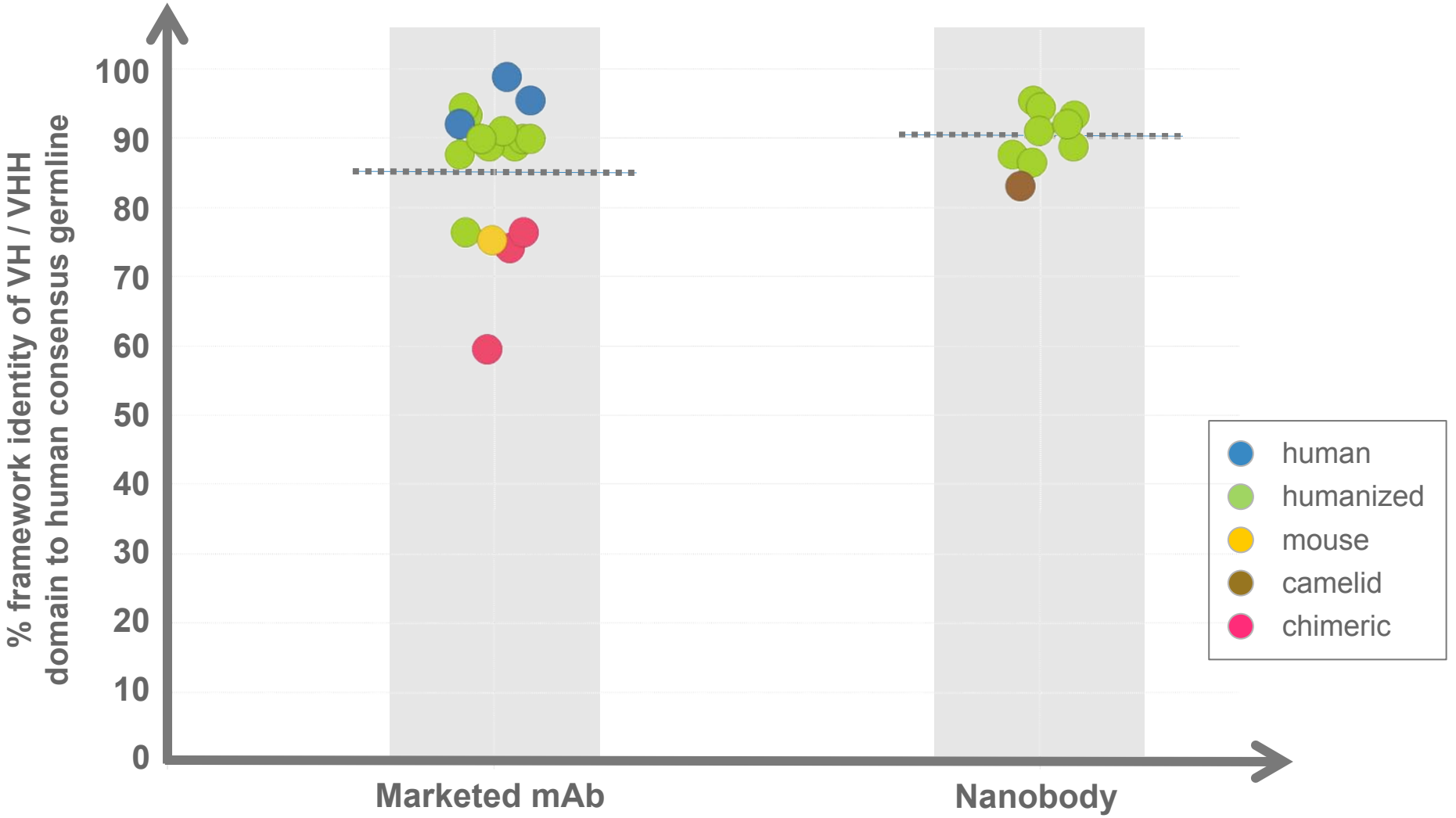
Outline

- Introduction
- Nanobodies - low immunogenicity by design
- Nonclinical experience
- Clinical experience
- Conclusion

Nanobodies – low immunogenicity by design
Features known to influence immunogenicity

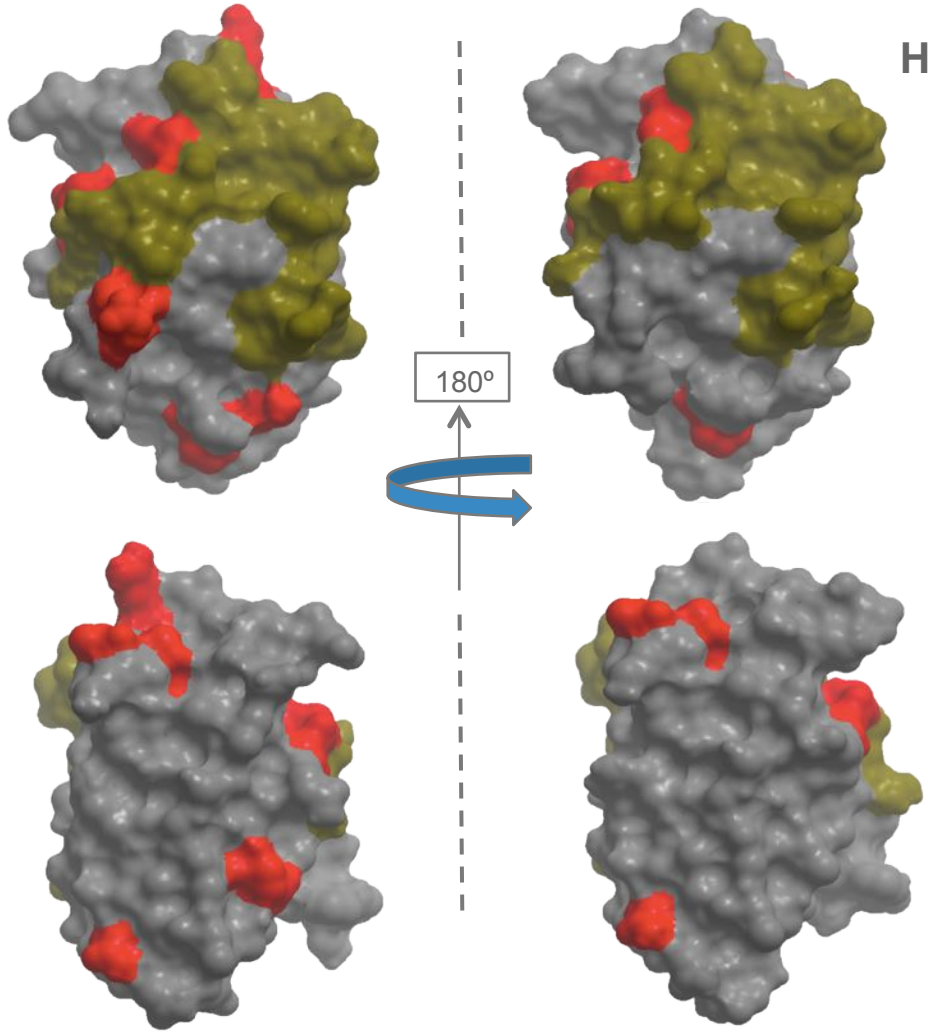
- Sequence homology
- T-cell epitopes present
- Aggregation in formulation
- Protein structure and post-translational modifications
  - size and folding
  - glycosylation

Nanobodies – low immunogenicity by design
Sequence homology



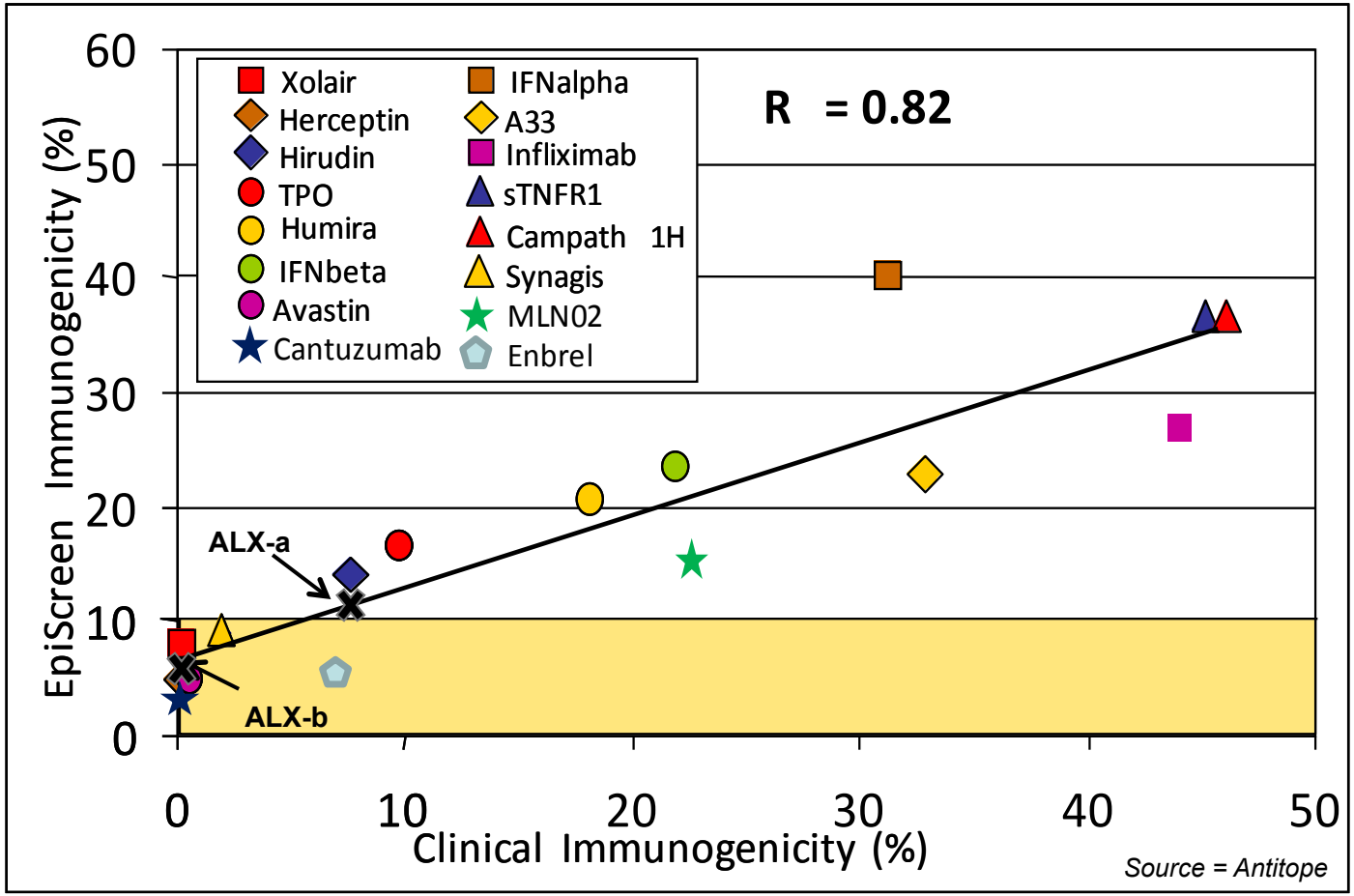
Nanobody sequence homology comparable to human/humanized mAb

Nanobodies – low immunogenicity by design
Exposed residues: identity to human VH surface



Nanobody engineering minimizes exposure of non-human residues

Nanobodies – low immunogenicity by design
T-cell proliferation assay (EpiScreen™, Antitope)



Limited T-cell epitopes in Nanobodies – expect low immunogenicity risk

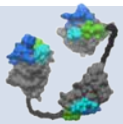
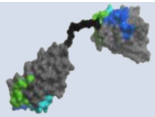
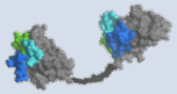
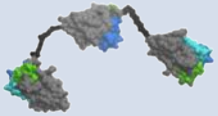
Nanobodies – low immunogenicity by design
Features known to influence immunogenicity

- Sequence homology
  - ✓~ 90% framework identity
- T-cell epitopes present
  - ✓ predictions place Nanobodies in “low immunogenicity” category
- Aggregation in formulation
  - ✓concentrations exceeding 100-150 mg/ml
  - ✓production batches essentially free of aggregates
- Protein structure and post-translational modifications
  - ✓ relative small size (<50kDa)
  - ✓ selected for robust and tight folding
  - ✓ removal of glycosylation sites in primary sequence:
    no N-glycosylation sites; very minor O-glycosylation if any

Outline

- Introduction
- Nanobodies - low immunogenicity by design
- Nonclinical experience
- Clinical experience
- Conclusion

Non-clinical immunogenicity
Observed benign immunogenicity for Nanobodies

22 non-clinical studies / dosing up to 26 weeks				
				
ALX-0081	ALX-0141	ALX-0061	ALX-0651	ALX-0171
Bivalent, monospecific	Trivalent, bispecific	Bivalent, bispecific	Biparatopic, monospecific	Trivalent monospecific
i.v. Subcutaneous	i.v. Subcutaneous	i.v.	i.v. Subcutaneous	i.v. inhaled
NHP, rodent	NHP	NHP	NHP	Rodent

Benign non-clinical immunogenicity profile

Routes of administration did not influence immunogenicity

0-37% of ADA positive animals (rodent/non-rodent species)

1/851 animals excluded due to clearing ADA

Outline

- Introduction
- Nanobodies - low immunogenicity by design
- Nonclinical experience
- Clinical experience
- Conclusion

Immunogenicity Assessment Strategy
Multi-tiered Approach

ADA assays

Screening assay

↓

Confirmatory assay
drug displacement assay

↓

End-point titration assay

↓

Characterization assay, e.g. neutralizing ADA assay

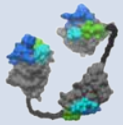
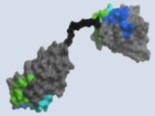
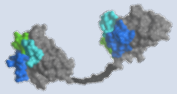
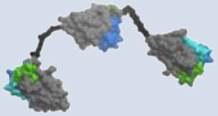
Data interpretation and risk assessment

Subject classification:
1) Negative
2) Treatment emergent

↓

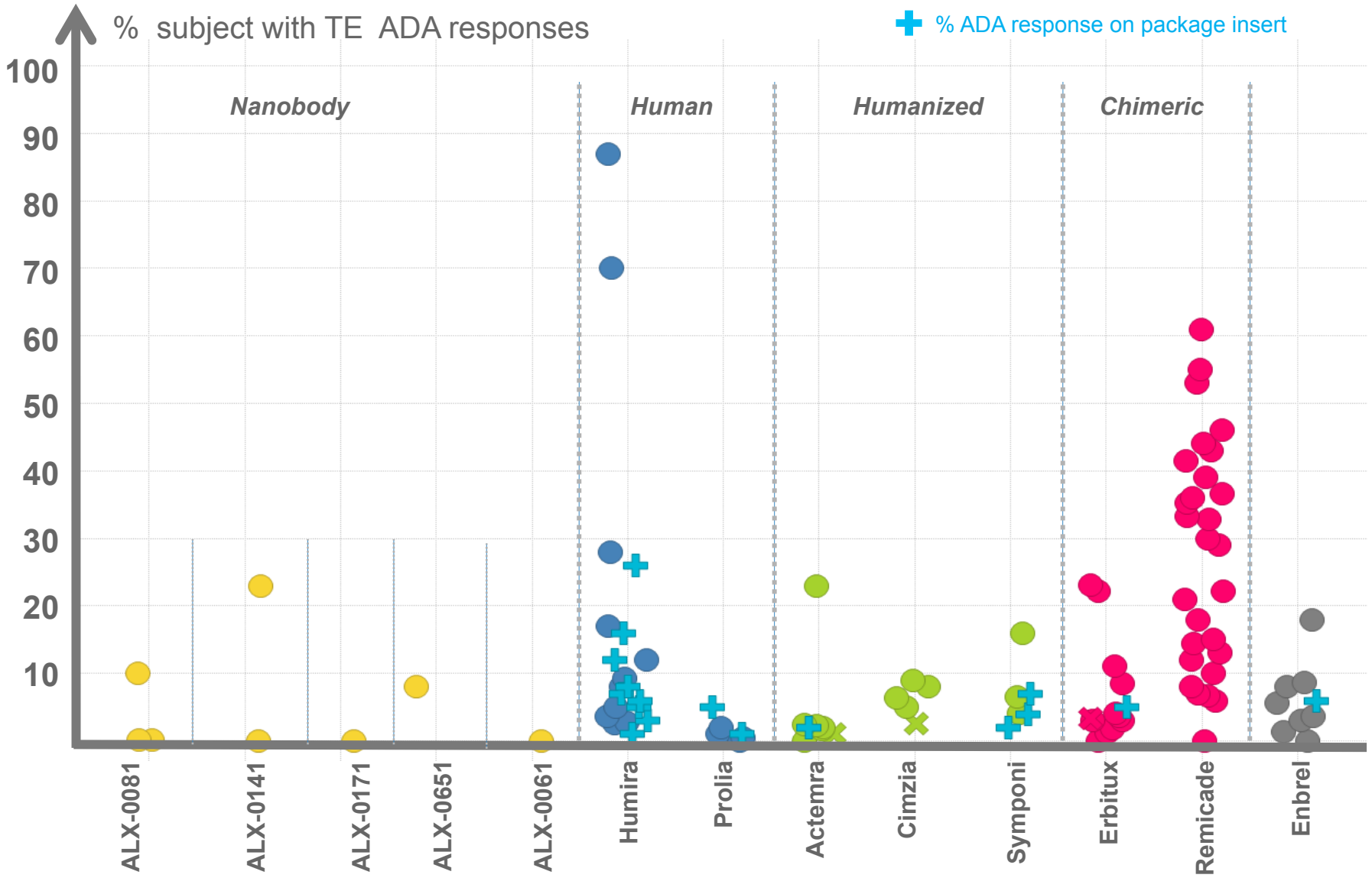
Correlation with PK, PD and safety

Clinical immunogenicity
Observed benign immunogenicity for Nanobodies

9 clinical studies / dosing up to 24 weeks				
				
ALX-0081	ALX-0141	ALX-0061	ALX-0651	ALX-0171
Bivalent, monospecific	Trivalent, bispecific	Bivalent, bispecific	Biparatopic, monospecific	Trivalent monospecific
i.v. Subcutaneous	Subcutaneous	i.v.	i.v.	Inhaled
Single dose Multiple dose	Single dose	Single dose Multiple dose	Single dose	Single dose Multiple dose

No link between administration route and immunogenicity seen so far

Clinical immunogenicity
ADA responses: Nanobodies in range with humanized mAbs



Case by case assay development
Nature of drug and clinical application determine ADA assay characteristics

- Clinical application
  - dosing regimen: single versus multiple dose, every day vs every 4/8 weeks
  - dose: 0,1 mg/kg vs 6 mg/kg
  - dosing route (*i.v., s.c., inhaled*)

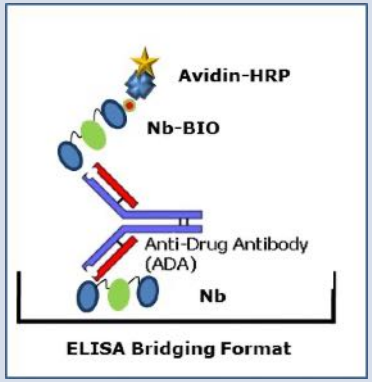
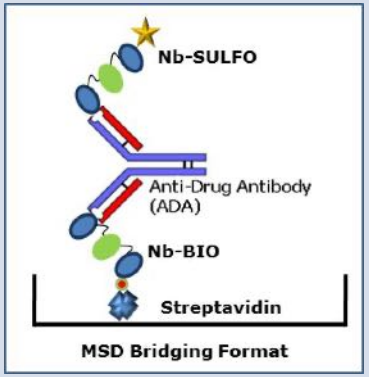
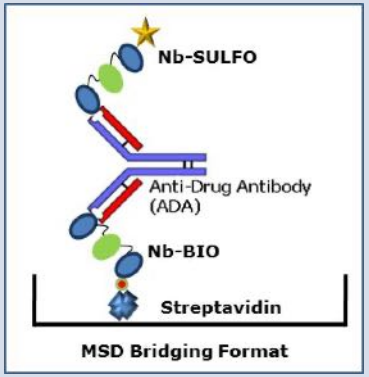
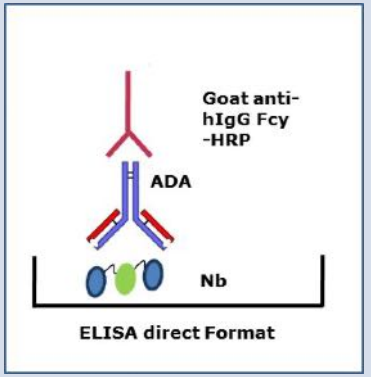
Different drug tolerance requirements

- Nature of Nanobody
  - half-life extended or not
  - soluble target or membrane target

Different drug tolerance requirements

Consider target interference

ADA assay experience in support of clinical trials

ADA assay format	ELISA bridging sequential	MSD bridging sequential	MSD bridging homogeneous	ELISA direct
pictogram				
#trials	4 trials	1 trial	4 trials	characterisation
Drug tolerance	-	++	+++	-
Target interference	yes	yes	yes	no

Strategies followed to improve drug tolerance

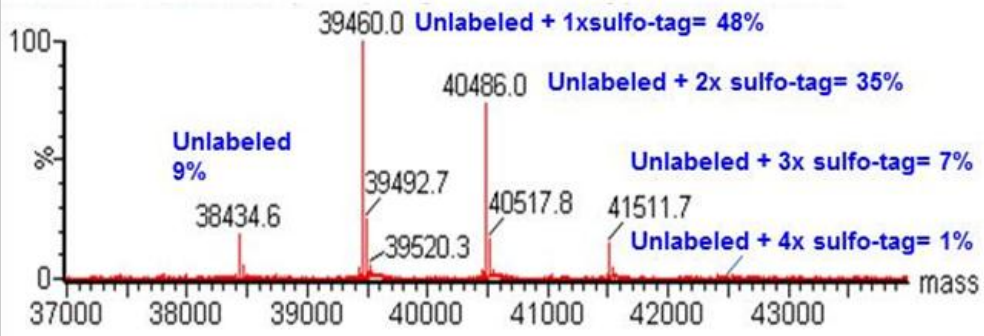
- Incubation time
  - increase contact time of sample with capture reagent or master mix: increase incubation from 1h/2 h to overnight
  - 2 examples:overnight incubation
- Concentration labeled reagents
  - Excess of labeled reagents (Bio/Sulfo) over free drug: 1 µg/ml to 4 µg/ml
  - 1 example with 4 µg/ml master mix
- Acid dissociation/neutralisation step
  - dissociate drug/ADA complexes with acid
  - Neutralize and re-equilibrium with bio-Nb/sulfo-Nb
  - drawback: ADA might be affected
  - 1 example with assay acid dissociation/neutralisation

Drug tolerance could be improved from ng/ml to 1-200 µg/ml

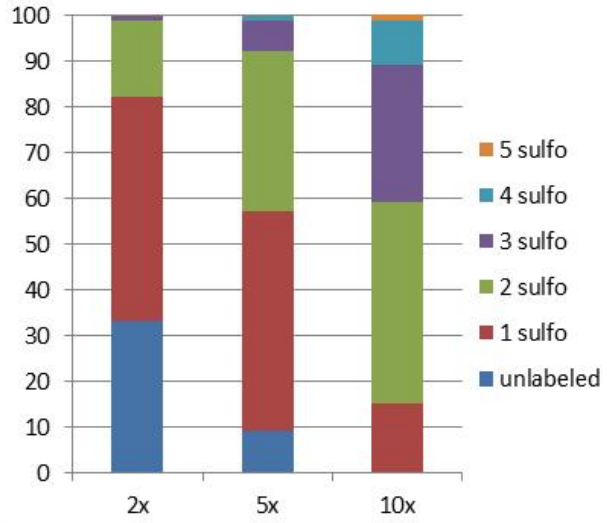
! Needs to be balanced with assay sensitivity – often inverse correlation

Points to consider during ADA assay development
Quality control of labeled reagents

LC-MS: - degree of unlabeled drug
- degree of labels incorporated



Mass of unlabeled nanobody: 38434.8 Da

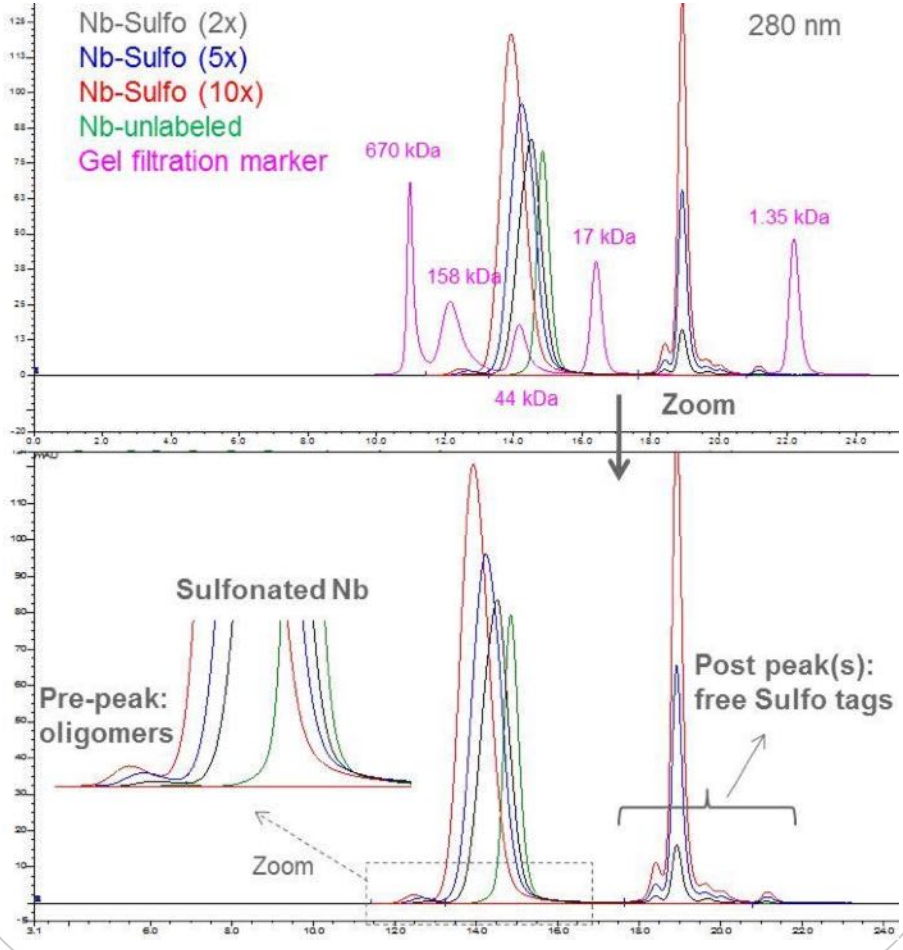


- Labeling conditions:
  - Molar excess label: 2 – 5- 10 fold
- Prevent excess of unlabeled material
- The higher the excess of molar label, the higher the degree of labeling

  → increase assay sensitivity
- QC check
  - Over time to guarantee stability
  - When re-labeling of assay reagents are required

Points to consider during ADA assay development
Quality control of labeled reagents

SEC: - degree of oligomers & aggregates
- presence of free label



- Labeling conditions:
  - Molar excess label: 2 – 5- 10 fold
- Prevent excess of free label

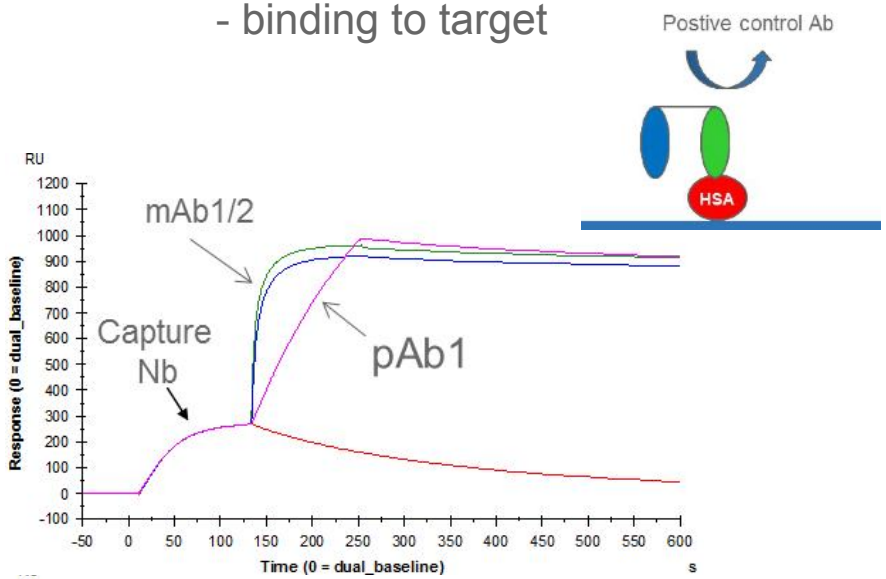
  → affects assay quality
- Monitor degree of aggregation

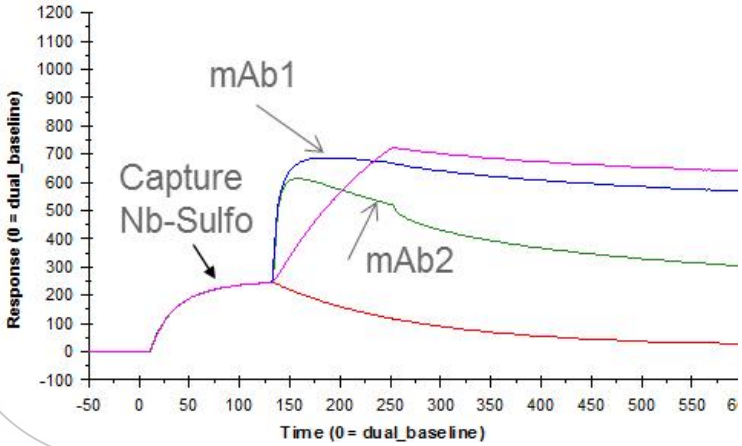
  e.g. 0,55% high molecular weight aggregates (>600 kDa) were sufficient to increase reactivity (Tatarewicz, 2010)
- QC check
  - Over time to guarantee stability
  - When re-labeling of assay reagents are required

Tatarewicz, et al. J Immunol Methods 2010, 357 (1-2):10-6

Points to consider during ADA assay development
Quality control of labeled reagents

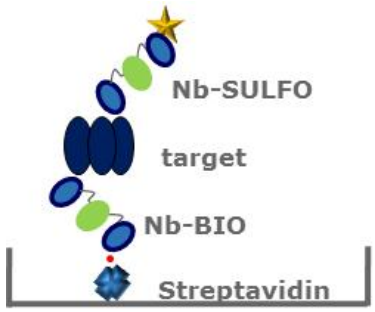
Biacore: - binding to positive control Ab
- binding to target



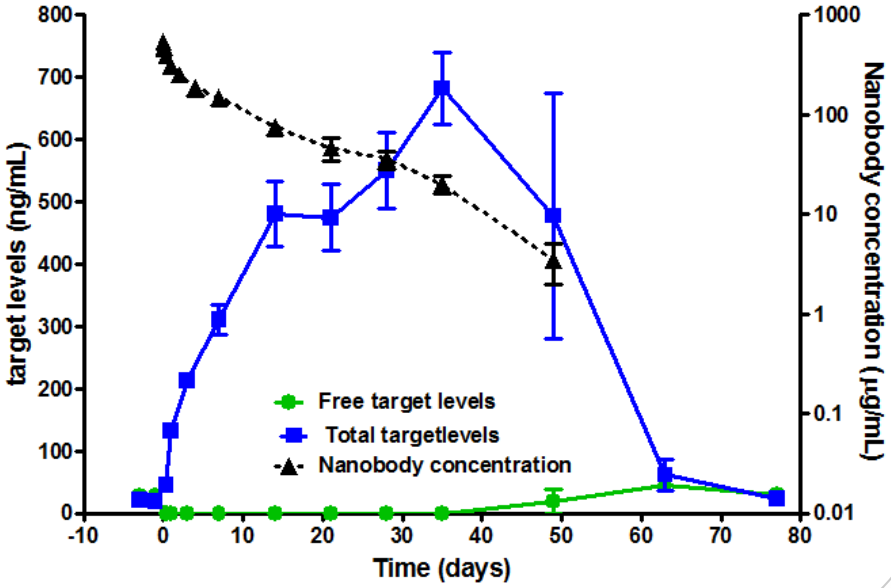


- Labeling conditions:
  - molar excess label: 2 – 5- 10 fold
- Binding on positive control antibody
  - assure optimal sensitivity
- Binding on target
  - Over-labeling might affect target binding
  - Assure detection of neutralizing antibodies

Investigation of target interference
A case study



MSD Bridging Format



- Circulating multivalent target capable of bridging between the Biotinylated and Sulfo-tagged Nb
- No interference observed at physiological relevant concentration: evaluated during method validation
- Target/drug complexes might accumulate upon treatment and reach superphysiological concentrations upon treatment
- No PK assay measuring target/drug complexes

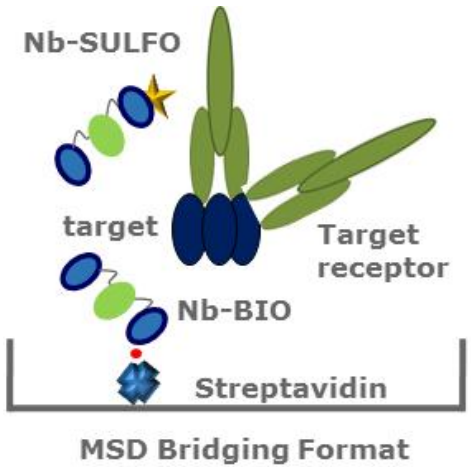
Investigation of target interference
A case study: spiking with target receptor or neutralising antibody

Inspiking with neutralizing Ab

	ECL signal	ECL after spiking NAb	% reduction
Mock target/drug complex samples			
50 ng/mL target	2782	222	**92%**
+ 0.5 µg/mL Nb	1355	167	**88%**
+ 3 µg/mL Nb	602	139	**77%**
200 ng/mL target	11267	449	**96%**
+ 0.5 µg/mL Nb	6690	293	**96%**
+ 3 µg/mL Nb	2601	230	**91%**
+ 7 µg/mL Nb	1143	205	**82%**
ADA samples			
Sample 1	1741	1887	**-8%**
Sample 2	3601	3870	**-7%**

Inspiking with target receptor

	ECL signal	ECL after spiking target receptor	% reduction
Mock target/drug complex samples			
200 ng/ml target + 10 µg/mL Nb	976	406	**58%**
ADA samples			
Sample 1	1609	1689	**-13%**
Sample 2	2391	2638	**-11%**

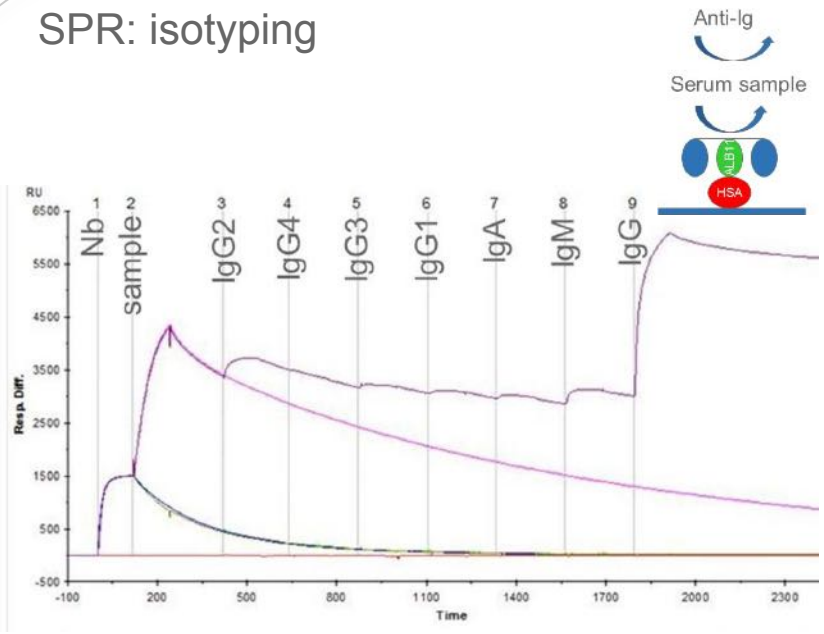


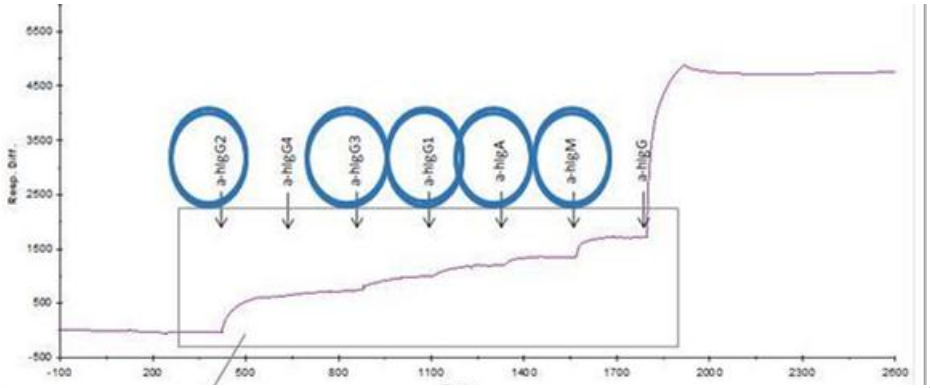
MSD Bridging Format

- Presence of target/drug complexes can be excluded by spiking neutralising antibody or target receptor

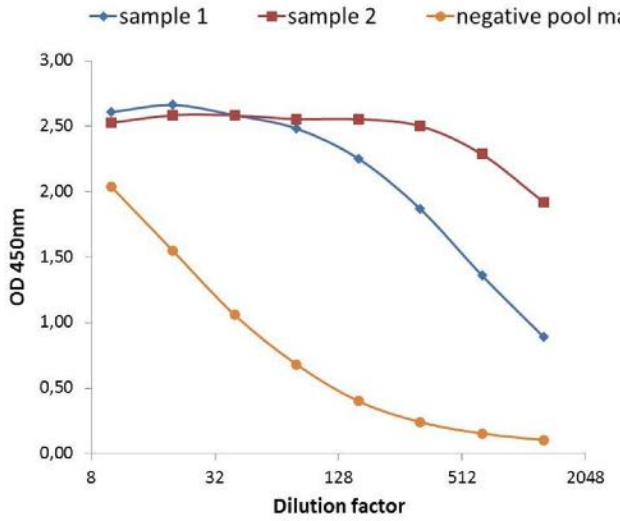
Characterisation of ADA responses

SPR: isotyping





Direct ELISA



- Direct ELISA detects IgG ADA whereas target does not interfere
- Surface plasmon resonance as powerful tool to perform isotyping, to pin point reactivity by testing on different building blocks, is used as quality control of ADA results

Outline

- Introduction
- Nanobodies - low immunogenicity by design
- Nonclinical experience
- Clinical experience
- Conclusion

Conclusions

- Nanobodies possess intrinsic “low immunogenicity risk”
- Nanobody platform allows to design and optimises molecules with low immunogenicity attributes
- Resulting observed immunogenicity in non-clinical studies was benign
- Interpretation of safety studies was not hindered
- Resulting observed clinical immunogenicity: generally low ADA incidence
- ADA assays are developed on case by case basis with special attention to drug tolerance, target interference and quality of the reagents

Acknowledgements

- Judith Baumeister
- Marie-Ange Buyse
- Carlo Boutton
- Peter Casteels
- Marie-Paule Bouche
- Ingrid Ottevaere
- Andreas Menrad
- Josi Holz
- Ablynx CMC department
- Ablynx Discovery department
- Ablynx Pharmacology department
- Ablynx Clinical department
- Ablynx Project management team
- Study subjects and patients