Immunogenicity monitoring during preclinical development of Nanobodies®: comparing assay formats and species matrices

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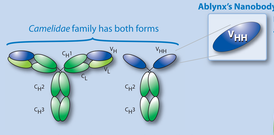
Introduction

- Ablynx develops antibody-derived therapeutic Nanobodies for the treatment of cardiovascular, bone and inflammatory diseases

Camelidae family has both forms



Conventional Antibody

Heavy and light chains

Both chains required for antigen binding and stability

Heavy-Chain Antibody

Only heavy chains

Full antigen binding capacity and very stable

Ablynx's Nanobody®

Based on the smallest functional fragment of a naturally occurring heavy-chain antibody

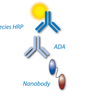
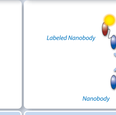
Therapeutic Nanobodies

ALX-0081	ALX-0141	ALX-0061
Clinical programme in phase II • therapeutic Nanobody targeting von Willebrand Factor (vWF) • may reduce risk of thrombosis in patients with acute coronary syndrome (ACS) and thrombotic thrombocytopenic purpura (TTP)	**Clinical programme in phase I** • therapeutic Nanobody targeting von receptor activator of NF-Kappa B ligand (RANK-L) • may reduce risk of fractures through highly potent inhibition of key driver of bone resorption in post-meno-pausal osteoporosis, rheumatoid arthritis and bone metastasis/cancer	**Programme entering IND phase (pre-phase I)** • therapeutic Nanobody targeting IL-6 receptor (IL-6R) • may reduce inflammation in rheumatoid arthritis
Bivalent target binding Nanobody (28kDa) • binds with high affinity to the A1 domain of vWF • blocks the interactions between vWF, platelets and vascular collagen • selectively prevents thrombus formation under high shear conditions	**Bivalent target binding Nanobody (41kDa)** • binds with high affinity to RANK-L • blocks the RANK-RANK-L interaction • tailored half life	**Monovalent target binding Nanobody (26kDa)** • binds with high affinity to mIL-6R and sIL-6R • blocks the interactions between IL-6 and IL-6R without cross-linking • tailored half life

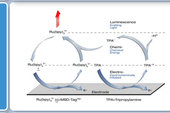
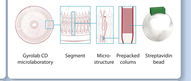
Purpose of immunogenicity monitoring

- Biopharmaceutical therapeutics are potentially immunogenic, which may
  - Influence pharmacokinetic behavior
  - Induce loss of efficacy and
  - Potentially lead to serious side effects
- Therefore immunogenicity of therapeutic proteins should be assessed during preclinical and clinical development
- Approach
  - Tiered approach
  - Risk based
  - Event driven
- Challenges
  - Assay formats and platforms
  - Drug interference
  - Acid dissociation
  - Validity of controls and established cut point
  - Clinical versus non clinical
  - Immunogenicity data interpretation

Assay formats

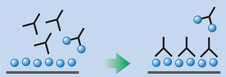
	Direct ELISA	Bridging ELISA
Setup		
Advantages	• Sensitive, less drug interference • No labeling • Commercial detection Abs	• Species independent • Highly specific • All isotypes
Disadvantages	• Species dependent • High background • Isotype dependence of detector Ab • Altered epitopes due to immobilization • Restricted detection of low affinity Abs	• Labeling of drug • Drug interference • Possible limited detection of IgG4 • Altered epitopes due to immobilization • Restricted detection of low affinity Abs

Assay platforms

	ECL bridging assay on MSD platform	Gyrolab™ BioAffy System
Setup		
Advantages	• Sensitive • Broad dynamic range • In solution phase kinetics/less washing steps → detection of low affinity Ab • High drug tolerance	• Small sample and reagent consumption • High throughput • Broad dynamic range • High drug tolerance
Disadvantages	• Single vendor • 2 labeled reagents • Expensive consumables	• Single vendor • 2 labeled reagents • Expensive consumables

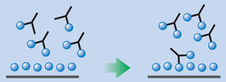
Drug interference

- Established drug interference levels will depend on
  - Assay format/assay platform
  - Positive control



Low drug levels

- Acceptable drug interference levels will depend on
  - Dosing scheme
  - PK/PD parameters
- Drug interference can be tackled by
  - Drug removal step
  - Drug dissociation step
  - Affinity capture elution



High drug levels

Assay performance I

	ALX-0081
Drug concentrations in samples	Low drug concentrations in wash out samples due to short half life and dosing schedule
Assay format	Bridging ELISA / ECL bridging
Species	human / human
MRD	1/4 / 1/6
LOD [ng/mL]	50 / 125
Drug tolerance level [µg/mL]	< 0.5 / < 0.5
Acid treatment	No / Yes
Positive control	mAb / mAb

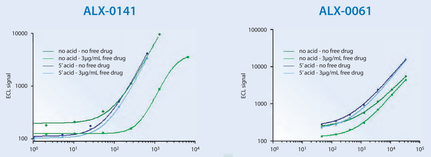
MRD: minimum required dilution • LOD: limit of detection

Assay performance II

	ALX-0141			ALX-0061	
Drug concentrations in samples	Higher drug concentrations for longer period of time due to long half life ***Need for assay optimization - different assay formats and platforms***				
Assay format	Bridging ELISA	Direct ELISA	ECL bridging	Direct ELISA	Bridging ELISA
Species	NHP	NHP	NHP	NHP	NHP
MRD	1/2	1/25	1/30	1/20	1/2
LOD [ng/mL]	385	506	662	970	385
Drug tolerance level [µg/mL]	0.005 - 0.05	0.5 - 5	20 - 50	0 - 3	0.005 - 0.05
Acid treatment	No	No	Yes	No	No
Positive control	pAb	pAb	mAb	pAb	pAb

Acid dissociation consequences ECL bridging I

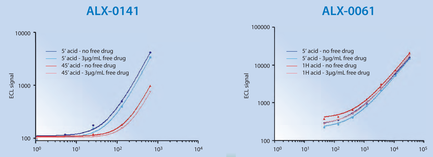
No acid dissociation versus short acid dissociation treatment



Acid dissociation step clearly improved drug tolerance levels without affecting the positive control

Acid dissociation consequences ECL bridging II

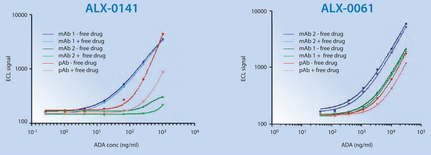
Short versus long acid dissociation treatment



Implementation of an acid dissociation step can have a deleterious effect on the study sample/positive control

Validity of control and validation samples

- Relevance of mouse monoclonal as positive control
  - Stability in human serum
  - Species specific control not always available
  - Limited availability of polyclonal control samples
- mAbs and pAbs can behave different in the assay
  - Affinity of mouse monoclonal will determine assay performance



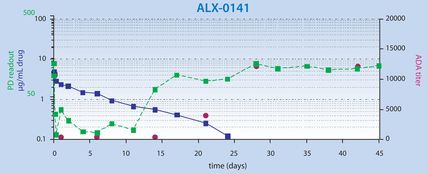
Preclinical versus clinical outcome

- Limited predictive power of animal models for immunogenicity of drug in clinical situation
  - Drugs are sequence optimized for human targets, not for animal targets
  - Differences in the immune system function
- Incidence of ADAs in preclinical studies does not necessarily increase the risk of antibody related clinical sequelae

Then why monitor immunogenicity in preclinical studies?

Preclinical immunogenicity

- Immunogenicity data are needed for correct interpretation of preclinical studies
  - To demonstrate exposure and/or explain altered PK/PD profiles
  - To demonstrate biological activity is not neutralized
- Correlate ADA/PK/PD/toxicology data



Conclusions

- Immunogenicity assay performance was determined in ***different species matrices*** and ***different formats*** were compared. Assay performance can vary significantly in different species matrices, even if a species independent assay format is used
- Acceptable ***drug tolerance levels*** are determined on a case-by-case basis
- ***Drug tolerance can be improved*** by implementing an acid dissociation step, using in solution phase kinetics or by switching assay format or platform
- The ***choice of positive control*** influences the outcome of validation, therefore it is advised to confirm the validation cut points using real study samples
- Although immunogenicity observed in preclinical studies cannot predict the immunogenicity potential of a drug candidate in the clinic, it is an ***essential tool*** in the interpretation of preclinical results

Acknowledgements

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