Ablynx

Nanobodies®
Innovative therapeutics

An innovative approach for detecting NAb directed to antibody-derived therapeutics based on the bridging ADA assay

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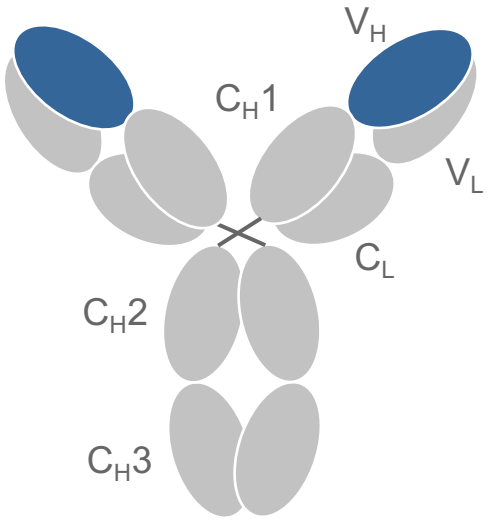
Outline

- Background
  - Ablynx's Nanobodies
- Clinical immunogenicity testing
  - ADA and NAb assay: current challenges in data interpretation
- Innovative assay approach for detecting NAb
  - based on the bridging ADA assay format
  - no pre-treatment step
  - NAb detected at same sensitivity as ADA, allowing unambiguous data interpretation
  - easy straightforward assay development especially for multi-functional antibody formats

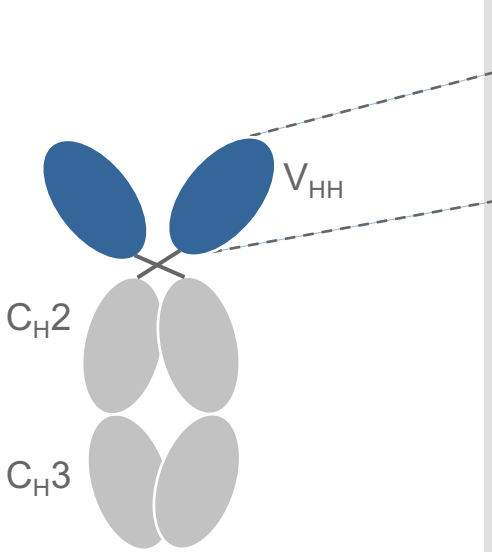
Nanobodies

Derived from heavy-chain only antibodies

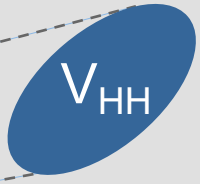
- *Camelid* heavy-chain only antibodies are stable and fully functional
- Nanobodies represent the next generation of antibody-derived biologics



Conventional antibodies



Heavy chain only antibodies



12-15kDa

Ablynx's Nanobody

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

Clinical Immunogenicity testing

Detection of anti-drug antibodies

- Anti-drug antibody (ADA) assay: monitor presence of ADA over time
  - guarantee sensitive detection of ADA in presence of drug
  - correlate with PK, PD, efficacy and safety read-outs

- Neutralizing antibody (NAb) assay: evaluate neutralizing potential
  - correlate with PD and efficacy
  - choice of NAb assay format (CLBA vs functional) depends on MOA
  - extensive pre-treatment steps often needed to obtain required drug/target tolerance

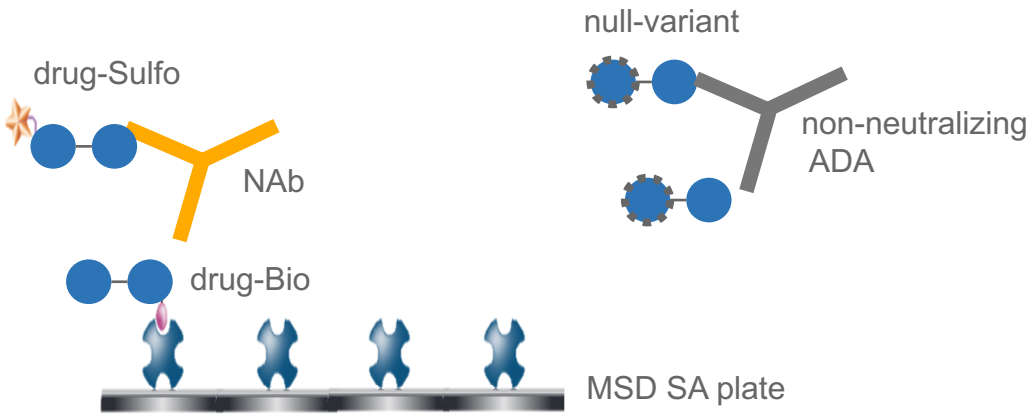
    => may introduce sensitivity difference between ADA and NAb assay

- Sensitivity gap complicates data interpretation as discrepancy between ADA and NAb incidence, or negative NAb results, can reflect
  - presence of non-neutralizing antibodies only
  - neutralizing antibodies that are left undetected in the NAb assay

Clinical Immunogenicity testing

An innovative approach for detecting NAb

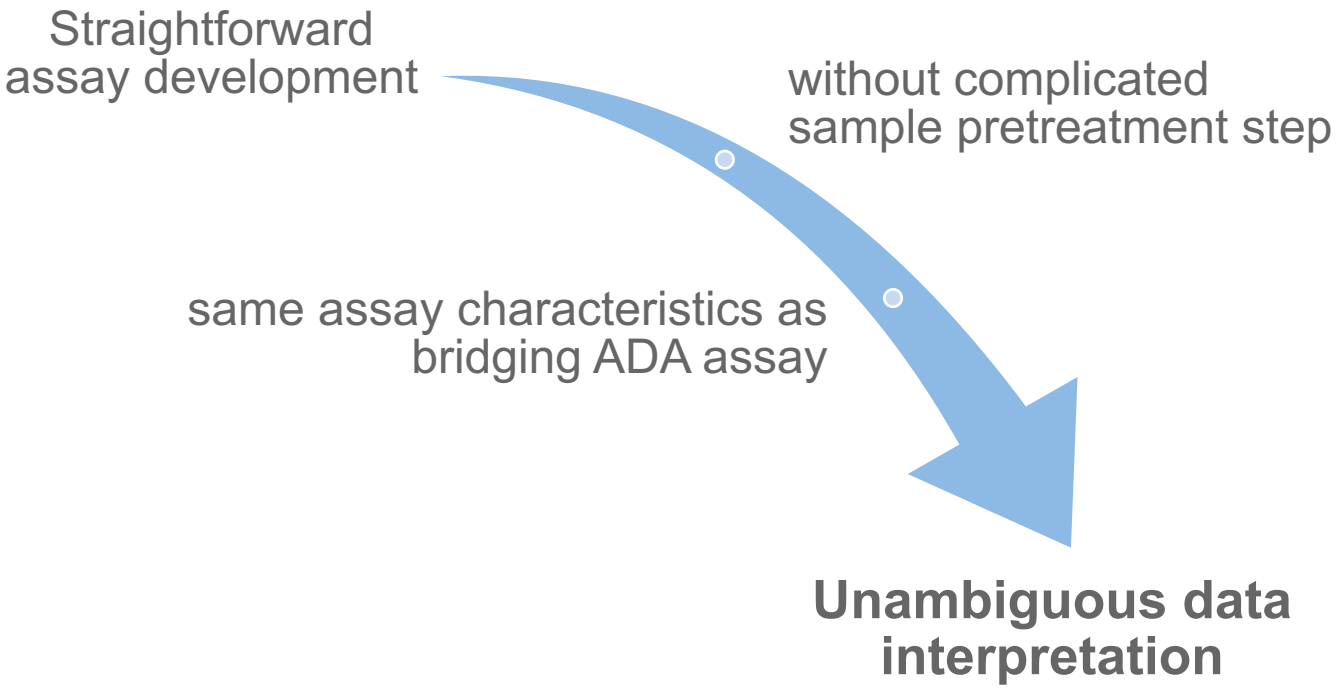
- Alternative NAb assay format based on conventional bridging ADA assay, without sample pretreatment
  - non-neutralizing antibodies are complexed with null variant and therefore not detected
  - null variant is identical to the drug except for altered CDR's of target binding domain
  - excess amount of null variant is added to biotinylated/sulfo-labelled reagent master mix
  - positive assay signals reflect antibodies with neutralizing potential only



alternative NAb assay format

Clinical Immunogenicity testing

An innovative approach for detecting NAb



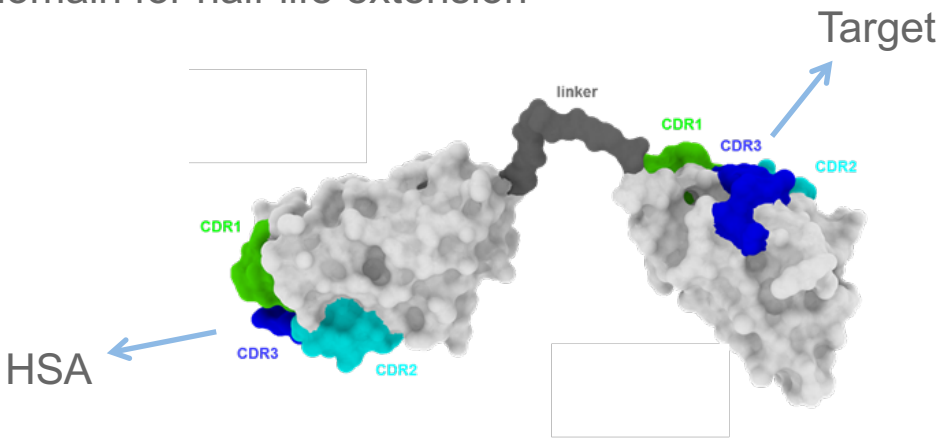
especially in early clinical development since higher risk that NAb is left undetected as responses are expected to be lower

allows determination of the neutralizing fraction within an ADA response

Case study

Introduction

- Bispecific Nanobody
  - domain for target binding (monomeric target)
  - HSA binding domain for half-life extension

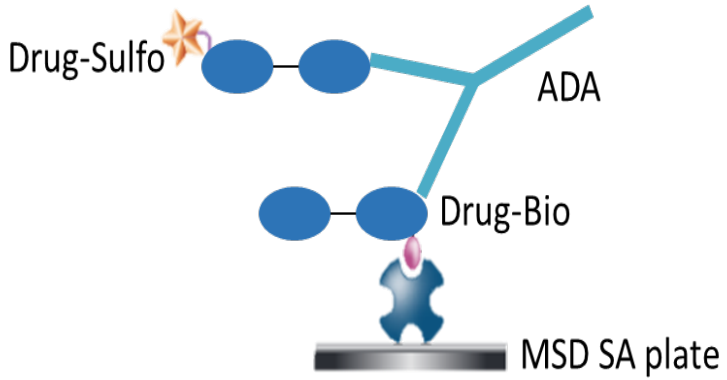


- Drug and target tolerance requirements of ADA and NAb assay to allow detection under treatment
  - up to 30 µg/ml drug at C_{trough} values
  - up to 1500 ng/ml target

Case study

Immunogenicity testing strategy

- Monitoring presence of ADA via homogeneous bridging ADA assay (MSD)
  - sufficiently drug tolerant
    - < 100 ng/ml ADA at anticipated C_{trough} for different dose groups
  - no target interference
    - no false positive results: monomeric target with no possibility to bridge between reagents
    - no false negative results: Bio- and Sulfo-labeled drug in master mix at sufficiently high concentration

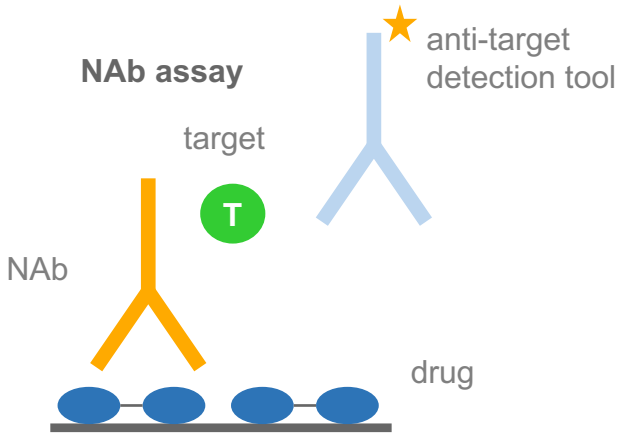


Case study

Immunogenicity testing strategy

- Evaluation of neutralizing potential via competitive ligand binding assay (CLBA) based on Nanobody-target receptor interaction

- NAb assay format justified during early clinical development as
  - MoA of Nanobody depends on “simple” binding/blocking of the target receptor
  - antagonistic function
  - no endogenous counterpart
  - no risk for change into agonistic functionality



Case study

Conventional NAb assay

- CLBA assay format: complex pre-treatment step needed to achieve drug and target tolerance
  - drug binds to soluble target with target accumulation (drug/target complexes) upon dosing
  - free drug and target cause false negative results

- Flow scheme

BEAD= Biotin-drug Extractin with Acid Dissociation

Case study

Conventional NAb assay

- Sensitivity gap between ADA assay and conventional NAb assay

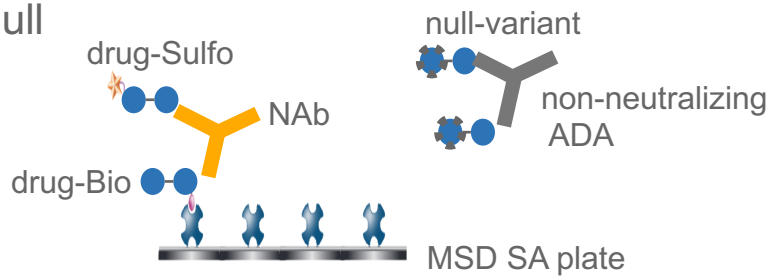
[drug]	NAb assay sensitivity (ng/mL)	ADA assay sensitivity (ng/mL)	Sensitivity difference ADA versus NAb assay
60 µg/mL	1890	<36	At least 50-fold
30 µg/mL	1374	<36	At least 40-fold
15 µg/mL	779	<36	At least 20-fold
600 ng/mL	417	<36	At least 10-fold
30 ng/mL	429	<36	At least 10-fold
0	521	<36	At least 10-fold

Neutralizing potential of ADA present at low levels cannot be determined

Case study

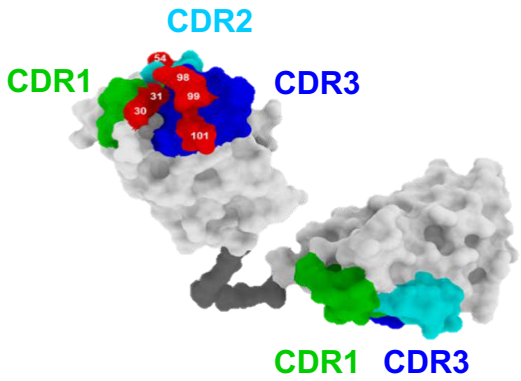
Alternative NAb assay

- Alternative NAb assay based on the conventional bridging ADA assay format
  - non-neutralizing antibodies are complexed with null variant and therefore not detected
  - positive assay signals reflect antibodies with neutralizing potential only



alternative NAb assay format

- Null variant of the Nanobody contains significant mutations in the CDRs (CDR1, 2 and 3) of the target binding domain, generated in different rounds of mutations
  - to abolish ability for target binding completely
  - to abolish binding of neutralizing antibodies (as determined by CLBA)
  - without introducing conformational changes and retaining binding of framework binding ADA

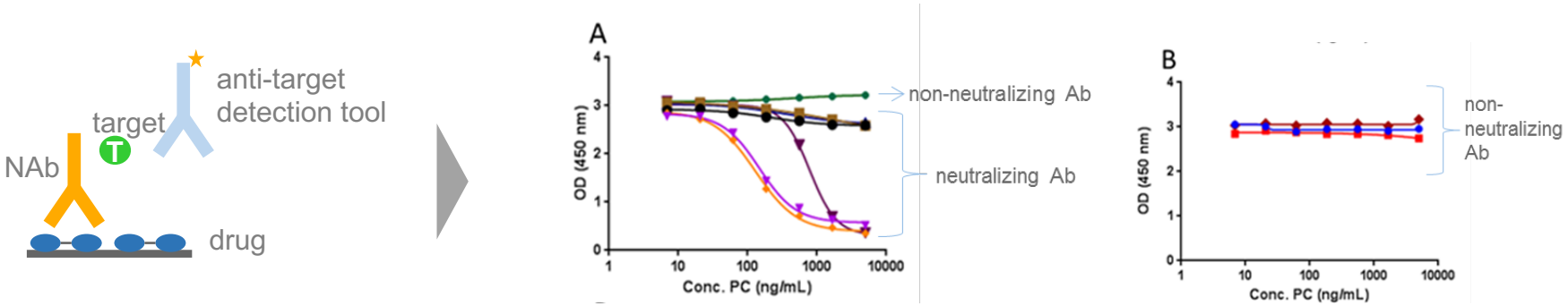


null variant of Nanobody

Case study

Alternative NAb assay

- Fit for purpose using panel of neutralizing and non-neutralizing mAbs
  - neutralizing potential defined as ability to block target interaction using competitive ligand binding assay (CLBA) in buffer without pre-treatment



  - binding region characterized (SPR/epitope mapping)
    - all neutralizing antibodies were found to bind to the CDRs of the target binding domain
    - framework binding ADA directed to different regions of the Nanobody (both on target and HLE domain) were all found to be non-neutralizing, i.e. are not sterically hindering target binding (despite small size of the Nanobody)

Case study

Alternative NAb assay

- Neutralizing Ab are detected at the same sensitivity as compared to the ADA assay: no false negative results

- Non-neutralizing Ab are left undetected
  - some residual binding can be detected at very high Ab concentrations, however these levels are not expected to be clinically relevant

		Assay response (ECL) at mAb concentration (ng/mL)									
Neutralizing Ab	Assay	20000	4000	1000	500	250	125	63	0	Sensitivity	Sensitivity in conventional NAb assay
mAb 1	ADA	19714	4122	1140	599	375	242	178	109	< 63 ng/mL	
	NAb	23151	4633	1266	640	383	246	173	92	< 63 ng/mL	556 ng/ml
mAb 2	ADA	55587	10405	2786	1405	754	448	273	106	< 63 ng/mL	
	NAb	62575	12217	3097	1596	854	466	278	89	< 63 ng/mL	556 ng/ml
mAb 3	ADA	646	214	139	122	118	117	110	116	400 ng/mL	
	NAb	671	203	127	105	103	99	94	94	543 ng/mL	> 5 µg/mL
mAb 4	ADA	757	250	147	136	125	119	116	107	165 ng/mL	
	NAb	807	251	140	127	117	108	103	95	111 ng/mL	> 5 µg/mL
Non-neutralizing Ab											
mAb 5	ADA	14731	5182	1811	935	530	301	209	102	< 63 ng/mL	
	NAb	128	101	94	88	88	89	92	96	6898 ng/mL	N/A
mAb 6	ADA	1624	435	206	165	139	129	131	120	< 63 ng/mL	
	NAb	96	92	95	93	96	97	99	97	> 20 µg/mL	N/A
mAb 7	ADA	1197	344	170	141	128	116	120	113	211 ng/mL	
	NAb	96	89	90	93	94	93	94	95	> 20 µg/mL	N/A
mAb 8	ADA	106020	20679	5109	2625	1314	733	446	110	< 63 ng/mL	
	NAb	117	99	92	90	93	94	90	89	11022 ng/mL	N/A

Case study

Alternative NAb assay

- Fit for purpose using polyclonal positive controls
  - neutralizing antibody fraction detected by titer and/or sensitivity determination
    - rabbit pAb (generated by immunization to be used as NAb positive control Ab)
    - pre-clinical study samples (rhesus monkey) originating from a disease model prone to development of ADA and shown to contain neutralizing activity via PD and efficacy markers

		Assay response (ECL) at pAb concentration (ng/mL)								Sensitivity	Sensitivity in conventional NAb assay
		4000	1000	500	125	31	13	5	0		
Rabbit pAb	ADA	4694	1192	659	230	135	117	114	105	<5.0 ng/mL	
	NAb	1520	416	267	129	98	88	95	92	31 ng/mL	5 µg/ml
Rhesus Monkey study samples		Assay response (ECL) at pAb dilution									
		100	400	1600	6400	25600	102400	409600	Log10 (titer)		
Sample 1	ADA	253499	25133	4526	812	236	121	105	5.1		
	NAb	95876	15281	3032	581	176	106	92	5.0		
Sample 2	ADA	714810	61990	9795	1711	389	155	110	5.5		
	NAb	268224	41932	7437	1363	309	123	99	5.4		
Sample 3	ADA	61076	8754	1496	333	144	112	98	4.9		
	NAb	35866	6523	1197	274	125	96	87	4.8		

Case study

ADA and Alternative NAb assay qualification

- Alternative NAb assay has same sensitivity and drug tolerance as ADA assay
  - compliant to current regulatory guidelines: < 100 ng/mL positive control in presence of highest anticipated drug levels

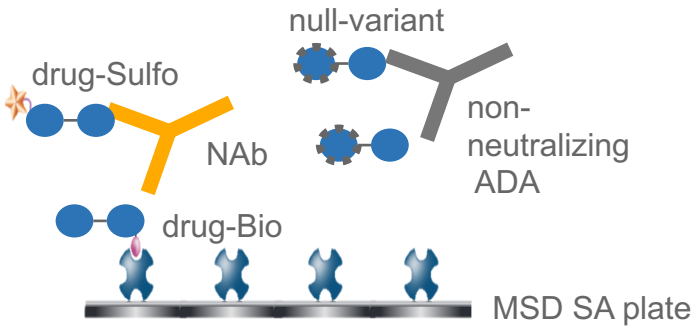
	ADA - Mean responses (ECL)					Alternative NAb - Mean responses (ECL)				
	Concentration drug					Concentration drug				
Conc. mAb 2 (ng/mL)	30.0 µg/mL	15.0 µg/mL	0.6 µg/mL	0.03 µg/mL	No drug	30.0 µg/mL	15.0 µg/mL	0.6 µg/mL	0.03 µg/mL	No drug
20000.0	27386	25746	41832	38100	34203	32166	38126	64889	67705	66243
5000.0	7264	9463	10162	10956	12297	7329	8712	13118	16188	16898
500.0	701	881	858	1210	1470	858	966	1164	1649	1747
250.0	441	525	512	545	745	456	482	619	817	1044
72.0	185	220	228	207	316	197	205	250	268	346
36.0	139	148	145	146	180	137	147	160	182	212
0.0	94	99	94	98	103	94	86	96	92	88
Sensitivity	<36.0 ng/mL	<36.0 ng/mL	<36.0 ng/mL	<36.0 ng/mL	<36.0 ng/mL	<36.0 ng/mL	<36.0 ng/mL	<36.0 ng/mL	<36.0 ng/mL	<36.0 ng/mL

- Target tolerance characteristics similar as the ADA assay: in case of a monomeric target, this NAb format is target tolerant
- Intra-run and inter-assay precision: ≤ 20%

Alternative NAb assay format

Conclusion

- **Format based on the conventional bridging ADA assay format**
  - positive signals reflect antibodies binding to the CDR region and having neutralizing potential by blocking target binding
  - non-neutralizing Ab are left undetected since these are saturated with the null variant of therapeutic drug



- **Straightforward assay development**
  - no pretreatment needed
  - once null variant available, easy straightforward assay development especially for multifunctional antibody formats, allowing evaluation of NAb against different functional domains

- **Allows unambiguous immunogenicity data interpretation as potential NAb are detected with the same sensitivity as ADA**
  - Titer levels can be determined and associated to PD, efficacy and safety to determine clinical relevant titer level, i.e. threshold level for clinical impact

Acknowledgments

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