COVA322 case study:

development of a novel anti-TNF/IL-17A bispecific FynomAb

7th OPEN SCIENTIFIC EIP SYMPOSIUM ON

IMMUNOGENICITY OF BIOPHARMACEUTICALS

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25 February, 2015

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Outline

- Introduction to Fynomer/FynomAb technology
- **COVA322 case study**
  - Design of COVA322 and rationale for dual cytokine targeting
  - Primary and safety pharmacology
  - **Pharmacokinetics and Immunogenicity**
  - Toxicology

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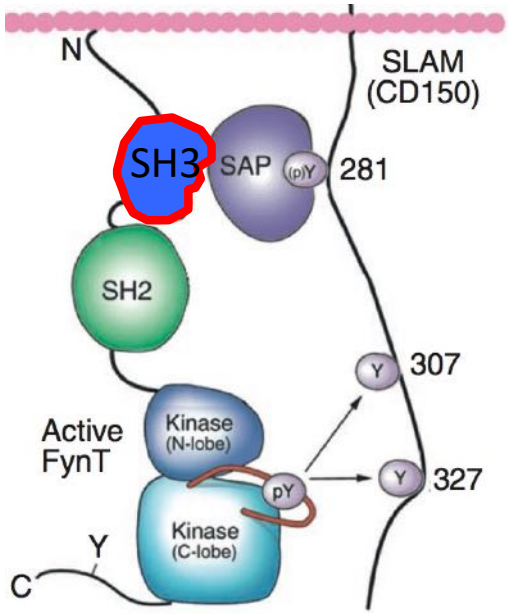
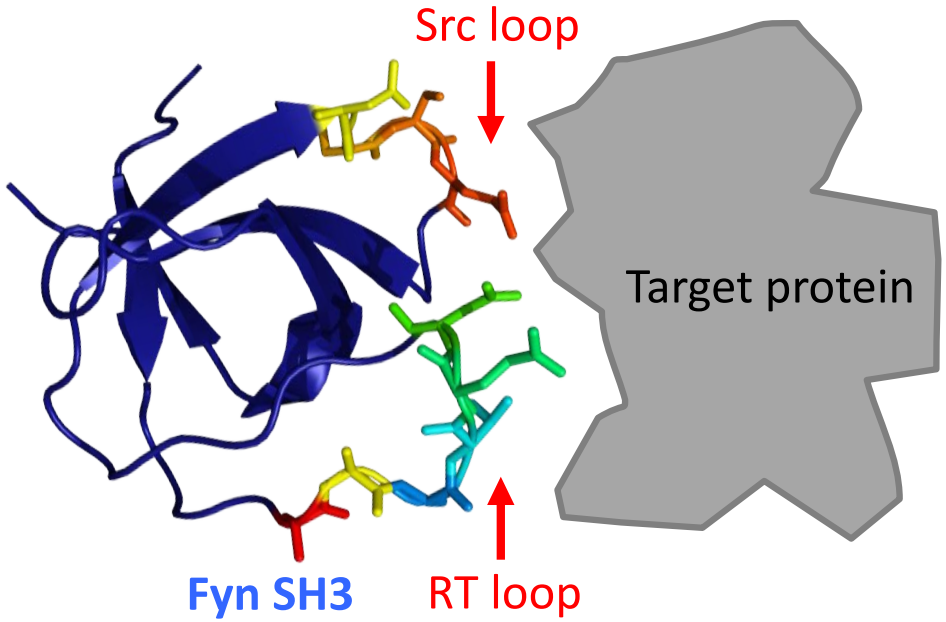
Fynomer technology: Fyn SH3 - the origin

- Fynomers are binding proteins derived from the SH3 domain of the Fyn kinase
- The SH3 domain contains two flexible loops (SRC and RT) that interact with the target

SH3 domain of Fyn kinase



SH3 interaction with target protein



from: Chan et al. (2003) *Nat Cell Biol*., 5, p. 155; Li, S.S. (2005) *Biochem J*, 390, p. 641.

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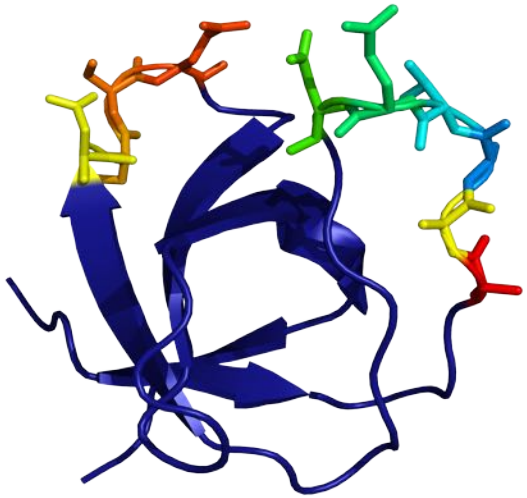
Fynomer technology

By randomly mutating the SRC and RT loops of the Fyn SH3 domain, Covagen has created a large phage display library from which Fynomers of virtually any target specificity and affinity can be isolated.

Fynomer characteristics at a glance:

- Derived from **fully human** SH3 protein domain
- SH3 domain is **fully conserved** (100% sequence homology) across different species
- **Monomeric** form, low complexity
- **No cysteine** residues
- **Small** size (7 kDa)
- **High** stability

Fynomer library



>8 x 10^{10} different library members

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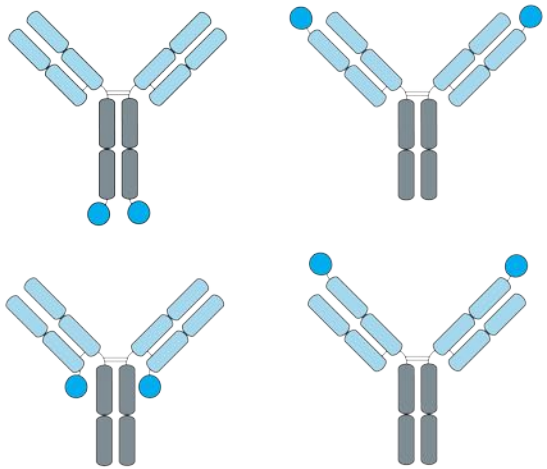
FynomAb technology

Fusion of Fynomers to monoclonal antibodies results in the generation of bi- or multispecific molecules called "FynomAbs"

FynomAb characteristics at a glance:

- FynomAbs are **stable** in vivo
- Both, Fynomers & antibody **retain their activity**
- Different fusion sites possible (light/heavy chain and N- or C-terminus) to allow for **maximal flexibility** in architecture
- Fc-mediated effector functions are **maintained**
- **Antibody-like** PK profiles
- **High productivity:** FynomAbs retain productivity of their parental antibody

Highly flexible architecture



Highly reproducible

>300 FynomAbs produced to date

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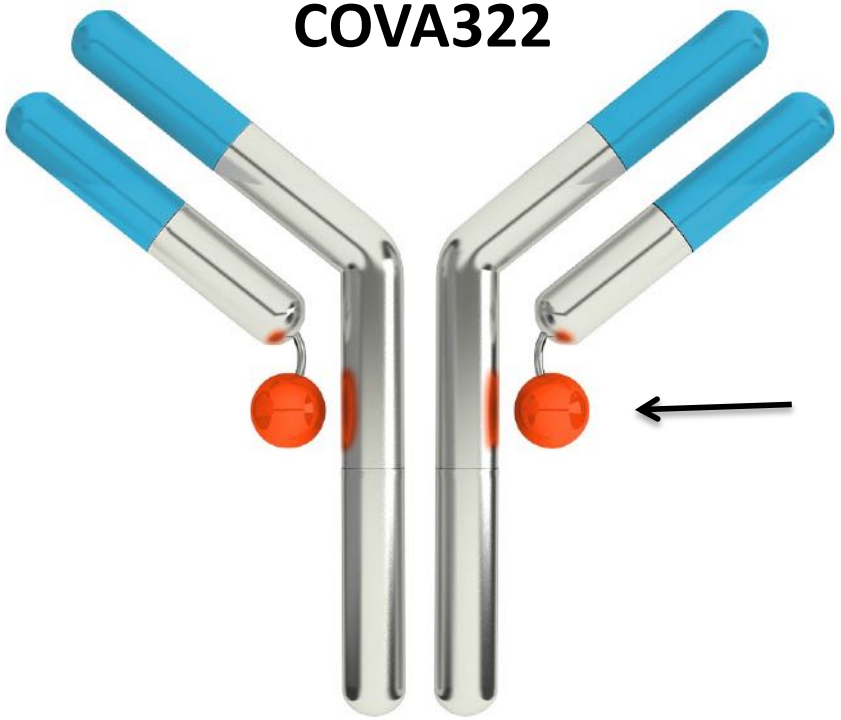
COVA322

Combining two clinically validated pathways in one molecule



Validated TNF antibody **adalimumab;** reduction of development risk

Anti-**IL-17A** selective Fynomer fused to C-terminus of the light chain

Designed for superior efficacy vs. mono-pathway treatment of inflammatory diseases due to synergistic effects of TNF and IL-17A

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Pharm-Tox package

- **Pharmacology**
  - Primary pharmacology: binding characterization, *in vitro* and *in vivo* bioactivity
  - Safety pharmacology: part of the 4-week repeat-dose toxicity study in Cyno

- **Pharmacokinetics (PK), toxicokinetics (TK) and immunogenicity (IM)**
  - Pilot PK studies (COVA322 and adalimumab) in mice and Cynomolgus monkeys
  - COVA322 single dose PK and dose range finding (DRF) study in Cynomolgus monkeys
  - TK profiles as part of the 4-, 13- and 26-week toxicity study in Cynomolgus monkeys

- **Toxicology**
  - GLP 4-, 13- and 26-week repeat-dose toxicity study in Cynomolgus monkeys
  - GLP tissue cross-reactivity study with human and Cynomolgus tissues
  - Cytokine release study with human whole blood cells

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COVA322 inhibits TNF as good as adalimumab, and IL-17A at least as good as secukinumab

Binding	COVA322 properties
Affinity for human IL-17A	K_D = 36 pM (better than secukinumab)
Affinity for Cynomolgus IL-17A	K_D = 63 pM (better than secukinumab)
Affinity for human TNF-alpha	K_D = 129 pM (as adalimumab)
Affinity for Cynomolgus TNF-alpha	K_D = 120 pM (as adalimumab)
Bioactivity	**COVA322 properties**
IC_{50} values *in vitro* (IL-17A inhibition) HT-29, HT-1080 cell line, NHDF primary cells	**IC_{50} =** 121 to 1084 pM (better than secukinumab)
IC_{50} value *in vitro* (TNF-alpha inhibition) L929 cell line	**IC_{50} =** 429 pM (as adalimumab)

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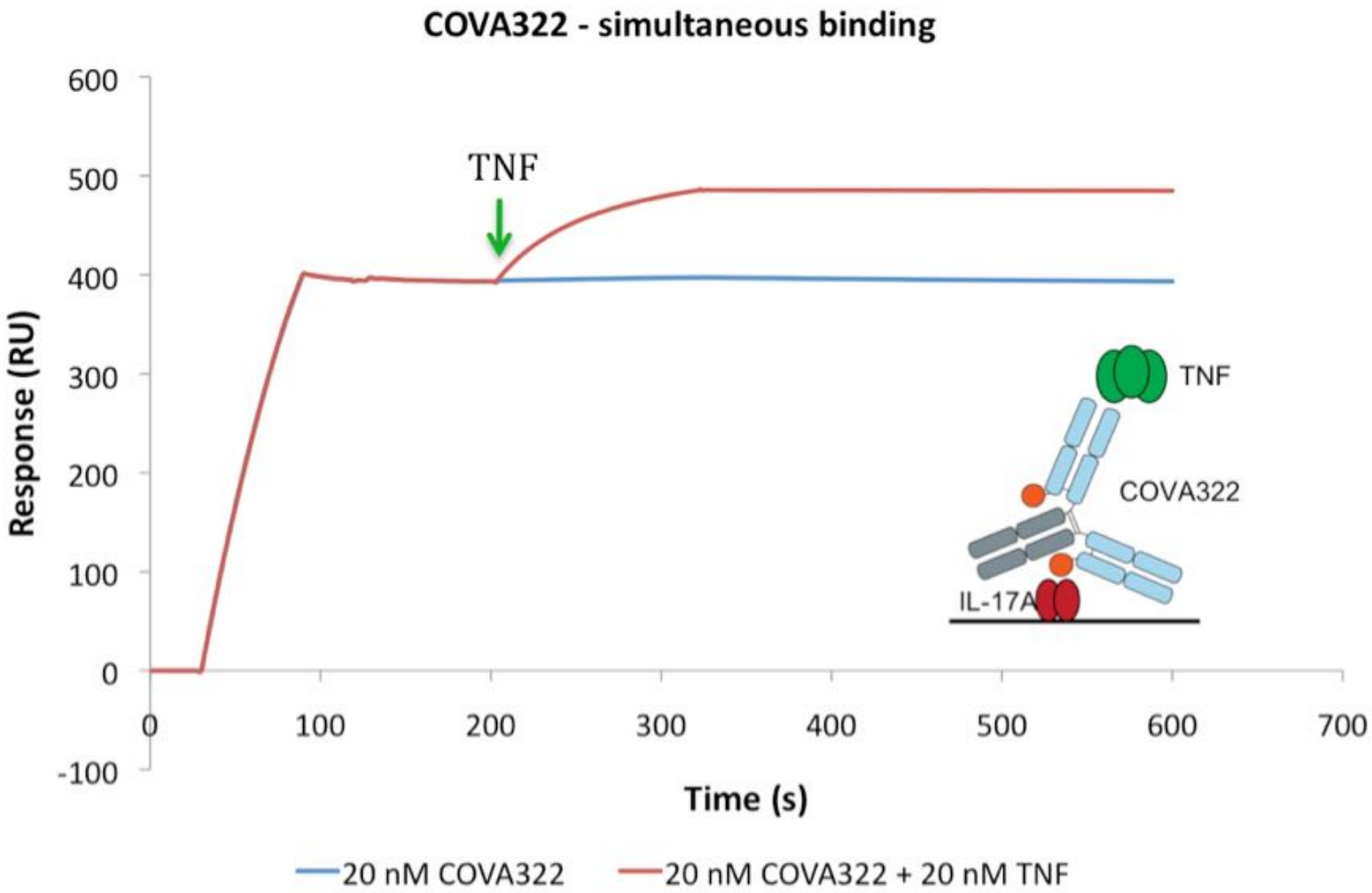
COVA322 simultaneously binds to TNF and IL-17A



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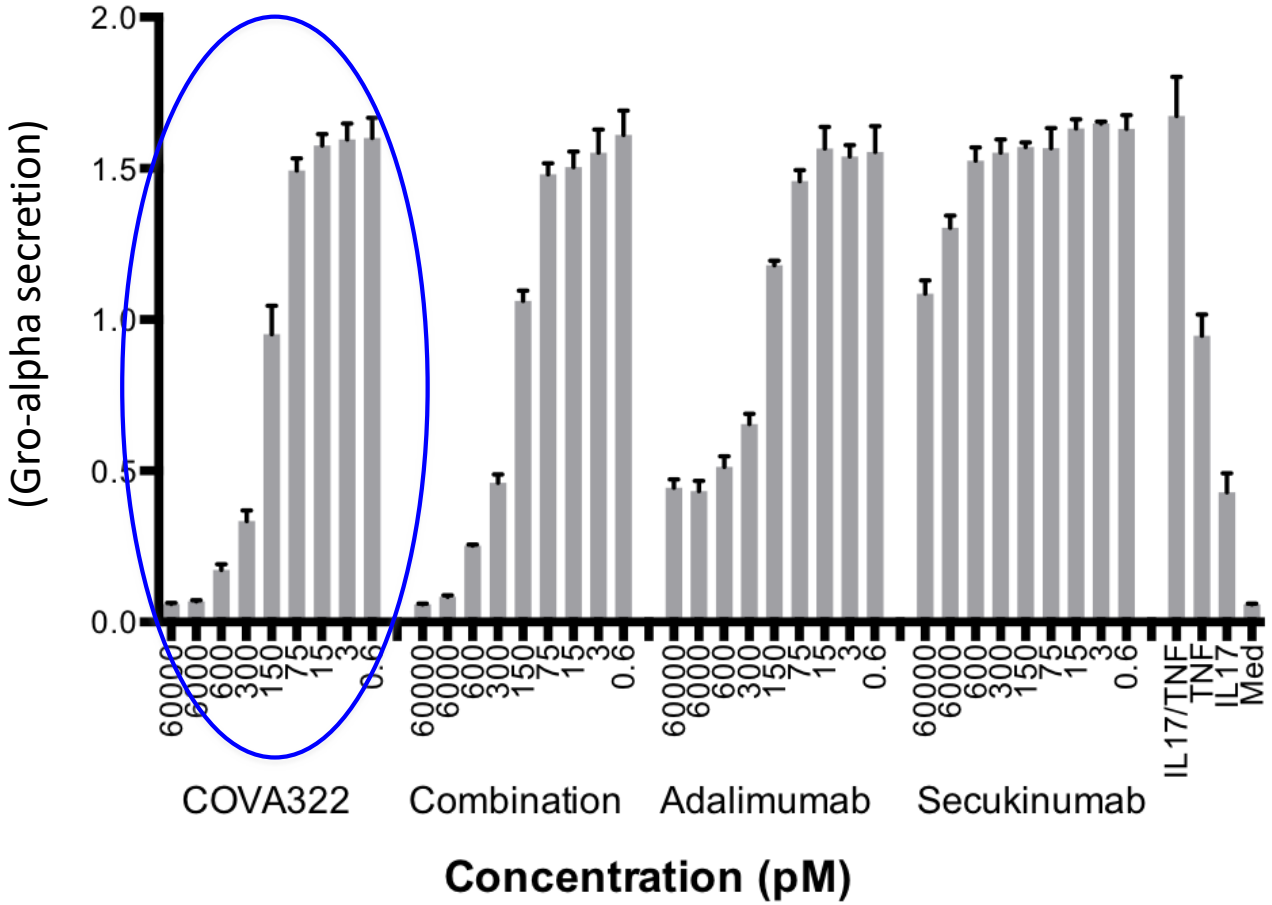
COVA322 simultaneously inhibits TNF & IL-17A



- HT-29 cells were stimulated with TNF and IL-17A
- COVA322, adalimumab, secukinumab or combination of adalimumab and secukinumab were added at different concentrations
- Gro-alpha levels were measured in tissue culture supernatants

COVA322 is as efficacious as the combination of adalimumab and secukinumab

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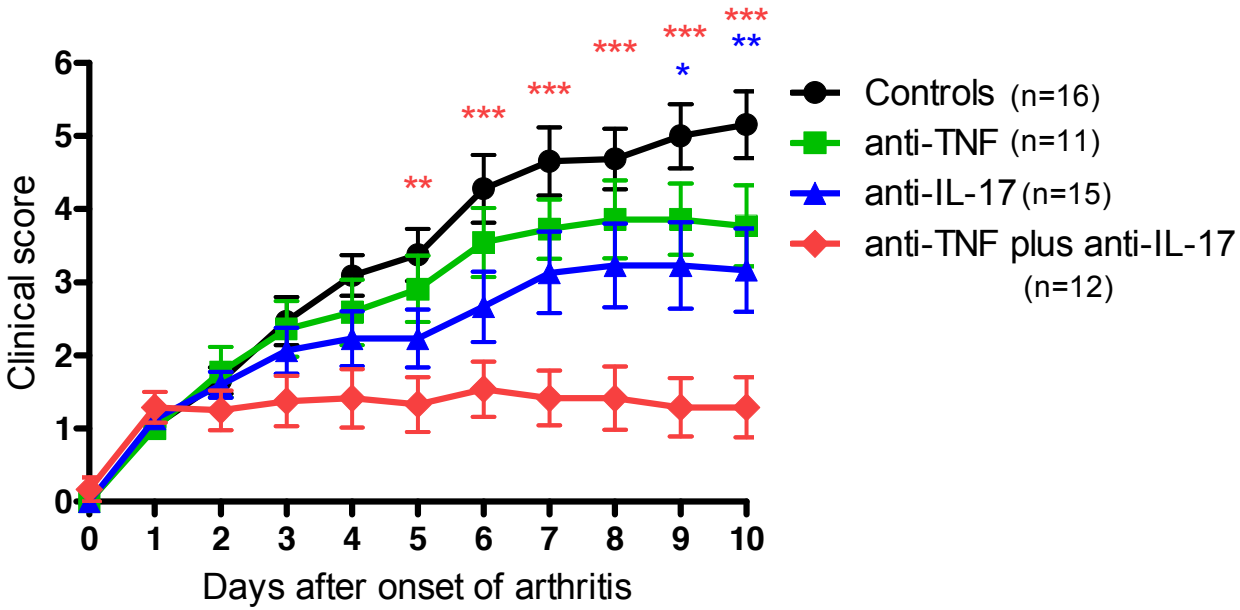
Dual TNF/IL-17 blockade more efficacious than monotherapy



CIA mouse model:

- Dual inhibition of TNF and IL-17A with antibodies at low doses is more effective than each of the monotherapies
- Dose: 50 µg of anti-TNF Ab and/or 50 µg of anti-IL-17 Ab per mouse

Synergistic effects at sub-therapeutic (single agent) dose levels

Alzabin S. et al. (2012) *Ann Rheum Dis*, 71, p. 1741

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Pharmacokinetics and Immunogenicity

- Assay set up (PK and IM)
  - PK (mono- and bifunctional) assay
  - Anti-drug antibody (ADA) assay
- PK and IM test strategy
- Single dose PK and IM
- Repeat-dose PK/TK and IM
  - 4-and 13-week repeat-dose tox
- Specificity analysis of anti-drug antibodies

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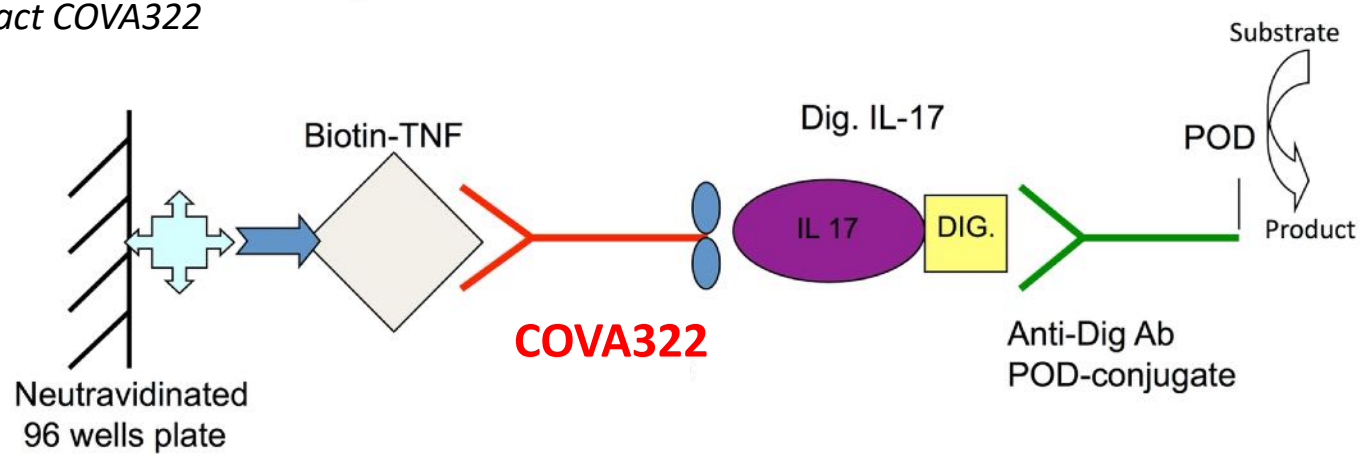
Two PK assays validated for Cynomolgus monkey

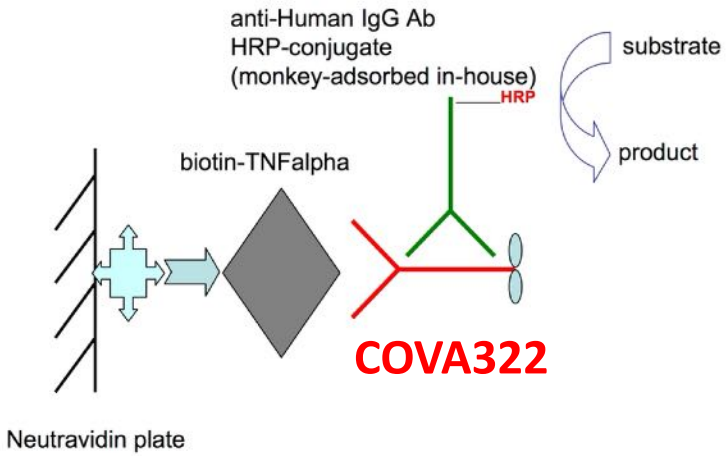
Bi-functional ELISA:

Detection of intact COVA322



Mono-functional ELISA:

Detection of anti-TNF moiety



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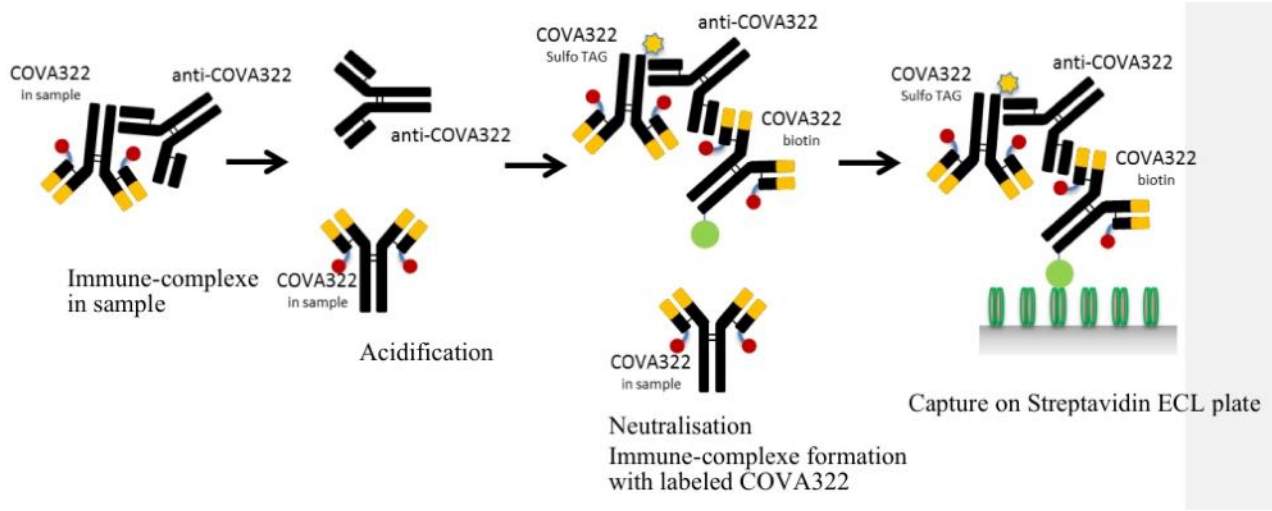
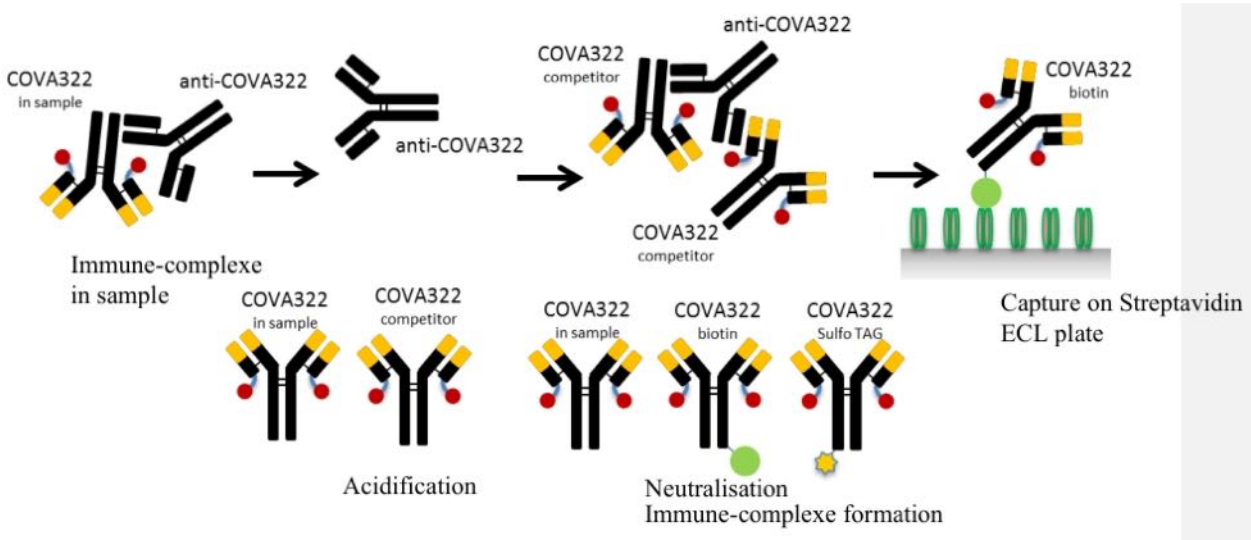
Anti-drug antibody (ADA) assay validated

Screening assay



Confirmatory assay



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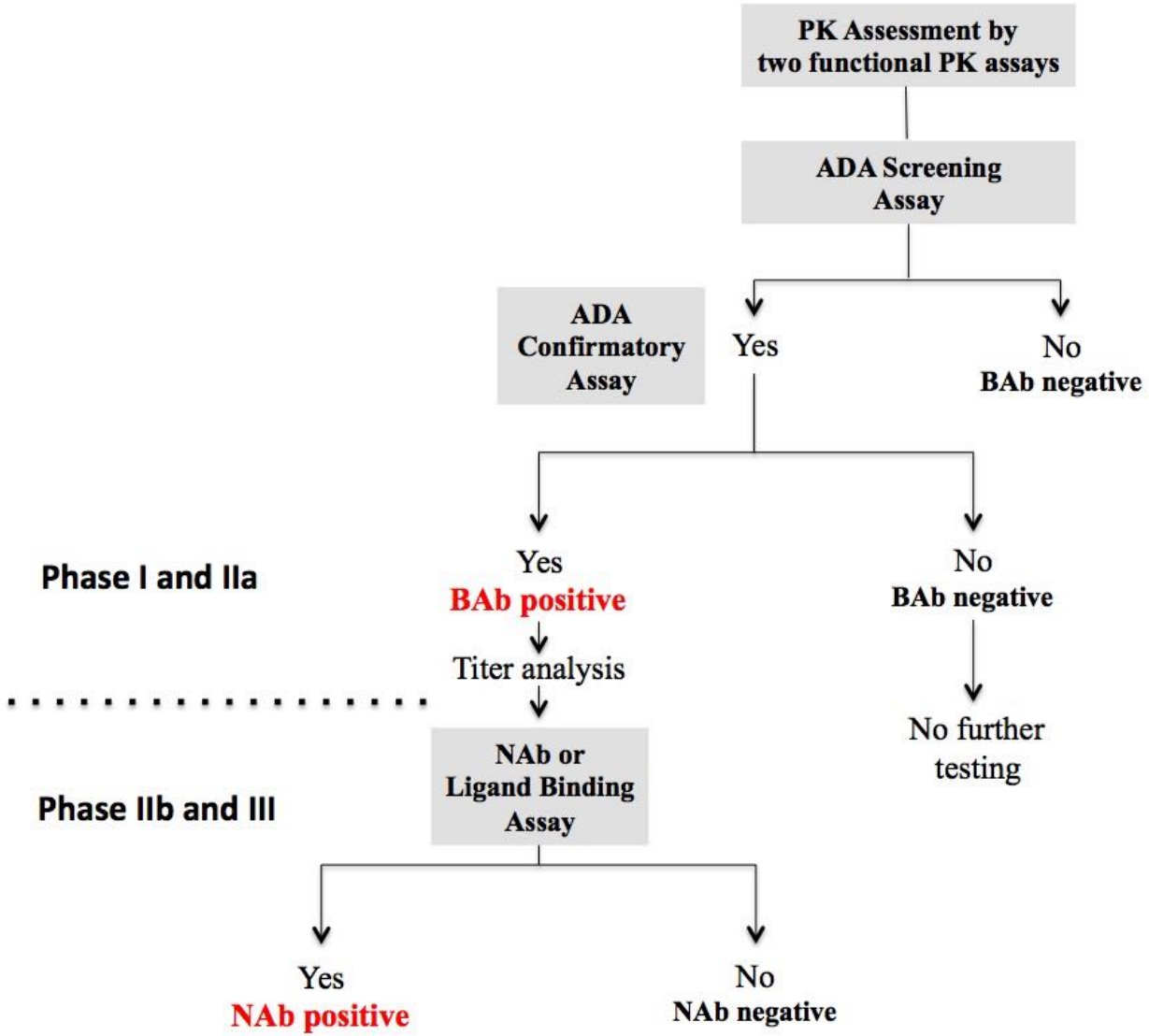
PK and Immunogenicity testing strategy



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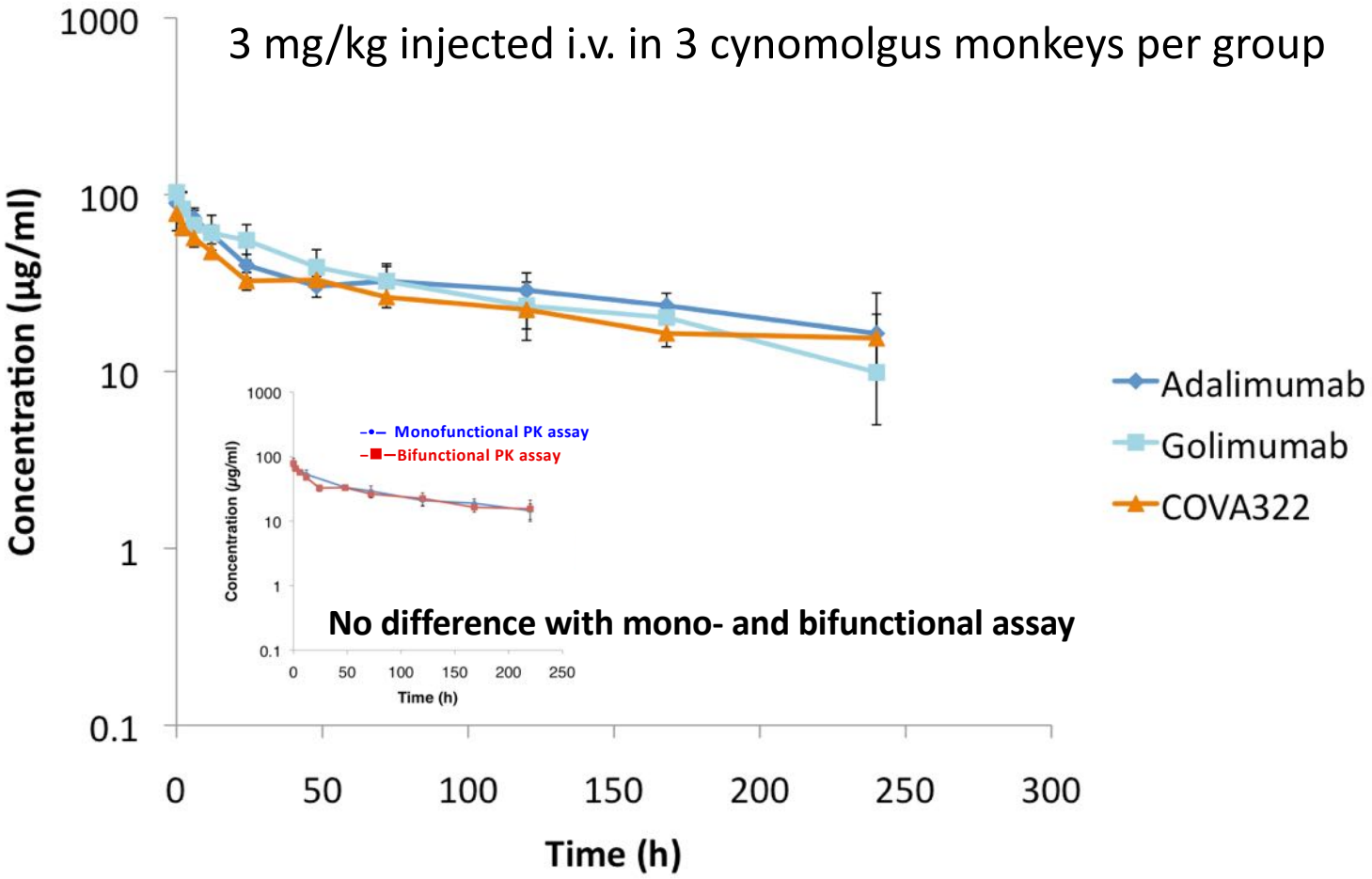
Single dose: COVA322 has an IgG-like PK profile in cyno



COVA322 plasma concentration-time curve is similar to that of adalimumab

No degradation of Fynomer entity in pilot PK studies

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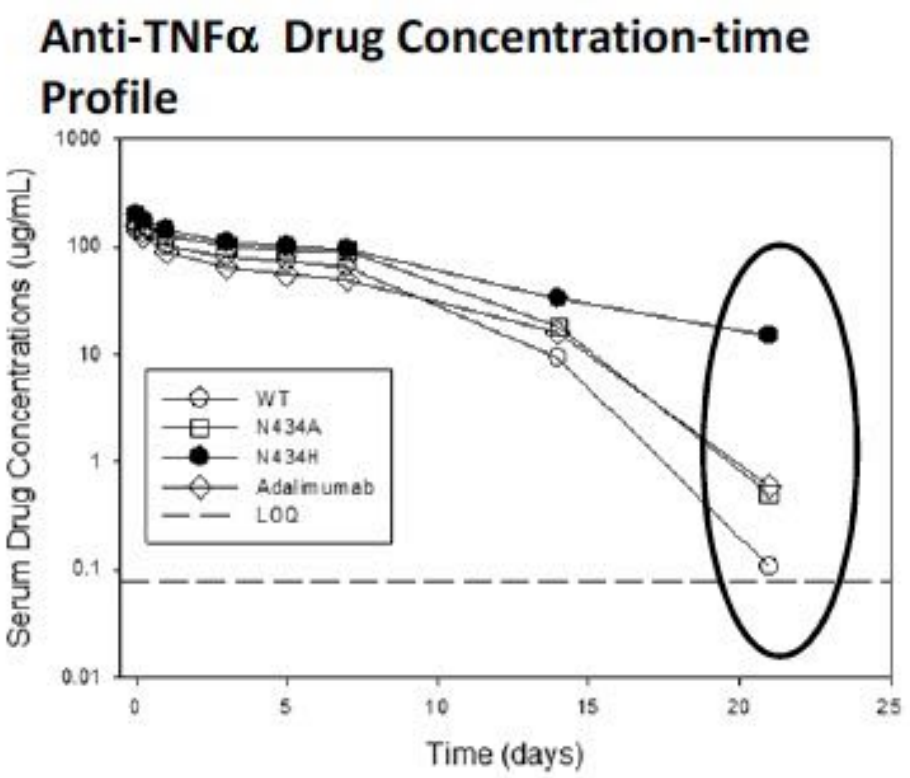
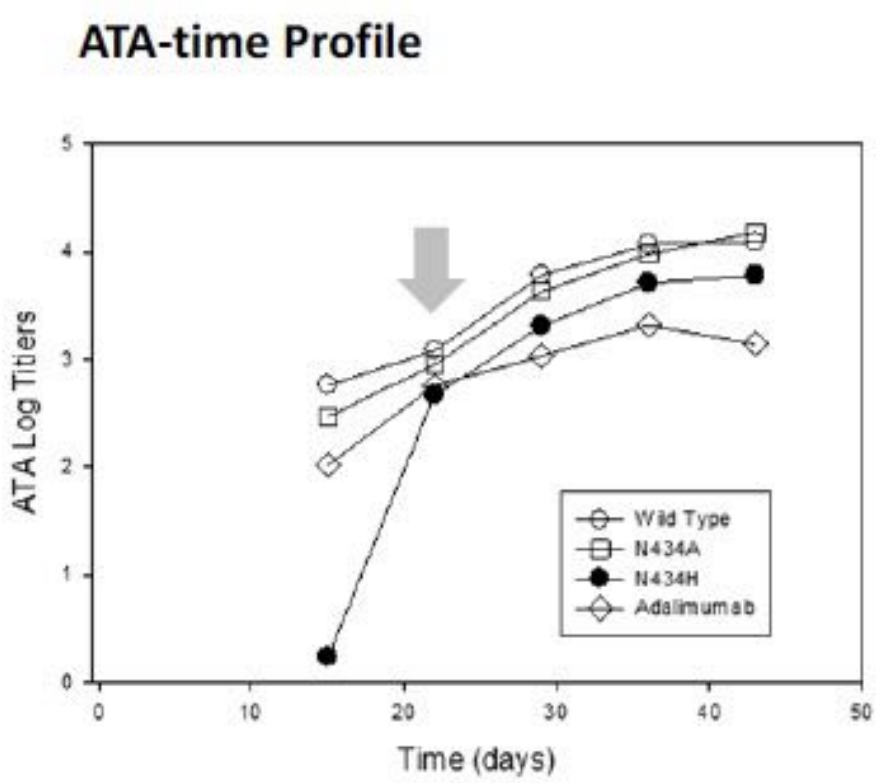
Adalimumab in known to be immunogenic in Cyno following single IV dose of 5 mg/kg





- Adalimumab induced anti-drug antibodies (ADAs) around day 14 even after a single intravenous infusion (Deng et al.)

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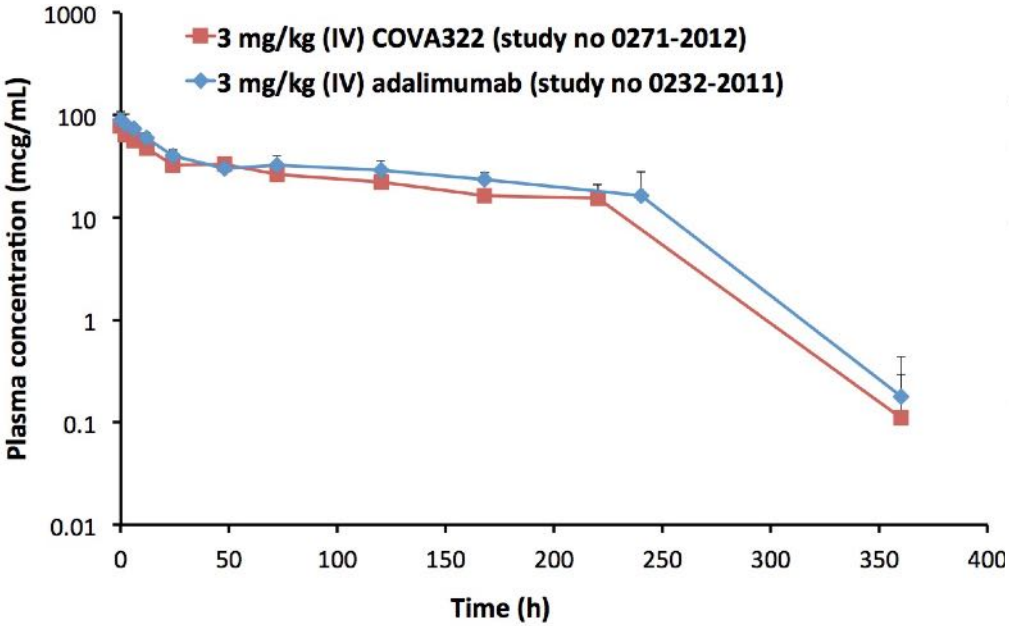
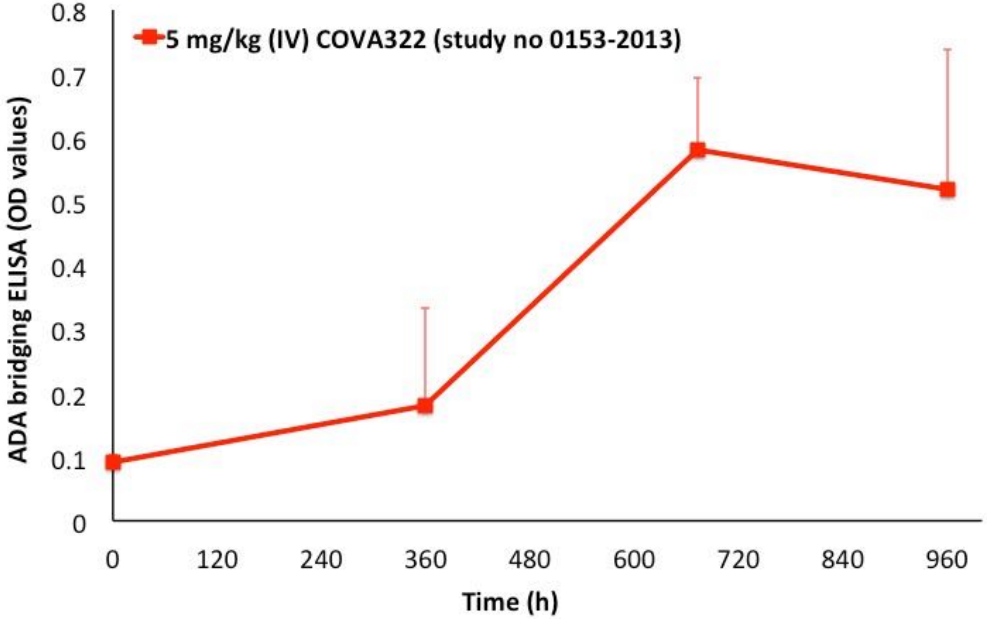
COVA322 has a comparable PK/immunogenicity profile to adalimumab in the Cynomolgus monkey

Same sharp drop in plasma concentration between 240 and 360 h



Same kinetic of ADA development starting at day 14



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Toxicokinetics and immunogenicity assessment in GLP 4-Week repeat-dose toxicity study

Study design: weekly IV dosing at 5, 25 and 100 mg/kg for 4 weeks

Group number	Group description	Dose level (mg/kg)	Dose volume* (mL/kg)	Animals/group		Necropsy after ...	
				Male	Female	4 weeks	17 weeks
1	Control i.v.	0	10	4	4	2 M / 2 F	2 M / 2 F
2	Low i.v.	5	10	3	3	3 M / 3 F	-
3	Intermediate – i.v.	25	10	3	3	3 M / 3 F	-
4	High – i.v.	100	10	5	5	3 M / 3 F	2 M / 2 F

PK

- 1^{st}, 4^{th} dose profile and peak to trough level at days 8, 15 and 29 during main study
- High dose (100 mg/kg) recovery animals: recovery weeks 1, 4, 8, 12 and 16

IM

- Predose: day 1, 15, 29
- Recovery weeks 4, 8, 12 and 16

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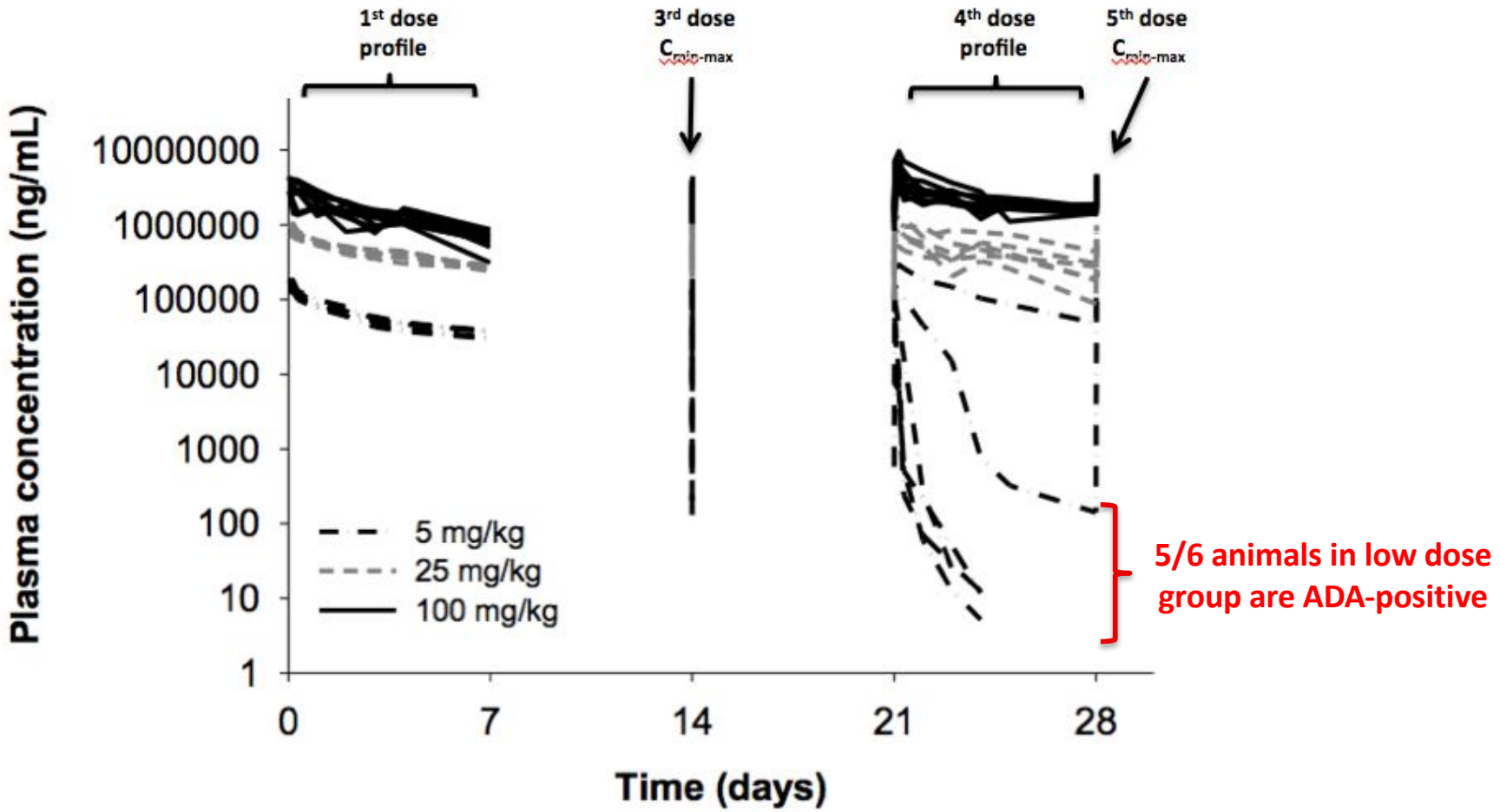
No ADA effect on exposure in high dose group following weekly IV doses: "Dosing through"



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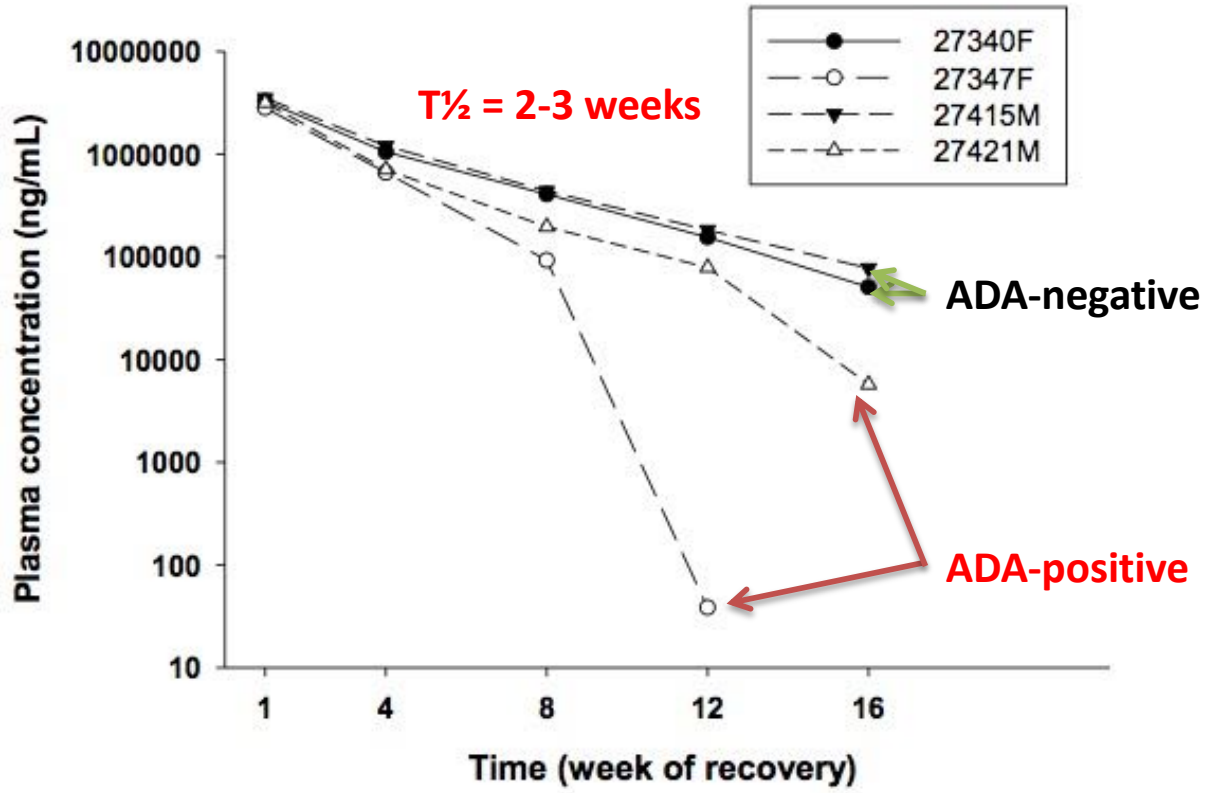
Long-lasting exposure in 100 mg/kg recovery animals



- Recovery period (4-months) could be considered as additional exposure period
- Terminal elimination half-life is 2-3 weeks as described for adalimumab

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13-week Tox study (IV)

Study design & current status

Study design: weekly IV dosing at 25 and 100 mg/kg for 13 weeks

Group number	Group description	Dose level (mg/kg)	Dose volume* (mL/kg)	Animals/group		Necropsy after ...	
				Male	Female	13 weeks	4-month recovery
1	Control i.v.	0	2	4	4	2 M / 2 F	2M / 2F
2	Low – i.v.	25	2	4	4	4 M / 4 F	
3	High – i.v.	100	2	6	6	4 M / 4 F	2M / 2F

PK

- 1st, 12th dose profile and peak to trough level at days 22, 36, 50, 64, 85 during main study
- High dose (100 mg/kg) recovery animals: recovery weeks 1, 4, 8, 12 and 16

IM

- Predose: day 1, 22, 36, 50, 64 and 85
- Recovery weeks 4, 8, 12 and 16

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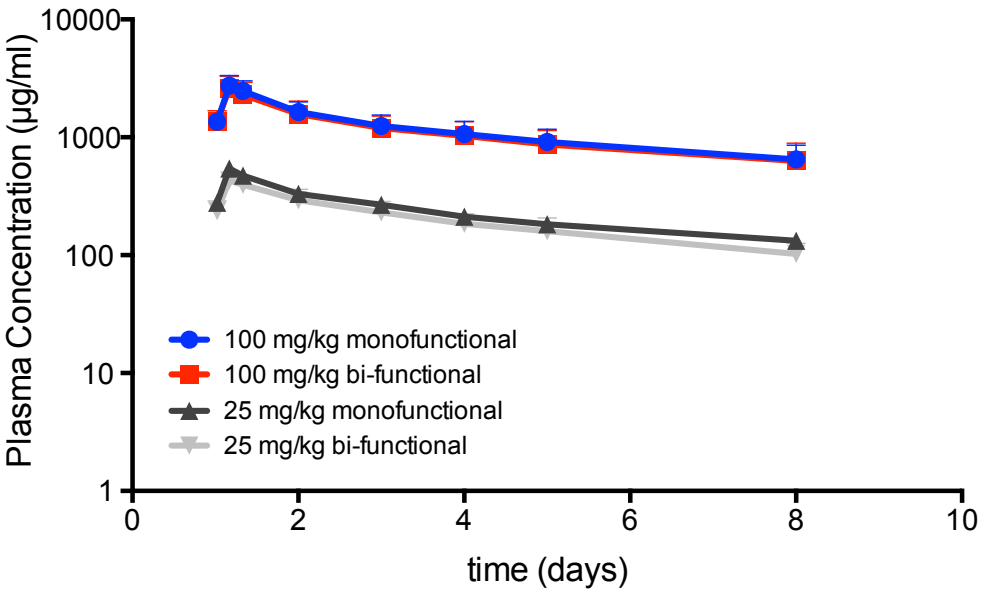
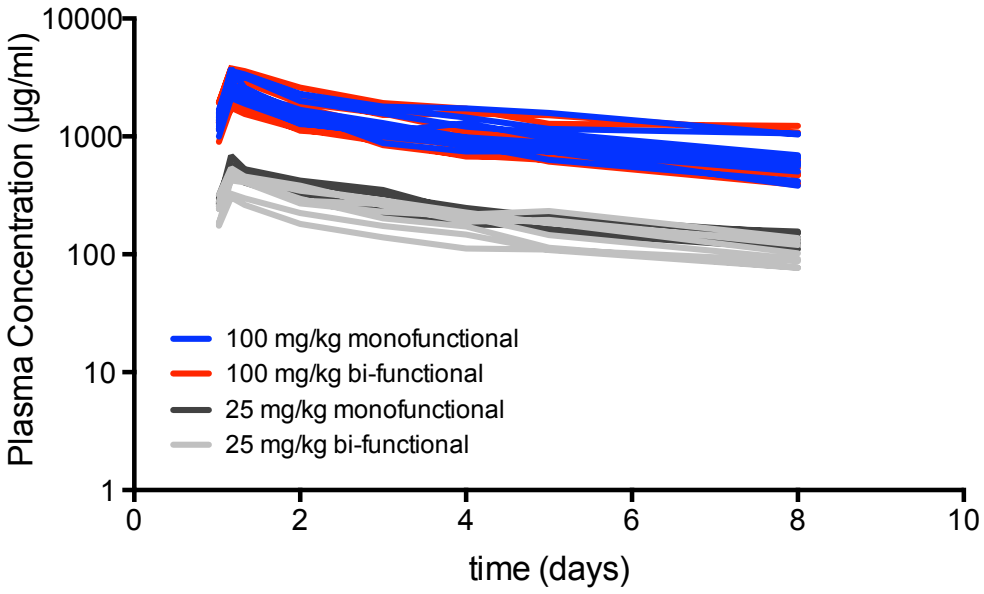
Same exposure measured by mono- and bifunctional assay

1st dose profile

Mean (+ SD)



Individual



1st dose profiles derived from 13-week study: low dose = 25 mg/kg (N=8); high dose = 100 mg/kg (N=12)

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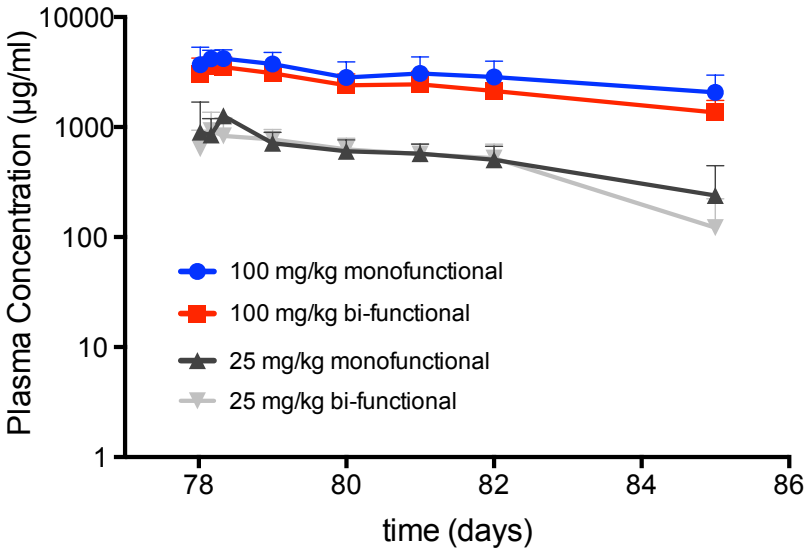
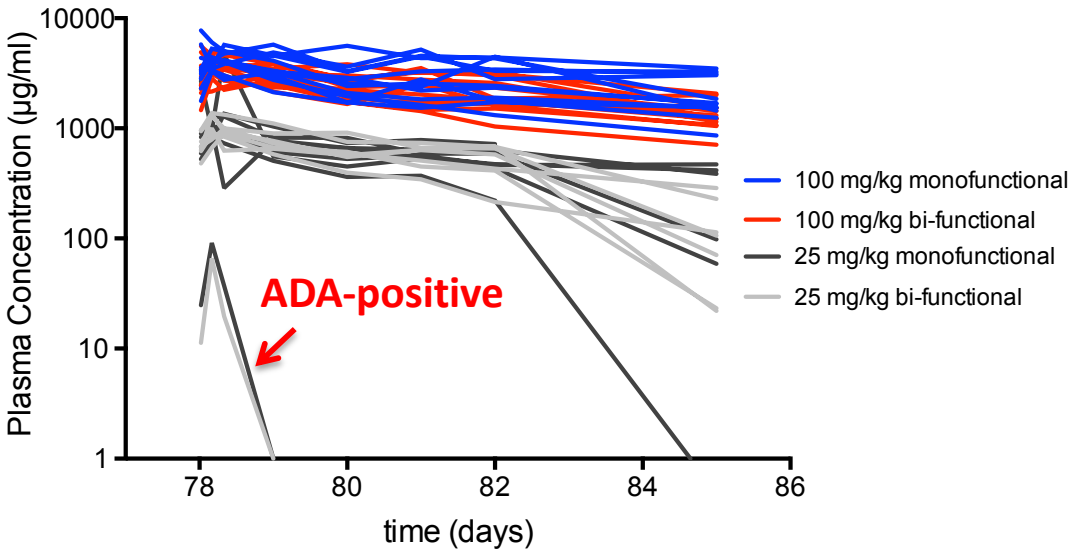
Same exposure measured by mono- and bifunctional assay

12th dose profile

Mean (+ SD)



Individual



12th dose profiles derived from 13-week study: low dose = 25 mg/kg (N=8); high dose = 100 mg/kg (N=12)

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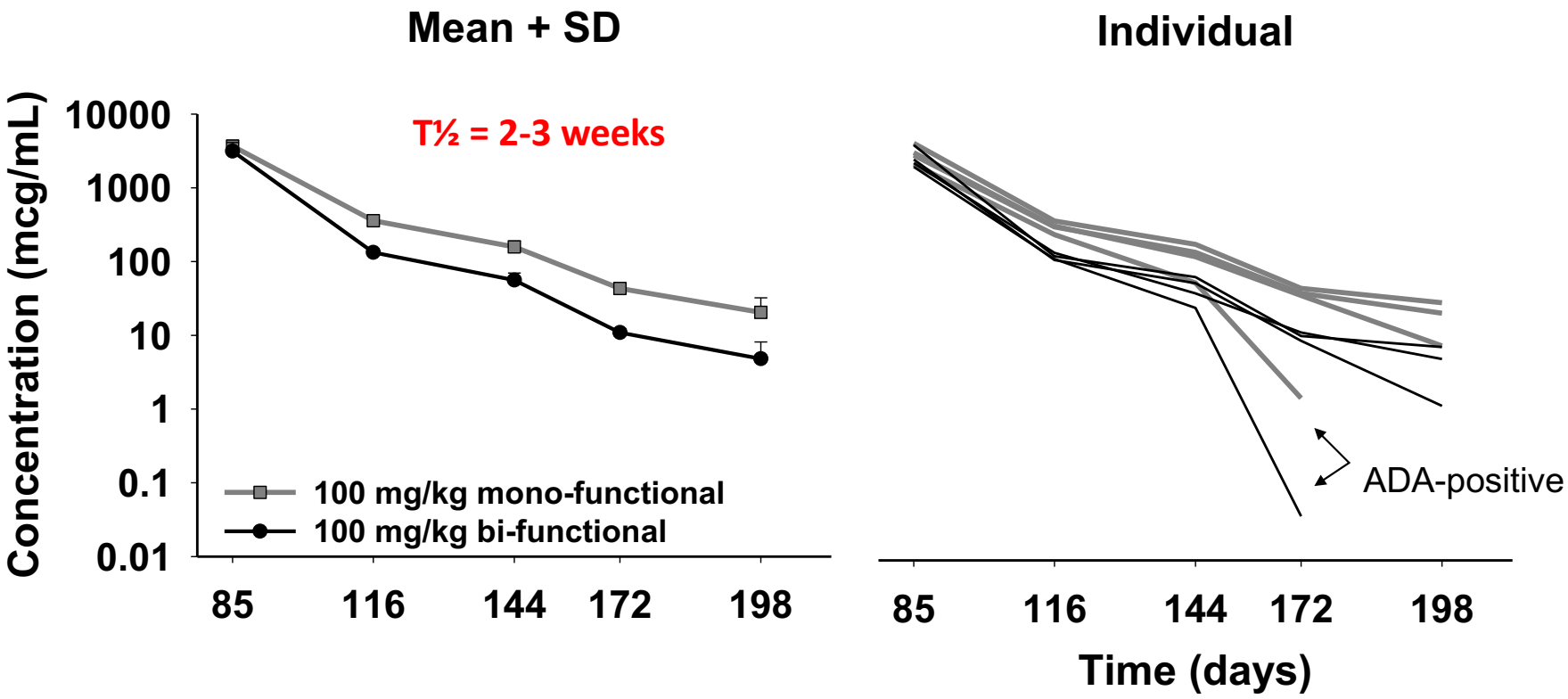
Long-lasting exposure during 16-week recovery period



Almost parallel curve progression with mono/bifunctional assay

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Exposure measured by mono- and bifunctional assay grossly comparable

	Parameter	Assay	Group 2 (n=8)* 25 mg/kg		Group 3 (n=12) 100 mg/kg	
			Mean	±SD	Mean	±SD
First dose	AUC(1-8d) (µg.h/mL)	mono-	39000	3900	193000	45300
		bi-	33200	7050	185000	53200
	Fold difference (mono- versus bi-functional assay)		1.17		1.04	
			Mean	±SD	Mean	±SD
Last dose	AUC(78-85d) (µg.h/mL)	mono-	92500	26400	493000	150000
		bi-	88500	20500	385000	90100
	Fold difference (mono- versus bi-functional assay)		1.05		1.28	
					Group 3 (n=3)	
					Mean	±SD
Recovery	AUC(inf) (recovery) (µg.h/mL)	mono-			1780000	338000
		bi-			1310000	376000
	Fold difference (mono- versus bi-functional assay)				1.38	

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Specificity assessment of Anti-drug-antibodies (ADA) against COVA322

- Assay principle to assess ADA specificity
- Positive control serum and assay reagents
- Results and preliminary conclusions

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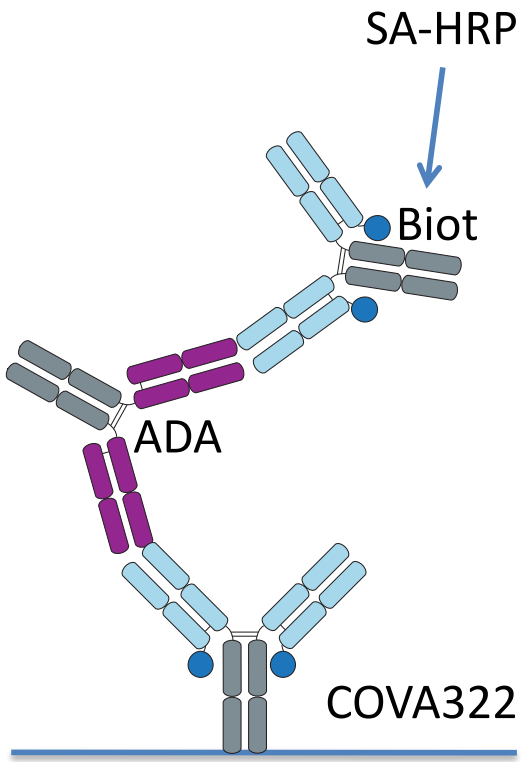
Assay principle to dissect Anti-drug-antibody (ADA) specificity*

Assay Principle
Sandwich ELISA

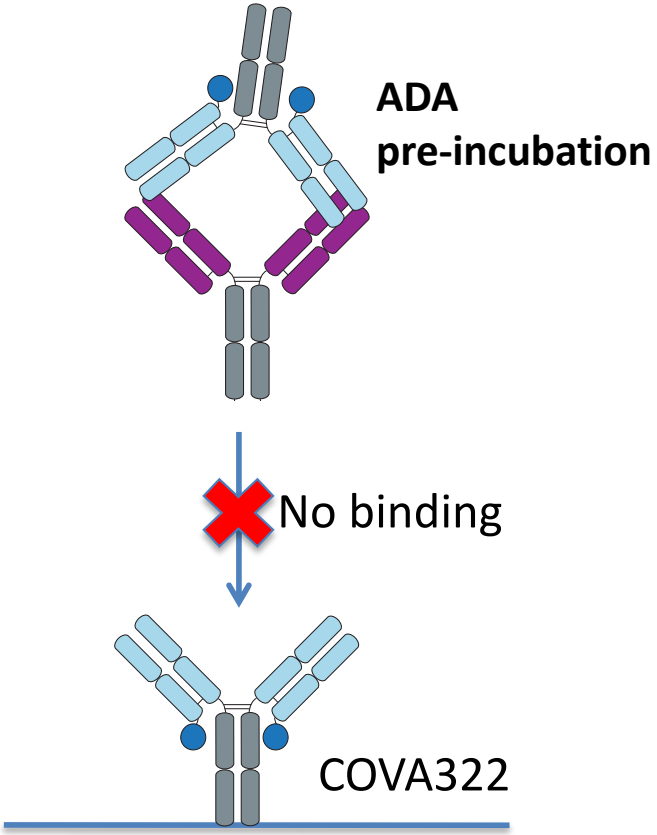


Detection

Sample (ADA)

Coating

Specificity Principle
Competitive Binding



*Protocol adapted from Hart et al., 2011

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Different reagents utilized to evaluate whether COVA322-specific ADAs are directed against adalimumab backbone, Fynomer entity or linker structure

Positive control:

- Monkeys **subcutaneously immunized** (4xq2w) with COVA322 (100 µg) formulated in Montanide ISA (adjuvant formulation) to generate high ADA titer (1:100 000)

Reagents used for competitive binding analyses:

- **COVA322**
- **Adalimumab** (TNF-specific human IgG1 backbone of COVA322)
- **B6 Fynomer** (IL-17-specific Fynomer entity of COVA322)
- **Fynomer control** (Fyn SH3 wildtype Fynomer) and **Human IgG1 control**
- **Fynomab control_ada** (adalimumab backbone, same linker and same domain arrangement as COVA322 but wildtype Fynomer)
- **Fynomab control_B6** (B6 Fynomer, same linker and same domain arrangement as COVA322 but human IgG1 control backbone)

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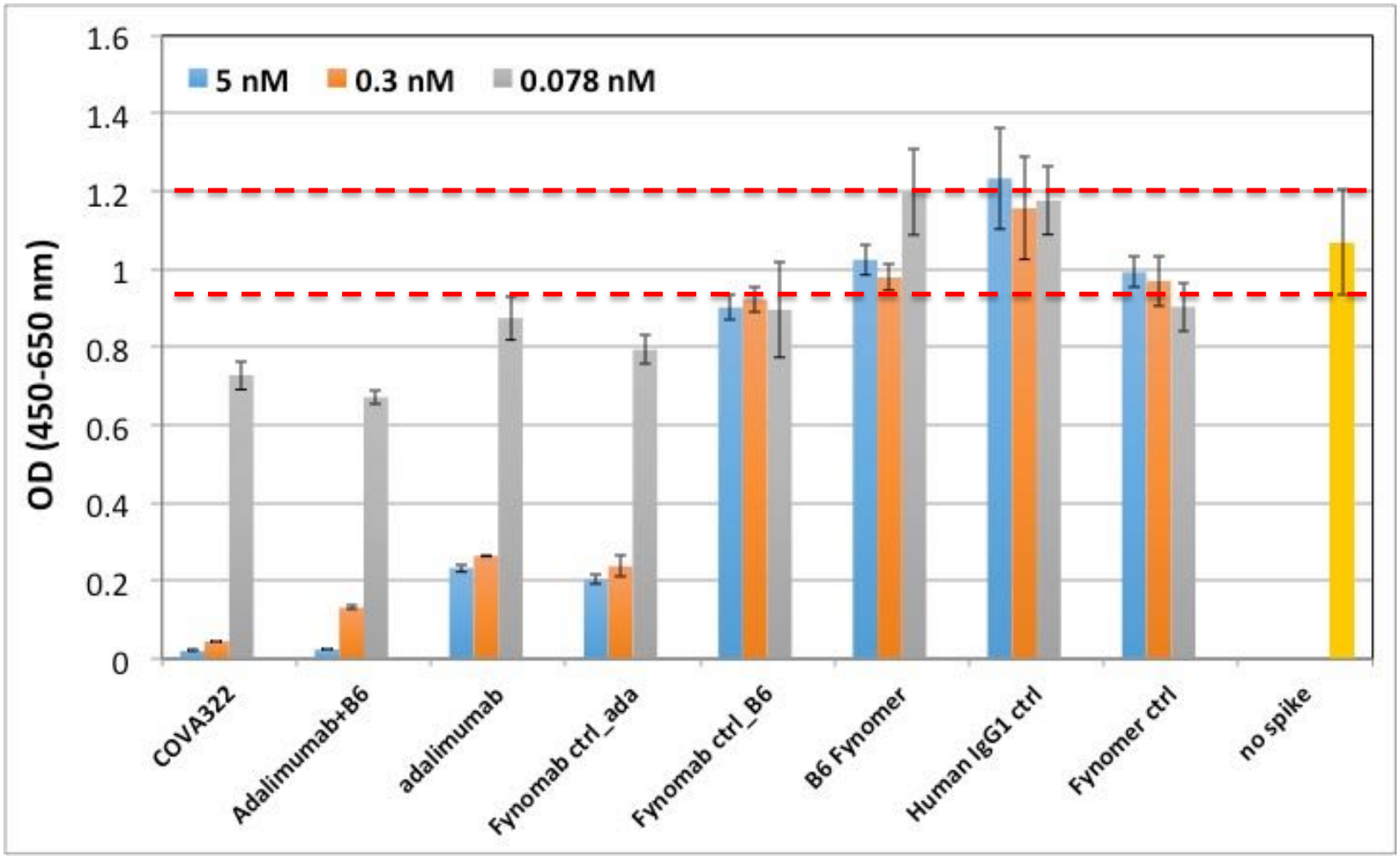
ADAs are predominantly directed against the adalimumab backbone



STDEV range

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Conclusions ADA specificity

- ADAs derived from immunized Cynomolgus monkeys (positive control) are predominantly directed against adalimumab backbone
- The ADA titer against the B6 Fynomer entity of COVA322 is low in the immunized monkeys
- There are no ADAs present that recognize the wildtype Fyn SH3 domain (origin of Fynomer technology)

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Overall, no indication that COVA322 has different PK characteristics as compared to adalimumab

- Dose proportional PK (Cmax and AUC) following intravenous administration
- IgG-like PK comparable to that of adalimumab (e.g. terminal disposition half-life = 2-3 weeks as described for adalimumab)
- Exposure measured by mono- and bifunctional assay considered similar given the assay variability
- No indication for meaningful Fynomer degradation
- Immunogenicity incidence considered in a similar range to that of adalimumab

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Acknowledgements

Discovery team

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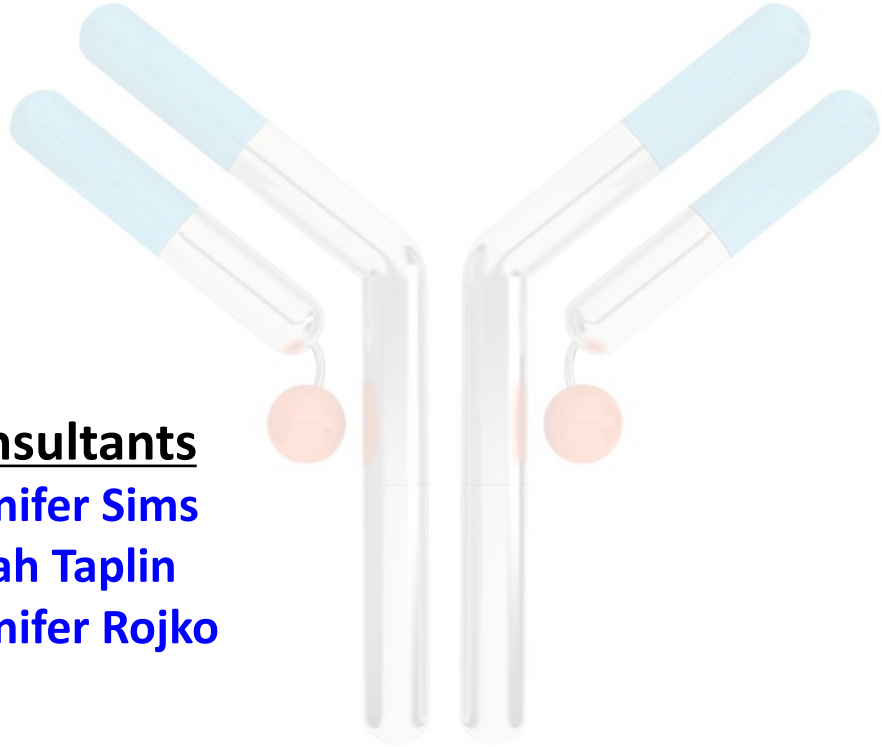

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Questions???

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