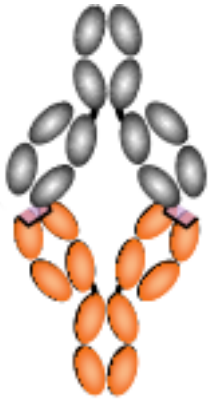
Immunogenicity of therapeutic antibodies



From % towards understanding

Gertjan Wolbink Rheumatologist Amsterdam rheumatollogy centre and Sanquin

Immunogenicity

- Assays for immunogenicty

  -Drug interference

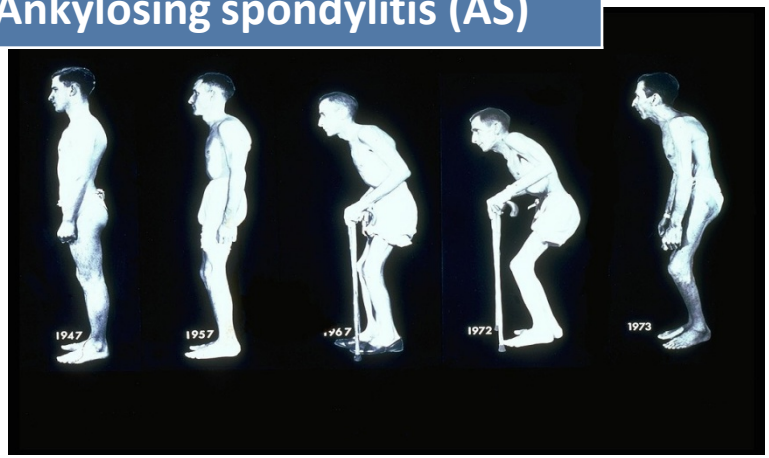
- Characterization of anti drug antibodies

- Clinical relevance
  - Side effects
  - Effects on PK

Amsterdam READE cohort

Long-term clinical and serological follow-up of 2000 patients on biologicals*

Ankylosing spondylitis (AS)

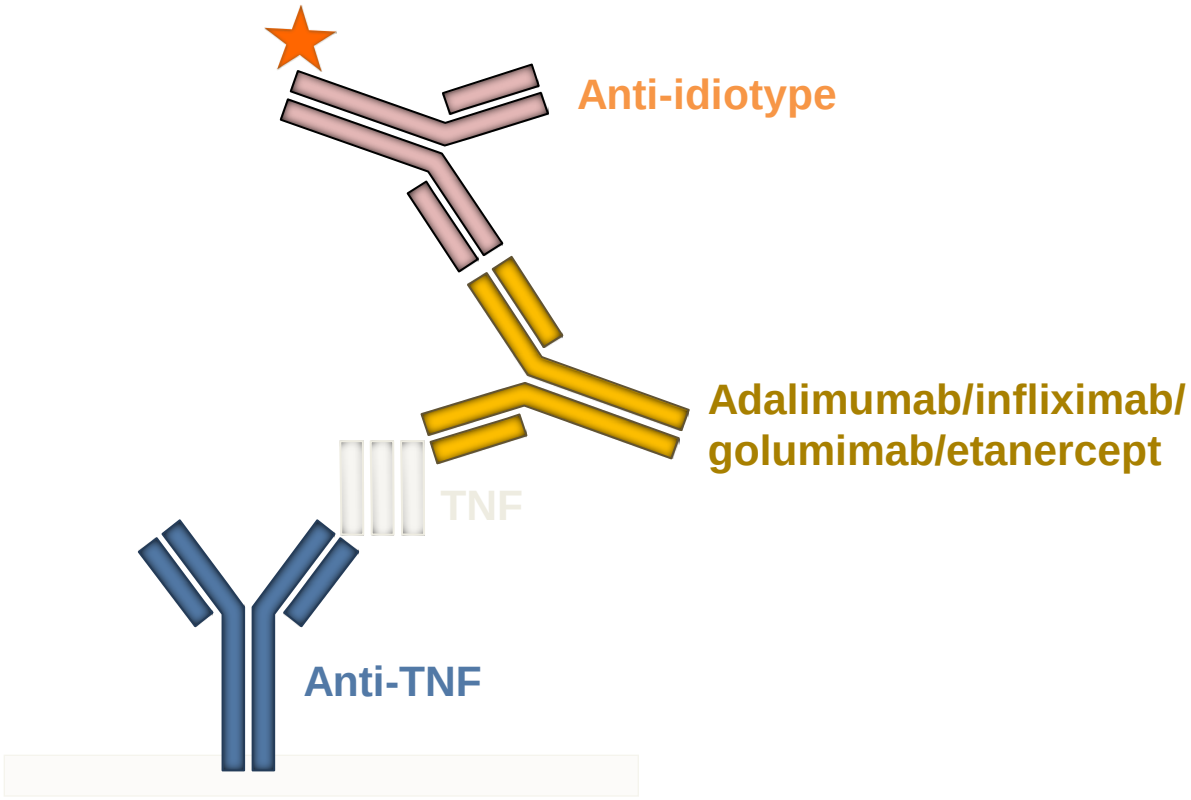


Rheumatoid Arthritis (RA)

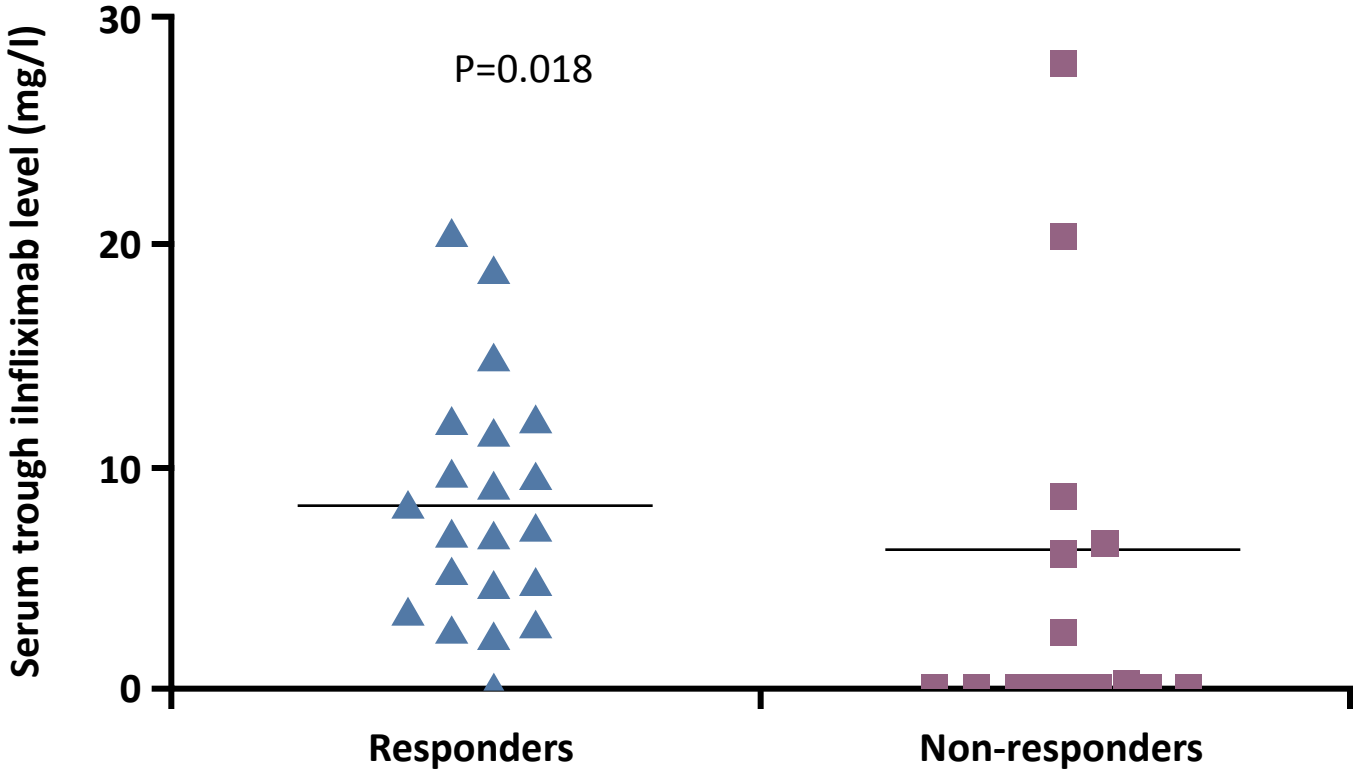
Psoriatic Arthritis (PsA)

*infliximab/adalimumab/etanerceptabatacept/golimumab/tocilizumab/rituximab

Pharmacokinetic assay (drug level test anti-TNF)



Infliximab
interindividual variation in drug concentration



Serum trough infliximab level for responders (n=21; 8.2mg/l) and non-responders (n=17; 6.3mg/l) according to the ASAS-20 response criteria, at week 54 (P=0.018)

de Vries MK, et al. *Ann Rheum Dis.* 2007;66:1252–1254

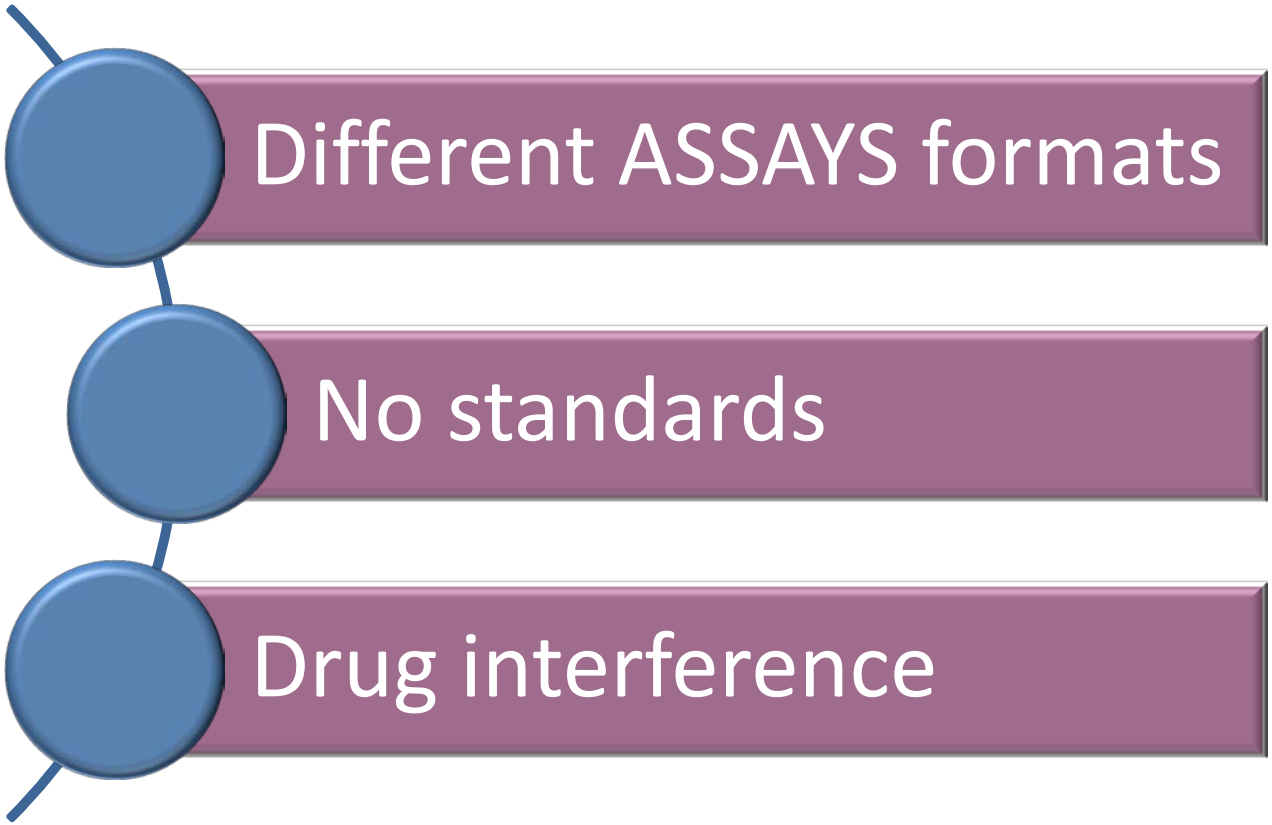
Patients with an allergic reaction to infliximab have low serum levels of infliximab

Infliximab concentration (Mg/L)	2 wk	6 wk	14 wk
Mean all patients n=105	23.9	16.0	4.6

Pt S reaction at wk 14	17.4	0.5	0.00
Pt R reaction at wk 14	37.1	2.8	0.00

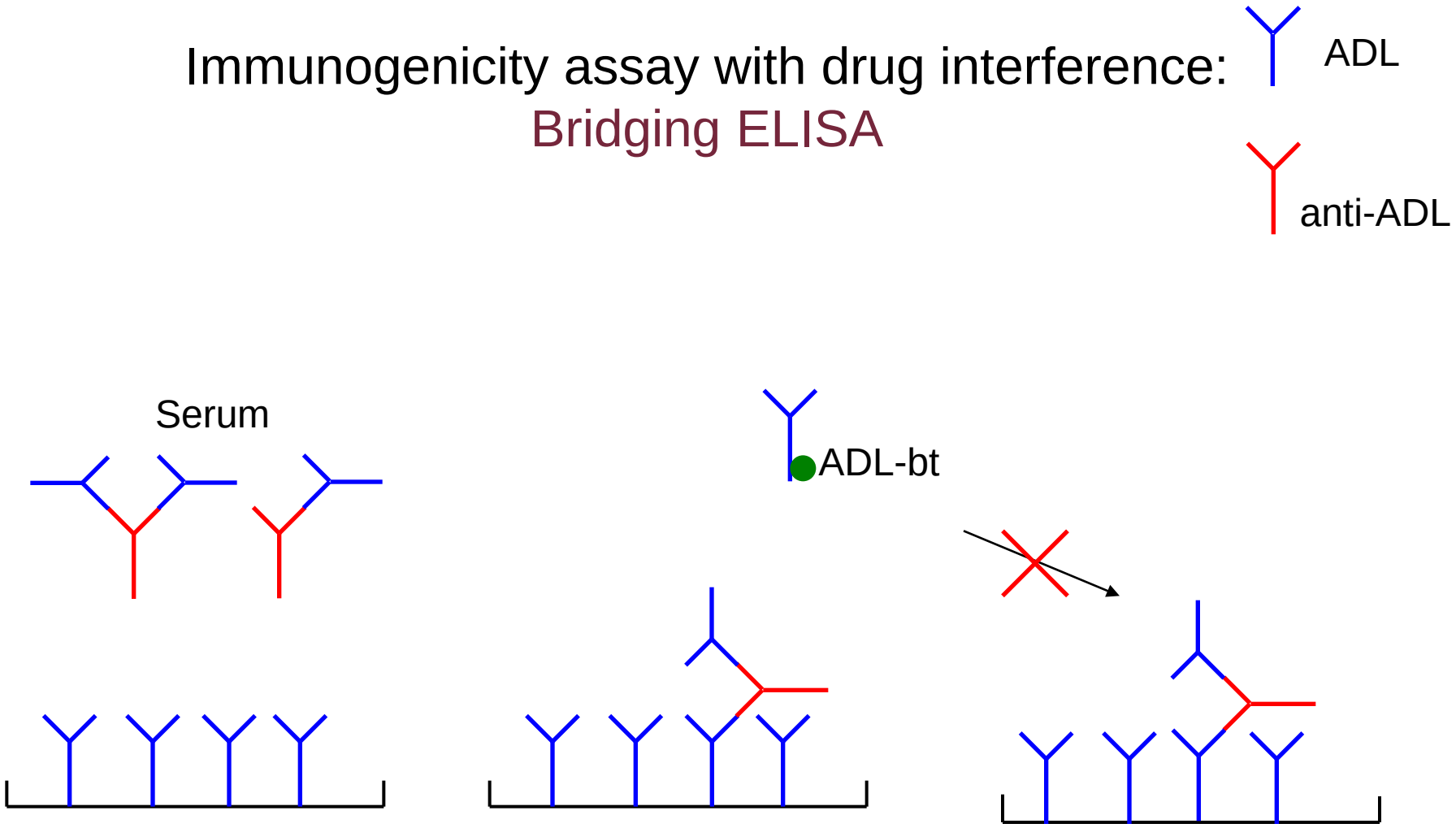
Wolbink GJ, et al. *Ann Rheum Dis.* 2005;64:704–707

Detection of antibody formation against therapeutic antibodies

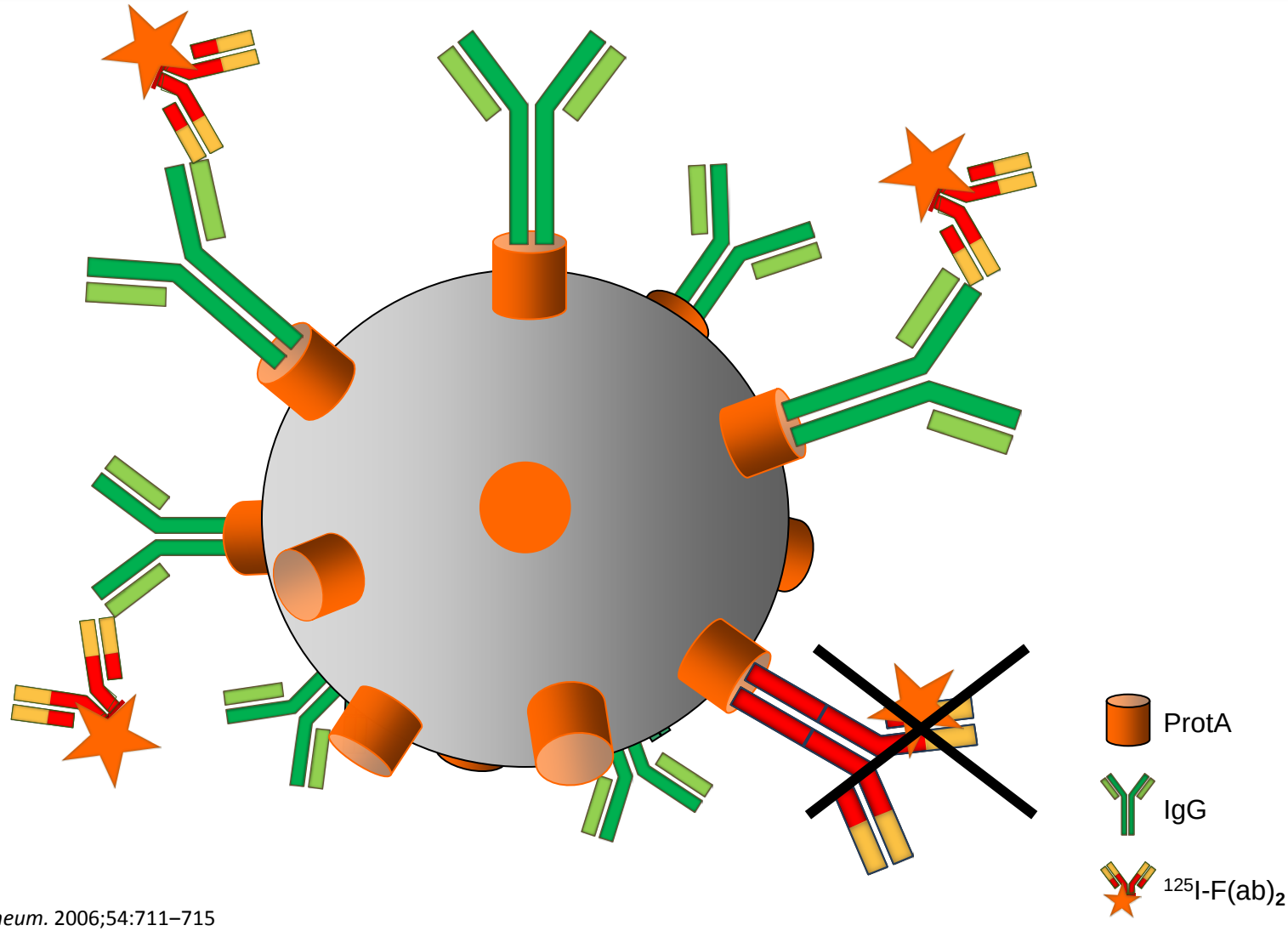


Aarden L, et al. *Curr Opin Immunol.* 2008;20:431–435

Immunogenicity assay with drug interference:
Bridging ELISA



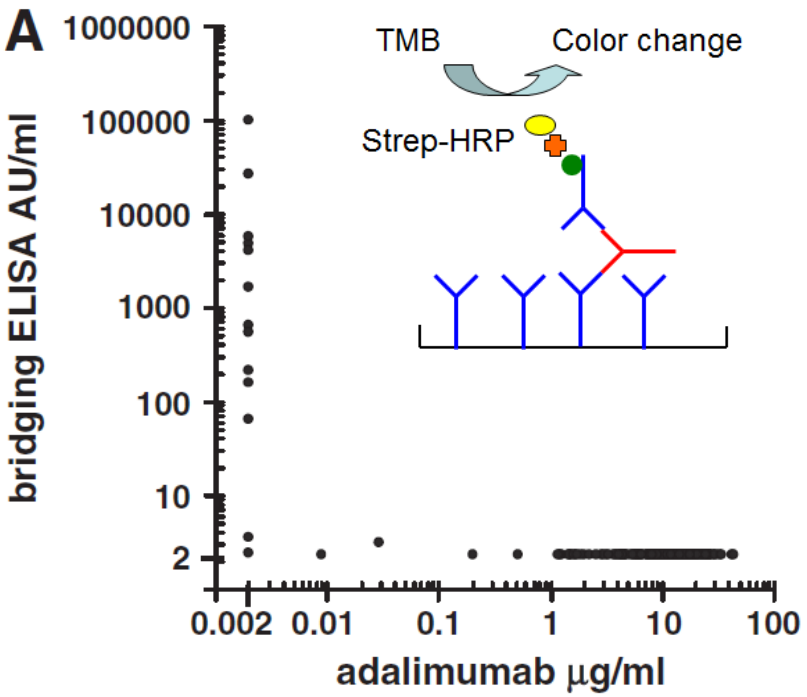
ABT monoclonal therapeuticals less sensitive to drug interference



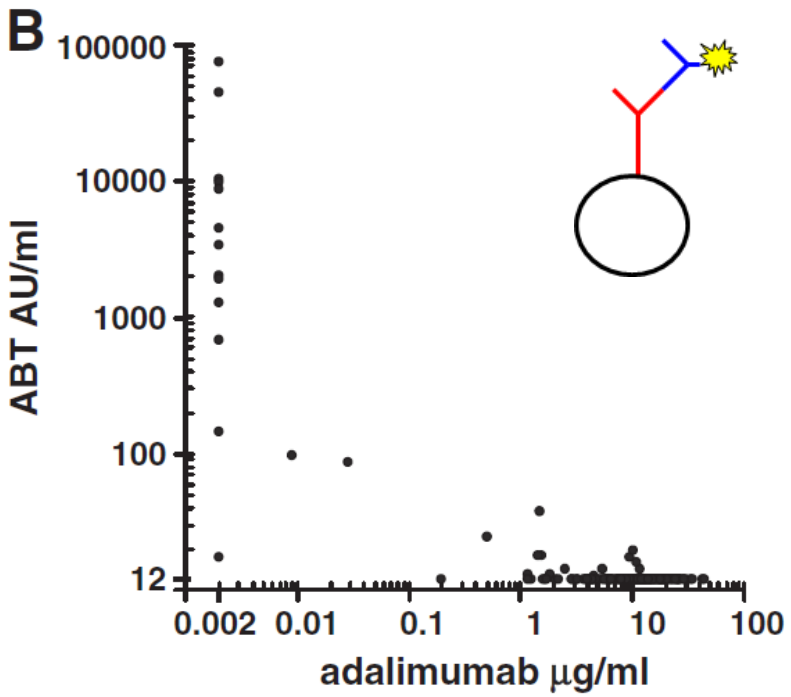
Wolbink GJ, et al. *Arthritis Rheum.* 2006;54:711–715

Drug interference in different assays

Bridging ELISA

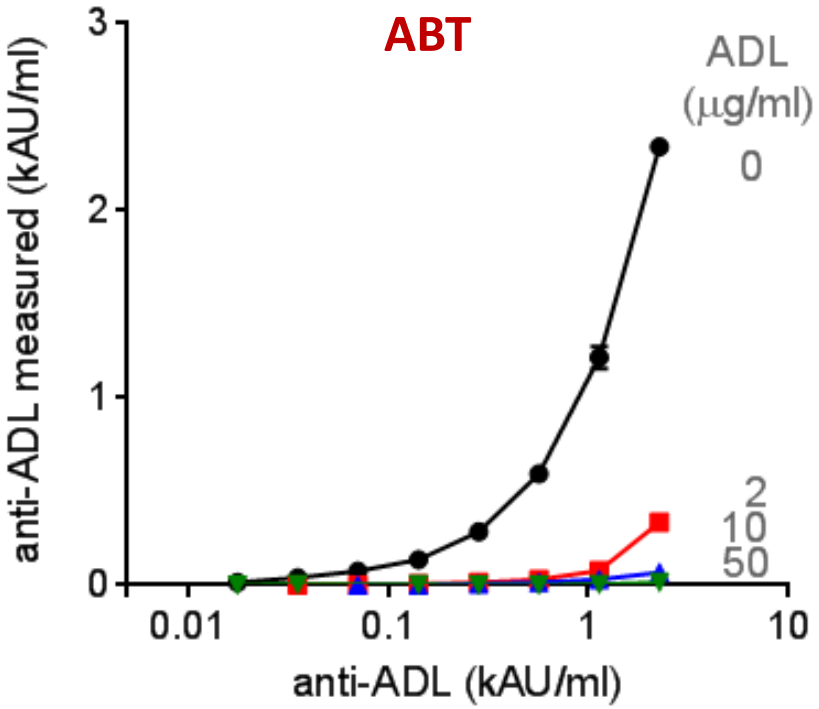


ABT/RIA

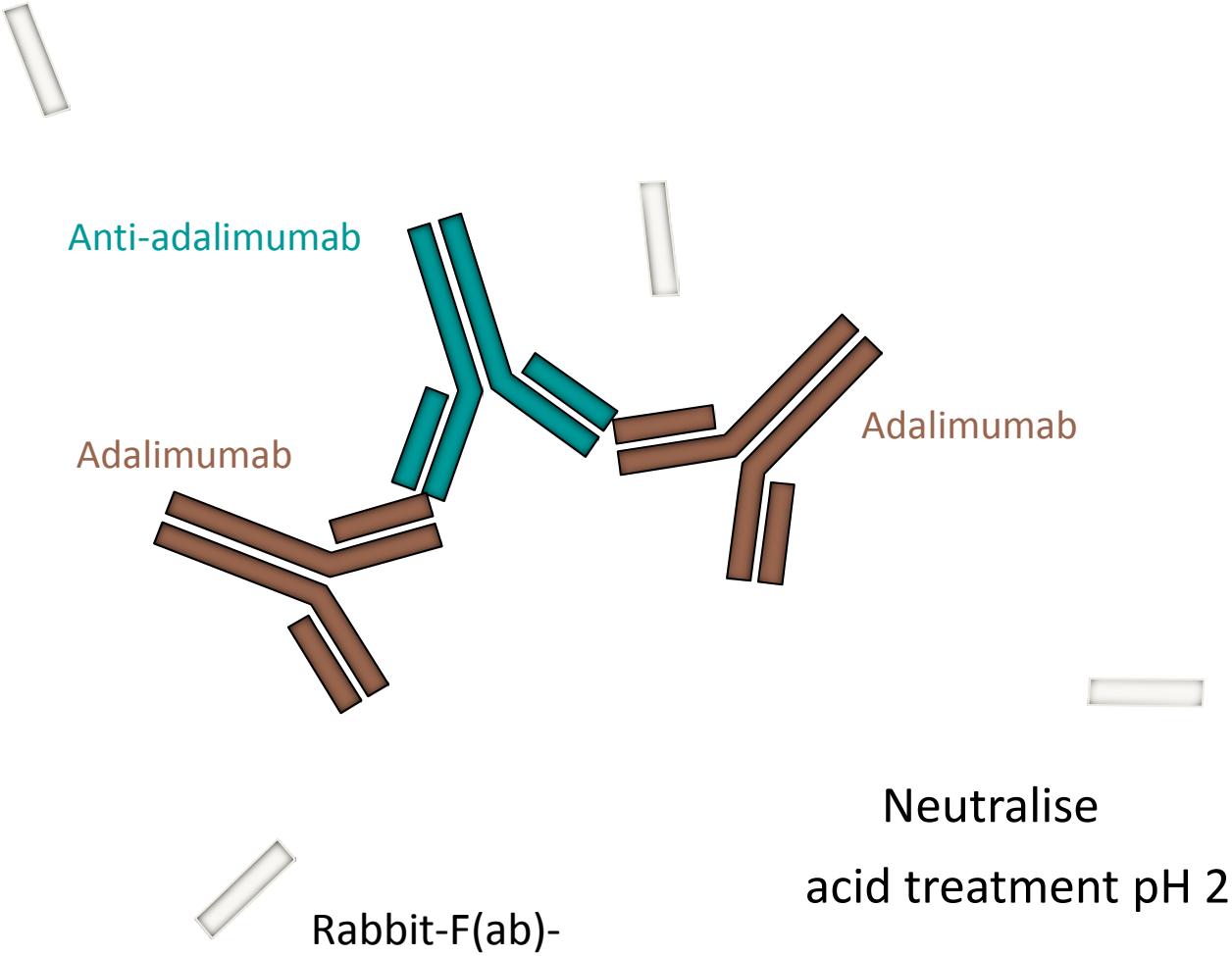


Hart *et al.*, J. Immun. Methods 2011

Antibody detection in ABT is hampered in the presence of drug

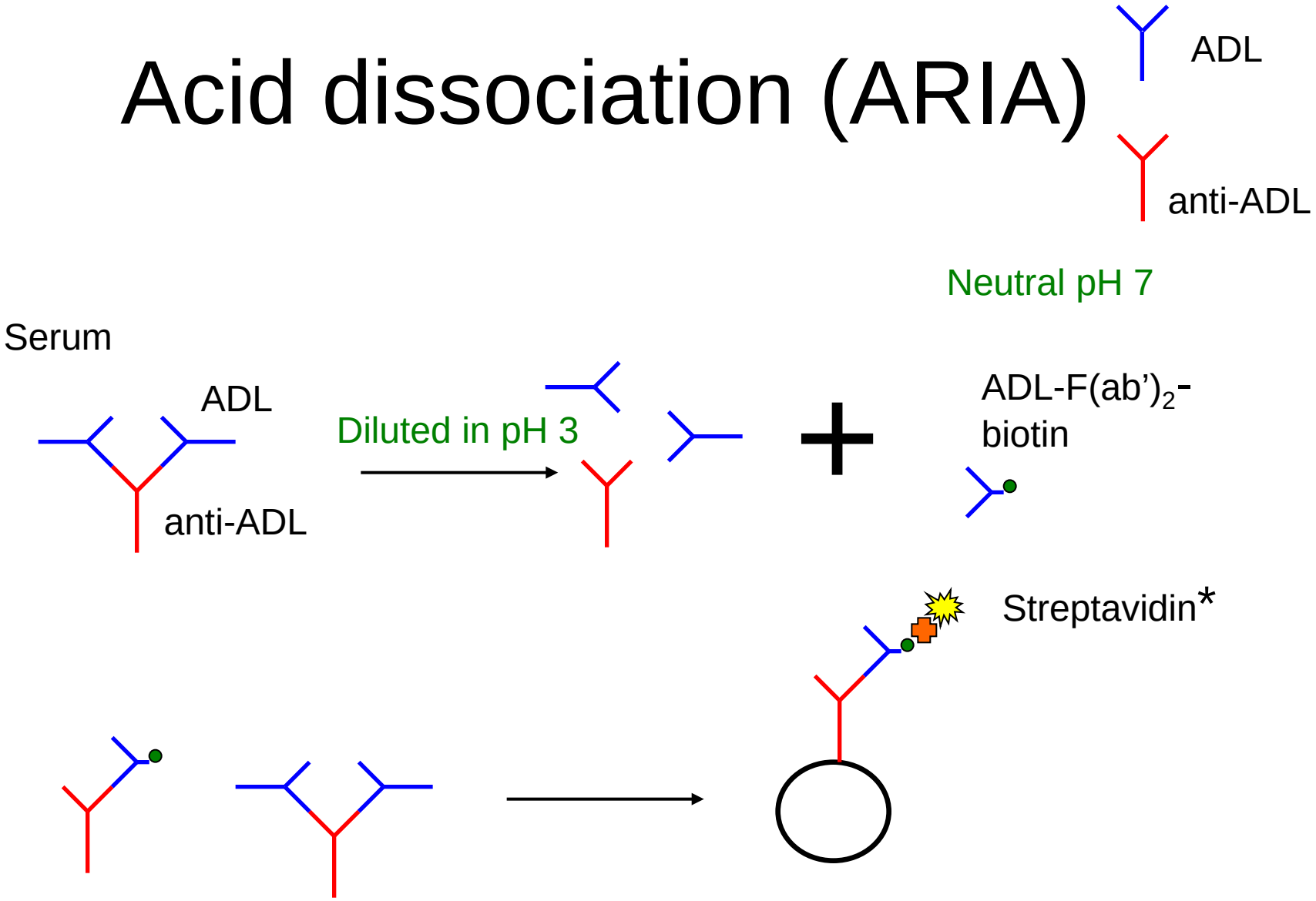


Bloem *et al.*, J. Immun. Methods 2015

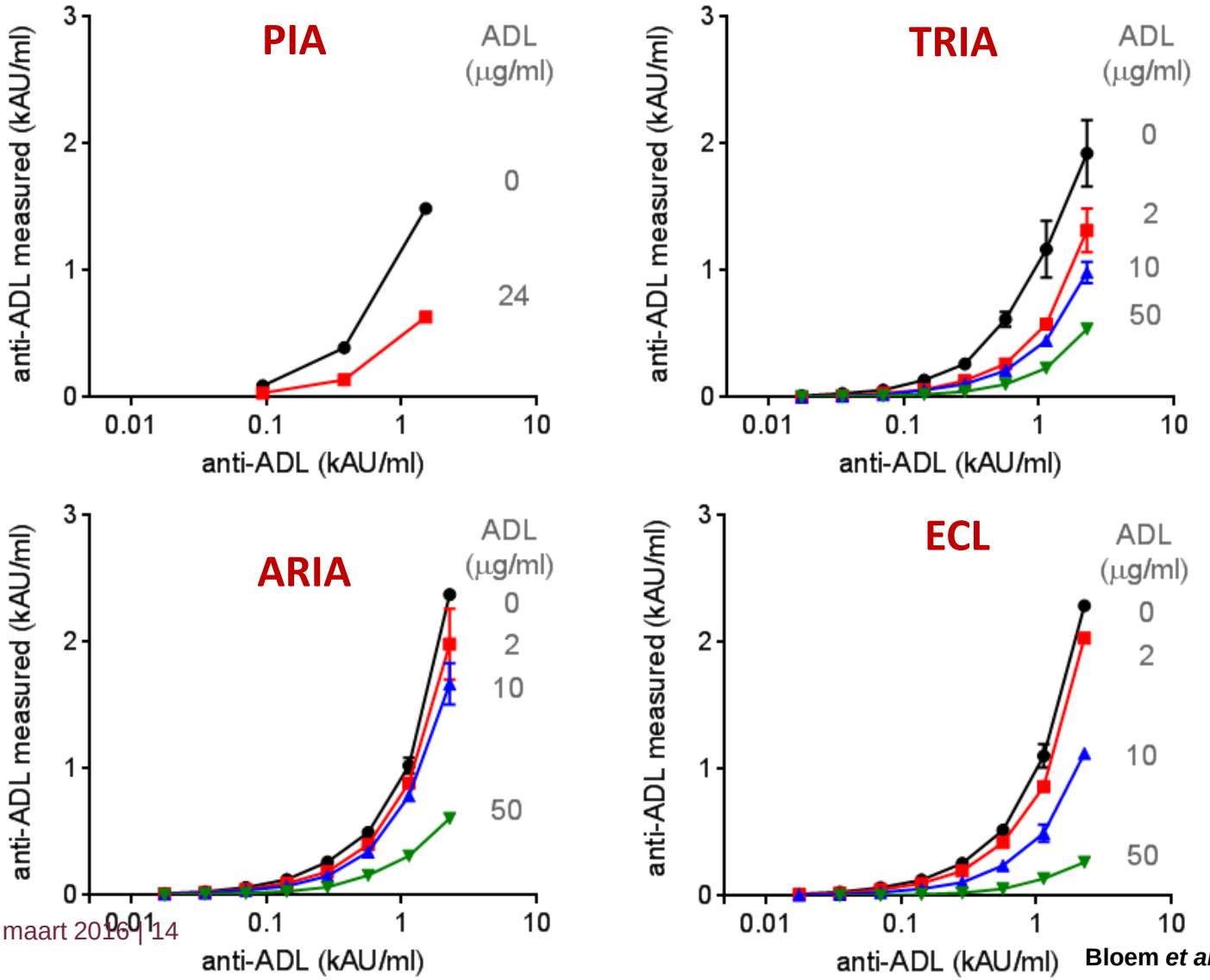


pH shift anti-idiotype
Antigen Binding Test

Acid dissociation (ARIA)

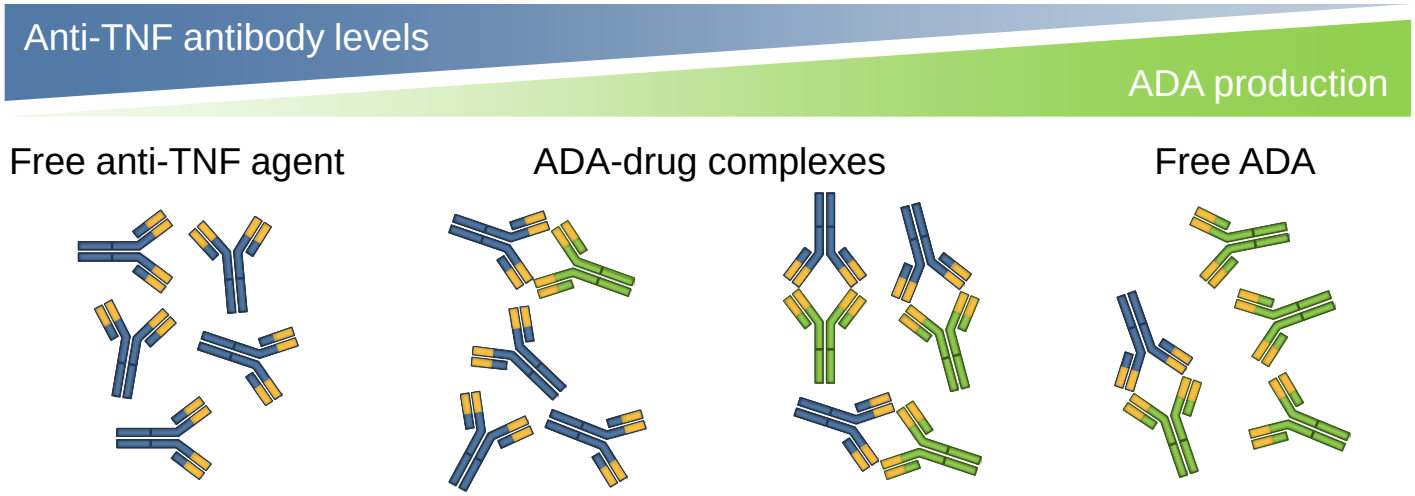


Drug tolerant assays detect antibodies in the presence of physiological amounts of drugs



Bloem *et al.*, J. Immun. Methods 2015

Detection of anti-drug-antibodies (ADA)

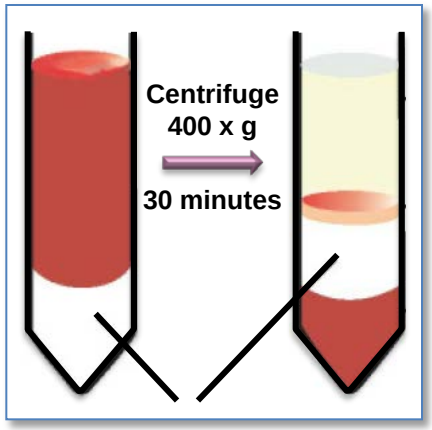


ADA detection method				
ELISA	–	–	–	+
ABT	–	–	+/–	+
PIA	–	+/–	+	+
Pharmacokinetic assay (TNF capture)	++	+	+/–	–

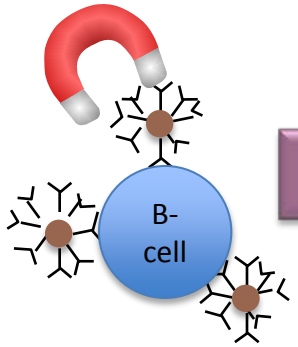
van Schouwenburg PA, et al. *Nat Rev Rheumatol.* 2013;9:164–172

Characterization of Anti-Drug Antibodies

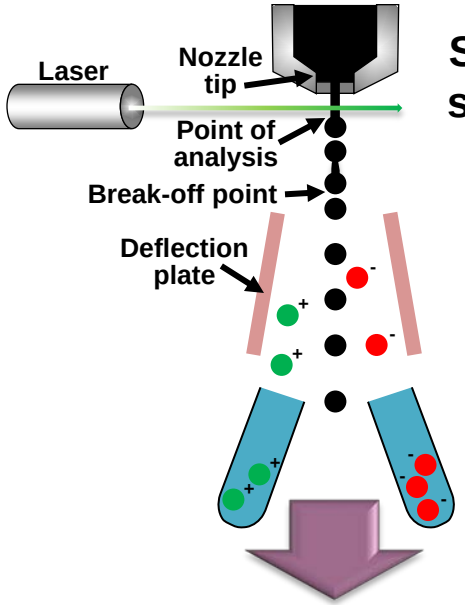
Generation of human monoclonal antibodies



Isolate PBMCs



Isolate B cells

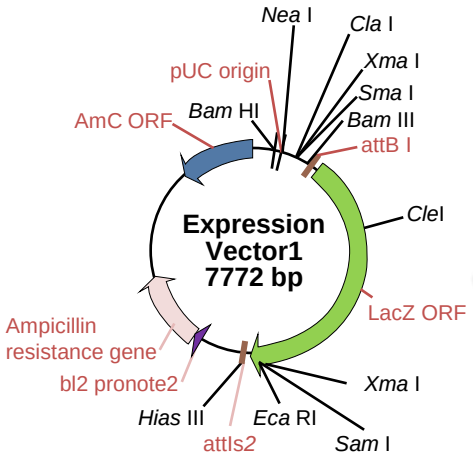


Sort antigen-specific cells

Culture 1 cell/well; screening

...AGGCATATCGA

Isolate RNA, determine sequence for VH/VL



Recombinant expression monoclonal antibodies

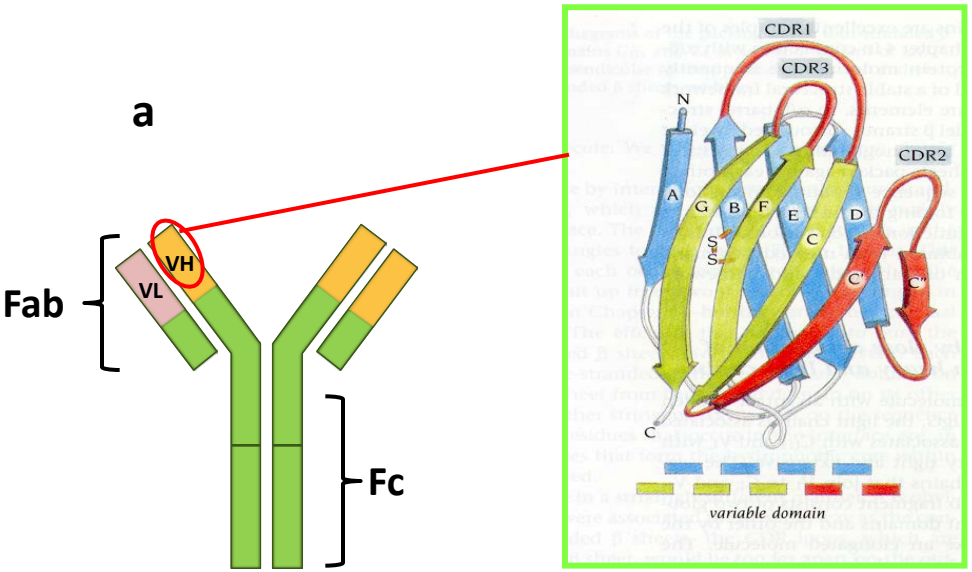
van Schouwenburg PA et al. *Ann Rheum Dis* 2013;72:104-9.

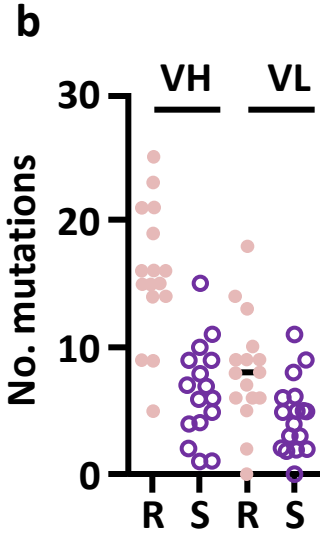
All monoclonal antibodies are derived from different precursor B-cells

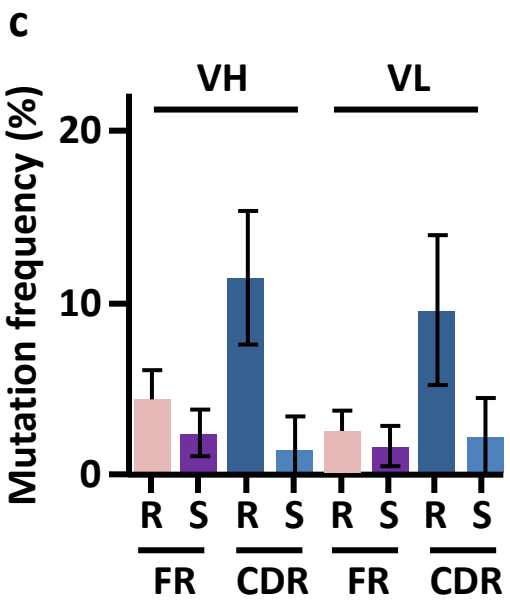
clone	isotype	V gene	D gene	J gene	CDR3-IMGT	length	R	S
1.1	IgG1	1-03	2-02	4	ARDIVVVPVAMHPDY	15	16	4
1.2	IgG4	1-02	2-15	5	ARDKWGPAAQYPDNWFDS	18	9	7
1.3	IgG1	1-18	1-14	4	AREPYDYSGTADY	13	15	4
2.1	IgG1	1-03	3-09	4	ASEGLLTGFPLDY	13	16	15
2.2	IgG4	1-69	3-10	6	ARLAIPWFGEAVFSYHYDMDV	21	15	9
2.3	IgG4	4-31	6-13	3	AREPAATGPSGDAFDI	16	21	5
2.4	IgG1	1-03	3-16	3	ARMGERGLDV	10	19	7
2.5	IgG4	4-59	6-13	3	ARQTLLMAADGDDAFDI	17	16	11
2.6	IgG1	4-39	1-26	4	ARRSVAAFDY	10	14	6
2.7	IgG1	4-34	1-26	3	AREGKNSGSYYVRLGDTFDI	20	5	1
2.8	IgG4	1-69	6-19	5	ARDQKGQWFDP	11	21	2
2.9	IgG1	3-48	2-21	6	ARVKDDIVVPTGLGMDV	17	23	9
2.10	IgG4	1-03	2-21	5	AELASSGLFDP	11	15	6
2.11	N.D.	1-69	6-19	4	ARLHSRGWSDFDY	13	14	8
2.12	IgG4	1-18	2-21	6	AREIAPGDMDE	11	25	10
2.13	IgG1	3-48	5-5	3	ARTGGHSHGPGGFDI	15	9	1

van Schouwenburg PA, et al.

Monoclonal antibodies undergo extensive affinity maturation



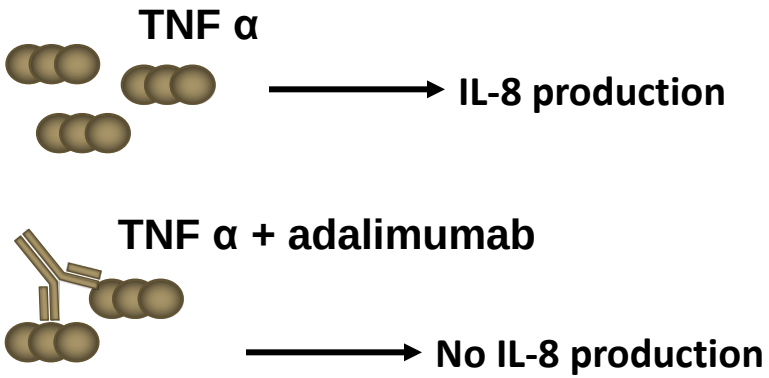


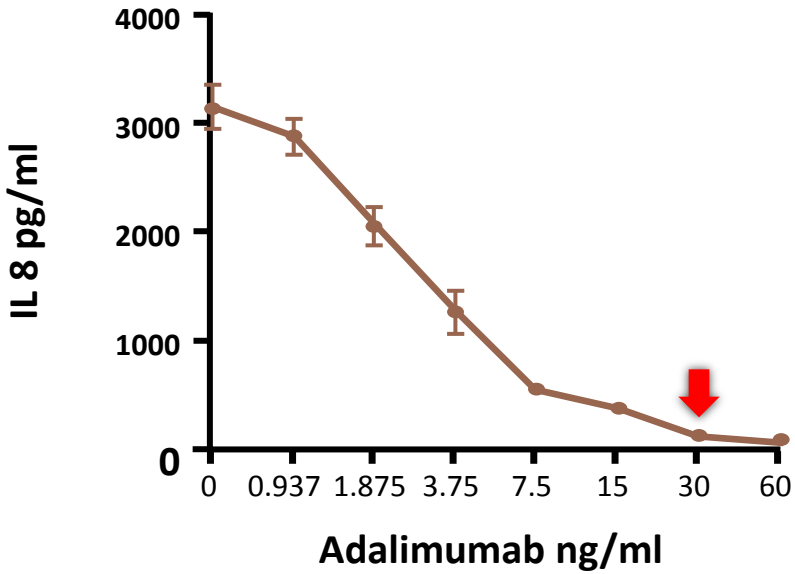


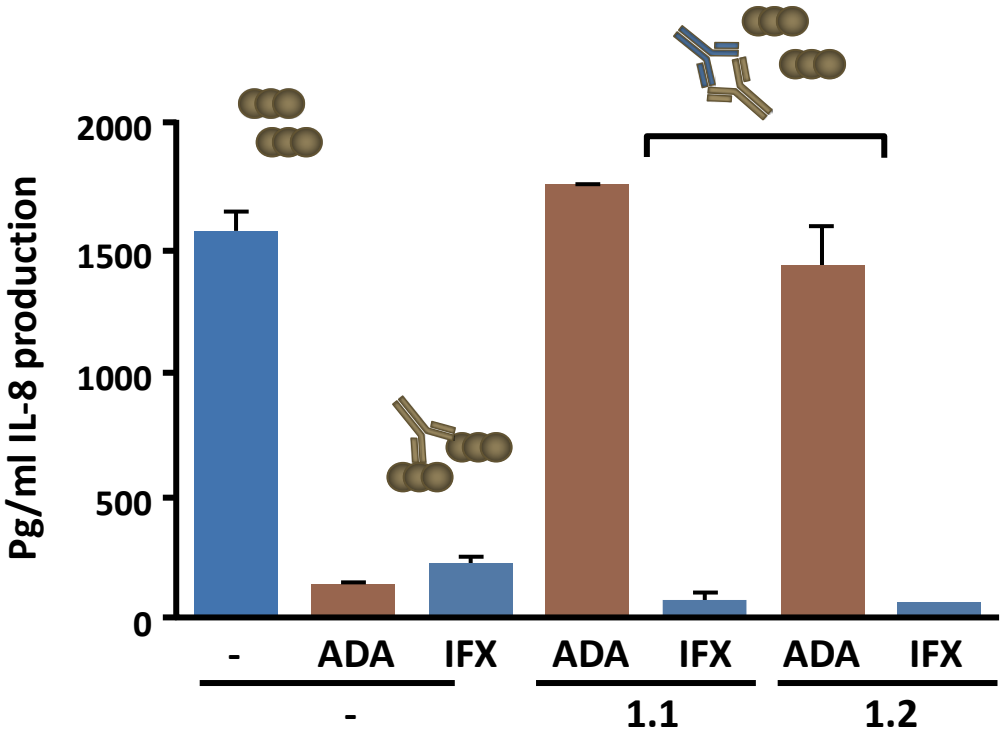
Clone	K_d (pM)
1.2	9.3 ± 2
1.3	21 ± 2
2.1	2.6 ± 0.1
2.2	0.78 ± 0.08
2.4	50,000
2.6	233 ± 33
2.7	195 ± 5
2.8	24 ± 1
2.9	82 ± 23
2.10	115 ± 15
2.12	0.64 ± 0.16

van Schouwenburg PA, et al.

ECRF bio-assay: TNF-sensitive human endothelial cell line

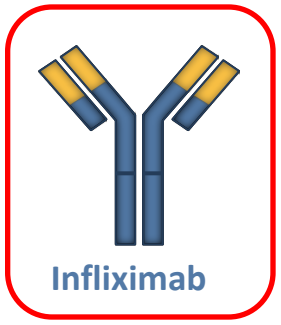


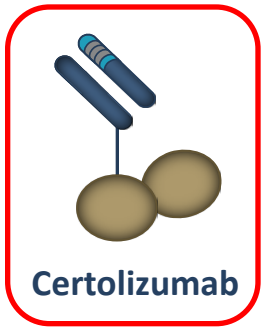


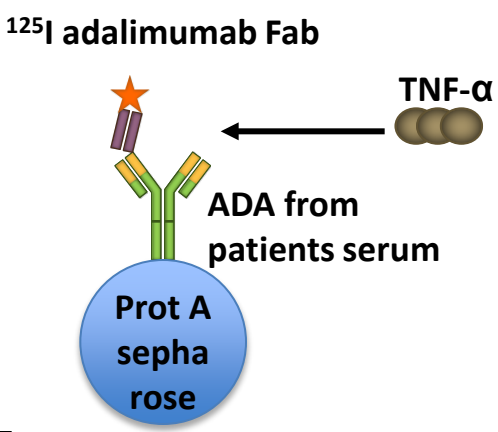


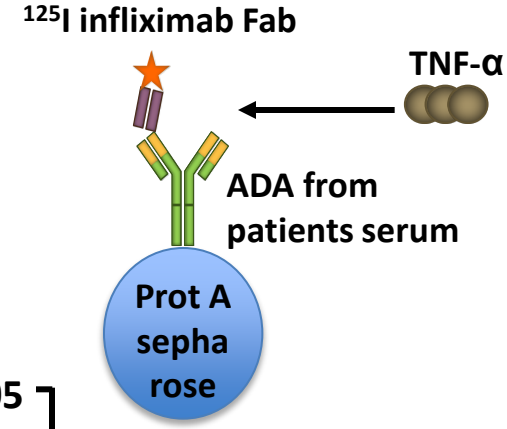
van Schouwenburg PA, et al. *Ann Rheum Dis.* 2013;72:104–109

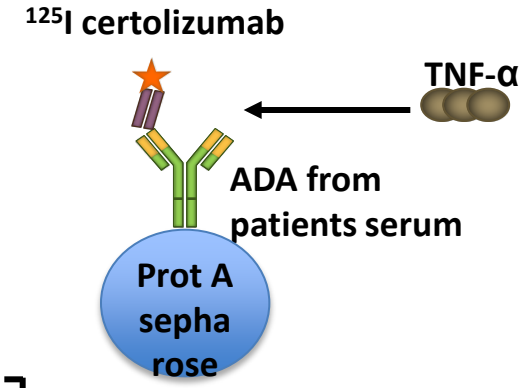
TNF inhibition

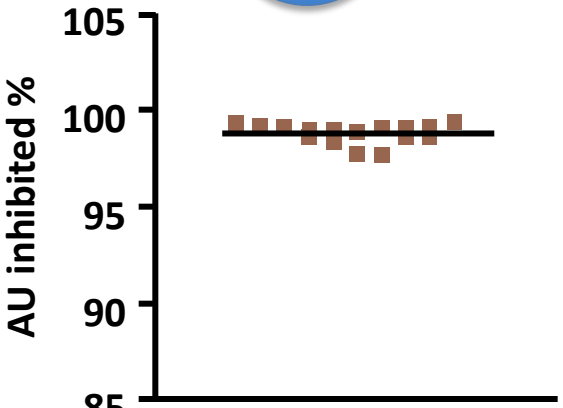


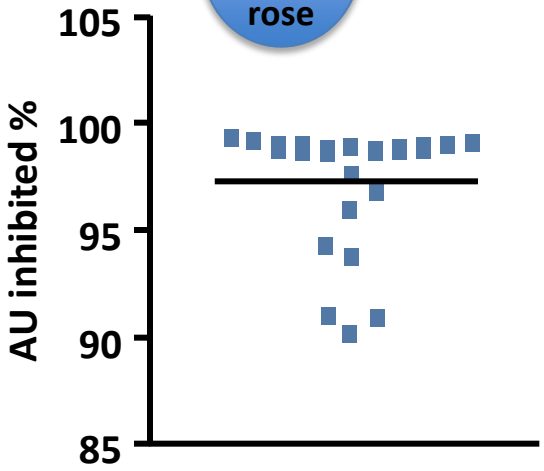


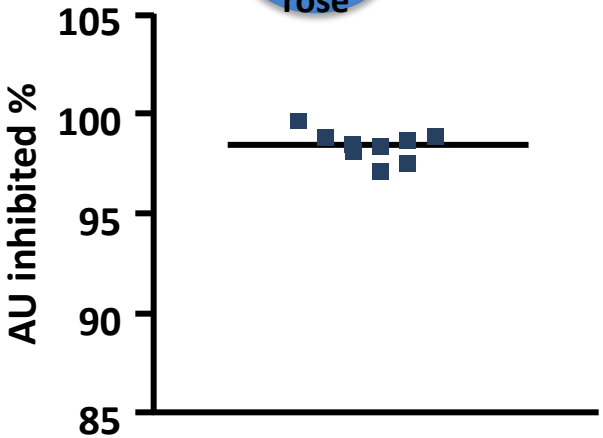






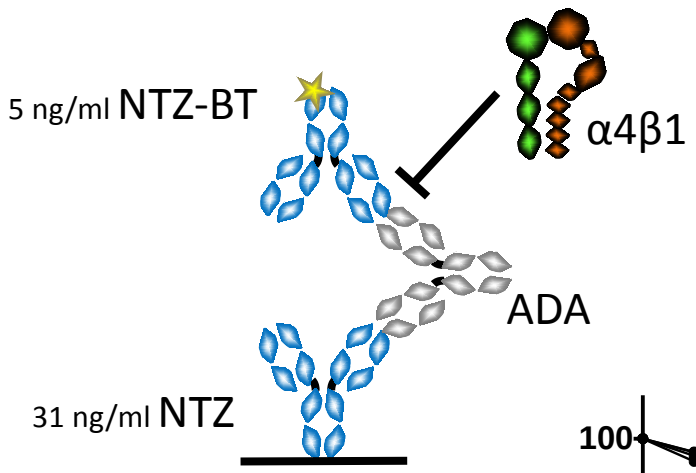


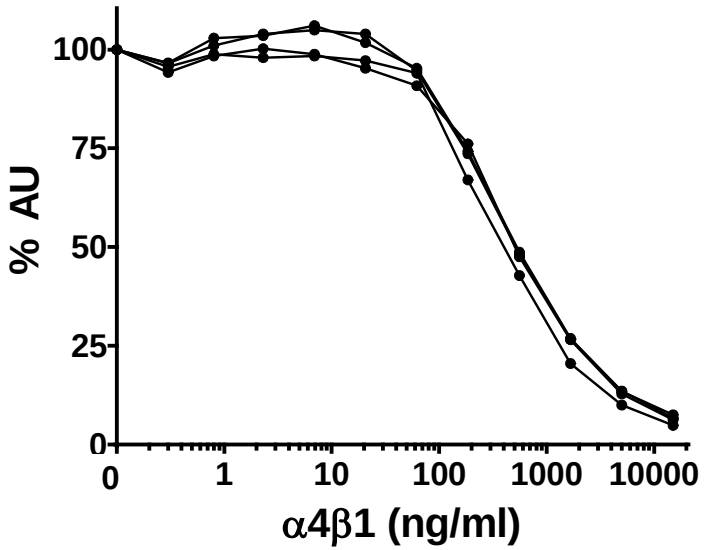


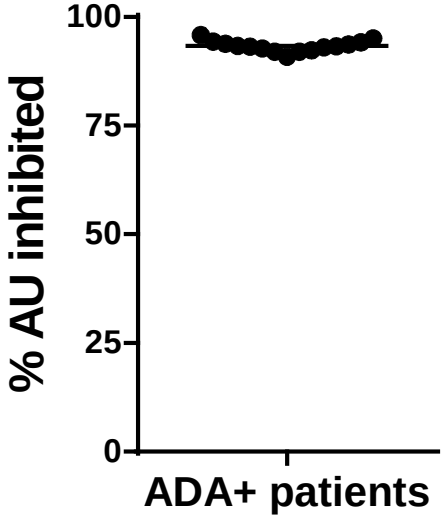


Wolbink, data on file

To what extent do patient ADA neutralize natalizumab?



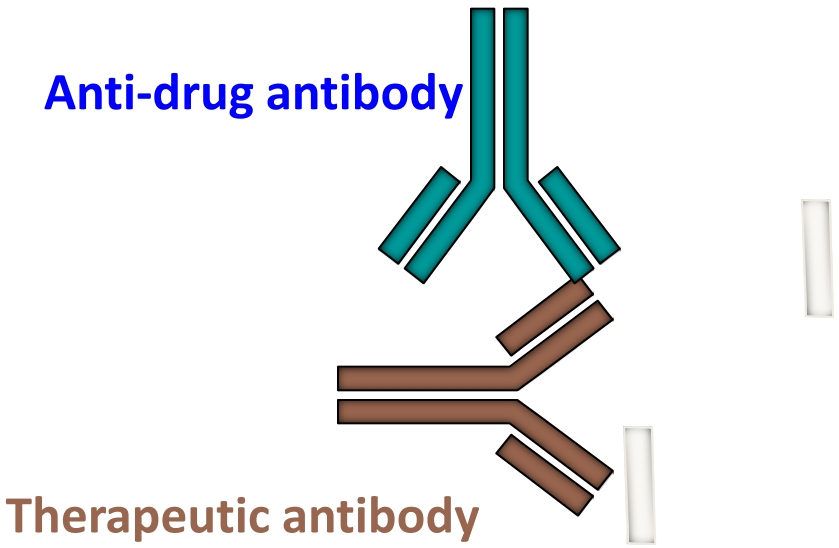




>90.9% of patient ADA is inhibited from binding natalizumab using recombinant α4β1 as blocker.

N=15

Anti-drug antibodies are anti-idiotypic and interfere with target binding



Wolbink GJ, et al. *Arthritis Rheum*. 2006;54:711–715

Clinical Relevance

- Side effects
- Effects on PK

Immune complexes

- In ADA+ adalimumab patients, small immune complexes (2 antibodies) are found weeks after adalimumab administration.

- In 1 ADA+ infliximab patient, large immune complexes (>6 antibodies) were found directly after infliximab infusion
  - ADA+ patient experienced an infusion reaction

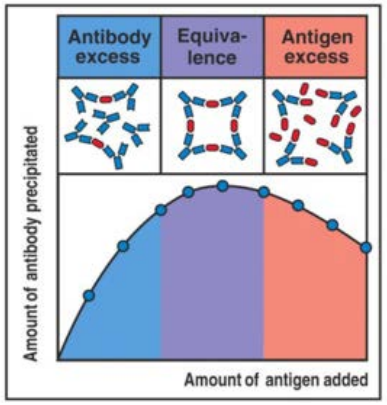
<u>Hypothesis</u>

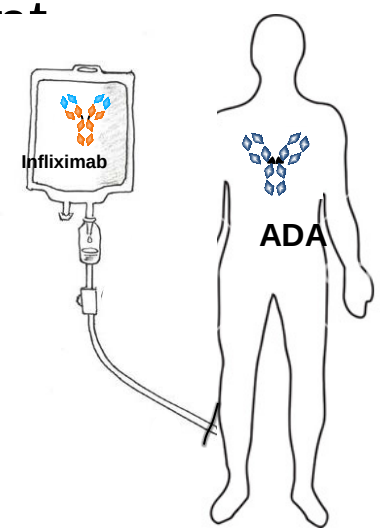
Infusion reactions are mediated by large immune complexes

van Schouwenburg et al., Ann Rheum Dis, 2012
van der Laken et al., Ann Rheum Dis, 2007

What influences immune complex size?

- Immune complex size is ratio dependent





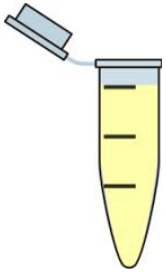
- Effect of concentration on complex size is unknown
  - Infliximab can be administered in various infusion speeds
  - Patients make various amounts of ADA

>100 µg/mL infliximab

Immunobiology, Janeway

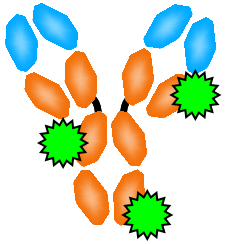
Influence of concentration ADA and IFX

Patient serum
140.000 AU/ml
34.000 AU/ml
12.000 AU/ml
3.400 AU/ml



serum

+



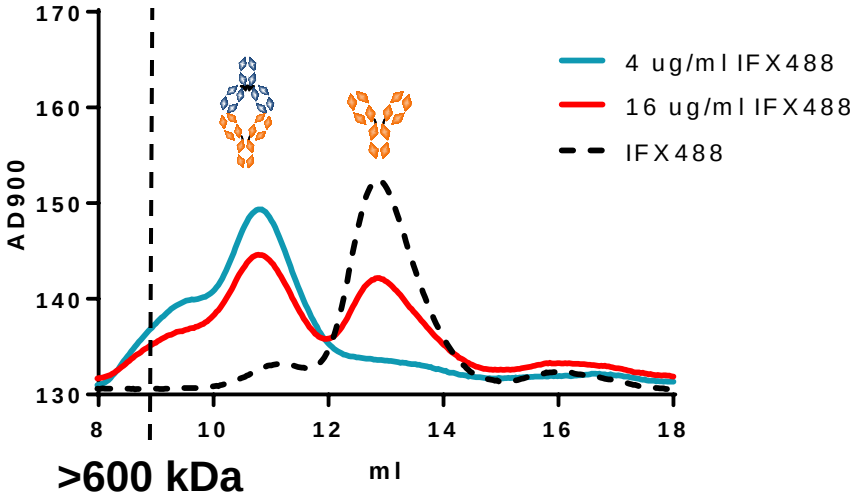
IFX-488

1.5 hour incubation

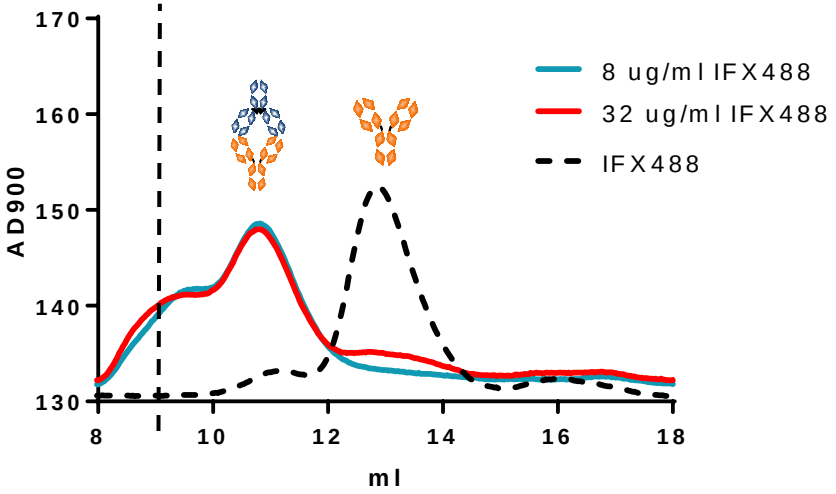
HP-SEC

Cpmplexes are small however Higher concentration of ADA and IFX gives larger complexes

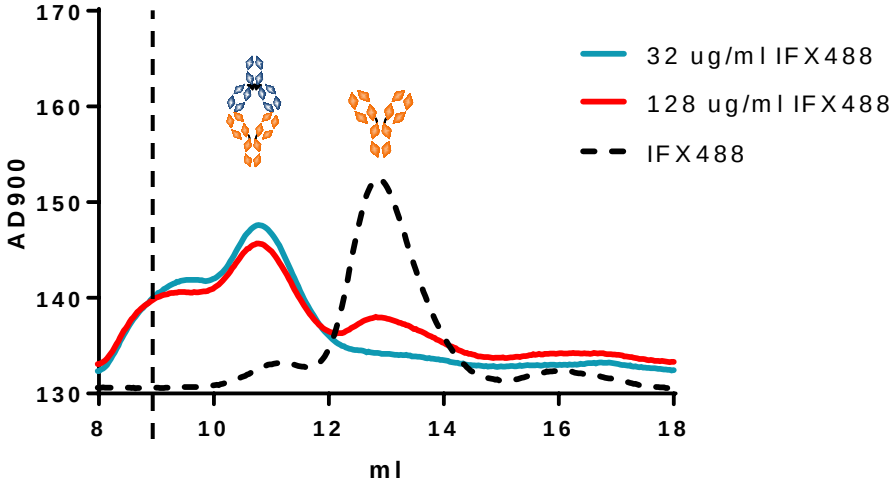
3.400 AU/ml



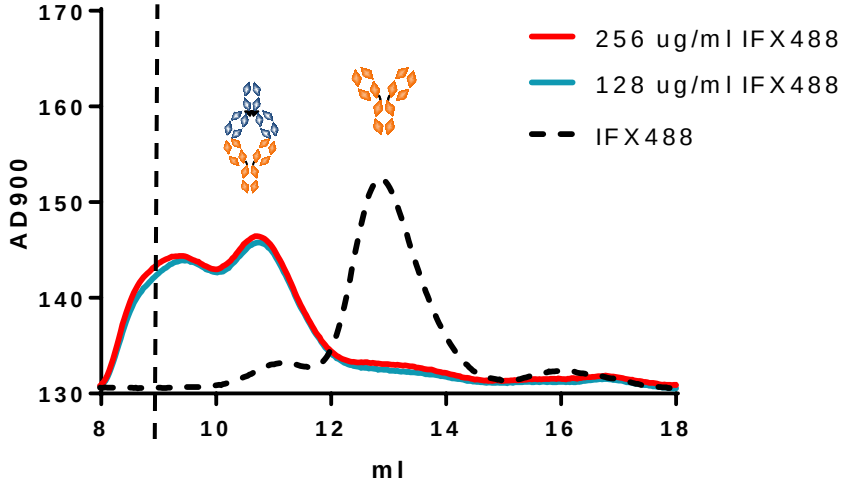
12.000 AU/ml



34.000 AU/ml

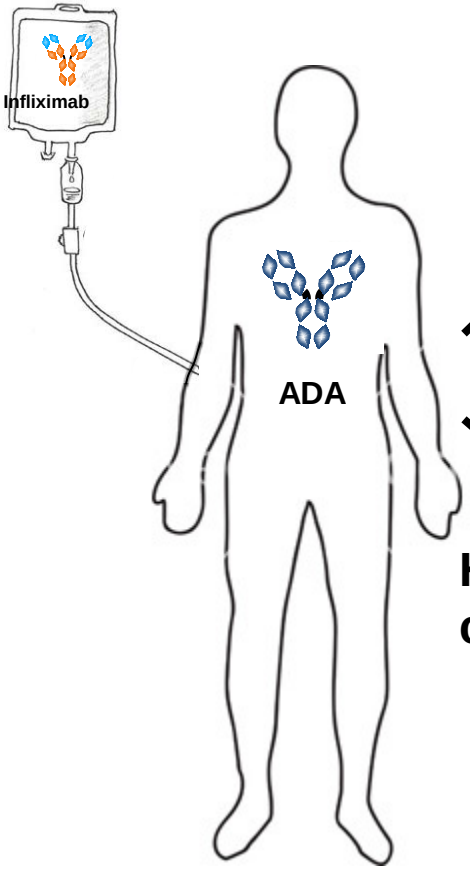


140.000 AU/ml



Summary

- Immune complex size is dependent on concentration of ADA and drug
  - The higher the concentration, the bigger the complexes
  - But in general they are small
- Risk factor for infusion reactions is a high ADA titer



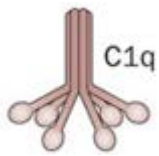
Low ADA/drug concentration

No clear adverse effects

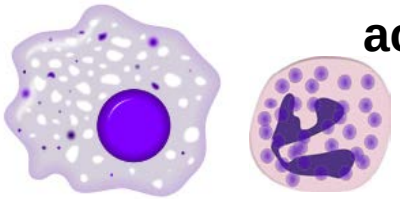
High ADA/drug concentration

Infusion reaction

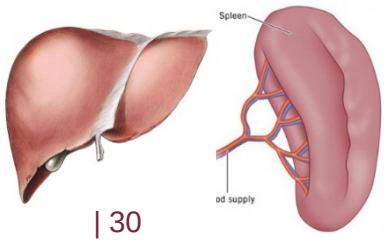
Complement activation



Immune cell activation



Clearance by liver&spleen



Immunogenicity in a long-term follow-up cohort of adalimumab treated rheumatoid arthritis patients

Bartelds GM, et al. *JAMA.* 2011;305:1460–1468

Patients & methods

1. 272 consecutive RA patients with active disease treated with adalimumab in a prospective observational cohort study
2. Disease activity monitored at baseline and 4, 16, 28, 40, 52, 78, 104, 130 and 156 weeks using the DAS28 score
3. Trough serum samples were obtained at all visits
4. Serum adalimumab concentrations and anti-adalimumab antibody (AAA) titres determined retrospectively at the end of follow-up using an ELISA and ABT (Sanquin Research, Amsterdam)

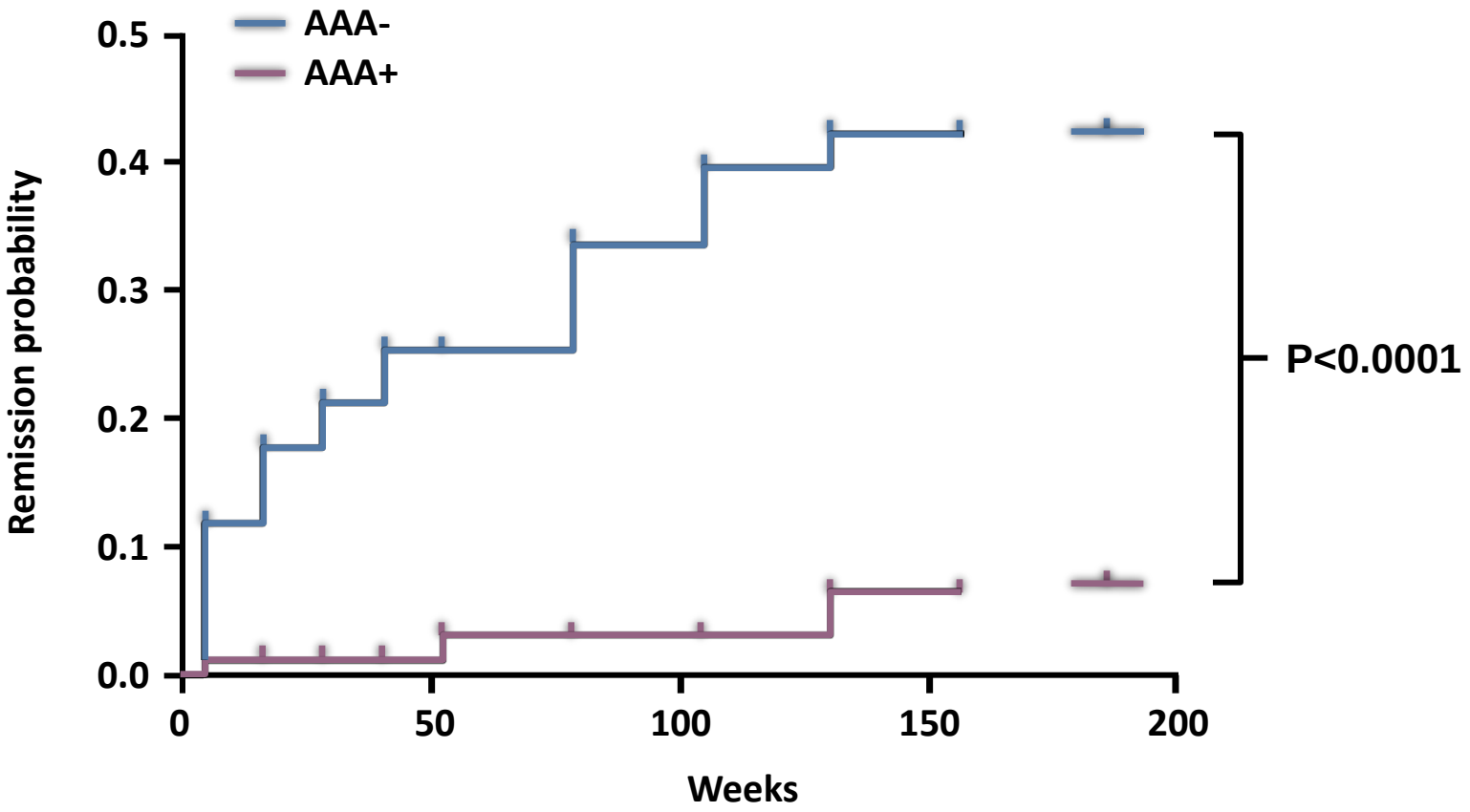
Bartelds GM, et al. *JAMA.* 2011;305:1460–1468

Results: baseline characteristics

	Total	Patient with AAA detected with ABT	Patients without AAA
	n=272	n=76	n=196
Age, years	54 ± 12	53 ± 13	54 ± 11
Female, no. (%)	219 (81)	62 (82)	157 (80)
RF, no. (%)	196 (72)	57 (75)	139 (71)
Prior DMARDs	3.1 ± 1.4	3.4 ± 1.5*	3.0 ± 1.3*
MTX use, no. (%)	202 (74)	41 (54)*	161 (82)*
MTX dose (mg/wk)	25 (15–25)	18 (10–25)*	25 (15–25)*
No DMARD, no. (%)	51 (19)	28 (37)*	23 (12)*
Disease duration (years)	8 (3–17)	12 (5–18)*	8 (3-16)*
Erosive disease, no. (%)	201 (74)	63 (83)*	138 (70)*
ESR (mm/h)	23 (11–42)	35 (18–60)*	21 (11–39)*
CRP (mg/L)	12 (5–29)	19 (7–46)*	11 (4–22)*
DAS28	5.2 ± 1.2	5.5 ± 1.1*	5.1 ± 1.3*

Bartelds GM, et al. *JAMA.* 2011;305:1460–1468

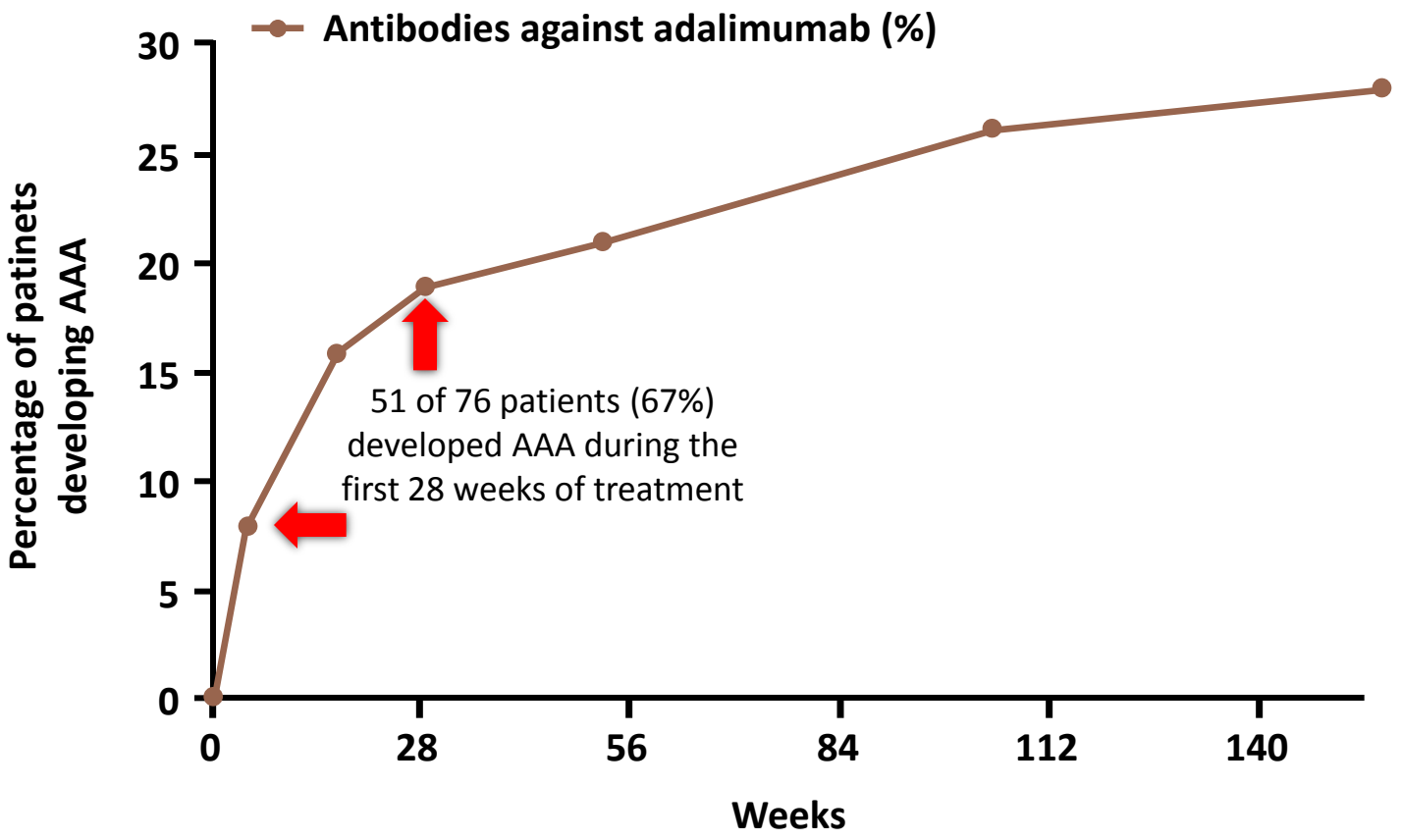
Sustained remission (DAS28 <2.6) correlates with absence of AAA detected



After adjustment for confounding variables MTX dosage, ESR and CRP (HR: 3.6; 95% CI 1.8–7.2, P<0.0001)

Bartelds GM, et al. *JAMA.* 2011;305:1460–1468

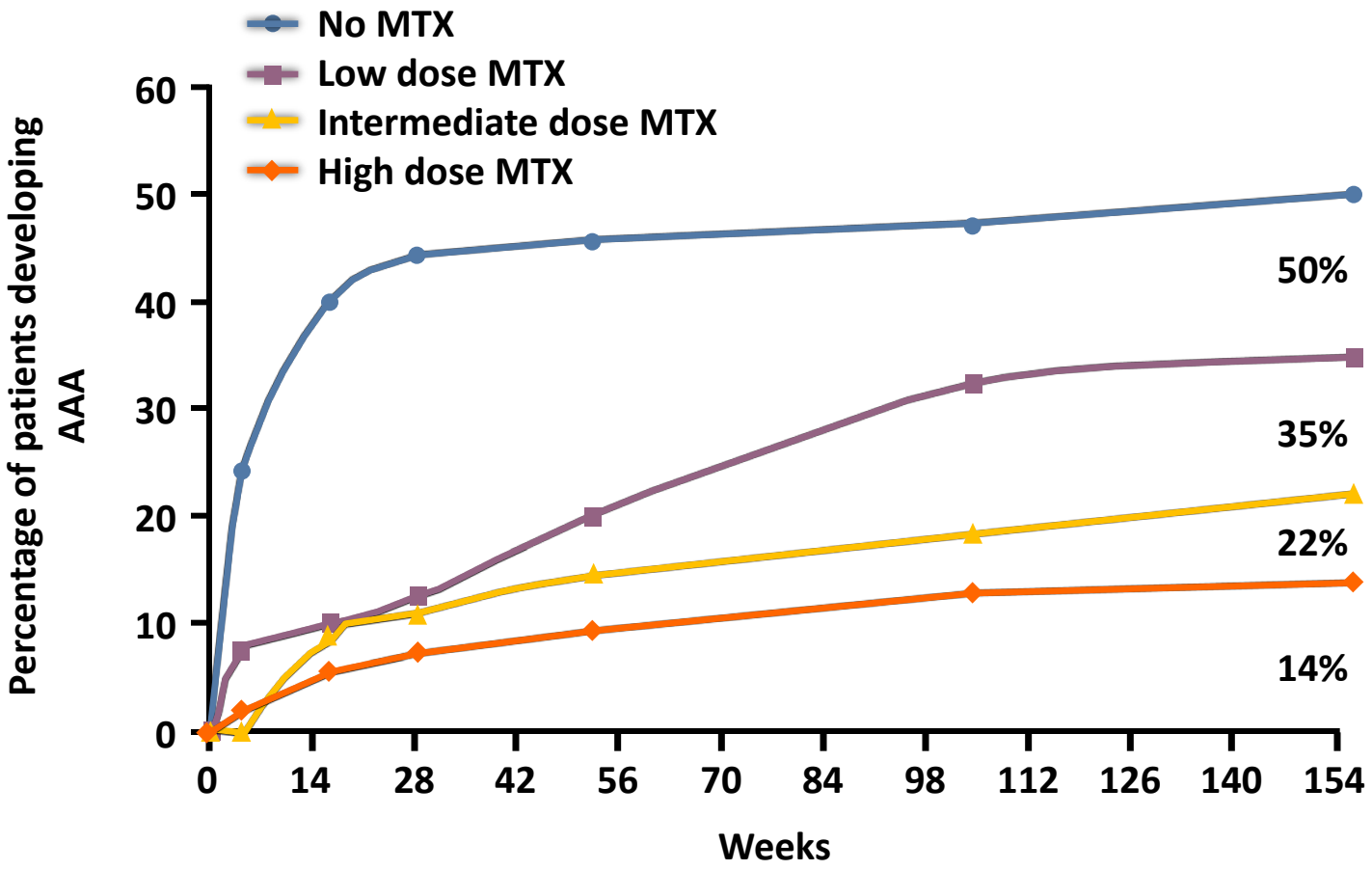
Percentage of patients developing detectable anti-adalimumab antibodies over three years



During 156 weeks follow-up, anti-adalimumab antibodies were detected (ASSAY IV:ABT) in 76 (28%) patients

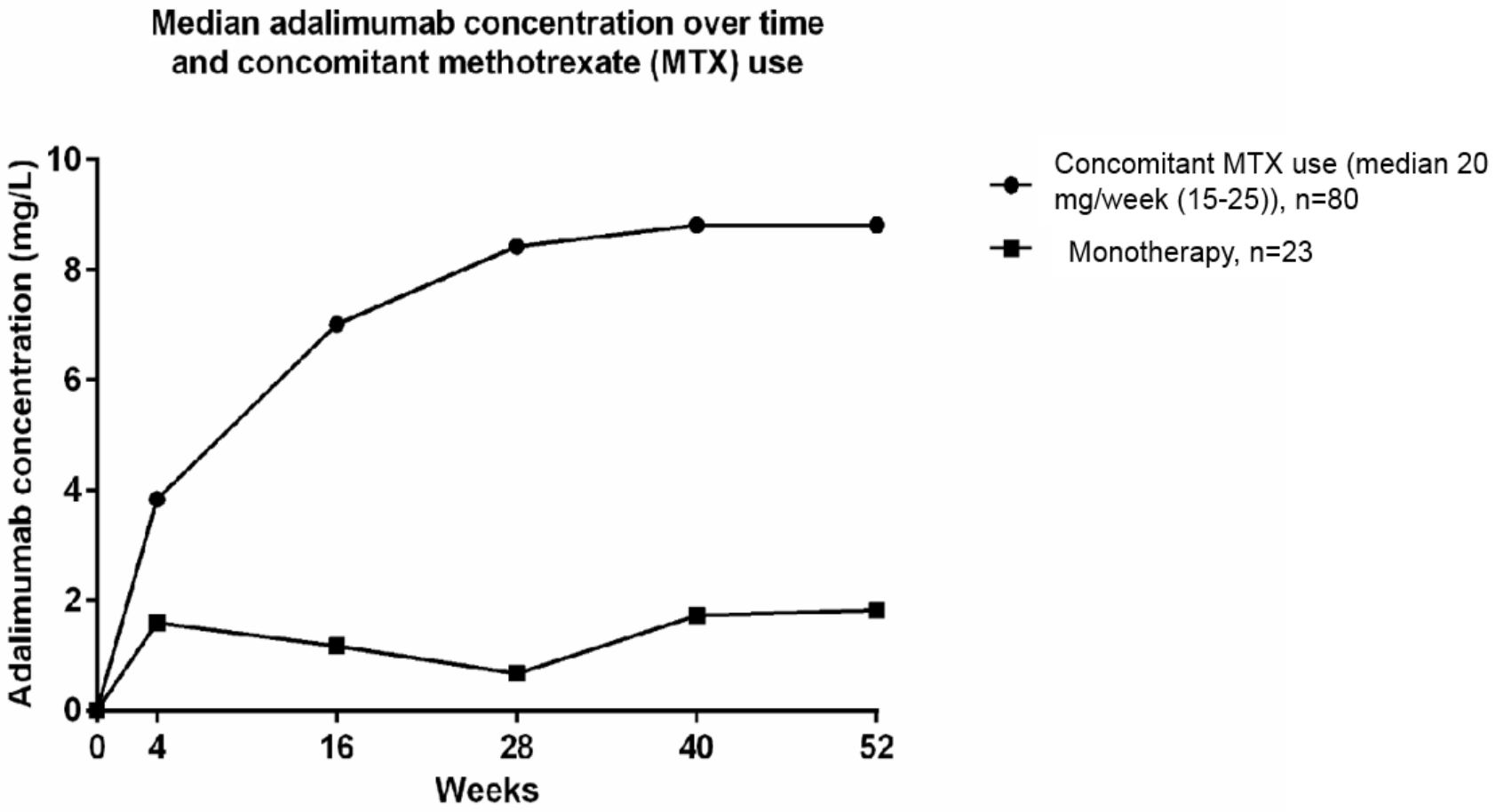
Bartelds GM, et al. *JAMA.* 2011;305:1460–1468

Methotrexate reduces immunogenicity in adalimumab-treated RA



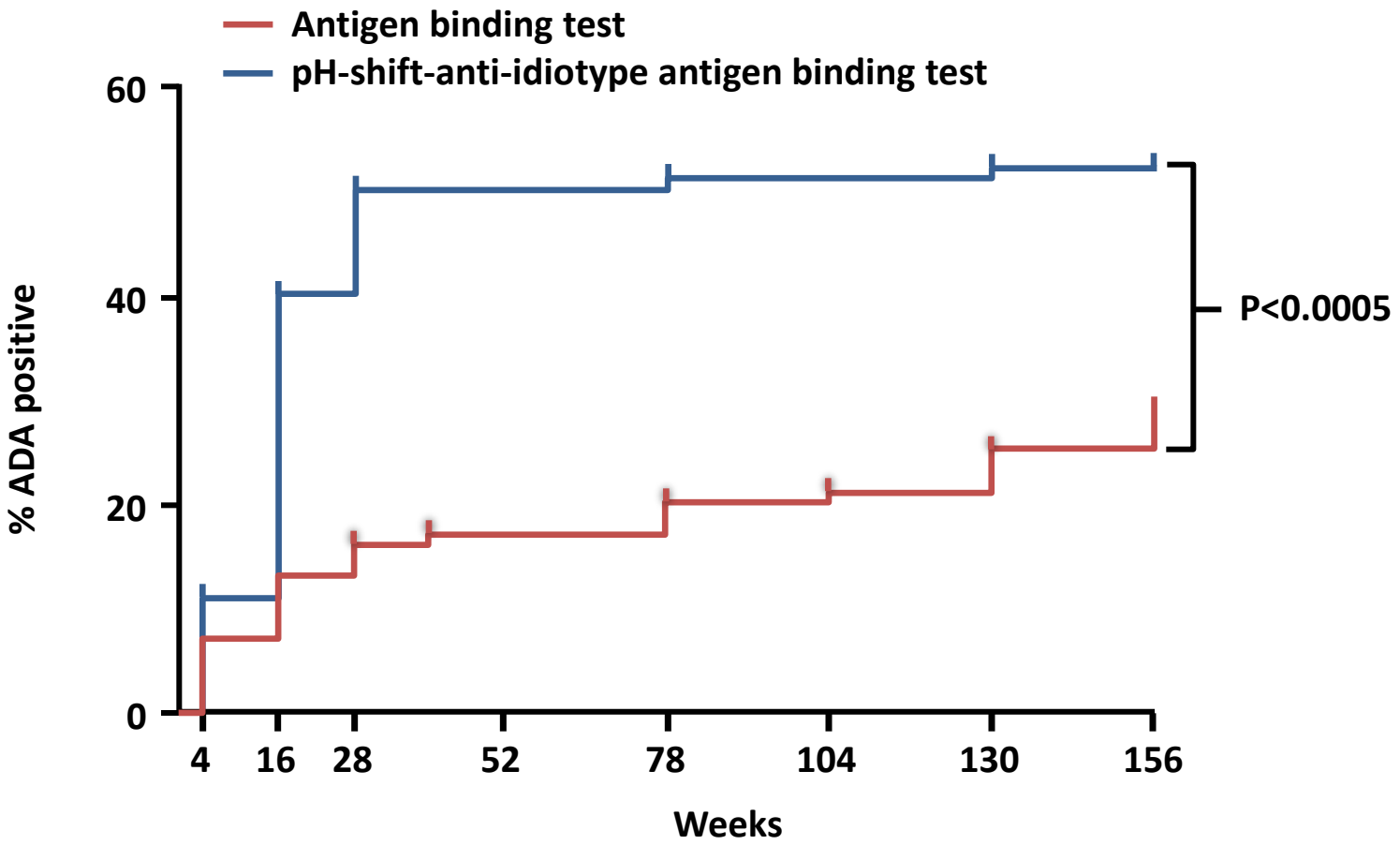
Krieckaert CL, et al. *Ann Rheum Dis.* 2012;71:1914–1915

Concomitant methotrexate (PsA)



Vogelzang et al ARD 2014 online first

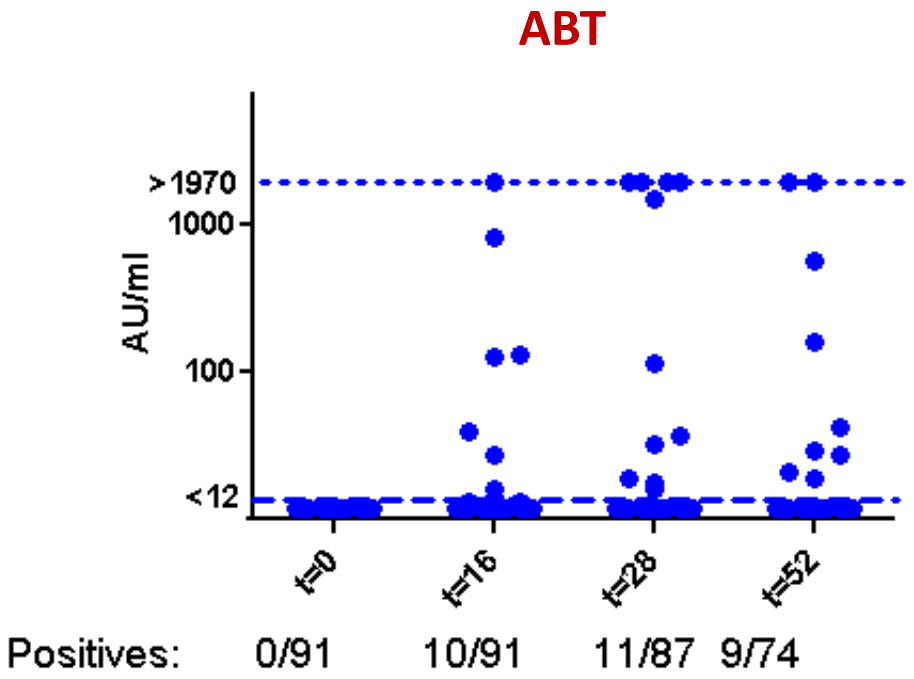
The accumulative percentage of ADA positive patients depends on assay method used



Accumulative percentage of patients positive for ADA assessed by pH-shift-anti-idiotype antigen binding test and antigen binding test

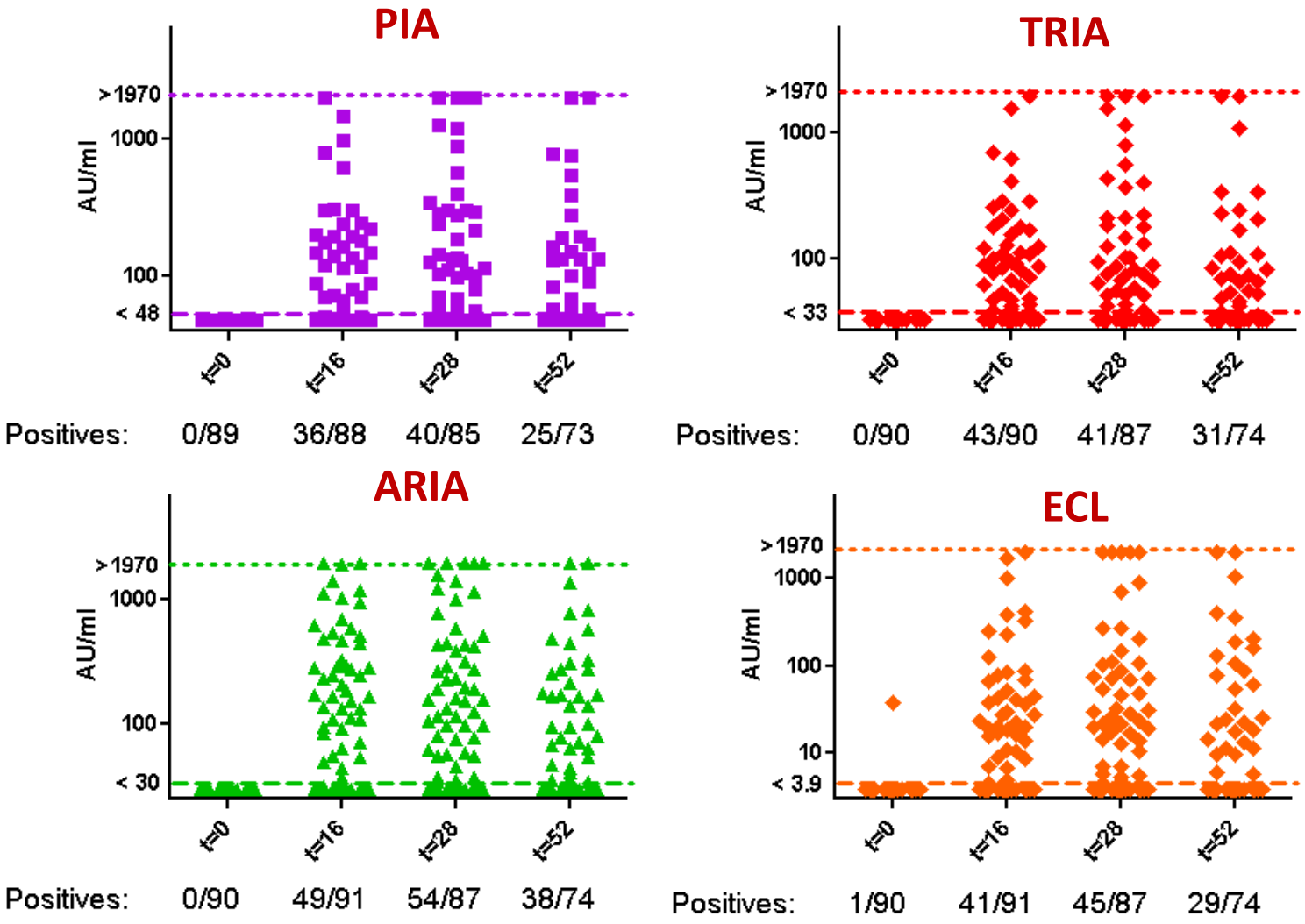
van Schouwenburg PA, et al. *Ann Rheum Dis.* 2013;72:1680–1686

Antibody detection in a small proportion of RA-treated patients using the ABT



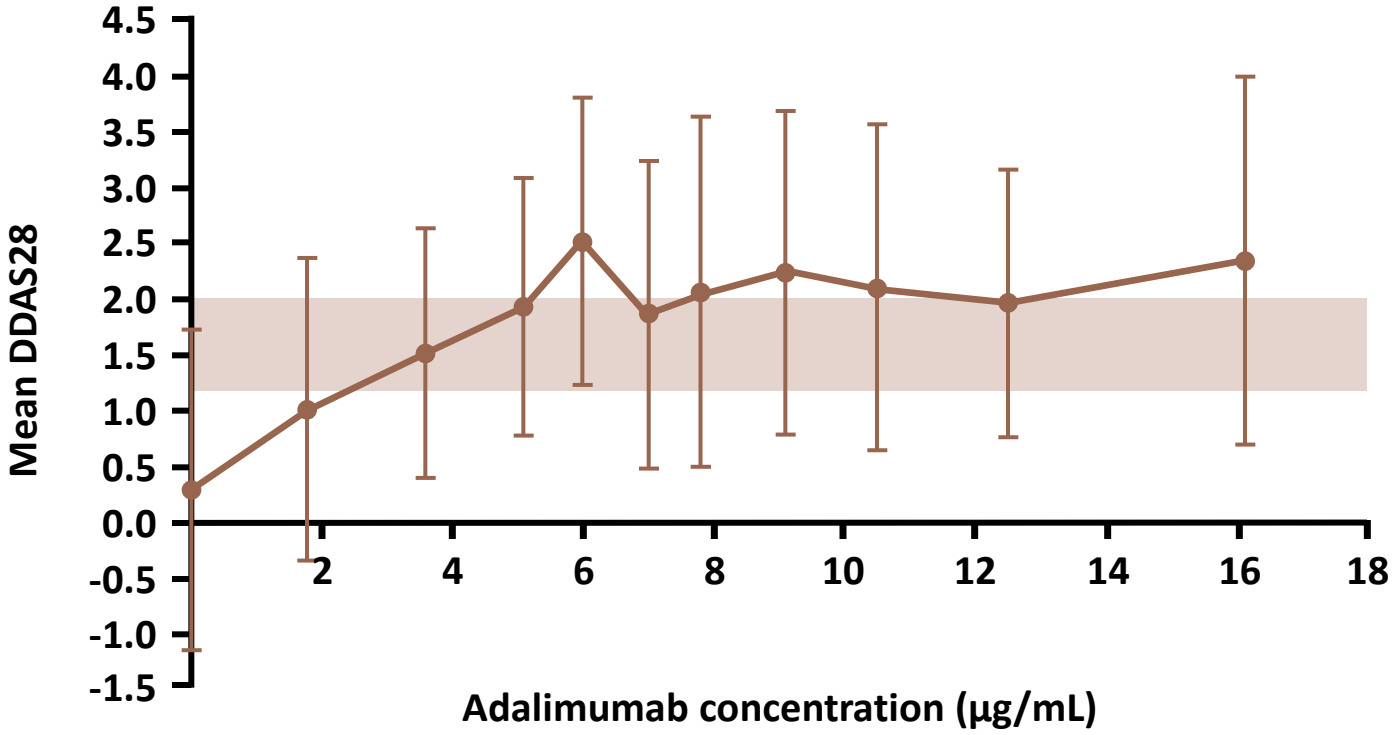
Bloem *et al.*, J. Immun. Methods 2015

Antibody detection in a substantial amount of the patient samples using drug tolerant assays



Bloem *et al.*, J. Immun. Methods 2015

Adalimumab concentration correlates with treatment efficacy

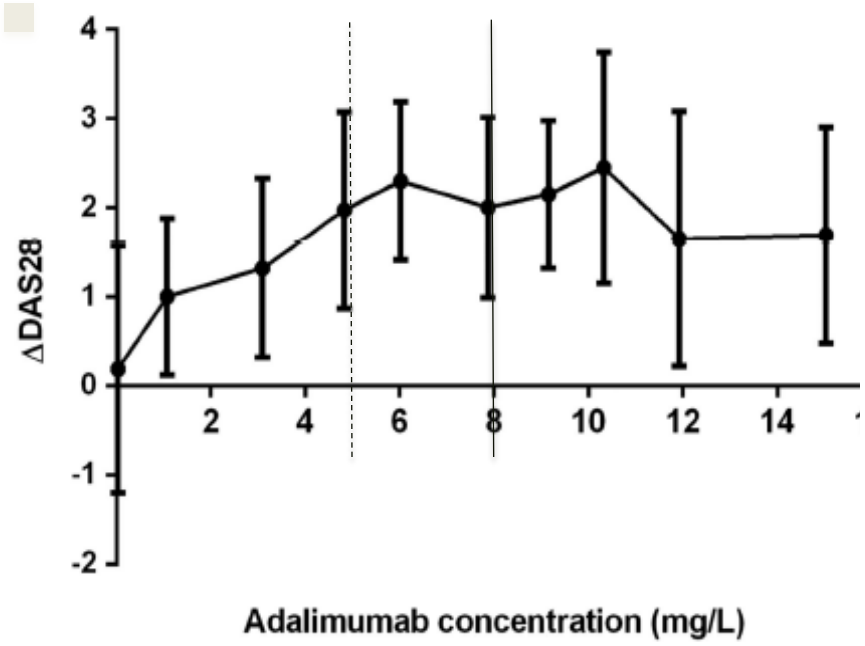


Mean delta DAS and adalimumab levels per 20 patients at week 28

Pouw M, et al. *Ann Rheum Dis.* 2013 [ePub]

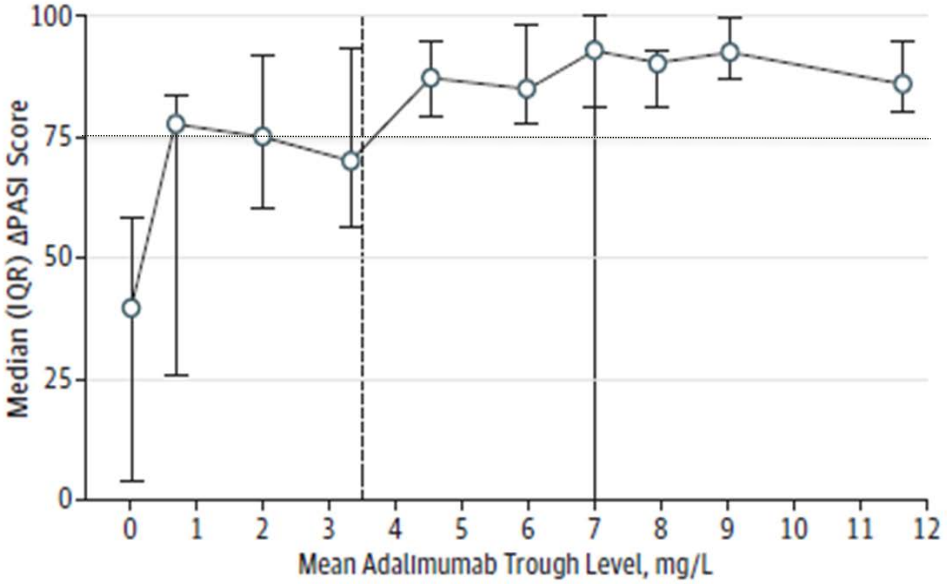
Concentration-effect curve adalimumab

Psoriatic arthritis (n=103)



Last observation carried forward

Psoriasis (n=135)



Without last observation carried forward

Vogelzang et al ARD 2014 online first; Menting et al JAMA dermatol 2015 online first

Conclusions

- Anti drug antibodies to therapeutic antibodies are common and anti-idiotypic
- Detection is highly dependend on the assay and testing strategy
- The clinical relevance is within the PK
- Immuuncomplexes formed are small and often do not mediate side effects

Discussion

- Availability of PK assays
- Availabilty of information on the concentration effect relationship
- Availability of immunogenicity assays