




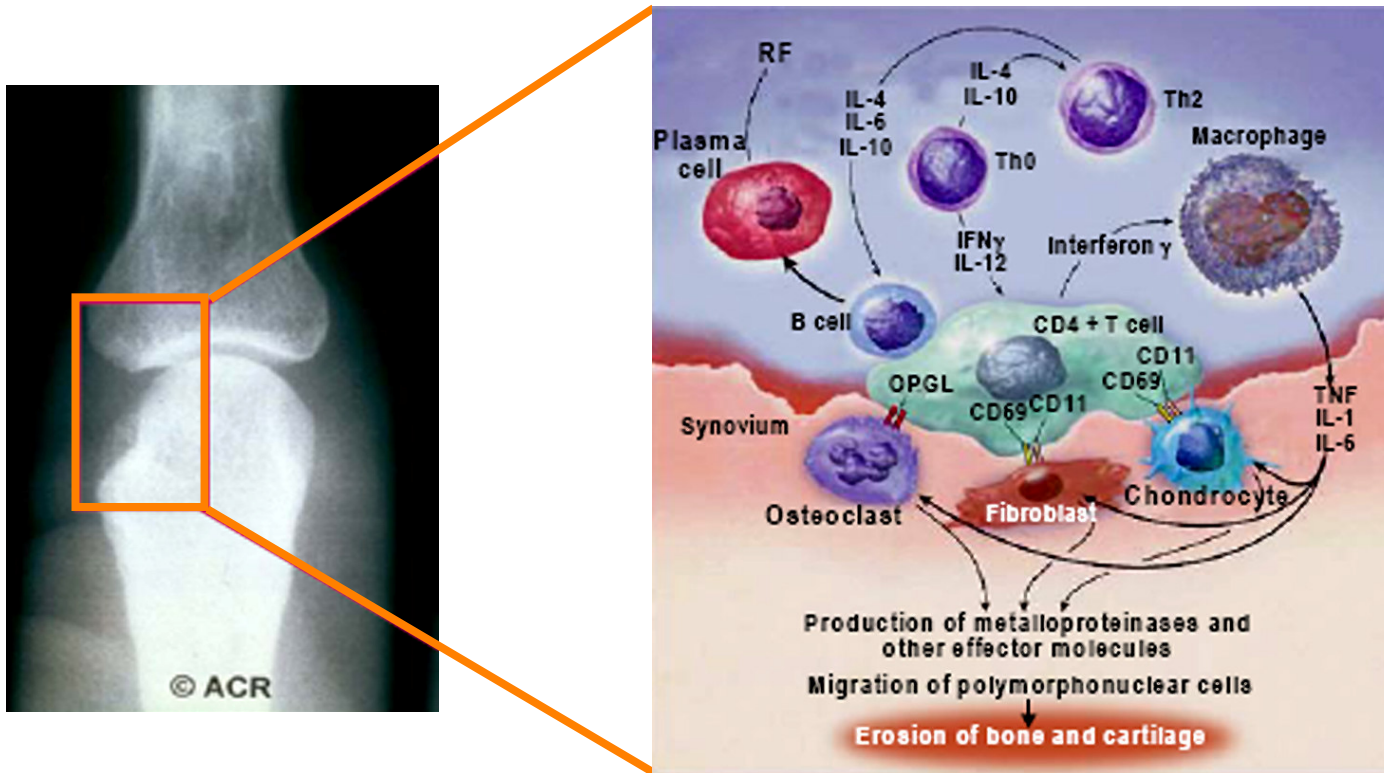
Immunogenicity of therapeutic antibodies Clinical Significance

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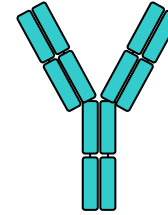
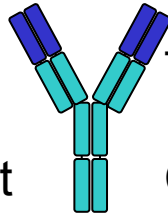
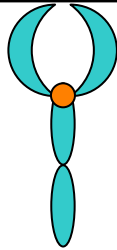
Cytokine network in RA

Cytokine signaling pathways involved in RA



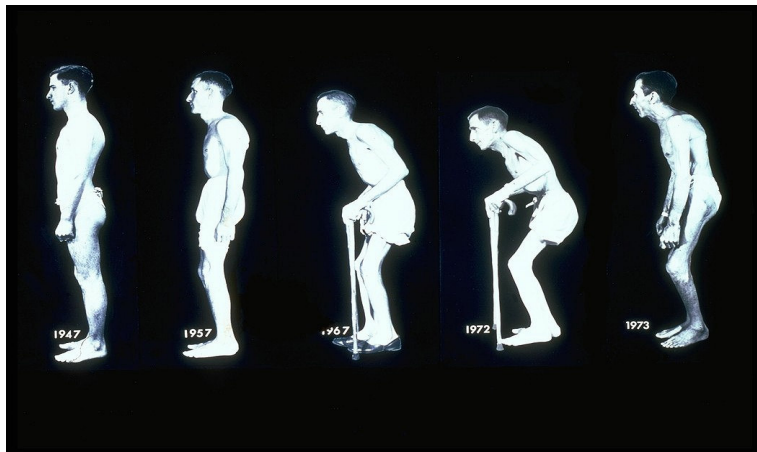
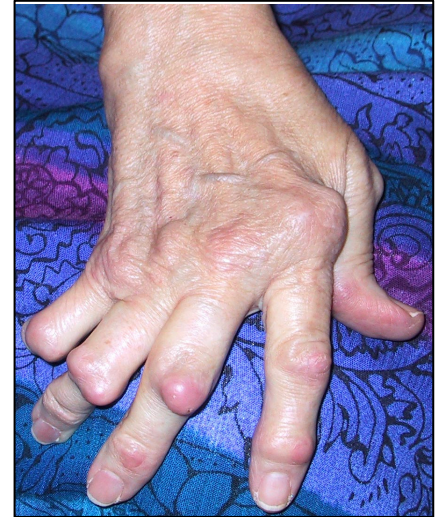
TNF alpha blockers

Characteristic	Etanercept (ENBREL [®])	Infliximab (REMICADE [®])	Adalimumab (HUMIRA [®])
Class	sTNFR	TNF MAb	TNF MAb
Construct	Recombinant fusion protein	Chimeric MAb	Recombinant human MAb
Half-life	4 days	8-10 days	10-20 days
Binding target	TNF/LT α	TNF	TNF
Administration	50mg SC Once weekly	3-10 mg/kg IV with MTX Every 4-8 weeks	40mg SC Every other week*



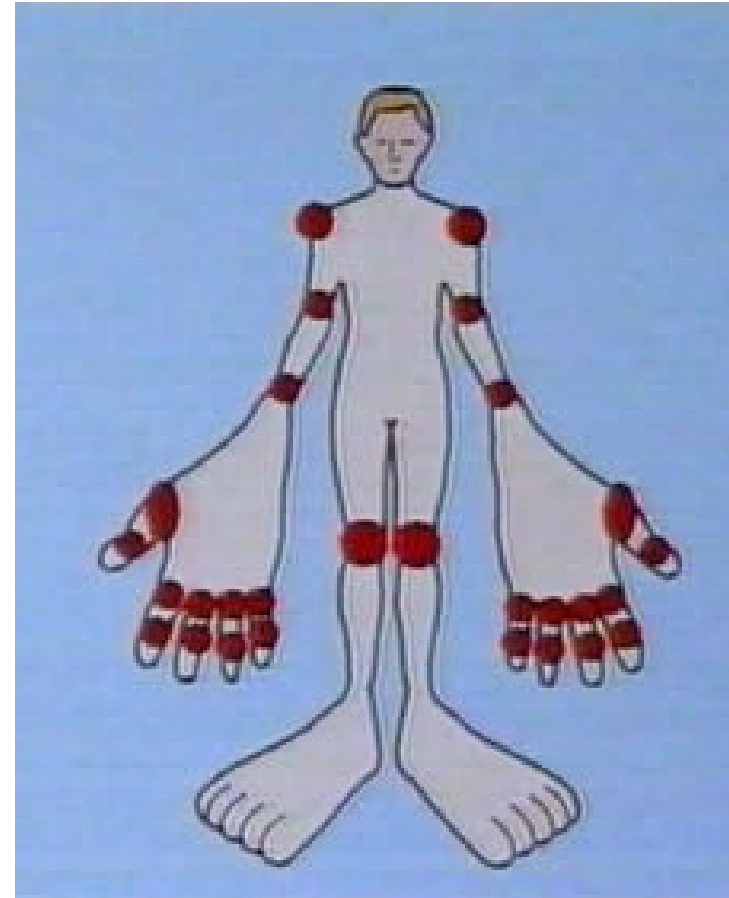
**Amsterdam-cohort:1000 patients on biologicals
infliximab/adalimumab/etanercept
Longterm clinical and serological follow-up**

- **Rheumatoid Arthritis (RA)**
- **Psoriatic Arthritis (AS)**
- **Ankylosing spondylitis (PsA)**



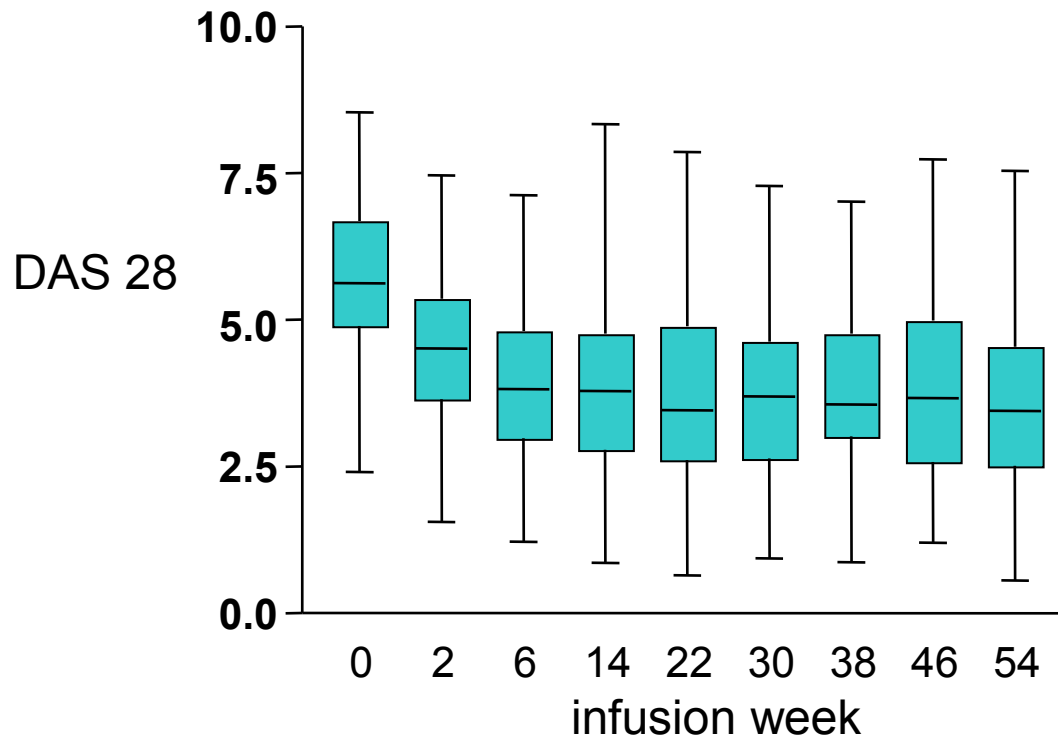
Disease activity: DAS 28 score

- Swollen joints
- Tender joints
- General health
- ESR



Anti-TNF Responses

Disease activity in
Infliximab-treated patients




- 60% response
- 40% persistent disease

Persistent disease



**TNF- Independent
or
incomplete blocking of TNF**



RA Cohort patients treated with infliximab

- Infusion 3 mg/kg wk 0, 2, 6, 14 + 8 wk
- DAS 28 (EULAR response)
- Serum infliximab

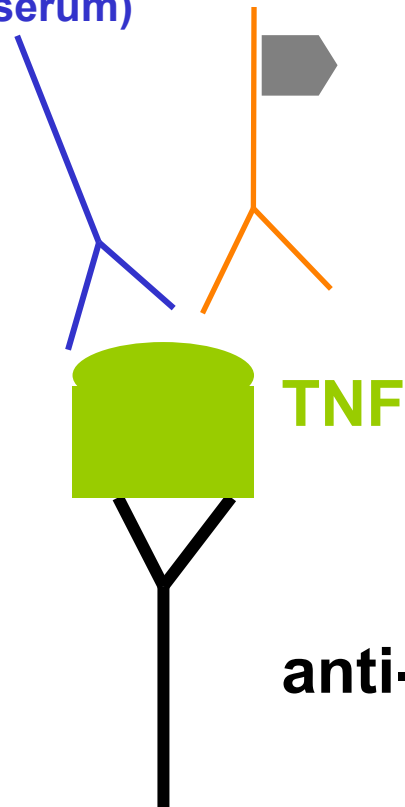
Sanquin: Functional Infliximab-ELISA

anti-idiotypic as “detecting antibody”

infiximab
(patient serum)



Streptavidine-HRP



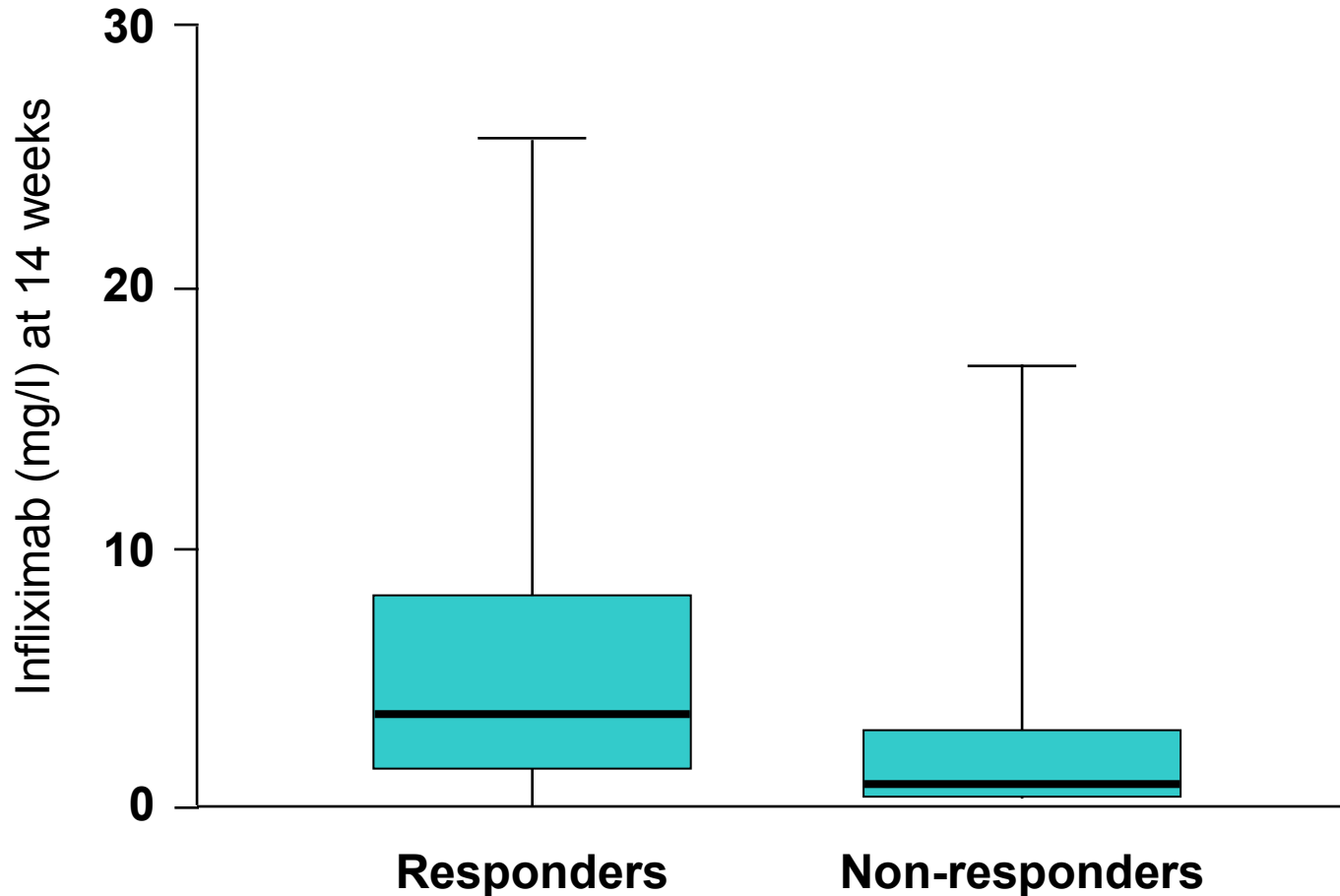
Detection with anti-idiotypic, 1 h

Incubation, patient serum, 1 h

Incubation rTNF, 1 h

coat: anti-TNF7, o/n

Infliximab trough levels and response at wk14

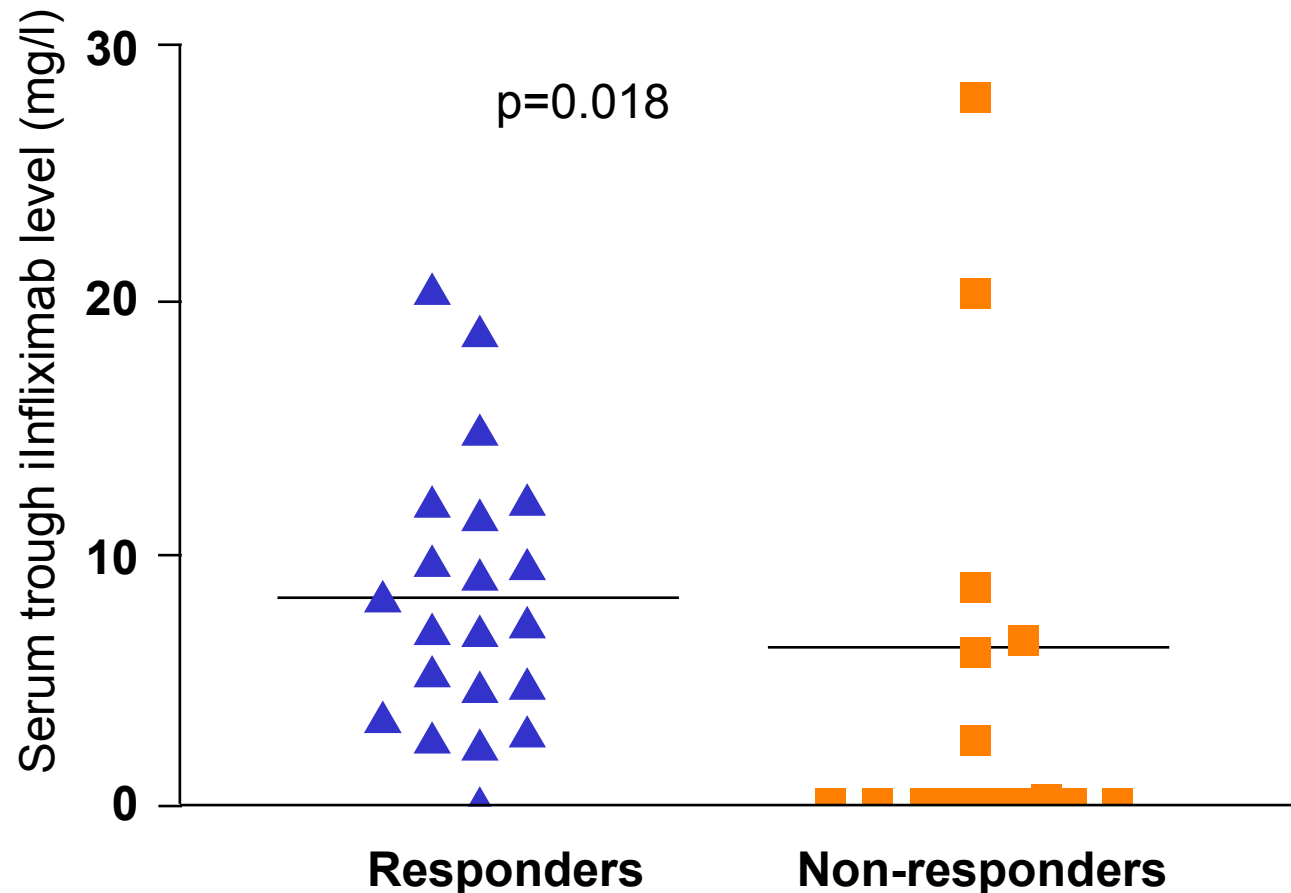


Ankylosing spondylitis

- Infliximab 5 mg/kg wk 0, 2, 6 + every 6 wks
- Disease activity: ASAS criteria
- Serum infliximab

Ankylosing spondylitis : infliximab levels and response

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



Conclusion:

- Variation in drug levels
- Related to clinical response
- Different types of non-responders ?

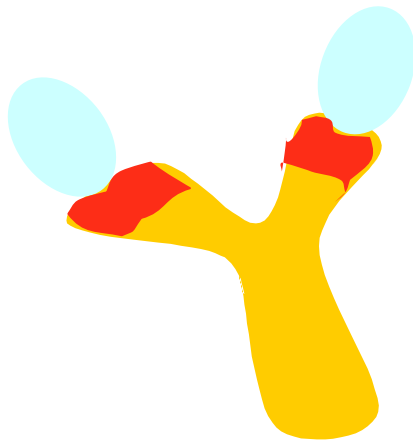
Clinical observation: Patients with an allergic reaction to infliximab have low levels

Infliximab concentration (MG/L)	2 wk	6wk	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

Immunogenicity?

Infliximab = a chimeric antibody



30% mouse

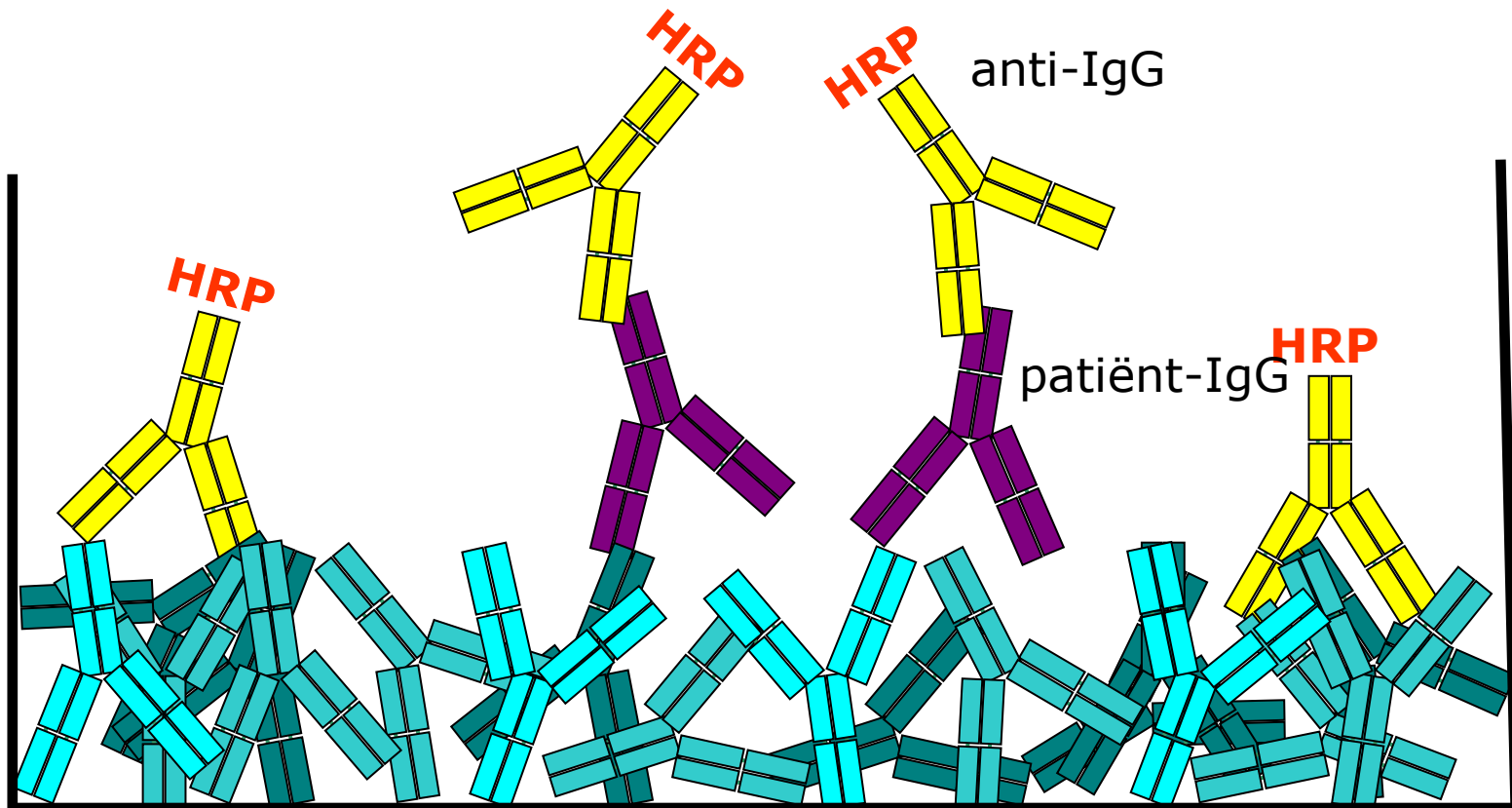
ARTHRITIS & RHEUMATISM
Vol. 50, No. 4, April 2004, pp 1051-1065
DOI 10.1002/art.20159
© 2004, American College of Rheumatology

Sustained Improvement Over Two Years in Physical Function, Structural Damage, and Signs and Symptoms Among Patients With Rheumatoid Arthritis Treated With Infliximab and Methotrexate

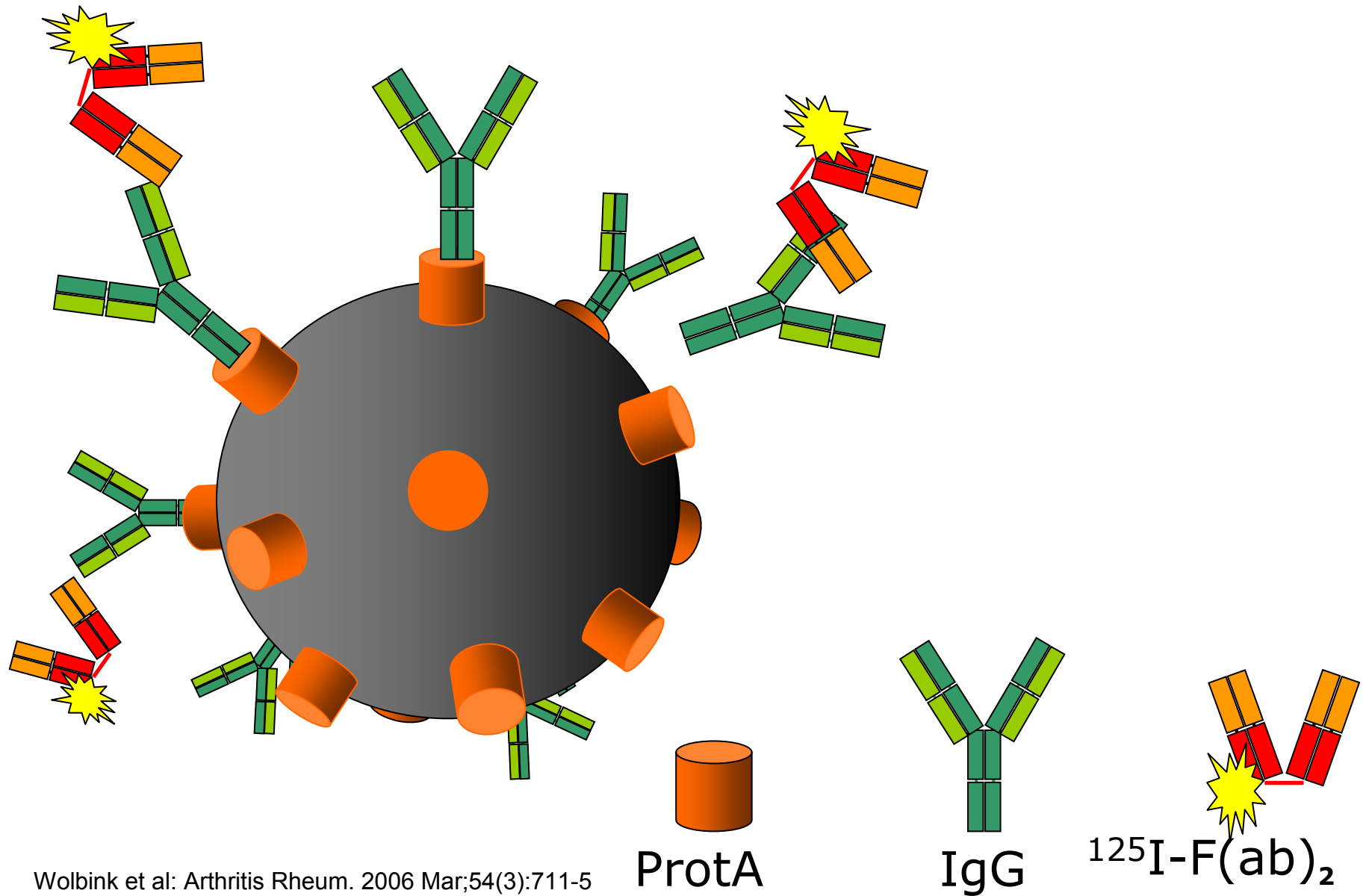
R. N. Maini,¹ F. C. Breedveld,² J. R. Kalden,³ J. S. Smolen,⁴ D. Furst,⁵ M. H. Weisman,⁶ E. W. St.Clair,⁷ G. F. Keenan,⁸ D. van der Heijde,⁹ P. A. Marsters,⁸ and P. E. Lipsky,¹⁰ for the Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group

Eight percent of patients receiving the infliximab plus MTX regimens developed antibodies to infliximab during the 102-week study period. Despite the presence of antibodies, similar proportions of patients with antibodies to infliximab (40%) and without antibodies to infliximab (38%) achieved an ACR20 response.

anti-antibody ELISA



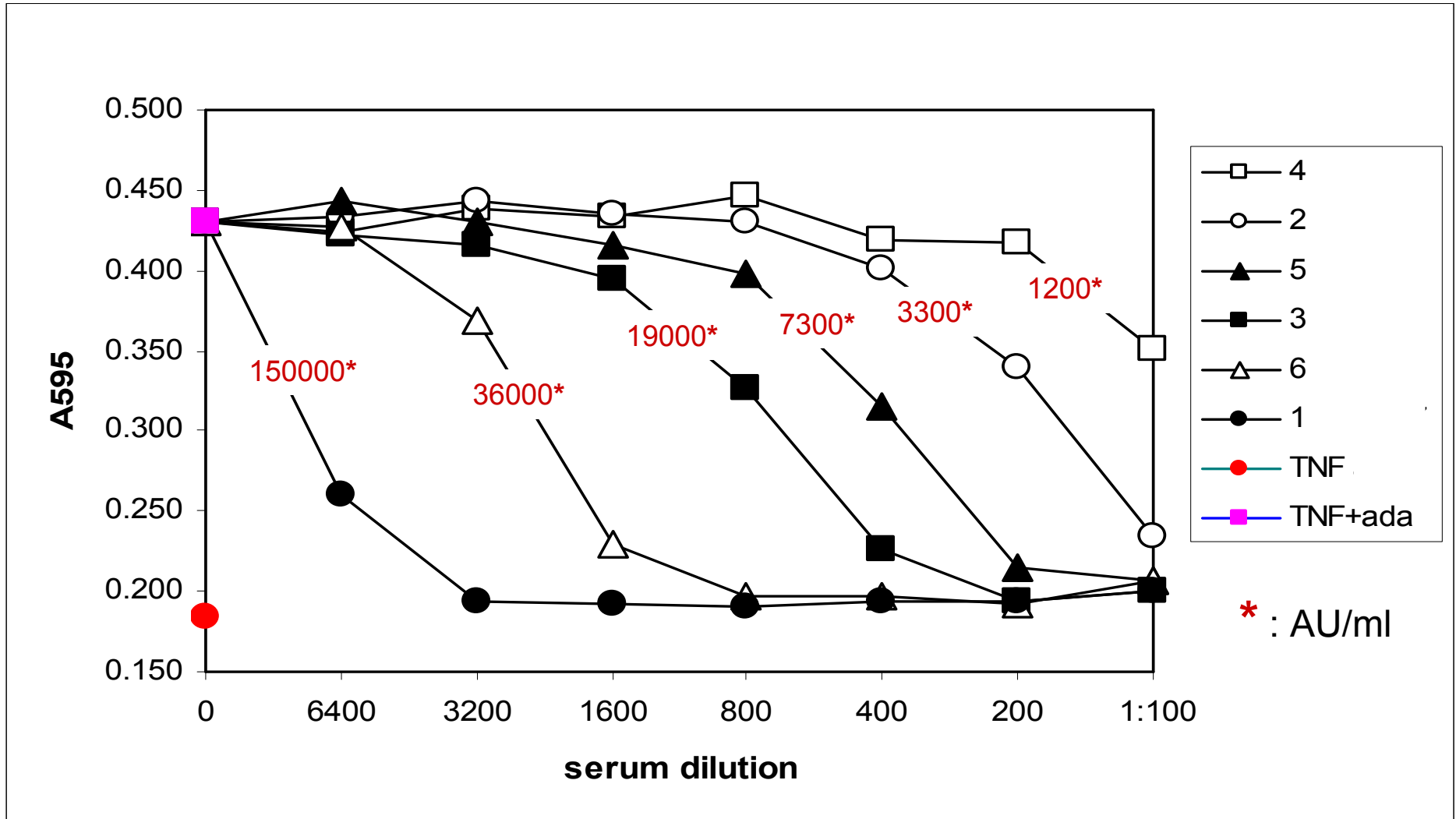
Antigen binding test for monoclonal therapy



Wolbink et al: Arthritis Rheum. 2006 Mar;54(3):711-5

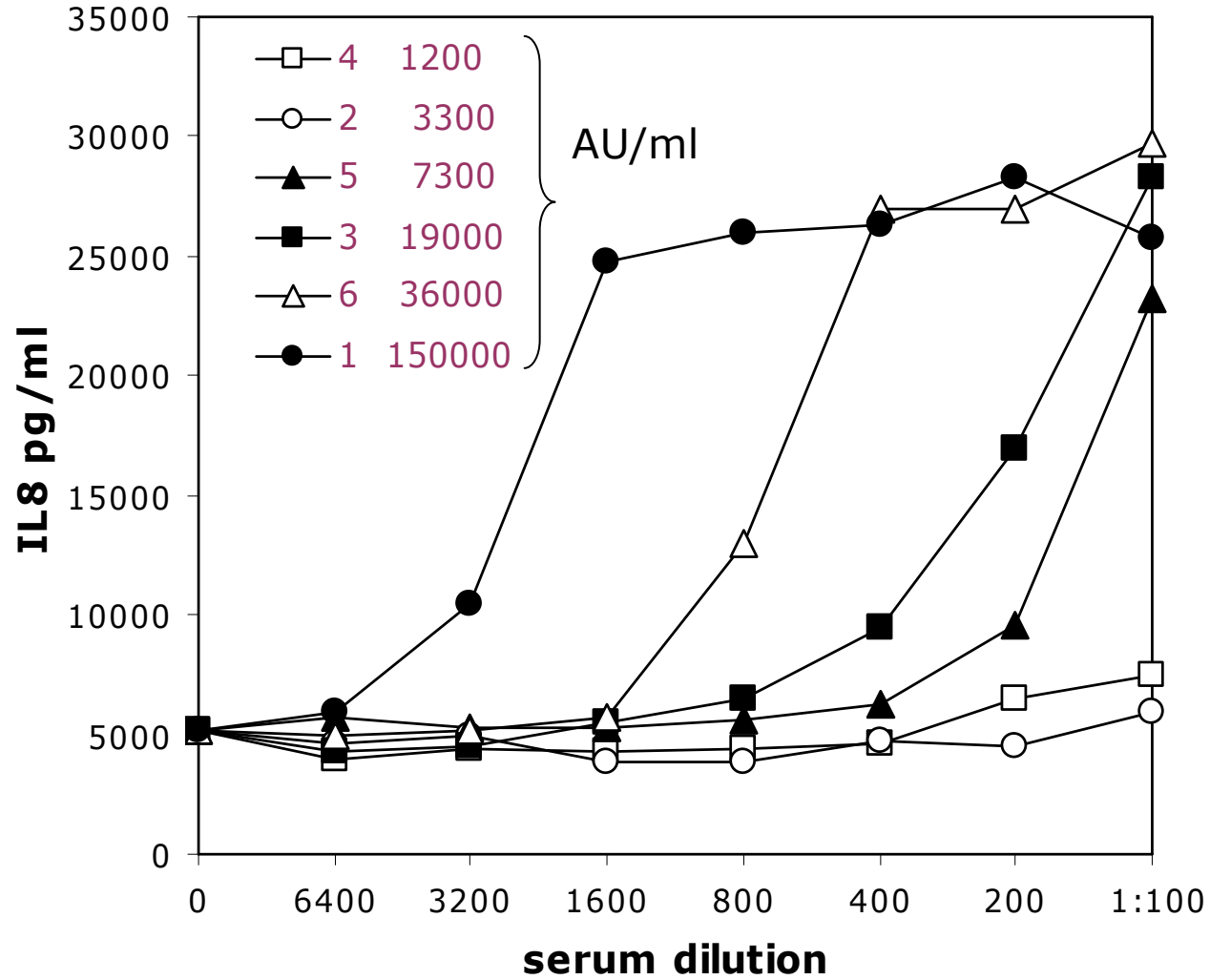
Inhibition of TNF neutralization (adalimumab) by RA patient serum

WeHI -TNF mediated killing

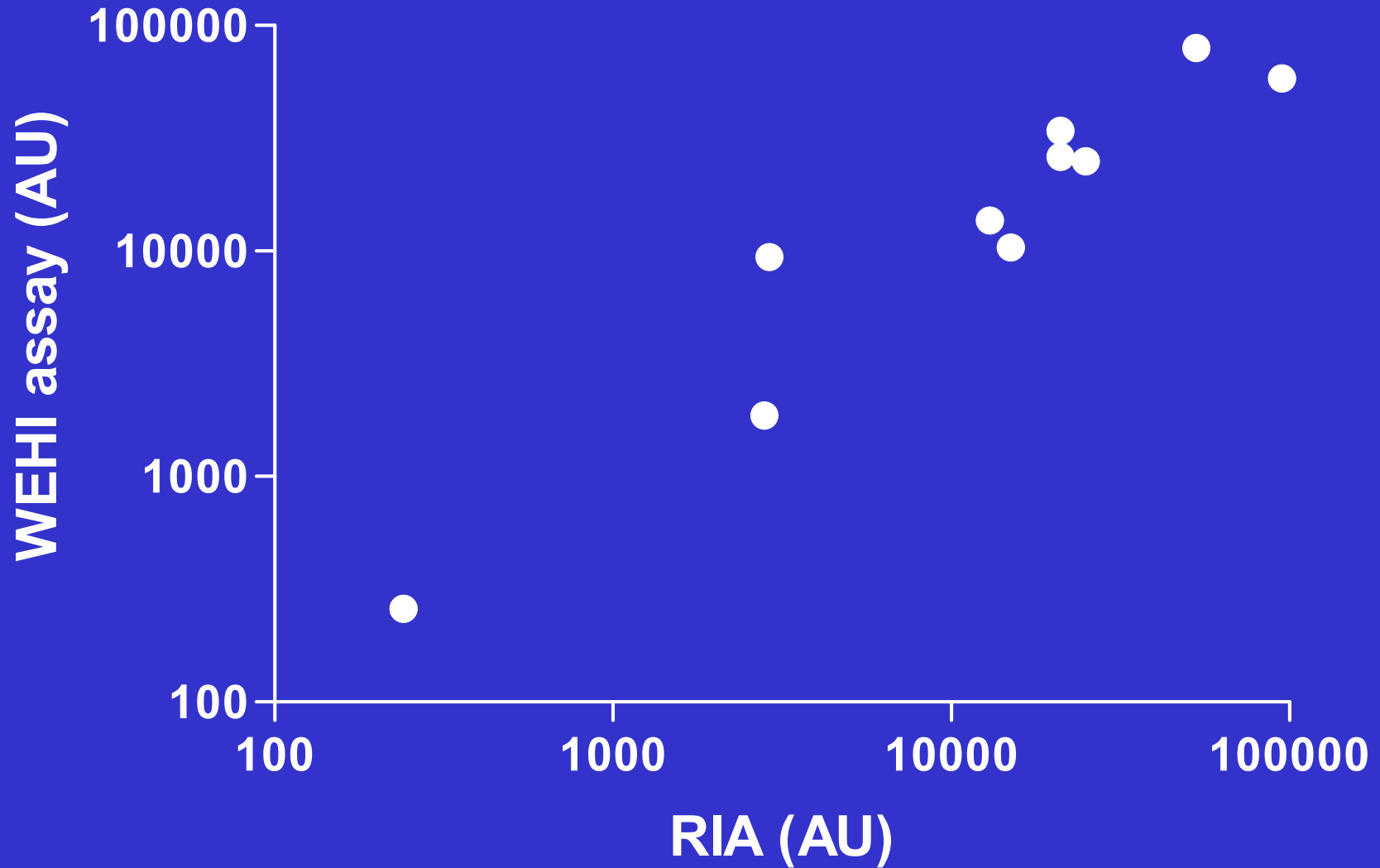


ECRF test:

IL-8 production and anti-adalimumab serum samples



correlation WEHI assay – infliximab ABT



antibody/antigen-complexes:

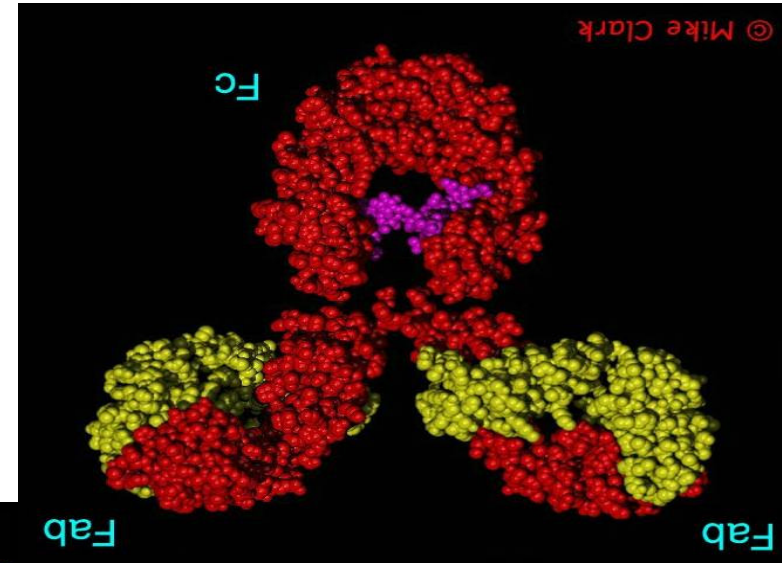
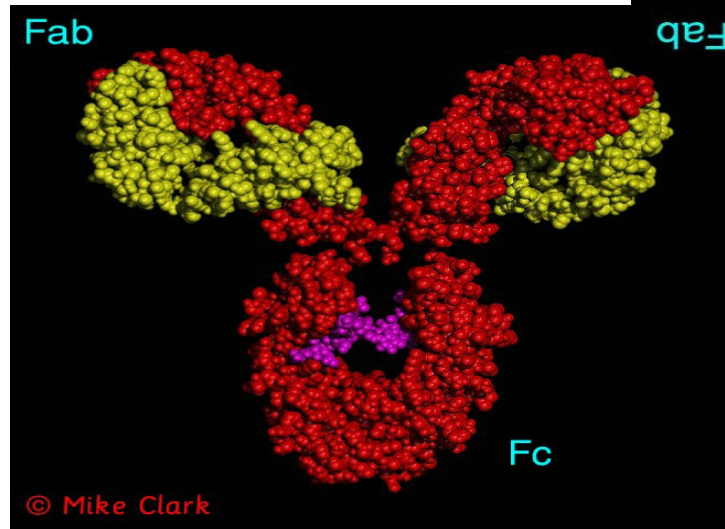
rapid clearance of therapeutic antibody

IgG1 half life in blood: \pm 3 weeks

Half life immune complex: \pm minutes!??

Antibodies against Antibodies

Infliximab

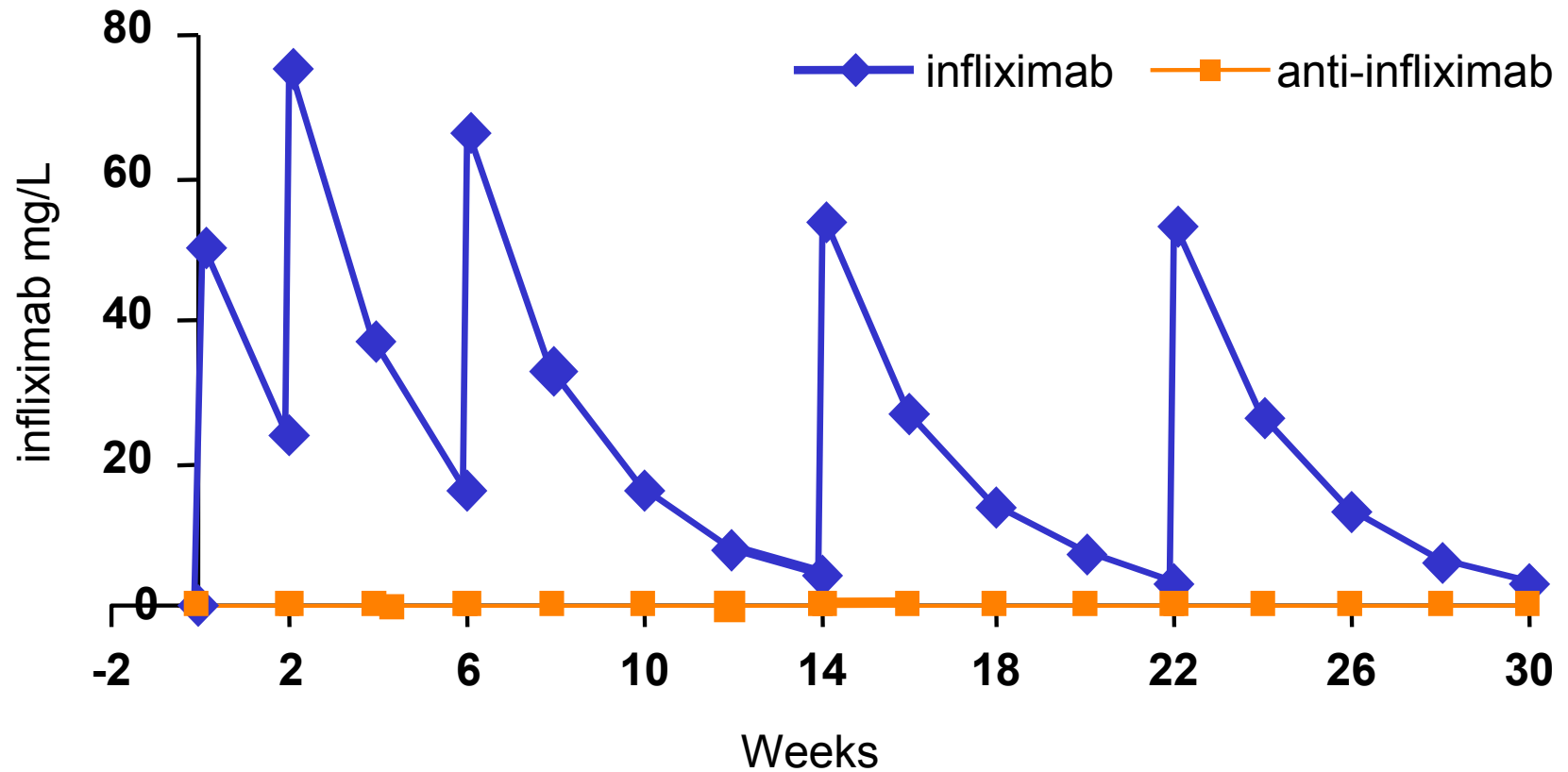


anti-infliximab

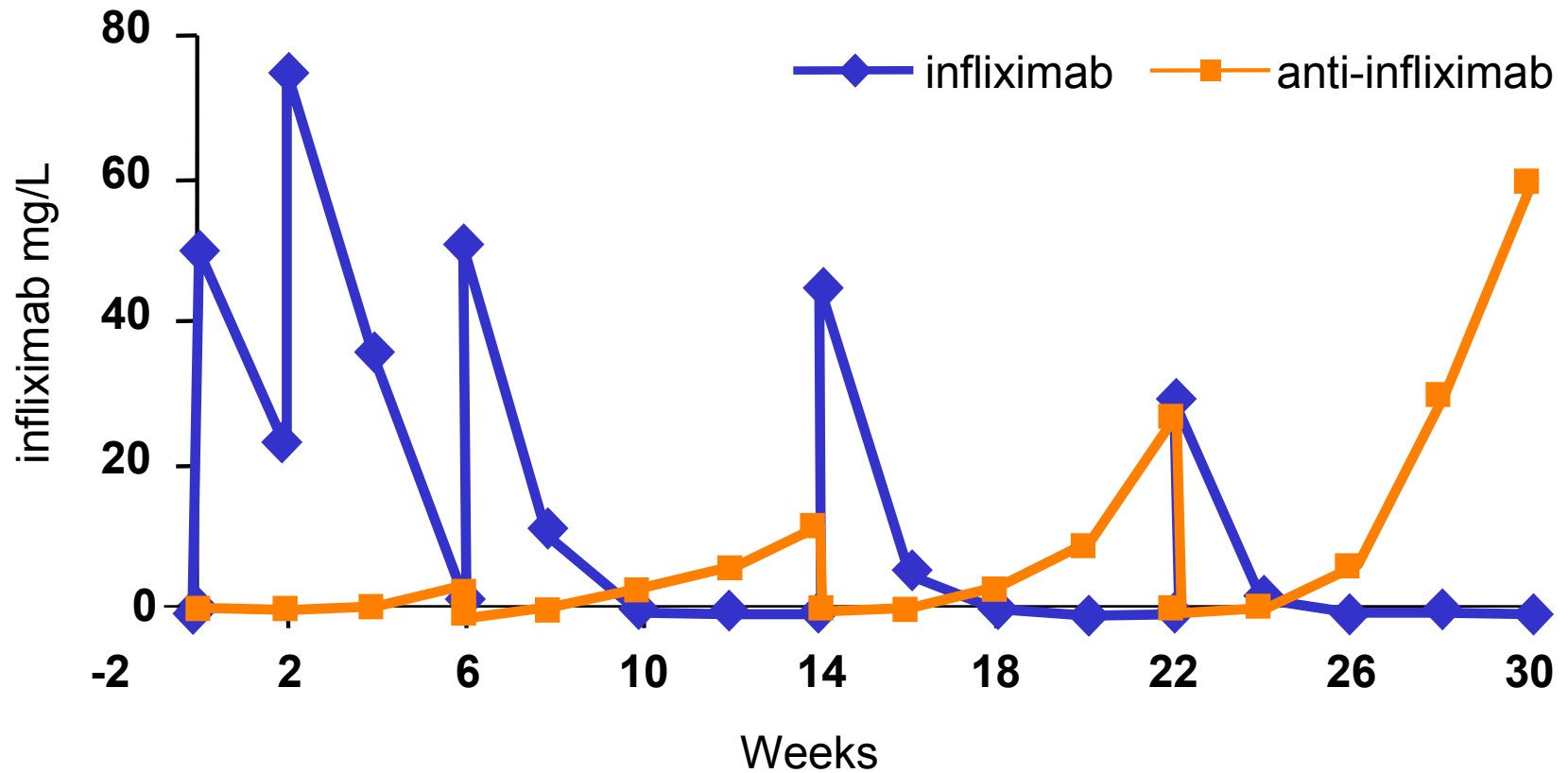
Infliximab=anti-anti-infliximab

anti-Infliximab=anti-anti-anti-infliximab

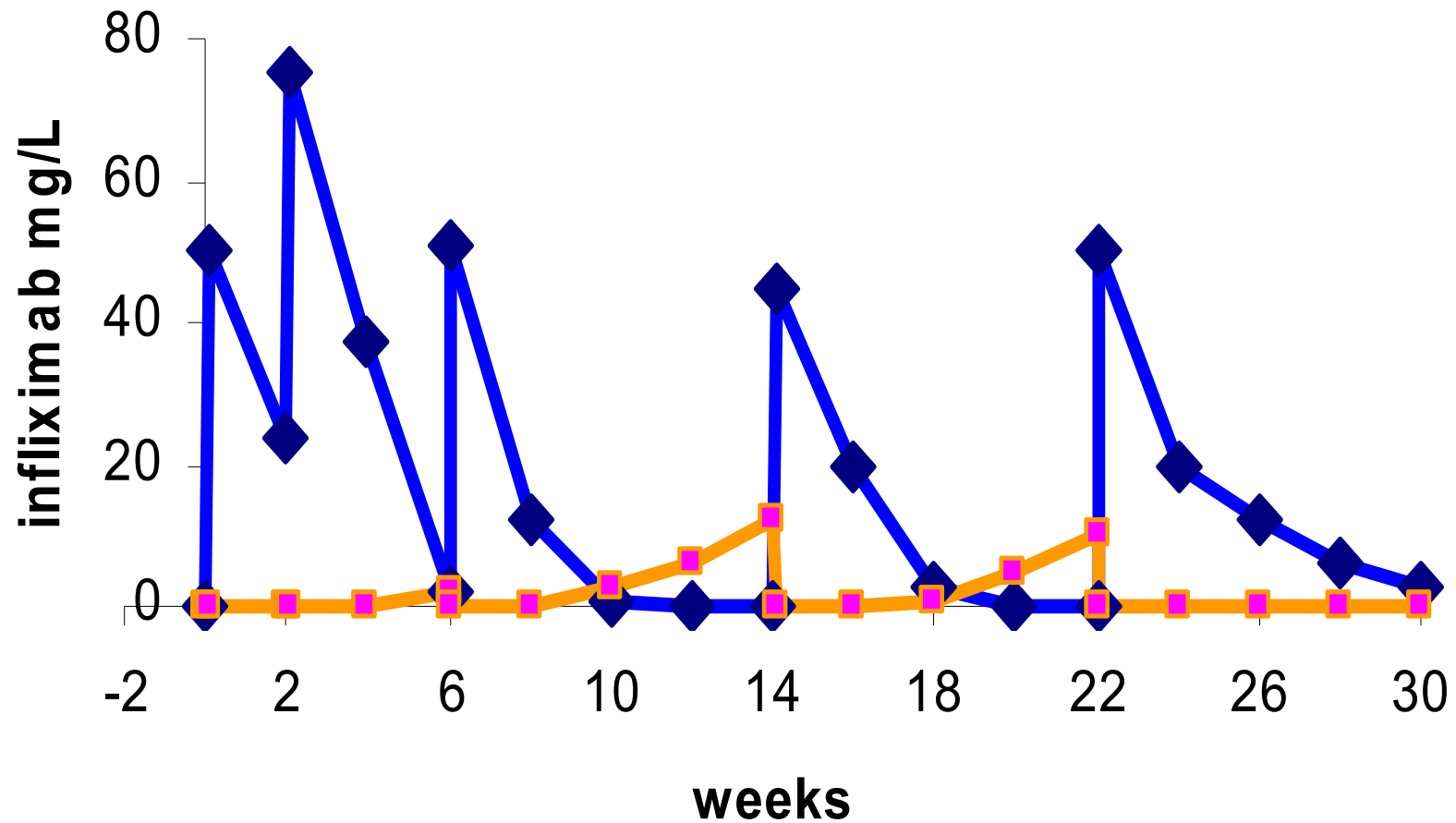
PK model no anti-infliximab detected



PK model anti-infliximab detected

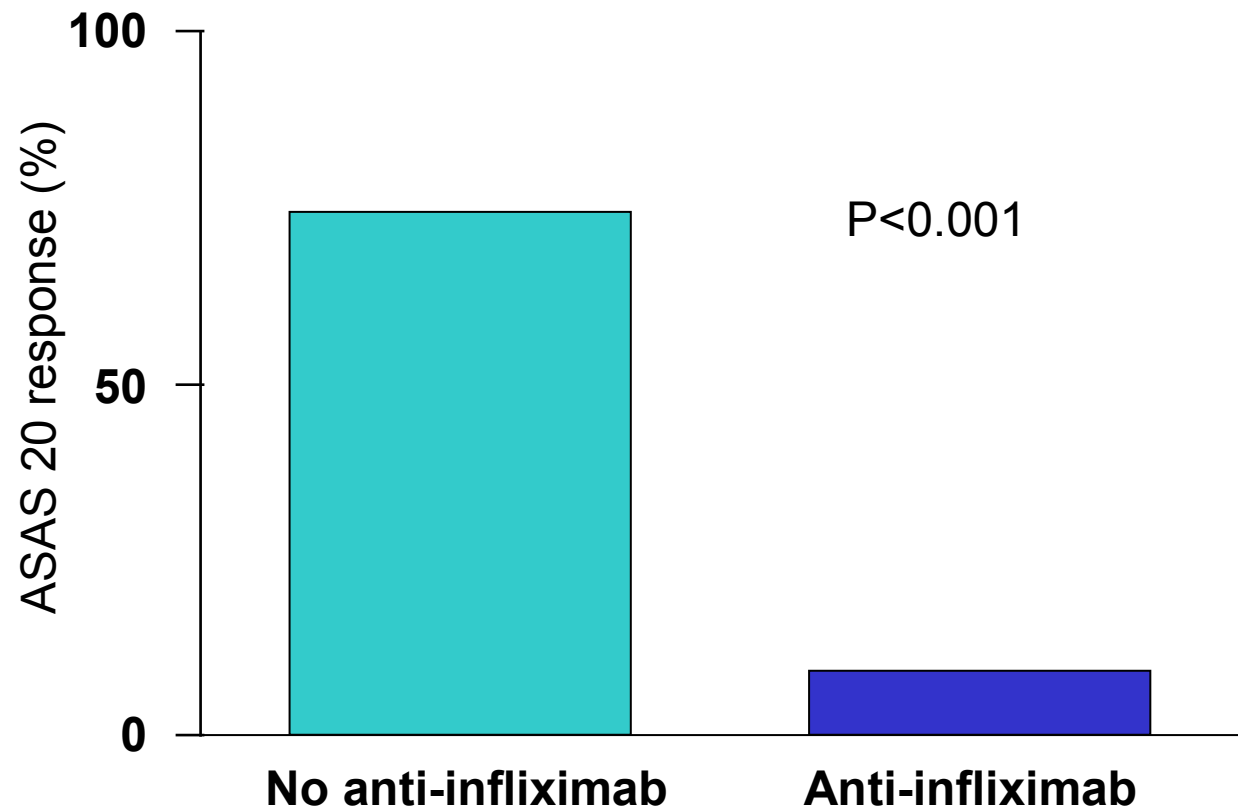


PK model anti-infliximab detected transient antibody formation



Ankylosing spondylitis

Percentage of patients (n = 38) with (9%) and without (74%) ATI fulfilling ASAS-20 response criteria at week 54



Conclusions infliximab

Anti-infliximab clinical significance

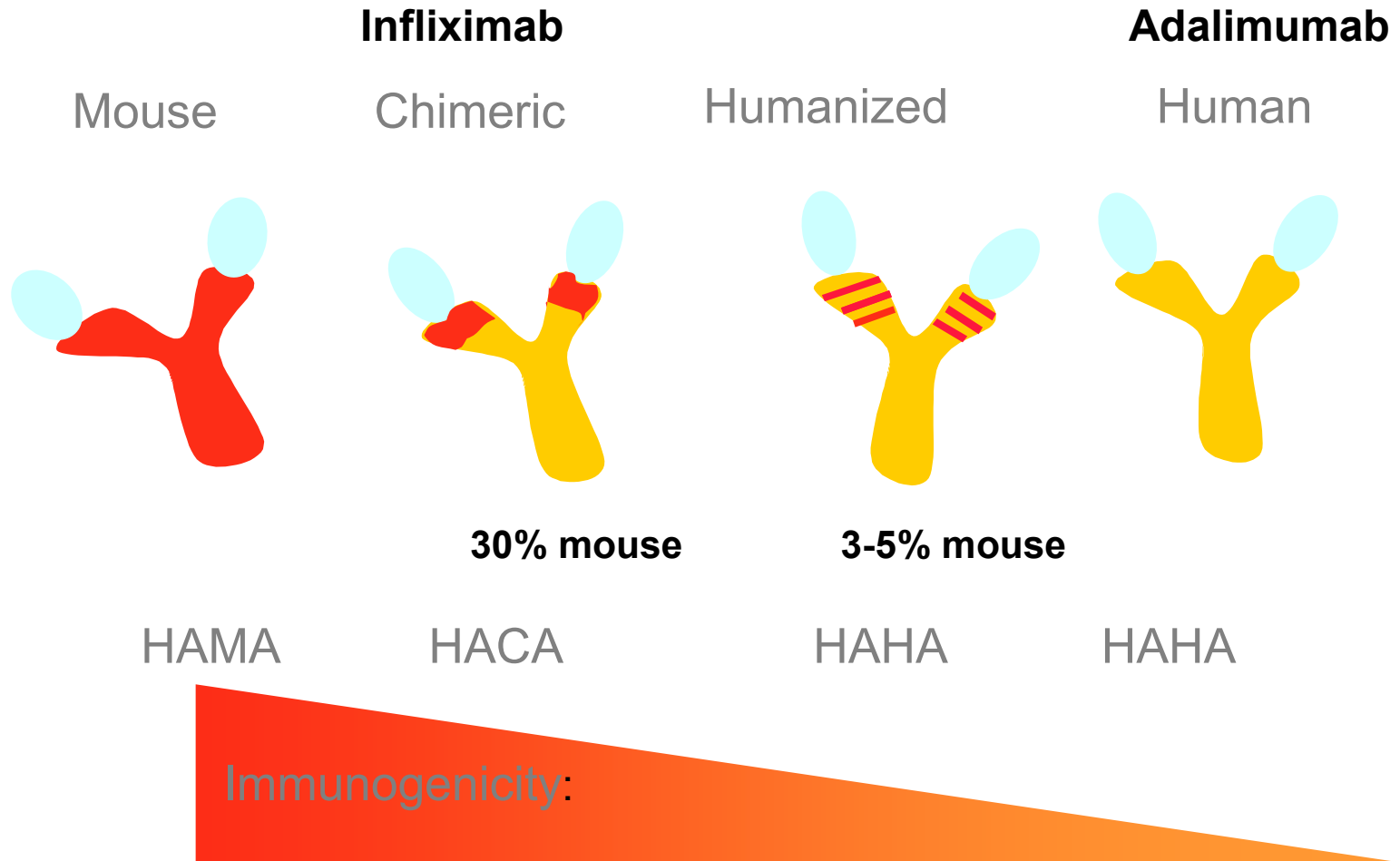
- Enhanced clearance of infliximab
- Reduced response to treatment

- Different types of non-responders
- Associated with allergic reactions
- Transient and persistent

Important for measuring anti-infliximab

- Type of assay
- Timing sample collection in relation PK

Immunogenicity therapeutic antibodies



Adalimumab study

Ann Rheum Dis 2007

Adalimumab

Human anti-TNF

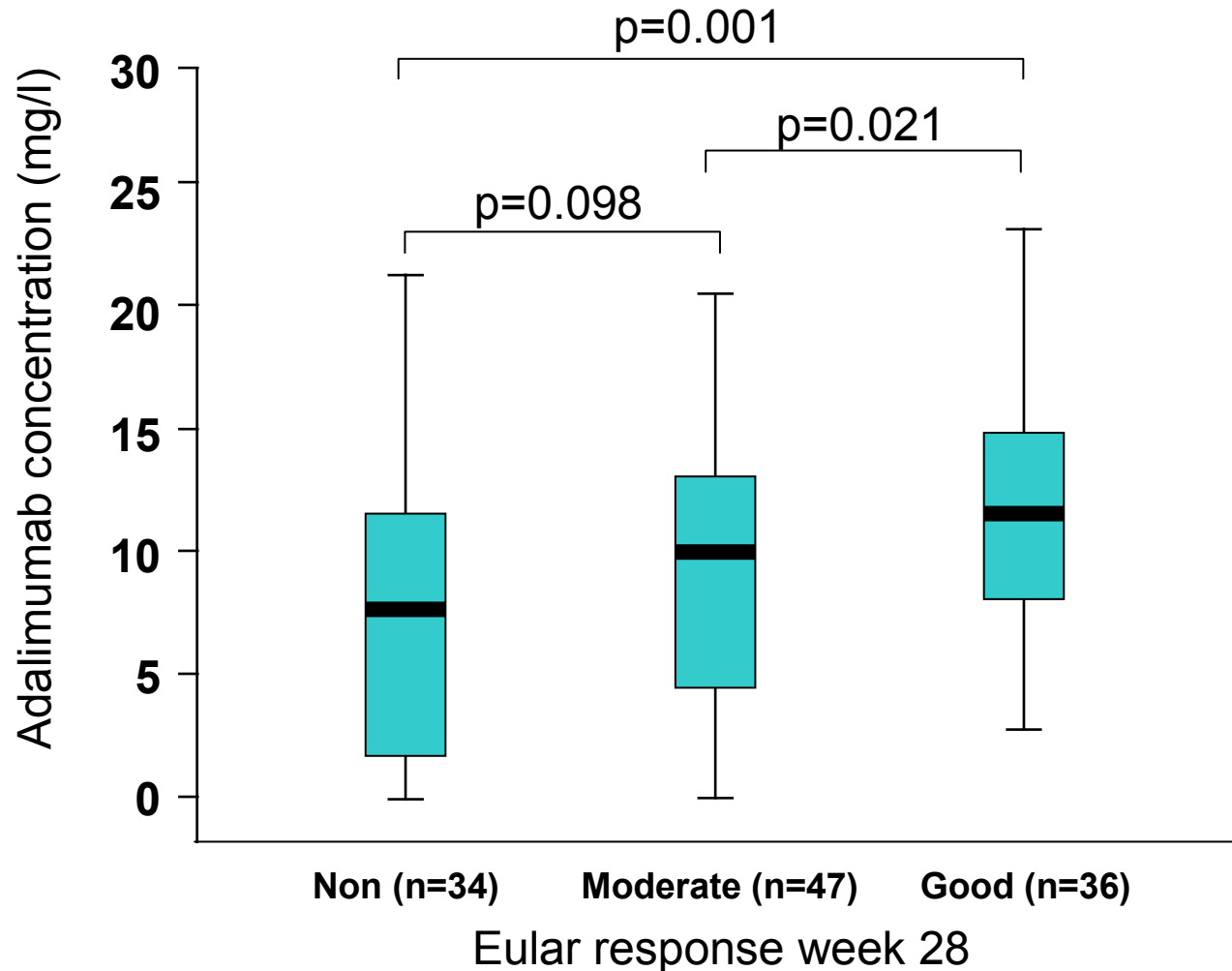


121 RA patients treated with adalimumab S.C 40mg/2wk
clinical and serological follow-up to 28 weeks

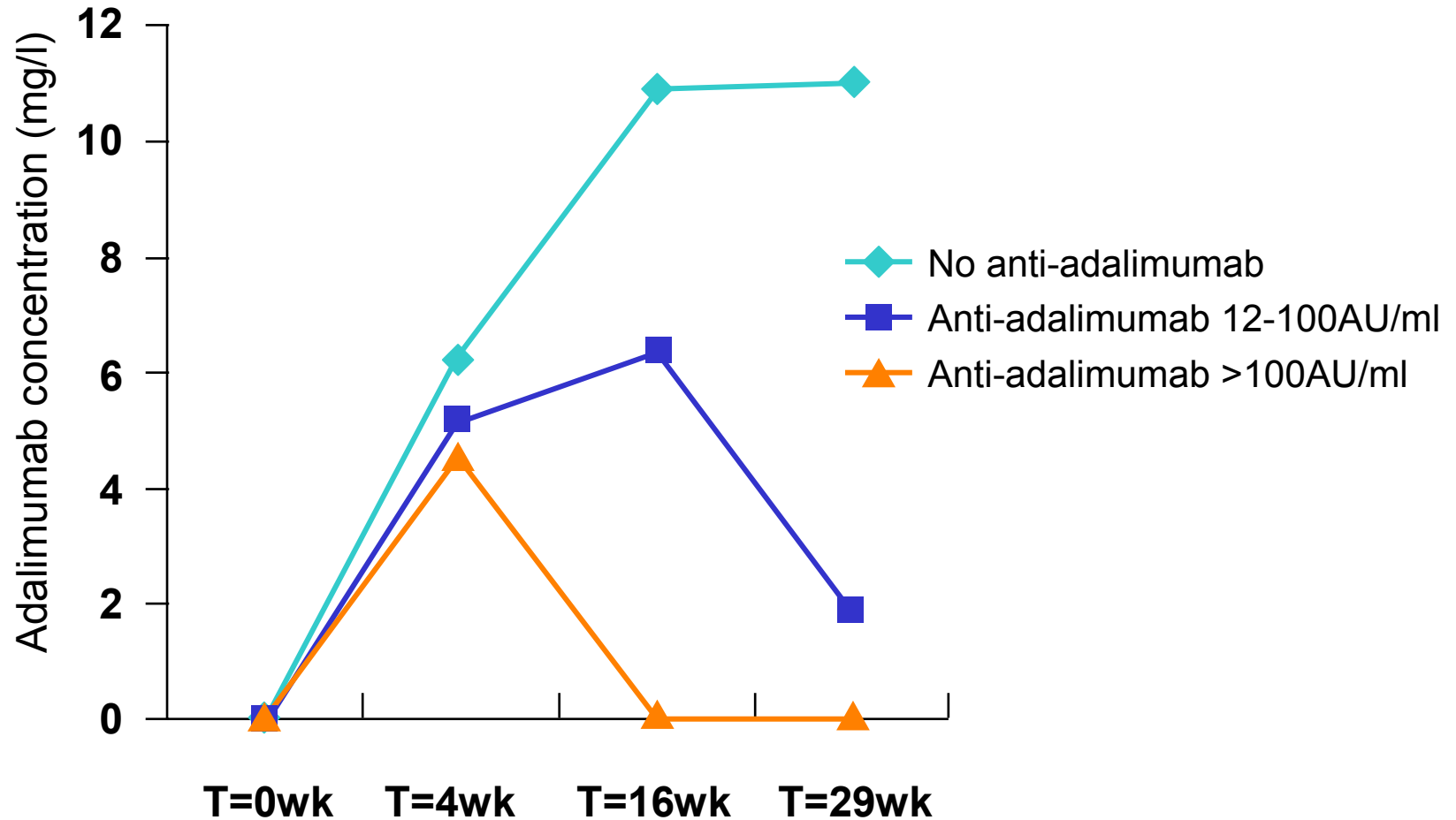
Basic clinical characteristics

	All 121	HAHA+ N=21	HAHA- N=100
MTX use	79%	52%	84%
RF+	77%	76%	77%
Crp	24	34	22
DAS28	5.3	5.5	5.3

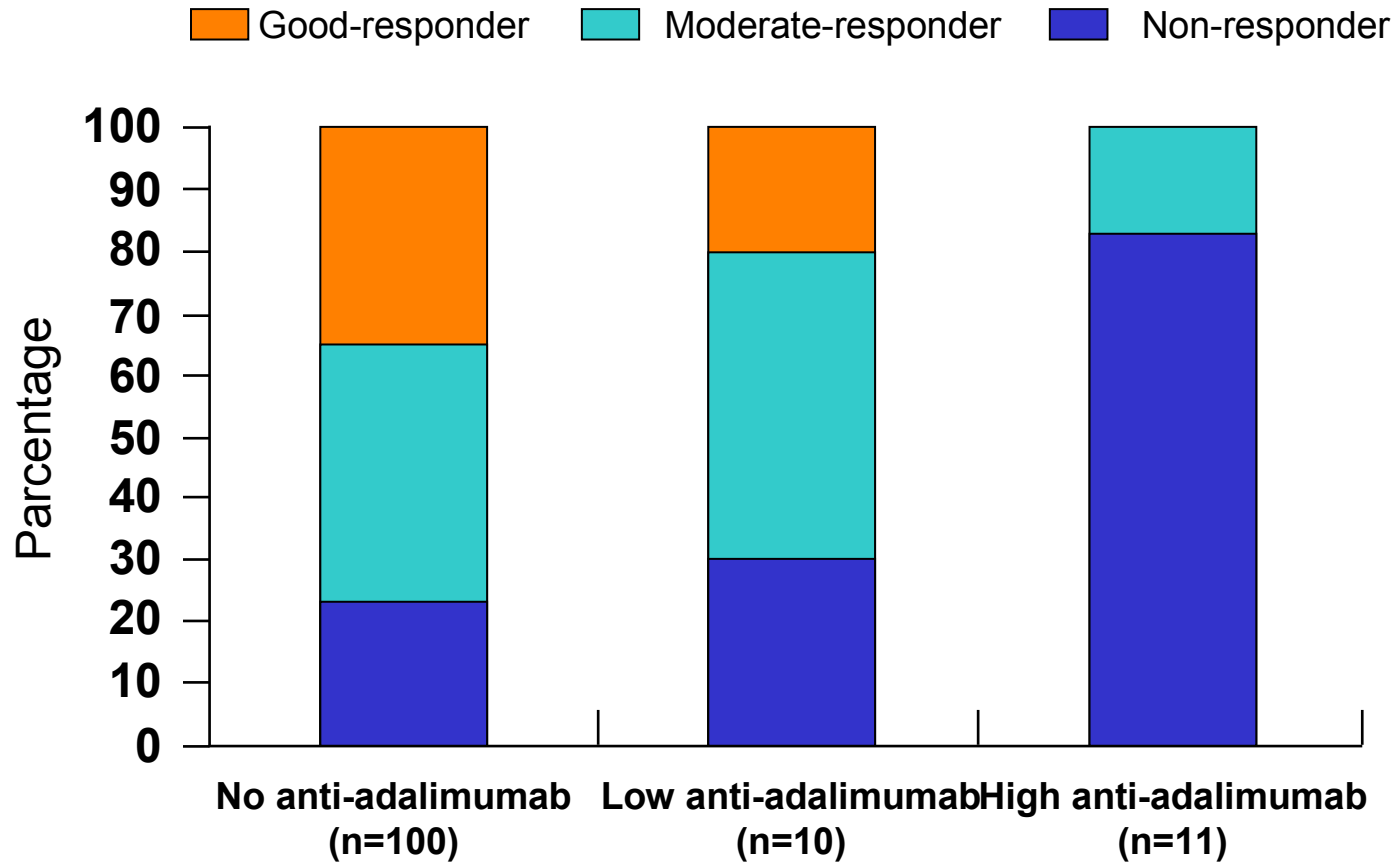
Serum adalimumab concentrations and response



Relationship between anti-adalimumab and drug levels



Relationship between EULAR response and presence or absence of anti-adalimumab antibodies



Factora influencing immunogenicity


- Dosis
- MTX
- Genetics
 - IL-10 polymorfisms
 - Allotypes

IL-10 promotor polymorphisms and anti-adalimumab

Table 1. *IL10* promoter polymorphisms and anti-adalimumab antibodies in the 192 rheumatoid arthritis study patients*

SNP, genotype	Anti-adalimumab antibodies		OR (95% CI)	<i>P</i>
	No. (%) negative (n = 167)	No. (%) positive (n = 25)		
-2849 AA or GA	78 (47)	17 (68)	0.41 (0.17–1.01)	0.05
GG	89 (53)	8 (32)		
-1082 GG or GA	121 (73)	25 (100)	0.05 (0.003–0.86)	0.001
AA	46 (28)	0 (0)		
-819 TT or TC	77 (46)	4 (16)	4.49 (1.48–13.65)	0.004
CC	90 (54)	21 (84)		

* SNP = single-nucleotide polymorphism; OR = odds ratio; 95% CI = 95% confidence interval.



29 patients with Psoriasis treated with
adalimumab (monotherapy),
serum adalimumab concentrations
and anti-adalimumab antibodies


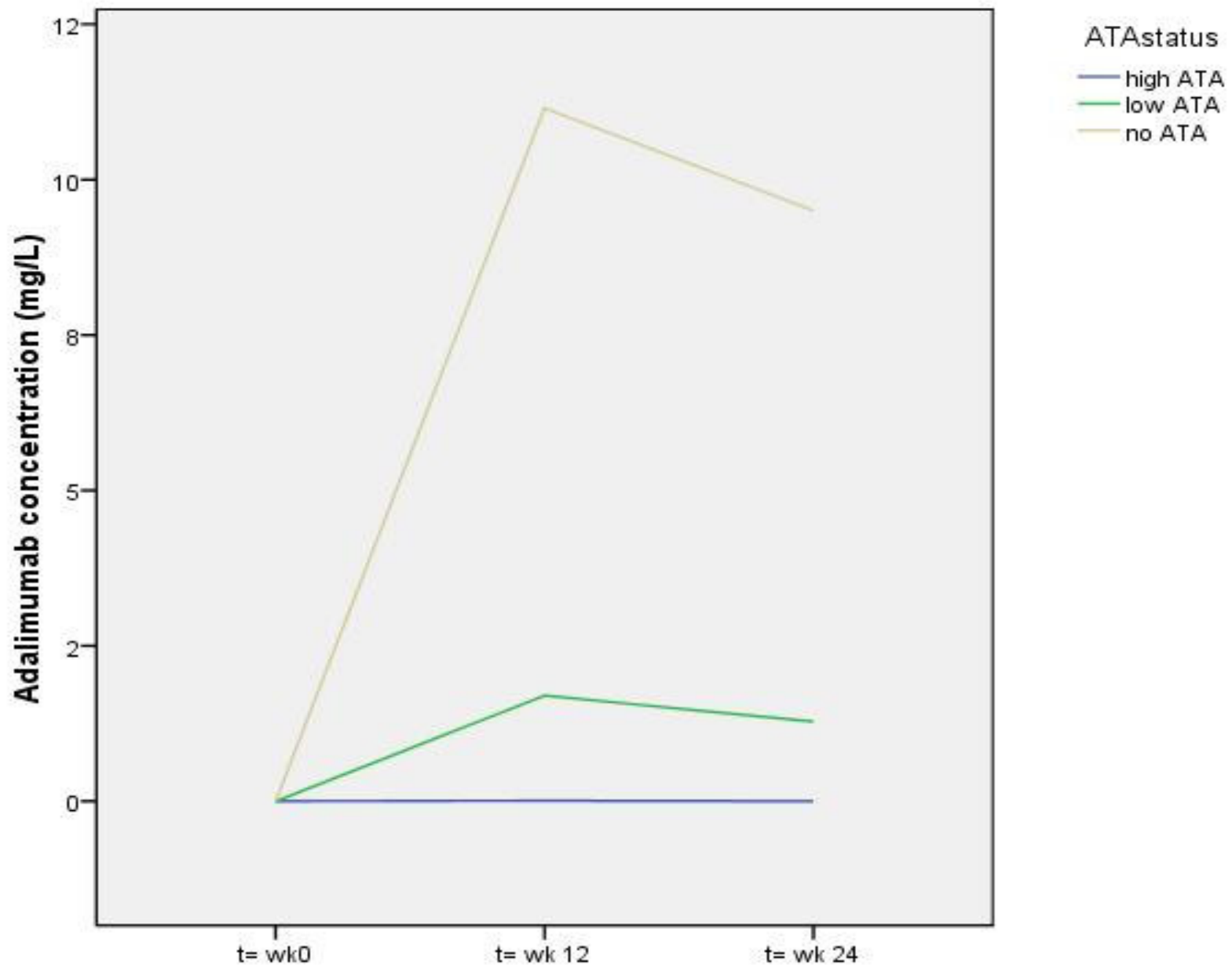


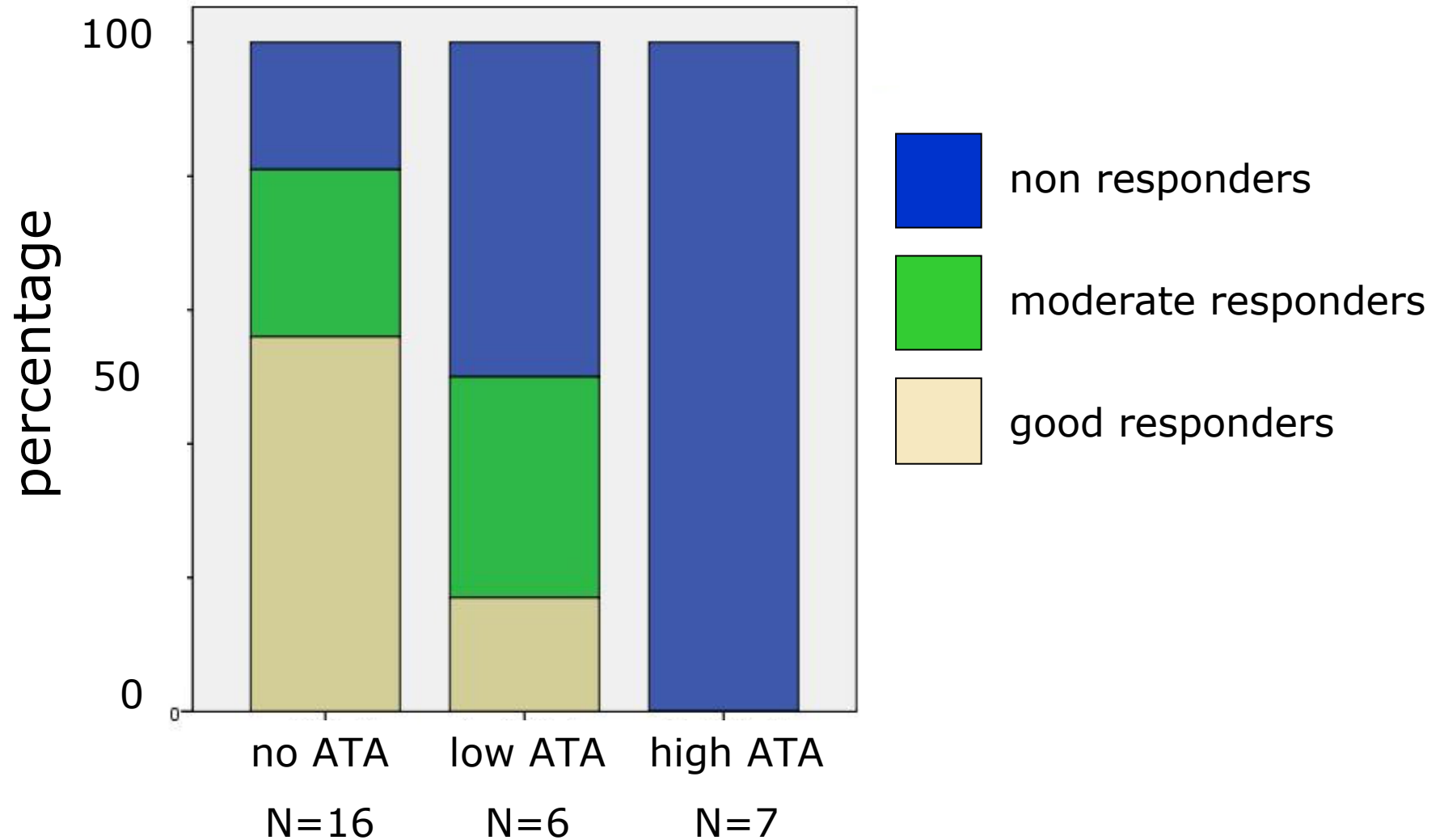
Table 1 Baseline and clinical characteristics of patients at baseline

	Total population (n = 29)	Patients with Anti-ADA (n = 13)	Patients without Ant-ADA (n = 16)
Age mean in yrs	44 ± 11	47 ± 13	42 ± 10
Male n (%)	20 (69)	8 (62)	12 (75)
PASI at baseline mean	15.5	16.2	14.9
Diagnosed with PsA n (%)	5 (17)	3 (23)	2 (12)
Concomitant methotrexate n (%)	3 (11)	0 (0)	3 (20)
Previous methotrexate %	97	92	100
Previous cyclosporine %	66	70	63



antibodies to adalimumab in Psoriasis patients

Lecluse (thesis, 2009/ archives dermatology in press)



Anti-adalimumab associated with treatment failure

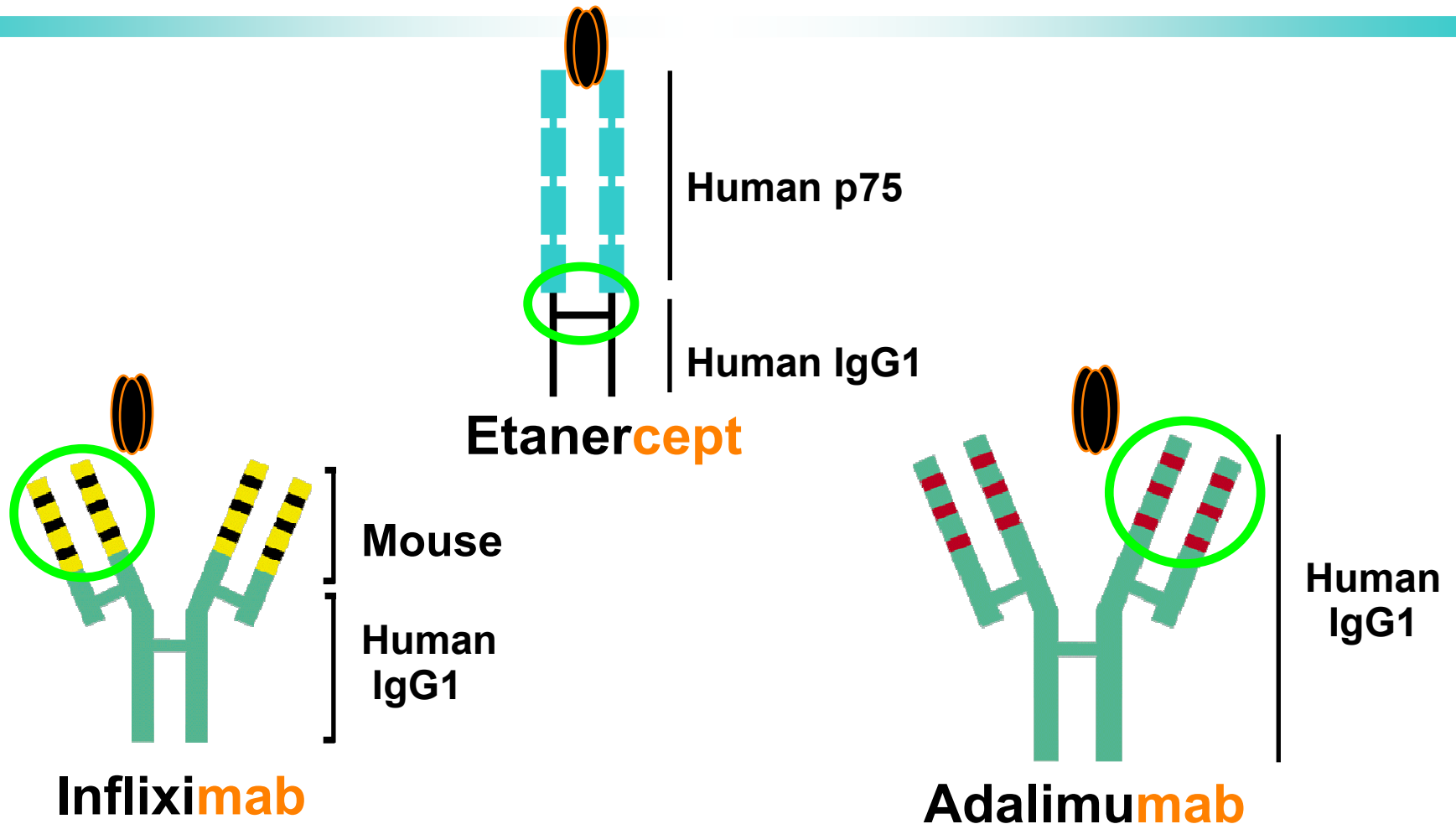
Recent conformation in other cohorts

- Crohn's (rutgeerts)
- Ankylosing spondylitis
- Psoriatic Arthritis

Etanercept in Ankylosing Spondylitis

- Etanercept 50 1X per week S.C.
- ASAS score responders/non-responders
- Serum
- Assays Sanquin

Molecular basis for differences Immunogenicity not/less relevant for etanercept ??



Response to Etanercept in RA

Naive vs Switchers

- First time anti TNF n=161 (anti-tnf naive)
- Switchers N=89
(From adalimumab or infliximab)
- 47(53%) switchers positive for anti-infliximab or anti-adalimumab
- Outcome: Response at 28 weeks

Naive versus Switchers to etanercept

unpublished Anna Jamnitski


	Delta DAS28
anti-TNF- α naïve patients (n=161)	2,1 (SD1,3)
Switchers without immunogenicity (n=42, 47%)	1,2 (SD1.3) **
Switchers with immunogenicity (n=47, 53%)	2,0 (SD1.3)

** p=0.017

Persistent disease



**TNF- Independent
or
incomplete blocking of TNF**



RA adalimumab – switchers

Bartelds ARD 2009

227 consecutive RA patients

Mean DAS28:5.2

Prospective cohort study

Serum and DAS28 at 28 weeks

-
- 175 patients anti-TNF naïve
 - 51 patients switched from IFX to ADA

Results:

- Anti-adalimumab in 46 pts (19%).
- Improvement DAS28:
 - 1.8 for patients without anti-adalimumab
 - 0.6 for patients with anti-adalimumab
($p < 0.001$)

Switchers

- 51 after treatment with infliximab
- 33/51 (67%) positive for anti-infliximab

Naive versus Switchers

	anti-ADA formation	Delta DAS28	Delta DAS28 anti-ADA-
anti-TNF-α naïve patients (n=175)	28 (16%)	1.8 (SD1.4)	2.0 (SD1.4)
prior IFX patients without anti-IFX (n=18)	2 (11%)	0.9 (SD1.5)	0.9 (SD1.4)
prior IFX patients with anti-IFX (n=33)	11 (33%)	1.2 (SD1.3)	1.6 (SD1.1)

IFX = infliximab

prior IFX patients = patients who were treated with infliximab before adalimumab treatment

Anti-IFX = anti-infliximab antibodies

Anti-ADA = anti-adalimumab antibodies

Delta DAS2* = Delta DAS28 with patients with anti-adalimumab excluded from analysis.

Conclusions

- Immunogenicity affects PK
- Is related to non-response
- Is related to side effects
- Differentiates non-responders
- Is associated with immunogenicity against other Moabs

www.biologicals.sanquin.nl

- Marijn Vis
- Magreet
- Anna Jaminski
- Pauline
- Diana
- Margret Bartelds
- Carla Wijbrandts
- Mirjam de Vries
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- Ben A C Dijkmans
- Paul Peter Tak
- Lucien Aarden
- Els de Groot
- Gertjan Wolbink



Future

- Standardization of assays
- Availability of assays
- Therapeutic drug monitoring part of treatment algorithms
- EIP - Clinical Group