



## Antibody response against adalimumab upon long term treatment appears to be IgG4 related

Confidential

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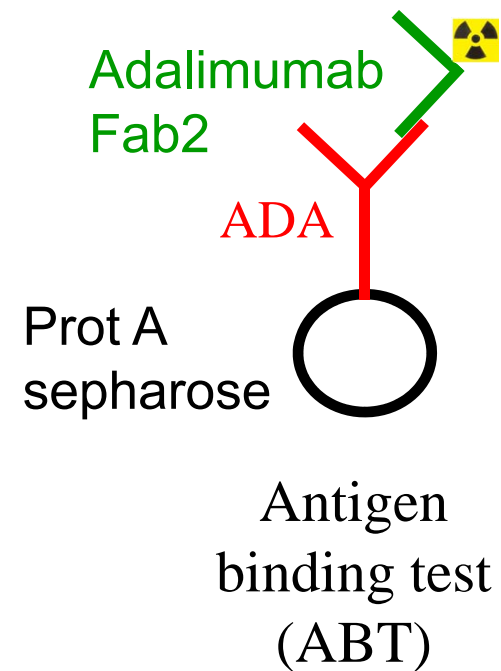
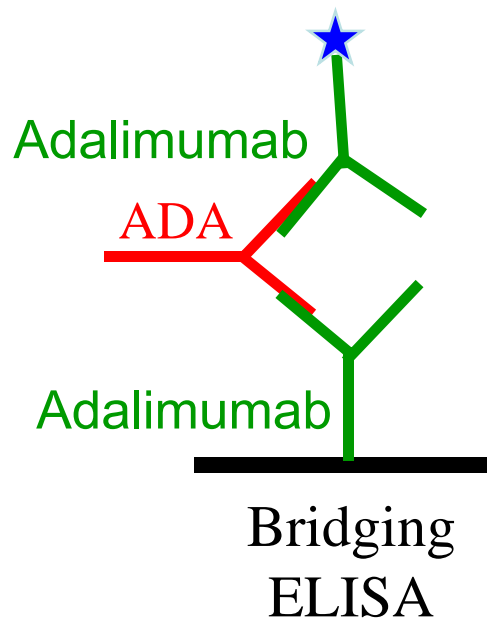


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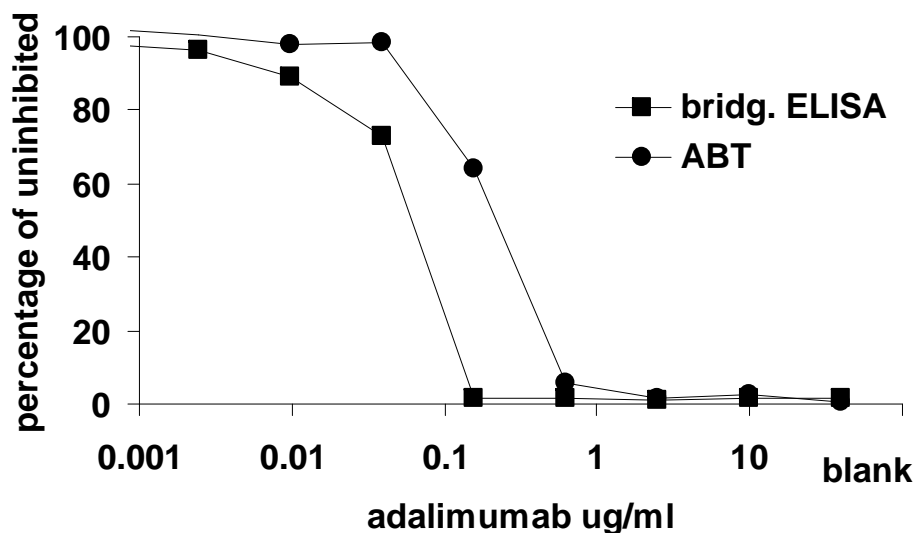
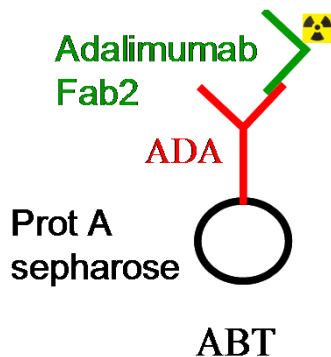
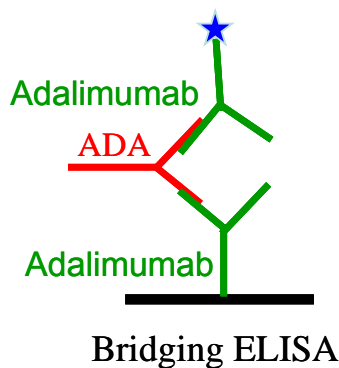
## Adalimumab

- Therapeutic antibody directed against TNF $\alpha$
- Fully human IgG1
- Used for treatment of autoimmune diseases such as rheumatoid arthritis
- One of the causes of non-response is the formation of Anti Drug Antibodies (ADA)
- In three year follow-up 28% of the patients have free ADA, which are linked to low adalimumab levels and clinical non-response Bartelds et al, JAMA, 2010

# Different assays for the measurement of ADA



# Drug interference in ADA measurements



Both the ABT and bridging ELISA are sensitive for drug interference

Only free ADA can be measured in patients sera



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## Overcoming drug interference

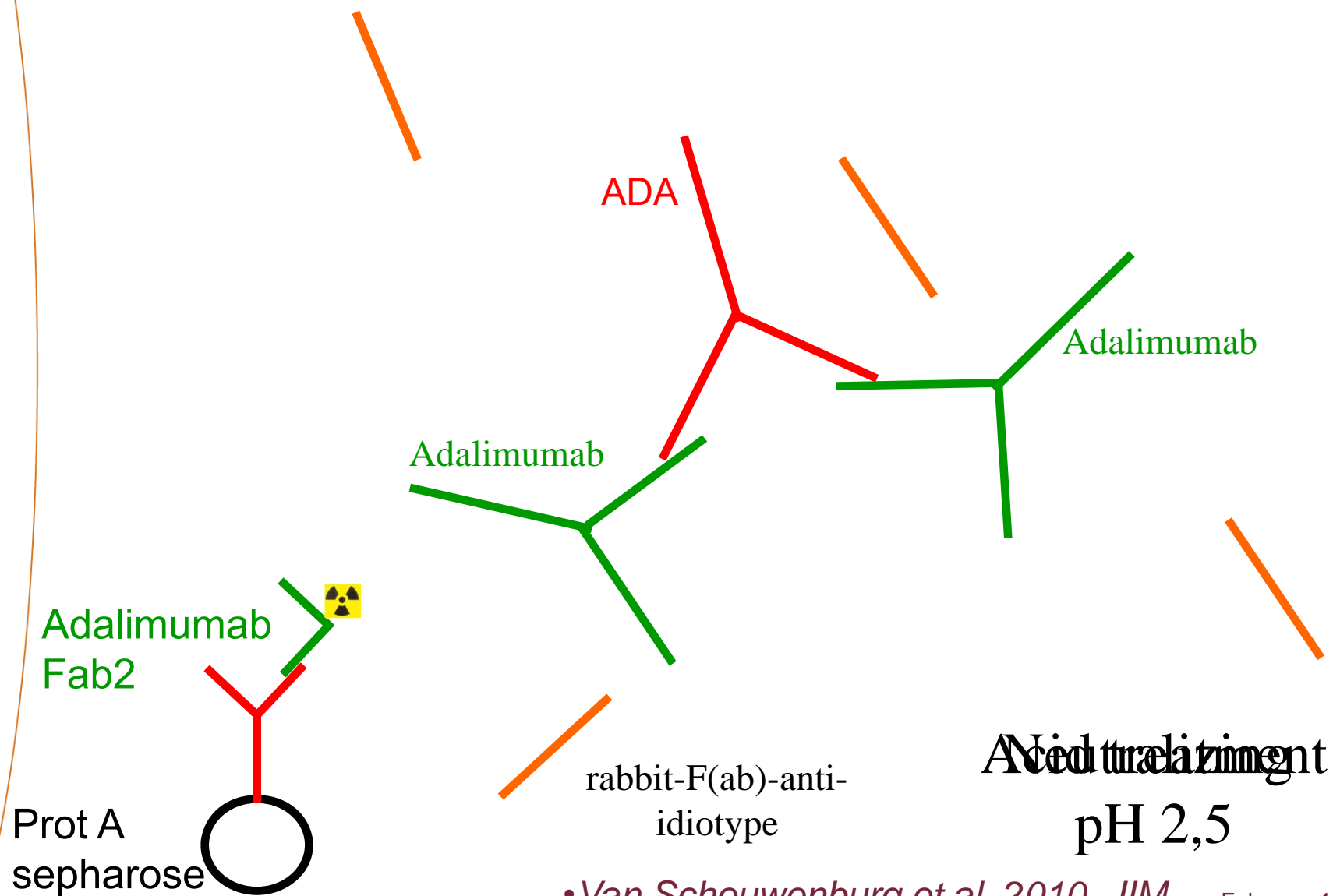
- Drug interference leads to false negative results in the bridging ELISA and ABT
- Most probably this is caused by the formation of drug-ADA complexes in vivo



## Overcoming drug interference

- An assay less sensitive for drug interference allows for
  - A better insight in the frequency and timing of ADA production
  - Studying immune complexes
- Most assays described are based on acid dissociation of complexes (*Bourdage et al., 2007; Lofgren et al., 2006; Lofgren et al., 2007; Patton et al., 2005; Schmidt et al., 2009; Sickert et al., 2008*)
- We set up an assay based on acid dissociation and blocking of complex reformation using anti-idiotypic F(ab) fragments

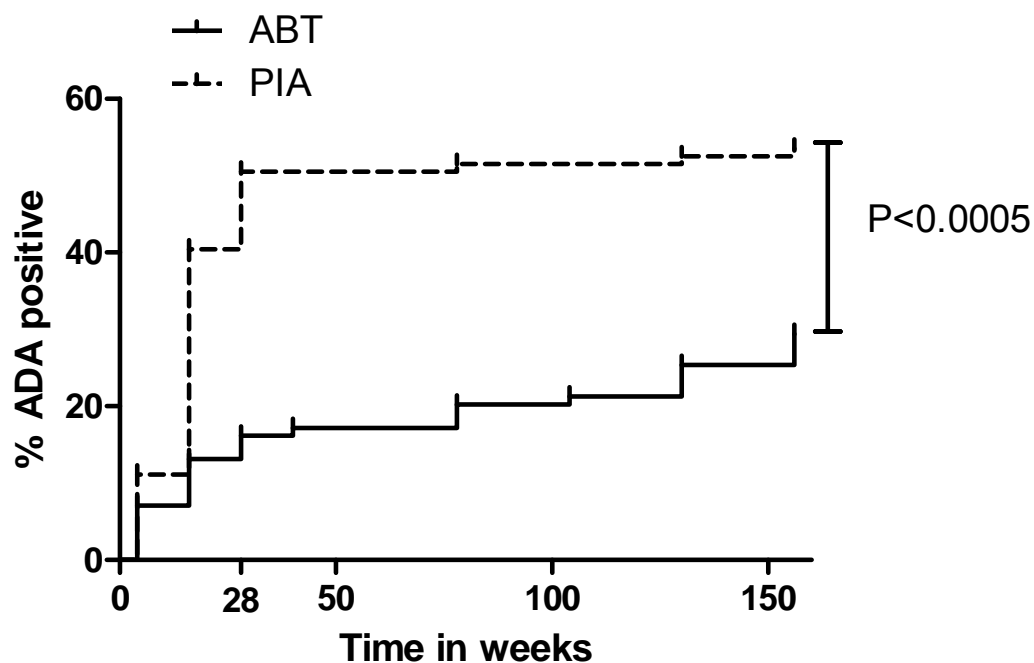
# pH-shift-anti-idiotypic Antigen binding test (PIA)



• *Van Schouwenburg et al, 2010, JIM*

## Long term measurement with PIA

- Measure 3 year follow-up data of 99 RA patients



54% of the patients develop ADA

94% of ADA+ patients develop ADA in the first 6 months

Many patients have ADA which are in complex with adalimumab



# ADA-Drug complexes

- What do these complexes look like?
  - Size of immune complexes
  - Composition of immune complexes



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## ADA-Drug complexes

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## The size of ADA-drug complexes

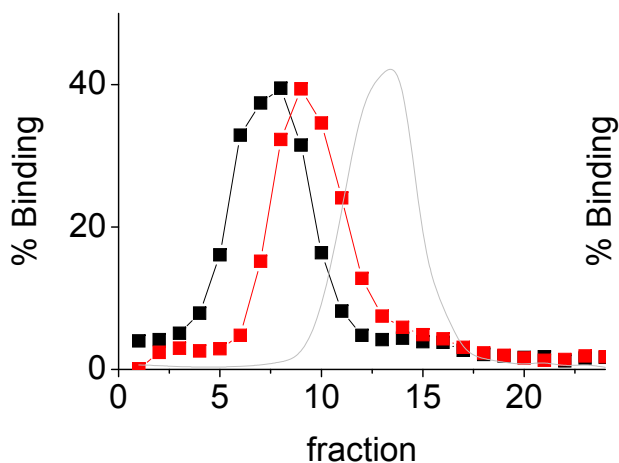
Sucrose gradients 6 patients with various amounts of adalimumab and ADA as measured in the ABT

	Adalimumab	IgG ABT	IgG PIA
adalimumab, no ADA (in ABT)	10800	< 12	555
	3990	< 12	617
adalimumab, low ADA (in ABT)	6375	14	
	6146	17	
no adalimumab, high ADA	< 2	471	
	8	417	

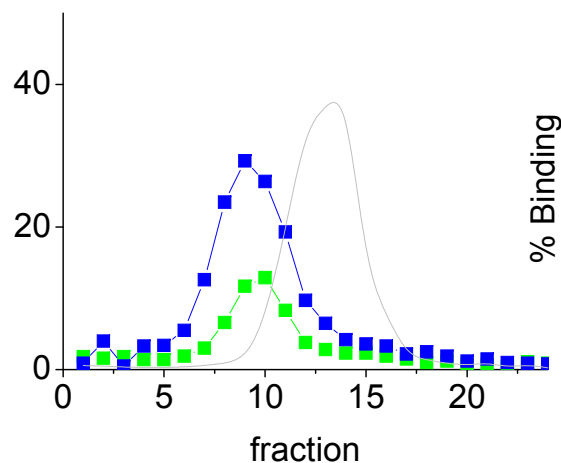
Measure ADA in the different fractions using the PIA

# The size of drug-anti-drug complexes

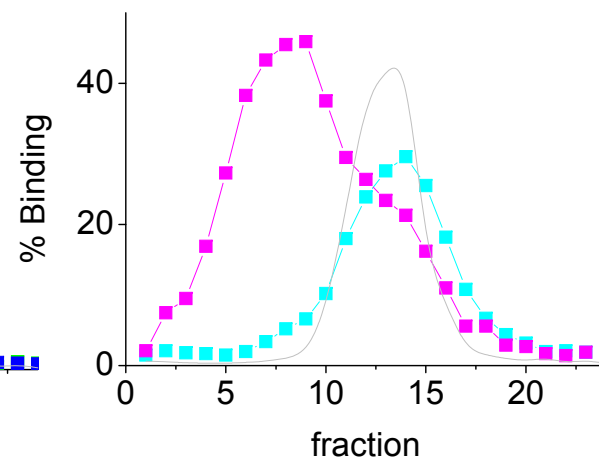
**adalimumab,  
no ADA (in ABT)**



**adalimumab,  
low ADA (in ABT)**



**no adalimumab,  
high ADA (in ABT)**



Most sera contain complexes

Measurement of marker proteins revealed that the complexes are the size of IgG dimers.

These complexes are not rapidly cleared

In total 14 patients tested, only small immune complexes were found

## ADA-Drug complexes

- What do these complexes look like?
  - Size of immune complexes
    - Most immune complexes are the size of dimers
      - Is this the result of IgG4 production
        - IgG4 has limited effector function and results in small immune complex formation
  - Composition of immune complexes

## ADA-Drug complexes

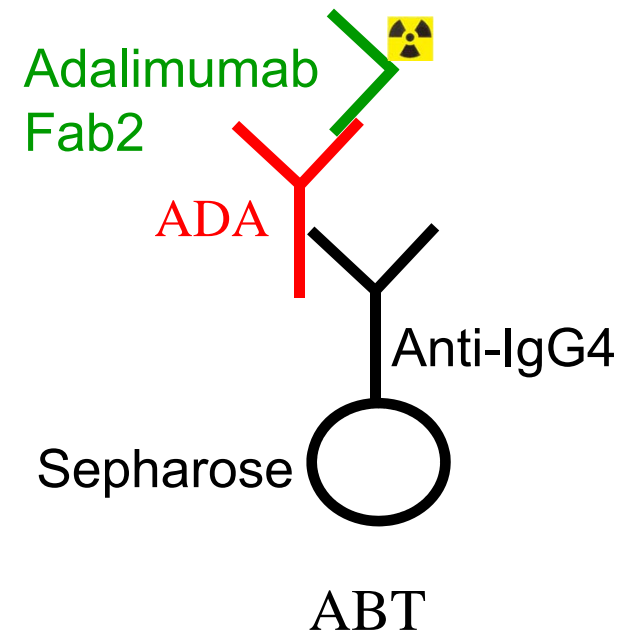
- What do these complexes look like?
  - Size of immune complexes
    - Most immune complexes are the size of dimers
      - Is this the result of IgG4 production
        - IgG4 has limited effector function and results in small immune complex formation
  - Composition of immune complexes
    - Do patients produce IgG4 against adalimumab?
    - Is IgG4 responsible for the formation of small immune complexes?

## IgG4 and anti-drug responses

- For other therapeutic proteins IgG4 production has been described
  - Factor VIII treatment in hemophilia patients (*van Helden et al 2008*)
    - Mainly production of IgG1 and IgG4
    - Suggested link between high IgG4 levels and failure of tolerance induction
  - For infliximab it has been shown that a significant part of the ADA is IgG4 (*Svenson et al 2007*)
  - For adalimumab there are little data

## Measurement of IgG4

- 271 RA patients treated with adalimumab
- 3 year follow-up
- Measurement of ADA
- Measurement of IgG4 ADA

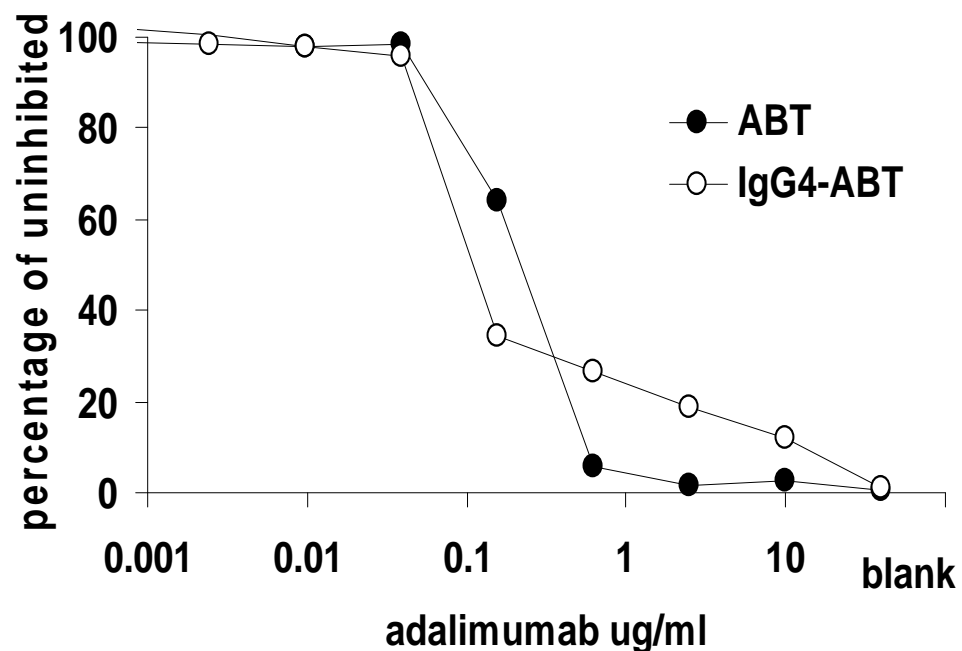




## Differences in drug interference

Measurement of total  
ADA and IgG4 ADA

Increasing amounts  
of adalimumab



The IgG4-ABT is less sensitive for drug interference

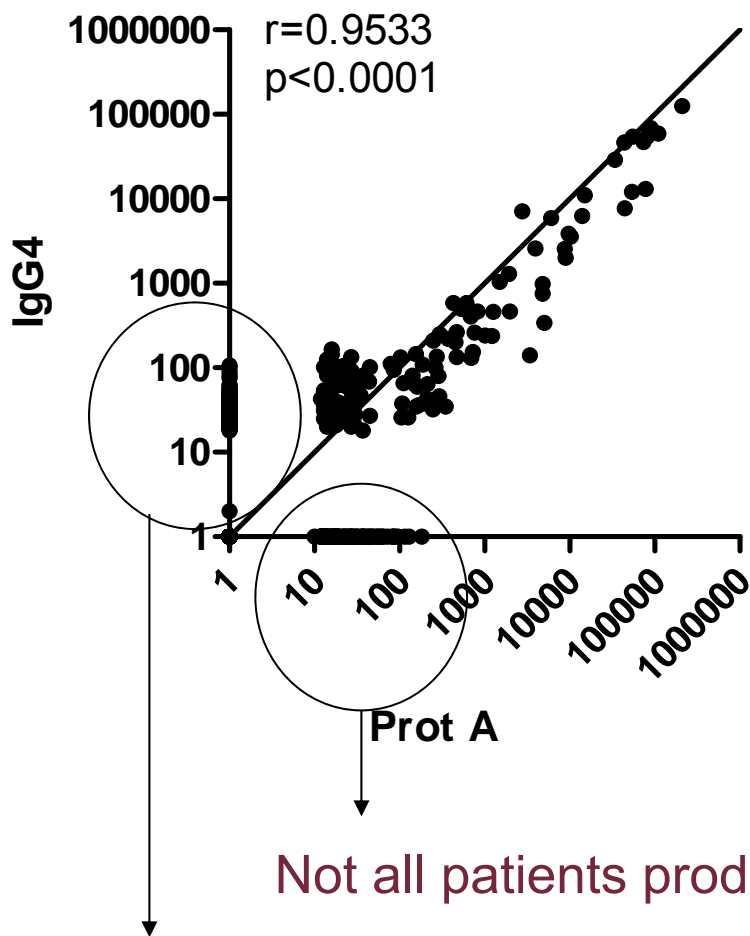
## IgG4 ADA in 271 RA patients

	IgG4 negative	IgG4 positive
Prot A negative	167	17
Prot A positive	25	62

71% of the ADA positive patients produce IgG4 antibodies against adalimumab

Does the production of IgG4 correlate with total ADA levels?

# Production of IgG4 correlates with total ADA



IgG4 ADA correlates with total ADA

IgG4 production varies a lot between patients

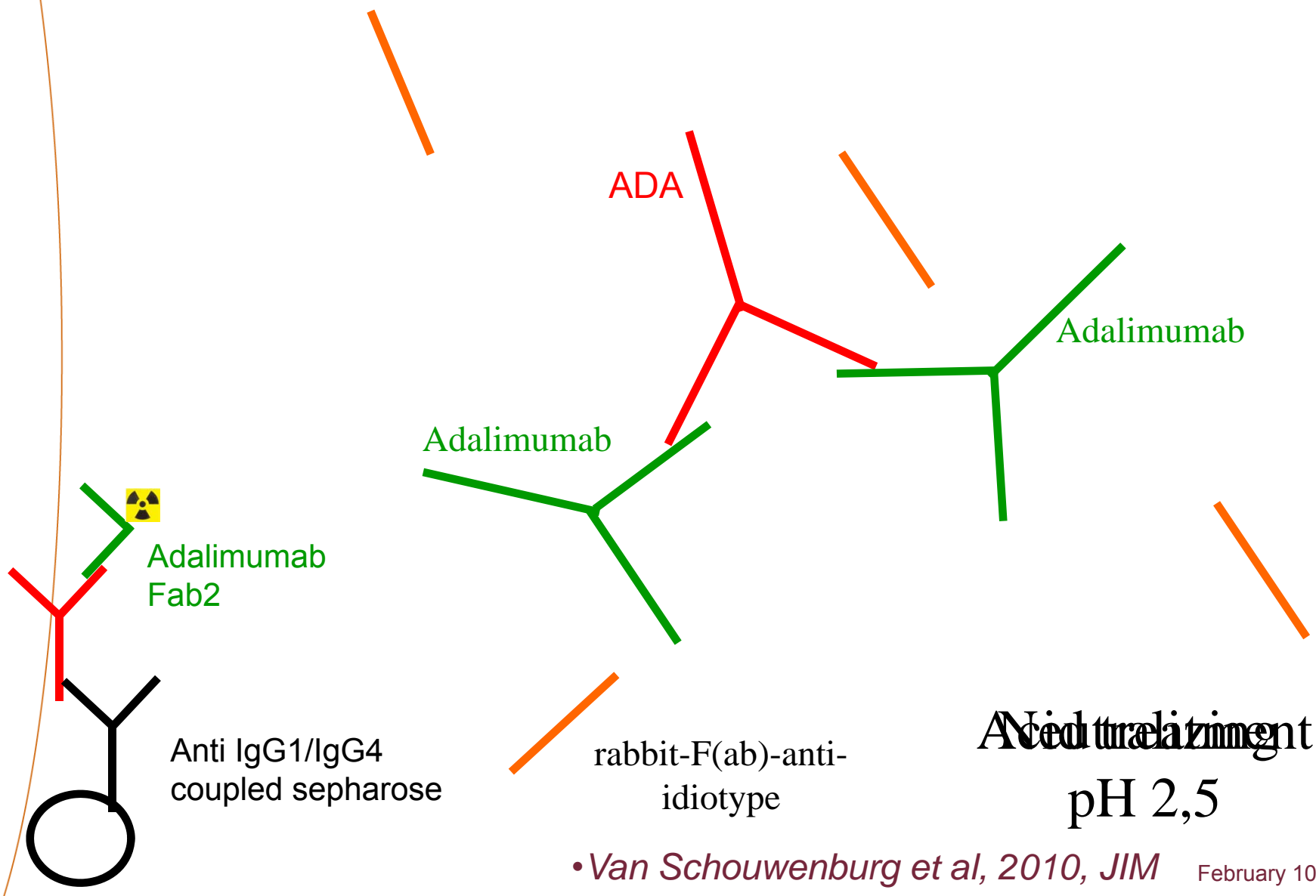
The IgG4 assay is less sensitive for drug interference

## Study the composition drug-anti-drug complexes

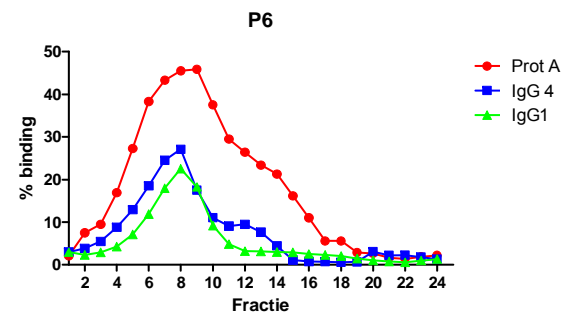
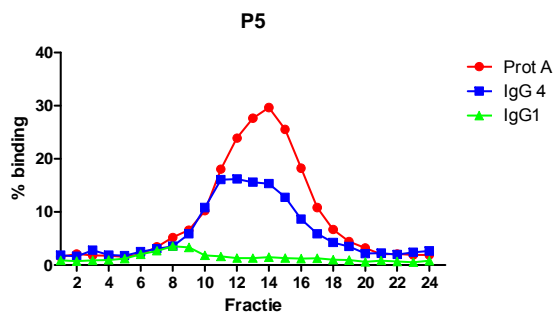
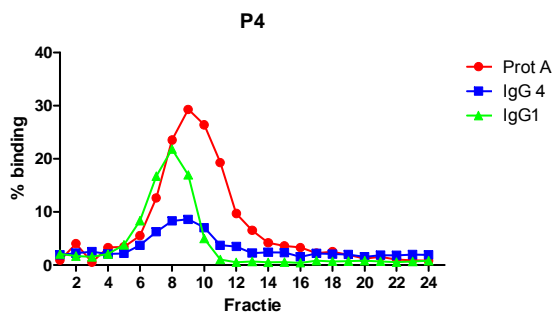
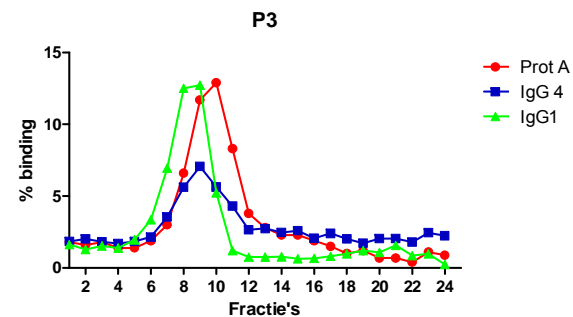
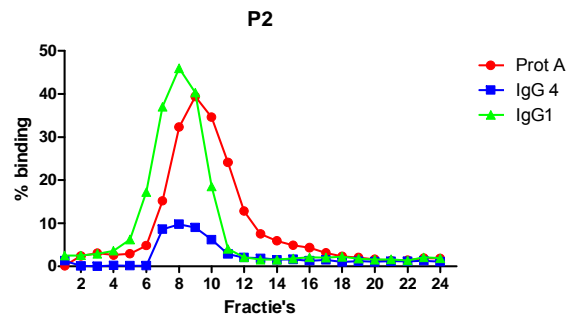
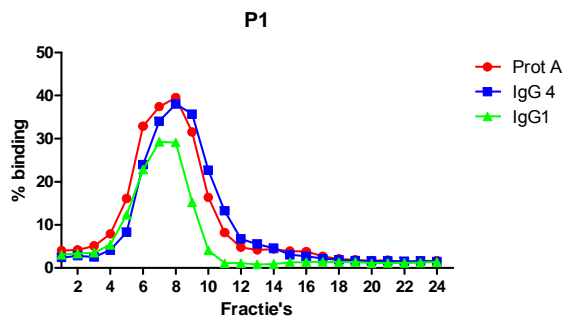
- Most patients make a considerable amount of specific IgG4
- Do the immune complexes between adalimumab and ADA contain mainly IgG4 ADA?

→ develop an IgG4 PIA and an IgG1 PIA

# pH-shift-anti-idiotypic Antigen binding test (PIA)



# Composition of drug-anti-drug complexes



Immune complexes consist of both IgG1 ADA and IgG4 ADA  
The ratio IgG1/IgG4 in complex varies between patients

## Conclusions

- 54% of the adalimumab treated RA patients develop ADA, as measured by PIA
- In many patients IgG4 ADA are produced
- Often ADA are present in the form of drug-ADA complexes
- Drug-ADA complexes are small (the size of dimers)
- Drug-ADA complexes of both IgG1 and IgG4 ADA



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## Conclusions

- Many patients are exposed longterm to circulating immune complexes
- What would be the clinical consequences of these circulating complexes?
  - Adverse effects
  - Trombo embolic events Korsewagen et al, 2011





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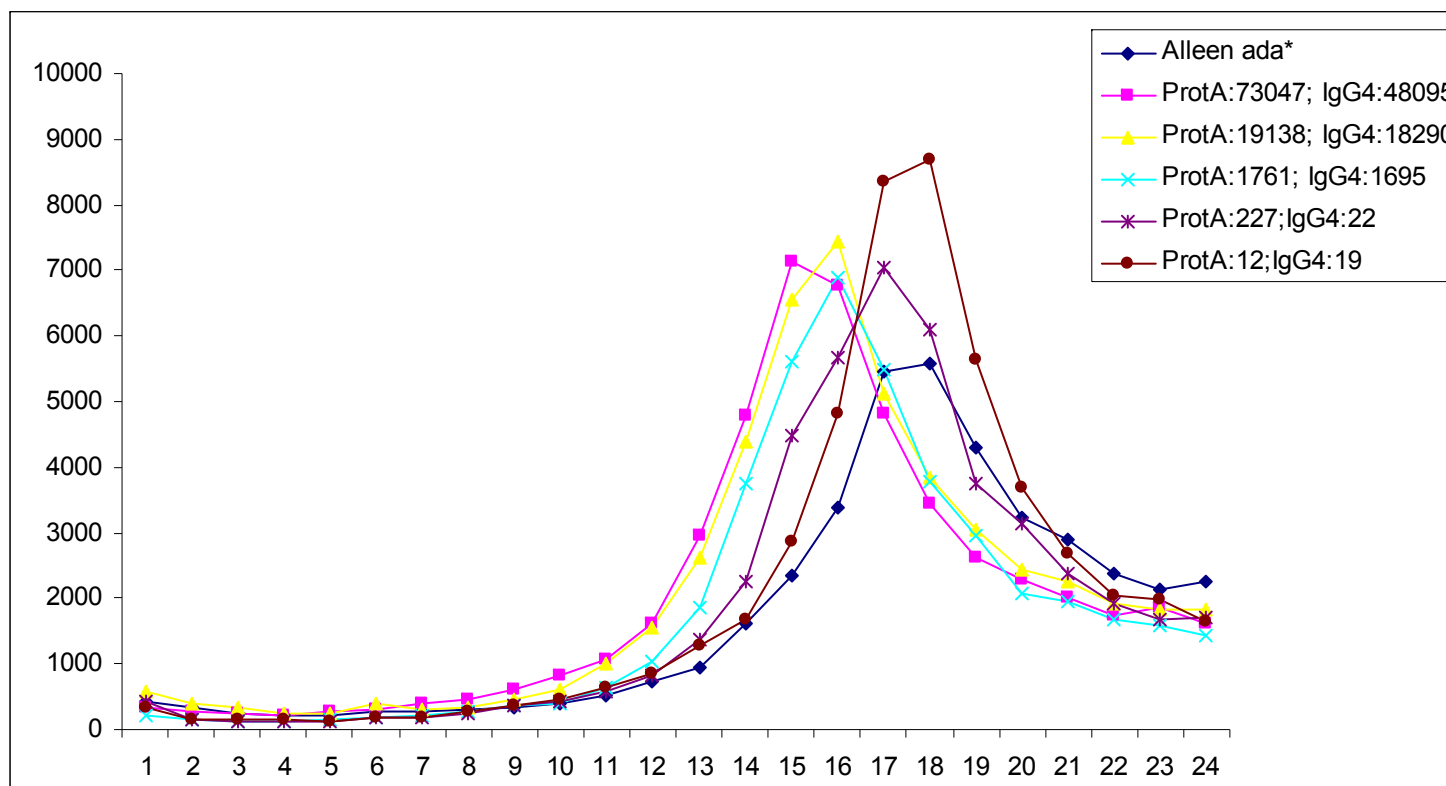
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## In vitro made complexes

5 patients sera

Adding 125I labeled adalimumab



In vitro made complexes: also small immune complexes