



Sanquin

HEALTH SOLUTIONS

Clinically relevant ADA testing for monoclonal antibody biologics

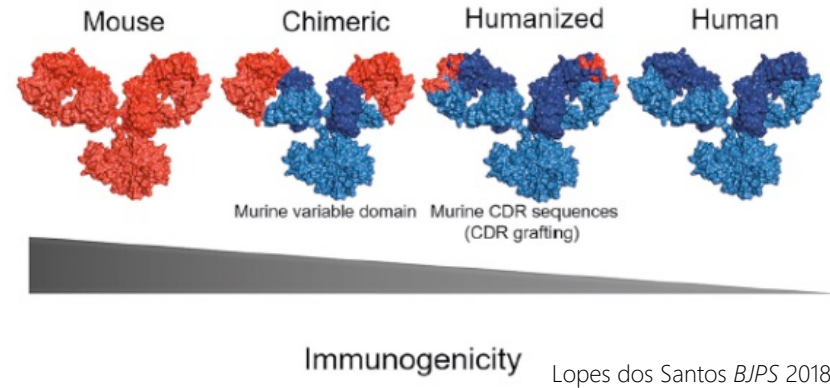
*Karien Bloem PhD
Principal Scientist
Sanquin Diagnostic Services*

For Life.



Immunogenicity of therapeutic mAbs

- Development of anti-drug antibodies (ADA) towards the therapeutic mAbs
- Theoretically reduced immunogenicity with humanized and human mAbs





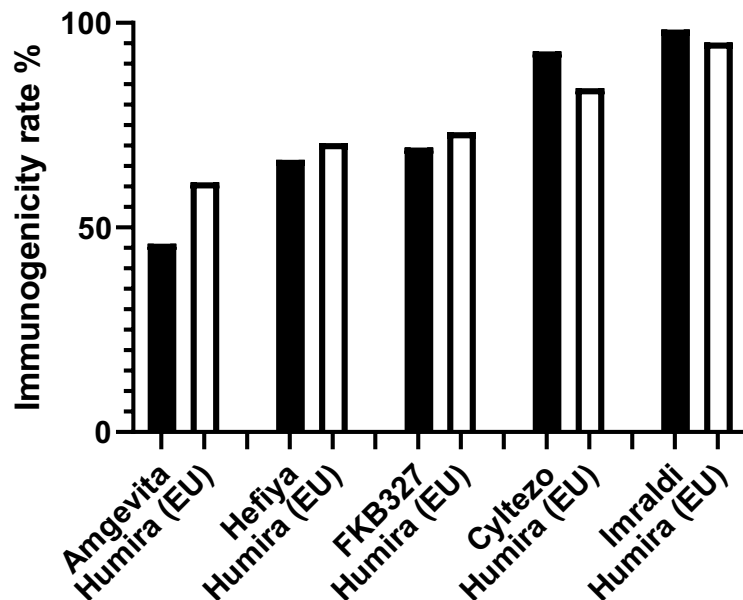
| Antibody INN (Trade name) | Antibody type (Generation Technique) | Target | Observed adverse events | Anti-drug antibodies | References |
|---|--|--------------|---|-------------------------|---|
| Antibodies targeting cytokines | | | | | |
| Adalimumab (<i>Humira</i>) | Human (phage display) | TNF | Infections, fever, diarrhea, rash | ++++ Neutralizing | Bender, et al. 2007; ⁴⁸ Coenen, et al. 2007 ⁴⁹ |
| Golimumab (<i>Simponi</i>) | Human (transgenic mouse) | TNF | Infusion reactions, nausea, infections | + Non-neutralizing | Shealy, et al. 2010, ⁵⁰ Kay, et al. 2010, ⁵¹ Kay, et al. 2008 ⁵² |
| Certolizumab pegol (<i>Cimzia</i>) | Humanized Fab | TNF | Abdominal pain, diarrhea, injection site reactions, infection | + Neutralizing | Baker 2009, ⁵³ Lichtenstein, et al. 2010 ⁵⁴ |
| Briakinumab | Human (phage display) | IL12/IL23p40 | Infections, fever, diarrhea, malignancies | Unknown | Gandhi, et al. 2010 ⁵⁵ |
| Ustekinumab (<i>Stelara</i>) | Human (transgenic mouse) | IL12/IL23p40 | Fatigue, headache, cardiac toxicity, infections | + Neutralizing | Gandhi, et al. 2010, ⁵⁵ Cingoz 2009 ⁵⁶ |
| Canakinumab (<i>Ilaris</i>) | Human (transgenic mouse) | IL1 | Infections | None Described | Dhimolea 2010, ⁵⁷ Lachmann, et al. 2009 ⁵⁸ |
| Tocilizumab/ Atlizumab (<i>Actemra</i>) | Humanized | IL6 receptor | Infusion reactions, infections, malignancy, anaphylaxis | + Neutralizing | Sharma, et al. 2008 ⁵⁹ |
| Lerdelimumab | Human (phage display) | TGFβ | Eye based infusion-Cataracts, pain, conjunctivitis | + Non-neutralizing | Khaw, et al. 2007 ⁶⁰ |
| B-E8 | Murine | IL6 | Headache, vomiting, fever, thrombocytopenia | + Non-neutralizing | Rossi, et al. 2005, ⁶¹ Emilie, et al. 1994 ⁶² |
| CB6 | Murine | TNF | Infections, headache, vomit- ing, fever, infusion reactions | +++++ | Fisher, et al. 1993 ⁶³ |
| B-N10 | Murine | IL10 | Infusion reactions | +++++ Neutralizing | Llorente, et al. 2000 ⁶⁴ |
| Afelimomab | Murine Fab | TNF | Infections, headache, vomit- ing, fever, infusion reactions | ++ Non-neutralizing | Panacek, et al. 2004, ⁶⁵ Reinhart, et al. 2001 ⁶⁶ |
| Nerelimumab | Murine | TNF | Serum sickness, hypotension | +++++ | Cohen and Carlet 1996 ⁶⁷ |

Getts *moAbs* 2010



Differences in immunogenicity of same drug

Phase I/II adalimumab biosimilars, single dose 40mg, 72d follow-up



Difference in anti-drug antibody ratio despite

- Same technique (MSD ECL)
 - Same assay design (homogeneous bridging, acid dissociation)
 - Same capture/detection reagents (labeled biologic)
 - Small difference is reagent concentrations, and the properties of surrogate positive control determines cut-off for positivity
-
- Immunogenicity rate in initial clinical studies of Humira in different diseases are much lower: 2 % - 26 %



Requirements from regulatory agencies

Development of different assays:

1. Screening assay
2. Confirmation assay
3. Titre assay
4. Neutralization assay

Important requirements

- Detection of all (clinically) relevant antibodies
- Low false positive rate is desirable, false negative results are unacceptable
- Drug tolerant



Requirements from regulatory agencies

Development of different assays:

1. Screening assay
2. Confirmation assay
3. Titre assay
4. Neutralization assay

Important requirements

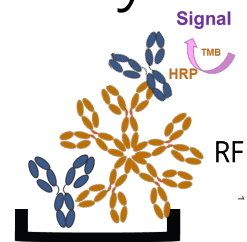
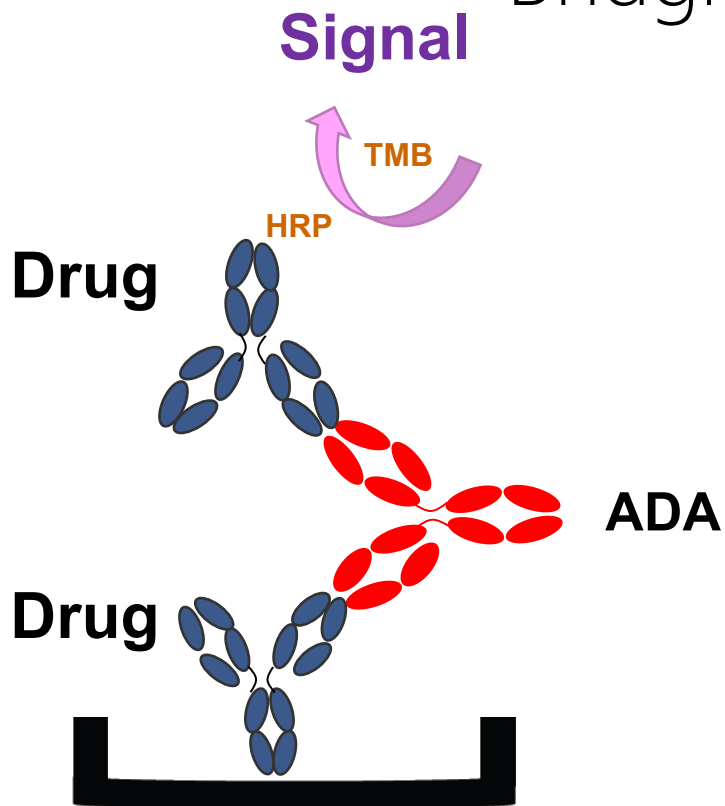
- Detection of all (clinically) relevant antibodies
- Low false positive rate is desirable, false negative results are unacceptable
- **Drug tolerant**
- **Only applicable for monoclonal antibodies**



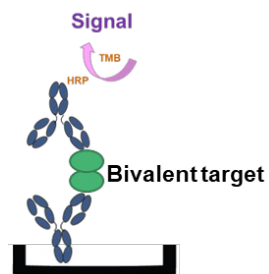
ADA testing in the clinic

- Patients studies of approved drug
 - Adalimumab (anti-TNF)
 - Infliximab (anti-TNF)
 - Ustekinumab (anti-IL-12)
 - Natalizumab (anti- $\alpha 4\beta 1$ integrin)
- Anti-drug antibodies (ADA) to help physician in decisions on treatment
- Clinical relevance of ADA

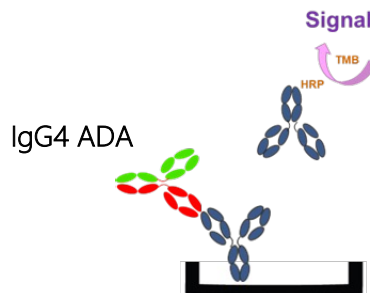
Bridging ADA assay



False positive



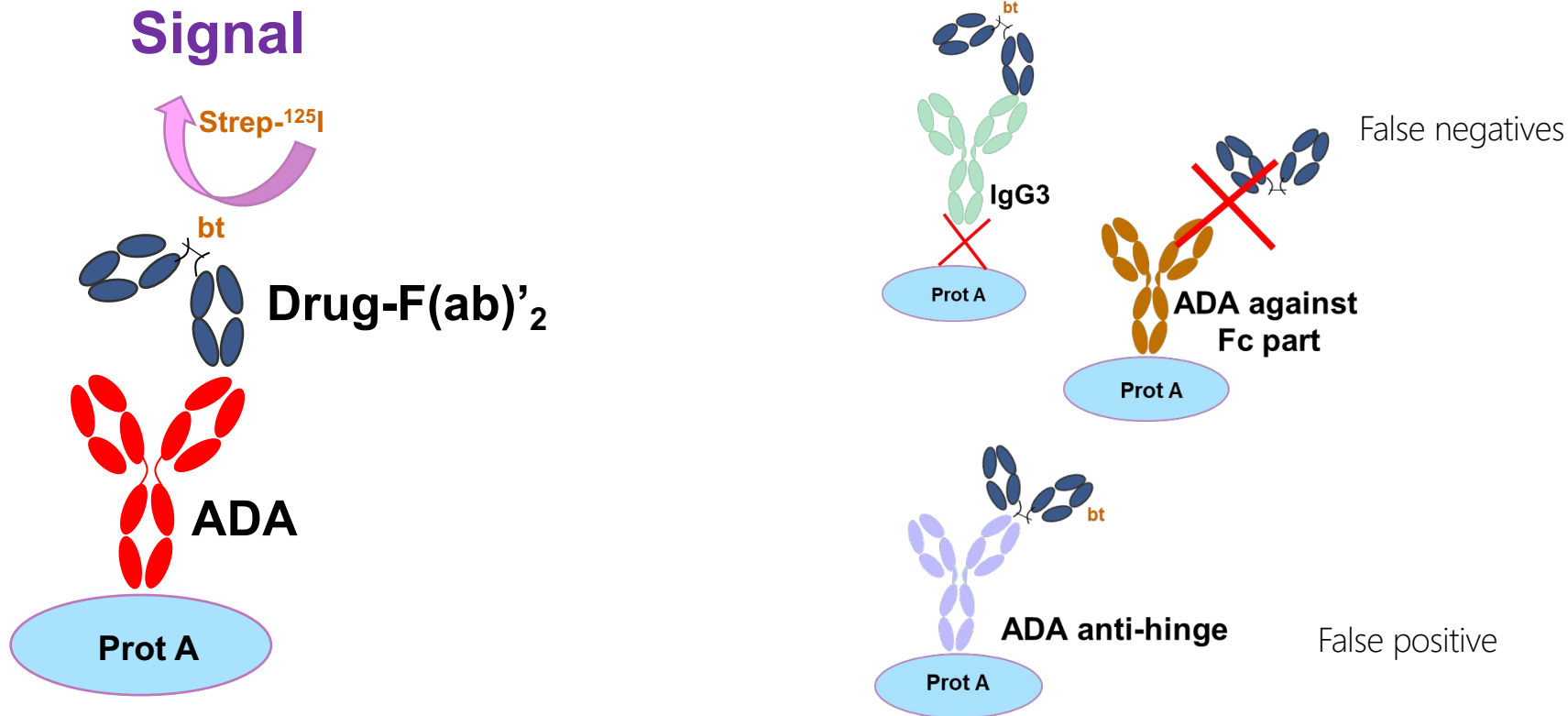
False positive



False negative

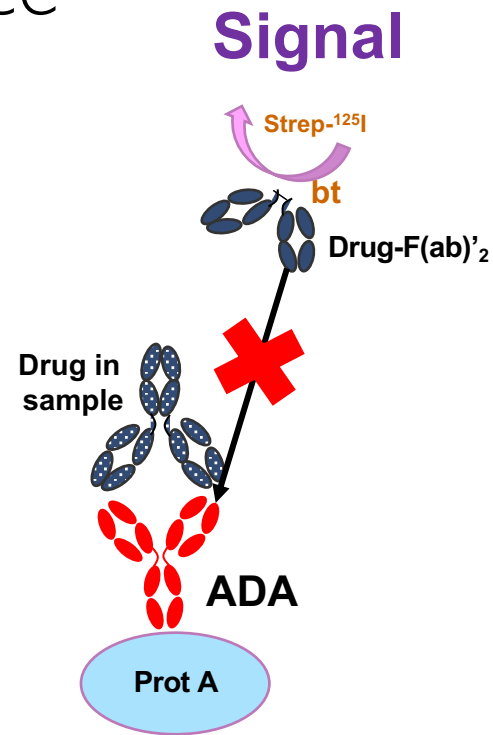
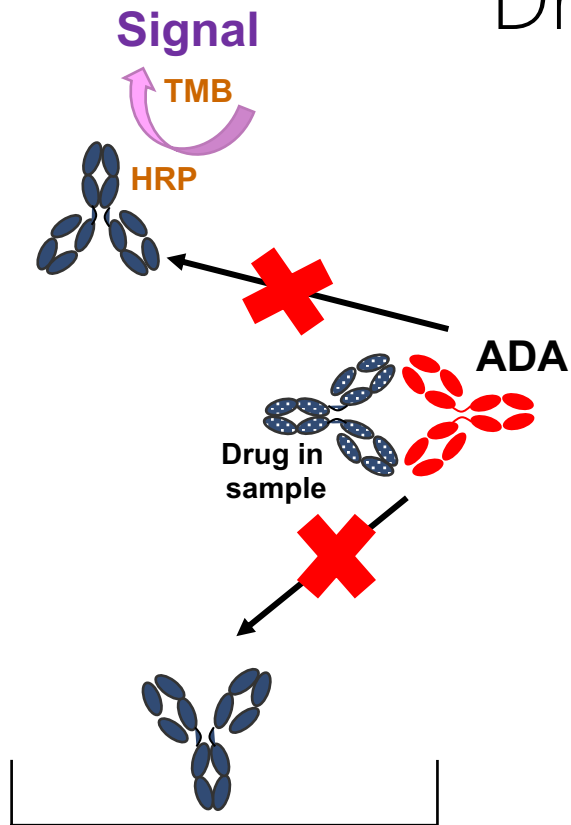


Antigen binding test (ABT)

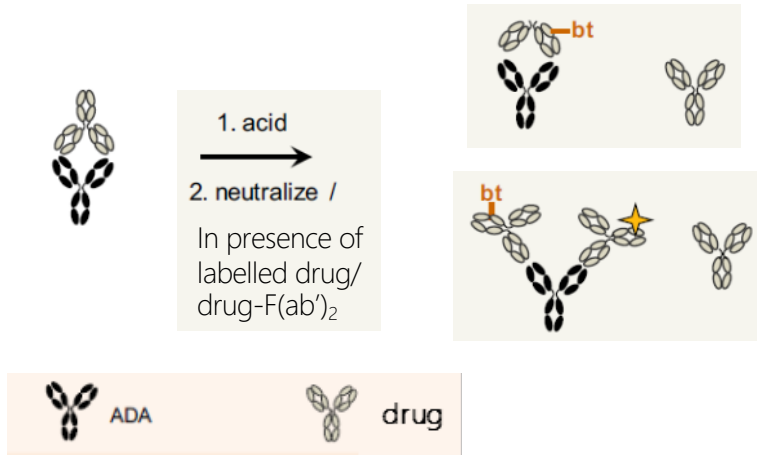


*: depending on the capture reagent used, here specified for protein A

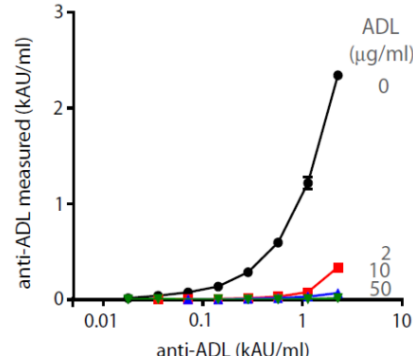
Drug interference



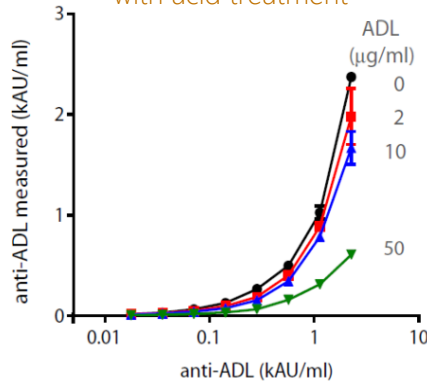
Drug tolerance of the assays is improved by acid dissociation



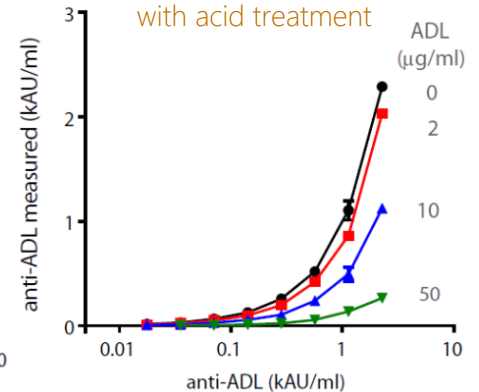
Antigen binding test



Antigen binding test with acid treatment



ECL with acid treatment



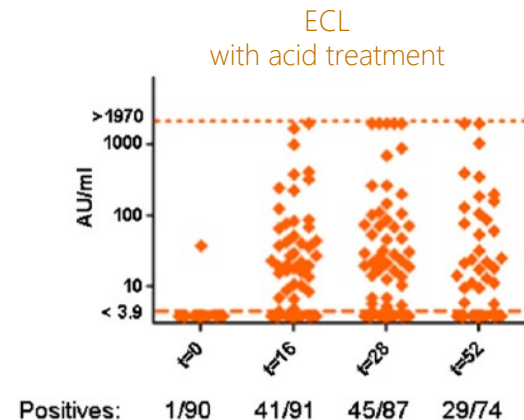
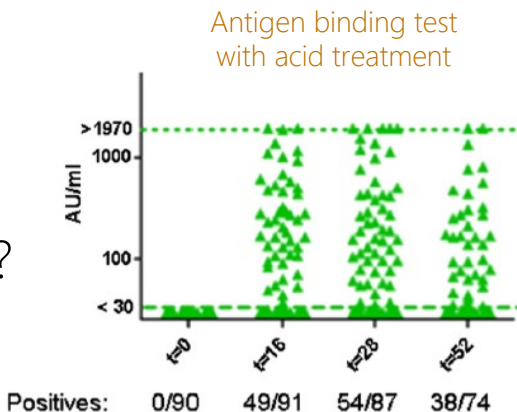
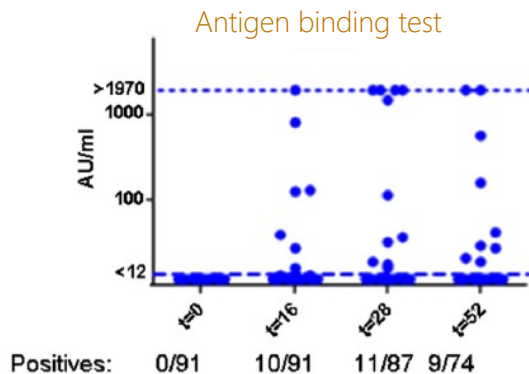
Adalimumab: ADL
human anti-TNF moAb



Increased ADA detection in drug tolerant assays

- 94 adalimumab treated RA patients
- 40 mg every other week SC
 - 20 patients 80 mg
- Samples taken before the next administration

However, which assay is correlated with clinical relevance?





Development of Antidrug Antibodies Against Adalimumab and Association With Disease Activity and Treatment Failure During Long-term Follow-up

JAMA. 2011;305(14):1460-1468

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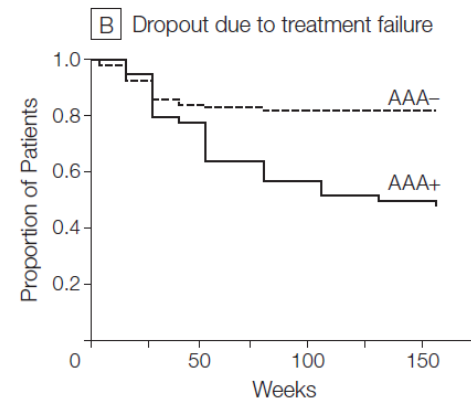
Gerrit Jan Wolbink, MD, PhD

Context Short-term data on the immunogenicity of monoclonal antibodies showed associations between the development of antidrug antibodies and diminished serum drug levels, and a diminished treatment response. Little is known about the clinical relevance of antidrug antibodies against these drugs during long-term follow-up.

Objective To examine the course of antidrug antibody formation against fully human monoclonal antibody adalimumab and its clinical relevance during long-term (3-year) follow-up of patients with rheumatoid arthritis (RA).

Design, Setting, and Patients Prospective cohort study February 2004–September 2008; end of follow-up was September 2010. All 272 patients were diagnosed with RA and started treatment with adalimumab in an outpatient clinic.

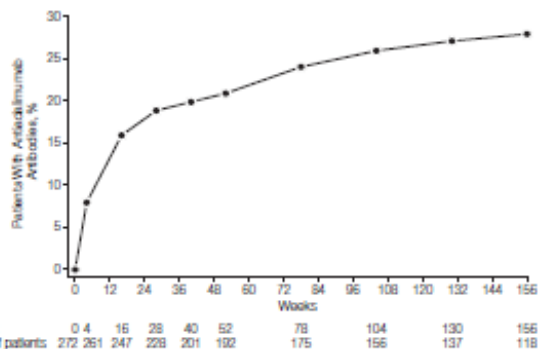
Main Outcome Measures Disease activity was monitored and trough serum samples



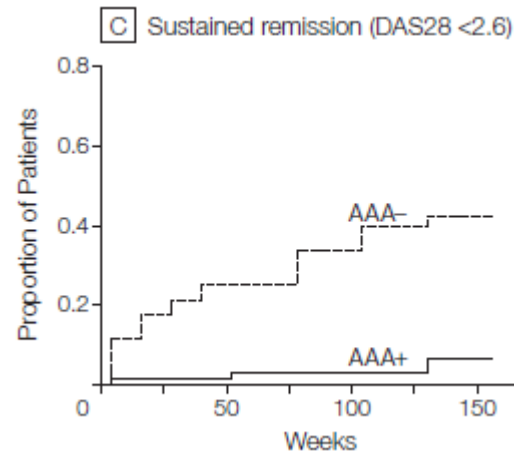
No. at risk

| | | | | |
|------|-----|-----|-----|-----|
| AAA- | 196 | 151 | 135 | 118 |
| AAA+ | 76 | 59 | 43 | 29 |

Figure 1. Percentage of Antiadalimumab Development Over Time



Number of patients with available serum samples are shown.

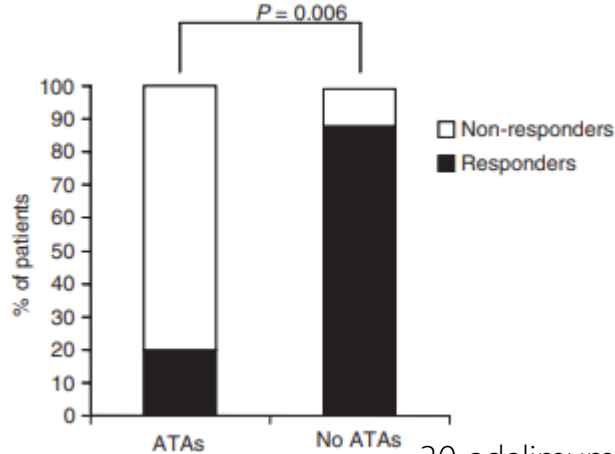




Correlation ADA and clinical efficacy in other diseases

Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease

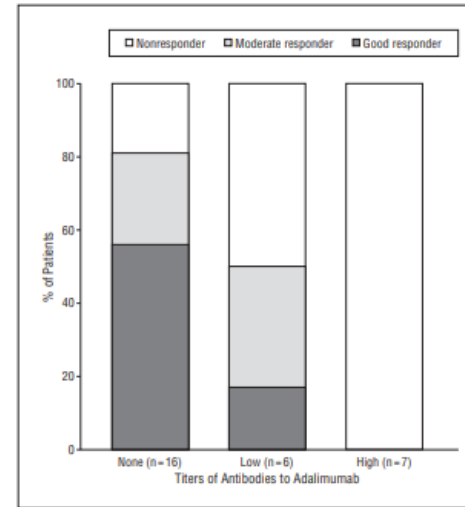
R. L. WEST*, Z. ZELINKOVA*, G. J. WOLBINK†, E. J. KUIPERS*,‡, P. C. F. STOKKERS & C. J. VAN DER WOUDE*



30 adalimumab treated patients with Crohn's disease

Extent and Clinical Consequences of Antibody Formation Against Adalimumab in Patients With Plaque Psoriasis

Lidjan L. A. Lecluse, MD; Rieke J. B. Driessen, MD; Phyllis I. Spuijs, MD, PhD; Elke M. G. J. de Jong, MD, PhD; Steven O. Stapel, PhD; Martijn B. A. van Doorn, MD, PhD; Jan D. Bos, MD, PhD, FRCP; Gert-Jan Wolbink, MD, PhD

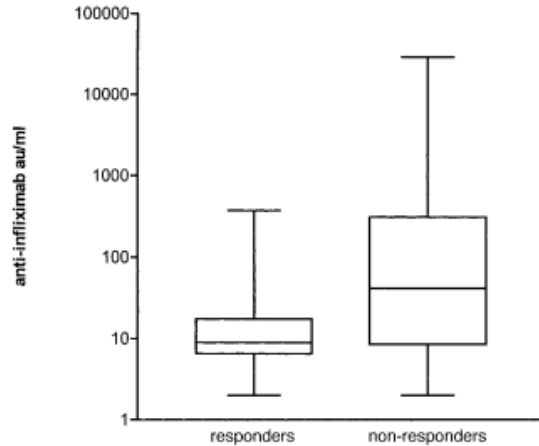


29 adalimumab treated patients with plaque psoriasis

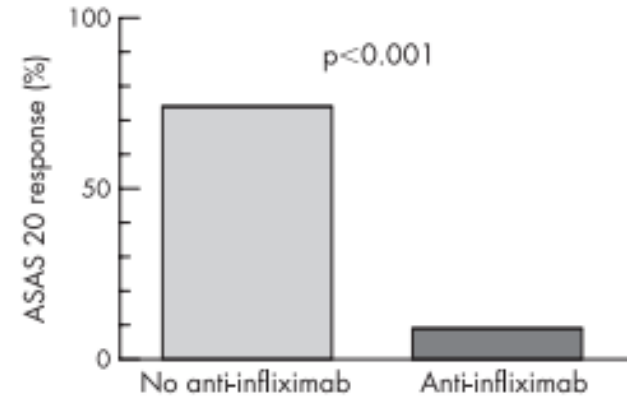
Lecluse et al. Arch dermatol 2010



Correlation ADA and clinical efficacy with other anti-TNF mAbs



52 infliximab treated patients
with rheumatoid arthritis



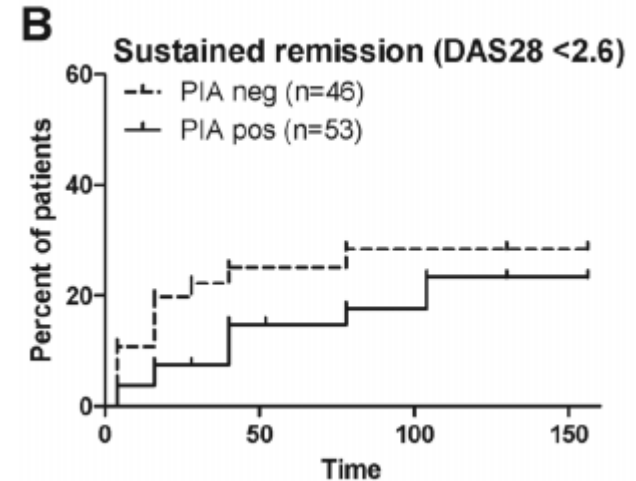
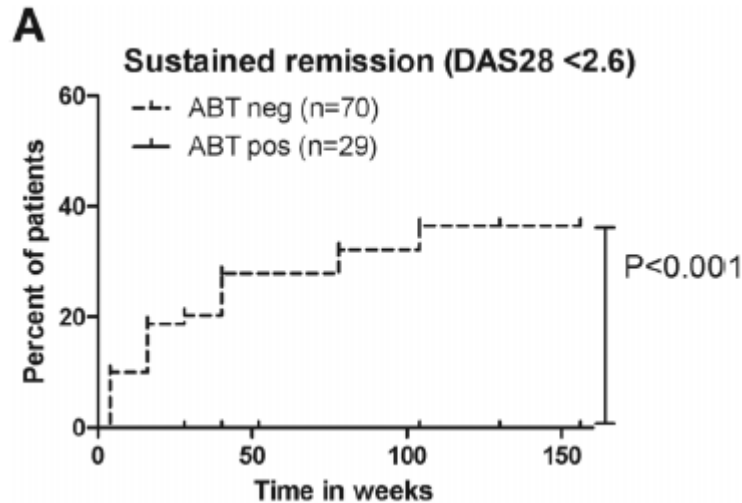
38 infliximab treated patients
with ankylosing spondylitis



Correlation with clinical outcome depends on ADA assay

ABT: drug sensitive

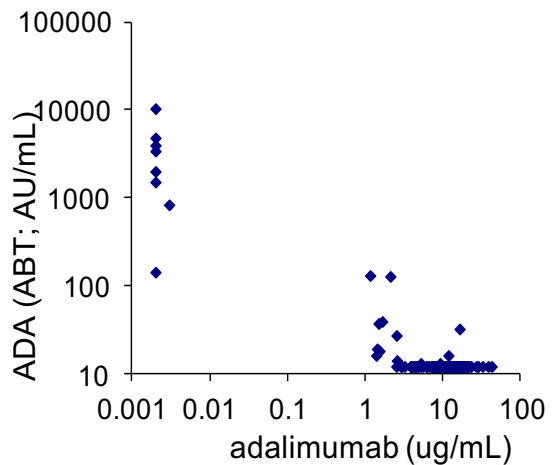
PIA: drug tolerant



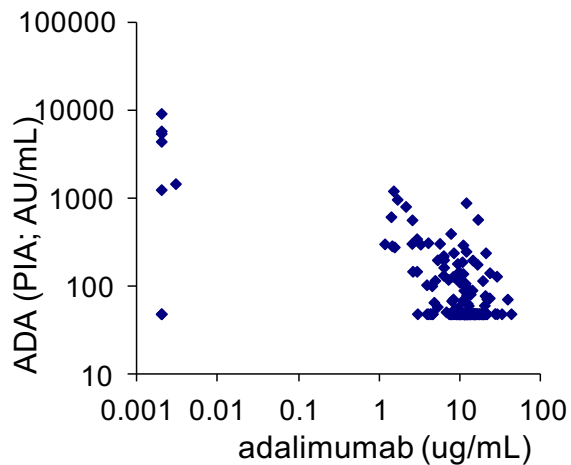


ADA and drug levels

Drug sensitive ADA assay

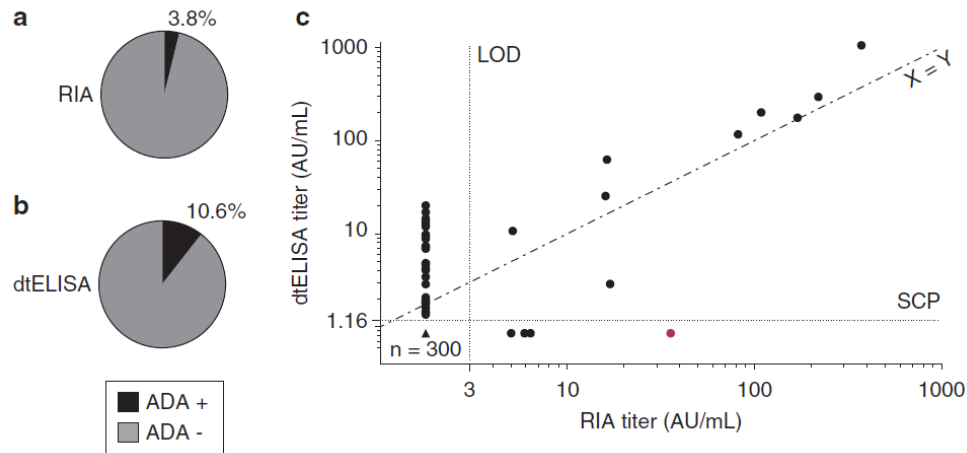


Drug tolerant ADA assay

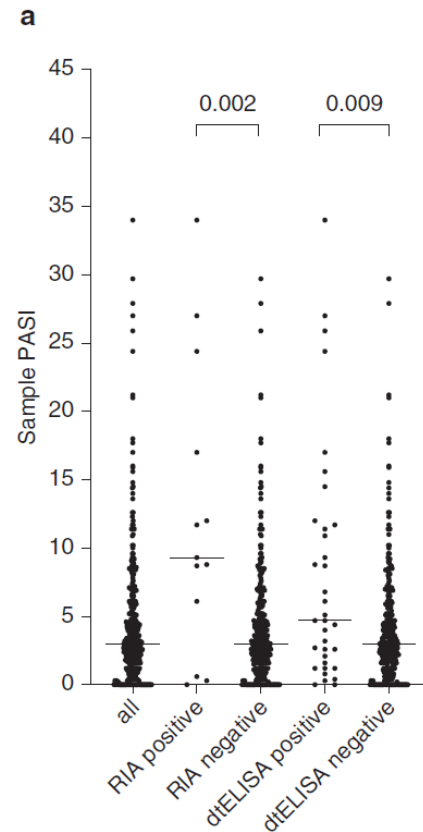




Clinical relevant ADA for ustekinumab

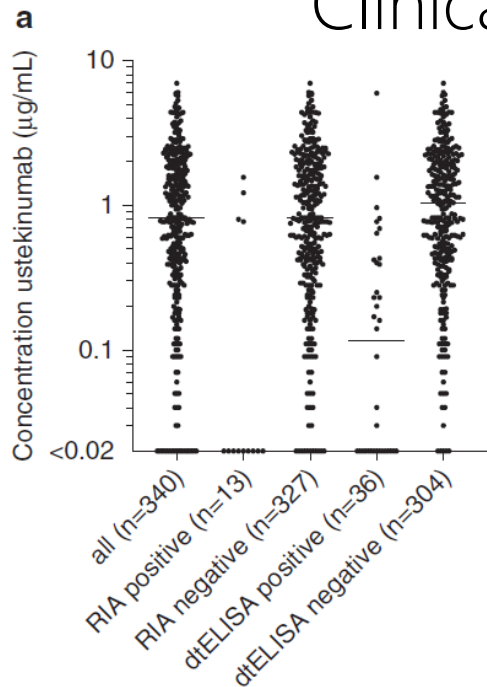


Ustekinumab: anti-IL-12 (p40 subunit)
 340 patients with psoriasis
 RIA: drug sensitive
 dtELISA: drug tolerant



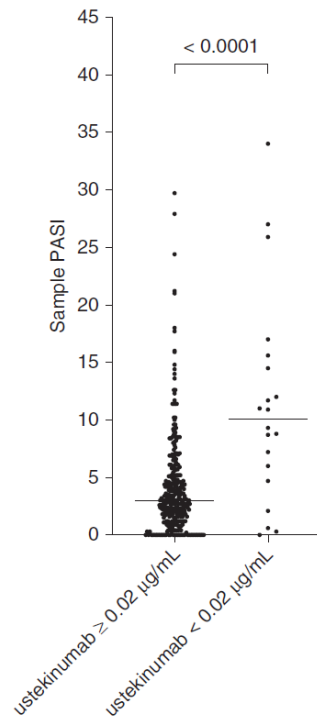
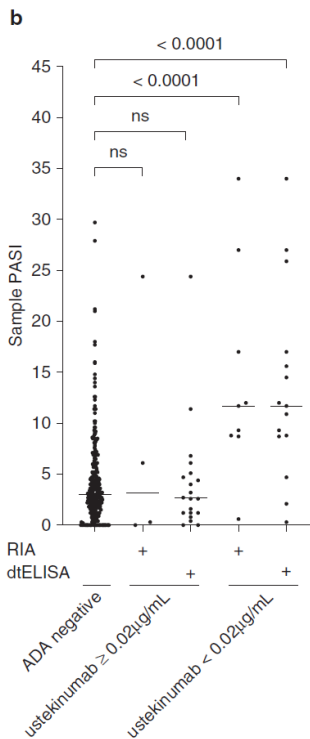


Clinical relevant ADA for ustekinumab



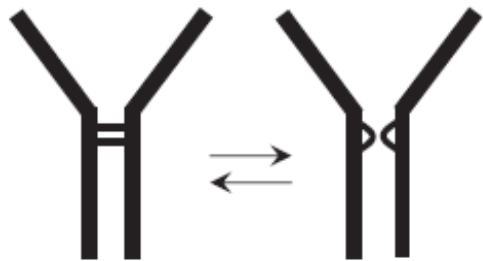
RIA: drug sensitive
dtELISA: drug tolerant

Loeff et al. *J Invest Derm* 2020

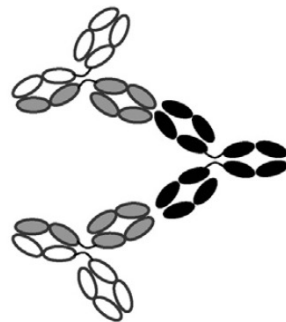




Higher drug tolerance for natalizumab (IgG4 moAb)



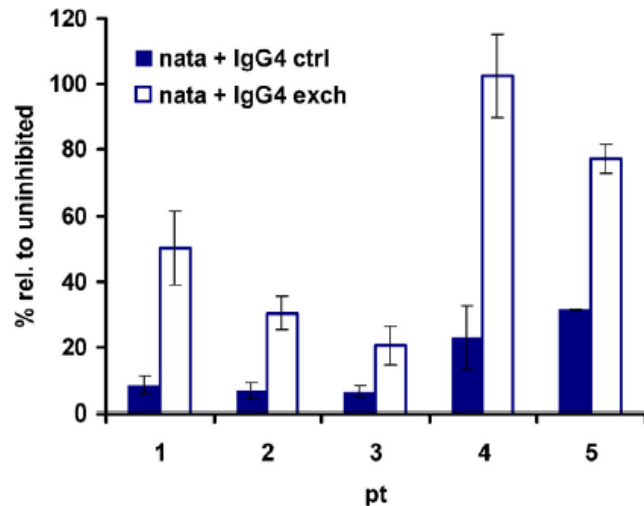
IgG1



IgG4



Natalizumab binds $\alpha 4$ chain of $\alpha 4\beta 1$ integrin and blocks the migration of leukocytes across the blood-brain barrier into the central nervous system

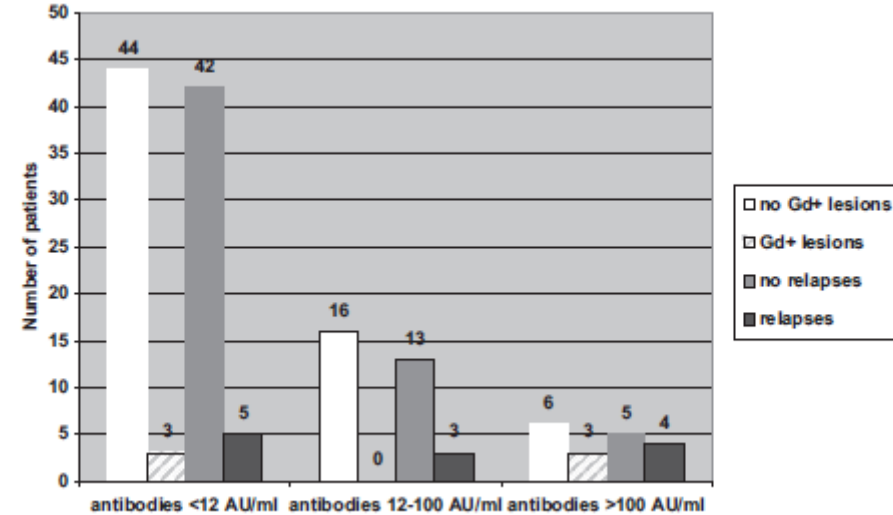
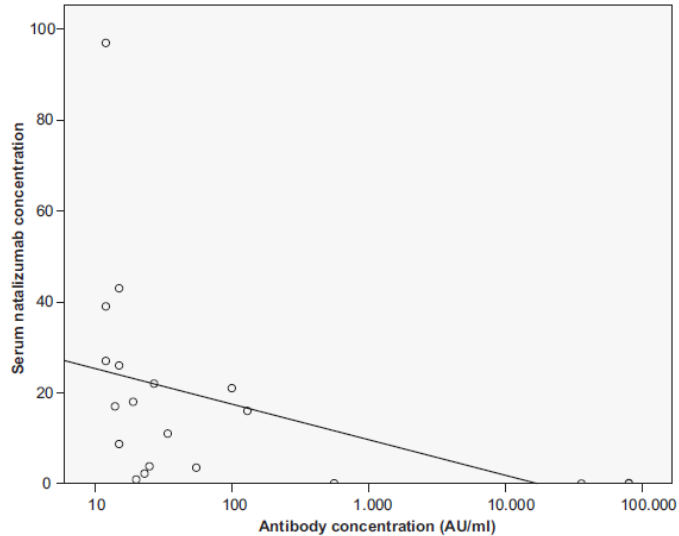


IgG4 can exchange half molecules with other IgG4
Resulting in bi-specific antibodies

Reduced drug interference of IgG4 moAb biologics



High levels of anti-natalizumab antibodies reduce efficacy

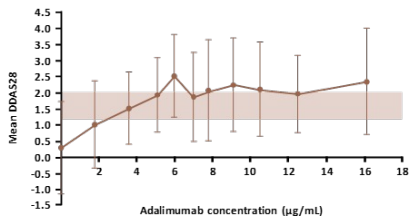


73 natalizumab treated patients with multiple sclerosis

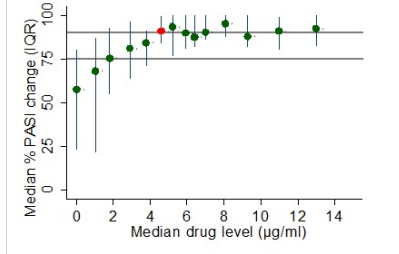


Drug concentration is the main driver in clinical efficacy

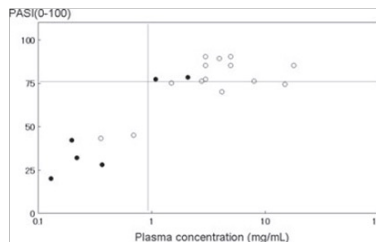
RA - adalimumab



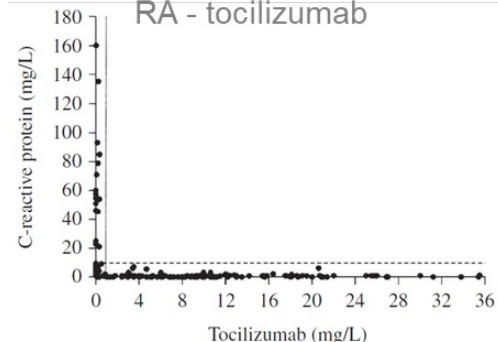
Psoriasis - adalimumab



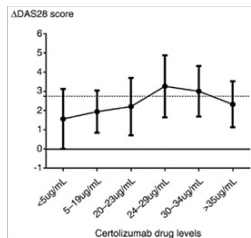
Psoriasis - infliximab



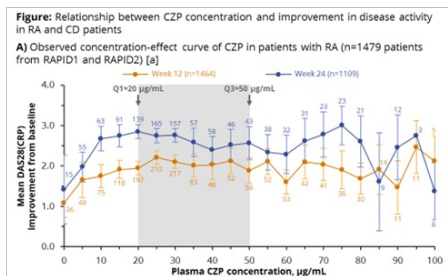
RA - tocilizumab



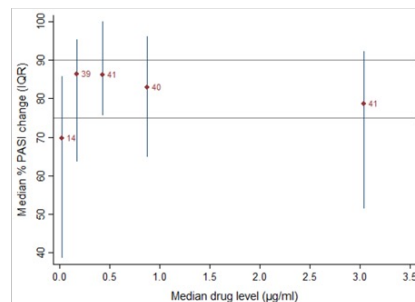
RA - certolizumab



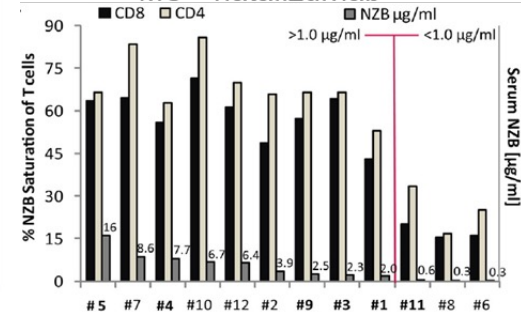
RA - certolizumab



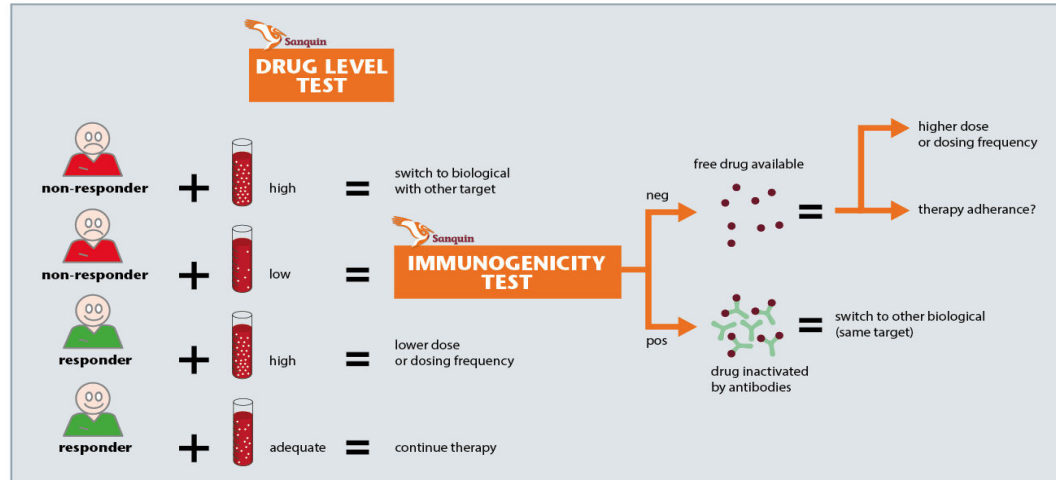
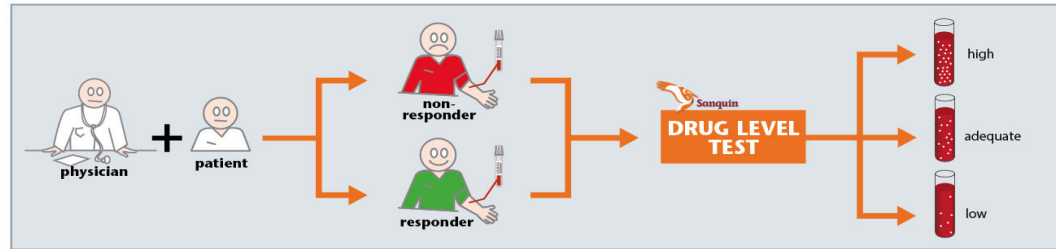
Psoriasis - ustekinumab



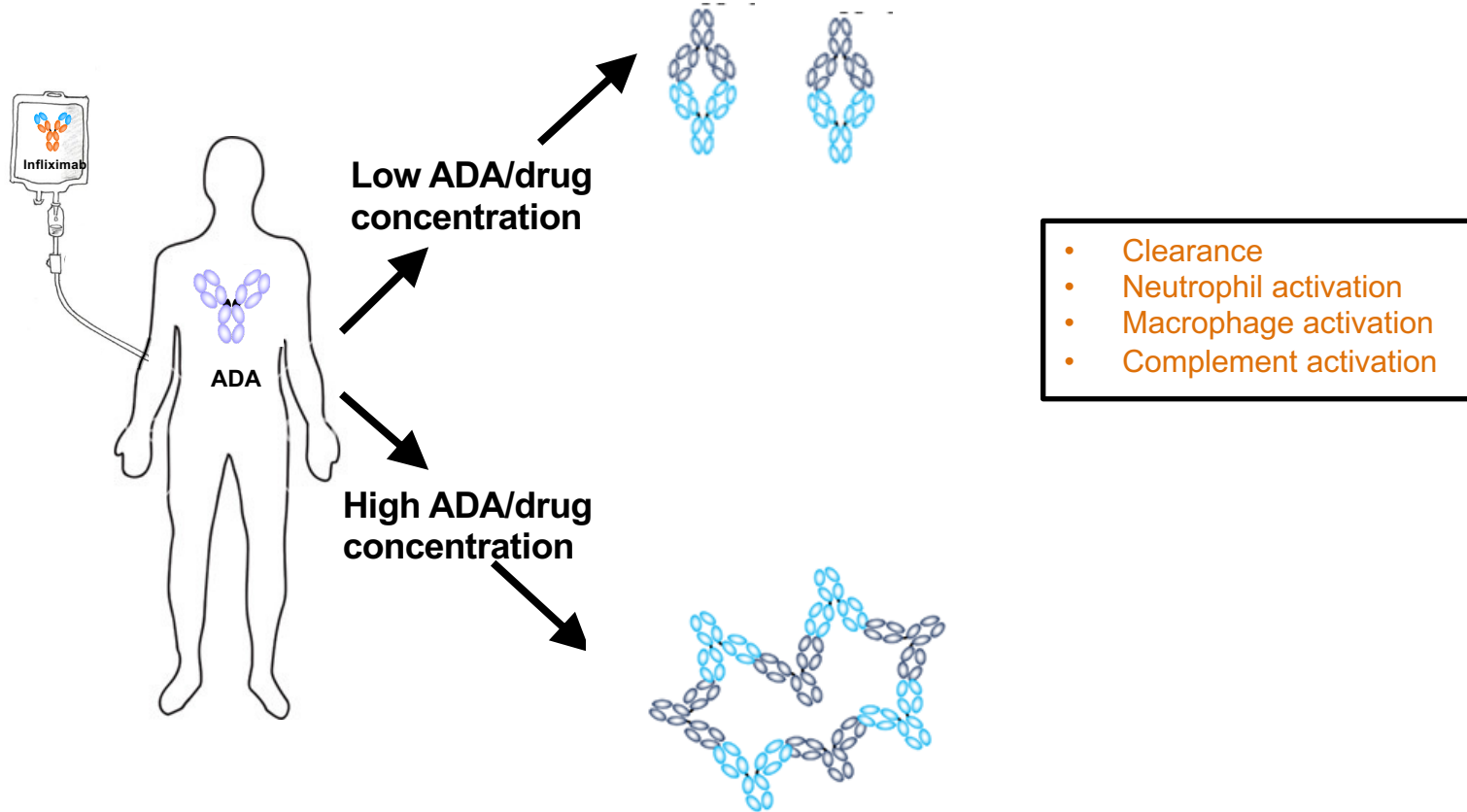
MS - natalizumab



PK is driving our testing advice

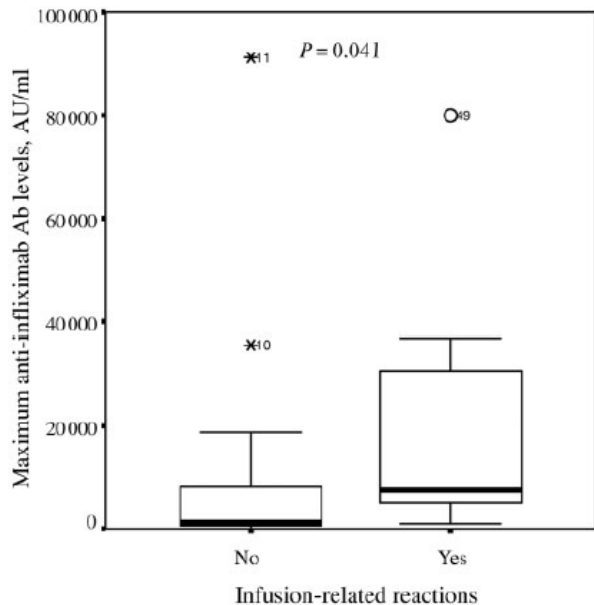


Link between ADA and side effects

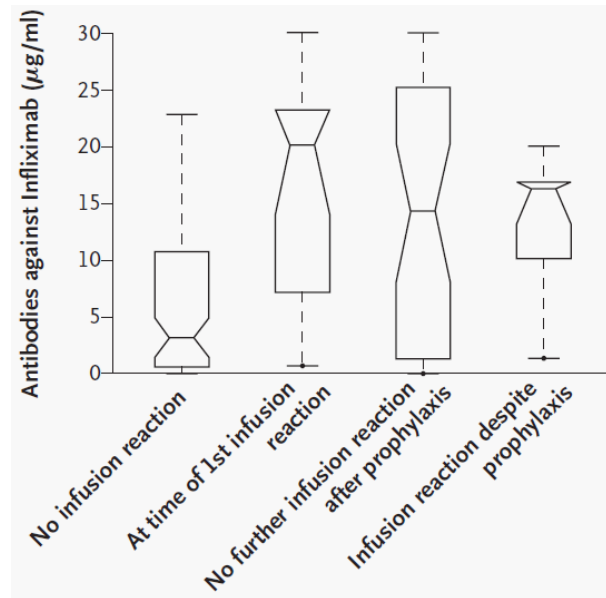




Infusion related reactions are linked to ADA level



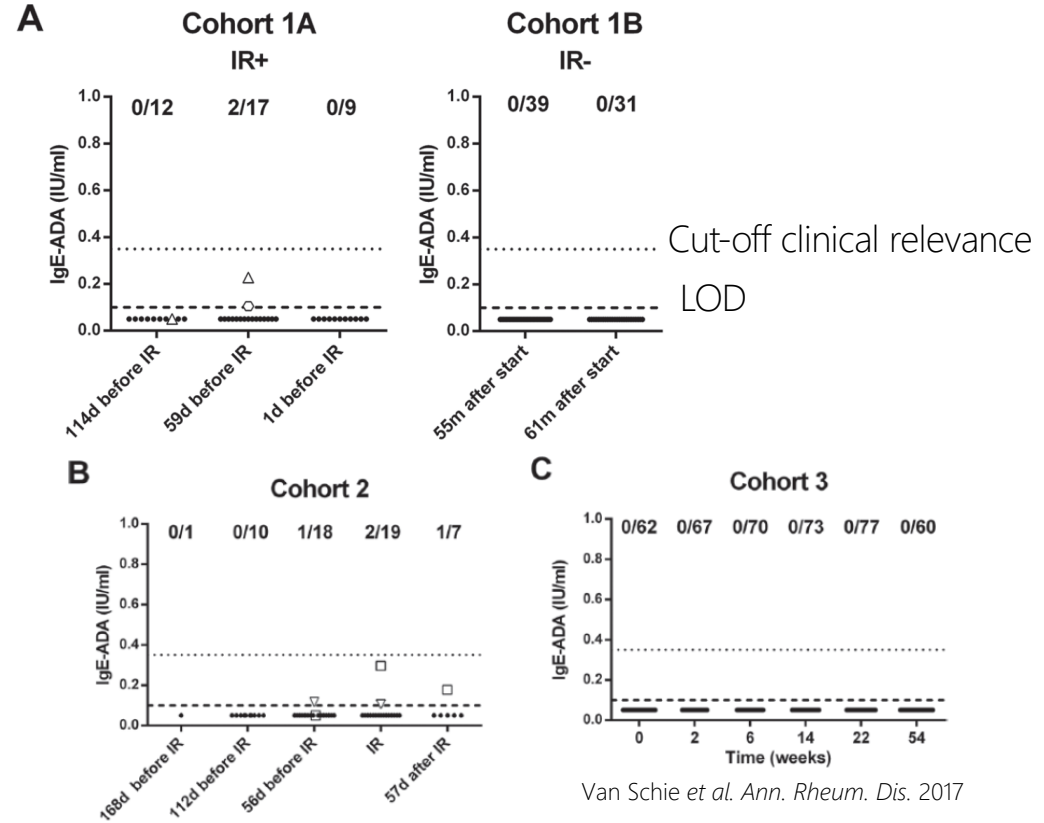
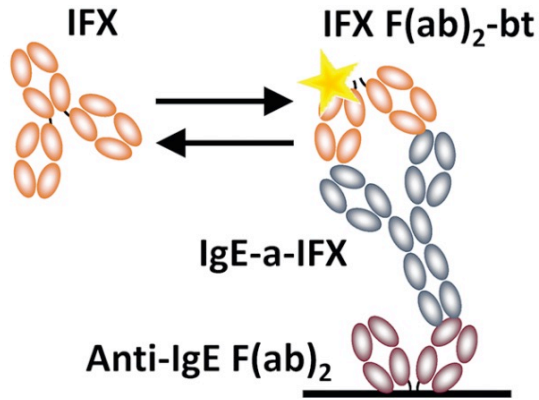
85 infliximab treated patients with RA measured in drug sensitive assay



125 infliximab treated patients with Crohn's disease measured in drug sensitive assay



Very limited IgE detection





Summary

- Increased sensitivity of ADA assays is not always needed (or even wanted)
- Drug tolerant assays are not preferred when clinically relevant ADA needs to be detected (at trough)
- Increased sensitivity and drug tolerant assays can confound effect of ADA on efficacy
- Side effects can be caused by ADA, but mostly related to high ADA levels
- Drug level is the main driver in treatment efficacy



Acknowledgements



Sanquin Diagnostic Services

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