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Clinical perspectives on immunogenicity of biotherapeutics

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Clinical Impact =

Severity of Consequences
X
Probability of Occurrence

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Immunogenicity

Immunogenicity is the ability of a particular substance, such as an antigen or epitope, to provoke an immune response in the body of a human or animal. In other words, **immunogenicity is the ability to induce humoral and/or cell-mediated immune responses**.

Wanted immunogenicity is typically related with vaccines, where the injection of an antigen (the vaccine) provokes an immune response against the pathogen (virus, bacteria...) aiming at protecting the organism. Vaccine development is a complex multistep process, immunogenicity being at the center of the vaccine efficiency.

Unwanted immunogenicity is an immune response by an organism against a therapeutic antigen (ex. recombinant protein, or monoclonal antibody). **This reaction leads to production of anti-drug-antibodies (ADAs)** inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects. The prediction of the immunogenic potential of novel protein therapeutics is thus a challenge in biotherapy

https://en.wikipedia.org/wiki/Immunogenicity

Overview

Drug	Indication	Type of product	ADA frequency	ADA consequences
IFNb	Multiple Sclerosis	Cytokine, native protein	1-30%	Breakthrough disease – alternative Rx
F VIII	haemophilia	Coagulation factor, native protein	20-30%	Breakthrough disease – Removal of ADA
Acid alpha-glucosidase (GAA)	Mb. Pompe	Enzyme, native protein	Depends on CRIM status	Breakthrough disease – Removal of ADA
Epo	anaemia	Enzyme, native protein	Eprex only	PRCA
Adalimumab	RA, IBD	Human mAb, anti-TNF	10-25%	Breakthrough disease – alternative Rx
Infliximab	RA, IBD	Chimeric mAb, anti-TNF	10-50%	Mostly IRA
Natalizumab	Multiple Sclerosis	Humanized mAb, anti VLA-4	5%	IRA, breakthrough disease – alternat. RX
Alemtuzumab	MS	Humanized mAb, anti-CD52	93%	IRA?
Bevacizumab	Cancers	Monoclonal, anti-VEGF	?	?

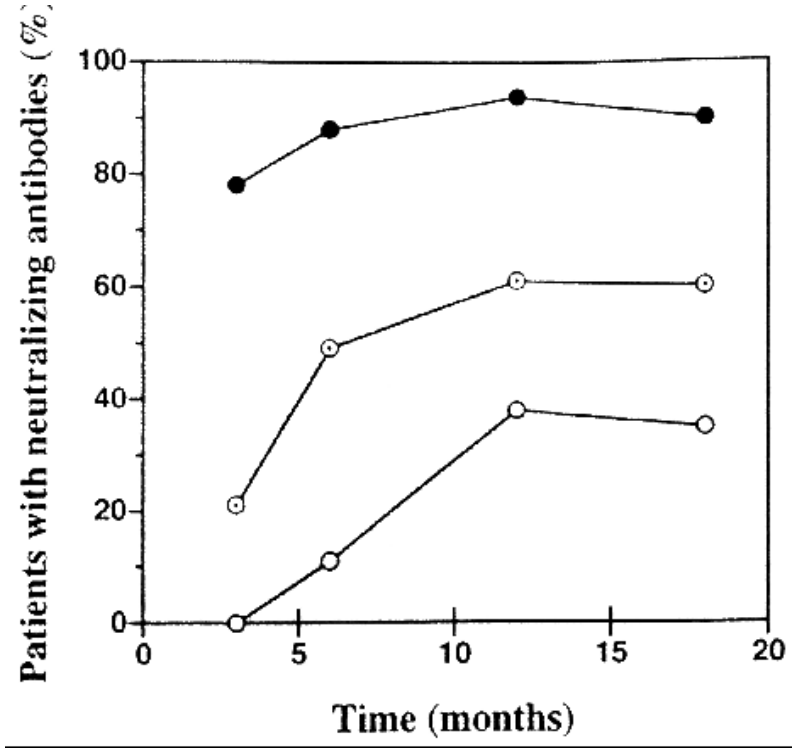
- → Anaphylaxis type 1
- → Infusion reaction
- → Cross-reactivity
- → Adverse events
- → Altered PK
- → Loss/lack of efficacy
- → No effect detected

Wide range of ADA in rheumatology

	Biological Agent	Abbreviation	ADAs% Min–Max	Nabs% Min–Max	Ref
TNFi	Infliximab	IFX	0–83.0	Not reported	[5,42]
	Adalimumab	ADL	0–54.0	Not reported	[5,42]
	Golimumab	GLM	0–19.0	Not reported	[5]
	Etanercept	ETA	0–18.3	Not reported	[5,42]
	Certolizumab pegol	CZP	3–37.0	Not reported	[5]
B cell-targeting biologics	Rituximab	RTX	23.1–50.0	Not reported	[49,50]
	Belimumab	BLM	0–4.8	Not reported	[51]
T cell activation inhibitor/co-stimulation modulator	Abatacept	ABT	0.9–4.1	0–0.4	[52–54]
IL-6R inhibitors	Tocilizumab	TCZ	0.7–2.0	0.8–1.3	[55,56]
	Sarilumab	SLM	1.4–12.3	0–10.8	[57,58]
IL-17A inhibitors	Secukinumab	SCK	0–1.0	Not reported	[5,59–61]
	Ixekizumab	IXK	1.7–9.0	Not reported	[62]
	Brodalumab	BDL	1.4–2.7	0	[59,63]
IL-12/23 and IL-23p19 inhibitors	Ustekinumab	UTK	1.0–11.0	Not reported	[5,42]
	Guselkumab	GKM	4.1–14.7	0.1–0.6	[64]
	Risankizumab	RZM	14.1–31.0	2.1–16.0	[64]
	Tildrakizumab	TZM	6.51–18.0	2.5–3.2	[64]
IL-1 inhibitors	Anakinra	ANA	<1	Not reported	[65,66]
	Canakinumab	CKM	3.1	0	[67]

J. Clin. Med. 2023, 12, 3271.

Sensitivity Of NAB Assay Depends On IFN Used In The System



- 3 LU/ml (high sensitivity)
- 10 LU/ml (medium sensitivity)
- 100 LU/ml (low sensitivity)

N=40, Betaferon 8MIU

Ross et al. Ann Neurol. 2000

Known factors (1/2)

ADA development has a complex multifactorial etiology

- **Genetic factors** influence the threshold for developing ADA
  - HA:
    - ✓ FVIII gene mutation type:
      - large deletions, nonsense mutations, and gene inversions => higher risk
      - missense mutations => lower risk Ref.: Miller et al. 2012; Oldenburg et al. 2002
    - ✓ Polymorphisms in immune response genes: IL-10, TNF-α, CTLA-4, HMOX-1 Ref.: Astermark et al.; Repessé et al. 2013
    - ✓ HLA class I and HLA class II alleles DQ and DR15 Ref.: Hay et al. 1997; Oldenburg et al. 1997
    - ✓ Family history of inhibitors => higher risk Ref.: Astermark et al. 2001, Chalmers et al. 2007, Goudemand et al. 2006
    - ✓ Ethnicity: Blacks and Hispanics => higher risk Ref.: Astermark et al. 2005; Miller et al. 2012; Ragni et al. 2009
  - MS:
    - ✓ HLA class II haplotypes (HLA-DRB1) Ref.: Hoffmann et al. 2008; Buck et al. 2011

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Known factors (2/2)

- **Environmental factors** determine whether sufficient immune activation occurs to overcome the threshold for ADA formation
  - HA
    - ✓ Periods of intensive treatment (bleeds, surgery) => higher risk — Ref.: ter Avest et al. 2008; Gouw et al. 2007
  - RA / IBD
    - ✓ Concomitant treatment with immunosuppressant => lower risk — Ref.: Bendtzen et al. 2006; Lee et al. 2012

- **Disease-related factors,** themselves influenced by genetic and environmental factors, may also mediate the risk of ADA development:
  - MS
    - ✓ Oligoclonal immunoglobulin G (IgG) band-negativity => lower risk — Ref.: Lundkvist et al. 2010
  - RA
    - ✓ High disease baseline activity => higher risk — Ref.: Bartelds et al. 2011
  - Pompe
    - ✓ CRIM status — Kishnani PS et al Mol Genet and Metab 2010

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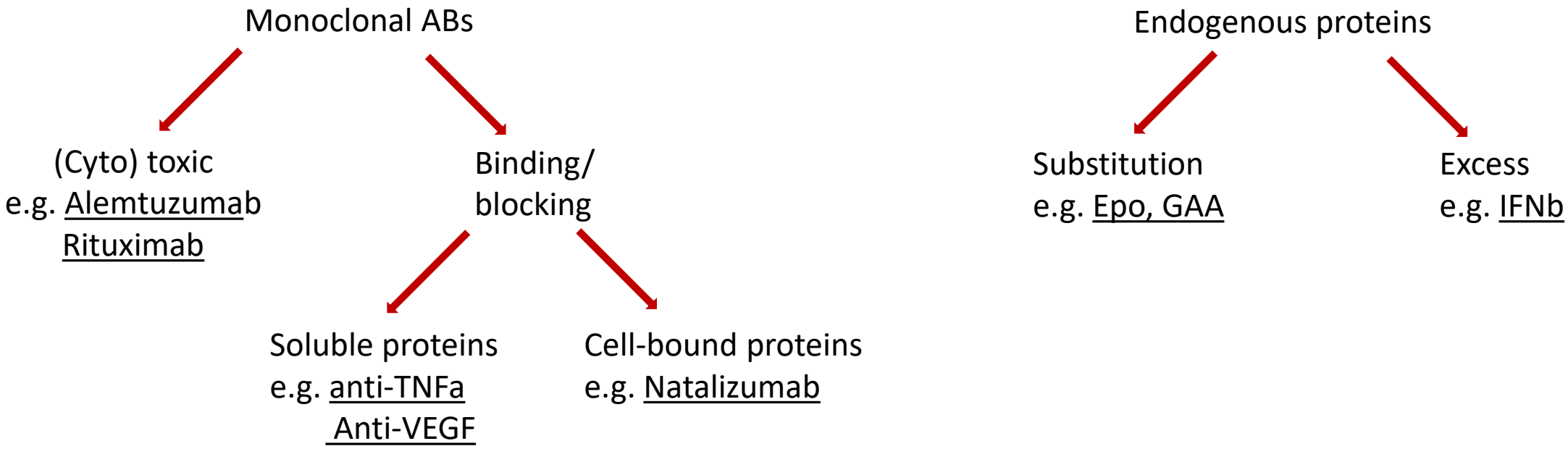
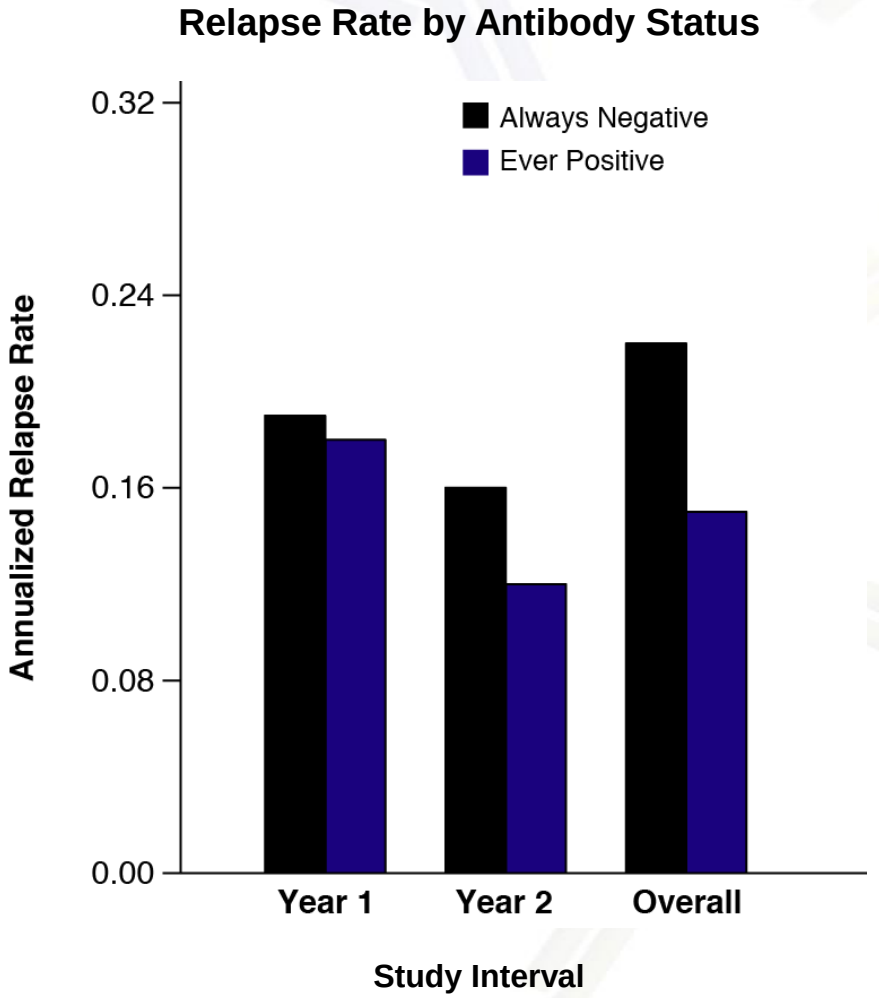
Biologicals by MoA



Anti-alemtuzumab Antibodies in CARE-MS I

- Anti-alemtuzumab antibodies are known to develop in response to alemtuzumab administration
  - Anti-drug antibodies have negatively impacted some MS treatments
- 87% of patients were “ever positive” for anti-alemtuzumab antibodies
- **6.7% of patients with anti-alemtuzumab antibodies were “always negative” for inhibitory antibodies**
- Correlations sought with anti-alemtuzumab status and titer
  - Pre-treatment
  - Peak within-treatment
- Anti-alemtuzumab antibody status was not correlated with relapse rate (Figure), risk of sustained accumulation of disability (SAD), imaging endpoints, safety outcomes, or pharmacodynamics



Data on file, Genzyme Corporation.

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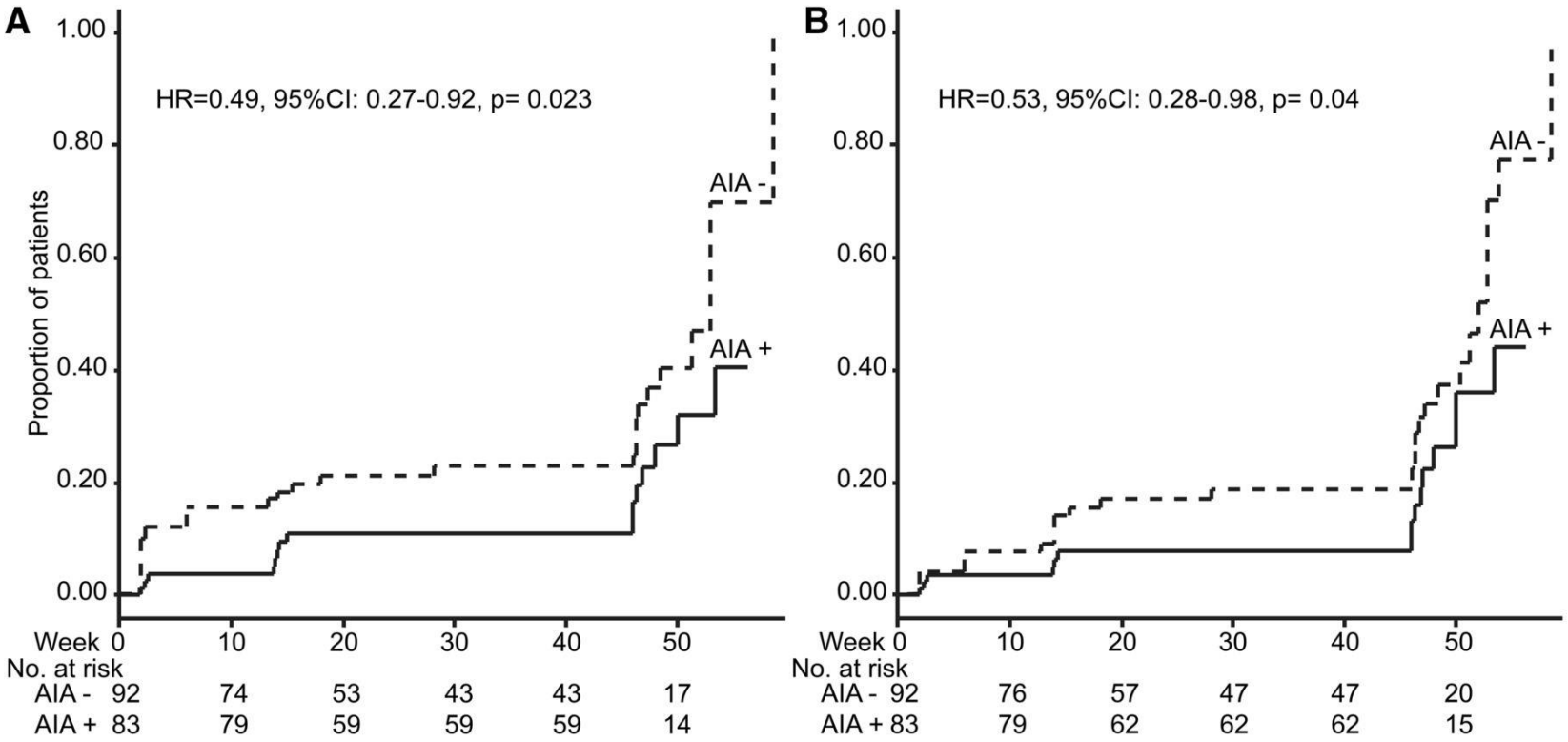
Rheumatoid arthritis infliximab:

Sustained minimal disease activity and sustained remission.

Sustained minimal disease activity (A) and sustained remission (B) in patients classified according to anti-infliximab status during the 52-week follow-up.



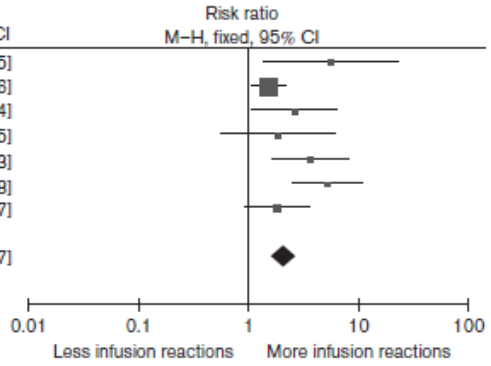
Sophine B. Krintel et al. Rheumatology 2013;52:1245-1253

Clinical consequences of anti-Infliximab Abs in IBD

Fig. 3

Study or subgroup	ATI +ve Events	ATI +ve Total	ATI –ve Events	ATI –ve Total	Weight	Risk ratio M-H, fixed, 95% CI
Farrell *et al.* [18]	10	25	2	28	3.5%	5.60 [1.35, 23.15]
Hanauer *et al.* [11]	29	80	71	297	56.4%	1.52 [1.06, 2.16]
Maser *et al.* [4]	11	22	5	26	8.6%	2.60 [1.07, 6.34]
Miele *et al.* [12]	4	12	4	22	5.3%	1.83 [0.56, 6.05]
Rutgeerts *et al.* [14]	5	14	21	215	4.8%	3.66 [1.62, 8.23]
Rutgeerts *et al.* [14]	6	12	17	176	4.1%	5.18 [2.51, 10.68]
Sands *et al.* [15]	13	44	13	80	17.3%	1.82 [0.93, 3.57]
Total (95% CI)		209		844	100.0%	2.07 [1.61, 2.67]
Total events	78		133			

Heterogeneity: $\chi^2 = 13.32$, $d.f. = 6$ ($P = 0.04$); $I^2 = 55\%$
Test for overall effect: $Z = 5.67$ ($P < 0.00001$)

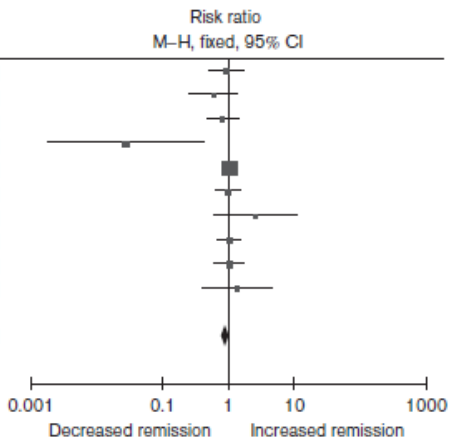


Rates of infusion reactions.

Fig. 4

Study or subgroup	Positive ATI Events	Positive ATI Total	Negative ATI Events	Negative ATI Total	Weight	Risk ratio M-H, fixed, 95% CI
Ainsworth *et al.* [20]	10	18	9	15	4.9%	0.93 [0.52,1.66]
Candon *et al.* [21]	3	6	11	13	3.5%	0.59 [0.26,1.36]
Colombel *et al.* [9]	8	14	12	17	5.4%	0.81 [0.47, 1.40]
Farrell *et al.* [18]	0	25	30	43	11.3%	0.03 [0.00, 0.43]
Hanauer *et al.* [11]	62	80	223	297	47.0%	1.03 [0.90, 1.18]
Maser *et al.* [4]	14	22	17	26	7.7%	0.97 [0.64, 1.48]
Rutgeerts *et al.* [14]	3	14	3	36	0.8%	2.57 [0.59, 11.26]
Rutgeerts *et al.* [14]	11	19	45	79	8.7%	1.02 [0.66, 1.56]
Sands *et al.* [15]	14	44	25	80	8.8%	1.02 [0.59, 1.75]
Seow *et al.* [3]	8	44	3	22	2.0%	1.33 [0.39, 4.54]
Total (95% CI)		286		628	100.0%	0.90 [0.79, 1.02]
Total events	133		378			

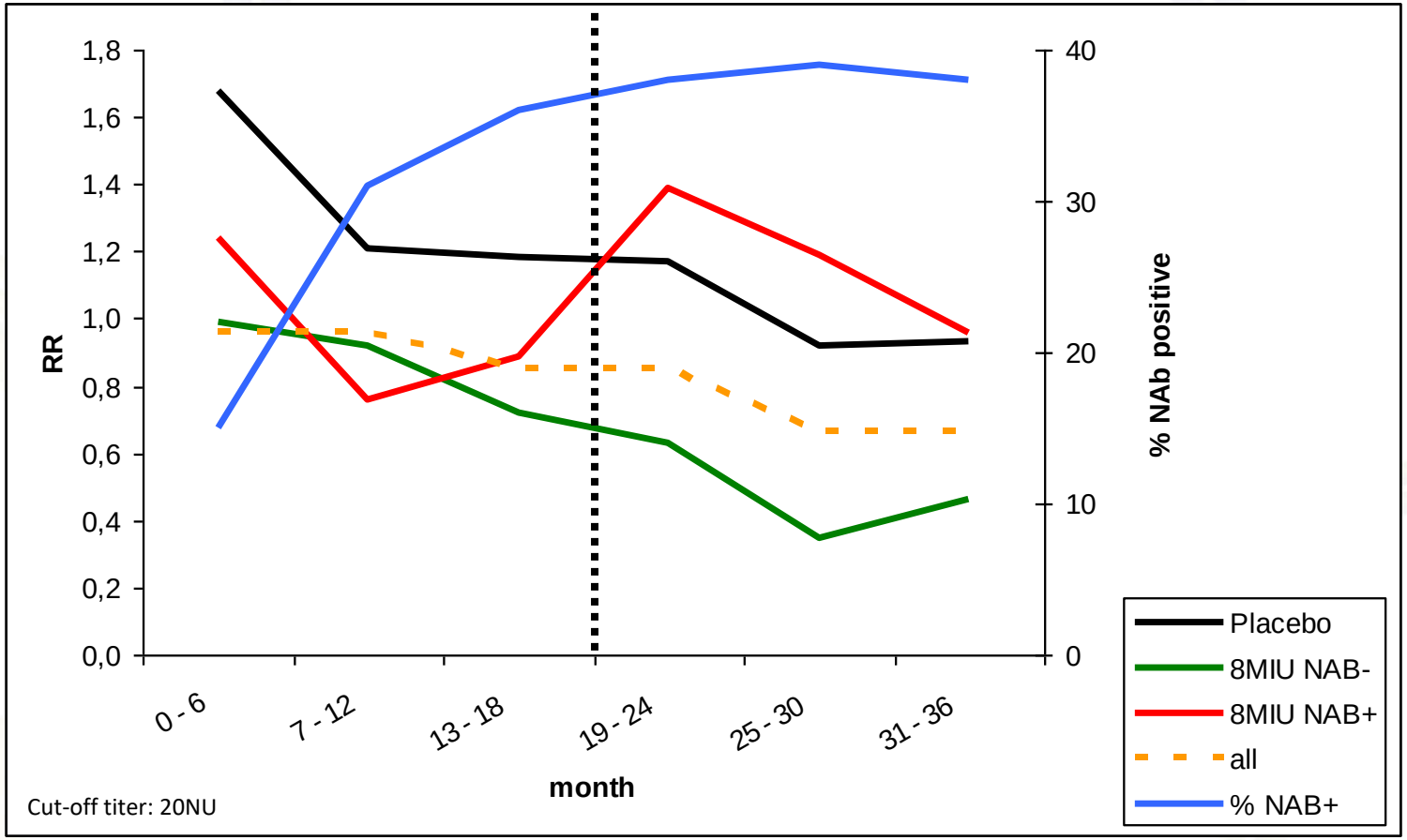
Heterogeneity: $\chi^2 = 14.32$, $d.f. = 9$ ($P = 0.11$); $I^2 = 37\%$
Test for overall effect: $Z = 1.64$ ($P = 0.10$)



Rates of clinical remission. ATI, anti-infliximab antibodies.

European Journal of Gastroenterology & Hepatology 2012, 24:1078–1085

NAB timeline (Betaferon trial)



IFNB study group
Neurology 1996;47:889-894

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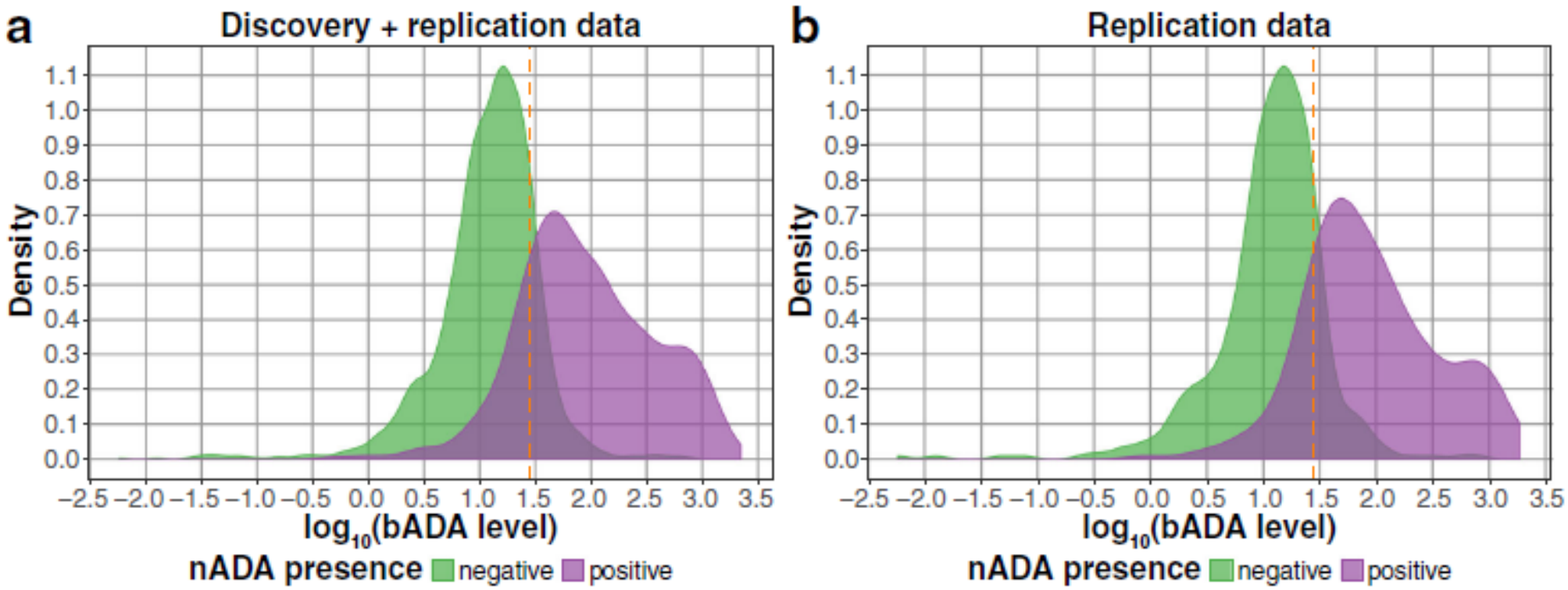
NABs and BABs



Andlauer et al. BMC Medicine (2020) 18:298

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Titre-dependency of clincal effects

*Table 4 Percentage increase in relapse rates for eventually NAB+ subgroups in low- and high-NAB+ periods relative to NAB− periods**

	Increase in relapse rate					
	Low NAB+, cutoff titer 20			High NAB+		
Cutoff titer	% Increase	95% CI	*p* Value	% Increase	95% CI	*p* Value
"Once positive, always positive"						
100, n = 57	48	10, 98	0.01	39	0, 94	0.05
200, n = 45	41	5, 90	0.02	70	21, 140	0.002
400, n = 33	41	6, 89	0.02	115	54, 200	<0.001
"All switches considered"						
100, n = 57	29	−5, 75	0.10	21	−10, 62	>0.20
200, n = 45	23	−9, 65	0.17	46	7, 99	0.02
400, n = 33	22	−8, 62	0.17	82	37, 140	<0.001

Patient counts refer to patients with high-NAB+ status, which differ from counts in eventually NAB+ subgroups for respective cutoff titers as no confirmation was required.

* Definition of low-NAB+ periods (confirmation required) refers to cutoff titer of 20. Definition of high-NAB+ periods (no confirmation required) refers to respective cutoffs.

NAB = neutralizing antibodies.

Neurology 2003;60:37-43.

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Time of First Positive Result

In Patients Who Developed Any Antibodies

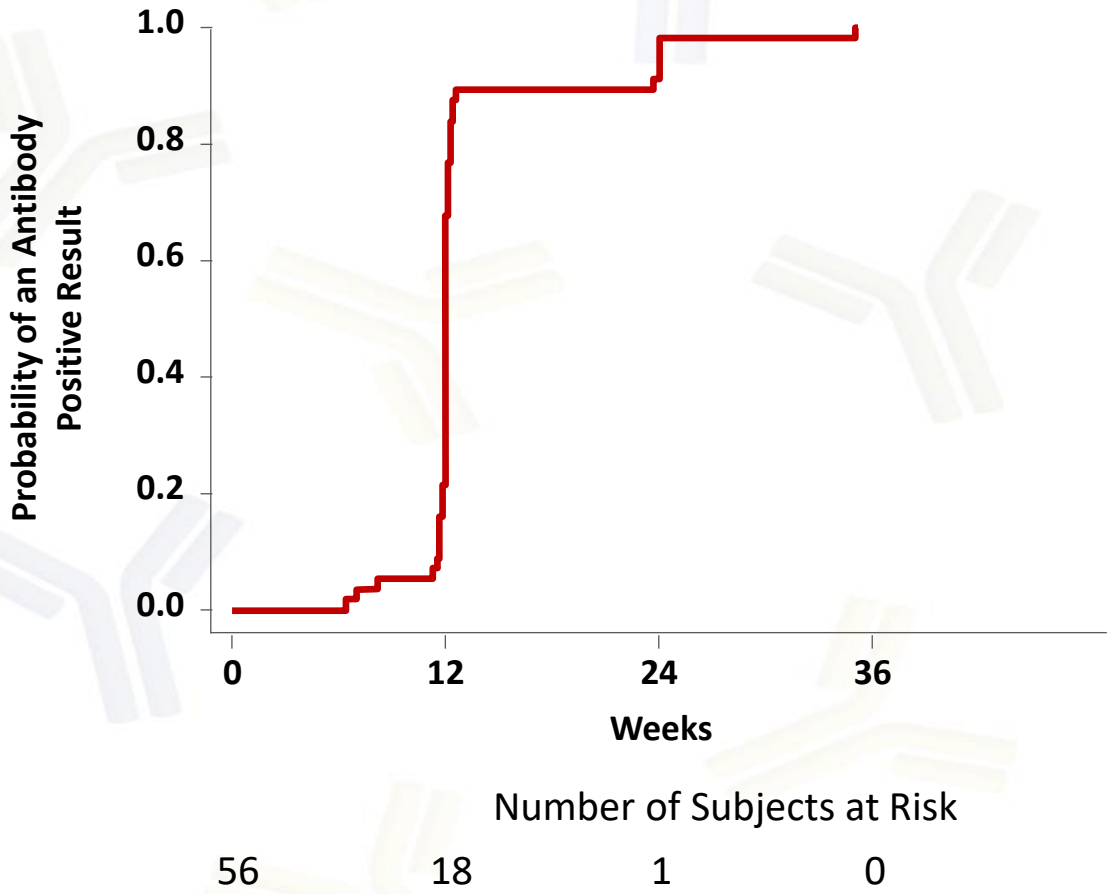

Table 3. The antibody titres (in ATU) of 30 antibody-positive patients at three months (± 0.2) time points.

Patient number	ATU (three months)	Positivity
1	4	Transient
2	7	Transient
3	8	Transient
4	8	Transient
5	9	Transient
6	10	Persistent
7	10	Transient
8	12	Transient
9	16	Transient
10	18	Transient
11	25	Transient
12	36	Persistent
13	55	Transient
14	113	Persistent
15	134	Transient
16	156	Persistent
17	176	Persistent
18	393	Persistent
19	418	Persistent
20	433	Persistent
21	887	Persistent
22	929	Persistent
23	1002	Persistent
24	1040	Persistent
25	1523	Persistent
26	2212	Persistent
27	2236	Persistent
28	2442	Persistent
29	2677	Persistent
30	3293	Persistent

Mult Scler. 2012 Oct;18(10):1493-9

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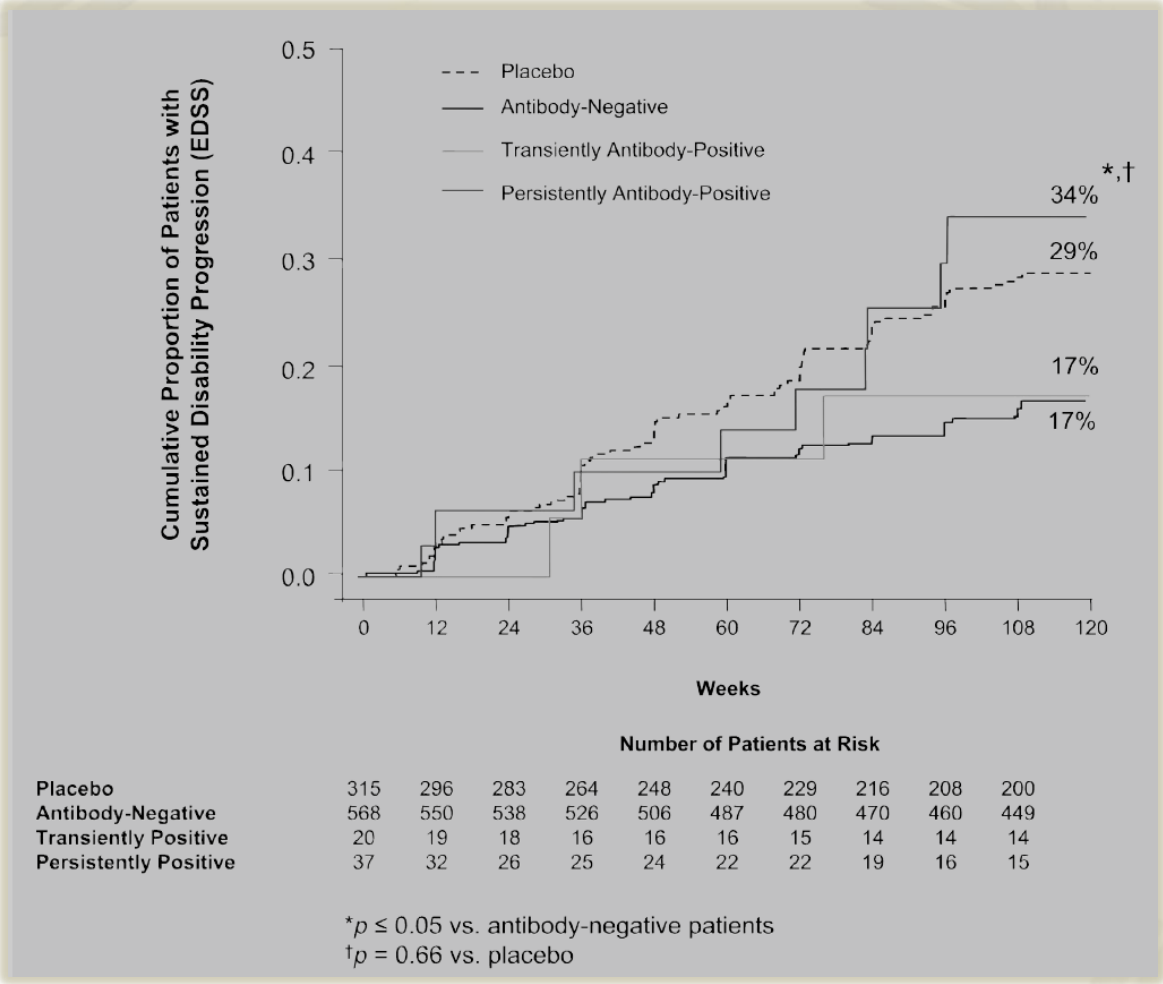
EDSS progression (95% CI) by antibody status over 2 years of natalizumab treatment



Neurology® 2007;69:1391–1403

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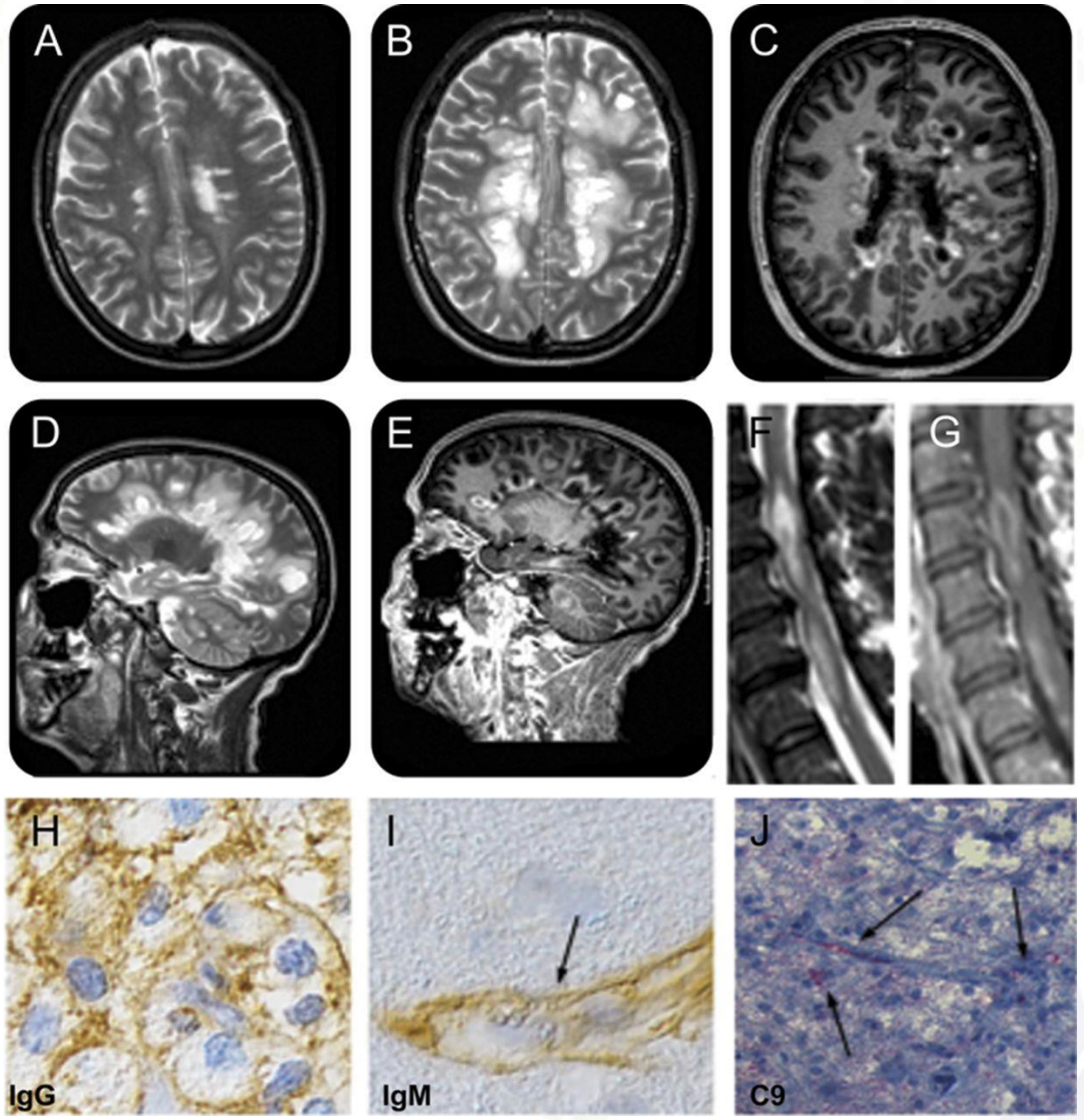
Rare severe consequences of ADA in Natalizumab treatment of MS

Patient developed ADA against Natalizumab within 2 months and severe disease rebound after 6 months followed by fatal course of brain inflammation.

Figure Imaging and immunostaining(A–G) MRIs showing aspects of the malignant transformation.



Anders Svenningsson et al. Neurology 2013;80:965-967

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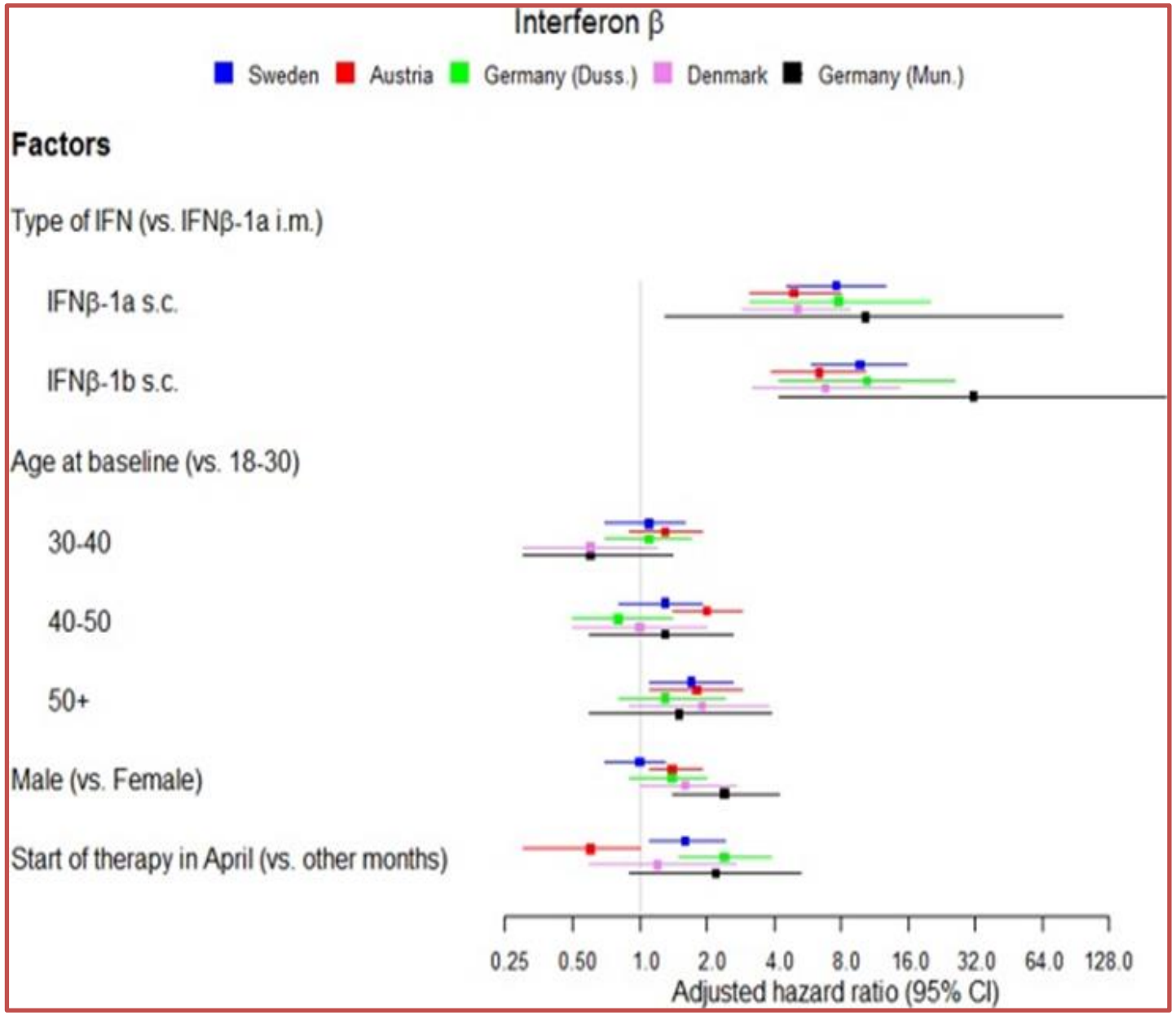
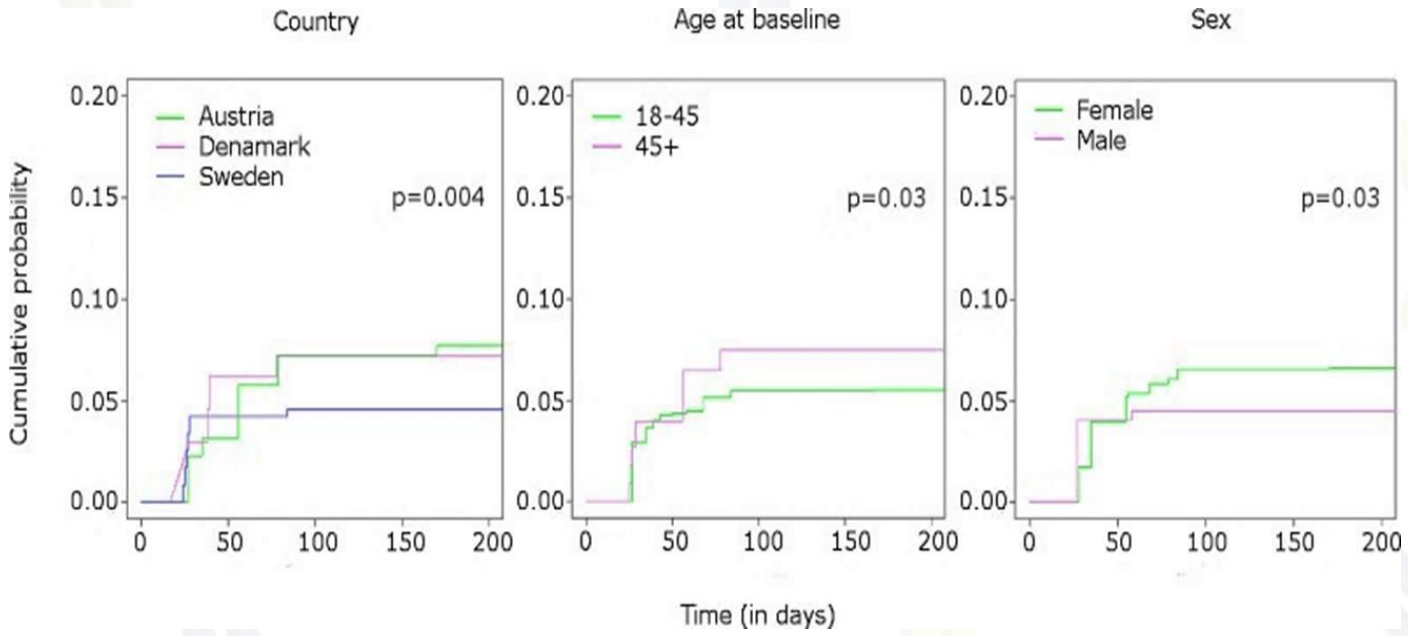
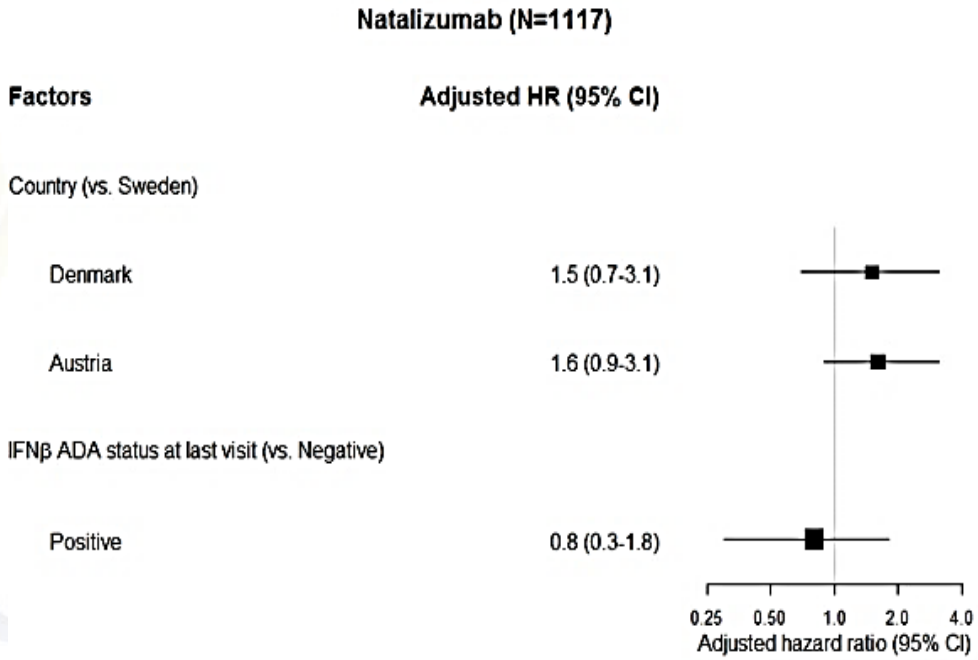


PLoS One. 2016; 11(11): e0162752.

IFNb ADA and risk for anti-Natalizumab ADA





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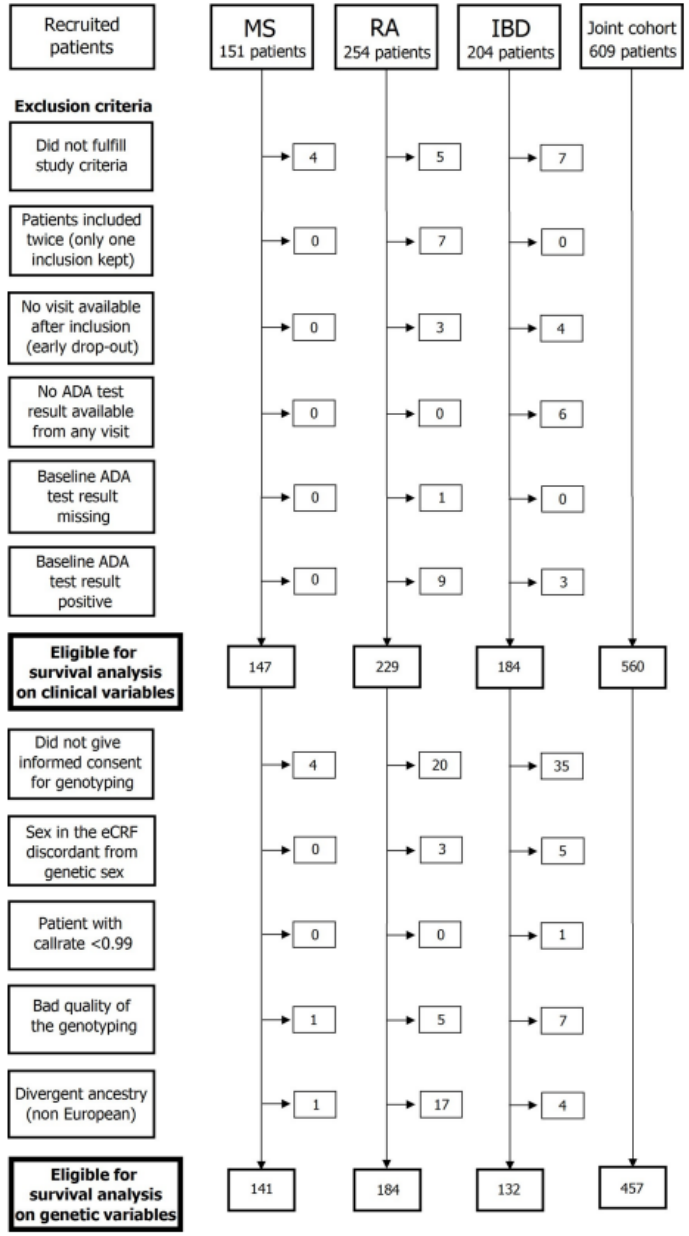
Common factors prospective study

Table 1. Demographics of the ABIRISK cohorts. Abbreviations: ABIRISK, Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK; BMI, body mass index; IBD, inflammatory bowel disease; IQR, interquartile range; MS, multiple sclerosis; RA, rheumatoid arthritis; SD, standard deviation.

		IBD, N = 184	MS, N = 147	RA, N = 229
Age, mean (SD)		36.9 (13.7)	35.1 (9.7)	54.2 (13.7)
Sex, N (%)	Female	89 (48.4)	103 (70.1)	176 (76.9)
	Male	95 (51.6)	44 (29.9)	53 (23.1)
Smoke, N (%) (cigarettes per day)	0	125 (68.3)	106 (72.6)	169 (74.8)
	1–10	34 (18.6)	18 (12.3)	33 (14.6)
	11–40	24 (13.1)	22 (15.1)	24 (10.6)
BMI, N (%)	Underweight	17 (9.3)	5 (3.4)	6 (2.7)
	Normal	114 (62.3)	84 (57.9)	110 (49.3)
	Overweight	40 (21.9)	29 (20)	56 (25.1)
	Obese	12 (6.6)	27 (18.6)	51 (22.9)
Country, N (%)	Austria	0 (0)	29 (19.7)	0 (0)
	Belgium	6 (3.3)	0 (0)	0 (0)
	Czech Republic	0 (0)	50 (34)	0 (0)
	France	150 (81.5)	0 (0)	136 (59.4)
	Germany	0 (0)	27 (18.4)	0 (0)
	Israel	28 (15.2)	0 (0)	0 (0)
	Italy	0 (0)	0 (0)	9 (3.9)
	Netherlands	0 (0)	0 (0)	65 (28.4)
	Spain	0 (0)	22 (15)	0 (0)
	Sweden	0 (0)	10 (6.8)	0 (0)
	Switzerland	0 (0)	9 (6.1)	0 (0)
	United Kingdom	0 (0)	0 (0)	19 (8.3)
Follow-up, days; median (IQR)		338.0 (63.5)	358.0 (175.0)	357.5 (158.25)

https://doi.org/10.1371/journal.pmed.1003348.t001



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Timing and frequency of ADA

Table 2. ADA occurrence during 12 months, stratified by disease and by BP therapy. ADA, antidrug antibody; BP, biopharmaceutical product; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; IM, intramuscular; MS, multiple sclerosis; RA, rheumatoid arthritis; SC, subcutaneous; TNF, tumor necrosis factor.

Total N (ADA positive N)		IBD	MS	RA
TNF inhibitors	etanercept			84 (3)
	infliximab	86 (13)		15 (3)
	adalimumab	98 (38)		55 (26)
IFN-beta	IFNb-1a IM		38 (0)	
	IFNb-1a SC		68 (11)	
	IFNb-1b SC		41 (26)	
Anti-CD20	rituximab			31 (16)
Anti-IL6-R	tocilizumab			44 (4)

https://doi.org/10.1371/journal.pmed.1003348.t002

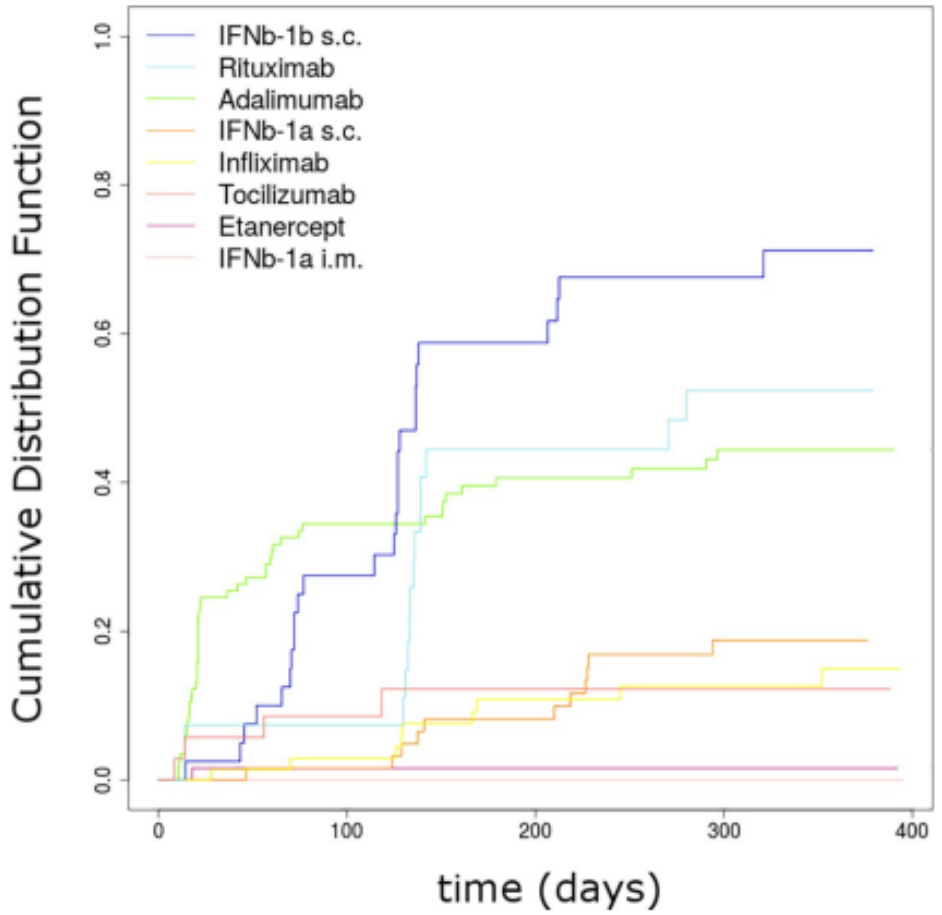

Fig 3. ADA occurrence by BP treatment. ADA, antidrug antibody; BP, biopharmaceutical product; IFNb, interferon beta; IM, intramuscular; SC, subcutaneous.

https://doi.org/10.1371/journal.pmed.1003348.g003

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Multivariate Analyses

Table 5. Results of multivariate Cox regression of time to ADAs on infections, immunosuppressants, antibiotics, tobacco smoking, rs10508884 SNP, and HLA (DQA1*05) with stratification on the disease status. ADA, antidrug antibody; CI−, lower 95% confidence interval; CI+, upper 95% confidence interval; HLA, Human Leukocyte Antigen; HR, hazard ratio; SNP, Single-Nucleotide Polymorphism.

N = 457		HR	CI− (0.95)	CI+ (0.95)	p-Value
Tobacco (heavy smokers)	yes	2.150	1.319	3.503	0.002
Infections	yes	2.757	1.616	4.704	0.0002
Immunosuppressants	yes	0.408	0.253	0.657	0.0002
Antibiotics	yes	0.121	0.0437	0.333	4.5×10^{-5}
rs10508884	[Aa]	1.901	1.254	2.883	0.0025
	[aa]	3.804	2.139	6.764	5.4×10^{-6}
HLA (DQA1:05)	[Aa]	1.474	0.983	2.211	0.0605
	[aa]	3.900	1.923	5.976	2.4×10^{-5}

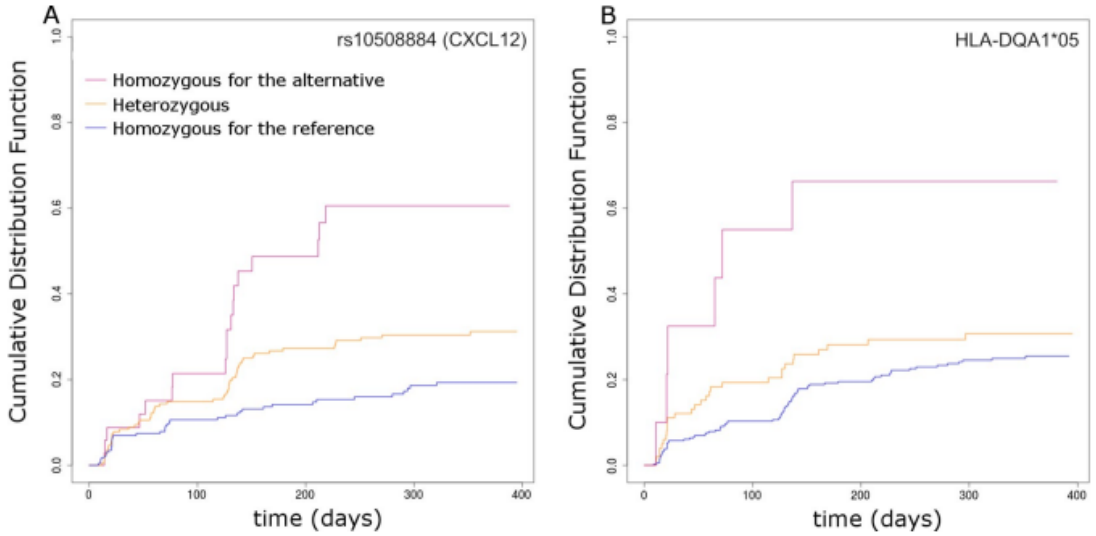

Fig 5. ADA occurrence according to (A) rs10508884 (*CXCL12*) or (B) *HLA-DQA1*05*. ADA, antidrug antibody; HLA, Human Leukocyte Antigen.

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Summary

Wide variation of clinical consequences of ADA

Wide variation of ADA frequency

Interference between factors driving disease and factors driving immunogenicity

Assay set up

Underlying disease, age sex

Timing

Common denominators: Smoking, infections, co-medication, some preliminary genetic factors

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