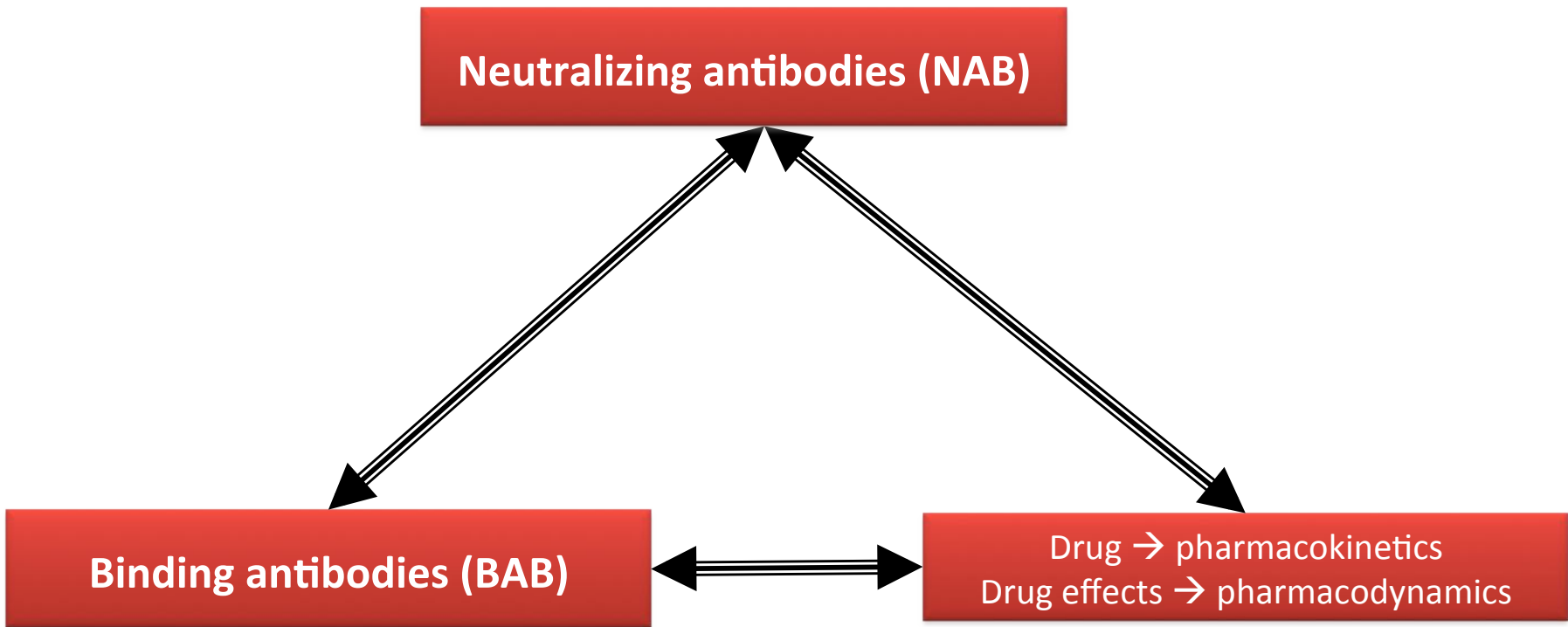


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# **Multiple Sclerosis: Immunogenic potential of interferon-beta and physicochemical properties of anti-drug antibodies**



# NAbs, BAbs, PK/PD

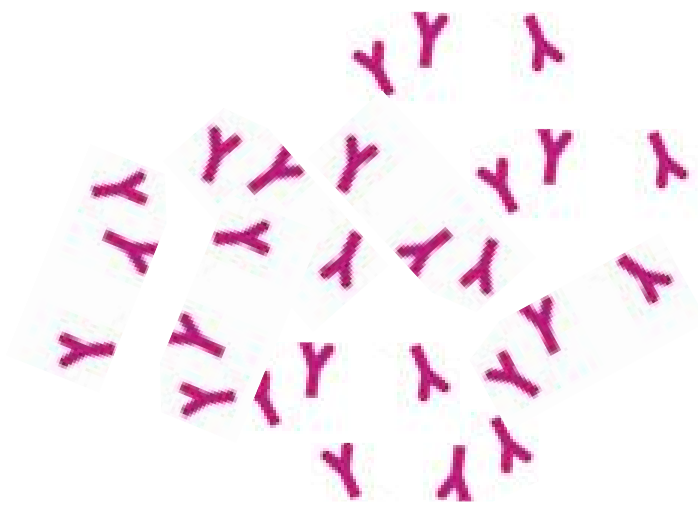


Anti - Drug - Antibodies

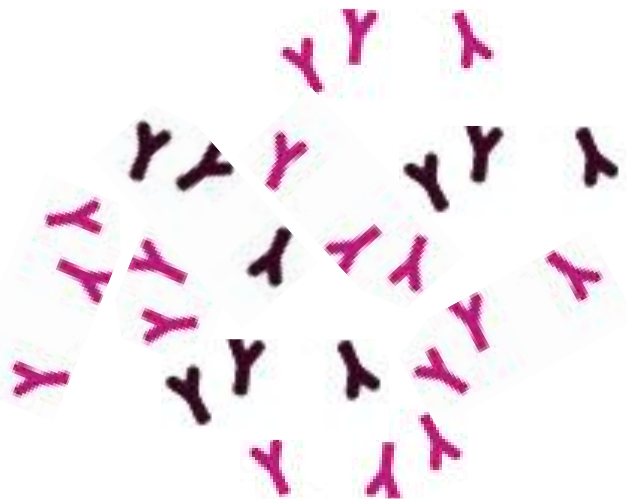
# Reality of antibody terminology

- BAB (binding antibodies)
- NAB (neutralizing antibodies)
- NNAB (non-neutralizing antibodies)
- ADA (anti-drug antibodies)
- ATA (antibodies to adalimumab or anti therapeutic antibodies)
- Etc.....

# NAbs, BAbs, PK/PD



# NAbs, BAbs, PK/PD



# BAB and NAB

- Is neutralization of a drug a quantitative or a qualitative phenomenon, or both?
- $BAB = NAB$
- $BAB \neq NAB$
- $BAB > NAB$

# Interferons

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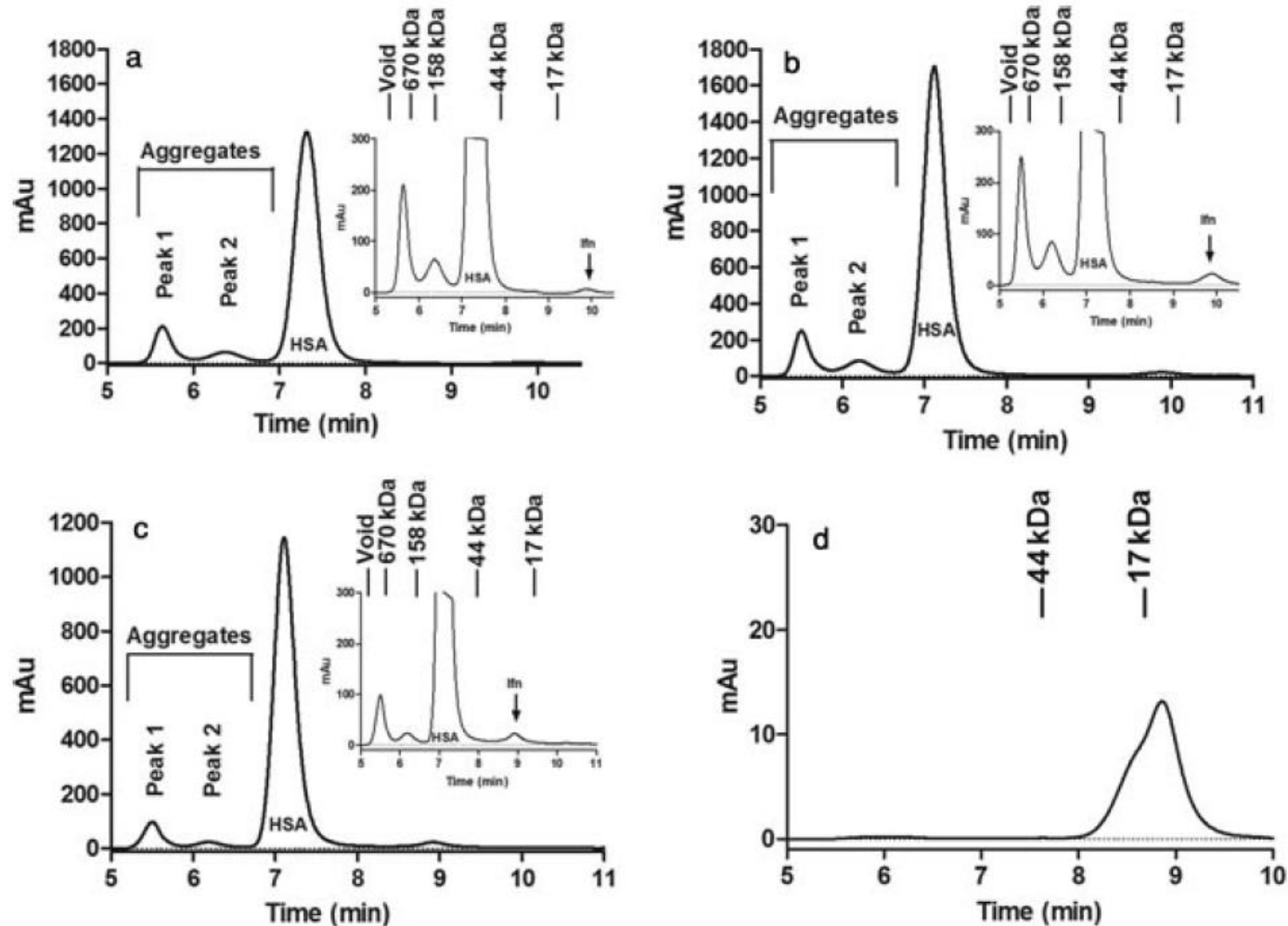
# Frequency of NAb

## Exogenous factors

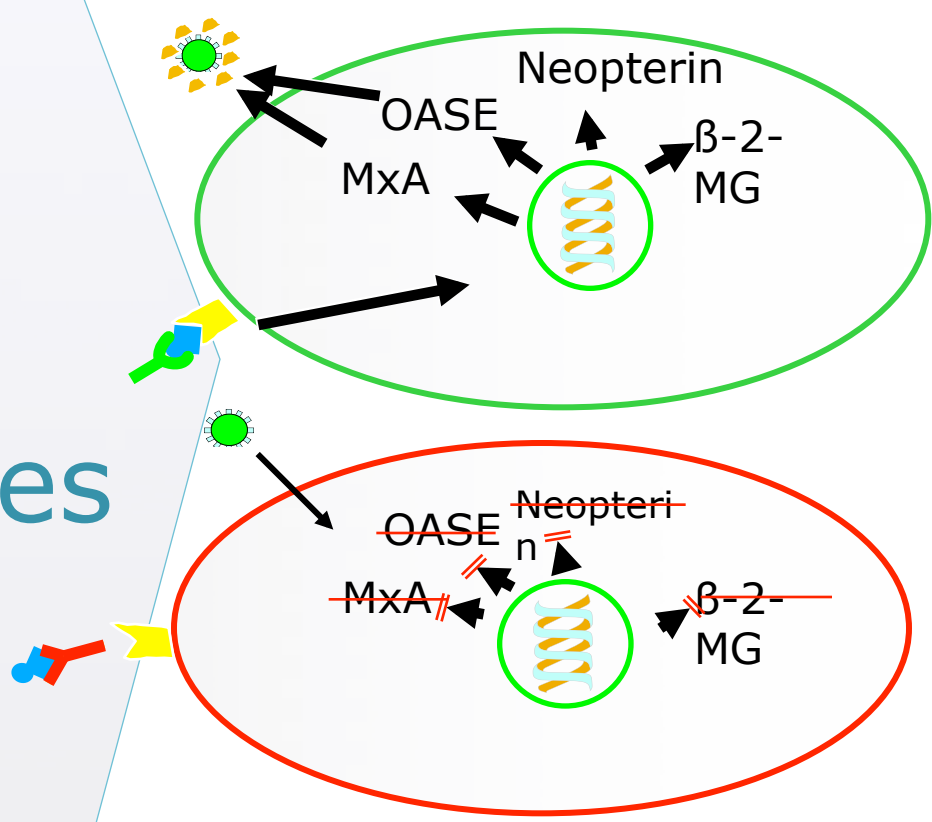
Preparation	Patients tested for NAb	NAb positive N (%)	High titer N (%)
IFN $\beta$ -1b	239	74 (31%)	43 (18%)
IFN $\beta$ -1a i.m.	202	11 (5%)	9 (4%)
IFN $\beta$ -1a s.c.	405	118 (29%)	83 (20%)
Total	846	203 (24%)	135 (16%)

$\chi^2$ :  $p < 0.0001$

# Drug aggregates and NABs



# Neutralizing Bioassays



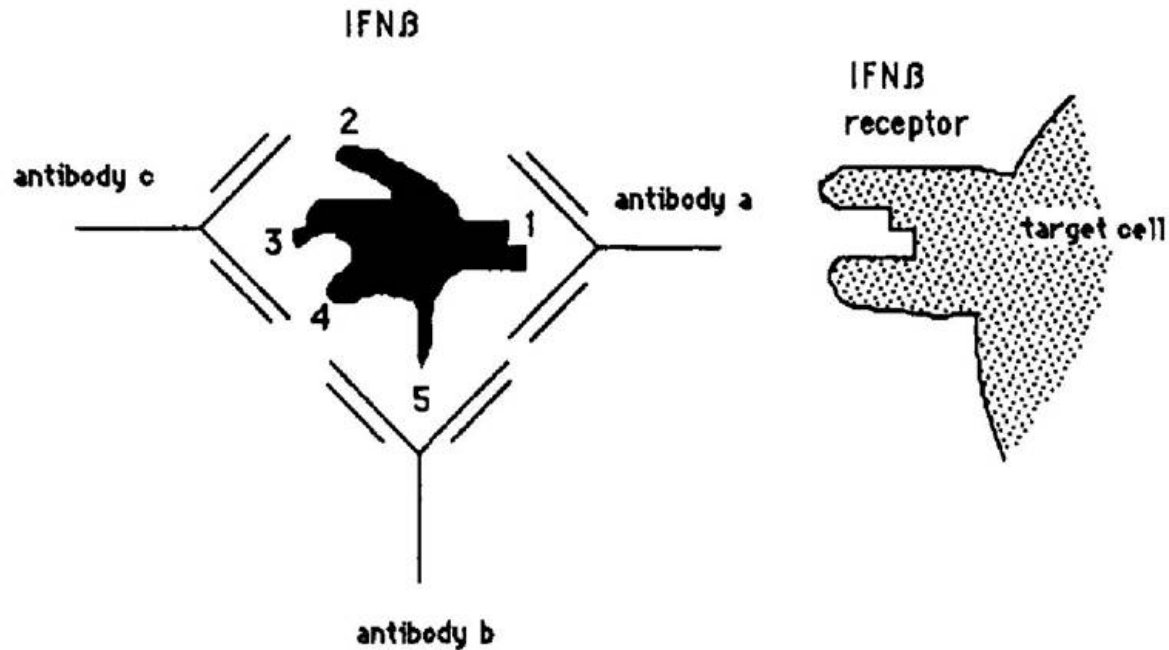
## Antibodies

Binding assays

# Distribution of Ig Subclasses in NAB and NNAB positive patients

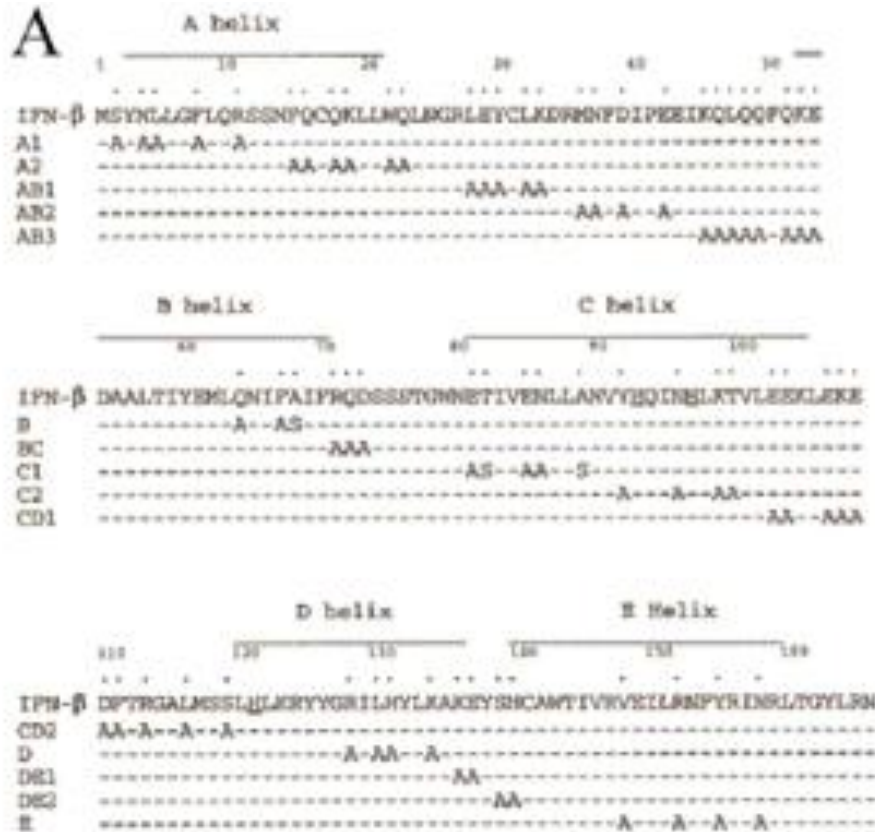
	N	IgG1 N (%)	IgG2 N (%)	IgG3 N (%)	IgG4 N (%)	IgA N (%)	IgM N (%)	Average age (years)	Mean duration of therapy (months)
NNAB	39	39 (100)	1 (3)	6 (15)	7 (18)	2 (5)	10 (26)	37.9	19.3
NAB	20	20 (100)	6 (30)	4 (20)	11 (55)	3 (15)	7 (35)	39.1	17.4
P value			<b>0.005</b>	0.72	<b>0.003</b>	0.32	0.45	0.65	0.39

# Epitope specificity



*Figure. Anti-IFN $\beta$  antibodies a, b, and c bind to different IFN $\beta$  epitopes.*

# Epitope specificity of NAB



32 peptides with 12 AA  
Offset 5 AA

Peptide 1: res 1-12  
 Peptide 2: res 6-17  
 Peptide 3: res 11-22

- .
- .
- .
- .
- .

# Distribution of binding sites

## NAB vs NNAB

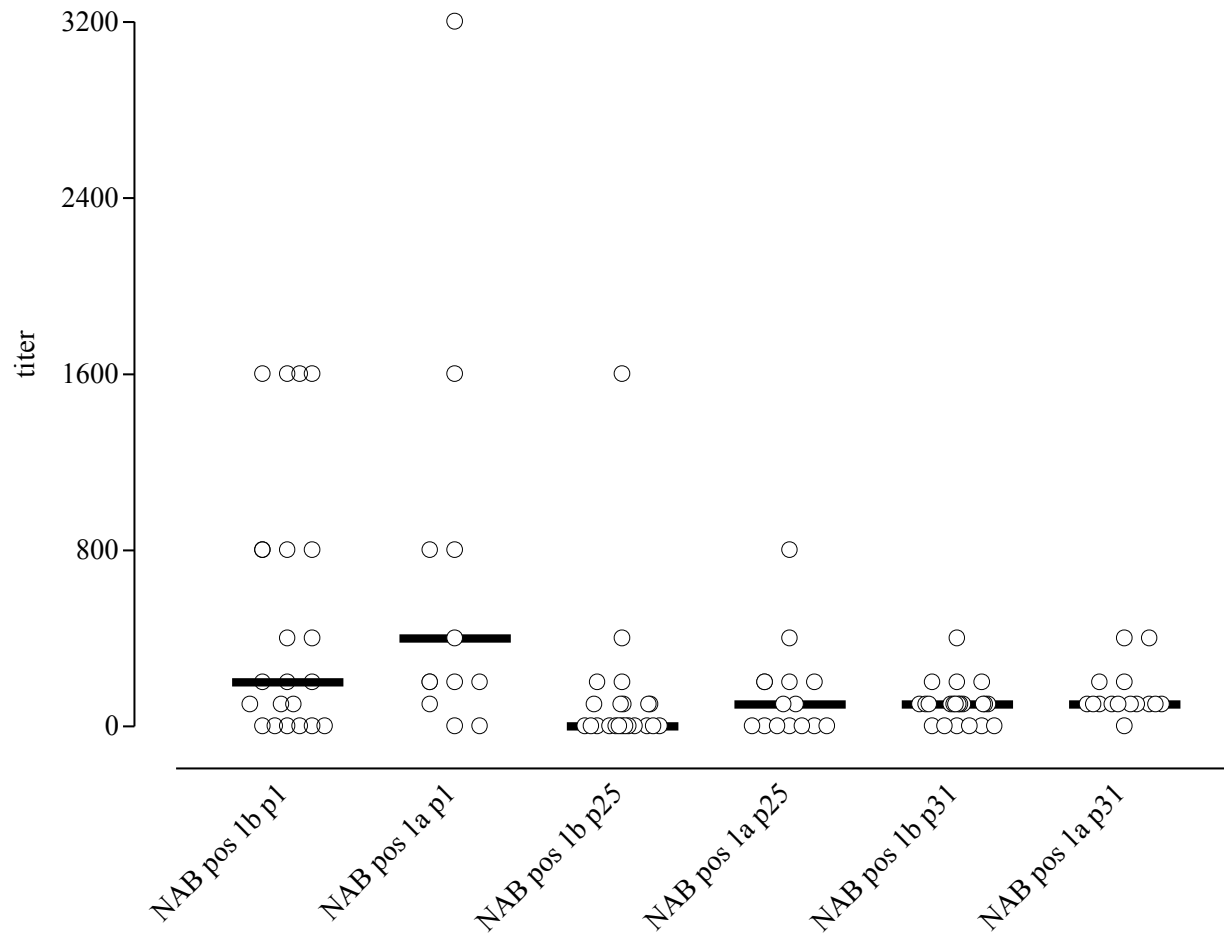
	Residues		
	1 - 12	121-132	151-162
NAB positive samples (n=37)	15 (41%)	23 (62%)	9 (24%)
NAB negative samples (n=34)	5 (15%)	24 (71 %)	2 (6%)
p value	0.02	ns	0.048

# Correlation of NAB titers with epitope specific titers

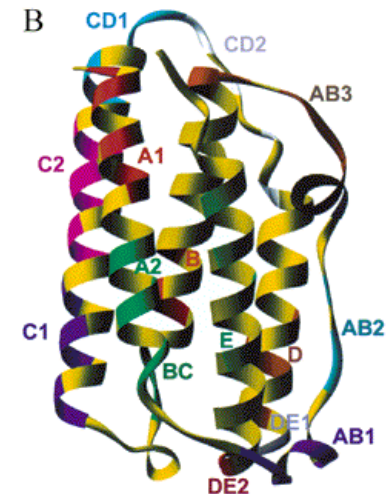
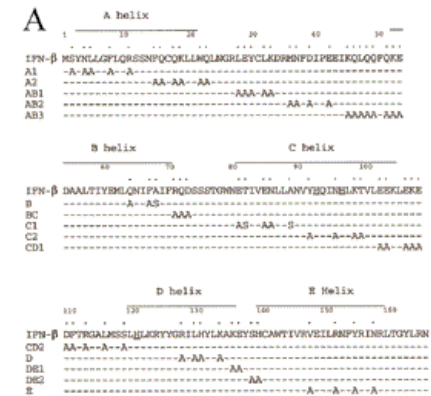
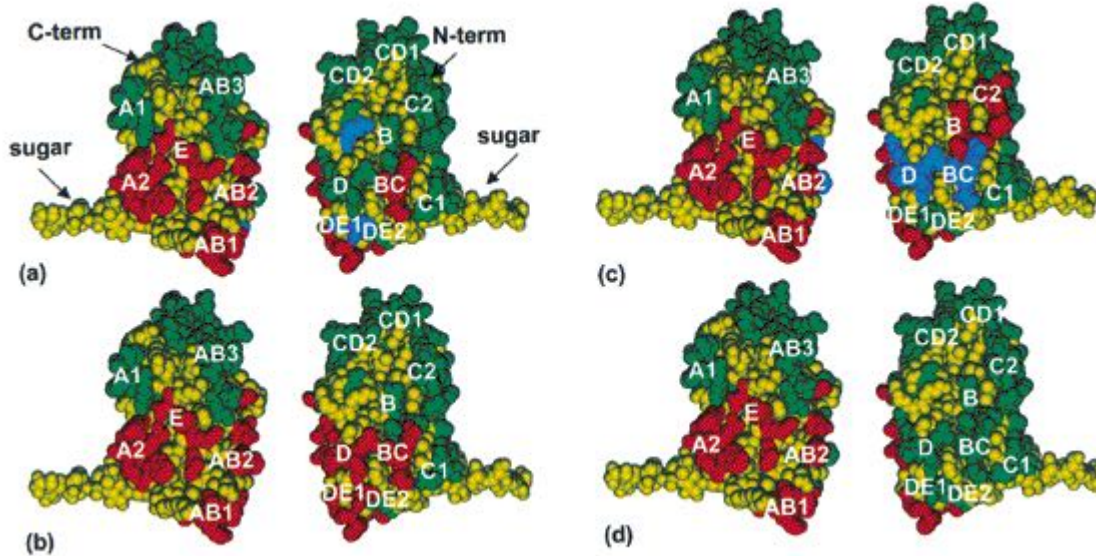
NAB-titer vs.	BAB-titer	1 - 12	121 - 132	151 - 162
$r$	0.27	<b>0.55</b>	0.1	0.17
$p$	0.1	<b>0.0005</b>	0.55	0.31



# Epitope specific titers 1a vs 1b in NAB positive patients

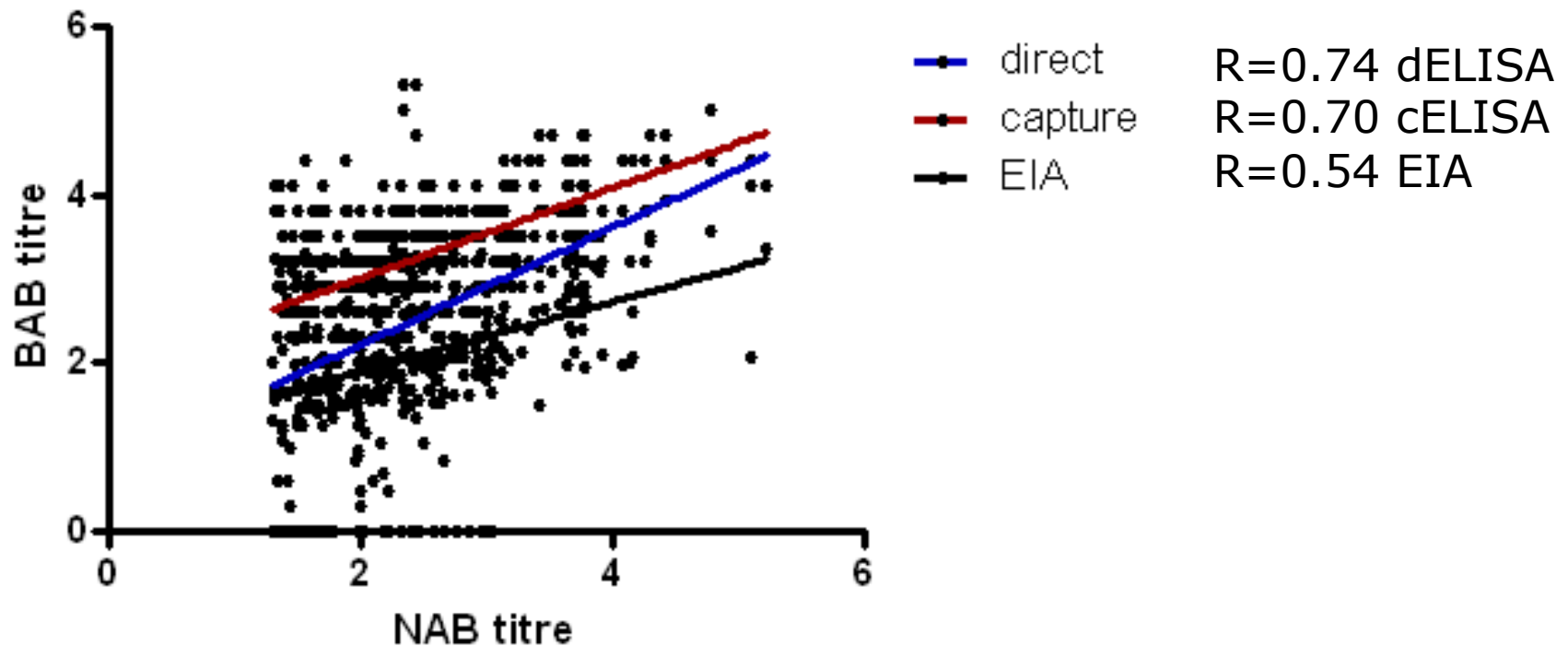


# Receptor binding and bioactivity of IFN $\beta$ in different AA-substitutions

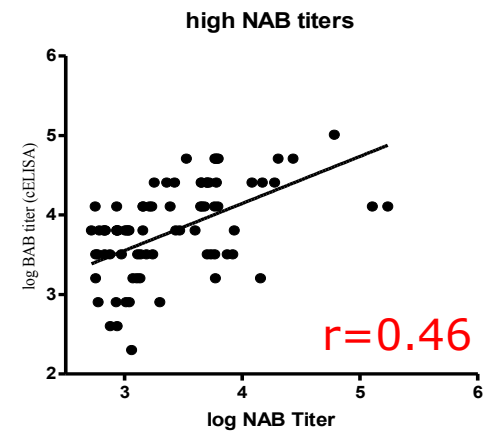
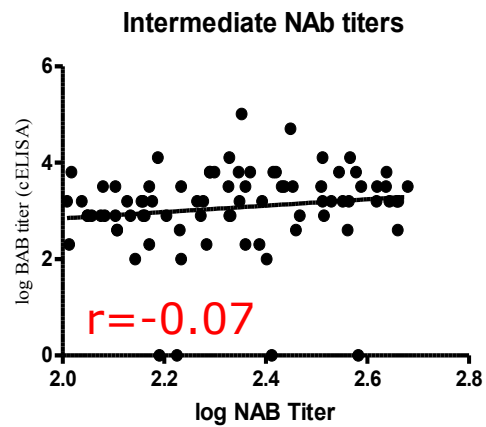
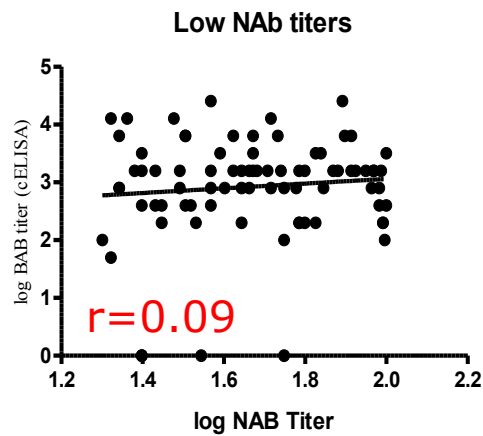


Green: no change of binding and loss of activity  
 Blue: slightly changed binding (a-c)  
 Red: clear change of binding and activity

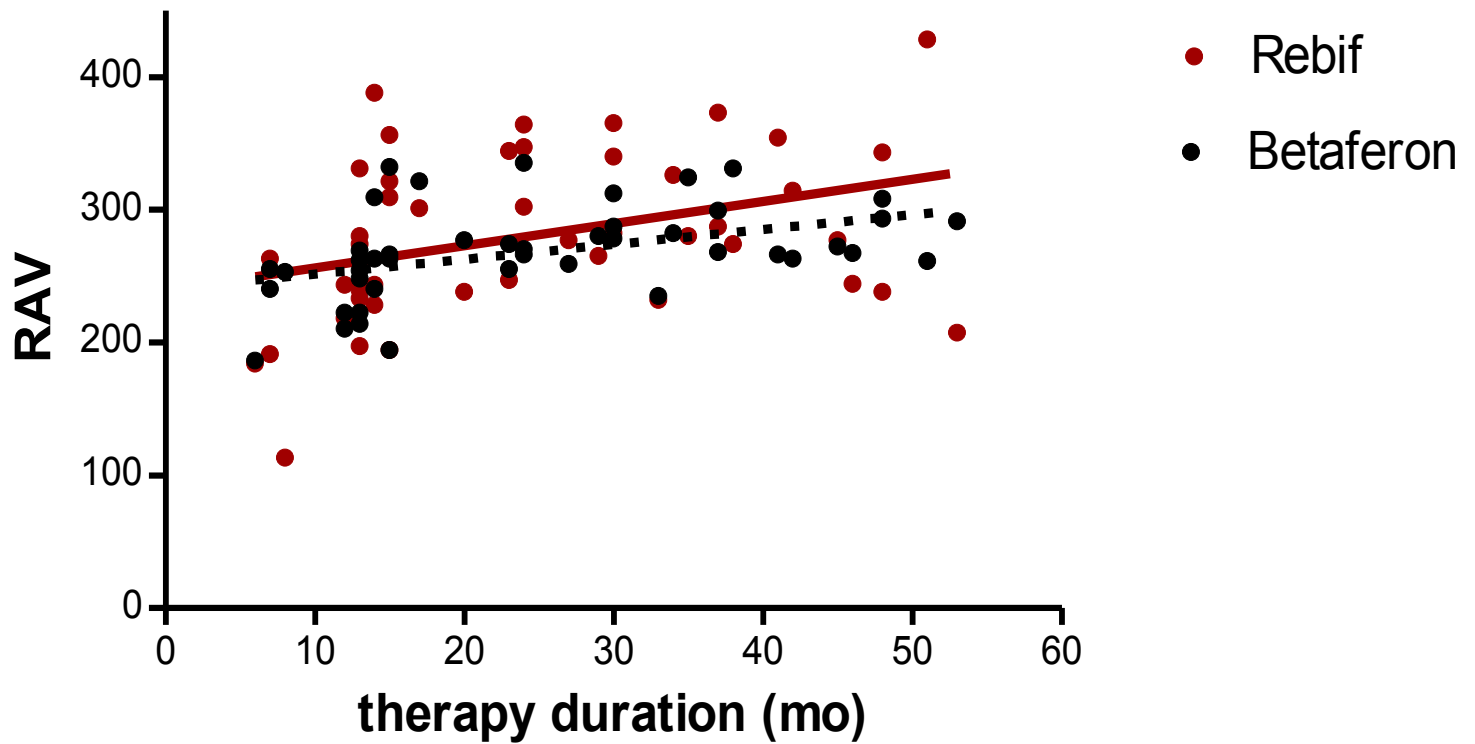
# Correlation NAB vs various BAB titres



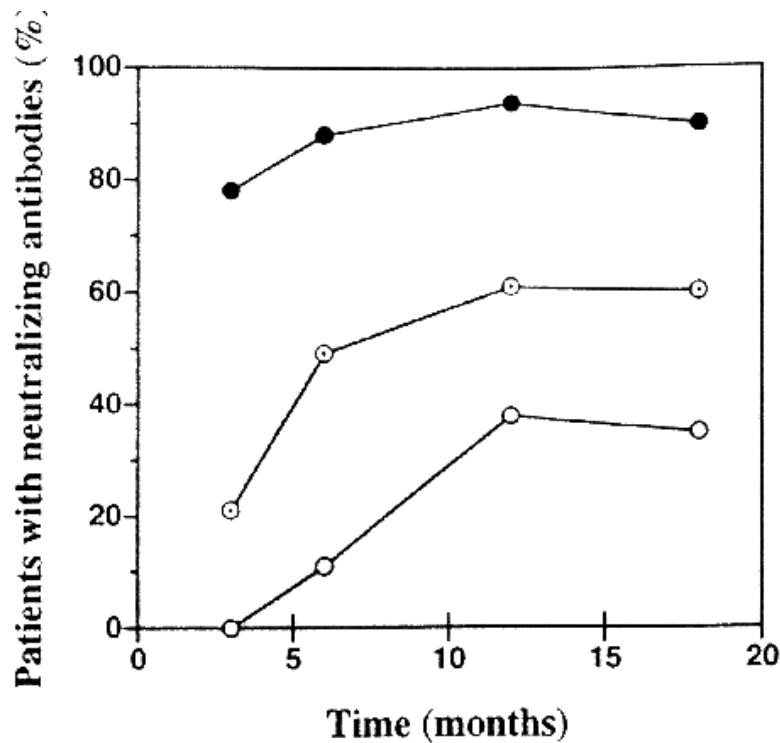
# BAB and NAB correlation depending on NAB titre



# Affinity maturation IFN $\beta$ 1a vs 1b



# Sensitivity Of NAB Assay Depends On IFN Used In The System



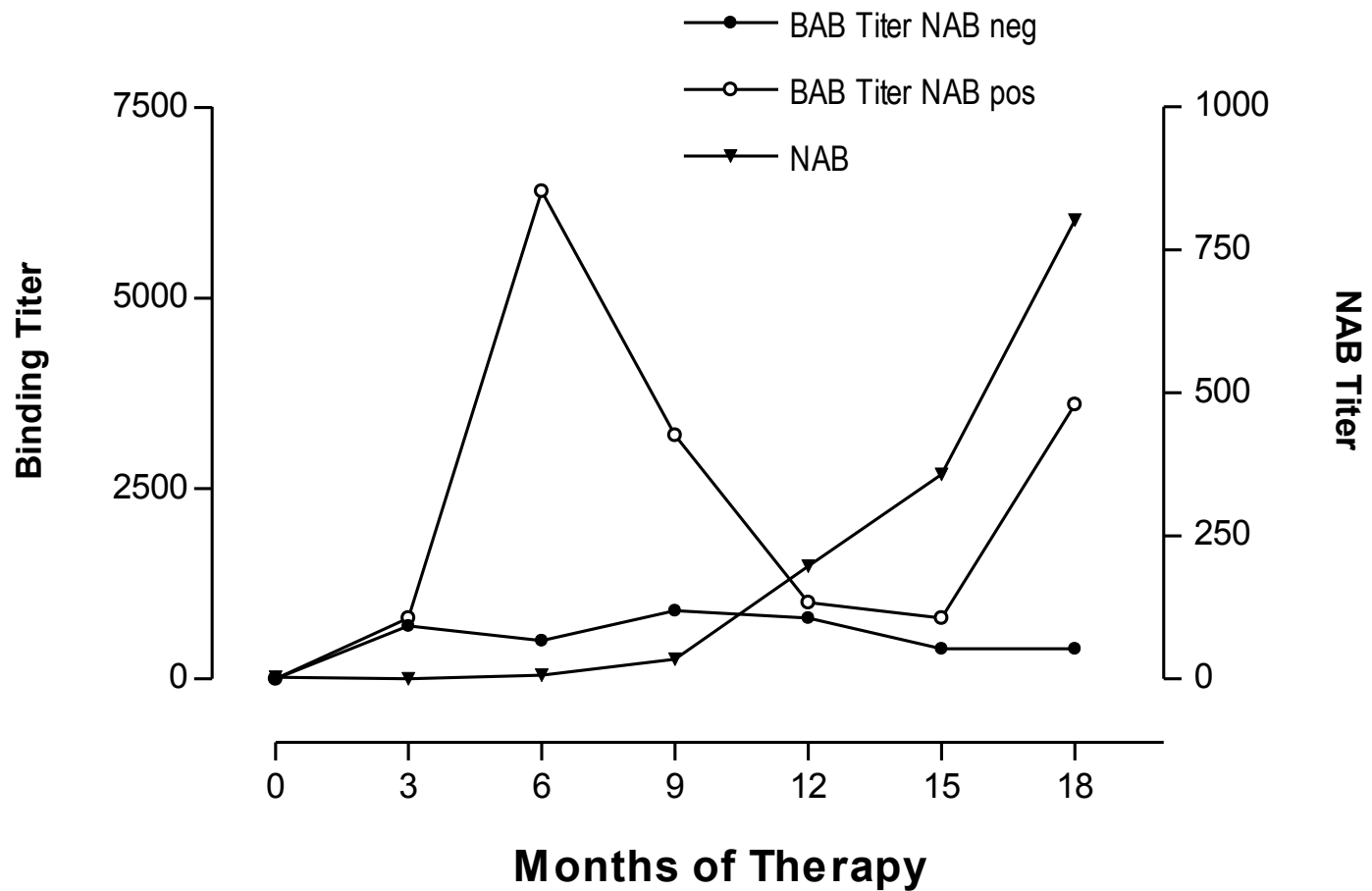
➤ 3 LU/ml (high sensitivity)

➤ 10 LU/ml (medium sensitivity)

➤ 100 LU/ml (low sensitivity)

N=40, Betaferon 8MIU

# Binding titers in NAB+ and NAB- patients

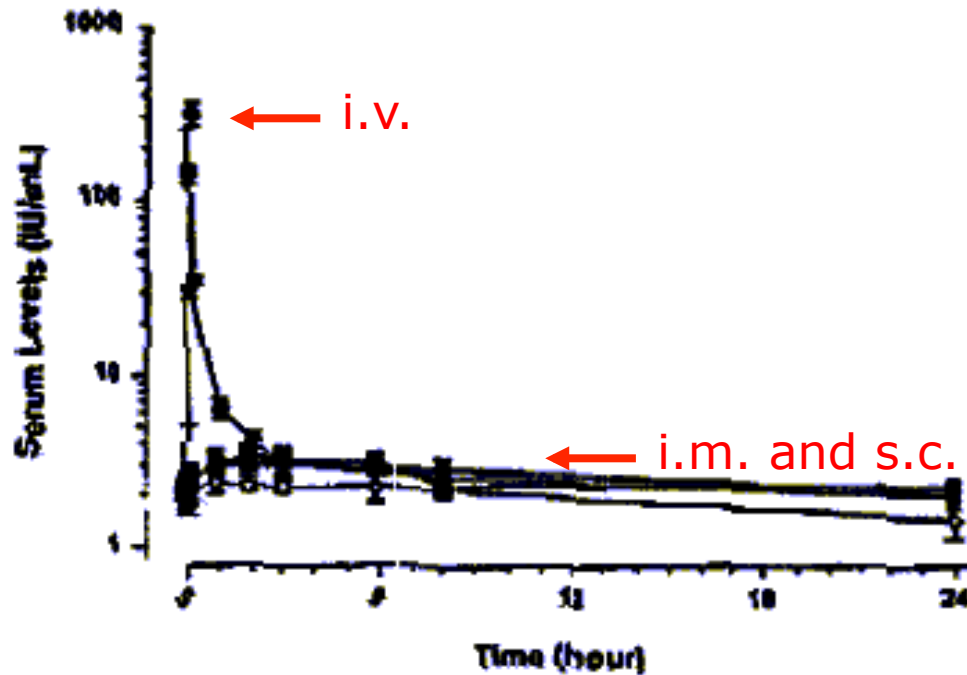


# NAB and PK/PD

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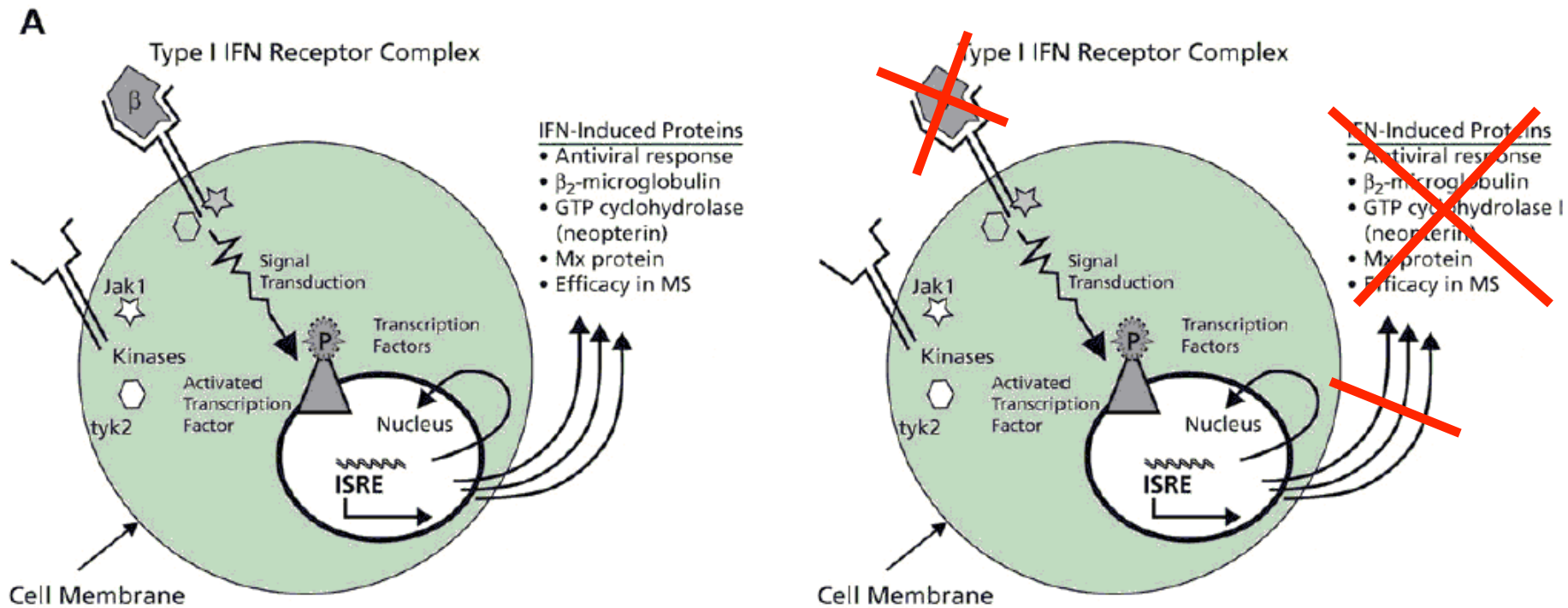
# IFN $\beta$ PK



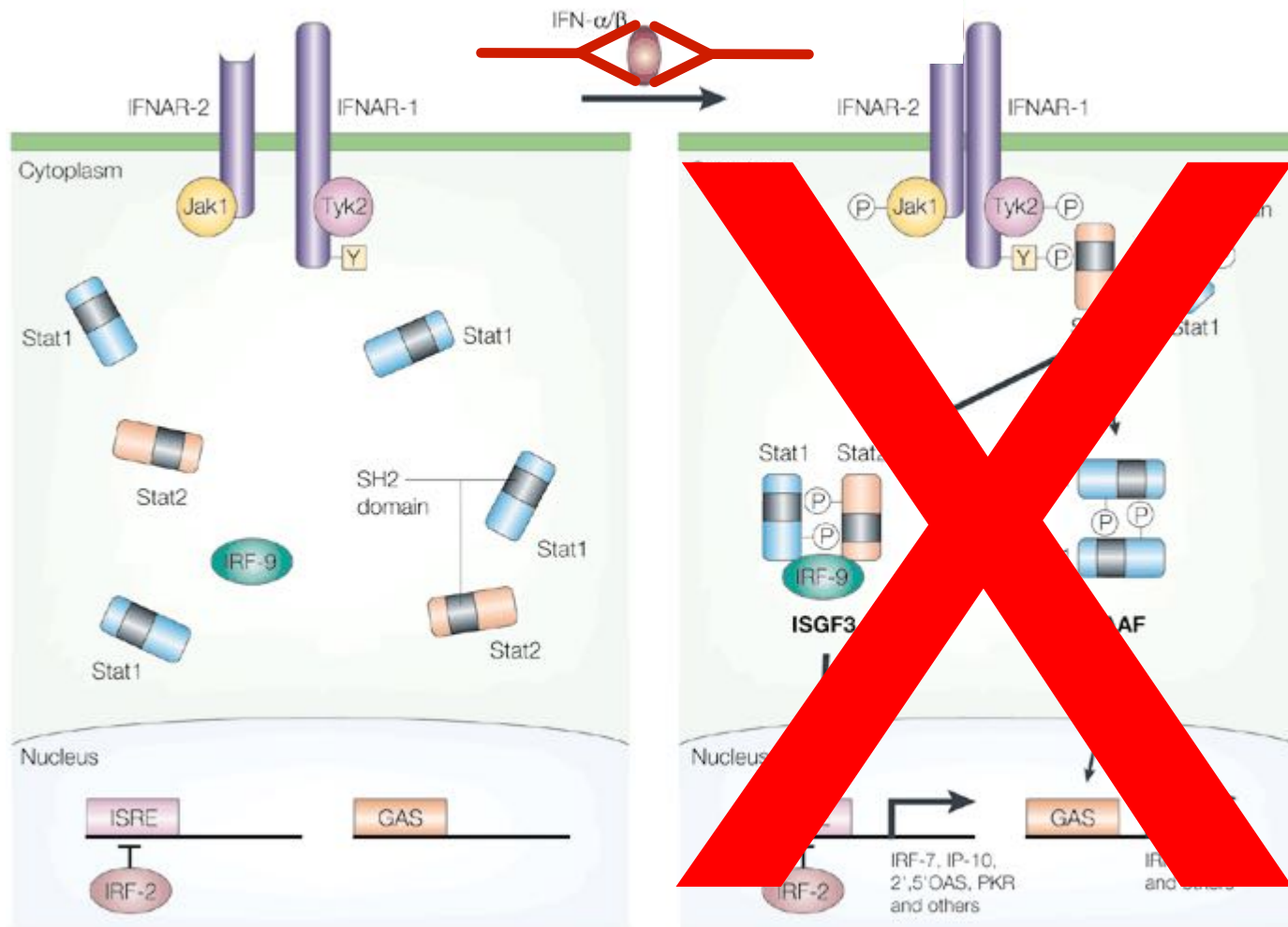
**FIG. 1.** Mean ( $\pm$ SEM) concentration-time profiles of IFN- $\beta$  of 12 healthy volunteers after intravenous ( $\bullet$ ), intramuscular ( $\circ$ ), subcutaneous ( $\Delta$ ), and placebo ( $\square$ ) administration of 6 MIU of rHuIFN- $\beta$ 1a.

# IFN- $\beta$ Mechanism of action

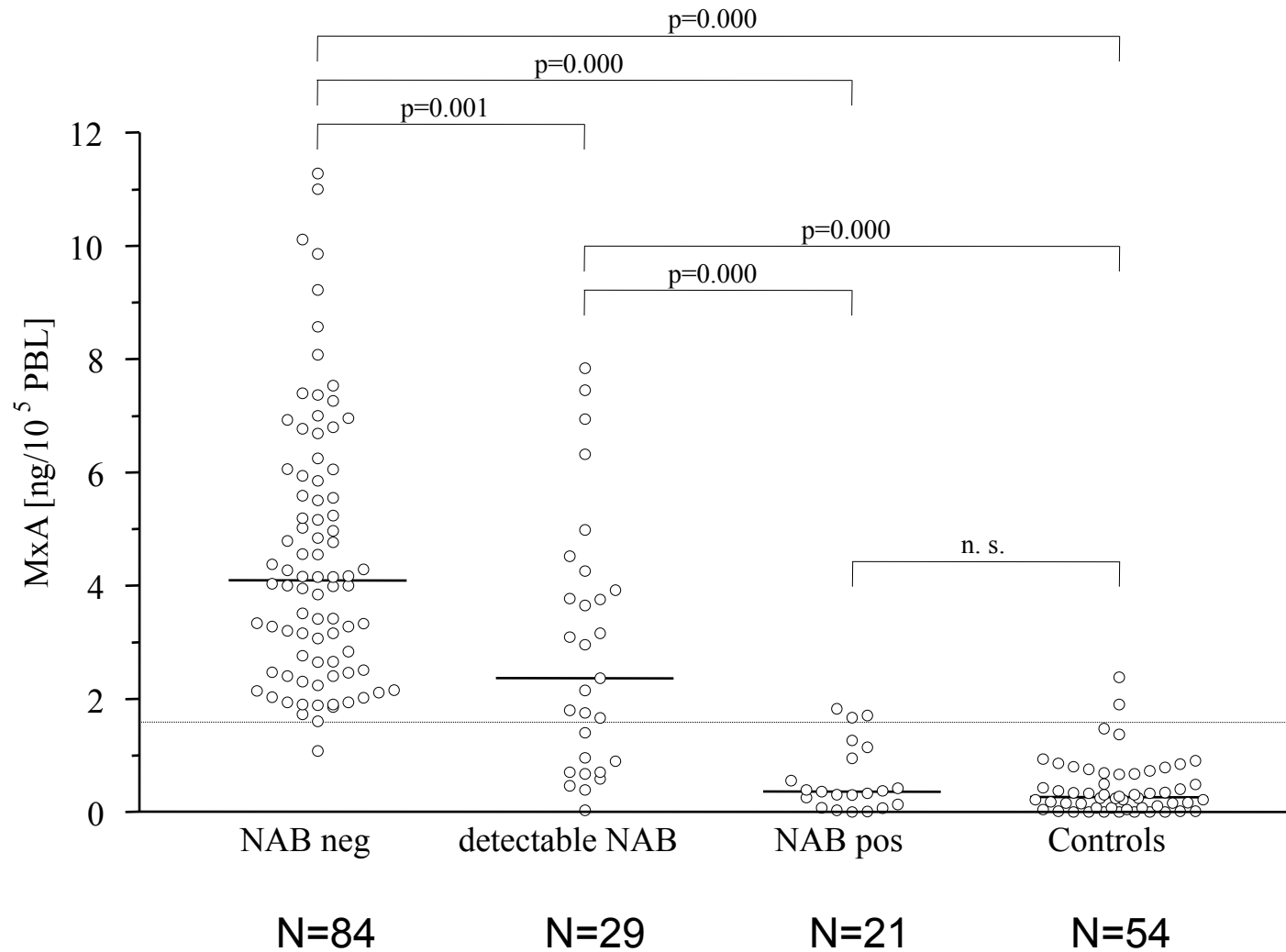
## Influence of NABs



# Class 1 IFN cascade

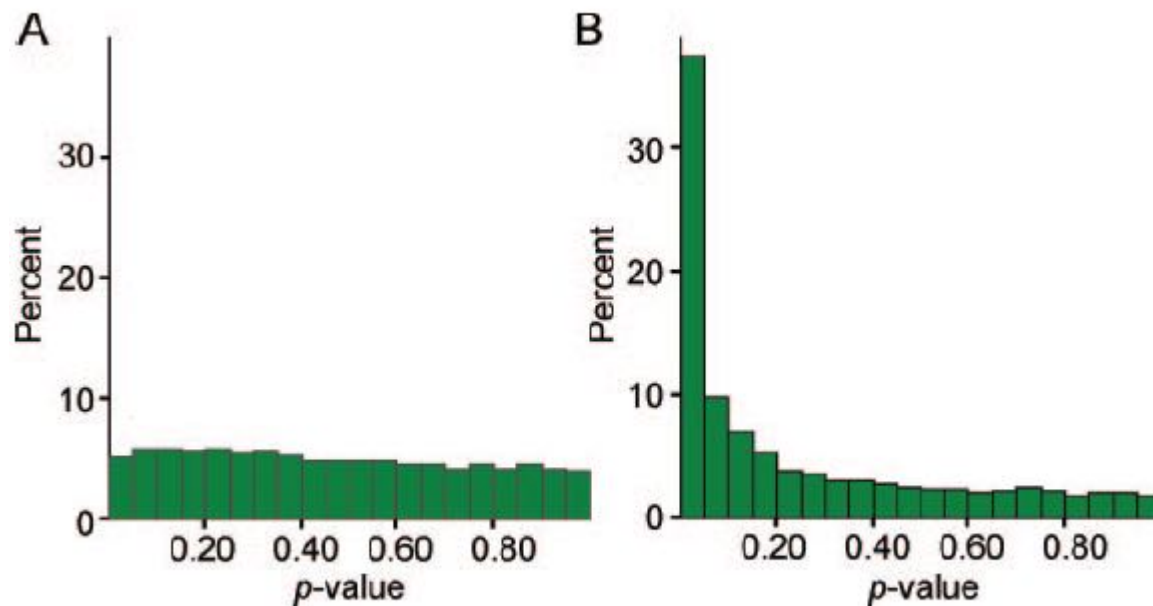


# Bioactivity of IFN in NAB+ and NAB- patients



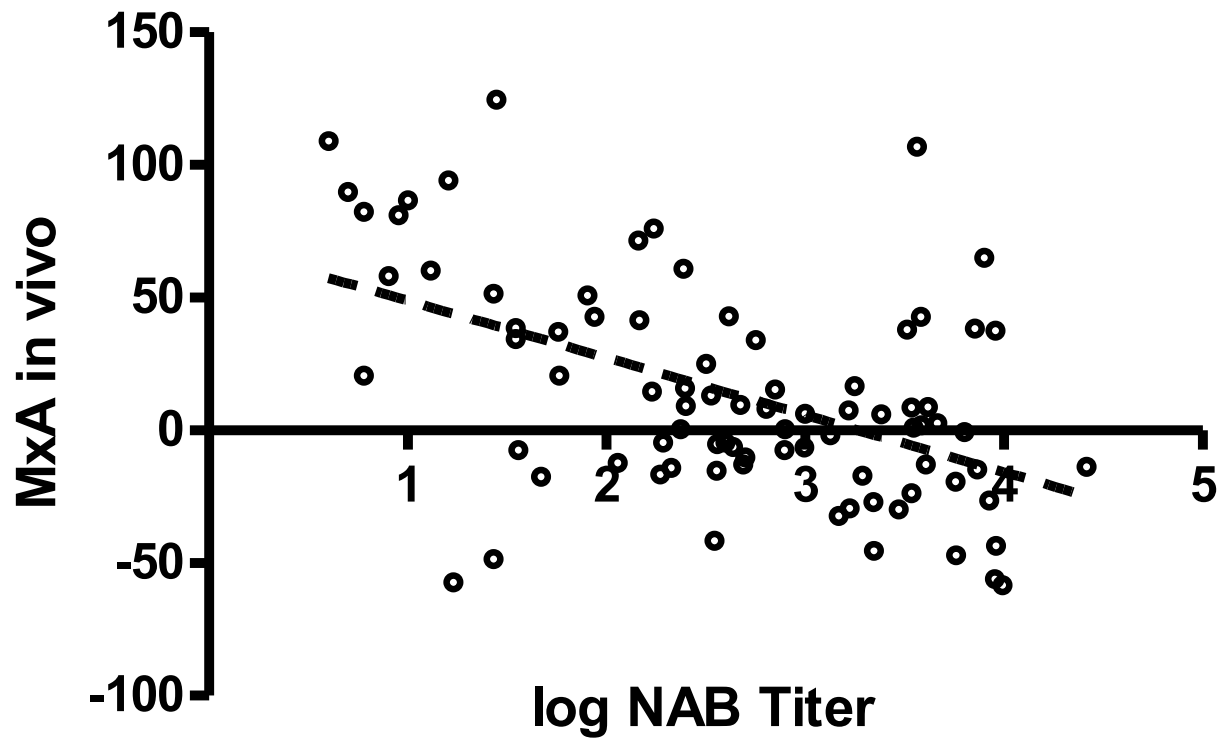
# Expression of 5593 IFN $\beta$ -induced genes in NAB+ and NAB- patients

**Figure 1** The  $p$  value distribution reflecting interferon (IFN)  $\beta$ -regulated genes



The  $p$  values from 5,593 paired  $t$  tests of detectable genes on Affymetrix. The  $p$  values are grouped in intervals of 0.05. (A) Neutralizing antibody (NAb)-positive patients and (B) NAb-negative patients.

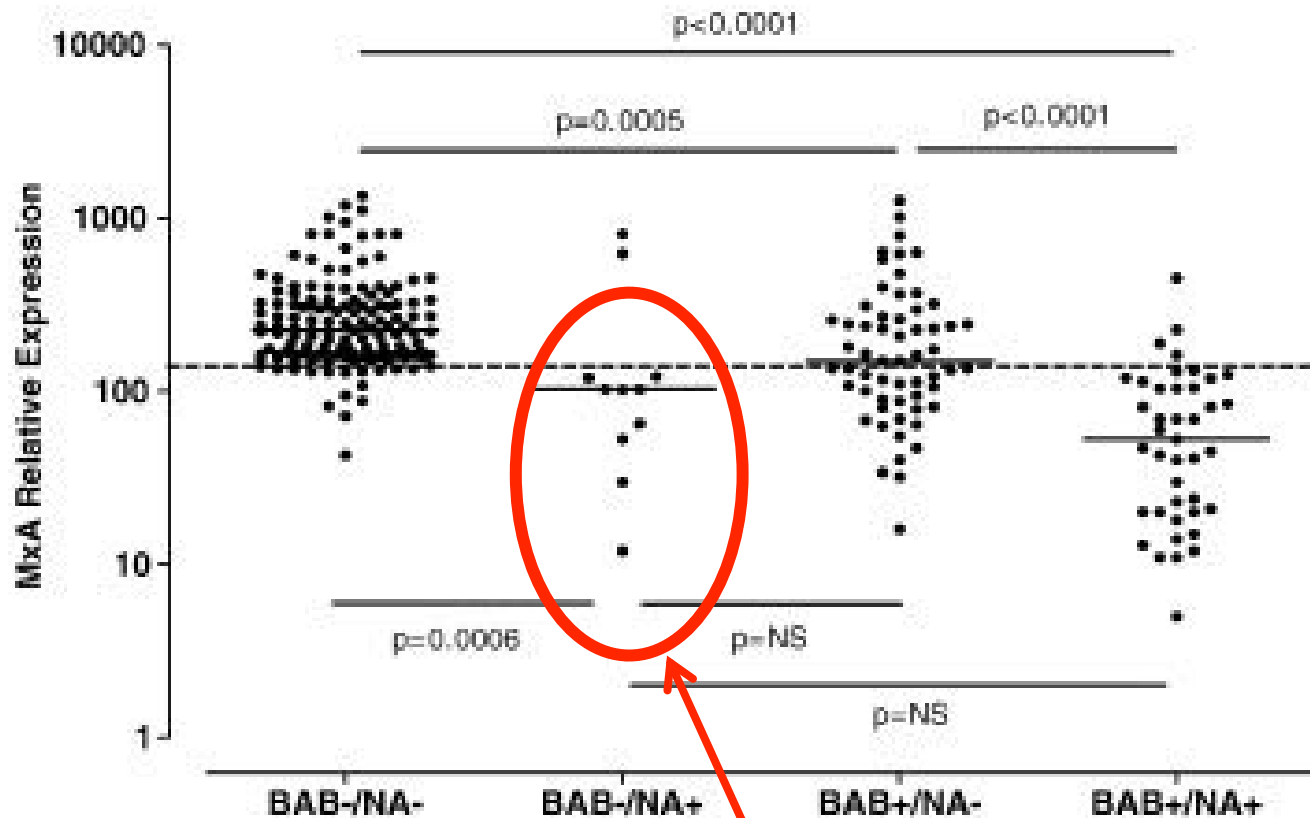
# NAB titer and IFN bioactivity



**Table II.** Bioavailability of interferon (IFN)- $\beta$  depending on neutralizing antibody (NAb) titres

Study	Marker	Level of expression	Antigen in NAb assay	NAb titre in partial biomarker response	NAb titre in strongly reduced/no biomarker response
Rudick et al. <sup>[40]</sup>	Neopterin	Protein	IFN $\beta$ -1a	5–19 NU	$\geq$ 20 NU
	$\beta_2$ -MG			ND	$\geq$ 5 NU
Deisenhammer et al. <sup>[69]</sup>	MxA	Protein	IFN $\beta$ -1b	1–19 NU	$\geq$ 20 NU
Cook et al. <sup>[70]</sup>	Neopterin	Protein	IFN $\beta$ -1a	<60 NU	$\geq$ 60 NU
			IFN $\beta$ -1b	ND	$\geq$ 60 NU
Vallittu et al. <sup>[71]</sup>	MxA	Protein	IFN $\beta$ -1a	40–160 NU	>160 NU
Pachner et al. <sup>[72]</sup>	MxA	RNA	IFN $\beta$ -1a/b	ND <sup>a</sup>	>200 NU
	OAS			ND	>200 NU
Bertolotto et al. <sup>[73]</sup>	MxA	RNA	IFN $\beta$ -1a/b	20–44 NU	$\geq$ 45 NU
Gilli et al. <sup>[74]</sup>	MxA <sup>b</sup>	RNA	IFN $\beta$ -1a/b	ND	$\geq$ 20 NU
Sorensen et al. <sup>[75]</sup>	Neopterin	Protein	IFN $\beta$ -1a/b	20–79% NC	$\geq$ 80% NC
	$\beta_2$ -MG			20–79% NC	$\geq$ 80% NC
Gilli et al. <sup>[76]</sup>	MxA	RNA	IFN $\beta$ -1a/b	$\geq$ 20 NU	ND
	TRAIL			$\geq$ 20 NU	ND
	XAF-1			$\geq$ 20 NU	ND
Pachner et al. <sup>[77]</sup>	MxA	RNA	IFN $\beta$ -1a/b	20–100 NU	>100 NU
	Viperin			20–100 NU	>100 NU
	OAS			20–100 NU	>100 NU
Santos et al. <sup>[78]</sup>	MxA	RNA	IFN $\beta$ -1a	1–19 NU	ND
	MxB			1–19 NU	ND
	STAT-1			1–19 NU	ND
	TRAIL			1–19 NU	ND
	$\beta_2$ -MG			1–19 NU	ND
Scagnolari et al. <sup>[79]</sup>	Neopterin	Protein	IFN $\beta$ -1b	20–200 NU	>200 NU
	$\beta_2$ -MG	Protein		ND	$\geq$ 20 NU
	MxA	RNA		20–200 NU	>200 NU
	PKR	RNA		20–200 NU	>200 NU
Sominanda et al. <sup>[80]</sup>	MxA	RNA	IFN $\beta$ -1a	150–600 NU	>600 NU
	TRAIL			150–600 NU	>600 NU

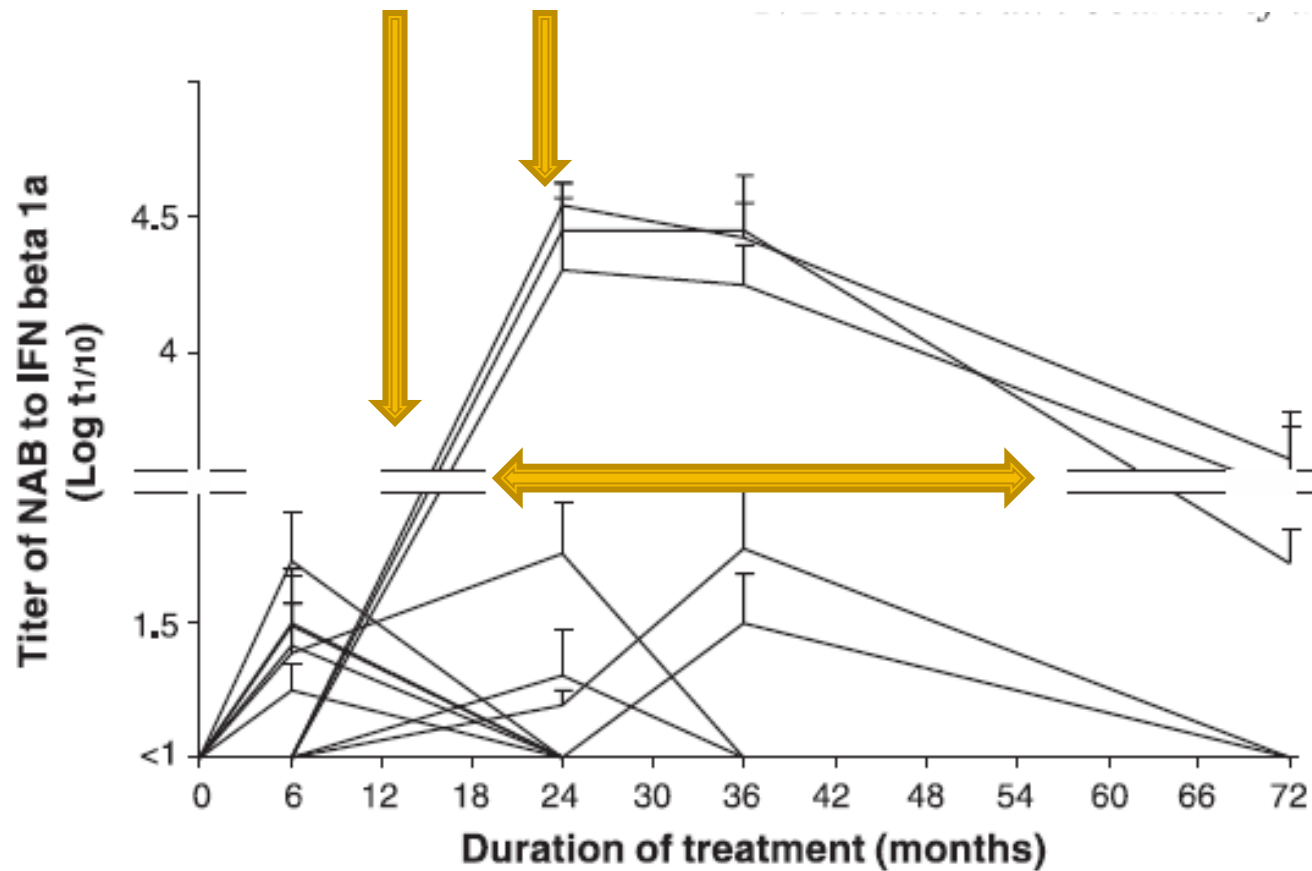
# Non antibody-mediated neutralization of IFN $\beta$



soluble IFN $\beta$  receptors detected

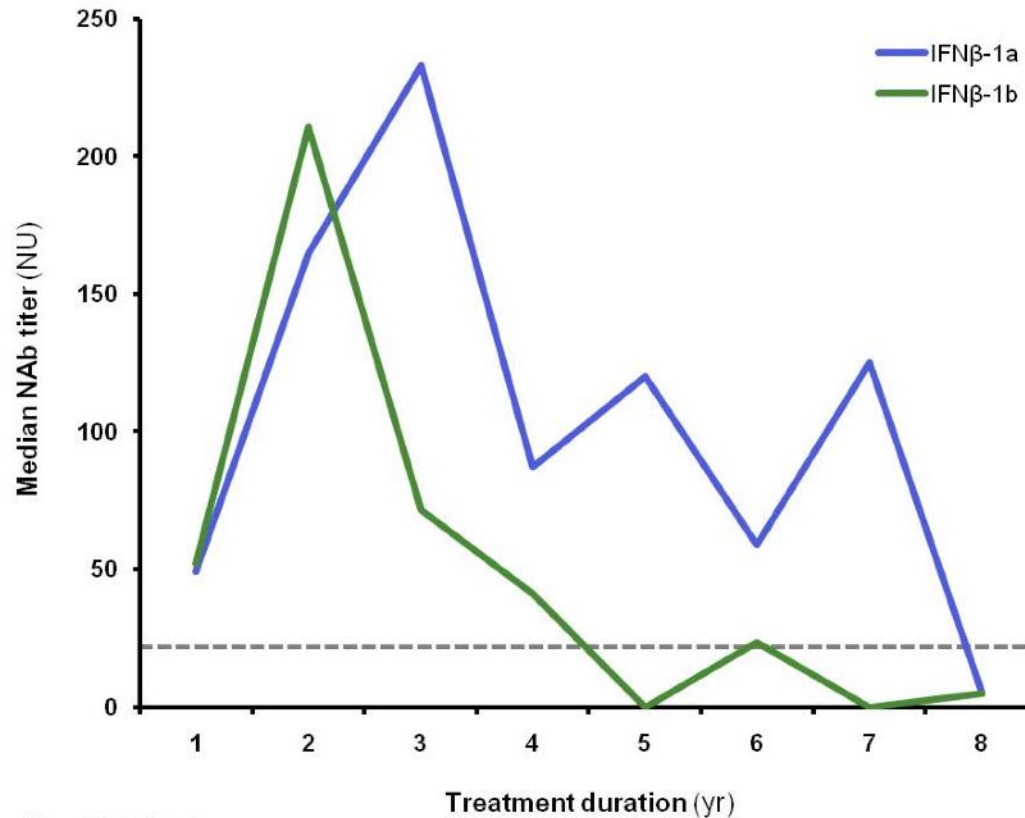


# NAB persistency depends on titer





# Long-term development of NABs



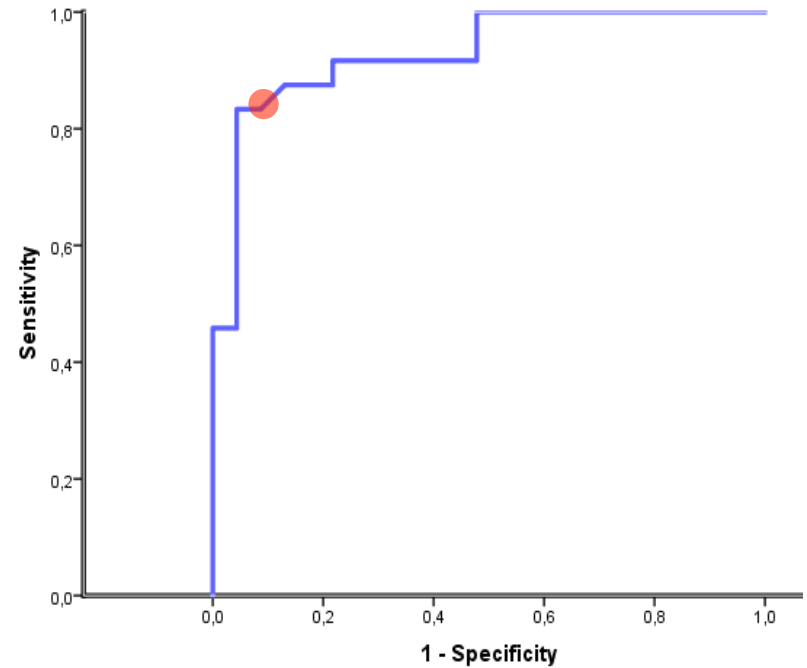
No. of patients

IFNβ-1a	11	14	23	14	17	10	4	4
IFNβ-1b	15	16	18	14	17	10	7	2



# Predictive NAb cut-off titers

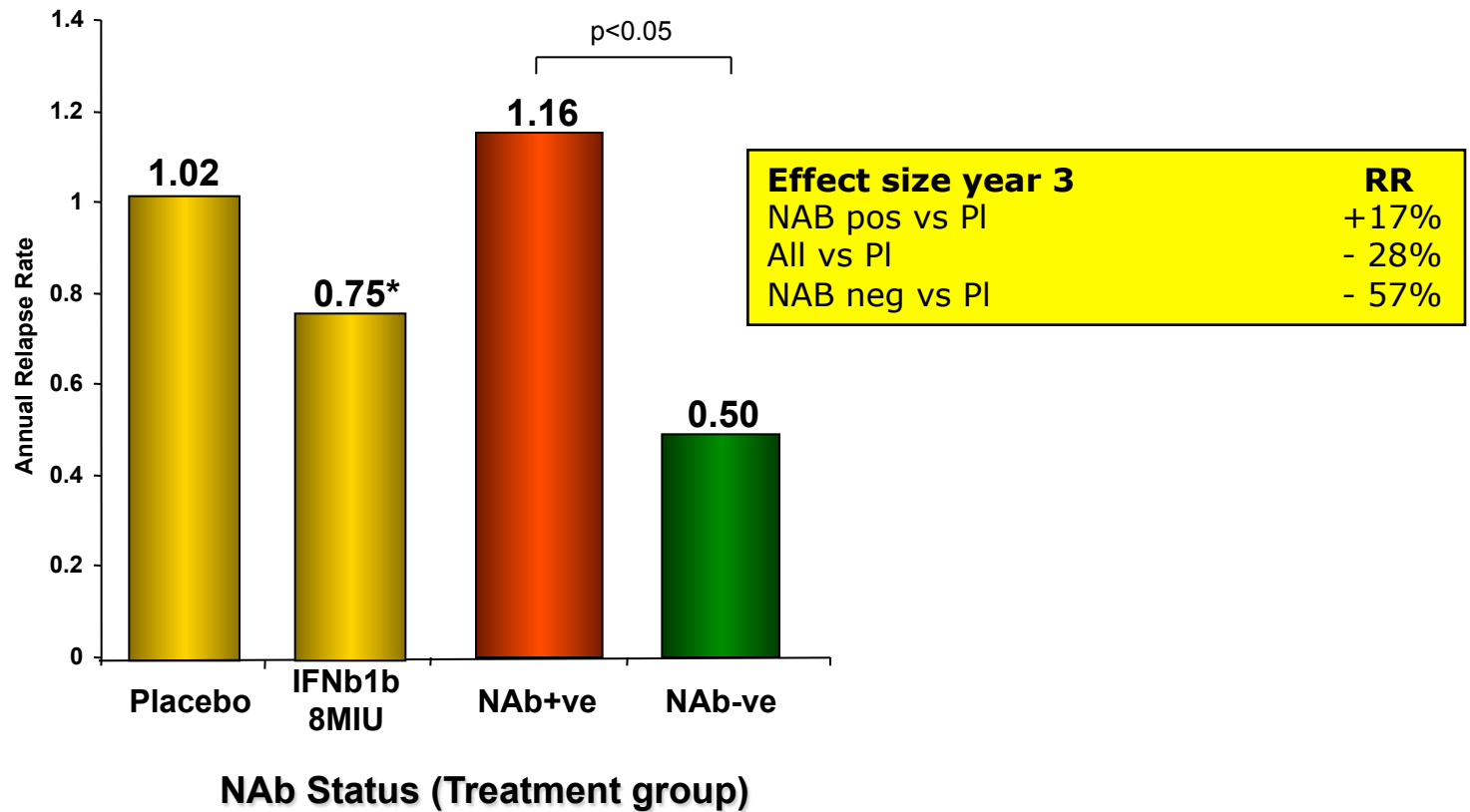
	All IFN $\beta$	IFN $\beta$ -1a	IFN $\beta$ -1b
Sensitivity (%)	83.3	81.3	100
Specificity (%)	91.3	90.9	91.7
<b>Cut-off NAb titer (NU)</b>	> 344	> 258	> 460



# Summary

- Common terminology and according definitions are needed
- IFNs BAB and NAB are associated
- There is a complex interaction between antibodies and PK/PD
- Measuring only one component doesn't show the whole picture
- On the individual level NAB tests are the most useful single test
- On the group level the relationship between antibody test and PD should always be evaluated
- Timing is a confounder regarding titers, binding strength, Ab kinetics, interpretation of test results (reporting)

# IFN $\beta$ - Clinical Impact



# PRISMS-4: NABs and relapse rates

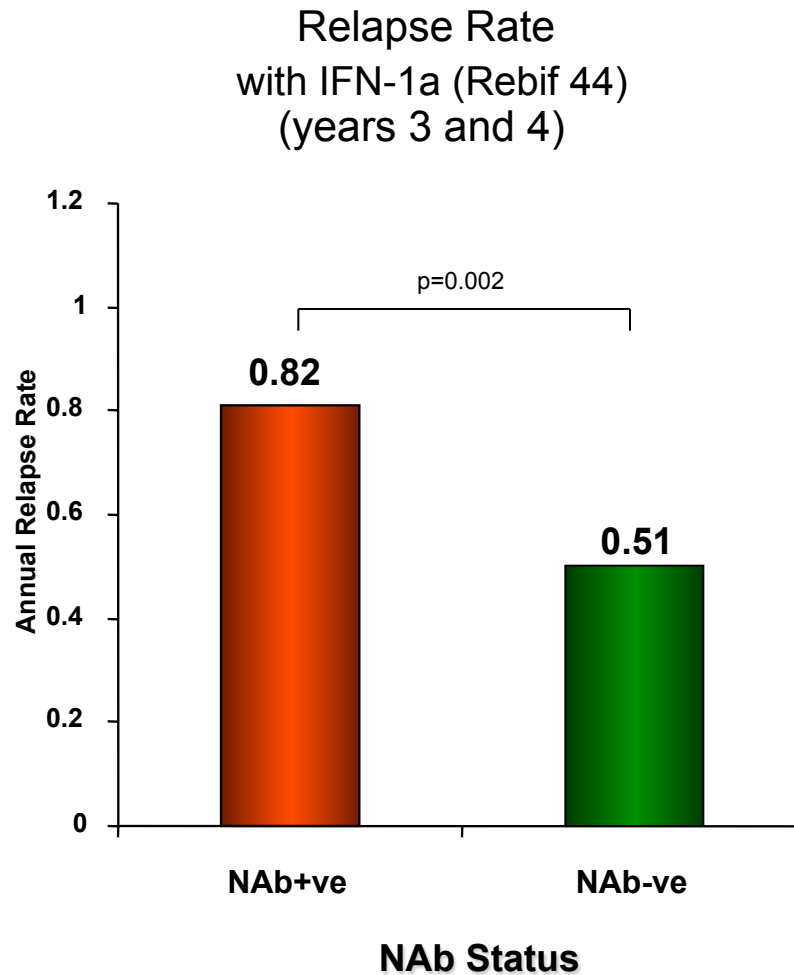
**Table 2** Relapse rate based on NAb status: PRISMS 4-year data

Time	Mean annualized relapse rate		Adjusted relapse rate ratio NAb+/NAb- (95% CI)	p Value
	NAb- (22 and 44 µg) n = 278	NAb+ (22 and 44 µg) n = 90		
<b>“Anytime positive” method*</b>				
Years 1–4	0.74	0.82	1.00 (0.82–1.22)	0.98
Years 1–2	0.94	0.83	0.81 (0.65–1.01)	0.06
Years 3–4	0.51	0.82	1.41 (1.12–1.78)	0.004
<b>“Interval positive” method†</b>				
Years 1–4	0.74	0.86	1.21 (1.03–1.43)	0.02
Years 1–2	0.92	0.88	1.04 (0.84–1.28)	0.73
Years 3–4	0.52	0.85	1.60 (1.29–1.97)	<0.001

\* Patients remain in same category throughout, regardless of when Ab first detected.

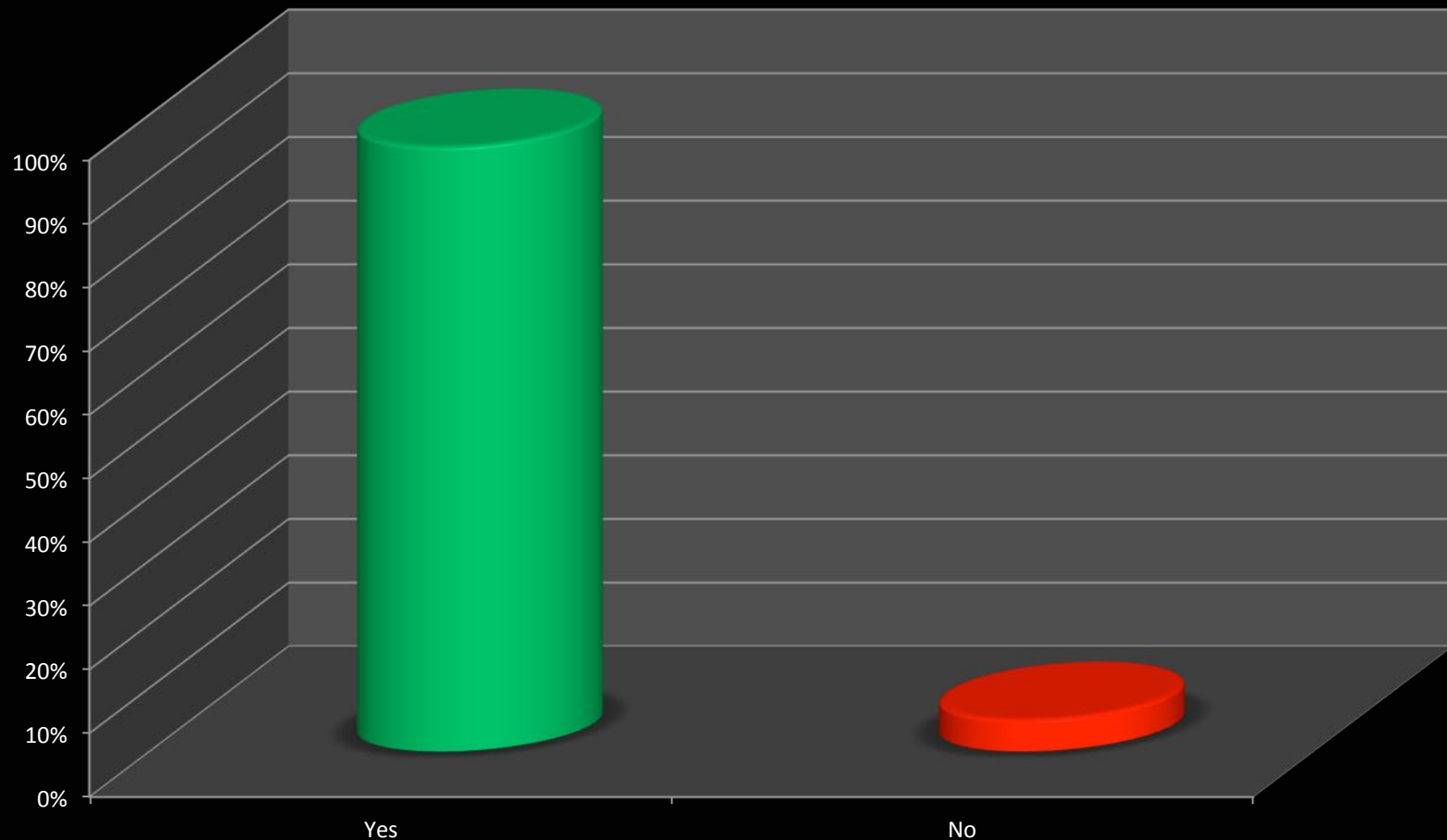
† Patients may change category if Ab status changes.

# IFN $\beta$ - Clinical Impact



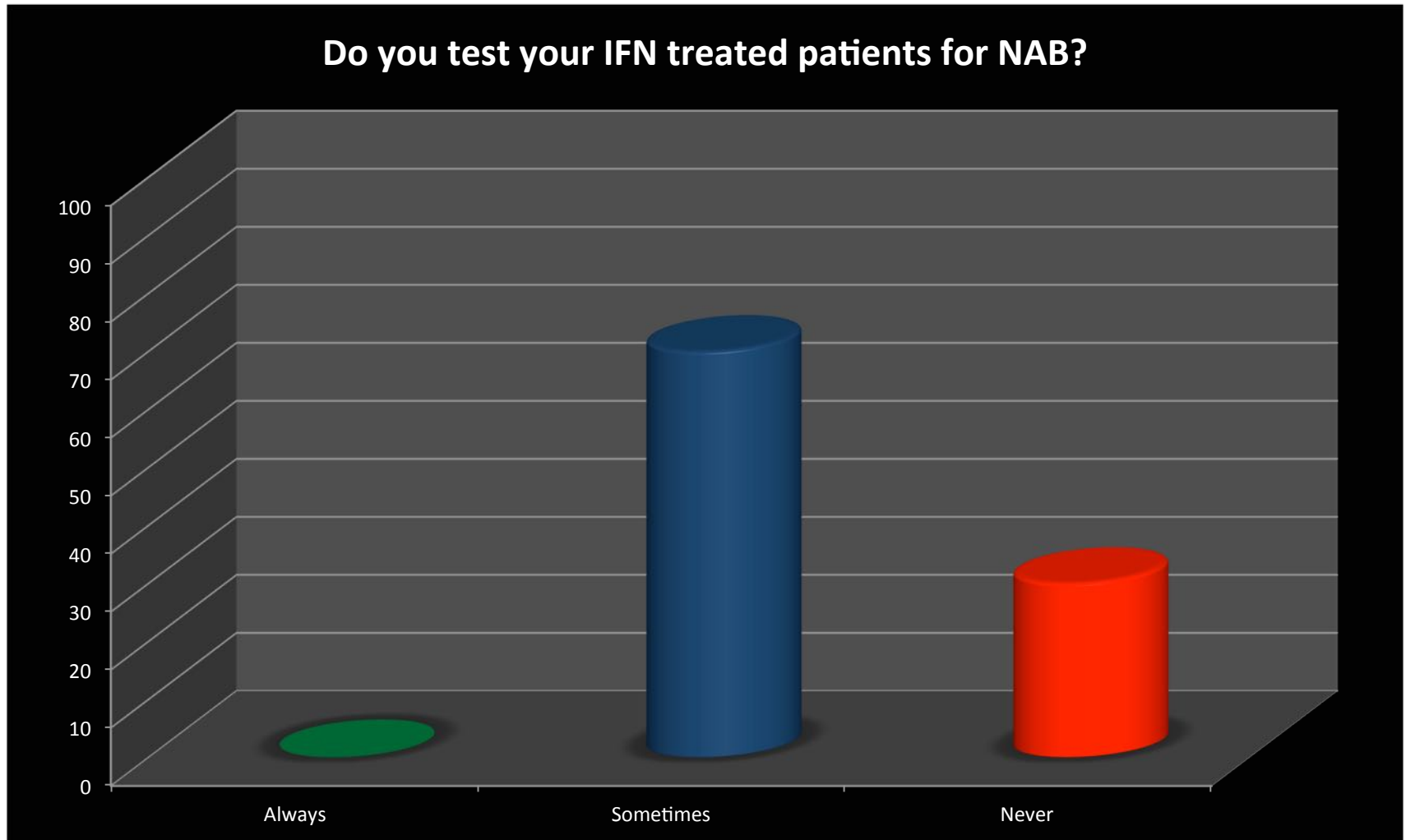
# Do you believe that NAB have an impact on efficacy of IFNs?

Do you believe that NAB have an impact on efficacy of IFNs?





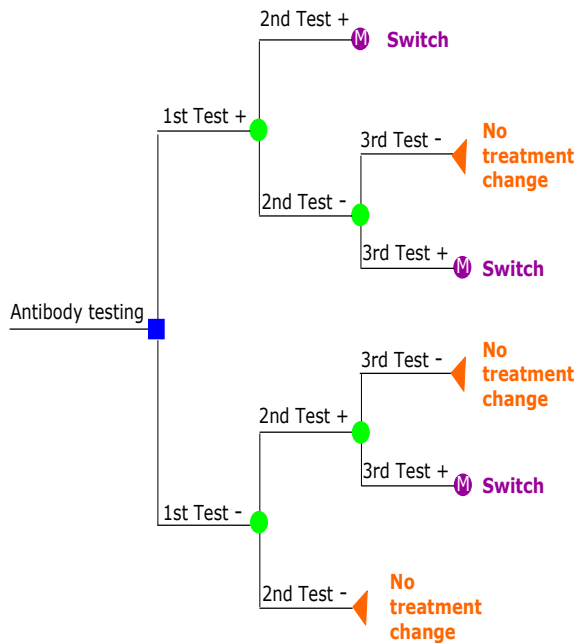
# Do you test your IFN treated patients for NAB?



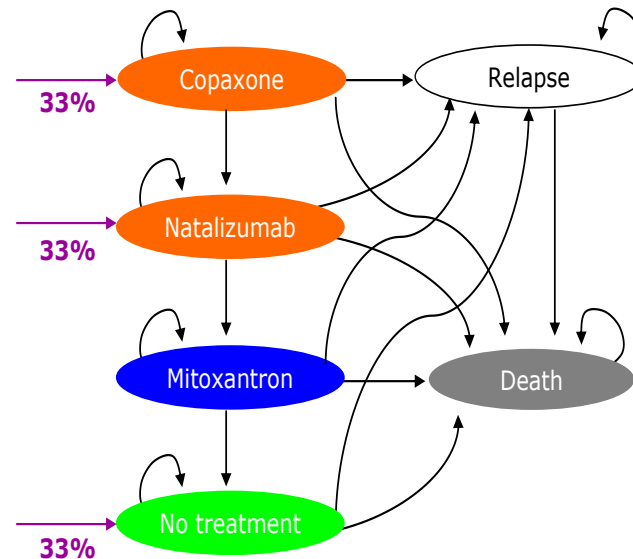
# Antikörpertest Algorithmus und Markov Modell

## Zeitlicher Rahmen: 5,5 Jahre

### Decision tree



### Markov model



Source: developed by IPF

No antibody testing → continuous IFNb therapy

# Results

## With NAB Tests: 1400 relapses avoided!

Table 2: cost-effectiveness analysis

<b>Cost with NAB testing</b>	<b>per Patient</b>	<b>3,590 Patients</b>
<b>Cost per Relapse avoided within 5.5 years</b>	<b>€ 124,261</b>	<b>€ 446,096,393</b>
Per year	€ 22,593	€ 81,108,435
Effectiveness (relapses avoided)	0.420	
Cost of Illness and Markov with testing	€ 52,190	€ 187,360,485
<b>Cost without testing</b>		
<b>Cost per Relapse avoided within 5.5 years</b>	<b>€ 151,629</b>	<b>€ 544,347,609</b>
Per year	€ 27,569	€ 98,972,293
Effectiveness (relapses avoided)	0.324	
Cost of illness w/o testing	€ 49,117	€ 176,331,610
Difference effectiveness	0.096	
<b>Difference cost of relapse avoided per year</b>	<b>€ -4,976</b>	<b>€ -17,863,858</b>

# Anti-Biopharmaceutical Immunization: Prediction and Analysis of Clinical Relevance to Minimize the Risk



Innovative Medicines Initiative



**Thanks.....**

**to my team and for your attention**

