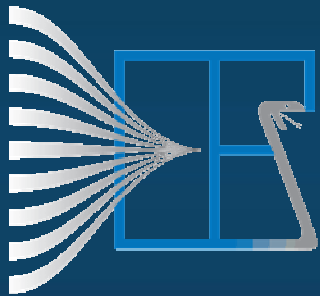


Antibodies and immunological memory induced by aggregated interferon beta in a transgenic immune tolerant mouse model

Miranda van Beers



Division of Drug Delivery Technology
Leiden/Amsterdam Center for Drug Research (LACDR)

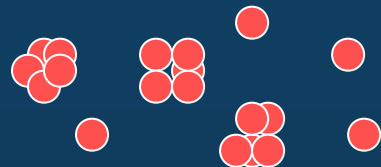
UIPS Utrecht Institute for
Pharmaceutical Sciences

Recombinant human interferon beta (IFN β)

Product	Percentage of patients developing antibodies (after 12 months)
Avonex [®] (IFN β -1a)	10 %
Rebif [®] (IFN β -1a)	25 %
Betaferon[®] (IFN β -1b)	90 %

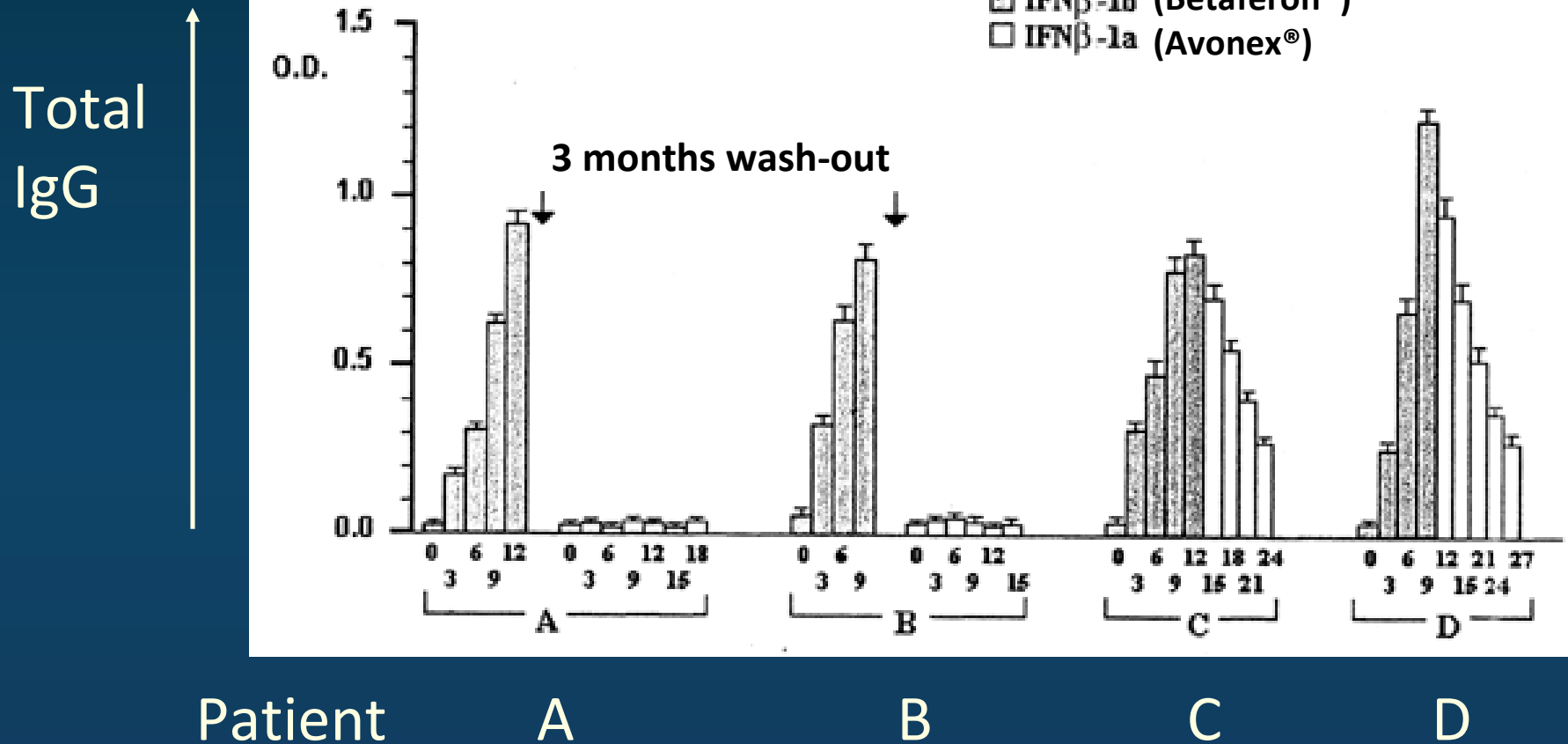
Perini et al. 2001

Contains 60% aggregates!



Switching from IFN β -1b to IFN β -1a

- Decrease in BAB levels after switch to Avonex[®]
- No immunological memory?



Switching from IFN β -1a to IFN β -1b

- Decrease in NAB levels after high-dose i.v. Betaferon[®]
- No immunological memory?

NAB titers after Betaferon[®] re-challenge

		8 MIU s.c.	8 MIU i.v.	16 MIU i.v.
Patient 1	Baseline	118	126	104
	3 h	166	43	<10
	12 h	145	45	<10
	24 h	134	68	14
Patient 2	Baseline	426	122	130
	3 h	344	152	36
	12 h	318	101	34
	24 h	207	110	34
Patient 3	Baseline	42	125	61
	3 h	39	26	<10
	12 h	81	32	<10
	24 h	92	42	<10
Patient 4	Baseline	2618	2748	1797
	3 h	2167	1067	1479
	12 h	2557	1722	1875
	24 h	2875	1120	1849
Patient 5	Baseline	4482	6875	11,773
	3 h	6408	8790	10,926
	12 h	4732	8811	11,027
	24 h	6014	9651	13,624

Aims

1 Study the effect of aggregation on the immunogenicity of Avonex-IFN β -1a

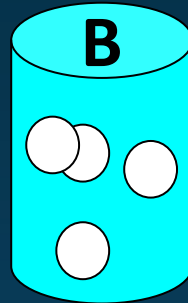
2 Study the presence of immunological memory in wildtype versus transgenic mice

- Wildtype mice - classical immune response – memory?
- Transgenic mice - breaking of tolerance - no memory?

Three interferon beta samples

Bulk IFN β -1a

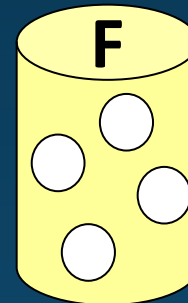
- Avonex bulk
- In PBS pH 7.2
- 270 ug/ml



Medium aggregate content

Formulated IFN β -1a

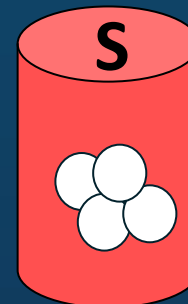
- Dialyzed and filtered Avonex
- In NaAc pH 4.5
- Formulated with Tween 20 and ArgHCl
- ~ 160 ug/ml



Low aggregate content

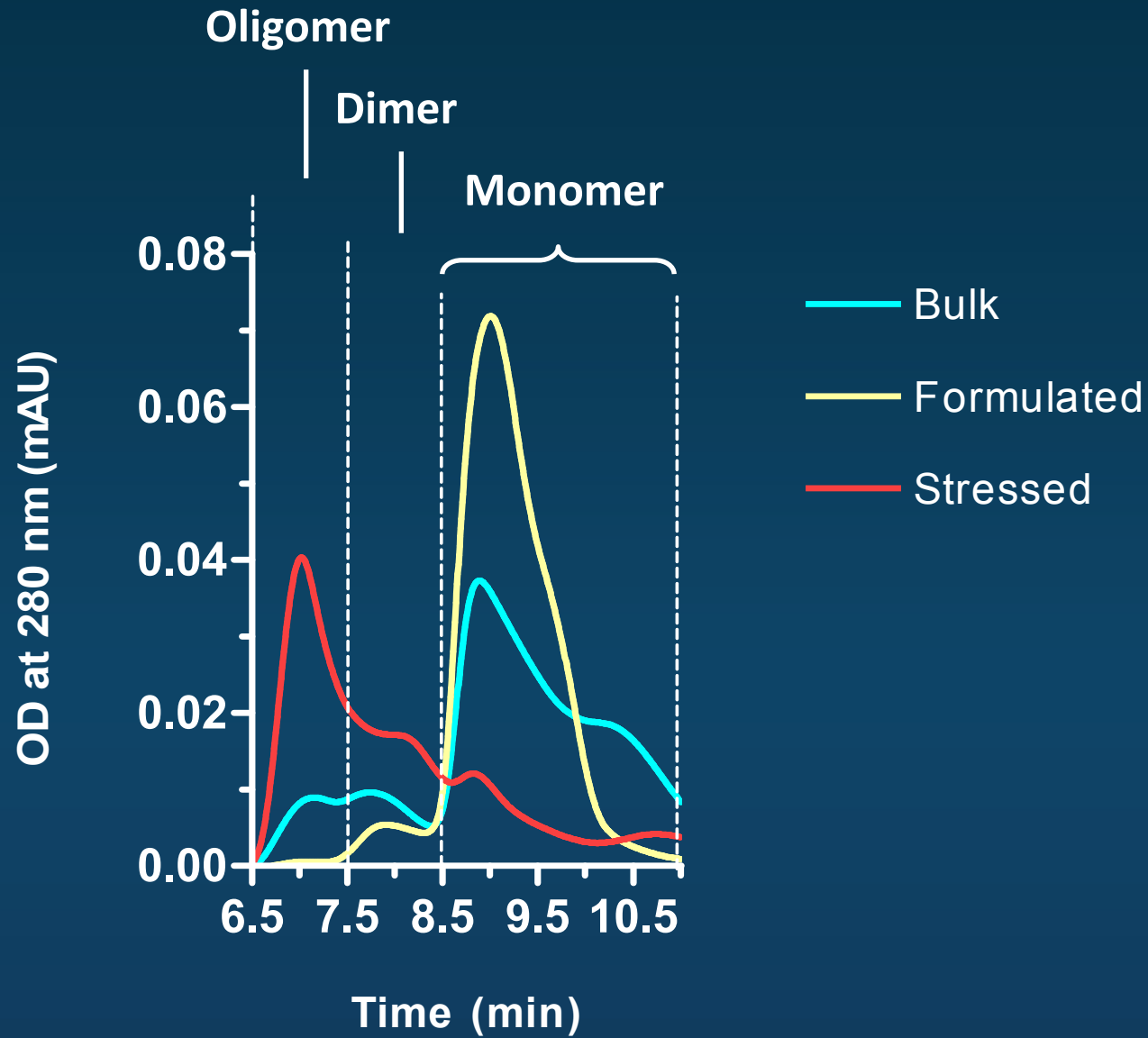
Stressed IFN β -1a

- Avonex incubated at pH 2 + 1 M NaCl
- Purification by SEC-HPLC
- In PBS pH 7.2
- 110 ug/ml



High aggregate content

Aggregate analysis by SEC-HPLC

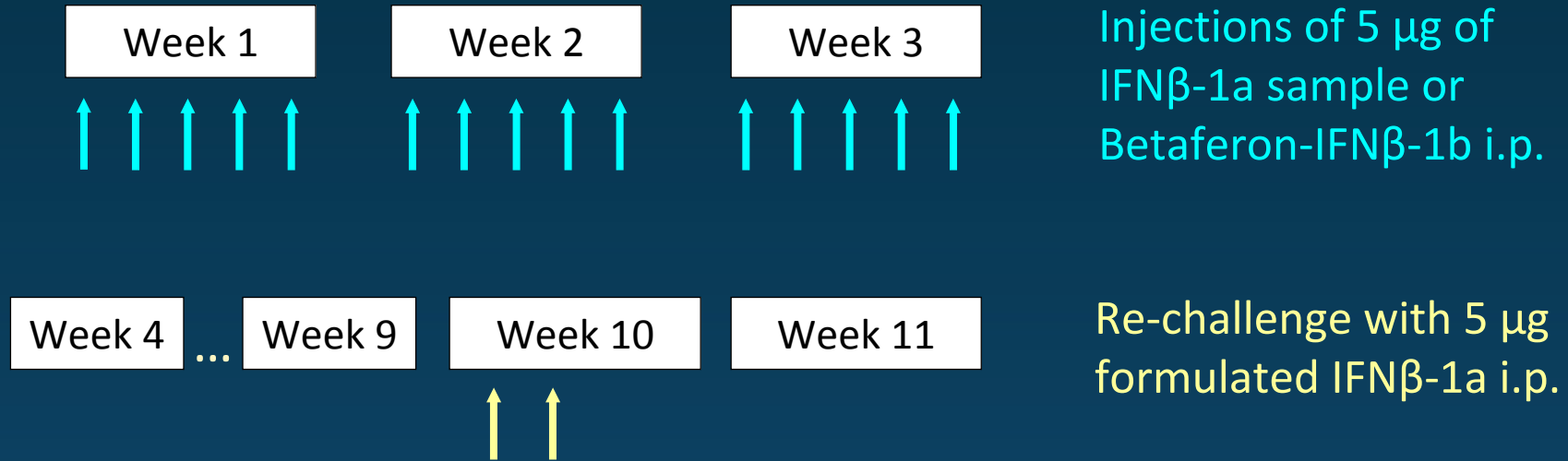


Aggregate percentage by SEC-HPLC

	Soluble fraction (%)			Non-recovered fraction (%)
	Monomer	Dimer	Oligomer	
Bulk	69	10	8	13
Formulated	87	6	0	7
Stressed	19	21	31	29

Immunogenicity???

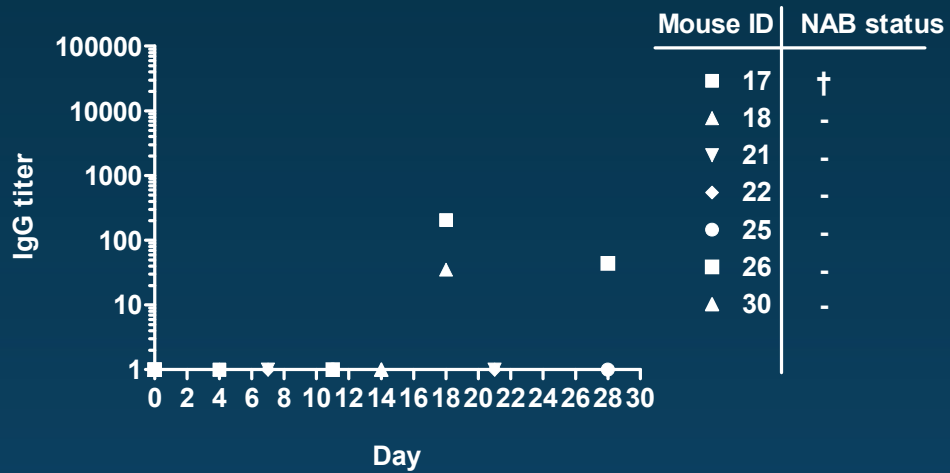
In vivo injection schedule



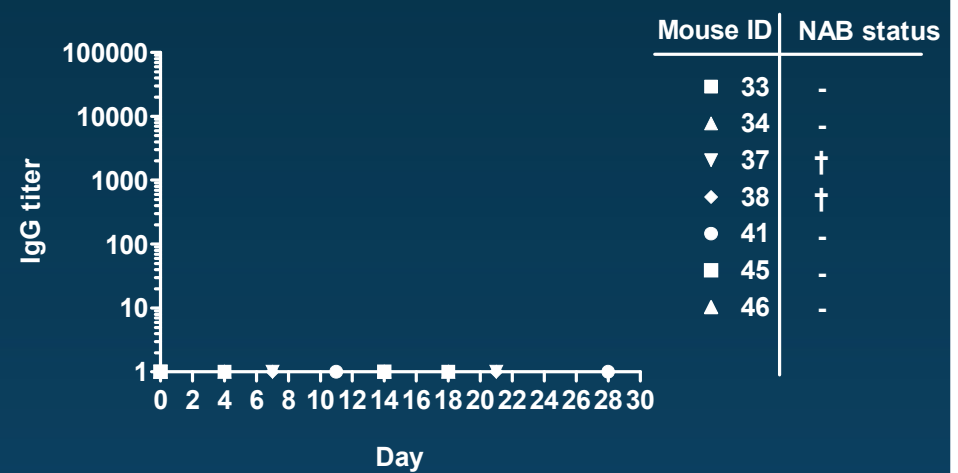
- Testing in wildtype and transgenic mice
- Follow Ab formation in time during 11 weeks
- Measure binding Abs with ELISA for total IgG
- Measure neutralizing Abs with bioassay

Immunogenicity in transgenic mice

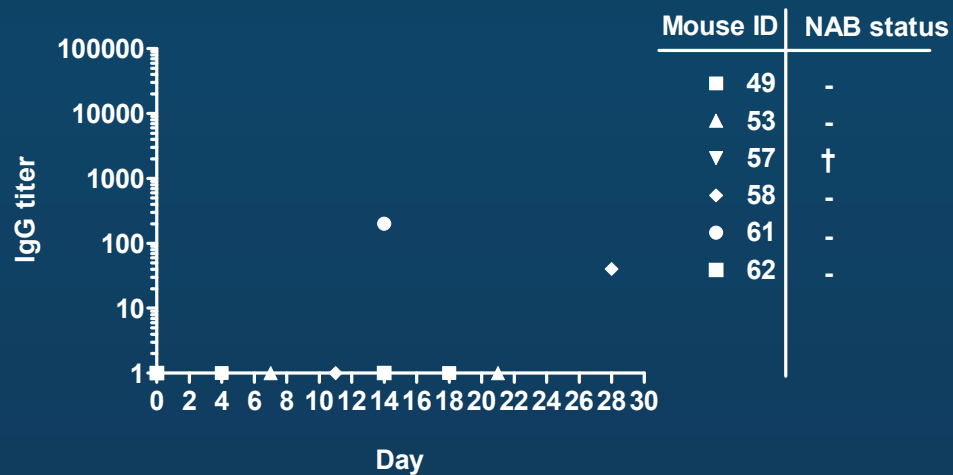
Bulk



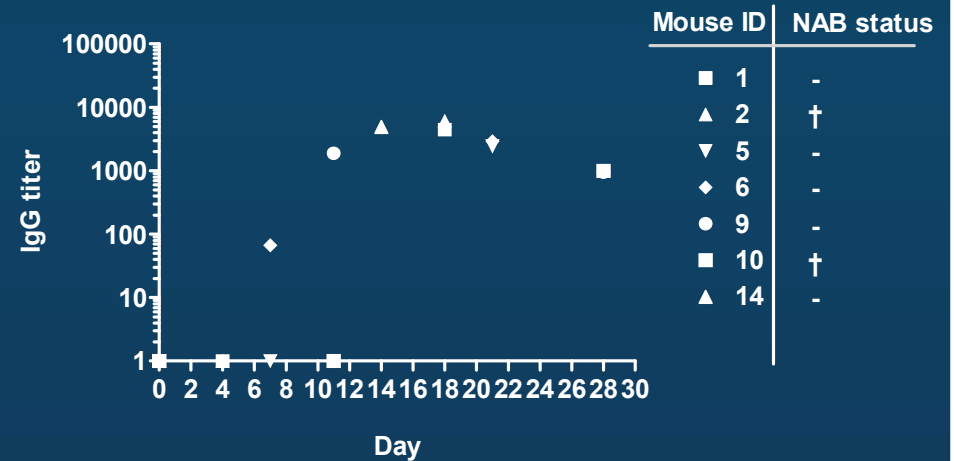
Formulated



Stressed

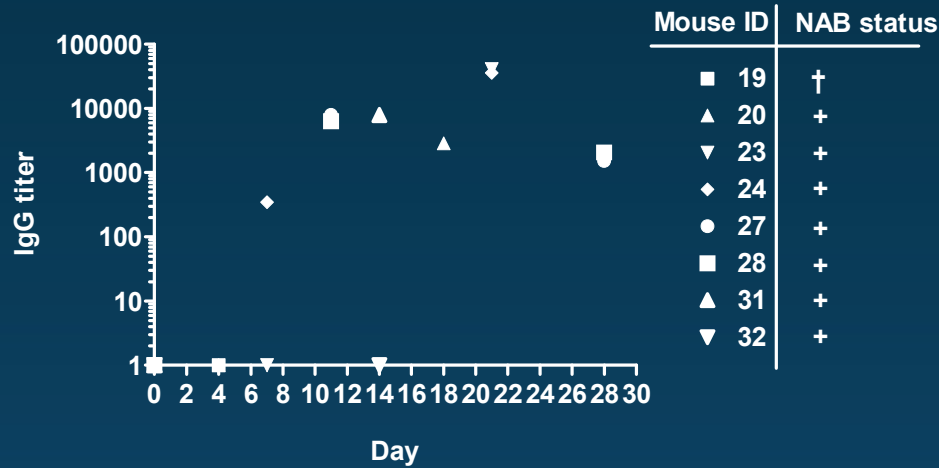


Betaferon®

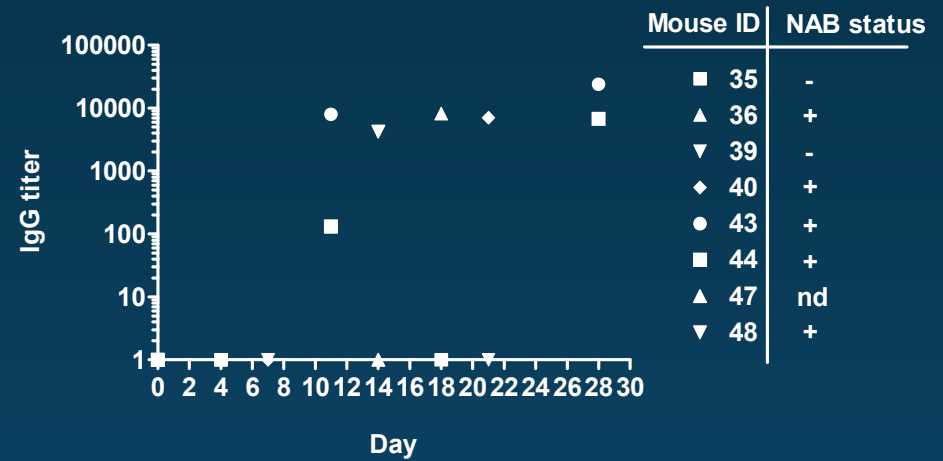


Immunogenicity in **wildtype** mice

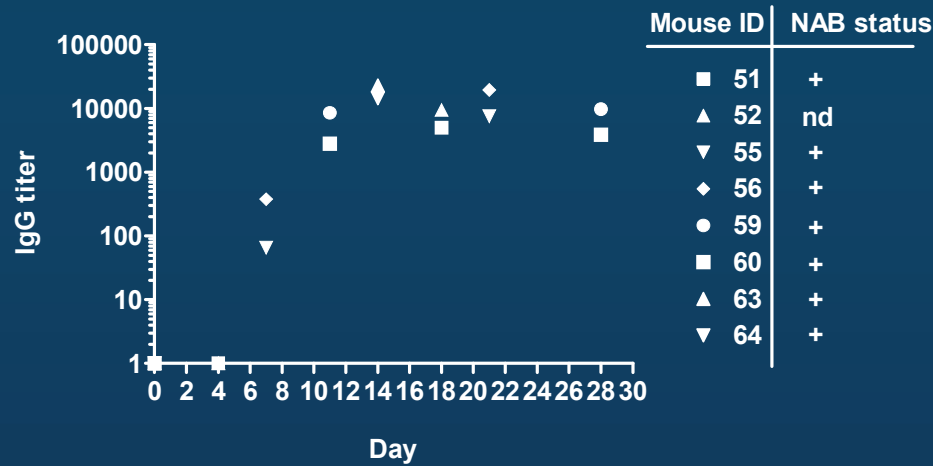
Bulk



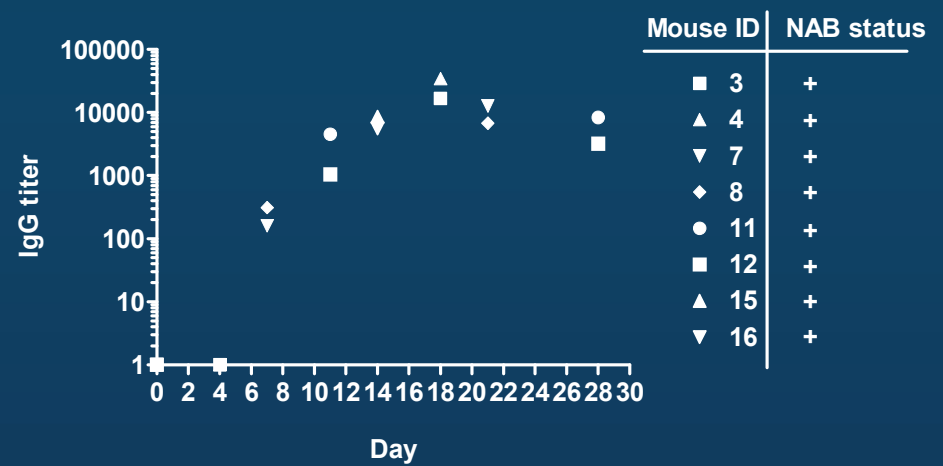
Formulated



Stressed



Betaferon®

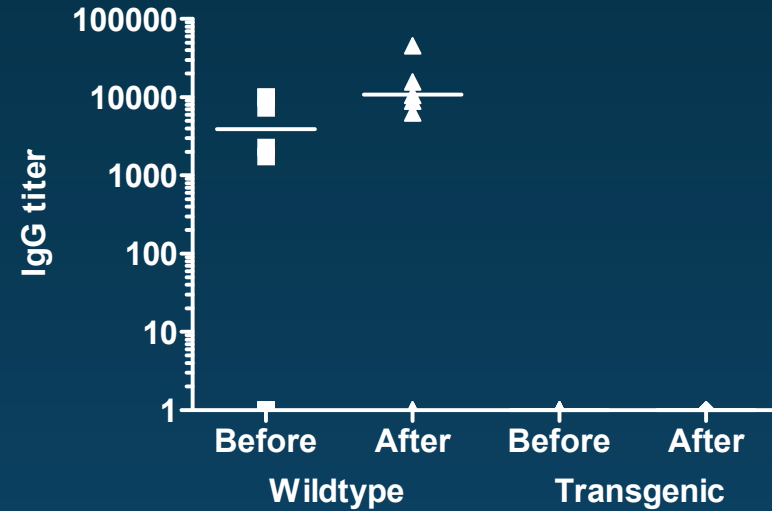


Memory: titers before and after re-challenge

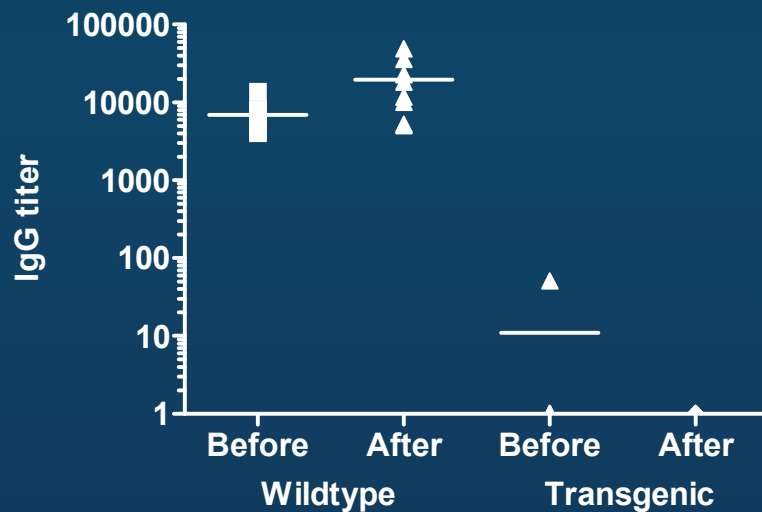
Bulk



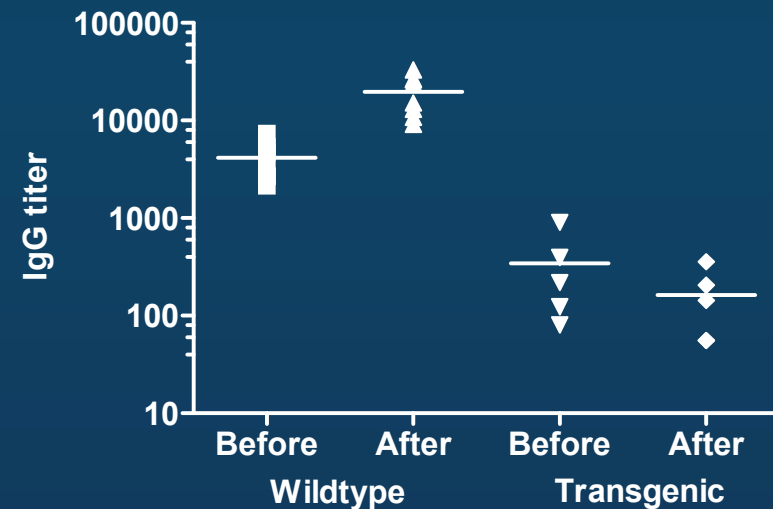
Formulated



Stressed



Betaferon®



Conclusions

Binding Abs

- Wildtype mice do not discriminate between products
- Transgenic mice indicate immunogenicity of products:
Formulated < Bulk = Stressed < Betaferon®
- Aggregate characteristics are important

Neutralizing Abs and memory

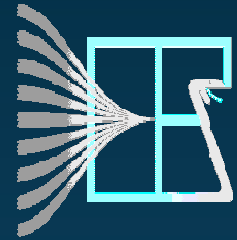
- Wildtype mice form BABs + NABs, transgenic mice only BABs
- Transgenic mice do not develop memory for IFN β

Immune mechanism???

Acknowledgements

Protein material

Biogen Idec (Cambridge, USA)



In vivo work

Melody Sauerborn (Department of Pharmaceutics, Utrecht)

Vera Brinks (Department of Pharmaceutics, Utrecht)

NAB assay

Francesca Gilli (Clinical Neurobiology, Turin)

UIPS Utrecht Institute for
Pharmaceutical Sciences

Supervisors

Wim Jiskoot (Drug Delivery Technology, Leiden)

Huub Schellekens (Department of Pharmaceutics, Utrecht)



Questions?

