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

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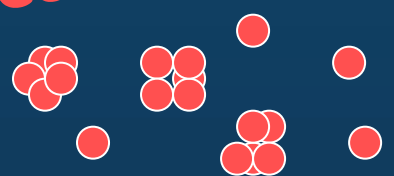
# Recombinant human interferon beta (IFNβ)

| Product | Percentage of patients developing antibodies (after 12 months) |
|---|---|
| **Avonex®** (IFNβ-1a) | 10 % |
| **Rebif®** (IFNβ-1a) | 25 % |
| **Betaferon®** (IFNβ-1b) | 90 % |

Perini et al. 2001

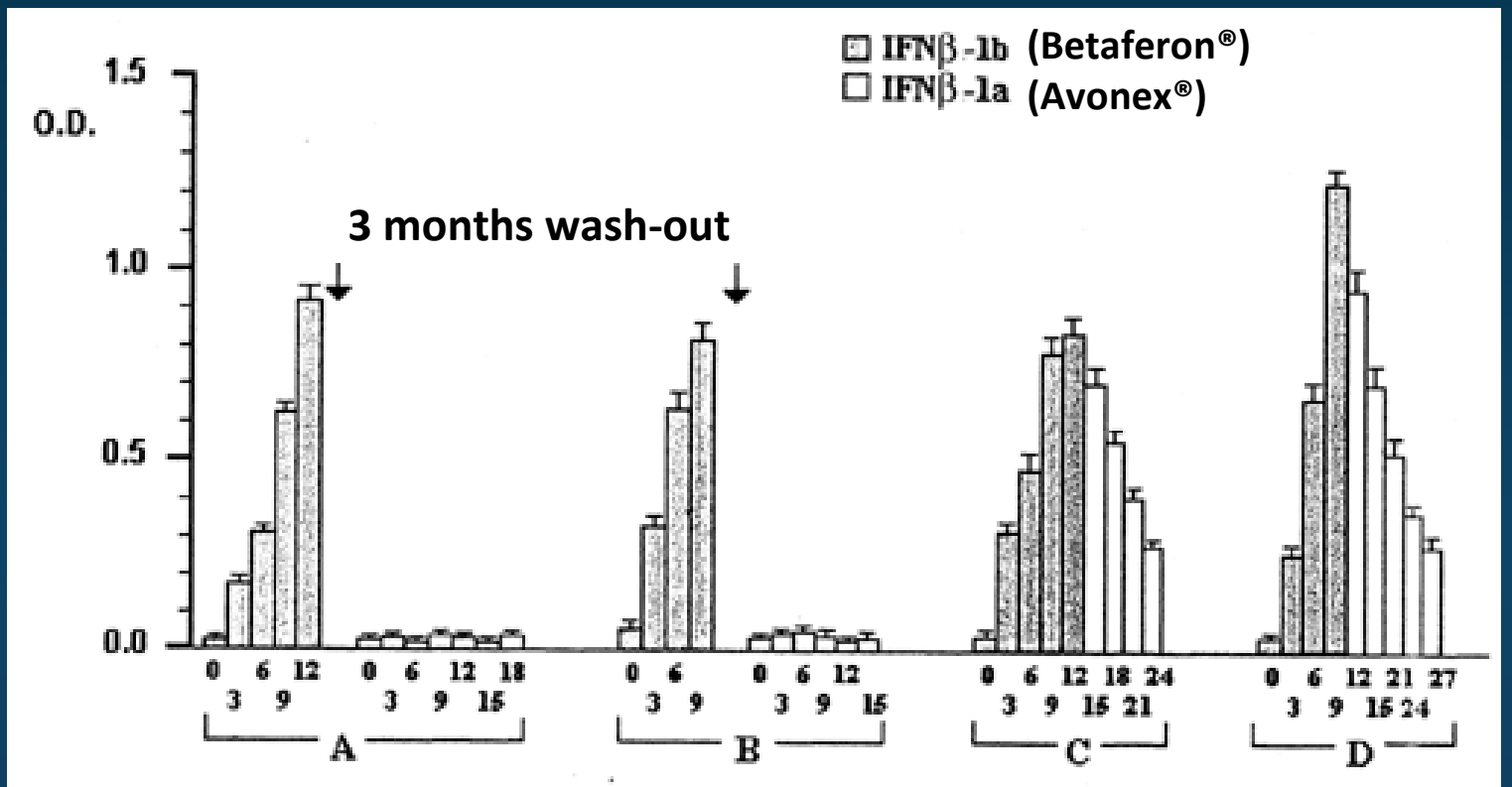
**Contains 60% aggregates!**



# Switching from IFNβ-1b to IFNβ-1a

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

Total IgG




Patient A B C D

Perini et al. 2001

# Switching from IFNβ-1a to IFNβ-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 |

Millonig et al. 2009

# Aims

**1 Study the effect of aggregation on the immunogenicity of Avonex-IFNβ-1a**

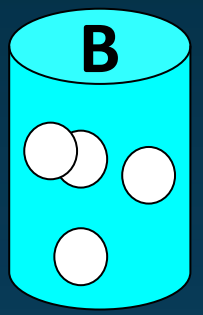
**2 Study the presence of immunological memory in wildtype versus transgenic mice**

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

# Three interferon beta samples

## Bulk IFNβ-1a

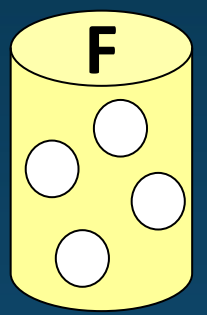
- Avonex bulk
- In PBS pH 7.2
- 270 ug/ml




Medium aggregate content

## Formulated IFNβ-1a

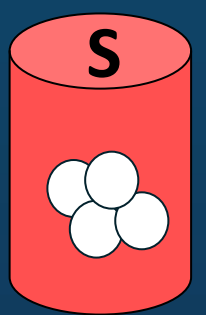
- Dialyzed and filtered Avonex
- In NaAc pH 4.5
- Formulated with Tween 20 and ArgHCl
- ~ 160 ug/ml




Low aggregate content

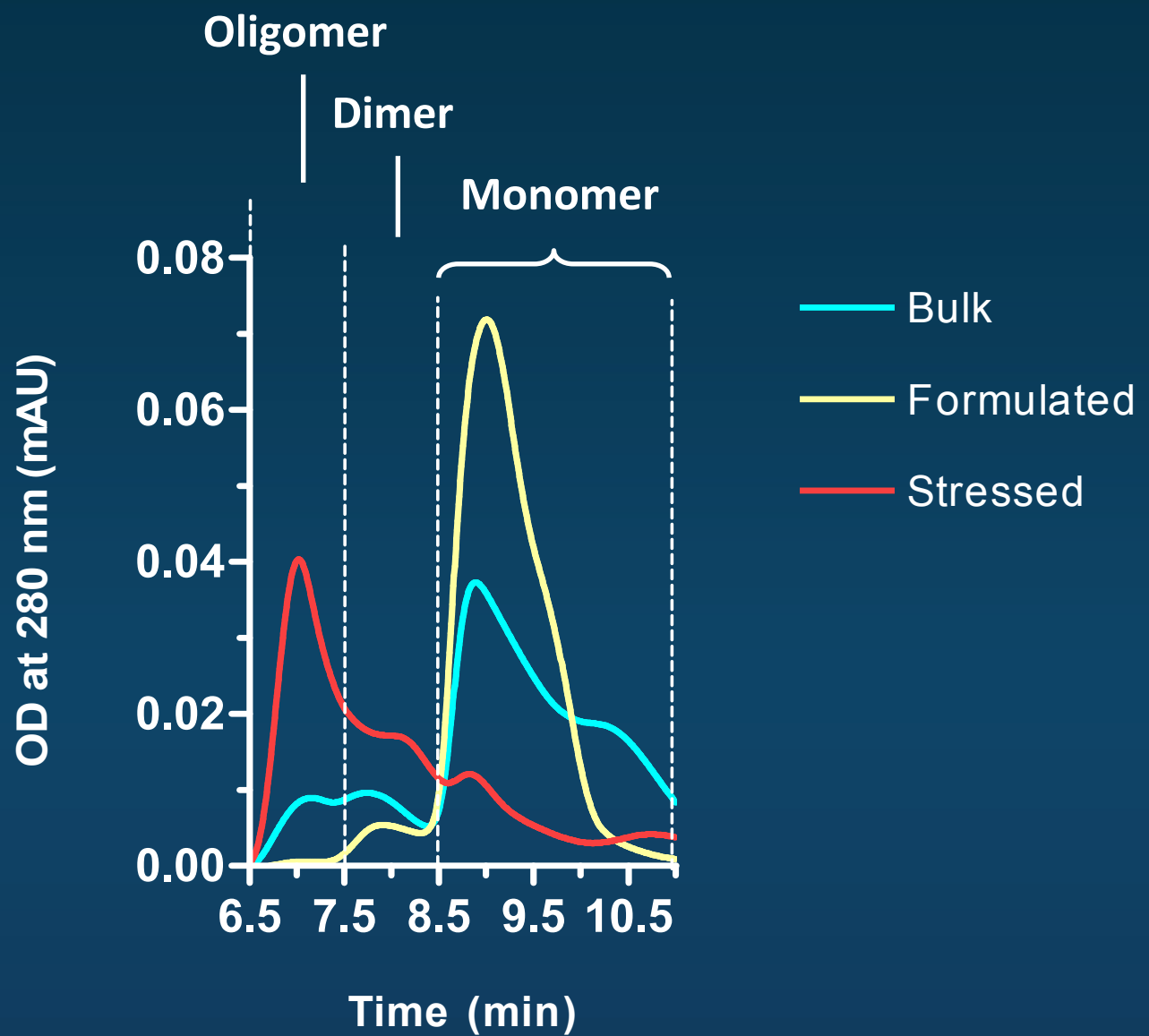
## Stressed IFNβ-1a

- 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

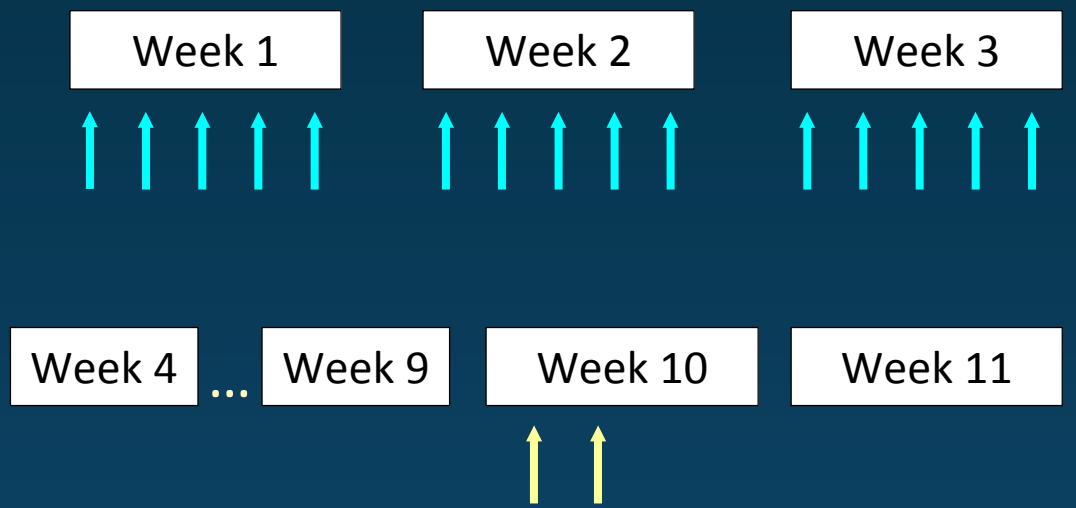


# Aggregate percentage by SEC-HPLC

| | 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



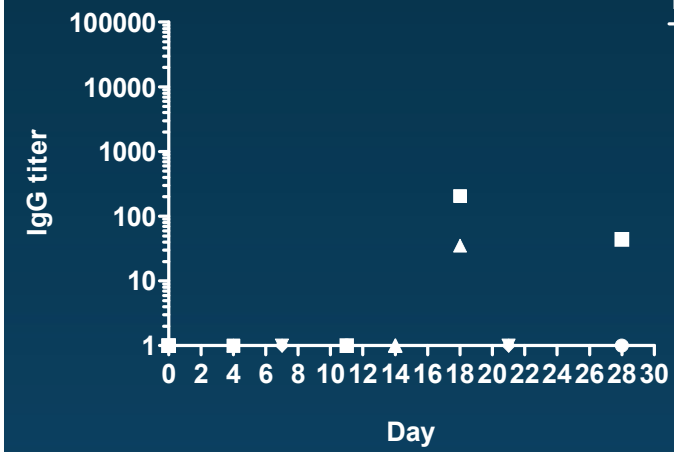

Injections of 5 µg of IFNβ-1a sample or Betaferon-IFNβ-1b i.p.

Re-challenge with 5 µg formulated IFNβ-1a i.p.

- 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

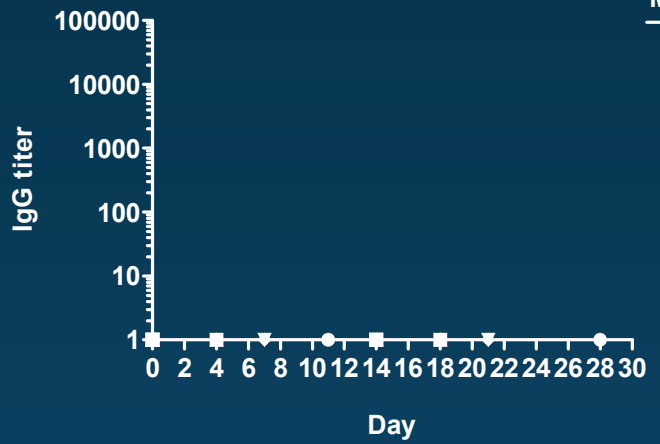
# Immunogenicity in **transgenic** mice

## Bulk



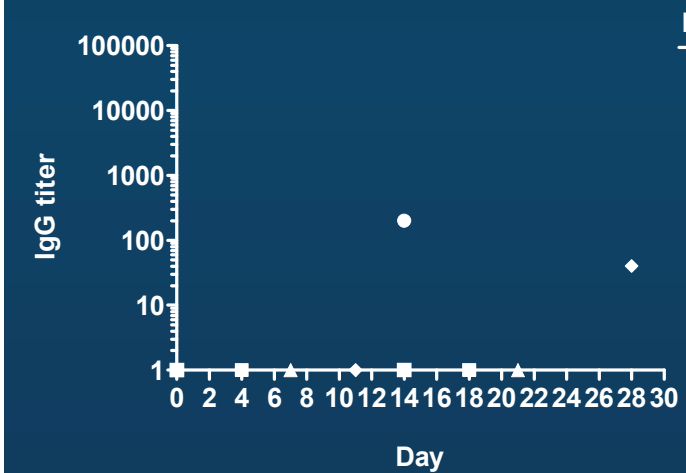

| Mouse ID | NAB status |
|---|---|
| 17 | † |
| 18 | - |
| 21 | - |
| 22 | - |
| 25 | - |
| 26 | - |
| 30 | - |

## Formulated



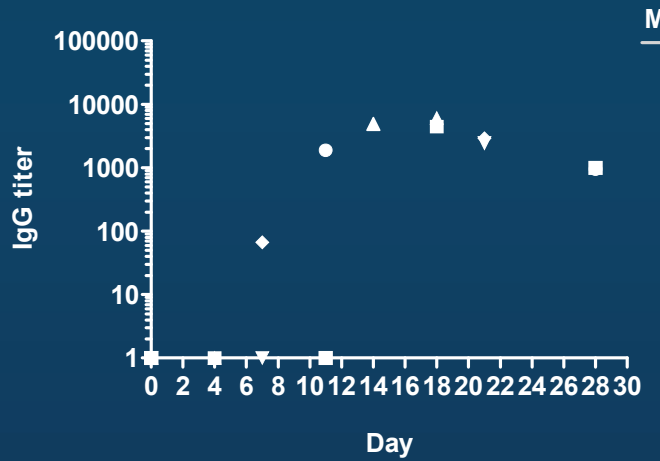

| Mouse ID | NAB status |
|---|---|
| 33 | - |
| 34 | - |
| 37 | † |
| 38 | † |
| 41 | - |
| 45 | - |
| 46 | - |

## Stressed




| Mouse ID | NAB status |
|---|---|
| 49 | - |
| 53 | - |
| 57 | † |
| 58 | - |
| 61 | - |
| 62 | - |

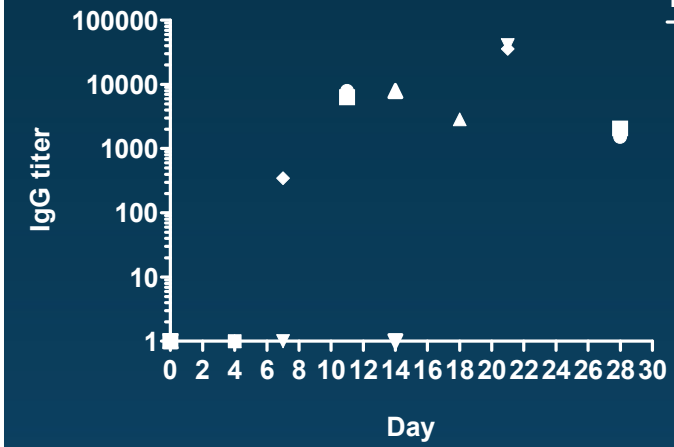
## Betaferon®




| Mouse ID | NAB status |
|---|---|
| 1 | - |
| 2 | † |
| 5 | - |
| 6 | - |
| 9 | - |
| 10 | † |
| 14 | - |

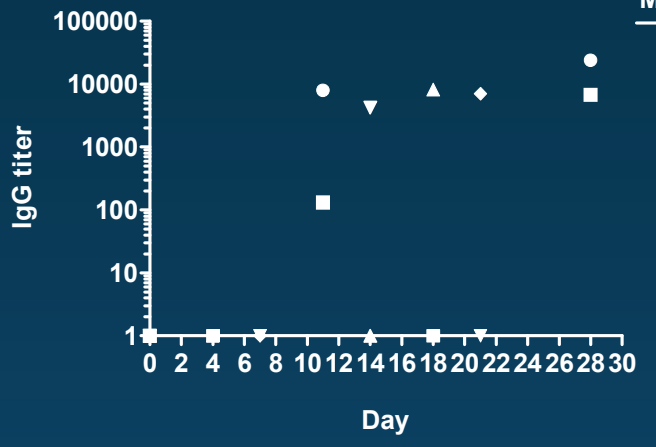
# Immunogenicity in **wildtype** mice

## Bulk



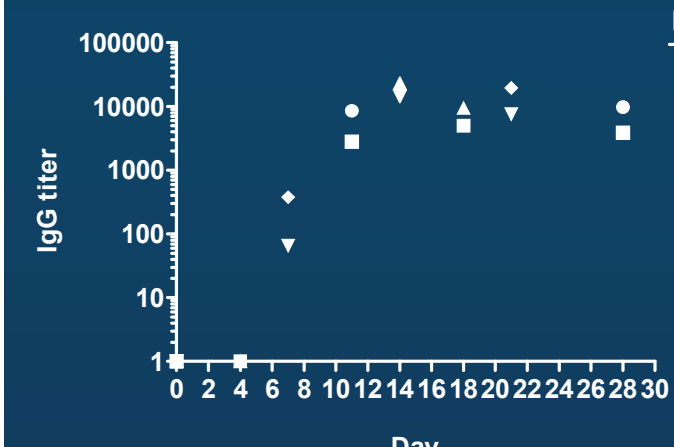

| Mouse ID | NAB status |
|---|---|
| 19 | † |
| 20 | + |
| 23 | + |
| 24 | + |
| 27 | + |
| 28 | + |
| 31 | + |
| 32 | + |

## Formulated



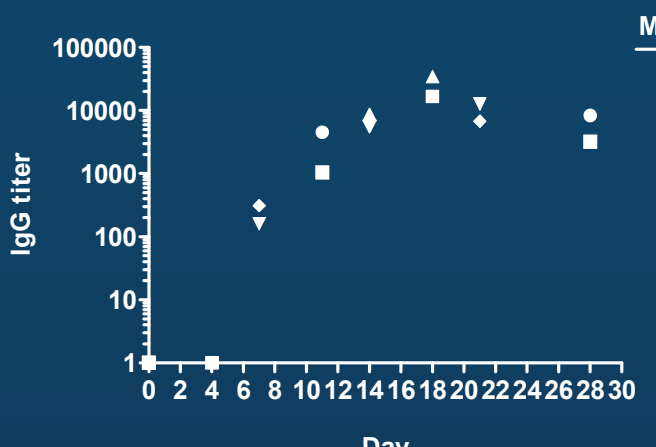

| Mouse ID | NAB status |
|---|---|
| 35 | - |
| 36 | + |
| 39 | - |
| 40 | + |
| 43 | + |
| 44 | + |
| 47 | nd |
| 48 | + |

## Stressed




| Mouse ID | NAB status |
|---|---|
| 51 | + |
| 52 | nd |
| 55 | + |
| 56 | + |
| 59 | + |
| 60 | + |
| 63 | + |
| 64 | + |

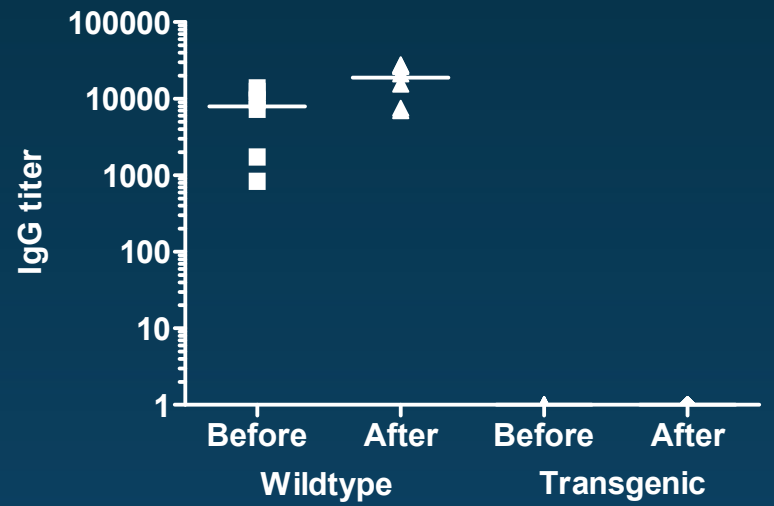
## Betaferon®



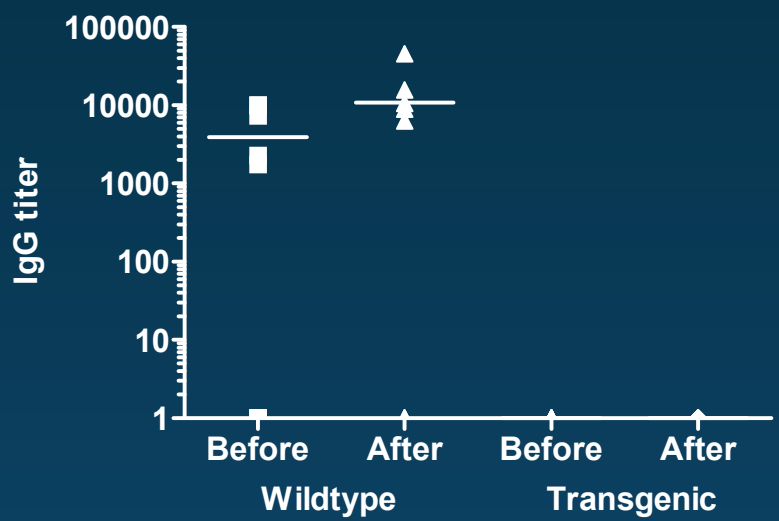

| Mouse ID | NAB status |
|---|---|
| 3 | + |
| 4 | + |
| 7 | + |
| 8 | + |
| 11 | + |
| 12 | + |
| 15 | + |
| 16 | + |

# Memory: titers before and after re-challenge

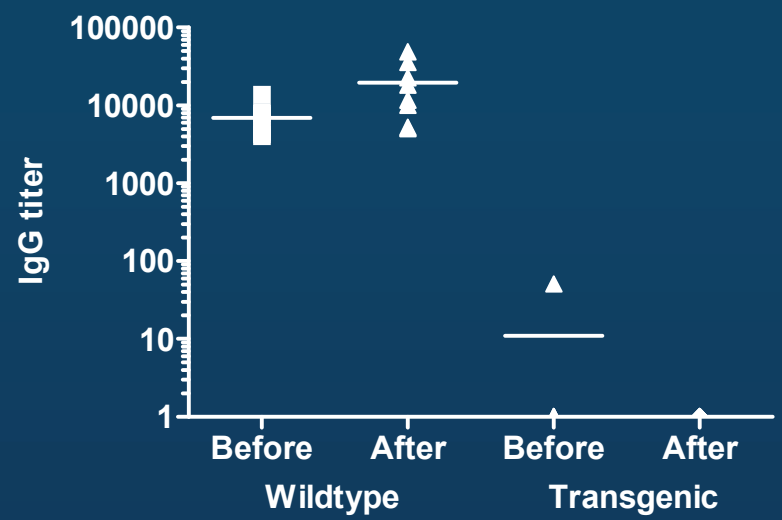
## Bulk



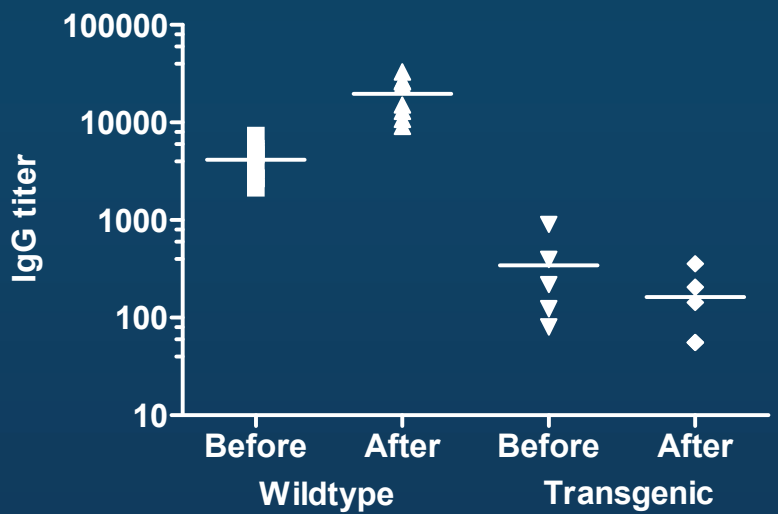

## Formulated




## 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 IFNβ

**Immune mechanism???**

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Biogen Idec (Cambridge, USA)

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UIPS Utrecht Institute for Pharmaceutical Sciences

Neutralizing antibodies on Interferon beta in Multiple Sclerosis
NABINMS

# Questions?