Institute for Research in Biomedicine

EIP 2020 Lisbon

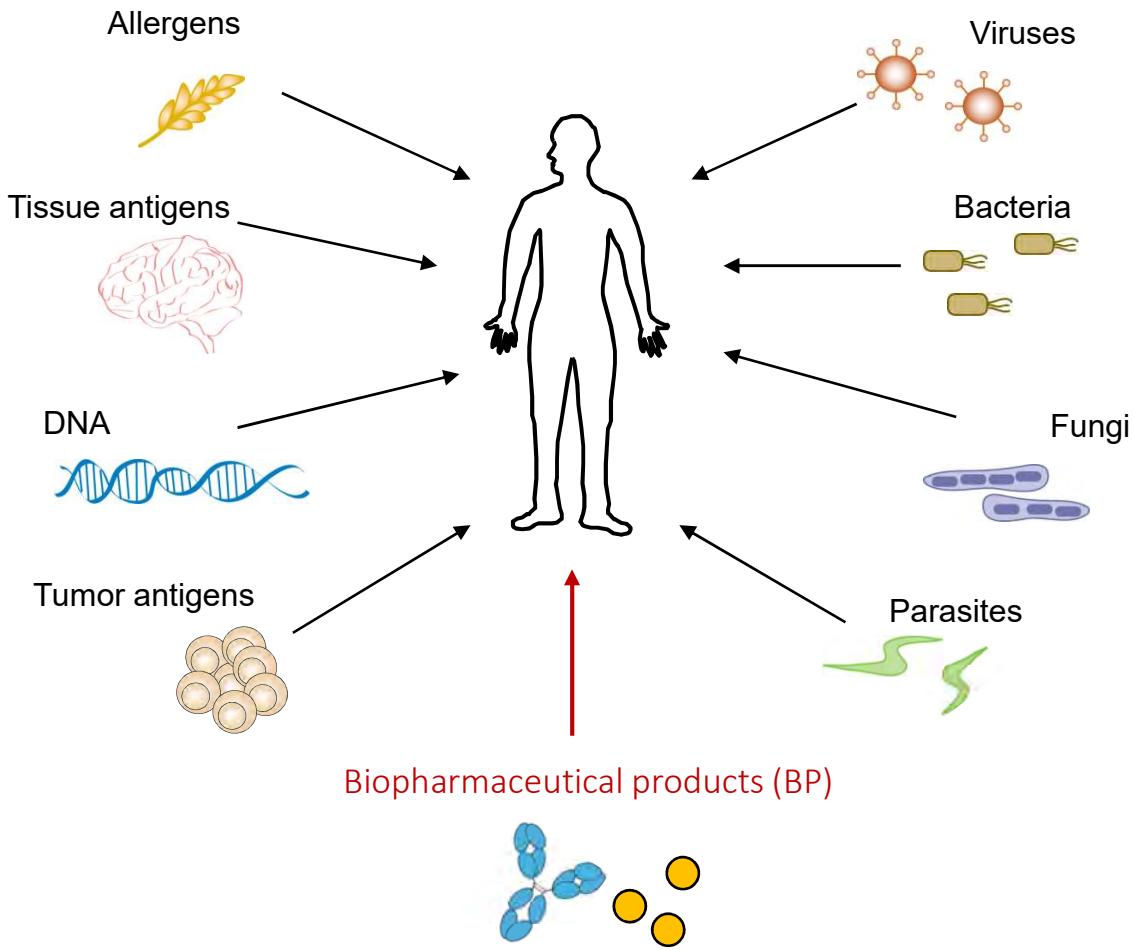
# T-B collaboration in the generation of anti-drug antibody response

Luca Piccoli

Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona, Switzerland
luca.piccoli@irb.usi.ch

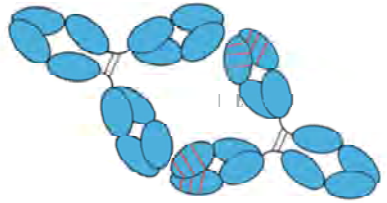
# Unique properties of the human adaptive immune system

- Specificity → large number of immune receptors (TCRs and BCRs)
- Self/non-self recognition
- Long-term memory



# BP may induce anti-drug immune response

A major limitation to the use of BP is the development of immunogenic responses that can affect safety in a subset of patients with induction of:



- Infusion reactions
- Hypersensitivity reactions
- Anti-drug antibodies (ADAs) causing
  - **Autoimmune syndromes** due to cross-reactivity with endogenous counterparts of BP
  - **Decreased efficacy** related to neutralization of the BP's biological activity or increasing its clearance.

It is important to detect and monitor ADA levels for a proper evaluation of the anti-drug response.

# Categories of biopharmaceutical products

| | |
|---|---|
| **Recombinant blood factors** | •Factor VIII **(Hemophilia A)**<br>•Other blood factors **(Hemophilia B, Congenital factor XIII A-subunit deficiency)** |
| **Recombinant thrombolytics, anticoagulants and other blood-related products** | •Tissue plasminogen activator **(Myocardial infarction)**<br>•Hirudin and others **(Venous thrombosis, angioedema)** |
| **Recombinant hormones** | •Insulin **(Diabetes mellitus)** •Growth hormone **(Growth failure/growth hormone deficiency)**<br>•Follicle-stimulating hormone **(Infertility/subfertility)** •Glucagon and other hormones **(Hypoglycemia)** |
| **Recombinant growth factors** | •Erythropoietin **(Anemia/Anemia associated with chronic renal failure)** •IGF-1 **(Growth failure)**<br>•Colony-stimulating factors **(Neutropenia/Chemotherapy-induced neutropenia)** |
| **Recombinant interferons, interleukins and tumor necrosis factors** | •Interferon-α **(Chronic hepatitis C)** •Interferon-γ **(Chronic granulomatous disease)**<br>•Interferon-β **(Multiple sclerosis, Relapsing/remitting multiple sclerosis)** •IL-1 **(Rheumatoid arthritis)** |
| **Recombinant vaccines** | •Immunizations against hepatitis A and B, influenza, diphtheria, tetanus, pertussis, H. influenzae b, hepatitis B and polio **(Infections)** •Immunization against papillomavirus **(Prevention of cervical cancer)** |
| **Monoclonal antibody (mAb)-based products targeting** | •α4β7 integrin **(Ulcerative colitis, Crohn's disease, Relapsing/remitting multiple sclerosis)**<br>•IL-6 **(Rheumatoid arthritis)** •CD20 **(Non-Hodgkin's lymphoma)** •CTLA-4 **(Melanoma)** •VEGF-2 receptor **(Gastric cancer, Metastatic colorectal cancer, Glioblastoma, Metastatic renal carcinoma)**<br>•TNF-α **(Arthritis, colitis, Crohn's disease, psoriasis, ankylosing spondylitis)** •IgE **(Asthma)**<br>• HER2 **(Breast cancer)** •Prostrate-specific membrane antigen **(prostate adenocarcinoma)** |
| **Other recombinant products** | •Bone morphogenetic proteins **(Posterolateral lumbar spinal fusion, Acute tibia fractures)**<br>•Enzymes **(Mucopolysaccharidosis, Gaucher disease, Pompe disease, Fabry disease, Cystic fibrosis)** |
| **Fusion proteins** | •CTLA4-IgG Fc **(Prophylaxis of organ rejection following kidney transplant)**<br>•rTNF receptor-IgG Fc **(Rheumatoid arthritis)** •IL-2-diphteria toxin **(Cutaneous T-cell lymphoma)** |
| **Gene therapy and nucleic acid-based** | •2'-O-(2-methoxy) ethyl–modified ribose antisense oligonucleotide **(Familial hypercholesterolemia)**<br>•h LPL gene housed in an engineered AAV1 vector **(Lipoprotein lipase deficiency)** |

*Adapted from Walsh. Biopharmaceutical benchmarks 2018. Nat Biotec (2018)*

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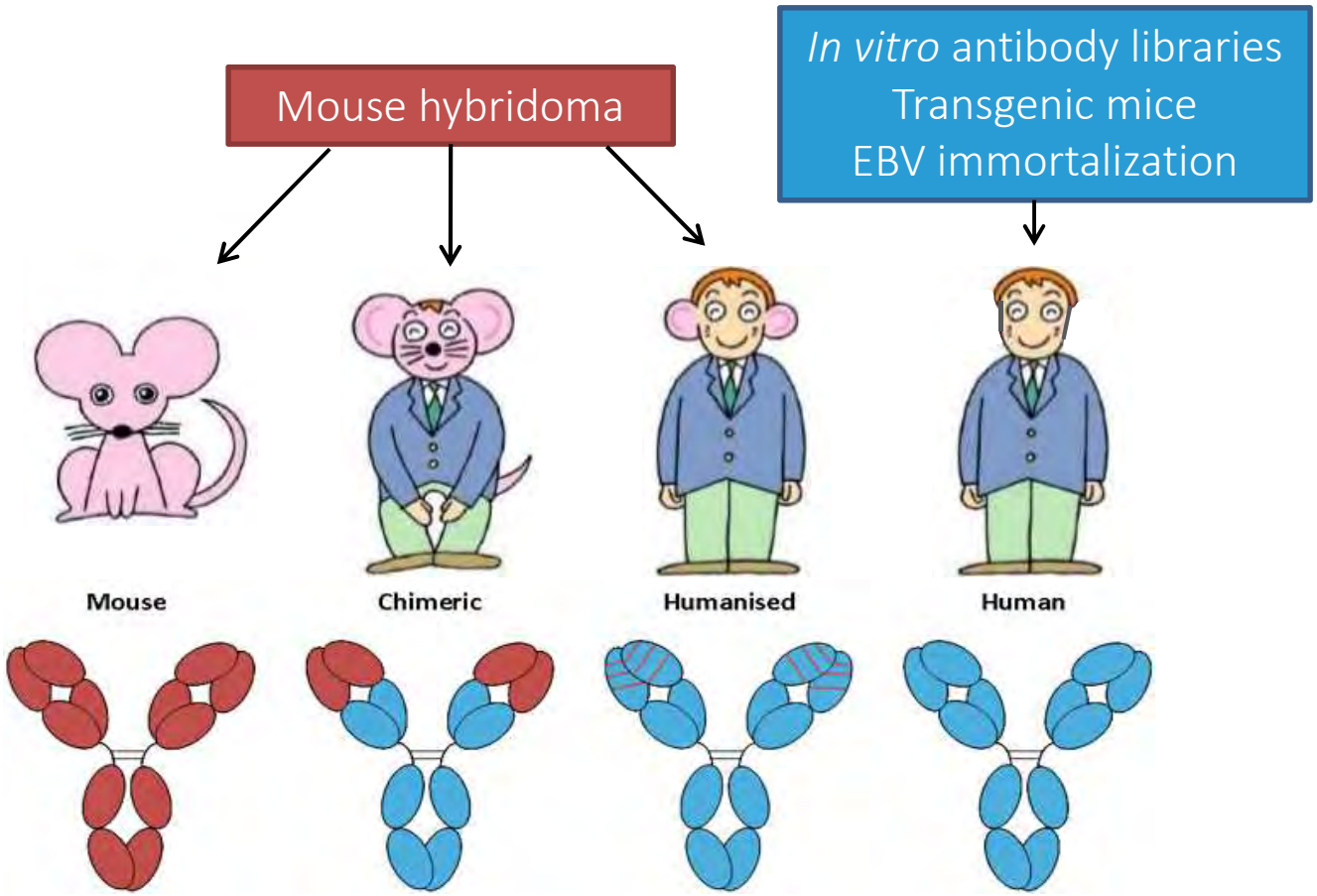
*Adapted from Walsh. Biopharmaceutical benchmarks 2018. Nat Biotec (2018)*

# Therapeutic monoclonal antibodies and ADAs

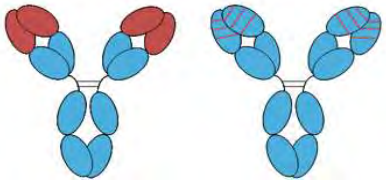



| | | | | |
|---|---|---|---|---|
| Mouse sequence | 100% | 33% | 10% | 0% |
| Example | Anti-CEA | Rituximab | Natalizumab | Nivolumab |
| ADA$^{+}$ patients | ≠ tumors: 64-100% | NHL: 1%<br>RA: 4-11%<br>SLE: 26-65% | MS: 6-9% | Melanoma: 12.7% |

# The generation of ADAs is sustained by CD4$^+$ T cells

Chimeric mAbs:

epitopes of FRs and CDRs



Humanized and fully human mAbs:

epitopes of all CDRs

frontiers in Immunology

## Characterization of CD4 T Cell Epitopes of Infliximab and Rituximab Identified from Healthy Donors

*Moustafa Hamze[1], Sylvain Meunier[1], Anette Karle[2], Abdelaziz Gdoura[1], Amélie Goudet[1], Natacha Szely[3], Marc Pallardy[3], Franck Carbonnel[4], Sebastian Spindeldreher[2], Xavier Mariette[5], Corinne Miceli-Richard[5] and Bernard Maillère[1]**



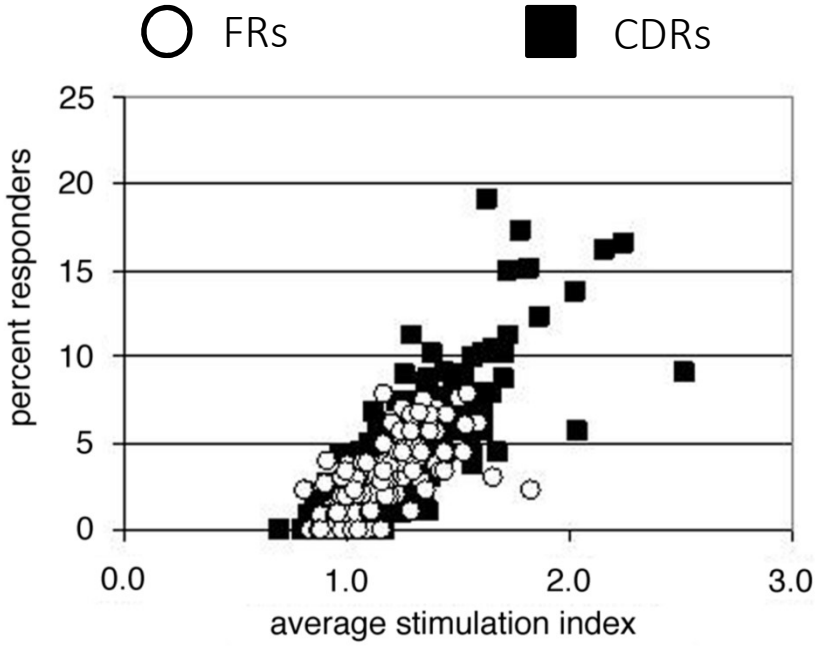

*Adapted from Fig. 5*

mAbs 2:3, 256-265; May/June, 2010; © 2010 Landes Bioscience

## The immunogenicity of humanized and fully human antibodies

### Residual immunogenicity resides in the CDR regions

Fiona A. Harding,* Marcia M. Stickler, Jennifer Razo and Robert B. DuBridge




*Adapted from Fig. 3*

# Not all the ADAs are neutralizing (NAb)

| mAb | Patients *N*, regimen, tumor type | ADA assay | ADA % | NAb % | Reference | Year |
|---|---|---|---|---|---|---|
| Anti-PD-1 | | | | | | |
| Nivolumab | *N* = 1086, pool of 6 clinical studies | ECL | 12.7 | 0.8 | Agrawal et al. | 2017 |
| | *N* = 2085 | ECL | 11.2 | 0.7 | US PI | 2018 |
| | Nivo 3 mg/kg followed by ipi 1 mg/kg Q3W | NR | 23.8-26<br>ADA to nivo | 0.5–1.9<br>nivo NAb | US PI | 2018 |
| | Nivo 1 mg/kg followed by ipi 3 mg/kg Q3W | NR | 37.8<br>ADA to nivo | 4.6<br>nivo NAb | US PI | 2018 |
| | Nivo followed by ipi | NR | 4.1–8.4<br>ADA to ipi | 0–0.3<br>ipi NAb | US PI | 2018 |
| Ipilimumab | *N* = 1024, melanoma | ECL | 1.1 | 0 | US PI | 2018 |
| | *N* = 144, melanoma[c] | ECL | 4.9 | 0 | US PI | 2018 |
| | Nivo and ipi, *N* = 499, RCC and mCRC | NR | 5.4<br>ADA to ipi | 0<br>ipi NAb | US PI | 2018 |

*Adapted from Table 2. Davda et al. J immTher (2019)*

What is the basis of the ADA response and the mechanism of ADA-mediated neutralization?

# The ABIRISK project

Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK

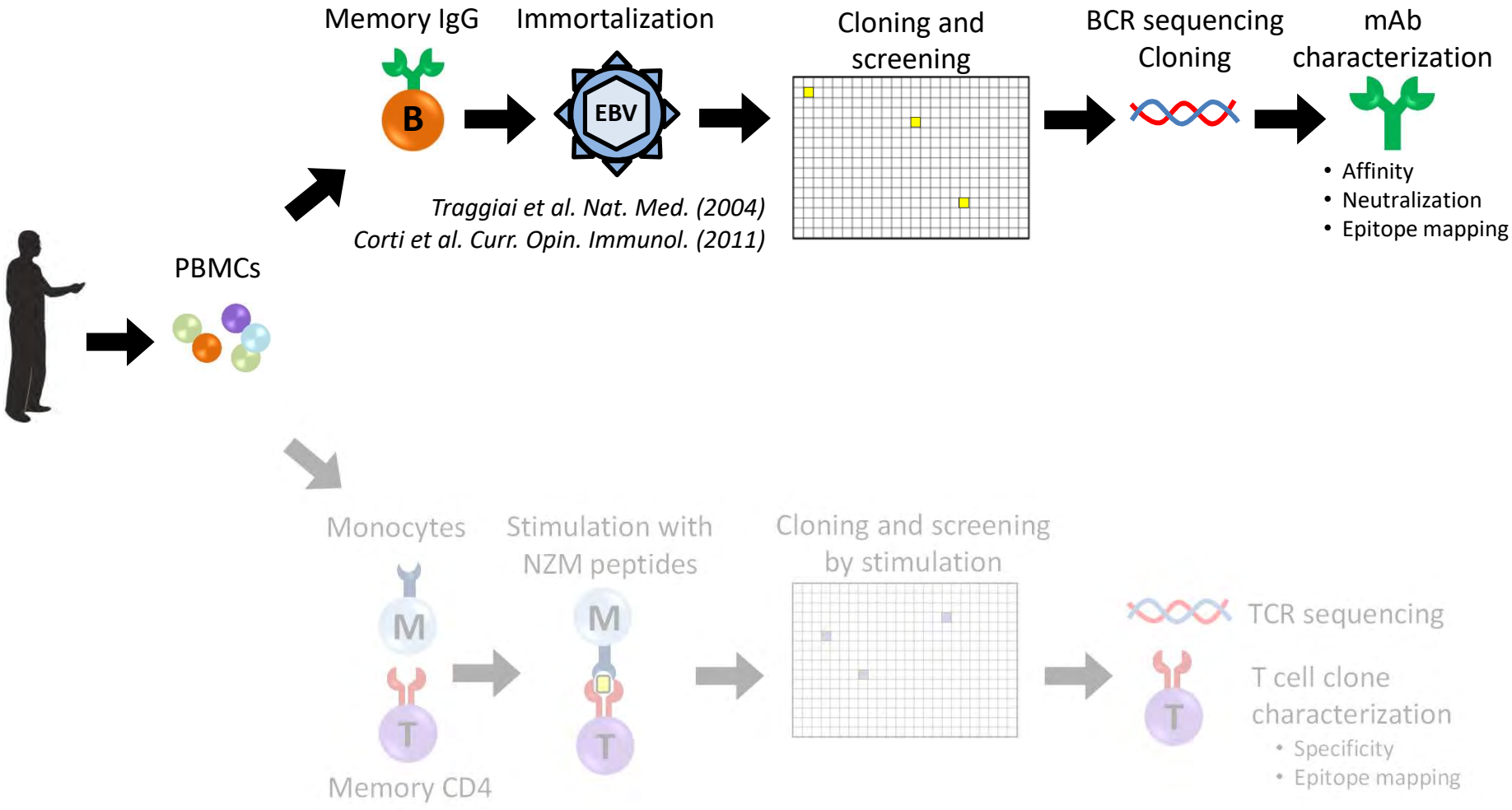
- The ABIRISK project aimed to provide an integrated approach to **predict, prevent, measure** and cure anti-drug immunogenicity.
- Evaluation of the **immunogenicity** of existing BPs for Hemophilia A, Multiple Sclerosis, and Inflammatory Diseases.
- Development of standardized **ADA assays**, including Neutralizing Antibody (NAb) Assays, for each BP.




**Our aim: isolation and characterization of monoclonal anti-drug antibodies:**

- to understand the mechanism of BPs neutralization by ADA.
- to provide positive controls for the development of ADA detection assays

# High-throughput screens and cell cloning to isolate human monoclonal antibodies and T cells



# Monoclonal ADAs as positive controls for ADA detection assays

We isolated several B cell clones producing anti-drug monoclonal antibodies from patients treated with different biopharmaceutical products.

| BP target | Disease | Nr. specific B cell clones isolated | Nr. of monocl. ADA produced | Properties of the monoclonal antibodies |
|---|---|---|---|---|
| Interferon-β | MS | 5 (from 1 out of 4 pts) | 3 | High-affinity binding; 2 epitopes recognized<br>Potent IFNβ neutralization (in combinations) |
| Rituximab | Pemphigus | 3 (from 1 pt) | 3 | High-affinity binding<br>Strong inhibition of binding to CD20 |
| Natalizumab | MS | 40 (from 2 pts) | 4 | High-affinity binding<br>No or variable levels of inhibition of binding to α4 integrin; inhibition dependent on dissociation rate |
| Infliximab | IBD | 12 (from 1 pt) | 4 | High-affinity binding<br>No or variable levels of inhibition of binding to TNFα |
| Adalimumab | Psoriatic arthritis | 26 (from 1 pt) | 4 | Low- to high-affinity binding<br>No or variable levels of inhibition of binding to TNFα |

ABIRISK

Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK

SCALE UP

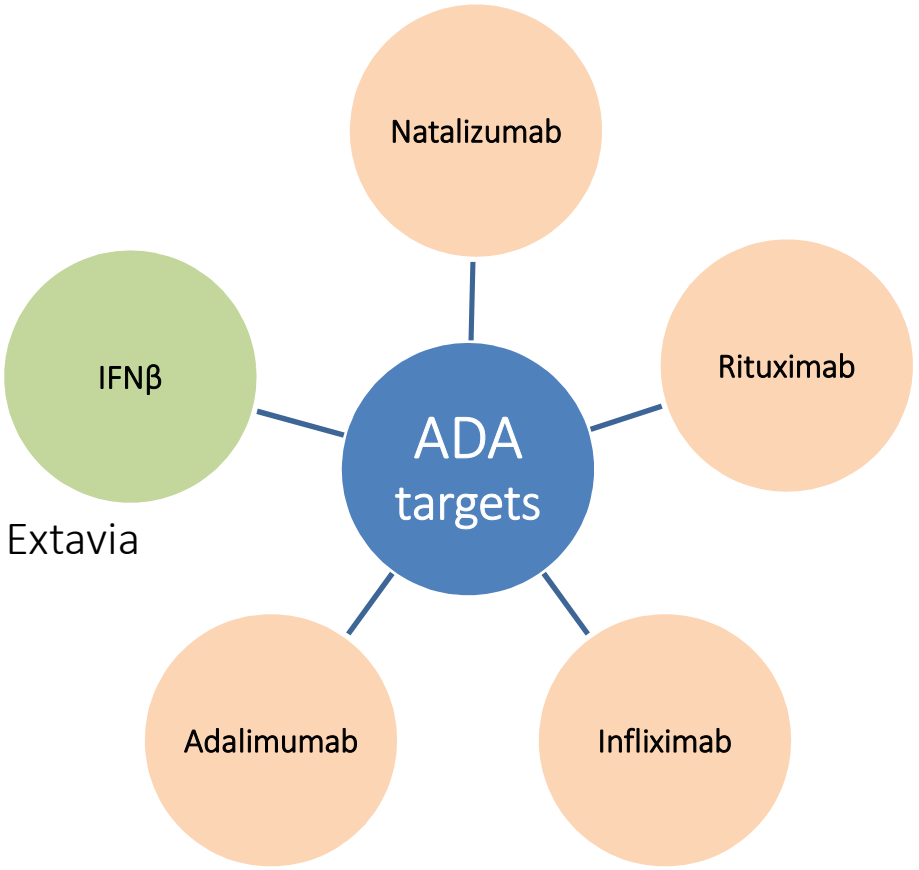
imi efpia

Selected monoclonal antibodies have been produced by Sanofi and lyophilized by the National Institute for Biological Standards and Control (NIBSC)

# ADA response to interferon-β in multiple sclerosis

IFNβ is a recombinant human cytokine used for relapsing/remitting MS → it balances the expression of pro- and anti-inflammatory agents in the CNS, and reduces the number of inflammatory cells that cross the blood brain barrier

1. Glycosylated (IFNβ1a): Rebif and Avonex
2. Non-glycosylated (IFNβ1b): Betaferon and Extavia

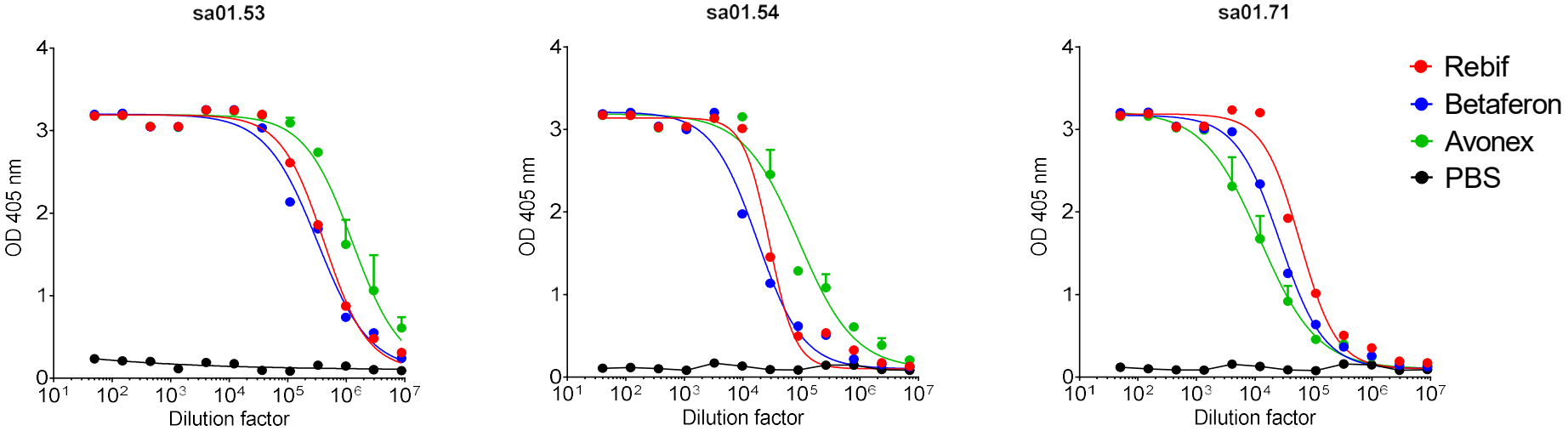



Screening of 3 MS patients with Rebif:
→ 5 anti-IFNβ mAbs isolated
(3 different clones)
→produced for PC selection

| NAME | ISOTYPE HC | LC | VH | D | JH | %VH | %JH | VL | JL | %VL | %JL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| sa01.6 | IgG4 | k | VH 3-23 | D3-10 | JH 4 | 97.57 | 85.4 | VK 1-39 | JK 1 | 95.34 | 97.4 |
| sa01.53 | IgG2 | k | VH 4-39 | D3-10 | JH 1 | 90.03 | 86.5 | VK 1-5 | JK 2 | 94.62 | 94.9 |
| sa01.54 | IgG4 | k | VH 3-23 | D3-10 | JH 4 | 97.22 | 85.4 | VK 1-39 | JK 1 | 95.7 | 92.1 |
| sa01.62 | IgG4 | k | VH 3-23 | D3-10 | JH 4 | 94.1 | 83.3 | VK 1-39 | JK 1 | 93.91 | 97.4 |
| sa01.71 | IgG1 | λ | VH 2-70 | D3-10 | JH 4 | 97.94 | 97.9 | VL2-8 | JL2 | 96.13 | 91.9 |

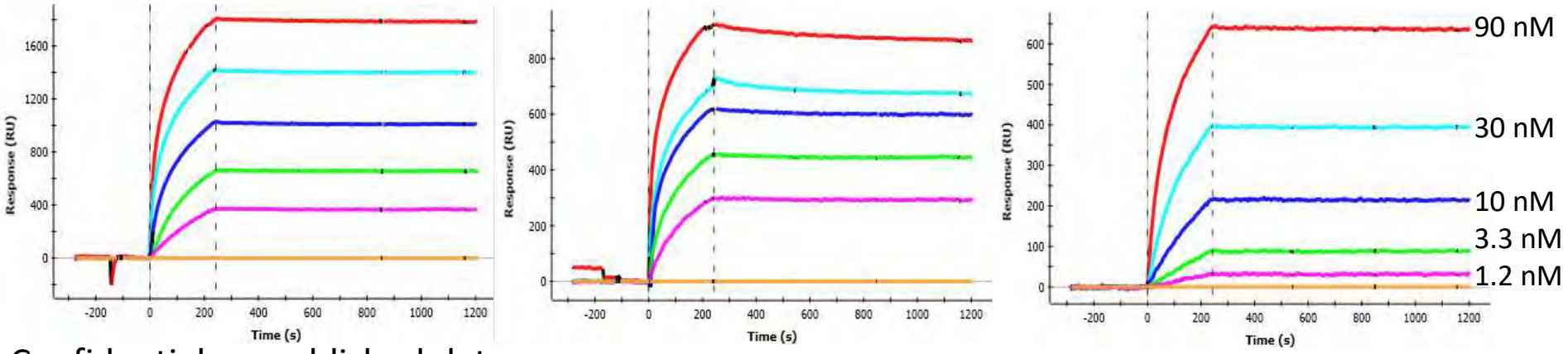
# Monoclonal ADAs bind to IFNβ drugs with high affinity

ELISA



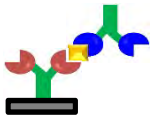

Surface Plasmon Resonance:

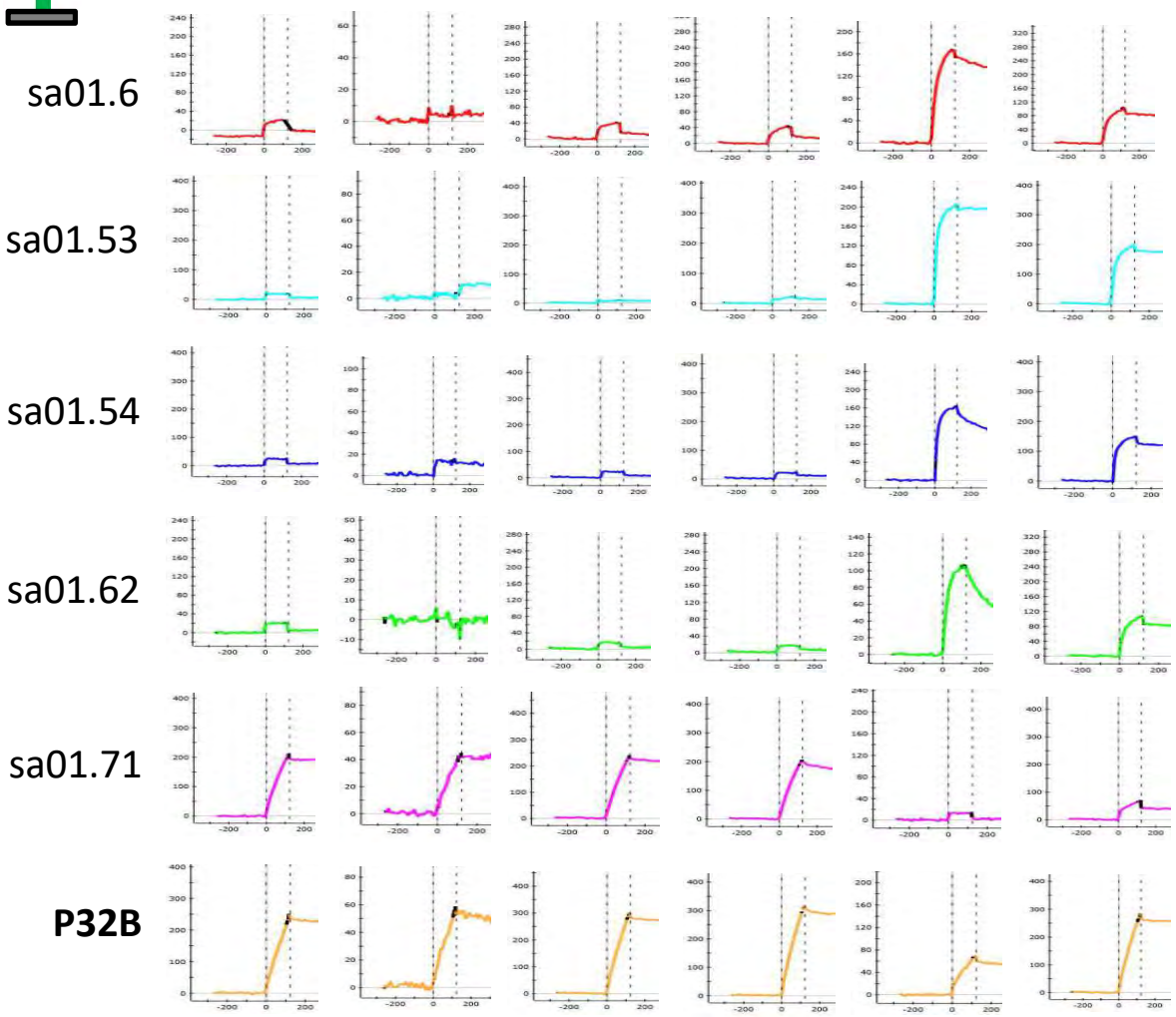
| | Ka (1/Ms) | Kd (1/s) | KD (M) |
|---|---|---|---|
| Sa01.53 | 1.60E+06 | 3.74E-06 | 2.34E-12 |
| Sa01.54 | 2.63E+06 | 2.09E-05 | 7.95E-12 |
| Sa01.71 | 3.78E+05 | 2.51E-06 | 6.64E-12 |

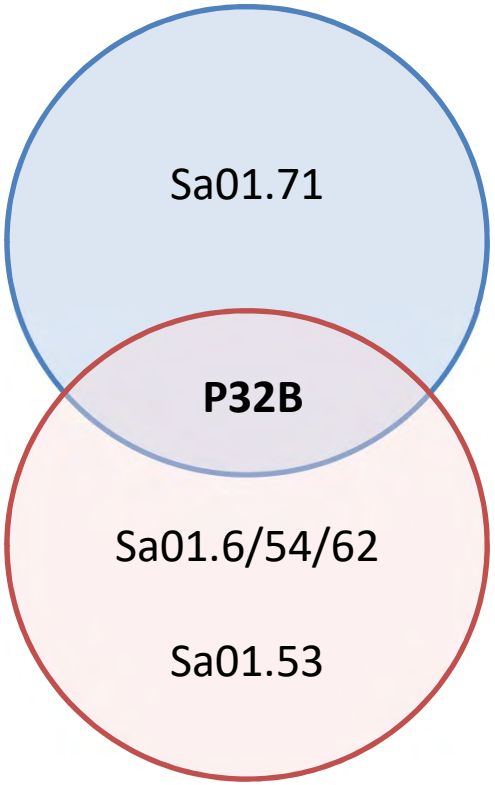



Confidential, unpublished data

# The antibodies recognize 2 different epitopes on IFNβ



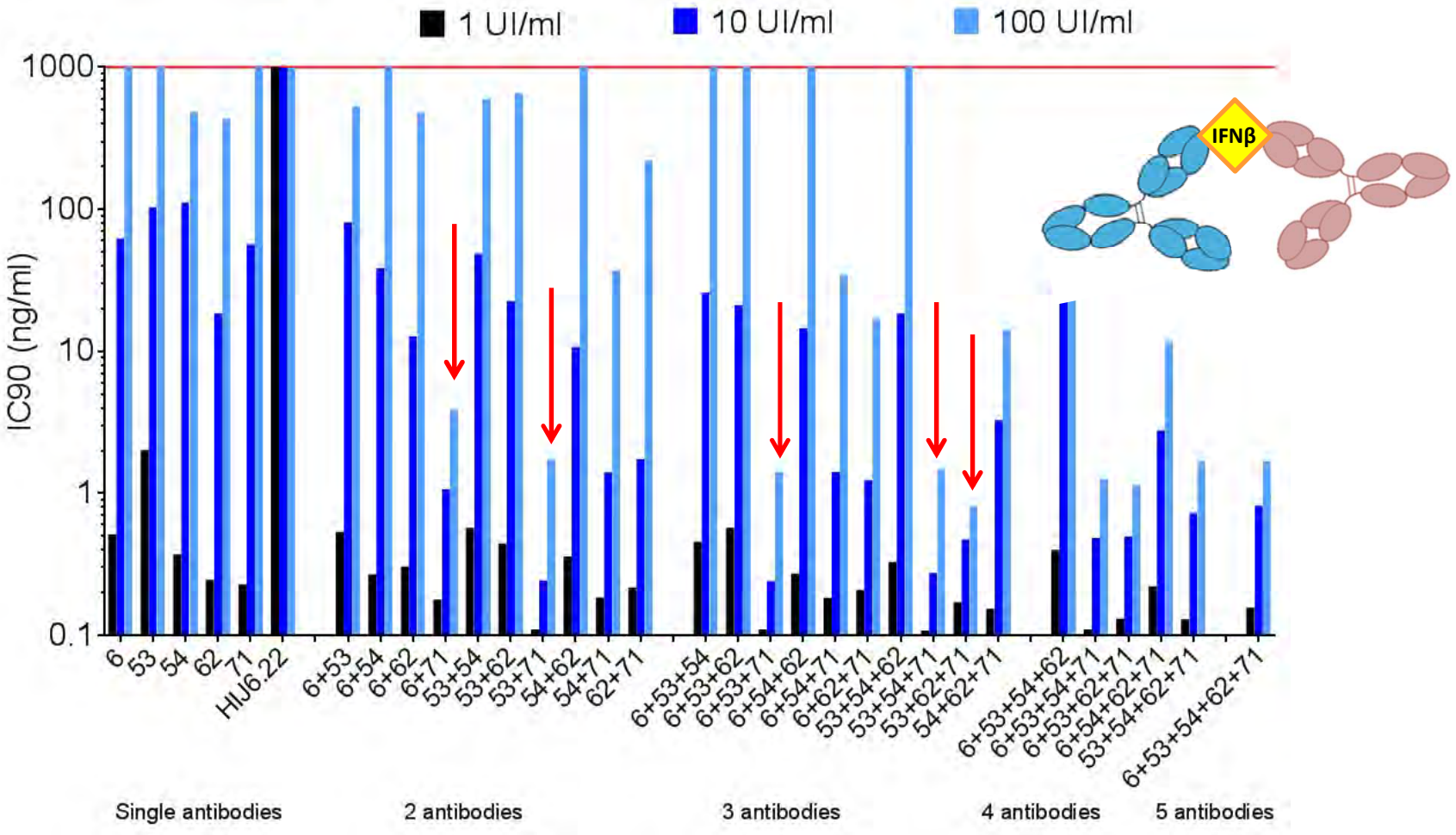



Confidential, unpublished data

# Combinations of ADAs completely neutralize IFNβ activity

To test neutralization we used a cell line (**HEK-Blue IFNa/β from Invivogen**) that expresses a reporter gene (secreted embrionic alkaline phosphatase, SEAP) after IFNβ-dependent activation of the JAK-STAT pathway.

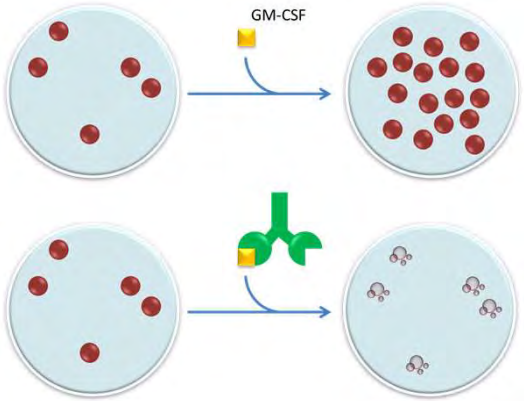


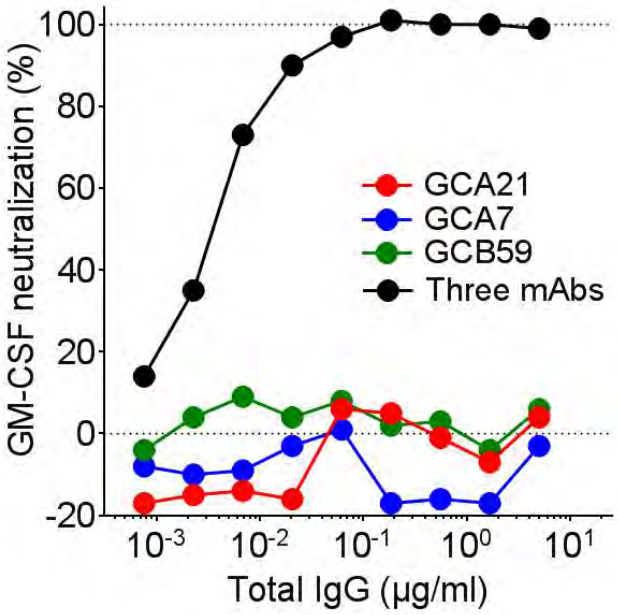

Confidential, unpublished data

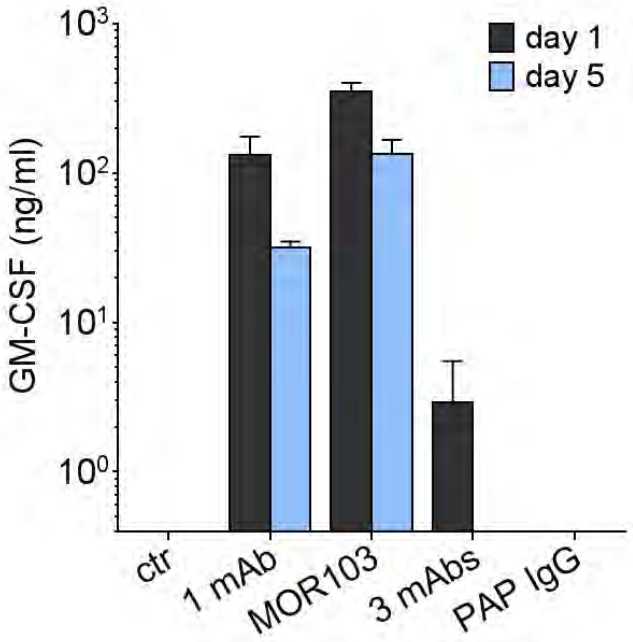
# GM-CSF is neutralized and cleared by autoantibodies in PAP

- Pulmonary alveolar proteinosis (PAP) is a rare and severe autoimmune disease caused by the production of autoantibodies that neutralize GM-CSF
  → inhibition of terminal differentiation and functions of alveolar macrophages.

- A combination of three non-cross-competing antibodies completely neutralizes GM-CSF activity *in vitro,* and *in vivo* promotes the rapid degradation of GM-CSF-containing immune complexes in an Fc-dependent manner.




Piccoli *et al.*, *Nat. Commun.* (2015)

# Conclusions (I)

- Effective neutralization of a cytokine activity requires the formation of immune complexes with antibodies targeting different epitopes.

- Such immune complexes can be cleared from the body (Fc-dependent mechanism).

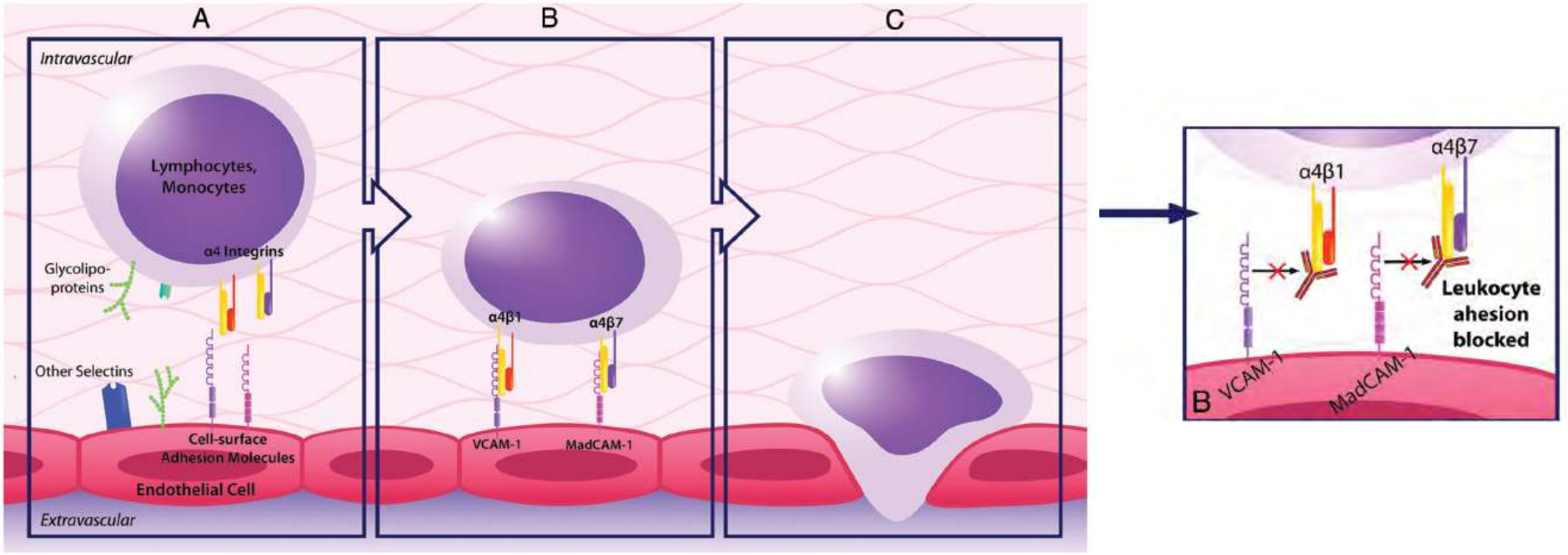
LETTERS
https://doi.org/10.1038/s41591-019-0568-2
nature medicine

# A single T cell epitope drives the neutralizing anti-drug antibody response to natalizumab in multiple sclerosis patients

Antonino Cassotta[1,2], Vincent Mikol[3], Thomas Bertrand[3], Stéphanie Pouzieux[3], Josiane Le Parc[3], Paul Ferrari[3], Jacques Dumas[3], Michael Auer[4], Florian Deisenhammer[4], Matteo Gastaldi[5], Diego Franciotta[5], Chiara Silacci-Fregni[1], Blanca Fernandez Rodriguez[1], Isabella Giacchetto-Sasselli[1], Mathilde Foglierini[1,6], David Jarrossay[1], Roger Geiger[1], Federica Sallusto[1,2], Antonio Lanzavecchia[1] and Luca Piccoli[1*]

# Immunogenicity of natalizumab in multiple sclerosis

- Natalizumab (Tysabri) is a second-line therapy for patients with relapsing-remitting multiple sclerosis that do not respond to treatment with first-line drugs such as IFNβ.
- It selectively binds to the α4-integrin component of adhesion molecules found on leukocytes preventing their migration into the CNS and reducing inflammation.




Adapted from Selewsky et al, AJNR Am J Neuroradiol (2010)

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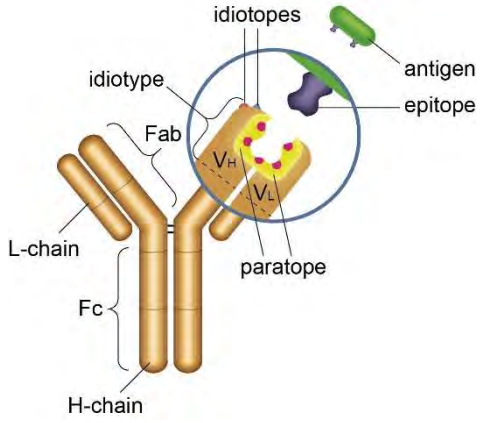
| | Natalizumab |
|---|---|
| Drug name | Tysabri |
| Properties | Humanized $IgG4_K$ monoclonal antibody |
| Administration | at specialized infusion centers for 1 hour with 1-hour monitoring (TOUCH program) |
| ADA frequency | ~9% with 3% that are transient |
| Side effect | Progressive multifocal leukoencephalopathy (PML): CNS infection by JC virus |



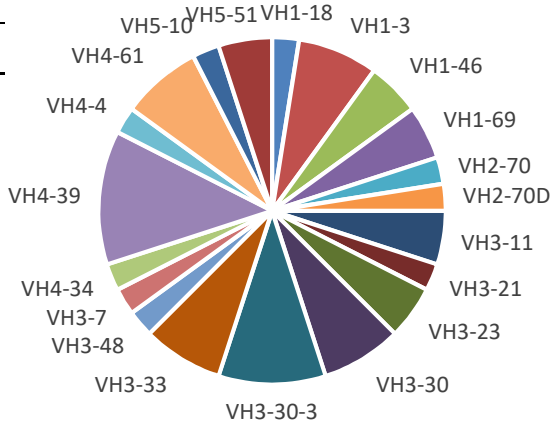


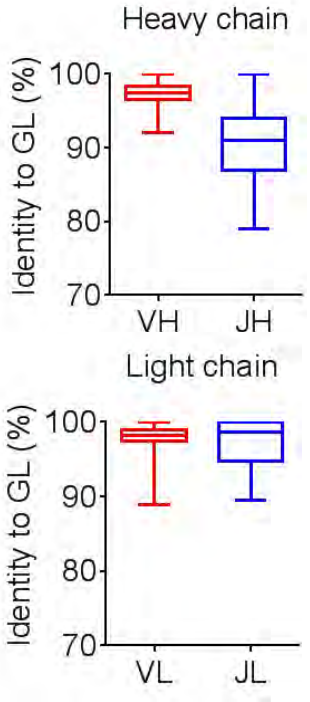

- As an humanized antibody, natalizumab contains immunogenic epitopes (idiotopes) for the induction of ADA → anti-idiotypic response.

*Chataway et al. Natalizumab Therapy for Multiple Sclerosis. Neurotherapeutics (2013) Selewsky et al, AJNR Am J Neuroradiol (2010) Bachelet et al. Occurrence of Anti-Drug Antibodies against Interferon-Beta and Natalizumab in Multiple Sclerosis: A Collaborative Cohort Analysis. PlosONE (2016)*

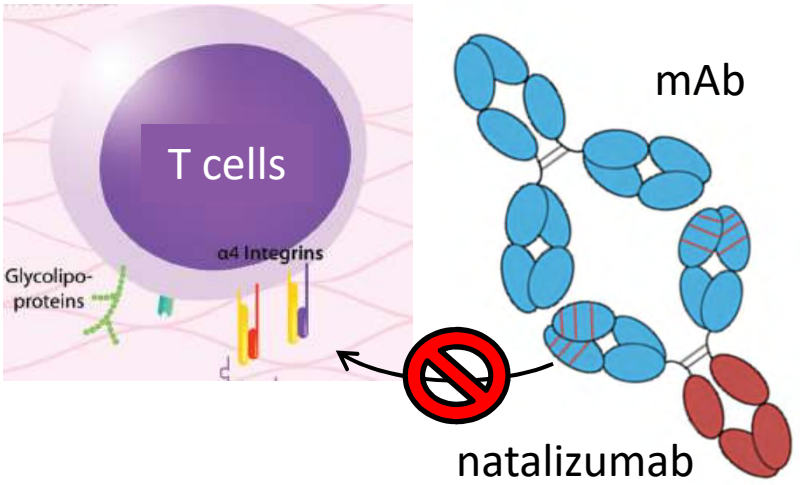
# NZM induces a polyclonal antibody response

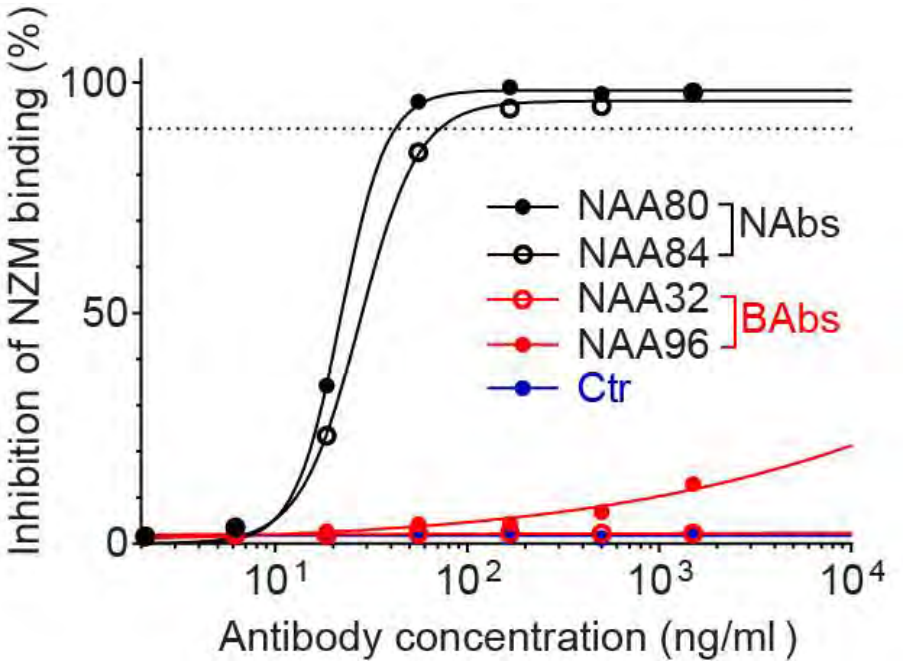
| MS patient | B cell clone | Isotype | | Heavy chain VDJ genes (% identity to germline) | | | | | Light chain VJ genes (% identity to germline) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | NAA2 | IgG1 | κ | VH1-46 | (97.6) | D4-23 | JH4 | (87.5) | VK3-11 | (98.6) | JK3 | (91.4) |
| | NAA5 | IgG1 | λ | VH4-39 | (96.6) | D3-22 | JH3 | (94) | VL2-8 | (98.3) | JL2 | (100) |
| | NAA6 | IgG1 | λ | VH3-30-3 | (97.2) | D1-26 | JH3 | (96) | VL3-19 | (97.5) | JL3 | (100) |
| | NAA7 | IgG1 | κ | VH4-4 | (97.5) | D3-3 | JH6 | (79) | VK1-5 | (97.9) | JK1 | (100) |
| | NAA9 | IgG3 | λ | VH3-23 | (99) | D7-27 | JH4 | (91.7) | VL1-51 | (98.6) | JL2 | (94.4) |
| | NAA20 | IgG1 | λ | VH4-34 | (97.5) | D6-13 | JH4 | (87.5) | VL2-8 | (97.9) | JL1 | (94.7) |
| | NAA32 | IgG1 | κ | VH1-3 | (97.9) | D2-15 | JH2 | (98.1) | VK3-15 | (100) | JK4 | (97.4) |
| | NAA33 | IgG1 | λ | VH5-51 | (97.2) | D6-6 | JH5 | (90.2) | VL3-1 | (95.3) | JL2 | (94.7) |
| | NAA34 | IgG3 | κ | VH3-23 | (98.6) | D2-15 | JH4 | (100) | VK1-9 | (98.6) | JK3 | (100) |
| | NAA36 | IgG1 | λ | VH3-30-3 | (95.5) | D1-1 | JH4 | (85.4) | VL2-11 | (97.9) | JL2 | (94.3) |
| | NAA40 | IgG1 | λ | VH4-61 | (99.7) | D6-13 | JH5 | (90.2) | VL1-44 | (99) | JL2 | (94.7) |
| | NAA44 | IgG1 | λ | VH4-39 | (96.9) | D5-12 | JH5 | (82.4) | VL2-8 | (97.5) | JL2 | (92.1) |
| | NAA45 | IgG1 | λ | VH4-61 | (97.9) | D3-16 | JH4 | (93.8) | VL1-44 | (99) | JL3 | (100) |
| | NAA49 | IgG1 | λ | VH3-33 | (98.6) | D5-24 | JH4 | (89.6) | VL3-21 | (98.9) | JL2 | (100) |
| | NAA59 | IgG1 | λ | VH1-69 | (97.9) | D1-1 | JH3 | (96) | VL3-19 | (98.6) | JL2 | (94.7) |
| | NAA62 | IgG3 | κ | VH3-11 | (97.6) | D4-17 | JH4 | (91.7) | VK3-11 | (97.5) | JK5 | (100) |
| | NAA80 | IgG1 | λ | VH3-48 | (96.9) | D2-15 | JH6 | (85.5) | VL3-19 | (98.9) | JL3 | (97.1) |
| | NAA84 | IgG1 | λ | VH3-21 | (97.6) | D3-22 | JH3 | (94) | VL3-19 | (97.5) | JL2 | (92.1) |
| | NAA92 | IgG3 | λ | VH3-30 | (99.3) | D3-22 | JH4 | (91.7) | VL4-69 | (97.3) | JL2 | (97.4) |
| | NAA94 | IgG2 | λ | VH1-3 | (96.9) | D3-22 | JH5 | (96.1) | VL1-40 | (100) | JL3 | (100) |
| | NAA96 | IgG3 | λ | VH4-61 | (98.3) | D3-3 | JH4 | (85.4) | VL1-44 | (98.3) | JL3 | (100) |
| | NAA104 | IgG1 | κ | VH2-70D | (100) | D6-19 | JH3 | (98) | VK3-11 | (98.2) | JK2 | (97.4) |
| | NAA105 | IgG1 | λ | VH1-46 | (96.5) | D1-20 | JH3 | (94) | VL3-19 | (98.9) | JL2 | (97.1) |
| | NAA110 | IgG1 | κ | VH3-30-3 | (96.9) | D2-15 | JH6 | (83.9) | VK1-5 | (98.2) | JK1 | (100) |
| | NAA113 | IgG1 | κ | VH3-33 | (96.2) | D1-14 | JH4 | (89.6) | VK3-11 | (100) | JK4 | (100) |
| | NAA114 | IgG1 | λ | VH4-39 | (98.5) | D4-17 | JH4 | (87.5) | VL1-51 | (99.3) | JL3 | (100) |
| | NAA116 | IgG1 | κ | VH3-30-3 | (97.6) | D2-15 | JH6 | (90.3) | VK3-20 | (100) | JK2 | (100) |
| | NAA119 | IgG1 | κ | VH1-3 | (96.5) | D6-19 | JH1 | (94.2) | VK3-20 | (98.6) | JK3 | (94.7) |
| | NAA125 | IgG1 | λ | VH4-39 | (95.2) | D2-21 | JH6 | (85.5) | VL1-40 | (98.6) | JL1 | (100) |
| | NAA128 | IgG1 | κ | VH1-69 | (96.9) | D4-11 | JH4 | (97.9) | VK3-11 | (100) | JK4 | (100) |
| B | NAE125 | IgG1 | λ | VH4-39 | (97.6) | D1-7 | JH4 | (81.3) | VL1-47 | (98.3) | JL3 | (97.3) |
| | NAE194 | IgG1 | κ | VH3-11 | (94.1) | D6-19 | JH4 | (79.2) | VK1-12 | (94.6) | JK4 | (91.7) |
| | NAE197 | IgG1 | κ | VH3-30 | (100) | D3-16 | JH4 | (100) | VK1-39 | (95.3) | JK1 | (97.4) |
| | NAE199 | IgG1 | λ | VH3-7 | (97.2) | D7-27 | JH3 | (94) | VL3-19 | (100) | JL1 | (97.4) |
| | NAE203 | IgG3 | λ | VH2-70 | (97.6) | D3-16 | JH3 | (100) | VL3-9 | (98.2) | JL2 | (100) |
| | NAE205 | IgG3 | λ | VH5-51 | (94.8) | D4-11 | JH6 | (87.1) | VL3-10 | (96.4) | JL1 | (86.8) |
| | NAE206 | IgG1 | κ | VH1-18 | (94.8) | D3-22 | JH5 | (96.1) | VK3-20 | (93.6) | JK2 | (100) |
| | NAE207 | IgG1 | λ | VH5-10 | (100) | D2-2 | JH4 | (75) | VL4-60 | (99.7) | JL3 | (97.4) |
| | NAE208 | IgG1 | κ | VH3-30 | (89.9) | D3-9 | JH3 | (90) | VK3-15 | (93.9) | JK1 | (94.7) |
| | NAE210 | IgG1 | κ | VH3-33 | (95.1) | D3-22 | JH5 | (84.3) | VK1-5 | (96.8) | JK1 | (100) |



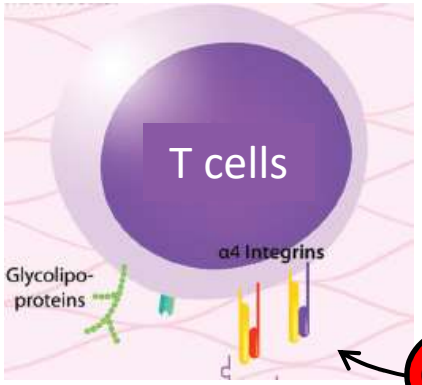
# Binding (BAbs) and neutralizing (NAbs) antibodies

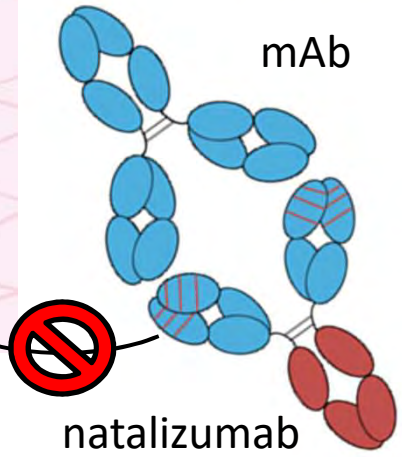



| Patient | Antibody | Type | IC90 (ng/ml) | EC50 (ng/ml) |
|---|---|---|---|---|
| A | NAA104 | BAb | 100,000.0 | 35.1 |
| | NAA32 | BAb | 100,000.0 | 68.1 |
| | NAA40 | BAb | 100,000.0 | 103.0 |
| | NAA94 | BAb | 100,000.0 | 69.8 |
| | NAA96 | BAb | 100,000.0 | 32.6 |
| | NAA36 | BAb | 29,485.0 | 83.6 |
| | NAA2 | BAb | 24,849.0 | 59.2 |
| | NAA20 | BAb | 23,763.0 | 99.0 |
| | NAA49 | BAb | 7,965.0 | 51.8 |
| | NAA110 | BAb | 7,843.0 | 65.9 |
| | NAA9 | BAb | 2,859.0 | 78.6 |
| | NAA34 | BAb | 1,611.0 | 51.1 |
| | NAA59 | NAb | 271.1 | 31.0 |
| | NAA5 | NAb | 222.3 | 51.0 |
| | NAA33 | NAb | 194.6 | 58.3 |
| | NAA7 | NAb | 187.9 | 69.5 |
| | NAA62 | NAb | 171.2 | 47.4 |
| | NAA116 | NAb | 147.3 | 44.0 |
| | NAA114 | NAb | 133.3 | 48.0 |
| | NAA105 | NAb | 88.8 | 50.5 |
| | NAA84 | NAb | 69.1 | 36.4 |
| | NAA92 | NAb | 60.8 | 33.2 |
| | NAA6 | NAb | 58.6 | 51.2 |
| | NAA113 | NAb | 57.7 | 30.2 |
| | NAA45 | NAb | 49.4 | 31.4 |
| | NAA44 | NAb | 48.0 | 43.8 |
| | NAA119 | NAb | 45.5 | 52.7 |
| | NAA80 | NAb | 43.7 | 46.0 |
| | NAA128 | NAb | 41.4 | 39.8 |
| | NAA125 | NAb | 17.1 | 48.4 |
| B | NAE125 | BAb | 100,000.0 | 24.5 |
| | NAE194 | BAb | 100,000.0 | 25.6 |
| | NAE197 | BAb | 100,000.0 | 823.3 |
| | NAE199 | BAb | 100,000.0 | 23.3 |
| | NAE205 | BAb | 100,000.0 | 736.3 |
| | NAE206 | BAb | 100,000.0 | 710.0 |
| | NAE207 | BAb | 100,000.0 | 972.6 |
| | NAE210 | BAb | 100,000.0 | 17.5 |
| | NAE208 | BAb | 100,000.0 | 6,433.0 |
| | NAE203 | BAb | 63,640.0 | 18.5 |

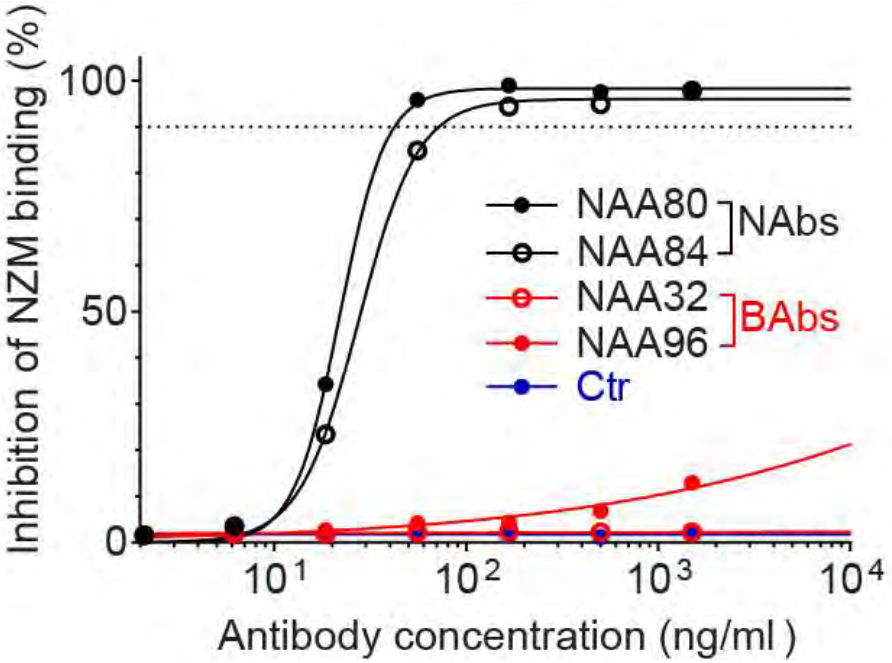
# Neutralizing antibodies carry a high load of replacement mutations in the CDRs

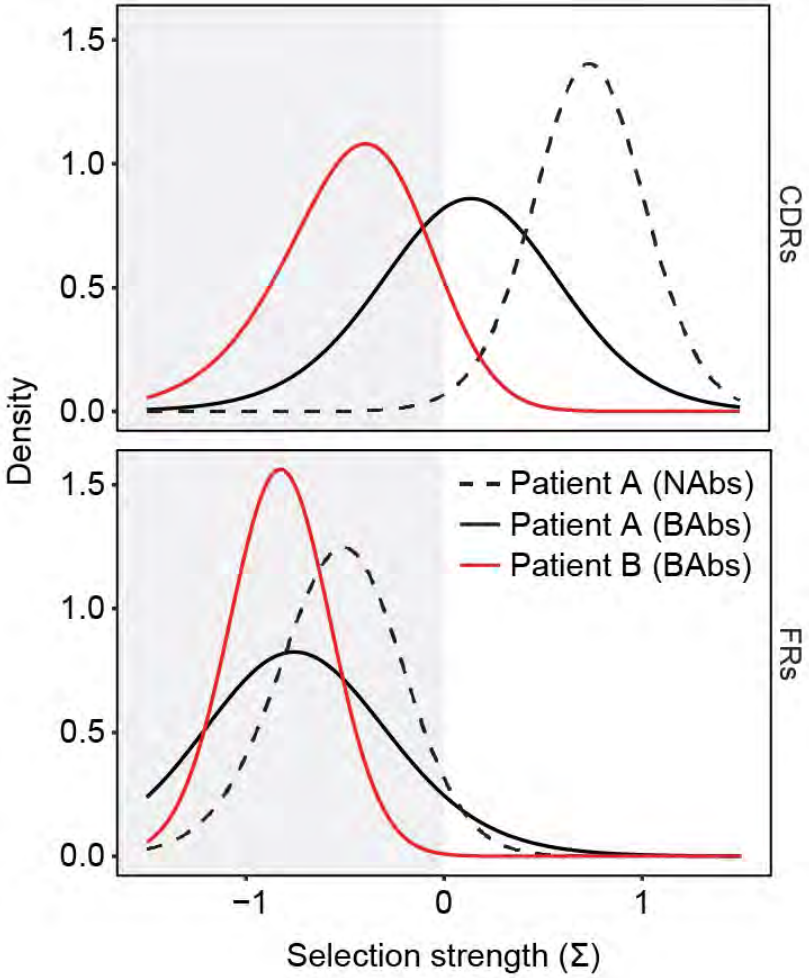



NAb = neutralizing antibodies

BAb = binding antibodies



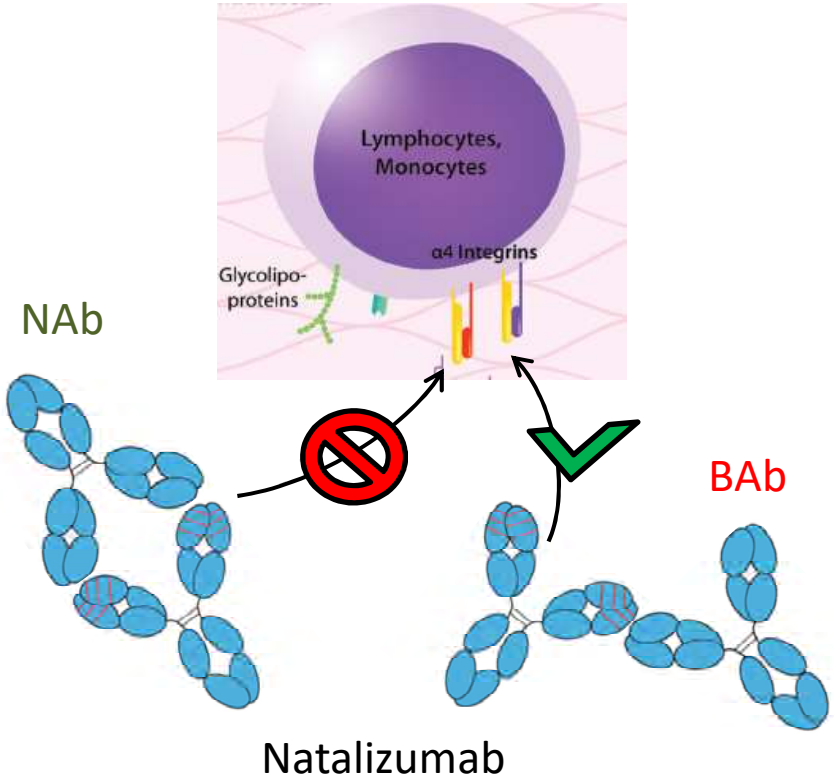
# Anti-natalizumab NAbs vs BAbs: what makes the difference?

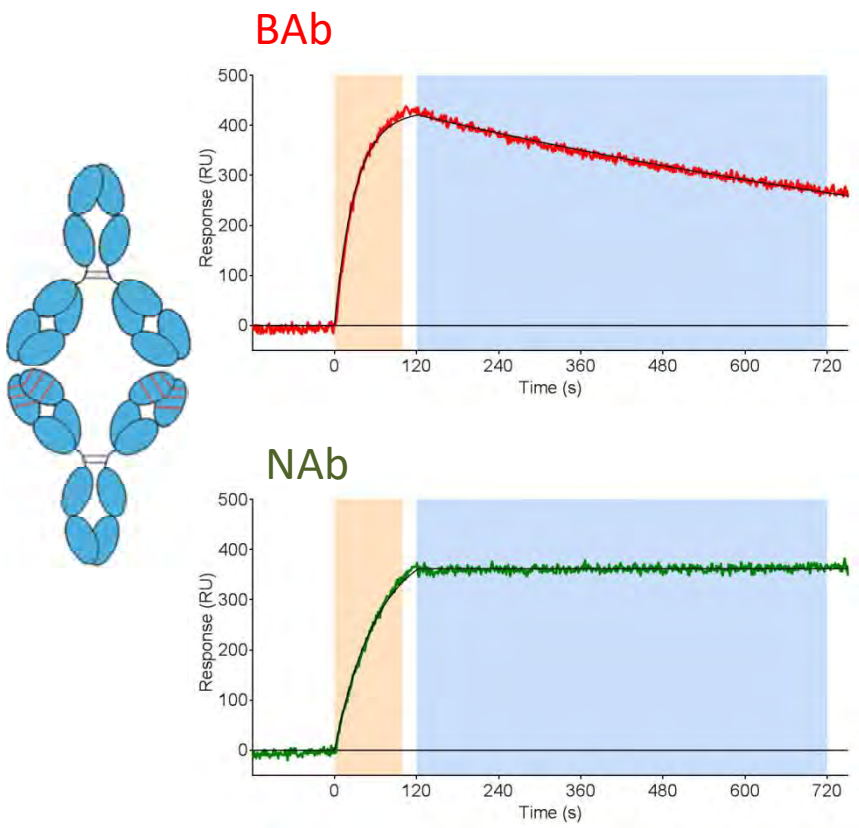
Hypothesis 1

**Steric hindrance**: NAbs bind to idiotopes important for binding to α4-integrin

Hypothesis 2

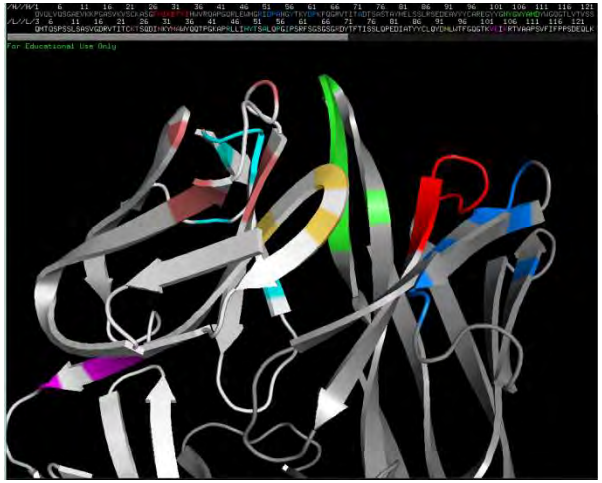
**Different kinetics**: BAbs have a faster dissociation from Natalizumab than Nabs

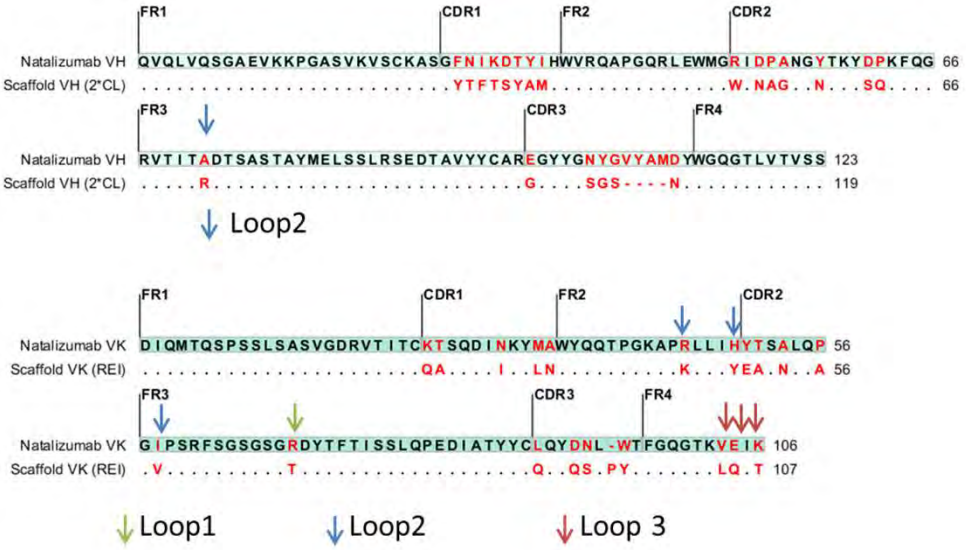


# Generation of 64 different natalizumab CDR variants

Natalizumab H & L variable domains






Natalizumab HCDR1→ scaffold HCDR1
Natalizumab HCDR2→ scaffold HCDR2
Natalizumab HCDR3→ scaffold HCDR3

Natalizumab LCDR1 → scaffold LCDR1
Natalizumab LCDR2 → scaffold LCDR1
Natalizumab LCDR3 → scaffold LCDR1

| VH | VH scaffold CDR1 | VH scaffold CDR2 | VH scaffold CDR3 | VH scaffold CDR1-2 | VH scaffold CDR 1-3 | VH scaffold CDR2-3 | VH scaffold CDR1-2-3 |
|---|---|---|---|---|---|---|---|

×

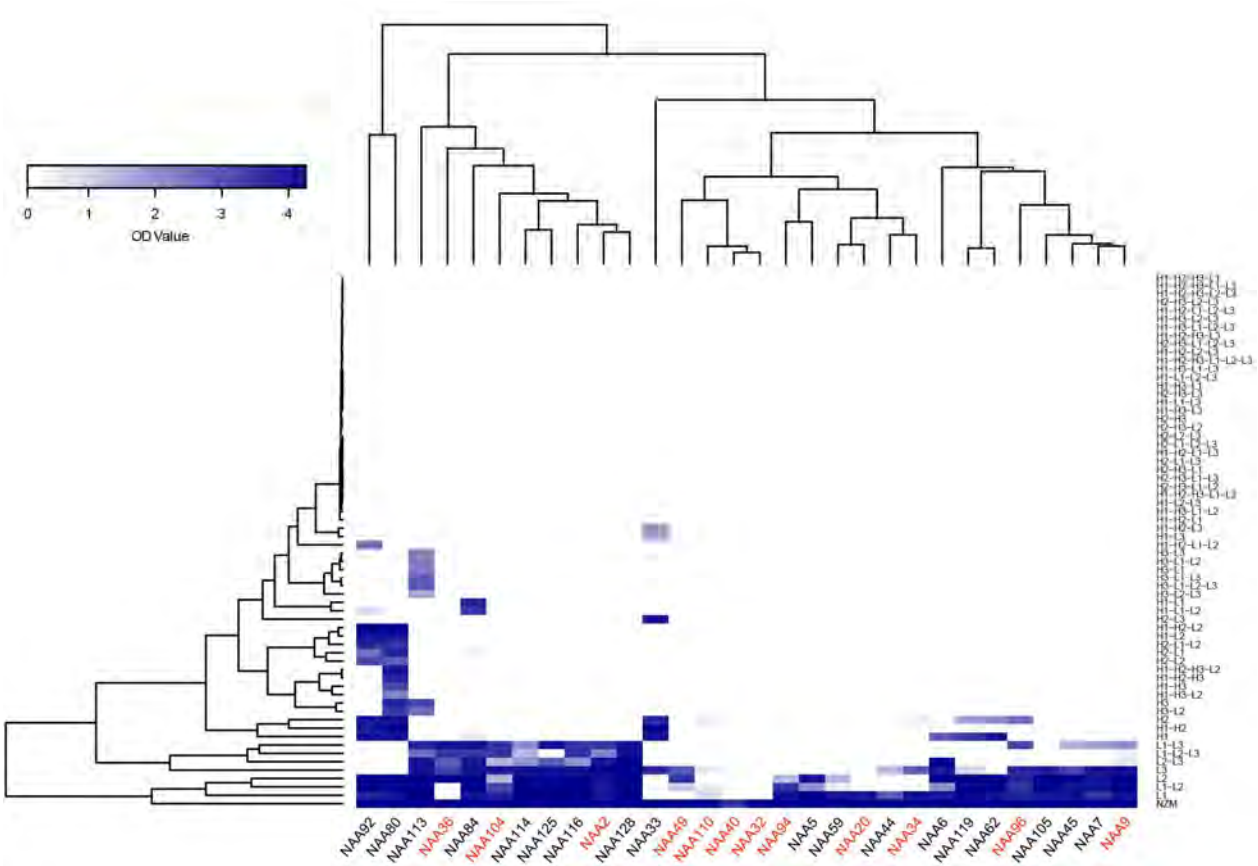
| VL | VL scaffold CDR1 | VL scaffold CDR2 | VL scaffold CDR3 | VL scaffold CDR1-2 | VL scaffold CDR 1-3 | VL scaffold CDR2-3 | VL scaffold CDR1-2-3 |
|---|---|---|---|---|---|---|---|

=

**64 Natalizumab variants**

# NAbs and BAbs target different epitopes of the NZM idiotype

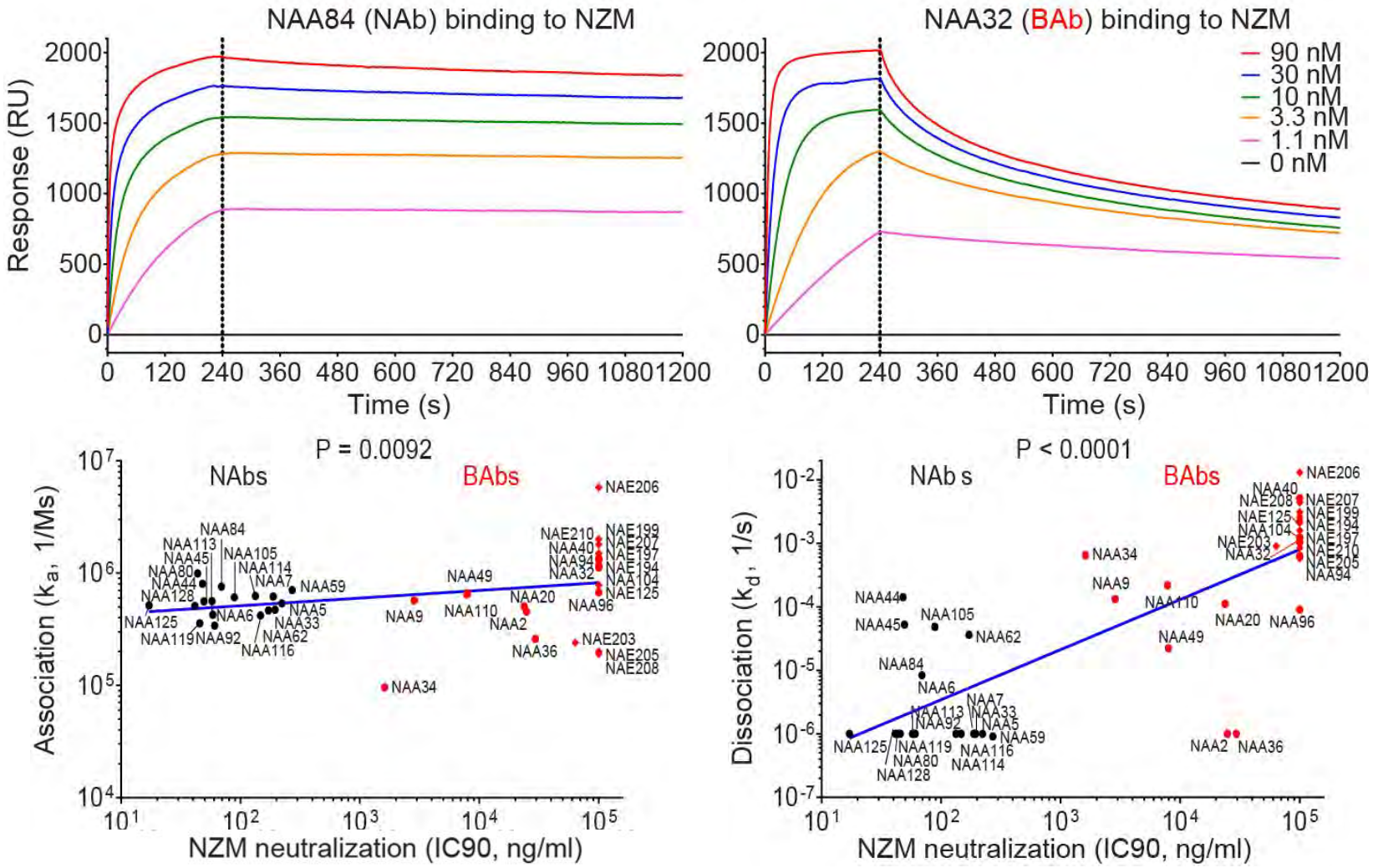
- Different patterns of binding to the 64 NZM variants.
- Most antibodies recognize idiotopes composed of all HCDRs and 0 to 3 LCDRs.
- NAb and BAb antibodies cluster independently of the binding pattern to NZM variants.
- The idiotopes overlap in the antigen binding site of NZM.




| MS patient | B cell clone | Binding to NZM CDR-variants H1 | H2 | H3 | L1 | L2 | L3 |
|---|---|---|---|---|---|---|---|
| A | NAA2 | | | | | | |
| | NAA5 | | | | | | |
| | NAA6 | | | | | | |
| | NAA7 | | | | | | |
| | NAA9 | | | | | | |
| | NAA20 | | | | | | |
| | NAA32 | | | | | | |
| | NAA33 | | | | | | |
| | NAA34 | | | | | | |
| | NAA36 | | | | | | |
| | NAA40 | | | | | | |
| | NAA44 | | | | | | |
| | NAA45 | | | | | | |
| | NAA49 | | | | | | |
| | NAA59 | | | | | | |
| | NAA62 | | | | | | |
| | NAA80 | | | | | | |
| | NAA84 | | | | | | |
| | NAA92 | | | | | | |
| | NAA94 | | | | | | |
| | NAA96 | | | | | | |
| | NAA104 | | | | | | |
| | NAA105 | | | | | | |
| | NAA110 | | | | | | |
| | NAA113 | | | | | | |
| | NAA114 | | | | | | |
| | NAA116 | | | | | | |
| | NAA119 | | | | | | |
| | NAA125 | | | | | | |
| | NAA128 | | | | | | |
| B | NAE125 | | | | | | |
| | NAE194 | | | | | | |
| | NAE197 | | | | | | |
| | NAE199 | | | | | | |
| | NAE203 | | | | | | |
| | NAE205 | | | | | | |
| | NAE206 | | | | | | |
| | NAE207 | | | | | | |
| | NAE208 | | | | | | |
| | NAE210 | | | | | | |

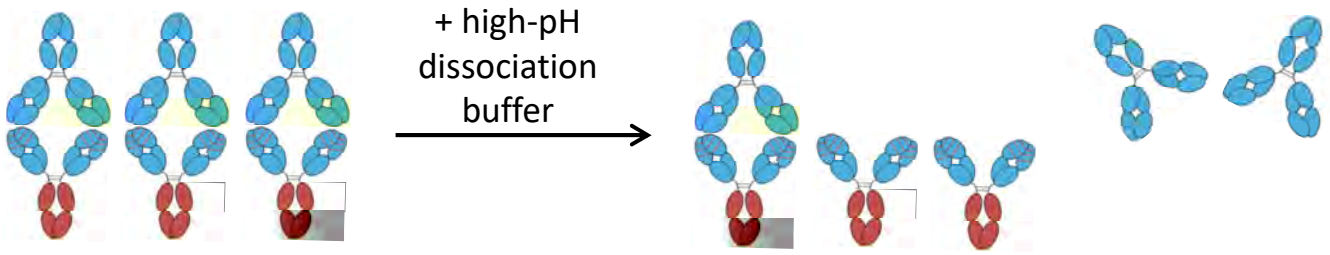
# The neutralizing activity correlates with a slow dissociation rate

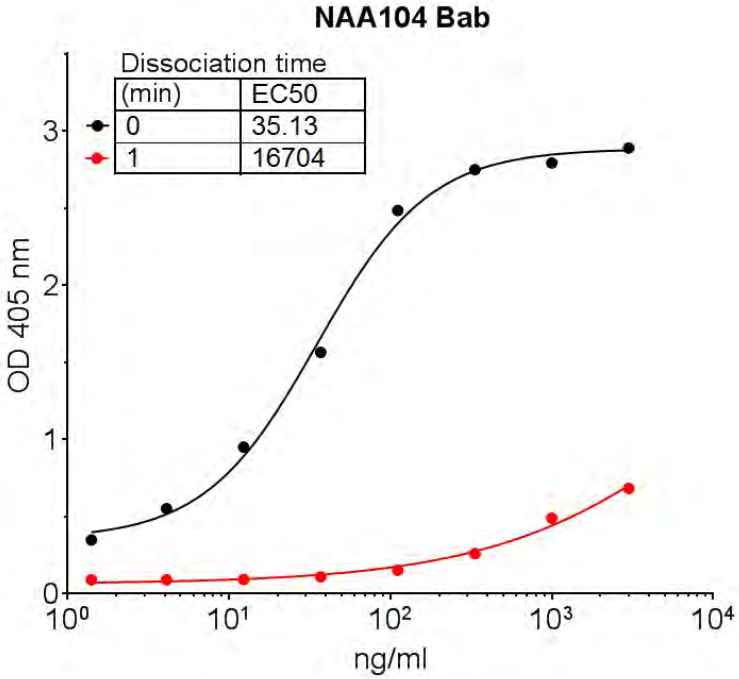
Surface plasmon resonance

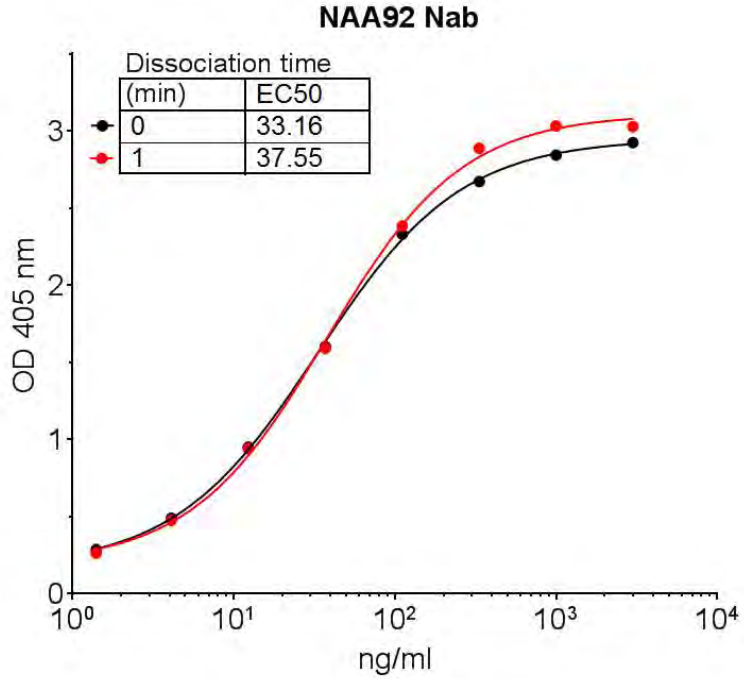


# BAbs easily dissociate from NZM

ELISA dissociation assay



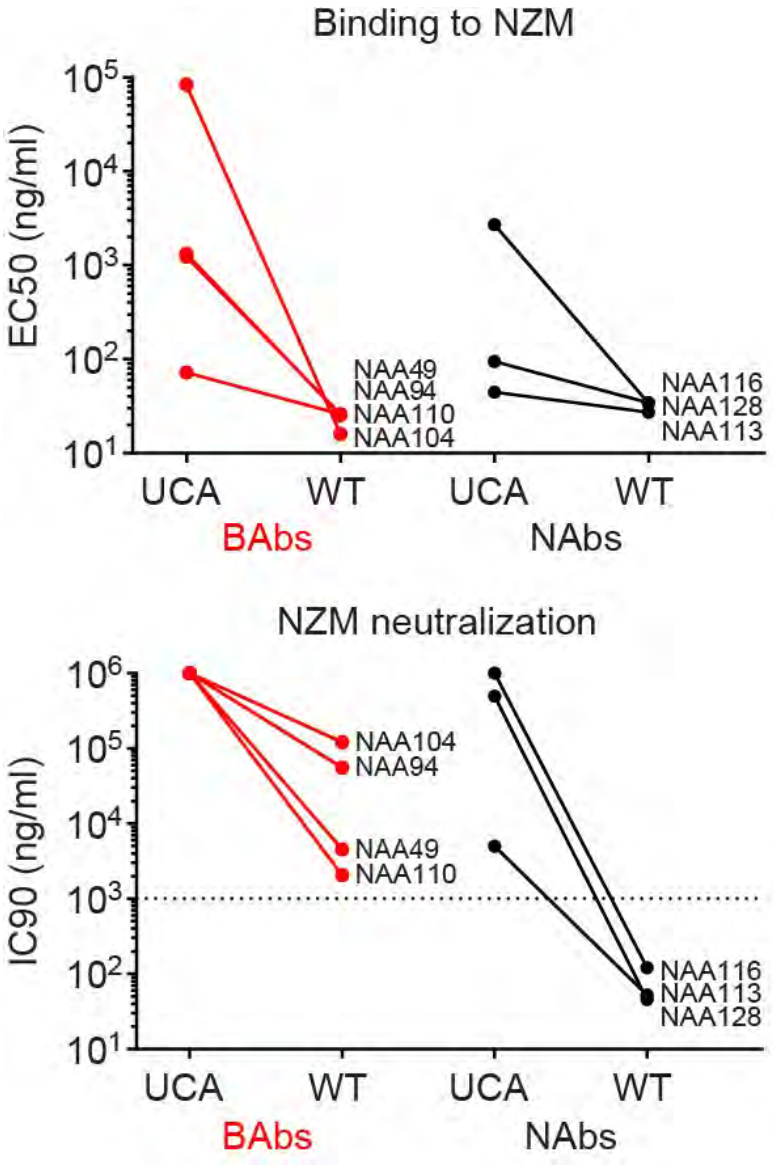



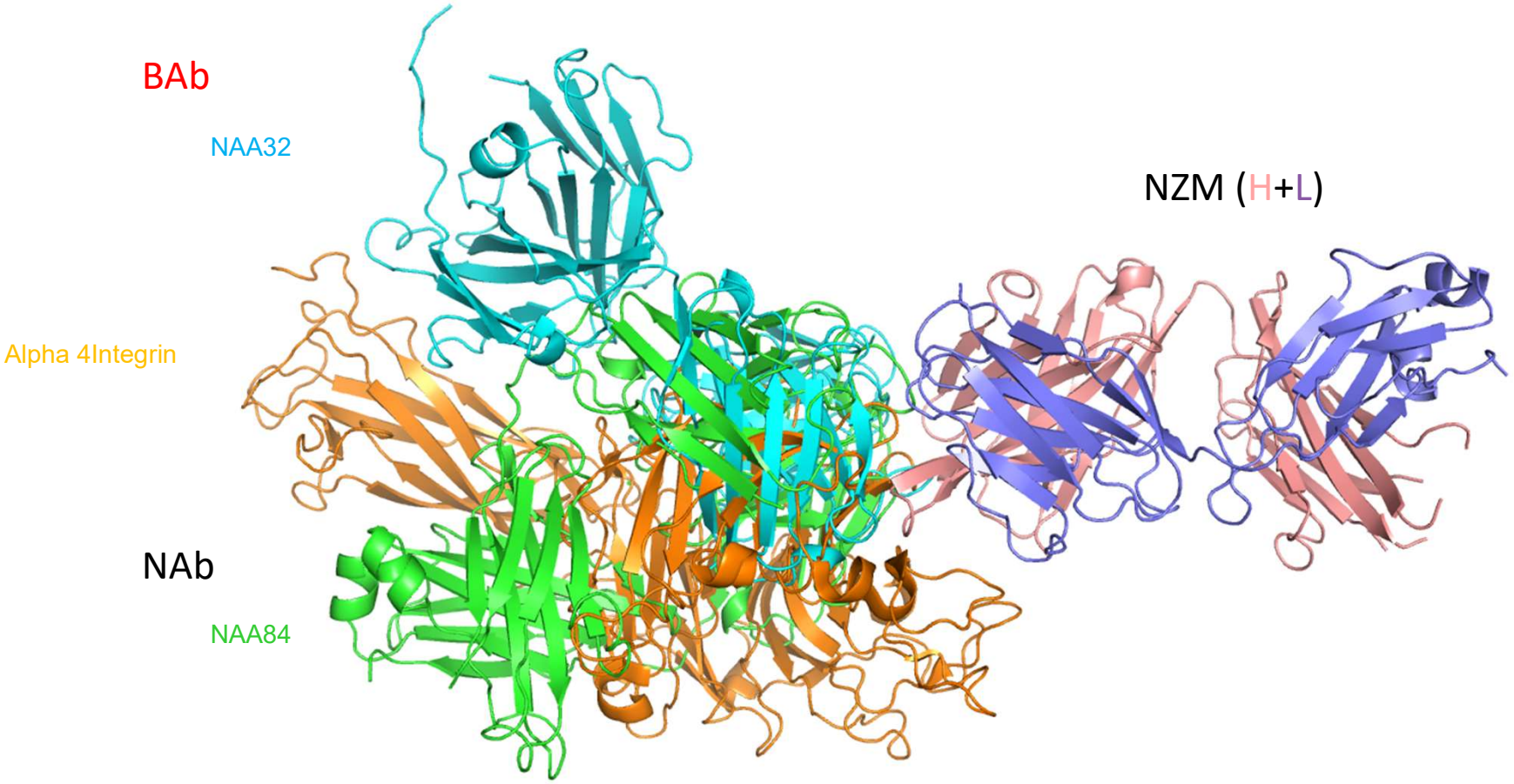
# The neutralizing activity is acquired through somatic mutations

Removal of somatic mutations to generate the **unmutated common ancestor (UCA)**

- Somatic mutations increase affinity of BAbs antibodies to natalizumab.
- Germline versions of **NAbs** antibodies bind to natalizumab with high affinity.
- **NAbs** antibodies gain their ability to highly neutralize natalizumab by acquiring somatic mutations.



# Two antibodies recognize the CDRs region of NZM, but engage NZM in a different orientation from one another

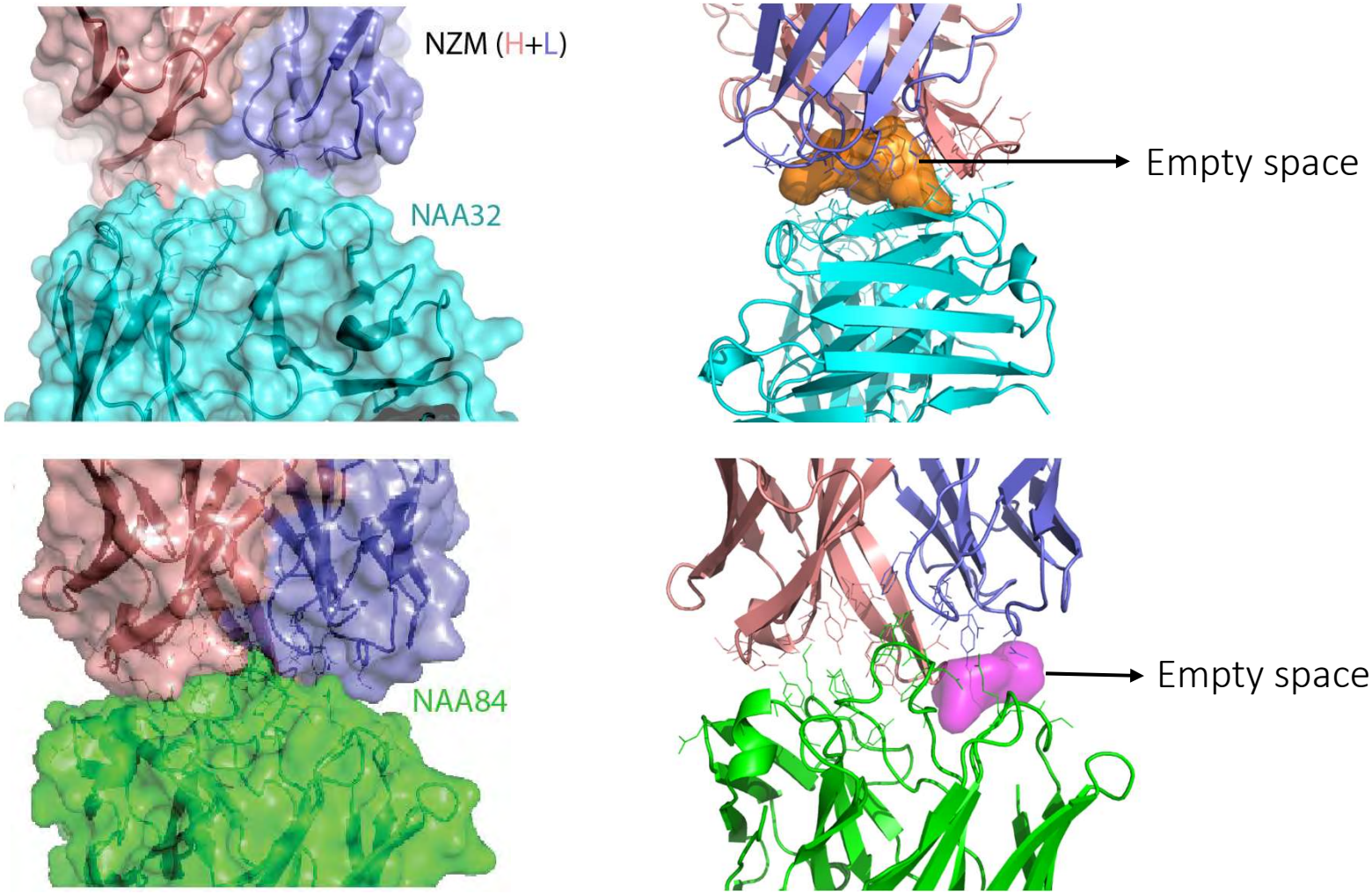



Both antibodies occlude α4 integrin binding site, but neither of them provide a molecular mimicry of α4 integrin.

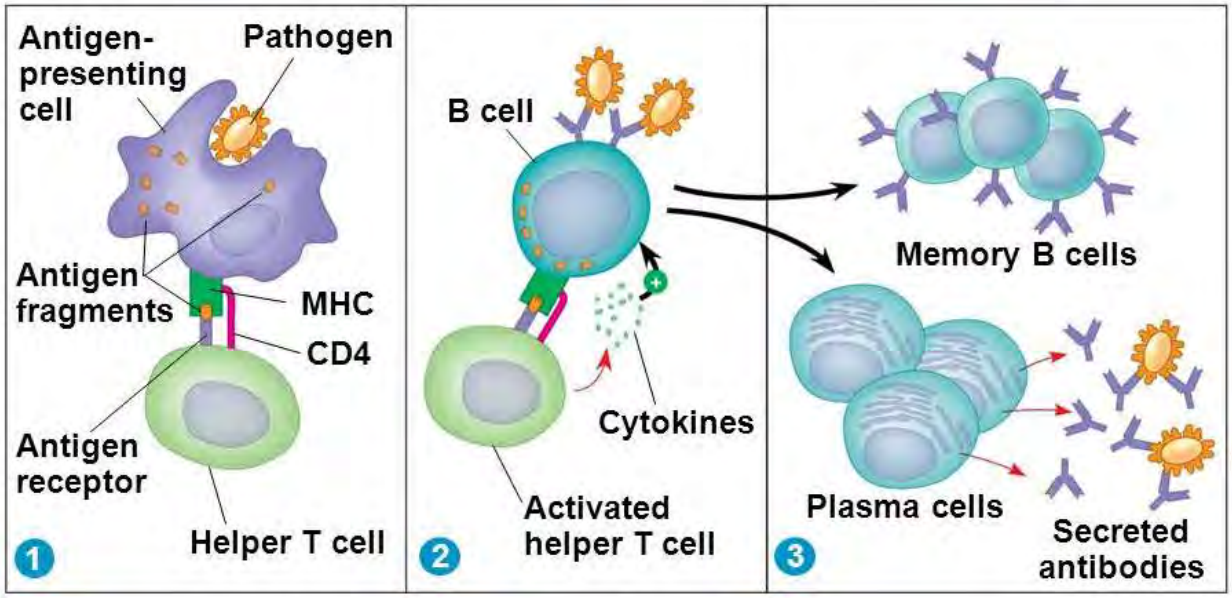
# NZM-NAb show tight residue contacts and better surface complementarity

BAb

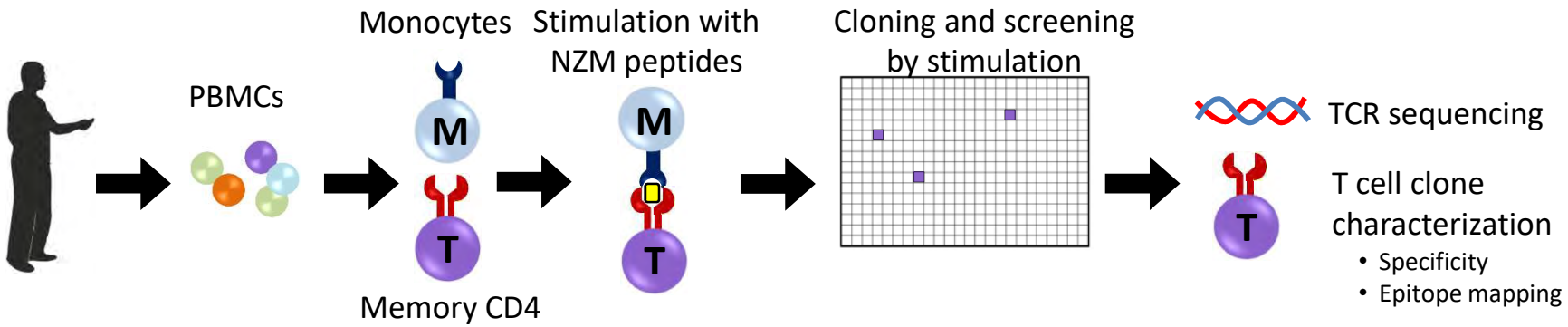
NAb

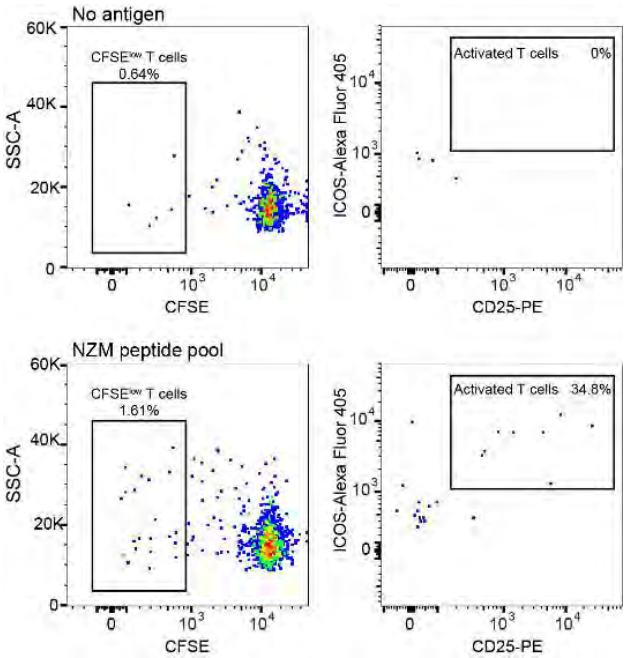


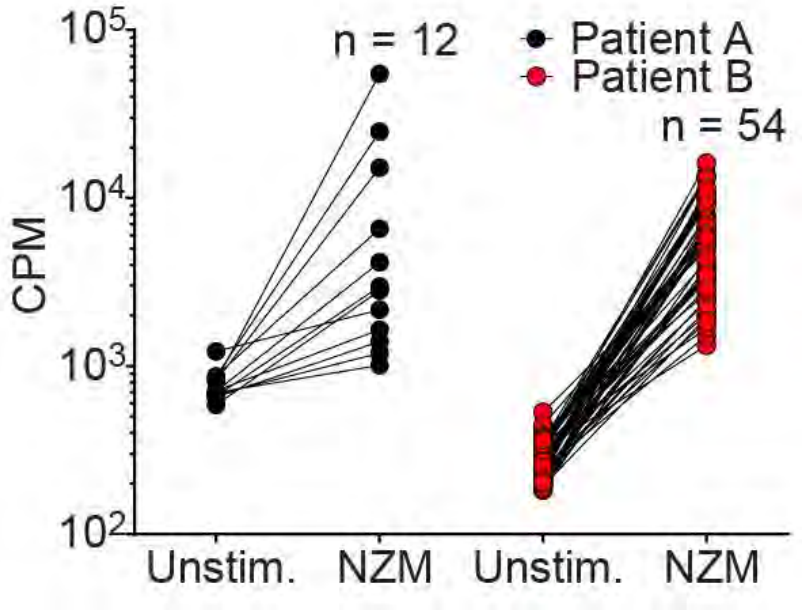
# What's the role of T cells to sustain the generation of neutralizing anti-drug antibodies?



# Isolation of memory CD4⁺ helper T cell clones reactive to NZM

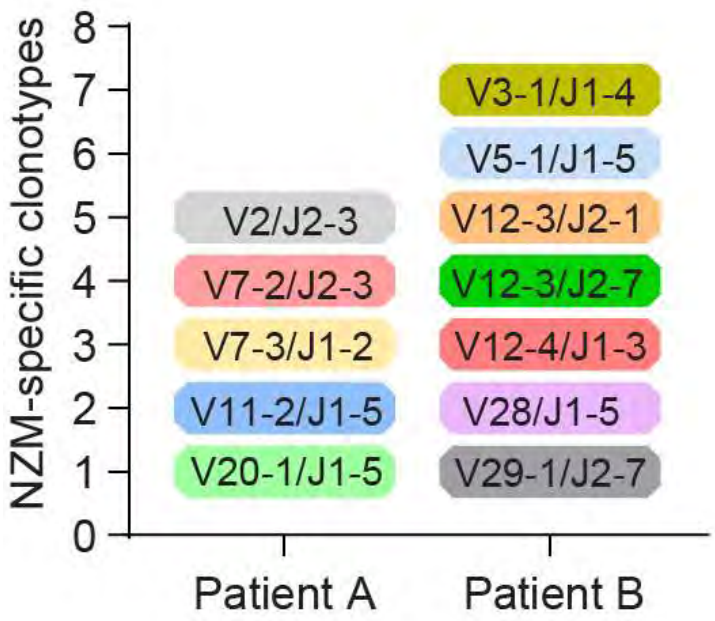


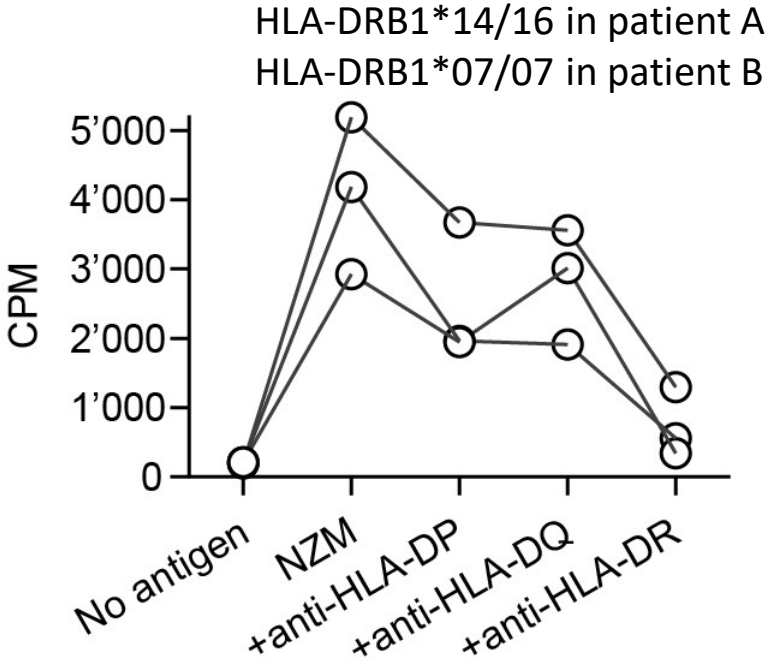



# T cell clonality and MHC-II restriction

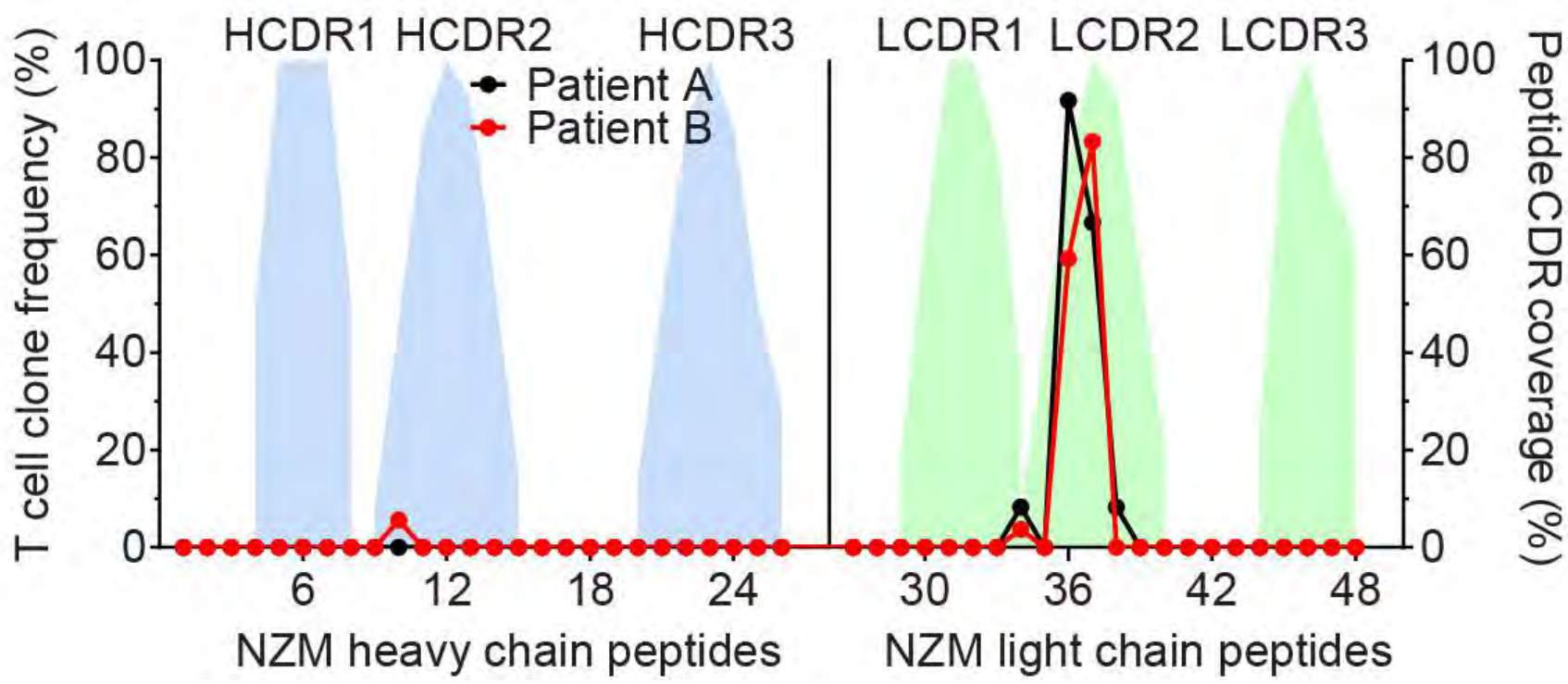
TCRβ sequencing



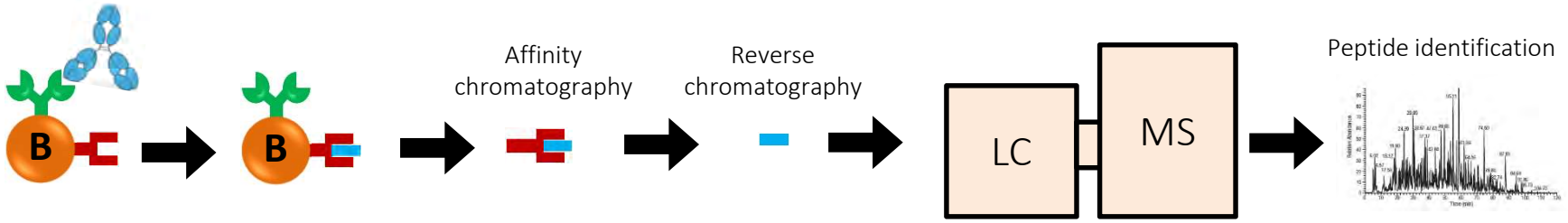
# The T cell clones recognize a single T cell epitope spanning the FR2-CDR2 region of NZM light chain

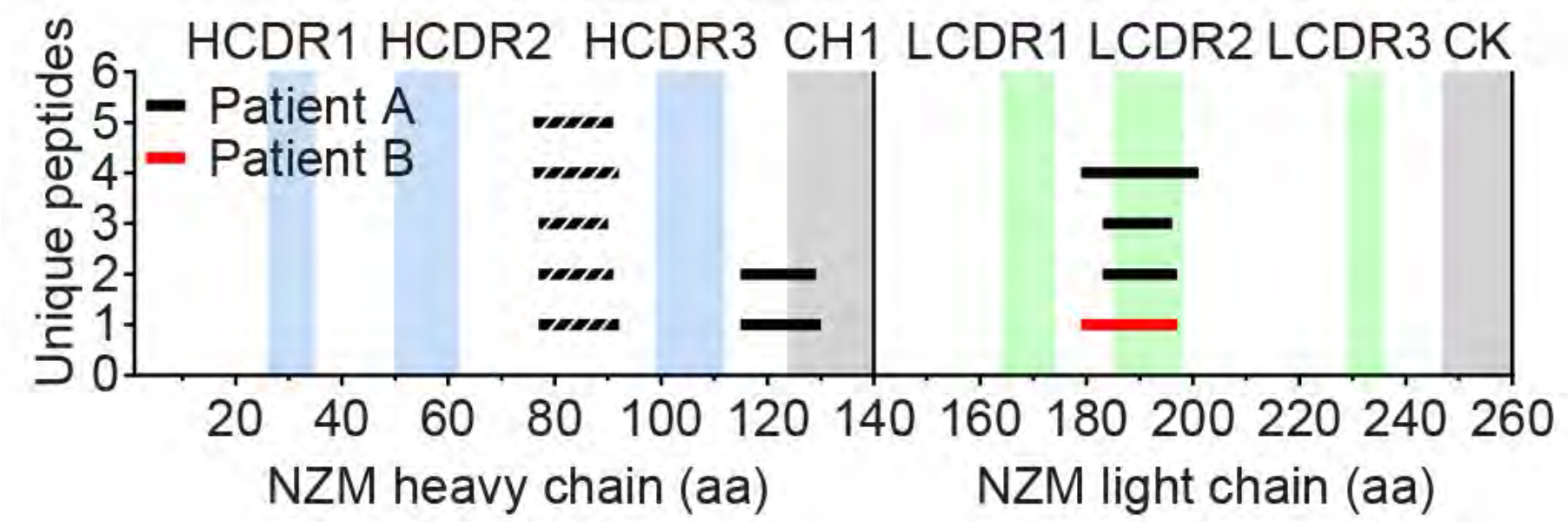
Epitope mapping by stimulating NZM-reactive T cell clones with single overlapping peptides covering the NZM VH and VL regions.



# Peptides spanning the immunodominant NZM-LC$_{FR2\text{-}CDR2}$ epitope are naturally presented by APCs

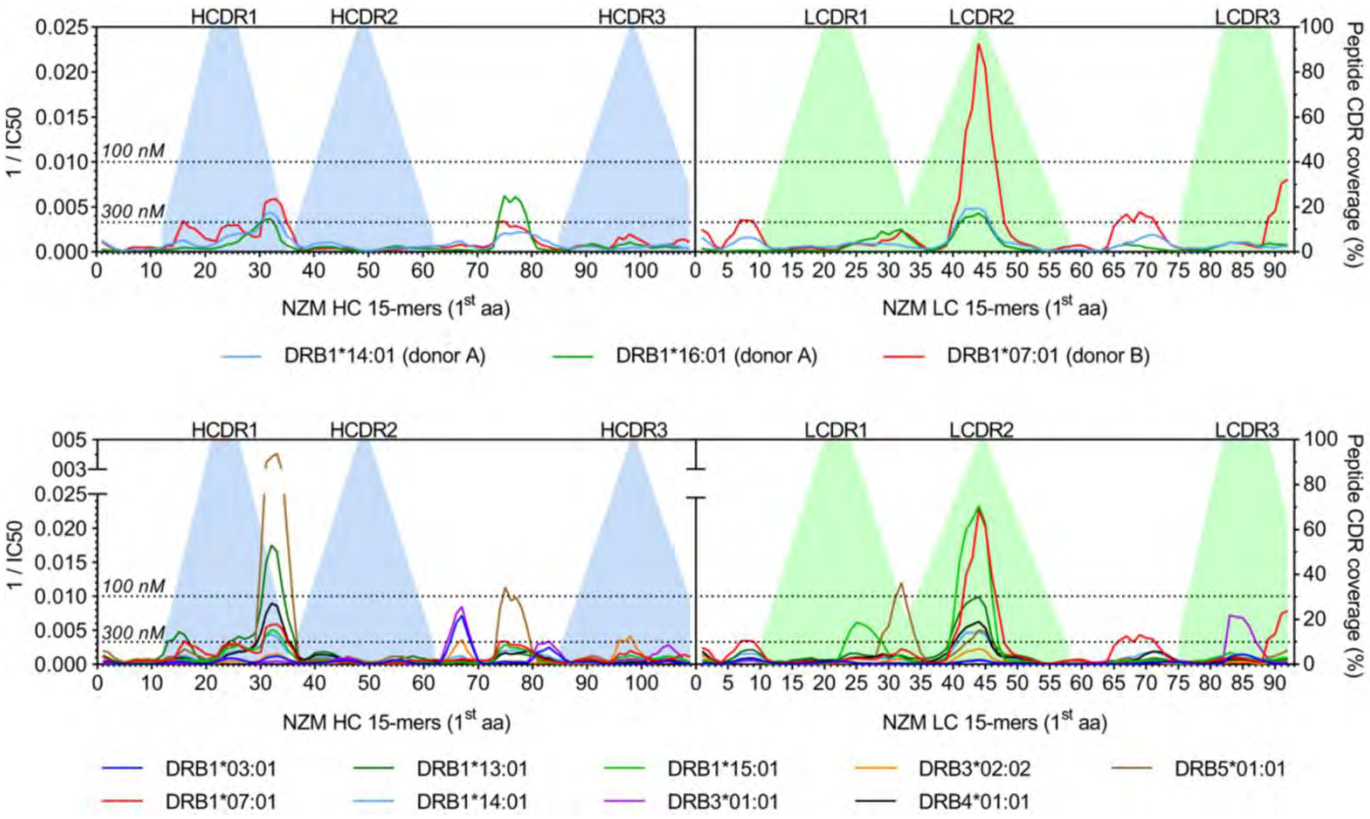
Mass spectrometry-based peptidomics of MHC-II-bound peptides processed by NZM-specific B cells.



# *In silico* prediction of NZM peptide binding to different HLA-DR alleles





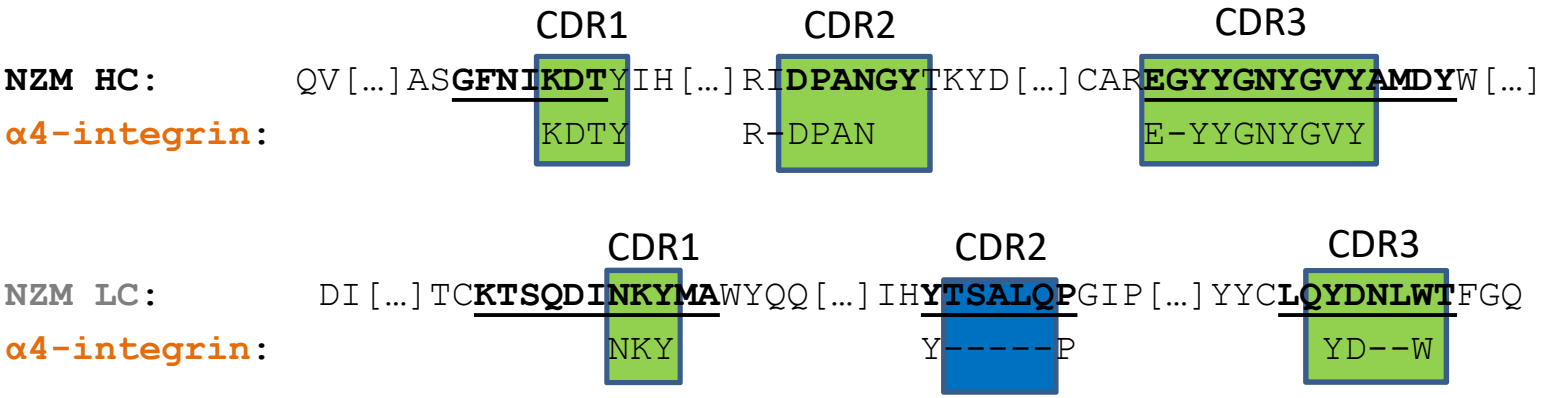

Reference panel of nine DRB1 and DRB3/4/5 alleles

Only one of the potentially immunogenic peptides encoded by the six CDRs of NZM was a naturally presented T cell epitope able to generate a polyclonal CD4+ T-cell response

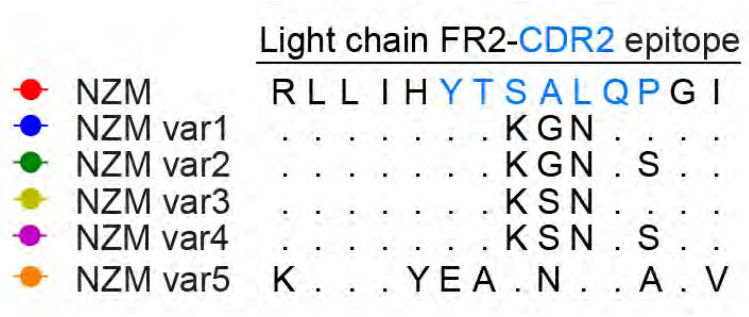
# How can we reduce the immunogenicity of therapeutic antibodies?

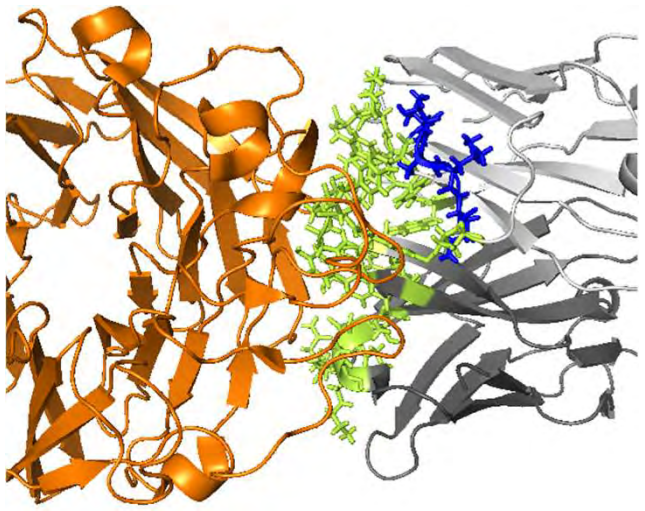
# Structure-guided design to engineer a “deimmunized” version of NZM

- Identification of residues of NZM light chain CDR2 that were not engaging α4 integrin binding
- Modelling of different mutants with the constraint to preserve the conformation of the CDR2 and the specificity of NZM

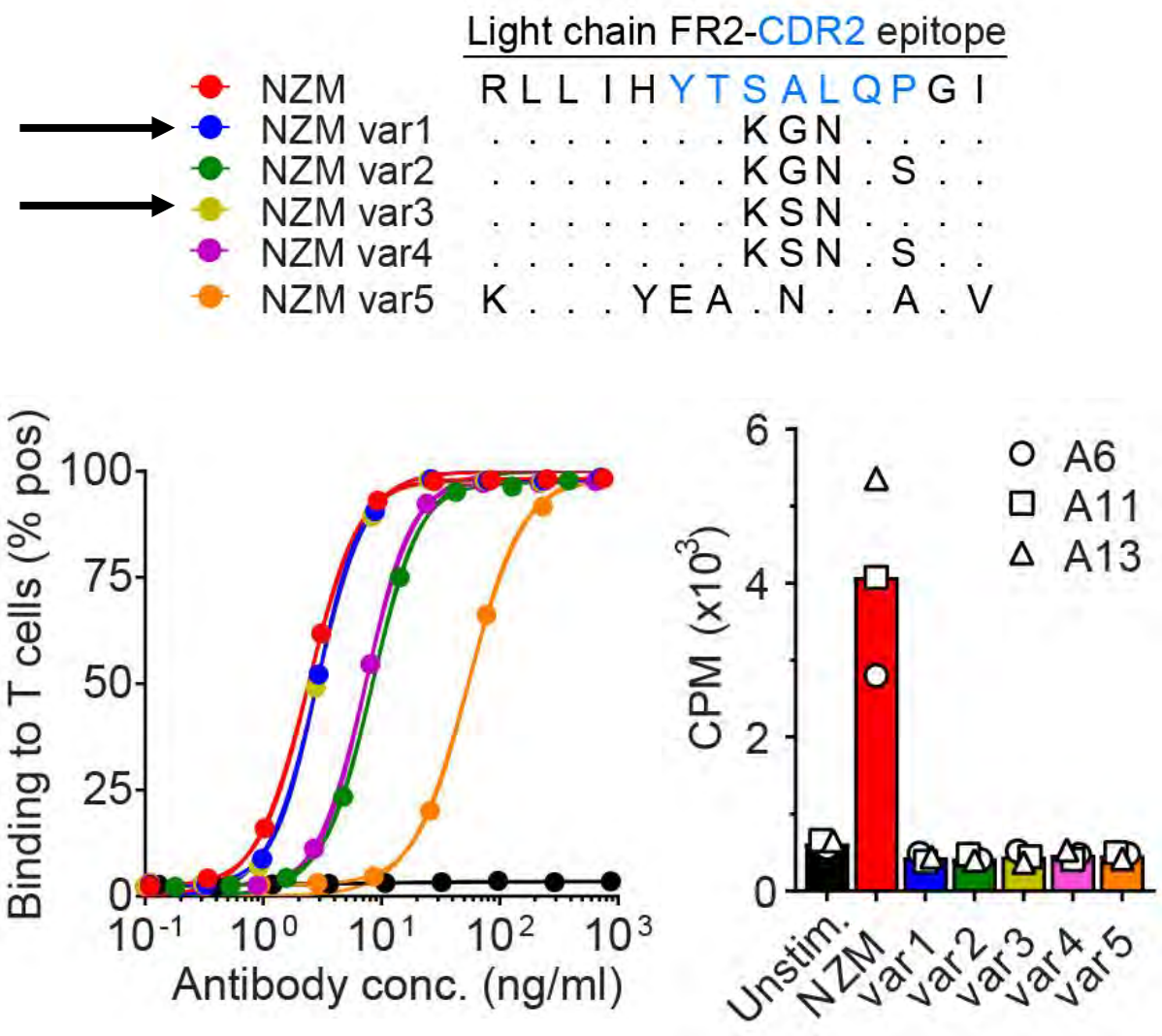



Cloning and expression of 5 NZM variants

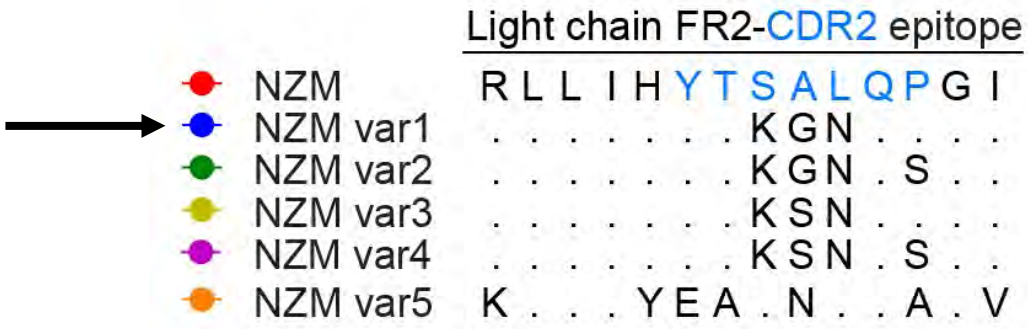


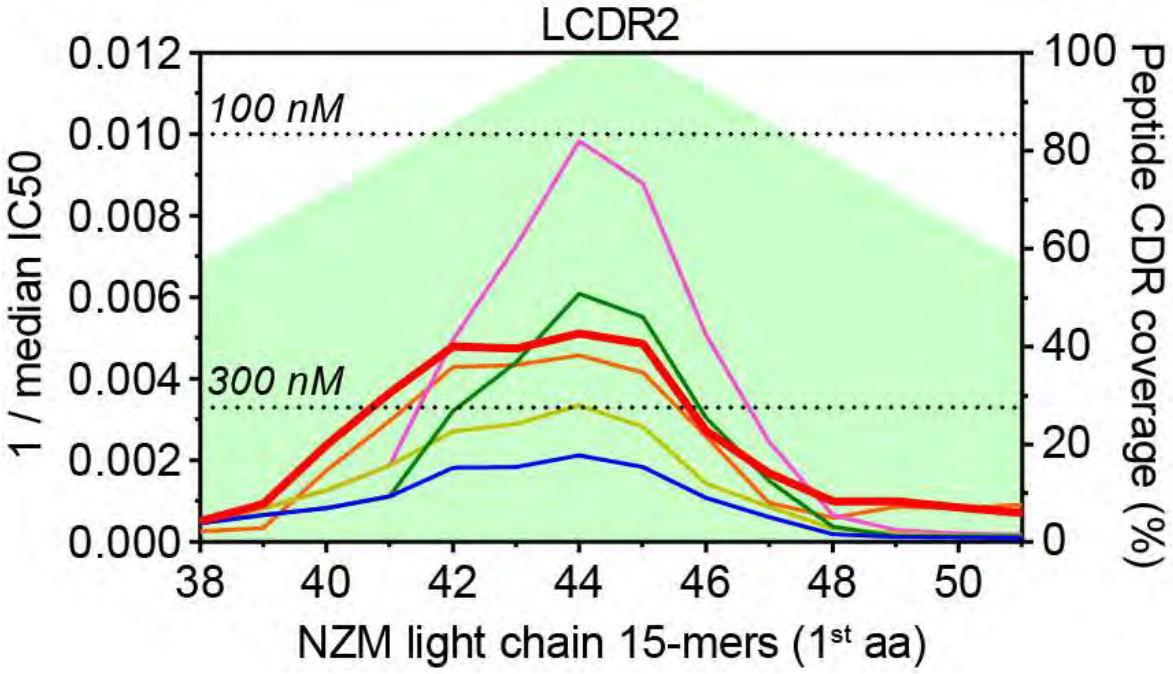



# Two $LC_{CDR2}$-modified versions of NZM were not recognized by T cells, while retaining binding to α4 integrins



# NZM variant 1 is predicted to be less immunogenic




Peptides from NZM variant 1 are predicted to have a drastically reduced binding affinity to MHC-II molecules

# Conclusions (II)

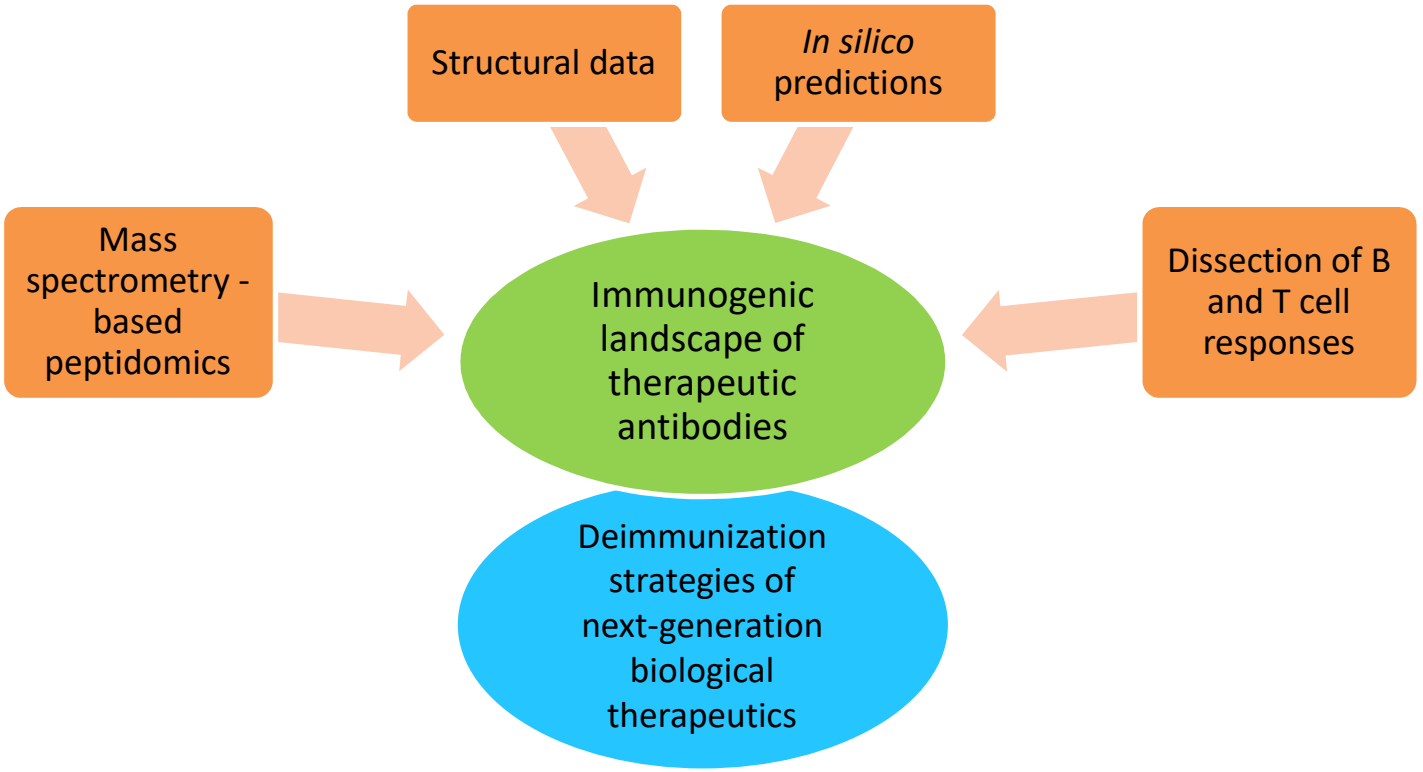
- The **integration** of peptidomics, structural data, *in silico* predictions and dissection of the specific B and T cell responses represents a powerful approach to define the immunogenic landscape of therapeutic antibodies.

- Neutralizing antibodies show high-level replacement mutations in the CDRs and low dissociation rate, suggesting **that B cell selection was driven by decreased off-rate** rather than increased on-rate.

- The **highly diverse anti-idiotypic response** is consistent with the presence of multiple B cell epitopes recognized by naïve B cells and contrasts with the **T cell response that is largely limited to a single epitope** that we mapped to the FR2-CDR2 region of NZM light chain.

- The finding that the **NZM-LC$_{FR2\text{-}CDR2}$ peptide is naturally presented** in the context of different HLA-DR alleles suggests that this peptide is a **major source of T cell help** driving the anti-idiotypic B cell response to NZM and offers the **possibility for the deimmunization of the drug** for a more safe treatment.

# Conclusions (II)




Our analysis delineates an approach to **guide the deimmunization strategies** of next-generation biological therapeutics for autoimmune, cancer and infectious diseases.

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Department of Neurology, Innsbruck Medical University, Innsbruck, Austria

Florian Deisenhammer
Michael Auer

Laboratory of Neuroimmunology, IRCCS Mondino Foundation, Pavia, Italy

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