



Biomarkers used in clinical practice for monitoring biological drugs

Practical cases

COST CLINIMARK TRAINING SCHOOL
Approaches for Biomarker Discovery and Validation
September 23rd to 27th 2019
Spetses, Greece

Begoña Oliver Martos, PhD

Neuroimmunology and neuroinflammation Group
Regional University Hospital of Malaga
Biomedical Research Institute of Málaga (IBIMA)



Outline

- **Biological drugs**
- **Immunogenicity. Anti-drug antibodies (ADAs)**
- **Clinical consequences of immunogenicity**
- **Anti-drug antibodies (ADAs) as biomarker for monitoring treatment response**
 - **Antibodies against NATALIZUMAB**
 - **Antibodies against IFN β**

Biological drugs. Definition

Biological drugs/ biologics/ biopharmaceuticals/ therapeutic proteins

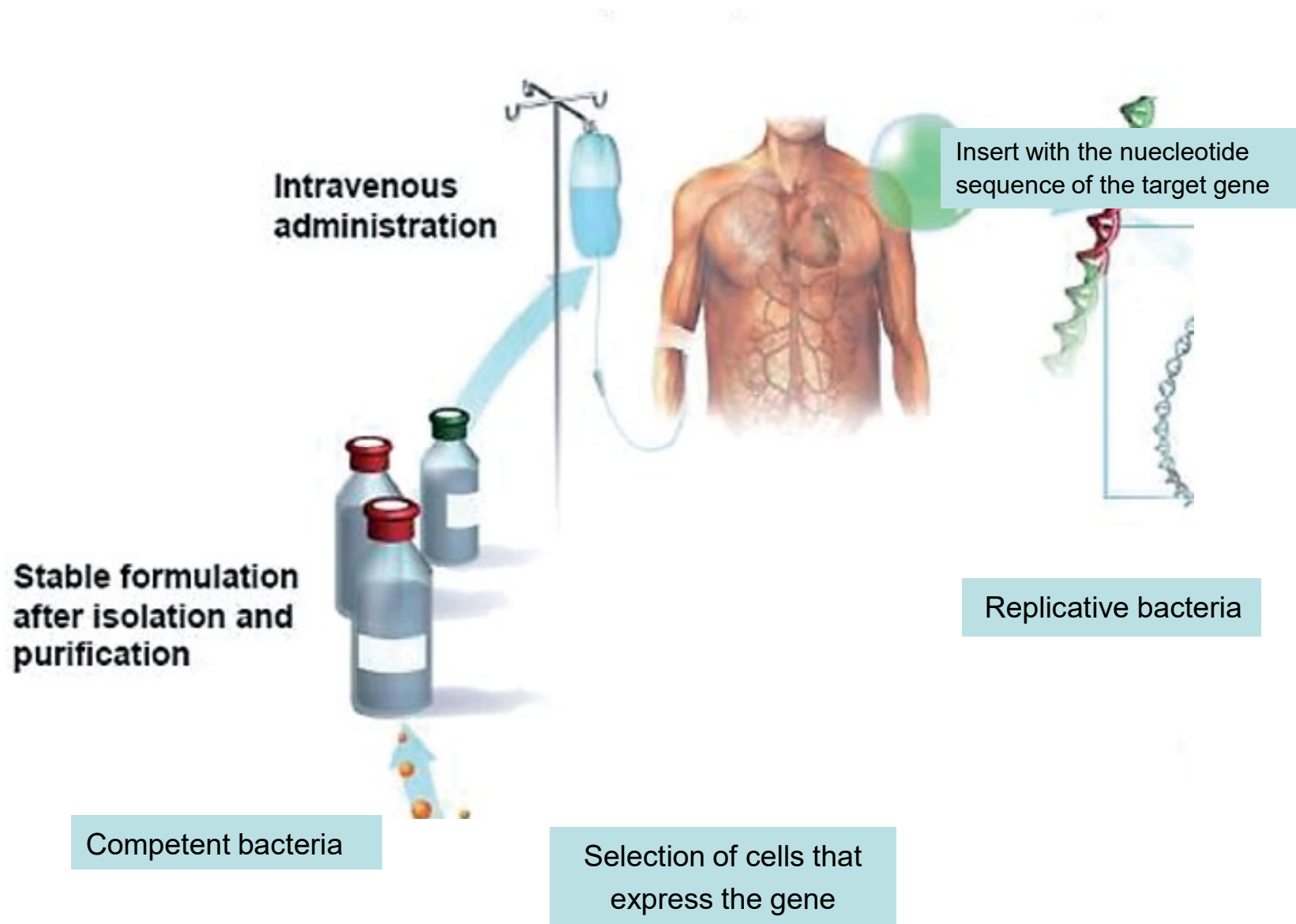
➤ Drugs synthesized using **biotechnology** and **genetic engineering**

➤ **Therapeutic revolution** for many diseases. **Greater efficacy**

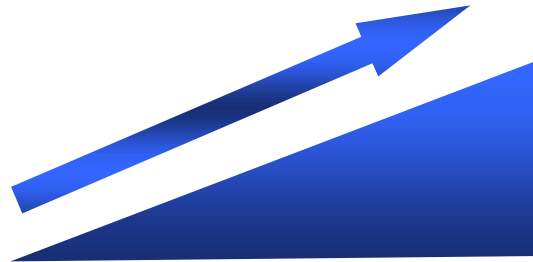
- **Monoclonal antibodies** for in-vivo use
- **Cytokines, growth factors, enzymes, immunomodulators; and thrombolytics**
- **Proteins intended for therapeutic use**

➤ Produced with the help of **genetically modified organisms** for this purpose (bacterial or mammalian cells)

Biological drugs. Production



Biopharmaceuticals represent an increasing percentage of new drug approvals



1982

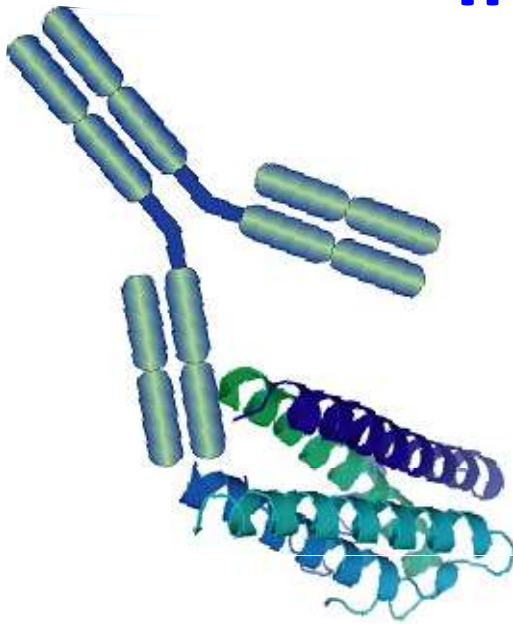
First FDA approval for a recombinant DNA product:
Insuline (Humulin1; 1982)

2019

A total of 322 therapeutic proteins approved by FDA

- Neurological diseases (multiple sclerosis)
- Chronic diseases (rheumatoid arthritis, inflammatory bowel disease)
- Cancer

Immunogenicity



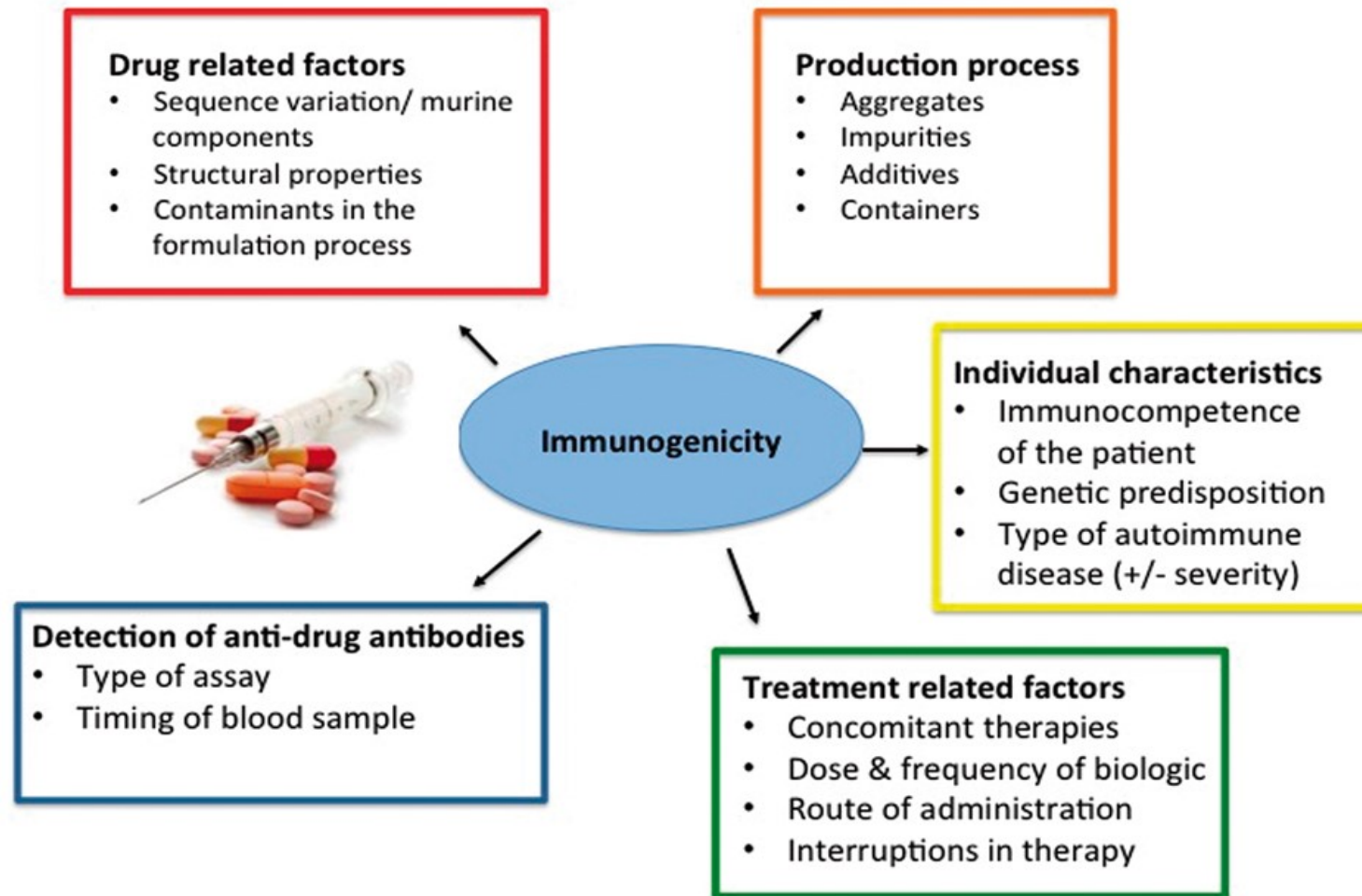
One major limitation:

Anti-drug antibodies (ADAs)

Can be **recognised by the immune system as foreign molecules** and generates an immune response against them in the form of ADAs

- adverse effects
- decrease the effectiveness of the drug

Factors that may influence the development of an immune response against a therapeutic protein



Clinical consequences of immunogenicity



Biologic drug



Anti-drug antibodies production



No effect



**Consequences on
Efficacy**



**Consequences
on Safety**

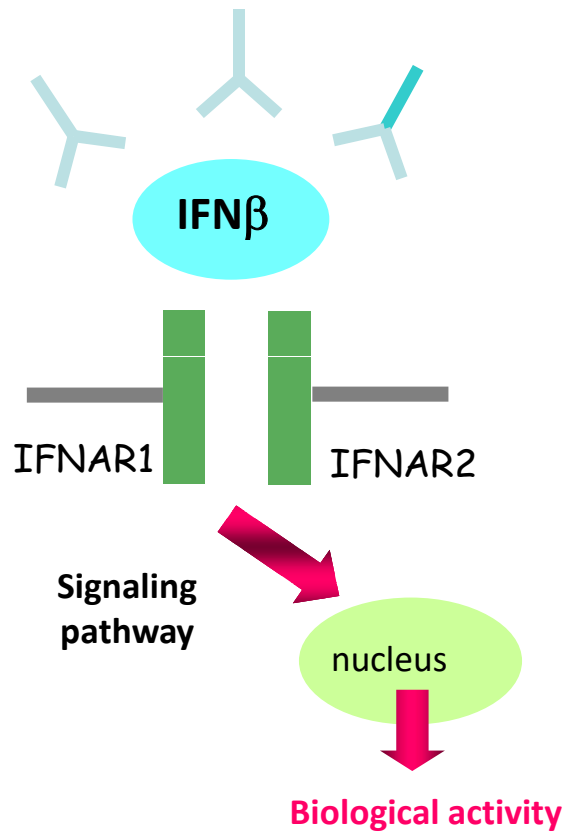
Clinical consequences of immunogenicity

Consequences on Efficacy

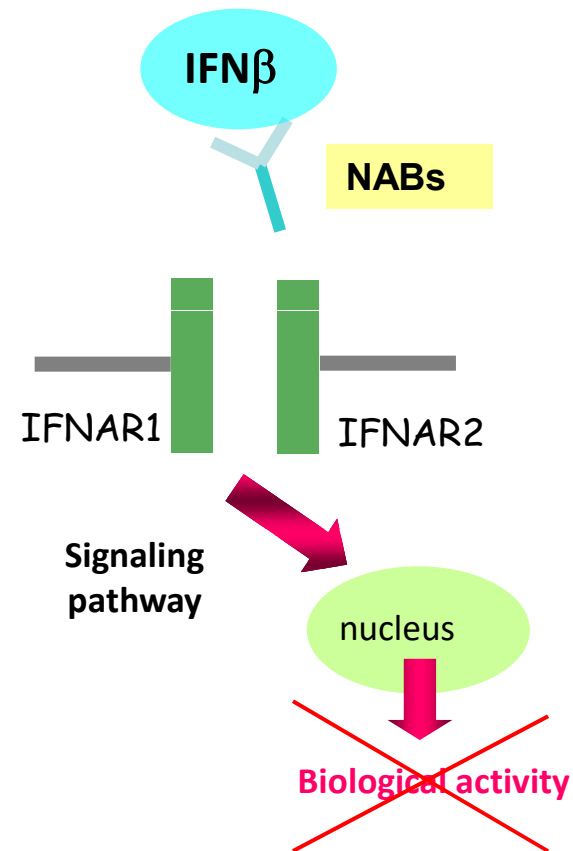
- ADAs may change the exposition of the therapeutic protein by either increasing or decreasing its clearance. **Binding antibodies**
- ADAs can affect the interaction between the therapeutic protein and its receptor. **Neutralising antibodies**

Antibodies against IFN beta

Binding antibodies



Neutralising antibodies



Clinical consequences of immunogenicity

Consequences on Safety

Infusion reactions

Hyperacute / acute reactions

anaphylactic/anaphylactoid reactions

Delayed reactions

(T cell-mediated) hypersensitivity and immune complex-mediated reactions

Autoimmunity

Cross-reactivity with the native protein

Therapeutic drug monitoring (TDM)

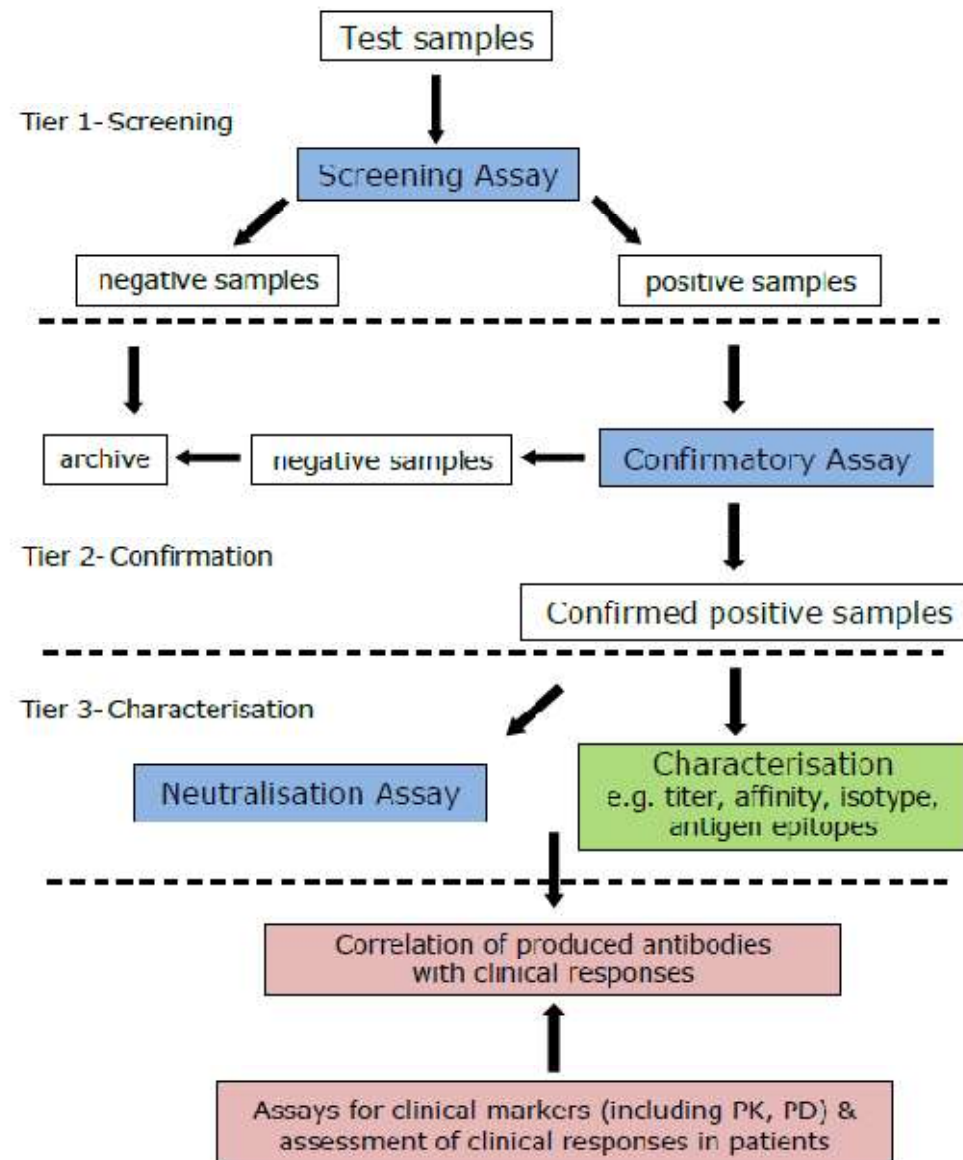
TDM: clinical decision-making tool that enables to adjust the dose of the drug, based on clinical and laboratory measurements, to reach the highest possible response rate

✓ Drug levels

✓ ADAs

Useful biomarkers for
monitoring biological
drugs in clinical practice

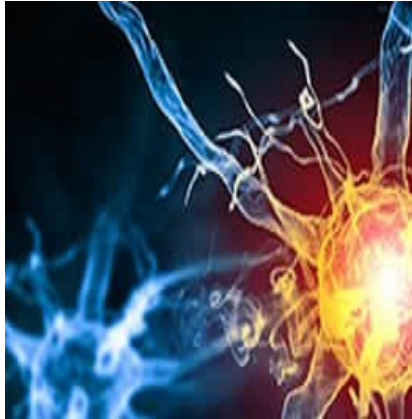
Strategy for immunogenicity assessment



Biological drugs with relevant assessment tests

- Adalimumab (Humira)
- Infliximab (Remicade)
- Etanercept (Enbrel)
- Rituximab (Rituxan)
- Interferon- β (Avonex, Rebif, Betaferon)
- Natalizumab (Tysabri)
- Ustekinumab (Stelara)
- Vedolizumab (Entyvio)
- Golimumab (Simponi)
- Certolizumab pegol (Cimzia)
- Basiliximab (Simulect)
- Eculizumab (Soliris)
- Tocilizumab (Actemra)
- Cetuximab (Erbix)
- Panitumumab (Vectibix)
- Trastuzumab (Herceptin)
- Bevacizumab (Avastin)
- Nivolumab (Opdivo)
- Secukinumab (Cosentyx)

Multiple sclerosis (MS)



✓ Chronic, inflammatory, demyelinating disease of the central nervous system



✓ Major cause of disability in young adults

Molecular Biomarkers in multiple sclerosis

Strength of evidence	
Exploratory biomarkers	
Cytokines (D, DA, IFN β -R, GA-R) Adhesion molecules (D, DA, IFN β -R, NZ-R) Chemokines and receptors (D, DA, IFN β -R) MMPs and inhibitors (D, DA, IFN β -R) Proteomics ¹³⁻¹⁵ (D, DA, IFN β -R) Cystatin C ¹⁶ (D) microRNA ¹⁷ (D, DA, GA-R) C31/C4b (D, DA) sCD146 (DA) sCD14 (D, DA) sHLA I and sHLA II (D, DA, IFN β -R) sHLA-G ¹⁸ (D) sNogo-A ¹⁹ (D, DA) Anti-Nogo-A (D, DA) Anti-MBP ²⁰ (D, DA) Anti-MOG ²¹ (D, DA) Anti-HHV6 (DA) Anti-proteasome (D) Anti-CD46 and anti-CD59 (DA) Lipocalin 2 (DA) VEGFA (DA) AMCase ²² and Chit (D, DA) Fetuin-A (D, DA, NZ-R) APRIL (DA) CSF cells (D, DA) 5'GPL (P, D) HMGB1 (D) TGB1 ²³ (D) S100B and ferritin (D, DA) Isoprostanes (P, D, DA) Oxysterols (D, DA) Pentosidine (D, DA) Tau ^{6,7,24} (D, DA) 14-3-3 (D, DA) NAA and NSE (D, DA) Anti-TUB and 3-TUB (D, DA) Anti-NEF ^{25,26} (DA) Neurotrophic factor (D, DA) Tregs (DA) KCNK5 ²⁷ (D, DA) FGF2 and PDGF-AA (DA) gMS classifier.1 (D, DA) Myelin MVs (D, DA) sAPP, A β peptides (D, DA) Apoptosis-related molecules (D, DA, IFN β -R) Co-signalling molecules (DA, IFN β -R) GWAS genes ^{28,29} (IFN β -R) Candidate genes (IFN β -R, GA-R) CHTA (IFN β -R) APLA (IFN β -R) IL17F ³⁰ (IFN β -R) ABCB1, A3CG2 (MT-R) IL21 (AL-R)	
Validated biomarkers	
Anti-EBNA ³¹⁻³³ (*P, D, DA) KFLC ³⁴⁻³⁶ (D) IgM OB ³⁷⁻⁴⁰ (*D, *DA, IFN β -R, NZ-R) NCAM1 ⁴¹⁻⁴⁴ (*D, DA) NO metabolites ⁴⁵⁻⁴⁸ (D, *DA) MMP9 ⁴⁹⁻⁵² (D, *DA, IFN β -R) MBP ⁵³⁻⁵⁵ (D, *DA) SPP1 ⁵⁶⁻⁵⁸ (D, *DA) CXCL13 ⁵⁹⁻⁶² (D, *DA) GFA ⁶³⁻⁶⁴ (D, *DA) BDNF ⁶⁵⁻⁶⁷ (D, *DA, IFN β -R, GA-R) KCNJ10 ⁶⁸ (D) MRZ reaction ^{69,70} (*D, DA) CH3L1 ⁷¹ (*D, DA, NZ-R) Complement factor H ⁷² (DA) Type I IFN β ⁷³ (DA, *IFN β -R) GPCS ⁷⁴ (IFN β -R) HLA-DRB1*04:01, HLA-DRB1*04:08 ⁷⁵ (IFN β -R) IL17 ⁷⁶ (D, *DA) BAFF ^{77,78} (D, *DA, IFN β -R) TNF, IL12, IL23 ^{79,80} (D, *DA) GWAS genes ²⁸⁻²⁹ (P, *D) NEFH ^{81,82-85} (DA) NEFL ^{87,88,89} (D, *DA, NZ-R) 25(OH) vit D ⁹⁰⁻⁹² (P, D, *DA, IFN β -R) CD56 ^{93,94} NK cells ^{13,95} (*D-C-R, IFN β -R)	
Clinically useful biomarkers	
Anti-NZ ⁹⁶⁻⁹⁹ (NZ-R) NAb ¹⁰⁰⁻¹⁰² (IFN β -R) IgG OB ¹⁰³⁻¹⁰⁴ (D) IgG index ¹⁰⁴ (D) Anti-AQP4 ¹⁰⁵⁻¹⁰⁷ (D) Anti-JCVirus ¹⁰⁸ (NZ-R) Anti-VZV ¹⁰⁹ (F-R)	

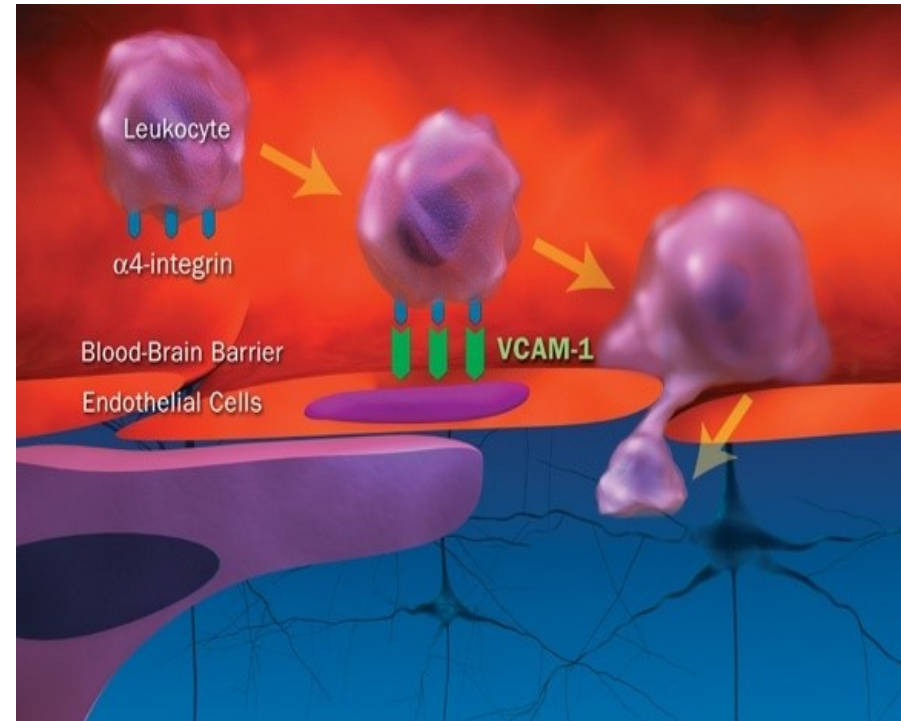
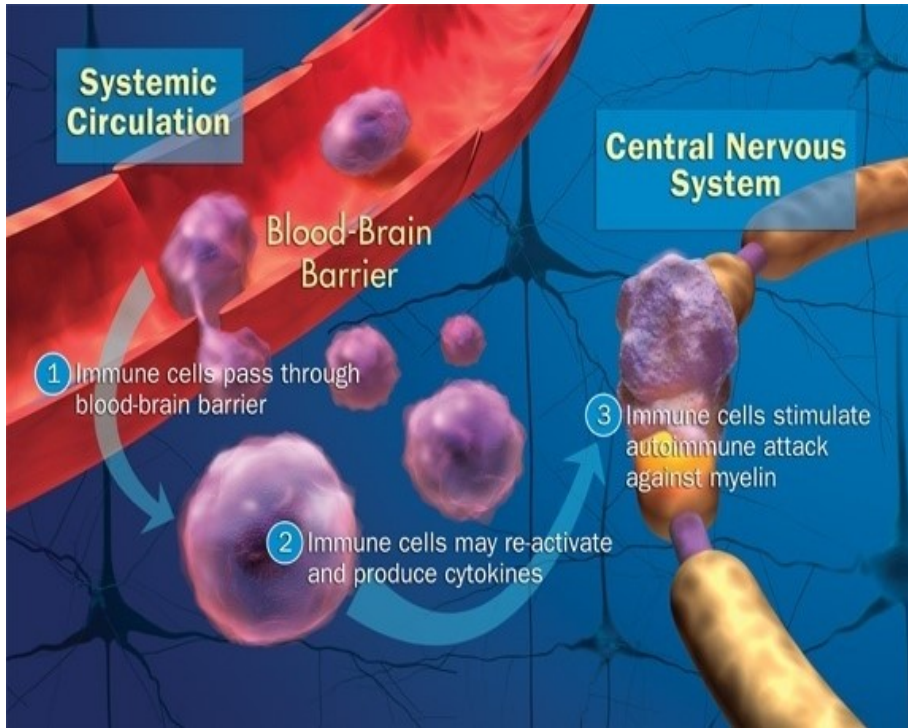
Antibodies against NATALIZUMAB
as biomarker for monitoring
treatment response

Tysabri (Natalizumab) Biogen



- Indicated for very **active relapsing remitting multiple sclerosis**
- Administration: intravenous infusion **once every 4 weeks**

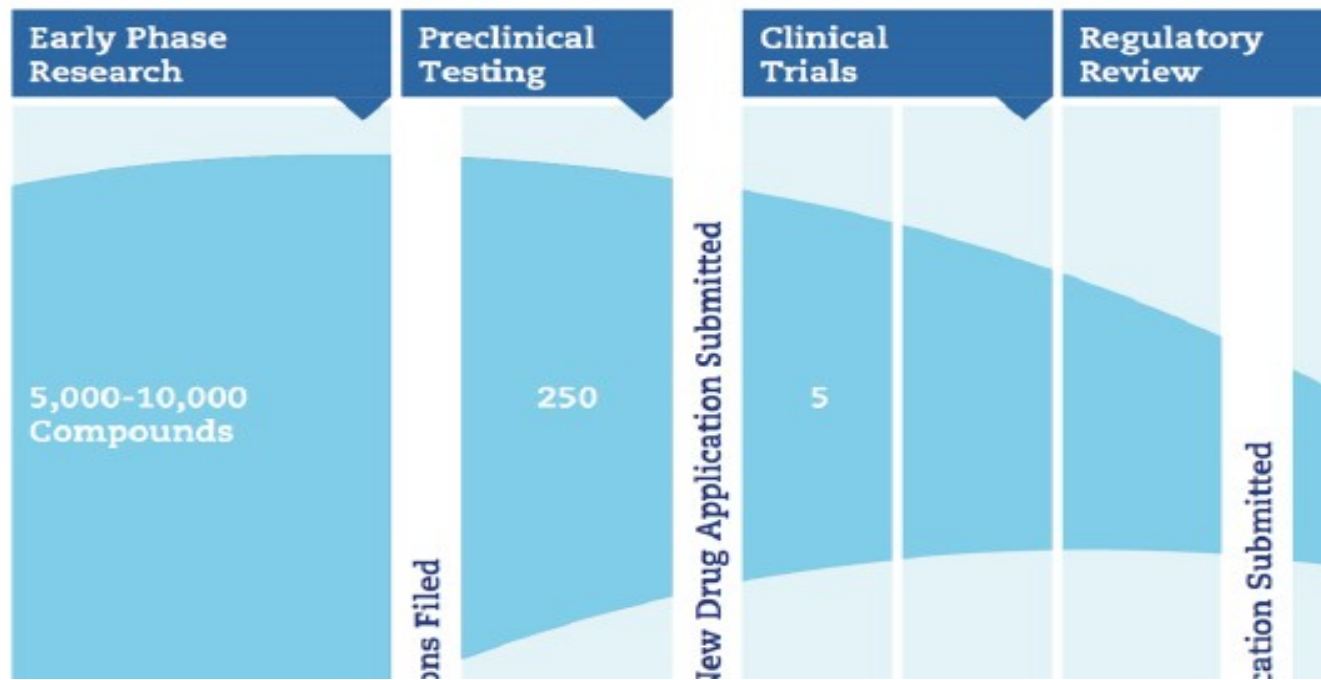
MoA Tysabri (Natalizumab)



Monoclonal antibody that block the union of VLA-4 to VCAM-1 interfering with lymphocyte trafficking into the CNS, thus reducing the pathogenic processes of MS



Drug development



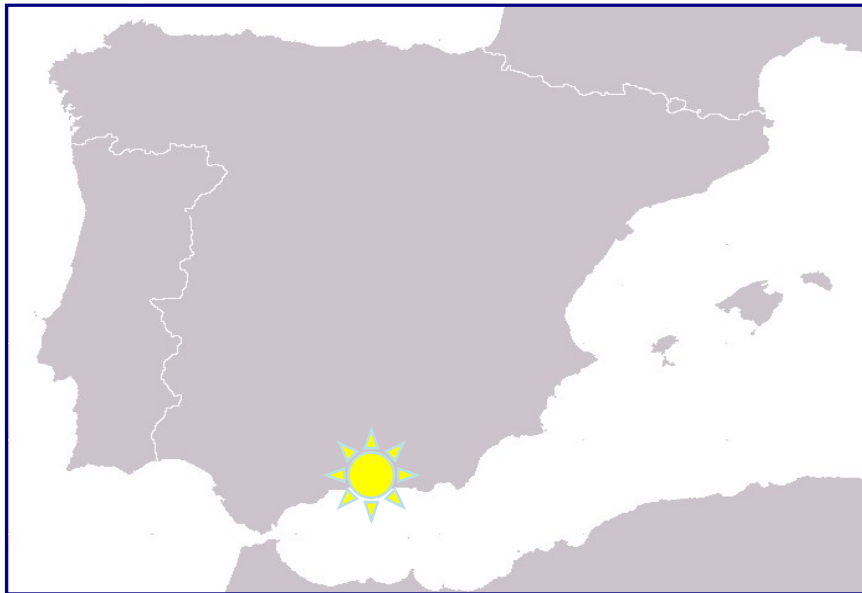
Major issue: Immunogenicity prediction in preclinical and clinical phases, and postmarketing surveillance

Therapeutic drug monitoring

- ✓ Development of a methodology for the determination of Natalizumab antibodies
- ✓ **Six laboratories** were **accredited** world wide



Reference laboratory for Spain and Portugal



Implementation of the assay to determine Natalizumab immunogenicity

Phase I - Assay Demonstration

Phase II – Reagent Qualification

Phase III – Validation parameters

Methodology for the determination of Natalizumab antibodies

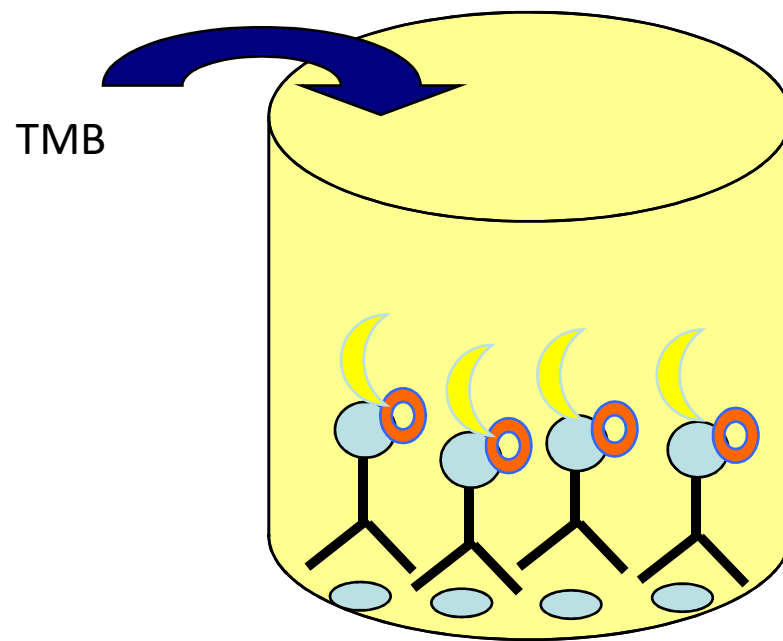
Bridging ELISA:

Screening test: detection of the presence of antibodies

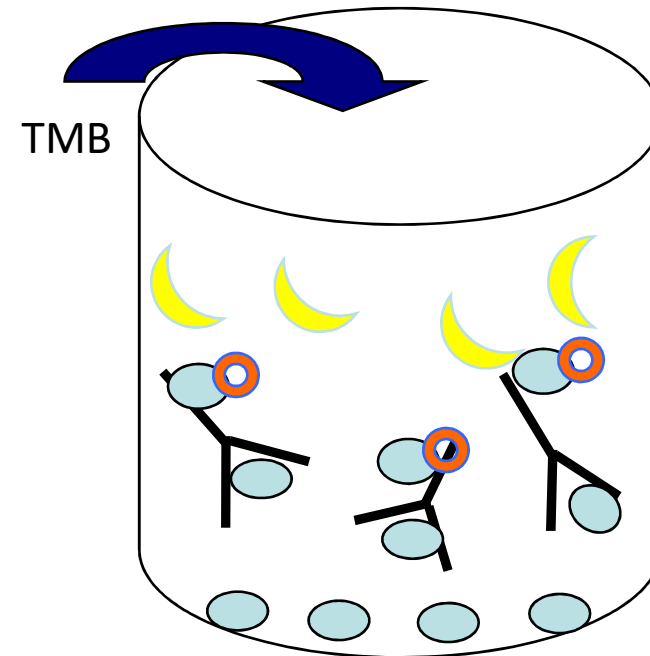
Confirmation test: evaluates the specificity of the antibodies

S1	S1	C1	C1	S8	S8	C8	C8	S15	S15	C15	C15
S2	S2	C2	C2	S9	S9	C9	C9	S16	S6	C16	C16
S3	S3	C3	C3	S10	S10	C10	C10	S17	S17	C17	C17
S4	S4	C4	C4	S11	S11	C11	C11	S18	S18	C18	C18
S5	S5	C5	C5	S12	S12	C12	C12	S19	S19	C19	C19
S6	S6	C6	C6	S13	S13	C13	C13	S20	S20	C20	C20
S7	S7	C7	C7	S14	S14	C14	C14	S21	S21	C21	C21
	QC1			QC2			NC			QC1C	

Methodology for the determination of Natalizumab antibodies



Screening



Confirmación

- Patient serum
- Biotinylated Natalizumab
- SA-HRP (streptavidine horseradish peroxidase)
- TMB

Validation of the assay to determine Natalizumab immunogenicity (II)

Phase II – Reagent Qualification

New lot of critical reagents will be run in parallel with the previous lot concurrently to establish acceptable performance before use in clinical testing.

Validation of the assay to determine Natalizumab immunogenicity (III)

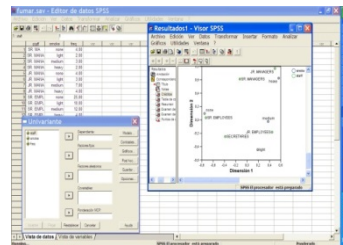
Phase III – Validation parameters:

- ✓ Precision:
 - ✓ Repeatability
 - ✓ Intermediate precision
- ✓ Specificity of the screening assay
- ✓ Specificity of the confirmation assay
- ✓ Robustness (inter lab)
- ✓ Control range

Workflow



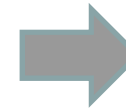
Sample Reception



Registration of samples
in the database



Encode samples and
aliquot them



Sample storage at -20°C



Screening and
confirmation test



HOJA DE COMUNICACION DE RESULTADO DETERMINACIÓN DE ANTICUERPOS AN
Nombre del paciente: JLRB
Nº de Historia Clínica: 667767
Fecha de nacimiento: 14/08/1971
MUESTRA RECIBIDA el día: 29/06/2018
Dr. Martínez Sánchez
Resultado: Evidencia de anticuerpos anti-natalizumab en suero: NEGA
Fecha de emisión resultado: 03/07/2018

Reports

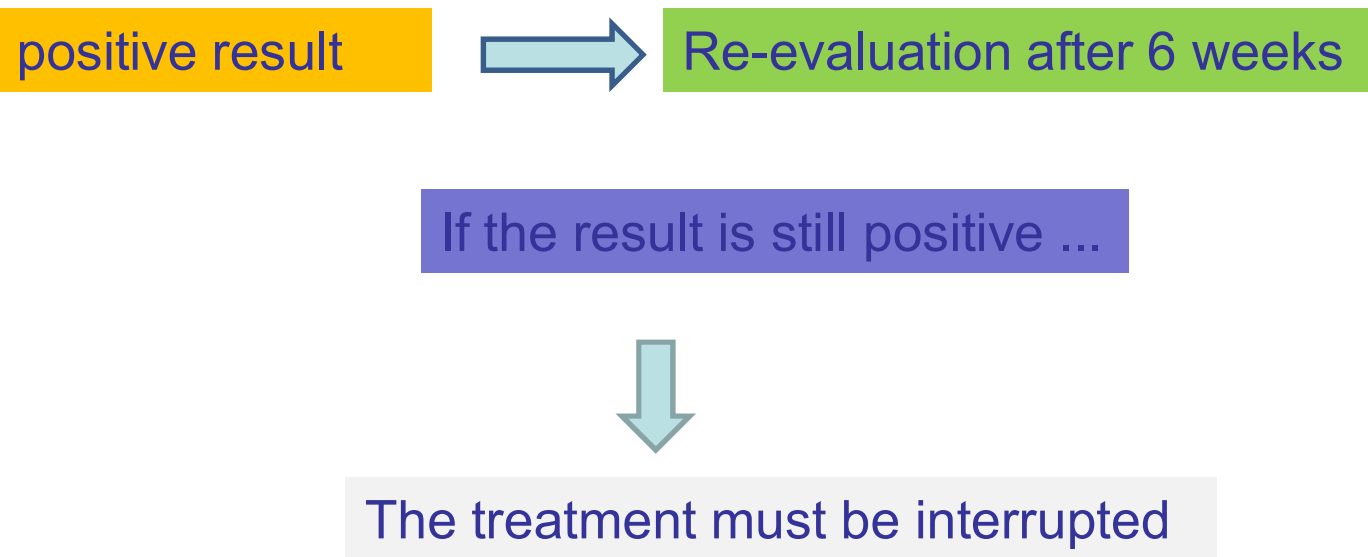


Results submission

Recommendations for clinical practice

The determination of antibodies against NTZ should be performed in the following situations:

- worsening disease
- infusion reactions
- hypersensitivity reaction



Natalizumab Immunogenicity

Disease exacerbations or infusion related events **may indicate the development of antibodies against natalizumab.** In these cases **the presence of antibodies should be evaluated and if these remain positive in a confirmatory test after at least 6 weeks, treatment should be discontinued,** as persistent antibodies are associated with a substantial decrease in efficacy of TYSABRI and an increased incidence of hypersensitivity reactions (see section 4.8).

Our experience

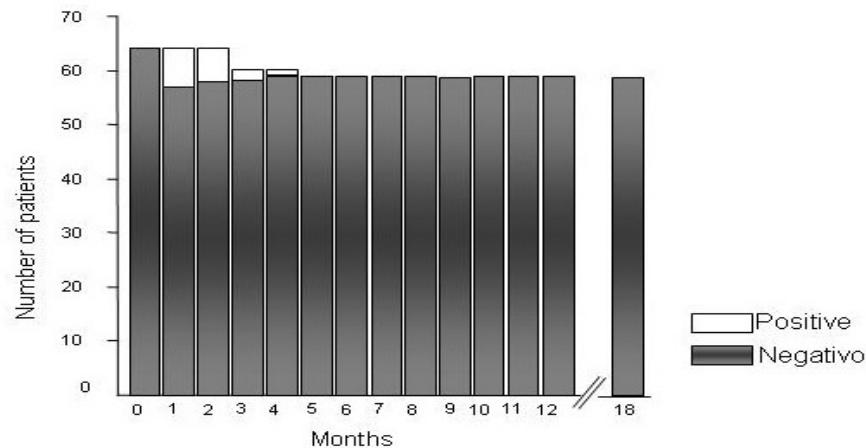
Research Paper

MULTIPLE
SCLEROSIS
JOURNAL

Kinetics and incidence of anti-natalizumab antibodies in multiple sclerosis patients on treatment for 18 months

Multiple Sclerosis Journal
9(03) 1-4
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DOI: 10.1177/1352458510385508
msj.sagepub.com
SAGE

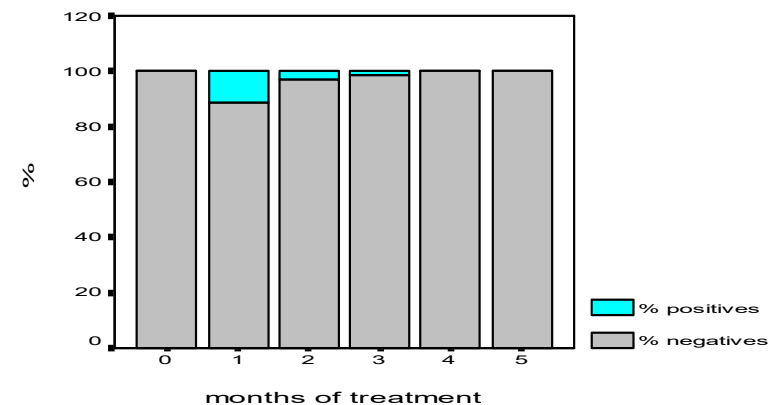
Begoña Oliver², Oscar Fernández¹, Teresa Órpez²,
Marcos Papais Alvarenga¹, María Jesús Pinto-Medel²,
Miguel Guerrero¹, Antonio León¹, José Carlos Lopez-Madróna¹,
Rafael Maldonado-Sánchez², Juan Antonio García-León²,
Gloria Luque¹, Victoria Fernández¹ and Laura Leyva²



Early development of anti-natalizumab antibodies in MS patients

B. Oliver-Martos, T. Órpez-Zafra,
P. Urbaneja, R. Maldonado-Sanchez,
L. Leyva & O. Fernández

Journal of Neurology
Official Journal of the European
Neurological Society
ISSN 0340-5354
J Neurol
DOI 10.1007/s00415-013-6991-2



9% of permanent positives patients

Most of positives patients are positives after the first infusion of NTZ

Antibodies against IFN beta (NABs)

IFN beta



First treatment approved for MS (1993)

- Reduce the number of relapses /CNS lesions
- Slows the progression of the disease



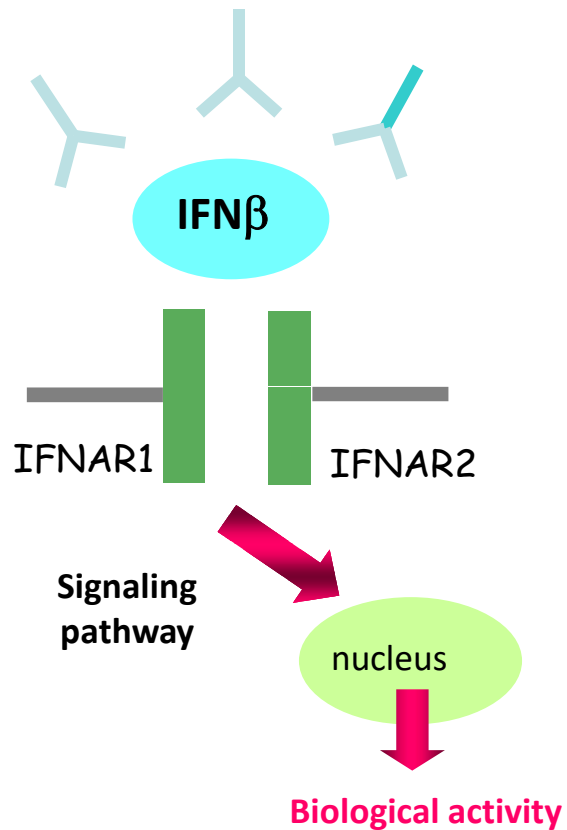
40% of patients are considered non responders



Development of NABs

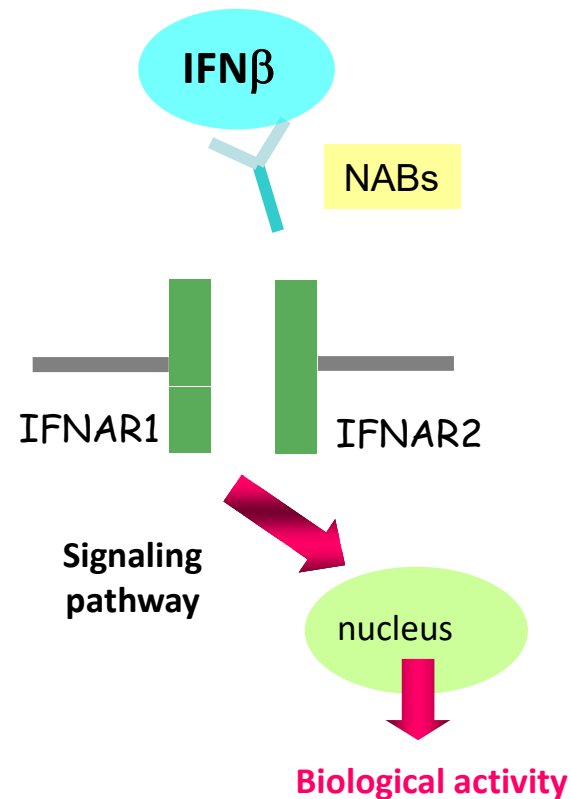
Antibodies against IFN beta

Binding antibodies



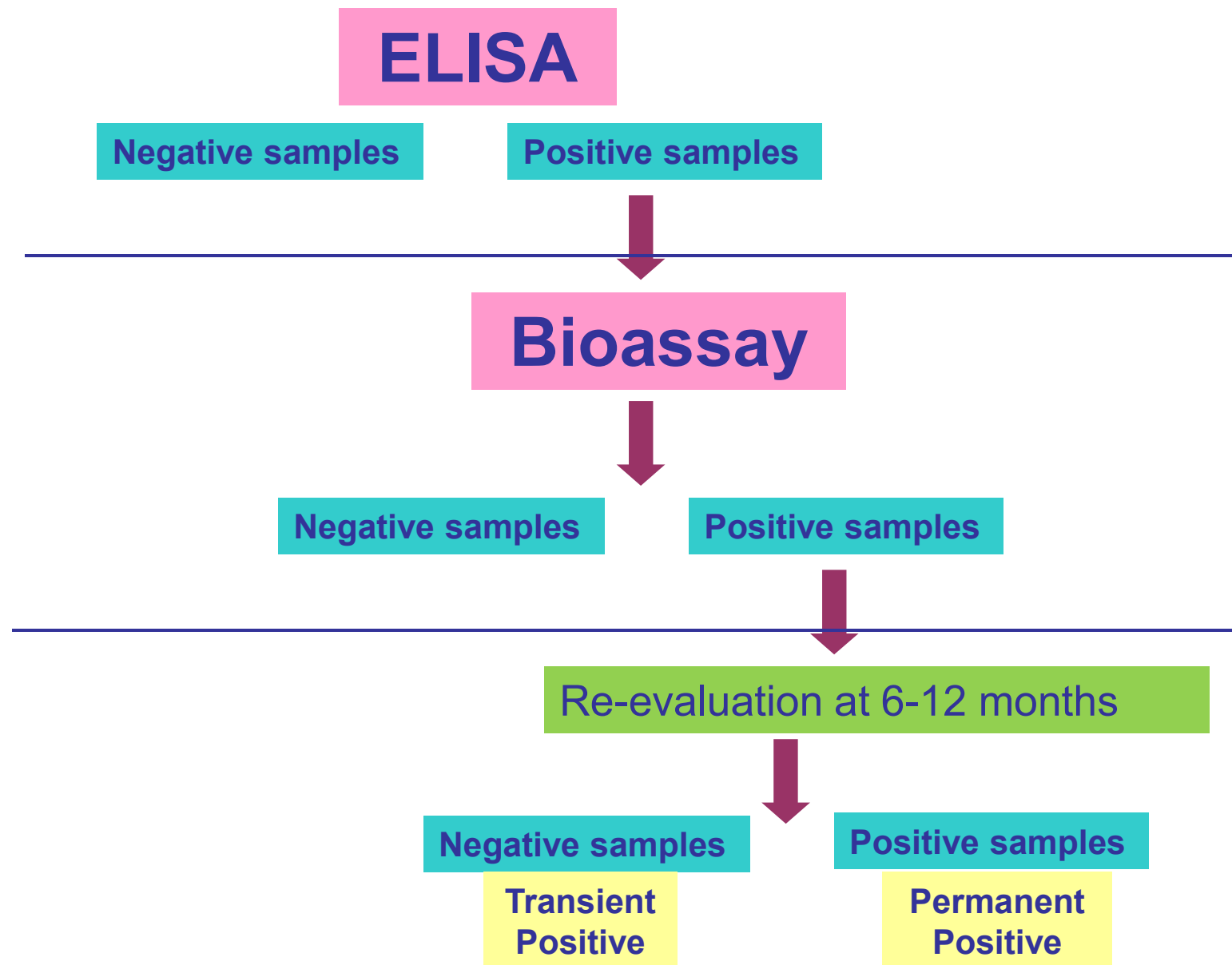
ELISA

Neutralising antibodies

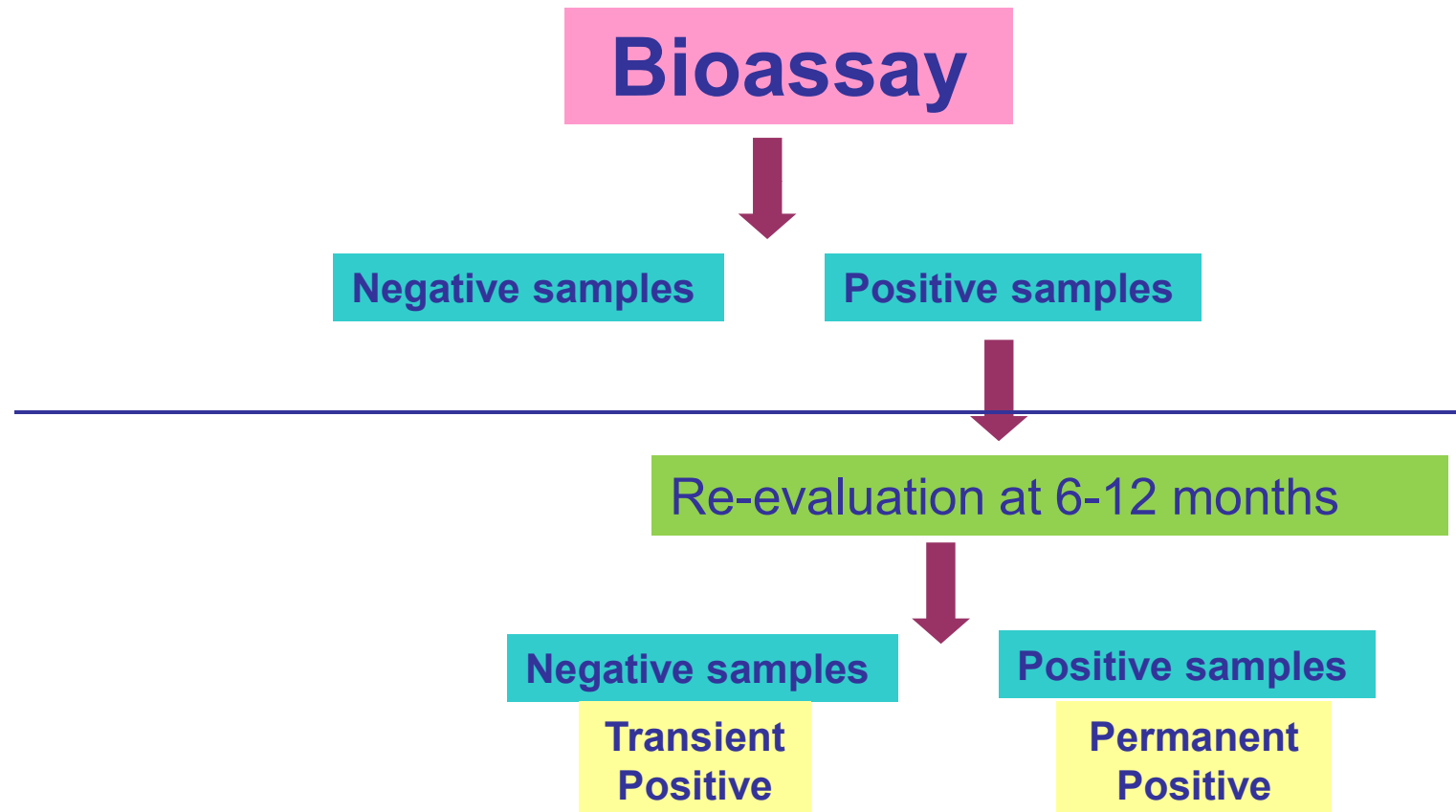


Bioassay

Strategy for immunogenicity assessment

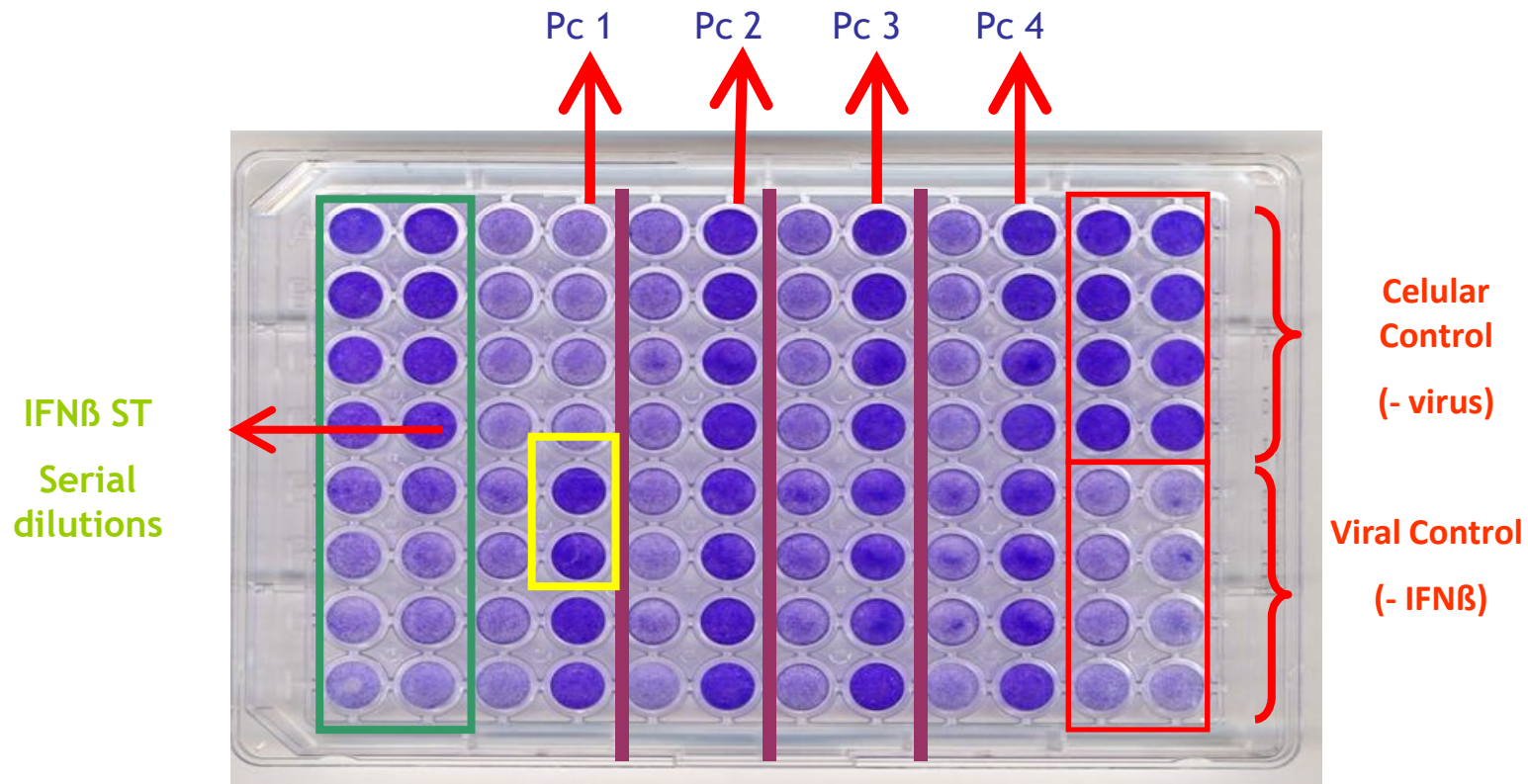


Strategy for immunogenicity assessment



Bioassays at IBIMA laboratory (Málaga)

- ✓ More than **20 year experience** in conducting Bioassay for determining NABs, according to the **Technical Report of WHO**
- ✓ Based on the **antiviral ability IFN β** : A cell culture (A549) infected with a specific virus (encephalomyocarditis virus) will be protected by the presence of IFN β (Blue = Cells alive)



A549: human lung cancer cell line
Encephalomyocarditis virus

Communication of results

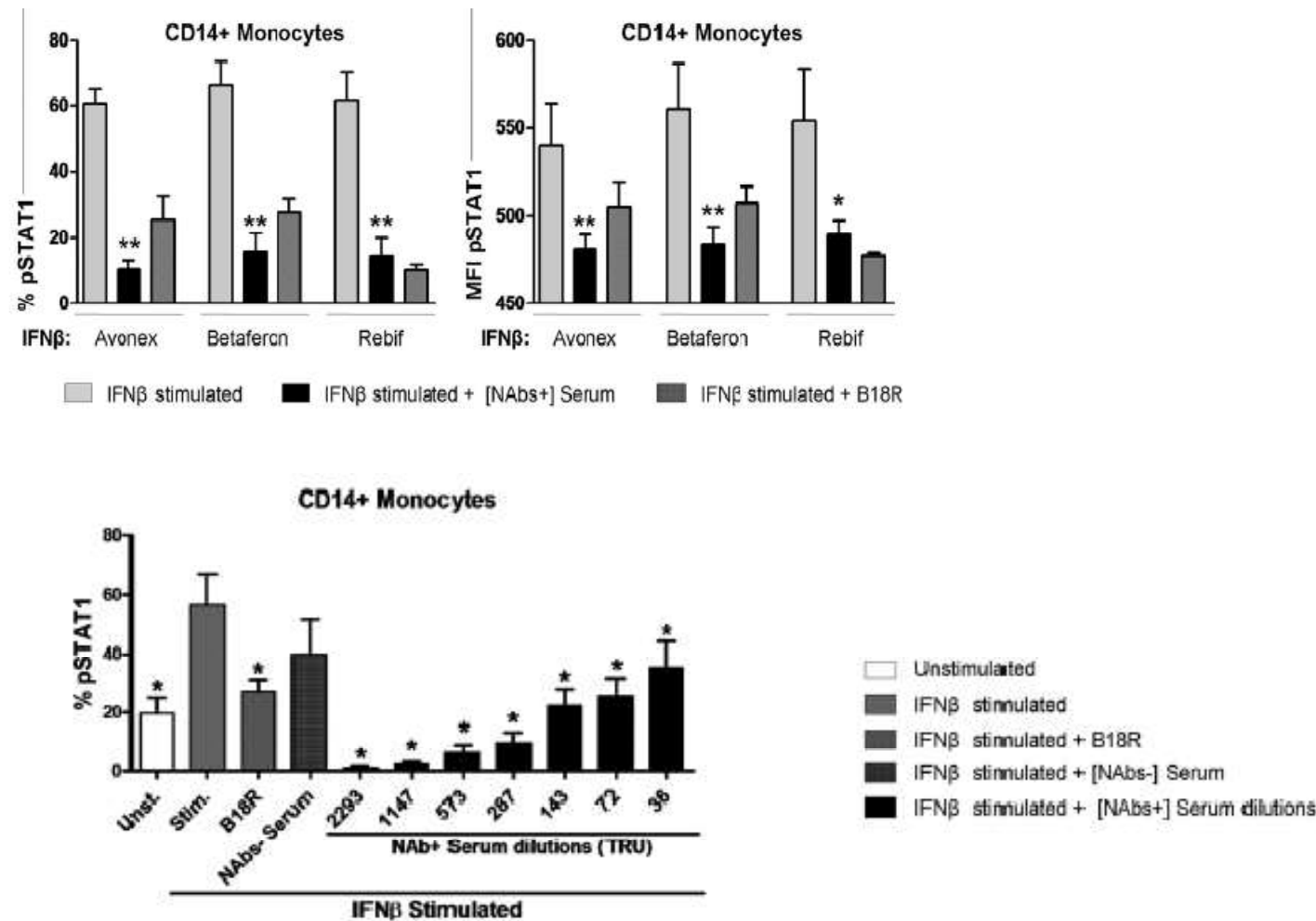
Name	Date of birth	Betaferon	Avonex	Rebif
xxxx	20/05/1956	Positive (1455 TRU)	Positive (1223 TRU)	Positive (3875 TRU)
xxxx	12/07/1970	Negative	Negative	Negative

OPEN

Cross-reactivity of antibodies against interferon beta in multiple sclerosis patients and interference of the JAK-STAT signaling pathway

Received: 8 August 2017
Accepted: 17 November 2017
Published online: 29 November 2017

Isaac Hurtado-Guerrero¹, María Jesús Pinto-Medell^{1,2}, Patricia Urbaneja^{1,2}, José Luis Rodríguez-Bada^{1,2}, Jesús Ortega-Pinazo¹, Pedro Serrano^{1,2}, Óscar Fernández¹, Laura Leyva^{1,2} & Begoña Oliver-Martos^{1,2}



<https://ki.se/en/cns/biopia>



BIOPIA

BIOPIA is a non-profit collaborative effort of European laboratories with expertise in biopharmaceutical pharmacokinetics and immunogenicity. It is an initiative that aims to raise awareness about biopharmaceuticals and their immunogenicity, with the aim of integrating testing of these factors in order to improve the care and overall health of patients. BIOPIA involves several labs from all across Europe that specialise in biotherapeutic immunogenicity and assessing drug serum levels in many diseases. Through this website, we will work together to:

- Provide easy, accessible information about ADA and drug level testing
- Create a site for clinicians to help them assess biologic responses in their patients and choose the correct treatment for each person
- Connect European labs and hospitals together, with the goal of implementing routine, clinical testing for immunogenicity and drug



Overview BIOPIA

For information about biopharmaceuticals, immunogenicity, and why clinical assessment needs to include tests for ADAs. ➤



Labs locations

Find the lab nearest you that assesses drug and ADA levels ➤



Biopharmaceuticals

Find your biologic of interest from a list of commercially available drugs



Drug & ADA Tests

Determine the best test for patients, where to order it, and how to interpret results



Publications

See major publications by each lab around Europe that specialise in biopharmaceuticals and assessing their immunogenicity



Summary

- ✓ One major limitation of biological drugs is the induction of Anti-drug antibodies
- ✓ These are related with a lower efficacy of the drug and with the presence of adverse events
- ✓ The therapeutic drug monitoring is highly recommended for the safety of the patients and the optimization of the resources

Thank you for your
attention!

Begoña Oliver
begoliver@gmail.com