Amsterdam UMC
Universitair Medische Centra

Signal transduction molecules as targets for therapy

Prof. dr. Sander W. Tas, internist-rheumatologist

Systeemziekten Symposium 3-2-2023

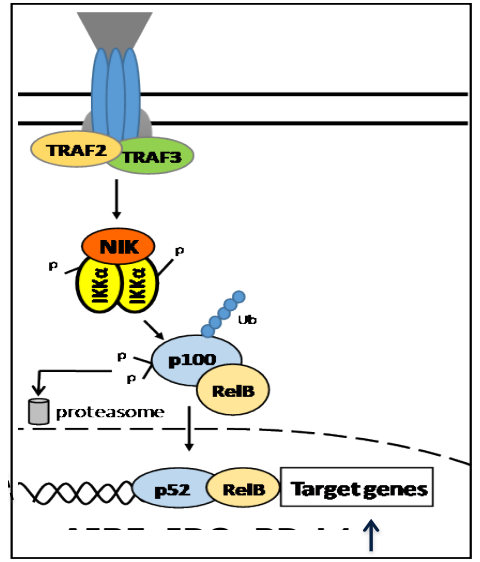
Disclosures

(potentiële) belangenverstrengeling	Zie hieronder
Voor bijeenkomst mogelijk relevante relaties met bedrijven	Bedrijfsnamen
• Sponsoring of onderzoeksgeld • Honorarium of andere (financiële) vergoeding • Aandeelhouder • Andere relatie, namelijk …	• AstraZeneca, UCB, Celgene, Lilly, Galvani bioelectronics, • MSD, AbbVie, BMS, Pfizer, Sobi, Roche, UCB, Arthrogen • •

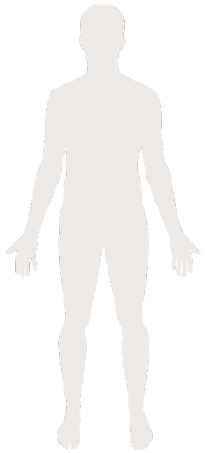
Translational immunology research

Research focus:

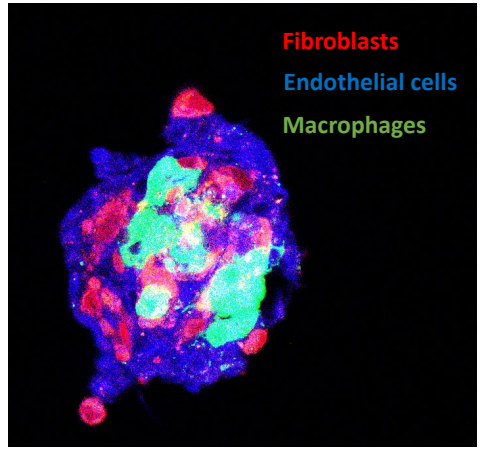
- NF-κB signaling and other signal transduction pathways in chronic inflammatory diseases
- Regulatory mechanisms in chronic inflammation



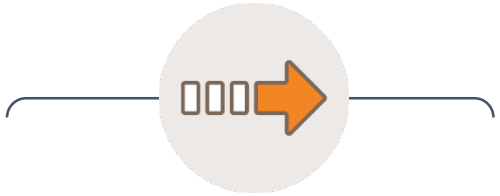
Different approaches



- Target discovery via unique patient materials (a.o. scRNAseq)
- Functional *in vitro* assays and *in vivo* studies (a.o. PBMC/B cell assay, synovial tissue organoids, animal models, 3D ultramicroscopy)
- 'Mechanism of action' studies and strategy trials in IMIDs
- Innovative novel treatment strategies:
  - *In vivo* DC vaccination (DC4Balance)
  - N. splenica plexus stimulation (Galvani bioelectronics)



Technological developments: more insight into the pathogenesis of autoimmune diseases



Starting point

Pathophysiology of autoimmune diseases not fully elucidated

Serological biomarkers hitherto rather disappointing for prediction of treatment response

Tissue is the issue

Profiling of different compartments of the immune system: more insight into pathophysiology

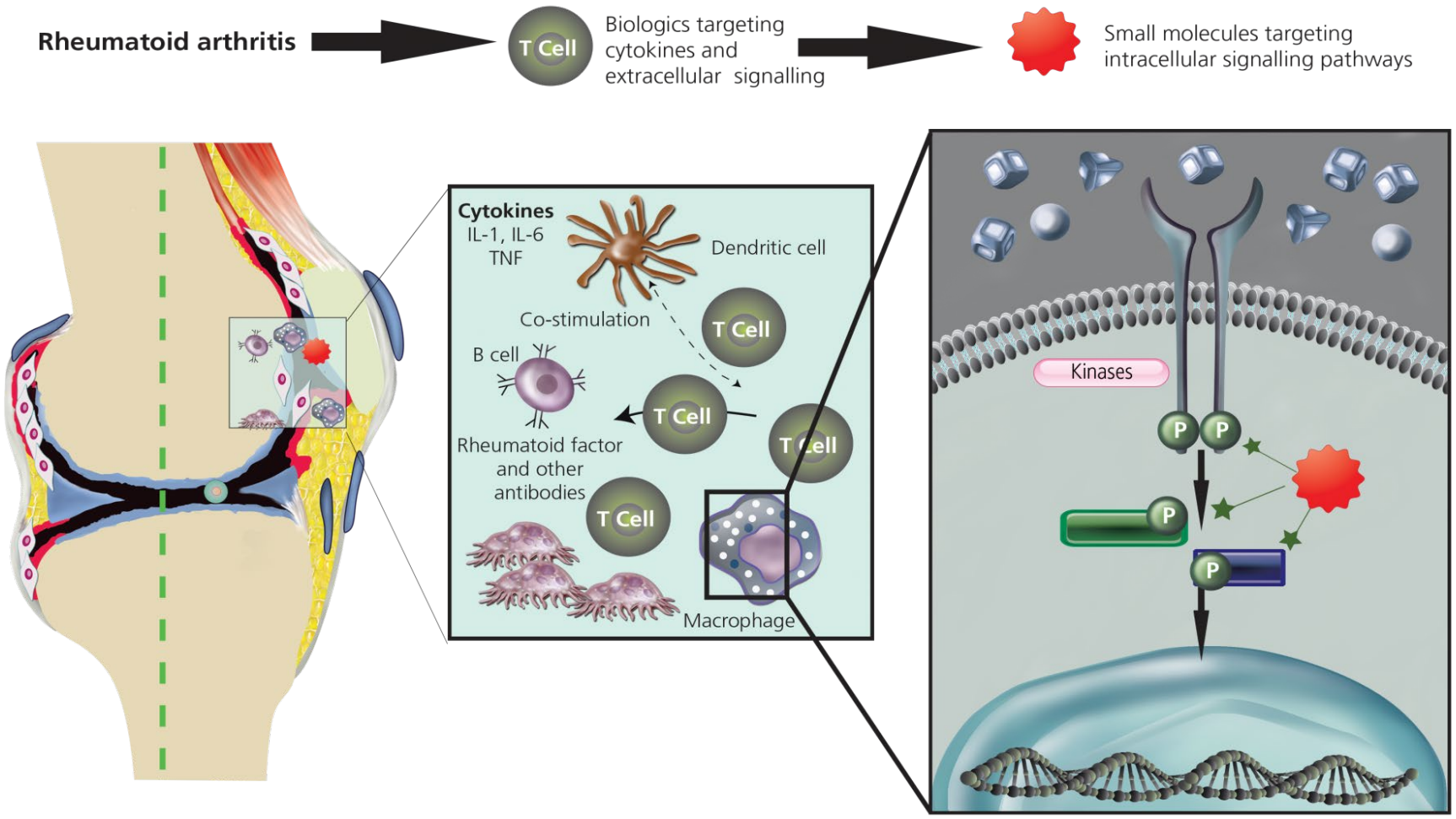
Synovial pathotype/molecular signature (RNAseq): prediction of treatment response in individual patients

Strategy trials

Stratified treatment based on unique tissue characteristics (pathotype and/or gene signature)

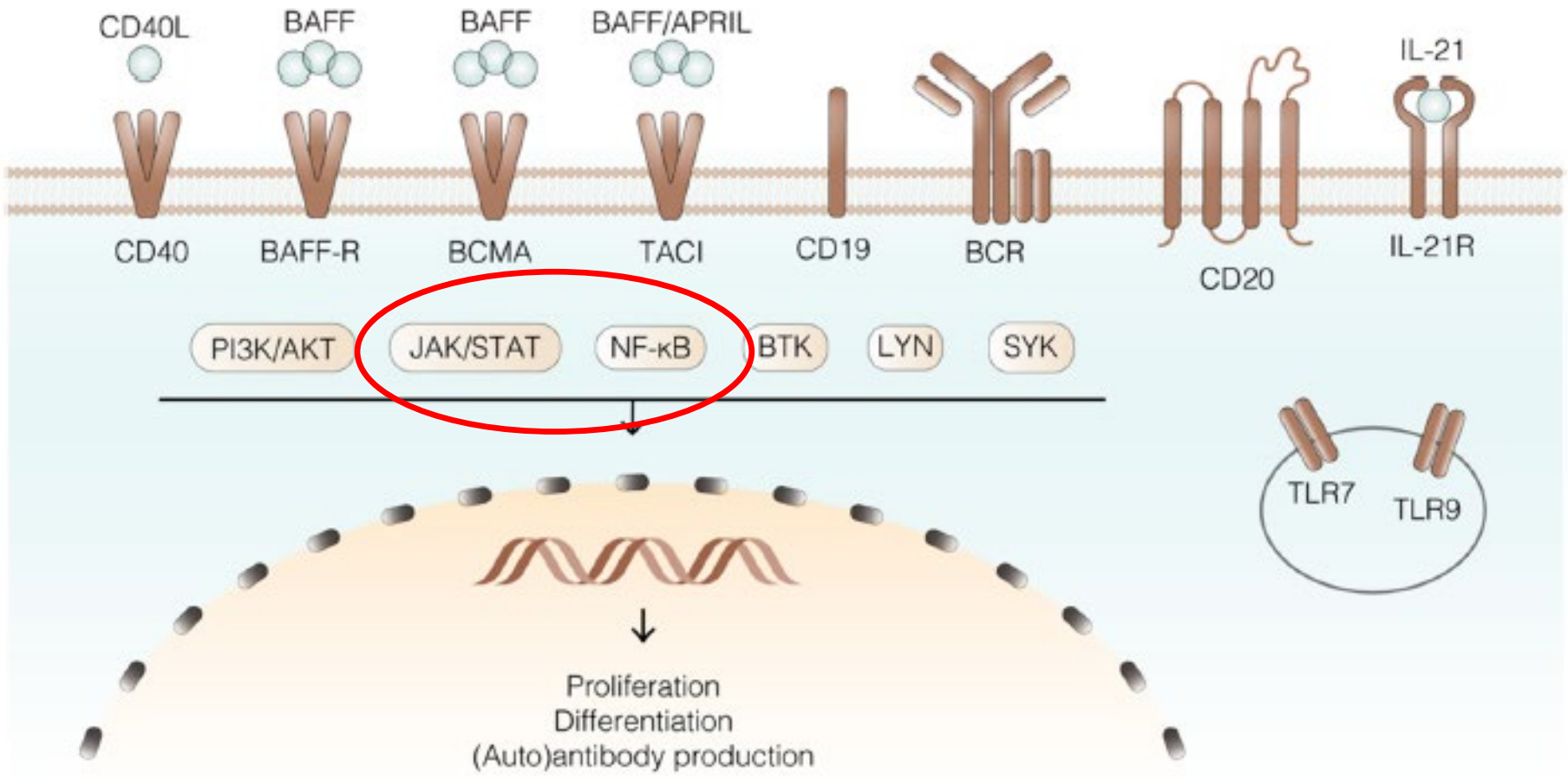
Non-invasive techniques to determine characteristics of the inflammatory process
→ optimal treatment choice

Extra- and intracellular targets in rheumatology



1. Smolen JS et al. *Nat Rev Drug Discov* 2003;2:473–488.
2. van Vollenhoven RF. *Nat Rev Rheumatol* 2009;5:531–541.
3. Ghoreschi K et al. *Immun Rev* 2009;228:273–287.

Intracellular signalling pathways



Merino-Vico et al. Int J Mol Sci. 2021;23(1):387

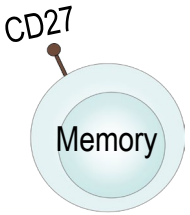
Part I:

NF-κB signalling in ANCA-associated vasculitis

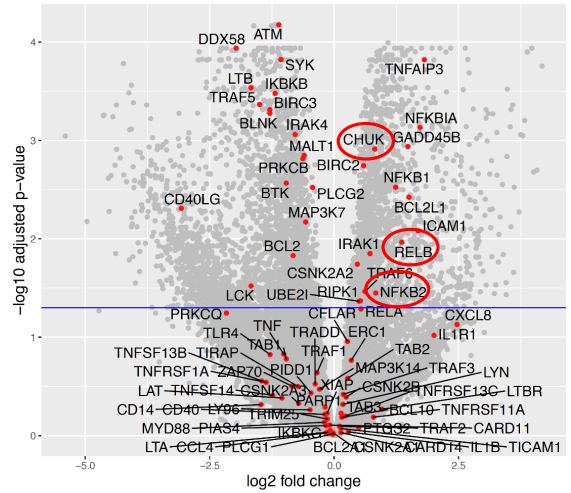
Pathophysiology and target discovery: B cells in AAV

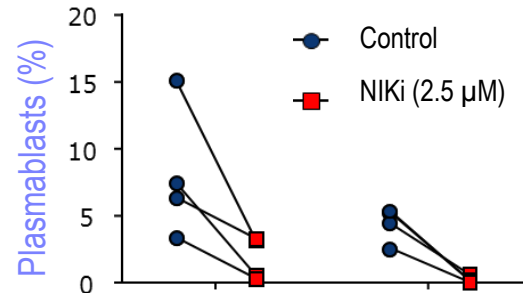
Blood sample

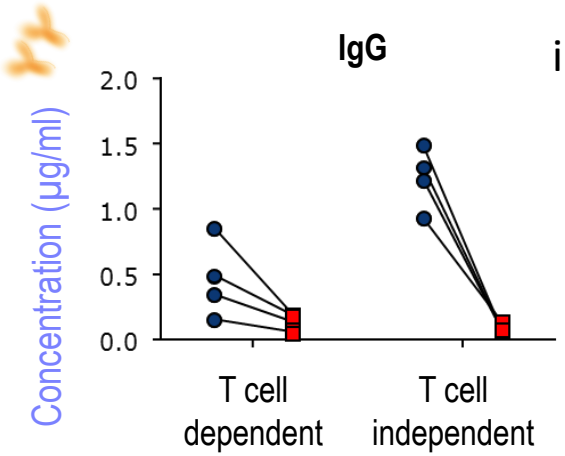
RNAseq
B cells



B mem - ACT vs REM



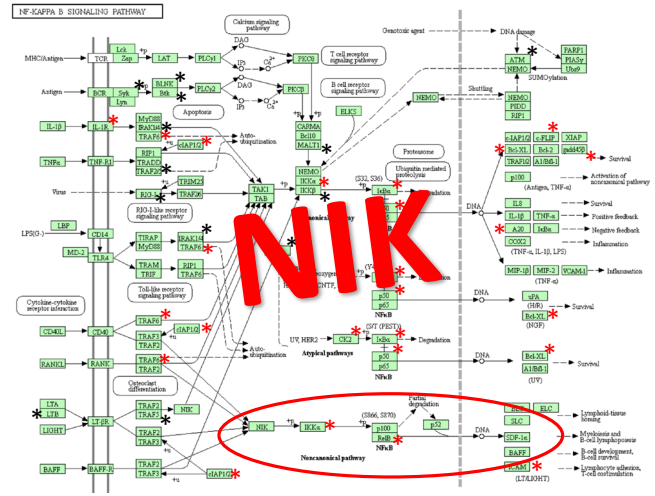




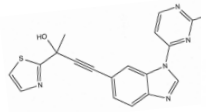
AAV

Target validation: inhibition of B cell responses

Target identification



NIK inhibitor



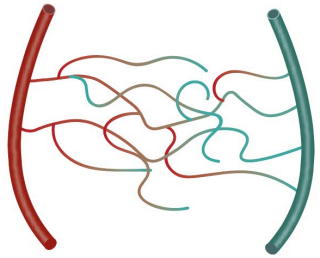
AstraZeneca

Merino Vico et al, *manuscript in preparation*

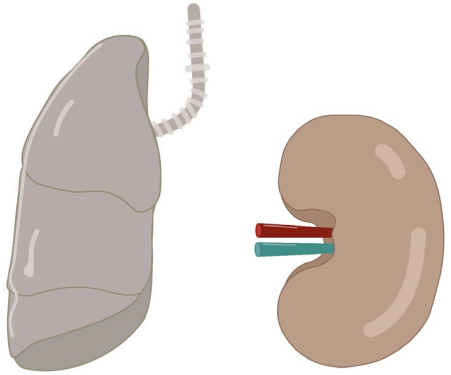
ANCA-associated vasculitis

Rare, systemic autoimmune disease →

Small-size blood vessels



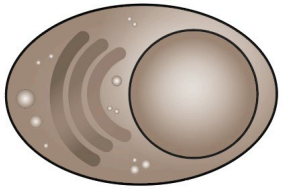
→



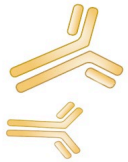
Pulmonary haemorrhage & kidney failure

ANCAs (anti-neutrophil cytoplasmic antibodies)

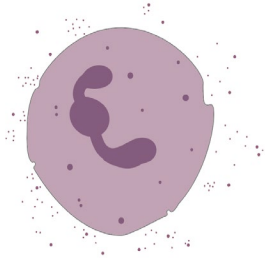
Plasma cells



ANCAs



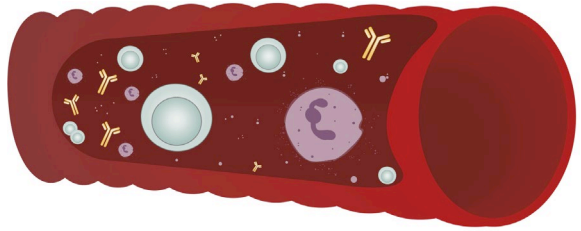
Neutrophil activation →



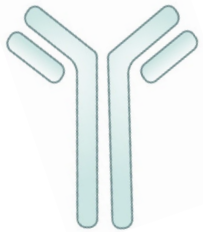
- Inflammatory cytokines
- Lytic enzymes

→

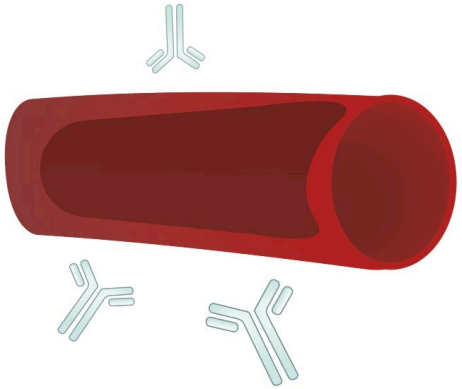
Endothelial damage



Rituximab (anti-CD20)



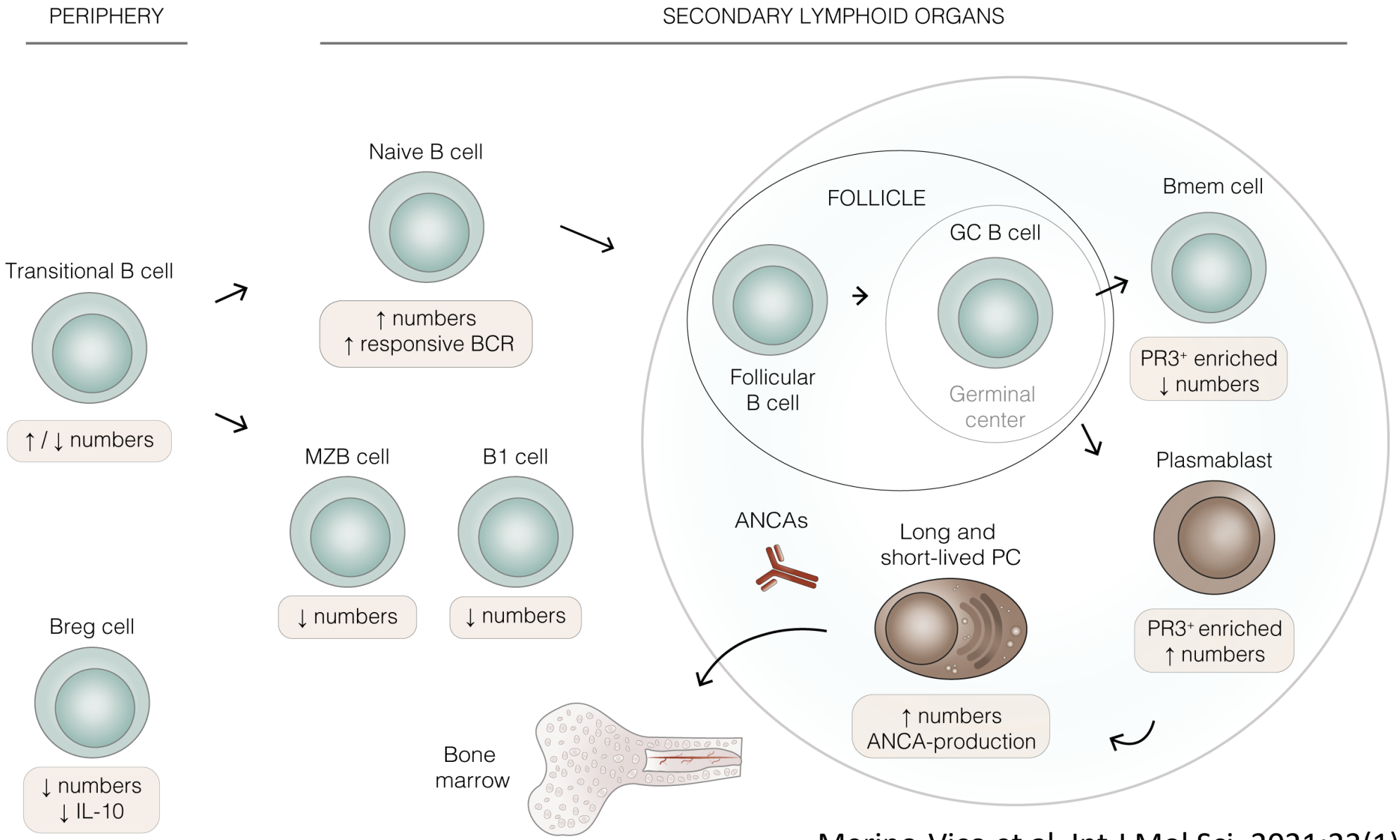
Circulating B cell depletion →



→ Disease remission

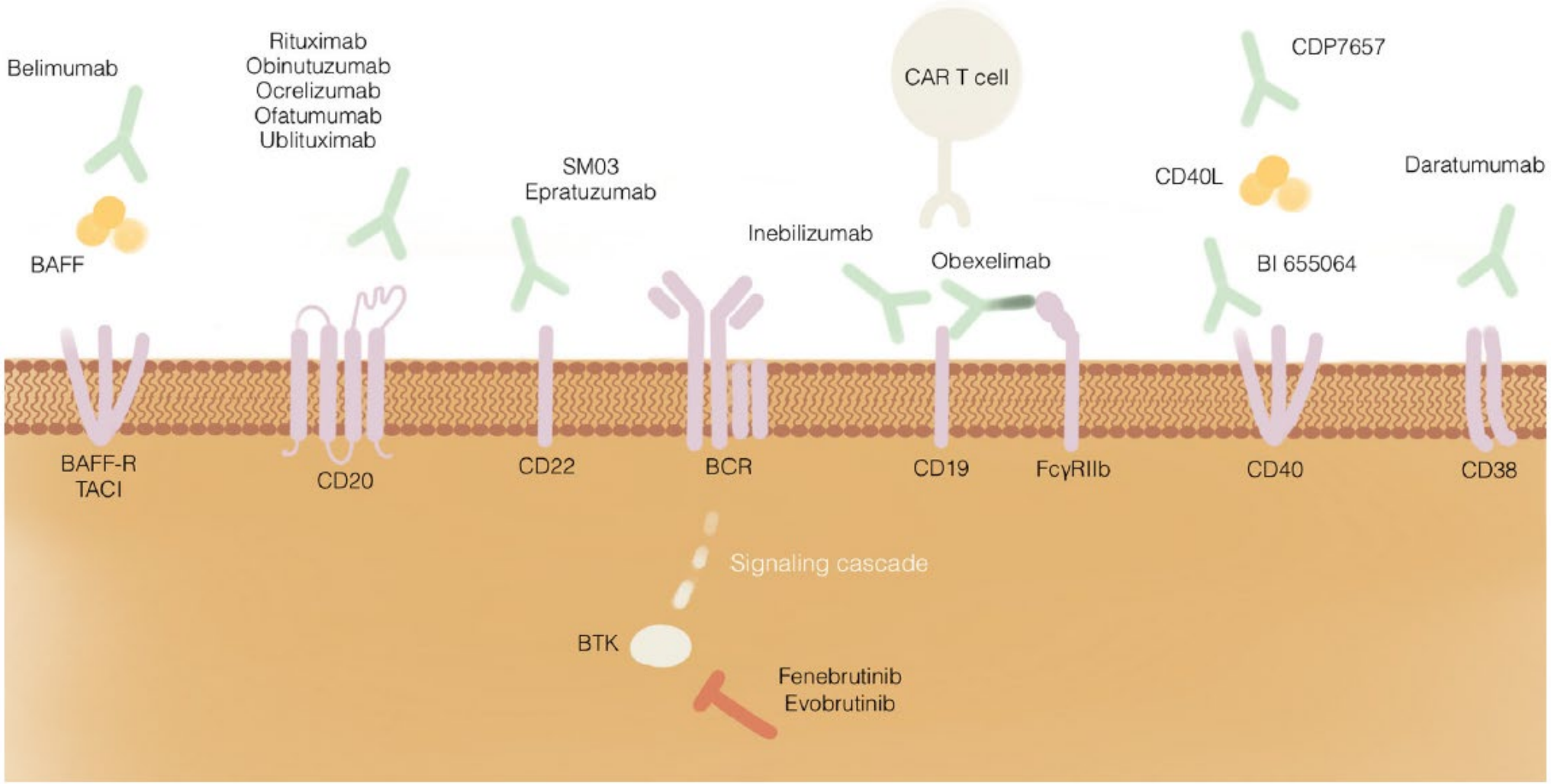
Rituximab is not perfect → Improved treatment needed

B cell populations are dysregulated in AAV



Merino-Vico et al. Int J Mol Sci. 2021;23(1):387

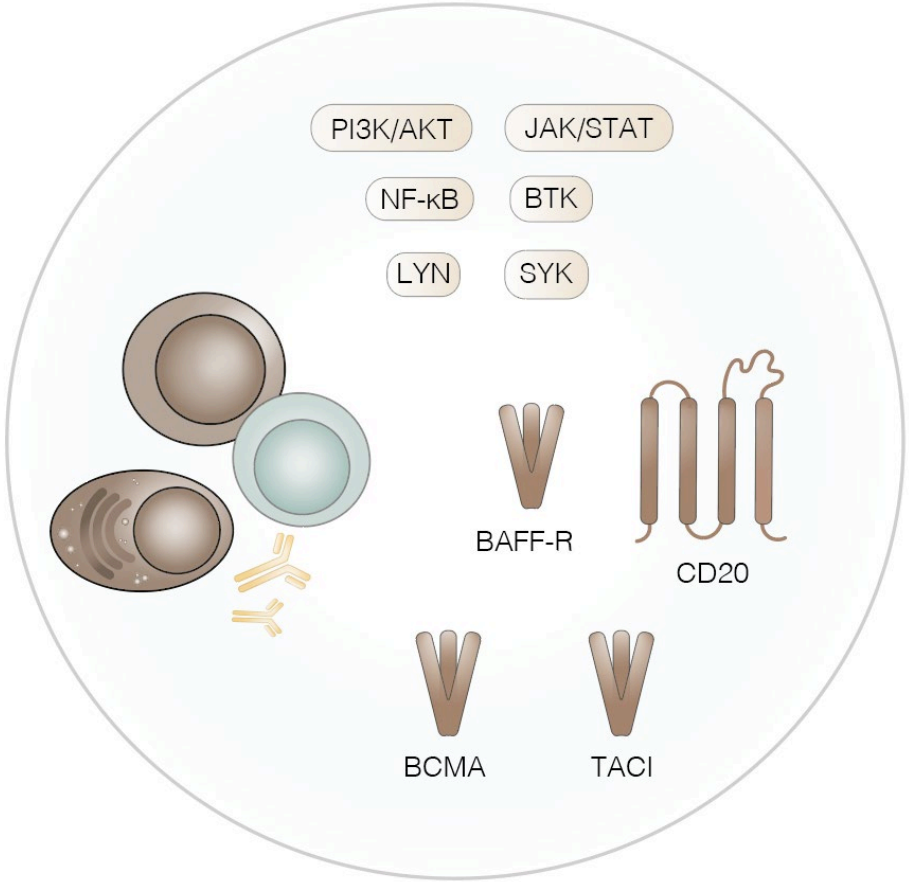
Targeting B cells and plasma cells



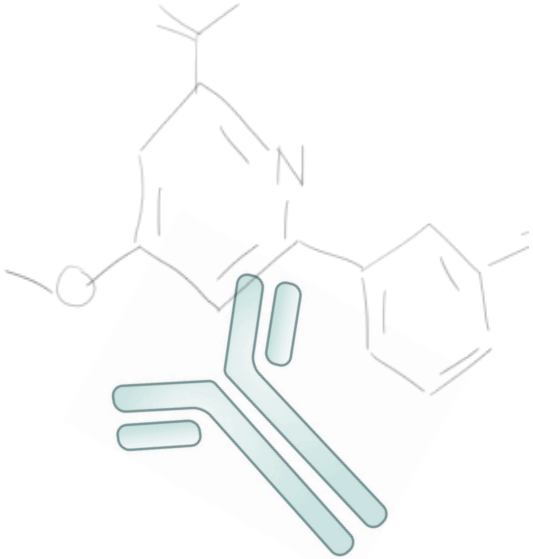
Merino-Vico et al. Eur J Immunol. 2023;53(1):e2149675

Are B cells and their signaling pathways functionally different in AAV patients compared to healthy controls?

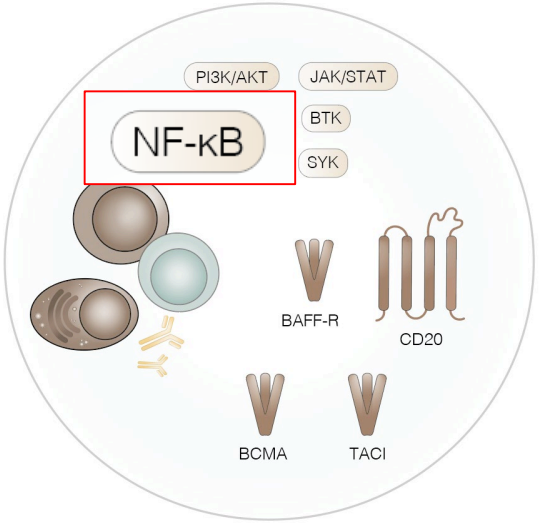
Can we modulate their intracellular signaling more specifically?



More specific targets?



NF-κB associated genes are upregulated in memory B cells of patients with AAV and active disease

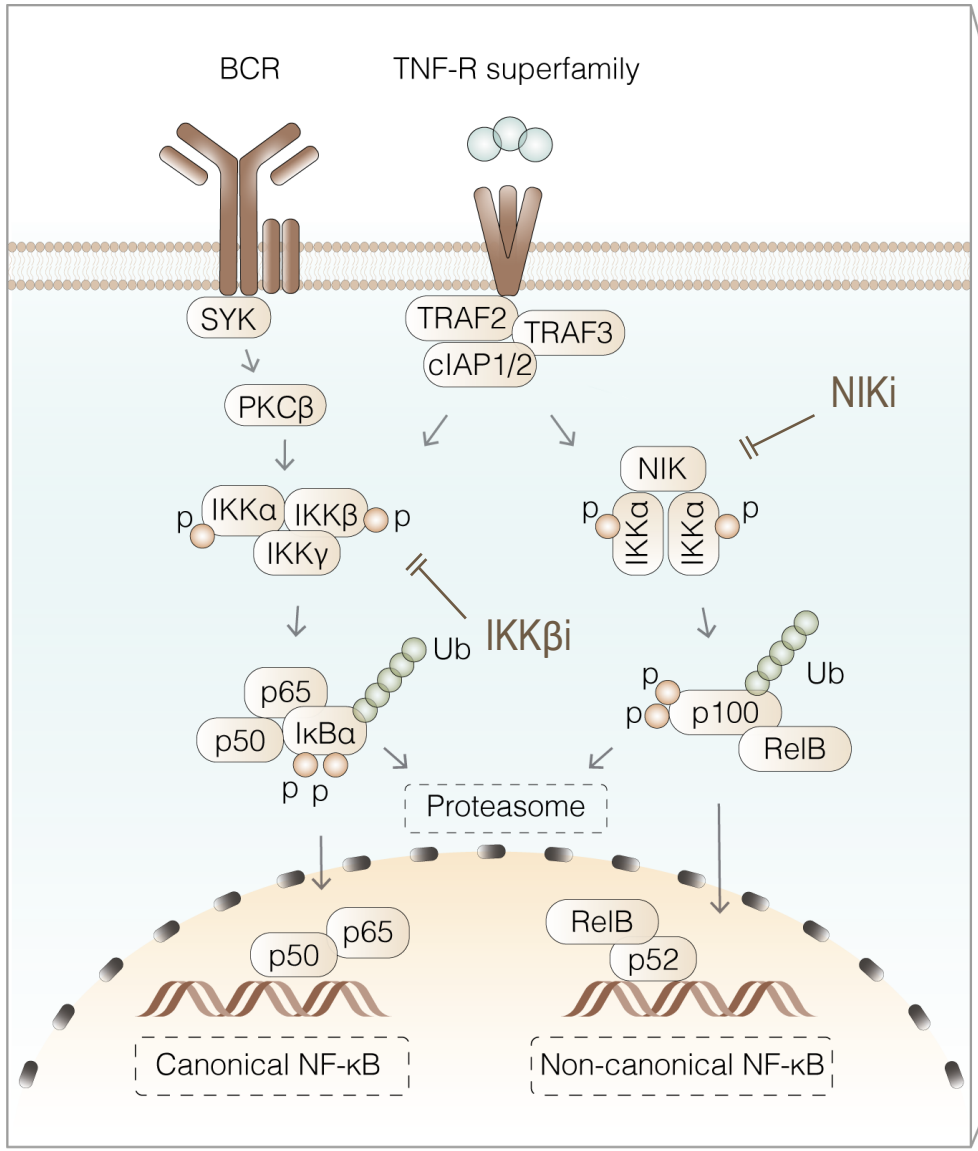


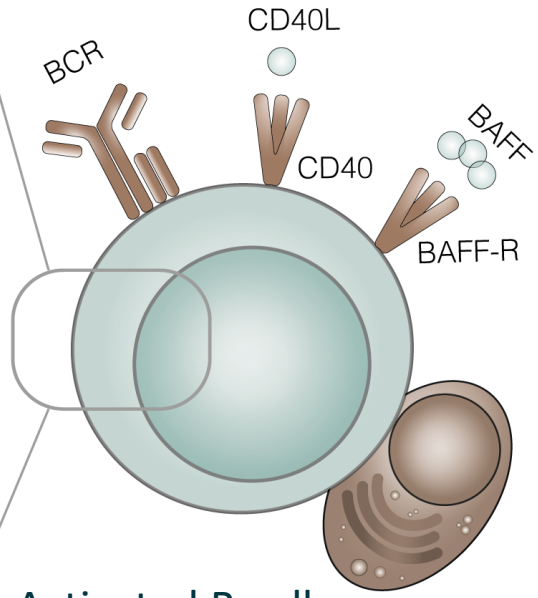
→ Can we modulate B cell response using small molecule inhibitors (SMI) of NF-κB?

Advantages:

- *No long-term B cell depletion*
  (anti-CD20 or other cell-depleting therapies)
- *SMI can reach long-lived PCs in the bone marrow*
- *Relatively short half-life: potentially less side-effects*

ANCA-associated vasculitis: targeting NF-κB signaling



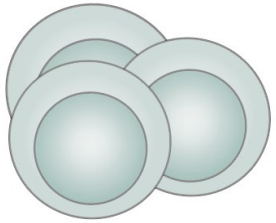


Activated B cell
or plasma cell

Adapted from: Tas & Baeten. Methods Mol Biol. 2016

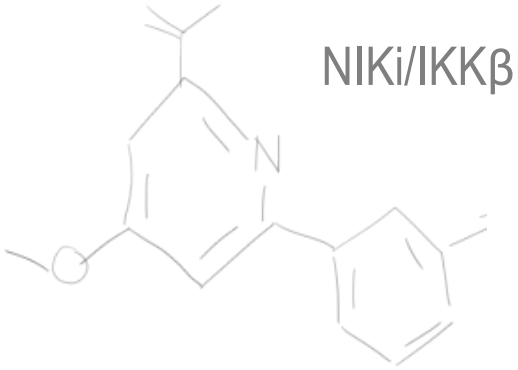
Evaluating functional B cell responses

Functional B cell assay



Healthy controls
GPA patients

\+

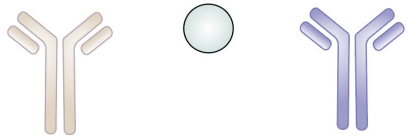


NIKi/IKKβi

Stimulation

2h (37°C)

T cell-dependent
(aCD40 + IL-21 + aIgM)



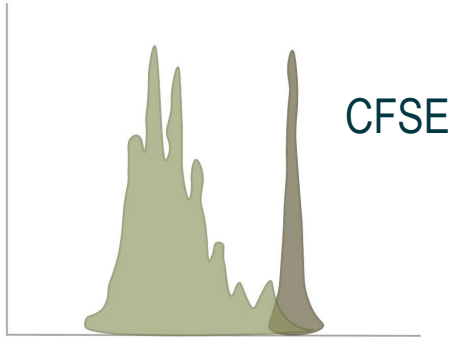
T cell independent
(IL-2 + CpG)

T cell stimulation
(aCD3/aCD28)

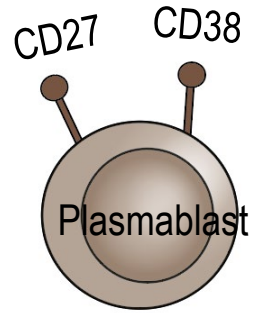
6-day
culture

- Flow cytometry
- ELISA

- Proliferation



- Differentiation



- Antibody production

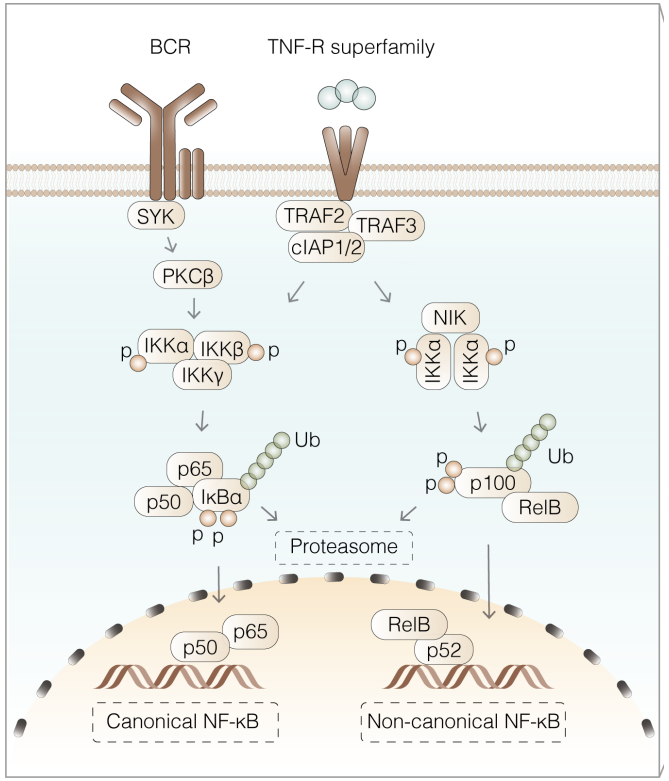
Effects of targeting NF-κB in B cells from AAV patients?

Functional assays with PBMCs from patients

Conclusions I

- Targeting NIK and IKKβ *in vitro* inhibits:

- B cell proliferation
- Plasmablast differentiation
- (Auto)antibody production



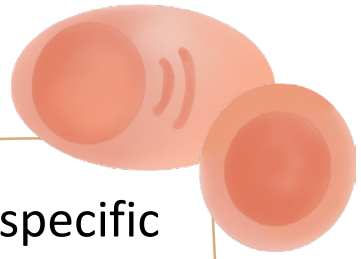
- NF-κB inhibitors, and in particular NIKi, may be potential therapeutics for B cell modulating therapy in autoimmune diseases such as AAV

- Inhibition of other important B cell signaling molecules may also have potential therapeutic effects that need to be studied

Part II:

JAK/STAT signalling in myositis

B cell-directed therapies: JAK inhibition using tofacitinib



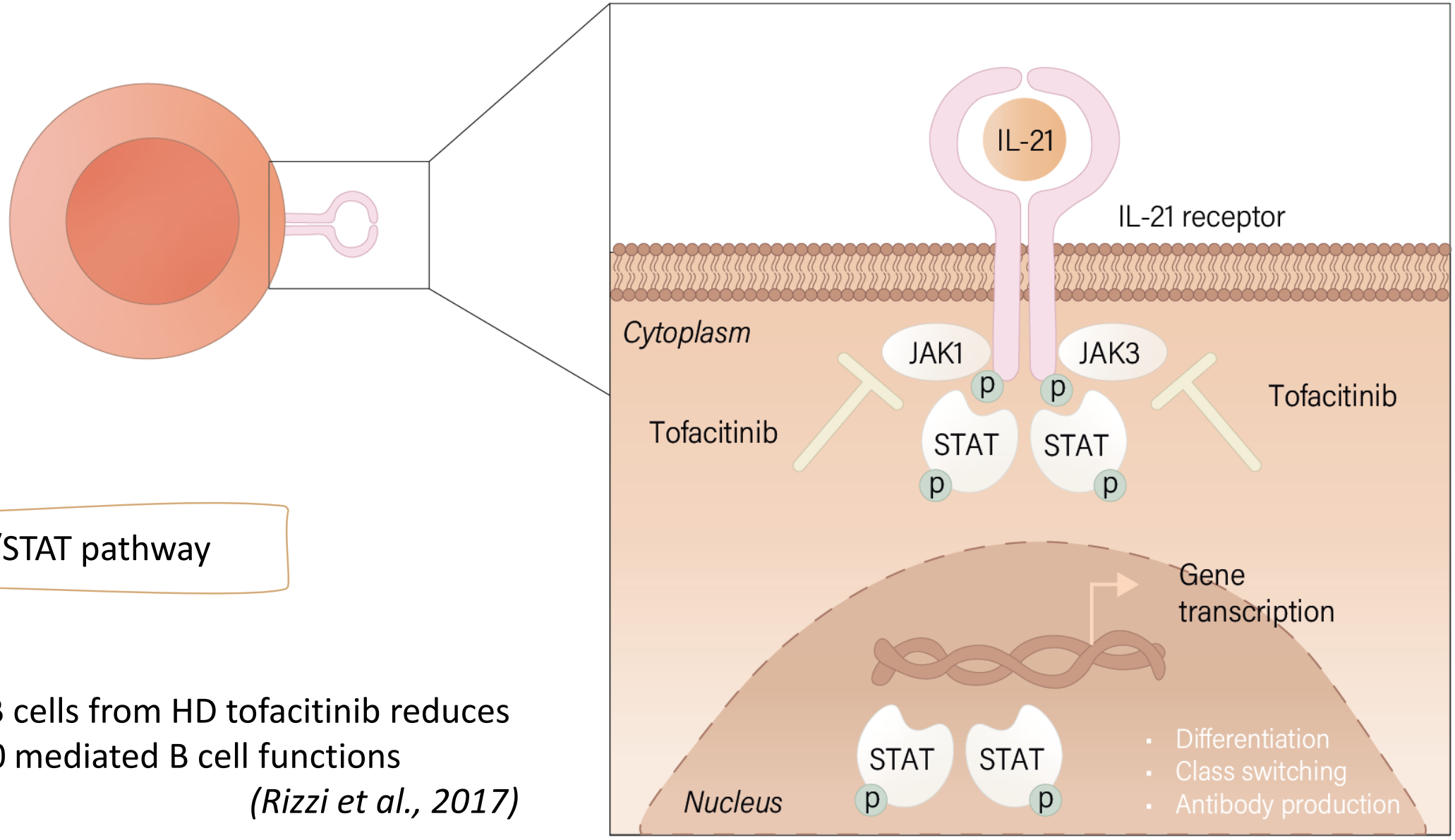
Find novel B cell-specific therapeutic targets

Janus kinase (JAK)/signal transduction and activator of transcription (STAT) pathway

¿?

- Therapeutic target in RA
- Altered in myositis/SLE
- Case reports/series in IIM

B cell-directed therapies: JAK inhibition using tofacitinib



JAK/STAT pathway

In purified B cells from HD tofacitinib reduces IL-21/aCD40 mediated B cell functions

(Rizzi et al., 2017)

Hypothesis

JAK/STAT signalling in autoreactive B lineage cells is essential for their differentiation and autoantibody production, thereby contributing to disease activity in myositis

Objectives

- **Characterize** the mechanism of action of **tofacitinib** *in vitro*
- Analyse the **effects** of inhibiting JAK **on pathogenic B cell responses in myositis** using tofacitinib in a functional assay *in vitro*
- **Characterize B cell populations** in PBMCs from patients with **myositis**

Conclusions II

1. The effects of **tofacitinib** on B cell responses are **predominantly** present upon **IL-21 mediated activation of JAK/STAT** signalling

2. **Targeting IL-21 activated JAK** with tofacitinib in **myositis** PBMCs **inhibits**:

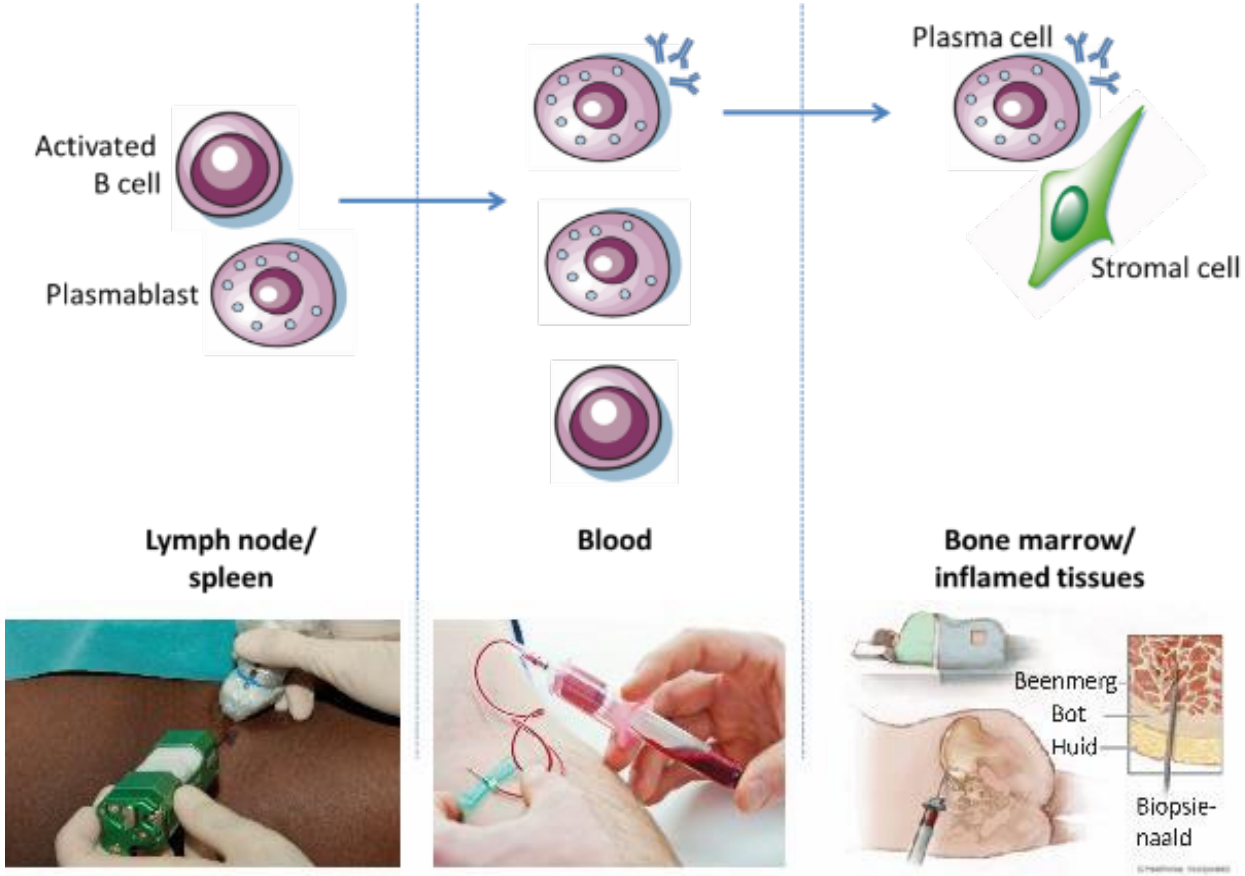
    - *Plasmablast **differentiation***
    - ***Immunoglobulin** production*

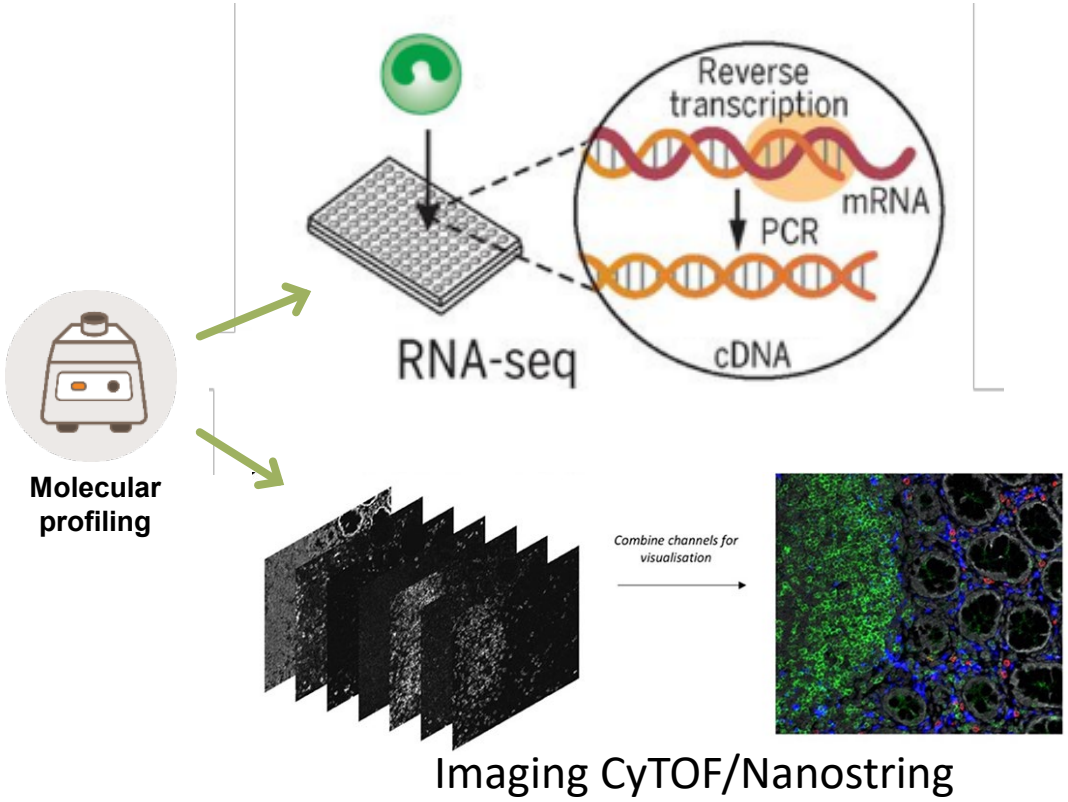
3. Targeting the JAK/STAT pathway may offer a novel treatment modality in myositis

Vacancy physician-scientist / PhD student!

- ReumaNederland project: **“Profiling of autoreactive B lineage cells in idiopathic inflammatory myopathies”**

Profiling of the B cell lineage in AAV and IIM





Fiechter RH et al, *Arthritis Res Ther.* 2022

Take home messages

- Signal transduction molecules are powerful targets for therapy:
  - NF-κB signalling in ANCA-associated vasculitis
  - JAK/STAT signalling in myositis
  - More to come!
- Small molecule inhibitors can also impact on long-lived PCs in the bone marrow and have a relatively short half-life: potentially less side-effects and can be quickly stopped in case of infections
- Profiling of different compartments of the immune system may yield new insights (i.e. on autoreactive B cells), including novel targets for treatment

Acknowledgements

Amsterdam UMC

Department of Rheumatology & Clinical Immunology

Department of Experimental Immunology

Yik Yang Kan
Charlotte van Rooijen
Giulia Frazzei
Lisanne van Rooijen
Guus van Laar
Ana Merino Vico
Eva Philippon
Jan Piet van Hamburg

Aram Al-Soudi
Linda van der Weele
Dornatien C. Anang
Niek de Vries

Amsterdam UMC
Department of Pediatrics

Paul Tuijnenburg
Machiel Jansen
Taco Kuijpers

Amsterdam UMC
Department of Neurology

Joost Raaphorst
Anneke van der Kooi
Eleonora Aronica

Imperial College
London UK

Charles D. Pusey
Stephen P. McAdoo

AstraZeneca
Sweden

Paulina Kucharzewska
Henric Olsen

UMCG
Groningen

Peter Heeringa
Bram Rutgers
Wayel Abdulahad
Carlo Bonasia
Frans Kroese
Gwenny Verstappen
Hendrika Bootsma

'This Project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 847551'

Questions?

s.w.tas@amsterdamumc.nl