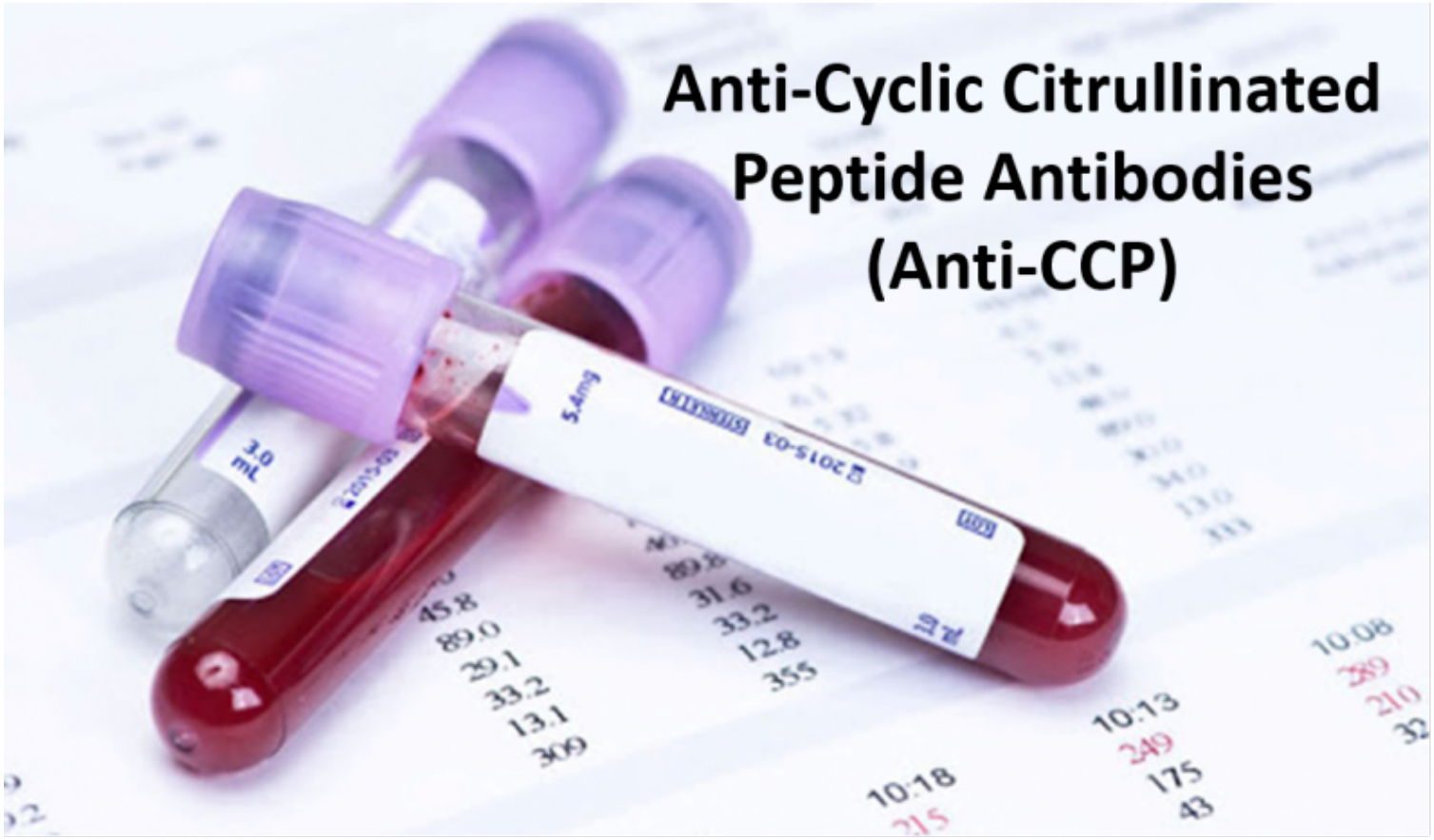
Rheumatoid Arthritis and Citrullinated Proteins



Alina Chen

MacMillan Group Meeting

Literature Talk

March 8th, 2022

Outline

What is Rheumatoid Arthritis

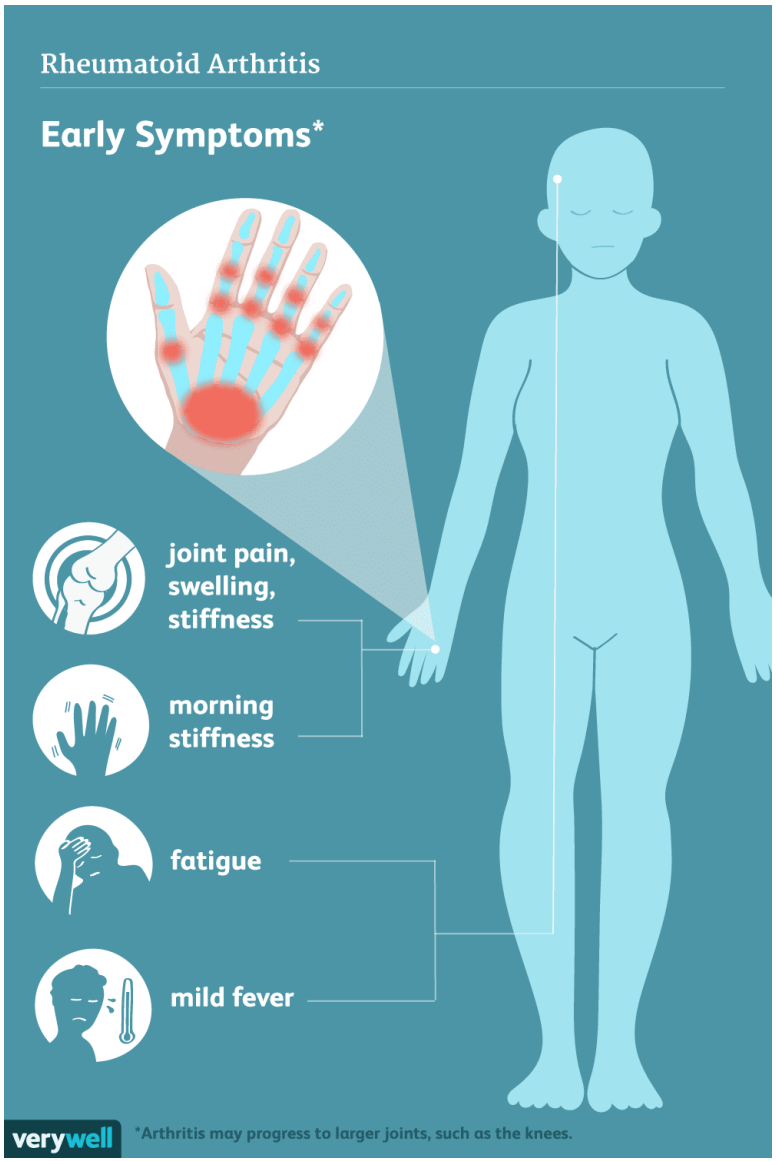
Citrullination & Citrullinated Peptides

Anti-CP antibodies (ACPA's)

Potential therapeutics

What is Rheumatoid Arthritis?

symptoms & causes



Rheumatoid arthritis

- A chronic, inflammatory, autoimmune disease
- Affects joints in hand, wrists, knee
- Common symptoms: swollen joints, weight loss, fatigue
- Specific causes unknown

risk factors

genetics **smoking** **age**

sex **obesity**

Deane, K.D.; *et al. Arthritis & Rheumatology* **2021,** 73, 2, 181.

Bullock, J.; et. al.; *Med Princ Pract.* **2019,** 27, 6, 501.

What is Rheumatoid Arthritis?

symptoms & causes

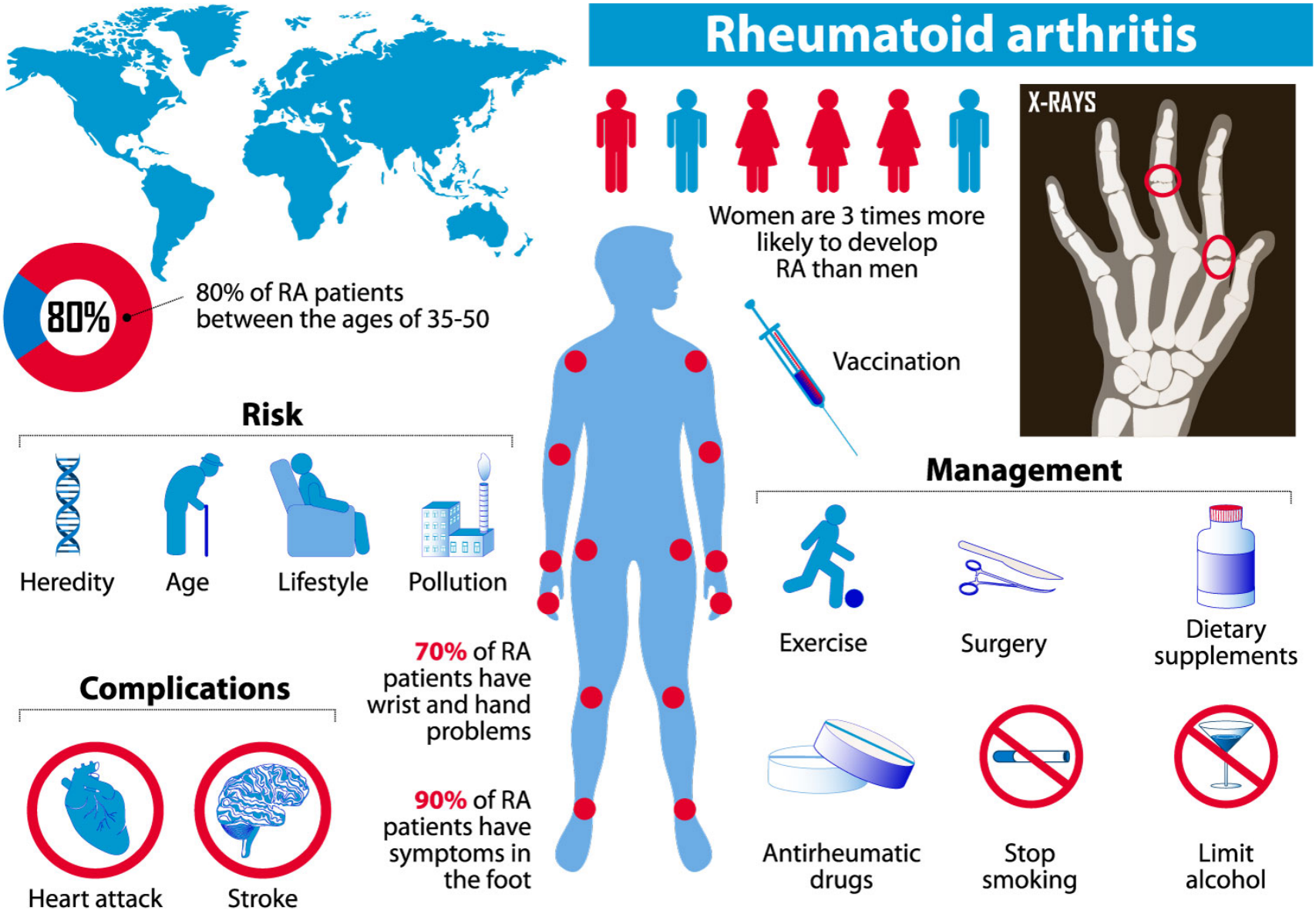
eventually leads to cartilage damage and bone erosion

also affects internal organs: skin, eyes, heart, etc.

Bullock, J.; et. al.; *Med Princ Pract.* **2019,** 27, 6, 501.

What is Rheumatoid Arthritis?

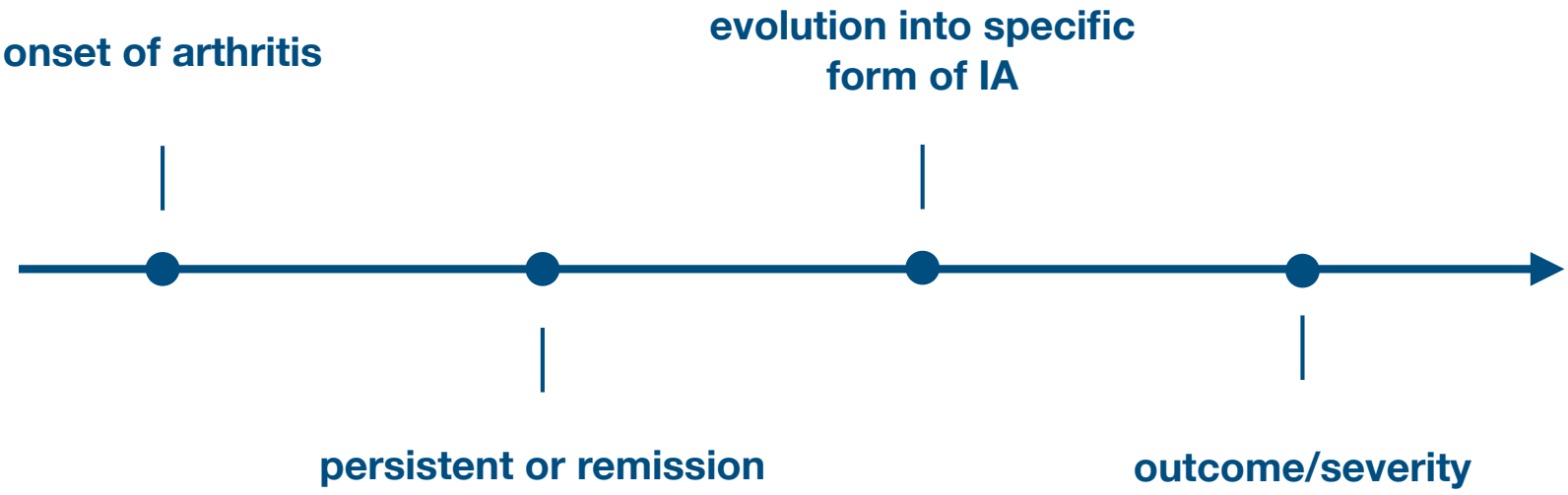
symptoms & causes



Arthritis Research | Arthritis National Research Foundation, 7 Sept. 2019.

What is Rheumatoid Arthritis?

diagnosis



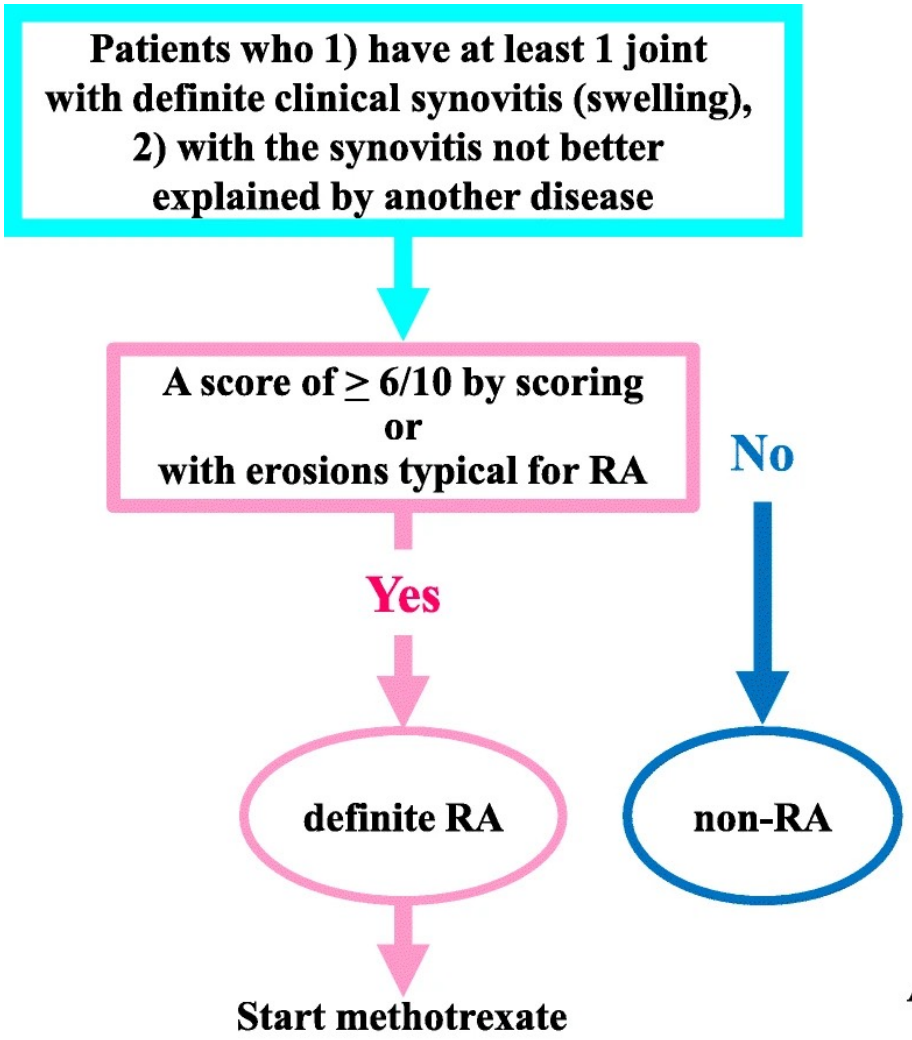
- **May proceed rapidly, or take months/years**
- **Earlier treatment prevents development of RA**
- **"*Early*" stage of RA not defined or universally agreed**

Heidar, B. *Caspian J. Intern. Med.* **2011** 2, 1, 161.

What is Rheumatoid Arthritis?

diagnosis

2010 American College of Rheumatology/Eroean League against Rheumatism



	score
Joint involvement	
=1 large joint	0
>1 large joint	1
1-3 small joints	2
4-10 small joints	3
>10 joints (small joint≧1)	5
Serology	
Negative RF and ACPA	0
Low positive RF or ACPA	2
High-positive RF or ACPA	3
Duration of symptoms	
<6 weeks	0
≧6 weeks	1
acute-phase reactants	
Normal CRP and ESR	0
Abnormal CRP or ESR	1

A score of ≥ 6/10 is needed for classification of a patient as having definite RA

Tanaka, Y. *Caspian Inflamm Regener* **2020** 40, 20.

What is Rheumatoid Arthritis?

Treatment

First line: relieve pain and decrease inflammation

Nonsteroidal anti-inflammatory drugs (NSAIDs)

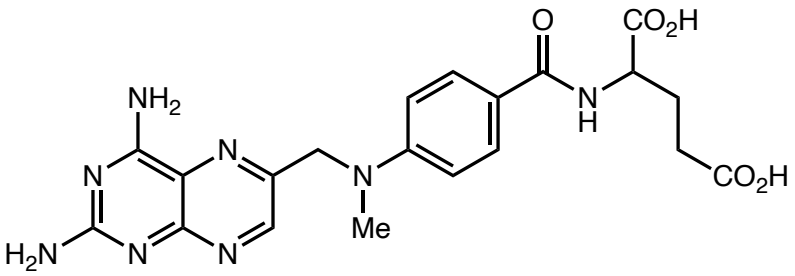
Steroids

Bullock, J. *et al Med Princ Pract.* **2019**, 27, 6, 501.

What is Rheumatoid Arthritis?

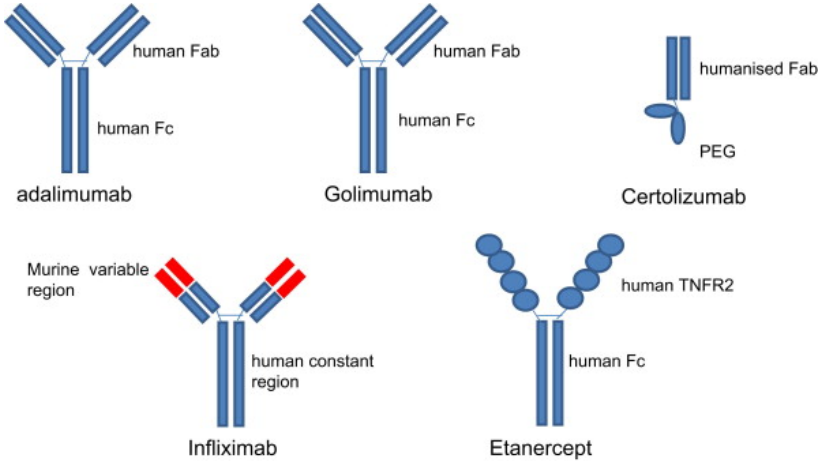
Treatment

Second line: promote remission



Methotrexate

Disease-modifying anti-rheumatic drugs (DMARDs)



TNFα inhibitor

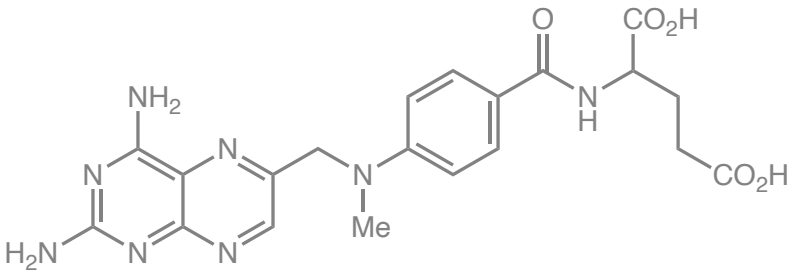
B-cell depletion and inhibition antibodies

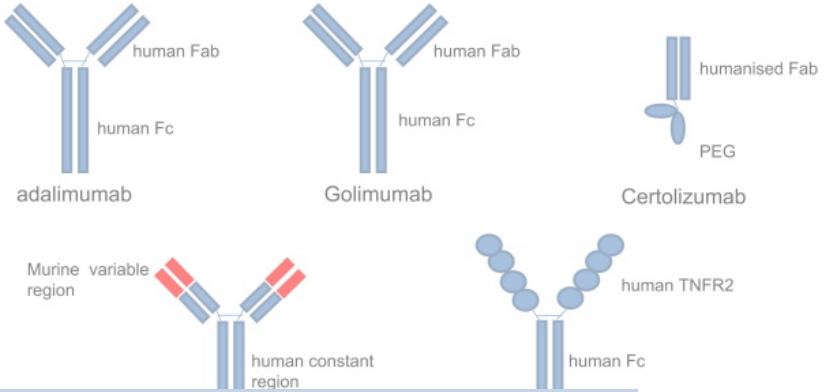
Gun, Q.; *et al Bone Res.* **2018**, 6, 15.

What is Rheumatoid Arthritis?

Treatment

Second line: promote remission





No cure for rheumatoid arthritis so far

Disease-modifying anti-rheumatic drugs (DMARDs)

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Outline

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Potential Therapeutic Targets

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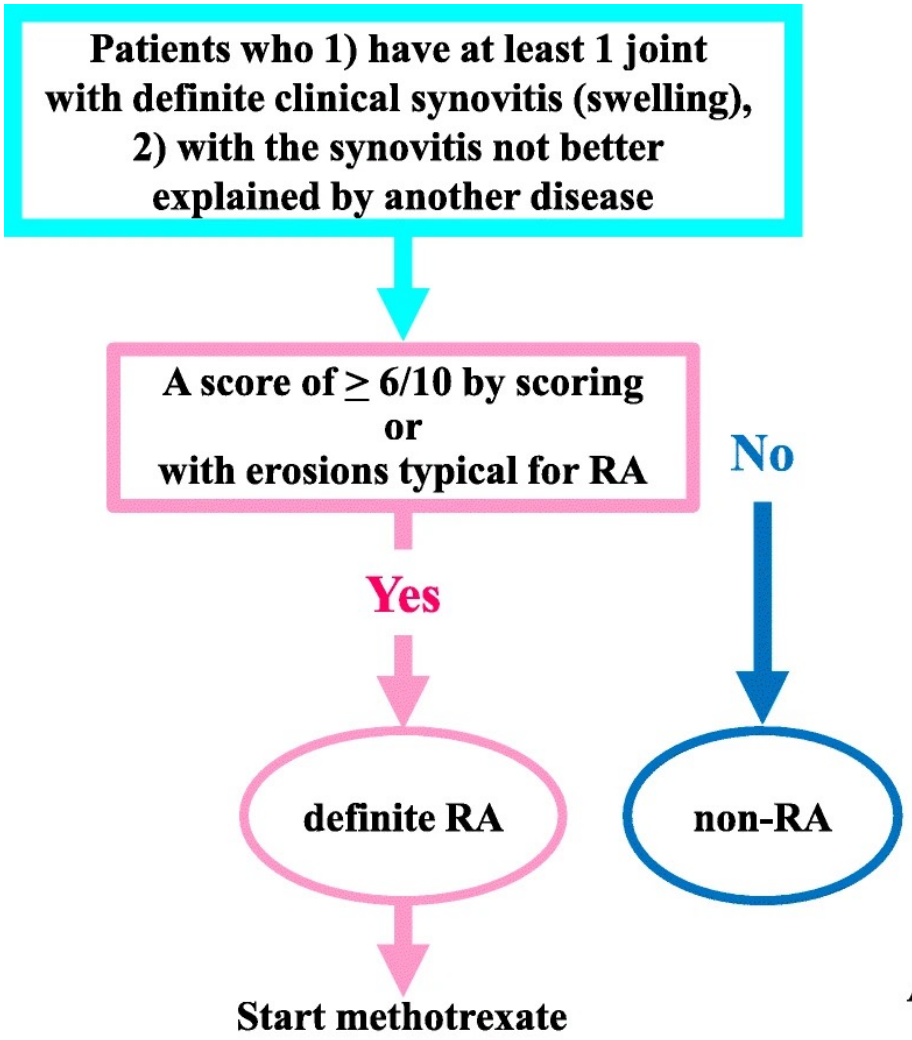

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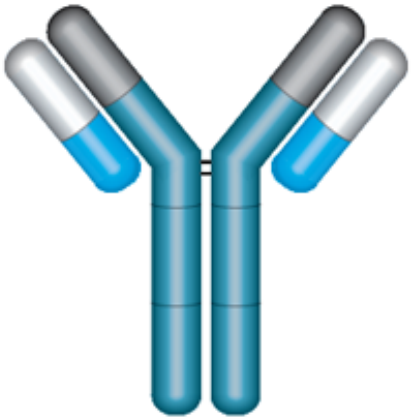
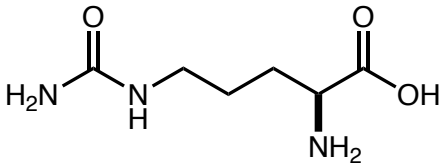
Tanaka, Y. *Caspian Inflamm Regener* **2020** 40, 20.

Citrullination and Citrullinated Peptides

anti-citrullinated peptide/protein antibodies (ACPA)





citrulline

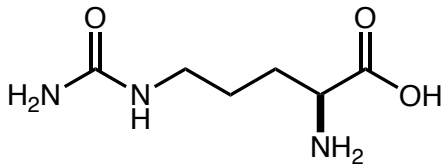
- ***95~98% specificity to RA patients***
- ***precede onset of clinical symptoms by years***
- ***related to more severe and erosive phenotype***

Wu, C.; *et al. Int. J. Mol. Sci* **2020**, 21, 4015

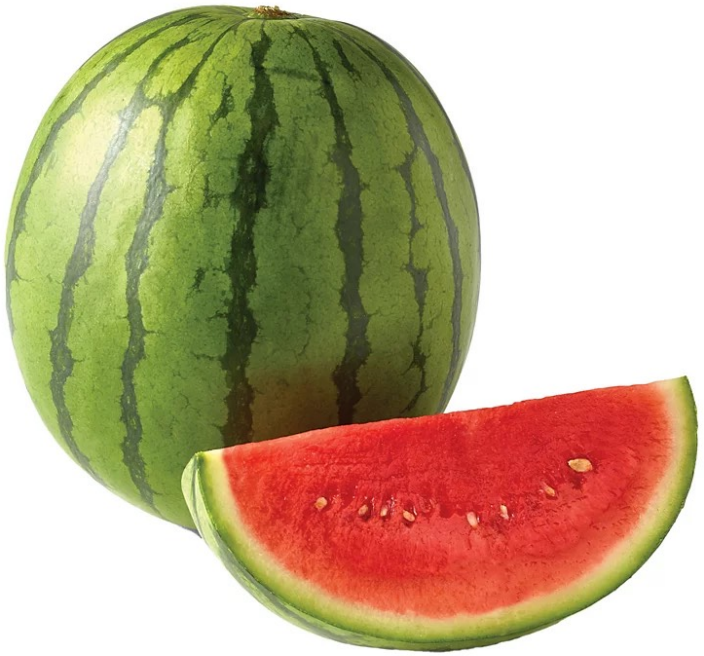
Baker, J.F. "Diagnosis and Differential Diagnosis of Rheumatoid Arthritis." *UpToDate*, 14 Oct. 2021.

Citrullination and Citrullinated Peptides

history



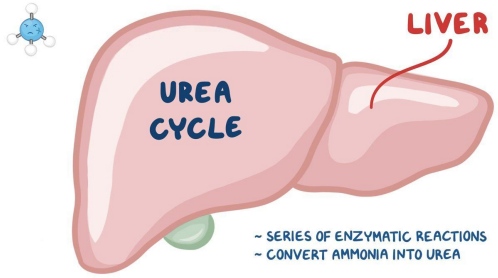
citrulline

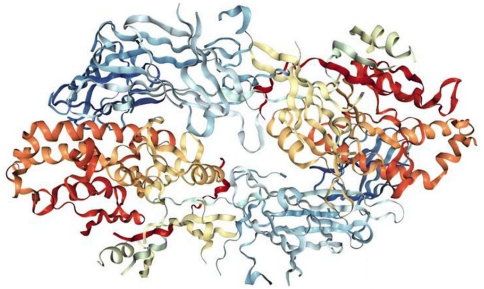


Koga, Y.; Ohtake, R. *J. Tokyo. Chem. Soc.* **1914**, 35, 519.

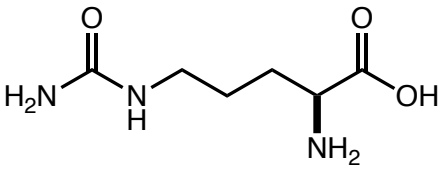
Citrullination and Citrullinated Peptides

history





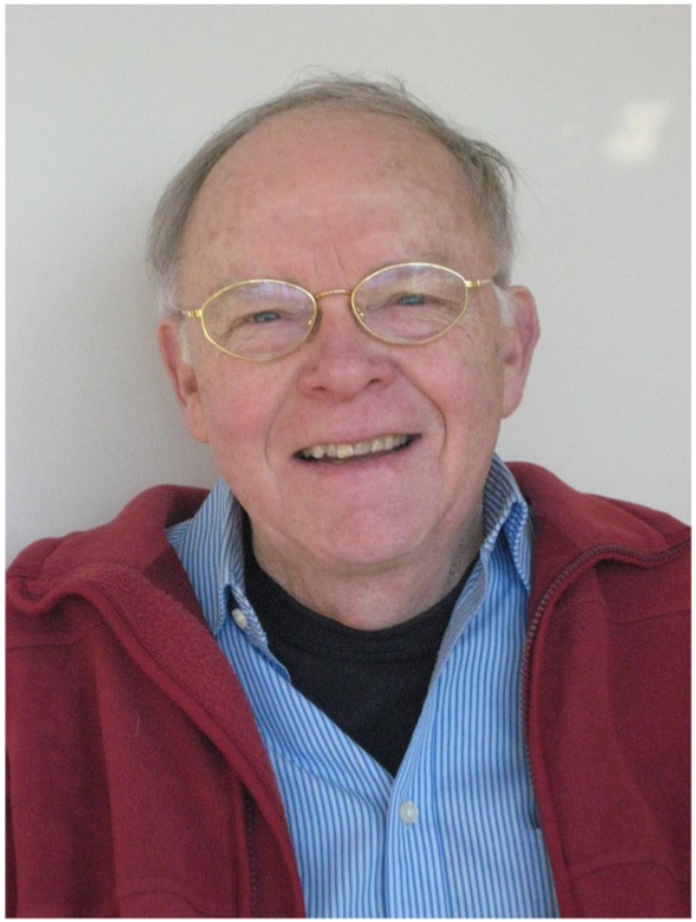
1914 ***1930*** ***1958*** ***1981*** ***1998***



Van Venrooij, W.J.; *et al. J. Clin. Invest.* **1998**, 101, 1, 273.

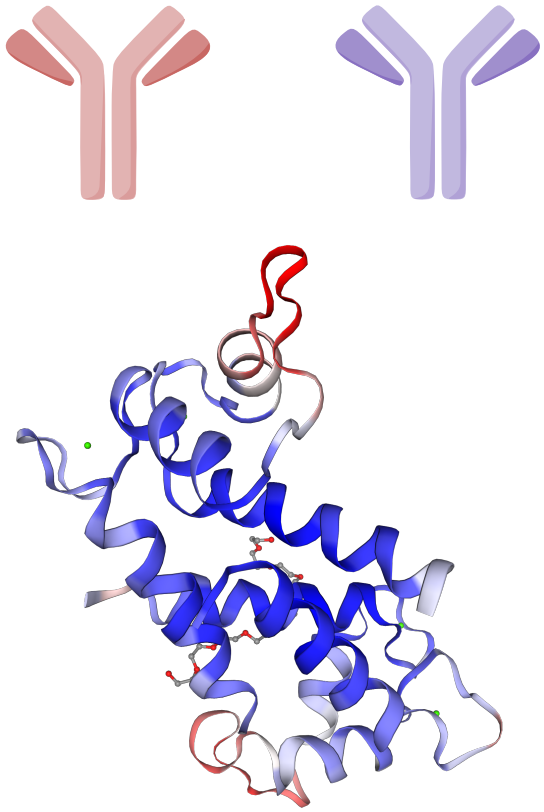
Citrullination and Citrullinated Peptides

citrullinated peptides & RA



Prof. Walther J. Van Venrooij

APF **AKA**



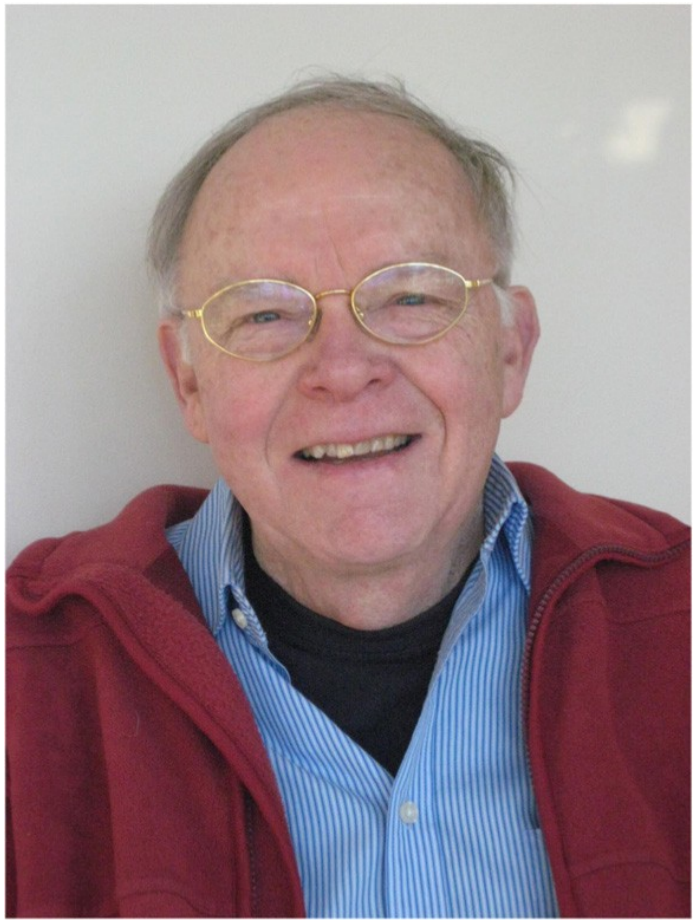
Filaggrin

Swiss-Model: 4PCW

Van Venrooij, W.J.; *et al. J. Clin. Invest.* **1998**, 101, 1, 273.

Citrullination and Citrullinated Peptides

citrullinated peptides & RA



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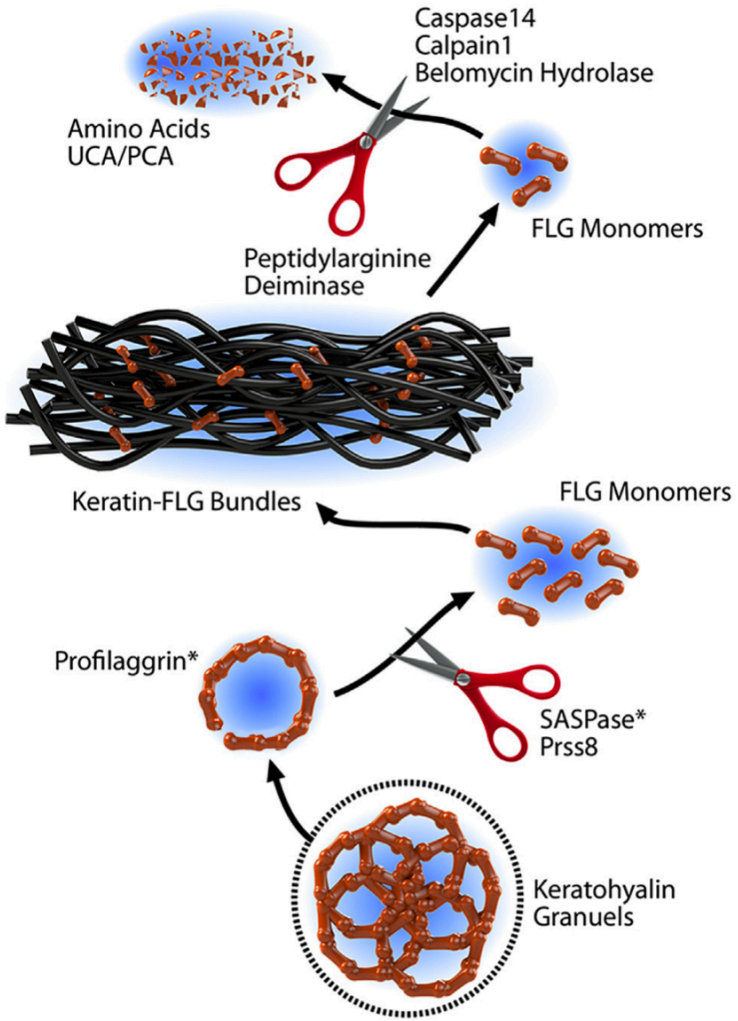
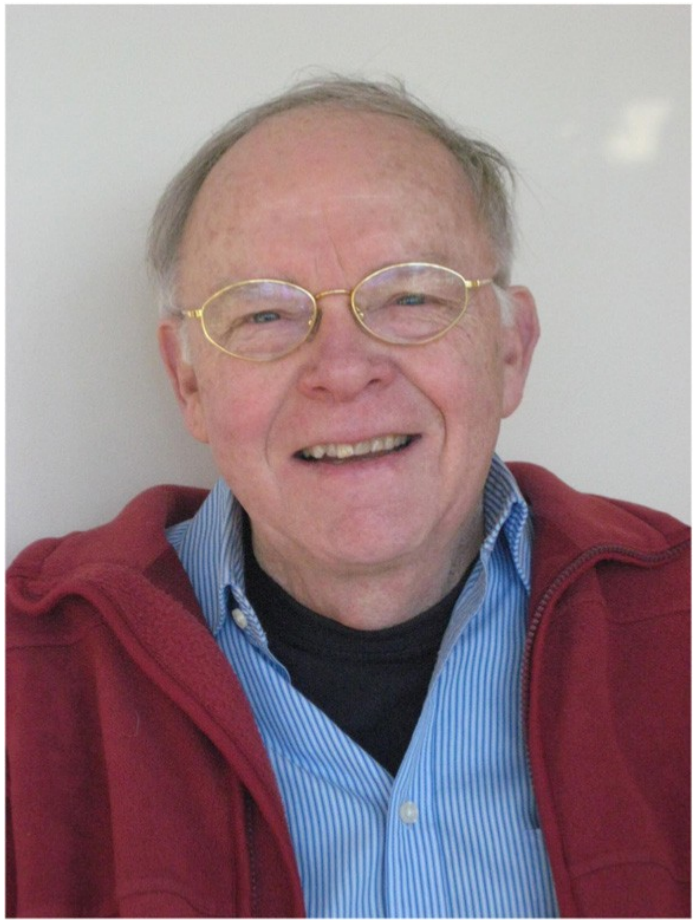


Egawa, G.; *et al. J. Allergy Clin. Immunol.* ***2016***, 138, 2, 350.

Van Venrooij, W.J.; *et al. J. Clin. Invest.* **1998**, 101, 1, 273.

Citrullination and Citrullinated Peptides

citrullinated peptides & RA



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Citrullination and Citrullinated Peptides

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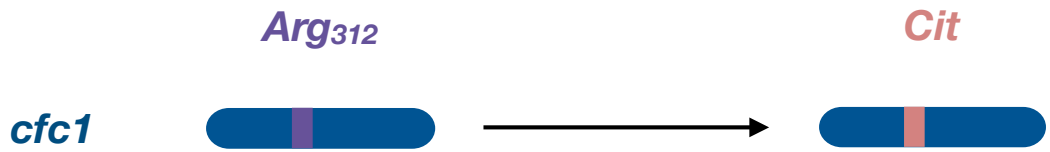
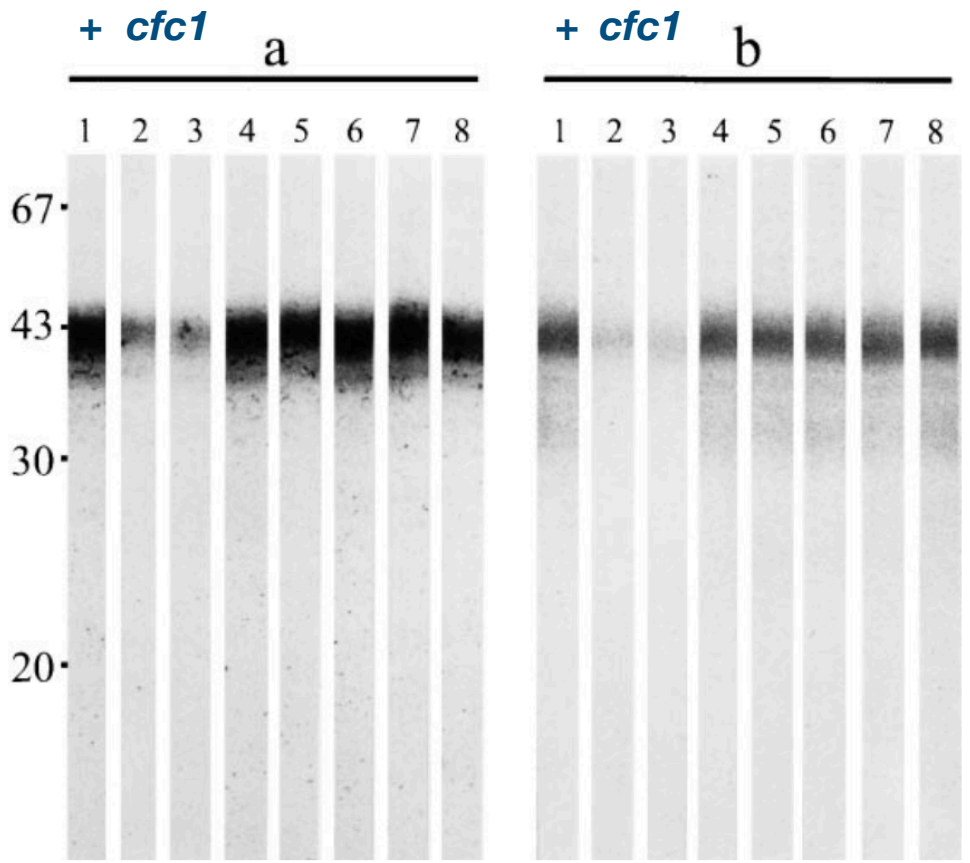


Collected sera from 288 patients
134 RA patients, 154 controls

Blot contains fliggarin

Citrullinated peptides competitively binds to IgG over fliggarin



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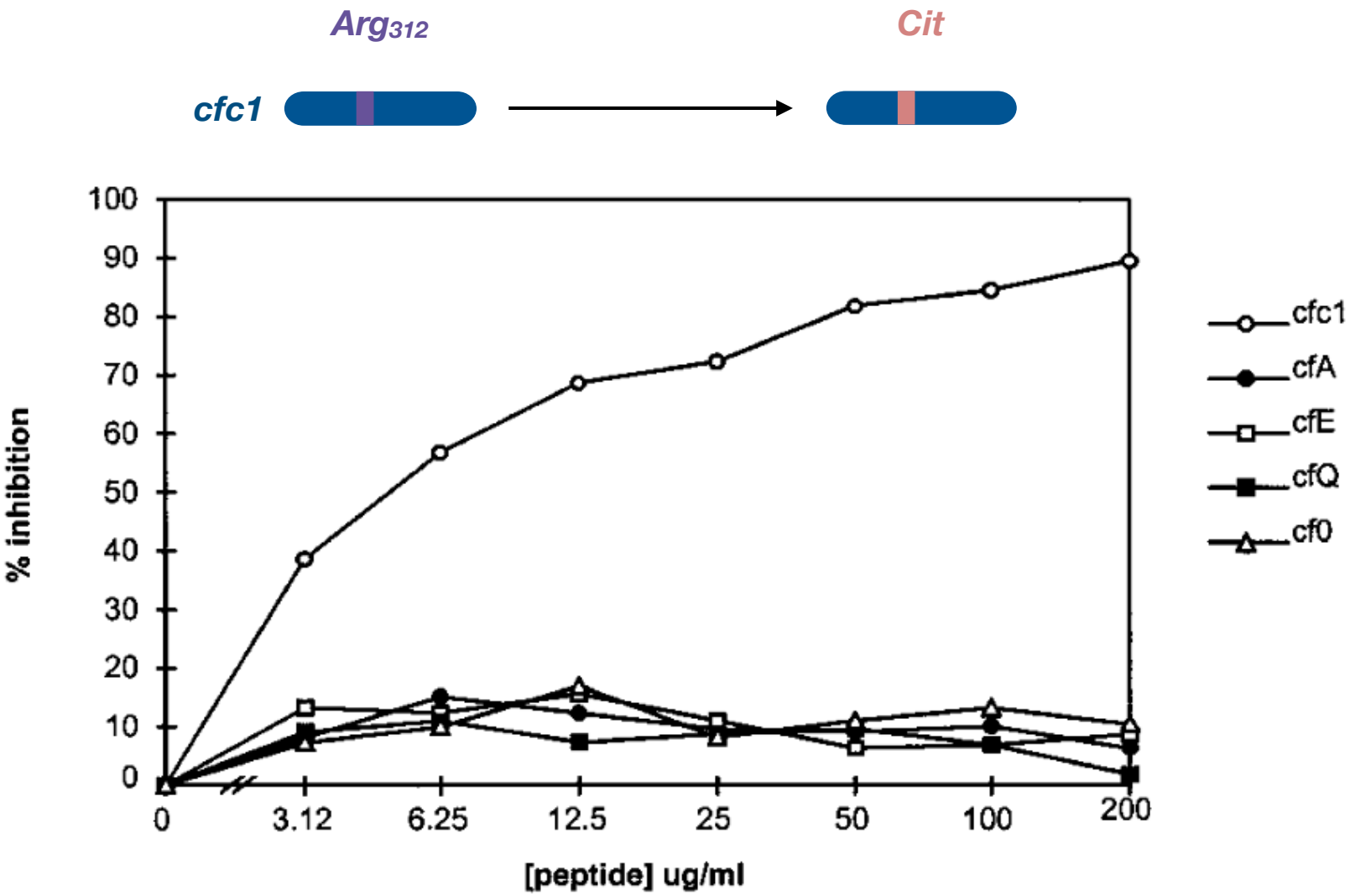
Citrullination and Citrullinated Peptides

citrullinated peptides & RA

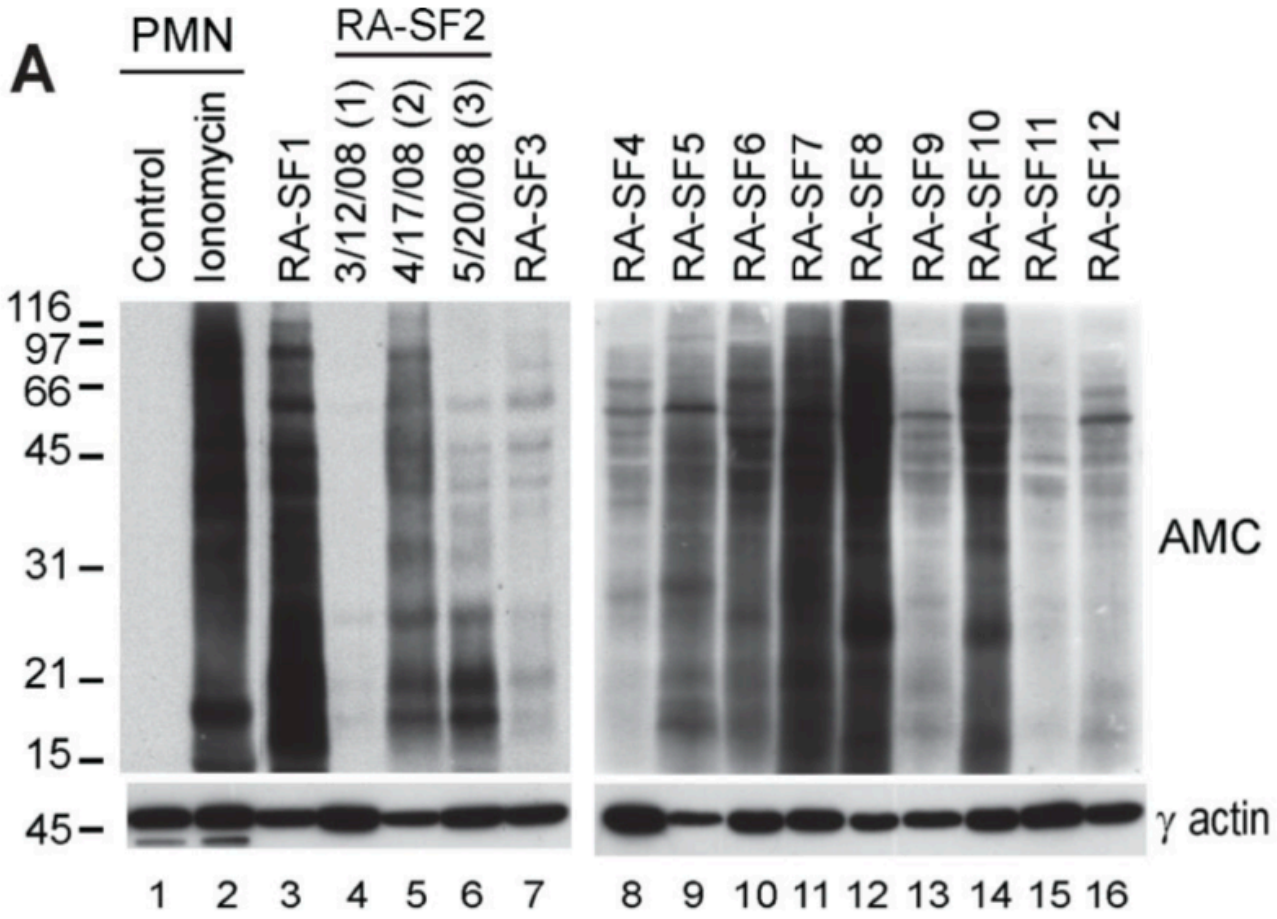


Is fliggarin the only CP associated with RA?

Van Venrooij, W.J.; *et al. J. Clin. Invest.* **1998**, 101, 1, 273.

Citrullination and Citrullinated Peptides

hypercitrullination in RA patients



Protein citrullination probed across 12 RA patients synovial tissues and SF

Hypercitrullination seen across almost all

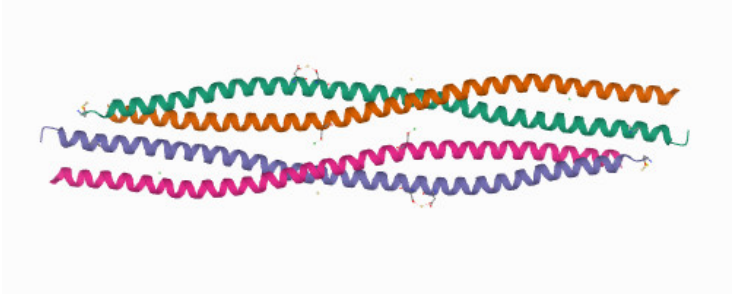
Romero, V.; *et al. Sci Transl Med.* **2013**, 5, 209, 209ra150.

Citrullination and Citrullinated Peptides

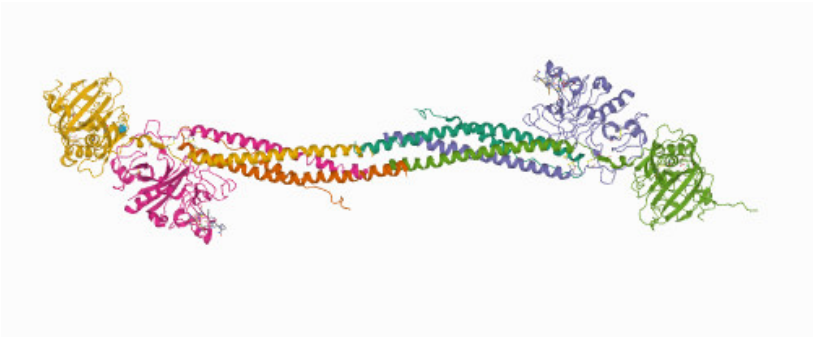
scope of citrullinated proteins

>100 citrullinated proteins identified

Keratin



Fibrinogen



Collagen

How does hypercitrullination occur?

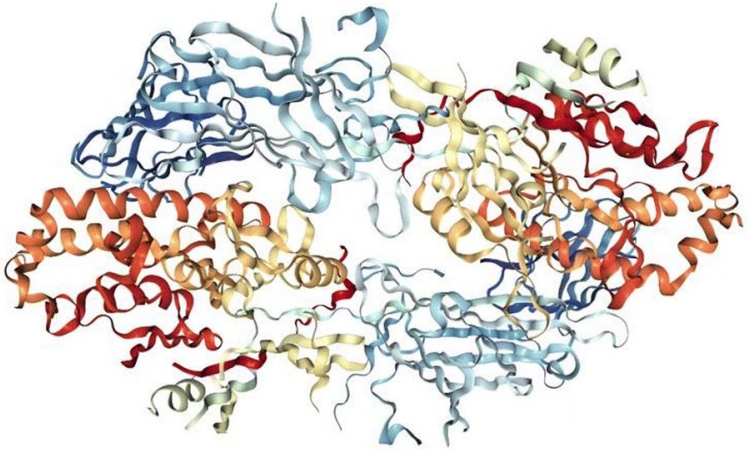
RCSB PDB: 6EC0; 1LWU; 5CVA

Darrah, E.; *et al*. *Curt Opin Rheumatol.* **2018** 30, 1, 72.

Citrullination and Citrullinated Peptides

Citrullination by PAD

Peptidylarginine deiminase (PAD)



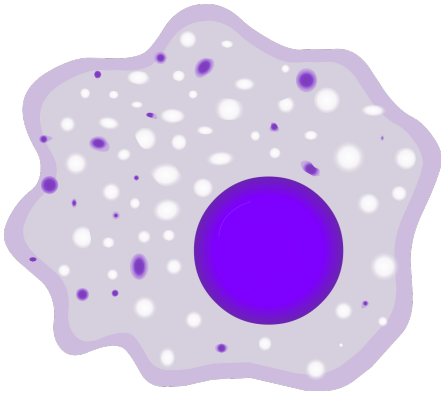
Alghamdi, M.; *et al. Cell. Mol. Life Sci.* ***2019***, 76, 4635.

Citrullination and Citrullinated Peptides

Citrullination by PAD

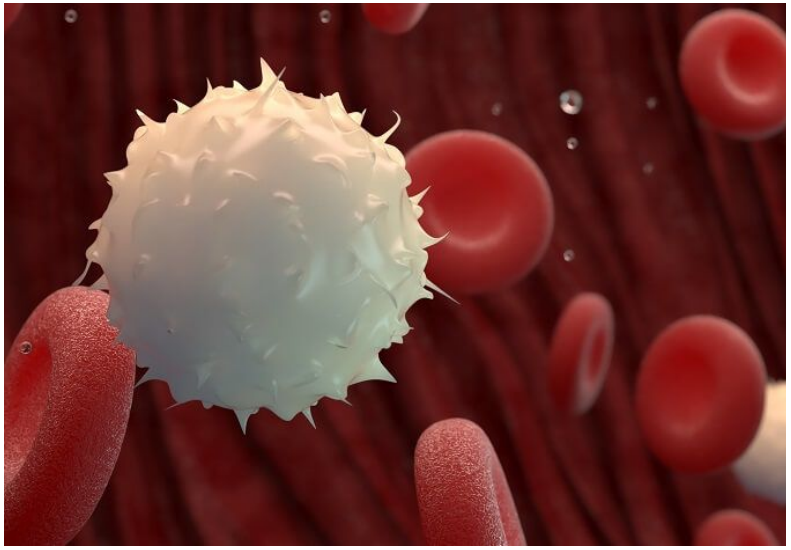
The PAD Family

PAD2



- *expressed in: skeletal muscles, brain, spleen, secretory glands,* ***macrophages***
- *Regulated by Ca^{2+} and partially regulated by hormones*

PAD4/5



- *expressed in:* ***white blood cells***
- *resides in cell nucleus*
- *citrullinates histones*

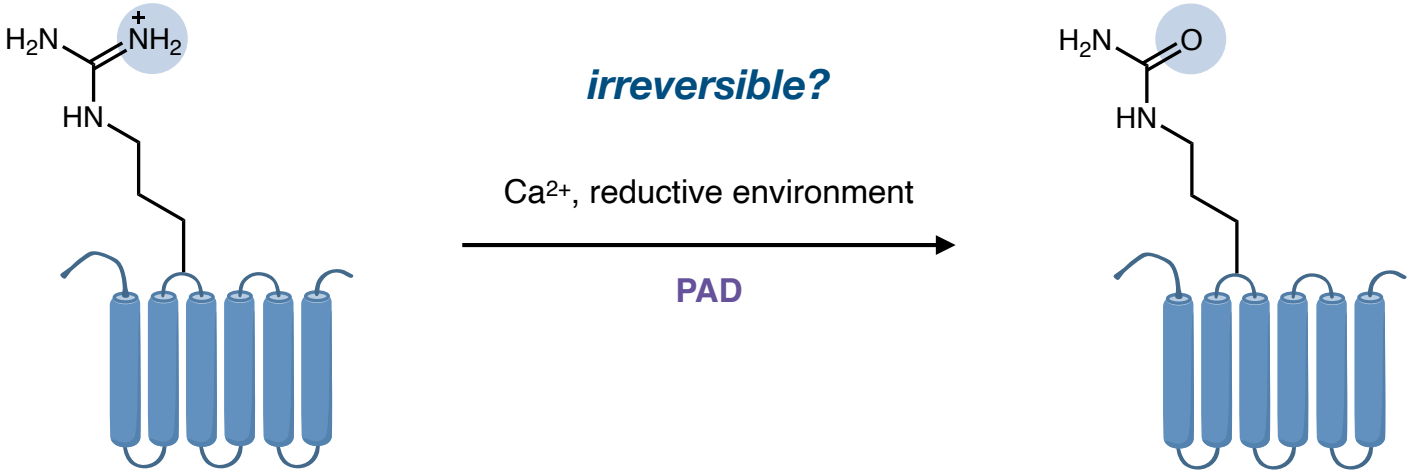
Vossenaar, E.R.; *et al. BioEssays* **2003**, 25, 11, 1106.

Citrullination and Citrullinated Peptides

Citrullination by PAD

positively charged

neutral



peptide arginine

peptide citrulline

Van Venrooij, W.J.; *et al*. *Ann Rheum Dis* **2004** 63, 373.

Citrullination and Citrullinated Peptides

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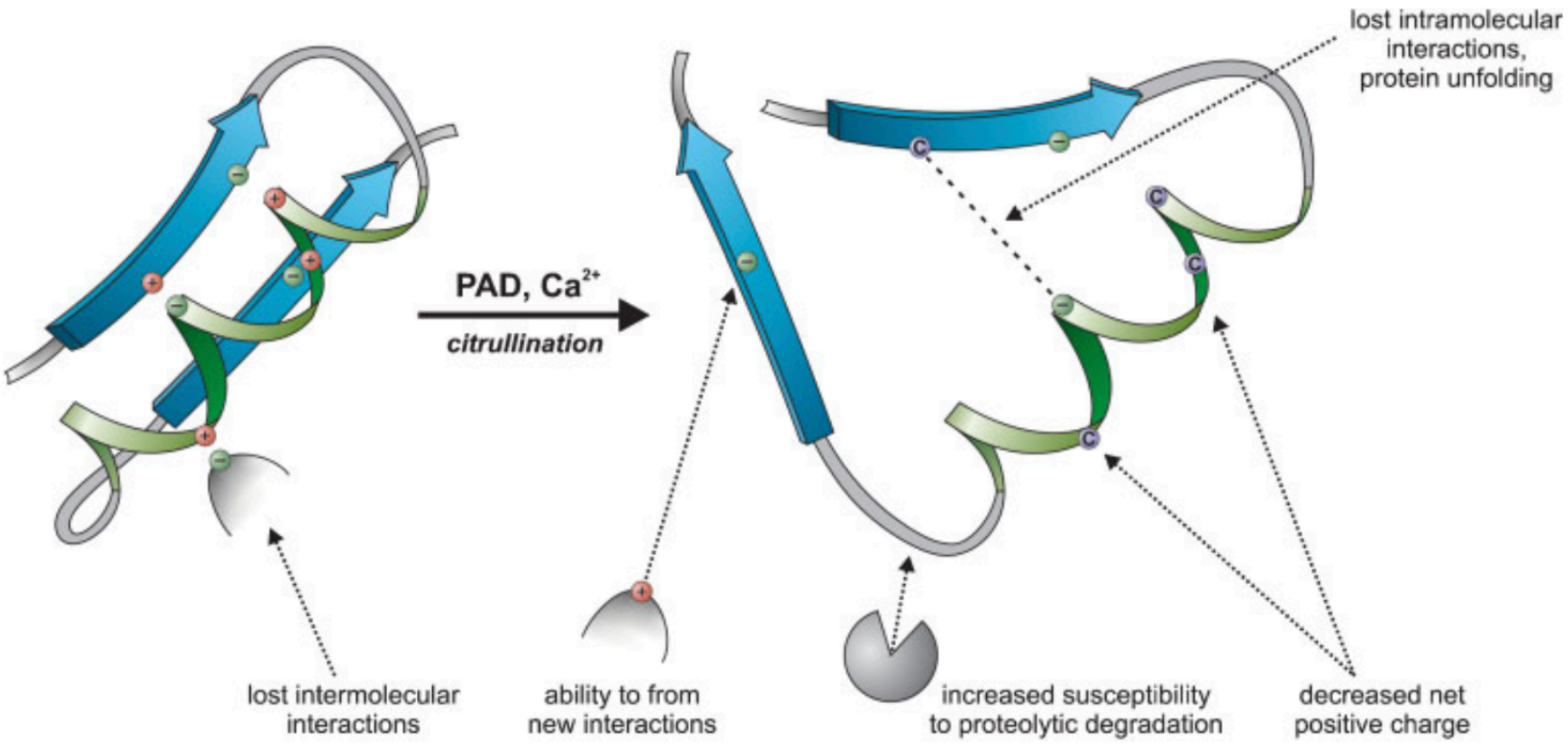

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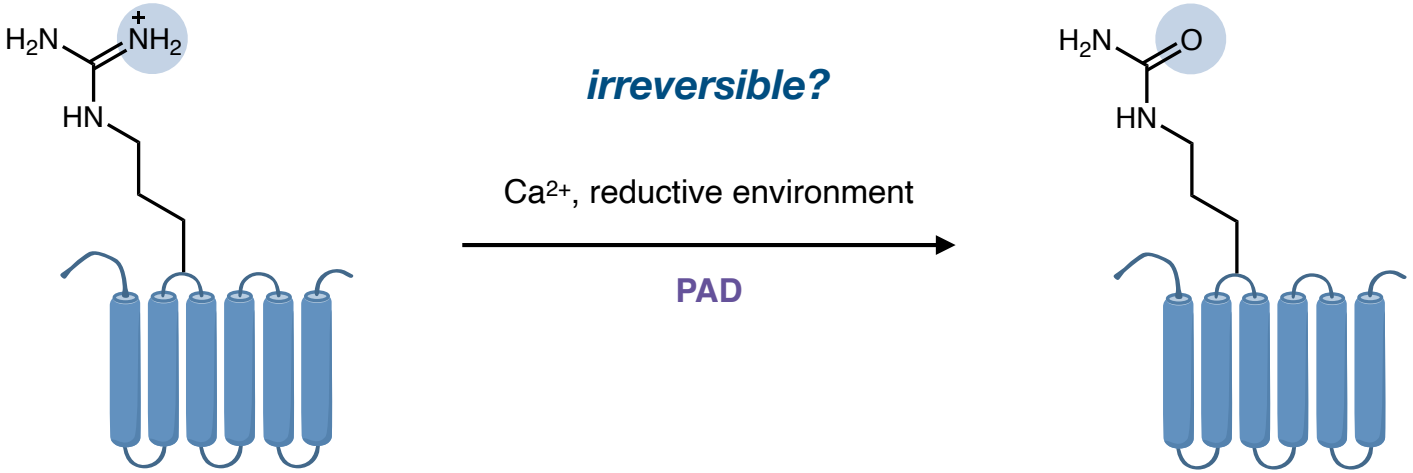

peptide arginine ***peptide citrulline***

PAD activity Ca^{2+} threshold: 10 μM

cytosolic Ca^{2+}: 0.1 μM

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Citrullination and Citrullinated Peptides

Citrullination by PAD

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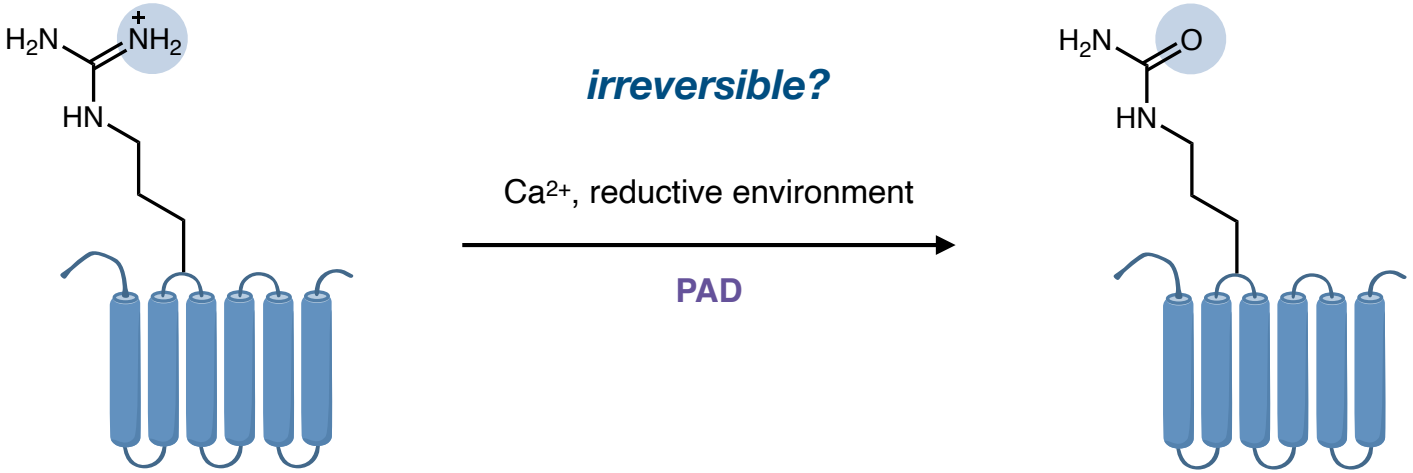

peptide arginine ***peptide citrulline***

How can PAD be activated?

Why do we find PAD extracellularly?

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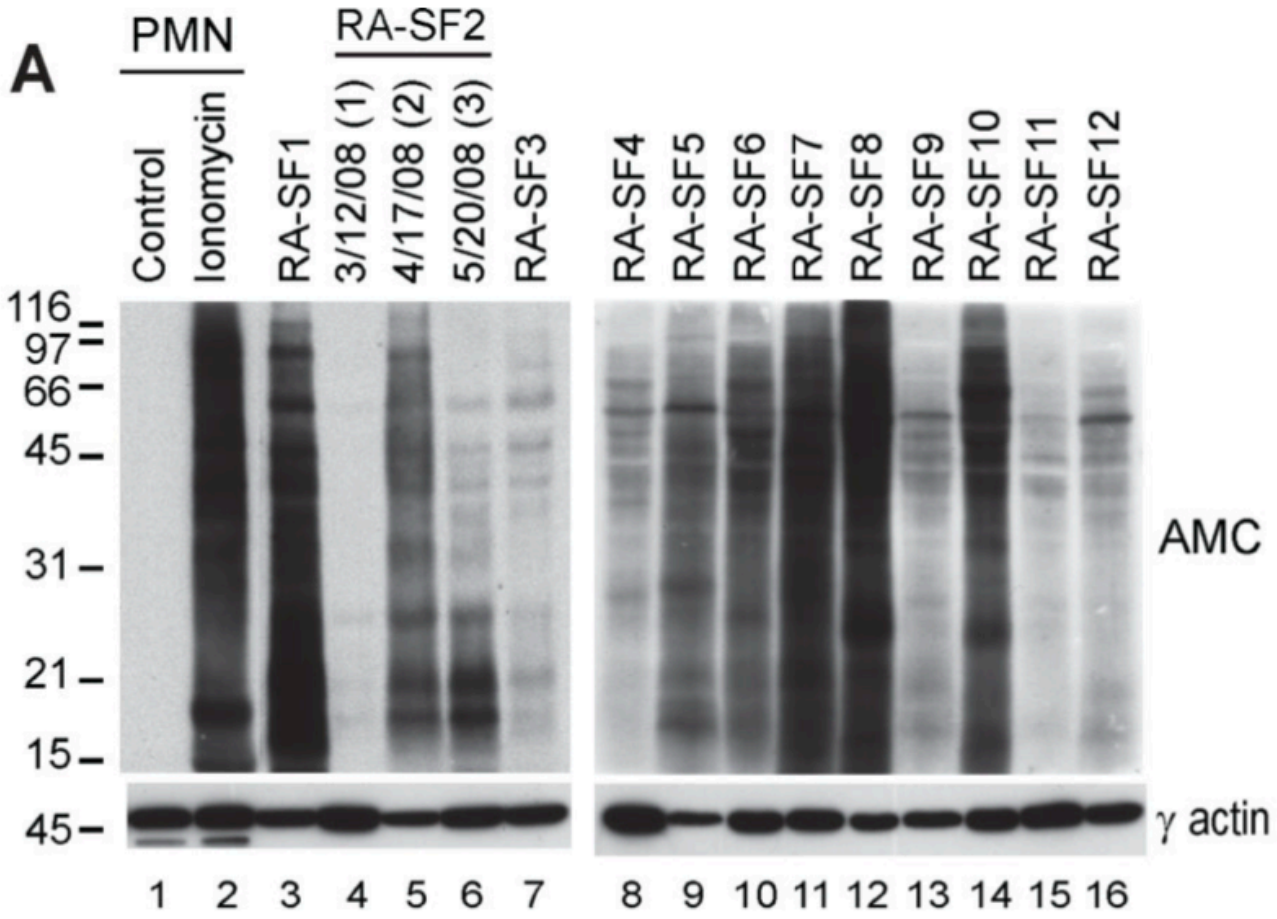
Citrullination and Citrullinated Peptides

hypercitrullination in RA patients



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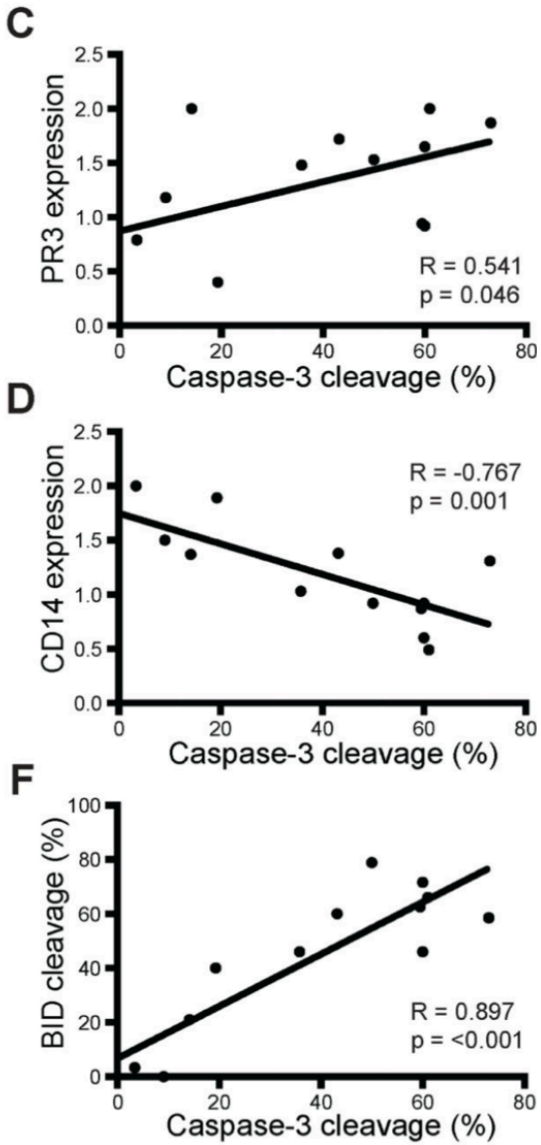

Caspase-3 cleavage: indication of apoptosis

PR3: neutrophil marker

CD14: monocyte marker

BID: marker for apoptosis activated through extracellular activation

Romero, V.; *et al. Sci Transl Med.* **2013**, 5, 209, 209ra150.

Citrullination and Citrullinated Peptides

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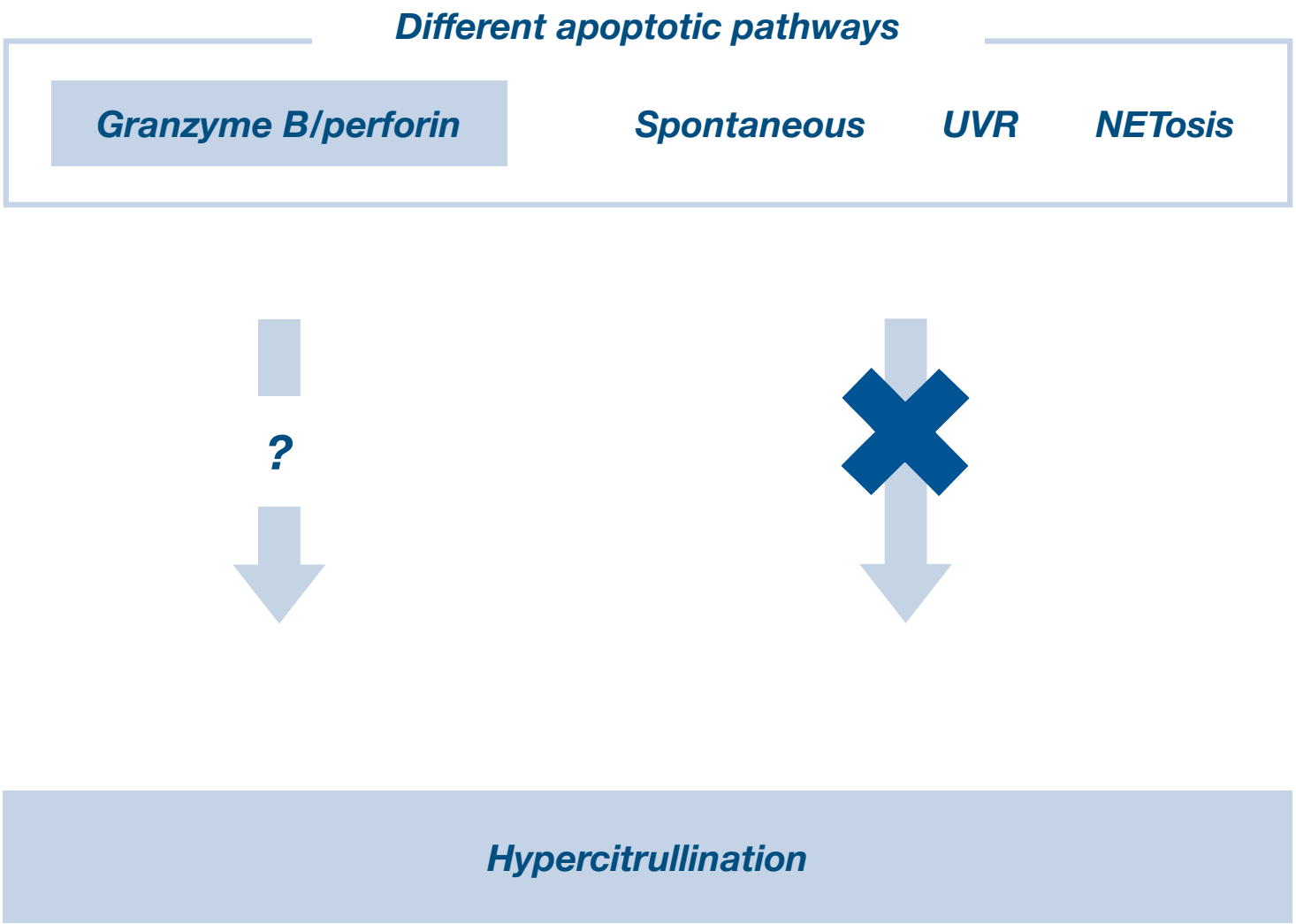

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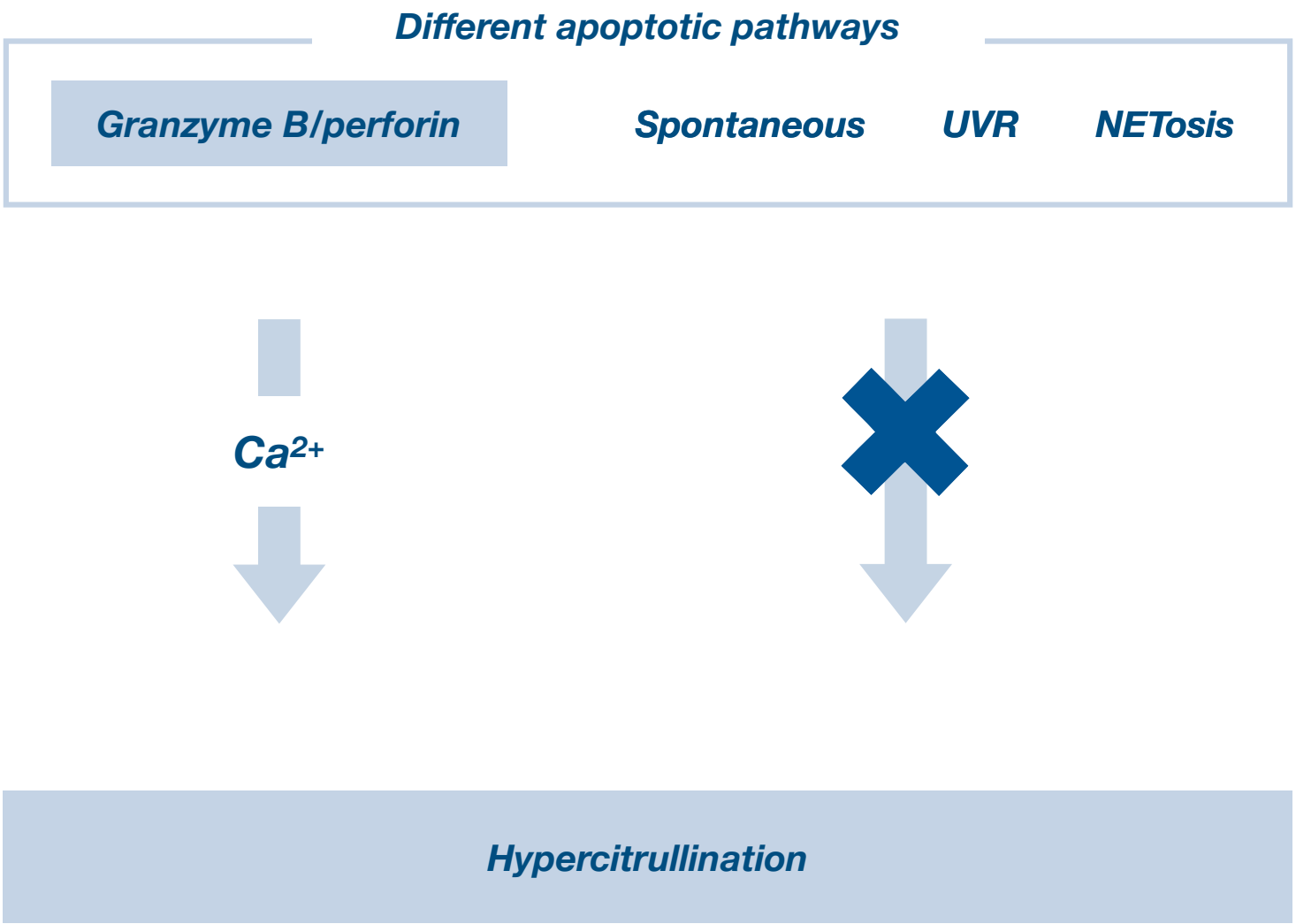

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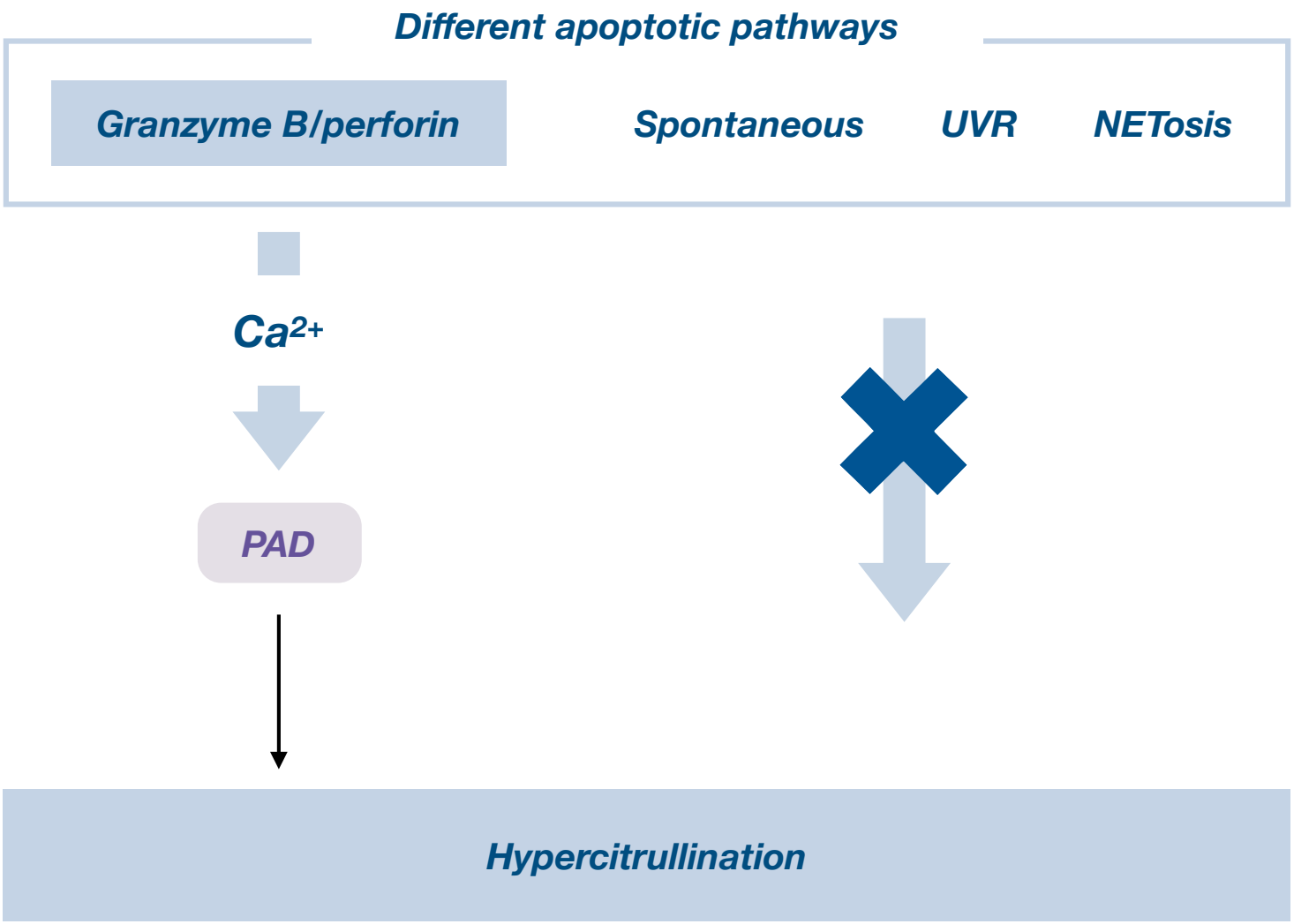

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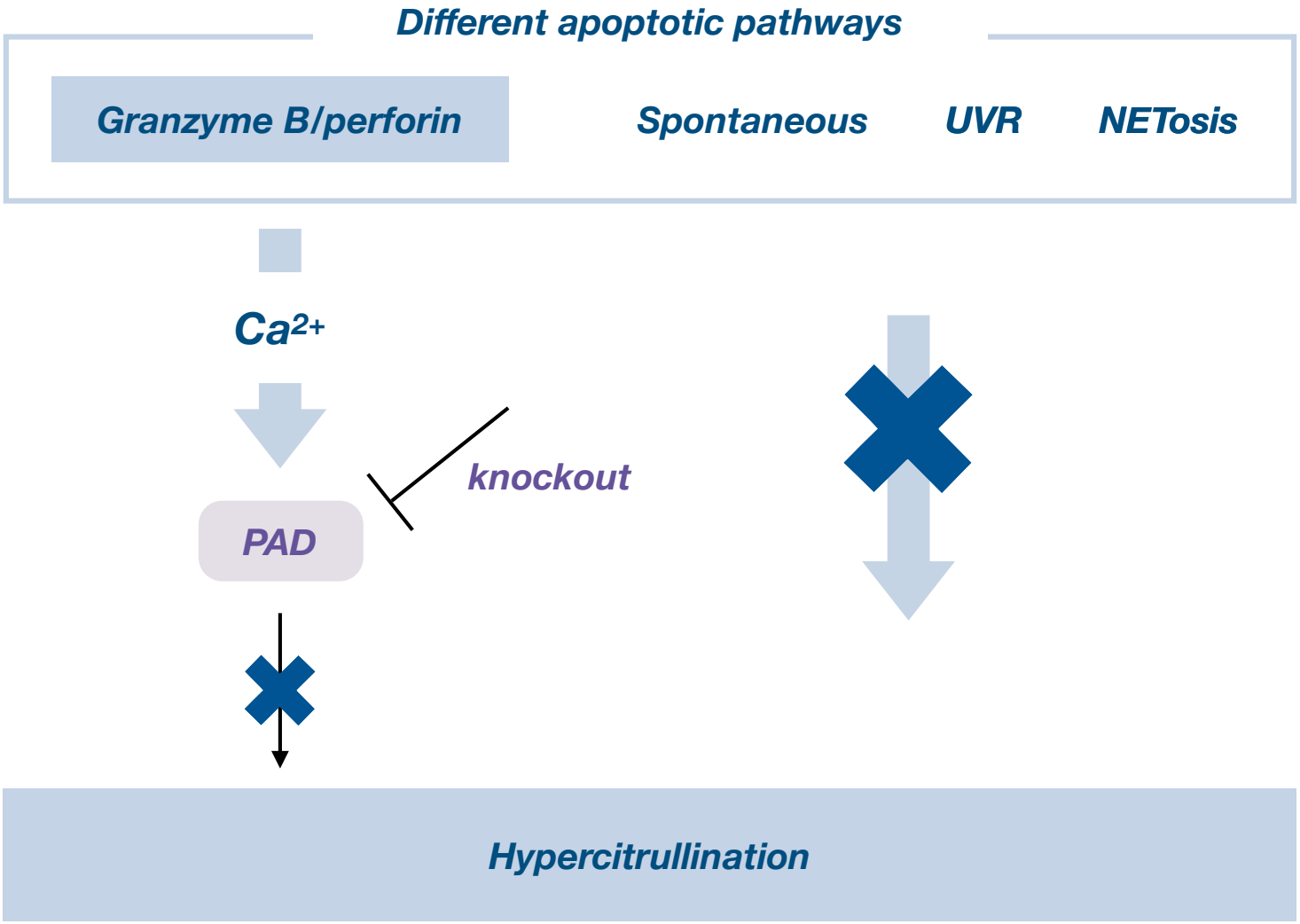

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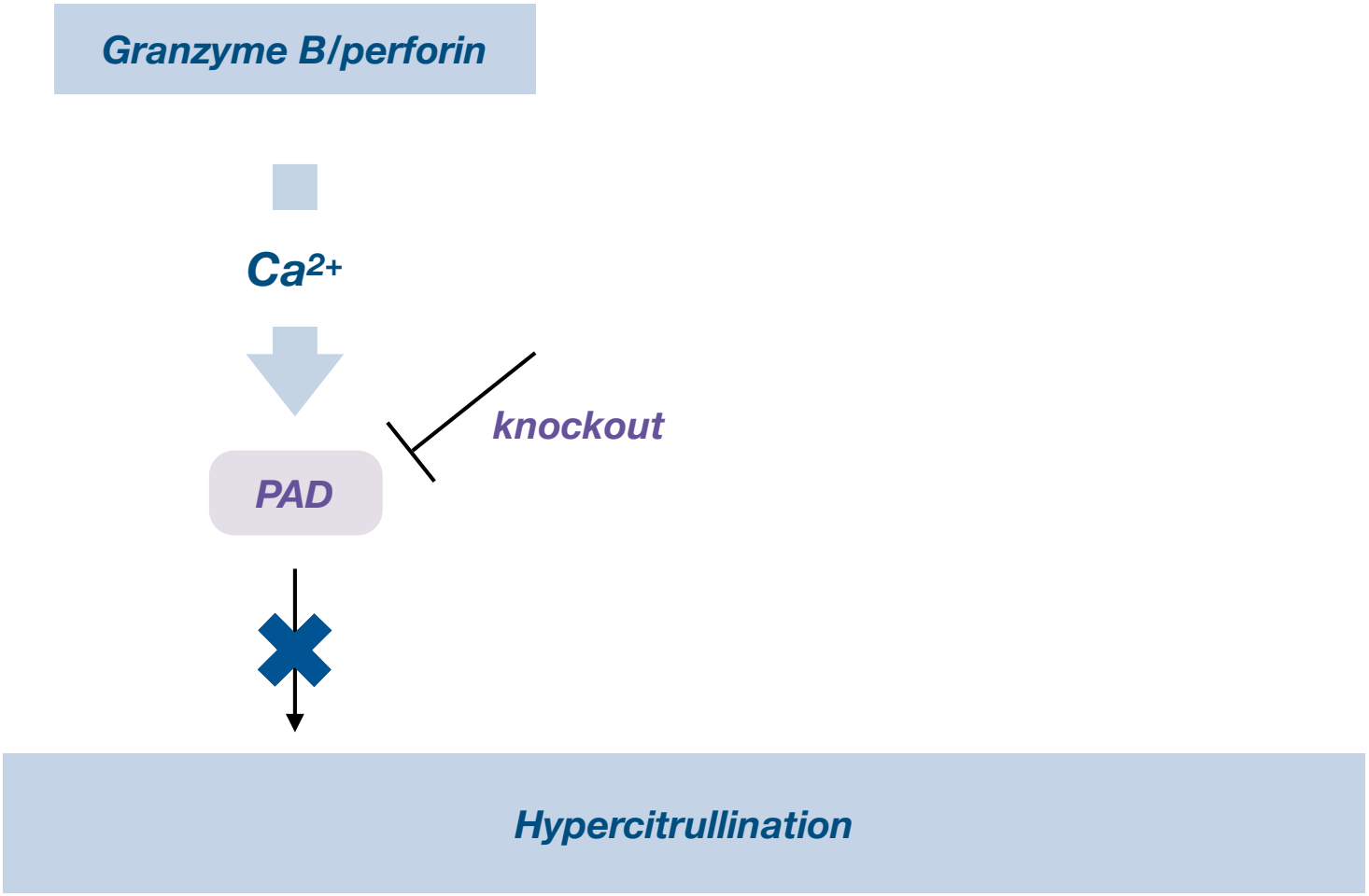
Citrullination and Citrullinated Peptides

Citrullination by PAD



Romero, V.; *et al. Sci Transl Med.* **2013**, 5, 209, 209ra150.

Citrullination and Citrullinated Peptides

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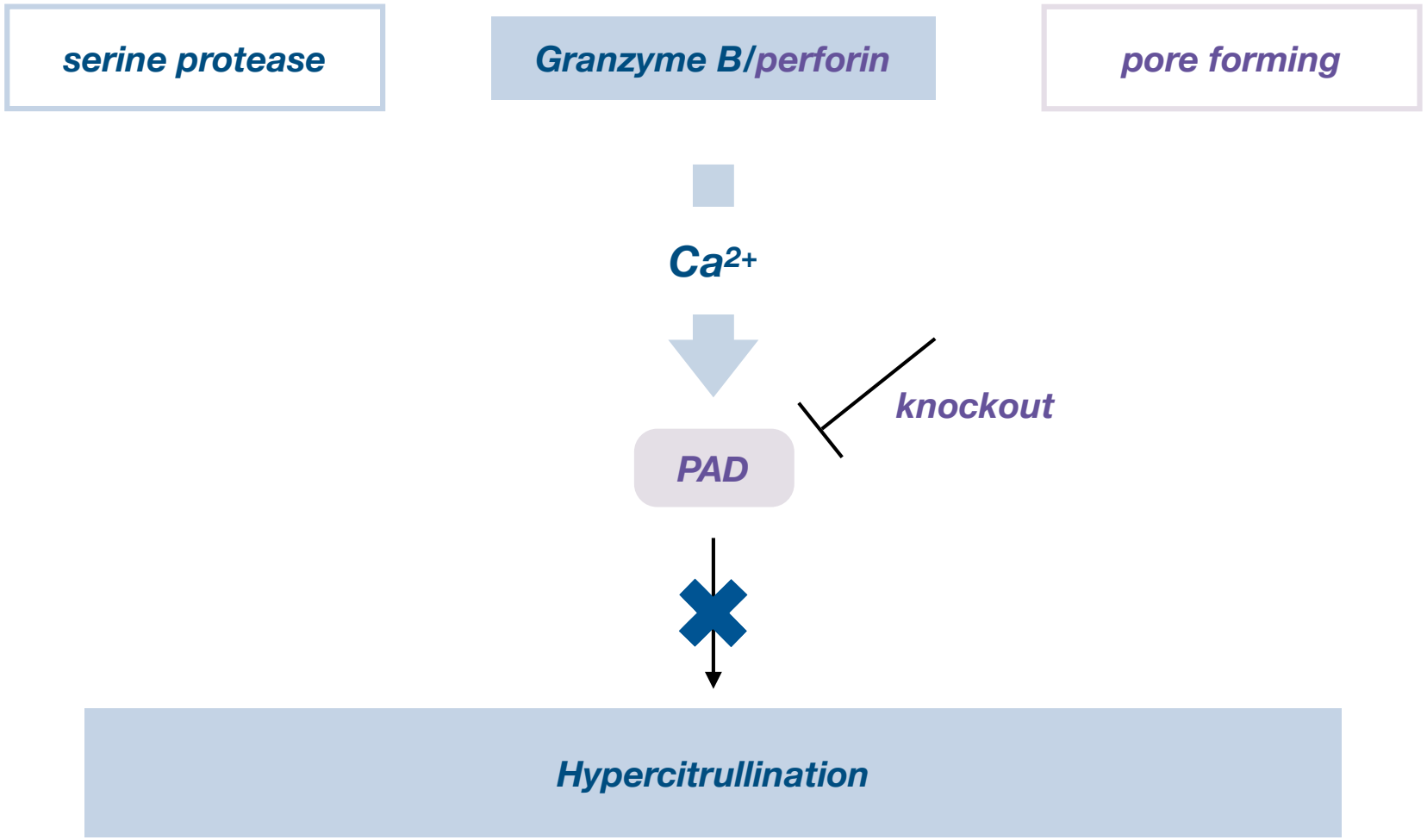
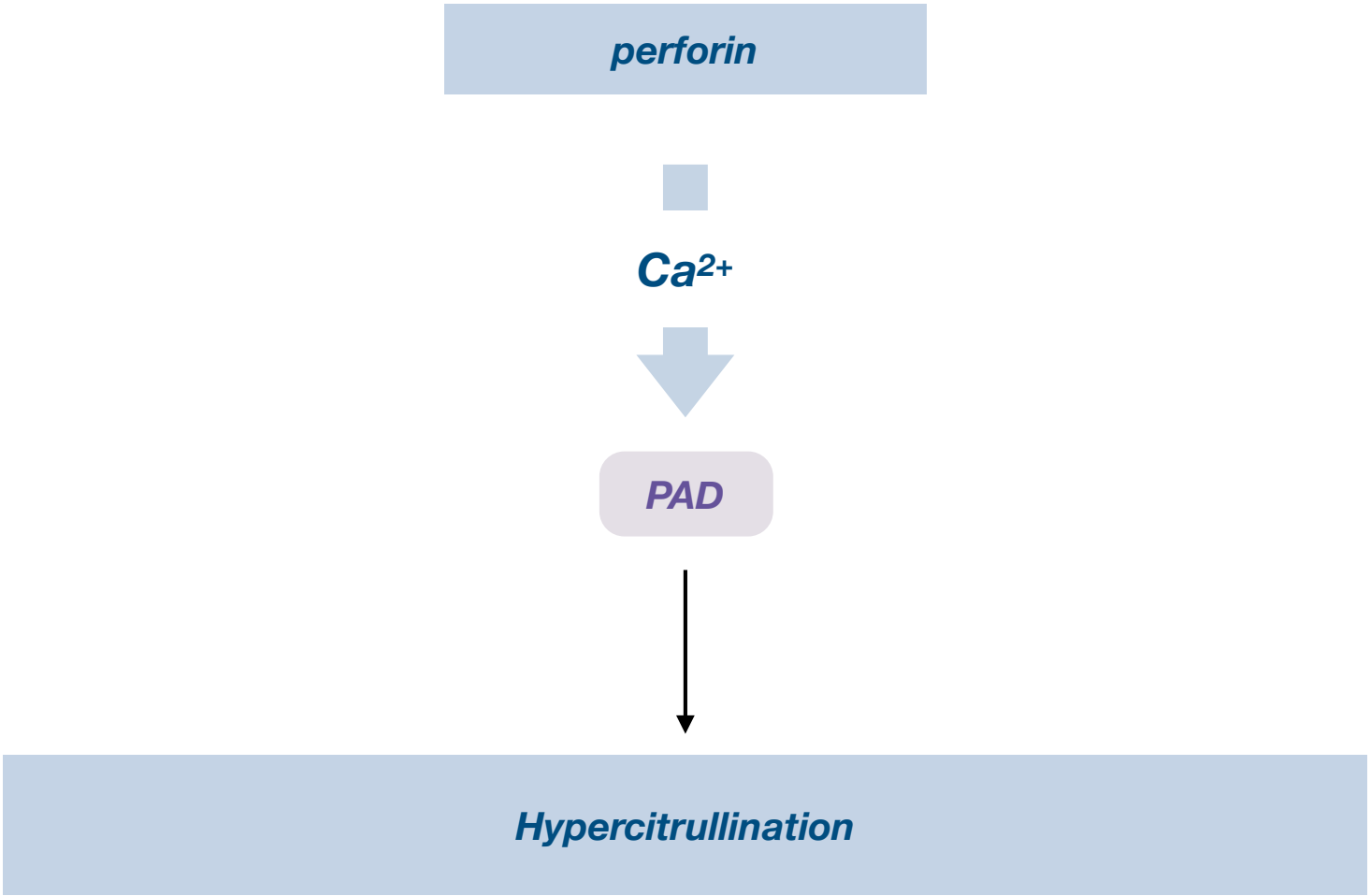


Romero, V.; *et al. Sci Transl Med.* **2013**, 5, 209, 209ra150.

Citrullination and Citrullinated Peptides

Citrullination by PAD

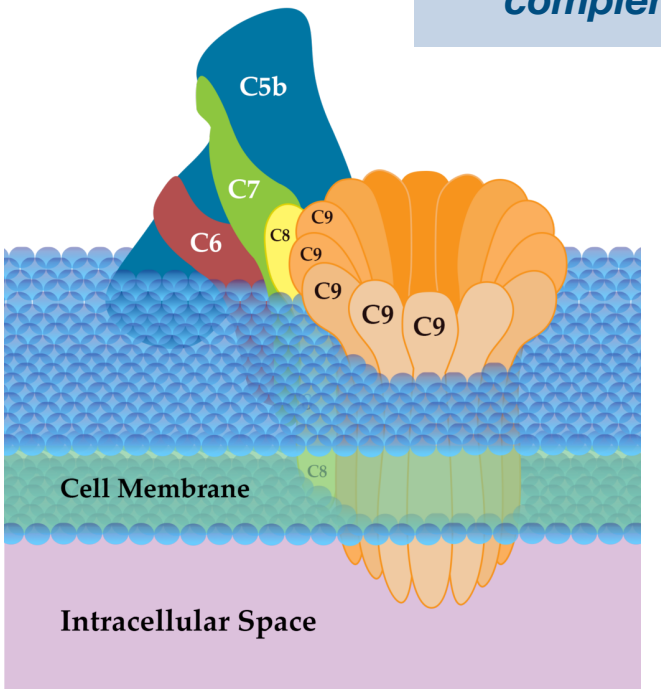


Romero, V.; *et al. Sci Transl Med.* **2013**, 5, 209, 209ra150.

Citrullination and Citrullinated Peptides

Citrullination by PAD

complement membrane attack complex



Ca^{2+}

PAD

Hypercitrullination

Membrane lesion induced Ca^{2+} influx is most responsible for hypercitrullination

Romero, V.; *et al. Sci Transl Med.* **2013**, 5, 209, 209ra150.

Outline

What is Rheumatoid Arthritis

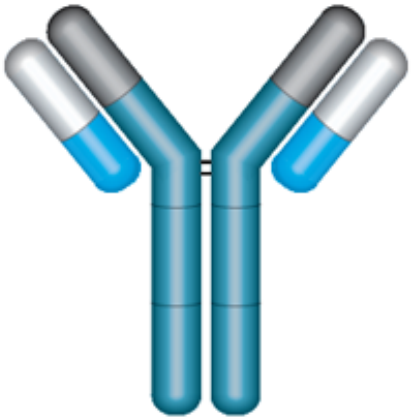
Citrullination & Citrullinated Peptides

Anti-CP antibodies (ACPA's)

Potential Therapeutic Targets

Anti-Citrullinated Peptide/Protein Antibodies

ACPA

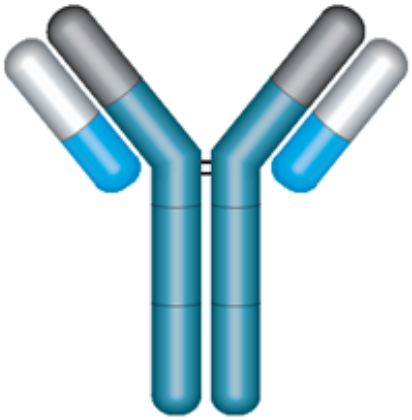


- ***95~98% specificity to RA patients***
- ***precede onset of clinical symptoms by years***
- ***related to more severe and erosive phenotype***

Wu, C.; *et al. Int. J. Mol. Sci.* **2020**, 21, 4015.

Anti-Citrullinated Peptide/Protein Antibodies

ACPA



recognizes >100 citrullinate proteins

promiscuous ACPA

- *No specific function*
- *Recognize different CP's*

private ACPA

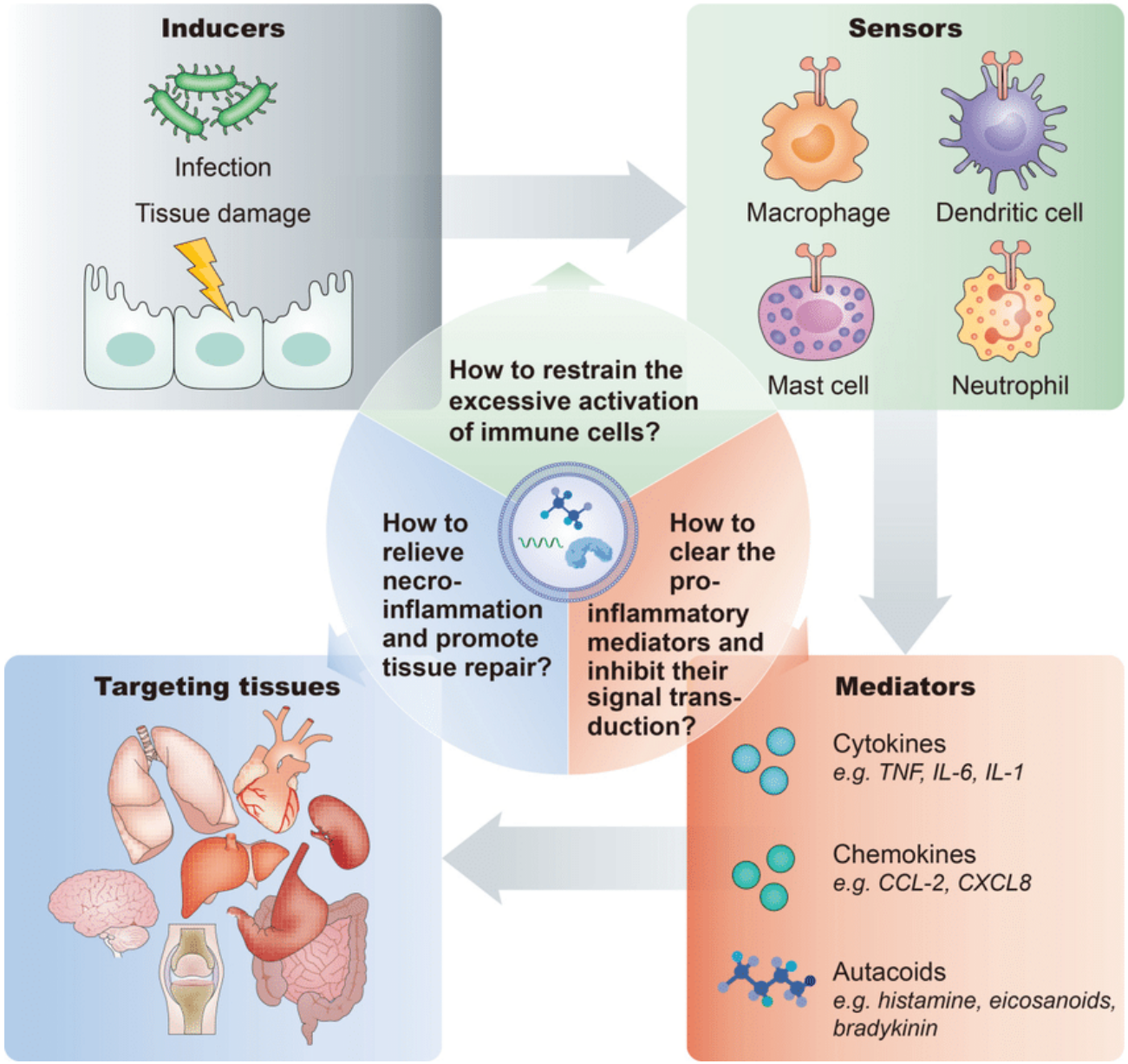
- *Target specific CP's*
- *May be arthritogenic*

ACPA evolves overtime to become less specific

Wu, C.; *et al. Int. J. Mol. Sci.* **2020**, 21, 4015.

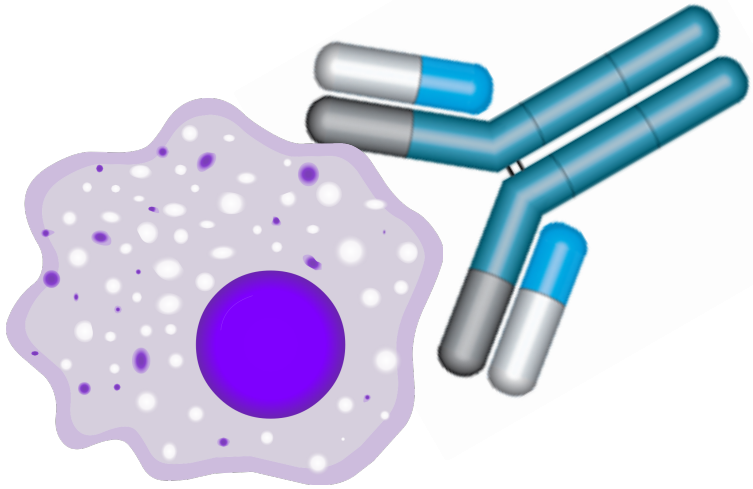
Anti-Citrullinated Peptide/Protein Antibodies

general immune response pathways



Anti-Citrullinated Peptide/Protein Antibodies

macrophage activation



ACPA

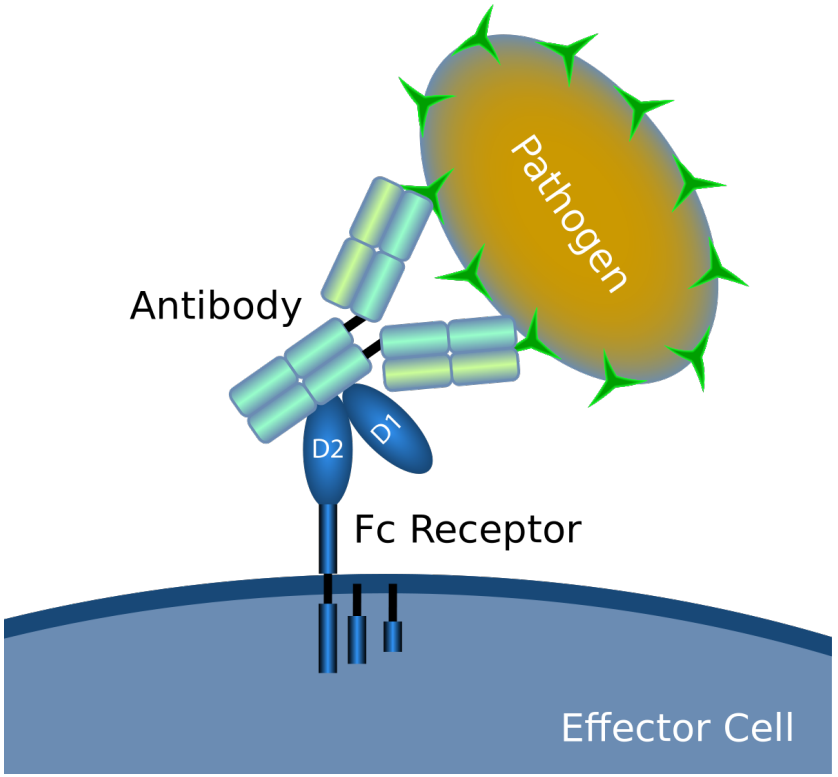
monocyte activation

- ***Binds to citrullinated proteins cell surface***
- ***Activates macrophages, monocytes, lymphocytes***
- ***Increases TNFα (cytokine) production***
- ***Alternatively, it activates TLR4***

Liu, M.; *et al. Arthritis & Rheumatism* **2010** 62, 1213

Anti-Citrullinated Peptide/Protein Antibodies

neutrophil activation



neutrophil

- ***Immune complex binds to Fcγ receptors***
- ***Activates neutrophils***
- ***Neutrophile degranulation, ROS release***
- ***Initiate cytokine & chemokine cascade***

Wu, C.; *et al. Int. J. Mol. Sci.* **2020**, 21, 4015.

Anti-Citrullinated Peptide/Protein Antibodies

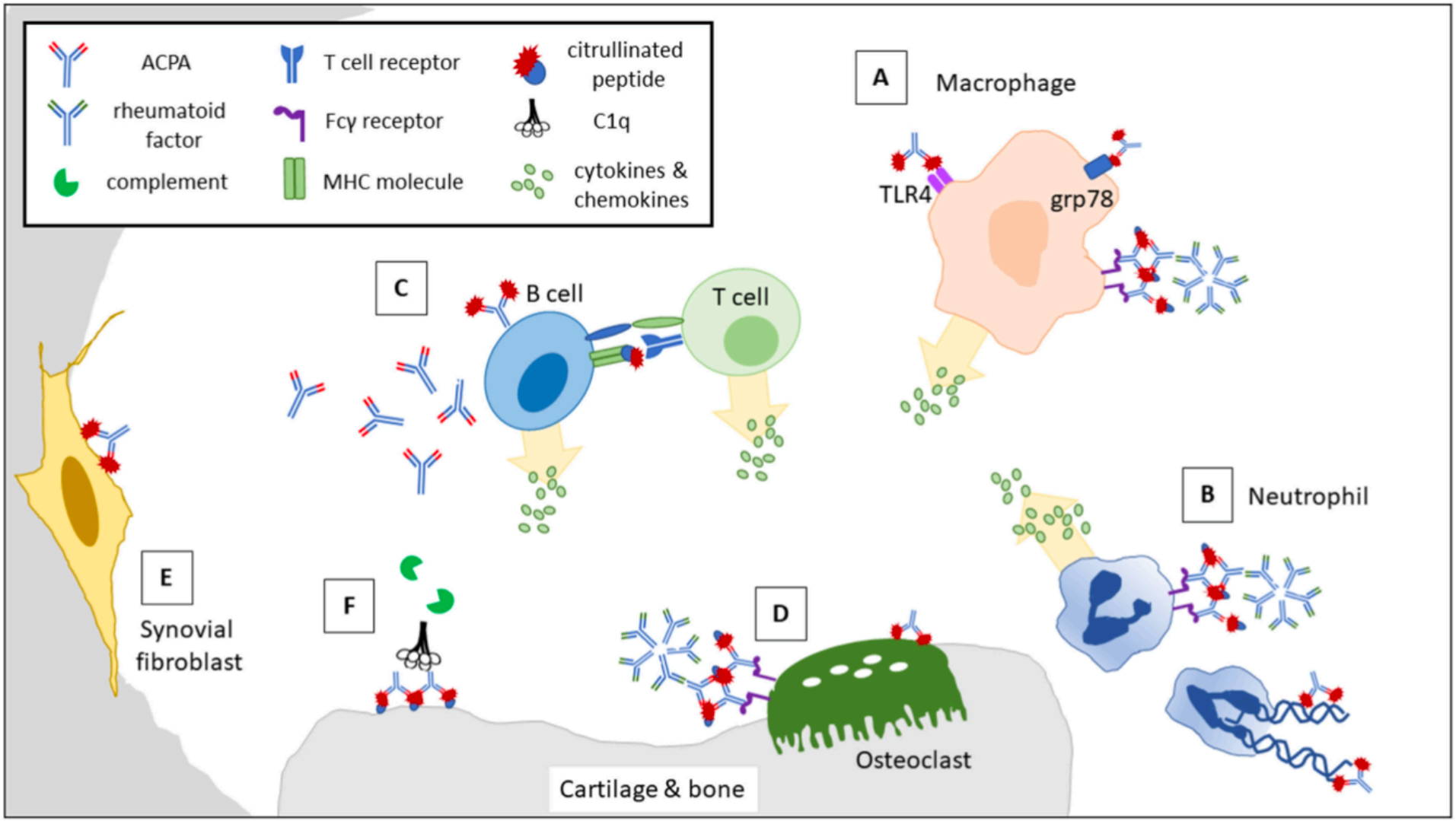
Interaction with osteoclast

osteoclast

- ***Large multinucleated cell***
- ***Causes bone dissolution and absorption***
- ***ACPA promotes osteoclast activation & deffrentiation***
- ***May be independent of antigen binding***

Wu, C.; *et al. Int. J. Mol. Sci.* **2020**, 21, 4015.

Anti-Citrullinated Peptide/Protein Antibodies



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Outline

What is Rheumatoid Arthritis

Citrullination & Citrullinated Peptides

Anti-CP antibodies (ACPA's)

Potential Therapeutic Targets

Potential Therapeutics

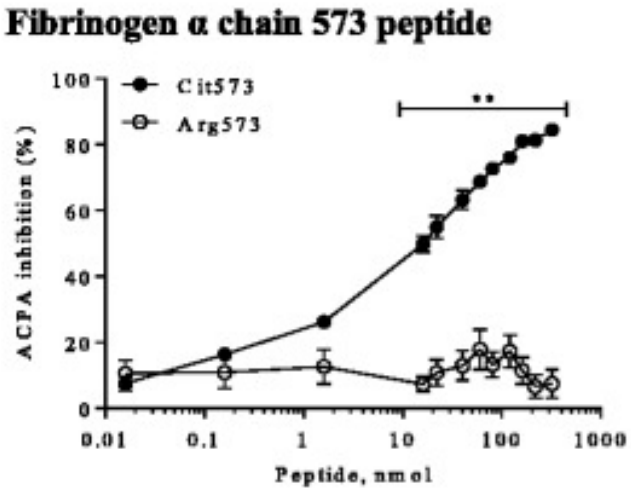
peptide inhibition of ACPA

- ***Tested against sera from 927 RA patients and 461 healthy controls***
- ***98% specificity was aimed for***
- ***ACPA were isolated from patients and pre-incubated with fibrinogen***
- ***Performed anti-CCP2 competitive ELISA***

Fernandes-Cerqueira, C.; *et al*. *Arthiritis Research& Therapy.* ***2015****, 17, 155.*

Potential Therapeutics
peptide inhibition of ACPA

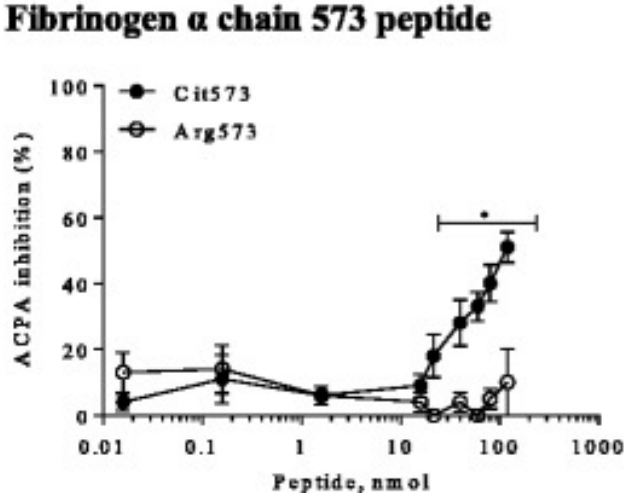
a ACPA pool I



84% inhibition

IC_{50} : 59 $\mu M \pm 8$

b ACPA pool II



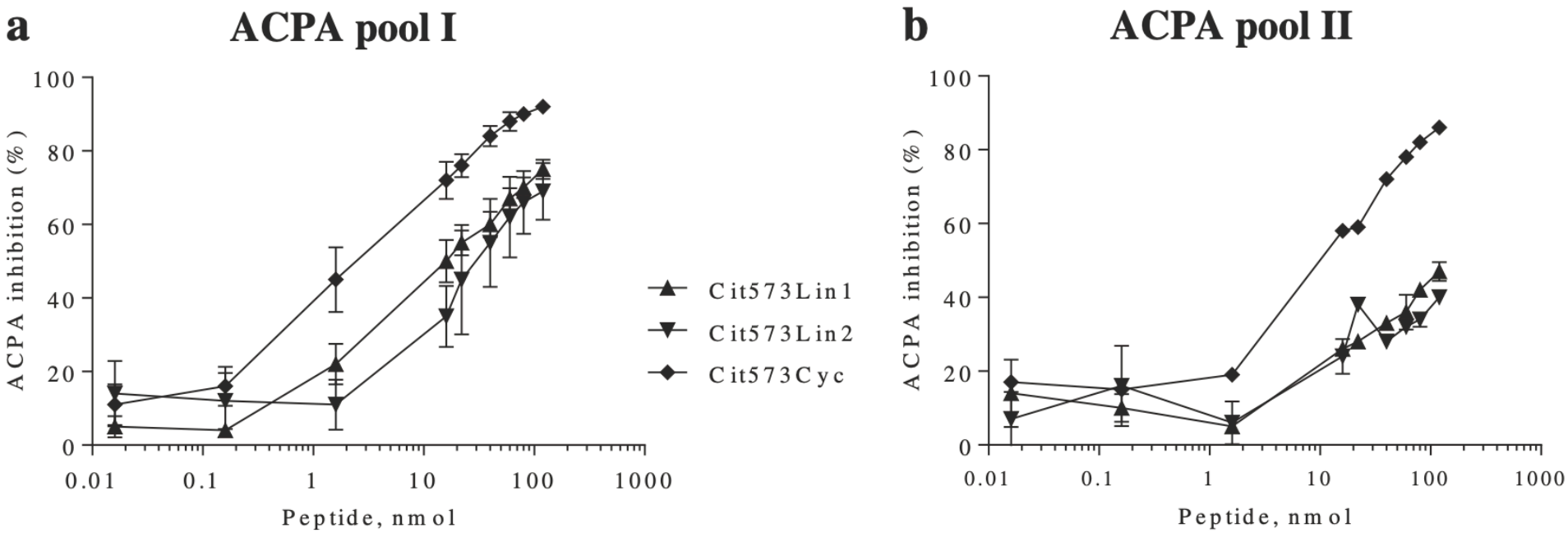
50% inhibition

IC_{50} : 548 $\mu M \pm 100$

Fernandes-Cerqueira, C.; *et al*. *Arthiritis Research& Therapy.* ***2015****, 17, 155.*

Potential Therapeutics

peptide inhibition of ACPA



92% inhibition

IC_{50} : 28 $\mu M \pm 5$

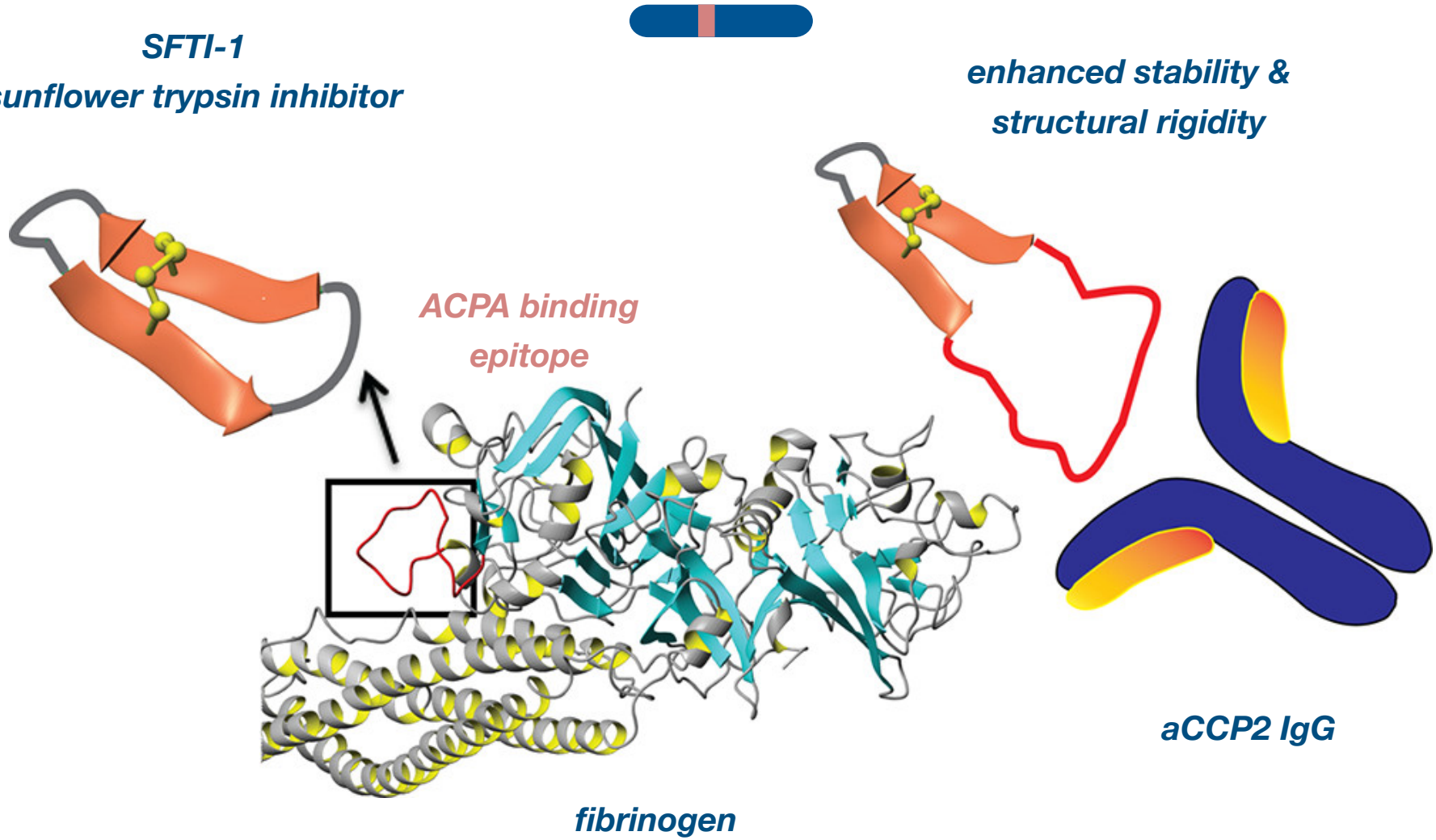
Cyclic citrullinated peptide showed enhanced reactivity

Fernandes-Cerqueira, C.; *et al*. *Arthiritis Research& Therapy.* ***2015****, 17, 155.*

Potential Therapeutics

peptide inhibition of ACPA

[Cit573]fibrinogen(563–583)



Gunasekera, S.; *et al*. *ACS Chem. Biol.* ***2018****, 13, 1525.*

Potential Therapeutics

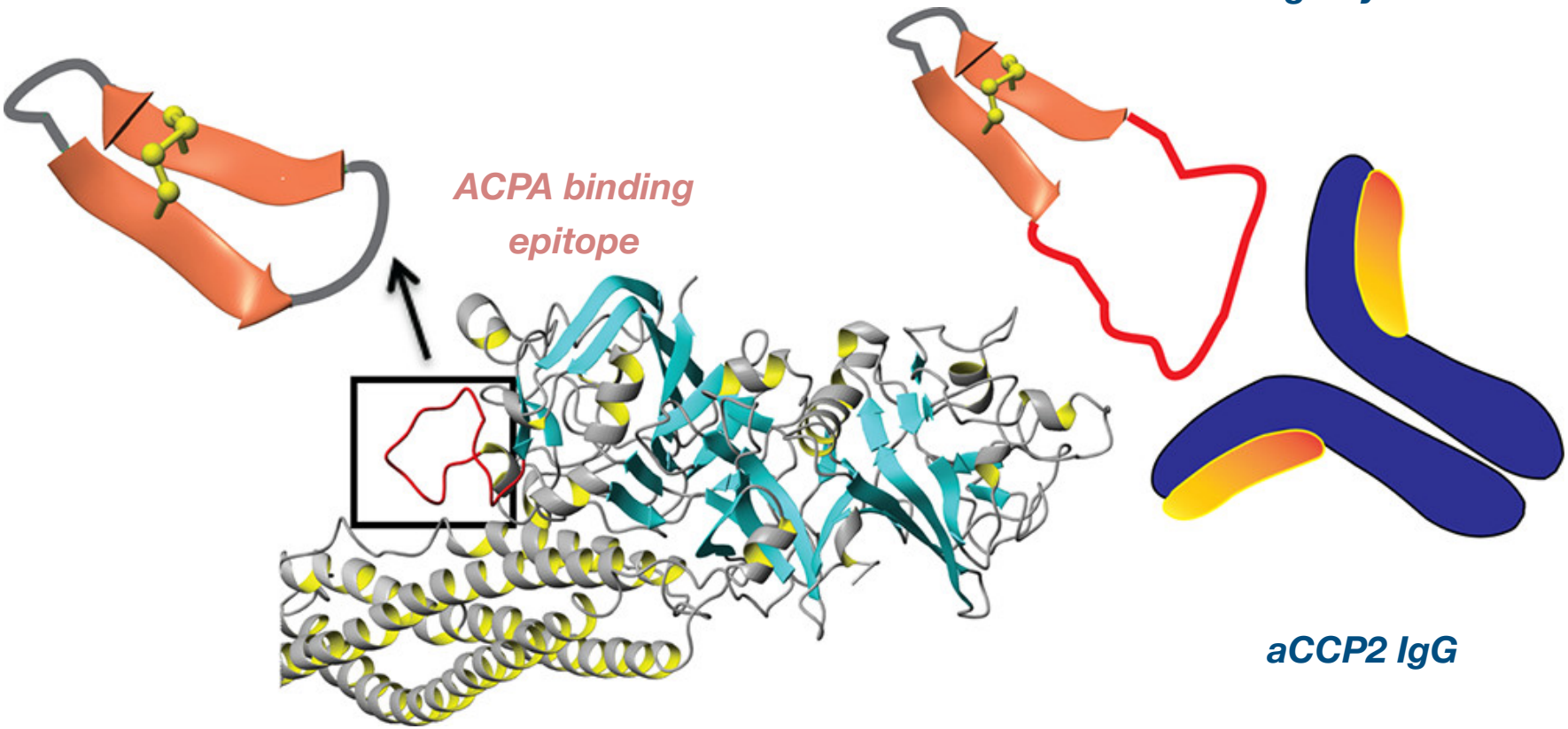
peptide inhibition of ACPA

ACPA pool I: 79% inhibition
IC_{50}: 20μM

ACPA pool II: 61% inhibition
IC_{50}: 87μM

SFTI-1
sunflower trypsin inhibitor

enhanced stability &
structural rigidity



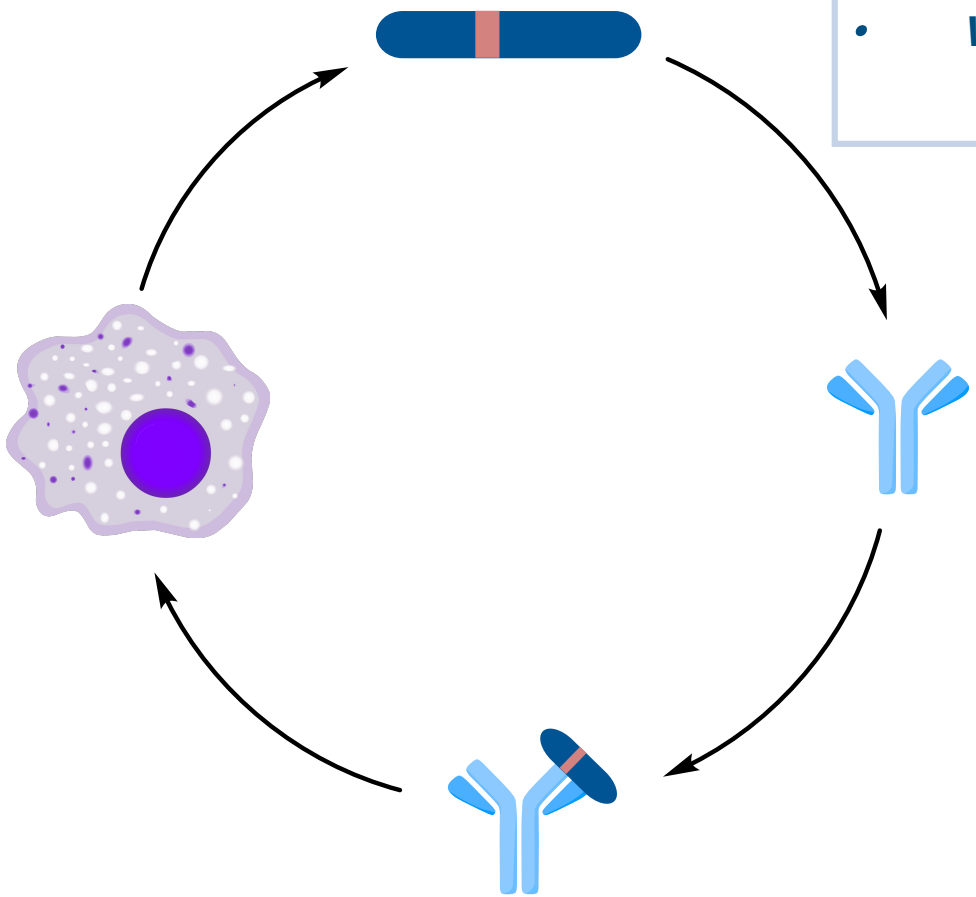
Gunasekera, S.; *et al*. *ACS Chem. Biol.* ***2018***, *13, 1525.*

Rheumatoid Arthritis and Citrullinated Proteins

outlook

hypercitrullination

- ***Is neutrophil apoptosis the cause of hypercitrullination?***
- ***What caused massive apoptosis?***



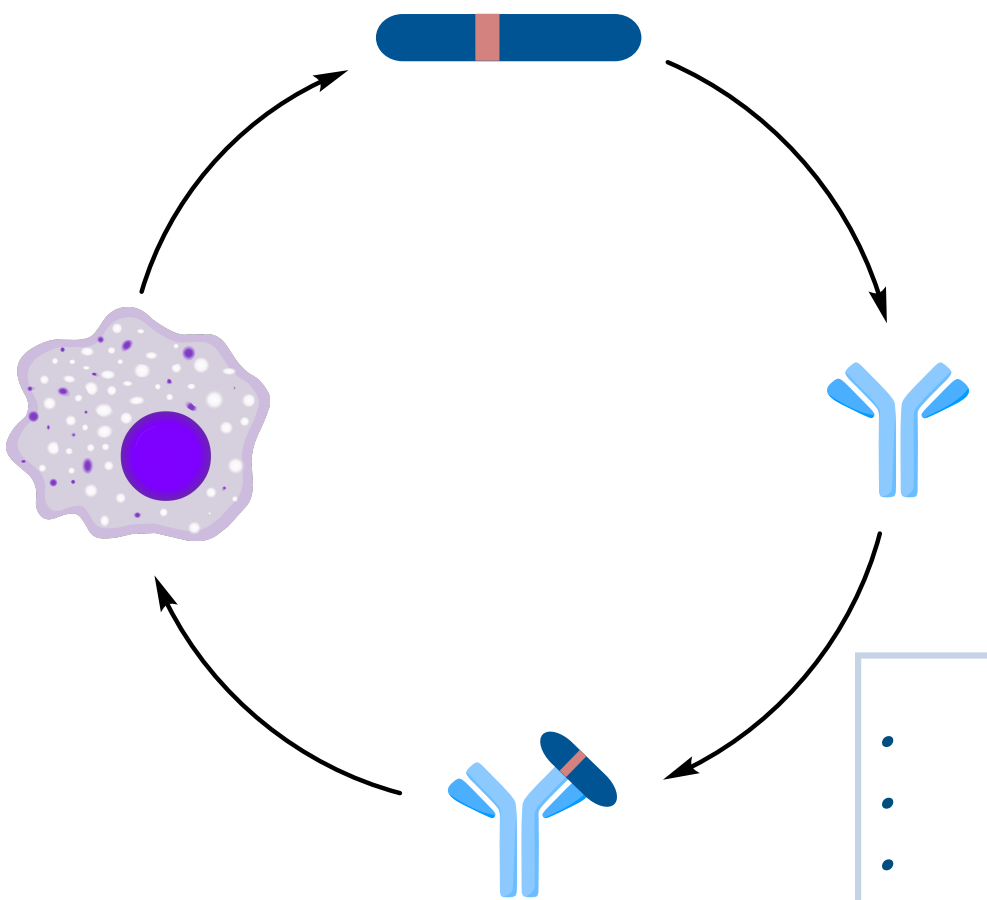
ACPA generation

formation of IC

downstream activation of inflammation pathway

Rheumatoid Arthritis and Citrullinated Proteins

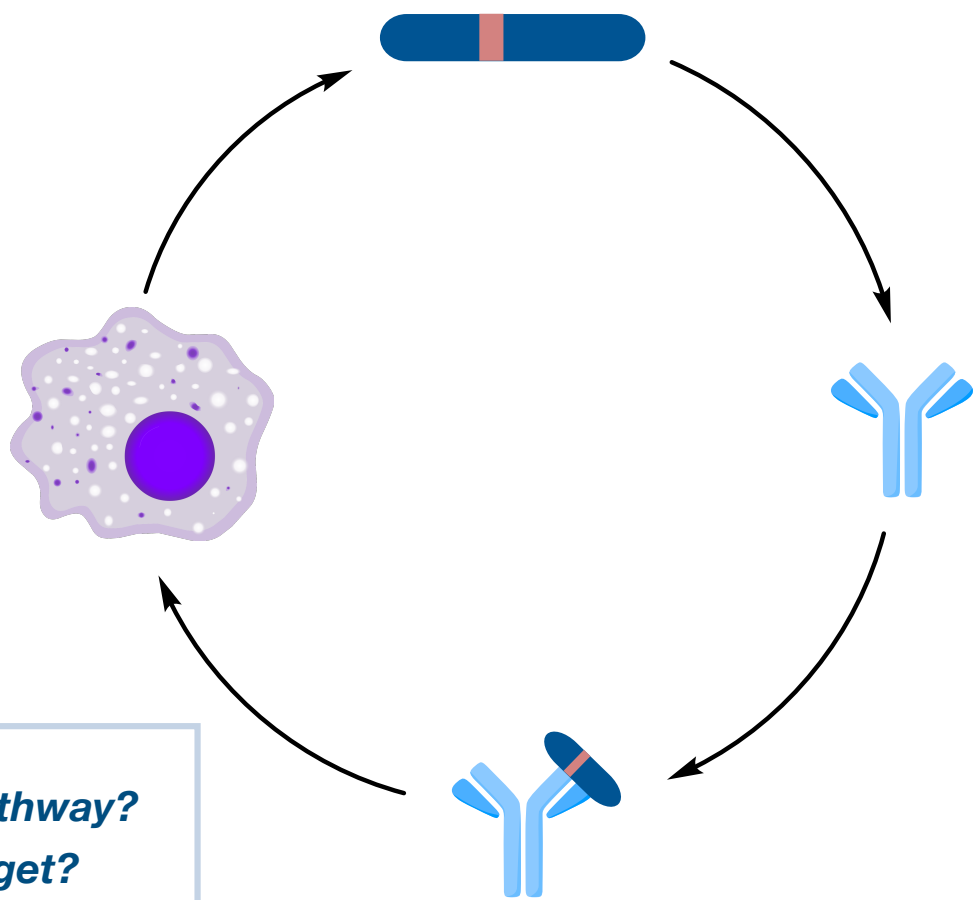
outlook



- ***Origin of ACPA?***
- ***Pathogenic?***
- ***Why preced RA?***

Rheumatoid Arthritis and Citrullinated Proteins

outlook

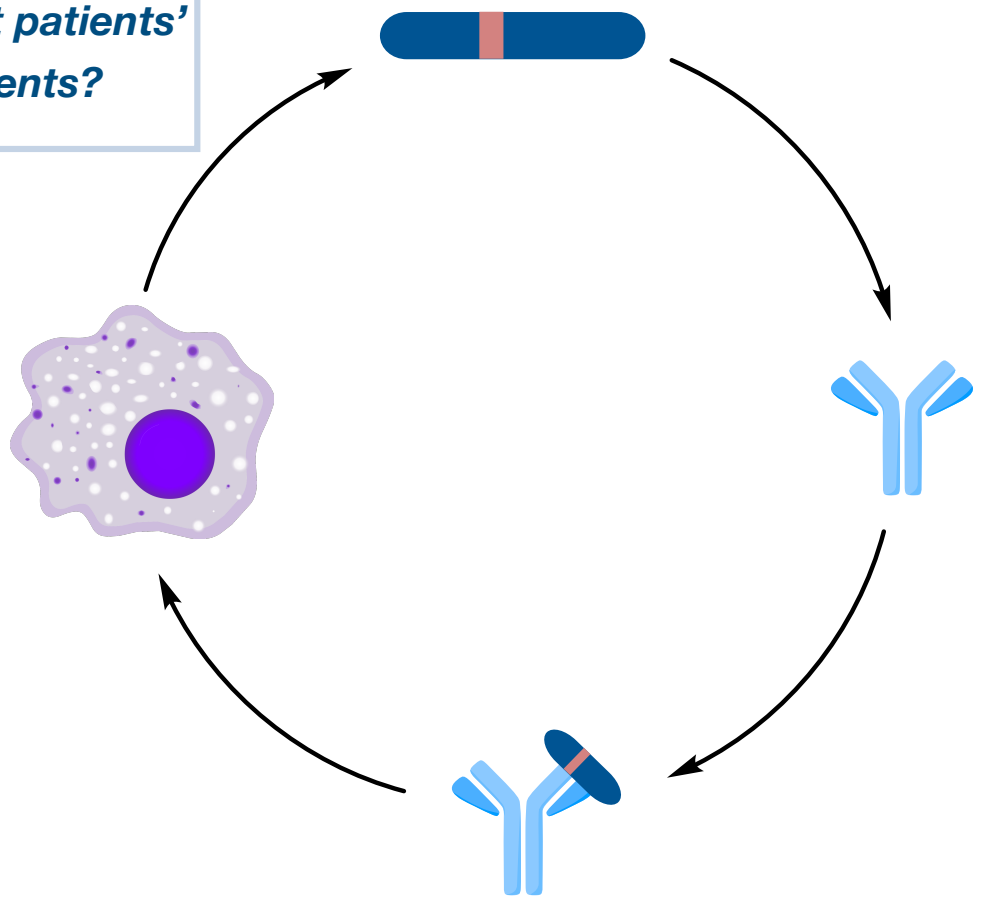


- *More activation pathway?*
- *Therapeutic target?*

Rheumatoid Arthritis and Citrullinated Proteins

outlook

- ***ACPA is only present in 2/3 of patients***
- ***Biomarkers that predict patients' response to treatments?***



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