



INTERNÍ KLINIKA 1. LF UK a ÚVN
Kardiologické oddělení



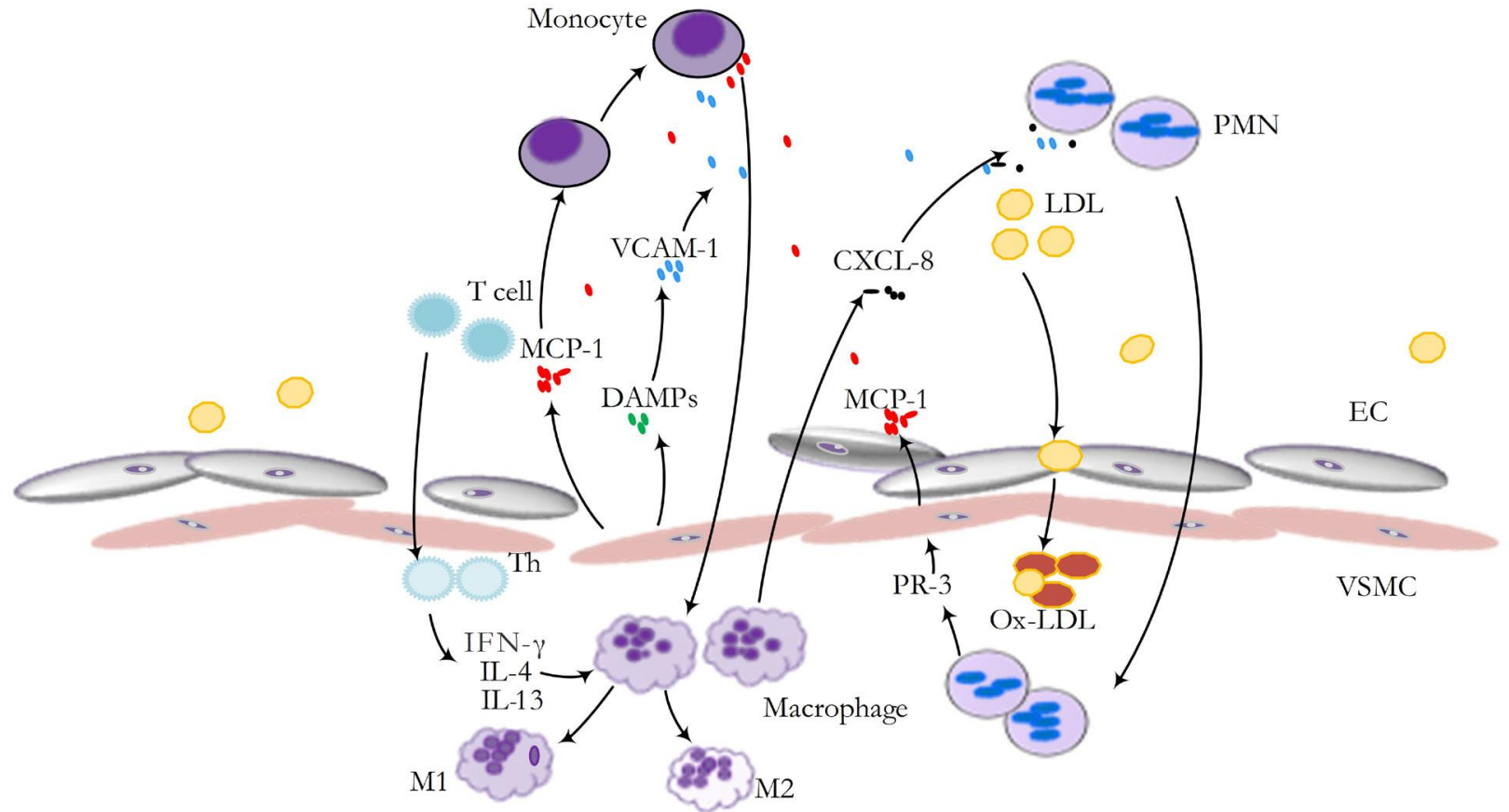
In vitro assessment of anti-inflammatory and cytotoxicity properties of colchicine

Malý M, Viktorová J, Strand O, Ruml T, Stránská M.


■ CZECH CARDIOVASCULAR
RESEARCH AND
INNOVATION DAYS **2022**
November 28-29, 2022 | Hermitage Hotel Prague



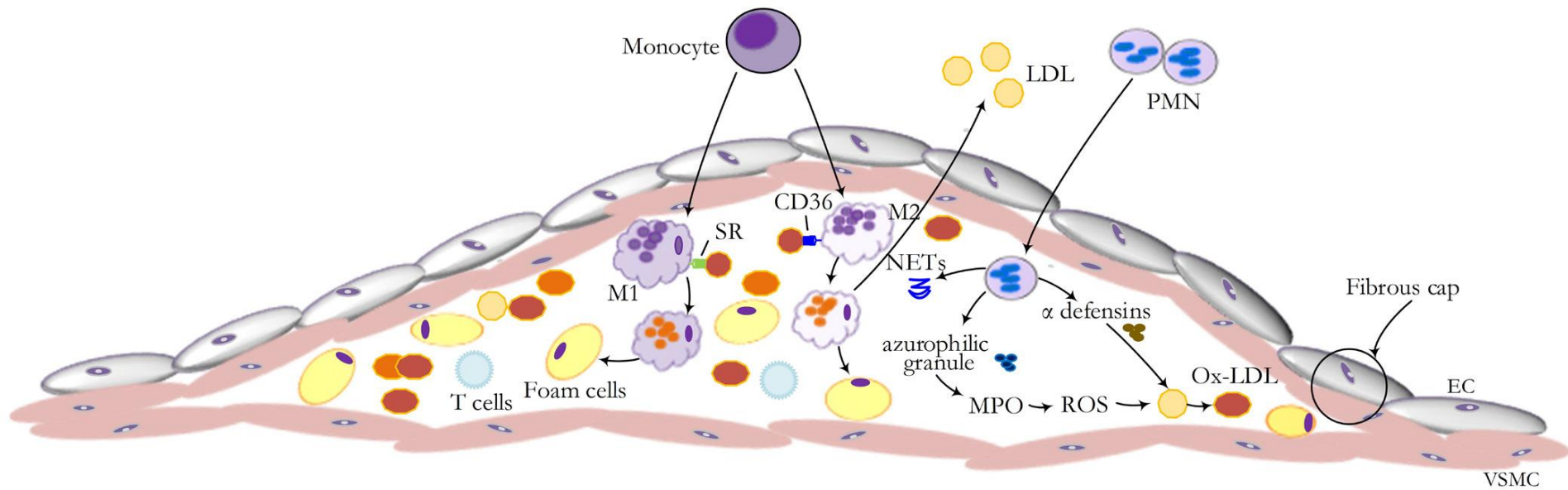
Initialization



J Transl Intern Med 2022; 10: 36-47.



Progression



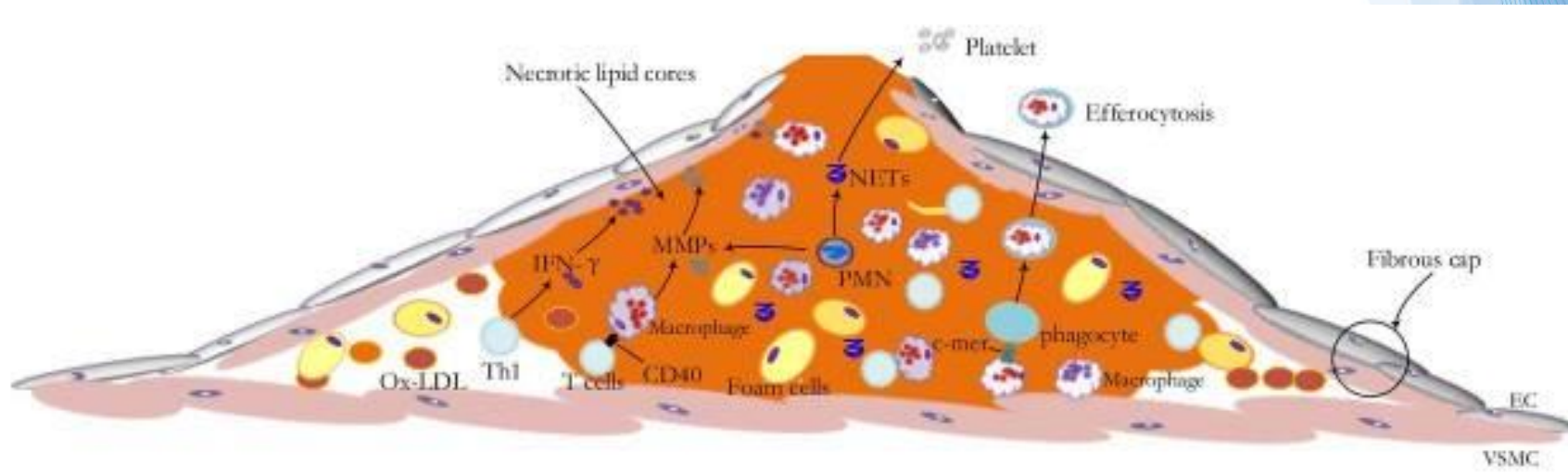
J Transl Intern Med 2022; 10: 36-47.



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Advanced lesions



J Transl Intern Med 2022; 10: 36-47.



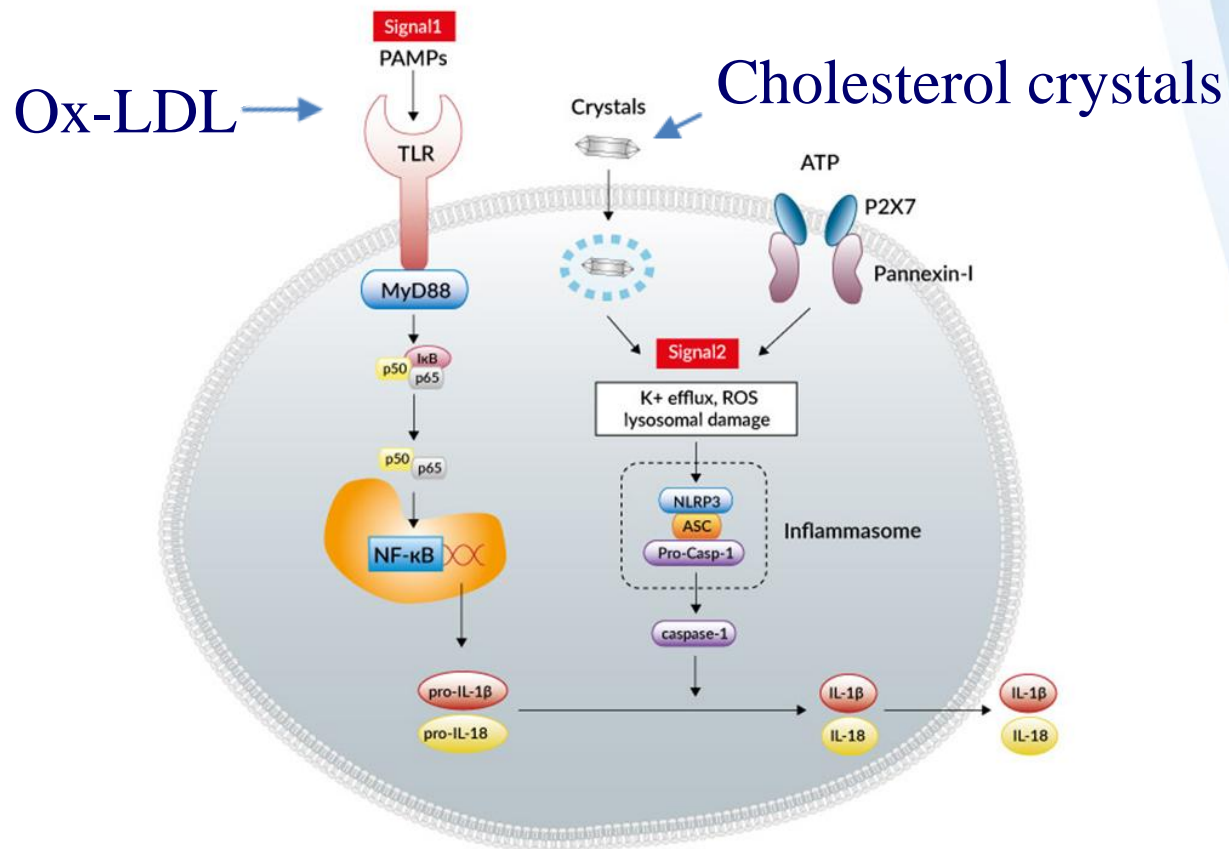
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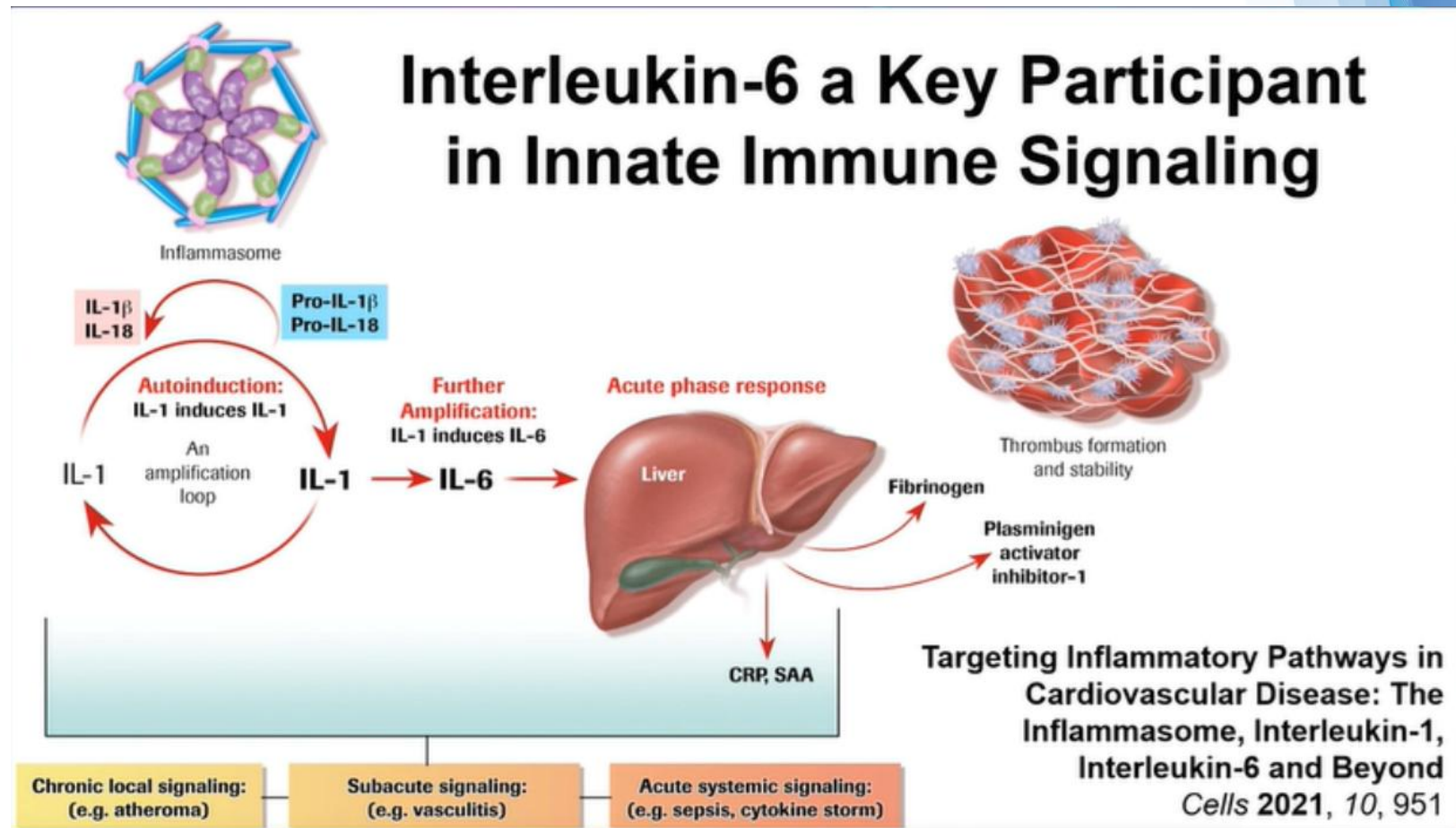
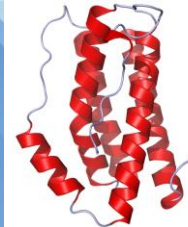
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NRLP3 (nucleotide binding domain leucine rich repeat) Inflammasome





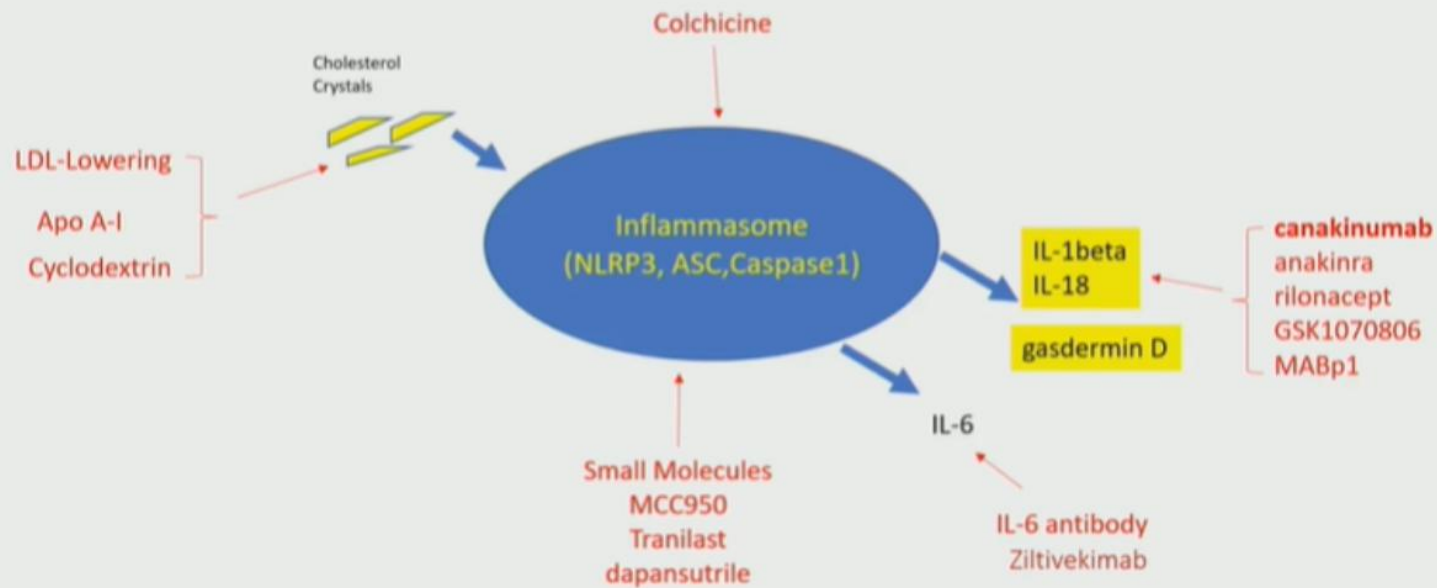
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NLRP3 Inflammasome as a Target for Atherosclerosis Treatment



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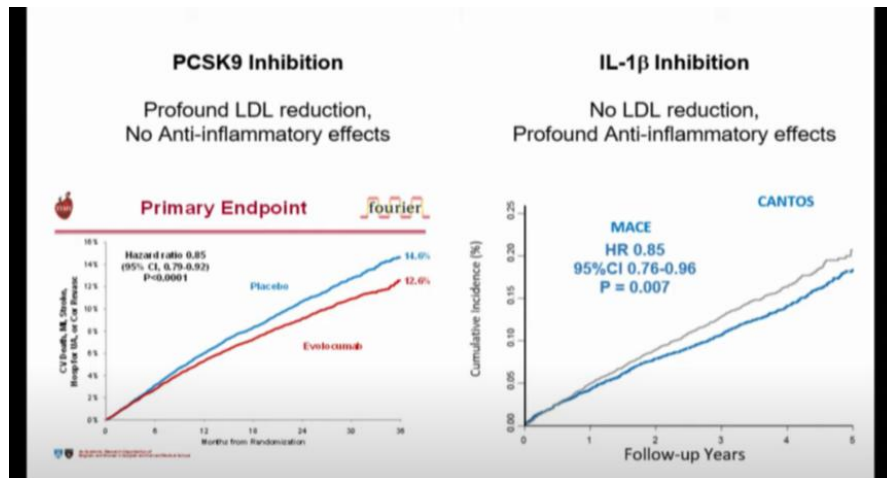
Clinical Anti-inflammatory trials

Antibodies

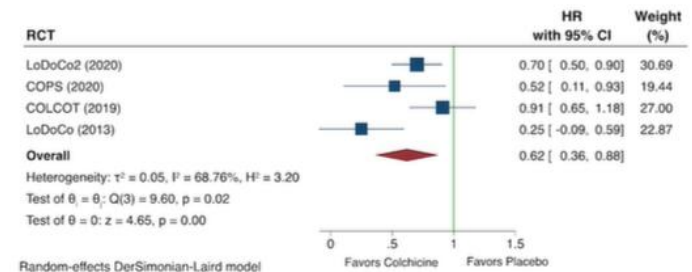
- 2017 CANTOS – IL1
- 2022 ZEUS – IL6 ongoing

Colchicine

- 2019 COLCOT
- 2020 LoDoCo



Meta-analysis of colchicine studies in CAD Myocardial infarction



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Colchicine in Patients with Chronic Coronary Disease

Stefan M. Nidorf, M.D., Aernoud T.L. Fiolet, M.D., Arend Mosterd, M.D., John W. Eikelboom, M.D., Astrid Schut, M.Sc., Tjerk S.J. Opstal, M.D., Salem H.K. The, M.D., Xiao-Fang Xu, M.D., Mark A. Ireland, M.D., Timo Lenderink, M.D., Donald Latchem, M.D., Pieter Hoogslag, M.D., [et al.](#), for the LoDoCo2 Trial Investigators*

The LoDoCo2 trial has several limitations. The percentage of women in the trial was lower than would be expected given the percentage of women with chronic coronary disease in the general population. We did not collect blood-pressure or lipid levels at baseline or during the trial, and we cannot report outcomes according to risk-factor control. [REDACTED]
[REDACTED]
[REDACTED] However, the effects of treatments were consistent across the majority of clinical subgroups examined.

N Engl J Med 2020; 383:1838-1847



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Induction of inflammation

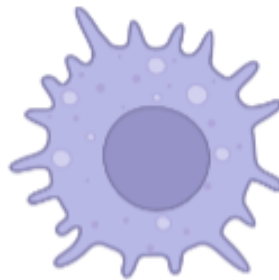
Suspension circulating in the blood
monocyte



PMA



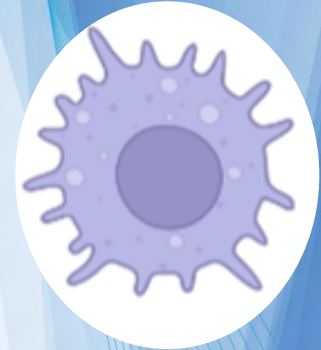
Adherent attached to the affected area
macrophage



MO

20 ng/ml IL-4
24 h

M2

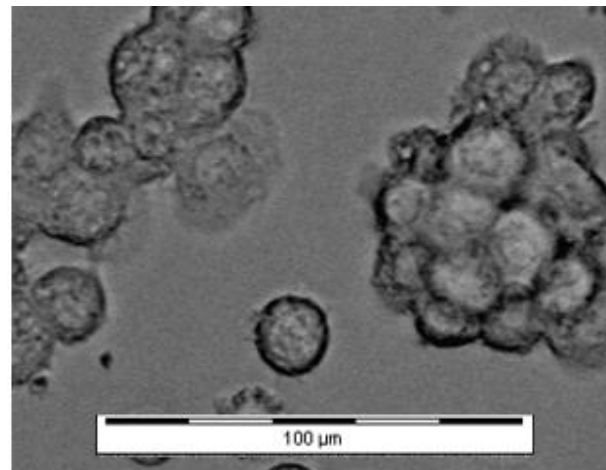
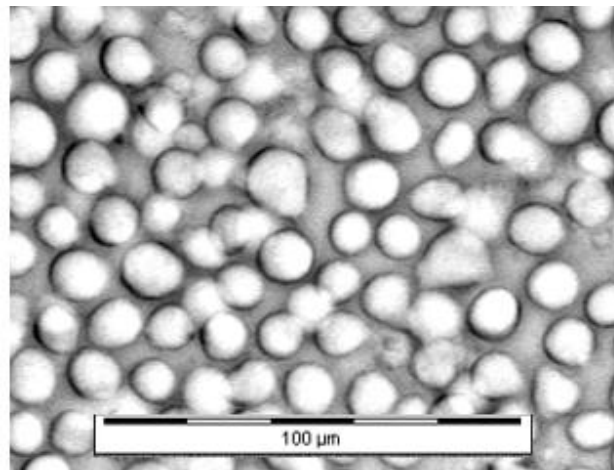


20 ng/ml IFN- γ
1 μ g/ml LPS
6 h

M1



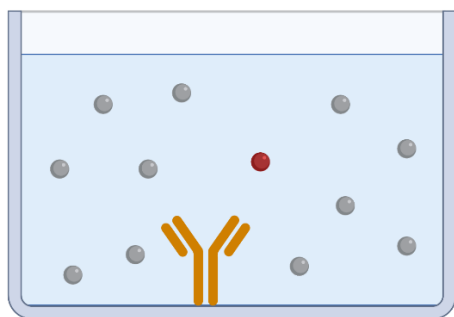
IL-1 β , IL-6, IL-8, IL-10, TNF- α
activation of NF- κ B



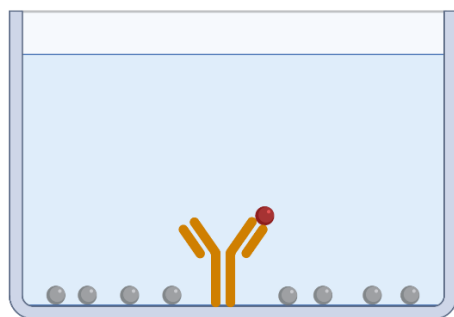
ELISA

enzyme-linked immuno sorbent assay

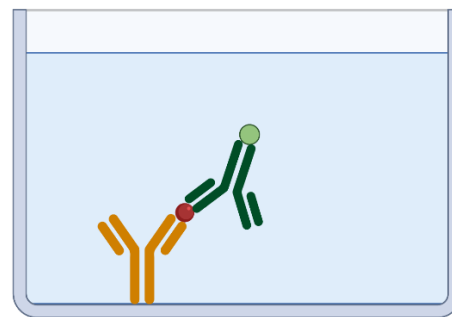
- The advantage of cytokines – secretion into the environment
- Home-made ELISA with limit of detection approx. 10 µg/L
- Quantification of IL-6, TNFα, IFNγ



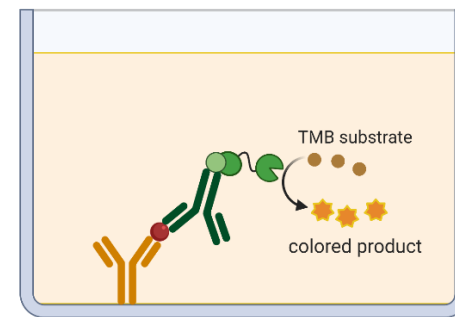
① Wells are pre-coated with capture antibody and sample is added



② Primary antibody binds to antigen with high specificity



③ Biotin labeled antibody binds to the immobilized analyte



④ Streptavidin-HRPO binds to biotin and catalyzes an enzymatic color reaction



Cell viability – resazurin assay

THP-1 (ATCC, TIB-202)

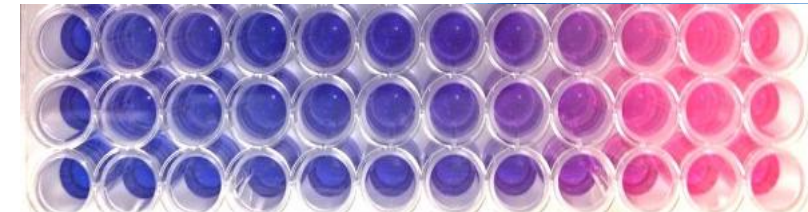
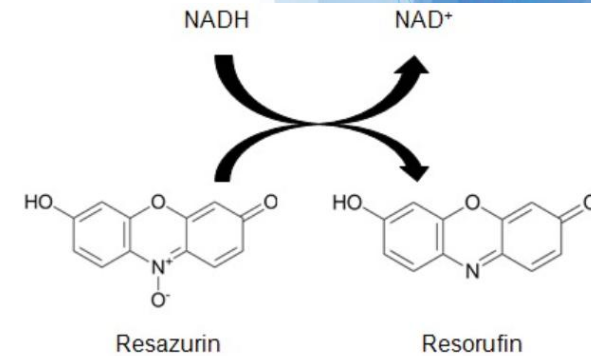
- Monocytes isolated from peripheral blood from a one-year-old patient with acute monocytic leukemia
- Differentiation into macrophages phorbol-12-myristate-13-acetate
- Polarization lipopolysaccharide (LPS), oxidized LDL

CORONARY ARTERY ENDOTHELIAL CELLS (Innoprot, P10460)

- Endothelium isolated from a healthy human coronary artery
- Primary cell line

RESAZURIN ASSAY

- Mitochondrial oxidoreductases reduce resazurin to resorufin (1-2 h)
fluorescence (560/590 nm)



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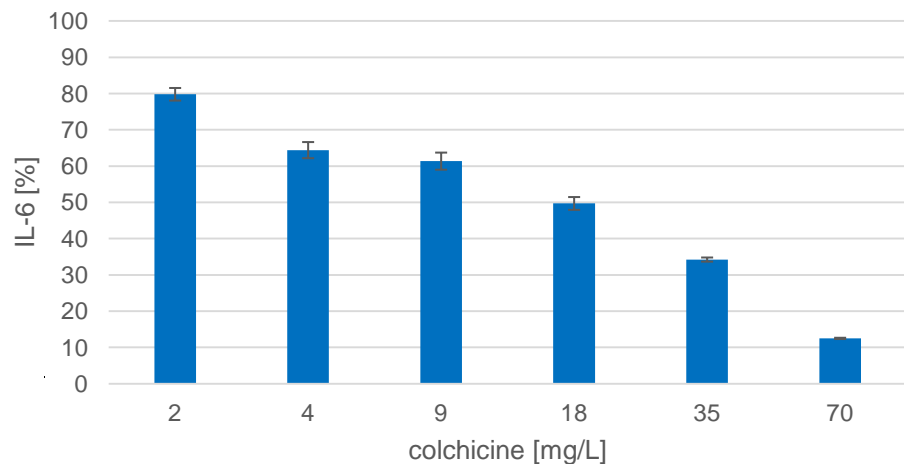
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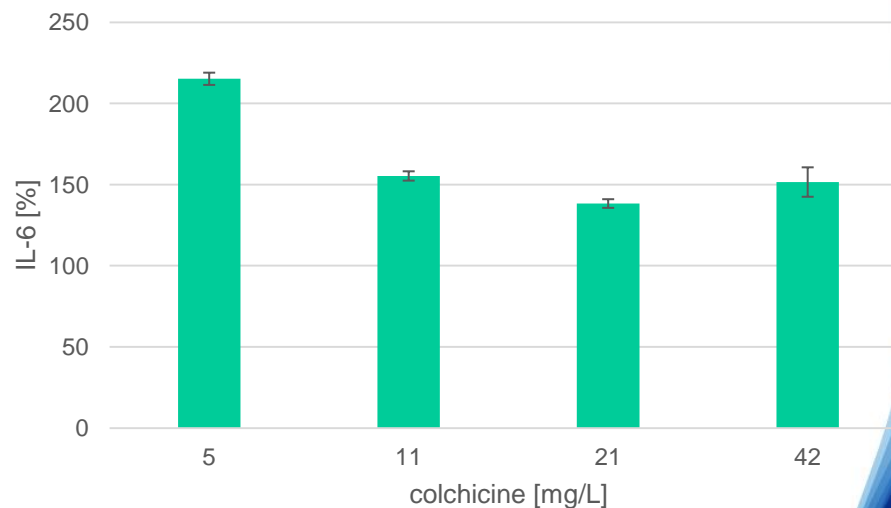
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Anti-inflammatory activity

HUMAN CORONARY ARTERY ENDOTHELIAL CELLS



MACROPHAGES



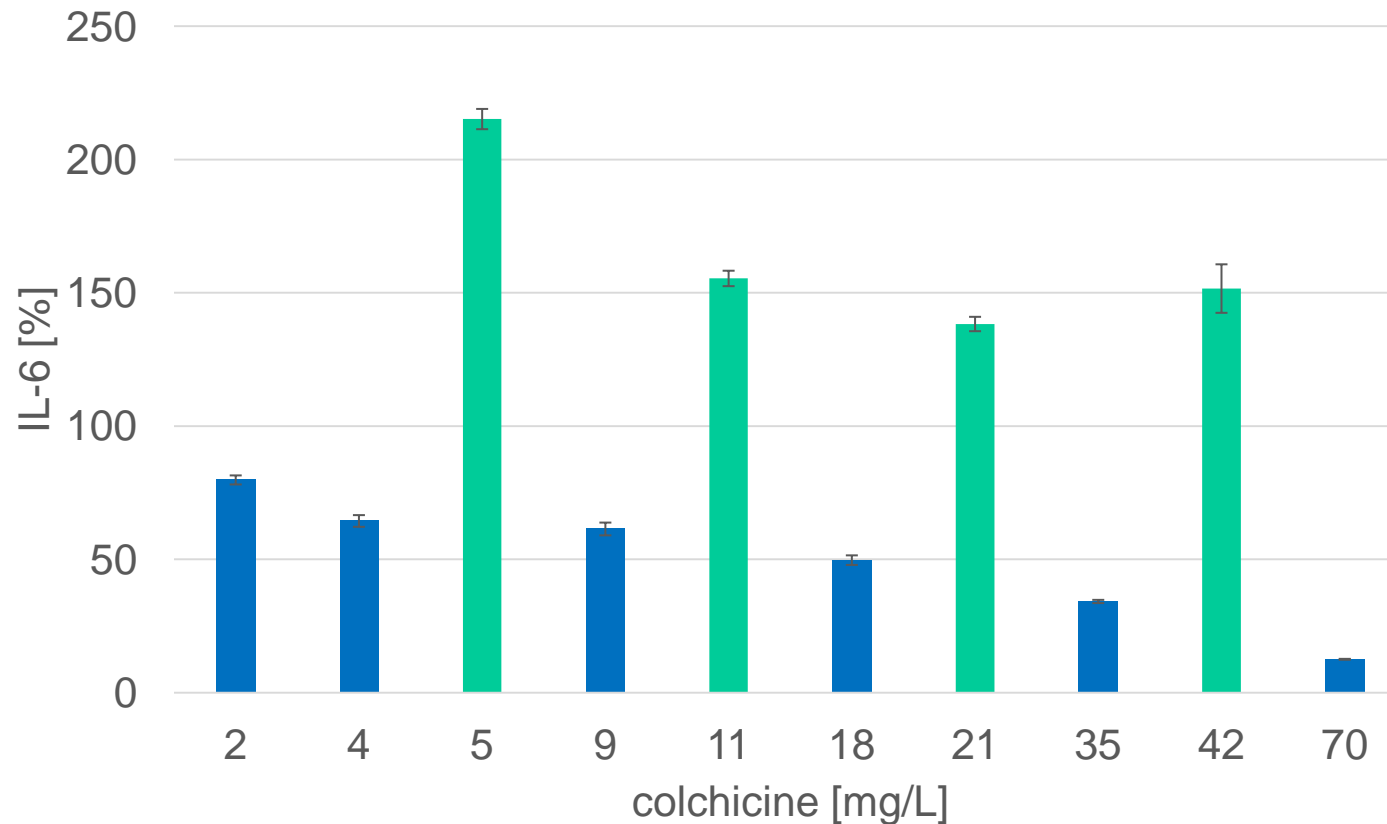
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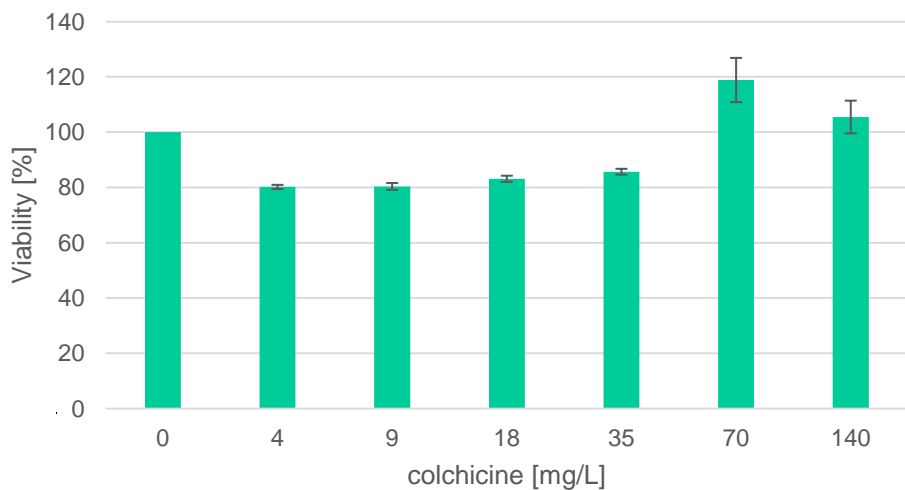
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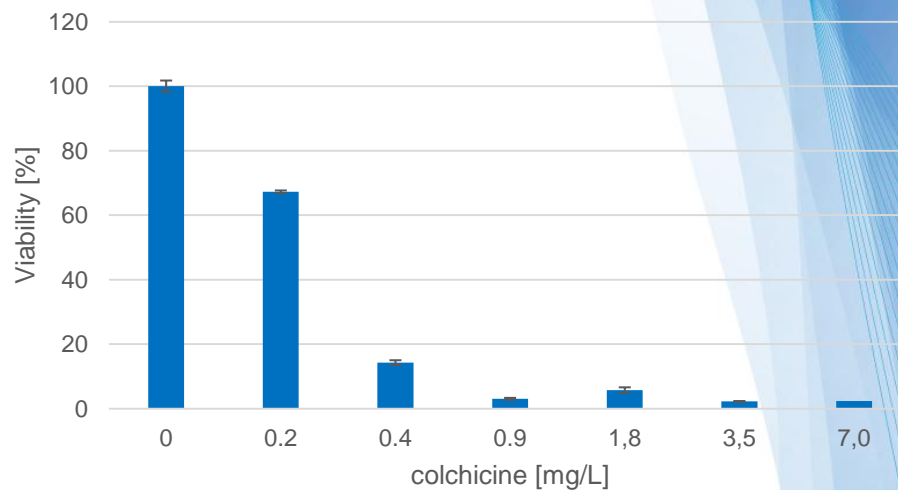


Cytotoxicity

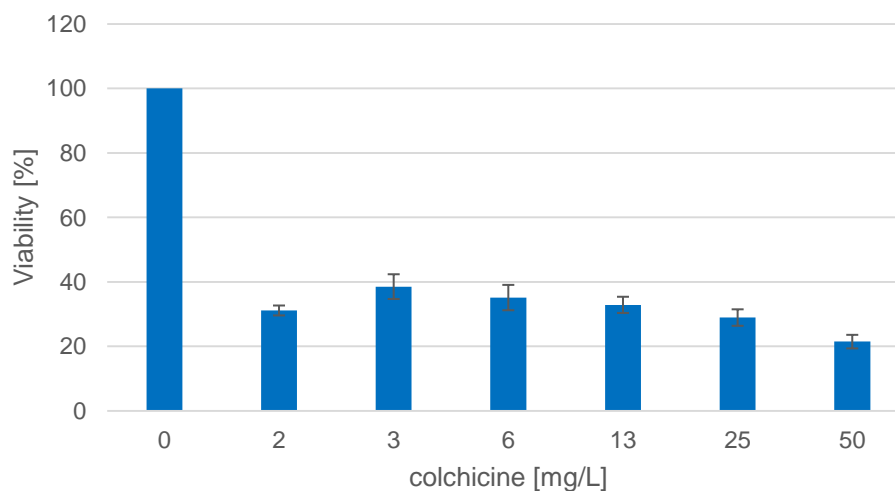
Macrophages, 72 h



Monocytes, 72 h



Endothelium, 72 h



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Conclusion

- “You need a little bit of fuel” — in this case, cholesterol — to get an atherosclerotic plaque started
- “Inflammation is definitely the fire.”

Erin Michos - Johns Hopkins University, Baltimore, Maryland



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