Paracrine factors released by γ-irradiated peripheral blood mononuclear cells inhibit neutrophil extracellular trap formation

Katharina Klas

PhD Defense, 13.02.2023

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Introduction

Study Aim and Study Design

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Discussion and Conclusion

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- Study Aim and Study Design
- Results
- Discussion and Conclusion

2014-2017

BSc
IMC FH Krems
Bachelorarbeit: Assoc. Prof. Pekka Katajisto, University of Helsinki
„Involvement of basement membrane laminins in proliferation and migration of mammary epithelial cancer cells"

2017-2019

MSc
Veterinärmedizinische Universität Wien
Masterarbeit: Prof. Dr. Erwin Tschachler, MedUni Wien - **Dermatologie**
„Distinct distribution of RTN1A in Mouse Skin and lymphoid organs"

2019-2023

PhD
Medizinische Universität Wien
Dissertation: Assoc.-Prof. Dr. Hendrik Jan Ankersmit, MedUni Wien
„Paracrine factors released by γ–irradiated PBMCs inhibit Neutrophil extracellular traps"

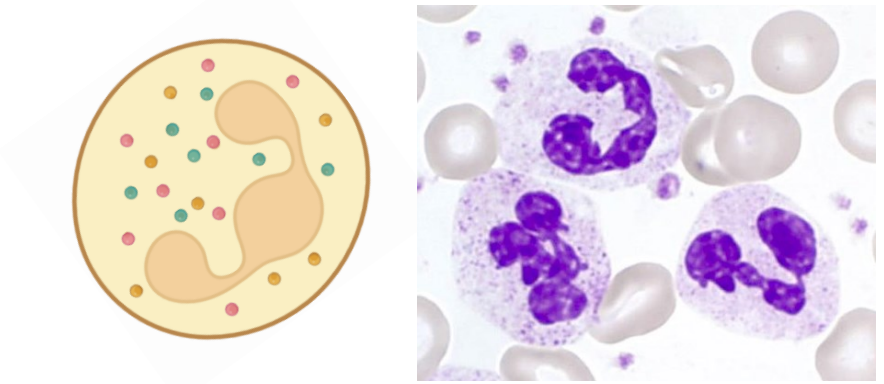
Parallel Forschung: MedUni Wien - **Dermatologie**
Mentor: Assoc. Prof. PD. Dr. Michael Mildner
Transkriptionelle Analyse von Sebozyten & Talgdrüsen, *Transkriptionelle Analyse von Hautalterung (Fokus Stammzellen in der Hautalterung)*

09/2022
–
to date

sanofi ***Medical Scientific Liaison Immunology***
Type 2 Inflammatroy SKIN Diseases

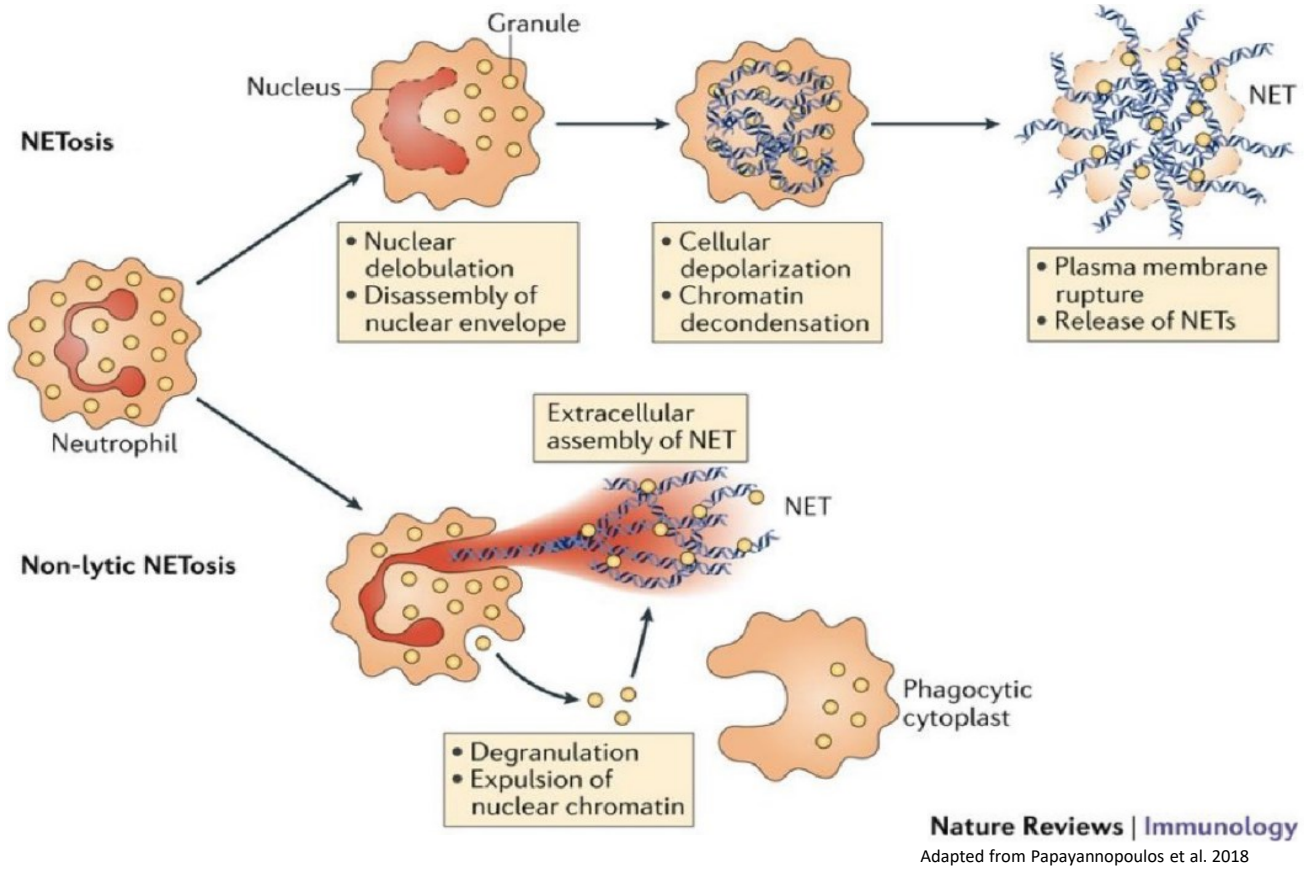
Neutrophils and NETs

- polymorphonuclear leukocyte
- most abundant granulocyte
- enriched cytoplasm with granules & secretory vesicles
- typically the first leukocyte recruited to inflammatory site
- capable of eliminating pathogens by multiple mechanisms



Kraus et al. 2021, Front Immunol.

Neutrophils and NETs



Adapted from Papayannopoulos et al. 2018

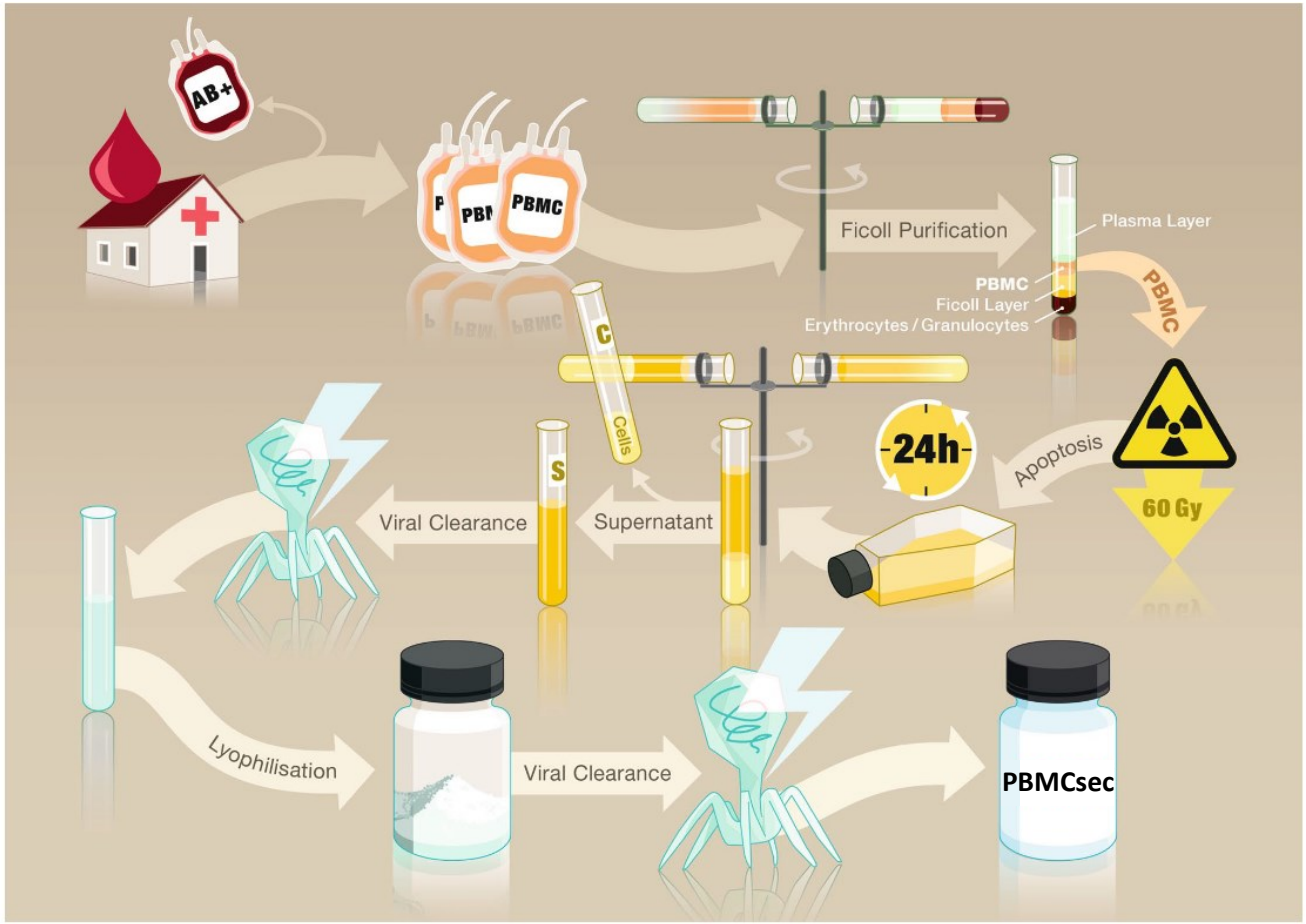
condensed chromatin and DNA, histones, diverse set of granule proteins, cytosolic proteins

Secretome of γ-irradiated peripheral blood mononuclear cells - PBMCsec

Secretome of γ-irradiated peripheral blood mononuclear cells - PBMCsec

Secretome of γ-irradiated peripheral blood mononuclear cells - PBMCsec

- preconditioning (stress-inducing stimuli) improve tissue-regenerative & beneficial effects of secretome
- irradiation-induced cell death (apoptosis, necroptosis)
- extracellular vesicles, lipids, proteins, DNA



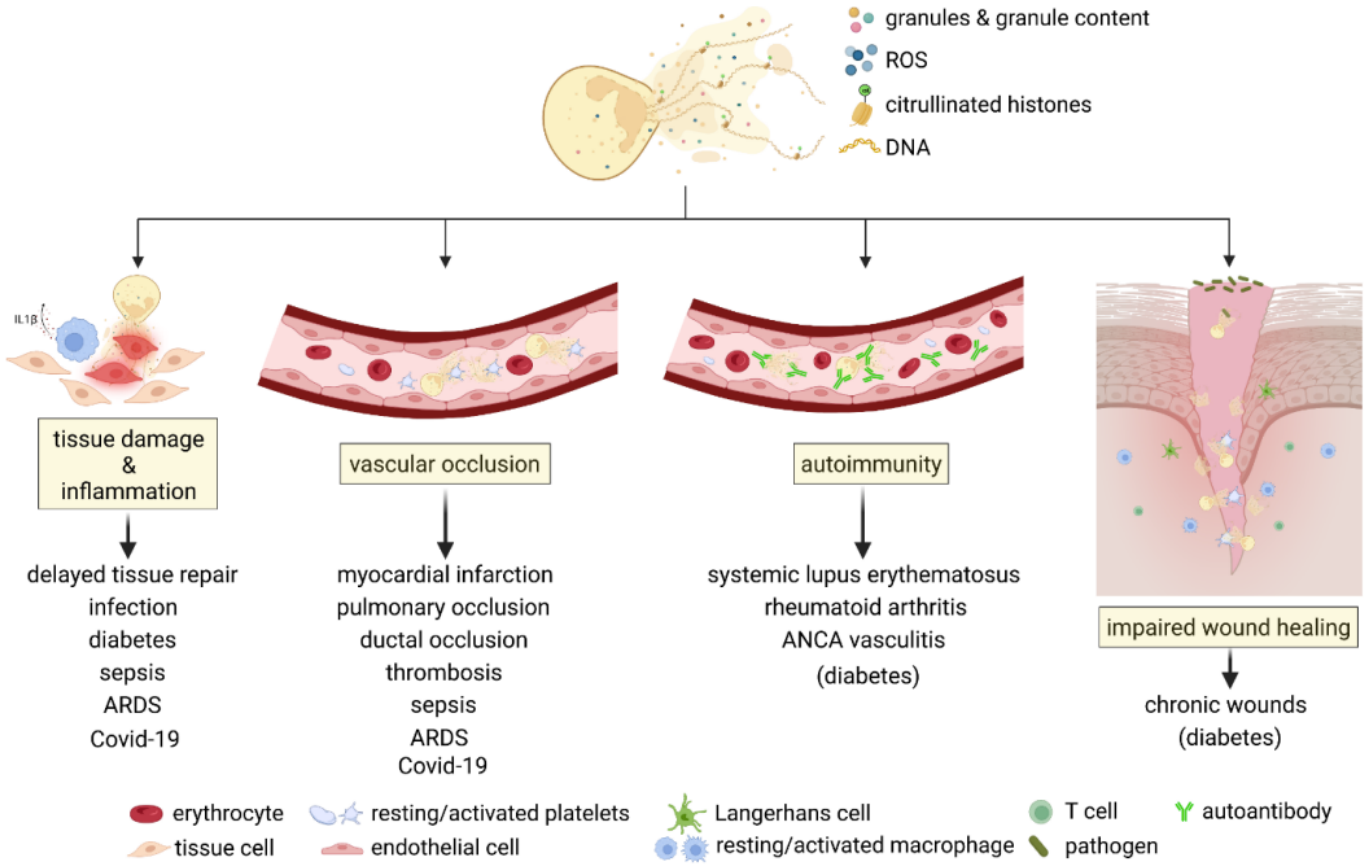
Adapted from Beer et al. 2016, Apoptosis

Project relevance

potent prothrombotic & pro-inflammatory properties of NETs

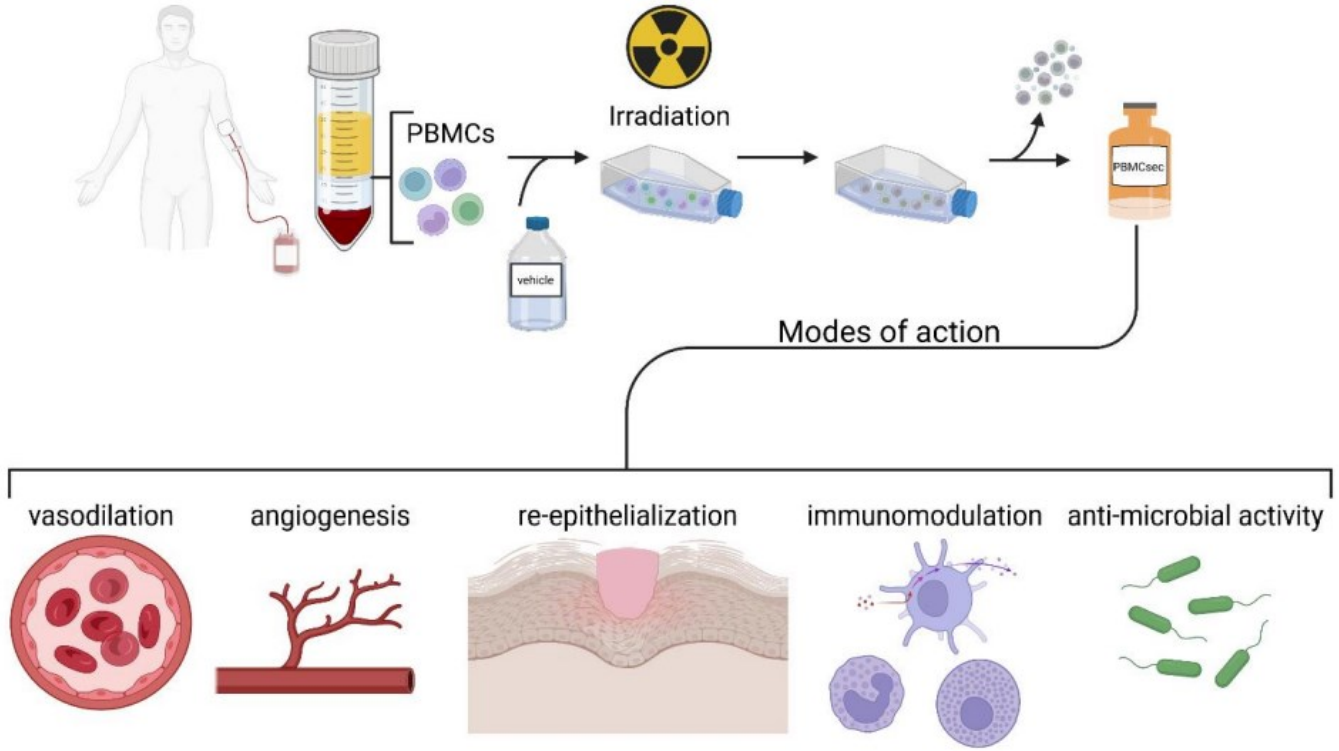
correlation w/ increased infarct size in ST-elevation myocardial infarction

impact on cardiac remodelling (interplay of Neutrophils & Monocytes)



NETs highly involved in broad range of autoimmunity (psoriasis, systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes mellitus) and autoinflammatory diseases & metabolic diseases

Project relevance

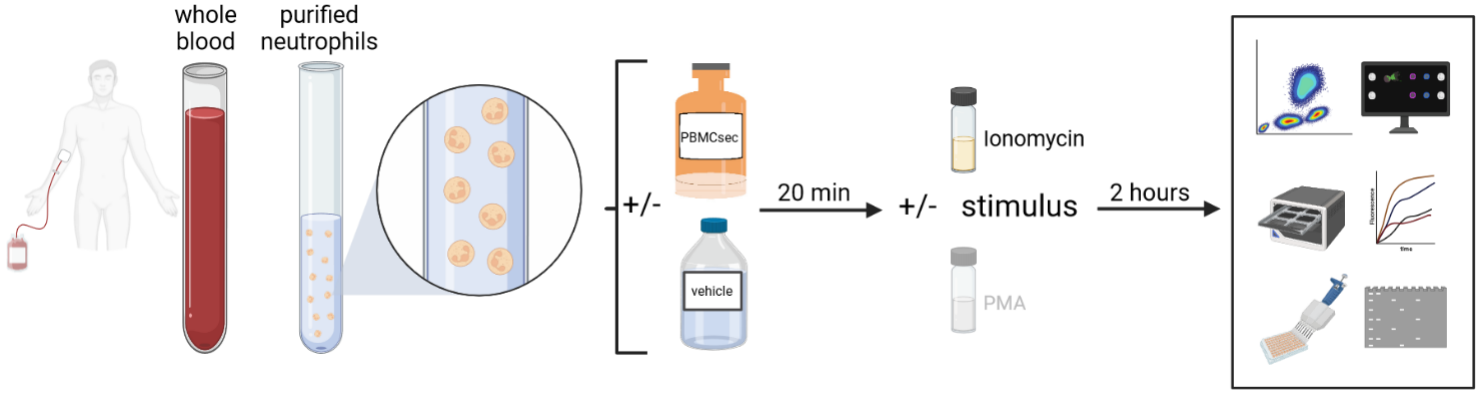


Study aim & design

Determine the effect of PBMCsec on NET-formation

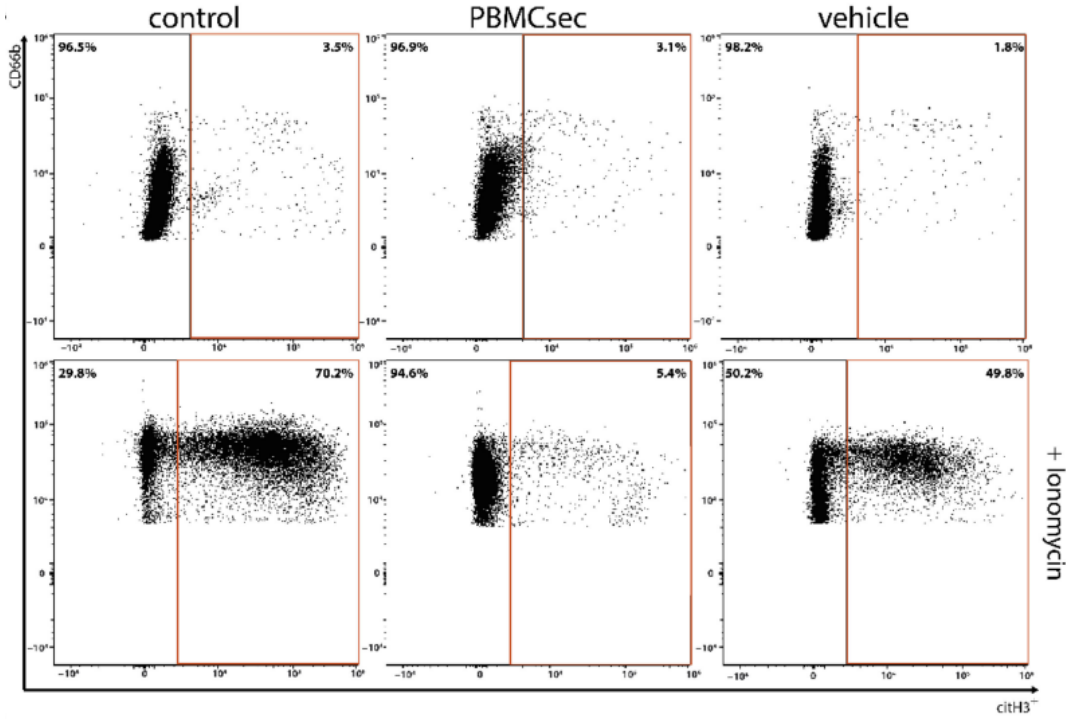
Unravel potential deviations in the potency of PBMCsec subfractions influencing NET-formation

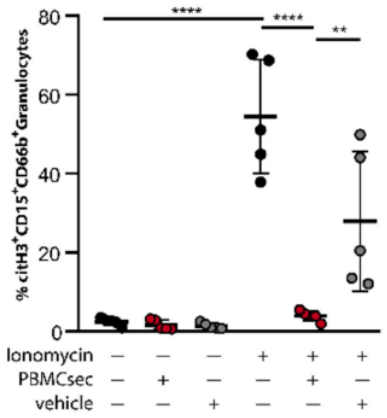
Identify the mode of action by which PBMCsec influences NET-formation



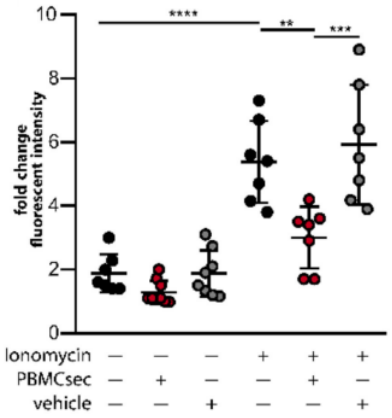
PBMCsec inhibits NET formation

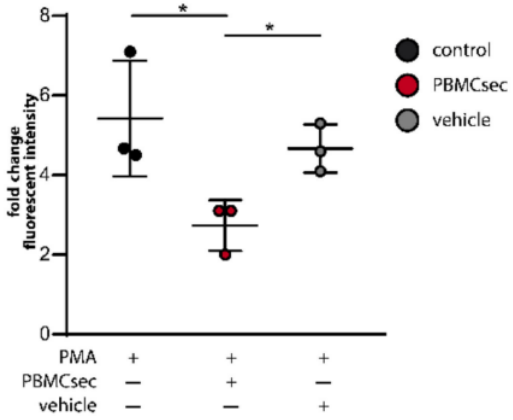
Flow cytometry





Cytox staining





PBMCsec inhibits NET formation

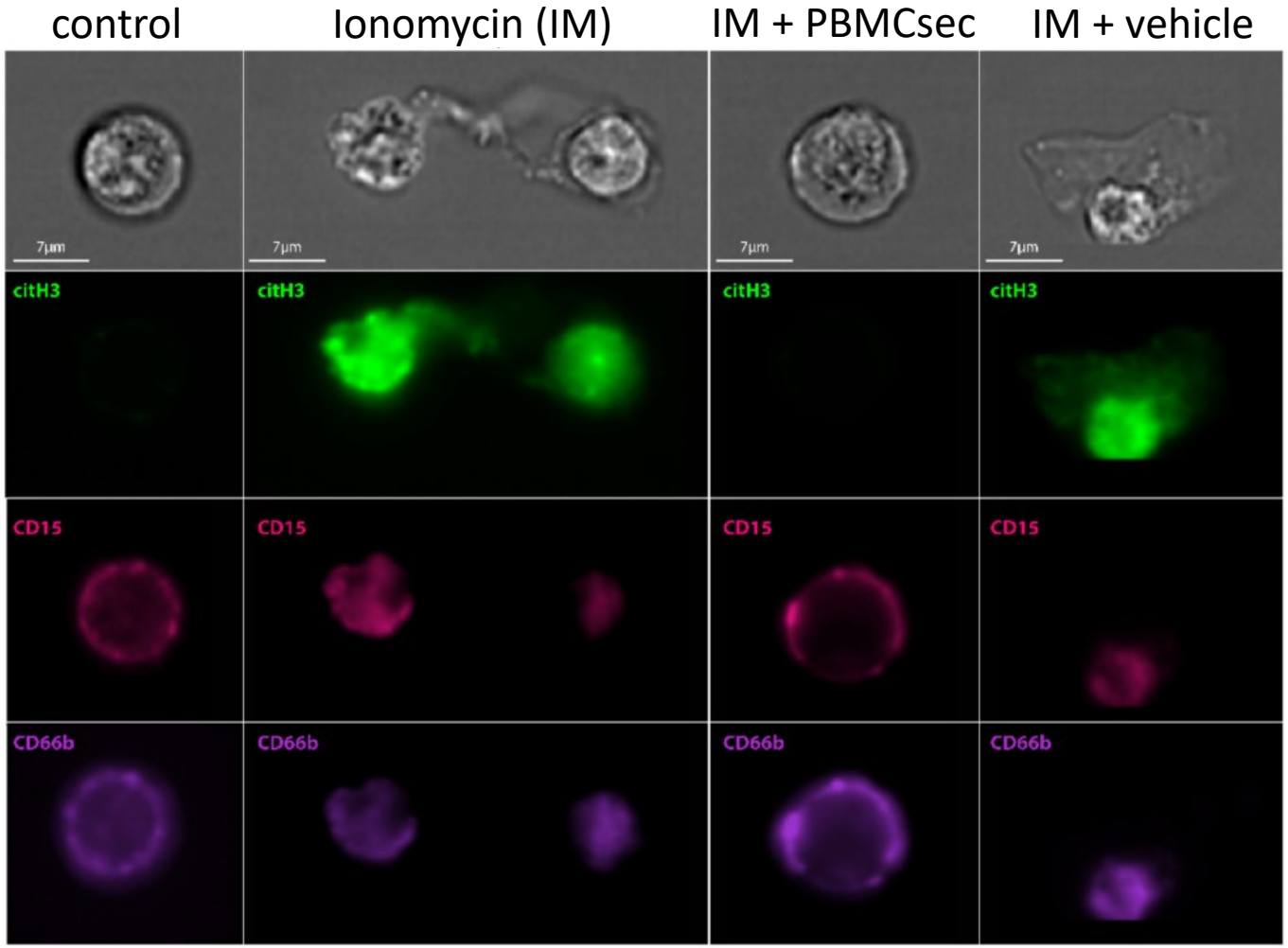
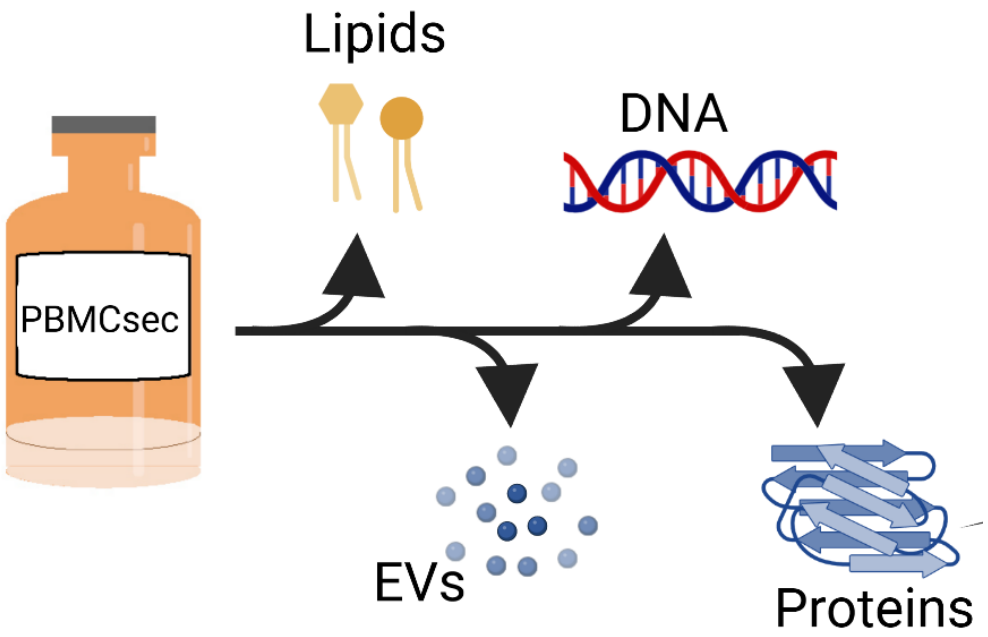
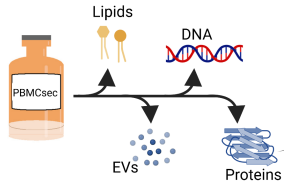


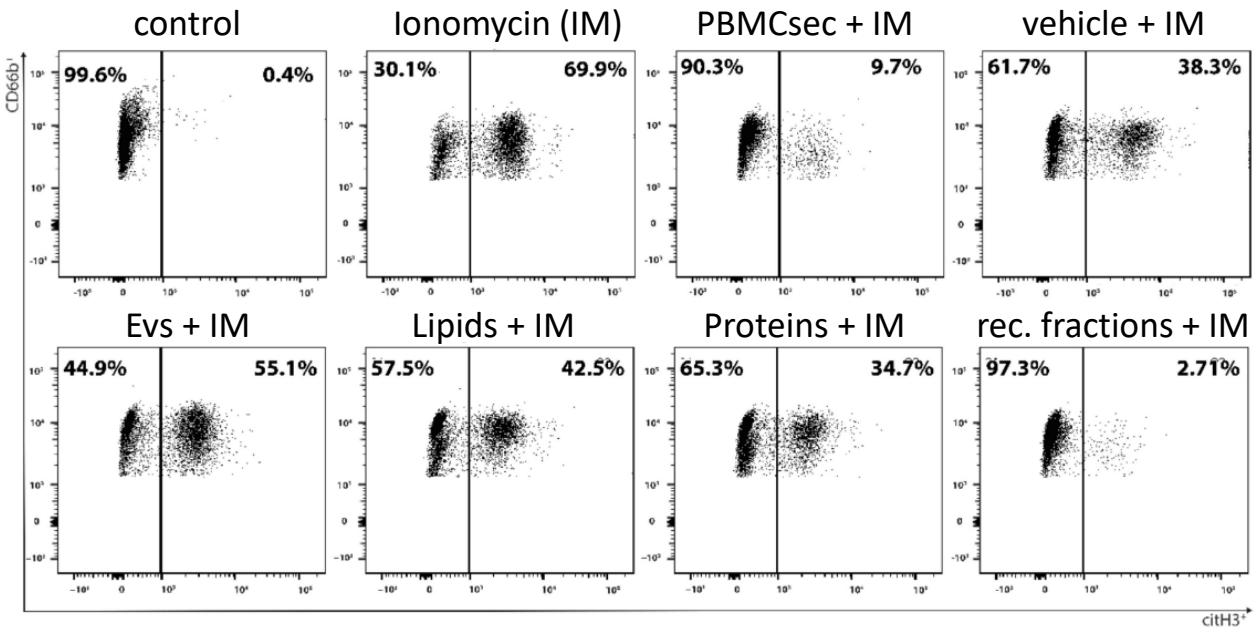
Image stream

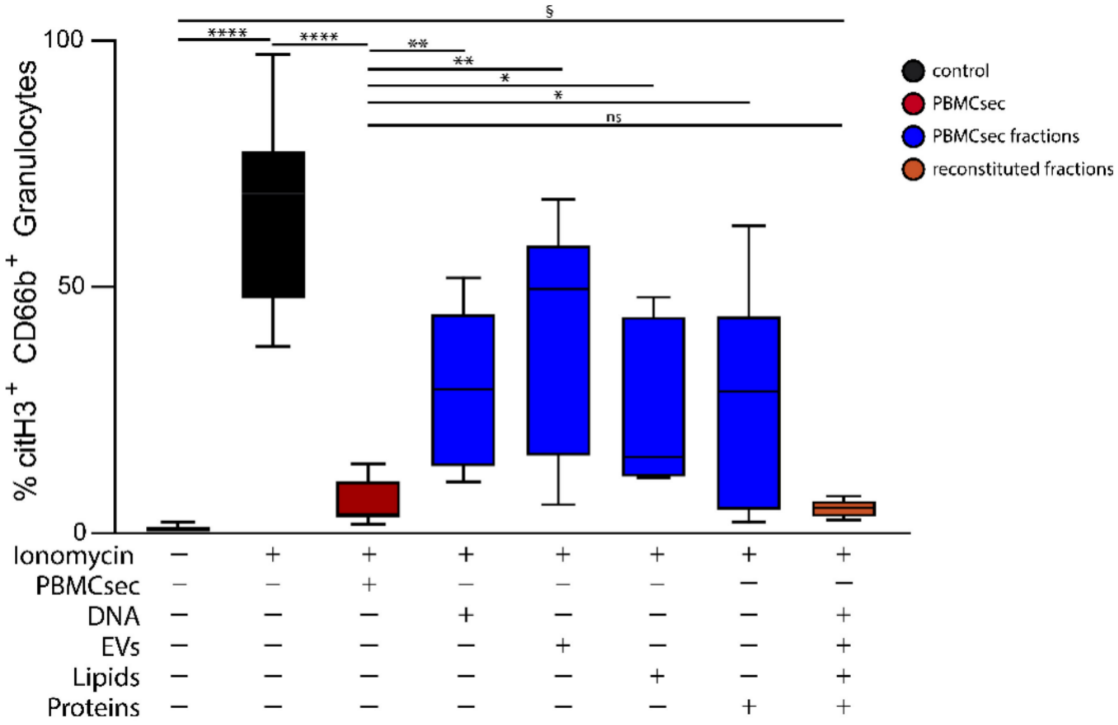
Isolated substance classes of PBMCsec



Isolated substance classes of PBMCsec show synergistic effect on NET-inhibition



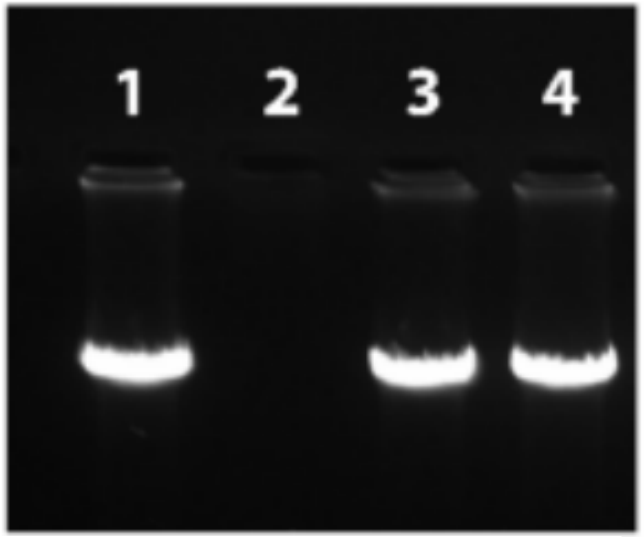




Flow cytometry

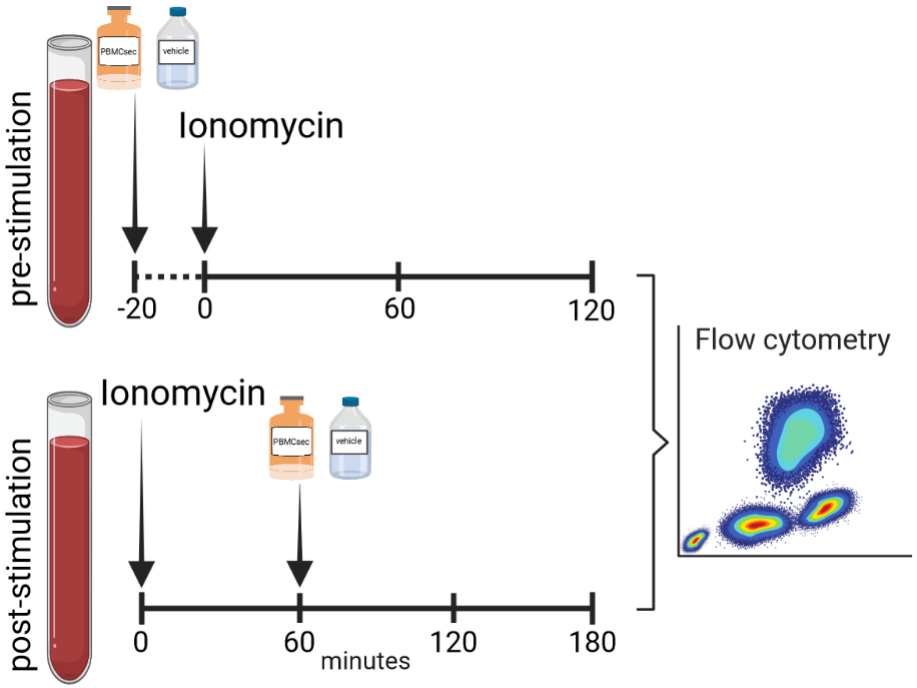
PBMCsec inhibits NET formation by DNase-independent mode of action

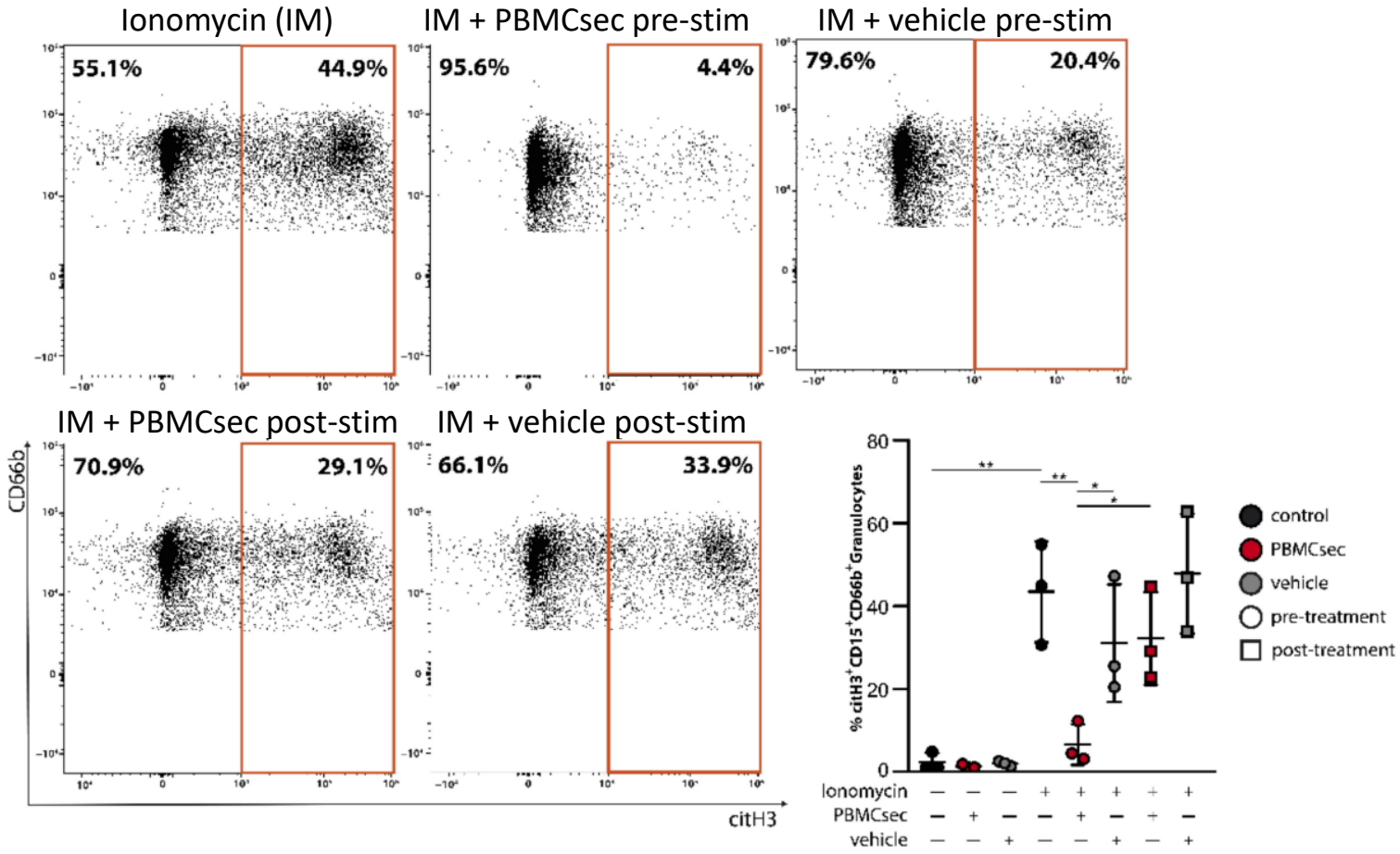
DNase activity – cell free assay



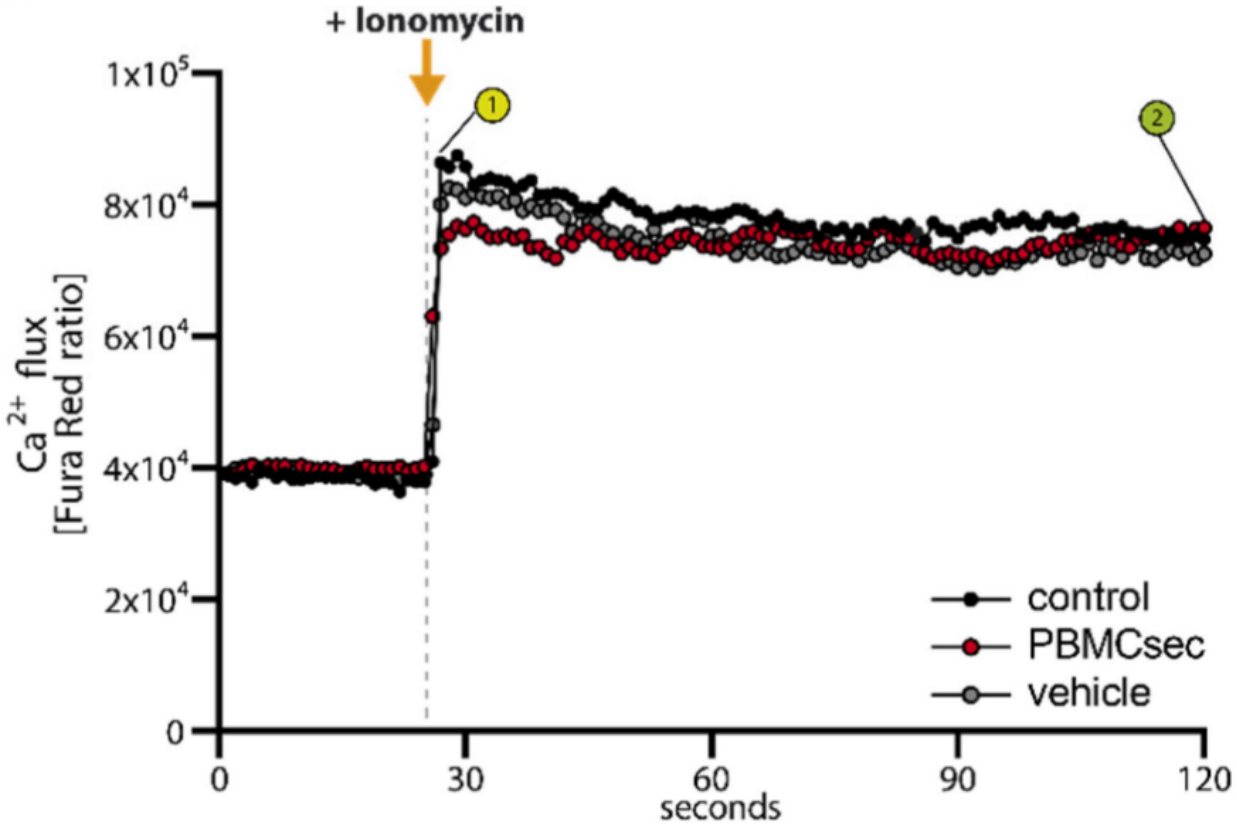
	1	2	3	4
λDNA	+	+	+	+
DNase I	-	+	-	-
PBMCsec	-	-	+	-
vehicle	-	-	-	+

PBMCsec inhibits NET formation by DNase-independent mode of action





PBMCsec inhibits NET formation without interfering with Ca-flux

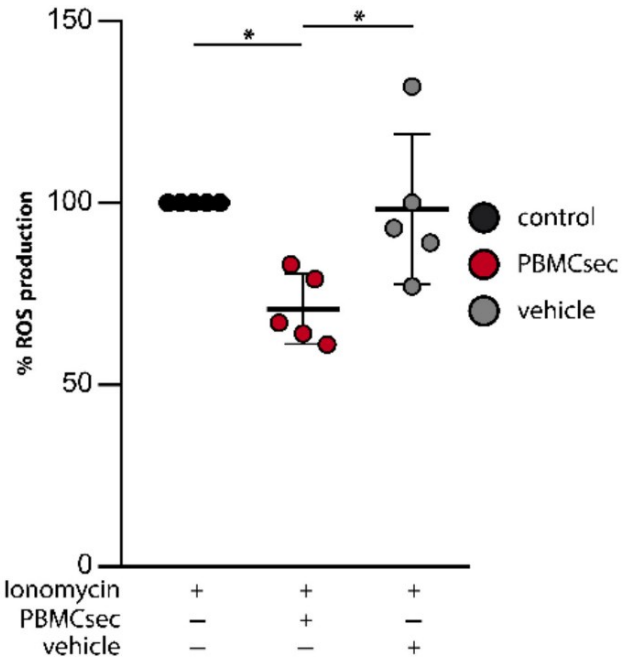


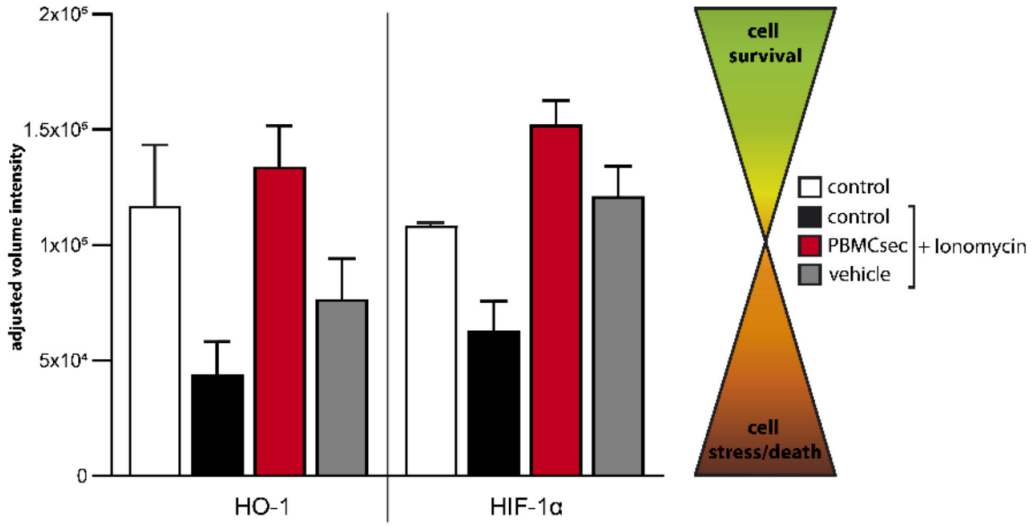
1 — stimulation with Ionomycin

2 — endpoint

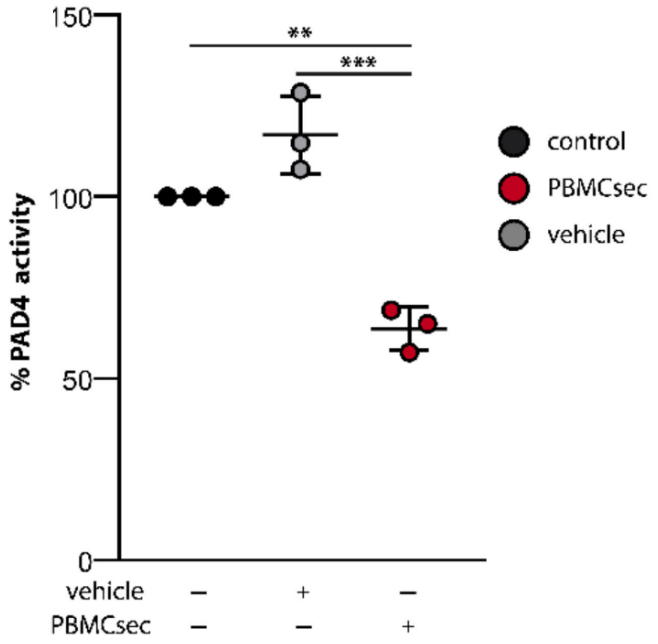
PBMCsec prevents ROS production and PAD4 activity

ROS assay





PAD4 activity – cell free assay



Discussion & Conclusion

Determine the effect of PBMCsec on NET-formation

- PBMCsec inhibits NET formation *ex vivo* upon Ionomycin- or PMA stimulation

Unravel potential deviations in the potency of PBMCsec subfractions influencing NET-formation

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Determine the effect of PBMCsec on NET-formation

- PBMCsec inhibits NET formation *ex vivo* upon Ionomycin- or PMA stimulation

Unravel potential deviations in the potency of PBMCsec subfractions influencing NET-formation

- Isolated & purified PBMCsec fractions show different efficacy in NET inhibition
- PBMCsec fractions exert synergistic effect on NET inhibtion

Identify the mode of action by which PBMCsec influences NET-formation

Discussion & Conclusion

Determine the effect of PBMCsec on NET-formation

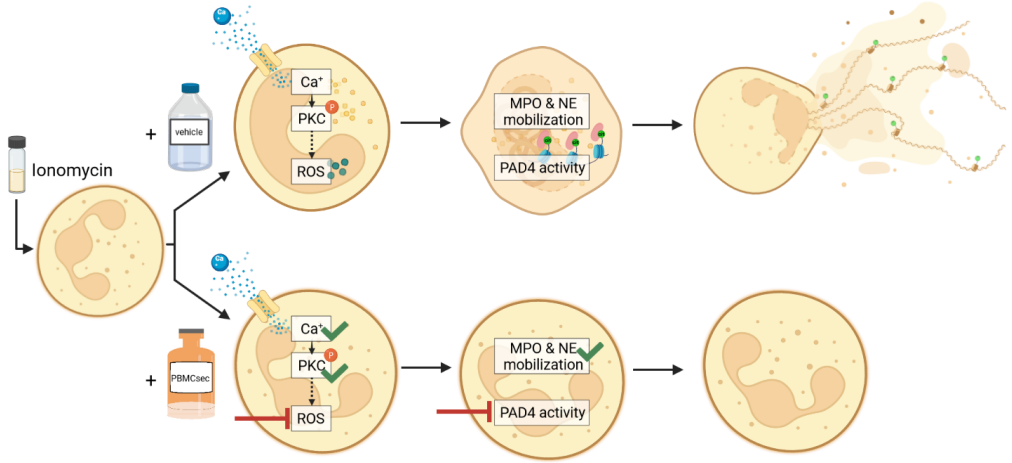
Unravel potential deviations in the potency of PBMCsec subfractions influencing NET-formation

Identify the mode of action by which PBMCsec influences NET-formation

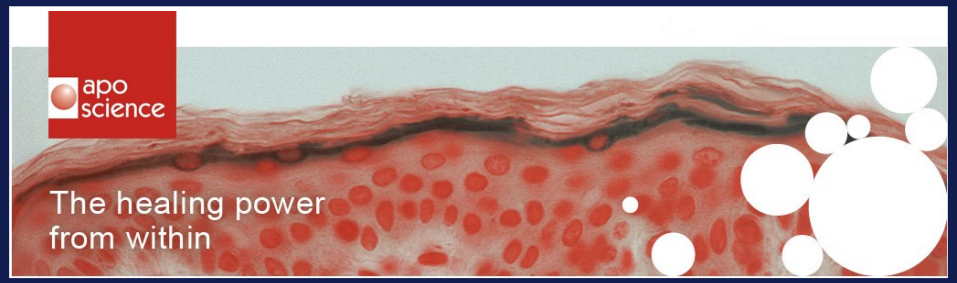
- PBMCsec inhibits NET formation *ex vivo* upon Ionomycin- or PMA stimulation

- Isolated & purified PBMCsec fractions show different efficacy in NET inhibition
- PBMCsec fractions exert synergistic effect on NET inhibtion

- PBMCsec inhibits NET formation in a DNase- independent mode of action
- PBMCsec does not interfere with Ca-flux
- PBMCsec reduces ROS production in activated neutrophils
- PBMCsec reduces PAD4 activity



Acknowledgments



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