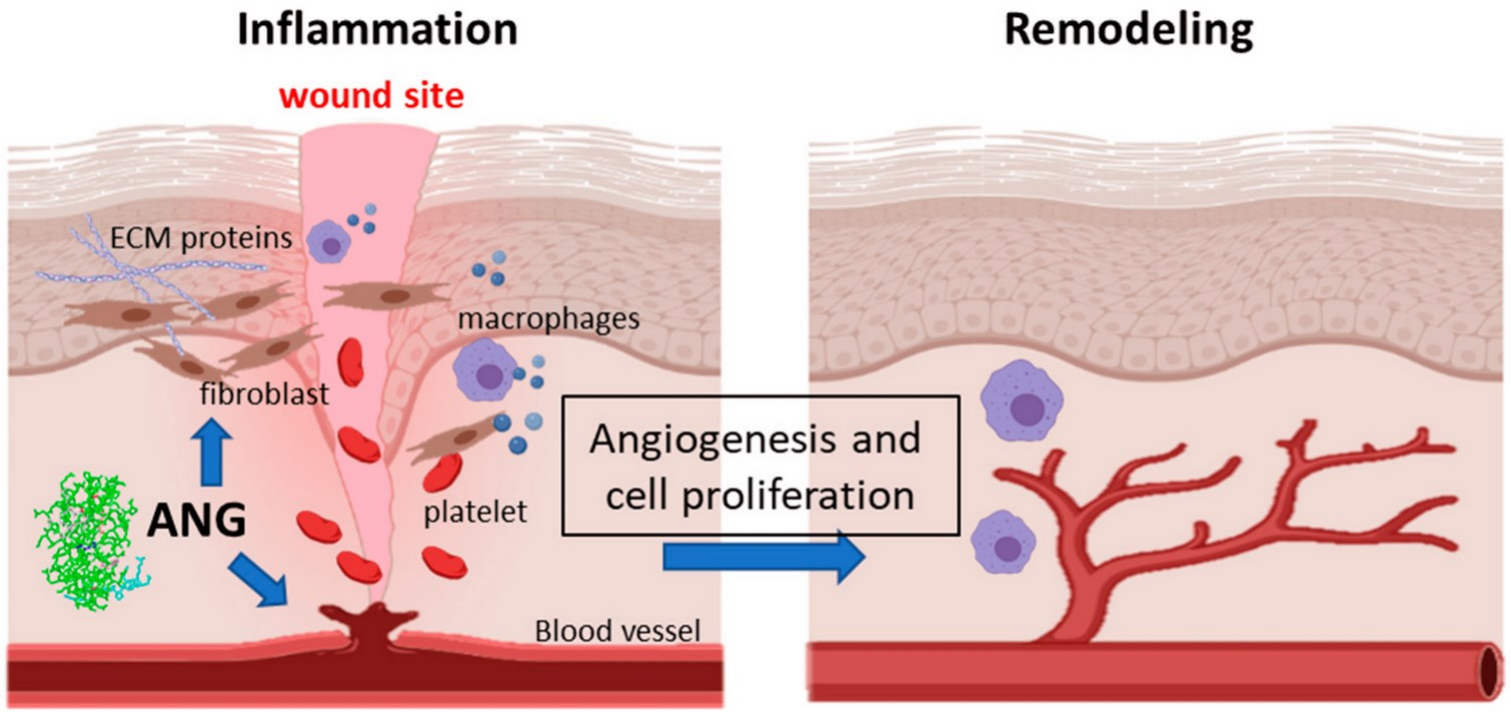
The role of the apoptotic and necroptotic peripheral blood mononuclear cell secretome in angiogenesis and wound healing

Doctoral viva

Dr. med. univ. Elisabeth Simader

July 15th 2024

The importance of wound healing



Dysfunctional wound healing leads to:

Infections, scars, loss of function, amputation, economic burden

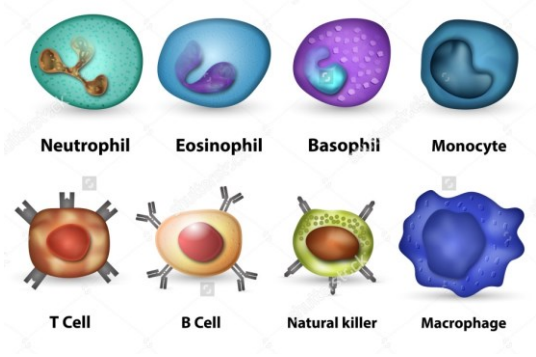
Cucci et.al. Int. J. Mol. Sci. PMID: 34639045

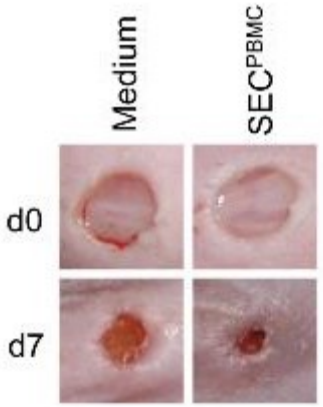
PBMC supernatant ameliorates wound closure

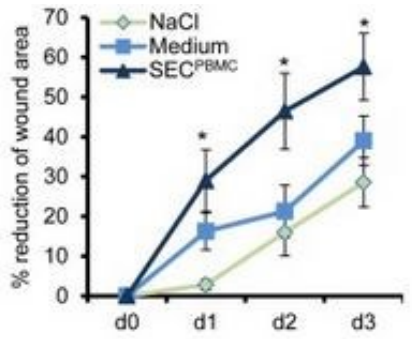
Secretome of Peripheral Blood Mononuclear Cells Enhances Wound Healing

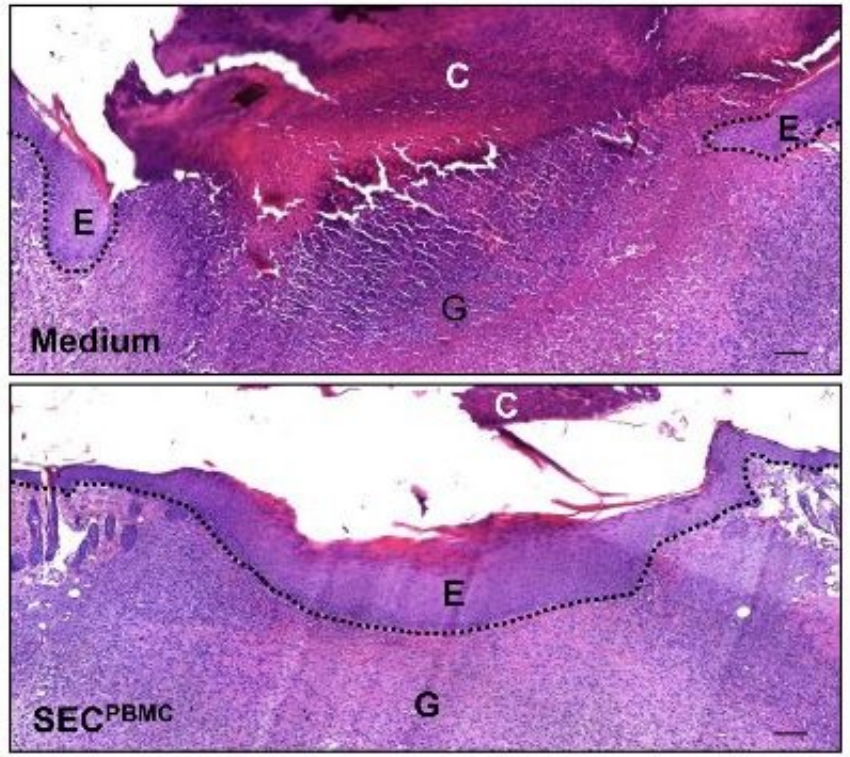
Michael Mildner[1,*], Stefan Hacker[2,3,*], Thomas Haider[3,4], Maria Gschwandtner[1], Gregor Werba[5], Caterina Barresi[1], Matthias Zimmermann[3,4], Bahar Golabi[3,4], Erwin Tschachler[1,6], Hendrik Jan Ankersmit[3,4,*]

1 Department of Dermatology, Medical University Vienna, Vienna, Austria, **2** Department of Plastic Surgery, Medical University Vienna, Vienna, Austria, **3** Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Vienna, Austria, **4** Department of Thoracic Surgery, Medical University Vienna, Vienna, Austria, **5** Department of Surgery, Medical University Vienna, Vienna, Austria, **6** Centre de Recherches et dInvestigations Epidermiques et Sensorielles (CE.R.I.E.S.), Neuilly, France

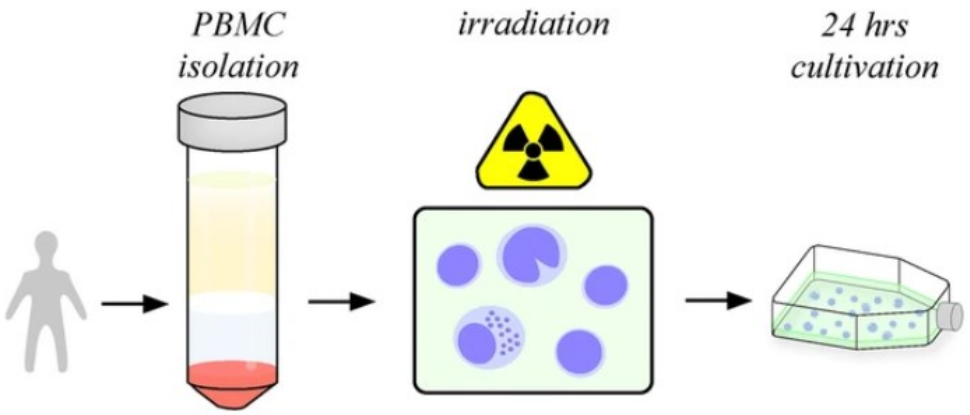


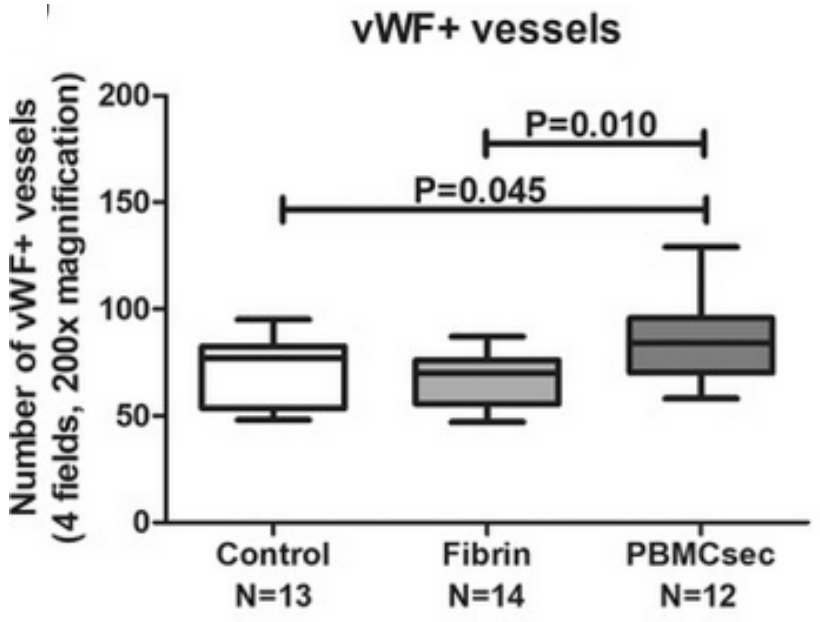






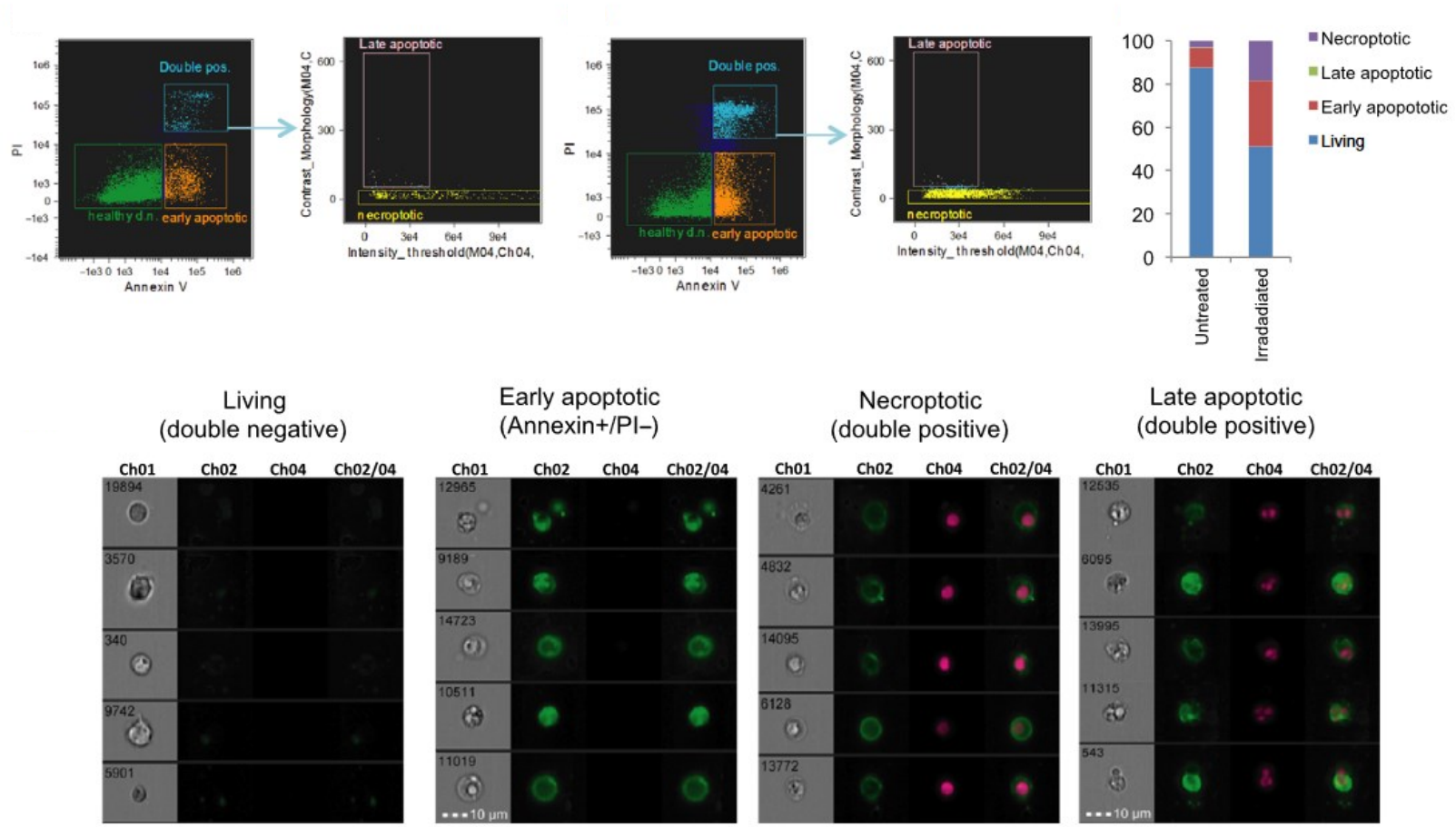
Enhancement of pro-angiogenic factors by irradiation





Laggner et al. Mol Ther Methods Clin Dev. PMID: 33768126 ; Hacker et.al. Bioeng Trans. Med.. PMID: 33532586

γ-irradiation induces cell death: apoptosis and necroptosis



Apoptosis:

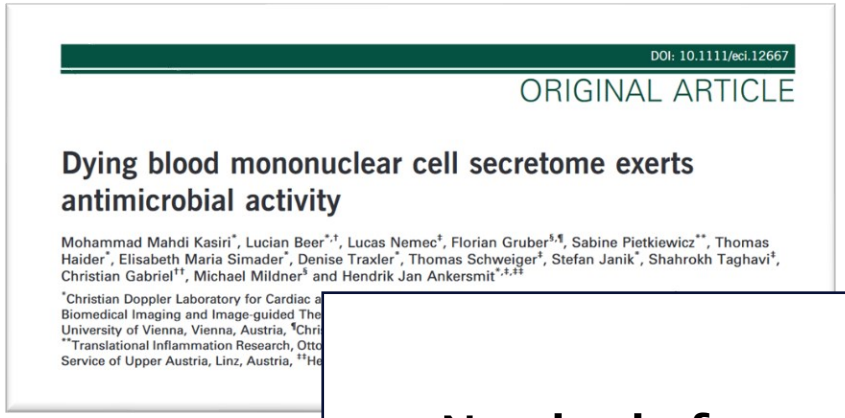
- Karyorrhexis
- Fractured nuclei
- Intact cell membrane

Necroptosis:

- Oncosis
- Intact nuclei
- Pore formation in the cell membrane

Kasiri et.al. Eur J Clin Invest. PMID:27513763

What causes this effect?

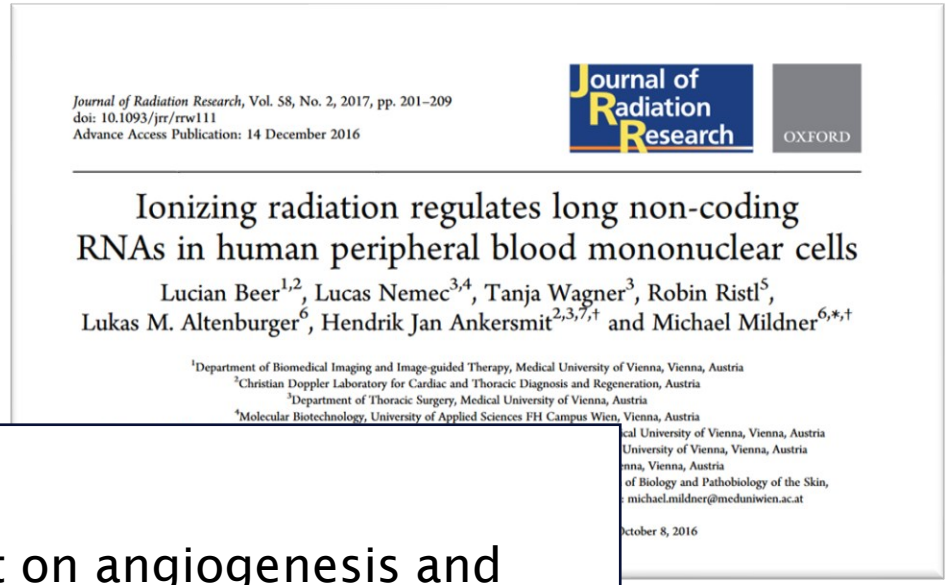

DOI: 10.1111/eci.12667

ORIGINAL ARTICLE

Dying blood mononuclear cell secretome exerts antimicrobial activity

Mohammad Mahdi Kasiri*, Lucian Beer*,†, Lucas Nemec‡, Florian Gruber§,¶, Sabine Pietkiewicz**, Thomas Haider*, Elisabeth Maria Simader*, Denise Traxler*, Thomas Schweiger‡, Stefan Janik*, Shahrokh Taghavi‡, Christian Gabriel††, Michael Mildner§ and Hendrik Jan Ankersmit*,‡,‡‡

*Christian Doppler Laboratory for Cardiac a
Biomedical Imaging and Image-guided The
University of Vienna, Vienna, Austria, ¶Chri
**Translational Inflammation Research, Otto
Service of Upper Austria, Linz, Austria, ††He


Journal of Radiation Research, Vol. 58, No. 2, 2017, pp. 201–209
doi: 10.1093/jrr/rrw111
Advance Access Publication: 14 December 2016

Journal of Radiation Research

OXFORD

Ionizing radiation regulates long non-coding RNAs in human peripheral blood mononuclear cells

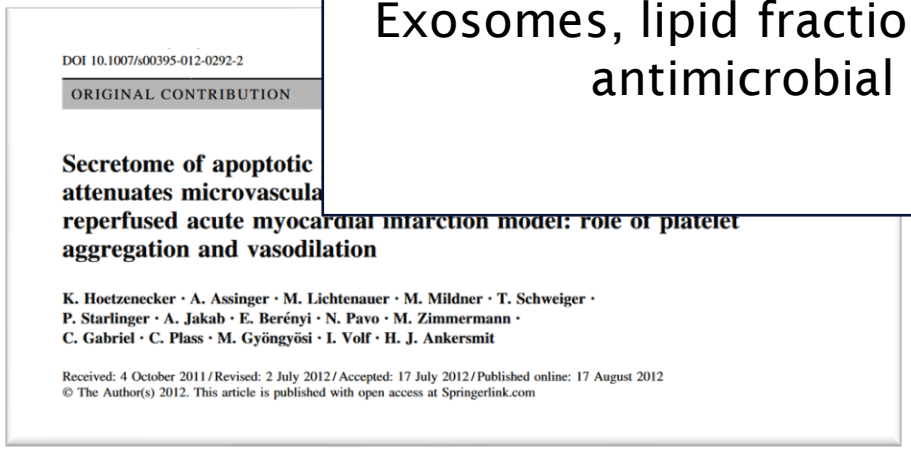
Lucian Beer[1,2], Lucas Nemec[3,4], Tanja Wagner[3], Robin Ristl[5], Lukas M. Altenburger[6], Hendrik Jan Ankersmit[2,3,7,†] and Michael Mildner[6,*,†]

[1]Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, Vienna, Austria
[2]Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Austria
[3]Department of Thoracic Surgery, Medical University of Vienna, Austria
[4]Molecular Biotechnology, University of Applied Sciences FH Campus Wien, Vienna, Austria
cal University of Vienna, Vienna, Austria
University of Vienna, Vienna, Austria
nna, Vienna, Austria
of Biology and Pathobiology of the Skin,
michael.mildner@meduniwien.ac.at

October 8, 2016

No single factor for the beneficial effect on angiogenesis and cytoprotection was found

Exosomes, lipid fractions, microvesicles, long non-coding RNAs and antimicrobial peptides alone were no responsible

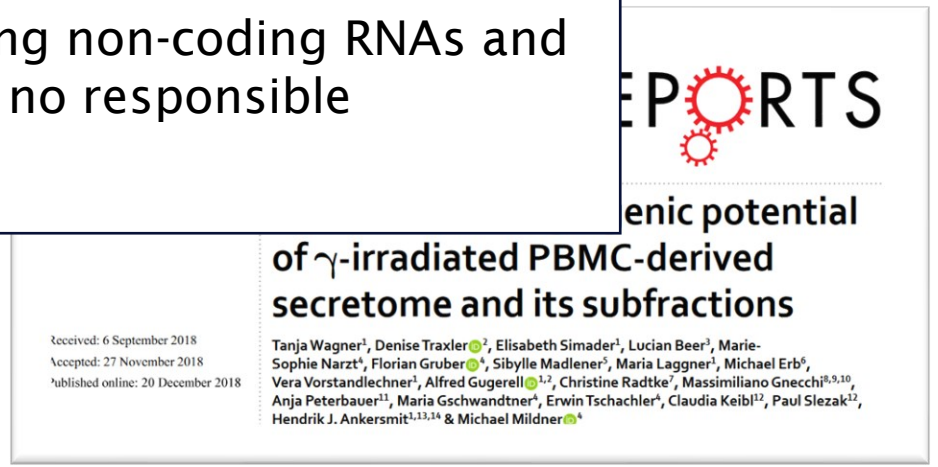

DOI 10.1007/s00395-012-0292-2

ORIGINAL CONTRIBUTION

Secretome of apoptotic attenuates microvascula reperfused acute myocardial infarction model: role of platelet aggregation and vasodilation

K. Hoetzenecker · A. Assinger · M. Lichtenauer · M. Mildner · T. Schweiger · P. Starlinger · A. Jakab · E. Berényi · N. Pavo · M. Zimmermann · C. Gabriel · C. Plass · M. Gyöngyösi · I. Volf · H. J. Ankersmit

Received: 4 October 2011 / Revised: 2 July 2012 / Accepted: 17 July 2012 / Published online: 17 August 2012
© The Author(s) 2012. This article is published with open access at Springerlink.com


EPORTS

enic potential

of γ-irradiated PBMC-derived secretome and its subfractions

Received: 6 September 2018
Accepted: 27 November 2018
Published online: 20 December 2018

Tanja Wagner[1], Denise Traxler[2], Elisabeth Simader[1], Lucian Beer[3], Marie-Sophie Narzt[4], Florian Gruber[4], Sibylle Madlener[5], Maria Laggner[1], Michael Erb[6], Vera Vorstandlechner[1], Alfred Gugerell[1,2], Christine Radtke[7], Massimiliano Gnecchi[8,9,10], Anja Peterbauer[11], Maria Gschwandtner[4], Erwin Tschachler[4], Claudia Keibl[12], Paul Slezak[12], Hendrik J. Ankersmit[1,13,14] & Michael Mildner[4]

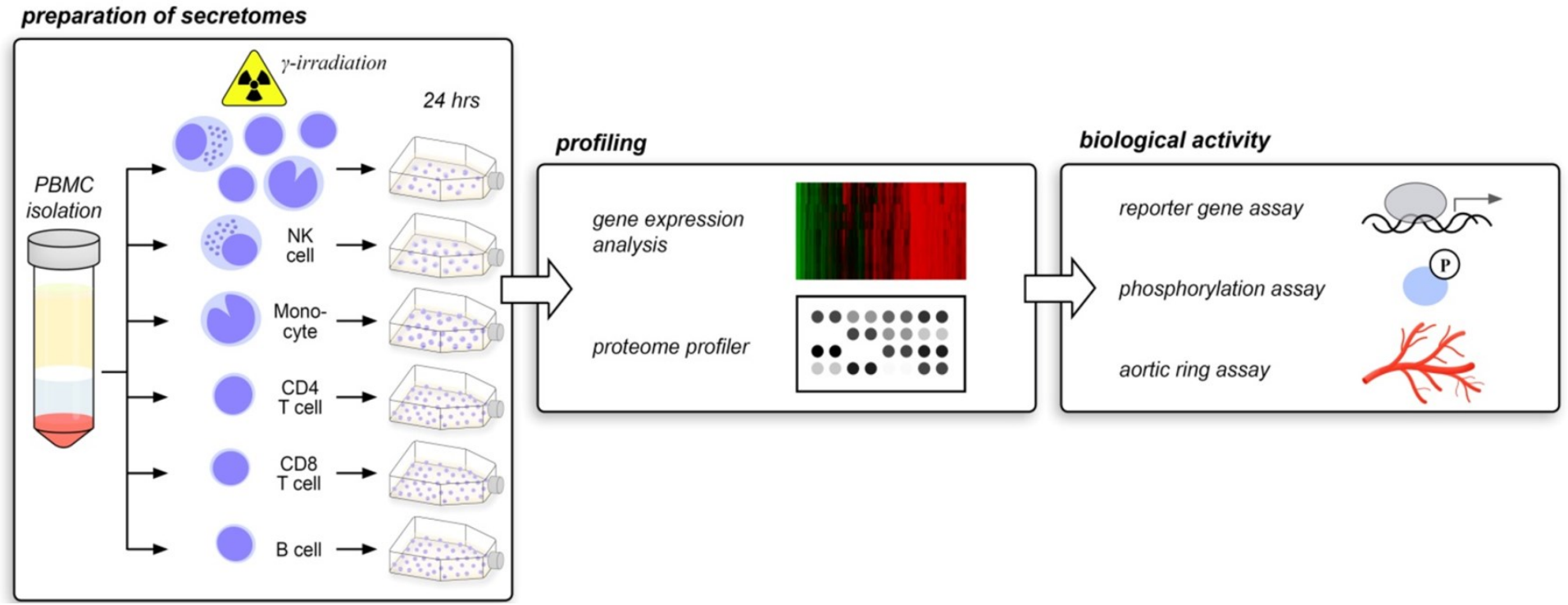
Scientific aims:

1. What is the role of PBMC subsets in angiogenesis?

2. Does the type of cell death have an effect on angiogenesis?

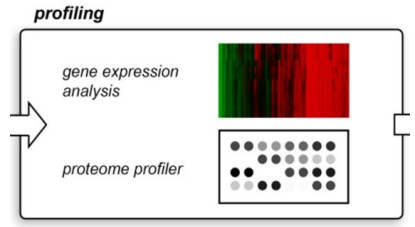
3. Translation of PBMC secretome into clinics - safety of topical application.

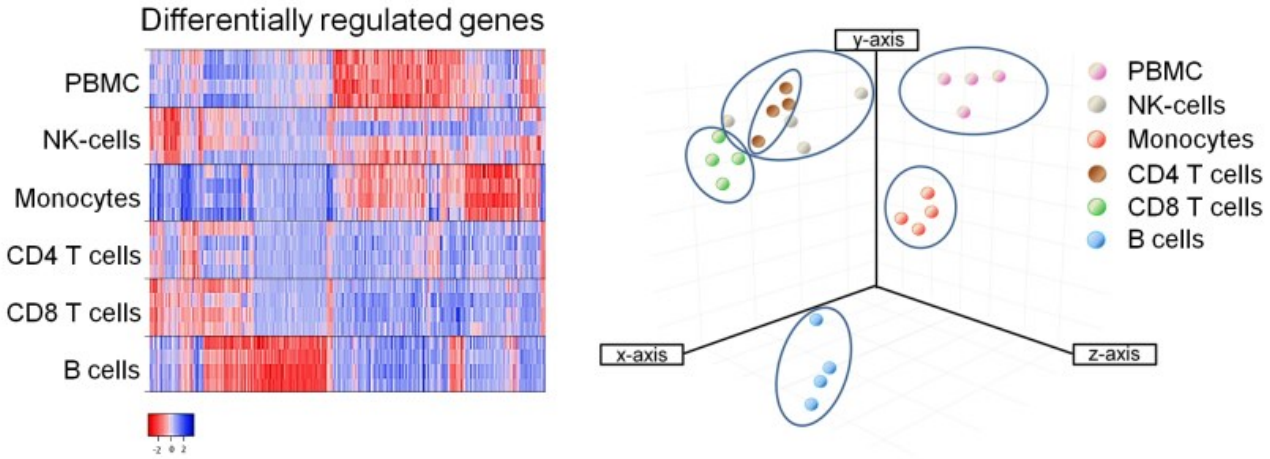
1. What is the role of PBMC subsets in angiogenesis?



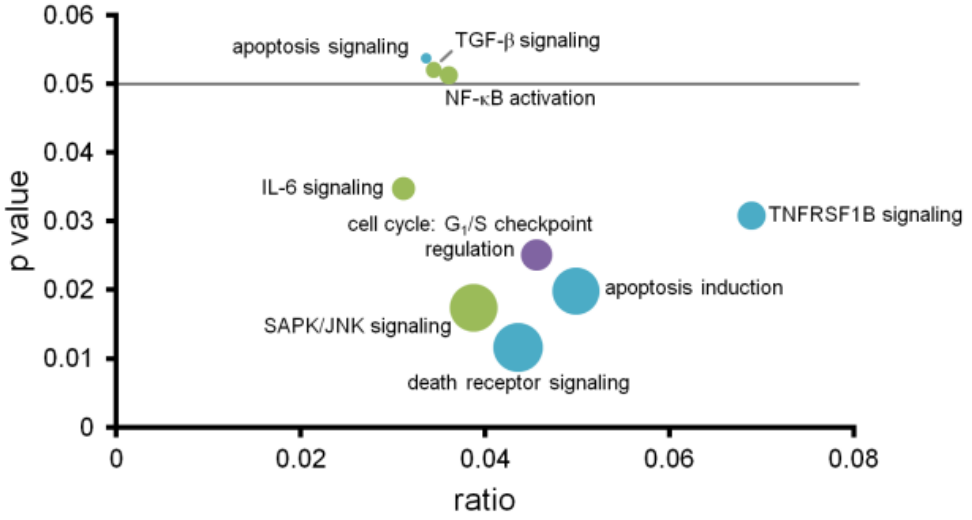
Experimental approach

Genetic profiling of PBMC subsets



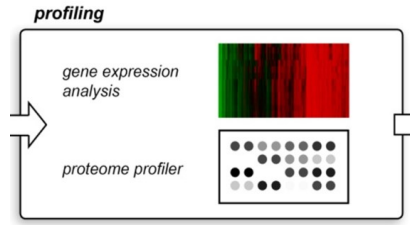


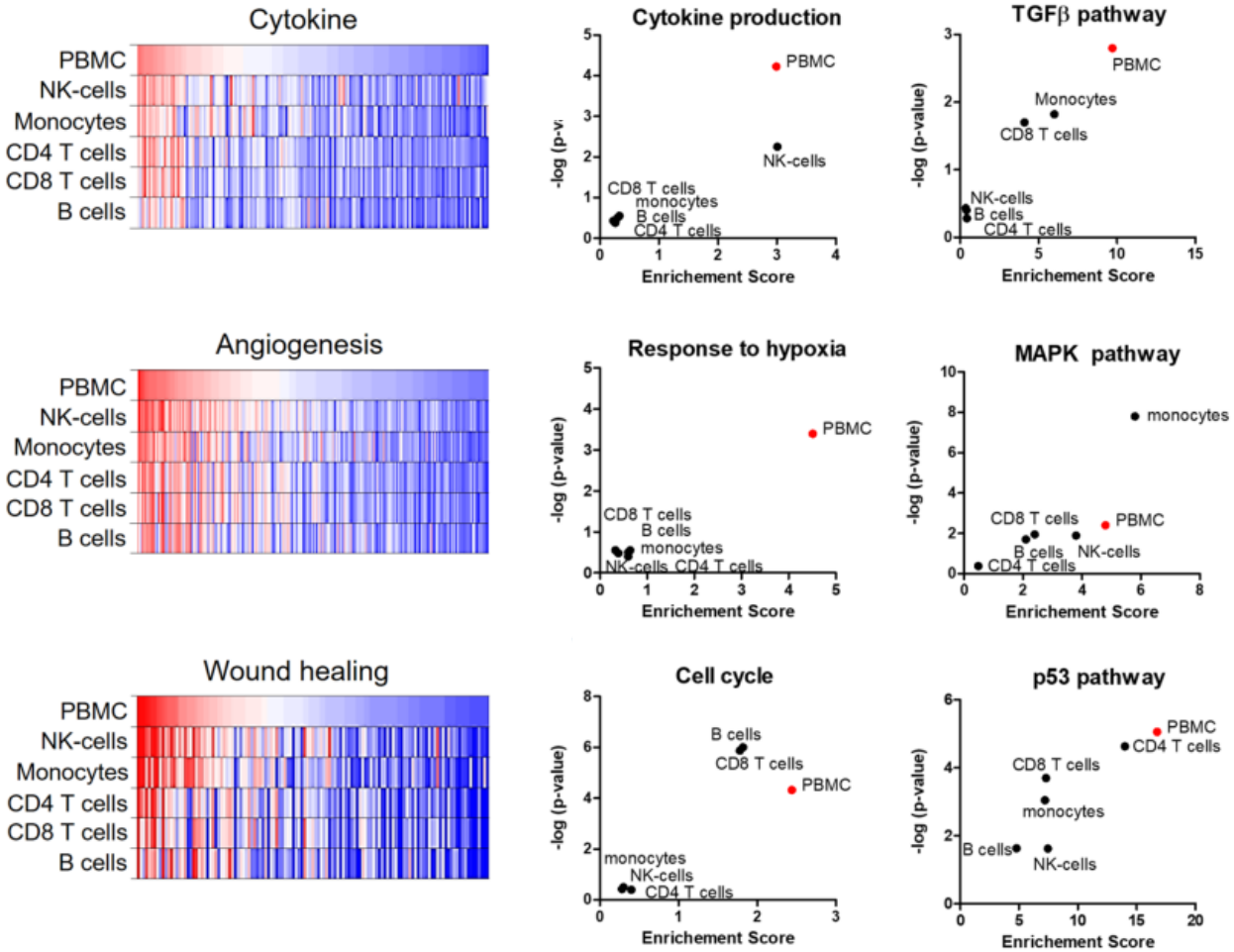
Whole PBMCs show distinct expression profiles as compared to PBMC subsets after irradiation



GO term analysis highlights genes associated with cell death

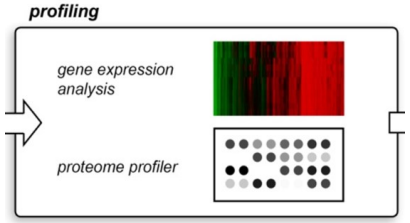
Whole PBMCs vs. PBMC subsets

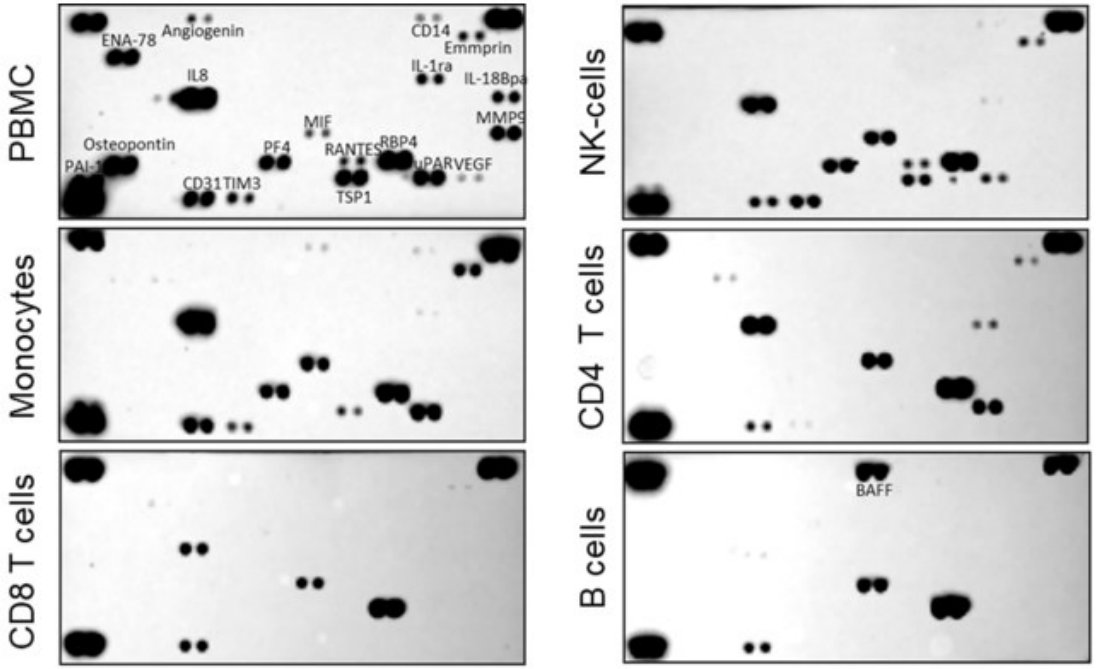




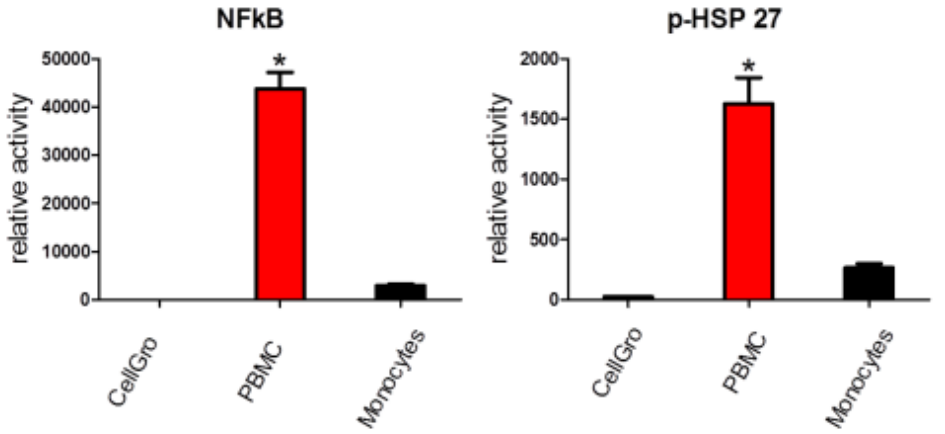
Simader et.al. Cell Death & Dis. PMID: 31570701

Protein secretion and phosphorylation assay





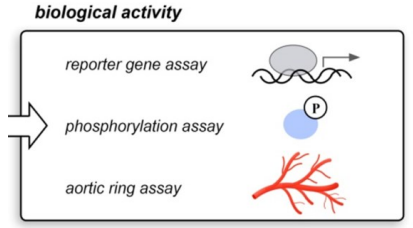
Whole PBMC supernatants comprised of specific cytokines and pro-angiogenic factors

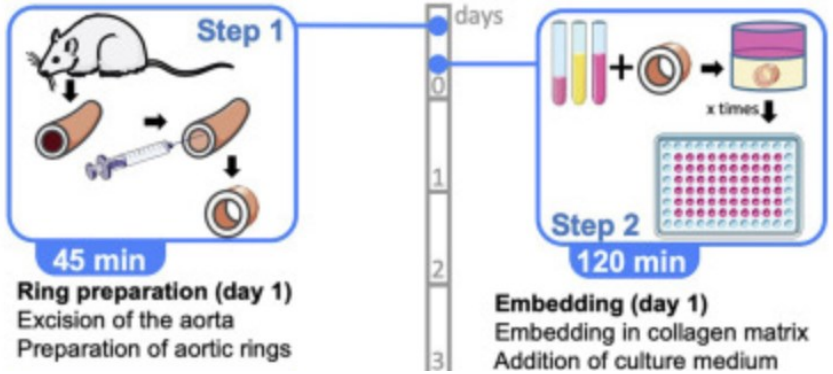


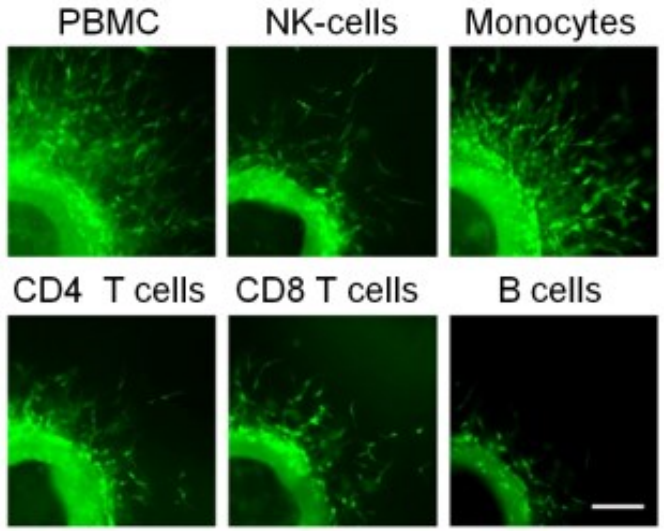
Activation of NFκB and HSP-27 upon stimulation with PBMC supernatant

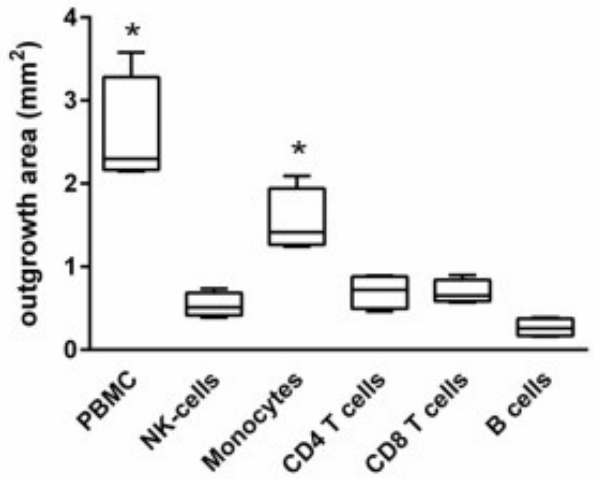
Simader et.al. Cell Death & Dis. PMID: 31570701

In vivo angiogenic potential of the PBMC secretome









PBMC and monocyte supernatants show the most pro-angiogenic potential

Blot et al. Star protocols PMID: 33532730 & Simader et.al. Cell Death & Dis. PMID: 31570701

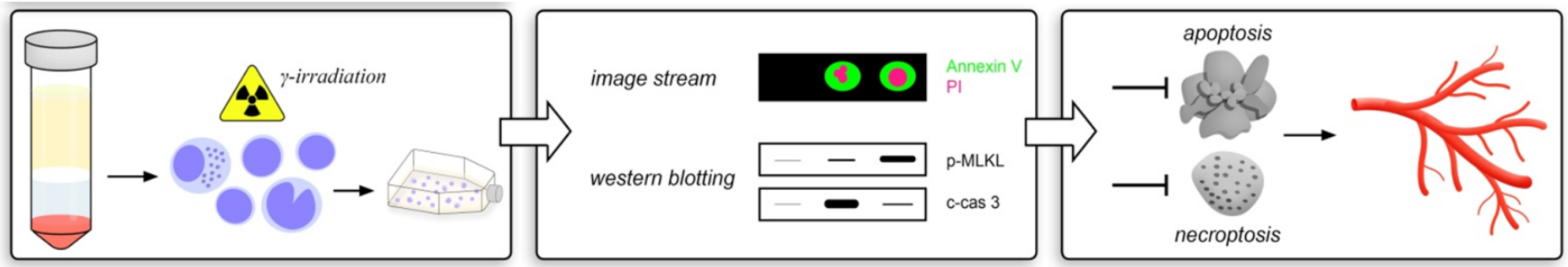
Scientific aims:

1. What is the role of PBMC subsets in angiogenesis?

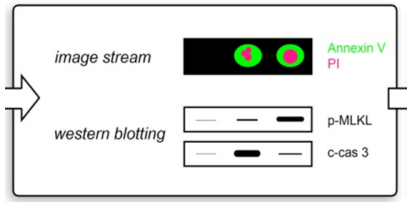
2. Does the type of cell death have an effect on angiogenesis?

3. Translation of PBMC secretome into clinics - safety of topical application.

Aim 2: Role of cell death type on angiogenesis?



Cell death induction after irradiation



Scanning electron microscopy of human PBMC after irradiation

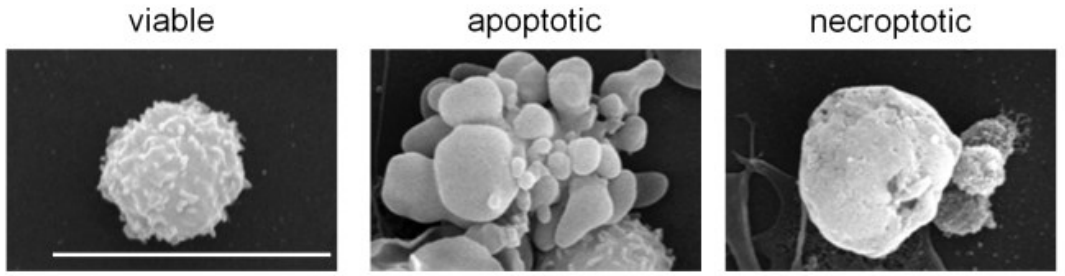
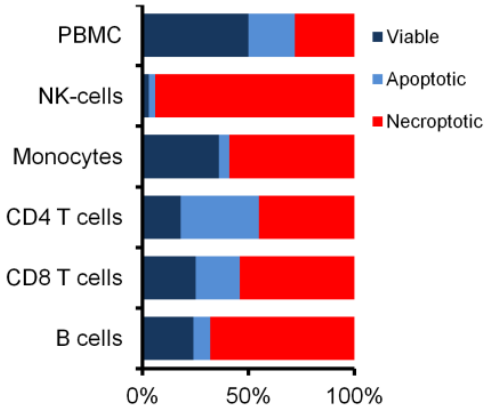
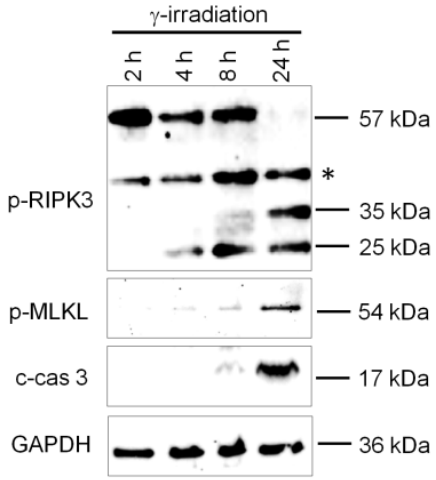
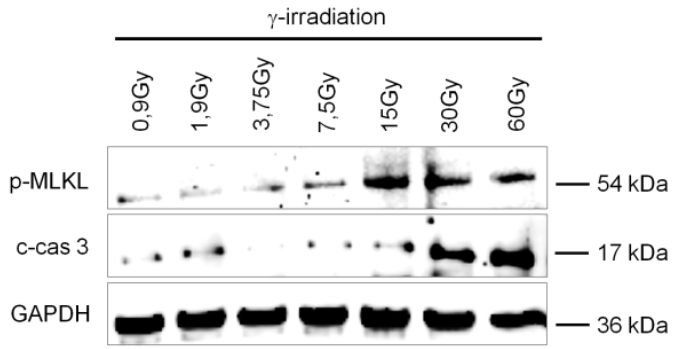


Image stream analysis

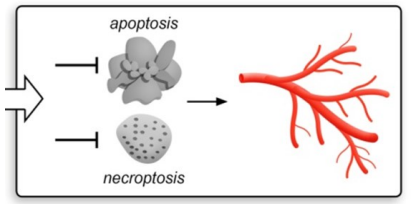




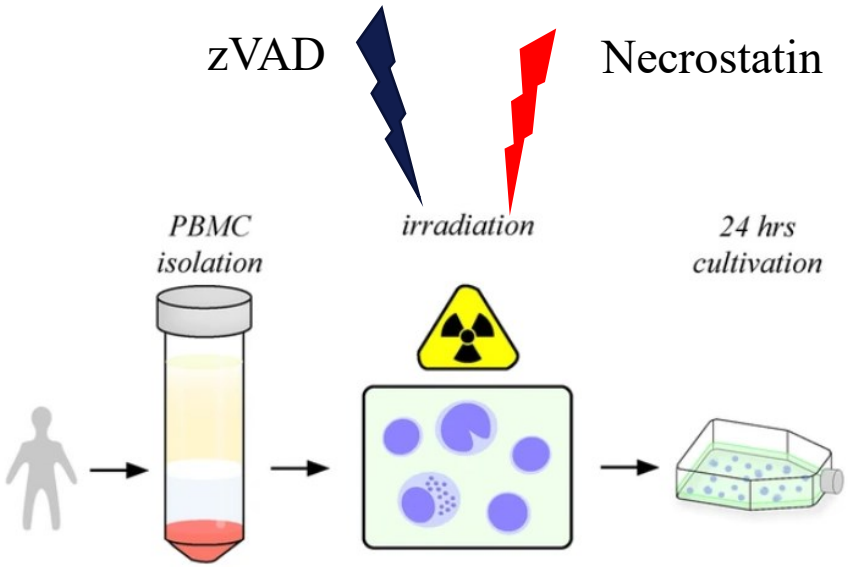


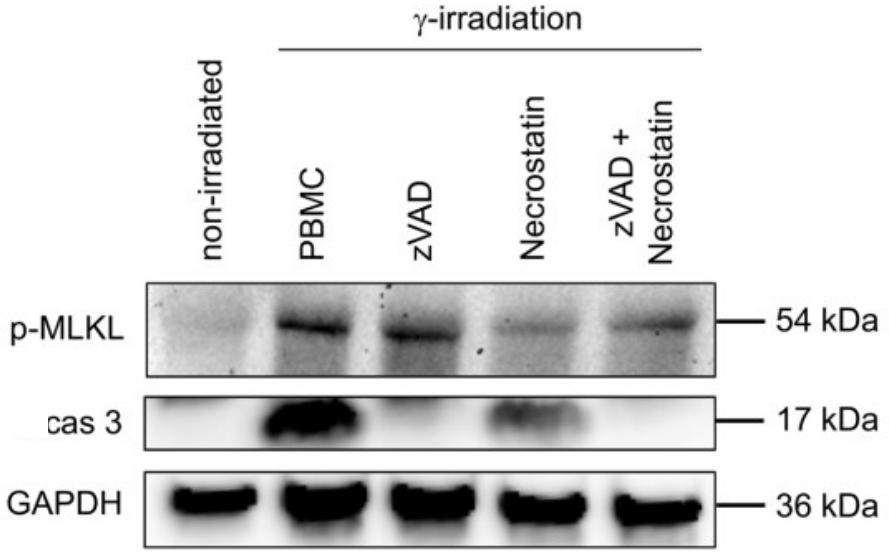
Simader et.al. Cell Death & Dis. PMID: 31570701

Is the type of cell death affecting angiogenesis?



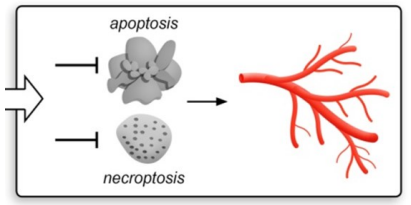
- **zVAD** – inhibition of apoptosis
- **Necrostatin** – inhibition of necroptosis



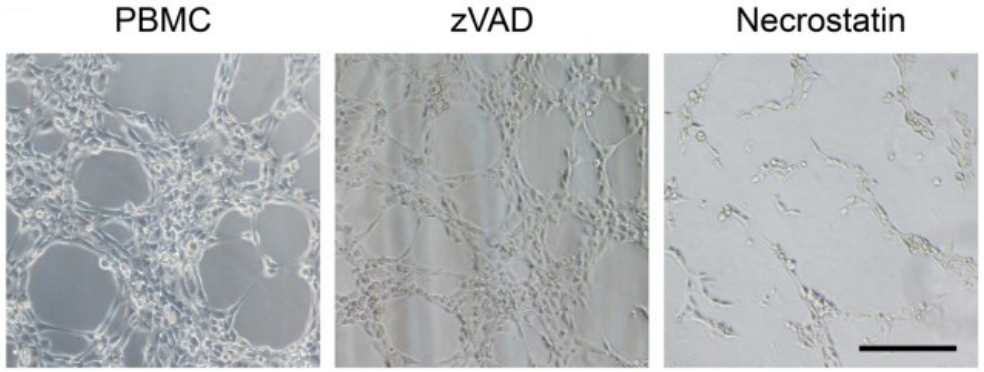


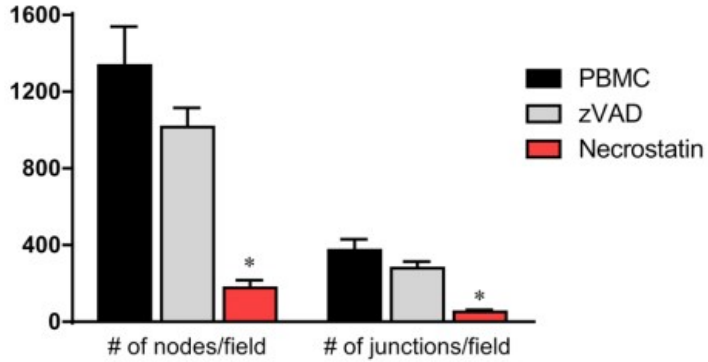
Simader et.al. Cell Death & Dis. PMID: 31570701

Is the type of cell death affecting angiogenesis?

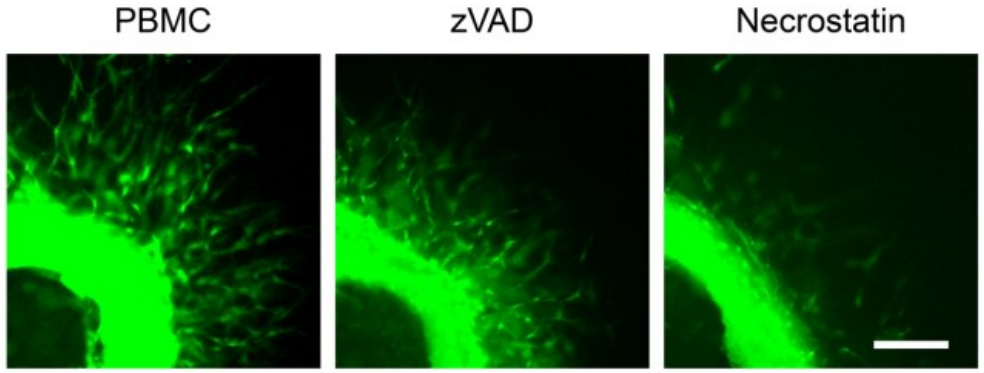


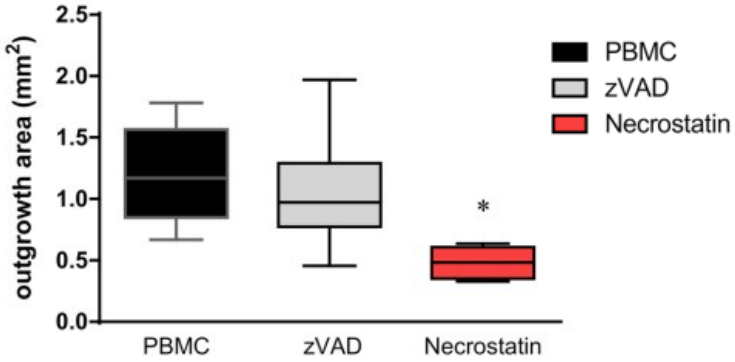
HUVEC based tube formation assay





Aortic ring sprouting assay





Simader et.al. Cell Death & Dis. PMID: 31570701

Conclusion

- Necroptosis rather than apoptosis seems to have a pro-angiogenic effect.
- Monocytes are the most capable among PBMC for inducing of angiogenesis
- Co-culture of PBMC may have a synergistic effect on angiogenesis

Scientific aims:

1. What is the role of PBMC subsets in angiogenesis?

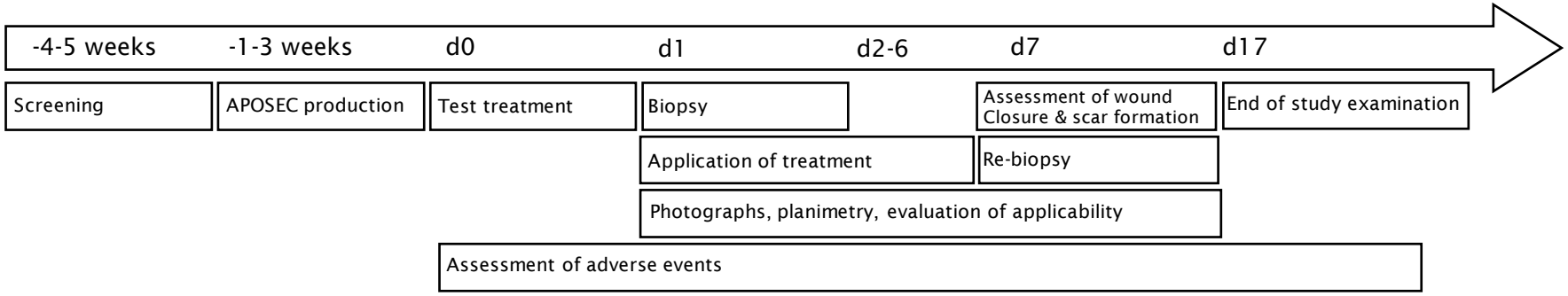
2. Does the type of cell death have an effect on angiogenesis?

3. Translation of PBMC secretome into clinics - safety of topical application.

Clinical phase I trial:
Autologous PBMC secretome on artificial wounds

- 10 healthy probands were included
- Two dose groups of autologous PBMC secretome (APOSEC)

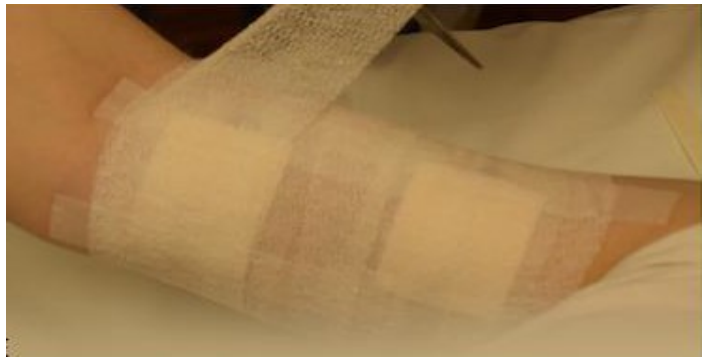
  - (25 Mio/ml or 12,5 Mio/ml)
- ECG, physical examination and laboratory results for safety evaluation

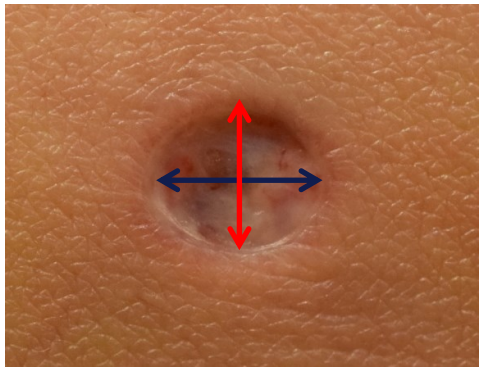


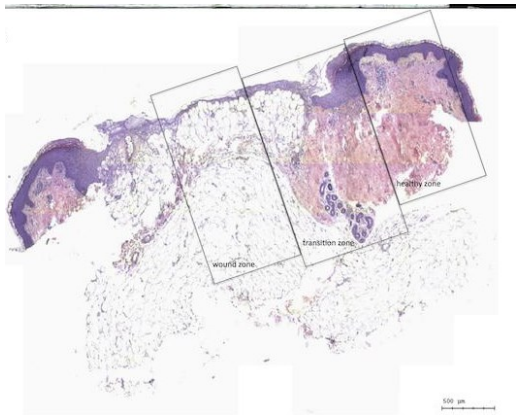
Simader et.al. Sci. Rep. PMID: 28740204

Clinical phase I trial: MARSYAS

- Primary endpoint: Safety of topical application
- Secondary endpoint: Faster wound closure in treatment groups







Simader et.al. Sci. Rep. PMID: 28740204

Safety

No serious adverse events were found at any of the screened subjects

Laboratory results: No abnormalities were detected at any of the subjects

AES reported:

- Mild erythema (2)
- Itching (1)
- Hematoma after punch biopsy (1)
- Small bleeding (1)
- Muscle tension (1)
- Sore throat (1)

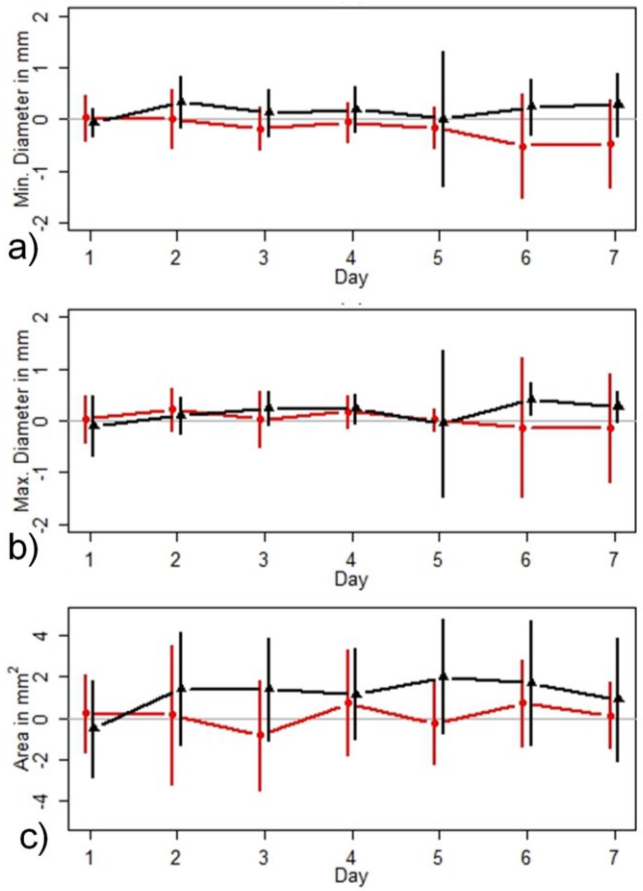
Simader et.al. Sci. Rep. PMID: 28740204

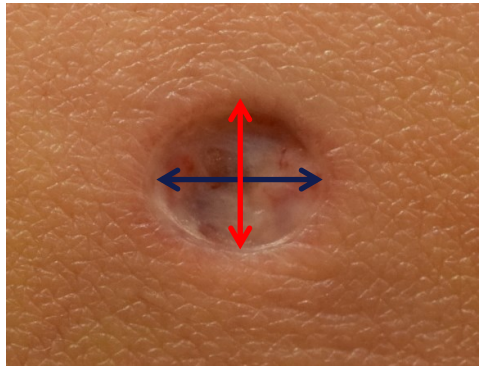
Efficacy

Planimetry:
- Minimal diameter
- Maximal diameter
- Area in mm^2

Black: High dose
Red: low dose

Normalized to Placebo





No significant difference between placebo, low dose and high dose group was assessed

Simader et.al. Sci. Rep. PMID: 28740204

Conclusion

- The application of APOSEC was safe on wounds
- No significant difference in wound closure between wounded areas in the high dose group, low dose group or placebo was found
- Limitations: short study duration of 7 days, low number of participants

Acknowledgements

Hendrik Jan Ankersmit

Michael Mildner

Lucian Beer

Maria Laggner

Tanja Wagner

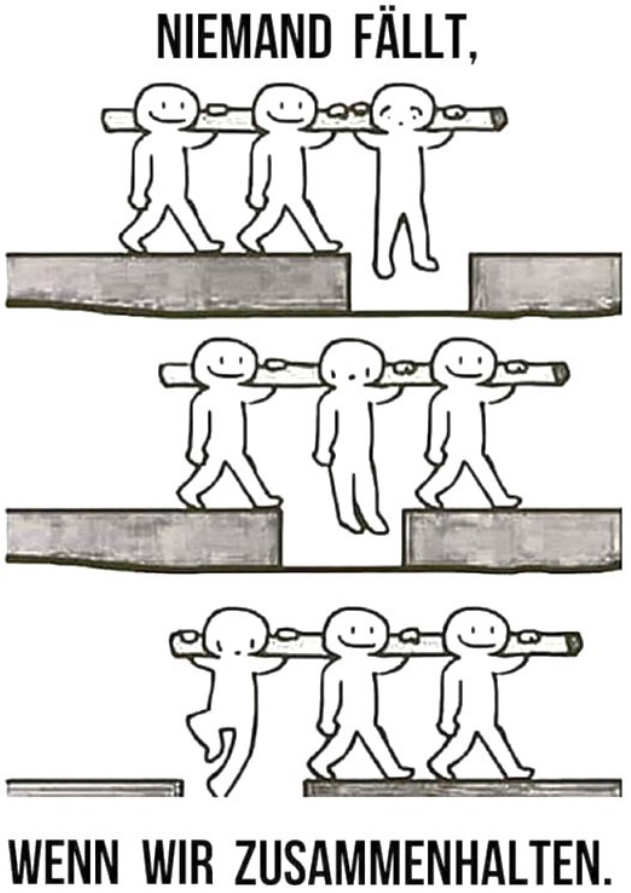
Vera Vorstandlechner

Alfred Gugerell

Dragan Copic

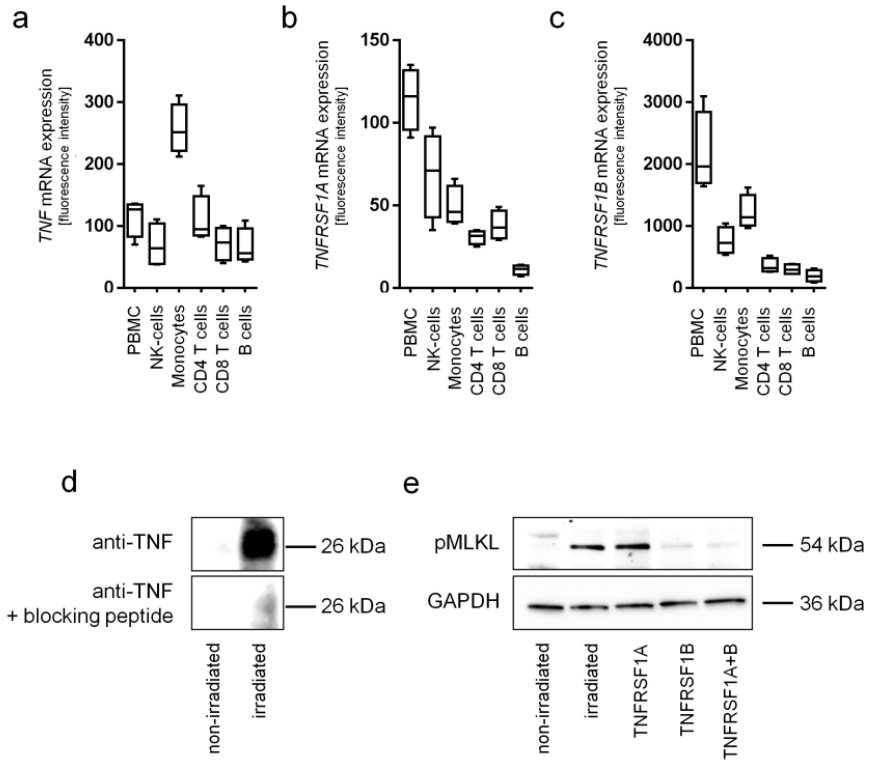
Katharina Klas

Martin Direder

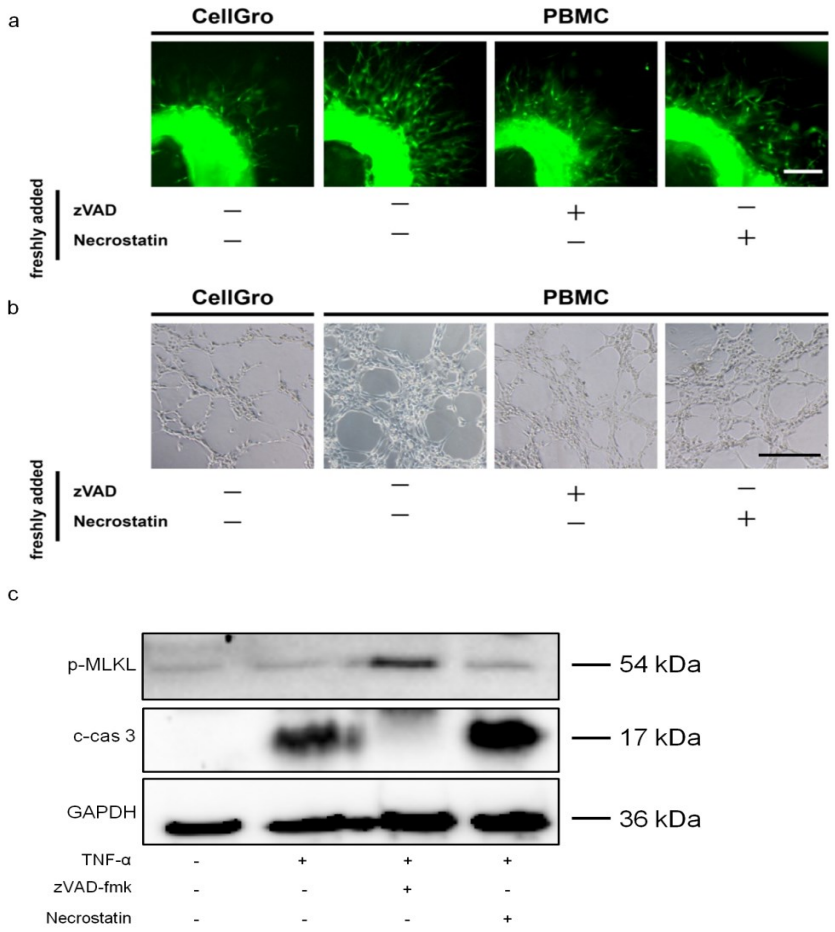


Activation of necroptosis by TNFRSF1B

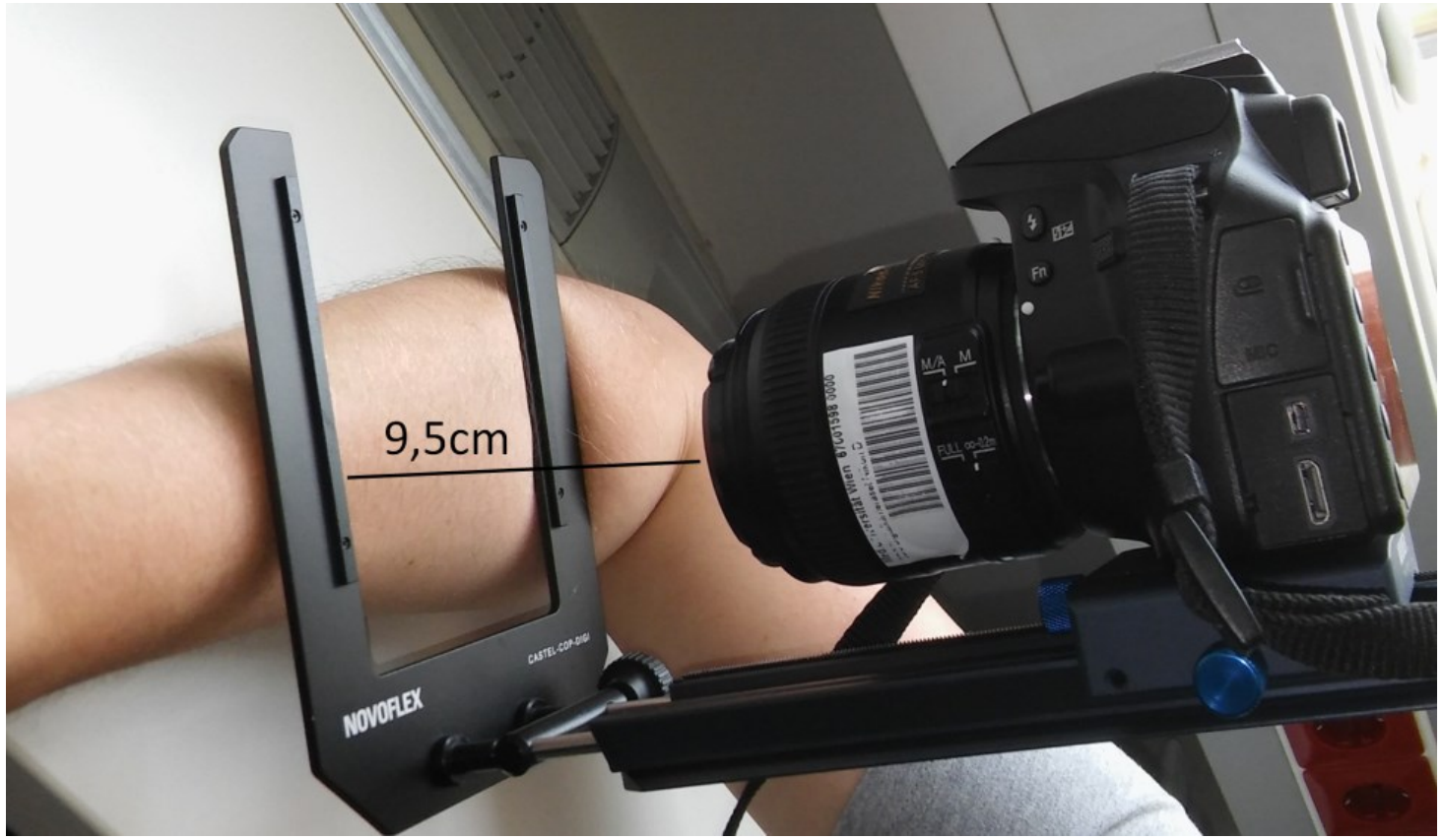
Figure 7



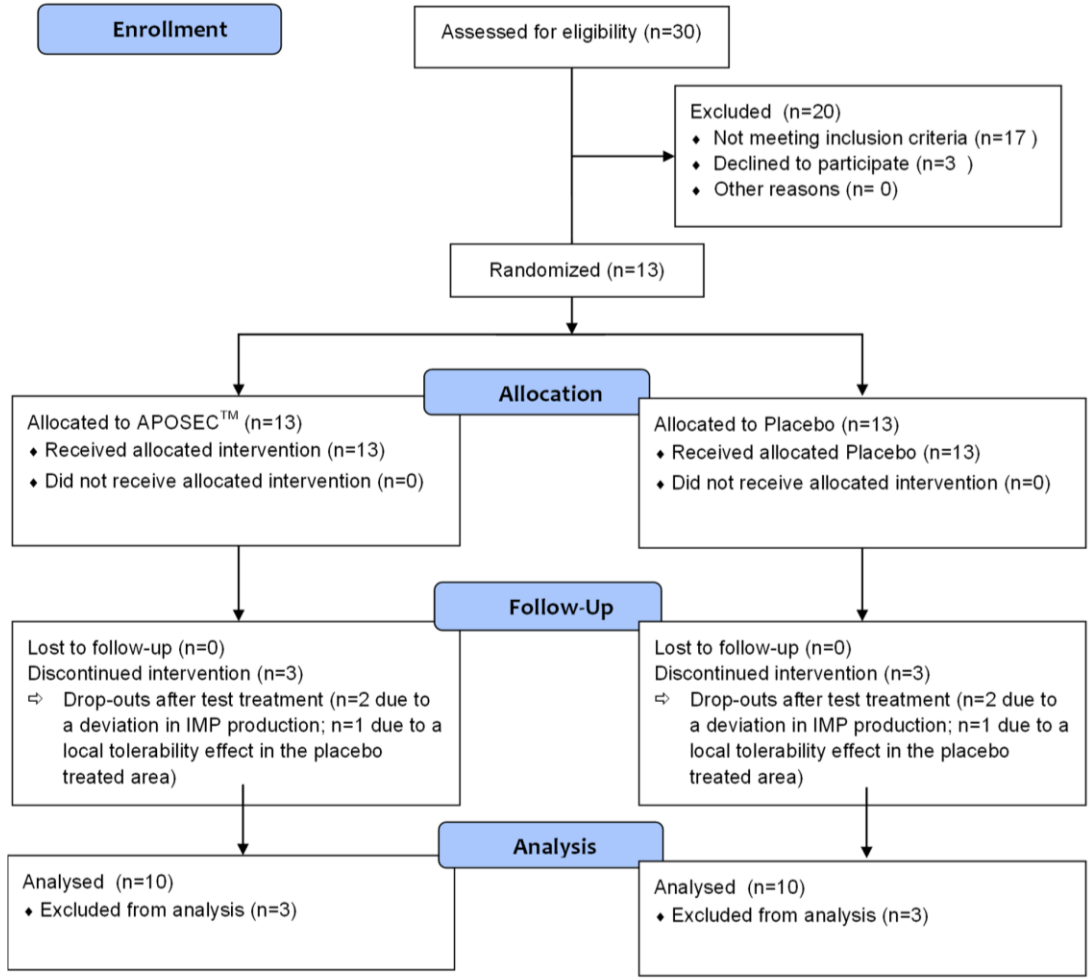
Control groups



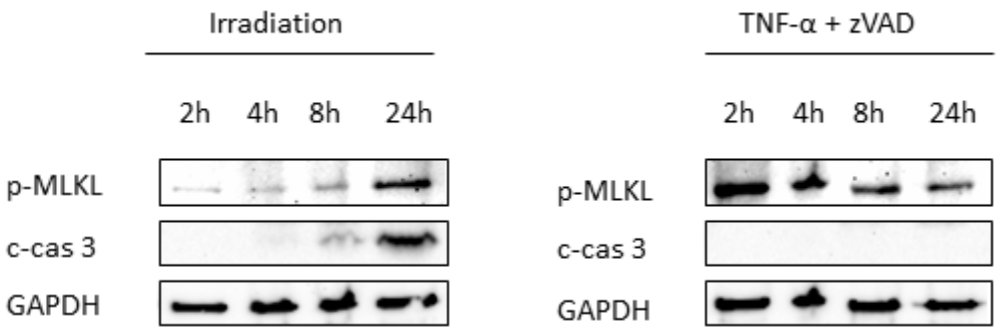
Planimetry

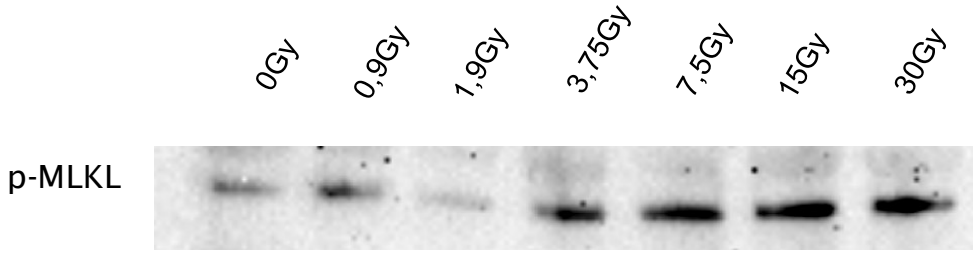


Inclusion

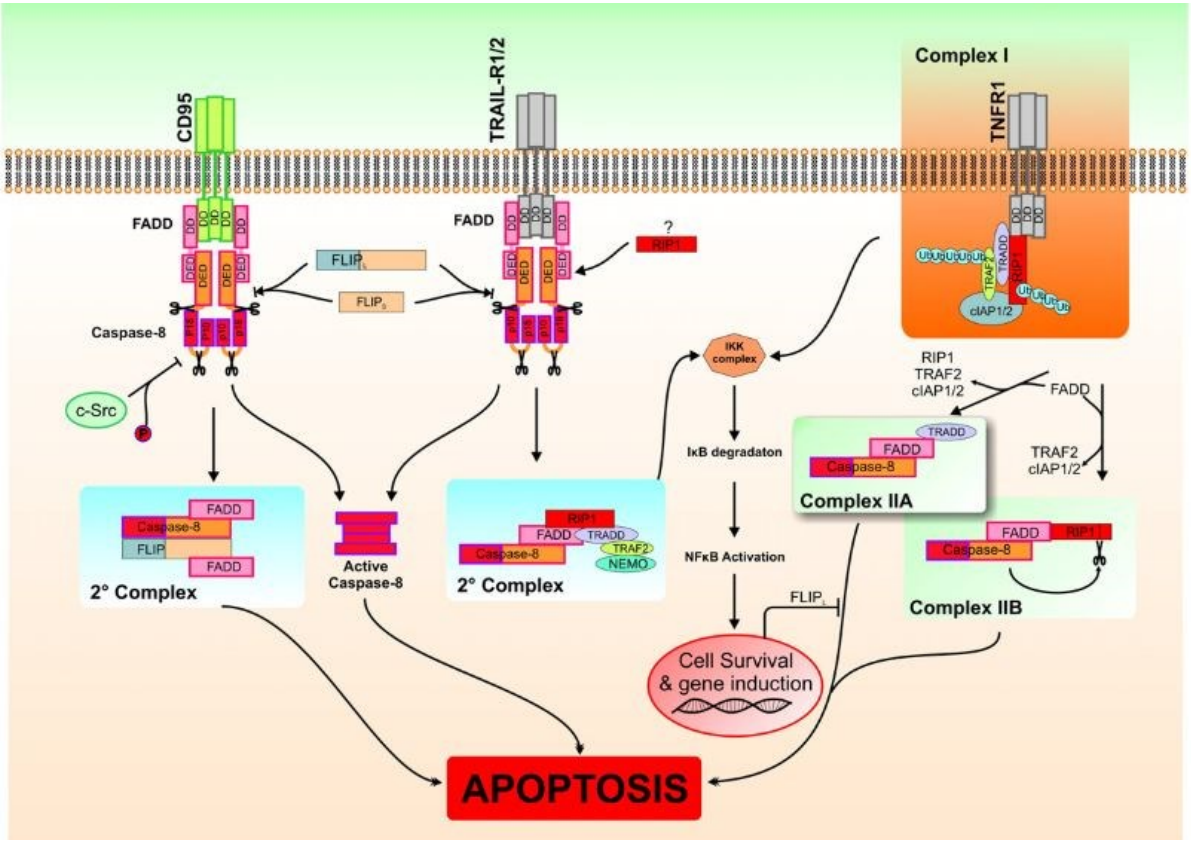

Enrollment
Assessed for eligibility (n=30)
Excluded (n=20)
Not meeting inclusion criteria (n=17)
Declined to participate (n=3)
Other reasons (n= 0)
Randomized (n=13)
Allocation
Allocated to APOSEC™ (n=13)
Received allocated intervention (n=13)
Did not receive allocated intervention (n=0)
Allocated to Placebo (n=13)
Received allocated Placebo (n=13)
Did not receive allocated intervention (n=0)
Follow-Up
Lost to follow-up (n=0)
Discontinued intervention (n=3)
Drop-outs after test treatment (n=2 due to a deviation in IMP production; n=1 due to a local tolerability effect in the placebo treated area)
Lost to follow-up (n=0)
Discontinued intervention (n=3)
Drop-outs after test treatment (n=2 due to a deviation in IMP production; n=1 due to a local tolerability effect in the placebo treated area)
Analysis
Analysed (n=10)
Excluded from analysis (n=3)
Analysed (n=10)
Excluded from analysis (n=3)

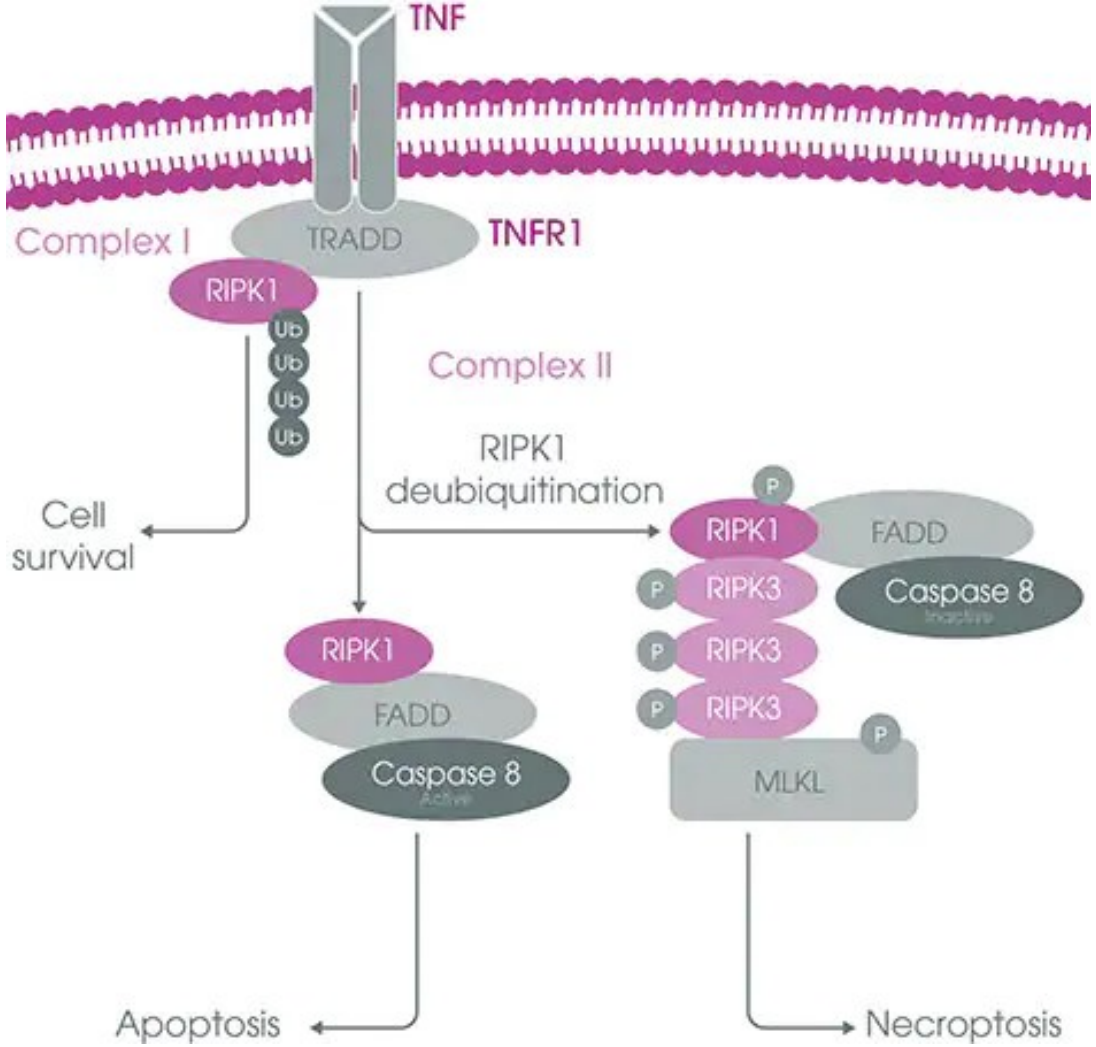
Time and dose-dependent effects



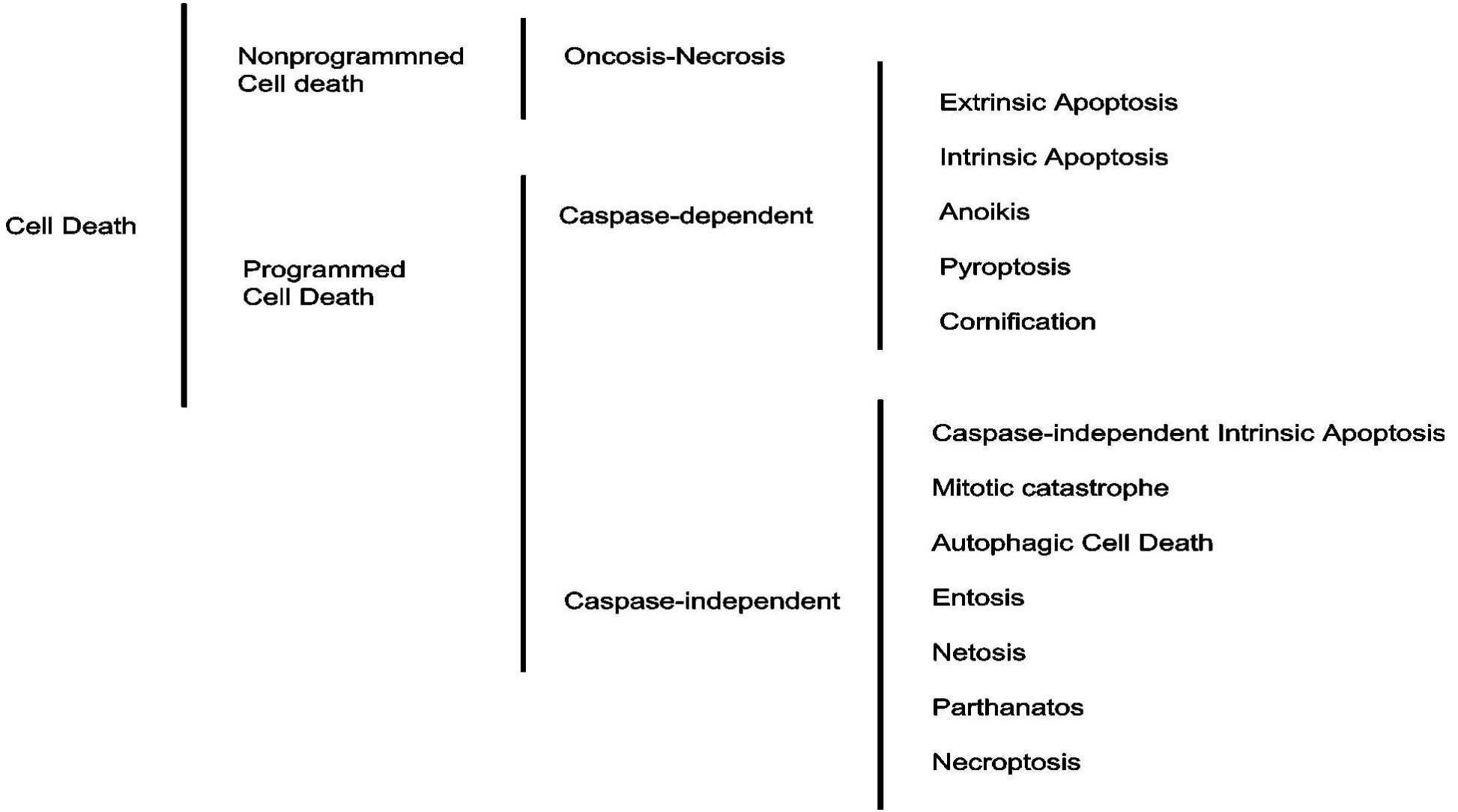


Cell death initiation





Cell death


Cell Death
Nonprogrammned Cell death
Oncosis-Necrosis
Programmed Cell Death
Caspase-dependent
Extrinsic Apoptosis
Intrinsic Apoptosis
Anoikis
Pyroptosis
Cornification
Caspase-independent
Caspase-independent Intrinsic Apoptosis
Mitotic catastrophe
Autophagic Cell Death
Entosis
Netosis
Parthanatos
Necroptosis