

Functional studies on inborn errors of innate immunity

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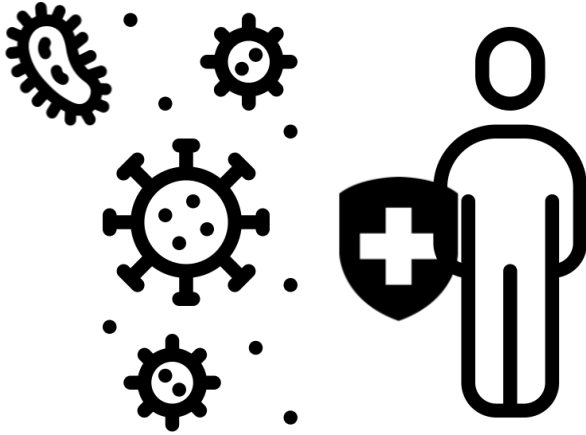
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ESCCA 2023 Utrecht
Disclosure commercial conflict of interest

X	No, nothing to disclose
	Yes, as specified below:

Company Name	Specification

The immune system

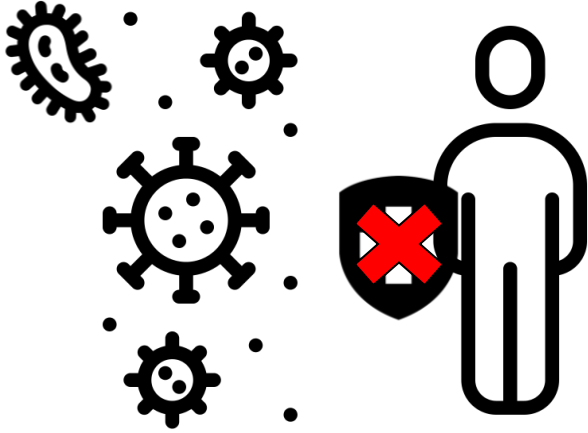


1st line of defence: physical and chemical barriers

2nd line of defence: innate immunity (non-specific)

3rd line of defence: adaptive immunity (specific)

Immunodeficiency



Acquired (secondary) immunodeficiency

- Chemotherapy
- AIDS

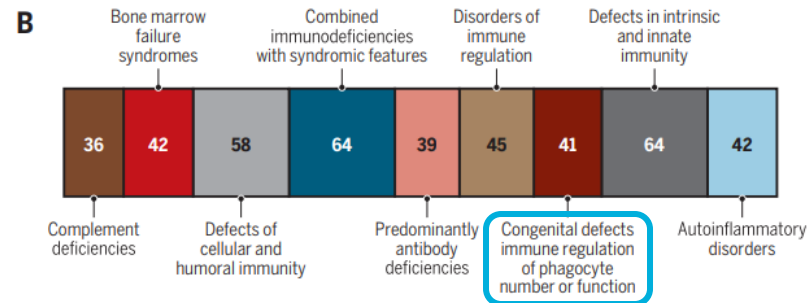
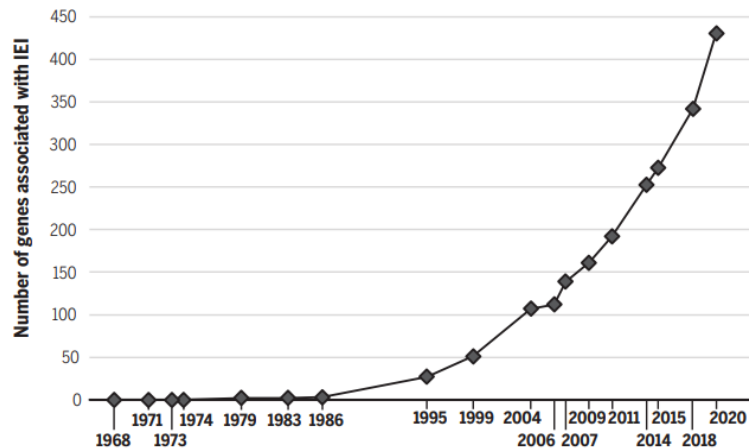
Primary immunodeficiency

- Rare: 4 per 100.000

Genetic defect: 'Inborn errors of immunity'



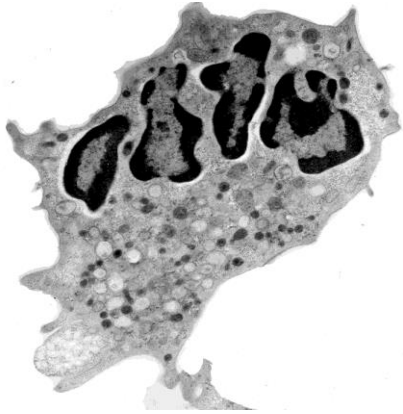
Inborn errors of immunity



41 genes associated with phagocyte number and function

- Congenital neutropenias
- Defects of motility
- Defects of respiratory burst
- Other non-lymphoid defects

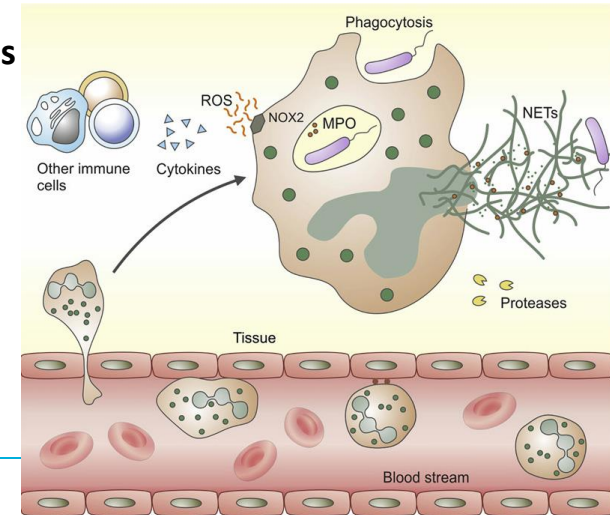
Neutrophilic granulocyte



- **Most abundant innate immune cells**
 - 50-70% of circulating leukocytes
- **Maturation in the bone marrow**
 - From stem cell to neutrophil: \pm 2 weeks
 - 10^{11} neutrophils per day
- **Important effector cells in the host defense against invading micro-organisms**
 - First immune cells recruited to the site of infection

Host defence mechanisms:

- Phagocytosis
- Degranulation
- Reactive oxygen species (ROS) production
- Neutrophil extracellular traps (NETs) formation

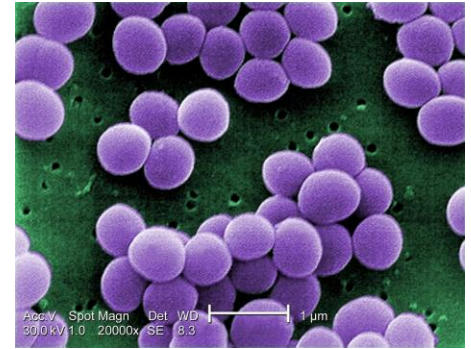


Suspicion for neutrophil disorders

Symptoms often occur from a young age

- Recurrent and severe bacterial and fungal infections
 - Respiratory tract, lymph nodes, skin
- Tissue and organ abscesses
- Delayed separation of the umbilical cord
- Abnormal wound healing

- Neutropenia (ANC $< 0,5 \times 10^6/\text{mL}$)



Staphylococcus aureus



Aspergillus fumigatus

Phagocyte function defects

2. Defects of Motility						
Disease	Genetic defect	Inheritance	OMIM	Affected cells	Affected function	Associated features
Leukocyte adhesion deficiency type 1 (LAD1)	<i>ITGB2</i>	AR	600065	N + M + L + NK	Adherence, Chemotaxis, Endocytosis, T/NK cytotoxicity	Delayed cord separation, skin ulcers, periodontitis, leukocytosis
Leukocyte adhesion deficiency type 2 (LAD2)	<i>SLC35C1</i>	AR	605881	N + M	Rolling, chemotaxis	Mild LAD type 1 features with hh-blood group, growth retardation, developmental delay
Leukocyte adhesion deficiency type 3 (LAD3)	<i>FERMT3</i>	AR	607901	N + M + L + NK	Adherence, chemotaxis	LAD type 1 plus bleeding tendency
Rac2 deficiency	<i>RAC2</i>	AD LOF	608203	N	Adherence, chemotaxis O ₂ ⁻ production	Poor wound healing, leukocytosis
β actin deficiency	<i>ACTB</i>	AD	102630	N + M	Motility	Mental retardation, short stature
Localized juvenile periodontitis	<i>FPR1</i>	AR	136537	N	Formylpeptide induced chemotaxis	Periodontitis only
Papillon-Lefèvre Syndrome	<i>CTSC</i>	AR	602365	N + M	Chemotaxis	Periodontitis, palmoplantar hyperkeratosis in some patients
WDR1 deficiency	<i>WDR1</i>	AR	604734	N	Spreading, survival, chemotaxis	Mild neutropenia, poor wound healing, severe stomatitis, neutrophil nuclei hemiate
Cystic fibrosis	<i>CFTR</i>	AR	602421	M only	Chemotaxis	Respiratory infections, pancreatic insufficiency, elevated sweat chloride
Neutropenia with combined immune deficiency due to MKL1 deficiency	<i>MKL1</i>	AR	606078	N + M +L + NK	Impaired expression of cytoskeletal genes	Mild thrombocytopenia

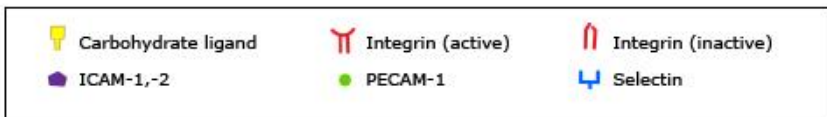
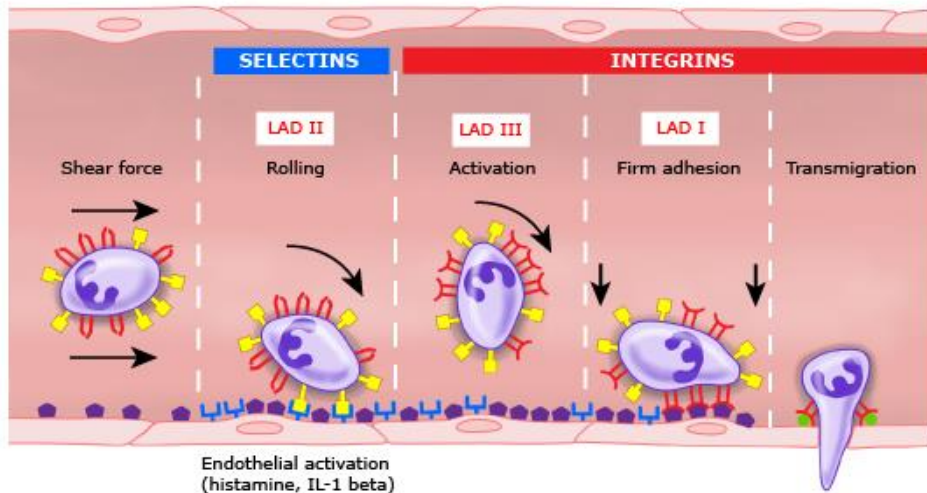
Functional assays to detect neutrophil motility defects

- Adherence
- Actin polymerization
- Chemotaxis
- Rolling, transendothelial migration
-

Leukocyte adhesion deficiency (LAD)

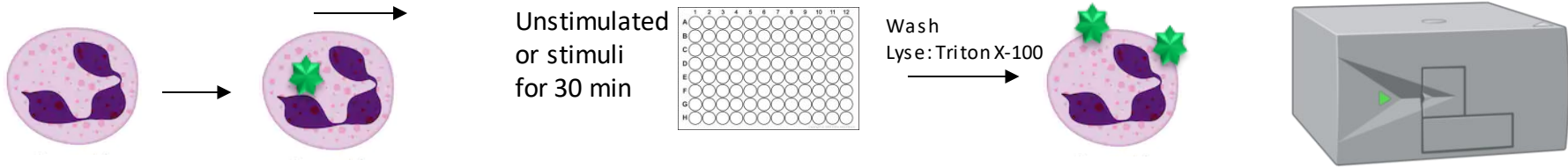
Neutrophil adhesion defect:

An inability of neutrophils to exit the bloodstream and enter tissues to kill microorganisms



	Normal	LAD-I	LAD-II	LAD-III
Integrins	Present	Absent or decreased β_2 integrins	Normal	Defective inside-out signaling of $\beta_1, \beta_2, \beta_3$ integrins
Selectin ligands	Present	Normal	Defective fucosylation	Normal
Functional defects	None	Tight adhesion, emigration	Rolling	Integrin activation, adhesion, emigration, others
Mutations	None	<i>ITGB2</i>	<i>SLC35C1</i>	<i>FERMT3</i>

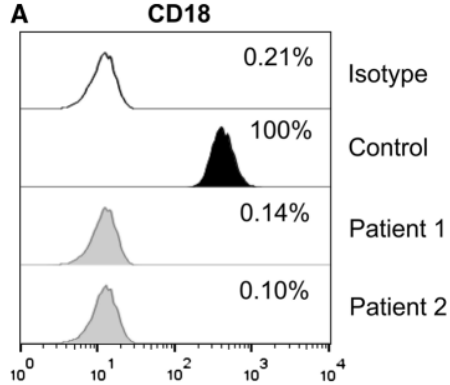
LAD1 and LAD3 – adhesion assay



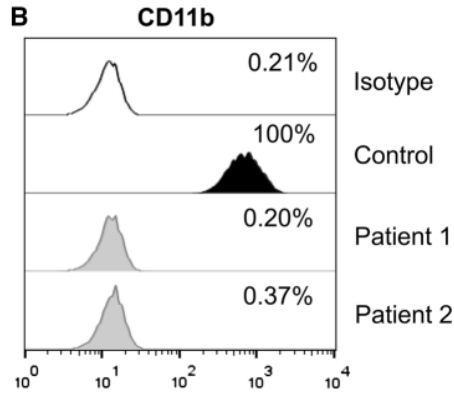
- **Essential for extravasation of neutrophils from blood vessel**
- Binding to polystyrene plate
- Multiple stimuli (chemotactic stimuli, TLR-ligands, grow factors)
 - No response, any stimulus: absence/dysfunctional integrin CD11b/CD18
 - Leukocyte adhesion deficiency (LAD1 or LAD3)
 - Lowered response to one or several stimuli
 - Defect in receptor or signaling protein (e.g. IRAK4)

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LAD1 – β_2 integrin detection by flow cytometry



beta2-subunit



alpha chain

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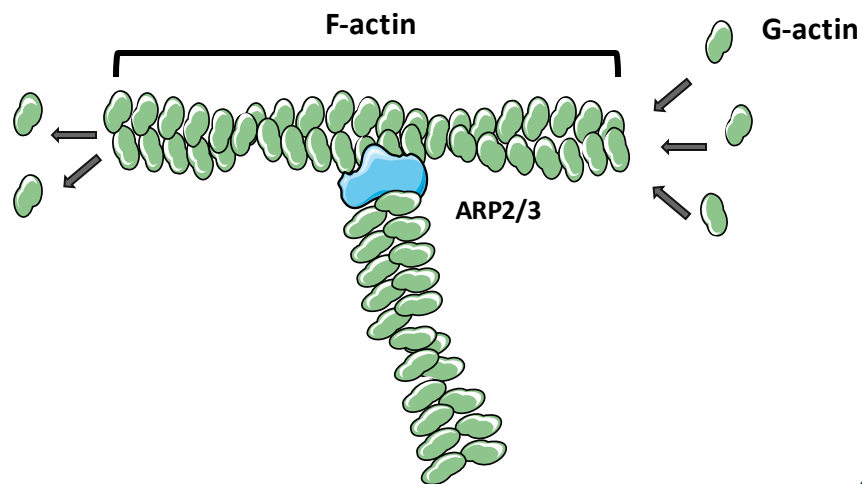
MKL1 deficiency

Megakaryoblastic leukemia 1 – transcriptional coactivator, important for actin regulatory gene transcription

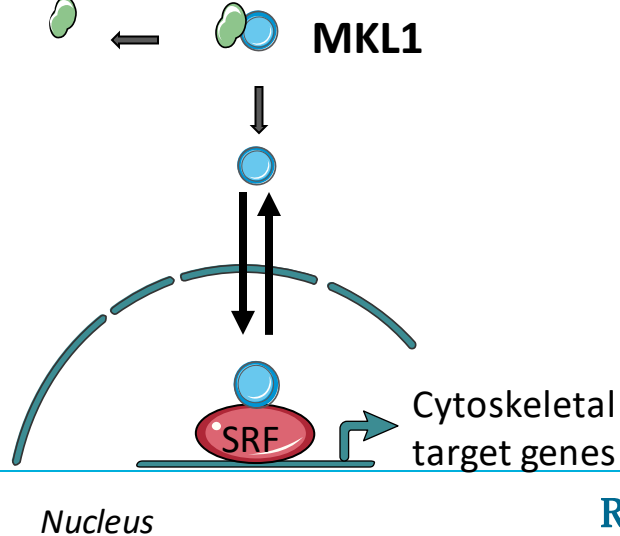
Cytoplasm

- End (depolymerization)

+ End (polymerization)



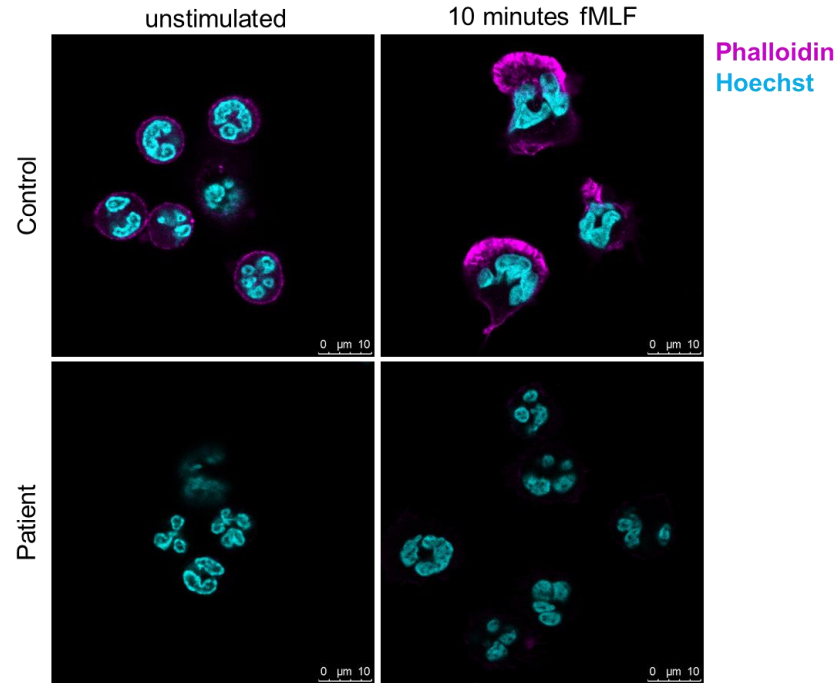
3 patients (2 sibs) described to date
Severe infections, potentially fatal



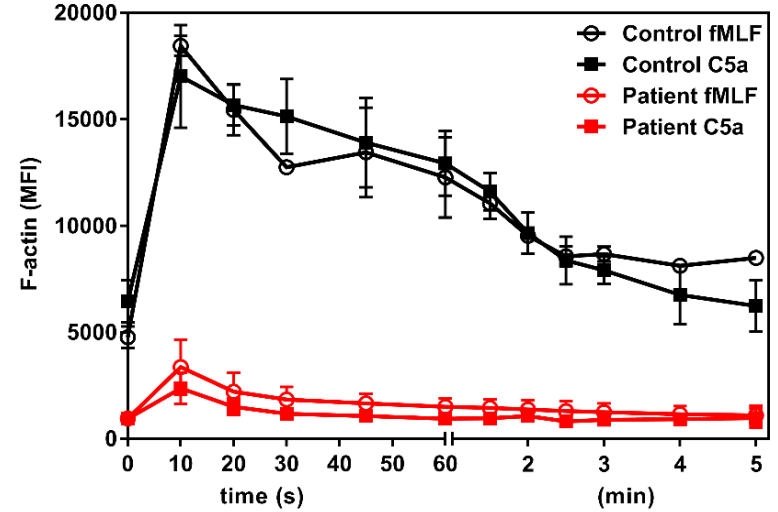
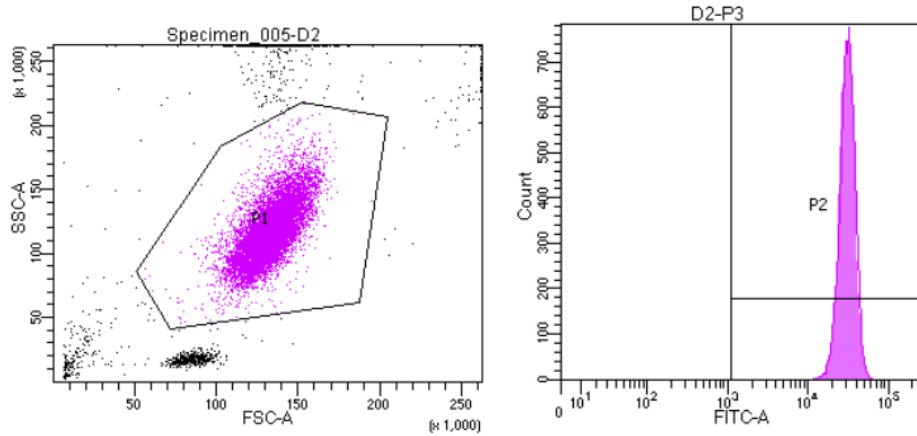
Made with Servier Medical Art

Sprenkeler, Henriët et al., Blood (2020)

Important for essential cellular functions

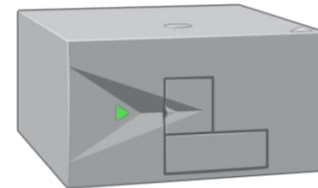
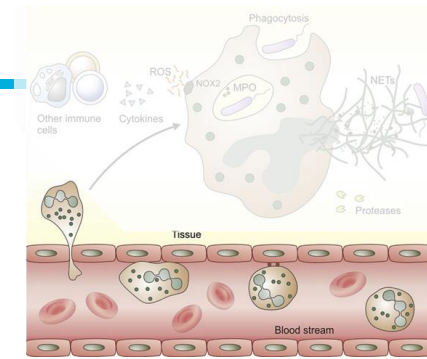
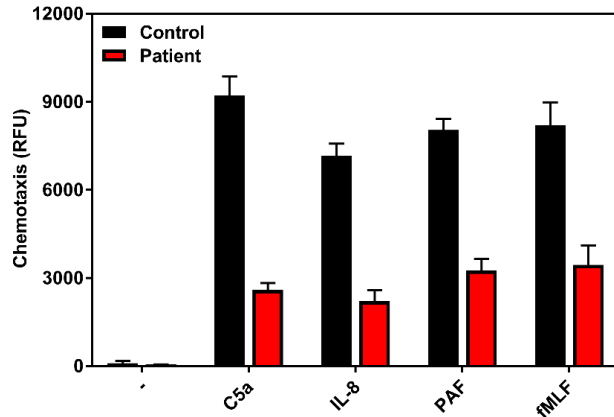


Important for essential cellular functions



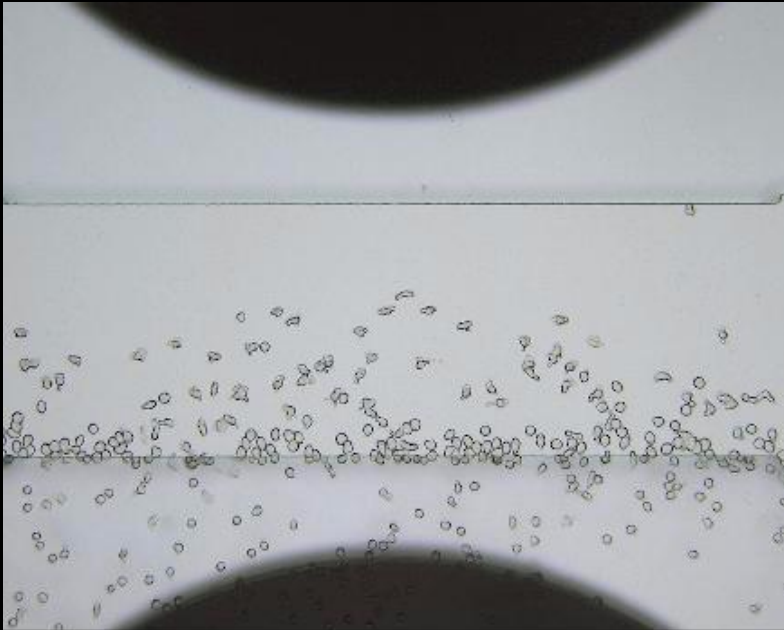
MKL1 deficiency - chemotaxis

- Essential for extravasation of neutrophils from blood vessel
- Transwell assay
- Chemotactic stimuli
 - C5a
 - fMLF
 - IL-8
 - Platelet-activating factor (PAF)

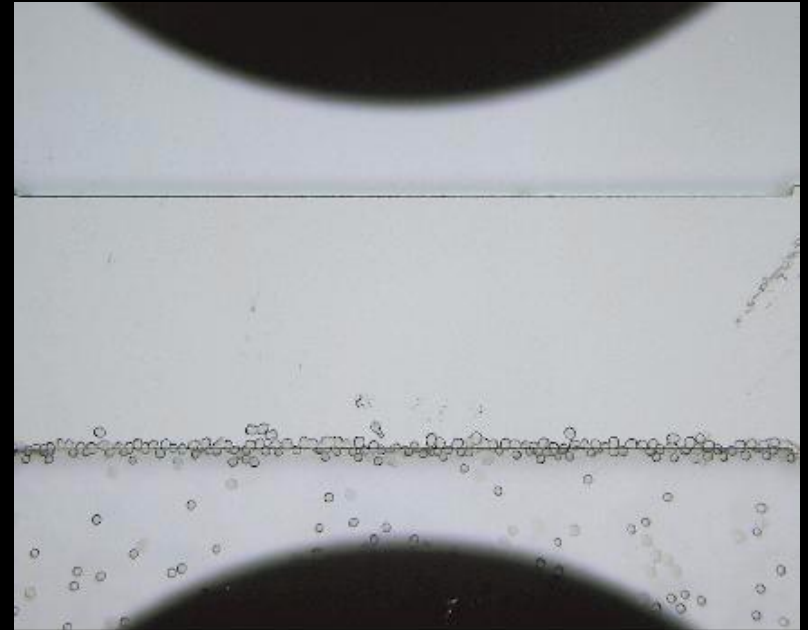


MKL1 deficiency - Taxiscan

Chemoattractant C5a

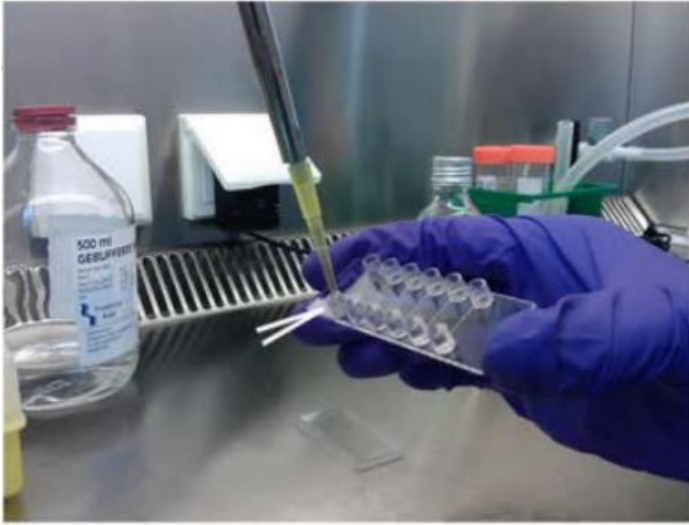


Control neutrophils

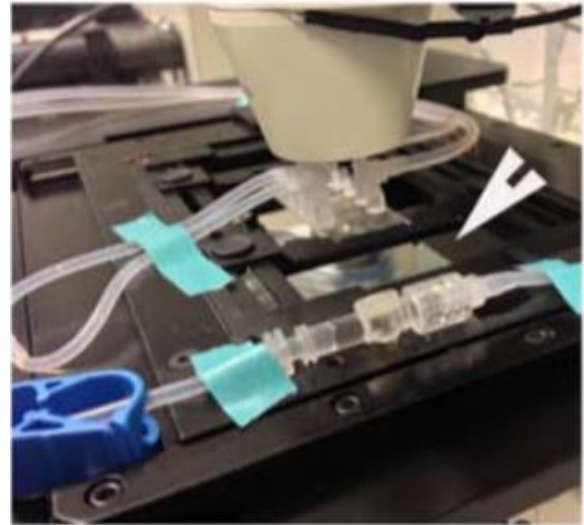


Patient neutrophils

Transendothelial migration under flow (HUVECs)

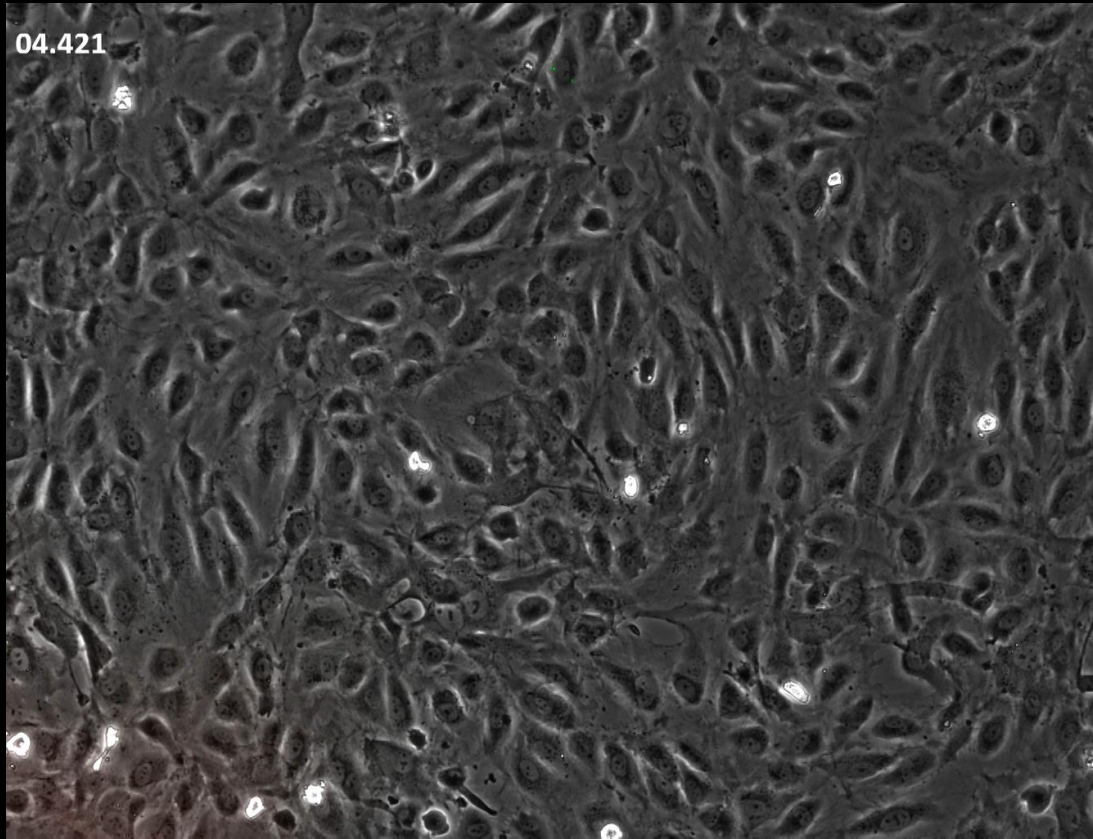


- *Grow monolayer HUVECs on fibronectin in in vitro flow chambers*
- *Stimulate endothelium with TNF α → upregulation ICAM-1 and VCAM-1*



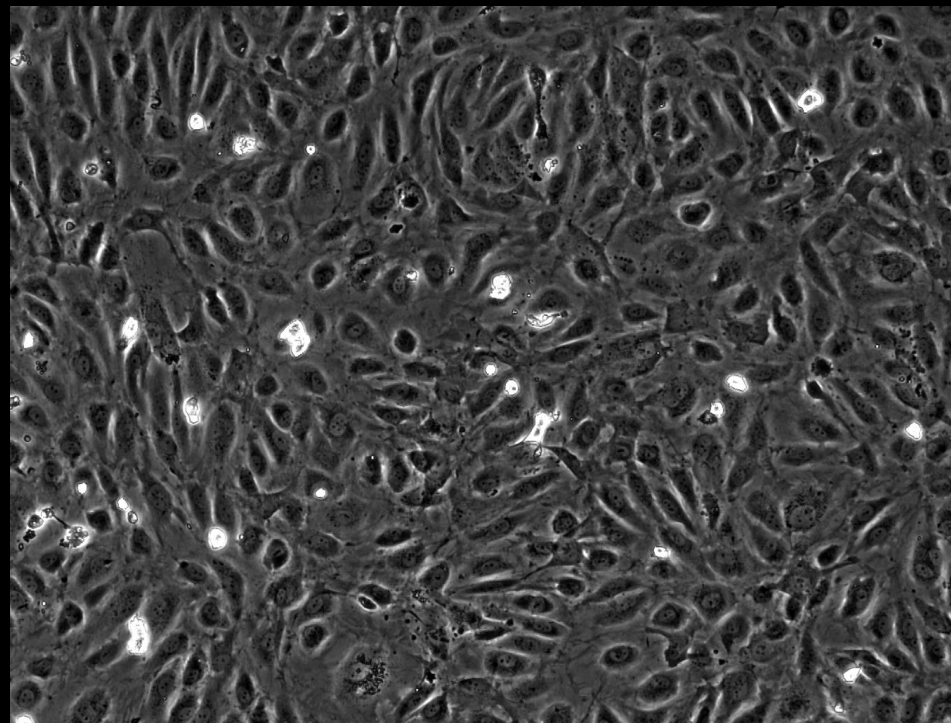
- *Isolate and fluorescently label neutrophils, Flow over endothelium under physiological flow conditions*

Control
Patient*

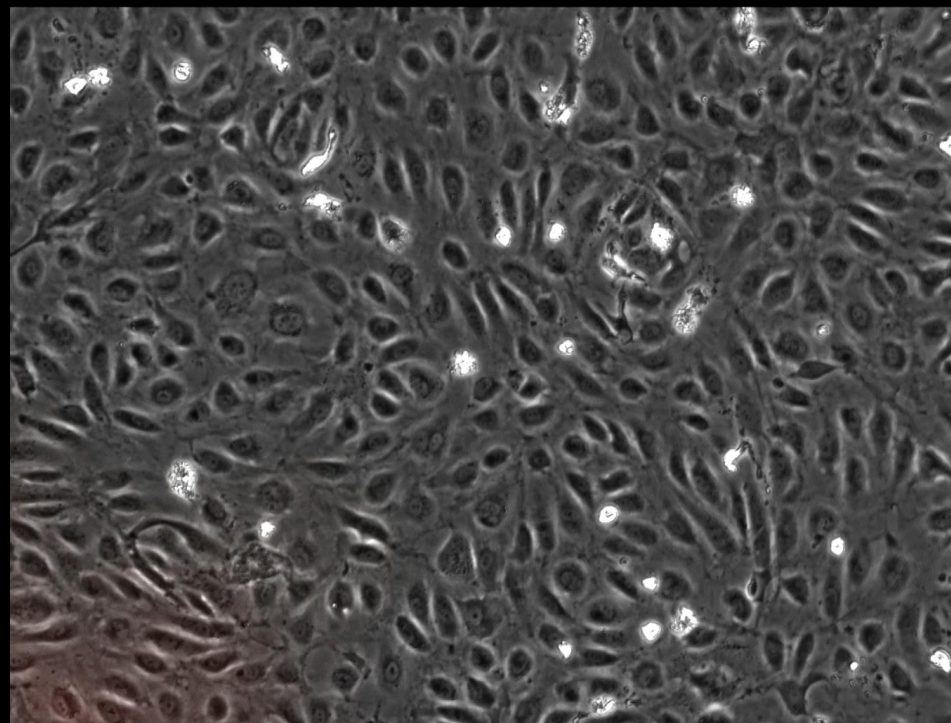


*actinopathy patient

Control



Patient

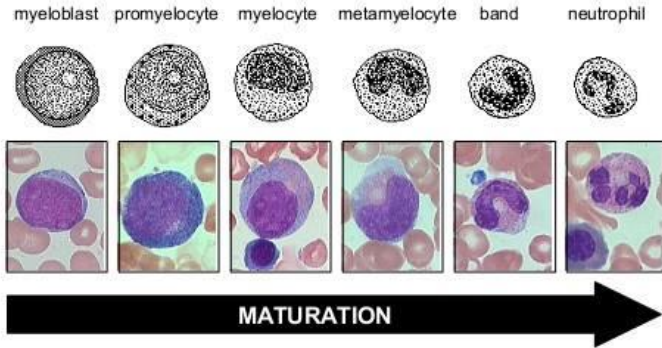


Decreased subendothelial motility

*actinopathy patient

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Neutropenia



Numbers count!

myeloid progenitors

- *CSF3R* (SCN5)
- *RUNX1* (AML1 / FDP)

transcription factors / granule formation

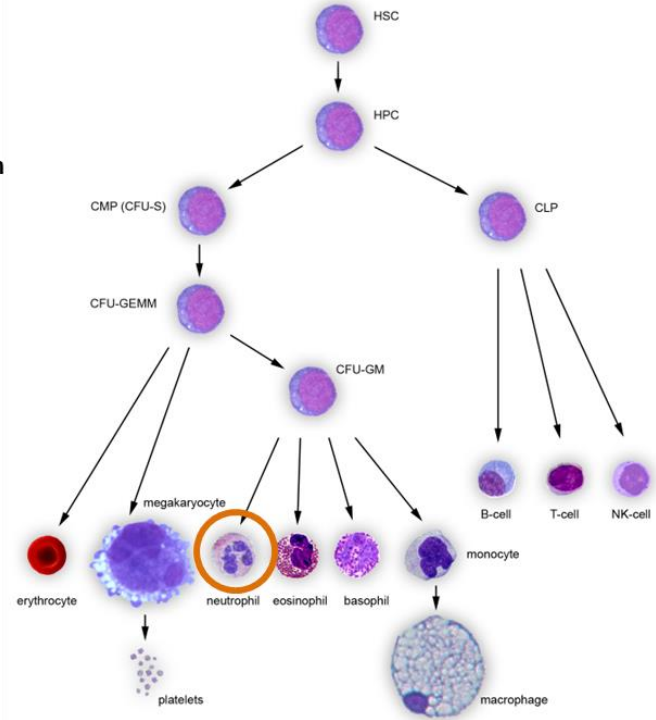
- *CEBPA*
- *GFI1* (SCN2)
- *GATA2* (MonoMac)
- *ELANE* (SCN1)
- *CEBPE* (SGD)
- *JAGN1* (SCN6)

neutrophil metabolism / signaling

- *HAX1* (SCN3)
- *G6PT1* (GSD1b)
- *WASP* (XL-SCN / XLT / WAS)
- *SBDS* (Shwachman)

release

- *CXCR4* (WHIM)
- *G6PC3* (SCN4)



Loss-of-function mutations in *CSF3R*

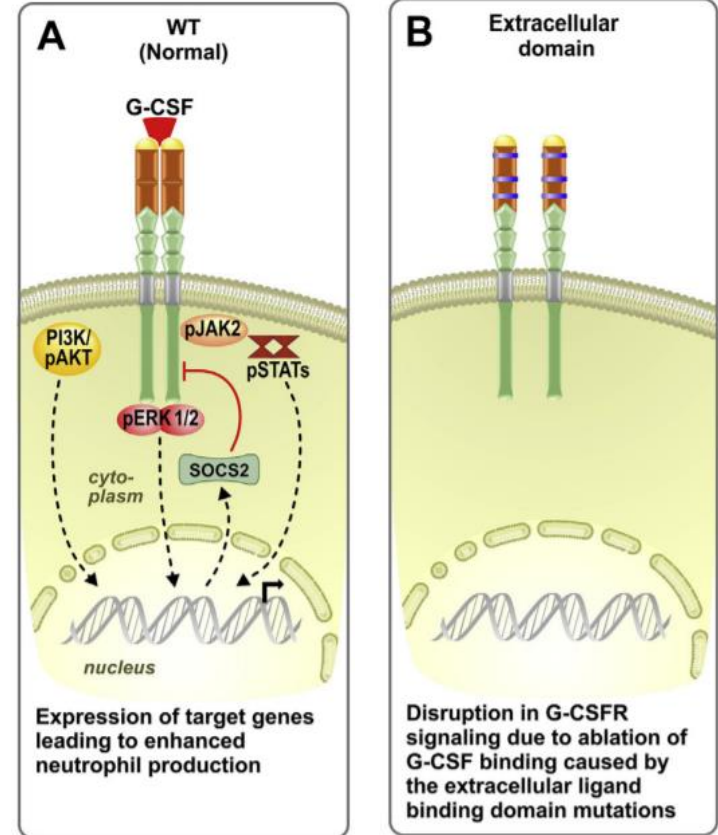
G-CSF:

- Growth factor
- Key regulator of neutrophil development
- Proliferation and differentiation of myeloid progenitors
- Emergency granulopoiesis in case of infection

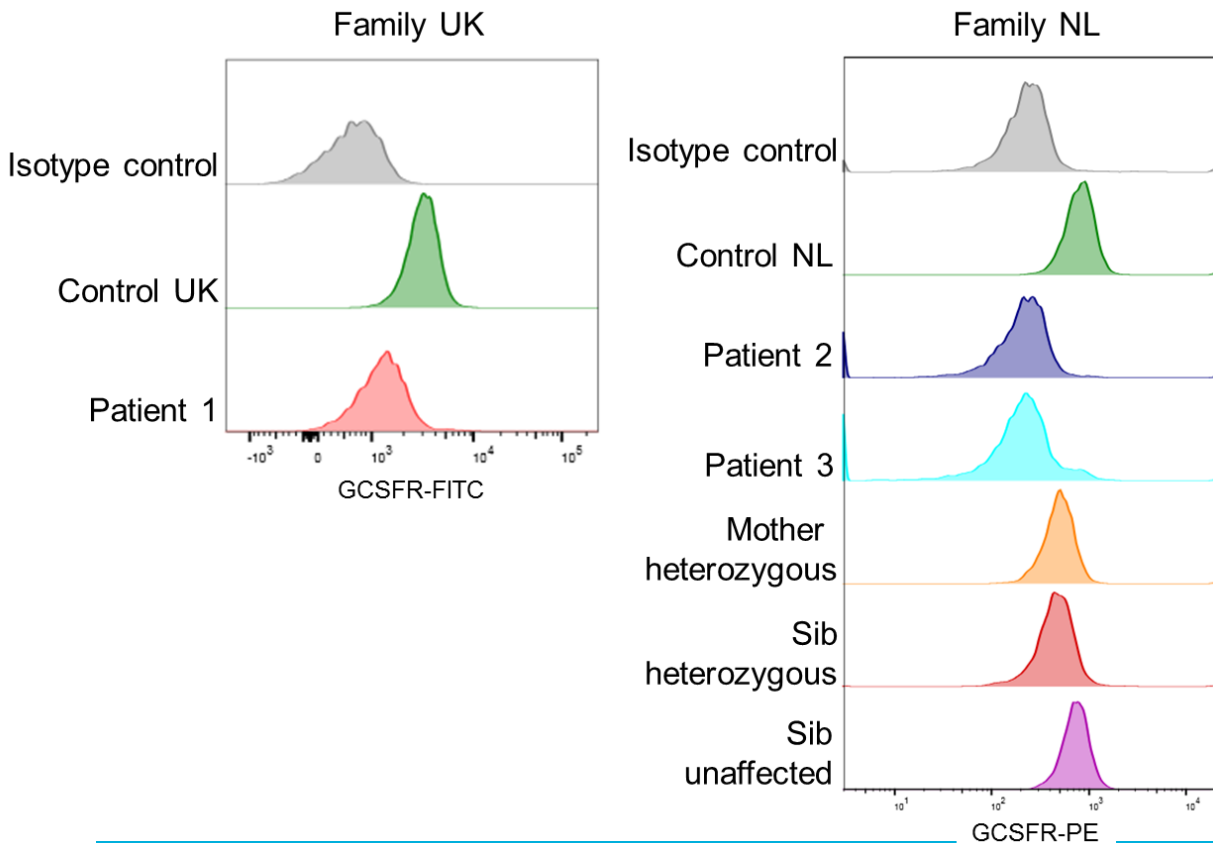
- Priming capacity & inhibits neutrophil apoptosis

- Treatment for severe congenital neutropenia (SCN)
- Absolute neutrophil count (ANC) < 500/ μ L

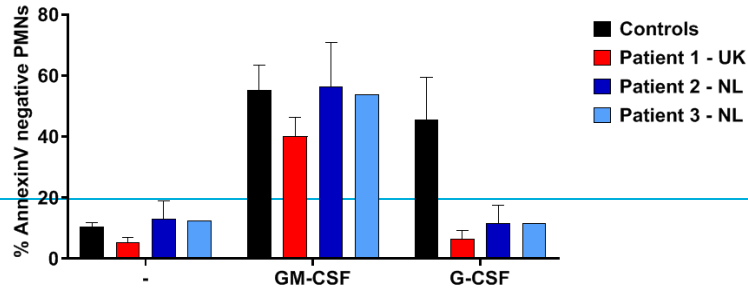
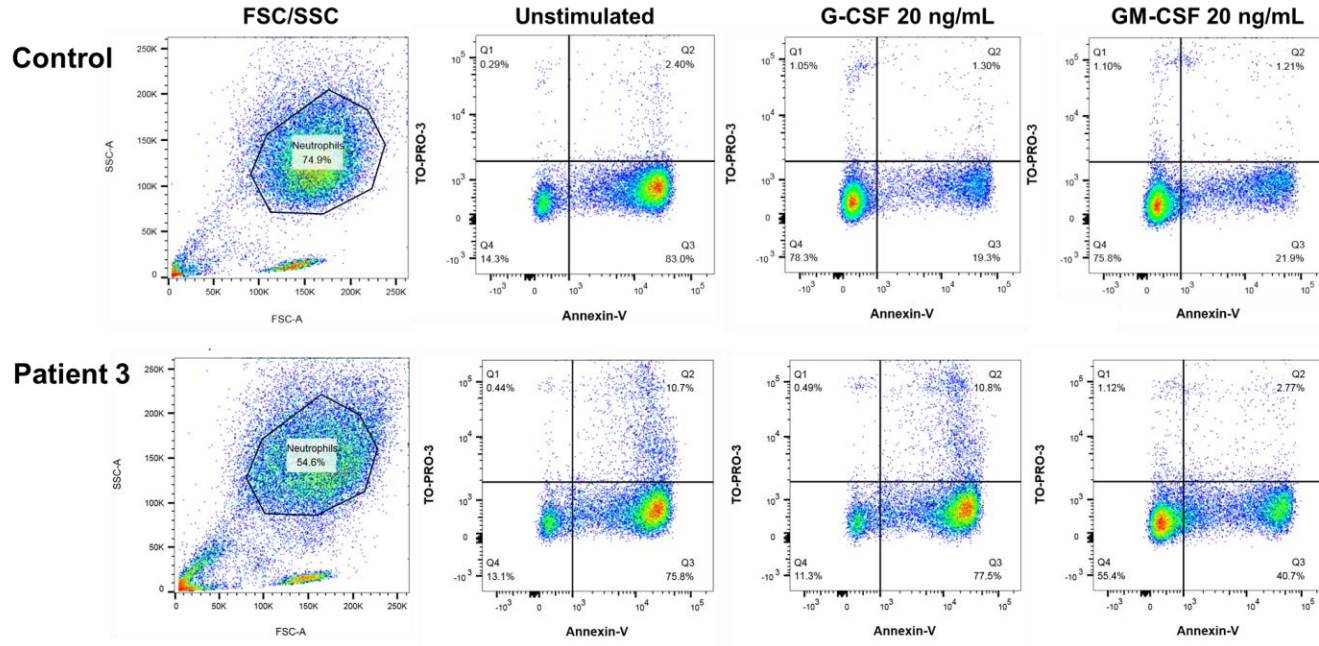
- **Neutropenia**
 - Recurrent bacterial infections
 - Non-responsive to G-CSF treatment



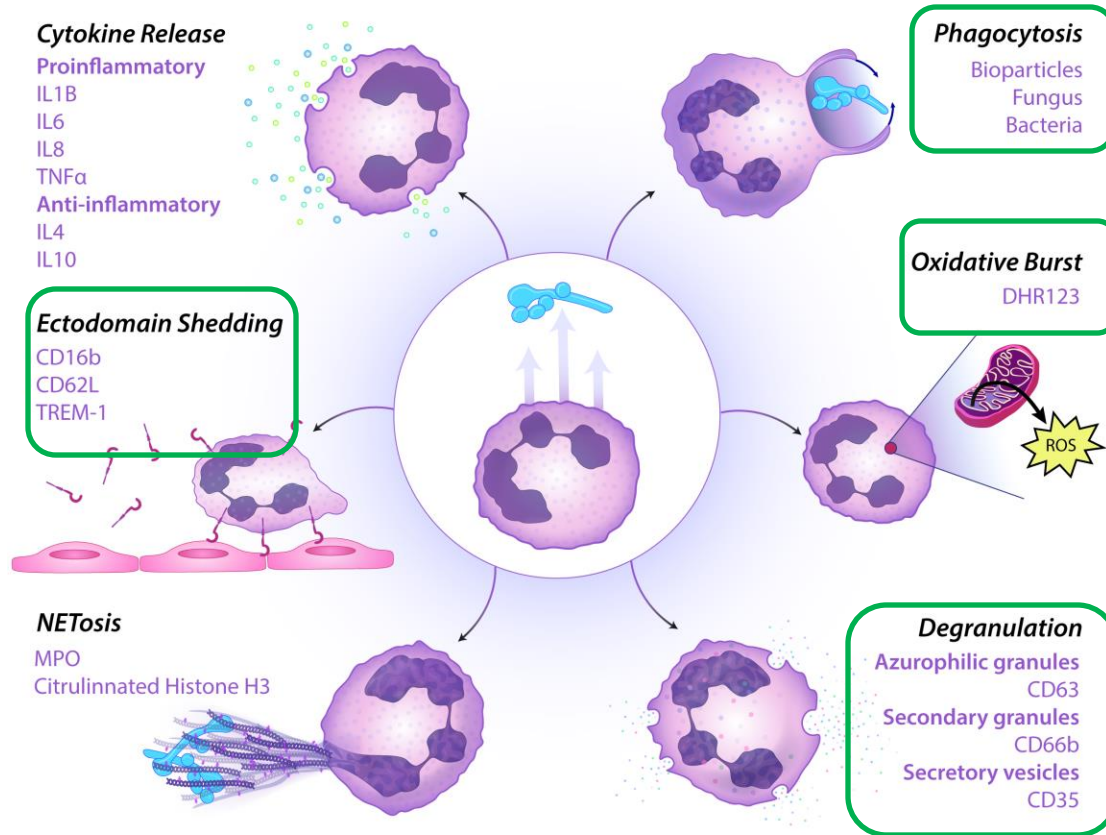
Lack of G-CSF-R expression on patient neutrophils



G-CSF stimulation of patient neutrophils does not prolong survival



And many more...



Acknowledgments

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Anton Tool

Paul Verkuijlen

Judy Geissler

Taco Kuijpers



Questions?

