



Neutrophil oxidative burst capacity and NK cell function assays in cellular diagnostics

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Central Diagnostic Laboratory (CDL), 29th September 2023



UMC Utrecht

ESCCA 2023 Utrecht
Disclosure commercial conflict of interest

<input checked="" type="checkbox"/>	No, nothing to disclose
<input type="checkbox"/>	Yes, as specified below:

Company Name	Specification

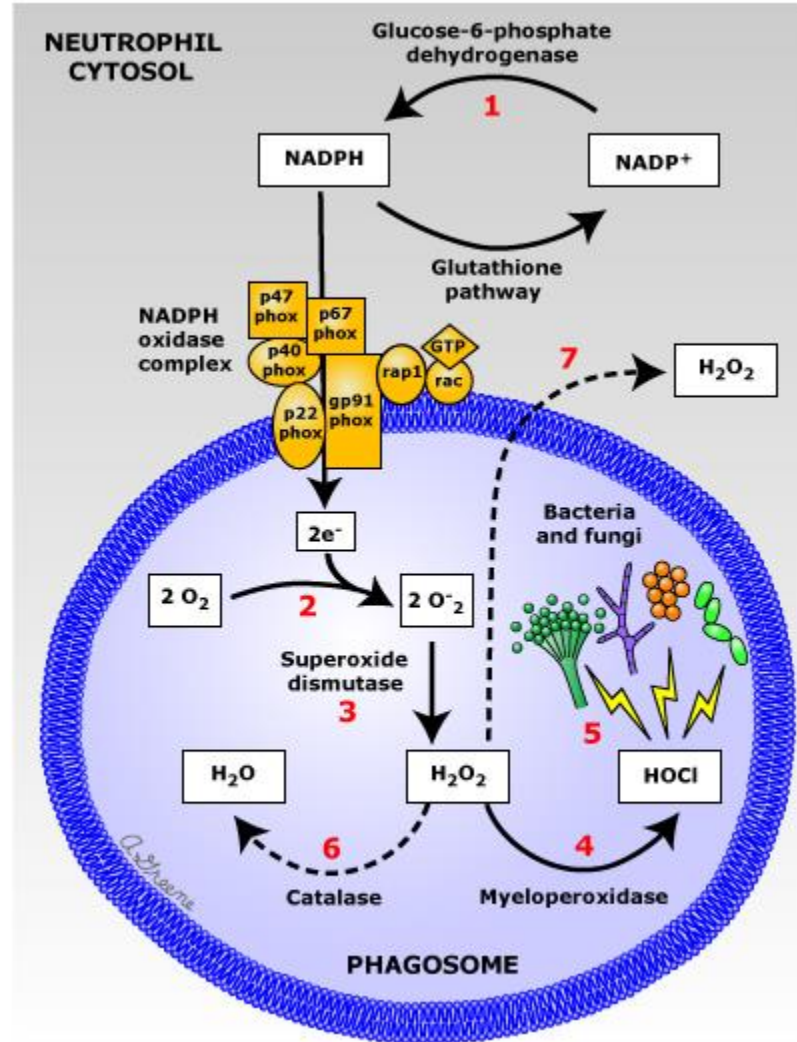
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1. Oxidative burst assay
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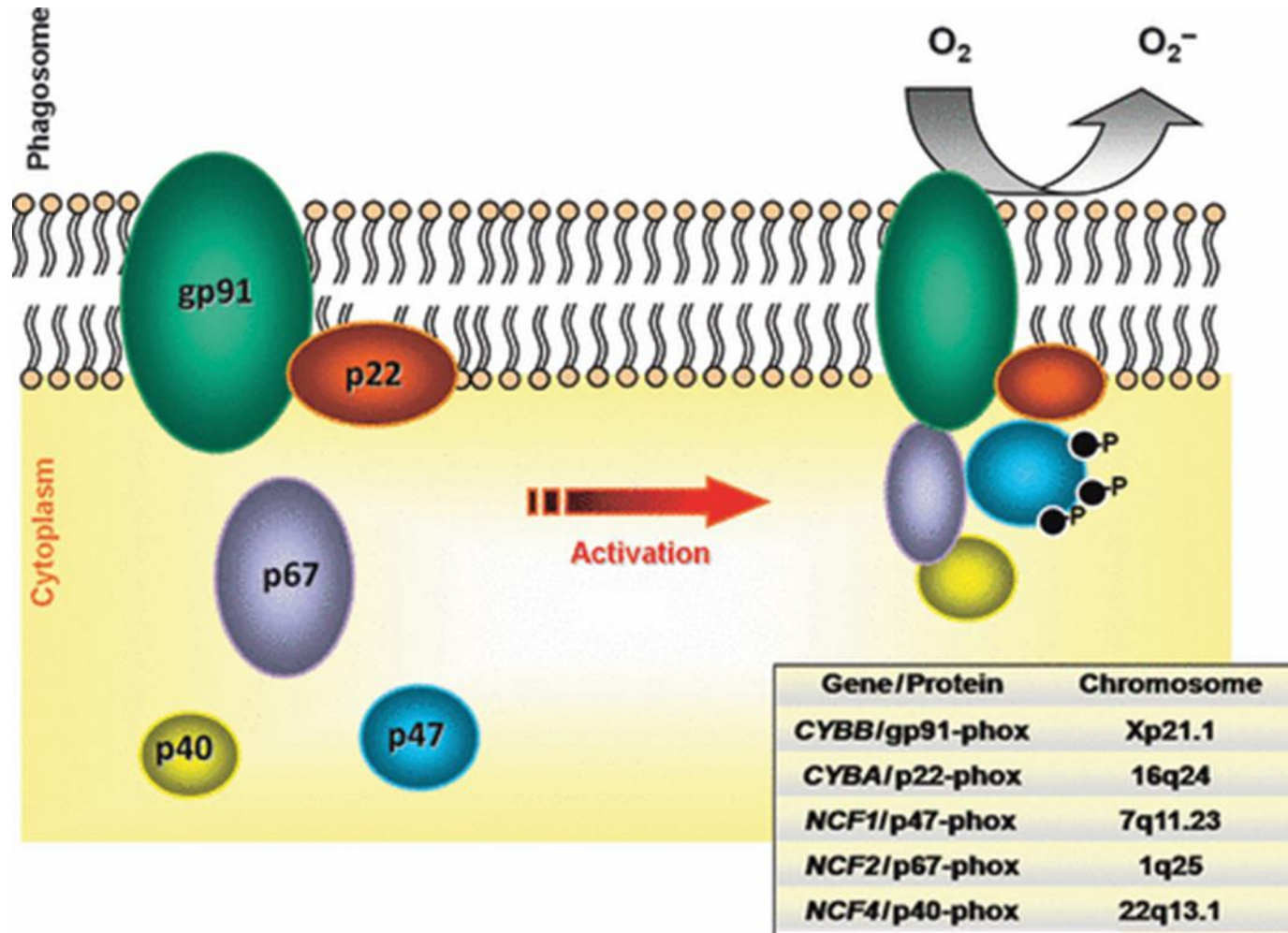
Chronic granulomatous disease (CGD)

- Patients with a history of recurrent or persistent infections, particularly infections caused by uncommon species such as *Aspergillus*, *Staphylococcus aureus*, *Serratia marcescens*, *Nocardia* and *Burkholderia cepacia*
- Genetic defects result in the inability of phagocytes (neutrophils, monocytes, and macrophages) to destroy certain microbes
- ~40% of patients with CGD have gastrointestinal involvement presenting as inflammatory bowel disease (IBD)
→ might be difficult to distinguish from Crohn's disease

Neutrophil function testing; superoxide production



The phagocyte NADPH Oxidase system



de Oliveira-Junior, 2011

Chronic Granulomatous Disease (CGD)

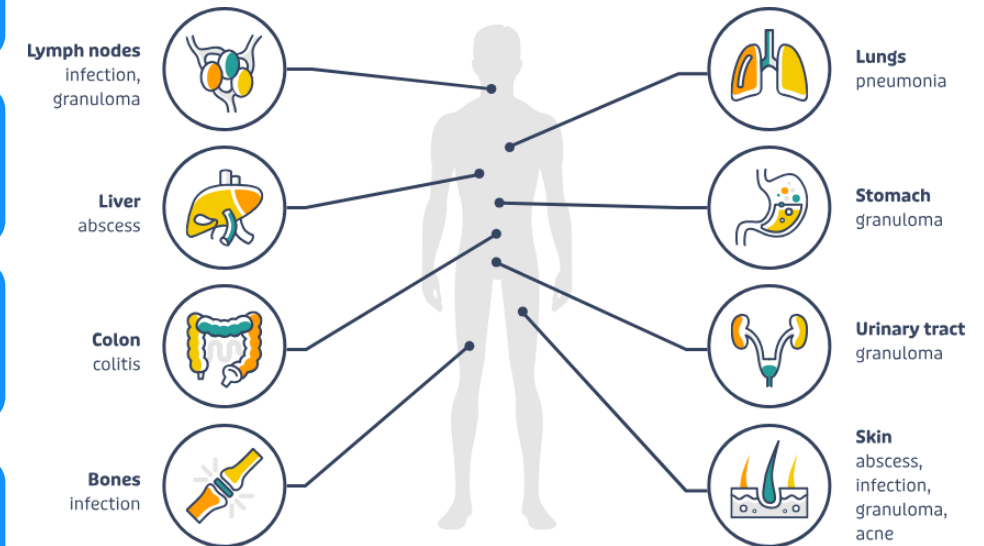
CYBB/gp91phox: XL 60-70%

CYBA/p22phox: AR 5%

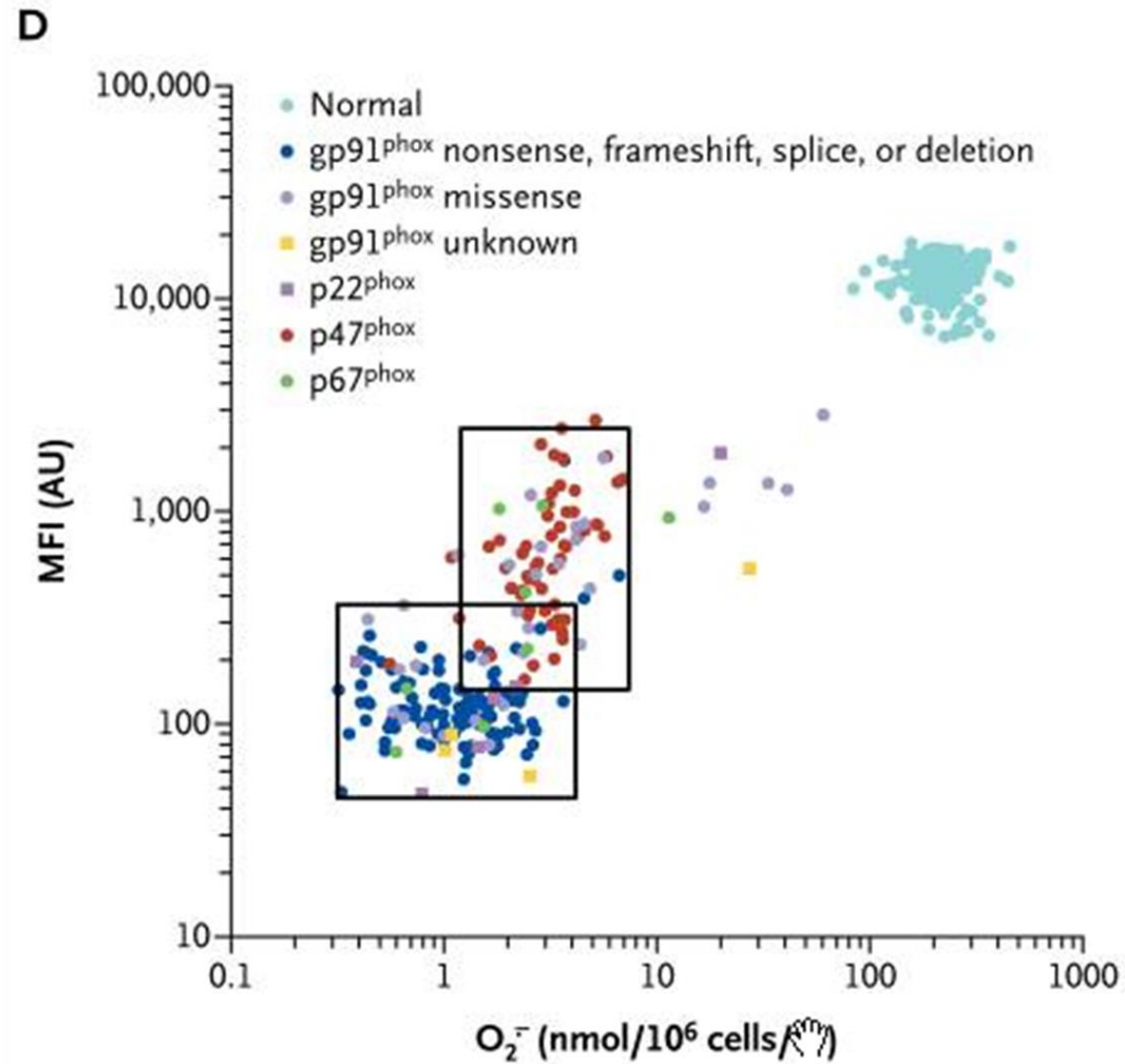
NCF1/p47phox: AR 20-30%

NCF2/p67phox: AR 5%

NCF4/p40phox: AR <1%



Production of reactive oxygen



Screening superoxide production

Oxidative burst assay

Use human heparinized whole blood



Neutrophil granulocytes are stimulated with E. Coli bacteria / PMA



NADPH oxidase is activated

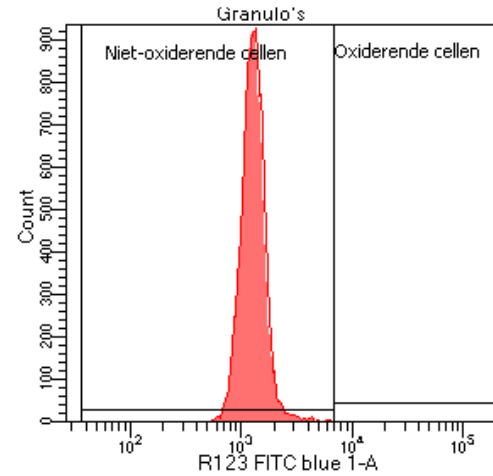
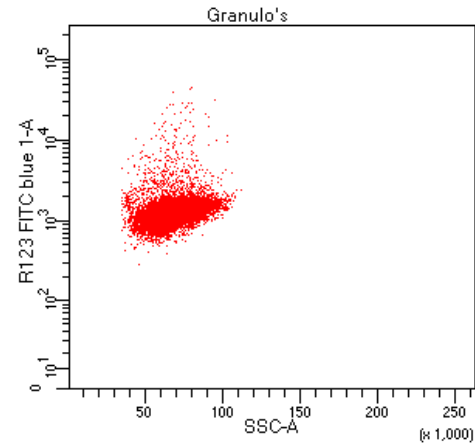
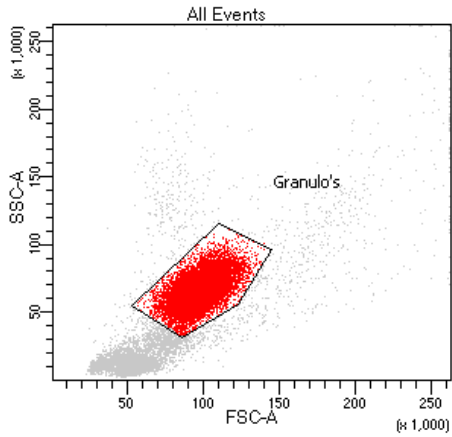


Respiratory burst



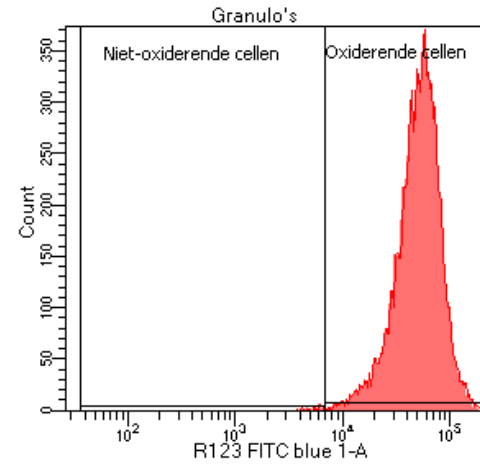
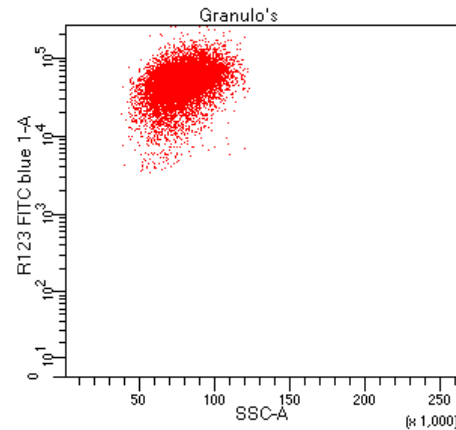
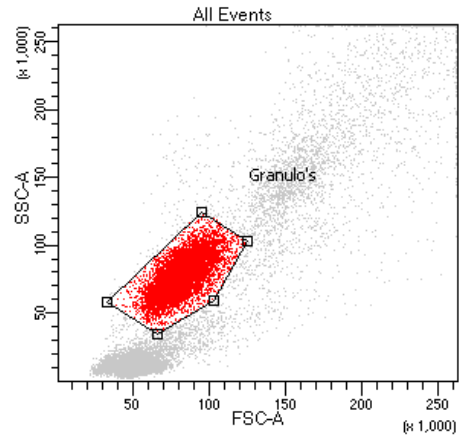
Dihydrorhodamine 123 (DHR123) is oxidized into fluorescent rhodamine 123 which is detected by a flow cytometer

Normal oxidative burst₁



No Stimulation

MFI mean: 1290

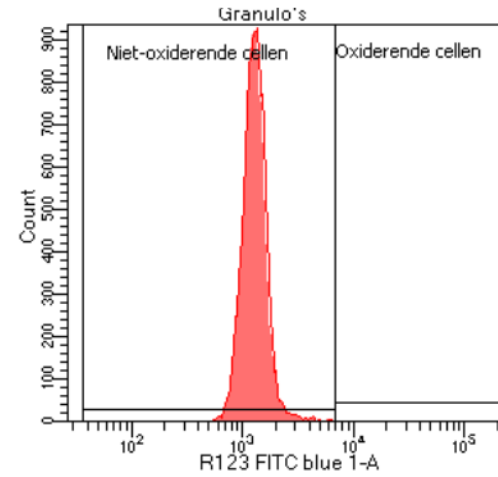
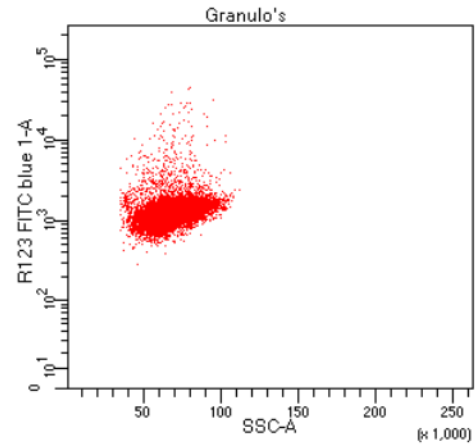
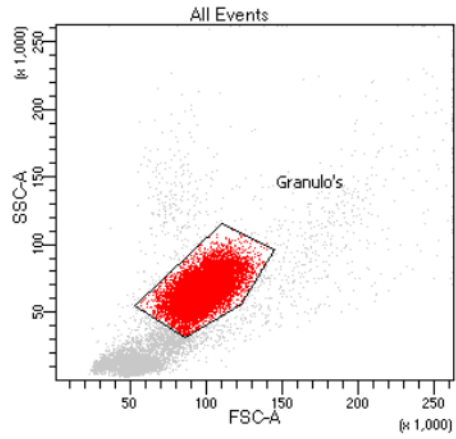


Stimulation with E. Coli bacteria

MFI mean: 54.061

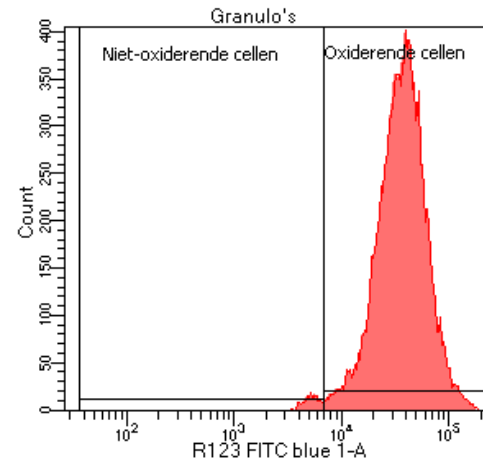
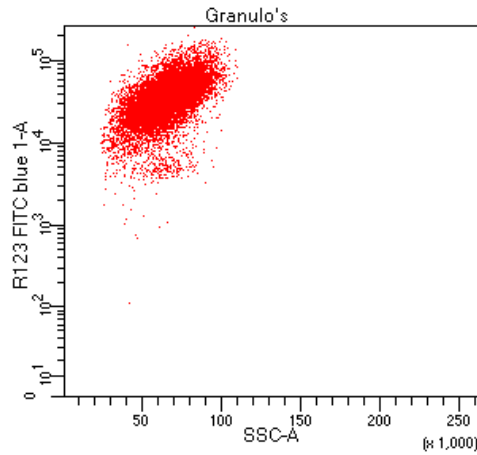
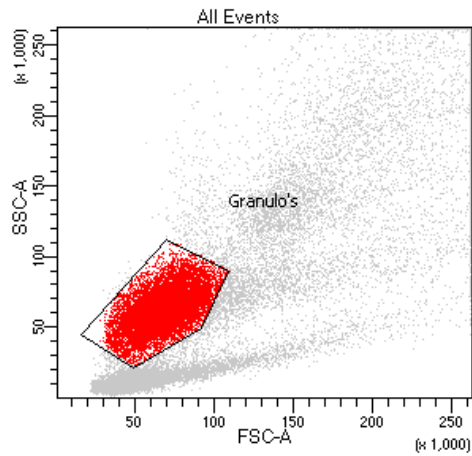
Stimulation Index:
 $54,061/1290 = 42$

Normal oxidative burst₂



No Stimulation

MFI mean: 1290



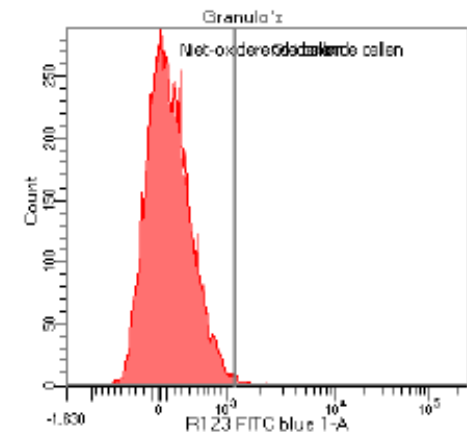
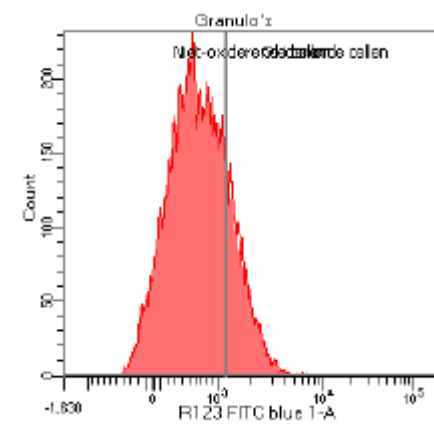
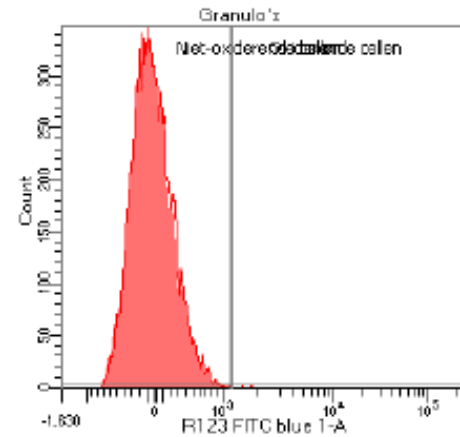
Stimulation with PMA

MFI mean: 40.234

Case 1

Female 57 years

- Treatment for Crohn's disease no effect
- IBD
- Increased CRP
- Normal leucocytes
- Liver abscesses
- Poor wound healing



Neg

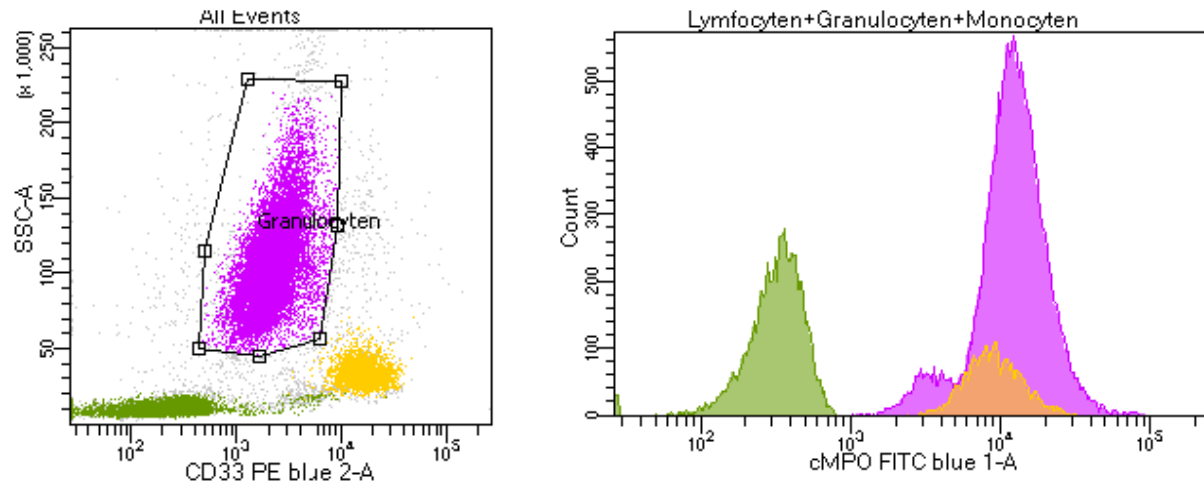
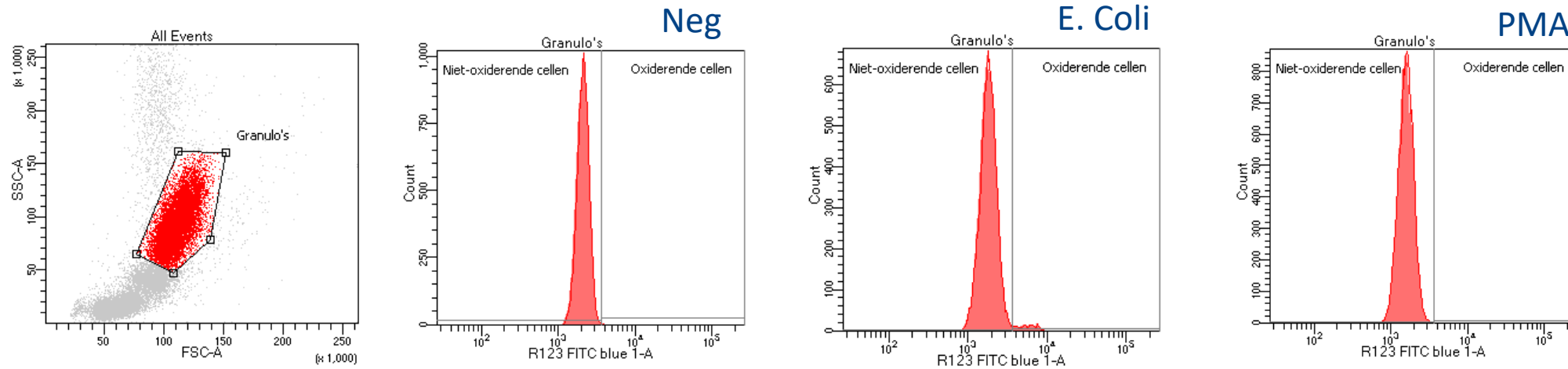
E. Coli

PMA

Deficiency confirmed by mutation analysis:
NCF1/p47 phox;
c.579G>A, p.Trp193* (homozygous)

Case 2

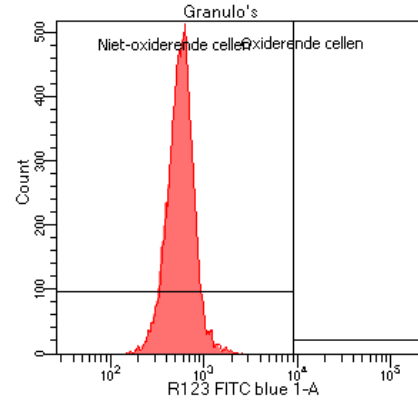
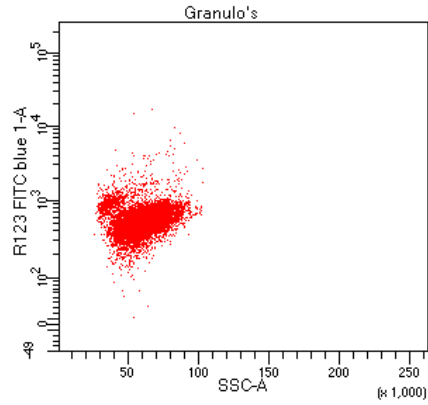
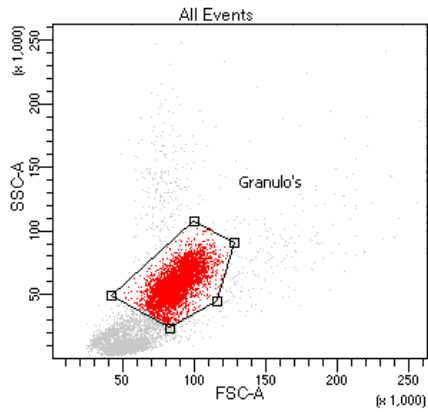
Newborn boy; normal burst?
Mother carrier CYBB mutation



CYBB deficiency confirmed

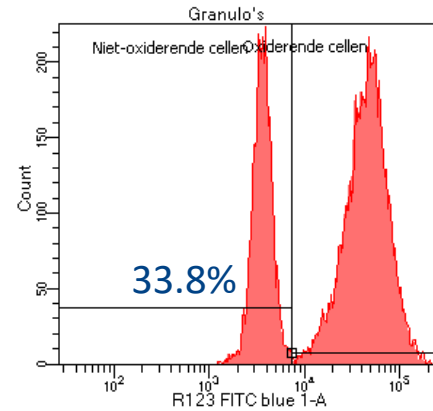
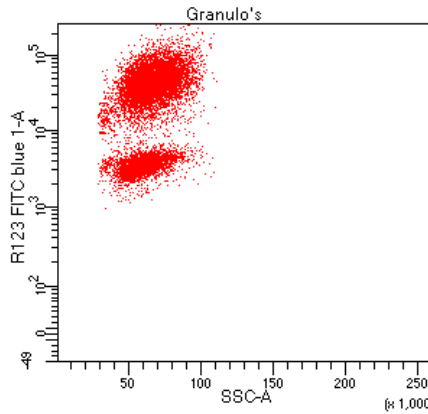
Case 3

XL-CGD (CYBB) carrier (41 years)
Skin back, acne



Neg

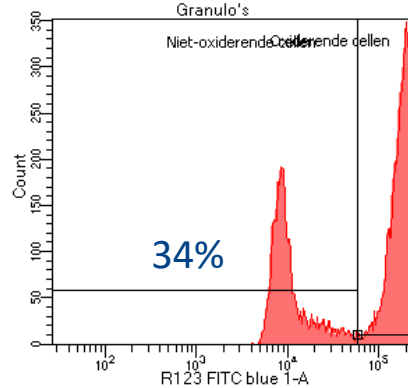
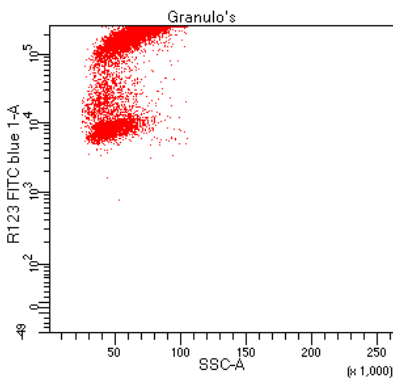
MFI 581



E. Coli

66.2% pos; MFI 49.328

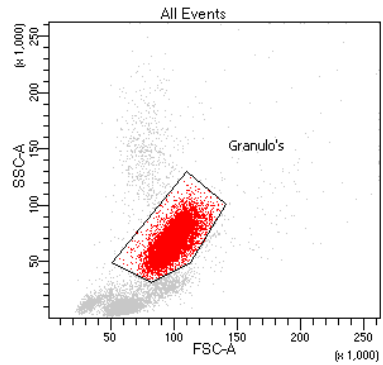
SI: 85



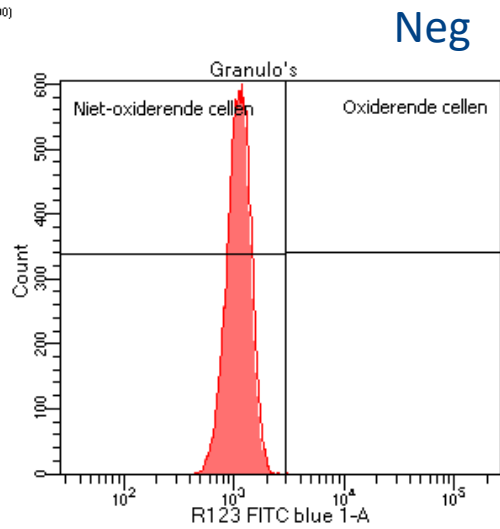
PMA

66% pos; MFI 188.935

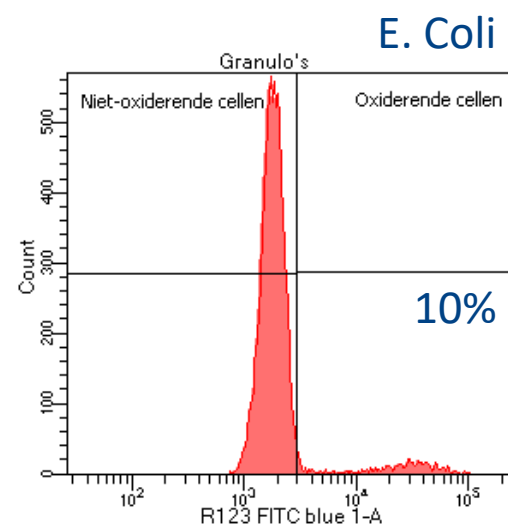
Case 4



XL-CGD (CYBB) carrier (69 years)
Recurrent skin and respiratory infections

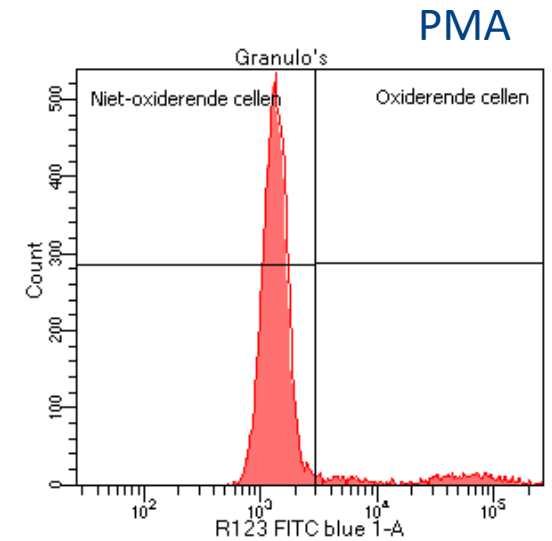


MFI mean: 1076



MFI mean: 29.917

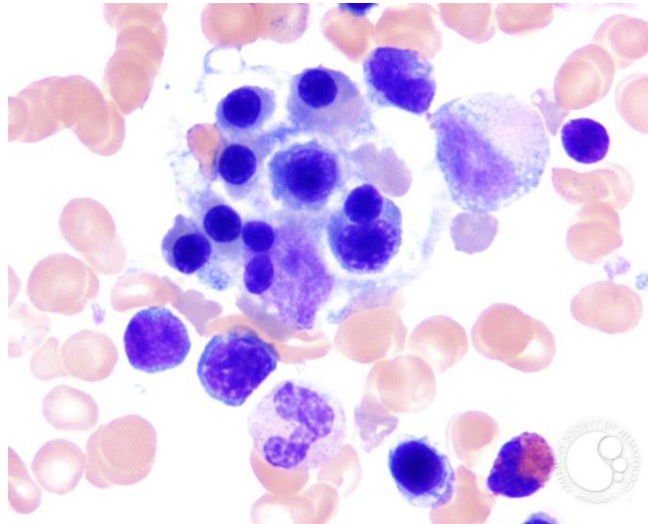
SI: 27.8



MFI mean: 53.190

Goal NK cell function assays

Confirm or exclude NK cell deficiency
in the context of
hemophagocytic lymphohistiocytosis (HLH)



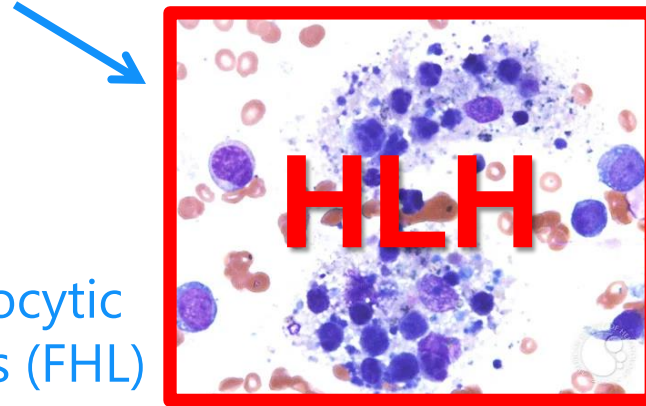
<https://imagebank.hematology.org/image/2975/hemophagocytic-syndrome>

HLH₁

- Aggressive and life-threatening syndrome of excessive immune activation
- Incidence of 1 in 50.000
→ probably underestimated
- Infants from birth to 18 months of age
but also observed in children and adults of all ages
- Clinical presentation is often hard to recognize
- Often a delay in diagnosis

Primary HLH
underlying genetic
cause (~25%)

familial hemophagocytic
lymphohistiocytosis (FHL)



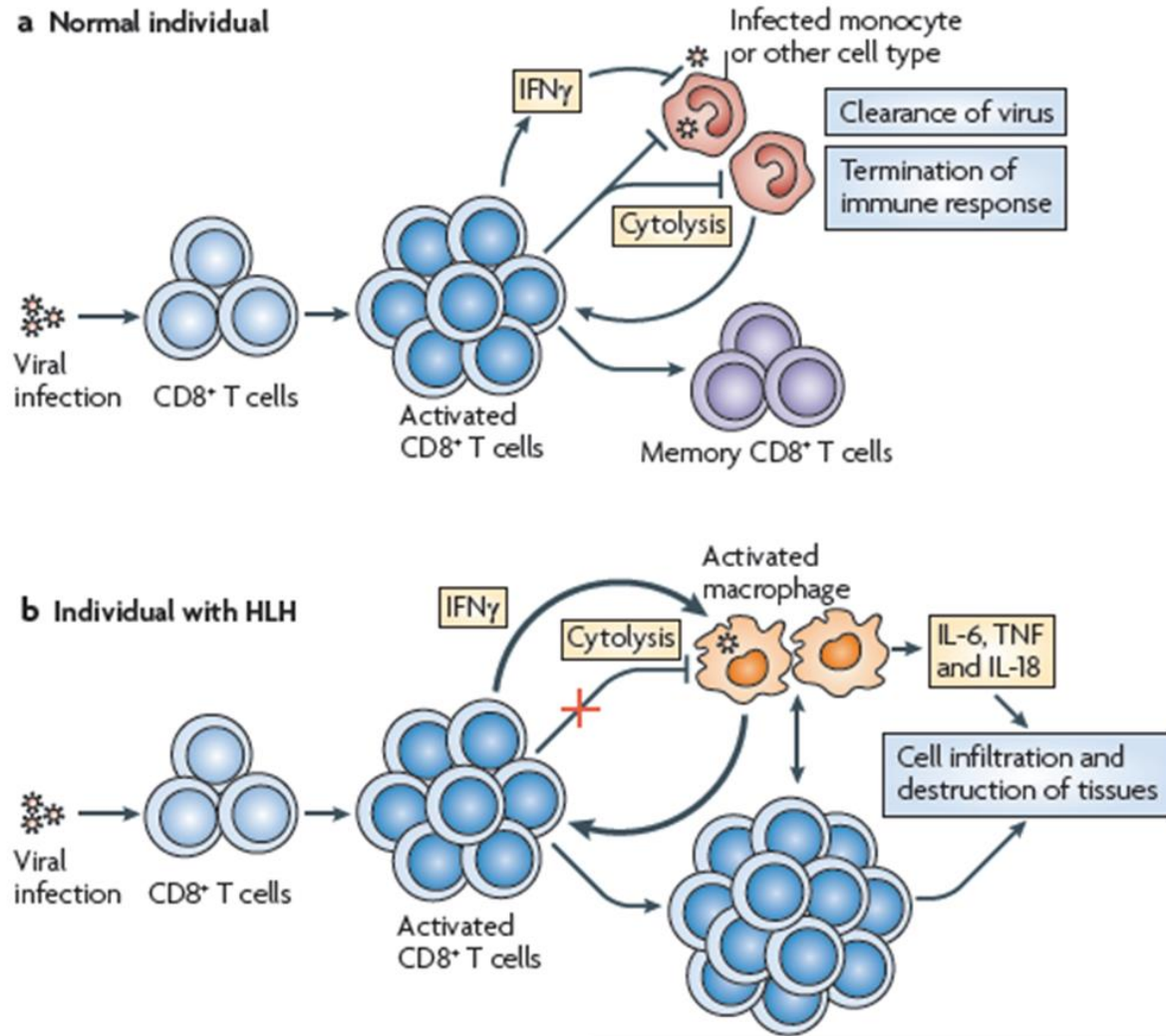
Secondary HLH
alternative source of
pathologic immune
activation

Reactive or required
HLH

HLH is a syndrome of excessive inflammation and
tissue destruction due to abnormal immune activation

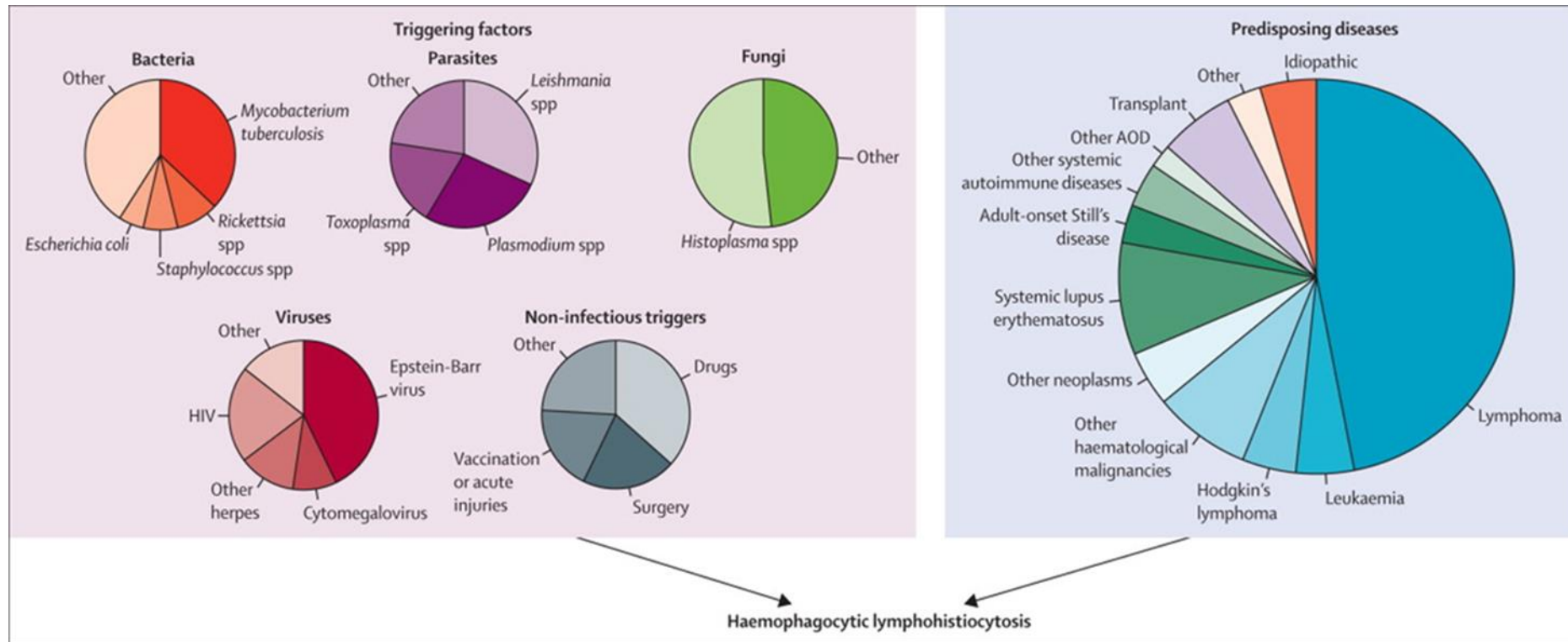
Different causes lead to the same clinical presentation

Primary HLH₃



De Saint Basille G., 2010

Reactive/secondary HLH

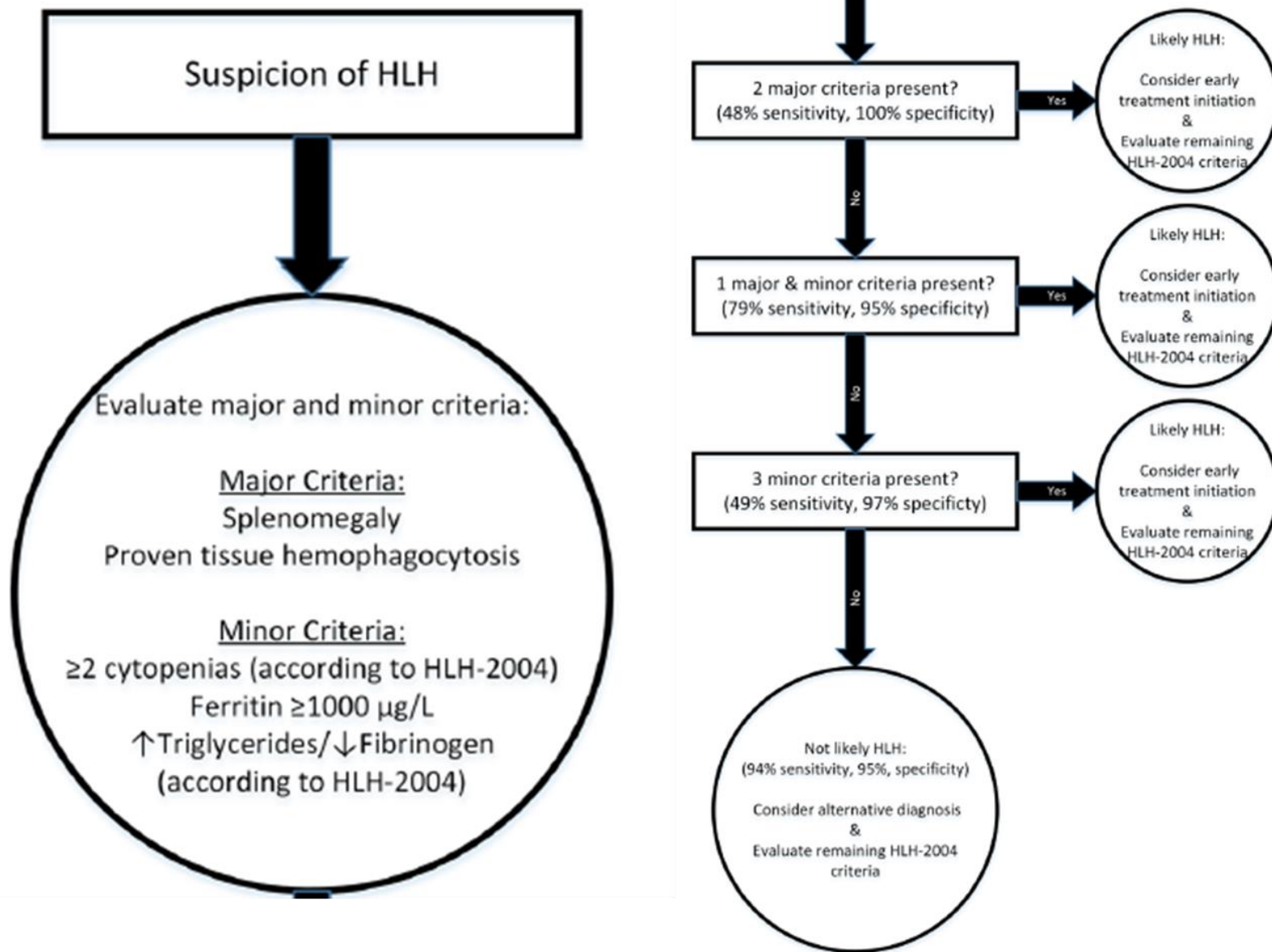


Ramos-Casals M. 2014

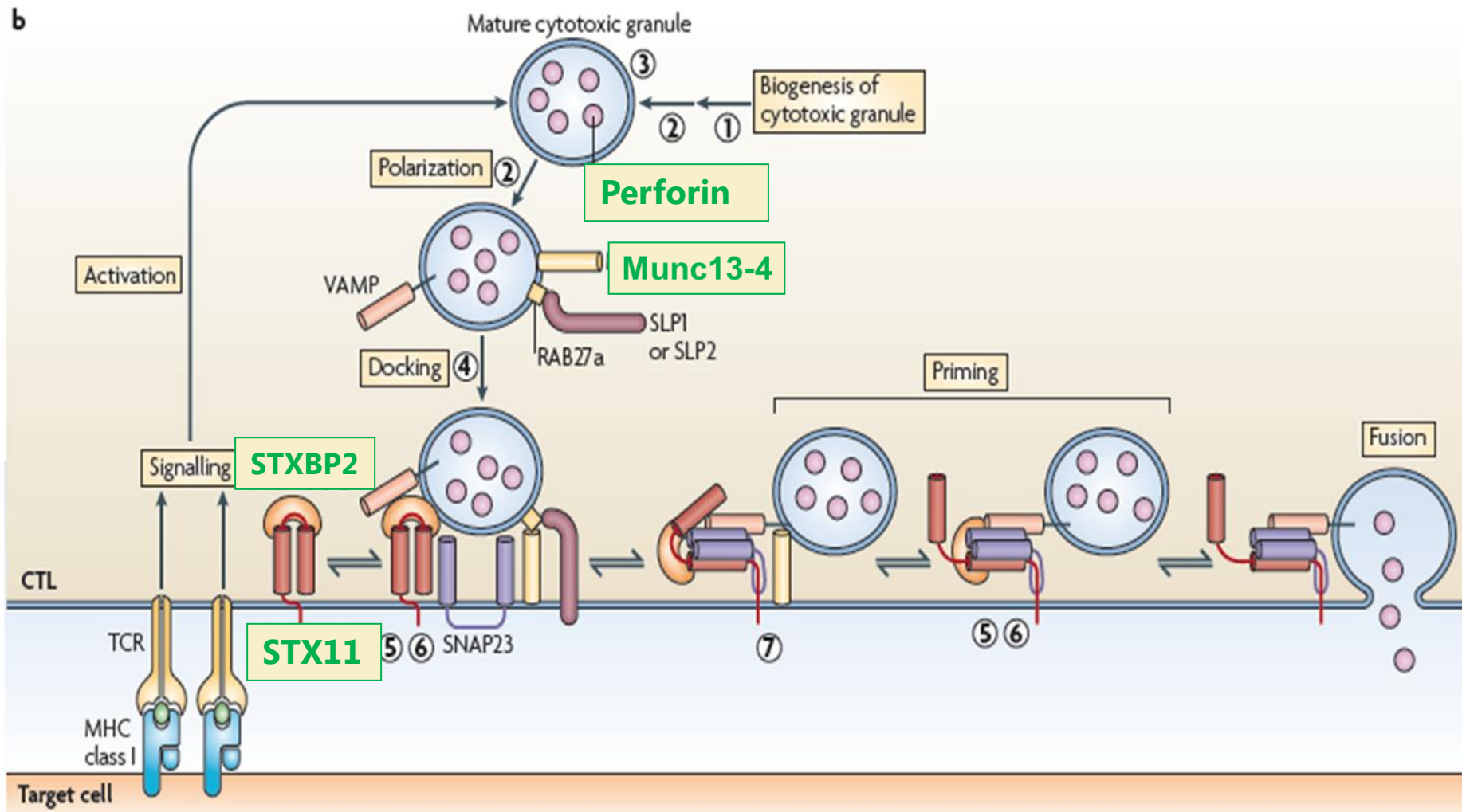
Criteria to diagnose HLH

- Fever (> 7 dagen)
- Splenomegaly
- Cytopenia (2/3 cellines)
- Hypertriglyceridemia and/or hypofibrinogenemia
- Hemophagocytosis (BM)
- NK cell activity absence/decreased
- Ferritin > 500 ug/L (>3000 ug/L)
- sIL2R (sCD25) elevation (2 x sd > ref. age)





Biogenesis and exocytosis of cytotoxic granules

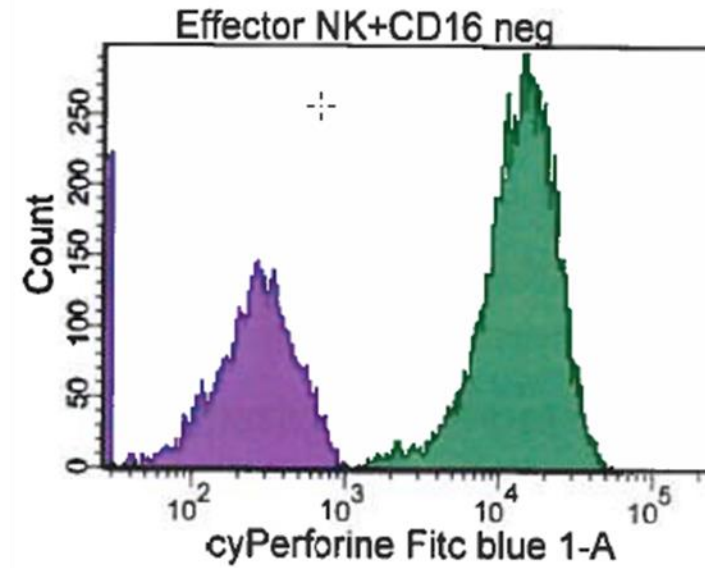
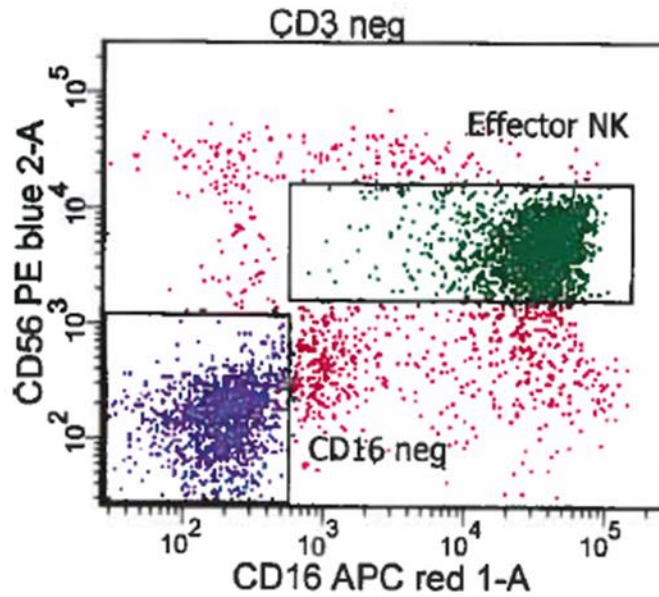
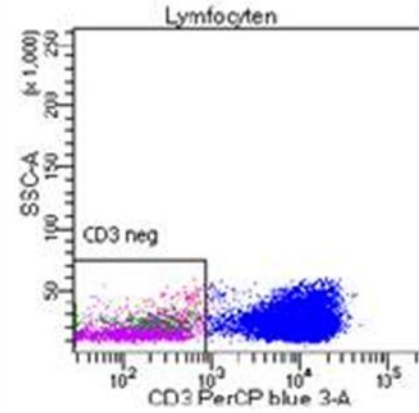
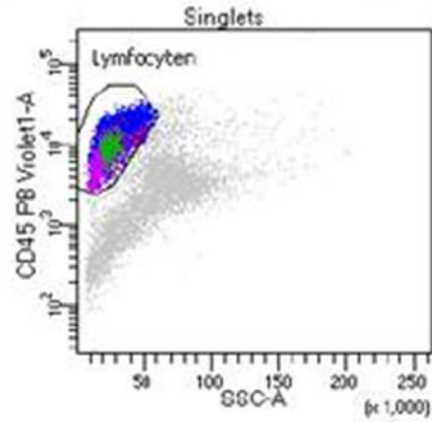


De Saint Basille G., 2010

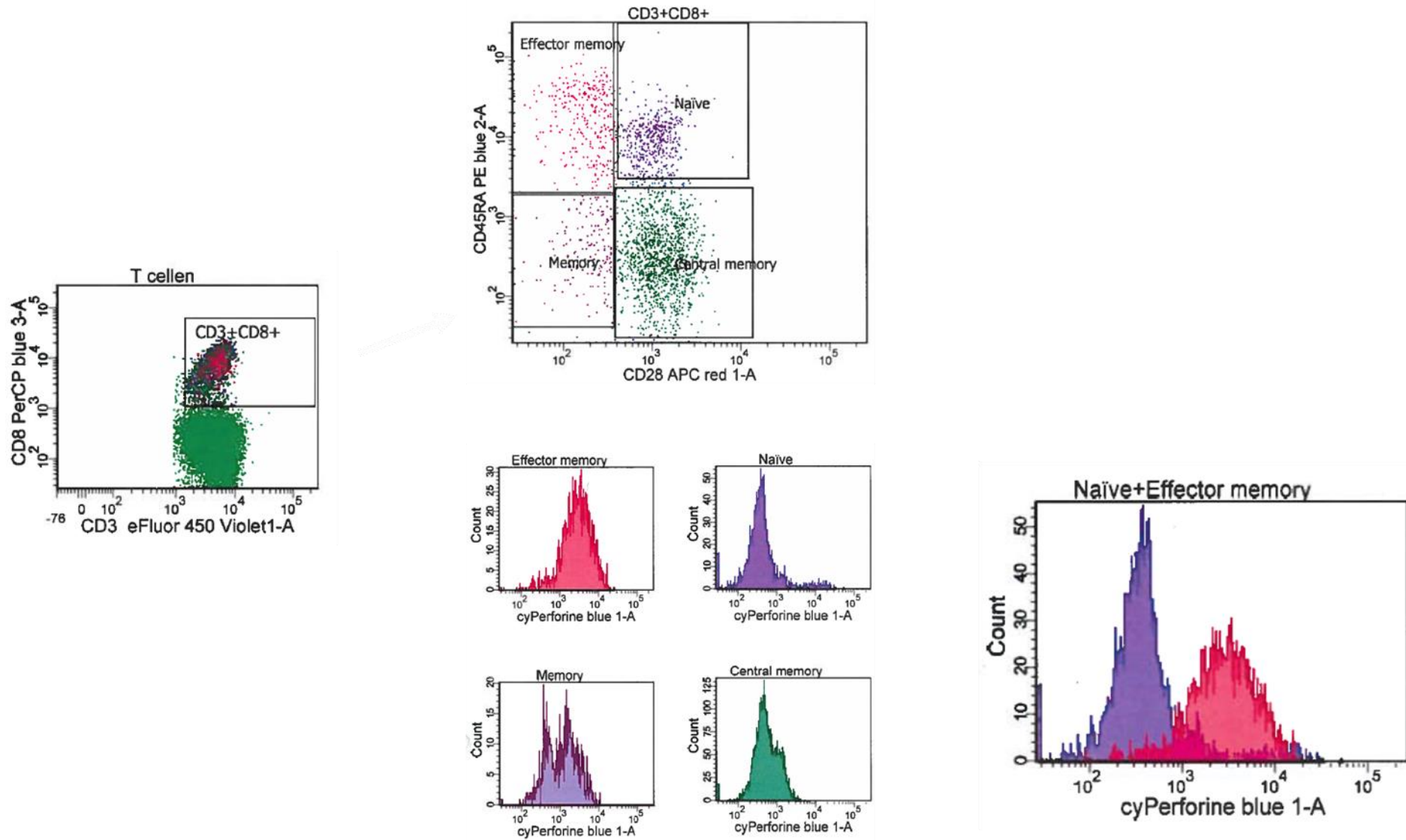
HLH diagnostics

- Cellular
 - Immunophenotyping lymphocyte subsets
 - Intracellular perforin expression
 - Intracellular SAP/XIAP expression
- Serologic
 - Soluble IL2-receptor (sCD25)
- Functional
 - NK cell lysis test
 - Degranulation assay (CD107a assay)
- Molecular
 - Mutation analysis of HLH-associated genes

Perforin expression in NK cells

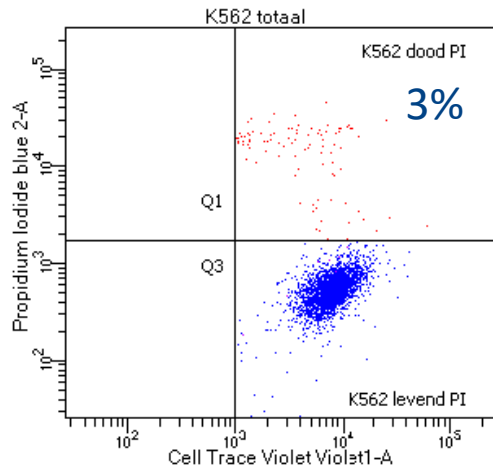
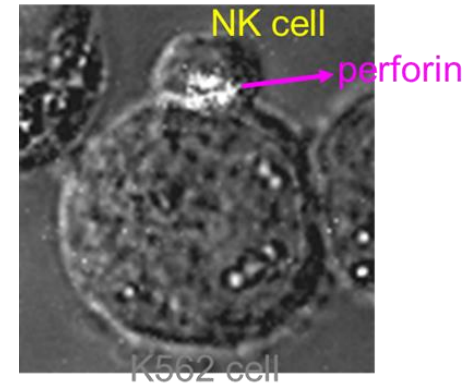


Perforin expression in CD8+ T cells

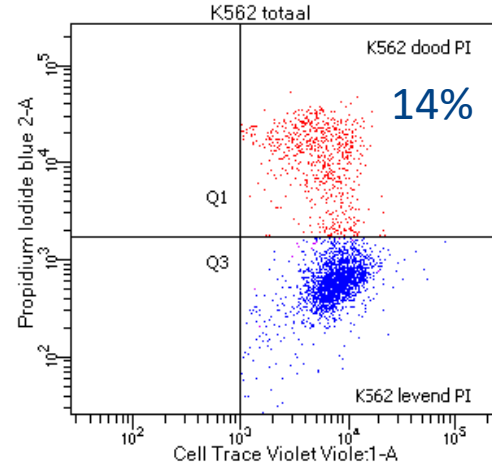


NK cell lysis test

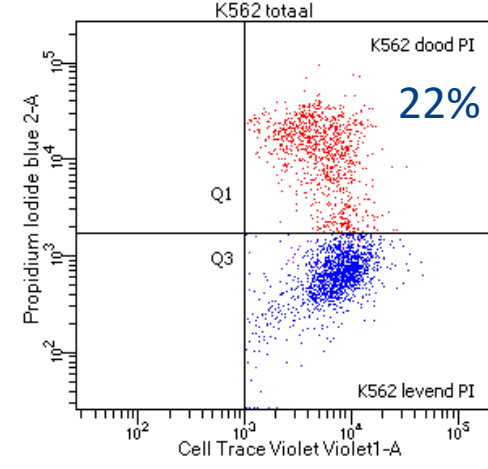
NK cells + Target cells (K562)
Measure % dead



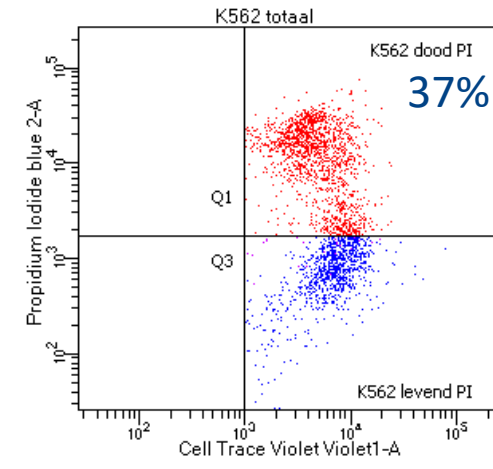
Medium



E:T ratio 1:1



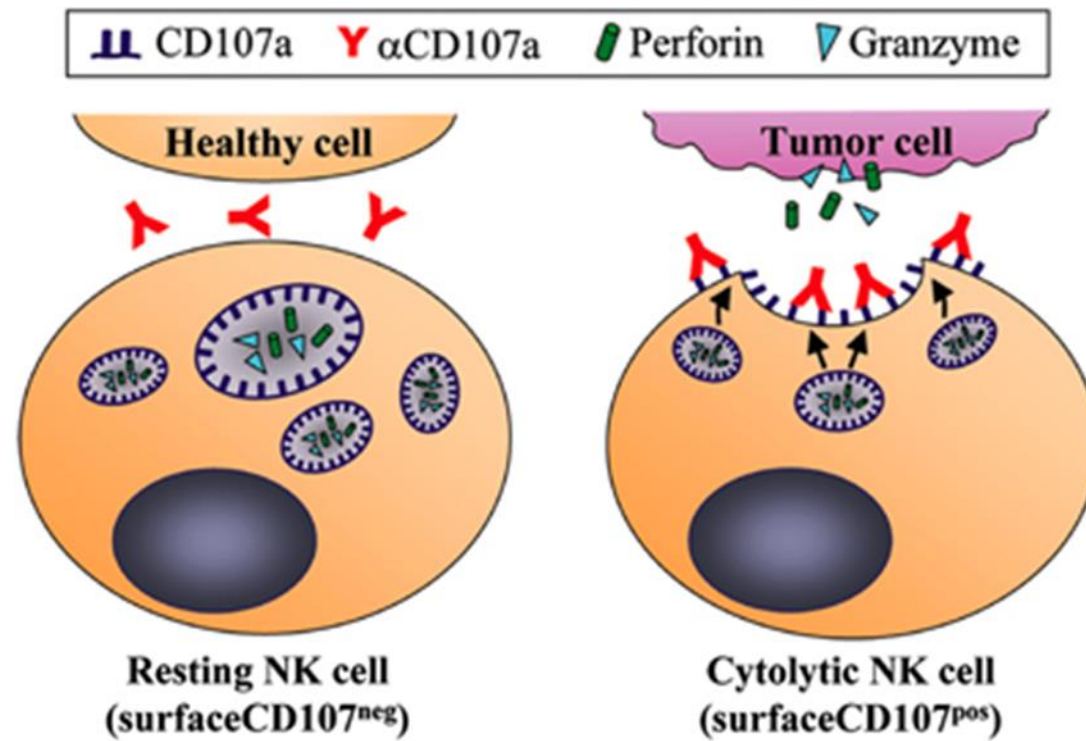
E:T ratio 2:1



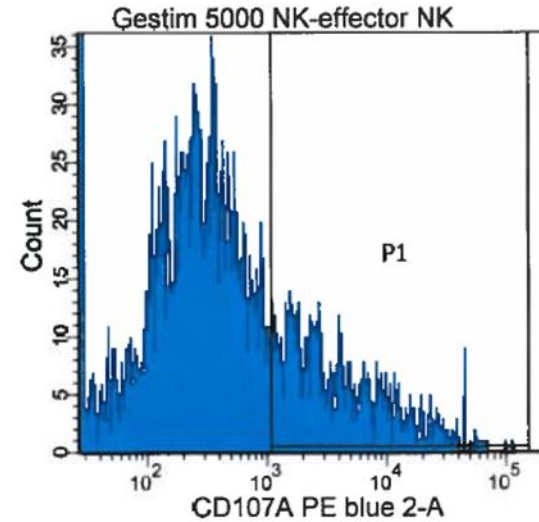
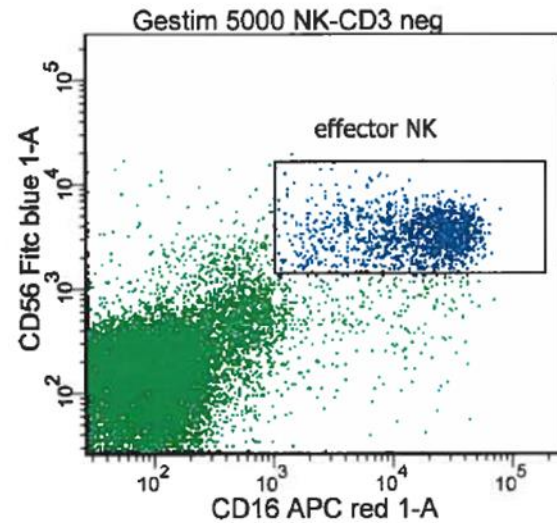
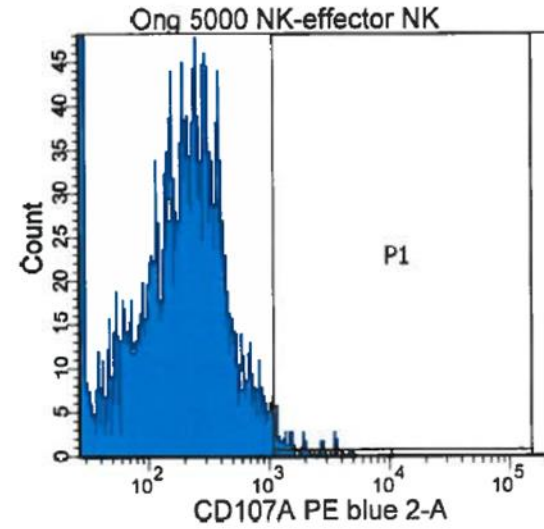
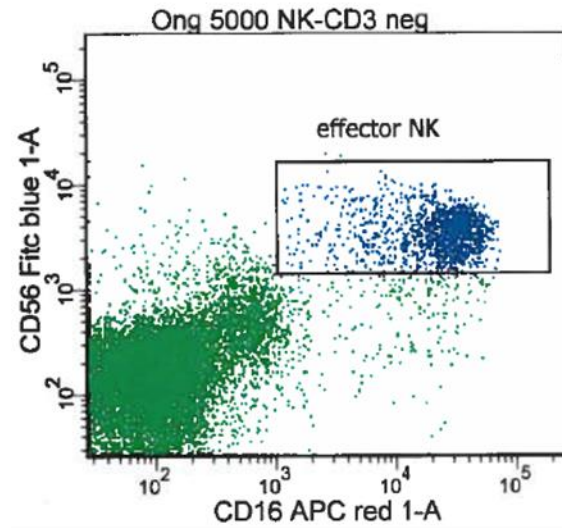
E:T ratio 4:1

CD107a degranulation assay

Target cells (K562) + NK cells
→ CD107a expression measure by flowcytometry



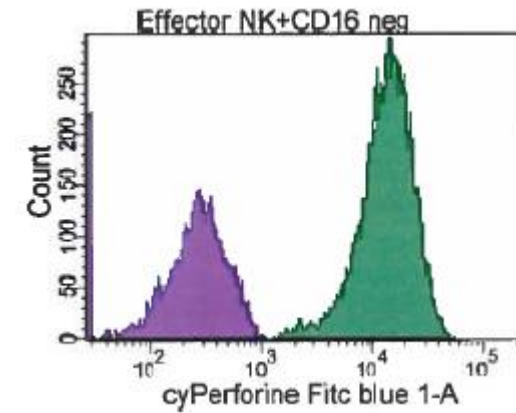
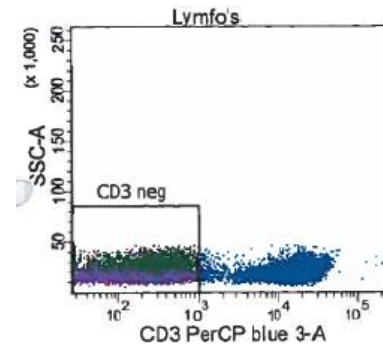
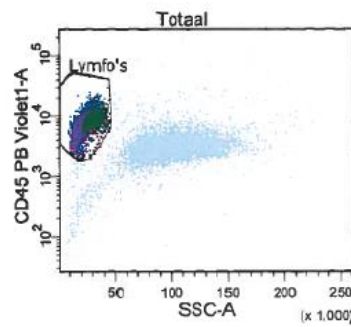
CD107a degranulation assay



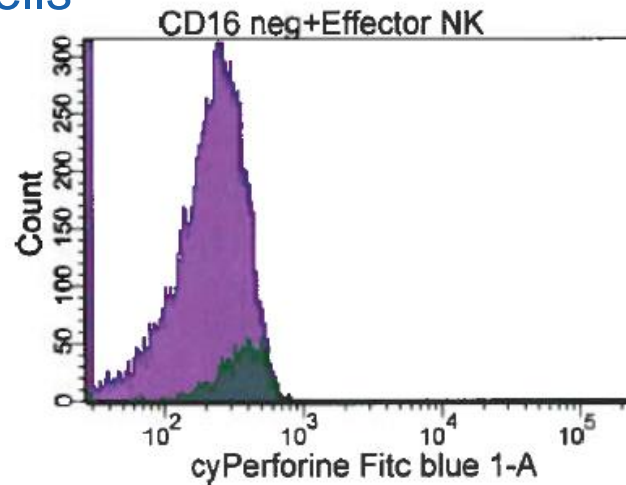
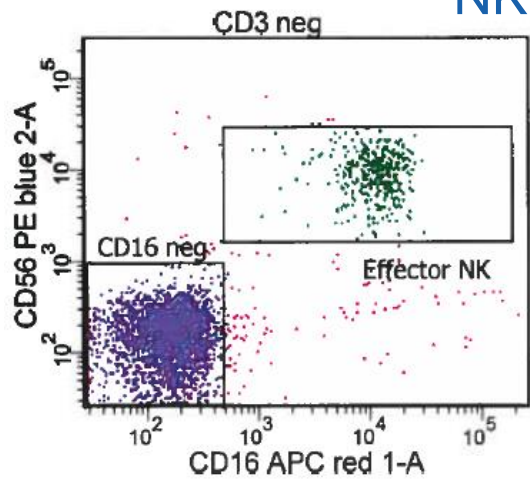
22%

Case 1a

Girl 2 months of age
Persistent fever, not responding to AB (sepsis),
Splenomegaly, cytopenia

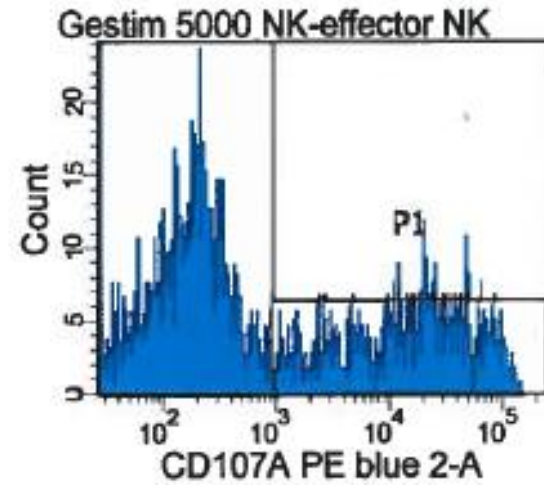
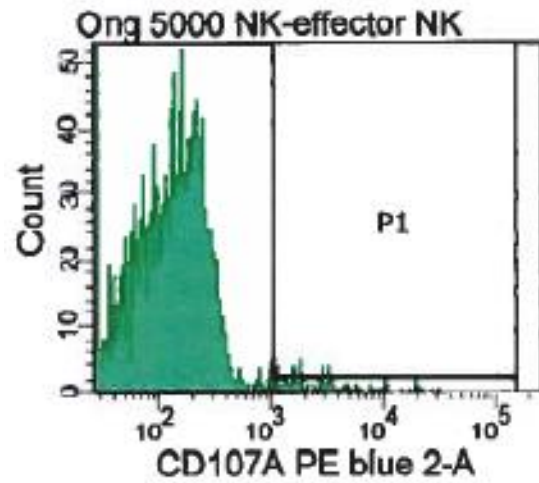


NK cells

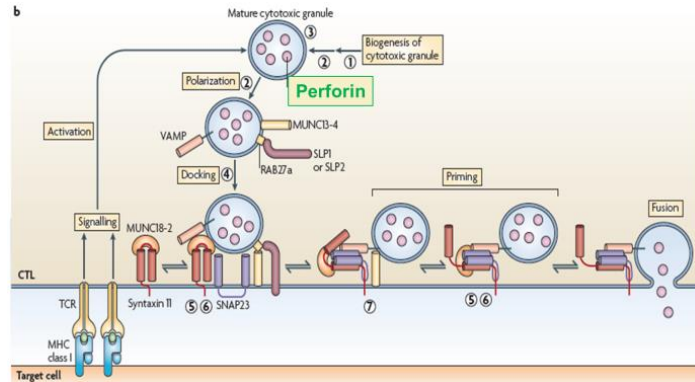


Control

Case 1b



31%



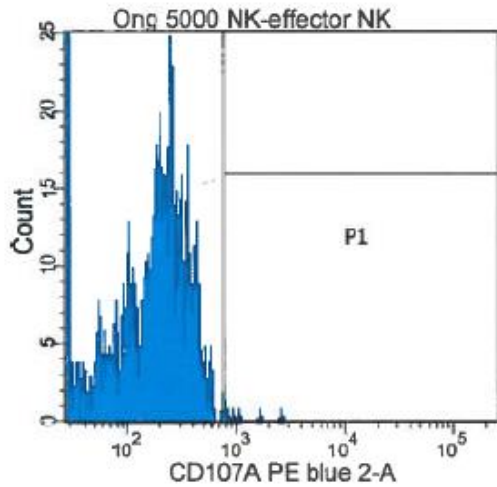
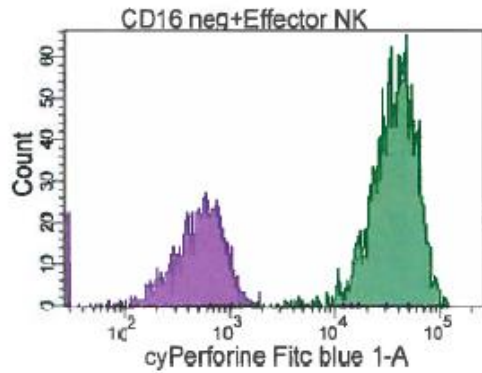
PRF1:

c.445G>A, (p.Gly149Ser)

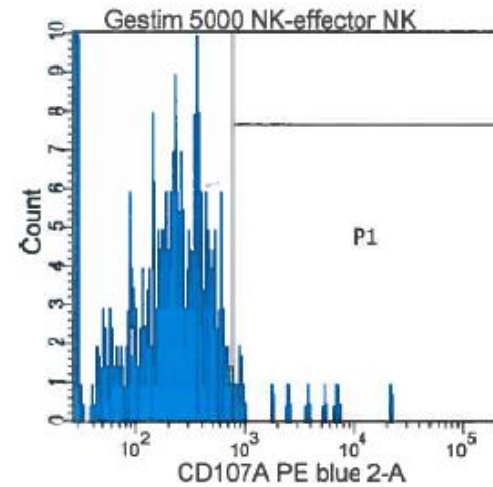
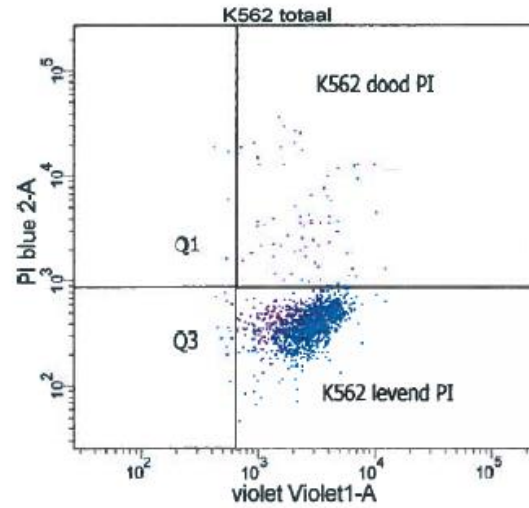
c.757G>A, (p.Glu253Lys)

Case 2

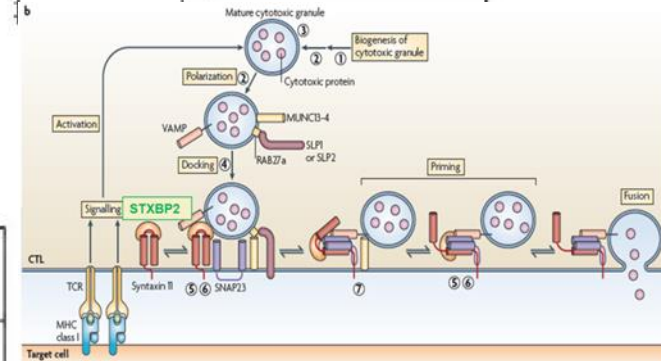
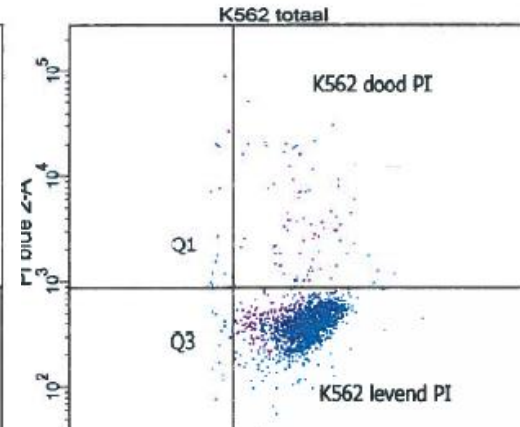
Boy, 2 months of age
dd HLH



E:T ratio 1:1



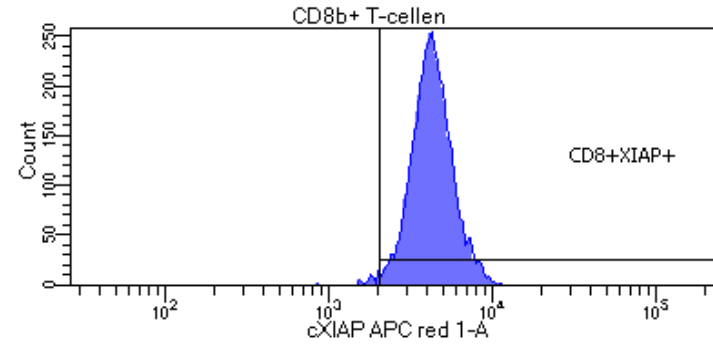
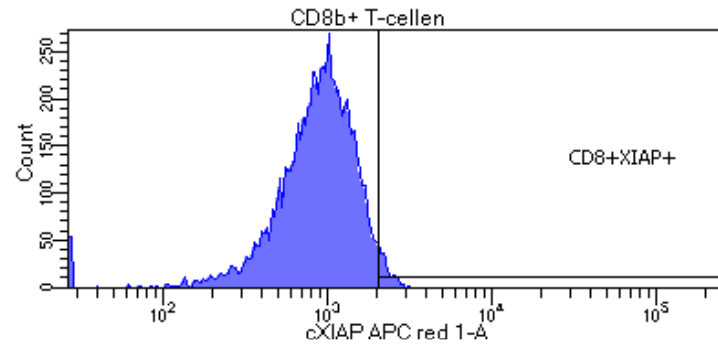
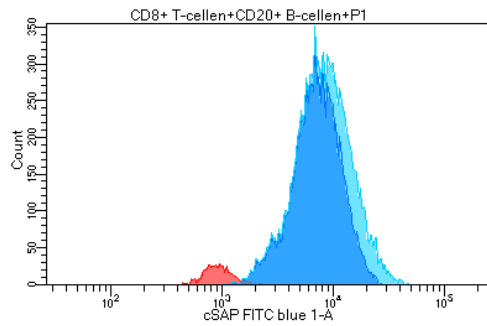
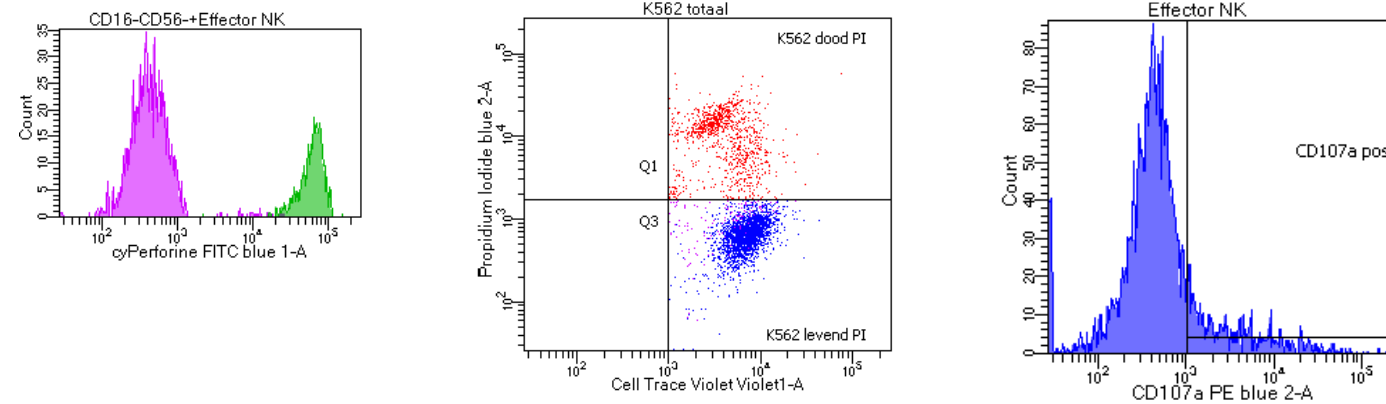
E:T ratio 2:1



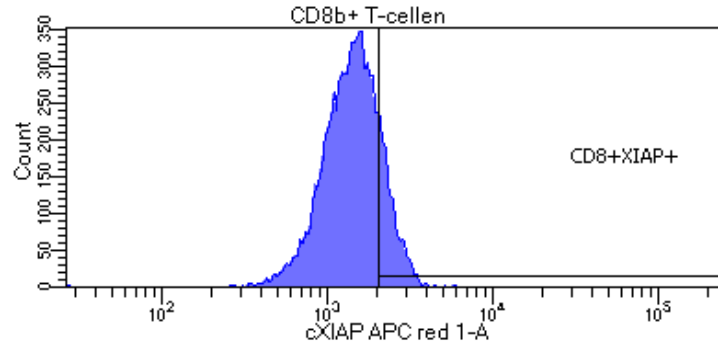
STXBP2: c.902+5G>A (homozygous)

Case 3

Male, 23 y
HLH
Primo EBV infection



Control



XIAP deficiency

Thanks to

Unit Celdiagnostics (CDL)

Department of Genetics