# HLH AND OTHER HISTIOCYTIC DISEASES



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# Histiocyte

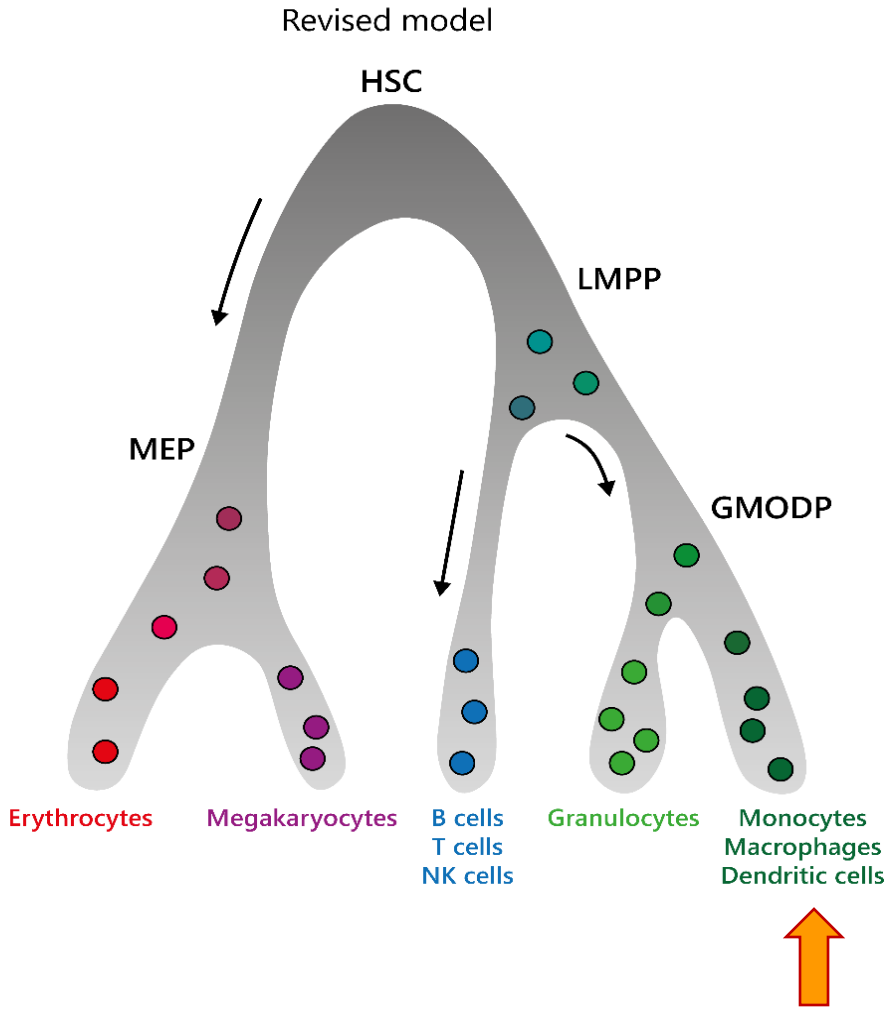
Article Talk

From Wikipedia, the free encyclopedia

A **histiocyte** is a vertebrate cell that is part of the mononuclear phagocyte system (also known as the reticuloendothelial system or lymphoreticular system). The mononuclear phagocytic system is part of the organism's immune system. The histiocyte is a tissue macrophage[1] or a dendritic cell[2] (**histio**, diminutive of **histo**, meaning *tissue*, and **cyte**, meaning *cell*). Part of their job is to clear out neutrophils once they've reached the end of their lifespan.

## Development [ edit ]

Histiocytes are derived from the bone marrow by multiplication from a stem cell. The derived cells migrate from the bone marrow to the blood as monocytes. They circulate through the body and enter various organs, where they undergo differentiation into histiocytes, which are part of the mononuclear phagocytic system (MPS).

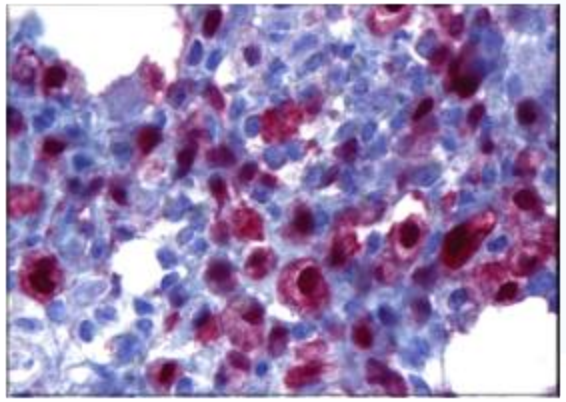


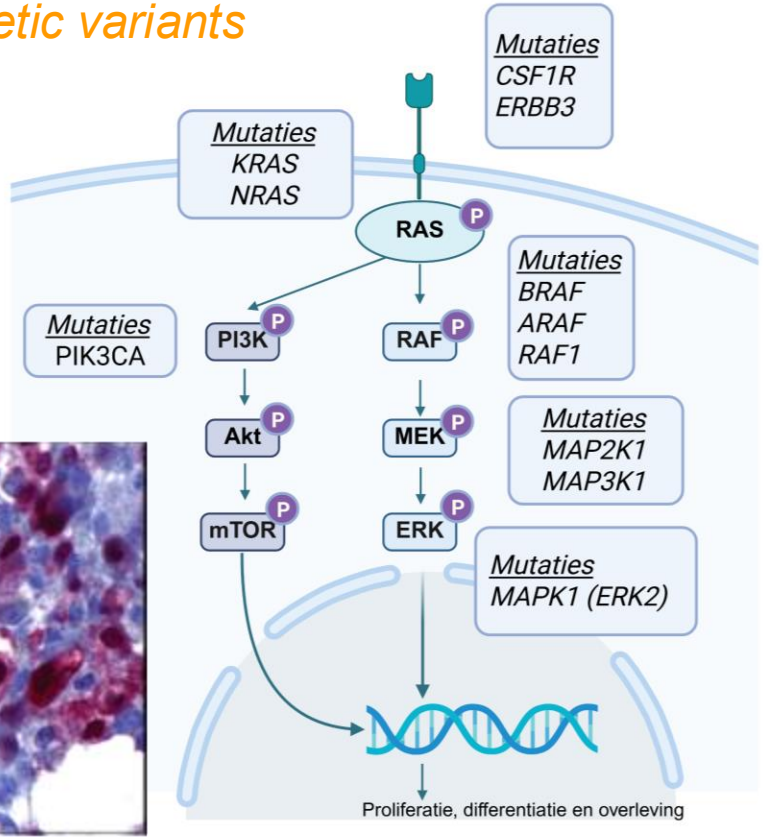
# histiocytic disorders are the result of tissue (hyper)inflammation caused by functionally derailed myeloid cells



## DC and histiocytic neoplasms

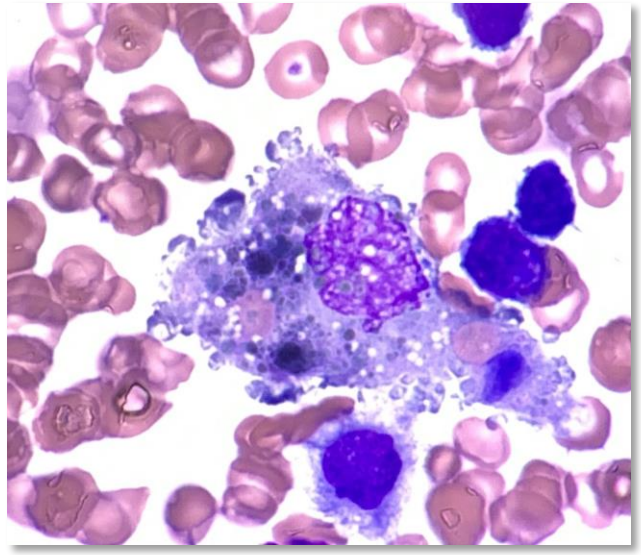
*85% somatic genetic variants*





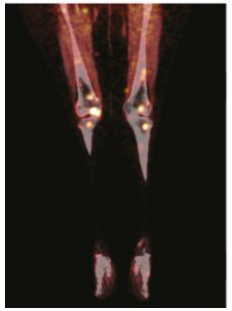

## hemophagocytic lymphohistiocytosis (HLH)

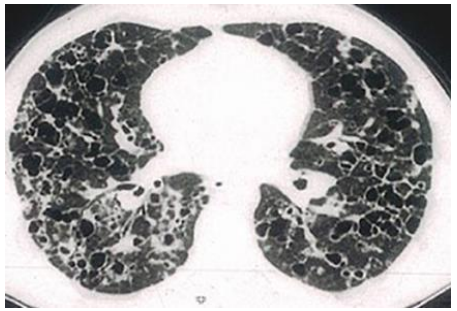
*<10% germline genetic variants*

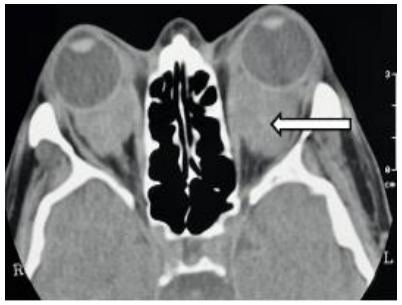


# Clinical features of histiocytic neoplasms



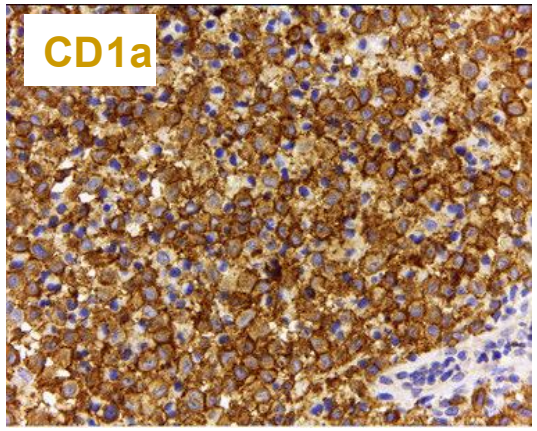


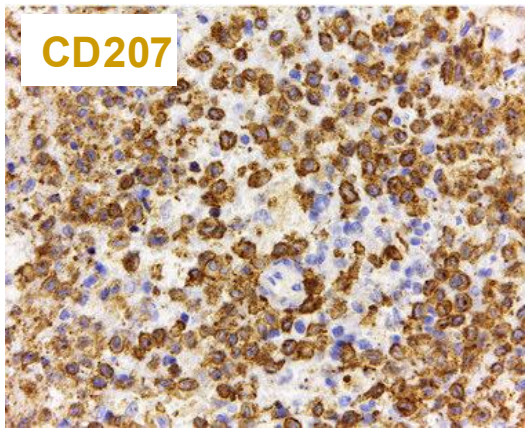





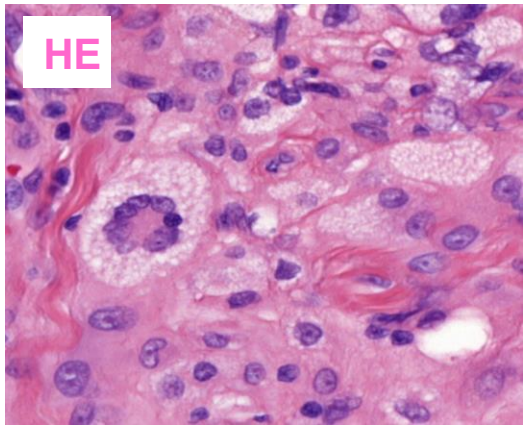
Langerhans cell / dendritic cell features

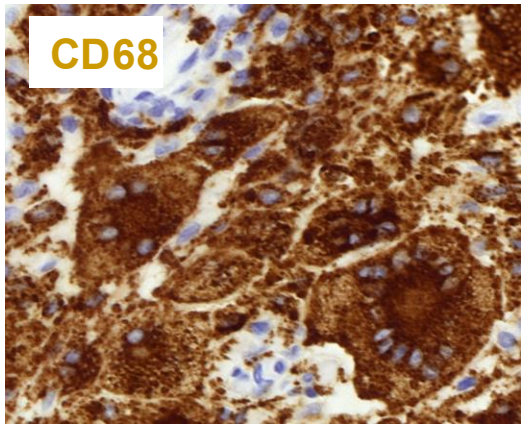
monocyte-macrophage features






Langerhans cell histiocytosis (LCH)
Langerhans cell sarcoma






Erdheim Chester disease (ECD)
Xanthogranuloma (XG)
Rosai Dorfman disease (RDD)
Malignant Histiocytosis

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# LCH

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| self-healing skin | √ | | | | | | | | | wait and watch |
| unifocal bone/skin | | √ | √ | √ | √ | √ | √ | √ | √ | wait and watch / local |
| unifocal lung | | | | | √ | √ | √ | √ | √ | stop smoking |
| multifocal bone | | √ | √ | √ | √ | √ | √ | √ | √ | chemotherapy ± steroids |
| multi-system RO- | | √ | √ | √ | √ | √ | √ | √ | √ | chemotherapy ± steroids |
| multi-system RO+ | √ | √ | | | | | | | | chemotherapy + steroids, MAPKi |
| mixed histiocytosis | | | | | | | | √ | √ | MAPKi |
| LCH + 2nd malignancy | | | | | | | | √ | √ | chemotherapy |

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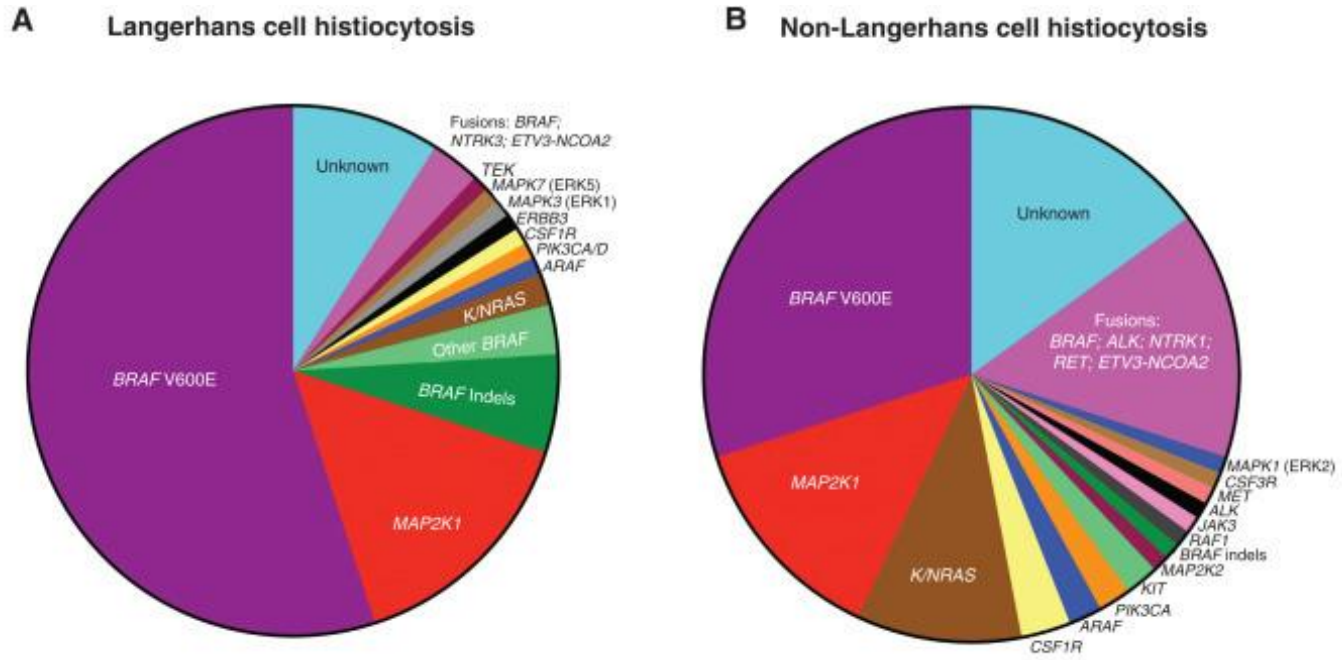
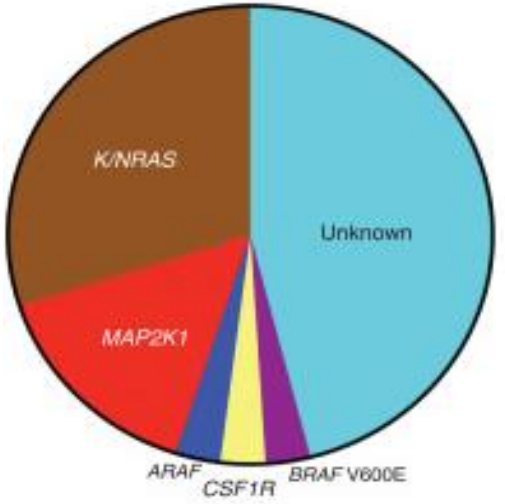
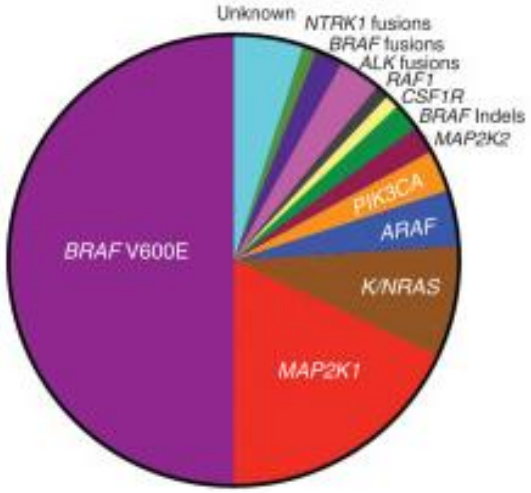
# Mutational spectrum of histiocytic neoplasms



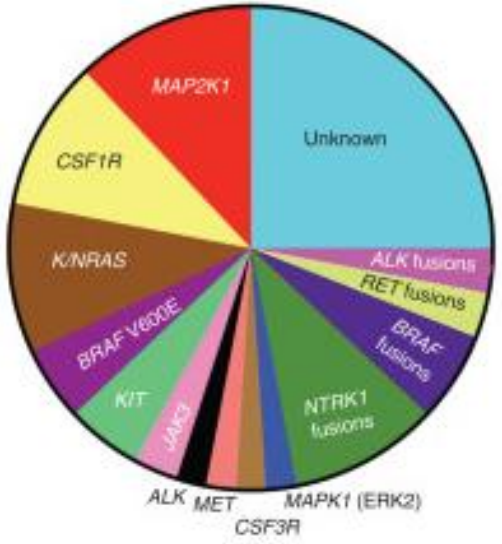



**Rosai–Dorfman–Destombes disease**




**Juvenile/adult xanthogranuloma**

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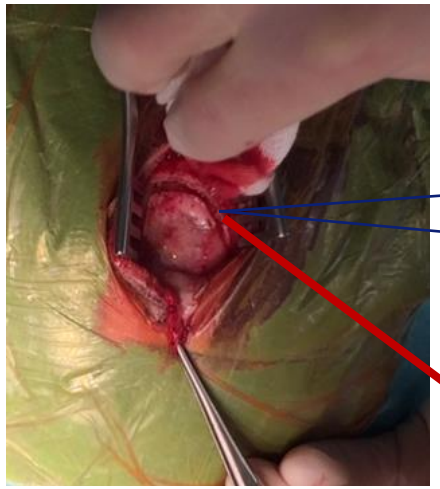
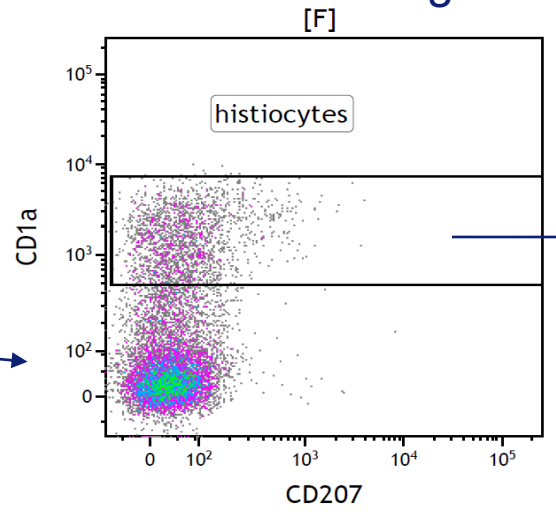
# Diagnostic tissue handling



fresh or
cryopreservation

bulk sorting

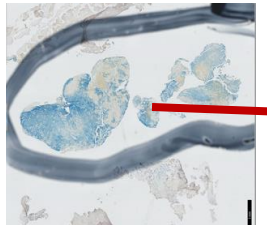



RNA or DNA

snap-frozen

IHC

manual
microdissection



RNA or DNA

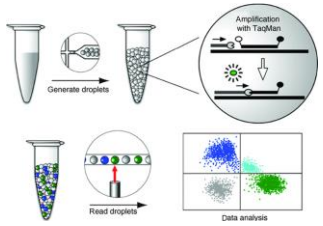
targeted sequencing

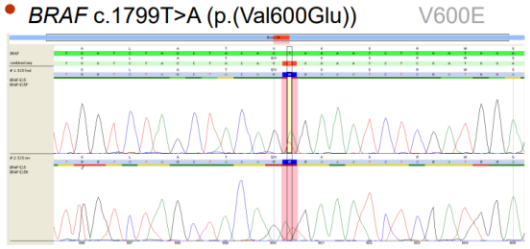
plasma
for ccf-DNA

ficol

PBMC for DNA

granulocytes

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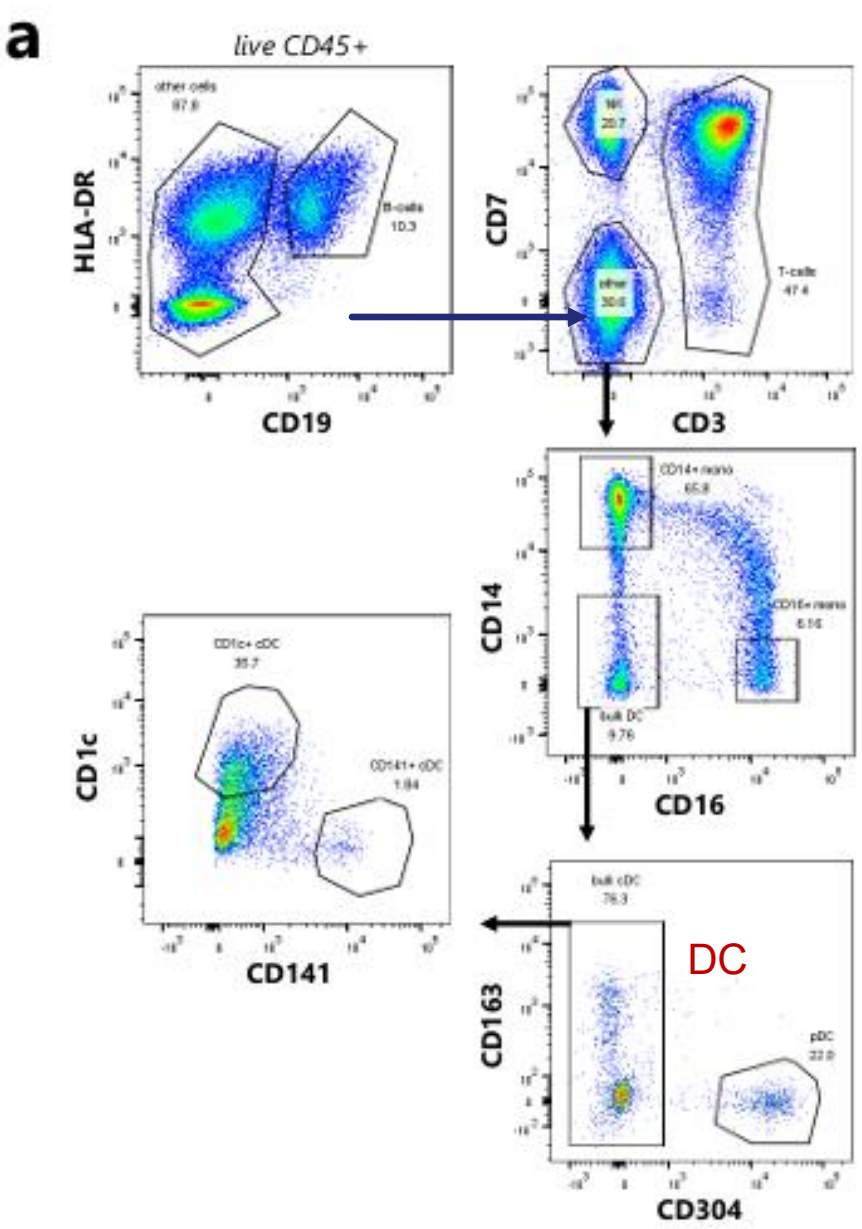
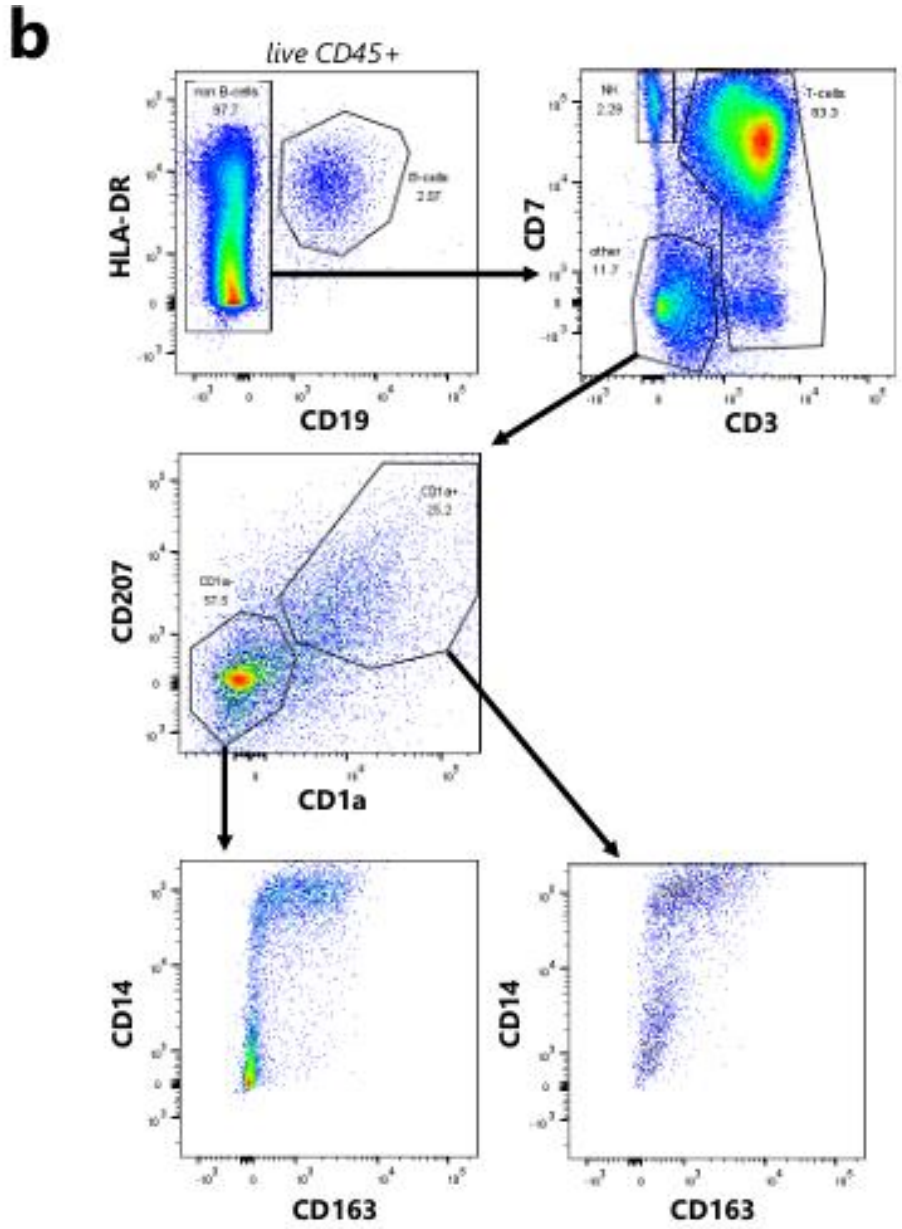
# Gating strategies for blood (a) and tissue specimen (b)






CD45 APC-H7

HLA-DR V500

CD3 FITC

CD7 R718

CD19 BV786

CD1a APC

CD207 PE

CD163 BV711

CD14 PE-CF594

CD16 Pacific blue

CD11c PE-Cy7

CD207 PE / CD304 PE

# Mutation distribution in adults with active histiocytosis



| Patient | Disease (extent) | Mutation | Sorted cells: Histiocytes | T | B | NK |
|---|---|---|---|---|---|---|
| 10 | AOX (SS) | *KRAS* p.G12R | 40,4 | 0,4 | 0 | 3,8 |
| 14 | LCS (SS) | *KRAS* exon 2 indel | 28,2 | 0 | 3,6 | NT |

% mutant cells: ≥ 15 … 0.002

Tissue

| Patient | Disease (extent) | Mutation | PBMCs | Sorted myeloid cells: Gran | CD14$^+$ mono | CD16$^+$ mono | DC | Sorted lymphoid cells: T | B | NK |
|---|---|---|---|---|---|---|---|---|---|---|
| **L-group** | | | | | | | | | | |
| 1 | LCH (SS) | *BRAF* p.V600E | 0 | 0 | 0,01 | NT | 0 | 0 | 0 | 0,005 |
| 2 | LCH (MS) | *BRAF* p.V600E | 0 | 0,039 | 0,03 | 0 | 0,023* | 0 | 0,007* | 0 |
| 3 | ECD/LCH (SS) | *BRAF* p.V600E | 0,22 | NT | 1,00 | 1,42 | 0 | NT | NT | 0,48 |
| 4 | ECD/LCH (MS) | *BRAF* p.V600E | 0 | NT | 0 | 1,02 | 0,56 | NT | NT | 0 |
| 5 | ECD/LCH (MS) | *BRAF* p.V600E | 0,18 | NT | 0 | 0 | NT | NT | NT | 0 |
| 6 | ECD (MS) | *BRAF* p.V600E | 0,06 | NT | 0 | 0 | 0 | NT | NT | 1,6* |
| 7 | ECD (MS) | *BRAF* p.V600E | 0 | NT | 0 | 0 | 0,26 | NT | NT | 0,34 |
| 8 | ECD (MS) | *BRAF* p.V600E | 0 | NT | 0 | 0 | 0 | NT | NT | 0,80 |
| 9 | ECD (MS) | *BRAF* p.V600E | 0 | NT | 0,46 | 0,64 | 0 | NT | NT | 1,34 |
| **C-group** | | | | | | | | | | |
| 10 | AOX (SS) | *KRAS* p.G12R | 2,12 | 0,76 | 10,60 | 11,00 | 6,00 | 0,15 | 2,50 | 8,20 |
| 11 | AOX (SS) | *BRAF* p.N518S | NT | 8,80 | 13,10 | 12,60 | 10,60 | 0,09 | 9,10 | 5,40 |
| 12 | AOX (SS) | *KRAS* p.Q61H | NT | NT | 4,10 | 0,70 | 1,80 | 0,54 | 1,80 | 0,18 |
| **R-group** | | | | | | | | | | |
| 13 | RDD/ECD (MS) | *MAP2K1* p.K57N | 0,05 | 0,004 | 0,02 | NT | 0,05 | 0 | 0,03* | 0,25 |
| **M-group** | | | | | | | | | | |
| 14 | LCS (SS) | *KRAS* exon 2 indel | 0 | 0 | 0 | NT | 0 | 0 | 0 | 0 |

Blood

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AGS van Halteren, *Blood Advances* 2025;2:1

# Mutation distribution in children with first onset LCH

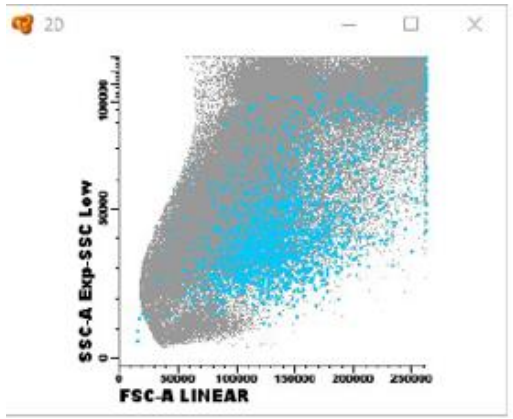
(+++) >10%
(++) 1 - 10%
(+) 0.1 – 1%
(+/-) 0.03 - 0.1%

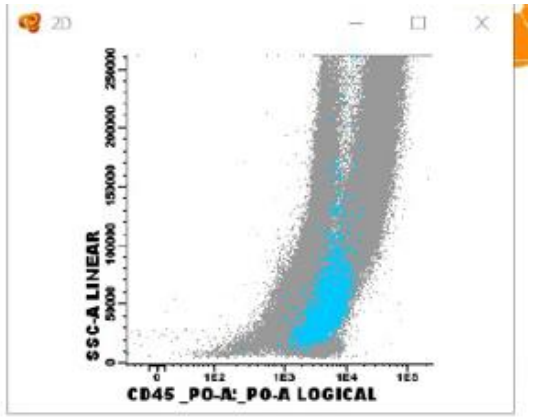
| | MS RO+ | | | | | MS RO- | | | MFB | | UF | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LCH714 | LCH110 | LCH344 | LCH442 | LCH886 | LCH785 | LCH354 | LCH572 | LCH135 | LCH578 | LCH450 | LCH469 | LCH555 | LCH586 | LCH556 | LCH692 |
| HLA-DR$^{pos}$ CD14$^{dim\text{-}to\text{-}neg}$ DC | +++ | ++ | +++ | ++ | - | ++ | - | +/- | - | + | - | - | - | - | - | - |
| HLA-DR$^{pos}$ CD14$^{bright}$ Mono | +++ | ++ | ++ | + | + | ++ | - | +/- | - | +/- | - | - | - | +/- | - | +/- |
| HLA-DR$^{pos}$ CD19$^{pos}$ B cells | + | + | +/- | - | +/- | ++ | - | - | - | n/a | - | - | - | | - | - |
| CD3$^{pos}$ CD7$^{neg\ or\ pos}$ T cells | +/- | - | - | - | - | +/- | - | - | - | n/a | - | - | - | - | - | - |
| CD3$^{neg}$ CD7$^{pos}$ NK cells | + | n/a | n/a | n/a | + | ++ | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | +/- |

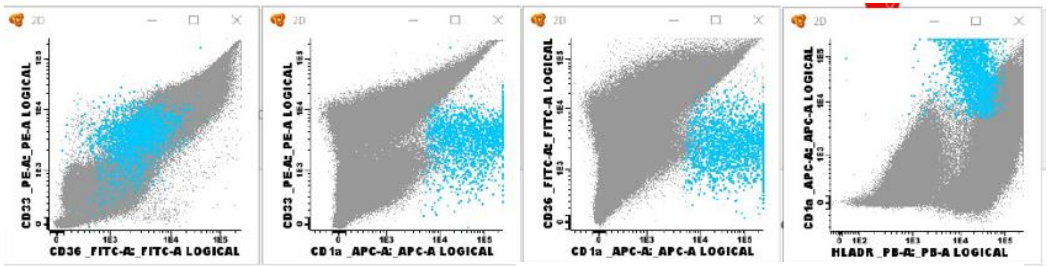
Adapted from A.G.S. van Halteren et al. *Blood* 2020;136:2188

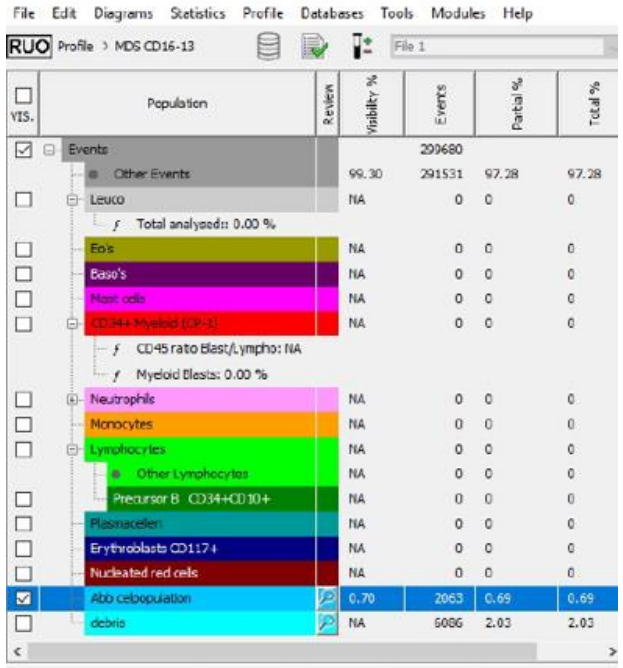
# Combining flow and mutational analysis on broncho-alveolar lavage of patient suspected of pulmonary LCH =>

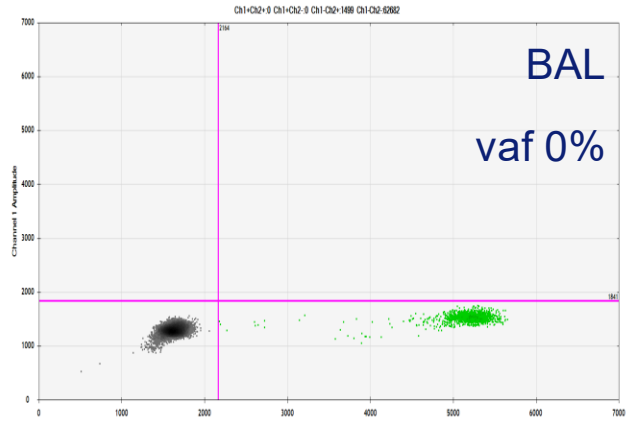


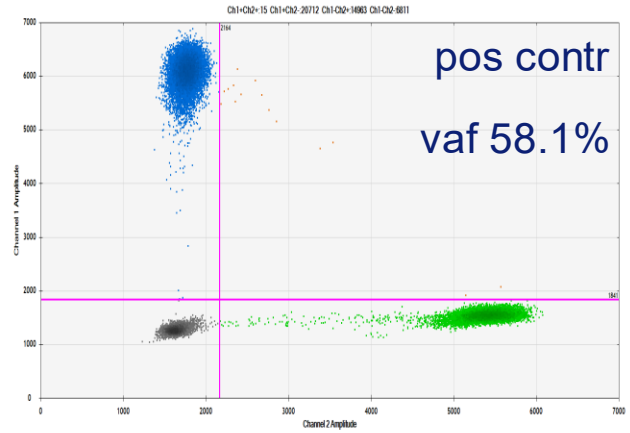


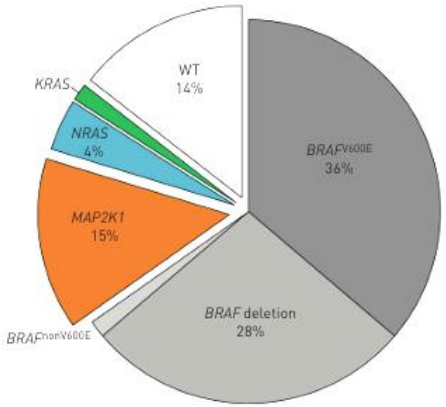




F. Jouenne et al. Eur Respir J 2020;55:1901190

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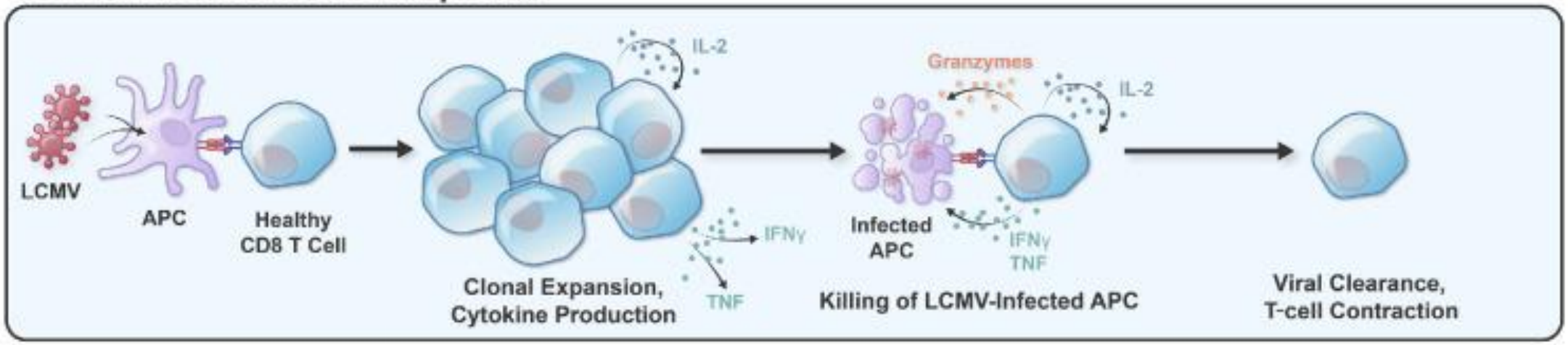
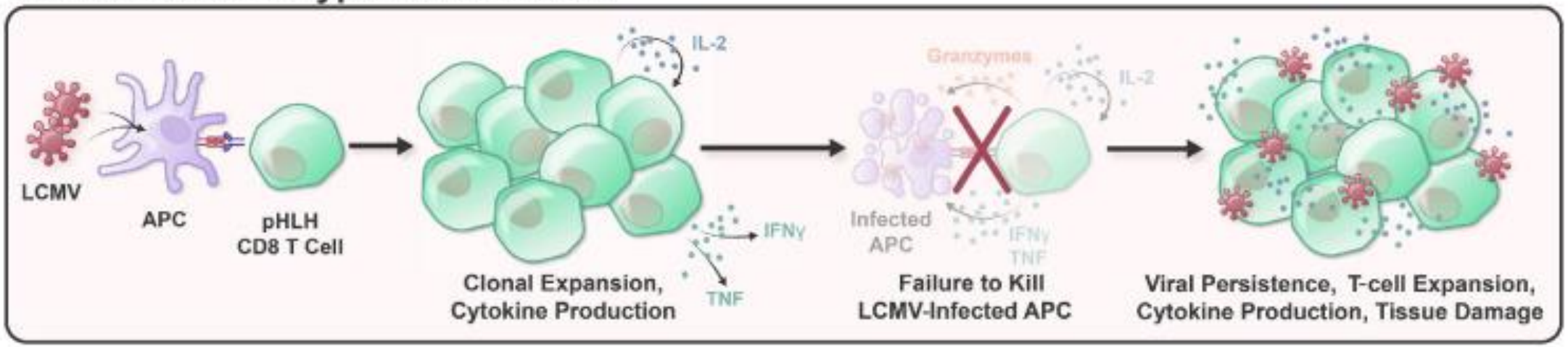
# HLH



## Normal Host Immune Response

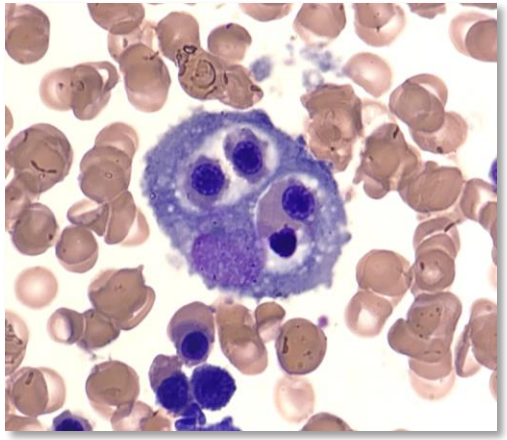



## HLH-Associated Hyperinflammation

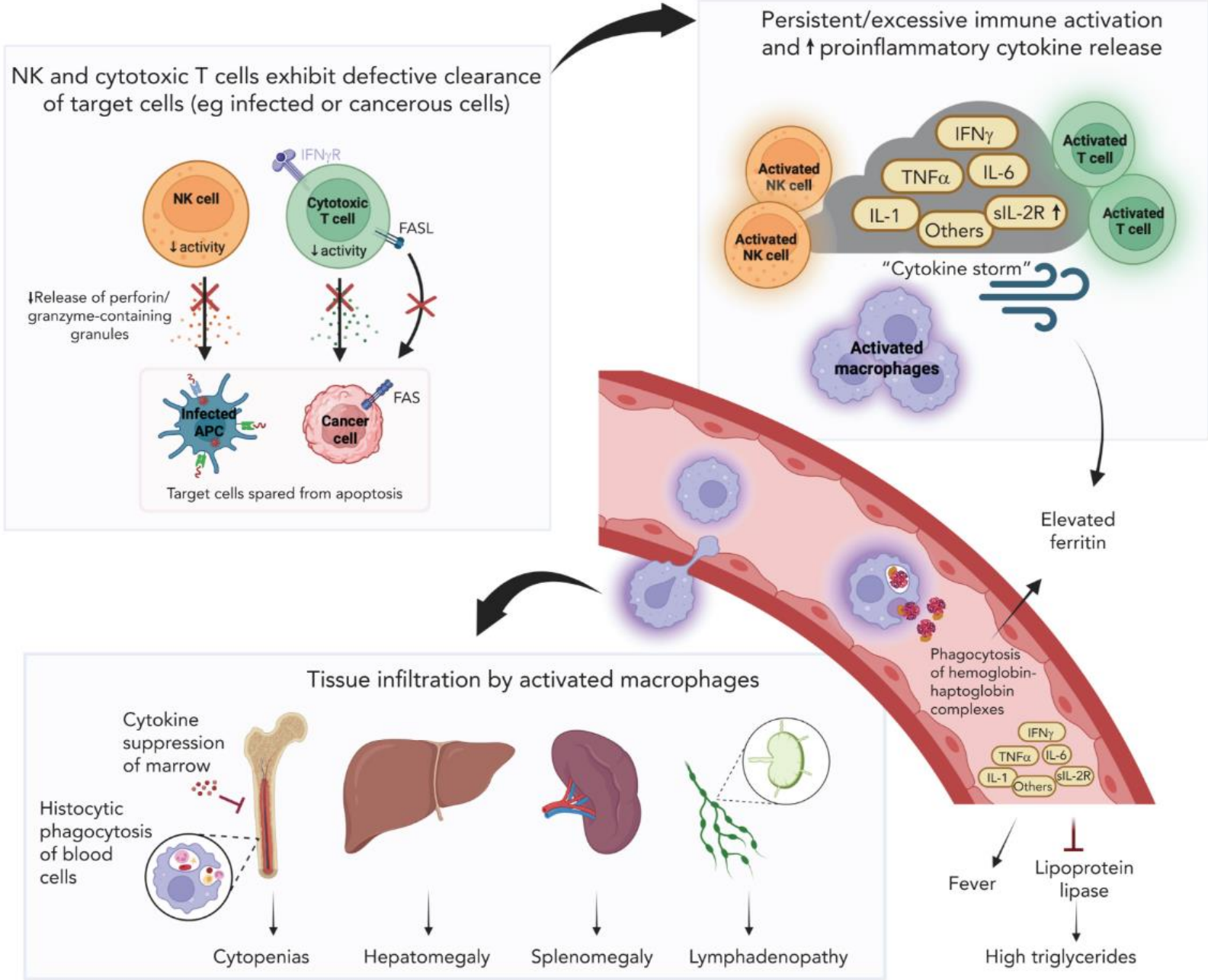


# Pathophysiology of HLH





JI Hsu et al. *Blood* 2026;147:1037



# 8 diagnostic criteria (HLH-2004)



**Table 3** | Diagnostic criteria from the HLH-04 study protocol.[86]

| Criteria |
|---|
| Fever |
| Splenomegaly |
| Cytopenias (affecting ≥2 of 3 lineages)<br>*Hemoglobin <90 g/L*<br>*Platelets <100x10$^9$/L*<br>*Neutrophils <1.0x10$^9$/L* |
| Hypertriglyceridemia and/or hypofibrinogenemia<br>*Fasting triglycerides ≥3.0 mmol/L*<br>*Fibrinogen ≤1.5 g/L* |
| Hemophagocytosis in bone marrow or spleen or lymph nodes |
| Low or absent NK-cell activity |
| Ferritin ≥ 500µg/L |
| sIL-2 receptor ≥2400 U/ml |

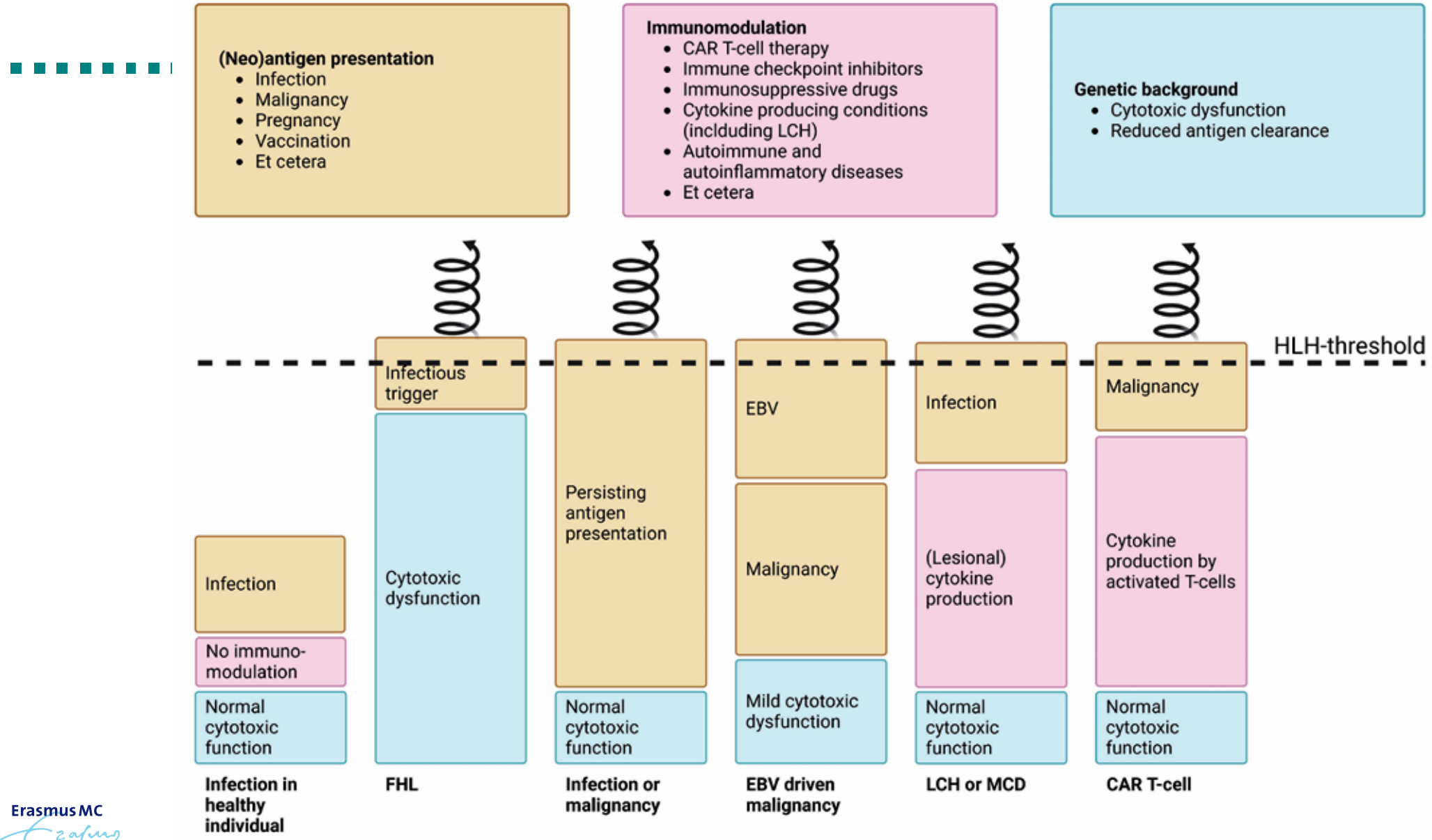
The diagnosis is established if five out of eight diagnostic criteria are fulfilled. Alternatively, a diagnose can be set if a genetic mutation is found that is consistent with HLH.



JI Henter et al. *Pediatr Blood Cancer* 2007;48:124

# Etiologic elements that can cause HLH



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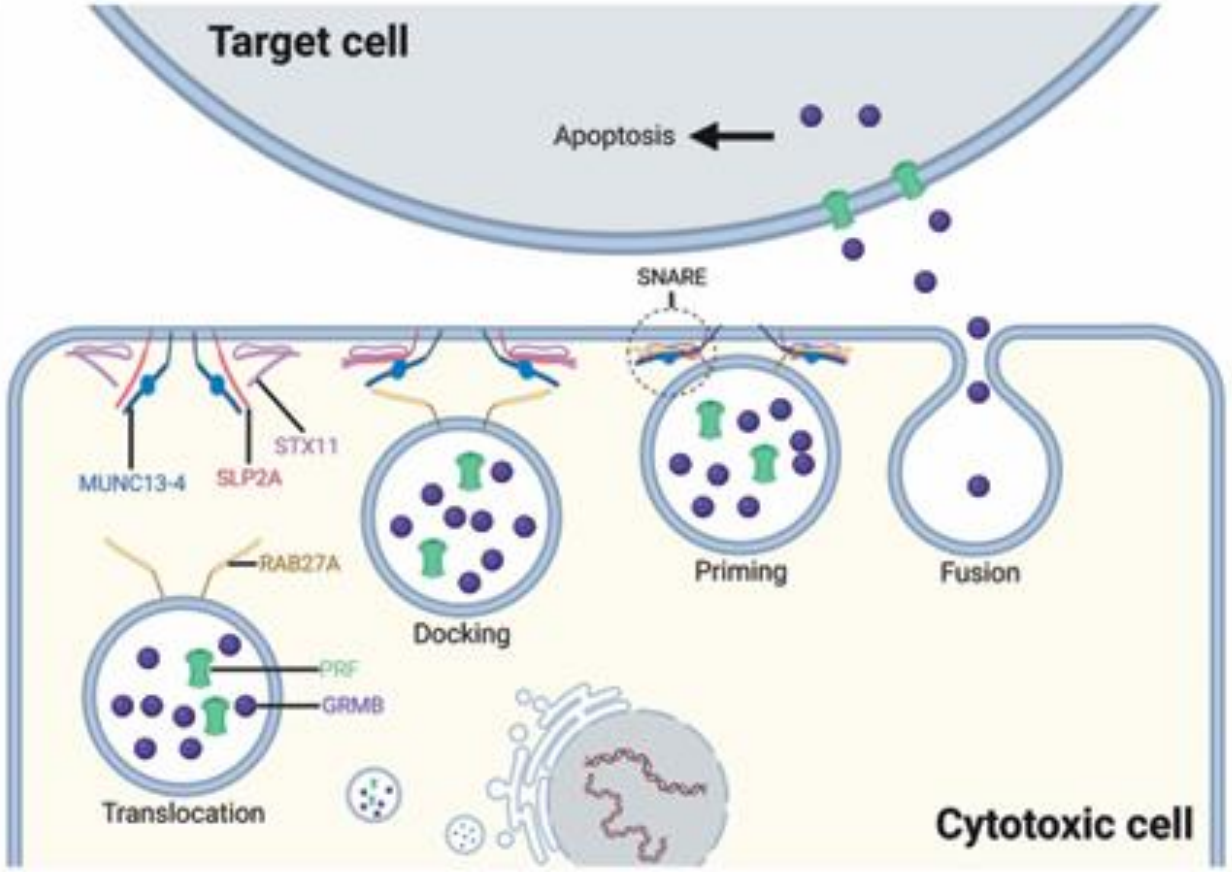
TCE Zondag, *PhD thesis* 2023

# Primairy or familial HLH results from loss-of-function gene variants affecting the cytotoxic pathway in $CD8^+$ T-cells and NK-cells at various levels

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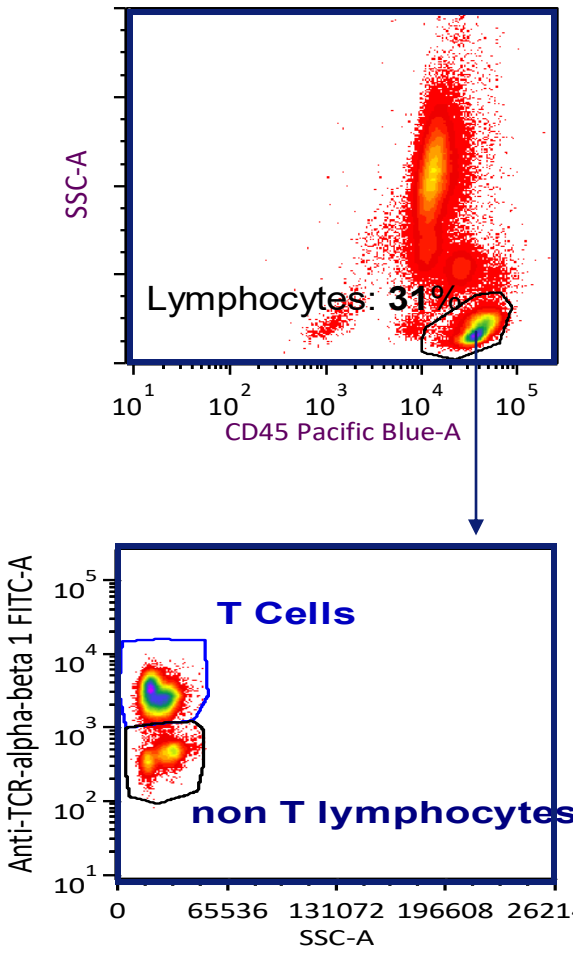
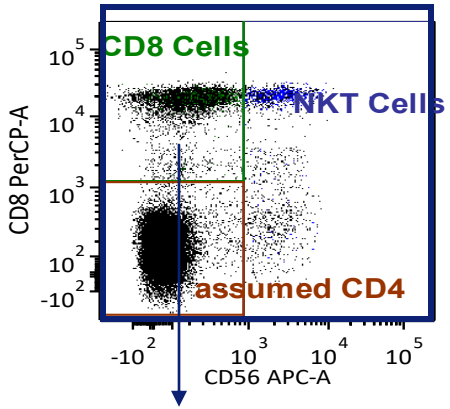
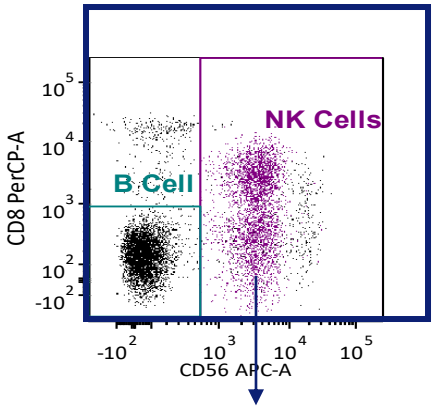
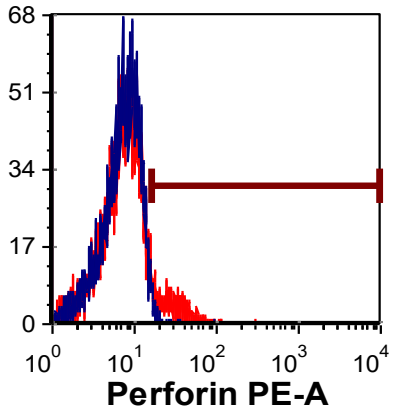
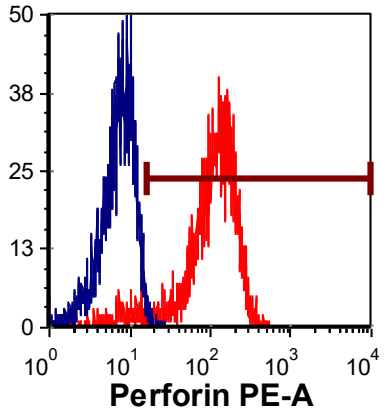
TCE Zondag, *PhD thesis* 2023



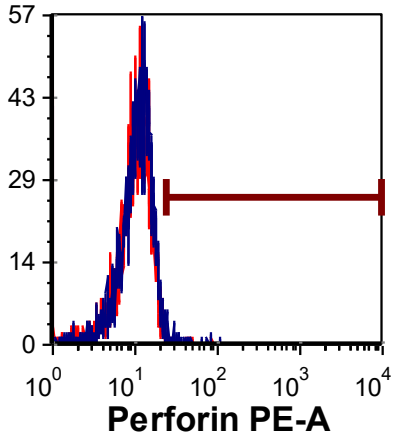



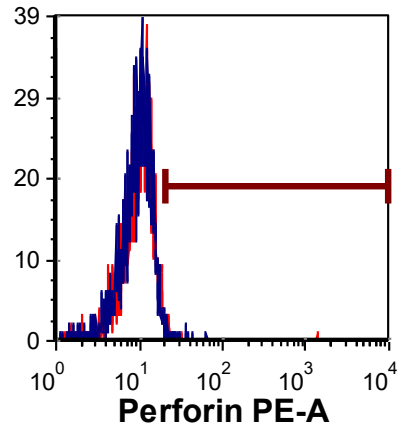




aged matched HC




patient with perforin-deficiency

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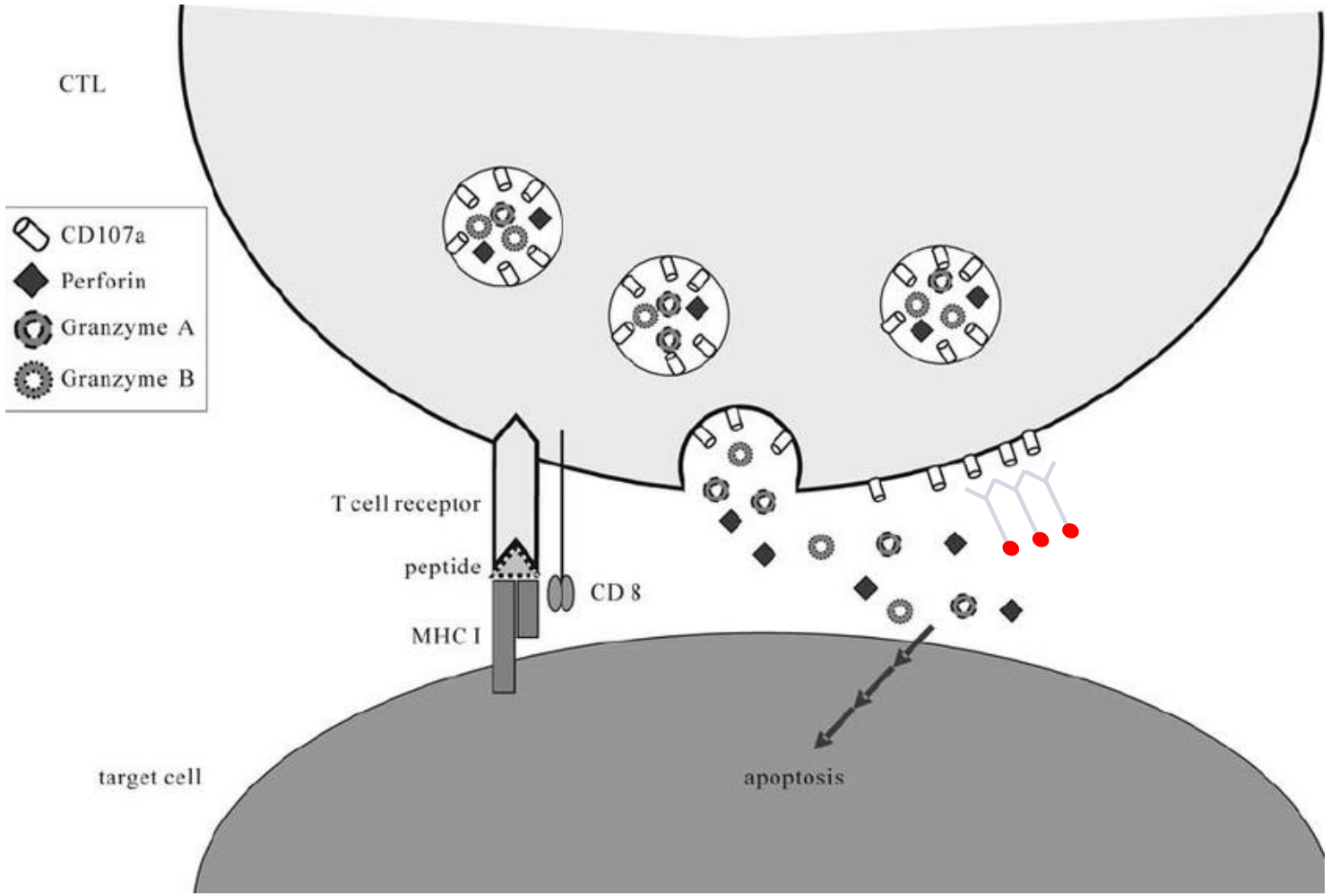
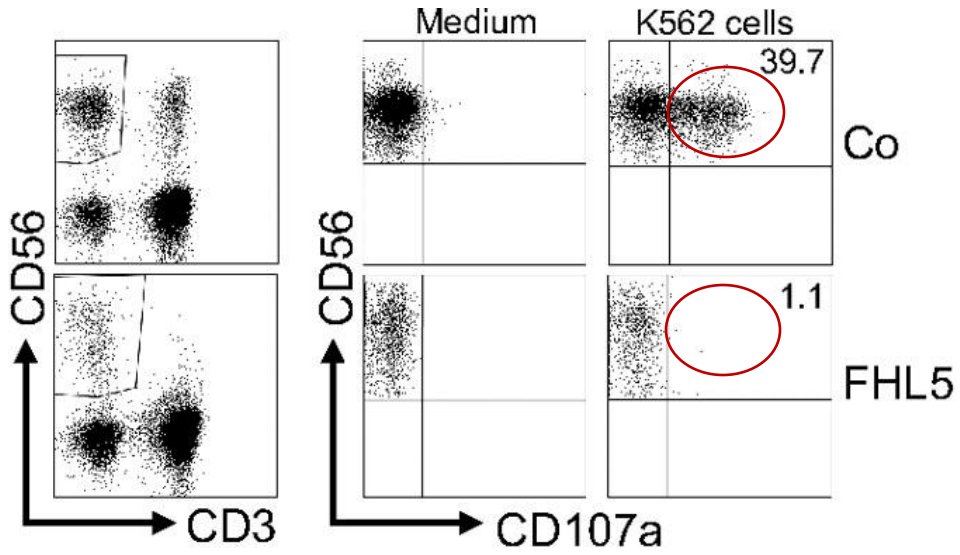
# Flowbased CD107a assay to address degranulation potential






antibody against CD107a

YT Bryceson et al. *Blood* 2012;119:2754

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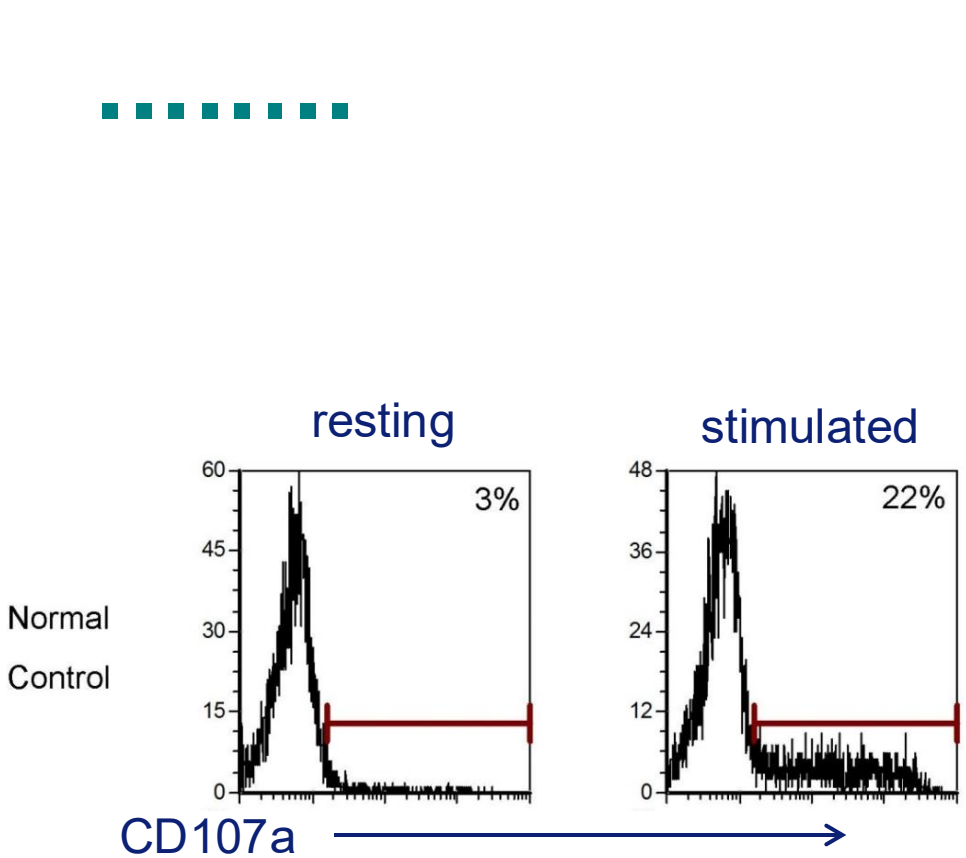
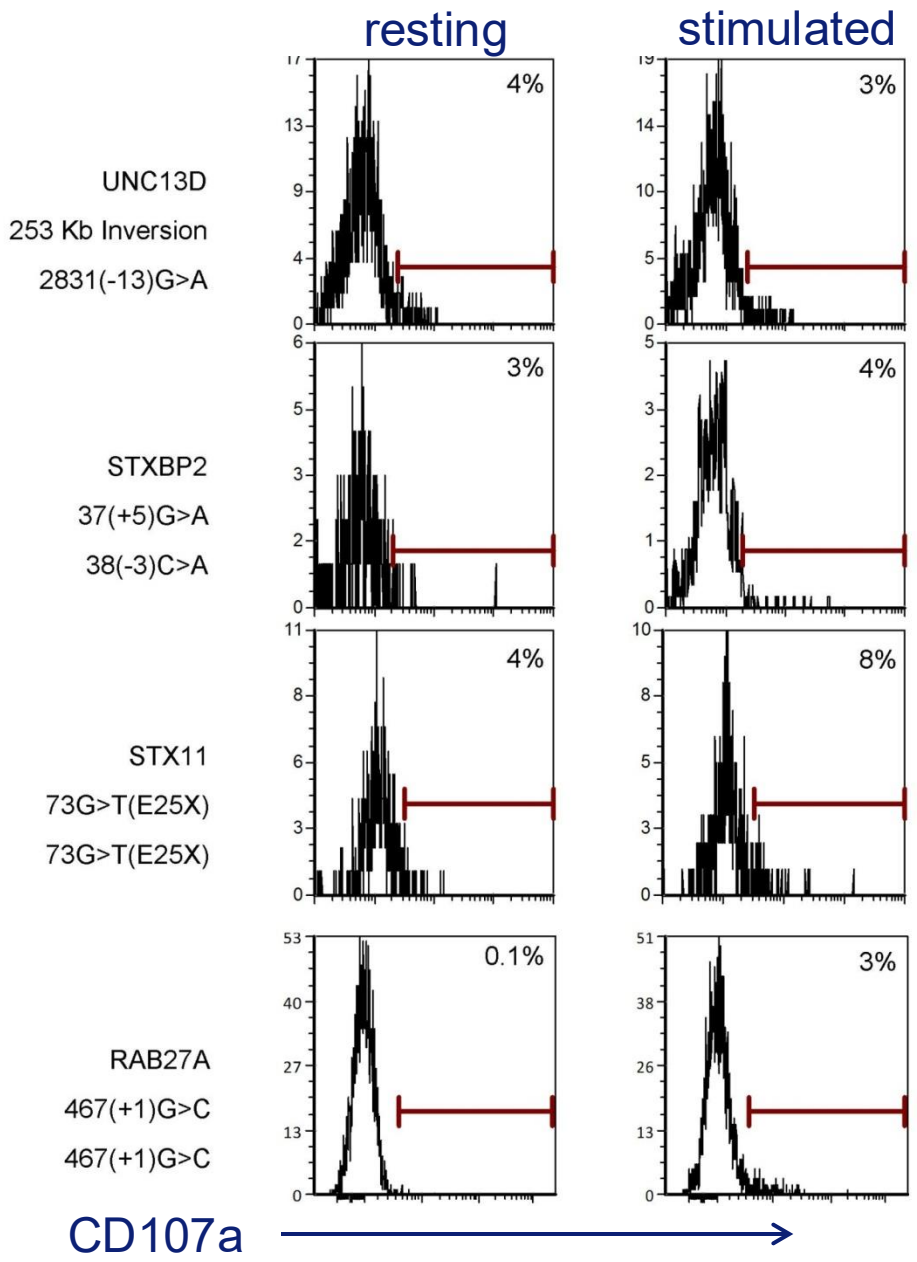
# HLH as a consequence of failure to degranulate



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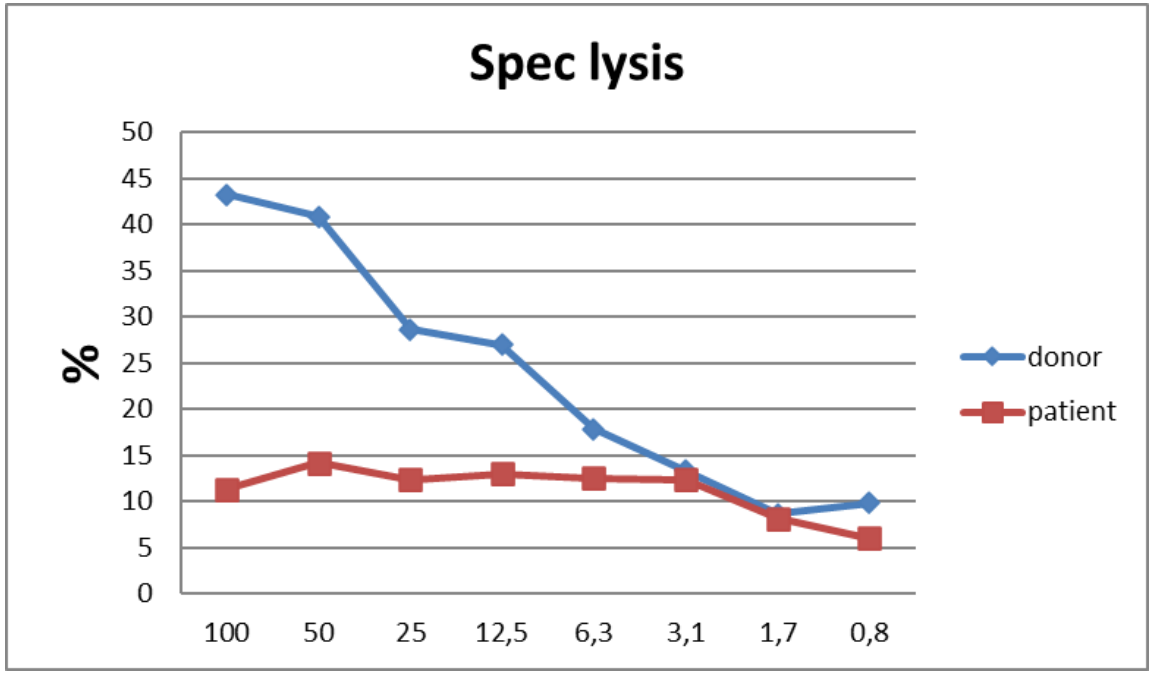
# Combined flowcytometric and functional degranulation assay (LMI)

*Method*

- PBMC isolation from blood
- K562 target cell labeling with Calcein AM
- 3 hours co-incubation at different E:T ratios in presence of monensin and cytochalasin
- Harvest supernatant for measuring Calcein AM released by lysed target cells
- Harvest cells for cytoplasmic CD107a staining

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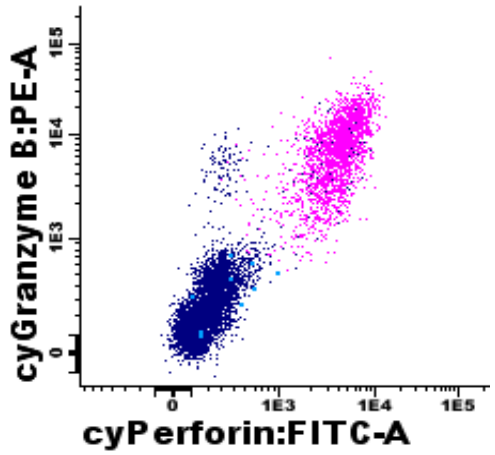
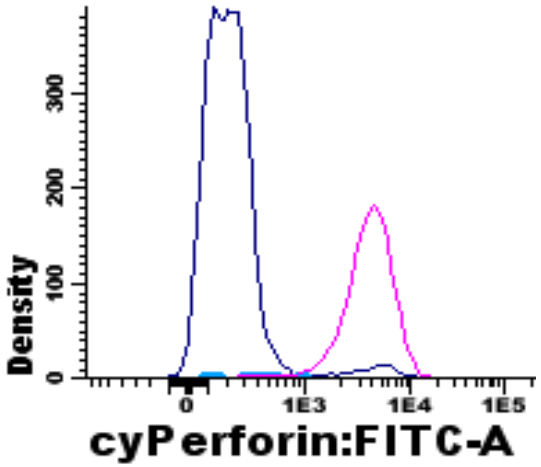
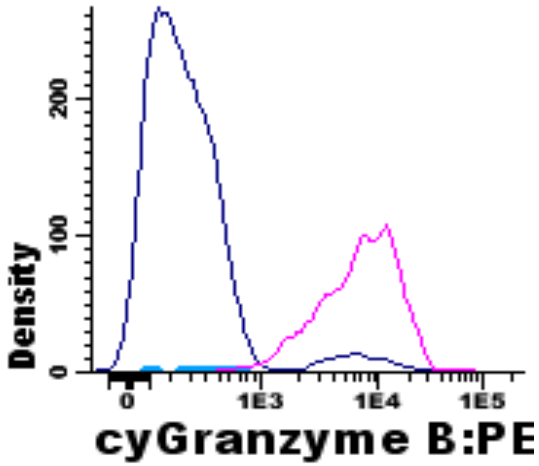
# Patient with perforin deficiency (LMI)

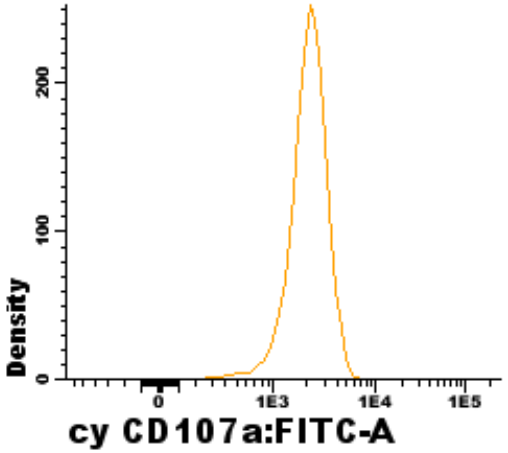
CD8⁺ T cells

HD

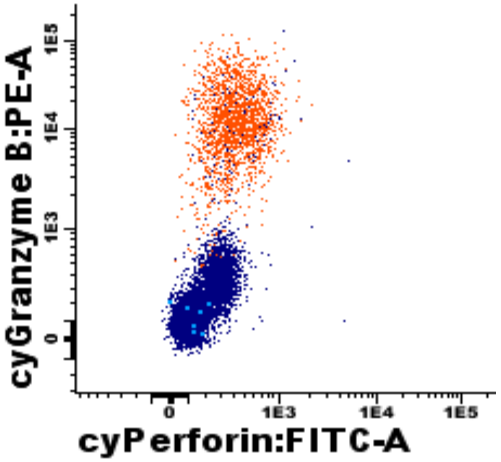


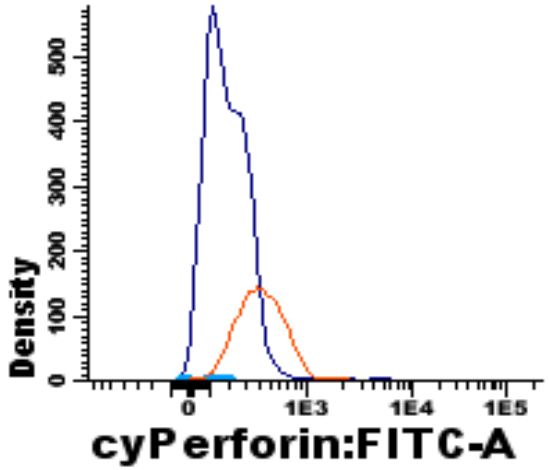



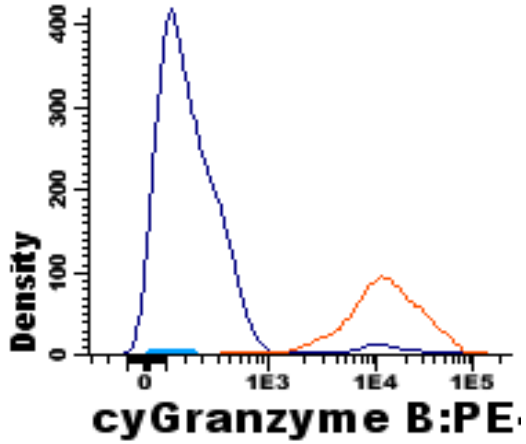



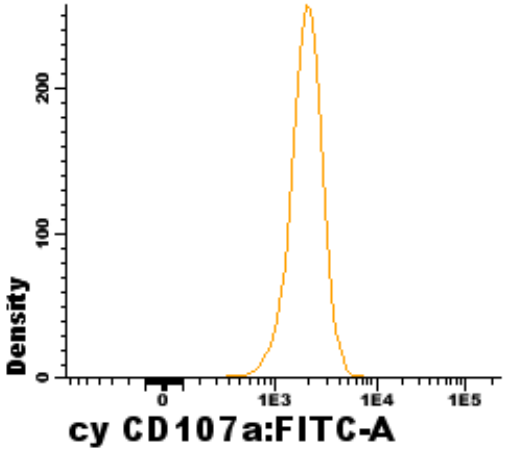




patient

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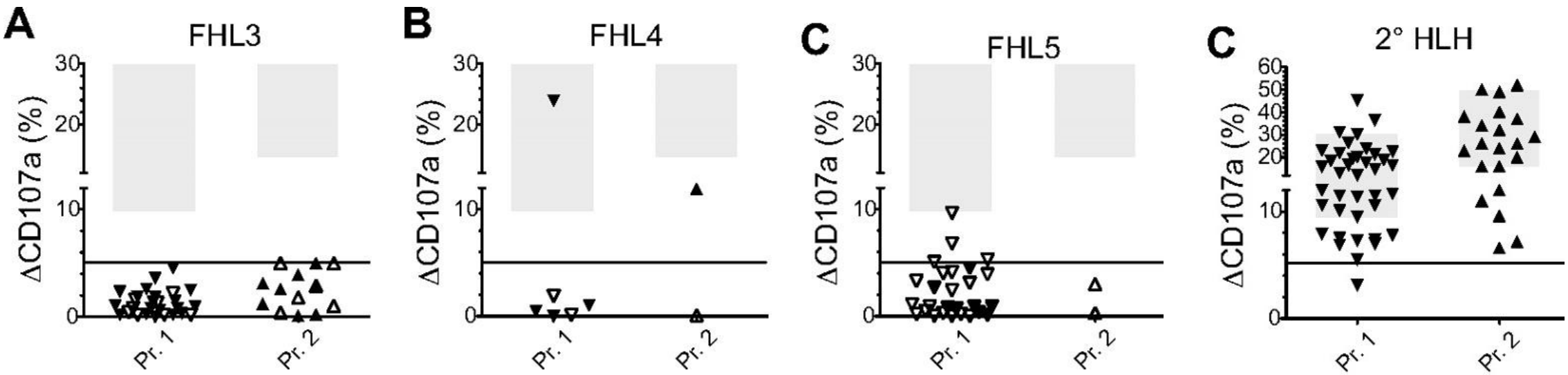
# NK stimulation assay using K562 target cells

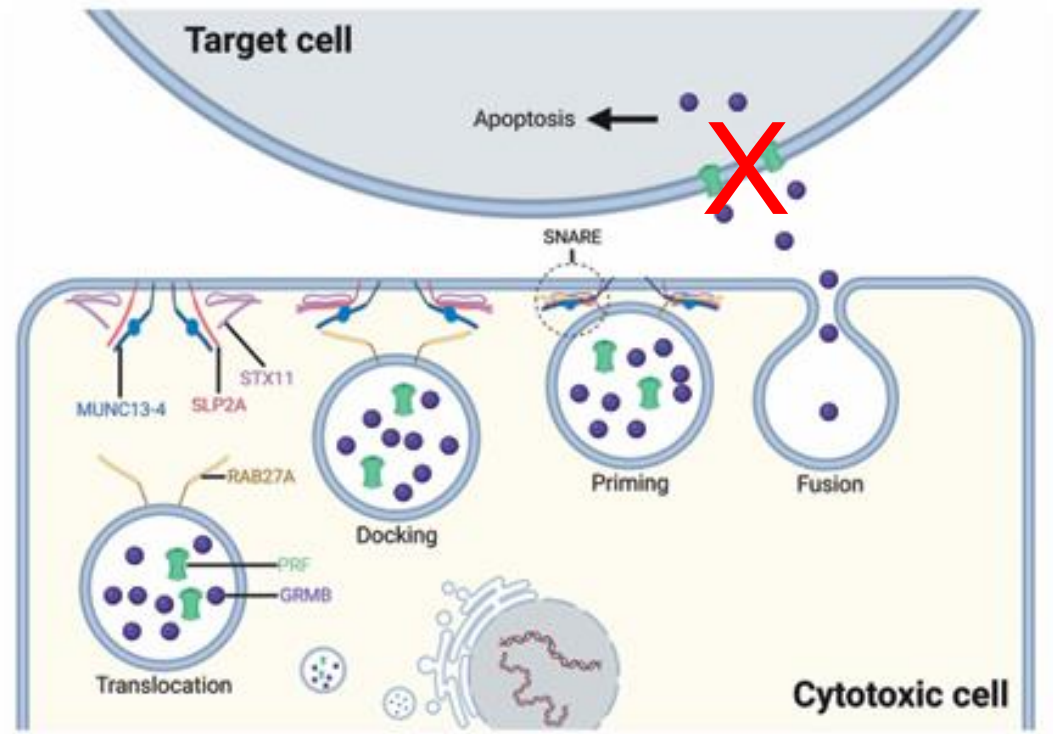


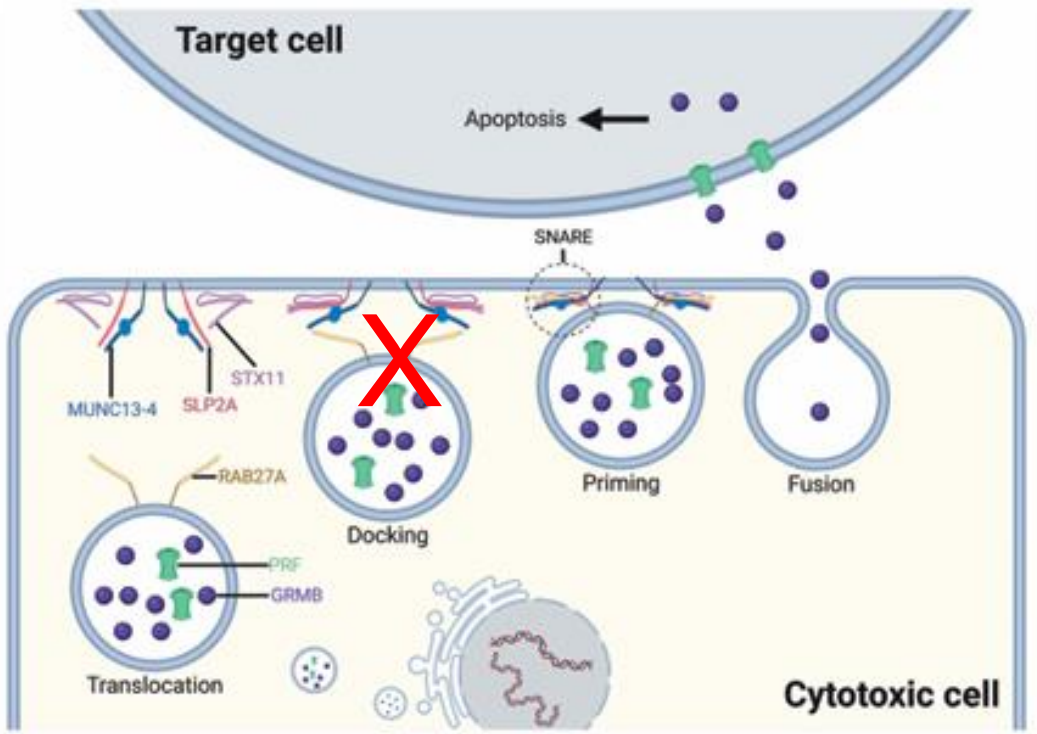



adapted from Y.T. Bryceson et al. *Blood* 2012;119:2754

# The most prevalent mechanisms underlying failure to clear target cells

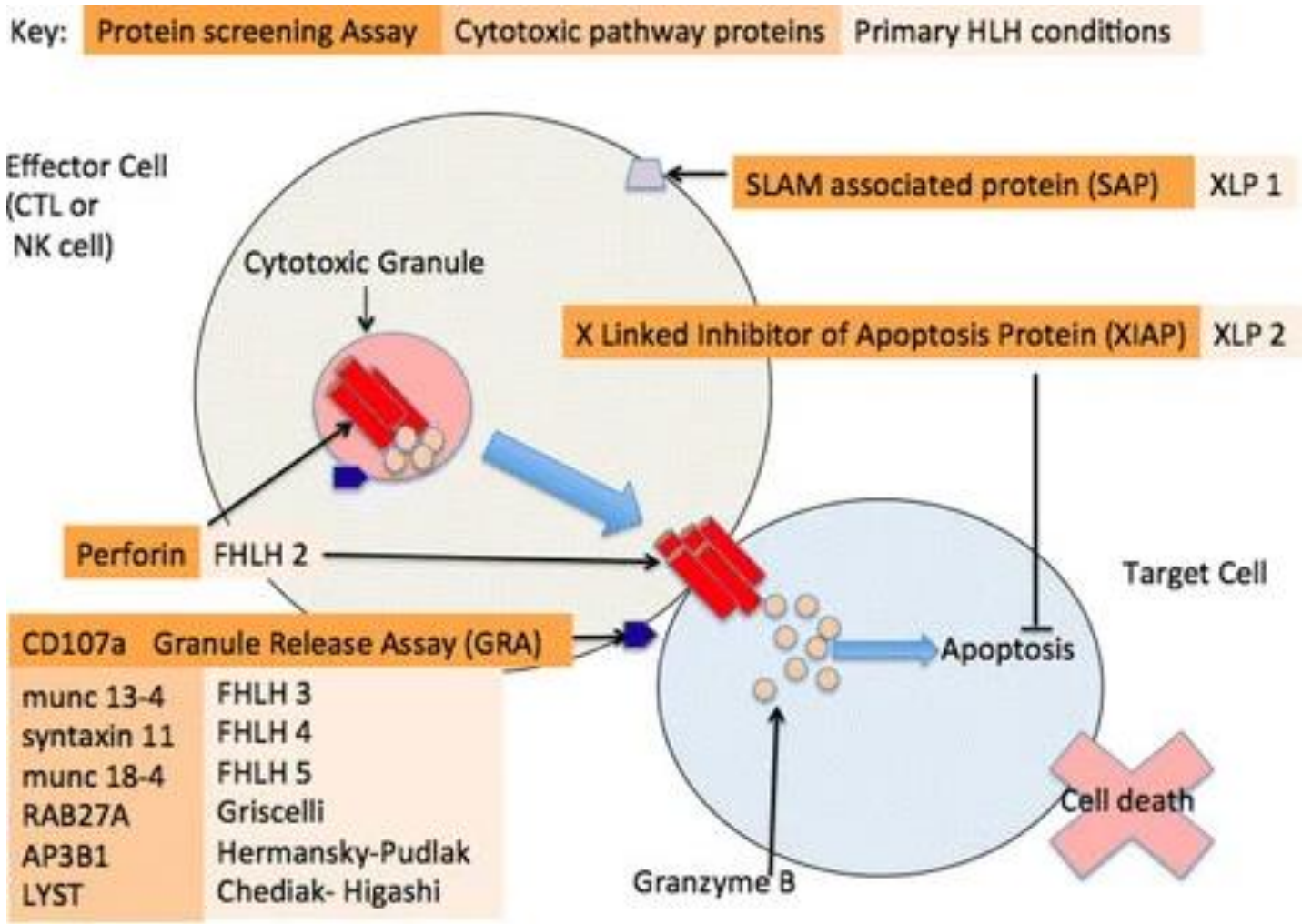




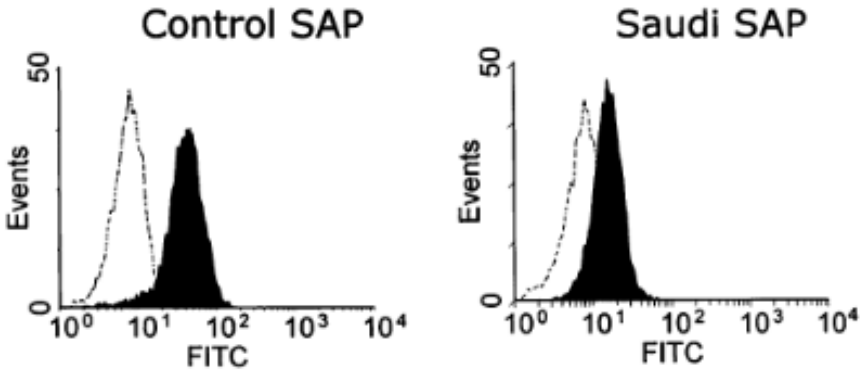
# Protein screening in children presenting with HLH

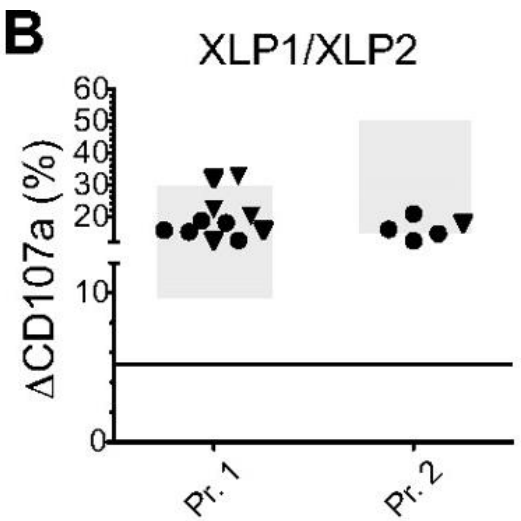





M. Cruikshank et al. *Pediatr Rheumatol* 2015;13:48

A. Alangari et al. *Eur J Pediatr* 2006;165:165



# HLH caused by apopotosis inhibitor deficiency (XIAP) and X-LPD



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https://doi.org/10.1093/cei/uxaf020
Advance access publication 25 March 2025
**Research Article**

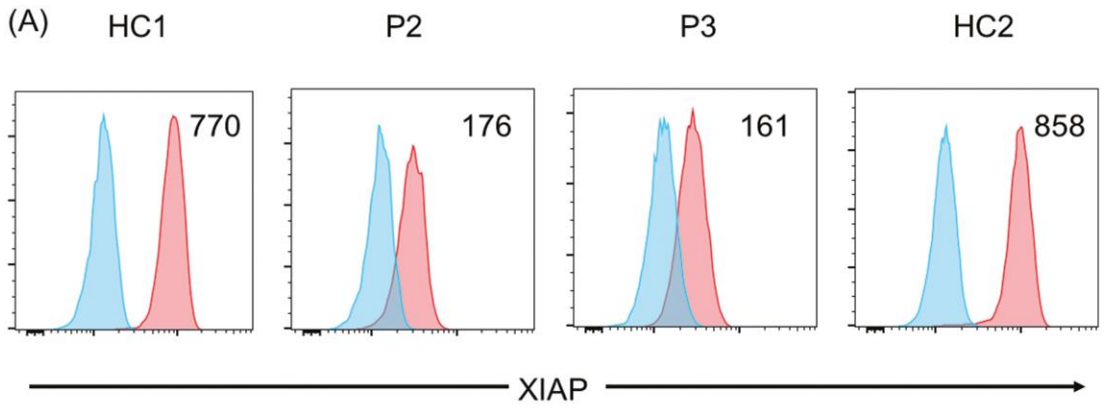
Clinical & Experimental IMMUNOLOGY

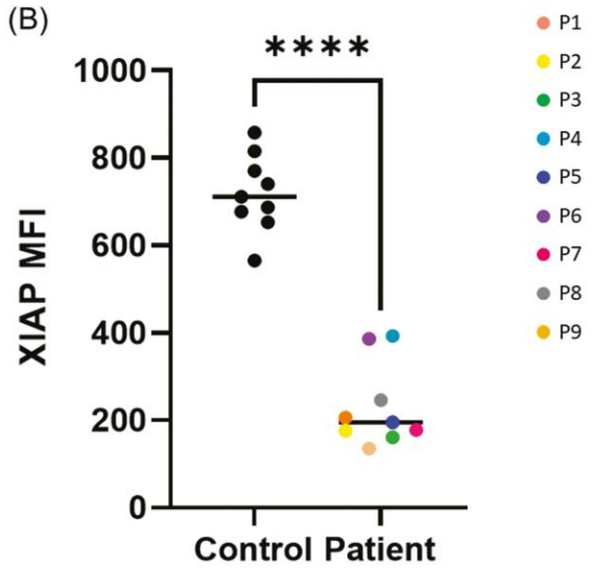
OXFORD

Research Article

## Comprehensive flow cytometry-based diagnosis of XIAP deficiency

Dan Tomomasa[1], Madoka Nishimura[1,2], Ayami Ohya[1,3], Kay Tanita[1,4], Ryosuke Wakatsuki[1], Ryohei Watanabe[1], Satoshi Miyamoto[1], Akihiro Hoshino[5], Takahiro Kamiya[1,6], Takeshi Isoda[1], Shuya Kaneko[1], Masaki Shimizu[1], Atsushi Hijikata[7], Katsuhide Eguchi[8], Masataka Ishimura[8], Yukako Maeda[9], Kazushi Izawa[9], Takaaki Meguro[10], Kosuke Fujimoto[11], Etsuko Ishikita-Murayama[12,13], Kyogo Suzuki[14], Eri Okura[15], Tomoko Uehara[16], Tomotada Takayama[17], Satoshi Okada[18], Masatoshi Takagi[1], Tomohiro Morio[1,19], Rebecca A. Marsh[20] and Hirokazu Kanegane[5,*]

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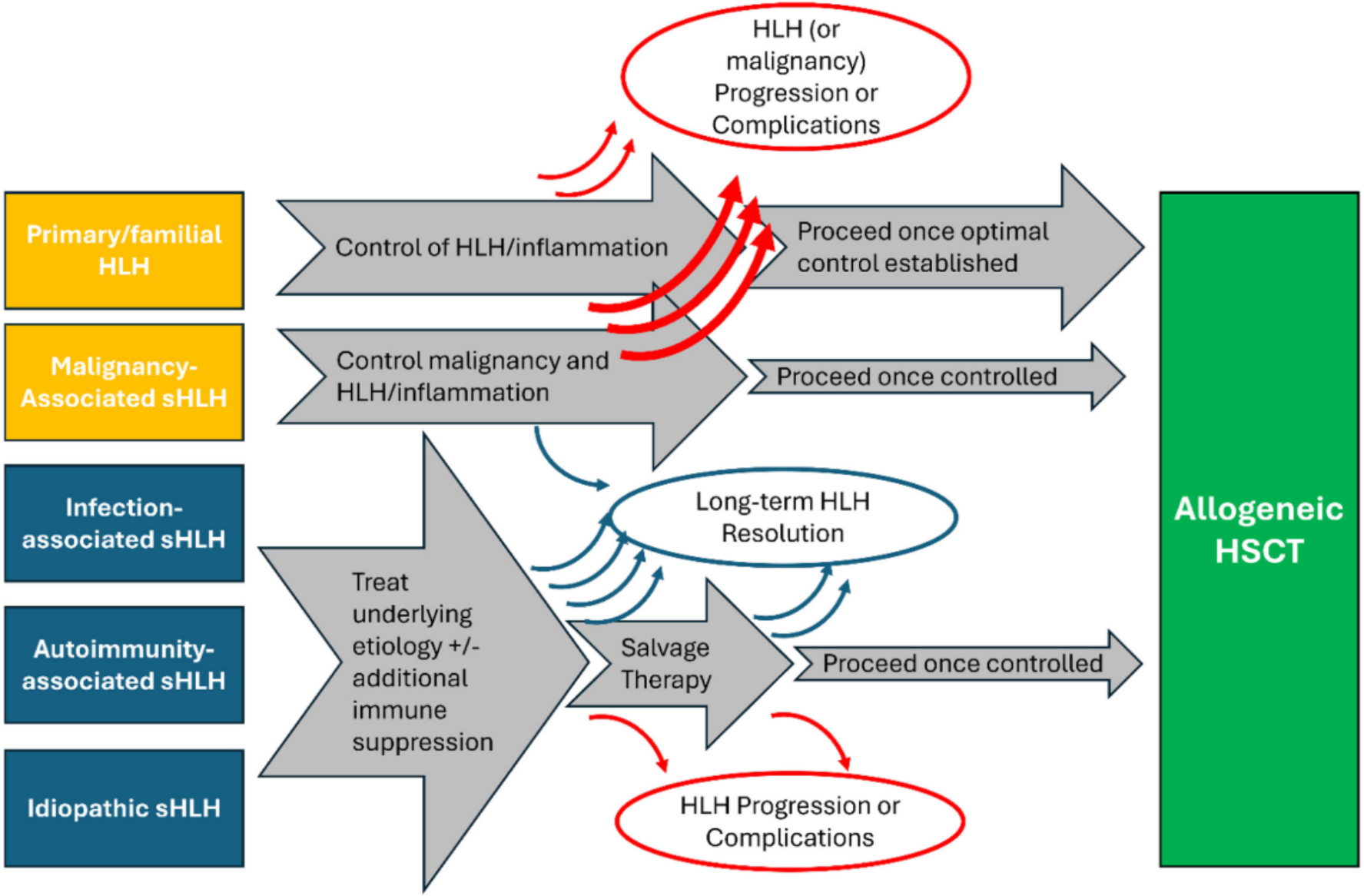
# Rapid diagnostics and start of HLH treatment are key for saving lives!



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S Nikiforow et al. *Hematol Oncol Clin N Am* 2025;39:645

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