Aieop
Associazione Italiana
Ematologia Oncologia Pediatrica

Linfoistiocitosi emofagocitica familiare

M. Aricò

PERCORSI PEDIATRICI IN VAL DI NOTO:
IL PEDIATRA E LA GENETICA

Sala Congressi Ospedale R. Guzzardi, Vittoria (RG)
31 gennaio 2015

Robert

- Genitori sani
- Maschio, 9 settimane
- Bene fino a 11 giorni prima: apatia
- Da due giorni: febbre e vomito

- E.O. febbre, epatosplenomegalia
- LND normali

Archive Disease Child 1952; 519-525

Robert: laboratorio

- Anemia (52% di 13.8 g/dl)
- Neutropenia
- Piastrinopenia
- Colture negative

Archive Disease Child 1952; 519-525

Robert: decorso

- Febbre remittente
- Citopenia
- Insufficienza epatica con ittero
- Esito rapidamente fatale

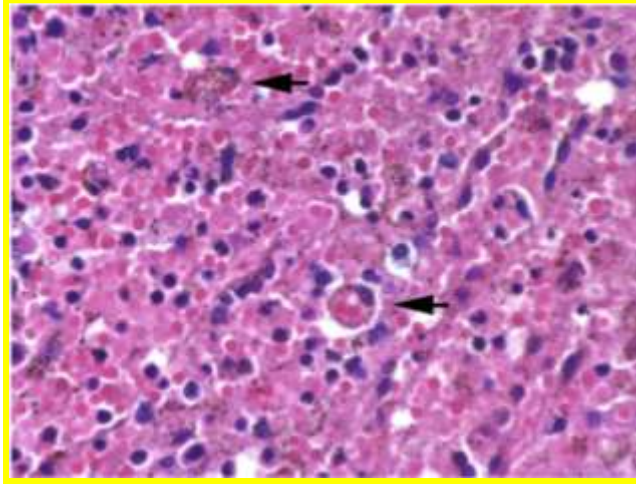
Archive Disease Child 1952; 519-525

Rachel, sorellina di Robert

- Età 9 settimane
- Da 5 giorni diarrea, da 2 vomito
- Febbre
- Epatosplenomegalia
- Citopenia
- Migliora, dopo ACTH, per 12 gg.
- Muore 94 giorni dopo l'esordio

Archive Disease Child 1952; 519-525

Rachel, necropsy



- Lymphnodes: proliferation of histiocytes, with phagocytosis of red and white cells

- Spleen: histiocytes with hemophagocytosis
- Liver: cellular proliferation with lympho- and histiocytes, hemophagocytosis

- BM: cellularity++, hemophagocytosis

Archive Disease Child 1952; 519-525

FAMILIAL HAEMOPHAGOCYTIC RETICULOSIS

BY

JAMES W. FARQUHAR and ALBERT E. CLAIREAUX
From the Departments of Child Life and Health and Pathology, University of Edinburgh

(RECEIVED FOR PUBLICATION MAY 3, 1952)

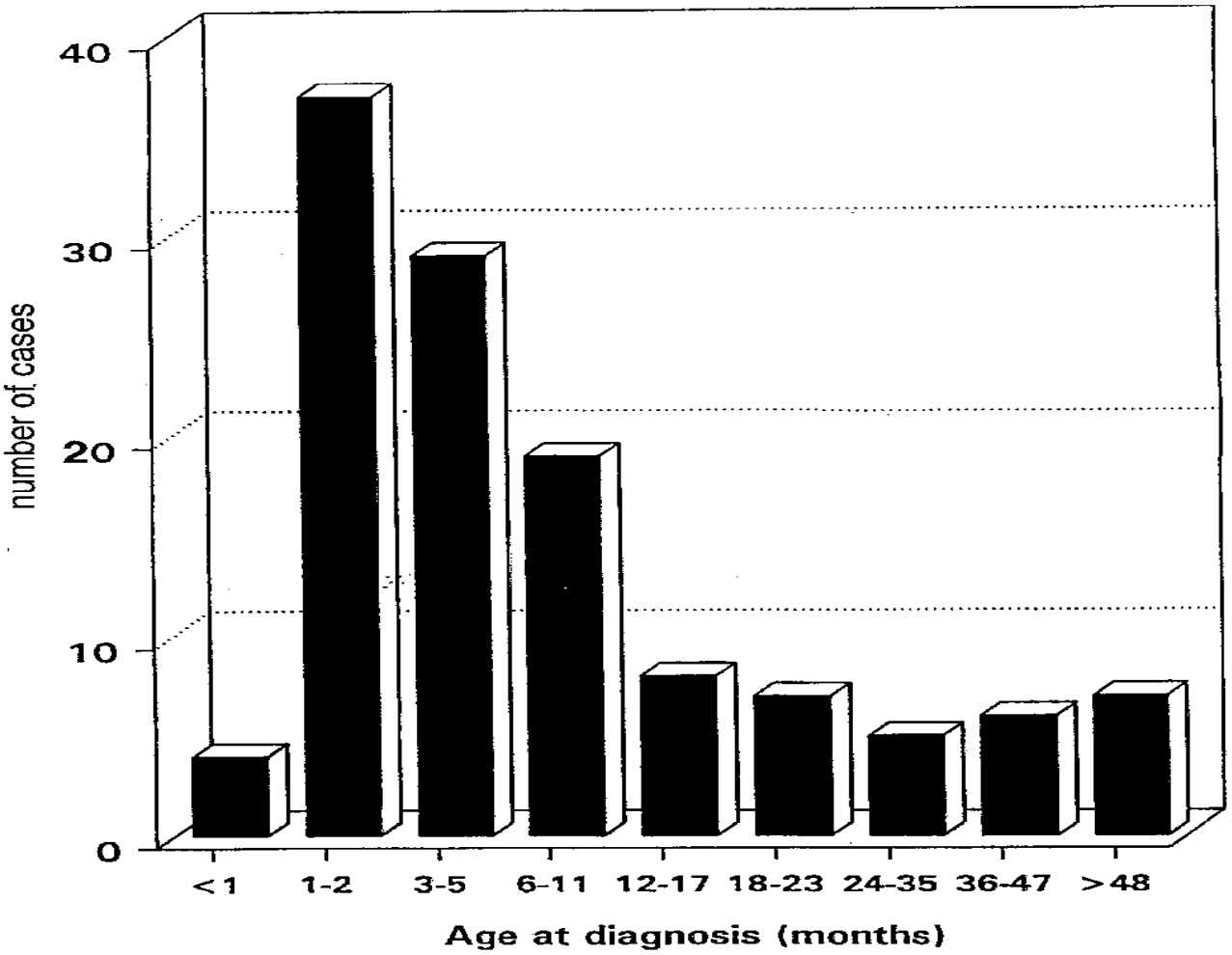
- Non-lipoid reticulosis of young infants
- Differs from "Letterer-Siwe" because:
  - is familial
  - no granulomas
  - no bone defects
- The cause of the histiocytic proliferation is unknown and treatment is of no avail

Leukemia (1996) 10, 197–203

CLINICAL REVIEW

Hemophagocytic lymphohistiocytosis. Report of 122 children from the International Registry

M Aricò[1], G Janka[2], A Fischer[3], J-I Henter[4], S Blanche[3], G Elinder[4], M Martinetti[5] and MP Rusca[6] for the FHL Study Group of the Histiocyte Society



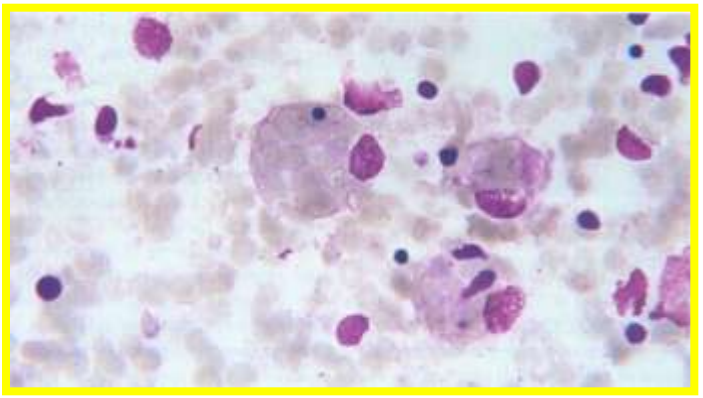
HLH - Principali caratteristiche cliniche

• M/F	1:1
• Febbre	114/122 (93%)
• Epato-Splenomegalia	119/122 (97%)
• Linfoadenomegalia	39/122 (31%)
• Rash cutaneo	30/122 (24%)

HLH - Principali alterazioni degli esami di laboratorio

- Trombocitopenia 104 / 122 (85%)
- Ipertrigliceridemia 78 / 97 (80%)
- Ipofibrinogenemia 66 / 101 (65%)
- Neutropenia 73 / 122 (59%)
- Pleiocitosi liquorale 55 / 94 (58%)
- ALT elevate 55 / 104 (53%)

HLH: difficoltà diagnostiche



- Clinica: sembra una leucemia..
- Laboratorio: non specifico
- Emofagocitosi: suggestiva, ma…
  - non è specifica !
  - nel 40% dei casi non è presente alla prima valutazione del midollo osseo. Questo NON deve fare escludere la diagnosi!

Diagnostic guidelines for HLH - revised 2004

- Fever
- Splenomegaly
- Cytopenia (affecting ≥ 2 of 3 lineages)
  - Hemoglobin (< 90 g/L),
  - platelets (<100 x 10^9/L),
  - neutrophils (<1.0 x 10^9/L)
- Hypertriglyceridemia (fasting ≥2.0 mmol/L or ≥3 SD)
- and/or hypofibrinogenemia (≤1.5 g/L or ≤3 SD)
- Hemophagocytosis (bone marrow, spleen, lymph nodes) and no evidence of malignancy

! **Low or absent NK activity**

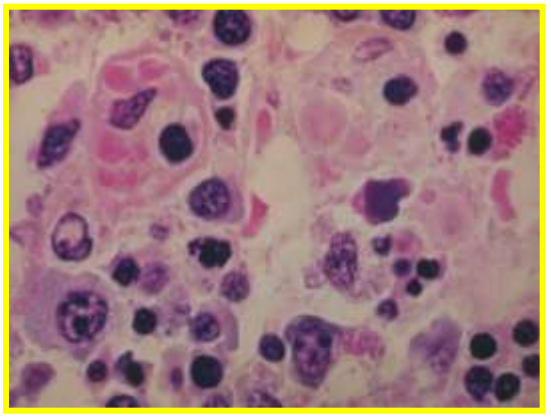
! **Ferritin** >500 μg/L

! **Soluble CD25** (IL2 receptor) >2400 U/ml

HLH-2004: Diagnostic and Therapeutic Guidelines for Hemophagocytic Lymphohistiocytosis

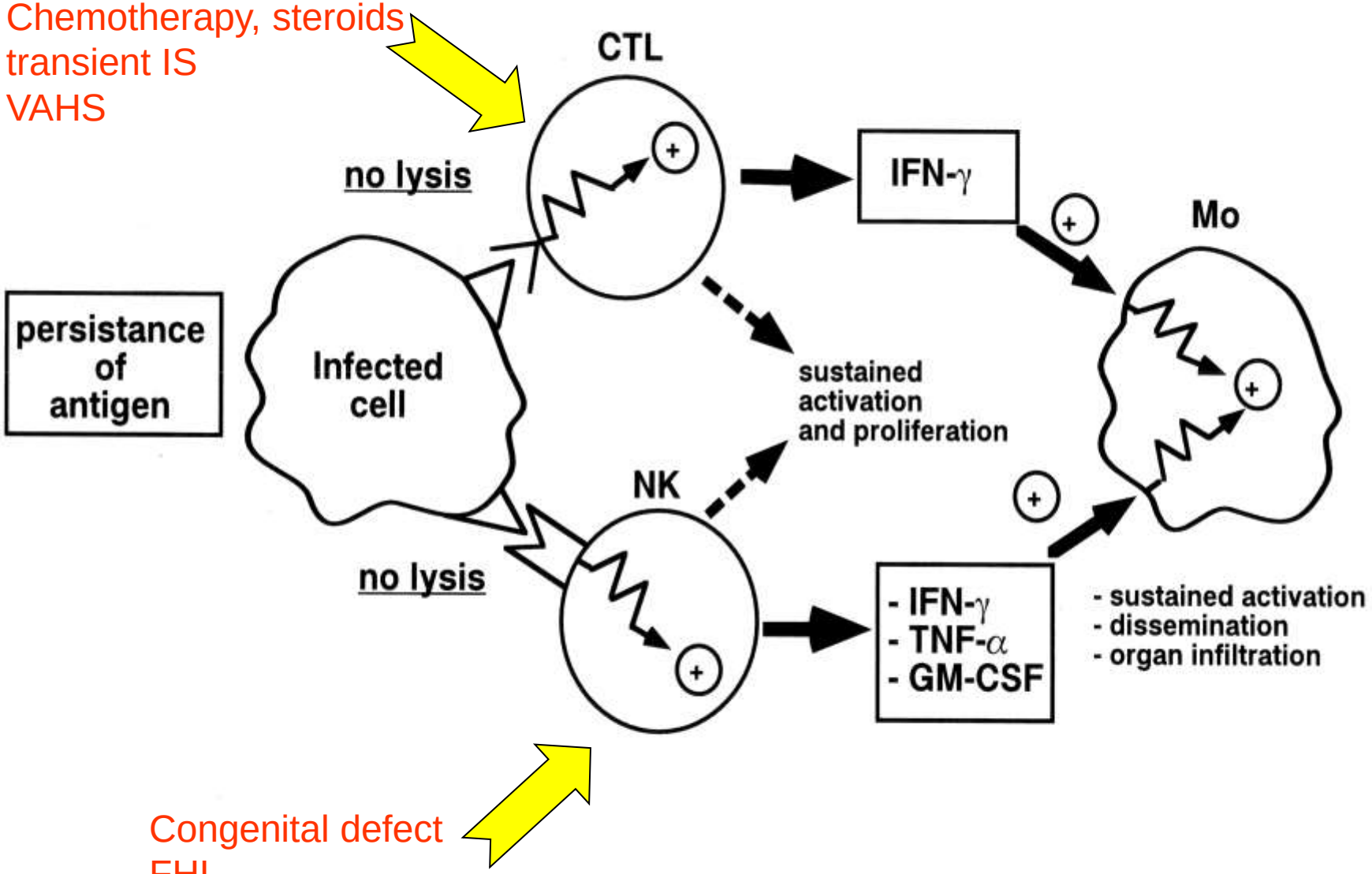
Jan-Inge Henter, MD, PhD,[1*] AnnaCarin Horne, MD,[1] Maurizio Aricó, MD,[2] R. Maarten Egeler, MD, PhD,[3] Alexandra H. Filipovich, MD,[4] Shinsaku Imashuku, MD,[5] Stephan Ladisch, MD,[6] Ken McClain, MD, PhD,[7] David Webb, MD,[8] Jacek Winiarski, MD, PhD,[9] and Gritta Janka, MD, PhD[10] for the Histiocyte Society

What is HLH ?



A clinical syndrome resulting from cytokine overproduction due to inefficient immune response

SCHEMATIC REPRESENTATION OF THE CYTOLYTIC EFFECTOR CELL DEFECT IN HLH



M. Aricò & L. Moretta. Br J Haematol, 2002

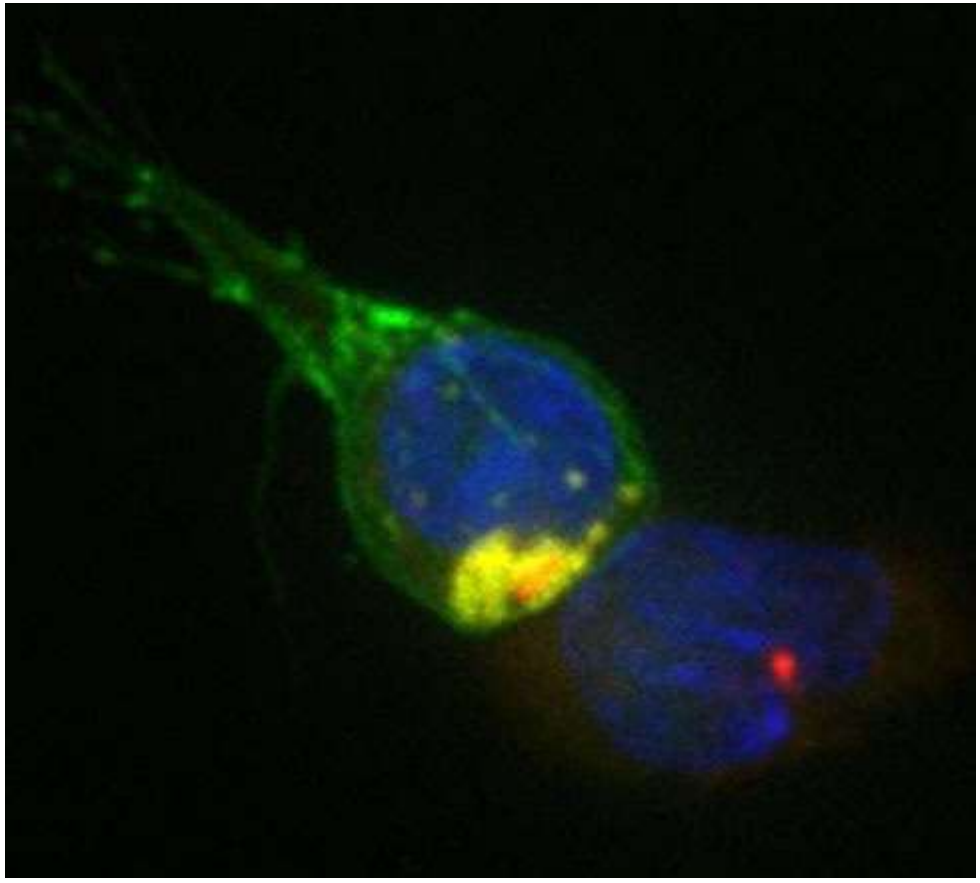


Fig. 3 Rab27a-deficient patient CTL stained with CD8 (green) recognizing, but not killing a target cell with granules stained with LAMP1 (yellow). The centrosomes and nuclei of both target and killer are stained with g-tubulin (red) and Hoechst (blue)

Cell. Mol. Life Sci.
DOI 10.1007/s00018-011-0835-y

Cellular and Molecular Life Sciences

MULTI-AUTHOR REVIEW

Familial hemophagocytic lymphohistiocytosis: a model for understanding the human machinery of cellular cytotoxicity

Elena Sieni · Valentina Cetica · Elena Mastrodicasa · Daniela Pende · Lorenzo Moretta · Gillian Griffiths · Maurizio Aricò

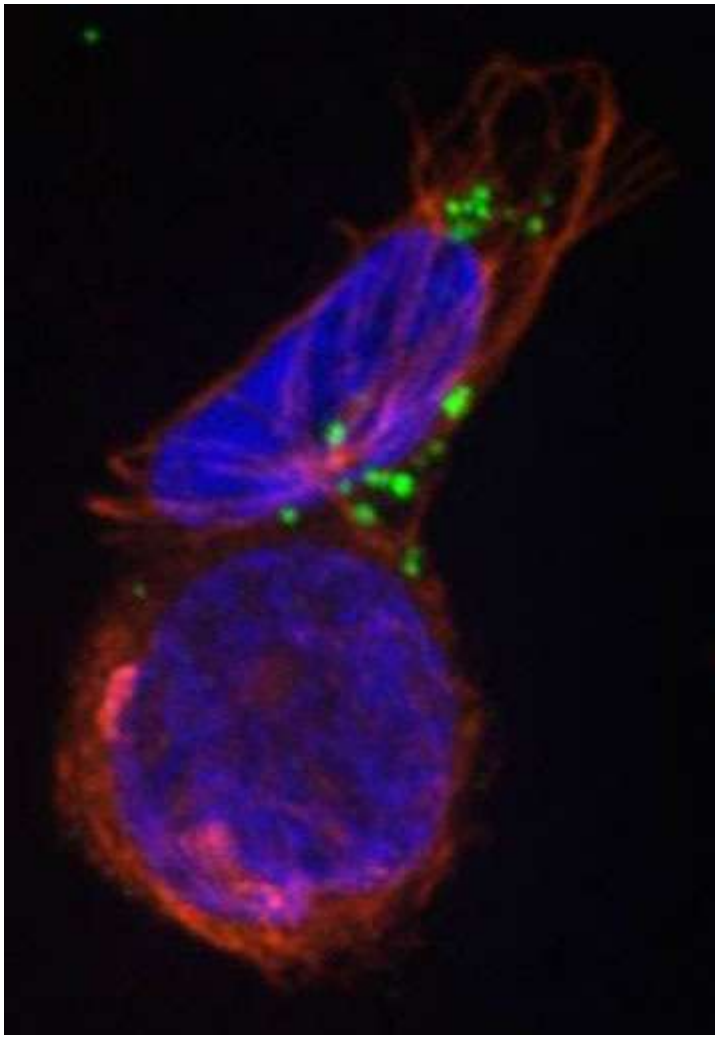


Fig. 1 NK cell recognizing a target and polarizing its cytoskeleton (tubulin, red) and lytic granules (perforin, green) towards the target. Nuclei are stained blue with Hoechst

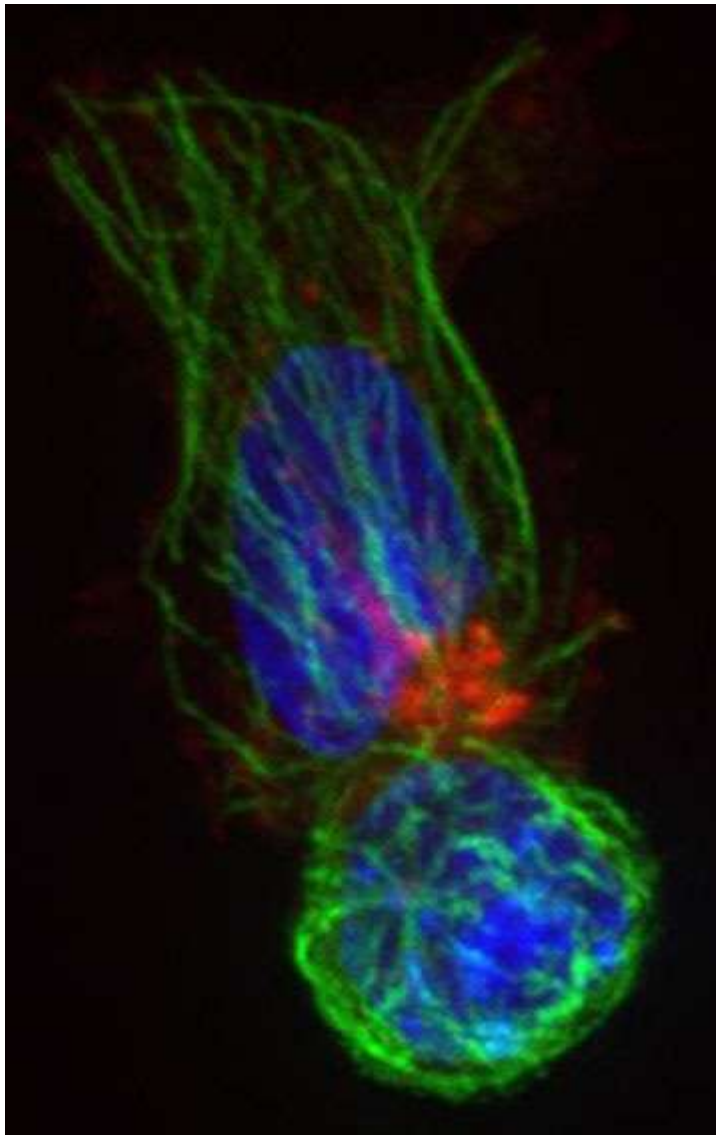


Fig. 2 Munc18-2-deficient CTL (FM) with microtubules (green) and perforin granules (red) polarized towards the target. Nuclei are labelled in blue with Hoechst

GENETICS OF FAMILIAL HLH

A story of granules ?

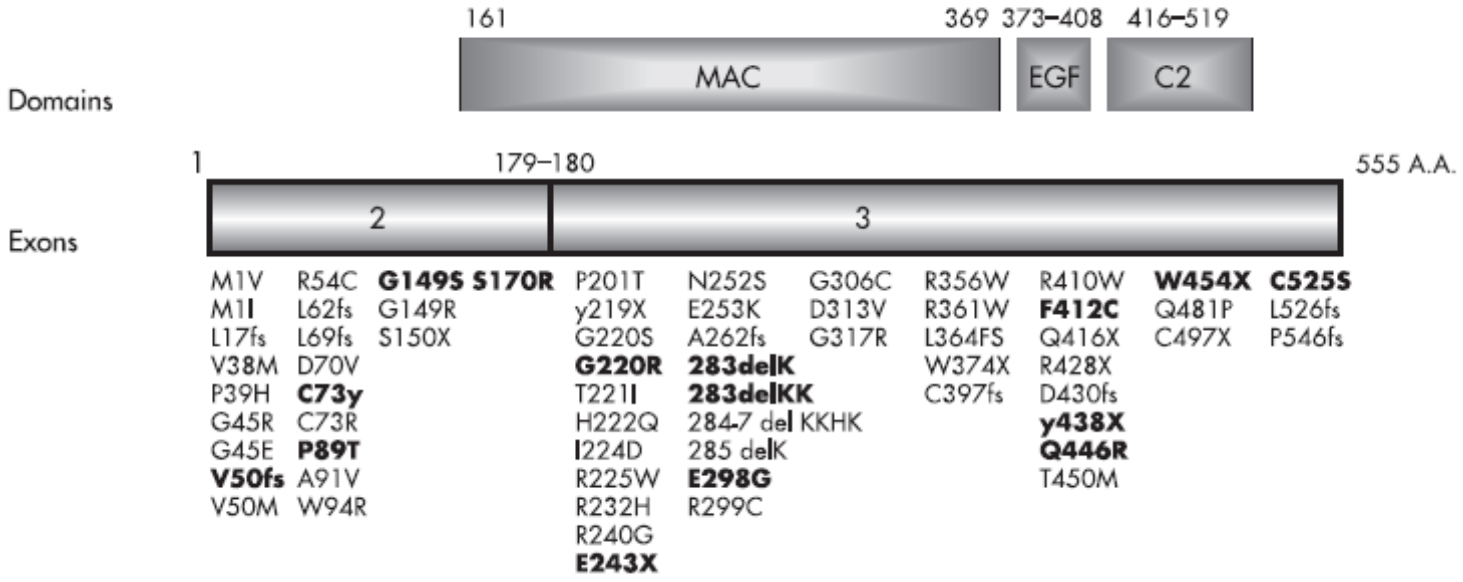
www.sciencemag.org SCIENCE VOL 286 3 DECEMBER 1999

Perforin Gene Defects in Familial Hemophagocytic Lymphohistiocytosis

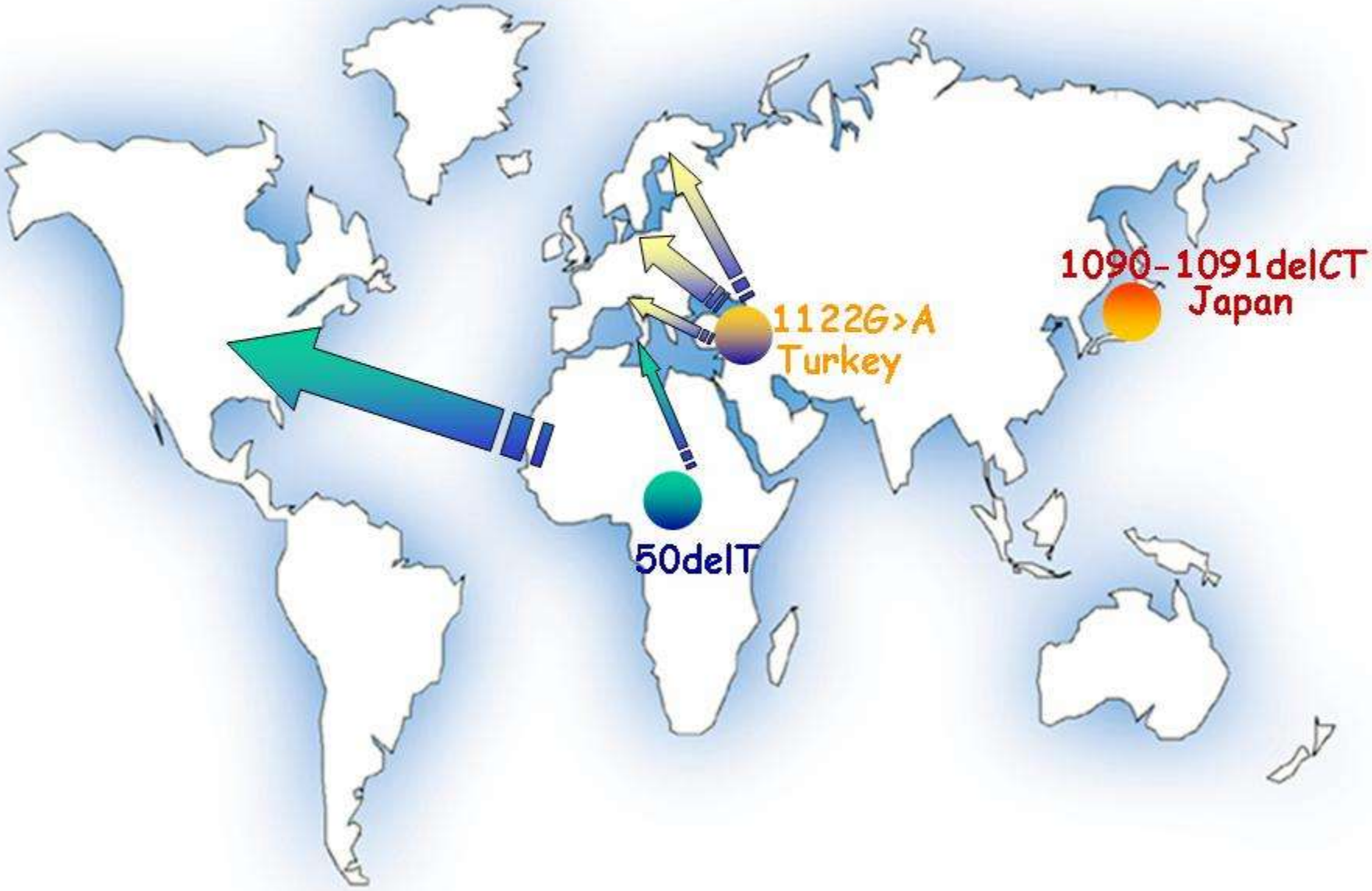
Susan E. Stepp,[1] Rémi Dufourcq-Lagelouse,[2] Françoise Le Deist,[2,3] Sadhna Bhawan,[1] Stéphanie Certain,[2] Porunelloor A. Mathew,[4] Jan-Inge Henter,[5] Michael Bennett,[1] Alain Fischer,[2,3] Geneviève de Saint Basile,[2*†] Vinay Kumar[1*]

Genotype–phenotype study of familial haemophagocytic lymphohistiocytosis due to perforin mutations

A Trizzino,[1] U zur Stadt,[2] I Ueda,[3] K Risma,[4] G Janka,[2] E Ishii,[5] K Beutel,[2] J Sumegi,[4] S Cannella,[1] D Pende,[6] A Mian,[7] J-I Henter,[8] G Griffiths,[9] A Santoro,[1,10] A Filipovich,[4] M Aricò,[1] for the Histiocyte Society HLH Study group



J Med Genet 2008;**45**:15–21. doi:10.1136/jmg.2007.052670


1090-1091delCT
Japan
1122G>A
Turkey
50delT

Cell, Vol. 115, 461–473, November 14, 2003, Copyright ©2003 by Cell Press

Munc13-4 Is Essential for Cytolytic Granules Fusion and Is Mutated in a Form of Familial Hemophagocytic Lymphohistiocytosis (FHL3)

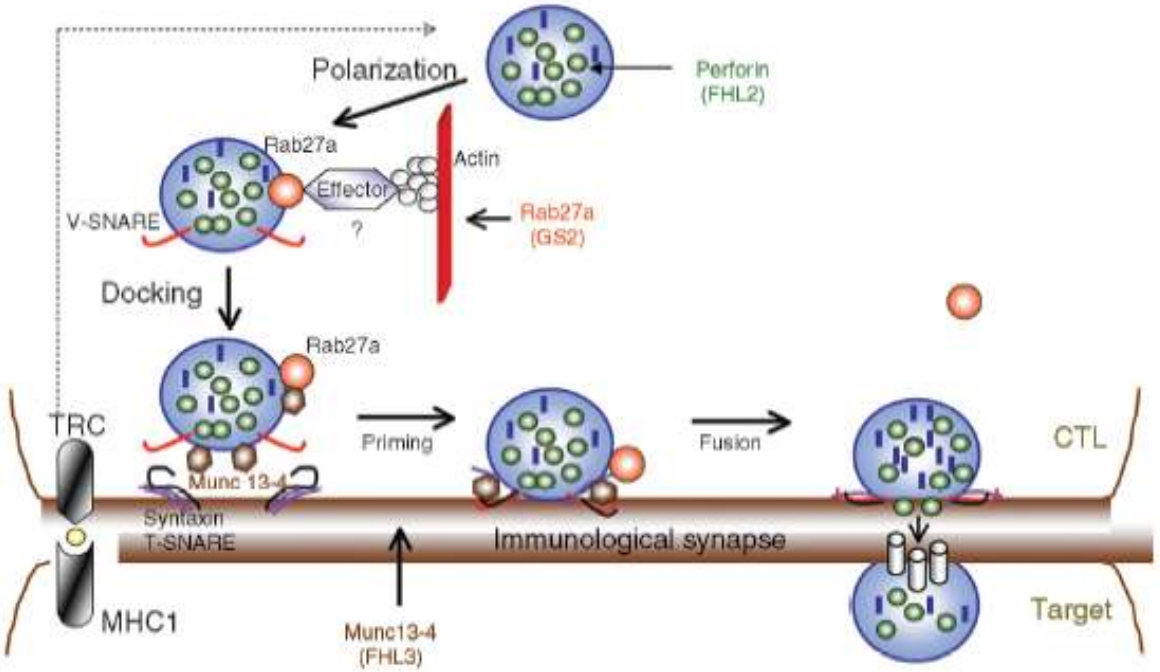
Jérôme Feldmann,[1] Isabelle Callebaut,[2] Graça Raposo,[3] Stéphanie Certain,[1] Delphine Bacq,[4] Cécile Dumont,[1] Nathalie Lambert,[1] Marie Ouachée-Chardin,[5] Gaëlle Chedeville,[5] Hannah Tamary,[6] Véronique Minard-Colin,[5] Etienne Vilmer,[7] Stéphane Blanche,[5] Françoise Le Deist,[1] Alain Fischer,[1,5] and Geneviève de Saint Basile[1,*]

cytotoxic granules at the immunological synapse. HMunc13-4 is therefore essential for the priming step of cytolytic granules secretion preceding vesicle membrane fusion.

Introduction

Cytotoxic T lymphocytes (CTL) and natural killer (NK)

MUNC13-4 and FHL-3



PRIMING:

- Munc13-1 Binds SNAREs and stabilize them in an open state allowing SNARE to form a complex

-Munc13-4 has the same role in non neuronal secretory cells

-Mutations of the Munc13-4 gene cause FHL-3 (Feldman, Cell 2003)

Novel Munc13–4 mutations in children and young adult patients with haemophagocytic lymphohistiocytosis

A Santoro, S Cannella, G Bossi, F Gallo, A Trizzino, D Pende, F Dieli, G Bruno, J C Stinchcombe, C Micalizzi, C De Fusco, C Danesino, L Moretta, L D Notarangelo, G M Griffiths, M Aricò

J Med Genet 2006;**43**:953–960. doi: 10.1136/jmg.2006.0

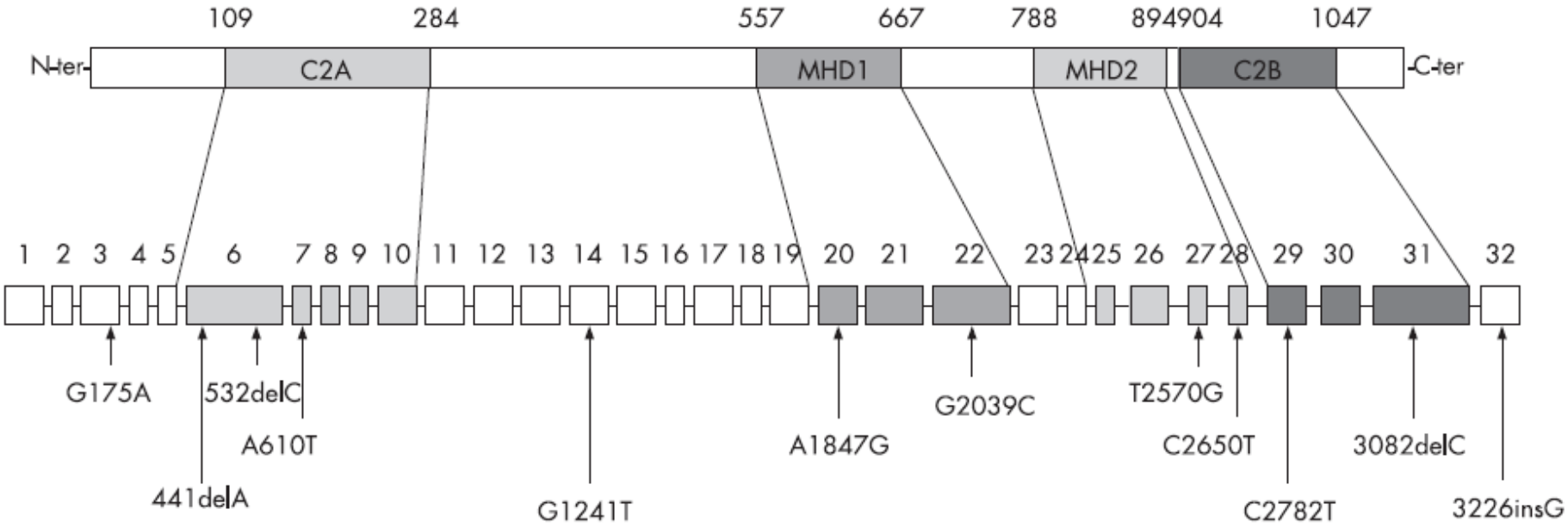


Figure 1 Sites of novel mutations described in this paper relative to exon structure and protein domains: C2 (C2A and C2B) and Munc homology domains (MHD1 and MHD2).

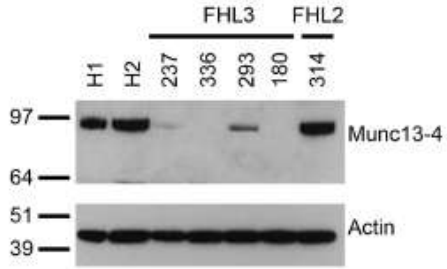


Figure 2. Munc13-4 expression in control and FHL NK cells. Cell extracts from polyclonal NK cells of healthy donors (H1, H2), patients with FHL3 (UPNs 237, 336, 293, 180), and patient with FHL2 (UPN 314) were analyzed by Western blot with anti–Munc13-4 antibody. Blots were reprobed with antiactin antibody. Molecular weight standards are shown on the left (kDa).

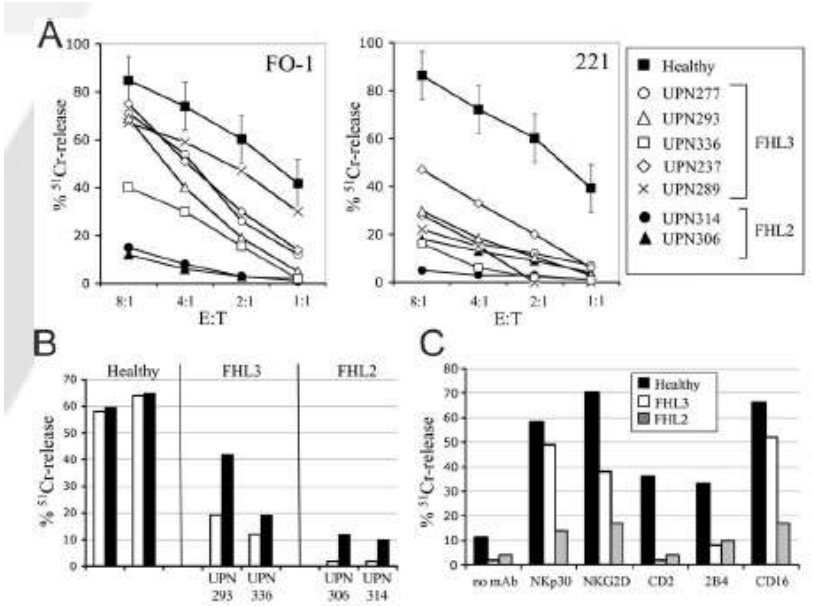


Figure 3. Cytotoxic activity in patients with FHL. Using the standard ^{51}Cr-release

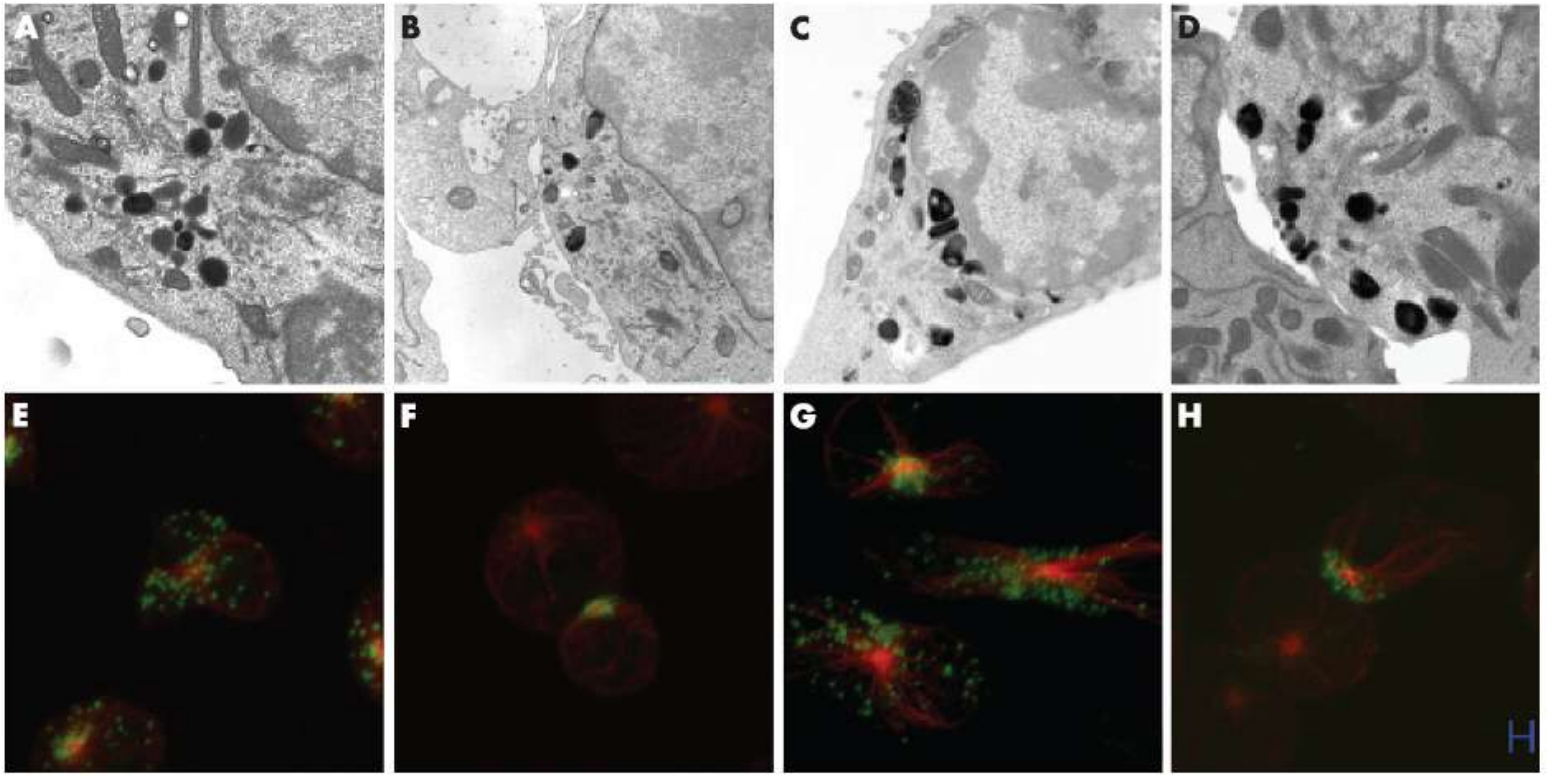


Figure 4 Granule polarisation in control and patients with FHL3. Electron microscopy (A–D) and confocal microscopy (E–H) of control (A, B, E, F) and FHL3 (C, D, G, H) cytotoxic T lymphocyte (CTL). Single CTL (A, E, C, G) shows granules distributed throughout the cells and CTL conjugated to target cells (B, F, D, H). FHL3 cells from patients 7 (C, D) and 9 (G, H). Scale bar = 10μm. Tubulin (red), lamp (green).

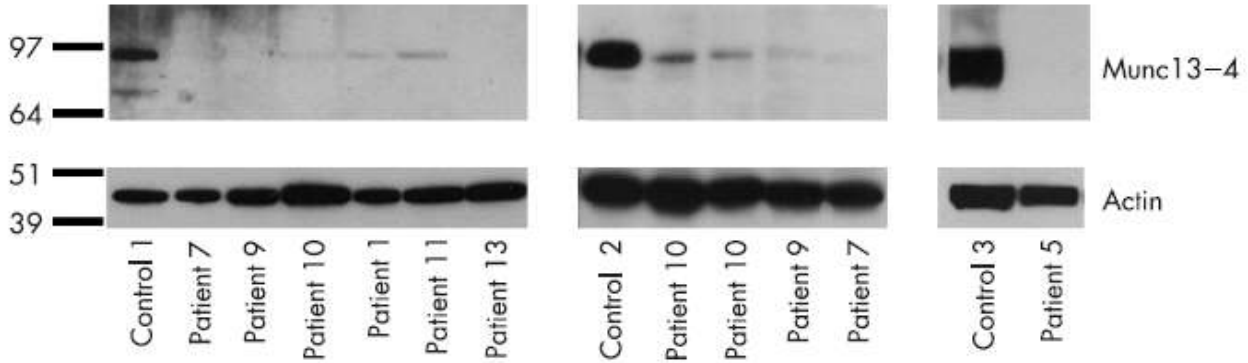
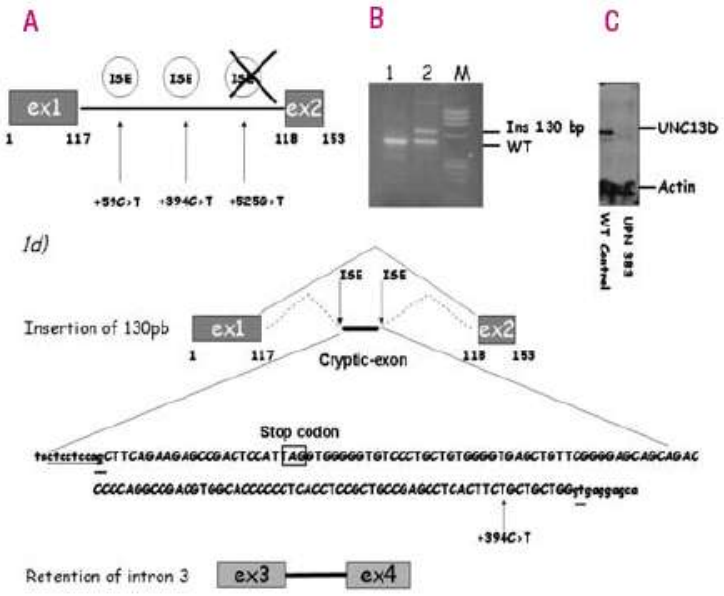


Figure 3 Munc13–4 protein expression is greatly reduced or absent in many cells. Western blot of natural killer cells (left and right) or CD8+CTL (middle panels) lysates from healthy donors (controls 1–3) and patients as indicated, probed with anti-Munc 13–4 and re-probed with anti-actin. Molecular weight markers (kD) are shown on the left.

Mutations affecting MRNA splicing are the most common molecular defect in patients with familial hemophagocytic lymphohistiocytosis type 3

Alessandra Santoro,[1,2] Sonia Cannella,[2] Antonino Trizzino,[2] Giuseppa Bruno,[2] Carmen De Fusco,[3] Luigi D. Notarangelo,[4] Daniela Pende,[5] Gillian M. Griffiths,[6] Maurizio Aricò[2,7]

From the [1]Divisione di Ematologia I, A.O. V. Cervello, Palermo; [2]Onco Ematologia Pediatrica, Ospedale dei Bambini "G. Di Cristina", Palermo; [3]Onco Ematologia Pediatrica, Ospedale Pausilipon, Napoli; [4]Division of Immunology, Children's Hospital, Harvard Medical School; [5]Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy; [6]Cambridge Institute for Medical Research, Addenbrooke's Hospital, Cambridge, UK; [7]Oncoematologia Pediatrica, A.O.U. Meyer, Firenze



Genotype—phenotype study of familial haemophagocytic lymphohistiocytosis type 3

Elena Sieni,[1] Valentina Cetica,[1] Alessandra Santoro,[2] Karin Beutel,[1,3] Elena Mastrodicasa,[4] Marie Meeths,[5,6] Benedetta Ciambotti,[1] Francesca Brugnolo,[1] Udo zur Stadt,[7] Daniela Pende,[8] Lorenzo Moretta,[9] Gillian M Griffiths,[10] Jan-Inge Henter,[5] Gritta Janka,[3] Maurizio Aricò[1]

- Esplorare la variabilità delle manifestazioni cliniche
- Esplorare la eterogeneità delle mutazioni di UNC13D
- Correlare le manifestazioni cliniche con le diverse mutazioni
- Confrontare FHL 3 vs FHL2 (*Trizzino A et al. J Med Genet 2007*).

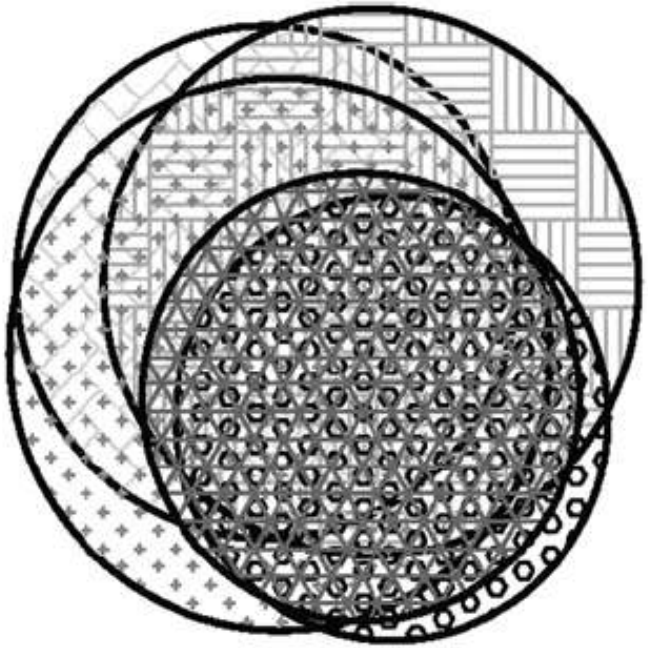
Sieni E, Cetica V, Santoro A, *et al. J Med Genet* (2011). doi:10.1136/jmg.2010.085456

JMG

Genotype–phenotype study of familial haemophagocytic lymphohistiocytosis type 3

Elena Sieni, Valentina Cetica, Alessandra Santoro, et al.

J Med Genet published online January 19, 2011
doi: 10.1136/jmg.2010.085456



Fever	89%
Splenomegaly	96%
Thrombocytopenia	94%
Haemophagocytosis	78%
Hyperferritinaemia	77%
Fever + Spleromegaly	88%
Fever + Splenomoegaly + Thrombocytopenia	84%
Fever + Splenomegaly + Thrombocytopenia+ Hyperferrtinaemia	71%
Fever + Splenomegaly + Thrombocytopenia+ Haemophagocytosis	69%
Fever + Splenomegaly - Thrombocytopenia+ Haemophagocytosis + Hyperferritinaemia	57%

Figure 1 Distribution of the frequency of some diagnostic parameters for haemophagocytic lymphohistiocytosis and pattern of their association.

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Genotype–phenotype study of familial haemophagocytic lymphohistiocytosis type 3

Elena Sieni, Valentina Cetica, Alessandra Santoro, et al.

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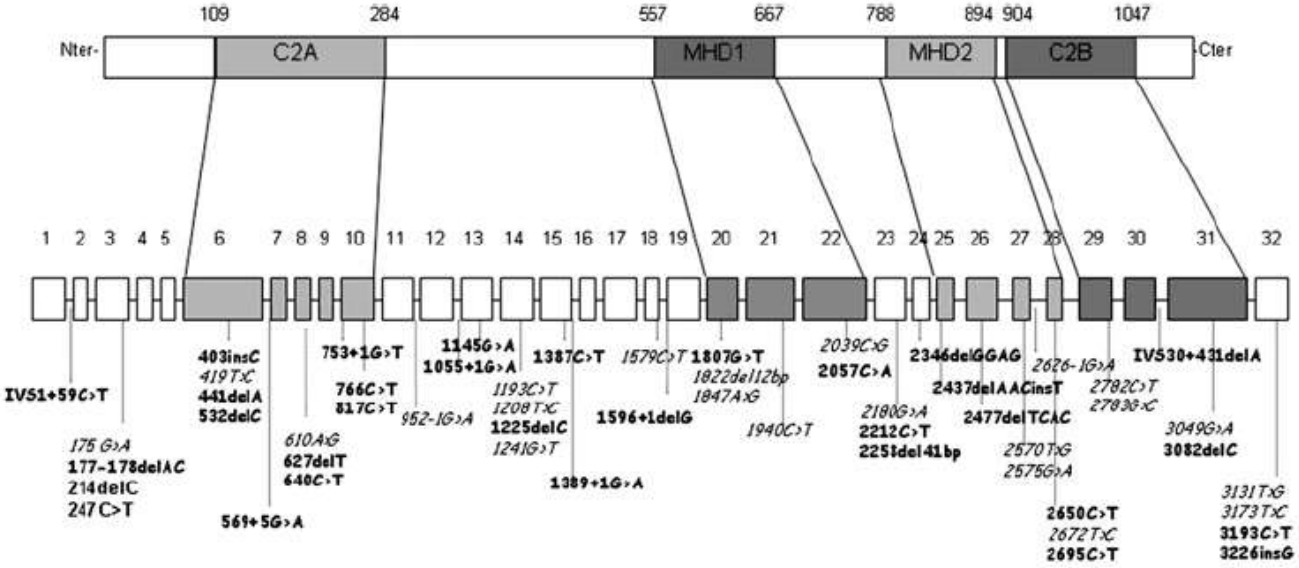

Figure 2 Details of 54 different *UNC13D* mutations observed in 84 patients with familial haemophagocytic lymphohistiocytosis type 3 (FHL3) from 69 families. Mutations are spread over the entire gene. Disruptive mutations are indicated in bold.

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Genotype–phenotype study of familial haemophagocytic lymphohistiocytosis type 3

Elena Sieni, Valentina Cetica, Alessandra Santoro, et al.

J Med Genet published online January 19, 2011
doi: 10.1136/jmg.2010.085456

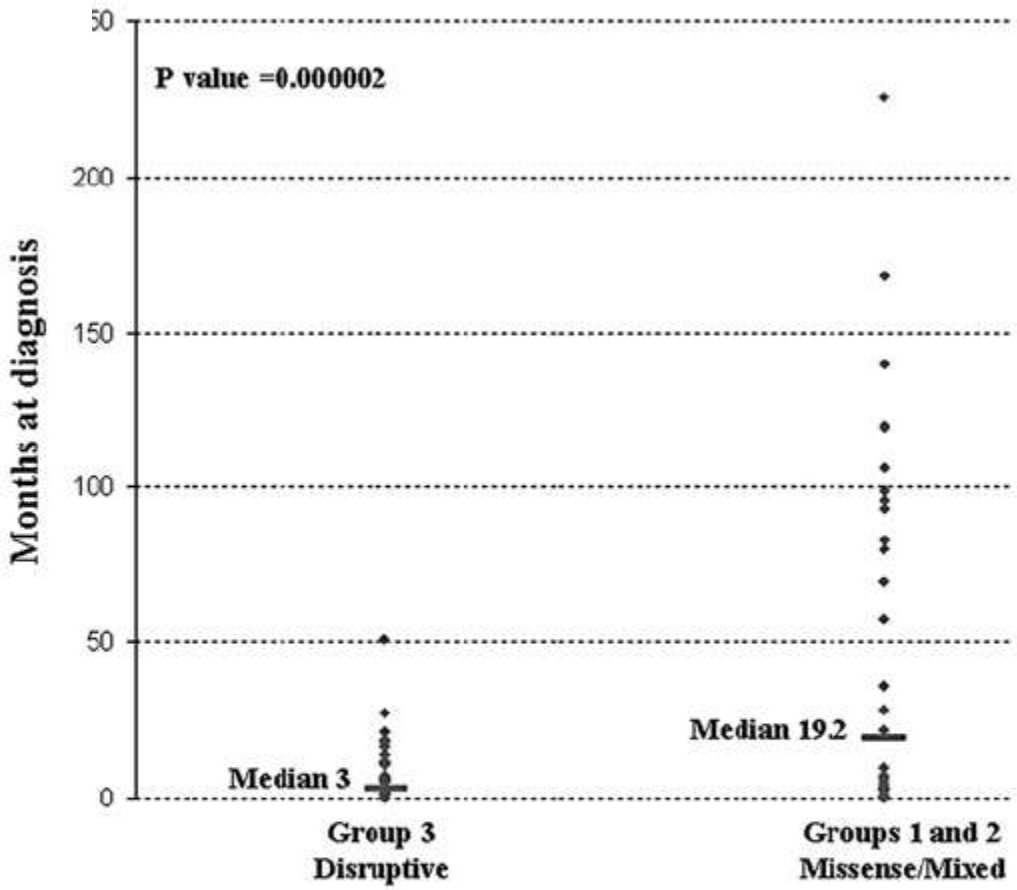


Figure 3 Age at onset of familial haemophagocytic lymphohistiocytosis type 3 (FHL3) depends on the functional impact of the *UNC13D* mutations. Patients with two disruptive mutations had a significantly younger age at diagnosis than patients with two missense mutations. Each point represents one person, lines indicate mean values.

STXBP2 mutations in children with familial haemophagocytic lymphohistiocytosis type 5

Valentina Cetica, Alessandra Santoro, Kimberly C Gilmour, et al.

J Med Genet 2010 47: 595-600
doi: 10.1136/jmg.2009.075341

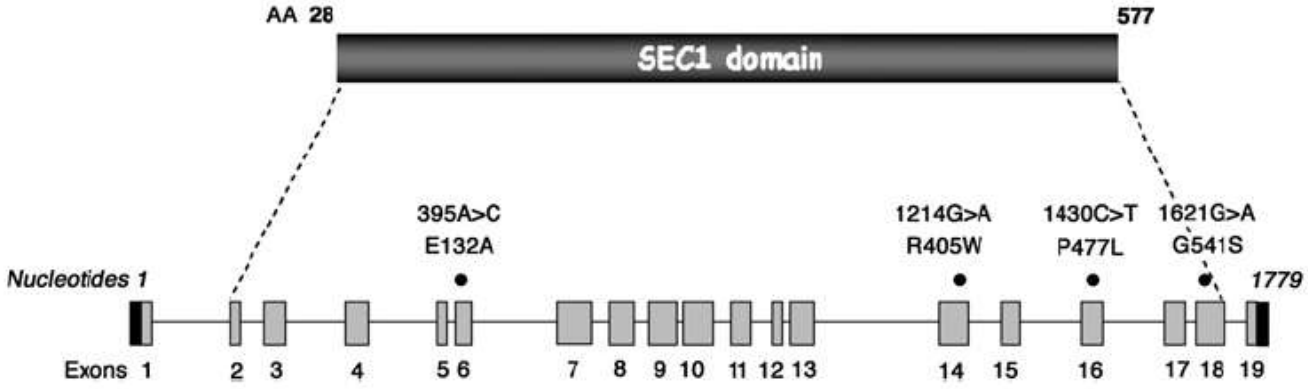


Figure 1 Structure of the *STXBP2* gene and location of the observed mutations in four patients with FHL-5.

JMG

STXBP2 mutations in children with familial haemophagocytic lymphohistiocytosis type 5

Valentina Cetica, Alessandra Santoro, Kimberly C Gilmour, et al.

J Med Genet 2010 47: 595-600
doi: 10.1136/jmg.2009.075341

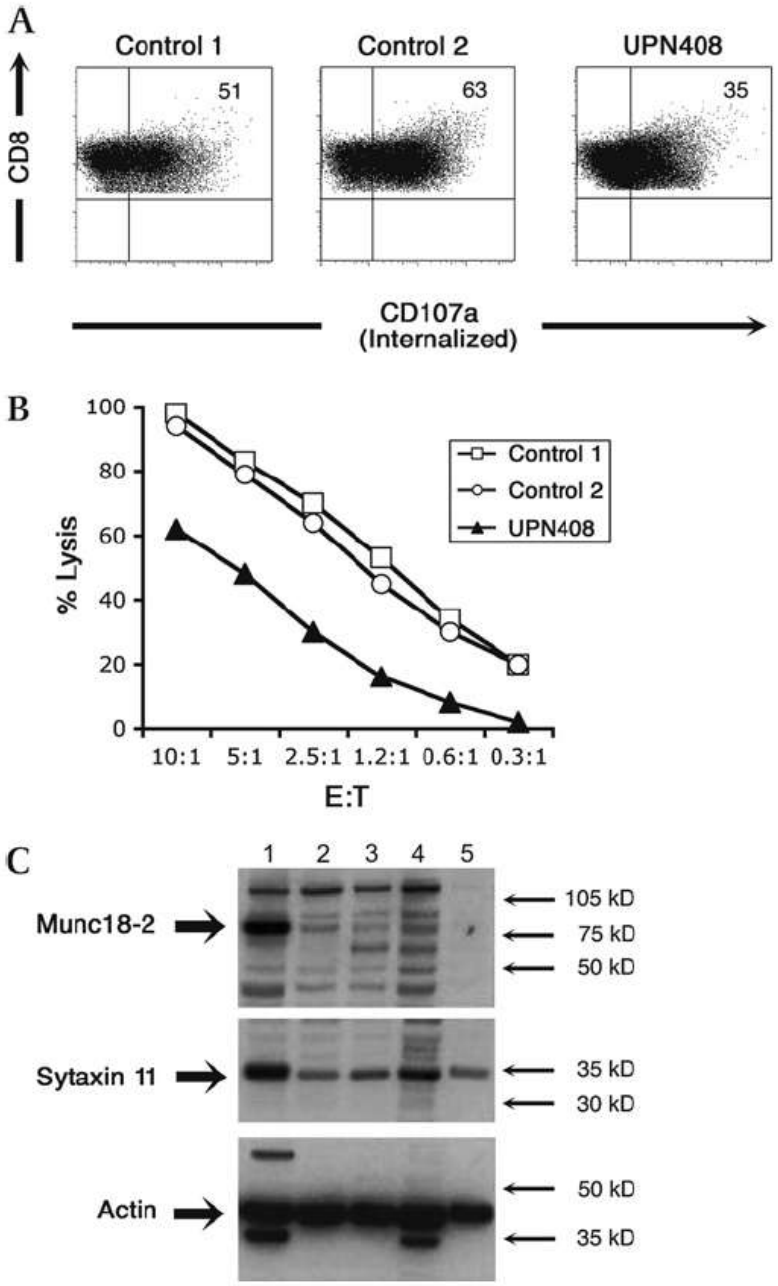
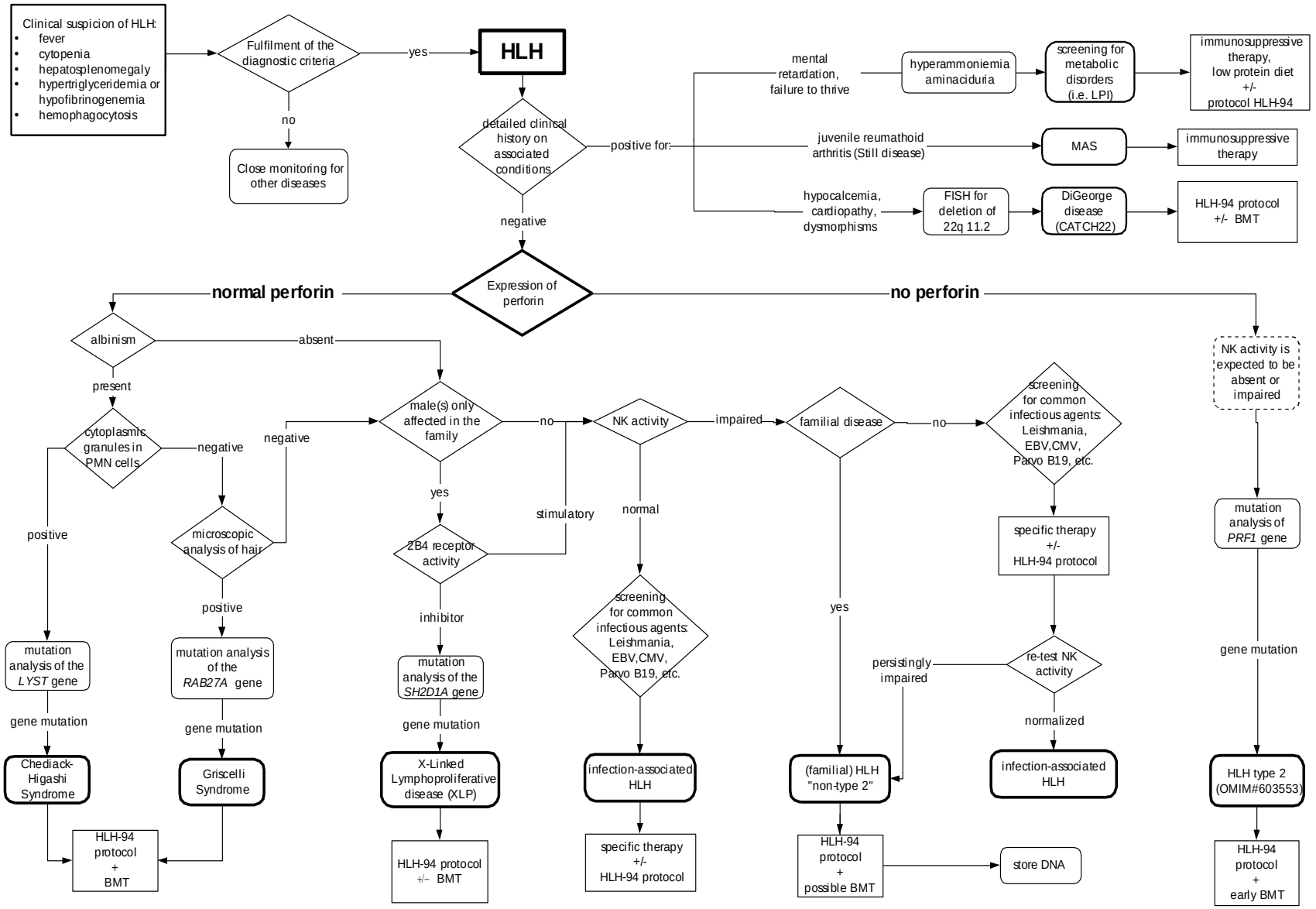


Figure 4 CTL degranulation and cytotoxicity and protein study of patient UPN 408. Degranulation assay showing CD3 stimulated internalisation of CD107a (panel A), detected with PE-CD107a mAb present throughout the 4 h incubation, from CTL lines from two healthy donors (controls 1 and 2) compared to those from UPN408. Cells were stained for CD8 (y axis) and CD107a-PE (x axis). Cellular cytotoxicity of CTL lines from UPN408 (shaded triangles) compared to those from healthy donors (open symbols) measured using redirected lysis of P815 target cells at different E:T ratios (panel B). Data points were performed in triplicate with standard deviations of $<2.5\%$ for all E:T ratios. Western blot of cell lysates from CTL lines (1—4) or phytohaemagglutinin (PHA) blasts (5) from (1) Healthy donor, (2) FHL-5 patient with Pro477Leu mutation (see reference 24), (3—4) UPN408 clones, and (5) UPN408 PHA blasts probed with antibodies to Munc18-2, syntaxin 11 and reprobed with actin to show loading levels (panel C). Molecular weight markers are shown.

DIAGNOSING FAMILIAL HLH

Such a nightmare?

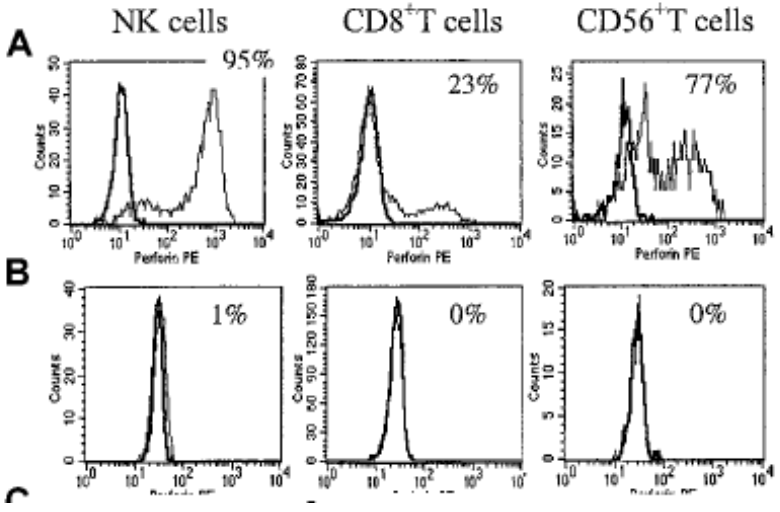


Aricò M, Allen M, Brusa S, Clementi R, Pende D, Maccario R, Moretta L, Danesino C. Br J Haematol. 2002

Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members

Kazuhiro Kogawa, Susan M. Lee, Joyce Villanueva, Daniel Marmer, Janos Sumegi, and Alexandra H. Filipovich

Mutations in the perforin gene have been described in some patients with hemophagocytic lymphohistiocytosis (HLH), but the role of perforin defects in the pathogenesis of HLH remains unclear. Four-color flow cytometric analysis was used to establish normal patterns of perforin expression for control subjects of all ages, and patterns of perforin staining in cytotoxic lymphocytes (natural killer [NK] cells, CD8+ T cells, CD56+ T cells) from patients with HLH and their family members were studied. Eleven unrelated HLH patients and 19 family members were analyzed prospectively. Four of the 7 patients with primary HLH showed lack of intracellular perforin in all cytotoxic cell types. All 4 patients showed mutations in the perforin gene. Their parents, obligate carriers of perforin mutations, had abnormal perforin-staining patterns. Analysis of cytotoxic cells from the other 3 patients with primary HLH and remaining family members had normal percentages of perforin-positive cytotoxic cells. On the other hand, the 4 patients with Epstein-Barr virus–associated HLH typically had depressed numbers of NK cells but markedly increased proportions of CD8+ T cells with perforin expression. Four-color flow cytometry provides diagnostic information that, in conjunction with evidence of reduced NK function, may speed the identification of life-threatening HLH in some families and direct further genetic studies of the syndrome. (Blood. 2002;99: 61-66)



…what about Munc?

Analysis of natural killer–cell function in familial hemophagocytic lymphohistiocytosis (FHL): defective CD107a surface expression heralds Munc13-4 defect and discriminates between genetic subtypes of the disease

Stefania Marcenaro, Federico Gallo, Stefania Martini, Alessandra Santoro, Gillian M. Griffiths, Maurizio Aricó, Lorenzo Moretta, and Daniela Pende

Natural killer (NK) cells from patients with familial hemophagocytic lymphohistiocytosis because of *PRF1* (FHL2, n = 5) or *MUNC13-4* (FHL3, n = 8) mutations were cultured in IL-2 prior to their use in various functional assays. Here, we report on the surface CD107a expression as a novel rapid tool for identification of patients with Munc13-4 defect. On target interaction and degranulation, FHL3 NK cells displayed low levels of surface CD107a staining, in contrast to healthy control subjects or perforin-deficient NK cells. B-EBV cell lines and dendritic cell targets reveal the FHL3 NK-cell defect, whereas highly susceptible tumor targets were partially lysed by FHL3 NK cells expressing only trace amounts of Munc13-4 protein. Perforin-deficient NK cells were completely devoid of any ability to lyse target cells. Cytokine production induced by mAb-crosslinking of triggering receptors was comparable in patients and healthy control subjects. However, when cytokine production was induced by coculture with 721.221 B-EBV cells, FHL NK cells resulted in high producers, whereas control cells were almost ineffective. This could reflect survival versus elimination of B-EBV cells (ie, the source of NK-cell stimulation) in patients versus healthy control subjects, thus mimicking the pathophysiologic scenario of FHL. (Blood. 2006;108:2316-2323)

CD107a study: which lesson?

- Defective expression of LAMP (CD107a) heralds defective degranulation as in Munc 13-4 deficiency
- This is not specific for FHL3
- CD107 is normal in FHL2

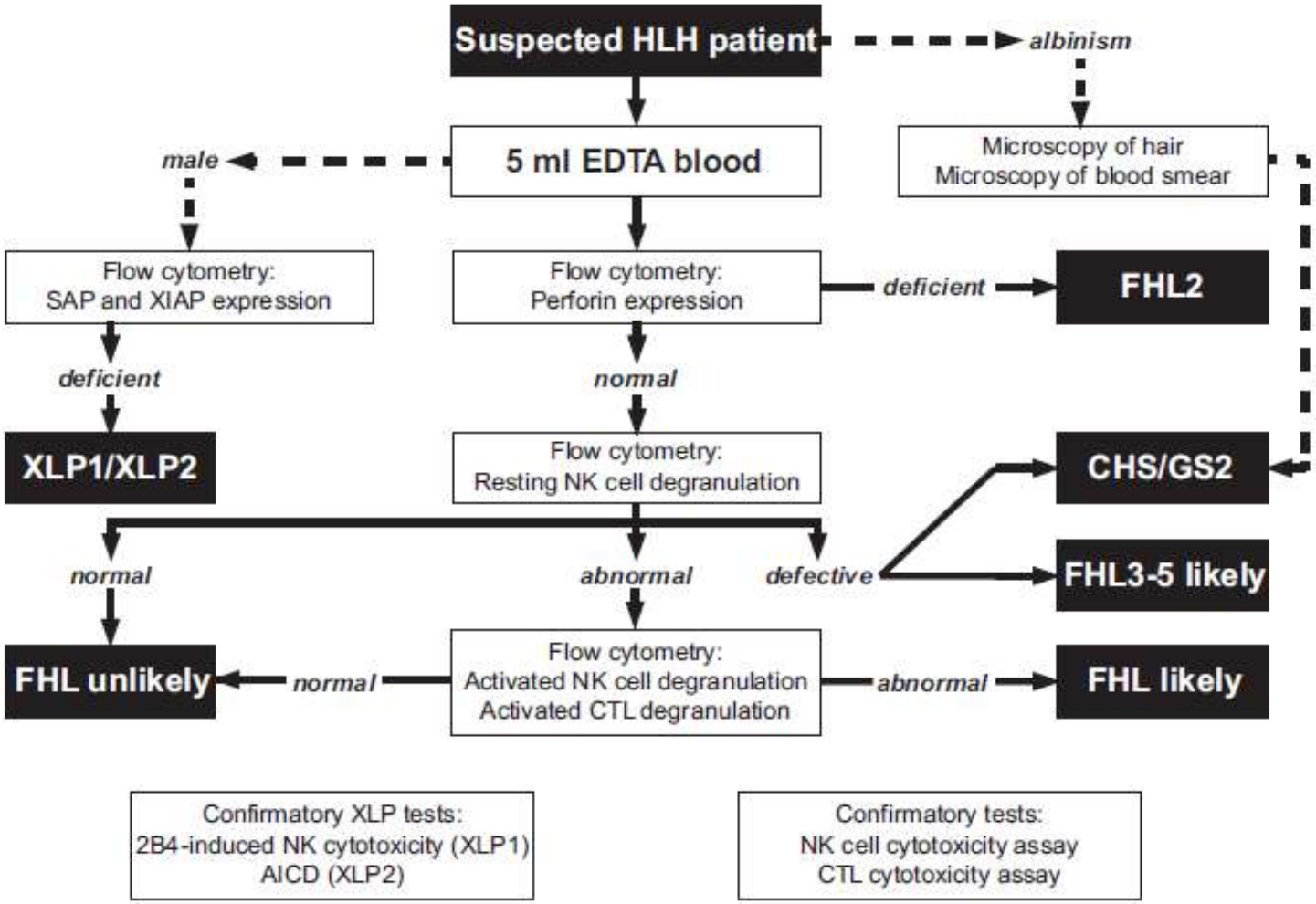


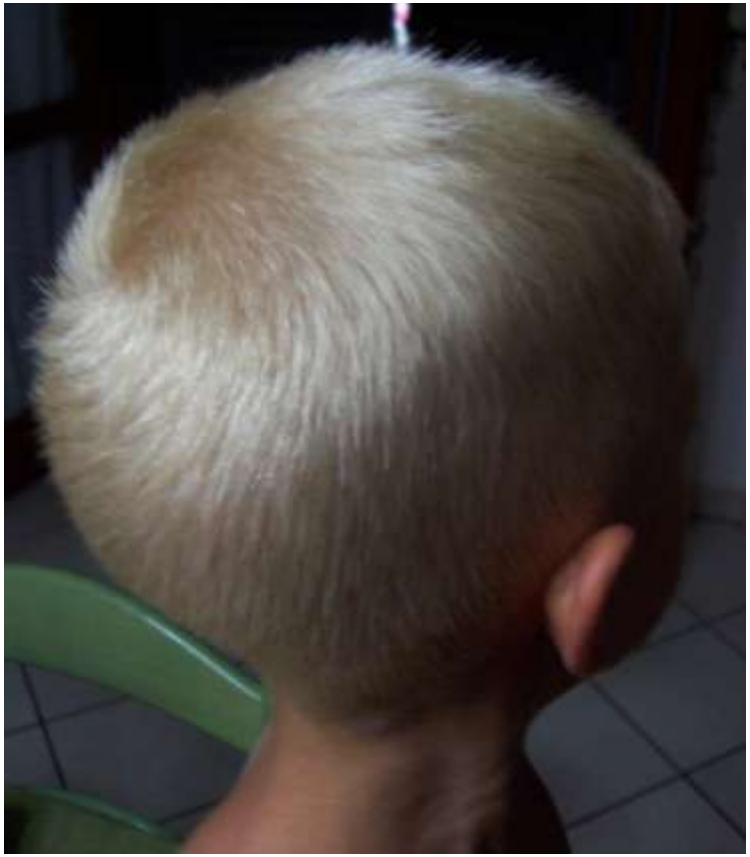
Figure 7. Proposed laboratory diagnostic algorithm based on degranulation assays for patients presenting with HLH. Normal values have to be determined in the diagnostic laboratory for the evaluation of resting NK-cell degranulation. For the centers participating in this study, resting NK-cell degranulation was considered defective if $< 5\%$, abnormal if 5%-10%, and normal if $> 10\%$, cutoffs that have proven to be useful. Analyses of activated NK-cell degranulation is recommended for all patients. AICD indicates activation-induced cell death.

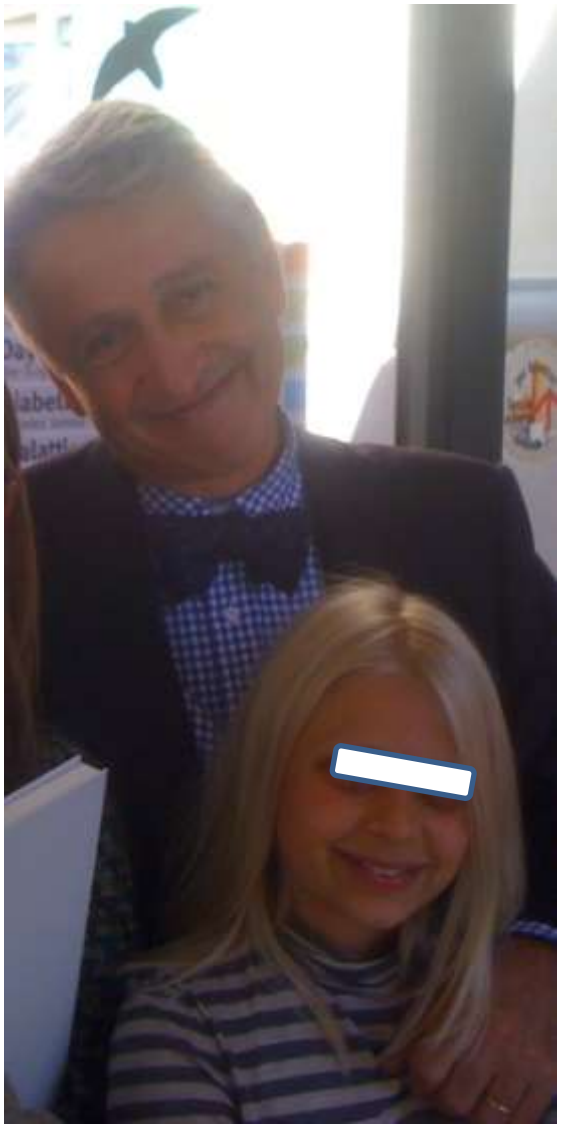
Hemophagocytic lymphohistiocytosis due to germline mutations in *SH2D1A*, the X-linked lymphoproliferative disease gene

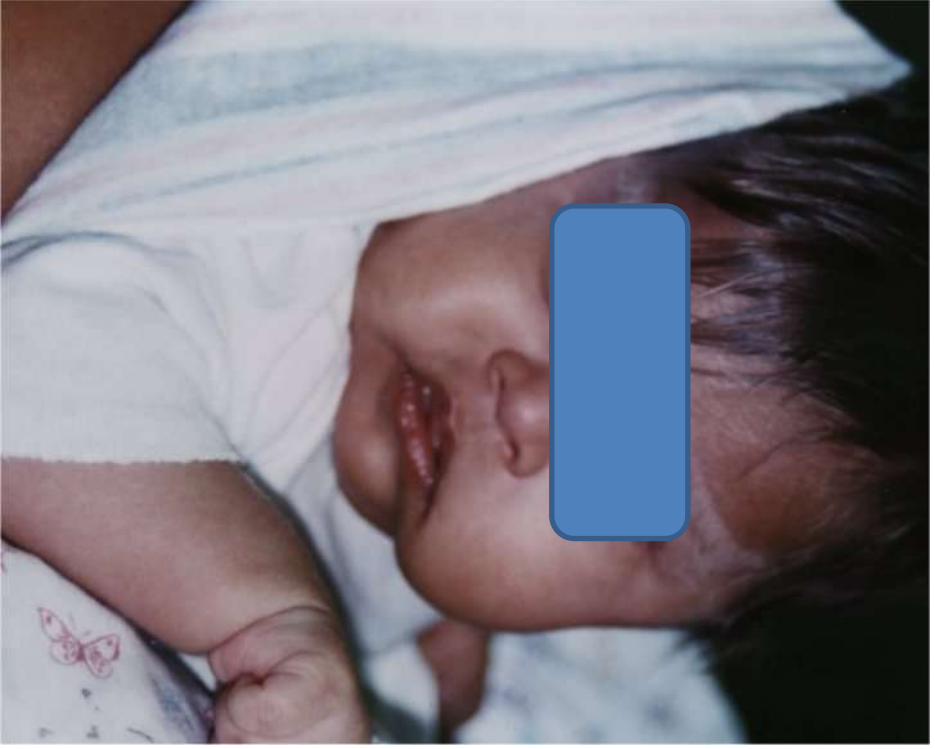
Maurizio Arico, Shinsaku Imashuku, Rita Clementi, Shigeyoshi Hibi, Tomoko Teramura, Cesare Danesino, Daniel A. Haber, and Kim E. Nichols

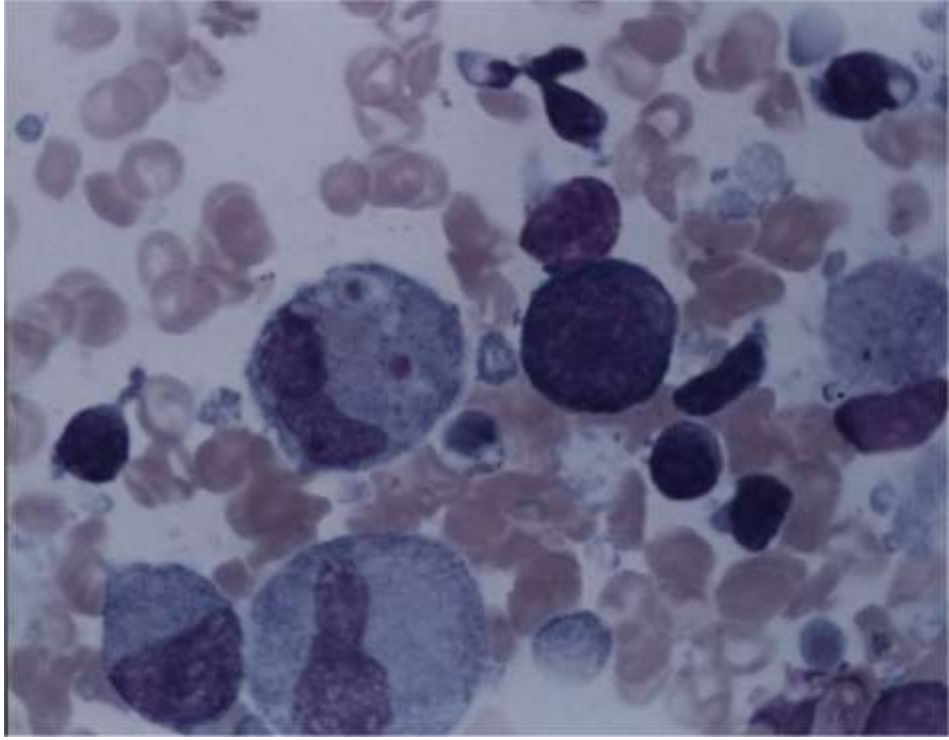
The hemophagocytic lymphohistiocytoses (HLH) comprise a heterogeneous group of disorders characterized by dysregulated activation of T cells and macrophages. Although some patients with HLH harbor perforin gene mutations, the cause of the remaining cases is not known. The phenotype of HLH bears a strong resemblance to X-linked lymphoproliferative disease (XLP), an Epstein-Barr virus (EBV)-associated immunodeficiency resulting from defects in SH2D1A, a small SH2 domain-containing protein expressed in T lymphocytes and natural killer cells. Here it is shown that 4 of 25 male patients with HLH who were examined harbored germline *SH2D1A* mutations. Among these 4 patients, only 2 had family histories consistent with XLP. On the basis of these findings, it is suggested that all male patients with EBV-associated hemophagocytosis be screened for mutations in *SH2D1A*. Patients identified as having XLP should undergo genetic counseling, and be followed long-term for development of lymphoma and hypogammaglobulinemia. (Blood. 2001; 97:1131-1133)

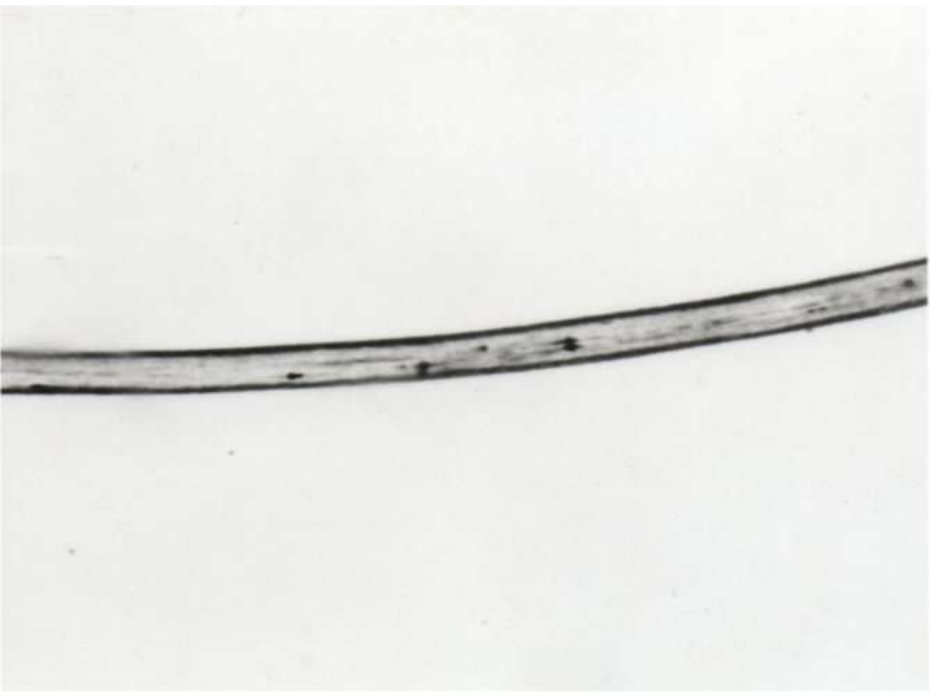
Di che colore ha i capelli questo bambino…. ?

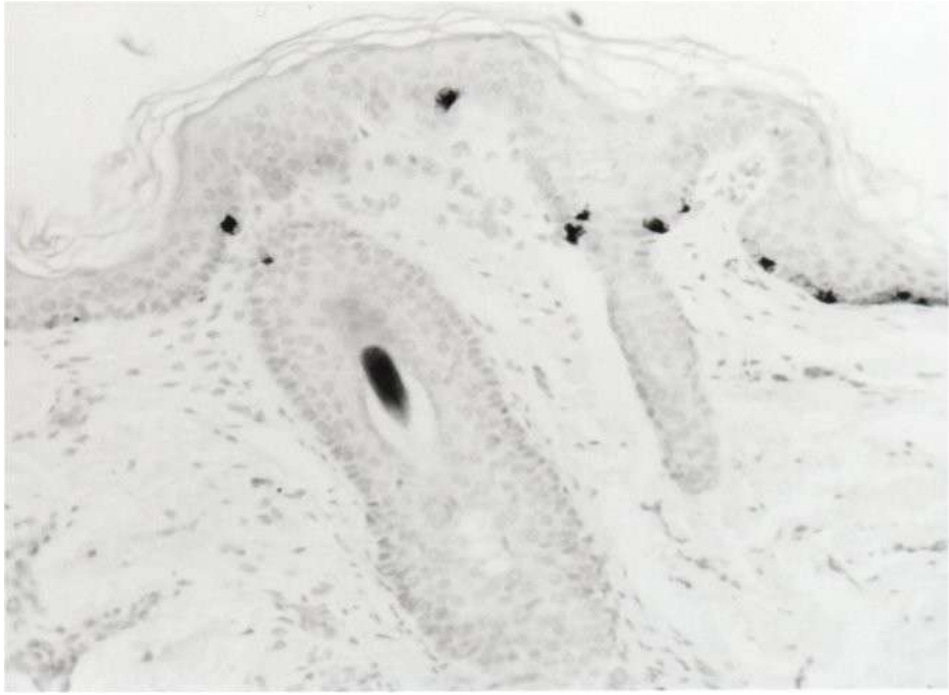












HISTIOCYTIC MEDULLARY RETICULOSIS

BY RONALD BODLEY SCOTT, M.A., D.M. Oxfd, M.R.C.P.

FIRST ASSISTANT IN THE MEDICAL PROFESSORIAL UNIT, ST. BARTHOLOMEW'S HOSPITAL, LONDON; ASSISTANT PHYSICIAN TO THE WOOLWICH WAR MEMORIAL HOSPITAL; AND

A. H. T. ROBB-SMITH, M.A. Oxfd, M.D. Lond.

NUFFIELD READER IN PATHOLOGY IN THE UNIVERSITY OF OXFORD AND ASSISTANT DIRECTOR OF PATHOLOGY AT THE RADCLIFFE INFIRMARY

(*With Illustrations on Plate*)

myeloid and fibril-forming elements. A suggested classification of the reticuloses and reticulosarcomata on a histological basis has recently been published by one of us (Robb-Smith 1938).

In this paper we are recording four examples of histiocytic medullary reticulosis and surveying the published reports of cases which appear to us similar. These cases will be seen to present a clear-cut clinical and pathological picture and give force to our contention that detailed analysis of the reticuloses will show the existence of unsuspected clinico-pathological entities. These cases illustrate what we have come to regard as the typical clinical course of the disease: fever, wasting and generalised lymphadenopathy are associated with splenic and hepatic enlargement and in the final stages jaundice, purpura and

HLH: what have we learned decades after the pioneers?

FAMILIAL HAEMOPHAGOCYTIC RETICULOSIS

BY

JAMES W. FARQUHAR and ALBERT E. CLAIREAUX

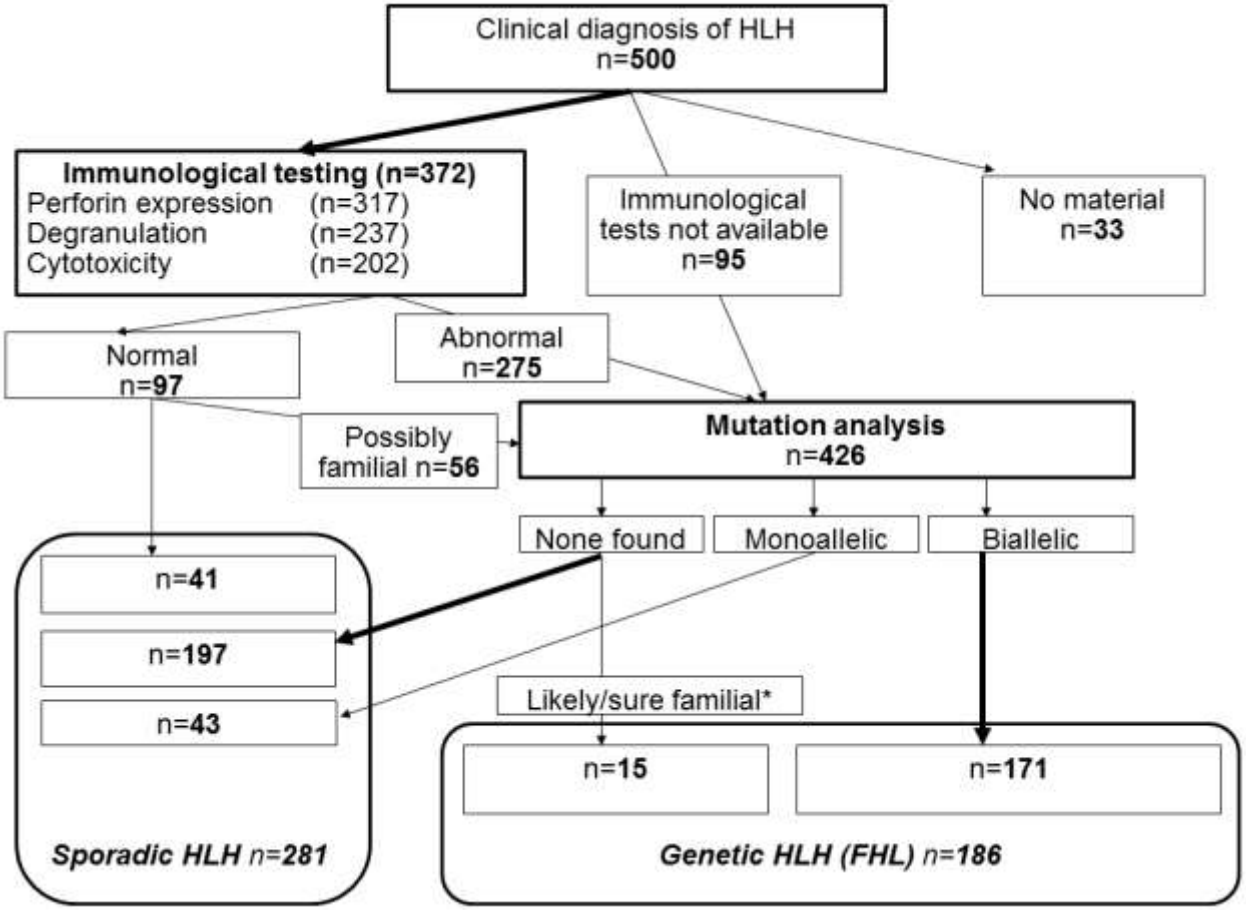
From the Departments of Child Life and Health and Pathology, University of Edinburgh

(RECEIVED FOR PUBLICATION MAY 3, 1952)

Table 1. Number of cases of HLH reported to the Italian National registry by time interval.

Time interval	Total number of patients (mean number per year)	Patients with biallelic mutation (mean number per year)	Sporadic patients (mean number per year)	Biallelic/ sporadic ratio
1989-1994*	53 (8.8)	28 (4.6)	25 (4.1)	1.12
1995-1999	46 (9.2)	28 (5.6)	18 (3.6)	1.55
2004-2004	67 (13.4)	32 (6.4)	35 (7.0)	0.91
2005-2009	127 (25.4)	37 (7.5)	90 (18.0)	0.41
2010-2014**	207 (48.7)	48 (11.2)	159 (37.4)	0.30
Total	**500**	**173**	**327**	**0.52**

One third of HLH patients have FHL



Cetica et al, submitted

Table 2. Correlation of perforin expression and degranulation assays in 372 patients with HLH in whom at least one of the immunological assays could be performed.

	Degranulation				Total
Perforin expression	normal	reduced	complete defect	not performed	
normal	106 [8]	34 [8]	29 [29]	46 [13]	215 [58]
reduced	26 [5]	10 [1]	3 [3]	33 [8]	72 [17]
absent	12 [12]	1 [1]	0	17 [17]	30 [30]
not performed	11 [2]	2 [0]	1 [1]	41* [25]	55 [28]
Total	**155 [27]**	**47 [10]**	**33 [33]**	**137 [63]**	**372 [133]**

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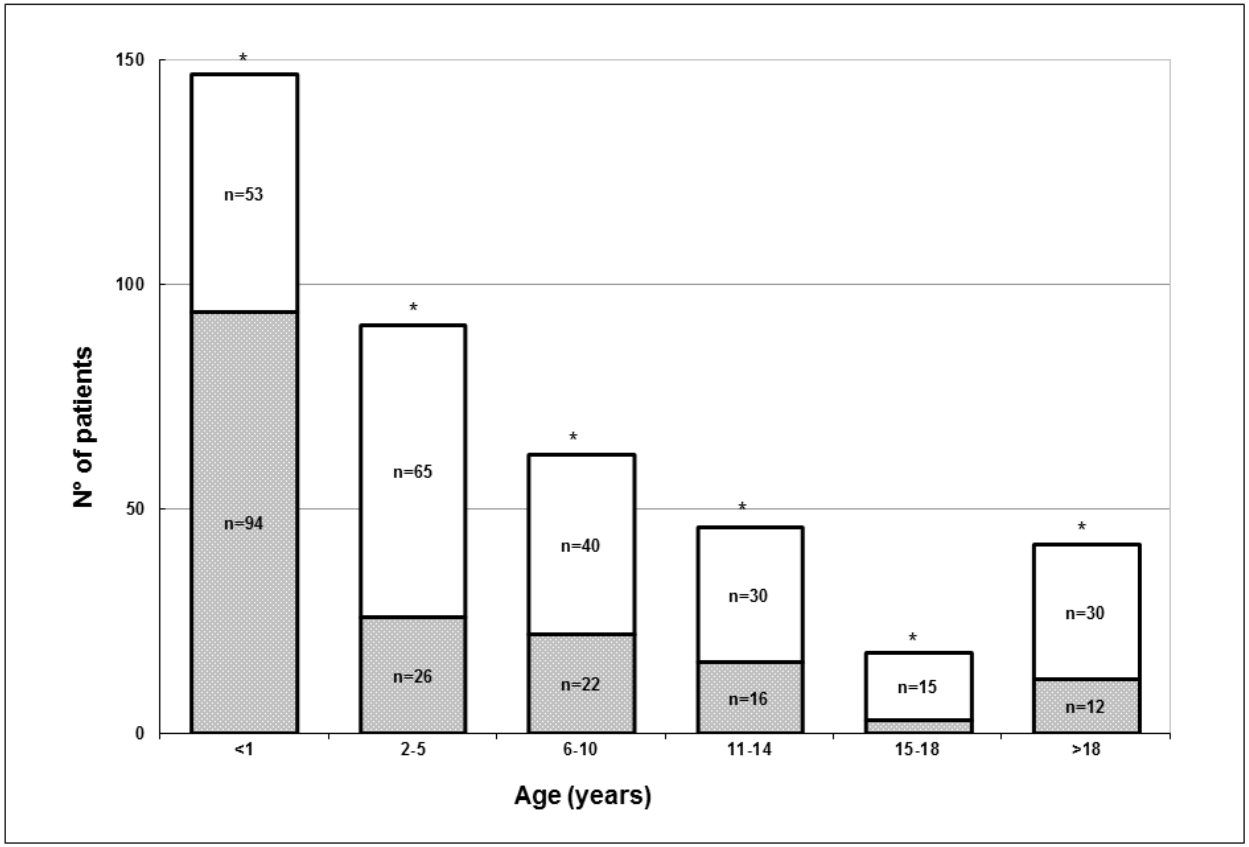
Total	**155 [27]**	**47 [10]**	**33 [33]**	**137 [63]**	**372 [133]**

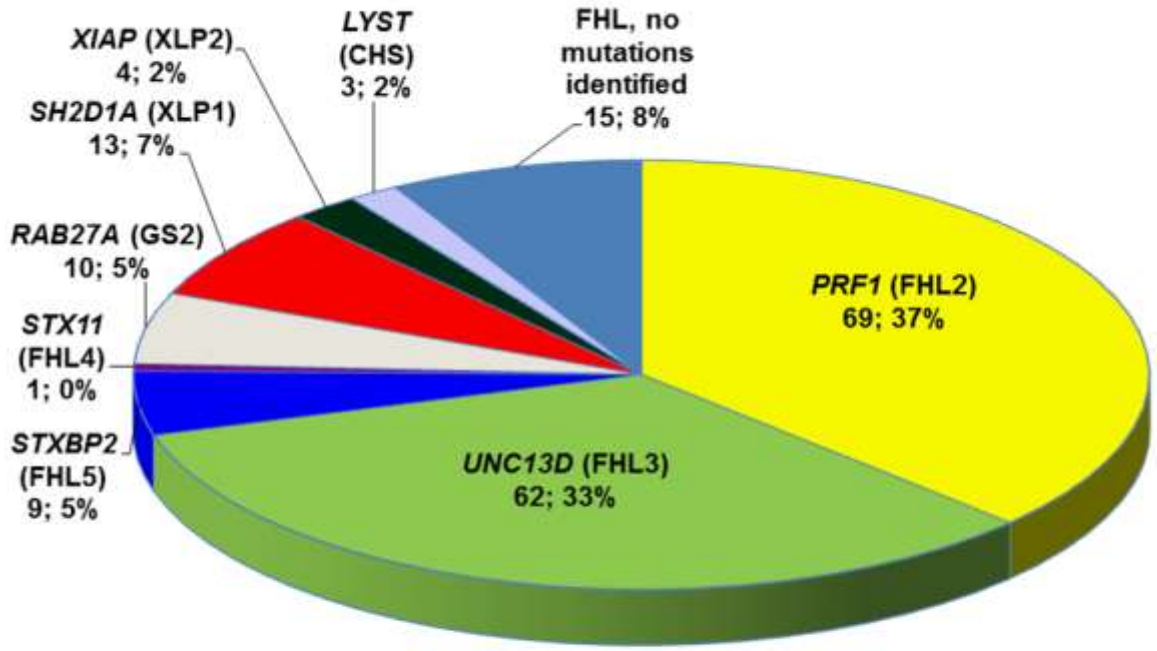
Children <1 year with HLH likely have FHL (64% chance)



**Chi-square test, p-value <0.001.*

Cetica et al, submitted

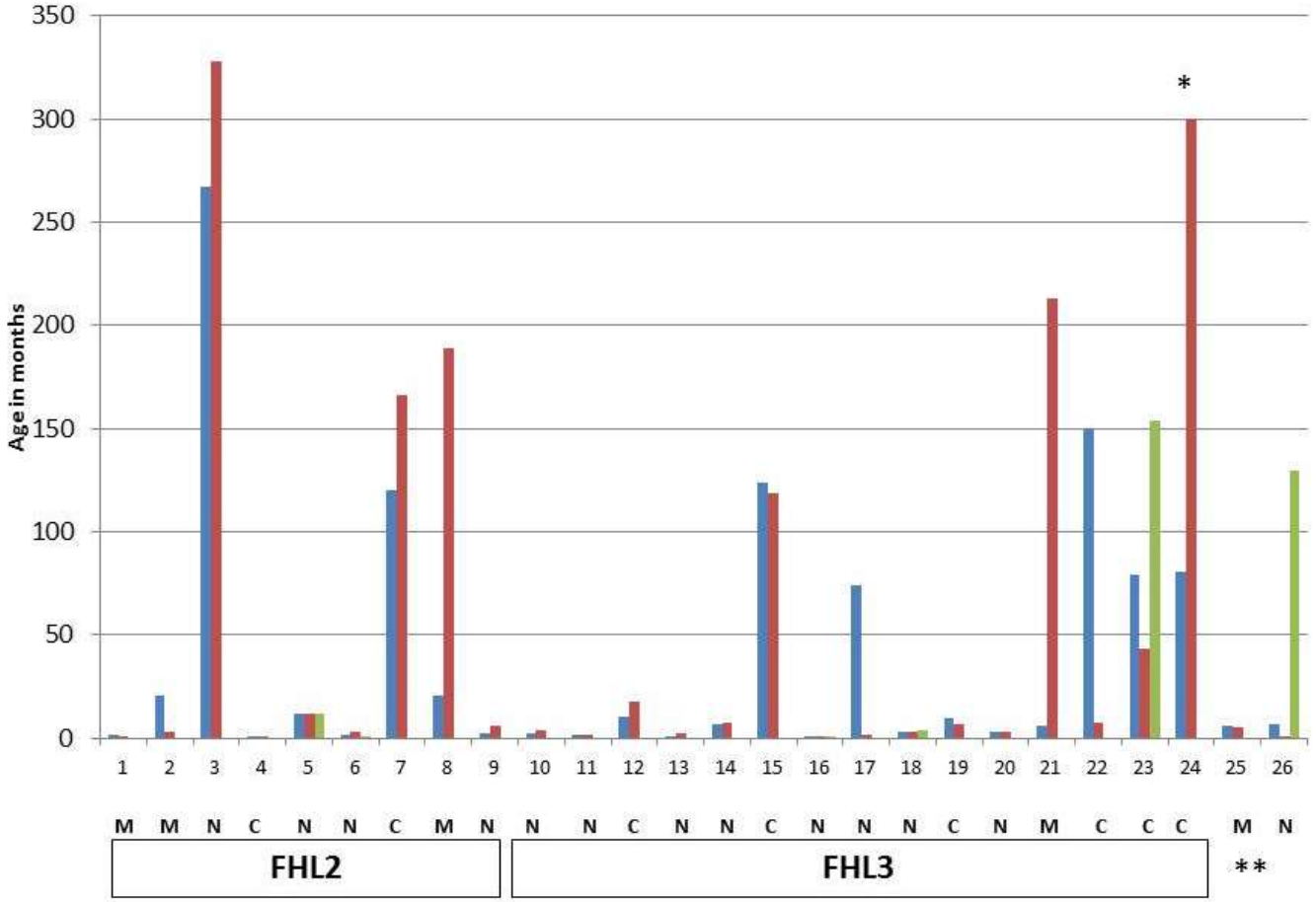
In over 90% of patients with FHL the gene responsible has been identified



N=186

Cetica et al, submitted

Intrafamilial variation of the age at the onset of FHL in 26 families with more than one affected case.

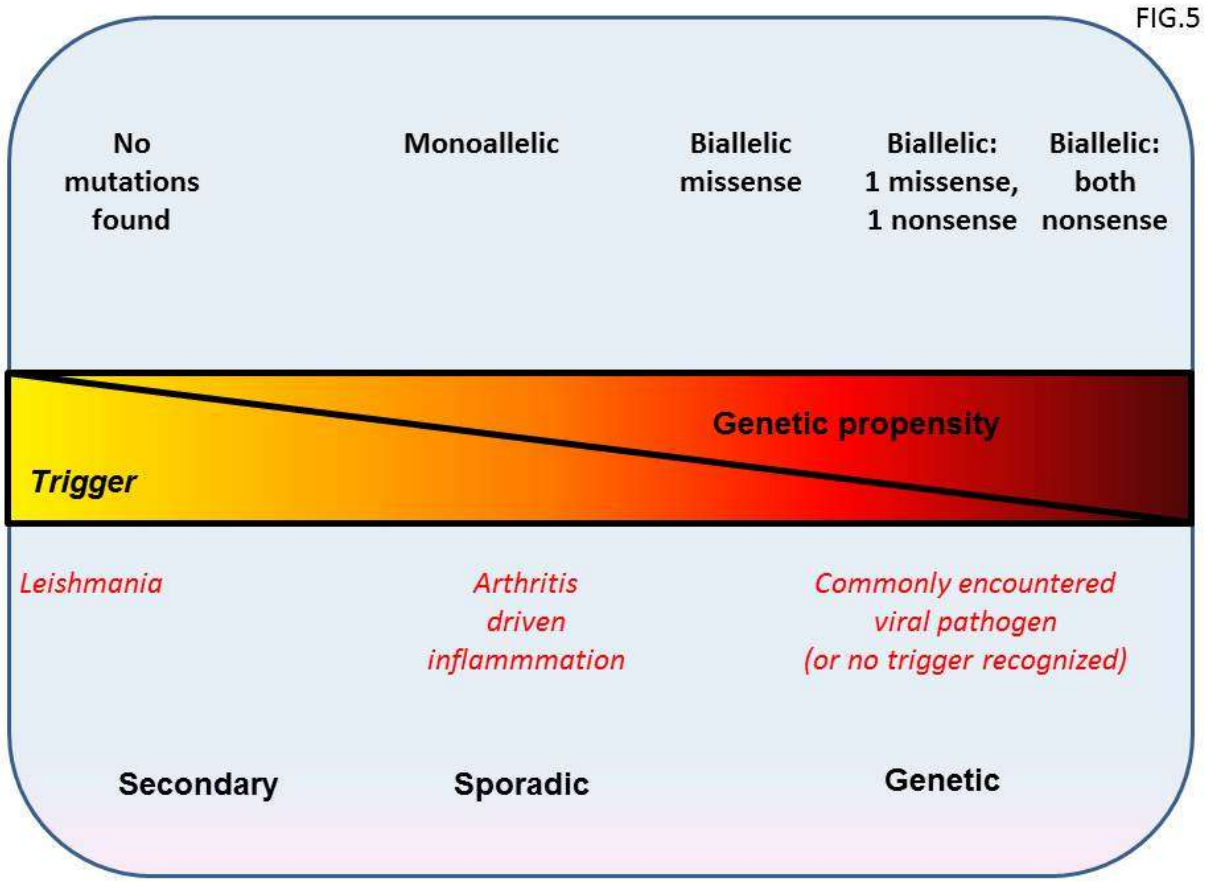


**This sib of patient #24 is now 25 years of age and has not yet developed the disease.*

***Family #25, FHL5; family #26, GS2*

N, biallelic nonsense mutations; M, biallelic missense mutations; C, combination of one nonsense and one missense mutations.

The risk of developing HLH results from the interaction of a predisposed genotype and environmental triggering factor(s).



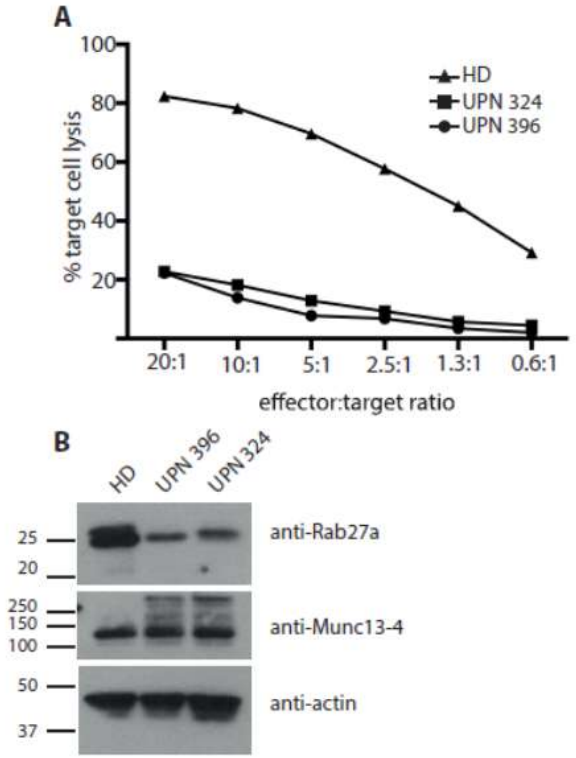
GRISCELLI SYNDROME PATIENTS WITH NORMAL PIGMENTATION IDENTIFY RAB27A MUTATIONS THAT SELECTIVELY DISRUPT MUNC13-4 BINDING

Valentina Cetica, PhD, Yvonne Hackmann, PhD*, Samantha Grieve*, Elena Sieni, MD, Benedetta Ciambotti PhD , Maria Luisa Coniglio PhD, Daniela Pende, PhD, Kimberly Gilmour MD, Paolo Romagnoli MD, Gillian M. Griffiths, PhD , Maurizio Aricò, MD*

Main features of 6 patients from 3 families with HLH but no pigment dilution, in whom GS2 was diagnosed based on RAB27A mutations

UPN	Gender/ Age	GRA/ Cytotoxicity	Mutation	Hair*	Clinical outcome
154	M/6.8 m	NP	c.422_424delGAG p.R141_V142delinsI **		Died from disease
155	M/0.5 m	NP/Absent	c.422_424delGAG p.R141_V142delinsI **		Reactivated after MRD SCT; died from disease
313	F/10.7 y	Reduced/ Reduced	c.422_424delGAG p.R141_V142delinsI **	Mild blond; small and medium size granules	Reactivated; 16 year, cured after UD SCT
324	F/4 y	Absent/Absent (Figure 1)	c.422_424delGAG p.R141_V142delinsI c.487A>C p.S163R		Reactivated, 13 year, cured after UD SCT
396	M/7.3 y	Absent/Absent (Figure 1)	c.227C>T p. A76V c.476A>G p. Y159C	Mild blond; small size granules	Reactivated; 14 year, cured after UD SCT
226	M/5 y	Reduced/ Reduced	c.514_518delCAAGC p.Q172Nfs c.422_424delGAG p.R141_V142delinsI		17 year, cured after SCT

CTL from patients UPN396 and UPN324 show severely reduced ability to kill target cells



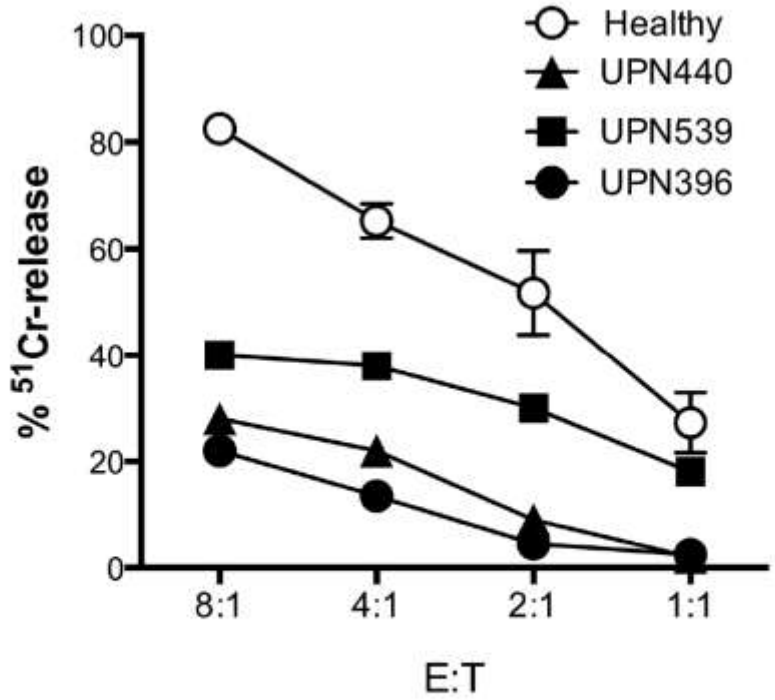
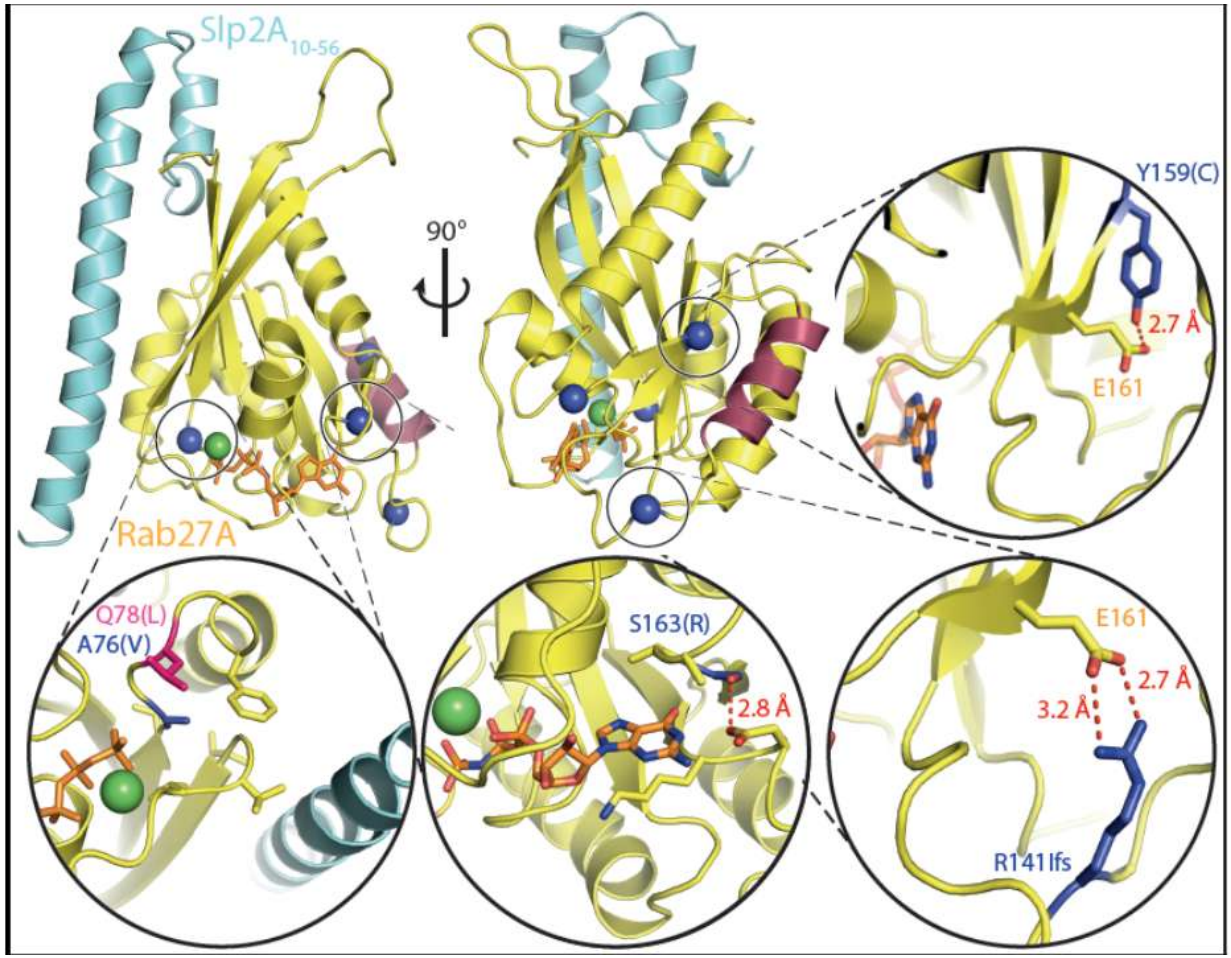


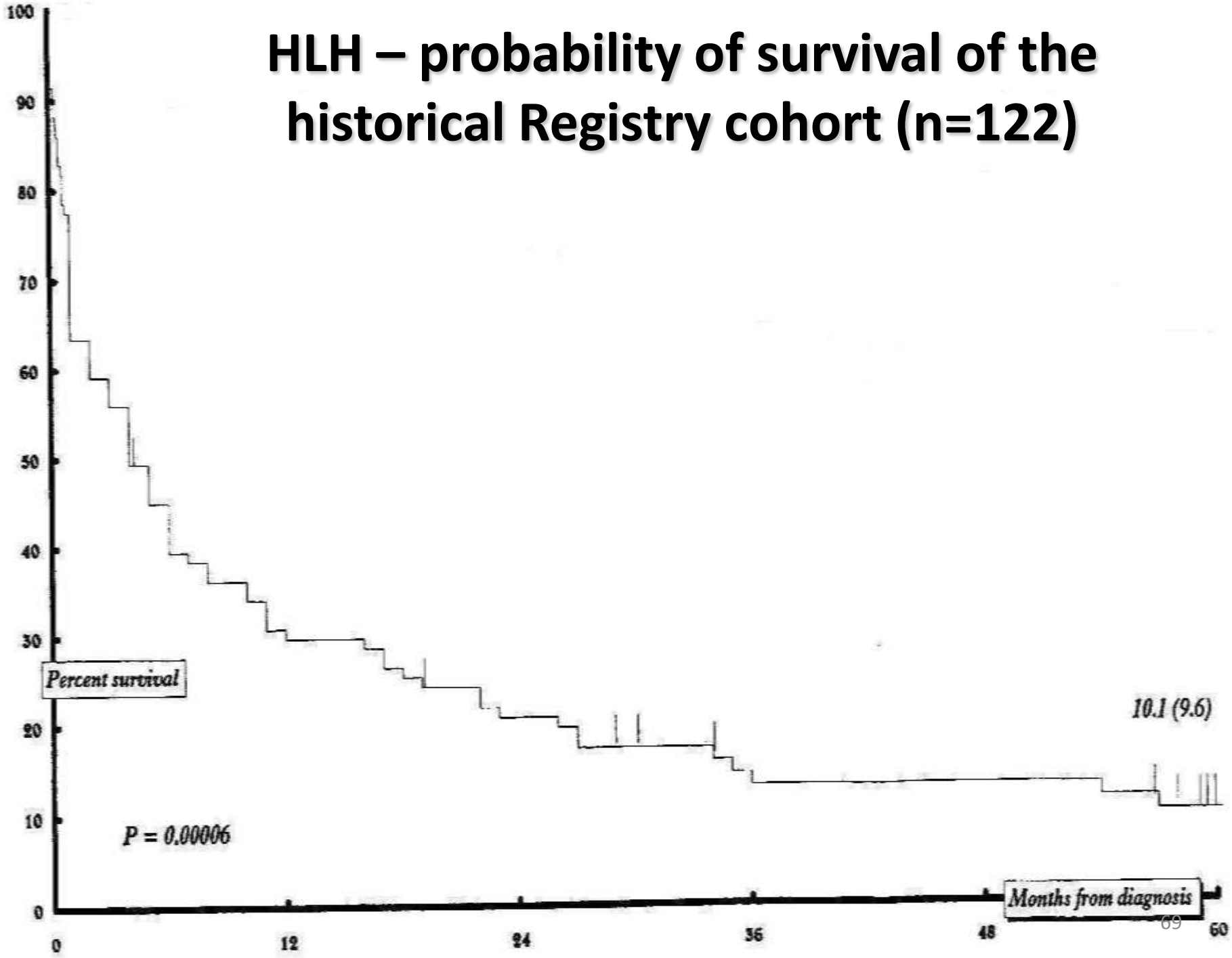
FIG E1. Cytolytic activity of polyclonal activated NK cells from the three representative patients UPN440 (CHS), UPN539 (GS2 with pigment dilution) and UPN396 (GS2 without pigment dilution), in comparison with healthy controls (mean ± SEM), against 221 B-EBV target cell line.

Location of amino acid mutations in patients UPN394 and UPN326



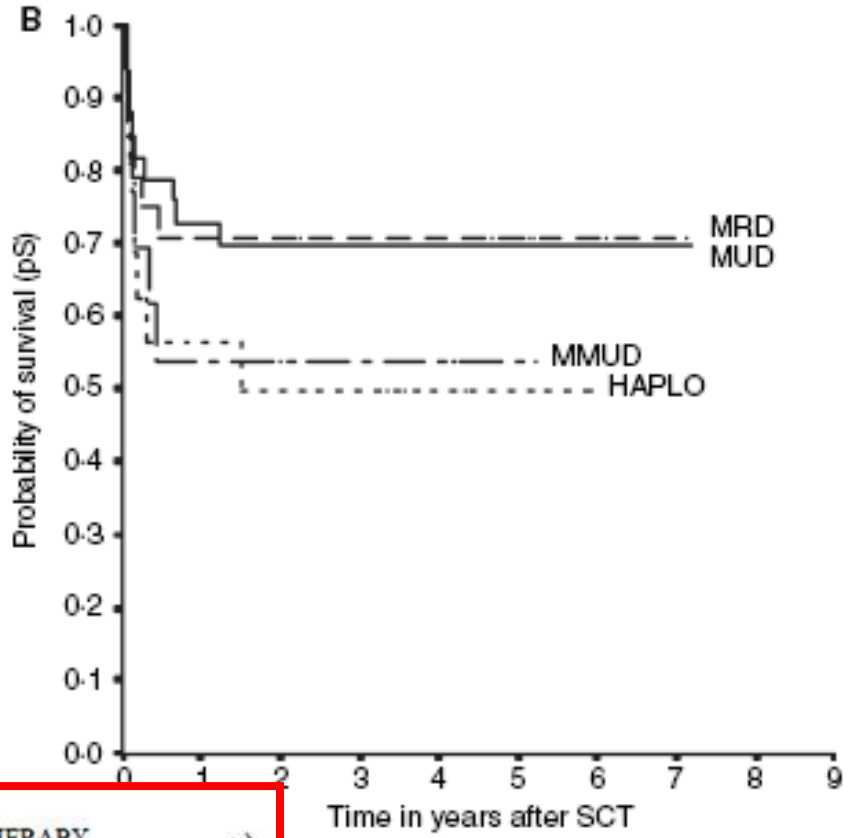
Treatment of HLH

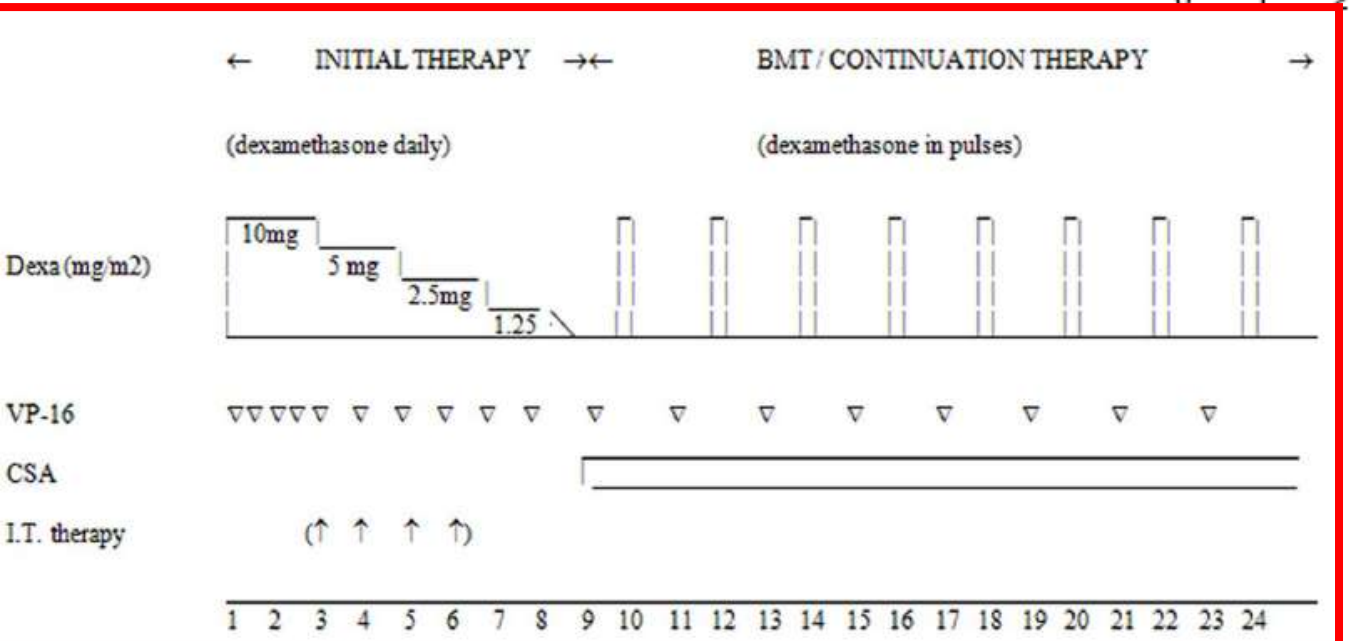
HLH – probability of survival of the historical Registry cohort (n=122)



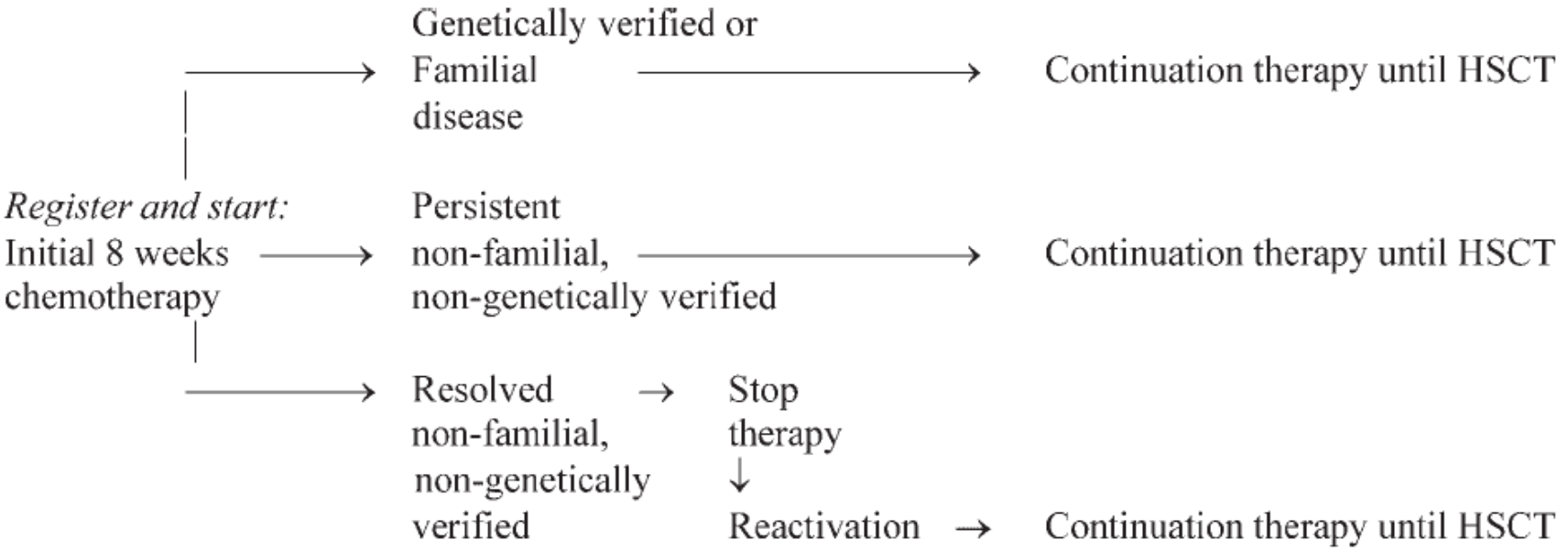
Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation

Jan-Inge Henter, AnnaCarin Samuelsson-Horne, Maurizio Aricò, R. Maarten Egeler, Göran Elinder, Alexandra H. Filipovich, Helmut Gadner, Shinsaku Imashuku, Diane Komp, Stephan Ladisch, David Webb, and Gritta Janka, for the Histiocyte Society

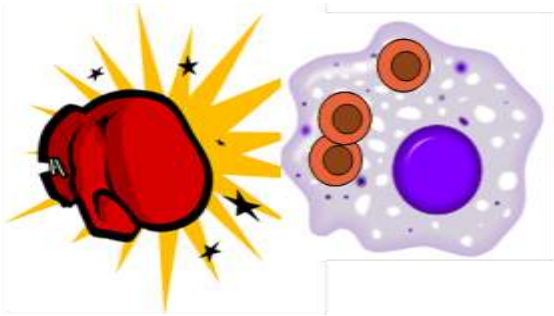


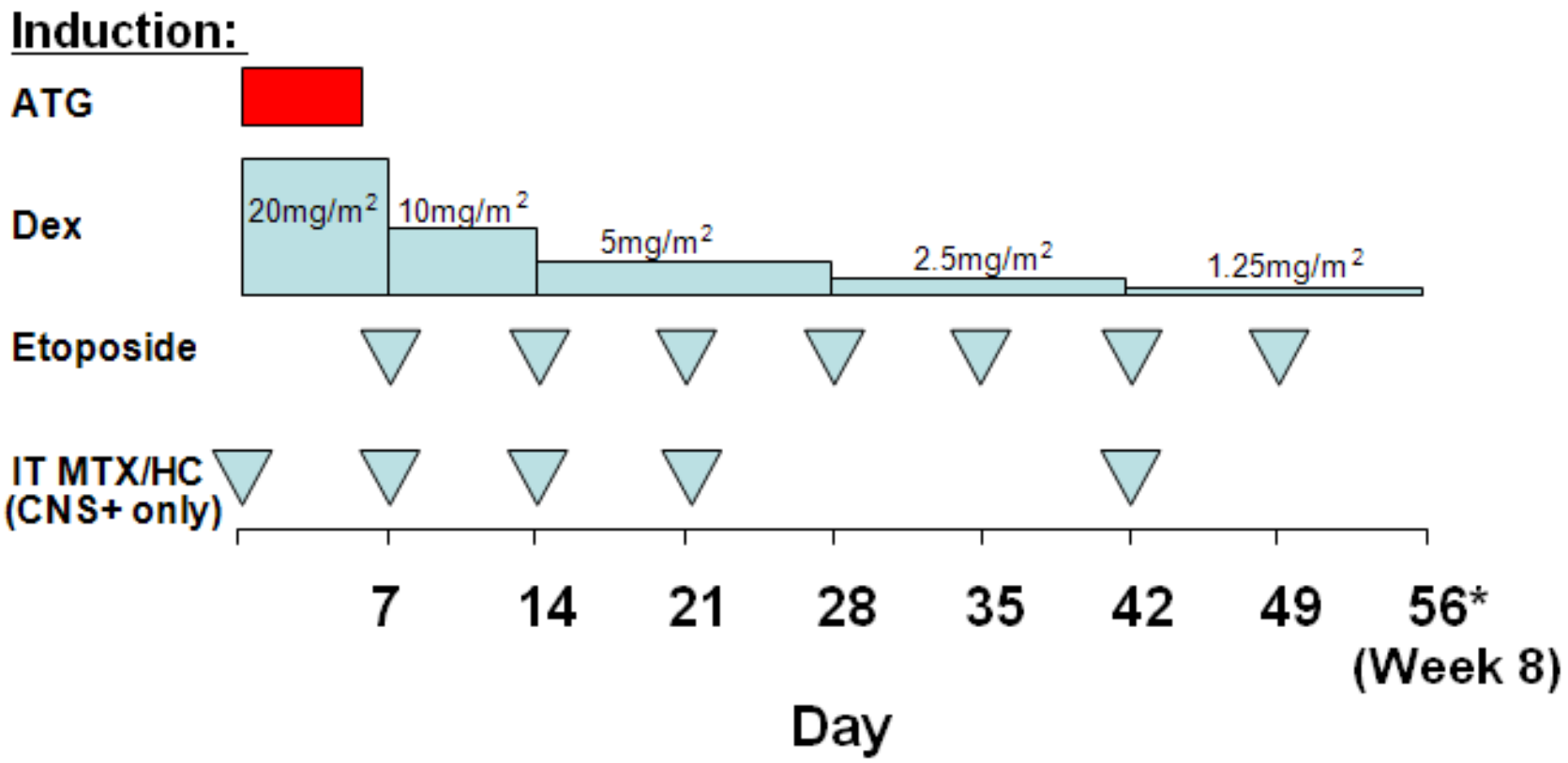


HLH: SCT for everybody?



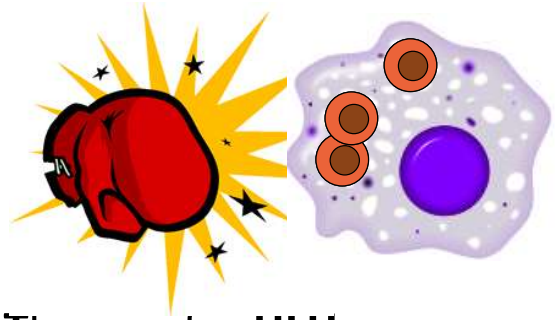
EURO-HIT-HLH




Induction:
ATG
Dex
20mg/m2
10mg/m2
5mg/m2
2.5mg/m2
1.25mg/m2
Etoposide
IT MTX/HC
(CNS+ only)
7
14
21
28
35
42
49
56*
(Week 8)
Day

EURO-HIT-HLH

EUDRACT number 2011-002052-14



European cooperative study for testing Hybrid **I**mmuno**T**herapy for **HLH**

PARTICIPATING COUNTRIES & Reference Centres

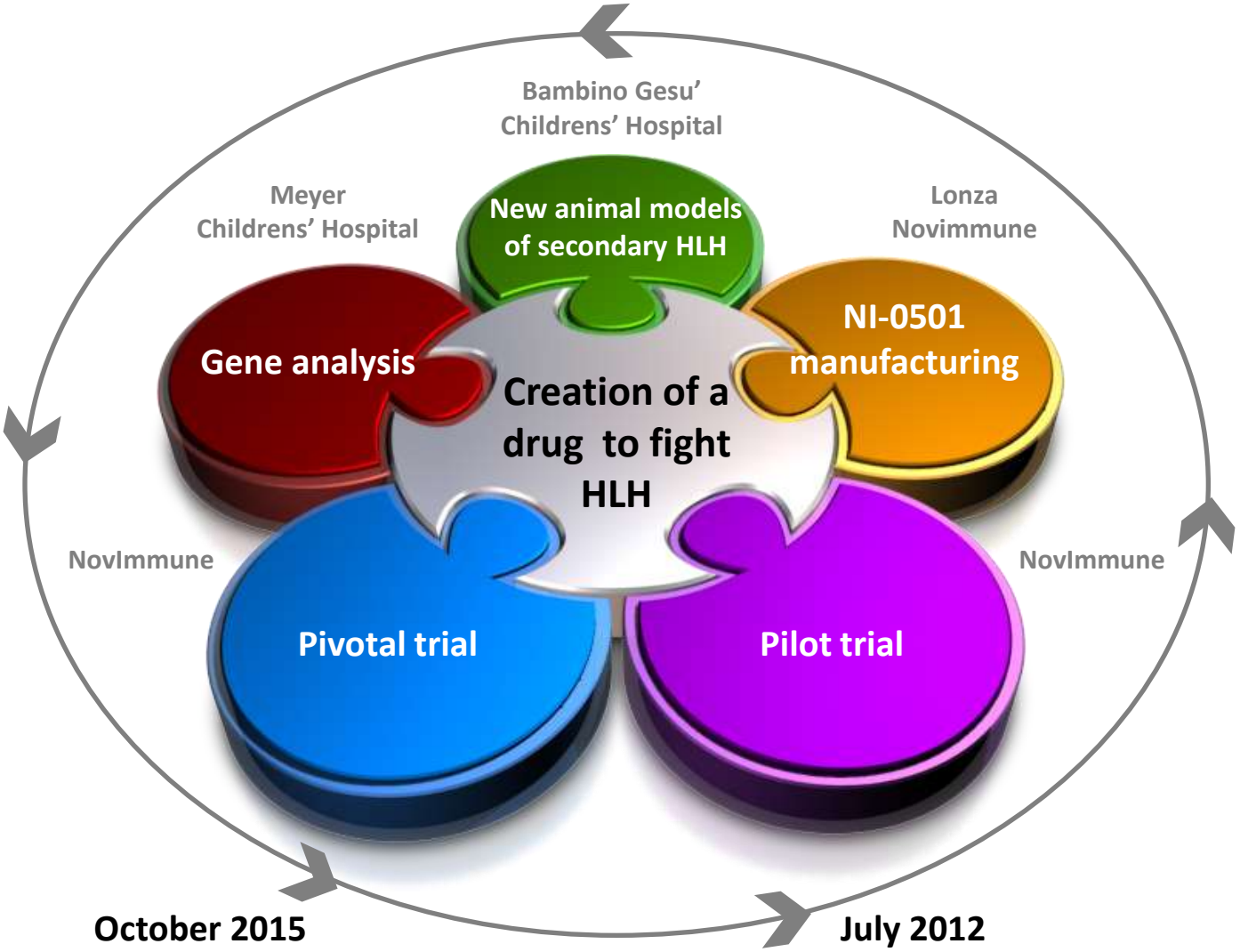
- **Italy, PI M.Aricò (open in xx centres)**
- **Czech Republic, Prague** (open)
- **Germany, Hamburg (start Nov 2013)**
- **Spain, Bilbao (start Oct. 2013)**
- **Austria, Wien (start Nov 2013)**

The study is run in parallel with the **HIT-HLH** chaired in Cincinnati by M.Jordan with L. Filipovich

HLH:
what's next?

"FIGHT HLH"

An FP7 grant awarded to NovImmune, July 1, 2012. 6 M Euros



Perforin (prf) KO mice: a model of primary HLH

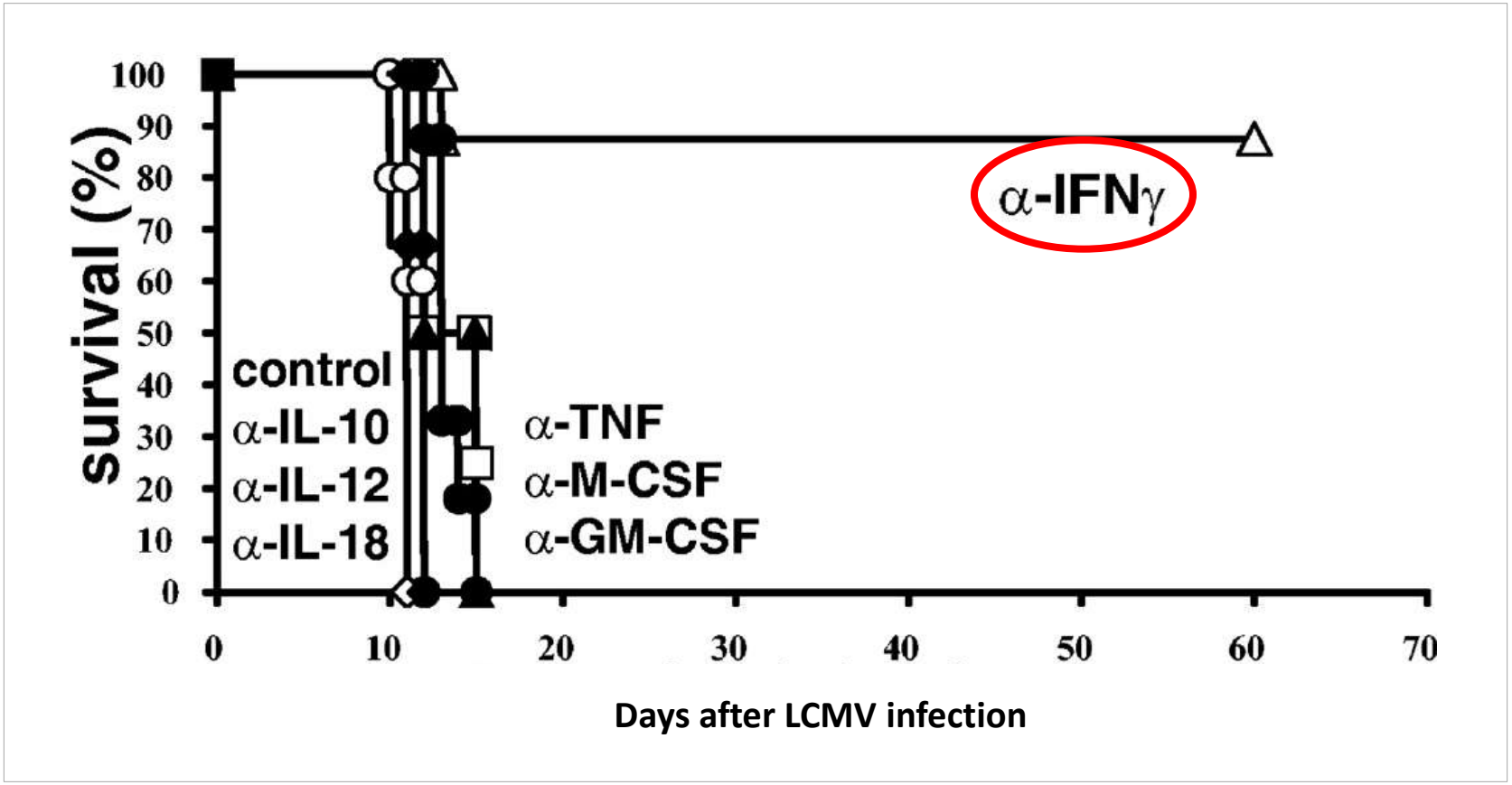
- Mice are healthy
- 100% mortality after experimental infection with lymphocytic choriomeningitis virus (LCMV)

– Clinical and laboratory features:
  – Fever
  – Splenomegaly
  – Cytopenia
  – Hyperferritinemia, hypertriglyceridemia, hypofibrinogenimia
  – Haemophagocitosis
  – Lymphohistiocytic infiltrate, bone marrow hypoplasia, meningeal infiltrate
  – Hypercytokinemia

Jordan et al, Blood 2004

Pachlopnik-Schmid et al, EMBO Mol Med 2009

IFNγ blockade leads to survival of prf KO mice



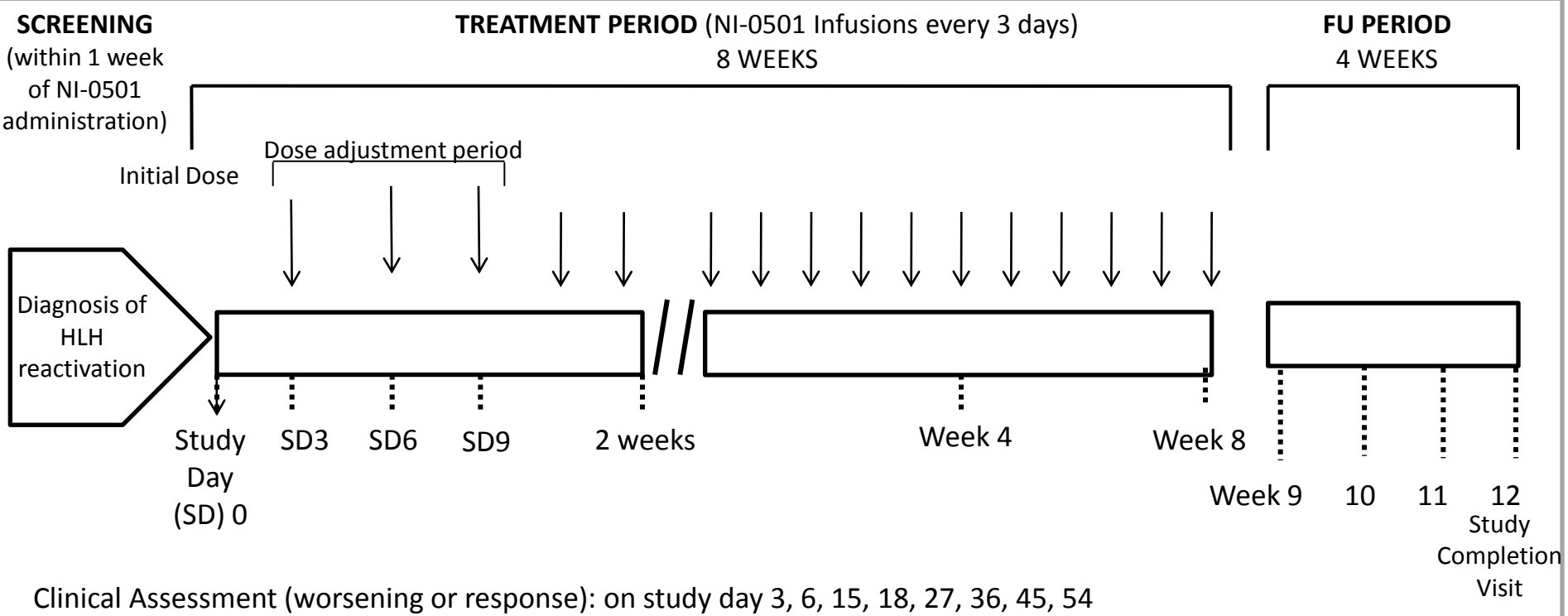
Jordan et al, Blood 2004

NI-0501 pilot study design

EudraCT : *2012-003632-23, approved IRB AOU Meyer 08.10.2012*

Study PI: M. Aricò, Florence

14 Centres: Italy (n=7), Germany (Munster), UK (GOS), Spain (n=3), Austria, Czech



Clinical Assessment (worsening or response): on study day 3, 6, 15, 18, 27, 36, 45, 54 as well as at week 9, 11, 12 and on unscheduled visits (if any)

⚠ At any time during the study, if the patient's condition is worsening, s/he will be withdrawn (rescue therapy)

HLH Registry and studies

Primary participants: Italian AIEOP centers

Main cooperations:

Genova CBA/Gaslini:	D.Pende, L.Moretta
Boston:	L.D.Notarangelo
Cambridge:	G.M.Griffiths
London:	K.Gilmour
Rome O.P. B. Gesù:	F.De Benedetti

MEYER

Azienda Ospedaliero-Universitaria

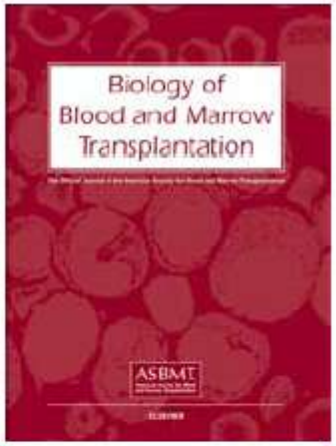
Valentina Cetica, Elena Sieni

B.Ciambotti, M.L.Coniglio, M.DaRos

PERFORIN

HLH, last minute news!!!

Accepted Manuscript



An intermediate alemtuzumab schedule reduces the incidence of mixed chimerism following reduced intensity conditioning hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis

Rebecca A. Marsh, Mi-Ok Kim, Chunyan Liu, Denise Bellman, Laura Hart, Michael Grimley, Ashish Kumar, Sonata Jodele, Kasiani C. Myers, Sharat Chandra, Tom Leemhuis, Parinda Mehta, Jack J. Bleesing, Stella M. Davies, Michael B. Jordan, Alexandra H. Filipovich

PII: S1083-8791(13)00394-7

DOI: 10.1016/j.bbmt.2013.09.001

Reference: YBBMT 53192

To appear in: *Biology of Blood and Marrow Transplantation*

Received Date: 14 June 2013

Accepted Date: 2 September 2013

SCT: a «really final» solution for everybody?

- We describe large-scale changes in the intestinal and renal epithelium causing severe osmotic diarrhea and renal proximal tubular dysfunction even after successful treatment of HLH by HSCT.

- Clinical manifestations in FHL5 patients despite successful HSCT may therefore be related to defective membrane trafficking in the gut and kidney.

Persistent defective membrane trafficking in epithelial cells of patients with FHL-5.
Stepensky et al., PBC, in press

Perforina e…….

- Fenotipi varia
- Frequenza di
- Modello anin

A proportion of patients with lymphoma may harbor mutations of the perforin gene

Rita Clementi, Franco
Cesare Danesino, Mar

Genes and Immunity (2008), 1–7
© 2008 Nature Publishing Group All rights reserved 1466-4879/08 $30.00
www.nature.com/gene

Perforin mutations
strated in a proporti
nosed with the fam
phagocytic lymphohi
the present study, w
some patients with
clinical characteristi
harbor mutations of t
analyzed 29 patient
patients, who develo
or non-Hodgkin lym
mutations of the perforin gene. One of

ORIGINAL ARTICLE
Variation
sclerosis

G Cappellano[1,1]
A Cometa[4], R C
FR Guerini[6], D C
F Monaco[1,2] and

Acute Lymphoblastic Leukemia

A single amino acid change A91V in perforin: a novel, frequent predisposing factor to childhood acute

ARTHRITIS & RHEUMATISM
Vol. 58, No. 2, February 2008, pp 567–570
DOI 10.1002/art.23199
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Mutations of the
Hemophagocytic Lymphohistiocytosis–Associated Gene *UNC13D*
in a Patient With Systemic Juvenile Idiopathic Arthritis

Melissa M. Hazen,[1] Amy L. Woodward,[2] Inga Hofmann,[3] Barbara A. Degar,[3] Alexei Grom,[4] Alexandra H. Filipovich,[4] and Bryce A. Binstadt[5]

haematologica 2005; 90:697-698
http://www.haematologica.org/journal/2005/5/697.html)

carrier of a *PRF1* mutation compared with healthy controls, suggesting a possible predisposing role. *Cancer* 2007;109:2566–71. © 2007 American Cancer Society.

MONOALLELIC MUTATIONS OF THE PERFORIN GENE MAY REPRESENT A PREDISPOSING FACTOR TO CHILDHOOD ANAPLASTIC LARGE CELL LYMPHOMA

Ciambotti B. Dr[1], Mussolin L. PhD[2,3], D'Amore E.S.G. MD[4], Pillon M. MD[3], Sieni E. MD[1], Coniglio M.L. Dr[1], Da Ros M. Dr[1], Cetica V. PhD[1], Aricò M MD[1], Rosolen A MD[3,5].

1.U.O. Oncoematologia Pediatrica, A.O.U. Meyer, Firenze, Italia.
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3.Hematoly and Oncolgy Clinic, Department of Pediatrics , Padua Italy
4.Department of Pathology, San Bortolo Hospital, Vicenza, Italy
5.Department of Pediatrics, University of Udine, Udine Italy

Case No.	Mutation	In silico	Age/Sex	IHC
1	c.272C>T p.A91V*	Prob. Dam	7F	3+
3	c.272C>T p.A91V*	Prob. Dam	11F	N.P.
4	c.368G>A p.R123H	Poss. Dam.	14M	N.P.
10	c.272C>T p.A91V*	Prob. Dam	8F	2+
17	c.272C>T p.A91V*	Prob. Dam	10M	3+
24	c.272C>T p.A91V*	Prob. Dam	11F	N.P.
26	c.272C>T p.A91V*	Prob. Dam	7M	3+
27	c.529C>T p.R177C	Prob. Dam	1M	N.P.
28	c.1741G>A p.D491N	Prob. Dam	12M	N.P.
40	c.1262T>G p.F421C**	Benign	13M	N.P.
43	c.272C>T p.A91V*	Prob. Dam	10M	2+
44	c.272C>T p.A91V* c.695G>A p.R232H*	Prob. Dam Prob. Dam	13F	N.P.
72	c.82C>T p.R28C***	Prob. Dam	5F	N.P.
49	c.272C>T p.A91V*	Prob. Dam	2M	3+
61	c.272C>T p.A91V*	Prob. Dam	4M	N.P.
65	c.272C>T p.A91V*	Prob. Dam	6M	N.P.
70	c.368G>A p.R123H	Poss. Dam.	2M	1+
46	c.632C>T p.A211V*	Benign	2M	N.P.
52	c.755A>G p.N252S*	Benign	4F	N.P.
54	c.755A>G p.N252S*	Benign	8M	N.P.
69	c.1349C>T p.T450M*	Prob. Dam	6M	1+

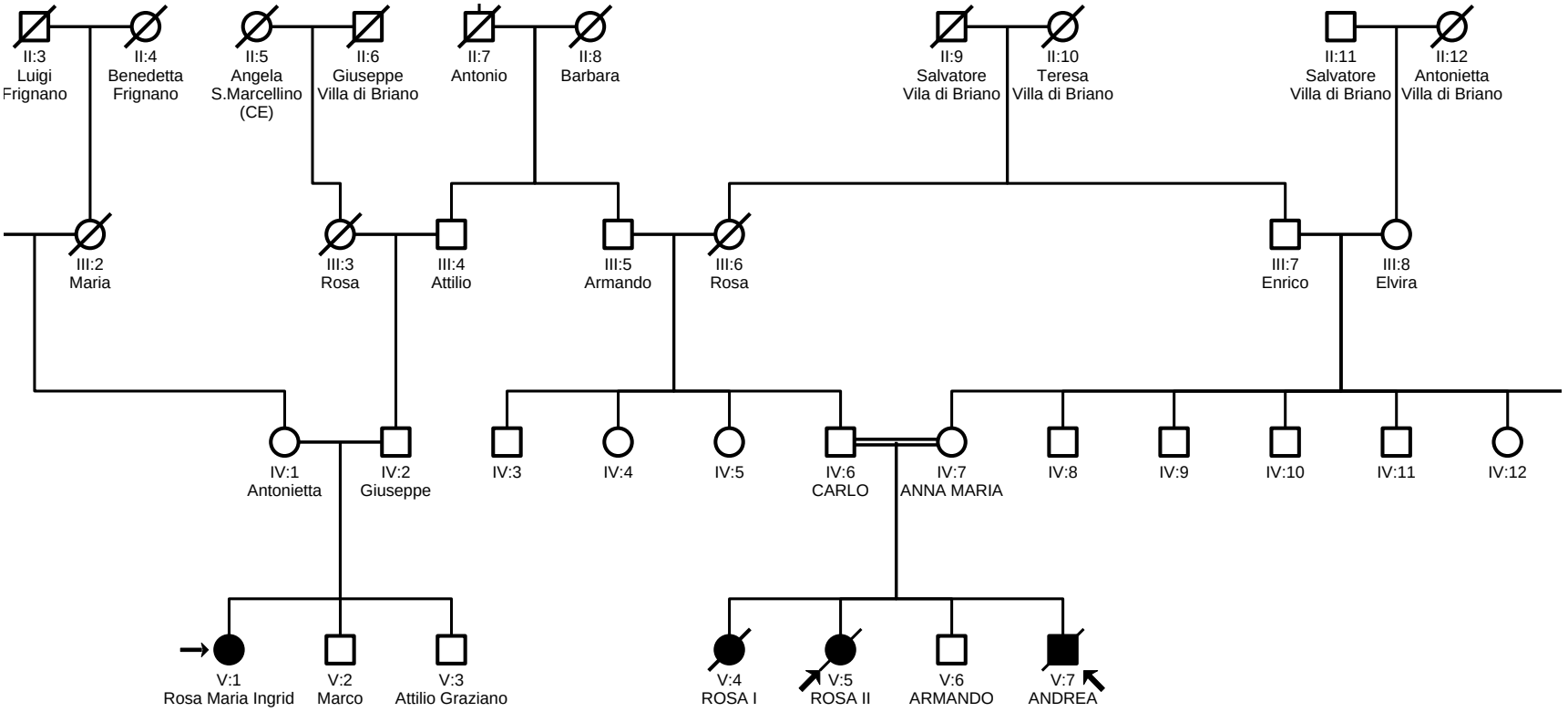
UNC13D

In totale sono state identificate 2 varianti missenso in 2 pazienti (2.3%).

Case No.	Mutation	In silico	Age/Sex	IHC
35	c.1555A>T p.I519F***	Prob. Dam	6F	N.P.
66	c.2191G>A p.V731M*	Prob. Dam	4F	N.P.

La mutazione c.2191G>A, p.V731M è stata precedentemente riportata in pazienti con FHL3.

FHL, LCH…….? Are we sure enough?



Riv. Ital. Ped. (I.J.P.), 11, 421-424, 1985

La linfoistiocitosi familiare eritrofagocitica. Descrizione di due nuovi casi clinici

The erythrophagocitic lymphoistiocitosis. Description of two new cases

A. Verrotti, M. R. Sisto, G. Bracali, M. Ramenghi

Divisione di Pediatria
Divisione di Pediatria Neonatale
Servizio di Istologia e Anatomia Patologica
Ospedale Civile di Pescara

FAMILIAL AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME CAUSED BY HOMOZYGOUS FAS LIGAND (FASLG) MUTATION MIMICKING FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS.

Valentina Cetica[1], Elena Sieni[1], Maria Luisa Coniglio[1], Martina Da Ros[1], Paolo Di Bartolomeo[2], Giulia Sindici[3], Maurizio Aricò[1,4]

Pediatrics. 2013 Sep 16. [Epub ahead of print]

Mutation of FAS, XIAP, and UNC13D Genes in a Patient With a Complex Lymphoproliferative Phenotype.

Boggio E, Aricò M, Melensi M, Dianzani I, Ramenghi U, Dianzani U, Chiocchetti A.

Interdisciplinary Research Center of Autoimmune Diseases and Department of Health Sciences, "A. Avogadro" University of Eastern Piedmont, Novara, Italy;

Abstract

This article presents a case report for a child presenting with mixed clinical features of autoimmune lymphoproliferative syndrome (ALPS), familial hemophagocytic lymphohistiocytosis (FHL), and X-linked lymphoproliferative (XLP) disease. From 6 months, he exhibited splenomegaly and lymphoadenopathy and from 4 years, he showed recurrent severe autoimmune hemocytopenia and sepsislike bouts of fever, from which he eventually died at the age of 12. Intriguingly, the patient carried mutations in FAS, XIAP, and UNC13D genes, which are involved in ALPS, XLP disease, and FHL, respectively. These mutations were inherited from the mother, who had rheumatoid arthritis but no signs of ALPS. A role for other modifying genes was suggested by the finding that the healthy father exhibited defective Fas function, without mutation of the FAS gene, and had transmitted to the patient an osteopontin (OPN) gene variant previously associated with ALPS. Therefore, several genes might influence the disease outcome in this family. In vitro analyses revealed that the FAS and the XIAP mutations decreased expression of the corresponding proteins, and the UNC13D mutation decreased granule secretion and Munc interaction with Rab-27a. These findings suggest that overlap may exist between ALPS, FHL, and XLP disease, in accordance with the notion that FHL and XLP disease are due to defective natural killer (NK)/NK T-cell function, which involves Fas. Therefore, we propose that NK cell defects should be evaluated in patients with ALPS-like characteristics, and hematopoietic stem cell transplantation should be considered in individuals with severe refractory cytopenia and FHL-like manifestations.

KEYWORDS: ALPS, FAS, FHL, MUNC13-4, XIAP, XLP

PMID: 24043286 [PubMed - as supplied by publisher]

OPEN ACCESS Freely available online

PLOS ONE

Variations of the *UNC13D* Gene in Patients with Autoimmune Lymphoproliferative Syndrome

Maurizio Aricò[1], Elena Boggio[2,3], Valentina Cetica[1], Matteo Melensi[2,3], Elisabetta Orilieri[2,3], Nausicaa Clemente[2,3], Giuseppe Cappellano[2,3], Sara Buttini[2,4], Maria Felicia Soluri[2,3], Cristoforo Comi[2,4], Carlo Dufour[5], Daniela Pende[6], Irma Dianzani[2,3], Steven R. Ellis[7], Sara Pagliano[8], Stefania Marcenaro[5], Ugo Ramenghi[8], Annalisa Chiocchetti[2,3]*, Umberto Dianzani[2,3]

1 Department of Pediatric Hematology Oncology, Meyer Children Hospital, Firenze, Italy, **2** Interdisciplinary Research Center of Autoimmune Diseases (IRCAD), "A. Avogadro" University of Eastern Piedmont, Novara, Italy, **3** Department of Health Sciences, "A. Avogadro" University of Eastern Piedmont, Novara, Italy, **4** Department of Translational Medicine, "A. Avogadro" University of Eastern Piedmont, Novara, Italy, **5** Istituto Giannina Gaslini, Genova, Italy, **6** IRCCS AOU San Martino-IST, Genova, Italy, **7** Department of Biochemistry and Molecular Biology, University of Louisville, Louisville, Kentucky, United States of America, **8** Department of Pediatrics, University of Torino, Torino, Italy

Abstract

Autoimmune lymphoproliferative syndro
characterized by lymphadenopathy/sple
expansion is absent in the ALPS varia
addition to the causative mutations, the g
suggested a disease-modifying role for
(FHL). The *UNC13D* gene codes for Mu
thus, another candidate for a disease-mo
ALPS and 20 DALD patients and compa
multiple sclerosis (MS) patients. We de
carrying p.Cys112Ser, p.Val781Ile, and
the mutant cDNAs into HMC-1 cells sh
construct. An additional rare missense v
did not decrease Munc13-4 function. Th
factors for ALPS development.

Table 1. Gene variations detected in patients with ALPS or DALD.

Patients (gender)	Diagnosis	Fas function*			*FAS*		*UNC13D*	
	Pt	Pt	F	M	Variation	Inh†	Variation	Inh†
Pt.1 (female)	ALPS-FAS	D	D	N	p.Gln273His	F	p.Arg928Cys	M
					(c.819G>C)		(c.2782C>T)	or F‡
Pt.2 (male)	ALPS-FAS	D	ND	ND	p.Glu261Lys	F	p.Cys112Ser	M
					(c.755G>A)		(c.335C>G)	
Pt.3 (female)	ALPS-U	D	D	D			p.Ala59Thr	M
							(c.175G>A)	
Pt.4 (male)	ALPS-U	D	D	D			p.Ile848Leu	M
							(c.2542A>C)	
							p.Ala995Pro	M
							(c.2983G>C)	
Pt.5 (female)	ALPS-U	D	ND	ND			p.Val781Ile	ND
							(c.2342G>A)	
Pt.6 (male)	DALD	D	D	D			p.Ala59Thr	F
							(c.175G>A)	
Pt.7 (female)	DALD	D	D	D			p.Arg928Cys	M
							(c.2782C>T)	
Pt.8 (male)	DALD	D	D	ND			p.Arg928Cys	ND
							(c.2782C>T)	

* D = defective, N = normal, Pt = patient, F = father, M = mother

† Inheritance, F = father, M = mother; ND = not determined; no parent displayed ALPS, DALD, XLP, or FHL; Pt.1's mother had rheumatoid arthritis.

‡ both parents carried the variation.

Molecular basis of familial hemophagocytic lymphohistiocytosis

Valentina Cetica,[1] Daniela Pende,[2] Gillian M. Griffiths,[3] Maurizio Aricò[1]

[1]Department Pediatric Hematology Oncology, Azienda Ospedaliero-Universitaria Meyer, Florence, Italy. [2]Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy; [3]Cambridge Institute for Medical Research, Addenbrooke's Hospital, Cambridge, CB2 0XY, UK
E-mail: m.arico@meyer.it. *doi:10.3324/haematol.2009.019562*

Table 1. OMIM classification of familial hemophagocytic lymphohistiocytosis.

267700: Hemophagocytic lymphohistiocytosis, familial, 1; FHL1 (gene map locus 9q21.3-q22)
603553: Hemophagocytic lymphohistiocytosis, familial, 2; FHL2 (gene map locus 10q22)
608898: Hemophagocytic lymphohistiocytosis, familial, 3; FHL3 (gene map locus 17q25.1)
603552: Hemophagocytic lymphohistiocytosis, familial, 4; FHL4 (gene map locus 6q24)
613101: Hemophagocytic lymphohistiocytosis, familial, 5; FHL5

Based on current knowledge, it seems that there are at least two additional FHL-related genes to be identified: one (or more) could explain the subset of patients with defective NK activity and an unassigned defect, while at least one more gene is expected to underlie FHL in a subset of families with normal NK activity (*M. Aricò; unpublished data*). In this regard, functional tools, such as the degranulation assay, confocal microscopy and protein studies, appear to be of paramount importance for pinpointing novel defects. Beyond providing a confirmation of the diagnosis and a genetic marker for family studies, the information gained during the investigation of patients with FHL has turned out to be of great value in improving our knowledge of some aspects of the immune system.

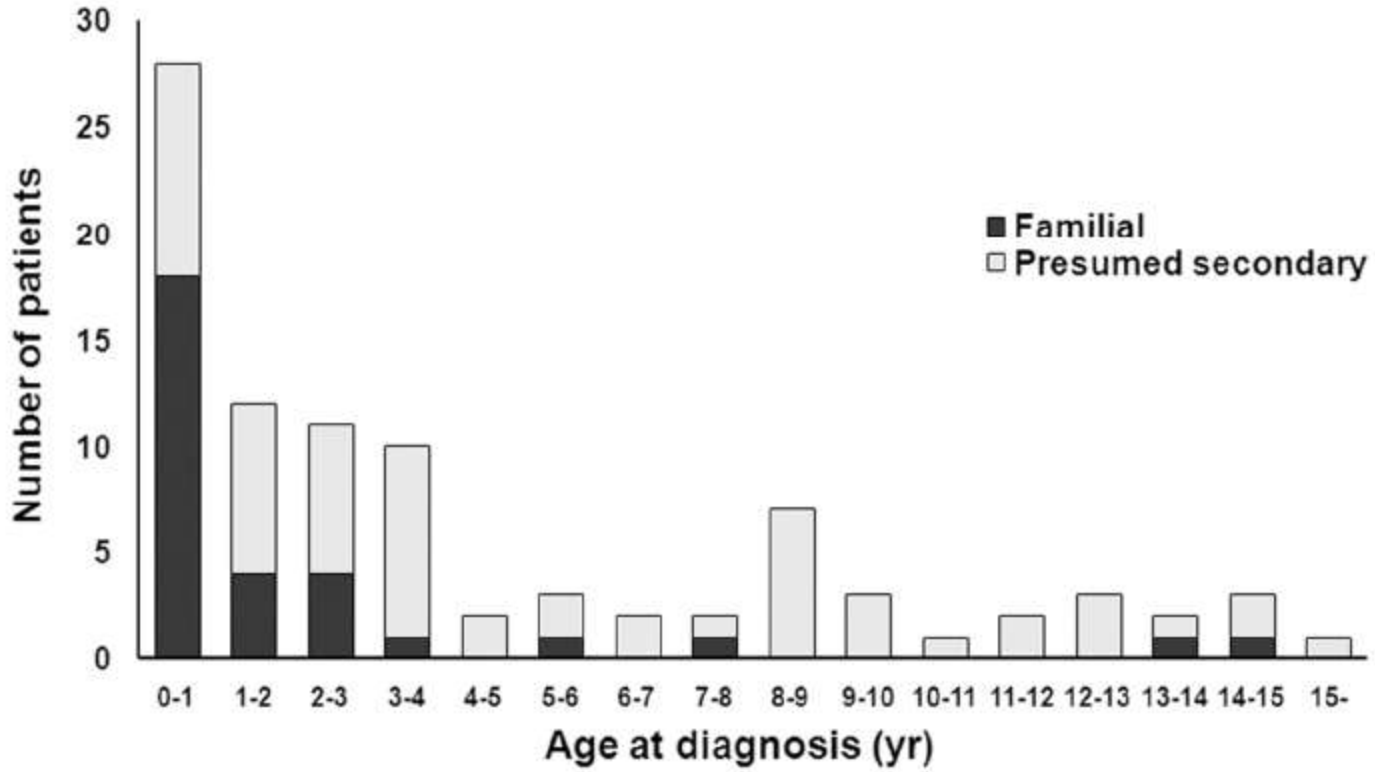


Fig. 1. Distribution of familial and presumed secondary HLH by age for 92 classifiable patients.
73x41mm (300 x 300 DPI)

Macrophage activation syndrome (MAS)

- A serious, potentially fatal complication of rheumatic diseases seen most frequently in systemic juvenile idiopathic arthritis (sJIA) and in its adult equivalent, adult-onset Still disease

- Increasingly reported in other pediatric inflammatory disorders: juvenile SLE, Kawasaki disease, periodic fever syndromes.

- MAS is similar to the autosomal recessive disorders collectively known as familial hemophagocytic lymphohistiocytosis (FHL)

Genes and Immunity (2012) **13,** 289 - 298

www.nature.com/gene

REVIEW

Macrophage activation syndrome as part of systemic juvenile idiopathic arthritis: diagnosis, genetics, pathophysiology and treatment

A Ravelli[1], AA Grom[2], EM Behrens[3] and RQ Cron[4]

Table 1. Main clinical, laboratory and pathological features of macrophage activation syndrome

Clinical features
Non-remitting high fever
Hepatomegaly
Splenomegaly
Lymphadenopathy
Hemorrhages
Central nervous system dysfunction
Laboratory features
Cytopenia
Abnormal liver function tests
Coagulopathy
Decreased erythrocyte sedimentation rate
Hypertriglyceridemia
Hyponatremia
Hypoalbuminemia
Hyperferritinemia
Elevated sCD25 and sCD163
Histopathological features
Macrophage hemophagocytosis in the bone marrow
Increased CD163 staining of the bone marrow

Repeated TLR9 stimulation results in macrophage activation syndrome–like disease in mice

Edward M. Behrens,[1] Scott W. Canna,[1] Katharine Slade,[1] Sheila Rao,[2] Portia A. Kreiger,[3] Michele Paessler,[4] Taku Kambayashi,[5] and Gary A. Koretzky[6,7]

[1]Division of Rheumatology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA. [2]Immunology Graduate Group, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [3]Department of Pathology, Alfred I. duPont Hospital for Children, Wilmington, Delaware, USA. [4]Department of Pathology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA. [5]Department of Pathology, [6]Department of Medicine, and [7]Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Hemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (MAS) are 2 similar diseases characterized by a cytokine storm, overwhelming inflammation, multiorgan dysfunction, and death. Animal models of HLH suggest that disease is driven by IFN-γ produced by $CD8^+$ lymphocytes stimulated by persistent antigen exposure. In these models and patients with "primary" HLH, the antigen persists due to genetic defects, resulting in ineffective cytotoxic responses by $CD8^+$ T cells and poor pathogen clearance. However, infectious triggers are often not identified in patients with MAS, and some patients with HLH or MAS lack defects in cytotoxic T cell killing. Herein, we show that repeated stimulation of TLR9 produced an HLH/MAS-like syndrome on a normal genetic background, without exogenous antigen. Like previous HLH models, TLR9-induced MAS was IFN-γ dependent; however, unlike other models, disease did not require lymphocytes. We further showed that IL-10 played a protective role in this model and that blocking IL-10 signaling led to the development of hemophagocytosis. IL-10 may therefore be an important target for the development of effective therapeutics for MAS. Our data provide insight into MAS-like syndromes in patients with inflammatory diseases in which there is chronic innate immune activation but no genetic defects in cytotoxic cell function.

Arthritis Rheum. 2012 May;64(5):1680-8. doi: 10.1002/art.33496.

Amplification of the response to Toll-like receptor ligands by prolonged exposure to interleukin-6 in mice: implication for the pathogenesis of macrophage activation syndrome.

Strippoli R, Carvello F, Scianaro R, De Pasquale L, Vivarelli M, Petrini S, Bracci-Laudiero L, De Benedetti F.

Ospedale Pediatrico Bambino Gesù, Rome, Italy.

Abstract

OBJECTIVE: To investigate whether prolonged exposure to interleukin-6 (IL-6) affects the inflammatory response induced by Toll-like receptor (TLR) ligands.

METHODS: IL-6-transgenic mice and wild-type mice were stimulated with different TLR ligands; survival rates, blood cell counts, and biochemical parameters were analyzed. Murine splenic mononuclear cells and peritoneal macrophages were stimulated with lipopolysaccharide (LPS), lipoteichoic acid, poly(I-C), or CpG. Human macrophages were cultured for 4 days in the presence of IL-6 and then stimulated with LPS. Inflammatory cytokine expression was measured by enzyme-linked immunosorbent assay or reverse transcription-polymerase chain reaction. Activation of STAT-3, ERK-1/2 (MAPK), and p65 NF-κB was evaluated by Western blotting or confocal analysis.

RESULTS: Treatment of IL-6-transgenic mice with TLR ligands led to an increased fatality rate and elevated levels of IL-1β, tumor necrosis factor α (TNFα), IL-6, and IL-18. Macrophages from IL-6-transgenic mice produced increased levels of inflammatory cytokines, which were associated with increased phosphorylation of STAT-3 and ERK-1/2 and with increased NF-κB nuclear translocation. Human macrophages treated with IL-6 and then stimulated with LPS showed elevated levels of cytokines and similarly elevated signaling pathway activation. After LPS administration, IL-6-transgenic mice showed an increase in ferritin and soluble CD25 levels, as well as a decrease in platelet and neutrophil counts and in hemoglobin levels compared to wild-type mice.

CONCLUSION: Our findings indicate that prolonged exposure to IL-6 in vivo and in vitro leads to an exaggerated inflammatory response to TLR ligands. Hematologic and biochemical abnormalities in IL-6-transgenic mice treated with LPS show striking similarities to macrophage activation syndrome.

HLH Registry
Patients with MAS (n=47)

Ethnic group:

- Caucasian, n=42; Indian, n= 5

Gender

- M: 32, F: 15; Ratio 2.1

Age at diagnosis

- Median 8 anni
- Range: 1 day – 34 years
- Quartiles: 2 years, 8 years, 12 years

HLH Registry
Patients with MAS (n=47)

	N/total* (%)
Fever	**33/37 (89)**
Splenomegaly	21/37(57)
CNS symptoms	6/32 **(19)**
Anemia	15/25 (60)
Neutropenia	5/23 (22)
Thrombocytopenia	**11/23 (48)**
Hypofibrinogenemia	5/15 **(33)**
Hypertriglyceridemia	17/21 (81)
Hyperferritinemia	**23/24 (96)**
Hemophagocytosis	12/37 (32)
Defective perforin expression°	*7/28 (25)*
Defective GRA°	*6/23 (26)*

* Total of evaluable patients

HLH Registry
Patients with MAS (n=47)

Diagnosis	N
JIA	22
SLE	4
Connectivitis	2
Dermatomiositis	1
“Autoimmune disease”	1
Not futher specified	17

MAS & FHL-related genes mutation

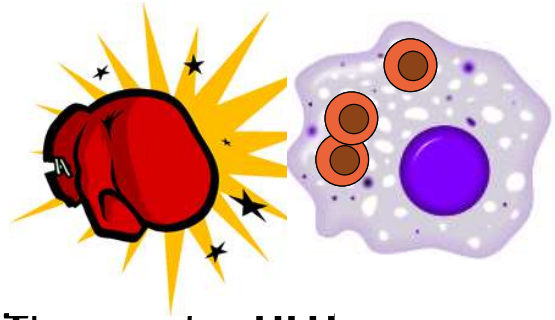
- **10/36 (28%)** patients with MAS have monoallelic mutation

UPN	*Age (mos)	PRF expr	GRA	Mutation	Gene
238	6	ND	NORM	**c.272T (p.A91V)**	*PRF*
429	32	NORM	NORM	c.610A>G (p.M204V)	*UNC-13D*
431	53	ND	ND	**c.272T (p.A91V)**	*PRF*
527	120	NORM	NORM	c.799G>A (p.V267M)	*STX 11*
579	95	**RID**	**RID**	c.1034C>T (p.T345M)	*STXBP2*
654	186	NORM	**RID**	**c.272T (p.A91V)**	*PRF*
661	6	**RID**	**RID**	**c.272T (p.A91V)**	*PRF*
717	106	ND	**RID**	c.1357G>A (p.V453M)	*PRF*
738	40	ND	ND	c.1681T>C (p.W561R)	*STXBP2*
746	1	NORM	NORM	c. 610G>A (p.A210K)	*STXBP2*

***Median age 3,6 years**

EURO-HIT-HLH

EUDRACT number 2011-002052-14



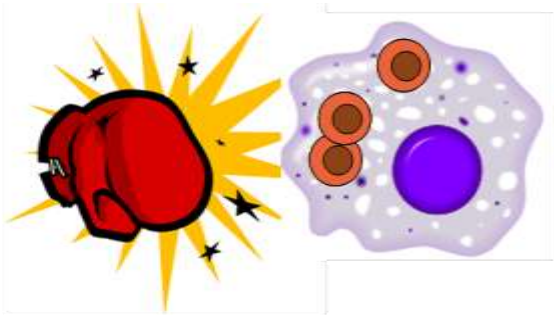
European cooperative study for testing Hybrid **I**mmuno**T**herapy for **HLH**

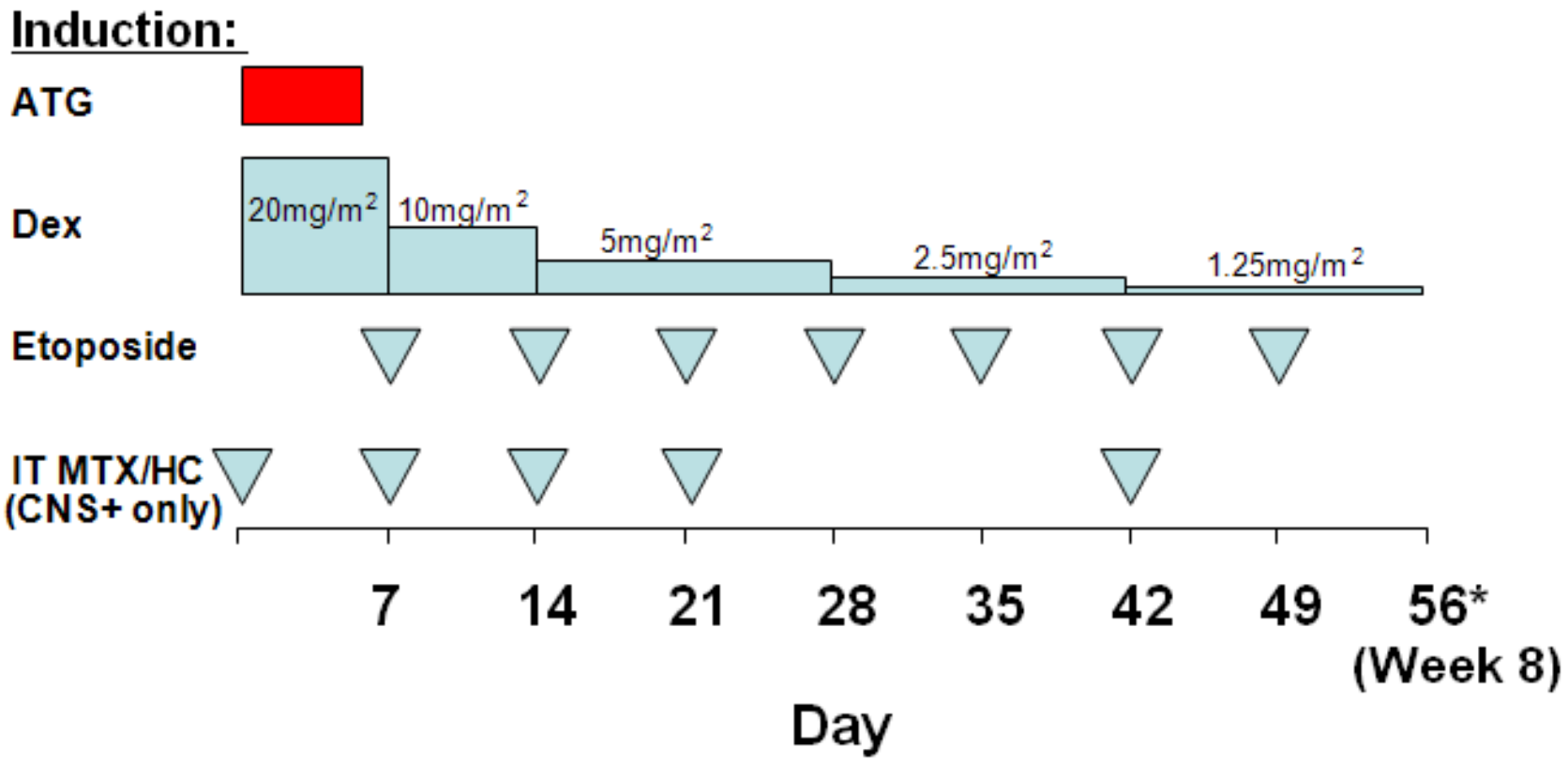
PARTICIPATING COUNTRIES & Reference Centres

- **Italy, PI M.Aricò (open in 10 centres)**
- **Czech Republic, Prague (open)**
- **Germany, Hamburg (open since Nov 2013)**
- **Austria, Wien (open in 4 centers)**
- **Spain, Bilbao (unable to open)**

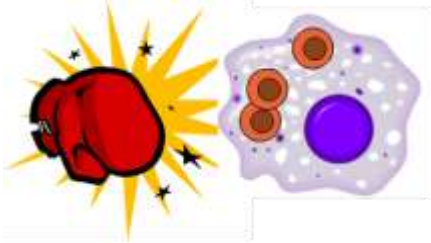
The study is run in parallel with the **HIT-HLH** chaired in Cincinnati by M.Jordan with L. Filipovich

EURO-HIT-HLH




Induction:
ATG
Dex
20mg/m2
10mg/m2
5mg/m2
2.5mg/m2
1.25mg/m2
Etoposide
IT MTX/HC
(CNS+ only)
7
14
21
28
35
42
49
56*
(Week 8)
Day

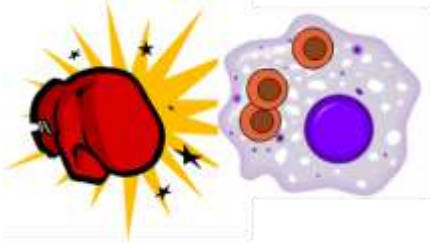
EURO-HIT-HLH: ACCRUAL



http://euro-hit-hlh.unipa.it

- A total of 10 patients have been enrolled
  - Italy: 4 patients
  - Germany: 4 patients
  - Czech Republic/Prague: 2 patients
  - Austria: 0 patients

EURO-HIT-HLH: ACCRUAL



http://euro-hit-hlh.unipa.it

Id	Insert Date	Name	Centre	Status
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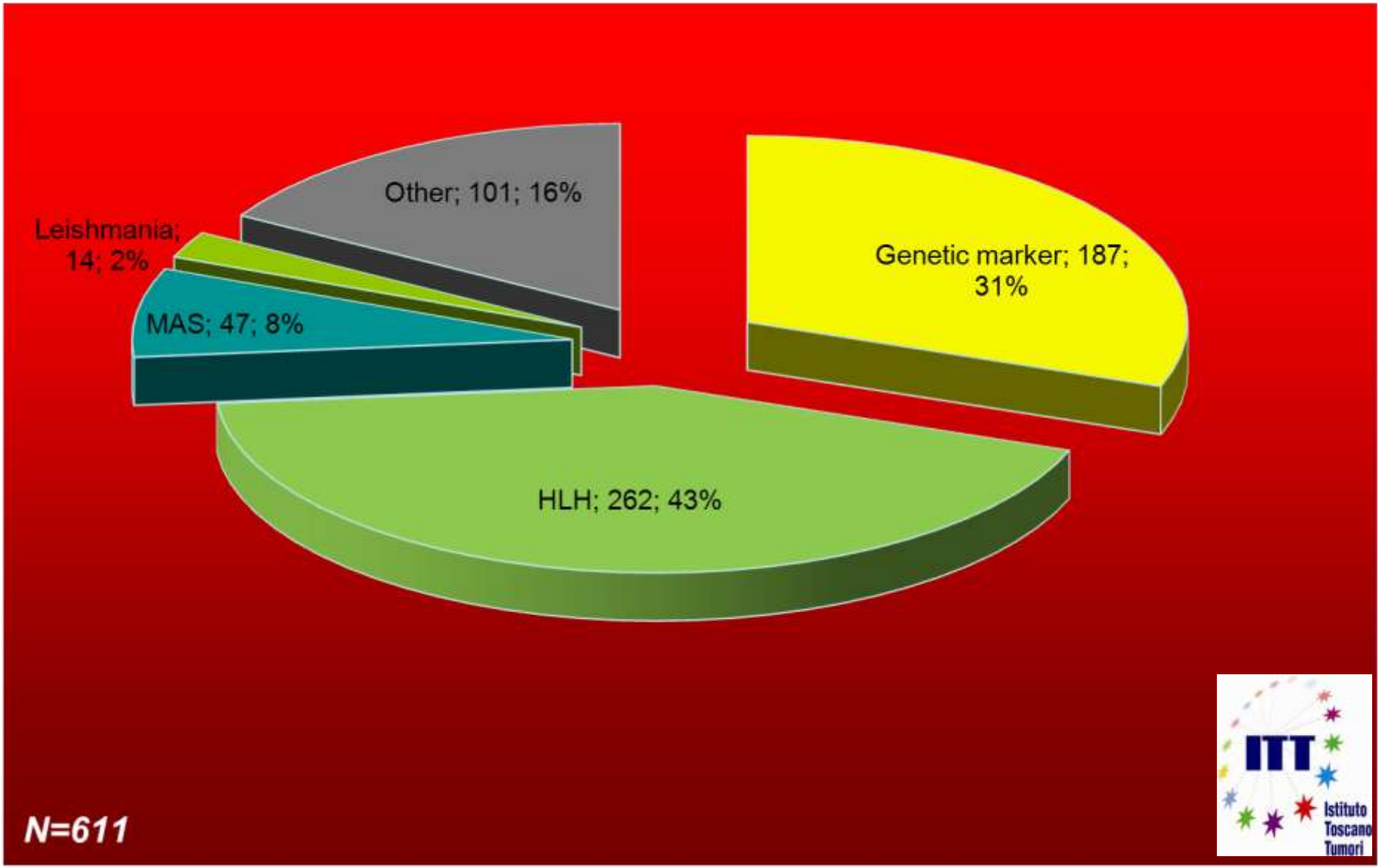
9	14-05-2014	GERMANY003 003	GPHO	valid
8		GERMANY001 002	GPHO	to validate
7	07-05-2014	GERMANY001 001	GPHO	valid
6	20-02-2014	V.C.	Dipartimento di Pediatria Università di Padova	valid
5	11-10-2013	S.L.	S.C. Oncoematologia Pediatria e Centro Trapianti OIRM Torino	valid
4	09-07-2013	B.B.	U.O. Pediatria A.O. Annunziata	valid
3	04-07-2013	N.B.	S.C. Oncoematologia Pediatria e Centro Trapianti OIRM Torino	valid
2	29-05-2013	M.A.	Department Pediatric Hematology-Oncology Charles University Prague and University Hospital Motol	valid
1	14-05-2013	C.M.	Department Pediatric Hematology-Oncology Charles University Prague and University Hospital Motol	valid

The universe of HLH



M.Aricò, unpublished data 2012

HLH: PRIMARY OR "SECONDARY"?

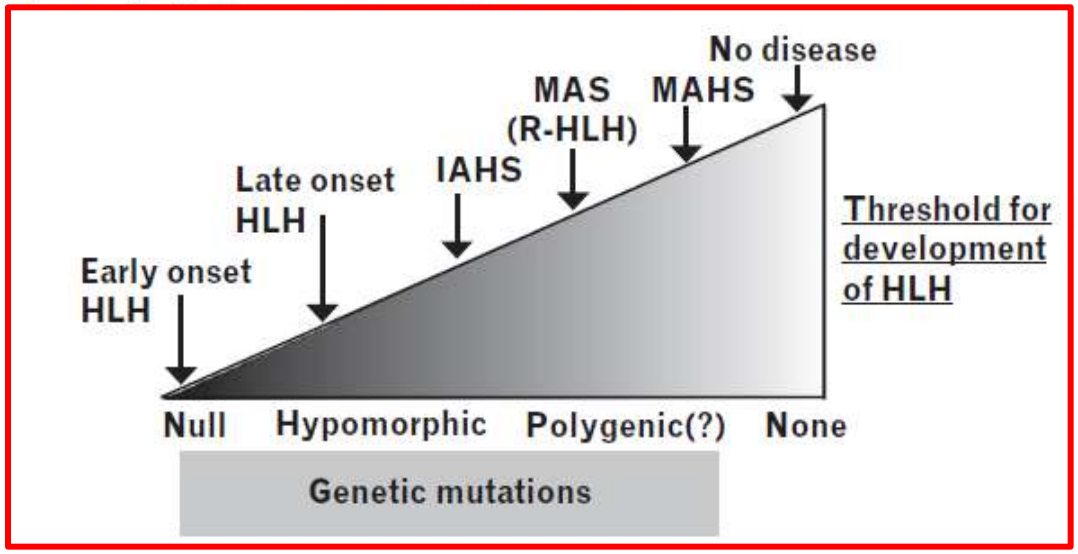
	FHL n (%)	HLH non genetic / secondary (%)	p
Fever	135/186 (72%)	187/289 (64%)	p=0.091
Splenomegaly	135/186 (**72%**)	165/289 (57%)	p=0.000
Thrombocytopenia	127/151 (**84%**)	134/204 (65%)	p=0.000
Ferritin (high) *- Quartiles (mg/L)*	65/82 (79%) 519; 1.639; 7.270; 74.860	81/92 (88%) **1.000; 2.601; 10.213; 140.000**	p=0.172
Fever *+Splenomegaly* *+Thrombocytopenia* *+Ferritin (high)*	**50/79 (63%)**	44/83 (52%)	P=0.244

Sieni E, Cetica V, Ciambotti B, Coniglio M.L., De Benedetti F, De Fusco C, Micalizzi C, Fagioli F, Putti C, Santoro N, Aricò M, in preparation

Evolving concepts in pathophysiology and clinical definition of HLH: a continuum of risk

- Due to the increasingly blurred distinction between primary and secondary HLH, the clinical spectrum of HLH appears as a continuum of risk
- Genetic variance in genes that cause HLH, depending on their types and penetrance, affect the threshold for the development of HLH

(b) Emerging view of HLH: a continuum of risk



IAHS: infection-associated hemophagocytic syndrome
MAHS: malignancy associated hemophagocytic syndrome
MAS: macrophage activation syndrome (R-HLH: rheumatic HLH)

- Any significant residual function of proteins involved in cytotoxic mechanisms protect the individual from early-onset of HLH

Risma & Jordan, Curr Opin Pediatr, 2012