Langerhans Cell Histiocytosis: An Overview

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LCH a very long story…

Achievements

Case description (Hand, 1893)

Unifying concept (Lichtenstein, 1953)

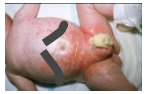
Origin arguments (Nezelof, 1973)

Clonality (Willman, 1994)

BRAF Mutation (Badalian-Very, 2010)

Myeloid neoplasia (Beres, 2013)

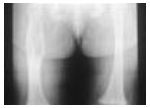
Hand-Schüller-Christian Syndrome



Letterer-Siwe disease

Histiocytosis X

Langerhans Cell Histiocytosis



Eosinophilic granuloma

1890 1900 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2015

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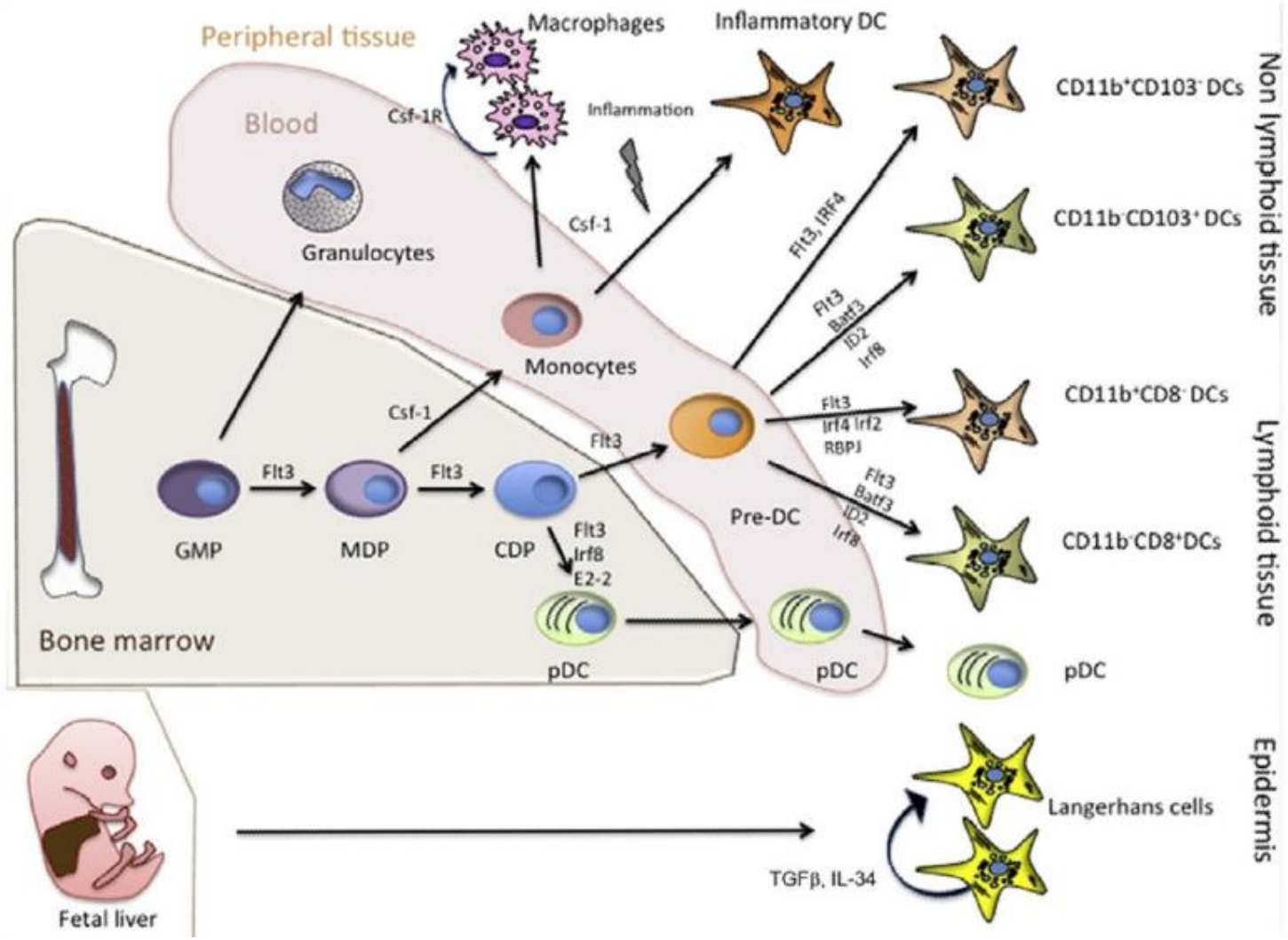
DAL HX 83/90 Studies (1983-1991)
LCH I-II-III Studies (1991-1998)
LCH-IV Study since 2012

What was Known About LCH until recently?

- Abnormal proliferation and dissemination of clonal dendritic cells, which carry much of the surface antigen profile of **normal Langerhans cells**
- Any organ (bone, skin, liver, lungs, spleen, lymph node, brain, etc.) can be involved
- Wide spectrum of clinical presentation with variable course
- Granuloma formation (various proportions of T-lymphocytes, granulocytes, monocytes and eosinophils) and abundance of cytokines evidence inflammatory reaction

The Mononuclear Phagocyte System



Berres ML et al., Adv Immunol, 2013, 120: 127-161

Are the LCH cells really LCs?

Cell-Specific Gene Expression in Langerhans Cell Histiocytosis Lesions Reveals a Distinct Profile Compared with Epidermal Langerhans Cells

Carl E. Allen, Liunan Li, Tricia L. Peters, Hon-chiu Eastwood Leung, Alexander Yu, Tsz-Kwong Man, Sivashankarappa Gurusiddappa, Michelle T. Phillips, M. John Hicks, Amos Gaikwad, Miriam Merad and Kenneth L. McClain

J Immunol 2010; 184:4557-4567; Prepublished online 10 March 2010;
doi: 10.4049/jimmunol.0902336
http://www.jimmunol.org/content/184/8/4557

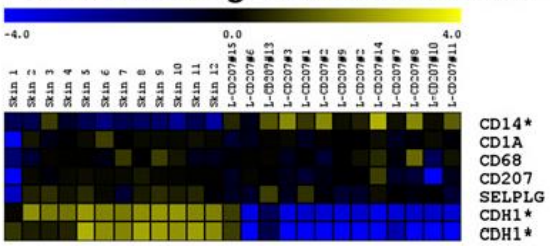
Ref.: Allen CE et al., J Immunol, 2010, 184: 4557-4567

Are the LCH cells really LCs?

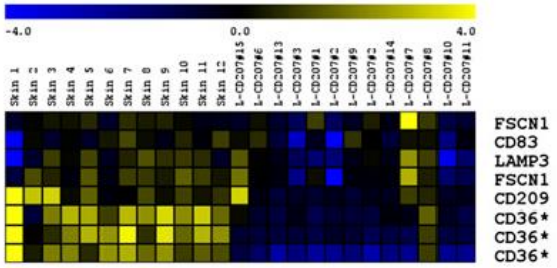
Table I. *Comparison of real-time PCR and microarray results: LCH CD207 versus control skin CD207*

Gene Symbol	Real-Time PCR	Array
SPP1	162.6	22.5–37.0
CEACAM6	76.7	10.1
CDK2NA	73.9	2.1–4.9
JAK3	46.1	1.9–7.1
VNN1	44.0	5.2–7.7
SMYD3	34.8	9.3–12.4
AFF3	24.7	3.1–11.3
HOXB7	20.7	6.6–6.8
DUSP4	15.7	3.4–10.3
MMP9	15.5	7.6
MMP1	11.5	7.2
CCR1	8.6	4.5–5.7
CCL5	8.6	4.6
NRP1	4.8	4.5–9.5
TNFRSF9	4.6	5.8–6.4
DCAL1	3.2	5.1–6.6
CD36	−28.1	−(1.4–14.4)
S100A8	−60.2	−(22.5–26.2)
EpCAM	−204.4	−38.1
CDH1	−269.6	−(32.2–123.6)
PERP	−1,172	−(3.9–227.5)
S100A7	−14,201	−(1.7–74)
IL-2	NA	1.0
IL-17	NA	1.1

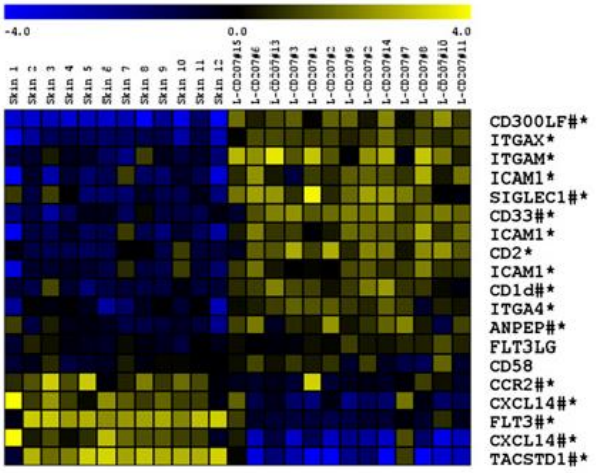
Immature Langerhans Cell Markers



Mature Langerhans Cell Markers

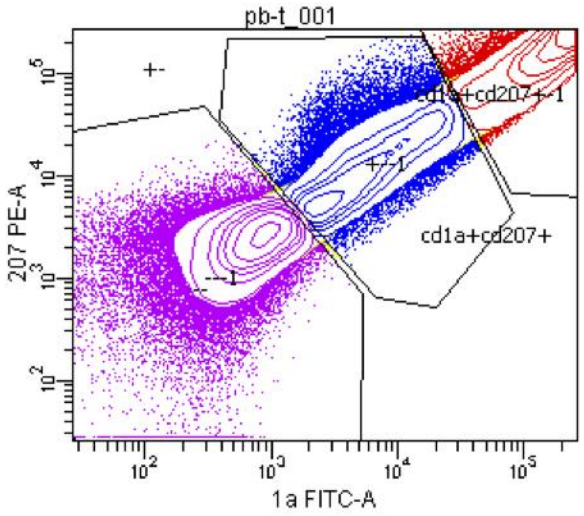


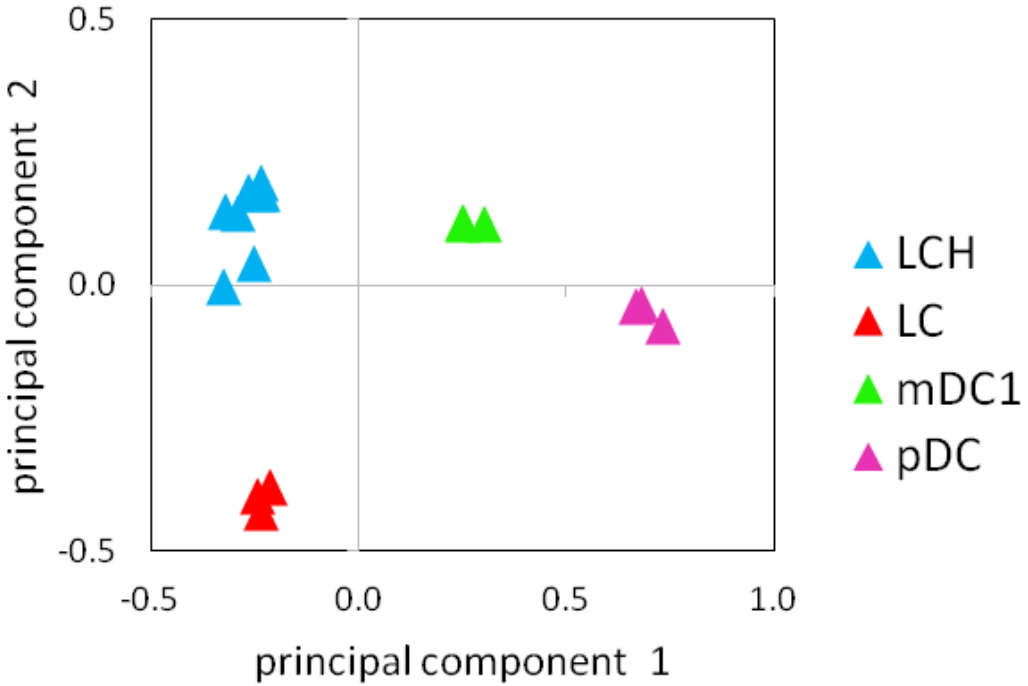
Myeloid Dendritic Cell Maturation



Ref.: Allen CE et al., J Immunol, 2010, 184: 4557-4567

LCH cells are a distinct entity





starting material: fresh biopsy samples (few mm^3)

Hutter et al. Blood 2012;120:5199-5208

The first somatic mutation in LCH

blood

2010 116: 1919-1923
Prepublished online Jun 2, 2010;
doi:10.1182/blood-2010-04-279083

Recurrent BRAF mutations in Langerhans cell histiocytosis

Gayane Badalian-Very, Jo-Anne Vergilio, Barbara A. Degar, Laura E. MacConaill, Barbara Brandner, Monica L. Calicchio, Frank C. Kuo, Azra H. Ligon, Kristen E. Stevenson, Sarah M. Kehoe, Levi A. Garraway, William C. Hahn, Matthew Meyerson, Mark D. Fleming and Barrett J. Rollins

Langerhans cell histiocytosis (LCH) has a broad spectrum of clinical behaviors; some cases are self-limited, whereas others involve multiple organs and cause significant mortality. Although Langerhans cells in LCH are clonal, their benign morphology and their lack (to date) of reported recurrent genomic abnormalities have suggested that LCH may not be a neoplasm. Here, using 2 orthogonal technologies for detecting cancer-associated mutations in formalin-fixed, paraffin-embedded material, we identified the oncogenic BRAF V600E mutation in 35 of 61 archived specimens (57%). TP53 and MET mutations were also observed in one sample each. BRAF V600E tended to appear in younger patients but was not associated with disease site or stage. Langerhans cells stained for phospho-mitogen–activated protein kinase kinase (phospho-MEK) and phospho-extracellular signal-regulated kinase (phospho-ERK) regardless of mutation status. High prevalence, recurrent *BRAF* mutations in LCH indicate that it is a neoplastic disease that may respond to RAF pathway inhibitors. (*Blood*. 2010;116(11):1919-1923)

And even more genes and mutations…

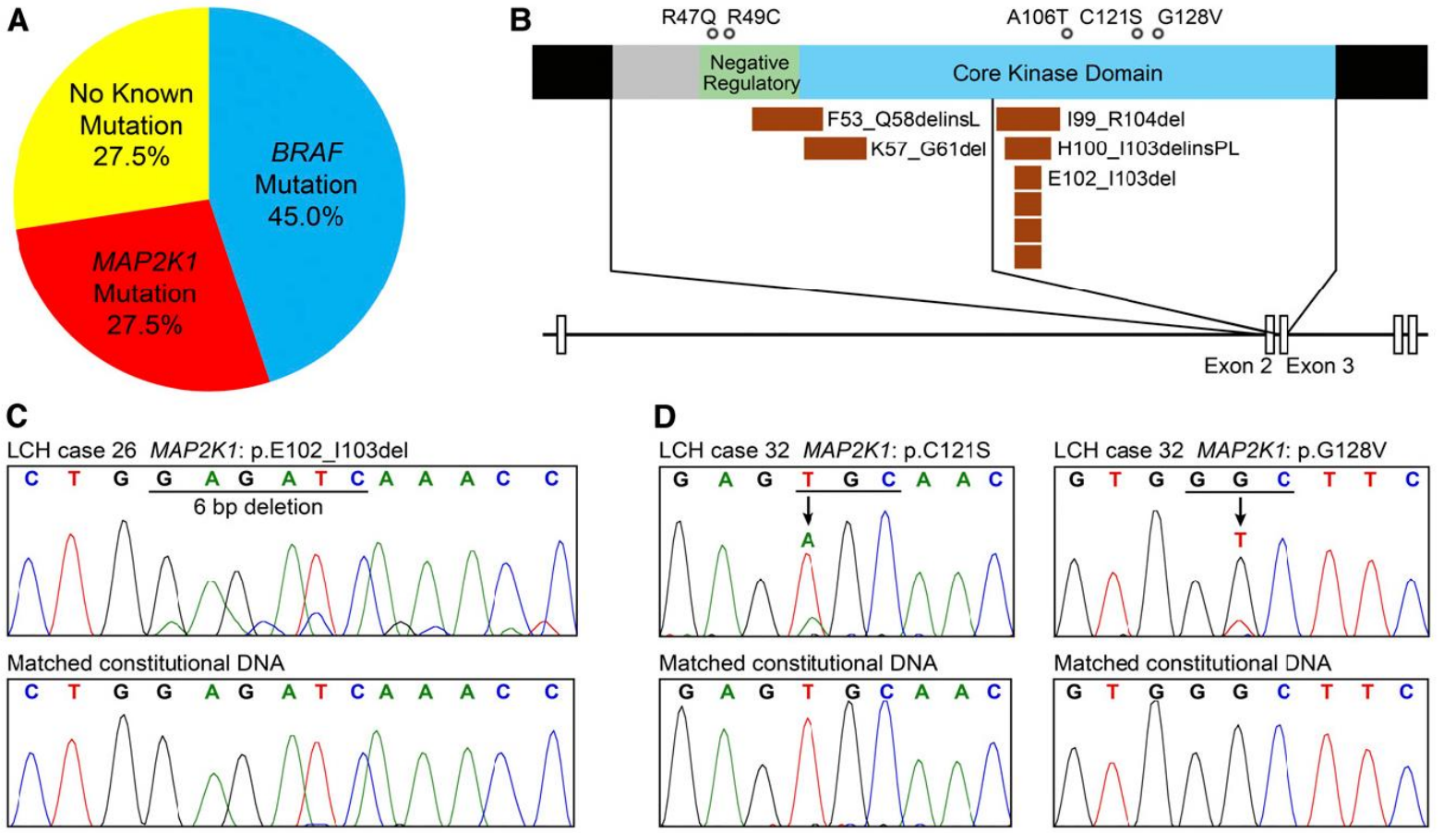


Figure 1. Somatic *MAP2K1* mutations in LCH. (A) The frequency of mutually exclusive *BRAF* and *MAP2K1* mutations in LCH is shown. (B) A portion of the *MAP2K1* gene including exons 2 and 3 is depicted at the bottom, and regions of the MEK1 protein encoded by exons 2 and 3 are depicted above. Somatic mutations in LCH involve the N-terminal negative regulatory region encoded by exon 2 and the catalytic core encoded by exon 3.

Ref.: Brown NA et al., Blood, 2014, 124: 1655-1658

Mutations in LCH: Summary

Genes	Mutations	Pathway	Frequency	References
BRAF	**V600E**	MAPK	35/ 61 (57%) 64/100 (64%) 22/ 42 (52%) 9/ 16 (56%)	Badalian-Very G. et al., 2010 Berres ML et al., 2014 Bubolz AM et al., 2014 Satoh T et al., 2012
	600DLAT (T599A)		2/ 16 (12%) 1/ 16 (6%)	
	V600D		Case report	Kansal R et al., 2013
ARAF	F351L / Q347_A348del T70M / BRAF V600E	MAPK	Case report 1/ 41 (2%)	Nelson DS et al., 2014 Chakraborty R et al., 2014
MAP2KI	R47Q F53_Q58delnsL K57_G61 del R49C / A106T **E102_I103del** H100_I103delinsPL I99_K104del C121S / G128V	MAPK	1 1 1 1 **4** 1 1 1	Brown NA et al., 2014 11/40 (28%) of all 11/22 **(50%) of BRAF^{wt}**
	E102_I103del **Q58_E62del** F53_Q58delnsL Q56P		2 3 1 1	Chakraborty R et al., 2014 7/41 (17%) of all 7/21 **(33%) of BRAF^{wt}**
	C121S / G128D Q56_G61del>R C121S		1 1 1	Nelson DS et al, 2015 3/30 (10%) of all 3/20 **(15%) of BRAF^{wt}**
MAP3KI	T799fs LI48Ifs EI286V		1 1 1	Nelson DS et al., 2015 3/30 (10%) of all 3/20 (15%) of BRAF^{wt}
PIK3CA	E542K	PI3K/AKT MAPK	1/ 86 (1.2%)	Heritier S et al., 2015

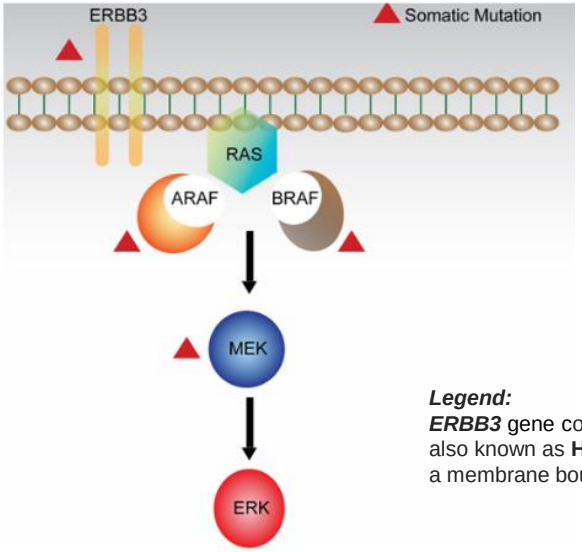
Activated pathways

MYELOID NEOPLASIA

Mutually exclusive recurrent somatic mutations in *MAP2K1* and *BRAF* support a central role for ERK activation in LCH pathogenesis

Rikhia Chakraborty,[1] Oliver A. Hampton,[2,3] Xiaoyun Shen,[1] Stephen J. Simko,[1,4] Albert Shih,[1] Harshal Abhyankar,[1] Karen Phaik Har Lim,[1,5] Kyle R. Covington,[2,3] Lisa Trevino,[2,3] Ninad Dewal,[2,3] Donna M. Muzny,[3] Harshavardhan Doddapaneni,[3] Jianhong Hu,[3] Linghua Wang,[2,3] Philip J. Lupo,[1,4] M. John Hicks,[1,4,6] Diana L. Bonilla,[7] Karen C. Dwyer,[7] Marie-Luise Berres,[8-10] Poulikos I. Poulikakos,[8,9,11] Miriam Merad,[8-10] Kenneth L. McClain,[1,4] David A. Wheeler,[2,3] Carl E. Allen,[1,4,5] and D. Williams Parsons[1-5]

[1]Texas Children's Cancer Center, Texas Children's Hospital, Houston, TX; [2]Department of Molecular and Human Genetics, [3]Human Genome Sequencing Center, [4]Division of Pediatric Hematology-Oncology, Department of Pediatrics, [5]Program in Translational Biology and Molecular Medicine, and [6]Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX; [7]Stem Cell Transplantation and Cellular Therapy, University of Texas MD Anderson Cancer Center, Houston, TX; and [8]Department of Oncological Sciences, [9]Tisch Cancer Institute, [10]Immunology Institute, and [11]Department of Dermatology, Icahn School of Medicine, New York, NY



Legend:
ERBB3 gene codes for **Receptor tyrosine-protein kinase erbB-3**, also known as **HER3** (**human epidermal growth factor receptor 3**), a membrane bound protein

Ref.: Chakraborty R et al., Blood, 2014, 124: 3007-3015

Mutations and Maturation Stage

MYELOID NEOPLASIA

e-Blood

BRAFV600E mutant protein is expressed in cells of variable maturation in Langerhans cell histiocytosis

Felix Sahm,[1,2] David Capper,[1,2] Matthias Preusser,[3] Jochen Meyer,[2] Albrecht Stenzinger,[4] Felix Lasitschka,[4] Anna-Sophie Berghoff,[5] Antje Habel,[1] Marion Schneider,[6] Andreas Kulozik,[7] Ioannis Anagnostopoulos,[8] Leonhard Müllauer,[9] Gunhild Mechtersheimer,[4] and Andreas von Deimling[1,2]

[1]Department of Neuropathology, Institute of Pathology, Ruprecht-Karls-Universität Heidelberg, Heidelberg, Germany; [2]Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [3]Department of Medicine I and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; [4]Department of General Pathology, Institute of Pathology, Ruprecht-Karls-Universität Heidelberg, Heidelberg, Germany; [5]Institute of Neurology and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; [6]Experimental Anaesthesiology, University Hospital Ulm, Ulm, Germany; [7]Department of Pediatric Oncology, Hematology and Immunology, University Children's Hospital of Heidelberg, Heidelberg, Germany; [8]Institute of Pathology, Charité-University Medicine Berlin, Berlin, Germany; and [9]Institute of Pathology, Medical University Vienna, Vienna, Austria

Langerhans cell histiocytosis (LCH) is a clinically and histologically heterogeneous disorder. Its classification as either reactive inflammatory or neoplastic has been a matter of debate. However, the recent finding of frequent BRAFV600E mutations in LCH argues for the latter. The exact cell type that harbors the mutation and is responsible for proliferation remains to be identified. We here apply a BRAFV600E mutation-specific antibody to detect the *BRAF* mutant cells in lesions from 89 patients with LCH. We found BRAFV600E mutations in 34 of 89 (38%) lesions. In lesions with the BRAFV600E mutation, the majority of cells coexpressing S-100 and CD1a harbored mutant BRAFV600E protein. These cells also expressed CD14 and CD36, whereas various fractions exhibited CD207. On the other hand, CD80 and CD86 expression was also present on BRAFV600E-positive cells. Thus, cells of variable maturation, exhibiting an immunohistochemical profile compatible either with myeloid cell or with dedifferentiated Langerhans cell antigens, carry the BRAFV600E mutation. In conclusion, we identify and characterize the neoplastic cells in LCH with BRAFV600E mutations by applying a mutation-specific marker and demonstrate feasibility for routine screening. (*Blood*. 2012;120(12):e28-e34)

Ref.: Sahm F et al., Blood, 2012, 120: e28-e34

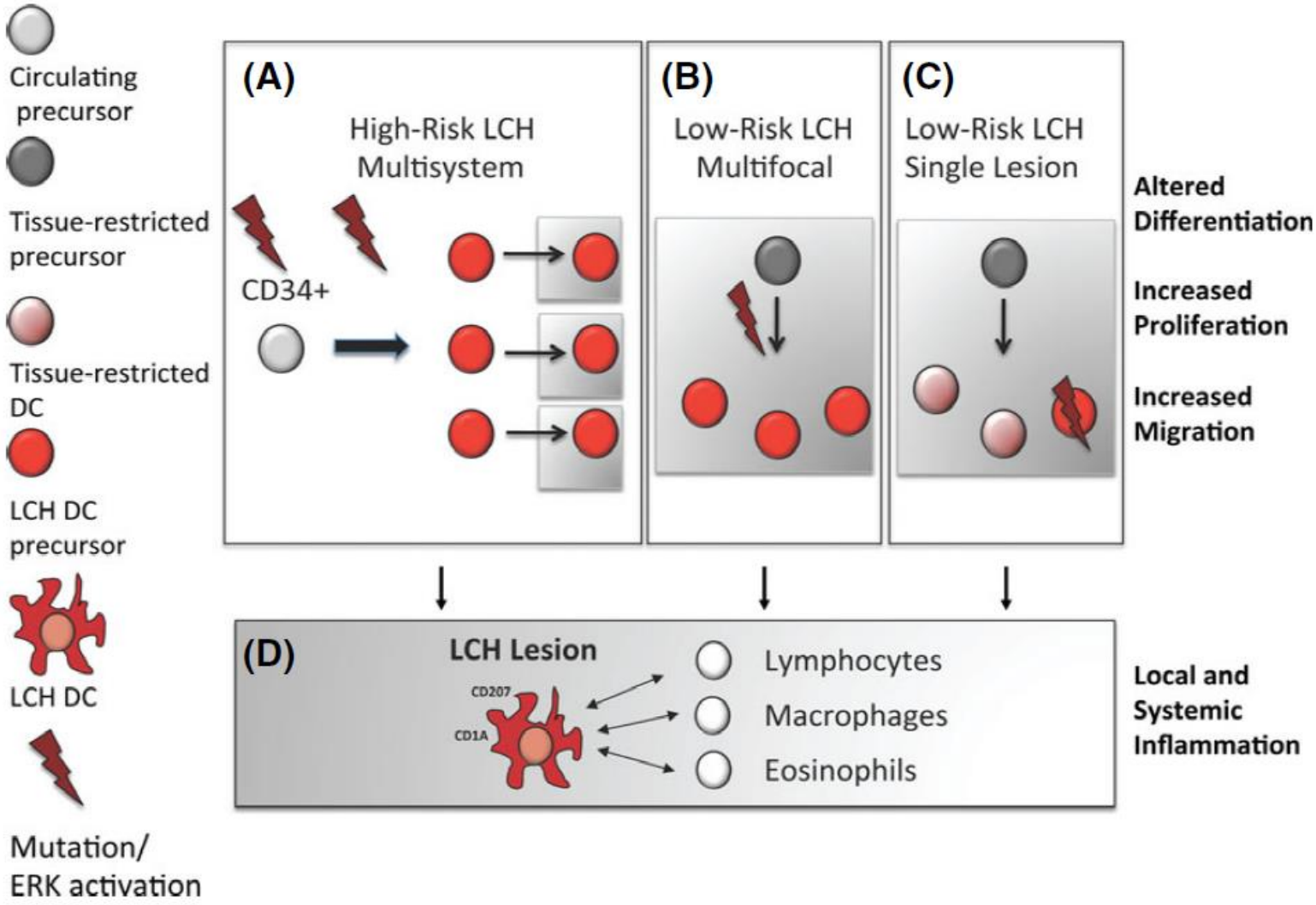
BRAF-V600E expression in precursor versus differentiated dendritic cells defines clinically distinct LCH risk groups

Marie-Luise Berres,[1,2,3] Karen Phaik Har Lim,[4] Tricia Peters,[5] Jeremy Price,[1,2,3] Hitoshi Takizawa,[7] Hélène Salmon,[1,2,3] Juliana Idoyaga,[8,9,10] Albert Ruzo,[8] Philip J. Lupo,[4,6] M. John Hicks,[5] Albert Shih,[4] Stephen J. Simko,[4,6] Harshal Abhyankar,[4,6] Rikhia Chakraborty,[4,6] Marylene Leboeuf,[1,2,3] Monique Beltrão,[3] Sérgio A. Lira,[3] Kenneth M. Heym,[11] Venetia Bigley,[12] Matthew Collin,[12] Markus G. Manz,[7] Kenneth McClain,[4,6] Miriam Merad,[1,2,3] and Carl E. Allen[4,6]

Langerhans cell histiocytosis (LCH) is a clonal disorder with elusive etiology, characterized by the accumulation of CD207+ dendritic cells (DCs) in inflammatory lesions. Recurrent *BRAF-V600E* mutations have been reported in LCH. In this study, lesions from 100 patients were genotyped, and 64% carried the *BRAF-V600E* mutation within infiltrating CD207+ DCs. *BRAF-V600E* expression in tissue DCs did not define specific clinical risk groups but was associated with increased risk of recurrence. Strikingly, we found that patients with active, high-risk LCH also carried *BRAF-V600E* in circulating CD11c+ and CD14+ fractions and in bone marrow (BM) CD34+ hematopoietic cell progenitors, whereas the mutation was restricted to lesional CD207+ DC in low-risk LCH patients. Importantly, *BRAF-V600E* expression in DCs was sufficient to drive LCH-like disease in mice. Consistent with our findings in humans, expression of *BRAF-V600E* in BM DC progenitors recapitulated many features of the human high-risk LCH, whereas *BRAF-V600E* expression in differentiated DCs more closely resembled low-risk LCH. We therefore propose classification of LCH as a myeloid neoplasia and hypothesize that high-risk LCH arises from somatic mutation of a hematopoietic progenitor, whereas low-risk disease arises from somatic mutation of tissue-restricted precursor DCs.

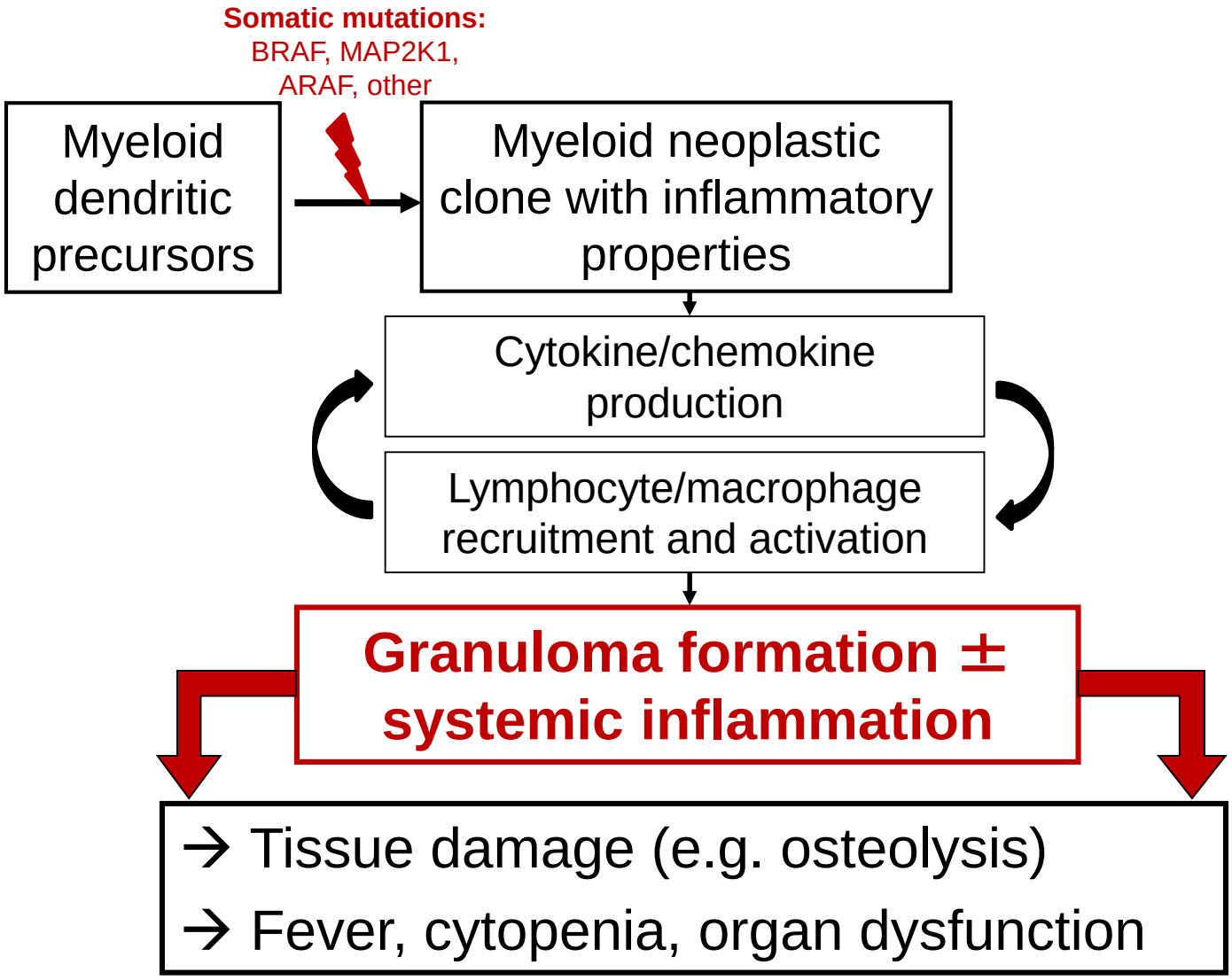
Ref.: Berres ML et al., J Exp Med, 2014, 211: 669-683

Putative Model on LCH Pathobiology



Ref.:
Berres ML et al., BJH, 2014, 120: 127-161

Current View on LCH Pathogenesis

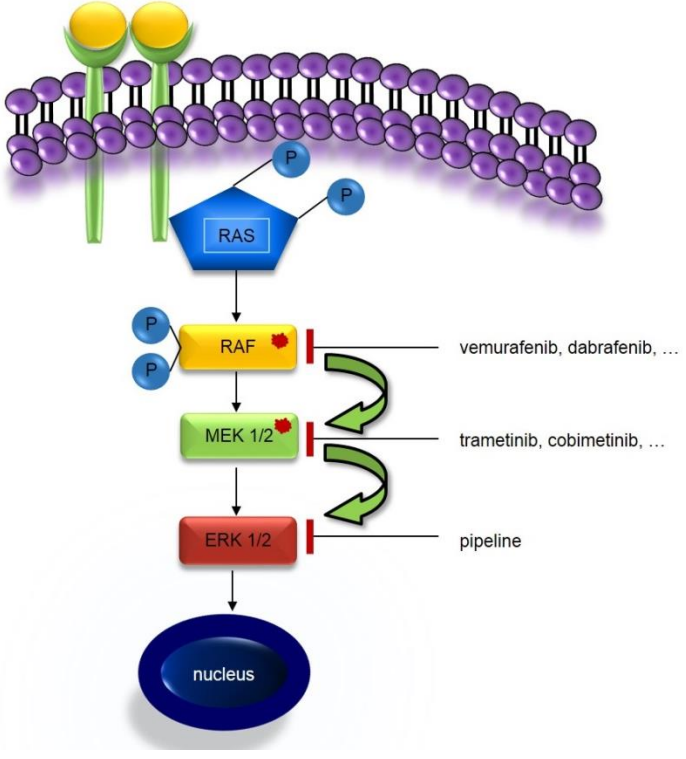


(Modified from Arceci RJ, „Atypical cellular disorders“, Hematology 2002, 297 ff)

Implications of the New Knowledge

- Liquid biopsies?
- Assessment of disease burden (staging)?
- Response evaluation (MRD)?

- Rational (targeted) therapy

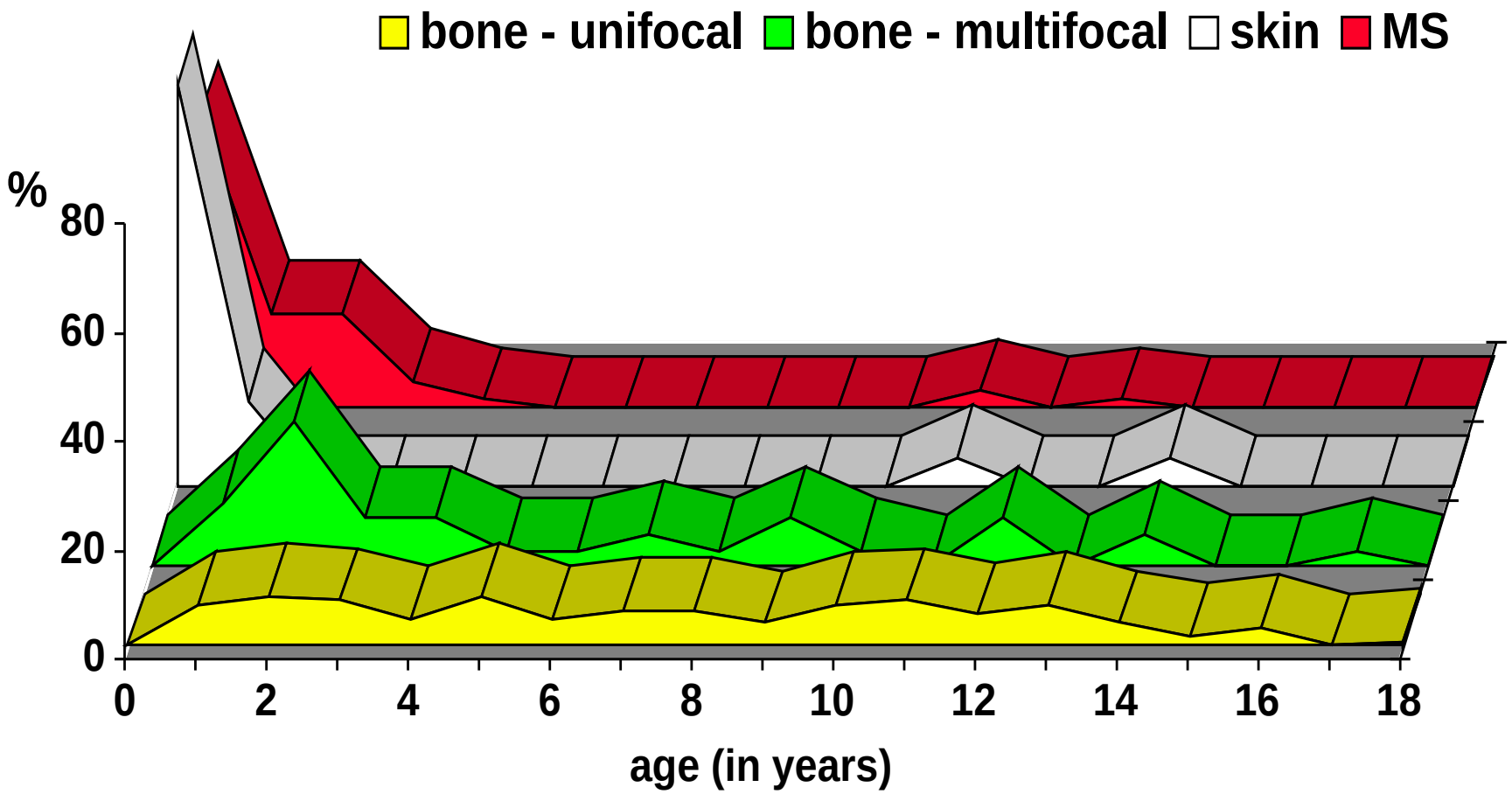


EPIDEMIOLOGY

- any age group
- incidence 0.1-1/ 100 000 children / year

  (1/ 20 000 children by age 15)
- incidence in adults ?
- male:female 1:1 to 2:1

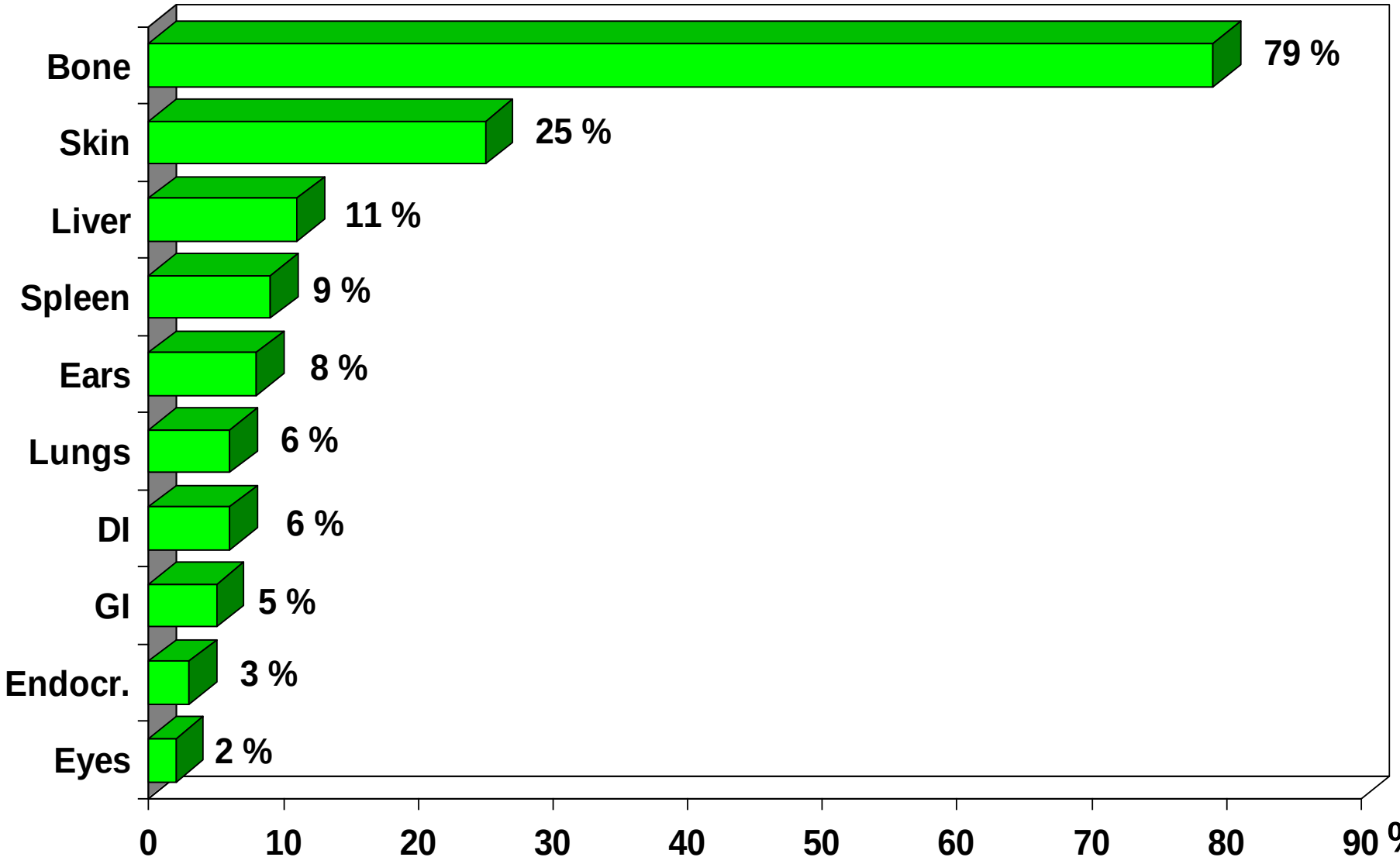
AGE DISTRIBUTION OF LCH



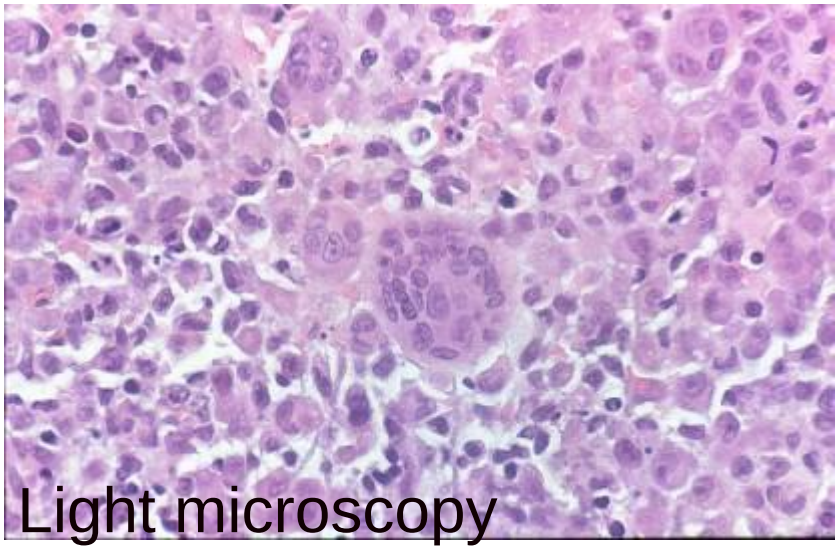
SIGNS AND SYMPTOMS OF LCH

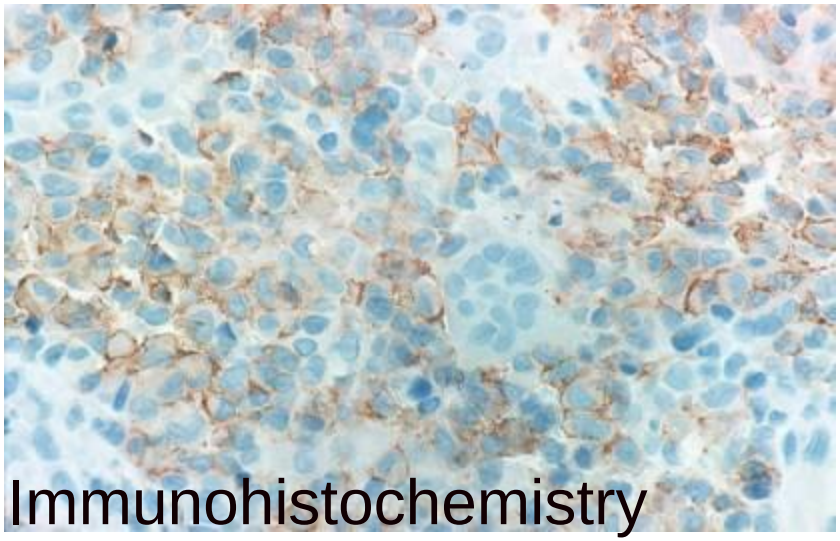
- PAIN, LOCAL SWELLING, MOTION DEFICIT
- SKIN RASH
- NEUROLOGICAL SYMPTOMS
- AURAL DISCHARGE
- POLYURIA, POLYDIPSIA
- ENDOCRINOPATHIES
- FEVER
- COUGH, DYSPNEA
- DIARRHEA; FAILURE TO THRIVE
- TEETH LOSS

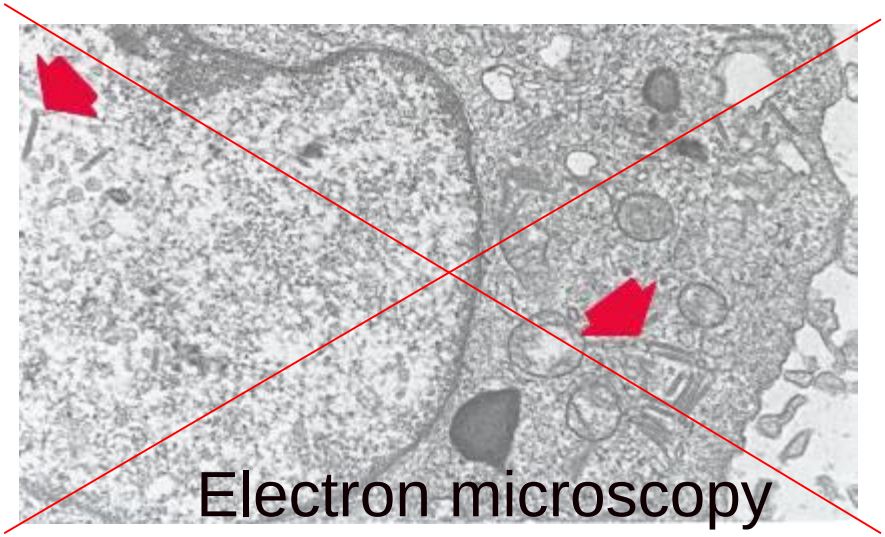
ORGAN INVOLVEMENT IN LCH
n=275



HISTOPATHOLOGICAL DIAGNOSIS OF LCH







DEFINITIVE DIAGNOSIS

CD1a +

~~Birbeck granules~~

CD207 + (Langerin)

OBLIGATORY CLINICAL AND LABORATORY EVALUATION

Physical examination	Complete examination, special attention to: skin and mucosal lesions, floating teeth, proptosis, chronic otitis, aural discharge, cough, dyspnea, enlarged liver, spleen, or LN
Laboratory tests	CBC, ALT, AST, γ-GT, ALP, bilirubin, total protein, albumin, coagulation (aPTT, TT, fibrinogen), urine specific gravity and osmolality
Imaging	Skeletal survey, chest x-ray, abdominal ultrasound

EVALUATION UPON INDICATION

Test	Indication
HLA-typing and donor search	Involvement of risk organs
Bone marrow tap and trephine biopsy	Anemia, leukopenia, thrombocytopenia
Respiratory function test, HRCT	Dyspnea, changes on thorax x-ray
Lung biopsy (if BAL negative)	Dyspnea, changes on thorax x-ray (Cave opportunistic infections)
Gut endoscopy (biopsy?)	chronic, diarrhea, malabsorption, weight loss
Liver biopsy	Liver dysfunction (discrimination between active disease and cirrhosis)
Panoramic x-ray of the jaw	Floating teeth, lumps or mucosal lesions
Skull CT	Suspected orbital, temporal or other skull base lesion
MRI of the brain	Neurological or endocrine abnormalities
Consult endocrinologist	DI, growth failure, other endocrine abnormalities
Consult neurologist/psychologist	DI, neurological signs and symptoms, abnormal MRI
Consultation ENT specialist, audiogram	Chronic otitis, otorrhea, hearing loss
Consult ophthalmologist	Proptosis, orbital lesions

CLINICAL CLASSIFICATION

SINGLE SYSTEM DISEASE

Single or Multiple Site

Bone

Skin

Lymph node

(CNS, Lungs, Thyroid)

MULTI SYSTEM DISEASE

> 2 organs involved

± risk organ involvement

TREATMENT
OF
SINGLE SYSTEM LCH

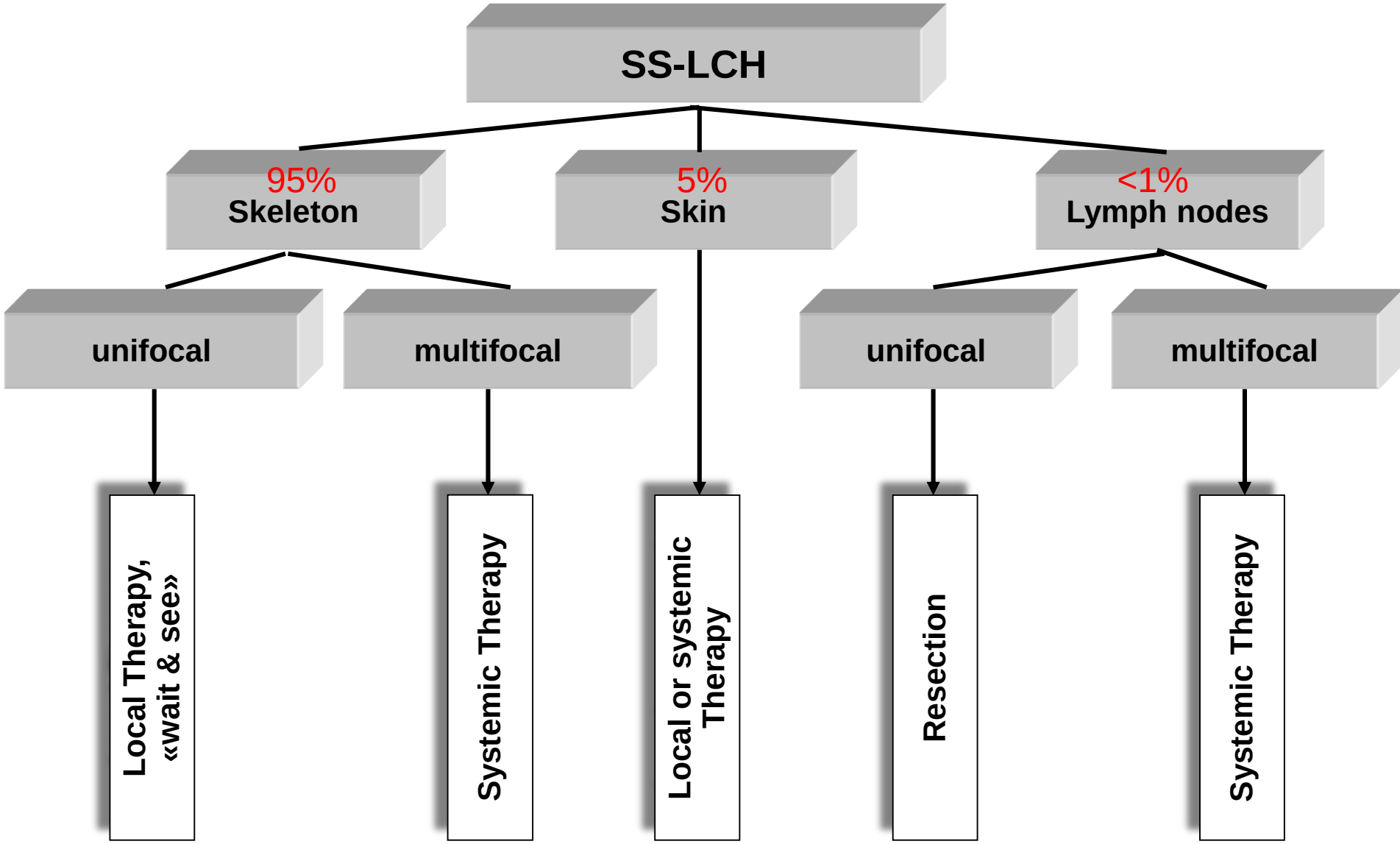
DATA OF THE DAL-HX STUDIES on single-system LCH

Overall survival:	**100%**
Event-free survival:	**82%**
Reactivations	**18%**
Permanent consequences:	**25%***

*** 50% of the PC present at diagnosis**

Ref.: Titgemeyer C. et al., MPO, 2001, 37:108-114

CURRENT RECOMMENDATIONS



TREATMENT
OF
MULTISYSTEM LCH

PROSPECTIVE CLINICAL TRIALS OF THE HISTIOCYTE SOCIETY

- LCH I Study 1991-1995
- LCH II Study 1996-2000
- LCH III Study 2001-2008

STUDY CENTER
ST. ANNA CHILDREN ‘S HOSPITAL
VIENNA, AUSTRIA

GENERAL THERAPEUTIC APPROACH

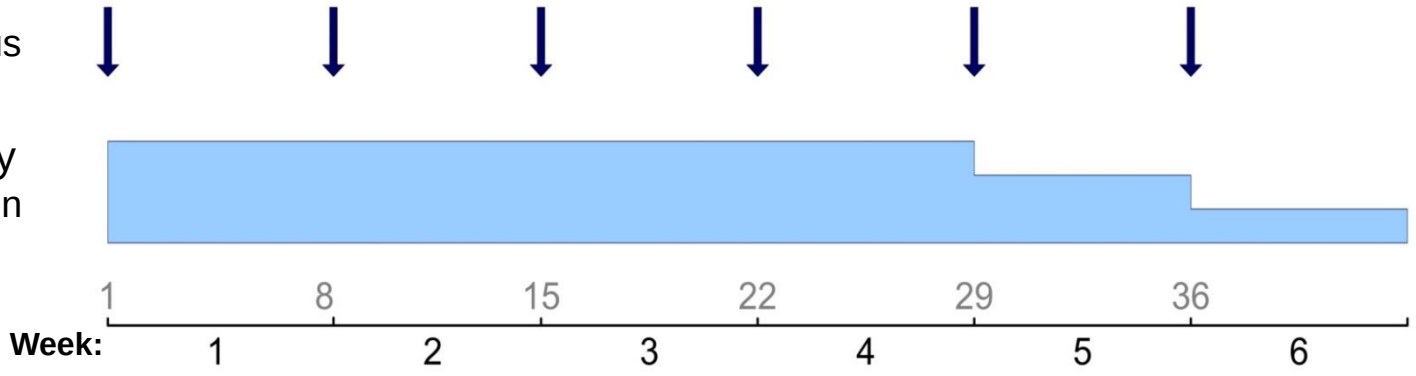
- **INITIAL THERAPY**
  - intensive, duration 6-12 weeks

- **CONTINUATION THERAPY**
  - non-intensive, duration at least 12 months

STANDARD INITIAL THERAPY

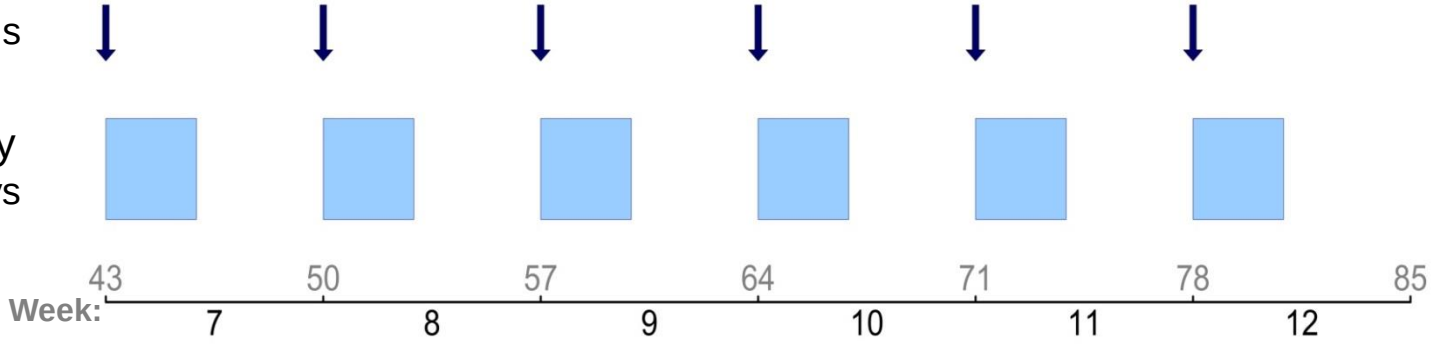
VBL 6 mg/m^2 i.v.bolus

PRED 40 mg/m^2/day
orally; weekly; reduction after week 4



VBL 6 mg/m^2 i.v.bolus

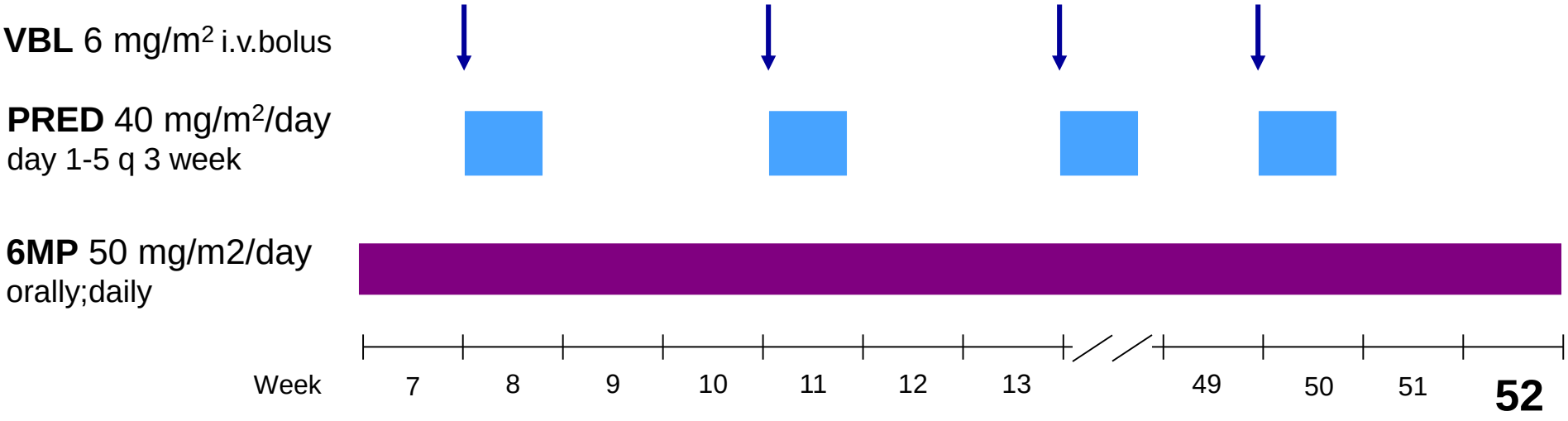
PRED 40 mg/m^2/day
orally; weekly for 3 days



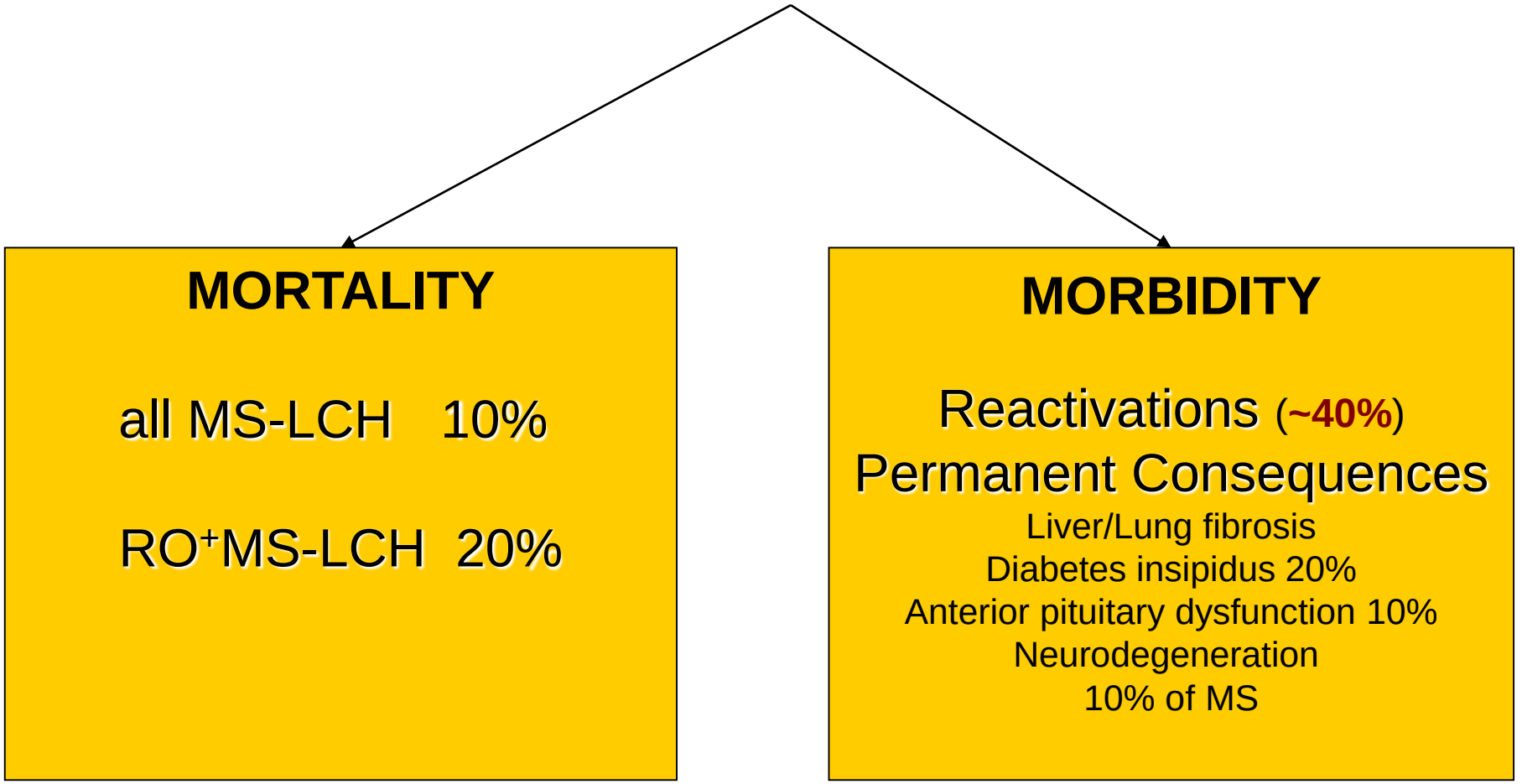
STANDARD CONTINUATION THERAPY

PRED/VBL Pulses

± mercaptopurine



CURRENT LCH PROBLEMS



LCH-IV

International Collaborative Treatment Protocol for Children and Adolescents with LANGERHANS CELL HISTIOCYTOSIS

EudraCT Nr.: 2011-001699-20

International Sponsor: St. Anna Kinderkrebsforschung
(Children's Cancer Research Institute)
Vienna, Austria

Protocol Version 1.0, April 13th, 2011

(corrected version, November 25th, 2012)

Protocol Code Number: 042011

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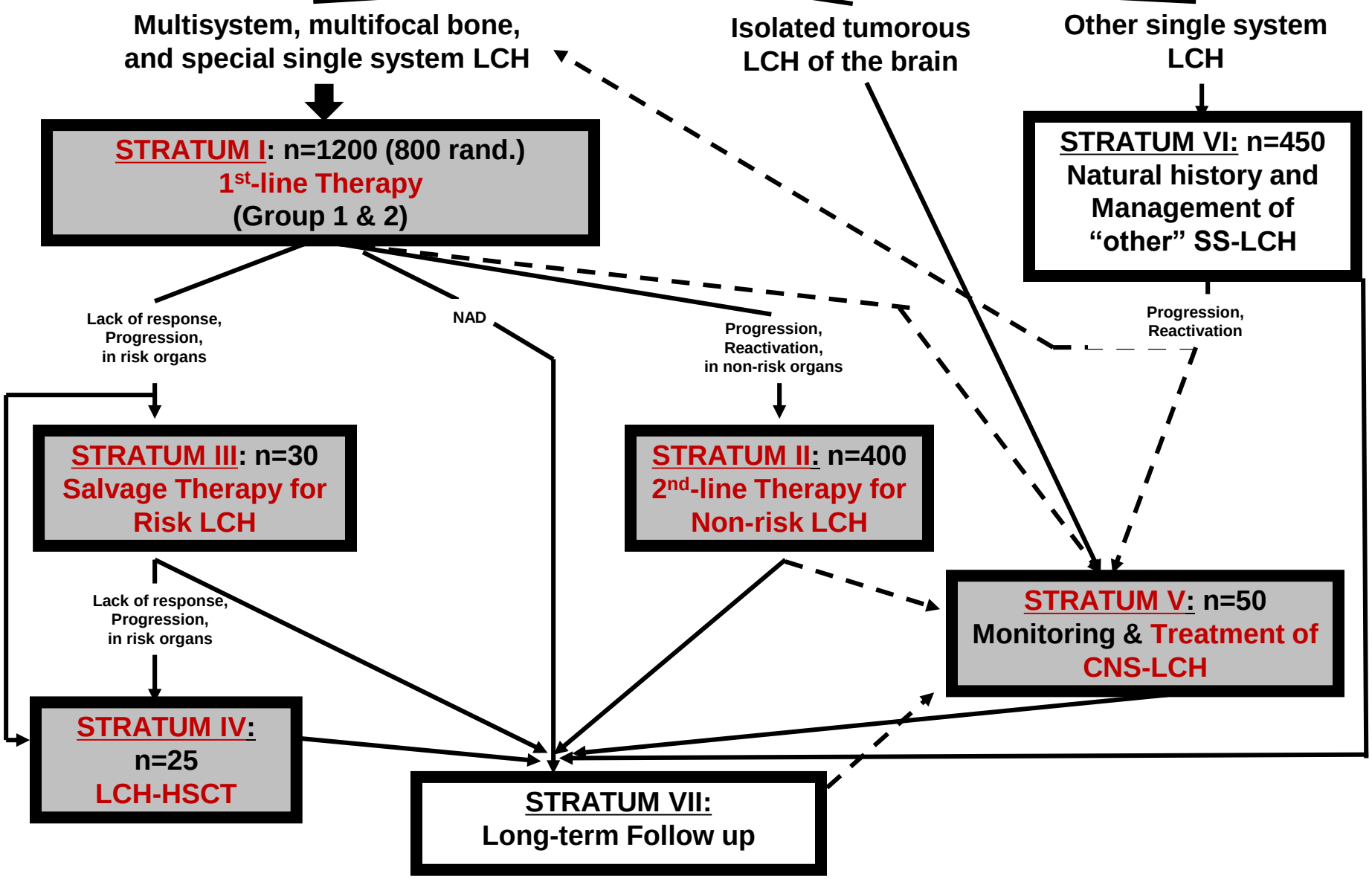
(http://www.histiocytesociety.org)

04/12/2012

Start of the Study: December 1st, 2012

LCH IV Registry & Stratification



LCH-IV: Teilnehmende Länder