LCH - III

(2nd Version: January 2002)

Treatment Protocol of the

Third International Study

for

LANGERHANSCELL HISTIOCYTOSIS

START OF THE STUDY: April 2001

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2 BACKGROUND

Langerhans cell histiocytosis (LCH) is a rare disease that may affect any age group. It is regarded as a clonal accumulation and proliferation of abnormal bone marrow derived Langerhans cells. These dendritic cells along with lymphocytes, eosinophils and normal histiocytes form infiltrates typical for the disease which may be found in various organs and at different extent[1]. LCH includes a wide range of clinical presentations comprising the clinical pictures of eosinophilic granuloma, Hand-Schüller-Christian syndrome or Letterer-Siwe disease. The course of disease is unpredictable, varying from spontaneous regression and resolution to rapid progression and death or repeated recurrence and recrudescence with the risk of permanent consequences, defined as irreversible long-term disabilities which are directly linked, predictable and permanent results of the disease upon the patient[2].

Patients with disease that is localized (skin, bone or lymph node) have a good prognosis and are felt to need minimum or even no treatment. In contrast, multiple organ involvement, which is particularly frequent in young children under 2 years, carries the risk of a poor outcome[3-6]. Patients with multi-system disease benefit from therapy with cytotoxic drugs and/or steroids, either alone or in combination as demonstrated in early prospective multicentric studies for disseminated LCH[7,8]. On 1st April 1991, the Histiocyte Society initiated LCH I - the first international clinical trial for the treatment of multisystem LCH. It was the goal of this randomized prospective study to compare the efficacy of monotherapy with vinblastine and etoposide (VP-16) with respect to response, failure and morbidity. Therapy response was assessed according to the following newly defined criteria: complete resolution of disease (no active disease, NAD), disease regression (active disease, AD-better), intermediate response with regression of some and reappearance of other lesions (AD-intermediate, mixed) or unchanged disease (AD- intermediated, stable) and progression of the disease (AD-worse). By the end of the study on August 15th, 1995 447 patients with LCH were registered onto LCH I. 143 patients with multi-system disease were randomized on the clinical trial, 74 patients were assigned to treatment arm A (VBL), 69 patients to treatment arm B (VP-16). After 6 weeks of treatment (i.e. 2 treatment courses) 53% of the patients were judged as responders (NAD or AD-better), 17% showed a progression

of the disease after 2 courses of treatment and were classified as nonresponders. Reactivations of the disease after complete response (NAD) occurred with a probability of 58% after a median of 9 months from NAD. After a median observation time of 4y 11m (range 2y 10m – 7 y 2m) the overall probability of survival was 78%, but was 91% for the responders in contrast to only 34% for the nonresponders to the initial treatment. This finding clearly indicated the impact of response to initial treatment (Fig.1).

The comparison of the two treatment arms showed that there was no significant difference between monotherapy with VP-16 or vinblastine, neither with respect to initial response and the probability of reactivations, nor with respect to mortality[9].

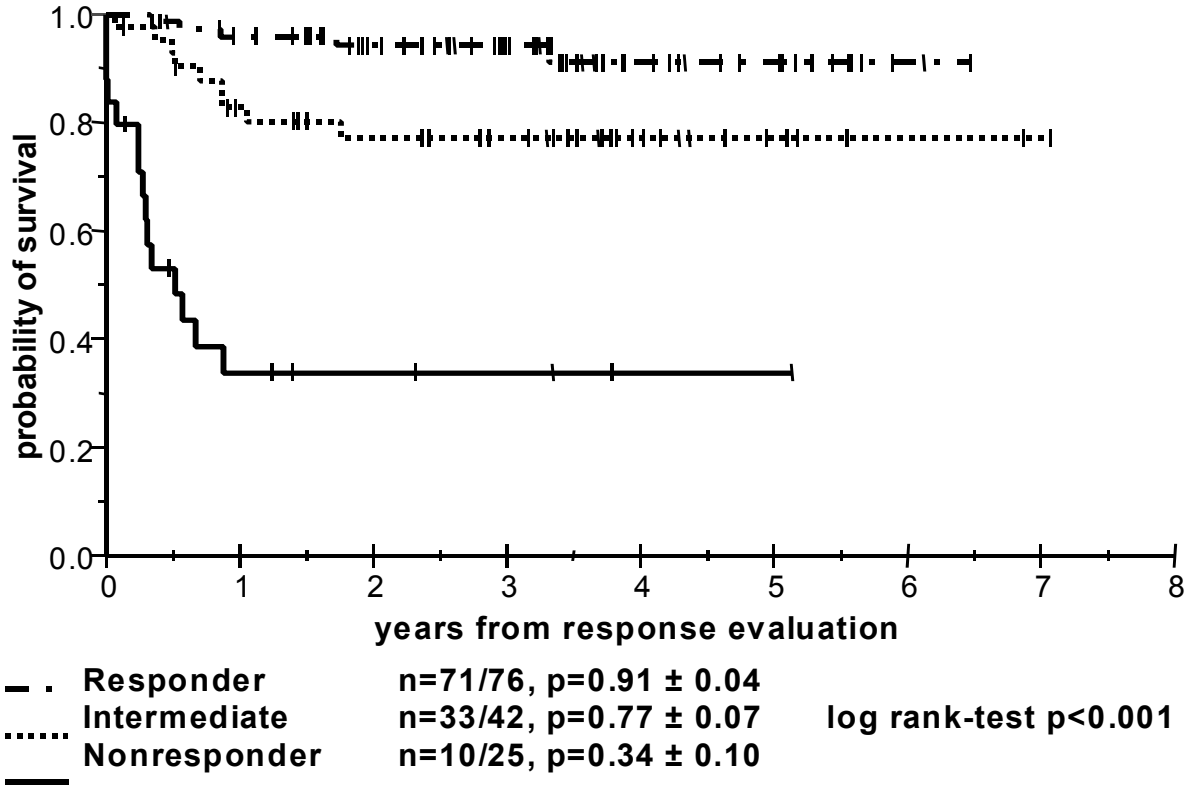

Figure 1. LCH- I: Survival by response at week 6

The results of the LCH-I study were compared with the results of the preceding DAL HX-83 and DAL HX-90 studies, two consecutive multicentric clinical trials, which had been run in Austria, Germany, Netherlands and Switzerland between 1983 and 1990. In these non randomised studies the risk-adapted polychemotherapy protocol included an initial treatment with continuous oral prednisone (PDN) for 6 weeks in combination with vinblastine (VBL) and etoposide (VP-16), followed by a continuation treatment with

continous oral mercaptopurine and 3-weekly pulses of prednisone, VBL, VP-16, and methotrexate for multisystem patients with organ dysfunction. In the 63 evaluable patients with multi-system disease the initial response rate was 79%, 14% were nonresponders. The probability of reactivation was 36%, and the probability of survival was 83%.

The comparison of the LCH I and DAL HX-83/90 results showed a clear superiority of combination therapy given for one year with respect to initial response and rate of reactivation as compared to monotherapy for six months. The mortality rate of ~ 20% did not significantly differ between the 2 studies.

It was the goal for the next international trial, LCH II, to match the results of the DAL HX-studies, and to clarify the question of the value of the addition of VP-16 to prednison and vinblastine by comparing two treatment arms, with or without VP-16 in a randomized way. The continuation therapy included mercaptopurine (6-MP) but the duration was limited to 24 weeks as given in LCH I (Fig.2).

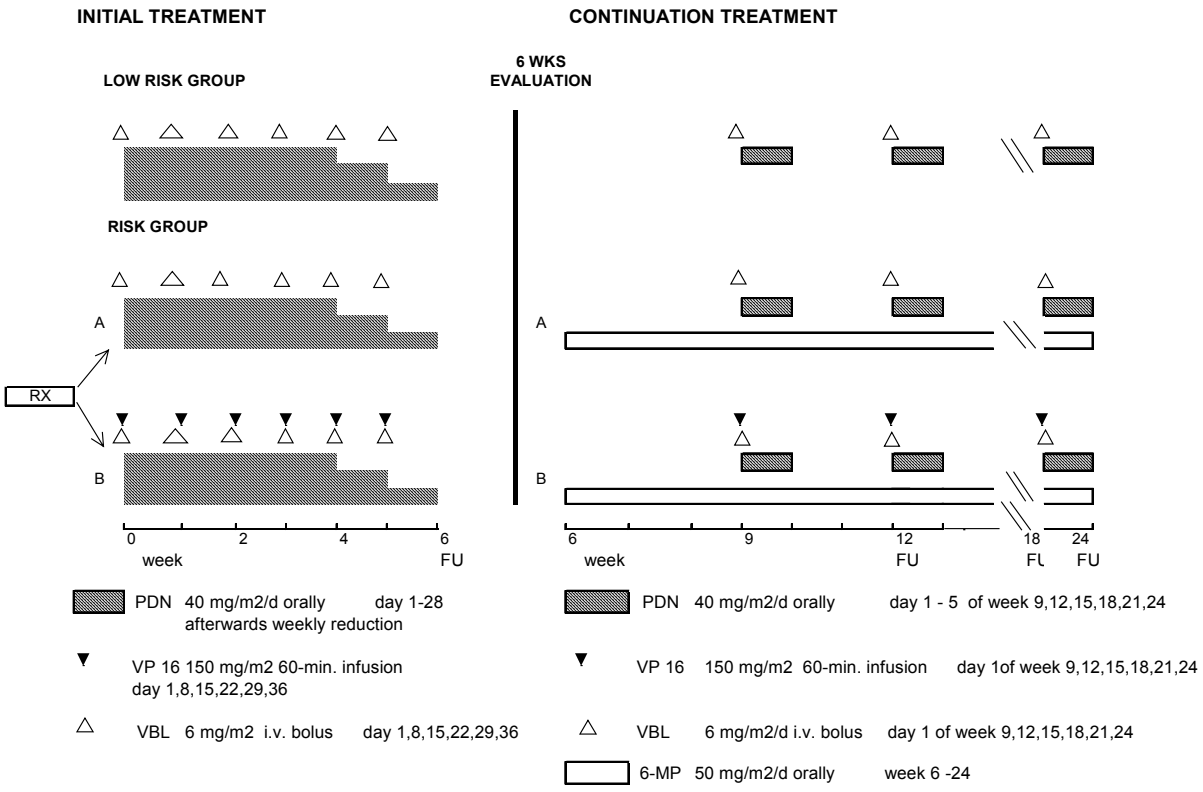

Figure 2. Treatment plan LCH II

A new stratification system was adopted, distinguishing between “RISK“ patients with involvement of “RISK“ organs like liver, spleen, lungs, hematopoetic system or age under 2 years, and ”LOW RISK” patients without such organs involved and age beyond 2 years. “RISK” patient were eligible for the randomisation between the 2-drug and the 3-drug arm, “LOW RISK” patients received initial treatment according to the 2-drug arm only, and a continuation therapy without 6-MP.

Since the start of the LCH II study on 1st May 1996, 697 patients were registered on the LCH II Study. 321 patients had multisystem disease, 87 (27%) of these were stratified as ”LOW RISK“ patients, 233 (73%) patients were classified as “RISK” patients. Overall the compliance of the participating subcenters and clinics was not completely satisfying. Only 176 of these “RISK” patients were randomised (76%), 88 each to arm A and arm B. 66 (37%) of the “RISK“ patients were under 2 years of age without involvement of “RISK“ organs, of these only 41 (62%) were randomised. This points towards a poorer acceptance of the randomisation for this particular group of patients.

The results in the “LOW RISK“ group were satisfying. There were 89%

responders, only one nonresponder at week 6, and no fatalities. Among 170 randomized “RISK” patients, in whom the response at week 6 was available, 113 (66%) were judged as responders. This compares favourably to the 6-week responder rate of 44% in the LCH I study, but is less than the 76% rate of responders in the DAL HX studies.

Interestingly, the overall probability of survival of the multisystem patients did not differ significantly between the 3 studies - DAL HX, LCH I and II, and was around 80%. This observation indicates that there is a “High RISK” population of about 20% of the multisystem patients which cannot be rescued with standard treatment including VBL and PDN with or without VP-16. In LCH II among the 118 randomised “RISK“ patients with risk organ involvement (any age) 22% showed progressive disease at week 6, 35% of the remaining patients did not achieve a further improvement within the next 6 weeks of treatment. This means that by week 12 50% of the patients with “RISK” organ involvement had not shown a response to treatment, but still had intermediate active or progressive disease. For these patients the probability of mortality after 12 weeks of treatment is about 75% (Fig.3), whereas the probability of becoming free of disease is less than 20% (Fig.4).

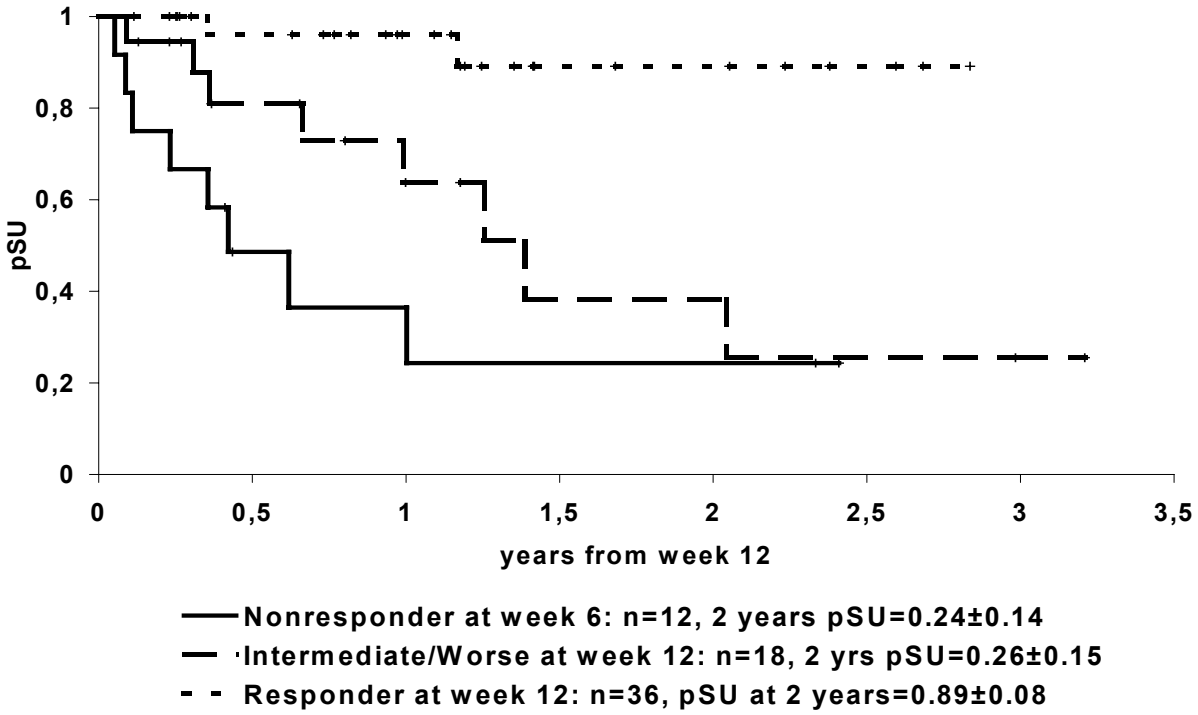

Figure 3: LCH II – Probability of survival by response at week 6 and 12 in patients with "RISK" organs.

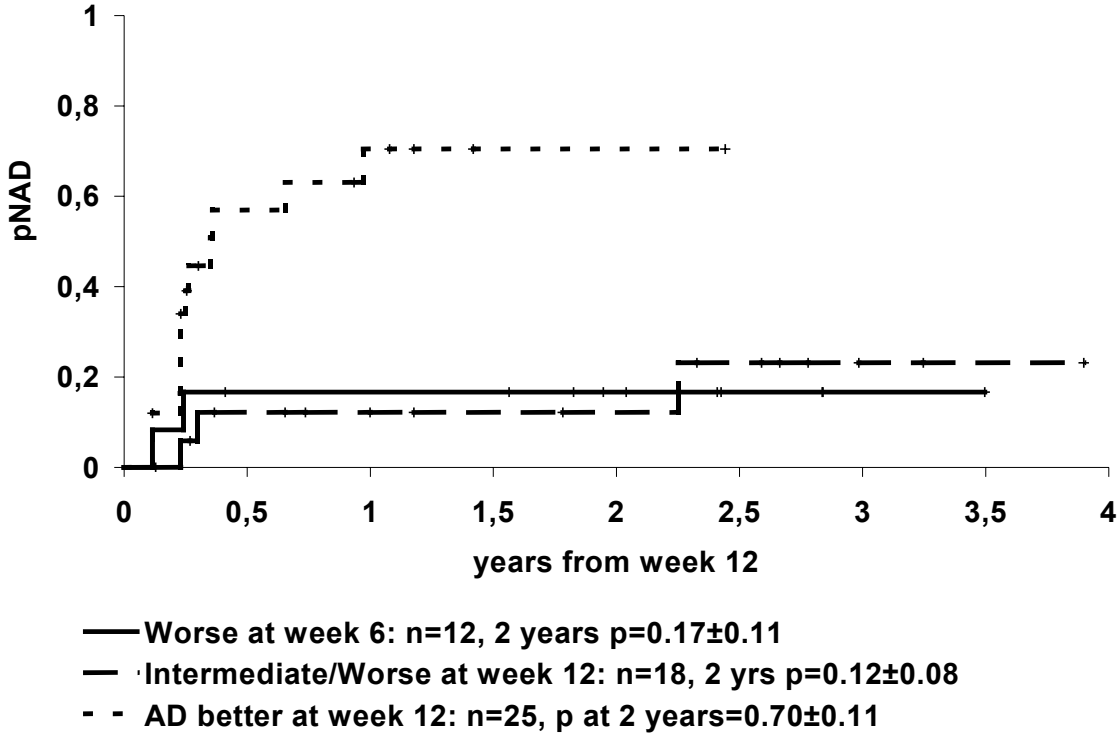

Figure 4: LCH II - Probability of becoming free of disease (NAD) by response at week 6 and 12 in patients with "RISK" organs

Thus, patients with involvement of "RISK" organs, who do not show disease regression by week 12 of therapy have high risk of poor outcome. This is the patient group we need to focus on to improve their outcome in the next study. These patients may benefit from new agents in the initial treatment and obviously rapidly need to be switched to alternative salvage treatment strategies.

Notably, all of the patients who died in LCH II and in LCH I had involvement of "RISK" organs. Therefore, it seems justified to regard risk organ involvement and response to initial treatment as the most important prognostic factors, whereas young age under 2 years did not prove not anymore considered to be of independent prognostic importance.

Overall the probability to become free of disease (NAD) was 84% for the "LOW RISK" patients, and 57% for the "RISK" patients. Interestingly, the speed of response was equal in both groups. The reactivation rate after complete response to therapy (NAD) was 56% in the "LOW RISK" patients and 64% in the "RISK" patients after 2 years.

The comparison of the reactivation frequency for all multisystem patients in the 3 studies showed a similar probability of reactivations in the responders of LCH I (53%) and LCH II study (62%), which both had a treatment duration of only 6 months, whereas the probability of reactivation was only 27% in the DAL-HX study with a therapy duration of 12 months. (Fig.5) Similar results were seen when we looked at the "RISK" and "LOW RISK" groups separately. These observations indicate a potential benefit of prolonged treatment duration.

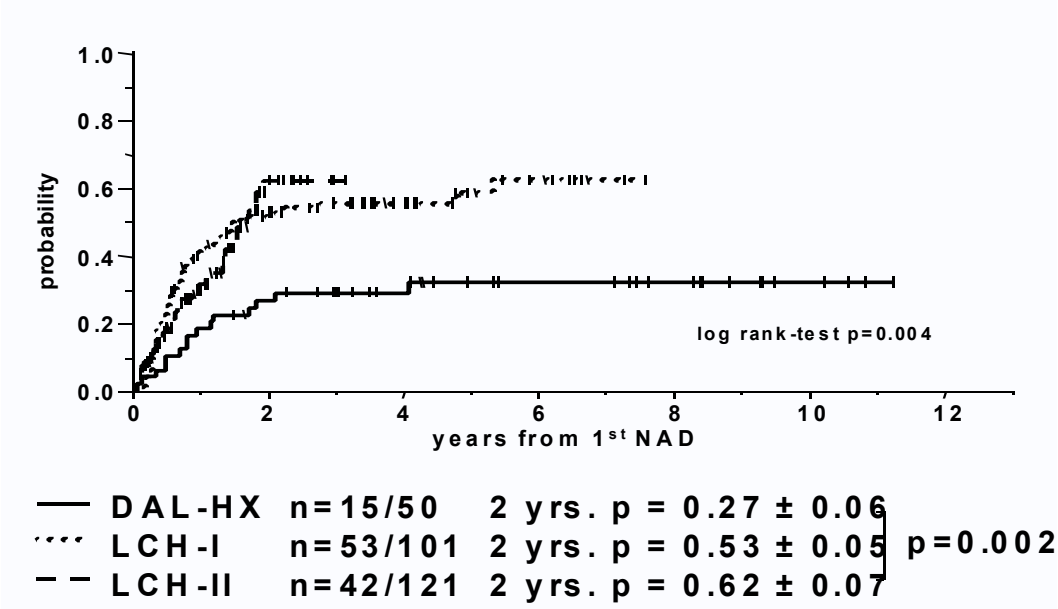

Figure 5. Reactivations after NAD

So far, the comparison of the two treatment arms of LCH II, i.e, the 2-drug arm A with PDN and VBL and the 3-drug arm B with PDN, VBL and VP-16 has not shown any significant difference with respect to initial response, survival and reactivation free survival.

In the LCH I study, toxicity was seen in about 50% of the multisystem patients, and was almost equal in both the treatment arms with vinblastine and VP16. Mild to moderate leukopenia (WHO score I-II) was the most frequently observed event. Severe thrombocytopenia or anemia, as well as hepatic dysfunction was seen only in patients with initial involvement of "RISK" organs, and it was not possible to differentiate treatment related toxicity from disease related dysfunction. Importantly, none of the patients had to be withdrawn of the study because of toxicity.

Preliminary analysis revealed toxic events in 15/89 treatment courses in LCH II, including mild to moderate leukopenia, nausea or vomiting.

The data on permanent consequences in LCH II will be evaluated within the next year. So far, it can be stated that the probability to develop diabetes insipidus is 14% which is about the same as in the previous study.

2.1 CONCLUSIONS OF LCH II

Risk organ involvement and poor response to initial treatment proved to be the most important prognostic factors.

Patients with "RISK" organ involvement who do not achieve a response to initial treatment and are AD worse or intermediate by week 12 carry an about 75% risk of fatal outcome. The probability of becoming free of disease is with standard therapy is less than 20% for such patients with standard therapy.

Age under two years at diagnosis without "RISK" organ involvement is not associated with a poor outcome, and will therefore not be considered for the initial stratification.

So far, VP16 has not shown any additional therapeutic benefit with respect to response, survival or reactivation frequency, neither as monotherapy nor in combination with VBL and PDN. Therefore, VP-16 will not be included in the standard initial treatment of LCH III, considering its potential leukemogenicity.

The fatality rate was around 20% in all 3 studies which were using combination therapy, monotherapy, or 2-drug and 3-drug regimen including PDN, VBL, and VP-16. This observation points towards a need of new agents in the treatment for patients with "RISK" organ involvement.

The retrospective comparison of the DAL HX, LCH I and II studies indicates that prolonged duration of treatment may reduce the rate of reactivations.

2.2 RATIONALE FOR THE USE OF METHOTREXATE IN "RISK" PATIENTS

For years methotrexate (MTX) has proven to be an effective agent in the treatment of LCH[10,11]. In 1974 Jones et al were comparing combination therapy with vincristine (2mg/m2/week i.v.) and PDN versus MTX (30mg/m^2 twice weekly orally) and PDN for a minimum of 4 weeks in a randomized way and found a superior response rate in the MTX group, even at this low dose. Their study also showed a clear benefit of prolonged maintenance therapy[12].

In the prospective DAL HX-83 study 14/21 Arm C multisystem patients (with organ dysfunction) who had responded to the initial therapy, were given intermediate dose MTX (500mg/m^2 q 3 weeks) during the continuation therapy[8]. 7 pts experienced a reactivation, 2 of these a fatal disease progression. 12 (86%) patients finally became free of disease. The small number of patients in this study precludes final conclusions on the effect of MTX in LCH.

2.3 SITUATION IN PATIENTS WITH MULTIFOCAL BONE DISEASE AND "SPECIAL SITES" OF DISEASE

2.3.1 MULTIFOCAL BONE DISEASE (MFB)

The LCH II Study Protocol did not include recommendations for treatment of single system patients with multiple bone lesions. In the past there has been a controversy on how to treat MFB. During the last two years information on 104 patients with MFB was collected in a retrospective survey at the study center. Thirty-six patients initially did not receive any systemic therapy, but were only observed after biopsy (n=13), or treated with surgery (n=20), irradiation (n=4), or intralesional steroids (n=1). Sixty-eight patients

were treated with systemic therapy, which was monotherapy in 17 patients (PDN n=3, VBL n=10, VP16 n=3, 2-CDA n=1). Twenty-two patients received either a combination of VBL + PDN (n=17) or VP16 + PDN n=5. Combination therapy according to the DAL-HX 83/90 protocol was applied in 25 patients, and one patient received a combination therapy consisting of cytosin-arabinoside, VCR and PDN[13]. Independent of the initial treatment strategy, regression or resolution of the disease was seen in about 90% of the patients. However, there was a significant difference with respect to the frequency of reactivation between the different treatment groups. The probability of remaining free of reactivation was 48% for local treatment and 55% for monotherapy, whereas it was 80% for the 2-drug regimen, and 91% for combination therapy. These data confirm the observation of the DAL-HX 83 study, in which only 18% of reactivations were observed in multifocal disease, which was equal to unifocal bone disease and much less than reported in the literature[14]. No statistical impact could be detected with request to the duration of treatment.

Based on these observations, it was decided to offer an initial treatment according to the "LOW RISK" arm of LCH III consisting of PDN and VBL with a treatment duration of 6 months to patients with multifocal bone disease.

2.3.2 "SPECIAL SITES"

2.3.2.1 "CNS-RISK" lesions

A retrospective analysis based on 1524 patients registered in the DAL HX-83/90, the LCH I and LCH II studies revealed that involvement of the facial bones or anterior or middle cranial fossa (temporal, sphenoidal, ethmoidal, cygomatic bone, orbital bones) with intracranial tumour extension carry an about 3-fold risk for the development of diabetes insipidus (DI) which is the hallmark of central nervous system involvement in LCH and therefore are called "CNS-RISK" lesions. This was not true for vault lesions.

Based on these data it was concluded that patients with "CNS-RISK" lesions as the only site of disease activity should not be regarded as simple single system disease, because there is usually bone disease with soft tissue tumor and sometimes infiltration of the meninges. Local therapy is usually problematic in such location and these patients should rather receive systemic treatment. The LCH III study protocol offers therapy with

PDN and VBL to these patients.

2.3.2.2 VERTEBRAL LESIONS

Vertebral lesions sometimes present with significant soft tissue masses that may lead to spinal cord compression, which can be now adequately assessed by MRI. Also in such locations surgery might be too risky. Irradiation may be considered or systemic therapy as offered in the LCH III study protocol should be initiated immediately even if the lesions represent the only site of disease.

3 PATIENT'S ELIGIBILITY FOR LCH III

All newly diagnosed patients who meet the following criteria are eligible to be enrolled and followed in the study:

- Definitive diagnosis of LCH
- Age under 18 years
- No prior treatment for LCH

4 LCH III STUDY REQUIREMENTS

Confirmation of a definitive histopathological diagnosis according to the criteria defined by the Histiocyte Society. Mandatory review by the local reference pathologist in case of presumptive diagnosis or provisional diagnosis.

Adoption of uniform clinical, laboratory and radiographic baseline and follow up evaluations as given in the study protocol.

4.1 HISTOPATHOLOGICAL DIAGNOSTIC CRITERIA

(modified according to the Writing Group of the Histiocyte Society[15].)

4.1.1 Definitive diagnosis

requires the demonstration of CD1a antigenic determinants on the surface of lesional cells (by immunocytology or immunohistology) or the finding of Birbeck granules in lesional cells by electron microscopy.

4.1.2 Provisional diagnosis

is justified when the lesion has characteristic morphology and phenotype to an experienced pathologist and the cells express S100 and at least one of the following: ATPase, alpha-D-mannosidase, peanut lectin. Unstained slides from a provisional diagnosis should be immediately sent before treatment is instituted to the regional study pathologist for definitive diagnosis.

4.2 BASELINE DIAGNOSTIC EVALUATIONS

(modified according to Clinical Writing Group of the Histiocyte Society[16])

4.2.1 Clinical evaluation

4.2.1.1 Complete history:

Fever, pain, irritability, failure to thrive, loss of appetite, diarrhea, polydipsia, polyuria, recurrent otitis, skin rashes, activity level, behavioural changes, neurological changes

4.2.1.2 Complete physical examination:

Measurement of temperature, height, weight, head circumference, pubertal status
Skin and skalp rashes, purpura, bleeding
Jaundice, pallor
Aural discharge
Orbital abnormalities
Gum and palatal lesions, dentition
Soft tissue swelling, lymphadenopathies
Dyspnea, tachypnea, intercostal retractions
Liver and spleen size, ascites, edema
Neurological examination (including papilledema, cranial nerve abnormalities, cerebellar dysfunction)

4.2.2 Laboratory and radiographic evaluation

4.2.2.1 Mandatory minimum baseline evaluations for all patients:

Hemoglobin and/or hematocrit

Ferritin, iron, transferrin

White blood count and differential

Platelet count

Erythocyte sedimentation rate (ESR)

Renal function test (including creatine clearance, mandatory in “Risk” patients randomized on Arm B, prior to MTX infusion)

Liver enzymes and function tests (SGOT, SGPT, γ-GT, alkaline phosphatase, bilirubin, total protein, albumin)

Coagulation studies (PT, PTT, fibrinogen)

Chest radiograph, p.a. and lateral

Skeletal radiograph survey (radionuclide bone scan is not as sensitive as the skeletal radiograph survey in most patients)

Urine osmolality (measurement after overnight water deprivation)

4.2.2.2 Mandatory for multi-system patients

Bone marrow aspiration and trephine with CD1a staining

HLA-typing (for “RISK” patients only, as soon as possible)

4.3 EVALUATIONS REQUIRED UPON SPECIFIC INDICATION

(modified according to the Clinical Writing Group of the Histiocyte Sciety[16])

Indication	**Test**
Abnormal chest radiograph, tachypnea, intercostal retractions	High resolution – CT, Pulmonary function test (if age appropriate)
Patients with abnormal pulmonary high resolution-CT: to yield a diagnosis in case of isolated lung involvement or to exclude infection	Lung biopsy, Bronchoalveolar lavage
Unexplained chronic diarrhea or failure to thrive, evidence of malabsorption	Endoscopic biopsy

Indication	Test
Liver dysfunction: to differentiate active LCH of the liver from sclerosing cholangitis	Sonography, Liver biopsy
Visual or neurologic abnormalities	MRI of brain with i.v. gadolinium – DTPA, Neurological evaluation, psychological tests
Polyuria, polydipsia, short stature, growth failure, hypothalamic syndromes, galactorrhea, precocious or delayed puberty	Endocrine evaluation including water deprivation test, dynamic tests of the anterior pituitary, MRI of brain with i.v. gadolinium - DTPA
Gingiva involvement, loose teeth	Panoramic dental radiography and computed tomography of mandible and maxilla, oral surgery consultation
Aural discharge, deafness	Otolaryngology consultation and audiogram, MRI of brain with i.v. gadolinium - DTPA

4.4 DEFINITION OF ORGAN INVOLVEMENT

4.4.1 "RISK" organs

Hematopoetic involvement: With or without bone marrow Involvement*	Anemia: hemoglobin <10 g/dl, infants <9 g/dl (exclusion of iron deficiency) Leukocytopenia: leukocytes <4,0 x 10^9/l, Thrombocytopenia: platelets < 100 x 10^9/l

*Bone marrow involvement is defined as demonstration of CD1a positive cells on bone marrow smears. The clinical significance of CD1a positivity in the bone marrow remains to be proven. Hypocellularity, hemophagocytosis, myelodysplasia, and/or myelofibrosis may be regarded as secondary phenomena. Hemophagocytosis may be prominent in severe progressive cases.

Spleen involvement:	enlargement ≥ 2 cm below costal margin (proven by sonography)
Liver involvement:	enlargement > 3 cm below costal margin (proven by sonography) and/or liver dysfunction (hyperbilirubinemia, hypoproteinemia, hypalbuminemia, elevated γGT, alkaline phosphatase, elevated transaminases, ascites, edema) and/or histopathological diagnosis
Lung involvement:	typical changes on high resolution computed tomography (HR-CT) and/or histopathological diagnosis

4.4.2 "CNS RISK" lesions

Lesions in the orbital, temporal/ mastoid, sphenoidal, zygomatical, ethmoidal bones, maxilla, sinuses or anterior or middle cranial fossa, with intracranial soft tissue extension demonstrated on magnetic resonance imaging (MRI). Vault lesions are not regarded as "CNS Risk" lesions.

5 STRATIFICATION

5.1 GROUP 1 - MULTISYSTEM "RISK" PATIENTS

Multisystem patients WITH involvement of one or more "RISK" organs i.e. hematopoetic system, liver, spleen or lungs

Patients with **single system lung** involvement are not eligible for randomisation

5.2 GROUP 2 - MULTISYSTEM "LOW RISK" PATIENTS

Multisystem patients with multiple organs involved but WITHOUT involvement of "RISK"

organs

5.3 GROUP 3 - SINGLE SYSTEM “MULTIFOCAL BONE DISEASE” AND LOCALIZED “SPECIAL SITE” INVOLVEMENT

Patients with multifocal bone disease, i.e. lesions in 2 or more different bones;

Patients with localized special site involvement, like “CNS-RISK” lesions with intracranial soft tissue extension or vertebral lesions with intraspinal soft tissue extension;

6 GOALS FOR LCH III

Like in the LCH II study the overall aims of this study are:

- to deliver risk-adapted therapy according to the extent and severity of the disease
- to evaluate the response in the different patient groups
- to evaluate the rate of failure in the different treatment groups, i.e. nonresponse to therapy or disease reactivation during therapy
- to assess morbidity, i.e. evaluation of therapy toxicity and evaluation of the incidence of permanent consequences in the different treatment groups.

The specific goals for the 3 patient groups are as follows:

6.1 GROUP 1: MULTISYSTEM “RISK” PATIENTS

6.1.1 To decrease mortality

- by decreasing the rate of nonresponders to initial treatment at week 6 by introducing MTX as a new agent. The effect of MTX in addition to the initial standard therapy with VBL and PDN will be assessed in a randomized way.
- by decreasing the rate of patients who do not achieve a response (NAD or AD better) in “RISK” organs at week 12 by applying a second course of initial therapy.
- by encouraging an early switch to salvage therapy for nonresponders at week 6 or 12.

6.1.2 To decrease morbidity

- i.e. the rate of reactivation and permanent consequences by prolonging the continuation treatment to one year.

6.1.3 To assess acute and late treatment toxicity

6.2 GROUP 2: MULTISYSTEM "LOW RISK" PATIENTS

6.2.1 To decrease morbidity

- by reducing the rate of reactivation and permanent consequences. The value of prolonged continuation therapy for responders to initial treatment and the efficacy of 6 versus 12 months continuation treatment will be tested in a randomized way.

6.2.2 To increase the response rate

- by applying a second course of initial treatment course according to arm A of the "RISK" group in patients with an insufficient response (intermediate and worse) to initial treatment at week 6.

6.2.3 To assess acute and late treatment toxicity

6.3 GROUP 3: SINGLE SYSTEM MULTIFOCAL BONE AND LOCALIZED "SPECIAL SITES"

6.3.1 To reduce morbidity

i.e. the rate of reactivation and permanent consequences (as compared to the historical control group of the DAL HX 83/90 and LCH I and II studies).

7 STUDY DESIGN

LCH III is an international, multicentric, prospective clincial study comprising

- a randomized clincial trial for multisystem "RISK" patients and

- a randomized clincial trial for multisystem “LOW RISK” patients and
- a pilot study for patients with single system MFB and localized “SPECIAL SITES”

8 REGISTRATION AND RANDOMIZATION

8.1 REGISTRATION

After confirmation of the diagnosis of a new patient, the registration form and the diagnostic evaluation forms have to be sent to the study subcenter or study reference center by fax or e-mail without delay together with the completed and signed consent form.

8.2 RANDOMISATION

8.2.1 Eligibility for randomization group 1 “RISK” patients

- eligibility for the study
- multisystem disease with involvement of “RISK” organs

8.2.2 Eligibility for randomization 2 “LOW RISK” patients

- eligibility for the study
- multisystem disease without involvement of “RISK” organs
- response to initial therapy, i.e. regression (AD better) or resolution (NAD) after 6 weeks of initial treatment course 1

Randomisation will be performed by the study subcenter or study reference center promptly after the reception of the registration, diagnostic evaluation or follow up and consent forms. The information on the assigned treatment arm and the randomisation number will be forwarded to the participant by e-mail or fax without delay.

9 TREATMENT

9.1 GROUP 1: MULTISYSTEM “RISK” PATIENTS

consists of an initial treatment of one or two 6 week courses (according to

response) and a continuation treatment. The overall therapy duration is 12 months.

9.1.1 Treatment arm A

9.1.1.1 Initial reatment course 1

Continuous oral prednisone (PDN) 40mg/m^2 daily in 3 doses as a 4-week course, tapering over a period of 2 weeks.

Vinblastine (VBL) 6 mg/m^2 i.v. bolus, day 1 of week 1, 2, 3, 4, 5, 6.

9.1.1.2 Initial treatment course 2

(starting without delay after course 1 for patients who are AD better or intermediate after course 1. Patients who are NAD after course 1 proceed to continuation treatment.).

Oral prednisone (PDN) 40mg/m2 in 3 divided doses for 3 days every week, from week 7-12.

Vinblastine (VBL) 6 mg/m^2 i.v. bolus, day 1 of week 7, 8, 9, 10, 11, 12.

9.1.1.3 Continuation treatment

(starting after initial treatment at day 1 of week 7 in pts who are NAD after course 1 of initial treatment, or at day 1 of week 13 in pts who are NAD or AD-better after course 2 of initial treatment.

Continuous oral 6-mercaptopurine (6-MP) 50mg/m^2 daily until the end of month 12 from therapy start.

Pulses of oral prednisone PDN 40mg/m^2 in 3 doses, day 1-5 q 3 weeks, starting at day 1 of week 7 in patients NAD after course 1 or at day 1 of week 13 in patients NAD or AD better after course 2 until the end of month 12.

Vinblastine (VBL) 6mg/m^2 i.v. bolus, day 1 q 3 weeks, starting at day 1 of week 7 in patients NAD after course 1 or at day 1 of week 13 in patients NAD or AD better after course 2 until the end of month 12.

9.1.2 Treatment arm B

9.1.2.1 Initial treatment course 1

Continuous oral prednisone (PDN) 40mg/m^2 daily in 3 divided doses as a 4-week course, tapering over a period of 2 weeks.

Vinblastine (VBL) 6 mg/m^2 i.v. bolus, day 1 of week 1, 2, 3, 4, 5, 6 given before the MTX infusion.

Methotrexate 500 mg/m^2 24 hours-infusion with folinic acid (leucovorin) rescue, day 1 of week 1, 3, 5. 1/10 of the dose as i.v. bolus over 30 min, followed by 9/10 of the dose as 23.5 hours infusion with 2000ml/m^2 hydration.

Folinic acid 12mg/m^2 orally is given 24 hours and 30 hours after the stop of the MTX infusion (id est: 48 and 54 hours after start of MTX therapy).

9.1.2.2 Initial treatment course 2

(starting without delay after course 1 for patients who are AD better or intermediate after course 1. Patients who are NAD after course 1 proceed to continuation treatment).

Oral prednisone (PDN) 40mg/m2 in 3 divided doses for 3 days every week, from week 7-12.

Vinblastine (VBL) 6 mg/m^2 i.v. bolus, day 1 of week 7, 8, 9, 10, 11, 12 given before the MTX infusion.

Methotrexate 500 mg/m^2 24 hours-infusion with folinic acid (leucovorin) rescue, day 1 of week 7, 9, and 11. 1/10 of the dose as i.v. bolus over 30 min, followed by 9/10 of the dose as 23.5 hours infusion with 2000ml/m^2 hydration.

Folinic acid 12mg/m^2 orally is given 24 hours and 30 hours after the stop of the MTX infusion (id est: 48 and 54 hours after start of MTX therapy).

9.1.2.3 Continuation treatment

Continuous oral 6-mercaptopurine (6-MP) 50mg/m^2 until the end of month 12 from therapy start.

Pulses of oral prednisone PDN 40mg/m^2 in 3 doses, day 1-5 q 3 weeks, starting at day 1 of week 7 in patients NAD after course 1 or at day 1 of week 13 in patients NAD or

AD better after course 2 until the end of month 12.

Methotrexate 20mg/m^2 orally, once weekly until the end of month 12.

Vinblastine (VBL) 6mg/m^2 i.v. bolus, day 1 q 3 weeks, starting at day 1 of week 7 in patients NAD after course 1 or at day 1 of week 13 in patients NAD or AD better after course 2 until the end of month 12.

9.2 GROUP 2: "LOW RISK" GROUP

Treatment consists of an initial treatment of 6 weeks (a second course is given only to patients with persistent or progressive disease) and a continuation treatment.

The overall therapy duration is 6 or 12 months as randomly assigned.

9.2.1.1 Initial treatment course 1

Continuous oral prednisone (PDN) 40mg/m^2 daily in 3 doses as a 4-week course, tapering over a period of 2 weeks.

Vinblastine (VBL) 6 mg/m^2 i.v. bolus, day 1 of week 1, 2, 3, 4, 5, 6.

9.2.1.2 Initial treatment course 2

(Only in patients with intermediate or worse response after course 1)
Oral prednisone (PDN) 40mg/m2 in 3 divided doses for 3 days every week , from week 7-12.
Vinblastine (VBL) 6 mg/m^2 i.v. bolus, day 1 of week 7, 8, 9, 10, 11, 12.

9.2.1.3 Continuation treatment

The overall therapy duration is 6 months for Arm LR 6 or 12 months for Arm LR 12 .

Pulses of oral prednisone PDN 40mg/m^2 in 3 doses, day 1-5 q 3 weeks, starting at day 1 of week 7 in patients NAD after course 1 or at day 1 of week 13 in patients NAD or AD better after course 2 until the end of month 6 or 12 from therapy start.

Vinblastine (VBL) 6mg/m^2 i.v. bolus, day 1 q 3 weeks, starting at day 1 of week 7 in patients NAD after course 1 or at day 1 of week 13 in patients NAD or AD better after course 2 until the end of month 6 or 12 from therapy start.

9.3 GROUP 3: "MULTIFOCAL BONE DISEASE" AND "SPECIAL SITES"

Treatment consists of an initial treatment of 6 weeks and a continuation treatment. A second course is given only to patients with progressive disease. The overall therapy duration is 6 months.

9.3.1 Initial treatment

Continuous oral prednisone (PDN) 40mg/m^2 daily in 3 doses as a 4-week course, tapering over a period of 2 weeks.

Vinblastine (VBL) 6 mg/m^2 i.v. bolus, day 1 of week 1, 2, 3, 4, 5, 6.

9.3.2 Continuation treatment

(starting after initial treatment at day 1 of week 7 in pts who are NAD after course 1 of initial treatment or at day 1 of week 13 in patients who are NAD or AD-better after course 2 of initial treatment)

Pulses of oral prednisone PDN 40mg/m^2 in 3 doses, day 1-5 q 3 weeks, starting at day 1 of week 7 in patients NAD after course 1 or at day 1 of week 13 in patients NAD or AD better after course 2 until the end of month 6 from therapy start.

Vinblastine (VBL) 6mg/m^2 i.v. bolus, day 1 q 3 weeks, starting at day 1 of week 7 in patients NAD after course 1 or at day 1 of week 13 in patients NAD or AD better after course 2 until the end of month 6 from therapy start.

9.4 SUPPORTIVE CARE GUIDELINES

Pneumocystis carinii prophylaxis

Oral sulphamethoxazole/trimethoprime, 5 mg/kg/day of the trimethoprime, divided into 2 doses/day, on 3 days per week throughout the study period and for 12 weeks thereafter (must be stopped during MTX-infusion).

Antiemetics should be given as necessary.

Transfusions of red cells and platelets

Blood cell components should be filtered blood products and preferably irradiated (25 Gy), for prevention of GvHD.

G-CSF

In case of prolonged neutropenia, G-CSF may be given subcutaneously or intravenously. The use of GM-CSF is not recommended.

Intravenous immunoglobulin

may be given in cases of hypoimmunoglobulinemia.

9.5 TOXICITY

(please fill in Toxicity sheet, Appendix)

6-Mercaptopurine

Myelosuppression, hepatic dysfunction (elevated transaminases and cholestatic jaundice), mucositis, dermatological manifestations, interaction with allopurinol, nausea, vomiting

Methotrexate

Myelosuppression (leukopenia, anemia, thrombocytopenia), nausea, vomiting, mucositis (ulcerative stomatitis, diarrhea), alopecia, skin rashes, nephrotoxicity, hepatic dysfunction, liver fibrosis, encephalopathy, pneumonitis

Prednisone

Increased appetite, centripedal obesity, fluid retention, hyperglycemia, immunosuppression, myopathy, osteoporosis, aseptic necrosis, peptic ulceration, pancreatitis, mental alteration, cataracts, hypertension, precipitation of diabetes, growth failure, amenorrhea, impaired wound healing, atrophy of subcutaneous tissue

Vinblastine

Peripheral neuropathy: paresthesia, dysphagia, hoarseness, bone pain (esp. mandible), constipation, paralytic ileus, convulsions, myelosuppression (leukopenia, anemia, thrombocytopenia), alopecia, inappropriate ADH secretion, local pain and necrosis if

extravasated, nausea, vomiting.

9.5.1 Serious Adverse Events

Any serious adverse event (death or grade III - IV non-haematological life threatening toxicity) must be reported immediately (i.e. within the next working day) by the treating institution to the the study reference center (and relayed to the local subcenters and DSMB), for further reporting according to local practice (use form in appendix).
The toxicity criteria will be the same for all participating groups (WHO-score) and appear in Appendix.

9.6 THERAPY MODIFICATIONS

Try to avoid dose-reductions or delays.

Pancytopenia

at presentation may be disease related and is then not an indication for reduction of the initial dosage.

Infants with body weight under 10 kg:

Drug doses are calculated based on body surface area (BSA) only and dose is adjusted for age as follows:

< 6 months	50% of dose calculated form BSA
> 6 months < 12 months	75% of dose calculated from BSA
> 12 months	100% of dose calculated from BSA

Bone marrow toxicity:

In case of good response to therapy it is recommended to wait for hemapoetic recovery. An absolute neutrophil count greater than 1.0 x 10^9/l and a platelet count greater than 100 x 10^9/l are essential before starting each course of therapy except for the first dose. In case of persistent disease activity it is recommended to continue protocol regardless of the hematologic values.

Hepatotoxicity:

Samples for determination of liver enzymes (ALT/GOT, AST/GPT) must be

drawn immediately prior to a course of i.v. MTX. If values are 10-20N (i.e. 10-20 times higher than normal values) wait 48 hrs and recheck to ensure that the levels are decreasing. Discontinue TMP/SMZ if the transaminase elevation persists or increases, and withhold chemotherapy until the value is <10N, then resume full dose chemotherapy. Resumption of TMP/SMZ prophylaxis is left to the investigator's discretion. Should therapy be withheld for elevated transaminases during initial therapy, resume therapy at point of interruption. Transaminase values of 20N mandate holding therapy until the level returns to <10N. Persistence of values >20N for >2 weeks requires an evaluation including: bilirubin, alkaline phosphatase, coagulation tests, albumin, total protein and hepatitis serologies. A liver biopsy should be considered before additional therapy is given to help to distinguish hepatic toxicity from LCH involvement or sclerosing cholangitis. Under such circumstances, please contact the local coordinator.

Nephrotoxicity

For patients with a GFR by creatinine clearance of less than 60 ml/min/1.73m^2 delay MTX and repeat clearance after hydration.

Gastrointestinal toxicity

In case of severe mucositis or diarrhea, therapy should be discontinued until recovery and then reinstituted without dose reduction. To prevent constipation in patients treated with vinblastine regular administration of mild laxatives is recommended. Serious constipation with paralytic ileus requires cessation of vinblastine administration.

Neurotoxicity

In the event of significant toxicity (extensive weakness, severe paresthesia, severe ileus), VBL may be temporarily discontinued and resumed at 50% dose when toxicity resolves. Contact the local study coordinator before discontinuing VBL. Increase to maximum tolerated dose (not exceed protocol dose) as soon as possible.

Oral 6-mercaptopurine and oral methotrexate

if neutrophil count falls below 500/μl, treatment will be held until recovery above these levels and then resumed as tolerated. If neutrophil count falls below 500/μl on >2 occasions during continuation, discontinue TMP/SMZ and decrease dose of 6-MP or

MTX by 25% on alternating basis upon resumption of therapy. Begin by reducing the 6-MP dose. Should therapy be withheld for myelosuppression or elevated transaminase, resume therapy at the correct point chronologically.

10 ASSESSMENT OF TREATMENT RESPONSE

In contrast to leukemia or other malignancies the terms “remission” or “relapse” should be avoided. In accordance with the nature of LCH the following definitions should be applied to judge the effect of treatment.[9]

10.1 DEFINITION OF DISEASE STATE

NON ACTIVE DISEASE (NAD)	no evidence of disease	resolution of all signs or symptoms
	regressive disease	regression of signs or symptoms, no new lesions
ACTIVE DISEASE (AD)	stable disease	persistence of signs of symptoms, no new lesions
	progressive disease*	progression of signs or symptoms and/or appearance of new lesions

10.2 DEFINITION OF RESPONSE CRITERIA

There are three categories of response

BETTER	complete resolution	NAD
	regression	AD better
INTERMEDIATE	mixed	new lesions in one site, regression in another site
	stable	unchanged
WORSE	progression*	

*in isolated bone disease progression is defined as appearance of new bone lesions or

lesions in other organs

10.3 RESPONSE EVALUATION

Please send the follow up evaluation sheet to the study subcenter as soon as possible after the evaluation (fax or e-mail).

Clinical status and performance[17]
Blood count and differential
Requirement of blood products
Liver and spleen size
Liver enzymes and function tests
HR-CT and pulmonary function tests (if age-appropriate) for patients with lung disease
Skeletal radiograph of lesional sites only
MRI of the brain in patients with “CNS-RISK” lesions or intracranial lesions
Spinal MRI in patients with vertebral lesions
Histological proof of AD or NAD may be required in specific sites

10.3.1 Group 1: “RISK PATIENTS”

Any treatment decision must be based on the response in “RISK ORGANS” (i.e.R.O.)

INTERVAL	EVALUATION	RESPONSE	THERAPY
week 6	after initial treatment course 1	NAD	continuation treatment
		better	initial treatment course 2
		intermediate	initial treatment course 2
		worse	salvage (see appendix)
week 12	after initial treatment course 2	NAD	continuation treatment
		better	continuation treatment
		intermediate	salvage (see appendix)
		worse	salvage (see appendix)

week 24 or any time in case of event	during continuation treatment	NAD, better	continuation treatment
		intermediate without R.O. intermediate with R.O.	continuation treatment salvage (see appendix)
		worse without R.O.	arm A: re-start initial treatment arm B arm B: salvage (see appendix)
		worse with R.O.	salvage (see appendix)
month 12	end of continuation treatment	NAD	STOP (in case of persistent signs of disease activity, please discuss with local coordinator)

10.3.2 Group 2 "LOW RISK" patients

Please send the follow up evaluation sheet to the study subcenter IMMEDIATELY after the evaluation at week 6 (fax or e-mail) to have the randomization for the duration of continuation treatment performed!

Interval	**Evaluation**	**RESPONSE**	**CONTINUE**
week 6	after initial treatment course 1	NAD better	continuation treatment randomization: LR 6 or LR 12
		intermediate	initial treatment course 2
		worse	initial treatment course 2
week 12	after initial treatment course 2	NAD better	continuation treatment
		intermediate	continuation treatment
		worse	discuss with local coordinator
week 7 or 13-23	during continuation treatment	NAD, better	continuation treatment
		intermediate	continuation treatment
		worse	discuss with local coordinator

month 6	end of continuation treatment	NAD better	LR 6 STOP LR 12 continuation treatment
		intermediate	continuation treatment
		worse	re-start initial treatment arm B
month 6-12	during continuation treatment LR 12	NAD better	continuation treatment
		intermediate	continuation treatment
		worse	re-start initial treatment arm B
month 12	end of continuation therapy LR 12		STOP (in case of persistent signs of disease activity, please discuss with local coordinator)

10.3.3 Group 3 patients with "MFB" or "SPECIAL SITES"

Brain MRI (and/or CT scans) or spinal MRI must be performed to assess the treatment response in these critical sites. In case of residual "CNS-RISK" lesions on MRI treatment must not be stopped. A biopsy of such lesions to rule out residual active disease should be considered.

Interval	**Evaluation**	**RESPONSE**	**CONTINUE**
week 6	after initial treatment course 1	NAD better	continuation treatment
		worse	initial treatment course 2 (risk patients arm A)
week 12	after initial treatment course 2	NAD better	continuation treatment
		worse	discuss with local coordinator
week 7 or 13-23	during continuation treatment	NAD better	continuation treatment
		worse	discuss with local coordinator

month 6	after continuation treatment	NAD better	STOP (in case of clear regression stop therapy, repeat MRI after 3 months, in case of significant residual tumor consider biopsy and discuss with local coordinator)

11 OFF STUDY CRITERIA

In case of progression in “RISK organs” after initial treatment course 1 or course 2 switch to salvage (see appendix).

In case of evidence of residual active disease at the end of protocol therapy a biopsy of residual lesions to rule out persistent active disease is recommended, and the individual case should be discussed with the local co-ordinator.

If you have any questions regarding salvage therapy, please contact the local coordinator or the study reference center.

12 AUTOPSY

Autopsy is to be encouraged for fatal cases. A set of unstained slides (5-10) or blocks should be submitted to the regional study pathologist.

13 FOLLOW UP INVESTIGATIONS AFTER STOP OF THERAPY

	FOLLOW UP		
	With Risk Organ involvement	**Without Risk Organ involvement**	**Multifocal bone involvement, special site**
1. year			
Clinical examination	6 weekly	6 weekly	3 monthly
Height, weight, pubertal status	6 monthly	6 monthly	6 monthly
Lab-examinations Blood count, ESR, liver (renal function)tests, urine osmolality	3 monthly	3 monthly	6 monthly
Radiographs of bone lesions	3 monthly until signs of regression (or as clinically indicated)	3 monthly until signs of regression (or as clinically indicated)	3 monthly until signs of regression (or as clinically indicated)
HR-CT, pulmonary function tests (in case of pulmonary involvement)	6-monthly		
Sonography in pts with liver involvement	6-monthly		
Brain MRI in pts with DI or other endocrinopathies, in pts with CNS risk lesions	yearly, 3 monthly until no residual mass lesions or stable findings		
Neuropsychometric assessment (in case of CNS involvement)	yearly	yearly	yearly

2.- 5. year			
Clinical examination* *more frequently when clinically indicated	6 monthly*	6 monthly*	6 monthly*
Height, weight, pubertal status	6-monthly	6-monthly	6-monthly
Lab-examinations Blood count, ESR, liver and renal function tests, urine osmolality	6 monthly (or as clinically indicated)	6 monthly (or as clinically indicated)	6 monthly (or as clinically indicated)
Radiographs of bone lesions	only in case of suspected lesions	only in case of suspected lesions	only in case of suspected lesions
HR-CT and pulmonary function tests in pts with lung involvement	6 monthly (or as clinically indicated)	-	-
Brain MRI in pts with DI or other endocrinopathies, with CNS risk lesions	yearly (or more frequently when indicated)	yearly (or more frequently when indicated)	yearly (or more frequently when indicated)

14 DATA COLLECTION AND EVALUATION

The data questionnaires will be used both as initial patient registration form and as form for follow up evaluations during and after treatment.

Transmission of the data from collaborating institutions to the local subcenter will be made on data forms either by conventional mail, fax or by e-mail.

The subcenters and the study reference center will perform randomisation promptly after receiving the registration forms or the 6 week report forms from the referring institutions.

For evaluation, the completed questionnaire sheets are sent to the subcenters.

The local coordinator will be available for any clinical questions of the treating

physicians, and review the data sheets for completeness and correctness.

The data input into the uniform LCH III database will be performed at the local subcenter, if possible, or the revised data sheets will be transferred to the study reference center in Vienna.

For the regular study evaluations the local data bases will be transferred to the study reference center.

Access to common data from the study data base will be given only with the approval of the Scientific Committee and the permission of the Board of the Histiocyte Society.

15 DATA SAFETY MONITORING BOARD (DSMB)

An independant Data Safety Monitoring Board composed of 4 international experts will monitor the progress of the study on ethical and scientific grounds. The role of the DSMB will be

- to review accrual rate and
- to be involved in all interim analysis. Five sequential analysies are planned, the results will remain confidential. Bases on the results of the interim analysis the DSMB will recommentd whether the study can continue of whether it must be amended or stopped prematurely.
- to monitor toxicity- biannually the DSMB will review the toxicity reports together with the study committee.

The DSMB will be asked to review any major modification to the study proposed by the study committee prior to its implementation.

16 STATISTICAL CONSIDERATIONS

The study reference center in Vienna will carry out all analyses. The results and the data will be send to the Institute of Medical statistics in Mainz for an independent statistical review after the interim-analyses and three weeks before a study committee meeting. The randomisation lists will be provided by the statistical center in Mainz.

16.1 DESIGN

16.1.1 Group 1 “RISK” patients

The Group 1-Study of LCH-III is a randomized controlled clinical trial for patients with multi-system LCH WITH involvement of “RISK” organs (hematopoetic system, liver, spleen or lungs). The random assignment to the treatment arms will be done in blocks,. stratified according to local subcenters to ensure a balance within the local subcenters and within each pre-specified number of treatment assignments (=block). The randomization will be done in the local subcenter without delay after diagnosis.

16.1.2 Group 2 “LOW RISK” patients:

The Group 2-Study of LCH-III is a randomized controlled clinical trial for patients with multi-system LCH WITHOUT involvement of “RISK” organs (hematopoetic system, liver, spleen or lungs) and initial response at week 6 (NAD/AD better).

The random assignment to the treatment arms will be done in blocks stratified according to age at diagnosis (≤ 2 years, > 2 years) and local subcenters to ensure a balance within age groups, the local subcenters and within each pre-specified number of treatment assignments (=block). The randomization will be done in the local subcenter without delay after the first response evaluation 6 weeks after therapy start. Only patients with therapy response, i.e. regression (AD better) or resolution (NAD) after 6 weeks of initial treatment, will be eligible for randomization.

In addition, the impact of a prolonged initial treatment (= course2) for those patients who are not eligible for randomization is examined and compared with the historical control group of LCH-II patients.

16.2 ENDPOINTS

16.2.1 Primary endpoints

16.2.1.1 Group 1 “RISK” patients:

The primary aim of the study is to compare the therapeutic efficacy of **control arm A** (PDN+VBL) with the **experimental arm B** (PDN+VBL+MTX). The primary endpoint is

the proportion of **non-responder in risk organs to the initial treatment.**

Non-response to initial therapy is defined as:

- death within 12 weeks of initial treatment or
- progression (worse) in risk organs at week 6
- lack of response (=intermediate response or progression) in risk organs at week 12 as compared to the status of disease at week 6

If the null hypothesis is true, the two randomized treatment arms are equally effective in terms of non-response. If the alternative hypotheses is true, there is a difference between the two randomized arms in terms of efficacy.

16.2.1.2 Group 2 "LOW RISK" patients:

The primary aim of the study is to compare the reactivation free survival rate in initial responders at week 6 with continuation treatment for 6 months (Arm LR 6) versus 12 months (Arm LR 12) in those patients without disease reactivation within the first 6 months.

If the null hypothesis is true, the reactivation rate of both randomized arms are equal. If the alternative hypothesis is true, there is a difference between the two arms in terms of reactivation frequency.

16.2.2 Secondary endpoints

- Overall survival
- Proportion of responders (overall and in risk organs) at week 6
- Proportion of responders (overall and in risk organs) at week 12
- Reactivation free survival after response at week 12
- Time to NAD
- Incidence of permanent consequences
- Toxicity
- Reactivation free survival after NAD (overall and in risk organs) and for "RISK" patients a historical comparison of Arm A of LCH III with Arm A of LCH-II with respect to the frequency of reactivation to evaluate the impact of the

prolonged study duration in risk patients

- Vaule of a prolonged initial treatment (course 2) of non-responding “LOW RISK” patients compared to a historical control of LCH-II patients without “RISK” organ involvement

16.3 ANALYSES

The analyses of the primary and secondary endpoints will be done according to the intention-to-treat principle, i.e. the patients will be analyzed in their allocated treatment group, even in case of non-compliance or protocol violations.

The statistical analyses of the primary endpoint will be done with a two-sided significance level of 5 %. The statistical analyses of the secondary endpoints are exploratory. A separate analyses will be performed for each group (“RISK” and “LOW RISK”). In addition to the intention-to-treat analyses a secondary per protocol analyses will be done including all patients who were treated according to the originally assigned treatment arm without protocol violations (= unjustified dose modification, therapy delay and/or improper switch to another therapy within the first 12 months).

16.3.1 Analysis of primary endpoints

16.3.1.1 Group 1 “RISK” patients:

The Fisher’s exact test will be used to compare the proportion of non-responders in risk organs and the proportion of responders at week 6 and week 12.

16.3.1.2 Group 2 “LOW RISK” patients:

Reactivation (=progression in any organs) and death will be considered as events for the calculation of Reactivation Free Survival. The interval will start 6 months after therapy start, i.e. the time point when half of the patients are intended stop the continuation therapy (Arm LR6) whereas patients from the other randomized arm (Arm LR12) will further receive 6 months of continuation therapy. This means, randomized patients with reactivations within the first 6 months after randomization will not be included in the analyses. For censored patients the interval will be calculated until the date of the last response evaluation.

The proportion of reactivation free survival will be estimated according to the method of Kaplan-Meier and confidence intervals according to Dorey and Korn will be given for the reactivation free survival rate after 2 and 3 years.[18,19] The primary statistical evaluation of the treatment effect will be done by log rank-test.

As a secondary aim the question whether a prolonged therapy can slow down the speed of reactivation will be considered. Retrospective data from LCH-I and LCH-II and the DAL-studies indicate that the hazards between 6 and 12 months of continuation therapy are proportional and constant in time (i.e. exponentially distributed reactivation free survival times). Therefore, a Weibull accelerated failure time model will be fitted to evaluate an acceleration factor.[20] Moreover, a time dependent Cox regression model will be performed.[21]

16.3.2 Analysis of secondary endpoints

The overall survival time will be calculated from the date of randomization to death or the last response evaluation.

The reactivation free survival will be calculated from the date of initial response evaluation at week 12. Reactivation (=progression overall and in risk organs after response at week 12) and death will be considered as events. For censored patients the interval will be calculated until the date of the last response evaluation.

The time to non active disease (NAD) will be calculated from randomization to the date of NAD. For censored observation the interval will be calculated until the date of the last follow up information.

The reactivation free survival after NAD will be calculated from the date of NAD. Reactivation (reappearance or progression in any organ) and death will be considered as events. For censored patients the interval will be calculated until the date of last response evaluation.

The time to permanent consequences will be calculated from the date of randomization to the diagnoses of permanent consequences. Deaths without permanent consequences will be censored at the time of death. For all other censored patients the interval will be calculated until the date of the last response evaluation. For the comparison of treatment arms patients with permanent consequences which are already

present at therapy start will not be considered in the analyses.

The proportion of survival, reactivation free survival after response in week 12, reactivation after NAD, the time to NAD and the incidence of permanent consequences will be estimated by the method of Kaplan Meier. The comparison of the randomized arms will be done by log rank-tests.

The proportion of patients with severe organ toxicity (WHO score grade III-IV) within the first 12 weeks of treatment will be compared with Fisher's exact test.

To study the effect of prolonged initial therapy in Low Risk patients without response at week 6. the response rate at week 12 (compared to the status of disease at week 6) will be compared to the response rate at week 12 of the corresponding LCH-II patients. This will be done with Fisher's exact test.

16.4 INTERIM-ANALYSES

The primary aims of the two trials will be monitored according to a group sequential plan.

16.4.1 Group 1 "RISK" patients

The data will be monitored a total of 5 times in equally spaced intervals, i.e. after the response evaluation at week 12 of 20%, 40%, 60%, 80% and 100% of the total sample size.

Early stopping will be implemented either to reject the null hypothesis of no difference between the two randomized arms, or to retain the null hypotheses .[22] The design of the stopping boundaries is due to O'Brien-Fleming using the normal approximation of the binomial distribution.

16.4.2 Group 2 "LOW RISK" patients

The data will be monitored 5 times i.e. after 20%, 40%, 60%, 80% and 100% of the total number of events (see 13.4.1.2: Power Considerations) This schedule of interim analyses allows an equal distribution of the information between the interim analyses. Early stopping will be implemented either to reject the null hypothesis of no difference between the two randomized arms or to retain the null hypotheses[22]. The design of the stopping boundaries is according to O'Brien-Fleming.

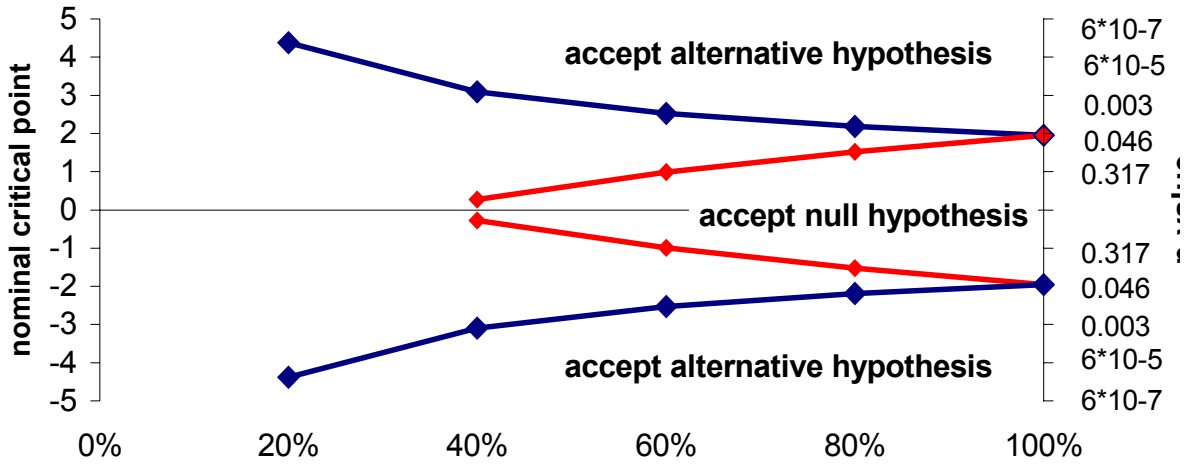

Figure 6: Design of the stopping boundaries

The O'Brien and Fleming boundary requires very strong evidence at the first interim analyses, whereas the criteria at the final test are rather close to those for a single sample design. The first opportunity for early stopping in favor of the null hypothesis will be at the second interim analyses.

In case the outer boundary is crossed during one interim analysis in one of the two trials the alternative hypothesis is accepted. If the inner boundary is crossed during one interim-analysis, enough evidence is collected to retain the null hypotheses of no difference between the two randomized arms in terms of the primary endpoint. In this case no ethical reason exists, which forces the closure of the trial. The results of the trial will be discussed by the study committee together with the independent statistical reviewers of the Institute of Medical Statistics and Documentation in Mainz. The decision to stop or continue the trial will include considerations on long term outcome, such as survival, reactivation and permanent consequences as well as toxicity. If it is decided to stop one trial because of futility, it is anticipated that the trial will be continued until the start of the subsequent trial.

16.5 POWER CONSIDERATION

16.5.1 Group 1 "RISK" patients:

The estimated proportion of non-responder in the control group is 50 %. With Arm B (with the addition of MTX) we aim to reduce the rate of non-response to 30%. To achieve a power above 80% we will need a total sample size of 202 patients to show this 20%-difference (two-sided α=5%. NQuery 3.0, Fisher's exact test). The group sequential design of the study will raise the maximum sample size to 228. If the null hypothesis is true, the expected sample size will be reduced to 143 patients. If the alternative hypothesis is true (i.e. a 20% difference in the proportion of non-responders between the two arms) the expected sample size is 158 patients[23].

In LCH-II we observed an average annual recruitment of 70 – 80 multi-system patients, of whom 60 % had risk organ involvement. This means that 42 – 48 patients will be eligible for randomization of arm A versus arm B . If the randomization rate is similar to LCH II i.e. 85% we will have approximately 35-40 randomizations a year. We assume that we will not be able to evaluate response at week 12 in 10 % of the patients because they will be lost to follow up. This means that we expect to reach the maximum sample size of 228 evaluable patients after a period of approximately 6 years. If the null hypothesis is true, the expected duration of the study will be 4.3 years. If the alternative hypothesis is true, this will be 4.7 years.

16.5.2 Group 2 "LOW RISK" patients:

Data from the previous LCH-II study indicate that the reactivation free survival rate of the control group (LR 6) is approximately exponential distributed with a 1-year reactivation free survival rate of about 65%. With this study we aim to show a 20% difference in the 1-year reactivation free survival rate (i.e. a 1-year reactivation free survival rate of 85% in patients with 12 month continuation treatment) with sufficient power.

To show a 20% difference at the 5% significance level with a power of 80% we would need a total of 36 events following the group sequential design (Jennison Turnbull).

85% of the patients with multi-system LCH without involvement of risk organs had an initial response. That means that with the expected annual recruitment of 40 multi-

system patients without risk organ involvement we can expect 34 patients per year who will be eligible for randomization. We expect that 85% of these patients will be randomized this means there will be 29 randomizations/year. About 5% of these patients will show a reactivation within 24 weeks of treatment, another 10% will possibly be lost to follow up within the first 24 weeks. Thus we will be able to recruit about 24 patients/year who will enter the analyses.

We considered a cautious estimated lost to follow up-rate in the calculation, which is assumed to be exponentially distributed with λ=0.664, i.e. about half of the patients are followed for more than one year. Given this lost to follow up rate and a minimum follow up of 2 years (period from entry of the last patients to the final analysis) following the group sequential design, we will a need a recruitment period of about 6 years and a maximum sample size of 148 patients.

16.6 STOPPING RULES

In patients without "RISK" organ involvement experience from LCH-II indicate that only a limited number of toxicities can be expected. Therefore for this subgroup no stopping rules will be implemented. Stopping rules for organ toxicity and early deaths are implemented only for patients with "RISK" organ involvement. In LCH patients the differentiation between treatment related toxicity and disease related organ dysfunction (liver, hematopoiesis, mucous membranes and skin) may present a diagnostic dilemma. For this reason organ dysfunction will be assessed according the WHO toxicity score already at diagnosis and throughout the follow up. Clear toxic events should be reported immediately. Interim analyses on severe organ toxicity (WHO score grade III-IV) within the first 12 weeks of therapy will be performed twice a year. The rate of severe organ toxicity within the first 12 weeks of therapy of both treatment arms will be compared by Fisher's exact test. A group sequential design according to Pocock will be applied to account for repeated significance testing to assure an overall significance level of α = 5%.[24] In addition we will monitor early deaths within the first 6 weeks of therapy. In arm A of the LCH II study, 5 early deaths within 12 weeks of therapy were observed among 50 patients (10%, 95% CI 5% – 23%). Based on this previous experience, we consider an early death rate below or equal to 10% as acceptable. An early death rate of 15% as unacceptably high. We will perform Wald's sequential ratio test.[25] The upper boundaries

we will use will be shown in figure 7.

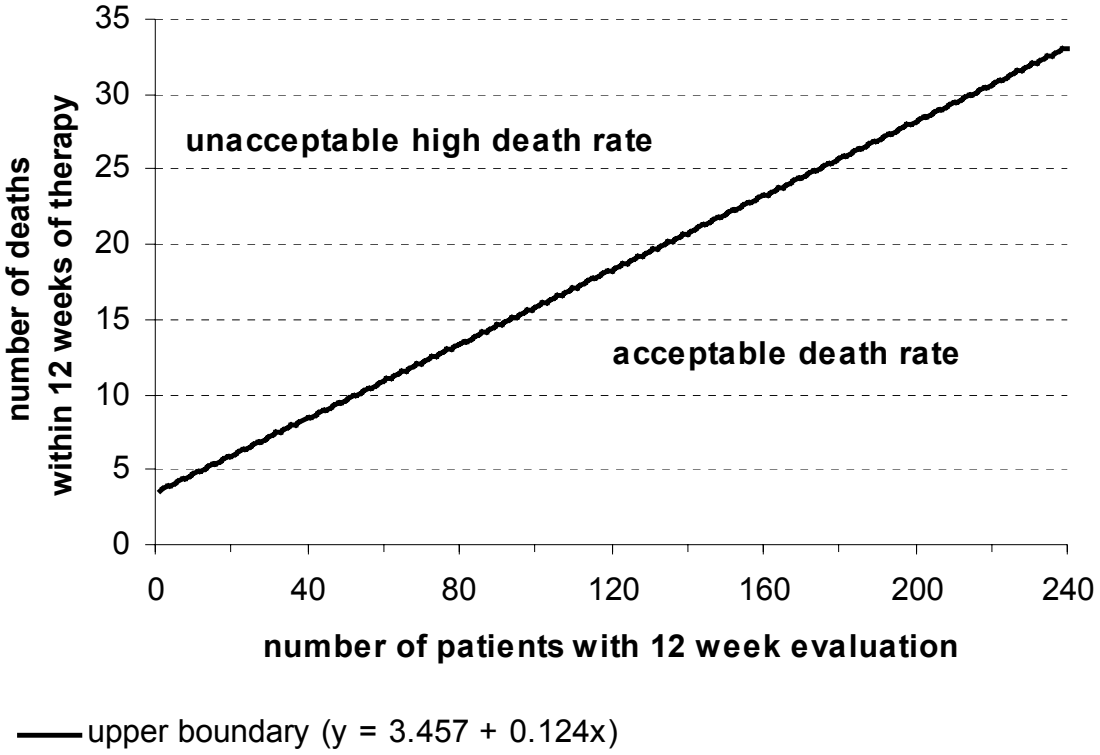

Figure 7: Boundaries for deaths within 12 weeks

Simulations show that with this boundary the risk to wrongly conclude that there is an excess of early deaths (whereas the real event rate is below or equal to 10%) is 13%. On the other hand, the power to detect an excess of early deaths of 15% or more will be 78%.

If we find significant differences in toxicities or if the number of early deaths observed, reaches the boundary defined by the sequential plan, a full analysis will be performed, and the results will be discussed among the members of the study committee together with the independent biometrical reviewers. The study committee together with the independent biometrical reviewers will decide, whether and how the study will proceed.

17 PUBLICATION

Publication of overall study data or projects arisen from the overall study population may be undertaken only with the agreement of the study committee.

Every subcenter or participating clinic may publish their own observations related to LCH patients or data on specific research projects not concerning questions of the overall study.

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