LCH – A1

First International Study

for

LANGERHANS CELL HISTIOCYTOSIS IN ADULTS

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1 BACKGROUND

1.1 INTRODUCTION

Langerhans cell Histiocytosis (LCH) (OMIM 604856) is a rare disorder characterized by the dysregulated growth, activity and trafficking of Langerhans cells (LC). 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 extents (1). LCH may affect any age group, from newborn to elderly people. For several reasons the disease has been better recognized in children and thus most of the available information concerning clinical features, pathogenesis, and treatment outcome derives from the pediatric experience which will be thus summarized below.

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. Patients with disease that is localized, or "*single system*" (SS) as it is often currently defined (skin, bone or lymph node) have a good prognosis and are felt to need minimum or even no treatment. In contrast, multiple organ involvement, ("*multisystem*", MS) which is particularly frequent in young children under 2 years, carries the risk of a poor outcome (2,3). 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 (4-6).

1.2 CLINICAL INFORMATION ON LCH IN ADULTS

Limited experience is available so far on LCH in adult patients. Only a few reports are available in which series of patients in which LCH was diagnosed in adulthood are described.

In 1991 Axiotis (7) reviewed 42 cases of LCH involving the female genital tract. This disease may involve the vulva, vagina, cervix, endometrium, and ovary. Four distinct patient groups, segregated on the basis of initial presentation and subsequent anatomic extent of disease, were categorized as follows: (1) "pure" genital LCH, (2) genital LCH with subsequent multi-organ involvement, (3) oral or cutaneous LCH with subsequent genital and multi-organ involvement, and (4) diabetes insipidus with subsequent genital and multi-organ disease. Involvement of the genital tract was most common in young adulthood; the major pathologic differential diagnosis is venereal and other inflammatory diseases. The pure genital form may have a distinct nosologic position in the spectrum of LCH similar to the "pure," self-limited cutaneous

histiocytosis seen in infants. There is no correlation between histologic findings and the outcome of the genital lesions. There is also no correlation between clinical presentation and/or the extent of involvement and outcome of genital lesions; complete regression, partial improvement, persistent lesions, and recurrences were seen in all four groups of patients. The treatment of genital LCH is not well defined and is highly individualized. Therapy has included surgery, radiation, topical corticosteroids, topical nitrogen mustard, systemic chemotherapy, and combination therapy; mixed results were obtained with all treatment modalities. Although no modality has been shown to yield a superior outcome, the authors suggested complete surgical excision as initial therapy.

In 1996 **Malpas & Norton** (8) described 47 well-documented patients with LCH, in which a slight female preponderance was observed, with onset as late as the ninth decade. The skin was the commonest site of presentation, but pulmonary and bone involvement was frequent. Patients with single-site disease did best. The worst prognosis was seen in the elderly or those with organ dysfunction. A high incidence of associated malignant disease was seen, which could precede, be coincidental with, or occur after a diagnosis of LCH.

In 1997 **Baumgartner** et al (9) reported 19 patients meeting the histopathologic criteria of presumptive LCH who were followed for 1.5-20 years (average 7.7 years). Most frequently, skeletal lesions (16 patients), diffuse interstitial lung infiltrates (seven patients), and pituitary gland involvement with diabetes insipidus (four patients) were present. Bone lesions of the skull and axial skeleton were associated with an infiltration of adjacent soft tissues in 10 of 16 patients. Liver, lymph node, and bone marrow involvement appeared sporadically. LCH was divided into localized or multifocal form. Localized disease took a benign course with remission of bone (n = 4) or lymph node lesions (n = 2). Also, in isolated pulmonary LCH (n = 2), spontaneous transition to inactive disease occurred. With the exception of isolated bone lesions (n = 27), which remained asymptomatic or showed a remission to treatment, multifocal LCH had a more aggressive course. Osseous lesions with adjacent soft tissue infiltration (n = 20) showed a relapse rate in excess of 80% independent of the treatment applied. Pulmonary involvement led to a more marked functional impairment compared to the isolated form, and systemic treatment yielded no convincing effect. In three patients with liver or bone marrow involvement, LCH showed a persistent, serious disease activity. One patient died of transition into acute monomyelocytic leukemia 18 months after diagnosis without preceding chemotherapy. Their conclusion was that in adults, LCH seems to be limited to a few organ systems, and multifocal LCH represents the more aggressive form with unfavorable prognosis in patients with bone lesions spreading into the adjacent soft tissue and liver or bone marrow involvement.

In 1999 Howarth et al (10) described information on 314 Mayo Clinic patients with histologically proven LCH, taken from the case history notes and tumor registry correspondence. The median time of follow-up was 4 years (range, 1 month to 47.5 years). The age of the patients ranged from 2 months to 83 years. Of the 314 patients, there were 28 deaths. Multisystemic LCH was found in 96 patients, 25 of whom had continuing active disease after treatment. Isolated bone LCH lesions were observed in 114 of the 314 patients, 111 of whom (97%) achieved disease free survival after treatment. The most common sites of osseous LCH were the skull and proximal femur. Of the 87 patients with isolated pulmonary involvement, only 3 were nonsmokers. After treatment with corticosteroids (with or without cyclophosphamide or busulphan), 74 patients achieved disease free survival, but 10 patients died. Diabetes insipidus was found in 44 patients. After treatment, 30 of these patients had disease free survival, but all required long term hormone replacement with desmopressin acetate. Lymph node involvement was found in 21 patients, and mucocutaneous involvement was found in 77 patients. Patients with isolated bone LCH lesions had the best prognosis compared with patients with LCH involvement of other systems. By contrast, 20% of patients with multisystem involvement had a progressive disease course despite treatment.

In 2000 **Kaltsas** et al (11) described Hypothalamo-pituitary abnormalities in 12 adult patients with histologically proven LCH and DI, followed up for a median of 11.5 yr (range, 3-28 yr) after the diagnosis of DI was made. Eleven patients received systemic treatment of different types, and 5 patients received external beam radiotherapy confined to the HPA. The median age at diagnosis of DI was 34 yr (range, 2-47 yr); DI was the presenting symptom in four patients, whereas the remaining eight each developed DI 1-20 yr (median, 2 yr) after the diagnosis of LCH. Eight patients developed one or more anterior pituitary hormonal deficiencies at a median of 4.5 yr (range, 2-22 yr) after the diagnosis of DI. All patients developed disease outside of the hypothalamus during the course of the study, and no fluctuation of disease activity in the HPA region was noted. They concluded that anterior pituitary hormonal deficiencies developed in 8 of 12 patients with hypothalamic LCH and DI, over the course of 20 years. Radiotherapy was useful in achieving local control of tumor, but established anterior, posterior pituitary, and other NEH dysfunctions did not improve in response to current treatment protocols.

1.3 PULMONARY LCH : AN INTRIGUING PROBLEM

Pulmonary LCH is an uncommon disorder occurring most often in young smokers. Histologically, the disease is characterized by granulomatous lesions containing LC that destroy distal bronchioles. The etiology of the disease remains unknown, but progress has been made in understanding its pathogenesis. Modifications in the bronchiolar epithelium induced by smoking, such as the increased secretion of GM-CSF by these cells, are probably responsible for the initial accumulation of large numbers of LC. However, given the rarity of pulmonary LCH compared with the frequency of smoking, an as yet unidentified genetic predisposition may also be necessary for the development of the disease. Although LC in LCH granulomas may be clonal in origin, several observations argue against the idea that the disease, which can regress spontaneously, is a malignant process. Cells of dendritic cell lineage (including LC), are potent antigen presenting cells, suggesting that pulmonary LCH results from an uncontrolled immune response initiated by LC. Consistent with this idea, LC and T-cells are the predominant cell populations found in the early lesions of pulmonary LCH, and unlike LC in the normal bronchial mucosa and those accumulating in other pathologic situations, LC in pulmonary LCH granulomas express surface molecules important for the activation of T-lymphocytes. A number of mediators are produced in the microenvironment of granulomas that probably influence the outcome of the local immune and inflammatory reaction. Ultimately, precise knowledge of the pathogenesis of this disorder should permit the development of specific treatment. In the interim, therapies aimed at modifying the state of activation of LC in the granulomatous lesions may prove useful (12).

In the attempt to define possible clonality of pulmonary LCH, Yousem et al (13) used the X-linked polymorphic human androgen receptor assay (HUMARA) locus to assess clonality in female patients with one or more discrete LCH cell nodules in open lung biopsies. LC were excised from formalin-fixed, paraffin-embedded tissue by microdissection to assure a relatively pure cellular population, and studies for differential methylation patterns at the HUMARA locus were performed. Twenty-four nodules in 13 patients were evaluated. Seven (29%) were clonal and 17 (71%) were nonclonal. Of six cases with multiple discrete nodules, three (50%) showed a nonclonal LCH cell population. In one biopsy with five nodules, two nodules were clonal with one allele inactivated, one nodule was clonal with the other allele inactivated, and two nodules were nonclonal. Thus, possibly at differnce with the systemic form, pulmonary LCH appears to be primarily a reactive process in which nonlethal, nonmalignant clonal evolution of LCH cells may arise in the setting of nonclonal LCH

cell hyperplasia. Cigarette smoking may be the stimulus for pulmonary LCH in contrast to other forms of LCH.

Whether or not accumulation of large numbers of LCs in the course of the disease depends on their proliferation or prolonged survival, remains controversial. In 1998 **Brabencova** et al (14) reported their study of the proportion of replicating LCs in biopsied granulomas from patients with localized or diffuse form of LCH by means of several histopathological techniques currently used in assessment of cell proliferation. They found that, except for proliferating cell nuclear antigen (PCNA), all parameters measured are low in all forms of the disease. They are similar to those of renewing epithelial cells and clearly less than those of neoplastic cells. They concluded that LCs in LCG granulomas are not a rapidly dividing cell population and that local LC replication makes only a minimal contribution to granuloma maintenance.

These data are apparently not in keeping with more recent data on expression of cell cycle related gene products in LCH. They showed that lesions of 30 LCH patients stained positive for Ki-67, p53-p21 and p16-Rb pathways. These data suggest that besides an hyeractive cell cycle, the LCH lesion is based on an imbalance between proliferation and apoptosis.

The above described persisting uncertainties on the pathogenesis of LCH have certainly limited current capability of treating the disease. Furthermore, while cooperative effort including the two randomised trials conducted in pediatric patients have produced a considerable amount of solid information, this is not yet available for those issues of the disease which are not shared by adults and children. This is the case of pulmonary LCH. Despite most of the adult patients with LCH do have pulmonary involvement, empirical approach prevented advances in this field.
In 1964 Lewis described 12 patients with LCH either had pulmonary involvement or isolated pulmonary disease (13)
In 1978 Basset et al provided the first review of pulmonary histiocytosis X (**14**). In 1981 Friedman et al (15) reviewed 100 cases of "eosinophilic granuloma" diagnosed by open lung biopsy. They were 60 women and 40 men, ages 18 to over 60 years. The outcome in 60 available cases was generally benign; the 16 asymptomatic patients remained well; 17 others had complete remission of symptoms, 22 had persistent symptoms, though half had partial improvement; 4 patients had progressive disease despite treatment, but only 1 patient died (of bilateral pneumothoraces complicating severe fibrosis). The more severe manifestations were found in young men, who had a higher incidence of pneumothorax, fibrosis and honeycombing, and diabetes insipidus. The effectiveness of treatment with steroids could not be assessed because of lack of controls; some individuals appeared to benefit, but relapse was very unusual in any case. The observation that smoking was far more common among these patients (97%

altogether; 80% current) than in the general population (about 35%) was considered, at that time, an unexplained finding.

Recurrent reports of improvement or even spontaneous resolution following smoking cessation (16**)** have supported the concept that this is a mild disease which does not request an aggressive approach. Furthermore, the concept that this is an inflammatory disorder have largely supported the empirical use of steroids, either in short pulse or long-term exposure, for treatment of pulmonary LCH.
In their recent review Vassallo et al. (17) reviewed the records of 102 adults with histopathologically confirmed pulmonary LCH to ascertain their vital status and whether cancer had been diagnosed. After a median follow-up period of 4 years (range, 0 to 23) there were 33 deaths, 15 of which were attributable to respiratory failure. Six hematologic cancers were diagnosed. The overall median survival was 12.5 years, which was significantly shorter than that expected for persons of the same sex and calendar year of birth (P<0.001). In a univariate analysis, variables predictive of shorter survival included an older age (P=0.003), a lower forced expiratory volume in one second (FEV1) (P=0.004), a higher residual volume (P=0.007), a lower ratio of FEV1 to forced vital capacity (P=0.03), and a reduced carbon monoxide diffusing capacity (P=0.001).
Since respiratory failure is a limiting factior in survival and quality of life of patients with pulmonary LCH, lung transplantation has been employed in children and in adults with advanced LCH. Although this has providede some benefit to some patients with limited pulmonary function, failures are reported due to local recurrence of LCH after transplantation (18-21)**.**

1.4 EXPERIENCE OF TREATMENT OF LCH IN CHILDREN

On April 1st, 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) occured 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 initial responders and only 34% for the nonresponders. This finding clearly indicated the impact of response to initial treatment.

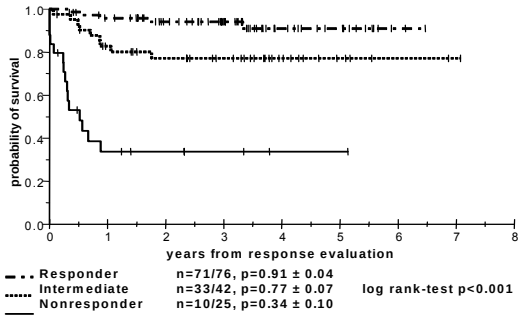


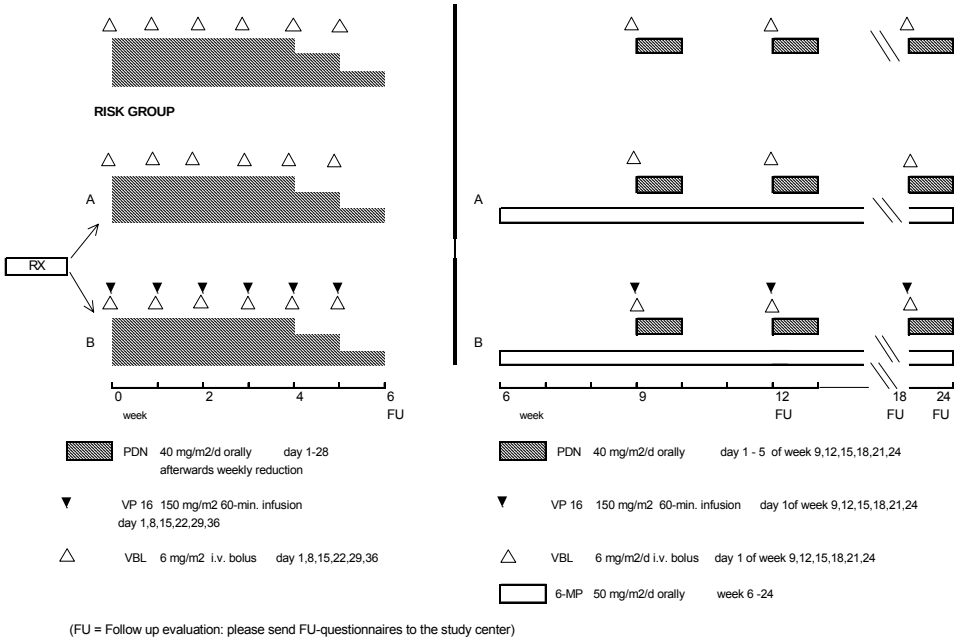
Figure 1. Survival by response at week 6

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 the initial response and the probability of reactivations, nor with respect to mortality (22-Gadner 2001).

The results of the LCH-I study were compared with the results of the preceding **DAL-HX83 - and DAL-HX90** 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 prednisonse, 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 HX83/90 results showed a clear superiority of combination therapy given for one year with respect to initial response and rate of reactivation. Surprisingly, the mortality rate did not significantly differ between the 2 studies.

It was the goal for the next internationl 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 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.
A new stratification system was adopted, distinguishing between "RISK" patients with involvement of "RISK" organs like liver,spleen, lungs, hematopoetic system or lungs 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.



(FU = Follow up evaluation: please send FU-questionnaires to the study center)

Figure 2. Treatment plan of LCH II

Between May 1st, 1996 and December 31st, 2001, 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. 176 of these were randomised (76%), 88 each to arm A and arm B. 66 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.

In the "LOW RISK" group there were 89% of responders, only one non-responder at week 6, and no fatality.
Among 170 "RISK" paitents, in whom the response at week 6 was available, 113 (66%) were judged as responders. This compares favourable to the 6-week reponder rate of 44% in the the LCH I study, but is less thean the 76% rate of responders in the DALHX studies (23).
Interestingly, the overall probability of survival of the multisystem patients did not differ significantly between 3 studies - DALHX, LCHI 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, in whom the response information was available, 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, i.e. that at week 12 still 50% of the patients with "RISK" organ involvement had not shown a response to treatment, but had intermediate active or progressive disease. The probabilty of mortality for these patients about 75%, whereas the probability of becoming free of disease is less than 20%.

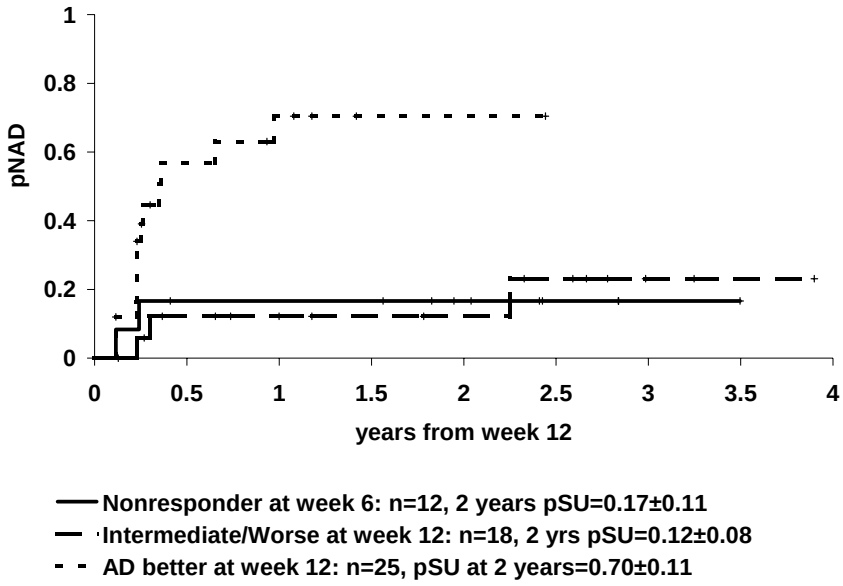


Figure 3: LCH II - Probability of becoming free of disease (NAD) in patients with "RISK" organs

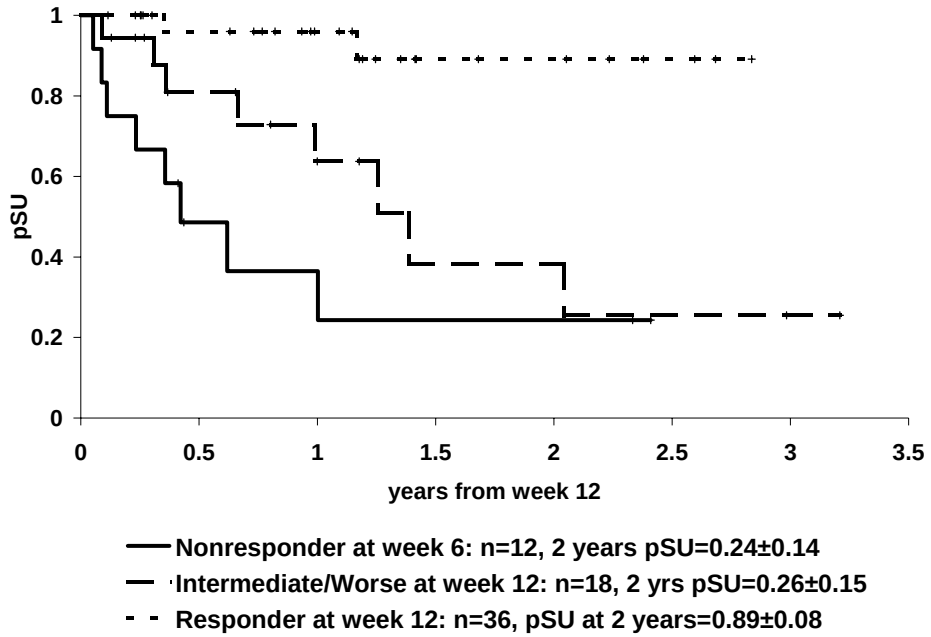


Figure 4: LCH II - Probability of Survival by response at week 12 in patients with "RISK" organs

Thus, patients with "RISK" organ involvement who do not show disease regression by week 12 of therapy have high risk of poor outcome. These patients may benefit from new agents in the initial therapy 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 therapy as the most important prognostic factors, whereas young age under 2 years is not anymore considered to be of independant 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 the response in patients who did respond 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. Similar results were seen when we looked at the "RISK" and "LOW RISK" groups seperately. These observations indicate a potential benefit of prolonged treatment duration.

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 showed no significant difference with respect to initial response, survival and event free survival (Gadner et al. for the Histiocyte Society, unpublished data). The conclusions of the LCH-II study were the following:

1. Risk organ involvement and poor response to initial therapy proved to be the most important prognostic factors
2. Patients with "RISK" organ involvement who do not achieve a response (NAD or AD better) to initial therapy by week 12 carry an about 75% risk of fatal outcome. The probability of becoming free of disease is less than 20% for such patients with standard therapy.
3. Age under two years at diagnosis without "RISK" organ involvement was not associated with a poor outcome and will not be considered for the initial stratification.
4. 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.
5. The fatality rate was around 20% in all 3 studies which were using combination therapy, monotherapy, or 2-drug and 3-drug regimen mainly including PDN, VBL, and VP-16. This observations points towards a need for new agents in the initial treatment for patients with "RISK" organ involvement.
6. The retrospective comparison of the DAL HX, LCH I and II studies indicates that prolonged duration of treatment may reduce the rate of reactivations (Fig.5).

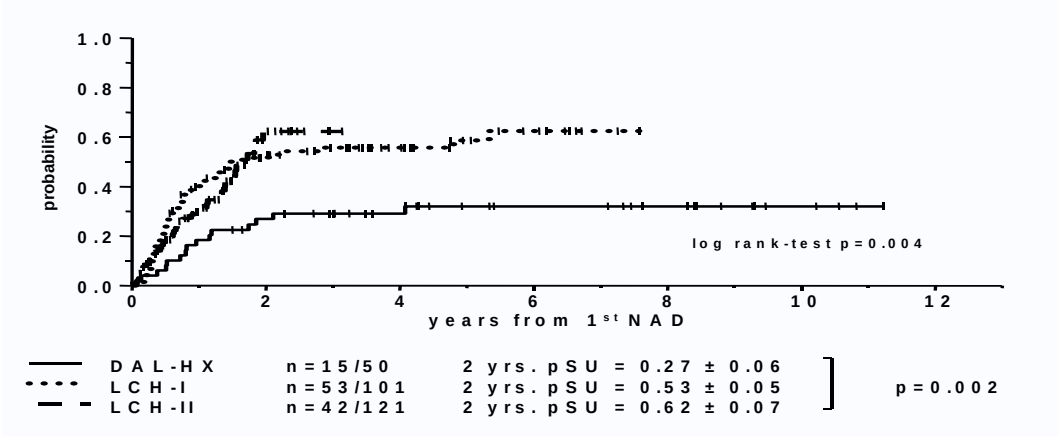


Figure 5. Reactivations after NAD in different studies for LCH

"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 and anterior and middle cranial fossa (temporal, sphenoid, ethmoid, cygomatic bone, the orbital bones) with intracranial tumour extension carry an about 3-fold risk for the development of diabetes insipidus (DI) which is one hallmark of central nervous system involvement in LCH. Based on these data it was concluded that for patients with one sngle, this 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 and these patients should be rather receive systemic treatment.

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. Systemic therapy should be initiated immediately even if the lesions represent the only site of disease.

1.5 CLINICAL INFORMATION ON TREATMENT OF DISSEMINATED LCH IN ADULTS

In 1992 **Tsele** et al (24) reported their evaluation of three adult patients with severe or resistant LCH (one single-system skin disease, two with multisystem disease), treated with etoposide, 100 mg/m2/day, for 3 days. This was repeated every 3 or 4 weeks for three or four cycles. All patients achieved clinical remission that persisted during a 12- to 14-month follow-up. No serious side effects were noted (REF).

In 1997 **Giona** et al (25) reported a single center retrospective study of 11 adult patients with LCH. Based on the sites and extent of disease at diagnosis, patients were divided into four categories. Group A was comprised of four patients with unifocal bone disease who had surgical curettage. At last follow-up only 1 patient was in continuous complete response, i.e. non active disease (NAD) at 29+ months. The other 3 patients recurred at 3, 12, and 30 months, respectively, after surgery and at last follow-up were found to be in NAD at 16+, 48+, and 124+ months, respectively, after therapy with VBL and high dose methylprednisolone (HDMP). Group B was comprised of three patients with multifocal bone disease. Two of these patients received VBL + HDMP; at last follow-up, 1 patient was in NAD 8 months after completion of therapy, and the other developed progressive disease 11 months later. The third patient was treated with interferon (IFN) and at last follow-up was in NAD at 35+ months. Group C was comprised of 2 patients with bone and visceral disease who were treated with VP-16 + HDMP; at last follow-up, 1 patient was in NAD at 42+ months and the other patient, who had isolated vulvar recurrence 16 months later, was in NAD with treatment with local IFN. Group D was comprised of two patients with lung and lymph node involvement, one of whom was treated with VP-16 + HDMP and the other with cyclophosphamide, doxorubicin, vincristine, and prednisone; at last follow-up, both were in NAD at 30+ and 71+ months, respectively. They concluded that VBL + HDMP showed efficacy in patients with bone disease, in particular those treated for recurrent LCH after surgery. Therapy with VP-16 and HDMP was successfully employed in patients with visceral disease. IFN was effective both for localized disease and in patients with multiple bone lesions.

Langerhans-cell histiocytosis (LCH) results from the accumulation of tissue histiocytes derived from the same progenitor cells as monocytes. Because cladribine is potently toxic to monocytes, **Saven & Burian** conducted at the Scripps Clinic a phase II trial of cladribine (26). Cladribine was administered to 13 LCH patients at 0.14 mg/kg per day by 2-hour intravenous infusion for 5 consecutive days, every 4 weeks for a maximum of six courses. Median age was 42 years (range, 19 to 72) and median pretreatment disease duration was 99 months (range, 6 to 252). One patient

was untreated, one had received prior prednisone only, one prior radiation only, six prior radiation and chemotherapy, and four prior surgery, radiation, and chemotherapy. Seven patients had cutaneous involvement, six multifocal osseous, six pulmonary, two each with soft tissue and nodal involvement, and four had diabetes insipidus. Of 13 patients, 12 were evaluable for response and all for toxicity. After a median of three courses (range, 1 to 6), seven (58%) patients achieved complete responses (two pathologic and five clinical) and two (17%) patients achieved partial responses; overall response rate, 75%. Median response follow-up duration was 33 months (range, 1 to 65). Seven patients experienced grade 3 to 4 neutropenia. Only one patient had a documented infection, dermatomal herpes zoster. At a median follow-up of 42 months (range, 5 to 76), 12 patients remain alive and one patient has died. Their conclusion was that cladribine has major activity in adult LCH and warrants further investigation as a single agent and in combination with other drugs.

RATIONALE FOR THE USE OF VINBLASTINE AND PREDNISONE IN ADULT PATIENTS WITH LCH

Adult patients with multisystem LCH appear to behave like children with MS disease. Evidence accumulated by the pediatric trials showed very clearly the advantage of treating MS disease. At present standard chemotherapy regimen for MS LCH in children is the combination of VBL + PDN given over a period of twelve months. We plan to apply the same chemotherapy regimen in adult patients to confirm its efficacy, so establishing in a multicenter international trial the standard treatment for MS adult LCH.

2 LCH-A1 STUDY GOALS

GOALS of the trial are:

- To define and implement an uniform initial evaluation and stratification of adult patients with LCH
- To define and implement an uniform treatment approach for patients with single system disease, excluding:
  - isolated osteolytic lesions which are no at risk of relevant orthopedic complications;
  - isolated lesions which have been completely excised;
  - CNS lesions
- To define a common therapeutic strategy for patients with multisystem or pulmonary isolated LCH

- To explore the therapeutic efficacy on adult patients of the standard regimen for multisystem LCH in children, i.e. the combination of vinblastine + prednisone. Endpoints will be survival, reactivation-free survival, permanent sequelae.
- To address in a randomized fashion the question whether an extended continuation therapy may reduce disease reactivations occurring after treatment completion in patients with multisystem LCH.
- To describe the natural history of isolated pulmonary disease and in particular the role of smoking cessation on the disease course.
- To explore the therapeutic efficacy of steroid monotherapy on adult patients with isolated pulmonary disease showing disease progression

3 PATIENT'S ELIGIBILITY

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

- **Age comprised between 18 and 50 years**

- **Definitive diagnosis of LCH**

- **No prior antiblastic treatment for LCH**

Exclusion criteria from this study will be the following:

- Patients with severe impairment of clinical condition including: severely impaired pulmonary function (for example TLC<60%, FEV1<30%, DLCO<30%, PaO2< 60 mmHg), long term oxygen therapy or cor pulmonale.
- Treatment with immune suppressive agents and/or biphosphonates within 4 weeks from baseline evaluation.
- Pregnancy
- Other associated conditions which in the opinion of the attending physician might significantly impair the possibility to deliver treatment or to evaluate its impact on the clinical course of the disease.

4 STUDY REQUIREMENTS

- Confirmation of the histopathological diagnosis according to the criteria defined by the Histiocyte Society for all patients who require treatment. Thus provisional "presumptive" diagnosis of LCH" based on clinical and radiological findings will be

accepted only for patients enrolled in a prospective observational arm. When these patients will need any treatment according to this protocol, histological diagnosis will be required.

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

- Insurance for this study has to be addressed according to the local policy. Unless specific funding will be available in the future, no central insurance is being provided by the study coordination. Please consider that in many countries this study has been considered as the best available clinical standard for such patients an for this reason the treating hospital may accept to keep this treatment under the insurance held by the hospital for general clinical practice.

4.1 HISTOPATHOLOGICAL DIAGNOSTIC CRITERIA

4.1.1. DEFINITIVE DIAGNOSIS requires the demonstration of CD1a antigenic determinants on the surface of lesional cells.

4.1.2. DIAGNOSIS is justified when the lesion is characteristic by light microscopy and the lesional cells show the presence of two or more of the following features: positive stain for ATPase, S-100 protein, or alpha-D-mannosidase, or characteristic binding of peanut lectin in lesional cells.

4.1.3. PRESUMPTIVE DIAGNOSIS is warranted when findings, on study of conventionally stained biopsy material alone, are merely "consistent" with those defined in the literature.

It is highly recommended to obtain, whenever possible and with the patient's informed consent, **additional biopsy material for research purposes as well as a peripheral blood sample to be used for extraction of constitutional nucleic acyds.**

4.2 BASELINE DIAGNOSTIC EVALUATIONS TO BE PERFORMED IN ALL PATIENTS

4.2.1 CLINICAL EVALUATION

4.2.1.1 Complete history:

- Family history (including ethnic origin, possible consanguinity and undiagnosed disorders compatible with LCH, thyroid disease, diabetes, autoimmune disorders)
- Cigarette smoking (number per day and duration) and exposition to environmental mineral or organic dust (particularly occupational++)
- General symptoms: fever, weight loss, loss of appetite, asthenia, night sweats
- Respiratory alterations: cough, dyspnea at exertion or rest (graded according to the NHYA classification), chest pain (rib lesion or more frequently pneumothorax), hemoptysis*, wheezing.

*NB: *: the presence of hemoptysis in an adult with pulmonary LCH should not be attributed to the underlying disease until other causes such as bronchogenic carcinoma have been excluded.*

- Diarrhea
- Polydipsia / polyuria
- Recurrent / refractory otitis or ear discharge
- Recurrent / refractory skin rashes

4.2.1.2 Complete physical examination including:

- measurement of temperature, height, weight, blood pressure, heart rate
- skin and skalp rashes, purpura, bleeding
- aural discharge,
- orbital abnormalities
- gum and palatal lesions, dentition
- soft tissue swelling, lymphadenopathies
- cough, dyspnea, crackles, wheezing, pneumothorax, clubbing, cor pulmonale
- liver and spleen size, ascites, edema, jaundice
- neurological examination (including papilledema, cranial nerve abnormalities, cerebellar dysfunction)
- ano-genital examination

4.2.2

LABORATORY AND RADIOGRAPHIC EVALUATION

4.2.2.1 Mandatory minimum baseline evaluations for ALL patients:

- Hemoglobin, White blood count and differential, Platelet count
- Erythocyte sedimentation rate (ESR), CRP
- Creatinine, BUN, Sodium and Potassium
- Liver enzymes and function tests (SGOT, SGPT, γ-GT, alkaline phosphatase, bilirubin, total protein, albumin)
- Coagulation studies (PT, PTT, fibrinogen)
- Fluid balance
- Skeletal radiograph survey. NB: radionuclide bone scan may be not as sensitive as the skeletal radiograph survey in most patients.
- Chest radiograph, p.a.; NB: Chest HR-CT is definitely useful in all patients, even with no evidence of alteration at chest film, but is mandatory in all cigarette smokers.
- Abdominal ultrasound

4.2.2.2 Mandatory minimum baseline evaluations for patients with pulmonary (isolated or combined) disease:

- Physical examination (see above)
- Blood count (see above)
- Serology for HIV (upon informed consent!)
- Exclusion of other conditions including: HIV (excavated pneumocystosis), mycobacterial or other infections, Wegener disease, excavated metastatic carcinoma, alveolar carcinoma, sarcoidosis, septic emboli, lymphangiomyomatosis (LAM) in women.
- Accurate evaluation of cigarette smoking, with number of cigarettes per day and time of exposure and exposition to environmental mineral or organic dust (particularly occupational++).
- Chest radiograph, p.a. and lateral
- Pulmonary HR-CT (with expiratory slices) (please consider to apply computerized system for calculation of cumulative cystic volume)
- Pulmonary function testing including: (plethysmography) TLC, VC, RV; FEV1; FEV1/FVC, flow/volume curve, TLCO, blood gases.
- Bronchoalveolar lavage: total cell counts, differential, CD1a immunostaining (see recommended procedure), exclusion of microorganisms.
- Six-minutes walk test (American thoracic society statement).
- Echocardiography (for evaluation of pulmonary hypertension)

IMPORTANT REMARK:

** please consider that pulmonary LCH cannot be diagnosed only by BAL. In most cases the finding of increased percentage of CD1a+ LC in the range of 1-4% should not be taken as specific enough, especially in patients who are cigarette smokers. Thus, especially for patients who deserv specific therapy,* ***lung biopsy is to be considered mandatory.*** *The only exception should be made for those patients in whom the lung cystic degeneration is so advanced as to support the suspect that lung biopsy might be harmful. Yet, in such rare cases the diagnosis is usually not difficult.* ***Most of these patients would present exclusion criteria from the protocol.*** *On the contrary, this study is aimed to describe the natural history of lung disease especially starting from the initial, oligosymptomatic stages, in which differential diagnosis from other conditions should fully explored.*

4.3 DEFINITION OF SPECIFIC ORGAN INVOLVEMENT

• **Hematopoetic involvement: with or without bone marrow involvement ***	anemia: hemoglobin <10 g/dl, (exclusion of iron deficiency or other causes) Leukocytopenia: leukocytes < 4,0 x109/l, Thrombocytopenia: platelets < 100 x 109/l

*** Important** : Bone marrow involvement is defined as the 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 myleofibrosis are regarded as secondary phenomena

• **Spleen involvement**:	enlargement **≥ 2 cm below costal margin** (exclusion of iron deficiency or other causes)
• **Liver involvement**: **an**d/or	enlargement **>3 cm below costal margin** total protein < 5,5 g/dl, albumen < 2,5 g/dl (not due to protein loosing enteropathy) ascites: edema hyperbilirubinemia: total bilirubin < 1,5 g/dl (exclusion of other causes including liver cirrhosis)
• **Lung involvement:**	typical changes on HR-CT with or without functional impairment and/or histopathologic diagnosis.

4.4 EVALUATIONS REQUIRED UPON SPECIFIC INDICATION

(modified according to the Clinical Writing Group of the Histiocyte Society (Med Ped Oncol, 17, 492-495, 1989)

INDICATION	TEST
Patients with pulmonary disease (symptomatic or radiological)	HR-CT Scan Pulmonary function Broncho-alveolar lavage (BAL); Echocardiography; Lung biopsy when appropriate
Unexplained chronic diarrhea or failure to thrive, evidence of malabsorption	Small bowel series and biopsy; stools culture and colonoscopy
Liver dysfunction to differentiate active LCH of the liver from sclerosing cholangitis	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, hypogonadism	Endocrine evaluation including water deprivation test, dynamic tests of the anterior pituitary MRI of brain with i.v. gadolinium - DTPA
Gingival 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
Vaginal inflammation, ulcer, discharge	Gynecological evaluation

5 STRATIFICATION

GROUP 1 :

- Patients with **single system** disease which requires systemic treatment and in particular :
  - Multifocal bone disease
  - Vertebral lesions which present with significant soft tissue masses that may lead to spinal cord compression
  - Single system "CNS-risk" disease, defined as involvement of the facial bones and anterior and middle cranial fossa (temporal, sphenoid, ethmoid, cygomatic bone, the orbital bones) with intracranial tumour extension

GROUP 2 :

- **Multisystem** patients

GROUP 3 :

- Patients with **Isolated pulmonary disease**

6 REGISTRATION AND RANDOMISATION

6.1 REGISTRATION

Immediately after confirmed diagnosis of a new patient the registration form and the diagnostic evaluation forms have to be sent to the study subcenter. In case a local subcenter was not defined, please notify the study reference center by fax (+39-091-6666-001) or email (arico@ospedalecivicopa.org).

6.2 RANDOMISATION

The study includes one randomized question on treatment duration in group 2 (multisystem disease, MS).

Randomisation procedures

It is possible that in the near future a study-specific website will be available for remote data-entry including randomization request.

Until then, randomization for patients enrolled in **group 2** (MS) will be performed and assigned by the study reference center. Requests may be addressed either by fax (+39-091-6666-001) or email (arico@ospedalecivicopa.org).

The information on the assigned treatment arm and the randomisation number will be returned to the participant by email or fax.

7 TREATMENT

7.1 GROUP 1 "SINGLE SYSTEM"

According to current knowledge, single system LCH does not require any specific therapy in many cases. In particular, all cases of isolated osteolytic lesions in non-weight-bearing bones can be considederd at low risk for orthopedic sequelae and thus not an indication for specific treatment. Excision of the involved bone is not recommended, as well a radiotherapy, since these procedures might be responsible for sequealae and morbidity even more than the disease itself. For the same reason also chemotherapy is considered not necessary.
On the contrary, single system disease of the bone with multifocal involvement is considered an indication for systemic chemotherapy. Some "special sites" have been defined above, including "CNS-risk" lesions and vertebral lesions (see page 16).

This may also occur during the follow-up of patients previously diagnosed and registered only, since their clinical status was considered as compatible with a "wait-and-see" policy or with local therapy only.

NB: Single system CNS disease is excluded from this study since it is object of another specific study.

7.1.1.1 Initial treatment

- **Prednisone** (PDN) : 1 mg/kg/day **(not to exceed 60 mg)**, as a 4-week course, tapering over a period of 2 weeks
- **Vinblastine** (VBL) 6 mg/m^2 i.v. bolus **(not to exceed 10 mg)**, day 1, 8, 15, 22,29, 36

7.1.1.2 Continuation treatment

starting at day 43 after initial treatment:

- **6-mercaptopurine** (6-MP): 30 mg/m^2 **(not to exceed 50 mg)** daily until completion of treatment
- **Prednisone** (PDN) : 1 mg/kg/day **(not to exceed 60 mg)** day 1-5 every 3 weeks until completion of treatment
- **Vinblastine** (VBL): 6 mg/m^2 i.v. bolus **(not to exceed 10 mg)** day 1 every 3 weeks until completion of treatment (starting 3 weeks after the last VBL injection of the initial treatment or intensification)

Total duration of treatment: six months.

7.2 GROUP 2 "MULTISYSTEM" GROUP

7.2.1.1 Initial treatment

- **Prednisone** (PDN) : 1 mg/kg/day **(not to exceed 60 mg)**, as a 4-week course, tapering over a period of 2 weeks
- **Vinblastine** (VBL) 6 mg/m^2 i.v. bolus **(not to exceed 10 mg)**, day 1, 8, 15, 22, 29, 36

7.2.1.2 Continuation treatment

starting at day 43 after initial treatment:

- **6-mercaptopurine** (6-MP): 30 mg/m^2 **(not to exceed 50 mg)** daily until completion of treatment
- **Prednisone** (PDN) : 1 mg/kg/day **(not to exceed 60 mg)**, day 1-5 every 3 weeks until completion of treatment
- **Vinblastine** (VBL): 6 mg/m^2 i.v. bolus **(not to exceed 10 mg)** day 1 every 3 weeks until completion of treatment (starting 3 weeks after the last VBL injection of the initial treatment or intensification)

Total duration of treatment: duration of treatment will be the object of a randomized study:

Arm A: duration of therapy : 6 months

Arm B: duration of therapy : 12 months

Randomisation will be performed by the study subcenter or study reference center immediately after the reception of the registration and diagnostic evaluation forms. The information on the assigned treatment arm and the randomisation number will be returned to the participant by email or fax.

7.3 GROUP 3 " ISOLATED PULMONARY DISEASE (PLCH)"

Population: The study population consists of male and female adult, aged 18 years or older and <75y, outpatients with PLCH, with onset of symptoms or diagnosis up to 48 months prior to screening. Pregnant or breast feeding subjects are not permitted in the trial.

Summary of the aims:

- To describe the natural course of the disease in patients in the first few months after the diagnosis and to record the possible beneficial effect of cessation of cigarette smoking.

- To assess the therapeutic efficacy of monotherapy with steroids in patients who during or after initial observation show progressive disease.

Study design for patients with PLCH:

- **Stage 1:** Initial observation after cigarette smoke withdrawal for 6 months. In case of progression of the symptoms or pulmonary dysfunction:
- **Stage 2:** Steroid monotherapy.

No chemotherapy regimen is included in this therapeutic strategy, at present.

Observation phase (Step 1)

All patients with isolated PLCH will be initially put into an observation phase for 6 months, provided that their clinical status is compatible with such policy. No specific therapy will be given in this phase except for counselling on smoking cessation and the prescription for appropriate smoking cessation therapies, including buproprion and nicotine patches, so that the potential beneficial effect of smoking withdrawal may be evaluated.

Thus inclusion criteria for the "step 1: wait-and-see" phase will be:

- Diagnosis of LCH. At this stage, also presumptive diagnosis may be accepted, provided this is consistent with all the following criteria:

- In women the presence of isolated cystic lesions on HR-CT must lead to the exclusion of LAM by lung biopsy or by demonstrating the presence of typical intra-abdominal angiomyolipomas by ultrasound or computed tomography.
- In case of pneumothorax, lung biopsy should be performed at the same time if videothoracoscopy is indicated for the specific treatment of pneumothorax.

- Diagnosis may alternatively be established by a TYPICAL or DIAGNOSTIC HR-CT if other diagnoses (including sarcoidosis, hypersensitivity pneumonitis) have been excluded by BAL or transbronchial biopsy.
- Cigarette smoking for smokers
- FEV1>30% of predicted value at baseline
- DLCO>30% of predicted valued at screening
- TLC>60% of predicted value at baseline
- PaO2≥60 mmHg on room air at baseline
- Able to understand and sign a written informed consent form and comply with the requirements of the study, particularly acceptance of the prescription of stop cigarette smoking. In the attempt to be sure that the patient has stopped smoking, monitoring urinary cotinin seems the best test.

Follow up investigations:
All patients should undergo thorough clinical, Chest X-ray, HR-CT Scan and functional pulmonary evaluation at 3 and 6 months from the protocol start. Appearance of additional MS-LCH lesions should be carefully considered at any clinical evaluation.

Steroid therapy (Step 2)

All patients with isolated PLCH show a disease progression during stage 1, will be put into under steroid therapy.

Progression of disease is defined by a decline in FEV1 or DLCO or FVC by >15%, or progression of symptoms (dyspnea, cough, constitutional symptoms) that cannot be explained by diagnoses other than PLCH (infection, heart disease and other clinical issues excluded by careful clinical evaluation and appropriate testing) or the occurence of more than 1 pneumothorax during this period, the patients will be considered as a failure of observation and progress to step 2 of the protocol. Moving to step 2 of the protocol does not depend on actual smoking cessation. Some patients will have stable disease in spite of continued smoking and some patients will have progressive disease even if they quit smoking. In addition, since no more than a third of patients could be realistically expected to quit, we will allow both persistent and ex-smokers to progress to step 2, if clinically indicated (i.e. have evidence of declining lung function and/or have progressive symptoms that cannot be attributed to any other cause than PLCH) as above.

Inclusion criteria for the "step 2: steroid therapy" will be a diagnosis of LCH. At this stage presumptive diagnosis may not be accepted any more and histological diagnosis will be required in all patients.
Although a value of CD1a+ cells ≥5% in BAL is highly suggestive of the disease, BAL will not be considered for diagnosis because of the technical pitfalls (a standardized procedure is however provided to evaluate prospectively the efficiency of BAL for the diagnosis of pulmonary LCH).

Prednisone (PDN) at the following dosage :

- 1 mg/kg/day **(not to exceed 60 mg)**, daily for 1 month
- 0.5 mg/kg/day, daily for 1 month
- 0.25 mg/kg/day, daily for 2 months
- 0.125 mg/kg/day, daily for 2 months (or another protocol of tapering)

Total duration of steroid therapy is 6 months.

At this dosage, gastroprotection is usually not be necessary but it will not be contraindicated; it shoud be recommended in case of comedication (especially non-steroidal anti-inflammatory agents).

During this treatment phase the patients should not receive any other specific therapy so that the potential beneficial effect of isolated steroid monotherapy may

be evaluated. All patients should undergo thorough clinical, Chest-X Ray, HR-CT Scan and functional pulmonary evaluation at 3 and 6 months from the phase start. In the presence of clinical progression of disease as defined by a decline in FEV1 or DLCO or FVC by >15%, or progression of symptoms (dyspnea, cough, constitutional symptoms) that cannot be explained by diagnoses other than PLCH (infection, heart disease and other clinical issues excluded by careful clinical evaluation and appropriate testing), the occurence of more than 1 pneumothorax during this period, the patients will be considered as a failure of steroid monotherapy and progress to stage 3 of the protocol.

NB: for several reasons, including current incomplete knowledge of the natural course of the disease, this study will not include a chemotherapy arm for patients with isolated pulmonary LCH. For study patients who experience progressive disease after the above described stages 1 and 2, chemotherapy could be considered but this will not be part of the present study. Study coordinator and individual countries PIs will be available for counselling on individual patients.

Additional monitoring studies:

- The 6 minutes walk test should be performed at the same time than the pulmonary function test, every 3 months.
- Echocardiography should be performed every 6 months, possibly by the same examinator.

All patients should be followed up for a minimum of 2 years. Much longer follow-up, at the end of the protocol, seems anyway advisable in order to better evaluate the natural history of PLCH.

Exclusion criteria

- Unacceptable toxicity
- Use of other immunomodulatory therapies or chemotherapeutic agents
- Non-compliance with study protocol
- Request to withdraw
- Pregnancy or breast-feeding
- Patients with severe impairment of clinical condition including: severely impaired pulmonary function (for example TLC<60%, FEV1<30%, DLCO<30%, PaO_2<60 mmHg), long term oxygen therapy or cor pulmonale.

ENDPOINT OF THE STUDY

Primary endpoints measurements:

- Time to progression of the disease during observation phase (Stage 1)
- Proportion of cases showing progression of PLCH defined by 15% decline in the FEV1 or DLCO or FVC during observation phase.
- The proportion of patients relapsing within 6 months of cessation of corticosteroids
- Proportion of cases showing progression of PLCH defined by 15% decline in the FEV1 or DLCO or FVC during steroid therapy.

Secondary endpoints measurements

- Change from baseline in lung function at rest (FVC,FEV1,DLCO) at the end of the study.
- Change in the 6-minute walking test
- Change from baseline in resting alveolar-arterial gradient at end of study.
- Change from baseline in HR-CT scans at end of study.
- Change in estimated pulmonary artery pressure on echocardiography at end of study.
- Mortality, progression to respiratory failure (defined as arterial PaO_2 <60 mmHg on room air) or progression to lung transplant at end of study.

7.4 SUPPORTIVE CARE GUIDELINES FOR ALL PATIENTS

Treatment of adult patients with LCH requires specific skillness by the attending physicians. Thus iit is recommeneded that all patients arw cared at, or at least under the supervision, of reference centers. The supportive care which is necessarym, in particular for chemotherapy, will be delivered according to the individual centers' regimens.
We provide some guidelines:

- **Pneumocystis carinii prophylaxis.** This is necessary for patients under chemotherapy and might be useful also for patients under long-lasting steroid therapy. Oral sulphamethoxazole/trimethoprim, 5 mg/kg/day of the trimethoprim, divided into 2 doses/day, on 2-3 days per week throughout the study period and for 12 weeks thereafter.

- **Antiemetics** should be given only when necessary.

- **Transfusions of red cells and platelets.** Blood cell components should be filtered and irradiated (prevention of GvHD) according to the local protocol of the centers.

- **G-CSF.** Only in case of severe infection during 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.

TOXICITY

6-Mercaptopurine

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

Prednisone

increased appetite, centripedal obesity, fluid retention, hyperglycemia, immunosuppression, myopathy, osteoporosis, aseptic necrosis, peptic ulceration, pancreatitis, psychiatric disorders, 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, coma, myelosuppression (leukopenia, thrombocytopenia), alopecia, inappropiate ADH secretion, local pain and necrosis if extravasated

THERAPY MODIFICATIONS

Try to avoid indiscriminate dose-reductions or delays. The indicated drug dosages have been decided keeping in mind the specific characteristics of adult patients with LCH. Lack of compliance might easily jeopardize the evaluation of the study results. Give each patient the recommended dose at the appropriate time.

Bone marrow toxicity:
Pancytopenia may occasionally be seen at presentation of multisystem LCH. In such cases this is disease related and should not be taken as an indication for reduction of the initial dosage.
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. Decrease the dosage of VBL rather than delay the administration until these criteria are met!

Hepatotoxicity:
If serum liver enzymes (SGOT, SGPT) increase >10 times the normal value, decrease drug doses by 50% until values return to normal.

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 a dose reduction of vinblastine by 25-50%.

8 ASSESSMENT OF TREATMENT RESPONSE

In contrast to leukemia or other malignacies the terms "remission" or "relapse" should be omitted. In accordance with the nature of LCH the following definitions should be applied to judge the effect of treatment.

8.1.1 DEFINITION OF DISEASE STATE

NON ACTIVE DISEASE (NAD)	no evidence of disease	resolution of all signs or symptoms

ACTIVE DISEASE (AD)	regression of the disease	regression of signs or symptoms, no new lesions
	stable disease	persistence of signs or symptoms, no new lesions
	progressive disease*	progression of signs or symptoms and/or appearance of new lesions

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

8.1.2 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	

8.2 DEFINITION OF THERAPY RESPONSE

8.2.1 GROUP 1 (SINGLE SYSTEM)

The following parameters must be assessed to evaluate the response beyond clinical evaluation:

- Skeletal radiography of lesional sites only. Radionuclide bone scan is not as sensitive as the skeletal radiography survey in most cases.
- Sonography of lymphnode or other soft tissue lesions
- Clinical evaluation of measurable lesions.

8.2.2 GROUP 2 (MULTISYSTEM)

Please send the follow-up evaluation sheet to the study subcenter IMMEDIATELY after the INITIAL evaluation to have the randomization performed!

- **All involved sites should be re-evaluated after week 6 with the same techniques adopted at diagnosis.** This is aimed to evaluation of response to initial therapy, which in childhood LCH studies proved to be a very effective prognostic factor.
- Patients who fail to achieve any response by week 6 are considered as failures and should be addressed to an alternative salvage therapy.
- Patients who show a response at week 6 should be re-evaluated every 3 months during treatment (i.e. month 3, 6 and 9 when appropriate) and then at treatment completion.

8.2.3 GROUP 3 (PULMONARY)

On the basis of the study design, definition of failure, i.e. progressive pulmonary disease, is pivotal.

Progressive disease is defined on the finding of

- **documented functional deterioration** (i.e decline >15% of FEV1 or DCLO or FVC) or
- **progression of symptoms** (dyspnea, cough, constitutional symptoms) that cannot be explained by diagnoses other than PLCH (infection, heart disease and other clinical issues excluded by careful clinical evaluation and appropriate testing) or >1 pneumothorax.

9 OFF STUDY CRITERIA

Criteria for the stop of protocol therapy

- **Resistant disease: in case of progression after initial therapy**, the patients should be addressed to alternative therapy. In such cases alternative chemotherapy regimens, in particular **2-CDA should be considered for treatment of multisystem, refractory patients. Please contact local subcenter for report of treatment failure aand also for appropriate counseling.**

- **Reactivation:** in the case of reactivation under therapy, continuation therapy must be stopped. In most cases reactivation may be re-treated according to the same initial schedule with good chances of response. **Please contact local subcenter for report of treatment failure aand also for appropriate counseling.**

- **Persistent disease:** in case of evidence of residual active disease at the end of protocol therapy treatment must not be stopped. A biopsy of residual lesions to assess persistent active disease may be recommended and the individual case should be discussed with the local coordinator.

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

10 FOLLOW UP INVESTIGATIONS AFTER STOP OF THERAPY

After completion of treatment all patients should undergo re-evaluation of the involved sites, with the same techniques adopted at diagnosis :

- at least every 6 months for the first 3 years
- at least once a year during the following 3 years

11 DATA COLLECTION AND EVALUATION

The specific registration forms will be used for initial notification and for follow-up evaluations, during and after treatment completion.
Transmission of the data from collaborating institutions to the local subcenter will be made on data forms either by conventional mail or fax or by email.
The study reference center will perform randomization immediately after receiving the registration forms from the referring institutions.
The local coordinator will be available for any clinical questions of the treating physicians, and review the data sheets for completeness and correctness.

NB: hopefully during the study course a study-specific website will be opened, allowing remote data-entry and randomization, initially only by national/regional coordinators. When this will be applicable, all involved people will be timely and adequately informed and new guidelines will be circulated.

12 STATISTICAL CONSIDERATIONS (Catherine KLERSY and Sylvie CHEVRET)

13.1 Group 1 "Single system"

Binomial proportion will be computed together with its exact 95% confidence interval (95% CI) to estimate prevalence of the described end-points and incidence (total number of events/total person time) and its 95% CI to estimate rates over time.

13.2 Group 2 "Multisystem"

13.2.1 Descriptive part

Binomial proportions will be computed together with their exact 95% CI to estimate response rate at 6 week evaluation and acute toxicity.
Reactivation probability by the end of treatment and late toxicity will be estimated by means of Kaplan Meier method.

13.2.2 Randomized study

Based on currently available information derived from single center or limited series, we may assume a proportion of reactivation of disease between 40 and 50% in arm A (6 months therapy) within 6 months from stop of therapy. We expect the prolonged treatment (12 months-arm B) to reduce this proportion by 20%. Thus the following table for sample size (per arm) can be computed, based on a 2-sided test, a type I error of 5% and a power of 80%:

Test significance level	0.05	0.05
1 or 2 sided p	2	2
Proportion of patients who fail in arm A at 6 months after stop of therapy	0.40	0.50
Proportion of patients who fail in arm B at 6 months after stop of therapy	0.32	0.40
HR	0.804	0.756
power	80	80
N per arm	**521**	**372**

If we consider 0.50 and 0.40 to be the most reliable guesses for the expected proportions of reactivation in the 2 groups, 372 patients per group will to be required. After accounting for a dropout rate of 10%, a total of 409 patients needs to be enrolled for the comparison study.
For the analysis of treatment effect on time to reactivaton a Cox model for event-free survival will be fitted, in cluding treatment effect, Center effect and Center per treatment interaction if needed. The analysis will be performed according to the intention to treat principle.

13.3 Group 3 "Isolated Pulmonary disease"

13.3.1 Endpoints

The endpoint is to estimate the prevalence of progression during the observation phase 1 and during steroid monotherapy (phase 2). At present, these data are not available from the literature.
We are aware that a randomization study would have better addressed the question whether steroid monotherapy may really alter the natural course of the disease, in particular it may reduce the rate of disease progression in patients treated because of progression during observation. Yet we are not confident that the expected number of patients may be sufficient to answer the randomized question. Thus, at least in this first experience, we conduct an uncontrolled study. Furthermore, follow-up observation after steroid monotherapy may provide us an additional surrogate endpoint: if a significant number of patients that were stabilized or improved under corticosteroid develop progression of the disease soon after treatment withdrawal, than we can infere that the treatment had been efficient; this because in adult patients with pulmonary LCH it is very unfrequent to have spontaneous recurrence of the disease once stabilized or improved.

13.3.2 Sample size estimate

For the phase 1 of this study (observation phase) the sample size is **385 patients**, based on a progression rate of 0.20 with a 0.04 precision for a two-sided confidence interval extending from 0.16 to 0.24.

As for the sample size required for the phase II uncontrolled study in treated patients, **77 treated patients** (i.e., 20% of the 385 patients enrolled in the phase I study) would allow to detect a difference in reactivation rate from 10% (null hypothesis) to 30% (alternative hypothesis) with a type I error of 0.05 and a power of 0.99, using a two-sided statistics.

13 PUBLICATION

Publication of common study data may be undertaken only with the agreement of the study committee.
With the permission of the Histiocyte Society every center may publish their own observations related to study patients or data on specific research projects only if they do not concern questions of this study, after the overall study has been closed and submitted for publication.

ASSOCIATED SCIENTIFIC RESEARCH PROJECTS. All the participants to the study who are interested in these projects are encouraaged to contact the project coordinator.

This cooperative, prospective study offers an unique opportunity for research on adult patients with LCH. All the physicians and researchers are warmly invited to submit research proposals. They will be evaluated by the study committee in order to be considered as "LCH-A1 associated research studies".
All centres participating in the LCH-A1 study will be very much encouraged to contribute to these scientific research projects with biologic material from the patients. Each scientific research projects remain entirely under the responsibility of the individual study coordinator. No funding will be available from the LCH-A1 study committee for the associated research projects. Application for funding through the HAA grants is encouraged. A statement that the project has been promoted as an "LCH-A1 Associated scientific research project" will be available from the LCH-A1 study coordinator when applicable.
The responsability for shipping, preserving and analyzing biologic material for a research project relies entirely on the project coordinator,

The attending physicians and participating centers are allowed to enroll study patients in any local research program provided this will not include treatment modification which may alter the clinical course or response to treatment.
The study coordinator retains the right to exclude from the study those patients who will be recognized in such situation. If an adequate information and follow-up will be available, those patients will be analyzed and evaluatd separately.

Provisional list of associated research projects:

- *Microarray study* - An initial screening using a prototype array to assess gene expression in adult patients with different clinical manifestations of LCH. *Project coordinator:* Maurizio Aricò (Palermo), Giuseppe Basso (Padova).

- *Chromosome analysis* – Chromosome analysis of peripheral blood lymphocytes from adult patients with different clinical manifestations of LCH, and during different stages of the clinical course. *Project Coordinators: Maurizio Aricò (Palermo), Salvatore Feo (Palermo).*

- *Familial disease* – All patients with LCH affecting more than one subject in the same family will be enrolled in a prospective study clinical, cytogenetic and molecular study. *Project Coordinators: Maurizio Aricò (Palermo), Cesare Danesino (Pavia).*

- *Cytokine and growth factor gene analyis in adult with Langerhans cell histiocytosis* – We have established a consistent method of isolating CD1a+ Langerhans cells and CD3+ T cells from frozen selctions of LCH biopsies. RNA is isolated from several hundred cells, amplified then hybridized to a membrane cytokine array containing 96 ctokins and growth factors genes. Debsitometric measure ment of hybridization intensity and normalization to "housekeeping" genes is done. The relative differences in quantity of hybridization has shown distinct differences in the different clinical subgroups. Similar analysis of adult tissue specimens will be done to characterize the possible similarities /differences between sinlge system, multisystem and pulmonary isolated presentations of adult and childhood LCH. Frozen tissue can be sent to Kenneth Mc Clain MD, PhD, Texas Children Cancer Center, 6621 Fannin St. CC 1410 Houston, TX 77030 USA. Email kmclain@txccc.org Phone +832-822-4208; fax +832-825-1503

- *Function of pro-inflammatory cytokines in the pathogenesis of pulmonary LCH* - Using imunohistochemical techniques, the distribution of chmokines and chemokine-receptors inside the lesions of PLCH i sto be characterized. The aim of the study is to assess the type of chemokines mostly involved in pulmonary LCH and their impact on migration and accumulation of LC and other leukocytes. Furthermore, the role of pigmented macrophages, found in large numbers within the lesions of PLCH, in the pathogenesis of the disease, will be explored. *Project Coordinator: K.M. Muller (Bochum, Germany).*

- *Characterization of S100 subtypes in LC* - The specific distribution of the 19 known subtypes of the S100 protein inside different clls is not characterized, yet. Using specific antibodies against the S100 subtypes A1, A2, A4, A6, A8/9 and B, the aim is t figure out which S100 subtypes are expressed by LC in LCH patients and thus poteantially to define new specific markers for these cells. *Project Coordinator: K.M. Muller (Bochum, Germany).*

- *Evaluation and validation of LCH activity scores in a prospective study* – The French group has recently developed and published a disease acivity score which may be useful for asssessing the disease course. Although the studdy committe acknowledge that it may be ddifficult at the present stage to use it for clinical decision, it is considered of great interest to test it in a prospective mannner. All te participants are encourahed to use this sscore in their patientss whenever it is considered feasible. Forms for score assessment are available through the study committe. *Project Coordinator:* M.Aricò, A.Tazi.

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LCH-A1 Initial therapy outline

Patient: …… Weight kg. …… Height cm..... B.S.: ……m^2
Date of treatment start:……

- PDN (prednisone) 1 mg/kg (max. 50 mg) individual dose: ..... mg
- VBL (vinblastine) 6 mg/m^2 (max. 10 mg) individual dose: .....mg

PDN						
Dose (mg) administered						
VBL	▼	▼	▼	▼	▼	▼
Dose (mg) administered						
week	1	2	3	4	5	6
Date						

- PDN (prednisone) 1 mg/kg day p.o. day 1-28, then tapered (see text)
- VBL (vinblastine) 6 mg/m^2 i.v. (not to exceed 10 mg) weekly for 6 weeks

LCH-A1 Continuation therapy outline (6 months)

Patient: …… Weight kg. …… Height cm..... B.S.: ……m^2
Date of treatment start:…… Date of start of continuation therapy: ……

- PDN (prednisone) 1 mg/kg (max. 50 mg) individual dose: ..... mg
- VBL (vinblastine) 6 mg/m^2 (max. 10 mg) individual dose: ..... mg
- 6-MP (mercaptopurine) 30 mg/m^2 (max. 50 mg) individual dose: ..... mg

PDN	■	■	■	■	■	■	■
Dose (mg) administered							
VBL	▼	▼	▼	▼	▼	▼	▼
Dose (mg) administered							
6-MP							
week	9	12	15	18	21	24	27
Date							

- PDN (prednisone) 1 mg/kg day p.o. day 1-5 q 3 weeks
- VBL (vinblastine) 6 mg/m^2 i.v. (max.10 mg)
- 6-MP (mercaptopurine) 30 mg/m^2 p.o. (max. 50 mg) daily

LCH-A1 Continuation therapy outline (12 months)

Patient: …… Weight kg. …… Height cm..... B.S.: ……m^2
Date of treatment start:…… Date of start of continuation therapy: ……

- PDN (prednisone) 1 mg/kg (max. 50 mg) individual dose: ..... mg
- VBL (vinblastine) 6 mg/m^2 (max. 10 mg) individual dose: ..... mg
- 6-MP (mercaptopurine) 30 mg/m^2 (max. 50 mg) individual dose: ..... mg

PDN	■	■	■	■	■	■	■	■
Dose (mg) administered								
VBL	▼	▼	▼	▼	▼	▼	▼	▼
Dose (mg) administered								
6-MP								
week	30	33	36	39	42	45	48	51
Date								

- PDN (prednisone) 1 mg/kg day p.o. day 1-5 q 3 weeks
- VBL (vinblastine) 6 mg/m^2 i.v. (max. 10 mg)
- 6-MP (mercaptopurine) 30 mg/m2 p.o. (max. 50 mg) daily

LCH-A1 STUDY

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH PROJECT

TITLE OF PROJECT: LCH-A1 - Treatment Protocol of the First International Study for Langerhans Cell Histiocytosis - Group 2 - MULTISYSTEM DISEASE

NAME OF INSTITUTION:_____

PRINCIPAL INVESTIGATOR:_____

INTRODUCTION

We invite you to participate in the research study named above at the center named _____.
Before you can decide whether or not to volunteer for this study, you must understand the purpose, how it may help you, any risks to you, and what is expected of you. This process is called informed consent.

This consent form gives you information about the study which will be discussed with you. Once you understand the study, and if you agree to participate, you be asked to sign this Informed Consent document. You will be given a copy of this document to keep. Before you learn about the study, it is important that you know the following:

- Your participation is entirely voluntary;
- You may decide not to participate or to withdraw from the study at any time without penalty;
- If the study is changed in any way which could affect your participation, you will be told about the changes and may be asked to sign a new informed consent.

A. PURPOSE OF STUDY

You have been told that you have Langerhans cell histiocytosis. This is a rare, tumor-like disease that has an unpredictable course and can be fatal. The cause of this disease is unknown.

Patients with Langerhans cell histiocytosis who have more than one site of disease involvement or disease in many different parts of the body may have a poor prognosis. These patients are usually treated with chemotherapy.

The previous studies of the Histiocyte Society in children LCH I and II have shown that monotherapy with the two drugs, Vinblastine and etoposide (VP 16) seems to be equally effective in the treatment of Langerhans cell histiocytosis without causing serious side effects. However, combination therapy of Prednisone and Vinblastine yielded superior results with respect to therapy response. The addition of VP-16 as a third drug has not yielded any additional benefit. Analysis of risk factors revealed that patients with involvement of liver, spleen, hematopoetic system or lung are “RISK” patients with an about 60% risk of fatal outcome, when they do not respond to initial therapy.

- The goals of this study are to (1) To define and implement an uniform initial evaluation and stratification of adult patients with LCH (2) to uniform the treatment of adult patients with

Langherans Cell Histiocytosis (3) to improve treatment results with respect to survival, therapy response, prevention of disease recurrence, and late effects.

B. TREATMENT PLAN

If you agree to participate in this study, and you have Langerhans cell histiocytosis with multiple organ involvement you will be treated with a program which will utilize Vinblastine and Prednisone and Mercaptopurine.

Prednisone is given orally as tablets, daily during the initial 6 weeks, then as 5 daily pulses every 3 weeks during the continuation treatment. Mercaptopurine is given as daily oral tablets during the continuation therapy. Vinblastine is given intravenously as an injection. The treatment duration will be 12 months.

C. POTENTIAL RISKS AND DISCOMFORTS

Potential side effects of prednisone are increased appetite, obesity, immunosuppression, myopathy, osteoporosis, impaired wound healing, atrophy of subcutaneous tissue, peptic ulceration, pancreatitis, psychiatric disorders, hypertension, precipitation of diabetes mellitus, growth failure, amenorrhea. The side effects associated with vinblastine include loss of hair, muscle weakness, bone pain, nausea, vomiting, constipation, skin and soft tissue irritation and burns if the drug escapes the vein during administration. The side effects associated with mercaptopurine comprise myelosuppression, hepatic dysfunction (elevated transaminases and cholestatic jaundice), mucositis; or dermatological manifestations, interaction with allopurinol.
During treatment with either drug there is an increased risk of infection. This is because of the depression of the blood counts which leads to immunosuppression.

D. POTENTIAL BENEFITS

The potential benefit to be gained from participation in this research study is control of your disease. Information will be gained that will be useful to researchers studying this disease.

E. ALTERNATIVES TO PARTICIPATION

Alternatives to participation in this research study include receiving treatment with other chemotherapeutic agents and/or radiation therapy.

F. QUESTIONS

If you have questions, or complaints, about your rights as a research participant, you may contact the Chief of our Unit, Dr/Prof._____.

This research study has been reviewed by the Institutional Review Board (IRB) of our institution which is an independent board composed of physician's and staff members, and representatives of the community. The IRB has reviewed this study, evaluated the potential benefits and risks, and has granted approval for the solicitation of participants. The hospital maintains a Multiple Project Assurance of Compliance, a document that explains how the hospital provides for protection of human subjects. You will receive a copy of this assurance if so requested.

G. CONFIDENTIALITY

As far as the law allows, your research and medical records will be kept confidential. However, the Health Authorities have the right to inspect your medical records relating to this research for the purpose of verifying data. All information gathered in the study will be completely confidential to the research staff. Identifying information, collected in order to locate persons for follow-ups if applicable, will only be accessible to research staff.

H. COMPENSATION

Neither our institution nor the Principal Investigator can guarantee or assure that the stated risks, or other unknown consequences will not occur. In the event that injury or illness is caused that you believe is directly related to participation in this research study, our institution requests that you contact the Sanitary Direction and ask for appropriate procedures. The hospital will carefully investigate each reported circumstance to determine whether medical treatment or some other form of compensation is required. However, you will receive any immediate emergency treatment necessary.

VOLUNTARY AUTHORIZATION:

Before giving your consent by signing this document, the methods, inconveniences, risks and benefits, and alternatives have been explained and your questions have been answered. It is understood that you may ask questions at any time and that you are free to withdraw from the study at any time without causing bad feelings or affecting your medical care. Your participation may be ended by the Principal Investigator or by the sponsor for reasons that would be explained. New information developed during the course of this study that may affect your willingness to continue in this research study will be given to you as it becomes available. You understand that signed copies of this consent document will be: (1) retained on file by the Principal Investigator, (2) filed with you medical record and chart, and (3) given to you to keep. You understand that you do not give up any of your legal rights by signing this document.

Printed Name of Participant:_____

Medical Record Number:_____

INVESTIGATOR'S AFFIDAVIT: This statement should be included: I certify that I have explained to the above individual(s) the nature and purpose of the study, potential benefits, and possible risks associated with participation in this study. I have answered any questions that have been raised and have witnessed the above signature on the date indicated below.

Printed Name of Individual Obtaining Consent: _____

Date: _____

Title: _____

Signature: _____

I have witnessed the explanations made by the investigator and heard the responses to questions. I have no conflicting interest in the activity proposed.

Printed Name & Signature of Witness:_____

LCH-A1 STUDY

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH PROJECT

TITLE OF PROJECT: LCH-A1 - Treatment Protocol of the First International Study for Langerhans Cell Histiocytosis - Group 2 - MULTISYSTEM DISEASE

NAME OF INSTITUTION:

PRINCIPAL INVESTIGATOR:

A. This study has been explained to me ☐ Yes ☐ No

B. I understand this information ☐ Yes ☐ No

C. I agree to participate in this research ☐ Yes ☐ No

Acknowledgement of Assent:

Printed Name of Participant: _____

Signature of Participant: _____

Printed Name of Individual Obtaining Assent: _____

Date: _____

Title: _____

Signature: _____

I have witnessed the explanations made by the investigator and heard the responses to questions. I have no conflicting interest in the activity proposed.

Printed Name of Witness: _____

Signature of Witness: _____

LCH-A1 STUDY

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH PROJECT

TITLE OF PROJECT: **LCH-A1 - Treatment Protocol of the First International Study for Langerhans Cell Histiocytosis - Patients with Singlesystem MULTIFOCAL BONE or localized "SPECIAL SITE" INVOLVEMENT**

NAME OF INSTITUTION:

PRINCIPAL INVESTIGATOR:

INTRODUCTION

We invite you to participate in the research study named above at our center named_____. Before you can decide whether or not to volunteer to volunteer for this study, you must understand the purpose, how it may help you, any risks to you, and what is expected of you. This process is called informed consent.

This consent form gives you information about the study which will be discussed with you. Once you understand the study, and if you agree to participate, you be asked to sign this Informed Consent document. You will be given a copy of this document to keep. Before you learn about the study, it is important that you know the following:

- Your participation is entirely voluntary;
- You may decide not to participate or to withdraw from the study at any time without penalty;
- If the study is changed in any way which could affect your participation, you will be told about the changes and may be asked to sign a new informed consent.

A. PURPOSE OF STUDY

You have been told that you have Langerhans cell histiocytosis. This is a rare, tumor-like disease that has an unpredictable course and can be fatal. The cause of this disease is unknown.

Patients with Langerhans cell histiocytosis who have more than one site of disease involvement or disease in many different parts of the body may have a poor prognosis. These patients are usually treated with chemotherapy.

The previous studies of the Histiocyte Society in children LCH I and II have not included recommendation of the treatment of single-system patients with multifocal bone lesions or special disease sites. A retrospective analysis of the Histiocyte Society has shown that systemic therapy with standard drugs like Prednisone and Vinblastine can reduce the rate of reactivations and thereby reduce the frequency of late effects and improve the quality of life.
Based on previous studies it has also been shown that patients with disease in the craniofacial bones with intracranial tumor extension have an increased risk to develop endocrinological problems like diabetes insipidus (hormone loss with excessive water drinking and urination). Patients with lesions in the spine with soft tissue extension in the spinal canal, there is danger of neurological damage. Such patients may benefit from systemic therapy. Thus, in the current LCH III study for children, patients with these features are currently treated with a similar strategy.

The goals of this study for single-system patients with multifocal bone lesions or special disease sites are to decrease the rate of reactivation and permanent consequences as compared to the historical control group of such patients registered on the previous studies.

B. TREATMENT PLAN

If you agree to participate in this study, and you have Langerhans cell histiocytosis with multiple bone involvement or with lesions in the craniofacial bones with intracranial tumor extensions or in the vertebral bones with intraspinal tumor extension, you will be treated according to the treatment program for Group 1, which will utilize Vinblastine and Prednisone and Mercaptopurine.

In the initial therapy of 6 weeks Prednisone is given orally as tablets, daily and Vinblastine is given intravenously as a weekly injection. During the continuation treatment Prednisone is given as 5 daily pulses and Vinblastine as an intravenous injection every 3 weeks.
Continuation therapy will consist of 3 weekly pulses of oral Prednisone for 5 days, and one intravenous injection of Vinblastine; Mercaptopurine is given as daily oral tablets. The treatment duration will be 6 months.

C. POTENTIAL RISKS AND DISCOMFORTS

Potential side effects of prednisone are increased appetite, obesity, immunosuppression, myopathy, osteoporosis, impaired wound healing, atrophy of subcutaneous tissue, peptic ulceration, pancreatitis, psychiatric disorders, hypertension, precipitation of diabetes mellitus, growth failure, amenorrhea. The side effects associated with vinblastine include loss of hair, muscle weakness, bone pain, nausea, vomiting, constipation, skin and soft tissue irritation and burns if the drug escapes the vein during administration. The side effects associated with mercaptopurine comprise myelosuppression, hepatic dysfunction (elevated transaminases and cholestatic jaundice), mucositis; or dermatological manifestations, interaction with allopurinol.
During treatment with either drug there is an increased risk of infection. This is because of the depression of the blood counts which leads to immunosuppression.

D. POTENTIAL BENEFITS

The potential benefit to be gained from participation in this research study is control of your disease. Information will be gained that will be useful to researchers studying this disease.

E. ALTERNATIVES TO PARTICIPATION

Alternatives to participation in this research study include receiving treatment with other chemotherapeutic agents and/or radiation therapy.

If you have questions, or complaints, about your rights as a research participant, you may contact the Chief of our Unit, Dr/Prof._____.

This research study has been reviewed by the Institutional Review Board (IRB) of our institution which is an independent board composed of physician's and staff members, and representatives of the community. The IRB has reviewed this study, evaluated the potential benefits and risks, and has granted approval for the solicitation of participants. The hospital maintains a Multiple Project Assurance of Compliance, a document that explains how the hospital provides for protection of human subjects. You will receive a copy of this assurance if so requested.

G. CONFIDENTIALITY

As far as the law allows, your research and medical records will be kept confidential. However, the Health Authorities have the right to inspect your medical records relating to this research for the purpose of verifying data. All information gathered in the study will be completely confidential to the research staff. Identifying information, collected in order to locate persons for follow-ups if applicable, will only be accessible to research staff.

H. COMPENSATION

Neither our institution nor the Principal Investigator can guarantee or assure that the stated risks, or other unknown consequences will not occur. In the event that injury or illness is caused that you believe is directly related to participation in this research study, our institution requests that you contact the Sanitary Direction and ask for appropriate procedures. The hospital will carefully investigate each reported circumstance to determine whether medical treatment or some other form of compensation is required. However, you will receive any immediate emergency treatment necessary.

VOLUNTARY AUTHORIZATION:

Before giving your consent by signing this document, the methods, inconveniences, risks and benefits, and alternatives have been explained and your questions have been answered. It is understood that you may ask questions at any time and that you are free to withdraw from the study at any time without causing bad feelings or affecting your medical care. Your participation may be ended by the Principal Investigator or by the sponsor for reasons that would be explained. New information developed during the course of this study that may affect your willingness to continue in this research study will be given to you as it becomes available. You understand that signed copies of this consent document will be: (1) retained on file by the Principal Investigator, (2) filed with you medical record and chart, and (3) given to you to keep. You understand that you do not give up any of your legal rights by signing this document.

Printed Name of Participant:_____

Medical Record Number:_____

INVESTIGATOR'S AFFIDAVIT: This statement should be included: I certify that I have explained to the above individual(s) the nature and purpose of the study, potential benefits, and possible risks associated with participation in this study. I have answered any questions that have been raised and have witnessed the above signature on the date indicated below.

Printed Name of Individual Obtaining Consent: _____

Date: _____

Title: _____

Signature: _____

I have witnessed the explanations made by the investigator and heard the responses to questions. I have no conflicting interest in the activity proposed.
Printed Name & Signature of Witness:_____

LCH III STUDY

THIRD INTERNATIONAL LCH TRIAL

ASSENT SHORT FORM TO PARTICIPATE IN A CLINICAL RESEARCH PROJECT

TITLE OF PROJECT: **LCH-A1 - Treatment Protocol of the First International Study for Langerhans Cell Histiocytosis - Patients with Singlesystem MULTIFOCAL BONE or localized "SPECIAL SITE" INVOLVEMENT**

NAME OF INSTITUTION:

PRINCIPAL INVESTIGATOR:

A. This study has been explained to me ☐ Yes ☐ No

B. I understand this information ☐ Yes ☐ No

C. I agree to participate in this research ☐ Yes ☐ No

Acknowledgement of Assent:

Printed Name of Participant:_____

Signature of Participant:_____

Printed Name of Individual Obtaining Assent: _____

Date: _____

Title: _____

Signature: _____

I have witnessed the explanations made by the investigator and heard the responses to questions. I have no conflicting interest in the activity proposed.

Printed Name of Witness:

_____

Signature of Witness: _____

LCH-A1 STUDY

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH PROJECT

TITLE OF PROJECT: **LCH-A1 - Treatment Protocol of the First International Study for Langerhans Cell Histiocytosis - Patients with Isolated pulmonary disease**

NAME OF INSTITUTION:

PRINCIPAL INVESTIGATOR:

INTRODUCTION

We invite you to participate in the research study named above at our center named_____.
Before you can decide whether or not to volunteer to volunteer for this study, you must understand the purpose, how it may help you, any risks to you, and what is expected of you. This process is called informed consent.

This consent form gives you information about the study which will be discussed with you. Once you understand the study, and if you agree to participate, you be asked to sign this Informed Consent document. You will be given a copy of this document to keep. Before you learn about the study, it is important that you know the following:

- Your participation is entirely voluntary;
- You may decide not to participate or to withdraw from the study at any time without penalty;
- If the study is changed in any way which could affect your participation, you will be told about the changes and may be asked to sign a new informed consent.

A. PURPOSE OF STUDY

You have been told that you have Langerhans cell histiocytosis. This is a rare, tumor-like disease that has an unpredictable course and can be fatal. The cause of this disease is unknown.

Patients with Langerhans cell histiocytosis affecting only the lung may have an un predictable disease course. Some of them are or have been cigarette smokers and this has been related to the developmentor of the disease or even to is natural course. These patients have been treated worldwide with different approaches and there is no conclusive evidence on (1) the characteristic of the disease which definitely suggest the need for a specific treatment and (2) what is the current standard therapy for this disease. Most of the available information is derived from report of small series of patients which were often reated not uniformely.

The goals of this study are: (1) to uniformely evaluate the patients with pulmonary isolated Langherans Cell Histiocytosis (2) to assess the role of cigarette smoking withdrawal alone in decreasing the severity of the disease (3) to define the criteria which qualify the individual patient for specific therapy (4) to assess the role of steroid monotherapy in reducing the signs and symptoms associated with the disease.

B. TREATMENT PLAN

If you agree to participate in this study, and you have Langerhans cell histiocytosis with isolatd pulmonary involvement, you will be treated according to the treatment program for Group 3, which will utilize according t the clinical stage, either a “wait-and-see” approach or steroid monotherapy.
We will observe patients during the first few months from the diagnosis to clarify whether their disease, at present not very aggressive, has a propensity to rapid progression or rather to an stable or even self-regressing course. If you have functional alteration, symptoms are alternateviley you show during the observation time a rapid deterioration, you will be immediately shifted to steroid monotherapy. We plan to clarify with this approach if (1) treatment is necessary for all patients and (2) if steroid monotherapy is effective in such condition.
During theobservational phase you will be asked to withdraw from cigarette smoking if you are (still) a smoker. Prednisone will be administered orally on a daily basis.

C. POTENTIAL RISKS AND DISCOMFORTS

Potential side effects of prednisone are increased appetite, obesity, immunosuppression, myopathy, osteoporosis, impaired wound healing, atrophy of subcutaneous tissue, peptic ulceration, pancreatitis, psychiatric disorders, hypertension, precipitation of diabetes mellitus, growth failure, amenorrhea.

D. POTENTIAL BENEFITS

The potential benefit to be gained from participation in this research study is control of your disease. Information will be gained that will be useful to researchers studying this disease.

E. ALTERNATIVES TO PARTICIPATION

Alternatives to participation in this research study include either being put ina an observation phase or being treated with steroids.
If you have questions, or complaints, about your rights as a research participant, you may contact the Chief of our Unit, Dr/Prof._____.

This research study has been reviewed by the Institutional Review Board (IRB) of our institution which is an independent board composed of physician's and staff members, and representatives of the community. The IRB has reviewed this study, evaluated the potential benefits and risks, and has granted approval for the solicitation of participants. The hospital maintains a Multiple Project Assurance of Compliance, a document that explains how the hospital provides for protection of human subjects. You will receive a copy of this assurance if so requested.

G. CONFIDENTIALITY

As far as the law allows, your research and medical records will be kept confidential. However, the Health Authorities have the right to inspect your medical records relating to this research for the purpose of verifying data. All information gathered in the study will be completely confidential to the research staff. Identifying information, collected in order to locate persons for follow-ups if applicable, will only be accessible to research staff.

H. COMPENSATION

Neither our institution nor the Principal Investigator can guarantee or assure that the stated risks, or other unknown consequences will not occur. In the event that injury or illness is caused that you believe is directly related to participation in this research study, our institution requests that you contact the Sanitary Direction and ask for appropriate procedures. The hospital will carefully

investigate each reported circumstance to determine whether medical treatment or some other form of compensation is required. However, you will receive any immediate emergency treatment necessary.

VOLUNTARY AUTHORIZATION:

Before giving your consent by signing this document, the methods, inconveniences, risks and benefits, and alternatives have been explained and your questions have been answered. It is understood that you may ask questions at any time and that you are free to withdraw from the study at any time without causing bad feelings or affecting your medical care. Your participation may be ended by the Principal Investigator or by the sponsor for reasons that would be explained. New information developed during the course of this study that may affect your willingness to continue in this research study will be given to you as it becomes available. You understand that signed copies of this consent document will be: (1) retained on file by the Principal Investigator, (2) filed with you medical record and chart, and (3) given to you to keep. You understand that you do not give up any of your legal rights by signing this document.

Printed Name of Participant:_____

Medical Record Number:_____

INVESTIGATOR'S AFFIDAVIT: This statement should be included: I certify that I have explained to the above individual(s) the nature and purpose of the study, potential benefits, and possible risks associated with participation in this study. I have answered any questions that have been raised and have witnessed the above signature on the date indicated below.

Printed Name of Individual Obtaining Consent: _____

Date: _____

Title: _____

Signature: _____

I have witnessed the explanations made by the investigator and heard the responses to questions. I have no conflicting interest in the activity proposed.

Printed Name & Signature of Witness:_____

LCH-A1 STUDY

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH PROJECT

TITLE OF PROJECT: **LCH-A1 - Treatment Protocol of the First International Study for Langerhans Cell Histiocytosis - Patients with Isolated pulmonary disease**

NAME OF INSTITUTION:

PRINCIPAL INVESTIGATOR:

A. This study has been explained to me ☐ Yes ☐ No

B. I understand this information ☐ Yes ☐ No

C. I agree to participate in this research ☐ Yes ☐ No

Acknowledgement of Assent:

Printed Name of Participant:_____

Signature of Participant:_____

Printed Name of Individual Obtaining Assent: _____

Date: _____

Title: _____

Signature: _____

I have witnessed the explanations made by the investigator and heard the responses to questions. I have no conflicting interest in the activity proposed.

Printed Name of Witness:

_____

Signature of Witness: _____