Histiocyte Society

HLH-2004
(UK No: LCH 2006 02)

Hemophagocytic Lymphohistiocytosis Study Group

Treatment Protocol of the Second International HLH Study 2004

Start of the Study: January 2004 (Europe)
Final MREC approval: 24 November 2005
Start of the Study: 1 March 2006 (UK)
EUDRACT Number: 2005-002187-28

Chairman: Jan-Inge Henter, M.D., Ph.D., Stockholm, Sweden
UK Co-ordinator: Vasanta Nanduri
CONTENTS

1: STUDY COMMITTEE .................................................................................................................................3
2: SYNOPSIS ..................................................................................................................................................5
3: GLOSSARY OF ABBREVIATIONS ...........................................................................................................5
4: Figures and Tables .......................................................................................................................................6
   FIGURE 1: Flow-sheet for Children with Hemophagocytic Lymphohistiocytosis (HLH) in HLH-2004 ..............................................6
   FIGURE 2: Treatment protocol overview for Hemophagocytic Lymphohistiocytosis (HLH-2004) ...........................................7
   FIGURE 3: Documentation Sheet for the Initial Therapy in HLH –2004 (week 1-8) ...............................................7
   FIGURE 4. Documentation Sheet for the Continuation Therapy in HLH-2004 week 9-24 .............................................8
   FIGURE 5. Documentation Sheet for the Continuation Therapy in HLH-2004 week 25-40 .............................................9
   FIGURE 6: Documentation Sheet for the Continuation Therapy in HLH-2004 week 25-40 ....................................10
   TABLE 1: Assessment for patients with HLH (in HLH-2004) ........................................................................10
   TABLE 1: Assessment for patients with HLH (in HLH-2004) ........................................................................11
5: GENERAL BACKGROUND .......................................................................................................................12
   5.1: Nomenclature ......................................................................................................................................12
   5.2: Therapeutic overview .........................................................................................................................13
   5.3: Introduction To Protocol ...................................................................................................................13
      5.3.1: Aims ............................................................................................................................................13
      5.3.2: Rationale .....................................................................................................................................13
      5.3.3: Hypotheses .................................................................................................................................13
   5.4: Summary of the HLH-94 Results .........................................................................................................13
6: DIAGNOSIS AND CLINICAL PRESENTATION .....................................................................................14
   6.1: Clinical Features ................................................................................................................................14
   6.2: Molecular diagnosis ............................................................................................................................14
   6.3: Clinical Diagnostic Guidelines ..........................................................................................................15
   6.3: TABLE 2. Diagnostic Guidelines For HLH-2004 .............................................................................16
7: THERAPEUTIC BACKGROUND ..............................................................................................................17
8: GENERAL STUDY DESIGN ....................................................................................................................20
   8.1: Overview .........................................................................................................................................20
   8.2: Declaration of intent ..........................................................................................................................20
   8.3: Brief protocol overview (see Figures 1-2) ........................................................................................20
   8.4: Patient Eligibility ..............................................................................................................................21
   8.5: Pre-Treatment Investigations ...........................................................................................................21
   8.6: Monitoring .......................................................................................................................................22
9: TREATMENT .............................................................................................................................................22
   9.1: Acute Management ............................................................................................................................22
      9.1.1: Initial Therapy .............................................................................................................................22
      9.1.2: Continuation Therapy ................................................................................................................23
9.1.3: Subsequent Therapy (in non-SCT patients) ................................................................. 23
9.2: Reactivation Therapy ................................................................................................. 24
9.3: Macrophage Activation Syndrome ............................................................................ 24
9.4: Salvage Therapy ....................................................................................................... 24
9.5: Ending Therapy ......................................................................................................... 24
10: DEFINITION OF DISEASE STATES ............................................................................. 25
11: STEM CELL TRANSPLANTATION (SCT) ................................................................. 26
11.1: SCT REGIMEN (Suggested Protocol) .................................................................... 26
12: DRUG INFORMATION AND TOXICITY .................................................................... 28
12.1: Therapy Modifications ........................................................................................... 28
12.2: Serious Adverse Event Reporting ............................................................................ 29
13: DATA COLLECTION AND EVALUATION ................................................................. 30
14: BIOLOGICAL STUDIES .............................................................................................. 31
14.1: Genetic And Expression Studies ............................................................................ 31
14.2: NK Cell And Cytotoxic T Cell Activity Studies ..................................................... 31
15: PUBLICATION ............................................................................................................. 32
16: REFERENCES .............................................................................................................. 33
APPENDIX I : Addresses for biological studies ............................................................... 39
APPENDIX II: PARENT/PATIENT INFORMATION SHEETS AND CONSENT FORMS .......... 40
1: STUDY COMMITTEE

Study Chairman
Jan-Ing Henter, MD, PhD
Child Cancer Research Unit
Department of Pediatrics
Karolinska Hospital, Q6:05
S-171 76 Stockholm, Sweden
Tel: +46 - 8 5177 2870 (secr),
Fax: +46 - 8 5177 3184
E: Jan-Inge.Henter@kbh.ki.se

Study Coordinator
AnnaCarin Horne, MD
Child Cancer Research Unit
Department of Pediatrics
Karolinska Hospital, Q6:05
S-171 76 Stockholm, Sweden
Tel: +46 - 8 5177 7098 (office)
Fax: +46 - 8 5177 3184
E: ACHorne@telia.com

Clinical Study Group
Maurizio Aricò, MD
Oncoematologia Pediatrica
Ospedale dei Bambini
90100 Palermo, Italy
Tel: +39 - 091-6666-131
Fax: +39 - 091-6666-001 (202)
arico@ospedalecivicopa.org

R Maarten Egeler, MD, PhD
Dept of Pediatrics, Rm J6-222
Leiden Univ Medical Center
PO Box 9600, 2300 RC Leiden, The Netherlands
Tel: +31 - 71 526-4131/2824
Fax: +31 - 71 524-8198
E: RM.Egeler@LUMC.nl

Lisa H Filipovich, MD
Children’s Hosp Medical Center
Div of Hematology/Oncology
3333 Burnet Avenue
Cincinnati, Ohio 45229, USA
Tel: +1 - 513 636-7206
Fax: +1 - 513 636-5845
Lisa.Filipovich@chmmc.org

Shinsaku Imashuku, MD, Dir,
Kyoto City Inst of Health and Environmental Sciences
1-2 Higashitakada-cho, Mibu
Nakagyo-ku, Kyoto, Japan 604
Tel: +81 - 75 312 4941
Fax: +81 - 75 311 3232
shinim95@mbox.kyoto-inet.or.jp

Prof Dr Gritta Janka-Schaub
Children's University Hospital
Dept of Ped Hematol Oncol
Martinistrasse 52
20246 Hamburg, Germany
Tel: +49 -40 42803-3796 (4270)
Fax: +49 - 40 42803-2580 (4601)
janka@uke.uni-hamburg.de

Stephan Ladisch, MD
Children's National Medical Cent
111 Michigan Avenue N.W.
Washington DC, 20010-2970
Tel: +1 - 202 884 3898
Fax: +1 - 202 884 3929
E: S.Ladisch@cnmc.org

Ken McClain, MD, PhD
Pediatric Hematology/Oncology
Texas Children’s Hospital
CC 1510.00, 6621 Fannin St,
Houston, TX 77030, USA
Tel: +1 - 832-822-4208
Fax: +1 - 832-822-1503
E: kmcclain@txccc.org

Vasanta Nanduri, MD
Department of Paediatrics
Watford General Hospital
Vicarage Road, Watford,
Hertfordshire, WD18 0HB
United Kingdom
Tel: +44 -1923 217992
Fax: +44 –1923 217279
vasanta.nanduri@whht.nhs.uk

BMT advisor
Jacek Winiarski, MD, PhD
Dept of Pediatrics
Huddinge University Hospital
S-141 86 Huddinge, Sweden
Tel: +46 - 8 5858 7336
Fax: +46 - 8 5858 7390
Jacek.Winiarski@klinpvet.ki.se

Biology Study Advisors
Bengt Fadeel, MD, PhD
Inst of Environmental Medicine
Nobels väg 13, Karolinska Inst
S-171 77 Stockholm, Sweden
Tel: +46 8 728 75 56
Fax: +46 8 32 90 41
E: Bengt.Fadeel@imm.ki.se

Marion Schneider, PhD
Sektion Experimentelle Anästhesiologie
Universitätsklinikum Ulm
Steinböwelstrasse 9
89075 Ulm, Germany
Tel: +49-731 500-27940 (7943)
Fax: +49-731 500-26755
E: marion.schneider@medizin.uni-ulm.de

LOCAL COORDINATORS

AUSTRIA:
Milen Minkov, MD
St Anna Children’s Hospital
Kinderspitalgasse 6
A-1090 Vienna, Austria
Tel: +43 - 1 40 170 250
Fax: +43 - 1 40 170 430
E: Minkov@ceri.univie.ac.at

SPAIN:
Itziar Astigarraga, MD, PhD
Pediatric Oncology Unit
Hospital de Cruces
Spain
Tel: + 34 94 6006331
Fax: + 34 94 6006155
iastigarraga@heru.osakidetza.net

SOUTH-AMERICA:
Jorge Braier, MD
Hem/Onc, Hospital Garrahan
Combate de los Pozos 1881
Buenos Aires 1245, Argentina
Tel:+54 11 43084 300
Fax:+54 11 4308 5325
E: jbraier@intramed.net.ar

UKCCSG CONTROLLED DOCUMENT COPY. EXPIRES END APRIL 2006. MAY BE SUBJECT TO AMENDMENT AT ANY TIME. PLEASE REFER TO THE UKCCSG WEBPAGE FOR LATEST VERSION OR TO THE DATA CENTRE BEFORE TREATING PATIENTS.
2: SYNOPSIS

Haemophagocytic Lymphohistiocytosis (HLH) is a disorder of the macrophage system which may either be inherited as an autosomal recessive condition or be secondary to infection, inflammation or malignancy. In its inherited form it is fatal without treatment.

HLH 2004 has been developed for the treatment of the genetic and severe acquired forms of the disease. The aim is to achieve remission and improve survival.

Diagnosis is made on Clinical criteria: fever, splenomegaly
Laboratory criteria: cytopenias, hypertriglyceridemia and/or hypofibrinogenemia
Histopathologic criteria: Hemophagocytosis in bone marrow, spleen or lymph nodes.

Inclusion criteria: - Patients who fulfil the diagnostic criteria of HLH, aged < 18 years at onset of therapy. No prior cytotoxic or cyclosporin A treatment for HLH.

The treatment protocol involves induction of remission with a combination of intravenous Etoposide, oral Dexamethasone and Cyclosporin A and intrathecal methotrexate and hydrocortisone, followed by maintenance with the same drugs. In the inherited form a stem cell transplant is needed to achieve a cure.

3. GLOSSARY OF ABBREVIATIONS

Terms relating to disease:
- HLH – Haemophagocytic Lymphohistiocytosis
- FEL – Familial Erythroid Lymphohistiocytosis
- FHL - Familial Hemophagocytic Lymphohistiocytosis
- MAS – Macrophage activating syndrome
- MAHS - Malignancy associated Haemophagocytic syndrome
- IAHS – Infection associated Haemophagocytic syndrome
- VAHS- Virus associated Haemophagocytic syndrome

Terms relating to investigations:
- ANC – Absolute neutrophil count
- CSF - Cerebrospinal Fluid
- IL 2 – Interleukin 2
- EBV – Ebstein Barr Virus
- HDL – High density Lipoprotein
- APTT – Activated Partial Thromboplastin time
- PT – Prothrombin Time
- NK cell – Natural killer cell
- GFR – Glomerular Filtration Rate
- VLDL- Very low density lipoprotein

Terms relating to treatment:
- CSA – Cyclosporin A
- Dexa – Dexamethasone
- VP 16 – Etoposide
- IVIG – Intravenous Immunoglobulin
- I.T. – Intrathecal
- HC - Hydrocortisone
- MTX – Methotrexate
- ATG – Anti thymocyte Globulin

Terms relating to transplant:
- GVHD – Graft-versus host disease
- MRD – Matched related donor
- MUD – Matched unrelated donor
- PBSCT – Peripheral blood stem cell transplant
- SCT – Stem Cell Transplant
4: Figures and Tables

FIGURE 1: Flow-sheet for Children with Hemophagocytic Lymphohistiocytosis (HLH) in HLH-2004

- Patients with HLH *
  - Initial 8 weeks chemotherapy
  - Register and start #:
    - Persistent non-familial, non-genetically verified → Continuation therapy until SCT
    - Resolved non-familial, non-genetically verified → Stop therapy
    - Reactivation non-familial, non-genetically verified → Continuation therapy until SCT

Genetically verified or Familial disease → Continuation therapy until SCT

* If there is a treatable infection it should be treated. However, this may not be sufficient and the patient may need HLH-treatment in addition. All severe forms should start HLH-treatment. If HLH is persistent or recurrent, consider that the patient may have an undiagnosed inherited disease. HLH may also develop secondary to a number of other diseases as malignancies, rheumatic diseases and metabolic disorders, requiring different treatment.

# Start therapy if the patient has genetically verified disease, a familial form of HLH, or if the disease is severe, persistent, or recurrent.
SCT = Stem cell transplant
FIGURE 2: Treatment protocol overview for Hemophagocytic Lymphohistiocytosis (HLH-2004)

<table>
<thead>
<tr>
<th>Dexamethasone (mg/m²)</th>
<th>Etoposide (VP-16)</th>
<th>Cyclosporin A (CSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg</td>
<td>⬇</td>
<td></td>
</tr>
<tr>
<td>5 mg</td>
<td>⬇</td>
<td></td>
</tr>
<tr>
<td>2.5 mg</td>
<td>⬇</td>
<td></td>
</tr>
<tr>
<td>1.25 mg</td>
<td>⬇</td>
<td></td>
</tr>
<tr>
<td></td>
<td>⬇</td>
<td></td>
</tr>
</tbody>
</table>

Go to SCT during continuation therapy as soon as an acceptable donor is available with:
- HLA identical related donor or
- Matched unrelated donor or
- Mismatched unrelated donor or
- Family haploidentical donor

(further SCT information: see text)

A donor search is suggested as soon as possible in familial and poorly responding patients, and must also be considered in infants.

Doses calculated per m² even if BW <10kg

* = EVALUATION, see Table 1
§ See Fig 1 for info on start of Continuation Therapy

Intrathecal therapy: $\uparrow$ = (Start only if there are progressive neurological symptoms, or if an abnormal CSF has not improved)
- Methotrexate doses by age: <1 year 6 mg, 1-2 years 8 mg, 2-3 years 10 mg, >3 years 12 mg each dose.
- Hydrocortisone doses by age: <1 year 6 mg, 1-2 years 8 mg, 2-3 years 10 mg, >3 years 12 mg each dose.
- Maximum four doses are suggested, but start only if progressive neurological symptoms or if an abnormal CSF has not improved.

Supportive therapy:
- Cotrimoxazole- orally twice daily 2 days per week. Dose 0.5 to 0.75m² – 240mg, 0.76 to 1m² – 360 mg, >1m² – 480mg (week 1 and onwards).
- An oral antimycotic from week 1 to week 9.
- IvIG (0.5 g/kg iv) q 4 weeks. Gastroprotection suggested week 1-9.
FIGURE 3: Documentation Sheet for the Initial Therapy in HLH –2004 (week 1-8)

(To be sent to local subcenter coordinator, with Follow-Up Report Sheet 2 months after onset of therapy)

<table>
<thead>
<tr>
<th>Year of diagnosis:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family name:</th>
<th>Given name:</th>
<th>DOB (yy/m/m/dd):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight:</th>
<th>Length:</th>
<th>Surface area:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dexa (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg Dexa administered (per day, each week)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Etoposide (VP-16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 mg iv/m²</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mg VP-16 (per dose)</th>
<th>Etoposide</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA week 1 – 8</td>
<td></td>
</tr>
</tbody>
</table>

| Mg CSA administered (per day, each week) |
| CSA plasma level (microgram/L) |

<table>
<thead>
<tr>
<th>Intrathecal Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Start only if progressive neurological symptoms, or if an abnormal CSF has not improved)</td>
</tr>
</tbody>
</table>

| Mg Mtx administered |
| Mg HC administered |

<table>
<thead>
<tr>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3*</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5*</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9*</td>
</tr>
</tbody>
</table>

* = EVALUATION, see Table 1

<table>
<thead>
<tr>
<th>Signature:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>
### FIGURE 4. Documentation Sheet for the Continuation Therapy in HLH-2004 week 9-24

(To be sent to local subcenter/coordinator, with Follow-Up Report Sheet 6 months after onset of therapy)

<table>
<thead>
<tr>
<th>Family name:</th>
<th>Given name:</th>
<th>DOB (yy/mm/dd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight:</th>
<th>Length:</th>
<th>Surface area:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year of diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Dexamethasone (Dexa)**

**10 mg/m²**

for 3 days in each pulse

<table>
<thead>
<tr>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>9*</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>17</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td>19</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>21</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>23</td>
</tr>
<tr>
<td>24</td>
</tr>
</tbody>
</table>

* = EVALUATION, see Table 1

<table>
<thead>
<tr>
<th>Mg Dexamethasone administered (per day, each pulse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Etoposide (VP-16)**

**150 mg/m²**

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date pulse started:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**CSA week 9-24**

<table>
<thead>
<tr>
<th>Mg CSA administered (per day, each week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**CSA plasma level (microgram/L)**

<table>
<thead>
<tr>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>17</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td>19</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>21</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>23</td>
</tr>
<tr>
<td>24</td>
</tr>
</tbody>
</table>

**Signature:**

Date:
FIGURE 5. Documentation Sheet for the Continuation Therapy in HLH-2004 week 25-40

(To be sent to local subcenter/coordinator, with Follow-Up Report Sheet 12 months after onset of therapy)

<table>
<thead>
<tr>
<th>Year of diagnosis</th>
<th>Family name:……………………………..</th>
<th>Given name:……………………….</th>
<th>DOB (yy/mm/dd) : ….../.……../…..</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight:………………………kg</td>
<td>Length:………………………cm</td>
<td>Surface area:………………m²</td>
</tr>
</tbody>
</table>

Dexa 10 mg/m²
for 3 days in each pulse

<table>
<thead>
<tr>
<th>Date pulse started:</th>
<th>Day</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg Dexa administered (per day, each pulse)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Etoposide (VP-16)
150 mg/m²

<table>
<thead>
<tr>
<th>Date:</th>
<th>Day</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administered dose mg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CSA week 25-40

<table>
<thead>
<tr>
<th>Mg CSA administered (per day, each week)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA plasma level (microgram/L)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Weeks

| 25 | 26 | 27* | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |

* = EVALUATION, see Table

Signature:                      Date:
### TABLE 1: Assessment for patients with HLH (in HLH-2004)

<table>
<thead>
<tr>
<th>Required evaluations</th>
<th>weeks of treatment</th>
<th>If re-activation</th>
<th>Pre-SCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, WBC, diff, platelets</td>
<td>once weekly</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>Ferritin, transaminases</td>
<td>every second week</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>Triglycerides, fibrinogen</td>
<td>every second week</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>Creatinine</td>
<td>every second week</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>CSA-levels</td>
<td>recommended initially weekly</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>APTT/PT/D-dimers</td>
<td>x (then follow as clinically indicated)</td>
<td>every second week</td>
<td>x x x</td>
</tr>
<tr>
<td>CSF (cells/protein)</td>
<td>x (x x x x)</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>Histology/cytology</td>
<td>x</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>Chest X-ray (or CT)</td>
<td>x</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>Abdominal ultrasound (or CT)</td>
<td>x</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>MR of the brain</td>
<td>x</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>NK-cell activity</td>
<td>x</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>Genetic analyses</td>
<td>x</td>
<td>every second week</td>
<td>x x x x</td>
</tr>
<tr>
<td>Soluble IL-2 receptor (sCD25)</td>
<td>(x)</td>
<td>every second week</td>
<td>(x) (x) (x) (x)</td>
</tr>
</tbody>
</table>

1. Additional pre-treatment investigations, with reticulocytes, serum electrolytes and, in particular, investigation for infection, see section 8.5. HLA-typing as soon as feasible (see page 21). Investigations in brackets are optional and are to be done only if there has been previous signs of involvement of the particular analysis.
2. Monoclonal antibody assay of whole blood.
3. Safe and available tissue, such as bone marrow, lymph node, liver or CSF.
4. Specific HLH-2004-laboratories suggested (for addresses (see Appendix).
5. Genetic analysis for perforin and hMunc gene defects or flow cytometry perforin screening is recommended. Specific HLH-2004-laboratories suggested (see Appendix).
6. Soluble IL-2 receptor (sCD25) is optional, since it is not readily available, but suggested if available. As a voluntary parameter, it may be analyzed more frequently.
7. The additional examinations after 40 weeks intended for patients stopping therapy at this point.
5: GENERAL BACKGROUND

5.1: Nomenclature
The term histiocytoses identifies a group of disorders that have in common proliferation of cells of the mononuclear phagocyte system, with the histiocyte as a central cell. Hemophagocytic lymphohistiocytosis (HLH) is a macrophage-related disorder (1-5). HLH comprises two different conditions that may be difficult to distinguish from each other (4-7):

(i) Familial hemophagocytic lymphohistiocytosis (FHL) (primary HLH)
FHL is an autosomal recessive disease, which in some patients is associated with decreased triggering of apoptosis (8). Mutations in the perforin gene (9-15), account for 20-40% of all affected FHL families (10), causing a defect in NK- and T cell cytotoxicity (12, 16-22). Mutations in the gene hMunc 13-4, essential for cytolytic granule fusion, may also cause FHL (23). Despite the name, familial HLH (also known as familial erythrophagocytic lymphohistiocytosis or FEL), there may be no family history, since the disease is recessive. The onset of FHL may be triggered by infections. The incidence of FHL has (in Sweden) been estimated to 1.2/1,000,000 children/year (around 1:50,000 live born) (24).

(ii) Secondary hemophagocytic syndrome (secondary HLH, sHLH)
Secondary HLH is a macrophage activation syndrome (MAS) with hemophagocytosis as a result of immunological activation triggered by a variety of conditions including infection, rheumatoid disorders, malignancies and metabolic disorders. Although most patients are not immuno-suppressed, the condition has been described in immuno-compromised hosts in association with viral infections and the term virus (infection)-associated hemophagocytic syndrome (VAHS, or IAHS) is also frequently used (5,25). Bacteria and parasites may induce secondary HLH (5,6), as well as rheumatoid disorders. sHLH may also develop during malignancies (malignancy-associated hemophagocytic syndrome, MAHS), in association with metabolic disorders, and following prolonged intravenous nutrition (fat overload syndrome) (5,6). Although sHLH may subside spontaneously, it may result in mortality (5). It is important to remember that some patients with no evidence of known mutations or familial disease might be affected due to other currently unknown genetic defects. In these patients evidence of persistently impaired NK activity should be considered important as there may be prognostic implications (26).

NOTA BENE 1: HLH traditionally, and theoretically, is divided into familial (primary) and secondary HLH. However, this distinction may not be possible in the initial clinical setting until molecular diagnosis is available. Proving an acute infection at the onset does not have major therapeutic importance, since both sHLH and FHL often feature a triggering infectious agent (27).

5.2: Therapeutic overview

(i) FHL is invariably a fatal disease, with a median survival of < 2 months after diagnosis if untreated (2). Survival and cure includes first an initial/continuation therapy, and thereafter a successful allogeneic stem cell transplant (SCT).

(ii) Patients with secondary HLH may also need treatment for HLH initially, as not all cases resolve spontaneously. The treatment may then have to be adapted depending upon the underlying cause of the disease.

The present treatment protocol HLH-2004 has been designed for the primary, inherited disease FHL and the severe acquired form of HLH, in patients aged <18 years.
5.3: Introduction To Protocol

5.3.1: Aims

Primary Aims:

1) To provide and evaluate a revised initial and continuation therapy, with a goal to achieve and maintain an acceptable clinical condition in order to perform a curative SCT, for patients with familial, relapsing, or severe and persistent HLH.

2) To evaluate and improve the results of SCT with various types of donors, and to evaluate the prognostic importance of the state of remission at the time of SCT.

3) To evaluate the neurological complications, with regard to early neurological alterations and CSF-findings.

Secondary Aim:

4) To improve understanding of the pathophysiology in HLH using biological studies of genetics and cytotoxicity in affected patients, including genotype-phenotype studies and the prognostic value of NK-cell-activity subtyping.

5.3.2: Rationale

Regarding the aims above:

The prognosis for HLH-children has improved with the HLH-94 protocol (28,29). However, although HLH-94 was successful in most of the affected children who were admitted to SCT, around 20-25% of the children died during the pre-SCT phase (29). An attempt to improve the protocol was suggested and since most of these deaths occurred during the first 2 months, this period is studied in more detail.

HLH-94 data suggest that although children who responded well to initial pre-SCT-induction fared best, active HLH may not automatically preclude performing SCT (30). It is important to clarify the prognostic importance of the disease activity at the time of SCT.

Neurological complications are the most important long-term sequelae in HLH.

Genotype and NK-cell-activity subtyping appear to have prognostic value (15), and patients with NK-type-3-deficiency appear most likely to require SCT to survive (22, 23).

5.3.3: Hypotheses

- The outcome of children with HLH may be further improved, as compared to HLH-94, by moderate modifications in the treatment protocol.
- Genotype and NK-cell-activity-subtypes may have prognostic value.

5.4: Summary of the HLH-94 Results

113 eligible patients aged ≤15 years from 21 countries started on HLH-94 between July 1, 1994 and June 30, 1998. They all had an affected sibling (n=25) and/or fulfilled the Histiocyte Society diagnostic guidelines (6). At a median follow-up of 3.1 years, the estimated 3-year probability of overall survival was 55% (95% confidence interval +/-9%) and in the familial cases 51% (+/-20%). Twenty enrolled children were alive and off-therapy for >12 months without SCT. For patients who were transplanted (n=65), died prior to SCT (n=25) or were still on therapy (n=3), the 3-year survival was 45% (+/-10%). The initial and continuation therapy was successful in 88/113 (78%) children, in that they were either admitted for SCT (n=65) or were alive at last follow-up (n=23). Also, 80% (20/25) of the patients with a positive family history received SCT. The 3-year probability of survival after SCT was 62% (+/-12%).

Survival as reported in the three largest publications on HLH.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Year</th>
<th>No. pts</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Janka (review) (2)</td>
<td>1983</td>
<td>121</td>
<td>5% (1-yr)§</td>
</tr>
<tr>
<td>Arico et al (3)</td>
<td>1996</td>
<td>122</td>
<td>22% (5-yr)*</td>
</tr>
<tr>
<td>HLH-94 (29)</td>
<td>2002</td>
<td>113</td>
<td>55% (5-yr)*</td>
</tr>
<tr>
<td>HLH-94 (familial cases) (29)</td>
<td>2002</td>
<td>25</td>
<td>51% (5-yr)</td>
</tr>
</tbody>
</table>

§ Out of 121 patients reviewed, 5/101 with follow-up data survived more than 12 months

* Probability of survival according to Kaplan-Meier estimate
6: DIAGNOSIS AND CLINICAL PRESENTATION

6.1: Clinical Features
The typical features of HLH are fever, hepatosplenomegaly and cytopenia. Other common findings include hypertriglyceridemia, coagulopathy with hypofibrinogenemia, liver dysfunction, elevated levels of ferritin and serum transaminases, and neurological symptoms that may be associated with a spinal fluid hyperproteinemia and a moderate pleocytosis (2-4, 6, 31). Other clinical findings include lymphadenopathy, skin rash, jaundice and oedema. Spontaneous partial remissions are common (32).

Histopathological findings include a widespread accumulation of lymphocytes and mature macrophages, sometimes with hemophagocytosis, affecting especially the spleen, lymph nodes (if enlarged), bone marrow, liver and CSF. In the liver, a histological picture similar to chronic persistent hepatitis is commonly found (33). Other frequent abnormal laboratory findings in HLH are low natural killer (NK) cell activity (12,16-22), and a hypercytokinemia in serum and the CSF (34-41), in particular elevated soluble interleukin-2 receptor (sIL-2R) levels (sCD25) (21,35,41).

Many conditions can lead to the clinical picture of HLH, including malignancies (leukemia, lymphoma, other solid tumors), infections (viral, bacterial or parasitic), and rheumatoid disorders. In addition, there are diseases which may resemble HLH at first sight, such as Langerhans cell histiocytosis, X-linked lympho-proliferative syndrome (XLP), and Chédiak-Higashi and Griscelli syndromes (5,6,42-48). Notably, XLP, Chédiak-Higashi syndrome and Griscelli syndromes have been successfully treated with the HLH-94 protocol. Other differential diagnoses are lysinuric protein intolerance (49), SCID (50), DiGeorge with HLH, and Omenn's syndrome (51).

In particular, difficulty exists in the differential diagnosis between primary and secondary HLH in non-familial cases. Viral infections, especially EBV, may trigger primary as well as secondary HLH (5). These patients may develop a severe, persistent non-familial HLH that can be treated with this protocol (52). In less severe sHLH cases, either no treatment or a short duration of therapy might suffice, but future studies are necessary to define these subsets, possibly with additional genetic markers.

If the disease is familial, relapsing, or severe and persistent even without family history, SCT from the best available donor is strongly recommended (29, 30).

6.2: Molecular diagnosis
In 1999, perforin gene (10q21) mutations were reported in FHL patients (9). Later analyses revealed that they affect 20-40% of FHL patients (10). Perforin, which is co-localized with granzyme B in granules in cytotoxic cells, is secreted from cytotoxic T lymphocytes and NK cells upon conjugation between effector and target cells. In the presence of calcium it is able to insert (perforate) into the membrane of the target cell, where it polymerizes to form a cell death-inducing pore (53-55). Pore formation is thought to lead to destruction of target cells by osmotic lysis and by allowing entrance to granzymes, which trigger apoptosis (56, 57), but perforin concentrations lower than the level necessary for pore formation together with granzyme B may induce cell death. Recent studies suggest that entry of granzyme B into target cells can also occur in a perforin-independent manner (58), but granzyme alone is not sufficient to induce toxicity.

In 2003, it was shown that mutations in hMunc 13-4 (17q25) can cause FHL (23). HMunc 13-4 is essential for the priming step of cytolytic granules secretion preceding vesicle membrane fusion and a deficiency results in defective cytolytic granule exocytosis.

In XLP, 60-70% of patients have mutations in the gene SAP (SLAM-associated protein), also termed SH2-DIA (SH2-domain containing gene 1A) or DSHP. This gene, located to Xq25, regulates a protein involved in signal transduction in T and NK cells. In T cells, the protein binds to Signaling Lymphocyte Activation Molecule (SLAM, known as CDw150) and in NK cells it binds to 2B4, an NK-cell-activating receptor (43-46). Chédiak-Higashi is linked to the LYST-gene (lyzosomal trafficking regulator gene, 1q42), Griscelli syndrome to two genes on
15q21, RAB27a (which is a key effector of cytotoxic granule exocytosis) and MYO5a (involved in organelle transport machinery) (47,48).

6.3: Clinical Diagnostic Guidelines

Diagnostic Guidelines for HLH were presented in 1991 by the FHL Study Group of the Histiocyte Society, see below (6), based on common clinical, laboratory and histopathological findings. For many patients, molecular diagnosis may not be available and clinical criteria need to be considered.

_____________________________________________________________________

The 1991 Diagnostic Guidelines for HLH* (adapted from ref 6)

Clinical criteria
* Fever
* Splenomegaly

Laboratory criteria
* Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood:
  Hemoglobin (< 90 g/L), Platelets (<100 x 10^9/L), Neutrophils (<1.0 x 10^9/L)
* Hypertriglyceridemia and/or hypofibrinogenemia
  (fasting triglycerides ≥2.0 mmol/L or ≥3 SD of the normal value for age, fibrinogen
  ≤1.5 g/L or ≤3 SD)

Histopathologic criteria
* Hemophagocytosis in bone marrow or spleen or lymph nodes.
  No evidence of malignancy

_____________________________________________________________________

Revision of Diagnostic Guidelines for HLH-2004

As mentioned already in the 1991 publication on Diagnostic Guidelines, HLH “may also have an atypical and insidious course in some patients, in whom all criteria not always are fulfilled” (6). Moreover, a number of patients may develop one or more of the diagnostic criteria late during the course of the disease (6, 59).

Based on these findings, and the added knowledge of molecular diagnosis, the diagnostic guidelines have been revised. Firstly, patients with a molecular diagnosis of primary HLH do not need to also fulfill the diagnostic criteria.

Second, additional criteria are introduced:
A. Low or absent NK-cell activity (according to local laboratory reference).
B. Ferritin >500 microgram/L
C. Soluble CD25 (i.e. soluble IL-2 receptor) >2400 U/ml.

Regarding the additional criteria above:

NK-cell activity: NK-cell activity is often low or absent in HLH (12, 16-22). Preliminary data indicate that almost all perforin deficient patients have abnormal NK cell activity.

Ferritin: In the HLH-94 study, 48 eligible children registered 1994-June 2002 had familial disease. Data on ferritin, an important diagnostic parameter (31), was available for 31 children, 26 of whom had ferritin levels >500 microgram/L (sensitivity 0.84) and 23 had ferritin >1000 microgram/L (sensitivity 0.74).

Soluble CD25: Soluble CD25 (>2400 U/ml) appears to be a valuable serum parameter in the diagnosis of HLH (12, 15-19). When compared with other diseases (sepsis, juvenile myelomonocytic leukemia, Langerhans cell histiocytosis), specificity was 1.0 and sensitivity 0.93. The corresponding values for CD95 ligand (>500 pg/ml) were 1.0 and 0.72 (60). These markers are not readily available for many patients.
The diagnosis of HLH can be established if one of either 1 or 2 below is fulfilled.

1. A molecular (genetic) diagnosis consistent with HLH.

2. Diagnostic criteria for HLH fulfilled (5 out of the 8 criteria below).

A) Initial diagnostic criteria (to be evaluated in all patients with HLH).

**Clinical criteria**

* Fever

* Splenomegaly

**Laboratory criteria**

* Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood: Hemoglobin (<90 g/L), Platelets (<100 x 10^9/L), Neutrophils (<1.0 x 10^9/L)
  (In infants <4 weeks: Hemoglobin <100 g/L)

* Hypertriglyceridemia and/or hypofibrinogenemia
  (fasting triglycerides ≥3.0 mmol/L (i.e. ≥265 mg/dL), fibrinogen ≤1.5 g/L)

**Histopathologic criteria**

* Hemophagocytosis in bone marrow or spleen or lymph nodes.
  No evidence of malignancy

B) New diagnostic criteria

* Low or absent NK-cell activity (according to local laboratory reference)

* Ferritin ≥500 microgram/L

* Soluble CD25 (i.e. soluble IL-2 receptor) ≥2400 U/ml

Comments:

1. If hemophagocytic activity is not proven at the time of presentation, further investigation for hemophagocytic activity is encouraged. If the bone marrow specimen is not conclusive, material may be obtained from other organs. Serial marrow aspirates over time may also be helpful.

2. The following findings may provide strong supportive evidence for the diagnosis:
   (a) Spinal fluid pleocytosis (mononuclear cells) and/or elevated spinal fluid protein,
   (b) Histological picture in the liver resembling chronic persistent hepatitis (biopsy).

3. Other abnormal clinical and laboratory findings consistent with the diagnosis are:
   Cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, skin rash. Hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, VLDL ↑, HDL ↓.

**NOTA BENE 2:** Not all patients fulfil all the diagnostic criteria presented in Table 2. Moreover, a number of patients may develop one or more of the diagnostic criteria late during the course of the disease (6,59). Thus, **therapy may sometimes have to be commenced on strong clinical suspicion of HLH**, before overwhelming disease activity results in irreversible damage and makes a response to treatment less likely. (Contact your local subcenter or local coordinator in case of questions).

**NOTA BENE 3:** There are no reliable criteria to distinguish primary and secondary HLH, clinically and histologically. The onset of FHL is most common in infancy, but has also been reported in adolescents and young adults (61,62). Secondary HLH can occur at all ages. In infants, a primary cause of HLH is more likely than a secondary cause.
7: THERAPEUTIC BACKGROUND

Chemotherapy: Without treatment, FHL is usually fatal with a median survival of two months (2,24). A number of treatments including cytotoxic agents were initially tried with no or moderate effect (2). Repeated plasma or blood exchange induced transient resolution in some patients (63). The use of the epipodophyllotoxin derivatives, etoposide (64) and later teniposide (65), in combination with steroids were both shown to induce prolonged resolution. A treatment protocol including etoposide, steroids, intrathecal methotrexate and cranial irradiation was shown to be successful in inducing resolution and prolonged survival (66). Later, a therapeutic regimen that also included guidelines for the maintenance therapy and reactivation was presented, based on similar drugs but the cranial irradiation had now been excluded (32). This treatment was effective in prolongation of survival in some patients >5 years after onset, but it has not been possible yet to cure any child with familial disease with chemotherapy alone (29).

The biology of the remarkably beneficial effects of etoposide in HLH, previously not well understood, may be explained by the recent findings that FHL is associated with a defective triggering of apoptosis, and that etoposide is known to be an excellent initiator of apoptosis (8, 67). Similarly, the effect of dexamethasone might be explained by its anti-inflammatory and pro-apoptotic properties, particularly valuable since the drug also penetrates well into the CNS, and CSA is known to reduce T-cell activity, which is increased in HLH. An epipodophyllotoxin derivative (etoposide) and corticosteroids (dexamethasone) were used in the HLH-94 protocol (29).

SCT: A major therapeutic breakthrough was achieved when allogeneic hematopoietic SCT was shown to induce not only a prolonged resolution but also cure (68). Allogeneic SCT is necessary to cure a child with FHL (68-72). Recent SCT series have reported data ranging from a 3-year probability of survival of 45 % (n=20) to an overall survival of 64% with HLA-nonidentical donors (n=14) and 100% in a single-center material with matched sibling donors and unrelated donors (n=12) (73-76).

CNS disease: Cerebral involvement may cause severe and irreversible damage (59,77-80) and intrathecal therapy has been used, although its therapeutic effect has neither been sufficient nor persistent. Children with HLH CNS disease at diagnosis, often resolve with systemic therapy whereas intrathecal therapy appears to be less effective. Therefore, systemic therapy including dexamethasone, which penetrates the blood-brain barrier well, was first line therapy in HLH-94, even in those with CNS involvement. Intrathecal therapy may be added in certain clinical situations, see pages 18 and 22.

Immunotherapy: The immunosuppressive drug cyclosporin A (CSA) has been shown to be effective in FHL (81, 82). ATG has also been successful in inducing resolution (82). However, a majority of patients who were not transplanted in the months following ATG treatment, relapsed in the CNS despite CSA therapy. In HLH-94, CSA was combined with steroids and etoposide (28, 29).

Virus-infections associated with onset of the disease: FHL is often triggered by an infection. Thus, the presence of a virus-infection, such as EBV, in a child with HLH does not rule out an inherited disease, i.e. FHL (27). In addition, clinical features of numerous EBV-associated cases are controlled only by continuous administration of chemotherapy (52, 83). The prognosis for children with HLH is poor whether a virus-infection is associated or not (6,27,84). Therefore, in HLH-94 all children with HLH were initially started on chemotherapy, whether a virus-infection was associated with the onset or not.

HLH-94: In HLH-94, the initial treatment was based on etoposide (initially twice weekly, then once weekly) and corticosteroids (in line with a previously presented regimen, 32) followed by a continuation therapy with etoposide and steroid pulses, in combination with CSA and, in selected cases, intrathecal methotrexate. In addition, SCT was suggested for children with persistent and reactivating disease (28, 29).
CONCLUSIONS FROM HLH-94

The survival results with the HLH-94 protocol exceeded expectations (29). More children than expected survived the intensive disease phase by using the initial and continuation therapy, and hence more children could be admitted to SCT. Moreover, more patients than expected survived the allogeneic SCT.

Overview of the outcome in HLH-94 during the first 4 years (from ref 29)

In children with an affected sibling, i.e., verified familial disease, the 3-year probability of survival (pSU) was 51% (95% confidence level ±9%) for eligible patients recruited during the 4-year period July 1994 - June 1998. (Eligibility was defined as no previous cytotoxic or CSA treatment, familial disease or all diagnostic criteria fulfilled, and HLH-94 therapy commenced prior to July 1, 1998).

At a median follow-up of 3.1 years, the estimated 3-year probability of survival overall was 55% (+/-9%) (n=113). Twenty enrolled children were alive and off-therapy for >12 months without SCT. For patients who were transplanted (n=65), dead prior to SCT (n=25) or were still on therapy (n=3), the 3-year survival was 45% (+/-10%). The 3-year probability of survival after SCT was 62% (+/-12%).

In brief, 25 of the eligible 113 patients (22%) died prior to SCT (for details see below). In addition, 25 children died after SCT. The present protocol is aiming to improve the results further.

Initial treatment (week 1-8)

Not surprisingly in a disease characterized by severe cytopenia and an immune deficiency, dose modifications in HLH-94 were common. In particular, the treatment of etoposide was decreased in a substantial number of the patients. For dexamethasone, the amount administered was increased in more patients than it was decreased.

During the first 4-years of analysis, 6 patients died during the first month of treatment and a further 6 during the second month of treatment. An increase in treatment intensity during the first 2 months of therapy, with a drug that does not induce increased myelotoxicity is suggested.

Proposed action:
• CSA, previously introduced after 8 weeks, is now initiated at the onset.

Neutropenia at onset of the Initial treatment (week 1)

In our opinion, neutropenia at onset is caused by the disease, and it does not therefore in itself justify dose reduction. Proposed action if ANC at onset of treatment is <0.5 x10^9/L and the bone marrow is hypocellular (which is only rarely the case): Consider omitting the first two doses of etoposide, and discuss the treatment with the local sub-center.

Neutropenia developing during the Initial treatment (week 2-8)

• If the disease has started to regress (fever subsides, platelet count improves), one or two doses of etoposide may be omitted if the bone marrow is hypocellular, during which period dexamethasone is administered at 10 mg/m2, and CSA as scheduled. Consider discussing the treatment with the local sub-center.

• If the disease has not started to regress at all: This is a very difficult situation, and is recommended to be discussed with the local sub-center. Consider the possibility of an ongoing (viral) infection triggering the immune system, and use appropriate therapy.
Continuation therapy (week 9-)

Of the six children who died during weeks 9-24 of the HLH-94 protocol, all were reported as dead due to disease, at least three of whom had CNS-involvement.

Proposed action:
- Since CNS reactivations may occur during continuation therapy, it is suggested to analyse CSF (at least for cells and protein, and cytopsin if CSF-pleocytosis) if there is a systemic reactivation or neurological symptoms, in order to detect reactivation in the CNS early. Additional information may be provided by cytokine analysis (as neopterin) (37). Brain MRI is also highly recommended.
- In case of reactivation during the continuation therapy, it is recommended to restart at week 2 of the protocol, see separate paragraph (page 22). In this case, the initial therapy period may be shorter, and the continuation therapy may be more intensive, and continuous dexamethasone 2.5 mg/ m² between the dexamethasone pulses may be considered.
- In addition, if the patient is a candidate for SCT it should be performed as soon as an acceptable donor is available. If no other donor is available, SCT with a haplo-identical family donor is suggested, to be performed at an experienced SCT center (see SCT chapter below).

Intrathecal therapy

With available HLH-94 data, it has not yet been possible to determine whether intrathecal therapy is beneficial or not, in addition to systemic HLH-94 therapy (29). It is the opinion of the Study Committee that systemic therapy, in particular with corticosteroids, will reduce CNS disease activity, in particular CNS activity at diagnosis. It cannot be ruled out that intrathecal therapy may have additional beneficial effects, at least in some patients. Intrathecal therapy may be beneficial in patients with CNS reactivation, and is suggested in case of CNS reactivation.

As in HLH-94, up to four intrathecal doses are recommended week 3, 4, 5 and 6, but only if the neurological symptoms are progressive during the first two weeks, or if an abnormal CSF at onset has not improved after two weeks. Having the beneficial effect of systemic corticosteroids in mind, it is suggested to add corticosteroids to the IT MTX when IT therapy is administered to the patients with CNS involvement.

Stem cell transplantation (SCT) (see also Section 9)

Analysis of SCTs performed 1995-2000 revealed an overall estimated 3-yr-survival post-SCT of 64% (+/-10%) (n=86); 71% (+/-18%) with matched related donors (MRD, n=24), 70% (+/-16%) with matched unrelated donors (MUD, n=33), 50% (+/-24%) with family haploidentical donors (n=16), and 54+/−27% with mismatched unrelated donors (n=13) (78). Univariate analysis (n=86) revealed a lower 3-yr-survival in children with active disease at SCT (54%, n=37) as compared to children with non-active disease (71%, n=49) (p=0.065). There was a non-significant trend towards better survival in children that had received etoposide as part of their conditioning (70% versus 58%, univariate analysis). In summary: 1) the cure rate with HSCT using MRD or MUD is not markedly different, and acceptable also with mismatched donors (considering that SCT is necessary for cure in FHL), 2) active disease should probably not automatically preclude performing SCT, and 3) inclusion of etoposide in the SCT-conditioning may improve survival further.
8: GENERAL STUDY DESIGN

8.1: Overview
For general overview, see Figure 1. The HLH-2004 protocol is designed for the primary, inherited disease FHL, but may be beneficial in patients with secondary HLH as well. The protocol is based on etoposide, steroids, cyclosporin A, intrathecal therapy in selected patients (methotrexate and hydrocortisone), and SCT. The major aim is to achieve a clinically stable resolution of the disease and to cure by SCT.

Following 8 weeks of initial therapy, all children with familial disease or with a diagnosis verified by molecular biology, as well as children with a non-familial disease that is severe and persistent, or reactivated, continue with etoposide/steroids in combination with cyclosporin A immunotherapy. SCT is performed as early as possible, when an accepted donor is available. In non-familial cases, treatment is stopped in patients with a complete resolution of disease after 8 weeks of initial therapy, in order to avoid SCT in a child with an HLH which may be an unrevealed secondary disease.

In children with secondary HLH, such as infection-associated HLH or malignancy-associated HLH, the underlying cause of the immune activation is treated first. If necessary, chemo-immunotherapy is also administered, as in HLH-2004.

8.2: Declaration of intent
In many children it may not be possible to determine whether the disease is a primary, inherited disease or a secondary HLH. If disease is severe and persistent, or reactivating, treatment according to HLH-2004 is suggested, initially for 8 weeks. Be aware that a viral infection, such as EBV and CMV, may trigger a primary HLH.

The intention with this protocol is that children with primary HLH will receive continuation therapy and SCT. In children with secondary HLH, first the cause of the immune activation is treated and, if necessary, HLH-2004 is also administered. If it is unknown whether the disease is primary or secondary and a thorough investigation has revealed no underlying malignancy, no bacterial or parasitic infection and no other cause of the immune-activation, the patient is administered initial therapy, whether a viral infection is associated or not. Treatment is stopped after 8 weeks, if the disease has had a complete resolution. If the disease is severe and persistent, or reactivating, continuation therapy and SCT is suggested.

8.3: Brief protocol overview (see Figures 1-2)
Initial therapy: At diagnosis many of the patients are critically ill and the major aims of the initial therapy are 1) to keep the patients alive and to reduce the number and degree of permanent complications during this critical period, and 2) to achieve a resolution of the disease. The initial therapy covers the first 8 weeks of treatment and includes etoposide, dexamethasone, CSA, and, in selected patients, intrathecal therapy (methotrexate and hydrocortisone) (see Section 9).

Continuation therapy: The major aim is to sustain the resolution of the disease. SCT is performed when an accepted donor is available. The therapy is intensive with a combination of etoposide, dexamethasone pulses and cyclosporin A in order to reduce the risk of reactivation. Since the disease activity is different in each child, the therapy may have to be further intensified in some patients, (see Section 9).

SCT: SCT is recommended as soon as an accepted donor is available and is preferably performed when the disease is in resolution. However, active HLH disease should probably not automatically preclude performing SCT (see Section 11) (30). The choice of performing SCT or not, as well as the choice of donor, is made by the treating physician.
8.4: Patient Eligibility

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

**Inclusion criteria:**
- Patients who fulfil the diagnostic criteria of HLH (Section 6.3)
- Age < 18 years at onset of therapy.
- No prior cytotoxic or cyclosporin A treatment for HLH.

Patients with HLH starting the HLH-2004 protocol who do not fulfil the diagnostic criteria or aged ≥18 yrs may also be registered in the study but will be studied separately. Patients with XLP, Chediak-Higashi syndrome, Griscelli syndrome, and similar syndromes, as well as patients with macrophage activation syndrome (MAS) secondary to known rheumatoid diseases may also be registered, and will be studied separately.

It must be emphasized that patients with active HLH may be extremely sick but that this is no reason to avoid treatment since the initial therapy commonly induces a rapid regression of the symptoms.

Any doctor or patient is free to withdraw from the study at any time.

**Exclusion criteria:** Any patient who does not fulfil inclusion criteria

8.5: Pre-Treatment Investigations

**Clinical**

* Complete history:
  Family history (consanguinity, previous childhood deaths in this family or relatives, late miscarriages of the mother), recent infections and vaccinations, previous bouts with similar symptoms, fever (duration and level), neurological symptoms (including irritability, ataxia, convulsions and others), edema, jaundice, skin rash.

* Complete physical examination:
  Temperature, height, weight, skin rashes, jaundice, purpura, bleeding, edema, tonsillitis, lymphadenopathies, dyspnea, tachypnea, liver size, spleen size, ascites, blood pressure, neurological examination incl cranial nerve abnormalities and cerebellar dysfunction.

**Laboratory and Radiographic**

Baseline evaluations for all patients:
- Hemoglobin, WBC and differential, platelet count, reticulocytes, ferritin
- Liver function (serum transaminases, bilirubin, albumin, LDH)
- Coagulation profile (fibrinogen, APTT, PT, D-dimers)
- Lipid evaluation (fasting triglycerides)
- Kidney function (creatinine) and serum electrolytes
- Soluble IL-2 receptor (sCD25) is not readily available, but suggested if available.
- Immunoglobulin levels (including also IgA)
- Spinal tap
  - cell and protein content (consider to add lactate and glucose)
  - morphological and immunological analyses (if cells in CSF)
- Infectious investigation including CMV, EBV, HIV, HSV, HHV6+8, rubella, varicella, parvovirus, adenovirus and other appropriate viruses. It is suggested that the investigations include PCR. Consider the diagnoses leishmaniasis, brucellosis, tuberculosis, mycoplasma, syphilis, among others.
- Bone marrow aspiration (hemophagocytosis, differential diagnosis evaluation)
  (consider a bone marrow biopsy in case of dry tap or a diluted marrow sample)
- Fine needle aspiration biopsy of an enlarged lymph node or a liver biopsy may also be valuable, (see also Diagnostic Guidelines, Section 6.3 ).
- NK-cell activity (studied at specific study laboratories, see Appendix).

- Molecular diagnosis (perforin, hMunc 13-4 and relevant other genes).
  Some institutions perform flow cytometry screening for perforin in NK cells and
cytotoxic T cells (12). For addresses of study laboratories, see Appendix.

- Glomerular filtration rate (because of cyclosporin therapy), as soon as feasible, at least
  in patients with elevated creatinine levels.

- Imaging
  - Abdominal ultrasound (or CT) (liver & spleen size, other abnormalities)
  - Chest X-ray (or CT of the chest) (pulmonary infiltrates)
  - MRI of the brain is recommended, since CNS involvement is common, and it
    is strongly recommended in patients with neurological symptoms

- HLA-typing of the patient and the family is performed as soon as feasible. We recommend a
  preliminary donor search in infants (< 6 mos at diagnosis) even if the patient has a full
  response and is eligible to stop therapy at 8 weeks.

8.6: Monitoring

• For monitoring and assessment, see Table I.

• Follow-up evaluations for the study are at 2 months, 6 months, 12 months and then once
  yearly. If SCT has been performed, change to SCT +100, SCT +1yr, and thereafter yearly
  for 5 years.

• The documentations sheets for the initial and the continuation therapy, as well as the
  follow-up sheets must be sent to the UKCCSG Data Centre.

9: TREATMENT

9.1: Acute Management

The initial therapy covers the initial 8 weeks of treatment. It includes etoposide,
dexamethasone, cyclosporin A (CSA), and in some patients, intrathecal therapy.
**The doses are calculated per m² even in children less than 10kg.**

Early supportive therapy:
* Maximal supportive care.
* Appropriate broad-spectrum antibiotics (until culture results are available).

Further and continuous recommended supportive therapy:
* Prophylactic cotrimoxazole, eq 5 mg/kg of trimethoprim, 2-3 times weekly.
* An oral antimycotic, at the choice of the physician, during the initial therapy.
* Consider antiviral therapy in patients with ongoing viral infections.
* IvIG (0.5 g/kg iv) once every 4 weeks (during the initial and continuation therapy).

9.1.1: Initial Therapy

1. **Etoposide**
   - 150 mg/m² iv twice weekly (week 1-2). Only in certain conditions, if absolute neutrophil
     count (ANC) <0.5 x10⁹/L and the bone marrow is hypocellular (which only rarely is the
     case), can these be omitted.
   - 150 mg/m² iv once weekly (week 3-8).

2. **Dexamethasone**
   - Dexamethasone 10 mg/m² daily, for the first 2 weeks (week 1-2).
   - Dexamethasone 5 mg/m² daily, for another 2 weeks (week 3-4).
   - Dexamethasone 2.5 mg/m² daily, for another 2 weeks (week 5-6).
   - Dexamethasone 1.25 mg/m² daily, for another week (week 7).
Steroids are tapered and discontinued during week 8. Gastroprotection with ranitidine or other gastroprotective agent is suggested.

3. **Cyclosporin A**
The blood levels determine the dosages, aim at levels around 200 microgram/L (trough value) (monoclonal antibody assay of whole blood). Start with 6 mg/kg daily (divided in 2 daily doses) in week 1, if kidney function is normal.

4. **Intrathecal injections with methotrexate and hydrocortisone**
The CSF is evaluated at diagnosis and after 2 weeks. If after 2 weeks there is clinical evidence of progressive neurological symptoms or if an abnormal CSF (cell count and protein) has not improved, additional CNS-therapy is initiated with 4 weekly intrathecal injections. Be aware that some patients may have increased intracranial pressure.
   - Methotrexate: <1 yr 6 mg, 1-2 yrs 8 mg, 2-3 yrs 10 mg, >3 yrs 12 mg.
   - Hydrocortisone: <1 yr 6 mg, 1-2 yrs 8 mg, 2-3 yrs 10 mg, >3 yrs 12 mg.

9.1.2: **Continuation Therapy**
The continuation therapy is a continuation of the initial therapy with the major aim to keep the disease non-active weeks 9-40. Increasing disease activity may make it necessary to intensify the treatment in some children. Patients with non-familial disease and no genetic evidence of HLH, are suggested to start continuation therapy only if the disease is active after the initial therapy (see flow-sheet - Fig 1).

1. **Etoposide**
   - 150 mg/m² iv, every second week.

2. **Dexamethasone**
   - Dexamethasone pulses every second week, 10 mg/m² for 3 days.

3. **Cyclosporin A**
   - Aim for blood levels around 200 microgram/L, as above. Monitor GFR.

9.1.3: **Subsequent Therapy (in non-SCT patients)**
In children with primary HLH, cure can be achieved only by SCT. Even in perforin deficient patients transient treatment-induced or even spontaneous resolution may be observed, but ultimately all these patients will end up in progressive disease. If a matched donor cannot be found, mis-matched donor SCT is suggested, as with a family haploidentical donor. The aim of the subsequent therapy is to sustain a resolution in patients where SCT has not been performed, if possible with a reduction of therapy. In secondary HLH, treatment should not continue beyond 40 weeks, usually only 8 weeks are necessary. Four treatment strategies are offered, and treatment can be tapered and stopped if there is no reactivation. The treating physician may choose either one:

1. Continue the continuation therapy as it is (as week 9-40).
2. Prolong the intervals between each etoposide infusion and dexamethasone pulse from 2 to 4 weeks, and continue CSA as previously. Thus, the patient will receive alternating treatment every second week (instead of weekly) with etoposide or dexamethasone pulse.
3. Exclude etoposide. Continue with CSA and dexta only, in doses and interval as week 9-40.
4. Exclude etoposide. Continue with CSA or dexta only.

It must be noted that many patients may have to go back to the initial continuation schedule, since a reduced treatment will not be enough to keep the disease non-active.
9.2: Reactivation Therapy

Reactivations may occur following immune response triggering, such as infections and vaccinations. In case of reactivation, consider broad-spectrum antibiotics, antiviral therapy, and antifungal therapy.

FHL is characterized by frequent reactivations, or even a more or less continuous disease activity. In particular, reactivation of the disease is common as the therapeutic intensity is reduced. Accordingly, a reactivation will commonly respond to an intensification of the ordinary therapy. Treatment of a reactivation has to be individualized for each patient.

Suggested action if the patient develops a reactivation:
1. It is recommended that therapy is intensified, such as - restart from wk 2, but the initial therapy may be less than 8 wks, and then continue with modified continuation therapy.
2. Add intrathecal therapy in case of CNS-reactivation.
3. Consider dexamethasone daily, also between the dexa-pulses, in continuation therapy, but be aware that it may lead to severe side-effects, and an early SCT is then suggested.
4. If inadequate response, contact your local sub-center.

9.3: Macrophage Activation Syndrome

Macrophage activation syndrome (MAS), a serious complication of rheumatoid arthritis and other childhood systemic inflammatory disorders, is thought to be caused by excessive activation and proliferation of T lymphocytes and macrophages. The recognition that MAS belongs to the secondary or reactive hemophagocytic syndromes has led to the proposal to rename it according to the contemporary classification of histiocytic disorders (85, 86). In addition to corticosteroids, CSA has been found effective in patients with corticosteroid-resistant MAS (87).

9.4: Salvage Therapy

The current protocol does not include a salvage protocol. An alternative approach of inducing remission, with a regimen including steroids (2 to 5 mg/kg/d methylprednisolone intravenously, followed by progressive tapering) and anti-thymocyte globulin (ATG) (82) is possible. There have only been a small fraction of patients that did not respond to some degree to the HLH-94 protocol, and many of these did not respond to ATG either. It is therefore suggested to discuss salvage therapy with the local sub-center. In case of reactivation after graft rejection, it is suggested to restart from wk 2. Note that early after SCT, immunosuppression may induce a secondary HLH picture, which may be due to late lymphocyte recovery, necessitating HLH therapy.

In brief: It is recommended to discuss non-responders with the local study coordinator.

9.5: Ending Therapy

Ending therapy is only recommended in children with resolution of the disease. Close follow-up including signs of reactivation are warranted (such as fever, hepato-splenomegaly, neurological abnormalities; hemoglobin, platelets, WBC, ANC, ferritin, transaminases).
10: DEFINITION OF DISEASE STATES

* Clinical response:

Criteria to be used during the induction therapy (at 2 weeks and 4 weeks) on whether to continue the therapy as outlined:

- No fever
- Reduction of spleen size
- Platelets ≥100x10^9/L
- Normal fibrinogen
- Decreasing ferritin levels (by 25%)

If not all criteria are fulfilled, contact your local sub-center to discuss further therapy.

* Non-active disease (resolution):

Criteria to be used at the decision-point on whether to continue therapy after 8 weeks:

- No fever
- No splenomegaly (isolated moderate splenomegaly may persist in some patients)
- No cytopenia (Hb ≥90 g/L, platelets ≥100x10^9/L, ANC ≥0.5x10^9/L)
- No hypertriglyceridemia (<3mmol/L, i.e. <265 mg/dL)
- No hyperferritinemia ≥500 µg/L
- Normal CSF (for previously CSF positive patients)
- (Decrease of sCD25 in case the test is available)

* Active disease:

Patients that do not have non-active disease, as defined above.

* Reactivation of disease:

Children that have achieved a remission, and then again develop ≥ 3 of these 8 signs:

- Fever
- Splenomegaly
- Platelets <100x10^9/L
- Hypertriglyceridemia (fasting level ≥3.0 mmol/L, i.e. ≥265 mg/dL)
- Hypofibrinogenemia ≤1.5 g/L
- Hemophagocytosis
- Increasing ferritin levels
- Soluble CD25 (i.e. soluble IL-2 receptor) ≥2400 U/ml

The development of new CNS symptoms are sufficient as a single criterion for reactivation.
11: STEM CELL TRANSPLANTATION (SCT)

In primary HLH, i.e. FHL, allogeneic SCT is the only curative therapy (29, 68-76). Some major problems are 1) to find an acceptable SCT-donor, and 2) to keep the patients alive and without sequelae until the SCT is performed.

In familial disease and in severe and persistent non-familial disease, SCT is performed, preferably when the disease is non-active, when an acceptable donor is available. In non-familial disease with complete resolution after the initial 8-week therapy, SCT is performed only if the disease has been reactivated (indicating a primary disease).

An HLA-identical donor is preferable. The risk of a sibling carrying the disease must be considered and is less likely if using an older sibling, but this age criteria cannot be used as an indicator for being non-affected. If a genetic marker (as perforin/hMunc) is not available, NK-cell activity has been considered as a surrogate marker of immune dysfunction, but recent data suggest that healthy siblings may also have low NK-cell activity (20). If an HLA-identical relative is not available, SCT with a matched unrelated donor is recommended. If there is no matched donor available, a mismatched donor (including a haploidentical family donor) or cord blood is suggested, upon the decision of the physician. The results with mismatched donors are improving (73-76). At the decision of the physician, PBSCT may be considered, particularly if marrow is not available.

### Outcome after SCT in HLH-94 (ref 29)

<table>
<thead>
<tr>
<th>SCT donor</th>
<th>All cases (n=65)</th>
<th>Alive (%) (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matched related donor</td>
<td>15</td>
<td>10 (67%)</td>
</tr>
<tr>
<td>Matched unrelated donor</td>
<td>25</td>
<td>17 (68%)</td>
</tr>
<tr>
<td>Mismatched unrelated donor</td>
<td>4</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>Family haploidentical</td>
<td>14</td>
<td>6 (43%)</td>
</tr>
<tr>
<td>Cord</td>
<td>5</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Incomplete data*</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

* Includes: related donor with match not reported (n=2, both alive)

The preparative treatment for SCT and the GVHD prophylaxis is up to the treating physician and the transplant unit. However, we would advise the inclusion of etoposide, in addition to busulfan and cyclophosphamide, in the conditioning regimen, is advised in accordance with previous experience (29-30, 73-76). A suggestion is provided below:

### 11.1: SCT REGIMEN (Suggested Protocol)

#### Preparative Regimen

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>-8</td>
<td>Busulfan 1mg/kg po, or equivalent iv, four times daily.</td>
</tr>
<tr>
<td>-7</td>
<td>Busulfan 1mg/kg po, or equivalent iv, four times daily.</td>
</tr>
<tr>
<td>-6</td>
<td>Busulfan 1mg/kg po, or equivalent iv, four times daily.</td>
</tr>
<tr>
<td>-5</td>
<td>Busulfan 1mg/kg po, or equivalent iv, four times daily.</td>
</tr>
<tr>
<td>-4</td>
<td>Etoposide 30 mg/kg iv (6 hr infusion) (maximum 1800 mg)</td>
</tr>
<tr>
<td>-3</td>
<td>Cyclophosphamide 60 mg/kg iv (1 hr infusion)</td>
</tr>
<tr>
<td>-2</td>
<td>Cyclophosphamide 60 mg/kg iv (1 hr infusion)</td>
</tr>
<tr>
<td>0</td>
<td>Marrow infusion (preferably ≥3 x 10^8 nucleated cells/kg, non T-cell-depleted).</td>
</tr>
</tbody>
</table>

Graft-vs-Host Disease (GVHD) Prophylaxis

1. Cyclosporin continuous infusion starting day -1 pre-transplant with 3 mg/kg until oral nutrition re-established, thereafter 12.5 mg/kg orally daily. CSA dosage is adjusted according to monitoring of CSA through concentration levels. The immunosuppression is discontinued after 6-12 months, if possible.
2. Short course methotrexate:
* Day +1 15 mg/m^2 iv
* Day +3 10 mg/m^2 iv
* Day +6 10 mg/m^2 iv

Methotrexate may be substituted by mycophenolate mofetil (MMF) (as in cord blood transplants and in patients with decreased liver function) 15 mg/kg x 2 daily orally, starting on day 0 and given until day 40 post transplant, then tapered to be discontinued.

Additional Treatment for Unrelated Donor Transplants

* ATG (Antithymocyte Globulin) (12 hour infusion iv) on days –3, -2 and –1. Adjust ATG dosage according to manufacturers recommendation.

(Methylprednisolone 2 mg/kg iv and clemastinhydrogenfumarate 1 mg iv 30 min prior to each ATG infusion.)

* Metronidazole 22 mg/kg daily (po or iv) from day –8 until discharge.

Supportive Care Guidelines

The supportive care is up to the treating physician and the transplant center. Suggestions:

* Monitoring of busulfan concentration
* Clonazepam administered before and parallel to busulfan as anticonvulsive prophylaxis
* Dexamethasone days –4, –3 to prevent etoposide induced anaphylactic-like symptoms
* Mesna for uroprotection in association with cyclophosphamide infusions
* Hydration iv 2-3 L/m^2/24h from start of conditioning through 24 hours after last dose of cyclophosphamide
* Trimethoprim/Sulfamethoxazole: pneumocystis prophylaxis 2-3 days/week
* Acyclovir prophylaxis day +1 until +100, at least to pat with high herpes simplex titers

Pre and Post Transplant Monitoring for the Study

* Disease activity status prior to SCT conditioning; at day +100, at 1-year, at 2-year, etc
* CNS disease activity within 2 weeks prior to SCT (raised cell count or high protein)
* MRI of the CNS prior to SCT conditioning, and at +1-year if abnormal pre-SCT
* NK and CTL-cell activity prior to SCT conditioning, and at day+100 and at +1-year
* Lansky play scale: at SCT, and at day+100, +1-year, etc
* Engraftment (first day with ANC >0.5 x 10^9/L)
* Chimerism analysis day +100, and at 1-year recommended.
* Staging for acute GVHD (ref 88)
* Staging for chronic GVHD (ref 89)

Additional Pre and Post Transplant Monitoring

* Hepatic veno-occlusive disease (VOD):
  Modified Seattle criteria (ref 90). Bilirubin ≥34.2 µ/L (before day 20) + weight gain ≥5%, liver ≥3cm more than baseline under the costal margin.
* Mucositis staging (ref 91)
* Capillary leak syndrome defined as:
  Generalized edema + weight gain ≥10% + pleural effusions or ascites
  (There is a potential risk for hypercytokinemia with engraftment +/- acute GVHD – an emergency that could be treated with very high doses of steroids).

Reduced intensity conditioning

There is as yet limited data available on reduced intensity conditioning in HLH. It is not possible at this time to make any firmly based suggestions on such regimens in HLH.
12: DRUG INFORMATION AND TOXICITY

Please refer to UKCCSG drug monographs produced by the Chemotherapy Standardisation group which are available on the UKCCSG website.

Etoposide

Dilute to a concentration of not more than 0.4 mg/ml in 0.9% sodium chloride or 5% dextrose. Administer as intravenous infusion over 1-3 hours.

Toxicity: Myelosuppression (leukopenia, thrombocytopenia), hypotension (if the drug is infused too rapidly), hepatocellular damage, nausea, vomiting, fever, headache, abdominal pain, diarrhoea, anorexia, alopecia, allergic reactions, and, rarely, second malignancies (leukemia/myelodysplastic syndrome). Second malignancies are very rare in HLH (three cases reported and one unpublished) (29,92,93). Etoposide is included in the protocol since it has shown to have such a positive effect in FHL, which without treatment is uniformly fatal. Etoposide has also been used in familial cases diagnosed and treated with chemotherapy in utero (94).

Dexamethasone

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

Cyclosporin A

Nephrotoxicity (usually dose dependent but may be irreversible), hypertension, hypertrichosis, nausea, vomiting, anorexia, gingival hyperplasia, liver affection with elevation of serum transaminases and bilirubin, tremor, hypomagnesemia, tiredness, paresthesia, edema, headache, convulsions (less common), diarrhoeas, rash, weight gain, elevation of serum potassium and uric acid, and anaphylactic reactions (rare).

12.1: Therapy Modifications

If <10kg: The dosages are calculated per m2 even in children less than 10kg.

HLH may cause a wide variety of symptoms and it may be difficult to evaluate whether a sign or symptom is due to the therapy or due to the disease. The treatment may have to be individualized.

Bone marrow toxicity: Cytopenia is a common sign of disease activity, particularly at onset, but myelotoxicity may occur following intensive etoposide treatment as well. Delayed administration or reduced doses may be necessary, in particular if no other signs of disease activity are available. The differential diagnosis between cytopenia induced by the disease or by etoposide myelotoxicity may be difficult. High serum ferritin, persistent thrombocytopenia, or a persistently elevated sCD25 strongly suggests persistence of basal disease rather than therapy-induced myelotoxicity. Consider bone marrow examination.

Nephrotoxicity: Cyclosporin A may cause irreversible kidney damage. In case of increasing creatinine and urea values or other signs of decreasing kidney function, a reduction of the cyclosporin dosage has urgently to be considered. Check GFR.

Hepatotoxicity: The disease itself may markedly affect liver function and a dose reduction due to the therapy is rarely necessary.
Neurotoxicity: Intrathecal methotrexate may cause neurotoxicity. Cyclosporin has been related to neurological complications. However, many patients with HLH also develop CNS-disease and this has to be urgently considered.

12.2: Serious Adverse Event Reporting
Any serious adverse event (death or Grade III-IV non-haematological life threatening toxicity) must be reported within 24 hours of knowledge of the event by the treating institution to UKCCSG, who will then relay the information to the Study reference center for relay to the DSMB and other national centers for further reporting according to local practice. The toxicity criteria are included in the Case Report Forms.
13: DATA COLLECTION AND EVALUATION

Data forms will be used both at the initial patient registration and at the follow-up evaluations. Data are transmitted to the study subcenters/local coordinators by completing and returning the forms. The chief investigator will be available to answer clinical questions from the treating physicians, will review the data sheets for completeness and correctness, and transfer the revised data sheets to the study reference center in Stockholm.

The final evaluation will be performed in the study reference center, in collaboration with the study subcenters. The study reference center in Stockholm will survey the data collection, perform the overall data input and prepare the final data evaluation. Access to common data from the study data base will be given only with the approval of the Scientific Committee and permission of the Board of the Histiocyte Society.

Study Reference Center
(Stockholm/Henter)

Study Subcenters / National Chief Investigators

<table>
<thead>
<tr>
<th>Germany/</th>
<th>Italy/</th>
<th>Japan/</th>
<th>Netherlands/</th>
<th>Sweden/</th>
<th>UK/</th>
<th>USA/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centr Europe/</td>
<td>S Europe/</td>
<td>Asia/</td>
<td>Benelux/</td>
<td>Scandinavia/</td>
<td>Gt Britain/</td>
<td>Canada/</td>
</tr>
<tr>
<td>Janka</td>
<td>Aricò</td>
<td>Imashuku</td>
<td>Egeler</td>
<td>Henter</td>
<td>Nanduri</td>
<td>HAA-New Jersey</td>
</tr>
</tbody>
</table>

In addition, there are local coordinators in Austria (Dr Minkov, Vienna), South-America (Dr Braier, Buenos Aires), and Spain (Dr Astigarraga, Bilbao). For addresses, see Section 1.

An independent Data Safety Monitoring Board (DSMB) composed of three international experts will monitor the progress of the study on ethical and scientific grounds, Drs Finn Wesenberg, Åke Jakobson and Jim Whitlock, for the duration of the study.

Follow-up reports

For evaluation of the initial therapy response, the Follow-up form 1 is sent to the local subcenter soon after completion of the initial therapy. The next Follow-up reports are scheduled at 2, 6 and 12 months after onset of therapy and later once yearly for 5 years, unless a SCT has been performed at that time. The Documentation Sheets for the Initial therapy, Continuation therapy week 9-24 and 25-40, respectively, are sent once completed.

If SCT has been performed, the SCT follow-up +100-days form is used, and thereafter the SCT+1-year form is delivered once yearly after SCT.

Endpoint

The endpoint in the study is survival. The initial intention is to have a minimum of a 4-yr recruitment. There will be yearly evaluations regarding the need of modifications.

Statistics

A power analysis is planned, comparing the results of this study to HLH-94. With 80% power, using 95% confidence intervals, there is a need for 250 patients from HLH–94 (with a pre-SCT mortality of 25%) and 288 patients from HLH-2004, assuming that pre-SCT mortality is reduced to 15%. With a projected recruitment of 80 patients per year, we presume the study will take 4-5 years to complete. In the UK we expect approximately 5 patients/year.

The review of statistical analyses will be performed by Dr Scott M Montgomery, Clinical Epidemiology Unit, Karolinska Hospital L1:00, SE – 171 76 Stockholm, Sweden; telephone: +46 - 8 5177 9325, facsimile: +46 - 8 5177 9304.
14: BIOLOGICAL STUDIES

The HLH-94 study had a number of associated biological studies, including analyses of NK cell and T cell cytotoxicity, preparation of DNA for genetic analyses, as well as EBV-associated studies. These studies have been successful and they have improved diagnostics and therapy, and increased the biological understanding of the disease as well as of normal human immune modulation. In order to improve the diagnoses, therapy and biological understanding further, participation in the biological studies associated with HLH-2004 is encouraged.

Recent studies have shown that the disease is associated with decreased apoptosis triggering (8, 67, 94). This causes the defect in the NK and T cell cytotoxicity that has been known for long (12, 16-22). Two underlying gene defects have been revealed, mutations in the perforin gene (9-15), which account for 20-40% of all affected FHL cases (10), and in the hMunc 13-4 gene (23). It is possible to identify individuals with perforin mutations by the use of flow cytometry for the perforin protein (12). Moreover, it has also recently been shown that the cytotoxicity defect can be grouped in four subtypes (21), and that group 3 patients will most likely need a SCT in order to survive (22).

The biological studies in HLH-2004 address these recent novel findings. The goals are to:

1. Study the correlation of genetic mutations and associated flow cytometry results.
2. Gather biological material in order to identify additional genetic defects.
3. Study genotype-phenotype associations.
4. Study the biological and clinical significance of cytotoxic subgroups.
5. Document the infectious triggering agent in genetic as well as secondary HLH.

It is therefore suggested to sample for genetic analysis including flow cytometry from the study patients. It is also suggested to sample for analysis of NK- and T cell cytotoxicity. The HLH-2004 Study Group does not provide any financial support for the above studies.

14.1: Genetic And Expression Studies

Analysis of perforin is part of routine diagnostics in HLH, using sequencing and/or flow cytometry. This can be performed at certain specified laboratories for patients in the study. Laboratories with this service are listed in the Appendix. Similarily, analysis of hMunc 13-4 may also be part of routine diagnostics, but this is not yet the case.

Gathering of material in order to identify additional genetic defects is important with the aim to identify other genetic defects than perforin and hMunc 13-4 mutations that are responsible for the development of HLH. These studies can be performed at each respective center, or as a collaborate effort upon the decision of each participating center.

Having genotype data and phenotype data together will also make it possible to perform important studies on genotype-phenotype correlations. This may hopefully provide information of value for the clinical care in the future.

14.2: NK Cell And Cytotoxic T Cell Activity Studies

Analysis of NK and cytotoxic T cell activity is part of routine diagnostics in HLH. This is not only of diagnostic value but it also appears that NK-cell activity may have a prognostic value (21, 22). NK cell activity can be analyzed at certain specified laboratories for patients in the study which are listed in Appendix I.
15: PUBLICATION

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

Every subcenter or participating clinic may present and publish their own data and observations, except treatment results, related to HLH patients that this particular center or clinic has reported to the Study, at any time.
16: REFERENCES


APPENDIX I : Addresses for biological studies

SCANDINAVIA

**Genetic Studies**
Kim Ericson
CMM L8:02
Karolinska Hospital
171 76 Stockholm
Sweden
Tel: +46 8 517 76538
Fax: +46 8 517 73620
Email: kim.ericson@cmm.ki.se

**NK Cytotoxicity Studies**
Chengyun Zheng, MD, PhD
CMM L8:02
Karolinska Hospital
171 76 Stockholm
Sweden
Tel: +46 8 517 76538
Fax: +46 8 517 73620
Email: chengyun.zheng@cmm.ki.se

GERMANY

**Genetic Studies**
Dr. Udo zur Stadt
Children's University Clinic
Pediatric Hematology/Oncology
Martinistr. 52
20246 Hamburg, Germany
Tel: +49 40-42803-2743
Fax: +49 40-42803-8931
Email: zurstadt@uke.uni-hamburg.de

**NK Cytotoxicity Studies**
Marion Schneider, PhD
Sektion Experimentelle Anästhesiologie
Universitätssklinikum Ulm
Steinhövelstrasse 9
89075 Ulm, Germany
Tel: +49-731 500-27940
Fax: +49-731 500-26755
Email: marion.schneider@medizin.uni-ulm.de

ITALY

**NK and Genetic Studies**
Maurizio Aricò,
Onco Ematologia Pediatrica,
Ospedale dei Bambini “G. Di Cristina”,
Via Benedettini 1
90144 Palermo, Italy
Tel. +39-091-6666-131
Fax: +39-091-6666-001
Email: arico@ospedalecivicopera.org

SPAIN

**NK Cytotoxicity Studies**
Marta Rinon, MD, PhD
Laboratorio de Immunologia
Hospital de Cruces
48903 Baracaldo, Vizcaya
Spain
Tel: +34 94 6006144
Fax: +34 94 6006052
Email: mrinon@hruc.osakidetza.net

UNITED KINGDOM

**Genetic Studies**
Dr H Kempski
Dept of Molecular Haematology
Camelia Botnar Laboratories
Great Ormond Street Hospital
London WCIN 3JH
Tel: +44- 207 905 2314
Fax: +44- 207 813 8100
Email: H.Kempski@ich.ucl.ac.uk

USA

**NK and Genetic Studies**
Lisa H Filipovich, MD

Children’s Hosp Medical Center
Div of Hematology/Oncology
3333 Burnet Avenue
Cincinnati, Ohio 45229, USA
Tel: +1 - 513 636-7206
Fax: +1 - 513 636-5845
Email: Lisa.Filipovich@chmcc.org

JAPAN

Shinsaku Imashuku, MD
Consultant
Email: shinim95@mbox.kyoto-inet.or.jp

Eiichi Ishii, MD
Regional study-in-Chief and new genetic study
Saga Medical School, Saga
TEL 0952-34-2314
FAX 0952-34-2061
Email: ishiei/post.saga-med.ac.jp

Akira Morimoto, MD and Ikuyo Ueda, MD
Perforin study
Kyoto Prefectural University of Medicine, Kyoto
TEL 075-251-5571
FAX 075-252-1399
Email: akiramorimoto@hotmail.com

Masaki Yasukawa, MD
CTL study
Ehime University of Medicine
TEL 089-960-5296
FAX 089-960-5299
Email: yasukawa@m.ehime-u.ac.jp
APPENDIX II: PARENT/PATIENT INFORMATION SHEETS AND CONSENT FORMS

Parent/Guardian Information Sheet
Young person aged 16+ Information Sheet
Child aged 13 – 15 Information Sheet
Child aged 8 – 12 Information Sheet
Child Under 8 Information Sheet
GP Information Sheet
Parent/Child Consent Form
Young Person Consent Form aged 16+

INFORMATION SHEET FOR PARENTS/GUARDIANS
(Version 2.0, November 2005)

Dear Parent
You are being invited to allow your child to take part in a research study looking at the treatment of hemophagocytic lymphohistiocytosis. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please do not hesitate to ask us to explain anything that is not clear or if you would like more information. Take time to decide whether or not you want to take part in this study.

Thank you for reading this.

1. What is the purpose of the study?
Hemophagocytic lymphohistiocytosis (HLH) is a very rare and severe disease in which the patient has too many infection-fighting cells called lymphocytes and histiocytes. These cells are not working properly and collect in the body’s organs causing damage. The cause of HLH is not known but there are main two forms. One, known as primary HLH or familial hemophagocytic lymphohistiocytosis (FHL), is inherited (genetic). The other, secondary HLH develops secondary to problems of the immune system such as infection. It may not be possible to tell whether or not the disease is inherited. HLH is a life threatening disease. Without treatment, the primary inherited form has a very poor outlook and even with treatment, cure may not be possible. The secondary form may recover spontaneously but can also have a very poor outcome. This is a study of treatment for children and adolescents with either the primary inherited form, or a severe secondary form of HLH, which aims to improve the survival and reduce the long-term side effects resulting from the disease and its treatment. As this is a very rare disease only a few patients will be diagnosed each year in the UK. This is an international study so that we can collect information from a large number of patients on the nature and course of the disease and find out more about its cause.

2. Why has my child been chosen?
You are being asked to allow your child take part because he/she has been diagnosed as having hemophagocytic lymphohistiocytosis. The study is a clinical trial (a research study involving human patients) which only involves patients who wish to take part.

3. Does my child have to take part?
If you decide that you do not want your child to take part in the clinical study or any of the scientific studies this is entirely up to you. It will not affect the standard of care that your child receives or your relationship with your child’s doctor. He/she will try to do what is best for your child. If you change your mind once the treatment has started, let your child’s doctor know and the treatment might be changed. If you decide to allow your child to take part you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

4. What will happen to my child if he/she takes part?
Your child will be treated according to a treatment programme developed by a group of international specialists working together in the Histiocyte Society. The treatment programme consists of two parts. The initial treatment aims to get the disease controlled, that is, into remission. This control is temporary in the primary inherited form (FHL). The best chance of cure is a bone marrow transplant. Whilst looking for a suitable donor, continuation treatment uses the same drugs but less
frequently. In secondary HLH, remission may only be temporary and a bone marrow transplant may also be required.

The initial treatment lasts 8 weeks and consists of dexamethasone, a steroid, given daily by mouth or into a vein, etoposide given twice weekly for 2 weeks and then once weekly into a vein, and cyclosporin given daily by mouth or into a vein. Your doctors may also need to give some drugs into the fluid around the spine by performing a lumbar puncture.

Depending on your child’s response to this treatment, the doctors will decide whether the treatment can be stopped or if your child needs to continue on the chemotherapy and may need a bone marrow transplant.

5. What do I have to do?
During the whole of your child’s treatment the team looking after him/her will advise you as to the precautions to be taken. You will have to be aware of the risks of infection due to the side effects of chemotherapy.

Your child will need to have regular blood tests to monitor the effects of the chemotherapy and in particular the levels of the cyclosporin. Your child will also need to have his/her kidney function monitored closely.
In order to give the treatment safely, your child will need to have an operation to insert a tube into a vein (Hickman line or Portacath). This is a routine procedure for patients receiving chemotherapy.

6. What is the drug or procedure that is being tested?
The treatment will involve a combination of chemotherapy drugs as described above. It is hoped that the initial and continuation treatment in this protocol will ensure that more patients are well enough to undergo a bone marrow transplant. It is also hoped that the results of these transplants may be improved.

7. What are the alternatives for diagnosis or treatment?
If you decide not to allow your child to take part in this study, it will not affect the medical care that he/she receives. Your child will be given the same treatment which is currently thought to be the best available. The only difference between what we would normally do and what we are doing in this study is that we are collecting data from all children and putting it together to try and improve treatment in the future.

8. What are the side effects of any treatment received when taking part?
All drugs have a number of side effects. However, your child will not necessarily experience all of them. Most side effects can be managed and the doctors looking after your child will discuss this with you.

The more common side effects of dexamethasone include high blood pressure, increased appetite, weight gain, fluid and salt retention (oedema), a bloated appearance and stomach irritation. Your child may be more miserable and bad tempered during this time. Less common problems are muscle weakness, softening of the bones, high blood sugar, inflammation of the pancreas and very rarely convulsions.

The more common side effects of cyclosporin A include high blood pressure, decreased kidney function, changes of blood salt composition, headache, nausea, vomiting, loss of appetite, tiredness, diarrhoea and hairiness.. Other side effects are rash, weight gain, liver problems, gum swelling, oedema and rarely convulsions and severe allergic reactions. Regular blood samples will be needed to monitor the blood concentration of the drug.

The side effects of etoposide include reduction of blood counts, which may require treatment with blood or platelet transfusions or antibiotics in case of infection, and nausea and vomiting which usually respond to treatment with anti sickness medication. Your child may lose his/ her hair, but this is temporary as it grows back after treatment is completed. There may also be abdominal pain, diarrhoea, loss of appetite, short term liver function abnormalities and rarely allergic reactions or low blood pressure (if given too fast). Patients treated with etoposide have been reported to be at risk of developing a second cancer (leukaemia). Etoposide is a useful drug in many childhood cancers and in the treatment of children with HLH is on balance more useful than harmful.
9. Are any tissue samples going to be stored?
Your child will need to have blood tests and other tissue samples such as bone marrow taken before the study begins. These are used to diagnose the condition and to assess how sick your child is. If after all tests are completed there is extra tissue left over, this will then be stored for any future use. This may include use for research into the cause and treatment of the condition.

10. What are the possible disadvantages and risks of taking part?
The possible disadvantages and risks of taking part in this study relate to the side effects listed above. However, even if you decide not to take part in the study, your child might still receive the same treatment, as it is currently considered the best available.

11. What are the possible benefits of taking part?
Your child may not directly benefit from this study. However, we hope that by improving the treatment and achieving control of the disease, some children may be able to have curative treatment in the form of a bone marrow transplant. We also hope to be able to reduce the long-term effects of the disease itself.

12. What if new information becomes available?
Sometimes during the course of a research project, new information becomes available about the drug/treatment that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw from this study, your doctor will make arrangements for your child’s care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

13. What happens when the research study stops?
This study is likely to continue for at least five years and will be published when the research stops.

14. What if something goes wrong?
If taking part in this research project harms your child, there are no special compensation arrangements. If your child is harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

15. Will my taking part in this study be kept confidential?
We will need to collect data on your child and his/her full name will be used. All information that is collected about your child during the course of the research is kept strictly confidential in the UK. Information on the treatment and progress of all patients entered into this study will be sent to the United Kingdom Children’s Cancer Study Group (UKCCSG) Data Centre in Leicester. The UKCCSG operates a strict code of conduct on confidentiality. From the UKCCSG the information will be sent to the main study coordinating centre at Karolinska Hospital in Stockholm, Sweden, where again the data will be stored carefully with attention to maintaining confidentiality. Some of the information may however be passed on to researchers or regulatory authorities in other countries some of whom might not have the same protection as the UK. With your permission we would also inform your child’s general practitioner of your intention to participate in this study and keep him/her informed of your child’s progress.

16. What will happen to the results of the research study?
The results of this research study will be published in a medical journal once the study has been completed. Your child would not be identified in any publication and it will be impossible to identify him/her in any of the information presented.
Interim data may be presented at national and international conferences. The data collected from this study is regularly reviewed by a group of international experts in the Histiocyte Society.

17. Who is organising and funding the research?
The research is being organised by the Histiocyte Society and the United Kingdom Children’s Cancer Study Group (UKCCSG). This group of experts from around the world have considerable experience in the treatment of hemophagocytic lymphohistiocytosis.

18. What if I have any concerns?
If you have any concerns or other questions about this study or the way it has been carried out, you should contact the local investigator (name etc), the Chief Investigator (Dr Vasanta Nanduri, Consultant Paediatrician, Watford General Hospital, tel: 01923 217992), or you may contact the hospital (name etc) complaints department.

If you agree to allow your child to take part in this study we will need you to sign a consent form. You will be given a copy of the consent form and this information sheet to keep. Also, with your consent we will be informing your child’s GP about your participation in this study.

Thank you for taking the time to read this information sheet.

INFORMATION SHEET FOR YOUNG PERSON AGED 16+
(Version 2.0, November 2005)

Dear Patient,
You are being invited to take part in a research study looking at the treatment of hemophagocytic lymphohistiocytosis. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please do not hesitate to ask us to explain anything that is not clear or if you would like more information. Take time to decide whether or not you want to take part in this study.

Thank you for reading this.

1. What is the purpose of the study?
Hemophagocytic lymphohistiocytosis (HLH) is a very rare and severe disease in which the patient has too many infection-fighting cells called lymphocytes and histiocytes. These cells are not working properly and collect in the body’s organs causing damage. The cause of HLH is not known but there are main two forms. One, known as primary HLH or familial hemophagocytic lymphohistiocytosis (FHL), is inherited (genetic). The other, secondary HLH develops secondary to problems of the immune system such as infection. It may not be possible to tell whether or not the disease is inherited. HLH is a life threatening disease. Without treatment, the primary inherited form has a very poor outlook and even with treatment, cure may not be possible. The secondary form may recover spontaneously but can also have a very poor outcome.
This is a study of treatment for children and adolescents with either the primary inherited form, or a severe secondary form of HLH, which aims to improve the survival and reduce the long-term side effects resulting from the disease and its treatment.
As this is a very rare disease only a few patients will be diagnosed each year in the UK. This is an international study so that we can collect information from a large number of patients on the nature and course of the disease and find out more about its cause.

2. Why have I been chosen?
You are being asked to take part because you have been diagnosed as having HLH.
The study is a clinical trial (a research study involving human patients). Clinical trials involve only patients who choose to take part.

3. Do I have to take part?
If you decide that you do not want to take part in the clinical study or any of the scientific studies this is entirely up to you. It will not affect the standard of care that you receive or your relationship with your doctor. He/she will try to do what is best for you. If you change your mind once the treatment has started, let your doctor know and the treatment might be changed.
If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

4. What will happen to me if I take part?
You will be treated according to a treatment programme developed by a group of international specialists working together in the Histiocyte Society.
The treatment programme consists of two parts. The initial treatment aims to get the disease controlled, that is, into remission. This control is temporary in the primary inherited form (FHL). The best chance of cure is offered by a bone marrow transplant.
Whilst looking for a suitable donor, continuation treatment uses the same drugs but less frequently. In secondary HLH, remission may only be temporary and a bone marrow transplant may also be required.

The initial treatment lasts 8 weeks and consists of dexamethasone, a steroid, given daily by mouth or into a vein, etoposide given twice weekly for 2 weeks and then once weekly into a vein, and cyclosporin given daily by mouth or into a vein. Your doctors may also need to give some drugs into the fluid around the spine by performing a lumbar puncture. Depending on your response to this treatment, your doctors will decide whether the treatment can be stopped or if you need to continue on the chemotherapy and may need a bone marrow transplant.

5. What do I have to do?
During the whole of your treatment the team looking after you will advise you as to the precautions to be taken. You will have to be aware of the risks of infection due to the side effects of chemotherapy.

You will need to have regular blood tests to monitor the effects of the chemotherapy and in particular the levels of the cyclosporin. You will also need to have your kidney function monitored closely.

In order to give the treatment safely, you will need to have an operation to insert a tube into a vein (Hickman line or Portacath). This is a routine procedure for patients receiving chemotherapy.

6. What is the drug or procedure that is being tested?
It is hoped that the treatment in this protocol will ensure more patients are well enough to undergo a bone marrow transplant. It is also hoped that the results of these transplants may be improved.

7. What are the alternatives for diagnosis or treatment?
If you decide not to take part in this study, it will not affect the medical care that you receive. You will be given the same treatment which is currently thought to be the best available. The only difference between what we would normally do and what we are doing in this study is that we are collecting data and putting it together to try and improve treatment in the future.

8. What are the side effects of any treatment received when taking part?
All drugs have a number of side effects. However, you will not necessarily experience all of them. Most side effects can be managed and the doctors looking after you will discuss this with you.

The more common side effects of dexamethasone include high blood pressure, increased appetite, weight gain, fluid and salt retention (oedema), a bloated appearance and stomach irritation. You may feel more miserable and bad tempered during this time. Less common problems are muscle weakness, softening of the bones, high blood sugar, inflammation of the pancreas and very rarely convulsions.

The more common side effects of cyclosporin A include high blood pressure, decreased kidney function, changes of blood salt composition, headache, nausea, vomiting, loss of appetite, tiredness, diarrhoea and hairiness. Other side effects are rash, weight gain, liver problems, gum swelling, oedema and rarely convulsions and severe allergic reactions. Regular blood samples will be needed to monitor the blood concentration of the drug.

The side effects of etoposide include reduction of blood counts, which may require treatment with blood or platelet transfusions or antibiotics in case of infection, and nausea and vomiting which usually respond to treatment with anti sickness medication. You may lose your hair, but this is temporary as it grows back after treatment is completed. There may also be abdominal pain, diarrhoea, loss of appetite, tiredness, short term liver function abnormalities and rarely allergic reactions or low blood pressure (if given too fast). Patients treated with etoposide have been reported to be at risk of developing a second cancer (leukaemia). Etoposide is a useful drug in many childhood cancers and in the treatment of children with HLH is on balance more useful than harmful.
9. Are any tissue samples going to be stored?
You will have had blood tests and other tissue samples such as bone marrow taken before the study begins. These are used to diagnose the condition and to assess how sick you are. If after all tests are completed there is tissue left over, this will be stored for future use. This may include use for research into the cause and treatment of the condition.

10. What are the possible disadvantages and risks of taking part?
The possible disadvantages and risks of taking part in this study relate to the side effects listed above. However, even if you decide not to take part in the study, you might still receive the same treatment, as it is currently considered the best available.

11. What are the possible benefits of taking part?
You may not directly benefit from this study. However, we hope that by improving the treatment and achieving control of the disease, some children and young people may be able to have curative treatment in the form of a bone marrow transplant. We also hope to be able to reduce the long-term effects of the disease itself.

12. What if new information becomes available?
Sometimes during the course of a research project, new information becomes available about the drug/treatment that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw from this study, your doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

12. What happens when the research study stops?
This study is likely to continue for at least five years and will be published when the research stops.

13. What if something goes wrong?
If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

14. Will my taking part in this study be kept confidential?
We will need to collect information about you and will use your full name. All information, which is collected, about you during the course of the research will be kept strictly confidential. Information on the treatment and progress of all patients entered into this study will be sent to the United Kingdom Children’s Cancer Study Group (UKCCSG) Data Centre in Leicester. The UKCCSG operates a strict code of conduct on confidentiality.
From the UKCCSG, the information will be sent to the main study coordinating centre at Karolinska Hospital in Stockholm, Sweden where again the data will be stored carefully with attention to maintaining confidentiality. Some of the information may however be passed on to researchers or regulatory authorities in other countries some of whom might not have the same protection as the UK.
With your permission we would also inform your general practitioner of your intention to participate in this study and keep him/her informed of your progress.

15. What will happen to the results of the research study?
The results of this research study will be published in a medical journal once the study has been completed. You would not be identified in any publication and it will be impossible to identify you in any of the information presented.
Interim data may be presented at national and international conferences. The data collected from this study is regularly reviewed by a group of international experts in the Histiocyte Society.

16. Who is organising and funding the research?
The research is being organised by the Histiocyte Society and the United Kingdom Children’s Cancer Study Group (UKCCSG). This group of experts from around the world have considerable experience in the treatment of hemophagocytic lymphohistiocytosis.

17. What if I have any concerns?
If you have any concerns or other questions about this study or the way it has been carried out, you should contact the local investigator (name etc), Chief Investigator (Dr Vasanta Nanduri, Consultant Paediatrician, Watford General Hospital, tel: 01923 217992), or you may contact the hospital (name etc) complaints department.

If you agree to take part in this study we will need you to sign a consent form. You will be given a copy of the consent form and this information sheet to keep. Also, with your consent we will be informing your GP about your participation in this study.

Thank you for taking the time to read this information sheet.
Dear Patient,

You are being invited to take part in a research study looking at the treatment of hemophagocytic lymphohistiocytosis (HLH). Before you decide, it is important for you to understand why the research is being done and what it involves. Please read the following information carefully and discuss it with your parents or others if you wish. Please do not hesitate to ask us to explain anything that is not clear or if you would like more information. Take time to decide whether or not you want to take part in this study.

Thank you for reading this.

1. What is the purpose of the study?

HLH is a very rare and serious disease in which you have too many infection-fighting cells which are not working properly and cause damage to the body. The cause of HLH is not known. There are two main forms: One, primary HLH or familial hemophagocytic lymphohistiocytosis (FHL), is inherited. The other, secondary HLH develops after another condition such as infection. It may not be possible to tell which type you have. In this study, we are trying to improve the cure of HLH and reduce the long-term problems due to the disease and treatment.

As this is a very rare disease only a few patients will be diagnosed each year in the UK. This is an international study so that we can collect information from a large number of patients around the world and find out more about the cause of the disease.

2. Why have I been chosen?

You are being asked to take part because you have HLH. This study is a clinical trial (research study) which only involves patients who wish to take part.

3. Do I have to take part?

It is up to you and your parents to decide whether or not to take part in this study. If you decide that you do not want to take part in this study this is entirely up to you and your parents. It will not affect the way you are looked after or your relationship with your doctor. He/she will try to do what is best for you. If you change your mind once the treatment has started, let your doctor know and the treatment might be changed.

If you decide to take part you will be given this information sheet to keep and you and your parents will be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

4. What will happen to me if I take part?

You will be treated on a programme developed by a group of international specialists. The treatment programme consists of two parts. The initial treatment aims to get the disease controlled, that is, into remission. As this control is temporary you might need a bone marrow transplant to cure you. While the doctors are looking for a bone marrow donor you will continue to receive treatment.

The initial treatment lasts 8 weeks and consists of 3 medicines (chemotherapy) - dexamethasone, etoposide and cyclosporine. These medicines may be given into a vein or by mouth. Your doctors may also need to give some drugs into the fluid around the spine by performing a lumbar puncture. Depending on your response to this treatment, your doctors will decide whether the treatment can be stopped or if you need to continue on treatment and may need a bone marrow transplant.
5. What do I have to do?
During the whole of your treatment the team looking after you will advise you and your parents as to the precautions to be taken. You need to be careful as you might be more prone to infections. You will need to have regular blood tests to monitor your kidney function and the effects of the chemotherapy and to check levels of the cyclosporine. You will need to have an operation to insert a tube into a vein (Hickman line or Portacath) to give you your treatment and to do the blood tests.

6. What is the drug or procedure that is being tested?
It is hoped that the treatment in this protocol will ensure more patients are well enough to undergo a bone marrow transplant. We also hope that these transplants may be improved.

7. What happens if I decide not to take part?
If you decide not to take part in this study, it will not affect the care that you receive. You will be given the same treatment which is currently thought to be the best available.

8. What are the side effects of any treatment received when taking part?
All drugs have a number of side effects. However, you might not experience all of them. Most side effects can be managed and the doctors looking after you will discuss this with you. These are some of the things that might happen to you:
- **Feeling sick** - you will be given medicine to try and stop this.
- **Lose your hair** – Unfortunately this happens with all chemotherapy. But this is only temporary. When the treatment is finished your hair will grow back again.
- **Anaemia** – reduction in the number of red blood cells, treated with a blood transfusion.
- **Reduction in white blood cells** – this will reduce your body’s ability to fight infection. This may require admission to hospital to treat the infection.
- **Reduction in platelets** – these are cells in the blood that help blood to clot making you more likely to bleed and get bruises. This can be treated with a platelet transfusion.
- **Dexamethasone** - will make you hungry all the time and make you put on weight
  - can make you feel sad and bad tempered
- **Cyclosporin A** - can affect your kidneys

9. What are the possible disadvantages and risks of taking part?
The possible disadvantages and risks of taking part are due to side effects listed above.

10. What are the possible benefits of taking part?
You may not directly benefit from this study. However, we hope that by improving the treatment of the disease, some children may be able to have curative treatment in the form of a bone marrow transplant. We also hope to be able to reduce the long-term effects of the disease and treatment.

11. What if new information becomes available?
Sometimes during the course of a research project, new information becomes available about the drug / treatment that is being studied. If this happens, your research doctor will tell you and your parents about it and discuss with you whether you want to continue in the study. If you decide to withdraw from this study, your doctor will make arrangements for your care to continue. If you decide to continue in the study you and your parents will be asked to sign an updated consent form.

12. What happens when the research study stops?
This study will be published when the research stops.

13. What if something goes wrong?
If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone’s negligence, then your parents may have grounds for a legal action. If you or your parents wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this...
study, the normal National Health Service complaints mechanism is available to you and your parents.

14. **Will my taking part in this study be kept confidential?**
We will need to collect information about you while you’re on the study and will use your full name. All information that is collected about you during the course of the research will be kept strictly confidential both in the UK and in the main study coordinating centre at Karolinska Hospital in Stockholm, Sweden. With your permission we would also inform your general practitioner of your intention to participate in this study and keep him/her informed of your progress.

15. **What will happen to the results of the research study?**
The results of this research study may be presented at national and international conferences and will be published in a medical journal once the study has been completed. Your name would not be used in any publication and it will be impossible to identify you in any of the information presented.

16. **Who is organising and funding the research?**
The research is being organised by the Histiocyte Society and the United Kingdom Children’s Cancer Study Group (UKCCSG). This group of experts from around the world have considerable experience in the treatment of HLH.

17. **What if I have any concerns?**
If you have any concerns or other questions about this study you can always ask the doctors or nurses who are taking care of you.

If you agree to take part in this study we will need your parents to sign a consent form. You will be given a copy of the consent form and this information sheet to keep. Also, with your consent we will be informing your GP about your participation in this study.

Thank you for taking the time to read this information sheet.
Dear Patient
You are being invited to take part in a research study. Although we have to have your parent’s permission for you to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read this sheet and talk to others about it if you wish. Ask us if there is anything that is not clear or if you would like more information.

1. What is research?
Research means finding out more about something.

2. What is this research study about?
The tests have shown that you are poorly because you have too many infection-fighting cells, which are not working properly, collecting in parts of your body. Doctors call this problem HLH, which stands for hemophagocytic lymphohistiocytosis.
If we do not give you medicines, the HLH will make you very poorly so the doctors need to try and get rid of it, this is called treatment.
Only a few children get HLH and doctors have to treat them very specially. Because of this doctors from all over the world are working together on a project to find out the best treatment for HLH. When we study a treatment we call it a Clinical Study.

3. Why have I been chosen?
Because you have hemophagocytic lymphohistiocytosis (HLH).

4. Do I have to take part?
Your parents have to make the decision about you taking part but they may also like to know how you feel about it. They do not have to say yes, they can say no and this will not affect the way the doctors treat you. If they do say yes, they can always change their minds later on. You can also say tell us if you do not want to take part in the study.
5. What will happen to me if I take part?
The treatment will be medicines called chemotherapy. We use the three best medicines that we have used before for treating HLH. They are called Dexamethasone, Etoposide and Cyclosporin.
We will give you these medicines for 8 weeks to try to get the illness under control. Depending on how your illness responds to this treatment, your doctors will decide if the treatment can be stopped or if you need to have more treatment. The doctors will talk to you and your parents about this.

6. What do I have to do?
You will need to have an operation to insert a tube into a vein (Hickman line or Portacath) so the treatment can be given safely.
You will need to come into hospital for some of your treatment. Sometimes you may feel poorly between treatments and need to be in hospital until you are feeling better. The doctors and nurses looking after you will tell you more about this.
You will need to have regular blood tests and kidney tests to check how the medicines are affecting you.

7. What is the treatment being tested in the trial?
We know that Dexamethasone, Etoposide and Cyclosporin are good treatments for HLH. We hope that in this trial we can use them to make the treatment better.

8. What if I don’t join the study?
We will look after you just as well even if you decide not to take part in the study. You will be treated with the same treatment, which is the best for HLH that we have at the moment. Your doctor will talk to you about it.

9. Can the treatment cause me any harm?
All chemotherapy medicines have side effects and your doctor will tell you more about them.
Here are some of the side effects you may have:
Feeling sick - you will be given medicine to try and stop this.
Lose your hair - Unfortunately this happens with all chemotherapy. But when the treatment is finished your hair will grow back again.
Anaemia - reduction in the number of red blood cells, which can be treated with a blood transfusion.
Reduction in white blood cells - this will reduce your ability to fight infection. This may require admission to hospital to treat the infection.
Reduction in platelets - these are cells in the blood that help blood to clot making you more likely to bleed and get bruises. This can be treated with a platelet transfusion.

Dexamethasone will make you hungry all the time and put on weight  
- can make you feel sad and bad tempered  
Cyclosporin A can affect your kidneys

10. What are the risks of taking part in the study?  
These will be all the side effects of the medicines that we have already talked about.

11. What could be good about taking part in the study?  
We hope that more children with HLH will get better with the treatment used in this study. We also hope that the information learned from this study may help other children in the future.

12. What if we learn more about the treatment during the study?  
Sometimes during a clinical study we learn more about the treatment as the study goes along and may need to make changes. If this happens, your doctor will tell you and your parents about it and discuss whether you want to continue in the study. Whatever happens you will still have treatment.

13. Will the information about me be kept private?  
All information that is collected about you during the course of the study is kept confidential (private). We will send the information to Leicester and Sweden where it will be collected together with that of other children treated for HLH and looked at by the doctors and researchers dealing with the study.

14. What will happen to the results of the research study?  
The results of the research will be made into a report, published in a Medical Journal and presented at medical conferences. However nobody will be able to tell from this information that you took part in this study. The information from the study may help in the development of further treatments in the future.

15. What if I am worried about the study?  
If you are worried or have questions about the study, you should talk to your doctor or nurse, or ask your parents to talk to them. We are there to help and are happy to answer any questions.
If you and your parents agree for you to join the study we will need you and your parents to sign a consent form. You will be given a copy of the consent form and this letter to keep.

Thank you very much for reading this letter.
The tests we have been doing in hospital show that you are poorly because you have too many infection-fighting cells, which are not working properly and are collecting in your body. This is called HLH. It can make you very ill so we need to give you medicines to try to get rid of it. This is called treatment.

For the first part of the treatment we will give you medicines to try to make the HLH go away. These medicines are called chemotherapy or “chemo”. There are different types of chemo and they all have different names. The ones you will get are called Dexamethasone, Etoposide and Cyclosporin A.

Although the chemo is good at treating the HLH, it can do bad things too, called side effects. We can do some things to help. The doctors will talk to you about this.

You will need to come into hospital to get the treatment. Sometimes you may feel poorly between treatments and need to be in hospital until you are feeling better. The doctors and nurses looking after you will talk to you more about this.

We know you may have some worries about having your treatment and we want to help you. Please ask us about anything you want to know or ask your mum and dad so they can ask us.

INFORMATION SHEET FOR GENERAL PRACTITIONERS (Version 2.0 November 2005)

Dear Colleague

Whilst in our care the parent/guardian of your patient ………………… (DOB……………….) has given permission for their child to take part in the clinical study named above. The following information is to help you understand the purpose of the study and what is expected of your patient if they take part in the study.

Your patient has been diagnosed with HLH and fulfils the eligibility criteria for the study.

Hemophagocytic lymphohistiocytosis (HLH) is a life threatening disease. Without treatment, the primary inherited form has a very poor outlook and even with treatment, cure may not be possible. The secondary form may recover spontaneously but can also have a very poor outlook.

This is a study of treatment for children and adolescents with either the primary inherited form, or a severe secondary form of HLH, which aims to improve the survival and reduce the long-term side effects resulting from the disease and its treatment. As this is a very rare disease only a few patients will be diagnosed each year in the UK and we are therefore taking part in a collaborative international study run under the auspices of the International Histiocyte Society.

The treatment programme consists of two parts. The initial treatment aims to get the disease into remission. This control is temporary in the primary inherited form (FHL). The best chance of cure is offered by a bone marrow transplant. Whilst looking for a suitable donor, continuation treatment uses the same drugs but less frequently. In secondary HLH, remission may only be temporary and a bone marrow transplant may also be required.

The initial treatment lasts 8 weeks and consists of dexamethasone, given daily orally or i.v., etoposide given twice weekly for 2 weeks and then once weekly i.v., and cyclosporin given daily orally or i.v.. For some patients intrathecal therapy may be beneficial. Depending on your patient’s response to this treatment, a decision will be made on whether the treatment can be stopped or if your patient needs to continue on the chemotherapy and may need a bone marrow transplant.

If you would like further information, please don’t hesitate to get in touch.
TO BE PRINTED ON INSTITUTION HEADED NOTE PAPER

Centre Number:
Patient Identification Number for this trial:

CONSENT FORM FOR YOUNG PERSON AGED 16+ YRS
(Version 2.0 November 2005)

Title of Project:  International Hemophagocytic Lymphohistiocytosis Study (HLH 2004)
(LCH 2006 02)

Name of Researcher:

Please initial box:

1. I confirm that I have read and understand the information sheet(s) (Version ….,
dated ….) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at
any time, without giving any reason, without my medical care or legal rights
being affected.

3. I understand that sections of any of my medical notes may be looked at by
responsible individuals from the UKCCSG or from regulatory authorities where
it is relevant to my taking part in research. I give permission for these
individuals to have access to my records and understand that my full name will
be used on data forms.

4. I agree to take part in the above study.

5. I agree that my GP may be notified about participation in the above study.

6. I agree for samples to be stored and used in the biological research studies
integrated into this study and for future studies.

7. I agree that data about me relating to this study may be sent to countries that
might not have data protection laws that are similar to those in the UK.

Name of patient                   Date                   Signature

_________________________ ________________                  ____________________
Name of person taking consent (if different from researcher) Date                     Signature

_________________________ ________________                  ____________________
Researcher                           Date                   Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes
TO BE PRINTED ON INSTITUTION HEADED NOTE PAPER

 Centre Number:  
 Patient Identification Number for this trial: 

 PARENT/CHILD CONSENT FORM  
 (Version 2.0 November 2005)  

 Title of Project:  International Hemophagocytic Lymphohistocytosis Study (HLH 2004)  
 (LCH 2006 02)  

 Name of Researcher:  

 **Please initial box**  
 **(delete words as necessary)**  

 1. I confirm that I have read and understand the information sheet(s) dated (Version … Date …) for the above study and have had the opportunity to ask questions. 

 2. I understand that my/my child’s participation is voluntary and that I am/he/she is free to withdraw at any time, without giving any reason, without my/his/her medical care or legal rights being affected. 

 3. I understand that sections of any of my/my child’s medical notes may be looked at by responsible individuals from the UKCCSG or from regulatory authorities where it is relevant to my/my child’s taking part in research. I give permission for these individuals to have access to my/my child’s records. 

 4. I agree/agree for my child to take part in the above study. 

 5. I agree that my/my child’s GP is notified about participation in the above study. 

 6. I agree for samples to be stored and used in the biological research studies integrated into this study and for future studies. 

 7. I agree that data about me/my child relating to this study may be sent to countries that might not have data protection laws that are similar to those in the UK. 

 ______________________  ______________________  ______________________  
 Name of patient  Date  Signature  

 ______________________  ______________________  ______________________  
 Name of parent/guardian  Date  Signature  

 ______________________  ______________________  ______________________  
 Name of person taking consent  Date  Signature  
 (if different from researcher) 

 ______________________  ______________________  ______________________  
 Researcher  Date  Signature  

 1 for patient; 1 for researcher; 1 to be kept with hospital notes