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INTERFANT-06

INTERNATIONAL COLLABORATIVE TREATMENT PROTOCOL FOR INFANTS UNDER ONE YEAR WITH ACUTE LYMPHOBLASTIC OR BIPHENOTYPIC LEUKEMIA

SKION
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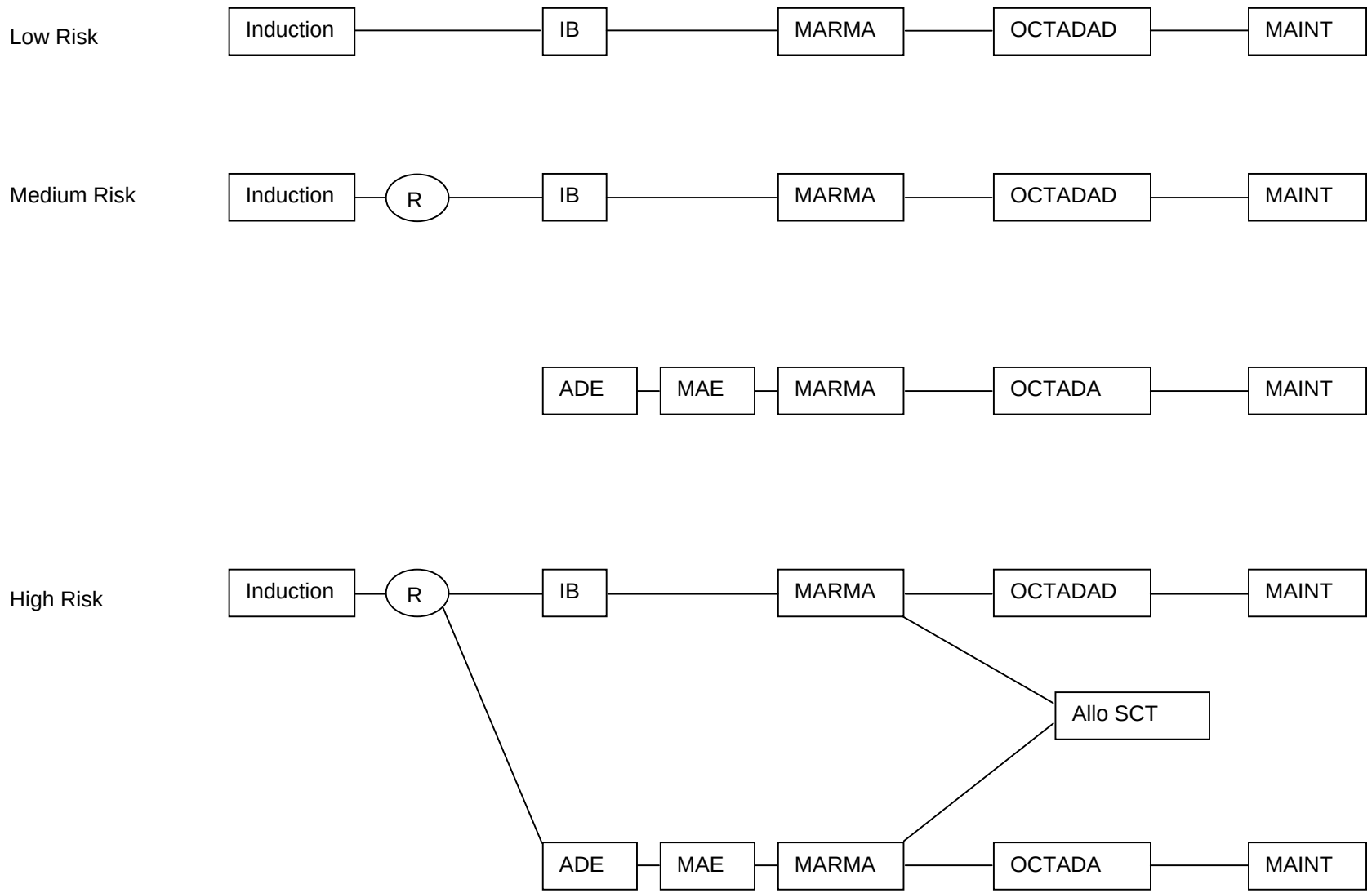
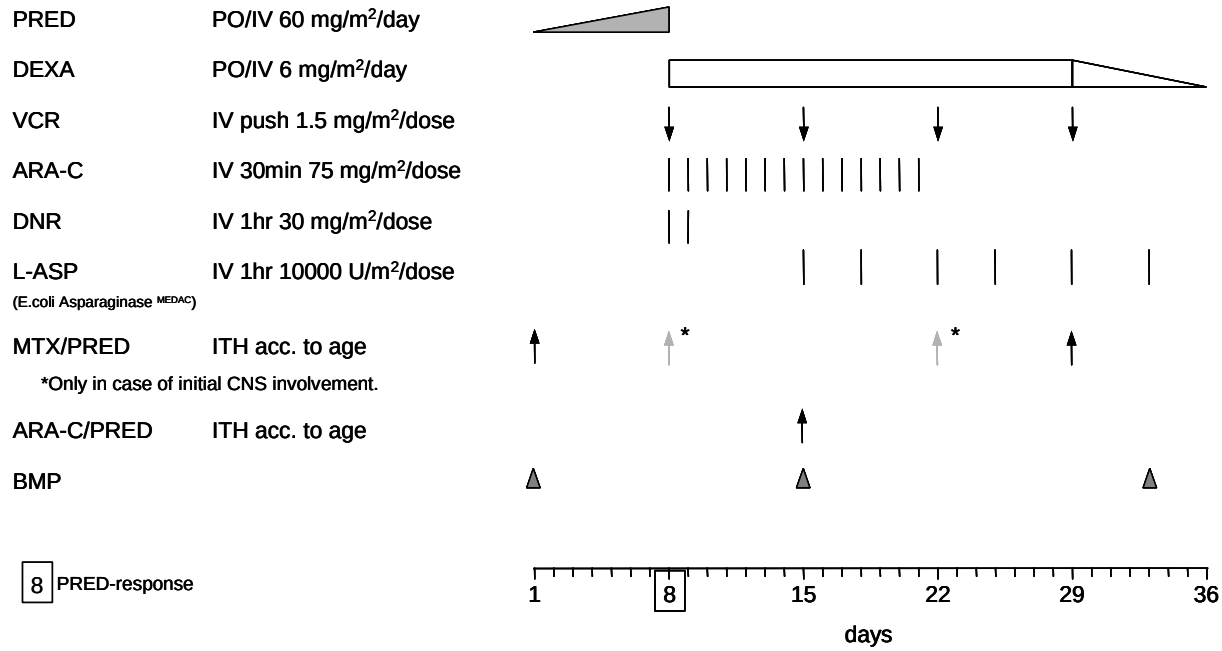
EudraCT Number: 2005-004599-19

INTERFANT-06



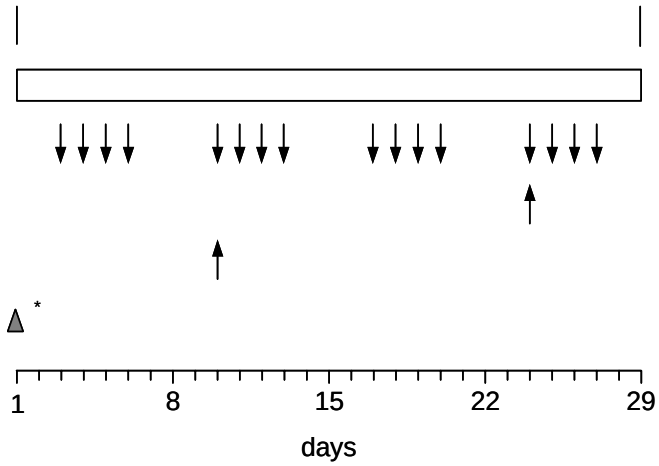
INDUCTION



!! Please be aware of the dose adjustments according to age !!
Calculate the surface area at the start of each treatment block and then adjust
Children <6 months of age: *2/3 of the calculated dose based on surface area*
Children 6 through 12 months of age: *3/4 of the calculated dose*
Children >12 months of age: *full dose*
Dose reductions are for all drugs including glucocorticoids, but not for intrathecal drugs.
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

PROTOCOL IB

CPM	IV 1hr 1000 mg/m^2/dose
6-MP	PO 60 mg/m^2/day
ARA-C	IV push 75 mg/m^2/dose
MTX/PRED	ITH acc. to age
ARA-C/PRED	ITH acc. to age
BMP (only if BMP at day 33 is inconclusive)	



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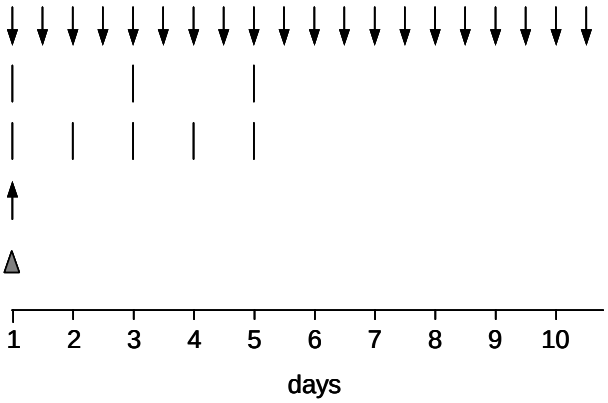
ADE

ARA-C	IV push 100 mg/m^2/dose
DNR	IV 1hr 50 mg/m^2/dose
Etoposide	IV 4hrs 100 mg/m^2/dose
ARA-C/PRED	ITH acc. to age
BMP	



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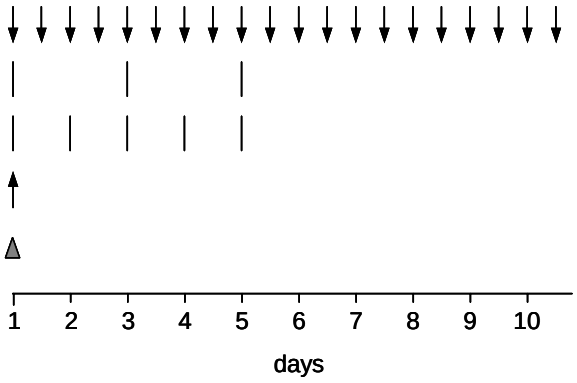
MAE

ARA-C	IV push 100 mg/m^2/dose
Mitoxantrone	IV 1hr 12 mg/m^2/dose
Etoposide	IV 4hrs 100 mg/m^2/dose
MTX/PRED	ITH acc. to age
BMP	



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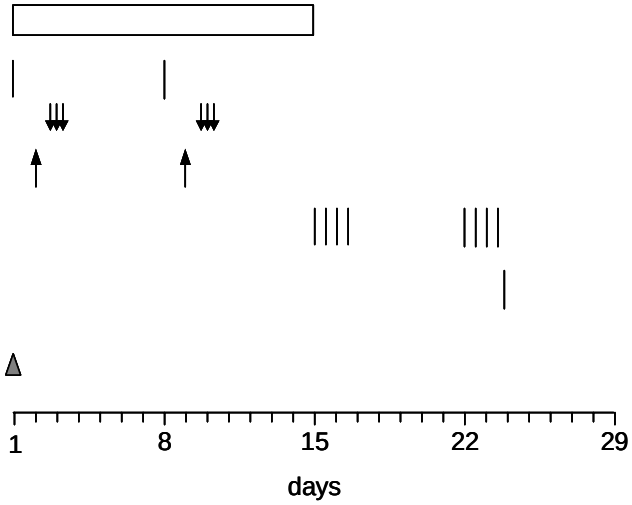
MARMA

6-MP	PO 25 mg/m^2/day
HD-MTX	IV 24hrs 5000 mg/m^2/dose
Leucovorin-Rescue	PO/IV 15 mg/m^2/dose
MTX/PRED	ITH acc. to age
ARA-C	IV 3hrs 3000 mg/m^2/dose
PEG-ASP (ONCASPAR)	IV 1hr 2500 U/m^2/dose
BMP	



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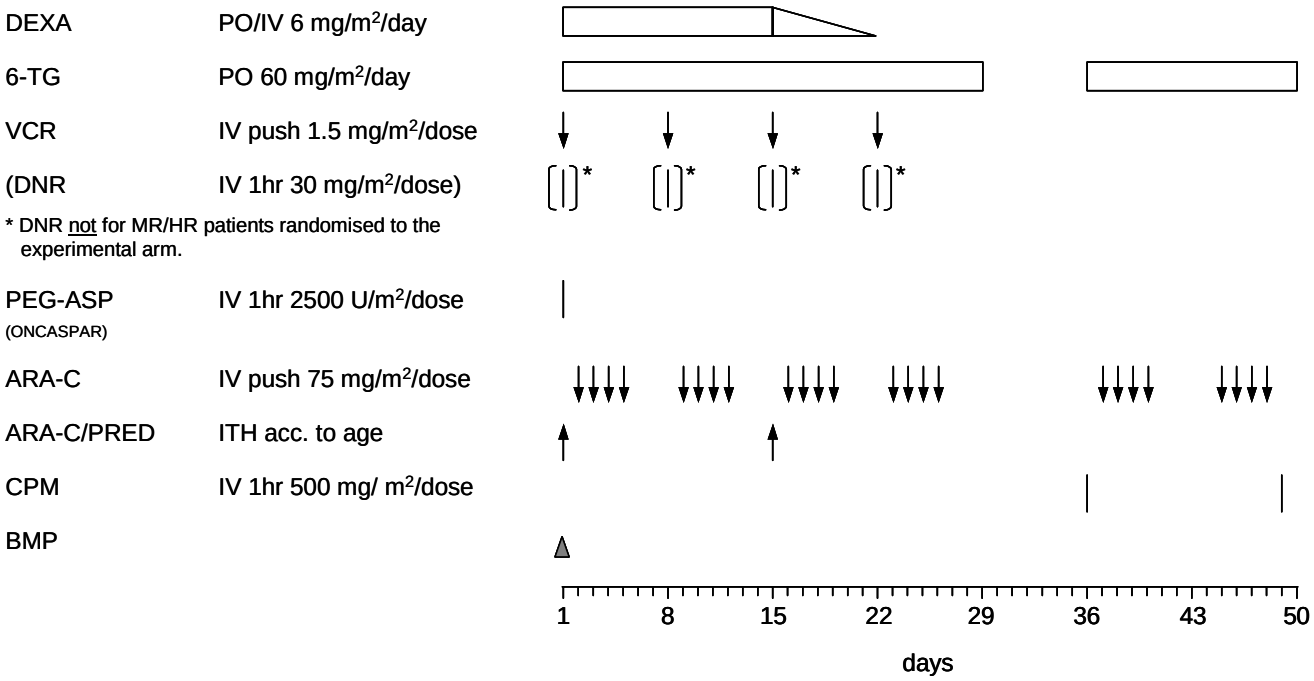
OCTADA(D)



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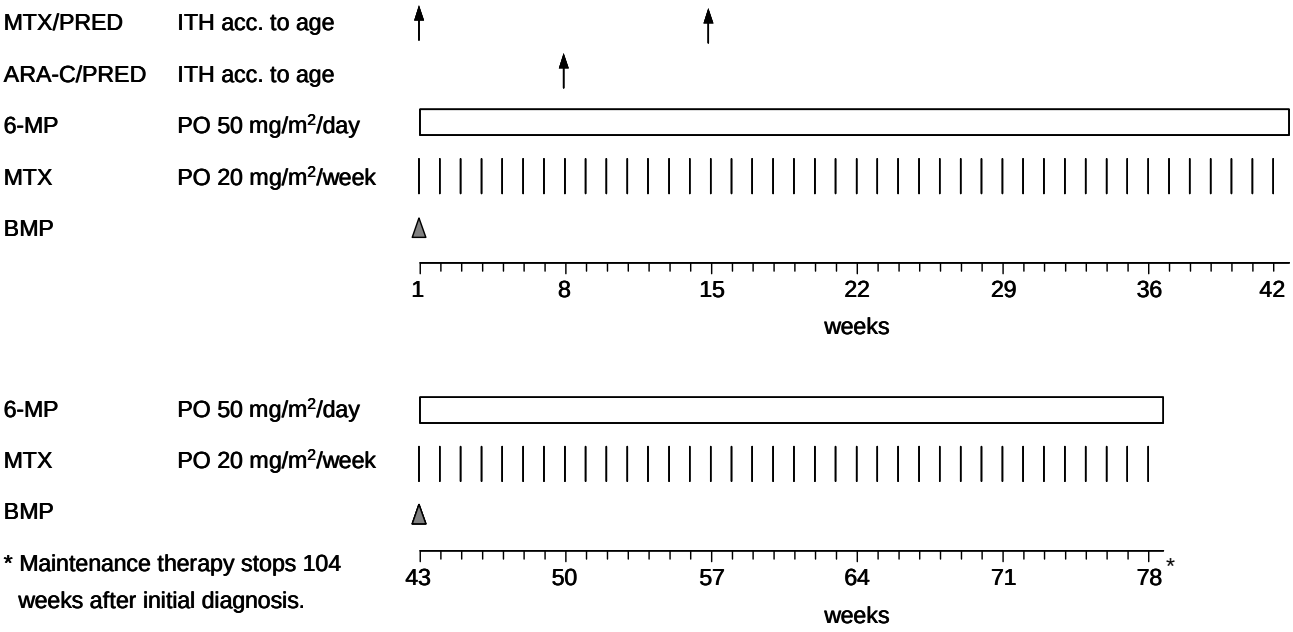
MAINTENANCE



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1 Background

1.1 Treatment results

Infant acute lymphoblastic leukemia (ALL) is a rare disease and comprises about 4% of childhood ALL. Whereas the outcome of older children with ALL has improved to 80-85% event-free survival (EFS) infants with ALL have a worse prognosis. Published treatment results in infant ALL of the major study groups are shown in Table 1. In 1999, a large international collaborative study group was started to develop common treatment protocols for infant ALL to try to improve the outcome for these very young children. The Interfant-99 protocol was the first treatment protocol of this collaborative group that consisted of all major European study groups and several study groups and large pediatric oncology centers outside Europe. This led to the largest trial for infant ALL known so far: the interim analysis reported in May 2004 included 331 cases. At that analysis, the median follow-up time was 2 years and the overall outcome of the Interfant-99 protocol was comparable to that of the most favourable historical control series with sufficient patient numbers (BFM, CCG) and better than historical results from most other study groups. Because 90% of all events occur in the first 2 years after diagnosis in infant ALL these results are close to the final outcome results.

Table 1. Treatment results in infant ALL

Group	Outcome	N	Reference
DFCI 85-01	4yr EFS 54%	23	Silverman 1997
Interfant-99	*2yr EFS 53%*	*331*	*Interim analysis*
BFM	4yr EFS 43%	105	Dordelmann 1999
EORTC-CLCG	4yr EFS 43%	25	Ferster 1994
CCG-1883	4yr EFS 39%	135	Reaman 1999
CCG-107	4yr EFS 33%	99	Reaman 1999
UKALL-92	5yr EFS 33%	86	Chessels 2002
POG 8493	4yr EFS 28%	82	Frankel 1997
POG (alt. Drugs)	5yr EFS 17%	33	Lauer 1998

1.2 Detailed results of Interfant-99

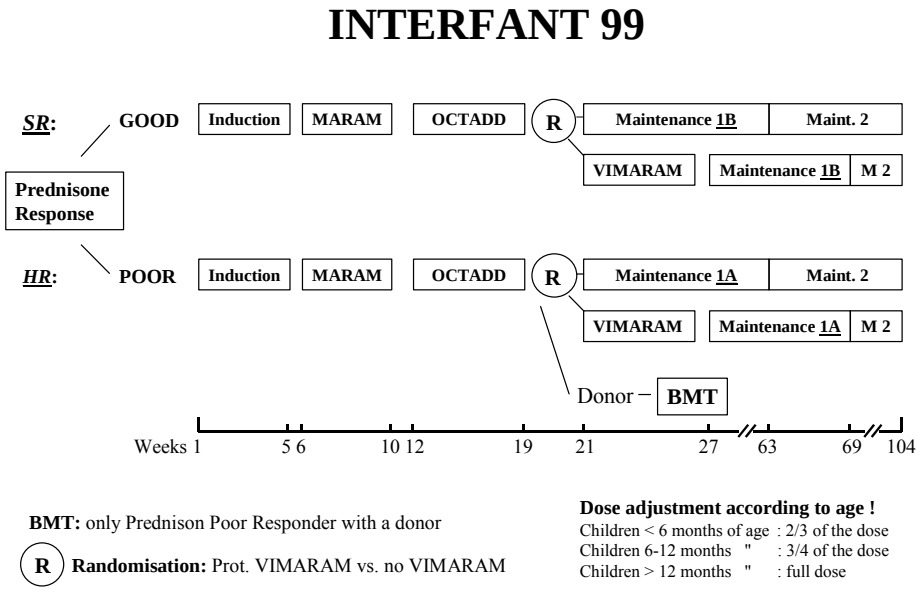
1.2.1 Overall outcome and randomisation in Interfant-99

The overall outcome (2-yr 53% EFS) of the Interfant-99 is satisfying as mentioned above. The 2-year EFS and 2-year survival for the groups originally participating in Interfant-99 were 54.9% and 63.9% respectively. Treatment results of groups that participated at a later stage were 45.6% and 62.8% respectively.

The CR rate was 95%, and at a median of 2-years follow-up time the death rate in CCR was 5.9% and the relapse rate was 30.8%. The far majority of relapses were BM relapses (24.9%), followed by combined BM+CNS relapses (2.6%), isolated CNS relapse (2.3%) and others (1%).The median time to relapse was 8 months (range 0-34 months) which indicates that very early BM relapse is still the major problem in treatment of infant ALL.

Inclusion of patients for the randomised question whether addition of VIMARAM was of benefit was stopped before the target number of patients was reached because both arms of the study were so close to each other that it was highly unlikely that any difference would have been found. It was concluded that addition of this intensification block at a later stage did not improve the outcome.

figure 1. Scheme Interfant-99



1.2.2 Prognostic factors in Interfant-99

ALL in infancy is associated with a high white blood cell count (WBC) at presentation, a high frequency of an immature precursor B-lineage characterized by the lack of CD10 expression and the presence of MLL gene (11q23) rearrangements. These 3 factors and also age < 3 or 6 months have been associated with a poor prognosis (Biondi 2000, Pieters 2003). In addition, Reiter et al (1994) reported that a poor in vivo response to prednisone was of prognostic value in infants, which was confirmed by an update of the BFM86/90 studies before the start of the Interfant-99 protocol: 6-yr EFS was 58% for infants with a good prednisone response (GPR) versus only 16% for infants with a poor prednisone response (PPR). Because all these prognostic factors are highly interrelated and the numbers of patients were small in the reported studies, an important aim of the Interfant-99 protocol was to determine which factors have independent prognostic value.

A Cox regression analysis was performed to investigate the role of sex, age at diagnosis, WBC at diagnosis, immunophenotype (CD10 expression), presence of any MLL rearrangement and Prednisone response on 246 Interfant99 patients. A regression tree approach based on Cox results, was used to produce the stratification into three risk groups to be used in the new Interfant-06 protocol. Age at diagnosis, WBC at diagnosis and MLL status appeared to have prognostic value in the Interfant-99 study, while sex, CD10 expression and Prednisone response did not. In particular, age at diagnosis <6 months, WBC at diagnosis ≥ 300 x 109/L and MLL rearrangement seemed to be correlated with a worse prognosis. This analysis did not consider separately the different MLL rearrangements, as univariate analyses showed that t(4;11) positive cases and cases with MLL rearrangements other than t(4;11) had a superimposable outcome (EFS).

Infants with a MLL germline ALL treated with Interfant-99 have a 2 years EFS of 87.0%. This percentage might drop when follow-up gets longer because it is known from older patients with MLL germline ALL that about half of the relapses occur after the first 2 years. ALL infants with germline MLL will be stratified as low risk in the Interfant-06 protocol and will include 15-20% of all patients.

For patients with MLL rearranged ALL almost all events tend to occur within the first 2 years after diagnosis so the EFS at 2-yr follow up for these patients is close to the final EFS. The 2-year EFS for patients with MLL gene rearranged ALL in the Interfant-99 study was 45%. Further subdivision

of this group showed that infants with MLL rearranged ALL and also both other high risk features (i.e. age <6 months and WBC ≥ 300 at diagnosis) have a 2-year EFS of only 15.9%. These patients will be stratified as high risk in the new Interfant-06 protocol and will include about 15% of all patients. The remaining MLL rearranged patients have a 2-yr EFS of 49.5% and will be stratified as medium risk in the new protocol and will include about 2/3 of all patients. Patients whose MLL status was not fully known in the Interfant-99 protocol also had an intermediate outcome.

1.2.3 Stem cell transplantation (SCT) in Interfant-99

In Interfant-99 only the prednisone response was used for stratification of patients: PGR patients and PPR patients received the same blocks of intensive therapy but PPR patients were eligible for allogenic SCT. Patients with a PPR have an improved outcome (2-yr EFS 40%) when compared to the historical control patients (EFS 16%) but the numbers in the historical control were small. The Interfant study was not designed to analyse whether this improved outcome was because part of them received SCT. However, when compared by performed treatment, adjusted by waiting time to transplant, the EFS curve of PPR patients who received SCT (n=16) and the EFS curves of PPR who did not receive SCT (n=56) were not significantly different.

1.3 Outlines of the Interfant-06 protocol

1.3.1 Stratification

Stratification into 3 risk groups will be based upon the MLL status, age and WBC as argued above. The LR group will consist of all MLL germline cases, including MLL germline patients with a PPR. HR patients are MLL rearranged, < 6 mths and WBC ≥ 300. MR patients are all others.
The risk groups LR, MR and HR will contain ~20%, ~65% and ~15% of all patients based upon numbers with a known MLL status in Interfant-99. In about 25% of patients in Interfant-99 the MLL status was unknown because determination of this was not mandatory. In the theoretical case that again 25% would be "MLL unknown" in the Interfant-06 study then the percentages of patients in the LR, MR and HR groups will be respectively ~15%, ~75% and 10-15%. However, determining the MLL status by split signal FISH in Interfant-06 will be mandatory for participating groups.

1.3.2 Induction

All patients get the same induction therapy as in Interfant-99, because CR rate was very satisfying.

1.3.3 Asparaginase

The way asparaginase was used in the Interfant-99 protocol could be improved according to current insights about pharmacokinetics of asparaginase. In induction, Coli Asparaginase dose will be increased from 5.000 to 10.000 U/m^2. The two doses of native Coli Asparaginase during MARAM will be replaced by one dose of 2.500 U/m^2 Oncaspar at the end of MARAM (now called MARMA). This will lead to 2-4 weeks of asparagine and glutamine depletion in the serum. Because the reinduction course OCTADD did not contain any asparaginase, one dose of Oncaspar 2500 U/m^2 will be added at the start of OCTADD (now called OCTADAD). This will be done for all patients.

1.3.4 Randomisation for MR and HR

The outcome of MR and especially HR patients needs to be improved. Therefore, in these patients a randomised question will be asked whether two blocks of AML induction chemotherapy will improve outcome. AML blocks are used because infant MLL has myeloid characteristics. The AML blocks are well tolerated by infants with AML (Webb 2001). The courses that will be randomised will be introduced early, after induction, because early relapse is still the major problem in treatment of infant ALL. Standard Interfant-99 therapy is however considered not to be effective enough as control arm. Therefore the standard arm of Interfant will be changed by the addition of

the regular BFM protocol IB (6-MP, AraC, Cyclofosfamide) directly after induction as is commonly used for older children with ALL and for infants in BFM-like schedules before 1999. This IB block has shown to be effective in reducing the leukemic load measured by minimal residual disease in recent BFM studies. Also, the course IB has been used extensively in infant ALL by e.g. EORTC and BFM study groups without excessive toxicity. The randomisation question will be the comparison of protocol IB (standard arm) against two AML-blocks, i.e. ADE and MAE (experimental arm) in MR and HR patients. Because of the cumulative dose of anthracyclines in the experimental arm, OCTADAD will be without anthracyclines (called OCTADA) in this latter arm.

1.3.5 SCT for HR

HR patients will follow the same protocol and randomisation as MR patients. The only difference is that they will be eligible for allogenic SCT. Time of SCT will be after MARMA so before OCTADA(D) or during OCTADA(D). Donor selection, conditioning regimen and graft versus host prophylaxis will be performed as advised by Christina Peters.

1.3.6 LR

LR patients should be treated according to the modified standard Interfant protocol (so identical as the control arm of the MR/HR patients).

1.3.7 Dose reductions

Dose reductions should be as in Interfant-99 for all drugs, including glucocorticoids (including the prednisone prephase) but excluding intrathecal drugs.

1.3.8 Central Nervous System and Testicular Involvement and Therapy

The CNS relapse rate in Interfant-99 was low without CNS irradiation. Therefore, CNS directed therapy is unchanged. This means for central nervous system (CNS) involvement at diagnosis (CNS+) that weekly intrathecal doses in induction are scheduled, at least two but more if needed to clear the spinal fluid from blasts. In case the central nervous system is not involved at initial diagnosis, the total number of intrathecal therapeutic injections is 12. For CNS+ cases the number of intrathecal injections is at least 14. For definitions of CNS involvement, CNS status and traumatic lumbar punctures (TLP) see chapter 3. Patients with CNS2 or TLP+ are not defined as CNS+ but should be treated as CNS+ so these patients also get at least two extra intrathecal doses of therapy.
In case of enlarged testes at diagnosis, this should be normalized after induction therapy. If not, a testicular biopsy is indicated.

1.3.9 Stem cell transplantation (SCT)

The role of high dose chemotherapy and SCT in infant ALL is unclear. There are no data to support extensive use of SCT in infants. No randomised studies have been performed that studied the role of SCT in infant ALL. Two recent publications might suggest that the use of SCT contributed to a favourable outcome in infant ALL but these studies did not have a control arm in which patients only received chemotherapy and the data were not corrected for waiting time to SCT and in one of these studies total body irradiation was used which leads to severe late effects in infants (Sanders 2005, Kosaka 2004). After correction for waiting time to SCT, the Interfant-99 data did not show a difference in outcome for infants with a poor prednisone response who received either SCT or maintenance chemotherapy. Data from a large retrospective intergroup analysis also did not show differences between infant MLL rearranged cases who did or did not receive SCT (Pui 2002).
In view of the uncertainties about the efficacy and risks of SCT only infants of the newly defined HR category in Interfant-06 will be eligible for SCT. All HR patients will undergo high resolution HLA allele typing. Eligible donors are HLA identical sibling donors (MSD) or very well matched related or unrelated donors (MD) – HLA compatible in 10/10 or 9/10 allels (determined by 4 digit/allel high resolution typing). Umbilical cord blood (UCB) might be an option if HLA-identical or closely

matched. All HR patients with a suitable matched donor are scheduled for SCT after MARMA and before or after part of OCTADA(D), provided they are in CR1 at that time. Conditioning (as for t(4;11) patients) consists of Busulfan, Cyclophosphamide and Melphalan. Total body irradiation is not used because of its neurotoxic side effects in these young children.

2 Aims of the study

The primary aim of the study is:

1. To assess the role of an early intensification of two "AML" induction blocks versus protocol Ib directly after induction, in a randomized way in MR and HR patients.

Secondary aims are:

2. To assess the role of an early intensification of two "AML" induction blocks versus protocol Ib directly after induction, in a randomized way in MR and HR patients, separately.
3. To assess the overall outcome of the Interfant-06 protocol compared to the historical control series, especially the Interfant-99.
4. To assess the outcome of LR, MR and HR patients as compared to the historical control series in Interfant-99.
5. To study which factors have independent prognostic value.
6. To assess the role of SCT in HR patients.

3 Eligibility criteria and definitions

3.1 Eligibility criteria

3.1.1 Inclusion criteria

The criteria for entry to the study are:

1. Children aged 365 days or less with newly diagnosed acute lymphoblastic leukaemia (ALL) or biphenotypic leukemia according to EGIL criteria. Children with CNS or testicular leukemia at diagnosis are eligible.
   It is important that all infants with ALL less than 1 year of age, including those infants who are eligible but are not treated according to the protocol are registered so that any selection bias can be determined.
2. Morphological verification of the diagnosis, confirmed with cytochemistry and immunophenotyping. In case a bone marrow aspiration results in a "dry tap", a trephine biopsy is advised unless it is possible to confirm the diagnosis by peripheral blood examination.
3. Informed consent of the parents or other legally authorized guardian of the patient.

3.1.2 Exclusion criteria

Patients are excluded from the study if at least one of the following exlusion criteria applies:

1. Mature B-ALL, defined by the immunophenotypical presence of surface immunoglobulines or t(8;14) and breakpoint as in B-ALL.
2. The presence of the t(9;22) (q34;q11) or bcr-abl fusion in the leukemic cells (if these data are not known, the patient is eligible).
3. Age > 365 days.
4. Relapsed ALL.
5. Systemic use of corticosteroids less than 4 weeks before diagnosis. Patients who received corticosteroids by aerosol are eligible for the study.

3.2 Definitions

3.2.1 CNS-status and CNS involvement

a) CNS status is defined as follows:
   - CNS1: nontraumatic puncture, ≤ 5 WBC/µl CSF without leukemic cells after cytocentrifugation.
   - CNS2: nontraumatic puncture, ≤ 5 WBC/µl CSF with identifiable leukemic cells.
   - CNS3: nontraumatic puncture, > 5 WBC/µl CSF with identifiable leukemic cells.
   - TLP+: traumatic puncture with leukemic cells.
   - TLP-: traumatic puncture without leukemic cells.

   A traumatic lumbar puncture (TLP) is defined as 10 or more erythrocytes/µl CSF or as CSF macroscopically contaminated with blood.

b) CNS involvement is defined as follows:
   - CNS3 status OR
   - Intracerebral or meningeal mass seen on the MRI or CT scans OR
   - Cranial nerve palsy (irrespective of CSF or imaging findings) OR
   - Retinal Involvement (irrespective of CSF findings).

3.2.2 Testicular involvement

Testicular involvement is defined as leukemic infiltration of the testis, documented by biopsy.

3.2.3 Mediastinal mass

Mediastinal mass is defined as a mass of > 1/3 thoracic diameter at the level of the 5th thoracic vertebra.

4. Risk group stratification and randomisation

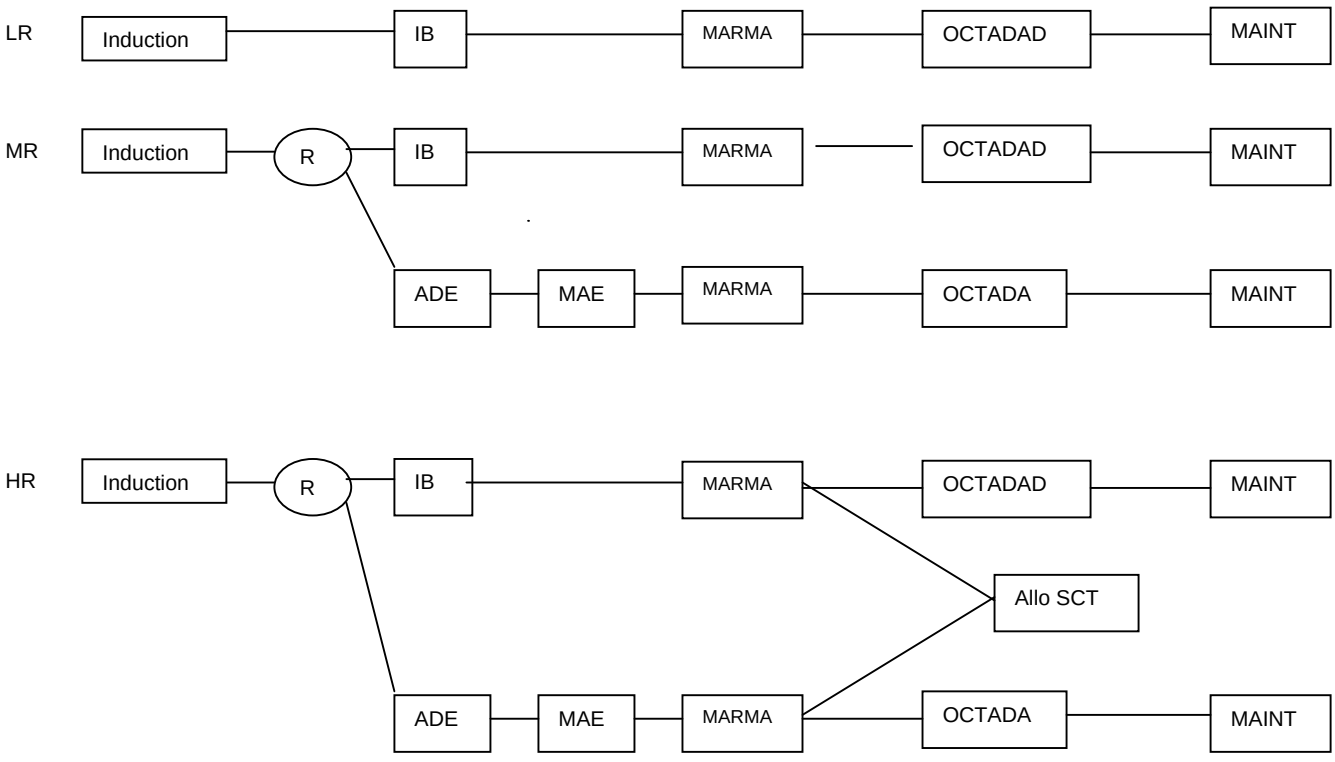
Low risk (LR): MLL germline
High risk (HR): MLL rearranged AND
Age at diagnosis < 6 months (i.e. <183 days) AND
WBC ≥ 300 x 10^e9/L
Medium risk (MR): all other cases so including those with:

- MLL status unknown (see Section 9.1 point 3.3) OR
- MLL rearranged AND age > 6 months OR
- MLL rearranged AND age < 6 months AND WBC < 300 x 10^e9/L

The **standard (control) arm** of therapy consists of the following blocks: induction. IB, MARMA, OCTADAD and maintenance.
The **experimental arm** of therapy consists of the following blocks: induction, ADE, MAE, MARMA, OCTADA and maintenance.

All **low risk** patients receive the **standard** arm.
Medium risk and **high risk** patients are **randomised** to receive **the standard or experimental** arm (see Section 11.1).
Only high risk patients are **eligible for SCT**, provided that the donor criteria as defined in the paragraph on SCT are fulfilled. In that case HR patients receive SCT after MARMA so before the start of OCTADA(D) or after receiving part of OCTADA(D).

In case **no informed consent** is obtained for randomisation, patients will be treated according to the **standard arm**.

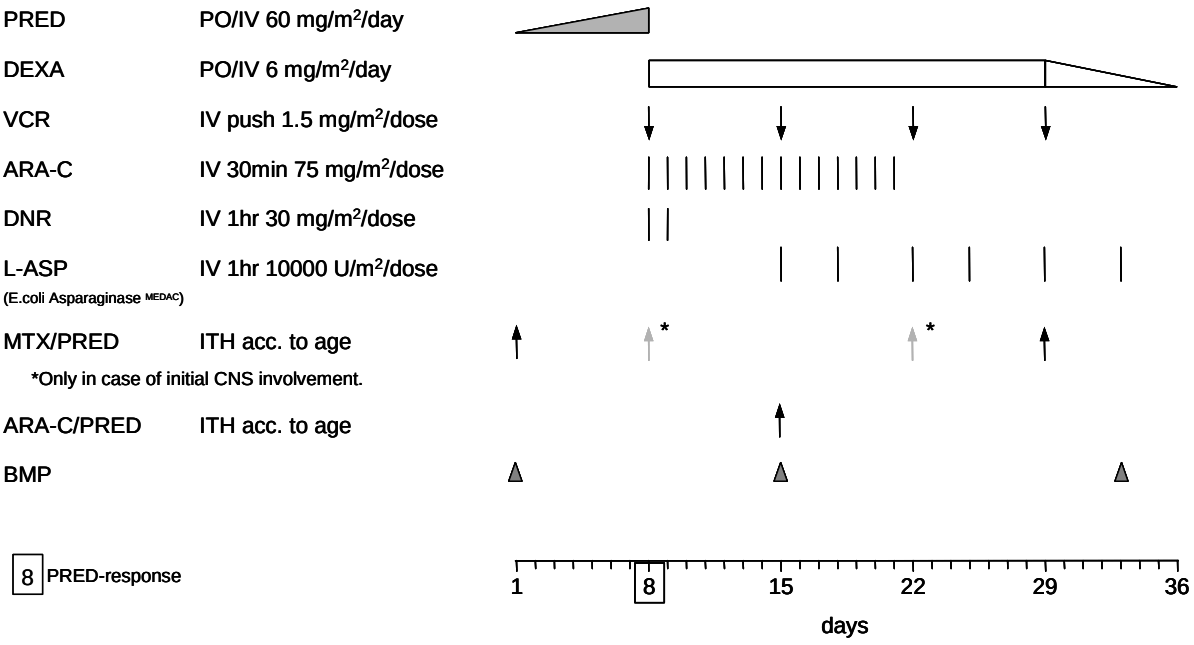


5 Chemotherapy Schedule

!! Please be aware of the dose adjustments according to age !!
Calculate the surface area at the start of each treatment block and then adjust
Children <6 months of age: 2/3 of the calculated dose based on surface area
Children 6 through 12 months of age: 3/4 of the calculated dose
Children >12 months of age: full dose
Dose reductions are for all drugs including glucocorticoids, but not for intrathecal drugs.
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

5.1 Induction

INDUCTION



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Calculate the surface area at the start of each treatment block and then adjust
Children <6 months of age: 2/3 of the calculated dose based on surface area
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Children >12 months of age: full dose
Dose reductions are for all drugs including glucocorticoids, but not for intrathecal drugs.
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

Prednisone Phase

- Prednisone: 60 mg/m2 daily divided into 3 doses orally or iv on 7 consecutive days, i.e day 1-7. In case of a high risk of tumor lysis it is advisable to start at a lower dose which is increased each day:

Initial WBC	Advised Starting dose of Prednisone at day 1:
50-100x 109/L	15 mg/m2 daily
>100x109/L	6 mg/m2 daily

The total dose of prednisone in 7 days should be at least 200 mg/m2 (optimal 420 mg/m2). Previous experience with the BFM regimens shows that sometimes a rise in WBC can be seen during the first 2 days of treatment with prednisone, followed by a decrease thereafter. If the patient remains in good condition there is no need to introduce other drugs. If the patient's condition deteriorates or if the leucocyte count continues to rise after three days then the other induction drugs should be started.

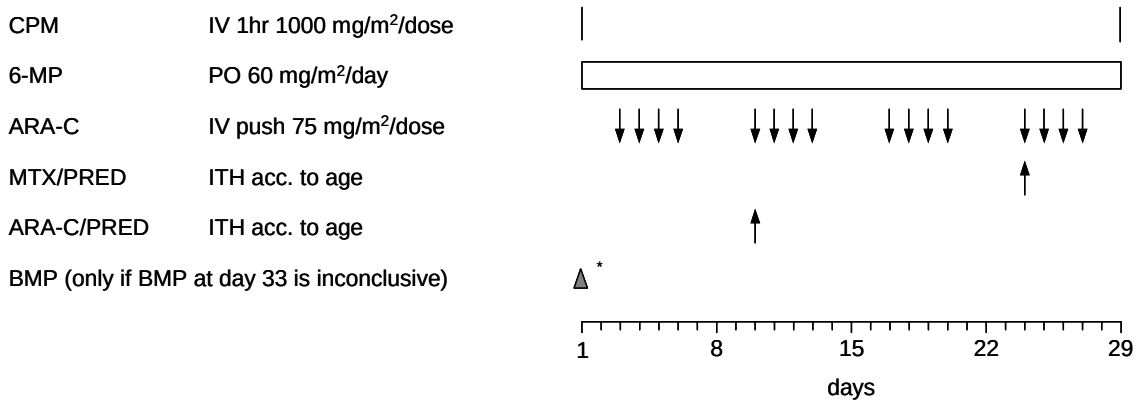
- Intrathecal methotrexate and prednisolone is given on day 1 (see below for dose)
- Appropriate management of tumor lysis according to local policy. See for advise paragraph 8.2.

Rest of Induction

- Dexamethasone: 6 mg/m2 daily divided into 3 doses iv or orally on 21 consecutive days, i.e. day 8-28, followed by one week in which the drug is reduced stepwise to zero.
- Vincristine: 1.5 mg/m2 daily iv push on 4 days at day 8, 15, 22, 29.
- Cytarabine: 75 mg/m2 daily iv in 30 min on 14 consecutive days from days 8-21.
- Daunorubicin: 30 mg/m2 daily iv in 60 min on 2 consecutive days at day 8 and 9. If local protocols advise other infusion times of daunorubicin, it is acceptable to give it iv over a minimum of 30 min up to a maximum of 6 hrs.
- L-Asparaginase: 10.000 U/m2 daily iv in 1 hr or im on day 15, 18, 22, 25, 29, 33. The L-asparaginase to be used is a Coli preparation preferably from Medac because of its prolonged asparagine depletion. If only Elspar Coli Asparaginase is available, the dose should be adjusted to 20.000 U/m2. If only Erwinase is available, the dose should be adjusted to 20.000 U/m2, 3 times a week so a total of 9 doses.
- Intrathecal MTX: at day 1 and at day 29. The intrathecal dose of 6 mg methotrexate when age is < 1 yr, 8 mg when age is ≥ 1yr. In case of CNS involvement at initial diagnosis extra intrathecal doses of MTX should be given at day 8 and 22. If CNS leukemia is still present at day 29 then weekly intrathecal MTX until the CNS is free of leukemia.
- Intrathecal cytarabine: at day 15 intrathecal dose of 15 mg araC when age is < 1 yr, 20 mg when age is ≥ 1yr.
- Intrathecal prednisolone: When intrathecal MTX or intrathecal araC is scheduled combine this with intrathecal prednisolone: 6 mg when age is < 1 yr, 8 mg when age is > yr. If prednisolone is not available for intrathecal use, this can be replaced by 12 mg or 16 mg hydrocortisone respectively.

5.2 Protocol IB

PROTOCOL IB



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Calculate the surface area at the start of each treatment block and then adjust
Children <6 months of age: 2/3 of the calculated dose based on surface area
Children 6 through 12 months of age: 3/4 of the calculated dose
Children >12 months of age: full dose
Dose reductions are for all drugs including glucocorticoids, but not for intrathecal drugs.
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

Protocol IB starts at day 36 (day counts follow induction).

Requirements for the start of protocol IB

- Complete remission at day 33 (see paragraph 10.3)
- Good clinical condition without serious infections
- Creatinine within normal limits according to age
- Neutrophil count > 0,5 x 10^9/l
- Platelets > 50 x 10^9/L

Requirements starting each block of cytosine arabinoside (ARA-C)

- WBC > 0.5 x 10^9/L
- Platelets > 30 x 10^9/L

Requirements for the second cyclophosphamide dose at day 64

- WBC > 1 x 10^9/L
- Neutrophil count > 0.3 x 10^9/L
- Platelets > 50 x 10^9/L

If possible, the ARA-C blocks should not be interrupted. If nevertheless an ARA-C block has to be postponed or interrupted because of clinical problems, the 6-mercaptopurine should also be interrupted. Omitted 6-mercaptopurine doses should be made up until the planned cumulative total dose of 1680 mg/m2 (28 x 60 mg/m2) is reached.

CPM Cyclophosphamide 1,000 mg/m2/dose, i.v. over 1 hour, day 1 and 29
Requirements during administration:

- Hydration and cystitis prophylaxis: 3,000 ml/m^2 fluid/24 hr for a minimum of 6 hours.
- Mesna (Uromitexan®): 400 mg/m^2/dose i.v. before and 3 and 6 hours after the start of the CPM-infusion.
- In case of (microscopic) hematuria: increase i.v. fluid and Mesna.
- Furosemide 0.5 mg/kg i.v., 6 hours and 12 hours after CPM only if required for diuresis.

6MP 6 Mercaptopurine 60 mg/m^2/day p.o., days 1-28 (28 days in total).

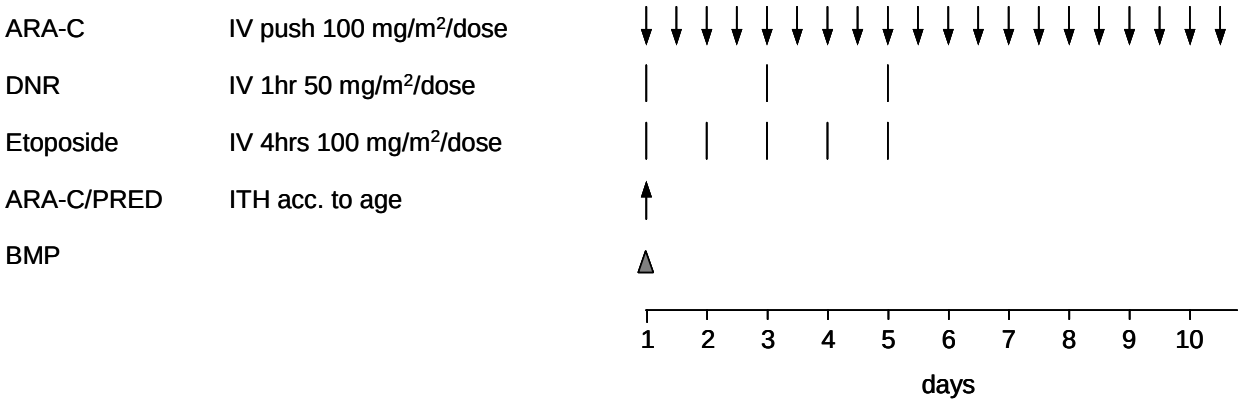
- Administration: with empty stomach, in the evening, not with milk.
- Omitted 6 MP-doses should be made up until the planned cumulative total dose of 1680 mg/m^2 (28 x 60 mg/$m^{2)}$ is reached.

ARA-C Cytosine Arabinoside 75 mg/m^2/dose i.v. push in four blocks, of 4 days each:
Days 3, 4, 5, 6
Days 10, 11, 12, 13
Days 17, 18, 19, 20
Days 24, 25, 26, 27

Intrathecal MTX at day 24: 6 mg when age is < 1 yr or 8 mg when age ≥ 1 yr.
Intrathecal cytarabine at day 10: 15 mg AraC when age is < 1 yr, 20 mg when age is ≥ 1 yr.
Intrathecal prednisolone at day 10 and 24: 6 mg when age is < 1 yr, 8 mg when age is ≥ 1 yr. If prednisolone is not available for intrathecal use, this can be replaced by 12 mg or 16 mg hydrocortisone respectively.

5.3 ADE

ADE



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Children <6 months of age: 2/3 of the calculated dose based on surface area
Children 6 through 12 months of age: 3/4 of the calculated dose
Children >12 months of age: full dose
Dose reductions are for all drugs including glucocorticoids, but not for intrathecal drugs.
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

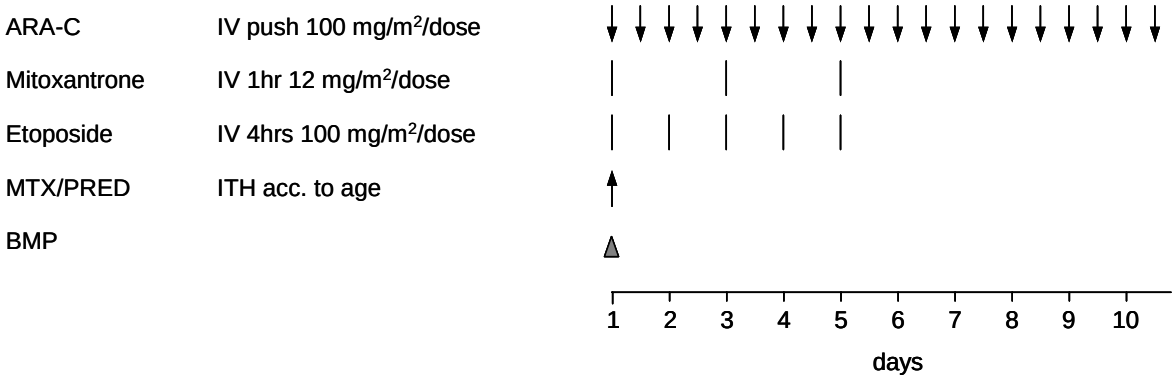
ADE starts at day 36 (day counts follow induction).

Requirements for the start of ADE
Neutrophil count > 0,5 x 10^9/l and platelets > 50 x 10^9/l and both are rising.

- ARA-C: 100 mg/m^2, push i.v. every 12 hr day 1-10 (20 doses).
- Daunorubicin: 50 mg/m^2 over 1 hour IV on day 1, 3 and 5 (3 doses).
- Etoposide 100 mg/m^2 over 4 hrs iv on day 1-5 (5 doses).
- Intrathecal therapy on day 1:
  - Inthrathecal cytarabine: 15 mg araC when age is < 1 yr, 20 mg when age is $\geq$ 1 yr.
  - Inthrathecal prednisolone: 6 mg when age is < 1 yr, 8 mg when age is $\geq$ 1 yr. If prednisolone is not available for intrathecal use, this can be replaced by 12 mg or 16 mg hydrocortisone respectively.

5.4 MAE

MAE



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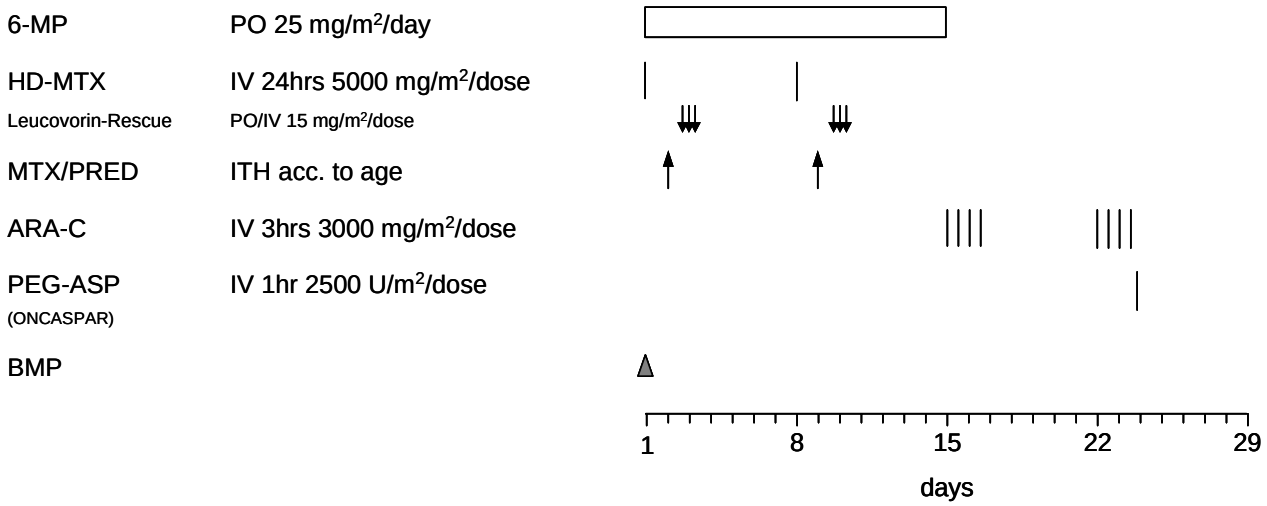
Children 6 through 12 months of age: 3/4 of the calculated dose
Children >12 months of age: full dose
Dose reductions are for all drugs including glucocorticoids, but not for intrathecal drugs.
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

Requirements for the start of MAE
Neutrophil > 0,5 x 10^9/l and platelets > 50 x 10^9/l and both are rising.

- ARA-C: 100 mg/m^2, push iv every 12 hrs on day 1-10 (20 doses).
- Mitoxantrone: 12 mg/m^2 over 1 hr iv on day 1, 3 and 5 (3 doses).
- Etoposide: 100 mg/^{2} over 4 hrs iv on day 1-5 (5 doses).
- Intrathecal therapy on day 1:
  - Intrathecal MTX: 6 mg methotrexate when age is < 1 yr, 8 mg when age is ≥ 1 yr.
  - Intrathecal prednisolone: 6 mg when age is < 1 yr, 8 mg when age is ≥ 1 yr. If prednisolone is not available for intrathecal use, this can be replaced by 12 mg or 16 mg hydrocortisone respectively.

5.5 MARMA

MARMA



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Children >12 months of age: full dose
Dose reductions are for all drugs including glucocorticoids, but not for intrathecal drugs.
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

Requirements for the start of MARMA
Neutrophil count > 0.5 x 109/l and platelets > 50 x 109/l and rising.

- 6MP 6-Mercaptopurine: 25 mg/m2 daily in 1 dose orally on 14 consecutive days, i.e. day 1-14. If day 8 MTX is delayed due to toxicity then stop 6-MP and recommence with second dose to complete 14 days.
- MTX Methotrexate: 5000 mg/m2 iv as 24 hour infusion on day 1 and 8; 10% (500 mg/m2) of the dose in 30 minutes iv followed by 90% (4500 mg/m2) of the dose in 23.5 hrs. Cotrimoxazole should be stopped from 48 hours prior to methotrexate and until methotrexate plasma level is <0.2 uM. The second dose of HD-MTX may be given regardless of the blood count but not regardless of the condition of the patient, e.g. mucositis.
- Leucovorin rescue: 15 mg/m2 orally/ iv at 42, 48 and 54 hrs after the start of the MTX infusion. Plasma levels of MTX should be determined 24 hrs and 48 hrs after the start of the MTX infusion. If the plasma MTX level is > 0.2 uM at 48 hrs after the start of MTX infusion then continue the leucovorin doses every 6 hours until MTX plasma level is <0.2 uM.

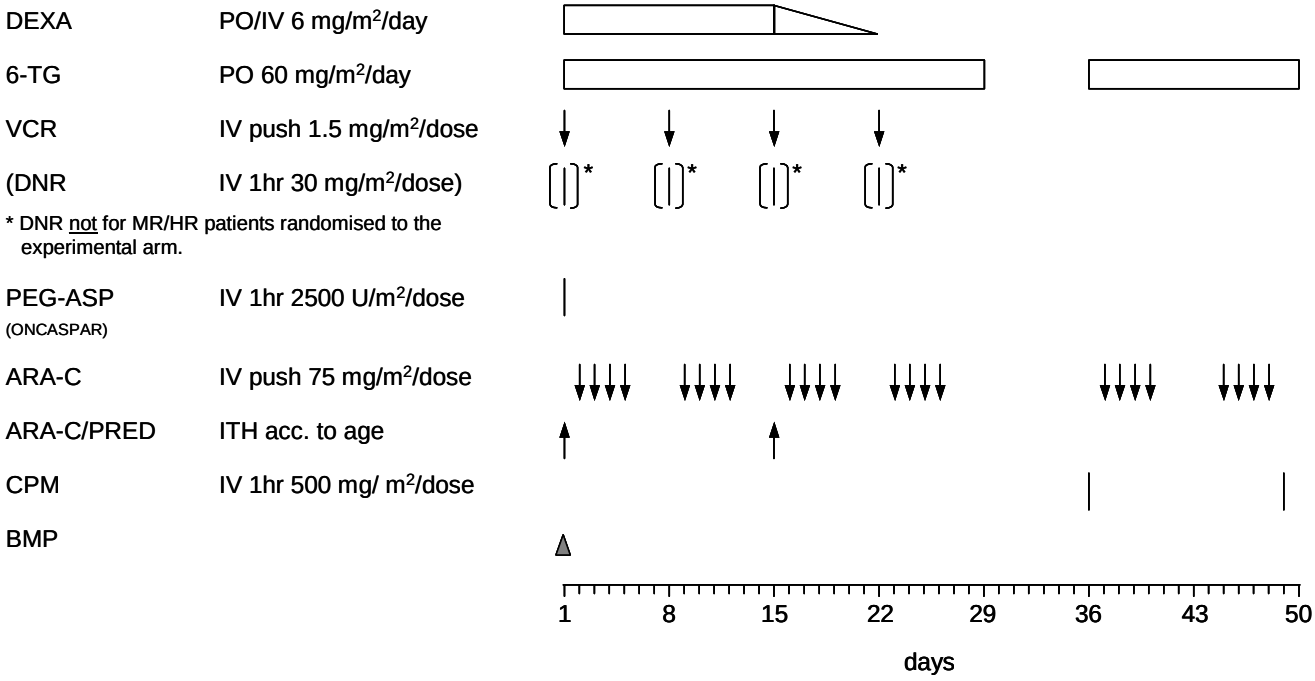
- Intrathecal MTX: At the end of the 24 hr MTX infusion, i.e. at day 2 and 9: intrathecal dose of 6 mg methotrexate when age is < 1 yr, 8 mg when age is ≥ 1 yr.
- Intrathecal prednisolone at day 2 and 9: 6 mg when age is < 1 yr, 8 mg when age is ≥ 1 yr. If prednisolone is not available for intrathecal use, this can be replaced by 12 mg or 16 mg hydrocortisone respectively.

The second phase of MARMA consisting of high dose cytarabine and asparaginase may start only when there is no mucositis and when the neutrophil count > 0.5 x 109/l and platelets > 100 x 109/l. *The high dose cytarabine at day 22 can be started irrespective of the blood counts.*

- AraC: 3000 mg/m2 iv in 3 hrs infusion twice daily with 12 hrs interval on 4 days, i.e. day 15, 16, 22, 23.
- PEG-Asparaginase: 2500 U/m2 on day 23 iv in 1 hr or im. The asparaginase is given 3 hrs after completion of the last araC infusion on day 23 because of its supposed synergistic effects. The asparaginase should not be given before or during araC infusion because of supposed antagonistic effects in that case. The L-asparaginase to be used is the PEG-Asparaginase preparation from Medac because of its prolonged asparagine depletion.

5.6 OCTADA(D)

OCTADA(D)



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Children <6 months of age: *2/3 of the calculated dose based on surface area*
Children 6 through 12 months of age: *3/4 of the calculated dose*
Children >12 months of age: *full dose*
Dose reductions are for all drugs including glucocorticoids, but not for intrathecal drugs.
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

Requirements for the start of OCTADA(D)
OCTADA(D) starts when neutrophil count > 0.5 x 109/l and platelets > 50 x 109/l and both are rising but should not start earlier than 2 weeks after the end of MARMA. *The first part of this course takes 4 weeks after which there is one week without chemotherapy.* Neutrophils and platelets should be measured at the start of each week. Application of VCR, DNR and the start of an ARA-C bloc should be delayed and 6TG interrupted when neutrophils drops < 0.3x109/L and/or platelets < 50x109/L but if a 4-day course of cytarabine has started, then this should not be interrupted.

First part

- DEXA Dexamethasone: 6 mg/m2 daily divided into 3 doses orally on 14 consecutive days, i.e. day 1-14, followed by one week in which the drug is reduced stepwise to zero at day 21.
- 6-TG 6-Thioguanine: 60 mg/m2 daily in 1 dose orally on 28 consecutive days, i.e. day 1 to 28.
- VCR Vincristine: 1.5 mg/m2 iv push on 4 days at day 1, 8, 15, 22.
- DNR Daunorubicin: 30 mg/m2 iv over 60 min on 4 days at day 1, 8, 15, 22. If local protocols

advise other infusion times of daunorubicin, it is acceptable to give it iv over a minimum of 30 min up to a maximum of 6 hrs. Note: Daunorubicin should not be given to MR/HR who are randomised to the experimental arm because of cumulative dose of anthracyclines; DNR only for LR patients and MR/HR patients randomised to the standard arm.

- PEG-Asp PEG-Asparaginase: 2500 U/m2 iv in 1 hr or im on day 1.
- ARA-C Cytarabine: 75 mg/m2 daily iv push on day 2-5, day 9-12, day 16-19 and day 23-26.
- Intrathecal cytarabine at day 1 and 15: 15 mg when age is < 1 yr, 20 mg when age is ≥ 1 yr.
- Intrathecal prednisolone at day 1 and 15: 6 mg when age is < 1 yr, 8 mg when age is ≥ 1 yr. If prednisolone is not available for intrathecal use, this can be replaced by 12 mg or 16 mg hydrocortisone respectively.

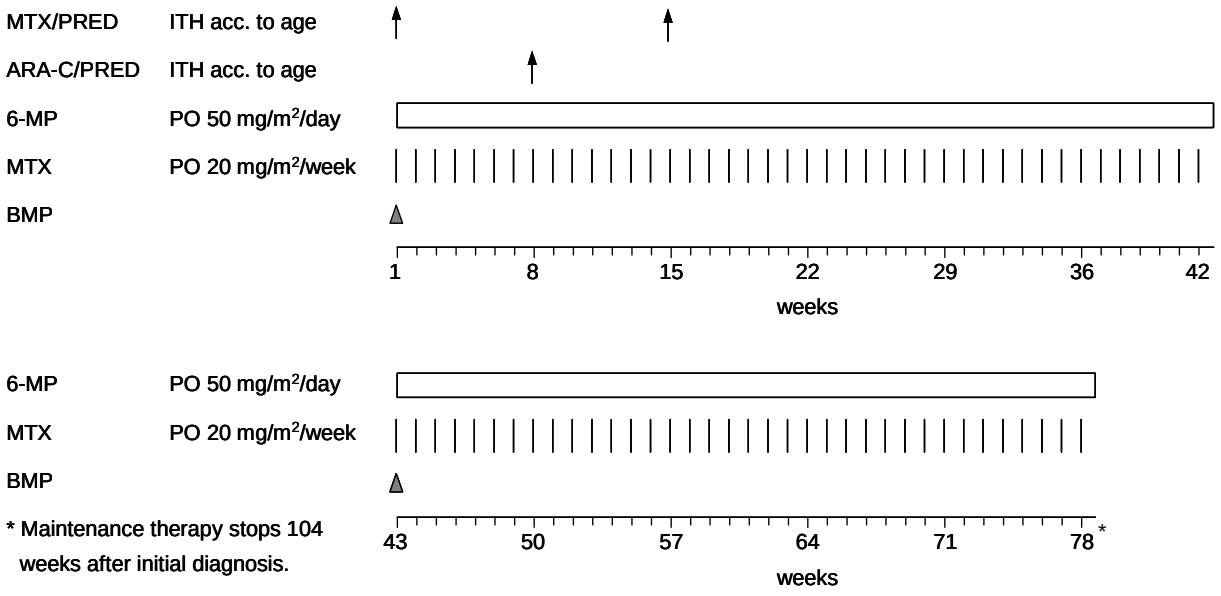
Second part

The second part of this course should only start when neutrophils > 0.5 x 109/l and platelets > 50 x 109/l.

- 6-Thioguanine: 60 mg/m2 daily in 1 dose orally on 14 consecutive days, i.e. day 36-49.
- Cytarabine: 75 mg/m2 daily given iv push on day 37-40 and day 45-48.
- Cyclophosphamide: 500 mg/m2 in 1 hr iv on day 36 and 49.

5.7 Continuing Treatment (Maintenance)

MAINTENANCE



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Children >12 months of age: full dose
Dose reductions are for all drugs including glucocorticoids, but not for intrathecal drugs.
Intrathecal doses are according to age as indicated in the schemes and do not depend on surface area.

Requirements for the start of maintenance
This phase starts when the neutrophil count > 0.5 x 109/l and platelets > 50 x 109/l and rising but not earlier than 2 weeks after the end of the previous course of chemotherapy.
This part of the maintenance consists of daily 6-MP plus weekly MTX and 3 administrations of intrathecal medication (week 1, 8, 15).

- Intrathecal MTX in week 1 and 15: 6 mg when age is < 1 yr or 8 mg when age ≥ 1 yr.
- Intrathecal AraC in week 8: 15 mg when age is < 1 yr, 20 mg when age is ≥ 1 yr.
- Intrathecal prednisolone in week 1, 8 and 15: 6 mg when age is < 1 yr, 8 mg when age is ≥ 1.
- yr. If prednisolone is not available for intrathecal use, this can be replaced by 12 mg or 16 mg hydrocortisone respectively.
- 6-MP 6-Mercaptopurine: 50 mg/m2 daily in 1 dose orally in the evening, on an empty stomach avoiding milk products.
- MTX Methotrexate: 20 mg/m2 once a week orally on the same day of each week.

Maintenance therapy stops 104 weeks after initial diagnosis. The duration of this phase varies according to the length of previous consolidation.

Dose adjustments during maintenance
During maintenance the doses of 6-MP and MTX should be adjusted upward (with no upper dose limit) to obtain a total white blood cell count below 3.0 x 109/L. The drugs should be reduced in dosage or withdrawn if the white blood cell count falls below 1.5 x 109/L, the absolute neutrophil count below 0.3 to 0.5 x 109/L, or the platelet count below 50 x 109/L.

Routine measurements of liver function are not necessary in patients without symptoms. In case of symptoms, dose reductions should be based on a rise in bilirubin to more than three times the upper normal limit or aminotransferase levels more than 10 times the upper normal limit and rising. In such cases, other causes such as viral hepatitis or Gilbert syndrome should be considered. In case of low leucocytes, neutrophils or trombocytes also consider to stop cotrimoxazole temporarily.

6 Stem cell transplantation

6.1 Background and indication for SCT

See also paragraph 1.3.9. In view of the uncertainties about the efficacy and risks of SCT only infants of the newly defined HR category in Interfant-06 will be eligible for SCT and only if the donor criteria are met.
The primary eligibility criteria for SCT are:

- Age at diagnosis : < 6 months (i.e. <183 days)
- MLL Rearrangement
- Initial WBC ≥ 300 x 10*9/L
- First complete remission

6.2 Time scheduled for SCT

SCT should be performed after MARMA so before OCTADA(D) or during OCTADA(D) **but not later than 8 months after initial diagnosis**. The conditioning regimen should start as soon as the patient has recovered from MARMA or OCTADA(D). If infection or toxicity requires that the start of conditioning is postponed, patients should receive risk adjusted chemotherapy, to bridge the time until transplantation.

6.3 HLA typing and donor selection

In HR patients, HLA typing of the patient, parents and sibling(s) should be done as soon as possible. The SCT centre should be contacted in time in order to perform SCT within the given time frame.
Minimum requirements for HLA typing are as follows:
The loci A, B, C, DRB1 and DQB1 are to be determined. For the patient the HLA class I and the HLA class II will be determined by high resolution methods (4 digit/10 allels). For possible suitable sibling donors the definition of the HLA-I features via so-called “medium resolution” methods could be sufficient. The HLA typing of unrelated donors and possible family donors needs to be high-resolution typed in class I and class II (4 digits/10 allels).

Eligible donors are HLA identical sibling donors (MSD) or very well matched related or unrelated donors (MD) – HLA compatible in 10/10 or 9/10 allels (determined by 4 digit/allel high resolution typing). Umbilical cord blood (UCB) might be an option if HLA-identical or closely matched. Donor hierarchy is thus as follows:

Priority	HLA-typing result	Transplantation group
1	HLA-identical sibling	MSD
2	10/10 identical unrelated or family donor	MD
3	9/10 identical unrelated or family donor	MD
In case of 1 mismatch:		
1	allele-mismatch	MD
2	antigen-mismatch	MD
In case of 1 mismatch on different HLA loci:		
1	C-mismatch	MD
1	B-mismatch	MD
2	class II-mismatch	MD
3	A-mismatch	MD

In addition to the ranking according to HLA-typing the following features are considered:

- CMV-Status
- Sex
- Age of donor
- Stem cell source
- Availability of donor

6.4 Stem cell source and number of stem cells

The preferred stem cell source is bone marrow. However, if bone marrow harvest is not possible, peripheral blood stem cells (PBSC) (of G-CSF stimulated donors) or cord blood (CB) are acceptable exceptions.Unmanipulated bone marrow is the preferred stem cell source. A minimum nucleated cell (NC) of 3x108 /kg BW of the patient or 3x106/kg BW CD34+ cells should be available for the transplantation. Umbilical cord blood is accepted if a sibling donor is not able to donate bone marrow and UCB with a sufficient number of NCs (>1, 5x10*7/kg recipient BW) is cryopreserved. If no MD could be identified a highly matched unrelated UCB (> 7/8 matches identified by high resolution typing) may be used. The transplant analysis should include the number of transplanted nucleated cells, the number of CD34+ cells, as well as the number of CD3+ (if applicable CD4+, CD8+) cells.

6.5 Conditioning therapy

Before start of conditioning the remission status must be documented by a bone-marrow and lumbar puncture, which should not be older than 14 days. Busulfan (BU) is an established myeloablative alternative to total body irradiation (TBI). An irradiation free conditioning with busulfan, melphalan and cyclophosphamide that has shown good results in JMML and AML, will be used.

Conditioning regimen:

Matched sibling donors: MSD

Time	**Conditioning / Dosage**	
Day –7	BU	5mg/kg p.o.
Day –6	BU	5mg/kg p.o.
Day –5	BU	5mg/kg p.o.
Day –4	BU	5mg/kg p.o.
Day –3	CYCLO	60mg/kg i.v.
Day –2	CYCLO	60mg/kg i.v.
Day –1	MEL	140mg/m^2 i.v.
Day 0	Allogeneic HSCT	

Matched Donor: MD

Time	**Conditioning / Dosage**			
Day –7	BU	5mg/kg p.o.		
Day –6	BU	5mg/kg p.o.		
Day –5	BU	5mg/kg p.o.		
Day –4	BU	5mg/kg p.o.		
Day –3	CYCLO	60mg/kg i.v.	ATG	20mg/kg i.v.
Day –2	CYCLO	60mg/kg i.v.	ATG	20mg/kg i.v.
Day –1	MEL	140mg/m^2 i.v.	ATG	20mg/kg i.v.
Day 0	Allogeneic HSCT			

For very obese children, the conditioning should be calculated not according to the actual body weight, but to the 97th percentile of the current age.

Busulfan (BU)
Patients should receive a total dose of 20mg/kg. BU should be given orally at a dose of 1.25mg/kg respectively, at 6-hour intervals on 4 consecutive days. Blood levels of the drug should be measured if possible, and the dose should be adjusted accordingly. During BU-application a seizure prophylaxis is required. If intravenous busulfan is applied the given preparation and dose must be documented.

Cyclophosphamide (CY)
Cyclophosphamide is given at a dose of 60mg/kg as infusion over one hour on day –3 and -2. Balanced hydration has to be taken care of. The specific urinal weight should be kept below 1010g/l. In cases of haemorrhagic cystitis hydration should be increased, an adequate pain management should be initiated and MESNA-administration should be continued.

Melphalan (MEL)
MEL should be given at a dose of 140mg/m2 on day –1. Melphalan is to be applied at least 3 hours after dissolution as an one-hour infusion. MEL may only be dissolved in sodium chloride infusion, and may not be mixed with glucose.

ATG Fresenius (ATG) (only in case of MD/MFD)
ATG-Fresenius S is obtained from rabbits immunised with human T-lymphoblasts of the Jurkat cell-line. ATG-Fresenius S is administered at a dose of 20 mg/kg on three consecutive days (day –3 until day –1). Emergency medicines need to be ready for immediate intervention, and frequent examination of the vital signs is required.

6.6 GvHD-prophylaxis and – therapy
In MSD the GvHD-prophylaxis consists of Cyclosporin A (CsA) starting on day -1 given i.v. twice daily 1.5 mg/kg. As soon as oral intake is possible it can be switched to CsA per os. Oral CsA is administered twice daily at a total dose of 3mg/kg BW. CsA blood levels should be measured and CsA dose should be adjusted accordingly. In the absence of GvHD symptoms, CSA is tapered at day +60 (by app. 20% of initial dose each week)

In MD the GvHD-prophylaxis consists of CsA, MTX and ATG. CsA is administered as outlined for group MSD. MTX is given on days +1, +3 und +6 at a dose of 10mg/m2 i.v. On days +2, +4, +7 Leucovorin i.v. is given at 15mg/m2/dose. The application and dosage of ATG is described above.

6.7 Supportive care recommendations
The guidelines for supportive care differ between centres. Here, general recommendations are given:

- **Isolation**: At the onset of bone marrow aplasia latest, preferably at the beginning of chemo-conditioning, the patient should be nursed in a reverse isolation unit. For additional particle air filtration HEPA or laminar air-flow units are recommended.
- **Oral supportive measures**: The following supportive measures need to be undertaken starting one week before conditioning and continued until the end of severe neutropenia (ANC<500/ µl) or control of potential GvHD >II.
- Oral decontamination in accordance with local standards.
- Trimethoprim-Sulfomethoxazol as pneumocystis-carinii-prophylaxis from day +14 until 4 weeks after the end of immunosuppression on two days per week.
- Careful oral hygiene (including mucosa) in accordance with local standards.

- **Central-venous access**.
- **Aciclovir** (3x10mg/kg/day) as prophylaxis against herpes simplex starting by day +1 until at least day +100.
- Intravenous substitution of **immunoglobulins** on days –1 and +14 400mg/kg BW each, then adapted to target level (target level >500mg/dl) until normal values have been achieved.
- **Transfusion**: Substitution of packed red cells in cases of Hb levels below 9 g/dl; substitution of platelet concentrates in cases of platelet levels below 20,000/µl; in cases of haemorrhage or sepsis the number of platelets should be kept higher. All blood products should be irradiated, filtered and leukocyte depleted.
- **Hydration**: During the entire period of conditioning hydration (100ml/kg BW or 3 L/m²) and excretion need to be checked for sufficiency.
- **Nutrition**: Sufficient enteral and, if necessary, parenteral nutrition should be ensured in order to prevent catabolic metabolism. Low bacterial enteral nutrition is recommended according to local standards until neutrophil recovery and the absence of intestinal GVHD. In some cases a nasogastric tube is necessary.
- **Antiemetics, pain control**: Together with conditioning antiemetic therapy should be initiated in accordance with local protocols.
- **Monitoring of bacterial, viral and fungal infections**: Virus antibodies in the donor as well as recipient should be serologically determined (at least according to JACIE-standards: HIV, hepatitis A, B, C, CMV, EBV, VZV, Parvo, HTLV 1+2, Toxoplasmosis) before transplantation. The patient's CMV virus load should be evaluated at least once a week by means of PCR-VNS, as CMV may have a significant influence on the occurrence and course of GvHD. Especially during severe GVHD a long lasting severe immunodeficiency can be expected. Therefore close monitoring of viruses (esp. Adenoviruses, CMV, herpes viruses, EBV) is recommended. Bacterial and fungal surveillance cultures and prophylactic therapy should be performed during the treatment in accordance with local standards.
- **Pre-emptive therapy with Ganciclovir in CMV**-PCR positivity. CMV-VNS evidence in the serum (PCR: >1x10³ copies/ml) should initiate pre-emptive treatment with Ganciclovir or alternatively with Foscarnet. (26-30).
- **Infection therapy**: In the presence of fever and/or other signs of infection, empirical treatment with broad-spectrum antibiotics in accordance with local standards is necessary. If no improvement can be seen, appropriate systemic antifungal drugs, which also include the aspergillus species, should be used.

7 Modification for toxicity

L-asparaginase
If PEG-Asparaginase is not available in MARAM and OCTADA(D), then native E Coli Asparaginase from Medac should be administered at a dose of 5000 U/m2 once every 3 days for a total of 9 doses. As shown by the COALL group also one dose of 45000 U/m2 native Coli Asparaginase gives equally long asparagine and glutamine depletion as one dose of PEG-Asparaginase. L-asparaginase should be discontinued in the presence of clinically evident pancreatitis, which needs to be confirmed by raised serum amylase and/or ultrasonography. In most of the cases, hyperglycaemia in induction will be due to steroids rather than L-asparaginase.
If severe anaphylactic reactions (urticaria, hypotension, wheezing) occur, the E-coli asparaginase 10.000 U/m2 should be replaced by PEG-asparaginase 2.500 U/m2 once every 2 weeks or Erwinase 20.000 U/m2 3 times per week if available.
In case of clinically significant hemorrhagic or thrombotic complications, withhold asparaginase until laboratory examinations of coagulopathy are performed and exclude asparaginase as cause of problems.

Cyclophosphamide
It is unlikely that hematuria will occur at the dose of 500 mg/m2. If it does occur, hyperhydration and Mesna 500 mg/m2 continuous infusion for 24 hrs after a loading dose of 150 mg/m2 is advisable. See also chapter 13.

High Dose Cytarabine (AraC, Cytosar)
If during cytarabine nystagmus occurs as an isolated event, stop araC for 24 hours. If nystagmus and other cerebellar signs occur, stop cytarabine and do not proceed with this course. Conjunctivitis should be treated or prevented with prednisone eyedrops (e.g. 0.5% 2 hourly).

Daunorubicin
If cardiac function is low (left ventricular shortening fraction (LVSF) (repeatedly) lower than 27%) daunorubicin needs to be delayed to OCTADA(D) or ADE. When LVSF is 27%-30% or higher, the normal dose of daunorubicin can be given.

Dexamethasone and Prednisone
When clinical overt diabetes mellitus develops after introduction of steroids, use insulin. In case of hypertension, first use antihypertensive drugs and sodium restriction. If further treatment of hypertension is absolutely necessary, reduction of the dose with 30%-50% of the glucocorticoids may be indicated.

Etoposide (VP-16)
In hepatic dysfunction with bilirubin 26-51 micromol/L reduce the dose by 50%. The decision to administer if bilirubin > 51micromol/L is a clinical one.
For renal dysfunction dose can be reduced as:

Creatinine clearance (ml/min/1.73m2)	% dose given
46-60	85%
31-45	80%
<30	75%

Anaphylactoid reactions, including bronchospasm, have occurred with etoposide. Only in very severe anaphylactoid recations, exposure to etoposide is not recommended.

High Dose -Methotrexate (5000 mg/m2)

1. Stop co-trimoxazole 48 hours before HD-MTX until 24 hours after the plasma MTX level < 0.2 □M.
2. If creatinine is above the upper normal limit for age or increased >30% from baseline value, it is advised to measure the glomerular filtration rate (GFR) or creatinine clearance before giving MTX. If the GFR is below the upper normal limit for age, consider 50% dose reduction for MTX. If GFR < 30 ml/min/1.73m2 omit MTX. Subsequent renal function can be measured with plasma creatinine and correlating these with the creatinine, obtained at the first GFR measurement.
3. MTX levels: these will be determined at the end of the MTX infusion, i.e. 24hrs after the start of the MTX infusion (T24) , and 48 hrs after the start of the MTX infusion (T48). If the MTX level is > 0.2 □M at T48, repeat MTX level determinations every 6 to 24 hrs until the level is < 0.2 □M.
4. Hydration and alkalinization: Pre-hydration with e.g. glucose 5%/NaCl 0.45% (+ 50 mmol Sodium Bicarbonate/L) at the rate of 125 ml/m2/hour during 6 hours. Urine pH needs to be > 7.0. If urine pH < 7.0, increase Sodium Bicarbonate to 75 mmol/L infusion fluid. Hyperhydration and alkalinization should be continued during MTX infusion and after infusion until MTX plasma level is < 0.2 □M.
5. Leucovorin rescue: 15 mg/m2 orally or iv at 42 (T42), 48 (T48) and 54 (T54) hrs after the start of the MTX infusion. If the plasma MTX level is > 0.2 uM at T48, then continue these doses every 6 hours until MTX plasma level is <0.2 uM.

Mitoxantrone

If functional cardiac changes occur during treatment, monitor ECG or echocardiography more frequently but do not reduce the dose. Mitoxantrone is less cardiotoxic than daunorubicin and cumulative anthracycline dose should not exceed safe limits.

In severe hepatic failure or abnormal third spacing total clearance will be decreased. A dose reduction may be considered, in conjunction with the trial co-ordinators, in such patients.

Vincristine

The dose of vincristine may be reduced to 2/3 of the recommended dose, when severe paresis or constipation develop. If the symptoms disappear, re-introduce vincristine at a full dosage. In case of a dropping foot or when an ileus is present, vincristine is withheld until the clinical signs are completely resolved. Re-introduce vincristine at 2/3 of the recommended dose. Do not modify the dose for jaw pain but use analgesics.

8. Guidelines for supportive care

Supportive care is needed because of the likelihood of infections, metabolic disturbances and organ damage especially in the time period between diagnosis and start of maintenance therapy. Most participating centres will have their own supportive care protocols, which should be followed. Guidelines for the following areas should be clarified in advance to ensure that treatment related complications are minimised.

8.1 Venous access

All patients should get a central venous catheter, e.g. of the Hickman or Broviac type by an experienced pediatric surgeon.

8.2 Hyperleucocytosis and tumor lysis syndrome

Hyperleucocytosis (WBC >100X10^9/L) may be associated with intracranial and pulmonary bleeding and leucostasis and with tumor lysis syndrome. Leucapheresis can not be performed in the very small children. Blood exchange can be considered in some cases, if WBC above 500x10^9/l but is of limited efficacy. Red blood cell transfusions lead to increased viscosity and should be avoided in this case if possible.

Measures to prevent tumor lysis syndrome include:

- Hyperhydration: 3000 ml/m2/24 hours is introduced before treatment starts. The composition of the fluid is preferably Gluc 5%NaCl 0.45% without K+ during the first day. Potassium is added when serum potassium falls below 3.5 Meq/L and an adequate urinary output is obtained.
- Urine alkalinisation: maintain the urine pH between 7 and 8 by giving NaHCO3 100-125 mEq (mmol)/m2/24hr, until uric acid has been normalized. However, urine alkalinisation should be avoided in case of use of rasburicase.
- Allopurinol: 200-300 mg/m2 daily divided into 2-3 doses. Allopurinol should be replaced by urate-oxydase (Rasburicase from Sanofi) for patients with a WBC > 100x109/l and/or markedly elevated uric acid. This drug rapidly converts uric acid to the more soluble allantoin (hypoxanthine). The usual dosage is 0.2 mg/kg/day, once a day. In case of severe or persisting hyperuricemia, urate-oxydase can be given twice daily. Urine alkalinisation should be avoided if urate-oxydase is used, since hypoxanthine is less soluble at pH > 7. In most cases, one or two doses are sufficient. Administration of urate-oxydase can be stopped after normalisation of uric acid for 3 days or when the WBC is < 10.000/µl. In the first days, daily blood samples to determine uric acid should be put on ice immediately to prevent breakdown of uric acid by rasburicase in the tube. Because of the risk of anaphylactic reactions, the first infusion should be given in 30 min in 25-50 ml 0.9% saline. Following infusions can be given as bolus.
- Regular measurement of uric acid, electrolytes, calcium, phosphorus, creatinine and urea are mandatory.
- Hyperphosphatemia may lead to an increased P/Ca product and renal failure. Hyperphosphatemia can be managed by saline diuresis, diuretics and oral P binders. Correction of asymptomatic hypocalcemia must be avoided. Dialysis may be necessary in some cases. In case of renal failure, glucose and insulin may force translocation of intracellular phosphorus and this can be a temporizing measure and avoid dialysis.
- If necessary, diuresis (> 3 ml/kg/hr) is maintained with furosemide 1 mg/kg q4-8hr.

8.3 Nausea and vomiting

Daunorubicin and Cytarabine in the induction and during OCTADA(D) may induce sickness and vomiting. High dose Cytarabine in MARMA is emetogenic as well and in these cases anti-emetic treatment according to local protocols is recommended. As a guideline, one can use ondansetron 5 mg/m2 2-3 times a day, starting with one dose pre-chemotherapy, eventually together with

domperidon 0.4 mg/kg per dose 4-6 hourly. If vomiting persists after having given these drugs, metoclopramide 0.1-0.2 mg/kg/dose 4-6 hourly for 2-3 days is suggested.

8.4 Prevention and treatment of infections

This protocol is very intensive and meticulous attention and measures to prevent and treat infection are essential. Blood should be taken at diagnosis to determine the antibody status to common viruses and to act as a baseline in the event of subsequent infections. Cultures may be taken at the start of intensification blocks and during treatment according to local protocols.

Preventive measures

- Mouth and skin care should be provided, especially in the diaper region.
- Because of the high risk of Pneumocystis Carinii Pneumonitis (PCP), it is mandatory to start PCP prophylaxis not later than at day 28 of the induction therapy. The prophylaxis should be interrupted in the HD-MTX courses as indicated in section 4.2 and 4.4.
- Preventive measures against bacterial infections should be taken according to the local policy of each centre.
- During neutropenia after HD-ARA-C, infections with particularly the gram positive Streptococcus viridans can occur, complicated by ARDS. (Centres may wish to consider prophylaxis during the neutropenic phase after HD-AraC with oral penicillin).
- Prevention of fungal infection may be achieved using an oral anti-fungal drug, e.g. oral Amphotericin or nystatin suspension. When oral prophylaxis with antifungal suspensions is not possible or there is manifest thrush, alternatively Fluconazole 6 mg/kg/day orally twice a day may be given.
- Mouth care is important in the prevention of infection.
- Because of their young age and the intensive chemotherapy regimen, most of the children develop severe hypogammaglobulinemia that often lasts until the end of maintenance. It is recommended to monitor serum IgG levels monthly and to give replacement therapy (IVIg) to maintain IgG level above 5 g/L.
- A potential large number of infants will not have been infected previously with the Varicella-Zoster Virus (Herpesvirus Varicellae). If there has been an exposure of the patient with an individual with varicella, Varicalla Zoster Immunoglobulin (VZIG) needs to be administered within 72 hours of exposure. The administration of VZIG to a patient extends the incubation period to 18-21 days. Nevertheless specific VZIG is not available in many countries. In that case, chemoprophylaxis with acyclovir 40-80 mg/kg/day PO in 4 divided doses starting 7-9 days after exposure (second viremic phase) is recommended. In case of manifest varicella infection, complications with pneumonia or encephalitis can be avoided by prompt treatment with intravenous acyclovir (500 mg/m^2 q 8 hr IV). In case of active disease the chemotherapy should be stopped, till all lesions have dried. Vaccination of household members against varicella is recommended if they have not developed previous natural infection.
- Measles is potentially the most serious infection during treatment as measles is not treatable and leads to progressive interstitial pneumonia and death. The high level of immunisation in the population has rendered this problem less common. The non-immunised child should avoid contact at all cost. In case of contact, hyperimmune immunoglobulin, if available, or standard Ig preparation is strongly recommended. In case of measles, IV Ribavarin is recommended.

Treatment of infections

Prompt investigation of fever and neutropenia is essential and should be initiated according to local protocols. Broad-spectrum antibiotics including an anti-pseudomonas effective agent are recommended. In case of febrile neutropenia, after HD-AraC, it should be considered to use a drug

which has efficacy against streptococcus species (cefepime instead of ceftazidime or imipenem for instance). The use of ceftazidim plus vancomycin may also be considered.
Prolonged fever after adequate broad spectrum intravenous antibiotic treatment should be treated empirically with a systemic antifungal agent. Higher doses of the anti-fungal drug amphotericin-B can be achieved using liposomal amphotericin (3-5 mg/kg/day can be given). If the central venous line is suspected to be the cause of infection, Vancomycin (40 mg/kg/24hr in 4 divided doses) should be added.
Interstitial pneumonia may be due to pneumocystis, candida or viral infections. Bronchoalveolar lavage may be indicated. Prompt empirical treatment is indicated with high dose co-trimoxazole TMP/SMX 20/100/mg/kg/day in 3 divided doses. ARDS in the context of streptococcus infection may improve with corticosteroids

8.5 Transfusion of red cells and platelets

Blood cell components should be filtered blood products and irradiated (prevention of GvHD) according to the local protocol of the centres. Transfusion of red cells is recommended when the Hb is < 5 mmol/L (< 8 g/dl). Transfusion of platelets is recommended during induction treatment, when the platelet count is < 20x109/L, irrespective of the absence or presence of haemorrhagic diathesis (bruising or petechiae). A platelet count >50x109/L is recommended before performing a lumbar puncture. Before a central line is inserted, the platelet count should preferrably exceed 80x109/L. Later during treatment, platelet transfusion is recommended when the platelet count drops below 20x109/L.

8.6 General and nutritional support

During the intensive treatment of this protocol, which is mainly during the first 6 months adequate nutritional support is essential. Early introduction of naso-gastric tube feeding to provide sufficient calories and balanced nutrients is advised. Parenteral feeding may be indicated during periods when enteral feeding is impossible. However, enteral feeding is preferable. Weight needs to be checked regularly, at least once every week.

8.7 Organ toxicity

8.7.1 Nephrotoxicity

(see also section 7.2: tumor lysis syndrome)
The prevention of nephrotoxicity during high-dose Methotrexate is achieved by hyperhydration (3000 ml/m2/24hr), forced diuresis and alkalinisation of the urine (pH > 7). Prior to the first HD-MTX infusion in each block, the renal function should be known. Daily analysis of electrolytes is recommended.
Bladder toxicity is a potential side effect of Cyclophosphamide. Mesna and adequate hydration are recommended for prevention of haemorrhagic cystitis as indicated in the protocol when Cyclophosphamide dose is 1000 mg/m2 or higher.

8.7.2 Gastro-intestinal toxicity

Vincristine can cause constipation and paralytic ileus. The treatment of constipation is decided by the local policies, however, early start of laxitives (eg. lactulose) is recommended to prevent serious bowel obstruction.
Diarrhoea can be caused by mucositis after HD-ARA-C and HD-MTX. Unfortunately, this cannot be prevented. The importance lies in the fact that during neutropenia after the HD regimens, a necrotic enterocolitis may develop, and patients should be kept under close observation during this time. When severe diarrhoea or mucositis develops, treatment should be witheld until the condition of the patient has improved.

8.7.3 Cardiac toxicity

An echocardiogram to determine the left ventricular shortening fraction (LVSF) is recommended before treatment starts, before week 12 and 15, and in between if necessary (when LVSF < 27% or a drop of >10%). In case of cardiotoxicity, this should be discussed with the study coordinators.

9 Required observations

The observations mentioned below are essential for observation and monitoring. ***It is vitally important that every effort is made to ensure proper immunophenotyping and cytogenetics and analysis of MLL gene by split signal FISH on all patients.***

9.1 At initial diagnosis

1. History and physical examination (including length, weight, body surface, head circumference, performance status).
2. Hb, leucocytes and differential count, platlelets , reticulocytes, AB0 rhesus type.
3. Bone marrow and peripheral blood

3.1. Morphology and cytochemistry.

3.2. Immunophenotyping.

3.3. **Analysis of MLL gene rearrangements by split-signal FISH is mandatory**. This should be followed by techniques to identify the fusion partner of the MLL gene, e.g. by additional FISH or PCR, at least to analyze the presence or absence of t(4;11), t(9;11) and t(11;19). In case split-signal FISH is "not successful" or "not evaluable" or in general "not known", a positive result for t(4;11), t(9;11), t(11;19) or other translocation involving the MLL gene, obtained by PCR or FISH is accepted.

3.4. Cytogenetic analysis including standard karyotyping and molecular-genetic analysis of TEL/AML1, bcr-abl and ploidy status.

4. Cerebrospinal fluid: cell count, cytology.
5. Blood chemistry: BUN, creatinine, uric acid, Na, K, Ca, P, Cl, Mg, bilirubin, total protein, glucose, SGOT, SGPT, LDH, alkaline phosphatase, IgG.
6. Coagulation: PT, PTT.
7. Infection diagnostics: Antibodies against Varicella Zoster, hepatitis A, -B, and -C, CMV, Herpes Simplex virus, HIV.
8. Urine culture.
9. Cardiac evaluation: echocardiogram, ECG.
10. Imaging studies: chest X-ray, echography abdomen.

9.2 At day 8: Prednisone response

Day 1 is the first day that prednisone is given to the patient. Determine prednisone response on day 8: if possible, use EDTA-free peripheral blood (venous or capillary) or otherwise peripheral blood with little EDTA for leucocyte count and differential count in your own laboratory. Also send an unstained, non-EDTA peripheral blood smear to the reference laboratory of the individual group. From the WBC/ul and the % of blast cells, the blast cell count/ul is calculated. The response to prednisone is defined as "good" if this blast cell count/ul is < 1000 and defined as "poor" if this is equal to or >1000.

9.3 At day 15 and day 33

Bone marrow to determine day 15 response and to determine whether complete remission is achieved at day 33. If the peripheral blood shows pancytopenia at day 33 it is justified to postpone the BM puncture to the timepoint just before the start of protocol Ib or ADE. If the bone marrow at day 33 is hypocellular and one is therefore unable to determine CR or not, then the bone marrow puncture should be repeated before the start of protocol Ib or ADE.

9.4 During induction treatment

HLA typing of parents, siblings and patient should be done as soon as possible after diagnosis for HR patients. Bone marrow punctures need to be performed as indicated on the chemotherapy schedules. Cerebrospinal fluid examination for leucocytes and erythrocytes count with each scheduled lumbar puncture.

9.5 Specimen submission

The logistic systems routinely used by the participating groups should also be used for this study. This implies that the reference laboratories of these groups should be used for the specific objectives as cytogenetic analysis, immunophenotyping and determination of the prednisone reponse. The specimen submission requirements are as for other leukemia studies as described by the national groups.

9.6 Toxicity

Information about all toxic events, whether volunteered by the patient, discovered by the responsible investigator, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Toxicity Form provided by the Trial Data Centre. A toxic event is any undesirable sign, symptom or medical condition occurring after start of treatment, in any arm.

As far as possible, each toxic event will be reported according to the NCI/NIH Common Toxicity Criteria, severity grades 1–4.

9.7 Serious Adverse Events (SAE)

A Serious Adverse Event (SAE) is any undesirable sign, symptom or medical condition which:

- is fatal or life-threatening.
- requires prolonged hospitalisation.
- results in persistent or significant disability/incapacity.
- constitutes a congenital anomaly or a birth defect.
- is medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Life- threatening events are defined as:

- circulatory/cardiac insufficiency requiring catecholamines/positive inotropes;
- respiratory failure requiring intubation/ventilation;
- other clinical situations requiring *immediate* intervention, e.g.
    - gastrointestinal bleeding or perforation requiring surgery;
    - cerebral abscess/bleeding requiring immediate neurosurgical intervention.

Any SAE in the Interfant-06 protocol must be reported within 24 hours of learning of its occurrence (see below). Exceptions to immediate SAE reporting include:

- Hospitalization for i.v. antibiotic treatment due to uncomplicated infections (fever with neutropenia after chemotherapy). On the contrary, grade IV infections, systemical or invasive fungal infections or severe soft tissue infections must be immediately reported.
- Hospitalization for parenteral nutrition or i.v.-rehydratation due to mucositis, inappetence/anorexia or vomiting/diarrhea.

Such events should be documented on the regular Toxicity Form.

Events not considered to be SAE are hospitalizations occurring under the following circumstances:

- hospitalizations planned before entry into the clinical study
- elective treatment of a pre-existing condition
- hospitalizations on an emergency, outpatient basis that do not result in overnight hospitalization (unless fulfilling the criteria above)
- routine treatment not associated with any deterioration in condition.

Each SAE must be reported by the clinical centre to its own group contact person, within 24 hours of learning of its occurrence, even if is not felt to be treatment-related. Information about all SAE are to be collected and recorded on the SAE Form which is provided by the Trial Data Centre.

After ensuring that the SAE Form is accurately and fully completed, the clinician who is the group contact person must send it immediately to the Coordination Unit. Any relevant follow-up information about a reported SAE must also be reported timely with the same modalities.

9.8 Late side effects

9.8.1 Cardiotoxicity

Cardiotoxicity should be monitored by performing a cardiography at the following minimum time points (see form in paragraph 9.8.4):

- At diagnosis.
- 2 yrs from diagnosis.
- 4 yrs from diagnosis.

9.8.2 Growth

Patient height and weight should be monitored at the following time points (see form in paragraph 9.8.4):

- At diagnosis.
- 1 yr from diagnosis.
- 2 yr from diagnosis.
- 4 yr from diagnosis.

Father's and mother's height should be measured once.

9.8.3 Neuropsychological function

(see form in paragraph 9.8.4)

The neuropsychological analysis should be performed at the age of 4 years, preferably between the age of 4 yrs 5 months and 4 yrs and 8 months. Instructions for neuropsychological testing are as follows:

- Examiners should use the most appropriate Wechsler test editions, i.e. the latest available editions relevant for the country and language. For instance in the USA WISC-III (1991) and WPPSI-III (1989) should be used. Please indicate year of issue. Do not use previous editions with outdated test norms.
- Examiners should strictly adhere to the directions given in the test manual appropriate for their country. Test procedures, including sequence of subtests may significantly differ among countries. Hence, the sequence of subtests given on the datasheet may differ from the directions in your manual; please follow the manual appropriate for your country.
- Raw scores have to be converted into scaled scores appropriate to the age of the child and the country's norms.
- The child's verbal IQ is based on the sum of 5 administered verbal tests (as indicated on the data sheet), the performance IQ is based on the sum of 5 administered performance tests (as indicated on the data sheet).
- Full scale IQ is based on the 10 individual subtests.
- No other subtest may be used to calculate the IQ's; for instance "coding" cannot be replaced by "mazes".
- If only 4 subtest's scores are available, the sum of scaled scores on the affected scale must be prorated to obtain the performance or verbal IQ. Write "pro" (for "prorated") in the margin to indicate which specific subtest was not administered.
- The fullscale IQ must be based on at least 4 of the indicated verbal and 4 of the indicated performance subtests.

9.8.4 Late effects scoring form

Pt #:_____ Date of birth:_____ (ddmmyy)

GROWTH

Father's height: _____(cm) Mother's height: _____(cm)

Time point	Date(ddmmyy)	Patient's height (cm)	Patient's weight (kg)
at diagnosis	_____	_____	_____
1 yr from Dx	_____	_____	_____
2 yrs from Dx	_____	_____	_____
at 4 yrs of age	_____	_____	_____

CARDIAC

Time point	Date(ddmmyy)	Echo cardiac shortening fraction (%)
at diagnosis	_____	_____
2 yrs from Dx	_____	_____
at 4 yrs of age	_____	_____

NEUROPSYCHOLOGY AT 4 YEARS OF AGE (see instructions)
preferably at the age between 4.05.16 and 4.08.15 (years.months.days)

Pt #:_____ Date of birth:_____ (ddmmyy)
Test date:_____(ddmmyy) Examiner:_____
Site of examination:_____

Wechsler Preschool and Primary Scale of Intelligence; WPPSI
_ edition/year of issue:_____ (Latest edition appropriate for country)
_ age table:_____(years.months.days)

_ subtests:	rawscore:	scaled score (m=10±3):
* information	_____	_____
* comprehension	_____	_____
* arithmetic	_____	_____
* vocabulary	_____	_____
* similarities	_____	_____+
** SUM (5 subtests) →		_____ →
** **Verbal IQ** →		_____ (m=100±15)
* object assembly	_____	_____
* geo. design	_____	_____
* block design	_____	_____
* mazes	_____	_____
* picture cmpl	_____	_____+
** SUM (5 subtests) →		_____ →
** **Performance IQ** →		_____ (m=100±15)

_ full scale IQ (10 subtests): _____ (m=100+15)

10 Response criteria

10.1 Prednisone response

Prednisone response: determination of the number of leukemic blasts in peripheral blood on day 8 after 7 days of systemic treatment with prednisone and one dose of intrathecal methotrexate and prednisolone at day 1. Patients with ≥ 1000 leukemic blasts/µl blood at day 8 are defined as

prednisone poor responders (PPR) and those with <1000 leukemic blasts/µl blood at day 8 as prednisone good responders (PGR).

10.2 Bone marrow status

M1 status: < 5% leukemic cells.
M2 status: > 5% and < 25% leukemic cells.
M3 status: > 25% leukemic cells.

10.3 Complete remission

Bone marrow response will be determined at day 33 of induction therapy. Complete remission (CR) at day 33 is defined on morphological grounds by the presence of <5% leukemic blasts and by regenerating hematopoiesis and no evidence of disease at any other site.
Patients with hypoplastic bone marrow and no evidence of disease at any other site with:

- WBC ≥ 2 x 109/L and platelets ≥ 50 x 109/L are considered to be in complete remission.
- WBC < 2 x 109/L or platelets < 50 x 109/L should undergo an extra bone marrow puncture at the start of protocol IB or ADE (so in principle when WBC and platelets fulfill these criteria). In case the start of IB or ADE can not be further delayed AND the WBC and platelets are not fulfilling the criteria AND the repeated bone marrow puncture still shows insufficient hematopoiesis recovery, the BM performed at the start of the chemotherapy course following the randomized phase (i.e. following IB or ADE and MAE) will be used to determine whether CR has been achieved. These patients with persistent hypoplastic bone marrow are eligible for randomization.

In case there is no CR after induction therapy (induction failure) patients are not eligible for randomization. For these patients it is advised to continue treatment with the experimental arm of the HR arm so with protocol ADE and MAE and to evaluate CR after these courses.

10.4 Relapse

Relapse is recurrence of leukemia after CR has been documented. Relapse is defined as:

- > 25% leukemic blasts in the bone marrow by morphology at any time after remission induction. In case of doubt confirmation is needed by immunophenotyping or genotyping that these blasts are derived from the original leukemic clone. This confirmation is necessary because high percentages of normal regenerating blasts may occur in infants that may not be differentiated from leukemic blasts by morphology. AND/OR
- leukemic blasts in the peripheral blood by morphology, confirmed by immunophenotyping or genotyping AND/OR
- leukemic cells in the CSF by morphology, confirmed by immunophenotyping or genotyping. But if these leukemic cells in CSF are found within ≤ 5 WBC/µl CSF, an extra confirmation is needed by a repeated lumbar punction after about 4 weeks AND/OR
- leukemic infiltration elsewhere.

Patients will be off protocol in the event of relapse. Guidelines for treatment of relapse are beyond the scope of this protocol.

11 Statistical considerations

11.1 Randomization

Patients eligible to randomization: patients both in the MR and in the HR stratum who fulfill the following requirements.

Needed requirements:

1. MLL status as follows:
   1a. *Known* (rearranged) MLL status.
   1b. *Unknown* MLL status, as defined by Split-Signal FISH analysis either "not evaluable" or "not successful" or "not known" is accepted, but leads to randomization in a separate stratum of MR (unless the presence of t(4;11) or t(9;11) or t(11;19) or other translocations involving the MLL gene is documented by FISH/PCR).
2. Achievement of CR1. Treatment schedule allows only 3 days between CR1 evaluation (BM at day 33) and start of the subsequent randomized phase (day 36). Therefore, in order to ensure a timely application of the protocol, randomization before CR1 evaluation is accepted but recommendations reported below are to be closely followed.
3. Informed consent from parents or guardians.

When to randomize:

- When MLL status is defined as known or unknown, as specified above.
- Not before day 29 of Induction and as close as possible to the CR1 evaluation.
- Preferably after CR1 achievement is documented. Exceptions to this rule are allowed, but it is
- Recommended to postpone randomization in case CR1 evaluation itself is likely to be performed later than scheduled (see Section 10.3).

Modalities of randomization:

- By country: randomization will be performed by the data centre of each group, so that treatment arms will be balanced within each group.
- By phone: treating physicians will ask for randomization by telephoning to the data centre and the data centre will perform a check on the eligibility criteria before assignment. Therefore, at the time of randomization, at least the registration and the diagnosis with MLL status data should be available at the data centre.
- By blocks: the random assignment will be produced by an automatic procedure based on random permuted blocks. Due to the small number of infants entered by each group, it is reasonable to define blocks of small size and to randomize patients in the following randomization strata, only: MR with rearranged MLL status, MR with unknown MLL status and HR. The minimization technique, where available, could be used instead of the permuted blocks.

Randomization refusals: if parents or guardians do not agree with randomization, patients should be treated according to the standard arm.

11.2 Analysis

The primary aim of the study (randomized question) will be evaluated on Disease Free Survival (DFS) as the primary endpoint. DFS is defined as the time from randomization to relapse, second malignancy or death, whichever occurs first. Secondary endpoint will be survival from date of randomization to death from any cause. Assessment of the results of the randomized question will also be performed within MR and HR patients separately, as a secondary analysis with the same endpoints above.

Both primary and secondary analysis for MR and HR randomized patients in CR1 after Induction will be carried out according to the "intention to treat" principle, in order to ensure an unbiased estimation of treatment effect. Based on the assumption that SCT will be performed according to the protocol, i.e. in the HR group and upon suitable donor availability only, censoring at SCT in both arms would not bias the results. An analysis without censoring SCT will also be performed and the likelihood of this assumption will be evaluated. Comparison of the two arms accounting for deviations from the assigned treatment will also be added to this main analysis.
Another secondary aim of the study is the assessment of the overall outcome as compared to Interfant99. It will be evaluated by considering as main endpoint the Event Free Survival (EFS), i.e. the time from diagnosis to either one of the events below:

- Early death.
- Resistance to induction (no CR1).
- Relapse.
- Second malignancy.
- Death in CCR.

Also the outcome within the risk groups LR, MR and HR will be evaluated in terms of EFS.
The evaluation of outcome will also be performed in terms of survival time from diagnosis (endpoint is death for any cause).

The evaluation of SCT in HR patients will be done primarly according to the "intention to treat" principle, comparing the DFS in patients who have a suitable donor with those for whom no donor was found, regardless of whether they actually received SCT. Secondarily, analysis will also be done by treatment performed. This latter analysis will need to be adjusted by waiting time to SCT (with a time-dependent variable for the treatment indicator in a Cox model).

11.3 Sample size

This study compares the outcome of infants with ALL treated with two AML-like induction blocks, namely ADE and MAE, given after Induction versus protocol Ib given after Induction. As infant ALL is very rare, the study aims at recruiting patients for 5 years, in order to enter about 445 infants. Participating groups have the following expected recruitment per year (calculated on the basis of the recruitment observed in the Interfant99 protocol):

AIEOP (Italy)	10
ANZCSG (Australia, New Zealand)	1
ARGENTINA	7
BFM (Germany, Austria)	11
COALL (Germany)	4
CPH (the Czech Republic)	2
DCOG (the Netherlands)	5
DFCI Consortium (USA)	3
CLG (France, Belgium)	7
FRALLE (France)	7
HONG KONG	1
MD Anderson (USA)	3
NOPHO (Scandinavian Countries)	5
PINDA (Chile)	2
PPLLSG (Poland)	4
Seattle (USA)	3
SJCRH (USA)	3
UKCCSG (United Kingdom)	11
Total:	89 infant ALL cases per year

About 85% of the infants are expected to be stratified in the MR and HR groups. Based on the Interfant99 experience, the expected proportion of non–randomized patient is anticipated to be as high as 15% (because of refusals for any cause and occurrence of events prior to randomization). Thus the overall sample available for randomization would include 320 infants.

Different scenarios, in terms of possible DFS differences between the two arms and different baselines values of the DFS have been examined.
Three hypotheses are made in these scenarios:

1. No qualitative interaction between stratification criteria and the effect of the AML-like treatment block given after Induction: this means that it is clinically reasonable to assume that the effect of the AML-like treatment block will be in the same direction in both strata (either beneficial or not in both MR or HR infants).
2. The estimate of the effect of the AML-like treatment block given after Induction may be given in the three separate strata (MR with rearranged MLL status, MR with unknown MLL status and HR). However, the statistical test is performed on the pooled estimate of the effect, after stratification.
3. Compliance to the protocol directive on SCT (see Chapter 6). This will allow a meaningful comparison in HR group.

The overall DFS at 3 years of MR and HR infants, based on the Interfant99 experience, can be expected to be about 41%. The table below reports the calculated power of the final two-sided test, according to O'Brian and Fleming (1979), assuming the first type error □=0.05, with various absolute differences in the 3-year DFS.

Sample size: 320 patients
Baseline 3-year DFS: 41%

3-year DFS in Experimental Group	DFS Difference	Total Number of Events Needed	Power
51%	10%	173	45%
56%	15%	165	77%
57%	16%	163	82%

Other scenarios based on different estimates of the baseline DFS (e.g. 45%) lead to very similar findings. In conclusion, the power calculations show that only in the presence of a marked difference in the 3-year DFS of the two treatment arms, i.e. about 16% absolute difference, the study could have sufficient power to detect the difference.

The power of the test on the effect of AML-like treatment block was also calculated in the MR and HR stratum separately, with an expected baseline 3-year DFS of 49% and 16%, respectively. The study would have a good power to detect a 18% absolute difference in the MR group.

11.4 Interim analyses

Interim analysis evaluates the randomized question on treatment effect while the trial is still in progress. The aim is to avoid prolongation of the study beyond the time when clear superiority can be demonstrated for one of the randomized treatment schedules. The intermediate analyses are scheduled at intervals of one year from the start of the protocol. The significance levels of the interim tests, adjusted for the multiplicity of looks according to O'Brian and Fleming (1979), are calculated with a type-one error □=0.05 and a power of 82% (two-tailed test). They are:

Significance levels for interim analyses

	December 2007	December 2009	Final
p-level	0.00006	0.006	0.05
Years from 1st randomization	2	4	7

The number of events expected for the first and second interim analysis are approximately 1/3 and 3/4 of the total.

11.5 Methods of analysis

EFS, DFS and survival curves will be computed using the Kaplan-Meier estimator. The DFS in the two treatment arms will be compared with the log-rank test stratified by risk group and by participating group. A combined estimate of treatment effect will be given, adjusting by risk group and by participating group, if no significant heterogeneity of the effects will be detected.
A regression model such as the Cox model, if appropriate, will be applied to evaluate treatment effect adjusting for the candidate prognostic factors such as age (continuous), type of MLL rearrangement, WBC at diagnosis (continuous) and response to prednisone. This analysis will allow studying the relevance of the candidate prognostic factors included as covariates in the model. The interaction between treatment and main prognostic factors and risk group will be evaluated.

11.6 Early stopping guidelines for treatment related mortality

Guidelines are designed to ensure that the trial will be stopped as early as possible if its application is associated with a treatment-related mortality higher than acceptable in standard treatment of infant ALL. Treatment related mortality has been accounted for in terms of deaths in Induction (evaluated overall) and deaths in CCR (evaluated per arm) separately. In particular, guidelines for monitoring of deaths in CCR are necessary because, although all Interfant-06 chemotherapy blocks have extensively been used in infant leukemia, they have never been administrated in the proposed order, i.e. Interfant-99 Induction followed either by AML induction blocks or by protocol Ib.

The method applied in both cases follows a Bayesian approach (Mariani and Marubini, 1996), extending that of Metha and Caine (1984). In these guidelines, the maximum acceptable level of probability of treatment related death, say p_{max}, was considered. The number of failures, either deaths in induction or in CCR, is assumed to be taken from a Binomial distribution. The prior distribution for the probability of the endpoint of interest was taken as a Beta (1, 1), corresponding to an uninformative Uniform distribution. The stopping bounds reported in the following tables are the experimental results that give a posterior probability of 90% or more, of observing $p \geq p_{max}$. For mortality in Induction p_{max} has been set to 4% while, for mortality in CCR, p_{max} =10%.

The table below shows the overall minimum number of deaths in Induction at which the possibility of stopping the trial should carefully be evaluated.

Guidelines for early stopping due to mortality in Induction

No. of deaths in Induction	No. of subjects in study
2	13-27
3	28-44
4	45-61
5	62-79
6	80-98
7	99-117

The table below shows, for each set of patients enrolled in each arm separately, the minimum number of deaths in CCR at which investigators should carefully consider the possibility of stopping the application of the treatment arm itself.

Guidelines for early stopping due to mortality in CCR

No. of deaths in CCR	No. of subjects in study arm
3	11-17
4	18-24
5	25-31
6	32-39
7	40-47
8	48-55
9	56-63
10	64-71
11	72-79
12	80-87
13	88-96
14	97-104

11.7 Guidelines for study monitoring in the SR protocol

The SR protocol does not include a randomized question. In this observational study, treatment is very similar to that applied to SR patients in Interfant-99. Thus the outcome in this group will be monitored to ensure that the probability of failure with the Interfant-06 SR treatment does not exceed the historical Interfant99 corresponding figure. For this purpose, failure is defined as one of the following events, whichever occurs first:

- Early Death.
- Resistance to induction (no CR1).
- Relapse.
- Second malignancy.
- Death in CCR.

Failure rate during the first two years from diagnosis was 0.07 in SR patients treated with Interfant99. We wish to avoid continuation of Interfant-06 SR study if its outcome is likely to be inferior to the one observed in SR patients treated according to Interfant99. Thus, we define a

procedure based on the sequential probability ratio test (SPRT), which considers the following hypotheses:

$$H_0: \{\lambda \geq 0.32\}$$
$$H_1: \{\lambda \leq 0.07\},$$

where λ is the unknown failure rate for Interfant-06 SR study and 0.32 is the failure rate observed during the first two years from diagnosis in MR patients treated with Interfant99. SPRT boundaries calculations are based on the following choices:

- Exponential model for the time-to-failure, with constant failure rate λ.
- Type I error, $\alpha = 0.01$ and type II error, $ß = 0.20$.
- Log-likelihood ratio of H_0 versus H_1.

The table below shows the calculated sequential boundaries expressed in terms of failures and cumulative observation time (i.e. cumulative years of follow-up).

Guidelines for SR study monitoring

No. of failures	Cumulative years of follow-up	
	Lower boundary	Upper boundary
1	-	23.6
2	5.8	29.7
3	11.8	35.8
4	17.9	41.8
5	24.0	47.9
6	30.1	54.0
7	36.2	60.1
8	42.2	66.1
9	48.3	72.2
10	54.4	78.3
11	60.5	84.4
12	66.6	90.5
13	72.6	96.6
14	78.7	102.6
15	84.8	108.7
16	90.9	114.8
17	97.0	120.9
18	103.0	127.0
19	109.1	133.0
20	115.2	139.1
21	121.3	145.2
22	127.3	151.3
23	133.4	157.4
24	139.5	163.4
25	145.6	169.5
26	151.7	175.6
27	157.7	181.7
28	163.8	187.8
29	169.9	193.8
30	176.0	200.0

The focus of the application will be on the lower boundary: if, given the number of observed failures, the observed cumulative follow-up time (in years) is inferior to the lower boundary, then the outcome of the Interfant-06 SR protocol is judged to be poor or, in other words, more likely to be similar to the Interfant99 MR rather than SR outcome. In such a case, we conclude that evidence from accumulating data suggests that Interfant-06 SR study continuation should be discussed.

In general, these comparisons of observed data (failures and pooled follow-up time) with the theoretical boundaries serve as a guideline for discussing the continuation of Interfant-06 SR study, either because the data support H_0 (values below the lower boundary) or H_1 (values above the upper boundary). Values in between the boundaries are interpreted as no evidence in favour of either H_0 or H_1, so no evidence-based need for study re-consideration. (Piantadosi, 2005).

12 Organizational aspects and data managament

Each participating group will refer to the contact person of the group and to the usual network of clinical centres, data centre and experts (statistician, biologists, etc.) for the application of this protocol, the monitoring of data collection and data quality and for the randomization procedure. The International Study Coordinator, Vice-Coordinator and the Trial Data Centre will act as a Coordination Unit for the monitoring and exchange of information and for the pooling of the data. Contact details of the Coordination Unit can be found in Chapter 15.

12.1 Data Collection

Data collection is based upon:

- registration of each new infant diagnosed with ALL or biphenotypic leukemia;
- a common study database implemented on the Web, in which data for each patient who enters the protocol will be saved;
- common criteria for randomization.

The Trial Data Centre designs the forms for data collection and provides the Group Data Centres with a web-database specific for this study, so that all groups will use a common study database.

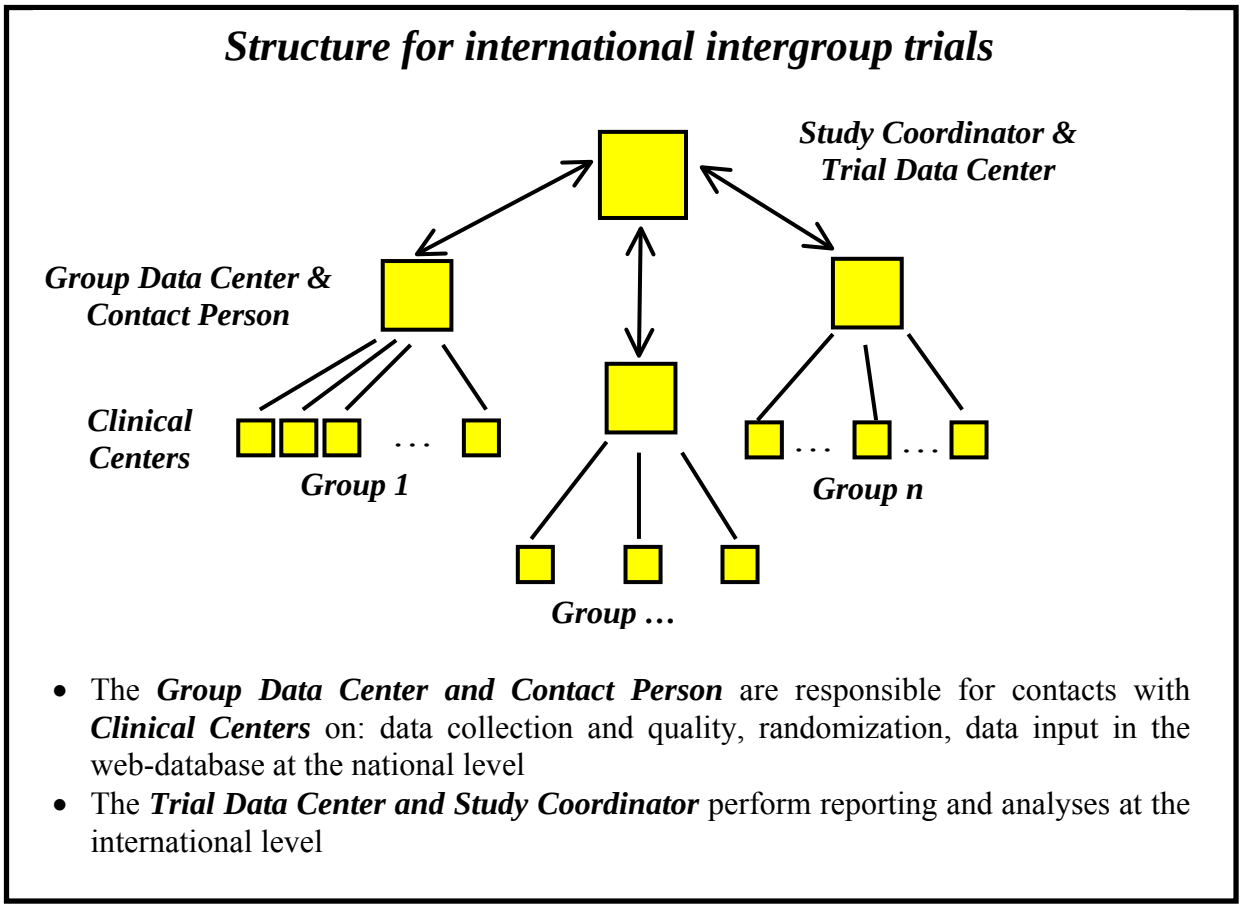
Each group will:

- use the data collection forms designed for this protocol (to be found in Appendix A);
- centralize the forms in its own Group Data Centre for quality checks and input, according to the approach routinely used in the group;
- collect their own data in the common study database provided by the Trial Data Centre;
- keep its own data in the common study database updated and provide periodic (yearly) update of follow-up .

In summary, each clinical centre of each group is required to:

1. register at its own Group Data Centre each new infant diagnosed with ALL or biphenotypic leukemia, regardless of whether this infant will subsequently enter the Interfant-06 protocol. This is necessary in order to know which percentage of eligible patients is treated according to the protocol. Registration should be done as soon as possible after diagnosis of ALL or biphenotypic leukemia;
2. report immediately each event (relapse, death, SMN) to its own Group Data Centre;
3. report each SAE to its own Group Contact Person, within 24 hours from learning of its occurrence;
4. send on a regular basis, to its own Group Data Centre, the forms on diagnosis, response, randomization, treatment and toxicity, as soon as they can be completed. Please recall that randomization can be obtained from the Group Data Centre only if registration, diagnosis with MLL status and preferably data on CR achievement are available;
5. up-date follow-up at the end of each calendar year.

For eligible infants registered but not included in the Interfant-06 protocol, follow-up data only might be routinely requested.



12.2 The study web-database and study management

The Trial Data Centre, in collaboration with the International Study Coordinator, the Contact Persons and the Statisticians of each group, will be responsible for maintaining the study web-database and evaluating the data according to the protocol aims.

The trial data are property of the participating groups and will be used under their responsibility for the trial aims, only.

Management and analysis of the trial data will be performed following these steps:

- Each group will make its own data available to the Trial Data Centre by routinely saving them in the web-database. At the beginning of each new calendar year, data of each group must be frozen and follow-up updated at December of previous year.
- Each group will be able to extract its own data from the web-database.
- A report will be produced each year on the study progress (recruitment, toxicity and so on) and the interim analyses performed when planned.
- Reports will be circulated by the Coordination Unit to the Contact Person of each group and to the Data Safety and Monitoring Committee (DSMC, see Section 12.4).
- Interim analyses will be submitted (blinded) to the contact person of each group and (unblinded, if specifically required) to the DSMC.

Access to the common study database will be granted on the Internet to the Data Centers and Contact Persons of each participating group as well as to the Coordination Unit, with different modalities. For this purpose, the web-site will be designed by the Trial Data Center in collaboration with the informatic staff at CINECA (Consorzio Interuniversitario per il Calcolo Automatico, Bologna, Italy). The web-site will provide the interface for data input and modification (electronic

CRF will reflect the common data collection forms), the archive of the protocol documents and a forum for communication among participating groups.

The web-site is implemented in such a way that data confidentiality and data security standards are met.

In particular, data confidentiality is ensured by:

- Separation of demography data from sensitive patient data. Only demography data pertinent to the study are collected (and in an anonymous form whenever possible).
- Data traffic with the server is encrypted with high grade of cryptography (up to 128 bit) and X.509 Certificate (SSL).
- Access to web-site is only possible through valid user identification (i.e. USERID) and associated password. Users may change his/her password at any time.

Data security is ensured by:

- Controlled access to the server data (see above).
- Appropriate daily backup of all data on electronic media, to allow restoration in case of loss or damage of the database. Protection against major disasters (fire, flooding, etc.) and Disaster Recovery Procedures are implemented.
- Operation tracking log (registration of any operation by any user) and electronic data audit trials (creation of a database of original entries/modifications with identification of date, time,source and user identity).

WEB-based system

The WEB-based system supports:

- ***Group Data Centers*** (randomization, data management)
- ***Trial Data Center*** (central monitoring, reporting)
- ***International Study Coordinator*** and ***Group Contact Persons***

by providing:

- Common study database
- Randomization procedure
- Archive of protocol documents (Trial Master File)
- Forum for communications

12.3 Ethics and Good Clinical Practice

The last revision of the Helsinki Declaration as well as the previsions of the Oviedo Declaration, provide the general framework for the ethical conduct of the study.

The study protocol is designed to ensure adherence to Good Clinical Practice principles and procedures (ICH/GCP, CPMP/ICH/135/95).

12.4 Data safety and monitoring committee (DSMC)

Members of the DSMC are experienced researchers not involved in the trial who will be responsible for providing the investigators with guidance on the trial conduction and, in case of problems, on whether the trial should be stopped, modified or continued.

The DSMC consists of dr. Bruce Camitta, pediatric hemato-oncologist (Medical College of Wisconsin, 8701 Watertown Plank Rod, Milwaukee WI 53226) and dr. Yaddanapudi Ravindranath, pediatric hemato-oncologist (Children's Hospital of Michigan, 3901 Beaubien Boulevard, Detroit, MI 48201).

13 Chemotherapeutic drugs

Anti-thymocyte globulin

Dose and administration:	Rabbit derived anti-T lymphocyte serum 15mg/vial. 20mg/kg/day for 3 days intravenously over a minimum of 4 hours in 0.9% sodium chloride infusion.
Storage:	Refrigerated.
Toxicity:	Hypersensitivity, rashes, anaphylaxis. Fever. Delayed onset serum sickness.
Precautions:	Premedicate with steroid and/or antihistamine according to local policy. Ensure emergency rescustitation medicines and equipment are available during and after the infusion.

L-Asparaginase

Dose and administration:	10.000 U/m2 (MedacR), 20.000 U/m2 (E Coli Elspar or Erwinia-Asparaginase) or 2500 U/m2 PEG-Asparaginase (MedacR). Intravenously in 1 hour or by intramuscular injection. Reconstitute with 50 ml 5% dextrose/0.45% saline or 0.9% sodiumchloride for intravenous use; infuse only if clear. For IM injection reconstitue with sterile water or sodiumchloride for injection.
Storage:	at 2o-8o, use within 8 hours, and only if clear
Toxicity:	hypersensitivity, anaphylaxis, coagulopathy, stroke, hypercholesterolaemia, lowered insulin secretion, pancreatitis, hepatotoxicity, encephalopathy.
Precautions:	The drug is given while there may still be thrombocytopenia. For IM injections, platelets may be necessary to cover the injection and extra local pressure may be needed.

Busulfan

Dose and administration:	20mg/kg divided into 16 doses (1.25mg/dose for 4 doses/day for 4 days). Patients must be starved for 2 hours pre each dose and 30 minutes post dose. Repeat doses vomited and monitor levels as per protocol.
Storage:	Refrigerate.
Toxicity:	Nausea, vomiting and diarrhoea. Mucositis, sterility, seizures, rashes. Adrenal insufficiency, veno-occlusive disease (VOD), pulmonary fibrosis.
Precautions:	Ensure adequate oral/IV hydration. Give seizure prophylaxis according to local policy.

Cyclophosphamide

Dose and administration:	1 gr/m² in Ib and 0.5gr/m² in OCTADA(D), intravenously over 1 hour in glucose 5%.
Storage:	Stable 7 days refrigerated or 4 days at room temperature.
Toxicity:	Bone marrow depression (nadir at 7-14 days), nausea and vomiting, alopecia, skin rash, facial flushing during injection, eosinophilia, inadequate secretion of antidiuretic hormone (ISADH). Hemorrhagic cystitis to be prevented by hyper-hydratation and concomitant administration of MESNA.

Precautions:	hydration post infusion of the drug, 125 ml/m2/hour during 6 hours after drug infusion, may prevent toxic effects. Requirements during administration: Hydration and cystitis prophylaxis: 3,000 ml/m2 fluid/24 hr for a minimum of 6 hours. Mesna (Uromitexan®): 400 mg/m2/dose i.v. before and 3 and 6 hours after the start of the CPM-infusion. In case of hematuria: increase i.v. fluid and Mesna. Furosemide 0.5 mg/kg i.v., 6 hours and 12 hours after CPM only if required for diuresis.

Cytarabine (Ara-C, Cytosar)

Dose and administration:	75 mg/m2 intravenously in 30 min in Induction, 3000 mg/m2 intravenously in 3 hr in MAR(A)M(A), 75 mg/m2 intravenously as bolus in OCTADA(D), 100 mg/m2 twice daily as bolus in ADE, 75 mg/m2 as bolus in IB, 15 or 20 mg intrathecally.
Storage:	At room temperature.
Toxicity:	Myelosuppression, nausea, vomiting, diarrhoea, gastro-intestinal inflammation and ulceration, abnormal liverfunction, fever, myalgia and arthralgia (flu-like syndrome), sepsis, abdominal pain, urticaria and skin ulcers, abnormal renal function, neuritis and CNS toxicity, headaches, pneumonia, shortness of breath, conjunctivitis.
Precautions:	Co-administration with steroids relieves “flu” symptoms. Prednisone eye drops prevent/relieve occular irritation at high doses > 1g/m2/day.

Daunorubicin

Dose and administration:	30 mg/m2 (or 50 mg/m2) in ADE) intravenously in 1 hour infusion in 25-50 ml 5% dextrose or 5% dextrose/0.45% saline.
Storage:	24 hour at room temperature and 48 hour refrigerated, in darkness.
Toxicity:	Myelosuppression, nausea, vomiting, diarrhoea, cardiac toxicity (early: SV arythmias, ST-wave abnormal, VT; late: cardiac decompensation), skin abnormalities (rash, dermatitis, hyperpigmentation skin/nails), alopecia.
Precautions:	Do not mix with heparin, monitor heart function before the first dose of daunorubicin and after each next 60 mg/m2 (not corrected for age); ==> ultrasound heart before week 1, week 12 and week 14: If LVSF < 27% (repeated): omit daunorubicin for this course. Avoid extravasation.

Dexamethasone and Prednisone

Dose and administration:	Dexamethasone 6 mg/m2 orally or intravenously as bolus injection in 3 divided doses; Prednisone 60 mg/m2 orally or intravenously as bolus injection in 3 divided doses; Prednisone 6 or 8 mg intrathecally.
Storage:	At room temperature.
Toxicity:	Obesity, hirsutism, fluid retention, hypertension, Cushing face, stomach and duodenal ulcers, decreased or increased appetite,

hyperglycemia, glucosuria, adrenocortical insufficiency, osteoporosis, avascular bone necrosis, irritability, psychosis.

Etoposide (VP-16)

Dose and administration: 100 mg/m² intravenously by 4 hours infusion on days 1-5 in ADE and MAE. Reconstitution with normal saline or glucose 5% to achieve a final concentration of 0.2 to 0.4 mg/ml. At concentrations above 0.4 mg/ml, precipitation may occur.

Storage: At room temperature for 48 hours at this concentration.

Toxicity: Myelosuppression, emesis, diarrhea, mucositis, anorexia, alopecia, hypertension following rapid intravenous infusion. Transient liver function abnormalities. Anaphylactic-like reaction with fever, chills, bronchospasm, dyspnea and tachycardia. Peripheral neuropathy.

6-Mercaptopurine

Dose and administration: 60 mg/m^2 in protocol IB; 25 mg/m^2 in MARMA; 50 mg/m^2 in maintenance. Orally in 1 daily dose in the evening, on an empty stomach avoiding milk products.

Storage: At room temperature.

Toxicity: Myelosuppression, hepatic toxicity.

Methotrexate

Dose and administration: 5000 mg/m2 (HD-MTX) in MARMA in 24 hours intravenously: 10% (500 mg/m2) of the dose is given in 0.5 hour and 90% (4500 mg/m2) of the dose is given subsequently in 23.5 hours.
20 mg/m2 once a week in maintenance orally.
6 or 8 mg intrathecally.

Storage: At room temperature in a dark place.

Toxicity: At low doses (oral): myelosuppression, mouth ulcers, skin rashes; At high doses (iv): myelosuppression, skin rashes, nausea, vomiting, mucositis, enteritis, rarely hepatotoxicity, neurotoxicity or acute renal failure; Intrathecal MTX: headache, vertigo, ataxia, convulsions, abnormal vision.

Precautions: HD-MTX can only be given in centers, with experience in the assessment of MTX levels in the blood.

Mitoxantrone (MTZ)

Dose and administration: 12 mg/m² daily over 1 hour in MAE in 5% dextrose or 5% dextrose/0.45 saline.

Storage: Stable at room temperature during 48h.

Toxicity: Myelosuppression, nausea, vomiting, cardiac toxicity.

Precautions: Monitoring of cardiac function (shortening fraction) should be performed before MAE and further administration of anthracyclines. Strict IV injection must be performed because of the risk of local necrosis if extravasation occurs.

6-Thioguanine

Dose and administration:	60 mg/m2 orally in 1 daily dose.
Storage:	At room temperature.
Toxicity:	Myelosuppression, hepatic toxicity, stomatitis, diarrhoea, neurotoxicity (peripheral neuropathy).

Vincristine

Dose and administration:	1.5 mg/m2, max 2 mg intravenously as bolus injection.
Storage:	At 2o-8o in refrigerator.
Toxicity:	Neuromuscular toxicity, with paresis, ataxia, sensory disturbances, muscle atrophia, systemic neurotoxicity with coma, focal cerebral, abnormalities and rarely convulsions, ataxia, paraesthesia, jaw-pain, constipation, ileus, inappropriate ADH secretion and hyponatriaemia, alopecia, psychosis, hallucination, depression, agitation, severe local necrosis if extravasation, very serious CNS toxicity when injected intrathecally (serious morbidity and death).
Precautions:	Avoid extravasation, avoid intrathecal administration. The drug should not be available in the same room to anyone who performs a lumbar puncture.

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16 Informed Consent (to be translated and adapted to the local situation)

Dear parent(s),

Your child is diagnosed with acute lymphoblastic leukemia (ALL) and will be treated according to the protocol Interfant-06. This international protocol is a guideline for treatment of very young children with ALL diagnosed in the first year of life (infants). It is developed by an international group of pediatricians who are experienced in treatment of children with ALL and is based upon the results of the earlier international treatment protocol Interfant-99. This treatment consists of different drugs active against leukemia, so-called combination chemotherapy.

It is known that infants with ALL respond less well to the regular therapy compared to older children. Therefore, specific treatment protocols has been designed for infants with ALL. The exact probability of cure for an infant with ALL depends on 3 so-called risk factors. These are age, number of leukemic cells in the blood and the presence or absence of a specific chromosomal abnormality (MLL gene abnormality) in the leukemic cells. Age above 6 months, a white blood cell count <300 and the absence of a MLL gene abnormality are favourable features respectively. Based upon these factors 3 different groups are defined with a lower or higher risk to be cured.

Infants with a low risk ALL have a relatively high probability to be cured. They will be treated with chemotherapy that is primarily based upon the results of the Interfant-99 protocol with some slight modifications. The therapy consists of 4 courses named INDUCTION, IB, MARMA and OCTADAD followed by maintenance chemotherapy.

Infants with medium risk ALL have an intermediate probability to be cured. For these children it will be investigated whether an intensified treatment will result in a better chance of survival. Therefore, children will be randomised into 2 groups. Half of the children will receive the slightly modified Interfant-99 chemotherapy as the infants of the low-risk group. The other half will receive intensified therapy: instead of course IB these children will receive two other courses of therapy: ADE and MAE. These 2 intensive courses have extensively been used for infants with another type of leukemia (AML) and do not contain “experimental” drugs. Because leukemic cells of infants with ALL show some characteristics also shown by AML it will be investigated whether the courses ADE and MAE will result in a better outcome than when giving course IB. The rest of the treatment is identical.

You will be asked for permission that your child participates in this study. If you do not want to participate this will in no way influence the quality of care given to you child. Participation means that your child will be allocated in one of the two groups by chance. Participation does not mean that you or your physician can choose one of the two groups. If you decide not to participate your child will be treated according to the standard therapy. If your treating physician thinks that it is better to adapt the treatment for your child, this will of course be done, irrespective of the fact whether your child participates in the study or not.

Infants with high risk ALL have a lower probability to be cured than the other infants. For these children the same strategy will be followed as for the medium risk group. So half of the patients will receive chemotherapy courses including protocol IB, the other half ADE-MAE courses instead of IB (see above). In addition, infants with high risk ALL are eligible for a bone marrow transplantation in case a suitable donor is available. This transplantation will be performed after the MARMA course and will be given instead of OCTADA and maintenance therapy.

17 Add-on studies

Project A:

Molecular characterization of infant ALL, aiming to develop innovative and more specific therapeutic strategies

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Introduction

In order to improve prognosis for infants diagnosed with acute lymphoblastic leukemia (ALL), new therapeutic strategies are urgently needed. Collaborative studies between the Dana Farber Cancer Institute (Boston, USA) and our laboratory have recently resulted in the identification of FLT3 as a drugable therapeutic target. Additional preliminary results from our laboratory led to the postulation of new hypotheses surrounding genes that may also represent potential therapeutic targets. The hereby proposed research project is designed to further gain insights into the aggressive nature of infant ALL and to develop more efficient treatments for these very young patients. For this we aim for the identification and characterization of:
Possible genomic defects underlying infant ALL (using array-CGH).
Possible infant ALL specific microRNAs.
Additional new drugable therapeutic targets for infant ALL (with a focus on the leukemic stem cell).

Background:

Array-comparative genomic hybridization (array-CGH) is a recently developed genome wide high resolution screening technique suitable for the detection of deleted or amplified chromosomal regions.1,2 The major advantage of array-CGH is its 10-20 fold higher resolution than spectral karyotyping or conventional metaphase CGH. To date, array-CGH has predominantly been used for screening of solid tumors and its application in childhood leukemias is limited.3-5 Since 2003 our laboratory has been applying the array-CGH technique in T-ALL. This has led to the identification of several new and previously unknown genetic abnormalities. A major example is the identification of a 9q34 duplication that occurs in one third of all T-ALL cases.6 Here we propose to use this technique to screen for possible unidentified genetic defects underlying infant ALL. Array-CGH profiling will be performed on a group of 40-60 infant ALL samples, and the data will be related to gene expression profiles, in vitro drug response and clinical outcome. For this, DNA isolated from untreated infant ALL cells and healthy human reference DNA will be fragmented and

labeled with Cy3 and Cy5 respectively, and vice versa (dye-swap experiments), and hybridized onto Spectral ChipTM 2600 BAC arrays (genomic resolution of about 1MB; Spectral Genomics, Houston, TX, USA). Upon hybridization and washing, chips will be scanned using ScanArray Express HT (Applied Biosystems). Individual spots will be quantified using Imagene 6.0 software for Cy3 and Cy5 fluorescence and analyzed by SpectralWare 2.0 software. All data points within a two standard deviation range of the mean ratio of al spots will be used to plot profiles among chromosome ideograms. Recurrent abnormalities will be further analyzed by FISH experiments on cytospin preparations, using BAC-clones containing the corresponding human chromosomal region obtained from BacPac Resources (Oakland, CA, USA). In order to reveal possible deregulated genes (or cellular pathways), gene expression profiles from leukemic samples carrying newly identified abnormalities will be compared to expression profiles from samples lacking these abnormalities. This way, novel targets may be identified that become (in)activated as a (in)direct consequence of possible genomic defects underlying infant ALL. Ideally, this approach will also be applied on 20-30 samples from relapsed infant ALL cases, in order to study whether certain genomic abnormalities may be associated with re-emergence of the leukemia.

MicroRNAs (miRNAs) are a recently discovered class of small non-coding RNA species that negatively regulate gene expression by binding the mRNA of target genes. Interestingly, several miRNAs appeared to be involved in B-cell development7, and have been shown to co-localize with known leukemia specific genetic abnormalities. In 2005, our laboratory initiated collaborative studies with the research group of Dr. Chang-Zheng Chen (Stanford University School of Medicine, Stanford, USA) in order to identify the role of miRNAs in childhood cancer. Inhibiting the synthesis of specific target proteins, the abundance of certain miRNAs may point to silenced tumor suppressor genes which may have allowed oncogenic transformation. In contrast, under-expressed or absent expression of certain miRNAs may provide a mechanism for proto-oncogene expression. Insights into miRNAs that are possibly associated with MLL translocations (which are found in ~80% of the infant ALL cases, and confer a poor prognosis) may lead to a better understanding of the biology and leukemogenesis of MLL rearranged infant ALL, and may eventually point to potential “drugable” target genes to direct therapy against. We here propose to screen MLL rearranged infant ALL samples for the expression of miRNAs. Accordingly, such miRNAs will be cloned, and the level of expression will be determined using Northern blotting8 and compared to other precursor B-lineage leukemia subtypes. Finally, these miRNA expression patterns will be correlated to the levels of expression of the corresponding targeted genes. Once identified, these target genes will be subjected to intensive studies validating them either as potential therapeutic targets or as key-players in leukemogenesis of MLL rearranged infant ALL.

In collaboration with Dr. Scott Armstrong (Dana Farber Cancer Institute, Harvard Medical School, Boston, USA) we have recently demonstrated that ALL patients carrying translocations of the MLL gene display a unique gene expression profile that distinguishes this leukemia from both acute lymphoblastic and acute myeloid leukemias carrying germ line MLL genes.9 Accordingly, this specific gene expression profile was used to identify FLT3 as a potential therapeutic target for the treatment of MLL rearranged infant ALL.10,11 These studies clearly emphasized the benefit of using gene expression profiling as a tool to discover novel therapeutic strategies. Meanwhile, we have been mining the unique gene expression profile for MLL rearranged ALL (MLL) for additional target genes. As a result from these analyses we are currently validating several genes as potential therapeutic targets, including MCL-1, CD44 and cyclin A1. To gain more specific expression profiles that may allow us to study more specific therapeutic targets, we currently are comparing and analyzing gene expression profiles from prednisone resistant and sensitive infant ALL samples. Possibly this may yield insights in what mechanisms underlie the remarkable resistance to prednisone as observed in infants with ALL12,13, and may point to strategies to abrogate prednisone resistance. In addition, we are analyzing gene expression profiles from infant ALL samples carrying different types of MLL translocations in order to explore the possibilities of developing MLL subtype specific therapy. Validation of potential therapeutic targets that come forth from these studies may eventually lead to the identification of innovative and more adequate

treatment strategies for infant ALL patients. Increasing evidence is emerging that the self-renewal properties of certain types of acute leukemias are sustained by the presence of a minor sub-population of leukemic stem cells. Hotfilder et al. (2005), demonstrated the presence of a lymphoid-restricted CD33/CD19 LSC in t(4;11) positive infant ALL samples, carrying the actual MLL translocation.14 The presence of leukemic stem cells may have serious consequences for the treatment of this aggressive type of leukemia, as these primitive, self-renewing stem cells usually are not prone (yet) to undergo apoptosis.15 Thus, initial therapy may kill the majority of leukemic cells, while few chemotherapy-resistant CD34+CD19– leukemic stem cells (LSCs) survive, and rapidly repopulate the bone marrow with the original leukemia as observed at diagnosis. Therefore, in order to more effectively treat t(4;11) positive infant ALL (and possibly infant ALL patients carrying other MLL translocations), it may be of utmost importance to develop therapeutic strategies that also target LSCs underlying MLL rearranged infant leukemia. Therefore, the requested material shall in part be used in experiments designed to validate the potential of several genes identified (in above described studies) as possible therapeutic targets in these stem cells. For this, in collaboration with Dr. Vormoor (University Children's Hospital, Münster, Germany), immature CD34+CD19– cells will be purified by cell sorting from infant ALL samples carrying different types of MLL translocations. This will reveal whether LSCs are present only in t(4;11) positive leukemias or whether the presence of LSCs are characteristic for MLL rearranged leukemia in general. From LSC positive samples, RNA will be extracted from the LSCs and subsequently the LSC specific gene expression profile will be determined using micro-array analyses. The obtained LSC expression profile will be compared to the profile associated with the bulk of the leukemic samples, and shall then be used to identify therapeutic targets to direct therapy against that not only target the bulk of CD19+ leukemic cells but also target the CD34+CD19– LSC population within these leukemias. Accordingly, the potential of these targets shall again intensively be validated in additional experiments.

Required material for this research proposal:

Given the usually very high white blood cell counts at presentation which is typical for infant ALL, 1-4 ml bone marrow and/or 5-10 ml peripheral blood sampled at diagnosis and reaching our laboratory within 24 hours after sampling, will provide sufficient material to adequately perform the here proposed research project. These samples not only provide sufficient viable leukemic cells, but also allows the extraction of enough RNA, DNA and protein needed for these studies. To be able to identify chromosomal abnormalities re-emerging in, similarly bone marrow and/or blood samples are required at relapse.

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INSTRUCTIONS FOR SAMPLING AND SHIPMENT

1. ANNOUNCEMENT	*	inform us one day before the sample will be taken or **as soon as possible** at the day of sampling
	*	call the research laboratory Pediatric Oncology in Rotterdam, the Netherlands: **+31 10 408 8340** (preferably), +31 10 408 8051 or +31 10 463 6233
2. SAMPLE	*	use **standard preheparinized tubes** with a **maximum of 20 IU heparin per ml bone marrow or peripheral blood**
	*	1-3 ml bone marrow and/or 5-10 ml peripheral blood
	*	keep the tubes stored at room temperature
	*	**caution**: do not use standard EDTA-containing tubes
3. SHIPMENT	*	the laboratory in Rotterdam will instruct TNT Express Worldwide to pick up the sample at your hospital
	*	the TNT service will contact you about the time and location of the pick up
	*	fill in the **invoice form** and **include 2 copies**
	*	fill in the **patient documentation form**
4. PAYMENT	*	transport costs: check the appropriate box on the patient documentation form

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INVOICE

Delivery: **PRIORITY** contents: **BLOOD samples** caution: **FRAGILE**

Date : ..... Invoice no. : .....

To: Research Laboratory of Pediatric Oncology
Sophia Children's Hospital
Dr. Molewaterplein 60
3015 GJ Rotterdam
The Netherlands

Deliver package at: **Reception-desk of main entrance Sophia Children's Hospital**
(portier Hoofdingang Sophia Kinderziekenhuis)
Dr. Molewaterplein 60
3015 GJ Rotterdam
The Netherlands

Message for the reception/receiver:

Bij aflevering s.v.p. direct het research-laboratorium KinderOncologie bellen:
Toestel 88340, 88051 of 36233
Contactpersoon: Mw. K.M. Kazemier
Pakje bij kamertemperatuur bewaren

From: Name of contact person : …….
Hospital :…..……
Department :…….…….
Address :……..
ZIPcode and City :……..
Phone :……..
Telefax :……..

Description of contents:

This package contains human blood samples for research purpose only

Value:	Dfl. 15.= (Euro 6.81)
Weight:	less than 0.2 kg
Country of origin	…….
Storage conditions	room temperature

(Please, enclose this form with the sample)

Patient Documentation

Name of the patient : ……

Date of birth : ……

Gender : male / female

Registration no : ……

Protocol patient : Interfant / other: ……

Leukemia Documentation

(please encircle)

* at initial diagnosis:
  did the patient receive any **treatment during two weeks before this sample was taken**, e.g Allopurinol, antibiotics, Prednisolon, other cytostatics:

  no / yes

  if yes, please indicate which agents have been given:
  ……

* time-point of sample taken:
  - at initial diagnosis
  - after non-response to induction therapy / at 1st relapse / at 2nd or later relapse

* white blood cell count of the sample : …….

REMARKS:
……...……...
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The costs of XP TNT priority delivery from your hospital to the research laboratory of Pediatric Oncology in Rotterdam, The Netherlands will be paid by:

□ sender □ research laboratory of Pediatric Oncology, Rotterdam

(please check appropiate box)

Project B:

Identification of New Partner Gene of MLL in Infant with ALL

Andrea Biondi

Background:

The *MLL* gene, located in 11q23 band, results being rearranged in almost 80% of Infant with ALL (results from Interfant 99 analyses). The presence of this rearrangement is statistically significant for the stratification of such patients, as the new Interfant protocol stratification showed. In 70% of the *MLL*-positive cases the partner gene is easily identifiable using FISH and RT-PCR techniques; in fact, the involvement of chromosome 4 (AF4 gene), chromosome 19 (ENL, EEN, ELL gene) or chromosome 9 (AF9 gene) are well established. In the other 10-20% of *MLL*-positive cases, we were enable to identify the Partner gene. New technical approaches were applied in two different laboratories (Dr Luca Lo Nigro (Italy) and Prof. Rolf Marschalek (Frankfurt)), which are now cooperating for this purpose.

Methods:

In Italy, in Luca Lo Nigro Lab will be analyzed the cases with the following characteristics:
RT-PCR screening for t(4;11)-t(11;19)-t(9;11) Negative & FISH x11q23 Positive
In Catania we will applied Panhandle PCR approaches using both DNA and RNA:
Regular PanHandle PCR (Leukemia 1998) for the genomic characterization of the MLL breakpoint at the level of der(11);
cDNA Panhandle PCR (PNAS 2001) for the identification of fusion transcripts;
Recombination PCR (PNAS 2000) for cloning and sequencing of Panhandle PCR products;
Repetitive region of MLL will be checked using the "Repeat Masker Program" at the Washington University Human Genome Center .
(http://ftp.genome.washington.edu/bin/mrs/mrs/reg).

Preliminary Data:

Using these techniques, we were able to identify three new partner genes in three different infant with AML:
MYO1F located at chromosome 19 band p13 (in which lie ENL, EEN and ELL) (Lo Nigro L et al. Blood Vol. 100 (11) ASH - abs 2080; pag 531a. 2002).
RPS3 located at chromosome 11 band 11q12.13 (Lo Nigro L et al. Blood Vol. 102 (11) ASH - abs 4455; pag 184b. 2003).
ARGBP12 located at chromosome 4 band q35 (Tonelli R., Lo Nigro L. et al. Blood Vol. 104 (11): abs 4331; pag 538a. 2004).

Goals:

To increase number of new partner genes of MLL in order to
Well characterize the leukemogenesis process;
Clarify the mechanism of this kind of translocation/rearrangement;
Confirm that the MLL rearrangement could be enough as single event to initiate the process of leukemogenesis;
Compare these results with data obtained from infants with AML and pediatric cases with more than 12 months of age, affected by MLL-positive leukemia.

Project C:

Detection of Minimal Residual Disease in Infant with ALL using Genomic Sequences of MLL Rearrangements

A. Biondi

Background:

Recent data (Brit. J. Haemat 2002; and Cazzaniga G at al. Blood Vol. 104 (11): abs 164; pag 51a. 2004), have showed that application of conventional methodology for the detection of MRD in infants with ALL is characterized by several troubleshooting leading to a false-negative results!! Main reasons are: a) low frequency of clonotypic marker; b) high frequency of clonal evolution (as IgH and TcR delta). In addition, detection of MRD using RT-PCR for known fusion transcripts can cover 50% of cases and will be based on amplification of RNA, which is well known to be a very unstable molecule, not for a Multicenter Study. Thus we need to use a more stable molecule as DNA and a more reliable technique as Panhandle PCR (Dr Luca Lo Nigro – Catania – Italy) and Inverse PCR (Prof Rolf Marschalek – Frankfurt – Germany). In these two labs a network for identification of genomic sequences involved in the MLL breakpoint rearrangements has been already started. Moreover, the MRD detection in the group of Intermediate Risk (new stratification – Interfant 05) will identify the subgroup of cases who, even in morphological CR, will be still in covert-leukemia status and will presumably need a bone marrow transplantation procedure!!! In fact, newly released papers (Kosaka Y Blood Nov 2004; Sanders J Blood prepub 2005) emphasize the crucial role of BMT performed in first CR!!

Methods:

In Italy (Luca Lo Nigro) and in Germany (Rolf Marschalek) will analyze the cases with the following characteristics:
RT-PCR screening for t(4;11)-t(11;19)-t(9;11) Positive & FISH x11q23 Positive
In Catania we will apply Panhandle PCR approaches using both DNA and RNA:
Regular PanHandle PCR (Leukemia 1998) for the genomic characterization of the MLL breakpoint at the level of der(11);
cDNA Panhandle PCR (PNAS 2001) for the identification of fusion transcripts in case of RT-PCR negative screening;
Repetitive region of MLL will be checked using the "Repeat Masker Program" at the Washington University Human Genome Center.
(http://ftp.genome.washington.edu/bin/mrs/mrs/reg).
MRD detection will be performed using a Real-time PCR methodology which is in the way of application in Monza (Dr Cazzaniga G.) and Catania (Dr Lo Nigro L.)

Preliminary Data:

Our network has been already able to rapidly characterize 14 out of 21 italian
MLL-positive infants with ALL, enrolled in Interfant 99. For the other 7, analyses are in progress. The detection of MRD for specific cases revealed that conventional methodology which generates negative results failed to identify a covert and/or persistent status of leukemia. Data collection is in progress.

Goals:

To detect of MRD using a stable, sensitive and highly specific marker of leukemia;
To identify a subgroup of cases who will benefit of changing in therapeutic strategy, as well as any kind of BMT or new drugs (?), during the course of front line treatment and in first morphological remission.

Project D:

Identification of clonogenic stem cells in infant acute leukaemias with t(4;11) / MLL-AF4 fusions

Mel Greaves PhD
Institute of Cancer Research, London

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Infant ALL with *MLL-AF4* fusions have, overall, a very poor prognosis. Within this group, high white cell count and age (<6 months) are additional adverse factors 1. Blast cells from such cases have phenotype features indicative of resistance to apoptotic stress and drug resistance that distinguish them from leukaemic cells in common forms of childhood ALL (i.e. in older children with hyperdiploidy and *TEL-AML1* fusion). Infant patients with *MLL-AF4* usually enter remission but this is often short-lived. These characteristics all suggest that the variant subtype of ALL in infants with *MLL-AF4* is a biologically distinct disease and may be driven and sustained by a unique clone of stem cells.

The nature of the stem cell in infant ALL may be key to unravelling the clinical intransigence of the disease. Some limited data is available suggesting that although the leukaemia is usually classified as pro-B in immunophenotype, it may be derived from a more primitive lympho-myeloid stem cell (Table 1). Patients in remission have residual *MLL-AF4* fusion positive cells that are CD34+/CD19- (Fig 1) and equivalent cells are detectable as a minor fraction at diagnosis 3.

We propose a more systematic analysis of stem cells in infant *MLL-AF4* positive ALL in which we will assess the following:

A more detailed immunophenotypic analysis of the CD34+/CD19- population that carry the *MLL-AF4* fusion gene. The methodology would involve immunomagnetic antibody-based sorting followed by combined two-colour immunophenotype/FISH staining 5. Additional antibodies that might generate informative data on the nature of this cell type include CD79a, IL7Rα, Sca-1/Thy-1, CD11b.

We will identify the immunophenotype of the leukaemic cells that can transfer leukaemia into NOD/SCID mice 6 using purified populations and intrafemoral injection.

At present, there is no in vitro clonogenic assay for infant ALL cells, though mouse stem cells transformed by *MLL* fusions regularly undergo replicate cloning in vitro 7. Such an assay, if available, would be the method of choice for analysing drug sensitivity. We will attempt to set up such an assay using cytokine cocktails and stromal monolayers.

In designing these experiments, we will compare the stem cell properties of very young, <6 months, versus older infants with *MLL-AF4* positive leukaemias since these might be distinctive. The experiments will be conducted on a modest number of cases (e.g. 5-10 of each age group). In preliminary experiments, we will compare the NOD/SCID repopulation capacity of blood versus bone marrow leukaemia cells. If there is little or no difference, all further experiments will be with blood-derived cells. Preliminary experiments will also assess the impact of cell storage in liquid nitrogen on clonogenic/stem cell function.

Specific objectives:

What is the clonogenic stem cell phenotype in infant ALL?

Does either the cell type/phenotype of stem cells in infants or the quantity of stem cells (in marrow or blood) vary according to age (and in relation to prognosis)?

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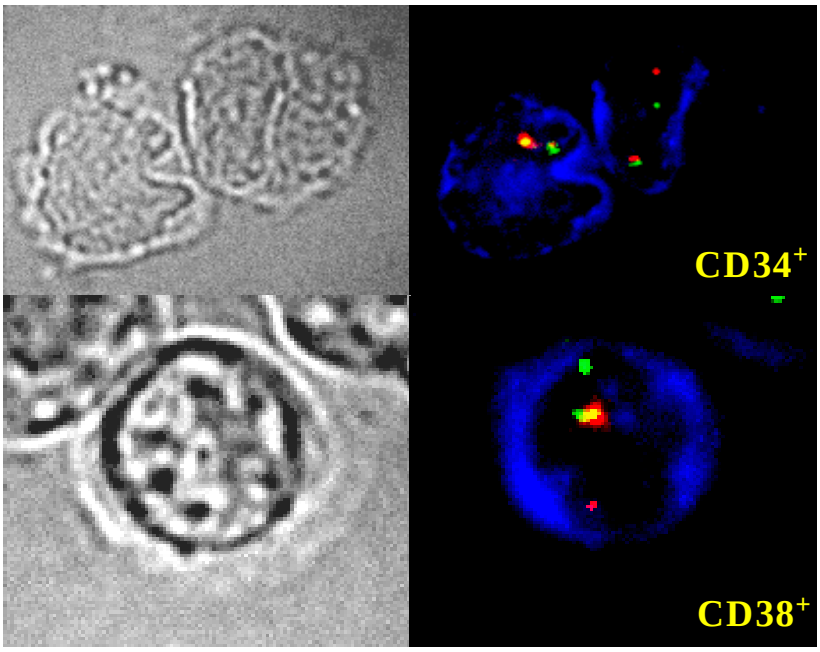
Table1

Unique phenotypic properties of leukaemic cells in infant ALL with *MLL-AF4* fusions

	Reference
Pro-B lympho-monocytic phenotype	1
Intraclonal pro-B to monocyte switches following induction chemotherapy	2
MLL-AF4 fusion gene present at diagnosis in cells that are CD34+/CD19-, i.e. earlier than committed B cell progenitors (= candidate stem cells?)	3
MLL-AF4 fusion gene present in residual cells in remission that are (exclusively) CD34+/CD19- (= candidate pre-leukaemic or leukaemic stem cells?)	4

Figure 1



Purified CD34+/CD19- progenitor/stem cells from bone marrow of infant ALL in remission. Blue = antibody with blue dye/AMCA. FISH probe for MLL is two colour red/green 'split apart' probe. Normal MLL gene registers as green + red (= yellow). Rearranged MLL gene scores as separate red and green spots (as in upper right cell and lower cell; upper left cell is normal).

Project E:

Health status and health-related quality of life in survivors of acute lymphoblastic leukemia in infancy.

R. Barr

Background

Acute lymphoblastic leukemia (ALL) in the first year of life is characterized by particular biological features that portend a much poorer prospect for survival than is the case in older children. In order to improve this circumstance, an international collaborative study has been undertaken with a uniform therapeutic strategy based on intensive chemotherapy.
The combination of this intensity and the early developmental status of the patients make it likely that there will be a considerable burden of treatment – related morbidity and mortality. While this will be self-evident during the administration of therapy, the late effects (long-term sequelae) in survivors are likely also to be notable in scope and severity.

Objective:

As a first step in assessing this "price of cure" it is proposed to measure the health-status and health-related quality of life (HRQL) in the population of survivors who have completed the protocol of chemotherapy.

Hypotheses :

1. The study subjects will have poorer HRQL than age-matched children in the general population.
2. The major burden of morbidity will be manifest in the attributes (domains/dimensions) of health encompassed by cognition, emotion and pain.

Study subjects:

All survivors who are over 5 years of age at July 1, 2006 will be eligible. From projections provided by the Interfant database and an estimated participation rate of 75% it is anticipated that approximately 200 subjects will be available for study.

Methods:

A cross-sectional survey will be undertaken using a mailed-out questionnaire for parental proxy assessments of the children's health status. This is a 15 item document that is available in multiple languages and takes less than 10 minutes to complete. From the current inventory of questionnaires in the specific format proposed for these studies, 9 language versions will cover 80% of the study sample. This represents all of the subjects in the following countries – Argentina, Australia, Austria, Belgium, Canada, Chile, France, Germany, Holland, Italy, New Zealand, Portugal, United Kingdom and United States. Minor modifications of existing instruments would allow the inclusion of all the countries in NOPHO (an additional 10% of the Interfant population).

The responses are converted by coding algorithms into the levels of two complementary multi-attribute health status classification systems which provide health state vectors for each subject. Multi-attribute utility functions generate single attribute utility scores and scores for overall HRQL.

These components are part of the Health Utilities Index (HUI) family of instruments that constitute a generic, preference-based approach to the measurement of health status and HRQL.

Significance:

Determination of the prevalence and severity of compromised health status and HRQL serves numerous purposes, including
Facilitation of communication between health care consumers and providers
Focus of attention on items of importance to patients and their families
Identification of issues requiring intervention
Definition of targets for amelioration in future studies
Provision of data essential for economic evaluation of treatment strategies

Appendix

Elaboration:

Assessments of patient-focused health status and HRQL are being recognized increasingly by clinicians, patient advocates, regulatory authorities, administrators and policy makers as primary measures of the need, efficacy, effectiveness and efficiency associated with health care services1. Functional health status and HRQL measures are important for a variety of reasons that complement conventional clinical measures2. HRQL is the more comprehensive concept and has been defined as " … the value assigned to the duration of life as modified by the impairments, functional states, perceptions and social opportunities that are influenced by disease, injury, treatment or policy"3.

Measures of HRQL may be classified as specific or generic4. The former focus on a specified health problem, disease or age group of subjects. The latter are applicable to a wide variety of clinical groups and general populations. These are two types of generic measures: health profiles, such as SF365, and preference-based instruments. Again, there are two types of preference-based instruments (which offer the advantage, over health profiles, of integrating measurements of morbidity and mortality in a single summary score): direct measurements, such as the standard gamble6, and multi-attribute classification systems with preference-based scoring functions7. Included in the latter are the Health Utilities Index (HUI)8,9, the Quality of Well-Being Scale (QWB)10 15D11, EQ5D from EuroQol12, AQOL13 and SF6D14. The 15 D and AQOL instruments have not been used widely outside of Finland and Australia respectively. SF6D has been developed only recently, so there is little experience on which to report.

Preferences are expressed either as utilities or values. The former include an element of risk attitude and are appropriate therefore for decision-making in the context of uncertainty. Value scores, as used in the QWB and EQ5D instruments, are preferences measured under conditions of certainty. Uncertainty is an important factor in health outcomes, so utility scores are more appropriate than value scores in this setting. These issues have been discussed in detail by Torrance et al. 6, 15. Given these considerations, it is proposed to use the HUI instruments in this study. The complementary systems HUI2 and HUI3 can identify 24,000 and 972,000 unique health states respectively; each health state being represented by a vector consisting of one level of function for each attribute, the number of health states reflecting the factorials of the number of levels (n=3-6) in all of the attributes in HUI2 (n = 7) and HUI3 (n = 8).

Single attribute utility scores range from zero (the lowest level) to 1.00 (no compromise of function). Overall HRQL scores are bounded by zero (equivalent to being dead) and 1.00

(equivalent to perfect health). Negative utility scores, as are measurable with HUI2 and HUI3, are assigned to health states worse than being dead9.

Among the measurement properties required of suitable HRQL instruments are validity, reliability and responsiveness; all of which have been demonstrated repeatedly for HUI9. Measurements have been shown to vary by assessment viewpoint. Although children as young as 7 years can complete interviewer-administered questionnaires reliably16, few children in this study will be > 7 years of age and it is not proposed that interviewers will be used. Mode of data collection is important and should be standardized across subjects, assessors and assessment points17 (of which there will be only one in this cross-sectional survey). These considerations underly the decision to use parental proxy assessors and mailed out questionnaires. HUI questionnaires exist for various recall periods. Relatively long periods can be used when the patients' health status is fairly stable, as may be assumed in a cohort of survivors who have completed therapy. We will use the versions of HUI for "usual health".

There are notable challenges to the measurement of HRQL in children18. These have been addressed particularly in the context of cancer19. A consensus definition formulated at the American Cancer Society workshop on Quality of Life in Children's Cancer defined HRQL as a multi-dimensional construct that includes the physical, social and emotional functioning of the child, measured from the perspective of both the child and his/her family, and sensitive to the changes that occur throughout development20. In a subsequent international workshop, organized by colleagues at McMaster University and St. Jude Children's Research Hospital, this topic was discussed in more detail 21. More recently, special attention has been devoted to the measurement of HRQL in children with acute lymphoblastic leukemia (ALL) 22. HUI instruments have been used in children with ALL, during23 and after therapy 24,25. The burden of morbidity was identified as occurring mainly in the attributes of cognition, emotion and pain.

The importance of qualifying conventional outcome measures of survival in children with cancer, by adjustments on the basis of HRQL measurements, has been emphasized26. Yet, despite earlier pleas for the incorporation of such measurements in clinical trails27, a recent review of the paediatric literature28 identified only 18 clinical trials in which HRQL was measured; and not one of them was in children with cancer. For much of the past decade we have been engaged in such an undertaking with the Dana Farber Cancer Institute Childhood ALL Consortium, of which McMaster University is a member. This involves serial measurement of HRQL from remission induction therapy through to long-term follow-up.

Accordingly, the proposal to assess the HRQL of survivors in the Interfant study, using HUI instruments, is well-supported. The established instruments can be used by parental proxy respondents for children as young as 5 years of age29. As the accrual target for the clinical trial is close to being met, by the time the HRQL study is initiated the majority of survivors with be > 5 years of age. An additional advantage of setting this lower limit is that it will allow a comparison of the HRQL data with assessments of educational performance; a comparison of particular relevance given the expected burden of morbidity in the attribute of cognition.

Assessment of the HRQL of 2-5 year old survivors could be undertaken by the PedsQL modular instrument30 which is not preference-based but incorporates a generic component and a module specific for cancer; or by an HUI affiliated instrument, the Comprehensive Health Status Classification System for Pre-school children (CHSCS – PS), which has been used in survivors of cancer in childhood31. However, the CHSCS – PS is available only in English and is not linked yet to a multi-attribute utility function.

Finally, HUI offers versions currently available in English, Chinese (Simplified and Traditional), Dutch, French (European and Canadian), German, Italian, Japanese, Portuguese (European and Brazilian), Russian, Spanish (European, Latin America and North America), and Swedish. Other language versions in development include Czech, Polish, Finnish, Norwegian and Danish29.

With respect to the proposed study, comparative data from HUI assessments are available from ALL populations in Canada23,24 and the United Kingdom25, as well as from Latin America32; and normative data are available from some 200,000 adolescents and adults in North America29 as well as from children in the general population of Canada33 and elsewhere.

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18 Appendix A: data collection forms