Treatment of MLL gene rearranged Acute Lymphoblastic Leukemia

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Introduction
Infant leukemia is defined as leukemia occurring in the first year of life. Whereas ALL has a higher incidence than ANLL in older children, the incidence of ALL and ANLL in infants is about equal. Infants account for about 4% of childhood ALL and differs from ALL in older children with respect to immunophenotypic, cytogenetic and molecular genetic features. In contrast to the predominance of male sex in older children with ALL, there is a slight predominance of girls in infant ALL. Trisomy 21 is a predisposing factor in the development of leukemia at young age, but if children with Down syndrome develop leukemia in the first year of life this is always ANLL and never ALL.

At least two independent mutations are necessary to cause leukemia. The first is thought to take place in utero for the majority of childhood leukemia cases in general whereafter postnatal events are required for the full development of ALL. However, in infant leukemia, all necessary genetic events may have occurred in utero. Indirect evidence for this hypothesis includes the sometimes very early onset of infant leukemia and the high rate of concordance of leukemia in identical monozygotic twins if one of the children developed leukemia in the first year of life. Direct evidence for the prenatal origin of infant ALL was demonstrated by Gale et al who detected unique MLL-AF4 fusion sequences in the Guthrie cards from infants who developed ALL at very young age.

Clinical Presentation and Biology
Compared to older children with ALL, infants have a high leukocyte count and an increased incidence of hepatosplenomegaly and central nervous system (CNS) involvement. In about half of the infants with ALL, the WBC is >100 x 10^9/L. About 15% of infant ALL cases have CNS involvement at diagnosis. Enlarged testes were seen in ~13% of male infants. The mediastinum is enlarged in less than 5% of infant ALL cases.

Immunophenotype
About two-thirds of infant ALL is classified as very immature CD10- negative B-lineage precursor ALL (proB ALL). The remaining group mainly consists of common/pre-B cases. Mature B-lineage ALL is an exceptional finding, while T-lineage ALL was diagnosed in only 4% of cases. Infant ALL cells are more likely to express myeloid-associated antigens. These data suggest that infant ALL arises from an immature precursor cell that is not fully committed to lymphoid differentiation. Intraclonal switch from B-lineage to monocytic lineage leukemia has been described in infants.

Genetics
Cytogenetic abnormalities that occur relatively frequent in older children, such as hyperdiploidy and the t(12;21) resulting in the TEL/AML1 fusion product, but also the Philadelphia translocation t(9;22) and the t(1;19), are rare in infant ALL. The most common chromosomal aberrations in infant ALL are translocations involving chromosome band 11q23 leading to translocation of the MLL gene. Cytogenetic analysis detects MLL gene rearrangements in ~50% of infant ALL cases, but this percentage rises to 80% when molecular techniques are used. The t(4;11)(q21;q23) is the most common and is found in about 50% of the MLL gene rearranged cases whereas the t(11;19)(q23;p13) in about 20% and the t(9;11)(p22;q23) in 10%. More than 40 other 11q23 partner chromosomes have been reported, all occurring at a very low frequency but together in about 10-20%. Therefore, most MLL gene rearranged cases will be detected if specific RT-PCR’s for the well-known t(4;11), t(9;11) and t(11;19) fusion
transcripts are performed but other translocations will be missed. The split-signal FISH method is an easy method to detect any types of MLL gene translocation in a single FISH experiment and is therefore advised as a first screening technique (6). It requires a low amount of material, but does not show which MLL gene rearrangement is present.

Prognostic factors
The presence of MLL gene rearrangements, the absence of CD10 expression, and a high WBC are highly correlated with each other and are inversely related to the age of the infant. About 20% of infants with ALL lack these features. The poor prognosis of infant ALL has been associated with the following factors in univariate analysis (1-7): age below 3 or 6 months, organomegaly, CNS involvement, a high WBC, CD10 negativity, myeloid antigen expression, MLL gene rearrangement and day-14 bone marrow or day 8 response peripheral blood response to therapy. Gender was not a prognostic factor. In multivariate analysis including these factors, the presence of MLL rearrangements often remains the most important factor predicting a poor outcome (8, 9, 10). The EFS for MLL rearranged infant cases (5–34%) is worse than for their MLL germ line counterparts (42–92%) (7). Some studies reported that only t(4;11) positive infant ALL patients experience a poor prognosis, whereas patients carrying other types of MLL rearrangements fare equally well as MLL germ line cases (15, 11). In a large meta-analysis, all types of MLL gene rearrangements were associated with a poor prognosis in infants (12). In a recent review (7) we concluded that the presence of MLL rearrangements and young age are probably the strongest independent prognostic factors. This is confirmed by preliminary results from the large collaborative Interfant-99 study.

Biology and drug resistance
Leukemic cells from infants with MLL gene rearranged ALL cells grew better on stromal cell layers in vitro (13), had a higher leukemic cell recovery when inoculated into SCID mice (14) and were more resistant to cell death resulting from serum deprivation in vitro (15) compared with cells from other children with ALL. Infant ALL cells were more resistant in vitro to prednisolone and L-asparaginase than cells from older children with ALL (16). This is in concordance with the finding that infants with ALL more frequently show a poor response to prednisone than older children with ALL do (17, 18). Infant ALL cells do not express higher levels of the multidrug resistance genes BCRP, MDR1, MRP1 and LRP/MVP than other ALL subtypes. (19)

Although relatively resistant to several chemotherapeutic drugs, infant ALL cells are more sensitive to the cytarabine (Ara-C) and the adenosine analogue 2-CdA (2-chlorodeoxyadenosine or cladribine) compared with cells from older children with ALL (16, 20). Sensitivity to Ara-C in infant ALL appeared not to be directly associated with rearrangements of the MLL gene, as both MLL rearranged and MLL germ line infant ALL cases appeared equally sensitive to this drug in vitro (21). The Ara-C sensitivity is most likely due to the high expression of the human equilibrative nucleoside transporter 1 (hENT1) (22), on which Ara-C is mainly dependent to permeate the cell membrane. However, at high-dose Ara-C regimens, Ara-C also enters the cell by passive diffusion. Improved outcomes have been reported for infant ALL patients when high-dose Ara-C was implemented during the consolidation phase (23, 24). Also, improved outcome for adult pro-B ALL cases was observed with intensified post-remission therapy including high-dose Ara-C/mitoxantrone (25). In 1999, the collaborative Interfant-99 treatment protocol for infant ALL was initiated that added the intensive use of both low and high-dose Ara-C on top of a ALL based chemotherapy schedule. These observations may also the use of the combination of Ara-C and 2-CdA in infant ALL. Moreover, given the sensitivity of infant ALL cells to nucleoside analogues, newly developed nucleoside analogues, e.g. clofarabine and troxacitabine, may be interesting candidate drugs for further analysis in infant ALL.

Treatment results
Whereas the overall cure rate for childhood ALL has risen to ~80% with contemporary treatment schedules, progress in the treatment of infant ALL has remained behind. We recently reviewed the results of published studies, showing an
overall EFS rate of 35-40%. The complete remission rate in infant ALL is about 94%. Toxicity after remission induction is not the major problem: --4% of infants die from therapy toxicity. The major cause of treatment failure is relapse: about half of the patients experience a relapse, which involved the bone marrow in 80% of cases, the CNS in 30% and the testes in 8%. The majority of relapses occur very early during the first year of treatment already. Outcome after these early relapses is very dismal. So early bone marrow relapse is the major cause of death in infant ALL.

Comparison of treatment protocols
Comparisons of different treatment protocols and outcome are difficult because most protocols differ in many details and the numbers of included patients are low, even in the larger published trials.

A study performed by several POG institutions, resulted in a 5-year EFS of only 17%. Unlike other protocols, this regimen did not contain dexamethasone, high-dose methotrexate (MTX), high-dose cytarabine (ara-c), cyclophosphamide or ifosfamide and L-asparaginase was used in the induction phase only. In another POG study (8493), the EFS rate was 27%, which is also lower than the results of other study groups. This protocol lacked dexamethasone, L-asparaginase, anthracyclines, high-dose ara-c and high-dose MTX in contrast to other protocols.

Protocols of MRC UKALL specified high-dose MTX dose and high-dose ara-c, but not dexamethasone, cyclophosphamide or ifosfamide. L-asparaginase was administered only in the induction phase. The overall EFS rate was 25%, lower than that reported by other groups. The Dana-Farber Cancer Institute (DFCI) consortium has intensified its treatment protocols since 1985, leading to a significant improvement in treatment results. The overall EFS rate for infants, 43%, is among the highest of reported results. A small study of the EORTC–CLG using a slightly modified BFM regimen also resulted in a 43% EFS. In both studies, cranial irradiation was given to a subgroup of the patients.

The CCG-1883 resulted in 39% EFS, which is comparable to the results reported by BFM and DFCI investigators. These outcomes were better than historical CCG control series in which less intensive systemic therapy was used. Importantly, the CNS relapse rates in the more recent studies, which relied on intensive systemic chemotherapy and intrathecal therapy, but without irradiation, were no higher than those in trials with radiation. Major difference between the CCG protocols described by Reaman compared to historical controls was the inclusion of high-dose ara-c, cyclophosphamide, and more L-asparaginase in the consolidation and reconsolidation phases.

An important finding was that cranial irradiation and intensive chemotherapy combined with intrathecal therapy result in the same CNS relapse rate, even in patients with CNS involvement at initial diagnosis. In particular, high-dose MTX, high-dose ara-c, dexamethasone, and intrathecal therapy may contribute to prevention of CNS relapses.

Another possible conclusion from a comparison of historical controls, intensive POG protocols, and more recent protocols of the CCG, BFM and DFCI is that intensive postinduction chemotherapy and the use of high-dose ara-c, high-dose MTX, L-asparaginase, dexamethasone and cyclophosphamide/ifosfamide are helpful in preventing early bone marrow relapses. In the context of the low incidence of infant ALL, large international collaborative studies are needed to study new
therapies for this disease. Two large collaborative efforts - COG and Interfant - are currently analyzing the efficacy of intensified therapy for infant ALL.

**Bone marrow transplantation**

The role of allogeneic bone marrow transplantation (BMT) in infant ALL is unclear and debatable. No proper randomized studies have been performed comparing allogeneic BMT with chemotherapy, so the published data are scant and selective. A relatively large meta-analysis (12) did not show a benefit for the use of allogeneic BMT from a matched donor in infant MLL gene rearranged ALL. Controlled studies are needed to determine the usefulness of BMT in this vulnerable group of patients. In the Interfant studies BMT is restricted to infants with a very high risk of relapse.

**Late effects**

Little is known about late effects of treatment for infant ALL, mainly because substantial numbers of infants did not survive until recently. In studies reported to date, learning disabilities and developmental delays were identified in the majority of irradiated infants. (23, 29) Obesity and short stature were found in ~25% irradiated cases. Asymptomatic echocardiographic abnormalities and stable congestive heart failure have been reported in single cases. (23, 29) In 30 nonirradiated infants who were treated with high-dose MTX as CNS-directed therapy, the neurodevelopmental outcome was normal. (30) Frankel (27) reported on one patient with a severe developmental disorder among 18 infants who were neither irradiated nor transplanted and remained in complete remission. As treatment becomes more effective for infants with leukemia, it will be important to incorporate prospective studies of late effects into all new protocols.

**Drug Dosage Adjustment**

A persistent problem are the rules for drug dosage adjustment in infants. (31) The total-body water content decreases from 75% at birth to 60% at 1 year, and the percentage of extracellular water decreases with age. (32) Drugs bind less avidly to serum proteins in newborns than in adults, leading to a higher unbound active fraction of drugs in infants. (33) The lower activity of P-450 enzymes in infants (34,35) can lead to reduced cytotoxic effects as well as increased cytotoxic effects. Drugs cleared by the kidneys may have increased systemic exposures in young infants because tubular and glomerular function reach adult levels by ~6 months of age (31). The volume of the CNS relative to body surface area or body weight, is larger in children compared to adults. Therefore, intrathecal chemotherapy should be calculated on age and not on body surface to avoid undertreatment of infants. (36) The ratio of body weight to body surface is lower in infants than in older children, which implies that if dosages are calculated on body weight, infants are exposed to lower amounts of drugs.

A small study in infants with ALL showed no decreased clearance of MTX compared to older children. (37) It has been suggested that infants show decreased ara-C clearance after high-dose therapy with this agent because of poorer conversion of ara-C to ara-U. (38) Others have not found a difference in ara-C clearance between infants and older children. (39) At the moment, current protocols rely on arbitrary calculations based on body weight, body surface area or one of these with a correction for age. Thus, pharmacokinetic studies together with toxicity measurements are urgently needed in infants with leukemia (and other types of cancer).

**New therapeutic targets**

Combinations of multiple new drugs will be required to cure infant MLL gene rearranged ALL patients who are not cured with current chemotherapies. Thus, several innovative treatment strategies are needed that either overcome resistance to conventional drugs or which involve alternative novel agents that more effectively target infant MLL cells. (7) Examples of these are mentioned below.

In addition to ara-C and 2CdA described above, a class of nucleoside analogue drugs that may be effective against MLL gene rearranged ALL cells are DNA demethylating cytidine analogues, such as 5-azacytidine, 5-aza-2'-deoxycytidine (decitabine), or the recently identified agent zebularine (40, 41) showed that MLL rearranged ALL cases had the highest number of methylated genes of all ALL subtypes. Thus, MLL gene rearranged ALL is characterised by aberrant DNA
hypermethylation. In concordance with this, we recently observed that the tumour suppressor gene FHIT was silenced by methylation of the promoter region in 100% of the infant MLL gene rearranged cases tested, whereas silencing of this gene was observed in only 50% of older children with ALL (42). We observed that ectopic expression of FHIT in MLL rearranged cells induced leukaemic cell death. Likewise, treatment with the demethylating agent decitabine resulted in re-expression of FHIT protein expression and induced apoptosis. Therefore, inhibition of DNA methylation may be an effective therapeutic strategy in the treatment of infant MLL, especially since decitabine depends on ENT1 to cross the cell membrane, which is highly expressed in infant ALL cells (22).

FLT3, the gene encoding Fms-like tyrosine kinase 3, is highly expressed in patients with MLL gene rearranged ALL (43). FLT3 is important in early B-lineage development and is highly expressed in immature B-cells (44). In AML the FLT3 gene is frequently subjected to mutations that activate this receptor (45). Constitutively activated FLT3 became a promising therapeutic target in AML and several small molecule inhibitors (e.g. CEP-701, PKC412 and SU5416) inactivate FLT3 and induce leukaemic cell death. This has led to the initiation of phase I/II clinical trials with these inhibitors in refractory adult AML, and so far the results are promising (46, 47, 48). Interestingly, constitutively activated FLT3 also occurs in MLL rearranged infant ALL patients carrying activating mutations, and in MLL rearranged infant ALL displaying high-level expression of wild-type FLT3 (21, 49). We and others demonstrated that high-level wild-type FLT3 expression in primary infant MLL rearranged ALL samples is associated with activated FLT3 and cytotoxic responsiveness to FLT3 inhibitors (21, 50). This showed that FLT3 inhibition may represent a novel therapeutic strategy for infant MLL that needs clinical testing.

Conclusions
Infant ALL shows a highly unfavorable outcome compared to that of older children with this disease subtype, which possesses unique clinical and biologic features. The major problem in treatment of infant ALL is the occurrence of early relapses, justifying early intensive chemotherapy. The role of allogenic bone marrow transplantation in infants is debatable. Large collaborative studies are the only way to investigate possible improvements of therapy for infants with ALL. Development of new innovative approaches are needed to increase the cure rate to the same rate as that in older children with ALL.

References


