Infant Acute Lymphoblastic Leukemia

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Introduction

Acute lymphoblastic leukemia (ALL) in infants under 12 months of age accounts 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 (1). In infant leukemia, all necessary genetic leukemogenic events may have occurred in utero, illustrated by the very early onset of infant ALL and the high rate of concordance of leukemia in monozygotic twins if one of the children developed leukemia during infancy. MLL-AF4 fusion sequences have been detected in the Guthrie cards from children who were diagnosed with ALL in infancy (2).

Infants have a higher tumor load (median white blood cell count at diagnosis of 100 x 10^9/L) and more often central nervous system (CNS) involvement (15%) than older children with ALL (1).

Two-thirds of infant ALL has the immature CD10-negative B-lineage precursor ALL (proB ALL). Mature B-lineage ALL is an exceptional finding; T-lineage ALL is present in only 4% of cases (1). Infant ALL cells often express myeloid-associated antigens. Intraclonal switch from B-lineage to monocytic lineage leukemia has been described in infants. (3) These data illustrate that infant ALL arises from an immature precursor cell that is not fully committed to lymphoid differentiation.

Genetics

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 myeloid leukaemia and never ALL. Cytogenetic abnormalities that occur relatively frequent in older children, such as hyperdiploidy and TEL/AML1 fusion, but also the Philadelphia translocation t(9;22) and the t(1;19), are rare in infant ALL. About 80% of infant ALL cases carry translocations of the MLL gene. The t(4;11)(q21;q23) is found in 50% of the MLL gene rearranged cases, the t(11;19)(q23;p13) in 20% and the t(9;11)(p22;q23) in 10% (1), (4). The t(9;11) occurs in older infants than the t(4;11) and t(11;19) (4) and is associated with a more mature immunoglobulin rearrangement pattern (5). Many other partner chromosomes have been reported, occurring together in 10-20% of cases. The split-signal FISH method detects any type of MLL gene translocation and is therefore advised as a first screening technique (6).

The MLL gene encodes a member of the trithorax protein family, regulating transcription mediated by various functional domains. Disruption of the MLL gene leads to deregulated gene expression (7). MLL-rearranged ALL displays a unique expression profile that is clearly distinguishable from other ALL subtypes (8), (9) Moreover, in a recent study we demonstrated that, apart from a fundamental signature shared by all MLL-rearranged infant ALL samples, each type of MLL translocation is associated with a translocation-specific gene expression signature. We also showed the existence of 2 distinct subgroups among t(4;11)-positive infant ALL cases characterized by the absence or presence of HOXA expression, and that patients lacking HOXA expression are at extreme high risk of disease relapse (10). Highly characteristic for MLL fusion proteins is the loss of the H3K4 methyltransferase (SET) domain, which results in aberrant histone modifications, and hence altered chromatin remodeling (11). (12) This results in abnormal promoter methylation patterns, and abnormal gene expression favoring malignant transformation. Recently performed genome-wide methylation studies in our laboratory showed that patients...
carrying t(4;11) or t(11;19) exhibit extensive abnormal promoter methylation, directly influencing gene expression (50). In contrast, infant ALL patients carrying wild-type MLL genes or t(9;11) displayed DNA methylation patterns that closely resembled normal bone marrow. Moreover, apart from an overlapping promoter methylation profile shared by t(4;11) and t(11;19)-positive infant ALL patients, we also found distinct DNA methylation patterns that specifically associate with either translocation t(4;11) or t(11;19). This is in line with a recently postulated model proposing that different MLL fusion proteins lead to varying histone modifications directed by the MLL fusion partner (11). These recent findings suggest that MLL-rearranged infant ALL can be considered an epigenetic malignancy, and that epigenetic therapies may be an attractive new therapeutic option (see below).

MicroRNAs (miRNAs) control the expression of protein-coding genes in normal hematopoietic cells. and, consequently, aberrant expression may contribute to leukemogenesis. To identify miRNAs relevant to MLL rearranged ALL, we recently cloned known and new miRNA genes expressed in patients' leukemia cells. Eight miRNAs were differentially expressed between MLL and non-MLL precursor B-ALL cases. The expression of miR-196b was 500-fold higher in MLL-rearranged ALL compared with the expression level in other precursor B-ALL cases whereas miR-708 was 500-fold lower expressed in MLL-rearranged ALL. The expression did not correlate with the maturation status of leukemia cells. (13). The miR-196b gene is located in the HOXA cluster at chromosome 7p15. It has been suggested that transcriptional activation of this cluster is caused by MLL binding and subsequent H3K4 methylation of associated histones. (14) In line with the fact that miR-196b is mapped between HOXA9 and HOXA10, we observed that miR-196b expression correlated with the expression of HOXA9 and HOXA10 in MLL-rearranged ALL. However, the high expression of miR-196b appeared not exclusively MLL-driven but was also found in other types of leukemia with aberrant activation of HOXA-genes. Since miR-196b has been shown to exert oncogenic activity in bone marrow progenitor cells, these observations imply a potential role for miR-196b in the underlying biology of all HOXA-activated leukemias (15).

**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. The poor prognosis of infant ALL has been associated with many factors in univariate analyses (1), (16). In the large Interfant study, multivariate analysis showed that the presence of MLL rearrangements and age < 6 months are the most important factor predicting a poor outcome followed by a WBC > 300 x 10^9/L and a poor prednisone response (4). Compared with the 9-12 month cohort, the hazard ratio for any event was 3.05, 2.25 and 1.64 for 0-3, 3-6 and 6-9 months cohorts, respectively. MLL rearranged cases showed a 3.1-fold increased risk of an event compared to MLL-germline cases. This increased risk was the same regardless of the type of MLL rearrangement (4). These risk factors are now used for stratification in the Interfant-06 study.

**Drug resistance**

Leukemic cells from infants with MLL gene rearranged ALL cells grow better on stromal cell layers in vitro (17), have a higher leukemic cell recovery when inoculated into SCID mice (18) and are more resistant to cell death resulting from serum deprivation in vitro (19) compared with cells from other children with ALL. Infant ALL cells are more resistant in vitro to prednisolone and L-asparaginase than cells from older children with ALL (20) and infant ALL more frequently shows a poor response to prednisone than ALL in older children (21). The mechanisms of resistance are not known but recently we showed that overexpression of MCL-1 may contribute to this. Inhibition of MCL-1 by shRNA or by drugs sensitized MLL rearranged ALL cells from infants to glucocorticoids (22), (23). Infant ALL cells do not express higher levels of the multidrug resistance genes BCRP, MDR1, MRP1 and LRP/MVP than other ALL subtypes (24).

Although relatively resistant to several chemotherapeutic drugs, infant ALL cells are more sensitive to cytarabine (Ara-C) and 2-CdA (2-chlorodeoxyadenosine or cladribine)
compared with cells from older children with ALL (20), (25). 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 (26). The Ara-C sensitivity is most likely due to the high expression of the human equilibrative nucleoside transporter 1 (hENT1) (27), 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 (28), (29). Also, improved outcome for adult pro-B ALL cases was observed with intensified post-remission therapy including high-dose Ara-C/mitoxantrone (30). Based upon these data, the collaborative Interfant-99 and Interfant-06 protocols added both low and high-dose Ara-C on top of a ALL based chemotherapy schedule (4).

**Treatment**

*Treatment results.* The results of studies on infant ALL published in the last decade show an EFS rate of −50% or lower (table 1). Studies included low patient numbers with the exception of the Interfant-99 study that included 500 patients from 22 countries and achieved a long-term EFS of 47% and survival of 55% (4). The complete remission rate in infant ALL is 93-97% (table 1). Toxicity after remission induction is not the major problem: 4% of infants die from therapy toxicity while being in remission (1), (16). 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 (4).

**Comparison of treatment protocols.**

Table 1. Outcome of infant ALL in Interfant-99 study and other published studies

<table>
<thead>
<tr>
<th>Study group</th>
<th>CR rate</th>
<th>EFS/survival timepoint</th>
<th>EFS (SE)</th>
<th>Survival (SE)</th>
<th>Number patients</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFCI (1985-95)</td>
<td>96%</td>
<td>4 yr</td>
<td>54% (11%)</td>
<td>-</td>
<td>23</td>
<td>(28)</td>
</tr>
<tr>
<td>MLL-96 and MLL 98</td>
<td></td>
<td>5 yr</td>
<td>50%</td>
<td>61%</td>
<td>102</td>
<td>(39)</td>
</tr>
<tr>
<td>Interfant-99</td>
<td>94%</td>
<td>4 yr</td>
<td>47% (2.6%)</td>
<td>55.3% (2.7%)</td>
<td>482</td>
<td>(4)</td>
</tr>
<tr>
<td>AIEOP-91/95</td>
<td>96%</td>
<td>5 yr</td>
<td>45% (95% CI 31-58%)</td>
<td>-</td>
<td>52</td>
<td>(35)</td>
</tr>
<tr>
<td>BFM</td>
<td>95%</td>
<td>6 yr</td>
<td>43% (5%)</td>
<td>48% (6%)</td>
<td>105</td>
<td>(21)</td>
</tr>
<tr>
<td>EORTC-CLCG</td>
<td>86%</td>
<td>4 yr</td>
<td>43% (95% CI 24-62%)</td>
<td>-</td>
<td>25</td>
<td>(34)</td>
</tr>
<tr>
<td>CCG-1953</td>
<td>97%</td>
<td>5 yr</td>
<td>42% (9%)</td>
<td>45% (6%)</td>
<td>115</td>
<td>(59)</td>
</tr>
<tr>
<td>CCG-1883</td>
<td>97%</td>
<td>4 yr</td>
<td>39% (4%)</td>
<td>51% (4%)</td>
<td>135</td>
<td>(29)</td>
</tr>
<tr>
<td>CCG-107</td>
<td>94%</td>
<td>4 yr</td>
<td>33% (5%)</td>
<td>45% (5%)</td>
<td>99</td>
<td>(29)</td>
</tr>
<tr>
<td>UKALL-92</td>
<td>94%</td>
<td>5 yr</td>
<td>33% (95% CI 23-44%)</td>
<td>46% (95% CI 35-57%)</td>
<td>86</td>
<td>(60)</td>
</tr>
<tr>
<td>POG 8493</td>
<td>93%</td>
<td>4 yr</td>
<td>28% (5%)</td>
<td>-</td>
<td>82</td>
<td>(32)</td>
</tr>
<tr>
<td>POG alternating drugs</td>
<td>94%</td>
<td>4 yr</td>
<td>17% (8%)</td>
<td>-</td>
<td>33</td>
<td>(31)</td>
</tr>
</tbody>
</table>

CR = complete remission; EFS = event-free survival

Comparisons of different treatment protocols and outcome are difficult because most protocols differ in many details and the reported patient numbers are often low.

A small study by several POG institutions, resulted in a 5-year EFS of only 17%. (31). Unlike other protocols, this regimen did not contain dexamethasone, high-dose methotrexate (MTX), high-dose ara-c, cyclophosphamide or ifosfamide whereas L-asparaginase was used in the induction phase only. In another POG study (32) 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. Protocols of MRC UKALL specified high-dose MTX dose and high-dose ara-c, but not dexamethasone, cyclophosphamide or ifosfamide. (33) L-asparaginase was administered only in the induction phase. The overall EFS rate was only 25%.

The Dana-Farber Cancer Institute (DFCI) consortium intensified its treatment protocols since 1985. This led to a significant improvement for infants with an EFS of 54% in a very small series of cases (28). The main difference with the historical control series was the use of a postinduction intensification course with high-dose MTX, high-dose ara-c, L-asparaginase, vincristine and 6-mercaptopurine. Dexamethasone, cyclophosphamide or ifosfamide, and epipodophyllotoxins were all excluded from this DFCI protocol but cranial irradiation was administered at the age of 1 year.

The CCG-1883 resulted in 39% EFS (24) which was higher than historical CCG control series in which less intensive systemic therapies were used. Major difference was the inclusion of high-dose ara-c, cyclophosphamide, and more L-asparaginase in the consolidation and reconsolidation phases. An important finding was that intensive chemotherapy combined with intrathecal therapy resulted in the same CNS relapse rate as earlier schedules including cranial irradiation, even in patients with CNS involvement at initial diagnosis (29). In particular, high-dose MTX, high-dose ara-c, dexamethasone, and intrathecal therapy may have contributed to reduction of CNS relapses.

Since 1983, BFM investigators stratified patients according to the prednisone response and leukemic cell burden, resulting in treatment of infants according to different arms of the protocols. In general, infants were over-represented in the higher-risk arms because of their high leukemic burden and high incidence of poor prednisone response. The overall EFS rate for infants on BFM protocols was 43% (21). Small studies of the EORTC–CLG (34) and AIEOP (35) that also used BFM regimens reached 43% and 45% EFS respectively. In these BFM based protocols, cranial irradiation was given to a subgroup of the patients and 30-60% of the infants were not treated by “regular” ALL therapy but by intensive high-risk chemotherapy courses of BFM (21), (34), (35). The 47% EFS achieved by the intergroup Interfant-99 study is comparable to the best reported outcomes in single group studies but Interfant protocol did not include intensive high risk courses such as in BFM protocols. Interfant-99 is based on a regular ALL protocol with addition of ara-C in different doses and schedules. On Interfant-99, prednisone-good responders had a similar outcome as reported by the BFM study whereas prednisone-poor responders obtained a 30% EFS compared with 15% in the BFM study (21), (34), (35). In Interfant-99 no irradiation was used, no alkylating agents and a low dose of anthracyclines was used and very few patients received BMT.

Bone marrow transplantation (BMT). No randomized studies have compared allogeneic BMT with chemotherapy; many small (single institution) and biased series have been published. A meta-analysis (38) did not show a benefit for the use of allogenic BMT from a matched donor in infant MLL gene rearranged ALL. The combined results of two consecutive Japanese studies using intensive chemotherapy blocks followed by BMT in case of MLL rearrangement resulted in a long-term EFS of 50% (39). This regimen resulted however in a significant number of serious late effects. Also, 8 out of 53 patients who underwent BMT died from toxicity and over half of the events occurred before instigation of BMT.

The Interfant-99 study did not show a significant benefit of BMT for prednisone poor responders (4) but more recent analyses indicates that high-
risk patients as currently defined in the Interfant-06 protocol, benefited from the use of BMT in the Interfant-99 protocol. This high risk group is defined by the presence of all 3 of the following risk criteria: (a) MLL rearrangement AND (b) age below 6 months at diagnosis AND (c) a poor prednisone response or a WBC >300x10e9/L (40). If patients did not fulfill all 3 criteria the outcome was not different between patients that received chemotherapy only or chemotherapy followed by BMT.

So, in conclusion, Interfant, BFM based regimens and Japanese protocols have achieved the best outcome results. The BFM strategy implied the use of intensive high risk courses for a large number of infants and the Japanese approach implied BMT for almost all MLL rearranged cases with substantial morbidity and mortality. In general, we can conclude that intensive postinduction chemotherapy and the use of high-dose ara-c, high-dose MTX, L-asparaginase, dexamethasone and cyclophosphamide or ifosfamide are probably helpful in preventing early bone marrow relapses. BMT should be reserved for a small group of selected high risk cases.

Late effects

Little is known about late effects of treatment for infant ALL, mainly because substantial numbers of infants did not survive until recently. Learning disabilities and developmental delays were identified in the majority of irradiated infants (28), (34). Obesity and short stature were found in ~25% of irradiated cases. Asymptomatic echocardiographic abnormalities and stable congestive heart failure have been reported in single cases. (28), (34). In 30 nonirradiated infants who were treated with high-dose MTX as CNS-directed therapy, the neurodevelopmental outcome was normal (41). Frankel (32) reported on one patient with a severe developmental disorder among 18 infants who were neither irradiated nor transplanted and remained in complete remission. The Japanese study group did not observe significant late effects in patients who did not receive BMT. However, several serious late complications were seen in a substantial proportion of patients who did receive BMT such as chronic graft versus host disease, hypothyroidism, skin abnormalities, ophthalmologic complications, pulmonary complications, dental abnormalities and neurocognitive problems (39). As treatment has become more effective for infants with leukemia nowadays, it is important to incorporate prospective late effects analyses.

Drug Dosage Adjustment and pharmacokinetics

A persistent problem are the rules for drug dosage adjustment in infants (42). In general, the total-body water content decreases from 75% at birth to 60% at 1 year, and the percentage of extracellular water decreases with age. Drugs bind less avidly to serum proteins in newborns than in adults, leading to a higher unbound active fraction of drugs in infants. The lower activity of P-450 enzymes in infants 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 (42). 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 (43). 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.

Three studies have looked at MTX pharmacokinetics in infants. The first and small study showed no decreased clearance of MTX in infants compared to older children (44) whereas a more recent report showed that MTX clearance was slightly lower in younger infants (0-6 months) than in older infants (7-12 months). Steady-state clearance for these older infants appeared to be comparable to values reported for older children. Very young infants (0-3 months) experienced a slightly higher incidence of renal toxicity but no difference in liver toxicity or mucositis (45). Very recently, the Interfant collaborative group reported on 103 infants at the time of their first treatment with methotrexate (5 g/m2) (46). In the Interfant-99 protocol, infants <6 months of age received two-third, children 6-12 months three-fourth, and children >12 months full dose calculated on body surface area.
The systemic clearance tended to increase with age. All infants tolerated the dose well enough to receive a second dose of MTX without further dose reduction. No significant effect on disease-free survival for MTX steady-state concentration, MTX clearance, or time to MTX level below 0.2 microM was found. Interestingly, male infants had higher clearance than female infants. So, younger infants have slightly lower MTX clearance than older infants and when using dose reduction rules as applied in Interfant-99, this leads to a comparable toxicity profile as for older infants. However, in view of the poor treatment results for especially young infants, one might also consider not to decrease the dose for these patients or to increase the dose for those who reach low plasma levels after the first MTX dose (46).

Hempel et al showed (47) daunorubicin clearance, central volume of distribution, apparent clearance of daunorubicinol and apparent volume of distribution showed no age-dependency. Consequently, due to the empirical dose reduction in Interfant-99 the overall exposure to daunorubicinol in infants was smaller than would be expected from older children. Patients aged <6 months experienced more infections in the induction phase than the group aged 6-12 months at diagnosis. Other toxicities were similar in both groups. The authors concluded that there was no age-dependency in the pharmacokinetics of daunorubicin.

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 (48). Others have not found a difference in ara-C clearance between infants and older children (49).

In general, pharmacokinetic studies in infants are very scarce while many protocols rely on arbitrary calculations based on body weight, body surface area or one of these in combination with arbitrary dose reductions by age. Thus, pharmacokinetic studies together with toxicity measurements are urgently needed in infants.

**New therapeutic strategies**

Combinations of multiple new drugs will be required to cure infant MLL gene rearranged ALL patients who are not cured with current chemotherapies. Thus, innovative strategies are needed that either overcome resistance to conventional drugs or which involve alternative novel agents that more effectively target infant MLL cells (16).

Given the sensitivity of infant ALL cells to nucleoside analogues such as araC and 2CdA as described above, newly developed nucleoside analogues may be interesting candidate drugs for further analysis in infant ALL. Clofarabine has been shown to be effective in refractory or relapsed ALL in childhood and is also transported by the ENT1 protein. So it seems worthwhile to investigate this drug in infant ALL.

Another class of drugs that may be effective against MLL gene rearranged ALL cells are demethylating cytidine analogues, such as 5-aza-2'-deoxycytidine (decitabine), or the recently identified agent zevalin. As mentioned above, especially t(4;11) and t(11;19) positive ALL are characterised by aberrant DNA hypermethylation. The degree of hypermethylation may influence outcome in infant ALL and that demethylating agents largely reverses the aberrant methylation pattern of MLL rearranged ALL cells, leading to apoptosis in these cells (50). In concordance with this, we 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 (51). 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. In conclusion, inhibition of DNA methylation may be an effective therapeutic strategy in the treatment of infant MLL, especially since several demethylating agents also depend on ENT1 to cross the cell membrane, which is highly expressed in infant ALL cells (27).

**FLT3**

FLT3, the gene encoding Fms-like tyrosine kinase 3, is highly expressed in patients with MLL gene rearranged ALL (8). FLT3 is important in early B-lineage development and is highly expressed in immature B-cells (52). In AML the FLT3 gene is frequently subjected to mutations...
that activate this receptor (53). 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 leukemic cell death. This has led to the initiation of clinical trials with these inhibitors in adult AML, and so far the results are promising. 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 (26), (54). 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 (55), (56). Also, the level of FLT3 expression has prognostic relevance (57). This showed that FLT3 inhibition represents a novel therapeutic strategy for infant MLL which has led to two ongoing clinical trials exploring this.

Infant ALL has myeloid characteristics as mentioned above and the fact that MLL stands for mixed lineage leukemia illustrates that the leukemic cells in which the MLL gene gets affected are very immature and may differentiate into different lineages or have biphenotypic features. Chemotherapy blocks as being used for acute myeloid leukemia may have value in infant ALL therefore. This is currently being explored in the Interfant-06 protocol which compares the use of two AML induction courses versus protocol IB of the BFM protocol after induction therapy.

Several studies in children and adults with ALL have shown that minimal residual disease (MRD) status is a strong prognostic factor. Data in infants are scarce. Very recently we evaluated the prognostic significance of MRD in ~100 cases of infant ALL (58). All patients with MRD levels >/=10^-4 after consolidation relapsed. These patients are now eligible for BMT in the current Interfant-06 protocol.

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 is the occurrence of early relapses, justifying early intensive chemotherapy whereas only a small selected subgroup of high risk patients may benefit from allogenic BMT. Large collaborative studies are the only way to investigate possible improvements of therapy for infants with ALL. New insights in the biology of MLL rearranged ALL have suggested new innovative approaches which will be tested in real life now and in the near future in an attempt to increase the cure rate to the same rate as that in older children with ALL.

References


