Acute leukaemia in children : aspects of diagnosis and treatment

Willemze, A.J.

1

General introduction and outline of this thesis

1

General introduction

1. Acute leukaemia in children and assessment of prognosis following chemotherapy

1.1. General prognostic factors

The vast majority of childhood leukaemia cases are acute and are characterized by rapid tumour cell proliferation and a predominance of blast cells. The most common form, acute lymphoblastic leukaemia (ALL) accounts for 75%-80% of all cases of acute leukaemia in children and adolescents, while acute myeloid leukaemia (AML) accounts for 15-20%. With modern intensive chemotherapy and supportive care measures the prognosis of ALL, and to a lesser extent of AML, in children has improved significantly over the past decades.

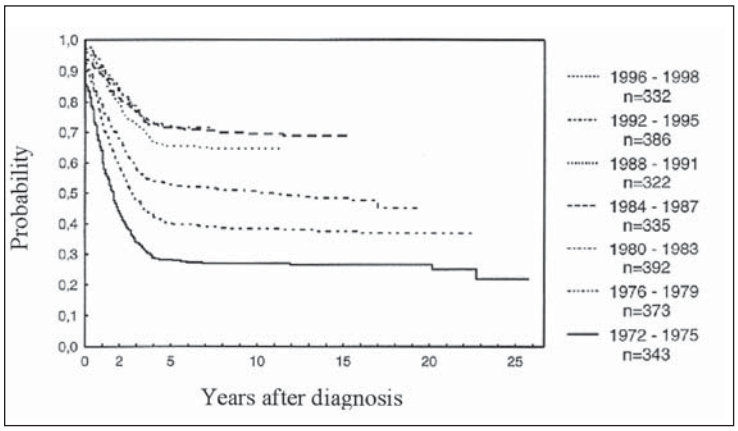

Figure 1. Disease free survival of children with ALL in the Netherlands (1972-1998); adapted from the revised SNWLK ALL-9 protocol with permission from the SKION.

To minimize the frequency of treatment-related deaths and sometimes severe late effects of chemotherapy, risk-group stratification is important. At the same time suboptimal therapy should be avoided. Therefore, paediatric collaborative study groups try to assess the relapse risk in individual patients, so that only high-risk cases are treated aggressively, while less aggressive therapy is reserved for patients at lower risk of relapse(1). To develop adequate risk stratification many prognostic parameters have been investigated. However, due to effective contemporary therapy improvements, several of the biological and clinical features,

which once were associated with poor prognosis, do not confer a poor outcome anymore. It is, therefore, necessary to evaluate risk factors within the context of contemporary treatment strategies and continue the development of new prognostic parameters.

The prognostic determinants for a patient with a malignant disease are heterogeneous and often tumor-type specific. In general, prognostic factors are related to the characteristics of the patient, the histopathology and biology of the tumour, and of the efficacy of therapy. However, the same variable may play a different prognostic role and may have diverse correlations with the other prognostic indicators in different malignancies(2).

In childhood leukaemia, patient's age and leukocyte count at diagnosis have consistently shown prognostic strength, regardless of the treatment regimen used. Although this impact can in part be explained by the association with specific tumour cytogenetic abnormalities, these genetic features do not entirely account for treatment outcome. One of the possible reasons for the unpredictable relationship between the biological characteristics of the disease and the response to treatment is the influence that pharmacodynamic and pharmacogenetic factors exert on the effectiveness of treatment. Many studies have indicated that the patient's early response to therapy (i.e. initial decrease in leukaemic blasts), reflecting both leukaemic cell characteristics and patient pharmacogenetics, is an important independent predictor of long-term outcome(3;4). The sequential monitoring of minimal residual disease (MRD), by polymerase chain reaction (PCR) analysis of clonal antigen-receptor gene rearrangements, provides an objective and more sensitive assay than formerly used bone marrow cytology to monitor the response to treatment and can improve the precision of risk assessment still further. Several clinical trials currently incorporate MRD assessment as part of risk group classification, adjusting the intensity of therapy based on MRD levels obtained at various time points early in treatment. We will need a longer follow-up to determine the prognostic impact of the therapeutic changes(5).

1.2. Specific prognostic factors

In addition to the patient's response to treatment as an important prognostic factor, many other strategies have been used to optimize risk stratification and subsequent therapy. This

chapter will highlight some of these prognostic factors.

One of the oldest classifications is the French-American-British (FAB) classification(6) system, that divided AML into 8 subtypes, M0 through to M7, based on the type of cell from which the leukemia developed and its degree of maturity. This is done by examining the appearance of the malignant cells under light microscopy and/or by using cytogenetics to characterize any underlying chromosomal abnormalities. The subtypes have varying prognoses and responses to therapy. The World Health Organization (WHO) classification of acute myeloid leukemia attempts to be more clinically useful and to produce more meaningful prognostic information than the FAB criteria. Although the WHO classification may be more useful, the FAB system is still widely used for AML. In ALL the FAB classification system(6) originally defined three subtypes (L1, L2, L3), based solely on morphologic features. Because of the importance of immunophenotypic, cytogenetic and molecular features of ALL in risk stratification and the lack of correlation between these features and the FAB subtypes, the FAB classification of ALL has been largely abandoned in practice(1).

Several cytogenetic abnormalities can be found in many patients with AML and ALL; the types of chromosomal abnormalities often have prognostic significance. Many of the rearranged genes encode tyrosine kinases, transcription factors or other proteins that have relevant activities during the cell cycle and apoptosis. The ultimate consequence of many of these rearrangements and mutations is the impairment of hematopoietic development and a “differentiation arrest.” For example, in acute promyelocytic leukemia, the t(15;17) translocation produces a PML-RARα fusion protein which binds to the retinoic acid receptor element in the promoters of several myeloid-specific genes and inhibits myeloid differentiation. In ALL, the abnormal karyotypes are subgrouped according to chromosome number (ploidy) and structural chromosomal changes. Current treatment protocols use the presence of specific cytogenetic abnormalities for risk stratification.

Drug cytotoxicity assays are used to test the responsiveness of leukaemic cells to different classes of both novel and already established cytotoxic drugs in vitro and can be used to stratify ALL patients in risk groups(7). Children with in vitro-resistant ALL cells at initial diagnosis have poorer long-term clinical outcome than do in vitro-sensitive patients(8).

In contrast, cellular drug resistance at initial diagnosis of paediatric AML has not been shown to be discriminative for long-term clinical outcome(9). Although the use of in vitro cellular resistance testing in the contemporary risk stratification has been limited so far, the generation of cellular models of drug resistance has been fundamental in unravelling the main effectors of resistance to traditional chemotherapy at the molecular level. Furthermore, recently a relatively small number of genes was found to be associated with both in vitro drug resistance and treatment outcome in childhood ALL by gene expression analysis(10), implicating that genetic profiling may identify patients with poor initial responses.

In acute leukaemia the immature malignant cells accumulate in the bone marrow. Soon after, they leave the bone marrow, populate the blood, and in some cases localize in extramedullary sites. In ALL, leukaemic cells have a tendency to migrate to the central nervous system (CNS), liver, spleen and lymph nodes. In AML, leukaemic spread occurs most commonly in the skin, gingiva, soft tissue, bone and the CNS. The soft tissue tumours are also referred to as chloromas, granulocytic sarcomas or myeloblastomas. Especially in childhood AML, extramedullary disease is frequently reported at diagnosis (in 23-40% of the cases) (11-13). There is conflicting evidence on the prognostic significance of extramedullary leukemia in patients with acute myeloid leukemia. In general, they are felt to confer a poorer prognosis, with a poorer response to treatment and lower survival rate; however, others have reported that extramedullary disease in childhood AML does not have independent prognostic significance(11).

Very rarely, chloroma or solid extramedullary tumor can occur without a known pre-existing or concomitant diagnosis; this is known as primary chloroma. In almost all reported cases of primary chloroma, acute leukemia has developed shortly afterward (median time to development of acute leukemia 7 months, range 1-25 months)(14). Therefore, primary chloroma should be considered an initial manifestation of acute leukemia, rather than a localized process, and should be treated as such.

More research on the mechanisms of cancer migration and the role of chemokine/ chemokine receptor interaction in acute leukaemia may offer insights into the development of metastasis and identification of potential therapeutic targets.

2. *Chemokines and their receptors*

2.1. Chemokines and chemokine receptors in cancer metastasis

Chemokines are a superfamily of small cytokine-like proteins that work by binding to specific G-protein-coupled receptors (chemokine receptors) on plasma membranes of target cells. Binding triggers intracellular G proteins to activate a signalling cascade that results in adherence of leukocytes to the endothelium before they extravasate into the tissue. Chemokines, along with adhesion molecules, are crucial during inflammatory responses for a timely recruitment of specific leukocyte subpopulations to sites of tissue damage. Chemokines and their receptors are also important in dendritic cell maturation, B and T cell development, Th1 and Th2 responses, and angiogenesis(15). In addition, they also seem to be involved in the process of tumour cell growth, migration, invasion, and metastasis(16).

During the last 10 years many new chemokines and chemokine receptors have been identified (Table 1). The small (8-10 kDa) chemokine proteins are classified into four highly conserved groups (CXC, CC, C and CX3C), based on the position of the first two cysteines that are adjacent to the amino terminus. More than 50 chemokines have been discovered so far and there are at least 19 seven-transmembrane-domain chemokine receptors(16).

Although metastases to organ sites such as lymph nodes, lung and liver are to a certain extent due to specific anatomic pathways such as lymphatic or vascular channels that mechanically direct tumour cell migration, it has been known since the early 1900s that some tumour cells have a propensity to metastasize to specific organs(17) and that they do not migrate randomly. In the last few years, the involvement of chemokines and their receptors in cancer, particularly metastasis, has been firmly established(18). The role of chemokines and their receptors in cancer include (i) providing directional clues for migration, (ii) shaping the tumour environment and (iii) providing survival and/or growth signals(16).

Table 1. Summary of chemokine receptors and some of their known ligands

Chemokine receptors*	Ligands*	Old nomenclature(15)
CXCR1	CXCL6, CXCL8	GCP-2, IL-8
CXCR2	CXCL1-3; CXCL5-8	Groα/Groβ/Groγ; ENA-78/GCP-2/NAP-2/ IL-8
CXCR3	CXCL9-11	Mig/IP-10/I-TAC
CXCR4	CXCL12	SDF-1
CXCR5	CXCL13	BLC
CXCR6	CXCL16	HCC-4
CXCR7	CXCL12	SDF-1
CCR1	CCL3-5; CCL7, CCL8, CCL13-16; CCL23	Groγ/PF4/ENA-78; NAP-2, IL-8, BLC/ Leukotactin/HCC-4; MPIF-1
CCR2	CCL2, CCL7, CCL8, CCL13	MCP-1, MCP-3, MCP-2, MCP-4
CCR3	CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL24, CCL26	RANTES, MCP-3, MCP-2, Eotaxin, MCP-4, Leukotactin, MPIF-2, SCYA26
CCR4	CCL17, CCL22	TARC, MDC
CCR5	CCL3, CCL4, CCL5. CCL8, CCL14	MIP-1α, MIP-1β, RANTES, MCP-2, HCC-1
CCR6	CCL20	MIP3α
CCR7	CCL19, CCL21	MIP3β
CCR8	CCL1, CCL4, CCL17	I-309, MIP-1β, TARC
CCR9	CCL25	TECK
CCR10	CCL26-28	SCYA26/CTACK/MEC
XCR1	XCL1-2	Lymphotactin/SCM-1β
CXCR3	CX3CL1	Fractalkine

*CXC, CC, C and CX3C reflect the four families of chemokine receptors based on the pattern of cysteine residues in the ligands. R (receptor) or L (ligand) is added to these letters accordingly.

When Müller et al. highlighted the role for chemokines in directing organ-specific metastasis, it became clear that chemokine receptor expression patterns on cancer cells and the localization of the corresponding ligands could provide clues for understanding directional metastasis(19). In many types of cancer the malignant cells were shown to exhibit an increased or aberrant expression of particular chemokine receptors relative to their normal counterparts(20), resulting in migration of the tumour cells by mechanisms similar to normal lymphocyte trafficking (Table 2).

Various reasons for altered chemokine receptor expression have been identified. The tumour microenvironment, mutant proteins or altered signalling in the cancer cell itself can affect

Table 2. Some of the chemokine receptors that are expressed on cancer cells(16;22)

Chemokine receptor	Cancer cell expression	Normal-cell expression
CXCR4	23 different haematopoietic and solid cancers	Haematopoietic stem cells, thymocytes, T cells, B cells, immature and mature dendritic cells, some endothelial cells, macrophages and neutrophils
CCR5	Breast cancer cell lines	Thymocytes, B lymphocytes, immature and mature dendritic cells, and macrophages
CCR7	Breast cancer, CLL, gastric cancer, non-small-cell lung and oesophageal cancer	B cells, T cells and mature dendritic cells
CCR10	Melanoma	Plasma cells and skin-homing T cells
CXCR2	Melanoma	Macrophages, eosinophils and neutrophils

chemokine receptor levels(16). For example, hypoxia induces up-regulation of CXCR4 transcription(21). Secondly, chemokines and their receptors play roles in shaping the tumour microenvironment by their ability to attract leukocytes, which promote angiogenesis and activate other enzymes and cytokines. Thirdly, expression of chemokine receptors on cancer cells can provide the cells with more than a mechanism for migration. Receptor signalling may also provide a survival advantage. It has been demonstrated that CXCL12 [formerly known as SDF-1α (stromal-cell-derived factor-1α)]

activates the specific signalling pathways that promote survival effects(23). These pro-tumourigenic pathways are likely to be particularly important for the ability of metastatic tumour cells to thrive in a foreign environment. Furthermore, chemokines that serve as migratory signals to control homing of lymphocytes to protective niches in the bone marrow and lymph nodes may also promote survival of leukaemic cells.

2.2. Chemokines and their receptors in acute leukaemia

To date most studies, aimed at the involvement of chemokines and their receptors in the migration and survival of leukaemic cells in humans, have concentrated on the interaction of CXCL12 and CXCR4(24). CXCL12 is a “homeostatic” chemokine that is constitutively secreted by marrow stromal cells. It signals through CXCR4, a receptor, which is broadly expressed by normal and malignant cells of haematopoietic and non-haematopoietic lineages. The CXCR4-CXCL12 axis is particularly important in the marrow microenvironment. Rapid

mobilization of haematopoietic stem cells by CXCR4 antagonists (AMD3100), as shown in recent clinical trials, supports the hypothesis that regulation of the homing and retention of haematopoietic progenitor cells in the distinct stromal niches of the marrow is one of its essential functions(25). It was generally thought that CXCL12 mediates its effects solely through CXCR4. However, recent studies characterized a novel receptor for CXCL12, termed CXCR7(26;27). CXCR7 is expressed on malignant cell types and foetal liver cells, but on few other cell types(27) Furthermore, it is highly expressed on tumour-associated blood vessels, but not on normal vasculature(28). Unlike many other chemokine receptors, ligand activation of CXCR7 does not cause Ca2+-mobilization or cell migration(27;29). However, interaction of CXCL12/SDF-1 with CXCR7 results in a proliferative effect, in contrast to CXCR4 which mediates chemotaxis. Further, CXCR7 has been attributed a potential role in tumour development because its expression provides cells with a growth and survival advantage and increased adhesion properties.

The CXCL12-CXCR4 interaction has been shown to be functional in ALL cells and important for marrow homing of ALL cells(24). Crazzolara et al reported that a higher expression level of CXCR4 on ALL cells, as determined by flow cytometry, correlated with more organ infiltration at diagnosis that was independent from the peripheral lymphoblast count(30). Schneider et al. detected a high expression of CXCR4 on ALL blasts of children with a high white blood cell count and organ infiltration at diagnosis. Higher levels of CXCR4 expression on blast cells at diagnosis did not constitute a prognostic factor of extramedullary relapse, but appeared to confer a poorer outcome in this small study(31).

Also, CCR5 was recently studied in relationship to acute lymphoblastic leukaemia. A polymorphism in the CCR5 genotype was positively correlated with the clearance of minimal residual disease, indicating that CCR5 may play a role in the early response to chemotherapy(32).

Also on myeloid cells, the CXCL12-CXCR4 interaction provides a strong chemotactic signal. Apart from this chemotactic activity, CXCL12 has also been suggested to suppress apoptosis and promote G0/G1 transition in myeloid cells. Furthermore, some evidence suggests that CXCL12 induces an arrest in myeloid differentiation. This mechanism provides a potential

way of leukaemia cell evasion from chemotherapy(33). This hypothesis is supported by the impact of high CXCR4 expression on AML cells, determined by flow cytometry, as an adverse prognostic indicator in AML, independent from other prognostic factors, particularly cytogenetics and white blood cell count(33;34). In addition, polymorphisms in the CXCL12 coding gene is associated with higher counts of circulating AML cells and a higher frequency of extramedullary disease(35).

Given the crucial roles of chemokines and their receptors in a wide array of physiological functions and their association with many malignant diseases, the receptors have become important targets for risk stratification and cancer treatment. Confirmation of the prognostic value of CXCR4 expression and investigation of the expression of other chemokine receptors in relationship to outcome in childhood leukaemia is needed and may lead to new risk stratification strategies. Moreover, to successfully develop therapeutic strategies for modulating chemokine action, we need to gain a detailed understanding of the mechanisms of chemotaxis mediated by chemokines and their receptors in acute leukaemia.

3. Treatment of acute leukaemia in children

3.1. Treatment of ALL in childhood

Childhood leukaemias are among the most drug-responsive of human malignancies. More than 70% of children with ALL can now be cured, largely by modern combinations of chemotherapeutic drugs, administered according to international protocols(36). For all ALL, except mature B-cell ALL, treatment includes remission induction (RI) followed by consolidation therapy to eliminate residual leukaemia, eradication or prevention of CNS leukaemia, and maintenance treatment to ensure continuation of remission. In most studies, patients are stratified into risk groups, based on cytogenetic abnormalities and response to RI treatment, although there is no consensus on the most valid criteria. However, measurement of minimal residual disease (MRD) by leukaemia-specific PCR-based quantitative techniques has emerged as a very powerful post-RI prognostic factor.

The goal of RI-therapy is to induce a complete remission by eradicating over 99% of the initial leukaemic cell population, and by restoring normal haematopoiesis. With modern

chemotherapy and supportive care, 97% to 99% of children can reach a complete remission (i.e. ≤5% blasts in the bone marrow on cytology). Patients who fail to achieve a cytological remission at the end of remission induction have a poor prognosis. These patients, as well as patients with more than 1% blasts identified by MRD studies as applied in some centres, should be candidates for allogeneic stem cell transplantation(1).

With restoration of normal hematopoiesis, patients in remission become candidates for intensification (consolidation) therapy. The most successful post-RI intensification regimens are generally administered continuously, whereas high-dose pulse therapy with long rest periods due to myelosuppression appears to be less effective. For reasons that are poorly understood, children with ALL require long-term continuation treatment. Attempts to shorten the duration of treatment have resulted in a high risk of relapse after cessation of therapy(37).

Response to treatment at relapse is much inferior to that in newly diagnosed ALL, because of drug resistance already present in subclones at diagnosis or acquired after exposure to antileukaemic drugs. Well-established prognostic factors in relapsed ALL are time from reaching complete remission to relapse, site of relapse, immunophenotype and specific cytogenetic translocations. In most children with relapsed ALL, second remissions can be induced. However, in many patients, chemotherapy is not sufficient to maintain this remission. Stem cell transplantation has therefore been used as intensive post remission treatment.

3.2. Treatment of AML in childhood

Although the outcome of treatment of children with AML has improved markedly over the last three decades(38;39), the cure rate continues to lag behind that of children with ALL.

Although for several years uncontrolled proliferation was considered the distinguishing property of any malignant disease, recent studies have shown that AML is initiated and sustained by a small, self-renewing population of leukemic stem cells (LSCs), which produce progeny consisting of a heterogeneous population of progenitor cells; the vast majority of the LSCs is in a quiescent state and, thus, is insensitive to the effects of most chemotherapeutic

agents. This latter feature explains, at least in part, the difficulties in eradicating such a cell population by conventional chemotherapy(41;42).

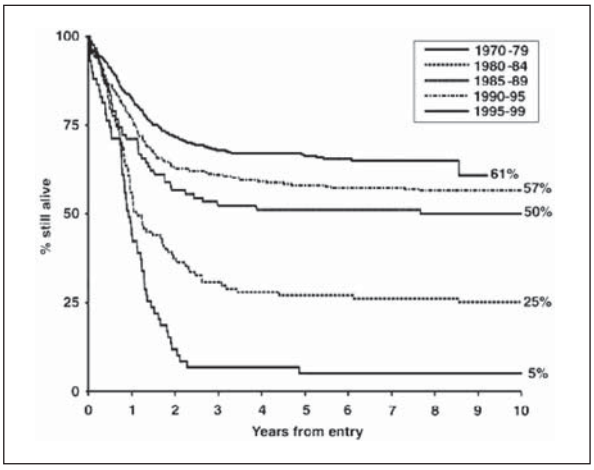

Figure 2. Overall survival of children younger than 15 years of age who had acute myeloid leukemia treated in MRC trials during the past 3 decades(40); with permission from Nature Publishing Group.

Clinical trials of AML feature intensive chemotherapy with or without subsequent stem cell transplantation(43). Therapy consists of a limited number of intensive courses of chemotherapy. Increasing the intensity of the remission induction has not significantly improved remission rates, but the induction "intensity" may well improve ultimate outcome(44). In contrast to ALL treatment, most cooperative study groups have abandoned the use of maintenance therapy in AML(1). Allogeneic stem cell transplantation is a feasible and effective alternative to chemotherapy as post-remission therapy for AML. Many studies have demonstrated that relapse-free survival probabilities for patients who have AML and a suitable family donor, irrespective if they undergo matched sibling donor (MSD-)SCT or not are better than those of patients who receive only intensive chemotherapy(44). However, others have shown no such advantage, primarily because of transplantation related mortality (38). Although MSD-SCT is often recommended, considerable controversy remains as to which patients in first remission should undergo this procedure. Currently, most European investigators agree that intermediate-risk and high-risk patients (based on cytogenetic abnormalities and response to

treatment) should undergo MSD-SCT during first remission of AML but do not recommend SCT for low-risk patients(45).

4. Haematopoietic stem cell transplantation

Over the last three decades, haematopoietic stem cell transplantation (HSCT) has become an important treatment modality for a wide range of life-threatening haematological and immunological disorders in both children and adults, with over 40,000 transplants worldwide each year. With increased understanding of histocompatibility and development of novel immunosuppressive drugs, HSCT has become a therapeutic option for an increasing number of patients with otherwise incurable leukaemias. Other indications include certain immune deficiencies, hemoglobinopathies and inborn errors of metabolism. On the following pages some background information will be given on a few basic aspects of allogeneic stem cell transplantations, which are relevant to this thesis.

4.1. Conditioning regimen and eradication of leukaemia

The chemotherapy or irradiation given immediately prior to a transplant is called the conditioning or preparative regimen, the purpose of which is to eradicate the residual leukaemic cells and provide adequate immunosuppression needed for successful engraftment of allogeneic haematopoietic stem cells. The most commonly used preparative regimens prior to allogeneic HSCT for leukaemia include various doses of total-body irradiation (TBI) and cyclophosphamide, without or with additional chemotherapeutical agents(46). Non-TBI-based regimens usually include busulphan and cyclophosphamide. Sometimes other chemotherapeutic agents are added for extra anti-leukaemic effect such as cytosine-arabinoside or etoposide.

TBI is also one of the most important causes of late effects after HSCT in children, such as interstitial pneumonitis and pulmonary fibrosis, cataract, stunted growth, endocrine dysfunction, and secondary tumours(47-49). In the paediatric population, besides the risk of diminished neuropsychological functioning, the damage of irradiation to growth plates and gonads has important effects on growth and gonadal development. For that reason an age-

dependent total dose is applied in the paediatric SCT centre of the LUMC(50).
In an attempt to reduce the effects of radiation-induced toxicity, most centres have replaced single fraction TBI by fractionated TBI. Fractionation of the total radiation dose allows higher total doses without increasing the damage to normal tissue. Some haematological malignancies, however, are not very radiosensitive, as was demonstrated by Cosset et al. (51), resulting in reduced tumour kill if fractionation is applied.

4.2. Graft-versus-Host Disease and Graft-versus-Leukaemia effect

Since the start of allogeneic HSCT prevention and management of acute Graft-versus-Host Disease (GVHD) has been an important challenge. GVHD is a complex immunological reaction of immunocompetent cells in the graft against tissues of the host. The incidence of this complication increases with increasing HLA mismatches between donor and recipient. Acute GVHD, per definition starting within 50 days after HSCT, is mostly first visible in the skin. Other major target organs are the liver and gastrointestinal tract, but other tissues can also be involved. A grading system based on signs and symptoms for acute GVHD was originally proposed by Glucksberg et al(52). To prevent acute GVHD, originally methotrexate (MTX) was used, later on cyclosporine A (CsA), a calcineurin inhibitor with an immune-modulating effect on donor T cells, was introduced(53), and the combination was clearly superior than single drugs in the prevention of GVHD after SCT (54). Nowadays, in case the graft is not depleted of T cells, a short course of MTX in combination with daily administration of CsA for several months is usual.
At the same time, HSCT provides an anti-leukaemic effect caused by a reaction of donor immune cells against residual leukaemic cells in the recipient. This effect is called the Graft-versus-Leukaemia (GVL) effect(55). Its effect differs for different types of leukaemia, and is most pronounced in chronic leukaemia. Unfortunately, GVL is mostly associated with GVHD. Measures to reduce or prevent GVHD, e.g. T-cell depletion of the graft, were associated with a higher relapse rate(56).
A lot of research is directed towards the development of strategies to exploit the GVL effect without increasing Graft-versus-Host Disease-related mortality. Optimizing the GVL effect

has been demonstrated by using a reduced dose of cyclosporine A, which was associated with a significant reduction in the leukaemia relapse rate in an prospective randomized trial in children with acute leukaemia (57). The intensity of post-transplant immunosuppression may well affect the GVL effect and, therefore, appears to be an important determinant for a potential relapse of leukaemia after HSCT. An alternative strategy to augment an anti-leukaemia immune response is the infusion of lymphocytes from the original stem cell donor (DLI) after the transplant.

5. Pharmacokinetics of HSCT-related drugs in children

5.1. Paediatric drug research

Most drugs that are currently prescribed for children have not been tested in children. Various approaches to determine paediatric drug dosing are being used. Many of these apply allometric principles (i.e., based on relative body size), that generally assume that there are predictable, linear relations between body weight and body-surface area among infants, children, adolescents and adults(58). However, since growth is not a linear process and the body composition changes through childhood, these simplified dosing strategies are not adequate to reach optimal treatment results or to achieve appropriate systemic exposure to drugs in case of a narrow therapeutic window and/or high interpatient variability. The testing of medications in children, however, presents a dilemma. Nonetheless, children may be harmed if they are given medications that have been inadequately studied. Therefore, future paediatric patients' clinical studies will be needed for a better understanding of the pharmacokinetics of drugs used in infants and children in order to optimize dosing and increase safety for children taking these drugs. There are several HSCT-related drugs, that need pharmacologic research when applied in children, e.g. busulfan, already investigated by us and others in the past(59-61) and CsA, only scarcely studied in the paediatric graft recipients(62).

5.2. Pharmacokinetic analysis

Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion of

drugs. Interpatient variability characterizes the disposition of many drugs. In drugs with a narrow therapeutic window, such variability is likely to affect either clinical efficacy and/or toxicity. Clinical pharmacology studies often need a series of measurements (e.g. serum concentration) in subjects at consecutive time points. Subsequently, pharmacokinetic (PK) analysis is performed without specific models (e.g. non-compartment models) or by estimating the parameters of mathematical models that describe that particular profile (e.g. compartmental methods)(63). Non-compartmental methods estimate the exposure to a drug by estimating the area under the curve (AUC) of a concentration-time graph (Figure 2).

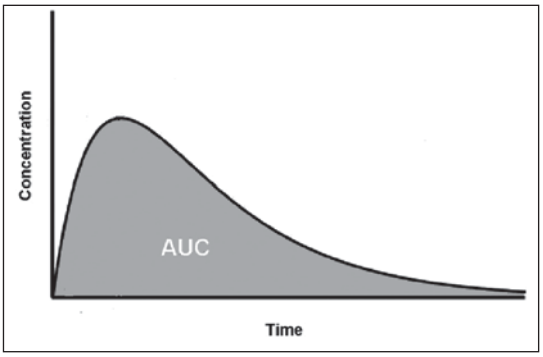

Figure 2. Example of a concentration-time graph.

The total drug exposure can be estimated with the trapezoid rule. This area estimation is highly dependent on the sampling schedule; i.e. the closer the sampling points are, the closer the trapezoids are to the actual shape of the concentration-time curve. This approach may be satisfactory if data are extensive for each subject and if there is only minor between-subject variability. In contrast, compartmental PK analysis uses kinetic models, similar to models used in other scientific disciplines such as chemical kinetics and thermodynamics, to describe and predict the concentration-time curve. Especially in the pediatric setting, compartmental population PK modeling offers several advantages. Rather than obtaining rich data from a selection of individuals, sparse data from many individuals can be analyzed. This approach generally can use data from the children being given the drug therapeutically. Furthermore, the model is flexible, does not depend on exact blood sampling time-points

and enables interpretation of sets with missing data. It is therefore useful in clinical practice. Consequently, a Bayesian maximum a posteriori fitting uses individual patient covariables and the information obtained from a prior population analysis, to estimate individual PK parameters(64).

Thus, population PK studies can describe variabilities in response and/or concentration, determine the dose that achieves a target concentration, derive maximum a posteriori Bayesian individual predictions during therapeutic drug monitoring and predict the best sampling protocols for future studies(63). Also in the setting of HSCT, children have benefitted and will continue to benefit from this approach.

5.3. Pharmacokinetics of cyclosporine A

CsA is a highly lipophilic drug whose pharmacokinetics are characterized by slow, incomplete and variable absorption, large volume of distribution, liver biotransformation via the 3A4 isoenzyme of cytochrome P450 (CYP) and low clearance (Cl)(65). Despite the extensive use of CsA for HSCT in adults and children, only limited information is available about its pharmacokinetics. The narrow therapeutic index of CsA and its high intra- and interindividual pharmacokinetic variability have made therapeutic drug monitoring essential(66). To prevent drug toxicity, trough level monitoring usually is applied. Systemic exposure, however, is poorly reflected by this measurement alone(67). Furthermore, in solid organ transplantation, systemic exposure of CsA, as measured by the area under the curve (AUC), has been shown to correlate well with clinical outcome(68;69). It has not been established yet how systemic exposure, as reflected by the AUC, correlates with the clinical effects of CsA following allogeneic SCT.

6. Chimerism after stem cell transplantation

In zoology, a chimera is an animal that has two or more different populations of genetically distinct cells that originated in different zygotes. In medicine, this condition is either natural (inherited or acquired through pregnancy), or artificial, resulting from a medical intervention such as an allogeneic HSCT(70).

Natural chimerism includes chimerism of blood cells caused by an exchange of hematopoietic stem cells via communications between the two fetal blood systems or resulting from passage of cells between maternal and fetal circulation. Fetal progenitor cells can survive for many years in maternal blood and vice versa. Based on certain similarities between GVHD and auto-immune diseases, microchimerism is considered to be a potential trigger for certain types of auto-immune diseases, however, the mechanisms responsible for this relationship remain unclear(71).

Allogeneic HSCT leads to complete, and sometimes incomplete replacement of the haematopoietic cell lineages(72). Chimerism of distinct haematopoietic cell lineages after HSCT is a critical observation because it provides information about the status of the graft. Accumulating evidence suggests that bone marrow-derived progenitor cells are capable of differentiating into other cells than strictly haematopoietic cells, e.g. endothelial cells(73-75). In recipients of allogeneic hematopoietic stem cells, donor-derived endothelial cells were detected in the skin and gut after SCT(76). Bone marrow-derived cells can also take on the phenotype of epithelial cells in multiple organs(77). However, these findings have generated controversy. One of the main concerns is the technical challenge to prove a cell is (i) donor-derived, (ii) expresses epithelial markers and (iii) to rule out overlay of cells. Furthermore, it is not clear which subpopulation of cells present in the graft is capable of engrafting as epithelial cells. Also little is known regarding the mechanism, kinetics, and potential clinical utility or pathology associated with this phenomenon(78). However, to successfully develop strategies for using of bone marrow-derived stem cells for therapeutic purposes e.g. regenerative medicine and drug delivery, we need to gain a detailed understanding of the exact subpopulation of the bone marrow precursor cells involved, the cell type into which they have ability to evolve and by which mechanism this occurs first.

1 Outline of this thesis

To gain a better understanding of the role that chemokine receptors play in the migration and survival of leukaemic blasts in paediatric ALL and AML, we investigated the pattern of chemokine receptor expression on these cells. In chapter 2, we describe the chemokine receptor expression on the leukaemic blast cells of 11 paediatric T-ALL patients at diagnosis in relationship to homing to the gut. Chapter 3 describes the chemokine receptor expression on AML blasts in children with and without spread of the leukaemic blasts to the skin, as well as the expression of the specific ligands at the site of migration.

To analyze prognostic factors for relapse after SCT, we evaluated, retrospectively, the outcome of 132 children with acute leukaemia that were treated with HLA-identical HSCT in the paediatric SCT centre of the Leiden University Medical Centre (LUMC) in chapter 4.

With the aim of improving therapeutic drug monitoring of Cyclosporine A (CsA) in children after SCT, we described in chapter 5 a pharmacokinetic model and developed a limited sampling strategy in order to determine the AUC of CsA. With this pharmacokinetic model, we retrospectively estimated the area-under-the-curve (AUC) of CsA in children transplanted for a haematological malignancy. Chapter 6 evaluates CsA systemic exposure (~AUC) during the early post HSCT period on clinical outcome i.e. occurrence of acute GVHD and relapse of the haematological malignancy after HSCT.

Although, the objective of allogeneic stem cell transplantation is to graft hematopoietic precursor cells in patients, recent evidence indicates that bone marrow-derived cells may have potential to fuse with or differentiate into endothelial and epithelial cells. The potential future therapeutic applications of these findings call for a better understanding of the mechanisms of chimerism induction and its context in the HSCT-setting. Therefore, in chapter 7, we investigated the appearance of donor-derived endothelial and epithelial cells after HSCT in relation to conditioning regimen, time course after SCT and occurrence of GVHD.

Finally, the results and implications of the different studies are discussed and summarized in chapter 8, followed by a summary in Dutch in **chapter 9**.

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