Acute leukaemia in children : aspects of diagnosis and treatment

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Summary and discussion

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1. Summary

This thesis describes clinical, cytological, immunological and pharmacological aspects of acute childhood leukaemia and allogeneic stem cell transplantation, with the emphasis on the analysis of potential improvements in risk stratification and possible treatment adaptation, in order to decrease relapse frequency and disease-related death.

Chapter 1 gives a general introduction to the possible involvement of chemokines and chemokine receptors in the migration and survival of malignant cells, and the modern treatment options of acute lymphoblastic and myeloblastic leukaemia in children, including the role of allogeneic stem cell transplantation. In addition, some background information on the basic aspects of the conditioning regimen, GVHD prophylaxis and pharmacokinetic research in children is given. Finally, the variability of chimerism following stem cell transplantation and its possible consequences for future interventions is mentioned.

Childhood acute lymphoblastic leukaemia (ALL) is often associated with extramedullary infiltration by leukaemic cells at diagnosis or at relapse. To understand the mechanisms behind the dissemination of T-ALL cells we investigated the homing receptor expression on the blast cells of 11 paediatric T-ALL patients at diagnosis **(Chapter 2)**. One patient revealed a unique profile with high expression of the chemokine receptor CCR9 and the integrin CD103 on the T-ALL cells. Both these molecules are specifically associated with homing to the gut. This finding was clinically significant as the patient later suffered a relapse which was confined to the gut. Immunohistochemistry revealed that the leukaemic cells in the gut still expressed CCR9 and co-localized with a high expression of the CCR9 ligand, CCL25. These findings suggest that the original expression of CCR9 and CD103 on the leukaemic cells contributed to the relapse location in the gut of this patient.

In order to study the role of chemokine receptor/ligand interactions in the context of extramedullary AML, we analysed skin histology, peripheral blood and bone marrow of 18 paediatric patients with skin involvement at diagnosis in **chapter 3**. Fifteen additional AML patients without skin involvement were used as controls. Flow cytometric analyses

on circulating AML cells showed significantly higher expression of CCR2 in patients with extramedullary leukaemia as compared to patients without skin involvement. Immunohistochemical staining of affected skin biopsies revealed expression of CCL3 and CXCL12; CCL2, one of the ligands of CCR2 could, however, not be detected. Skin-infiltrating tumour cells expressed CX3CR1, CCR5, CXCR4 and CXCR7. CXCR7 was shown to be important for blast cell survival. Our data suggest that CCR2 expression on circulating AML cells mediates migration to the skin in response to an as yet unidentified chemokine. While CCR5/CCL3 and CXCR4/CXCL12 interactions subsequently facilitate the retention of these cells in the skin, we hypothesise that CXCR7/CXCL12 signalling may provide an additional signal for cell survival. Understanding the homing and survival patterns of AML cells in extramedullary sites may yield new perspectives for improved treatment of AML patients.

Chapter 4 addresses the relapse frequency, transplant-related complications and survival in relationship to disease- and pre-treatment-related characteristics in a retrospective evaluation of the treatment results of 132 children, who consecutively received an allogeneic HLA-identical SCT for acute leukaemia in our centre. The 5-year probability of overall survival was 63%, 53% and 74% for ALL1, ALL2 and AML1, respectively (median follow-up 10.6 yrs). The overall transplant-related mortality was 6%. The incidence of acute GVHD was 17%; 6% was grade II-IV. A higher total biologically effective TBI dose (BED) resulted in a decreased relapse frequency and increased overall survival. AML patients with acute GVHD got no relapse; this was not the case in ALL patients.

Cyclosporine A (CsA) is commonly used as GVHD prophylaxis after HSCT. To prevent drug toxicity, trough level monitoring usually is applied. Systemic exposure, however, is poorly reflected by this measurement alone and the usefulness of dose adjustments based on CsA trough levels continues to be controversial. In order to optimize the use of CsA for adequate GVHD prophylaxis and to avoid drug toxicity, we investigated the pharmacokinetics of CsA in children after SCT. In **chapter 5**, we decribe a limited sampling strategy to determine AUC and to optimize therapeutic drug monitoring. Large interindividual variability in CsA clearance was found, which was independent of body weight; this finding justifies a dosing strategy distinct from that per kg body weight. This was a first step to investigate the

relationship between AUC and the clinical effects of CsA.

In solid organ transplantation, area-under-the-blood-concentration-versus-time-curve (AUC) correlates with clinical outcome. However, in HSCT it has not been determined whether the AUC is superior to trough level monitoring to optimize clinical efficacy of CsA therapy. Therefore, the aim of the study, described in chapter 6, was to identify the relationships between CsA trough levels and/or AUC early after HSCT, with clinical outcome. We included 91 children (1.1 to 17.3 years), treated consecutively with a HSCT for a haematological malignancy, receiving CsA and methotrexate for GVHD prophylaxis. CsA trough levels were obtained retrospectively and were used to estimate the AUC with nonlinear mixed effect modelling (NONMEM). Subsequently, these exposure parameters were correlated to occurrence of acute GVHD, relapse risk and overall survival. Low CsA trough levels were found to correlate with the occurrence of acute GVHD. In addition, AML patients with a CsA AUC over 3000 mcg*h/L had a significantly reduced overall survival, as well as a higher relapse risk compared to patients with lower CsA exposure. This was not the case for ALL patients. Thus, monitoring CsA exposure early after HSCT and adjusting the CsA dosing if needed may provide a tool to influence the GVHD/GVL balance.

To gain a better understanding of the mechanism of chimerism induction of endothelial and epithelial cells following allogeneic stem cell transplantation, the occurrence of endothelial and epithelial cell chimerism in relation to the conditioning regimen, time interval after SCT and development of acute and chronic Graft-versus-Host Disease (GVHD) was studied and decribed in **chapter 7**. Fifty-five skin biopsy samples from 35 female patients transplanted with a male donor were screened for donor-derived endothelial and epithelial cells using in-situ hybridisation for the Y chromosome in combination with immunohistochemical cell marking techniques. Endothelial cell chimerism was found in 25% of the biopsies and increased in time after SCT. Its appearance was increased in patients with acute GVHD more than two weeks prior to biopsy. Also, in most biopsies taken after or during chronic GVHD endothelial cells of donor origin were found. Epithelial cell chimerism was found in 85% of the biopsies. Appearance of epithelial cell chimerism was not correlated with the time interval after SCT nor with tissue damage due to GVHD.

2. *Discussion*

2.1. Chemokines and chemokine receptors in acute childhood leukaemia

The properties of chemokines and their receptors in controlling cell migration have made them clear candidates for involvement in cancer cell migration. However, the contribution of chemokines to other aspects of cancer, such as growth, proliferation, angiogenesis and cell survival, have also become areas of extensive investigation(1). The process by which leukaemic cells grow and migrate to extramedullary sites is complex. Firstly, leukaemic cells need to survive and grow in the primary site, mostly the bone marrow stroma. Subsequently, homing and adherence to an extramedullary site is required. Finally the leukaemic cells must survive and grow at the site of migration. The employed strategies often result from redirecting existing physiological migration and proliferation pathways, such as abnormal chemokine receptor expression, regulation or utilization.

In adult AML patients, high expression of surface CXCR4 on leukaemic blasts at diagnosis is strongly associated with poor prognosis(2). Although patients with high CXCR4 expression also had higher WBC counts at diagnosis, when considering CXCR4 expression as a risk factor for relapse, the presence of high WBC count at diagnosis lost its impact for risk of relapse. Because of this powerful prognostic value, CXCR4 expression may have an important role in the biology of this disease. It is hypothesized that the CXCL12-CXCR4 interaction in the protective stromal microenvironment, makes AML cells less susceptible to cytotoxic drugs. Confirmation of this prognostic value, also in childhood AML, may lead to new risk stratification strategies in AML.

The expression of CCR9 on T-ALL blasts of a patient who later experienced a relapse which was confined to the gut provides further evidence for the link between the expression of homing molecules and extramedullary organ infiltration by leukaemic cells(3). CCR2 expression on circulating AML cells seemed to mediate migration to the skin in response to an as yet unidentified chemokine (this thesis). These observations show that the analysis of the chemokine receptor repertoire on leukaemic blasts at diagnosis may identify a subset of patients with a high chance of extramedullary disease and a sustained growth of leukaemic blasts, leading to a worse prognosis.

In addition to the leukaemic cell-adhesion to the protective bone marrow environment, and the chemokine-directed migration of leukaemic blasts to extramedullary sites, the signalling between chemokine receptor and chemokine may provide a survival advantage, as is probably the case for the CXCR7-CXCL12 interaction in skin infiltrating AML blast cells. Since both chemokine receptor, as well as ligand, was highly expressed, and survival-assays showed that the CXCR7/CXCL12 pathway was important for the survival of AML blast cells, we hypothesise that CXCR7/CXCL12 signalling may provide an additional signal for tumour cell survival.

In conclusion, elucidation the signalling pathways activated by chemokines in acute leukaemia will be important to understand how chemokines influence disease progression; it also may reveal potential downstream targets for therapeutic intervention.

2.2. TBI and biologically effective dose

In the late fifties, total body irradiation (TBI) was introduced in the preparative regimen before allogeneic stem cell transplantation(4). In the seventies, the concept of fractionation of TBI, to obtain a similar antileukaemic effect, together with a better sparing of normal tissues, was developed(5). Fractionated TBI schemes were adopted in the majority of centres throughout the world. Even though some investigators question the way TBI works in the conditioning of patients before SCT(6), most agree that TBI is able to achieve significant leukaemia cell kill, which, together with other mechanisms, can lead to eradication of (residual) leukaemia. Despite the parallel use of a large choice of TBI schedules, none has proven to be superior. An essential issue in any analysis of TBI results is the “radiotherapeutical normalization”, because fractionation of TBI can lead to reduced leukaemia cell kill. To reduce the negative effects of fractionation on tumour cell kill and immunosuppression, it is important to calculate the biologically effective dose (BED) for each TBI schedule to ensure optimal leukaemia cell kill(7).

We found that a higher total dose of BED-TBI was associated with a significantly lower risk of relapse and a higher overall survival(8). In the young children, however, a possible decreased relapse rate, following higher BED-TBI will need to be weighed against the

increased susceptibility for neurodevelopmental and endocrinological adverse effects (9-12) and secondary cancer(13). To overcome these side effects, a fractionated TBI dose with higher biologically effective leukaemia cell kill may give superior suppression of malignant disease while limiting adverse effects on other tissues (5;7;14).

2.3. Cyclosporin A pharmacokinetics

Although there are reasons why children often do not participate in pharmacological trials, including ethical, scientific and commercial consideration, it is considered undesirable to treat children with drugs that have not been studied properly in young individuals. The goal of providing infants and children with safe and effective drug therapy must be kept clearly in mind, while conducting pharmacological trials in children.

Cyclosporin A, together with methotrexate, is the most frequently used drug combination for GVHD prophylaxis(15). Many factors may influence CsA pharmacokinetics, making comparative interpretation with other patient populations difficult(16). In our study, the correlation between the AUC, determined by pharmacokinetic modelling, using only the CsA trough concentration, and the reference AUC was remarkably good, in contrast to what has been reported by others in the setting of SCT(17) and solid organ transplantation(18;19). However, with the inclusion of more time points in the pharmacokinetic modelling, a more accurate estimate of systemic exposure (AUC) of CsA was obtained.

The pharmacological suppression of the allo-reactive immune response has played an important role in reducing the morbidity and mortality of HSCT recipients as a result of GVHD(20). At the same time, it has become clear that the intensity of post transplant immunosuppression is an important determinant of relapse frequency of leukaemic disease(21-23). Our results indicate that, in addition to the CsA dose, a close correlation of AUC with outcome exists and stresses the importance of CsA drug monitoring in SCT recipients to minimize GVHD and maximize GVL effect. The use of limited sampling in combination with population pharmacokinetics to determine accurately the systemic drug exposure (AUC) may augment our understanding of the relationship between CsA dose and adequate immunosuppression and (especially for AML) further improve the outcome following SCT of children with

high risk acute leukaemia. Future trials will need to include a prospective validation of the pharmacokinetic model and monitoring CsA exposure early after HSCT, adjusting the CsA dose on basis of a predefined target AUC.

2.4. Endothelial and epithelial cell chimerism following stem cell transplantation

Recent evidence indicates that bone marrow-derived precursor cells contribute to endothelial and epithelial cell renewal in recipients of an allogeneic stem cell transplantation (SCT) (24-30). Although bone marrow-derived cells can take on the phenotype of epithelial cells in multiple organs, these findings have generated controversy, mainly because of the technical difficulties for detection(29). Furthermore, it is not clear which subpopulation of cells present in the graft is capable of engrafting as epithelial cells. One of the potential mechanisms underlying the transition from bone marrow precursor cell to epithelial cell could be fusion of donor cells with recipient cells. Differentiation from a pluripotent precursor cell of donor origin or transdifferentiation from a hematopoietic precursor cell are other possible mechanisms that need further investigation.

From the results of our study, it seems that donor-derived endothelial cells are involved in maintenance of vascular homeostasis in a time-dependent way. However, injury due to the development of acute GVHD augmented the occurrence of endothelial cell chimerism and therefore, donor-derived endothelial cells most likely have a role in the repair of damaged endothelium as well. In contrast, epithelial cell chimerism follows a more uniform pattern of engraftment, not influenced by tissue damage.

2.5. Future perspectives

Remarkable progress has been made in the treatment of acute leukaemia in children over the past decades(31). However, we may have reached a plateau in the cure rate with conventional chemotherapy. The use of more aggressive therapy will likely reach a point of diminishing returns, where any therapeutic benefits will be outweighed by damage to normal vital tissues(32).

Further advances in the treatment of acute leukaemia in children and young adults will

require a greater understanding of the biology of the disease, improved risk stratification and risk-directed therapies, and the development of alternative treatment approaches, such as new chemotherapeutic agents, targeted therapy, and cellular therapies(33).

Recent technological advances, including DNA microarray technology, have allowed high throughput analysis of gene expression profiles. Progress in the molecular characterization of acute leukaemia will possibly lead to new insights into the underlying biology of different leukaemic subtypes and may lead to new targets for treatment(31).

Moreover, in the context of HSCT outcome will improve by refinement of the minimal residual disease based strategies to identify patients who are at risk for relapse. In addition, ways will have to be found to selectively inhibit GVHD and improve the GVL effect(31). Individualizing therapy, based on pharmacokinetic and pharmacogenetic principles, can further improve the outcome of children, treated for leukaemia. This will certainly be assessed by future studies.

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