Chapter 7

Summary

Acute lymphoblastic leukemia is a disease characterized by an uncontrolled proliferation and maturation arrest of lymphoid progenitor cells in the bone marrow, resulting in an excess of malignant cells. The disease has a peak incidence between the age of 2-5 years, and a low and steady rise from the age of 40. The prognosis in children with ALL is one of the most favorable of all disseminated cancers: more than 95% achieve complete remission and disease-free survival rates are 63-83%. Adults with ALL have a worse prognosis, with a complete remission rate of 75-89% and disease-free survival rates of 28-39%. From the age-dependent incidence of ALL and differences in characteristics and prognosis, it could be concluded that ALL in childhood and adults are distinct diseases. Recent evidence has underscored that the difference in characteristics and biology of adult versus childhood ALL might be the result of a different origin. According to the two-hit paradigm of Knudson to develop cancer, two genetic events are necessary. It has been suggested by Greaves et al., that in childhood ALL the first genetic event happens in the more mature lymphoid committed progenitor cells, whereas in adult ALL the first hit occurs in multipotent stem cells. In light of this hypothesis, it is assumed that the hematopoietic cells in which the transformation takes place, share some characteristics with their normal hematopoietic counterpart. The committed progenitors have a limited self-renewal capacity and are more sensitive to apoptosis induced by chemotherapy in contrast to the multipotent stem cells. Chapter 1 compares various patient characteristics, the extent of the disease, leukemic cell characteristics and treatment between childhood and adult ALL. This is discussed in relation to the hypothesis that the maturation stage of the cells, from which the leukemia arises, is responsible for the differential behavior of adult and childhood ALL.

Differences in intrinsic cellular differences, e.g. the expression of ABC-transporters, may be a result of this difference in origin. Chapter 2 describes the role of the ABC-transporters P-gp and MRP1-2 in ALL in children and adults. The functional activity of P-gp in leukemic blasts is determined in a flow cytometric assay, measuring the accumulation of rhodamine 123 with or without the P-gp inhibitor PSC833. For MRP activity, the carboxyfluorescein accumulation with or without the MRP-inhibitor MK-571 is determined. We have observed that most T-ALL patients with high P-gp activity are adults (89%). P-gp activity in B-lineage ALL is similar in children and adults. When determining the prognostic impact of P-gp activity, lower overall and event-free survival rates are found in patients with high P-gp activity. Therefore, high functional P-gp activity in leukemic blasts may be one of the factors explaining differences in treatment outcome between children and adults.
uncontrolled bone marrow, difference between childhood and adults
The prognosis for childhood ALL is more favorable compared to adults: more than 63% patients are cured. According to the French Society for Pediatric Cancer (SFP), the relapse rate of 75-89% for childhood ALL is similar to the incidence of ALL in adults. Gréaves et al. described that ALL in children might be the more immature progenitors to develop into T-ALL. The future lymphoid lineage development in multipotent hematopoietic cells in the bone marrow is determined by their normal self-renewal characteristics, the transition between childhood and adulthood, and the maturation of the B- or T-lineage.

C-transporters, members of the ABC-transporter family, play a role in multidrug resistance. MRP1-2, one of the ABC-transporters, expressed in our ALL cell line, is the ABC-transporter PSC833. P-gp activity, measuring the accumulation of the ABC-transporter P-gp, is present in childhood ALL. However, the role of P-gp in ALL cells is described in chapter 2. However, P-gp is also present in normal tissues, including the liver and kidney, and affects the blood-brain barrier. In chapter 3 we investigate the role of germline MDR-1 genetic variants in vincristine pharmacokinetics and side-effects in childhood ALL. From 52 out of 70 children, who participated in our previous study on vincristine pharmacokinetics, patient material was available for investigation of the MDR-1 genetic variants. The SNPs C3435T and G2677T are determined using restriction fragment length polymorphism polymerase chain reaction. We observed no association between C3435T or G2677T and vincristine pharmacokinetics. However, the significance is lost after Bonferroni correction for multiple testing. The haplotypes do not affect the additional pharmacokinetic parameters, such as clearance and area under the concentration-time curve, suggesting that the observed effect on elimination half-life is of very limited relevance. Moreover, SNPs in the MDR-1 gene do not identify patients with an increased risk for vincristine-induced constipation.

Overexpression of the Breast Cancer Resistance Protein, first identified in a multidrug resistant human breast cancer cell line, was shown to confer resistance to mitoxantrone, doxorubicin and daunorubicin, which are frequently used in the treatment of ALL. In chapter 4 an overview of the current knowledge of BCRP is given and specifically the role of BCRP in acute leukemia. The role of BCRP in hematopoietic stem cells is demonstrated by a “side population” phenotype in the hematopoietic stem cell compartment. This side population is based on the efflux of fluorescent dyes such as rhodamine 123 and Hoechst 33342. BCRP knock-out mice have an almost complete loss of “side population” cells. The knock-out mice also showed an increased sensitivity to mitoxantrone, implying a physiological role of BCRP in providing protection from cytotoxic substrates. The functional activity of BCRP can be determined by measuring the effect of BCRP inhibitor fumitremorgin C on the accumulation of the BCRP substrate mitoxantrone. Studies so far showed that B-lineage ALL patient samples have a higher BCRP activity compared to T-lineage ALL and AML patient samples. The impact of

with T-ALL. MRP1-2 activity shows a great variability in ALL cells, with quite high activities in children in comparison with adults. The activity of MRP has no prognostic impact on overall survival and event-free survival. However, in this small study population only few patients suffered from a relapse.
BCRP functional activity on clinical outcome needs to be studied in larger groups of patients in order to investigate whether these patients could benefit from the combination of BCRP inhibitors and chemotherapy.

In chapter 5, we investigate BCRP in de novo ALL samples of children and adults. BCRP expression is measured flow cytometrically with the BXP-34 monoclonal antibody. BCRP functional activity is determined flow cytometrically by measuring mitoxantrone accumulation (expressed as Median Fluorescence Intensity (MFI)) in combination with the BCRP inhibitor fumitremorgin C (FTC). We show a higher BCRP expression in B-lineage ALL compared to T-lineage ALL. BCRP functional activity can be detected in B-lineage ALL, whereas in T-lineage ALL this activity is less pronounced. The influence of FTC on mitoxantrone accumulation correlates with BCRP protein expression. In summary, we show that BCRP is expressed higher and functionally more active in B-lineage, than in T-lineage ALL.

The study described in chapter 6 focuses on the role of transporters of the MRP-family in ALL. The mRNA expression of MRP1-6 is determined by real-time quantitative PCR. Since numerous transporters can be functionally active in ALL, the connection between the expression of the six MRP genes is also addressed. In adult ALL a significantly higher expression of MRP1, MRP2 and MRP3 is observed than in childhood ALL. Interestingly, the expression of the MRPs is comparable in children and adults who eventually relapsed. High expression of one MRP in individual patients is correlated with increased expression of multiple MRPs. The ability to discriminate between patients who will remain in continuous complete remission and patients who will relapse was determined by receiver operating characteristic (ROC) curves. For children and adults, the area under the ROC curves show that MRP1, MRP2, MRP3, MRP5 and MRP6 predict relapse. Using median values as cutoffs for high versus low expression, high expression of all MRP genes, except MRP4, is significantly associated with reduced event-free survival in children and adults. In summary, we show that a subset of patients with high mRNA expression of multiple MRPs has an unfavorable prognosis independently of age. Early recognition of a profile with high MRP expression could identify patients with an increased risk for relapse that could benefit from treatment adaptations based on this knowledge.

General discussion and future perspectives

This thesis describes the role of ABC-transporters in ALL in children and adults. The worse survival rates of adult ALL patients compared to children and the differences in incidence of ALL depending on age has led to the hypothesis of Greaves\textsuperscript{1-2}. He suggested a different pathogenesis of ALL at a different time in life. A role for ALL in childhood could be the formation of TEL-ALL. However, this is not a certain role. To further study the role of subpopulations of BCRP and MRP, TEL-ALL has been found to contain at least one more CD4+ T lymphocyte source of ALL in childhood. The origin of this cell remains unknown. To further study the role of BCRP and MRP in lymphoid malignancies, we have developed a cell line model of BCRP and MRP in a non-lymphoid cell line. By demonstrating the functional expression of BCRP and MRP in non-lymphoid cells, we have been able to discriminate between MRP expression in childhood and adult ALL and non-lymphoid cells. These results suggest that the expression of MRP can be related to the expression of immunologically related pathways. Also, we have demonstrated that the subpopulation of BCRP and MRP in children is similar to that of BCRP in adults. More recently, we have studied the expression of MRP and BCRP in patients that were not found to have a subpopulation of BCRP and MRP in childhood.