Summary

Although the prognosis of pediatric AML patients has improved significantly over the last 30 years, 30-40% of patients relapse and 5-10% of patients die of the toxicity of treatment. Therefore, novel treatment approaches are needed. In the first part of this thesis (chapter 2-5), we described the poor prognosis of relapsed pediatric AML and we identified type I mutations in pediatric AML which might be used as targets for treatment. In the second part of this thesis (chapter 6-8), we examined the in vitro effects of targeted therapeutics and related sensitivity to these drugs to the expression and the presence of mutations of the specific targets.

In chapter 2 we studied the clinical outcome of all pediatric AML patients initially diagnosed between 1980 and 1998 who subsequently relapsed (N=113). Most patients (63%) relapsed within one year after reaching first complete remission (CR1). In 80% of patients reinduction therapy was given with curative intent. No uniform treatment protocol was used. CR2 was achieved in 63% of patients, however the probability of 5 year overall survival (10-year pOS) was only 16%. In univariate analysis a short CR duration (≤1 year) and FAB M4 were associated with poor survival, while patients with FAB M5 had a borderline significantly improved pOS. Stem cell transplantation (SCT) was performed in 25 patients after achieving CR2. In multivariate analysis, including SCT as a time-dependent variable, CR1 duration, FAB M4 and FAB M5, no factor was significantly associated with pOS. A significant proportion (24%) of children in CR2 who did not receive an allogeneic stem cell transplantation are long-term survivors. Half of the survivors suffered from late effects of treatment, especially the children who had been transplanted. This study shows that there are children with relapsed AML who can be cured without a SCT. It is important to identify these patients and spare them a SCT which is associated with frequent and severe long-term side-effects. The prognosis of relapsed AML is poor and collaborative studies with novel agents and new treatment schedules are necessary to improve outcome.

In the study described in chapter 3 we genotyped 150 pediatric AML samples for mutations in KIT (exons 8, 17), NRAS and KRAS (exons 1, 2) and FLT3/ITD.58 40% of children with AML had a mutation in KIT (11.3%), RAS (18%) or FLT3/ITD (11.1%). We found interesting non-random associations between type I and type II mutations. Seventy percent of the core binding factor (CBF) leukemia cases had a mutation in KIT or RAS. Mutations in RAS (30%) or FLT3/ITD (20%) were frequently found in association with a normal karyotype. Patients with a FLT3/ITD mutation had a significantly worse clinical outcome, but the presence of a KIT or RAS mutation did not significantly influence clinical outcome in our study. Small patient numbers precluded a meaningful analysis of outcome within the CBF AML subgroup. We demonstrated that KIT exon 8 mutations result in constitutive ligand-
independent kinase activation which could be inhibited by imatinib. Remarkable in our study was the low frequency of KIT, RAS and FLT3 mutations in FAB M5 compared to non-FAB M5 AML (10% vs. 45%, \(p=0.011\)). It was previously reported that PTPN11 mutations were frequent in FAB M5 AML. We therefore hypothesized that PTPN11 might be involved in the development of FAB M5 AML and investigated the prevalence of PTPN11 mutations in this patient cohort in chapter 4. We enriched this cohort with another 24 pediatric FAB M5 AML patients, for a total of 55 FAB M5 AML cases. The PTPN11 mutation prevalence did not differ significantly between the FAB M5 and the non-FAB M5 cohorts (7.3% vs. 5.0%, \(p=0.73\)). Overall, we found a KIT, RAS, FLT3/ITD or PTPN11 mutation in 44% of patients. There were large differences between subgroups, as in CBF AML we found a KIT, RAS, FLT3/ITD or PTPN11 mutation in 70% of patients, while in FAB M5 this was only 27%. For some of these mutated proteins, novel drugs are now available which might be useful in the treatment of AML.

In chapter 5 we determined FLT3 internal tandem duplications (FLT3/ITD) and D835 point mutations in paired initial and relapse samples from 80 pediatric and adult AML patients. Only one D835 point mutation was found, in an initial pediatric AML sample. A FLT3/ITD was present in 26% of newly diagnosed and 27% of relapsed AML samples. FLT3/ITD status changed between diagnosis and relapse in 14 cases. In 4 patients the FLT3/ITD became undetectable at relapse, in 5 patients FLT3/ITD was only detected at relapse, and in 5 patients the length or number of ITDs changed. FLT3/ITD positivity was related to a significantly shorter time to relapse, which was most pronounced when the ITD positive status was found at relapse. These results indicate that FLT3/ITD is not conserved between initial diagnosis and relapse and this makes it unsuitable as an MRD marker.

In the second part of this thesis we focused on targeted treatment of AML. Novel drugs are necessary to improve the prognosis of children with AML preferably without additional toxicity. Specifically killing the leukemic cells with targeted drugs is an appealing concept as this might limit the side-effects of treatment. In chapter 6 we compared the cytotoxicity of free daunorubicin (DNR) and liposomal daunorubicin (L-DNR) in acute leukemia, normal bone marrow (N BM) and peripheral blood (PB) samples. There was strong cross-resistance between DNR and L-DNR, and within AML and ALL there were no significant differences between the sensitivity to DNR or L-DNR. Leukemic samples were significantly more sensitive to DNR and L-DNR than N BM and N PB mononuclear cells, reflecting the therapeutic index of these drugs. In conclusion, this study has shown that there is no significant difference in cytotoxicity between DNR and L-DNR and that therefore, L-DNR might be more effective clinically, as it is employed in higher dosages and has a greater AUC.
As our knowledge of the genetic aberrancies in AML has increased dramatically, it is appealing to therapeutically target these aberrancies. In this thesis we have described 2 different drugs targeting type I mutations. In chapter 7, we investigated pediatric AML and ALL samples for in vitro sensitivity to Tipifarnib and compared these results to those obtained with N BM samples. Tipifarnib is an orally available farnesyl transferase inhibitor (FTI), specifically developed to target RAS-driven malignancies. AML samples were more sensitive to Tipifarnib compared to B-cell precursor ALL or N BM samples. Within AML, FAB M5 samples were most sensitive to Tipifarnib. T-ALL samples were significantly more sensitive than BCP ALL and N BM samples. RAS mutations were present in 32% of AML and 18% of ALL samples, but there was no correlation between RAS mutational status and sensitivity to Tipifarnib. Therefore, Tipifarnib is most effective in vitro in FAB M5 AML and T-cell ALL, but this sensitivity cannot be explained by the RAS mutational status.

In chapter 8, we investigated whether pediatric AML samples were sensitive to the tyrosine kinase inhibitor SU11657 in vitro, and whether sensitivity was related to expression of, and mutations in, FLT3 and KIT. SU11657 is very similar to SU11248 (also known as sunitinib or Sutent®) which is clinically available. Overall, SU11657 showed moderate cytotoxicity. FLT3 and KIT mutated samples were significantly more sensitive to SU11657 than WT KIT and FLT3 samples. Samples without KIT or FLT3 mutations, but with a high WT KIT expression were significantly more sensitive to SU11657 than samples with low KIT expression. About a third of FLT3 and KIT mutated samples was resistant to SU11657 in vitro and in contrast, a third of the FLT3 and KIT WT samples were relatively sensitive to SU11657. Therefore, SU11657 might be clinically useful in more than just KIT and FLT3 mutated patients. In this thesis we have shown that there still is a lot of work to do to improve the prognosis of children with (relapsed) AML and that not all patients with relapsed AML need a SCT to be cured. We can identify type I mutations in almost half of pediatric AML patients. Unfortunately, FLT3/ITD mutations are not conserved between initial diagnosis and relapse in many patients and cannot be used for MRD follow-up. We demonstrated that L-DNR is as effective in vitro as DNR. This is important as L-DNR possibly is less (cardio)toxic and therefore employment of L-DNR in stead of DNR might be as effective clinically but associated with fewer side-effects. Type I mutations can also be used as treatment targets using newly developed drugs. Our results with Tipifarnib and SU11657 demonstrate that although a drug is developed for a specific target, its efficacy does not always depend on these targets and therefore should also be investigated in patients without the intended target.

In the last few years much has been learned about the genetic abnormalities associated with AML and new drugs have been developed targeting theses
abnormalities. In the next years, clinical research with these new drugs will show whether they will be able to improve the prognosis of children with AML.

**General discussion**

With current chemotherapeutic agents and treatment schedules we have reached an unsatisfactory plateau in the outcome of children with AML. If we want to cure more children, we need to study the biology of AML and identify novel treatment targets.

In the first part of this thesis we reported the poor prognosis of Dutch children with recurrent AML, as only 18 of the 113 children with relapsed AML in our study are long term survivors. In the previously described pediatric relapsed AML cohorts the pOS for CR2 patients treated with chemotherapy alone ranged from 0 to 100%, and 22% of the children in our cohort treated with chemotherapy only are long-term survivors. Therefore, we question whether a SCT in CR2 is the only chance of cure for children with relapsed AML, as is commonly thought. A randomized clinical trial to prove this has never been performed. Especially when a matched donor is unavailable, intensive chemotherapy may be preferable over a mismatched or haplo-identical donor SCT, which is associated with significant morbidity and mortality. Future research should try to identify the characteristics of children who do not need a SCT after relapse for cure. Most importantly, as the survival after relapse in general is poor, initial treatment should be optimized further to prevent the occurrence of relapse. When a relapse does occur, optimal treatment in the setting of a clinical trial should be offered to all patients. The I-BFM-SG AML committee has opened the first international randomized pediatric relapsed AML study in 2001/2002, randomizing children between FLAG and FLAG-DNX. This initiative has been an important step forward in the treatment of children with relapsed AML, providing a broad platform for future studies.

In our study on the prevalence of KIT and RAS mutations in pediatric AML, we also investigated the prognostic significance of these mutations. As KIT mutations were most common in CBF AML, we studied the prognostic relevance of KIT mutations in this subgroup. In this relatively small group of patients (N=27) we could not show a prognostic significance for KIT mutations, but recently, different groups have demonstrated that children and adults with CBF AML and a KIT exon 17 mutation have a poor prognosis. The presence of KIT mutations in most CBF AML patients and the poor prognosis of patients with KIT exon 17 mutations, make...