Chapter 10

General discussion & future perspectives

INTRODUCTION

Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is basically a curative treatment in various hematologic malignancies and non-malignant diseases in both adult and pediatric patients, it may be accompanied by various complications, such as recurrence or persistence of the initial disease, severe infections, graft-versus-host disease (GvHD) and both acute and late toxicity related to the conditioning regimen. Pharmacotherapy is an essential part in the allo-HSCT procedure and therefore optimization of pharmacotherapy can help to reduce the risks of adverse events in allo-HSCT. Busulfan and treosulfan are both alkylating agents, which are widely used and play an important role in the conditioning regimen prior to allo-HSCT. Busulfan exposure has been related to allo-HSCT outcome and toxicity and therapeutic drug monitoring is often applied in clinical practice to target the exposure in the individual patient.[1–3] In addition, several population pharmacokinetic (pop-PK) models have been developed to describe busulfan PK. However, significant interpatient variability remains, caused by unknown variables. In this thesis the role of genetic markers in busulfan PK is explored.

Treosulfan was only recently introduced as myeloablative agent in conditioning prior to allo-HSCT. It is a promising drug with strong myeloablative and immunosuppressive capacity and a mild toxicity profile.[4–6] It is currently unresolved whether treosulfan therapy could be optimized when targeting exposure, similar to busulfan therapy. Treosulfan PK in adults is linear, however, there are limited data in pediatric patients showing a wide interpatient variability.[7] In this thesis further research on treosulfan PK in pediatric patients was performed, by development of a bioanalytical method and pop-PK model.

A major risk after allo-HSCT is the occurrence of aGvHD, which is accompanied with significant morbidity and mortality.[8] Despite improvements in donor matching and prophylaxis still approximately 25% of the patients will develop aGvHD requiring treatment with systemic high-dose glucocorticoids (GCs) as the first line therapy.[9,10] Unfortunately, about half of the patients do not respond to GCs and require alternative therapy.[11,12] In other diseases non-responsiveness towards GCs has been observed too.[13,14] Therefore it is suggested that GC-unresponsiveness is an intrinsic property of a subset of the population and differences in genetic make-up might contribute to this phenomenon.[13,14] Further insight in biological factors causing GC resistant aGvHD will be instrumental in designing the most effective aGvHD treatment for the individual patient.

BUSULFAN

In this thesis it was investigated whether genetic markers could be identified to explain the interpatient variability in busulfan clearance. Genetic markers were studied in both an adult and a pediatric population. In adults, the absence of maturation effects and relative small variability in body weight makes the association of busulfan clearance and genetic markers probably more straight forward. In **chapter 3** we studied the effect of SNPs in three genes encoding for glutathione-S-transferases (GSTA1, GSTM1, and GSTP1). These enzymes are involved in conjugation of busulfan with glutathione, with GSTA1 being the predominant enzyme.[15] A SNP (rs3957357) in *GSTA1* was associated with busulfan clearance and could explain 18% of the interpatient variability in this adult study population. No association between *GSTM1*, *GSTP1* and busulfan exposure was observed, nor could any relation between *GSTA1* genotype and clinical outcome be found in our study population. The latter is most probably due to the application of busulfan in a non-myeloablative regimen resulting in very low toxicity levels. The association of *GSTA1* genotype with busulfan PK parameters has been observed in a few studies in adults as well.[16,17] However, Krivoy *et al*.[18] did not observe an association with *GSTA1*, but found an association with *GSTP1* and two SNPs in *ABCB1* in combination with *GSTM1* and busulfan clearance. However, in this study the patients received busulfan orally and therefore other factors might contribute to this contradictious finding.

In **chapter 4** an exploratory pharmacogenetic approach was applied to investigate whether the remaining interpatient variability in busulfan clearance in adults could be explained. This analysis included a wide range of genes involved in drug ADME (absorption, distribution, metabolism and excretion). Two cohorts were composed; an exploratory and a validation cohort. Patients in the exploratory cohort were genotyped with the DMET array. This array contains 1936 SNPs in 225 genes involved in drug metabolism and transport. This array is a comprehensive platform to identify potential new genetic markers involved in busulfan PK. Again, the role of the *GSTA1* in association with busulfan clearance was confirmed both in the exploratory and validation cohort and no additional genetic markers were identified explaining the remaining part of the variability in busulfan PK. This could have been due the relative small cohort sizes resulting in low statistical power to detect rare variants or variants with a small effect size. Although it has been suggested that transporters are involved in busulfan metabolism, we could not identify any genetic markers in a wide variety of transporters affecting busulfan metabolism.

Clinical relevance of the *GSTA1* genotype in busulfan-based allo-HSCT in adults is minor. The effect of *GSTA1* genotype is potentially of more interest in pediatric patients, since in children

a high interpatient variability in exposure is observed, pediatric patients receive more often a myeloablative conditioning and a tighter control of exposure is aimed for in clinical practice.

However, the effect of genetic markers on busulfan PK in pediatric patients seems to be more complicated to determine, which is illustrated by several studies in pediatric patients presenting conflicting results. Either no association between *GSTA1* genotype and busulfan PK[19,20] was observed or the association was found only in a subset of the study cohort receiving busulfan orally.[21] However, there are also a number of positive studies,[22–25] including **chapter 5** of this thesis.[26] To our opinion, several factors might account for the discrepancies between the positive and negative studies towards the effect of genetic variation in *GSTA1* on busulfan clearance in children, such as sample size,[20] correction of busulfan clearance for body size[19,20] or administration route.[21]

Interpatient variability of busulfan PK in children is higher in comparison with adults and several mechanisms from birth until adulthood will affect busulfan PK and with that the effect of genetic markers on busulfan PK. Young children have a higher clearance (expressed per unit of body weight) in comparison to adults, which is partially caused by an increased liver size to body weight ratio. To capture the effect of physiological growth and liver size, busulfan clearance should be expressed as function of allometric scaled body weight or body surface area.[27,28] However, when clearance is expressed by body surface area there is still an increased clearance in younger children (< 4 years). This is most probably caused by an increased GST expression.[29] Furthermore, in the earliest years after birth, maturation of enzyme function occurs as demonstrated by a 1.7 fold increase in busulfan clearance in the first 2 years after birth.[28]

A genotype of each individual is set at conception. However, one's phenotype is affected by environmental factors and can change over time. *GSTA1* genotype affects GST activity and with that busulfan metabolism, however, the magnitude of effect of the genotype on busulfan metabolism is also determined by other contributing factors, such as administration route, co-medication and age of the patient. Furthermore, the expression of the *GSTA1* gene changes with age (as described above) and with that the relative contribution of this genotype to busulfan metabolism will change. A schematic overview of factors influencing busulfan PK is given in Figure 10.1. We hypothesize, that when there is abundant GST activity, which is the case at the age of 2–4 years, a less functional allele in *GSTA1* gene will have limited effect on busulfan PK. This was demonstrated in **chapter 5** of this thesis; a larger effect of *GSTA1* genotype was observed in children under 2 years of age in comparison to the older children; explaining 20% versus 5% of interpatient variability, respectively. Therefore, genotyping of *GSTA1* in pediatric patients seems only to be clinical relevant in a certain subpopulation based on age.

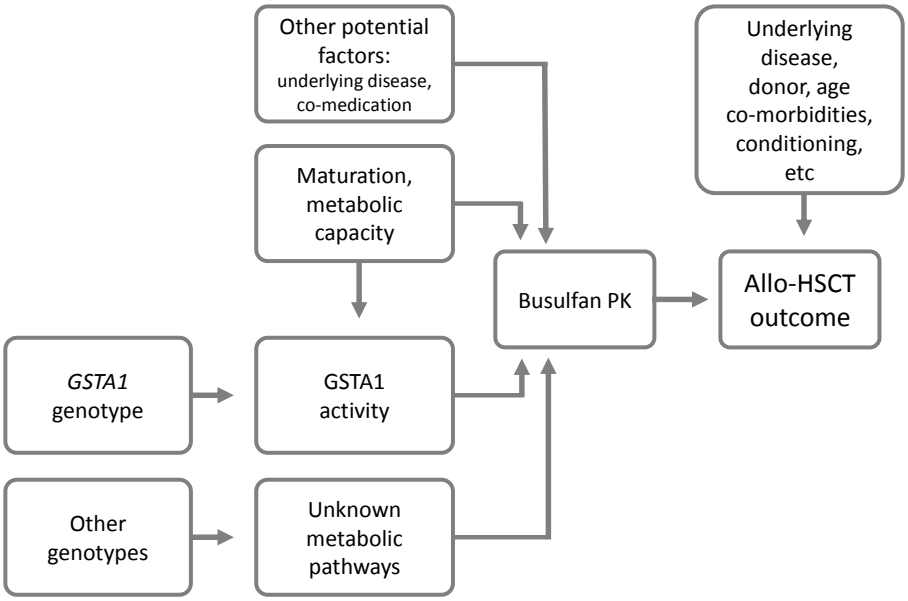

Figure 10.1 Factors affecting busulfan pharmacokinetics in pediatric patients. Allo-HSCT: allogeneic hematopoietic stem cell transplantation, PK: pharmacokinetics.

In **chapter 5** also a novel genetic marker (*CYP39A1*) was associated with busulfan clearance in pediatric patients. The role of this genetic marker in general and in busulfan metabolism in particular is unclear yet and functional analysis should be performed. Furthermore, the effect of *CYP39A1* in combination with *GSTA1* needs to be replicated in a second cohort.

In this thesis we searched for a genetic explanation of interpatient variability in busulfan PK in adults and in children. Investigating genetic markers in adults and children demonstrated that the effect of genetic variants is relative small to other patient related factors, such as age, maturation and body weight. Furthermore, not all the interpatient variability in busulfan PK cannot be explained by genetic markers involved in drug transport and metabolism.

TREOSULFAN

Treosulfan is an interesting alternative for busulfan in the conditioning prior to allo-HSCT. It has a mild toxicity profile and strong myeloablative capacity. It is most often applied in reduced toxicity or reduced intensity conditioning regimens. Data on PK of treosulfan in pediatric

patients is limited.[7] To assess the PK profile of treosulfan in pediatric patients we developed and validated a bioanalytical method for determination of treosulfan in serum, a pop-PK model and a limited sampling strategy, described in **chapter 6** of this thesis. Furthermore, in **chapter 7** we analyzed the PK profile of treosulfan in 21 pediatric allo-HSCT patients receiving treosulfan as part of their conditioning regimen.

The presented population PK model performs adequately, which is mainly due to the predictive PK profile of treosulfan. We have demonstrated in **chapter 6** and **7** that the interpatient variability in treosulfan exposure is relative small (14%) in pediatric patients above the age of 1 year. Furthermore, we could not relate early clinical outcome and toxicity to treosulfan exposure, This could be due to several reasons. First, such a relationship could be non-existent. Second, the interpatient variability may be too limited to observe such a relationship in this heterogeneous cohort of children. At first, a larger cohort and single-disease subgroups should be studied to more precisely assess the interpatient variability and the relationship of exposure with clinical outcome. Furthermore, it should be investigated whether treosulfan-based conditioning is more effective when increasing the dose of treosulfan while keeping its beneficial toxicity profile. The optimal dose of treosulfan for the youngest and smallest patients remains to be established, since we were only able to study patients above the age of 1 year and with a body weight of more than 10 kg, when developing the limited sampling strategy. One patient was much younger (2 months and weighing 5 kg) and received a lower adjusted dose of 10 g/m^2. Treosulfan exposure was assessed with the limited sampling strategy and the exposure of this young patient was similar to the mean exposure observed in the older patients. This suggests that younger patients may need a lower dose (based on body surface area). However, this is based on the finding in one patient and more data on the efficacy and toxicity profile in infants is required.

All patients above 1 year of age received 14 g/m^2 of treosulfan and a decrease of treosulfan exposure with age was observed. However, the higher exposure in the younger patients was not related to increased toxicity and this implicates that dose escalation in the older patients is possible. Treosulfan-based conditioning was relatively well tolerated and effective, as demonstrated by the mild toxicity profile in comparison to other conditioning regimens, a high overall survival (90%) and good disease free survival (74%). However, chimerism levels after 1 year and primary engraftment could be improved, especially in diseases where effective myeloablation and donor chimerism are mandatory, such as hematologic malignancies. It is therefore warranted to continue the research of treosulfan PK in relation to clinical outcome, in order to identify the optimal exposure and dosing regimen.

GLUCORTICOID RESPONSIVENESS IN AGVHD

Acute GvHD is one of the major complications after allo-HSCT which occurs when donor cells engraft and mature donor T-cells react with recipient immune cells. In malignant diseases a beneficial effect of aGvHD has been demonstrated, since it may also be accompanied with graft-versus-leukemia (GvL) effect. However, the right balance between the beneficial GvL effect and the adverse event of aGvHD is difficult to achieve. In patients diagnosed with aGvHD grade 2 or higher, first line treatment with high-dose glucocorticoids remains the gold standard. However, approximately only half of the patients responds adequately to this therapy. Non-responsiveness to GCs is associated with high morbidity and mortality. Furthermore, non-responsiveness will delay adequate treatment of the aGvHD, very often resulting in worsening of the disease. Overall, non-responsiveness towards GCs in aGvHD treatment is of major clinical concern. In **chapter 8** of this thesis 7 expression quantitative trait loci (eQTLs) were identified in healthy volunteers and these 7 eQTLs and an eQTL of *RBMS3* were investigated for association with GC non-responsiveness in pediatric patients with aGvHD. In this study it was demonstrated that genetic markers are involved in GC responsiveness and that donor genotype has a predominant effect on responsiveness. This finding is in line with the physiological process of aGvHD where alloreactive donor T-cells interact with immune cells of the recipient and GC therapy is considered to mainly target donor lymphocytes activity.

The pharmacologic effect of GCs is complicated and is affecting multiple targets and gene expression. Several studies sought to indentify genetic markers for association with GC-responsiveness and most of them were based on a candidate gene approach and mainly studied SNPs in genew encoding for the GC receptor and transcription factors. Combining genome wide genotyping data and multiple levels of phenotypic data provides the opportunity to identify polymorphisms that interact with GC treatment and influence response. These polymorphisms are therefore excellent candidates. The strength of the study in **chapter 8** is that findings from genome wide information were combined into a candidate gene approach study on a retrospective patient cohort.

PHARMACOGENETIC TESTING IN ALLO-HSCT RECIPIENTS

In this thesis pharmacogenetic testing was applied in 2 widely used drugs in allo-HSCT. When pharmacogenetic testing is more broadly applied in allo-HSCT patients it is essential to choose the right biospecimen for collecting DNA as we described in **chapter 9**. Patient material should preferably be acquired before the allo-HSCT. When genotyping patients with malignant disease

it is important to obtain germline DNA, therefore a blood sample in remission is a good option. Furthermore, we have demonstrated in **chapter 9** that after allo-HSCT, mixed chimerism in a patient could affect genotyping outcomes, emphasizing the need for proper quality control procedures.

FUTURE PERSPECTIVES

Busulfan conditioning can be optimized with therapeutic drug monitoring and evidence has been provided that tight control of exposure results in a better outcome.[3,30] It is essential to identify the optimal target exposure giving the optimal balance between efficacy and toxicity for different conditioning regimens. Research on the optimal busulfan exposure should be performed in more homogenous cohorts; patients receiving the same conditioning regimen and having the same underlying disease. This may result in disease and regimen specific recommendations.

The same accounts for treosulfan-based conditioning, however, the experience with treosulfan-based conditioning is limited and with that the knowledge on PK of treosulfan. First, the optimal dose and combination of drugs in treosulfan-based conditioning should be investigated. We hypothesize that the efficacy of treosulfan-based conditioning can be improved if a higher dose of treosulfan is administered and keeping its beneficial toxicity profile. At this point, busulfan is considered the most effective myeloablative conditioning regimen, whereas treosulfan appears to have a more favorable toxicity profile. Therefore, in pediatric patients, treosulfan is preferentially used in patients with co-morbidities or in non-malignant diseases. In malignant diseases a strong myeloablative conditioning is required. Treosulfan effectiveness might be improved at a higher dose. Therefore, it would be interesting to expand research on treosulfan PK and whether higher exposure translates into improved outcome. Ultimately, this may lead to a dose-escalation study in patients requiring a potent myeloablative conditioning regimen. Another advantage of treosulfan is the relative small interpatient variability in exposure. To further optimize the clinical use of both alkylating drugs, a direct comparison is warranted. Busulfan- and treosulfan-based conditioning should be compared prospectively in a large homogenous cohort in patients with the same underlying disease and disease status. A good example of such a prospective randomized study is the recently started ALL SCTPED 2012 FORUM study (EudraCT: 2012-003032-22) in which busulfan- and treosulfan-based conditioning regimens are directly compared to a traditional total body irradiation-based regimen in pediatric patients with ALL.

The finding that genetic markers are involved in non-responsiveness towards GCs in aGvHD is very promising. At first, the results should be replicated in a second and larger cohort. This will

lead to a more accurate measure of the predictive value of the two eQTLs, giving the possibility to study the effect of the eQTLs prospectively in pediatric patients with aGvHD. Furthermore, the physiological effect of the SNPs in *TMEM71* and *DPYSL3* should be further elucidated. At first, the SNPs should be studied in an *in vitro* model which resembles immune response in aGvHD accurately. When the SNPs remain to be associated with GC non-responsiveness in aGvHD, it could be of great interest to study them in other diseases such as asthma, inflammatory bowel disease and rheumatoid arthritis. For allo-HSCT patients ultimately, HSCT donors should be genotyped for the SNPs related to GC non-responsiveness and these biomarkers can be used to identify patients that will not benefit from GC therapy when encountering aGvHD. When aGvHD occurs in these patients, an alternative aGvHD therapy can be started directly and more stringent aGvHD prophylaxes can be applied in these patients. In addition, anti-thymocyte globuline (ATG) dose may be adapted based on the genotype of the donor, to reach the optimal balance between T-cell depletion to reduce the risk of aGvHD and rapid immune reconstitution. Furthermore, PK-models for estimation of ATG exposure are being developed and matching of ATG exposure to donor genotype could even be more beneficial.

Pharmacologic research in the field of allo-HSCT is often focused on one agent and one mechanism to optimize the studied drug. However, during allo-HSCT multiple factors, such as drugs, patient and donor characteristics, have also an effect on outcome. For example, the conditioning regimen can affect the risk of aGvHD. Therefore, future pharmacological studies should emphasize on the effects of combination of drugs, together with specific patient conditions, in order to further improve allo-HSCT outcome.

CONCLUSION

In this thesis, studies on three different pharmacological agents applied in pediatric and adult allo-HSCT have been performed. The goal of these studies was to elucidate mechanisms causing interpatient variability and thereby optimize current therapies for each individual patient. We demonstrated that *GSTA1* is involved in busulfan PK in adults and pediatric patients and in children a novel genetic marker, *CYP39A1*, was demonstrated to affect busulfan PK. Interpatient variability in treosulfan PK in pediatric patients is not as large as observed with busulfan, which could be a beneficial aspect of treosulfan. We also demonstrated that pharmacogenetic markers in HSCT donors are involved in GC responsiveness of patients with aGvHD. The procedure of allo-HSCT is very complex and pharmacotherapy plays an important role. Studies on the optimization of current drug therapies and combining results on different agents could have potentially great impact on morbidity and mortality rates in allo-HSCT patients.

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