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Individualized dosing of serotherapy in allogeneic hematopoietic cell transplantation - a delicate balance

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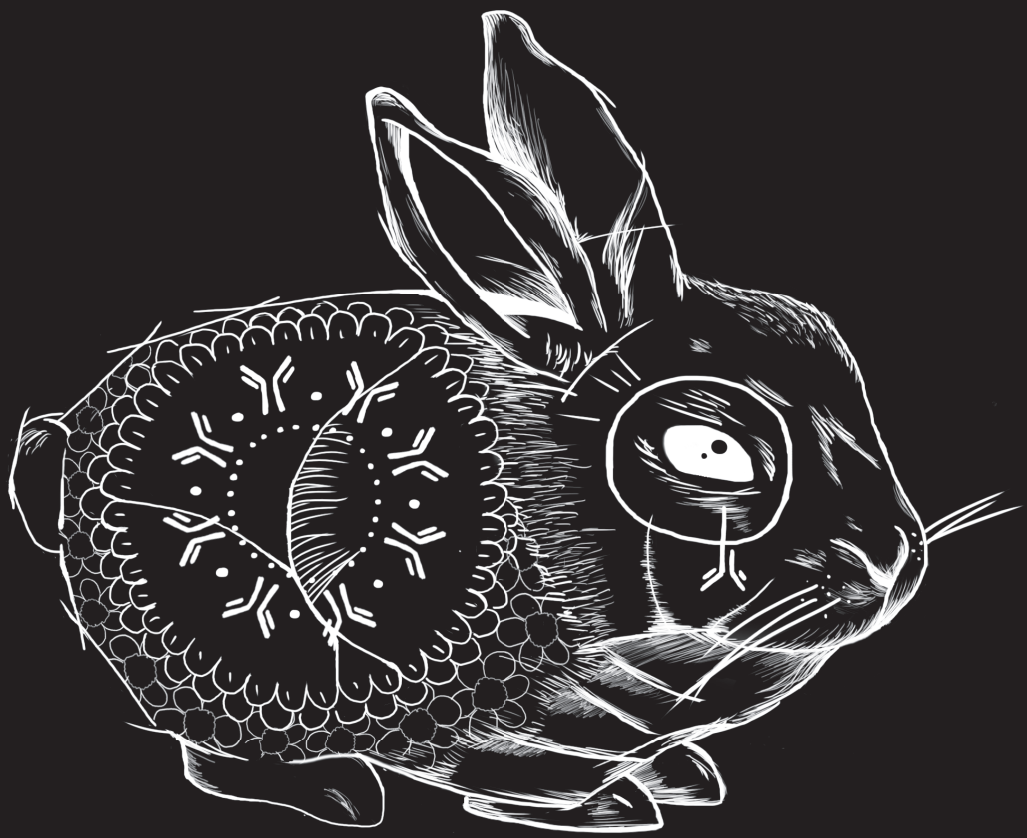
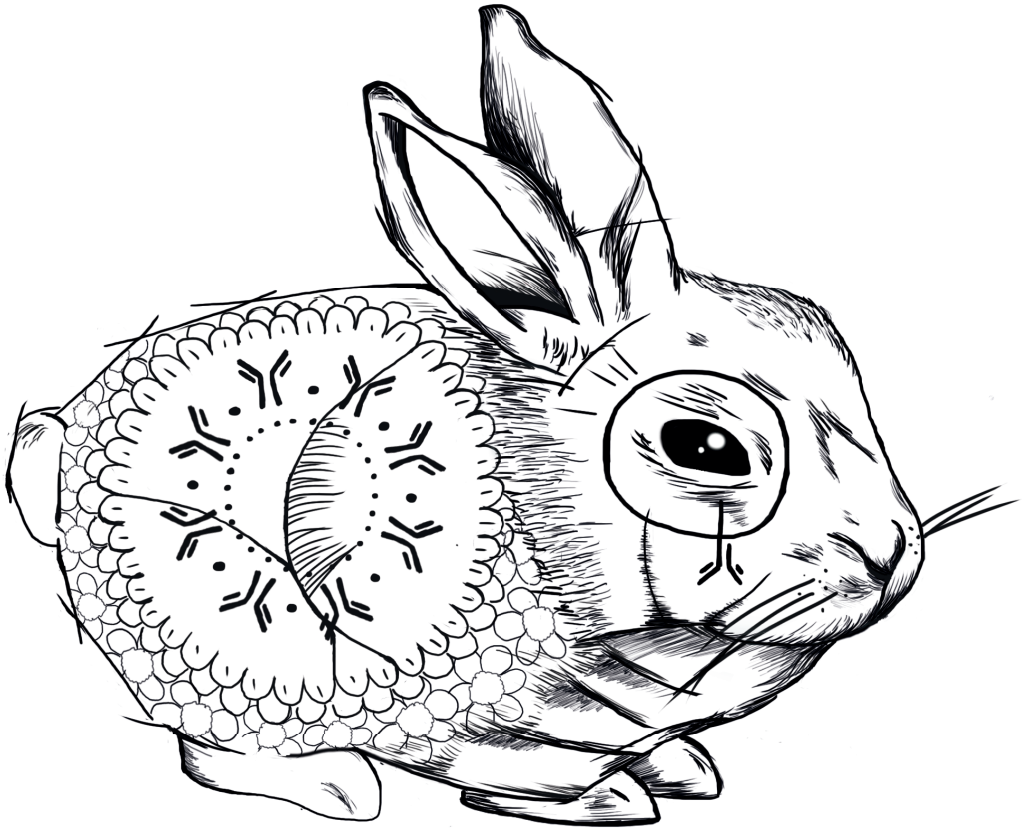


PART VI

Conclusions and Perspectives



Chapter 12

Individualized Dosing of Serotherapy in Allogeneic Hematopoietic Cell Transplantation: Summary, Conclusions and Perspectives

SUMMARY AND CONCLUSIONS

Allogeneic hematopoietic cell transplantation (HCT) is a potentially life-saving procedure to treat malignant and non-malignant disorders. The limitations of HCT include graft-versus-host-disease (GvHD) and rejection, for which serotherapy (anti-thymocyte globulin [ATG] or alemtuzumab) is introduced to the conditioning regimen. However, serotherapy may cause delayed or absent T-cell immune reconstitution following HCT, potentially leading to lethal viral reactivations or relapse of malignancy. The balance between efficacy and safety of serotherapy may be influenced by the highly variable relationship between dose and exposure (pharmacokinetics). Additionally, the optimal exposure to ATG to prevent GvHD and rejection but to promote immune reconstitution is largely unknown (pharmacodynamics).

The aim of this thesis is to develop individualized dosing regimens for serotherapy agents on the basis of PK/PD modeling in children and adults. The focus in this thesis is on ATG in children, the most frequently used drug in HCT. Due to the major changes in pharmacokinetics, children are especially at risk for under- or overdosing. The overarching aim of this thesis is to enhance the safety and efficacy profile of serotherapy, and thereby contribute to the improvement of outcomes of HCT. To reach this goal, the dose-exposure-effect relationships of serotherapy in allogeneic HCT are thoroughly investigated in patients ranging from neonates to adults. The role of immune reconstitution on clinical outcomes, potentially hampered by over-exposure to serotherapy, is explored. Additionally, this thesis generates an insight into the developmental pharmacokinetics of antibodies.

Part I: Introduction

Currently, all serotherapy drugs are dosed in a linear fashion with body weight (i.e. mg/kg) in both adults and children. Such dosing assumes a linear increase in pharmacokinetic parameters (e.g. clearance, volume of distribution) with body weight. Using per kg doses, the assumption is made that the dose to achieve comparable concentrations increases in a linear fashion with weight. However, changes in PK due to development and growth are mostly non-linear, therefore linear dosing can lead to over- or underdosing in certain age groups. Therefore, the relation between dose and exposures need to be described to assure equal drug exposure in all patients. Using population PK-modeling, pharmacokinetics can be described as well as the explanatory covariates for variability in PK such as body weight.

Chapter 2 presents an outline on performing a population PK/PD study and translating these results into rational dosing regimens, with the development and prospective evaluation of PK/PD derived evidence-based dosing regimen being discussed. Examples on amikacin, morphine and busulfan are provided, showing how PK(/PD) modeling not only led

to optimization and individualization in pediatric clinical care for the specific drugs but also to insight in maturation of organ systems involved. It is shown that the latter results can subsequently be used as a basis for dosing of other drugs eliminated through the same pathway. Ultimately, these efforts should lead to predictable drug efficacy and safety across all age groups.

Part II: pharmacokinetics of serotherapy

In **chapter 3**, the population pharmacokinetics of active ATG (Thymoglobulin) are described based on a cohort of 267 patients aged 0.2-23 years, receiving ATG as part of the conditioning regimen in the pediatric HCT-units of the university hospitals of Utrecht (UMCU) and Leiden (LUMC), the Netherlands. On the basis of over 3000 concentration samples collected between 2004 and 2012, a population PK model was developed. A two-compartment model with parallel linear and saturable clearance pathways best described the data. Additionally, under-prediction during the distribution phase of ATG led to the inclusion of saturable inter-compartmental distribution towards the peripheral compartment in the model. Body weight, was a predictor for both linear clearance and volume of distribution, and was included as covariate in a power-function. Moreover, peripheral blood lymphocyte counts before the first infusion of ATG was found to predict clearance. Simulation studies showed that the currently used dosing regimen, cumulative 10 mg/kg ATG over 4 days, leads to increasing exposure with increasing body weight, and is therefore suboptimal. This PK-model serves as a basis for individualized dosing of ATG in patients receiving a HCT.

The population pharmacokinetics of alemtuzumab, a monoclonal anti-CD52 antibody, are described in **Chapter 4**. For this study, data from patients receiving alemtuzumab (Campath) in the pediatric HCT programs of the LUMC Leiden, the Netherlands and Great Ormond Street Hospital in London (GOSH), United Kingdom, were analyzed. A total of 1146 concentration samples from 206 patients aged 0.2-19 years were collected. Alemtuzumab PK could be well described using a two-compartment model with parallel linear and saturable clearance. Body weight was a covariate for both linear clearance and volume of distribution. The relationship between linear clearance and body weight was best described using a so-called bodyweight-dependent exponent model (BDE), in which the exponent changes from 1.94 in children weighing 5 kg to 0.61 in those weighing 60 kg. Interestingly, although CD52 is mainly expressed on lymphocytes, our data did not support alemtuzumab clearance being impacted by lymphocyte counts. This may be due to a relative excess of alemtuzumab compared the amount of CD52 on lymphocytes.

Part III: Exposure-response relationship of serotherapy

Where Part II focused on the dose-exposure relationship of serotherapy, Part III describes the exposure-effect relationship of ATG and alemtuzumab in children and adults. Determination of the pharmacodynamics reveals the therapeutic window, therewith identifying the optimal target exposure for individualized dosing.

In **chapter 5**, the relationship between ATG exposure and clinical outcomes including T-cell immune reconstitution was investigated in a large cohort of 251 receiving a first HCT. Patients from the LUMC and UMCU were included, and no restrictions were applied based on stem cell source, conditioning regimen or ATG dose. Different ATG exposure measures were calculated using the validated PK-model described in chapter 3, including the maximum concentration, concentration at time of HCT, time to reach concentrations below 1 AU/mL, area under the curve (AUC) of the concentration-time plot, and AUC before and after infusion of the graft. AUC after infusion of the graft, i.e. AUC after HCT, proved to be a powerful predictor for CD4+ T-cell immune reconstitution (IR) after HCT, while acute GvHD was not impacted by AUC after HCT. Subsequently, successful CD4+ IR was a predictor for improved survival as well as lower treatment related mortality and relapse related mortality in patients with acute myeloid leukemia (AML). In subgroups, low AUC of ATG after HCT led to improved survival, however the optimal AUC was lower for cord blood recipients compared to bone marrow and peripheral blood stem cell recipients. On the other side, exposure to ATG before HCT was a predictor for acute GvHD, chronic GvHD and graft failure. This analysis shows that exposure to ATG before HCT seems to be responsible for the desired pharmacological effects, while exposure after HCT leads to adverse effects. The determined optimal exposures, combined with the validated PK-model, have led to an individualized dosing regimen for ATG in children. This regimen is currently being evaluated in a prospective clinical trial (see Perspectives).

Exposure to ATG seemed most stringent in cord blood recipients, who were therefore analyzed in a larger cohort in **chapter 6**. In a total of 137 patients receiving a cord blood transplantation as a first HCT at the pediatric ward of the UMCU were included that were homogeneously treated in terms of conditioning and supportive care. The optimal ATG exposure after HCT was determined to be even lower compared to the results from chapter 5, again showing the detrimental effect of ATG exposure after HCT on CD4+ T-cell immune reconstitution. Low exposure to ATG after CBT led to higher chances on event free survival, defined as survival without relapse or graft failure. In line with this, successful CD4+ T-cell immune reconstitution predicted improved overall and event free survival, as well as reduced treatment related mortality and relapse related mortality, thereby confirming the results found in chapter 5.

Chapter 7 describes the pharmacokinetics and pharmacodynamics of ATG in 146 adult patients with acute leukemia receiving a peripheral blood stem cell (PBSC) transplant following reduced intensity conditioning (RIC). First, in order to describe the population pharmacokinetics of ATG in an adult population, data from the adult RIC-population was analyzed with the data from the pediatric population described in chapter 3 as well as data from an additional 81 adult patients treated with other conditioning regimens and/or cell sources. In this PK-model, a so-called body-weight dependent (BDE) parameterization best described the relationship best, with the exponent ranging from 1.33 in children of 5 kg body weight to a value of 0.06 in patients weighing 80 kg. The exponent is below 0.1 in patients weighing 50 kg and above, implying that no further increase in clearance is observed beyond this body weight. Peripheral blood lymphocyte counts before the first dose of ATG were however found to impact ATG clearance, and should be taken into account in dosing.

Next, to assess the optimal therapeutic window in this adult RIC-PBSC cohort, the different ATG exposure measures were related to outcome. The AUC of ATG after HCT proved to be the most powerful predictor for overall survival. There seemed to be an optimal exposure to ATG after HCT: below optimal exposure led to increased treatment-related mortality, above optimal exposure led to more relapse. Patients with below-optimal exposure after HCT had higher incidences of severe acute and chronic GvHD. No relationship was found between GvHD or graft failure and exposure to ATG before HCT, possible because the vast majority had an AUC before HCT above the optimal exposure as determined in chapter 5.

Finally, evaluation of multiple currently used dosing guidelines resulted in poor target attainment, while 96% of patients groups with individualized dosing reached optimal exposure. The individualized dosing regimen for adult RIC-PBSC patients may yield an improvement of outcomes in HCT.

A recently published paper showed a relation between peri-transplant alemtuzumab concentrations (±3 days) and the incidence of GvHD and mixed chimerism[1]. **Chapter 8** explores the therapeutic window of alemtuzumab in a cohort of 176 pediatric patients receiving a first HCT with alemtuzumab as part of the conditioning, treated in the LUMC Leiden and Great Ormond Street Hospital, London. The impact of alemtuzumab concentrations, as calculated with the population PK-model from chapter 4, on clinical outcomes was assessed. Alemtuzumab concentrations on the day of transplantation were markedly higher compared to the previous report where alemtuzumab was dosed more distally and subcutaneously. While alemtuzumab concentrations predicted the incidence of grade 2-4 and grade 3-4 GvHD, no impact on survival, mixed chimerism, viral reactivations or T-cell immune reconstitution could be identified. The lack of an exposure-toxicity relationship combined with a moderate exposure-effect relation suggests a relative overdose in a major part of patients using proximal intravenous dosing.

Part IV: Immune Reconstitution as a Predictor for Clinical Outcomes

As discussed before, overexposure to serotherapy after HCT may lead to delayed or absent early T-cell reconstitution. As a consequence, patients may have impaired cellular immunity leaving them at risk for relapse and viral reactivations. Part IV describes the role of immune reconstitution on relapse and viral reactivations.

Early CD4+ T-cell reconstitution was associated with less relapse in acute myeloid leukemia (AML), but not in acute lymphoid leukemia (ALL) after cord blood transplantation (CBT). This finding was further investigated in **chapter 9** in a larger cohort comprising patients who received ATG as well as patients who did not. Patients receiving a CBT in UMCU and GOSH for acute leukemia were included resulting in a cohort of 87 patients of whom 41% received ATG as part of the conditioning. Leukemia-free survival was significantly impacted by CD4+ T-cell reconstitution in patients with myeloid leukemia, while this effect was absent in patients with lymphoid leukemia's. Treatment-related mortality was however reduced by CD4+ T-cell reconstitution in all patients.

In **chapter 10**, viral reactivations and associated outcomes were investigated in the context of immune reconstitution. First, reconstitution of CD3+, CD4+ and CD8+ T-cells as well as B-cells and NK-cells were investigated for their predictive value for reactivations of adenovirus (AdV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV6) and BK-virus (BK). CD4+ T-cell reconstitution was the only predictor for viral reactivations; higher CD4+ T-cell counts led to a lower chance on reactivations of AdV, EBV and HHV6. CMV and BK were not impacted by any immune reconstitution marker. When exploring the effect of viral reactivations on clinical outcomes, AdV reactivation proved to be a powerful predictor for survival. When further investigating the detrimental effect of AdV, patients with AdV who at the same time had CD4+ T-cell reconstitution performed comparably to those not having AdV, while patients with AdV not having CD4+ immune reconstitution have a poor prognosis. An increased incidence of GvHD was observed in patients with EBV and HHV6 reactivations. CMV and BK did not impact any clinical outcome measure. This data shows the importance of early CD4+ T-cell reconstitution, not only in reducing the incidence of viral reactivations, but also in preventing virus-associated mortality.

Part V: Clinical implementation of individualized dosing

Individualized conditioning regimens in cord blood transplantation are reviewed in **chapter 11**, focusing on current clinical outcomes, unmet needs, and future directions in individualized therapy in cord blood transplantation.

In **chapter 12** the conclusions are summarized and perspectives of this thesis are discussed. First, the clinical implications of the results on ATG in pediatric HCT are presented. Based on chapters 3 and 5, an individualized dosing regimen for ATG in children was developed, which is currently being evaluated in a prospective clinical trial. Furthermore, the possibility of therapeutic drug monitoring (TDM) for ATG is discussed.

The second part focuses on the observation that exposure of ATG before infusion of the graft impacts GvHD rather than after infusion. The depletion of host-derived antigen-presenting cells is hypothesized to be an important mechanism of action of ATG.

In the third part, the main differences between two available agents for serotherapy, ATG and alemtuzumab are discussed from a PK/PD perspective.

The final part of the perspectives focuses on individualized dosing as the future of pediatric pharmacotherapy. Currently, the majority of drugs is dosed in an off-label or unlicensed manner, mostly using empirical (mg/kg) dosing. This practice is in dire need of revision, especially for drugs with a critical therapeutic window.

PERSPECTIVES

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment option for indications including leukemia, immune deficiencies, benign hematological diseases and inborn errors of metabolism. To reduce the chance of developing GvHD and graft rejection, serotherapy is included in the preparative conditioning regimen. Serotherapy mainly depletes T-lymphocytes of the recipient and graft, which are important mediator cells in GvHD and rejection. However, in line with the mode of action, too rigorous T-cell depletion of the graft may result in delayed or absent T-cell reconstitution after HCT, leading to an increased risk for viral reactivations and relapse of malignancy. Serotherapy therefore has a central role in outcome after HCT, and appears to have a delicate balance between its efficacy and toxicity.

This thesis describes the pharmacokinetics and pharmacodynamics of ATG and alemtuzumab as a platform for individualized dosing. Furthermore, as hampered immune reconstitution is the major toxicity of serotherapy, the role of early immune reconstitution on clinical outcomes is investigated. Finally, an insight is provided in population pharmacokinetic modeling of antibodies in children.

Individualized dosing for ATG: Clinical Implementations

Dosing of ATG was traditionally based on extrapolations of adult dosing. Children mostly received a dose of 10 mg/kg, irrespective of age or body weight, and started 5 days before infusion of the graft. Here, the assumption is made that both pharmacokinetics (PK) and

pharmacodynamics (PD) show a linear increase with body weight. However, the results from chapter 3 indicate that the PK of ATG is non-linear and dependent on body weight and lymphocyte counts. In addition, the PD is dependent on stem cell source, and influenced by the starting day of ATG relative to graft infusion. Therefore, individualized dosing as described in chapter 2 seems an attractive possibility for ATG. In individualized dosing, the dose for individual patients is adjusted based on patient characteristics aiming for optimal exposure[2–5]. In case of ATG, the dose and timing of ATG can be amended per patient so that exposure before and after HCT is most optimal, potentially leading to superior outcomes. Therapeutic drug monitoring (TDM), preferably combined with individualized dosing, can be used for further control of drug exposure[6–8]. With TDM, drug concentrations following the first doses are measured in order to adjust subsequent doses within the same patient. Both approaches have been implemented for ATG in clinical care.

Based on the results from the pharmacokinetic and pharmacodynamic analyses of ATG in pediatric HCT (chapters 3 and 5), an individualized dosing regimen was developed. The optimal dose for each patient is calculated based on three factors: 1) body weight, 2) baseline lymphocyte counts, and 3) stem cell source. Patients with higher body weights, lower lymphocyte counts and CB transplants are proposed to receive a lower dose depicted in mg/kg compared to patients with lower body weights, higher lymphocyte counts and BM/PBSC. In addition, the first infusion of ATG is given more distal to the HCT in order to increase the exposure before HCT and decrease the exposure after HCT. The first dose of ATG is given on day -9 before HCT.

The efficacy of this individualized dosing regimen is currently assessed in a prospective, open label, phase II clinical trial entitled "Prospective Analysis of an individualized dosing Regimen of ATG (Thymoglobulin) in Children undergoing HCT: redUcing Toxicity and improving Efficacy – a single arm phase II study", in short PARACHUTE. This study is registered in the Dutch Trial Register under number NTR4960. The main objective of this study is to assess whether individualized dosing of ATG leads to improved CD4+ T-cell immune reconstitution. Secondary endpoints include overall survival, GvHD, rejection, relapse, viral reactivations, and validation of the developed PK-model. Outcomes of the PARACHUTE-study will be compared to a previously treated cohort of children receiving the traditional dose of ATG. All children receiving an allogeneic HCT with ATG as part of the conditioning, treated in the two pediatric blood and marrow transplantation programs in the Netherlands, are eligible for this study. The total study cohort of 53 patients is expected to be included early 2017; final results will be obtained after 1 year of follow-up.

Other research groups have also adopted the individualized dosing regimen for ATG. It is implemented in the CHAMP study (BMT-CTN 1502), investigating optimized conditioning for aplastic anemia in children and young adults. The cord blood arm receives individual-

ized ATG according to our results. In this study, ATG concentrations will be measured in the lab of the UMCU in the blood samples collected in this study, and together with the PK-model as described in chapter 3 actual exposures will be calculated. This study will give another prospective validation of the pharmacokinetic and pharmacodynamic results in chapters 3 and 5. Moreover, various major pharmaceutical industries investigating cellular therapies in cord blood transplantation have scheduled to collect samples for ATG measurements. Finally, several clinical groups worldwide performing HCT have consulted us for dosing advises for ATG in clinical care for individual patients. Currently, we have supplied dosing advise for ATG in over 50 patients based on the previously derived PK-model from chapter 3 and the therapeutic window aiming for optimal immune reconstitution and survival (chapter 5).

For the specific subgroup of patients with a high risk for graft failure, GvHD, or ongoing infections who receive a cord blood transplantation, ATG with therapeutic drug monitoring (TDM) was introduced. Patients with an increased inflammation with intact or activated T-cell function were considered. Using the PK-model of chapter 3, dosing of ATG for these children was chosen to target very high exposure before infusion of the graft, with minimal exposure after infusion. Individual pharmacokinetics are determined based on actual concentration samples collected during the first doses of ATG. Subsequent doses were adjusted if necessary based on these. In all children, actual PK is highly in line with predicted PK and few dose adjustments were necessary. This procedure has now been performed in 7 very high-risk children with promising results.

Preventing GvHD after HCT: the role for antigen presenting cells

Prevention of GvHD is one of the main reasons for including ATG or alemtuzumab in the conditioning regimen. It is given for in-vivo lymphodepletion of the T-cells infused with the graft, although some immune-modulatory properties have also been attributed to ATG[9]. In line with its production process, ATG harbors epitopes directed to a variety of cell surface markers, including but not limited to those found on T-cells. While most epitopes of ATG are directed to markers found on T-cells, it also targets markers found on other cell types including natural killer (NK) cells, monocytes and dendritic cells (DC). Alemtuzumab on the other hand is a monoclonal antibody directed against CD52, which is mainly expressed on T-cells, B-cells and NK-cells, and some DC's[10]. Hence, although both serotherapy products mainly target T-cells, other cell types that play a role in the development of GvHD may also be targeted.

The pathophysiology of acute GvHD is proposed to be a three-phase model[11]. The first phase consists of activation of host antigen-presenting cells (APC) present in tissues, mostly DC's. The conditioning and underlying disease are the main causes of APC activation. In the second phase, donor T-cells are activated by the host APC's, followed by differentiation, proliferation and migration of these T-cells. Human leukocyte antigen (HLA) is among the

most important host proteins that are targeted in this response. Mismatches in HLA therefore lead to a more severe reaction compared to matched donor-recipient pairs[12]. The third phase is target cell destruction by cellular and inflammatory effectors, mainly occurring in gut, skin and liver. The third phase will also further enhance the first phase, creating a vicious circle. Most therapies, both prophylaxis and treatment, aim to reduce the T-cell response in the second phase. Approaches include inhibition of calcineurin, a T-cell activation marker (cyclosporin A, tacrolimus), reduction of T-cell proliferation (prednisolone, mycophenolate mofetil), purine synthesis (methotrexate) and T-cell depletion (serotherapy).

The depletion of donor T-cells is beneficial in the prevention of acute GvHD[13–15]. However, depletion of graft-infused T-cells also abrogates peripheral expansion. This leads to low or absent T-cell counts until thymopoiesis commences, this may take months to years. Donor derived T-cells are pivotal for resolving viral reactivations, which after HCT may cause significant morbidity and mortality (chapter 10). In addition, T-cells are important for the graft-versus-leukemia effect, a donor-driven anti-leukemic response (chapters 5, 6 and 9). This is reflected by large trials, showing a decrease in incidence of GvHD after inclusion of serotherapy, but no improvement in survival[16,17], probably due to increased mortality due to relapse and viral reactivations[13,18,19]. This leaves physicians with a dilemma: inclusion of serotherapy may have beneficial as well as harmful effects.

In the first large trial investigating ATG exposure measures as a predictor for clinical outcomes (chapter 5), we observed that the exposure to ATG after HCT was not a predictor for the incidence of acute GvHD in children. In other words, the amount of in vivo lymphodepletion of the graft-infused T-cells was not associated with the occurrence of acute GvHD. The probability of T-cell reconstitution was indeed impacted by exposure to ATG after HCT. On the other side, the incidence of acute GvHD was impacted by the exposure to ATG before infusion of the graft. It could therefore be hypothesized that, in children, the main working mechanism of ATG to prevent GvHD is not donor T-cell depletion, but rather is related to the depletion of host APC's. However, these results are restricted to myeloablative bone marrow and cord blood transplantation.

Compared to donor derived APC's, host APC's are significantly more potent in inducing acute GvHD[20–22]. Host APC's can be found in blood (hematopoietic APC) and peripheral tissues (non-hematopoietic APC). Of these, hematopoietic APC's are less likely to cause GvHD compared to the non-hematopoietic APC's, and are mostly depleted by chemotherapy before infusion of the graft[20,23]. Therefore, non-hematopoietic host APC's appear to have a central role in the development of acute GvHD. This is in line with our findings: high exposure to ATG before infusion of the graft prevents acute GvHD, potentially by depleting non-hematopoietic APC's. In animal studies, radioactive labeled alemtuzumab has been shown to distribute to peripheral tissues, including the manifesting organs of acute GvHD[24]. Moreover, labeled rabbit anti-dog IgG administered to dogs reaches higher concentrations in liver compared to blood[25]. Importantly, host APC's seem to have no or minor impact in

the graft-versus-leukemia effect[26,27]. Therefore, sufficient exposure to serotherapy before infusion of the graft seems to be an attractive approach to prevent acute GvHD. Combination of high exposure before HCT with low exposure after HCT to ensure T-cell reconstitution may give the best of both worlds: protection against GvHD, and powerful defense against relapse and viral reactivations.

The hypothesis described above does not seem to apply for adult patients receiving a peripheral blood stem cell (PBSC) transplant after reduced intensity conditioning (chapter 7). In these patients, acute GvHD is mainly impacted by the exposure to ATG after HCT, albeit being exposed to sufficiently high exposure of ATG before HCT. In other words, ample depletion of APC's is not enough to prevent GvHD in this adult setting; some in-vivo T-cell depletion of the graft is still needed. This may be due to the moderately toxic chemotherapy, the high numbers of graft-infused T-cells, or a combination of these. To be able to distinguish these factors, more research is needed in patients receiving a PBSC with full intensity conditioning and other stem cell sources with reduced intensity conditioning.

In conclusion, the working mechanism of serotherapy in preventing GvHD may include the depletion of host-derived antigen-presenting cells, most notably those residing in peripheral tissues. This challenges the current dogma that in-vivo T-cell depletion of the graft is the primary goal of serotherapy for preventing acute GvHD. However, this may not be true in all patients, including reduced intensity conditioning with PBSC grafts. Further research is needed to fully reveal the therapeutic window for all possible patient-donor-conditioning combinations.

ATG versus alemtuzumab: a PK/PD perspective

The choice between the two available products for serotherapy is mostly based on center or physician preferences. Some studies are available investigating the differences between ATG and alemtuzumab, in which the incidence of GvHD is lower after alemtuzumab[28–30,14,31]. This benefit is abrogated by very poor immune reconstitution compared to ATG[28,32], making survival after alemtuzumab-based serotherapy comparable to ATG[29,14,31]. However, most studies only compare the use of alemtuzumab versus ATG, and dose or exposure are not investigated as a predictor for outcome as we did in our analyses.

There are however significant differences between ATG (Thymoglobulin) and alemtuzumab in terms of PK and PD. Most striking is the difference in population clearance, which is 10 times (linear clearance) and 2 times (maximum elimination rate in saturable clearance) lower for alemtuzumab when compared to ATG (chapters 3 and 4). Unexplained between-patient variability is also considerably larger for alemtuzumab (chapters 3 and 4). Furthermore, the clearance of ATG is higher in patients with higher lymphocyte counts, while this is not a predictor for alemtuzumab PK. In terms of PD, the so-called lympholytic level is significantly lower in alemtuzumab (0.1 μg/mL) than ATG (1.0 AU/mL), as indicated with the dashed horizontal lines in figure 1[33,34]. Therefore, due to the lower clearance, the

fraction of exposure to alemtuzumab occurring after infusion of the graft is higher after standard dosages and uniform starting days. Therefore, alemtuzumab may be longer present at lympholytic levels compared to ATG, and pharmacokinetics are less predictable.

The exposure to alemtuzumab has minor impact on the outcomes of HCT (chapter 8) in terms of the incidence of GvHD. No impact of alemtuzumab exposure on survival or T-cell reconstitution was found. In addition, very few of the patients described in chapter 8 had an optimal alemtuzumab concentration on day 0 in terms of immune reconstitution[1]. Combined, these suggest that most patients are over-exposed to alemtuzumab using the current dosing regimen (0.5-1 mg/kg starting day -8).

Since T-cell reconstitution is a powerful predictor for survival, dosing of serotherapy should therefore be chosen aiming for minimal in-vivo T-cell depletion. Taking into account the clearance, the variability, its predictability, and the potency, ATG seems to be a more attractive therapeutic than alemtuzumab when used in an optimized, individualized dosing regimen.

Individualized dosing: the future of pediatric pharmacotherapy

Growth in children causes a change in pharmacokinetics and pharmacodynamics, and may impact the effects of drugs[35]. These changes are mostly not linear with age or body weight. Still, the majority of the pediatric dosing regimens are based on a fixed dose depicted in mg/kg, thereby assuming a linear relationship between body weight and PK/PD parameters. Additionally, the majority of drugs in children, especially in an academic setting, are used in an unlicensed or off-label manner[36]. For these drugs, few or no pharmacological studies have been performed, and pediatric dosing is a relatively uninformed extrapolation of adult dosing. This empirical dosing can lead to serious under- or overdosing in parts of the pediatric population, and thereby cause unpredictable and undesirable effects. Individualized dosing based on PK/PD modeling will lead to an important improvement in pediatric pharmacotherapy, and thereby potentially clinical outcomes[2,4,8].

As described in this thesis, empirical dosing can be detrimental in terms of safety and efficacy. ATG has been used in pediatric HCT for decades in a dose of 10 mg/kg, irrespective of any other variable other than body weight, and leading to very high exposures in older children. The dose of ATG in adult HCT varies, however all available regimens are based on body weight only. The presented results demonstrate that these fixed dosing regimens based on body weight lead to highly variable ATG exposure. This is particularly relevant because the exposure to ATG is a strong predictor for survival of the procedure. Optimal exposure to ATG leads to a survival of > 90% at 5 years, while survival in patients who are over-exposed is only 57-76% (chapter 5). To put this into perspective, these improvements in survival are in line with some of the major advances in hematology and oncology[37,38], although the results presented in this thesis have to be validated in a prospective study. Individualized dosing targeted to optimal exposure is expected to give a survival benefit after allogeneic

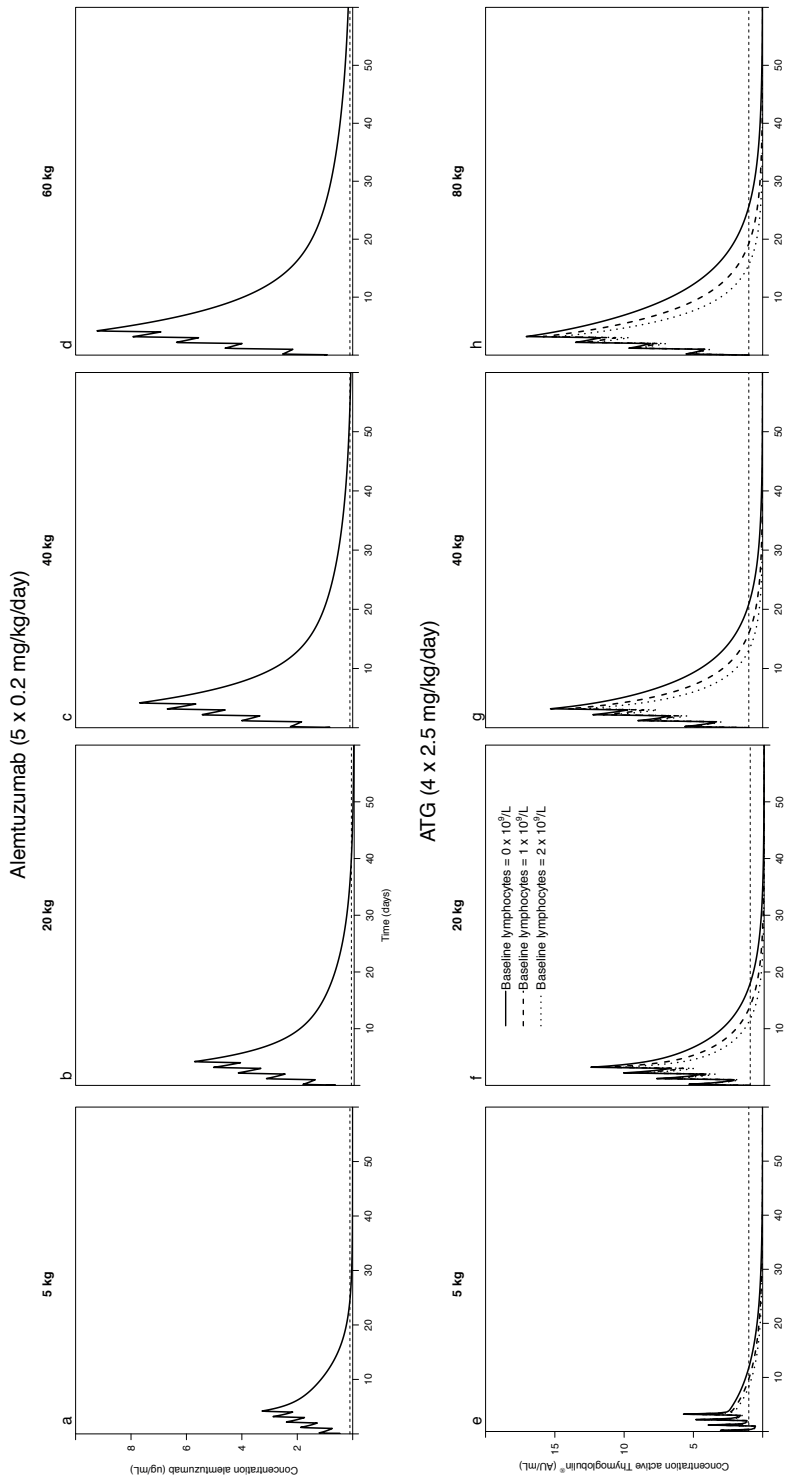


Figure 1. Simulated exposure after standard dosing of alemtuzumab (1 mg/kg cumulative over 5 days, panels a-d) and ATG (10 mg/kg cumulative over 4 days, panels e-h) in children weighing 5, 20, 40 and 80 kg. ATG exposures are stratified for lymphocyte counts. Dashed lines: lympholytic levels

HCT. Furthermore, the enhanced safety of HCT may also impact the selection of patients. For indications where HCT is currently part of the therapy, for instance leukemia and severe aplastic anemia, HCT may be considered more upfront in the treatment plan. Also, HCT may become a safe treatment option for diseases where HCT is currently not indicated, like steroid and immunotherapy resistant autoimmune disease and milder phenotypes of inborn errors of metabolism.

Further research should focus on the individualized dosing of other drugs with a critical therapeutic window. These include other drugs used in HCT (fludarabine, prednisolone) but also chemotherapy and tyrosin kinase inhibitors (TKI) in pediatric oncology and biologicals used in pediatric immunology. For all these drugs, little or no solid evidence is available for its currently used dose in these settings, while they form the backbone of the treatment. Dose individualization for these agents can significantly improve outcomes. In addition, pharmacotherapy in the fields of HCT, oncology and immunology frequently involves multiple agents, who combined determine the clinical effects. Therefore, a multi-agent PK/PD model is currently developed for all drugs used in the conditioning for pediatric HCT. This model takes into account any interactions and synergies between the different agents, both in terms of pharmacokinetics but also in pharmacodynamics.

Dose individualization will result in highly variable doses, which can potentially lead to dosing errors. Possible solutions include the use of dosing tables[39] or graphs, however the most likely solution is a computerized dosing system, either web-based or integrated in the hospital information system. This will be a change in prescription behavior for physicians, who have to be properly trained in using these systems. Albeit the technical implementation might take some effort, convincing physicians of the need for individualized dosing may be the biggest hurdle for its introduction. It is major change in pediatric pharmacotherapy compared to current standard of care. This starts in the training of registrars, where attention should be paid to the changing pharmacokinetics by growth and development and its impact on drug exposure and effects. Pediatricians should actively participate in the development of individualized dosing regimens to increase the involvement of the professionals. Finally, funds should be made available to support this research. Costs for the development of evidence based individualized dosing regimens are relatively low, and can be done in a limited amount of time. Since the direct clinical benefits can be significant, PK/PD modeling is an attractive field to financially support.

In conclusion, these examples stress the importance of individualized dosing, especially in pediatrics. Individualized dosing can enhance the safety of efficacy of drugs, and thereby improve clinical outcome.

GENERAL CONCLUSION

Unregistered and off-label use of drugs is common practice in pediatric care, often without available evidence based dosing guidelines. This also applies to anti-thymocyte globulin (ATG) and alemtuzumab, both used for lymphodepletion in hematopoietic cell transplantation (HCT) to prevent graft-versus-host-disease (GvHD) and graft failure. Main toxicities include absent or slow immune reconstitution, especially of T-cells with consequences with respect to morbidity and morality. This thesis investigates the population pharmacokinetics (PK) and pharmacodynamics (PD) of ATG and alemtuzumab in order to derive evidence based dosing regimens for both agents.

The pharmacokinetics of ATG and alemtuzumab in children can be well described using population PK-modeling, in which parameters were not found to increase linearly with age or body weight. Therefore, current weight-based dosing of ATG and alemtuzumab leads to highly biased exposures across the different age groups in the pediatric population. We found that exposure to ATG and alemtuzumab can be predicted and targeted based on body weight and peripheral blood lymphocyte counts (the latter only in ATG). Furthermore, ATG clearance was not found to increase with increasing body weight in patient over 50 kg (i.e. adolescents and adults). This indicates that fixed dose rather than mg/kg dosing in adults and individuals over 50 kilograms will result in equal exposures.

Timely CD4+ T-cell immune reconstitution after HCT is essential for reducing viral reactivations and relapse following HCT, and thereby improves survival chances. High exposure to ATG after infusion of the graft diminishes chances for CD4+ T-cell reconstitution. Therefore, exposure to ATG has a major impact on the clinical outcomes including survival following HCT in children and adults. T-cell reconstitution is generally poor using standard doses of ATG following cord blood transplantation. However, provided the exposure to ATG after HCT is very low, CD4+ immune reconstitution after cord blood transplant proved excellent.

The protective effect of ATG for GvHD and graft failure is determined by the exposure before infusion of the graft in children. However, in adults receiving mild chemotherapy combined with high number of infused cells (peripheral blood stem cell grafts), some exposure to ATG after graft infusion is needed to prevent GvHD. Exposure to alemtuzumab on the other hand impacts the incidence of acute GvHD, but not toxicity endpoints, possibly indicating a relative overdose using current dosing regimens.

We conclude that individualizing dosing and timing of ATG potentially makes HCT a safer and more effective treatment option, and will lead to improved survival chances. Individualized dosing regimens for ATG in children have been designed based on the results in this thesis, and are currently being evaluated in prospective clinical trials for efficacy and safety.

REFERENCES

1. Marsh RA, Lane A, Mehta PA, et al. Alemtuzumab levels impact acute GVHD, mixed chimerism, and lymphocyte recovery following alemtuzumab, fludarabine, and melphalan RIC HCT. *Blood*. 2015;127(4):503–513.
2. Knibbe CAJ, Krekels EHJ, Danhof M. Advances in paediatric pharmacokinetics. E*xpert Opin Drug Metab Toxicol*. 2011;7(1):1–8.
3. Ince I, Wildt SN De, Tibboel D, Danhof M, Knibbe CAJ. Tailor-made drug treatment for children: creation of an infrastructure for data-sharing and population PK – PD modeling. *Drug Discov Today*. 2009;14:316–320.
4. Admiraal R, van Kesteren C, Boelens JJ, Bredius RGM, Tibboel D, Knibbe C a J. Towards evidence-based dosing regimens in children on the basis of population pharmacokinetic pharmacodynamic modelling. *Arch Dis Child*. 2014;99(3):267–72.
5. De Cock RFW, Piana C, Krekels EHJ, Danhof M, Allegaert K, Knibbe C a J. The role of population PK-PD modelling in paediatric clinical research. *Eur J Clin Pharmacol*. 2011;67 Suppl 1:5–16.
6. Mould D. Why therapeutic drug monitoring is needed for monoclonal antibodies and how do we implement this? *Clin Pharmacol Ther*. 2016;99(4):351–354.
7. Lalmohamed A, Bartelink I, van Reij L, et al. Studying the Optimal Intravenous Busulfan Exposure in Pediatric Allogeneic Hematopoietic Cell Transplantation (alloHCT) to Improve Clinical Outcomes: A Multicenter Study. *Biol Blood Marrow Transpl*. 2015;21(2):S102–S103.
8. Mould DR, D'Haens G, Upton RN. Clinical Decision Support Tools: The Evolution of a Revolution. *Clin Pharmacol Ther*. 2016;66(5):732–40.
9. Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. *Leukemia*. 2007;21(7):1387–94.
10. Kanda J, Lopez RD, Rizzieri DA. Alemtuzumab for the prevention and treatment of graft-versus-host disease. *Int J Hematol*. 2011;93(5):586–593.
11. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;373(9674):1550–1561.
12. Loiseau P, Busson M, Balere ML, et al. HLA Association with Hematopoietic Stem Cell Transplantation Outcome: The Number of Mismatches at HLA-A, -B, -C, -DRB1, or -DQB1 Is Strongly Associated with Overall Survival. *Biol Blood Marrow* Transplant. 2007;13(8):965–974.
13. Lindemans CA, Chiesa R, Amrolia PJ, et al. Impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune reconstitution and clinical outcome. *Blood*. 2014;123(1):126–32.
14. Veys P, Wynn RF, Ahn KW, et al. Impact of immune modulation with in vivo T cell depletion and myleoablative total body irradiation conditioning regimen on outcomes after unrelated donor transplantation for acute lymphoblastic leukemia in children. *Blood*. 2012;119(25):6155–6162.
15. Booth C, Veys P. T cell depletion in paediatric stem cell transplantation. *Clin Exp Immunol*. 2013;172(2):139–47.
16. Finke J, Bethge WA, Schmoor C, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol*. 2009;10(9):855–64.
17. Theurich S, Fischmann H, Chemnitz J, Holtick U, Scheid C, Skoetz N. Polyclonal anti-thymocyte globulins for the prophylaxis of graft-versus-host disease after allogeneic stem cell or bone marrow transplantation in adults. *Cochrane Database Syst Rev*. 2012;(9).

18. Seidel MG, Fritsch G, Matthes-Martin S, et al. Antithymocyte Globulin Pharmacokinetics in Pediatric Patients After Hematopoietic Stem Cell Transplantation. *J Pediatr Hematol Oncol*. 2005;27(10):532–536.
19. Bacigalupo A, Lamparelli T, Bruzzi P, et al. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood*. 2001;98(10):2942–2947.
20. Koyama M, Kuns RD, Olver SD, et al. Recipient nonhematopoietic antigen-presenting cells are sufficient to induce lethal acute graft-versus-host disease. *Nat Med*. 2011;18(1):135–142.
21. Shlomchik WD, Couzens MS, Tang CB, et al. Prevention of Graft Versus Host Disease by Inactivation of Host. *Science* 1999;285(July):412–415.
22. Duffner UA, Maeda Y, Cooke KR, et al. Host Dendritic Cells Alone Are Sufficient to Initiate Acute Graft-versus-Host Disease. *J Immunol.* 2004;172(12):7393–7398.
23. Li H, Demetris AJ, McNiff J, et al. Profound depletion of host conventional dendritic cells, plasmacytoid dendritic cells, and B cells does not prevent graft-versus-host disease induction. *J Immunol*. 2012;188(8):3804–11.
24. De Decker M, Bacher K, Thierens H, Slegers G, Dierckx R a, De Vos F. In vitro and in vivo evaluation of direct rhenium-188-labeled anti-CD52 monoclonal antibody alemtuzumab for radioimmunotherapy of B-cell chronic lymphocytic leukemia. *Nucl Med Biol*. 2008;35(5):599–604.
25. McAfee JG, Gagne G, Subramanian G, Schneider RF. The localization of indium-111-leukocytes, gallium-67-polyclonal IgG and other radioactive agents in acute focal inflammatory lesions. *J Nucl Med*. 1991;32(11):2126–31.
26. Teshima T, Ordemann R, Reddy P, et al. Acute graft-versus-host disease does not require alloantigen expression on host epithelium. *Nat Med*. 2002;8(6):575–581.
27. MacDonald KP, Shlomchik WD, Reddy P. Biology of Graft-versus-Host Responses: Recent Insights. *Biol Blood Marrow Transplant*. 2013;19(1 SUPPL.):S10–S14.
28. Willemsen L, Jol-van der Zijde CM, Admiraal R, et al. Impact of Serotherapy on Immune Reconstitution and Survival Outcomes After Stem Cell Transplantations in Children: Thymoglobulin Versus Alemtuzumab. *Biol Blood Marrow Transplant*. 2015;21(3):473–482.
29. Marsh JC, Pearce RM, Koh MBC, et al. Retrospective study of alemtuzumab vs ATG-based conditioning without irradiation for unrelated and matched sibling donor transplants in acquired severe aplastic anemia: a study from the British Society for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2014;49(1):42–8.
30. Soiffer RJ, Lerademacher J, Ho V, et al. Impact of immune modulation with anti – T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies Impact of immune modulation with anti – T-cell antibodies on the outcome of reduc. *Blood*. 2011;117(25):6963–70.
31. Norlin A-C, Remberger M. A comparison of Campath and Thymoglobulin as part of the conditioning before allogeneic hematopoietic stem cell transplantation. *Eur J Haematol*. 2011;86(1):57–66.
32. Park SH, Choi S-M, Lee D-G, et al. Infectious complications associated with alemtuzumab use for allogeneic hematopoietic stem cell transplantation: comparison with anti-thymocyte globulin. *Transpl Infect Dis*. 2009;11(5):413–23.
33. Waller EK, Langston A a, Lonial S, et al. Pharmacokinetics and pharmacodynamics of anti-thymocyte globulin in recipients of partially HLA-matched blood hematopoietic progenitor cell transplantation. *Biol Blood Marrow Transplant*. 2003;9(7):460–471.

34. Morris EC, Rebello P, Thomson KJ, et al. Pharmacokinetics of alemtuzumab used for in vivo and in vitro T-cell depletion in allogeneic transplantations: Relevance for early adoptive immunotherapy and infectious complications. *Blood*. 2003;102(1):404–406.
35. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DW, Leeder JS, Kauffman RE. Developmental pharmacology—drug disposition, action and therapy in infants and children. *new Engl J Med drug Ther.* 2003;349(12):1157–1167.
36. Kimland E, Odlind V. Off-label drug use in pediatric patients. *Clin Pharmacol Ther*. 2012;91(5):796–801.
37. Yu AL, Gilman AL, Ozkaynak MF, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med*. 2010;363(14):1324–1334.
38. Maude SL, Frey N, Shaw PA, et al. Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia. *N Engl J Med*. 2014;371(16):1507–1517.
39. Bartelink IH, van Kesteren C, Boelens JJ, et al. Predictive performance of a busulfan pharmacokinetic model in children and young adults. *Ther Drug Monit*. 2012;34(5):574–83.