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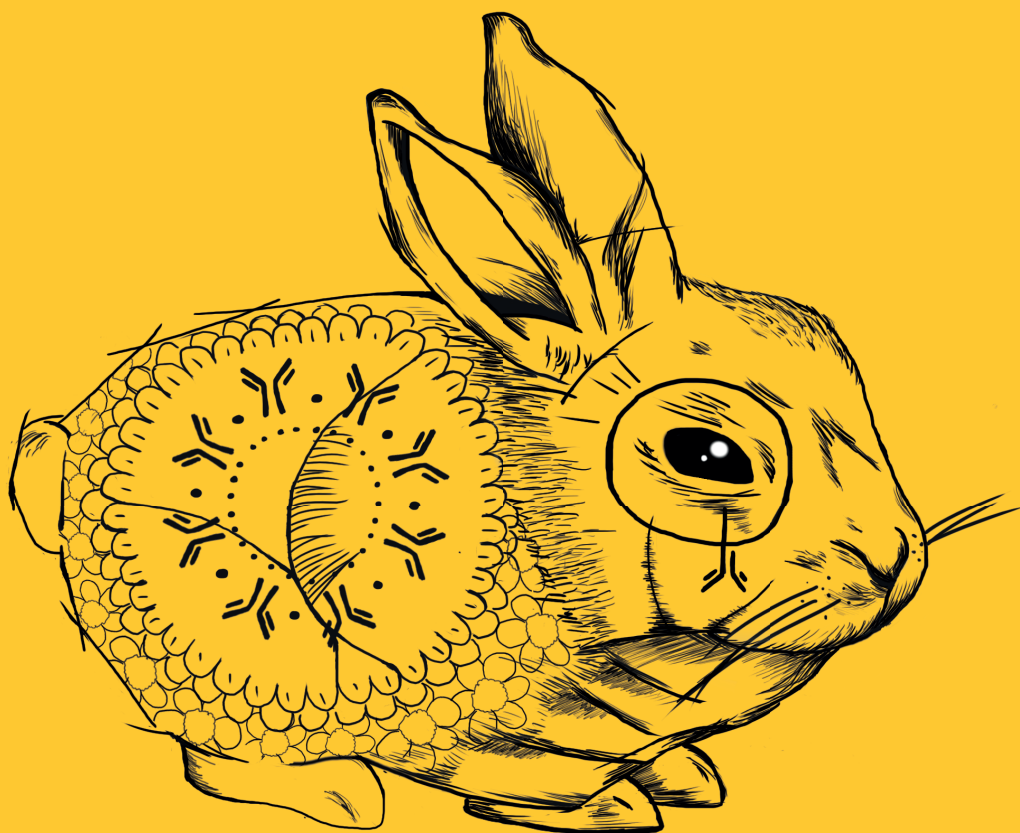**Issue Date**: 2017-03-15

Rick Admiraal

Individualized Dosing of Serotherapy in Allogeneic Hematopoietic Cell Transplantation

A Delicate Balance

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A delicate balance

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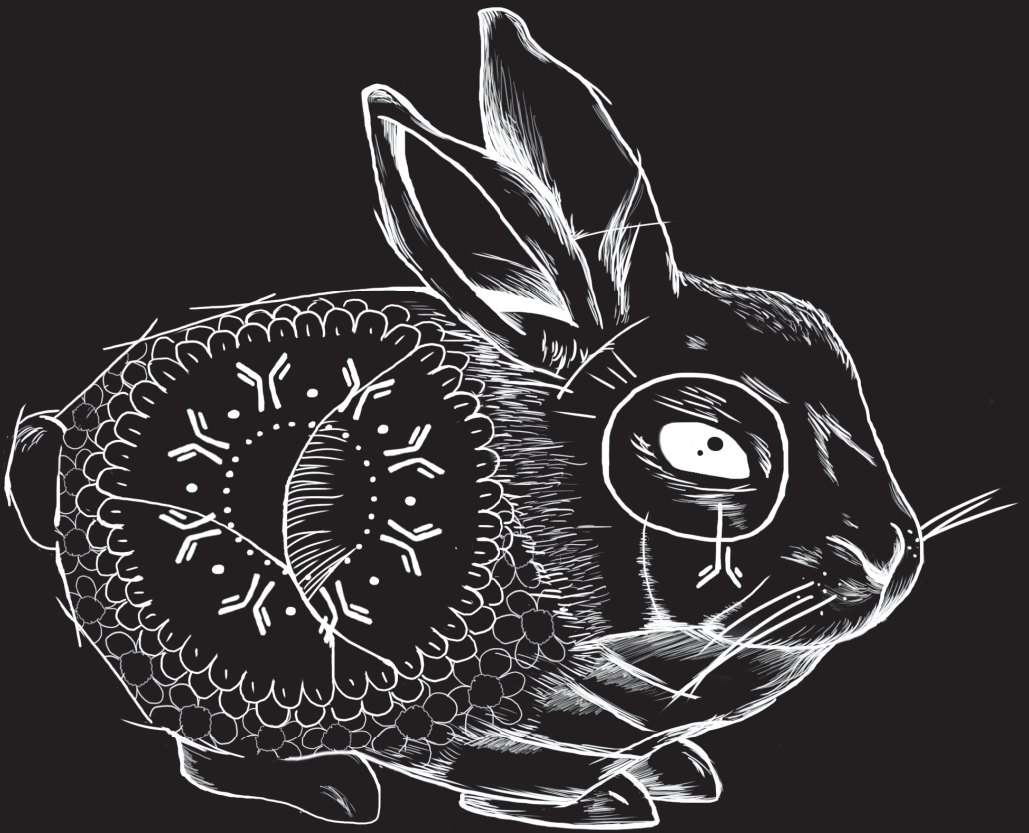Rick Admiraal

geboren te Beverwijk
in 1984

CONTENTS

PART I

Introduction

Chapter 1

Individualized Dosing of Serotherapy in Allogeneic Hematopoietic Cell Transplantation: Scope and Intent of the Investigations

GENERAL INTRODUCTION

Allogeneic hematopoietic cell transplantation

Allogeneic hematopoietic cell transplantation (HCT) is a potentially life-saving procedure by transplanting donor-derived hematopoietic stem cells and lymphocytes into to a patient. The technique is also referred to as stem cell transplantation, however this is not fully correct due to co-infusion of lymphocytes and other hematopoietic cells. Indications for HCT include malignant (leukemia, lymphoma) and non-malignant disorders (primary immune deficiencies, bone marrow failure, inborn errors of metabolism and hemoglobinopathies)[1–3]. During this procedure, the diseased bone marrow and cellular immune system is replaced by a healthy, donor-derived hematopoietic system.

The donor cells can be harvested from a donor in several ways, and can be either from related and unrelated donors. Historically, an identical sibling was the most predominant stem cells source used in HCT. As two siblings only have a 25% chance of being human leukocyte antigen (HLA) identical, expansion of the donor pool was needed to be able to offer HCT to more patients. Bone marrow donor registries for unrelated donors were established, the first registry was introduced in 1973 in the United Kingdom[4]. During the late 1980's, better HLA-typing expanded the possibilities to use grafts from both related and unrelated donors[4,5]. Nowadays, transplanted donor cells can be either derived from bone marrow (BM), mobilized peripheral blood stem cells (PBSC), or umbilical cord blood (CB), from either related or unrelated donors. Each source has its advantages and disadvantages. Compared to BM, the main advantage of PBSC includes the harvesting of cells that can be performed without anesthesia and sedation[6]. Cord blood on the other hand has less stringent HLA-matching criteria, and has the advantage to be promptly available[5]. However, the number of cells are lower in CB and BM when compared to PBSC, although the latter is associated with a higher incidence of chronic graft-versus–host-disease[7].

In 2014, approximately 1 in 40.000 United States inhabitants received an allogeneic HCT[8]. In the Netherlands a total of 350 first HCT's are performed annually, of which approximately 80-90 in children[9].

Principles of HCT

The treatment plan for HCT depends on the disease, age, comorbidities, previous treatments, stem cell source and local protocols, and can therefore vary considerably between patients. Still, the main components for any HCT are the same, and are depicted in figure 1.
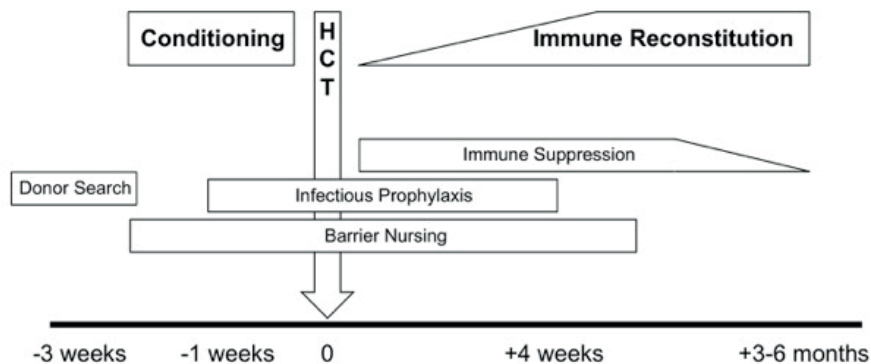
Figure 1. Overview of treatment plan for HCT

The donor search starts when a patient becomes eligible for HCT and is registered to the HCT unit. Based on center preference and donor availability, HLA-matching and donor cell counts, the most optimal donor is selected[5].

The conditioning phase starts approximately one week before infusion of the stem cells, however some centers including the UMC Utrecht start conditioning earlier[10]. The main goal of the conditioning is to deplete the bone marrow and suppress the host immune system. Additionally, in case of malignancy, the conditioning regimen depletes any re-sidual leukemic cells. Bone marrow depletion, or myeloablation, is mostly performed using chemotherapy, while some patients receive chemotherapy combined with total body irra-diation (TBI)[11,12]. Chemotherapy-based conditionings mostly consist of an alkylating agent (busulfan, melphalan, treosulfan) combined with a second cytostatic drug (fludarabine, cyclophosphamide)[13–15]. The alkylator mainly gives myeloablation, while fludarabine and cyclophosphamide are used for immunosuppression and immunoablation. Clofarabine, a purine antinucleotide, can be added to the conditioning regimen for malignant indications[16]. In TBI-containing regimens, TBI is used for myeloablation as well as immunosuppression, and is combined with a cytostatic drug. In recent years, non-myeloablative regimens or reduced intensity conditioning (RIC) has been increasingly used for older patients (>60 years) and those in poor clinical condition[17–19]. These patients usually receive low dose TBI, cyclophosphamide, or low dose busulfan or thiotepa, all combined with fludarabine.

Serotherapy is another important component of the conditioning regimen, introduced to prevent graft-versus-host disease (GvHD) and rejection[20]. The main mechanism of action of serotherapy is in-vivo lymphodepletion, mainly of T-cells, although it is thought to have some immune-modulatory properties as well[21]. Anti-thymocyte globulin (ATG) and alemtuzumab (Campath®) are the two drugs used for this indication[22–24]. ATG is the product of vaccinating rabbits or horses with human lymphocytes or whole thymus tissue, and is

therefore a polyclonal non-humanized IgG antibody with many epitopes directed various human cell-bound targets[21,25]. Alemtuzumab on the other hand is a monoclonal humanized anti-CD52 IgG antibody.

Starting some days before HCT, immune suppression is given as GvHD prophylaxis. The cornerstone of GvHD prophylaxis is cyclosporin A (CsA), a calcineurin inhibitor, which is combined with prednisolone, methotrexate or mycophenolate mofetil (MMF) depending on the stem cell source[4]. In case of CsA toxicity, patients are switched to tacrolimus or sirolimus[26]. Immune suppressive therapy is given up to 3-4 weeks after HCT, after which it is carefully tapered.

Supportive care consists of infectious prophylaxis (standard antifungals, antivirals, and pneumocystis jiroveci prophylaxis) and selective gut decontamination, as well as treatment in high-efficiency, particle-free, positive pressure rooms[4,27,28]. All medical and nursing staff perform barrier nursing during the admission of any patient.

Immune reconstitution following HCT can be separated in neutrophil recovery and lymphocyte reconstitution. When focusing on neutrophil recovery, patients will experience a phase of neutropenia starting approximately 14 days after the first dose of busulfan or TBI, which reflects the transit time for neutrophils[29,30]. From this moment onwards, the patient will depend on donor-stem cell derived neutrophils, which will enter the peripheral blood around day 14-25 after HCT[31]. Patients are highly susceptible for bacterial and fungal infections during this time of neutropenia. Lymphocytes on the other hand are mainly depleted by serotherapy, which causes a rapid decline in peripheral blood lymphocyte counts, and to a lesser extend of tissue lymphocytes[22,32]. Within the lymphocyte compartment, reconstitution of NK-cells occurs parallel to neutrophil reconstitution, while B-cells start to be detectable on day +40 after HCT[32].

Reconstitution of T-cells following HCT is markedly different compared to other lymphocytes[22,33,34]. Two distinct routes of T-cell reconstitution can be identified: peripheral expansion and thymopoiesis. Under the influence of interleukin (IL)-7, IL-15, IL-21 and tumor growth factor (TGF) β, graft-infused T-cells divide to give rise to a relatively oligoclonal T-cell population[33,35]. However, although this T-cell population has a skewed T-cell receptor (TCR) repertoire, these cells seem effective in clearing viral infections, which is most pronounced in CB[35,36]. Depending on thymic function, output of naïve T-cells through thymopoiesis commences 3-6 months after HCT[37,38]. Several factors negatively influence thymic function, including steroid use, GvHD and age[37]. In light of the relatively long time-window between HCT and thymopoiesis, patients fully depend on peripheral expansion during the most critical time after HCT in terms of mortality[31,35,39]. Hence, the graft-infused T-cells are crucial, and must be protected against rigorous depletion[33,40–42]. Exposure of donor T-cells

to serotherapy and immuno-ablative cytotoxic agents as fludarabine can potentially result in severe lymphodepletion, thereby abrogating early T-cell immune reconstitution. Serotherapy is more potent and has a significantly longer half-life compared to fludarabine[43–46], and therefore has a greater influence on T-cell immune reconstitution following HCT.

Limitations of HCT

The major limitations of HCT include 1) transplant-related mortality, 2) relapse of disease, and 3) late effects.

1) The main causes of transplant-related mortality include alloreactivity and infections. Alloreactivity in HCT can manifest as either GvHD or graft rejection. GvHD can present acutely, manifesting in skin, gut or liver, or in a more chronic way, mainly in skin, mucous membranes, lungs and as cytopenias[47–49]. A three-step model is mostly used to describe the pathophysiology of acute GvHD[50]. First, tissue damage, either pre-existing or caused by the conditioning regimen, leads to antigen presenting cell (APC) activation. Next, host APC's activate donor T-cells, which finally give rise to an inflammatory reaction. This process leads to tissue damage, followed by more APC activation, resulting in a self-reinforcing process. The pathophysiology of chronic GvHD on the other hand is poorly understood. The main treatment for acute and chronic GvHD is steroids, steroid refractory GvHD has abominable outcome.

As opposed to GvHD, graft rejection is an immunological reaction of host cells towards the donor. Here, host T-cells give rise to a cellular response against the donor stem cells[51]. Outcome following graft rejection are negatively impacted by infections as well as a high chance on developing a second graft failure[52].

The main predictor for GvHD and rejection is HLA-disparity between donor and recipient, however many other factors including viral reactivations, the gut microbiome and pharmacotherapy may also play a role[50,51,53,54].

Infections are another important contributor to morbidity and mortality. Following the conditioning regimen, patients will go through a period of 2-3 weeks of neutropenia dependent on rate of engraftment, leaving the patient vulnerable for bacterial and fungal infections[55,56]. During and after this neutropenic period, cellular immunity may be hampered up to months after HCT depending on the level of immunosuppression and T-cell depletion[22,31,57]. The main effector cells for cellular immunity are lymphocytes, including T-cells, B-cells and NK-cells. This puts patients at risk for reactivations of previously encountered viral infections, including adenovirus, cytomegalovirus and Epstein Barr virus[58–62], but also relapse.

2) Relapse of the underlying malignancy is another major limitation of HCT, occurring in 10-30% of patients[63–65]. Disease status, remission status and tumor burden before HCT

expressed in minimal residual disease (MRD) are predictors for relapse[66–69]. The main mechanisms for tumor control by HCT include high doses of myeloablative chemotherapy and the so-called graft-versus-leukemia (GvL) effect, a donor T-cell-driven response against residual leukemic blasts[70]. This stresses the importance of T-cell reconstitution after HCT for preventing relapse[39].

3) With the higher survival rates after HCT, late effects become increasingly important. Late effects may have a significant impact on the quality of life, which particularly in children is pivotal. Chronic GvHD requiring systemic immune suppression is associated with infections, poor quality of life and premature death. Growth and cognitive capabilities may be impaired in children following HCT, the latter mainly following central nervous system irradiation[71]. Fertility may be hampered in patients receiving a HCT as a child due to ovarian dysfunction or decreased spermatogenesis[72–74]. Secondary malignancies as a result of any chemotherapy-treatment and/or radiation, is a rare but serious late effect.

In recent years, HCT has become a safer procedure through less toxic conditioning regimens, novel therapeutic options for treatment and prevention of relapse and GvHD, improvements in donor selection, promising alternative donor sources, and better supportive care[2,3,75,76]. However, therapy- and relapse related mortality as well as long-term morbidity remains to be a limitation of HCT. Further enhancement of the safety of the procedure as well as getting better disease control can further improve the outcomes of HCT[77]. As pointed out above, the number of characteristics introduced to the treatment is significant, including patient, donor, conditioning and supportive care. A uniform treatment plan for all patients may therefore lead to under- or overtreatment in certain part of patients. Therefore, a promising approach to improve outcomes is by individualizing the treatment. This includes risk stratification for treatment intensity, individualized dosing of agents used in the conditioning regimen, and adjuvant cellular therapies targeting specific tumor markers[68,78,79]. Besides improved outcomes, safer and more effective treatment may extend the indications for HCT towards lower risk malignancies and milder phenotypes of benign disease.

As discussed above, timely immune reconstitution is an important predictor for infectious disease and relapse. Serotherapy, given in the conditioning regimen prior to transplantation in order to prevent GvHD, may significantly delay immune reconstitution.

This thesis will focus on the pharmacokinetics and pharmacodynamics of serotherapy, both ATG and alemtuzumab, in order to derive an individual dosing regimen for both agents.

History of serotherapy: ATG and alemtuzumab

Currently, two agents are used for serotherapy: ATG and alemtuzumab.

ATG was introduced in the late 1960's to prevent rejection following solid organ transplant and graft-versus-host disease in HCT[80,81]. At that time, ATG was mainly referred to as anti-lymphocyte serum (ALS), and consisted of immunized rabbit serum as opposed to currently used purified IgG. Currently, several products of ATG are on the market, which however are not biosimilar. ATG is made by immunizing rabbits with whole thymus tissue (Thymoglobulin®, Genzyme, Cambridge, MA, USA) or the Jurkat human T-lymphocyte cell line (ATG-Fresenius® S, Neovii Biotech, Munich, Germany), or by immunizing horses with human T-cells (ATGAM®, Pfizer, NY, USA). As ATG consists of purified rabbit or horse IgG, all preparations are polyclonal antibodies with varying numbers of epitopes for potential binding[21]. Additionally, the number of IgG-molecules targeted against human markers (referred to as active ATG) may differ from animal to animal[21]. Therefore, IgG from many immunized animals is pooled aiming for a stable and comparable product. Additionally, the percentage active ATG differs between the different ATG products. In Thymoglobulin, the most commonly used ATG preparation in HCT, approximately 9% of total rabbit IgG is directed to human markers[46,82].

In 1983, first reports on alemtuzumab (Campath) were published[83]. At first, Campath was a monoclonal rat-anti-human IgM antibody, and was later humanized. This served as the basis for the currently used drug alemtuzumab, an anti-CD52 IgG antibody[84]. CD52 is mainly expressed on cells originating from the lymphoid lineage, and is not expressed on hematopoietic stem cells. In 1991, alemtuzumab was approved as a treatment for chronic lymphatic leukemia and as serotherapy in HCT. Nowadays it is most frequently used in the United Kingdom and in selected treatment protocols. In recent years, alemtuzumab (marketed as Lemtrada®, Genzyme, Cambridge, MA, USA) has been introduced as a treatment for remitting-relapsing multiple sclerosis (RR-MS), and has subsequently been withdrawn for all other indications. However, alemtuzumab is still available for HCT through a compassionate use program.

Pharmacology of antibodies

All drugs used for serotherapy are antibodies, which often display pharmacokinetics (PK) that are distinctively different compared to small molecules, comprising the majority of drugs on the market. The most striking difference is the size of the drugs: the molecular weight of antibodies is in the order of 150 kDa, a 1000 times the molecular weight of a drug like acetaminophen. This has a major impact on absorption, distribution and elimination of antibodies.

Oral dosing is not possible due to denaturation of proteins in the acidic gastric environment; therefore administration will be confined to intravenous and subcutaneous dosing[85]. Bio-availability of antibodies following subcutaneous administration is however relatively low (50-80%) due to proteolytic degradation in the lymphatic system[86].

The distribution of antibodies is mainly confined to the intravascular space due to size and polarity[87], however some distribution towards peripheral tissues may occur.

Elimination of antibodies is very different compared to small molecules. Hydrophobic small molecules undergo metabolism, followed excretion in bile or urine, while hydrophilic drugs are mainly excreted unchanged in urine. Elimination of antibodies comprehends neither renal nor hepatic involvement; main elimination routes include target binding and non-specific degradation (proteolysis and endocytosis). Target binding, mainly referred to as target mediated drug disposition (TMDD), is both the main mechanism of action of antibodies as well as the main elimination route. Antibodies have a high affinity to their target, which can be divided into cell-bound and soluble targets. For cell-bound targets, following binding of the antibody, the host cell is killed either by complement-dependent cytotoxicity (CDC), antibody dependent cell-mediated cytotoxicity (ADCC) or signaling-induced apoptosis[88]. Phagocytic cells such as macrophages will clear remains of the target cells, including antibody-target complexes. Soluble targets, after formation of an antibody-target-complex, bind to Fcγ-receptors present on macrophages and monocyte, are internalized and degraded[89]. Target binding, both to soluble and cell-bound targets, is dependent on the amount of target available. This makes the clearance of antibodies dependent on target concentrations: high concentrations lead to high clearance, while clearance is usually very low with a low target concentration.

Besides target mediated drug disposition, therapeutic antibodies undergo non-specific degradation comparable to endogenous IgG through fluid-phase endocytosis by phagocytic cells. Binding to the FcRn (or neonatal Fc-receptor, Brambell-receptor) salvages IgG after endocytosis by redirection to the cell-surface. At the cell surface, the physiological pH breaches the binding with IgG resulting in a recycling of the molecule. This recycling mechanism applies to antibodies as well, and is an explanation for the relatively long half-lives seen in antibodies. However, the efficacy of recycling is determined by the affinity to FcRn, which depends on the species from which the Fc-region of the antibody is derived. Finally, a third method of antibody elimination may occur after the development of anti-drug-antibodies (ADA). Clearance through ADA is comparable to TMDD of soluble targets, however here patient-derived antibodies bind to a therapeutic antibody. Development of ADA significantly shortens half-life of the therapeutic antibody, making it largely ineffective[85,87,90]. The incidence of ADA depends on the immunogenity of the antibody: chances are

smaller in fully humanized antibodies compared to non-humanized, and to a lesser degree in chimeric antibodies.

The highly variable PK of antibodies due to multiple mechanisms of clearance, most strikingly TMDD, results in a highly variable exposure to serotherapy between patients.

The role of serotherapy in GvHD, graft failure and T-cell immune reconstitution

Serotherapy is among the most potent drugs available to prevent graft failure and GvHD following HCT through in-vivo lymphodepletion of the graft[20,34,91–94]. Additionally, serotherapy depletes antigen-presenting cells (APC's), including dendritic cells (DC), residing in gut, skin and lungs. Depletion of APCs may contribute to abrogate the first step in the development of GvHD[21].

Currently, all serotherapy agents are dosed empirically, i.e. a fixed mg/kg dose in all children irrespective of age or body weight. Dosing of serotherapy in the pediatric population is neither based on scientific evidence nor does it take into account the complex pharmacokinetics of antibodies. This is mostly true for adults as well, as sophisticated techniques for dose selection were not common practice at the time of registration for the two respective agents.

The therapeutic window of serotherapy is limited by T-cell immune reconstitution[33]. Due to the very long half-life of ATG and alemtuzumab (7-14 days), patients may be exposed to ATG or alemtuzumab both before and after infusion of the graft[44,46,95–100]. In line with the mechanism of action, exposure of graft-infused T-cells to serotherapy may give depletion, thereby diminishing chances on early T-cell reconstitution through peripheral expansion. This makes the therapeutic window for serotherapy critical: under-exposure may lead to GvHD and graft failure, while over-exposure results in delayed or absent T-cell reconstitution. In addition, the starting day relative to infusion of the graft also impacts the proportion of exposure before and after graft infusion.

This is in line with most clinical outcomes: while the inclusion of ATG leads to a significant decrease in acute and chronic GvHD, no survival advantage has yet been demonstrated[20,91]. In these large trials, immune reconstitution and viral reactivations are mostly not reported, however it is plausible that the decrease in GvHD-related mortality is balanced by increased mortality due to poor T-cell reconstitution. As such, it seems that the beneficial properties of ATG, and potentially also of alemtuzumab, are abrogated by deleterious side effects.

Several explanations for the non-superiority in survival after introduction of ATG can be hypothesized. A first possibility may be that the optimal serotherapy exposure for prevent-

ing GvHD and rejection is overlapping with the optimal ATG for promoting immune reconstitution. In this scenario, no optimal exposure can be defined that leads to both efficacy and safety. Alternatively, there may be an optimal exposure, but due to the high variability in PK[44–46,96], the actual exposure to ATG is similarly variable[101]. Therefore some patients will be under-exposed, some over-exposed, and some have optimal exposure, which overall will not lead to improved survival. For ATG and alemtuzumab however, an optimal dose or optimal exposure has not yet been determined, especially in pediatric populations[87,101]. This optimal dose may also depend on transplant-related factors like stem cell source, HLA-mismatch etcetera.

Towards individualized dosing in children and adults

Historically, the vast majority of drug development studies were performed in adults. Many drugs are not evaluated in children, contributing to off-label or unlicensed use in as high as 49–87% of drugs used in tertiary care hospitals[102,103]. Pediatric dosing regimens are often empirical, linearly extrapolated from adult dosing based on body weight. When using a per kilogram dose, the assumption is made that the PK (e.g. clearance, volume of distribution) also increase linearly with body weight in order to reach comparable concentrations. In addition, the assumption is made that the concentration-effect relationship is comparable between children and adults. However, since developmental changes are mostly non-linear[104], empirical dosing can lead to underdosing or overdosing. This is especially true in the very young children and adolescents, thereby introducing toxicity or reduced efficacy[103,105]. In order to reach optimal exposure in all patients, the PK and pharmacodynamics (PD) need to be described, including the influence of predictors such as body size on PK and PD. With these models, the optimal dose for any individual patient can be predicted to reach optimal exposure. This approach has been demonstrated in pediatric HCT[106]. While most cytostatic agents used in HCT are dosed using a fixed mg/kg or mg/m^2 dose for all patients, busulfan dose is fully individualized and controlled using therapeutic drug monitoring (TDM)[106]. Recent work has shown that actual exposure to busulfan impacts outcome in terms of toxicity, graft failure and relapse[107–109].

The population approach, using advanced non-linear mixed effects modeling and high computing power, is the preferred method for PK analyses according to both the FDA and EMEA guidelines[110,111]. Previously, the so-called two-step approach was the method of choice. In this approach, PK-parameters are individually determined for which full sampling is required in all patients. Next, descriptive statistics are applied to the PK-parameters in the whole population. In the population approach, data from all patients is pooled to estimate a population mean for all PK-parameters[112]. Next, based on individual concentrations inter-individual variability and residual error are calculated for each patient. Main advantage of the population approach is the ability to use sparsely sampled and unbalanced (differences

in number of samples and sample times between patients, as often the case) data[113]. This makes the population approach particularly attractive in pediatrics, where few samples are available and the absolute dose varies significantly between children. Additionally, the estimation of PK-parameters is more robust as the software is able to differentiate between real inter-individual variability and residual error (a combination of incorrect sample times, measurement errors and model misspecification)[114]. All together, from an ethical, practical and methodological point of view, the population approach is the preferred method for PK analyses.

After describing the population pharmacokinetics, the relationship between concentrations or exposure and effects or toxicity (PD) needs to be determined. The PD-analysis will give further insight into the therapeutic window, and will set an optimal target exposure. Next, an individualized dosing regimen can be designed using the population PK model, aiming for optimal exposure. The proposed individualized dosing regimen should be evaluated in a prospective trial, both for external validation of the PK-model and the clinical safety and efficacy[115].

CONCLUSION

HCT provides a final and potentially curative treatment option for a number of malignant and benign disorders. However, there is a need for improved survival chances after HCT, which may be accomplished by improving disease control and reducing the toxicity of the procedure. Serotherapy plays an important role in clinical outcomes following HCT, both in preventing GvHD and graft failure as well as enabling timely T-cell immune reconstitution. T-cell reconstitution has a crucial role in preventing viral disease and relapse following HCT, and therefore potentially has an impact on survival. However, although serotherapy seems to be of vital importance in HCT, the most optimal dose has not yet been defined. In fact, the pharmacokinetics of serotherapy are highly variable and poorly understood. In addition, the therapeutic window for both ATG and alemtuzumab is not known. There is a stringent need for an evidence-based, individualized dosing regimen for both serotherapy agents.

To address this issue, a more thorough insight is required in the pharmacokinetics and pharmacodynamics of ATG and alemtuzumab. The population PK of ATG and alemtuzumab will be determined for both serotherapy agents in different populations. Next, characterization of the pharmacodynamics will unravel the most optimal exposure to serotherapy, setting a target for dosing. Based on these, an individualized dosing regimen can be derived, aiming for improved and predictable immune reconstitution following HCT. Improved immune

reconstitution will result in improved disease control and reduced toxicity, which will augment survival chances.

OBJECTIVES OF THIS THESIS

The objective of this thesis is to develop individualized dosing regimens for serotherapy in children and adults on the basis of PK/PD modeling. The focus in this thesis will be on ATG in children, as this is the most frequently used drug in HCT. Due to the major changes in pharmacokinetics, children are at higher 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 following HCT. To reach this goal, the dose-exposure-effect relationships of serotherapy in allogeneic HCT will be thoroughly investigated in patients ranging from neonates to adults. Additionally, this thesis may generate an insight into the developmental pharmacokinetics of antibodies.

OUTLINE OF THIS THESIS

Chapter 1 gives a general introduction on the subjects discussed in this thesis. The concept of individualized dosing on the basis of population PK/PD modeling will be further discussed in chapter 2. In chapter 3, the population pharmacokinetics of ATG in children are studied. Chapter 4 presents the population pharmacokinetics of alemtuzumab in children. Chapter 5 investigates the relationship between ATG exposure and clinical outcomes including T-cell immune reconstitution in pediatric HCT. In Chapter 6, clinical outcomes following cord blood transplantation are studied in relation with ATG exposure and T-cell reconstitution. Chapter 7 describes the pharmacokinetics and pharmacodynamics of ATG in adult patients receiving reduced intensity conditioning. The therapeutic window of alemtuzumab in pediatric patients is explored in Chapter 8. Chapter 9 describes the difference in relapse according to CD4+ T-cell immune reconstitution in acute myeloid leukemia versus acute lymphoid leukemia following pediatric cord blood transplantation. In chapter 10, viral reactivations and associated outcomes were investigated in the context of immune reconstitution. Chapter 11 reviews individualized conditioning regimens in cord blood transplantation. The conclusions and perspectives in chapter 12 summarizes this thesis and presents the implications of the results of our studies for clinical care, and proposes further research.

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Chapter 2

Towards evidence-based dosing regimens in children on the basis of population pharmacokinetic pharmacodynamic modelling

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ABSTRACT

When growing up, the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of drugs change, which may alter the effect of drugs. To ensure optimal drug efficacy and safety in paediatric care, PK and PD relationships of drugs need to be explored in children. This article 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) modelling not only led to optimization and individualization in paediatric 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.

INTRODUCTION

Many drugs used in daily paediatric practice lack an evidence-based dosing regimen. A recent review shows 13–30% of drugs used in primary care setting and 49–87% in hospitals are prescribed in an off-label or unlicensed manner.[1] Off-label doses in children are often empirical, based on body weight in a linear manner, and derived from an extrapolated adult dose. Using per kg doses, one assumes that the dose to achieve comparable concentrations increases in a linear fashion with weight. In addition, the assumption is often made that children and adults have a comparable concentration-response relationship. Since developmental changes are mostly non-linear, empirical dosing can lead to overdosing or underdosing, especially in specific age groups such as neonates, in particular extreme low birth weight infants, thereby possibly introducing toxicity or reduced efficacy.[2]

In order to prescribe drugs in children in an evidence-based manner a thorough understanding of the pharmacological profile in children is needed, since the response to drugs may vary highly between children and adults. When growing up, among others, body composition changes, enzyme pathways and renal function mature thereby influencing the pharmacokinetics (PK) of drugs. Maturation also occurs in the expression and function of proteins and receptors, which may alter the effect of drugs (pharmacodynamics (PD)). Also, maturation rates are known to vary between organs and within organs between metabolic path- ways.[3] To describe these processes, the PK as well as the PD need to be investigated in a wide age range.[4,5] Subsequently, evidence-based dosing regimens can be derived.[6–8] The so-called population approach has highly facilitated PK/PD modelling in children because it enables the analysis of sparse sampling datasets and/or datasets derived from clinical practice in which different doses have been applied.[9–11]

This article presents an outline how to perform a population PK/PD study and how to translate these results into evidence-based dosing regimens. The approach to reach individualised dosing guidelines in children based on population PK/PD modelling is explained, after which examples are presented and clinical implications as well as perspectives of this approach are discussed.

PK/PD Modelling on the Basis of a Population Approach

The concentration-time profile of a drug in blood is determined by several processes such as absorption, distribution, metabolism and elimination, and the parameters characterising these processes such as clearance and bioavailability can be calculated from this profile. Since individuals show variability in concentration-time profiles and thus PK parameters, concentration-time profiles of different individuals are needed, resulting in a mean value for each parameter with a distribution.

If all parameters were to be estimated in each patient separately, a substantial number of samples per patient are required in order to describe the entire profile. This obviously is not an ethically justifiable approach in paediatric and in particular neonatal medicine. Also, the dosing regimen and the number of samples have to be roughly the same to allow for comparison between patients. Another disadvantage of this approach is the inability to distinguish between-subject variability (BSV) and error, a variable containing information on measurement errors, wrong notation of sample times and model misspecification. This may result in over-prediction of BSV, leading to large confidence intervals of parameters.

Nowadays, using advanced software in combination with high computing power, the population approach is the preferred method of PK/PD analysis.[12] Using this method, instead of estimating parameters individually followed by a statistical analysis, all available data from all individuals are pooled to estimate a population mean for all parameters. Subsequently, based on individual concentrations, the BSV and error are estimated separately. Here, BSV is a percentage indicating the difference between population mean and the individual value for each parameter. Since the whole dataset is used to estimate the population means, sparse and unbalanced (unequal distribution of samples over time and/or per patient, as is often the case) sampling can be done.

In summary, population PK/PD is the preferred choice, for ethical and practical reasons, as well as the more accurate estimation of model parameters.[9–11]

Designing an Individualized Dosing Regimen

When performing a PK/PD study, drug concentrations and outcome/effect data are needed from each patient. Besides drug concentrations and effects, factors potentially influencing the PK or PD should be collected. These so-called covariates are important variables determining part of the BSV, and a crucial part of the individualised dosing regimen. Covariates may include patient related (weight, age, creatin clearance), disease related (severity, progression, duration) and/or treatment related (route of administration, co-medication) factors.

Development of an evidence-based individual dosing regimen through population PK/PD modelling is optimally achieved using a multistep approach (figure 1).[4] This approach consists of the following steps:

1. Optimal study design based on preliminary data
2. Development and internal validation of PK/PD model
3. External validation of the PK/PD model
4. Prospective validation in clinical study
5. Proposed individualised dosing regimen
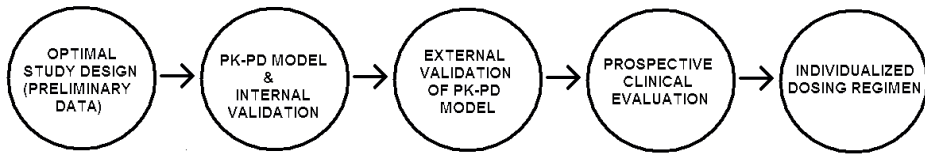
Figure 1. Proposed multistep approach for modeling and simulation using non-linear mixed effects model-
ing for the optimization of drug dosing in children. The four steps that are proposed are (1) optimization of
clinical trial designs based on simulations using preliminary data; (2) development and internal validation of
population PK-PD models using sparse data; (3) external validation of the population PK-PD models using
independent data; and (4) prospective clinical evaluation of the PK- PD model-based dosing regimen. PK:
pharmacokinetics; PD: pharmacodynamics. Printed with permission from: Ince I et al. Drug Discovery Today
2009;14:316–20.

In the first step, based on literature or previous experience, a study has to be designed,
optimising the number of patients[13] and timing of the sampling windows. Since popula-
tion PK/PD modelling can be done on unbalanced and sparse data, the number of samples
per patient is of less importance. Bearing in mind that all samples of all individuals are
analysed simultaneously, it can be anticipated that when all samples are taken on the same
pre-set times after dosing, crucial information may be lacking between these concentrations
thereby complicating the analysis.[14]

Second, with data generated from the performed study, a population PK model is developed,
starting with the identification of a structural model. This comprehends a model adequately
describing the data, typically not including covariates. Then a statistical model characteris-
ing BSV is selected, after which covariates are tested and selected. For example, in most
paediatric studies, weight is a covariate for clearance and/ or volume of distribution. The
selected covariates need to be clinically relevant and feasible. The ultimate goal of the model
is not only describing PK and PD in the population used to develop the model, predicting
dosing in future patients is of much greater importance. In these patients, dosing can be
adjusted based on the value of the included covariates.[11] The above steps will be repeated for
the PD model to describe the relationship between drug concentrations and effects.

During and after developing a PK/PD model, the different aspects of the performance of a
model have to be tested by an internal validation procedure. Validation procedures are of
particular relevance when sparse datasets are analysed.[15] For example, predictions need to
be accurate in low and high con- centration ranges and in different weight ranges despite
the limited number of observations. Predictions of concentrations are tested using the diag-
nostic plots, where predictions are plotted against measured concentrations. On the other
hand, to test for subgroups, which might influence the performance, internal validation can
be performed using resampling and predictive techniques.[15–17]

As a third step, external validation is executed using another dataset when available. Here, the developed model is evaluated using another dataset, the performance of the model is checked and parameter values are compared with the values of the original dataset. The external dataset should be comparable to the original in terms of the included covariates as extrapolations may introduce bias.

With the model being internally and externally validated, a new dosing regimen will be proposed based on the model. In this fourth step, data simulation is a helpful tool. Here, the different parameters (i.e., clearance, volume of distribution) can be fixed to their estimated value. Now, when varying the dose, blood concentrations over time can be simulated, making it possible to select the optimal dosing regimen for each individual child with a given body weight and age.

In a subsequent prospective study performed as a proof of concept, the developed individualised dosing regimen is evaluated. The goal of this evaluation is to verify whether predicted outcomes match observed outcomes in terms of PK (exposure) and/or PD (drug effects) across all age groups studied.

Examples of Population PK-Derived Evidence-Based Dosing Regimens

Amikacin

An example in which population PK-modelling has lead to an individualised dosing regimen is a study on the PK of amikacin, which is an antibiotic drug that is almost entirely eliminated through glomerular filtration, in a large dataset of more than 800 preterm and term neonates.[18] Amikacin clearance proved related to postnatal age and birth weight, that is, children with higher age and weight having a faster maturation of clearance. Another covariate included in this model was the co-administration of ibuprofen, which reduces amikacin clearance. With this model, an evidence-based dosing regimen was proposed by performing simulations with the developed model. In contrast with most current paediatric dosing regimens, the proposed dosing regimen uses birth weight in combination with postnatal age to calculate the appropriate dose instead of weight.[18] When comparing simulated concentrations both of the new regimen as well as various current dosing regimens, including the Red Book,[19] the proposed evidence-based model was shown to be superior to all other regimens in terms of achievement of target peak and trough concentrations (figure 2). Thus, in contrast to established dosing regimens, the model- based individualised dosing regimen may be anticipated to prevent toxicity while remaining efficacious.[18] Currently, the results of a prospective study in more than 600 neonates in which the individualised dosing regimen[18] is evaluated are being analysed.
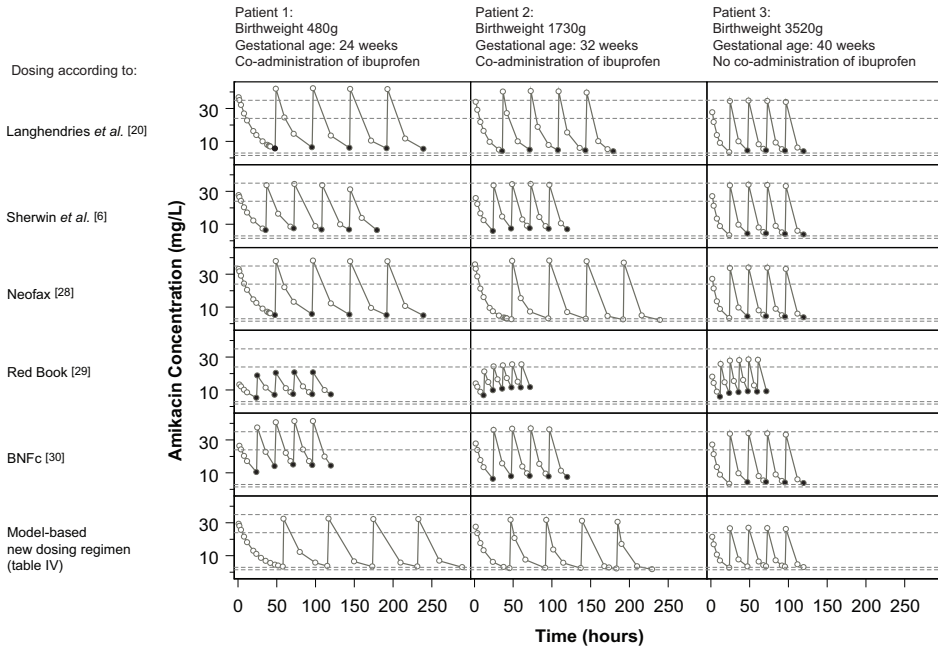
Figure 2. Model-based predicted concentration-time profiles of amikacin for three typical neonates, using five different currently used dosing guidelines[19,34–37] and according to the new model-based dosing regimen. Open dots: concentrations within target range, black filled dots: Cmax over target C_{max}, grey filled dots: C_{min} under target C_{min}. C_{max}=maximum concentration; C_{min}=minimum concentration. Long dash line: Limit of target C_{max} range; Short dash line: limit of target Ctrough range. Adapted from De Cock RFW et al. Maturation of the glomerular filtration rate in neonates, as reflected by amikacin clearance. Reproduced from Cock R. F.W. De et al. Maturation of the glomerular filtration rate in neonates, as reflected by amikacin clearance. Clinical pharmacokinetics 2012;51:105–17 with permission from Adis (© Springer International Publishing AG 2012. All rights reserved.)

Morphine

Morphine is a commonly used opioid in children for which the feasibility of PK/PD-models to derive evidence-based dosing regimens was shown.[9] Modelling of morphine in children of 0–3 years old including premature neonates showed the increase of clearance through uridine glucuronosyltransferase (glucuronidation) to be higher in older children than in neonates.20 In addition, neonates younger than 10 days proved to have a 50% lower glucuronidation clearance. Using the non- linear dosing regimen based on this model, a narrow range of serum concentrations of morphine can be achieved in this age group, despite a broad variation in clearance[20] (figure 3).

This model was externally validated using six datasets which were not used for the development of the model.[21] Using this model, an evidence-based dosing regimen was developed, which was prospectively validated in a double-blinded clinical controlled trial (table 1).[22] In this clinical study, patients post- operatively received a morphine loading dose followed
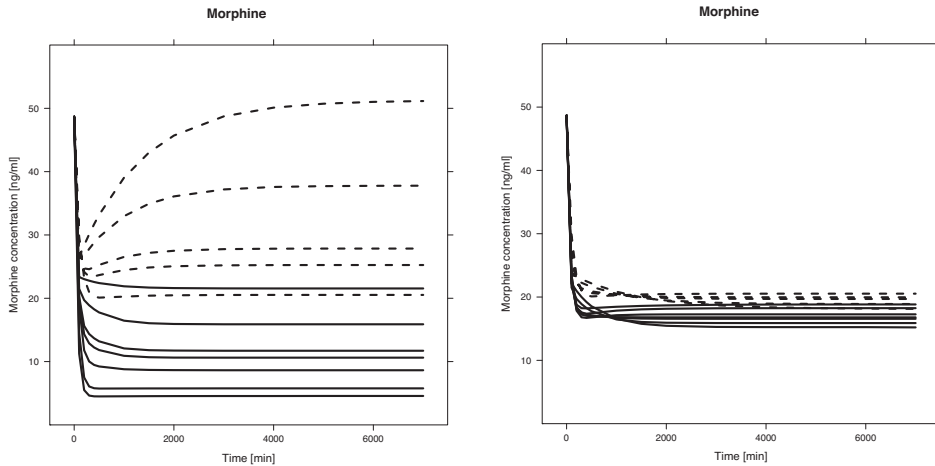
Figure 3. Simulation of morphine concentrations in children weighing 0.5 kg, 1 kg, 2 kg, 2.5 kg and 4 kg with a postnatal age <10 days (dotted lines) and children weighing 0.5 kg, 1 kg, 2 kg, 2.5 kg, 4 kg, 10 kg and 17 kg with a postnatal age of >10 days (solid lines) based on a dosing scheme containing a loading dose of 100 µg/kg followed by a maintenance of 10 µg/kg/hr (A) or 10 µg/kg$^{1.5}$/hr with a 50% dose reduction in children <10 days old (B). Reproduced from Knibbe, CAJ et al. (2009). Morphine glucuronidation in preterm neonates, infants and children younger than 3 years. Clinical pharmacokinetics 2009;48:371–85 with permission from Adis (© Springer International Publishing AG 2012. All rights reserved.)
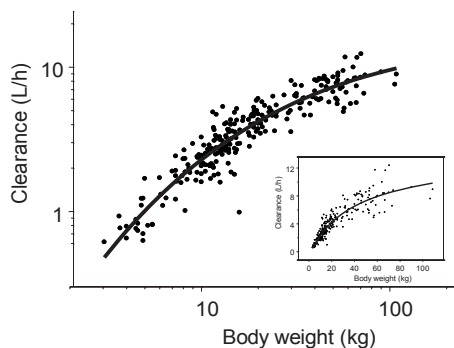
Figure 4 Individual predicted (post hocs, presented as dots) and population predicted (lines) values for busulfan clearance versus body weight. Data is presented on a log scale and on a normal scale (insert). Printed with permission from Bartelink IH, van Kesteren C, Boelens JJ, et al. Predictive performance of a busulfan pharmacokinetic model in children and young adults. Therapeutic Drug Monitoring 2012;34:574–83.

by either paracetamol or morphine infusion. In case of pain, morphine rescue doses were given in both treatment arms. Morphine dosing in both the morphine treatment arm as well as rescue medication were given according to the individual dosing regimen, with children younger than 10 days receiving a 50–75% lower dose compared with current standards, while older children received a higher dose. Using this dose, efficacy was maintained in the majority of children, while the risk of overdosing was reduced, especially in young neonates.

	Model-based dosing algorithm		Traditional dosing algorithm
	PNA < 10 days 2.5 mg*bodyweight$^{1.5}$ per h	PNA > 10 days 5 mg*bodyweight$^{1.5}$ per h	10 µg*bodyweight per h
Bodyweight [kg]	[µg/h]	[µg/h]	[µg/h]
0.5	0.88	-	5
1	2.5	5.0	10
1.5	4.6	9.2	15
2	7.1	14.1	20
2.5	9.9	19.8	25
3	13.0	26.0	30
3.5	16.4	32.7	35
4	20.0	40.0	40
4.5	23.9	47.7	45
5	28.0	55.9	50
5.5	32.2	64.5	55
6	36.7	73.5	60
6.5	-	82.9	65
7	-	92.6	70
7.5	-	102.7	75
8	-	113.1	80
8.5	-	123.9	85
9	-	135	90
9.5	-	146.4	95
10	-	158.1	100
11	-	182.4	110
12	-	207.8	120
13	-	234.4	130
14	-	261.9	140
15	-	290.5	150

Table 1. Dosing table for individualized maintenance dose for children based on the developed evidence based dosing regimen and the dosing based on the current regimen. Reproduced from Knibbe, CAJ et al. (2009). Morphine glucuronidation in preterm neonates, infants and children younger than 3 years. *Clinical pharmacokinetics*, 48(6), 371–85 with permission from Adis (© Springer International Publishing AG 2012. All rights reserved.)

However, although blood concentrations were comparable, rescue doses were frequently needed in the relatively older children, suggesting a difference in PD relation in different age groups. This may be caused by a difference in sensitivity to morphine and its active metabolites or a difference in distribution to the target sites. To date, the morphine target concentration to achieve adequate pain relief is unknown and may vary between children

in different age groups. Incorporating this PK-PD relationship and the effect of age on this may improve the proposed model and thus the dosing regimen.

Busulfan

A third example is the development of an individualised dosing regimen used in paediatric haematopoietic cell transplantation (HCT). It has been shown before that individualisation of dosing of the various drugs used in this procedure, including chemotherapy, can improve morbidity and mortality.[23–25] Busulfan is one of the chemotherapeutic drugs used in paediatric HCT as preparative chemotherapy. This drug has a narrow therapeutic window, with underdosing as well as overdosing leading to significant morbidity and mortality. An international cohort of 245 patients receiving busulfan in HCT was included in a retrospective PK/PD study.[23] Ages varied from 1 month to 26 years, and patients received HCT for various indications. In this model, body weight proved an important factor influencing volume of distribution and clearance (figure 4).

The dosing regimen based on this model is expected to lead to an optimised exposure in all body weight ranges when compared with the approved dosing, because it is aiming for an AUC that previously has shown to result in the highest event-free survival.[26] After external validation of this model,[27] the dosing regimen is now used for dosing busulfan in current clinical practice (table 2).

DISCUSSION AND PERSPECTIVES

In children, with their changing body composition and maturation in function of metabolising and/or eliminating organs and receptors, evidence-based dosing regimens are crucial. With empirical dosing, which is used in a substantial portion of drugs prescribed in paediatric practice, drug effects may vary over age and weight. This may potentially lead to a decrease in drug effect or toxic doses with an increase in side effects in one or more specific age groups.

Population PK/PD-modelling is a validated tool for developing evidence-based dosing schemes.[8,10] Using this technique, provided a proper internal validation procedure is performed, a model can be built based on sparse and unbalanced data, which is common in paediatric practice due to practical and ethical restrictions of frequent blood sampling.[11,12] With this model, the relationship between exposure and effects of drugs can be precisely predicted to ensure a constant dose-effect relation in a population, including children in varying age groups including neonates.[9]

| Body weight | | Model-based individualized dosing nomogram | | Approved dose in SPC | |
| | | Myeloablative dose 4 days, 1dd, mg/kg target AUC_{day0-4} 90mg*h/L[a] | | 4 days, 1dd, mg/kg | |
kg	Dose (mg)	Dose (mg/kg)	± % deviation of target AUC	Dose (mg/kg)	± % deviation of target AUC
3	11	3.8	0%	4.0	5%
5	24	4.7	0%	4.0	-15%
7	36	5.1	0%	4.0	-22%
8	41	5.2	0%	4.0	-23%
9	47	5.2	0%	4.8	-8%
11	58	5.2	0%	4.8	-9%
13	68	5.2	0%	4.8	-8%
15	77	5.1	0%	4.8	-6%
16	81	5.1	0%	4.4	-13%
20	97	4.9	0%	4.4	-9%
23	108	4.7	0%	3.8	-19%
25	115	4.6	0%	3.8	-17%
30	130	4.3	0%	3.8	-12%
35	143	4.1	0%	3.2	-22%
40	156	3.9	0%	3.2	-18%
45	167	3.7	0%	3.2	-14%
50	177	3.5	0%	3.2	-10%
55	187	3.4	0%	3.2	-6%
60	195	3.3	0%	3.2	-2%
65	204	3.1	0%	3.2	2%

Table 2. The model-based individualized dosing table for busulfan, aiming for a myeloablative (AUCday0–4 of 90 mg*h/L in combination with fludarabine), compared to the approved dose in the summary of product characteristics (SPC). Printed with permission from Bartelink IH, van Kesteren C, Boelens JJ, *et al.* Predictive performance of a busulfan pharmacokinetic model in children and young adults. *Therapeutic Drug Monitoring* 2012;**34**:574–83.

When conducting a study to develop a dosing regimen through PK/PD, the multistep approach (figure 1) is the preferred choice.[4] Using this approach, an informative database is derived on which a PK/PD model can be built after which model performance is extensively validated. Nonetheless, a robust PK/PD model can still be built with limited information. This approach has proven its value in past studies resulting in solid evidence-based dosing regimens.[18,20,23] It should be noted that, when completing the steps in this approach, there may still be room for optimisation of the model and the proposed dosing regimen. With increasing amounts of data, special populations (i.e., renal impairment, liver disease) and other conditions resulting in outliers can be identified, after which dose corrections for these groups can be implemented in the regimen.

Besides the development of individualised dosing regimens, a validated PK model may also be used to understand metabolic pathways. Acquired knowledge on a biological system on the basis of a paradigm drug can be used to translate an existing model to other drugs that use the same metabolic or elimination pathway.[9,10] System-specific parameters (maturation of clearance as quantified in the identified covariate model) can be obtained from previous work on paradigm drugs, so that only drug- specific parameters (population value of volume of distribution or clearance) have to be estimated, which drastically reduces the number of patients and samples needed.[9,10] Interaction with Physiologically Based PK (PBPK) modelling groups[28] is of great value in this context which has resulted in successful predictions of weight-related changes in glucuronidation clearance of zidovudine and potentially many other uridine glucuronosyltransferase substrates on the basis of the modelling results in morphine.[29,30] Also, the amikacin model characterising weight and age-related changes in amikacin clearance in preterm and term neonates[18] was able to precisely reflect maturation of glomerular filtration and thus predict the dosage regimens of other renally excreted drugs by glomerular filtration in neonates.[31,32] These examples demonstrate how the development of evidence-based dosing regimens can be accelerated in a sophisticated manner.

However, it should be noted that, although the development of evidence-based dosing regimens using PK models is often a major improvement compared with empirical dosing, more emphasis should be put on the characterisation of the PD relation in children.[5] It is both the dose-concentration and concentration-effect relationship across the paediatric age range that should be explored in order to achieve predictable efficacy and safety in all individuals, as well as the effect of covariates such as age on these relationships, which may lead to further improvement of dosing regimens. In the examples presented in this paper, surrogate PD end points (i.e., target peak and trough concentrations associated with efficacy and toxicity, respectively) were available for amikacin[18] and target AUCs associated with optimised efficacy with acceptable toxicity were available for busulfan.[26] Further research should therefore focus on PD relations in children, with the field of pain research as an example where many efforts have been put on the validation and use of age-related PD end points that may serve as a basis for the PK/PD studies in children.[33]

In the future, evidence-based derived dosing regimens in paediatrics need to become the norm rather than the exception. In order to achieve this, paediatric PK/PD studies need to be conducted in both the development of new drugs and in drugs that have been on the market for a short time or a long time. The examples shown here demonstrate how PK (/PD) modelling may lead to optimisation and individualisation in paediatric clinical care for the specific drugs but also for other drugs eliminated through the same pathway. While this could potentially accelerate the development of dosing guidelines for many drugs, ultimately, these and other efforts should lead to predictable drug efficacy and safety across all age groups.

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PART II

Pharmacokinetics of Serotherapy

Chapter 3

Population Pharmacokinetic Modeling of Thymoglobulin in Children Receiving Allogeneic-Hematopoietic Cell Transplantation:
Towards Improved Survival Through Individualized Dosing

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ABSTRACT

Background and Objectives

To prevent graft-versus-host disease and rejection in hematopoietic cell transplantation (HCT), children receive Thymoglobulin®, a polyclonal antibody acting mainly by depleting T cells. The therapeutic window is critical as over-exposure may result in delayed immune reconstitution of donor T cells. In this study, we describe the population pharmacokinetics of Thymoglobulin® as a first step towards an evidence-based dosing regimen of Thymoglobulin® in pediatric HCT.

Methods

Serum active Thymoglobulin® concentrations were measured in all pediatric HCTs performed between 2004 and 2012 in two pediatric HCT centers in The Netherlands. Population pharmacokinetic analysis was performed using NONMEM® version 7.2.

Results

A total of 3,113 concentration samples from 280 pediatric HCTs were analyzed, with age ranging from 3 months to 23 years old. The cumulative Thymoglobulin® dose was 10 mg/kg in 94% of the patients given in 4 consecutive days. A model incorporating parallel linear and concentration-dependent clearance of Thymoglobulin® was identified. Body weight [for linear clearance (CL) and central volume of distribution] as well as lymphocyte counts pre-Thymoglobulin® infusion (for CL) were important covariates. As such, the current dosing regimen results in higher exposure in children with a higher bodyweight and/ or a lower lymphocyte count pre-Thymoglobulin® infusion.

Conclusion

This model can be used to develop an individual dosing regimen for Thymoglobulin®, based on both body weight and lymphocyte counts, once the therapeutic window has been determined. This individualized regimen may contribute to a better immune reconstitution and thus outcome of allogeneic HCT.

INTRODUCTION

Since the 1970s, anti-thymocyte globulin (ATG) has been widely applied as serotherapy in order to prevent acute graft-versus-host disease (GvHD) and graft rejection in patients undergoing non-HLA-identical hematopoietic cell transplantations (HCTs)[1–4]. The introduction of ATG to the conditioning regimen has indeed led to a significant decrease in severe acute and chronic GvHD[5,6]. How- ever, too high doses of ATG impair the required immune reconstitution after HCT. As such, there is a delicate balance between prevention of GvHD on one side and the promotion of immune reconstitution on the other.

Theoretically, the incidence of GvHD or rejection versus delayed immune reconstitution may result from a variable exposure of ATG. Prolonged and/or high exposure to ATG leading to lymphodepletion with delayed or absent immune reconstitution may result in relapse of the malignancy or viral infections, while a short or absent ATG- related lymphodepletion may result in limited or no protection against acute GvHD[7,8]. During the first weeks to months after transplantation, T cell reconstitution depends on peripheral expansion, the division of mature T cells infused with the graft. Hereafter, depending on the thymus function, definitive repopulation of the T cells will take place through thymopoiesis[9–11]. As peripheral expansion is restricted by circulating ATG, excess ATG due to relative overdosing leads to delayed or even absent reconstitution of T cells, possibly resulting in lethal viral reactivations and relapse[12,13].

Thymoglobulin® is the most commonly used preparation of ATG in pediatric HCT. Children usually receive an empirically derived cumulative Thymoglobulin® dose of 10 mg/kg, given in 4 days, starting 4–6 days before transplantation. Thymoglobulin® is not registered in pediatrics, and as such its use is off-label. Particularly in children, the pharmacokinetics of a drug may differ as a result of changes in body composition and maturation in organ function[14]. This variability in pharmacokinetics influences drug exposure, which in turn determines the drug response of pharmacodynamics. In order to maintain efficacy while reducing adverse effects of drugs across the entire pediatric age range, identification of the pharmacokinetic/ pharmacodynamic relationships and the effect of growth and maturation on the different pharmacokinetic and pharmacodynamic parameters involved are crucial[15–17]. This has been shown before in pediatric HCT, where individualized busulfan dosing has led to a significant decrease in toxicity whilst remaining effective in terms of relapse and engraftment[18,19].

To date, only a limited number of papers on the pharmacokinetics of ATG (Thymoglobulin® and other preparations) have been published, with very few in children[20–26]. The half-life of Thymoglobulin® is reported to be 7–14 days[20,26]. The available evidence for Thymo-

globulin® shows that pharmacokinetics change during childhood, with varying exposures possibly leading to different outcomes[25,26]. Therefore, the aim of this study was to develop a population pharmacokinetic model for active Thymoglobulin® as a first step in describing Thymoglobulin® pharmacokinetics/ pharmacodynamics and thus developing a basis for an individualized dosing regimen. The focus was on active Thymoglobulin®, which is the fraction that is directed against human targets and that can be held responsible for its pharmacological action in humans[27]. Since Thymoglobulin® pharmacokinetics are pivotal both in preventing acute GvHD and rejection as well as assuring a successful and timely immune reconstitution, individualizing the dosing may improve survival in pediatric HCT.

METHODS

Study Design and Patients

For this pharmacokinetic analysis, all patients receiving an HCT between April 2004 and December 2012 with Thymoglobulin® as part of the conditioning in the pediatric hematopoietic stem cell transplantation programs of the University Medical Center Utrecht (UMCU) and the Lei- den University Medical Center (LUMC), The Netherlands, were included. In case of multiple transplants in one patient, concentrations of Thymoglobulin® in all transplants were included, provided no serotherapy (i.e., ATG, alemtuzumab) was given in the last 3 months before second/third transplantation. Patients receiving serotherapy other than Thymoglobulin® within a period of 3 months before HCT were excluded. Informed consent was given by the child and/or the parents (the former when over 12 years old). All data were collected after written informed consent was obtained in accordance with the Declaration of Helsinki and institutional ethical committee approval for sample and data collection, trial numbers METC 05/143 and METC 11/063-k (UMCU) and P01.028 (LUMC).

Patient characteristics including transplantation details of the studied patients are shown in Table 1. Patients typically received Thymoglobulin® over 4 h in a cumulative dose of 10 mg/kg divided over 4 consecutive days starting 5 days before HCT. At the discretion of the treating team of physicians, some patients received a lower dose of Thymoglobulin® (7.5 mg/kg) and/or received Thymoglobulin® earlier (day –9). Some patients with hemophagocytic lymphohistiocytosis received a higher dose of up to 20 mg/kg. Pharmacokinetic samples were avail- able in the elimination phase of Thymoglobulin® in all patients, whilst in patients treated in the LUMC, peak and trough concentrations (collected 15 min after and 15 min before 4-h infusion, respectively) were also available, which in light of the very long half-life can be seen as the true peak and trough concentrations. Generally, samples were taken weekly (Mondays/Thursdays in UMCU, Monday/Wednesday/Friday in LUMC) until 12 weeks after transplantation. Collected samples were centrifuged upon drawing; plasma was

Characteristics	Leiden	Utrecht	Total
Number of patients (n)	153	114	267
Number of HCTs (n)	159	121	280
Male sex (%)	67	57	62
Age (years)	6.5 (0.4-19)	5.9 (0.2-23)	6.5 (0.2-23)
Actual body weight (kg)	21 (4.7-75)	20 (3.7-96)	21 (3.7-96)
BSA (m^2)	0.84 (0.28-1.95)	0.82 (0.14-2.1)	0.83 (0.14-2.1)
Number of samples [n (mean per patient)]	2352 (15)	761 (6)	3113 (11)
Starting day Thymoglobulin® (days before transplantation)	5 (3-15)	5 (1-19)	5 (1-19)
Lymfocyte count at first dose Thymoglobulin® (x 10^9)	0.1 (0.01-4.5)	0.71 (0.01-10.4)	0.29 (0.01-10.4)
Cumulative Thymoglobulin® dose (%) <9 mg/kg	3	5	4
9-11 mg/kg	97	89	94
>11 mg/kg	0	6	2
Diagnosis (%)			
Malignancy	50	42	47
Immune deficiency	16	24	19
Bone marrow failure	4	10	6
Metabolic disease	0	21	9
Benign hematology	30	1	18
Auto-immune disease	0	2	1
Stem cell source (%)			
Bone marrow	63	29	49
Peripheral blood stem cells	23	5	15
Cordblood	14	60	34
Cordblood plus haplo or 2nd cordblood	0	6	2
Conditioning regimen (%)			
Reduced intensity	0	7	4
Chemotherapy-based	72	78	74
TBI-based	28	15	22

Table 1. Patient characteristics. Values are shown as median (range) unless otherwise specified. BSA: body surface area, TBI: total body irradiation

stored to be analyzed in batches. Conditioning regimens were given according to (inter) national protocols. Gut decontamination and infection prophylaxis were given according to local protocols. Patients were treated in high-efficiency, particle-free, air-filtered, positive-pressure isolation rooms. Patients received clemastine combined with either di-adreson-F aquosum (2 mg/ kg) or prednisolone (2 mg/kg) before and during Thymoglobulin® infusion.

Measurement of Active Thymoglobulin® and Anti-Thymoglobulin® Antibodies

Active Thymoglobulin®, defined as Thymoglobulin® capable of binding to HUT-78 cells, was measured using a quantitative flow cytometry assay[25], based on a method described

by Rebello[28]. In short, HUT-78 T cells (PHACC, Porton Down, UK) were incubated with fourfold dilutions of patient serum, followed by washing and incubation with Alexa Fluor 647 labeled with goat anti-rabbit IgG (Biosource, Life Invitrogen, Carlsbad, CA, USA). To prepare standards with predefined active Thymoglobulin® concentrations, Thymoglobulin® was serially diluted two- fold in triplicate to produce a range of Thymoglobulin® standards ranging from 5 to 0.005 AU/mL. Active Thymoglobulin® is expressed in arbitrary units (AU): Thymoglobulin® 5 mg/mL is arbitrarily set to contain a concentration of 5,000 AU/mL of active Thymoglobulin®. The lower limit of quantification was 0.01 AU/mL. Cells were washed and analyzed by flow cytometry on a FACS scan (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA); mean fluorescence intensities of standard dilutions were plotted against the active Thymoglobulin® concentrations and the reference curve was used to determine the concentration of active Thymoglobulin® in patient samples. Samples were tested in duplicate with an accepted coefficient of variation of 0.2, both in high and low ranges.

Anti-Thymoglobulin® antibodies in classes IgA, IgM, and IgG were measured using an ELISA. After blocking, patient sera were applied to Thymoglobulin®-coated plates. Anti-ATG antibodies were detected using alkaline phosphatase-conjugated rabbit-anti-human (IgG; Jackson ImmunoResearch Europe, Newmarket, UK) or goat-anti- human (IgM, IgA; Jackson) antibodies[25], adsorbed for rabbit IgG. As literature shows IgG anti-ATG antibodies to significantly influence Thymoglobulin® pharmacokinetics[25], samples with IgG anti-Thymoglobulin® antibodies were marked. While the decline in concentration after occurrence of these antibodies was variable, these samples (n = 13 from six patients) were excluded from the analysis.

Population Pharmacokinetic Analysis

To allow analysis of sparse and unbalanced data, the population approach was applied for pharmacokinetic modeling[15,16]. This method uses data from all patients simultaneously to estimate pharmacokinetic parameters for individual patients as well as the whole cohort. For this purpose, the non-linear mixed effects modeling software NONMEM version 7.2 (Icon, Hanover, MD, USA)[29] was used, with Pirana version 2.8.1[30] and R version 3.0.1[31] for visualization of data. The estimation method used was first-order conditional estimation with interaction (FOCE-I). Active Thymoglobulin® concentrations measured as AU/mL, were logarithmically transformed, and fitted simultaneously. Observations below limit of quantification (BLQ) were set at half the limit of quantification, with following samples being deleted[32]. Other methods for handling BLQ were investigated (M3 and removal of BLQ[32]); this did not result in an improvement of the model. Modeling of data was performed in four steps: (1) selection of a structural and statistical model; (2) selection of an error model; (3) covariate analysis and selection; and (4) internal validation of the model. Individual

pharmacokinetic parameters (post hocs) such as clearance of the individual patient were estimated using the POSTHOC option in NONMEM, according to Eq. 1:

$$P_i = P_{pop} \cdot e^{\eta_i}$$

(Eq. 1)

where P_i is the individual or post hoc value of the parameter in the ith individual, P_{pop} the population value for that parameter, and η_i the inter-individual variability of the *i*th person samples from a distribution with a mean zero and variance of ω^2 with a log-normal distribution.

A proportional error model was used, so that for the *j*th observation in the *i*th individual, the observations are described using Eq. 2:

$$Y_{i,j} = C_{pred,i,j} \cdot (1 + \epsilon)$$

(Eq 2)

where $Y_{i,j}$ is the observed concentration, $C_{pred,i,j}$ is the predicted concentration for *j*th observation in the ith individual, and ϵ is the error samples from a distribution with a mean zero and variance of σ^2.

In the model-building process, several criteria were applied. A decrease in objective function value (OFV) over 3.84 points between two hierarchical (sub) models was considered statistically significant; this correlates with $p<0.05$ based on a Chi-squared (χ^2) distribution for 1 degree of freedom. In addition, goodness-of-fit plots [observed vs. both individual and population predictions of concentrations as well as conditional weighted residuals (CWRES) versus time and observed concentrations] were evaluated with emphasis on the population predictions. Furthermore, confidence intervals of parameter estimates, g-shrinkage, and visual improvement of the goodness-of-fit plots were used to evaluate the models. Inter-occasion variability on the different parameters was tested for the subsequent doses to assess changes in pharmacokinetic parameters between doses.

Covariate Selection

Possible covariates, including, among others, patient characteristics and disease- and treatment-related variables, were studied. Inter-individual variability as well as post hocs, weighted residuals (WRES) and CWRES were independently plotted against covariates to evaluate possible relationships. While categorical covariates such as sex and treatment center were tested as a fraction for each category, continuous covariates were tested in linear and power functions (Eqs. 3 and 4):

$$P_i = P_{pop} \cdot \left(\frac{Cov_i}{Cov_{median}} \right)^k \qquad\qquad \text{(Eq. 3)}$$

$$P_i = P_{pop} \cdot \left(1 + \left(\frac{Cov_i}{Cov_{median}} \right) \cdot l \right) \qquad\qquad \text{(Eq. 4)}$$

where P_i and Cov_i are the value for parameter and covariate for the ith individual, respectively, P_{pop} is the population mean for parameter P, and Cov_{median} is the standardized value of the covariate. In the power function, the scaling factor is depicted by k. For Eq. 4, l represents the slope of the linear function.

Covariate model building was analogous to structural model building. Potential variables were evaluated using forward inclusion and backward elimination with a level of significance of <0.005 (-7.9 points in OFV) and <0.001 (-10.8 points in OFV), respectively. In addition, inclusion of a covariate in the model had to result in a decline in unexplained inter-individual variability before it was included in the final model[33,34].

Model Evaluation

Since the model will be used for future dose selection, proper internal validation of the model is of utmost importance[35]. To assess the predictive properties of the developed model, the proposed model was internally validated using bootstrap techniques. Here, a new dataset is repeatedly created through resampling using the individuals in the original dataset. One thousand replicated datasets were run using the bootstrap option in Perl speaks NONMEM (PsN) version 3.5.3[36]. Medians as well as the 2.5th and 97.5th percentiles were compared with parameter values estimated using the original dataset to check for discrepancies.

Another validation technique used was normalized prediction distribution errors (NPDE), simulating the prediction discrepancies while taking into account the predictive distribution and the correlation between observations in the same individual. The R-package NPDE was used for normalized prediction of errors[37].

Model-Based Simulations

With the evaluated model, simulations were performed on the basis of the currently recommended dose in children a cumulative dose of 10 mg/kg given in 4 h infusions in 4 consecutive days (2.5 mg/kg/day)] to visualize concentration–time profiles in representative children based on body weight and baseline lymphocyte counts.

RESULTS

Patients and Data

Active Thymoglobulin® concentrations obtained from 267 patients undergoing 280 HCTs were included from the two study centers. A total of 3,113 concentration samples were available with a median of 11 samples (range 1–32) per patient. A total of 13 observations from six patients were excluded due to anti-Thymoglobulin® IgG antibodies. Patient characteristics, other than diagnosis (benign hematology versus inborn errors of metabolism) and stem cell source, were equally distributed between the two centers (Table 1).

Structural Pharmacokinetic Model

Active Thymoglobulin® pharmacokinetics could be well- described using a two-compartment model (Fig. 1), which yielded a good description of the data in all age groups (Fig. 2). A two-compartment model was superior over a one-compartment model for statistical reasons [decrease in OFV of 382 points (p<0.001)] and improvement of goodness-of-fit plots (data not shown). A three-compartment model was tested and proved to be unstable with inaccurate parameter estimates. A proportional residual error was incorporated; adding an additive error did not significantly improve the model.
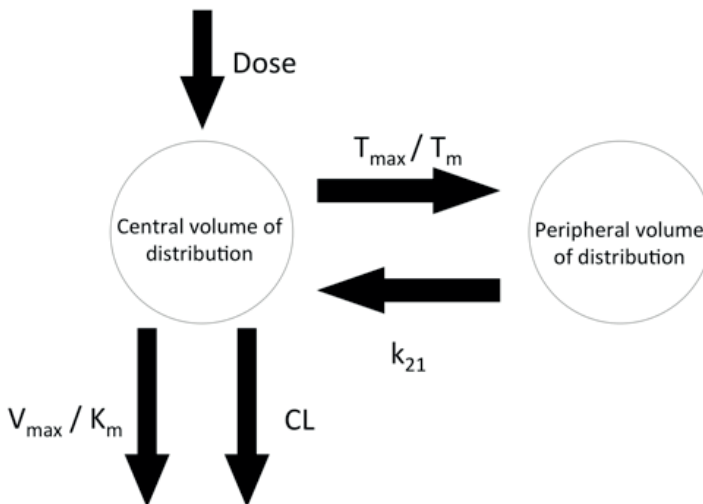
Figure 1. Model overview. Tmax: maximum rate of transport towards the peripheral compartment. Tm: concentration in central volume of distribution at 50% saturation of Tmax. K_{21}: rate of transport from the peripheral compartment to the central compartment. CL: linear clearance. V_{max}: maximum rate of elimination. K_m: concentration in central volume of distribution at 50% saturation of V_{max}.

Individual concentration–time plots indicated non-linear clearance, which was accounted for by a model with both linear clearance (CL) and saturable clearance, defined as the quotient of maximum elimination rate V_{max} and the Michaelis–Menten constant K_m (Fig. 1). This was previously described for antibody kinetics[38,39], and was found to lead to an improved description of the observations.

In addition, due to an under-prediction of concentrations during and shortly after the infusion, saturable distribution towards the peripheral compartment (T_{max}/T_m; Fig. 1) was included. This saturable distribution was parameterized as a coefficient of maximum transport rate (T_{max}) and the Michaelis–Menten constant (T_m). Figure 3 shows a structural
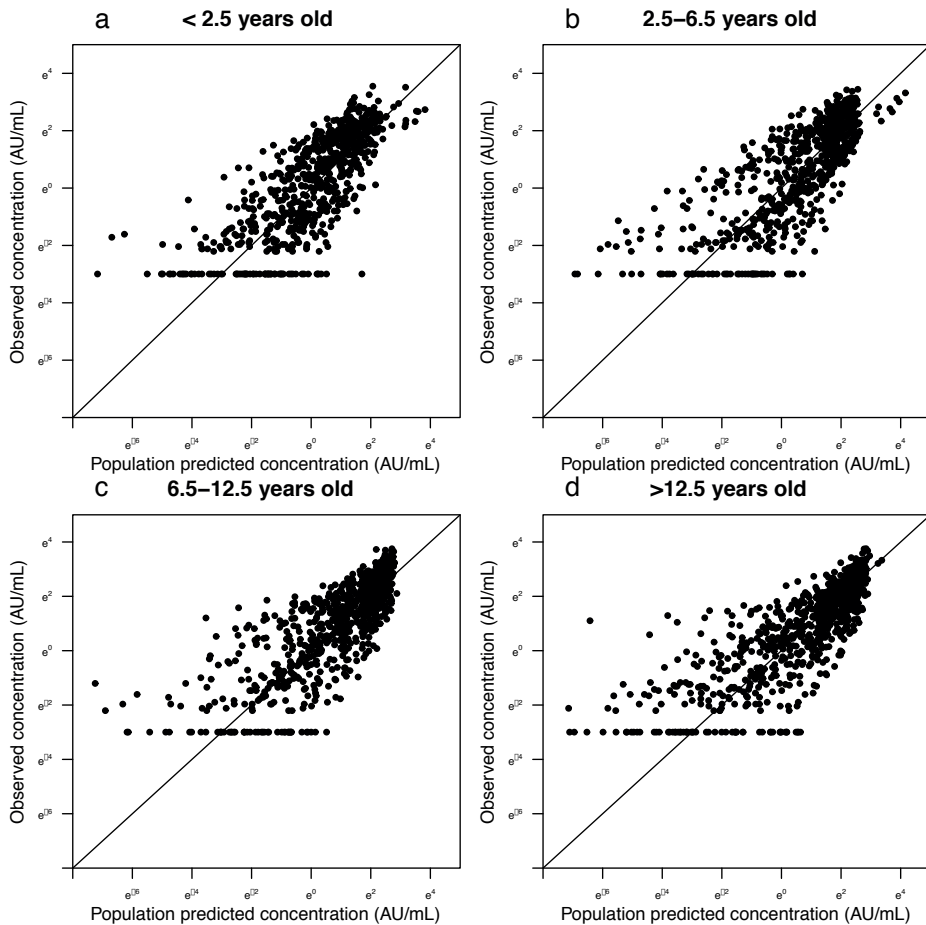
Figure 2. Diagnostic plots of the final model: Observed versus population predicted active Thymoglobulin® concentrations split by quartiles of age. Panel a: <2.5 years, panel b: 2.5-6.5 years, panel c: 6.5-12.5 years, panel d: >12.5 years old. Dots: individual concentration versus population predicted value. Lines: x=y

under-prediction that can be seen at 3–7 days after start of Thymoglobulin® dosing (upper panel) and is corrected after inclusion of the saturable distribution (lower panel).

The addition of both of these non-linear functions to the final model as depicted in Fig. 1 led to a significant decrease in OFV [202 and 103 points (p<0.001), respectively] as well an improvement in goodness-of-fit plots. The Michaelis-Menten constants K_m and T_m were estimated in the observed concentrations range. Inter- occasion variability on CL and central volume of distribution (V_1) was tested for the subsequent doses; this yielded no significant improvement in model performance.

Without saturable intercompartmental transport

With saturable intercompartmental transport
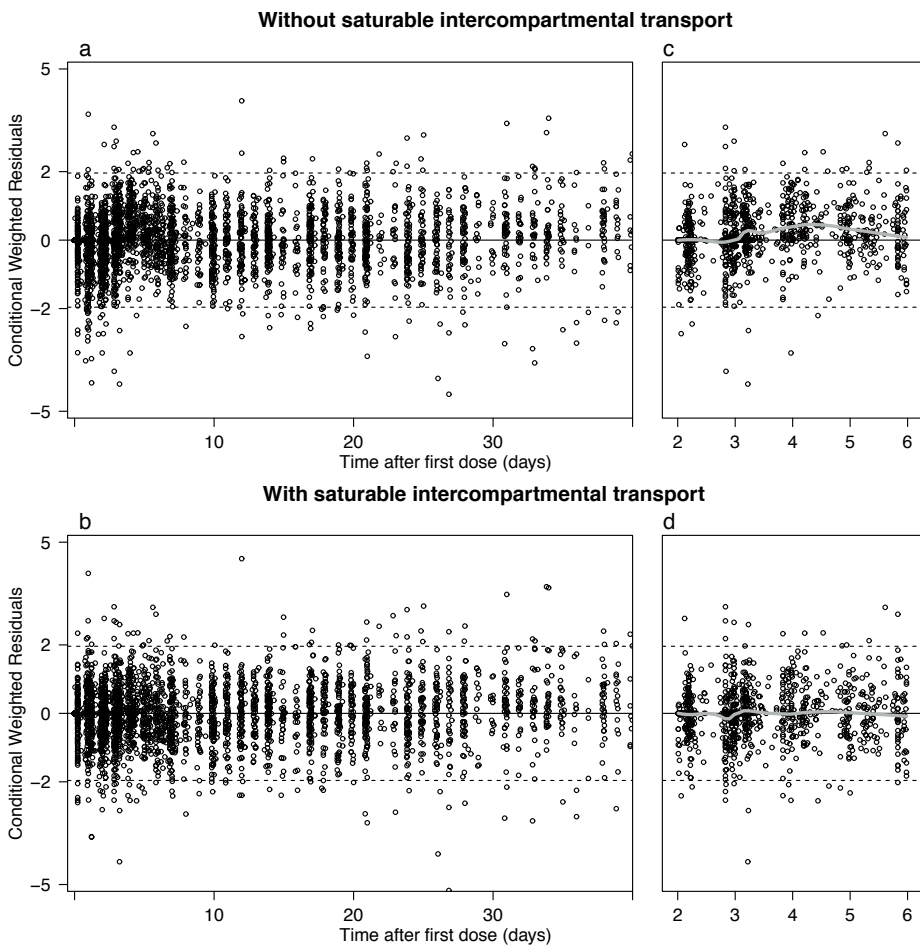
Figure 3. A trend in conditional weighted residuals (CWRES) versus time can be seen before introduction of saturable intercompartmental transport (panel a+b), which is accounted for after introduction (panel c+d). Panels a+c: all data; panels b+d: zoomed in to 2-6 days. Dots: CWRES per concentration sample, solid line CWRES=0, dashed lines ± 2SD, curved lines: spline regression.

Figure 4 shows how total clearance, defined as the sum of linear (CL) and saturable clearance (V_{max}/K_m) depends on the serum concentration of active Thymoglobulin®. At low concentrations, saturable clearance represents almost half of the total clearance. Above a certain concentration, the non-linear pathway becomes saturated, as seen in the decreasing saturable clearance. At concentrations above 10 AU/mL, the non-linear pathway is fully saturated, and total clearance is mostly dependent on the CL, with only a small contribution of the saturable clearance. From a clinical perspective, a concentration of 1 AU/mL is thought to be the lympholytic level in an in vitro setting[20].

	Dataset	Shrinkage	1000 bootstrap replicates (98% successful)	
	[estimate (RSE)]	(%)	Median	2.5th-97.5th percentile
Structural model				
$CL_i = CL_{pop} * \left(\dfrac{WT}{WT_{median}}\right)^k * \left(1 + \left(\dfrac{BL}{BL_{median}}\right) * l\right)$				
CL_{pop} (L/day)	2.2 (6%)		2.1	1.8-2.4
k	0.61 (13%)		0.62	0.45-0.80
l	0.12 (36%)		0.12	0.04-0.22
$V_{1,i} = V_{1,pop} * \left(\dfrac{WT}{WT_{median}}\right)^m$				
$V_{1,pop}$ (L)	7.8 (6%)		7.8	6.9-9.0
m	1.1 (7%)		1.1	0.92-1.3
$K_{21,pop}$	1.2 (18%)		1.2	0.79-1.8
$T_{max,pop}$ (AU/day)	156 (15%)		161	98-241
$T_{m,pop}$ (AU/L)	7.6 (21%)		7.5	4.9-13
$V_{max,pop}$ (AU/day)	1.8 (21%)		1.9	1.2-3.1
$K_{m,pop}$ (AU/L)	1.1 (21%)		1.1	0.67-1.8
Random variability				
Inter-individual variability on CL (%)	86 (5%)	5	85	76-94
Inter-individual variability on V_1 (%)	59 (7%)	20	58	49-67
Inter-individual variability on T_m (%)	106 (7%)	18	107	93-123
Inter-individual variability on V_{max} (%)	70 (12%)	54	70	49-93
Inter-individual variability on K_m (%)	177 (12%)	36	177	117-227
Proportional residual error (%)	32 (12%)	13	31	28-36

Table 2. parameter estimates and bootstrap results. WT: body weight (kg), WT_{median}: median population body weight (21 kg), BL: baseline (before first Thymoglobulin® infusion) lymphocyte count (x10^9 lymphocytes/L), BL_{median}: median baseline lymphocyte count (0.29 x 10^9 lymphocytes/L). CL: linear clearance; V_1: central volume of distribution, K_{21}: constant depicting distribution from the peripheral to the central compartment, T_{max}: maximum transport rate towards in saturable distribution towards peripheral compartment, T_m: Michaelis-Menten constant saturable distribution towards peripheral compartment, V_{max}: maximum transport rate for saturable clearance pathway, K_m: Michaelis-Menten constant saturable distribution for saturable clearance pathway.

Covariate Analysis

The covariate analysis showed actual body weight, body surface area (BSA, Mosteller formula) and age to influence CL and V_1, while lymphocyte count at first dose of Thymoglobulin® was a covariate on CL only. Actual body weight was the best predictor of CL and V_1 when compared with other body size parameters such as BSA and age, in terms of the decrease in OFV as well as improvement in goodness-of-fit plots. The decrease in OFV was 52 and 124 points, respectively ($p<0.001$). The relationship between bodyweight and both CL and V1 was best described by a power function (Eq. 3), with the exponent k being 0.61 in the relationship with CL, and 1.1 in the relationship with V_1. Lymphocyte count was tested as a covariate since it is a target for Thymoglobulin® mediating its clearance.

Lymphocyte counts were available both before chemotherapy and shortly before (1–4 h) the start of Thymoglobulin® infusion, which was after 1–2 days of chemotherapy. As the chemotherapy in the conditioning might be expected to cause a drop in the lymphocytes, both lymphocyte counts were evaluated. Both covariates were comparable in predicting
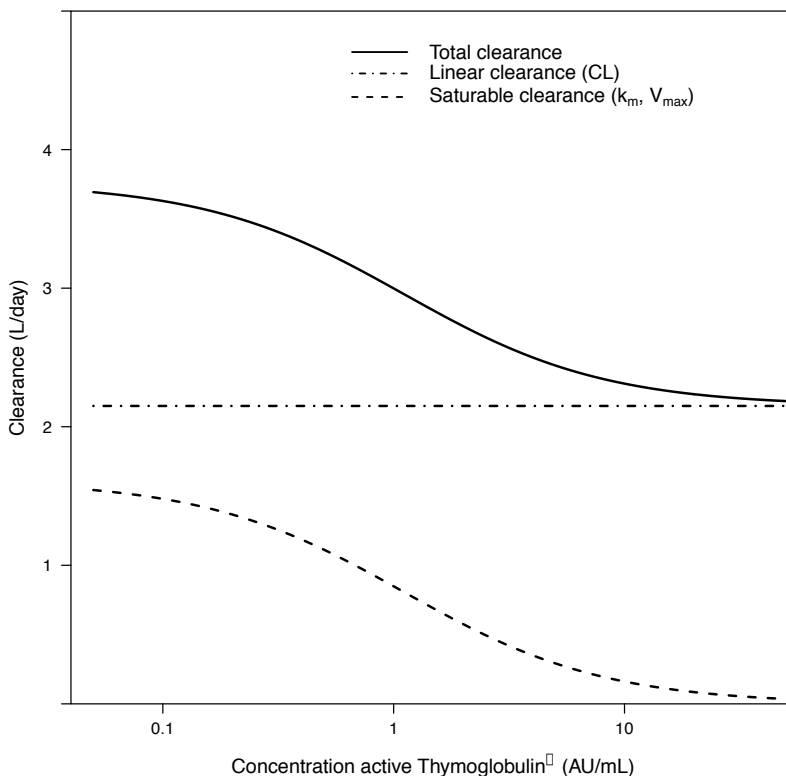
Figure 4. Total Thymoglobulin clearance, the sum of linear and saturable clearance, is dependent on active Thymoglobulin® concentrations.

inter-individual variability in CL. As the lymphocyte count before the first dose of Thymoglobulin® best reflects the current amount of available targets, this covariate was chosen. It was included as a linear relationship with CL (Eq. 4). Inclusion of this covariate led to a decrease in OFV of 54 points (p<0.001). As lymphocyte counts were not available after starting Thymoglobulin®, time was used as a surrogate for decreasing lymphocyte counts to take into account the decreasing lymphocyte counts after dosing of Thymoglobulin®. This did not result in an improvement of the model.

Diseases with a high peripheral lymphocyte burden were marked; these were no covariate on either clearance path- ways. No other covariates were identified, including treatment center, treatment year, and underlying disease.

Internal Validation of the Final Model

The final model with inclusion of all of the above-described covariates seemed stable in the bootstrap analysis with 98% successful runs. Median parameter values as well as the 2.5th and the 97.5th percentiles were in line with the model estimations and standard errors (Table 2). The NPDE showed a normal distribution of errors, with no trends in the NPDE versus time and NPDE versus predictions (Supplemental Figure 1).

Simulations

Figure 5 shows simulated Thymoglobulin® concentrations over time for patients with a body weight of 5, 20, and 40 kg, and lymphocyte counts at the first dose of Thymoglobulin® of 0,
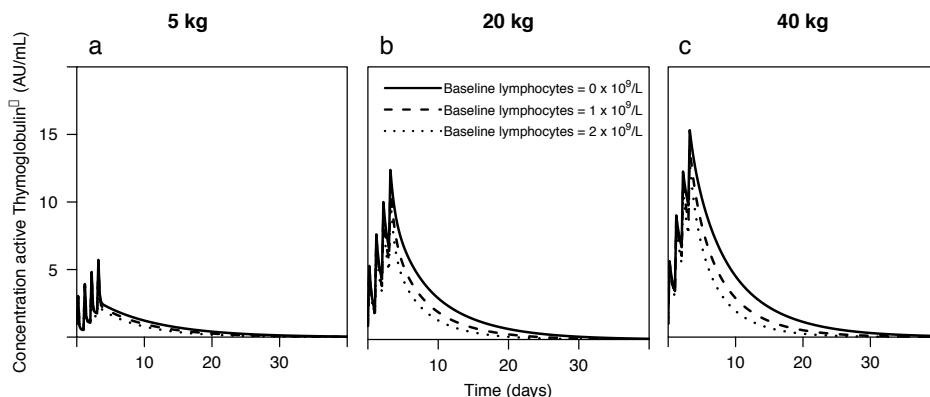
Figure 5. Model based simulation results of active Thymoglobulin® concentrations showing the population predictions of three representative individuals receiving Thymoglobulin® according to the current dosing regimen (cumulative dose of 10 mg/kg in 4 consecutive days), weighing 5 kg (panel a), 20 kg (panel b) and 40 kg (panel c). Solid lines: baseline lymphocytes 0x109/L, dashed lines: baseline lymphocytes 1x109/L, dotted lines: baseline lymphocytes 2x109/L.

1, and 2 x 10^9 cells/L, respectively, using the final model. The figure illustrates that, while using the currently approved dosing regimen, exposure increases with higher weight and/or lower baseline lymphocytes.

DISCUSSION

Thymoglobulin® is considered to play a pivotal role both in preventing GvHD and rejection as well as the occurrence of successful and timely immune reconstitution in HCT. We described the population pharmacokinetics of active Thymoglobulin® in a pediatric population.

Concentrations could be well predicted using this extensively validated model. Body weight and lymphocyte count at first dose of Thymoglobulin® proved to be predictors of CL and V_1. Simulation studies showed the current dosing regimen to be suboptimal, with patients with a higher body weight and/or a lower baseline lymphocyte count having a higher exposure.

In the proposed model, parallel linear and non-linear clearance was identified, which is frequently used for describing the pharmacokinetics of antibodies with non- soluble targets[38–40]. A true target-mediated drug disposition (TMDD) model was not possible due to the non-soluble targets, for which no concentrations were available, combined with the diversity of potential targets for Thymoglobulin® due to its polyclonal nature. This result is in line with the mechanism of action since active Thymoglobulin®, as all antibodies, is cleared through two mechanisms: target binding and non-specific degradation. Target binding is unique for the active fraction, whereas non-specific degradation occurs both in the active and the non-active fraction. The final model was developed based on the assumption that target binding is only possible when sufficient targets are available, resulting in a limited specific clearance in the case of a low number of targets. This also holds true for active Thymoglobulin®, although it has a great number of potential targets including T cells, B cells, natural killer cells, monocytes, and endothelium due to its manufacturing process in rabbits. To further unravel active Thymoglobulin® CL, the total Thymoglobulin® pharmacokinetics may be taken into consideration. Introducing information from total Thymoglobulin® pharmacokinetics, which is approximately 93% not reactive to human targets[27], more information may be gathered on non-active pharmacokinetics, which could sophisticate the current model.

The covariate model building showed actual body weight to be the best predictor for size on both CL and V_1. Lymphocyte count before the first dose of Thymoglobulin® proved to be a predictor for CL. Lymphocyte counts before conditioning (i.e., chemotherapy or

radiotherapy) as well as before the first dose of Thymoglobulin®, which were mostly just days apart, were available. Still, lymphocyte counts dropped between the two measurements in some patients, while other patients had a stable lymphocyte count. This effect appeared not to be related to the type of chemotherapy used. Nonetheless, pre-conditioning and pre-Thymoglobulin® lymphocyte counts were comparable predictors for inter-individual variability on CL. As pre- Thymoglobulin® lymphocyte counts best reflect the current amount of available targets, and therefore the potential for clearance through target binding, pre-Thymoglobulin® lymphocyte counts were used in the covariate model.

Several varieties of ATG are on the market: both horse (Atgam®, Pfizer, New York, NY, USA) and rabbit derived, with the latter being produced using human thymoid tissue (Thymoglobulin®, Sanofi (previously Genzyme), Lyon, France) or a Jurkat T cell leukemia line (ATG-Fresenius® S, Fresenius Biotech, Gräfelfing, Germany) as immunogen[5]. This pharmacokinetic model for Thymoglobulin® might to some extent reflect pharmacokinetics of other varieties of ATG, bearing in mind that the active fraction and its profile of specificities most likely differ[41]. For these products, in analogy to Thymoglobulin®, a population pharmacokinetic model should be developed, which could be partly based on this model.

One pediatric study has been published on the population pharmacokinetics of active Thymoglobulin® in HCT[26]. This study was performed in a relatively small cohort of 13 children, fitted using a two-compartment model with linear pharmacokinetics, where body weight was a covariate for both CL and V_1. Only the mean population value for CL was given, which is roughly in line with our results, although no parallel clearance was incorporated making interpretation difficult. No internal or external validation was performed in that study. Some other studies have been performed in children[21,25], adults[20,23,24], or both[22] based on the standard two-stage approach[23] or non-compartmental analyses[20–22,24,25], reporting on active or total ATG of different brands. The population approach, which we applied here, is considered superior over the standard two-stage approach and non-compartmental analysis[15–17,42]. Therefore, our results are not easily comparable to these results because of differences in methods.

With the developed pharmacokinetic model, active Thymoglobulin® exposure can be predicted in the entire pediatric age range. Dosing can be adjusted so that each child, irrespective of body weight and lymphocyte count, will have a predictable exposure. This is a major improvement when compared to the current dosing regimen, where exposure increases with body weight and/or decreasing baseline lymphocyte count, without evidence being available to support the rationale for this, and possibly even leading to significant side effects such as delayed or absent immune reconstitution. The next step in the development of an individual dosing regimen will be the determination of the therapeutic window.

Pharmacokinetic endpoints such as AUC can be related to clinical outcome measures as survival and the incidence of GvHD, rejection, relapse, and successful immune reconstitution, which have been shown to depend on ATG exposure[22,43,44]. These studies will result in an optimal active Thymoglobulin® exposure, which may vary based on transplant-related factors. The optimal exposure, combined with covariates influencing pharmacokinetics, will deter- mine the Thymoglobulin® dose for each individual patient.

Predictable exposure leads to predictable immune reconstitution, which is paramount for giving adjuvant cellular therapies for consolidation of the treatment for malignancies. Likewise, therapeutic decisions such as starting antivirals or tapering GvHD prophylaxis may also depend on immune reconstitution. All together, this is expected to result in an improvement of outcome after pediatric HCT.

CONCLUSION

We developed and evaluated a population pharmacokinetic model incorporating non-linear distribution and elimination, which accurately describes active Thymoglobulin® pharmacokinetics in the entire pediatric age range. Body weight and baseline lymphocytes were the most predictive covariates influencing the pharmacokinetics of active Thymoglobulin®, which could explain a major part of the inter-individual variability. The current dosing regimen is shown to be suboptimal, leading to varying exposures across age. Once the therapeutic window has been deter- mined, this model can be used to develop an individual dosing regimen for Thymoglobulin®, based on both body weight and lymphocyte counts. This individualized regimen may contribute to a better immune reconstitution, and thus outcome, of allogeneic HCT.

3

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SUPPLEMENTALS
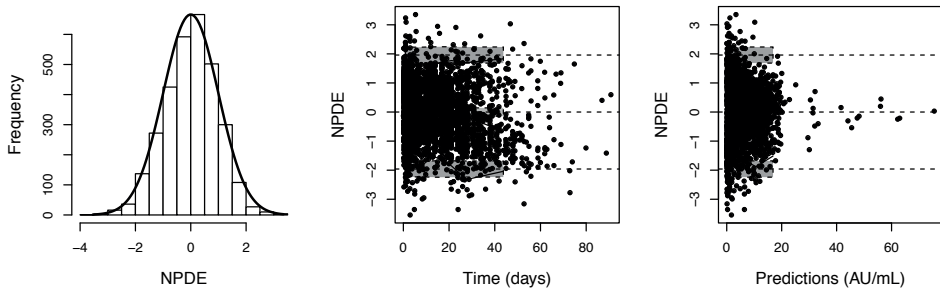
Figure S1. Normalized prediction distribution of errors (NPDE). Panel a: histogram of the NPDE with the solid line representing a normal distribution with a mean of 0 and variance of 1. Panel b: NPDE versus observations, panel c: NPDE versus predictions. Grey blocks: 95% confidence interval of NPDE.

Chapter 4

Population Pharmacokinetics of Alemtuzumab (Campath) in Pediatric Hematopoietic Cell Transplantation: Towards Individualized Dosing to Improve Outcomes

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ABSTRACT

Introduction

Alemtuzumab (Campath®), a humanized anti-CD52 monoclonal antibody, is used to prevent graft-versus-host-disease and graft failure following pediatric hematopoietic cell transplantation (HCT). The therapeutic window is critical, with overexposure being associated with delayed immune reconstitution after HCT, potentially leading to viral reactivations and increased relapse chances. Individual exposure of alemtuzumab is unpredictable due to highly variable pharmacokinetics (PK). Therefore, patients treated with a comparable dose of alemtuzumab may have different drug exposure and thereby clinical outcomes. Describing the population pharmacokinetics in children is the first step towards evidence-based individualized dosing of alemtuzumab.

Methods

Serum alemtuzumab concentrations were measured in all children receiving a HCT with alemtuzumab as part of the conditioning regimen between January 2003 and July 2015, in two pediatric transplant centers. Population PK-analyses were performed using NONMEM 7.3.0. The current dosing regimen, a cumulative dose of 1 mg/kg, will be evaluated using simulation studies.

Results

A total of 1146 concentration samples from 206 patients were included, with age ranging from 2.4 months to 19 years old. Alemtuzumab PK could be best described using a 2-compartment model with parallel saturable and linear elimination pathways. Body weight was a predictor for central volume of distribution and clearance, a body weight dependent exponent was implemented in the latter. No relationships between baseline lymphocyte counts and pharmacokinetics were found. Simulations of the current dosing regimen showed an increase in exposure with increasing body weight.

Conclusion

The pharmacokinetics of alemtuzumab increase in a non-linear fashion with body weight. Therefore, any mg/kg-based dosing will lead to highly variable alemtuzumab exposure in children. Following determination of the therapeutic window, the proposed model can be used to develop an individualized dosing regimen for alemtuzumab, taking into account body weight. Individualized dosing of alemtuzumab may improve outcome following pediatric HCT.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment option for children with a variety of underlying diseases including malignancies, immune deficiencies and bone marrow failure. While the success rates of this procedure in terms of survival have been improving in the last decades, treatment related mortality (TRM) and recurrence of disease remain to be significant hurdles. Approaches to reduce mortality are essential, including the prevention of graft-versus-host-disease (GvHD), which contributes to both short-term and long-term morbidity and mortality following HCT.

Alemtuzumab (Campath®, Genzyme, MA, USA), a humanized anti-CD52 monoclonal antibody, was introduced as serotherapy in HCT to prevent GvHD but also graft failure by in-vivo depletion of lymphocytes. Other drugs used as serotherapy include the poly-clonal antibody anti-thymocyte globulin (ATG). The choice of serotherapy is dictated by center preferences, and previous treatment with ATG due to the potential development of anti-ATG-antibodies at second exposure. Inclusion of alemtuzumab in the conditioning regimen significantly reduces the incidence of both acute and chronic GvHD[1–3], for which an exposure-dependent relationship between alemtuzumab concentrations and occurrence of acute GvHD was reported[4]. On the other hand, higher doses of alemtuzumab have been associated with delayed immune reconstitution (IR) by excessive lymphodepletion[5,6]. IR, especially of T-cells, is dependent on peripheral expansion of graft-infused cells during the first months after HCT; depletion of these T-cells may leave patients with no or little IR. This could potentially lead to increased viral reactivations as well as less graft-versus-leukemia effect, thereby abrogating the beneficial effect on GvHD reduction. Despite a reduced incidence of GvHD, the absence of improvement in survival chances with the inclusion of alemtuzumab[1,2,4,6–9] may be due to absence of T-cell IR. Moreover, most studies report on adult populations; few studies investigate alemtuzumab in pediatric populations.

While evidence suggests a relationship between the use of alemtuzumab and clinical out-comes in adult populations, individual exposure of alemtuzumab is unpredictable due to highly variable pharmacokinetics (PK)[10–13]. As a consequence, patients treated with a com-parable dose of alemtuzumab may have significant differences in drug exposure and thereby clinical outcome. Part of this variability in pharmacokinetics of alemtuzumab may be due to non-linearity in clearance[7,10], where elimation changes from a first-order to a zero-order process after complete binding of targets (e.g. CD52 presented on cells)[14,15]. In addition, only descriptive pharmacokinetics of alemtuzumab are available in pediatric populations, while variability in PK is often most substantial in children[16,17]. The variable PK and fre-quent associations with outcome underline the need for predictable exposure to antibodies

between patients[10,18–21]. In line, the importance of dose individualization and/or therapeutic drug monitoring (TDM) of monoclonal antibodies is increasingly recognized[22,23].

Therefore, there is a great need for a population PK-model for alemtuzumab in pediatric patients receiving a HCT, in order to understand and explain the variability in pharmacokinetics and also to be able to adjust dosing on an individual level aiming for optimal alemtuzumab exposure in the future. In the current study, we describe the population PK of alemtuzumab in children receiving an HCT as a first step to develop an individualized dosing regimen.

PATIENTS AND METHODS

Study Design and Patients

Patients receiving a HCT with alemtuzumab as part of the conditioning, treated at the pediatric wards of the Leiden University Medical Center (Leiden, the Netherlands; LUMC) and Great Ormond Street Hospital (London, United Kingdom; GOSH) between January 2003 and July 2015, were included. In case of multiple HCT's per patient, all transplantations were included. Patients using other serotherapy drugs (anti-thymocyte globulin; ATG) within the same conditioning regimen were excluded. Additionally, patients who received any type of serotherapy in a 3-month period before this HCT were excluded from this analysis. No restrictions were applied on the timing and dose of alemtuzumab, or any patient, disease or transplantation related factors. Data were collected and samples were taken after informed consent was given through the parents and/or the child in accordance with the declaration of Helsinki. Ethical committee approval was acquired through trial numbers P01.028 (Leiden) and V0904 (London).

Alemtuzumab (Campath, Genzyme, Cambridge, MA, USA) was given as an intravenous infusion, usually starting 6-8 days before HCT for 4-5 consecutive days. In London, alemtuzumab was the standard choice for serotherapy, in Leiden, it was reserved for patients with patients with inflammation (selected immune deficiencies) and myelodysplastic syndrome, and for a short period of time standard serotherapy. Patients with hemophagocytic lymphohistiocytosis (HLH) received alemtuzumab 15 days before transplantation. Although the dose of alemtuzumab varied, most patients were given a cumulative dose of 1 mg/kg (5 x 0.2mg/kg/day), with a substantial number of patients receiving alemtuzumab at a cumulative dose of 0.5 mg/kg at the treating physician's discretion. Few included patients received in vitro lymphodepletion of the graft by adding 20mg of alemtuzumab to the infusion bag containing the graft some hours before infusion of the graft, either following a course of alemtuzumab or without receiving prior serotherapy. Patients receiving alemtuzumab

following allergic reactions to ATG in the same conditioning were excluded from this analysis. Conditioning regimens were given according to (inter)national protocols. GvHD-prophylaxis consisted of cyclosporin A (controlled with therapeutic drug monitoring at trough levels of 150-250 μg/L) combined with either prednisolone (cord blood transplants), methotrexate (matched unrelated donor). Patients receiving an identical related donor transplantation or CD34 selected graft did not receive any additional GvHD prophylaxis. All patients received selective gut decontamination and were treated in positive pressure, particle free air-filtered isolation rooms.

Samples for pharmacokinetic measurements were taken before and after each infusion, followed by one sample weekly in the patients from Leiden until approximately +70 days after HCT, while in London, three samples were available per patient: on the day of HCT (day 0), and +14 and +28 days after HCT. The samples around infusion were taken at ±15 minutes before and after infusion, respectively, which in light of the long half-life of alemtuzumab can be seen as true trough and peak levels. Samples were prospectively collected and measured in batches.

Measurement of alemtuzumab concentration and anti-alemtuzumab antibodies

Q-FACS assay
The laboratory in London, measuring part of the London population, used a Q-FACS assay. Alemtuzumab levels were measured using quantitative flow cytometry assays (Q-FACS), in modifications of the method described[24]. In short, 1×10^6 HUT-78 T-cells were incubated using fourfold dilutions of patients serum in PBS, followed by washing and incubation with conjugated secondary antibodies (Alexa Fluor 647 labeled goat-anti-human IgG [Life Technologies]). To construct a reference curve, HUT-cells were incubated with known amounts of alemtuzumab (range 10-0.01 μg/ml), containing 25% human serum. Cells were washed and MFI was measured on a FACS Calibur machine (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA). The lower limit of detection for alemtuzumab in this assay was 0.1 μg/mL.

ELISA assay
All concentration samples from Leiden and part of the samples from London were measured in Leiden using an ELISA-based assay. Microtitre plates (Corning Corporation, Corning, NY, USA) were coated with CD52 anti-idiotype (Geoff Hale Developments, D003p56) diluted in PBS at a concentration of 0.5 μg/mL by incubating overnight at 4°C after blocking with 2% casein in PBS. Samples, controls and a diluted standard range of alemtuzumab (25 ng/mL-0.1 ng/mL, diluted in 10% pooled human serum) were applied and incubated for 1 hr at room temperature (RT). After washing, bound alemtuzumab was detected with biotin-

labeled NC [anti-idiotype antibody (Geoff Hale Developments, D003p95, 0.2 µg/mL), 1 hr at RT, followed by streptavidin poly-HRP (Sanquin, 8000145253, 2 µg/mL), 30 min. The lower limit of detection was 0.01 µg/mL.

In both assays alemtuzumab spiked sera were used as controls. The results of 146 samples tested with both ELISA NC anti-idiotype and Q-FACS were compared. For the correlation only samples with a measured alemtuzumab concentration >0.1 µg/mL in QFACS were used. The correlation between the two used assays was good (R^2 0.89).

Population Pharmacokinetic Analysis

For analysis of the PK-data, non-linear mixed effects modeling was employed using NON-MEM 7.3.0 (Icon, Hanover, MD, USA). R version 3.2.3 and Pirana version 2.8.2 were used for preparation and visualization of data. First order conditional estimation (FOCE) with interaction was used throughout model development. Alemtuzumab concentrations were logarithmically transformed and simultaneously fitted. Samples that were reported to be below the limit of quantification (BLQ), which only occurred in the tail end of concentration, and were set at half the BLQ with subsequent samples being removed in accordance with method M6[26]. Inter-individual variability on PK-parameters was assumed to follow a log-normal distribution, and were implemented in the model according to equation 1:

$$P_i = P_{pop} * e^{\eta_i}$$
(Eq. 1)

where P_i is the individual or post-hoc value of the parameter in the ith individual, P_{pop} is the population mean for this parameter, and η_i the inter-individual variability of the ith person, which samples from a normal distribution with a mean of 0 and a variance of ω^2. An additive error model was used, which due to logarithmically transformed data should be seen as a proportional error model. Here, the jth observation for the ith individual was described using equation 2:

$$Y_{i,j} = C_{pred,i,j} + \varepsilon$$
(Eq. 2)

where $Y_{i,j}$ is the observed concentration, $C_{pred,i,j}$ the jth predicted concentration for individual i, and ε the error, sampled from a normal distribution with a mean of 0 and a variance of σ^2.

Several criteria applied in the process of model building and selection. A decrease in objective function value (OFV) over 3.84 points between nested models was considered statistically significant, this correlated with $p<0.05$ based on a Chi-squared distribution with 1

degree of freedom. Goodness of fit plots were evaluated, including observed versus both individual and population predicted concentrations, as well as conditional weighted residuals (CWRES) versus time and observed concentrations. Additionally, parameter uncertainty and η-shrinkage were evaluated to assess model performance.

Inter-occasion variability (IOV) was tested to assess changes in parameters between the respective doses according to equation 3:

$$P_i = P_{pop} * e^{\eta_i + \kappa_m}$$

(Eq. 3)

where, compared to equation 1, κ_i is the inter-occasion variability for the *m*th occasion. Individual pharmacokinetic parameters (post-hocs) were estimated using the POSTHOC option in NONMEM

The elimination of antibodies is often dependent on the concentration of substrate[32,33], therefore non-linear elimination pathways were explored. No data was available on target concentrations over time (i.e. CD52, or lymphocytes), therefore full TMDD-models as previously described were not persued[33,34]. Instead, non-linear elimination pathways were explored by incorporating clearance described by Michaelis-Menten kinetics:

$$V = \frac{V_{max} * C}{K_m + C}$$

(Eq. 4)

where V is the elimination rate, V_{max} the maximum elimination rate, C the concentration alemtuzumab, and K_m the Michaelis-Menten constant; the concentration at which 50% of maximum elimination rate is reached.

Covariate Model
Patient characteristics, including body-size parameters, and transplant- and disease specific variables were studied as a possible covariate for their relation with PK-parameters. In line with previous reports, the role of lymphocyte counts on alemtuzumab pharmacokinetics was also investigated, as CD52 is almost exclusively expressed on these cells. Cell counts drawn before the first infusion of alemtuzumab were available; the lymphocyte counts are greatly reduced after the first dose in most patients and were therefore not available. Therefore, we considered lymphocyte counts drawn within 48 hours before infusion of the first alemtuzumab dose as a covariate.

To assess the covariate relations, post-hocs, inter-individual variability and CWRES were plotted against covariates, both before and after inclusion of the covariates, to evaluate potential relationships. Lastly, only those covariates where a physiological or pharmacological mechanism could be hypothesized were included. Continuous covariates such as age and body weight were tested in a linear and power function (equations 5 and 6):

$$P_i = P_{pop} * \left(1 + \left(\frac{Cov_i}{Cov_{median}}\right) * l\right) \qquad \text{(Eq. 5)}$$

$$P_i = P_{pop} * \left(\frac{Cov_i}{Cov_{median}}\right)^k \qquad \text{(Eq. 6)}$$

where P_i and Cov_i are the parameter and covariate value for the ith individual, P_{pop} the population mean for this parameter, Cov_{median} the standardized value for the covariate. In the linear relationship equation (eq. 5) l represents the slope factor of the linear function, while in the power-relationship equation (eq. 6) k is the scaling factor. Additionally, more complex variations of equation 6 were explored, where k is dependent on the covariate value of the ith individual, as proposed by Wang et al[27], and implemented in several other models[28,29]. Evaluated variations included an E_{max} approach and a power function according to:

$$k = k_0 - \frac{k_{max} * BW_i{}^{\Upsilon}}{k_{50}{}^{\Upsilon} + BW_i{}^{\Upsilon}} \qquad \text{(Eq. 7)}$$

$$k = a * BW^b \qquad \text{(Eq. 8)}$$

where k is the exponential scaling factor in equation 6, k_0 the value for the exponent for an individual with a hypothetical bodyweight of 0 kg, k_{max} the maximum decrease of the exponent, k_{50} the bodyweight at which 50% of k_{max} is reached, and Υ the hill coefficient determining the steepness of the sigmoidal decline. In a power function, a represents the coefficient and b is the exponent.

Potential covariates were evaluated using forward inclusion and backward elimination with a significance level of <0.005 (-7.9 points in OFV) and <0.001 (-10.8 points in OFV), respectively. Building of the covariate model was comparable to the development of the structural model. In addition, after inclusion of a covariate, a decline in unexplained inter-individual variability had to be achieved before inclusion into the final model[30].

Model Evaluation

As the main goal for this model is to guide future dosing in children, the model has to be thoroughly evaluated for its robustness. To assess the predictive performance of the model, bootstrap analyses were performed, stratified on treatment center. One thousand datasets were created using random selection from the original dataset; the final model was fit to each data set. For each parameter, median values from the thousand fits for each parameter as well as 95% confidence intervals were compared to parameter estimates of the final model.

In addition, a normalized prediction distribution of errors (NPDE) was performed, where the prediction discrepancies are simulated taking into account the correlation between observations in the same individual and the predictive distribution[31]. Finally, prediction-corrected visual predictive checks (VPC) were created to assess the predictive performance of the final model as compared to the measured concentrations.

Dose Simulations

To evaluate the current most frequently used dosing regimen for alemtuzumab (cumulative dose of 1 mg/kg over 5 days; 0.2 mg/kg/day), we performed simulation studies. Patients were selected based on representative covariate values; simulations were performed taking into account the full random effects model with 500 patients per group being simulated. Median as well as 95% confidence intervals of concentration over time are shown.

RESULTS

Patients

A total of 206 patients receiving 212 HCT's were included from the two treatment centers (Table 1). Median age was 4.8 years old, median body weight was 17.2 kilogram. Fifty-four percent of patients received a cumulative alemtuzumab dose of 0.9 to 1.1 mg/kg, while 35% received a cumulative dose of less than 0.9 mg/kg. Median starting day relative to the infusion of stem cells was -8 days, ranging from 0 days (alemtuzumab added to the bag containing the stem cells) to 21 days before transplantation. Most patients (52%) received a HCT to treat an immune deficiency; the most frequently used stem cell source was bone marrow. A total number of 1146 concentration samples were available for this analysis (median 5.4 samples per patient; Figure 1). The majority of the samples (84%, collected in 136 patients) were measured in Leiden.

Structural Pharmacokinetic Model

A two-compartment model best described the pharmacokinetics of alemtuzumab (Table 2; Figure 2). Compared to a one-compartment model, the two-compartment was superior in

terms of goodness-of-fit (GOF) plots and objective function value (253 points decrease in OFV; p<0.001). Three patients in the lowest body-weight group were significantly under-predicted in low concentrations (Figure S2). However, large residual standard errors in the parameters associated with distribution were observed, as well a high dependency on initial values. To address this issue, model simplification was applied with peripheral volume of

	London	Leiden	Total
Number of patients (n)	139	67	206
Number of HCTs (n)	139	73	212
Male sex (%)	66	67	67
Age (years)	4.0 (0.4-15)	7.3 (0.2-19)	4.8 (0.2-19)
Weight (kg)	16.0 (4.0-60)	21.0 (2.6-80)	17.2 (2.6-80)
Number of samples [n (mean per patient)]	343 (2.5)	803 (11.0)	1146 (5.4)
Location of Concentration Measurements (% of samples)			
Leiden	47	100	84
London	52	0	16
Starting day alemtuzumab (days before transplantation)	8 (5-21)	6 (0-16)	8 (0-21)
Lymphocyte count before conditioning (x 10^9)	0.74 (0.00-9.3)	0.54 (0.03-7.5)	0.74 (0.00-9.3)
Cumulative dose (mg/kg) [%]			
<0.9 mg/kg	37	31	35
0.9-1.1 mg/kg	50	62	54
>1.1 mg/kg	13	7	11
Diagnosis (%)			
Hematologic Malignancy	17	40	25
Immune deficiency	62	34	52
Bone marrow failure	15	25	18
Metabolic disease	5	0	4
Benign hematology	1	1	1
Stem cell source (%)			
Bone marrow	61	60	61
Peripheral blood stem cells	39	32	36
Cordblood	0	8	3
Conditioning regimen (%)			
Reduced intensity (NMA)	43	66	51
Chemotherapy-based (MA)	51	29	43
TBI-based (MA)	6	5	6

Shown as median (range) unless otherwise specified

Table 1. Patient Characteristics. HCT: hematopoietic cell transplantation; TBI: total body irradiation; NMA: non-myeloablative; MA: myeloablative
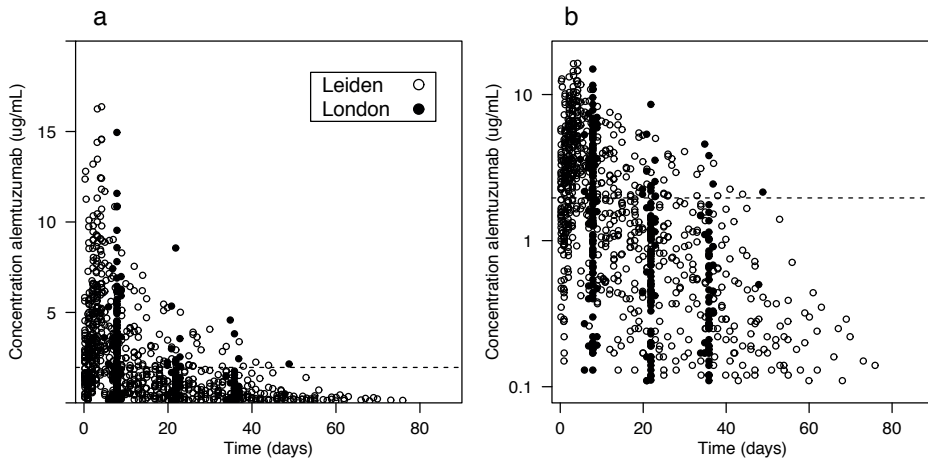
Figure 1. Concentration-time plots of all patients from LUMC Leiden (open circles) and GOSH London (dots) on a normal scale (panel a) and a log scale (panel b). Dashed line: Michaelis-Menten constant K_m.

	Dataset [estimate (RSE)]	Shrinkage (%)	1000 bootstrap replicates (99.1% successful)	
Parameter			Median	95% confidence interval
Structural model				
$$Cl_i = CL_{pop} * \left(\frac{WT}{WT_{med}} \right)^{(a*WT)^b}$$				
CL_{pop} (L/day)	0.20 (27%)		0.21	0.12-0.33
a	0.048 (33%)		0.047	0.022-0.102
b	-0.47 (30%)		-0.55	-2.53 - -0.2
$$V_{1,i} = V_{1,pop} * \left(\frac{WT}{WT_{med}} \right)^{c}$$				
$V_{1,pop}$ (L)	1.89 (9%)		1.87	1.49-2.3
c	0.72 (11%)		0.72	0.56-0.91
$V_{2,pop}$ (factor of V1)	0.82 (20%)		0.89	0.59-1.24
Q_{pop} (L/day)	0.33 (28%)		0.34	0.21-0.98
$V_{max,pop}$ (mg /day)	0.60 (30%)		0.63	0.28-0.99
$K_{m,pop}$ (mg/L)	1.96 (36%)		1.87	0.97-4.1
Random variability				
Inter-individual variability on CL (%)	117 (14%)	17	117	92-145
Inter-individual variability on V_1 (%)	56 (11%)	22	58	45-70
Inter-individual variability on K_m (%)	144 (9%)	30	140	111-180
Proportional residual error (%)	34 (9%)	18	33	28-39

Table 2. Parameter Estimates and Bootstrap Results. *Cl* linear clearance, *WT* body weight (kg), WT_{median} median population body weight (17.3 kg). V_1 central volume of distribution, V_2 peripheral volume of distribution, *Q* intercompartmental clearance, V_{max} maximum transport rate for saturable clearance pathway, K_m Michaelis–Menten constant saturable distribution for saturable clearance pathway, RSE relative standard error
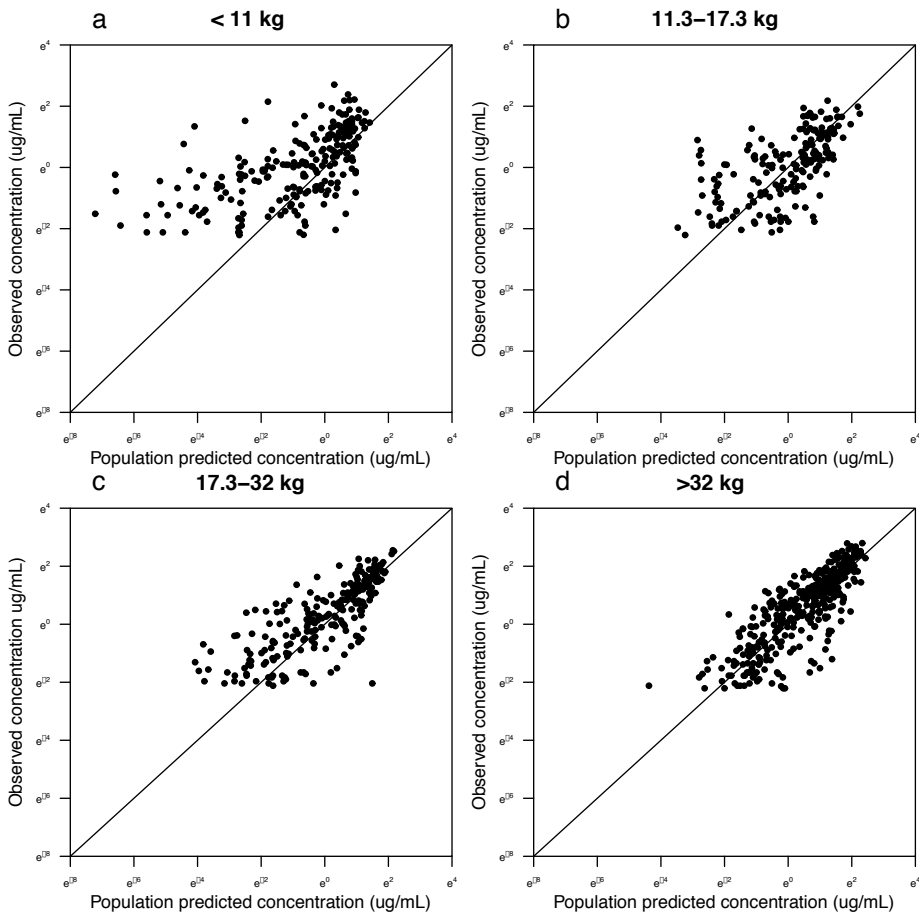
Figure 2. Goodness-of-fit plots of the final model: population predicted versus observed concentrations of alemtuzumab in all patients, split by quartiles of body weight. Panel A: < 11kg; Panel B: 11-17.3kg; Panel C: 17.3-32kg; Panel D: >32kg. Lines: line of unity (x=y). In the <11 kg group, three individuals are under-predicted.

distribution (V2) being estimated as a factor of central volume of distribution (V1), which made the model more stable and independent on initial values. This model yielded a decrease of 158 points in OFV compared to the one-compartment model (p<0.001), and showed comparable GOF plots compared to the full two-compartment model. A three-compartment model proved unstable, showing inaccurate parameter estimates. A proportional error model was incorporated in the model.

Looking at the individual concentration-time profiles, non-linear pharmacokinetics could be identified (Figure 1). Models with only non-linear clearance as well as models with parallel linear and non-linear clearance were evaluated. Here, compared to only linear clearance,
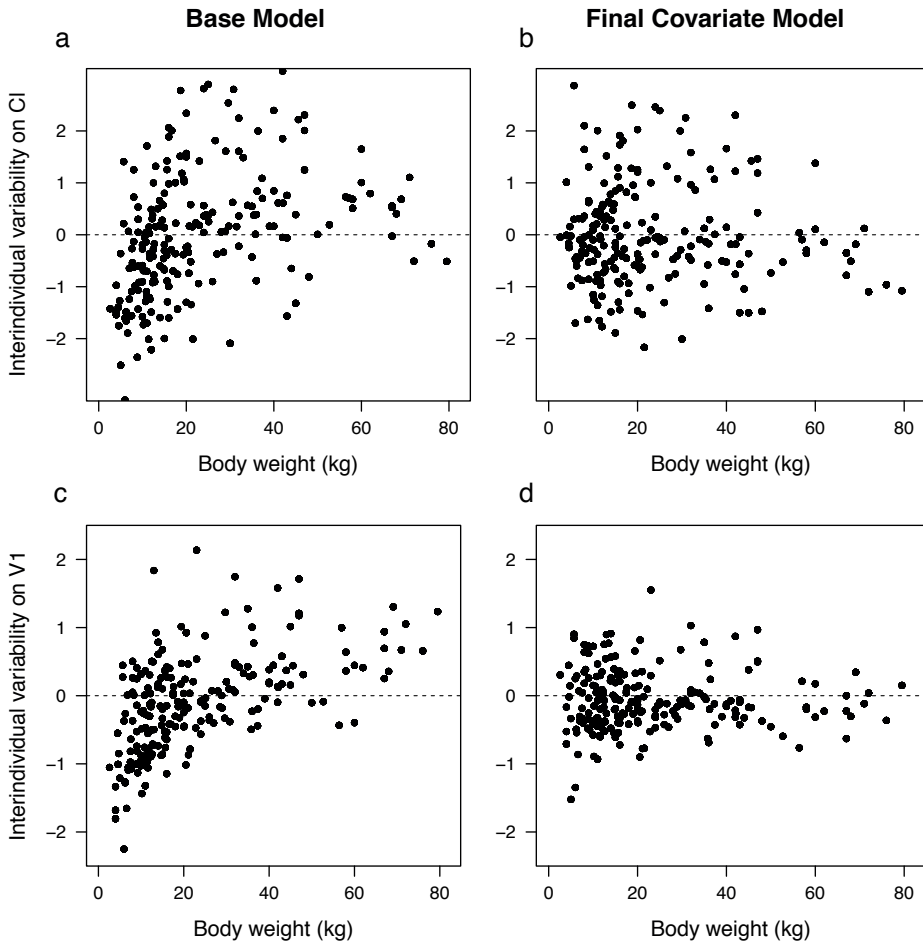
Figure 3. Interindividual variability on clearance (upper plots) and central volume of distribution (lower plots), both before (left plots) and after (right plots) inclusion of body weight as a covariate.

both models resulted in a significant decrease in OFV, however the model with parallel clearance pathways was clearly superior (-39 and -99 points in OFV for only non-linear and parallel clearance with three and four additional parameters, respectively). Therefore, alemtuzumab elimination was described using linear clearance (CL) and non-linear clearance, which was parameterized using the quotient of maximum elimination rate V_{max} and Michaelis-Menten constant K_m, depicting the concentration at which the elimination rate was 50% of Vmax. Besides a decrease of 99 points in OFV (four additional parameters, p<0.001), the addition of non-linear clearance to the linear clearance model resulted in an improvement in GOF-plots. The Michaelis-Menten constant could be well estimated and fell within the observed concentration range (Figure 1). No improvement of the model in
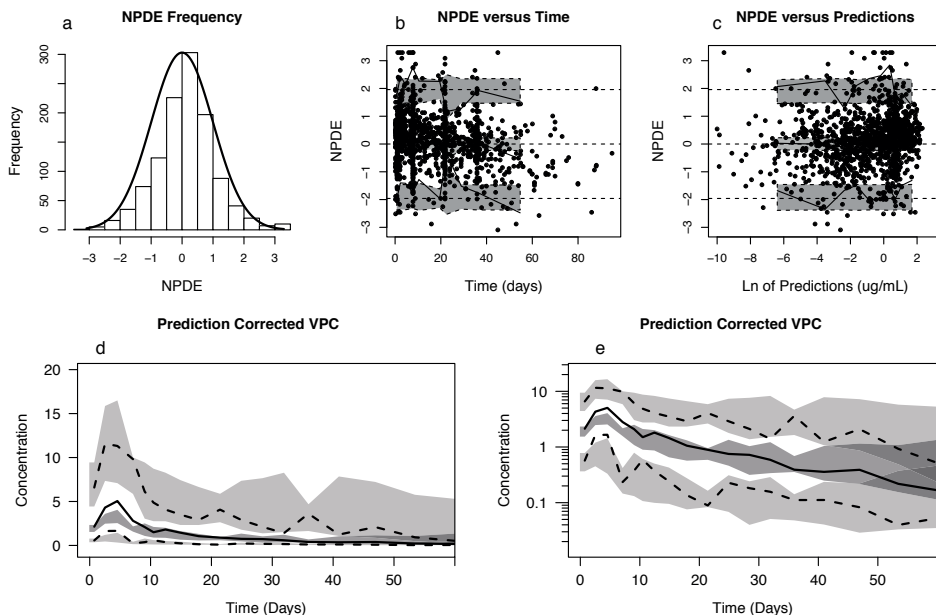
Figure 4. Validation studies. Panels A-C: Normalized Prediction Distribution of Errors (NPDE). Panel A: Histogram of the NPDE with the solid line representing a normal distribution with a mean of 0 and variance of 1. Panel B: NPDE versus time; Panel C: NPDE versus predictions. Grey blocks: 95% confidence interval of NPDE. Panels D-E: Prediction corrected visual predictive check (VPC) on a normal axis (panel D) and logarithmically transformed axis (panel E). Solid line: median of data, dashed lines: 95% confidence intervals of data, dark grey blocks: median of simulations, light grey blocks: 95% confidence intervals of simulations.

terms of OFV and goodness-of-fit-plots was observed when including IOV on any of the parameters.

Covariate Model

According to the predefined criteria, the covariate analysis showed that actual body weight and age were correlated with both central volume of distribution and linear clearance. Actual body weight proved the best predictor for both parameters both in terms of decrease in OFV and improvement of GOF plots (Figure 3). Inclusion of body weight as a power function (eq. 4) on V1 and CL yielded a decrease in OFV of 92 and 43 points, respectively. In addition, the effect of body weight on CL was parameterized as a body-weight dependent exponent (BDE), in which the exponent (k in eq. 4) differs according to body weight (Figure 3)[28,29]. Including a BDE parameterization on clearance gave a better description of the relation with body weight, especially in the smaller children, as seen in plots of interindividual variability on CL versus body weight. The exponent in this model varied from 1.94 in children of 5 kg bodyweight to 0.54 in patients weighing 80 kg. Inclusion of
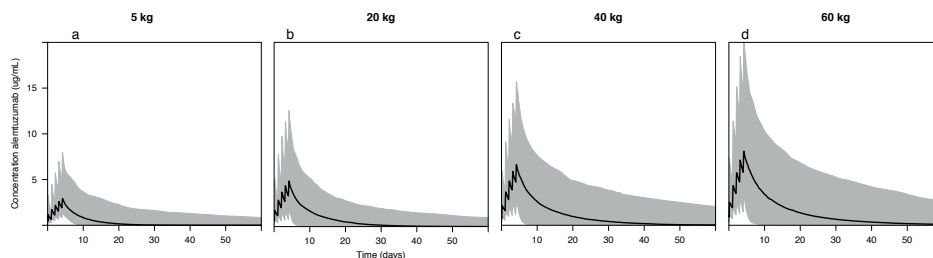
Figure 5. Simulation studies showing median (lines) and 75% confidence intervals (grey areas) of concentration over time after a cumulative dose of 1 mg/kg divided over 5 consecutive days (5 x 0.2 mg/kg/day).

a BDE-parameterization gave an additional decrease of 9 points in OFV (one additional parameter) as compared to a non-changing exponent.

Next, lymphocyte counts were evaluated as a covariate for alemtuzumab elimination. Data on lymphocyte counts were missing in 56 patients, these were set at median to give a gross covariate value of 1. Baseline peripheral blood lymphocyte counts did not influence any PK-parameter, including linear and non-linear elimination.

Internal validation

The final model with body weight on volume of distribution and in a BDE-parameterization on linear clearance was stable in bootstrap analysis (99.1% successful). The bootstrap was stratified on treatment center to account for the density of sampling. Median and 95% confidence intervals were in line with the model estimations and residual standard errors (Table 2). The NPDE-analysis showed normally distributed errors, with no major trends in NPDE versus time or NPDE versus predictions. The prediction corrected VPC shows model simulations to be well in line with model predictions, both in high and low concentrations (Figure 4).

Simulations

Concentrations over time profiles were simulated for patients with a body weight of 5, 20, 40 and 60 kg; medians as well as 95% confidence intervals are shown (Figure 5). Simulation studies show that, while using the same cumulative mg/kg dose, alemtuzumab exposure increases, proving the current dosing regimen to be suboptimal. Additionally, the unexplained variability in alemtuzumab pharmacokinetics is substantial, as seen in the confidence intervals.

DISCUSSION

Alemtuzumab plays an important role in preventing GvHD and relapse following pediatric HCT as well as the occurrence of early T-cell immune reconstitution. In this large cohort of children, we describe the population pharmacokinetics of alemtuzumab in a HCT setting. The proposed model adequately describes the observed concentrations, and was extensively validated. Actual body weight was found to be a predictor for clearance and central volume of distribution, and should therefore be taken into account for dosing of alemtuzumab. The most frequently used dosing regimen is shown to lead to escalating exposure with increasing body weight, of which the implications are yet unknown.

In the developed model, alemtuzumab elimination was best described using a parallel linear and saturable clearance pathway. This is in line with antibody pharmacology, where both target binding and non-specific degradation are the major elimination pathways. The implemented parameterization with Michaelis-Menten kinetics is often used, and particularly when the antibody targets a non-soluble protein[32]. As lymphocytes harbor the vast majority of CD52, the lymphocyte count was considered as a covariate for elimination. However, no impact of lymphocyte counts on any PK parameter was found. A possible explanation could be that a vast excess of drug is introduced in relation to the amount of CD52, thereby minimizing the effect of target availability. This should, however, be kept in mind when significantly decreasing the administered dose, as lymphocyte counts are known to influence alemtuzumab clearance at lower dosages.

One previous study by Mould et al. described the population pharmacokinetics of alemtuzumab in a population of adults treated for chronic lymphatic leukemia (CLL)[10]. Here, alemtuzumab PK was described using a two-compartment model, incorporating saturable clearance. White blood cell (WBC) count on V_{max} was found to be the only covariate predicting PK, indicating a higher maximal clearance rate in patients harboring more targets for alemtuzumab. Although the population and treatment setting in the current study is significantly different, our parameter estimates in terms of total clearance and central volume of distribution are roughly in line with their results. Importantly, the doses used in the present study (0.5-1 mg/kg) are significantly higher than those in the CLL-study, where the majority of patients received a dose of 3-30 mg (corresponding with 0.04 to 0.4 mg/kg bases on a 70-kg weighing adult). This may explain why the CLL-study did indeed find cell counts to impact elimination, while in an HCT-setting, using high doses of alemtuzumab; the role of cell counts on the PK is minor.

Few studies have investigated the dose-effect or exposure-effect relationship of alemtuzumab in terms of immune reconstitution. Nonetheless, T-cell reconstitution, especially of CD3+

and CD4+ T-cells, is suggested to be slower following higher exposures of alemtuzumab[4,6,35]. In terms of clinical outcome parameters, higher doses of alemtuzumab have been associated with a lower incidence of GvHD[1,2,4,7,9,36,37]. In one study investigating alemtuzumab concentration rather than dosage, those patients with higher concentrations on the day of HCT had less acute GvHD, but more mixed chimerism and poor immune reconstitution, however no impact on survival was demonstrated based on the concentrations on the day of HCT. The authors suggest an optimal day 0 concentration of 0.2-0.4 mcg/mL, however the simulation studies show that a majority of patients will have higher day 0 concentrations when a cumulative dose of 1 mg/kg (Figure 5).

The available evidence for the therapeutic window of alemtuzumab is still minor, and fully based on single concentrations as a predictor for outcome. Using the presented PK-model, full concentration-time profiles can be estimated for all included patients, after which multiple alemtuzumab exposure measures can be related to outcome. In previous work on the polyclonal anti-thymocyte globulin in pediatric HCT, exposure before and after HCT was found to be a powerful predictor for outcome[18]. These exposure measures may be more predictive for outcome compared to single concentrations on day 0. Following the determination of the therapeutic window, the proposed model may serve a basis for individualized dosing of alemtuzumab to ensure optimal outcome.

Besides alemtuzumab, ATG is a drug that is frequently used as serotherapy in HCT. Comparative studies between ATG and alemtuzumab show patients treated with alemtuzumab to have significantly slower immune reconstitution compared to ATG[35]. Still, alemtuzumab is associated with a lower incidence of acute and chronic GvHD when compared to ATG[38,39] but not with survival[38–41]. Albeit most centers prefer ATG over alemtuzumab, there still is a place for alemtuzumab in the conditioning of second transplants due to the possibility of anti-drug-antibody development after receiving a course of rabbit-derived ATG.

In recent years, alemtuzumab (marketed as Lemtrada®) was introduced as a treatment modality for relapsing-remitting multiple sclerosis (RRMS), where it is superior when compared to standard treatment with interferon-β in terms of relapse and disease progression[42–44]. In order to expand the economical market value for the indication RRMS, alemtuzumab was withdrawn from the market for all other indications by the manufacturer, including the brand Campath® which was registered for the treatment of CLL, prevention and treatment of solid organ transplant rejection, and as serotherapy in HCT. However, the manufacturer still has a compassionate use program for Campath making it available for use in HCT.

CONCLUSION

We have developed and extensively validated a population pharmacokinetic model, which adequately describes alemtuzumab PK over the entire pediatric age range. This model incorporates parallel linear and non-linear elimination pathways, reflecting TMDD as frequently observed in antibody kinetics. Actual body weight was identified as a covariate on clearance and volume of distribution, the former as a bodyweight-dependent exponent. Although CD52 is mainly expressed on lymphocytes, no relationship between lymphocyte counts and alemtuzumab elimination was found. Evaluation of the current dosing regimen showed that exposure varies across age and is therefore suboptimal.

This model can be used for further studies to investigate optimal alemtuzumab exposure, and subsequently serve as the basis for an individualized dosing regimen for children receiving a HCT. Using this regimen, optimal alemtuzumab exposure can be achieved, potentially improving clinical outcome in these children.
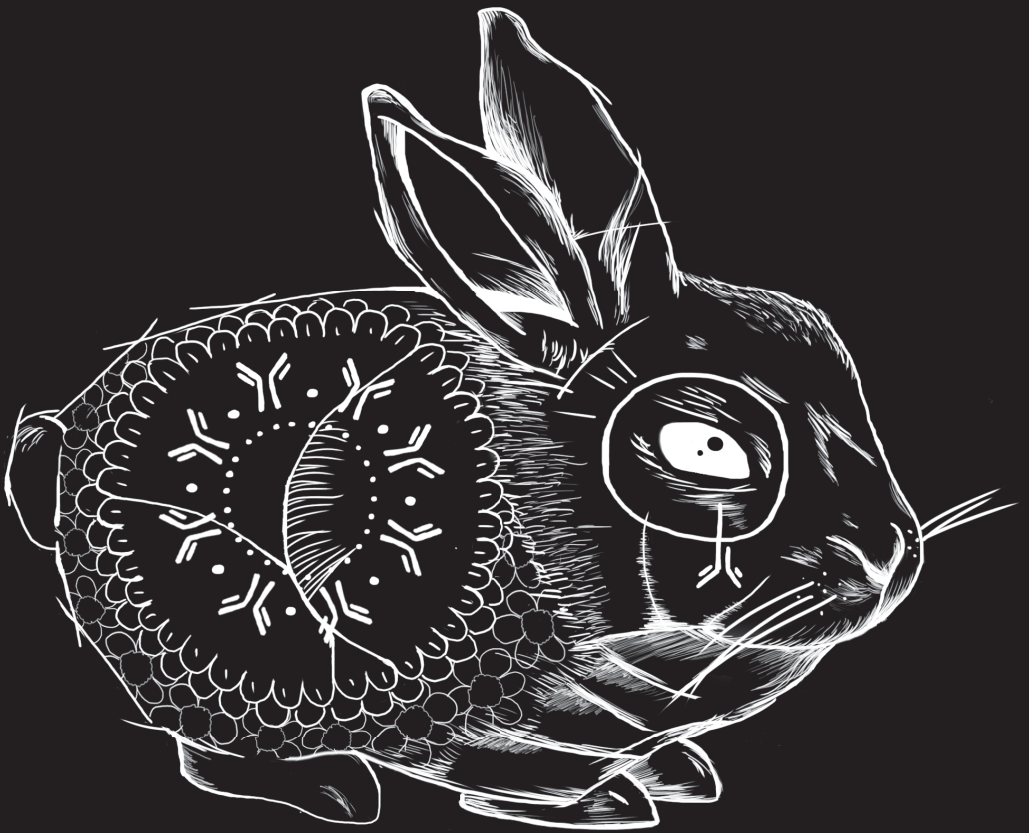
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PART III

Exposure-Response Relationships
of Serotherapy
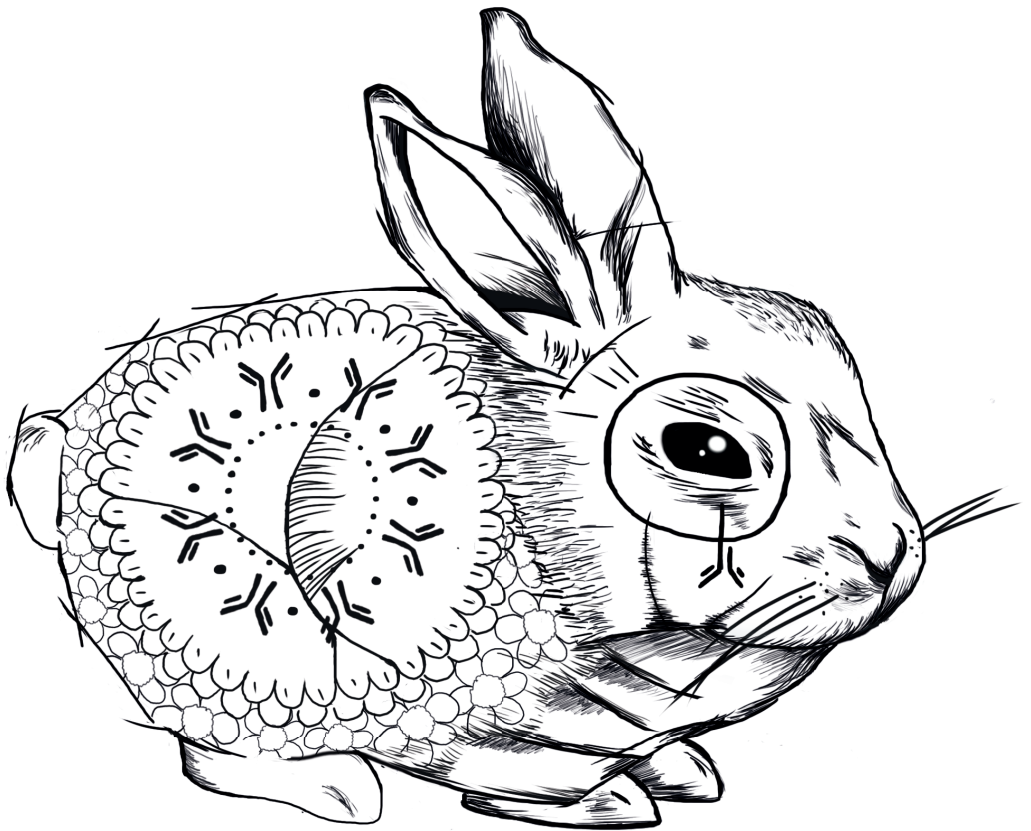
Chapter 5

Association between Anti-Thymocyte Globulin Exposure and CD4+ Immune Reconstitution in Paediatric Haematopoietic Cell Transplantation: a Retrospective Pharmacodynamic Cohort Analysis

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SUMMARY

Background

Anti-thymocyte globulin (ATG) was introduced into the conditioning regimen in haemopoietic cell transplantation (HCT) to prevent graft-versus-host-disease (GvHD) and graft failure. However, ATG can also cause delayed immune reconstitution of donor T cells. We studied the relation between exposure to active ATG and clinical outcomes in children.

Methods

In this retrospective analysis, all patients (age 0·2–23 years) receiving their first HCT between April 1, 2004, and April 1, 2012, who received ATG (thymoglobulin) in two Dutch paediatric HCT programmes were included. The cumulative dose of ATG was chosen according to local protocols and was given intravenously over 4 days consecutively. ATG exposure measures (maximum concentration, concentration at time of HCT, clearance, days to reach a concentration below the lympholytic concentration of one arbitrary unit [AU] per mL, total area under the curve [AUC], AUC before HCT, and AUC after HCT) were calculated using a validated population pharmacokinetic model. The main outcome of interest was immune reconstitution (defined as CD4+ T cells $>0·05 \times 10^9$ cells per L in two consecutive measurements within 100 days). Other outcomes of interest were survival, acute and chronic GvHD, and graft failure. We used Cox proportional hazard models, logistic regression models, and Fine-Gray competing risk regressions for analyses.

Findings

251 patients were included. The chance of successful immune reconstitution decreased as the ATG AUC after HCT increased (odds ratio 0·991, 95% CI 0·987–0·996; p<0·0001). Within the cord blood group, we noted decreased immune reconstitution above the lowest AUC quartile ($\geq$20 AU × day/mL; p=0·0024), whereas in the bone marrow or peripheral blood stem cell group, decreased immune reconstitution was noted only in the highest quartile ($\geq$100 AU × day/mL; p=0·0024). Successful immune reconstitution by day 100 was associated with increased overall survival (hazard ratio [HR] 0·49, 95% CI 0·29–0·81; p=0·0047) caused by reduced non-relapse mortality (0·40, 0·21–0·77; p=0·0062), and relapse-related mortality in myeloid leukaemia (0·25, 0·08–0·76; p=0·015). An AUC before transplantation of at least 40 AU × day/mL resulted in a lower incidence of acute GvHD (grade 2–4 HR 0·979, 95% CI 0·963–0·994; p=0·0081; and grade 3–4 0·975, 0·952–0·998; p=0·033), chronic GvHD (0·983, 0·968–0·998; p=0·029), and graft failure (0·981, 0·965–0·997; p=0·020) compared with an AUC of less than 40 AU × day/mL.

Interpretation

These results stress the importance of improving the efficacy and safety of ATG in HCT by amending dosage and timing. Individualised dosing and timing of ATG to aim for optimum exposure before and after HCT could result in improved outcomes after paediatric HCT.

Funding

INTRODUCTION

Haemopoietic cell transplantation (HCT) is a curative treatment for various underlying malignancies and benign disorders in children. To reduce the risk of graft failure and graft-versus-host disease (GvHD), in-vivo lymphodepletion through serotherapy, such as by anti- thymocyte globulin (ATG), was introduced into conditioning regimens.[1] Although serotherapy has led to a decreased incidence of GvHD and graft failure, side- effects such as viral reactivation and a loss of graft- versus-leukaemia effect have emerged, caused by delayed or absent early T-cell reconstitution after HCT.[2–6]

Although ATG is a commonly used serotherapy drug, its optimum therapeutic window and the timing of treatment have not been defined. Nevertheless, the dosage of ATG has been suggested to affect outcome in terms of GvHD, graft failure, and immune reconstitution.[3,7–9] Since ATG has a half-life of 5–14 days, which means patients are exposed to ATG both before and after HCT,[8–11] the timing of ATG relative to the HCT (i.e., starting day –9 or day –5) also has an effect on outcome.[2] The variable and unpredictable exposure to ATG after comparable doses of ATG further complicates this treatment.[9,12,13] Moreover, T-cell reconstitution[7,14] and the risk of GvHD[2,3,15] differs between cord blood, bone marrow, or peripheral blood stem cell sources, suggesting that optimum exposure might vary between graft types.

Furthermore, different products of ATG are not biosimilar; in this study, we focus on thymoglobulin (Genzyme, Cambridge, MA, USA) because it is the most frequently used type of ATG in HCT. When treating children, developmental pharmacokinetics also needs to be taken into account. The commonly used dosing regimen of thymoglobulin in HCT is a dose of 2·5 mg/kg for 4 days consecutively starting on day –5. This results in markedly different exposure to ATG between age groups, with older children having a disproportionately higher exposure because clearance per kg is lower in older than in younger children.[16] Also, a low lymphocyte count at the time of ATG infusion, which is the target for ATG and therefore its elimination pathway, leads to high ATG exposure.[16]

We aimed to assess the relation between ATG exposure, calculated using a recently developed pharmacokinetic model,[16] and clinical outcome. To achieve this, we did a retrospective analysis to relate different exposure measures of the pharmacologically active fraction of ATG (hereafter referred to as ATG) to various outcome parameters of HCT, such as immune reconstitution, GvHD, graft failure, and survival.

METHODS

Study design and patients

In this analysis, we included all patients (age 0·2–23 years) who received allogeneic HCT with ATG (thymoglobulin only) as part of the conditioning regimen who were enrolled at two paediatric HCT centres in the Netherlands (University Medical Center Utrecht [UMCU], Utrecht, and Leiden University Medical Center [LUMC], Leiden) from April 1, 2004, to April 1, 2012. Only patients undergoing their first HCT were included; there was no restriction on the indication, cell source used, or dose of ATG used. Consecutive patients were included. We excluded patients who were in receipt of serotherapy other than thymoglobulin within 3 months before HCT and those who developed neutralising IgG anti-ATG antibodies within 1 month after HCT. Clinical data were collected prospectively and registered to the clinical database. Additionally, blood samples were prospectively collected for measurement of ATG concentrations.

Minimum follow-up for surviving patients was 6 months. Patients were included and data collected after written informed consent was obtained in accordance with the Declaration of Helsinki. Institutional ethical committee approval for sample and data collection was obtained through trial numbers 05/143 and 11/063-k (UMCU) and P01.028 (LUMC).

Procedures

According to national and international protocols, patients typically received a cumulative dose of 10 mg/kg ATG (thymoglobulin); the infusion of the first dose was started a median of 5 days (range 1–19) before transplantation. The daily dose was administered as a continuous 4 h infusion at constant rate (UMCU) or as an infusion with an increasing rate over 4–5 h (LUMC). According to the local protocol, patients weighing over 40 kg who were treated from 2010 onwards in UMCU received a lower dose of 7·5 mg/kg ATG. Patients treated from 2010 onwards with a cord blood transplant in UMCU received ATG at day –9 instead of day –5. According to local protocol, patients with haemophagocytic lymphohistiocytosis received higher doses of ATG.

Conditioning regimens were given according to national and international protocols. For busulfan-containing regimens, which were given intravenously, therapeutic drug monitoring was used to aim for an area under the curve (AUC) of 75–95 mg × h/day in a myeloablative setting.[17,18] Reduced-intensity conditioning with ATG was reserved for patients with severe aplastic anaemia and Fanconi's anaemia. Patients who were receiving a reduced intensity conditioning for other indications received alemtuzumab as serotherapy and were therefore not included in this study. Patients received gut decontamination, infection prophylaxis, and GvHD prophylaxis according to local protocols, as described previously.[12,18] GvHD prophylaxis mostly consisted of cyclosporin A, with a target trough concentration of 150–250 µg/L controlled by therapeutic drug monitoring, combined with either prednisolone 1 mg/kg per day for patients receiving a cord blood transplant; or mycophenolate mofetil 15 mg/kg per day or methotrexate 10 mg/m² (on days 1, 3, and 6 after transplantation) for patients receiving an unrelated donor transplant. GvHD prophylaxis was given intravenously and cyclosporin A, mycophenolate mofetil, and prednisolone were switched to oral at discharge. Patients were treated in high-efficiency, particle-free, air-filtered, positive-pressure isolation rooms. Patients received clemastine and prednisolone (2 mg/kg) intravenously before and during ATG infusion.

After reaching a leucocyte count of at least 0.3×10^9 cells per L, lymphocyte subsets, including CD3+, CD4+, and CD8+ T cells, B cells, and natural killer cells, were measured by flow cytometry at least every other week up to 12 weeks after HCT and monthly thereafter up to 6 months after HCT.

Serum ATG concentrations were measured in blood samples collected before and after each infusion in LUMC only and every other week thereafter in both centres.[16] These data were used to develop and validate a population pharmacokinetic model for ATG.16 Using these findings and this model, concentration–time profiles including all ATG exposure measurements of interest could be accurately calculated in all patients. ATG exposure measures of interest were maximum concentration, concentration at time of HCT, clearance, days to reach a concentration below the lympholytic concentration of one arbitrary unit (AU) per mL,[11] total AUC, AUC before HCT, and AUC after HCT. All ATG exposure measures were calculated using NONMEM 7.2.0.

Outcomes

The main outcome of interest was immune reconstitution, defined as repopulation of CD4+ T lymphocytes. This definition was based on their central role in adaptive immunity and in activating phagocytic cells, as well as their relation with survival.[7] A CD4+ T-lymphocyte count of at least 0.05×10^9 cells per L in two consecutive measurements within 100 days after HCT was deemed successful immune reconstitution. This count was chosen because

counts under this limit are associated with a higher probability of viral reactivations.[2,19] Patients who died before 100 days of follow-up were assessed until the date of death.

We were also interested in the association between ATG exposure and overall survival, event-free survival, non-relapse and relapse mortality, acute and chronic GvHD, graft failure, and effect of graft type. We were also interested in the effect of immune reconstitution on the outcomes of interest. Overall survival was defined as the time from transplantation to last follow-up or death. Event-free survival was defined as survival from HCT to last contact whereby graft failure, relapse of disease, or death were regarded as events. All surviving patients were censored at date of last contact. Non-relapse mortality was defined as death due to causes other than relapse of a malignancy; relapse-related mortality was defined as death due to relapse of a malignancy. Acute GvHD (grade 2–4 and grade 3–4) was classified according to the Glucksberg[20] criteria and chronic GvHD (extensive vs no or limited) was classified according to the Shulman[21] criteria. Graft failure was defined as non- engraftment (i.e., autologous reconstitution) or graft rejection (i.e., secondary loss of donor chimerism). In case of non-engraftment, the time of non-engraftment was set at 60 days after HCT. Additionally, we analysed the association between CD3+, CD8+, and natural killer reconstitution and clinical endpoints.

Statistical analysis

Duration of follow-up was the time to the last assessment for patients who were alive at the end of the study, or death. We assessed the association between outcome and patient-related variables (age at transplant, sex, and cytomegalovirus status); disease (malignancy, primary immune deficiency, bone marrow failure, or benign non-primary immune deficiency); donor factors (HLA disparity and cytomegalovirus status); conditioning regimen (myeloablative or reduced intensity conditioning); and ATG exposure measures (maximum concentration, concentration at time of HCT, days to reach a concentration below the lympholytic concentration of 1 AU/mL, total AUC, AUC before HCT, and AUC after HCT). Clinical outcomes were analysed in subgroups in terms of the different ATG exposure or patient characteristics, including stem cell sources.

Variables associated with a p value less than 0·05 by univariate analysis were selected for testing in a multivariate analysis. Probabilities of event-free survival and overall survival were calculated using the Kaplan- Meier estimate; we used the two-sided log-rank test for univariate comparisons. Time-dependent outcomes were analysed using Cox proportional hazard models. For the endpoints non-relapse mortality, relapse-related mortality, acute GvHD, chronic GvHD, and graft failure we used Fine-Gray competing risk regressions.[22] For dichotomous variables, univariate and multivariate logistic regression analyses were done. Statistical analyses were done using R version 3.0.1.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. RA and JJB had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

251 patients were included: 142 at LUMC and 109 at UMCU (table 1). Bone marrow (118 [47%] of 251) and cord blood (91 [36%]) were the main stem cell sources, and 116 (46%) patients had a malignant disease as the indication for HCT. Six (2%) patients (three from each institute) were excluded from the analysis because of neutralising IgG anti-ATG antibodies. Median follow-up was 111 weeks (IQR 32–209). A median of 11 (range 1–32) ATG serum samples were available per patient.

The AUC of ATG after transplantation was predictive of successful immune reconstitution of CD4+ T cells. With increasing exposure after HCT (ranging from 0 to 480 AU × day/mL), the chance of successful immune reconstitution before day 100 decreased for every one-point increase in the AUC (odds ratio 0·991, 95% CI 0·987–0·996; p<0·0001; figure 1A). In multivariate analyses, low AUC after HCT, a matched donor, and bone marrow or peripheral blood source of stem cells were associated with successful immune reconstitution. Table 2 lists a summary of the results of multivariate analyses of the effect of ATG exposure on clinical outcome parameters; the AUC after HCT affected immune reconstitution, whereas the AUC before HCT affected acute and chronic GvHD and graft failure.

We also examined the effect of graft type on immune reconstitution and survival. Figure 2 shows the effect of AUC after transplantation using ATG on CD4+ T-cell reconstitution and overall survival in all patients, those who received cord blood transplants, and those who received bone marrow or peripheral blood stem cell transplants. Few patients who had peripheral blood stem cell transplants were included in this study; therefore, bone marrow and peripheral blood stem cells were analysed as one group since outcomes, including immune reconstitution, were similar (data not shown). We divided the cohort into four groups according to exposure after HCT; cut-off values were chosen according to the quartiles of exposure after HCT (20, 50, and 100 AU × day/mL, respectively). Reconstitution of CD4+ T cells was markedly different between stem cell sources. Within the cord blood group, we noted decreased immune reconstitution above the lowest AUC quartile (<20 AU × day/mL vs ≥20 AU × day/mL; p=0·0024; figure 2B), whereas in the bone marrow or peripheral blood stem cell group, decreased immune reconstitution was noted only in the highest quartile (<100 AU × day/ mL vs ≥100 AU × day/mL; p=0·0024 figure 2C). The amount of CD4+

	Leiden	Utrecht	Total
Number of patients (n)	142	109	251
Male sex [n (%)]	96 (68)	61 (56)	157 (63)
Age (years)	6.2 (0.4-19)	5.9 (0.2-23)	6.2 (0.2-23)
Starting day ATG (days before transplantation)	5 (3-9)	5 (1-19)	5 (1-19)
Cumulative dose [n (%)]			
<9 mg/kg	4 (3)	5 (5)	9 (4)
9-11 mg/kg	136 (96)	97 (89)	233 (92)
>11 mg/kg	2 (1)	7 (6)	9 (4)
Number of concentration samples (mean per patient)	15	6	11
Diagnosis [n (%)]			
Malignancy	69 (49)	47 (43)	116 (46)
Immune deficiency	23 (16)	28 (26)	51 (20)
Bone marrow failure	6 (4)	9 (8)	15 (6)
Benign disorders	44 (31)	25 (23)	69 (28)
Stem cell source [n (%)]			
Bone marrow	89 (63)	29 (27)	118 (47)
Peripheral blood stem cells	30 (21)	12 (11)	42 (17)
Cordblood	23 (16)	68 (62)	91 (37)
Conditioning regimen* [n (%)]			
Reduced intensity	0 (0)	6 (5)	6 (2)
Chemotherapy-based myelo-ablative	103 (73)	88 (81)	191 (76)
TBI-based myelo-ablative	39 (27)	15 (14)	54 (22)
Positive CMV status of recipient [n (%)]	70 (49)	56 (51)	126 (50)
Positive CMV status of donor [n (%)]	57 (40)	19 (17)	76 (30)
Follow up (weeks)	126 (3-427)	84 (1-382)	111 (1-427)

Shown as median (range) unless otherwise specified

Table 1: Patient characteristics. Myelo-ablative chemo is defined as busulfan-based regimens (Targeting to a myelo-ablative exposure: > 70mg(-100)*h/L or TBI > 7Gy unfractioned or > 10Gy fractioned, as per the international guidelines. RIC (Reduced Intensity Conditioning) contained Cyclophosphomide and Fludarabine. Values are shown as median (range) unless otherwise specified. TBI: total body irradiation, CMV: cytomegalovirus.

T-cell reconstitution over time in the lowest exposure group in cord blood (<20 AU × day/mL) was similar to that noted with bone marrow or peripheral blood stem cells (p=0·54).

Successful immune reconstitution at day 100 was associated with increased overall survival (hazard ratio [HR] 0·49, 95% CI 0·29–0·81; p=0·0047; figure 1B, table 2). In multivariate analyses, diagnosis group and mismatched donor were associated with worse survival. In all patients, overall survival was significantly different in the four post-transplant ATG

Variable	Univariate	Multivariate		
	p	HR	95% CI	p-value
Post-HCT AUC				
CD4+ Immune reconstitution	<0.00001	0.995	(0.991-0.998)	0.0049
Overall survival	<0.00001	1.001	(0.998-1.004)	0.50
Acute GvHD grade 2-4	<0.00001	0.992	(0.984-1.001)	0.078
Acute GvHD grade 3-4	<0.00001	0.995	(0.985-1.006)	0.40
Chronic extensive GvHD	<0.00001	0.998	(0.990-1.005)	0.57
Pre-HCT AUC				
Acute GvHD grade 2-4	<0.00001	0.979	(0.963-0.994)	0.0081
Acute GvHD grade 3-4	<0.00001	0.975	(0.952-0.998)	0.033
Chronic extensive GvHD	<0.00001	0.983	(0.968-0.998)	0.029
Graft failure	<0.00001	0.981	(0.965-0.997)	0.020
Immune reconstitution				
Overall Survival	0.0002	0.489	(0.295-0.809)	0.0047
Non-relapse mortality	0.0002	0.403	(0.213-0.774)	0.0062
Relapse mortality in myeloid leukaemia	0.024	0.248	(0.082-0.761)	0.015
Relapse mortality in lymphoid leukaemia	0.74	4.833	(0.931-25.091)	0.061

Table 2. Multivariate analysis. Overview of multivariate analyses using a Cox proportional hazard model.

exposure groups; there was weak evidence of improved survival in the group with lowest AUC after HCT in the cord blood group compared with other AUC groups (p=0·079, figure 2E). In the bone marrow and peripheral blood stem cell group, patients with the highest exposure showed worse survival compared with the other exposure groups (p=0·00021, figure 2F). Successful immune reconstitution by day 100 was also associated with increased event-free survival (HR 0·45, 95% CI 0·29–0·69; p<0·0001; figure 1B). Non-relapse mortality, relapse, and relapse-related mortality (all patients with relapse died) were affected by CD4+ reconstitution within the first 100 days (figure 3). The incidence of non-relapse mortality was lower in patients with successful than in those with unsuccessful immune reconstitution by day 100 (HR 0·40, 95% CI 0·21–0·77; p=0·0062; figure 3A, table 2). Part of the non-relapse mortality was caused by infectious deaths, which was also affected by immune reconstitution (p=0·010). Having a mismatched donor was associated with decreased non-relapse mortality.

Successful immune reconstitution by day 100 was associated with decreased relapse-related mortality in patients with myeloid malignancy (HR 0·25, 95% CI 0·08–0·76; p=0·015; figure 3B, table 2), but not in lymphoid malignancy (4·83, 0·93–25·09; p=0·061, figure 3C). Since no patients with relapse after HCT survived, data on relapse incidence were similar to those
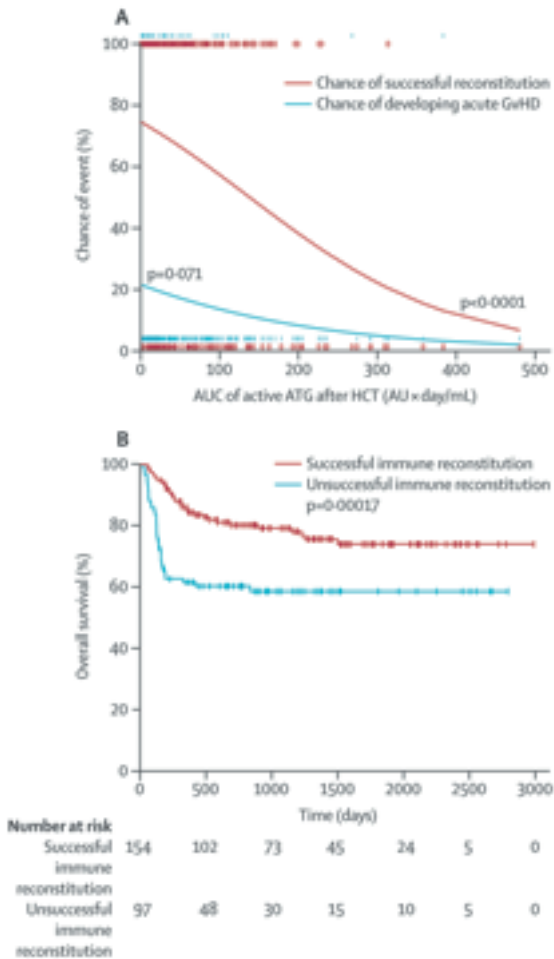
Figure 1. Chance of successful reconstitution, incidence of acute graft-versus-host disease, and overall survival (A) Successful CD4+ T-cell reconstitution before day 100 (red 0's) and grade 2–4 acute GvHD (blue I's) versus AUC of active ATG after HCT. The logistic regression lines show the chance of successful reconstitution versus the AUC after HCT (red line) and the chance of developing acute GvHD of at least grade 2 versus the AUC after HCT (blue line). Every I or O represents a patient with their respective AUC after HCT (x axis) and whether they had an event (y axis, either yes [1; top] or no [0; bottom]). Therefore, the patient with an AUC after HCT of 480 AU × day/mL had no immune reconstitution and no GvHD. (B) Kaplan-Meier survival curve of overall survival according to successful CD4+ T-cell immune reconstitution. ATG=antithymocyte globulin. AU=arbitrary units. AUC=area under the curve. GvHD=graft-versus-host disease. HCT=haemopoietic cell transplantation.

of relapse-related mortality. Similar analyses were done for CD3+ and CD8+ T-cell, B-cell, and natural killer cell reconstitution; these subsets were less predictive for overall survival than CD4+ reconstitution (data not shown).

To identify the optimum therapeutic window for targeted and individualised dosing, we did subgroup analyses within the two stem cell source groups. We noted a direct association between the ATG AUC after transplant and overall survival in a subgroup analysis of 53 patients who received a cord blood transplantation for a benign disorder and 105 who received HLA-matched bone marrow and peripheral blood stem cells. In patients who received a cord blood transplant and had benign underlying disease, the AUC after HCT above or below the median (20 AU × day/mL) affected overall survival (HR 5·1, 95% CI 1·2–23·1, p=0·035; figure 4A). We noted no other associations with overall survival
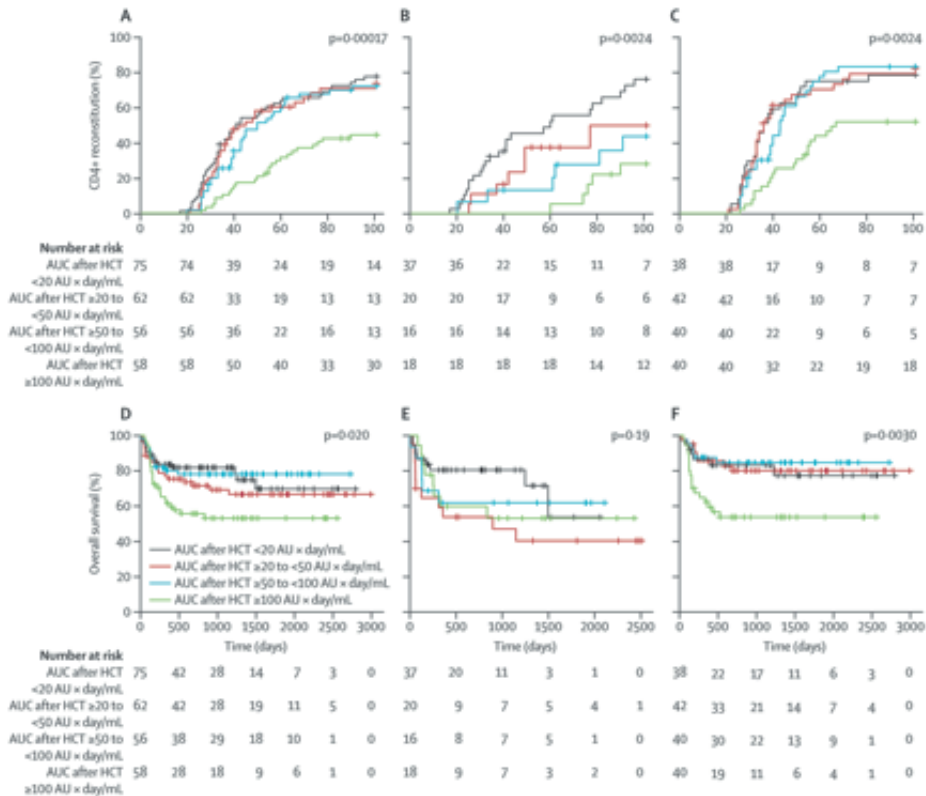
Figure 2. CD4+ T-cell reconstitution and overall survival according to area under the curve after haemopoietic stem cell transplantation by stem cell source The effect of AUC of ATG after HCT on immune reconstitution in all patients (A), those who received cord blood transplants (B), and those who received bone marrow and peripheral blood stem cell transplants (C). The effect of AUC of ATG after HCT on overall survival in all patients (D), those who received cord blood transplants (E), and those who received bone marrow and peripheral blood stem cell transplants (F). P-values are for the comparison between all four groups (log-rank test). ATG=anti-thymocyte globulin. AU=arbitrary units. AUC=area under the curve. GvHD=graft-versus-host disease. HCT=haemopoietic cell transplantation.

in subgroup analyses (data not shown). In patients who received cord blood transplants for a malignant indication, exposure after HCT did not affect overall survival. However, the number of patients with an AUC less than 20 AU × day/mL after HCT in this group was low (n=9), probably because of the low lymphocyte count before conditioning, which leads to low clearance and thus high exposure.[16] In patients who received a matched bone marrow transplant or peripheral blood stem cells, a cut-off of 50 AU × day/mL (median AUC 45 AU × day/mL) gave the best predictive value of overall survival (HR 4·19, 95% CI 1·24–14·18; p=0·021; figure 4B). We identified no multivariate predictors for this analysis. In non-matched bone marrow and peripheral blood stem cells, survival at 5 years was 52%

Figure 3. Cumulative incidence of non- relapse-related mortality and relapse- related mortality Cumulative incidence curve of non-relapse-related mortality (A), relapse-related mortality in myeloid leukemia (B), and relapse-related mortality in lymphoid leukemia (C) according to successful CD4+ T-cell immune reconstitution.

(95% CI 37–72) when compared with the matched bone marrow and peripheral blood stem cells, irrespective of AUC after HCT.

The estimated probability of acute GvHD grade 2–4 was not affected by AUC after HCT in a logistic regression analysis (odds ratio 0·999, 95% CI 0·996–1·003; p=0·78, figure 1A). ATG exposure after transplantation, divided in quartiles of exposure (<20, ≥20 to <50, ≥50 to <100, and ≥100 AU × day/mL; appendix p8) did not affect acute GvHD grade 2–4
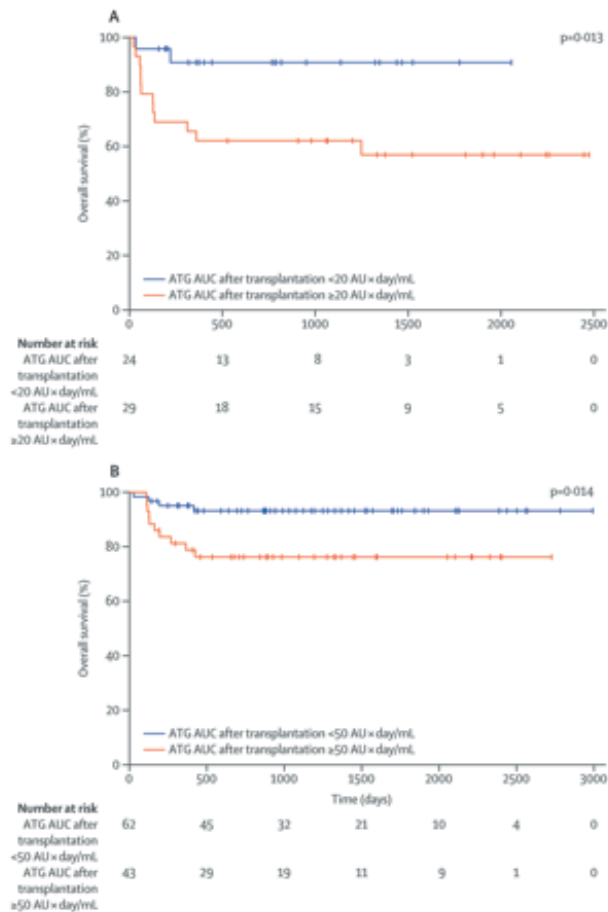
Figure 4. Survival curves according to area under the curve after transplantation in subgroups (A) Overall survival in patients with a benign underlying disease who had cord blood transplants and had an AUC after HCT of 20 AU × day/mL or higher (red line) or below 20 AU × day/mL (blue line). (B) Overall survival in patients who received bone marrow and peripheral blood stem cell transplants from a fully matched donor and who had an AUC after HCT of 50 AU × day/mL or higher (red line) or below 50 AU × day/mL (blue line). ATG=anti-thymocyte globulin. AU=arbitrary units. AUC=area under the curve. GvHD=graft-versus-host disease. HCT=haemopoietic cell transplantation.
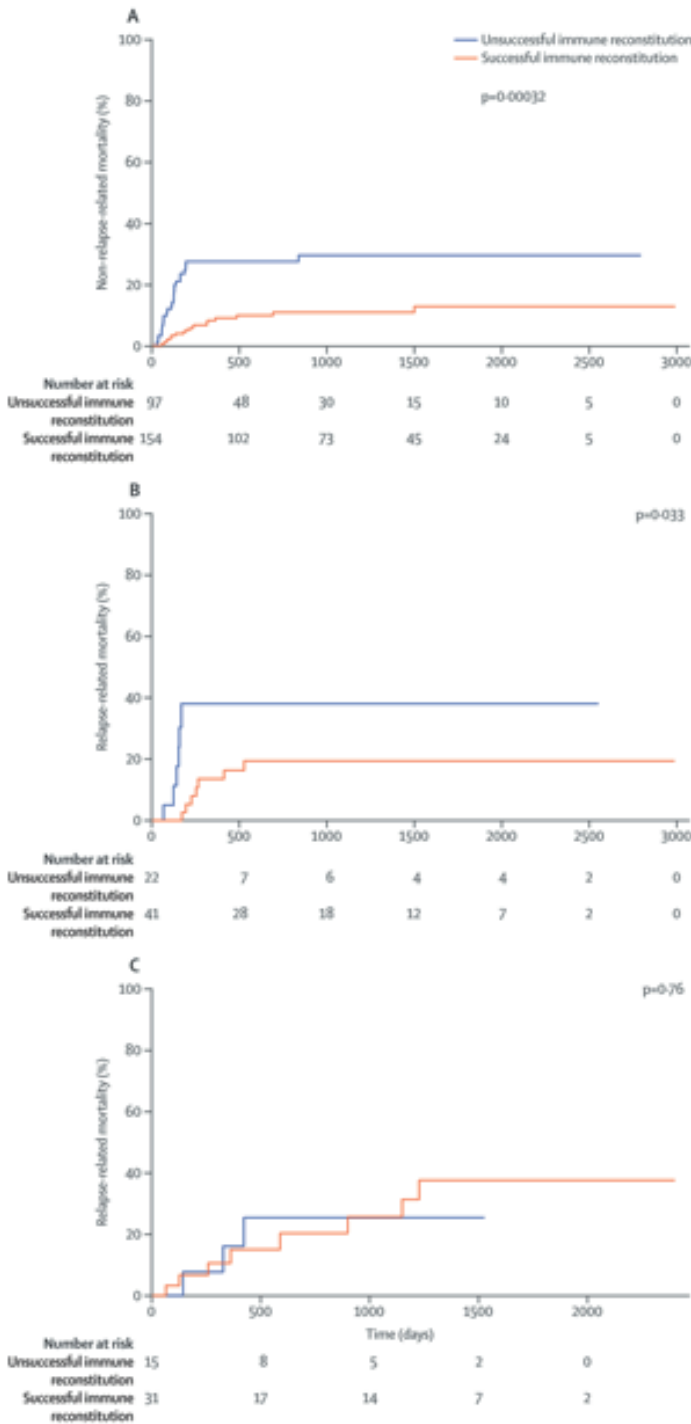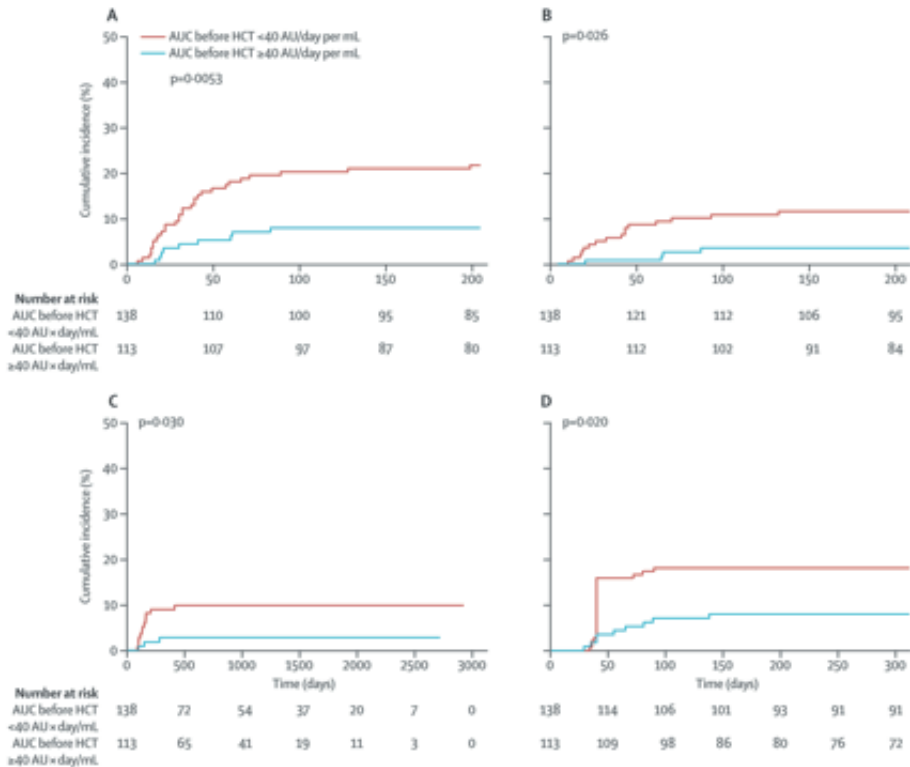
Figure 5. Cumulative incidence curves for acute and extensive chronic graft-versus-host disease and graft failure according to area under the curve before transplantation Cumulative incidence curves of acute GvHD grade 2–4 (A), acute GvHD grade 3–4 (B), extensive chronic GvHD (C), and graft failure (D) according to active ATG AUC before HCT below 40 AU × day/mL (red line) or 40 AU × day/mL or higher (blue line). ATG=antithymocyte globulin. AU=arbitrary units. AUC=area under the curve. GvHD=graft-versus-host disease.

(p=0·85), acute GvHD grade 3–4 (p=0·84), or chronic GvHD (p=0·23) in bone marrow and peripheral blood stem cell recipients, whereas in cord blood recipients, exposure after transplantation affected acute GvHD grade 2–4 (p=0·0050), but not acute GvHD grade 3–4 (p=0·15) or chronic GvHD (p=0·60).

However, the association between AUC before HCT and acute and chronic GvHD and graft failure was the strongest association among ATG exposure measures (figure 5). Pre-transplant exposure above 40 AU × day/mL, which was about the median AUC before HCT (37 AU × day/mL) was associated with a significantly lower incidence of grade 2–4 (HR 0·979, 95% CI 0·963–0·994; p=0·0081; figure 5A) and grade 3–4 acute GvHD (0·975, 0·952–0·998; p=0·033; figure 5B). Besides the AUC before HCT, malignant disease and male sex were associated with acute GvHD. A higher AUC before HCT also led to a lower incidence of extensive chronic GvHD (HR 0·983, 95% CI 0·968–0·998; p=0·029; figure 5C)

and graft failure (0·981, 0·965–0·997; p=0·020; figure 5D). The incidence of graft failure in the multivariate analysis was affected by malignant disease. Similar results were found when stratified by cell source.

DISCUSSION

To our knowledge, this is the first large pharmacokinetic and pharmacodynamic study of ATG in children to investigate the relation between exposure and clinical outcome (panel); this study was done with the aim of identifying the optimum therapeutic window of ATG to optimise ATG dosing and thereby to improve outcome, including survival chances, of paediatric HCT. With the limitations of a retrospective cohort study taken into account, our data suggest that active ATG exposure has an effect on the occurrence of successful immune reconstitution and thereby overall survival, and on the prevention of graft failure and GvHD. Low exposure after HCT was most important for ensuring early CD4+ T-cell reconstitution, especially in cord blood transplants, whereas immune reconstitution improved overall survival by reducing both relapse and non- relapse mortality. However, the AUC after HCT had less effect on the prevention of grade 2–4 or grade 3–4 acute and extensive chronic GvHD compared with the AUC before HTC, which were more affected by exposure to ATG before HCT. High exposure before HCT also led to significantly reduced graft failure. The described optimum range of exposure should be used to study future individualised dosing of ATG.

Most published studies on ATG pharmacokinetics reported the concentration at single time-points rather than the more informative exposure of ATG before and after HCT. Because AUC contains information on the whole concentration–time curve rather than the concentration at discrete time-points, it usually gives a better prediction of drug effects.[24] As expected, immune reconstitution was mostly affected by ATG exposure to the graft—i.e., the AUC after HCT. In the first 6–9 months after HCT, patients' T-cell counts are dependent on thymus-independent peripheral expansion of T lymphocytes infused with the graft.[3,25] Adequate immune reconstitution in this period led to fewer viral reactivations and relapses and subsequently improved survival.[2,6,7] We showed that low exposure after HCT was associated with better immune reconstitution, probably because in-vivo lymphodepletion was reduced, as had been suggested on the basis of active ATG concentrations shortly after transplantation.[13] Additionally, this effect was more profound in cord blood transplants than in bone marrow and peripheral blood stem cells, possibly because of the lower number of infused T lymphocytes in the graft combined with improved binding and lysing of the predominant subpopulation of naive cells in cord blood.[7] Although reconstitution worsened with increasing AUC after HCT in cord blood, patients with bone marrow and peripheral

blood stem cells in the three lower exposure groups had equally good reconstitution. Immune reconstitution in cord blood with a low ATG exposure after HCT was comparable to that in bone marrow and peripheral blood stem cells, although a mean of 1 log fewer T cells are infused in cord blood,[3] which is in line with the higher proliferative potential of cord blood cells.[26]

The price for lower exposure to ATG after transplantation might be an increased incidence of GvHD. However, in most studies, no association was found between post- transplantation ATG concentrations and acute or chronic GvHD was found,[8–10,27] whereas omission of serotherapy did result in a higher incidence of both acute and chronic GvHD.[2,4,5,28–31] Our results are mainly in line with these findings. However, we showed that both acute and chronic GvHD were affected by exposure before HCT, and suggest that this was possibly through depletion of antigen-presenting cells, which are pivotal to the induction of acute GvHD after HCT, and the reduction of inflammatory processes before transplantation, which might also be a trigger for induction of acute GvHD.[2] This theory is in line with the absence of association between successful immune reconstitution and acute GvHD. So far, no studies have investigated the role of ATG concentrations before HCT on outcome.

Furthermore, total body irradiation has been suggested to give protection against GvHD;[32] however, we could not find an additional effect of total body irradiation with a high AUC of ATG before HCT in our study. The data in this study suggest the optimum AUC after transplantation in cord blood transplants should be very low—less than 20 AU × day/mL—whereas in bone marrow and peripheral blood stem cell transplants the target AUC after HCT should be less than 50 AU × day/mL.

Exposure before transplantation should be above 40 AU × day/mL, irrespective of the stem cell source. To achieve these target exposures, not only does the dose of ATG need to be revised, the timing is also of importance. Because of the long half-life of active ATG, a low AUC after HCT in cord blood can only be achieved by giving ATG earlier before HCT and possibly at a lower dose depending on weight and lymphocyte count before ATG dosing. More predictable ATG concentrations and predictable immune reconstitution are also important when considering adjuvant cellular treatments, such as donor lymphocyte infusions, or engineered cell treatments, such as cell-based vaccinations and T cells with chimeric antigen receptors. Controlled ATG exposure by dose individualisation is probably also of importance in reduced intensity conditioning in both adults and children. Optimisation of exposure before and after HCT would probably help to reduce the risk of rejection and improve the ability to have fast immune reconstitution, which is of importance for improving the graft-versus-leukaemia effect in a reduced intensity conditioning setting. Studies investigating ATG pharmacokinetics and pharmacodynamics in adults (i.e., myelo-ablative

and reduced intensity conditioning) are being done by our group. To ensure early and robust immune reconstitution in patients transplanted with cord blood for haematological malignancies, some centres have abandoned ATG, because the risk of rejection is low in these patients. In view of our results, this approach seems feasible; early immune reconstitution reduces both non-relapse and relapse mortality, but the latter only in myeloid leukaemia. Still, event-free survival is comparable in this patient group when ATG is compared with no ATG.[2] Future studies are necessary to study the effects of individualised ATG dosing versus no ATG in reducing morbidity (e.g. GvHD) and mortality.

In conclusion, by using an ATG dose that aims to reach the target exposure before and after transplantation, we expect that optimum immune reconstitution and a low incidence of GvHD and graft failure can be achieved, which would probably lead to improved survival.

5

Panel: Research in Context

Systematic review

We searched Medline on Aug 15, 2014, using the search term "bone marrow transplantation AND antilymphocyte serum". We selected studies that compared antithymocyte globulin (ATG) versus no ATG, diff erent doses of ATG, and diff erent starting days of ATG before transplantation, and studies that related outcome to concentrations of active ATG. No language restrictions were applied. We identified three systematic reviews, seven randomised controlled trials, 12 clinical controlled trials, and two case series. Although in most studies no effects of ATG on overall survival were found, immune reconstitution had a dose-related effect with ATG, whereby a lower dose led to a better immune reconstitution, which translated into less viral reactivations. The incidence of acute and chronic graft-versus-host disease (GvHD) was reduced when ATG was introduced to the conditioning regimen. Also, there seemed to be a concentration- dependent effect, whereby a higher plasma concentration of ATG leads to a lower incidence of acute GvHD, but this was not as evident in chronic GvHD. No studies have reported on effects of ATG on relapse and rejection. Although this review of the published work included randomised controlled trials, clinical controlled trials, and case series, these results are in line with the available systematic reviews, which only included randomised controlled trials that compared ATG versus no serotherapy.

Interpretation

No previous studies have investigated the relation between several exposure measures of ATG and clinical outcome in children. In our study, low ATG exposure after haemopoietic cell transplantation (HCT) led to improved early CD4+ T-cell immune reconstitution, which was associated with increased survival through improved transplant-related mortality and relapse mortality. The incidence of GvHD and graft failure could be reduced by ensuring a sufficiently high ATG exposure before HCT. These exposures before and after HCT determine the therapeutic window of ATG, providing a target for individualised dosing that needs to be confirmed in a prospective study.

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Chapter 6

Excellent T-cell Reconstitution and Survival Depend on Low ATG exposure after Pediatric Cord Blood Transplantation

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ABSTRACT

Successful immune reconstitution (IR) is associated with improved outcomes following pediatric cord blood transplantation (CBT). Usage and timing of anti-thymocyte globulin (ATG), introduced to the conditioning to prevent graft-versus-host-disease and graft failure, negatively influences T-cell IR. We studied the relation between ATG exposure, IR and clinical outcomes.

All pediatric patients receiving a first CBT between 2004-2015 at the University Medical Center Utrecht were included. ATG-exposure measures were determined with a validated PK-model. Main outcome of interest was early CD4+ IR, defined as CD4+ T-cell counts over 50×10^6/L twice within 100 days after CBT. Other outcomes of interest included event free survival (EFS). Cox proportional-hazard and Fine-Gray competing-risk models were used.

A total of 137 patients, median age of 7.4 years (range 0.2-22.7), were included, of whom 82% received ATG. Area under the curve (AUC) of ATG after infusion of the CB transplant predicted successful CD4+ IR. Adjusted probability on CD4+ IR was reduced with 26% for every 10 points increase in AUC after CBT (hazard ratio (HR) 0.974, p<0.0001). Chances on EFS were higher in patients with successful CD4+ IR (HR 0.25, p<0.0001) and lower ATG exposure after CBT (HR 1.005, p=0.0071).

This study stresses the importance of early CD4+ IR after CBT, which can be achieved by reducing the exposure to ATG after CBT. Individualized dosing of ATG to reach optimal exposure, or in selected patients omission of ATG, may contribute to improved outcomes in pediatric CBT.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) has been introduced as a curative option for diseases including malignancies, immune deficiencies, bone marrow failure and selected metabolic disease. Since the late eighties, umbilical cord blood has become available as an alternative donor source in pediatric HCT[1]. Compared to bone marrow (BM) or peripheral blood stem cells (PBSC), umbilical cord blood transplantation (CBT) has the advantage of less stringent human leukocyte antigen (HLA) matching requirements, increasing the chances of finding a suitable donor and is rapidly available[2]. Furthermore, CBT shows at least comparable survival compared to BM and PBSC[3–6], lower rates of chronic GvHD and is suggested to have a more potent anti-leukemic effect compared to the traditional stem cell sources[7,8].

When focusing on early T-cell immune reconstitution (IR) (i.e. within 3 months after HCT), CBT is suggested to have a slower IR compared to non-T-cell depleted BM or PBSC[9–11]. Anti-thymocyte globulin (ATG), introduced to the conditioning regimen to prevent graft-versus-host disease (GvHD) and graft failure, is suggested to influence IR after CBT more profound compared to BM and PB [6,12–14]. This more profound in vivo depletion may be due to the fact that ATG is produced in rabbits using infant human thymus, which may result in more antibodies against naïve receptors, which are more present on CB T-cells[15,16].

Successful immune reconstitution following HCT is associated with improved survival chances following HCT[9,11,15,17,18], making efforts to improve IR imperative. Strategies to enhance IR may include optimizing the dosing and thereby the exposure to ATG. Using the same linear dosing regimen in mg/kg for individuals of all sizes, exposure to ATG has been highly variable between patients[19–23], especially in pediatric populations. This is mainly due to large variability in clearance, which depends on both body weight and recipient lymphocyte counts[20,23]. Individualized dosing of ATG targeted at a predefined optimal exposure, thereby reducing variability in exposure for all patients, may subsequently lead to better predictable and improved IR. However, for individualized dosing of ATG, the optimal target exposure (i.e. the therapeutic window) needs to be further investigated in cord blood recipients.

Studies investigating the relationship between ATG exposure and either IR or clinical outcome are scarce, especially for CBT, where it may be most relevant[15,20]. We recently described the relationship between ATG exposure and outcome, however the CB patients in this analysis were heterogeneous in terms of conditioning regimens, GvHD-prophylaxis and supportive care. In the current study, these limiting aspects will be overcome by including a large cohort with uniformly treated children receiving a CBT in an experienced CBT center

to study the relationship between ATG exposure, IR and clinical outcomes. An identified therapeutic window may be used as the target for individualized dosing.

METHODS

Study design and Patients

In this analysis, we included all patients receiving a single-unit CBT treated in the pediatric Blood and Marrow Transplantation Program of the University Medical Center Utrecht, the Netherlands between December 2004 and October 2015. Cord blood was selected in patients where an identical sibling donor was not available, or based on indication (high risk acute myeloid leukemia [AML], inborn errors of metabolism, primary immune deficiencies). Only first allogeneic HCT's were included. There was no restriction on the underlying disease or dose of ATG used. Consecutively treated patients were included. Patients developing IgG anti-ATG antibodies were excluded[24]. Data were collected prospectively in a local clinical database. Blood samples were collected weekly; ATG concentrations were measured in batches. Patient inclusion and data collection started after informed consent was obtained in accordance to the declaration of Helsinki. For data and sample collection, institutional ethical committee approval was obtained through trial numbers 05/143 and 11/063-K.

Procedures

UCB units were obtained from (inter)national cord blood banks. Human Leukocyte Antigen (HLA)-matching was performed on HLA-A, HLA-B and HLADRB1, and had to be at least 4/6 matched to the patient. The minimum total nucleated cell dose required for the transplant was 2.5, 3, or $5x10^7$ nucleated cells per kilogram in case of a 6/6, 5/6, or 4/6 HLA-matched UCB-unit, respectively. In rare cases, a 3/6 matched UCB unit was chosen with a minimum of $5x10^7$ nucleated cells per kilogram. Grafts were thawed and diluted 1:1 with NaCl 0.9% before infusion, no washing was performed. Conditioning regimens were selected according to national and international study protocols open in the program, most frequently busulfan-based myeloablative regimens. Busulfan was given intravenously with therapeutic drug monitoring aiming for a target cumulative area under the curve (AUC) of 85-95 mg*h/L in a myeloablative setting[25,26]. ATG (Thymoglobulin®, Genzyme, MA, USA) was administered at a dose of 10 mg/kg in 4 consecutive days according to (inter)national protocols. From 2010 onwards, patients with a body weight over 40 kg received a reduced dose of 7.5 mg/kg. Additionally, the first infusion of ATG relative to the CBT was moved from day -5 (before 2010) to day -9 (2010 onwards). Starting from 2013, patients treated for acute myeloid leukemia (AML) or high-risk acute lymphoid leukemia (ALL) did not receive ATG as part of the conditioning regimen to ensure early T cell reconstitution[27].

Patients not receiving ATG were included in the analysis to investigate the full spectrum of ATG exposures, including no exposure. GvHD-prophylaxis consisted of cyclosporin A with a target trough concentration of 200-250 µg/L combined with prednisolone 1 mg/kg. Prednisolone was tapered in 2 weeks starting 4 weeks post-HCT in benign disorders, in malignant disorders in one week after engraftment. Cyclosporin A was continued until 3 months (malignant disease) or 6 months (benign disorders) after HCT. Patients were prophylactically treated with aciclovir; treatment for viral reactivations of adenovirus, cytomegalovirus and Epstein–Barr virus was started after reaching 1000 copies/mL. All patients received gut decontamination and pneumocystis jiroveci prophylaxis according to local protocol as previously described[15]. Patients were treated in high-efficiency, positive pressure, particle-free isolation rooms.

ATG concentrations

Active ATG concentrations were measured in serum every week following the first infusion of ATG[23]. Using a validated pediatric population pharmacokinetic model[23], full concentration-time curves could be estimated, and pharmacokinetic exposures measures were calculated. These included the total AUC, the AUC before (defined as the AUC from start of first infusion up to the infusion of the graft) and after (defined as the AUC from start of infusion to infinite time, i.e. total elimination of all ATG) CBT, maximum concentration and concentration at the time of infusion of the graft. ATG exposure measures were calculated using NONMEM 7.3.0 (Icon, Ireland).

Lymphocyte subsets

Lymphocyte subsets, consisting of CD3+, CD4+ and CD8+ T-cells, B-cells (CD19+) and NK-cells (CD56+CD3-), were measured after reaching a leucocyte count of > 0.4 x 10^9 cells/L. Cell counts were followed every other week up to 12 weeks after CBT, thereafter monthly.

Outcomes

Main outcome of interest was CD4+ T-cell immune reconstitution, defined as having > 50 x 10^6 CD4+ T-cells in 2 consecutive measurements within 100 days. The definition was based on the previously demonstrated association with survival as well as the central role of T-helper cells in adaptive immunity[6,9,12,15]. Patients who deceased within 100 days were evaluated until date of death. Other outcomes of interest were overall survival (OS), defined as days to death of any cause or last follow up, event free survival (EFS), defined as days to first event (death due to any cause, relapse, or graft failure) or last follow up. Non-relapse mortality (NRM) and relapse related mortality (RRM) were defined as death due to any cause other than relapse and death due to relapse, respectively, or last follow up. Acute and chronic GvHD were classified according to the Glucksberg[28] and Shulman[29] criteria.

Graft failure was defined as non-engraftment or secondary rejection. Viral reactivations of cytomegalovirus (CMV), adenovirus (AdV) and Epstein-Barr virus (EBV) were defined as >1000 copies/ml in blood. Both AUC of ATG before and after CBT, and CD4+ immune reconstitution were investigated as predictor for clinical outcomes. Additionally, other immune reconstitution markers including CD3+, CD4+, B- and NK-cell counts were evaluated as a predictor for outcome. All patients were censored at the date of last contact.

Statistical analyses

Duration of follow-up was defined as the time from CBT to last contact or death. Factors considered as predictors for outcome included patient related variables (age, sex, cytomegalovirus [CMV] serostatus and Epstein-Barr virus [EBV] serostatus), disease variables (malignancy, primary immune deficiency [PID], bone marrow failure syndromes, or benign non-PID), donor related variables (HLA-disparity, CMV serostatus, EBV serostatus), treatment period (before or after median year of transplantation), ATG exposure measures (AUC before and after CBT) and CD4+ immune reconstitution. Immune reconstitution was considered as a time-varying predictor. Variables with a 2-sided p-value of < 0.05 in univariate analysis were considered as a predictor in multivariate analysis. Probabilities of survival were determined using the Kaplan Meier estimation; p-values were calculated using a two-sided log-rank test. Cox proportional hazard and logistic regression models were used. For the endpoints acute and chronic GvHD, NRM and RRM, Fine-Gray competing risk models were used. For finding optimal cut-off values for the primary outcome, receiving-operator-characteristic (ROC) curves were used. The cut-off with the maximum sum of sensitivity and specificity, and therefore the most accurate, was selected. Statistical analyses were performed using R version 3.2.3, with the packages cmprsk, survival, and ROCR.

RESULTS

Patients

A total of 137 patients with a median age of 7.4 (range 0.2-22.7) were included in this analysis (Table 1, and split for ATG exposure in table S1). Of these patients, 66 patients (48%) were included in a previous analysis[15]. Most (82%) patients received ATG as part of their conditioning regimen; ATG was omitted in 17/30 patients with AML and 6/20 patients with ALL. One patient (0.7%) was excluded due to the development of anti-ATG antibodies. Busulfan-based conditioning regimen was the most frequently used regimen (89%) either combined with fludarabine alone (67%) or with fludarabine and clofarabine (22%). The indication for CBT was a non-malignant disorder in 58% of the patients. Median follow-up was 36 months (0.3-131).

Main outcome of interest

Low exposure to ATG after infusion of the CB transplant was found to be the best predictor for successful CD4+ immune reconstitution. In multivariate (MV) analysis, chance on CD4+ IR was reduced 26% with every 10 points increase in AUC after CBT (hazard ratio (HR) 0.974, 95% confidence interval (CI) 0.962 - 0.986, p<0.0001; Table 2, Supplemental Table S2). Next, the optimal cut-off in exposure to ATG after CBT for CD4+ IR was investigated using ROC curves. The most optimal cut-off was found to be 16 AU*day/mL, with a specificity of 85% with a sensitivity of 65% (Supplemental Figure S1). Therefore, the patients were divided in groups of no ATG, low AUC after CBT exposure (<16 AU*day/mL) and high AUC after CBT (≥16 AU*day/mL). Omitting ATG resulted in 100% CD4+ IR, while patients that did receive ATG had lower chances of reaching IR (81±6%, p=0.008 and 33±6%, p<0.0001 in no ATG versus low AUC and versus high AUC, respectively; Figure 1). No other multivariate predictors for CD4+ IR were found (Supplemental Table S1). When investigating whether the optimal ATG exposure differed based on donor, recipient or

	Total
Number of patients (n)	137
Male sex [n(%)]	82 (60)
Age at transplant (years)	7.4 (0.2-22.7)
Patients receiving ATG [n(%)]	112 (82)
Diagnosis [n(%)]	
Malignancy	56 (41)
Acute Lymphoid	22 (16)
Acute Myeloid	30 (22)
Lymphoma	4 (3)
Primary Immune Deficiency	33 (24)
Bone Marrow Failure	7 (5)
Benign non-PID	41 (30)
Conditioning regimen [n(%)]	
Bu-Flu	92 (67)
Bu-Flu-Clo	30 (22)
TBI based	10 (7)
Cy-Flu	5 (4)
Matchgrade [n(%)]	
6/6 matched	55 (40)
5/6 matched	63 (46)
4/6 matched	18 (13)
3/6 matched	1 (1)
Follow-up (months) [mean (range)]	44 (0.2-143)

Table 1. Patient Characteristics

Variable	HR	95% CI	p	
Post-HCT AUC of ATG (continuous)				
CD4+ Immune Reconstitution	0.974	(0.962-0.986)	<0.0001	****
Event Free Survival	1.005	(1.001-1.009)	0.0071	**
Overall Survival	1.005	(1.001-1.009)	0.026	*
Non-relapse Mortality	1.005	(1.001-1.009)	0.028	*
Use of ATG (no ATG is reference)				
Acute GvHD grade 2-4	0.878	(0.353-2.185)	0.78	
Acute GvHD grade 3-4	0.268	(0.083-0.862)	0.027	*
Chronic Extensive GvHD	1.132	(0.137-9.383)	0.91	
Immune reconstitution (no IR is reference)				
Event Free Survival	0.264	(0.156-0.447)	<0.0001	****
Overall Survival	0.516	(0.279-0.955)	0.035	*
Non-relapse mortality	0.358	(0.154-0.829)	0.017	*
Relapse mortality in myeloid leukemia	0.134	(0.03-0.595)	0.008	**

Table 2. Multivariate analysis

transplant characteristics, no variables could be identified, indicating the optimal exposure after CBT is <16 AU*day/mL, irrespective of disease, age and HLA match-grade.

Other outcomes of interest according to ATG exposure after CBT

Event-free survival was comparable in patients not receiving ATG and having low ATG exposure after CBT, while those with high AUC after CBT ATG had a significantly lower EFS compared to each of the other 2 groups (Figure 2). However, patients not receiving ATG are not comparable to those that do: ATG is omitted only in AML and high-risk ALL. In multivariate analysis, high ATG exposure after CBT (HR 1.005, 95% 1.001-1.009, p=0.0071) and positive recipient CMV serostatus were multivariate predictors for inferior EFS (HR 2.01, 95% CI 1.113 - 3.64, p=0.021). Here, ATG exposure after CBT was introduced as a continuous covariate to use the full statistical power of the data and to minimize bias. Overall survival chances were comparably improved with lower ATG exposure after CBT (HR 1.005, 95% CI 1.001-1.01, p=0.022). This indicates that every 10 points increase in ATG exposure after CBT results in 5% lower survival probability. Causes of death in the three exposure groups can be found in table S3. RRM and relapse incidence was not impacted by ATG exposure, but the incidence of NRM was significantly reduced in patients with a lower ATG exposure after CBT (HR 1.005, 95% CI 1.001-1.009, p=0.036). A trend for lower incidence of viral reactivations was seen in no ATG and low ATG exposure (figure S2). GvHD was not impacted by exposure to ATG after CBT (p=0.38, p=0.21 and p=0.15 for grade 2-4 acute GvHD, grade 3-4 acute GvHD and extensive chronic GvHD, respectively).

CD4+ IR according to post–CBT AUC
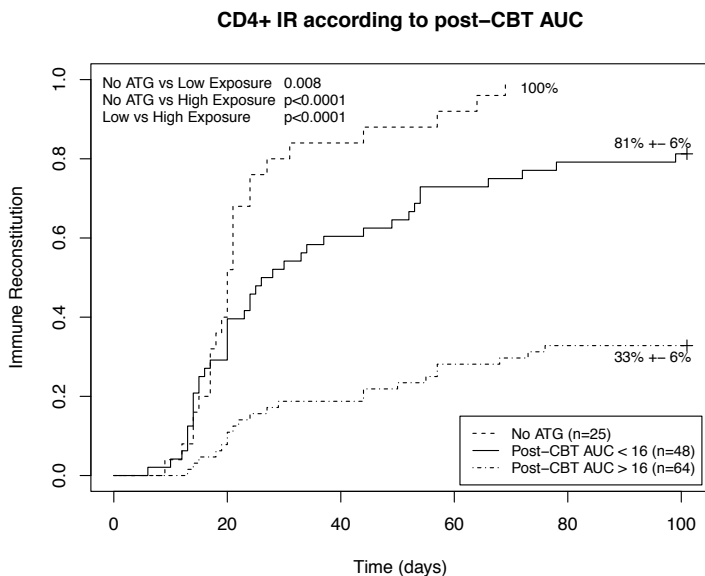
Figure 1. Cumulative incidence of CD4+ IR within 100 days according to exposure to ATG after CBT. Orange line: No ATG; Black line: Exposure to ATG after CBT < 20 AU*day/mL; Red line: Exposure to ATG > 20 AU*day/mL.

Other outcomes of interest according to use of ATG

No difference in GvHD incidence was found based on ATG exposure levels. Therefore, we investigated whether the use of ATG impacted GvHD. Here, we found that although the use of ATG did not predict the incidence of grade 2-4 GvHD (p=0.74), patients receiving ATG had a significantly lower incidence of grade 3-4 acute GvHD compared to those not receiving ATG (HR 0.27, 95% CI 0.08-0.86, p=0.027). No differences were found in incidence of chronic GvHD and graft failure according to the use of ATG, which may be due to the low number of events (5% and 11% for chronic GvHD and graft failure, respectively).

Other outcomes of interest in the context of CD4+ IR

Within the immune reconstitution markers, we found CD4+ IR to be the strongest and only predictor for the outcomes of EFS, OS, NRM and RRM.

Patients with successful early CD4+ IR at day +100 had better chances on EFS (HR 0.25, 95% CI 0.14-0.43, p<0.0001; Figure 3a). Besides CD4+ IR, negative CMV serostatus before transplantation was a multivariate predictor for improved chances on EFS. Overall survival was comparably improved with successful CD4+ IR (HR 0.40, 95% CI 0.21-0.77, p=0.0070, Figure 3b). Moreover, successful CD4+ IR lowered the chances on NRM (Figure 3c) and

RRM (Figure 3d), the latter only in myeloid leukemia's and in a small subgroup of patients. In the multivariate models for NRM and RRM, CD4+ IR was the only predictor (HR 0.28, 95% CI 0.12-0.67, p=0.004; HR 0.18, 95% CI 0.04-0.88, p=0.034, respectively). In patients

Event Free Survival according to post–CBT AUC
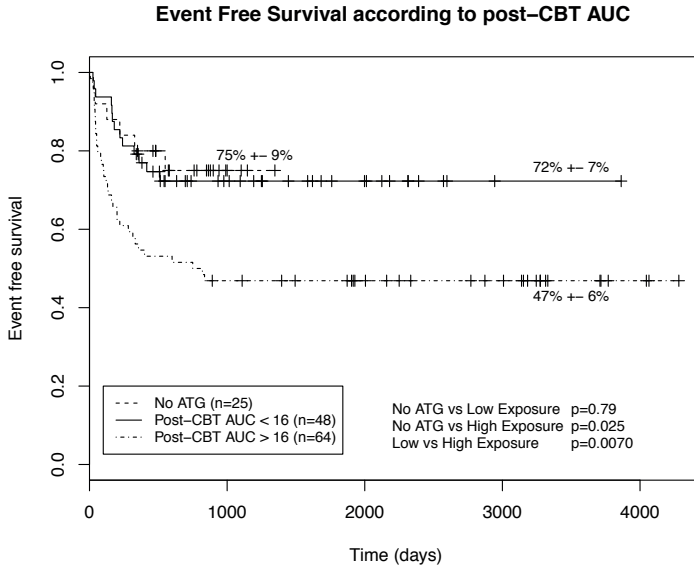
25	4	0	0	0
48	21	10	1	0
64	29	22	16	0

Figure 2: Event-free survival according to exposure to ATG after CBT. Orange line: No ATG; Black line: Exposure to ATG after CBT < 20 AU*day/mL; Red line: Exposure to ATG > 20 AU*day/mL.

not reaching CD4+ IR, infectious disease was the most common cause of NRM: 11/52 patients, compared to 5/85 patients with CD4+ IR. Other causes of NRM were comparable between the groups.

DISCUSSION

To our knowledge this is the largest pediatric single center study comprehensively investigating the relation between ATG exposure and CD4+ T-cell IR and clinical outcomes. We show that even very minimal exposure of ATG after CBT has a detrimental effect on early CD4+ immune reconstitution. We also found that both ATG exposure and CD4+ IR were predictors for clinical outcome, including EFS, OS, and NRM. The use of ATG was associated with a lower incidence of grade 3-4 acute GvHD but not grade 2-4 or chronic GvHD, the latter possibly due to low incidence. While recognizing the limitations of a retrospective cohort
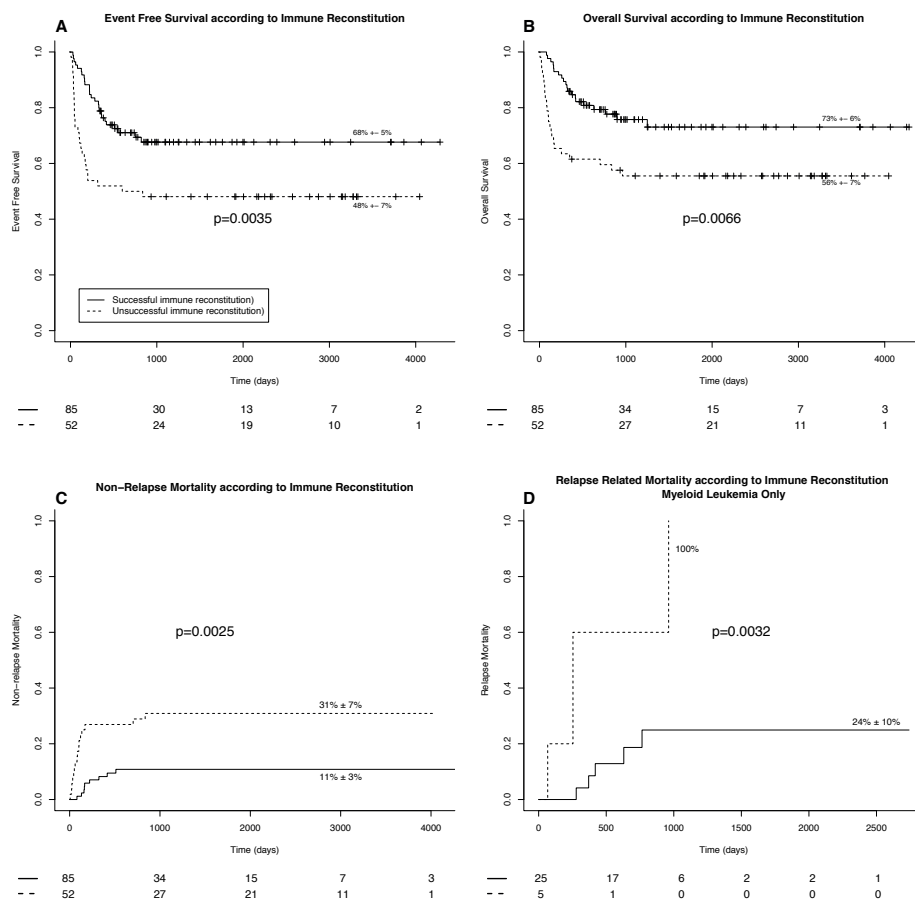
Figure 3. Event-free survival (Panel A), overall survival (Panel B) and non-relapse mortality (Panel C) according to successful CD4+ IR in all patients. Panel D: Relapse Related Mortality according to successful CD4+ IR in myeloid leukemia's only. Black lines: successful CD4+ IR; red lines: no successful CD4+ T-cell IR.

study, the strengths of this study include the homogeneous group of patients analyzed, e.g. conditioning regimens, GvHD-prophylaxis, supportive care. Additionally, the inclusion of consecutive patients with prospective data collection minimizes the risk for potential bias. Taken together, these results stress the importance to aim for optimal ATG exposure in pediatric cord blood transplantation; both for achieving early immune reconstitution and ensuring improved survival chances.

Outcomes of CBT have improved significantly over the last decade. In the nineties and early 2000's, lower engraftment rates were a significant disadvantage of CBT, however the current use of higher cell dosed units has significantly reduced this issue[4]. NRM by infectious causes however is still reported to be a serious limitation of CBT, while relapse incidence

on the other hand is suggested to be lower in CBT[4]. As timely immune reconstitution seems essential in preventing relapse- and non-relapse mortality, strategies to improve immune reconstitution to increase protection against viral disease and relapse of malignancy are warrented[7,15,27,31]. T-cell immune reconstitution has been associated with use, dose and timing of serotherapy, all indicating that a higher dose of serotherapy shortly before infusion of the graft is detrimental for timely and robust immune reconstitution[6,14,27,32,33]. Thus, in-vivo exposure of the graft-infused T-cells to serotherapy results in significant depletion of T cells and limits the exceptional proliferative capacity of CB T-cells as recently shown[15]. The present study confirms this: very low exposure to ATG has dramatic effects on T cell reconstitution potential, while no ATG shows exceptional IR. The IR potential in CBT is possibly even better than other stem cell sources as suggested before[27].

In the major landmark studies by Rocha et al. and Eapen et al. comparing cord blood to other stem cell sources, most cord blood patients received ATG[3,30]. Taking the results of the current study into account, when reducing in-vivo exposure of ATG to the graft in these studies the cord blood groups may have performed superior.

Moreover, the presented results are likely true in adult CBT, and potentially at a greater magnitude. Due to the higher body weights in adults compared to children, the number of T-cells per kilogram is lower. Additionally, from a pharmacokinetic perspective, ATG clearance is markedly higher in smaller (younger) children compared to older children, and is likely to be even lower in adults. Therefore, current dosages of ATG in adult CBT may result in very high ATG exposure after infusion of the graft, and fully deplete the even lower amount of graft-infused T-cells. This will negatively impact outcomes, both in terms of CD4+ IR and EFS in line with the results presented in this paper.

Both successful CD4+ IR and lower ATG exposure after CBT were associated with survival due to both lowering non-relapse and relapse mortality in AML. This is in line with previous studies, where CD4+ IR was associated with lower incidence of viral reactivations and relapse, and better survival chances[6,9,15,27,31]. Few studies however report on the association between ATG PK and survival, where PK is evaluated either as single concentration measurements or as exposure. Studies investigating single concentrations at a certain time-point could not find a correlation with survival, while actual exposure of ATG before and after transplantation was associated with a variety of outcomes[15,19–21,34]. This discrepancy may be due to a lower number of patients included in the concentration-studies, however ATG exposure (AUC) more likely harbors more information compared to single concentration samples and therefore is a stronger predictor[35]. Wide scale implementation of ATG PK as a variable in outcome following CBT depends on an assay for measuring ATG concentrations and skilled pharmacology staff. This should be achievable in larger centers.

In the current study, the use of ATG was found to lead to a lower incidence of grade 3-4 acute GvHD, which is in line with previous studies [6,36–38]. No correlation was found however between the use of ATG and grade 2-4 GvHD, extensive chronic GvHD and graft failure, which in previous work were related to exposure of ATG before transplantation. The absence of a correlation may be due to the relatively low incidence of chronic GvHD and graft-failure in this cohort (5% and 11% for extensive chronic GvHD and GF, respectively). Also the incidence of acute GvHD grade 2-4 was relatively low compared to previous studies where ATG was omitted[27]. This difference may be due to different GvHD-prophylaxis regimens: the current study used prednisolone while Chiesa et al. used mycophenolate mofetil[27].

Strategies for improved and predictable T-cell IR are warranted and may lead to improved survival chances following HCT. This is especially important in CBT, where even very low exposure after CBT has a major impact on CD4+ IR probability. Omission of ATG in a CBT-setting for all indications may contribute to improved IR, however is less feasible in immune-competent recipients in whom omitting ATG could potentially lead to more graft-failure or GvHD [6,27]. All chemo-naïve patients (e.g. benign disorders) received ATG in the current analysis. Therefore no inferences can be made on omitting ATG in these patients based on this study. Individualizing ATG is an alternative strategy: dosing is based on patient characteristics (e.g. weight and absolute lymphocyte count) aiming to reach the optimal exposure before and after CBT. The advantages of individualized dosing include optimal immune reconstitution while having protections against GvHD and graft-failure, thereby giving the best of both worlds. This approach is currently being investigated in a prospective clinical trial (Dutch trial register NTR4960). This individualized dosing regimen is relatively easy to use, also in centers not able to do ATG PK. In our opinion, both malignant and non-malignant indications can be treated with individualized ATG in a CBT-setting, while in AML, ATG can be omitted to add more safety. The T-cell repertoire in CBT following successful CD4+ IR may give an additional advantage over BM and PBSC[39]. Not only do CB derived T-cells mediate a more potent anti-leukemic effect[7], the high number of naïve cells make CBT a powerful platform for adjuvant cellular therapies (e.g. cell vaccinations)[40,41].

In conclusion, this study shows the importance of adequate targeting of ATG exposure after CBT to ensure early CD4+ T-cell reconstitution. By omitting ATG or using an individualized dosing and timing of ATG aiming for optimal target exposure, T-cell IR will improve. Our data indicate that promoting CD4+ IR will likely improve survival chances by lowering both relapse- and non-relapse mortality.
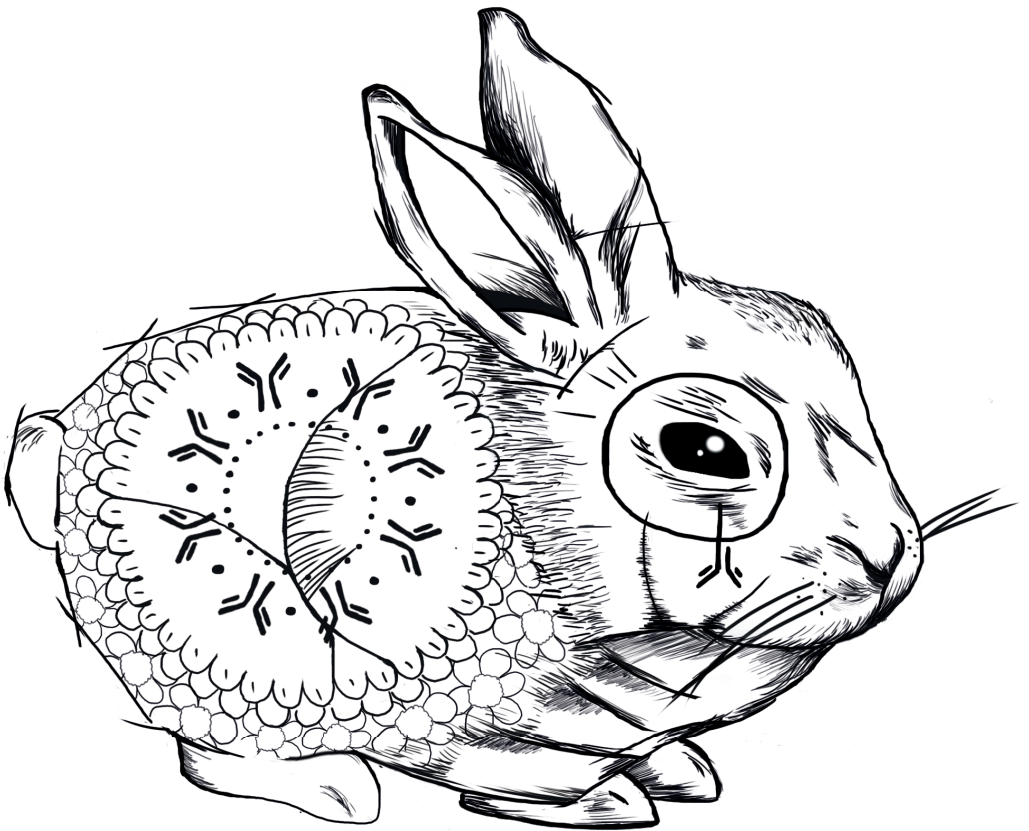
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Chapter 7

Optimizing Anti-Thymocyte Globulin Exposure to Improve Survival Chances

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ABSTRACT

Background

Anti-thymocyte globulin (ATG) is used to prevent graft-versus-host-disease (GvHD) following allogeneic hematopoietic cell transplantation (HCT). However, ATG can also cause delayed immune reconstitution, negatively influencing survival. We studied the relation between exposure to ATG and clinical outcomes in adult patients with acute leukemia and myelodysplastic syndrome.

Methods

In a retrospective analysis, consecutive patients receiving a T-cell repleted allogeneic peripheral blood stem cell-HCT with ATG (Thymoglobulin) as part of reduced intensity conditioning were included (March-2004 to May-2015). Active-ATG levels were measured using a validated bioassay and pharmacokinetic exposure measures were calculated with a validated population PK-model. Main outcome of interest was overall survival (OS); other outcomes were relapse- and non-relapse mortality, acute- and chronic-GvHD and evaluation of current and optimal dosing. Cox proportional-hazard models and Fine-Gray competing risk models were used.

Results

146 patients were included. ATG exposure after HCT was found the best predictor for 5-year OS. Optimal exposure after HCT (60-95 AU*day/mL; 69±8%) yielded superior OS compared to below (32±8%, hazard ratio [HR] 3.36, 95% confidence interval [CI] 1.69-6.68, p=0.00057) and above-optimal exposure (48±6%, HR 2.5, 95%-CI 1.29-4.84, p=0.0064). Above-optimal exposure led to higher relapse-related mortality (HR 2.66, p=0.027). Below-optimal exposure increased non-relapse mortality (HR 4.17, p=0.0060), grade 3-4 acute-GvHD (HR 3.09, p=0.035) and chronic-GvHD (HR 2.56, p=0.048). Dosing based on absolute lymphocyte counts led to higher optimal target attainment compared to weight-based dosing.

Conclusions

Exposure to ATG impacts survival following HCT in adults, stressing the importance of optimal ATG dosing. Individualizing dosing of ATG, based on lymphocyte counts rather than body weight, may improve survival chances after HCT.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment option for high-risk or relapsed acute leukemia's and myelodysplastic syndrome (MDS). Immunological rejection of residual tumor by donor-derived immune cells (graft-versus-leukemia; GvL) should give disease control including life-long anti-tumor immune surveillance[1]. Graft-versus-host disease (GvHD) remains a severe complication of HCT, leading to significant morbidity and mortality. As a strategy to prevent GvHD following HCT, anti-thymocyte globulin (ATG) was introduced to the conditioning regimens applied before HCT[2]. Although the use of ATG was associated with a decreased incidence of acute-[3–5] and chronic-GvHD[4,6], most studies fail to show a survival advantage[5–7]. This is probably due to unpredictable ATG-induced in vivo T-cell depletion of the graft resulting in delayed or absent early T-cell immune reconstitution[5,8,9]. Poor immune reconstitution abrogates the GvL-effect and antiviral activity resulting in increased relapse- and non-relapse mortality[10].

In this delicate balance between preventing GvHD and timely T-cell immune reconstitution, ATG has a pivotal role as T cell depleting antibody of host and donor T cells. A variety of studies have tried to determine the optimal dose of ATG by performing dose-effect studies[11–14]. A major drawback of these dose-effect studies is the lack of incorporation of the high inter-patient variability in pharmacokinetics (PK) of ATG. Therefore, using standard dosing for all patients and not taking into account exposure, makes the results difficult to interpret[15,16]. Furthermore, as patients are exposed to ATG before and after transplantation due to the long half-life of 5-14 days[17–19], the timing of ATG is an additional important variable[5]. It would be better to correlate actual exposure to outcome rather than dosage. Population PK-modeling can be used to describe pharmacokinetics and determine individual exposure, and is the standard for reporting pharmacokinetic data according to current FDA and EMA guidelines[20,21]. Although ATG has been used since the early 1980s, the pharmacokinetic of ATG has not been thoroughly described[17,19,22–25].

We recently described that ATG exposure impacted outcome in pediatric HCT receiving either bone marrow or cord blood after myeloablative conditioning. High exposure to ATG after HCT was associated with a poor immune reconstitution probability and lower survival, while high exposure before HCT was associated with less GvHD and graft failure[22]. No studies are available investigating the optimal ATG exposure in adult HCT using mobilized peripheral blood stem cells (PBSC) after reduced intensity conditioning (RIC)[26,27]. Therefore, we aimed to assess the relation between ATG exposure and clinical outcomes in this RIC-PBSC-setting. To achieve this, the available pharmacokinetic model for ATG in children and young adults was expanded and validated for adults. We subsequently performed a retrospective cohort analysis of consecutive patients to relate different exposure

measures of the pharmacologically active fraction of ATG (hereafter referred to as ATG) to various clinical outcomes of HCT, such as GvHD, relapse and survival.

METHODS

Study design and Patients

We included patients transplanted for an acute lymphoid leukemia (ALL), acute myeloid leukemia (AML) or MDS receiving their first HCT between March 2004 and May 2015 within the adult blood and marrow transplantation unit at the University Medical Center in Utrecht, the Netherlands. Only patients receiving a T-repleted PBSC graft with ATG (Thymoglobulin) as part of RIC conditioning were included. Clinical data and serum samples for ATG concentration measurements were collected prospectively; consecutive patients were included. Minimal follow-up for surviving patients was 6 months. Patients were included and data were collected after informed consent was acquired according to the declaration of Helsinki. Ethical committee approval was given through trial number 11/063.

Procedures

Patients underwent a RIC containing a cumulative dose of 8 mg/kg ATG divided over 4 days, starting on day -8 before HCT, fludarabine 90 mg/m2 (day -3, -2 and -1) and 200 cGy total body irradiation (TBI) on day 0. Actual dose of ATG was rounded upwards to 25 mg so that patients received only full vials. Clemastine, paracetamol and 100 mg prednisolone were given prior to ATG infusion. GvHD-prophylaxis consisted of cyclosporin A (CsA) and mycophenolate mofetil (MMF). Start dose of CsA was 4.5 mg/kg/day until day +84 or +120 (target trough levels 200-350 mg/L). Hereafter, CsA was tapered if no GvHD was present. Patients received 15 mg/kg/day MMF (maximum of 3 g/ day) until day +28 or +84, also followed by tapering in the absence of GvHD[28]. Ciprofloxacin and fluconazole were given as selective gut decontamination, trimethoprim/sulfomethoxazole and valaciclovir were used for infectious prophylaxis until 12-15 months after HCT.

ATG pharmacokinetics and exposure

To describe ATG pharmacokinetics from young children to adult patients, an adult dataset for ATG was combined with a previously published dataset of children and young adults[15]. In the adult population, samples were collected weekly after HCT, while samples during infusions were available in 35 patients. The complete development and validation of the population PK-model is described in the Supplemental Methods.

Following the development of the population PK-model, full concentration-time curves were estimated for each individual patient. Based on these, individual pharmacokinetic

exposure measures could be calculated. The pharmacokinetic exposures of interest included the maximum concentration (C_{max}), concentration at time of infusion of the graft (C_{HCT}), time to reach a concentration of 1 AU*day/mL ($T_{C<1}$)[18], the area under the curve (AUC) and the AUC before and after HCT (Figure S9).

To study the most predictive PK-measure for the main outcome of interest, all PK exposure measures were split in four groups according to quartiles. Predictive PK-measure models were selected based on the lowest Akaike Information Criterion (AIC), a criterion to select the best predicting model; in this case the proportional hazard model. Additionally, selection was based on the distribution in estimated survival between groups in Kaplan Meier plots.

Outcomes

Main outcome of interest was 5-year overall survival (OS), defined as days to death of any cause or last follow up. Other outcomes of interest included non-relapse mortality (NRM) and relapse related mortality (RRM), which were defined as days to death due to any cause other than relapse and days to death due to relapse, respectively, or last follow up. Event free survival (EFS) was defined as the days to death, relapse, graft failure (GF), or last follow up, whichever occurred first, while relapse incidence was defined as time to relapse or last follow up. Acute and chronic GvHD were classified according to the Glucksberg[29] and Shulman[30] criteria. GF was defined as non-engraftment or secondary graft rejection. As we were interested in the predictive power of the various PK exposure measures, we related the outcomes of interest with these PK measures.

Statistical analyses

Duration of follow-up was defined as the time from HCT to last contact or death. Patients were censored at the date of last contact. Factors considered as predictors for outcome included patient variables (age, sex, Epstein-Barr virus [EBV] and cytomegalovirus [CMV] serostatus), disease variables (ALL, AML, MDS), donor related variables (HLA-disparity, EBV and CMV serostatus), year of treatment (before or after median year of HCT), and ATG exposure measures.

Probabilities of survival were determined using the Kaplan Meier estimation; p-values were calculated using a two-sided log-rank test. Variables with a p-value < 0.05 in univariate analysis were included as a predictor in multivariate analysis. For the endpoints OS and EFS, Cox proportional hazard models were used. For the endpoints TRM, RRM, relapse and acute and chronic GvHD, Fine-Gray competing risk models were used[31]. Statistical analyses were performed using R 3.2.4, with packages cmprsk, survival and rms.
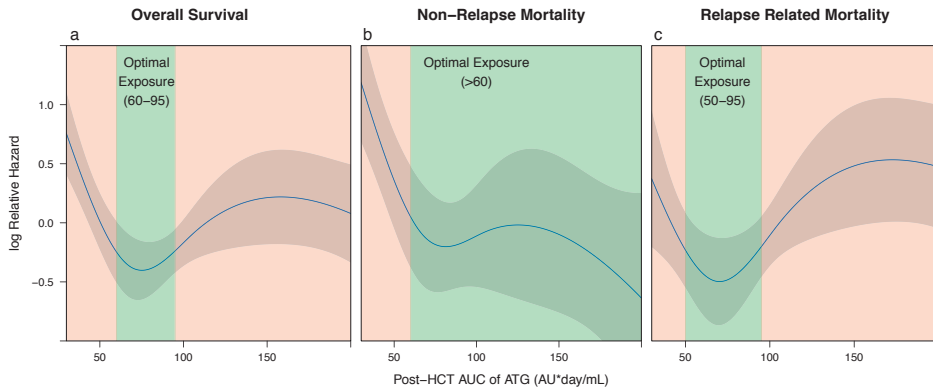
Figure 1. Log relative hazard for overall survival (panel A), non-relapse mortality (panel B) and relapse mortality (panel C) according to ATG exposure after HCT. Blue line: log relative hazard; shaded area: 95% CI for log relative hazard; green areas: optimal exposure range; red areas: sub- optimal exposure range.

	Total
Number of patients (n)	146
Male sex [n(%)]	84 (58)
Baseline lymphocyte count (x 10^9 /L)	0.7 (0.0-3.3)
Age at transplant (years)	49.8 (18.1-69.9)
Cumulative dose of ATG (mg/kg)	8 (4.1-9.1)
Starting day of ATG (days before HCT)	8 (4-12)
Number of blood samples taken per patient	4 (1-12)
Diagnosis [n(%)]	
Acute Myeloid Leukaemia	74 (50)
Myelodysplastic Syndrome	36 (25)
Acute Lymphoid Leukaemia	36 (25)
Stem Cell Source [n(%)]	
Peripheral Blood Stem Cells	146 (100)
Conditioning Regimen [n(%)]	
Flu-TBI	146 (100)
Match grade [n(%)]	
Matched	111 (76)
Mismatched	35 (24)
Follow-up (months)	37 (0.6-139)

Table 1. Patient Characteristics. Values are depicted as median (range), except when otherwise specified. Baseline lymphocyte count: Absolute peripheral blood lymphocyte count before 0-7 days before the first dose of ATG; Flu: fludarabine; TBI: total body irradiation

Optimal dosing regimen

Following the determination of the most predictive PK-exposure measure and the sub-

sequently determined optimal range of exposures, available ATG dosing regimens were evaluated for target attainment. Investigated regimens included current local dosing (8 mg/kg over 4 days, starting day -8), the European society for Bone Marrow Transplantation (EBMT) protocol[32] (7.5 mg/kg over 3 days, starting day -3), and a recently published regimen in adult RIC-PBSC[33] (4.5 mg/kg, starting day -2). Additionally, an optimal dosing regimen will be designed and subsequently evaluated using the same approach. Groups of patients were selected based on the predictors for PK. Concentration-time profiles were simulated using the validated PK-model, incorporating 1000 virtual patients in each group,
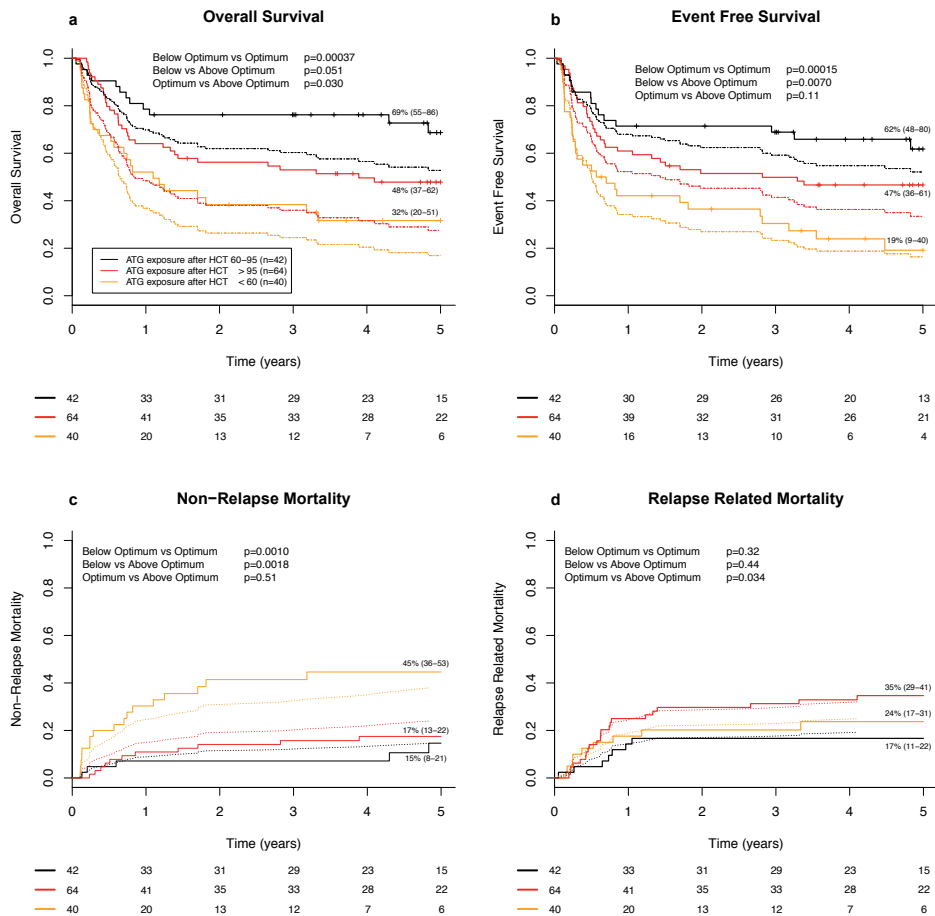
Figure 2. Unadjusted (solid lines) and adjusted (dashed lines) estimations of clinical outcomes according to groups of ATG exposure after HCT. Black lines: optimal exposure; Orange lines: below optimal exposure; Red lines: above optimal exposure. Panel A: Overall Survival; Panel B: Event Free Survival; Panel C: Non-relapse Mortality; Panel D: Relapse Mortality. Adjusted estimations are to be interpreted as the expected outcomes if all ATG exposure groups were the same, on average, with respect to all multivariate predictors (diagnosis [all], age [OS, EFS, NRM] and EBMT risk score [OS, EFS]). P-values are derived from the two-sided log-rank test. .

while taking into account full interindividual variability. For each group, median exposure after HCT was compared to the optimal therapeutic window.

RESULTS

Patients

A total of 146 patients were included; 74 with AML, 36 with ALL and 36 with MDS as indication for HCT (Table 1). Median age was 50 years (range 18.1-69.9 years); 111 patients (76%) received a 10/10 matched graft. Median follow-up of all patients was 37 months (range 0.6-139 months).

Pharmacokinetic analyses

A population pharmacokinetic model was developed that accurately described concentration data and was extensively validated (Figure 1). In the model, body weight was found a predictor for ATG clearance for body weights below 50 kilogram. Above this weight, no increase in clearance was observed with increasing body weight (Figure S5). Absolute lymphocyte counts (ALC) before the first dose of ATG also predicted clearance, which is in line with the pharmacological properties of ATG. Higher number of lymphocytes harbor
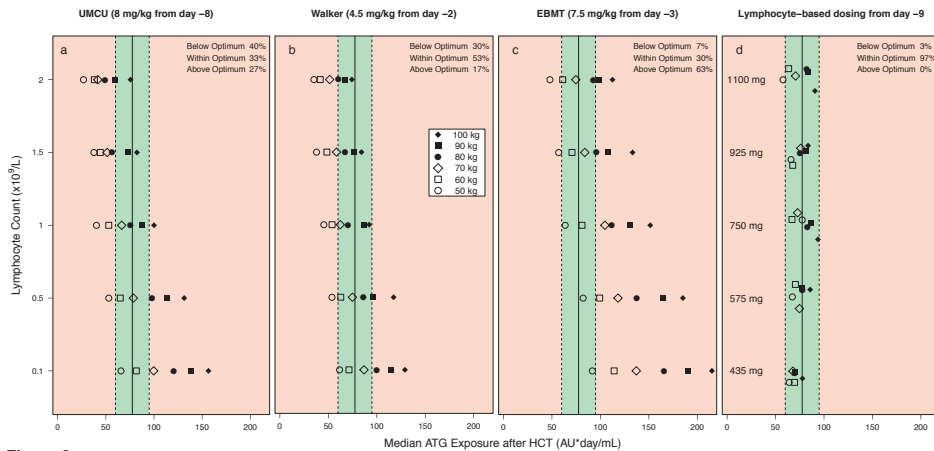
Figure 3. Evaluation of currently used ATG dosing regimens and optimal thymoglobulin dosing regimen, showing median ATG exposure after HCT for several lymphocyte/body weight groups. Panel A: Dosing regimen used for the current cohort; cumulative dose of 8 mg/kg over 4 days, starting day -9. Panel B: Dosing regimen according to Walker et al; cumulative dose of 4.5 mg/kg, 0.5 mg/kg on day -2, 2 mg/kg on day -1, and 2 mg/kg on day +1. Panel C: Dosing regimen proposed by European Bone Marrow Transplant Society; cumulative dose of 7.5 mg/kg over 3 days, starting day -3. Panel D: Dosing regimen based only on lymphocyte count, given over 4 days, starting on day -9. Absolute cumulative dose is given for each lymphocyte level. Symbols depict body weights; open circles: 50kg; open squares: 60kg; open diamonds: 70kg; filled circles: 80kg; filled squared: 90kg; filled diamonds: 100kg.

more targets for ATG binding, leading to increased clearance (Figure S5). ATG exposure measures could be accurately calculated for all patients using the validated PK-model.

Main outcome of interest

Exposure to ATG after HCT was found to be the best predictor for OS. The AIC for exposure after HCT was lowest (Supplemental Table S2), indicating the best fit of the Cox proportional hazard model, as well as the largest distribution of survival curves.

To evaluate the most optimal range of exposure to ATG after HCT, the hazard ratio (HR) for OS, NRM and RRM was plotted against ATG exposure after HCT (Figure 2). The optimal AUC after HCT was determined to be between 65 and 90 AU*day/mL (Figure 2A); below this threshold increased risk for non-relapse mortality was observed (Figure 2B), while both below and above this threshold chances on relapse mortality were higher (Figure 2C). Estimated 5-year overall survival after optimal exposure (69±8%), was significantly higher than in the groups below (32±8%; p=0.00037) and above (48±6%; p=0.030) optimal exposure (HR 3.36, 95% confidence interval [CI] 1.69-6.68, p=0.00057 and HR 2.5, 95% CI 1.29-4.84, p=0.0064 for below and above optimum, respectively; Figure 3a, Table 2).

Other outcomes of interest

RRM was higher in patients with above optimal exposure after HCT (HR 2.66, 95% CI 1.12-6.31, p=0.027; Figure 3d). Patients with a below optimal exposure had an increased risk for NRM compared to optimal exposure (HR 4.17, 95% CI 1.51-11.54, p=0.0060; Figure 3c). EFS was comparably influenced by the exposure groups as OS; patients in the optimal exposure groups have higher EFS chances than those below or above optimal exposure (HR 2.88, 95% CI 1.53-5.43, p=0.0011 and HR 1.97, 95% 1.07-3.61, p=0.029, respectively) (Figure 3b). The incidence of grade 3-4 was higher in patients with below-optimal exposure compared to the optimal exposure group (HR 3.09, 95% CI 1.08-8.78, p=0.035). Probability on chronic GvHD were higher in the lower exposure group (HR 2.56, 95% CI 1.01-6.48, p=0.048) compared to optimal exposure.

Optimal dosing regimen

Current thymoglobulin dosing regimens and a novel ALC-based nomogram thymoglobulin were evaluated; groups of patients with body weights of 50-100 kg and ALC before first infusion of ATG of $0.1\text{-}2.0\text{x}10^9$/L were simulated for three current thymoglobulin dosing regimens (Table S5). Simulated ALC values were in line with the actual lymphocytes of included patients (median $0.72\text{x}10^9$/L, range 0.01-3.29). The dosing regimen used in our center is representative for most Dutch centers and affiliated centers participating in HOVON studies[28]. It showed high variability in ATG exposure after HCT; median exposure was on target in 33% of the groups (Fig 4a). Practices from other centers utilizing thymoglobulin

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Variable	HR	95% CI	p-value	
Overall survival				
Optimal ATG Exposure after HCT	1			
Below Optimal ATG Exposure after HCT	3.36	(1.69-6.68)	0.00057	***
Above Optimal ATG Exposure after HCT	2.5	(1.29-4.84)	0.0064	**
Event free survival				
Optimal ATG Exposure after HCT	1			
Below Optimal ATG Exposure after HCT	2.88	(1.53-5.43)	0.0011	**
Above Optimal ATG Exposure after HCT	1.97	(1.07-3.61)	0.029	*
Non-relapse mortality				
Optimal ATG Exposure after HCT	1			
Below Optimal ATG Exposure after HCT	4.17	(1.51-11.54)	0.006	**
Above Optimal ATG Exposure after HCT	1.63	(0.58-4.62)	0.35	
Relapse mortality				
Optimal ATG Exposure after HCT	1			
Below Optimal ATG Exposure after HCT	1.45	(0.55-3.83)	0.46	
Above Optimal ATG Exposure after HCT	2.66	(1.12-6.31)	0.027	*
Relapse Incidence				
Optimal ATG Exposure after HCT	1			
Below Optimal ATG Exposure after HCT	1.28	(0.57-2.86)	0.55	
Above Optimal ATG Exposure after HCT	1.79	(0.89-3.61)	0.11	
Incidence of grade 2-4				
Optimal ATG Exposure after HCT	1			
Below Optimal ATG Exposure after HCT	1.44	(0.75-2.76)	0.28	
Above Optimal ATG Exposure after HCT	0.79	(0.41-1.51)	0.48	
Incidence of grade 3-4				
Optimal ATG Exposure after HCT	1			
Below Optimal ATG Exposure after HCT	3.09	(1.08-8.78)	0.035	*
Above Optimal ATG Exposure after HCT	1.07	(0.35-3.24)	0.91	
Incidence of chronic GvHD				
Optimal ATG Exposure after HCT	1			
Below Optimal ATG Exposure after HCT	2.56	(1.01-6.48)	0.048	*
Above Optimal ATG Exposure after HCT	1.03	(0.41-2.57)	0.95	

Table 2. Multivariate analyses. Multivariate analyses using Cox proportional hazard and Fine-Gray competing risk models. * p<0.05; ** p<0.01; *** p<0.001

with a different timing and dosing (0.5 mg/kg on day-2, 2 mg/kg on day -1, and 2 mg/kg on day +1)[33] demonstrated an optimal target attainment in 53% of groups (Fig 4b), and the current EBMT-recommended dose (7.5 mg/kg in 3 days, starting day -3) of thymoglobulin for unrelated donors[32] led to an optimal target attainment in 30% of groups only (Figure 4c). Based on the developed PK-model, the optimal dosing should be based on ALC, as ATG

clearance in patients (> 50 kg) was not influenced by weight. When targeting to the optimal ATG exposure after HCT, cumulative ATG dosage is calculated using the following formula:

$$\text{Cumulative dose} = 400 + 350 * \text{lymphocyte count (in } 10^9/\text{L)}$$

This cumulative dose should be given over 4 days starting day -9. Simulations of this dosing regimen led to optimal exposure in 97% of groups (Fig 4d); higher than any of the regimens analyzed in current study[28,32,33].

DISCUSSION

To our knowledge, this is the first study investigating the pharmacokinetics and pharmaco-dynamics of ATG in a large, consecutive cohort of adult patients receiving PBSC after RIC for acute leukemia's and MDS. We aimed to determine the therapeutic window of ATG in this setting. Taking into account the limitations of a retrospective study, the data show that the exposure to ATG after HCT impacts survival as well as acute and chronic GvHD. There seems to be an optimal window of exposure to ATG after HCT (60-95 AU*day/mL); lower exposure increases chances on mortality, mostly associated with GvHD, while an exposure above the optimum was related with more relapse-related mortality. The PK-model showed that ALC was found the only relevant predictor for PK. Therefore, ALC-based dosing will result in achieving the optimal AUC in >95% of the simulated patient groups. This may subsequently result in higher survival chances after RIC HCT receiving PBSC.

The importance of T-cell immune reconstitution following HCT is increasingly recognized in recent years. The use of ATG, more particularly exposure of the graft to ATG, has been associated with poor immune reconstitution early after HCT[5,13,18,22]. In the early phase after HCT, until thymic output, patients depend on graft-infused T-cells undergoing peripheral expansion[34]. Therefore, in-vivo depletion of these T-cells may result in prolonged T-cell lymphopenia, leaving patients vulnerable for relapse and viral reactivations[2]. Restoration of thymic function can be hampered by age, GvHD, chemotherapy and steroids[34]. In the current cohort, few data on immune reconstitution shortly following HCT was available. The increased relapse mortality following high ATG exposure however suggests that one of the causes of relapse after HCT may be poor immune reconstitution, as was found in the previous studies[22,35,36].

Higher concentrations of ATG following infusion of the graft have been associated with a lower incidence of GvHD, although these studies only investigated single concentra-

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tions[19,25]. However, in a more comprehensive study investigating ATG PD in children, too low exposure before HCT was associated with higher chances on GvHD, while exposure after HCT did not impact GvHD[22]. This is in contrast with our current results in adults, where ATG levels before HCT did not impact the clinical outcome and a very low exposure to ATG after HCT was associated with an increased incidence of grade 3-4 acute GvHD and chronic GvHD. These differences might be the consequence of the fact that the majority of patients already had a very high exposure to ATG before HCT and we consequently had no power to address the question of levels of exposure before HCT and clinical outcome. In addition, the profound effect of high ATG exposure after HCT on GvHD in the current analyses presumably reflects the at least one log higher numbers in T cells used in PBSC, compared to CB and BM[37].

The clearance of ATG in adults is not influenced by bodyweight. In contrast to the current practice, ATG should therefore not be dosed in mg/kg but rather as a fixed dose based on ALC prior to ATG. This is illustrated by the simulation studies: the currently used weight-based dosing regimens for ATG result in poor optimal target attainment of 30-53%. An ALC-based dosing regimen leads to optimal exposure in 97% of patients. However, this proposed dosing nomogram should be evaluated in a prospective study. Moreover, the dosing regimen is only valid for Thymoglobulin, as the various ATG preparations are not biosimilar.

In conclusion, our data show that survival after HCT is highly impacted by ATG exposure after HCT. By using a dosing regimen that aims for optimal ATG exposure, which can best be achieved using an ALC-based dosing, outcomes following HCT could be improved, resulting in higher survival chances.

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SUPPLEMENTAL METHODS

Patients in pharmacokinetic analysis

In order to derive an individualized dosing regimen for ATG for patients of all ages, the pharmacokinetics need to be described. For this purpose, data from adult HCT patients was pooled with data from a previously published PK-analysis in children and young adults[1].

The adult population consisted of all patients treated in the adult hematopoietic cell transplantation program of the University Medical Center in Utrecht between April 2004 and May 2015 who received an HCT with ATG as part of the conditioning. No restriction applied in terms of conditioning regimen, type of donor and underlying diagnosis. Dose and starting day of ATG varied based on the treatment protocol; most patients received a cumulative dose of 8 mg/kg starting day -8 or -12 before HCT given over 4 days. In all patients samples were collected weekly after HCT, while in 35 patients samples before and after infusions were available.

The previously described PK-model consisted PK data of children and young adults receiving a HCT with ATG as part of the conditioning for any indication and conditioning regimen[1]. Patients treated in the pediatric hematopoietic cell transplantation programs of the University Medical Center in Utrecht (UMCU) and the Leiden University Medical Center (LUMC) in Leiden, both in the Netherlands, between April 2004 and December 2012 were included. Most patients received a cumulative dose of 10 mg/kg ATG given over 4 days, starting day -5 before HCT, however selected patients received other dosages at different starting times. In patients treated in the LUMC, samples were collected before and after each dose, and weekly thereafter, while patients treated in the UMCU only weekly elimination phase samples where available.

Any patients receiving serotherapy in the 3 months prior to the current HCT was excluded, as well as patients developing IgG anti-ATG antibodies.

Measurements of active ATG concentrations

Active ATG, defined as the fraction of ATG capable of binding to human lymphocytes, was measured in a flow-cytometry based assay[2].

The samples collected in adult patients were measured in the laboratory for translational immunology in the University Medical Center Utrecht (UMCU). In this assay, the EDTA plasma was filtered and added in different dilutions to Jurkat cell suspensions (ATCC) in 96-well plates (100.000 cells/well). Dilutions of ATG were used to prepare a standard curve and allow quantitative measurements. After 30 min incubation cells were washed and incubated with biotinylated goat anti-rabbit IgG (Jackson ImmunoResearch) and subsequently with streptavidin conjugated to phycoerythrin (BD Biosciences). Acquisition was performed on a FACS CANTO and results were analyzed with BD FACSDiva software (version 8).

The assay used in the previously described population were performed in the pediatric immunology laboratory of the Leiden University Medical Center (LUMC) in the Netherlands, has been previously published[1,3]. In short, HUT-78 T cells (PHACC, Porton Down, UK) were incubated with patient serum, followed by incubation with goat anti-rabbit IgG labeled with Alexa Fluor 647 (Biosource, Life Invitrogen, Carlsbad, CA, USA). Standards were made by serially diluting ATG in triplicate in a range from 5 to 0.005 AU/ml. Active ATG is expressed in arbitrary units, 1 μg/mL is arbitrarily set to 1 AU/mL. After washing, cells were analyzed by flow cytometry on a FACS-scan (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA).

Population pharmacokinetic analysis

The population approach was used for pharmacokinetic analyses to allow for analysis of sparse and unbalanced data (differing samples times and number of samples per patient). For this purpose, the non-linear mixed effects software package NONMEM version 7.2.0 (Icon Hanover, MD, USA) was used, with Pirana 2.8.2 and R 2.3.4 for workflow management and data visualization, respectively[4,5]. Within NONMEM, the first order conditional estimation option with interaction was used (FOCE-I). Active ATG concentrations were logarithmically transformed and simultaneously fitted. Concentrations below the limit of quantification for the respective assays (which were only found in the tail end of the concentration-time curve), were set at half the limit of quantification, with any subsequent measurements below the limit of quantification being removed according to method M6[6].

For the structural model one, two and three compartment models were tested. In addition, as antibodies are mostly eliminated by target binding or protein degradation[7] with the former being responsible for the pharmacological effects, also non-linear clearance parameters being dependent on the concentration substrate available were tested[8,9]. Non-linear elimination pathways were explored by incorporating clearance described by Michaelis-Menten kinetics.

Inter-occasion variability was tested for the subsequent doses on all parameters involved in elimination to detect potential time-dependency.

Several criteria applied in the development of the structural and statistical pharmacokinetic model. First, the inclusion of a parameter had to result in a significant improvement of the model fit. This was evaluated using the objective function value (OFV), the sum of all squared differences between observed and predicted values, which is assumed to follow a chi-squared distribution. Lower numbers of OFV represent a better fit of the model; a decrease of 3.84 points in OFV corresponds to a p-value of <0.05. In addition to a significant decline in OFV, the goodness-of-fit plots, which are used to detect any model misspecification,

needed to improve. In these plots, observed concentrations are plotted against individual predictions and population predictions, and conditional weighted residuals (CWRES) is plotted against time and observed concentrations. Finally, the residual standard errors of the parameters and shrinkage of interindividual variability were assessed[10].

Inter-individual variability was assumed to follow a log-normal distribution and was therefore implemented into the model as:

$$P_i = P_{pop} * e^{\eta_i} \qquad \text{(Eq. 1)}$$

where P_i depicts the individual or post-hoc value of the parameter in the ith individual, P_{pop} the population mean for the parameter, and η_i the inter-individual variability of the ith person, sampled from a normal distribution with a mean of 0 and a variance of ω^2.

The residual variability was described using a proportional residual error models. Due to the logarithmical transformation of the data, residual error will be inserted into the model as an additive error, however this should be read as a proportional error:

$$Y_{i,j} = C_{pred,i,j} + \varepsilon \qquad \text{(Eq. 2)}$$

where $Y_{i,j}$ is the observed concentration, $C_{pred,i,j}$ the jth predicted concentration for individual i, and ε the error, sampled from a normal distribution with a mean of 0 and a variance of σ^2.

Covariate selection

Following development of the structural and statistical pharmacokinetic model, potential predictors (covariates) for pharmacokinetic parameters such as clearance were evaluated. Potential covariates included patients related (body weight, lean body weight (LBW), ideal bodyweight (iBW), body surface area, age, lymphocyte counts, underlying disease), donor related (HLA match, stem cell source) and treatment related (conditioning regimen) and laboratory factors (assay used). Lymphocyte counts were assessed as a potential covariate because it harbors most targets for ATG, and therefore may influence ATG clearance[1]. As absolute lymphocyte counts rapidly drop after the first dose of ATG, only samples before the first infusion (-72h – 0h before first infusion) were used. Lean body weight (LBW) was calculated using the formulas developed by Janmahasatian et al. (adults) and Peters et al (children)[11,12]. IBW was calculated based on actual length and the ideal BMI of 22.5 for adults, while for children, the 50th percentile for the growth charts was used.

To investigate to evaluate the relationship between a potential covariate and a pharmaco-kinetic parameter such as clearance, potential covariates were plotted against individual predictions of the parameter involved (posthoc values), inter-individual variability, and conditional weighted residuals, both before and after inclusion of a covariate. Continuous covariates such as body weight and lymphocyte counts were tested in a linear and power function (equations 4 and 5):

$$P_i = P_{pop} * \left(1 + \left(\frac{Cov_i}{Cov_{median}}\right) * l\right)$$

<div align="right">(Eq. 4)</div>

$$P_i = P_{pop} * \left(\frac{Cov_i}{Cov_{median}}\right)^k$$

<div align="right">(Eq. 5)</div>

where P_i and Cov_i are the PK-parameter and covariate value for the ith individual, P_{pop} the population mean for the parameter for a patient with a median value for the covariate in the population, and Cov_{median} the median value for the covariate in the population. In the linear relationship equation (eq. 5) l is a fraction of the population value representing the increase of the parameter with the covariate, while in the power-relationship equation (eq. 6) k is the scaling factor. Additionally, more complex variations of equation 5 were explored for body weight as covariate, including a previously published bodyweight-dependent exponent (BDE) for k [13,14]:

$$k = a * BW^b$$

<div align="right">(Eq. 6)</div>

where k is the scaling factor in equation 5, a is the coefficient and b the exponent. Using a BDE, the exponent k can vary with bodyweight, leading to a sigmoidal relation between bodyweight and the parameter[13,14].

Improvement of the model after inclusion of a covariate was evaluated for significance using forward inclusion and backward elimination[10]. A significance level of p<0.005 (-7.9 points in OFV) was used for the forward inclusion, and p<0.001 (-10.8 points in OFV) for backward elimination. In other respects, building of the covariate model was comparable to the development of the structural PK-model, with specific emphasis on goodness of fit plots split for age to assure that the model predicts equally well in different age groups. In addition, inclusion of a covariate had to result in a decline in unexplained inter-individual variability and plots for interindividual variability versus the covariate involved needed to improve[10].

Model Evaluation Techniques

As the developed model is used as the basis for dosing in future patients, the model was extensively evaluated for the robustness. Several validation techniques are performed, all in accordance with EMEA and FDA guidelines for population pharmacokinetic analyses[15,16]. To assess the predictive power and accuracy of the model, a bootstrap analysis was performed. In this validation technique, 1000 replicates of the dataset were created using random sampling with replacement, and the model was fit using each dataset. For each parameter, median and 95% confidence intervals were compared to the parameter estimate and uncertainty in the final model.

In addition, a normalized prediction distribution of errors (NPDE) was performed. The prediction discrepancies between the final model and 1000 simulations of the model were evaluated, taking into account the correlation between observations in the same individual and the predictive distribution[17].

Finally, prediction-corrected visual predictive checks (VPC) were created to assess the predictive performance of the final model as compared to the measured concentrations. The prediction-corrected VPC can handle a large variability in absolute dosing, as is the case in the dataset. In this analysis, the observed concentration data and its median and 95% confidence intervals was compared to the median and 95% confidence intervals of 1000 simulations of the model.

SUPPLEMENTAL RESULTS

Patients and Samples for Pharmacokinetic Analyses

A total of 227 adult patients were included receiving 242 HCT's. Median age was 50 years, all patients received reduced intensity conditioning and the dose of ATG was 7.5-9 mg/kg over 4 days starting at day 7 (range 4-12). A total of 1964 ATG concentrations samples (Figure S1) were available, with a median of 8 samples per patient.

The pediatric cohort consisted of 267 children and young adults receiving 280 HCT's[1]. Median age was 6.5 years of age (range 0.2-23 year) and 74% received myeloablative conditioning. Most (83%) patients received a dose of 10 mg/kg over 4 days starting at day -5 before HCT. A total number of 3113 ATG concentration samples were available, mean number of samples per patient was 11[1]. The combined cohort existed of 494 adult and pediatric patients (Table S1).

Structural Pharmacokinetic Model

As testing for the best structural model was complicated by the broad range of body sizes resulting in unstable models, body weight was included as a covariate using a power function (equation 5, single exponent) on both clearance and volume of distribution during this phase of model development. Compared to a one-compartment model, the two-compartment model was superior in terms of goodness-of-fit plots and OFV (decrease of 889 points, 4 additional parameters, p<0.0001). A three-compartment model was unstable, giving inaccurate parameter estimates and being highly dependent on initial values. A proportional error model was used. Next, non-linear elimination pathways were explored. Inclusion of a saturable elimination pathway next to the linear elimation resulted in a decline in OFV of 191 points (4 additional parameters, p<0.0001) and improvement in GOF, especially in the lower concentration range. A model with only saturable clearance was unstable. In line with the PK-model in children, in the adult population was also a discrepancy noted in CWRES vs time. To address this under-prediction of concentrations during and shortly after the final infusions of ATG, saturable distribution towards the peripheral compartment was included in the model. This was parameterized using Michaelis-Menten kinetics with the parameters T_{max} (maximum rate of transport) and T_m (concentration at half maximum rate; Figure S2). Inclusion of saturable intercompartmental transport improved the CWRES vs time, and yielded a decrease of 342 points (2 parameters, p<0.0001; Figure S4). Details of the final structural and statistical model are presented in Figure S2, Figure S3 and Table S2.

Covariate Model

The influence of body weight on clearance and central volume of distribution was further tested using the evaluation of different parameterizations for the influence of bodyweight (see equation 5 and 6) and with the use of different bodyweight metrics such as LBW and iBW. Of these covariates and functions, the use of a Bodyweight Dependent Exponent for k (equation 6) as covariate function for bodyweight for clearance resulted in the best results. Compared to the model with a single exponent k (equation 5), a decrease in OFV of 19 points was noted (p<0.005 considering 1 additional degree of freedom) (Table S2). Of the other covariates tested, lymphocyte counts were found to impact linear clearance with an increase in the absolute lymphocyte count before the first dose of ATG yielding an increase in clearance (decrease in OFV of 37 points (p<0.0001)). No other covariates were identified on any other parameter (P> 0.05). Figure S5 shows how clearance changes with bodyweight given different baseline lymphocyte counts for the final model. The figure illustrates that clearance does not change with bodyweight in the adult weight range (> 50 kg) while the influence of baseline lymphocyte counts on clearance are substantial across the population.

After inclusion of body weight and lymphocyte counts, no trends could be observed in the plots of inter-individual variability on clearance and volume of distribution versus the

included covariates (Fig S6, panels a-c). In addition, after correcting for body weight in the model, no effect of age could be identified on any of the PK-parameters (Figure S6, panels d-f). The parameter estimates of the final (covariate) model are included in Table S2.

Model Validation

The final model with body weight and lymphocyte counts before the first dose of ATG was stable in a bootstrap analysis, with 96.1% of runs being successful. Bootstrap medians as well as the 95% confidence interval were well in line with the model estimates and residual standard errors (Table S2). The prediction corrected VPC shows the median and 95% confidence interval of the observed data to follow the predictions of the model (Figure S7). The NPDE-analysis showed a normal distribution, and no trends were observed in the NPDE versus time or predictions (Figure S8).

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7

SUPPLEMENTAL TABLES

	Adults	Children and young adults[1]	Total
Number of patients (n)	227	267	494
Number of HCTs (n)	242	280	522
Male sex (%)	60	62	61
Age (years)	50 (17.6-70)	6.5 (0.2-23)	17 (0.2-70)
Weight (kg)	74 (43-123)	21 (3.7-96)	55 (3.7-123)
BSA (m2)	1.91 (1.34-2.56)	0.83 (0.14-2.1)	1.55 (0.14-2.56)
Number of samples [n (mean per patient)]	1964 (8)	3113 (11)	5077 (10)
Starting day ATG (days before transplantation)	7 (4-12)	5 (1-19)	7 (1-19)
Lymfocyte count before conditioning (x 10^9)	0.72 (0-10.9)	0.29 (0-10.4)	0.64 (0-10.9)
Cumulative dose (mg/kg)			
<7.5 mg/kg	22	3	12
7.5-9 mg/kg	78	4	38
9-11 mg/kg	0	83	45
>11 mg/kg	0	10	5
Diagnosis (%)			
Malignancy	0	47	72
Immune deficiency	0	19	11
Bone marrow failure	0	6	3
Metabolic disease	0	9	5
Benign hematology	0	18	9
Auto-immune disease	0	1	0
Stem cell source (%)			
Bone marrow	13	49	32
Peripheral blood stem cells	86	15	47
Cordblood	1	34	19
Cordblood plus haplo or 2nd cordblood	0	2	2
Conditioning regimen (%)			
Reduced intensity	55	4	27
Chemotherapy-based	17	74	48
TBI-based	28	22	25

Shown as median (range) unless otherwise specified

**Table S1.** Patient Characteristics in Pharmacokinetic Analysis

	Dataset [estimate (RSE)]	Shrinkage	1000 bootstrap replicates (96.1% successful)	
			Median	95% confidence interval
Structural model				

$$CL_i = CL_{pop} * \left(\frac{WT}{WT_{med}}\right)^{a*WT^b} * \left(1 + \frac{BL}{BL_{med}} * m\right)$$

	Dataset [estimate (RSE)]	Shrinkage	Median	95% confidence interval
CL_{pop} (L/day)	2.0 (9%)		2	1.6-2.5
a	0.16 (18%)		0.16	0.1-0.28
b	-1.1 (28%)		-1.1	-2.4--0.5
m	0.32 (26%)		0.33	0.18-0.53

$$V_{1,i} = V_{1,pop} * \left(\frac{WT}{WT_{med}}\right)^{l}$$

	Dataset [estimate (RSE)]	Shrinkage	Median	95% confidence interval
$V1_{pop}$ (L)	16.6 (13%)		16.6	13.2-29.7
l	0.86 (18%)		0.87	0.62-1.43
$K_{21,pop}$ (L-1)	0.67 (19%)		0.68	0.45-1.6
$T_{max,pop}$ (AU/day)	187 (15%)		187	101-266
$T_{m,pop}$ (AU/L)	9.5 (16%)		9.4	5.3-12.5
$V_{max,pop}$ (mg/day)	4.7 (18%)		4.7	2.3-6.6
$K_{m,pop}$ (mg/L)	2.3 (19%)		2.2	1.2-3.3
Random variability				
Inter-individual variability on CL (%)	72 (4%)	15	72	65-79
Inter-individual variability on V1 (%)	57 (11%)	39	57	45-74
Inter-individual variability on T_m (%)	150 (5%)	27	150	118-164
Inter-individual variability on V_{max} (%)	67 (12%)	56	67	50-90
Inter-individual variability on K_m (%)	184 (8%)	38	181	143-219
Proportional residual error (%)	32 (5%)	15	31	28-35

Table S2. Pharmacokinetic Parameter Estimates and Bootstrap Analysis. RSE: residual standard error; CL: clearance; WT: body weight; WTmed: median bodyweight of 55 kg; BL: baseline lymphocyte count before first dose of ATG; BL_{med}: median baseline lymphocyte count (0.64×10^9/L); V_1: central volume of distribution; K_{21}: distribution constant towards the central compartment; T_{max}: Maximum rate of distribution towards the peripheral compartment; AU: arbitrary units; T_m: Michaelis-Menten constant of distribution towards the peripheral compartment; V_{max}: Maximum rate of non-linear elimination; K_m: Michaelis-Menten constant of non-linear elimination.

7

Predictor	AIC
AUC after HCT	507.75
Concentration at time of HCT	508.48
Total AUC	509.02
Time to reach Concentrations < 1 AU/mL	509.88
Maximum Concentration	510.18
AUC before HCT	510.64

Table S3. Akaike Information Criterion (AIC) for all PK-predictors

SUPPLEMENTAL FIGURES
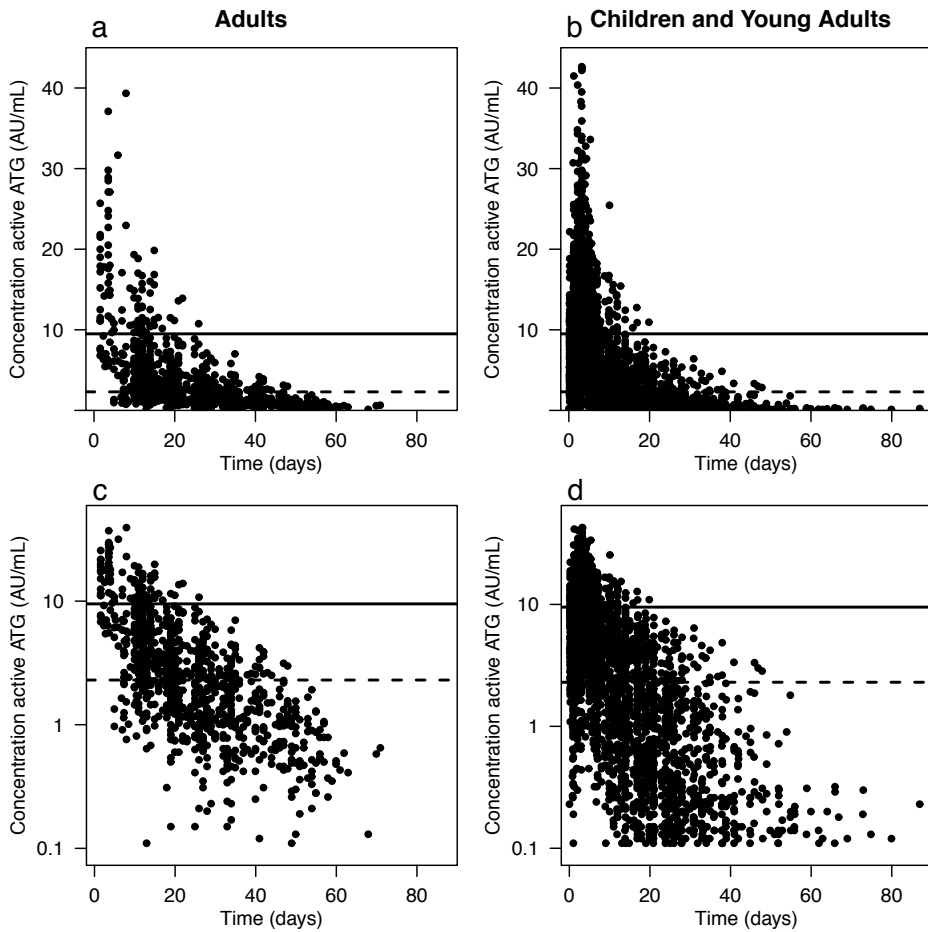
Figure S1. Available active ATG concentration data. Data on linear scale (upper panels) and logarithmic scale (lower panels) over time for adult (left) and children and young adults (right) cohorts. Solid lines: T_m, dashed lines: K_m.
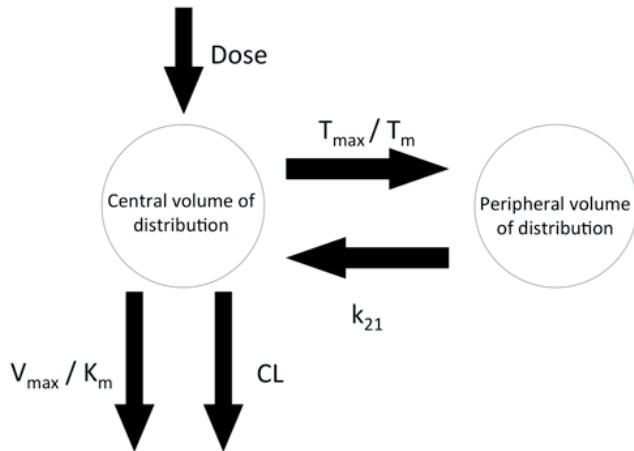
Figure S2. Overview of the final pharmacokinetic model. CL: clearance; V_{max}: Maximum rate of non-linear elimination; K_m: Michaelis Menten constant of non- linear elimination; T_{max}: Maximum rate of non-linear distribution; T_m: Michaelis Menten constant of non-linear distribution; K_{21}: distribution constant towards central compartment.

Individual Predictions

a **< 6.7 years old**

Observed concentration (AU/mL)

e^4 e^2 e^0 e^{-2} e^{-4} e^{-6}

e^{-6} e^{-4} e^{-2} e^0 e^2 e^4

Individual predicted concentration (AU/mL)

b **6.7–18 years old**

Observed concentration (AU/mL)

e^4 e^2 e^0 e^{-2} e^{-4} e^{-6}

e^{-6} e^{-4} e^{-2} e^0 e^2 e^4

Individual predicted concentration (AU/mL)

c **18–50 years old**

Observed concentration (AU/mL)

e^4 e^2 e^0 e^{-2} e^{-4} e^{-6}

e^{-6} e^{-4} e^{-2} e^0 e^2 e^4

Individual predicted concentration (AU/mL)

d **>50 years old**

Observed concentration (AU/mL)

e^4 e^2 e^0 e^{-2} e^{-4} e^{-6}

e^{-6} e^{-4} e^{-2} e^0 e^2 e^4

Individual predicted concentration (AU/mL)

Figure S3.

Population Predictions

Figure S3. Goodness-of-Fit plots of final model. Individual predictions (panels A-D) and population predictions (panels e-h) for patients < 6.7 years (panels A and E), 6.7-18 years (panels B and F), 18- 50 years (panels C and G) and >50 years (panels D and H). Lines: lines of unity (x=y)
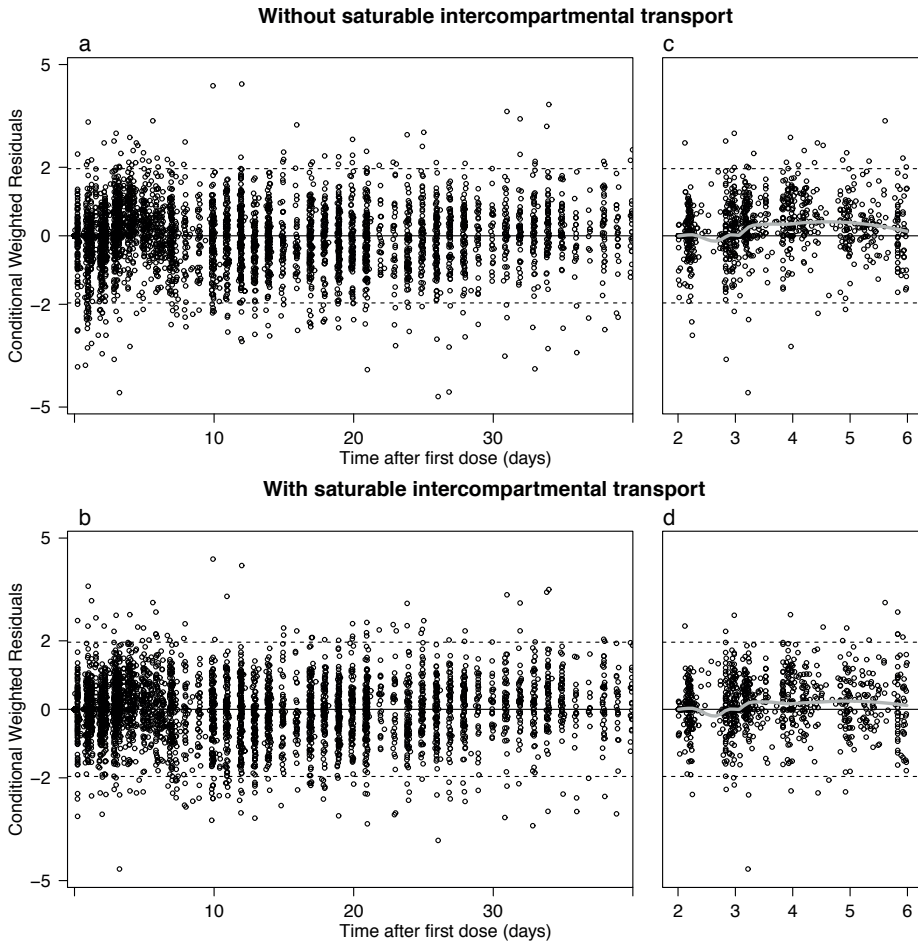
Figure S4. Saturable Intercompartmental Transport. A trend in conditional weighted residuals (CWRES) versus time can be seen before introduction of saturable intercompartmental transport (a+b), which is accounted for after introduction (c+d). a+c: All data; b+d: Zoomed in to 2–6 days. Dots show the CWRES per concentration sample, the solid line shows where CWRES = 0, the dashed lines indicate ±2 standard deviations, and the curved lines show spline regression
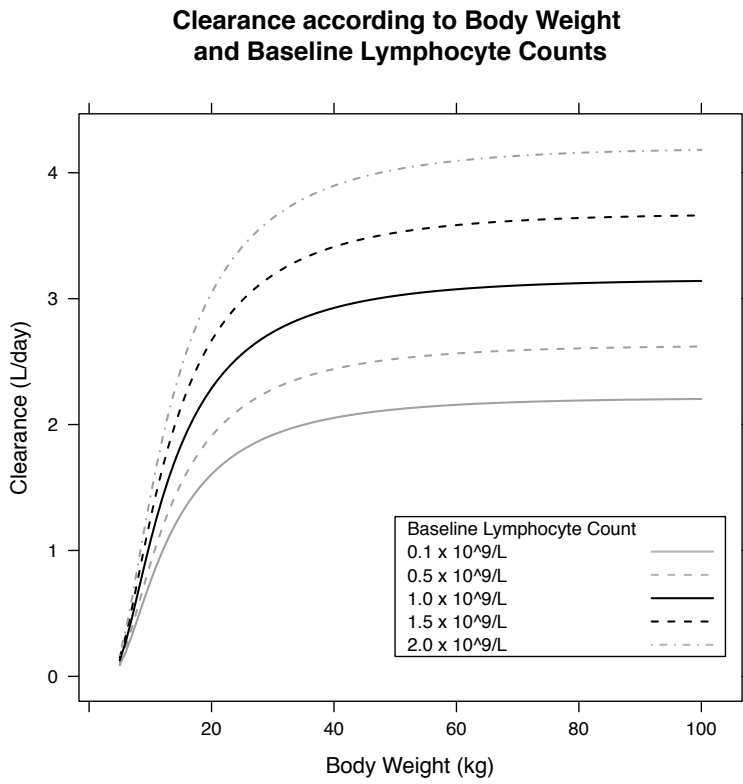
Figure S5. Impact of Body Weight and Lymphocyte Counts on Clearance
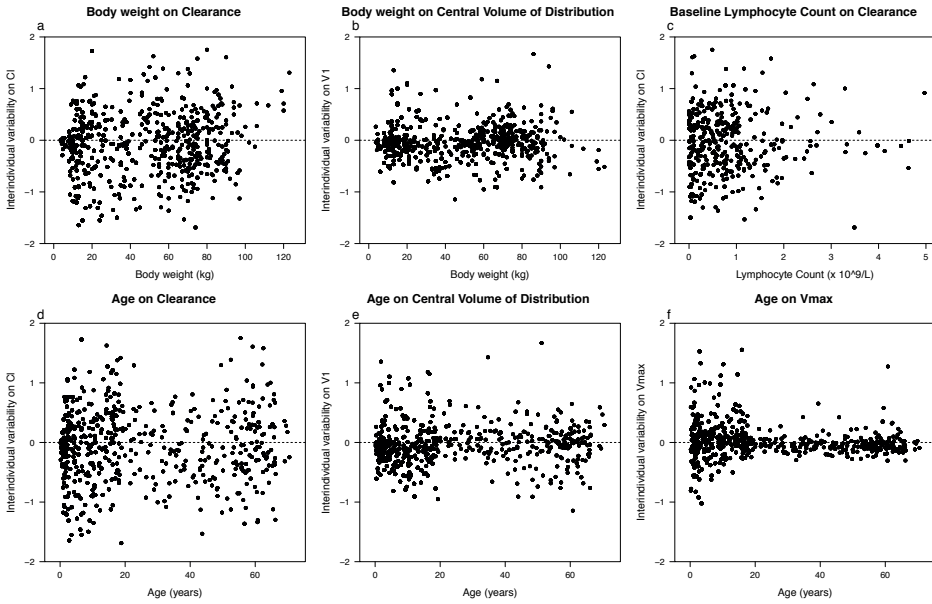
Figure S6. Inter-individual variability versus covariates in final model. Lines: inter-individual variability=0.

Prediction Corrected Visual Predictive Check
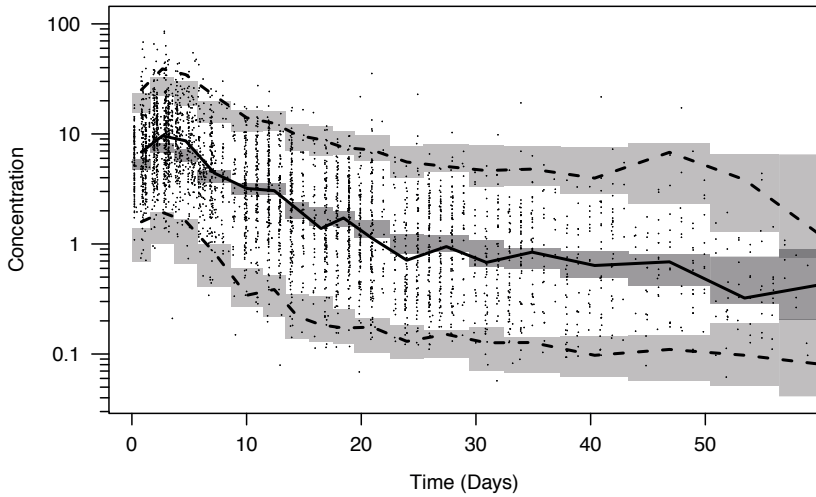
Figure S7. Correction-predicted Visual Predictive Check. Dots: actual concentration data. Solid line: median concentration over time; dashed lines: 2.5 and 97.5% quartiles of concentration of time. Dark grey bars: 95% confidence interval (CI) of median predictions; light grey bars: 95% CI of 2.5 and 97.5% predictions.
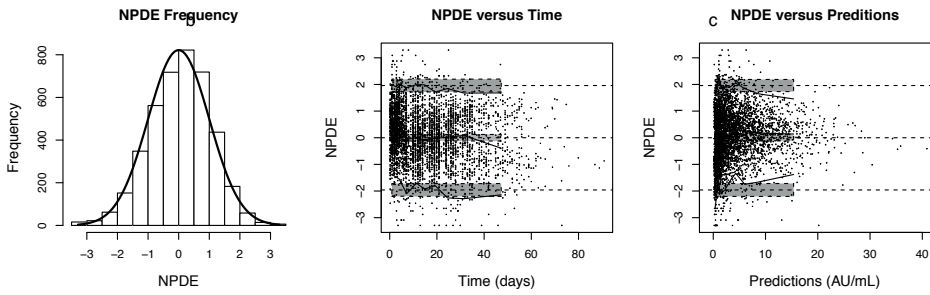
Figure S8. NPDE validation. Panel a: histogram of the NPDE with the solid line representing a normal distribution with a mean of 0 and variance of 1. Panel b: NPDE versus observations, panel c: NPDE versus predictions. Grey blocks: 95% confidence interval of NPDE.
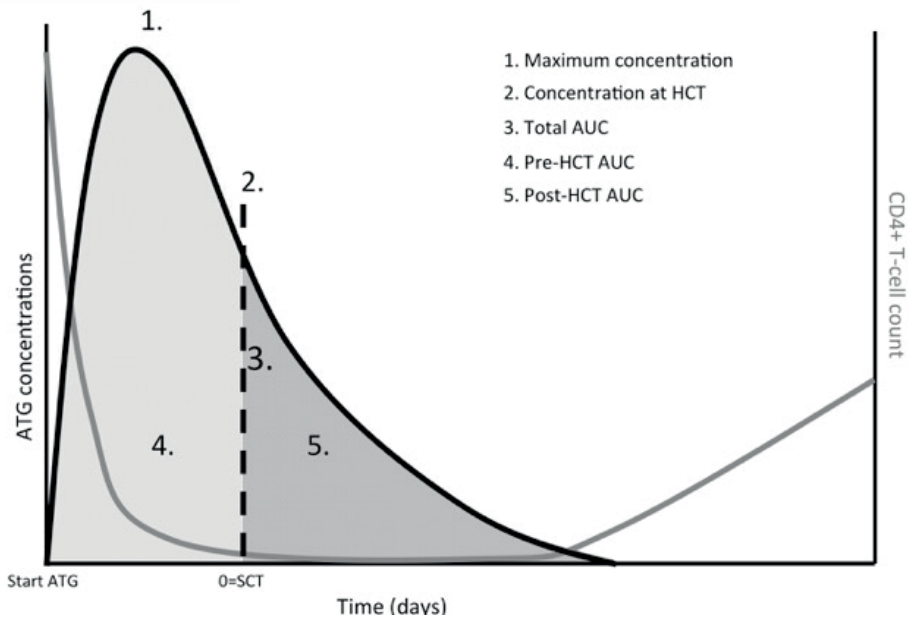
Figure S9. Overview of PK exposure measures

Chapter 8

Impact of alemtuzumab exposure on clinical outcomes of hematopoietic cell transplantation: are we overdosing children using I.V. dosing close to transplantation?

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To the editor,

Alemtuzumab (Campath®, Genzyme, MA, USA) is used as serotherapy in hematopoietic cell transplantation (HCT) to prevent graft-versus-host-disease (GvHD) and graft failure by in-vivo depletion of lymphocytes. Inclusion of alemtuzumab in the conditioning regimen significantly reduces the incidence of both acute and chronic GvHD[1-3]. Higher doses of alemtuzumab have been associated with delayed immune reconstitution (IR) by excessive lymphodepletion[4-6]. Poor IR could potentially lead to increased viral reactivations as well as less graft-versus-leukemia effect, thereby abrogating the beneficial effect on GvHD reduction.

A recent publication by Marsh and colleagues[7] in Blood described the impact of peri-transplant alemtuzumab concentrations on clinical outcomes. Low concentrations were associated with acute GvHD, while higher concentrations led to poor lymphocyte reconstitution and increased mixed chimerism.

We aim to evaluate these results in a larger cohort of children. We use a pharmacologically and methodologically stronger approach by calculating the alemtuzumab concentrations on the basis of a validated pharmacokinetic model[8]. This exactly estimates concentrations at the beginning of infusion of the graft rather than peri-transplant concentrations (±3 days) and eliminates some of the uncertainty incorporated in a single concentration measurement.

Children receiving their first HCT in the Leiden University Medical Center (Leiden, the Netherlands; LUMC) and Great Ormond Street Hospital (London, United Kingdom; GOSH) with alemtuzumab as part of the conditioning were included. Patients using other serotherapy drugs (anti-thymocyte globulin; ATG) within the same conditioning regimen were excluded. Data was collected after informed consent; ethical committee approval through trial numbers P01.028 (Leiden) and V0904 (London).

Alemtuzumab (Campath, Genzyme, USA) was dosed at 0.5 or 1 mg/kg (5 x 0.1-0.2 mg/kg/day) in most children. Details about the samples and alemtuzumab assay is available in the supplements. Conditioning regimens were given according to (inter)national protocols.

176 patients were included between January 2003 and July 2015 with a median age of 4.8 years (range 0.2-19 years; Table 1). Full description on the definitions and statistical approach can be found in the supplements. Fifty-four percent of patients received a cumulative dose of 1 mg/kg over 5 doses, 35% received a dose of <0.9 mg/kg. Median starting day was day -8 (-21 to -3). Immune deficiency was the most frequent indication for HCT. Median follow-up for surviving patients was 64 months (range 16.9-149).

	London	Leiden	Total
Number of patients (n)	125	51	176
Male sex (%)	64	67	65
Age (years)	4.0 (0.4-15)	8.1 (0.2-19)	4.8 (0.2-19)
Cumulative dose (mg/kg) (%)			
<0.9 mg/kg	36	29	35
0.9-1.1 mg/kg	50	65	54
>1.1 mg/kg	14	6	11
Starting day alemtuzumab (days before transplantation)	8 (5-21)	6 (3-16)	8 (3-21)
Number of samples [n (mean per patient)]	309 (2.5)	557 (10.9)	866 (4.9)
Diagnosis (%)			
Malignancy	14	41	22
Immune deficiency	65	37	57
Bone marrow failure	15	20	16
Metabolic disease	5	0	4
Benign hematology	1	2	1
Stem cell source (%)			
Bone marrow	35	65	44
Peripheral blood stem cells	65	27	54
Cordblood	0	8	2
Conditioning regimen (%)			
Reduced intensity	45	63	50
Chemotherapy-based	50	31	45
TBI-based	5	6	5
Follow-up surviving patients (months)	59 (17.3-130)	84 (16.9-149)	64 (16.9-149)

Shown as median (range) unless otherwise specified

Table 1. Patient characteristics

A clear difference in C_{graft} was observed compared to peri-transplant concentrations as reported by Marsh et al[7]. In our cohort, 6 patients (3%) had a very low C_{graft} < 0.155 µg/mL, while C_{graft} was very high (>4.36 µg/mL) in 38 patients (21%; Figure S1). In the Marsh report, of 18% and 8% had very low and very high concentrations, respectively[7]. Other groups were comparable. Patients were analyzed in groups with a C_{graft} < 1, 1-2, 2-3 and >3 µg/mL.

The incidence of grade 2-4 acute GvHD was impacted by C_{graft}. Patients with high C_{graft} showed the lowest incidence, while the three groups with lower C_{graft} performed worse (figure 1a). In multivariate analysis (MV), high C_{graft} was associated with a lower incidence of acute GvHD in both grades 2-4 and 3-4 (hazard ratio [HR] 0.79, 95% confidence interval
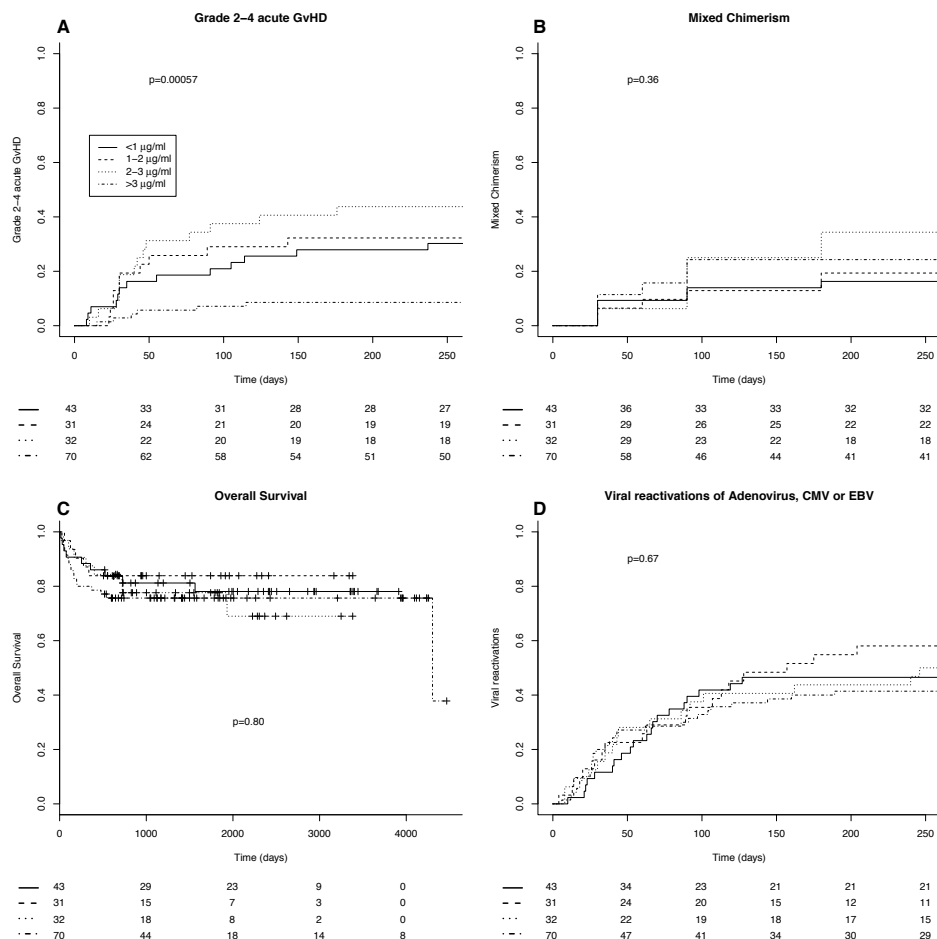
Figure 1. Cumulative incidence of acute GvHD (panel A), mixed chimerism (panel B) and viral reactivations (panel D) and Kaplan-Meier overall survival curve (panel C) in groups of alemtuzumab concentrations at time of graft infusion. Orange: <1 μg/mL; black: 1-2 μg/mL; red: 2- 3 μg/mL; blue: >3 μg/mL.

[CI] 0.67-0.93, p=0.0046 and HR 0.58, 95% CI 0.38-0.87, p=0.0081, respectively; Table S1 and S2). No other multivariate predictors were identified. Chronic GvHD was not impacted by C_{graft} (p=0.32 in MV analysis).

Mixed chimerism, defined as < 95% donor chimerism in 2 whole blood samples, did not differ between groups (figure 1b). In multivariate analysis, no significant impact of C_{graft} on mixed chimerism could be identified (HR 1.03, 95% CI 0.93-1.13, p=0.60).

Overall survival was not impacted by C_{graft}, both in univariate (fig 1c) and in multivariate analysis (HR 0.99, 95% CI 0.88-1.13, p=0.97). However, treatment after 2009 (median treat-

ment year in this cohort) was a multivariable predictor for improved survival (p=0.046, Table S2).

We also investigated viral reactivations of Epstein-Barr virus (EBV), cytomegalovirus (CMV) and adenovirus, defined as a > 1000 viral copies/mL in 2 subsequent measurements. No difference in viral reactivations was found between groups of C_{graft} (HR 0.96, 95% CI 0.88-1.06, p=0.40; figure 3d).

Finally, the relation between C_{graft} and CD3+ T-cell counts was investigated. CD3+ immune reconstitution (IR) was defined as a CD3+ T-cell count >100 x 10^6/L in 2 samples before 100 days, as adapted from literature[10]. While there were significant differences between groups of C_{graft}, the distribution of CD3+ kinetics not ordered in terms of C_{graft} (figure S2a). This is reflected in the multivariate analysis, where only a trend was found between C_{graft} and CD3+ IR (HR 1.14, 95% CI 0.99-1.31, p=0.060). The absolute CD3+ count at day 100 was not significantly different between groups (p=0.064; figure S2b).

These data suggest that high alemtuzumab concentrations during graft infusion may reduce the incidence of acute GvHD. Other outcome parameters including survival, mixed chimerism, viral reactivation and T-cell recovery are not impacted by alemtuzumab concentrations.

Compared to previous the study by Marsh et al[7], the alemtuzumab concentrations in this cohort are relatively high. Part of this difference may be due to differences in underlying disease and conditioning regimen. The dosing and route of administration are most likely larger contributors to the difference in alemtuzumab concentrations. The alemtuzumab dose used by Marsh (1mg/kg, or fixed dose of 33 mg [<10 kg] or 48 mg [>10kg]) is generally higher than was used in this cohort (0.5-1 mg/kg in most patients). However, the starting day was more proximal (i.e. closer to graft infusion) in the current study, and 66% of patients received subcutaneous dosing in the Marsh-study[7]. Subcutaneous dosing leads to a high first-pass metabolism, and results in a slower release, both reducing alemtuzumab concentrations in blood[11]. Still, taking into account the higher C_{graft} in this cohort, the results are generally comparable. The most optimal therapeutic window has not been set convincingly.

From a pharmacological perspective, the most optimal dose for a drug shows the desired effects with minimal toxicity[12]. For alemtuzumab, the desired effect is the prevention of GvHD and mixed chimerism, while toxicities include poor T-cell reconstitution and subsequent viral reactivations[13]. In the current study, a moderate exposure-response effect can be identified for GvHD, not for mixed chimerism. Of note, stable mixed chimerism can be allowed in subgroups of patients. The C_{graft} however is not correlated to toxicity. It can be

concluded that a vast majority of patients had a supratherapeutic alemtuzumab exposure. This is in line with the final conclusion by Marsh, who reports the optimal C_{graft} for alemtuzumab to be between 0.2-0.4 µg/mL[7].

Therapeutic drug monitoring (TDM) in combination with individualized dosing may be useful in assuring the most optimal alemtuzumab exposure[14,15]. However, the long time window (±10 days) between the infusions and the graft infusion, combined with the narrow therapeutic window during graft infusion, may complicate this approach. Still, taking into account the very slow clearance[16–18] and low lympholytic level[7] of alemtuzumab, approaches to target exposure will be difficult. Anti-thymocyte globulin has a faster clearance[19,20] and higher a lympholytic level[20], and therefore may be a more attractive therapeutic option to prevent GvHD and graft failure[21–24].

In conclusion, alemtuzumab concentrations at graft infusion after intravenous doses of 0.5-1 mg/kg starting 10-6 days before HCT results in supratherapeutic exposures. Dosing should be reduced, given earlier or administered subcutaneously.

8

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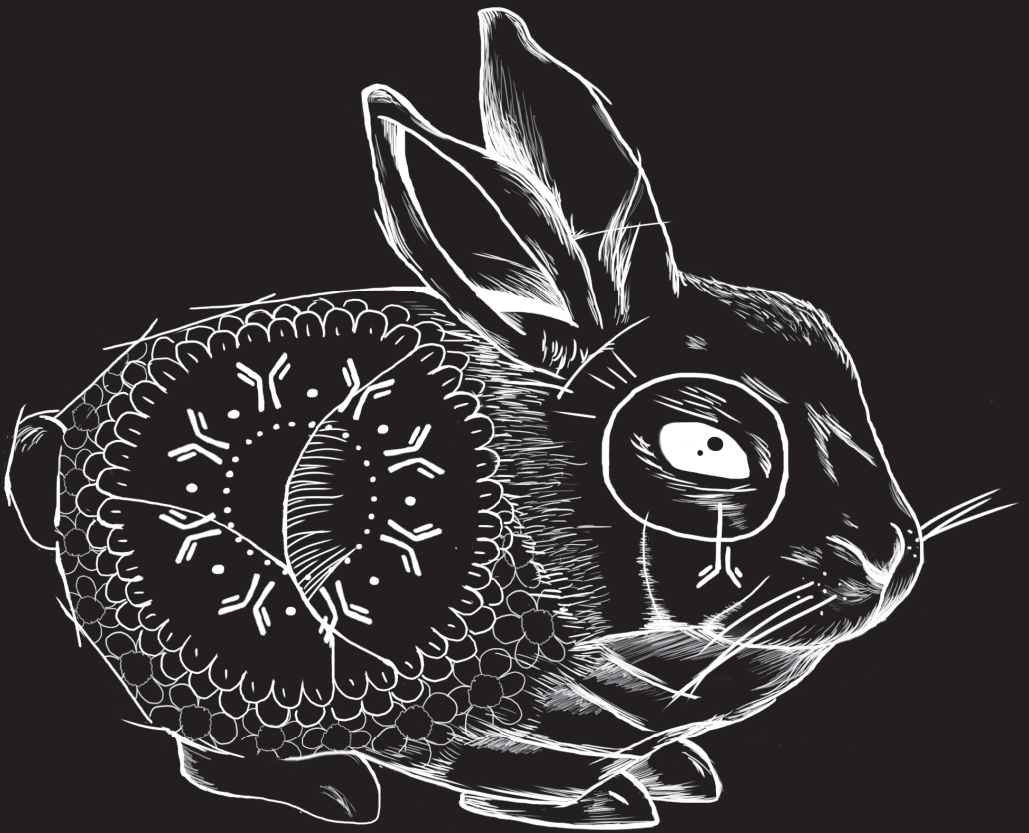
PART IV

Immune Reconstitution
Predicting Clinical Outcome

Chapter 9

Leukemia-free Survival in Myeloid Leukemia, but not in Lymphoid Leukemia, is Predicted by Early CD4+ Reconstitution following Unrelated Cord Blood Transplantation in Children: a Multicenter Retrospective Cohort Analysis

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Hematopoietic cell transplantation (HCT) is a potentially curative option for chemotherapy-resistant leukemia. In addition to either high-dose chemotherapy or TBI, the advantages of HCT include clearance of residual disease by GvL effect.[1] This should lead to a durable CR.

Unrelated umbilical cord blood (CB) has been increasingly used as an alternative cell source during the last decades. However, T-cell immune reconstitution (IR) in CB can be delayed when compared with matched unrelated donors or sibling transplants,[2] which may be caused by the lower number of infused T cells combined with a potentially higher susceptibility of CB T cells to serotherapy (for example, anti-thymocyte globulin; ATG).[3] Sufficient IR has subsequently been related to a reduced relapse incidence, indicating that reduction of in vivo lymphodepletion of the graft is of utmost importance.[4] For this reason, some centers decided to remove ATG from the conditioning regimen in malignant indications.[5]

Here, we aim to investigate CD4+ T-cell IR as a predictor of leukemia-free survival (LFS), being the main outcome of interest. Children receiving a cord blood transplantation (CBT) in two dedicated CB transplantation centers, the University Medical Center Utrecht (UMCU) in Utrecht, The Netherlands, and Great Ormond Street Hospital in London, UK, were included. In both centers, CB was the preferred cell source when a matched sibling donor was not available. IR was defined as reaching a CD4+ T-cell count of 50×106 cells/L within 100 days after CBT in two consecutive measurements that was previously described as being the best predictor.[3,6] We also took the effect of exposure to ATG on T-cell reconstitution into account, as ATG may significantly hamper CD4+ IR. Patients were divided into three groups: no ATG, low exposure to ATG after CBT (<20 arbitrary units (AU)days/mL) and high exposure (>20 AU×days/mL). We also related CD4+ IR to other outcomes of interest: overall survival (OS), non-relapse mortality (NRM) and GvHD.

The conditioning regimens in this cohort were given according to (inter)national protocols, mostly consisting of myeloablative busulfan-based regimens. The majority of patients did not receive ATG: only those patients treated at the UMCU before 2013 and those with ALL treated after 2013 received ATG. ATG was given at a cumulative dose of 10 mg/kg over 4 consecutive days, starting from day −5 in patients treated before 2009, while patients treated from 2009 onwards received the same dose of ATG starting from day −9. From 2010 onwards, patients with a body weight 430 kg received a lower cumulative dose of ATG (7.5 mg/kg), and a 50% dose reduction was given when pre-conditioning lymphocyte counts were less than 300×10^{6}/L.

Patients received infection prophylaxis including gut decontamination and GvHD prophylaxis according to local protocols. GvHD prophylaxis consisted of cyclosporin A aiming at a trough level of 200–250 µg/L (therapeutic drug monitoring controlled), combined with

prednisolone 1 mg/kg (Utrecht, The Netherlands) or mycophenolate mofetile (London, UK).

We included a total of 87 consecutively treated children between 1 January 2004 and 1 August 2014 (Table 1). Of these patients, 40 percent (n=35) of children had lymphoid leukemia (acute lymphoid leukemia), 55% (n=48) of patients suffered from myeloid leukemia (acute and chronic myeloid leukemia myelodysplastic syndrome) and 4 patients (5%) had a lymphoma. This cohort includes 30 patients who have been described in a previous report.[3] Forty-one percent of the treated children received ATG in the conditioning regimen. The median follow-up for surviving patients was 48 months (range 9.4–139 months).

	GOSH	UMCU	Total	
Number of patients (n)	33	54	87	
Transplants [n(%)]				p=0.08
First transplants	29 (88)	51 (94)	80 (92)	
Second transplants	4 (12)	1 (2)	5 (6)	
Third transplants	0 (0)	2 (4)	2 (2)	
Male sex [n(%)]	19 (58)	35 (65)	54 (62)	p=0.65
Age at transplant (years)	5.4 (0.7-12.7)	9.7 (1-18.1)	8 (0.7-18.1)	p=0.6
Patients receiving ATG [n(%)]	0 (0)	36 (67)	36 (41)	p<0.001
Diagnosis [n(%)]				p=0.11
Lymphoid leukemia	10 (30)	25 (46)	35 (40)	
Myeloid leukemia	22 (67)	26 (48)	48 (55)	
Other malignancies	1 (3)	3 (6)	4 (5)	
CR status [n(%)]				p=0.00022
No CR	9 (27)	0 (0)	9 (10)	
CR 1	10 (30)	18 (33)	28 (32)	
CR 2	14 (42)	36 (67)	50 (57)	
Remission status [n(%)]				p=0.0029
Refractory disease	11 (33)	6 (11)	17 (20)	
CR, MRD positive	9 (27)	34 (63)	43 (49)	
CR, MRD negative	13 (39)	14 (26)	27 (31)	
Conditioning regimen [n(%)]				p=0.0018
Busulfan based	17 (52)	40 (74)	57 (66)	
Treosulfan based	9 (27)	1 (2)	10 (11)	
TBI based	6 (18)	13 (24)	19 (22)	
Other	1 (3)	0 (0)	1 (1)	
Follow-up (months) [mean (range)]	34 (1.2-81)	32 (0.2-139)	32 (0.2-139)	p=0.73

Table 1. Patient characteristics. GOSH: Great Ormond Street Hospital; UMCU: University Medical Center Utrecht; ATG: Anti-thymocyte globulin; CR: Complete Remission; MRD: Minimal Residual Disease; TBI: Total Body Irradiation.

When analyzing the data, we found a strong correlation between ATG exposure after CBT and probability of CD4+ IR (Kaplan–Meier estimated incidence of 89±4%, 50±18%, and 32±9% in patient with no ATG, low exposure and high exposure, respectively, P<0.0001). Successful CD4+ IR resulted in a higher probability of LFS (hazards ratio (HR) 0.44, 95% confidence interval (CI) 0.23–0.84, P=0.013); when subdividing leukemia's, this effect was only observed in myeloid leukemia's (HR 0.24, 95% CI 0.10–0.61, P=0.003, figure 1a), while in lymphoid leukemia's, LFS is not significantly influenced by CD4+ IR (figure 1b). In multivariate analysis, CD4+ IR was found to be a significant predictor of LFS (HR 0.44, 95% CI 0.23–0.84, P=0.013), along with CR status.

Corresponding with findings for LFS, OS was higher in patients reaching successful CD4+ IR (HR 0.32, 95% CI 0.16–0.64, P=0.0014). This difference was found only in children with myeloid leukemia (HR 0.16, 95% CI 0.06–0.45, P=0.00044), while in lymphoid leukemia, patients with or without successful CD4+ IR performed similar. In a multivariate model, CR status was also identified as a predictor of OS. In the whole cohort, NRM was lower in patients who achieved early CD4+ IR (HR 0.20, 95% CI 0.06–0.65, P=0.0072; Figure 1C), with no other multivariate predictors found then CD4+ IR. Relapse incidence in myeloid leukemia was significantly reduced by successful CD4+ IR (HR 0.31, 95% CI 0.10–0.95, P=0.041), while CD4+ IR did not predict relapse incidence in lymphoid leukemia's. The incidence of GvHD, both acute and chronic, was not impacted by CD4+ IR.

Taking into account the limitations of a retrospective cohort study and potential center differences, these data suggest successful CD4+ IR, resulting from no or low ATG exposure after CBT, improve LFS in myeloid leukemia. In lymphoid leukemia's, however, CD4+ IR did not predict LFS. NRM was reduced by successful CD4+ IR in all patients. These results

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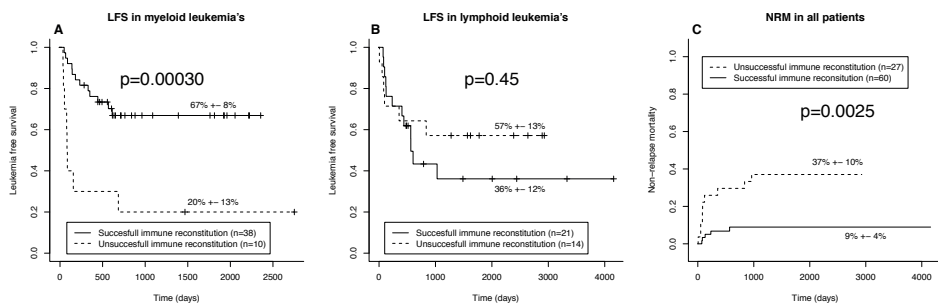
Figure 1. Kaplan–Meier curves of leukemia-free survival (LFS) according to successful CD4+ T-cell reconstitution in myeloid leukemia's (a) and lymphoid leukemia (b); non-relapse mortality (NRM) according to successful CD4+ T-cell reconstitution in all patients (c). Solid lines: successful CD4+ reconstitution, dashed lines: unsuccessful CD4+reconstitution.

stress the importance of achieving an early immune reconstitution in order to generate a GvL effect, especially in myeloid leukemia, and reduce the NRM for all patients.

CB as emerging alternative cell source shows promising results in terms of LFS,[7] anti-leukemic effect[8] and reduction of viral reactivations[5] as compared with bone marrow. Outcome may, however, be further improved by promoting or achieving predictable early immune reconstitution. Timely reconstitution of CD4+ T cells has been shown to be associated with higher survival chances.[3] Other studies comparing conditioning without ATG in a CB-setting as compared with ATG showed mixed results in terms of OS, NRM and relapse; either having no effect,[5] a lower incidence of relapse but not OS,[9] or lower incidence of NRM and OS, but not relapse.[10]

These data suggest a difference in susceptibility between myeloid leukemia and lymphoid leukemia to early CD4+ T-cell reconstitution in terms of relapse. The mechanism behind this difference is not clear, although this finding seems to be in line with the observed effect of donor-lymphocyte infusions, which is suggested to be stronger in AML compared with lymphoid leukemias.[11] Also in a recent CIBMTR study, no difference in relapse and LFS were seen in children with ALL with or without ATG in the conditioning regimen.[12] It can be speculated that this difference in GvL effect in favor of myeloid leukemia may be due to the TCR repertoire, which may harbor more targets to myeloid blasts compared with lymphoblasts. In addition, ATG itself may be able to target residual lymphoblasts, and therefore high exposure to ATG in ALL may have anti-leukemic effects itself. This may potentially result in neutralizing the beneficial effect of low ATG exposure after CBT on NRM. In vitro studies have shown tumor lysis by ATG on tumor cell lines.[13]

This study stresses the importance of early immune reconstitution to prevent relapse and NRM after CBT. Omission of serotherapy seems a feasible option in a CBT setting for malignancies. However, omission of serotherapy is associated with an increased incidence of GvHD.[14,5] Another strategy to prevent GvHD may be to optimize mycophenylate mofetil dosing. With recent understanding of the pharmacokinetics and pharmacodynamics of ATG in pediatric CBT,[3,15] individualized dosing (and timing) of ATG may yield benefits over omission of ATG.

On the basis of this study, designing a conditioning regimen that facilitates early CD4+ IR after CBT may optimize LFS in AML and reduce NRM for all CBT recipients. Moreover, early and predictable CD4+ IR opens the possibility for adjuvant immunotherapies, which depend on a functional adaptive immune system. All together, optimizing early immune reconstitution is likely to improve outcomes in patients receiving a CBT transplant for malignant indications.

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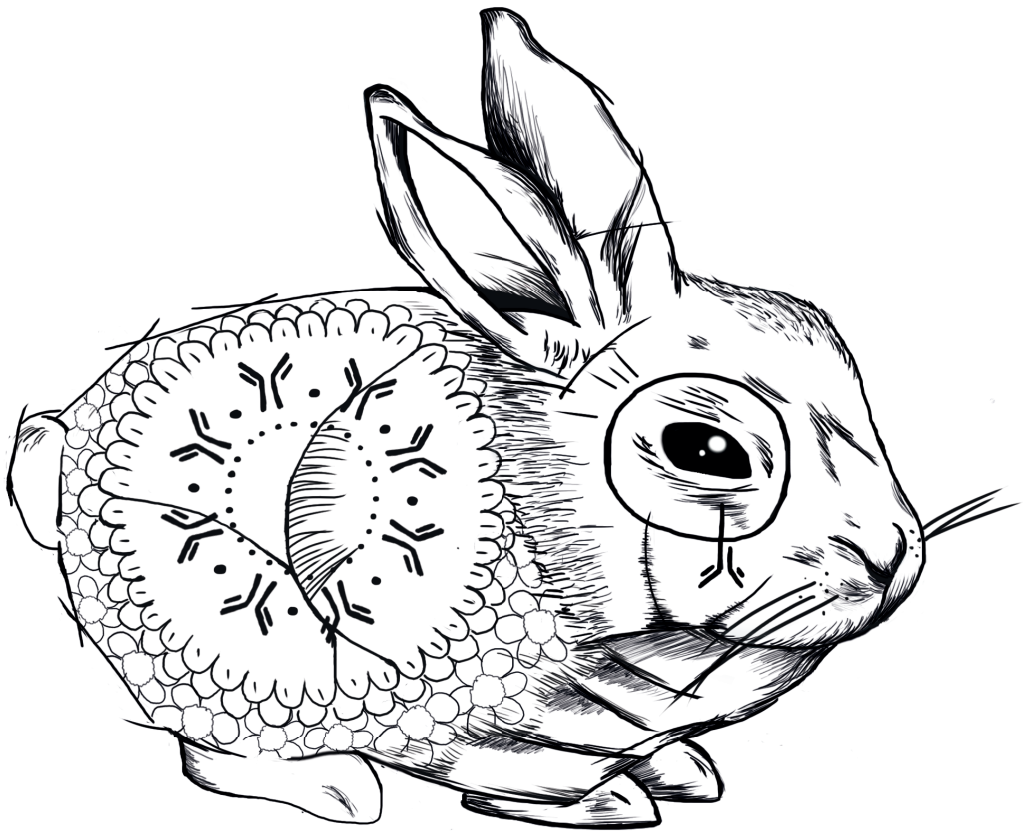
Chapter 10

Viral Reactivations and Associated Outcomes in Immune Reconstitution after Pediatric Hematopoietic Cell Transplantation

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ABSTRACT

Background

Viral reactivations (VR) following hematopoietic cell transplantation (HCT) contribute to significant morbidity and mortality. Timely immune reconstitution (IR) is suggested to prevent VR. We studied the relation between IR (as a continuous over-time-predictor) and VR (as time-varying-predictor), and the relation between VR and other clinical outcomes.

Patients and Methods

In this retrospective analysis, all patients receiving a first HCT between January-2004 and September-2014 were included. IR (CD3/CD4/CD8 T-cells, NK- and B-cells) was measured bi-weekly until 12 weeks, and monthly thereafter. Main outcomes of interest were VR of adenovirus (AdV), Epstein-Barr-virus (EBV), human-herpesvirus 6 (HHV6), cytomegalo-virus (CMV), and BK-virus, screened weekly. Clinical outcomes included overall-survival (OS), event-free-survival, non-relapse-mortality (NRM), and graft-versus-host-disease (GvHD). Cox-proportional-hazard- and Fine-Gray-competing-risk-models were used.

Results

273 patients (0.1-22.7 years; median follow-up 58 months) were included. CD4-reconstitution predicted reactivation of AdV (HR 0.995; p=0.022), EBV (HR 0.994, p=0.029), and HHV6 (HR 0.991, p=0.012), but not CMV (p=0.31) and BK (p=0.27). Duration of AdV-reactivation was shorter with timely CD4-reconstitution, defined as $\geq50*10^6$ cells/L within 100-days. AdV-reactivation predicted lower OS (HR 2.17, p=0.0039) and higher NRM (HR 2.96, p=0.0008). Concomitant CD4-reconstitution abolished this negative effect of AdV-reactivation: OS (p=0.67) and NRM (p=0.64). EBV- and HHV6-reactivations were predictors for occurrence of GvHD, while CMV- and BK-reactivations did not predict clinical outcomes.

Conclusion

These results stress the importance of timely CD4-reconstitution. Strategies to improve CD4-reconstitution may improve HCT-outcomes, including survival, and reduce the need for toxic anti-viral therapies.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative therapy for various hematological malignancies and benign disorders in children. The success of HCT is hampered by relapse of malignancy and transplantation related mortality due to HCT-associated complications, which include opportunistic infections (e.g. viral reactivations; VR) and graft-versus-host disease (GvHD). The majority of these limitations are due to absent or delayed T-cell reconstitution.[1,2]

Early CD4+ T-cell reconstitution in particular is suggested to be important for better survival chances after HCT.[2–7] Delayed immune reconstitution (IR) after HCT was previously found to be a predictor for VR,[7] which may subsequently contribute to acute GvHD (aGvHD), graft failure (GF), and increased mortality.[8] Early and improved prediction of HCT-related complications provides the opportunity for earlier initiation of pre-emptive treatment, which subsequently could improve survival chances.

Although VR and IR are related and are both associated with clinical outcome,[7–10] their joint time-dependent relation as predictors for clinical outcome remains to be studied. Most studies investigating the role of IR-markers as a predictor for VR only include very limited measurements at relative late time-points after HCT. As most VRs occur early after HCT, this is not optimal. Early IR-markers assessed as a continuous value over time may provide better predictors, which can also be used in clinical practice for decision-making. The identification of early IR-related predictors may guide us in the initiation of anti-viral therapies or targeted anti-viral cellular therapies.

We aimed to assess the relation between IR and VR and other clinical outcomes. To achieve this we did a large retrospective cohort analysis in which clinical outcomes, such as survival, GvHD, and GF, were related to VR. In this analysis, VR was uniquely evaluated as a time-varying predictor. Additionally, this is the first study that related various immune reconstitution markers as continuous over-time variables to viral reactivations. This enabled us to determine the effect of immune reconstitution on VR-associated clinical outcomes.

PATIENTS AND METHODS

Study design and patients

In this analysis we included pediatric patients receiving an allogeneic HCT, between January 2004 and September 2014 at the University Medical Centre in Utrecht, The Netherlands. All consecutive patients undergoing their first transplantation were included. The minimum

follow-up for surviving patients was 6 months, and data were collected and registered prospectively. Patients were enrolled and data were collected only after written informed consent in accordance with the Helsinki Declaration. The study was approved by the local ethical committee (trial numbers 05-143 and 11-063k).

Procedures

Conditioning regimens were applied according to (inter-)national protocols. For myeloablative busulfan-containing protocols (administered intravenously) therapeutic drug monitoring was used to aim for an area under the curve (AUC) of 75-95 mg*h/L. Anti-thymocyte globulin (ATG; Thymoglobulin) was given at a dose of 10 mg/kg starting 5 days before HCT from 2004 to 2010. From 2010 onwards, patients weighing over 40 kg received a lower dose of ATG (7.5 mg/kg). A 50% dose reduction was given when pre-conditioning lymphocyte counts were less than $300*10^6$ T-cells/L. Those receiving a cord blood transplant from 2009 on received ATG starting 9 days before HCT. Starting 2013, patients receiving a cord blood transplant for malignant indications did not receive ATG. Patients received gut decontamination and infection prophylaxis according to local protocols. GvHD-prophylaxis consisted of cyclosporin A (CsA; targeted at trough levels of 150-250 μg/L for all patients), combined with either prednisolone 1 mg/kg (cord blood) or methotrexate 10 mg/m^2 (on day +1, +3, and +6; unrelated donor). CsA was continued for at least 3 months or 6 months after HCT in patients with benign and malignant indication, respectively. Prednisolone was tapered after 28 days in benign disorders, and after engraftment in malignancies. Cord blood recipients were treated with filgrastim from day +7 after HCT until neutrophils were above 2000 cells/μL. Patients were treated in high-efficiency, particle-free, air-filtered, positive-pressure isolation rooms.

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) serostatus was assessed in all patients and donors prior to HCT. Following transplantation, all patients underwent weekly PCR viral screening for Human Herpesvirus 6 (HHV6), EBV, Adenovirus (AdV), CMV, and BK-virus irrespective of serostatus and clinical conditions. Viral reactivation, in which no distinction was made between reactivation or primo infection, was defined as having a viral load of >1000 copies/mL. From 2012 onwards, EBV and CMV were reported in IU/mL, for which the threshold was set at 5000 and 500 IU/mL, respectively, according the conversion factor in the same viral laboratory. Patients showing an EBV-, CMV- or AdV-reactivation received anti-viral treatment. Patients with HHV6-reactivation were treated only in high clinical suspicion of HHV6-associated disease (e.g. encephalitis, bone marrow suppression). Rituximab was used for treating EBV-reactivation; CMV was treated with either ganciclovir or foscarnet, HHV6 with foscarnet, and AdV-reactivation with cidofovir. Patients with BK-virus associated hemorrhagic cystitis received hyperhydration (3 L/m^2/day) as supportive care. Treatment for viral reactivations was stopped when viral load was

undetectable in two subsequent samples, or in case of severe toxicity after acquiring low viral loads (<1000 copies/mL).

After reaching a leukocyte count of at least $0.3*10^9$/L, absolute numbers of lymphocyte subsets, including overall T-cells (CD3+), T helper-cells (CD3+CD4+CD8-), cytotoxic T-cells (CD3+CD8+CD4-), B-cells (CD19+), and NK-cells (CD3-CD16+CD56+), were measured by flow cytometry at least every other week up to twelve weeks post-HCT and monthly thereafter up to six months post-HCT on EDTA-treated whole blood using TruCOUNT technology (BD Biosciences, Erembodegem, Belgium).

Outcomes

Main outcome of interest: Viral reactivations, of AdV, EBV, HHV6, CMV, and BK, defined as viraemia with >1000 copies/mL.

Other outcomes of interest: Overall survival (OS) was defined as time from transplantation to death or last follow-up. Event-free survival (EFS) was defined as time from HCT to last contact whereby graft-failure, relapse of disease, or death were regarded as events. All surviving patients were censored at date of last contact. Non-relapse mortality (NRM) was defined as death due to a cause other than relapse of malignancy. Relapse related mortality (RRM) was defined as death due to relapse of a malignancy. Acute- and chronic GvHD (aGvHD, cGvHD) were classified according to the Glucksberg[11] and Shulman[12] criteria, respectively. Graft failure was defined as non-engraftment (autologous reconstitution) or graft rejection (secondary loss of donor chimerism). In case of non-engraftment, the assessment date was +60 days after HCT. Interstitial pulmonary syndrome (IPS) and bronchitis obliterans (BO) were defined according to international accepted criteria as previously described.[13]

Statistical analysis

Duration of the follow-up was defined as the time from HCT to the last assessment for surviving patients or death. Actual cell counts for CD3+, CD4+ and CD8+ T-cells, as well as NK- and B-cells, considered as continuous values over time, were evaluated as predictors for viral reactivation. For the clinical endpoints, viral reactivations of AdV, EBV, HHV6, CMV, and BK were evaluated as predictors. Additionally, reconstitution of CD4+ T-cells was assessed as a co-predictor for clinical outcome. This was chosen in line with previous findings.[2,10,14,15] CD4+ T-cell reconstitution (CD4+ IR) was defined as reaching $\geq 50*10^6$ CD3+CD4+ cells/L in 2 consecutive measurements within 100 days post-HCT, and considered as time-varying covariate, in accordance with previous publications.[14] In multivariate analysis, predictors considered for outcome were patient-related variables (age at transplant, gender, CMV and EBV serostatus), disease (malignant/primary immune deficiencies; PID/bone marrow failure/benign non-PID), donor factors (HLA disparity, CMV and EBV serostatus), and conditioning regimen (myeloablative or reduced intensity conditioning).

As immune suppression was similar for all patients during the first 3 months after HCT, this was not considered as a multivariate predictor. Variables associated with a p-value <0.05 by univariate analysis were selected for testing in a multivariate analysis. Probabilities of events were calculated using the Kaplan-Meier estimate; the two-sided log-rank test was used for univariate comparisons. Time-dependent outcomes were analyzed using Cox proportional hazard models. For the endpoints NRM, RRM, aGvHD, cGvHD, and GF, Fine-Gray competing risk regressions were used. Here, competing events were RRM, NRM, death not due to aGvHD or cGvHD, and death not due to GF, respectively. Statistical analyses were performed using R 3.0.1 using the packages survival and cmprsk.

Role of the Funding Source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. RA and JJB had full access to all the data in the study and RA, CdK, and JJB had final responsibility for the decision to submit for publication.

RESULTS

A total of 273 patients were included with a median age of 8.4 years (range 0.1-22.7). Patient characteristics are summarized in Table 1. Busulfan combined with fludarabine and ATG (Thymoglobulin) was the most frequently used conditioning regimen. Cord blood (52%, n=142) and bone marrow (45%, n=123) were the most frequently used cell sources. Median time to follow-up was 58 months (range 0.2-130). Cumulative incidence of viral reactivations was 27%, 18%, 15%, 13% and 11% for HHV6, CMV, AdV, BK and EBV, respectively (Fig S2).

We only identified CD4+ IR as a predictor for VR. No associations between CD3+, CD8+ T-cells, NK-, or B-cell IR and VR were found (Supplemental Table S1). In AdV, the chance on reactivation is reduced 5% with every increase of 10/μl CD4+ T-cell counts (hazard ratio [HR] 0.995, 95% confidence interval [CI]: 0.99-0.999, p=0.022; Table 2). CD4+ IR similarly predicted EBV (HR 0.994, 95% CI: 0.988-0.999, p=0.029), and HHV6 (HR 0.991, 95% CI: 0.985-0.998, p=0.012). No relation with CMV-reactivation (p=0.31) and BK-reactivation (p=0.27) was identified.

Interestingly, patients with successful early CD4+ IR had shorter treatment duration of AdV (Figure 1). The median treatment duration of was only 14 days for patients with timely CD4+ IR, compared to 47.5 days for patients without recovery of CD4+ IR (p=0.011). Early CD4+ IR did not influence the duration of CMV, EBV and HHV6 (Supplemental Figure S3). Timely CD4+ IR, however, did also not affect duration of treatment for CMV (p=0.29; Supplemental Figure S3a).

	All patients
Number of patients (n)	273
Male sex (%)	59
Age at transplant (years)	8.4 (0.1-22.7)
Recipient/Donor EBV Serostatus	
-/-	19
+/-	60
-/+	3
+/+	18
Recipient/Donor CMV Serostatus	
-/-	37
+/-	48
-/+	3
+/+	12
Diagnosis (%)	
Malignancy	53
Primary immune deficiency	12
Bone marrow failure	16
Benign non-PID	18
Stem cell source (%)	
Bone Marrow	45
Cord Blood	52
Peripheral blood stem cells	3
Conditioning regimen (%)	
Busulfan based	83
TBI based	17
Patients receiving serotherapy (%)	71
Follow-up (months)	58 (0.2-130)

Table 1. patient characteristics

We next investigated the effects of viral reactivations on clinical outcome parameters. In this analysis, patients were considered as having a VR only from the day of reaching >1000 viral copies/mL in plasma, giving a robust estimation of the hazard associated with the different VR's (Figure S1). Here, we identified AdV, EBV, and HHV6 as VR-predictors for various clinical outcomes (Table 3). Reactivation of AdV was a predictor for lower OS (HR 2.17, 95% CI 1.28-3.68, p=0.0039; Table 3, Figure 2A) and EFS (HR 1.77, 95% CI 1.05-2.99, p=0.032; Table 3, Figure 2C) chances compared to patients not experiencing an AdV-reactivation (HR 2.17, 95% CI 1.28-3.68, p=0.0039; Table 3, Figure 2A). We did not find any VR associated with RRM (Table S4). NRM was associated with both AdV- (HR 2.95, 95% CI 1.57-5.57, p=0.0008; Table 3, Figure 2E) and EBV-reactivations (HR 2.03, 95% CI 1.01-4.11, p=0.049, respectively; Figure S4A).

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Endpoint	HR	95% confidence interval	p-value	Significance level
CMV-reactivation				
CD4+ T-cell counts (continuous over time)	0.998	0.993 – 1.002	0.312	
HHV6-reactivation				
CD4+ T-cell counts (continuous over time)	0.991	0.985 – 0.998	0.012	*
BK-virus-reactivation				
CD4+ T-cell counts (continuous over time)	0.996	0.99 – 1.003	0.271	
AdV-reactivation				
CD4+ T-cell counts (continuous over time)	0.995	0.99 – 0.999	0.022	*
EBV-reactivation				
CD4+ T-cell counts (continuous over time)	0.994	0.988 – 0.999	0.029	*

Table 2. Multivariate analysis of CD4+ IR versus VR. IR was considered as a continuous time-varying predictor. VR was defined as having a viral load >1000 copies/mL in plasma. * Indicates p<0·05. HR are presented as the hazard ratio for each point increase in CD4+ T-cell counts.

As early CD4+ IR predicted both the incidence and duration of VR, we subsequently investigated whether CD4+ IR influenced the negative effect of VR on clinical outcome. Here, we found that OS was similar in patients with AdV-reactivation and concurrent CD4+ IR compared to those patients without an AdV-reactivation (68±3% vs 66±12%, p= 0.67; Figure 2B). Patients with AdV-reactivation without CD4+ IR performed considerably worse (OS of 32±10; p=0.0045 in MV analysis for CD4+ IR; Table 3). For EFS and NRM, we found comparable results: patients with AdV-reactivation without CD4+ IR had lower EFS (63% and 62% versus 32% for AdV-, AdV+/IR+, and AdV+/IR-, respectively; p=0.0021 in MV analysis for CD4+ IR; Table 3) and higher NRM (18% and 28% versus 54% for AdV-, AdV+/IR+, and AdV+/IR-, respectively; p=0.0005 in MV analysis for CD4+ IR; Table 3, Figure S4B) compared to those with CD4+ IR in presence or absence of AdV.

While AdV was associated with all survival-related parameters, HHV6 and EBV were predictors for the occurrence of GvHD. HHV6 was found to be a predictor for a higher chance on aGvHD grade 2-4 (HR 3.47, 95% CI 2.11-5.7, p<0.0001; Table 3, Figure S5A). Diagnosis was an additional multivariate predictor with bone marrow failure and primary immune deficiencies having the lowest chance on grade 2-4 aGvHD (Table S3). For grade 3-4 aGvHD, both HHV6 (HR 2.74, 95% CI 1.22-6.15, p=0.015; Table 3, Figure S5B) and EBV (HR 4.8, 95% CI 1.31-17.62, p=0.018; Table 3, Figure S5C) were predictors, with age

being an additional predictor (Table S3). Extensive chronic GvHD (cGvHD) probability was predicted by EBV-reactivation (HR 3.61, 95% CI 1.13-11.53, p=0.03; Table 3, Figure S5D). Receiving cord blood as cell source was an additional predictor for lower chance on cGvHD (Table S3). For the outcomes GF, lung injury, IPS, and BO, we did not identify any VR-predictors.
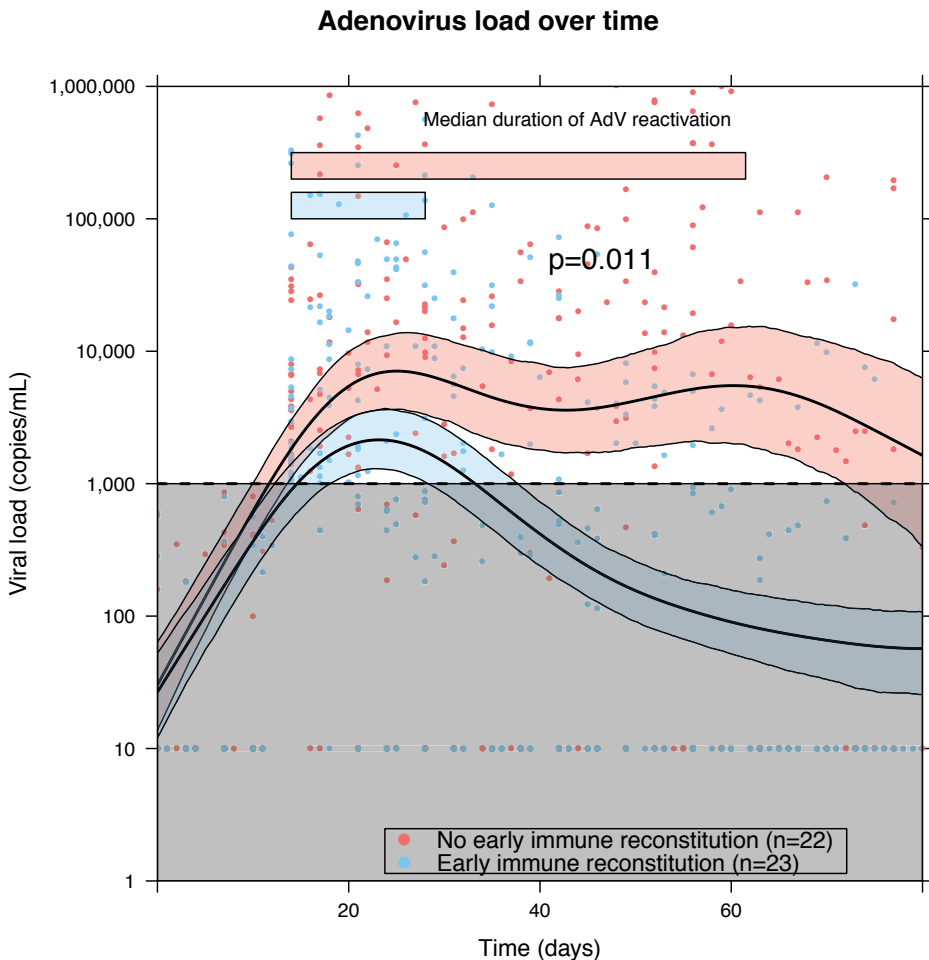
Figure 1. Viral load in patients with AdV reactivation, according to early reconstitution of CD4+ T- cells. Spline regression analysis (5 degrees of freedom) of AdV load (bold solid lines with 95% CI as shaded area) over time (normalized to 14 days before reaching a viral load of 1000) in patients with AdV-reactivation, with (blue area/dot/bar) or without (red are/dot/bar) early CD4+ T-cell reconstitution, showing longer AdV-reactivation and higher AdV loads in those without early reconstitution. Median duration of AdV-treatment was 14 days for patients with early immune reconstitution, and 47.5 days for the no early immune reconstitution group. Dots: individual raw AdV load. Bars: median duration of AdV treatment. Grey background: clinically insignificant viral load.

OS, EFS and NRM according to Adenovirus Reactivations
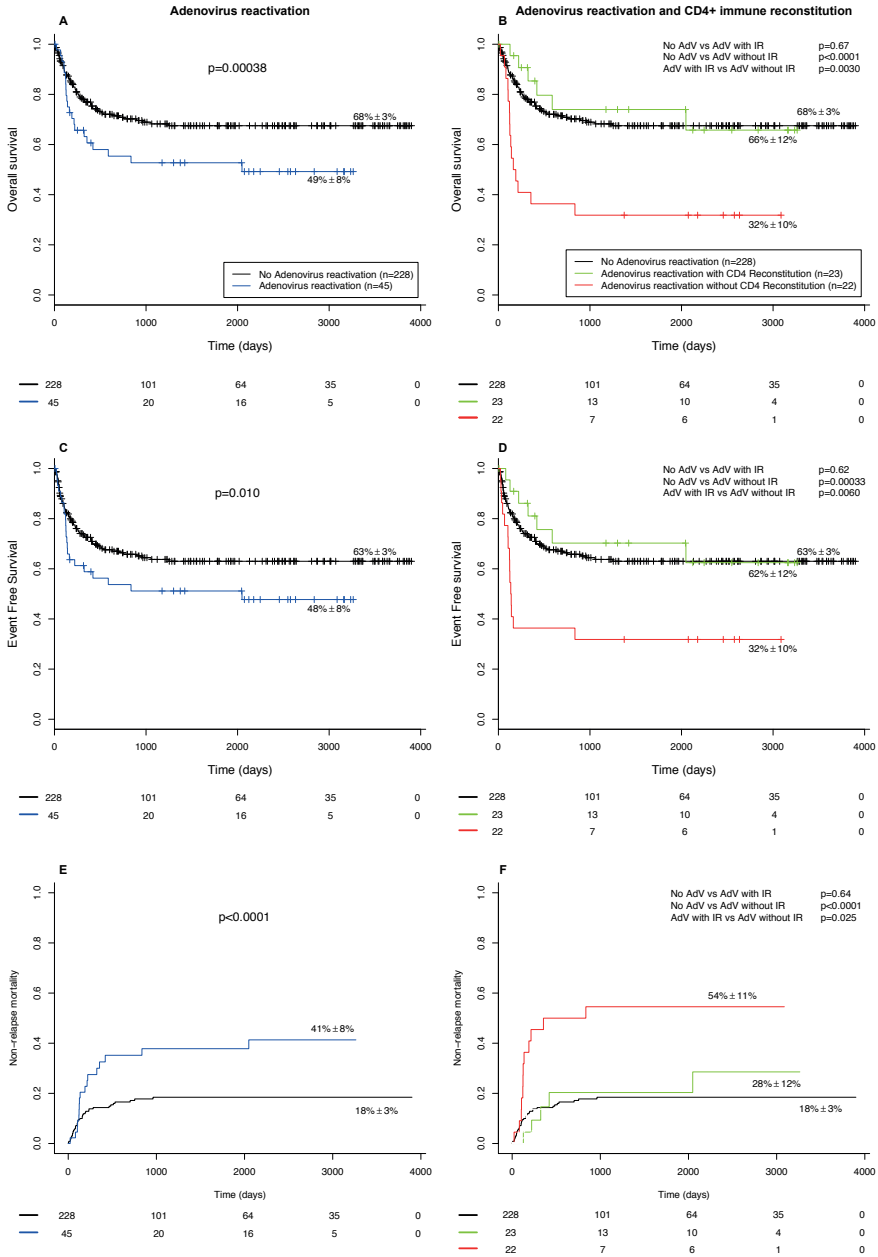
Figure 2. Clinical outcome according to viral reactivation and immune reconstitution. Plots on the left show the incidence of A: overall survival (OS), C: Event Free Survival (EFS), and E: Non-relapse Mortality (NRM), in patients without AdV-reactivation (black lines) versus patients with AdV- reactivation (blue lines). Viral reactivation was defined as having a viral load >1000 copies/mL. Plots on the right show the effect of AdV and CD4+ T-cell reconstitution on the incidence of B: OS, D: EFS, and F: NRM, in patients without AdV-reactivation (black lines) versus patients with AdV-reactivation; subdivided into patients having CD4+ T-cell reconstitution (green) versus patients not having CD4+ T-cell reconstitution (red)

Variable	Univariate	Multivariate			
	p-value	HR	95% CI	p-value	
Overall Survival					
AdV-reactivation >1000 copies/mL	0.00038	2.173	(1.282-3.684)	0.0039	**
Early CD4+ T-cell reconstitution	0.002	0.52	(0.332-0.816)	0.0045	**
Event Free Survival					
AdV-reactivation >1000 copies/mL	0.0101	1.771	(1.05-2.988)	0.032	*
Early CD4+ T-cell reconstitution	0.00079	0.515	(0.337-0.786)	0.0021	**
Non-Relapse Mortality					
AdV-reactivation >1000 copies/mL	<0.0001	2.955	(1.567-5.571)	0.0008	***
EBV-reactivation >1000 copies/mL	0.0013	2.028	(1.001-4.109)	0.049	*
Early CD4+ T-cell reconstitution	0.00017	0.344	(0.189-0.629)	0.0005	***
Grade 2-4 acute GvHD					
HHV6-reactivation >1000 copies/mL	<0.0001	3.472	(2.113-5.704)	<0.0001	****
Grade 3-4 acute GvHD					
EBV-reactivation >1000 copies/mL	0.0085	4.799	(1.307-17.616)	0.018	*
HHV6-reactivation >1000 copies/mL	0.014	2.74	(1.22-6.155)	0.015	*
Extensive chronic GvHD					
EBV-reactivation >1000 copies/mL	0.0298	3.611	(1.131-11.534)	0.03	*

Table 3. Multivariate analysis of VR and CD4+ IR versus outcome. VR and IR were considered as time varying predictors. VR was defined as having a viral load >1000 copies/mL in plasma. * Indicates p<0.05, ** p<0.01, *** p<0.001, and ****p<0.0001.

DISCUSSION

To our knowledge this is the first study to investigate the relations between various immune reconstitution markers, occurrence and duration of VR, and clinical outcome in a large pediatric cohort. Here, we uniquely considered all predictors in a time-dependent manner, making the findings more robust. With the limitations of a retrospective cohort study taken into account, our data show that from all evaluated immunological markers, timely CD4+ T-cell reconstitution predicted reactivations of AdV, EBV, and HHV6 best. Furthermore, AdV predicted lower OS and EFS, AdV and EBV predicted higher NRM, while EBV- and HHV6-reactivation predicted GvHD. Importantly, early CD4+ IR completely abolished the adverse effect of VR on survival parameters. Moreover, CD4+ IR did not influence reactivations of CMV and BK, nor were these VR's predictive for clinical outcome.

Our finding that absent of timely CD4+ IR predicts VR is in line with previous findings.[2–7,14] A previously suggested relation between CD8+ T-cell recovery and VR could not be identified[16], nor could a correlation between T-cell recovery and CMV-reactivation be confirmed.[7,15] These discrepancies may be due to the fact that we considered IR as a

continuous, time-dependent variable as early as 2 weeks after HCT, rather than binary and time-independent at certain time-points as done in most previous analyses. By taking time-dependency into account, a more precise insight into the predictive value of the various the various immune-markers will be obtained. Nonetheless, in the implemented models, an effect of viraemia on CD4+ T-cell counts is not accounted for, although most patients do not show an increase in CD4+ count after VR.

Considering VR as predictors for clinical outcome, our findings are in partly line with other studies showing that AdV-reactivation is associated with lower survival,[17] EBV is associated with lower survival and GvHD,[18] and HHV6-reactivation is associated with a higher risk for aGvHD in myeloablative HCT recipients.[19,20] Some conflicting data exist as well, since others did find a relation between HHV6 and higher mortality rates,[20,21] or between HHV6- or EBV-reactivation and aGvHD.[22,23] These discrepancies may most likely be explained by differences in treatment protocols, age, or VR definition. Again here, considering the time-dependency of the variables may also have had a significant influence the found associations.

With respect to CMV-reactivation in relation to clinical outcome, we did not find any association, while CMV has previously been associated with the occurrence of aGvHD and lower survival.[8,15] This may be due to the pre-emptive treatment in our cohort controlling the negative effects that are associated with CMV. However, most available data on the impact of CMV-reactivations is acquired through retrospective multi-center registry studies, where CMV monitoring may differ between the centers: e.g. when CMV-load is only measured when suspicion of CMV-disease, there may be an under-reporting of actual CMV reactivations. While a significant effect of CMV on survival was shown in large registry studies, the absolute effect size, and thereby the clinical relevance, was relatively small (difference of 1.8% in OS and ~5% in NRM in studies by EBMT,[24] and CIBMTR,[25] respectively. Also, BK-reactivation was found not to predict any of the clinical outcome parameters assessed in this study, however we did not consider hemorrhagic cystitis as an outcome measure. Regardless of the fact that BK associated cystitis can be a very painful and a long lasting complication requiring prolonged hospitalization,[26] according to our findings it does not affect survival or any other clinical outcome analyzed after HCT.

Data from this study imply that anti-viral therapies to increase survival chances in case of VR might not be needed in patients with timely CD4+ IR, especially taking toxicities of anti-viral drugs (e.g. nephrotoxicity, bone marrow suppression) into account. Thus, anti-viral treatment may be delayed and possibly omitted in cases with sufficient CD4+ T-cell recovery at the time of reactivation. Early monitoring of CD4+ IR can be an important tool

to identify patients at risk. The effect and safety of omitting anti-viral therapy in patients with CD4+ IR should, however, be carefully evaluated first.

Although this association between absence of CD4+ IR and lower survival was described previously,[1,3,5,6] it has never been shown before that CD4+ IR impacts VR-associated mortality. Therefore, an important strategy to prevent mortality associated with VR would be to target for a predictable and certain early CD4+ IR after HCT. As recent findings by our group suggest that high exposure of ATG after HCT significantly delays CD4+ IR,[14] individualized ATG dosing regimens, aiming at optimal exposure, may fasten CD4+ IR, and subsequently enhance CD4+ T-cell predictability. Other strategies that might stimulate CD4+ IR are currently being tested in clinical settings; e.g. sex hormone inhibitors,[27] low-dose interleukin-2 treatment,[28] keratinocyte growth factor,[29] and Thymosin alpha 1 treatment.[30] The effects of these treatment options on early CD4+ T-cell recovery, however, remain to be explored.

In conclusion, the results obtained by this study stress the importance of having timely CD4+ IR, and provide insight in morbidities and mortality associated with developing a viral reactivation. Strategies to better predict CD4+ IR are of utmost importance to improve survival chances after HCT. These novel strategies should, preferably, be tested in the context of harmonized clinical trial design and standardized immuno-monitoring to better compare different strategies, as recently reviewed.[31] Moreover, identifying patients with early CD4+ IR, and thus at lower risk for viral reactivation-related morbidity and mortality, may limit the need for toxic anti-viral drugs. On the other hand, the identification of at risk patients with a delayed CD4+ IR provides the opportunity to pre-emptively intervene with anti-viral (cell) therapies. Altogether, finding strategies that lead to a better predictable CD4+ T-cell reconstitution and subsequently the prevention of viral reactivation may lead to lower morbidity and better survival chances for HCT patients.

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10

PART V

Clinical Implementation of
Individualized Dosing in HCT

Chapter 11

Individualized Conditioning Regimens in Cord Blood Transplantation: Towards Improved and Predictable Safety and Efficacy

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ABSTRACT

Introduction
The conditioning regimen used in cord blood transplantation (CBT) may significantly impact the outcomes. Variable pharmacokinetics (PK) of drugs used may further influence outcome. Individualized dosing takes inter-patient differences in PK into account, tailoring drug dose for each individual patient in order to reach optimal exposure. Dose individualization may result in a better predictable regimen in terms of safety and efficacy, including timely T cell reconstitution, which may result in improved survival chances.

Areas Covered
Conditioning regimens used in CBT varies significantly between and within centers. For busulfan, individualized dosing with therapeutic drug monitoring has resulted in better outcomes. Anti- thymocyte globulin (ATG), used to prevent rejection and GvHD, significantly hampers early T-cell reconstitution (IR). Timely IR is crucial in preventing viral reactivations and relapse. By individualizing ATG, IR is better predicted and may prevent morbidity and mortality. Expert

Opinion
Individualization of agents used in the conditioning regimen in CBT has proven its added value. Further fine-tuning, including new drugs and/or comprehensive models for all drugs, may result in better predictable conditioning regimens. A predictable conditioning regimen is also of interest/importance when studying adjuvant therapies, including immunotherapies (e.g. cellular vaccines or engineered T-cell) in a harmonized clinical trial design setting.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) can be a potentially curative treatment option for a variety of diseases including leukemia, bone marrow failure (BMF), inborn errors of metabolism (IEM), and primary immune deficiencies (PID). Its success is limited by complications like graft-versus-host- disease (GvHD), rejection, relapse, and (viral) infections. These complications can be serious threats to patients undergoing HCT, hampering survival chances. Fortunately, over the last decades, many steps have been made toward reducing mortality and morbidity; better HLA-typing, centralized care, monitoring for infectious complications, and new agents in conditioning regimens and GvHD-prevention became avail- able. Furthermore, the use of alternative stem-cell sources such as unrelated (mismatched) donors, haplo-identical donors, and cord blood (CB) donors made HCT available for almost all patients. As all these sources contain different number and phenotypes of cells the optimal conditioning regiment may be different to enable the best effect for each source of cells. Over the last decade, individualized dosing and therapeutic drug monitoring (TDM) has been introduced in the field of HCT aiming to optimize the outcomes.

Differences in pharmacokinetics (PK) of agents, which are part of the conditioning, can be associated with variable myeloablation and immune suppression before and after CBT resulting in different outcomes. By relating the exposure of these agents to clinical outcomes, the most optimal exposures of each drug can be determined. Individualized dosing takes inter-patient differences into account and tailors the drug dose for each individual patient to optimal exposures. This may mediate a predictable regimen in terms of safety and efficacy. Also, immune reconstitution (IR), crucial in preventing relapse and viral reactivation may be better predictable. Such a better predictable regimen may subsequently result in improved survival chances. In the current paper, we reviewed literature on individualized conditioning regimens in umbilical cord blood transplantation (CBT) aiming for better predictable regimens in terms of safety and efficacy, including IR, to optimize the survival chances after CBT.

Umbilical CBT

Umbilical CB was first introduced as an alternative stem-cell source in allogeneic HCT by Gluckman et al. treating a patient with Fanconi anemia.[1] In the following decades, the use of CB has significantly increased and is currently considered a good alternative cell source when suitable HLA-identical related and unrelated donors are lacking.

Compared to the more traditional cell sources such as bone marrow (BM) or peripheral blood stem cells (PBSC), the advantages of using CBT include the reduced incidence of

GvHD,[2] while maintaining anti-leukemic effect.[3–6] Due to banking and HLA-matching before storage, CB is promptly available donor, and less stringent HLA-matching criteria apply, resulting in suitable donors for most patients.[7] Disadvantages include the lower nucleated cell- and CD34+ cell dose/kg, which may lead to prolonged neutropenia (and associated problems, including higher probability on graft failure), and the higher costs of a CB unit compared to other stem-cell sources. Moreover, early T-cell reconstitution (i.e. within 3–12 months after transplantation) is suggested to be slower in UCB when compared to BM or PBSC,[8–11] leading to an increased incidence of viral reactivations associated with increased transplant-related mortality. Despite this higher TRM in some reports, interestingly the probability on overall and leukemia-free survival was similar to BM and PBSC, suggesting a stronger graft-versus-leukemia effect.[12–14]

Clinical results: CBT versus other stem cell sources

Several studies have compared CBT to other donor cell sources for patients with hematological malignancies and benign disorders. Unfortunately, well-designed randomized controlled studies are lacking. Despite this, there is growing evidence of comparable results for patients with malignant and benign disorders.

In benign disorders, the use of CBT is increasing, and in some diseases such as inborn errors of metabolism (IEM) and primary immune deficiencies (PID) is even becoming the preferred HCT source. CBT allows for faster availability, which is of utmost importance for certain life-threatening rapidly progressive dis- eases (e.g. PID, metabolic disorders) and avoids invasive procedures for donors. In Hurler's disease (MPS-1), Boelens et al. showed that patients receiving a CBT, matched sibling donor (MSD) or matched unrelated donor (MUD) transplant had similar event-free survival (EFS) probabilities.[15] While EFS was lower in 4/6 HLA-matched CBT with low cell dose, those with high cell dose had similar EFS probability as either 5 or 6/6 matched units. Those with a mismatched MUD had inferior survival probability. Interestingly, full donor chimerism and normal enzyme levels after HCT were significantly more frequent in patients with CBT, despite similar busulfan (Bu)-based myeloablative conditioning. For PID (severe combined immune deficiency), Pai et al. reported on a cohort of children receiving a HCT.[16] Survival was highest among those with a MSD, while other stem-cell sources including CBT perform comparably.

For patients with hematological malignances both in a myeloablative and non-myeloablative setting, the use of CBT is a valuable alternative source of HCT when a suitable donor is lacking, as several studies found similar probabilities on overall and leukemia-free survival.[2,12,14,17] In studies by Rocha, Laughlin, and Eapen, all comparing donor sources in patients with leukemia, relapse rates in CBT were lower or comparable to BM and PBSC. Transplant-related mortality in the more historical studies was higher after CBT, which

partly may be associated to lower cell dosed CB units as this appears to be a predictor for worse clinical outcomes.[2,18] An interesting and important observation in these cells source comparison studies are that lower chronic GvHD is reported in the CB group. This is important, as the complication is associated with substantial lower quality of life. Nowadays, some centers regard CB to be the preferred cell source for patients at high risk of relapse with or without minimal residual disease pre-transplant.[19] This is supported by the lower probability of relapse in majority of studies, even in very high-risk, MRD-positive patients.[2,17] The less stringent HLA-matching resulting in more HLA-disparity[20] may explain this lower probability of relapse observed in CBT when compared to MUD and mismatched unrelated donors (MMUD). Also, a recent report showed that CB T cells mediate stronger anti-leukemic activity compared to adult cells.[13] In addition, novel donor CB selection strategies based on non-maternal inherited antigen (NIMA)[21] and/or numbers of predicted indirectly recognizable HLA epitopes (PIRCHES)[22] may further reduce the risk of relapse after CBT. Over the last years haplo-identical transplantation has become a popular and cheaper alternative cell source for patients lacking identical donors. Especially, the post-HCT cyclophosphamide (Cy) strategy has become the alternative option of interest for some centers. The early outcomes look promising but longer-term follow-up is needed as well as comparison of early and late outcomes to other cell sources including CB. This should also include chronic GvHD-free-EFS comparisons.

In summary, these landmark papers reviewed above showed that CBT can be considered as an alternative cell source with similar estimated survival chances as HLA-identical related and unrelated donors, for benign disorders and malignant indications for HCT. Important to consider is that most studies included historical patients (treated >10 years ago), which may have influenced the outcomes. Cell-dose selection criteria have improved outcome and HLA-typing on high resolution, as well as selection for preferred mismatches (e.g. NIMA, PIRCHES), may improve the outcomes of CBT as well. Moreover, further improvement may be achieved by improving lymphocyte reconstitution, in particular T-cell reconstitution.

Unmet needs in CBT

The unmet needs in CBT, and likewise in HCT in general, include reduction of the short- (e.g. viral disease, GvHD) and long-term toxicity (including chronic GvHD) of the procedure as well as getting better disease control (efficacy). Most safety and efficacy problems are associated with absence of timely and balanced IR (neutrophils and T-cell reconstitution), which may even be more hampered after CBT; therefore, strategies aiming for predictable T-cell IR may result in better survival chances. This may be achieved by individualization of treatment, making a transformation from one-size-fits-all to tailored, individualized transplantation.

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Neutrophil engraftment

Historically, engraftment was a major drawback of using CB as a stem-cell source. With lower numbers of infused cells, both total nucleated cells (TNC) as well as CD34+ cells, neutrophil engraftment was slower compared to BM or PBSC.[2,12,14,17] Advantages have been made in improving engraftment, including a larger inventory of stored CB units. Nowadays, the stored units contain higher numbers of TNC (and CD34+). Additionally, stimulation of neutrophil proliferation with granulocyte colony-stimulating factor is routinely used following CBT. These interventions have improved neutrophil engraftment following CBT when compared to BM or PBSC. In more recent studies, where a lower limit of acceptable cell dose/kg was taken into account, CBT performs comparably to other sources in terms of engraftment, especially in children.[15,16]

If a single unit is not containing sufficient number of cells, a strategy to overcome the limiting cell dose is infusion of 2 CB units or combining a single CB unit with other cell source graft: e.g. CD34+ selected haplo-identical cells (haplo-CB transplantation). These strategies are mainly applied in the adult transplantation setting, where body weights are generally higher compared to children. Both strategies rely on a higher number of infused TNC and/or CD34+ to ensure early engraftment from one winning units (double cord) or initially from the haplo-donor (haplo-cord), followed by gradually increasing single cord-blood donor chimerism. In pediatrics and young adults, the use of double CB transplant gave equal survival chances between single (with sufficiently high cell dose) and double-cord transplants with a higher probability on acute GvHD 3–4 and chronic GvHD in the double cord group.[23] Using these strategies, steps have been made to make CBT safer, aiming for similar survival chances as after related or unrelated donor transplantation.[23–30] More recently, strategies such as the use of ex-vivo-expanded CD34+ cells have led to a significant improvement in time to neutrophil engraftment.[31,32] Various strategies have been used, such as Notch, StemRegenin- 1, and nicotinamide.[33–35] Currently, various clinical trials are running and phase III randomized controlled studies are planned.

T-cell IR

With temporary absent or delayed reconstitution of the humoral immunity not being a major obstacle in current clinical practice of CBT (also because this can be easily substituted), restoration of cellular immunity is a significant hurdle. Delayed reconstitution of lymphoid lineages, most importantly T cells, has been associated with inferior survival chances. Viral reactivations[36–38] and relapse of malignancy[9,39–41] both depend on adequate T-reconstitution and influences survival chances.[8,9,42]

In most literature, CBT has been associated with delayed or very poor T-cell IR.[9–11,43,44] Important to realize is that in these studies, anti-thymocyte globulin (ATG) was used

frequently, which is suggested to be the main predictor influencing early T-cell reconstitution. Many reports are available showing a relationship between dosage and timing of ATG and early T- cell IR.[9,37,42,45–47] Relatively low exposure of ATG to the graft already leads to a significant delay in T-cell IR while acute- GvHD was not influenced by exposure after CBT. This suggests that exposure after graft infusion should be avoided in CBT.[9] If a patient lacks early T-cell IR (no ability of peripheral expansion of the infused T cells), the IR needs to come from thymic output which can take at least 6–9 months, but more likely up to several years after transplant.[48] This leaves the patients vulnerable for viral reactivations and relapse.

More recently, several centers have omitted ATG (or other serotherapy) from the conditioning regimen in CBT. This strategy has been shown to be feasible in malignancies and is occasionally used in primary immune deficiencies. Results from these studies show excellent early T-cell IR, possibly even superior to BM or PBSC.[10,11,45,46] Hiwarkar et al. compared viral reactivations between cell sources: patients receiving CB without ATG in the conditioning showed lowest incidence of viral reactivation (cytomegalovirus, Epstein–Barr virus, adenovirus), even lower than BM without ATG.[36] Additionally, CB-derived T cells mediate a more powerful anti-leukemic effect[13] and tumor responses compared to adult T cells.[2,6,19] A drawback of this strategy may be an increased incidence of GvHD. This was described in other manuscript from same group, but this GvHD was mainly acute and not chronic and did not influence TRM.[46] Although ATG exposure after transplantation did not influence the probability of aGvHD (and significantly hampers CD4+ IR), sufficient exposure before transplantation was associated with lower probability on aGvHD.[9] Furthermore, not using in vivo T-cell depletion of the host may also be a problem in immune- competent recipients (e.g. BMF or IEM and hemoglobinopathies) in preventing rejection. Thus, by individualizing the dosing and timing of ATG before infusion of donor graft, the chances on aGvHD and rejection may be reduced, while timely T-cell IR is promoted resulting in better survival chances.

Currently used conditioning regimens and outcomes

In current daily practice, many conditioning regimens are used in allogeneic HCT, including CBT-setting. Most of the regimens used in CBT were simple copies from regimens used in transplantation using the conventional sources: related and unrelated BM or PBSC. The huge variety of regimens makes comparisons between centers, and even between stem cell sources, difficult as the conditioning regimen used can influence the outcomes. Furthermore, a conditioning regimen used for BM may not be optimal in CBT as the number of cells and the phenotype of cells is different. The currently used conditioning regimens and GvHD- prophylaxes in centers performing a substantial number of CBT's on a yearly basis are shown in Table 1.[9,11,15,37,41,49–59]

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Centre	Diagnosis				
	Adult Leukaemia	*Paediatric Malignancies*	PID	Inborn errors metabolism	GvHD prophylaxis
MSKCC New York, USA[50]	HD: TBI 1350, Cy, Flu **OR** Clo, Thio, Mel; ID: TBI 400, Cy, Flu, Thio; RIC: Mel, Flu; NMA: Cy, Flu, TBI 200	N/A	N/A	N/A	CsA, MMF
Great Ormond Street Hospital London, UK[38,42]	N/A	TBI 1200, VP16; Bu, Cy, Mel; Treo, Flu	Treo, Flu	Flu, Bu	CsA, MMF
Hospital Universitario La Fe Valencia, Spain[51]	Bu, Thio, Cy, ATG; Bu, Thio, Flu, ATG	N/A	N/A	N/A	CsA, MMF OR CsA pred
University of Minnesota, USA Minnesota[52]	MAC: Flu, Cy, TBI 1320; RIC: Flu, Cy, TBI 200	Flu, Cy, TBI 1300	N/A	Cy, TBI	CsA, MMF
Royal Children's Hospital Manchester, UK[15]	N/A	Bu, Cy, Mel; Bu, Cy	Treo, Flu; Flu, Bu	Bu, Flu, ATG	CsA, pred
Fred Hutchinson Cancer Center Seattle, USA[53]	Cy, Flu, TBI 200	Bu, Cy; TBI, Cy	?	?	??
University Medical Center Utrecht, the Netherlands[9,42]	Cy, Flu, TBI 400	Bu, Flu, (Clo)	Bu, Flu, ATG	Bu, Flu, ATG	CsA, Pred
Duke University Durham, USA[11,15,55]	TBI1320, Flu + (Cy or Thio)	Bu, Cy, Flu; TBI-base	Flu, Mel, Thio, Camp	Bu, Cy, ATG	CsA, MMF (since 2006, before CsA, Pred)
MD Anderson/Texas Children's Hospital, Houston, USA	Bu, Clo, Thio; Flu, Bu, Clo, TBI 200	?			
COBLT study Varying sites, USA[56-58,60]	Bu, Mel, ATG **OR** TBI 1350, Cy, ATG	TBI 1350, Cy, eATG; Infant: Bu, Mel, ATG		Bu, Cy, ATG	CsA, pred

Table 1. Currently used conditioning regimens in HCT centers performing large numbers of cord blood transplantation. MSKCC: Memorial Sloan Kettering Cancer Center; USA, United States of America; UK: United Kingdom; HD: high dose; TBI: total body irradiation; Cy: cyclopho- sphamide; Flu: fludarabine; Clo: clofarabine; Thio: thiotepa; Bu: busulfan; Mel: melphalan; ID: intermediate dose; RIC: reduced intensity conditioning; NMA: Cy, Flu, TBI 200; ATG: antithymocyte globulin; MAC: myeloablative conditioning; RIC: reduced intensity conditioning; VP16, etoposide; Treo, treosulfan; CsA: cyclosporin A; MMF: mycophenolate mofetil; Pred, prednisolone.

In adults, where the main indication is leukemia, most centers use a total body irradiation-based conditioning, mostly combined with Cy and/or fludarabine (Flu). Other centers use Bu-based conditioning.[60] In children, indications for CBT are more diverse; we focused on conditioning regimens used in CBT for leukemia, PID and IEM. In general, most centers use a chemotherapy-based conditioning for all indications, mostly Bu- or Flu-based. No center uses the same conditioning in all pediatric indications, nor were the same conditioning regimens for adults and children with the same indication. Historically, decision-making was mainly based on expert opinion not on clear evidence.

While most patients received ATG containing regimens, nowadays most centers choose not to use ATG or other serotherapy, such as alemtuzumab in CBT, neither in adults nor children. The main reason for this is that exposure to the graft of ATG (and alemtuzumab) significantly depletes the T cells in the CB-graft. Early T-cell reconstitution relies in the first months mainly on peripheral expansion of the infused T cells. Therefore, over- exposure to serotherapy may lead to delayed or absent T-cell reconstitution during the first 6–9 months after CBT, making a patient at risk for viral reactivations and relapse. When serotherapy is not part of the conditioning regimen, IR following CBT can result in a restoration of adaptive immunity within 2 months after transplantation.[46,61] A drawback, however, of omitting ATG is the higher incidence of acute GvHD as discussed above. Developing better balanced individualized conditioning regimens is a current excitement in the field of HCT.

Interestingly, as mentioned above the choice of conditioning regimen over the last decades was mostly expert opinion based. No solid evidence existed showing that one of conditioning regimens was superior to others. Most centers balance the necessity for intense conditioning, which may be needed for disease control and myeloablation, with toxic effects, both short term (e.g. GvHD, veno-occlusive disease, idiopathic pneumonia syndrome) and long term (growth in children, endocrinopathies, fertility, chronic GvHD including bronchitis obliterans). In this decision-making, possible inter-patient variability of PK is not taken into account. As PK measures may also influence PD end- points, the outcomes may be optimized by studying the PK and PD in cohorts of patients to determine the optimal exposure of each component of the conditioning regimen. Over the last decades, there has been an increasing interest in pharmacological research in agents used in HCT (e.g. cyclosporine, tacrolimus, ATG, Busulfan). With these findings, a more evidence- based decision can be made on how to design the most optimal conditioning regimens (in term of exposure and combination of agents), which may result in the best predictable outcomes.

Toward individualized conditioning

As mentioned above, it is well recognized that differences in conditioning regimen may contribute to differences in out- come. Even within patients receiving the same condition-

ing regimen with comparable doses, outcomes may not be the same, due to variability in PK and PD of agents used in the conditioning (Figure 1). Variables such age, body size, organ function, and concomitant medications can influence the PK profile resulting in variable PD outcomes.[62–65]

From a pharmacological perspective, drug exposure (i.e. drug concentration over time), rather than the drug dosing, is driving the effects. Many drugs used in the conditioning regimens are dosed in mg/kg or mg/m2, assuming a linear increase in clearance with increasing weight or body surface area, while this most often is untrue.[66–68] Also, other factors potentially influencing PK such as renal function, obesity, or age are not taken into account. As a consequence, using the same dose (expressed in mg/kg or mg/m^2) for all patients, a vast variability in drug exposure is introduced, which may lead to under- or overdosing of certain patients (Figure 1a). It would be preferable to adjust dosing, taking into account all factors influencing PK and PD (i.e. individualized dosing), resulting in comparable drug exposure and drug effects for all patients (Figure 1b). These individualized dosing regimens may contribute to better predictable regimens in terms of safety and efficacy and in the context of HCT also predictable IR which reflects safety and efficacy.[62,69,70]
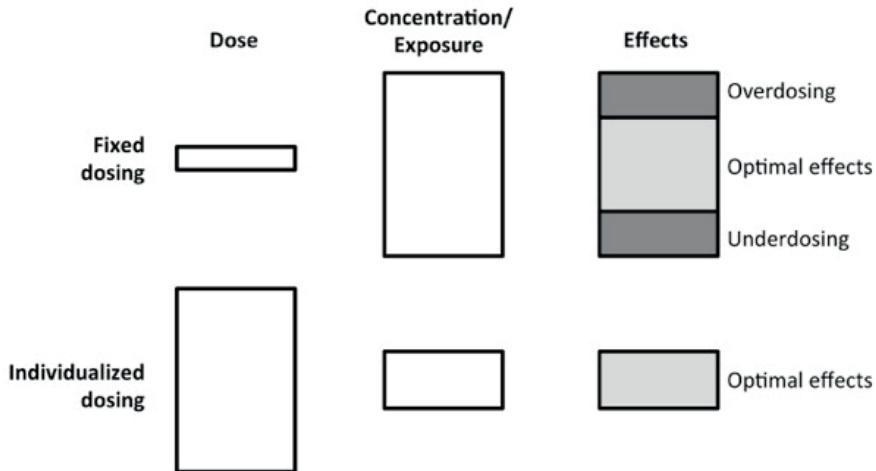
Figure 1. Schematic representation of fixed dosing (top row) and individualized dosing (bottom row). In fixed dosing, most variability is found in concentration/ exposure and drug effects, leading to under- and overdosing in a part of the population. In individualized dosing, the variability is found in the dose, thereby accounting for differences in PK, leading to more on-target exposure and drug effects. Adapted with permission from: Steeghs N, Best Practice: TDM in oncology. Where there is evidence. Presented at the IATDMCT 2015 in Rotterdam, the Netherlands

To develop individualized dosing regimens, the PK and PD of a drug of interest needs to be described in a population PK/ PD model. With the PK and PD described, dose can be calculated to reach optimal effects, taking into account all factors influencing PK and PD. To finalize, a proposed individualized dosing regimen should be prospectively validated to assess its performance both in reaching optimal exposure and effects (Figure 2). Ultimately, PK and PD of all components of the conditioning can be modeled in a multi-agent PKPD model. As the individual components of the conditioning regimen can each have their own optimal effect and can influence each other, modeling in a multi-agents model may even further optimize drug efficacy and safety. Such a multi-agent PKPD modeling has so far not been studied in the context of HCT.

TDM may further fine-tune the optimal drug exposure and is complementary to individualized dosing. This may be of value when significant variability in drug exposure remains following implementation of an individualized dosing regimen due to unpredictable PK. TDM can also be used without individualized dosing; however, substantial dosing adjustments and the additional costs are drawbacks. Additionally, PK and optimal exposure need to be described in order to perform TDM; therefore, the additional effort to design an individualized dosing regimen is minimal.
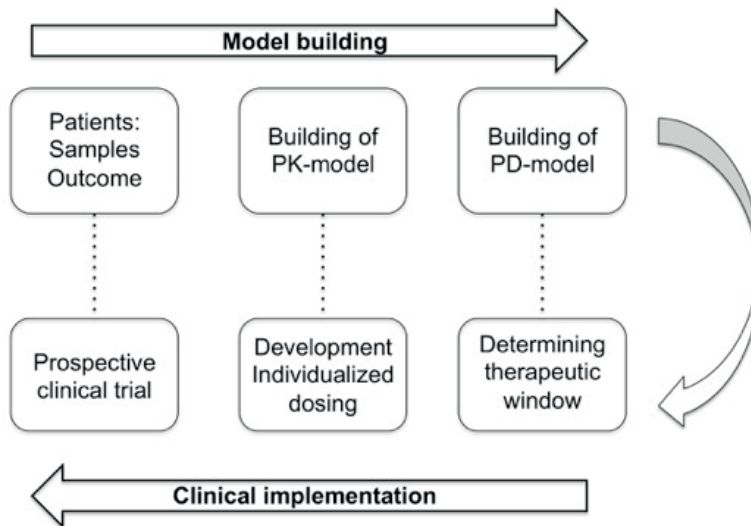
Figure 2. The development of individualized dosing. First, in the model-building phase, samples are collected from the population of interest. Next, the PK and PD are described in this population. In the clinical implementation phase, using the PD-model, the therapeutic window is determined. Knowing the target exposure, the optimal dosing is calculated using the PK-model. This optimal dosing is evaluated in prospective clinical trial, potentially leading to a validated individualized dosing regimen.

By individualizing the dose of all agents used in CB transplantation, the efficacy of the treatment may be improved while reducing unwanted toxicity, which may result in improved survival chances. A predictable conditioning is also of importance when harmonizing clinical trial design, where various novel therapies or interventions can be better com- pared as recently reviewed by an international consortium.[71]

Examples of individualized dosing in HCT

Although PK/PD modeling has been around for decades,[72] implementation of PK/PD-based individualized dosing regimens used prior to HCT including CBT, is scarce. This may have been due to lack of communication between physicians and pharmacometricians (PK/PD specialists), a difference in scope of research (i.e. more descriptive versus predictive models), and little confidence in the advantages of individualized dosing by physicians. However, the field of HCT has been a pioneer in the use of individualized dosing aiming to optimize the survival chances.[63,64,67,73,74] Currently, most centers target to trough levels of cyclosporin A or tacrolimus in the context of GvHD prophylaxis and are using individualized Bu-dosing with TDM to aim for optimal exposure.[75,76] More recently, in some (inter)national studies a newly developed individualized ATG regimen aiming for improved and predictable T-cell IR was implemented.[9,37,77]

Busulfan PK has been studied by several groups (adult and pediatric), which has led to several PK-models, mainly developed in cohorts of infants and children but also in some adult cohorts.[66,67,78–80] The optimal therapeutic window has been established in multiple reports [75,76,81,82] (Figure 3). This optimal exposure appears to be independent on cell source, match grade, indication, and concomitant conditioning agents.[75,76] Although the optimal exposure was similar among 1, 2, and 3 alkylators, patients receiving only Busulfan combined with Flu had lowest toxicity and superior survival chances (due to lower toxicity: e.g. VOD, GvHD, and IPS). The optimal cumulative target for Bu $AUC_{0-4\ days}$ was found to be 90 mg h/L (equivalent to 5600 µmol min per day over 4 days), for all cell sources, including CB. Although conflicting data are present,[83–85] most (larger) studies including a meta-analyses suggest that the outcomes may be further optimized by combining Bu with Flu rather than with Cy and/or melphalan, mainly by reducing toxicity.[53,76,86–88] For a definite answer, best would be to study individualized dosing regimens in randomized controlled trials.

To better predict the T-cell IR, some groups have described the PK and PD of ATG (Thymoglobulin®).[9,42,89–93] From these PK models we have learned that dosing should be dependent on bodyweight as well as on the lymphocyte count prior to ATG, as these determine ATG clearance.[89] Variations in levels of ATG or exposure to ATG before and after HCT were associated with various outcomes, such as CD4+ IR, TRM, relapse, and GvHD.[9,42,91,92] Most studies investigated concentrations at single time points as a predictor of outcome rather
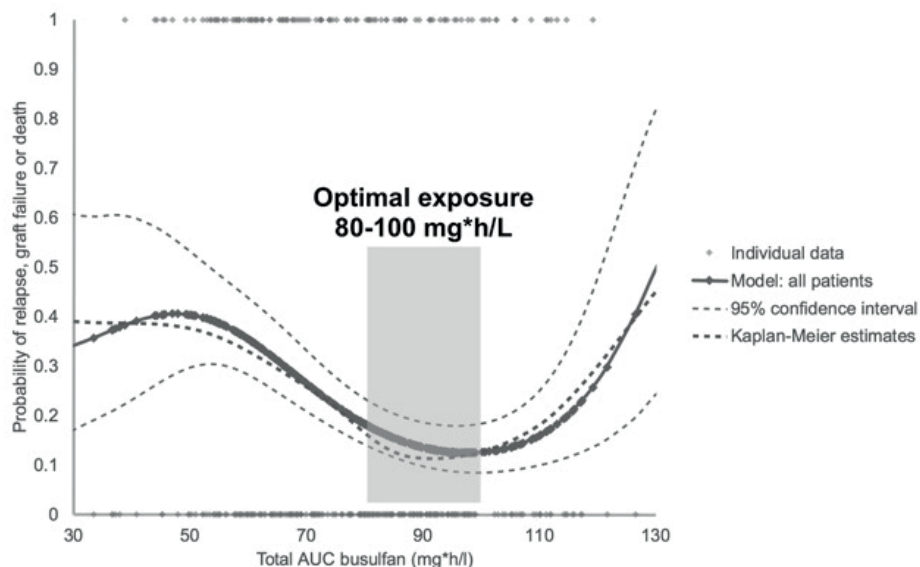
Figure 3. Weibull model of busulfan exposure in relation to EFS for all patients, showing the optimal exposure to be between 80-100 mg*h/L. Solid and dashed blue lines: Weibull model with 95% confidence intervals. Red dashed line: Kaplan Meier estimate. Reprinted from: Lalmohammed et al, Studying the Optimal Intravenous Busulfan Exposure in Pediatric Allogeneic Hematopoietic Cell Transplantation (alloHCT) to Improve Clinical Outcomes: A Multicenter Study, Biology of Blood and Marrow Transplantation 2015;21(2):S102-S103, with permission from Elsevier.

than total exposure (before and/or after HCT), making some reports hard to interpret. In the largest report in children, high exposure to ATG after transplantation was associated with lower chances on timely CD4+ IR (defined as twice over 50×10^6 per liter within 100 days after CBT: found to be the best predictor for outcomes), which was associated with lower survival and higher TRM and relapse rates[9] (Figure 4). Of note, in CBT even very low exposure of ATG after transplantation results in poor or even absent T-cell reconstitution during the first 6–9 months after transplantation (Figure 5), while omitting ATG results in very fast CD4+ IR as described by various groups.[30,46,94] Furthermore, the incidence of GvHD and rejection was mainly influenced by sufficient ATG exposure before infusion of the graft, not after. Taking these observations into account, an individualized dosing regimen was established, which is currently being investigated in a prospective study (PARACHUTE- trial; Dutch Trial Register identifier NTR4960).

Taken together, the therapeutic windows of Busulfan and ATG are narrow and critical. Dose individualization appears to be essential for reaching optimal drug exposure. This may result in a better predictable conditioning regimen in terms of safety and efficacy associated with better survival chances.
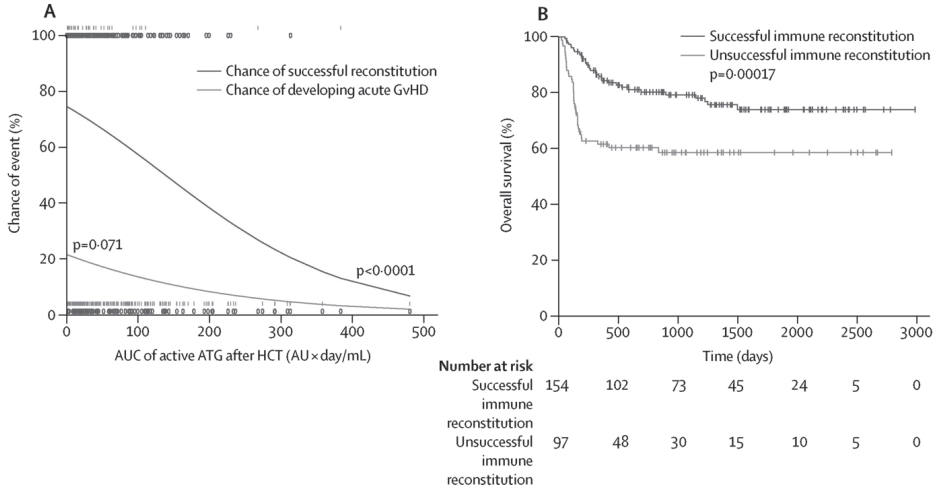
Figure 4. Chance of successful reconstitution, incidence of acute graft-versus-host disease, and overall survival (A) Successful CD4+ T-cell reconstitution before day 100, defined as twice > 50 x 106/L (red 0's) and grade 2–4 acute GvHD (blue I's) versus AUC of active ATG after HCT. The logistic regression lines show the chance of successful reconstitution versus the AUC after HCT (red line) and the chance of developing acute GvHD of at least grade 2 versus the AUC after HCT (blue line). Every I or O represents a patient with their respective AUC after HCT (x axis) and whether they had an event (y axis, either yes [1; top] or no [0; bottom]). Therefore, the patient with an AUC after HCT of 480 AU × day/mL had no immune reconstitution and no GvHD. (B) Kaplan-Meier survival curve of overall survival according to successful CD4+ T-cell immune reconstitution. Reprinted from: Lancet Haematology, Volume 2, Issue 5, Admiraal et al, e194-e203. Copyright (2014), with permission from Elsevier.

Future of CBT

The two most important upcoming developments in the field of CBT are designing conditioning regimens that better predict early CD4+ IR and CB-based adjuvant cellular therapies. A predictable CD4+ IR is essential for an optimal effect of adjuvant cellular therapies, such as cell vaccines. In addition, new indications for CB therapy are being explored, including cerebral palsy and hypoxic ischemic brain injury: promising results have been reported for these new indications.[95,96]

Predictable early T-cell IR

As described above, there is apparently a delicate balance present between ATG (or other serotherapy: e.g. Campath) in CBT and prevention of GvHD and graft failure on one side, and T-cell IR on the other. Omitting ATG may be feasible in heavily chemotherapy pre-treated or immune-compromised patients (leukemia's, primary immune deficiencies) but is associated with higher probability on severe acute GvHD, but not chronic GvHD or TRM.[37] Adding ATG to the conditioning may lead to delayed or absent early CD4+ IR by peripheral expansion, due to too high exposure of ATG after CBT.[9]

Our group has worked on describing the PK and PD of ATG, the most commonly used drug for serotherapy in the Netherlands, in order to develop an evidence-based, individualized dosing regimen (see also Section 7). With this regimen, dosing and starting day can be chosen for each patient to ensure optimal ATG exposure before (and after) CBT. This individualized regimen has the advantages of sufficient recipient T-cell depletion and other immune cells with targets for antibodies in the ATG (e.g. antigen presenting cells) for the prevention of GvHD and graft failure, while exposure to ATG after infusion of cells is very low to prevent (too deep) T-cell depletion. When used in this individualized way, both early and late CD4+ IR following CBT will be at least similar (Figure 5), but probably better than BM or PBSC transplants as suggested by various groups.[9,46,94] The normal functionality of these cells is shown in some cell source comparison studies for probability on viral reactivation and relapse.[2,13,36] Currently, a prospective clinical trial is recruiting in the Netherlands, investigating individualized ATG compared to historical fixed–dose ATG (see above). The primary end- point is achieving CD4+ IR within 100 days.

More recently we initiated research into Flu, a purine analogue used commonly next to Bu in CBT conditioning. Flu inter- acts with lymphocyte proliferation; therefore, exposure to Flu may play a role in T-cell IR.[97,98] The project is aimed at describing the population PK and PD of Flu in order to derive individualized dosing nomograms.

With the PK and PD of Bu, Flu and ATG described, a conditioning platform consisting of individualized drugs will be available. As a final step, a comprehensive model, integrating all agents, combined with patient and donor variables, will give a fully predictable and adjust-
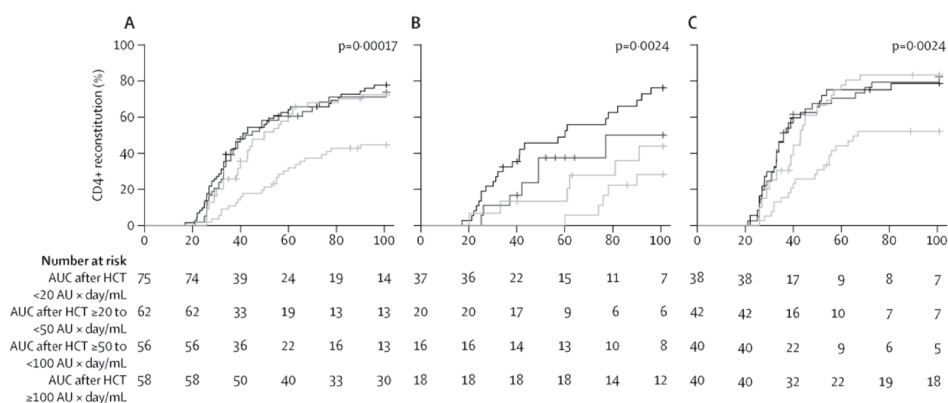
Figure 5. CD4+ T-cell reconstitution and overall survival according to area under the curve after haemopoietic stem cell transplantation by stem cell source. The effect of AUC of ATG after HCT on immune reconstitution in all patients (A), those who received cord blood transplants (B) and those who received bone marrow and peripheral blood stem cell transplants (C). Reprinted from: Lancet Haematology, Volume 2, Issue 5, Admiraal et al, e194-e203. Copyright (2014), with permission from Elsevier.

able Bu–Flu–ATG backbone. Designing a platform that is predictable with regards to safety and toxicity (including CD4+ IR) is essential also in the context of harmonized clinical trial design to study effect of novel interventions such as adjuvant immunotherapies.[71]

Targeted therapies, including adjuvant cellular therapies, after CBT

Targeted therapies, including adjuvant cellular therapies, given post-transplantation, are strategies being used and developed to get better disease control in patients receiving a HCT, including CBT for malignant disease as this remains an unmet need for certain indications. For a very selected group of patients, those with Philadelphia-positive ALL or CML, targeted small molecule therapies with tyrosine kinase inhibitors (TKI: e.g. imatinib, dasatinib) are given after HCT aiming to prevent disease relapse.[99] More kinase inhibitors, when identified to have a role in the biology of leukemia and lymphoma, may be used post-HCT in future. Currently, no CB specific studies are known. Regarding cellular therapies, historically (and still in some protocols) unmanipulated lymphocyte infusions are given, while nowadays also more specific cell therapies are being developed, such as engineered T cells and cellular vaccines.

As relapse remains the main obstacle even after potentially curative HCT, novel combinational immunotherapeutic strategies are being developed aiming at preventing relapse after HCT. Currently, the most widely used type of additional immunotherapy combined with allogeneic HCT is donor lymphocyte infusion, where alloreactive T cells may help to eradicate residual tumor cells. Unfortunately, this 'non-specific' strategy suffers from severe toxic side effects, such as GvHD.[100] Other approaches aim to increase innate or adaptive antitumor responses by transferring ex vivo-generated cells, such as chimeric antigen receptor (CAR)-modified tumor-specific cytotoxic T lymphocytes (CTL) or natural killer cells.[101–107] Although initial results seem promising, these procedures are often time-consuming (up to months) and may have limitations, such as HLA-restriction and uncertain functionality. Additionally, there is a highly variable induction of immunological memory upon transfer in the patient, which may restrict the broad eligibility of these treatments. Although CAR modified T cells looks promising, at least on the short term, several drawback, such as (life)-long B-cell lymphopenia in CAR T cells against CD19. Also, duration of effect, which may reflect the life span of these engineered T cells, remains unclear.

Another intriguing option is development of cell vaccines: increased antigen presentation provided by a DC-vaccine combined with the intrinsic increased proliferative capacity of the grafted CB cells may result in fast differentiation and proliferation of tumor-specific CTL early after CBT.[108,109] This early and mass expansion of tumor-specific CTL may subsequently result in clearance of minimal residual disease and prevention of relapses in cancer patients. Naïve CB-T cells display exceptional proliferative capacities, suggesting that ef-

ficient priming of these cells using a tumor-specific DC- vaccine will provide powerful antitumor activity. This may result in clearance of, and long-term immunological memory against, tumor cells. That CB T cells mediates a stronger anti-leukemic activity compared to adult cells was recently shown in a study by Hiwarkar et al.[13]

For all these adjuvant immunotherapies, predictable T-cell IR is essential as the effect relies on absence of circulation ATG but also on presence of adequate number of T cells to mediate the desired antitumor effect.

CONCLUSION

CB is an emerging alternative cell source. In historical, mainly registry studies CB showed similar probabilities on survival compared to MUD, MMUD, and in some studies even compared to MSD transplantation. Some of the disadvantages such as prolonged neutrophil and platelet reconstitution have been overcome using higher cell-dosed units, double-cord transplants and more recently with expanded CB products. Furthermore, individualization of Bu has influenced donor-cell engraftment, relapse, and limited toxicity. More recently, the significant influence of exposure of ATG after CBT on T-reconstitution was recognized, while expo- sure before transplantation appears to be important in the prevention of rejection and GvHD. Naïve T cells from CB have been recognized to mediate powerful antiviral and anti-leukemic properties early after transplantation. To get the most potent effect, individualizing ATG dosing to improve T-cell IR after transplantation seems to be essential. Although a variety of regiments have been used, it seems that individualized ATG–Bu–Flu backbone used for CBT suggests being associated with the best predictable outcomes. Dose individualization is essential to optimize the effects. A predictable conditioning backbone is also essential for studying the effect of future adjuvant immune therapies or new agents to get better disease control, within a harmonized clinical trial setting (Figure 6).

Expert opinion

Outcomes of CBT are reported to be highly variable. In addition to variables such as cell dose, underlying disease, co-morbidities of the patients, the conditioning regimen used is recognized to impact the outcomes. Furthermore, differences in PK can be associated with variable myeloablation and immune suppression before and after CBT. Dose individualization of agents used in the conditioning regimen in CBT has proven its added value in terms of enhancing safety and efficacy. Further fine-tuning of individualized conditioning regimens, including all used agents and/or finding the optimal combination of agents, may result in better predictable conditioning regimens in terms of safety and efficacy including predictable T-cell IR. Furthermore, such a predictable conditioning regimen in CBT in the

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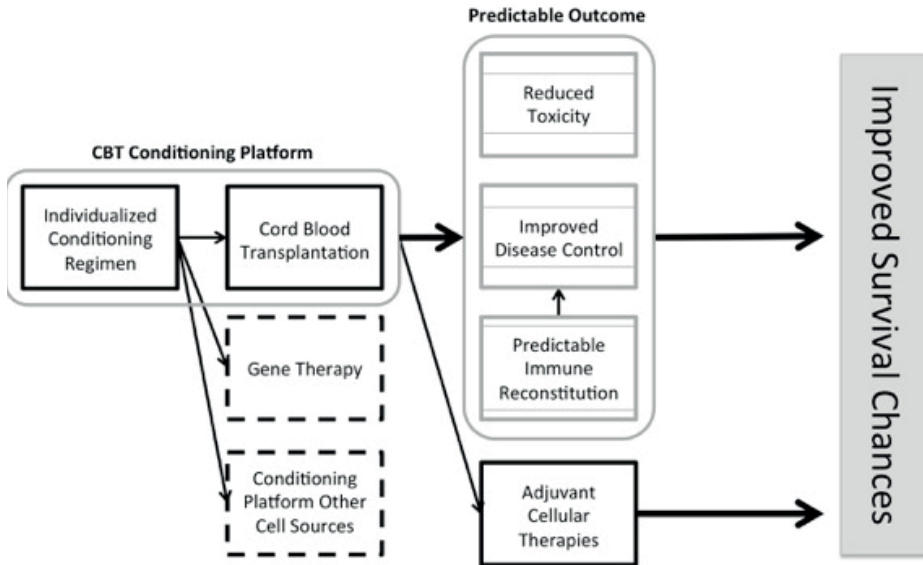
Figure 6. Summarizing figure showing the implications of individualized dosing in the conditioning of hematopoietic cell transplantation.

context of harmonized clinical- trial design is also of interest/importance to study the effect of adjuvant immunotherapies on CBT platforms, such as cellular vaccines, engineered T-cell therapies.

Currently, a large number of conditioning regimens are being used in allogeneic CBT. Besides differences in choice of drugs, the actual drug exposure varies due to variability in PK and PD between patients. The use of regular dosing regimens, with a linear increase in dose with body weight (mg/kg) or body surface area (mg/m^2) leads to a highly variable expo- sure, with some patients being under- or overdosed. By using individualized dosing regimens, this variability in PK and PD is accounted for, resulting in more patients reaching optimal drug exposure and thereby drug effects.

While this may have major implications for patient care, research in comparing drugs or drug doses may also be impaired due to a skewed distribution in PK or PD.

An individualized dosing regimen is available and being used in clinical care for Bu.[53] Additionally, exposure of ATG before and after HCT has shown to have impact on the outcomes. Individualized dosing regimen for ATG seems, there- fore, crucial to influence the outcomes. To our best knowledge, these are the only drugs used in the conditioning regimen in allogeneic HCT for which individualized dosing regimens are available. How- ever, efforts have been made to characterize the PK and PD of other drugs used including

Flu,[98,110] treosulfan[68] and Cy,[111] which however did not yet result in practical guidelines or dosing recommendations.

There are however some hurdles in designing individualized drug dosing. As described above, the development of a dosing regimen includes describing drug PK and PD. In terms of PK, blood needs to be collected to determine drug concentration levels, finally resulting in a population PK model. Besides the possible logistical issues, collecting blood samples and accurate documentation of taking these samples, is a challenge in a pediatric setting, where observational studies are difficult due to ethical constraints. On the other hand, describing the PD and determination of the therapeutic window, that is the target exposure poses most challenges. This is especially true for drugs with effects for which no direct bio-marker is available, or is also being influenced by concomitant medication (e.g. lymphocyte counts for ATG and Flu). Additionally, in drugs where the time between drug exposure and clinical effect is large (i.e. hysteresis), for instance incidence of relapse following clofarabine as conditioning, the exposure–effect relationship is hard to estimate.

Currently, cost effectiveness is playing an increasingly larger role in healthcare decision-making. In this perspective, we hypothesize that dose individualization may be quite cost-effective, especially in the field of HCT/CBT where all complications are very costly (e.g. treatment of GvHD, expensive anti- viral drugs, VOD, graft failure).[53,112] Additionally, the costs of the development of individualized dosing regimens are relatively low.

In the last years, dose individualization has proven its added value in terms of enhancing safety and efficacy. In the coming years, we expect to see more individualized dosing regimens emerging in the field of CBT, especially in pediatric transplantations, where differences in PK are major. In our view, we need fully individualized conditioning regimens including all drugs used. This way, outcome will be predictable and adjustable based on individual patients' needs. Additionally, other drugs used in CBT may need individualization as well, including GvHD-prophylaxis and the treatment and prophylaxis for infectious diseases. Finally, the currently available models may be further sophisticated, describing not only PK or PD, but rather the complete spectrum of drug treatment, including dose, PK, biomarker response, clinical efficacy, and toxicity in one comprehensive model. We expect development and implementation of individualized dosing to take place in the next 10 years, thereby improving the knowledge and efficacy of clinical drug therapy, and improving clinical outcome following CBT. With individualized dosing, unwanted variability in drug exposure will be reduced, leading to predictable, adjustable, and improved outcome of CBT (Figure 6). Such a predictable conditioning regimen can also be used as a transplantation platform in the context of harmonized clinical trial design to study the effects of adjuvant therapies: for example concomitant chemotherapy in conditioning or adjuvant immunotherapies.

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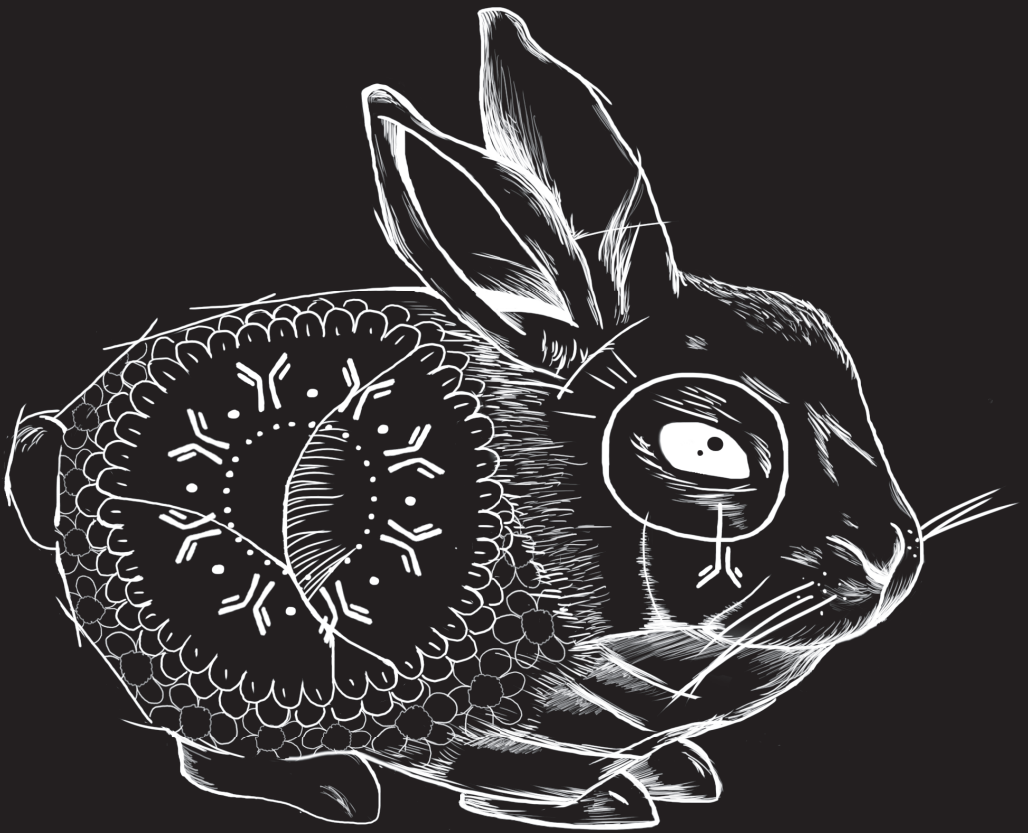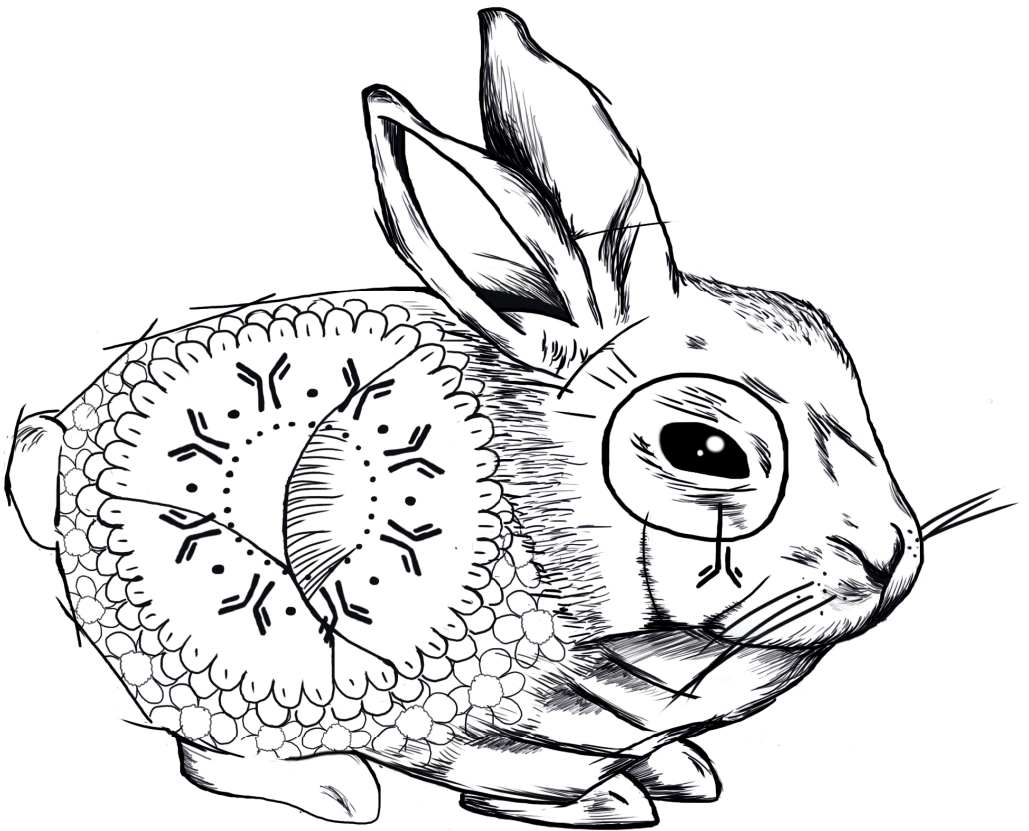
PART VI

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.

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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

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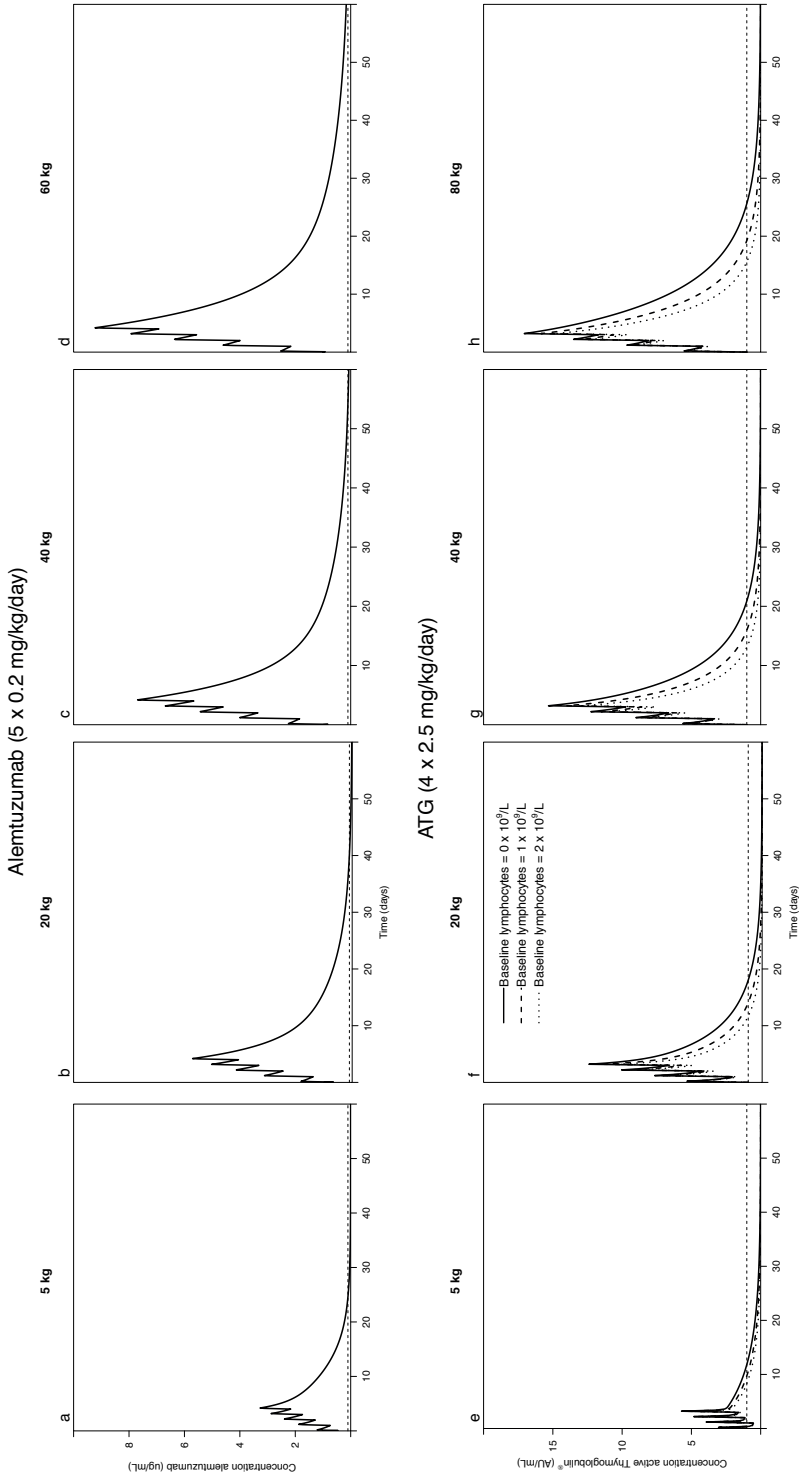
Figure 1. Simulated exposure after standard dosing of alemtuzumab (1 mg/kg cumulative over 5 days, panels a-d) and ATG (10 mg/kg cumulative over 4 days, panels e-h) in children weighing 5, 20, 40 and 80 kg. ATG exposures are stratified for lymphocyte counts. Dashed lines: lympholytic levels

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.

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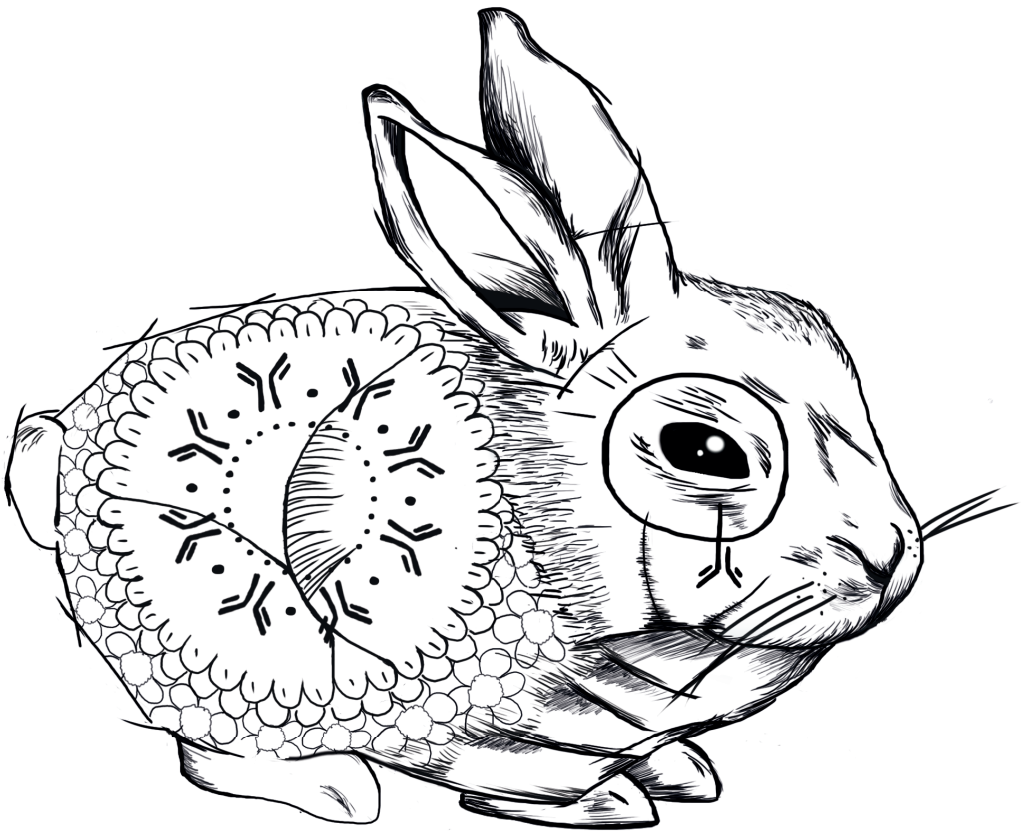
We conclude that individualizing dosing and timing of ATG potentially makes HCT a safer and more effective treatment option, and will lead to improved survival chances. Individualized dosing regimens for ATG in children have been designed based on the results in this thesis, and are currently being evaluated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

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Chapter 13

Nederlandse Samenvatting /Dutch Summary

GEÏNDIVIDUALISEERDE DOSERING VAN SEROTHERAPIE IN ALLOGENE HEMATOPOIETISCHE CELTRANSPLANTATIE – EEN DELICATE BALANS

Deel I: Achtergrond en introductie

Allogene stamceltransplantatie (SCT) is een potentieel levensreddende behandeling voor ernstige ziektes waaronder leukemie, immuundeficiënties en beenmergfalen. Bij deze behandeling ontvangt de patiënt bloedstamcellen van een donor, welke vervolgens uitgroeien tot een gezond bloed- en afweersysteem. De bloedstamcellen zijn meestal afkomstig uit het beenmerg van een donor. Hiernaast kan navelstrengbloed van niet-gerelateerde donoren worden gebruikt. Dit zou normaliter weg worden gegooid. Een derde optie, voornamelijk gebruikt bij volwassenen, zijn gemobiliseerde en uit bloed gewonnen stamcellen. Voordat de nieuwe bloedstamcellen gegeven kunnen moet ruimte worden gemaakt in het beenmerg. Hiervoor ontvangen patiënten chemotherapie of lichaamsbestraling. Na de SCT kan een afweerreactie ontstaan vanuit de patiënt gericht tegen de donor (afstoting), of van de donor tegen de patiënt (transplantatieziekte). Om deze afweerreacties te voorkomen worden patiënten voor de transplantatie behandeld met serotherapie (anti-thymocyten globuline [ATG] of alemtuzumab). Het nadeel van serotherapie is dat het zowel de afweercellen van de ontvanger als van de donor vernietigt; dit laatste kan resulteren in matig tot geen afweerherstel na de transplantatie. Zonder deze afweercellen hebben patiënten een hoger risico op infecties en een eventueel recidief van de leukemie. De dosering serotherapie is dus cruciaal; te weinig kan leiden tot transplantatieziekte of rejectie, te veel geeft slecht immuunherstel. De relatie tussen de dosering serotherapie en de concentraties in het bloed (farmacokinetiek; PK) is grotendeels onbekend en hoogst variabel. Hiernaast is ook de optimale blootstelling aan serotherapie (farmacodynamiek; PD) niet goed onderzocht. Het doel van deze thesis is het beschrijven van de PK en PD van ATG en alemtuzumab in kinderen en volwassenen teneinde een evidence based, geïndividualiseerde dosering te ontwikkelen voor beide middelen.

Deel II: Farmacokinetiek van serotherapie

In de huidige praktijk worden beide middelen voor serotherapie rechtlijnig met lichaamsgewicht (in mg per kg) gedoseerd in zowel kinderen als volwassenen. Dit doseerregime impliceert dat de onderliggende farmacokinetische processen, bijvoorbeeld klaring en distributievolume, ook rechtlijnig toenemen met gewicht. Echter, doordat groei en ontwikkeling vaak niet rechtlijnig zijn met gewicht, leiden de rechtlijnige doseerregimes voor serotherapie tot onder- en overdosering in bepaalde patiëntengroepen. Hiernaast zijn ATG en alemtuzumab beide antilichamen, waarvan beschreven is dat de PK hoogst variabel kan zijn. Het is daarom belangrijk om de PK van serotherapie te beschrijven en zodoende een gelijke blootstelling te verkrijgen in alle patiënten. Een veel gebruikte techniek is het zogenaamde populatie PK/PD modelleren. Hiermee wordt in een model de PK en diens

relatie met de PD beschreven. Met deze modellen kan men op een geïnformeerde wijze een dosering berekenen om een zo optimaal mogelijk effect van behandeling te bereiken. In **hoofdstuk 2** wordt het concept van geïndividualiseerde dosering op basis van PK/PD modelleren verder besproken teneinde de behandeling te verbeteren.

In **hoofdstuk 3** wordt de populatie PK van ATG (Thymoglobulin) beschreven in een cohort van 267 patiënten (0.2-23 jaar oud) die een SCT hebben gekregen op de kinderafdeling van het Universitair Medisch Centrum Utrecht (UMCU) en het Leids Universitair Medisch Centrum (LUMC). In meer dan 3000 bloedmonsters die zijn afgenomen tussen 2004 en 2012 is de ATG-concentratie bepaald, deze zijn gebruikt om een PK-model te ontwikkelen. Bijzonder aan dit model is de wijze van klaring van dit geneesmiddel. Deze vindt plaats door zowel normale (lineaire) klaring, zoals in de meeste geneesmiddelen wordt gezien, alsmede een verzadigbare klaring, die afhankelijk is van de concentratie ATG in het bloed. Bij hoge concentraties is deze component verzadigd en hierdoor weinig bijdragend, bij lagere concentraties speelt de verzadigbare klaring een relatief grotere rol. Vervolgens werden eventuele voorspellers van de PK geanalyseerd, welke een rol zullen spelen in de dosering van toekomstige patiënten. Lichaamsgewicht was een voorspeller van zowel lineaire klaring als verdelingsvolume, terwijl het aantal lymfocyten voor de eerste dosis ATG alleen de lineaire klaring beïnvloed. Dit laatste is te verklaren door het werkingsmechanisme van ATG: het bindt aan receptoren die voornamelijk op lymfocyten tot expressie komen waarna het uit de circulatie verdwijnt. Meer lymfocyten leidt dus tot een hoger aanbod van receptoren, waardoor meer ATG kan binden en de klaring sneller wordt. Met het finale model is de huidige dosering ATG bij kinderen geëvalueerd door middel van simulatiestudies. Hieruit blijkt dat kinderen met laag lichaamsgewicht (jonge kinderen) een significant lagere blootstelling aan ATG hebben vergeleken met zwaardere (oudere) kinderen. In een nieuw doseerregime, waarvoor dit PK-model de basis vormt, moet de dosis aangepast worden zodat alle kinderen gelijke blootstelling hebben.

De populatie PK van alemtuzumab in kinderen wordt beschreven in **hoofdstuk 4**. Voor deze analyse is data verzameld van 206 patiënten van 0.2 tot 19 jaar oud die een SCT op de kinderafdeling van het LUMC en het Great Ormond Street Hospital (GOSH) in Londen, Verenigd Koninkrijk, hebben gekregen. Het PK-model was structureel vergelijkbaar met dat van ATG; ook hier werden zowel lineaire klaring als verzadigbare klaring geïdentificeerd. Lichaamsgewicht was een voorspeller voor lineaire klaring en distributievolume, waarbij de relatie tussen gewicht en klaring werd beschreven met een zogenoemde lichaamsgewicht-afhankelijke exponent. Deze exponent maakt dat de relatie tussen gewicht en klaring een sigmoidaal verband heeft waarmee de klaring in zowel kleinste kinderen als de adolescenten goed wordt voorspeld. Interessant genoeg was het aantal lymfocyten geen voorspeller voor de klaring van alemtuzumab.

Deel III: Farmacodynamiek van serotherapie

Waar nadruk in de hoofdstukken 2, 3 en 4 voornamelijk lag op de relatie tussen dosis en blootstelling zullen hoofdstukken 5 tot en met 8 voornamelijk de relatie tussen blootstelling en klinische effecten beschrijven, de farmacodynamiek. Beschrijving van de farmacodynamiek werpt een licht op de therapeutische breedte. Dit zal de optimale benodigde blootstelling voor geïndividualiseerde dosering bepalen.

In **hoofdstuk 5** wordt de relatie tussen de blootstelling aan ATG en zowel immuunherstel als klinische uitkomsten wordt bestudeerd in een cohort van 251 patiënten die een eerste SCT ondergaan. In deze studie werden alle patiënten geïncludeerd die behandeld werden op de kinderafdeling van het LUMC en UMCU. Met behulp van het PK-model werden diverse maten voor blootstelling berekend, waaronder de oppervlakte onder de concentratie-tijd grafiek (area under the curve; AUC). De AUC na de infusie van het transplantaat (AUC na SCT) was een sterke voorspeller voor immuunherstel van het afweersysteem, in het bijzonder een subset van zogenoemde T-cellen, de T-helper cellen. Deze cellen brengen het eiwit Cluster of Differentiation 4 (CD4) tot expressie, en worden dan ook CD4+ T-cellen genoemd.

De AUC na SCT had geen invloed op het optreden van transplantatieziekte. Herstel van CD4+ T-cellen was vervolgens een sterke voorspeller voor verbeterde overleving; het verminderde de kans op overlijden door zowel een recidief leukemie alsmede mortaliteit door de behandeling (infecties, transplantatieziekte, afstoting et cetera). In subgroepen werd een directe relatie tussen AUC na SCT en overleving gezien, waarbij de optimale AUC na SCT lager was bij patiënten die navelstrengbloed ontvingen in vergelijking met beenmerg.

De effectiviteit van ATG in het voorkomen van transplantatieziekte en afstoting werd voornamelijk bepaald door de blootstelling aan ATG vóór infusie van de stamcellen (AUC voor SCT), deze moest voldoende hoog zijn. Dit onderzoek toont aan dat de blootstelling aan ATG voornamelijk voor de SCT belangrijk is voor het farmacologisch effect, terwijl blootstellen aan ATG na de transplantatie nadelige effecten veroorzaakt. De optimale blootstelling die in deze studie werd aangetoond, gecombineerd met het PK-model uit hoofdstuk 2, hebben geleid tot een geïndividualiseerd doseerregime voor ATG in kinderen die op het moment wordt geëvalueerd.

De blootstelling van ATG lijkt meest belangrijk na een navelstrengbloed transplantatie, welke verder werd geanalyseerd in een groter cohort in **hoofdstuk 6**. Een cohort van 137 kinderen die een eerste SCT met navelstrengbloed ontvingen in het UMCU werd onderzocht, die een uniforme behandeling ondergingen wat betreft chemotherapie en ondersteunende behandeling als antibiotica. De optimale blootstelling aan ATG na de transplantatie lijkt iets lager dan wat voorgesteld werd in hoofdstuk 5, hogere blootstelling leidt tot langzaam of afwezig immuunherstel. Lage blootstelling na SCT leidt tot betere event free survival,

gedefinieerd als overleving zonder relapse of rejectie. Hiernaast werd ook het belang van CD4+ T-cel immuunherstel benadrukt: patiënten met immuunherstel hadden een betere overleving en event free survival, hiernaast was mortaliteit door zowel recidief leukemie alsmede de behandeling lager. Deze studie valideert de resultaten uit hoofdstuk 5.

Hoofdstuk 7 beschrijft de farmacokinetiek en farmacodynamiek van ATG in 146 volwassen patiënten met acute leukemie die een perifeer bloed transplantatie ontvingen na milde chemotherapie in het UMC Utrecht. Eerst werd de farmacokinetiek beschreven, waarbij data van deze 146 patiënten werd gecombineerd met de pediatrische data uit hoofdstuk 3 en 81 volwassenen met andere diagnoses en/of intensieve chemotherapie. Dit PK-model kwam overeen met de resultaten zoals beschreven in hoofdstuk 2, waarbij slechts de parameter-schattingen alsmede de wiskundige relatie tussen gewicht en lineaire klaring verschilde. In het kindermodel werd deze met een enkele exponent werd beschreven, terwijl in het gecombineerde model een lichaamsgewichts-afhankelijke exponent de beste beschrijving van de data gaf. De exponent bereikte een asymptoot van 0 vanaf een gewicht van 50 kg, wat bekent dat de lineaire klaring van ATG vanaf 50 kg niet toeneemt op basis van gewicht. Het aantal lymfocyten in het bloed voordat de eerste gift ATG wordt gegeven blijft echter de lineaire klaring beïnvloeden. Vervolgens werden diverse maten voor blootstelling aan ATG berekend met het PK-model welke werden gerelateerd aan klinische uitkomsten. De blootstelling aan ATG na de SCT (AUC na SCT) was een sterke voorspeller van overleving. Er lijkt een optimale blootstelling aan ATG na SCT te zijn bij deze patiëntengroep: te lage blootstelling resulteerde in hogere mortaliteit door de behandeling, terwijl te hoge blootstelling meer recidieven van leukemie tot gevolg had. Ook hadden patiënten met een te lage blootstelling veel transplantatieziekte, wat de verhoogde sterfte door de behandeling kan verklaren. Er werd geen optimale blootstelling voor de SCT gevonden, mogelijk doordat vrijwel alle patiënten een AUC voor SCT hadden die ruim boven het optimum zoals bepaald in hoofdstuk 5. Afsluitend werden diverse bestaande doseringsschema's voor ATG vergeleken op het behalen van optimale blootstelling. Hiernaast werd ook een nieuw doseerregime ontworpen en onderzocht dat gebaseerd is op het gecombineerde PK-model. Omdat de klaring niet toeneemt boven de 50 kg is dit doseerregime alleen gebaseerd op het lymfocytengetal, niet op gewicht. Van alle huidige doseerschema's, gebaseerd op een mg/kg dosering, behaalde slechts maximaal 30-53% van de patiëntengroepen de optimale blootstelling, terwijl dit voor het geïndividualiseerde regime op 93% lag. Dit geïndividualiseerde doseerregime voor ATG zou een verbetering in overleving van de behandeling kunnen bewerkstelligen.

Een recente publicatie beschreef een relatie tussen peri-transplantatie concentraties van alemtuzumab (±3 dagen) en de incidentie van GvHD en gemengd chimerisme[1]. In **hoofdstuk 8** wordt de therapeutische breedte en optimale blootstelling van alemtuzumab verkend

in een cohort van 176 kinderen die een stamceltransplantatie ondergaan in het LUMC in Leiden en Great Ormond Street Hospital in London. Alemtuzumab concentraties op de dag van transplantatie werden berekend met het PK-model uit hoofdstuk 4, en werden geëvalueerd als voorspeller voor klinische uitkomsten. Vergeleken met de eerdere studie, waar een alemtuzumab subcutaan en verder van de transplantatie werd gegeven, waren de concentraties alemtuzumab op de dag van transplantatie opvallend hoger. De incidentie van graad 2-4 en graad 3-4 acute GvHD werd beïnvloed door de concentratie alemtuzumab, er werd echter geen invloed op overleving, gemengd chimerisme, virale reactivaties of immuunherstel van T-cellen gevonden. Het ontbreken van een blootstelling-toxiciteit-relatie in combinatie met een matige blootstelling-effectiviteit-relatie zou kunnen wijzen op een relatieve overdosering in een groot deel van de patiënten wanneer intraveneuze alemtuzumab kort voor de stamceltransplantatie wordt gegeven.

Deel IV: De rol van immuunherstel op klinische uitkomsten

Zoals hierboven besproken leidt te hoge blootstelling aan ATG na de SCT tot vertraagd of afwezig immuunherstel. Hierdoor lopen patiënten verhoogd risico op een recidief leukemie en virale reactivaties, welke beiden een dodelijke afloop kunnen hebben. De hoofdstukken 9 en 10 beschrijven de rol van immuunherstel op een recidief van leukemie en virale reactivaties.

Vroeg herstel van CD4+ T-cellen na navelstrengbloedtransplantatie was een voorspeller van een recidief leukemie, echter alleen in acute myeloïde leukemie (AML). In acute lymphoïde leukemie (ALL) werd geen relatie tussen immuunherstel en een recidief gevonden. De voorspellende waarde van slecht immuunherstel voor een recidief werd verder onderzocht in een groter cohort in **hoofdstuk 9**. Hier werden kinderen geïncludeerd die ATG hebben gekregen of geen serotherapie hadden gehad. In totaal bestond het cohort uit 87 kinderen die behandeld waren in het UMCU en GOSH, van wie 41% ATG had ontvangen. De overleving vrij van leukemie was significant lager in patiënten met CD4+ T-cel immuunherstel in AML, echter dit was niet het geval in ALL. De sterfte door de procedure was echter verminderd door immuunherstel in alle patiënten. Deze studie valideert de resultaten uit hoofdstuk 5.

In **hoofdstuk 10** worden virale reactivaties en geassocieerde uitkomsten onderzocht in het licht van immuunreconstitutie in een cohort van 273 patiënten uit het UMCU. Virale reactivaties die bij gezonde mensen slechts milde griepachtige symptomen veroorzaken kunnen bij SCT-patiënten ernstige ziekte veroorzaken. In het eerste deel van dit onderzoek werd het immuunherstel van diverse afweercellen (CD3+, CD4+ en CD8+ T-cellen, B-cellen en NK-cellen) bekeken als voorspeller van virale reactivaties van adenovirus (AdV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), humaan herpesvirus 6 (HHV6) en BK-virus

(BK). Herstel van CD4+ T-cellen voorspelde virale reactivaties; hogere cel-aantallen leidden tot minder reactivatie van AdV, EBV en HHV6. CMV en BK waren met geen enkele immuunherstel-marker geassocieerd. In de volgende stap werden virale reactivaties na SCT gerelateerd aan klinische uitkomsten. Hier bleek dat AdV een belangrijke voorspeller voor overlijden na SCT was. Echter, het nadelige effect van AdV op overleving wordt teniet gedaan door CD4+ immuunherstel: patiënten met AdV én CD4+ T-cel herstel hadden een vergelijkbare overleving als patiënten zonder AdV, terwijl patiënten met AdV zonder CD4+ herstel een slechte prognose hebben. De incidentie van transplantatieziekte was hoger na een EBV of HHV6 reactivatie, terwijl CMV en BK geen enkele klinische uitkomst beïnvloedden. Deze data onderstreept het belang van CD4+ T-cel immuunherstel, zowel in het reduceren van de kans op virale reactivaties als in het voorkomen van virus-geassocieerde mortaliteit.

Deel V: Klinische implementatie van geïndividualiseerde dosering

In **hoofdstuk 11** wordt een overzicht gegeven van het gebruik van geïndividualiseerde conditionering (chemotherapie en serotherapie) in navelstrengbloedtransplantaties. Hierbij ligt de nadruk op de huidige klinische uitkomsten, de behoeftes, en toekomstperspectieven van geïndividualiseerde behandeling in navelstrengbloedtransplantaties.

Deel VI: Conclusies en perspectieven

Allogene stamceltransplantatie is een potentieel curatieve behandeling voor onder andere leukemie, afweerstoornissen, hematologische ziektes en metabole ziekten. Om transplantatieziekte en afstoting te voorkomen wordt serotherapie (anti-thymocyten globuline [ATG] of alemtuzumab) gebruikt in de voorbereidende behandeling. Inherent aan het werkingsmechanisme van serotherapie, namelijk depletie van T-cellen, kan overmatige blootstelling aan serotherapie het immuunherstel na de transplantatie vertragen of zelfs volledig tegengaan. Traag immuunherstel maakt patiënten kwetsbaar voor virale infecties en een recidief van de leukemie.

In dit proefschrift werden de farmacokinetiek en de farmacodynamiek van serotherapie in allogene hematopoietische stamceltransplantatie bestudeerd om uiteindelijk tot een evidence-based doseeradvies te komen. Hiernaast wordt de rol van traag immuunherstel, de belangrijkste toxiciteit van serotherapie, onderzocht als voorspeller van uitkomst van de transplantatie. Als laatste wordt inzicht gegenereerd in de farmacokinetiek van antilichamen in kinderen.

Geïndividualiseerde dosering van ATG: klinische implementatie

De dosering van ATG was traditioneel gebaseerd op extrapolatie van de volwassen dosering. Kinderen kregen over het algemeen een dosis van 10 mg/kg, ongeacht de leeftijd of lichaamsgewicht, en kregen de eerste dosis ATG 5 dagen voor de infusie van de stamcellen. Door op

deze manier te doseren wordt verondersteld dat de farmacokinetiek (pharmacokinetics; PK) en farmacodynamiek (pharmacodynamics; PD) rechtlijnig toenemen met lichaamsgewicht. Echter, de resultaten uit hoofdstuk 3 laten zien dat de PK van ATG niet lineair toeneemt met gewicht, en hiernaast afhankelijk is van het aantal lymfocyten in het bloed voor de eerste infusie van ATG. Hiernaast is de PD van ATG afhankelijk van de stamcelbron, en wordt de blootstelling aan ATG na transplantatie beïnvloed door de startdag van ATG ten opzichte van infusie van de stamcellen. Een geïndividualiseerd doseerregime zoals geïntroduceerd in hoofdstuk 2 lijkt een aantrekkelijke optie voor ATG. In een geïndividualiseerd doseer-regime wordt de dosis voor een individuele patiënt bepaald door de karakteristieken van die patiënt om zodoende tot de meest optimale blootstelling en effecten te komen[2–5]. Voor ATG moeten zowel de dosering als de timing van ATG individueel worden aangepast om zo tot een optimale blootstelling voor en na infusie van het transplantaat te komen. Om de blootstelling verder te optimaliseren zou gebruik kunnen worden gemaakt van zogenaamd therapeutic drug monitoring (TDM)[6–8]. Hierbij worden de concentraties die na de eerste doses gemeten wordt gebruikt om de volgende doses aan te passen indien nodig. Beide hierboven beschreven methodes zijn inmiddels geïmplementeerd voor ATG in de klinische zorg op basis van de resultaten uit dit proefschrift.

Op basis van de farmacokinetische en farmacodynamische analyses uit de hoofdstukken 3 en 5 werd een doseerregime opgesteld voor ATG in kinderen. De optimale dosering hangt hierbij af van 3 factoren te weten 1) lichaamsgewicht, 2) het aantal lymfocyten in het bloed voor de eerste infusie van ATG, en 3) de stamcelbron. Patiënten met een hoger lichaams-gewicht, lager lymfocytengetal, of die navelstrengbloed (cord blood, CB) als stamcelbron hadden kregen een lagere dosis in uitgedrukt mg/kg dan patiënten met lager lichaamsge-wicht, hogere lymfocytengetallen en beenmerg (BM) of perifeer bloed stamcellen (PBSC) als stamcelbron. Hiernaast wordt de eerste gift ATG 9 dagen voor de transplantatie gegeven in plaats van de voorheen gebruikelijke 5 dagen, dit om de blootstelling aan ATG voor de SCT te verhogen terwijl de blootstelling na de SCT wordt verlaagd.

De effectiviteit van dit doseerregime wordt momenteel geëvalueerd in een prospectieve, open-label, fase II klinische studie getiteld "**P**rospective **A**nalysis of an individualized do-sing **R**egimen of **A**TG (Thymoglobulin) in **C**hildren undergoing **H**CT: red**U**cing **T**oxicity and improving **E**fficacy – a single arm phase II study", kortweg de PARACHUTE-studie. Deze studie is geregistreerd in het Nederlands Trial Register onder nummer NTR4960. Het primaire doel in deze studie is te beoordelen of geïndividualiseerde dosering van ATG leidt tot een verbetering in immuunherstel van CD4+ T-cellen. Secundaire eindpunten zijn de overleving, transplantatieziekte, afstoting, recidieven van leukemie, virale infecties en validatie van het PK-model. De uitkomsten van deze studie zullen worden vergeleken met een recent behandeld cohort in dezelfde centra. Alle kinderen die in de twee Nederlandse

transplantatiecentra worden behandeld kunnen meedoen aan deze studie. De resultaten worden in 2018 verwacht.

De resultaten uit deze thesis worden ook door andere onderzoeksgroepen gebruikt. Zo is het geïndividualiseerde doseerregime gebruikt in de CHAMP-studie (BMT-CTN 1502), een Amerikaanse studie die de meest optimale conditionering onderzoekt in kinderen en jong volwassenen met aplastische anemie. Ook in deze studie worden bloedmonsters van de kinderen verzameld om later het PK-model verder te ontwikkelen. Hiernaast wordt het belang van optimale ATG-blootstelling erkend in de ontwikkeling van cellulaire therapieën na navelstrengbloedtransplantatie. Als laatste wordt frequent advies gegeven aan centra wereldwijd voor de dosering ATG in individuele patiënten. Op moment van schrijven hebben meer dan 50 patiënten, behandeld buiten de bovengenoemde studies, ATG ontvangen middels een geïndividualiseerde dosering.

Voor hoog-risico patiënten op rejectie, transplantatieziekte en/of doorlopende ernstige infecties die een navelstrengbloed transplantatie ontvangen is geïndividualiseerde ATG met TDM geïntroduceerd. Dit wordt momenteel gebruikt voor patiënten met inflammatie met een intacte of geactiveerde T-cel functie. Deze patiënten krijgen een dosering ATG die leidt tot een zeer hoge blootstelling voor infusie van het transplantaat, met een zeer lage blootstelling na de infusie. Momenteel werd deze manier van doseren gebruikt in 9 patiënten met een zeer hoog risicoprofiel met veelbelovende resultaten.

Voorkomen van transplantatieziekte na SCT: de rol van antigeen presenterende cellen

Het voorkomen van transplantatieziekte is een van de belangrijkste redenen voor het gebruik van ATG en alemtuzumab in de conditionering. Het wordt vooral gebruikt van *in-vivo* depletie van T-cellen die met het transplantaat worden geïnfundeerd, hoewel ook immuun modulerende effecten aan ATG worden toegeschreven[9]. Door het productieproces bevat ATG epitopen gericht tegen een groot aantal markers op het celoppervlak waaronder die op T-cellen. De meeste epitopen richten zich tegen markers op T-cellen, echter ook andere celtypen kunnen worden herkend, waaronder natural killer (NK) cellen, monocyten en dendritische cellen. Alemtuzumab aan de andere kant is een monoklonaal antilichaam dat bindt aan CD52, wat voornamelijk tot expressie komt op T-cellen, B-cellen en NK-cellen[10]. Hoewel serotherapie dus voornamelijk gericht is op depletie van T-cellen worden andere cellen betrokken bij de ontwikkeling van GvHD ook gedepleteerd.

De pathofysiologie van transplantatieziekte laat zich beschrijven door een drie-fasen-model[11]. In de eerste fase worden antigeen presenterende cellen (APC) geactiveerd, dit betreft voornamelijk dendritische cellen (DC). De conditionering en de onderliggende ziekte zijn de meest genoemde oorzaken voor deze activering. In de tweede fase worden donor T-cellen geactiveerd door de DC's, waarna deze differentiëren, prolifereren en migreren.

Een van de belangrijkste eiwitten in deze activatie is human leukocyte antigen (HLA). Een mismatch in HLA leidt tot een ernstigere reactie in vergelijking met gematchte HLA-paren tussen donor en ontvanger[12]. De derde fase betreft de destructie van weefsel in longen, lever en darmen door een combinatie van cellulaire en inflammatoire effectoren. De derde fase leidt tot toegenomen weefselbeschadiging wat de eerste fase verder versterkt, en dus leidt tot een vicieuze cirkel. Het merendeel van de therapieën richt zich op de tweede fase van transplantatieziekte, waaronder donor T-cel depletie door ATG.

De depletie van donor T-cellen is effectief in het voorkomen van transplantatieziekte[13–15]. Deze depletie heft echter ook de perifere expansie van deze T-cellen op. Dit leidt tot ver-traagde of afwezig immuunherstel van T-cellen tot thymopoiese aanvangt, dit kan maanden tot jaren duren. Donor T-cellen zijn belangrijk in het voorkomen van virale reactivaties welke tot ernstige ziekte en dood kunnen leiden (hoofdstuk 10). Hiernaast hebben donor T-cellen een centrale rol in het zogenaamde graft-versus-leukemie effect, waarin donor T-cellen een anti-leukemische respons bewerkstelligen (hoofdstukken 5, 6 en 9). Dit wordt gereflecteerd door grote klinische trials, waarin de inclusie van serotherapie leidt tot een vermindering in GvHD, maar geen verbetering in overleving[16,17]. Dit laatste zou veroorzaakt kunnen door toegenomen sterfte door recidieven leukemie en virale reactivaties door slecht T-cel herstel[13,18,19]. De behandelaar staat voor een dilemma: de toevoeging van serotherapie in de conditionering heeft zowel positieve als negatieve effecten.

In de eerste grote analyse naar de blootstelling aan ATG als voorspeller van klinische uit-komsten (hoofdstuk 5) was de blootstelling aan ATG na de infusie van het transplantaat geen voorspeller voor de incidentie van acute transplantatieziekte in kinderen. Met andere woorden: de mate van depletie van donor T-cellen is geen voorspeller van het ontstaan van transplantatieziekte. Aan de andere kant werd immuunherstel wel degelijk beïnvloed door de blootstelling aan ATG na de transplantatie. Een hogere blootstelling voor infusie van het transplantaat leidde echter wel tot een verminderde incidentie van transplantatieziekte. Onze hypothese was dan ook dat niet donor T-cel depletie, maar depletie van APC's van de ontvanger belangrijk is het voorkomen van transplantatieziekte.

Vergeleken met donor APC's zijn de APC's van de ontvanger meer potent in het induceren van acute transplantatieziekte[20,21]. APC's van de ontvanger kunnen onder meer gevonden worden in het bloed (hematopoietische APC's) en in andere organen (niet-hematologische APC's). Binnen de APC's zijn hematopoietische APC's een minder belangrijke speler in de inductie van transplantatieziekte, en worden hiernaast veelal gedepleteerd door de voorbe-reidende chemotherapie[20–23]. De non-hematopoietische APC's hebben hierom een centrale rol in het ontwikkelen van acute transplantatieziekte. Dit is in lijn met onze resultaten: hoge blootstelling aan ATG voor de infusie van de stamcellen voorkomt transplantatieziekte,

potentieel door het depleteren van de non-hematopoietische APC's in darm, long en lever. In dierstudies is aangetoond dat alemtuzumab distribueert naar deze organen[24]. Tevens worden in hondenstudies hogere concentraties van anti-hond konijnen immuunglobu- line, vergelijkbaar met ATG in mensen, gevonden in de lever in vergelijking met bloed[25]. Hiernaast is het belangrijk te noemen dat APC's van de ontvanger geen rol hebben in het graft-versus-leukemie effect[26,27]. Een ruime blootstelling aan ATG voor de infusie van de stamcellen lijkt dus een aantrekkelijke manier om acute transplantatieziekte te voorkomen. Gecombineerd met een lage blootstelling na de transplantatie kan dit het beste van twee werelden geven: bescherming tegen transplantatieziekte gecombineerd met snel immuun- herstel en dus bescherming tegen virale infecties en recidieven.

De hierboven beschreven hypothese lijkt niet op te gaan voor volwassen patiënten die een PBSC transplantatie krijgen na mildere conditionering (hoofdstuk 7). In deze setting wordt de incidentie van acute transplantatieziekte vooral bepaald door de blootstelling aan ATG na de transplantatie. Wel moet worden gezegd dat vrijwel alle patiënten een voldoende ruime blootstelling voor de transplantatie hadden. Ruime depletie van APC's lijkt dus niet voldoende in deze volwassen setting; er lijkt nog steeds wat *in-vivo* depletie van donor T- cellen nodig te zijn. Dit zou kunnen worden veroorzaakt door de milde chemotherapie, het hoge aantal T-cellen wat bij dit soort transplantaties wordt geïnfundeerd, of een combinatie van beiden. Meer onderzoek is nodig om dit verschil in optimale blootstelling tussen de beide cohorten te onderzoeken.

Concluderend zou de depletie van APC's van de ontvanger een van de werkingsmechanis- mes van ATG kunnen zijn. Dit gaat in tegen de huidige gedachte dat *in-vivo* lymfodepletie van donor T-cellen het primaire doel van serotherapie is. Deze hypothese lijkt echter niet op te gaan voor alle patiënten, waaronder milde conditionering en PBSC transplantaties. Meer onderzoek is nodig om voor alle patiënt-donor-conditionering-combinaties.

ATG versus alemtuzumab vanuit een PK/PD-perspectief

De keuze tussen de twee beschikbare geneesmiddelen voor serotherapie is veelal gebaseerd op een voorkeur van het centrum of de behandelend arts. Er zijn enkele studies beschikbaar die de beide middelen vergelijken, waarin de incidentie van transplantatieziekte minder lijkt te zijn na alemtuzumab[14,28–31]. Dit voordeel wordt echter tenietgedaan door zeer matig immuunherstel in vergelijking tot ATG[28,32] waardoor de overleving vergelijkbaar is na behandeling met de twee middelen[14,29,31]. De meeste studies vergelijken echter het gebruik van ATG versus alemtuzumab, doseringen of blootstelling werd niet onderzocht in dit onderling vergelijk.

Er zijn aanzienlijke verschillen tussen ATG (Thymoglobulin) en alemtuzumab wat betreft farmacokinetiek en farmacodynamiek in kinderen. Het meest in het oog springende verschil tussen de beide middelen is het verschil in klaring, welke 10 maal (lineaire klaring) en 2 maal (maximale eliminatiesnelheid van de verzadigbare klaring) lager voor alemtuzumab in vergelijking met ATG (hoofdstuk 3 en 4). De onverklaarde variabiliteit tussen patiënten is ook groter voor alemtuzumab (hoofdstuk 3 en 4). Hiernaast is de klaring van ATG hoger in patiënten met een hoog aantal lymfocyten in het bloed, terwijl dit de klaring van alemtuzumab niet beïnvloed. Wat betreft farmacodynamiek is de zogenaamde lymfolytische concentratie lager van alemtuzumab (0.1 μg/mL) dan die van ATG (1 AU/ml), zoals aangegeven in de onderstaande figuur 1[33,34]. Door de lagere klaring zal de blootstelling aan alemtuzumab na de infusie van de stamcellen relatief groter zijn dan bij ATG na standaard dosering en vergelijkbare startdag. Hierdoor zullen patiënten langer blootgesteld worden aan lymfolytische concentraties bij gebruik van alemtuzumab. Hiernaast is de farmacokinetiek minder voorspelbaar in vergelijking met ATG.

De blootstelling aan alemtuzumab heeft een beperkte invloed op de klinische uitkomsten van de SCT wat betreft transplantatieziekte (hoofdstuk 8). Er werd geen invloed van alemtuzumab blootstelling op overleving of T-cel reconstitutie gevonden. Hiernaast bereikten weinig patiënten de eerder in de literatuur beschreven optimale concentratie op de dag van de infusie van stamcellen[1]. Deze observaties suggereren dat de in de twee studiecentra gebruikelijke dosering alemtuzumab (0.5-1 mg/kg/dag vanaf dag -8) leidt tot een te hoge blootstelling.

Aangezien vroege T-cel reconstitutie een belangrijke voorspeller is voor overleving zouden de doseringen van serotherapie zo moeten worden gekozen dat de *in-vivo* lymfodepletie tot een minimum wordt beperkt. Gezien de relatief langzame en matig voorspelbare klaring en de hogere potentie van alemtuzumab lijkt ATG een aantrekkelijkere keuze te zijn in kinderen bij gebruik van een geïndividualiseerd doseerregime.

Geïndividualiseerd doseren: de toekomst van farmacotherapie bij kinderen

De groei van een kind maakt dat de farmacokinetiek en de farmacodynamiek veranderd, wat zijn weerslag kan hebben op de farmacologische effecten van geneesmiddelen[35]. Deze veranderingen zijn veelal niet rechtlijnig met leeftijd of lichaamsgewicht. Desondanks wordt het merendeel van de geneesmiddelen voor kinderen gebruikt op basis van een vaste dosering uitgedrukt in mg/kg. Hierbij gaat men uit van een lineaire relatie tussen lichaamsgewicht en PK/PD parameters. Hiernaast wordt, vooral in een academische setting, de ruime meerderheid van de geneesmiddelen in kinderen gebruikt zonder dat deze voor deze indicaties of überhaupt voor kinderen zijn geregistreerd[36]. Voor deze geneesmiddelen is weinig of geen onderzoek in kinderen verricht, en de dosering voor deze geneesmiddelen is vaak een extrapolatie van de volwassen dosis. Dit soort empirische doseeradviezen kan leiden tot ernstige over- of onder-dosering in de verschillende leeftijdscategorieën van kinderen, en
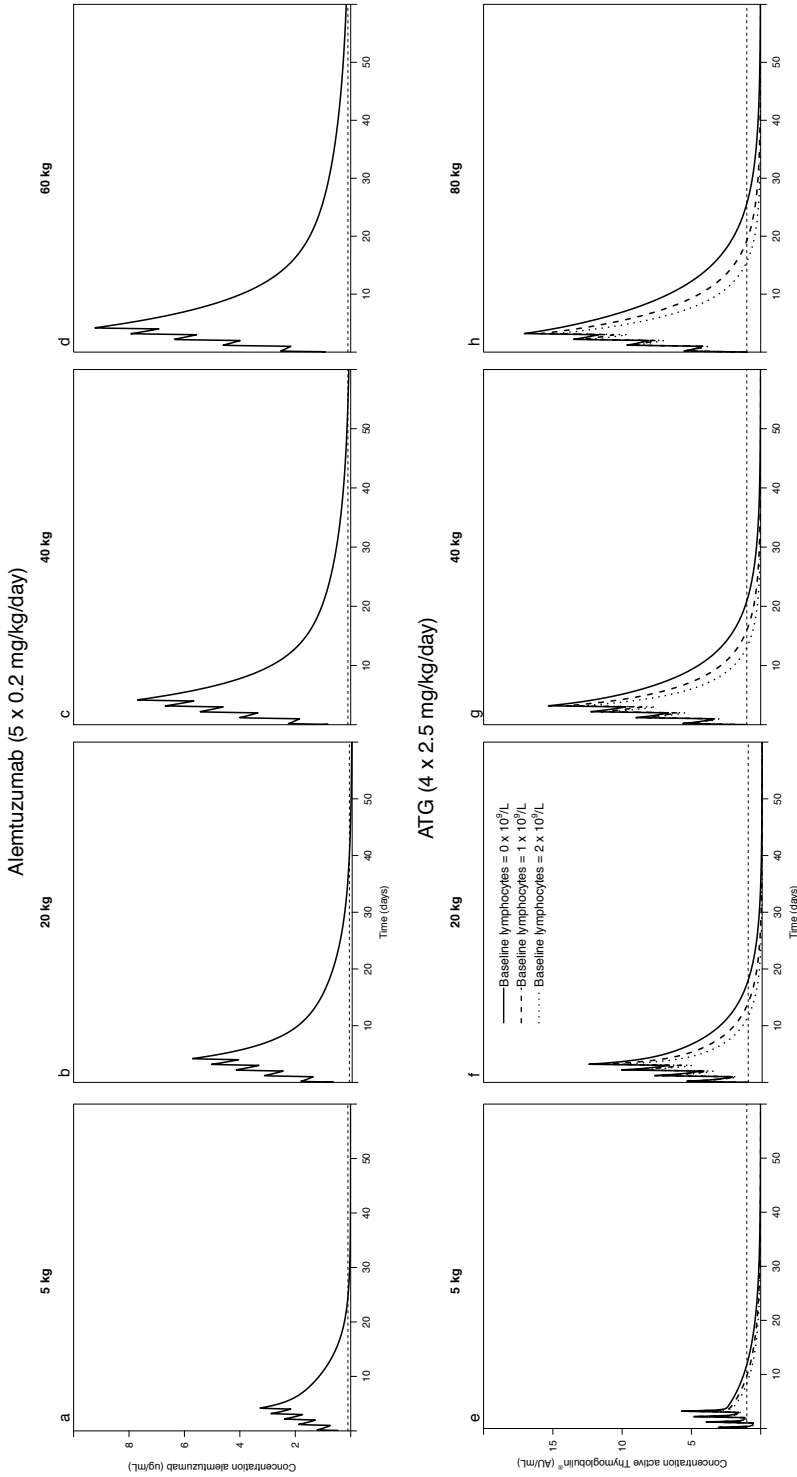
Figuur 1. Gesimuleerde blootstelling na standaard dosering van alemtuzumab (cumulatief 1 mg/kg gedurende 4 dagen, paneel e-h) in kinderen met een gewicht van 5, 20, 40 en 80 kg. ATG blootstelling is gestratificeerd voor lymfocyten in het bloed. Gestreepte lijnen: lymfolitische concentraties.

kan leiden tot ongewenste en onvoorspelbare geneesmiddeleffecten. Geïndividualiseerde dosering gebaseerd op PK/PD modelleren zal leiden tot een belangrijke verbetering in farmacotherapie bij kinderen, en zal daarmee de uitkomsten van behandeling verbeteren[2,4,8].

Empirisch doseren kan ernstig negatieve effecten hebben zoals beschreven in deze thesis. ATG werd decennia gebruikt in een dosering van 10mg/kg bij kinderen, waarbij alleen grof rekening wordt gehouden met lichaamsgewicht. Dit leidt tot ernstige overdosering bij de grote kinderen en de kinderen met lage lymfocytenaantallen voor SCT. De dosering ATG in volwassenen varieert, hoewel alle doseeradviezen een vaste dosering in mg/kg voorschrijven. Ook bij volwassenen hebben wij laten zien dat vaste dosering per kilogram lichaamsgewicht leidt tot een sterk variabele blootstelling. Dit is belangrijk gezien de aangetoonde relatie tussen blootstelling aan ATG na de SCT en overleving van de procedure. In kinderen leidt een optimale blootstelling aan ATG tot over 90% overleving na 5 jaar, terwijl dat bij overmatige blootstelling slechts 57-76% bedraagt (hoofdstuk 5). Om dit verschil in perspectief te plaatsen: deze verschillen in uitkomsten zijn vergelijkbaar met de recente grote interventies in hematologie en oncologie[37,38], hoewel de resultaten van deze studie gevalideerd moeten worden in een prospectieve studie. Wij verwachten dat geïndividualiseerde dosering van ATG gericht op een optimale blootstelling een verbetering in overleving zal geven. Verder zal een verbeterde veiligheid van de procedure ook invloed kunnen hebben op de selectie van patiënten. Zo kan SCT eerder in de behandeling worden ingezet van ziekten waar het nu al geïndiceerd is (bijvoorbeeld aplastische anemie, leukemie). Hiernaast kan een veiligere SCT een behandeling vormen voor ziektes waar de risico's te groot zijn in de balans tussen voor- en nadelen. Hierbij kan gedacht worden aan auto-immuunziektes die niet reageren op steroïden en immunotherapie, en mildere fenotypes van metabole ziektes.

Verder onderzoek moet zich richten op geneesmiddelen met een kritieke therapeutische breedte. Hieronder vallen andere geneesmiddelen die binnen SCT worden gebruikt (fludarabine, prednison), maar ook chemotherapie en thyrosine kinase inhibitors (TKI) in kinderoncologie en antilichamen zoals gebruikt worden in de kinderimmunologie. Voor veel van deze middelen is geen evidence-based doseerrichtlijn beschikbaar, terwijl ze een belangrijk onderdeel van de behandeling vormen. Hiernaast zijn dit complexe ziekten waar verschillende middelen naast elkaar gebruikt worden die ook invloed op elkaar kunnen hebben. Een PK/PD model waarin verschillende geneesmiddelen naast elkaar worden gemodelleerd en samen uitkomst te voorspellen is daarom nodig; deze wordt momenteel gemaakt voor SCT.

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Individualisering van doseringen leidt tot sterke variabiliteit in de uiteindelijke dosis, wat mogelijk kan leiden tot doseerfouten. Oplossingen hiervoor kunnen gezocht worden in doseertabellen[39] of doseergrafieken, hoewel de meest voor de hand liggende oplossing een

digitaal systeem zal zijn. Dit kan zowel online als geïntegreerd in de ziekenhuisinformatie-systemen. Dit is een grote verandering in de manier van voorschrijven van medicatie, artsen moeten dan ook goed getraind worden in deze systemen. De technische implementatie van de systemen kan ingewikkeld zijn, de grootste uitdaging ligt echter bij het overtuigen van artsen van het nut van geïndividualiseerd doseren. Het is een grote verandering in farmacotherapie in kinderen in vergelijking met de huidige praktijk. Kennis over farmacologie in kinderen moet een grotere rol krijgen in de opleiding van artsen, vooral waar het de relatie tussen een opgroeiend kind en de effecten van geneesmiddelen betreft. Kinderartsen moet actief betrokken worden bij de ontwikkeling van geïndividualiseerde doseerregimes, zowel voor het includeren van patiënten alsmede vroege participatie en implementatie in de kliniek. Als laatste moeten gelden beschikbaar komen om dit type onderzoek te financieren. De kosten voor de ontwikkeling van geïndividualiseerde dosering zijn relatief laag en kunnen in beperkte tijd worden verricht. De klinische impact kan groot en belangrijk zijn, wat dit type onderzoek een aantrekkelijk veld maakt in een kosten-baten analyse.

Concluderend benadrukken deze voorbeelden het belang van geïndividualiseerde dosering, vooral bij kinderen. Geïndividualiseerde dosering kan de veiligheid en de effectiviteit van geneesmiddelen verbeteren, en hierdoor klinische uitkomst verbeteren.

ALGEMENE CONCLUSIE

Veel geneesmiddelen worden ongeregistreerd en off-label gebruikt bij kinderen, vaak zonder goed gefundeerd doseeradvies. Dit is ook het geval bij anti-thymocyten globuline (ATG) en alemtuzumab, welke worden gebruikt voor lymfodepletie in hematopoietische stamceltransplantatie (SCT) ter voorkoming van transplantatieziekte en afstoting. Traag of afwezig immuunherstel, vooral dat van T-cellen, is de belangrijkste bijwerking, en heeft een belangrijk aandeel in de morbiditeit en mortaliteit van deze behandeling. Deze thesis beschrijft de populatie farmacokinetiek (pharmacokinetics; PK) en farmacodynamiek (pharmacodynamics; PD) van ATG en alemtuzumab teneinde geïndividualiseerde doseeradviezen met voorspelbare effectiviteit en veiligheidsbalans te ontwikkelen voor beide middelen.

De farmacokinetiek van ATG en alemtuzumab kan goed worden beschreven met de door ons ontwikkelde populatie PK-modellen. In beide modellen namen de farmacokinetische parameters niet lineair toe met leeftijd of lichaamsgewicht. De huidige op lichaamsgewicht gebaseerde dosering van ATG en alemtuzumab leidt hierom tot grote variatie in blootstelling tussen de verschillende leeftijdscategorieën op de kinderleeftijd. Wij toonden aan dat de blootstelling aan ATG en alemtuzumab wordt bepaald door lichaamsgewicht en het aantal

lymfocyten voor de eerste dosis (laatstgenoemde alleen bij ATG). Hierbij zagen wij dat de klaring van ATG in patiënten boven de 50 kg (adolescenten en volwassenen) niet verder toeneemt met lichaamsgewicht. Dit laat zien dat in patiënten boven 50 kg beter een vaste dosis gebruikt kan worden dan een dosis op basis van mg/kg teneinde een vergelijkbare blootstelling te krijgen.

Tijdig immuunherstel van CD4+ T-cellen na SCT is belangrijk in het voorkomen van virale reactivaties en recidieven van leukemie, en vergroot hierdoor de kans op overleving. Hoge blootstelling aan ATG na de infusie van het transplantaat verkleint de kans op immuun-herstel van CD4+ T-cellen. Hierdoor heeft de blootstelling aan ATG een grote impact op de klinische uitkomsten, inclusief overleving, na SCT bij kinderen en volwassenen. Verder is T-cel herstel vaak traag bij navelstrengbloed-transplantatie wanneer normale doseringen van ATG worden gebruikt, wat als nadeel van dit type transplantatie wordt gezien. Het immuunherstel na navelstrengbloed-transplantatie is echter uitstekend wanneer de bloot-stelling aan ATG na de SCT erg laag is.

Het beschermende effect van ATG voor transplantatieziekte en afstoting is bepaald door de blootstelling aan ATG vóór de infusie van de graft, althans in kinderen. Echter, bij volwassenen die na milde chemotherapie een SCT met hoge aantallen T-cellen (perifeer bloed stamceltransplantatie) krijgen is een bepaalde hoeveelheid blootstelling na de SCT noodzakelijk. Blootstelling aan alemtuzumab aan de andere kant bepaalt de incidentie van acute transplantatieziekte, maar niet de toxiciteits-eindpunten, mogelijk wijzend op een relatieve overdosis bij de huidige doseringen.

Wij concluderen dat individualisering van de dosering en de timing van ATG de stamcel-transplantatie potentieel veiliger en effectiever maakt, en de kans op overleving vergroot. Geïndividualiseerde doseerregimes voor ATG bij kinderen zijn ontwikkeld op basis van de resultaten uit deze thesis, en worden momenteel geëvalueerd voor effectiviteit en veiligheid in prospectieve klinische trials.
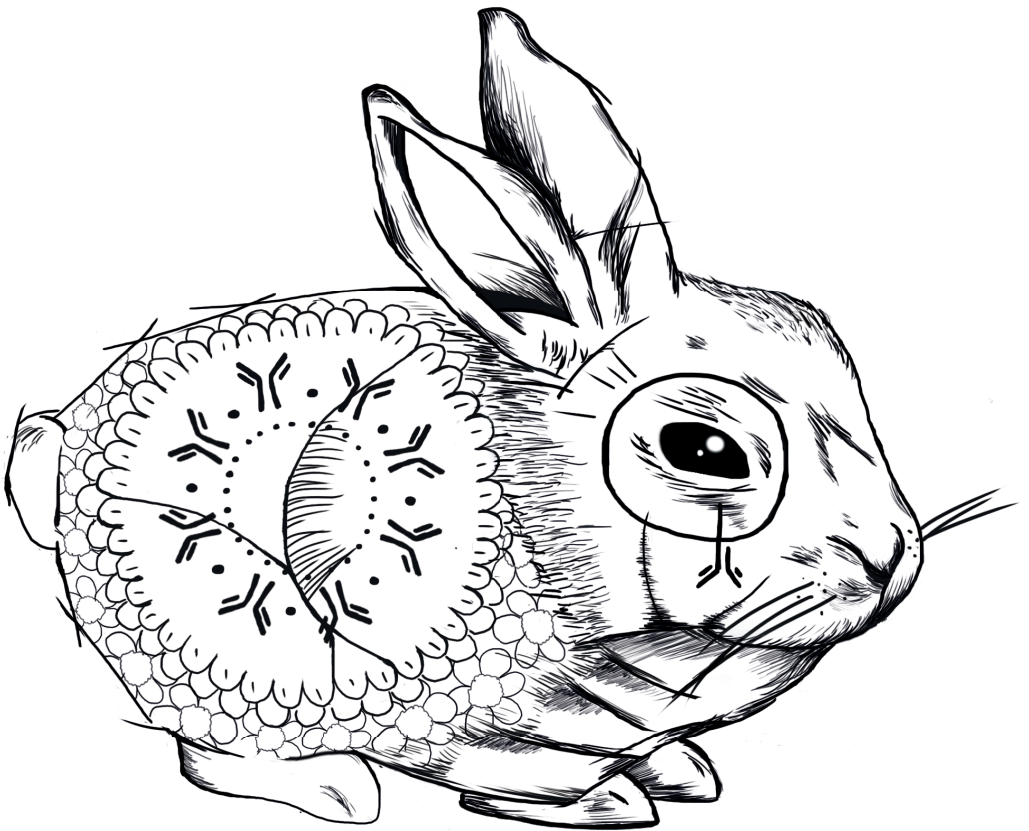
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Chapter 14

Appendices

Curriculum Vitae
List of Publications
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Dankwoord/Acknowledgements

CURRICULUM VITAE

Rick Admiraal was born on February 1st 1984 in Beverwijk, the Netherlands. He attended secondary school at the Jac. P. Thijssecollege in Castricum where he graduated in 2002. That year, he started his medical studies at the University of Amsterdam. He did his research internship at the Department of Pediatric Oncology. As part of his clinical rotations, he did a 5-month elective internship in pediatric oncology at the Women's and Children's Hospital in Adelaide, Australia. After obtaining his medical degree in 2011, he worked as a resident at the department of pediatrics at Tergooi Ziekenhuizen in Blaricum. In 2012, he was appointed PhD-student at the University Medical Center Utrecht (UMCU), the Leiden Academic Centre for Drug Research (LACDR) and the Leiden University Medical Center (LUMC) under the supervision of prof. dr. Catherijne Knibbe, dr. Jaap Jan Boelens and dr. Robbert Bredius. His studies focus on individualized dosing of serotherapy in hematopoietic cell transplantation, and have resulted in this thesis. He received the best abstract award at the annual meeting of the American Society for Bone Marrow Transplantation in 2013 and 2015 (pharmacology) and at the International Cord Blood Symposium 2016. During his PhD, he was trained as a clinical pharmacologist in the UMC Utrecht. In his spare time, he volunteers at a sailing camp for children with cancer, where he later joined the board. He currently works as a resident pediatrics at the Meander Medisch Centrum in Amersfoort. From 2017, he starts his residency in pediatrics at the University Medical Center Utrecht. Rick lives in Amsterdam together with his partner Jacqueline van Gorp.

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LIST OF ABBREVIATIONS

ADA	Anti-Drug Antibodies
ADCC	Antibody-Dependent Cell Mediated Cytotoxicity
AdV	Adenovirus
AIC	Akaike Information Criterion
ALC	Absolute Lymphocyte Count
ALL	Acute Lymphoid Leukemia
AML	Acute Myeloid Leukemia
APC	Antigen-Presenting Cell
ATG	Anti-Thymocyte Globulin
AU	Arbitrary Unit
AUC	Area Under the Curve
BDE	Bodyweight-Dependent Exponent
BK	BK-virus
BLQ	Below the Limit of Quantification
BM	Bone Marrow
BMF	Bone Marrow Failure
Campath	Registered brand of alemtuzumab
CAR	Chimeric Antigen Receptor
CB	Cord Blood
CBT	Cordblood Transplantation
CD4+ IR	Immune reconstitution of CD4+ T-cells (T-helper cells)
CDC	Cell-Dependent Cytotoxicity
C_{graft}	Peri-transplant concentrations
C_{HCT}	Concentration at the time of HCT
CL	Clearance
CLL	Chronic Lymphoid Leukemia
C_{max}	Maximum Concentration
CMV	Cytomegalovirus
CsA	Cyclosporin A
CTL	Cytotoxic T-lymphocyte
CWRES	Conditional Weighted Residuals
DC	Dendritic Cell
EBV	Epstein-Barr Virus
EFS	Event-Free Survival
ELISA	Enzyme-Linked Immuno Sorbent Assay
EMA	European Medicines Agency

FDA	Food and Drug Administration
FOCE-I	First Order Conditional Estimation with Interaction
GF	Graft Failure
GOF	Goodness of Fit plots
GvHD	Graft-versus-Host-Disease
GvL	Graft-versus-Leukemia
HCT	Hematopoietic Cell Transplantation
HHV6	Human Herpes Virus 6
HLA	Human Leukocyte Antigen
HR	Hazards Ratio
iBW	Ideal Body Weight
IEM	Inborn Errors of Metabolism
IgG	Immunoglobulin G
IQR	Interquartile Range
IR	Immune Reconstitution
K_m	Michaelis-Menten constant
LBW	Lean Body Weight
MMF	Mycophenolate Mofetil
MMUD	Mismatched Unrelated Donor
MPS	Mucopolysaccharidosis
MRD	Minimal Residual Disease
MSD	Matched Sibling Donor
MUD	Matched Unrelated Donor
NONMEM	Non-Linear Mixed Effects Modeling (software)
NPDE	Normalized Prediction Distribution Errors
NRM	Non-Relapse Mortality
OFV	Objective Function Value
OR	Odds Ratio
OS	Overall Survival
PARACHUTE	Prospective trial to evaluate individualized ATG dosing
PBPK	Physiology-Based Pharmacokinetics
PBSC	Peripheral Blood Stem Cells
PD	Pharmacodynamics
PID	Primary Immune Deficiency
PIRCHES	predicted indirectly recognizable HLA epitope
PK	Pharmacokinetics
PsN	Pearl speaks NONMEM (software)
Q-FACS	Quantative Fluorescence-Activated Cell Sorting
RIC	Reduced Intensity Conditioning

RRM	Relapse-Related Mortality
TBI	Total Body Irradiation
TCR	T-cell Repertoire
TDM	Therapeutic Drug Monitoring
TGF	Tumor Growth Factor
Thymoglobulin	Brand of ATG
TMDD	Target-Mediated Drug Disposition
TNC	Total Nucleated Cells
V_1	Central volume of distribution
V_{max}	Maximum elimination rate
VPC	Visual Predictive Check
WBC	White Blood Cell Count
WRES	Weighted Residuals

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