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

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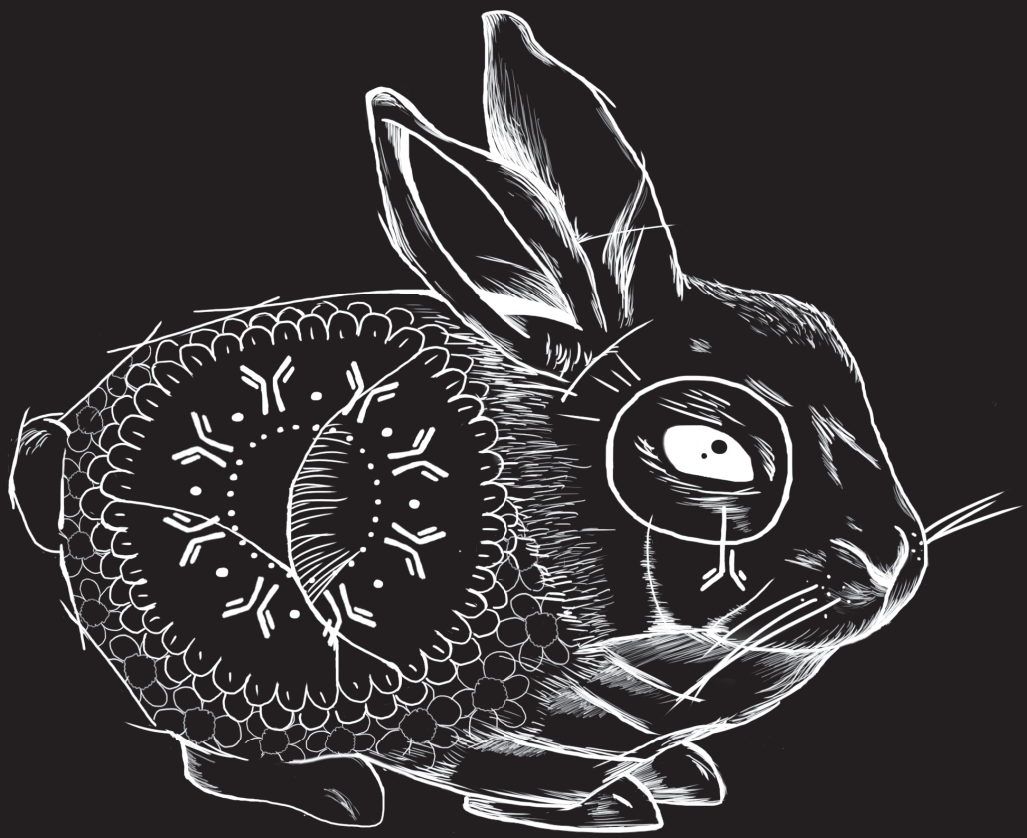
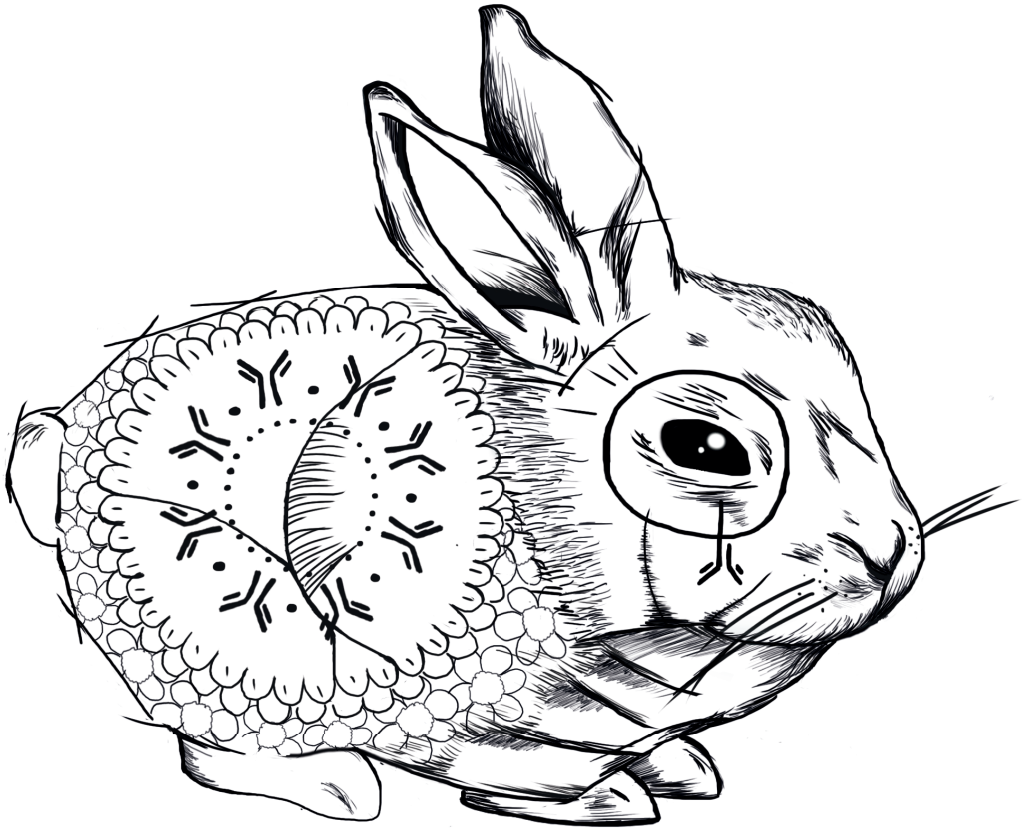


PART I

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.

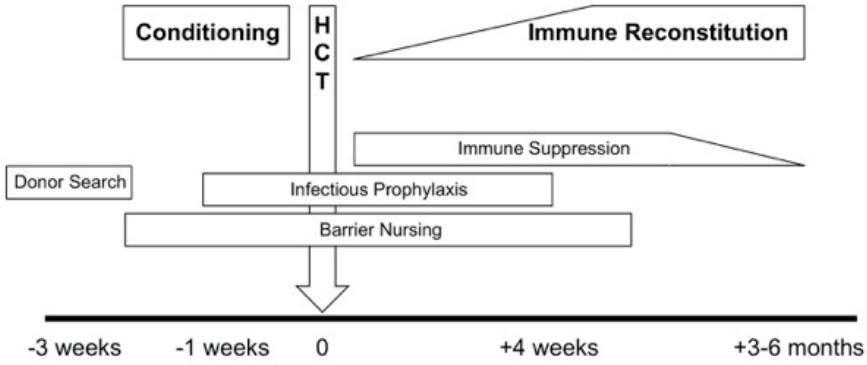


Figure 1. Overview of treatment plan for HCT

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 residual leukemic cells. Bone marrow depletion, or myeloablation, is mostly performed using chemotherapy, while some patients receive chemotherapy combined with total body irradiation (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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