

Review of the Clinical Pharmacokinetics and Pharmacodynamics of Alemtuzumab and Its Use in Kidney Transplantation

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Abstract Alemtuzumab is a humanized monoclonal antibody against CD52 and causes depletion of T and B lymphocytes, monocytes, and NK cells. Alemtuzumab is registered for the treatment of multiple sclerosis (MS) and is also used in chronic lymphocytic leukemia (CLL). Alemtuzumab is used off-label in kidney transplantation as induction and anti-rejection therapy. The objective of this review is to present a review of the pharmacokinetics, pharmacodynamics, and use of alemtuzumab in kidney transplantation. A systematic literature search was conducted using Ovid Medline, Embase, and Cochrane Central Register of controlled trials. No pharmacokinetic or dose-finding studies of alemtuzumab have been performed in kidney transplantation. Although such studies were conducted in patients with CLL and MS, these findings cannot be directly extrapolated to transplant recipients, because CLL patients have a much higher load of CD52-positive cells and, therefore, target-mediated clearance will differ between these two indications. Alemtuzumab used as induction therapy in kidney transplantation results in a lower incidence of acute rejection compared to basiliximab therapy and comparable results as compared with rabbit

anti-thymocyte globulin (rATG). Alemtuzumab used as anti-rejection therapy results in a comparable graft survival rate compared with rATG, although infusion-related side effects appear to be less. There is a need for pharmacokinetic and dose-finding studies of alemtuzumab in kidney transplant recipients to establish the optimal balance between efficacy and toxicity. Furthermore, randomized controlled trials with sufficient follow-up are necessary to provide further evidence for the treatment of severe kidney transplant rejection.

Key Points

Alemtuzumab, a monoclonal antibody against CD52, is registered for the treatment of multiple sclerosis, but is used off-label in patients with chronic lymphocytic leukemia and as induction and anti-rejection therapy after kidney transplantation.

Alemtuzumab causes a rapid and profound depletion of T and B lymphocytes, as well as various cells of the innate immune system. Reconstitution of cells from the innate immune system is faster (within 6 months) than that of T and B lymphocytes, which may take more than 1 year.

No pharmacokinetic studies of alemtuzumab exist for kidney transplant recipients. The results of the pharmacokinetic studies performed in patients with chronic lymphocytic leukemia could not be extrapolated directly to the kidney transplant population because patients with chronic lymphocytic leukemia have a much higher load of CD52-positive (tumor) cells.

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1 Introduction

Alemtuzumab (Campath-1H) is a humanized, rat monoclonal IgG₁ antibody with a molecular weight of approximately 150 kDa, directed against CD52. The depletion of donor T lymphocytes from stem cell transplants to eliminate graft-vs.-host disease was developed in the laboratory of Herman Waldmann and Geoff Hale at the University of Cambridge, UK [1]. The first anti-CD52 antibody developed was of the IgM class (Campath-1M), which was very effective in eliminating T lymphocytes *in vitro*. *In vivo*, there was a depletion of blood lymphocytes in stem cell transplant recipients, but there was no depletion of lymphocytes in the bone marrow and no effect on solid lymphoma masses or splenomegaly [1, 2]. This fueled further research and led to the development of a new IgG1 antibody (Campath-1G), which was found to result in long-lasting depletion of lymphocytes from both blood and bone marrow. A few years later, this antibody was humanized (Campath-1H) to reduce the anti-globulin responses (Fig. 1) [2–4].

In 2001, the US Food and Drug Administration and the European Medicines Agency approved alemtuzumab for the treatment of chronic lymphocytic leukemia (CLL) under accelerated approval regulations [5]. Later, alemtuzumab was also approved by the Food and Drug Administration (2014) and the European Medicines Agency (2013) for the treatment of relapsing-remitting multiple sclerosis (RRMS) and is currently marketed for this indication under the name Lemtrada[®] (Sanofi-Genzyme, Cambridge, Massachusetts, United States) [6].

Following the market approval of Lemtrada[®], the approval for the treatment of CLL was withdrawn (Fig. 1). However, alemtuzumab remains available for patients with CLL via the worldwide Campath Distribution Program [7]. In addition, alemtuzumab has also been used off-label for a variety of other diseases and conditions, including the prevention and treatment of acute rejection after solid organ transplantation (SOT).

In recent years, there has been a renewed interest in the use of alemtuzumab in SOT. In this review, we discuss the pharmacokinetics and pharmacodynamics of alemtuzumab, its use as induction and anti-rejection therapy in kidney transplantation, and strategies to improve the outcomes of alemtuzumab therapy.

2 Methods of Literature Search

A systematic literature search was performed (8 February, 2017) of Ovid MEDLINE, EMBASE, and the Cochrane Central Register of controlled trials. The search terms included ‘alemtuzumab’, ‘campath’, ‘pharmacokinetics’, ‘pharmacodynamics’, ‘induction therapy’, ‘rejection therapy’, and ‘adverse effects’ (see Electronic Supplementary Material). The search revealed 1668 articles. After exclusion of irrelevant articles (after reading the title and abstract), 730 articles remained, of which the relevant articles were included in this review. Examination of the reference list of the included studies identified further studies. There were no restrictions with regard to publication date. Only papers published in English were included.

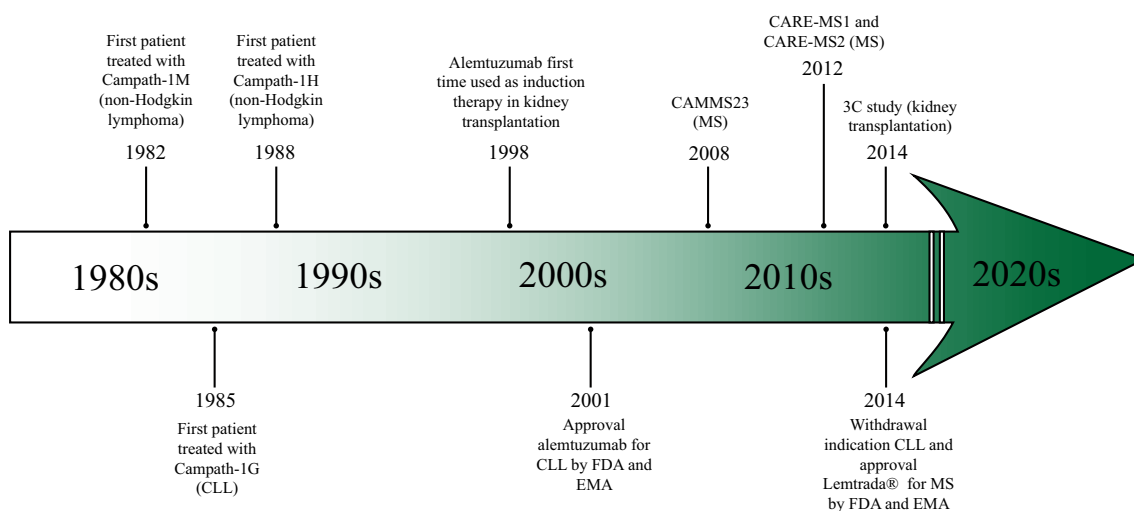


Fig. 1 Timeline of alemtuzumab. In the 1980s, alemtuzumab was called Campath and mainly used in hematology patients. Around 20 years later, alemtuzumab was approved for the treatment of chronic lymphocytic leukemia (CLL) and for the first time in kidney transplantation. A decade later, the registration of alemtuzumab for

CLL was withdrawn and alemtuzumab was approved as Lemtrada[®] for the treatment of multiple sclerosis (MS). In 2014, a large randomized controlled trial compared alemtuzumab induction therapy with basiliximab induction therapy. *EMA* European Medicines Agency, *FDA* US Food and Drug Administration

3 Pharmacodynamics of Alemtuzumab

CD52 is a 21–28 kDa cell surface glycoprotein attached to the cell membrane by a glycosylphosphatidylinositol anchor of 12 amino acids. CD52 is one of the most abundant membrane glycoproteins on T and B lymphocytes and is also expressed on natural killer (NK) cells, monocytes, macrophages, dendritic cells, and eosinophilic granulocytes and to a lesser extent on neutrophilic granulocytes [1, 8]. CD52 is not expressed on erythrocytes, platelets, and hematopoietic progenitor cells [9]. The exact function of CD52 is unknown but it is suggested that the molecule may be involved in T lymphocyte co-stimulation, the induction of regulatory T lymphocytes, and T lymphocyte migration and adhesion [10, 11].

Administration of alemtuzumab causes a profound depletion of T and B lymphocytes, NK cells, dendritic cells, granulocytes, and monocytes by three mechanisms: complement-dependent cytotoxicity (through C1q activation and subsequent generation of the membrane attack complex), antibody-dependent cellular cytotoxicity (after the activation of NK cells and macrophages through their IgG fragment C receptor), and induction of apoptosis (Fig. 2) [12, 13]. Depletion of peripheral lymphocytes occurs within 1 h after alemtuzumab administration. Lymphocyte depletion from secondary lymphoid tissues occurs over 3–5 days [14]. Alemtuzumab administration significantly depletes peripheral monocytes and NK cells [15].

Alemtuzumab has a long-lasting depletion effect. In kidney transplant recipients receiving alemtuzumab as induction therapy (40-mg total dose), B lymphocytes recovered after 12 months. In contrast, T lymphocyte numbers recovered to approximately 50% of baseline 36 months after alemtuzumab administration. CD8⁺ T lymphocytes repopulated more rapidly than CD4⁺ T lymphocytes [16]. Cells of the innate immune system reconstitute faster than cells of the adaptive immune system. After 1 month, more than half of the peripheral lymphocytes consists of NK cells and the number of NK cells returns to 60–80% of baseline by 6 months [17]. Monocytes are only mildly depleted and recover after 3 months [16]. Dendritic cells recover to baseline levels 6 months after alemtuzumab treatment [18].

Immunological reconstitution of T lymphocytes, either partial or complete, appears to occur predominantly through homeostatic proliferation of residual CD4⁺CD25⁺Forkhead box P3⁺ (FoxP3⁺) regulatory lymphocytes, as well as memory T lymphocytes and not by thymopoiesis [19]. Normally, levels of FoxP3⁺ regulatory T lymphocytes in kidney transplant recipients make up 3–4% of the total CD4⁺ population. After alemtuzumab treatment, a relative increase

of FoxP3⁺ regulatory T lymphocytes is seen (up to 12%), which persists for 2 years [20]. During immunological reconstitution, skewing of the immune system to a more anti-inflammatory pattern is observed: an increase in the percentage of the anti-inflammatory cytokines interleukin (IL)-10 and transforming growth factor- β 1 (produced by CD4⁺ and CD8⁺ cells), an increased percentage of IL-4-producing T-helper 2 cells, and decreased levels of proinflammatory cytokines IL-17 and interferon (IFN)- γ [21].

Anderson et al. described the reconstitution of T lymphocytes 12 years after treatment with alemtuzumab because of rheumatoid arthritis [22]. Twenty patients treated with alemtuzumab were compared with 13 age-matched patients with rheumatoid arthritis. Total CD4⁺ lymphocyte counts were lower in the alemtuzumab group compared with the controls (median $0.55 \times 10^9/L$ vs. $0.85 \times 10^9/L$; $p = 0.0014$). The naïve and central memory CD4⁺ lymphocytes were significantly reduced in the alemtuzumab-treated patients [$0.09 \times 10^9/L$ vs. $0.21 \times 10^9/L$ ($p = 0.0007$) and $0.1 \times 10^9/L$ vs. $0.36 \times 10^9/L$ ($p < 0.0001$), respectively]. However, effector memory CD4⁺ lymphocyte counts were not different. Total CD8⁺ lymphocytes were similar in both groups, but the naïve and central memory CD8⁺ lymphocytes were significantly lower in the alemtuzumab-treated patients [$0.05 \times 10^9/L$ vs. $0.07 \times 10^9/L$ ($p = 0.0061$) and $0.02 \times 10^9/L$ vs. $0.04 \times 10^9/L$ ($p = 0.0342$)] [22].

B lymphocyte reconstitution in patients treated with alemtuzumab coincides with a high level of the cytokine B lymphocyte activating factor (BAFF, also known as TALL-1, BLYS, THANK, and zTNF4), which persists for over 12 months [23, 24]. From the second month after alemtuzumab administration, B lymphocytes start to repopulate. First, the transitional B lymphocytes dominate, followed by Bm2⁺ (mature naïve) B lymphocytes [25]. Differentiation to memory B lymphocytes is slow and reaches 25% of baseline after 12 months [23, 24]. After alemtuzumab induction therapy, there is an increased risk of formation of de novo donor-specific anti-HLA antibodies (DSA) compared with basiliximab or anti-thymocyte globulin (ATG), which can lead to chronic humoral immune responses against graft alloantigens and subsequent graft failure [25, 26]. The authors hypothesized that the spared alemtuzumab-resistant memory cells in the presence of alloantigens can rapidly convert to plasmablasts and secrete donor-specific antibodies [25].

4 Pharmacokinetics of Alemtuzumab

4.1 Administration

Alemtuzumab is available as a solution for intravenous or subcutaneous administration. A vial contains 30 mg in

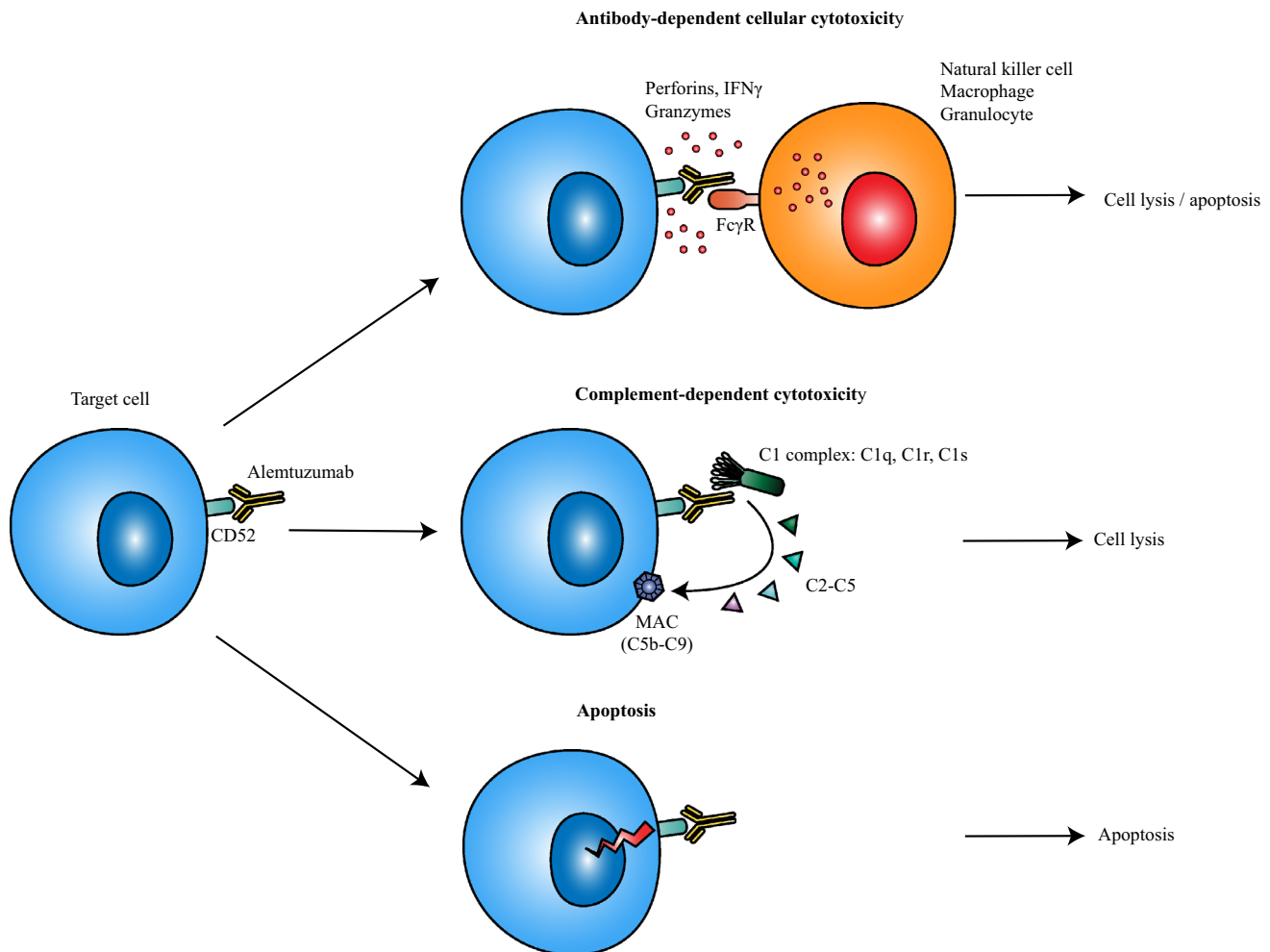


Fig. 2 Mechanism of action of alemtuzumab. Alemtuzumab binds to CD52 on target cells [T and B lymphocytes, natural killer (NK) cells, monocytes, granulocytes, and dendritic cells] and via three pathways depletion of the target cells occur. The antibody-dependent cellular cytotoxicity involves the IgG fragment C receptor (Fc γ R) on NK cells, macrophages, and granulocytes. The Fc γ R recognizes the Fc region of alemtuzumab and binds to it. The NK cell, macrophage, or

granulocyte releases perforins and granzyme B, which causes lysis and apoptosis of the target cell. In complement-dependent cytotoxicity, the C1 complex (consisting of C1q, C1r, and C1s) binds to alemtuzumab and this initiates the complement activation cascade and subsequently the formation of the membrane attack complex (MAC). Finally, binding of alemtuzumab to CD52 induces apoptosis directly. *IFN* interferon

1 mL, or in the case of Lemtrada[®] 12 mg in 10 mL. The recommended dose depends on the indication for alemtuzumab. In RRMS, the initial treatment is 12 mg/day intravenously for 5 consecutive days (cumulative dose of 60 mg) followed at 12 months by a second treatment course with 12 mg/day for 3 consecutive days (cumulative dose of 36 mg) [27]. For the indication CLL, it is advised to start with a maximum dose of 3 mg, intravenously, a second dose which is increased to 10 mg, which is followed by a third dose of 30 mg. Thereafter, the recommended alemtuzumab dose is 30 mg/day administered three times weekly for a maximum of 12 weeks (maximum cumulative dose 1080 mg) [28]. Dose recommendations have also been made for the reduced-intensity hematopoietic stem cell transplantation setting for non-malignant hematologic disease [29]. A typical dosing scheme of

alemtuzumab in SOT is 1 or 2 gifts of 30 mg intravenously or subcutaneously [30–32]. This dose is empirical and has been deducted from the maximum dose used in hematology. No formal dose-finding studies have been performed in SOT recipients. It is recommended that patients are pre-medicated with glucocorticoids, acetaminophen, and antihistamines immediately prior to the administration of alemtuzumab to diminish infusion-related reactions [33, 34].

4.2 Absorption

No pharmacokinetic studies of alemtuzumab have been performed in SOT recipients, whereas in patients with CLL and MS only a few such studies have been conducted. By definition, the bioavailability of alemtuzumab is 100%

after intravenous administration. In one study, the maximum plasma concentration (C_{\max}) of intravenously administered alemtuzumab was evaluated in 216 patients with RRMS [34]. Administration of 12 mg per day for 5 consecutive days resulted in a mean C_{\max} of 3014 ng/mL directly after the last administration on day 5. In patients with CLL, C_{\max} of 2800–26,400 ng/mL (mean 10,700 ng/mL) were measured after intravenous administration of 30 mg three times a week for 8 weeks [35].

Alemtuzumab can also be administered subcutaneously. Subcutaneous administration is more convenient and causes fewer infusion-related reactions as compared with intravenous administration [36, 37]. The bioavailability of subcutaneously administered alemtuzumab was studied in cynomolgus monkeys. Doses of 1, 2, and 3 mg/kg were slowly absorbed from the site of injection and the time to reach C_{\max} was around 48 h. The bioavailability after subcutaneous administration was approximately 47% [28]. In humans, Hale et al. [35] compared blood concentrations from patients with CLL treated either intravenously or subcutaneously (30 mg three times weekly). The highest measured pre-dose concentrations were similar between the two routes of administration (mean 5400 ng/mL). To reach a pre-dose concentration of 1000 ng/mL (an arbitrary threshold known to be potentially lympholytic), a higher cumulative dose was required when the drug was given subcutaneously as compared with intravenous administration (1106 and 146 mg, respectively).

Induction therapy with alemtuzumab in simultaneous pancreas-kidney transplant (SPKT) recipients showed no clinical difference between subcutaneous or intravenous therapy. Total lymphocyte and CD3⁺ lymphocyte depletions were not significantly different and the incidence of acute rejection episodes, as well as patient survival, were comparable in the two groups [31].

4.3 Distribution

Because of its size, alemtuzumab is not likely to cross cell membranes and is therefore expected to distribute between the plasma and interstitial space. In patients with MS, the volume of distribution was reported to be 14.1 L [34]. To measure the volume of distribution in patients with CLL, Mould et al. [38] pooled the data of 67 patients from four studies. This resulted in a steady-state volume of distribution of 11.3 L.

In addition to being expressed on the cell surface, CD52 also exists in a soluble form. Soluble CD52 can bind alemtuzumab, form immune complexes, and thereby reduce the amount of free and bioactive drug. Soluble CD52 levels are likely to be lower in patients with MS and recipients undergoing SOT compared with patients with CLL [39]. Higher plasma levels of soluble CD52 may

require higher doses of alemtuzumab for sufficient efficacy [40]. There are no data on the binding of alemtuzumab to other plasma proteins.

4.4 Metabolism and Elimination

The half-life of alemtuzumab depends on the concentration of its target. In the case of a high concentration of CD52, such as in patients with CLL with a large tumor burden, the half-life of alemtuzumab is short because binding of alemtuzumab to CD52 leads to cytotoxicity of malignant cells and rapid receptor-mediated clearance from the blood. When CD52 levels decrease (following successful treatment), the half-life of alemtuzumab increases. Therefore, patients with CLL will require a higher cumulative dose than patients treated for another indication. The half-life of alemtuzumab in patients with CLL is 6.1 days and in stem cell transplant recipients it is 8–21 days [35, 41, 42]. The half-life of alemtuzumab in patients with RRMS (12 mg on 5 consecutive days) was approximately 4–5 days and low or undetectable serum concentrations were measured within 30 days after completion of the course [34].

The mechanism of clearance of alemtuzumab from the circulation and interstitial space is not well understood. In a study of patients with CLL, alemtuzumab showed time- and concentration-dependent pharmacokinetics with (non-linear) clearance with large inter-patient variability [38]. This is probably explained by a difference in tumor burden. It is not known whether individual variations in factors such as hepatic function or macrophage activity affect the elimination rate of alemtuzumab [43]. No studies of the pharmacokinetics of alemtuzumab have been performed in patients with renal insufficiency or hepatic impairment.

It is also unknown if alemtuzumab binds to the neonatal Fc-receptor like some other monoclonal antibodies. The Fc-receptor is expressed on endothelial cells and influences the half-life of IgG₁ by internalization of immunoglobulins and protection from lysosomal degradation [44].

The expected metabolic pathway of alemtuzumab is degradation to small peptides and individual amino acids by widely distributed proteolytic enzymes. Classical bio-transformation studies have not been conducted but are unlikely to be relevant for alemtuzumab clearance [34].

There is no known antidote available in the case of an accidental overdose and treatment consists of supportive measures [34]. The effect of hemodialysis on the plasma concentration of alemtuzumab is unknown. However, it is unlikely that alemtuzumab is removed with hemodialysis because of its size (150 kDa). Likewise, no studies investigated if alemtuzumab is removed by plasmapheresis. For the monoclonal antibody rituximab, it is known that plasmapheresis removes an important proportion of the drug if performed within the first 72 h after administration

[45]. Like rituximab, alemtuzumab has a small volume of distribution and it is therefore likely that plasmapheresis can reduce the plasma concentration of alemtuzumab. However, the depletion effect on peripheral lymphocytes is seen in the first hour after alemtuzumab administration.

4.5 Immunogenicity

Alemtuzumab is a recombinant humanized protein with a variable framework with constant regions from a human IgG₁ immunoglobulin and six complementarity-determining regions from a rat IgG_{2a} antibody. The humanization of alemtuzumab has reduced the risk of antiglobulin responses [46]. However, anti-drug antibodies are still observed after administration of alemtuzumab [35, 46].

In patients with CLL, no patient developed anti-alemtuzumab antibodies in the group treated with intravenous alemtuzumab ($n = 30$), whereas two patients in the group given subcutaneous alemtuzumab developed such antibodies ($n = 32$). The antibodies likely inactivated alemtuzumab because these two patients did not show a significant reduction in lymphocyte count following alemtuzumab administration [35].

The phase III studies CARE MSI (Comparison of alemtuzumab and Rebif[®] efficacy) and CARE MSII (trials performed in patients with MS) showed a much higher percentage of anti-alemtuzumab antibodies. These antibodies were detectable in 29% of patients just before the second course of alemtuzumab (12 months after the last alemtuzumab gift) and in 81–86% of patients 1 month after the second course. Although the presence of anti-alemtuzumab antibodies was associated with a lower alemtuzumab concentration after the second course, the clinical outcome, lymphocyte depletion, and repopulation were not influenced [47, 48]. Rebello et al. described 12 patients treated with alemtuzumab because of kidney transplant rejection. No anti-alemtuzumab antibodies were detected [46].

Many factors possibly influence the immunogenicity of alemtuzumab including the dose and length of treatment, the route of administration, prior exposure to chemotherapy, and the concomitant use of other immunosuppressive drugs [46, 49]. Additionally, the incidence of anti-alemtuzumab antibodies is dependent on the sensitivity and specificity of the assay that is used.

5 Therapeutic Drug Monitoring

Pharmacokinetic monitoring is performed by three assays to measure alemtuzumab concentrations: an enzyme-linked immunosorbent assay, an indirect

immunofluorescence method with flow cytometry detection, and liquid chromatography-tandem mass spectrometry [50–52].

For enzyme-linked immunosorbent assay, serum samples are added to microtiter plates that contain rabbit anti-rat IgG antibodies that recognize the remaining rat sequence in the alemtuzumab molecule [50]. After incubation, the plates are washed and incubated with peroxidase-conjugated, affinity purified rabbit anti-human Fc. After washing, the substrate (3,3′-5,5′-tetramethylbenzidine; Dako, Carpinteria, CA, USA) is added. The reaction is stopped with hydrochloride and the signal is measured with a spectrophotometer. No significant difference was seen between serum or plasma. The lower limit of detection of the assay is 0.05 µg/mL [50].

Alemtuzumab can also be measured by means of flow cytometry. For this technique, a HUT-78 cell line is used. This CD8⁺ T-cell line is derived from a patient with Sézary syndrome and expresses high levels of CD52 [53]. The cell line is incubated with the serum of the patient treated with alemtuzumab. After washing, fluorescein isothiocyanate-labeled polyclonal anti-human Ig Fc antibodies are added and fluorescence is measured by flow cytometry. The lower limit of detection is 0.15 µg/L and the lower limit of quantification is 0.25 µg/L [51]. Recently, Marsh et al. used flow cytometry with normal donor, peripheral blood mononuclear cells instead of the HUT-78 cell line to measure alemtuzumab concentrations [54]. The lower limit of detection was 0.02 µg/mL, which is lower than that of the HUT-78 cell line-based assay [54].

Mass spectrometry has been described as a method to measure alemtuzumab [52]. It is currently not frequently used for the measurement of alemtuzumab. However, liquid chromatography-tandem mass spectrometry might become an important method to measure the blood concentrations of monoclonal antibodies in the future [55]. Pharmacodynamic monitoring is mainly performed by flow cytometry to quantify the numbers of circulating T and B lymphocytes and NK cells.

From the above, it is clear that measuring the serum or plasma concentration of alemtuzumab is possible. However, these assays are not widely available, technically demanding, and difficult to standardize. In SOT, no formal dose-finding studies exist and at present there are no tests that support specific alemtuzumab target concentrations, with an optimal balance between efficacy and toxicity. Such studies have been performed in patients undergoing hematopoietic stem cell transplantation, and pharmacokinetic-pharmacodynamic model target concentrations for this specific population have been proposed (personal communication, R. Admiraal, Leiden University Medical Center, Leiden, The Netherlands).

6 Clinical Use of Alemtuzumab in Kidney Transplantation

Alemtuzumab is not registered for SOT indications. However, the drug has been used off-label for both the prevention and treatment of acute allograft rejection in kidney, pancreas, intestinal, and lung transplantation.

6.1 Alemtuzumab as Induction Therapy

6.1.1 Kidney Transplantation

In many transplant centers, induction therapy is used to reduce early rejection rates. Two types of induction therapy are recognized: T lymphocyte-depleting antibody therapy and antibody therapy directed against IL-2 receptor. Basiliximab is a non-depleting monoclonal antibody directed against the IL-2 receptor, whereas ATG and alemtuzumab are depleting antibodies. Alemtuzumab was first used as induction therapy in 1998 in a case series of 13 kidney transplant recipients. The patients received induction therapy with alemtuzumab (two doses of alemtuzumab 20 mg intravenously on day 0 and 1) followed by low-dose ciclosporin as maintenance therapy. In the 6- to 11-month follow-up, only one patient experienced acute rejection [56].

Following this initial experience, the efficacy of alemtuzumab to prevent acute rejection following kidney transplantation was compared with IL-2 receptor antibodies in randomized controlled trials (RCTs). A systematic review of five of these RCTs described a reduced risk of acute rejection using alemtuzumab as compared with an IL-2 receptor antagonist at 12 months after kidney transplantation [659 patients; relative risk = 0.54; 95% confidence interval (CI) 0.37–0.79; $p < 0.01$] [57]. No significant difference was seen in graft loss, delayed graft function, or patient survival.

Recently, the results of the first phase of the ‘Campath, calcineurin inhibitor reduction and chronic allograft nephropathy’ (3C) study were published. The hypothesis of this RCT was that a more potent induction therapy at the time of transplantation allows for minimization of tacrolimus exposure without an increased risk of acute rejection. An immunosuppressive regimen with reduced exposure to the nephrotoxic tacrolimus could potentially lead to better renal function and longer graft survival. In the 3C study, induction therapy with alemtuzumab (30 mg on days 0 and 1, subcutaneously or intravenously) was compared with basiliximab (20 mg intravenously on days 0 and 4). A total of 852 patients were included ($n = 426$ in the alemtuzumab and $n = 426$ in the control arm). Patients in the alemtuzumab arm were co-treated with low-dose

tacrolimus (aiming for pre-dose concentrations of 5–7 ng/mL) and mycophenolate sodium (360 mg twice daily) without glucocorticoids. In the control arm, basiliximab-treated patients were co-treated with a standard-dose tacrolimus (target pre-dose concentrations 5–12 ng/mL), mycophenolate sodium (540–720 mg twice daily), and glucocorticoids (15–20 mg prednisone, withdrawn in accordance with local practice).

The primary endpoint of the 3C study was the incidence of biopsy-proven acute rejection (BPAR) at month 6 after transplantation. Induction therapy with alemtuzumab in combination with low-dose tacrolimus and mycophenolate sodium without glucocorticoids significantly reduced the incidence of BPAR: 26 (6.1%) vs. 65 (15.3%; $p < 0.0001$, hazard ratio 0.37; 95% CI 0.23–0.58), for the alemtuzumab and control arms, respectively. No significant difference was seen in the occurrence of biopsy-proven antibody-mediated rejection (ABMR): 8 (1.9%) vs. 5 (1.2%) ($p = 0.41$, hazard ratio 1.59; 95% CI 0.52–4.86). There was no difference 6 months after randomization between the two groups in terms of graft function (mean eGFR 50.1 mL/min per 1.73 m² in the alemtuzumab-treated patients vs. 49.8 mL/min per 1.73 m² in the basiliximab-treated group), the incidence of graft failure, mortality, or serious infection [58]. Limitations of the 3C study were the short follow-up duration of 6 months and no blinding of the induction therapies. In addition, the difference in tacrolimus exposure was limited: The average pre-dose concentration of tacrolimus in the alemtuzumab-treated patients was 6.9 ng/mL and in basiliximab-treated patients was 8.3 ng/mL [59].

Hanaway et al. compared alemtuzumab induction therapy (a single shot of 30 mg, intravenously) with basiliximab induction therapy (in patients with low risk of acute rejection) or with rabbit ATG (rATG) induction therapy in high-risk patients. A high risk of acute rejection was defined as a panel-reactive antibody (historical or current) above 20%, repeat transplantation, or black ethnicity. There were 139 high-risk patients; 70 received alemtuzumab and 69 received rATG. In the low-risk group, 335 patients were included; 164 received alemtuzumab and 171 patients received basiliximab. Basiliximab was given on day 0 and days 3, 4, or 5 (20 mg per gift). The total dose of rATG was 6 mg/kg (divided over four gifts). All patients received tacrolimus (target pre-dose concentration of 7–14 ng/mL in the first 3 months after transplantation, and 4–12 ng/mL after month 3), mycophenolate mofetil (1000 mg twice daily), and glucocorticoids (withdrawn on post-operative day 5). The rate of BPAR at 12 months in the alemtuzumab group was lower than in the basiliximab-treated patients (3 vs. 20%, $p < 0.0001$). No significant difference in BPAR after month 12 was observed between

alemtuzumab and rATG in the high-risk group (10 vs. 13%, $p = 0.53$) [60].

A systematic review with a meta-analysis compared induction therapy with alemtuzumab to rATG. A total of 446 patients was included and a comparable incidence of BPAR (relative risk = 0.79; 95% CI 0.52–1.21; $p = 0.28$) was seen. There was also no significant difference in graft loss and overall survival [57]. A recent Cochrane systematic review also showed comparable rates of BPAR between alemtuzumab and rATG in a total of six studies (446 patients; RR 0.68; 95% CI 0.44–1.05; $p = 0.66$). However, rates of BPAR after alemtuzumab induction were lower in four studies with early glucocorticoid withdrawal (360 patients; RR 0.57; 95% CI 0.35–0.93; $p = 0.025$). Rabbit ATG plus glucocorticoid continuation vs. alemtuzumab plus early glucocorticoid withdrawal showed no difference between the two groups (two studies; 86 patients, relative risk (sometimes RR is used and other times relative risk) 1.27, 95% CI 0.5–3.19; $p = 0.57$) [61].

Although no higher rejection rate was seen after alemtuzumab induction therapy in the studies described above, higher rates of acute ABMR have been described in a few studies [62–64]. LaMattina et al. [64] compared in a retrospective study induction therapy with alemtuzumab ($n = 632$), basiliximab ($n = 690$), or rATG ($n = 125$). Alemtuzumab was given one or two times (30 mg), basiliximab was administered on postoperative day 0 and 4 (20 mg), and the total dose of rATG was 6–8 mg/kg. Maintenance immunosuppression consisted of tacrolimus or ciclosporin in combination with mycophenolate mofetil and glucocorticoids (tapered to 5–10 mg/day after the first post-operative month). No significant difference was seen in overall frequency of BPAR; however, ABMR was significantly increased in the group of patients treated with alemtuzumab induction therapy compared with the group treated with rATG or basiliximab induction therapy. The 1-, 3-, and 5-year cumulative incidence of alemtuzumab-treated patients was 18.8, 23.8, and 26.5% respectively, vs. 11.3, 15.2, and 17.6% for the group receiving rATG or basiliximab ($p < 0.0001$). The higher incidence of ABMR could have been caused by a higher incidence of DSA after alemtuzumab treatment; however, this study did not test for the presence of DSA.

6.1.2 Simultaneous Pancreas-Kidney Transplantation

Adding a pancreas allograft to a kidney transplant seems to increase the risk of acute rejection. Over 90% of SPKT recipients receive antibody induction, with nearly 80% receiving a T lymphocyte-depleting antibody [65].

In a single-center RCT, 28 SPKT recipients treated with alemtuzumab induction were compared with 18 SPKT patients treated with rATG. Alemtuzumab induction

consisted of a single dose of 30 mg intravenously or rATG (cumulative dose 5–6 mg/kg). All patients received maintenance immunosuppression consisting of tacrolimus, mycophenolate mofetil, and glucocorticoids (with complete withdrawal on post-operative day 5). Patients identified as being of high immunological risk remained on maintenance glucocorticoids. In this underpowered study, no significant difference was seen in the frequency of rejection after 1 year (18 and 39%, respectively, for alemtuzumab and rATG; $p = 0.17$) and 5 years (21 and 44%; $p = 0.12$). Total patient survival after 5 years was not significantly different (82 vs. 89% for alemtuzumab and rATG, respectively). Furthermore, after 5 years, no significant difference was seen in kidney graft survival (78.6 vs. 66.7%) and pancreas graft survival (64.3 vs. 55.5%) [66].

Alemtuzumab induction therapy was compared with basiliximab in a retrospective cohort study of 136 SPKT recipients. All patients received maintenance immunosuppression with tacrolimus, mycophenolate mofetil, and glucocorticoids. Basiliximab was given to 39 patients and alemtuzumab (30 mg, on days 0 and 1) was given to 97 patients. Acute cellular rejection of the kidney was significantly less frequent in the alemtuzumab-treated patients (3.1 vs. 15.4%, $p = 0.017$). The occurrence of ABMR was not different between the two groups (18 vs. 14.4%, $p = 0.6$, for alemtuzumab and basiliximab, respectively). After 3 years, no significant difference was seen in patient survival or allograft survival of the kidney (86.2% for alemtuzumab and 91.8% for basiliximab) or pancreas (88.6% for alemtuzumab and 81.8% for basiliximab) [67].

Taken together, alemtuzumab is frequently used as an induction agent in SOT. Compared with basiliximab induction therapy, alemtuzumab results in a lower incidence of acute rejection. However, when compared with rATG no difference in the risk of acute rejection was observed. Graft survival and patient survival are mostly comparable between induction therapy with alemtuzumab and basiliximab or rATG.

6.2 Alemtuzumab as Anti-Rejection Therapy

In most centers, the first-line treatment of BPAR after a kidney transplant is pulse therapy with glucocorticoids. In the case of glucocorticoid-resistant rejection or severe (histological grade) rejection, depleting antibody therapy is indicated [68]. The standard depleting antibody is rATG [69]. However, treatment with ATG has limitations. First, ATG must be administered via a high-flow intravenous access (often a central venous catheter) or an arteriovenous fistula to avoid thrombophlebitis. Second, administration of ATG can cause cytokine release syndrome immediately after infusion. Cytokine release syndrome is characterized

by fever, hypotension, pulmonary edema, nausea, tachycardia, rash, or chills. Furthermore, anti-rabbit antibodies can form after rATG administration. In the case of subsequent exposure to rATG, this can lead to diminished activity and adverse reactions such as serum sickness [70]. An alternative treatment would be necessary in these patients.

Alemtuzumab has incidentally been used as a treatment of BPAR after kidney transplantation [30, 32, 46, 71–73]. No RCTs investigating this application have been performed. Clatworthy et al. [73] described the long-term outcome of first-line treatment of BPAR with alemtuzumab. Of the 15 patients described in this retrospective case series, 12 patients were diagnosed with an acute cellular rejection, one with an ABMR, and two with a mixed-type rejection. Alemtuzumab was administered intravenously and the first six patients were treated with 10 mg per day for 7 days (cumulative dose of 70 mg). The remaining nine patients received alemtuzumab in a dose of 6 mg/day for 4–10 days. The control group consisted of 25 patients with an acute rejection treated in the same period with intravenous methylprednisolone (1000 mg/day for 3 consecutive days). Of the 25 biopsies, 22 showed acute cellular rejection and three mixed-type rejections. Maintenance immunosuppressive therapy consisted of ciclosporin, azathioprine, and glucocorticoids. Baseline characteristics were comparable in both groups. All rejection episodes were treated successfully, as shown by a fall in serum creatinine within 3–10 days of treatment. Long-term transplant survival and allograft function were similar in both groups. There was no excess rate of cytomegalovirus infection, malignancy, autoimmunity, or post-transplant lymphoproliferative disorder in the alemtuzumab-treated patients. Serious infections during the first year were noted in 47% of patients treated with alemtuzumab and three patients died in the first year because of infection. In conclusion, this study demonstrates that treatment of acute rejection with alemtuzumab results in comparable long-term outcomes as with methylprednisolone pulse treatment; however, with an excess of infection-related death in the first year after treatment [73].

Alemtuzumab has also been used as second-line treatment in glucocorticoid-resistant or severe acute rejection. Basu et al. described 40 patients with glucocorticoid-resistant rejection (29 patients) or severe rejections (Banff 1B or worse, 11 patients). No control group was included. The patients were treated with alemtuzumab intravenously (30 mg, one to four doses). All patients had previously received induction therapy consisting of rATG or alemtuzumab followed by tacrolimus monotherapy as maintenance immunosuppression. Graft survival after a mean duration of 453 ± 163 days was 62.5%. In 14 patients, infectious complications occurred. Two patients died: one

patient developed post-transplant lymphoproliferative disorder and the other patient died because of an intraabdominal abscess [72]. The authors concluded that the outcome after treatment with alemtuzumab is comparable to the outcome of other antibody preparations (indirect comparisons with RCTs). However, infectious complications were frequent [72].

Another retrospective study compared alemtuzumab with rATG for the treatment of glucocorticoid-resistant rejection. Eleven patients were treated with 15–30 mg of alemtuzumab (subcutaneously) for 1–2 consecutive days. The reason for treating these patients with alemtuzumab were as follows: fluid overload, positive test for anti-rabbit IgG antibodies, treatment with ATG after previous transplantation, and cardiac ischemia. Three patients had no contra-indication for ATG. The control group consisted of 20 patients treated with rATG (2.5–4.0 mg/kg for 10–14 days). These historical controls consisted of patients with a glucocorticoid-resistant rejection and were matched for date after transplantation. The endpoint of this small study was a composite endpoint named ‘treatment failure’ after 3 months, which was defined as graft loss, the need for additional anti-rejection therapy, or the lack of improvement of renal allograft function (drop of less than 25% of serum creatinine at 3 months after treatment with alemtuzumab or rATG). The incidence of treatment failure was comparable in both groups (alemtuzumab 27% vs. rATG 40%, $p = 0.89$) [30].

Taken together, anti-rejection therapy with alemtuzumab results in a comparable graft survival compared with rATG. However, head-to-head RCTs with a rATG control and with longer follow-up are necessary to support this conclusion.

6.3 Alemtuzumab in Pediatric Kidney Transplantation

Reducing the toxicity of immunosuppressive drugs is of paramount importance in pediatric kidney transplant recipients. In particular, the minimization of glucocorticoids, which can cause, among others growth retardation, post-transplant diabetes mellitus, and weight gain, is an important goal in this population. Induction therapy with alemtuzumab has been used incidentally to avoid glucocorticoids and reduce calcineurin inhibitor exposure but no prospective, randomized controlled clinical trials comparing different induction therapies have been performed in children [74–83]. Several reasons may exist why limited studies have been performed with alemtuzumab in children. First, most children are unsensitized at the time of transplantation because most patients did not have prior kidney transplantations or pregnancies. Second, physicians may be concerned for the development of primo-

cytomegalovirus and Epstein–Barr virus (EBV) infections and EBV-related post-transplant lymphoproliferative disease (PTLD) after alemtuzumab administration.

The first experience with alemtuzumab as induction therapy in pediatric kidney transplant recipients was described in 2005 [75]. Four patients ranging from 20 months to 16 years of age received alemtuzumab intraoperatively (one dose of 30 mg in three patients and two doses of 30 mg in one patient). Three patients also received a calcineurin inhibitor, mycophenolate mofetil with or without corticosteroids as maintenance immunosuppressive therapy. In the fourth patient, calcineurin inhibitor therapy was withheld because of concerns for the recurrence of Factor H, deficiency-associated hemolytic uremic syndrome. In the short follow-up period of 5–12 months, three children experienced acute rejection (of which two were C4d positive, suggesting an antibody-mediated rejection) without graft loss. No serious infections or PTLD occurred [75]. White blood cell counts were measured by flow cytometry in one patient and demonstrated that CD3⁺, CD4⁺, CD8⁺, and CD20⁺ lymphocyte counts had recovered to 50% of baseline 1 year after administration. Monocytes recovered to baseline level by month 3 [75].

After this initial and disappointing experience, better results were obtained in a larger case series of 42 pediatric kidney transplant patients (mean age 9.0 years) treated with alemtuzumab induction therapy (in a dose of 0.4–0.5 mg/kg intravenously) followed by tacrolimus monotherapy [76]. The mean follow-up was 24.1 months. The aim of tacrolimus dosing was a pre-dose concentration of 8–12 ng/mL in the first 6 months. In the case of no rejection and in the absence of the development of de novo DSA and graft dysfunction, the tacrolimus dose was lowered to every other day. This strategy was successful in 12 patients. Only two patients experienced an episode of an acute cellular rejection and no cases of acute antibody-mediated rejection were observed. The 4-year graft survival rate was 85.4%. No cases of cytomegalovirus infection were seen and two patients were diagnosed with BK viremia. No PTLD or serious infections occurred. Two children died: one of an unknown cause and one because of a disconnected tracheostomy at home [76].

A larger case series of 101 pediatric kidney transplant patients (mean age 10.7 years) described a different outcome regarding the incidence of rejection and infection [77]. The patients were treated with two 30-mg doses of alemtuzumab: the first dose 12–29 days before transplantation and the second dose on the day of transplantation. The mean follow-up was 3.8 years. Maintenance therapy consisted of a calcineurin inhibitor (tacrolimus or ciclosporin) in combination with mycophenolate mofetil. Glucocorticoids were discontinued around day 5 if the graft

function was acceptable and target calcineurin blood concentrations were reached. The incidence of acute rejection (including subclinical rejections) was 37%. In four patients, rejection led to graft loss. Overall graft survival was 89.1% after 3 years. Cytomegalovirus and BK viremia occurred mostly during the first 3 months (30 and 25%, respectively). Twenty percent of patients experienced EBV viremia by year 2. No patients developed PTLD. Eight patients died (range 26–1457 days) of which five were because of an infection [77].

In a phase II multicenter prospective analysis, 35 pediatric kidney transplant patients were treated with one gift of alemtuzumab (0.3 mg/kg, maximum 20 mg) as induction therapy [84]. The primary aim of this study was to characterize the reconstitution of lymphocyte subsets in pediatric renal transplant recipients after alemtuzumab induction therapy followed by calcineurin inhibitor withdrawal. The patients were unsensitized and were first-time recipients with living donors. Maintenance immunosuppressive therapy consisted of tacrolimus and mycophenolate mofetil. Tacrolimus was switched to sirolimus after 2–3 months. In the follow-up period of 2 years, six patients developed acute rejection. Two patients experienced graft loss: one to focal and segmental glomerulosclerosis and one to non-adherence of medication. Fourteen children experienced infectious episodes. The reconstitution of the lymphocytes in these patients mimicked the pattern seen in adults. CD8⁺ T lymphocytes recovered faster than CD4⁺ lymphocytes: after 24 months, CD8⁺ T lymphocytes recovered to 60% of baseline and CD4⁺ lymphocytes to 25% of baseline ($p = 0.014$). No significant difference was seen in the recovery of CD4⁺ naïve and memory lymphocyte subsets and CD8⁺ naïve and memory lymphocytes. In the CD4⁺ memory lymphocyte population, the effector memory lymphocytes recovered faster than the central memory lymphocytes (44 vs. 24% after 24 months, respectively; $p = 0.027$). No significant difference was seen in the recovery of CD8⁺ central memory and effector memory lymphocytes. At baseline, 4% of CD4⁺ lymphocytes were CD4⁺CD25⁺FoxP3⁺ regulatory T lymphocytes. Three months after alemtuzumab, there was relatively less depletion of regulatory CD4⁺ lymphocytes (around 10% of the CD4⁺ cells had a regulatory T lymphocyte phenotype) and this effect persisted for 12 months of alemtuzumab treatment [84].

Alemtuzumab has also been successfully used as part of the induction therapy in highly sensitized, pediatric kidney transplant patients in two small case series [85, 86]. To our knowledge, only one paper has described the use of alemtuzumab as anti-rejection therapy in pediatric kidney transplant recipients [87]. Three patients were treated with alemtuzumab (0.3 mg/kg, intravenously) because of five episodes of a late (i.e., more than 3 months after

transplantation) glucocorticoid-resistant acute rejection. All patients were treated with ATG on two previous occasions. The first 14-year-old patient experienced recurrent rejection because of non-adherence. The first two episodes [acute cellular rejection (ACR) Banff type 1B] responded well to alemtuzumab. The third episode (ACR Banff type 1A) did not respond and the patient experienced graft loss soon thereafter. The second patient (14 years old) received one gift of alemtuzumab because of an ACR Banff type 1B. The serum creatinine concentration dropped from 292 to 150 $\mu\text{mol/L}$ 1 week after the administration of alemtuzumab. Two months after the alemtuzumab treatment, the patient experienced a borderline rejection with good response to high-dose glucocorticoids. The serum creatinine concentration stabilized around 175 $\mu\text{mol/L}$. The absolute lymphocyte count recovered to baseline level after 23 months. After 10 months, there was an asymptomatic rise in serum EBV load with spontaneous resolution. The third patient (5 years old) experienced an ACR Banff type 1B-2A. He was treated unsuccessfully with methylprednisolone, ATG, rituximab, intravenous immunoglobulins, and finally alemtuzumab, after which he lost his graft. In the year after the anti-rejection treatment, this patient experienced multiple serious infections probably related to the severe leukopenia, which required treatment with granulocyte colony-stimulating factor [87]. In conclusion, alemtuzumab reversed three of five rejection episodes in pediatric patients with a late glucocorticoid- and ATG-resistant rejection. However, it did not prevent graft loss in two of the three patients.

In summary, alemtuzumab is sometimes used as induction therapy and rarely as anti-rejection therapy in pediatric renal transplant recipients. The results are variable and different dosing schemes (some are weight adjusted and some not) of alemtuzumab are used. Prospective randomized controlled trials comparing different induction therapies (such as basiliximab, ATG, and alemtuzumab) are needed to establish the efficacy and long-term safety of alemtuzumab in pediatric renal transplant recipients.

7 Complications of Alemtuzumab Administration

7.1 Infusion-Associated Reactions

Acute infusion-related reactions occur in 70–80% of patients during treatment with alemtuzumab when given intravenously. These reactions are caused by cytokine release from lysed immune cells. These reactions are mostly mild to moderate and include headache, rash, nausea, hypotension, rigors, and pyrexia. Following subcutaneous administration, infusion-related reactions occur less

frequently, although local injection-site reactions do occur [34, 37].

7.2 Infections

Alemtuzumab results in a prolonged depletion of T and B lymphocytes (usually for over 12 months). This profound immunosuppression predisposes patients to infections. However, no depletion of the neutrophilic granulocytes typically occurs and reconstitution of the cells of the innate immune system is faster: monocytes typically recover after 3 months (although repopulation may occur in as little as 1 month) and NK cells return to 60–80% of baseline after 6 months).

Prophylaxis with an oral anti-herpes agent and prophylaxis against *Pneumocystis jirovecii* should be started directly after administration of alemtuzumab and be continued for a minimum of 2 months after the last alemtuzumab gift or until the CD4^+ T lymphocyte count is ≥ 200 cells/ μL [33, 34]. In our center, we do not routinely screen kidney transplant recipients for adenovirus or EBV, whereas we do for BK virus.

Published data on the occurrence of opportunistic infections after alemtuzumab treatment are limited. BK virus infection is more common after alemtuzumab induction in kidney transplantation compared with ATG induction [32]. Cytomegalovirus and opportunistic and non-opportunistic infections were not more common when comparing alemtuzumab with ATG induction therapy [32]. In contrast to induction therapy with alemtuzumab, anti-rejection therapy with alemtuzumab is associated with a higher risk of opportunistic infections (4.5 vs. 21% $p < 0.001$). The higher incidence of opportunistic infections may be directly related to the alemtuzumab treatment, but could also be owing to the fact that after rejection the maintenance immunosuppressive therapy is also intensified [88].

7.3 Malignancy

Long-term data linking alemtuzumab treatment with malignancy are scarce and the risk of developing malignancy is poorly defined. In a single-center retrospective analysis among 1350 kidney transplant recipients, no increased cancer incidence 4 years after induction therapy with alemtuzumab (2.8%) compared with ATG (5.4%) or no induction therapy (3.3%) was seen (across all groups; $p = 0.234$). This study did not include non-melanoma skin cancer [89].

In contrast, another study using US transplantation and cancer registries data to explore the relationship between induction therapy and cancer after transplantation came to a different conclusion [90]. A total of 111,857 kidney

transplant recipients were available for inclusion with a median follow-up of 3.5 years. Of the total group, 3394 patients received alemtuzumab induction therapy. Alemtuzumab induction, compared with no induction therapy, was associated with an increased risk of non-Hodgkin lymphoma [$n = 15$, adjusted incidence rate ratio (aIRR), 1.79; 95% CI 1.02–3.14; $p = 0.04$] and all virus-related tumors such as non-Hodgkin lymphoma, Hodgkin lymphoma, human papilloma virus-related cancers, Kaposi sarcoma, and liver cancer ($n = 19$, aIRR 1.84; 95% CI 1.11–3.03; $p = 0.02$). Alemtuzumab induction was also associated with increased colorectal cancer ($n = 7$, aIRR 2.46; 95% CI, 1.03–5.91; $p = 0.04$) and thyroid cancer ($n = 10$, aIRR 3.37; 95% CI 1.55–7.33; $p = 0.002$). Alemtuzumab induction was not associated with an increased risk of lung or kidney cancer, or melanoma [90]. No direct comparison between alemtuzumab and polyclonal depleting induction therapy was made.

Three RCTs compared alemtuzumab with IFN- β -1a in RRMS. In both the phase II (CAMMS223) and III trials (CARE-MSI and CARE-MS-II), malignancy was not more frequent after alemtuzumab compared with IFN- β -1a [47, 48, 91]. In the CAMMS223 trial, malignancy was observed in 2.8% of patients treated with alemtuzumab (one patient with cervical cancer and one patient with breast cancer) and 0.9% of patients taking IFN- β -1a (colon cancer) after a follow-up of 3 years. In the extension part of this trial, one patient in the alemtuzumab group died of sepsis following chemotherapy for Burkitt's lymphoma [91]. In CARE-MSI, two patients (0.5%) in the alemtuzumab group developed thyroid papillary carcinoma. It is not clear whether these cases were induced by alemtuzumab or were an incidental finding on ultrasound investigation of patients with thyroid dysfunction after screening. No patients in the IFN- β -1a group developed a malignancy [47]. In CARE-MSII, malignancy rates for alemtuzumab- vs. IFN- β -1a-treated patients were 0.6% vs. 1.5%, respectively, after 24 months of follow-up. These malignancies included one case of papillary thyroid cancer, basal cell carcinoma (two patients), cervical cancer (one patient), and colon cancer (one patient) in the alemtuzumab-treated group. In the IFN- β -1a-treated group, two malignancies were observed (one patient with a basal cell carcinoma and one case of acute myeloid leukemia) [48]. No further malignancies were observed in the long-term open-label follow-up (median 7 years, range 33–144 months) [92].

Occurrence of EBV-positive large-cell lymphoma has been described after administration of alemtuzumab in patients with CLL. In a study to investigate the efficacy and safety of alemtuzumab in patients with CLL with residual disease, 3 of 41 patients developed EBV-positive large-cell lymphoma. Two of three patients had spontaneous resolution without therapy and one patient

was treated with immunoglobulins and anti-viral medication [93]. A case report described the development of an EBV-positive lymphoma in an 80-year-old patient with CLL treated with chemotherapy and alemtuzumab [94].

In conclusion, alemtuzumab results in an increased risk of malignancy as compared with no induction therapy in kidney transplantation. In contrast, no increased risk of malignancy was associated with the use of alemtuzumab in patients with MS when compared with IFN- β -1a.

7.4 Autoimmunity

Secondary autoimmune events have been reported after alemtuzumab treatment. Interleukin-21 seems to play a role in the development of this autoimmunity. Interleukin-21 is involved in the proliferation of cytotoxic T lymphocytes, the inhibition of regulatory T lymphocytes, and the differentiation of B lymphocytes into antibody-producing plasma cells [95]. Pre-treatment levels of IL-21 in patients with MS were twofold higher in patients developing autoimmunity after alemtuzumab treatment compared with patients without autoimmunity [96].

Most commonly, the thyroid gland is affected. Autoimmune thyroid disorders, especially hypothyroidism and hyperthyroidism (Graves' disease) tend to occur between 6 and 61 months, peaking in the third year post-treatment in patients with MS. In kidney transplantation, Graves' disease has also been observed after alemtuzumab administration [97]. The total incidence of thyroid events, described in CAMMS223, CARE-MSI, and CARE-MSII, ranged between 16 and 30% [47, 48]. In the patients treated with IFN- β -1a, the incidence of thyroid events was 3–6%. It is advised that thyroid function tests should be obtained prior to initiation of treatment and tested on a regular basis until 48 months after the last infusion [34].

Immune thrombocytopenia (idiopathic thrombocytopenic purpura) as a side effect of alemtuzumab treatment was first described in the CAMMS223 study. A patient presented with intracranial hemorrhage and died. The incidence of idiopathic thrombocytopenic purpura was 1–2% in the CAMMS223 and CARE-MS studies [47, 48, 91]. Furthermore, four cases of glomerulopathy (0.3%) were described after alemtuzumab treatment in the CAMMS223, CARE-MSI, and CARE-MSII trials. Two patients developed anti-glomerular basement membrane disease and two patients developed membranous glomerulopathy. The onset of kidney disease ranged from 4 to 39 months after alemtuzumab administration [47, 48, 91]. One case of Guillain-Barre syndrome was reported in a patient treated with alemtuzumab because of a T lymphocyte prolymphocytic leukemia [98].

7.5 Fertility and Pregnancy

Alemtuzumab has been assigned to pregnancy category C by the Food and Drug Administration, meaning that animal production studies have shown an adverse effect on pregnancy outcomes but that no adequate studies have been performed in humans [34]. Immunoglobulin G molecules, such as alemtuzumab, are known to cross the placental barrier and may potentially affect the fetus.

Six months after delivery, concentrations of infliximab and adalimumab can be detected in the baby [99]. The administration of live vaccines (such as Bacillus Calmette-Guerin, rotavirus, varicella zoster, mumps, measles, and rubella) in the first 6 months after delivery to babies of mothers treated with infliximab can be life threatening [100]. It is not known whether alemtuzumab can cause fetal harm when administered to pregnant women or whether it can affect reproductive capacity. In the Cambridge long-term follow-up study of MS patients, a total of 15 babies were born to 12 women treated with alemtuzumab after a median interval from most recent treatment of 26 months (range 13–86 months). All deliveries and births were uncomplicated [92].

CD52 is expressed in the male reproductive system, including the epididymis, vas deferens, seminal vesicles, and mature spermatozoa [101]. Although CD52 antibodies agglutinate and inactivate sperm *in vitro*, reproductive problems have not been reported following therapy with alemtuzumab, although available data are limited. A long-term follow-up study reported six male individuals fathering seven live births, a median of 14 months (range 8–44 months) from most recent treatment to conception [92]. Another (sub)study ($n = 13$) showed that at baseline, and 1, 3, and 6 months post-alemtuzumab treatment, there was no evidence of aspermia, azoospermia, motility disorders, or depressed sperm counts [102].

It is unknown if alemtuzumab is excreted in human breast milk, but it has been detected in the milk of lactating mice. Therefore, breastfeeding should be discouraged to women for at least 4 months following treatment [34].

8 Summary and Future Directions

Alemtuzumab is frequently used off-label in kidney transplantation as induction therapy and less frequently as anti-rejection therapy. No pharmacokinetic studies have been performed in SOT recipients, probably because alemtuzumab has never been registered for this indication. Most pharmacokinetic and pharmacodynamic studies have been performed in patients with CLL and MS. However, the pharmacokinetics of alemtuzumab in the latter two patient populations may be very much different from SOT recipients. The alemtuzumab dose used in induction and anti-rejection therapy (30 mg one to two times) is not based on

formal dose-finding studies in SOT recipients, but is based on experience in CLL and MS. The duration of depletion of immune cells of the innate and the adaptive immune system is much longer after alemtuzumab treatment compared with rATG [103]. It may therefore be possible that lower doses of alemtuzumab will result in the same effect on graft survival, though with less toxicity. Subcutaneous administration showed the same outcomes compared with intravenous administration, but with less adverse events, although anti-alemtuzumab antibody formation may be more frequent.

Induction therapy with alemtuzumab in kidney transplantation shows comparable results in terms of graft and patient survival as compared with basiliximab and rATG. However, induction therapy with alemtuzumab is more effective in preventing acute rejection as compared with induction therapy with basiliximab. Results are comparable to induction therapy with rATG. The use of alemtuzumab induction therapy may facilitate minimization of the exposure to nephrotoxic immunosuppressive drugs, which may possibly lead to better long-term graft survival. Alemtuzumab used as anti-rejection therapy has shown some promising results. Replacement of rATG by alemtuzumab for this indication could lead to less infusion-related adverse events, shorter hospital stay, and a reduction in costs. However, long-term adverse events such as infection, autoimmunity, malignancies, and a higher frequency of ABMR may be more frequent among alemtuzumab-treated patients compared with rATG.

Although alemtuzumab is used off-label in kidney transplantation, it can be an additional treatment option to the drugs now used as induction or anti-rejection therapy. We should start the discussion with a pharmaceutical company to expand the indication for alemtuzumab to SOT, and thus more clinical studies can be performed. There is an unmet need to optimize alemtuzumab dosing in patients undergoing SOT and we believe dose-finding studies are needed. Furthermore, RCTs are required to compare the effectiveness and long-term results of alemtuzumab with rATG for the treatment of acute rejection.

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Compliance with Ethical Standards

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