

REVIEW

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Alemtuzumab for multiple sclerosis: the new concept of immunomodulation

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Abstract

Alemtuzumab (Lemtrada®) is a humanized anti-CD52 IgG1 monoclonal antibody that depletes CD52-expressing cells from the circulation. Robust clinical and radiologic data, derived from clinical trials and long-term observational studies, indicate that alemtuzumab induces a marked immunosuppression related to the depletion of circulating T and B lymphocytes. However, recent advances suggest that the long-term clinical effects of alemtuzumab are probably due to unique *qualitative* changes in the process of lymphocyte repopulation of the immune system. This leads to a particular rebalancing of the immune system. In this paper we review the immunomodulatory mechanisms underlying the therapeutic effect of alemtuzumab in pre-clinical models and in patients with relapsing remitting multiple sclerosis (RRMS), and stress the importance of a monoclonal antibody-based immunosuppression for treating the severe forms of RRMS. Alemtuzumab has many features of the ideal immunomodulatory drug: rapid biological and clinical actions and long-lasting benefit. Alemtuzumab can be used as rescue therapy or as first line drug in severe-onset MS. Thus, the availability of alemtuzumab constitutes a significant step forward in the therapy of MS.

Keywords: Alemtuzumab, Multiple sclerosis, Immunomodulation

Background

Alemtuzumab, previously known as Campath-1H, is a recombinant, humanised, monoclonal immunoglobulin IgG1 kappa, that targets the cell-surface glycoprotein CD52 [22, 23]. Alemtuzumab induces antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and activates pro-apoptotic pathways on CD52-expressing cells. One phase-2 trial (CAMMS-223) and two phase-3 studies (CARE-MS1 and CARE-MS2) have clearly demonstrated the efficacy of alemtuzumab in the treatment of naive patients and have established its superiority over high-dose high-frequency interferon beta-1a (IFNβ-1a) in patients who continue to experience relapses despite of first-line therapy [9–11]. Alemtuzumab primary safety concern is the development of secondary autoimmunity, that occurs up to five years after the first cycle of infusions and peaks at two years. Indeed, about 30% of patients develop thyroid autoimmunity and 1% idiopathic thrombocytopenic purpura (ITP). In addition, 4 out of

1486 patients (<0.3%) enrolled in phase II and II trials developed autoimmune glomerulonephritis.

In September 2013, the European Medicine Agency (EMA) approved alemtuzumab as a first-line therapy for adults with active relapsing remitting multiple sclerosis, under the trade name Lemtrada. Lemtrada is now also approved as a treatment of multiple sclerosis in US, Canada, Australia, Switzerland, Israel, and many countries in South America. This review focuses on the mechanisms of action of alemtuzumab on the immune system (Table 1) and tries to explain the long lasting biological and clinical effects of this monoclonal antibody on multiple sclerosis (MS).

CD52, the target

CD52 is a short peptide of 12 amino acids highly expressed on T and B lymphocytes, and at very low levels on natural killer cells, monocytes and macrophages (Fig. 1), while neutrophils, dendritic cells and bone marrow stem cells don't express this molecule (Buggings et al., 2002). Further, CD52 is found within the male genital tract and is present on the surface of mature sperm cells. Since it is highly negatively charged it has been supposed that its

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Table 1 Biological effects of Alemtuzumab

Effects	Mechanisms	References
Fast and long-lasting immune suppression	Rapid and complete depletion of circulating T and B lymphocytes; limited effect on lymphoid organs. Differentiated CD20, CD4 and CD8 lymphocyte repopulation (B before T, T4 before T8) Long-lasting re-modelling of the lymphocyte network: earlier appearance of regulatory B and T cells, later appearance of Th1 and Th17. Anti-inflammatory changes in the cytokine network. More tolerogenic immune network during reconstitution.	Hu et al., 2009 [29] Thompson et al., 2010 [38] Jones et al., 2009 [30] Zhang et al., 2013 [42] Turner et al., 2013 [40] Havari et al., 2015 [25] Wang et al., 2015 [43] Wang et al., 2015 [43] Watanabe et al., 2006 [44]
Decrease in brain inflammation and neurodegeneration	Almost complete suppression of brain inflammation. Increased production of neurotrophic/growth factors by antigen-specific T cells.	Jones et al., 2010 [31] Turner et al., 2015 [41]
No impact on immune competence	Immunocompetent cells in the lymphoid organs are largely preserved. B and T cell response against bacterial and viral antigens are maintained during therapy. Innate immunity is not affected.	Hu et al., 2009 [29] Buggins et al., 2002 [6] McCarthy et al., 2013 [34] Clark et al., 2012 [7] Turner et al., 2013 [40]
Cytokine release syndrome	Transient, cytotoxicity-related release of pro-inflammatory acute-phase cytokines (TNF, IL-1, IL-6).	Breslin et al., 2007 [4] Wing et al., 1996 [45] Bugelski et al., 2009 [5]

function consisted in hampering cell adhesion, thus allowing cells to freely move around (see the review of [23]). Although a recent study has found that activated CD4 T cells expressing high levels of CD52 may have regulatory activities on T effector lymphocytes [2] the function of CD52 remains partially unknown.

Human and mouse antigen-activated T cells with high CD52 expression were demonstrated to suppress other T cells. CD4 + CD25 + CD52^{high} T cells are distinct from CD4 + CD25^{high}Foxp3+ regulatory T cells inasmuch as they suppress auto-reactive T cell subsets by releasing soluble CD52 (CD52s). CD52s binds to the inhibitory receptor sialic acid-binding immunoglobulin-like lectins-

10 (Siglec-10) thus impairing the phosphorylation of the T cell receptor (TCR)-associated kinases Lck and Zap70 (Fig. 2). The final result is a down regulation of TCR assembly and, consequently, T cell activation. Humans with autoimmune diabetes were found to have a lower frequency and diminished function of CD4 + CD25 + CD52^{high} T cells responsive to the autoantigen GAD65. In diabetes-prone mice of the non-obese diabetic (NOD) strain, transfer of lymphocyte populations depleted of CD4 + CD25 + CD52^{high} cells results in a more severe disease [2].

Beside the CD52-Siglec-10 ligand-receptor mechanism, the CD52 molecule may down-regulate T cell activation

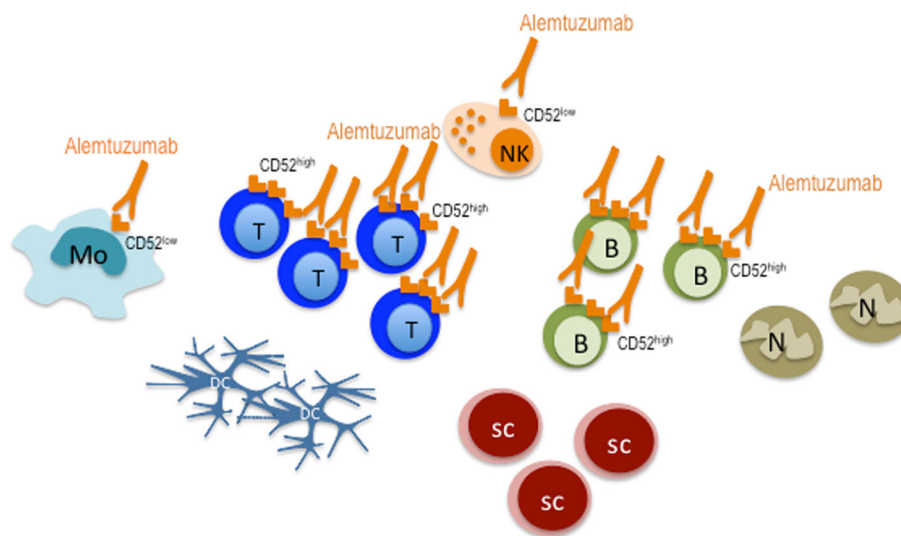
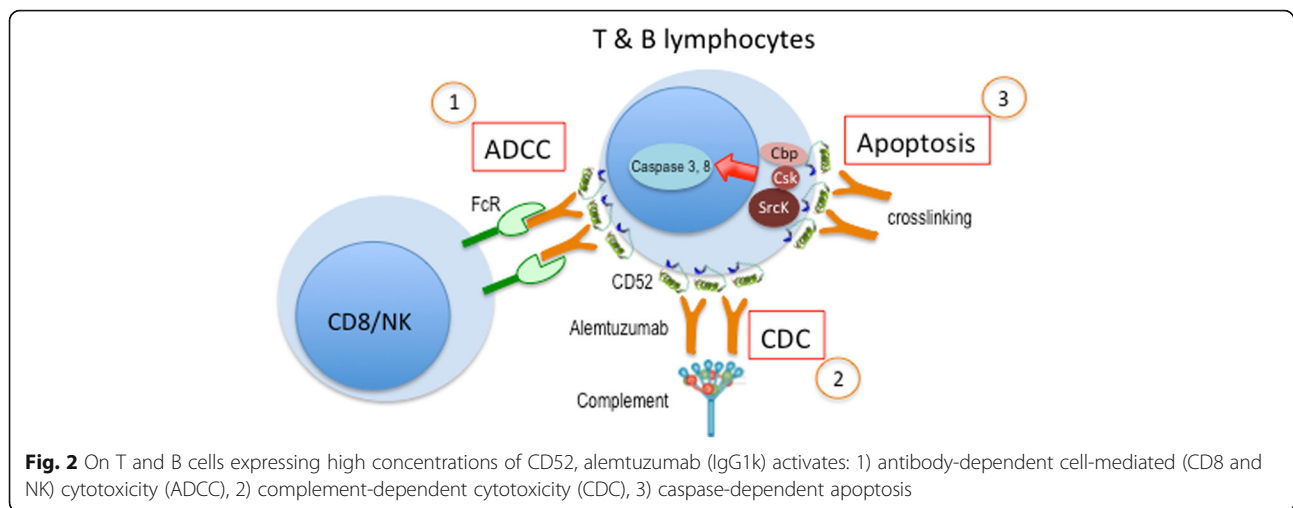


Fig. 1 CD52 is a short peptide of 12 amino acids highly expressed on T and B lymphocytes, and at very low levels on natural killer cells (NK), monocytes and macrophages (Mo), while neutrophils (N), dendritic cells (DC) and bone marrow stem cells (SC) do not express this molecule

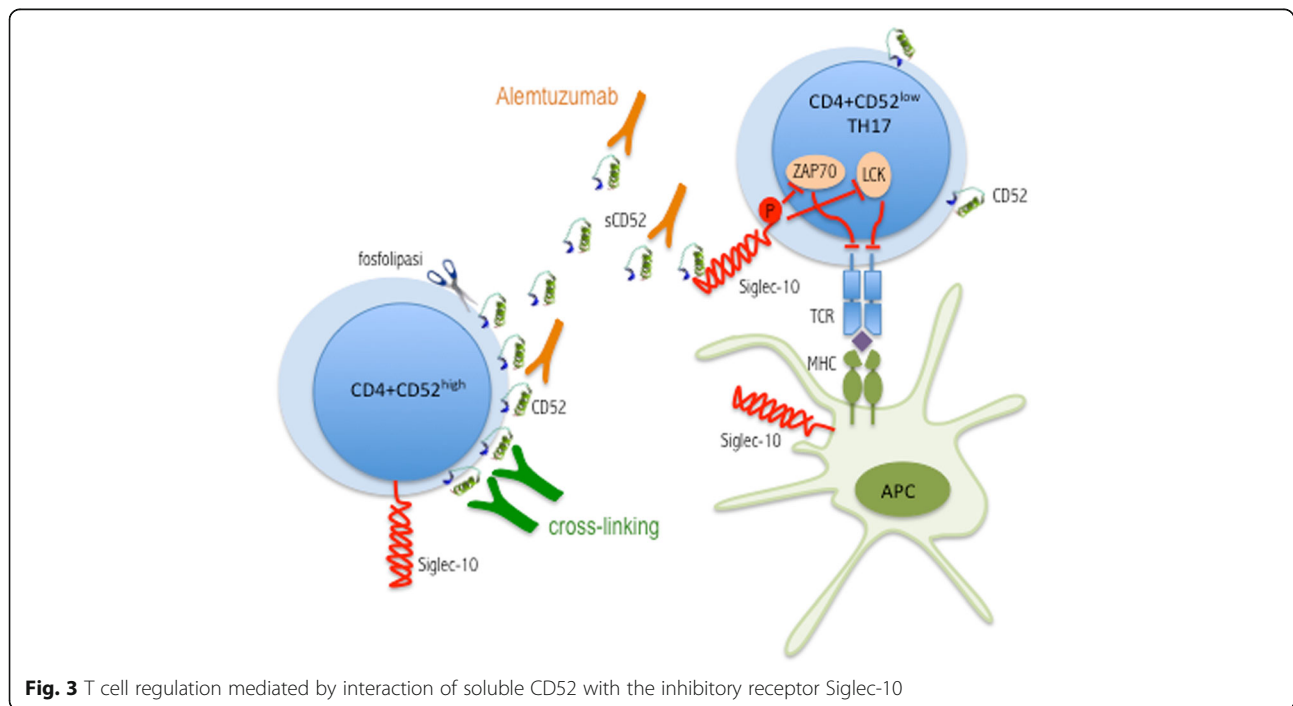


by molecular cross-linkage mediated by an as yet unidentified endogenous ligand that, in vitro, might be mimicked by a bivalent anti-CD52 antibody [3]. These two (cytotoxic and pro-apoptotic) mechanisms may operate in a synergistic manner.

Since one third of alemtuzumab-treated patients develop autoimmune diseases, in particular autoimmune thyroiditis [17, 18], it might be possible that the long-lasting deletion of CD4 + CD25 + CD52^{high} T cells following therapy needs may account for these adverse events.

However, the impairment of the CD4 + CD25 + CD52^{high}-mediated T-cell regulation following alemtuzumab administration does not constitute a concern

with regard of MS since alemtuzumab exerts a strong immunosuppressive activity by activating (see below) the ACDC and CDC mediated lysis of T and B lymphocytes and inducing caspase-dependent cell apoptosis, leading to a marked global lymphocyte depletion that includes autoreactive pathogenic lymphocytes. The density of CD52 on cell surface is believed to influence the susceptibility of lymphoid cells to alemtuzumab-induced ACDC and CDC [37], but other factors, e.g. the level of expression of complement-inhibitory proteins, were also suggested to play a role especially in protecting cell lysis in the lymphoid organs [Genzyme, EU summary of product characteristics, 2013; [36] (Fig. 3).



Pre-clinical studies

Transgenic hu-CD52 mice

Since alemtuzumab does not cross-react with mouse CD52, a transgenic mouse model expressing the human CD52 molecule (huCD52) was created to assess, in pre-clinical experimental/animal settings, the effects of alemtuzumab on lymphocyte subsets in lymphoid organs [29]. The administration of a single 10 mg/kg dose of alemtuzumab to huCD52-transgenic mice replicated the depletion of B and T lymphocyte circulating populations observed in human studies, with little effect on cells of the innate immune system (neutrophils and NK cells). Lymphocyte depletion in lymphoid organs (spleen, thymus, lymph nodes) was less profound than in the blood and was mediated primarily through ADCC exerted by neutrophils and NK cells, and largely independent of CDC [29]. At higher alemtuzumab doses, the degree of lymphopenia in the lymphoid organs (especially in spleen and lymph nodes) was definitely less profound compared to that observed in the peripheral circulation. This can be explained by the fact that, at the therapeutic dosage, alemtuzumab is largely absorbed by circulating lymphocytes, thus making its penetration in the lymphoid organs very limited. Moreover, it might be possible that the effector mechanisms responsible for cell depletion are less abundant or effective in lymphoid organs compared with peripheral blood. Interestingly, a relative sparing of T cells with a regulatory phenotype (CD4 + CD25^{high}FoxP3+) compared to total CD4+ T cell population was observed in both the blood and spleen even though both populations express equivalent levels of CD52 on their surface. The mechanism and significance of this apparent sparing of T cells with a regulatory phenotype remain unclear but could be a relevant factor in determining the long term suppression of inflammation obtained by alemtuzumab therapy.

In hu52 mice, lymphocyte repopulation was also very similar to that observed in humans. Namely, B lymphocytes returned to baseline levels by 7–10 weeks post-treatment while CD4+ and CD8+ T cells recovered more slowly and were still below the baseline levels at weeks 25. One possible explanation for the differential timing of T and B cell repopulation in the blood might be that alemtuzumab did not significantly affect the bone marrow thus allowing for the rapid recovery of B lymphocytes, while the effect on thymus determined a partial depletion of thymocytes, which could account, at least partly, for the slower recovery of T lymphocytes. Indeed, examination of the thymus of the transgenic mouse disclosed a partial depletion of single-positive and double-positive thymocytes (reaching 50% at the dose of 10 mg/kg of alemtuzumab). However, while the partial loss of thymocytes may, at least partly, explain the delay in T-cell recovery, the

explanation for the long-lasting duration of T-cell lymphopenia is still missing.

In the huCD52-transgenic mice it was also possible to replicate the transient increase in serum cytokine levels observed in humans, and responsible for the so-called 'cytokine released syndrome' and mainly related to the acute liberation of pro-inflammatory cytokines, such as TNFalpha, IL-1 beta, IL-6.

The functional properties of immune cells that remained following alemtuzumab treatment in huCD52 mice were also evaluated [29]. T lymphocytes showed in vitro proliferation rates and cytokine production in response to anti-CD3 Mab stimulation very similar to that observed in vehicle-treated mice. The T-cell receptor repertoire and the efficiency of T-cell response against a viral challenge were also maintained after alemtuzumab treatment. The primary T-cell response against viruses was explored by challenging the huCD52 transgenic mice with the adenovirus vector Ad2 at 7, 21, or 35 days after alemtuzumab treatment. T-cell responses were reduced at 7 and 21 days post alemtuzumab treatment, but comparable to that observed in control animals after five weeks later, before T-cell counts returned to pre-treatment levels. The primary B-cell response was also modestly affected. The antibody response to a T-dependent antigen was attenuated 3 days after alemtuzumab treatment (the nadir of lymphocyte depletion), but was similar to vehicle-treated controls at 21 days [29]. Preserved immune responses in alemtuzumab-treated animals may be due, in part, to the activity of residual B and T lymphocytes within peripheral lymphoid organs.

These observations were further confirmed in a study aimed at evaluating the impact of alemtuzumab treatment on number and function of innate immune cells in the same murine [40]. Following alemtuzumab administration, a transient decrease in primary adaptive immune responses was observed, but there was little effect on memory responses. Namely, the antibody response to a T-independent antigen was not significantly affected by the treatment suggesting that the remaining B lymphocytes were still capable to mount a normal antibody response. On the contrary, the antibody response to a T-dependent antigen was impaired when immunization was performed few days after alemtuzumab treatment, but returned to control levels by day 21 post-therapy. A mild, transient decline on primary T cell responses was also observed in mice immunized with adenovirus Ad2, but the difference with not treated mice did not reach statistical significance. Interestingly, T cell response to Ad2 returned to control levels by despite peripheral T lymphocyte counts were still suppressed.

Data in hu-CD52 transgenic mice and in experimental autoimmune encephalomyelitis (EAE) suggest that the effect of alemtuzumab on the immune network is

multifaceted, and probably includes a disequilibrium in the lymphocyte numbers, alterations in the functional properties of some lymphocyte subsets, and a transient effects on components of innate immunity. Particular intriguing is the observation that, after alemtuzumab treatment, naïve CD4+ and CD8+ T cells in the spleen were the most susceptible to depletion, whereas central and effector memory T cells, as well as Tregs, were depleted to a lesser extent, despite equivalent levels of CD52 expression on their surfaces.

All together, the observations done in Hu-CD52 transgenic mice suggest that protective levels of T cell immunity against infection can still be present despite the marked T and B cell lymphopenia. Animal data agree with the clinical data in humans showing that the highest risk of infection occurs in the first month post-alemtuzumab treatment and declines thereafter even though blood T cell counts remain below the normal range [12]. This may be partly explained by the recovery of sufficient numbers of lymphocytes in the lymphoid organs that can participate in the development of immune responses.

Experimental autoimmune encephalomyelitis

The effect of anti-CD52 therapy was tested in three murine models of the brain autoimmune inflammation, the EAE, that reproduces some of the histological features of MS: EAE induced by MOG_{35–55} (myelin oligodendrocyte glycoprotein) in C57BL/6 mice, which represents a standard acute model of disease primarily driven by peptide-specific CD4+ lymphocytes; chronic EAE induced by MOG_{1–121}, that is believed to involve the contribution of both T and B lymphocytes in the pathogenesis; and relapsing remitting EAE induced by PLP_{139–151} (proteolipid protein) in SJL mice [40]. Treatment with a single course of anti-muCD52 in mice with early symptoms (day 12–16 from immunization) achieved long term control of disease in all 3 models demonstrating efficacy against the different aspects of disease pathogenesis. In the MOG_{35–55} model, the delayed administration of anti-muCD52 at the time the mice attained more significant disability, with a disease score of 2 that corresponds to a full tail paralysis and hind limb paresis, was still efficacious and resulted in the almost complete reversal of disease symptoms. Interestingly, the control of disease was maintained up to ~day 90 without further retreatment in spite of complete lymphocyte repopulation in the blood and lymphoid organs.

Histological examination disclosed a significant reduction of immune cell infiltration in all regions of the spinal cord in anti-muCD52-treated mice vs control mice. This was accompanied by a significant reduction in the degree of spinal cord demyelination and axonal damage. Administration of anti-muCD52 not only

reduces the number of circulating T lymphocytes, but also reduces the number of infiltrating T lymphocytes in the CNS of EAE mice, decreases the number of MOG-reactive CD4 T cells and overall cytokine production and strongly inhibited the development of CNS inflammation and concomitant demyelination and axonal damage. Electrophysiological assessment showed preservation of axonal conductance in the spinal cord suggesting that anti-CD52 therapy may help to preserve CNS integrity [41]. Taken all together, these results demonstrate the robust general and local immunosuppressive action of anti-muCD52 treatment in several models of EAE and parallel the long term control of disease observed in the majority of RRMS patients treated with alemtuzumab.

Thus, experimental studies in vitro and in animal models have 1) confirmed the mechanism of action of alemtuzumab, 2) reproduced the kinetics of lymphocyte depletion and repopulation observed in humans and 3) correlated the strong, rapid and long-lasting immunosuppression to a significant reversion/amelioration of autoimmune-mediated brain inflammation.

Studies in humans

Alemtuzumab-treated patients exhibited a nearly complete depletion of circulating CD4(+) lymphocytes within few hours after administration with a nadir between day 3 and 7. The reconstitution of the circulating lymphocyte repertoire was found to follow peculiar kinetics. In the CARE-MS-I and -II studies, alemtuzumab was associated with a decrease in circulating lymphocyte counts after each treatment course, but had few or transient effects on other leukocytes (neutrophils, monocytes, eosinophils, basophils and natural killer cells) [8, 11, 12]. The total lymphocyte count returned to the lower limit of the normal values by month 6 in about 40% of patients and by month 12 in about 80% of patients. In one study, however, CD4+ lymphocytes remained significantly decreased for up to 24 months [42], while in another study both CD4+ and CD8+ lymphocyte counts remained below baseline levels for a median of 61 and 30 months, respectively [13]. B cell numbers returned to baseline by 3 months, and in some cases their number increased beyond baseline levels at 27 months (but rarely exceeded the upper limit of normal values) [13, 19].

In a follow-up study in 36 MS patients (384 person years), aimed at analysing the long term effects of a single course of alemtuzumab (100 mg over 5 infusion days, a dose higher than that currently use in MS) on lymphocyte reconstitution, the geometric mean recovery time of total lymphocyte counts to the lower limit of the normal range ($\geq 1.0 \times 10^9$ cells/L) was 12.7 months. For B cells the recovery time was 7.1 months, while CD8+ and CD4+ lymphocytes returned within the normal ranges in

20 and 35 months, respectively, but only in a minority of the patients they returned within the baseline values (30% and 21%, respectively). In spite of prolonged lymphocyte depletion, no infective safety concerns were observed [28].

The lymphocyte reconstitution profile that follows alemtuzumab-induced lymphocyte depletion appear very similar to that observed after marked immunosuppression obtained with other therapies, for instance haematopoietic stem cell transplantation, and suggests that it might be driven by common homeostatic mechanisms, such as proliferation of mature lymphocytes that escaped depletion, or by ex-novo lymphocyte production from precursors in the thymus [19, 32].

During the reconstitution phase of the circulating lymphocyte repertoire, CD4(+)CD25(+)CD127(low) regulatory T cells preferentially expanded within the CD4(+) lymphocyte population, reaching peak expansion at month 1. The increase in the percentage of TGF- β 1, IL-10, and IL-4 producing CD4(+) cells (Th2 phenotype) reached the higher level at month 3, whereas Th1 and Th17 Th cells increased much slowly and returned within the normal values at months 12. However, a significant decrease in the percentages of Th1 and Th17 cells was still detected at months 12 and 24 in comparison with the baseline values. A gradual increase in serum IL-7 and IL-4 and a decrease in IL-17A, IL-17 E, IL-21, IL-22, and IFN- γ levels were also demonstrated following treatment. These observations are in line with in vitro studies that disclosed that IL-7 induced an expansion of CD4(+)CD25(+)CD127(low) regulatory T cells and a decrease in the percentages of Th17 and Th1 cells, and further indicates that the differential reconstitution of T cell subsets and selectively delayed CD4(+) T cell repopulation following alemtuzumab-induced lymphopenia may partly contribute to its long-lasting suppression of disease activity [42] (Fig. 4).

In a recent 24-month multicentre study aimed at evaluating Th and Treg repopulation and function during alemtuzumab therapy in 29 MS patients, CD4+ cell percentage remained significantly lower compared to baseline, while a significant increase in Treg percentage was observed at month 24 and was accompanied by an increase in Treg suppressive activity against myelin basic protein-specific Th1 and Th17 cells [20]. Although the meaning of the increase in percentage and function of Treg in the late phase of alemtuzumab therapy is unclear and need to be investigated in larger numbers of patients, these findings further suggest that CD52 depletion induces a transient re-arrangement of the T cell network.

With regard to the analysis of lymphocyte subsets, data coming from CARE-MS-I and II showed that the relative proportion of CD4+ naive T cells decreased (from 36 to 5% [CARE-MS-I] and from 37 to 2% [CARE-MS-II]) and the relative proportion of CD4+ memory T cells increased (from 63 to 94% and from 63 to 97%, respectively) by month 1 and returned to near-baseline levels by month 12, while the relative proportions of CD4 + CD25+ regulatory T cells increased by month 1 (from 3 to 14% and from 4 to 13%, respectively) and remained elevated at month 12 [24]. A similar pattern was observed for CD8 + T cells. Moreover, the relative proportion of mature naive B cells decreased and the relative proportion of immature B cells increased by month 1 and approached baseline levels by month 6. It has been suggested that early T-cell reconstitution occurs via the expansion of existing cells which have escaped depletion, rather than via generation of new T cells in the thymus [32]. This may, at least partly, contribute to the development of secondary autoimmunity following alemtuzumab treatment, as peripheral expansion favours immune populations that respond to self-antigens [32]. However, as above mentioned, human and

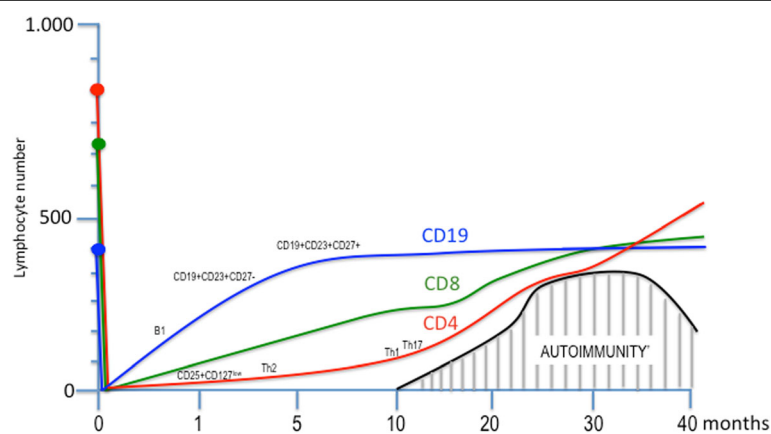


Fig. 4 Time-course repopulation of lymphocyte subpopulations in alemtuzumab-treated multiple sclerosis patients

mouse antigen-activated CD4⁺ T cells with high expression of CD52 on their surface (CD4 + CD52^{high}) were demonstrated to exert a suppressive action on other T cells by releasing CD52s. The demonstration that humans with autoimmune diabetes have a lower frequency and diminished function of CD52^{hi}CD4⁺ T cells responsive to the autoantigen GAD65 is particularly worth of interest, since it suggests a role of this T cell subpopulation in human autoimmunity. While these findings identify a CD52-mediated T cell regulatory mechanism that may protect from autoimmune diseases, they suggest that the impairment of this mechanism may contribute, at least partly, to the appearance of autoimmune disorders in alemtuzumab-treated individuals [2].

Alemtuzumab administration is associated with the acute induction of several pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF α), interleukin-6 (IL-6) and interferon gamma (IFN γ). This is due to the cross-linking of natural killer cells and to the lysis of lymphocytes and cells of the monocyte/macrophage lineage with subsequent release of pro-inflammatory cytokines that peaks 2-6 h after infusions and causes the so-called "cytokine-release syndrome).

However, the long-lasting effect of alemtuzumab on the cytokine network consists in the down-regulation of pro-inflammatory interleukins that may also be considered a major mechanism underlying the therapeutic effect of the drug. Indeed, in a study investigating cytokine production by ex vivo stimulated T cells obtained from MS patients treated for 12 months with alemtuzumab a long-lasting decrease in the secretion of IL-17 and IL-22 (produced by Th17 cells) was observed [27].

The immune-competence following alemtuzumab therapy was assessed in a pilot case-control study involving 24 patients [34]. The antibody responses to 3 vaccines (1. diphtheria, tetanus, and poliomyelitis vaccine, 2. Haemophilus influenzae type b and meningococcal group C conjugate vaccine, and 3. pneumococcal polysaccharide vaccine). The antibody titres against common viruses (mumps, rubella, varicella-zoster, and Epstein-Barr virus) were measured before alemtuzumab treatment and 1 and 9–11 months after treatment. Serum antibodies against common viruses remained detectable after treatment, and vaccine responses were normal to T-cell-dependent recall antigens (tetanus, diphtheria, and polio), to T-cell-dependent novel antigen (meningococcus C), and to T-cell-independent antigens (pneumococcal). No evidence for a diminished response to vaccinations in 5 patients studied within 6 months of alemtuzumab treatment was observed. Thus, alemtuzumab treated individuals retain 1) humoral immunologic memory (in the form of antibodies against common viruses and response to recall antigens), and 2) the ability to mount a humoral immune response against a novel

antigen. These data indicate that patients with relapsing remitting multiple sclerosis appear immunocompetent after treatment with alemtuzumab.

Alemtuzumab treatment was found to significantly reduce the risk of relapse and accumulation of disability compared with high-dose, high-frequency interferon beta 1a. The post hoc subgroup analyses of the CAMMS223 trial disclosed that among patients with no clinical disease activity immediately before treatment, or any clinical or radiological disease activity on-trial, disability improved after alemtuzumab but not following interferon beta. These observations led to the hypothesis that disability improvement after alemtuzumab was not only the result of the anti-inflammatory activity of the drug, but could in part be due to a sort of 'protective' effect mediated by neurotrophic factors possibly produced by lymphocytes. Indeed, after alemtuzumab, and only when specifically stimulated with myelin basic protein, peripheral blood mononuclear cell (PBMC) cultures produced increased concentrations of brain-derived neurotrophic factor, platelet-derived growth factor and ciliary neurotrophic factor [31]. Analysis by reverse transcriptase polymerase chain reaction of cell separations showed that the increased production of ciliary neurotrophic factor and brain-derived neurotrophic factor after alemtuzumab was attributable to increased production by T lymphocytes. Media from these post-alemtuzumab PBMC cultures promoted survival of rat neurones and increased axonal length in vitro, effects that were partially reversed by neutralizing antibodies against brain-derived nerve growth factor and ciliary neurotrophic factor. This conditioned media also enhanced oligodendrocyte precursor cell survival, maturation and myelination. The hypothesis that alemtuzumab-induced lymphocyte-derived neurotrophins may play a neuro-protective role in vivo in MS patients needs further investigations.

Pharmacokinetic data

The pharmacokinetics of alemtuzumab were investigated in a study involving 216 patients with RRMS who received a first treatment course of intravenous infusions of alemtuzumab 12 or 24 mg/day on 5 consecutive days, followed by a second course with the same dosage on 3 consecutive days one year later. The highest serum concentration of alemtuzumab was observed following the last infusion of each treatment course; serum concentration increased with each consecutive dose within the course. The mean maximum serum concentration (C_{max}) was 3014 ng/mL on day five of the initial treatment course, in recipients of alemtuzumab 12 mg; the mean C_{max} was 2,276 ng/mL on day three of the second treatment course.

Classical biotransformation studies have not been conducted for alemtuzumab, as yet. The alpha elimination half-life was $\approx 4\text{--}5$ days in both treatment courses, and low or undetectable serum concentrations occurred within ≈ 30 days of the end of each treatment course. No sufficient data are available to draw conclusion on the effect of race or sex on alemtuzumab pharmacokinetics and no formal drug interaction studies have been conducted for alemtuzumab at the recommended dosage in patients with MS (Genzyme Therapeutics Ltd. LemtradaTM, EU summary of product characteristics. 2013. [21] <http://www.ema.europa.eu/docs>).

Clinical trials and extension data

The effects of alemtuzumab on clinical and magnetic resonance parameters in patients with active RRMS have been investigated in one Phase II (Campath-1H in Multiple Sclerosis, CAMMS223) and two Phase III (Comparison of Alemtuzumab and Rebif efficacy in Multiple Sclerosis, CARE-MS-I and CARE-MS-II) randomized, rater-blinded, active comparator-controlled, multinational trials. Five years extension data have been made recently available from all these studies ([8, 10–12], Coles et al., 2013).

In CAMMS223 (enrolment period: December 2002–July 2004), 334 treatment naive RRMS patients with a disease duration <math><3</math> years were randomized to receive 1) treatment with i.v. infusion of alemtuzumab 12 ($n = 113$) or 24 ($n = 110$) mg/day on 5 consecutive days, followed by a second treatment course at the same dosage on 3 consecutive days at months 12 and 24, or 2) subcutaneous interferon beta-1a 44 mcg tiw ($n = 111$). On September 2005, the trial was suspended for the appearance of three cases (one death) of alemtuzumab-related immune thrombocytopenic purpura (ITP). Interferon beta-1a treatment continued as planned. In 2008, the dosing suspension was lifted, an option redosing was given to the patients and a safety monitoring programme (ended in January 2010) was planned and carried on. Compared to IFN β -1a, alemtuzumab 12 mg/day was significantly more effective in reducing the relapse rate (-67% $p < 0.0001$), in increasing the number of relapse-free patients (76% vs. 50% , $p < 0.0001$) and in decreasing the sustained accumulation of disability (SAD) (8% vs. 27% , $p = 0.0006$). The MRI findings were also impressive: compared to IFN β , alemtuzumab-treated patients had a lower increase in T2WM lesion volume ($p = 0.005$) and a lower reduction in brain volume on T1 images ($p = 0.05$).

In CARE-MS-I naïve RRMS patients were randomized 2:1 to treatment with i.v. alemtuzumab 12 mg (on days 1–5 at baseline and on days 1–3 in a second course 12 months later) or s.c. IFN β -1a 44 mcg tiw.

In CARE-MS II, RRMS considered not responder to DMD were randomized 2 : 2 : 1 to alemtuzumab 12 or 24 mg (on days 1–5 at baseline and on days 1–3 in a second course 12 months later) or IFN β -1a 44 mcg tiw. Alemtuzumab 12 mg/day was more effective than IFN β -1a in the reduction of relapse rate (co-primary endpoint) in patients with treatment-naïve (54.9%) or previously treated (49.4%) RRMS ($p < 0.0001$ in both studies). Alemtuzumab 12 mg/day was also significantly ($p < 0.0001$) more effective than IFN β -1a with regard to time to first relapse in both treatment-naïve (HR 0.45 [95% CI 0.33–0.61]) and previously treated patients (HR 0.53 [95% CI 0.41–0.69]).

The number of patients who were relapse-free at 2 years was significantly ($p < 0.0001$) higher in the alemtuzumab 12 mg/day group than in the IFN-1a group in both studies. Similarly, alemtuzumab 12 mg/day was more effective than IFN β -1a in the reduction of 6-month confirmed sustained accumulation of disability rate (coprimary endpoint) in patients previously treated RRMS, but there was no significant between-group difference in patients with treatment-naïve RRMS. Alemtuzumab was associated with a risk reduction of 30% (not significant) in treatment-naïve and 42% ($p = 0.0084$) in previously treated patients for this endpoint. In previously treated patients (CARE-MS-II), significantly ($p = 0.0002$) more alemtuzumab 12 mg/day than interferon beta-1a recipients had a sustained reduction in disability score from baseline, confirmed over 6 months, in patients with baseline EDSS scores of >2.0 [HR 2.57 (95% CI 1.57–4.20)]. Between-group differences in the change in EDSS score were significant ($p < 0.0001$) in previously treated patients, but not in treatment-naïve patients.

Alemtuzumab 12 mg/day was significantly ($p < 0.05$) more effective than IFN β -1a with regard to the proportion of patients with Gadolinium-enhancing lesions at 24 months or new or enlarging T2 lesions, and the median change in brain parenchymal fraction in patients with treatment-naïve or previously treated RRMS. No significant between-group difference was observed in the median change in volume of hyperintense lesions in either study. Significantly more recipients of IFN β -1a had new T1 hypointense lesions than recipients of alemtuzumab in previously treated patients; the difference was not significant in treatment-naïve patients. Alemtuzumab was associated with a significantly ($p < 0.01$) greater proportion of patients who were clinically disease free (absence of both relapses and sustained accumulation of disability) and those who were both clinically disease free and MRI disease free (absence of both Gadolinium-enhancing lesions and new or enlarging T2 lesions) than IFN β -1a in both treatment-naïve and previously treated

patients with RRMS. In a sub-analysis of patients with highly-active RRMS in CARE-MS II (101 alemtuzumab and 42 IFN β -1a recipients), 33.3 and 0%, respectively, were disease activity-free at 1 year ($p < 0.0001$) and 24.2% of the alemtuzumab recipients remained disease activity-free at 24 months. At 24 months, 35.8 and 60.0%, respectively, had relapses, 7.4 and 17.5% had sustained accumulation of disability, 22.1 and 52.5% had gadolinium-enhancing lesion activity, and 60.0 and 92.5% had T2 -lesion activity.

Supportive analyses demonstrated that significantly fewer alemtuzumab 12 mg/day than interferon beta-1a recipients had severe relapses (reduction of 61 and 48%; both $p < 0.05$) or relapses leading to steroid treatment (reduction of 58 and 56%; both $p < 0.0001$) in both treatment-naïve and previously treated patients, respectively, and relapses that led to hospitalization in previously treated patients (reduction of 55%; $p = 0.0045$).

Data of the five-years extension study of Phase III Clinical Trials have been recently presented atECTRIMS 2015 [1, 14, 16, 25, 33, 39] The proportion of patients having no evidence of 6-months confirmed disability progression remained high through year 5: 80% in CARE-MS-I and 76% in CARE-MS-II compared to respectively 92 and 87% observed at the end of the trials (2 years). More than 50% of the patients of both studies maintained the NEDA (non evidence of disease activity) at the end of the follow-up. Interestingly, alemtuzumab continued to slow-down the loss of brain volume over the five years: the mean annual rate of brain volume loss ranged between 0.07% (CARE-MS-II) and 0.2% (CARE-MS-I), a rate similar to that observed in normal individuals (0.1–0.3%). Furthermore, the proportion of patients with sustained improvement in pre-existing disability (defined as ≥ 1 -point EDSS decrease over 6 month for patients with EDSS ≥ 2.0 at core study baseline) increased progressively through 5 years, and was respectively 33% in CARE-MS-I and 43% in CARE-MS-II. Finally, the cytokine-release syndrome became progressively milder with repeated administration and the rate of antibody-mediated autoimmunity (thyroid) decreased from 20% to 10% from year 3 to year 5.

Recently, data of 10-year follow-up of patients enrolled in the CAMMS-223 study have been presented at the American Academy Neurology annual congress [15]. Of the 60 patients who enrolled in the long-term follow-up, 20 (33%) received only the initial 2 courses over 10 years. Of the 39 (65%) patients receiving > 2 alemtuzumab courses, 26 (67%) only received 3 courses, 7 (12%) received 4 courses, and 6 (10%) received a total of 5 courses.

Through 10 years of follow-up, a low ARR (0.08) was maintained, while the mean EDSS score change from baseline (SD) was +0.12 over 10 years. Interestingly, EDSS remained stable or improved over 10 years

in the majority of patients, and most patients (76%) showed no evidence of 6-month confirmed disability worsening. These observations indicate that alemtuzumab may provide a unique treatment approach with durable efficacy in the absence of continuous treatment for RRMS patients through 10 years.

Safety profile of alemtuzumab

The adverse events (AE) complained by up to 90% of patients during or within 24 h after alemtuzumab infusion constitute the so-called 'cytokine release syndrome', and appear more frequently on the first day of infusion. The majority of these AE are mild to moderate in severity and are managed by premedication with steroids, anti-pyretics, and anti-histamines. The proportion of patients with AE is higher during the first course of alemtuzumab than the second.

In the RCT CAMMS223, CARE-MS-I and CARE-MS-II, infections were slightly more common with alemtuzumab than with sc IFN β -1a, but most were mild or moderate in severity. Serious infections were reported rarely (2–4% alemtuzumab versus 1% IFN β -1a). The most common infections were those of the respiratory and urinary tracts. Herpetic infections occurred in 16% of patients in the CARE-MS I and CARE-MS II studies. The risk of herpetic infection was greatest in the first month post-treatment, and could be reduced by prophylactic oral aciclovir (only 0.5% of patients receiving aciclovir developed a herpetic infection after the first course of alemtuzumab). Despite the marked immune-depression, no case of progressive multifocal leukoencephalopathy has been reported in alemtuzumab-treated MS patients up to date.

No statistically difference in the risk of cancers was observed after alemtuzumab compared to IFN β -1a in any of the trials (that, however, were not powered to pick up differences in low-frequency events).

Autoimmune AE constitute the most relevant risk of alemtuzumab therapy and have been associated with the characteristic time-course lymphocyte repopulation above described. Three major types of autoimmune disorders have been reported: thyroid diseases in about 30% of patients (Graves' hyperthyroidism, 65.8%, hypothyroidism, 20.5%, and subacute thyroiditis (12.3%), idiopathic thrombocytopenic purpura (ITP) in 1% and nephropathy due to anti-glomerular basement membrane antibodies in 0.3% [8, 12]. In addition, single case of autoimmune neutropenia, pancytopenia and haemolytic anaemia, have been reported in less than 0.5% of treated patients.

The monitoring schedule recommended in the Lemtrada's Summary of Product Characteristics should be rigorously applied to identify rapidly the first symptoms of autoimmune disease so as to institute appropriate therapy.

Table 2 Alemtuzumab meets almost all the criteria that are expected from the ideal disease-modifying immunosuppressive/immunomodulatory drug for multiple sclerosis

Criteria of the ideal immunosuppressive drug	YES	Partially	NO
Rapid biological action	X		
Rapid clinical effects	X		
Long-lasting effects	X		
Low frequency of injections/administrations	X		
Respect the patient's quality of life	X		
Acceptable costs		X	
No non-specific toxicity	X		
Low risk of opportunistic infections	X		
No increase risk in cancer	X		
No increase in antibody-mediated autoimmunity			X
No increase in severe antibody-mediated autoimmunity		X	
No increase in mortality	X		
Manageable cytokine-release syndrome	X		
Immune system re-modelling/re-shaping	X		

Reviews with more detailed information on safety profile of alemtuzumab are available in the literature (Havrdova et al., 2015; [24, 26, 35]).

Conclusions

Alemtuzumab can be considered an immunosuppressive drug having complex, yet unexplored, immunomodulatory activities (Table 2). Its biological and clinical actions are fast and, in a large proportion of patients, long-lasting. This allows a low frequency of administration, thus making acceptable the infusion-related side effects (the cytokine release syndrome) and the costs of the therapy. In front of an increased in antibody-related autoimmune disorders (mainly autoimmune thyroiditis), it has to be pointed out that the risk of severe, life-threatening adverse events is extremely low and actually manageable. Moreover, Alemtuzumab seems not to expose the patients to an increased risk of cancer, non-specific toxicity and mortality, and the risk of infectious diseases appears very low. All these features make alemtuzumab a significant step forward in the therapy of MS.

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