Alemtuzumab

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Alemtuzumab (Campath®, MabCampath®, Genzyme) is an IgG1k anti-CD52 humanized monoclonal antibody (mAb) that was first licensed in March 2001 by FDA. EMEA granted its approval in July 2001 and Health Canada in November 2005. The initial indication was limited to B-CLL previously treated and resistant to alkylating agents. Starting from 2007, alemtuzumab was approved also as first-line therapy of B-CLL. So far, it has been experienced in over 60 countries.

Initial therapeutic attempts, performed up to 1995 on 527 subjects and conducted by Burroughs Wellcome, regarded both leukemia/lymphoma and non-neoplastic conditions (rheumatoid arthritis, renal transplant rejection). They were aimed at taking advantage of the profound depletion of T cells caused by this mAb. In fact, CD52 is also highly expressed on T-CLL (100 %), HCL, ALL (79 %), and NHL (94 %) other than on B normal and neoplastic cells. Among 21 trials conducted by the end of 2004, seven related to CLL, six to lymphomas and other types of leukemia, four investigated rheumatoid arthritis, three were compassionate studies, and one was conducted on kidney transplant recipients. However, the encountered severe hematotoxicities led to discontinuation of many of these attempts and the approved indication remained restricted to B-CLL.

Pivotal studies for initial approval consisted in three single arm trials enrolling 149 patients, and in particular one Phase II study (CAM211) enrolling 93 CLL patients (86 with B-CLL), Study CAM009 on 24 CLL patients (22 B-CLL), and Study CAM005 on 32 B-CLL patients. All subjects had been previously treated with alkylating agents and were refractory to fludarabine. The subsequent approval for B-CLL first-line therapy was based on one Phase III trial (CAM307) enrolling 297 (149 exposed) patients [1–6]. In most cases alemtuzumab is administered intravenously (IV), while in a number of studies and in clinical care is also administered subcutaneously (SC).

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In August 2012, EMEA decided to withdraw the marketing authorization for alemtuzumab, allowing patients in need of treatment for B-CLL to receive it through specific access programs. Nonetheless, off-label applications are still frequent and relate to different neoplastic and non-neoplastic conditions. More recently, two main ongoing studies (CARE-MS I, CARE-MS II) have evaluated the efficacy on multiple sclerosis (MS), and a new application of alemtuzumab, under the name of Lemtrada, has been submitted during 2012 to FDA (accepted for review in January 2013) and to EMEA.

7.1 Mechanism of Action

CD52 is a non-modulating glycoprotein of 21–28 kDa expressed on virtually all normal and malignant T and B lymphocytes, NK cells (>50 %), most monocytes, macrophages, a portion of dendritic cells, and granulocytes (<5 %). In the bone marrow, lymphoid progenitors stain strongly with alemtuzumab, while uncommitted and myeloid-committed progenitors are weakly positive. Erythrocytes and platelets are negative. In other non-hemopoietic tissues, relevant binding is present on cutaneous dendritic cells and T lymphocytes. Additional positivity was also encountered in lymphoid primary and secondary organs, and on some male sexual organs (epididymis, seminal vesicles) and mature sperm cells. In lymph nodes the germinal centers stain weakly. These bindings were considered Fab-specific. However, non-specific Fc binding was also detected in a wide range of organs and tissues. An average expression of 5×10^5 CD52 molecules/cell has been reported for lymphocytes.

Alemtuzumab is an IgG1k anti-CD52 humanized monoclonal antibody binding to CD52 cell surface nonmodulating glycoprotein. Upon binding, there is a profound depletion of CD52+ cells. A transient loss of CD52 cell expression was also observed during treatment. In a study sub-group included in the CAM307 trial, 2/139 patients reported a complete loss of CD52, which recovered in both cases prior to disease relapse. Therefore, unstable negative clones seem to be produced by this treatment.

The proposed mechanisms of action, based on in vitro studies, involve antibody-mediated cell cytotoxicity (ADCC), complement dependent cytolysis (CDC), and apoptosis. The first is considered the most relevant effector function in vivo. Other actions such as opsonization, T cell activation, cytokine release, non-specific complement activation, and induction of T cell anti-tumor activity are considered less relevant for the specific therapeutic action, but may be important for AEs induction. With this respect, some pharmacokinetic aspects of alemtuzumab accumulation and clearance are important as well. The latter was shown to be nonlinear and dependent on the amount of CD52+ cells (i.e. tumor burden). Hence, the half-life increases with dosing, due to saturation of clearance pathways and progressive reduction of tumor mass capturing the mAb. Therefore, the specific mechanism of action and the pharmacodynamics of alemtuzumab are crucial for

AEs typology and expression not only during treatment, but also for a wide post-treatment phase, due to the mAb long lasting activity [7, 8].

After alemtuzumab discontinuation, B cell recovery tends to precede T cell recovery, the number of B cells may exceed baseline values, and CD8+ T cell subset tends to reappear before CD4+ lymphocytes. However, the physiological balancing may take a considerable length of time (years) to reach normal values. Therefore, emerging autoreactive B cell clones, either T-independent or as a consequence of the relative absence of regulatory T cells, may expand and raise autoimmune phenomena after monoclonal lymphocyte depleting regimens.

7.2 Immunogenicity

The initial IgG2a rat anti-CD52 monoclonal antibody was genetically engineered by inserting six complementary-determined regions (CDR) into a human IgG1k molecule to reduce its immunogenicity. Therefore, the immunoreactivity against alemtuzumab is expected to be relatively low. In fact, the raise of anti-alemtuzumab antibodies has been estimated either in first (8 %) or in second-line (2 %) B-CLL therapy, and appears to be not relevant for AEs induction. The production of neutralizing antibodies is usually a minor fraction and seems not to be involved in the generation of AEs, as well. However, a higher response (30–50 %) was observed in RA patients, mainly when treated with SC injections. Noteworthy, such route of administration is known to produce more effective sensitizations to antigens, possibly due to a better concentration and presentation by Langerhans cells to T lymphocytes in the local environment. Moreover, in other experiences, SC administration gave a lower rate of response, which was attributed to a more effective induction of anti-mAb response [9, 10].

7.3 Adverse Events

Adverse reactions to alemtuzumab in B-CLL, as well as in various off-label and new special trials, remain the most important therapeutic limitation.

The general safety profile is primarily based on data obtained from the mentioned three primary studies, including 149 B-CLL patients previously treated with alkylating agents, and from the additional study on 147 untreated patients, which was presented in 2007 for approval extension to naive B-CLL patients [4]. Additional information comes from previous three Phase I-II studies including 175 patients, compassionate programs conducted on 177 patients, seven open oncology studies, three studies on RA (140 patients), and from postmarketing reporting.

Alemtuzumab-related adverse events in B-CLL patients are mainly expressed as *acute infusion reactions*, *infections,* and prolonged *cytopenias*. *Tumor lysis syndrome* (TLS) and *progressive multifocal leukoencephalopathy* (PML), although rarely encountered in the postmarketing experience, are additional serious complications. TLS was first included in the 2004 label update, and a warning about

monitoring and treatment suspension in the presence of signs suggesting PML has been recently added [6].

Infusion reactions (pyrexia, rigors/chills, nausea, hypotension, urticaria, dyspnea, rash, emesis, bronchospasm) occurred at the highest rate during the first week of treatment. In clinical trials, severe reactions (≥grade 3) have been estimated to be 35 % in patients previously treated with alkylating agents, and 10 % in untreated subjects. Serious and fatal events observed in the postmarketing settings included ARDS, pulmonary infiltrates, cardiac functional and ischemic disorders, angioedema and anaphylactoid shock.

The overall absolute frequency of infusion reactions in controlled studies is difficult to assess, due to systematic albeit variable premedications routinely performed and to a non-systematic reporting of mild-moderate events. While all patients receive antipyretics and antihistamines, about half of them also receive glucocorticoids, which usually are not advised in oncologic patients under different immunosuppressive treatments. Therefore, serious events (SAEs) are better estimated in trial records, since they receive much attention in the study, and reporting is mandatory.

In some clinical trials and clinical care, alemtuzumab has been administered by subcutaneous (SC) injections, usually for a more prolonged period. Local reactions (erythema, edema, pruritus, pain), often associated with pyrexia, and systemic reactions may appear as well. They are usually observed during the first 2 weeks, the latter with lower frequency and milder expression in case of IV administration. Hypotension, cutaneous reactions, and a number of constitutional signs are virtually absent after SC administration. However, pyrexia remains frequent (70 vs. 85 %), although considerably reduced in severity (2 vs. 14 %) [11, 12].

It is known that CLL is accompanied by immunosuppression, inherent to the disease and worsened by cytostatic treatments. Bacterial and viral *infections* are therefore common, and are the major cause of death.

Serious and sometimes fatal bacterial, fungal, viral, and protozoal infections have been reported as related to alemtuzumab, either in trials or in postmarketing reporting. The overall incidence ranges 23–80 % in different studies, and SAEs reach 50 %, with no significant differences among previously treated or naive B-CLL. Opportunistic infections are also frequent (17–43 %) and include pneumocystis pneumonia (PCP), aspergillus, HZV, CMV, candidiasis, mucomycosis, and JC virus reactivation (PML) [5, 13]. Since the immunosuppressive effect is not strictly dose-dependent, infection may appear at any stage of treatment and post-treatment, with repeated episodes of different etiology. CMV reactivation and subsequent infections have been followed with particular interest in these patients. CMV viremia was found to be as high as 66 %, and consequent infections appeared to be surprisingly higher (16 %) in naive patients than in previously treated ones (6–8 %). However, in protocols applied to untreated subjects, CMV detection and infectivity reporting were mandatory, while in other pivotal studies they were mostly recorded only when classified as serious. In fact, when only serious events were compared, the incidence in the two groups was similar [3, 5, 6]. It must be stressed that, as it happens for symptomatic premedication of

infusion reactions, the potential sensitivity to infections is likely to be in part masked by routine anti-microbial prophylaxis. Remarkably, more than 70 % of all infections remained of unknown etiology in most studies. Overall, their average rate was estimated to be over 1.8 infections/patient. In SC treatments, rates of CMV and non-CMV infections were similar [12].

Due to the massive destruction of circulating WBC, *cytopenia* is the central phenomenon related both to therapeutic effect and to AEs genesis. In particular, it derives from the profound and prolonged lymphopenia induced by alemtuzumab. A massive destruction of T cells is present in almost 100 % of cases, producing a rapid and abundant release of cytokines that are mainly responsible of the acute infusion reaction, *cytokine release syndrome* (CRS) and of similar systemic syndromes (see Chap. 3). The profound lymphopenia impairs the immune resistance to infections, including the opportunistic ones, while the rapid destruction of the neoplastic cell burden (mainly represented by malignant B lymphocytes) causes the nephrotoxic TLS. Therefore, these AEs are strictly related to the specific action of adalimumab, and theoretically are difficult to be avoided. However, they can be mitigated through different strategies, such as premedication and anti-microbial prophylaxis (for infusion reactions and infections, respectively), or administration rules (subcutaneous injection; dose-graduation; tumor burden pre-reduction) to globally reduce their overall negative impact. Finally, a peculiar risk of severe and profound lymphopenia is related to potential *transfusion-associated graft versus host disease* (TA-GVHD), usually avoided by the previous radiation of transfused material [14].

Other cytopenias, mainly neutropenia present in 75–85 % of cases (febrile neutropenia 5–10 %), further increase the risk of infections (bacterial in 40 % of cases). During SC treatments, neutropenia occurred at lower levels (56 vs. 70 % IV). Thrombocytopenia (over 70 %) can be serious in 57 % of cases, causing purpura and infrequent hemorrhagic fatalities. General hematotoxicity, expressed by pancytopenia, bone marrow hypoplasia, and aplastic anemia, is rare although serious.

As for AEs/SOC typology, they mainly involve the immune system, and the respiratory and dermatological compartments; less frequently, although with occasional severity, the cardiovascular and gastrointestinal systems are involved.

As for AEs timing, immediate events (hours to days) mostly relate to infusion and hypersensitivity reactions, while delayed reactions (weeks to months) pertain to cardiac function (insufficiency, failure), neuro-psychiatric disturbances (GBS, depression), and secondary malignancies. Overall, the majority of alemtuzumab-related reactions appear as early (days to weeks) events.

Fatalities are mostly related to infections, being higher in pretreated patients (16 %) than in patients receiving alemtuzumab as first-line drug (2 %). Fatal infections include viral meningitis, listeria meningitis, legionella pneumonia, CMV, PCP, EBV, and associated lymphoproliferative disorder, appearing at any stage of treatment and long after therapy.

Overall, the safety profile in previously treated and naive B-CLL patients is similar, although a lower incidence to induce severe reactions—mainly as drug-related severe infections—among the latter suggests a milder occurrence [15].

It must be stressed that since data are heterogeneous, attempting to compare profiles of different studies is difficult and inconvenient.

Since SC alemtuzumab injection showed comparable efficacy with a lower toxicity in CLL, this method of administration has become the preferred one. However, Health Canada did not approve such route [12, 14, 16, 17].

7.4 Off-Label Experience

Alemtuzumab therapeutic interventions, although officially limited to B-CLL, continue to expand. A number of therapeutic uses have been experienced, both in neoplastic and non-neoplastic diseases, either in studies or in current clinical care. Alemtuzumab has been mainly employed in non-B CLL, T-PLL, ALL, TCL, and pTCL. Non-neoplastic experiences are reported in in hemopoietic stem cell transplantation (HSCT), immunosuppression, renal transplant, bone marrow conditioning, and various autoimmune disorders, including MS. The latter indication is currently under evaluation from FDA and EMEA, on the basis of efficacy and safety data collected in two trials (CARE-MS I; CARE-MS II).

7.4.1 Neoplastic Off-Label Experience

Although results on *T cell prolymphocytic leukemia* (T-PLL) remain poor, alemtuzumab effects against this aggressive form of leukemia have been repeatedly investigated, since CD52 antigen is highly expressed on its cells. At present, five ongoing trials have enrolled 185 patients, while previous experiences have been recently reviewed [18].

In a previous report on eight single arm studies [19], poor results were associated with a high risk of AEs. Most common encountered AEs were serious thrombocytopenia 32 %, serious neutropenia 17 %, infections 25 %, and mild infusion reactions. However, more recent reports experiencing alemtuzumab as single agent have showed more encouraging results and reduced AEs rates [20]. About 50 % of these patients were naive T-PLL; nine of them had been previously treated in a study of the same group investigating IV vs. SC routes. Although the latter induced less AEs, the former method was chosen on the basis of clinical response. Nonetheless, it was found that infections were lower (10 %) in these patients than in CLL (40 %) cured by the same institution. Antimicrobial prophylaxis and symptomatic premedications respectively reduced opportunistic infections and infusion reactions. They also showed that debulking strategies to reduce tumor burden were not advisable, because of the increase in hematotoxicity.

Therefore, it seems that a proper use of alemtuzumab as monotherapy, instead of second-line therapy, remarkably reduces frequency and severity of AEs.

In principle, T cell lymphomas, including *peripheral T cell lymphoma* (pTCL), should be particularly suitable for therapy with alemtuzumab due to high CD52

density expressed on their neoplastic cells. However, the rate of serious AEs resulted particularly relevant. AEs encountered in a pilot study on 14 patients with relapsed or chemotherapy-refractory pTCL were mild/moderate infusion reactions (64 %), including urticaria and bronchospasm (7 %), during the first infusion. However, hematotoxicity resulted much relevant, since four patients suffered pancytopenia (one resolved) and two of these cases developed hemophagocytosis. Interestingly, one of them was reverted by mAb therapy discontinuation. Infections were serious and included opportunistic complications (three cases fatal), and CMV reactivations (37 %), with pneumonitis (29 %). In particular, two pulmonary aspergillosis (one fatal), one HZV infection (fatal), and one infection associated with TB (fatal) were observed. Due to a total of five fatal AEs in a short period, considered to be drug-related (36 %), the study was halted [21].

In a second investigation, including 20 patients with pTCL treated with consistent doses of alemtuzumab, AEs during therapy consisted of a relevant number of infections (70 %), mostly serious (86 %), neutropenic fever (40 %), and CMV reactivation (35 %). One CMV disease (retinitis) developed 1 year after therapy discontinuation. Three patients developed secondary EBV-related lymphoma (two of them after two months and the other after one year), and one developed CMV retinitis after one year from the end of treatment [22]. Similar experiences were reported even with lower doses of alemtuzumab. Therefore, due to the high incidence of CMV reactivation (approximately 30 % of patients in the two reported studies) and pneumonitis, compared to various T-lymphomas not treated with this mAb, a specific role of alemtuzumab was suspected.

Interestingly, susceptibility to AT-GVHD after alemtuzumab administration seems higher than after rituximab. In fact, irradiation of transfused blood materials is only recommended with the former, thus indicating its stronger and prolonged lymphopenic effect [14].

As for other relevant AEs signs, pancytopenia was observed at an unexpected high rate (29 %) and severity (100 %) in pTCL, compared to B-CLL (5–6 %; serious 3 %) and other neoplastic forms treated with alemtuzumab [11].

Since hemopoietic stem cells (CD34+) do not express CD52, additional toxicity and/or peculiarities of this lymphoma may be postulated. Furthermore, hemophagocytosis is known to be associated with T-lymphoma, although at lower expected rates, and has been related to EBV reactivation. However, other EBV-related lymphomas (HL, BKL) are not associated with hemophagocytosis. EBV reactivation and induction of lymphoproliferative diseases has been also encountered in these patients after months from the end of therapy, as it happens also with other immunosuppressive therapies, in kidney transplant recipients treated with alemtuzumab. Therefore, despite this anti-CD52 mAb is expected to destroy also EBV+ B cells, the subsequent imbalance in reconstitution of various lymphocyte cell classes may result in a new outbreak of EBV+ cells, more prone to proliferate in the absence of fully operative immunosurveillance [22]. In conclusion, particularly severe *pancytopenia associated with hemophagocytosis,* and *viral reactivations* may represent additional specific signals of alemtuzumab treatment in T

cell lymphoma patients, apparently developing hematotoxic secondary effects, due to additional mechanisms of action.

7.4.2 Non-Neoplastic Off-Label Experience

Major information on AEs expression derive from the experience in non-neoplastic conditions, consisting in the treatment of some autoimmune diseases and in lymphoablative procedures mainly followed by autologous HSCT. The most relevant signal coming from these experiences is the insurgence of *secondary autoimmune disorders* (sAID), reported in the range of 5–30 %, and including autoimmune cytopenias, thyroiditis, rheumatoid arthritis, lupus-like syndrome, Factor VII and Factor VIII hemophilia, and myasthenia gravis.

In *myeloablative/lymphoablative treatments* with alemtuzumab prior to HSCT, sAID occurrence has been reported (2–5 %) after autologous and allogeneic HSCT for nonmalignant and malignant conditions, and cytopenia was the most frequently reported event.

One retrospective (1996–2006) study on 155 patients undergoing auto-HSCT for various autoimmune primary disorders identified six patients having SLE (3), MS (2), or SSc (1) as primary disease, developing sAID *distinct* from their underlying autoimmune diseases after a median time of 8.5 months; four of them (67 %) had been previously treated with alemtuzumab and developed autoimmune cytopenias. In particular, they developed autoimmune thrombocytopenia (2), hemolytic anemia (1), and neutropenia (1). The remaining 2 cases were treated with anti-thymocyte globulin (ATG) and developed Factor VIII hemophilia. Overall, sAID complications were 16 % with alemtuzumab (4/25), 2 % with ATG (2/102) and 0 % without lymphoablative treatments [23].

Interestingly, the underlying mechanisms seem paradoxical and complex. A genetic propensity to develop autoimmunity, the unbalanced lymphocyte reconstitution after HSCT, and the combination of such status with the mAb-dependent lymphocyte depletion seem to act as powerful inducers of sAID, even when the primary autoimmune disease seems to be controlled by therapy. In fact, all but one of these patients developed sAID despite achieving remission of the primary autoimmune disease. With this respect, a prolonged lymphocyte depression induced by alemtuzumab and a delayed T cell reconstitution compared to B cells, both induced by HSCT and mAb treatments, may facilitate the appearance of uncontrolled new auto-reactive clones. Since alemtuzumab also affects dendritic cells, monocytes, and in part NK cells (all sharing the CD52 targeted antigen), such profound immune dysregulation may greatly enforce the possibility of developing new and even rare autoimmune disorders. Noteworthy, when employed in the absence of HSCT, such as in solid organ transplantation (see below), the frequency of sAID related to alemtuzumab was not particularly evident, thus indicating a peculiar synergistic effect of HSCT and alemtuzumab treatment in inducing autoimmune disturbances, especially in genetically "autoimmune-prone" patients.

Although not approved by FDA and EMEA, off-label use of alemtuzumab as inducer agent in *solid organ transplants* represents approximately the 10 % of its overall use. Among these, the *kidney transplant* is the major representative class, since there is little evidence on the beneficial role of alemtuzumab in liver and pancreas/islet transplantation.

Some interest has grown in small-bowel and multi-visceral transplantation, given the morbidity of acute rejection in this field. However, data on AEs are anecdotal and have not evidenced substantial peculiarities.

Beyond rejections in the first two years after transplant, infections are the major challenge. However, it is still unclear whether alemtuzumab effectively increases the risk of infection in solid organ transplant recipients or not. Hematologic toxicity and infusion reactions in transplant recipients appear with low frequency and limited severity compared to hematologic patients. One reason is that the former only receive single-dose therapy, while heavy multi-dose regimens are used in hematologic malignancies [24].

In 1986, alemtuzumab was the first biomedicine employed in renal transplant as immunosuppressor. The first report appeared in 1990, and the first long-term one was published in 2005, after a five year follow-up in 33 renal transplants.

According to the second report, infections (33 vs. 18 %), and in particular HZV infections (15 %) were more frequent after treatment, but differences were not statistically significant.

Skin cancer (9 %) and two autoimmune disorders (hemolytic anemia, hypothyroidism) were also observed in this group of patients. Moreover, one subject developed a fatal plasmacytoid lymphoma (a type of PTLD) three years after transplantation. Therefore, the emerged safety data were considered to be consistent with the alemtuzumab standard profile observed in AID treatment, yet with a reduced frequency of serious infections and the unexpected insurgence of PTLD [25].

In a subsequent study, safety and induction efficacy of alemtuzumab were compared with basiliximab and with ATG. Serious infections resulted to be higher (35 %) in the former than in the comparator mAb (22 %). In patients at high risk of rejection, ATG showed a rate of infections higher (81 %) than alemtuzumab (60 %). However, the overall infections rates—as well as those of CMV, BKV, and EBV—were similar compared to conventional therapy. Interestingly, the degree of lymphocyte depletion was not correlated with the rate of encountered AEs, nor with type and site of infection [26].

A large retrospective study on infections after solid organ transplants (82 % kidney, 12 % kidney pancreas, 3 % liver, 2 % pancreas, 1 % liver-kidney) in 726 patients treated with alemtuzumab reported an overall rate of 33 %, equal to that reported with ATG treated subjects, while basiliximab-treated patients developed infections in 40 % of cases. Ten percent of the overall infections were fungal, being this rate reported also for the basiliximab-treated group. However, disseminated fungal infections were 68 % in alemtuzumab and 30 % in basiliximab. Therefore, while basiliximab induced a slightly higher number of infections than

the other drugs, alemtuzumab caused more systemic fungal infections (mostly candida) and a higher rate of CMV viremia [27].

In another large and recent meta-analysis, infections and PTLD appeared comparable to other immunosuppressive treatments. However, the time of observation for PTLDs insurgence was considered too short, since they usually appear after 10 years from transplant [28].

Similarly, a retrospective analysis on 357 *pancreas transplant* (alone or combined with kidney graft) recipients treated with alemtuzumab associated with daclizumab and MMF, or with ATG and muromonab, to eliminate CD52 negative T cells, detected severe infections (70 %) and cytologic abnormalities in the bone marrow, together with hematologic disorders (AIHA, ITCP, RCA) in 20 recipients (6 %) within two years from therapy initiation. Nine cases of AIHA and 11 cases of RCA were diagnosed. In the latter group, seven RCA patients had also an associated hemolytic component. Most patients had autoantibodies and complement bound to erythrocytes. Severe infections analyses were restricted to the 20 patients having hematological complications, and indicated as main causes CMV (50 %) and BKV (20 %). No Parvovirus B19 was detected [29].

Interestingly, potential synergistic effects between alemtuzumab, daclizumab, and MMF were implied in the impairment of T cell subsets balance leading to autoimmune hematological disorders and virus reactivation.

As for *liver transplants*, it has been shown that alemtuzumab-treated patients had significantly elevated levels of HCV replication, causing an increase in related mortality as a consequence of lymphocyte depletion [30].

Although the overall risk of infection does not seem to be increased with the use of alemtuzumab, as compared to other immunosuppressive treatments, the occurring infections appear to be more severe and more likely to be disseminated. When used at higher regimens, such as for the treatment of rejection, the risk of opportunistic and unusual infections was three fold higher than the background of solid transplant recipients.

However, compared to hematologic patients treated with the same mAb, infections were low and usually mild, possibly due to a lower intensity of treatment. Noteworthy, fungal infections were associated with an excess mortality that in alemtuzumab patients was high compared to ATG, and low compared to basiliximab.

Alemtuzumab, as other biomedicines, has been experienced in various forms of *uveitis, including Behçet's syndrome*, with alternate fortune. The main concerns derive from the induction of sAID, such as Grave's disease and ITCP.

In a study on 18 patients, moderate infusion reactions (28 %) and hypothyroidism (33 %) were observed.

More recently, a retrospective study on 20 patients treated with alemtuzumab since 1998, reported that 25 % of them developed infusion reactions, with only one drug-related discontinuation, while six patients developed new thyroid dysfunctions. Interestingly, no drug-related infections were registered. Altogether, the incidence of the thyroid disorder ranged around 30 % in the two studies [31–33].

Similarly, in *peripheral neuropathy* and *chronic inflammatory demyelinating polyneuropathy* (PNP, CIDP) alemtuzumab mainly promoted sAID. Among seven CIDP treated patients, one developed a severe rash and three developed sAID. In particular, two of them developed anti-thyroperoxidase (TPO) antibodies, associated in one case with anti-TSH R antibodies, followed by Grave's disease after three years. One patient developed a fatal AIHA 18 months after treatment, although the cause of death remained obscure. Neither ITCP cases nor typical infusion reactions were detected in these patients. However, the typical unbalance in CD4/CD8 lymphocyte reconstitution was observed [34].

Since 1990s, alemtuzumab has been used in refractory *rheumatoid arthritis* (RA) with some evidence of temporary efficacy. Four early trials and a number of studies have not definitively assessed a positive risk/benefit, but off-label occasional applications are still on course. AEs were similar to those experienced in other autoimmune diseases, with opportunistic leading infections. These studies constantly signaled a profound and long lasting lymphocyte depletion with unbalanced reconstitution of T and B cell compartments and of CD4/CD8 T cell subsets, as observed in the previously mentioned disorders. Therefore, the major concern relates to the long-term potential consequences of such dysregulation in the immune system. Interestingly, sAID observed after the administration of alemtuzumab are predominantly antibody-mediated, and they respond to B cell depletion therapies. Similar features have been also observed after HSTC. Possibly, the RA model may fit better then other pathologies to evaluate long lasting consequences of a drug-induced unbalanced immune system.

A recent report examined 20 RA patients, treated with alemtuzumab between 1991 and 1994, at 12 years after treatment [35]. These patients still had a significantly low total lymphocyte count mainly dependent from the CD+ T cell and NK cell subsets. Within the former set, naive and central memory subsets were reduced, while effector memory CD4+ cells seemed unaffected. A similar subsets pattern was observed for CD8+ cells. Total B cell levels were comparable to controls, except for the CD5+ subset, which was significantly reduced. Immunoglobulin levels and response to vaccines were within the range of controls. The role of CD5+ B cells is still under investigation and may be related to autoimmune disorders. In the case of RA, a low rate of autoimmune disorders seems to correlate with the reduction of this subset. However, a good response to vaccines and a low rate of infections in these patients suggest a reasonable reconstitution of immune reactivity even in the presence of persistent imbalance of some T and B cell subsets.

Much interest is devoted to the possible therapeutic effects of alemtuzumab on *multiple sclerosis* MS. Quite recently, two trials (CAM-MS I; CAM-MS II) completed their observation on relapsing-remitting multiple sclerosis (RRMS) with encouraging results, and applications to FDA and EMA have been submitted for the alemtuzumab/Lemtrada® approval. In these trials alemtuzumab was administered as one annual dose for two consecutive years. Most common reported AEs were infusion reactions (90 %; 3 % as SAEs) and infections (67–77 %), usually mild to moderate, with no life threatening or fatal events. However, about 20 % of cases developed autoimmune thyroid abnormalities. A consistent increase

in immune thrombocytopenia initially had raised concerns, causing temporary discontinuation of the investigation in 2003, but at the end of study resulted less concerning (1–2 %).

Previous reports repeatedly showed a consistent frequency of sAID in these patients exposed to alemtuzumab. A survey on 248 MS treated patients revealed that 22 % of them developed sAID, being thyroid the most frequent target (16 %). Hematologic, renal, and dermatologic autoimmune disorders, including anti-GBM renal disease, were recorded with a peak appearance at 12–18 months after treatment. No cases were observed over 60 months after treatment. However, in some cases signs of sAID persisted up to 5 years. No relation was found with the total dose or interval of administered mAb, or with sex and age. Asymptomatic autoantibodies were also detected [35]. ITCP raised particular interest for its frequency (6/1000 P/Y vs. 00.2-0.04/1000 P/Y of the general adult population) and delayed appearance (more than 10 months after therapy), although with a self-limited course in about 80 % of cases and a good response to conventional therapy [36]. Interestingly, the imbalance of T lymphocytes during reconstitution was different in MS and RA treated patients. In particular, MS patients showed a biphasic profile, with an anticipated raise of CD4+ memory/regulatory T cells—possibly driven by IL-7 levels—followed by a normalization phase, where naive T cells progressively raised [36–39].

Nonetheless, the peripheral lymphocyte reconstitution remained impaired for long time compared to other ablative treatments (after one year, CD4+ T cells were still 50 % of baseline levels).

7.5 Postmarketing Surveillance

In the FAERS database providing about 4,000 reports acknowledged by the end of 2012, viral infection was the first reported class of AEs (40 %), followed by any infections, WBC abnormalities, immune disorders, and fungal infections. CMV infections were included in 230 reports, followed by 45 ITCP, 34 TLS, and 27 PML.

Similarly, in the EUV database 18 % of the over 430 reports referred to viral infections, and 26 % of them related to CMV infections. Five TLS, three CLS, three ITCP, two cases of JCV infection, and three cases of anaphylaxis were also reported. Seven cases of GBS, and three cases of PNP were registered among the nervous system disorders.

7.6 Remarks

Alemtuzumab is a potent lymphocyte depletory agent, and has been widely used for B-CLL treatment as well as in a series of off-label diseases. The analysis of induced AEs has revealed a number of complex disturbances, mostly related to its

mechanisms of action and affecting various systems and organs. Rapid, profound, and long lasting cytopenia, mainly as lymphopenia, is the pivotal cause of infusion reactions and infections, to be considered the main expression of drug-related adverse events.

The lowering of hematotoxicity and of some constitutional signs in naive patients undergoing alemtuzumab monotherapy indicates that the previous chemotherapy and/or the different clinical condition of pretreated patients are involved in increasing the incidence of AEs, yet not of SAEs.

Off-label experience is particularly wide and prolonged, thus allowing the observation of additional drug-related AEs among which sAID is the most important and new issue. Although particularly violent and aggressive in a minority of cases, most AEs have shown to be manageable and in part preventable through accurate symptomatic pre-medication and anti-microbial prophylaxis. T cell destruction and imbalanced post-treatment reconstitution are respectively considered responsible for early acute cytokine-mediated reactions such as infusion reactions and CRS, and for secondary delayed interventions such as sAID related to improper reconstitution of B and T cell compartments. As for sAID insurgence, two pathogenetic mechanisms have been evoked. The first relates to a genetically determined abnormal production of IL-21, a potent inducer of T cell apoptosis and cell cycling, in some subjects detectable even before treatment [37]. The second observation relates to the kinetics of the lymphocytes compartment's reconstitution, which can be delayed and incomplete up until 12 years after treatment, with anticipated reappearance of some B cells (except for CD5+ subset) followed by CD8+ T cells, and a persistent scarcity of CD4+ lymphocytes. Altogether, they seem to establish a situation particularly favorable to let autoreactive B cell clones appear and expand [33].

As for future consideration, these data suggest that more selective and less persistent depletive effects may be more promising for further reducing the incidence and severity of these drug-related events. In fact, depletion of T cells in B-CLL treated patients does not favor the control of disease and enhances adverse and long lasting conditions.

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