Novel Immunosuppressive Strategies for Bone Marrow Failure Syndromes: A Focus on Alemtuzumab

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Abstract: Acquired bone marrow failure syndromes (BMFS) are a heterogeneous group of hematological disorders characterized by impaired bone marrow function and subsequent cytopenia of one or more blood cell lineages [1,2]. The well-accepted pathogenic mechanism of the typical bone marrow failure – aplastic anemia (AA) – is a T cell mediated immune attack targeting the hematopoietic tissue [3]. This pathogenic mechanism is at least partially shared by other bone marrow failure syndromes, such as lineage-restricted aplasias and some myelodysplastic syndromes. Thus, for these disorders immunosuppression (IS) is the pivotal etiologic treatment. While the standard IS regimen include the heterologous anti-thymocyte globulin [4], here we review the recent data on the anti-CD52 monoclonal antibody alemtuzumab as a novel IS agent for marrow failures. Alemtuzumab led to objective responses in aplastic anemia patients in 3 recent prospective studies, with overall response rates ranging between 37% and 72%. Adverse events were irrelevant, ruling out even the concerns about the risk of infectious complications. Alemtuzumab was effective even for the treatment of lineage-restricted marrow failure, with very acceptable toxicity and excellent response rates (as high as 80%). More recently, even patients suffering from myelodysplastic syndromes showed a remarkable hematological response to alemtuzumab-based IS treatment. Thus, alemtuzumab is a novel IS agent representing an excellent alternative to ATG for all immune-mediated marrow failure syndromes. Even if the dose and the schedule may still require further refining, the available data support the need of large prospective trials comparing alemtuzumab to current standard IS regimens.

Keywords: Bone marrow failure, aplastic anemia, alemtuzumab.

INTRODUCTION

Acquired bone marrow failure syndromes (BMFS) are a heterogeneous group of hematological disorders characterized by impaired bone marrow function and subsequent cytopenia of one or more blood cell lineages. In contrast to secondary BFMS, which usually are due the infiltration of malignant processes (either hematological or extra-hematological) or iatrogenic causes (i.e., radio- or chemotherapy) impairing normal hematopoiesis, primary BMFS share a functional impairment of the hematopoietic stem cell (HSC) compartment, which results from either a qualitative or a quantitative defect of HSCs. The paradigm of BMFS is aplastic anemia (AA), which is characterized by the disappearance of the normal hematopoietic tissue from the bone marrow, with subsequent pancytopenia in the peripheral blood. As we discuss below, AA is considered an immune-mediated disorder, where an aberrant immune response (likely T cell mediated) may damage normal HSCs, leading to a massive impairment of normal hematopoiesis. Thus, in AA HSCs are mostly considered normal; this immune pathophysiology is shared by other BMFS, such as lineage-restricted aplasias and some myelodysplastic syndromes (MDS). However, these latter likely combine a passive damage of HSCs with their possible intrinsic defect, eventually leading to progression to malignant hematopoietic disorders. Here we briefly discuss the biological evidences supporting the immune pathophysiology of AA and some related BMFS, providing the rationale for the utilization of immunosuppression (IS) for their treatment. Then we review the available data on the use of alemtuzumab as a novel IS agent for the treatment of AA and other related BMFS.

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APLASTIC ANEMIA

AA is typically characterized by an empty or a fatty bone marrow and peripheral blood pancytopenia [1,2]. Diagnostic criteria for severe AA (SAA) include a trephine biopsy with decreased cellularity (below 30%) associated with a severe cytopenia involving at least two blood lineages (neutrophils <500/μL, reticulocytes <60,000/μL, platelets <20,000/μL); AA patients with neutrophils <200/μL are classified as very severe AA. Constitutional forms of AA should be ruled out in all patients by chromosome breakage test, family history and other specific tests (if appropriate). All causes of secondary AA should be investigated, including possible infectious (mostly viral) agents and idiosyncratic reactions to specific drugs. In addition, karyotype analysis must be always performed for the definitive differential diagnosis with myelodysplastic syndromes; the presence of a concomitant paroxysmal nocturnal hemoglobinuria (PNH) population should be assessed by flow cytometry (possibly leading to the diagnosis of AA/PNH syndrome). Acquired idiopathic AA usually does not carry abnormalities of any

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other organ or apparatus, with the exception of the rare hepatitis-AA syndromes; however, the association with other autoimmune disorder is not uncommon (e.g., eosinophilis fasciitis).

Immune-Mediated Pathophysiology of AA

Three decades of clinical and experimental evidences support the concept that acquired AA harbors an immune-mediated pathophysiology [2]. Initial reports describing the hematopoietic stem cell compartment in AA patients came to the conclusion that the impairment is essentially quantitative. In fact, functional CD34+ cells are reduced in all AA patients (both committed and immature CD34+/c-kit- or CD34+CD38- progenitors) [5,6]; furthermore, the hematopoietic progenitor pool, as assessed by secondary colonies and by long-term culture-initiating cell (LTC-IC, the best *in vitro* surrogate for HSCs) assay, is markedly reduced in AA patients [7]. More recently, gene expression profiling of the few residual CD34+ cells suggest a signature typical of stressed, dying and immunologically activated target cells rather than of an intrinsically abnormal population. In fact, up-regulated genes included those involved in cytokine/chemokine signal transduction, stress defense/immune responses (such as the death receptors *Fas*, *DR3*, *DR5*, *TNFRII*, and *TRAIL*), while genes promoting cell cycle progression and proliferation were down-regulated [8]. Looking to the immune mechanism leading to the development of the disease, the pivotal role of T cells was initially demonstrated the late '70s, when marrow and peripheral blood lymphocytes from AA patients were shown able to suppress hematopoiesis *in vitro* [9]. In the following years, cytotoxic/suppressor CD8+ T cells were identified as the lymphocyte subset impairing hematopoiesis in AA patients [10]. The terminal immune mechanism of damage includes both cell-cell interaction (possibly through the Fas/Fas-R apoptotic pathway) [11], as well as the release of several inhibitory cytokines, such as IFN-γ, TNF-α and TGF-β [12-17]. A number of studies have dissected the role of T cells in AA, aiming to demonstrate an antigen (Ag)-driven immune response, and possibly to identify the target antigen(s) [18-20]. Different groups have employed T cell receptor (TCR) analysis to identify clonal T cells in AA patients, leading to the identification of oligoclonal T cells in AA patients – a surrogate marker of antigen-driven T cell responses. Some studies also directly demonstrated the pathogenic role either *in vitro* or *in vivo* [21,22]. being able to correlate the presence of clonal T cells with disease activity [22]. However, the search for the target antigen(s) was so far unfruitful. In fact, even if some groups have identified auto-antibodies (Ab) against distinct proteins (kinectin, diazepam-binding inhibitor-related protein 1 and moesin) [23-25] possibly detectable in AA patients, they seem to be neutral auto-antibodies reflecting the underlying broad immune derangement, without any specific pathogenic role. Indeed, T cells are considered the actual target for an effective IS therapy in AA patients.

THE STANDARD IMMUNOSUPPRESSION FOR APLASTIC ANEMIA: A BRIEF SUMMARY

The history of immunosuppressive therapy (IST) for AA patients starts with the initial observations showing that a few AA patients failing sustained engraftment after SCT may subsequently recover from the conditioning regimen with autologous hematopoiesis [26]. This raised the concept that IST itself, rather than engrafted allogeneic stem cells, may have contrasted the underlying bone marrow failure. Since then, IST was investigated as a treatment for AA patients, using the available anti-lymphocyte or anti-thymocyte globulins (ALG or ATG). After the pioneer experience by Dr. Speck [27] and the placebo-controlled prospective randomized trial [28], IST by ATG or ATG was utilized by many groups for the treatment of AA patients, with response rates ranging between 30% and 70% [29,30]. In the following years, in the aim of increasing the response rate and preventing possible relapses, a number of immunosuppressive agents were associated to ATG or ALG. Only the calcineurin inhibitor cyclosporine A (CyA), which impairs IL-2-dependent T cell activation and differentiation, has been proven effective in increasing the response rate, as initially demonstrated by the German Aplastic Anemia Group in a randomized trial [31]. In fact, the addition of CyA to ATG and high dose steroids (utilized as prophylaxis of serum-sickness) resulted in an increase in 6 month overall response rate from 46% to 70%; this benefit was confirmed by an improved failure-free survival in the long-term follow up of this study [32]. Since the early '90s, ATG + CyA was considered the standard IST for AA patients, with an expected 50-60% probability of response and 60% overall survival at one year [33-36]. These data seem to be confirmed regardless the specific preparation of ATG/ALG, even if most studies utilized horse ATG (either ATGAM® or Lymphoglobulin®) and data with rabbit ATG are less robust (Thymoglobulin®). However, this dogma seems to be exploded by the recent observation from NIH that Thymoglobulin is markedly inferior to ATGAM; in fact, in a randomized prospective trial, Thymoglobulin resulted in a response rate of 35% at 6 months in comparison to 69% achieved with ATGAM [37]. In summary, ATG (preferably from horse) associated to CyA is the currently the standard first-line treatment for SAA patients lacking a hematopoietic stem cell donor. The addition of myeloid growth factors (i.e., granulocyte-colony stimulating factor, G-CSF) has been considered a possible way to improve the outcome of SAA patients [38]; nevertheless, it did not result in any benefit in terms of response and survival in a very recent study [39]. However, the use of short-term G-CSF may still be considered as supportive care of ongoing infectious events, as well as to identify patients who have a low probability to achieve a hematological response to IS (who should evaluated for an early transplant procedure) [38].

Treatment-Failure After First-Line Immunosuppression

Even if 60-70% of SAA patients show a hematological response after a single course of ATG + CyA, treatment failure remains a major problem for SAA patients [40]. In fact, about one third of patients does not respond to ATG, requiring further treatment strategies. In addition, about half of the patients initially responding to IS may subsequently relapse of their disease or floating with a mild to moderate, chronic disease requiring maintenance IS by CyA. For all these patients, a further intensive IS course is a worthy treatment option [41-43]. In this context, rabbit ATG is often

preferred to horse ATG, to decrease the risk of serum-sickness and other side effects secondary to previous sensitization to horse proteins. A second course of ATG + CyA may result in remarkable response rates, especially in patients relapsing after a good hematological response [41-43]; nevertheless, patients refractory after the first ATG have low chance to respond to further courses [41-43]. However, ATG-based IS remains a burdensome treatment and in case of relapsing diseases alternative strategies (especially transplantation) should be considered. Thus, the current challenges in IS for SAA aim the goal of improving the feasibility of the current IS regimens, retaining (and possibly improving) their effectiveness.

IMMUNOSUPPRESSION BY ALEMTUZUMAB: THE RATIONALE

Alemtuzumab (Campath 1H) is a new anti-lymphocyte agent which specifically targets the CD52 antigen;[44] CD52 is widely present on all human lymphocytes, expressed on the surface membrane *via* a glycosyl-phosphatidyl-inositol (GPI) anchor. Alemtuzumab is a humanized anti-CD52 monoclonal antibody which kills all CD52 bearing cells, and in particular lymphocytes; the mechanism of action includes both antibody-dependent cellular cytotoxicity and complement-mediated lysis [44]. Alemtuzumab was initially investigated in lymphoid malignancies, where it showed an excellent lympholytic effect; in fact, in 2001 it was approved as second-line treatment for chronic lymphocytic leukemia. Initially administered by intravenous infusion, alemtuzumab was subsequently delivered as subcutaneous injection. A pharmacokinetic study comparing the two administration routes in refractory chronic lymphocytic leukemia patientsshowed that concentration peaks achieved after subcutaneous and intravenous administration were comparable, even if in the former it may take longer to reach the steady state [45]. The drug was administered by escalating doses, to minimize the risk of first-dose reactions, which may consist of fever, rigor and chills. The standard dose regimen of 30 mg i.v. thrice weekly for 4-12 weeks utilized for lymphoid malignancies was developed quite empirically, without any detailed pharmacodynamic or pharmacokinetic study. In fact, recent data suggest that even lower doses may be effective, possibly with reduced risk of infectious complications. To note, the lympholytic effect of alemtuzumab is not restricted to malignant lymphocytes; in fact, a number of experiences in the context of stem cell transplantation confirmed that alemtuzumab kills normal B and T lymphocytes, resulting in a remarkable immune-ablation [46,47]. Several conditioning regimens for reduced-intensity stem cell transplantation include alemtuzumab as *in vivo* depleting agent; the most utilized schedule consists of a total dose of 100 mg, administered over 5 days as 20 mg intravenous infusions [46,47]. It has to be remarked that pharmacokinetic and biodistribution of alemtuzumab are dominated by available binding sites, which differ between lymphoid malignancies with massive lymphocytosis and other conditions [48,49]. It has been estimated that there are approximately 10^{12} lymphocytes in a healthy adult, each one harboring about 5×10^5 CD52 molecules; thus, about 100 mg of alemtuzumab are needed to saturate all binding sites [50]. As anticipated by theoretical calculations, a dose of 100 mg of alemtuzumab demonstrated extreme immune-ablation *in vivo*, leading to a complete pre-transplant lymphocyte depletion; lymphoid recovery usually takes several months, particularly for the CD4+ subset. In this setting, alemtuzumab showed anti-lymphocyte activity at least comparable to ATG, with acceptable toxicity. Thus, alemtuzumab seems an ideal IS agents for the treatment of marrow failures, due to its powerful lympholytic action sparing hematopoietic stem cells (CD34+ cells do not express CD52 on their surface). This assumption was confirmed by preliminary *in vitro* studies, showing that incubation of normal human lymphocytes with alemtuzumab results in apoptosis, while CD34+ cells remain vital (Selleri *et al*, unpublished data).

ALEMTUZUMAB FOR APLASTIC ANEMIA

To date, a single study in the early '90s tested the efficacy of alemtuzumab in a heterogeneous population of autoimmune cytopenia, mostly B cell mediated [51,52]. Nevertheless, alemtuzumab has not been systematically investigated as treatment for bone marrow failure. The concerns to include alemtuzumab in the immunosuppressive regimen for marrow failure are mostly due to the risk of infectious complications, including possible marrow toxicity secondary to CMV reactivation (which is the most frequent complication patients with lymphoproliferative disorders) [53]. However, in the last few months 3 independent studies tested alemtuzumab as alternative IST for aplastic anemia patients. In a dose-escalating study from Korea alemtuzumab was tested at 2 different doses (60 and 90 mg) delivered in 3 consecutive days in a cohort of 17 AA patients, in combination with maintenance CyA [54]. The overall response rate was 35%, with 23% complete and 12% partial remissions; surprisingly, all responding patients received the lower doses (response rate 50%), while no response was observed in the 90 mg dose cohort. This pilot study did not raise any safety concern, showing a 2 year survival of 81%.

Starting in 2004, we have conducted a phase I-II study investigating alemtuzumab + low dose CyA for the treatment of aplastic anemia and related immune-mediated bone marrow failures (NCT00895739) [55]. In our study, alemtuzumab was administered as subcutaneous (s.c.) injections at the dose of 3-10-30-30-30 in 5 consecutive days (total dose 103 mg, or 73 mg in patients with moderate forms); low-dose CyA (1 mg/kg) was started from day 7. In absence of formal pharmacokinetic and dose finding studies, we choose empirically both the dose and the administration schedule, based on the previous experience in the field of transplantation and auto-immune (antibody-mediated) cytopenias. The s.c. administration route was preferred given its proved equivalence to the i.v. one and easier administration; in fact, no patient required hospitalization due to the treatment, which was usually performed on an out patient basis. Premedication included intravenous steroids, anti-histamines and oral paracetamol. We enrolled 11 AA patients, of whom 6 had no previous IST; all the patients completed the treatment without any relevant adverse event. The most frequent adverse events were mild infusion reactions (local rubor, chills and fever), usually self-limiting or easily managed by paracetamol. All patients showed complete lymphocyte depletion within 2-3 days, with

lymphocyte count below 0.1 x 10^3/μL. Eight of the 11 patients (72%) showed a hematological response (cumulative probability of response was 84% at 6 months), with 5 complete (45%) and 3 partial (27%) remissions; hematological improvement was seen at 3 months, even if most cases achieved their best response after 6 months. Patients receiving alemtuzumab as first-line treatment seem to have a higher chance to respond: out of 6 patients, 3 had a complete remission, 1 a very good partial remission, and the remaining 2 did not reach adequate follow up (1 died at 1 month because of infection and 1 received a transplantation at 3 months because of persistent life-threatening hemorrhages). Indeed, the initial efficacy of alemtuzumab as an IST regimen was impressive; however, irrespective of the persistent severe lymphocytopenia, relapses were frequent, despite chronic CyA maintenance treatment (even if at a very low dose). In fact, 6 out of 8 patients experienced a relapse; however, re-treatment by further alemtuzumab (even administered as single 30 mg dose in case of early relapses) always resulted in an additional hematological response (which was not sustained in 2 cases). The 3 year overall survival was 80%. These excellent data in our prospective trial were only partially confirmed in a retrospective survey conducted among the centers of the European Bone Marrow Transplantation Severe Aplastic Anemia Working Party (EBMT-SAAWP); among the 8 patients included, only 3 showed a partial hematological response. However, it has to be remarked that all these patients were heavily pretreated, having failed multiple lines of IST, consistent with the assumption that IS-refractory cases are unlikely to be cured by further intensification of IS [55].

Our study also showed that treatment-related toxicity was acceptable; in fact, most safety concerns about the infectious risk associated to alemtuzumab were ruled out. In our study, all patients received prophylaxis with valganciclovir, antifungal and antibiotics and cotrimoxazole [55,56]. When all the 25 patients enrolled in the study were considered (11 SAA, 12 PRCA and 2 PWCA, see below), after a cumulative observation of 433 patient-months infectious events were infrequent: 6 cases of pyrexia of unknown origin (one associated with fatal cardiac complication), and 8 viral infections (1 Varicella-Zoster with shingles, 2 Herpes Simplex and 5 flu) were reported, all resolving quickly.[55] No CMV disease, EBV-related disease or lymphoproliferative disorder was observed. CMV viremia (as assessed by a sensitive PCR method) remained negative in all patients on valganciclovir; even if 4 patients developed asymptomatic CMV reactivation after anti-CMV prophylaxis discontinuation; however, the viremia (just above the detection limit) promptly disappeared after pre-emptive valganciclovir [56]. One patient with pre-treatment occult HBV infection developed HBV reactivation (defined by HBs-Ag+ and HBV viremia) without laboratory signs of hepatitis; lamivudine therapy was started and was effective in prompt viral clearing. Other 3 patients with possible occult HBV infection received prophylactic lamivudine, without subsequent HBV viral reactivation. Non-infectious adverse events were rare, and included mild increase of liver enzymes in 4 patients and of serum uric acid in 2, which were transient in all cases. In addition, in patients with partially preserved blood cell counts at baseline (these data are mostly drawn from patient with single-lineage cytopenia), transient worsening of blood counts was observed [57], usually between 2 and 6 weeks after treatment (possibly managed by on demand G-CSF). In contrast with some precedent observation [58,59], alemtuzumab treatment did not result in the emergence of PNH clones, even if (likely pre-existing) CD52-negative lymphocyte may become evident in the early post-treatment period (and then diluted at the time of lymphocyte rise). The immune reconstitution was very slow in all patients, especially for the CD4+ compartment; median lymphocyte count was about 0.5 and 1 x 10^3/μL at 6 and 12 months, respectively. The lymphocyte subsets faster to repopulate were NK and B cell, followed by CD8+ T cells (which were about 0.2 and 0.5 x 10^3/μL at 6 and 12 months); in contrast, CD4+ lymphocytopenia lasted several months after treatment (about 0.1 and 0.2 x 10^3/μL at 6 and 12 months).

The attractive results of our study were confirmed in an additional study from Mexico [60], which included 14 untreated AA patients receiving subcutaneous alemtuzumab at the dose of 50 mg (10 mg per day in 5 consecutive days) associated to CyA 2 mg/kg twice a day. With a median follow up of 20 months, the response rate was 57%, with 14% complete and 43% partial responses; median time to response was 2.5 months, while median time to transfusion-independence was 4.5 months. In contrast to our study, no relapse was observed, possibly due to the higher CyA dose as maintenance IS; in addition, no late clonal evolution was observed. This study also confirmed the excellent safety profile, showing no CMV reactivation even using a less specific anti-viral prophylaxis by acyclovir. The overall survival was 71% at 38 months, with deaths due to hemorrhages or infectious complication in non responding patients. Indeed, single-course alemtuzumab may induce objective and sustained responses in AA patients, even at low dose (in comparison to that used for lymphoid malignancies), apparently without carrying any additional co-morbidity, including the feared increased risk of infectious complications.

ALEMTUZUMAB FOR OTHER IMMUNE-MEDIATED BONE MARROW FAILURE SYNDROMES

Lineage-Restricted Marrow Failures and Large Granular Lymphocyte Disorders

Beside the classic AA, where all blood lineages are affected, there are cases of marrow failures restricted to a single hematopoietic lineage: pure red cell aplasia (PRCA), pure white cell aplasia (PWCA) and acquired amegakaryocytic thrombocytopenia (AAT). These rare disorders are associated with other diseases (e.g., thymoma or auto-immune diseases), which may share (or even being the cause of) an underlying immune-mediated pathogenic mechanism. According to the established theory, here the targets in the hematopietic hierarchy are lineage-committed hematopoietic progenitors rather than multipotent HSCs (as for AA) [61]. Thus, even for single-lineage marrow failures, IST is a reasonable treatment option. However, due to their rarity, large clinical trials are lacking, and only small case series or anecdotic cases are reported. Mild IST is a common strategy for PRCA patients [61]. and in most cases employed

steroids and/or CyA, more rarely mycofenolate, azathioprine, cyclophosphamide or even ATG; recent studies report experiences with other antibodies, including daclizumab, rituximab or alemtuzumab [61]. Alemtuzumab was demonstrated effective in some anecdotic PRCA cases and more recently in small series [62-64]; in some of these patients PRCA was secondary to an underlying lymphoproliferative disorder [63,64]. Anecdotic cases of PWCA responding to alemtuzumab have been reported too.[65,66] However the dose and the treatment schedule were quite heterogeneous. In our prospective trial we have systematically investigated alemtuzumab as IST for patients suffering lineage-restricted marrow failures, namely PRCA and PWCA [55]. Twelve PRCA (9 untreated) and 2 PWCA (1 untreated) patients were enrolled; PRCA was often associated to other diseases, such as thymoma or connective tissue disorders, while one PWCA patient harbored a large granular lymphocyte population. PRCA and PWCA patients received a total dose of 73 mg administered subcutaneously in 4 consecutive days (3-10-30-30 mg). The response rate was excellent, with an overall response rate of 87% for PRCA (8 complete and 2 partial remissions) and of 100% for PWCA (2 out of 2 complete responders). One of the two non-responding PRCA revealed to have a non immune-mediated disease (she quickly developed a myeloproliferative disorder). In comparison to AA, responses were faster, likely due to the downstream target in the hematopoetic hierarchy; however, even relapses were earlier, affecting 5 of the 8 PRCA and 1 of the 2 PWCA. As for AA, re-treatment by alemtuzumab was easy to administer (in most cases by single 30 mg injections) and effective in all cases. Some patients developed subsequent relapses (despite low-dose CyA), with a recurrent disease course requiring periodical injections of alemtuzumab, working as a maintenance treatment. Even if the safety profile was excellent as for AA patients (see above for details), the 3 year overall survival was only 60%, with further worsening with longer follow up. This low survival rate, quite unexpected for a non malignant disease, was mostly due to the concomitant diseases, which largely drive the long-term outcome. In addition, two patients developed a late clonal evolution (one after an alemtuzumab-dependent response lasting about 5 years, the other 2 years after a partial response), possibly suggesting that some PRCA may hide underlying hematological clonal disorders (or carry an intrinsic risk of evolution to malignancy).

Beside clearly documented lineage-restricted marrow failures, cytopenia of one or more blood lineages are often associated to large granular lymphocyte leukemia, possibly as a direct cytotoxic effect of the aberrant lymphocyte population. Alemtuzumab has been reported effective for the treatment of LGL leukemia [67-71]. In a recent retrospective study [72], 8 patients with severe refractory LGL-associated cytopenia received alemtuzumab as salvage therapy, showing a global response rate of 50%. Alemtuzumab resulted effective regardless the affected blood lineage, with transfusion independency in 3 of 5 anemic patients and restoration of neutropenia or thrombocytopenia in 1 of 3 and 1 of 1 patients, respectively. Interestingly, this study also suggested that, in contrast with data from AA patients, lymphocyte population with decreased CD52 expression (pre-existing alemtuzumab therapy) may be selected after the treatment, possibly resulting in subsequent alemtuzumab-refractory disease [72].

Myelodysplastic Syndromes

A role for immune-mediated pathogenic mechanisms has been hypothesized in some forms of MDS, especially in low-risk MDS with hypocellular bone marrow [73]. Several groups have reported in MDS the presence of T cell oligoclonality [74], hyperproduction on the inhibitory cytokines IFN-γ, TGF-β and TNF-α, as well as the activation of the Fas/Fas-ligand pathway [75]. IS is not a common treatment strategy for MDS patients, being MDS usually characterized by an intrinsic defect of (possibly clonal) HSCs. Thus, immunosupression has been exploited, mostly with the aim of improving cytopenia; in fact, stable remission or even cure are not expected, due to the qualitative impairment of the HSC pool. Results were quite heterogeneous among different groups, likely due to patient selection; however, hematological response in low-risk patients may be even above 50% [76-78]. More intensive IS regimens based on ATG + CyA have been extensively investigated at NIH, showing in a series of 129 patients a response rate of about 30%, 40% in RA patients [79]. These data were in agreement with some European experiences [80], but not confirmed in other studies, again due to patient selection bias (patients with excess of blast are very unlikely to respond). In fact, an additional insight from the NIH studies is that a simple scoring system (based on age, months of transfusion dependency and presence of the HLA-DR15) may be useful to identify MDS patients with high probability of response to IST [81]. Based on these data, alemtuzumab was investigated as an alternative intensive IS regimen in a MDS patient cohort with a high chance to respond to IST [82]. In this recent NIH open-label phase I/II study alemtuzumab was administered i.v. at the dose of 10 mg in 10 consecutive days, as single agent. Preliminary data showed a response rate of 77% in 22 intermediate-1 and 57% in 7 intermediate-2 evaluable patients, with a median time to response of 3 months. Responses were sustained at 1 year, with 77% transfusion-independency and 56% complete remission rates in the 9 evaluable responder patients; of note, four of seven patients with cytogenetic abnormalities also achieved a cytogenetic remission. Indeed, as in aplastic anemia patients, alemtuzumab was safe, with an excellent toxicity profile, making this treatment option an attractive alternative to ATG in selected MDS patients likely to benefit from IST.

CONCLUSIONS

Acquired bone marrow failure syndromes share in most cases an immune pathophysiology, which requires an appropriate immunosuppressive treatment. While ATG and CyA are the standard IST, resulting in significant response rate, alemtuzumab is a novel immunosuppressive agent that has been recently investigated for this indication. Preliminary studies suggest that alemtuzumab may deliver an adequate immunosuppression, resulting in remarkable response rates without carrying any additional morbidity. Immunosuppression by alemtuzumab is easy to be administered, possibly allowing re-treatment in case of

relapse; lymphocyte depletion usually lasts several months, but apparently the risk of infectious complication is limited. Even if these preliminary data need to be confirmed in larger prospective clinical trials, alemtuzumab has to be considered a novel immunosuppressive agent which may have a role in the treatment of aplastic anemia and other immune-mediated marrow failures, including lineage-restricted failures, LGL-associated cytopenias and even some MDS. While subcutaneous administration will likely be the preferred route of administration, the best dose and treatment schedule may require further refinement, as well as possible maintenance treatment with other IS agents. Of course, its cost-to-benefit in comparison to standard immunosuppressive regimens will require further investigations, possibly within large prospective randomized clinical trials.

CONFLICT-OF-INTEREST DISCLOSURE

A.M.R. has received lecture fees from Genzyme.

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