bjh short report

Alemtuzumab is safe and effective as immunosuppressive treatment for aplastic anaemia and single-lineage marrow failure: a pilot study and a survey from the EBMT WPSAA

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*Bruno Rotoli deceased on May 19, 2009. This paper is dedicated in his memory, to honour his dedication as an outstanding physician, scientist and mentor.

Summary

An alemtuzumab-based experimental immunosuppressive treatment (IST) regimen was investigated in 35 patients with severe aplastic anaemia (SAA), pure red cell (PRCA) or pure white cell aplasia (PWCA). Alemtuzumab total dose was 73–103 mg s.c., followed by cyclosporine. No serious toxicity due to the regimen was observed. Adverse events were clinically irrelevant; infectious events were rare. The total response rate was 58%, 84% and 100% in SAA, PRCA and PWCA, respectively, with corresponding 6 months cumulative response probabilities of 84%, 84% and 100%. Subcutaneous alemtuzumab is a feasible and sufficiently safe IST regimen for patients suffering from immune-mediated marrow failures.

Keywords: alemtuzumab, aplastic anaemia, pure red cell aplasia, pure white cell aplasia, bone marrow failure.

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Immunosuppressive therapy (IST) is a standard treatment for acquired aplastic anaemia (AA) (Risitano *et al*, 2004) and other immune-mediated bone marrow failure syndromes (BMFS) characterised by defective production of a single lineage (i.e., pure red cell aplasia [PRCA], white cell aplasia [PWCA]). The current IST regimen for AA is antithymocyte globulin (ATG) with cyclosporine A (CyA) (Frickhofen *et al*, 2003; Rosenfeld *et al*, 2003; Maciejewski & Risitano, 2005), while in PRCA and PWCA, IST is usually less intensive. The major limitations associated with current IST regimens are the side effects (especially with ATG), as well as primary treatment failures or relapse requiring re-treatment or chronic IST (Frickhofen *et al*, 2003; Maciejewski & Risitano, 2005). A regimen retaining a marked immunosuppressive effect but with fewer side effects and ease of administration would be ideal. Alemtuzumab is a humanised monoclonal antibody that specifically kills CD52-bearing cells via both antibody-dependent cellular cytotoxicity and complement-mediated lysis. Hence, alemtuzumab is a perfect candidate for testing as IST in autoimmune diseases.

Here we report a preliminary experience with alemtuzumab for the treatment of AA, PRCA and PWCA. The study included a phase II prospective trial and cases collected retrospectively as reported to the Working Party for Severe Aplastic Anaemia (WPSAA) of the European Bone Marrow Transplantation (EBMT) group.

Materials and methods

Patients

This observational study included a total of 35 patients reported from EBMT centres (Table I). Twenty-five patients (11 severe aplastic anaemia [SAA], 12 PRCA and 2 PWCA) were enrolled in a prospective clinical trial conducted in Naples, approved by the local Institutional Review Board (IRB); among these, 15 (6 SAA, 8 PRCA and 1 PWCA) were untreated. Ten patients (8 SAA, 1 PRCA and 1 PWCA) were treated in other EBMT SAA Working Party centres according to the protocol, as recommended salvage IST; retrospective data were collected through an EBMT survey, complying with local IRB guidelines (Table I).

Prospective clinical trial

The pilot clinical trial (NCT00895739) was a phase II study for SAA patients failing first-line IST, subsequently amended to include single-lineage bone marrow failure patients, and first-line treatment was also permitted. Alemtuzumab was administered subcutaneously in a single course over 4 or 5 consecutive days at escalating dose of 3-10-30-30-(30) mg. Premedication included intravenous steroids, anti-histamines and oral paracetamol. The total dose was 103 mg for SAA patients, and 73 mg for AA/paroxysmal nocturnal haemoglobinuria (PNH) and PRCA/PWCA (and in two elderly SAA patients, Table I). Re-treatment by alemtuzumab (as complete courses or single dose) was allowed for relapse in responding patients. Low dose (1 mg/kg) oral cyclosporine A commenced from day 7, and then adjusted according to blood level (targeting 100–200 ng/ml using a monoclonal assay) at the time of immune reconstitution. An intensive anti-infectious prophylaxis was used (Table I, Risitano *et al*, 2009), which included anti-cytomegalovirus (CMV) prophylaxis by oral vanganciclovir and weekly monitoring of CMV viraemia, anti-Pneumocystis Jirovecii prophylaxis by oral trimethoprim-sulphametoxazole, anti-bacterial and anti-fungal prophylaxis in case of severe neutropenia ($<0{\cdot}5 \times 10^9$/l), and prophylactic lamivudine in patients with possible occult hepatitis B virus (HBV).

Response definition

Responses in SAA patients were classified according to established criteria (Camitta, 2000), as described in Table I. Cumulative incidences of survival and response were run using a Kaplan–Meier method.

Results and discussion

All 25 patients enrolled in the prospective trial completed the scheduled treatment without serious adverse events (AE). Including additional doses given for relapse, a total of 63 courses of alemtuzumab were administered. Treatment was administered on an outpatient basis, with the exception of patients requiring hospitalisation for clinical reasons (e.g. symptomatic thrombocytopenia). The most frequent AE was injection-related fever and/or cutaneous rash, which occurred in seven patients (28%, Table I), but they were usually mild. Even for patients already pancytopenic before treatment, transient worsening of blood counts [especially neutropenia, as already reported (Gibbs *et al*, 2005)] was observed in 10 cases (40%), 2–6 weeks after treatment. This was treated with G-CSF. Other AEs were increase of liver enzymes in four patients and serum uric acid in two, which were transient in all cases. No emergence of PNH clones was recorded. With a median follow up of 17 months, there were six deaths, all but one (a cardiac complication occurring in concomitance with an infectious event) considered independent of the treatment. Three deaths were due to pretreatment non-haematological comorbidities, one to additional therapy employed (stem cell transplantation), one to the underlying haematological disease (which were reclassified as myelodysplastic syndrome with erythroid hypoplasia); all causes of death are described in detail in Table I.

The major concerns regarding the safety of alemtuzumab include its associated infectious risk (Elter *et al*, 2009). With a median follow up of 17 months (cumulative 433 patient-months, including repetitive treatment in relapsing patients), infectious events were infrequent (Table I): six cases of pyrexia of unknown origin (PUO), one associated with fatal cardiac

Table I. Patients characteristics before and after treatment.

UPN	Age (years), gender	Bone marrow	Concomitant disease	Previous therapy	Total alemtuzumab dose (mg)	Injection-related adverse events	Haematological toxicity	Follow up (months)	Status	Infections	Other adverse events	Clinical response							
												3 months	6 months	12 months	Best	Time	Relapse	Re-treat	Courses (*n*)
SAA 1	38 M	Hypocallular, no MK		1	103	Mild	Yes (neutropenia)	40	Alive	Flu	None	NR	NR	PR	PR	10	yes	PR	5
SAA 2	43 M	Acellular		1	103	None	Yes (neutropenia, thrombocytopenia)	28	Alive	HSV, HSV, PUO	None	CR	CR	Rel	CR	3	yes	CR	7
SAA 3	52 F	Hypocellular, no MK		0	103	None	Yes (neutropenia)	9	Dead[1]	none	None	NR	n.r. (SCT)	n.r. (SCT)	NR	n.a.	n.a.	n.a.	1
SAA 4	56 F	Hypocellular, no MK	HBV (occult)	0	103	None	Yes (neutropenia, thrombocytopenia)	22	Alive	VZV	None	PR	CR	Rel	CR	4	yes	CR	3
SAA 5	50 M	Hypocellular, no MK		0	103	None	Yes (neutropenia)	19	Alive	CMV reactivation	None	PR	CR	Rel	CR	4	yes	CR	2
SAA 6	35 M	Erythroid hyperplasia	Subclinical PNH	0	73	None	No	18	Alive	none	Liver	NR	NR	PR	PR	10	no	n.a.	2
SAA 7	56 F	Hypocellular, no MK	HBV (occult)	1	73	Mild	No	12	Alive	Flu, Flu	Liver	NR	PR	Rel	PR	6	yes	PR	3
SAA 8	68 F	Hypocellular, no MK	Atrial fibrillation	0	73	None	n.a.	1	Dead[2]	PUO	Fatal arrythmia	n.r.	n.r.	n.r.	NR	n.a.	n.a.	n.a.	1
SAA 9	35 M	Acellular		0	103	None	n.a.	3	Alive	none	None	CR	n.r.	n.r.	CR	3	n.a.	n.a.	1
AA/PNH 1	29 F	Erythroid hyperplasia		2	73	None	Yes (neutropenia)	23	Alive	none	None	PR	CR	CR	CR	4	no	n.a.	1
AA/PNH 2	36 F	Hypocellular, no MK		1	73	None	No	19	Alive	none	None	NR	NR	n.r. (SCT)	NR	n.a.	n.a.	n.a.	1
PRCA 1	62 M	Erythroid aplasia	DVT, diabetes	1	73	Severe*	Yes (neutropenia)	38	Alive	Flu	Hyperuricemia	PR	CR	Rel	CR	4	yes	CR	10
PRCA 2	71 F	Erythroid aplasia	Thymoma, COBP, MGUS	0	73	None	No	29	Alive	CMV reactivation	Hyperuricemia	PR	CR	Rel	CR	4	yes	CR	3
PRCA 3	61 M	Erythroid hypoplasia	HBV (occult)	0	73	Moderate	No	26	Alive	Bronchitis	None	PR	PR	Rel	PR	4	yes	NR	3
PRCA 4	75 F	Erythroid hypoplasia		0	73	None	Yes (neutropenia)	8	Dead[3]	none	MPS/MDS	NR	NR	n.r.	NR	n.a.	n.a.	n.a.	1
PRCA 5	63 F	Erythroid aplasia	Connective tissue disease	1	73	None	No	7	Dead[4]	none	CTD relapse	CR	Rel	n.r.	CR	1	yes	not done	1
PRCA 6	45 F	Erythroid aplasia	Thymoma	0	73	None	No	17	Alive	CMV reactivation, PUO, PUO	Thymoma relapse	CR	CR	PR	CR	1	yes	CR	7
PRCA 7	37 F	Erythroid aplasia		0	73	Mild	Yes (neutropenia)	12	Alive	none	None	CR	CR	PR	CR	3	no	PR	2
PRCA 8	40 M	Erythroid aplasia		0	73	None	Yes (neutropenia)	12	Alive	PUO	Liver	NR	NR	NR	NR	n.a.	n.a.	n.a.	2
PRCA 9	82 F	Erythroid aplasia	MI, diabetes	0	73	None	No	9	Dead[5]	PUO	CVA	NR	PR	n.r.	PR	4	no	n.a.	1
PRCA 10	56 M	Erythroid aplasia	Thymoma, MI, ostheoporosis	1	73	None	No	3	Dead[6]	CMV reactivation	MI	CR	n.r.	n.r.	CR	1	no	n.a.	1
PRCA 11	43 F	Erythroid aplasia		2	73	None	No	5	Alive	none	None	CR	n.r.	n.r.	CR	1	no	n.a.	1
PRCA 12	87 F	Erythroid aplasia	Atrial fibrillation, arthrosis	0	73	None	No	2	Alive	none	Liver, neuropathy	n.r.	n.r.	n.r.	CR	1	no	n.a.	1
PWCA 1	57 F	Myeloid aplasia	HBV (occult)	4	73	None	n.a.	39	Alive	Flu, HBV reactivation	MGUS	CR	CR	CR	CR	1	yes	CR	2
PWCA 2	49 F	Myeloid hypoplasia + lymphoid metaplasia	T-LGL expansion	0	73	None	n.a.	26	Alive	none	None	CR	CR	CR	CR	1	no	n.a.	1
SAA S1	63 F	Severely hypocellular		3	103	None	n.a.	9	Dead[7]	Sepsis	None	NR	NR	NR	NR	na	n.a.	n.a.	1

Table I. (Continued).

UPN	Age (years), gender	Bone marrow	Concomitant disease	Previous therapy	Total alemtuzumab dose (mg)	Injection-related adverse events	Haematological toxicity	Follow up (months)	Status	Infections	Other adverse events	Clinical response							Courses (*n*)
												3 months	6 months	12 months	Best	Time	Relapse	Re-treat	
SAA S2	31 F	Severely hypocellular		3	103	None	n.a.	18	Dead[1]	PUO	None	NR	NR	NR	NR	na	n.a.	n.a.	1
SAA S3	31 F	Moderately hypocellular		3	103	None	n.a.	32	Alive	PUO	None	NR	NR	PR	PR	12	n.a.	not done	1
SAA S4	30 M			3			n.a.					NR	NR	NR	NR	n.a.	n.a.	n.a.	1
SAA S5	41 F	Acellular		2	103	None	n.a.	12	Alive	none	None	NR	PR	PR	PR	6	no	not done	1
SAA S6	72 M	Acellular	Benign Hypertrophy Prostate	1	100	Mild	n.a.	16	Alive	HSV	None	NR	NR	NR	NR	n.a.	n.a.	n.a.	1
SAA S7	34 M	Hypocellular		2	100	Mild	Yes (neutropenia)	7	Alive	CMV reactivation	None	NR	NR	n.r.	NR	n.a.	n.a.	n.a.	1
SAA S8	21 M	Hypocellular		1	103	None	Yes (neutropenia, thrombocytopenia)	12	Alive	CMV reactivation	None	PR	Rel	n.r.	PR	4	yes	not done	1
PRCAS1	53 M	Erythroid hypoplasia		1	73	None	no	7	Alive	none	None	CR	Rel	n.r.	CR	2	yes	not done	1
PWCA S1	21 F	Hypocellular with no myelopoiesis	SCT, graft failure, Pseudomonas sepsis	1	100	Severe†	no	28	Alive	None	PTLD	CR	n.r. 2nd SCT	n.r. 2nd SCT	CR	4	yes	not done	1

Diagnosis: Diagnosis was performed according to criteria established by the International Aplastic Anaemia and Agranulocytosis Study Group. Myelodysplastic syndromes were excluded based on karyotyping as well as on morphological and flow cytometry findings; no patient had a significant number of blast cells at presentation. The presence of a paroxysmal nocturnal hemoglobinuria (PNH) clone was sought in all patients by flow cytometry: 2 AA patients had large PNH clones within both red and white cells, with measurable heamolysis (AA/PNH), while an additional one had a small PNH population only within white blood cells, without measurable haemolysis (subclinical PNH). The size of these PNH clones did not change after treatment.

Follow up is expressed as months from the first day of treatment.

Concomitant diseases: HBV, Hepatitis B virus; DVT: deep vein thrombosis; COBP: chronic obstructive bronco-pneumopathy; MGUS: monoclonal gammopathy of unknown significance; CTD: connective tissue disease; MI: myocardial infarct; T-LGL: T-cell large granular lymphocyte syndrome; SCT, stem cell transplantation.

Injection-related Adverse Events: *, injection-related fever refractory to paracetamol and anti-histamines (steroids not utilised because of uncompensated diabetes) requiring doubling of interval between alemtuzumab administrations; †, fever and hypotonia (concomitant with pre-existing sepsis by Pseudomonas).

Infections; HSV, herpes simplex virus; PUO, pyrexia of unknown origin; VZV, varicella zoster virus; CMV, cytomegalovirus. All patients received intensive anti-infectious prophylaxis, which included: i. anti-CMV prophylaxis administered to all seropositive patients (25 out of 25) by oral valganciclovir (450 mg, bi-daily) for 3 months or up to $CD4^+ > 0{\cdot}1 \times 10^9$/l (anti-herpetic agents other than valganciclovir were used in patients not on protocol); ii. weekly monitoring of CMV viraemia by polymerase chain reaction; iii. anti-Pneumocystis Jirovecii prophylaxis by oral trimethoprim-sulphametoxazole (bi-daily thrice per week); iv. anti-bacterial and anti-fungal prophylaxis in case of severe ($< 0{\cdot}5 \times 10^9$/l) neutropenia. With the exception of PWCA1, all patients with occult HBV (as indicated by positive Hepatitis B (HB) core antibody IgG with negative HBsurface antigen and HBe-antigen) also received prophylactic lamivudine.

Other Adverse Events: PTLD: post-transplant lymphoproliferative disorder in a transplanted patient treated with alemtuzumab after a graft failure; MPS/MDS, myeloproliferative syndrome/myelodysplastic syndrome.

Clinical responses were classified according to established criteria (Camitta, 2000). Patients were defined as complete responders (CR) if they had blood counts in the normal range; patients no longer meeting criteria for severe disease and/or becoming transfusion independent were classified as partial responders (PR). PRCA patients were defined as PR when transfusion independent, or CR if Hb level also normalised.

Cause of death: 1. transplant-related complications ($n = 2$) (one patient underwent allogeneic stem cell transplantation because of lack of response at 3 months, with persistent life-threatening haemorrhages); patient SAAS1 died of sepsis after later HSCT; 2. cardiac arrest on underlying atrial fibrillation, in concomitance with fever of unknown origin developing about 1 month after alemtuzumab treatment (on an underlying long-lasting very severe neutropenia); 3. progression to a myelodysplastic/myeloproliferative disorder; 4. progression of a concomitant connective tissue disease; 5. myocardial infarct (the patient had a previous infarct and active thymoma); 6. cerebrovascular accident, in an elderly patient with previous cerebrovascular manifestations; 7. sepsis, at +9 months from treatment in absence of haematological improvement.

Courses: total number of courses received by each patient, as a result of retreatment due to relapse; on the 25 patients enrolled in the prospective trial, a total of 63 courses of alemtuzumab (25 as single injection) were given.

complication, and eight viral infections (one Varicella-Zoster virus [VSV] with shingles, two Herpes Simplex virus [HSV] and five flu) were reported, all resolving quickly. All VZV and HSV episodes occurred after valganciclovir discontinuation. No CMV disease, Epstein–Barr virus-related disease or lymphoproliferative disorders were observed. A sensitive polymerase chain reaction method confirmed negative CMV viraemia in all patients receiving prophylactic valganciclovir (Risitano *et al*, 2009); four patients developed asymptomatic CMV reactivation after the discontinuation of anti-viral prophylaxis, but viraemia (just above the detection limit) promptly disappeared with pre-emptive valganciclovir. Finally, one patient with pre-treatment occult HBV infection developed HBV reactivation (positive for Hepatitis B surface antigen and HBV viremia) without laboratory signs of hepatitis; lamivudine therapy was started and was effective in viral clearing. The other three patients with possible occult HBV infection received prophylactic lamivudine. This acceptable safety profile was confirmed in the 10 additional patients receiving alemtuzumab as salvage IST at other EBMT centres. Even in heavily pretreated patients (all had received one or two ATG courses), alemtuzumab treatment did not result in any specific toxicity. There was one fatal sepsis (9 months after treatment, in a non-responding patient), one CMV reactivation (in a patient receiving famaciclovir instead of valganciclovir), one HSV infection and one post-transplant lymphoproliferative disorder (PTLD; this patient had received a previous transplant and was treated with alemtuzumab after a primary graft failure).

Alemtuzumab led to complete lympho-ablation in all patients, with the lymphocyte count (which was normal or almost normal in all patients) falling close to 0×10^9/l within 2–3 d, and remaining markedly reduced for several months (especially for the CD4 subset). Within the prospective trial, there were 5 complete responses (CR) and 3 partial responses (PR) in the 11 SAA patients, and 8 CR and 2 PR in the 12 PRCA. Both PWCA patients achieved a CR. Among SAA patients receiving alemtuzumab as first IST, there were three CR and one PR (the two non-responders had a follow up of 1 and 3 months). In the EBMT survey, three of eight SAA patients (all heavily pretreated) achieved a PR, while both of the two PRCA and PWCA patients achieved a CR. When combined with the data from the prospective clinical trial, the total response rate was 58% in SAA, 84% in PRCA and 100% in PWCA. Responses were faster in PRCA and PWCA patients (range 1–4 months), compared with SAA (3–10 months). Survival and cumulative incidence of response analyses in the 25 patients in the prospective trial (Fig 1) were 73% and 89% respectively. Despite most patients receiving chronic low dose cyclosporine A (only four patients had to discontinue the drug due to intolerance), relapses were frequent and appeared earlier than observed after ATG (Frickhofen *et al*, 2003), irrespective of persistent lymphocytopenia; this was possibly related to the low dose of cyclosporine A utilised in the study. Relapse occurred in five of seven SAA and AA/PNH patients,

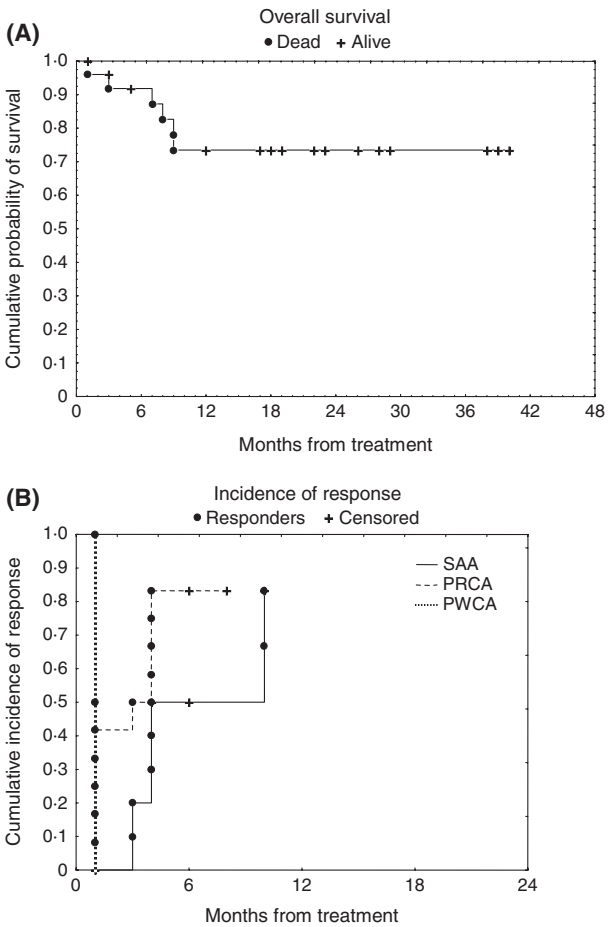

Fig 1. Overall survival and cumulative incidence of response of patients included in the prospective trial. (A) Overall survival in the whole population: with a median follow up of 17 months, the survival at 2 years was 73%. (B) Cumulative incidence of response according to the underlying disease: 84% in SAA, 84% in PRCA and 100% in PWCA.

five of eight PRCA and one of two PWCA patients. Alemtuzumab (administered as either single 30 mg injection or complete courses) was still effective even in patients experiencing multiple relapses (Table I), demonstrating easiness of re-treatment. In some patients, recurrent disease led to periodical (every 3–4 months) 30 mg injections, which worked as maintenance treatment to prevent further relapses.

Alemtuzumab has been reported to be effective in anecdotal cases (Killick *et al*, 1997; Alvares *et al*, 2004; Au *et al*, 2005; Schützinger *et al*, 2005) or small series (Willis *et al*, 2001; Gómez-Almaguer *et al*, 2009; Kim *et al*, 2009). Here we report the largest experience to date using alemtuzumab in SAA, PRCA and PWCA. In comparison to standard ATG, alemtuzumab was cheaper, simpler to administer (even without hospitalisation), and injection-related side effects were negligible. Safety concerns were assuaged given the low rate of infectious complications and acceptable toxicity profile, allowing its use even in diseases with a mild clinical course (i.e., PRCA and PWCA). Preliminary results on efficacy suggest response rates that were

not inferior to standard IST regimens, even if relapses were not infrequent. However, re-treatment for relapse was easy and effective. We provide evidence that alemtuzumab-based IST is feasible and manageable in patients suffering from immune-mediated BMFS, paving the way for systematic investigation in comparison to standard IS regimens.

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Contribution

A.M.R., C.S. and B.R. initially conceived the study, which was subsequently designed within the EBMT WPSAA in collaboration with H.S., J.P., J.M., A.B., G.S. and A.T. The prospective trial was conducted in Naples by A.M.R. with the contribution of B.S., under the supervision of B.R. and C.S. The following authors contributed to the retrospective study through the treatment of patients at their own Institutions: G.T., A.K, S.M., J.H., V.G, A.B., A.T. and J.M. All the authors contributed to the discussion and the interpretation of the data. The manuscript was written by A.M.R., J.M., J.P. and B.R.; all the authors critically revised the manuscript and contributed to the preparation in its final version.

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