Successful engraftment following allogeneic stem cell transplantation with busulfan as a single agent in very high-risk patients

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Background and Objectives. Busulfan is the most commonly used myeloablative alkylating agent, but is considered a poor anti-lymphocyte agent. Since engraftment of allogeneic stem cells depends not only on adequate immunosuppression but also on successful hematopoietic competition, and considering the fact that residual lymphocytes of host origin may play a beneficial role in preventing graft-versus-host disease (GVHD), we used low doses of oral busulfan as a single agent for conditioning prior to stem cell transplantation (SCT) in recipients of transplants from a variety of donors.

Design and Methods. Fifteen heavily pretreated high-risk patients (age 25-66, median 42 years) with hematologic malignancies were conditioned with busulfan alone, 4mg/kg/day for 2, 3, or 4 consecutive days. No additional pre- or post-transplant immunosuppressive agents were used in order to exploit the capacity of donor lymphocytes to induce graft-versus-malignancy (GVM) effects.

Results. Conditioning was well tolerated, trilineage engraftment was documented in all patients and none exhibited immune-mediated rejection. Time to recovery of absolute neutrophil count >0.5×10^9/L and 1.0×10^9/L was 12 - 38 (median 15) days and 12 - 41 (median 15) days, respectively. The time to platelet recovery ≥20 and ≥50×10^9/L ranged from 0 to 26 (median 11) days, and from 0 to 83 (median 14) days, respectively. Acute GVHD (≤grade I) occurred in 13/15 patients. Three patients benefited from long-term survival.

Interpretations and Conclusions. We suggest that using busulfan alone for the preparation of patients for SCT may be sufficient for engraftment, in very high-risk heavily pre-treated patients.

Key words: stem cell transplantation, graft-versus-malignancy effects, busulfan, reduced intensity conditioning, hematologic malignancies, leukemia.

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Busulfan, a sulfonic acid ester, is an alkylating agent with potent toxicity for hematopoietic and sinusoidal endothelial cells. The drug was initially introduced in 1952, as a treatment modality for patients with chronic myeloid leukemia.[1] It was only around 20 years later that this agent was first used as part of the conditioning regimen in bone marrow transplantation, substituting total body irradiation.[2] Considering the fact that busulfan was considered as a potent myeloablative rather than immunosuppressive agent, most transplant centers used the combination of busulfan and cyclophosphamide or other alkylating agents or total body irradiation with the aim of accomplishing maximal myeloablation as well as optimal immunosuppression prior to transplantation of allogeneic stem cells. Since then, the use of busulfan spread widely and it was also used in conjunction with autologous stem cell transplantation (SCT).[3-4] However, it was soon discovered that the use of busulfan could be associated with hepatic toxicity, mainly veno-occlusive disease, especially in patients undergoing allogeneic SCT.[5-6] Other toxicities of the drug included electroencephalographic abnormalities and an increased risk of convulsions due to the drug penetrating into the central nervous system,[7-8] inhibition of stromal cell function,[9-11] occasional pulmonary toxicity and an association with increased transplant-related mortality when used at high doses for allogeneic SCT.[12] In recent years, busulfex, an intravenous preparation of busulfan, has gradually been replacing oral busulfan in SCT regimens. Its main advantages are reduced liver toxicity and dosage assurance with predictable pharmacokinetics.[13-15] Given its known myeloablative properties, busulfan was considered in many centers as the treatment of choice for myeloid disorders, however, busulfan, was never recommended as a suitable single agent in a preparative regimen for allogeneic SCT because of its limited toxicity to mature lymphocytes. Combinations of busulfan with cyclophosphamide or other

agents were used not only to control mature lymphocytes in order to prevent rejection, but also to exploit synergistic activity against target tumors or genetically abnormal stem cells. The use of busulfan, or nowadays busulfex, avoiding the need for ionizing irradiation, is considered the preferred treatment for malignant and non-malignant indications especially in the pediatric age group. However, there are situations in which heavily pre-treated patients cannot safely receive extensive immunosuppressive and myeloablative conditioning; furthermore, in patients with resistant disease, it seems unlikely that all tumor cells can be eliminated by chemotherapy alone. We, therefore, investigated the feasibility of accomplishing durable and consistent engraftment of donor stem cells with low doses of busulfan alone, avoiding the use of post-transplant immunosuppression as GVHD prophylaxis in order to maximize induction of graft-versus-malignancy effects following tumor debulking with busulfan alone. Indeed, O'Brien and Goldman described the use of busulfan as a sole preparative agent in a group of patients with chronic myelogenous leukemia, but this was in the setting of autologous stem cell transplantation.[16] The present report summarizes our cumulative experience in a cohort of 15 patients who received allogeneic stem cell allografts after preparation with busulfan as a single agent.

Design and Methods

Fifteen high-risk patients with advanced and fully resistant disease, most of whom had been heavily pre-treated, were enrolled in this study between December 1997 and June 2000. Indications for transplantation and disease status are shown in Table 1. Patients were included if they had an absolute indication for allogeneic SCT but were not eligible for a standard transplant procedure, or if their potential to survive the standard protocol was judged to be low due to poor performance status, prior SCT or relapse after SCT, provided they had not previously been given busulfan. The series comprised 10 males and 5 females, ranging in age from 25-66 (median 42) years (Table 1). The mean Karnofsky performance status score was 88 (range 60-100). Each patient signed informed consent to the protocol which had been approved by the Institutional Review Board. Twelve patients were transplanted from fully matched HLA class I and II family members, two patients were transplanted from mismatched sibling donors (one from a haploidentical sibling and the other from a one-locus mismatched sibling). One patient received a marrow allograft from a fully matched unrelated donor (MUD); this graft was non-reactive in mixed lymphocyte culture, as shown in Table 2.

Table 1. Characteristics of patients undergoing stem cell transplantation following conditioning with busulfan as a single agent.

UPN	*Age (years)*	*Basic disease*	*Disease status at Tx.*	*No. of Tx.*	*Survival*	*Cause and time of death post Tx. (days)*
977	33	CML – Blastic transformation to AML-M7	Resistant blast Crisis s/p SCT	2nd	No	Infection and acute GVHD (83)
1170	47	MM	Resistant Disease s/p SCT	2nd	No	Disease progression (12)
1242	26	AML-M2	Resistant 1st Relapse after PR only	1st	Yes	Survived
1251	50	AML-M4	Resistant 1st Relapse after PR only	1st	Yes	Survived
1253	35	CML	2nd resistant blast crisis	1st	No	acute GVHD (16)
1283	59	AML*	2nd resistant relapse	1st	No	Disease progression (76)
1323	28	T-cell ALL	Resistant 1st relapse	1st	No	Disease progression (95)
1354	25	AML-M5	Resistant 1st relapse	1st	No	Infection and acute GVHD (34)
1358	44	Biphenotypic	Resistant disease – refractory to therapy	1st	No	acute GVHD (43)
1362	52	CML	2nd CP	1st	No	acute GVHD (75)
1374	46	AML-M5	Resistant disease - refractory to therapy	1st	No	Disease progression (120)
1378	36	CML	Resistant blast crisis	1st	Yes	Survived
1380	42	CML	2nd CP	1st	No	Disease progression (278)
1381	66	NHL	Resistant 1st Relapse after PR only	1st	No	acute GVHD (47)
1382	31	AML-M4	2nd resistant relapse s/p SCT	2nd	No	Infection and acute GVHD (110)

*UPN: unique patient number; Tx: transplantation; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; MM: multiple myeloma; NHL: Non-Hodgkin's lymphoma; HD - Hodgkin's disease; ALL: acute lymphoblastic leukemia; CP: chronic phase; PR: partial remission; GVHD: graft-versus-host disease; *:sub-classification not defined.*

Pre-transplant conditioning consisted of oral busulfan 4 mg/kg/day in four divided daily doses. The decision about the exact amount of busulfan each

Table 2. Characteristics of donors, donations and conditioning regimen.

UPN	Sex Recip./ Donor	Matching	Origin of donation	Dose of busulfan	CNS prophylaxis	Day* of ANC >1.0×10⁹	Day* of 100% chimerism
977	M/M	Full match	PBSC	4 mg×4	No	15	49
1170	M/MUD	Full match	PBSC	4 mg×2	No	12	NA
1242	F/F	Full match	PBSC	4 mg×4	No	17	17
1251	F/F	Full match	PBSC	4 mg×4	ARA-C	12	13
1253	M/M	Haplo.	PBSC	4 mg×4	No	13	13
1283	F/M	Full match	BM	4 mg×4	No	32	50
1323	M/F	Full match	PBSC	4 mg×4	No	13	22
1354	F/M	Full match	PBSC	4 mg×3	No	14	18
1358	M/F	Full match	PBSC	4 mg×4	ARA-C &MTX	12	18
1362	M/F	Full match	PBSC	4 mg×4	ARA-C &MTX	21	49
1374	M/F	1 locus mismatch	PBSC	4 mg×4	No	21	21
1378	M/M	Full match	PBSC	4 mg×4	ARA-C	16	43
1380	F/F	Full match	PBSC	4 mg×2	ARA-C	41	52
1381	M/M	Full match	PBSC	4 mg×2	No	15	21
1382	M/M	Full match	PBSC	4 mg×2	No	23	41

**Following transplantation; UPN: unique patient number; Recip.: recipient; F: female; M: male; Allo.: allogeneic transplantation; Auto.: autologous transplantation; BM: bone marrow; PBSC: peripheral blood stem cell; Haplo.: haploidentical mismatch; CNS: central nervous system; ARA-C: cytosar; MTX: methotrexate; NA: not achieved.*

patient was administered was made taking into account the treatment history and estimating each patient's ability to stand the protocol. Ten patients were treated for four consecutive days (total dose 16 mg/kg); one patient for three consecutive days (total dose 12 mg/kg); and four patients for only two consecutive days (total dose 8 mg/kg). Five of the patients also received central nervous system prophylaxis with 30 mg/m^2 cytosine arabinoside, administered intrathecally. Two of these patients were also given methotrexate intrathecally (12.5 mg and 15 mg) (Table 2). No additional pre- or post-transplant immunosuppression was administered. Fourteen patients were reconstituted with mobilized peripheral blood stem cells and one with bone marrow cells. Donors of the peripheral blood stem cells were injected subcutaneously with granulocyte-colony stimulating factor (G-CSF; Neupogen 5 μg/kg twice daily for 5 days) and the mobilized peripheral blood stem cells were collected on days 5 and 6. Bone marrow collection was done under epidural anesthesia. Prior to transplantation, all patients received trimethoprim/sulfamethoxazole (10 mg/kg/day trimethoprin) on days -8 to -2, acyclovir (500 mg/m^2×3/day) from day –8 until day +100, and allopurinol (300 mg/day) on days -8 to -1. Administration of trimethoprim/sulfamethoxazole (twice weekly) was reinstituted after recovery from neutropenia as a preventive measure against *Pneumocystis carinii* infection. Neutropenic patients with culture-negative fever received a combination of gentamicin, cefazolin and mezlocillin, as a first-line antibiotic protocol. Persisting fever was treated with amikacin and tazocin as a second-line protocol, while meropenem and vancomycin were used as the third-line protocol. In cases of persistent fever not responding to antibiotic therapy within 5 days, amphotericin B (1 mg/kg every other day) was added until the neutropenia resolved.

Starting on day -8, a DNA-polymerase chain reacton (PCR) test and, later during the follow-up period assay of pp65 antigenemia, was done weekly to detect cytomegalovirus. Two consecutive positive PCR results or one antigenemia assay with more then one cell positive for pp65 served as an indication for replacing acyclovir with ganciclovir (10 mg/kg/day), until a minimum of two negative tests were obtained. Patients were treated with reverse isolation in rooms equipped with HEPA filters, and received a regular diet. Additional supportive measures, such as blood components were administered as necessary.

Acute and chronic GVHD were graded according to Glucksberg's criteria.[17] Upon the appearance of signs and symptoms of acute GVHD >grade I, treatment was immediately started with methylprednisolone 2 mg/kg/day i.v. and cyclosporine 3 mg/kg/day i.v. in two divided doses for patients in the hospital and 6 mg/kg twice daily orally for outpatients.

In order to assess engraftment, degree of chimerism, minimal residual disease and early relapse, patients were monitored at regular intervals by cytogenetic analysis, and by donor and host-specific DNA markers as previously described, including male and female amelogenine gene PCR bands,[18] and by variable number of tandem repeats-PCR assay in patients with no sex mismatch.[19]

Results

During the conditioning the protocol used was well tolerated by all patients. All patients were fully mobile throughout the conditioning period. Two patients out of 15 died peri-transplantation, one on the day of engraftment due to progressive multiple myeloma disease, and the second, two days after documentation of engraftment, on day +16 because of acute GVHD. No patient had severe oral mucositis, facilitating maintenance by normal oral intake. Moderate or severe hepatic veno-occlusive disease did not occur in any of the patients. Neutropenic fever was noted in 13/15 patients (median of febrile episodes 1, range 0-2), with a median of four days of fever (range 0-15 days).

All patients displayed evidence of engraftment shortly after transplantation and none exhibited immune-mediated rejection. A transient period of mixed chimerism was detected in 11 patients. Of these patients, one developed 100% donor chimerism after receiving donor lymphocyte infusion (UPN 1283), a second patient (UPN 1170) died too early from progression of his basic disease, and all the others converted spontaneously to full 100% donor chimerism shortly thereafter. In the other four patients, rapid engraftment of 100% donor cells was confirmed without evidence of transient mixed chimerism (Table 2). The median nadir white cell count was 0.3×10^9/L (range 0.1 – 1.5×10^9/L). Time to recovery of absolute neutrophil count (ANC) above 0.5×10^9/L and 1.0×10^9/L was 12-38 (median 15) days and 12 - 41 (median 15) days, respectively. The median nadir platelet count was 8×10^9/L (range 2–37×10^9/L). Time for platelet recovery to values ≥20 $\times10^9$/L and ≥50×10^9/L ranged from 0 to 26 (median 11) days, and from 0 to 83 (median 14) days, respectively. In three patients the platelet count never dropped below 20×10^9/L throughout their hospitalization, whereas four of the patients did not reach a platelet count above 50×10^9/L before dying. Two of the patients did not need any platelet support during the entire transplantation course.

The overall mortality rate was high for a number of reasons, the first being the intentional selection of poor-risk patients who were not eligible for a standard transplant procedure but rather qualified for an experimental protocol. In addition, no anti-GVHD prophylaxis was used in order to exploit the capacity of donor lymphocytes to induce GVM effects, thus exposing the patients to the risk of uncontrolled GVHD. Consequently, only three patients benefited from long-term survival (UPN 1242, 1251, 1378, median follow-up 79 months [range 67-80 months]). Five of the 15 patients died of disease progression (median time to death 95 days [range 12-278 days]). Four patients died from acute GVHD (median time to death 45 days [range 16-75 days]). Three patients died from a combination of infection and GVHD (median time to death 83 days [range 34-110 days]).

Acute GVHD (≥grade I) occurred in 13/15 patients and in nine patients progressed to grade III-IV. In one patient acute GVHD progressed to chronic GVHD. As mentioned above, GVHD was the direct cause of death in four patients.

Discussion

We summarize the outcome of the first cohort of allogeneic SCT recipients conditioned with busulfan as a single agent, with an attempt to maximize GVM effects induced by alloreactive donor lymphocytes post transplantation. No anti-graft-versus host prophylaxis was given, thus intentionally maximizing GVM effects as the only possible modality to eliminate tumor cells resistant to all conventional anti-cancer agents. O'Brien and Goldman already described the use of busulfan as a sole preparative agent in a group of patients with chronic myeloid leukemia, but this was in the setting of autologous stem cell transplantation, in which no additional immunosuppression was indicated and no GVM effects were anticipated.[16] Myeloablative conditioning alone in preparation for allogeneic SCT, especially in heavily pretreated patients with poor performance status, carries a substantial risk of procedure-related toxicity and mortality.[20] Reduced intensity conditioning can decrease the incidence of some of these early complications.[21-22] Moreover, over the years aggressive preparatory regimens have not proven advantageous and have not improved disease-free survival in any of the disease categories in which SCT is indicated.[23-24] Therefore, when testing the feasibility of using busulfan alone for conditioning stem cell allograft recipients we used low doses of busulfan in order to identify the minimal dose that would allow durable engraftment and, subsequently, development of maximal GVM effects by alloreactive donor lymphocytes unperturbed by immunosuppressive agents used for anti-GVHD prophylaxis. It was also assumed that once engraftment had occurred in the absence of post-transplant immunosuppression, residual hematopoietic cells of host origin as well as leukemia, would likely be eliminated by donor lymphocytes due to induction of optimal GVM effects in the absence of cyclosporine or any other post-grafting anti-GVHD prophylaxis. The high incidence of relapse in recipients of T-cell-depleted allografts fol-

lowing myeloablative conditioning,[25] as well as in patients transplanted after myeloablative conditioning with no development of GVHD,[26-28] or recipients of stem cells obtained from an identical twin,[28-29] support the major role of alloreactive donor T cells on outcome following SCT. Furthermore, the negative effects of cyclosporine on GVM effects inducible by alloreactive donor lymphocytes has been well documented in preclinical animal models[30] and in humans.[31-32] Based on the above, the present study was designed with the aim of investigating the role of conditioning with a single myeloablative agent, busulfan, given in graded increments, according to the clinical condition of the poor-risk patients eligible for the study. The goal was to determine whether engraftment can be achieved with this alkylating agent, since once early engraftment has been accomplished, donor lymphocytes can be expected to continuously ablate residual hematopoietic cells of host origin enabling the development of 100% donor cell chimerism. Considering the *rule of balanced equilibrium*,[33] concerning the association between the intensity of pre-transplantation immunosuppression and the incidence and severity of GVHD, it was also interesting to investigate whether a protocol which consisted of a primarily myeloablative agent could also reduce the incidence of GVHD. Indeed, although fludarabine, cyclophosphamide and anti-lymphocyte antibodies, commonly used in reduced intensity conditioning protocols, were not used in the present study, no graft rejection was observed in any recipient of a minimum of two doses of busulfan 4 mg/kg/day. Moreover, all four patients who had a rapid, full engraftment of donor cells received four doses of busulfan 4 mg/day. On the other hand, all the other 11 patients, including 5 patients who also received all four doses of busulfan 4 mg/day, experienced a period of mixed chimerism before achieving 100% donor chimerism. Thus, it is not clear whether the dose of busulfan is of importance for chimerism. The same holds true concerning engraftment. The data (Table 2), are inconclusive on whether doses of busulfan between 8 mg to 16 mg are relevant to engraftment outcome. Our data from a small cohort of 15 patients show that durable engraftment and occasionally cure of the underlying malignancy can be accomplished with minimal conditioning based on the use of busulfan as a single oral agent, with a relatively low procedure-related toxicity, considering the severity of disease of this heavily pre-treated cohort of patients with a poor performance status, although exposing the patients to the risk of severe GVHD-related toxicity. From a theoretical point of view, this study seems to reiterate the importance of hematopoietic competition in engraftment following reduced intensity conditioning, as well as the efficacy of busulfan in preventing rejection.[34] Furthermore, it demonstrates the feasibility of overcoming fatal GVHD when non-myeloablative conditioning is used, which may be the consequence of a transient stage of mixed chimerism.[33]

Interestingly, consistent engraftment of donor stem cells was confirmed in all patients, including in one patient reconstituted with haploidentically mismatched stem cells and in another who received an allograft from a matched unrelated donor, while early marrow aplasia and pancytopenia were avoided or minimized, thus reducing the immediate transplant-related complications. However, late complications related primarily to uncontrolled GVHD were unavoidable. Our data suggest that busulfan, even at a dose of 8 mg/kg, can induce a sufficient degree of immunosuppression to ensure engraftment in patients who had already been heavily pretreated with chemotherapy. It is unclear whether durable engraftment could also be accomplished in previously untreated patients, since both prior treatment of the underlying leukemia as well as the malignant process itself could result in some degree of immunosuppression that could facilitate engraftment following subsequent conditioning with busulfan alone.

Taken together, our data suggest that reduced intensity conditioning, either using low doses of busulfan as shown here, or using escalated doses of cyclophosphamide and antithymocyte globulin,[35] may be sufficient to enable engraftment of HLA compatible stem cell allografts, at least in heavily pretreated patients. As pioneered in Jerusalem more than 18 years ago,[36,37] and supported by Kolb and colleagues,[38,39] successful engraftment of matched bone marrow allografts can enable subsequent induction of effective GVM effects by donor lymphocyte infusions, mediated by alloreactive donor lymphocytes.[36-39] More recently, in agreement with the aforementioned conclusions, it has shown that low doses of cyclophosphamide or busulfan with fludarabine with no myeloablative conditioning may be sufficient to eliminate all tumor cells in patients with hematologic malignancies,[21,22] thus paving the way to the successful use of stem cell transplantation following non-myeloablative conditioning. These data confirmed the efficacy of quality over quantity, or the need to improve immune regulation rather than to increase the intensity of conditioning. Furthermore, as shown earlier in a preclinical animal model of B- cell leukemia, mismatched allografts may induce curative graft-versus-leukemia effects with no GVHD following non-myeloablative conditioning.[40]

Although the overall outcome of the patients treated with busulfan alone did not appear to be impres-

sive, it should be remembered that the protocol was offered exclusively to patients with very advanced diseases not considered eligible for any alternative transplant program. Based on our data, it seems reasonable to assume that much better outcomes would be achieved in patients with minimal residual disease or with a better performance status who could be conditioned with a similar protocol on an outpatient basis, with no need for expensive components or radiation facilities. Such patients could benefit for a similar approach with additional anti-GVHD prophylaxis to minimize the risk of uncontrolled GVHD.

In conclusion, our observations on a small cohort of non-consecutive, poor risk patients may justify a larger study using busulfan (or busulfex for more consistent bioavailability of the drug), as a single agent for patients needing SCT who do not qualify for standard or non-myeloablative stem cell transplantation procedures. Further investigations in larger cohorts of patients will be required to investigate the efficacy of conditioning based on a single drug in comparison with that of combinations of non-myeloablative agents.

MB: writing the article; follow-up of patients; RO, MYS: treating patients, revision of article; IBR: revision of article;

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