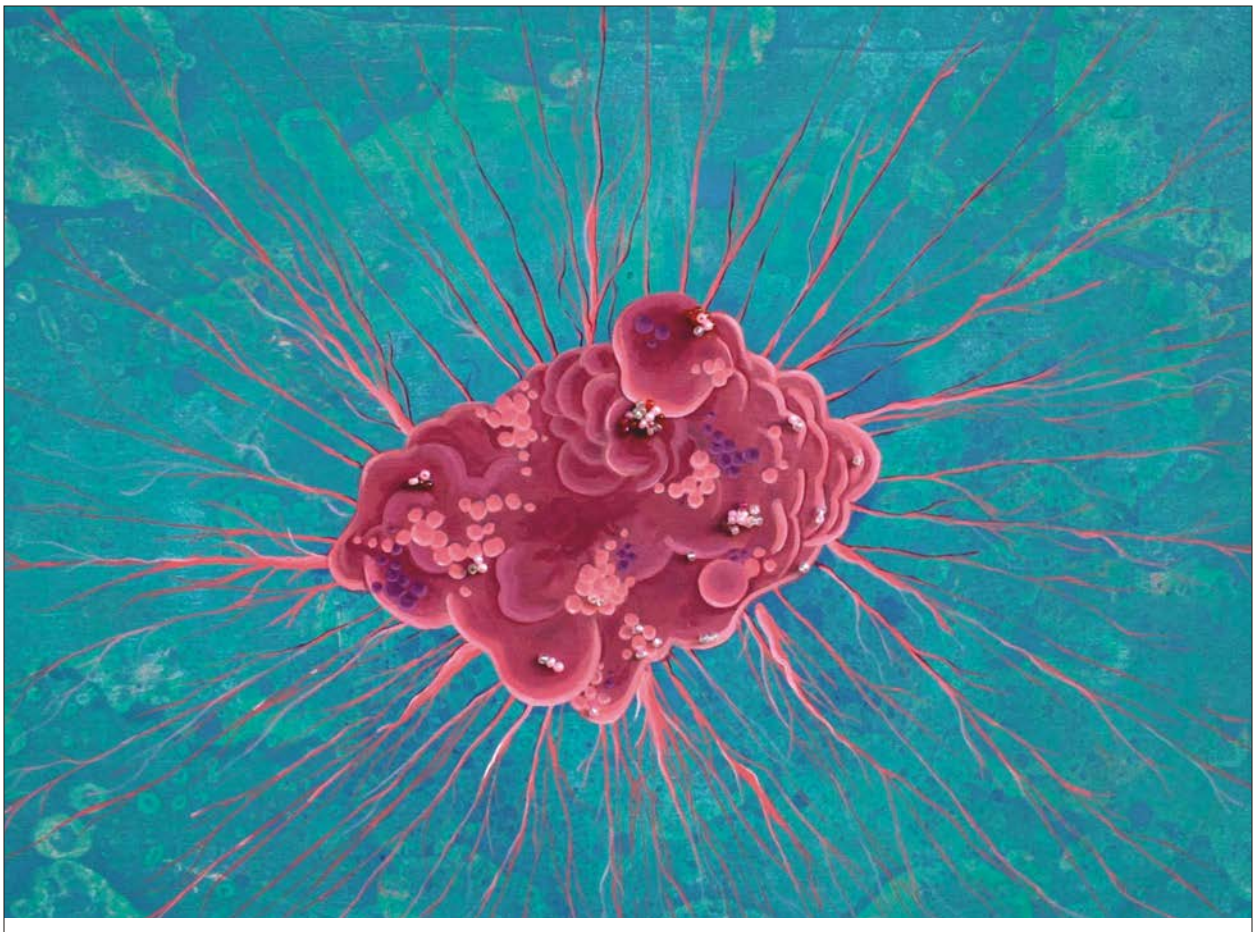

International Journal of Cancer Research

ISSN 1811-9727

Academic Journals Inc.

www.academicjournals.com

International Journal of Cancer Research 2 (2): 131-142, 2006
ISSN 1811-9727

Mitoxantrone, Ara-C and Pentostatin (Map Regimen): Phase I Study of a Novel Non-myeloablative Conditioning Regimen for Hematological Malignancies

Delong Liu, Karen Seiter, J.W. Chiao, Qiuhu Shi, Anila Qureshi, Woondoon Jeong
Lulu Lu, John Nelson and Tauseef Ahmed
New York Medical College and Westchester Medical Center,
Valhalla, NY 10595, USA

Abstract: We studied a new combination of mitoxantrone, ara C and pentostatin (MAP) as non-myeloablative conditioning regimen for allogeneic hematopoietic stem cell transplantation (NST). 25 patients with high-risk hematological malignancies were enrolled in this phase I study. Median age was 51 years (26-62). The MAP regimen consists of mitoxantrone (40 mg m^{-2} on day -6), cytarabine (2 g m^{-2} on day -6,-5,-4) and pentostatin. Pentostatin was given from 6 to 13 mg m^{-2} over 2 or 3 days in a dose-escalating manner. 18 patients received HLA-matched sibling grafts, 7 patients received unrelated grafts. Median donor chimerism at 1 month was 76% for related NST, 98% at 3 months. Four of seven patients with unrelated NST had primary graft failure but all had autologous recovery, the other three had full donor chimerism. Day 100 mortality is 8%. Dose-limiting toxicity was not observed, but two patients enrolled at level 5 died of sepsis and severe GVHD, respectively. Level 4 (pentostatin 13 mg m^{-2} over 3 days) is safe and chosen as phase II regimen. With a median follow-up of 28 months (12-1122 days), the median duration of overall and disease-free survival are 15 and 12 months, respectively. The estimated disease-free and overall survival rates at 2 years are 37 and 40%, respectively. In conclusion, Level 4 of the MAP regimen is safe and chosen as phase II regimen for further clinical investigation.

Key words: Pentostatin, mitoxantrone, map regimen, mini-transplant, non-myeloablative transplantation

Introduction

Allogeneic bone marrow transplantation (AlloBMT) is a potentially curable treatment option for patients with chronic myelogenous leukemia (CML), acute leukemia, myelodysplastic syndrome (MDS), aplastic anemia and other life-threatening hematologic disorders. The curative potential of allogeneic transplantation is in part due to immune-mediated graft-versus-malignancy (GVM) effect. GVM plays a significant role for a variety of diseases, particularly for chronic myeloid leukemia (Horowitz *et al*., 1990; Truitt *et al*., 1987; Fefer, 1999). Patients who develop graft-versus host disease (GVHD) following alloBMT are less likely to suffer a relapse of their underlying disease than those who do not develop GVHD (Horowitz *et al*., 1990; Truitt *et al*., 1987; Fefer, 1999). Conversely, there is a higher risk of relapse in patients who receive T-cell depleted or syngeneic grafts (Fefer, 1999).

Corresponding Author: Delong Liu, New York Medical College, Division of Oncology, Room 250, Munger Pavilion, Valhalla, NY 10595, USA Tel: 914-493-7514 Fax: 914-594-4420

Until a few years ago, myeloablative transplantation was considered to be standard of therapy for many malignant as well as non-malignant disorders. Non-myeloablative stem cell transplantation (NST) has been reported to induce the GVM effect yet with reduced toxicity. Several NST conditioning regimens have been reported. Virtually all of them contain the immunosuppressive nucleoside analog, fludarabine. These regimens also include low dose radiation and/ or additional agents, including cyclophosphomide, busulphan, melphalan, or CAMPATH-1H (Maris and Storb, 2003; Child *et al*., 2000; Giralt *et al*., 1997; Slavin *et al*., 1998; Khouri *et al*., 1998; Kottaridis *et al*., 2000; Carella *et al*., 2000; Milojkovic and Mufti, 2001; Badros *et al*., 2002; Taussig *et al*., 2003).

Pentostatin (2'-deoxycoformycin, DCF) is a potent inhibitor of adenosine deaminase (ADA). ADA inhibition is accompanied by a rise in intracellular deoxyadenosine triphosphate(dATP) and a depletion of adenosine triphosphate. The increased level of dATP can lead to inhibition of DNA synthesis and appears to be particularly toxic to lymphocytes (Carson *et al*., 1977; Mitchell *et al.,* 1983). In support of this view, elevated levels of dATP and deoxyadenosine have been detected in patients with severe combined immunodeficiency who have ADA deficiency (Donofrio *et al.,* 1978). Pentostatin has been used as a single agent to treat hairy cell leukemia (Seymour *et al*., 1997). Dose-limiting toxicity is neurotoxicity and the metabolite is excreted primarily from the kidneys (Major *et al*., 1981). The dose of pentostatin used as a single agent for hairy cell leukemia is 4 mg m^{-2} as a bolus infusion every two weeks (Grever *et al*., 1995). Doses of 5 mg m^{-2} for two consecutive days were well tolerated (Grever et al., 1981; Grever et al., 1985). Pentostatin has a more prolonged and potent effect of immunosuppression than fludarabine (Seymour *et al*., 1997), hence it is possible to use pentostatin for immunosuppression to enhance engraftment and establishment of chimerism. Pentostatin was combined with radiation and photophoresis in a reduced-intensity preparative regimen to allow allografting (Chan *et al*., 2003). Mitoxantrone is widely used for induction and salvage chemotherapy for acute leukemia as well as for lymphoma (Arlin *et al.,* 1990; Feldman *et al*., 2000). We report here a Phase I clinical trial of pentostatin in combination with mitoxantrone and cytarabine as a non-myeloablative regimen for allografting of hematopoietic stem cells in patients with hematological malignancies.

Materials and Methods

Patients aged 18 years and older with refractory and/or poor prognostic hematological malignancies were eligible. Adequate vital organ functions were required (Ejection Fraction>40%, DLCO>40%, Cr. <2.0 mg dL^{-1}, liver enzymes <2x normal limits). This Phase I clinical trial was approved by the Institutional Review Board at the New York Medical College and the Westchester Medical Center. The enrollment started from May 2000. The MAP regimen (Table 1) consists of mitoxantrone (40 mg m^{-2} in 50 mL saline over 20 min) on day -6, cytarabine (2 g m^{-2} in 250 mL saline over 3 h) daily on day -6,-5,-4. Pentostatin (Nipent, manufactured by Supergen) was given in 50 mL saline as a bolus over 20 min in a dose-escalating manner. The level I dose of pentostatin was 3 mg m^{-2} daily for two days on day -6 and -5, level II was 4 mg m^{-2} for two days and level III was 5 mg m^{-2} for two days. Level IV was 5 mg $m^{-2}$for two days, followed by 3 mg m^{-2} on day -4. Level V was 5 mg m^{-2} for two days, followed by 4 mg m^{-2} on day -4. Three patients were planned to be recruited to each level. An additional 3 patients were to be recruited if there were severe toxicity (NCI common toxicity grade 3 or 4).

Table 1: Phase I scheme of MAP regimen

Level	Patient (No.)	Mitoxantrone (mg m^{-2})	AraC (g m^{-2})	Pentostatin (mg m^{-2})
1	6	40 on day -6	2 on day -6, -5, -4	3 on day -6,-5
2	4	40 on day -6	2 on day -6, -5, -4	4 on day -6,-5
3	6	40 on day -6	2 on day -6, -5, -4	5 on day -6,-5
4	7	40 on day -6	2 on day -6, -5, -4	5 on day -6,-5; 3 on day -4
5	2	40 on day -6	2 on day -6, -5, -4	5 on day -6,-5; 4 on day -4

HLA-identical or one-antigen mismatched donors were required. Family donors received G-CSF (10 mcg kg^{-1}) from day -5 through day -1. Leukapheresis was done on day -1 and day 0. The target CD34+ cell d ose was 5 x 10^6 kg^{-1}. Unstimulated donor cells were also collected before G-CSF was started, aliquoted and cryopreserved for later infusion (donor leukocyte infusion, DLI). The target CD3+ cell dose was 1 x10^8 kg^{-1}. If there was evidence of engraftment (donor chimerism >10%) and there was no evidence of GVHD off immunosuppression for at least two weeks, DLI was given for low donor chimerism or for disease progression. DLI was given in a dose-escalating manner. The first dose was 5 x 10^6 kg^{-1}, second dose was 1 x 10^7 kg^{-1}, the third dose was 5 x 10^7 kg^{-1}. For unrelated donors, either bone marrow stem cells or G-CSF mobilized peripheral stem cells were allowed. Donor chimerism was evaluated by FISH analysis for unlike-sex grafts and by short-tandem repeat molecular analysis for like-sex grafts.

GVHD prophylaxis and immunosuppression was given as follows: Cyclosporin (CSA) 1.5 mg kg^{-1} IV Q12 h on day –1 to day +7, then 3 mg kg^{-1} po q 12 h (The dose may be rounded to the nearest 25 mg) from day +8 to day +28. IV CSA was continued if the patient was unable to tolerate po medication. CSA level was monitored daily and maintained between 400-500 ng dL^{-1}. In the absence of GVHD or graft rejection, CSA is tapered slowly starting at day 29. For recipients of transplants from an unrelated or HLA-mismatched donor, CSA was tapered slowly according to the patient's GVHD and /or chimerism status. Mycophenolate mofetil (MMF) w as given at 15 mg kg^{-1} po bid from day 0-27. The dose was rounded to the closest 250 mg (250 mg per tab). MMF was stopped without taper on day 28. The first few patients experienced graft failure after receiving unrelated grafts. Therefore, for subsequent mismatched and unrelated grafts, CSA and MMF were started on day -6 instead of on day -1 and 0, respectively. In addition, for mismatched and unrelated transplantation, ATG (equine antithymocyte globulin, 15 mg k g^{-1} iv daily on day -5, -4, -3, -2) was added to the NST regimen. Skin test for ATG anaphylaxis was done on every patient prior to first dose of ATG. Daily pre-medication for ATG included acetaminophen 650 mg po, diphenhydramine 50 mg IV and methylprednisolone 1mg kg^{-1} IV. Additionally, methylprednisolone at 1mg kg^{-1} was mixed with ATG, which was infused over 4-6 h.

Prophylaxis for microbial infection was as the following: itraconazole 200 mg po BID, acyclovir 200 mg po BID, pentamidine 300 mg iv every two weeks until platelets were over 50,000, at which time it was switched to trimethoprim /sulfamethoxazole (160/800 double strength) 1 tab po BID three times a week. If either the donor or the recipient had positive serology for CMV, valgancyclovir (Valcyte, manufactured by Hoffman-La Roche) 450 mg po BID three times a week was given when WBC was over 1,500 μL. For patients who could not take oral medications, IV gancyclovir at 5 mg kg^{-1} three times a week was used to replace valgancyclovir for CMV prophylaxis. The prophylaxis was continued to at least day 100 after transplantation.

Results

Patients

Twenty five patients were enrolled in the present study. The median age was 51 years (range 26 to 62 years). The patients either had acute myeloid leukemia with poor prognostic factors (relapsed, therapy-related, preceding myelodysplasia, or cytogenetic abnormalities), or had hematological malignancies refractory to chemotherapy, or relapsed after autologous stem cell transplantation (ASCT). Sixteen of the 25 patients had received 3 or more prior regimens; 13 had prior ASCT. Fourteen of the 25 patients had Hodgkin's/non-Hodgkin's lymphoma, 8 of the 25 had AML.

Four patients were enrolled initially at level 4 and had no severe adverse events. Two patients were treated at level 5 (Table 1). However, the first patient died of severe GVHD with aspergillosis and the second patient on level 5 died of sepsis with multi-organ failure. Neither death was attributed directly to any of the three chemotherapeutic agents. An analysis of the engraftment data showed satisfactory donor chimerism at level 4. Since the major goal of this phase is to find a safe combination regimen for successful allografting of donor stem cells, further dose escalation of pentostatin was abandoned. To ensure that donor chimerism was reproducible and toxicity was clearly acceptable at level 4, 3 additional patients were enrolled at level 4.

Engraftment and Chimerism

Eighteen patients received HLA-matched sibling grafts of G-CSF mobilized peripheral blood stem cells (PBSC), seven patients received unrelated grafts, three were PBSC and 4 bone marrow grafts. The median number of CD34+ cells was 5.98×10^6 kg^{-1}, the median number of CD3+ cells contained in the graft was 24.75×10^7 kg^{-1}. The median number of CD34+ and CD3+ cells in the unrelated grafts was much lower, as is expected from bone marrow grafts (Table 3). Five of the 25 patients received grafts from major ABO- mismatched donors. Volume reduction and red cell depletion were done for the bone marrow grafts with ABO mismatch.

GM-CSF was used after transplantation. Neutrophil recovery to ANC>500 mm^{-3} was reached at a median of 12 days (range 8-18 days), the median time to platelet count >20,000 mm^{-3} without transfusion at least for 72 h was 11 days (range 7-20 days). Median donor chimerism at the time of engraftment was 56% for related transplantations, with 3 patients achieving full donor chimerism (Table 4). One month after transplantation, donor chimerism continued to improve, with a median donor chimerism of 76%. The median donor chimerism reached 98% at 3 months. Three patients eventually had undetectable donor cells. One of them had rapid disease progression with plasma cell leukemia replacing the bone marrow. Two others lost the donor grafts despite donor leukocyte infusion. These patients all recovered autologous hematopoiesis. This confirms the non-myeloablative nature of the MAP conditioning regimen.

It was unclear at the beginning of the trial whether the immunosuppression should be increased or tapered rapidly to enhance donor chimerism. It is noteworthy that donor chimerism was increased with the rapid tapering of immunosuppression in majority of patients. However, immunosuppression needed to be increased (such as by adding or increasing doses of steroids) if donor chimerism decreased after initial tapering of immunosuppression.

For unrelated NST transplantation, the initial 2 patients rejected the donor graft rapidly and the third patient achieved full donor chimerism at two months post-transplantation after he received an infusion of donor leukocytes (Table 4). ATG was subsequently added to the pre-transplant

Table 2: Patient characteristics at non-myeloablative transplantation

ID	Sex/Age (year)	Disease	Dz Status	ASCT	#chemo	HLA
00801	F/26	HD	Refractory	yes	>3	6/6
00802	F/56	FML	Refractory	yes	>3	6/6
00803	M/39	DLCL	Refractory	yes	>3	6/6
00804	M/57	APL	CR2	no	2	6/6
00805	M/55	MCL	Refractory	yes	>3	6/6
00806	M/46	SLL(Richter's)	Refractory	no	>3	6/6
00807	M/41	DLCL	Refractory	yes	>3	6/6
00808	M/61	MDS/RAEBt	CR1	no	1	6/6
00809	M/36	tAML	CR3	no	3	6/6UD
00810	F/57	DML	Refractory	no	3	6/6
00811	F/41	DML	Refractory	yes	>3	5/6UD
00812	M/59	MM	Refractory	yesx2	>3	6/6
00813	M/56	CLL	Refractory	no	>3	6/6UD
00814	F/42	AML-M7	CR1	no	1	5/6UD
00815	F/59	tAML/HD	Refractory	yes x2	>3	6/6
00816	M/51	AML	CR1	no	1	6/6UD
00817	M/54	HD	Refractory	yes x2	>3	6/6
00818	M/35	HD	Refractory	yes x2	>3	6/6
00819	M/45	MCL	PR	no	1	6/6
00820	F/40	HD	Refractory	yes	>3	6/6
00821	M/53	AML	CR2	no	2	6/6UD
00822	M/53	MM	Refractory	yesx2	>3	6/6
00823	M/62	AML	CR1	no	1	6/6UD
00824	M/53	MCL	Refractory	yes	>3	6/6
00825	F/41	MCL	PR1	no	1	6/6

Dz: Disease; ASCT: Autologous Stem Cell Transplantation; HD: Hodgkin's Disease; FML: Follicular Mixed Small and Large Cell Lymphoma; DLCL: Diffuse Large B-cell Lymphoma; APL: Acute Promyelocytic Leukemia; MCL: Mantle Cell Lymphoma; SLL: Small Lymphocytic Lymphoma with Richter's Transformation; MDS: Myelodysplastic Syndrome; AML: Acute Myeloid Leukemia; DML: Diffuse Mixed Small and large cell lymphoma; MM: Multiple Myeloma; CLL; Chronic Lymophocytic Leukemia; UD: Unrelated Donor

Table 3: Donor and graft characteristics of non-myeloablated patients

	Sibling N = 18	Unrelated n = 7
PBSC/BMSC	18/0	3/4
CD34+ cells($x10^6$)*	5.98 (3.09-16.1)	2.1 (1.28-6.74)
CD3+ cells($x10^7$)*	24.75(10.46-52.05)	3.4 (1.99-23.5)
HLA Mismatch (5/6)	0	2
ABO Mismatch		
Major	3	2
Minor	1	0

* Median numbers

Table 4: Chimerism after non-myeloablative transplantation

	*At engraftment	1 month	3 months or later
Related (CSA day -1, MMF day 0) **			
	n = 10***	n = 16	n = 17
Full donor	3	7	11
Median	56% (7-97%)	76% (30-96%)	98% (0-100%)
Unrelated (CSA day -1, MMF day 0, two patients had ATGx4 days) **			
	n = 4***	n = 5	ND
Full donor	0	0	
Median	15% (10-25%)	0%(0-20%)	
Unrelated (CSA day -6, MMF day -6, ATG x 4 days) **			
	n = 2***	n = 2	n = 2
Donor	15%, 20%	70%, 95%	97%, 99%

*: When post-transplantation WBC>1000, **: Immunosuppression regimen used, ***: Number of patients who had chimerism done at the time point, Full donor: >=90%, Abbreviations: CSA: Cyclosporine; MMF: Mycophenolate Mofetil; ND: Not Done

conditioning to decrease the chance of graft rejection. However, graft failure still occurred in the next two patients who received bone marrow grafts. To further suppress graft rejection, cyclosporine and mycophenolate mofetil was started early on day -6. Two subsequent patients had good donor cell engraftment and became full donor chimerism at 3 months. It is noted that these latter two patients both received PBSC grafts.

Donor Leukocyte Infusions

Thirteen patients received Donor Leukocyte Infusions (DLI). In an attempt to minimize GVHD, the dose of CD3+ cells is started low and escalated to next dose level if there is no significant GVHD. A total of 34 DLI infusions were given. The median number of DLIs given was 2 (range 1 to 8). Five patients were given DLI for residual or progressive disease, 4 were given to increase donor chimerism and three were given for both reasons. Another was given for Kaposi's sarcoma and residual lymphoma. The patient who had Kaposi's sarcoma received one dose of DLI at 5×10^{6} kg^{-1} CD3+ cells and developed severe GVHD. The Kaposi's sarcoma lesions improved rapidly and significantly. The response correlated with the development of GVHD. To our knowledge, this is the first observation that Kaposi's sarcoma responds to GVM effect. Unfortunately, the patient later died of GVHD-related complications.

Among the 5 patients who had DLI to treat their malignancy, 4 had evidence of disease response. One of the four was rendered a complete resolution of residual lymphoma without evidence of GVHD after two doses of DLIs. Another one with mantle cell lymphoma entered unconfirmed complete response (CR) 16 months later. The third one with mantle cell lymphoma developed GVHD and had CR after three DLIs, but the GVHD resolved and the lymphoma relapsed a year later. Further DLI was given but there was no GVHD and the DLI only resulted in minimal response. Interestingly, this patient had pancytopenia due to multiple chemotherapy regimens and was not able to receive further therapy prior to mini-allogeneic transplantation. With normal hematopoiesis after allogeneic graft, this patient is able to receive further salvage chemotherapy and radiation therapy. The fourth had mild GVHD and transient response after one dose of DLI, but further DLIs did not result in GVHD. The patient eventually died of disease progression. The fifth patient had no GVHD after three doses of DLIs and there was no disease response. Therefore, GVHD is strongly correlated with disease response, though lack of it does not exclude the response.

Toxicity and Mortality

In this group of high-risk and /or heavily pretreated patients, the non-ablative conditioning regimen was well tolerated. Even at level 5 (the highest dose level reached), significant neurotoxicity and nephrotoxicity related to the MAP regimen were not observed. Major toxicities observed are summarized in Table 5.

Table 5: Major transplant-related toxicities after non-myeloablative transplantation

GVHD	
Acute	40% (10/25)
Chronic	28% (7/25)
Active Chronic*	12% (3/25)
GVHD post-DLI	46% (6/13)
TRM (day 100)	8% (2/25)
Liver(transient ALT elevation)	8%
Engraftment Syndrome	24%
CMV Antigenemia	32% (one with colitis)
Kaposi's Sarcoma	4% (1/25)

*: Chronic GVHD requiring immunosuppression

Transplantation-related Mortality

Two patients (8%) died without relapse within day 100, one of them died of sepsis with multi-organ failure at day +12. The other died of GVHD- related complications after unrelated marrow transplantation. Two patients were treated at level 5. The first patient died of severe GVHD and aspergillosis after unrelated PBSC transplantation at day +126. The second died of sepsis at day +12 as described above. Since satisfactory donor chimerism was achieved at level 4, further recruitment to level 5 was abandoned. Therefore, true maximal tolerated dose of pentostatin in the MAP regimen was unknown. 3 additional patients were recruited to level 4, including one at age 62 who received unrelated PBSC grafts. All 3 tolerated the regimen well. Therefore, level 4 was chosen as the Phase II regimen.

GVHD

Ten patients developed acute GVHD grade II or higher after transplantation, three of them were severe (Grade III-IV), seven evolved into chronic GVHD. Among the 7 with chronic GVHD, 1 died of relapse, 1 died of Aspergillosis, 2 had complete resolution and went off immunosuppression and 3 have limited GVHD mainly involving the liver. Six additional patients developed GVHD after donor leukocyte infusions. Three of them died of GVHD-related complications, the other 3 all recovered and are off immunosuppression. Therefore, GVHD after the mini-allograft still remains as one of the common major complications. It is nonetheless worthwhile to note that among the 16 patients who had GVHD history, 10 are alive. Among them, 8 are alive without disease, another one (00824) is currently in unconfirmed CR. Only 3 remain on immunosuppressive therapy for chronic GVHD of the liver.

Engraftment Syndrome

Six patients had a clinical syndrome around the time of engraftment, presenting with two or more of the findings of hypotension, fever, skin rash, interstitial pneumonitis and /or peripheral edema. Broad-spectrum antibiotics were used for all patients and vasopressors for 2 of them with limited effects. Methylprednisolone (1 mg kg^{-1} every 12 h) for two or three days was consistently successful in alleviating this clinical syndrome.

Transaminitis

Two patients had transient ALT /AST elevation during conditioning chemotherapy. The first patient was at level 1, the second was at level 3. The enzymes rapidly decreased to normal level following completion of chemotherapy. Both patients had history of the liver enzyme elevation with previous chemotherapy for their diseases.

Opportunistic Infections

CMV reactivation was detected in 8 patients. One of the patients also had CMV colitis. All the rest had only CMV antigenemia and never developed CMV disease. The CMV reactivation was seen in the early phase of the trial. Valgancyclovir is subsequently used for CMV prophylaxis in either patients or donors with pre-transplant history of CMV exposure (CMV IgG +, IgM -). It is started as soon as WBC is over 1500. CMV reactivation has not been seen since then. One patient had bilateral lower extremity skin lesions, pathology of which was reported to be Kaposi's sarcoma. Another

patient received additional treatment with Daclizumab (Zenapex, manufactured by Hoffman-La Roche) for severe GVHD. This patient later developed multiple brain lesions consistent and died of CNS aspergillosis.

Disease Response and Survival

With a median follow-up of 28 months (range 12 to 1122 days), 10 patients are alive, 8 of which are disease free with a Karnofsky performance status of 90-100%. Three of the 10 patients remain on cyclosporine, one of the three had unrelated graft. The remaining two patients with active disease had relapsed after prior ASCT. One of them continues to have disease improvement after DLI and is in unconfirmed CR. The median duration of overall survival and disease-free survival are 15 months (12-1122 days) and 12 months (86-1122 days), respectively (Fig. 1). The estimated overall and disease-free survival rates at 1 year is 48% (SE = 10) and 50% (SE = 10), respectively; and those at two years are 40% (SE = 10) and 37% (SE = 10), respectively.

In this patient population with high-risk diseases, 15 have died. Among them 10 died of rapid disease progression, 4 died of severe GVHD-related complications (2 also had disease), one died of sepsis with multi-organ failure.

Among the 25 patients transplanted, 14 had NHL or HD, ten of the 14 (71%) had failed prior ASCT. Seven of the 14 (50%) are alive and 5 (36%) are disease- free. The median duration of overall survival of the 14 patients has not been reached (Fig. 2). The estimated rates of overall survival of the 14 patients are 64% (SE = 13) at 1 year and 50% (SE = 13) at two years. It appears therefore that lymphoma patients, even heavily pre-treated, still were able to tolerate the procedure and perform exceptionally well.

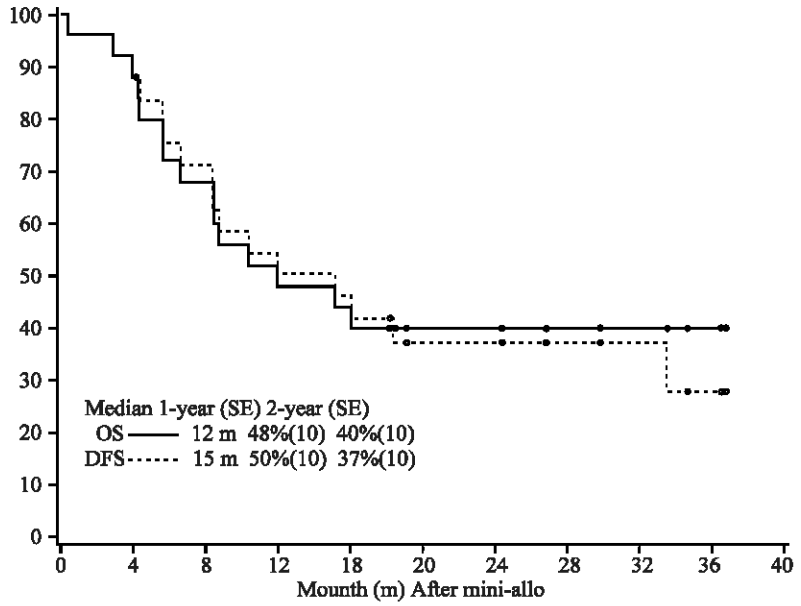

Fig. 1: Kaplan-Meyer Survival Curve after Mini-allografts. The estimated 1 year and 2 year overall (OS) and Disease-free Survival (DFS) are shown in percentage. Median: Median duration, SE: Standard Error, M: Month

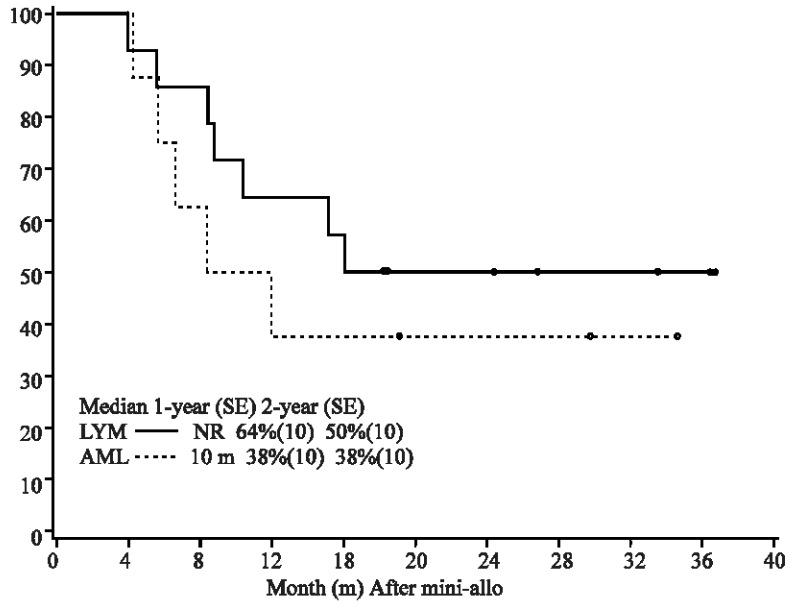

Fig. 2: Kaplan-Meyer Survival Curve for Lymphoma (Lym) and AML Patients after Mini-allografts. The estimated 1 year and 2 year overall survival rates are shown in percentage. Median: Median duration, SE: Standard Error, M: Month, NR: Not Reached

Eight of the 25 patients had AML. Two of them were therapy-related, one evolved from MDS with complex cytogenetic abnormalities including -5 and -7. Six of the 8 patients were in first CR, 1 in 2nd CR, another one in 3rd CR. Five of the 8 patients received unrelated grafts, three of them failed to engraft. The median duration of overall survival is 10 months (Fig. 2). The estimated rates of overall survival of the 8 patients are 38% (SE = 17) at both 1 year and two years. Three patients (38%) who had NST in 1st CR are alive and disease-free with follow-up of 583, 911 and 1058 days, respectively. Therefore, NST for AML in first CR is a viable option. Due to the limited number of patients, there was no statistical significance ($p = 0.42$) of the overall survival difference between patients with lymphoma and AML (Fig. 2).

Discussion

This phase I study shows that pentostatin at 13 mg m^{-2} (level 4) over three days in the MAP regimen is truely non-myeloablative and well tolerated, even in patients who have had heavy prior therapy and who have high-risk diseases. Although true MTD of MAP regimen is not reached, level 4 is safe and effective for establishing complete donor chimerism in the related transplantation. Since the MAP regimen is developed primarily for non-myeloablative allografting of hematopoietic stem cells, level 4 has therefore been chosen as Phase II regimen. With the addition of ATG and early start of cyclosporine and mycophenolate mofetil, four patients who received unrelated PBSC grafts and one patient who received one-locus mismatched related PBSC graft achieved full donor chimerism, but two who received unrelated bone marrow grafts had primary graft failure (unpublished observation).

Therefore, PBSC grafts are exclusively used for further MAP regimen –based NST from unrelated donors and mismatched donors.

Median dose of CD34+ cells in related transplants are 5.98 x 10^6 kg^{-1}. Although optimal dose is unknown, it appears that this dose level leads to rapid neutrophil (median 12 days) and platelets (median 11 days) engraftment. Donor chimerism was noted to be incremental over time, full donor chimerism was achieved around day 100. Patients who have had multiple chemotherapy regimens or had prior autologous transplantation rapidly became full donor chimerism. Those patients who had relatively fewer chemotherapy regimens (00808 and 00825) had secondary graft failure after initiation of tapering of immunosuppression. Therefore, close monitoring of chimerism and careful adjustment of immunosuppression medications according to donor chimerism are crucial to avoid graft failure in those subjects who have mixed donor chimerism. Immune status before and after transplantation is being studied to better delineate the factors important for donor cell engraftment.

GVHD remains the most significant complication of MAP-conditioned allografting. The rate of any form of GVHD is high (64%), though most of the acute GVHD was mild and resolved quickly on immunosuppression. Severe GVHD rate was only 24% and persistent chronic GVHD was 12%. It is noteworthy that the persistent chronic GVHD were all limited to the liver with isolated ALT elevation. GVHD is a good indicator for clinical response. Those patients who had transient GVHD showed disease progression after the resolution of the transient GVHD. This strongly suggests graft-vs-malignancy effect. However, transient GVHD may be beneficial in those patients who are in CR or have only minimal residual disease, since 3 patients (00803, 00814 and 00825) had transient GVHD, 2 of them had graft failure, yet all three remain disease-free. Limited chronic GVHD are correlated with slow but continuous clinical response. Patients 00802 and 00824 had limited cGVHD, their residual lymphoma finally entered CR at 24 and 16 months, respectively, after mini-transplantation without any further intervention.

The incidence and severity of GVHD post-DLI were not dose related. 3 of the 5 patients who had GVHD post-DLI infusion took place after first dose, 2 of the 3 severe GVHD had only one (lowest) dose of DLI. However, due to the limited number of patients who received DLI, it is not yet clear whether higher dose of DLI is correlated with better response. Among the 6 patients who had mixed donor chimerism and received DLIs, only 2 converted to full donor, 2 remained mixed and 2 lost donor cells. The two patients who were converted to full donor chimerism died of severe GVHD. Therefore, DLI alone does not seem to be particularly effective in converting mixed chimerism to full donor and may lead to severe GVHD.

The MAP regimen is an active combination toward lymphoma and leukemia. It seems that MAP regimen as NST conditioning regimen is particularly suited for lymphoma and for AML in first remission. Further phase II study focusing on these diseases are warranted. Autologous stem cell transplantation followed by MAP mini-transplantation is also a possible venue for further clinical investigation.

Acknowledgement

We thank Monica Fradkin, Judy Levy-Battaglia, Elsa Ferreira, Linda Major, Daniyal Ahmad Siddiqui for their important study contributions. We also thank the Oncology Fellows, Oncology Nursing Staff and the Hematotherapy Staff for their dedicated patient care. This study is supported in part by a grant from Supergen, Inc.

References

Arlin, Z., D.C. Case and J. Moore, 1990. Randomized multicenter trial of cytosine arabinoside with mitoxantrone or daunorubicin in previously untreated adult patients with acute nonlymphocytic leukemia (ANLL). Leukemia, 4: 177-183

Badros, A., B. Barlogie, E. Siegel, M. Cottler-Fox, M. Zangari and A. Fassas *et al.*, 2002. Improved outcome of allogeneic transplantation in high-risk multiple myeloma patients after nonmyeloablative conditioning. J. Clin. Oncol., 20: 1295-1303

Carson, D.A., J. Kaye and L.E. Seegmiller, 1977. Lymphospecific toxicity in adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency: Possible role of nucleoside kinases. Proc. Natl. Acad. Sci. USA, 74: 5677-5681

Carella, A.M., M. Cavaliere, E. Lerma, R. Ferrara, K Tedeschi and A. Roma *et al.*, 2000. Autografting followed by immunosuppressive chemotherapy and allogeneic peripheral blood hematopoietic stem cell transplantation as treatment of Hodgkin's disease and Non-Hodgkin's lymphoma. J. Clin. Oncol., 18: 3918-3924

Chan, G.W., G. Gorgun, K.B. Miller and F. Foss, 2003. Persistence of host dendritic cells after transplantation is associated with graft-versus-host disease. Biol. Blood Marrow Transplant., 9: 170-176

Childs, R., A. Chernoff, N. Contentin, E. Bahceci, D. Schrump and S. Leitman *et al.*, 2000. Regression of metastatic renal cell carcinoma after nonmyeloablative allogeneic peripheral blood stem cell transplantation. New England J. Med., 343: 750-758

Donofrio, J. and M.S. Coleman, J.J. Hutton, 1978. Overproduction of adenine deoxynucleosides and deoxynucleotides in adenosine deaminase deficiency with severe combined immunodeficiency disease. J. Clin. Invest., 62: 884

Fefer, A., 1999. Graft-versus-tumor Responses. In: E.D., Thomas, K. Blume and S. Forman (Eds.), Hematopoietic Cell Transplantation. Malden, Ma. Blackwell Sciences, pp: 316-326

Feldman, E.J., K. Seiter and F. Traganos, 2000. Phase II evaluation of a high-dose mitoxantrone based induction regimen in untreated adults with acute myeloid leukemia. Leuk. Lymphoma, 38: 309-315

Giralt, S., E. Estey, M. Albitar, K. van Besien, G. Rondón and P. Anderlini *et al.*, 1997. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. Blood, 89: 4531-4536

Grever, M.R., K. Kopecky, M.K. Foucar, D. Head, J.M. Bennett and R.E. Hutchison *et al.*, 1995. Randomized comparison of pentostatin versus interferon alpha-2a in previously untreated patients with hairy cell leukemia: An intergroup study. J. Clinl. Oncol., 13: 974-982

Grever, M.R., M.F. Siaw and W.F. Jacob, 1981. The biochemical and clinical consequences of 2'-deoxycoformycin in refractory lymphoproliferative malignancy. Blood, 57: 406-417

Grever, M.R., J.M. Leiby and E.H. Kraut, 1985. Low dose deoxycoformycin in lymphoid malignancy. J. Clin. Oncol., 3: 1196-1201

Horowitz, M.M., R.P. Gale,, P.M. Sondel, J.M. Goldman, J. Kersey and H.J. Kolb *et al.*, 1990. Graft-versus-leukemia reactions after bone marrow transplantation. Blood, 75: 555-562

Khouri, I.F., M. Keating, M. Korbling, D. Przepiorka, P. Anderlini, and S.O. 'Brien *et al.*, 1998. Transplant-lite: Induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor cell transplantation as treatment for lymphoid malignancies. J. Clin. Oncol., 16: 2817-2824

Kottaridis, P.D., D.W. Milligan, R. Chopra, K. Chakraverty, S. Chakrabarti and S. Robinson *et al*., 2000. *In vivo* CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. Blood, 96: 2419-2425

Major, P.P., R.P. Agarwal and D.W. Kufe, 1981. Clinical pharmacology of deoxycoformycin. Blood, 58: 91-96

Maris, M.B. and R. Storb, 2003. The transplantation of hematopoietic stem cells after non-myeloablative conditioning: A cellular therapeutic approach to hematologic and genetic diseases. Immunol. Res., 28: 13-24.

Mitchell, B.S., N.L. Ewards and C.A. Koller, 1983. Deoxyribonucleoside triphosphate accumulation by leukemic cells. Blood, 62: 419-424

Milojkovic, D. and G.J. Mufti, 2001. Extending the role of allogeneic stem cell transplantation. Lancet, 357: 652-654.

Seymour, J.F., M. Talpaz and R. Kurzrock, 1997. Response duration and recovery of CD4+ lymphocytes following deoxycoformycin in interferon-alpha-resistant hairy cell leukemia: 7-year follow-up. Leukemia, 11: 42-47

Slavin, S., A. Nagler, E. Naparstek, Y. Kapelushnik, Y. Aker and G. Cividalli *et al*., 1998. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood, 91: 756-763

Truitt, R., A. LeFever and C-Y. Shih, 1987. Graft-versus-leukemia Reactions: Experimental Models and Clinical Trials. In: R.P., Gale and R Champlin (Eds.): Progress in Bone Marrow Transplantation. New York, Ny. Alan R. Liss, pp: 219-232.

Taussig, D.C., A.J. Davies, J.D. Cavenagh, H. Oakervee, D. Syndercombe-Court and S. Kelsey *et al*., 2003. Durable remissions of myelodysplastic syndrome and acute myeloid leukemia after reduced-intensity allografting. J. Clin. Oncol., 21: 3060-3065.