

SPOTLIGHT

Stem cell transplantation with reduced-intensity conditioning regimens: a review of ten years experience with new transplant concepts and new therapeutic agents

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The realization in the 1990s that allogeneic stem cell transplants (SCT) have a potentially curative graft-versus-leukemia (GVL) effect in addition to the antileukemic action of myeloablative conditioning regimens was a major stimulus for the development of reduced-intensity conditioning (RIC) regimens, aimed primarily at securing engraftment to provide the GVL effect, while minimizing regimen-related toxicity. It is now over 10 years since RIC regimens were heralded as a new direction in the field of SCT. Over the last decade much has been learned about the ways in which the conditioning regimen can be tailored to provide adequate immunosuppression, and modulated to deliver a chosen degree of antimalignant treatment. The huge literature of clinical data with RIC transplantation now permits us to more clearly define the success and limitations of the approach. This review examines the origins of RIC SCT, explores the degree to which the initial expectations and purpose of the approach have been realized, and outlines some ways forward for the field.

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Introduction

When clinical bone marrow transplantations (BMT) for leukemia was introduced at the end of the 1960s, pretransplant conditioning regimens were used to deliver myeloablative antileukemic treatment with the immune suppression required to establish engraftment. The cyclophosphamide and total body irradiation (TBI) schedule pioneered by Thomas *et al.*¹ was a reliable way to achieve these objectives but did not prevent leukemic relapse.² Further intensification of conditioning was limited by a concomitant increase in regimen-related mortality (RRM). However, it became clear that allogeneic BMT also had a graft-versus-leukemia (GVL) effect, clearly identifiable in large patient cohorts and related to the infusion of allogeneic lymphocytes.³ Furthermore, the GVL effect of donor lymphocyte infusion (DLI) was sufficient to eliminate relapsed leukemia following BMT.⁴

Origin of reduced-intensity stem cell transplants

Given the power of the GVL effect, it became relevant to determine whether reduced-intensity conditioning (RIC) regi-

mens could be used to extend transplantation to elderly or debilitated patients, not normally considered for stem cell transplants (SCT) because of high RRM from a conventional BMT.^{5,6} It was argued that these safer transplants might be performed in the outpatient setting, with cost savings, and justify experimental transplantation seeking a graft-versus-tumor (GVT) effect in patients with metastatic solid tumors.^{7–9} An advantage of RIC transplantation was the possibility of modulating immune recovery by achieving a mixed chimeric state. Experimental transplantation in mice and pigs by Sachs and Sykes in Boston, and in dogs by Storb in Seattle indicated that reduced conditioning and post-transplant immunosuppression resulted in stable mixed chimerism without rejection or GVHD. Furthermore, full donor chimerism could be later restored by a DLI, to provide GVL reactivity.^{10–15} Such experimental models set the stage for RIC transplants in older or debilitated patients with hematological malignancies in Seattle,^{13,16–18} Boston¹⁹ and Jerusalem.^{14,20,21} A parallel strategy, introduced by Carella was a tandem autologous-allogeneic transplantation, where the patient's disease was debulked by a myeloablative stem cell autograft, followed after recovery by a RIC transplant to provide the graft-versus-malignancy effect.^{22,23} Thus, in the mid-1990s, transplant groups in the United States and Europe simultaneously introduced RIC regimens in HLA identical related and unrelated donor transplants for older or debilitated patients with hematological malignancies.^{24–27} In the last decade, the RIC approach has been widely adopted by transplant centers worldwide and over 10 000 RIC transplants, many in older individuals, have been reported. This review summarizes the results of RIC transplants in malignant diseases, examines how much the initial ideas and expectations of RIC transplants have been borne out, and defines limitations and future prospects for RIC transplantation.

Overview of RIC regimens

Published data on 39 reduced-intensity regimens used in over 2000 SCT are shown in Table 1. Of these, 35 (90%) use fludarabine (Flu) (125–240 mg/m²) as part of the conditioning. However, there is great diversity in the nature of the other agents used, and a spectrum of dose intensity from myeloablative regimens to those that are only immunosuppressive. Despite some overlap, regimens can be conveniently grouped into those that are relatively intensive, which employ either busulfan (Bu) at doses up to 10 mg/kg, melphalan (Mel) at doses up to 180 mg/m², or (iii) TBI at doses up to 8 Gy. Thiopeta in doses up to 10 mg/kg has also been included in some of these regimens.⁶³ The least intensive group of regimens employ cyclophosphamide (Cy) or thymic irradiation with various antibody combinations with or without Flu. Low-intensity RIC regimens carry an increased risk of graft failure – for example,

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Table 1 RIC regimens used in the last decade

Ref	Conditioning regimen	Drug dose and schedule (\pm ATG/antibodies)		TBI dose
		Flu (mg/m^2)	Alkylating agent	
28–37	Flu Bu	120–240	Bu 3.2–6.6 mg/kg i.v. Bu 8–10 mg p.o.	—
30,38–45	Flu Mel	90–180	Mel 70–180 mg/m^2	—
18,46–53	Flu TBI	90–120	+Cy 80–120 mg/kg	2–8 Gy
54–56	Cy TBI	—	80–120 mg/kg	4–5.5 Gy
40,57–62	Flu Cy	90–125	Cy 2–3.6 g/m^2 OR Cy 120–200 mg/kg	—
63	Flu Cy Th	60	Cy 60 mg/kg Th 10 mg/kg	—
64	Thymic radiation+Cy	—	Cy 150–200 mg/kg	—

Ref, reference number; ATG, antithymocyte globulin; TBI, total body irradiation; Flu, Fludarabine; Bu, busulfan, Mel, melphalan, Cy, cyclophosphamide; Th, thiotepa.

up to 17% of patients given low-dose TBI rejected the transplant,¹⁸ but the incidence was decreased to less than 3% with the addition of Flu.⁶⁵ Rejection rates for Flu-based grafts are generally less than 3%, suggesting that Flu given at doses between 125 and 150 mg/m^2 plays a central role in the establishment of engraftment in RIC transplantation. Flu, as the immunosuppressive component of the conditioning, has been successfully combined with wide variations in dose and type of partner agents (TBI and alkylating agents being the most popular) to create regimens of different intensity selected for particular indications. Of the alkylating agents, Cy has the most immunosuppressive action, making it a good partner with Flu for intensive immunoablation. Busulfan has emerged as a well-tolerated agent, especially by the intravenous route where its bioavailability is better controlled.^{29,34,35,66}

Engraftment dynamics

The need to understand engraftment with RIC regimens led to the development of lineage-specific chimerism assays, which have provided insights into the factors that separately regulate lymphoid and myeloid recovery. Using minisatellite probes to define donor and recipient cells sorted by T (CD3), myeloid (CD13/15), B (CD19) and NK lineages (CD56/16) Childs *et al.*⁶⁷ reported a consistent pattern of T-cell engraftment preceding myeloid engraftment. Donor B cell recovery was concurrent with donor myeloid recovery while grafted NK cells had an intermediate recovery pattern between T-cell and myeloid. Graft-versus-host disease (GVHD) and associated GVL effects only occurred after the establishment of full donor T-cell chimerism. The loss of residual host myeloid cells and B cells was also attributable to a graft-versus-marrow and graft-versus-B cell effect, respectively, revealing a role for donor T cells in myeloablation. This pattern of recovery established the Flu/Cy regimen as being highly immunoablative but not myeloablative, since the hematological recovery following transplant was initially recipient derived. In contrast, the Seattle group, using a low-dose TBI regimen reported the early establishment of donor myeloid engraftment, followed later by T-cell engraftment, demonstrating that the regimen was more myelosuppressive than immunosuppressive.^{18,53}

The choice of regimen may also affect immune reconstitution more subtly – Sykes and Spitzer present data suggesting that TLI with thymic irradiation provides tolerance through a mechanism not well understood.¹⁵ Recent promising results using very low-dose TLI regimens attribute the low incidence of GVHD in

transplants from donors other than HLA identical siblings to the persistence of host NKT cells with strong tolerizing properties.^{68–70}

Regimen-related toxicity

The dramatic reduction in the early post-transplant regimen-related toxicity from RIC transplants is undisputed. Mucositis and veno-occlusive disease are almost nonexistent after RIC transplants and early mortality is much lower than that observed after standard intensity SCT. 100-day mortality rates are also low, but more patients may die in the subsequent 100 days, mainly from GVHD (Tables 2–5), making the 200 day mortality a more reliable estimate of treatment failure. Nevertheless, older transplant recipients have greater co-morbidities and with increased age and disability more patients die from co-morbidities not directly associated with their underlying malignancy. The move to transplants in patients in their 6th 7th and even 8th decade has prompted transplanters to develop better ways to evaluate co-morbidity and determine the interaction of recipient frailty with RIC transplant outcome. In Seattle the Charleston comorbidity score applied to RIC transplant recipients has identified a direct relationship between score and outcome, which could help, in addition to the age factor, in improving outcome prediction for the individual.^{5,6,71}

GVHD

Acute and chronic GVHD remain the major limitations to the success of RIC transplants in older people. It was hoped that the reduced mucositis induced by the RIC regimen might translate into a reduced severity of GVHD, because of a reduction in the cytokine storm, believed to be initiated by gut-derived endotoxin leaking through damaged mucosa.^{72–75} With this assumption, many investigators using RIC regimens gave only modest immunosuppression post-transplant – for example, single agent cyclosporine⁷⁶ or cyclosporine plus mycophenolate mofetil.¹⁸ In the absence of comparative studies with standard regimens given similar post-graft immunosuppression, it is not clear that RIC transplants reduce the risk of GVHD. Recipient age is a further confounding factor: increasing age is associated with greater incidence and severity of GVHD.⁷⁷ There are many possible explanations for this – elderly recipients have less ability for tissue repair, they receive transplants from older donors who may have reduced numbers of regulatory T cells

Table 2 Busulfan-based RIC regimens

Ref	Year	Regimen	No.	Age range (median)	Disease	Donor type	GVHD prophylaxis	aGVHD % (Grade)	cGVHD (%)	NRM (%)	Outcome (%)
28	2006	Flu 180 mg/m ² Bu 8 mg/kg p.o., 6.4 mg/kg i.v. ± TBI (4 Gy for MUD)	23	42–67 (56)	AML MDS	MRD MUD	CSA MMF	48 (II–IV)	47.8	34.7	1 yr OS-48
29	2006	Flu 150–160 mg/m ² Bu 6.4 mg/kg ± ATG (MUD, mismatched)	41	18–70 (57)	AML MDS	MRD MUD	CSA MTX	8 (III, IV)	31	8	2 yr OS-47 2 yr DFS-43
30 ^a	2005	Flu 150 mg/m ² Bu 8 mg/kg Campath	38	12–66 (51)	Hem malignancies	MRD MUD Mismatch	CSA ± MTX	27 (I–IV)	62	17 (100 d); 33 (1 yr)	3 yr OS-43
31	2005	Flu 180 mg/m ² Bu 8 mg/kg ATG	33	26–60 (52)	AML	MRD	CSA MMF/MTX	24 (II–IV)	64	9	2 yr OS-79 2 yr DFS-76
32	2005	Flu 120 mg/m ² Bu 3.2 mg/kg	71	51–70 (58)	Hem malignancies	MRD MUD	CSA, steroid OR Tacrolimus, MTX	28 (II–IV); 20 (III–IV)	NA	6 (100 d)	32 1 yr OS-51 2 yr OS-39 1 yr PFS-40 2 yr PFS-27 1 yr OS-74 1 yr DFS-62
33	2004	Flu 150 mg/m ² Bu 8 mg/kg Campath	62	41–70 (56)	AML MDS	MRD MUD	CSA	0 (III, IV)-MRD 9 (III, IV)-MUD	NA	7 (100 d); 15 (1 yr)	1 yr OS-74 1 yr DFS-62
34	2003	Flu 120–180 mg/m ² Bu 8 mg/kg p.o., 6.4 mg/kg IV ATG	37	23–72 (55)	AML MDS	MRD MUD	CSA MTX	37 (II–IV) 17 (III–IV)	48	27	MRD: 3 yr OS-45 3 yr DFS-51 MUD: 3 yr OS-31 3 yr DFS-25 5 yr DFS-85
35	2003	Flu 180 mg/m ² Bu 8 mg/kg Campath	24	3–63 (35)	CML – 1st chronic phase	MRD MUD	CSA ± MTX	75 (I–IV)	55	12.5	
36	2002	Flu 150 mg/m ² Bu 10 mg/kg	37	22–66 (57)	AML MDS	MRD	CSA MTX	38 (I–IV)	1 yr – 43 (Ext)	5 (1 yr)	1 yr PFS-66
37	2001	Flu 125–240 mg/m ² Bu 6.6–12 mg/kg ± ARA-C	44	25–65 (52)	CML	MRD MUD	CSA ± MMF ± MTX ± steroid	61 (I–IV) 18 (I) 30 (II) 11 (III) 2 (IV)	NA	34	DFS-41 (median follow-up 562 d)

Ref, reference number; AML, acute myeloid leukemia; ATG, antithymocyte globulin; Bu, Busulfan; CSA, cyclosporine; d, days; DFS, disease-free survival; Flu, fludarabine; MDS, myelodysplastic syndrome; MMF, mycophenolate; MRD, matched related donor; MTX, methotrexate; MUD, matched unrelated donor; NA, not available; No, number of patients in the study; NRM, nonrelapse mortality; OS, overall survival; PFS, progression free survival; prophyl, GVHD prophylaxis; TBI, total body irradiation; yr, year.

^aIncludes patients received other types of conditioning regimens.

Table 3 Melphalan-based RIC regimens

Ref	Year	Regimen	No.	Age range (median)	Disease	Donor type	GVHD prophylaxis	aGVHD % (Grade)	cGVHD (%)	NRM (%)	Outcome (%)
38	2006	Flu 125–150 mg/m ² Mel 100–140 mg/m ²	56	19–65 (51)	Hem malignancies	MRD	CSA ± MTX	CSA 52 (I–IV) CSA+MTX 20 (I–IV)	CSA 64 CSA+MTX 26	16 (100 d) 24 (1 yr)	1 yr OS-70 1 yr PFS-63
39	2006	Flu 150 mg/m ² Mel 140 mg/m ² Campath	41	37–64 (54)	CLL	MRD MUD Mismatch	CSA	41 (I–IV)	33	5 (100 d) 26 (2 yr)	2 yr OS-51 2 yr PFS-45
30 ^a	2005	Flu 150 mg/m ² Mel 140 mg/m ² (FMC; n = 8) OR Flu 90 mg/m ² +BEAM (n = 9)+Campath	89	12–66 (51)	Hem malignancies	MUD Mismatch MRD	CSA ± MTX	F-BEAM 14 (I–IV) FMC 52 (I–IV)	62	17 (100 d) 33 (1 yr)	3 yr OS-43
40 ^a	2005	Flu 125 mg/m ² Mel 140–180 mg/m ²	26	26–59 (31)	HD	MRD MUD	Tacrolimus MTX	38 (II–IV) 10 (III–IV)	69	5 (100 d) 18 (18 mo)	18 mo OS-73 18 mo PFS-37
41	2004	Flu 90–180 mg/m ² Mel 70–160 mg/m ² ± ATG	120	31–65 (52)	MM	MRD MUD Mismatch (n = 4)	CSA MTX	46 (II–IV)	47 (Lim-25) (Ext-22)	18 (1 yr)	2 yr OS-59 2 yr EFS-39
42	2004	Flu 150 mg/m ² Mel 140 mg/m ² Campath	88	18–73 (48)	NHL	MRD MUD	CSA	15 (II–IV)	7	11–38 (3 yr)	3 yr OS-55 (low grade NHL)
43	2004	Flu 100–150 mg/m ² Mel 140–180 mg/m ²	62	22–75 (54)	AML MDS	MRD MUD Mismatch	Tacrolimus OR CSA+MTX	MRD 20 (II–IV) 8 (III–IV) All 39 (II–IV) 19 (III–IV) 49 (II–IV)	39	26 (100 d)	3 yr OS-34 3 yr PFS-32
44	2001	Flu 125 mg/m ² OR (cladribine 12 mg/ m ² × 5 days, n = 8) Mel 140–180 mg/m ²	86	22–70 (52)	Hem malignancies	MRD MUD Mismatch	Tacrolimus MTX	49 (II–IV)	68	37 (100 d)	2 yr OS-28 2 yr DFS-23
45	2000	Flu 150 mg/m ² Mel 140 mg/m ² Campath	44	18–56 (41)	Hem malignancies	MRD MUD	CSA ± MTX	10 (I–II) 0 (III–IV)	NA	11 (1 yr)	1 yr OS-73

Ref, reference number; AML, acute myeloid leukemia; ATG, antithymocyte globulin; CSA, cyclosporine; d, days; DFS, disease-free survival; Flu, fludarabine; MDS, myelodysplastic syndrome; Mel, melphalan; MMF, mycophenolate; MRD, matched related donor; MTX, methotrexate; MUD, matched unrelated donor; NA, not available; No, number of patients in the study; NR, nonrelapse mortality; OS, overall survival; PFS, progression free survival; prophyl, GVHD prophylaxis; TBI, total body irradiation; yr, year.

^aIncludes patients received other types of conditioning regimens.

Table 4 Low-dose TBI-based RIC regimes

Ref	Year	Regimen	No	Age range (median)	Disease	Donor type	GVHD prophylaxis	aGVHD % (Grade)	cGVHD (%)	NRM (%)	Outcome (%)
46	2006	TBI 2 Gy Flu 90 mg/m ²	32	36–68 (57)	Hem malignancies	MRD	Tacrolimus MMF	16 (II–IV) 3 (III–IV)	42 (Ext)	16	OS-63 PFS-50 DFS-40 (median follow-up 19 mo)
54	2005	TBI 5.5 Gy Cy 120 mg/kg	110	19–62 (44)	Hem malignancies	MUD	CSA MTX Steroid ± HCQ	30 (II–IV) 16 (III–IV)	59 (Ext) 11 (Limt)	19 (100 d) 30 (1 yr)	Good risk OS-47 PFS-40 Poor risk OS-25 DFS-21 (median follow-up 28 mo)
47	2005	TBI 2 Gy ± Flu 90 mg/m ²	64	44–69 (56)	CLL	MRD MUD	CSA MMF	55 (II–IV) 16 (III–IV)	50 (Ext)	22 (2 yr)	2 yr OS-60 2 yr RFS-52
48	2005	TBI 8 Gy Flu 120 mg/m ² ± ATG	71	20–66 (51)	AML	MRD MUD	CSA ± MTX	36 (II–IV) 17 (III–IV)	24 (Ext) 22 (Limt)	<i>Tx in CR</i> 3 (100 d) 8 (2 yr) <i>Tx not in CR</i> 20 (100 d) 37 (2 yr)	<i>Tx in CR</i> 2 yr OS-81 2 yr RFS-78 <i>Tx not in CR</i> 2 yr OS-21 2 yr RFS-16
55	2005	TBI 4 Gy Cy 80–120 mg/m ² ATG	75	19–66 (52)	AML MDS	MRD MUD Mismatch	CSA MMF	61 (I–IV) 24 (III–IV)	45 19 (Ext) 26 (Limt)	20 (100 d) 33 (1 yr)	2 yr OS-42 2 yr LFS-40
49	2004	TBI 2 Gy Flu 90 mg/m ²	33	33–70 (54)	MCL	MRD MUD	CSA MMF	57 (II–IV) 30 (III–IV)	64 (Ext)	24 (2 yr)	2 yr OS-65 2 yr DFS-60
50	2004	TBI 8 Gy Cy 80–120 mg/kg Flu 120 mg/m ² ATG	35	45–62 (51)	CML	MRD MUD	CSA MTX	48 (II–III) 0 (IV)	23 (Ext)	11 (100 d) 29 (1 yr)	OS-63 LFS-49 (median follow-up 30 mo)
51	2003	TBI 2 Gy Flu 90 mg/m ²	54	29–71 (52)	MM	MRD	CSA MMF	39 (II–IV)	64 46 (Ext)	2 (100 d) 15	OS-78 (median follow-up 18 mo)
52	2003	TBI 2 Gy Flu 90 mg/m ²	52	6–65 (48)	Hem diseases	MUD Mismatch	CSA MMF	63 (II–IV) 21 (III–IV)	30 (requiring treatment)	11 (100 d) 23 (19 mo)	OS-35 DFS-25 (median follow-up 19 mo)
53	2003	TBI 2 Gy ± Flu 90 mg/m ²	453	5–74 (55)	Hem malignancies	MRD MUD	CSA MMF	48 (II–IV)	44 (Ext)	22 (2 yr)	2 yr OS-51 2 yr PFS-37
56	2001	TBI 5.5 Gy Cy 120 mg/m ²	30	21–63 (47)	CML	MRD	CSA	17 (III–IV)	92	17	2 yr OS-83
18	2001	TBI 2 Gy ± Flu 90 mg/m ²	45	31–72 (56)	Hem malignancies	MRD	CSA MMF	47 (II–IV)	64	6.7	OS-67 (median follow-up 14 mo)

Ref, reference number; AML, acute myeloid leukemia; ATG, antithymocyte globulin; Bu, Busulfan; CSA, cyclosporine; d, days; DFS, disease-free survival; Flu, fludarabine; MDS, myelodysplastic syndrome; MMF, mycophenolate; MRD, matched related donor; MTX, methotrexate; MUD, matched unrelated donor; NA, not available; No, number of patients in the study; NRM, nonrelapse mortality; OS, overall survival; PFS, progression free survival; prophylaxis, GVHD prophylaxis; TBI, total body irradiation; yr, year.

Table 5 Fludarabine and cyclophosphamide combinations and other RIC regimens

Ref	Year	Regimen	No	Age range (median)	Disease	Donor type	GVHD PPX	aGVHD % (Grade)	cGVHD (%)	NRM (%)	Outcome (%)
57	2005	Flu 125 mg/m ² Cy 2000 mg/m ²	23	28–72 (59)	AML	MRD MUD Mismatch	Rapamycin Tacrolimus MTX	43 (II–IV)	77 46 (Ext) 31 (Limt)	0 (MRD)	2 yr OS-50 2 yr PFS-35
58	2005	Flu 120 mg/m ² Cy 3600 mg/m ²	28	31–69 (48)	NHL	MRD	CSA	68 (II–IV)	63	25	36 mo OS-49; 6 DFS-32
40 ^a	2005	Flu 125 mg/m ² Cy 3000 mg/m ² ± ATG	14	26–59 (31)	HD	MRD MUD	Tacrolimus MTX	38 (II–IV) 10 (III–IV)	69	30 (18 mo)	18 mo OS-39 18 mo PFS-21
59	2004	Flu 90 mg/m ² Cy 2250 mg/m ² ± Rituximab	17	44–73 (54)	CLL	MRD	Tacrolimus MTX	29 (II–IV) 12 (III–IV)	60	0 (100 d) 6 (1 yr) 22 (2 yr)	2 yr OS-80 2 yr PFS-60
60	2003	Flu 125 mg/m ² Cy 120 mg/kg	12	15–68 (43)	CML	MRD	CSA MMF	55 (II–IV) 27 (III–IV)	50	0	OS & DFS-67
61	2003	Flu 90 mg/m ² Cy 2250 mg/m ² ± ATG, ± Rituximab	18	46–64 (57)	MCL	MRD MUD Mismatch	Tacrolimus MTX	17 (II) 0 (III, IV)	36 (Ext)	0 (100 d) 15 (2 yr)	2 yr OS-86
62	2001	Flu 90–125 mg/m ² Cy 2000–2250 mg/m ² ± Rituximab (n = 9)	20	31–68 (51)	Indolent lymphoma	MRD	Tacrolimus MTX	20 (II–IV) 5 (III–IV)	64	10	2 yr OS & DFS-84
64	2000	Cy 150–200 mg/kg ATG thymic irradiation	21	22–62 (44)	Hem malignancies	MRD	CSA	29 (II–IV)	NA	10	OS-52 DFS-33 (median follow-up 15 mo)
7	2000	Flu 125 mg/m ² Cy 120 mg/kg ± ATG	19	37–65 (48)	RCC	MRD (+mismatch at single HLA locus)	CSA	53 (II–IV); 16 (III–IV)	21; 16 (Limt), 5 (Ext)	11	OS-47 (median follow-up 13 mo)

Ref, reference number; AML, acute myeloid leukemia; ATG, antithymocyte globulin; Bu, Busulfan; CSA, cyclosporine; d, days; DFS, disease-free survival; Flu, fludarabine; MDS, myelodysplastic syndrome; MMF, mycophenolate; MRD, matched related donor; MTX, methotrexate; MUD, matched unrelated donor; NA, not available; No, number of patients in the study; NRM, nonrelapse mortality; OS, overall survival; PFS, progression free survival; PPX, GVHD prophylaxis; TBI, total body irradiation; yr, year.

^aIncludes patients received other types of conditioning regimens.

and higher levels of proinflammatory cytokines and more Th1 cells – all factors promoting alloresponses. Tables 2–5 summarize the incidence of acute and chronic GVHD in several recent RIC transplant series in older patients or individuals with comorbidities. The high mortality from grades III–IV acute GVHD has been a stimulus to improve the prevention of this complication. The induction of a mixed T-cell chimerism does reduce GVHD or at least delay it until full chimerism is achieved.^{16,78,79} However, breaking the tolerant state with DLI can reintroduce the risk of severe GVHD.^{18,80,81} The administration of alemtuzumab during the preparative regimen is highly effective at reducing GVHD in Flu/Mel transplants.^{39,44,82,83} However, the dose and timing of the monoclonal is critical – too much too close to the time of transplant has led to a high incidence of treatment failure from infections (delayed immune reconstitution) or leukemic relapse.^{83–85} Doses of 10 mg given day –7 or –6 before transplant for 5 days appear to achieve better results.^{86,87} Chronic GVHD is the major cause of late mortality after RIC transplants.^{18,25,43,88,89} The incidence of chronic GVHD (Tables 2–5) ranges between 40 and 60%, leading to late deaths between 100 and 200 days post-transplant. Again it is difficult to ascribe the chronic GVHD incidence specifically to the use of RIC regimens since most RIC transplants are performed with G-CSF mobilized peripheral blood stem cell transplants (PBSCT), which have a significantly greater propensity to cause chronic GVHD than BMT.^{90,91} Moreover, after RIC transplants, delayed T-cell engraftment leads to development of GVHD after 100 days with features of both acute and chronic GVHD (the so-called ‘overlap syndrome’). Alemtuzumab (campath) is also effective at preventing this delayed form of GVHD (Tables 2 and 3).

Graft-versus-malignancy effects

It is now clear that the hypothesis that RIC transplants could be used to deliver an effective GVL effect is correct. Indeed, the typical delayed regression of malignant disease, long after any effect from the preparative regimen has passed, is proof of principle that RIC transplants exert strong and sometimes curative alloresponses against the recipient’s malignancy. The GVL effect after RIC transplants is most marked in CML,³⁵ chronic lymphocytic leukemia (CLL),^{92,93} mantle cell lymphoma,^{49,61,94} and low-grade lymphomas.^{62,94} It is also detectible in AML and MDS,^{18,44,63,76,95} multiple myeloma,^{22,96–100} and metastatic renal cell carcinoma.^{7,8,101} It has been described, but is less powerful in ALL,^{102,103} high-grade lymphomas,^{94,104} and other solid tumors including, ovarian carcinoma,⁸ breast cancer,^{8,9,23} and possibly pancreatic cancer.¹⁰⁵ Nevertheless, the RIC approach may have less ability to induce prolonged disease-free survivals than standard intensity regimens. For example, relapse rates after RIC regimens for AML are higher than relapse rates in comparable disease subtypes in (somewhat younger) patients receiving standard conditioning regimens.^{29,44,76,106} Even in CML, considered to be the most sensitive to GVL effects, the weakly myelosuppressive regimen Flu/Cy achieved disappointing results. In a series of CML patients receiving transplants from identical siblings, 4/5 patients transplanted in second chronic phase died in blast crisis and only 2/7 patients transplanted in first chronic phase achieved molecular remission (MR). The remaining chronic phase patients only achieved MR with multiple DLI, imatinib and interferon, or reconditioning with Cy/TBI 1200 cGy and a second transplant from the original donor (two patients).⁶⁰ More favorable results in CML have been reported with the more

myelosuppressive Flu/Bu regimen^{35,37} and also with a regimen consisting of dibromomannitol and cytosine arabinoside.¹⁰⁷ It thus appears that some leukemia bulk reduction may be helpful even in leukemias that are exceptionally sensitive to GVL effects. Whether favorable results could be achieved in CML by first reducing leukemia burden with imatinib followed by a nonmyeloablative transplant is unlikely to be known, given current reluctance to transplant older patients with CML who are responsive to this agent.

Results of RIC transplants

Because of the diversity of patients treated and differences in post-transplant management (indication for transplant, choice of donor, type of GVHD prophylaxis, use of DLI), only limited conclusions can be drawn about the relative merits of particular protocols. In Tables 2–5, results are summarized according to the major regimen categories listed in Table 1. The median age at transplant ranges from 31 to 59 years but is over 50 in most protocols. The inclusion of younger patients usually implies that they were selected for the RIC protocol because of comorbidities (often includes prior autologous SCT). Most series include family-matched and unrelated donor transplants for most hematological malignancies, but there is an overrepresentation of transplants for myeloid malignancies. The most frequently used GVHD prophylaxis is a standard combination of cyclosporine or tacrolimus with reduced-dose methotrexate. Nevertheless, acute and chronic GVHD incidence varies widely and profoundly impacts the wide spectrum of TRM encountered. There is a trend for the lowest TRM with the least intensive regimen, similar outcomes in TBI- and Bu-based regimens and somewhat higher TRM in Mel-based regimens. Overall survivals at 2 years or more range widely between 20 and 80% with somewhat lower figures for progression-free survival. It can be concluded that RIC regimens can achieve remarkably favorable outcomes in patients who would not normally be considered for SCT, but these regimens suffer from the same limitations as standard intensity transplants – relapse, GVHD and TRM from causes other than the regimen.

Current concerns

When to perform RIC versus standard intensity transplants? While it is clear that RIC transplants have opened the way to using allogeneic SCT in patients several decades older than the upper age limit of 55 years previously used as a cutoff for standard regimens, the superiority of the RIC approach cannot be assumed. Outcomes for standard transplant regimens have generally improved, newer myeloablative regimens of Flu with full-dose intravenous Bu achieve 100 day TRM below 10%, as do some current Cy TBI regimens.^{108–111} While older patients might suffer greater morbidity using such full-dose regimens, their TRM might be offset by lower relapse rates from a more intensive regimen, leading to a disease-free survival comparable to that achieved with less effective but safer RIC regimens. Indeed, some recent nonrandomized comparison of RIC versus standard intensity transplants found that despite lower TRM with RIC regimens, there was a higher relapse rate and comparable survival.^{29,32,112–114} These studies were carried out on a heterogeneous patient population. Given the variability of the GVL effect in different disease states, it would be necessary to perform comparisons of higher and lower intensity regimen transplants for specific disease states in order to find the best

compromise between efficacy and toxicity. Such studies are important because they may reveal that certain conditions with slowly progressive disease (e.g. CLL) are *always* best treated by RIC regimens, whatever the patient age; while conditions such as advanced MDS may require the maximum tolerated regimen intensity.^{115,116} RIC transplants have shown promises in myeloma,^{96–100} and advanced lymphomas.^{49,61,62,94}

What is a suitable age-limit for RIC transplants? Advancing age brings increasing risks from comorbidities, irrespective of the choice of transplant regimen. Population statistics indicate that the probability of 5-year survival in individuals aged 60–70 is 80% falling to 60% in the next decade and 50% in individuals over 80 years. To make sensible choices about which older patient to transplant, it is necessary to factor together the inherent age-related mortality of older patients, with the mortality risk of the individual's hematological malignancy.

Future developments

New RIC regimens

A developing area in preparative regimen design is the use of low-toxicity targeted therapies such as imatinib, bortezomib, rituximab to partner with RIC transplants to combine low-toxicity targeted treatment with a GVL effect.^{117,118,119} The newer tyrosine kinase inhibitor nilotinib (AMN105) and the Abl/Src inhibitor dasatinib (BMS 254825) have shown efficacy in imatinib resistant patients with CML¹²⁰ and might be useful to combine with RIC transplants as salvage treatment. The use of radiolabelled bone marrow seeking antibodies to target the leukemia is another strategy that could be usefully combined with RIC transplants.^{119,120}

RIC transplants as a platform for controlled immune reconstitution

Promising GVHD preventive therapy with sirolimus, pentostatin and photopheresis deserves exploration in RIC transplants as a more effective means of preventing GVHD. Much recent interest has focused on the potential of CD4+CD25+ regulatory T cells (T_{REG}) to suppress alloreacting T cells, and animal models indicate that this subset (which comprises <10% of circulating T cells in man) is extremely effective in preventing GVHD. Recently, Karakhanova *et al.* have developed a strategy to expand T_{REG} with IL-2 and IL-15 bringing closer the possibility of using T_{REG} to prevent GVHD.¹²²

It is not clear, however, that such improvements in GVHD can be achieved without a reduction in the GVL effect. New strategies are needed that control GVHD without compromising immunity to infection and the graft-versus-malignancy effect. Temporary depletion of dendritic cells (DC) by antibodies may be one way to achieve this. CMRF-44, a mouse monoclonal IgM can be used to specifically eliminate human DC.¹²³ A humanized antibody is being developed that could be used as an alternative to alemtuzumab in conditioning for SCT. The use of RIC transplants as a platform to enhance the GVL effect requires the selective prevention of GVHD without immunosuppression, so as to allow full immune recovery and an unmodulated GVL effect. Several approaches are already in clinical trial: Bonini's group¹²⁴ working with HLA mismatched donor–recipient pairs has transfected donor T lymphocytes with a retroviral vector expressing the herpes simplex virus-thymidine kinase (TK) gene, which confers sensitivity to ganciclovir. The infusion of donor lymphocytes without immunosuppression permits a GVL effect while at the onset of significant GVHD, the

administration of ganciclovir prevents GVHD progression by removing T cells carrying the suicide gene. We and others have prepared SCT depleted of GVHD reactivity by removing donor cells reacting to patient lymphocytes with an anti-CD25 immunotoxin.¹²⁵ Our results suggest that alodepletion of donor cells *ex vivo* is clinically feasible in older patients and may reduce the rate of severe acute GVHD. This technique should permit the safe withholding of immunosuppression, thereby facilitating an unrestricted immune response to the malignancy. Furthermore, a fully reconstituted, selectively depleted T-cell repertoire in the absence of immunosuppression, would allow the use of tumor vaccines in the early post-transplant period to further boost GVL effects. The development of peptide vaccines targeting leukemia such as PR1 and WT1 may eventually be used to boost GVL effects after RIC transplants.

Summary and conclusions

The introduction of RIC SCT to treat patients with hematological malignancies has revolutionized the field of SCT and has salvaged patients, who would hitherto have died from disease-progression without a transplant. The GVL effect is discernable in a variety of myeloid and lymphoid malignancies and RIC regimens can be successful in patients up to their 7th decade and in those with significant comorbidities. While the RRM is very low, the hope that GVHD would be better tolerated has not been borne out. Consequently, GVHD and disease relapse have emerged as the major limitations of RIC transplants and methods to separate GVHD from GVL are as relevant to RIC as to standard transplants. While it would be helpful to fully evaluate RIC SCT by comparison with standard SCT, there is little likelihood that informative trials will be performed because RIC transplants are largely applied to a different patient population than standard SCT. Furthermore, accumulating favorable experience with Flu-based regimens of all intensities adds justification to the argument that the revolution in conditioning regimens of the last decade is more to do with the use of purine analog-based regimens than the degree of regimen intensity *per se*.¹²⁶ Rather than attempt to compare standard with RIC transplants, it may now be timely to cautiously explore dose escalation with Flu-based regimens in older patients at high risk of relapse to find the best balance between disease control and regimen-related morbidity. The development of comorbidity scoring to predict transplant outcome offers the opportunity to more accurately select the older patient group most likely to suffer from morbidity and mortality after transplantation. This score could be used in future to select regimen intensity according to the patient's frailty rather than to biological age. Meanwhile, better prevention of GVHD and optimization of GVL awaits success with selective alodepletion approaches now being explored in both standard and RIC transplants.

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