

# Nonmyeloablative allogeneic hematopoietic cell transplantation

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## ABSTRACT

Most hematological malignancies occur in older patients. Until recently these patients and those with comorbidities were not candidates for treatment with allogeneic hematopoietic transplantation because they were unable to tolerate the heretofore used high-dose conditioning regimens. The finding that many of the cures achieved with allogeneic hematopoietic transplantation were due to graft-*versus*-tumor effects led to the development of less toxic and well-tolerated reduced intensity and nonmyeloablative regimens. These regimens enabled allogeneic engraftment, thereby setting the stage for graft-*versus*-tumor effects. This review summarizes the encouraging early results seen with the new regimens and discusses the two hurdles that need to be overcome for achieving even greater success, disease relapse and graft-*versus*-host disease.

## Introduction

Conditioning for allogeneic hematopoietic cell transplantation (HCT) in the treatment of hematologic malignancies has traditionally involved high doses of total body irradiation (TBI) and/or chemotherapy. The dual purpose of conditioning has been to reduce the patients' burden of malignant cells before HCT and suppress their immune system so that the allogeneic grafts are not rejected. The high intensity of the traditional regimens has precluded using allogeneic HCT in older patients or those with comorbidities because of unacceptable toxicities. This has been unfortunate, given that the median ages of patients at the time of diagnosis of most candidate malignancies, e.g. acute myelocytic leukemia (AML) or non-Hodgkin lymphoma (NHL), range from 65 to 75 years. The finding that the cure of hematologic malignancies not only results from intense conditioning but also in large part from the killing of tumor cells by transplanted donor immune cells, termed "graft-*vs*-tumor" (GVT) effect, set the stage for the development of reduced-intensity conditioning (RIC) regimens. Such regimens need to be immunosuppressive enough to allow sustained engraftment, thereby enabling GVT effects. The markedly reduced toxicities associated with these novel regimens have allowed for the extension of allogeneic HCT to include older and medically infirm patients. The relative intensities of individual conditioning regimens vary considerably as far as their immunosuppressive and myelosuppressive properties are concerned (Figure 1). The choice of a given regimen may, in part, be dictated by the nature of the underlying malignancy and, in part, by comorbidities. The results of trials using RIC or nonmyeloablative (NMA) regimens have been surprisingly encouraging. However, all the trials share two major problems that have limited trial outcomes. These are non-relapse mortality (NRM), mainly related to concurrent or preceding graft-*vs*-host disease (GVHD) and its treatment, and relapse mortality.

This review will describe the preclinical basis for some of the RIC and NMA regimens, address GVT effects, summarize trial results with HLA-matched and mismatched grafts, address the use of older sibling donors, and explore ways to reduce the risks of GVHD and relapse.

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### Pre-clinical studies

We used a canine model of major histocompatibility complex (MHC=DLA)-matched marrow grafts to develop a minimal-intensity or NMA conditioning regimen. We found that 2 Gy TBI either without postgrafting immunosuppression or with monotherapy using cyclosporine (CSP) did not enable consistently sustained engraftment.<sup>1</sup> However, when a short course of mycophenolate mofetil (MMF) was combined with CSP following 2 Gy TBI, synergism between the two drugs was noted, host T-cells were prevented from rejecting the donor marrow, and sustained engraftment was seen.<sup>2</sup> Similar synergism was observed with rapamycin used in lieu of MMF.<sup>3</sup> In other studies, which substituted 4.5 Gy irradiation targeted to the cervical, thoracic, and upper abdominal lymph node chain for 2 Gy TBI, we saw sustained engraftment in non-irradiated marrow and lymph node spaces, suggesting that the donor T-lymphocytes created space for grafts to home.<sup>4</sup> The results of the canine studies were the basis for the successful clinical introduction of an NMA regimen of 2 Gy TBI combined with fludarabine (FLU) before and MMF/calcineurin inhibitor after HLA-matched related and unrelated HCT.

Further canine work focused on replacing or augmenting 2 Gy TBI with radiolabeled monoclonal antibodies (mAbs).<sup>5</sup> Current clinical studies have already employed mAb to CD45 or CD20 coupled to beta-emitting radionuclides such as iodine-131 (<sup>131</sup>I)<sup>6</sup> or yttrium-90 (<sup>90</sup>Y);<sup>7</sup> however, the disadvantages of the beta-emitters became apparent, and included relatively long path lengths, long half-lives, and low energy. Therefore, we turned to alpha-emitting radionuclides, including bismuth-213 (<sup>213</sup>Bi)<sup>8</sup> and astatine-211 (<sup>211</sup>At).<sup>9</sup> <sup>211</sup>At coupled to an anti-CD45 mAb turned out to be more effective than <sup>213</sup>Bi.<sup>10</sup> Other advantages of <sup>211</sup>At include that it is produced at the University of Washington Cyclotron Facility, has a short half-life of 7.2 hours, has high energy, and, importantly, a very short path length of approximately 0.04-0.06 mm, thereby reducing the risk of off-target effects. Dose-finding toxicity studies in dogs have been completed, and DLA-identical marrow grafts successfully established using a <sup>211</sup>At-labeled anti-CD45 mAb.<sup>9</sup> Clinical studies are in preparation that are aimed at increasing tumor cell kill in patients with hematologic malignancies and replacing systemic chemo/radiation therapy in those with nonmalignant diseases.

In 1991 Japanese investigators showed that treating MHC-mismatched murine recipients with high-dose cyclophosphamide (CY) after HCT induced tolerance of the grafted lymphocytes to host tissues, while not impairing hematopoietic engraftment.<sup>11</sup> This has been possible since hematopoietic stem cells are protected against the toxic effect of CY metabolites by the presence within these cells of aldehyde dehydrogenase. These observations and those by investigators from Johns Hopkins Medical School<sup>12,15</sup> set the stage for the development of an effective HLA-haploidentical transplant protocol. The protocol utilized the basic FLU/2 Gy TBI NMA regimen with two additional small doses of CY for conditioning.<sup>14</sup> Patients were then given one or two high doses of CY on days 3 and/or 4 post-grafting, followed by MMF/calcineurin inhibitor.

### Clinical results

*HLA-matched related and unrelated HCT.* The choice of conditioning regimen intensity depends in part on the

underlying malignancy, disease burden, and comorbidities. The effects these variables can have on transplantation outcome are illustrated by results in 1,092 patients with advanced hematologic malignancies given a uniform NMA regimen of FLU/2 Gy TBI, which allowed for the purest assessment of GVT effects apart from conditioning and the best determination of GVHD not augmented by toxicities related to the regimen.<sup>15</sup> Patients were either older or had serious comorbidities. Their median age was 56 (range 7 to 75) years. Thirty-five percent of patients were older than 60 years. Six hundred and eleven patients had HLA-matched related donors and 481 had unrelated donors (one HLA allele-level mismatch was permitted). Diseases and disease stages are shown in Table 1. Twenty percent of patients had failed high-dose autologous or allogeneic HCT or had developed a secondary, usually myeloid malignancy after autologous HCT for another malignancy. Forty-five percent of patients had HCT-Comorbidity Index (CI) scores of 3 or greater. Cumulative incidence rates of acute GVHD were 37% for grade 2, 9% for grade 3, and 4% for grade 4, respectively; the rates were lower for related than for unrelated recipients. Table 1 divides patients based on low, standard, or high-risk of relapse as assessed by relapse rate per patient year. It is evident that disease and disease burden were major determinants for relapse risk. For example, patients with high-grade NHL in remission had a relapse rate of 0.16 per patient year in years 1-2, while those not in remission had a rate of 0.48. Similar findings were made for other diseases. These data suggested that reducing the tumor burden in certain diseases and disease stages before HCT might reduce the risk of relapse after HCT. Most relapses occurred in the first 2 years, and relapse rates in subsequent years were generally low. Five-year relapse mortality rates ranged from 18% to 50% depending on relapse risk (Figure 2). Of note, 5-year overall relapse mortality was the same among related and unrelated recipients, at 34.5% for both. Figure 2 also shows 5-year overall survivals which ranged from as low as 25% in patients with high relapse risk and high comorbidity scores to 60% in patients with low relapse risk and low comorbidity scores. Unrelated recipients had a significantly increased risk of GVHD-associated NRM compared to related recipients. Of note, a single HLA allele-level mismatch at class I did not adversely affect HCT outcome. Five-year overall NRM was 24% (20% related to preceding or concurrent GVHD), ranging from 14.7% (12% related to GVHD) among related recipients with low comorbidity scores to 36% (31.8% related to GVHD) among unrelated recipients with comorbidity scores of 3 and higher.

A phase II randomized clinical trial was carried out as part of an ongoing effort to optimize control of acute GVHD without reducing the GVT effect after unrelated HCT.<sup>16</sup> Patients were randomized between three different post-HCT immunosuppressive regimens. In arm 1, tacrolimus was administered for 180 days and MMF for 95 days (n=69). In arms 2 (n=71) and 3 (n=68), tacrolimus and MMF were administered for 150 and 180 days, respectively, with the addition of 80 days of sirolimus in arm 3. Grade II-IV acute GVHD rates in the 3 arms were 64%, 48% and 47% at day 150. Steroid use was significantly lower at day 150 in arm 3 (32% vs. 55% in arm 1 and 49% in arm 2; and the day 150 incidence of cytomegalovirus reactivation was significantly lower in arm 3 (arm 1, 54%; arm 2, 47%; arm 3, 22%) (Figure 3). Currently a 2-arm

phase III trial is ongoing using cyclosporine and MMF with and without sirolimus, in order to further evaluate the role of sirolimus.

Table 2 shows results with RIC or NMA regimens reported by registries or individual transplant centers. Most regimens used were more intense and relied less on GVT effects than the NMA regimen used in studies shown in Table 1 and Figures 2 and 3. Information on comorbidity scores were generally not provided. The two NMDP studies focused on results with unrelated donors. Five-year outcomes in the former of the two included 38% NRM, 42% relapse, and 23% overall survival.<sup>17</sup> The second study had a median follow-up of 3 years and showed that outcomes after RIC were comparable to those after NMA regimens, with approximately 34% NRM, 37% relapse, and 32% overall survival in both groups.<sup>18</sup> A large French registry study included slightly younger patients receiving grafts from related or unrelated donors.<sup>19</sup> Median follow-up was short at 1.75 years. Even though NRM was low at 15%, overall survival was only 42%. A Dana-Farber report included 433 related and unrelated recipients given RIC.<sup>20</sup> The median follow-up was 2 years. NRM rates were 6% for related and 8% for unrelated recipients, relapse rates were 65% and 52%, and overall survival rates were 50% and 56%, respectively. A large CIBMTR study of RIC and either T-replete or *in vivo* T-depleted (ATG or Campath) grafts from related or unrelated donors reported results with a median follow-up of 3 years.<sup>21</sup> NRM ranged from 21% to 26%, relapse from 38% to 51%, and survival from 38% to 50%, respectively, with slightly better outcomes seen with T-replete grafts. A

smaller single-center study from Marseille had a median follow-up of 5 years with grafts from related donors after RIC. NRM was 25%, relapse 22%, and survival 60%.<sup>22</sup> Among other comparisons, a second large CIBMTR study compared results with marrow and PBSC grafts after RIC to grafts after NMA conditioning.<sup>23</sup> Donors were either related or unrelated. With a median follow-up of 3 years, NRM ranged from 33.5% to 38%, relapse from 35% to 40%, and survival from 35% to 40%. An EBMT registry study in younger patients given either related or unrelated grafts after RIC, showed a 2-year NRM rate of 35%, relapse of 34% and event-free survival of 29%. In summary, the median follow-up in these studies was 3 (range, 1.75 to 5) years.<sup>24</sup> Across the studies the median event rates were 43% (range, 22–65%) for relapse, 34% (range, 6–38%) for NRM and 38% (range, 22–65%) for overall survival.

A phase III trial investigating conditioning intensity by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN)<sup>25</sup> randomized patients with MDS or AML to either a RIC regimen (FLU/BU2 or FLU/Mel) or a myeloablative conditioning (MAC) regimen (FLU/BU4, BU4Cy, or CyTBI). Inclusion criteria included <5% blasts, being between 18–65 years of age, an HCT-CI of < 4, both related and unrelated donors with 7/8 or 8/8 HLA loci matching, and either marrow or PBSC. The primary diagnosis was AML (80%) and 92% of patients received PBSC. The study was stratified by center. The primary endpoint was 18 months overall survival. The DSMB closed the study early at the second interim analysis after 272 patients were enrolled (MAC n=135; RIC n=137). Overall survival and

**Table 1. Relapse rates per patient year among 1,092 patients.<sup>15</sup>**

Diagnosis*	Stage	No. of Patients	Relapse Rate	
			Years 1 and 2	Years 3-5
<b>Low-risk</b>				
MPN	Any	18	0.10	0.00
CLL	CR	9	0.11	0.14
Waldenström's syndrome	Any	10	0.13	0.06
NHL	Any stage of mantle cell and low-grade; aggressive CR	140	0.16	0.02
ALL	CR1 <sup>†</sup>	28	0.17	0.04
MM	CR	38	0.19	0.06
<b>Standard-risk</b>				
CLL	No CR	113	0.24	0.05
CML	CP1	24	0.24	0.00
MM	No CR	179	0.32	0.17
AML	CR <sup>‡</sup>	191	0.33	0.02
MDS	RA/RARS	30	0.35	0.00
<b>High-risk</b>				
NHL	Aggressive; no CR	50	0.48	0.00
AML	No CR; evolved from MDS	98	0.65	0.04
HL	After failed autologous HCT	61	0.61	0.14
MDS	RAEB; CMML; second	62	0.65	0.04
CML	CP2; AP; BC	23	0.71	0.07
ALL	≥ CR2; no CR	18	1.03	–

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; AP: accelerated phase; BC: blast crisis; CLL: chronic lymphocytic leukemia; CML: chronic myelocytic leukemia; CMML: chronic myelomonocytic leukemia; CP: chronic phase; HCT: hematopoietic cell transplantation; HL: Hodgkin lymphoma; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasms; MM: multiple myeloma; NHL: non-Hodgkin lymphoma; RAEB: refractory anemia with excess blasts; RARS: refractory anemia with ring sideroblasts. \*There were 243 patients in the low-risk group (53% related and 47% unrelated donors); 537 patients in the standard-risk group (58% related and 42% unrelated donors), and 312 patients in the high-risk group (54% related and 46% unrelated donors). <sup>†</sup>Before HCT, 14% of patients had minimal residual disease. <sup>‡</sup>Before HCT, 13% of patients had minimal residual disease. Reprinted with permission. From: Storb R, et al. Graft-versus-host disease and graft-versus-tumor effects after allogeneic hematopoietic cell transplantation. *J Clin Oncol* 31(12), 2013; 1530-1538. ©2013 American Society of Clinical Oncology. All rights reserved.

progression-free survival at 18 months were 77.4% and 68.8% (MAC) and 67.7% and 47.3% (RIC), respectively ( $P=0.07$ ;  $P<0.01$ ). The incidences of both acute and chronic GVHD were significantly higher in MAC patients ( $P=0.024$  and  $P=0.019$ , respectively). The primary causes of death were GVHD in the MAC arm (52%) and relapse in the RIC arm (82%). The conclusion was that MAC remains the treatment of choice for younger patients with MDS or AML.

**HLA-mismatched unrelated HCT.** While many patients who would benefit from HCT have a HLA-matched donor, a substantial number will not, particularly those who do not have white European ancestry. An analysis was performed on data from the NMDP unrelated donor and cord blood registries to predict the likelihood of identifying suitable donors for U.S. patients.<sup>26</sup> The likelihood of finding an 8/8 HLA loci match ranged from 75% in white Europeans to 16-19% for Black/African racial groups. If one accepts a 7/8 HLA loci matched donor, the numbers increase to 97% and 66-76%, respectively. The CIBMTR compared outcomes in 563 recipients of a single HLA locus mismatch with 2,025 recipients of 8/8 HLA loci high-resolution matched unrelated RIC HCT.<sup>27</sup> There were more grades II-IV acute GVHD, higher NRM and lower disease free survival and overall survival in recipients of 7/8 HLA loci matched URD. Interestingly, there was no difference in chronic GVHD or relapse. The decreases in overall and disease free survival using a 7/8 HLA loci matched donor were slightly less than those in the myeloablative setting, suggesting a role of tissue damage in mortality following higher dose regimens. The findings in this large registry study are consistent with another smaller prospective study.<sup>28</sup>

Taken together these studies show that relapse and

NRM, mostly related to GVHD, represent the two major obstacles for patients given RIC or NMA regimens that need addressing in future trials.

**HLA-haploidentical HCT.** Many patients, particularly members of ethnic minorities, lack HLA-matched unrelated donors; however, most patients have a relative who is HLA-haploidentical. The development of low-toxicity regimens sufficient to overcome the immunologic barriers to engraftment is equally important for such patients. Johns Hopkins University and the Fred Hutchinson Cancer Research Center investigated a novel HLA-haploidentical marrow transplant trial using the fludarabine and 2 Gy TBI regimen and additional immunosuppression with CY both before and after HCT for the treatment of hematologic malignancies.<sup>14</sup> This regimen was well tolerated and, considering the strong immunological barriers that needed to be overcome, the rejection incidence was low. In addition, the incidences of severe acute and chronic GVHD were encouragingly low. These results were confirmed in a multi-site trial conducted by the BMT CTN<sup>29</sup> which also showed a relatively high relapse rate. A currently ongoing randomized study, BMT CTN Protocol 1101, compares HLA-haploidentical marrow vs. cord blood as a stem cell source.

A recent European publication noted a pronounced increase in the use of HLA-haploidentical family donors and a concurrent decrease in the use of cord blood donors.<sup>30</sup> More than twice the number of HLA-haploidentical grafts have been reported since 2010 compared to cord blood transplants. CIBMTR is reporting similar trends in North America.

A recent CIBMTR study compared outcomes in 2,174 patients with AML given grafts from HLA-matched unrelated ( $n=1,982$ ) or HLA-haploidentical related donors

**Table 2.** Results of retrospective analyses of transplantation outcomes in patients with hematologic malignancies after reduced intensity (RIC) or nonmyeloablative (NMA) conditioning.

Transplant Group (Reference)	# of Patients	Median age in Years (Range)	Donors	Conditioning Regimen	Median Follow-up (Years)	NRM	% Relapse	OS
NMDP (Giralt) <sup>17</sup>	285	53 (18–79)	URD	RIC	5	38	43	23
NMDP (Pulsipher) <sup>18</sup>	160	56 (1–75) 57 (17–73)	URD	RIC	3	34	37	32
				NMA	3	34	37	32
French Registry (Michallet) <sup>19</sup>	1,108	51 (1–72)	MRD > URD	RIC	1.75	15		42
Dana Farber (Ho) <sup>20</sup>	433	56 (18–73)	MRD	RIC	2	6	65	50
			URD	RIC		8	52	56
IBMTR (Soiffer) <sup>21</sup>	879 584 213	(21–69)	MRD	T-replete	3	23	38	46
			URD	ATG	3	26	49	38
				Campath	3	21	51	50
Marseille (Blaise) <sup>22</sup>	100	49 (18–64)	MRD	RIC	5	25	22	60
CIBMTR (Luger) <sup>23</sup>	273 768 407	51 (19–69)	MRD/URD	RIC (BM)	3	38	39	38
			MRD/URD	RIC (PBSC)	3	35	35	40
			MRD/URD	NMA	3	335	40	35
EBMT (Belkacemi) <sup>24</sup>	130	17–41	MRD/URD	RIC	2	35	34	29 (EFS)
Seattle (Storb) <sup>15</sup>	1,092	57 (7–75)	MRD/URD	NMA	5	24	34.5	25–60
CIBMTR (Ciurea) <sup>31</sup>	1,349 825	21–70	URD/Haplo	MAC	3	20/14	39/44	45/50
			URD/Haplo	RIC/NMA	3	23/9	42/58	37/37
Hopkins (McCurdy) <sup>34</sup>	372	55(18-75)	Haplo	NMA	3	11 (1 yr)	46	50



given regimens using post-HCT Cy (n=192).<sup>31</sup> The study included patients with myeloablative (unrelated n=1,245; HLA-haploidentical n=104) and RIC/NMA conditioning (unrelated n=737; HLA-haploidentical n=88). There was no difference in overall and disease free survival between the different donor types in either the myeloablative or RIC/NMA recipients (Table 2 and Figure 4). There was significantly more acute and chronic GVHD in recipients of unrelated grafts but a lower risk of NRM ( $P=0.01$ ), and a borderline increase risk of relapse ( $P=0.05$ ) in RIC/NMA-conditioned recipients of HLA-haploidentical related grafts. A similar CIBMTR study compared outcomes in 917 patients with NHL receiving HLA-haploidentical related versus HLA-matched unrelated HCT, the latter either with or without ATG.<sup>31</sup> There was no significant difference in overall survival between the 3 groups but there was inferior survival in those unrelated patients who received ATG. In a single center series of 372 patients, patients were stratified by the refined Disease Risk Index (DRI)<sup>32,33</sup> and evaluated for outcomes. By refined DRI, 3-year progression-free survival in low, intermediate and high/very high-risk groups were 65%, 37% and 22%, respectively (Table 2).<sup>34</sup> These results are similar to those historically seen with HLA-matched HCT, suggesting that prospective randomized trials are warranted to evaluate the use of alternative donors given the lower incidence of chronic GVHD seen after HLA-haploidentical HCT.

It has been suggested that the use of PBSC may reduce the risk of relapse among HLA-haploidentical recipients without increasing the risk of GVHD. Concurrent studies using PBSC were carried out at 4 centers and analyzed together.<sup>35</sup> Grades 2 and 3 acute GVHD developed in 53% and 8% of patients, respectively, and the 2 year incidence of chronic GVHD was 18%. The 2 year rates of NRM and

relapse were 23% and 28%, respectively, suggesting that PBSC can be substituted for marrow in HLA-haploidentical HCT. Other strategies to prevent, preempt or treat relapse include planned donor lymphocyte infusions.<sup>36</sup> A more novel approach includes preemptive infusions of donor NK cells. Thirty-six heavily pre-treated patients with hematologic malignancies, median age of 46 (range 8-75) years, were given donor NK cells on day 7 after HLA-haploidentical HCT.<sup>37</sup> Patients had a median time from cancer diagnosis to transplant of 2.1 (0.3 – 9.9) years, including 7 patients with prior autologous HCT and 6 patients with 1 or more prior allogeneic HCT. Overall and relapse-free survivals at 1 year of 74% and 69%, and at 2 years of 63% and 51% were observed, respectively.

**Engraftment kinetics and donor chimerism**

The overall goal in malignant disorders is to achieve high levels of or even complete donor T-cell chimerism early after HCT, as this has been associated with lower risks of graft rejection and relapse.<sup>38-40</sup> While complete donor chimerism develops rapidly following myeloablative allogeneic HCT, varying degrees of mixed donor host chimerism are seen initially following NMA conditioning, though the majority of patients will have full donor chimerism by day 100 after HCT. Many of the RIC regimens that are more myelosuppressive have kinetics of donor engraftment similar to those of myeloablative regimens. In addition to regimen intensity, other factors influencing the kinetics of engraftment including the use of PBSC and *in vivo* T-cell depleting agents (such as ATG or alemtuzumab) and HLA disparity between donor and recipient. Patients who received myelosuppressive chemotherapy or a preceding autologous HCT had a more rapid engraftment of donor T-cells. An association between high

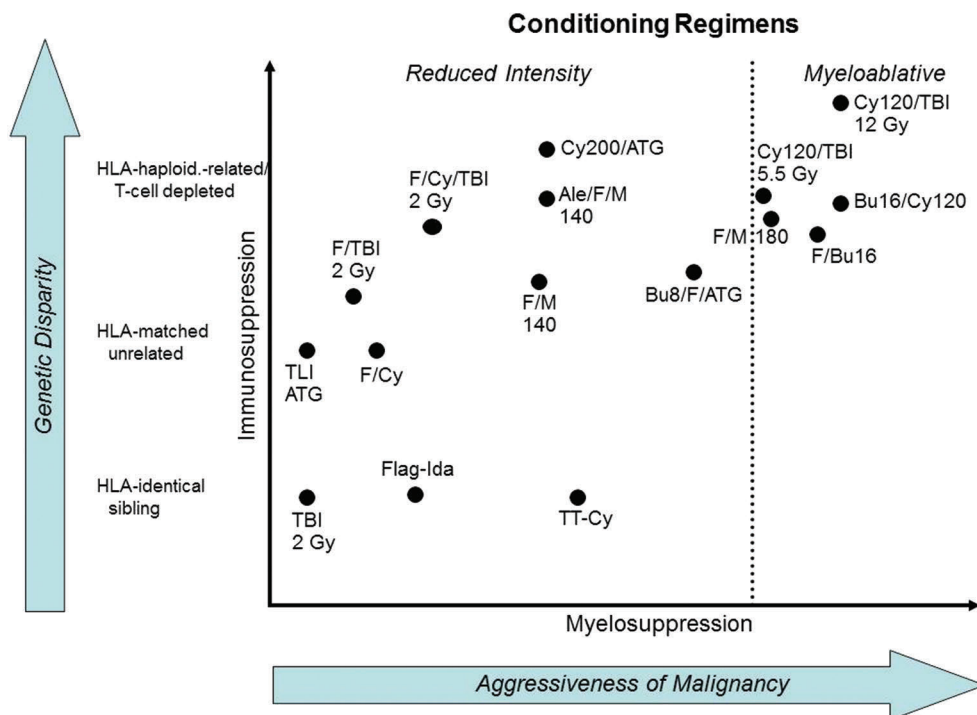
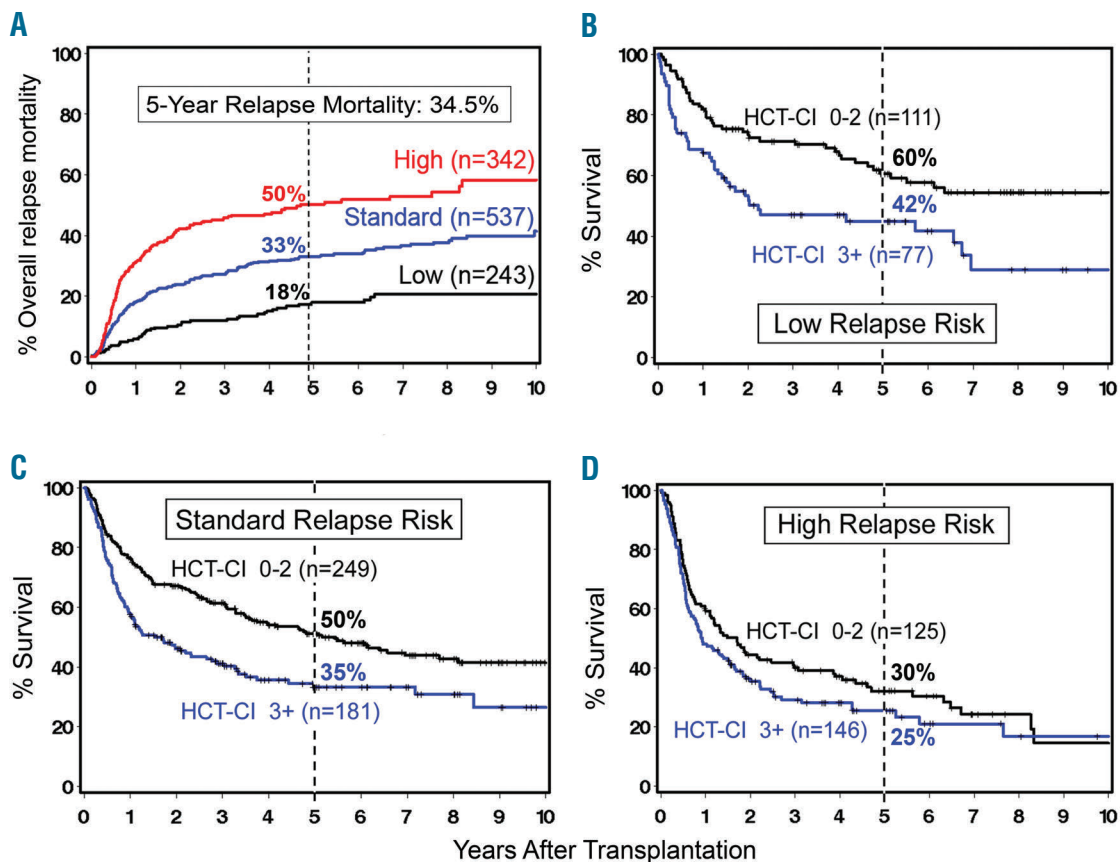


Figure 1. Reproduced from: Sandmaier BM, Storb R. Reduced-intensity allogeneic transplantation regimens, Chapter 21, In: Thomas' Hematopoietic Cell Transplantation, 5<sup>th</sup> Edition. Forman SJ, Negrin RS, Antin JH, and Appelbaum FR, Eds., ©John Wiley & Sons, Ltd., in press.



**Figure 2.** Five-year relapse mortality and overall survival of patients with advanced hematologic malignancies who were conditioned with FLU/2 Gy TBI before HLA-matched related or unrelated HCT and post-grafting immunosuppression with MMF/calcineurin inhibitor. Survival is shown depending on relapse risk and hematopoietic comorbidity scores (HCT-CI).

levels of donor T-cell chimerism and GVHD has been observed using different conditioning regimens.<sup>39,40</sup> When both NK and T-cell chimerism were modeled as continuous variables, only early donor T-cell chimerism was associated with acute GVHD, whereas high levels of NK chimerism were significantly associated with lower relapse rates but not with increased GVHD.<sup>41</sup> A phase III trial among patients treated with 2 Gy TBI alone vs. TBI with fludarabine 90mg/m<sup>2</sup> showed that adding fludarabine contributed to a more rapid T and NK cell chimerism and significantly less relapse (40 % vs. 55%), resulting in superior survival (60 % vs. 54% at 3 years).<sup>42</sup> This supported the previous observations of higher donor chimerism being protective for relapse.

### Toxicities and infections

High-dose conditioning is associated with higher NRM from organ toxicities and infectious complications. The former include hepatic sinusoidal obstruction syndrome/veno-occlusive disease (SOS / VOD) and idiopathic pneumonia syndrome (IPS). No cases of SOS were observed among 193 patients given NMA conditioning.<sup>43</sup> Acute renal failure (ARF) (defined as a >50% decrease in glomerular filtration rate) occurred less often in patients given NMA HCT compared to myeloablative conditioning (43% vs. 73%), despite greater age and comorbidities

among NMA recipients.<sup>44</sup> A separate multivariate analysis revealed that ARF during the first 100 days was associated with the development of chronic kidney disease (CKD). CKD was defined as at least a 25% reduction in GFR from baseline. Previous autologous HCT, long-term calcineurin inhibitor use and extensive chronic GVHD were independently associated with CKD. CKD following NMA HCT appears to be a distinct clinical entity and likely not related to radiation nephritis.<sup>45</sup> Pulmonary function was evaluated in patients before, at day 100, and 1 year after HCT.<sup>46</sup> Results suggested that, despite having worse pre-transplant lung function, NMA patients experienced less pulmonary toxicity than myeloablative patients. The incidences and outcomes of IPS among NMA (n=183) versus myeloablative (n=917) patients were compared. The cumulative incidence of IPS was significantly lower at 120 days after NMA conditioning (2.2% vs. 8.4%). IPS occurred early after transplant, progressed rapidly, and had a high mortality rate (75%) despite aggressive support. These findings support the concept that lung damage from conditioning regimen plays a crucial role in IPS after HCT.

Following NMA conditioning, patients have less cytopenias including less neutropenia. Significantly fewer NMA recipients (n=503) required platelet transfusions (25% vs. 99%) and red blood cell transfusions (64% vs. 96%) than myeloablative (n=1,353) recipients.<sup>47</sup> Among the NMA

patients, platelet and RBC transfusions were less frequent among related compared to unrelated recipients. Major/bidirectionally ABO-mismatched recipients required more RBC transfusions than ABO-matched recipients, though ABO-mismatching did not affect other NMA HCT outcomes. It was also hypothesized that NMA conditioning would be associated with less neutropenia after day 28 following engraftment. However, while NMA conditioning had protective effects on anemia and thrombocytopenia after day 28 there was no significant reduction of neutropenia either overall or in the context of ganciclovir use.<sup>48</sup> Elderly patients appear to be more prone to cumulative toxicities of post-HCT drug regimens, but NMA conditioning, optimized HLA matching, and higher doses of CD34<sup>+</sup> cell infusions reduced the risk of cytopenia after day 28.

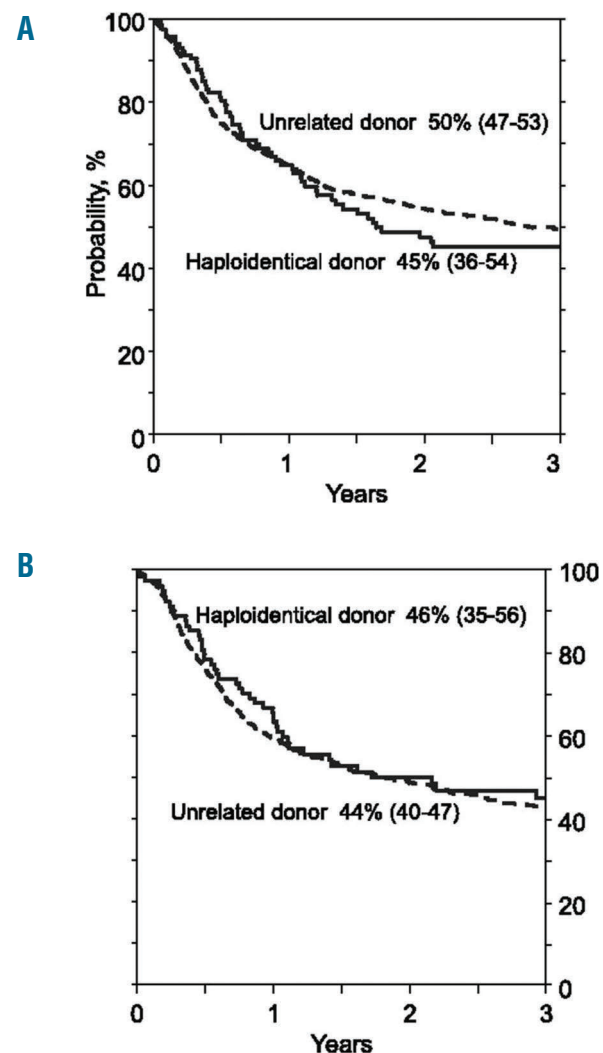
Multiple studies have shown that the incidence of infections early after HCT is reduced after RIC and NMA conditioning. There is less bacteremia in the first month presumably due to a lesser degree of neutropenias.<sup>49</sup> While the incidence of CMV infection is the same in CMV positive recipients, NMA-HCT was associated with a lower risk of high-grade CMV infection.<sup>50</sup>

#### Older donors

As the age of HCT recipients has increased, the age of their sibling donors has increased as well. Concern has been raised that increasing donor age might adversely affect the functional fitness of hematopoietic cells and thereby impair the marrow recovery after transplantation. Hematopoietic cells are subject to aging mechanisms such as accumulated DNA damage, telomere shortening, and epigenetic modification. However, studies on the effect of donor age on the function of hematopoietic cells have yielded controversial results, especially the work on stem cell aging in murine model systems. Dutch investigators commented on the variable results seen: “the discrepant conclusions of these studies, however, could be partly caused by (the different) mouse strains used, because strain-dependent increases or decreases in primitive hematopoietic cell frequency and function have been reported.”<sup>51</sup> Another concern is related to the longevity of hematopoietic stem cells which makes them ideal targets for mutagenic changes.<sup>52</sup> The theoretical possibility was raised that recipients of aged stem cells might be at an increased risk of developing malignant clonal disorders.

Published clinical results on the effects of aging on stem cells also vary. An NMPD study from 2001 reported inferior survival among patients given grafts from donors older than 45 years.<sup>53</sup> A French study initially saw no significant impact of donor age among MDS and AML patients undergoing transplantation.<sup>54</sup> In contrast, a later analysis by the French group found that donor age  $\geq 60$  years had a significant adverse impact on overall recipient survival.<sup>55</sup> A CIBMTR analysis from 2013 reported that outcomes were superior in recipients of grafts from HLA-identical sibling donors  $>50$  years old compared to those with grafts from HLA-matched unrelated donors  $<50$  years of age.<sup>56</sup> We analyzed the effects of donor age on the speed of hematopoietic engraftment and donor chimerism, acute and chronic GVHD, and NRM among 1,174 patients undergoing myeloablative and 367 patients undergoing NMA conditioning before HLA-matched related or unrelated HCT.<sup>57</sup> CD34 cell harvests were reduced in older (60-82 years) donors (median  $5.6 \times 10^6$  cells/kg) com-

pared to younger ( $<60$  years) donors (median  $7.7 \times 10^6$  cells/kg). However, sustained engraftment rates among recipients with older and younger donors were comparable. Sustained grafts were seen in 97% and 98% of patients given myeloablative and NMA conditioning, respectively, who had younger donors, and 90% and 100%, respectively, for those who had older donors. Also the tempo of neutrophil and platelet recoveries and donor chimerism did not show significant differences, except for an average 1.3-day delay in neutrophil recovery among myeloablative patients with older donors ( $P=0.04$ ). Moreover, aged stem cells did not convey an increased risk of donor-derived clonal disorders after HCT since none were seen. Both myeloablative and NMA recipients with older sibling donors had significantly less grade 2-4 acute GVHD compared to recipients with grafts from younger



**Figure 3. Overall survival.** (A) The probability of OS by donor type after myeloablative conditioning regimen, adjusted for age and disease risk index. (B) The probability of OS by donor type after reduced intensity conditioning regimen, adjusted for disease risk index and secondary AML. (Originally published in *Blood*. Ciurea SO, Zhang MJ, Bacigalupo AA, et al. Haploidentical transplant with posttransplant cyclophosphamide vs. matched unrelated donor transplant for acute myeloid leukemia. *Blood*. 2015;126(8):1033-1040. ©The American Society of Hematology).



unrelated donors. Rates of grade 3 and 4 acute GVHD, chronic GVHD, and NRM among recipients with older donors were not significantly different from those seen in recipients with younger donors. We concluded from this single-center study that grafts from donors  $\geq 60$  years of age did not adversely affect graft outcomes of HCT compared to grafts from younger donors  $< 60$  years of age.

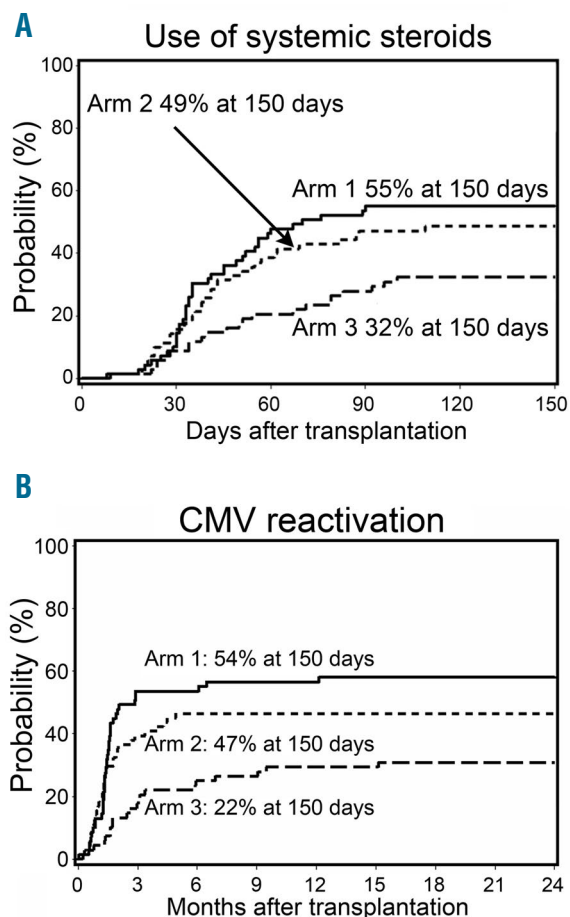
### Relapse

Relapse or progression of the underlying malignancy has remained the principal cause of failure of allogeneic HCT. This has been especially true in patients who for reasons of age or comorbidities have been conditioned with NMA regimens, where cure of malignancy depends almost entirely on GVT effects. The following sections will discuss outcomes with a minimal-intensity conditioning regimen and use the results as a basis for proposing ways to reduce relapse or progression.

We maintained the NMA FLU/low-dose TBI platform for patients with advanced hematologic malignancies because most of our patients either did not need or would not tolerate higher dose regimens, and because the regimen best defines the limits of GVT effects.<sup>15,58</sup> Powerful GVT effects were seen across all disease stages except for ALL in CR2 and beyond, where all patients progressed. As shown in Figure 2, between 45% and 75% of patients experienced sustained remissions depending on the nature and stage of the underlying malignancy. Overall 5-year relapse mortality was 34.5%. Seventy percent of relapse or progression occurred in year 1 and much of the remainder in year 2 after HCT. We hypothesize that early disease relapse or progression was due to blunted GVT effects from early post-transplantation immune compromise. Later, as the donor immune system was being built up and immunosuppressive drugs tapered and then discontinued, the “brakes were taken off” the immune cells, enabling GVT effects. This hypothesis is indirectly supported by former extensive immune function studies showing recovery of antibody responses to neoantigens, such as bacteriophage  $\phi X174$  and keyhole limpet hemocyanin, among others, as well as cellular immunity within 1-3 years after HCT.<sup>59</sup> Consistent with this hypothesis, relapse rates in most diseases were markedly reduced in years 3-5.

The options for decreasing the still existing relapse or progression risk are limited. Increasing the intensity, and thereby the toxicity, of the conditioning regimen may be problematic for at least two reasons. One is that a majority of patients did not relapse and, therefore, would be exposed to unnecessary toxicity. The other is that most patients were elderly and/or had comorbidities which pre-empted dose escalation. Also, more than one-fifth of patients had failed preceding high-dose HCT and another one-fifth had planned autologous HCT, and receiving another high-dose HCT regimen might be too toxic. Given these limitations, we envision two principal approaches for reducing the risk of relapse or progression in elderly or medically infirm patients.

One approach is based on the hypothesis that delaying disease relapse or progression until the grafted immune system is recovered sufficiently to generate GVT effects would increase cure rates. Such a delay would be accomplished with well-tolerated drugs or antibody-drug conjugates which, even though not curative on their own, would pave the way for curative GVT effects. An example



**Figure 4. Graft-versus-host disease and use of systemic steroids.** (A) Cumulative incidence of use of systemic steroids in arm 1 (n=69), arm 2 (n=71) and arm 3 (n=68). (B) Viral infections. Cumulative incidence of cytomegalovirus reactivation in arm 1 (n=69), arm 2 (n=71) and arm 3 (n=68). Originally published in *Haematologica* (Kornblit B, et al. A randomized phase II trial of tacrolimus, mycophenolate mofetil and sirolimus after non-myeloablative unrelated donor transplantation. *Haematologica* 2014; 99(10): 1624-1631. ©2014 Ferrata Storti Foundation).

of such an approach has been the treatment of patients with Ph1+ ALL in first remission with a tyrosine kinase inhibitor for one year after HCT.<sup>60</sup> The overall 5-year survival rate was 69% and 85% in the subgroup without MRD before HCT, which is impressively better than previous results without tyrosine kinase inhibitors. Candidate agents for patients with other malignancies include antibodies to CD20 (NHL) and CD30 (Hodgkin lymphoma), proteasome inhibitors (MM), and the FLT3 inhibitors (AML).

A second approach would be to reduce the tumor burden before HCT. One way to accomplish this is through the use of chimeric antigen receptor (CAR) T-cells in patients who have B cell lymphoid malignancies expressing CD19.<sup>61,62</sup> Another way is to increase the pre-transplant tumor cell kill by low-toxicity, targeted radiation therapy using a mAb to CD45 coupled to radionuclides used in addition to the basic FLU/2 Gy TBI regimen. One preliminary study summarized early results in 58 patients with advanced AML or high-risk MDS who were older



than 50 years and treated with the anti-CD45 mAb coupled to  $^{131}\text{I}$ .<sup>6</sup> One-year survival was 41%. Another study added  $^{90}\text{Y}$  coupled to an anti-CD20 mAb to FLU/2 Gy TBI in 40 patients with persistent, high-risk NHL. The estimated 30-month progression-free survival was 51%.<sup>7</sup> Several properties of the beta-emitting radionuclides  $^{131}\text{I}$  and  $^{90}\text{Y}$  limit their effectiveness including their long half-lives of 2.5 and 8 days, their relatively low energy of 0.7 and 2.3 MeV, and their long path lengths of 0.8-11.3 mm, respectively, which result in off-target effects. Additionally,  $^{131}\text{I}$  emits weak gamma radiation during its decay which necessitates placing patients in isolation rooms for several days. To get around these limitations, we have focused our attention on an alpha-emitting radionuclide,  $^{211}\text{At}$ , which has a half-life of 7.2 hours, high energy (5.9 MeV), and a path length of only 0.04-0.06 mm. This results in the unique ability of killing mAb-targeted cells while causing minimal damage to surrounding tissues. Moreover, the alpha particles cause multiple strand breaks, hence DNA repair mechanisms are inhibited, which reduces the risk of secondary cancer. An additional advantage of  $^{211}\text{At}$  is that it is relatively cheap compared to other alternatives. Our first clinical protocol has been firmly based on 15 years of

experience with alpha-emitting radionuclides in a canine allogeneic HCT model.

## Conclusions

Allogeneic HCT after RIC or MMA regimens to treat older or medically infirm patients with advanced hematological malignancies is feasible and effective. This is enabled in large part by GVT effects, and results in cures of appreciable numbers of malignancies. Increasing disease control and decreasing NRM, the latter mostly associated with or preceded by GVHD, will need to be addressed in future trials.

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