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Disease Risk and GVHD Biomarkers Can Stratify Patients For Risk of Relapse and Non-Relapse Mortality Post Hematopoietic Cell Transplant

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Abstract

The graft-versus-leukemia (GVL) effect after allogeneic hematopoietic cell transplant (HCT) can prevent relapse but the risk of severe graft-vs-host disease (GVHD) leads to prolonged intensive immunosuppression and possible blunting of the GVL effect. Strategies to reduce immunosuppression in order to prevent relapse have been offset by increases in severe GVHD and non-relapse mortality (NRM). We recently validated the MAGIC algorithm probability (MAP) that predicts the risk for severe GVHD and NRM in asymptomatic patients using serum biomarkers. In this study we tested whether the MAP could identify patients whose risk for relapse is higher than their risk for severe GVHD and NRM. The multicenter study population (n=1604) was divided into two cohorts: historical (2006–2015, n=702) and current (2015–2017, n=902) with similar non-relapse mortality, relapse, and survival. On day 28 post-HCT, patients who had not developed GVHD (75% of the population) and who possessed a low MAP were at much higher risk for relapse (24%) than severe GVHD and NRM (16% and 9%); this difference was even more pronounced in patients with a high disease risk index (relapse 33%, NRM 9%). Such patients are good candidates to test relapse prevention strategies that might enhance GVL.

Introduction:

Allogeneic hematopoietic cell transplant (HCT) is an important therapy for leukemia and lymphoma that is unresponsive to standard treatment. Its therapeutic benefit is mediated by a graft versus leukemia (GVL) effect in which T cells from the donor recognize histocompatibility antigens on malignant cells in the recipient[1]. When donor T cells recognize those same antigens on normal host tissues they cause graft versus host disease (GVHD), a major and potentially lethal toxicity[2]. Relapse of the primary malignancy occurs in approximately 25% of patients following HCT, whereas non-relapse mortality (NRM) (of which GVHD is the leading cause) occurs in another 20% of patients[3, 4]. Improvement in overall survival after allogeneic HCT requires maximizing GVL while minimizing GVHD.

All HCT patients receive some form of GVHD prophylaxis. The majority of patients receive intensive systemic immunosuppression to prevent GVHD, usually with two or more agents, including a calcineurin inhibitor (cyclosporine or tacrolimus) for the first several months after HCT, even though many, but not all studies, show that immunosuppression can blunt the GVL effect[5–8]. Unfortunately under-dosing or rapid tapers of prophylactic immunosuppression are more likely to lead to severe GVHD, the treatment for which is additional intensive immunosuppression[9–13]. Thus early rapid tapers can paradoxically lead to extended periods of immunosuppression which can impede or abrogate the desired GVL effect. Rapid tapers of prophylactic immunosuppression are usually reserved for patients whose malignancy is deemed highly likely to recur such as patients with minimal residual disease (MRD) or increasing percentage of recipient chimerism[14–16]. In patients whose malignancy has relapsed, a rapid taper of immunosuppression, while not always effective,

has been reported in several studies to amplify the GVL effect and produce durable remissions in up to 25% of patients[17–21]. Furthermore, a recent pilot study showed that in patients transplanted for refractory acute myeloid leukemia, a rapid taper of immunosuppression resulted in survival identical to patients transplanted in remission and who received a standard taper[22].

Several recent studies have shown that serum biomarkers can predict NRM caused by severe GVHD at the onset of GVHD symptoms[23–27]. The Mount Sinai Acute GVHD International Consortium (MAGIC) investigators developed and validated an algorithm that uses the concentrations of two serum biomarkers to predict long term outcomes at a number of timepoints following HCT. These two biomarkers, suppressor of tumorigenicity 2 (ST2) and regenerating islet-derived 3-α (REG3α), are derived primarily from the GI tract, the target organ most refractory to standard therapy. The algorithm generates a MAGIC algorithm probability (MAP), a value from 0.001 to 0.999 that predicts the probability of NRM within 6 months for individual patients. The same MAP identifies patients at high risk for severe GVHD and NRM before the onset of symptoms, at the onset of symptoms, and after treatment[25, 28].

The goal of this study was to investigate the ability of the MAP to identify patients at low risk of developing severe GVHD, but who remain at an increased risk of relapse. This population of patients might therefore benefit from early changes in immunosuppressive therapy in an effort to enhance the GVL effect, as their risk of severe GVHD is low.

Methods:

Study design and oversight

Patients from 18 centers in the Mount Sinai Acute GVHD International Consortium (MAGIC) underwent first allogeneic HCT for hematologic malignancy and provided blood samples for a biorepository according to an institutional review board approved protocol at each MAGIC participating center (Supplementary Table 1). All patients provided informed consent. Patients were divided into two cohorts based on date of HCT (Supplementary Figure 1). Patients in the historical cohort (n=702) underwent HCT from January 1, 2006 until June 30, 2015, whereas patients in the current cohort (n=902) underwent HCT from July 1, 2015 to May 1, 2017.

Clinical data, blood collection, and analysis

GVHD clinical staging was performed according to standardized published guidelines[29] and was adjudicated monthly beginning in 2013. Personnel at each MAGIC site were trained via webinars in the use of the guidelines and data entry personnel passed quality control tests prior to enrolling patient data. Patients were followed for one year post-HCT. Blood samples were collected prospectively 28 days after HCT (±3 days). Additional samples were collected from patients who developed GVHD at its onset. Samples were analyzed for ST2 and REG3α by ELISA in a central laboratory at Mount Sinai according to manufacturer protocols (R&D Systems-Human ST2 Lots: P109353 and P139040; MBL-Ab-Match Assembly Human PAP1 Kit/ Ab-Match Universal Kit Lots: 016FA and 017FA).

Non-relapse mortality was defined as a death from any cause, with relapse and second HCT treated as competing risks. The primary cause of death was assigned according to a published hierarchy[30]. For cause of death analysis, acute and chronic GVHD deaths were combined to form an overall GVHD mortality category. GVHD was considered the cause of death if the patient died from GVHD or a complication of GVHD treatment, e.g. infection while receiving systemic steroid treatment (≥10 mg prednisone equivalent per day). Infection was considered the cause of death if the center reported infection as the cause of death, the patient had not relapsed, and was on no more than minimal systemic steroid treatment for GVHD (<10 mg prednisone equivalent per day). Cause of death was centrally adjudicated and sites were queried to resolve cause of death for complicated cases. Early GVHD was defined as GVHD that occurred prior to or on day 28 post-HCT. Disease risk index (DRI) was determined for patients in the current cohort according to published criteria by an online calculator: https://www.cibmtr.org/ReferenceCenter/Statistical/Tools/Pages/DRI.aspx31. Patients with high or very high DRI were grouped together.

Statistical analyses

The MAGIC algorithm uses the concentrations of ST2 and REG3α to generate a predicted probability ($\hat{p}$), also termed the MAGIC algorithm probability or MAP, of six-month NRM: $\log[-\log(1-\hat{p})] = -11.263 + 1.844(\log_{10}\text{ST2}) + 0.577(\log_{10}\text{REG3}\alpha)$ as previously published[25, 28]. The MAP predicts the probability of NRM at 6 months for each patient, as well as additionally identifying patients at high risk of severe GVHD before the onset of symptoms. A threshold of 0.16 was used to demarcate high and low MAP groups in all patients without GVHD symptoms as previously described[25]. For samples obtained from patients at the onset of GVHD, patients were classified according to their Ann Arbor GVHD score (1–3) determined by biomarker probability, using the thresholds of 0.14 and 0.29, as previously described[25].

Cumulative incidences of NRM and relapse were calculated using the method of Fine and Gray[32] and compared between groups by Gray's test[33]. Overall survival was estimated by the Kaplan-Meier method and differences between groups were calculated using the log-rank test. Differences in proportions were calculated using chi-squared test. All analyses were performed using R statistical package version 3.4.0 (R Core Team 2017).

Results

We analyzed causes of death in the first year after HCT in 1604 patients from 18 sites of the MAGIC consortium, divided into two cohorts by date of HCT: historical (1/2006 – 6/2015) and current (7/2015 – 5/2017). The clinical characteristics of all patients are shown in Supplementary Table 2. The current cohort differed from the historical cohort in terms of donor type and HLA-match (more haploidentical donors), conditioning regimen (more reduced intensity conditioning), and intensive GVHD prophylaxis (more post-transplant cyclophosphamide, T-cell depletion, and ATG). These differences in cohorts reflected evolving transplant practices. The median day of GVHD onset was day 30 and 31 in the historical and current cohorts, respectively. The cumulative incidences of NRM, relapse and overall survival at one year for the historical cohort are 16%, 22%, and 72% respectively

(Figure 1A) and 13%, 21%, and 75% respectively for the current cohort (Figure 1B). GVHD grade II-IV developed in nearly 40% of patients in both cohorts (Supplementary Table 2).

As expected, relapse and GVHD were the primary causes of death in both cohorts (Figure 1). The proportion of deaths from GVHD is higher than the approximately 10–15% commonly reported by the Center for International Blood and Marrow Transplant Research (CIBMTR)[34, 35]. Up to 50% of the reported cause of death in the CIBMTR analyses are misclassified without central review[30, 35] whereas all causes of death in the MAGIC database undergo central review. Furthermore, CIBMTR guidance assigns infections as the primary cause of septic deaths during intensive steroid treatment of GVHD to infection as the primary cause, whereas MAGIC guidance assigns such deaths to GVHD.

A temporal analysis of major events in the first year after HCT showed little overlap in the onset of clinical GVHD and the onset of relapse: fewer than 3% of relapses occurred before day 28 whereas 50% of GVHD cases developed in this period (Figure 2). We therefore used day 28 to classify patients as either “early GVHD” or “no early GVHD” (all others). Although the number of new cases of GVHD rapidly declined after day 28, prophylactic immunosuppression routinely continued in the vast majority of patients for several more months, with 94% still receiving such prophylaxis on day 100. The contribution of relapse to mortality was higher in patients who had not developed early GVHD (Supplementary Figure 2) compared to those with early GVHD (Supplementary Figure 3). The vast majority of relapses occurred after the second month following HCT, suggesting that an immunologic intervention to enhance GVL effect, such as an early taper of prophylactic immunosuppression beginning on day 28, might be feasible.

Previous attempts at early tapers of prophylactic immunosuppression have resulted in spikes of severe GVHD with no overall improvement in DFS, dampening enthusiasm for this approach[12, 13]. We therefore sought to identify a subset of the patients who had not developed early GVHD and who were at low risk for severe GVHD but were at high risk for relapse and might benefit from efforts to enhance the graft-vs-leukemia effect. We analyzed biomarkers in serum samples on day 28 after HCT from 1,207 patients (Table 1) and used the previously validated MAP threshold of 0.16[25] to demarcate risk categories. We observed that a very large majority of patients (86% to 88%) were classified as low risk for severe GVHD and NRM (Table 2). Only one quarter of these low risk patients developed GVHD, and biomarkers classified the large majority of GVHD cases as low risk disease in both cohorts so that only 9% of these patients developed clinically severe (grade III/IV) GVHD (Table 2). Relapse was the primary cause of death in these patients and increased steadily after the second month, leading to suboptimal (77–78%) overall survival at one year (Figure 3). In patients whose malignancies were classified as high/very high DRI prior to HCT, the rate of relapse reached almost 35% by one year (Figure 4).

Patients with a high day 28 MAP represented a significant minority (12–14%) of the population of patients who did not developed early GVHD. Non-relapse mortality was common in these patients with a cumulative incidence of 31% by one year that was largely explained by the development of more severe GVHD measured by clinical symptoms at maximum grade (III/IV, 16%) and biomarker scores at onset (Ann Arbor 2/3, historical

cohort 69%, current cohort, 78%) (Table 2). But relapse rates also remained high in these patients resulting in poor one-year survival (~50%) (Supplementary Figure 4).

As already noted, 25% of patients developed GVHD early (Supplementary Table 3). The cumulative incidence of NRM, relapse, and one year OS for patients who developed early GVHD are shown in Supplementary Figure 3. In contrast to the deaths of patients who did not develop early GVHD, deaths of patients with early GVHD were due primarily to non-relapse causes rather than relapse (70% vs 30%, p=0.004). Patients who developed GVHD also exhibited increasing rates of NRM based on their Ann Arbor score at GVHD onset (Supplementary Figure 5). A majority of patients developed Ann Arbor 1 GVHD and were at low risk for NRM (<10%) at one year, a population of patients that might benefit from less intensive immunosuppressive treatment of GVHD.

Discussion:

Relapse of primary malignancy and GVHD are the major risks for mortality after HCT, and thus the separation of GVHD from the GVL effect has been a long-time goal for allogeneic HCT. However, such separation has proved highly elusive despite numerous attempts to improve GVHD immunosuppressive prophylaxis strategies. Because prophylactic strategies by definition have no specificity, patients who are not at risk for GVHD may experience toxicity such as increased serious infections or a diminished GVL effect, with no change in survival.

In this large dataset acute GVHD occurred earlier than relapse after HCT with few cases diagnosed after the second month. By contrast, relapses were practically nonexistent in the first month after HCT but then accelerated, with the vast majority of relapses after the second month. Because the risk for severe GVHD persists for months, most patients do not begin immunosuppression tapers until well into the risk period for relapse[36]. In this study we determined whether the use of predictive GVHD biomarkers could identify patients at minimal risk for severe GVHD and who would therefore be good candidates to test whether an early taper of immunosuppression might lead to an enhanced GVL effect and fewer relapses.

As presented here, a single measurement of the MAP at day 28 after HCT enables such a risk adapted approach. In this analysis of more than 1600 HCT patients at 18 different centers two-thirds had not developed GVHD and were categorized as low MAP on day 28. In these patients, the overall incidence of relapse greatly exceeds the incidence of NRM, and they might therefore benefit from an early taper of immunosuppression and more frequent monitoring for MRD. Furthermore, in those patients without GVHD after discontinuation of the immunosuppression, preemptive strategies to prevent relapse could also be tested, enabling clinicians to move beyond a “watchful waiting” posture for relapse. The safety of such an approach could first be evaluated in patients at greatest risk for relapse, such as high/very high DRI, but it should be noted that even in patients with low/intermediate DRI the risk for relapse is twice that for NRM. Patients with low MAPs who undergo a rapid taper and subsequently develop GVHD but maintain a low MAP would be eligible for risk-adapted treatment of GVHD with less intensive immunosuppression.

Other measures currently used to determine risk for relapse during the first few months post-HCT include serial measurements of donor chimerism and quantification of MRD. Both of these tools are highly specific but lack sensitivity because up to 50% of relapses occur in patients without either risk factor[15, 16, 37, 38]. This data set did not include serial monitoring of chimerism or MRD, but we speculate that the day 28 MAP could complement these assessments of the risk of relapse.

Severe GVHD was disproportionately high in the minority of patients with a high day 28 MAP. As a result, such patients would not be candidates for an early taper of immunosuppression. Unfortunately, the high incidence of severe GVHD and NRM was not offset by fewer relapses and there was no clearly dominant risk factor for mortality. Novel strategies to improve outcomes for these patients at high risk for both relapse and NRM are still needed.

As shown in Supplementary Figure 3, GVHD was the primary cause of death in patients who developed GVHD early. However, within this group of patients (25% of all patients), biomarkers predicted outcomes as recently reported[25, 26]. NRM was very low in the patients who developed Ann Arbor 1 GVHD but these patients also experienced significant rates of relapse and therefore they would also be eligible for testing the effectiveness of less intensive immunosuppression, such as steroid sparing agents or low dose steroids.

A strength of this multicenter study is the similarity in outcomes in both an earlier cohort and a large, recently transplanted cohort despite differences in practices. However, it should be emphasized that any risk adapted approach needs to be tested through carefully designed clinical trials. Because the strength of GVL varies among hematologic malignancies[39, 40], patient selection for initial studies should focus on populations who are at high risk for relapse and who are most likely to respond favorably to immunotherapy. This group could include patients with very high/high DRI pre-transplant but without overt relapse and whose malignancy has consistently shown a high response rate to immunologic interventions like donor leukocyte infusions (DLI) (e.g., AML or lymphoma)[41, 42]. A rapid taper of immunosuppression or other interventions, such as prophylactic DLI[42, 43] or pegylated interferon-2α[44], to enhance GVL can result in the development of acute GVHD or increase the incidence of chronic GVHD. Repeat monitoring of biomarkers beyond the schedule reported in this study may be able to offer further guidance regarding the appropriate intensity of immunosuppression throughout a patient's clinical course. Such trials could accelerate our attempts to balance the risks of relapse and GVHD and eventually increase our long term goal of improved survival following HCT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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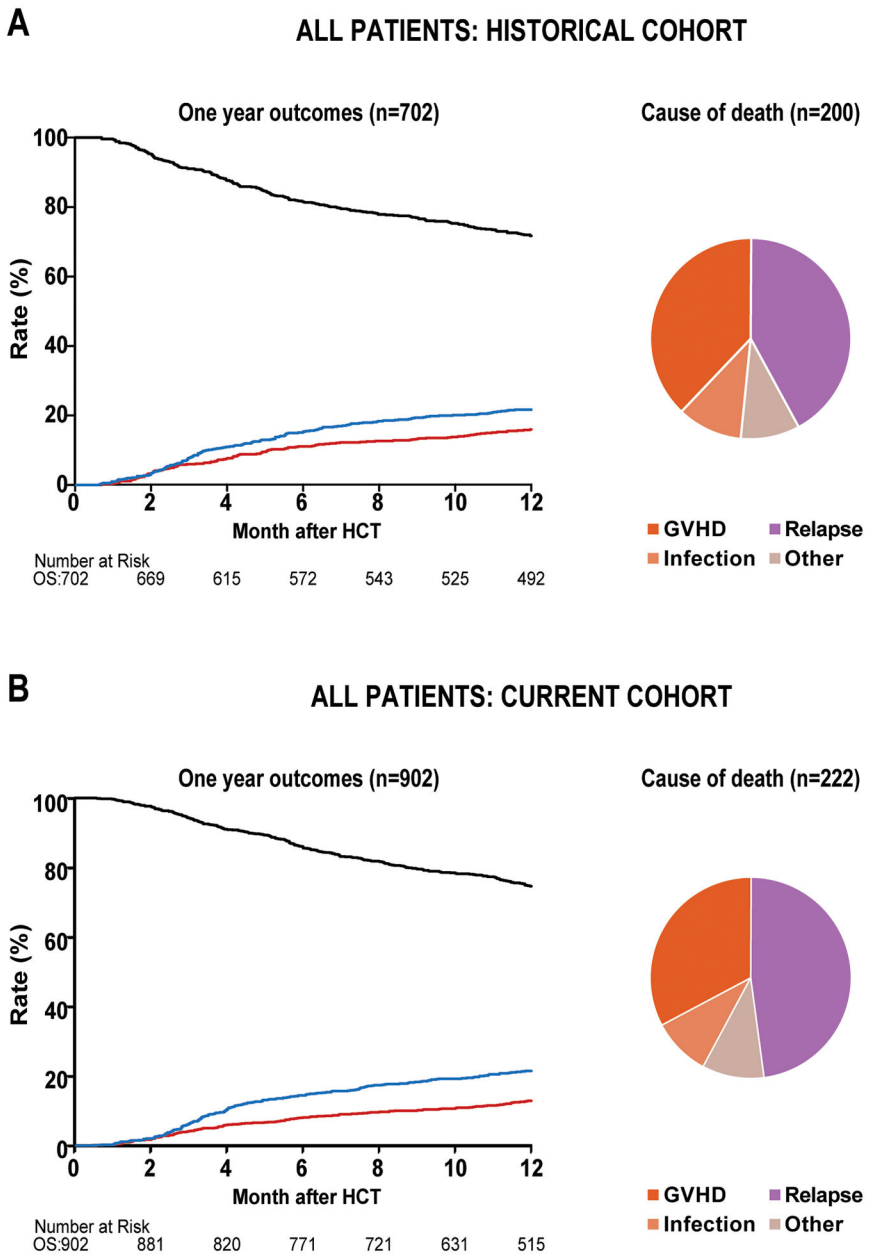

Figure 1. One year outcomes of the entire population.

A: Historical Cohort. 12-month cumulative incidence of NRM (red: 16%), relapse (blue: 22%), and OS (black: 72%) for all patients in the historical cohort (n=702) are displayed. Deaths (n=200) were categorized as GVHD (red), relapse (blue), infection (grey), and other (dark red). **B: Current Cohort**. 12-month cumulative incidence of NRM (red: 13%), relapse (blue: 21%), and OS (black: 75%) for all patients in the current cohort (n=902) are displayed. Deaths (n=222) were categorized as GVHD (dark orange), relapse (purple), infection (light orange), and other (beige). All causes of death are shown in Supplementary Table 4.

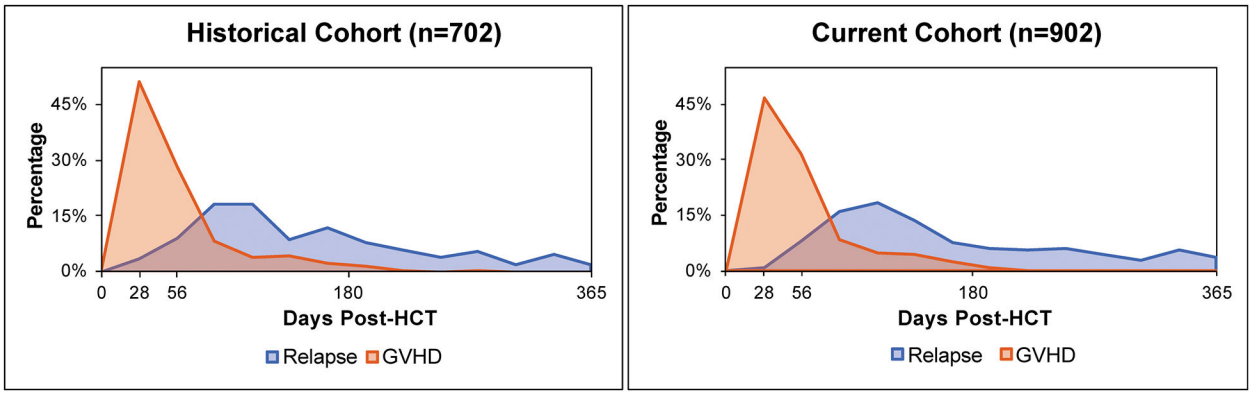

Figure 2. Distribution of grade II-IV GVHD and relapse within 1-year post-HCT.

A. Historical cohort. N=702. Maximum GVHD grades II-IV (orange) and relapse (blue) cases were counted in 28 day increments. **B. Current cohort**. N=902. Maximum GVHD grades II-IV (red) and relapse (blue) cases were counted in 28 day increments.

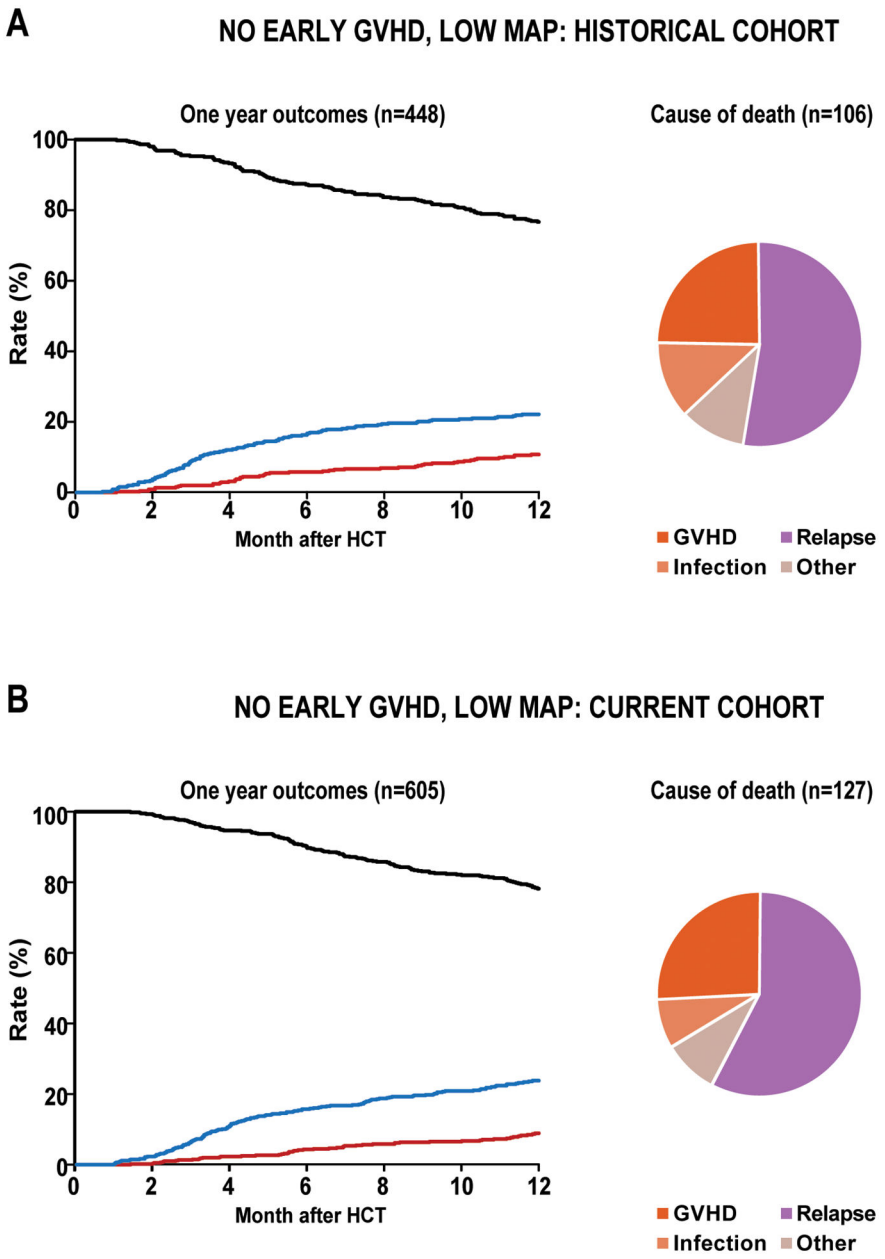

Figure 3. One year outcomes for patients with no early GVHD and low MAP.

A: Historical Cohort. 12-month cumulative incidence of NRM (red: 11%), relapse (blue: 22%), and OS (black: 77%) for this subset of patients in the historical cohort (n=448) are displayed. Deaths (n=106) were categorized as GVHD (dark orange), relapse (purple), infection (light orange), and other (beige). **B: Current Cohort**. 12-month cumulative incidence of NRM (red: 9%), relapse (blue: 24%), and OS (black: 78%) for this subset of patients in the current cohort (n=605) are displayed. Deaths (n=127) were categorized as GVHD (dark orange), relapse (purple), infection (light orange), and other (beige).

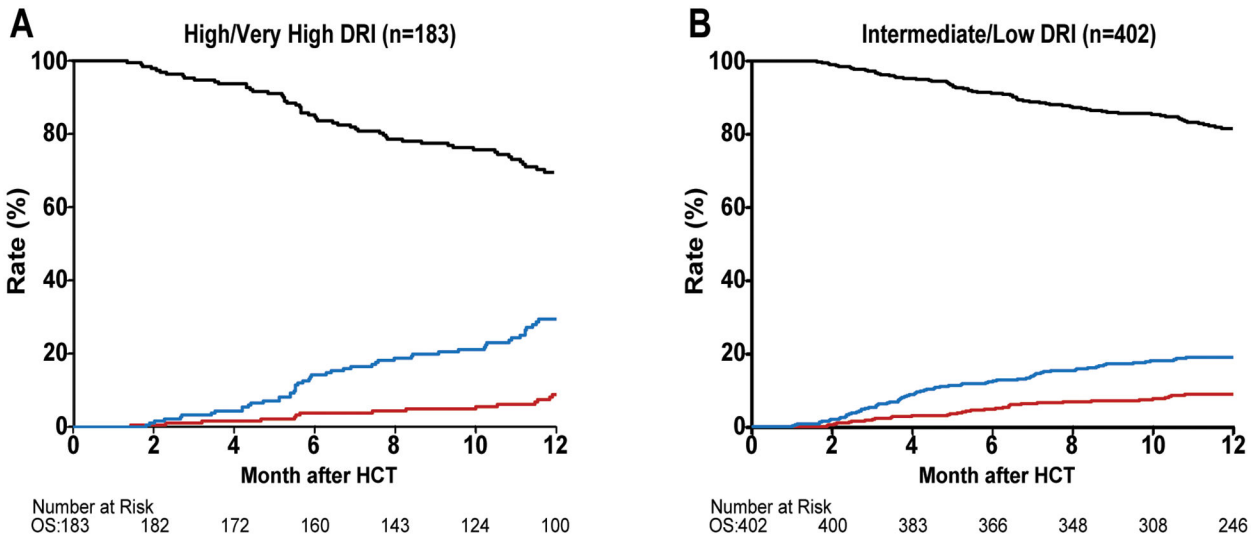

Figure 4. Relapse, NRM, and OS by DRI for current cohort patients without early GVHD and low MAP.

DRI was available for 585/605 patients. Patients were divided into two risk groups by DRI (high/very high, n=183; low/intermediate, n=402). Cumulative incidence curves for 12-month NRM, relapse, and OS were created for each risk group. **A. Very high/high DRI**: NRM (red: 9%), relapse (blue: 33%), and OS (black: 70%). **B. Low/intermediate DRI:** NRM: (red: 9%), relapse (blue: 19%), and OS (black: 83%).

Table 1.

Patient characteristics (no early GVHD)

Patient Characteristics	Historical Cohort (N=522)	Current Cohort (N=685)
Median Age - yr (range)	55 (1–76)	55 (1–78)
Indication for HCT - no. (%)		
Acute leukemia	308 (59%)	385 (56%)
MDS/MPN	133 (26%)	209 (31%)
Lymphoma	44 (8%)	58 (8%)
Other malignant	37 (7%)	33 (5%)
Donor type - no. (%)		
Related	220 (42%)	193 (28%)
Unrelated	293 (56%)	408 (60%)
Haploidentical	9 (2%)	84 (12%)
HLA-match - no. (%)		
Matched	436 (84%)	504 (74%)
Mismatched	77 (14%)	97 (14%)
Haploidentical	9 (2%)	84 (12%)
Stem cell source - no. (%)		
Marrow	88 (17%)	139 (20%)
Peripheral blood	407 (78%)	520 (76%)
Cord	27 (5%)	26 (4%)
Conditioning Regimen Intensity - no. (%)		
Full	372 (71%)	403 (59%)
Reduced	150 (29%)	282 (41%)
GVHD prophylaxis - no. (%)		
CNI/MTX ± other	355 (68%)	385 (56%)
CNI/MMF ± other	139 (26.6%)	137 (20%)
Tac + Sirolimus	5 (1%)	28 (4%)
Cyclophosphamide based	21 (4%)	100 (15%)
T cell depletion	0	28 (4%)
Other	2 (0.4%)	7 (1%)
GVHD serotherapy prophylaxis: no. (%)		
ATG	95 (18%)	247 (36%)
No ATG	427 (82%)	438 (64%)
Maximum GVHD grade no. (%)		
Grade 0	317 (61%)	404 (59%)
Grade I	77 (15%)	92 (13%)
Grade II	74 (14%)	123 (18%)
Grade III-IV	54 (10%)	66 (10%)
Disease Risk Index		

Patient Characteristics	Historical Cohort (N=522)	Current Cohort (N=685)
Very High/High	-	212 (31%)
Intermediate	-	414 (60%)
Low	-	34 (5%)
Unknown	-	25 (4%)

Table 2:

Outcomes in patients who did not develop GVHD by day 28, according to cohort and MAP on day 28

Historical (n=522)	**Low MAP (n=448, 86%)**		**High MAP (n=74, 14%)**	
Ann Arbor Score (n=108)	n	%	n	%
Ann Arbor 1	59	62%	4	31%
Ann Arbor 2	21	22%	6	46%
Ann Arbor 3	15	16%	3	23%
GVHD	n	%	n	%
Grades II-IV (n=128)	104	23%	24	32%
Grades III/IV (n=54)	42	9%	12	16%
12-month NRM	49	11%	23	31%
Current (n=685)	**Low MAP (n=605, 88%)**		**High MAP (n=80, 12%)**	
Ann Arbor Score (n=171)	n	%	n	%
Ann Arbor 1	110	72%	4	22%
Ann Arbor 2	26	17%	7	39%
Ann Arbor 3	17	11%	7	39%
GVHD	n	%	n	%
Grades II-IV (n=190)	167	27%	23	29%
Grades III/IV (n=67)	54	9%	13	16%
12-month NRM	52	9%	25	31%