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ARTICLE

Compatibility at amino acid position 98 of MICB reduces the incidence of graft-versus-host disease in conjunction with the CMV status

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Abstract

Graft-versus-host disease (GVHD) and cytomegalovirus (CMV)-related complications are leading causes of mortality after unrelated-donor hematopoietic cell transplantation (UD-HCT). The non-conventional MHC class I gene *MICB*, alike *MICA*, encodes a stress-induced polymorphic NKG2D ligand. However, unlike MICA, MICB interacts with the CMV-encoded UL16, which sequestrates MICB intracellularly, leading to immune evasion. Here, we retrospectively analyzed the impact of mismatches in MICB amino acid position 98 (MICB98), a key polymorphic residue involved in UL16 binding, in 943 UD-HCT pairs who were allele-matched at *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQB1* and *MICA* loci. *HLA-DP* typing was further available. *MICB98* mismatches were significantly associated with an increased incidence of acute (grade II–IV: HR, 1.20; 95% CI, 1.15 to 1.24; $P < 0.001$; grade III–IV: HR, 2.28; 95% CI, 1.56 to 3.34; $P < 0.001$) and chronic GVHD (HR, 1.21; 95% CI, 1.10 to 1.33; $P < 0.001$). *MICB98* matching significantly reduced the effect of CMV status on overall mortality from a hazard ratio of 1.77 to 1.16. *MICB98* mismatches showed a GVHD-independent association with a higher incidence of CMV infection/reactivation (HR, 1.84; 95% CI, 1.34 to 2.51; $P < 0.001$). Hence selecting a *MICB98*-matched donor significantly reduces the GVHD incidence and lowers the impact of CMV status on overall survival.

Introduction

Unrelated-donor hematopoietic cell transplantation (HCT) is an established treatment for a wide range of immunological and hematologic disorders, malignant, or otherwise [1]. Although more than 50,000 HCTs are performed annually worldwide [2, 3], adverse clinical outcomes occur frequently. One of the most common life-threatening complications is graft-versus-host disease (GVHD), which greatly hampers the successful outcome of this powerful and sometimes unique curative option. In GVHD, the donor's immune cells attack the patient's organs and tissues, impairing their ability to function and increasing the patient's susceptibility to infection. The organs/tissues most frequently targeted are the skin, the gastrointestinal tract, and the liver. Despite the availability of effective immunosuppressive drugs, the incidence of GVHD remains alarmingly high: up to 35% experience grade III–IV acute GVHD and 40% to 50% experience chronic GVHD [4–6].

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Cytomegalovirus (CMV) infection/reactivation represents another leading cause of morbidity and mortality in patients undergoing allogeneic HCT because it frequently causes serious complications, e.g., pneumonia, hepatitis, gastroenteritis, retinitis, and encephalitis [7–11]. Because of the immunosuppressive regimen, allogeneic HCT patients are indeed at a higher risk for CMV infection and/or reactivation. The incidence of CMV infection has been reported to vary between 40 and 80% in CMV seropositive allogeneic HCT patients not treated with anti-viral prophylaxis drugs, which currently represents most of the allogeneic HCT recipients [12–18]. In seronegative patients receiving a transplant from a seropositive donor, the rate of primo infection is ~30% [12]. Despite the implementation of prophylaxis, monitoring, and pre-emptive treatment of CMV reactivation/infection, cases of CMV seropositivity of the donor and/or the recipient show decreased survival rates compared to CMV-seronegative recipients who undergo allograft from CMV-seronegative donors [16, 19]. New strategies for preventing CMV reactivation/infection in transplant recipients therefore remain an important objective for the improvement of allogeneic HCT.

Increasing the degree of human leukocyte antigen (HLA) matching is one of the most important strategies to lower the risks of both GVHD and CMV infections. The former is a direct consequence of better HLA matching, whereas the latter is an indirect effect due to the well-described association of CMV infection with GVHD occurrence [20, 21]. However, even in genotypically HLA-matched donors and recipients, the incidence of grade III–IV acute GVHD and CMV reactivation/infection can be as high as 30% and 80%, respectively [13, 22]. For CMV infection/reactivation, other risk factors include age, source of stem cells, disease, and donor (D)/recipient (R) CMV serological status [23, 24].

The MHC-encoded non-conventional *MHC class I chain-related (MIC)* genes *A (MICA)* and *B (MICB)* encode polymorphic cell-surface proteins, which bind to NKG2D; an activating immune receptor expressed by cytotoxic T and NK cells [25, 26]. This interaction is seminal in defense both against infections and malignancies. Moreover, *MICB* [27, 28] happens to be one of the most promising candidates to explain, at least partially, GVHD and CMV complications that cannot be attributed to classical HLA genes or the related *MICA* gene incompatibilities [29–31]. *MICB* is indeed highly polymorphic, with 109 alleles reported to date (http://www.ebi.ac.uk/ipd/imgt/hla/stats.html). It encodes a cell-surface glycoprotein upregulated by cell stress [25, 32]. The gene is located 130 kb and 83 kb centromeric to *HLA-B* and *MICA*, respectively, and was discovered by us over 20 years ago [25]. *MICB* is highly similar to *MICA* in terms of sequence (83% shared amino acid sequence identity), linkage disequilibrium with *HLA-B*, protein structure (HLA-like structure without association to $ß_2$-microglobulin), and constitutive expression pattern (restricted to epithelial cells, fibroblasts, monocytes, dendritic cells and endothelial cells) [26, 33, 34]. MICB is a ligand for the activating NKG2D receptor expressed on the surface of cytotoxic $CD8^+$ αβ and γδ T lymphocytes and natural killer cells [35]. Interestingly, and in contrast to MICA, MICB binds the CMV protein UL16, which sequestrates MICB intracellularly in an immune escape mechanism [36]. Different *MICB* alleles are not equal with respect to binding to UL16. *MICB*008* has been shown to have a decreased binding capacity to UL16 compared to other alleles [37]. *MICB*008* is characterized by a polymorphism at amino acid position 98, causing an isoleucine (Ile) to methionine (Met) exchange in the α2 domain of the MICB protein. The variation Ile > Met is exclusively present in *MICB*008* and is the unique polymorphic position that is in direct contact with UL16 through hydrophobic contacts (distance < 4.0 Å) with leucine 161 of UL16 [38].

Several lines of evidence indicate that *MICB* could play a role in triggering GVHD and/or modulating CMV infection/reactivation: (1) the localized expression in epithelial cells of the gastrointestinal tract, whose damage during GVHD plays a major pathophysiologic role in the amplification of systemic disease [39]; (2) the common features with *MICA* that have repeatedly been shown to be involved in GVHD [29, 30, 40–42]; and (3) the binding of MICB to the UL16 protein [36]. The present study hence aims to show the effect of *MICB* matching at amino acid position 98, representing about 6% of transplantations, on the outcome of unrelated-donor HCT in a cohort of 943 donor/recipient pairs matched for *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQB1*, and *MICA*.

Patients and methods

Study design and oversight

This retrospective study was designed to test whether donor–recipient matching at amino acid position 98 of the MICB protein (*MICB98*) improves the outcome of unrelated HCT. Patients from six French and three Dutch centers and their donors were included; the unrelated donors originated from national or international donor registries. Genomic DNA samples and high-resolution *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQB1*, *-DPB1,* and *MICA* typing data were collected. Clinical information was made available by the SFGM-TC and the HOVON Data Center from the European group for Blood and Marrow Transplantation ProMISe patient database. All authors vouch for the accuracy and completeness of the results. This study, conducted under the auspices of SFGM-TC and the Dutch–Belgian Cooperative Trial Group for Hematology

Oncology (HOVON), was approved by institutional review boards of the participating centers and was performed according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Patients and donors

The study population consisted of 943 patients who underwent unrelated HCT for the treatment of blood disorders between 2005 and 2013. All patients received a first allogeneic transplant using bone marrow or peripheral blood stem cells, and donor–recipients were matched for 12 of the 12 possible alleles at *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQB1*, and *MICA* loci (Table 1).

MICB genotyping at amino acid position 98

The polymorphic nucleotide position 363 (C/G; rs3134900) causes an isoleucine (Ile) to methionine (Met) change at amino acid position 98 in the α2 domain of the MICB protein. Both patients and unrelated donors were genotyped for this position by Sanger sequencing of *MICB's* exon 3, following previously described protocols [43]. The sequences were analyzed using Seqscape v2.6 (Life Technologies, USA) to assign genotypes.

Definitions

Grading of acute and chronic GVHD was performed according to the classification of Glucksberg et al. [44]. For acute GVHD, severe corresponds to grades III and IV. CMV positivity of the donor and/or the recipient was defined by the presence of anti-CMV IgG in the serum of the donor and/or the recipient. CMV reactivation was defined as the time from transplantation to the first CMV infection episode. In addition to clinical examination, CMV infection/reactivation episodes were characterized at a molecular level by a viral load > 10^4 copies/ml as determined by quantitative PCR on whole blood. Overall survival (OS) was defined as the time from transplantation to death by any cause. Relapse-free survival (RFS) was defined as the time to relapse of primary disease or death by any cause, whichever came first. Non-relapse mortality (NRM) corresponds to mortality within the first complete remission of disease. Causes of death unrelated to transplantation included deaths related to relapse, progression of the original disease, secondary malignancy, and cell therapy (non-HCT). OS, RFS, NRM, GVHD, and CMV reactivation were censored at the time of the last follow-up. Incidences of clinical outcomes were defined as the cumulative probability of the outcomes at any given point.

Statistical analysis

After validating that the data meet requested assumptions, the distribution of each covariate between the *MICB98*-matched and mismatched groups was assessed by Pearson's Chi square test or Fisher's exact test for small sample sizes. The variances between the two groups were similar for the different variables assessed in our models and statistical tests (average variances in the matched and mismatched groups were 1.36 and 1.40, respectively). Multivariable competing risk regression analyses were performed for acute GVHD II-IV, acute GVHD III-IV, chronic GVHD, relapse, NRM, and CMV reactivation, using an extended Fine and Gray model [45–47]. For OS and RFS, Cox proportional regression models were used [48]. Competing events were defined as death without GVHD and relapse for GVHD endpoints (acute and chronic GVHD); death from any cause other than transplantation for NRM; relapse and death for CMV reactivation; and NRM for relapse. All statistical models were adjusted for center effect and covariates defining the European Society for Blood and Marrow Transplantation risk score: patient age, disease stage at transplantation, time to transplantation, and donor–recipient sex combination. In addition to these, the following relevant variables were included: *HLA-DPB1* matching (T-cell epitope matching level as defined by Fleischhauer et al. [49]), patient–donor serological status for CMV, year of transplantation, source of stem cells, conditioning regimen, GVHD prophylaxis, treatment with antithymocyte globulin or Alemtuzumab, and disease category. Interactions between patient–donor serological status for CMV and matching at amino acid position 98 of *MICB* were also assessed in the multivariable analyses [50, 51]. All models were checked for interactions and proportional hazards assumptions. All statistical analyses were conducted using the computing environment R [52].

Results

The demographics of the study population are shown in Table 1. The median posttransplant follow-up was 36 months (mean: 37 months; range: 1–105 months), and the median patient age was 53 years (mean: 48 years; range: 1–73 years). The patients suffered from both malignant and non-malignant diseases. Most transplants were performed with non-myeloablative/reduced intensity conditioning regimens (67%); in vivo T-cell depletion was performed in the majority of cases (73%), and peripheral blood was the main source for stem cells (79%). All donor/patient pairs were fully typed at high resolution (2nd field) for *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQB1*, *-DPB1* and *MICA* [29] and were matched for 12 out of 12 alleles at *HLA-A*, *-B*, *-C*, *-DRB1*,

Table 1 Demographics of the study population.

	Total transplants ($n = 943$)	MICB98 matched transplants ($n = 887$)	MICB98 mismatched transplants ($n = 56$)	P value[a]
Transplantation centers[b]				0.16
1	106 (11.2%)	100 (11.3%)	6 (10.7%)	
2	158 (16.8%)	142 (16%)	16 (28.6%)	
3	114 (12.1%)	109 (12.3%)	5 (8.9%)	
4	157 (16.6%)	153 (17.2%)	4 (7.1%)	
5	48 (5.1%)	47 (5.3%)	1 (1.8%)	
6	99 (10.5%)	90 (10.1%)	9 (16.1%)	
7	96 (10.2%)	91 (10.3%)	5 (8.9%)	
8	49 (5.2%)	46 (5.2%)	3 (5.4%)	
9	116 (12.3%)	109 (12.3%)	7 (12.5%)	
Age at transplant (years)				0.034
0–17	58 (6.2%)	57 (6.4%)	1 (1.8%)	
18–49	360 (38.2%)	333 (37.5%)	27 (48.2%)	
50–64	458 (48.6%)	430 (48.5%)	28 (50%)	
65 or older	67 (7.1%)	67 (7.6%)	0 (0%)	
Year of transplantation				0.97
2005–2008	360 (38.2%)	338 (38.1%)	22 (39.3%)	
2009–2013	583 (61.8%)	549 (61.9%)	34 (60.7%)	
Patient–donor sex				1.00
Male–Female	159 (16.9%)	150 (16.9%)	9 (16.1%)	
Other combinations	779 (82.6%)	732 (82.5%)	47 (83.9%)	
Missing	5 (0.5%)	5 (0.6%)	0 (0%)	
Patient–donor CMV status				0.082
neg.-neg.	357 (37.9%)	329 (37.1%)	28 (50%)	
pos.-neg./neg.-pos./pos.-pos.	560 (59.4%)	533 (60.1%)	27 (48.2%)	
Missing	26 (2.7%)	25 (2.8%)	1 (1.8%)	
Source of cells				1.00
Bone marrow	195 (20.7%)	183 (20.6%)	12 (21.4%)	
Peripheral blood stem cells	748 (79.3%)	704 (79.4%)	44 (78.6%)	
Conditioning regimen				0.79
Non-myeloablative/reduced intensity	635 (67.3%)	598 (67.4%)	37 (66.1%)	
Myeloablative without total-body irradiation	140 (14.8%)	130 (14.7%)	10 (17.9%)	
Myeloablative with total-body irradiation	167 (17.7%)	158 (17.8%)	9 (16.1%)	
Missing	1 (0.1%)	1 (0.1%)	0 (0%)	
GvHD prophylaxis				0.49
Cyclosporin only	183 (19.4%)	171 (19.3%)	12 (21.4%)	
Cyclosporin and Methotrexate	243 (25.8%)	231 (26%)	12 (21.4%)	
Cyclosporin and mycophenolate	360 (38.2%)	335 (37.8%)	25 (44.6%)	
Other combinations	135 (14.3%)	130 (14.7%)	5 (8.9%)	
Missing	22 (2.3%)	20 (2.2%)	2 (3.6%)	
In vivo T-cell depletion[c]				0.34
No	231 (24.5%)	214 (24.1%)	17 (30.3%)	

Table 1 (continued)

	Total transplants ($n = 943$)	MICB98 matched transplants ($n = 887$)	MICB98 mismatched transplants ($n = 56$)	*P* value[a]
Yes	690 (73.2%)	653 (73.6%)	37 (66.1%)	
Missing	22 (2.3%)	20 (2.3%)	2 (3.6%)	
Disease				0.99
Acute myeloid leukemia	240 (25.5%)	225 (25.4%)	15 (26.8%)	
Chronic myeloid leukemia	34 (3.6%)	32 (3.6%)	2 (3.6%)	
Acute lymphoblastic leukemia	121 (12.8%)	114 (12.9%)	7 (12.5%)	
Myelodysplastic syndrome	161 (17.1%)	152 (17.1%)	9 (16.1%)	
Non-Hodgkin lymphoma	127 (13.5%)	121 (13.6%)	6 (10.7%)	
Others[d]	260 (27.6%)	243 (27.4%)	17 (30.4%)	
Disease stage at transplantation[e]				0.97
Early	371 (39.3%)	348 (39.2%)	23 (41.1%)	
Late	507 (53.8%)	477 (53.8%)	30 (53.6%)	
Not applicable‖	44 (4.7%)	42 (4.7%)	2 (3.6%)	
Missing	21 (2.2%)	20 (2.3%)	1 (1.8%)	
Time until treatment				0.65
<12 months	440 (46.7%)	416 (46.9%)	24 (42.9%)	
>12 months	503 (53.3%)	471 (53.1%)	32 (57.1%)	
Non-Permissive HLA-DPB1 matching[f]				0.42
Matched	420 (44.5%)	392 (44.2%)	28 (50%)	
Mismatched	394 (41.8%)	374 (42.2%)	20 (35.7%)	
Missing	129 (13.7%)	121 (13.6%)	8 (14.3%)	

The results are presented as the number of patients and corresponding percentages of the study population. All clinical variables of the table were used for adjustment in the multivariate models.

HLA human leukocyte antigen.

[a]*P* values were determined with Pearson's Chi square test or Fisher's exact test for small sample sizes

[b]Patients received their transplant in six centers of the Francophone Society of Bone Marrow Transplantation and Cell Therapies (SFGM-TC) (1–6; $N = 682$) and in three Dutch centers that are part of the Europdonor operated by the Matchis Foundation network (7–9; $N = 261$).

[c]In vivo T-cell depletion was performed by the addition of antithymocyte globulin (ATG) or Alemtuzumab to the conditioning regimen.

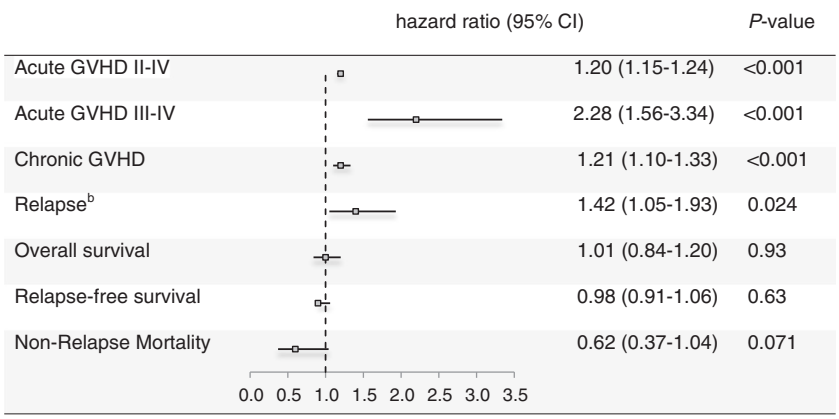
[d]Other diseases include multiple myeloma, Hodgkin lymphoma, Fanconi anemia, aplastic anemia, chronic lymphocytic leukemia, plasma cell leukemia, other acute leukemias, solid tumors (not breast), hemophagocytosis and inherited disorders.

[e]Early corresponds to diseases in the first complete remission or in the chronic phase. Late corresponds to second or higher complete remissions, accelerated phases, partial remissions, progressions, primary induction failures, relapses or stable diseases. Not applicable corresponds to bone marrow failure (aplastic anemia, Fanconi anemia), inherited disorders, hemophagocytosis and solid tumors.

[f]*HLA-DPB1* matching was defined at the T-cell-epitope matching level [49] with typing data at 2nd field resolution following the World Health Organization official nomenclature.

-DQB1 and *MICA* loci. Among the 943 transplantations, 394 (41.8%) had non-permissive *HLA-DPB1* mismatches. Fifty-six (5.9%) transplants were *MICB98* mismatched. The mismatch vectors of these 56 transplants were graft-versus-host ($n = 22$), host-versus-graft ($n = 33$) and bidirectional ($n = 1$). Except for the patient–donor CMV status, all relevant covariates for the analyzed clinical outcomes were equally distributed in the *MICB98*-matched and -mismatched patients (Table 1). Organ-specific sub-analyses showed that the *MICB98* matching effect was more important in the gut and the skin than in the liver (supplemental Fig. 1). *MICB98* mismatches were significantly associated with an increased incidence of acute GVHD (hazard ratio (HR) for grades II–IV: 1.20; 95% CI, 1.15 to 1.24; $P < 0.001$; for grades III–IV: 2.28; 95% CI, 1.56 to 3.34; $P < 0.001$) (Table 2). At day 100 post-HCT, the cumulative incidences of severe (grades III–IV) acute GVHD in *MICB98* mismatched vs. matched transplantations were 18.9% vs. 12.5%, respectively (Fig. 1a). Matching *MICB* at position 98 decreased the risk of chronic GVHD by 4% (40.9% vs. 36.9%) at 4 years post transplantation (HR, 1.21; 95% CI, 1.10 to 1.33; $P < 0.001$)

Table 2 Analysis of the impact of *xMICB* mismatches at amino acid position 98 on clinical outcomes after multivariate modeling[a].



GVHD graft-versus-host disease.

[a]All models were adjusted for patient's age, patient–donor sex, patient–donor serological status for cytomegalovirus, year of transplantation, time to transplantation, transplantation center, source of stem cells, conditioning regimen, GVHD prophylaxis, treatment with antithymocyte globulin or Alemtuzumab, *HLA-DPB1* matching status, disease category and severity at transplantation.

[b]Transplantations performed for non-malignant diseases were excluded from the analysis.

Results are presented as hazard ratios with 95% confidence intervals (CI).

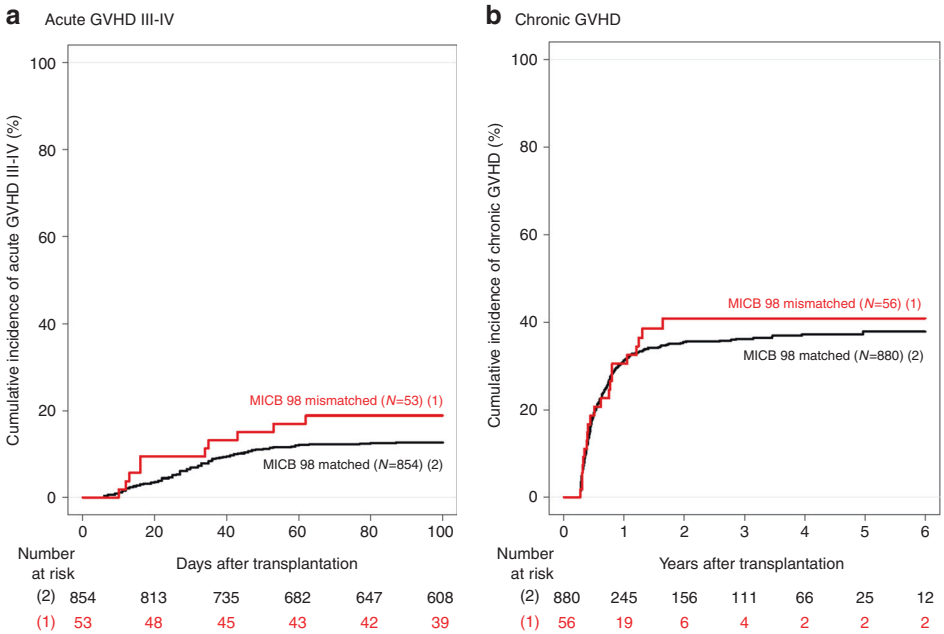


Fig. 1 Effect of *MICB98* matching on severe acute and chronic GVHD. The cumulative incidences of grades III–IV acute (**a**) and chronic GVHD (**b**) are shown for *MICB98* mismatched (1) versus matched (2) patients.

(Table 2 and Fig. 1b). In addition, *MICB98* mismatches were associated with a higher rate of relapse (HR, 1.42; 95% CI, 1.05 to 1.93; $P = 0.024$).

Knowing that amino acid position 98 is involved in the binding of MICB to the UL16 protein of the CMV, we assessed the interaction between *MICB98* mismatches and the CMV status in their effect on clinical outcomes. For this purpose, we performed multivariate analyses and included an interaction factor in the model. Table 3 represents the risks of various clinical outcomes associated with (1) *MICB98* mismatches when donor and recipients are negative for CMV, (2) CMV positivity in donor and/or recipients when *MICB98* is matched and (3) the interaction of *MICB98* matching with CMV status. A statistically significant value for the interaction factor indicates that the effect of *MICB98* matching depends on the category of CMV status and vice versa. When the HR of the interaction factor is <1 or >1, the HR of a variable (here, *MICB98*

Table 3 Analysis of the Impact of *MICB* Mismatches at position 98, CMV status and their interaction on clinical outcomes after multivariate modeling[a].

Outcomes and risk factors	Hazard ratio (95% CI)	*P* value
Acute GVHD II-IV		
MICB98 matching (mismatches)	1.47 (1.05–2.07)	0.025
CMV status (D+/R− or D-/R+ or D+/R+)[b]	1.18 (0.92–1.51)	0.2
Interaction: *MICB98* matching X CMV status	0.57 (0.29–1.10)	0.095
Acute GVHD III-IV		
MICB98 matching (mismatches)	3.63 (3.15–4.18)	<0.001
CMV status (D+/R− or D−/R+ or D+/R+)	1.50 (1.15–1.96)	0.003
Interaction: *MICB98* matching X CMV status	0.26 (0.17–0.40)	<0.001
Chronic GVHD		
MICB98 matching (mismatches)	1.26 (1.25–1.27)	<0.001
CMV status (D+/R− or D−/R+ or D+/R+)	1.34 (1.15–1.56)	<0.001
Interaction: *MICB98* matching X CMV status	0.91 (0.70–1.18)	0.48
Relapse[c]		
MICB98 matching (mismatches)	0.89 (0.78–1.01)	0.073
CMV status (D+/R− or D−/R+ or D+/R+)	0.77 (0.70–0.84)	<0.001
Interaction: *MICB98* matching X CMV status	2.61 (1.79–3.82)	<0.001
Overall survival		
MICB98 matching (mismatches)	0.80 (0.64–1.00)	0.054
CMV status (D+/R− or D−/R+ or D+/R+)	1.16 (1.14–1.19)	<0.001
Interaction: *MICB98* matching X CMV status	1.53 (1.38–1.69)	<0.001
Relapse-free survival		
MICB98 matching (mismatches)	0.78 (0.70–0.86)	<0.001
CMV status (D+/R− or D−/R+ or D+/R+)	1.09 (1.05–1.13)	<0.001
Interaction: *MICB98* matching X CMV status	1.57 (1.45–1.70)	<0.001
Non-relapse mortality		
MICB98 matching (mismatches)	1.14 (0.46–2.86)	0.78
CMV status (D+/R− or D−/R+ or D+/R+)	1.38 (1.12–1.70)	0.003
Interaction: *MICB98* matching X CMV status	0.41 (0.22–0.76)	0.005

Results are presented as hazard ratios with 95% confidence intervals (CI).

GVHD Graft-versus-host disease

[a]All models were adjusted for patient's age, patient–donor sex, patient–donor serological status for cytomegalovirus, year of transplantation, time to transplantation, transplantation center, source of stem cells, conditioning regimen, GVHD prophylaxis, treatment with antithymocyte globulin or Alemtuzumab, *HLA-DPB1* matching status, disease category and severity at transplantation.

[b]D and R stand for donor and recipient, respectively. The reference category for the CMV status is D−/R−.

[c]Transplantations performed for non-malignant diseases were excluded from the analysis.

matching or CMV status) is, respectively, lower or higher in the category at risk of its interacting variable compared to the reference category. For example, when the HR of the interaction factor is <1, the HR of *MICB98* mismatches is lower when the donor and/or recipient are positive for CMV (category at risk of the CMV status variable) and higher when both the donor and recipient are negative for CMV (reference category of the CMV status variable).

For acute GVHD III–IV, the HR of the interaction was <1 and was statistically significant (HR for acute GVHD III–IV, 0.26; 95% CI, 0.17–0.40; $P < 0.001$), indicating that the effect of *MICB98* mismatching on acute GVHD is more important when both the donor and the recipient are negative for CMV (acute GVHD III–IV HR, 3.63; 95% CI, 3.15–4.18; $P < 0.001$) compared to when the donor and/or the recipient are positive for CMV (acute GVHD III–IV HR, $3.63 \times 0.26 = 0.94$). This observation was confirmed by representing graphically cumulative incidences of acute GVHD III–IV in the above mentioned two CMV subgroups (Fig. 2a, b).

For OS, the interaction between *MICB98* mismatching and CMV status was statistically significant and was >1 (HR, 1.53; 95% CI, 1.38–1.69; $P < 0.001$). CMV positivity in the donor and/or recipient was associated with a slightly lower survival when *MICB98* was matched (HR, 1.16; 95% CI, 1.14–1.19; $P < 0.001$). However, because of the positive interaction with *MICB98* mismatches, this effect was higher when *MICB98* was mismatched (HR $1.16 \times 1.53 = 1.77$) (Table 3). The Kaplan–Meier estimates showing the higher impact of the CMV status on OS in *MICB98* matched and mismatched groups are presented in Fig. 2c, d, respectively. In other words, the risk of death associated with CMV positivity in the donor and/or recipient is lower in *MICB98* matched vs. mismatched groups.

Finally, to assess whether *MICB98* mismatches had a GVHD-independent effect on CMV infections in donor/recipients pairs at risk for CMV reactivation (i.e., the donor and/or recipient was positive for CMV pre-HCT), we performed a multivariate Fine and Gray analysis that included *MICB98* matching as well as the presence/absence of acute GVHD grades III-IV and chronic GVHD as time-dependent covariates in the model (Table 4). In accordance with the higher risk of death described above, *MICB98* mismatches were associated with a higher incidence of CMV infections (HR, 1.84; 95% CI, 1.34–2.51; $P < 0.001$) (Table 4 and Fig. 3). *MICB98* mismatches were not associated with EBV or HHV6 infections (Supplemental Table 1).

Discussion

This is the first study analyzing the role of *MICB* matching in transplantation (whether HCT or solid organ).

Here we report that HCT from a *MICB98* mismatched, but otherwise fully HLA 10/10 and *MICA* matched donor, carries a significantly increased risk of acute and chronic GVHD. Interestingly, the effect on GVHD was not accompanied by a decreased relapse rate. This unusual observation may be attributed to the CMV status that is not independent of the *MICB98* matching status. The significant interaction of *MICB98* matching with CMV status ($P < 0.001$) indicates that the CMV status has a strong positive impact on relapse when *MICB98* is mismatched (HR, $0.77 \times 2.61 = 2.01$) (Table 3).

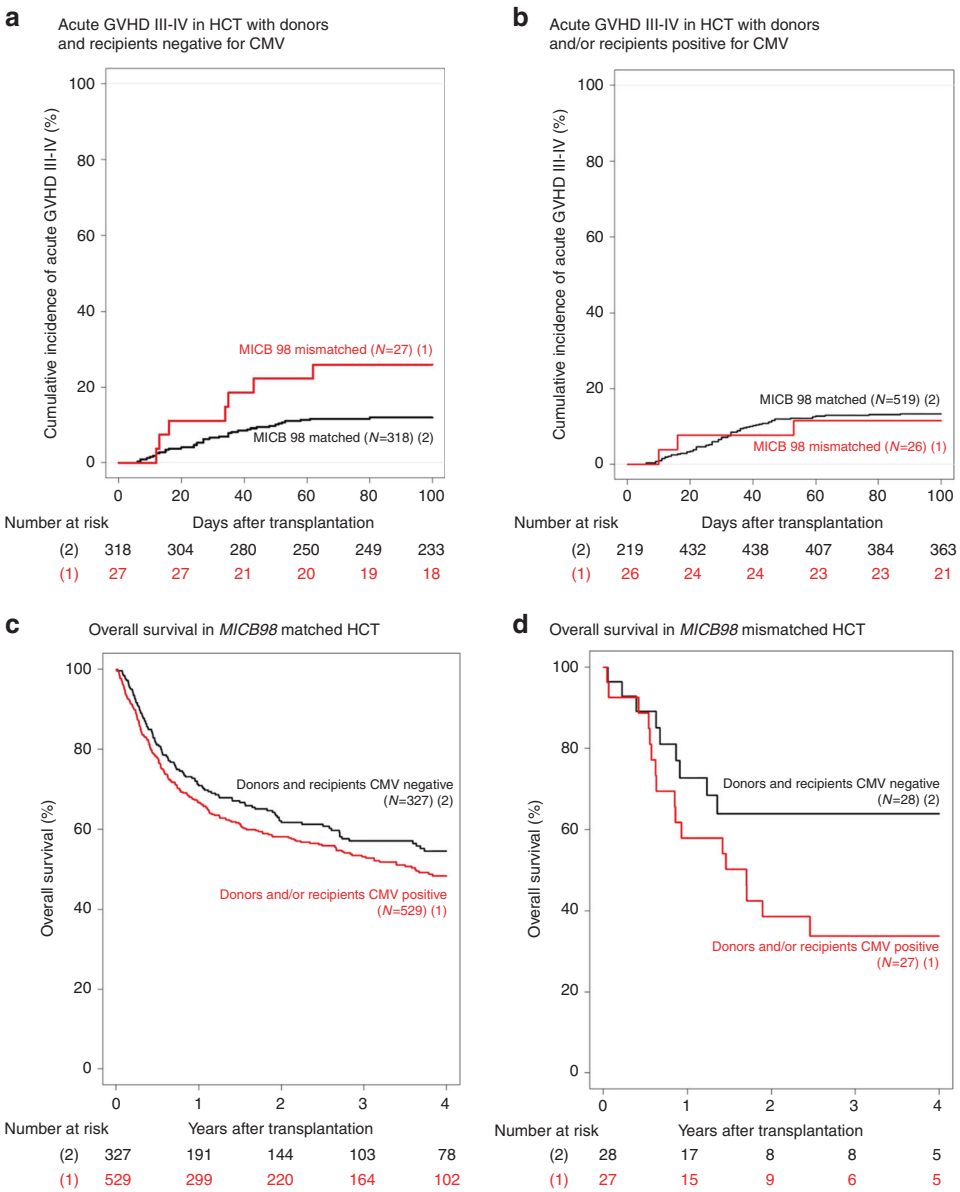


Fig. 2 Effect of *MICB98* matching and CMV status on GVHD and overall survival. Panels **a** and **b** represent the cumulative incidences of grades III–IV acute GVHD in HCT with donors and recipients negative for CMV (**a**) and HCT with donors and/or recipients positive for CMV (**b**). Panels **c** and **d** show the Kaplan–Meier estimates of overall survival in *MICB98*-matched (**c**) and mismatched (**d**) transplants.

CMV biology has been known to be linked to *MICB* for more than 15 years. Initially, Cosman et al. demonstrated that CMV infected cells can evade the immune system by the retention of MICB and ULBP-1 and -2 antigens in the cell via binding to the CMV protein UL16 [36]. This interaction hampers the ability of newly synthesized MICB proteins to mature and transit the secretory pathway [53]. By dissecting the molecular basis of MICB binding to UL16, Spreu et al. reported that the UL16-MICB interaction is dependent on helical structures of the MICB α2 domain [54]. Finally, more recently, it was shown that UL16 binding was not equivalent for all MICB alleles. The *MICB*008* allele in particular was shown to have a decreased binding activity compared to other alleles that do not have a methionine at position 98 in the MICB α2 domain [37]. Importantly, position 98 is the only polymorphic position of MICB that is known to be in direct contact with UL16 [38]. It is therefore not surprising that mismatches at this position have less impact on acute GVHD in the presence of CMV than in its absence. In the absence of CMV, the *MICB98* polymorphism may indeed not be able to modulate the expression of MICB at the cell surface through interaction with UL16 and consequently is not able to influence the alloreactivity that remains higher in the mismatch than in the matched situation. Another explanation for the higher *MICB*-mediated alloreactivity in the absence of CMV may be the absence of T-cell exhaustion that is known to be induced by CMV positivity [55]. Ultimately, this observation demonstrates that to lower the risk of acute GVHD in the absence of CMV (donor and recipient seronegative), a *MICB98* matched donor is a better choice than a *MICB98* mismatched donor.

Table 4 Effect of GVHD and *MICB98* matching on CMV infections.

	hazard ratio (95% CI)[a]	*P*-value
GVHD		
Chronic		
Absent ($n = 307$)	Ref.	–
Present ($n = 169$)	0.99 (0.83–1.19)	1.05
Acute III–IV		
Absent ($n = 388$)	Ref.	–
Present ($n = 78$)	1.12997 (1.1290–1.13)	<0.001
MICB98 matching		
Matched ($n = 437$)	Ref.	–
Mismatched ($n = 19$)	1.84 (1.34–2.51)	<0.001

Only the pairs in which the donor and/or the recipient was/were positive for CMV pre-HCT were included in the analysis. The results are presented as hazard ratios with 95% confidence intervals (CIs).

GVHD Graft-versus-host disease, *Ref.* Reference category.

[a]Multivariate Fine and Gray model including *MICB98* matching, acute GVHD III-IV and chronic GVHD as time-dependent covariates in the model. In addition, the model was adjusted for patient's age, patient–donor sex, patient–donor serological status for cytomegalovirus, year of transplantation, time to transplantation, transplantation center, source of stem cells, conditioning regimen, GVHD prophylaxis, treatment with antithymocyte globulin or Alemtuzumab, *HLA-DPB1* matching status, disease category and severity at transplantation.

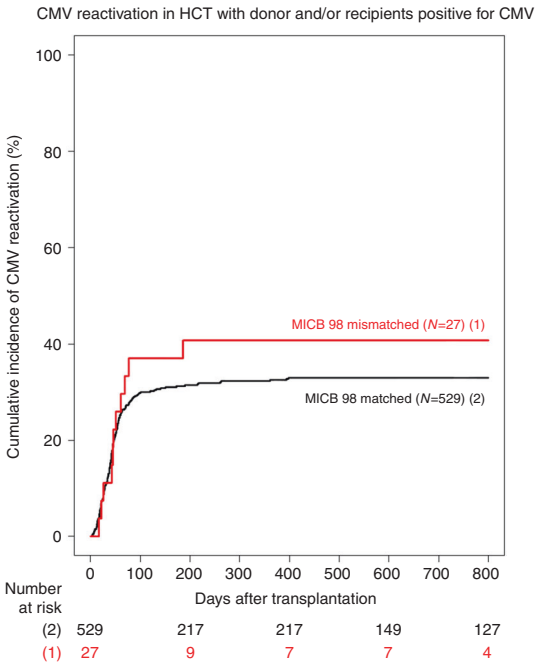


Fig. 3 Effect of *MICB98* matching on CMV reactivation/infection. The cumulative incidences of posttransplant CMV infection episodes in *MICB98* mismatched (1) versus matched (2) patients are shown.

CMV causes mortality in two ways: (1) directly by causing viral diseases, such as pneumonitis, a situation that is becoming rare (viral diseases represent less than 2% of deaths) thanks to pre-emptive therapies, or (2) indirectly by clinical events associated with virus seropositivity or the development of viral infections that are independent of the viral disease itself [56]. The indirect effects of CMV are recognized as a major cause of adverse outcomes after HCT, including GVHD and overall mortality [56–58]. Our dataset showed that the CMV effect on OS is amplified in *MICB98* mismatched HCT compared to *MICB98* matched HCT, indicating that matching donors at this position could be a useful strategy to decrease the risk of death related to CMV. Because *MICB98* mismatches were further shown to be associated with CMV infection episodes, and this independently of the occurrence of GVHD, deaths related to CMV may be due to CMV infections.

Collectively, these results suggest that pretransplantation *MICB98* typing may help in lowering the risk of both GVHD- and CMV-related mortality. In the absence of CMV, matching *MICB98* provides a means to lower the incidence of GVHD, whereas in the presence of CMV, it helps improve OS. Fortunately, the level of *MICB98* mismatching is only 5.9% in HLA 10/10 matched donor/patient pairs that are also matched for *MICA;* although in absolute terms, this represents several thousand patients per year. Therefore, finding a *MICB98*-matched donor should be relatively easy in clinical practice.

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Author contributions RC performed the experiments, designed the study, analyzed the data, and wrote the manuscript. SB designed the study, analyzed the data and wrote the manuscript. PS, AM, IK, CM, APi, and VR performed the experiments and analyzed the data. IA performed the statistics. PAB, DB, AC, DC, FC, KG, JK, JC, ML, PL, MMi, PM, MOu, APa, RPL, CPi, GS, ES, RT, AT, IY, VD and BH provided samples and clinical data, interpreted the clinical data and discussed the results. BL, MMo, AN, YM and CPa interpreted the clinical data and discussed results. FB, MMB, and BH analyzed the data and reviewed statistics. All authors contributed to the writing of the report and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest SB is the scientific founder and a (minority) shareholder of BIOMICA SAS. JK is the co-founder and chief scientific officer of Gadeta. He received personal fees from Gadeta. In addition, JK has a patent issued/pending. ES is the inventor of a patent application filed by the University Medical Center Utrecht on the

prediction of an alloimmune response against mismatched HLA (PCT/EPT2013/073386). All other authors declare no conflict of interests.

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References

1. Forman JS, Negrin SR, Antin HJ, Appelbaum RF. Thomas' hematopoietic cell transplantation, 5th ed.; Chichester: Wiley-blackwell; 2016.
2. Appelbaum FR, Thomas ED. Thomas' hematopoietic cell transplantation: stem cell transplantation, 4th ed. Wiley-Blackwell: Chichester; 2009.
3. Gratwohl A, Baldomero H, Aljurf M, Pasquini MC, Bouzas LF, Yoshimi A, et al. Hematopoietic stem cell transplantation: a global perspective. JAMA. 2010;303:1617–24. https://doi.org/10.1001/jama.2010.491.
4. D'Souza A, Fretham C. Current uses and outcomes of hematopoietic cell transplantation (HCT): CIBMTR Summary Slides; 2018. https://www.cibmtr.org. Accessed April 2019.
5. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med. 2010;363:2091–101. https://doi.org/10.1056/NEJMoa1004383.
6. Warren EH, Zhang XC, Li S, Fan W, Storer BE, Chien JW, et al. Effect of MHC and non-MHC donor/recipient genetic disparity on the outcome of allogeneic HCT. Blood. 2012;120:2796–806. https://doi.org/10.1182/blood-2012-04-347286.
7. Mori T, Kato J. Cytomegalovirus infection/disease after hematopoietic stem cell transplantation. Int J Hematol. 2010;91:588–95. https://doi.org/10.1007/s12185-010-0569-x. e-pub ahead of print 2010/04/24.
8. Paris C, Kopp K, King A, Santolaya ME, Zepeda AJ, Palma J. Cytomegalovirus infection in children undergoing hematopoietic stem cell transplantation in Chile. Pediatr Blood Cancer. 2009;53:453–8. https://doi.org/10.1002/pbc.22060.
9. Boeckh M, Nichols WG, Papanicolaou G, Rubin R, Wingard JR, Zaia J. Cytomegalovirus in hematopoietic stem cell transplant recipients: current status, known challenges, and future strategies. Biol Blood Marrow Transplant. 2003;9:543–58.
10. Boeckh M, Ljungman P. How we treat cytomegalovirus in hematopoietic cell transplant recipients. Blood. 2009;113:5711–9. https://doi.org/10.1182/blood-2008-10-143560. e-pub ahead of print 2009/03/21.
11. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis. 2002;34:1094–7. https://doi.org/10.1086/339329.
12. Ljungman P, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. Hematol/Oncol Clin N Am. 2011;25:151–69. https://doi.org/10.1016/j.hoc.2010.11.011.
13. Takenaka K, Nishida T, Asano-Mori Y, Oshima K, Ohashi K, Mori T, et al. Cytomegalovirus reactivation after allogeneic hematopoietic stem cell transplantation is associated with a reduced risk of relapse in patients with acute myeloid leukemia who survived to day 100 after transplantation: the Japan Society for Hematopoietic Cell Transplantation Transplantation-related Complication Working Group. Biol Blood Marrow Transplant. 2015;21:2008–16. https://doi.org/10.1016/j.bbmt.2015.07.019.
14. Manjappa S, Bhamidipati PK, Stokerl-Goldstein KE, DiPersio JF, Uy GL, Westervelt P, et al. Protective effect of cytomegalovirus reactivation on relapse after allogeneic hematopoietic cell transplantation in acute myeloid leukemia patients is influenced by conditioning regimen. Biol Blood Marrow Transplant. 2014;20:46–52. https://doi.org/10.1016/j.bbmt.2013.10.003. e-pub ahead of print 2013/10/15.
15. Jang JE, Kim SJ, Cheong JW, Hyun SY, Kim YD, Kim YR, et al. Early CMV replication and subsequent chronic GVHD have a significant anti-leukemic effect after allogeneic HSCT in acute myeloid leukemia. Ann Hematol. 2015;94:275–82. https://doi.org/10.1007/s00277-014-2190-1. e-pub ahead of print 2014/08/20.
16. Nichols WG, Corey L, Gooley T, Davis C, Boeckh M. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. J Infect Dis. 2002;185:273–82. https://doi.org/10.1086/338624. e-pub ahead of print 2002/01/25.
17. Sousa H, Boutolleau D, Ribeiro J, Teixeira AL, Pinho Vaz C, Campilho F, et al. Cytomegalovirus infection in patients who underwent allogeneic hematopoietic stem cell transplantation in Portugal: a five-year retrospective review. Biol Blood Marrow Transplant. 2014;20:1958–67. https://doi.org/10.1016/j.bbmt.2014.08.010.
18. Ichihara H, Nakamae H, Hirose A, Nakane T, Koh H, Hayashi Y, et al. Immunoglobulin prophylaxis against cytomegalovirus infection in patients at high risk of infection following allogeneic hematopoietic cell transplantation. Transplant Proc. 2011;43:3927–32. https://doi.org/10.1016/j.transproceed.2011.08.104.
19. Schmidt-Hieber M, Labopin M, Beelen D, Volin L, Ehninger G, Finke J, et al. CMV serostatus still has an important prognostic impact in de novo acute leukemia patients after allogeneic stem cell transplantation: a report from the Acute Leukemia Working Party of EBMT. Blood. 2013;122:3359–64. https://doi.org/10.1182/blood-2013-05-499830.
20. Petersdorf EW. Optimal HLA matching in hematopoietic cell transplantation. Curr Opin Immunol. 2008;20:588–93. https://doi.org/10.1016/j.coi.2008.06.014.
21. Miller W, Flynn P, McCullough J, Balfour HH,Jr, Goldman A, Haake R, et al. Cytomegalovirus infection after bone marrow transplantation: an association with acute graft-v-host disease. Blood. 1986;67:1162–7.
22. Flomenberg N, Baxter-Lowe LA, Confer D, Fernandez-Vina M, Filipovich A, Horowitz M, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. Blood. 2004;104:1923–30. https://doi.org/10.1182/blood-2004-03-0803.
23. Ruell J, Barnes C, Mutton K, Foulkes B, Chang J, Cavet J, et al. Active CMV disease does not always correlate with viral load detection. Bone Marrow Transplant. 2007;40:55–61. https://doi.org/10.1038/sj.bmt.1705671.
24. Castagnola E, Cappelli B, Erba D, Rabagliati A, Lanino E, Dini G. Cytomegalovirus infection after bone marrow transplantation in children. Hum Immunol. 2004;65:416–22. https://doi.org/10.1016/j.humimm.2004.02.013.
25. Bahram S, Bresnahan M, Geraghty DE, Spies T. A second lineage of mammalian major histocompatibility complex class I genes. Proc Natl Acad Sci USA. 1994;91:6259–63. https://doi.org/10.1073/pnas.91.14.6259.
26. Carapito R, Bahram S. Genetics, genomics, and evolutionary biology of NKG2D ligands. Immunological Rev. 2015;267:88–116. https://doi.org/10.1111/imr.12328.
27. Bahram S, Spies T. Nucleotide sequence of a human MHC class I MICB cDNA. Immunogenetics. 1996;43:230–3.
28. Bahram S, Shiina T, Oka A, Tamiya G, Inoko H. Genomic structure of the human MHC class I MICB gene. Immunogenetics. 1996;45:161–2.
29. Carapito R, Jung N, Kwemou M, Untrau M, Michel S, Pichot A, et al. Matching for the nonconventional MHC-I MICA gene significantly reduces the incidence of acute and chronic GVHD.

Blood. 2016;128:1979–86. https://doi.org/10.1182/blood-2016-05-719070.
30. Fuerst D, Neuchel C, Niederwieser D, Bunjes D, Gramatzki M, Wagner E, et al. Matching for the MICA-129 polymorphism is beneficial in unrelated hematopoietic stem cell transplantation. Blood. 2016;128:3169–76. https://doi.org/10.1182/blood-2016-05-716357.
31. Petersdorf EW, Hansen JA, Martin PJ, Woolfrey A, Malkki M, Gooley T, et al. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. N Engl J Med. 2001;345:1794–800. https://doi.org/10.1056/NEJMoa011826.
32. Groh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. Proc Natl Acad Sci USA. 1996;93:12445–50.
33. Wang WY, Tian W, Zhu FM, Liu XX, Li LX, Wang F. MICA, MICB polymorphisms and linkage disequilibrium with HLA-B in a Chinese Mongolian population. Scand J Immunol. 2016;83:456–62. https://doi.org/10.1111/sji.12437.
34. Liu X, Tian W, Li L, Cai J. Characterization of the major histocompatibility complex class I chain-related gene B (MICB) polymorphism in a northern Chinese Han population: the identification of a new MICB allele, MICB*023. Hum Immunol. 2011;72:727–32. https://doi.org/10.1016/j.humimm.2011.05.013.
35. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol. 2008;9:495–502. https://doi.org/10.1038/ni1581.
36. Cosman D, Mullberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, et al. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. Immunity. 2001;14:123–33.
37. Klumkrathok K, Jumnainsong A, Leelayuwat C. Allelic MHC class I chain related B (MICB) molecules affect the binding to the human cytomegalovirus (HCMV) unique long 16 (UL16) protein: implications for immune surveillance. J Microbiol. 2013;51:241–6. https://doi.org/10.1007/s12275-013-2514-1.
38. Muller S, Zocher G, Steinle A, Stehle T. Structure of the HCMV UL16-MICB complex elucidates select binding of a viral immunoevasin to diverse NKG2D ligands. PLoS Pathog. 2010;6:e1000723. https://doi.org/10.1371/journal.ppat.1000723.
39. Hill GR, Ferrara JL. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. Blood. 2000;95:2754–9.
40. Isernhagen A, Malzahn D, Viktorova E, Elsner L, Monecke S, von Bonin F, et al. The MICA-129 dimorphism affects NKG2D signaling and outcome of hematopoietic stem cell transplantation. EMBO Mol Med. 2015;7:1480–502. https://doi.org/10.15252/emmm.201505246.
41. Boukouaci W, Busson M, Peffault de Latour R, Rocha V, Suberbielle C, Bengoufa D, et al. MICA-129 genotype, soluble MICA, and anti-MICA antibodies as biomarkers of chronic graft-versus-host disease. Blood. 2009;114:5216–24. https://doi.org/10.1182/blood-2009-04-217430.
42. Parmar S, Del Lima M, Zou Y, Patah PA, Liu P, Cano P, et al. Donor-recipient mismatches in MHC class I chain-related gene A in unrelated donor transplantation lead to increased incidence of acute graft-versus-host disease. Blood. 2009;114:2884–7. https://doi.org/10.1182/blood-2009-05-223172.
43. Pellet P, Renaud M, Fodil N, Laloux L, Inoko H, Hauptmann G, et al. Allelic repertoire of the human MICB gene. Immunogenetics. 1997;46:434–6.
44. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18:295–304. https://doi.org/10.1097/00007890-197410000-00001.
45. Scheike TH, Zhang MJ. Analyzing competing risk data using the R timereg package. J Stat Softw. 2011;30:i02.
46. Scheike TH, Zhang MJ. Flexible competing risks regression modeling and goodness-of-fit. Lifetime Data Anal. 2008;14:464–83. https://doi.org/10.1007/s10985-008-9094-0.
47. Scheike T, Zhang M, Gerds T. Predicting cumulative incidence probability by direct binomial regression. Biometrika. 2008;95:205–20.
48. Therneau T, Grambsch P. Modeling survival data: extending the Cox model. New York: Springer; 2000.
49. Fleischhauer K, Shaw BE, Gooley T, Malkki M, Bardy P, Bignon JD, et al. Effect of T-cell-epitope matching at HLA-DPB1 in recipients of unrelated-donor haemopoietic-cell transplantation: a retrospective study. Lancet Oncol. 2012;13:366–74. https://doi.org/10.1016/S1470-2045(12)70004-9.
50. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. J Clin Epidemiol. 1995;48:1503–10. https://doi.org/10.1016/0895-4356(95)00048-8.
51. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol. 1996;49:1373–9. https://doi.org/10.1016/s0895-4356(96)00236-3.
52. Team RDC. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2010. http://R-project.org.
53. Wu J, Chalupny NJ, Manley TJ, Riddell SR, Cosman D, Spies T. Intracellular retention of the MHC class I-related chain B ligand of NKG2D by the human cytomegalovirus UL16 glycoprotein. J Immunol. 2003;170:4196–200.
54. Spreu J, Stehle T, Steinle A. Human cytomegalovirus-encoded UL16 discriminates MIC molecules by their alpha2 domains. J Immunol. 2006;177:3143–9.
55. Nikolich-Zugich J, Goodrum F, Knox K, Smithey MJ. Known unknowns: how might the persistent herpesvirome shape immunity and aging? Curr Opin Immunol. 2017;48:23–30. https://doi.org/10.1016/j.coi.2017.07.011.
56. de la Camara R. CMV in hematopoietic stem cell transplantation. Mediterranean J Hematol Infect Dis. 2016;8:e2016031. https://doi.org/10.4084/MJHID.2016.031.
57. Broers AE, van Der Holt R, van Esser JW, Gratama JW, Henzen-Logmans S, Kuenen-Boumeester V, et al. Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic T-cell-depleted stem cell transplantation. Blood. 2000;95:2240–5.
58. Cantoni N, Hirsch HH, Khanna N, Gerull S, Buser A, Bucher C, et al. Evidence for a bidirectional relationship between cytomegalovirus replication and acute graft-versus-host disease. Biol Blood Marrow Transplant. 2010;16:1309–14. https://doi.org/10.1016/j.bbmt.2010.03.020.

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