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

Running head: MICB, CMV and GVHD in HCT

Raphael Carapito^{1,2,3,4}, Ismail Aouadi^{1,2,3}, Angélique Pichot^{1,2,3}, Perrine Spinnhirny^{1,2,3}, Aurore Morlon^{2,5}, Irina Kotova^{2,5}, Cécile Macquin^{1,2,3}, Véronique Rolli^{1,2,3}, Anne Cesbron^{2,6,7,8}, Katia Gagne^{2,6,9}, Machteld Oudshoorn^{10,11}, Bronno van der Holt¹², Myriam Labalette^{13,14}, Eric Spierings¹⁵, Christophe Picard¹⁶, Pascale Loiseau^{2,7,17}, Ryad Tamouza^{2,17}, Antoine Toubert^{2,7,17}, Anne Parissiadis^{7,18}, Valérie Dubois¹⁹, Catherine Paillard^{1,2,7,20}, Myriam Maumy-Bertrand²¹, Frédéric Bertrand²¹, Peter A. von dem Borne²², Jürgen H.E. Kuball²³, Mauricette Michallet^{7,24}, Bruno Lioure^{7,25}, Régis Peffault de Latour^{2,7,26}, Didier Blaise^{7,27}, Jan J. Cornelissen²⁸, Ibrahim Yakoub-Agha^{7,14}, Frans Claas¹¹, Philippe Moreau^{7,29}, Dominique Charron^{1,2,17}, Mohamad Mohty^{7,30,31,32}, Yasuo Morishima³³, Gérard Socié^{2,7,25}, and Seiamak Bahram^{1,2,3,4}

1 Laboratoire d'ImmunoRhumatologie Moléculaire, INSERM UMR_S1109, Plateforme GENOMAX, Faculté de Médecine, Fédération Hospitalo-Universitaire OMICARE, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France.

2 Labex TRANSPLANTEX, Faculté de Médecine, Université de Strasbourg, Strasbourg, France.

3 INSERM Franco-Japanese Nextgen HLA laboratory, Nagano, Japan and Strasbourg, Strasbourg, France.

4 Laboratoire d'Immunologie, Plateau Technique de Biologie, Pôle de Biologie, Nouvel Hôpital Civil, Strasbourg, France.

5 BIOMICA SAS, Strasbourg, France.

28 6 Etablissement Français du Sang (EFS) Centre-Pays de la Loire, Laboratoire HLA, Nantes,
29 France.

30 7 Société Francophone de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC), Hôpital
31 Edouard Herriot, CHU, Lyon, France.

32 8 Société Francophone d'Histocompatibilité et d'Immunogénétique (SFHI), Paris, France.

33 9 INSERM 1232, CRCINA, Université Nantes-Angers, Nantes, France.

34 10 Europdonor operated by Matchis Foundation, Leiden, The Netherlands.

35 11 Department of Immunohematology and Blood transfusion, LUMC, Leiden, The
36 Netherlands.

37 12 HOVON Data Center, Department of Hematology, Erasmus MC Cancer Institute,
38 Rotterdam, The Netherlands.

39 13 Laboratoire d'Immunologie, CHRU de Lille, Lille, France.

40 14 LIRIC INSERM U995, Université Lille 2, Lille, France.

41 15 Laboratory for Translational Immunology, University Medical Center Utrecht, Utrecht, The
42 Netherlands.

43 16 Aix-Marseille Université, CNRS, EFS-PACA, ADES UMR 7268, Marseille, France.

44 17 Laboratoire Jean Dausset, INSERM UMR_S 1160, Hôpital Saint-Louis, Paris, France.

45 18 Etablissement Français du Sang (EFS) Grand-Est, Laboratoire HLA, Strasbourg, France.

46 19 Etablissement Français du Sang (EFS) Rhône-Alpes, Laboratoire HLA, Lyon, France.

47 20 Service d'Hématologie et d'Oncologie pédiatrique, Hôpitaux Universitaires de Strasbourg,
48 Strasbourg, France.

49 21 Institut de Recherche Mathématique Avancée, CNRS UMR 7501, LabEx Institut de
50 Recherche en Mathématiques, ses Interactions et Applications, Université de Strasbourg,
51 Strasbourg, France

52 22 Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands.

53 23 Department of Hematology, University Medical Center Utrecht, Utrecht, The Netherlands.

54 24 Centre Hospitalier Lyon Sud, Hématologie 1G, Hospices Civils de Lyon, Pierre Bénite,
55 Lyon, France.

25 Service d'Hématologie Adulte, Hôpitaux Universitaires de Strasbourg, Strasbourg, France.

26 Service d'Hématologie – Greffe, Hôpital Saint-Louis, APHP, Paris, France.

27 Institut Paoli Calmettes, Marseille, France.

28 Department of Hematology and ErasmusMC Cancer Institute, Erasmus University Medical Center, Rotterdam, The Netherlands.

29 Service d'Hématologie Clinique, CHU Hôtel Dieu, Nantes, France.

30 Hôpital Saint Antoine, Département d'Hématologie, Paris, France.

31 Université Pierre & Marie Curie, Paris, France.

32 Centre de Recherche Saint-Antoine, INSERM UMR_S 938, Paris, France.

33 Aichi Cancer Center Research Institute, Division of Epidemiology and Prevention, 1-1 Kanokoden, Chikusa-ku, Nagoya, Japan.

Correspondence: Seiamak Bahram and/or Raphael Carapito, both at Centre de Recherche d'Immunologie et d'Hématologie, 4 rue Kirschleger, 67085 Strasbourg Cedex France; e-mails: siamak@unistra.fr and/or carapito@unistra.fr

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 sequesters *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.

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 approximately 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 incompatibilities²⁹⁻³¹. *MICB* is indeed highly polymorphic, with 47 alleles reported to date (<http://www.ebi.ac.uk/ipd/imgt/hla/stats.html>). It encodes a cell-surface glycoprotein up-regulated 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 ago²⁵. *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 β_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⁺ $\alpha\beta$ and $\gamma\delta$ T lymphocytes and natural killer cells³⁵. Interestingly, and in contrast to MICA, MICB binds the CMV protein UL16, which sequesters MICB intracellularly in an immune escape mechanism³⁶. 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 alleles³⁷. *MICB*008* is characterized by a polymorphism at amino acid position 98, causing an isoleucine (Ile) to methionine (Met) exchange in the $\alpha 2$ 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 UL16³⁸.

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 disease³⁹; (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 protein³⁶. 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 EBMT (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-recipient 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 $\alpha 2$ 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 protocols⁴³. 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 model⁴⁵⁻⁴⁷. For OS and RFS, Cox proportional regression models were used⁴⁸. 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 non-relapse mortality 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 cytomegalovirus, 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 cytomegalovirus 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 R⁵².

RESULTS

The demographics of the study population are shown in Table 1. The median post-transplant follow-up was 36 months (mean: 37 months; range: 1 to 105 months), and the median patient age was 53 years (mean: 48 years; range: 1 to 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*, *-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 Figure 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 (Figure 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 and Figure 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 hazard ratio of the interaction factor is < 1 or > 1 , the hazard ratio of a variable (here, *MICB98* 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 hazard ratio of the interaction factor is < 1 , the hazard ratio 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 hazard ratio of the interaction was < 1 and was statistically significant (hazard ratio for acute GVHD III-IV, 0.26; 95% CI, 0.17 to 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 hazard ratio, 3.63; 95% CI, 3.15 to 4.18; $P < 0.001$) compared to when the donor

and/or the recipient are positive for CMV (acute GVHD III-IV hazard ratio, $3.63 \times 0.26 = 0.94$). This observation was confirmed by representing graphically cumulative incidences of acute GVHD III-IV in the above mentioned two CMV subgroups (Figure 2A and 2B).

For OS, the interaction between *MICB98* mismatching and CMV status was statistically significant and was > 1 (hazard ratio, 1.53; 95% CI, 1.38 to 1.69; $P < 0.001$). CMV positivity in the donor and/or recipient was associated with a slightly lower survival when *MICB98* was matched (hazard ratio, 1.16; 95% CI, 1.14 to 1.19; $P < 0.001$). However, because of the positive interaction with *MICB98* mismatches, this effect was higher when *MICB98* was mismatched (hazard ratio $1.16 \times 1.53 = 1.77$) (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 Figures 2C and 2D, 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 (hazard ratio, 1.84; 95% CI, 1.34 to 2.51; $P < 0.001$) (Table 4 and Figure 3). *MICB98* mismatches were not associated with EBV or HHV6 infections (Supplemental Table 1).

318

319 **DISCUSSION**

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

322 Here we report that HCT from a *MICB98* mismatched, but otherwise fully HLA
323 10/10 and *MICA* matched donor, carries a significantly increased risk of acute and
324 chronic GVHD. Interestingly, the effect on GVHD was not accompanied by a
325 decreased relapse rate. This unusual observation may be attributed to the CMV
326 status that is not independent of the *MICB98* matching status. The significant
327 interaction of *MICB98* matching with CMV status ($P < 0.001$) indicates that the CMV
328 status has a strong positive impact on relapse when *MICB98* is mismatched (HR,
329 $0.77 \times 2.61 = 2.01$) (Table 3).

330 CMV biology has been known to be linked to *MICB* for more than 15 years.
331 Initially, Cosman et al. demonstrated that CMV infected cells can evade the immune
332 system by the retention of MICB and ULBP-1 and -2 antigens in the cell via binding to
333 the CMV protein UL16³⁶. This interaction hampers the ability of newly synthesized
334 MICB proteins to mature and transit the secretory pathway⁵³. By dissecting the
335 molecular basis of MICB binding to UL16, Spreu et al. reported that the UL16-MICB
336 interaction is dependent on helical structures of the MICB $\alpha 2$ domain⁵⁴. Finally,
337 more recently, it was shown that UL16 binding was not equivalent for all MICB
338 alleles. The *MICB*008* allele in particular was shown to have a decreased binding
339 activity compared to other alleles that do not have a methionine at position 98 in the
340 MICB $\alpha 2$ domain³⁷. Importantly, position 98 is the only polymorphic position of MICB
341 that is known to be in direct contact with UL16³⁸. It is therefore not surprising that
342 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 positivity⁵⁵. 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.

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 preemptive therapies, or (2) indirectly by clinical events associated with virus seropositivity or the development of viral infections that are independent of the viral disease itself⁵⁶. The indirect effects of CMV are recognized as a major cause of adverse outcomes after HCT, including GVHD and overall mortality⁵⁶⁻⁵⁸. Our dataset showed that the CMV effect on overall survival 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 pre-transplantation *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 overall survival. 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. PAvdB, DB, AC, DC, FCI, KG, JK, JJ, MLaba, PL, MMi, NM, PM, MOu, APa, RP, CPi, GS, ES, RT, AT, IY and BvdH provided samples and clinical data, interpreted the clinical data and discussed the results. BL, MMo, AN and CPa interpreted the clinical data and discussed results. FB, YK, MMB, MOt, and

BvdH analyzed the data and reviewed statistics. All authors contributed to the writing of the report and approved the final version of the manuscript.

Ethics declarations

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 competing interests.

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FIGURE LEGENDS

Figure 1. Effect of *MICB98* matching on severe acute and chronic GVHD

The cumulative incidences of grades III-IV acute (panel A) and chronic GVHD (Panel B) are shown for *MICB98* mismatched (1) versus matched (2) patients.

Figure 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.

Figure 3. Effect of *MICB98* matching on CMV reactivation/infection

The cumulative incidences of post-transplant CMV infection episodes in *MICB98* mismatched (1) versus matched (2) patients are shown.

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TABLES

Table 1. Demographics of the Study Population

	Total transplants (n= 943)	MICB 98 matched transplants (n=887)	MICB 98 mismatched transplants (n=56)	P-value*
Transplantation centers†				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 ‡				0.34
No	231 (24.5%)	214 (24.1%)	17 (30.3%)	
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 §	260 (27.6%)	243 (27.4%)	17 (30.4%)	
Disease stage at transplantation ¶				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**				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%)	

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

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

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

‡ *in vivo* T-cell depletion was performed by the addition of anti-thymocyte globulin (ATG) or Alemtuzumab to the conditioning regimen.

§ 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.

¶ 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.

** *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 .

Table 2. Analysis of the Impact of *MICB* Mismatches at amino acid position 98 on Clinical Outcomes after Multivariate Modeling*

	hazard ratio (95% CI)	P-value
Acute GVHD II-IV	1.20 (1.15-1.24)	<0.001
Acute GVHD III-IV	2.28 (1.56-3.34)	<0.001
Chronic GVHD	1.21 (1.10-1.33)	<0.001
Relapse [†]	1.42 (1.05-1.93)	0.024
Overall survival	1.01 (0.84-1.20)	0.93
Relapse-free survival	0.98 (0.91-1.06)	0.63
Non-Relapse Mortality	0.62 (0.37-1.04)	0.071

Results are presented as Hazard Ratios with 95% confidence intervals (CI). GVHD: Graft-versus-host disease. * 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 anti-thymocyte globulin or Alemtuzumab, *HLA-DPB1* matching status, disease category and severity at transplantation. † Transplantations performed for non-malignant diseases were excluded from the analysis.

Table 3. Analysis of the Impact of *MICB* Mismatches at position 98, CMV status and their interaction on Clinical Outcomes after Multivariate Modeling*

Outcomes and risk factors	hazard ratio (95% CI)	P-value
Acute GVHD II-IV		
<i>MICB98</i> matching (mismatches)	1.47 (1.05-2.07)	0.025
CMV status (D+/R- or D-/R+ or D+/R+)‡	1.18 (0.92-1.51)	0.2
Interaction: <i>MICB98</i> matching X CMV status	0.57 (0.29-1.10)	0.095
Acute GVHD III-IV		
<i>MICB98</i> 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: <i>MICB98</i> matching X CMV status	0.26 (0.17-0.40)	< 0.001
Chronic GVHD		
<i>MICB98</i> 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: <i>MICB98</i> matching X CMV status	0.91 (0.70-1.18)	0.48
Relapse[†]		
<i>MICB98</i> 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: <i>MICB98</i> matching X CMV status	2.61 (1.79-3.82)	< 0.001
Overall survival		
<i>MICB98</i> 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: <i>MICB98</i> matching X CMV status	1.53 (1.38-1.69)	< 0.001
Relapse-free survival		
<i>MICB98</i> 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: <i>MICB98</i> matching X CMV status	1.57 (1.45-1.70)	< 0.001
Non-relapse mortality		
<i>MICB98</i> 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: <i>MICB98</i> 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. * 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 anti-thymocyte globulin or Alemtuzumab, *HLA-DPB1* matching status, disease category and severity at transplantation. † Transplantations performed for non-malignant diseases were excluded from the analysis. ‡ D and R stand for donor and recipient, respectively. The reference category for the CMV status is D-/R-.

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

	hazard ratio (95% CI)*	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
<i>MICB98</i> 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. * 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 anti-thymocyte globulin or Alemtuzumab, *HLA-DPB1* matching status, disease category and severity at transplantation.

Figure 1

Figure 2

A Acute GVHD III-IV in HCT with donors and recipients negative for CMV

B Acute GVHD III-IV in HCT with donors and/or recipients positive for CMV

C Overall Survival in MICB98 matched HCT

D Overall Survival in MICB98 mismatched HCT

Figure 3