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

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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].

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 incompatibilities [29-31]. *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 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 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-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 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 R [52].

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

293 and/or the recipient are positive for CMV (acute GVHD III-IV hazard ratio, 3.63 x 0.26
294 = 0.94). This observation was confirmed by representing graphically cumulative
295 incidences of acute GVHD III-IV in the above mentioned two CMV subgroups (Figure
296 2A and 2B).

297 For OS, the interaction between *MICB98* mismatching and CMV status was
298 statistically significant and was > 1 (hazard ratio, 1.53; 95% CI, 1.38 to 1.69; P <
299 0.001). CMV positivity in the donor and/or recipient was associated with a slightly
300 lower survival when *MICB98* was matched (hazard ratio, 1.16; 95% CI, 1.14 to 1.19;
301 P< 0.001). However, because of the positive interaction with *MICB98* mismatches,
302 this effect was higher when *MICB98* was mismatched (hazard ratio 1.16 x 1.53 =
303 1.77) (Table 3). The Kaplan-Meier estimates showing the higher impact of the CMV
304 status on OS in *MICB98* matched and mismatched groups are presented in Figures
305 2C and 2D, respectively. In other words, the risk of death associated with CMV
306 positivity in the donor and/or recipient is lower in *MICB98* matched vs. mismatched
307 groups.

308 Finally, to assess whether *MICB98* mismatches had a GVHD-independent
309 effect on CMV infections in donor/recipients pairs at risk for CMV reactivation (i.e.,
310 the donor and/or recipient was positive for CMV pre-HCT), we performed a
311 multivariate Fine and Gray analysis that included *MICB98* matching as well as the
312 presence/absence of acute GVHD grades III-IV and chronic GVHD as time-
313 dependent covariates in the model (Table 4). In accordance with the higher risk of
314 death described above, *MICB98* mismatches were associated with a higher
315 incidence of CMV infections (hazard ratio, 1.84; 95% CI, 1.34 to 2.51; *P* < 0.001)
316 (Table 4 and Figure 3). *MICB98* mismatches were not associated with EBV or HHV6
317 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 x 2.61 = 2.01) (Table 3).

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.

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 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 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, FCl, 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.

685 **TABLES**

686 **Table 1. Demographics of the Study Population**

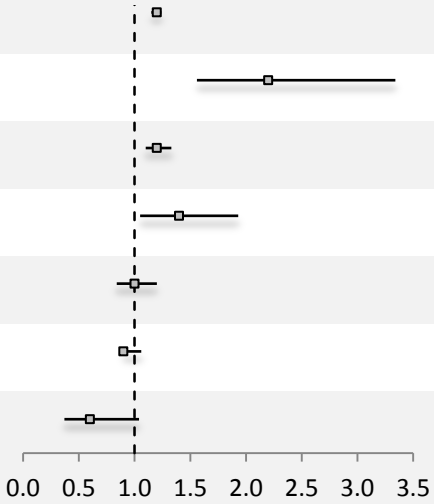
	Total transplants (n= 943)	MICB 98 matched transplants (n=887)	MICB 98 mismatched transplants (n=56)	*P-value**
Transplantation centers†				0.16
1	106 (11.2%)	100 (11.3%)	6 (10.7%)	
2	158 (16.8%)	142 (16%)	16 (28.6%)	
3	114 (12.1%)	109 (12.3%)	5 (8.9%)	
4	157 (16.6%)	153 (17.2%)	4 (7.1%)	
5	48 (5.1%)	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 applicablell	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%)	

687

688 The results are presented as the number of patients and corresponding percentages of the
689 study population. HLA: Human Leukocyte Antigen. All clinical variables of the table were
690 used for adjustment in the multivariate models.
691 * *P*-values were determined with Pearson's Chi square test or Fisher's exact test for small
692 sample sizes
693 † Patients received their transplant in six centers of the Francophone Society of Bone
694 Marrow Transplantation and Cell Therapies (SFGM-TC) (1 to 6; N =682) and in three Dutch
695 centers that are part of the Europdonor operated by the Matchis Foundation network (7 to 9;
696 N=261).
697 ‡ *in vivo* T-cell depletion was performed by the addition of anti-thymocyte globulin (ATG) or
698 Alemtuzumab to the conditioning regimen.
699 § Other diseases include multiple myeloma, Hodgkin lymphoma, Fanconi anemia, aplastic
700 anemia, chronic lymphocytic leukemia, plasma cell leukemia, other acute leukemias, solid
701 tumors (not breast), hemophagocytosis and inherited disorders.
702 ¶ Early corresponds to diseases in the first complete remission or in the chronic phase. Late
703 corresponds to second or higher complete remissions, accelerated phases, partial
704 remissions, progressions, primary induction failures, relapses or stable diseases. Not
705 applicable corresponds to bone marrow failure (aplastic anemia, Fanconi anemia), inherited
706 disorders, hemophagocytosis and solid tumors.
707 ** *HLA-DPB1* matching was defined at the T-cell-epitope matching level [49] with typing data at
708 2nd field resolution following the World Health Organization official nomenclature .
709

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		
MICB98 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: *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†		
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. * 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
***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. * 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

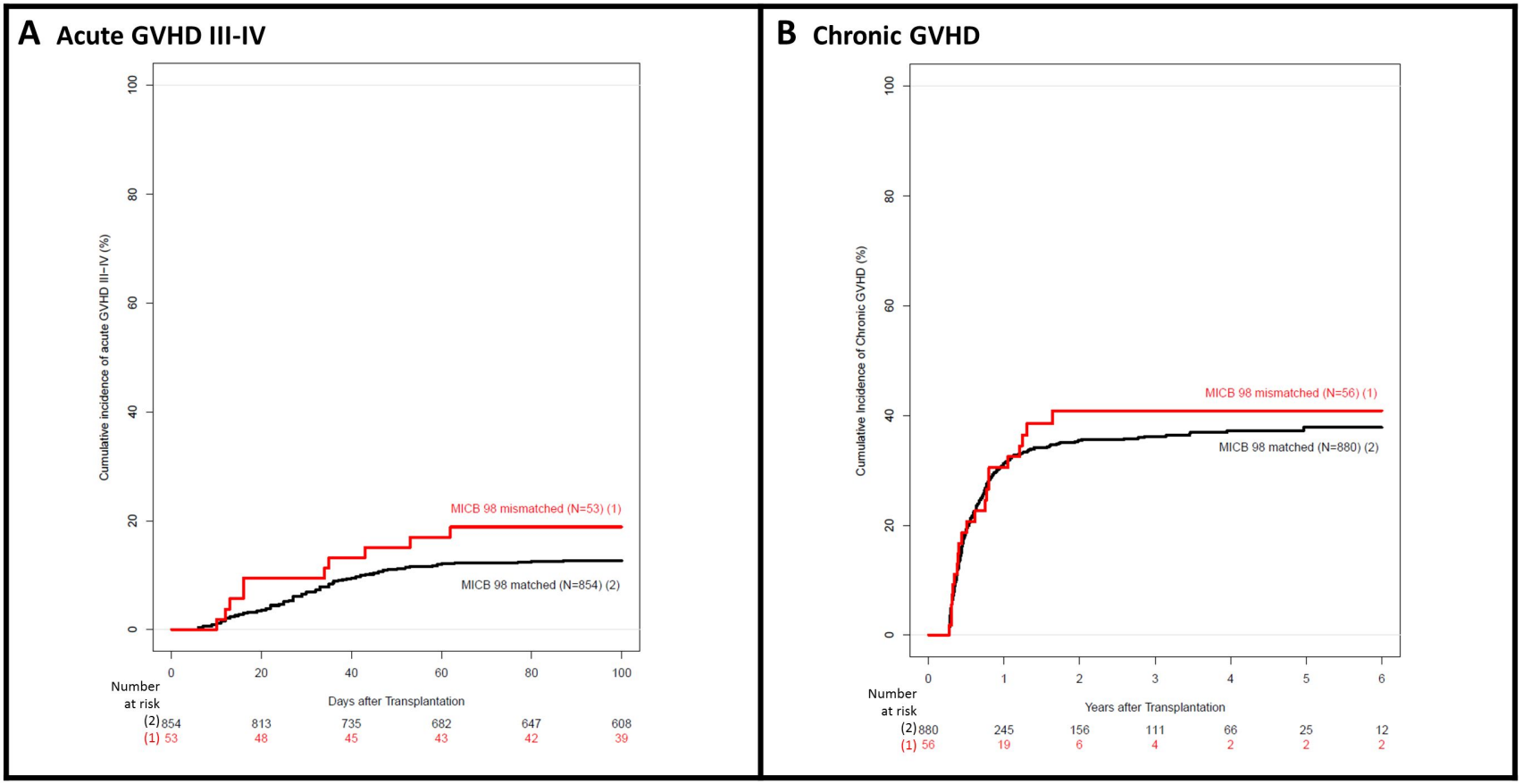

A Acute GVHD III-IV
Cumulative incidence of acute GVHD III–IV (%)
0
20
40
60
80
100
MICB 98 mismatched (N=53) (1)
MICB 98 matched (N=854) (2)
0
20
40
60
80
100
Days after Transplantation
Number at risk
(2) 854 813 735 682 647 608
(1) 53 48 45 43 42 39
B Chronic GVHD
Cumulative Incidence of Chronic GVHD (%)
0
20
40
60
80
100
MICB 98 mismatched (N=56) (1)
MICB 98 matched (N=880) (2)
0
1
2
3
4
5
6
Years after Transplantation
Number at risk
(2) 880 245 156 111 66 25 12
(1) 56 19 6 4 2 2 2

Figure 2

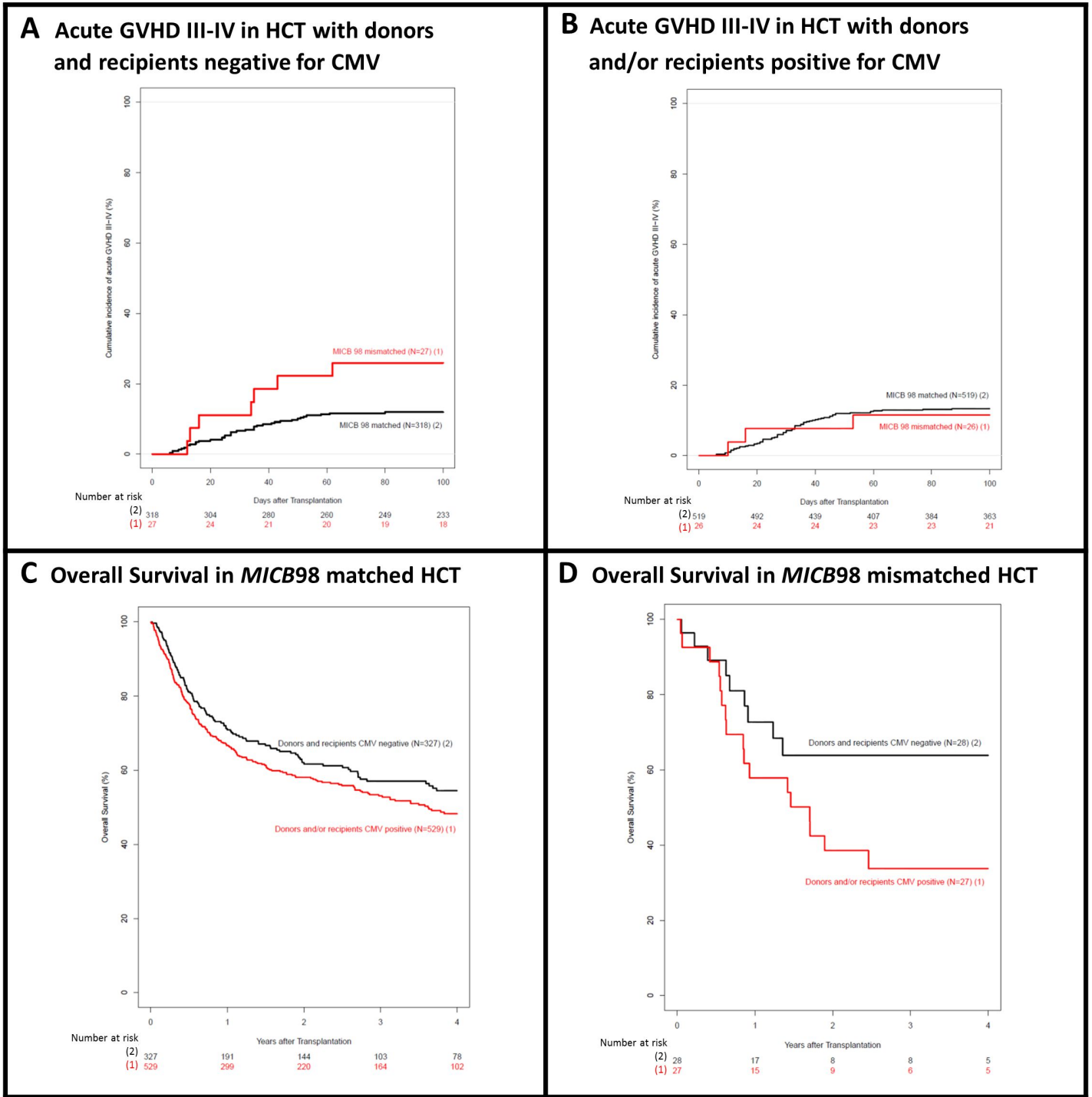
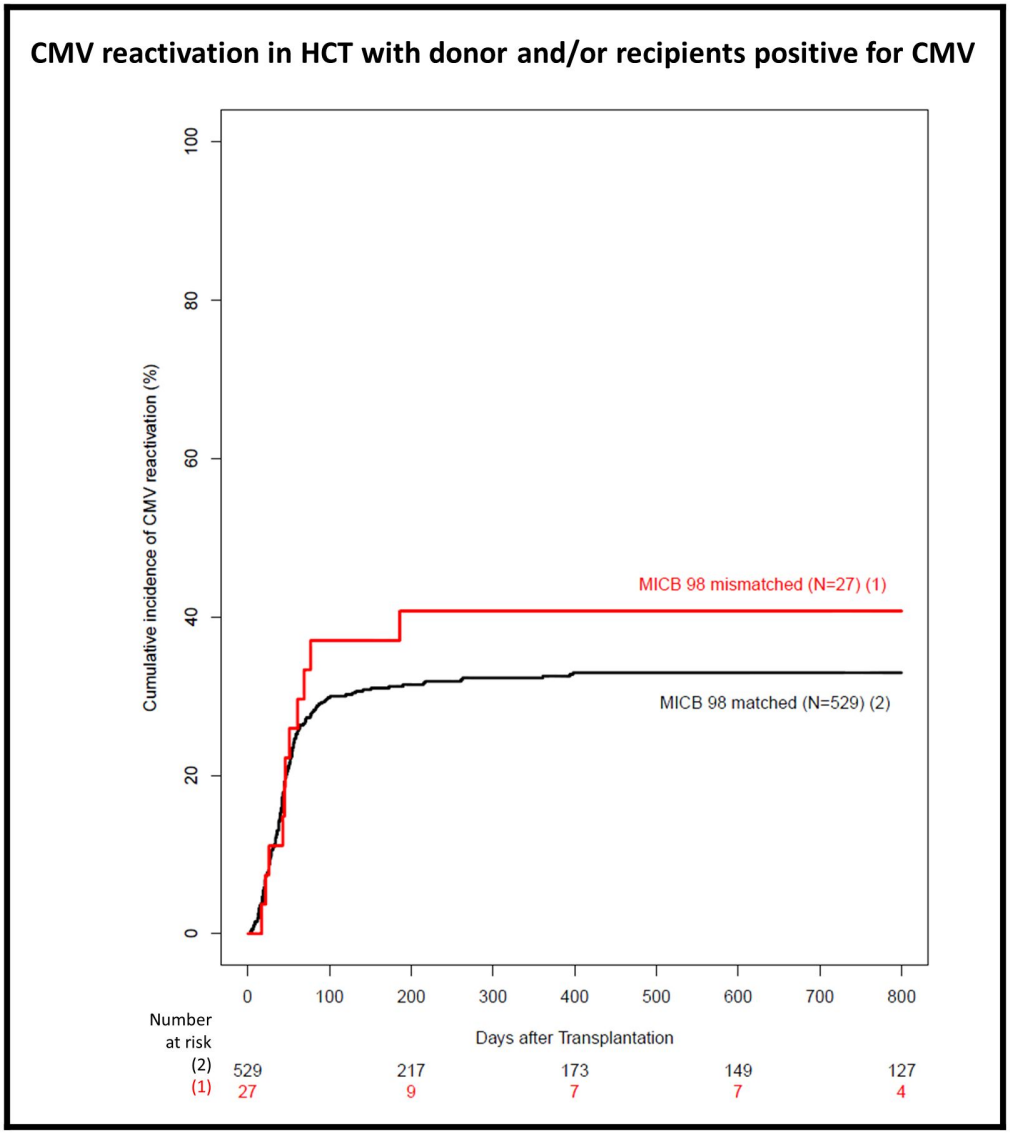

A Acute GVHD III-IV in HCT with donors and recipients negative for CMV
Cumulative incidence of acute GVHD III–IV (%)
MICB 98 mismatched (N=27) (1)
MICB 98 matched (N=318) (2)
Days after Transplantation
Number at risk
(2) 318 304 280 260 249 233
(1) 27 24 21 20 19 18
B Acute GVHD III-IV in HCT with donors and/or recipients positive for CMV
Cumulative incidence of acute GVHD III–IV (%)
MICB 98 matched (N=519) (2)
MICB 98 mismatched (N=26) (1)
Days after Transplantation
Number at risk
(2) 519 492 439 407 384 363
(1) 26 24 24 23 23 21
C Overall Survival in MICB98 matched HCT
Overall Survival (%)
Donors and recipients CMV negative (N=327) (2)
Donors and/or recipients CMV positive (N=529) (1)
Years after Transplantation
Number at risk
(2) 327 191 144 103 78
(1) 529 299 220 164 102
D Overall Survival in MICB98 mismatched HCT
Overall Survival (%)
Donors and recipients CMV negative (N=28) (2)
Donors and/or recipients CMV positive (N=27) (1)
Years after Transplantation
Number at risk
(2) 28 17 8 8 5
(1) 27 15 9 6 5

Figure 3


CMV reactivation in HCT with donor and/or recipients positive for CMV
Cumulative incidence of CMV reactivation (%)
0
20
40
60
80
100
MICB 98 mismatched (N=27) (1)
MICB 98 matched (N=529) (2)
0
100
200
300
400
500
600
700
800
Days after Transplantation
Number at risk
(2) 529 217 173 149 127
(1) 27 9 7 7 4