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

3

4 *Running head: MICB, CMV and GVHD in HCT*

5

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72

73

74 **ABSTRACT**

75 Graft-versus-host disease (GVHD) and cytomegalovirus (CMV)-related complications
76 are leading causes of mortality after unrelated-donor hematopoietic cell
77 transplantation (UD-HCT). The non-conventional MHC class I gene *MICB*, alike
78 *MICA*, encodes a stress-induced polymorphic NKG2D ligand. However, unlike *MICA*,
79 *MICB* interacts with the CMV-encoded UL16, which sequesters *MICB*
80 intracellularly, leading to immune evasion. Here, we retrospectively analyzed the
81 impact of mismatches in *MICB* amino acid position 98 (*MICB98*), a key polymorphic
82 residue involved in UL16-binding, in 943 UD-HCT pairs who were allele-matched at
83 *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQB1* and *MICA* loci. *HLA-DP* typing was further available.
84 *MICB98* mismatches were significantly associated with an increased incidence of
85 acute (grade II-IV: HR, 1.20; 95% CI, 1.15 to 1.24; $P < 0.001$; grade III-IV: HR, 2.28;
86 95% CI, 1.56 to 3.34; $P < 0.001$) and chronic GVHD (HR, 1.21; 95% CI, 1.10 to 1.33;
87 $P < 0.001$). *MICB98* matching significantly reduced the effect of CMV status on
88 overall mortality from a hazard ratio of 1.77 to 1.16. *MICB98* mismatches showed a
89 GVHD-independent association with a higher incidence of CMV infection/reactivation
90 (HR, 1.84; 95% CI, 1.34 to 2.51; $P < 0.001$). Hence selecting a *MICB98*-matched
91 donor significantly reduces the GVHD incidence and lowers the impact of CMV status
92 on overall survival.

93

94 **INTRODUCTION**

95 Unrelated-donor hematopoietic cell transplantation (HCT) is an established
96 treatment for a wide range of immunological and hematologic disorders, malignant or
97 otherwise ¹. Although more than 50,000 HCTs are performed annually worldwide ^{2, 3},
98 adverse clinical outcomes occur frequently. One of the most common life-threatening
99 complications is graft-versus-host disease (GVHD), which greatly hampers the
100 successful outcome of this powerful and sometimes unique curative option. In GVHD,
101 the donor's immune cells attack the patient's organs and tissues, impairing their
102 ability to function and increasing the patient's susceptibility to infection. The
103 organs/tissues most frequently targeted are the skin, the gastrointestinal tract and the
104 liver. Despite the availability of effective immunosuppressive drugs, the incidence of
105 GVHD remains alarmingly high: up to 35% experience grade III-IV acute GVHD and
106 40% to 50% experience chronic GVHD ⁴⁻⁶.

107 Cytomegalovirus (CMV) infection/reactivation represents another leading
108 cause of morbidity and mortality in patients undergoing allogeneic HCT because it
109 frequently causes serious complications, e.g., pneumonia, hepatitis, gastroenteritis,
110 retinitis, and encephalitis ⁷⁻¹¹. Because of the immunosuppressive regimen,
111 allogeneic HCT patients are indeed at a higher risk for CMV infection and/or
112 reactivation. The incidence of CMV infection has been reported to vary between 40
113 and 80% in CMV seropositive allogeneic HCT patients not treated with anti-viral
114 prophylaxis drugs, which currently represents most of the allogeneic HCT recipients
115 ¹²⁻¹⁸. In seronegative patients receiving a transplant from a seropositive donor, the
116 rate of primo infection is approximately 30% ¹². Despite the implementation of
117 prophylaxis, monitoring, and pre-emptive treatment of CMV reactivation/infection,
118 cases of CMV seropositivity of the donor and/or the recipient show decreased

119 survival rates compared to CMV-seronegative recipients who undergo allograft from
120 CMV-seronegative donors^{16, 19}. New strategies for preventing CMV
121 reactivation/infection in transplant recipients therefore remain an important objective
122 for the improvement of allogeneic HCT.

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

132 The MHC-encoded non-conventional *MHC class I chain-related (MIC) genes A*
133 (*MICA*) and *B* (*MICB*) encode polymorphic cell surface proteins which bind to
134 NKG2D; an activating immune receptor expressed by cytotoxic T and NK cells^{25, 26}.
135 This interaction is seminal in defense both against infections and malignancies.
136 Moreover, *MICB*^{27, 28} happens to be one of the most promising candidates to explain,
137 at least partially, GVHD and CMV complications that cannot be attributed to classical
138 HLA genes or the related *MICA* gene incompatibilities²⁹⁻³¹. *MICB* is indeed highly
139 polymorphic, with 47 alleles reported to date
140 (<http://www.ebi.ac.uk/ipd/imgt/hla/stats.html>). It encodes a cell-surface glycoprotein
141 up-regulated by cell stress^{25, 32}. The gene is located 130 kb and 83 kb centromeric to
142 *HLA-B* and *MICA*, respectively, and was discovered by us over 20 years ago²⁵.
143 *MICB* is highly similar to *MICA* in terms of sequence (83% shared amino acid

144 sequence identity), linkage disequilibrium with *HLA-B*, protein structure (HLA-like
145 structure without association to β_2 -microglobulin) and constitutive expression pattern
146 (restricted to epithelial cells, fibroblasts, monocytes, dendritic cells and endothelial
147 cells)^{26, 33, 34}. MICB is a ligand for the activating NKG2D receptor expressed on the
148 surface of cytotoxic CD8⁺ $\alpha\beta$ and $\gamma\delta$ T lymphocytes and natural killer cells³⁵.
149 Interestingly, and in contrast to *MICA*, MICB binds the CMV protein UL16, which
150 sequesters MICB intracellularly in an immune escape mechanism³⁶. Different
151 *MICB* alleles are not equal with respect to binding to UL16. *MICB*008* has been
152 shown to have a decreased binding capacity to UL16 compared to other alleles³⁷.
153 *MICB*008* is characterized by a polymorphism at amino acid position 98, causing an
154 isoleucine (Ile) to methionine (Met) exchange in the $\alpha 2$ domain of the MICB protein.
155 The variation Ile > Met is exclusively present in *MICB*008* and is the unique
156 polymorphic position that is in direct contact with UL16 through hydrophobic contacts
157 (distance < 4.0 Å) with leucine 161 of UL16³⁸.

158 Several lines of evidence indicate that *MICB* could play a role in triggering
159 GVHD and/or modulating CMV infection/reactivation: (1) the localized expression in
160 epithelial cells of the gastrointestinal tract, whose damage during GVHD plays a
161 major pathophysiologic role in the amplification of systemic disease³⁹; (2) the
162 common features with *MICA* that have repeatedly been shown to be involved in
163 GVHD^{29, 30, 40-42}; and (3) the binding of MICB to the UL16 protein³⁶. The present
164 study hence aims to show the effect of *MICB* matching at amino acid position 98,
165 representing about 6% of transplantations, on the outcome of unrelated donor HCT in
166 a cohort of 943 donor/recipient pairs matched for *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQB1*, and
167 *MICA*.

168

169 **PATIENTS AND METHODS**

170 **STUDY DESIGN AND OVERSIGHT**

171 This retrospective study was designed to test whether donor-recipient matching at
172 amino acid position 98 of the MICB protein (*MICB98*) improves the outcome of
173 unrelated HCT. Patients from six French and three Dutch centers and their donors
174 were included; the unrelated donors originated from national or international donor
175 registries. Genomic DNA samples and high-resolution *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQB1*,
176 *-DPB1* and *MICA* typing data were collected. Clinical information was made available
177 by the SFGM-TC and the HOVON Data Center from the EBMT (European group for
178 Blood and Marrow Transplantation) ProMISe patient database. All authors vouch for
179 the accuracy and completeness of the results. This study, conducted under the
180 auspices of SFGM-TC and the Dutch-Belgian Cooperative Trial Group for
181 Hematology Oncology (HOVON), was approved by institutional review boards of the
182 participating centers and was performed according to the principles of the Declaration
183 of Helsinki. Written informed consent was obtained from all participants.

184

185 **PATIENTS AND DONORS**

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

191

192 ***MICB* GENOTYPING AT AMINO ACID POSITION 98**

193 The polymorphic nucleotide position 363 (C/G; rs3134900) causes an isoleucine (Ile)
194 to methionine (Met) change at amino acid position 98 in the α 2 domain of the MICB
195 protein. Both patients and unrelated donors were genotyped for this position by
196 Sanger sequencing of *MICB*'s exon 3, following previously described protocols ⁴³.
197 The sequences were analyzed using Seqscape v2.6 (Life Technologies, USA) to
198 assign genotypes.

199

200 **DEFINITIONS**

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

217

218 **STATISTICAL ANALYSIS**

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

243

244 **RESULTS**

245 The demographics of the study population are shown in Table 1. The median
246 post-transplant follow-up was 36 months (mean: 37 months; range: 1 to 105 months),
247 and the median patient age was 53 years (mean: 48 years; range: 1 to 73 years).
248 The patients suffered from both malignant and non-malignant diseases. Most
249 transplants were performed with non-myeloablative/reduced intensity conditioning
250 regimens (67%); *in vivo* T-cell depletion was performed in the majority of cases
251 (73%), and peripheral blood was the main source for stem cells (79%). All
252 donor/patient pairs were fully typed at high resolution (2nd field) for *HLA-A*, *-B*, *-C*, *-*
253 *DRB1*, *-DQB1*, *-DPB1* and *MICA*²⁹ and were matched for 12 out of 12 alleles at *HLA-*
254 *A*, *-B*, *-C*, *-DRB1*, *-DQB1* and *MICA* loci. Among the 943 transplantations, 394
255 (41.8%) had non-permissive *HLA-DPB1* mismatches. Fifty-six (5.9%) transplants
256 were *MICB98* mismatched. The mismatch vectors of these 56 transplants were graft-
257 versus-host (n=22), host-versus-graft (n=33) and bidirectional (n=1). Except for the
258 patient-donor CMV status, all relevant covariates for the analyzed clinical outcomes
259 were equally distributed in the *MICB98* matched and -mismatched patients (Table 1).
260 Organ-specific sub-analyses showed that the *MICB98* matching effect was more
261 important in the gut and the skin than in the liver (supplemental Figure 1). *MICB98*
262 mismatches were significantly associated with an increased incidence of acute
263 GVHD (hazard ratio (HR) for grades II-IV: 1.20; 95% CI, 1.15 to 1.24; *P* < 0.001; for
264 grades III-IV: 2.28; 95% CI, 1.56 to 3.34; *P* < 0.001) (Table 2). At day 100 post-HCT,
265 the cumulative incidences of severe (grades III-IV) acute GVHD in *MICB98*
266 mismatched vs. matched transplantations were 18.9% vs. 12.5%, respectively
267 (Figure 1A). Matching *MICB* at position 98 decreased the risk of chronic GVHD by

268 4% (40.9% vs. 36.9%) at 4 years post-transplantation (HR, 1.21; 95% CI, 1.10 to
269 1.33; $P < 0.001$) (Table 2 and Figure 1B). In addition, *MICB98* mismatches were
270 associated with a higher rate of relapse (HR, 1.42; 95% CI, 1.05 to 1.93; $P = 0.024$).

271 Knowing that amino acid position 98 is involved in the binding of MICB to the
272 UL16 protein of the CMV, we assessed the interaction between *MICB98* mismatches
273 and the CMV status in their effect on clinical outcomes. For this purpose, we
274 performed multivariate analyses and included an interaction factor in the model.
275 Table 3 represents the risks of various clinical outcomes associated with (1) *MICB98*
276 mismatches when donor and recipients are negative for CMV, (2) CMV positivity in
277 donor and/or recipients when *MICB98* is matched and (3) the interaction of *MICB98*
278 matching with CMV status. A statistically significant value for the interaction factor
279 indicates that the effect of *MICB98* matching depends on the category of CMV status
280 and vice versa. When the hazard ratio of the interaction factor is < 1 or > 1 , the
281 hazard ratio of a variable (here, *MICB98* matching or CMV status) is, respectively,
282 lower or higher in the category at risk of its interacting variable compared to the
283 reference category. For example, when the hazard ratio of the interaction factor is $<$
284 1, the hazard ratio of *MICB98* mismatches is lower when the donor and/or recipient
285 are positive for CMV (category at risk of the CMV status variable) and higher when
286 both the donor and recipient are negative for CMV (reference category of the CMV
287 status variable).

288 For acute GVHD III-IV, the hazard ratio of the interaction was < 1 and was
289 statistically significant (hazard ratio for acute GVHD III-IV, 0.26; 95% CI, 0.17 to 0.40;
290 $P < 0.001$), indicating that the effect of *MICB98* mismatching on acute GVHD is more
291 important when both the donor and the recipient are negative for CMV (acute GVHD
292 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, 3.63×0.26
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 $1.16 \times 1.53 =$
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).

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

343 than in its absence. In the absence of CMV, the *MICB98* polymorphism may indeed
344 not be able to modulate the expression of MICB at the cell surface through interaction
345 with UL16 and consequently is not able to influence the alloreactivity that remains
346 higher in the mismatch than in the matched situation. Another explanation for the
347 higher *MICB*-mediated alloreactivity in the absence of CMV may be the absence of T-
348 cell exhaustion, that is known to be induced by CMV positivity⁵⁵. Ultimately, this
349 observation demonstrates that to lower the risk of acute GVHD in the absence of
350 CMV (donor and recipient seronegative), a *MICB98* matched donor is a better choice
351 than a *MICB98* mismatched donor.

352 CMV causes mortality in two ways: (1) directly by causing viral diseases, such
353 as pneumonitis, a situation that is becoming rare (viral diseases represent less than
354 2% of deaths) thanks to preemptive therapies, or (2) indirectly by clinical events
355 associated with virus seropositivity or the development of viral infections that are
356 independent of the viral disease itself⁵⁶. The indirect effects of CMV are recognized
357 as a major cause of adverse outcomes after HCT, including GVHD and overall
358 mortality⁵⁶⁻⁵⁸. Our dataset showed that the CMV effect on overall survival is amplified
359 in *MICB98* mismatched HCT compared to *MICB98* matched HCT, indicating that
360 matching donors at this position could be a useful strategy to decrease the risk of
361 death related to CMV. Because *MICB98* mismatches were further shown to be
362 associated with CMV infection episodes, and this independently of the occurrence of
363 GVHD, deaths related to CMV may be due to CMV infections.

364 Collectively, these results suggest that pre-transplantation *MICB98* typing may
365 help in lowering the risk of both GVHD- and CMV-related mortality. In the absence of
366 CMV, matching *MICB98* provides a means to lower the incidence of GVHD, whereas
367 in the presence of CMV, it helps improve overall survival. Fortunately, the level of

368 *MICB98* mismatching is only 5.9% in HLA 10/10 matched donor/patient pairs that are
369 also matched for *MICA*; although in absolute terms, this represents several thousand
370 patients per year. Therefore, finding a *MICB98*-matched donor should be relatively
371 easy in clinical practice.

372

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383

384

385 **AUTHOR CONTRIBUTIONS**

386 RC performed the experiments, designed the study, analyzed the data and wrote the
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388 AM, IK, CM, APi and VR performed the experiments and analyzed the data. IA
389 performed the statistics. PAvdB, DB, AC, DC, FCI, KG, JK, JJ, MLaba, PL, MMi, NM,
390 PM, MOu, APa, RP, CPi, GS, ES, RT, AT, IY and BvdH provided samples and
391 clinical data, interpreted the clinical data and discussed the results. BL, MMo, AN and
392 CPa interpreted the clinical data and discussed results. FB, YK, MMB, MOt, and

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

395

396 **Ethics declarations**

397 **Conflict of interest**

398 SB is the scientific founder and a (minority) shareholder of BIOMICA SAS. JK is the
399 co-founder and chief scientific officer of Gadeta. He received personal fees from
400 Gadeta. In addition, JK has a patent issued/pending. ES is the inventor of a patent
401 application filed by the University Medical Center Utrecht on the prediction of an
402 alloimmune response against mismatched HLA (PCT/EPT2013/073386). All other
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404

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668 **FIGURE LEGENDS**

669

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

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

673

674 **Figure 2. Effect of *MICB98* matching and CMV status on GVHD and Overall
675 Survival.**

676 Panels A and B represent the cumulative incidences of grades III-IV acute GVHD in
677 HCT with donors and recipients negative for CMV (A) and HCT with donors and/or
678 recipients positive for CMV (B). Panels C and D show the Kaplan-Meier estimates of
679 overall survival in *MICB98* matched (C) and mismatched (D) transplants.

680

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

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

684

685
686

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%)	

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

710 **Table 2. Analysis of the Impact of *MICB* Mismatches at amino acid position 98 on**
 711 **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

712 Results are presented as Hazard Ratios with 95% confidence intervals (CI). GVHD: Graft-
 713 versus-host disease. * All models were adjusted for patient's age, patient-donor sex, patient-
 714 donor serological status for cytomegalovirus, year of transplantation, time to transplantation,
 715 transplantation center, source of stem cells, conditioning regimen, GVHD prophylaxis,
 716 treatment with anti-thymocyte globulin or Alemtuzumab, *HLA-DPB1* matching status, disease
 717 category and severity at transplantation. † Transplantations performed for non-malignant
 718 diseases were excluded from the analysis.

719

720

721 **Table 3. Analysis of the Impact of *MICB* Mismatches at position 98, CMV status and**
 722 **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

723 Results are presented as Hazard Ratios with 95% confidence intervals (CI). GVHD: Graft-
 724 versus-host disease. * All models were adjusted for patient's age, patient-donor sex, patient-
 725 donor serological status for cytomegalovirus, year of transplantation, time to transplantation,
 726 transplantation center, source of stem cells, conditioning regimen, GVHD prophylaxis,
 727 treatment with anti-thymocyte globulin or Alemtuzumab, *HLA-DPB1* matching status, disease
 728 category and severity at transplantation. † Transplantations performed for non-malignant
 729 diseases were excluded from the analysis. ‡ D and R stand for donor and recipient,
 730 respectively. The reference category for the CMV status is D-/R-.

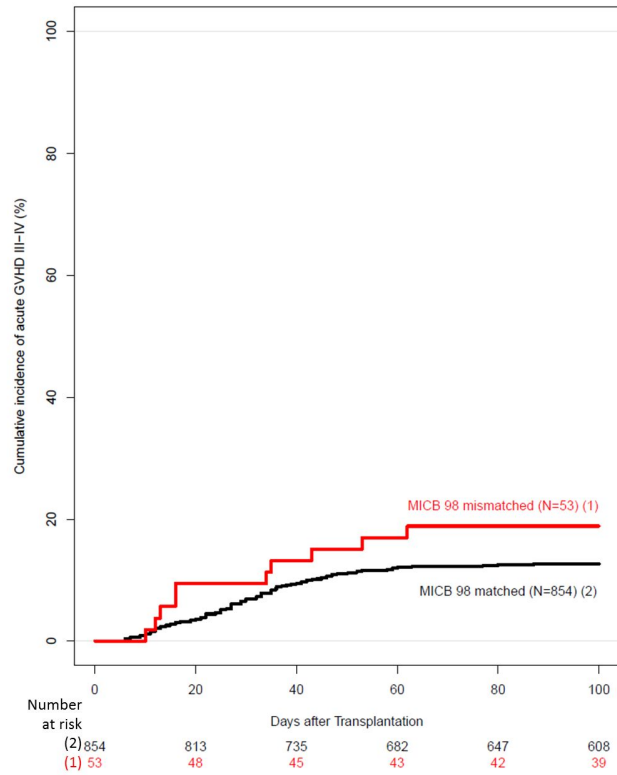
731 **Table 4. Effect of GVHD and *MICB98* matching on CMV infections**
 732

	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

733 Only the pairs in which the donor and/or the recipient was/were positive for CMV pre-
 734 HCT were included in the analysis. The results are presented as Hazard Ratios with
 735 95% confidence intervals (CIs). GVHD: Graft-versus-host disease. Ref.: Reference
 736 category. * Multivariate Fine and Gray model including *MICB98* matching, acute
 737 GVHD III-IV and chronic GVHD as time-dependent covariates in the model. In
 738 addition, the model was adjusted for patient's age, patient-donor sex, patient-donor
 739 serological status for cytomegalovirus, year of transplantation, time to transplantation,
 740 transplantation center, source of stem cells, conditioning regimen, GVHD prophylaxis,
 741 treatment with anti-thymocyte globulin or Alemtuzumab, *HLA-DPB1* matching status,
 742 disease category and severity at transplantation.

Figure 1

A Acute GVHD III-IV



B Chronic GVHD

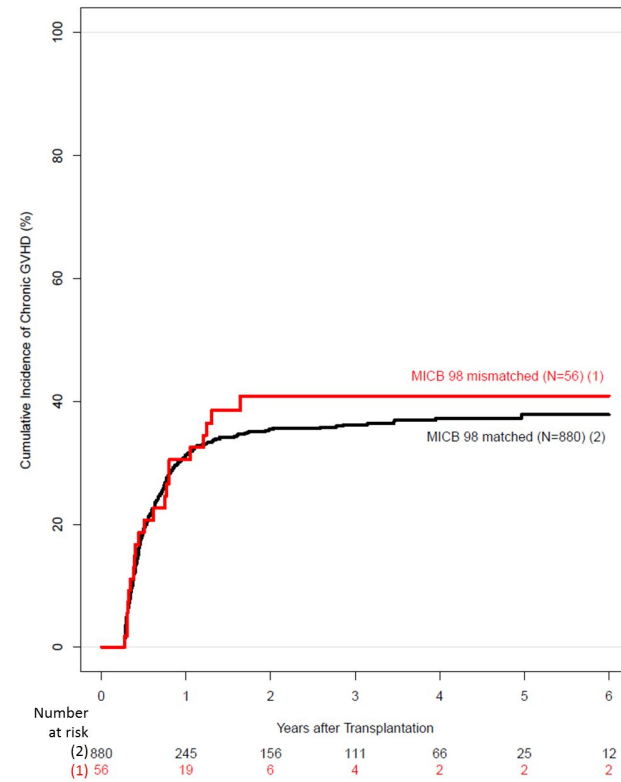
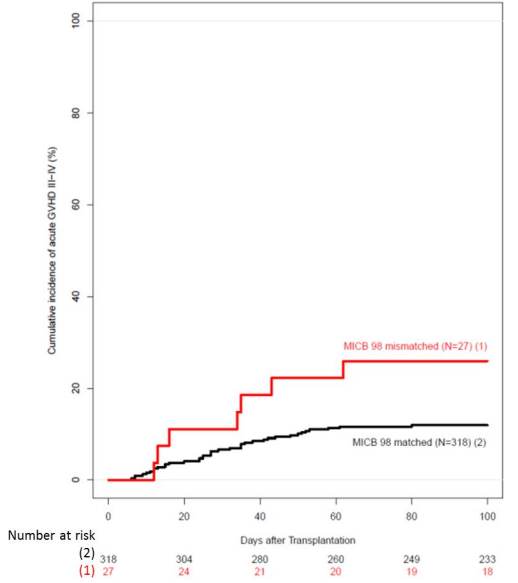
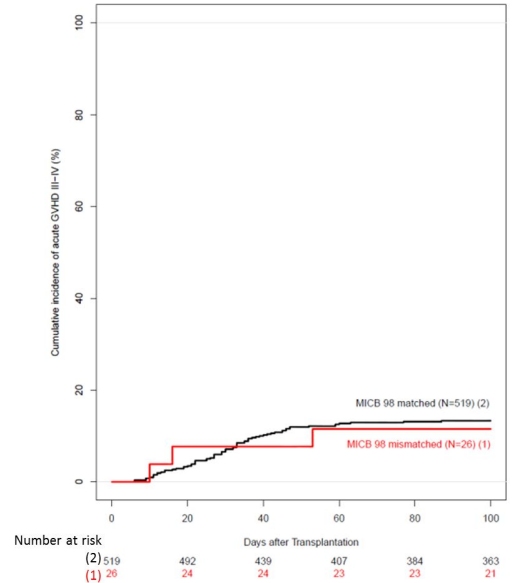


Figure 2

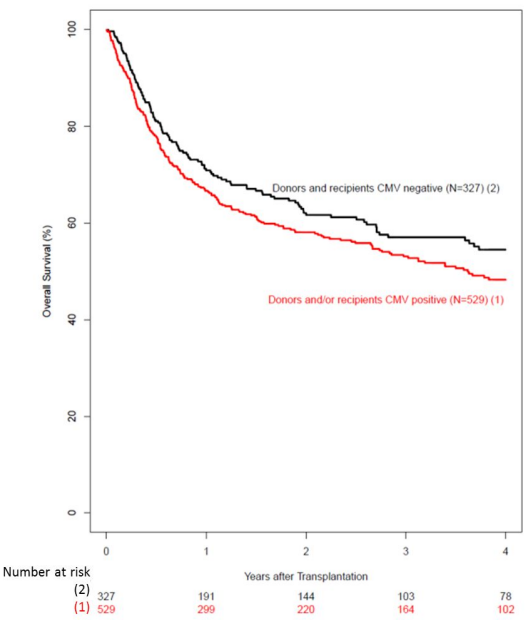
A Acute GVHD III-IV in HCT with donors and recipients negative for CMV



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

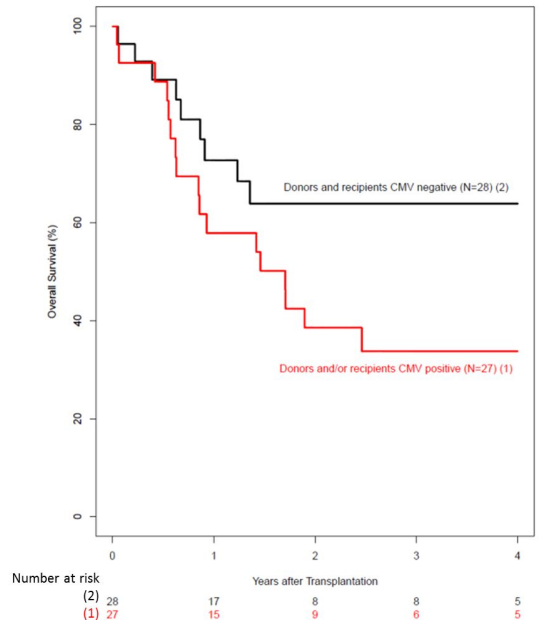


Figure 3

