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Clinical Research: Alternative Donors

Killer Cell Immunoglobulin-Like Receptor–Ligand Matching and Outcomes after Unrelated Cord Blood Transplantation in Acute Myeloid Leukemia

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The effect of killer cell immunoglobulin-like receptor (KIR)–ligand matching on outcomes after unrelated cord blood (CB) transplantation was studied in 461 patients with acute myeloid leukemia, categorizing KIR ligand for HLA-C groups C1 and C2 and Bw4. Donor–recipient HLA matching considered allele-level matching at HLA-A, -B, -C, and -DRB1. Separate analyses were conducted for 6-7/8 HLA-matched and 3-5/8 HLA-matched transplants because HLA matching confounded KIR-ligand matching (ie, KIR-ligand mismatching was less likely with better HLA matching). All patients received single CB unit and myeloablative conditioning. There were no significant differences in nonrelapse mortality (NRM), relapse, and overall mortality by KIR-ligand match status. However, among recipients of 3-5/8 HLA-matched transplants, NRM (HR, 2.26; $P = .008$) and overall mortality (HR, 1.78; $P = .008$) but not relapse were higher with KIR-ligand mismatched (host-versus-graft direction) compared with KIR-ligand matched transplants. These data do not support selecting CB units based on KIR-ligand match status for transplants mismatched at 1 or 2 HLA loci. Although transplants mismatched at 3 or more HLA loci are not recommended, avoiding KIR-ligand mismatching in this setting lowers mortality risks.

INTRODUCTION

Natural killer (NK) cell alloreactivity may play an important role in determining the outcome of patients given allogeneic hematopoietic stem cell transplantation (HSCT). NK cell function is controlled by an array of inhibitory and activating signals that are processed by cell surface receptors,

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including the inhibitory and activating killer cell immunoglobulin-like receptors (KIRs) [1,2]. Earlier models of NK alloreactivity in HSCT focused on the interactions between inhibitory KIRs and HLA class I ligands, in which the alloreactivity of donor NK cells is triggered by lack of self-HLA class I engagement of inhibitory KIRs [3,4]. In retrospective studies of outcomes after HSCT, particularly for patients with acute myeloid leukemia (AML), KIR-ligand incompatibilities have been associated with better survival, less relapse, and lower incidence of graft-versus-host disease (GVHD) [3,5-7]. This relationship is most evident in T cell–depleted HLA haplotype–disparate HSCT, in which recipients who lack the HLA ligand present in the stem cell donor benefit from lower relapse rates [3,6].

Most donor–recipient cord blood (CB) transplant pairs are HLA mismatched at 1 or 2 HLA loci, considering antigen-level (low-resolution) HLA matching at HLA-A and -B or at the allele level at HLA-DRB1. Matching at the HLA-C locus is not generally considered. Previous studies investigating the effect of KIR–HLA ligand matching on HSCT outcomes have shown varied and conflicting results. Although some studies showed a beneficial effect of KIR-ligand mismatching, others found deleterious effects or no effects [8-13]. These differing results may be explained by the heterogeneity of the patient cohorts studied, the degree of T cell alloreactivity secondary to HLA mismatch, and the different models used to determine KIR-HLA incompatibility [3,14-16].

To date, 4 retrospective studies investigating donor-versus-recipient alloreactivity after single- and double-unit unrelated CB transplantation (UCBT) using the ligand-incompatibility model have been reported, with conflicting results [17-20]. There are a number of significant differences between these studies, including sample size, population composition, and the definition of KIR-ligand matching assignment (inclusion of HLA-A–associated Bw4 ligands versus no inclusion). All these studies considered low-resolution typing and definitions of matching for HLA-A, -B, -C and high-resolution typing for HLA-DRB1, with a relatively small number of patients with heterogeneous diseases and conditioning regimens. We have shown that matching at the HLA-C locus lowers mortality after single-unit UCBT for acute leukemia [21,22], but whether selecting CB units on the basis of KIR-ligand matching improves survival and other outcomes is unclear. This analysis sought to study the KIR-ligand effect, using high-resolution HLA typing and matching of HLA-A, -B, -C, and -DRB1, in a large series of single-unit UCBT using myeloablative conditioning for AML.

METHODS

Patients

Data were obtained from the Center for International Blood and Marrow Transplant Research or Eurocord–European Group for Blood and Marrow Transplantation. Patients received a single UCB unit after a myeloablative conditioning regimen for treatment of acute leukemia with cyclosporine or tacrolimus-containing GVHD prophylaxis. All transplants were performed between 2000 and 2010. Those patients (n = 218) previously reported in a Eurocord report [17] were excluded. The Institutional Review Board of the National Marrow Donor Program and the Eurocord-Netcord scientific committee approved this study.

HLA Typing and KIR-Ligand Classification

Donor and recipient HLA typing at HLA-A, -B, -C, and -DRB1 was completed using molecular techniques with a minimum of antigen split-level resolution for HLA-A, -B, and -C and allele-level resolution at HLA-DRB1. Details of HLA-typing and imputation (Haplogic III developed by the National Marrow Donor Program) were previously reported [21]. Patients and donors were categorized by KIR-ligand expression for HLA-C group 1 or 2 and Bw4, as KIR-ligand matched or mismatched. We further classified the KIR-ligand mismatches as in the graft-versus-host (GVH) or host-versus-graft (HVG) direction. KIR-ligand mismatch in the GVH direction was present when the donor's KIR ligand was not shared by patients. KIR-ligand mismatch in the HVG direction was present when the patient's KIR ligand was not shared by donors and bidirectional mismatching was present when there were mismatches in both the GVH and HVG directions.

Outcomes

The primary endpoints were leukemia-free survival, defined as being alive and in remission (leukemia recurrence or death from any cause was considered an event; treatment failure, inverse of leukemia-free survival), and overall survival. Other outcomes evaluated were grades II to IV acute GVHD [23], chronic GVHD [24], nonrelapse mortality (NRM), and relapse.

Statistical Methods

The probability of NRM was calculated using the cumulative incidence estimator with relapse as the competing risk [25]. The probabilities of overall and leukemia-free survival were calculated using the Kaplan-Meier estimator [26]. HLA matching and KIR-ligand matching were confounded ($P < .0001$); recipients of better HLA-matched transplants were less likely to be KIR-ligand mismatched. Therefore, separate analyses were undertaken for recipients of 6-7/8 HLA-matched and 3-5/8 HLA-matched transplants to separate the effect of KIR-ligand match status from HLA disparity. The HLA-matching groups were defined based on the results of an earlier report that examined the effects of HLA matching at the allele level in UCBT recipients [21].

Cox regression models [27] were built for acute and chronic GVHD, NRM, relapse, overall mortality, and treatment failure and results reported as hazard ratios (HRs) with 95% confidence intervals (CIs). Proportional hazards assumption was tested for each covariate individually, and all covariates met this assumption. Multivariate models were built using a stepwise forward/backward model-building procedure, and variables that attained $P \leq .05$ were retained in the model. Interactions between the KIR ligand and the adjusted covariates were tested in each model, and no significant interactions were detected at the significance level of .05. All P values are 2-sided, and $P \leq .05$ were considered statistically significant. Analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

RESULTS

Patient, Disease, and Transplant Characteristics

The characteristics of patients who received 6-7/8 HLA-matched transplants are shown in Table 1. Fifty-seven percent of transplantations were KIR-ligand matched and 43% were mismatched. There were no differences in patient, disease, and transplant characteristics by KIR-ligand matching status. The characteristics of patients with AML and acute lymphoblastic leukemia who received 3-5/8 HLA-matched transplants are shown in Table 2. Consistent with the confounding effect of HLA disparity and KIR-ligand match status, 30% of transplantations were KIR-ligand matched and 70% mismatched. There were no differences in patient, disease, and transplant characteristics by KIR-ligand match status, except that in vivo T cell depletion with antithymocyte globulin was slightly more common for KIR-ligand mismatched transplants.

Transplantation Outcomes

KIR-ligand match status was not associated with treatment failure, overall mortality, NRM, relapse, or acute and chronic GVHD after 6-7/8 HLA-matched transplants (Table 3). A similar trend was observed when examining for the effects of KIR-ligand mismatched transplants in the GVH and HVG directions compared with KIR-ligand matched transplants (data not shown). However, for recipients of 3-5/8 HLA-matched transplants, KIR-ligand mismatching was associated with worse overall mortality and NRM but not relapse, treatment failure, or acute and chronic GVHD (Table 4, Figures 1 and 2). The adverse effect of KIR-ligand mismatching occurred with KIR-ligand mismatching in the HVG direction; for overall mortality (HR, 1.78; 95% CI, 1.16 to 2.74; $P = .008$) and for NRM (HR, 2.26; 95% CI, 1.23 to 4.16;

Table 1
Characteristics of Patients with AML Who Received 6-7/8 HLA-Matched Transplants

	KIR-Ligand Matched (n = 114)	KIR-Ligand Mismatched (n = 85)	*P*
Gender			.68
Male	53 (46%)	37 (44%)	
Female	61 (54%)	48 (56%)	
Age, yr			.87
≤16	82 (72%)	62 (73%)	
>16	32 (28%)	23 (27%)	
Cytomegalovirus serostatus			.38
Positive	66 (58%)	44 (52%)	
Negative	41 (36%)	38 (45%)	
Not reported	7 (6%)	3 (4%)	
Disease status at transplantation			.41
First complete remission	39 (34%)	37 (44%)	
Second complete remission	36 (32%)	23 (27%)	
Relapse	39 (34%)	25 (29%)	
Unit total nucleated cell dose			.79
$<3 \times 10^7$/kg	17 (15%)	10 (12%)	
$\geq 3 \times 10^7$/kg	95 (83%)	73 (86%)	
Unknown	2 (2%)	2 (2%)	
Donor–recipient HLA match A/B low resolution, DRB1			.22
6/6 HLA match	22 (19%)	12 (14%)	
5/6 HLA match	81 (71%)	69 (81%)	
4/6 HLA match	11 (10%)	4 (5%)	
Donor–recipient allele-level HLA match A/B/C/DRB1*			<.001
7/8 HLA match	65 (57%)	15 (18%)	
6/8 HLA match	49 (43%)	70 (82%)	
In vivo T cell depletion			.66
Yes	78 (68%)	62 (73%)	
No	33 (29%)	22 (26%)	
Not reported	3 (3%)	1 (1%)	
Conditioning regimen			.70
TBI-containing regimens	46 (40%)	32 (38%)	
Non-TBI regimens	68 (60%)	53 (62%)	
Transplant period			.44
2000-2004	28 (25%)	25 (29%)	
2005-2010	86 (75%)	60 (71%)	
Follow-up, surviving patients			
Median (range), mo	40 (9-120)	48 (13-100)	

TBI indicates total body irradiation.
* KIR-ligand matched HCT: 6 single mismatch at HLA-C locus and 1 double mismatch at HLA-C loci. Additional mismatches at HLA-C locus occurred with mismatches at HLA-A (n = 6), HLA-B (n = 11), and HLA-DRB1 (n = 6). KIR-ligand mismatched HCT: 2 single mismatch at HLA-C locus and 1 double mismatch at HLA-C loci. Additional mismatches at HLA-C locus occurred with mismatches at HLA-A (n = 7), HLA-B (n = 13), and HLA-DRB1 (n = 5).

Table 2
Characteristics of Patients with AML Who Received 3-5/8 HLA-Matched Transplants

	KIR-Ligand Matched (n = 29)	KIR-Ligand Mismatched (n = 183)	*P*
Gender			.85
Male	42 (53%)	95 (52%)	
Female	37 (47%)	88 (48%)	
Age, yr			.63
≤16	47 (59%)	103 (56%)	
>16	32 (41%)	80 (44%)	
Cytomegalovirus serostatus			.67
Positive	42 (53%)	102 (56%)	
Negative	36 (46%)	76 (42%)	
Not reported	1 (1%)	5 (3%)	
Disease status at transplantation			.64
First complete remission	32 (41%)	69 (38%)	
Second complete remission	25 (32%)	69 (38%)	
Relapse	22 (28%)	45 (25%)	
Unit total nucleated cell dose			.07
$<3 \times 10^7$/kg	7 (9%)	35 (19%)	
$\geq 3 \times 10^7$/kg	72 (91%)	146 (80%)	
Unknown		2 (1%)	
Donor–recipient HLA match A/B low resolution, DRB1			.20
6/6 HLA match	2 (3%)	3 (2%)	
5/6 HLA match	20 (25%)	40 (22%)	
4/6 HLA match	55 (70%)	134 (73%)	
3/6 HLA match	2 (3%)	6 (3%)	
Donor–recipient allele-level HLA match A/B/C/DRB1*			.71
5/8 HLA match	45 (57%)	96 (52%)	
4/8 HLA match	26 (33%)	70 (38%)	
3/8 HLA match	8 (10%)	17 (10%)	
Conditioning regimen			.21
TBI-containing regimens	39 (49%)	75 (41%)	
Non-TBI regimens	40 (51%)	108 (59%)	
In vivo T cell depletion			.04
Yes	51 (65%)	145 (79%)	
No	25 (32%)	35 (19%)	
Not reported	3 (4%)	3 (2%)	
Transplant period			.44
2000-2004	18 (23%)	50 (27%)	
2005-2010	61 (77%)	133 (73%)	
Follow-up, surviving patients			
Median (range), mo	38 (6-96)	41 (3-124)	

* KIR-ligand matched HCT: 3 single mismatch at HLA-C locus. Additional mismatches at HLA-C locus occurred with mismatches at HLA-A (n = 9), HLA-B (n = 14), and HLA-DRB1 (n = 3). KIR-ligand mismatched HCT: 4 single mismatch at HLA-C locus and 1 double mismatch at HLA-C loci. Additional mismatches at HLA-C locus occurred with mismatches at HLA-A (n = 11), HLA-B (n = 26), and HLA-DRB1 (n = 11).

$P = .008$) compared with KIR-ligand matched transplants. There were no significant differences in overall mortality and NRM with KIR-ligand mismatched (GVH direction) compared with KIR-ligand matched transplants (HR, 1.33; 95% CI, .87 to 2.02; $P = .18$ and HR, 1.72; 95% CI, .95 to 3.12; $P = .07$, respectively). Acute and chronic GVHD risks were not mediated by KIR-ligand match status (Tables 3 and 4).

DISCUSSION

This retrospective registry-based study analyzed a large series of patients with AML transplanted with a single CB unit who received a myeloablative conditioning regimen and identified several relevant factors when selecting CB units. First, KIR-ligand match status was confounded with HLA disparity in that KIR-ligand mismatching was more common with HLA disparity. Therefore, analyses were conducted separately for 6-7/8 and 3-5/8 HLA-matched transplants so we could distinguish the effects of HLA disparity from KIR-ligand match status. Second, KIR-ligand match status was not associated with outcomes after 6-7/8 HLA-matched transplants. Although donor–recipient HLA mismatching at 3 or more loci are not generally recommended, KIR-ligand mismatching was associated with higher overall and NRM. The adverse effect of KIR-ligand mismatching was shown to occur with mismatching in the HVG direction but not the GVH direction or bidirectional. Further, the adverse effect on survival AML relapse was not mediated by relapse or GVHD. These findings do not support selecting CB units based on KIR-ligand match status when HLA disparity is limited to 1 or 2 loci considering allele-level HLA matching. When contemplating HLA-mismatched transplants at 3 or more loci, avoiding KIR-ligand CB units mismatched in HVG direction lowers mortality risks. The results of the current analyses contradict some of the findings of an earlier Eurocord study in which KIR-ligand mismatch was associated

Table 3
Multivariate Analysis: 6/8 and 7/8 HLA-Matched Transplants

Variables	Events/Assessable	HR (95% CI)	*P*
Overall mortality*			
KIR-ligand matched	66/114	1.00	
KIR-ligand mismatched	51/85	.95 (.66-1.37)	.79
Treatment failure†			
KIR-ligand matched	71/113	1.00	
KIR-ligand mismatched	52/85	.87 (.61-1.25)	.45
Relapse‡			
KIR-ligand matched	45/113	1.00	
KIR-ligand mismatched	37/85	.96 (.61-1.51)	.87
NRM			
KIR-ligand matched	26/113	1.00	
KIR-ligand mismatched	15/85	.69 (.36-1.30)	.25
Grades II-IV acute GVHD§			
KIR-ligand matched	40/114	1.00	
KIR-ligand mismatched	30/85	.99 (.61-1.60)	.97
Chronic GVHD‖			
KIR ligand matched	15/114	1.00	
KIR ligand mismatched	21/85	1.66 (.85-3.24)	.14

* Model adjusted for disease status (second complete remission [CR2] vs. first complete remission [CR1]: HR, 1.91; 95% CI, 1.15 to 3.19; $P = .01$; relapse vs. CR1: HR, 4.82; 95% CI, 3.01 to 7.71; $P < .0001$).

† Model adjusted for disease status (CR2 vs. CR1: HR, 1.91; 95% CI, 1.15 to 3.19; $P = .01$; relapse vs. CR1: HR, 4.82; 95% CI, 3.01 to 7.71; $P < .0001$).

‡ Model adjusted for disease status (CR2 vs. CR1: HR, 2.80; 95% CI, 1.40 to 5.58; $P = .004$; relapse vs. CR1: HR, 10.04; 95% CI, 5.31 to 19.00; $P < .0001$) and sex (male vs. female: HR, 1.68; 95% CI, 1.08 to 2.63; $P = .02$).

§ Model adjusted for in vivo T cell depletion vs. none (HR, 1.79; 95% CI, 1.07 to 3.01; $P = .03$) and patient age (>16 vs. ≤16 years: HR, .50; 95% CI, .27 to .91; $P = .02$).

‖ Model adjusted for in vivo T cell depletion vs. none (HR, 2.35; 95% CI, 1.20 to 4.58; $P = .01$).

with better leukemia-free and overall survival for acute lymphoblastic leukemia and AML, and the effects were more pronounced for AML [17]. However, there are substantial differences between the current analyses and that report. The current analyses considers HLA matching at the allele level at HLA-A, -B, -C, and -DRB1, and the confounding between HLA disparity and KIR-ligand match status required the current analyses be conducted separately for transplants mismatched at 1 to 2 HLA loci and ≥3 HLA loci. Further, in the current analyses KIR-ligand match status did not consider Bw4 epitopes at HLA-A3 and -A11. Our findings also differ from a report from the Japanese registry that did not find an association between KIR-ligand matching and leukemia-free or overall survival in 643 recipients with acute leukemia transplanted with single CB units [18]. As with the Eurocord report, the Japanese registry also considered lower resolution HLA matching between CB units and their recipients. Conflicting results have also been reported in the setting of double UCBT [19]. Brunstein et al. [19] found that KIR-ligand incompatibility was associated with higher rates of acute GVHD and lower survival after reduced-intensity conditioning regimens. However, this effect was not seen in a similar study with 80 patients with various hematologic malignancies, including 31 patients with AML who received double UCBT [20].

Interestingly, in the setting of allogeneic HSCT using other stem cell sources, the results of KIR-ligand status are also somewhat contradictory among different studies [4,5,10,11,16,28-30]. To reconcile these discrepant results, it has to be underlined that from a biologic point of view, patients with KIR-ligand incompatibility are, by definition, at risk for donor T cell alloreactivity in unmanipulated transplantation; thus, in patients given a minimally T cell–depleted transplant, T cell alloreactivity often dominates and outweighs the effect of NK cells. This observation emphasizes the concept that proper studies need to be

Table 4
Multivariate Analysis: 3/8, 4/8 and 5/8 HLA-Matched Transplants

Variables	Events/Assessable	HR (95% CI)	*P*
Overall mortality*			
KIR-ligand matched	35/79	1.00	
KIR-ligand mismatched	109/183	1.51 (1.03-2.21)	.035
Treatment failure†			
KIR-ligand matched	40/79	1.00	
KIR-ligand mismatched	112/183	1.30 (.91-1.88)	.15
Relapse‡			
KIR-ligand matched	24/79	1.00	
KIR-ligand mismatched	47/183	0.86 (.53-1.42)	.57
NRM§			
KIR-ligand matched	16/79	1.00	
KIR-ligand mismatched	65/183	1.94 (1.12-3.36)	.019
Grades II to IV acute GVHD‖			
KIR-ligand matched	33/79	1.00	
KIR-ligand mismatched	50/183	.71 (.45-1.12)	.14
Chronic GVHD			
KIR-ligand matched	20/79	1.00	
KIR-ligand mismatched	43/183	1.27 (.73-2.19)	.39

* Model adjusted for disease status (CR2 vs. CR1: HR, 1.01; 95% CI, .65 to 1.55; $P = .97$; relapse vs. CR1: HR, 3.05; 95% CI, 2.05 to 4.54; $P < .0001$).

† Model adjusted for disease status (CR2 vs. CR1: HR, 1.03; 95% CI, .69 to 1.55; $P = .87$; relapse vs. CR1: HR, 2.92; 95% CI, 1.99 to 4.30; $P < .0001$).

‡ Model adjusted for disease status (CR2 vs. CR1: HR, 1.20; 95% CI, .66 to 2.19; $P = .55$; relapse vs. CR1: HR, 3.39; 95% CI, 1.90 to 6.04; $P < .0001$).

§ Model adjusted for disease status (CR2 vs. CR1: HR, 1.00; 95% CI, .57 to 1.76; $P = .99$; relapse vs. CR1: HR, 2.51; 95% CI, 1.49 to 4.23; $P = .0005$).

‖ Model adjusted for patient age (>16 vs. ≤16 years: HR, .46; 95% CI, .29 to .75; $P = .002$).

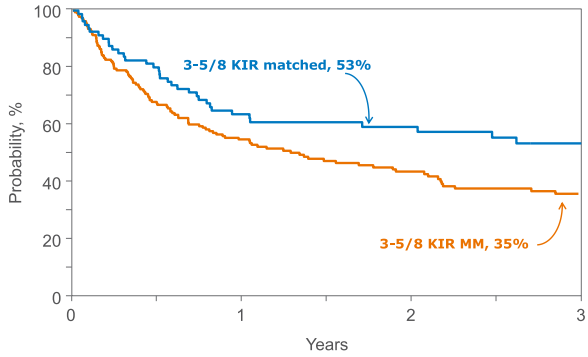

Figure 1. Overall survival after 3-5/8 HLA-mismatched transplantations by KIR-ligand match status.

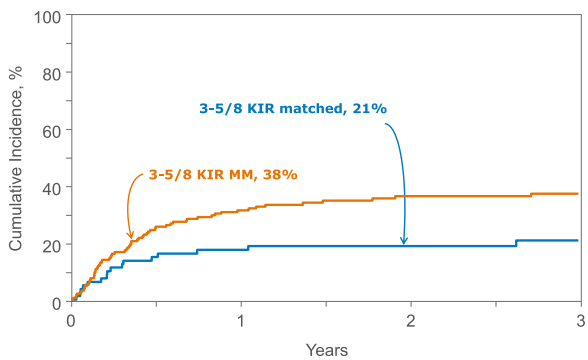

Figure 2. NRM after 3-5/8 HLA-mismatched transplantations by KIR-ligand match status.

conducted and analyzed to dissect and unveil the role played by the different components of the immune system in terms of protection against malignant recurrence. Methods that directly detect the donor KIR repertoire at the DNA, RNA, and surface protein expression levels are a more accurate measure of NK cell alloreactivity than that obtained using solely HLA-based KIR-ligand defining methods [15,31]. DNA-based methods for KIR analysis have the obvious advantage that a single DNA sample used for HLA genotyping can also be used for KIR typing. Another DNA-based approach is the KIR haplotype model, founded on the concept that the greater the number of activating KIRs the donor has, the larger the effect of NK cell alloreactivity [7,32-34]. Further support for the role of activating KIR genes in HSCT was reported by publications that have shown positive associations of individual activating KIR genes, namely *2DS1* with reduced relapse and *3DS1* with reduced acute GVHD, and improved survival [35,36]. However, others have shown that the number of activating KIR genes present in the donor graft or patients KIR genotype determined using the KIR haplotype model or assessing specific aKIRs led to poor outcomes [28,29,37,38].

Thus, the effects of KIR-ligand match status in the setting of UCBT remains controversial, and further studies using other models of NK alloreactivity including genotypes of donor and recipients are needed to elucidate the impact of KIR on outcomes after UCBT. Nevertheless, the current analyses suggest that KIR-ligand matching should not be considered in the setting of 6-7/8 HLA-matched transplants for AML. Generally, 3-5/8 HLA-matched transplants are not recommended, but in the event of such a transplant, avoiding KIR-ligand mismatch in the HVG direction may improve survival.

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