MYELOID NEOPLASIA

KIR gene haplotype: an independent predictor of clinical outcome in MDS patients

Kate Stringaris,¹ David Marin,² A. John Barrett,³ Robert Hills,⁴ Catherine Sobieski,² Kai Cao,² Jerome G. Saltarrelli,² May Daher,² Hila Shaim,² Nathaniel Smith,² David Linch,⁵ Rosemary Gale,⁵ Christopher Allen,⁵ Takuya Sekine,² Rohtesh Mehta,² Richard Champlin,² Elizabeth J. Shpall,² Hagop Kantarjian,⁶ Guillermo Garcia-Manero,⁶ and Katayoun Rezvani²

¹Department of Haematology, Hammersmith Hospital, Imperial College London, London, United Kingdom; ²Stem Cell Transplantation and Cellular Therapy, MD Anderson Cancer Center, Houston, TX; ³Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD; ⁴Centre for Trials Research, School of Medicine, Cardiff University, Cardiff, United Kingdom; ⁵Department of Haematology, UCL Cancer Institute, University College London, London, United Kingdom; and ⁶Leukemia Department, MD Anderson Cancer Center, Houston, TX

Key Points

 KIR haplotype A is an independent risk factor for the progression of MDS to AML. Myelodysplastic syndromes (MDSs) are a group of hematopoietic disorders affecting the myeloid lineage, characterized by cytopenias and clonal evolution to acute myeloid leukemia (AML). We hypothesized that natural killer (NK) cells and their activating killer immunoglobulin-like receptors (aKIRs) influence the immune surveillance and clinical outcome of patients with MDSs. Here, we first examined the distribution of *aKIR* genes and haplotype in 2 independent cohorts of MDS and AML patients. The median number of *aKIR*

genes was lower in MDS patients than healthy controls (2 vs 3 genes; P = .001), and lower in patients with secondary AML (progressed from MDSs) compared with de novo AML patients (2 vs 3; P = .008) and healthy controls (2 vs 3; P = .006). In a multivariate analysis, the presence of KIR haplotype A (characterized by low aKIR content 0-1) independently predicted a higher risk of conversion to AML (relative risk [RR] with 95% confidence interval [CI], 2.67 [1.13-6.71]; P = .02) and worse adjusted progression-free survival (RR with 95% CI, 2.96 [1.59-5.52]; P = .001) and overall survival (2.25 [1.17-4.31]; P = .02), compared with KIR haplotype B (multiple *aKIR* genes). These novel findings may help to identify MDS patients with a high risk of disease progression who would likely benefit from adoptive NK-cell therapy. (*Blood*. 2016;128(24):2819-2823)

Introduction

Immune surveillance, an important mechanism of cancer control, is partly mediated by natural killer (NK) cells, a key component of the innate immune system.¹ Recent studies implicate NK cells in the control of myelodysplastic syndromes (MDSs), a heterogeneous spectrum of clonal hematopoietic disorders affecting the myeloid lineage, characterized by cytopenias and transformation to acute myeloid leukemia (AML).^{1,2} Whereas higher NK-cell frequencies have been reported in patients with low-risk MDSs,³ in high-risk cases, NK cells are reduced, with decreased expression of activating receptors and impaired cytotoxicity.^{4,5}

Each NK cell can express both activating killer immunoglobulinlike receptors (aKIRs) and inhibitory KIRs that interact to regulate NK-effector function.^{2,6,7} There is striking heterogeneity in the number of inherited *aKIR* genes, varying from 0 to 6.⁸ Based on the number and distribution of *KIR* genes, individuals are classed along 2 broad haplotypes. Haplotype A comprises 5 inhibitory genes and the single activating gene *KIR2DS4*, whereas haplotype B incorporates various combinations of aKIR (up to 5) and inhibitory *KIR* genes. The number of *aKIR* genes inherited by individuals is linked to risk for cancer development.⁹⁻¹⁶ Prognostic systems used for MDSs rely on karyotypic and clinical features to stratify patients into risk groups. Because over half of all MDS patients have a normal karyotype and highly variable clinical phenotypes, we considered that the *aKIR* gene repertoire may help predict clinical outcome in this disease. Thus, we studied variations in *aKIR* gene content and haplotype in MDSs and their relationship to AML progression and survival in 2 independent patient cohorts.

Study design

Patients

MDS cohort. We studied 108 MDS patients treated at the MD Anderson Cancer Center (MDACC) from May 2008 to August 2013. Patients were classified according to the International Prognostic Scoring System (IPSS) for MDSs,¹⁷ cytogenetic risk group, and World Health Organization (WHO) classification (Table 1). Median age was 68.4 years (range, 18.1-88.4 years); 32% were female. Median follow-up for surviving patients was 33.3 months (range, 3-206 months). Controls were 139 healthy hematopoietic stem cell (HSC) donors at MDACC (supplemental Table 1, available on the *Blood* Web site).

AML cohort. A second study group consisted of 499 adults with AML, consecutively enrolled in the Medical Research Council (MRC-10/15)-AML trials in the United Kingdom between 1988 and 2009^{18,19} with available DNA (supplemental Table 2). Median age was 44 years (range, 13-69 years); 51% were

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Table 1. Four-year cumulative incidence of pro-	ogression to AML and 4-year probability	ity of PFS and OS according to	patient characteristics
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Variable		Univariate analysis 4-y probability		Multivariate analysis RR (95% CI)			
	n	AML	PFS	OS	AML	PFS	OS
Age, y*		P = .34	P = .03	P = .02			P = .02
≤70	53	27.2	55.8	57.0			1
>70	55	17.6	28.0	28.7			1.97 (1.08-3.59)
Sex		P = .79	P = .71	P = .91			
Female	34	20.3	44.2	43.6			
Male	74	23.6	41.7	43.6			
WHO type		P = .07	P = .01	P = .01			
RA	11	29.3	60.6	60.0			
RARS	8	0.0	85.7	85.7			
RCMD	35	11.9	44.9	47.5			
RCMD-RS	5	20.0	60.0	60.0			
RAEB-1	25	42.6	21.4	23.2			
RAEB-2	18	36.7	13.8	13.8			
MDS-U	4	0.0	100.0	100.0			
del(5q)	2	0.0	100.0	100.0			
WHO type		P = .07	P < .001	<i>P</i> < .001			
RAEB-1 and 2	43	36.3	18.5	18.7			
Others	65	13.0	59.8	61.0			
IPSS		P = .003	P < .001	<i>P</i> < .001		P = .01	P = .01
Low	48	5.6	71.3	74.0		1	1
Intermediate-1	22	34.2	39.5	38.4		2.34 (0.97-5.58)	2.35 (0.95-5.80)
Intermediate-2	10	22.2	22.2	22.2		5.08 (1.67-15.42)	4.39 (1.48-13.01)
High	28	40.9	4.3	4.8		6.10 (2.01-18.52)	5.74 (1.94-17.04)
Cytogenetic risk group		P = .001	P < .001	P < .001	P = .003	P = .03	P = .004
Low	69	12.1	62.6	63.9	1	1	1
Intermediate	14	30.8	15.9	11.9	2.87 (0.84-9.83)	3.76 (1.48-9.54)	5.46 (2.09-14.27)
High	25	47.3	4.8	5.7	5.29 (2.01-13.95)	2.07 (0.75-5.72)	2.40 (0.90-6.40)
No. of activating KIR genes		P = .87	P = .06	P = .09			
0-1	65	22.7	36.7	39.2			
≥2	43	22.6	52.5	51.3			
Activating KIR gene haplotype		P = .02	P = .02	P = .10	P = .02	P = .001	P = .02
Haplotype B†	80	16.8	48.9	48.2	1	1	1
Haplotype A	28	37.1	27.3	31.2	2.67 (1.13-6.31)	2.96 (1.59-5.52)	2.25 (1.17-4.31)
HLA-C group‡		P = .50	P = .78	P = .90			
HLA-C1/x§	93	24.4	44.2	44.4			
HLA-C2/2	13	15.4	35.2	39.6			

MDS-U, MDS unclassified; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ring sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, refractory cytopenia with multilineage dysplasia and ringed sideroblasts.

*The median age was 68.4 years (range, 18.1-88.4 years).

†Two patients had missing data.

‡Includes HLA-C1/C1 and HLA-C1/C2.

\$Haplotype B patients were further characterized as haplotype B centromeric and telomeric according to KIR gene content. They had similar outcomes, namely progression to AML (17.0% vs 16.0%; P = .96); PFS (45.2% vs 37.1%; P = .78); OS (45.8% vs 57.5%; P = .82).

female. The median follow-up for surviving patients was 88 months (range, 7-263 months). DNA from 253 HSC donors served as controls (supplemental Table 3).

All patients consented to the study in accord with the Declaration of Helsinki, and local ethics approval was obtained before sample collection. AML patients received anthracycline and cytarabine-based chemotherapy according to published protocols.^{18,19}

KIR genotyping

KIR genotyping to identify the presence or absence of *KIR* genes of interest was performed by polymerase chain reaction with sequence-specific primers.²⁰ Reverse specific oligonucleotide probe methodology was used for confirmatory typing. The KIR B haplotype was assigned if 1 or more KIR B defining loci were present (supplemental Table 4).

Statistical methods

Probabilities of overall survival (OS) and progression-free survival (PFS) were calculated by the Kaplan-Meier method.²¹ Probabilities of progression to AML were calculated by the cumulative incidence procedure. Associations between

categorical (KIR haplotype and number of *aKIRs*) and ordered variables (patient age group, presenting white blood cell count, cytogenetics) were tested with the Cochran-Armitage test. χ^2 tests were used to test associations between categorical (KIR haplotype and number of activating *KIRs*) and other categorical variables (sex, AML type, stem cell transplant). Variables found to be significant at the P < .15 level were included in the multivariate analysis, where OS and PFS were examined with a Cox regression model and AML progression by Fine-Gray regression analysis.^{22,23} Categorical data were compared with the Fisher exact test and quantitative data with the Mann-Whitney *U* test. Relative risks (RRs) are reported with 95% confidence intervals (CIs). All *P* values are 2-sided.

Results and discussion

MDS patients have lower numbers of aKIR genes

Because *aKIR* gene content determines both KIR haplotype and functional diversity among NK cells, and is linked to the pathogenesis





of childhood acute lymphoblastic leukemia,⁹ we first compared the number of *aKIR* genes in 108 MDS patients treated at MDACC with that in 139 HSC donors from the same institution. The median number of *aKIR* genes was significantly lower in MDS patients than controls (2 [range, 0-6] vs 3 [range 0-6]; P = .001). Interestingly, the KIR haplotype distribution did not differ significantly between these 2 groups (haplotype A, 28 of 108 [25.9] vs 27 of 139 [19.4] healthy donors; P = .28).

We next asked whether the lower number of aKIR genes observed in our study was characteristic of all myeloid malignancies or specific to MDSs. Thus, we compared the number of aKIR genes among healthy controls (n = 253), patients with secondary AML (transformed from MDS/previous therapy) (n = 37), and patients with de novo AML (n = 462) treated in the MRC-UK trials. The 37 patients with transformed AML had a median of 2 aKIR genes (range, 0-6) compared with a median of 3 (range, 0-6) for both the patients with de novo AML (P = .008) and healthy controls (P = .006). The distribution of KIR haplotype A was similar between the de novo AML cohort and healthy controls (141 of 462 [30.5] vs 85 of 253 [34.6]; P = .25), with no apparent influence of KIR haplotype on the probability of relapse (P = .3) or death (P = .8) in patients with de novo AML. In contrast, the proportion of patients with haplotype A was significantly increased in the transformed AML cohort (19 of 37 [51.4]; P = .01), suggesting a higher risk of AML progression in MDS patients with haplotype A. Of note, we found no significant associations between baseline patient or healthy donor characteristics and the number of aKIRs or KIR haplotype (supplemental Tables 1-3 and 5).

KIR haplotype is an independent predictor for MDS-AML transformation

We next examined the influence of KIR haplotype on the risk of AML transformation in the MDACC cohort of 108 MDS patients. The 28 KIR haplotype A patients had a higher 4-year probability of progression to AML and lower 4-year PFS and OS than the 80 KIR haplotype B patients: 37.1 vs 16.8 (P = .02), 27.3 vs 48.9 (P = .02), and 31.2 vs 48.2, (P = .10). Other factors impacting on these end points in univariate analysis were age, WHO type, IPSS, and cytogenetic risk group (Table 1).

Multivariate analysis including all variables with P < .15 identified only cytogenetic risk group and KIR haplotype as independent predictors of MDS progression to AML (Table 1). The relative risk of MDS-AML transformation in patients with haplotype A vs haplotype B was 2.67 (1.13-6.31) (P = .02). Similarly, only cytogenetic risk group, IPSS, and KIR haplotype independently predicted survival. MDS patients with haplotype A had worse adjusted PFS (RR with 95% CI, 2.96 [1.59-5.52]; P = .001) and OS (2.25 [1.17-4.31]; P = .02) compared with KIR haplotype B (multiple aKIR genes) (Figure 1).

Although the number of patients analyzed is limited, this study shows an intriguing and previously unrecognized association between *aKIR* gene content and MDS-AML transformation: patients with MDSs progressing to AML have fewer *aKIR*s than healthy controls or patients

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with de novo AML. Moreover, MDS patients with lower *aKIR* content, as seen in haplotype A, have a greater probability of disease progression and death than do those with haplotype B. Even after adjusting for competing covariates, such as cytogenetic risk group and IPSS, haplotype A was strongly associated with progression to AML and worse survival.

What biological mechanisms could account for these results? One possibility is that MDSs often follow an indolent course, beginning with genetic and epigenetic changes that may promote progression to AML.¹⁷ Thus, inefficient immune surveillance from NK cells with low *aKIR* gene content may fail to block the expansion of the transforming MDS clones, progressing eventually to overt high-risk MDSs or AML. By contrast, efficient immune surveillance may not play an equally important role in the control of diseases with rapid growth kinetics such as de novo AML, a notion supported by our observation of the same numbers of *aKIR* genes and haplotype distribution in de novo AML patients and healthy individuals. If confirmed in larger numbers of MDS patients with haplotype A, our findings would provide a compelling rationale for the use of adoptive NK-cell therapy for this subgroup.

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Authorship

Contribution: K.S. performed experiments and designed, interpreted, analyzed, and wrote the manuscript; D.M. interpreted, analyzed, and wrote the manuscript; C.S., K.C., J.G.S., M.D., H.S., N.S., and T.S. performed experiments and analyzed and commented on the manuscript; A.J.B., R.H., D.L., R.G., R.M., E.J.S., H.K., and G.G.-M. interpreted, analyzed, and commented on the manuscript; C.A. and R.C. supplied samples and commented on the manuscript; and K.R. designed and directed the study and wrote the manuscript.

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Correspondence: Katayoun Rezvani, Stem Cell Transplantation and Cellular Therapy, MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77004; e-mail: krezvani@mdanderson.org.

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