ONCOIMMUNOLOGY
2017, VOL. 6, NO. 12, e1307491 (9 pages)
https://doi.org/10.1080/2162402X.2017.1307491

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ORIGINAL RESEARCH

NKp46 expression on NK cells as a prognostic and predictive biomarker for response to allo-SCT in patients with AML

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ABSTRACT

NKp46 is a major determinant of natural killer (NK) cell function and it is implicated in tumor immune surveillance in acute myeloid leukemia (AML). The purpose of this study was to investigate the prognostic significance of NKp46 expression in an independent cohort of patients with AML, and to investigate the impact of NKp46 on clinical outcome after allogeneic stem cell transplantation (allo-SCT).

NKp46 expression was assessed at diagnosis on NK cells by flow cytometry (N = 180 patients). Clinical outcome was evaluated with regard to NKp46 expression. Patients with NKp46^{high} phenotype at diagnosis had better progression-free survival (PFS) and overall survival (OS) than patients with NKp46^{low} phenotype (74.3% vs. 46.6%, $p = 0.014$; 82.6% vs. 57.1%, $p = 0.010$, respectively). In multivariate analysis, high NKp46 was an independent factor for improved OS (HR = 0.409, $p = 0.010$) and PFS (HR = 0.335, $p = 0.011$). Subgroup analysis revealed that allo-SCT had a favorable impact on PFS in patients with NKp46^{high} phenotype ($p = 0.025$). By contrast, allo-SCT did not impact PFS in patients with low NKp46 expression ($p = 0.303$).

In conclusion, we validate the prognostic value of NKp46 expression at diagnosis in AML. However, the prognostic value of NKp46 expression is limited to patients treated with allo-SCT, thus suggesting that NKp46 status may be predictive for allo-SCT responsiveness.

ARTICLE HISTORY
Received 9 January 2017
Revised 20 February 2017
Accepted 12 March 2017

KEYWORDS
Allo-SCT; AML; NCR; NK; NKp46

Introduction

Acute myeloid leukemia (AML) is a hematologic disorder characterized by variable responsiveness to treatment. Induction chemotherapy based on cytarabine and anthracyclines induces complete remission (CR) in most patients; however, relapse concerns most patients.[1] In this context, precise and accurate patient stratification criteria are mandatory to enable identification of patients likely to benefit from allogeneic stem cell transplantation (allo-SCT). Therefore, discovery and validation of novel prognostic biomarkers is crucial for outcome prediction. However, most biomarkers lack formal validation on independent multicenter cohorts of patients, which is a challenging but mandatory step before clinical applications.[2,3]

Actual prognostic groups are based on cytogenetics,[4,5] and European LeukemiaNet (ELN) genetic classification.[6] These classifications define three groups of patients, i.e., favorable, adverse and intermediate prognosis; the benefits of post-remission therapy (PRT) with allo-SCT in patients with intermediate prognosis remain controversial,[7-9] and additional prognostic parameters are necessary to refine this classification. New molecular markers have been shown to impact prognosis and may be included in future revisions of ELN classification.[6,10,11] However, molecular markers do not account for the entire prognostic heterogeneity of AML and new markers are warranted.

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Supplemental data for this article can be accessed on the publisher's website.
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Beside genetic alterations, accumulating evidence highlights the microenvironment and, in particular, deficient immunity as factors strongly implicated in tumor progression and resistance to chemotherapy.[12] Thus, immune parameters are currently being extensively developed as prediction tools in solid tumors.[13-16] Natural killer (NK) cells are key components of the innate immunity and substantially contribute to antitumor immune responses.[17-19] In AML patients, NK cells play a major role in maintaining prolonged remission, especially in the context of allo-SCT.[20] Among crucial parameters linked to NK antitumor activity, NK-activating receptors expression such as natural cytotoxic receptors (NCR), notably NKp46 play a crucial role.[21-23] In line with this, our group previously reported that low NKp46 expression on NK cells was significantly associated with reduced overall survival (OS) in AML.[21] However, formal validation of this finding is an absolute prerequisite before considering any clinical application. Therefore, the aim of this study was to validate the prognostic value of NKp46 expression on clinical outcome in an independent multicenter cohort of patients with AML. A subgroup analysis in patients treated with allo-SCT in first complete remission (CR1) revealed that NKp46 expression impacts clinical response to allo-SCT.

Results

NKp46 expression at diagnosis

Baseline NKp46 expression on NK cells was assessed by flow cytometry. Patients were classified according to NKp46 rMFI. The threshold used to discriminate patients with NKp46^{low} and NKp46^{high} phenotype was based on dispersion criteria (Fig. S1), and defined as the intersection between the two Gaussian distributions among patients (rMFI = 43.5; see Patients and Methods for further details). Among 180 patients, 35 (19.4%) had high NKp46 expression on NK cells (NKp46^{high} phenotype), and 145 (80.6%) had low NKp46 expression on NK cells (NKp46^{low} phenotype) (Table 1). Frequency of patients with NKp46 high and low phenotype did not differ between age groups, cytogenetics or number of inductions (Table 1). Median follow-up after documentation of CR was 55.3 mo.

Prognostic value of NKp46 expression at diagnosis

In univariate analysis, 4-y progression-free survival (PFS) after CR was better in the NKp46^{high} group, with 74.3% (95%CI, 61.1% to 90.3%) versus 46.6% (95%CI, 38.8% to 55.9%) in the NKp46^{low} group ($p = 0.014$; Fig. 1A). Four-year OS after CR was better in the NKp46^{high} group, with 82.6% (95%CI, 70.8% to 96.3%) versus 57.1% (95%CI, 49.3% to 66.1%) in the NKp46^{low} group ($p = 0.010$; Fig. 1B).

High NKp46 expression at diagnosis was inversely correlated with cumulative incidence of relapse (CIR), with 20.0% (95%CI, 5.6% to 32.2%) versus 42.1% (95%CI, 33.1% to 49.9%) in the NKp46^{low} group ($p = 0.039$; Fig. 1C). There was no significant difference in non-relapse mortality between patients with high and low NKp46 expression ($p = 0.334$; Fig. 1D).

Multivariate Cox regression analysis was performed to assess the predictive value of NKp46 expression while adjusting for the prognostic factors in the population (age at transplantation, cytogenetics, white blood cells and number of inductions). In multivariate analysis, high NKp46 expression was significantly associated with improved PFS (HR = 0.409; 95%CI = [0.20–0.81]; $p = 0.010$) and OS (HR = 0.335; 95%CI = [0.14–0.78]; $p = 0.011$) (Table 2). Notably, in multivariate analysis, NKp46 status was more significantly associated with clinical outcome than cytogenetic risk group for both OS and PFS.

Prognostic value of NKp46 expression is restricted to patients treated with allogeneic haematopoietic stem cell transplantation (allo-SCT)

We then assessed the impact of NKp46 expression at diagnosis on clinical outcome after allo-SCT. Of 180 patients, 66 (36.7%) received allo-SCT in CR1. Four-year clinical outcome was assessed using Cox regression models after controlling for allo-

Table 1. Baseline patient characteristics.

Characteristic	All patients N = 180 Median		High NKp46 N = 35 (19.4%) Median		Low NKp46 N = 145 (80.6%) Median		p
Median Follow-up (months)	55.3		59.1		53.2		
Age (years)	47		47		48		0.328
WBC (10^9/L)	16		18		12		0.945
Time to allograft (months)	4.9		4.9		4.8		0.755
	Nb.	(%)	Nb.	(%)	Nb.	(%)	p
Sex							
Male	92	(51.1)	16	(45.7)	76	(52.4)	0.477
Female	88	(48.9)	19	(54.3)	69	(47.6)	
Cytogenetics							
Favorable	22	(12.2)	5	(14.3)	17	(11.7)	0.308
Intermediate	139	(77.2)	24	(68.6)	115	(79.3)	
Unfavorable	19	(10.6)	6	(17.1)	13	(9.0)	
Consolidation							
Allograft in CR1	66	(36.7)	14	(40.0)	52	(35.9)	0.648
No allograft in CR1	114	(63.3)	21	(60.0)	93	(64.1)	
Nb inductions							
1	139	(77.7)	29	(82.9)	110	(76.4)	0.410
2	41	(22.3)	6	(17.1)	35	(23.6)	

Abbreviations: CR1, first complete remission; Nb, number; WBC, white blood cells.

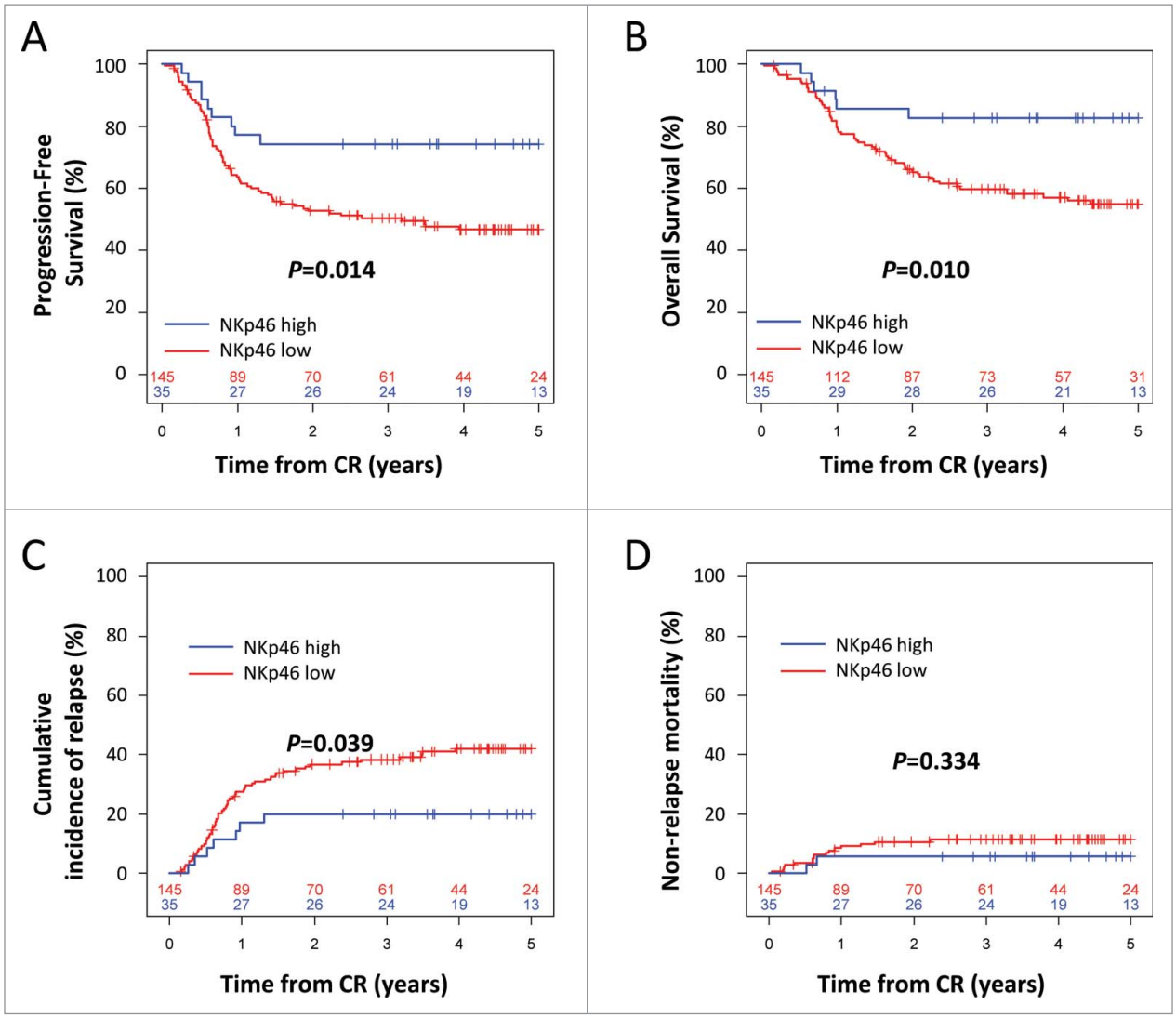

Figure 1. Kaplan–Meier estimates of progression-free survival (A) and overall survival (B) by NKp46 expression at diagnosis. Cumulative incidence of relapse (C) and non-relapse mortality (D) by NKp46 expression at diagnosis. The numbers at the bottom of each plot represent the number at risk at the beginning of each 12-mo period for each group of patients. CR: complete remission. Statistical analyses were performed using a log Rank tests. $p < 0.05$ was considered significant.

SCT as a time-dependent covariate. Patients were stratified by NKp46 expression and post-remission treatment.

In the group of patients with high NKp46 expression, allo-SCT significantly improved PFS, with PFS rates of 100% (95%CI, 100% to 100%) versus 60.4% (95%CI, 42.9% to 85.1%) in the conventional PRT group ($p = 0.025$; Fig. 2A). Consistently, allo-SCT was associated with improved OS with 100% (95%CI, 100% to 100%) versus 71.2% (95%CI, 54.0% to 93.8%) in the conventional PRT group (Fig. 2B). The significance for OS could not be tested due to the absence of death in the group of patients with high NKp46 expression, which precludes multivariate analyses (Fig. 2B).

Table 2. Multivariate analysis of PFS and OS.

	Multivariate HR for PFS			Multivariate HR for OS		
Variable	HR	95% CI	*p*	HR	95% CI	*p*
Cytogenetics						
Favorable	Reference			Reference		
Intermediate	1.552	0.69 to 3.45	0.280	2.432	0.86 to 6.82	0.091
Adverse	2.711	1.04 to 7.03	0.040	2.746	0.81 to 9.27	0.104
WBC (10^9/L)	1.002	0.99 to 1.01	0.292	1.001	0.99 to 1.01	0.740
Age	1.006	0.98 to 1.03	0.551	1.010	0.98 to 1.03	0.411
Nb inductions	1.297	0.78 to 2.14	0.306	1.575	0.92 to 2.69	0.096
NKp46 expression						
Low	Reference			Reference		
High	0.409	0.20 to 0.81	0.010	0.335	0.14 to 0.78	0.011

Abbreviations: CI: confidence interval; HR: hazard ratio; OS: overall survival; PFS: progression-free survival; WBC: white blood cells.

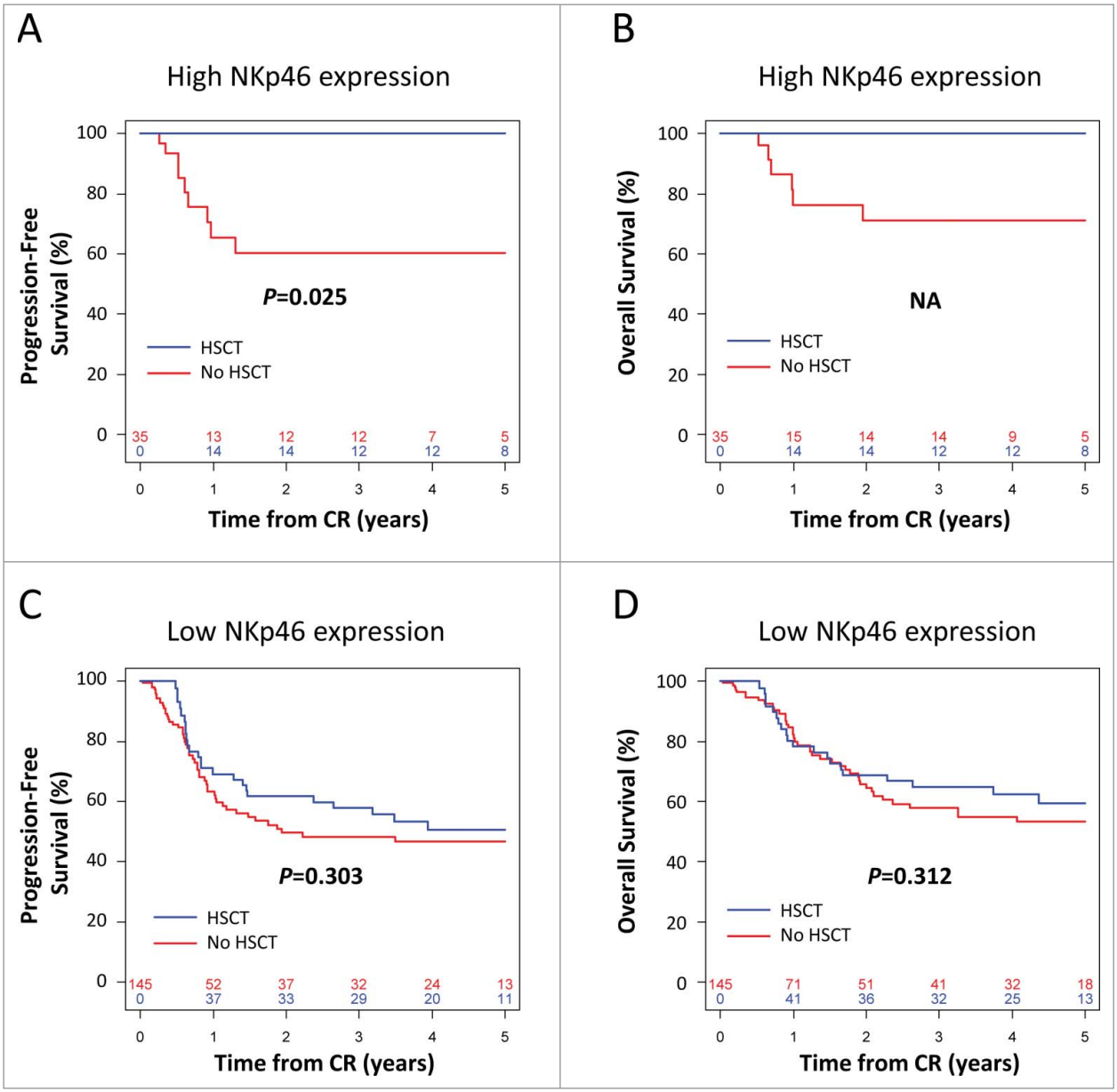

Figure 2. Kaplan–Meier estimates of progression-free survival (A, C) and overall survival (B,D) according to post-remission therapy in patients with low (A, B) or high (C, D) NKp46 expression at diagnosis. The numbers at the bottom of each plot represent the number at risk at the beginning of each 12-mo period for each group of patients. CR: complete remission. Statistical analyses were performed using a log Rank tests. $p < 0.05$ was considered significant.

By contrast, in the group of patients with low NKp46 expression, the impact of allo-SCT on PFS was not significant, with PFS rates of 50.8% (95%CI, 38.4% to 67.1%) versus 46.6% (95%CI, 37.0% to 58.6%) in the conventional PRT group ($p = 0.303$; Fig. 2C). Consistently, allo-SCT did not impact OS in the group of patients with low NKp46 expression, with OS rates of 62.3% (95%CI, 50.3% to 77.3%) versus 55.0% (95%CI, 45.2% to 66.8%) in the conventional PRT group ($p = 0.312$; Fig. 2D).

These results clearly suggest that the prognostic effect of NKp46 expression on survival observed in the total population is limited to the subgroup of patients with high NKp46 expression treated with allo-SCT. In addition, since allo-SCT selectively impacts survival in patients with high NKp46 expression, our data strongly suggest that NKp46 expression at diagnosis can be considered as a predictive biomarker of response to allo-SCT.

Discussion

NK cells are potent immune effectors that mediate graft-versus-leukemia effects.[24,25] Among NK activating receptors, NCR such as NKp46 are among the most important, acting by triggering cytolytic responses to tumor target cells.[21,25-28] The prognostic value of NKp46 expression at diagnosis in AML patients described in the present multicenter study is a formal validation of previous results published by our team.[21] Patients' distribution histograms confirmed the existence of two distinct populations of patients based on NKp46 intensity of expression (Fig. S1). In the present study, the threshold validation for NKp46 expression was based on objective dispersion criteria. The first group of patients characterized by a low expression of NKp46 was statistically associated with poor prognosis compared with the second group, which had a higher NKp46

expression. In addition, our study confirms that NKp46 is an independent prognostic biomarker at diagnosis. In contrast to our previous study with an exploratory cohort, we have included in our multivariate analyses all the currently admitted confounding variables (leukocytosis, age, cytogenetics, number of inductions), and NKp46 appeared as the most important risk factor compared with other variables in multivariate models.

We then assessed the impact of NKp46 expression at diagnosis on clinical outcome after allo-SCT. Our data suggest that the clinical outcome after allo-SCT is strongly dependent on NKp46 status at the time of diagnosis. Indeed, the clinical benefit of allo-SCT is exclusively observed in the subgroup of patients with high NKp46 expression, whereas in the subgroup of patients with low NKp46 expression, there was no significant effect of allo-SCT on survival and relapse. These results support the idea that NKp46 can be considered as a predictive biomarker for clinical outcome after allogeneic transplantation, since the observed benefit only occurs in case of treatment by allo-SCT. Interestingly, this biomarker is assessable at diagnosis, which is a great advantage compared with most surrogate biomarkers in allo-SCT assessed after transplantation.[29-32]

The impact of NKp46 expression on patients' NK cells at diagnosis on clinical outcome after transplantation is puzzling. Indeed, it is difficult to understand how the phenotype of NK cells at the diagnosis of AML can impact of clinical events taking place several months later and in an allogeneic context, where NK cells come from the donor.

NKp46 is a triggering receptor implicated in malignant cell recognition and destruction. Low NKp46 expression on NK cells is responsible for poor blast recognition,[21] and has been correlated with high minimal residual disease (MRD) after induction therapy in acute lymphoid leukemia.[33] In our study, the impact of NKp46 expression on NK cells at diagnosis on clinical outcome after transplantation may be explained, at least, by a higher MRD in patients with low NKp46 expression, favoring emergence of clones leading to relapse.

Beside relapse, failure of allo-SCT is mainly due to severe graft-versus-host disease (GvHD). Importantly, we noticed the absence of death by GvHD in the group of NKp46^{high} patients. In a recent study in a NKp46 knockout mouse model, it was evidenced an exacerbated GvHD in an experimental transplantation setting.[34] Although not directly transferrable to human setting, questions raise from this study concerning the potential of NKp46^{high} expression to prepare the immune cells to control GvH reaction. The number of patients with NKp46^{high} phenotype in our cohort did not enable us to analyze the impact of NKp46 expression on GvHD but should prompt further study in a larger cohort. If confirmed, this observation could provide additional arguments to stimulate NKp46 expression to enhance NK-mediated graft-versus-leukemia effect without inducing GvHD.

Mechanistically, the mechanisms of subversion of NK cells by leukemic cells remain challenging. This immune subversion has been observed in other cancer settings.[23,35]

Interestingly, in the group of NKp46^{high} patients, NKp46 expression was higher than that of healthy volunteers (HV) (median $\pm$ SD = 60.2 $\pm$ 16.0 vs. 29.2 $\pm$ 10.6, $p < 0.0001$, Fig. 3). This abnormal high expression may reflect some degree

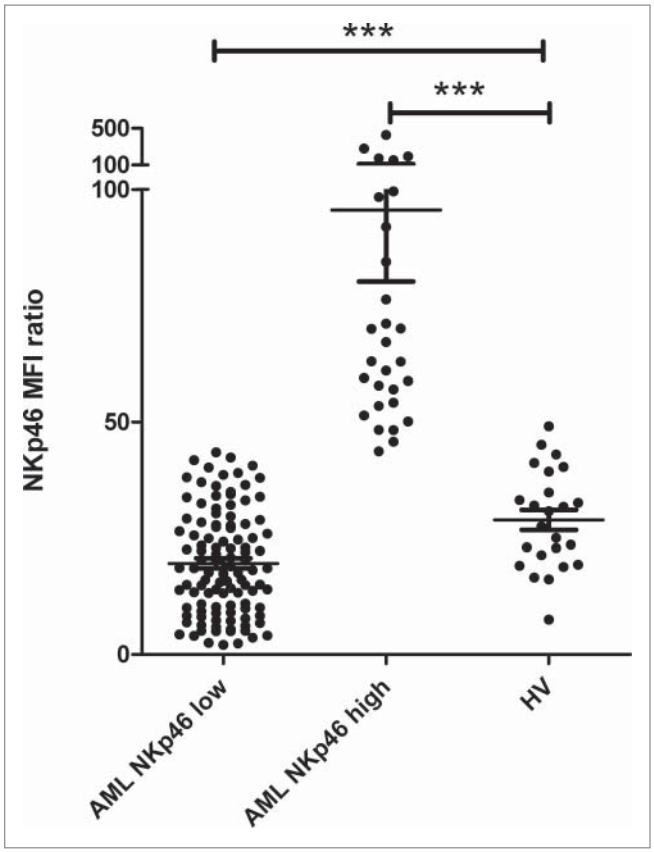

Figure 3. Comparison of NKp46 expression in AML patients and HV. NKp46 expression on NK cells was assessed by flow cytometry at diagnosis. Patients were stratified by NKp46 expression. NKp46 expression in each subgroup of patients (NKp46^{high} and NKp46^{low}) was compared with HV. Abbreviations: HV: healthy volunteers. Differences were assessed with a Student's *t* test. $p < 0.05$ was considered significant. $^{***}p < 0.0001$.

of activation, since activated NK cells display increase in activating receptor expression. This appeals for further investigation to test whether activation markers are also expressed in this group of patients. Additionally, a recent study have shown that NK cell gene expression, which was altered at diagnosis, was completely restored after in vitro activation and expansion.[36] Therefore, it would be interesting to analyze whether gene expression of these NKp46^{high} AML-NK corresponds to activated NK cells. Alternatively, a gene expression comparison between NKp46^{low} and NKp46^{high} patients may provide cues for membrane-bound or soluble factors provided by leukemic cells and responsible for NK cell defects. This strategy has been used by Khaznadar et al. in a recent study, and reveals that patients with a "normal" NK phenotype, gene data sets included pathways related to immune reaction, which is in line with our findings regarding NKp46^{high} patients.[37]

On the other hand, further investigations should focus on the different isoforms of NKp46. Indeed, four isoforms of NKp46 have been described, with isoform d having the highest activity,[38] and whose relative expression might be modulated by the microenvironment.[39] Moreover, recent studies demonstrated that NCR isoforms relative expression impact clinical outcome, as demonstrated for splice variants of NKp30 in gastrointestinal stromal tumors[35] as well as splice variants of NKp44 in AML.[40]

Beside applications for AML patients, a perspective of the present work is to investigate the generalization of our finding to other pathologies, such as refractory Hodgkin lymphoma or high-grade lymphoma. In the case of these pathologies, allo-SCT is a therapeutic option likely to be balanced with alternative treatment with anti-PD1 therapy. The possibility of the prediction of success of allo-SCT in these contexts would be highly relevant for clinical decision making.

In conclusion, our results formally validate the prognostic value of NKp46 expression in AML described in our previous work.[21] However, the prognostic value of NKp46 expression is limited to patients treated with allo-SCT, thus suggesting that NKp46 status may actually be predictive for allo-SCT responsiveness, as opposed to strictly prognostic.[41] Although this conclusion requires further validation on an independent cohort of patients, our study provides a strong rationale to develop interventional therapy to induce NKp46 expression after allo-SCT.

Patients and methods

Patients

All participants gave written informed consent in accordance with the Declaration of Helsinki. The entire research procedure was approved by the ethical review boards from the IPC and the GOELAMS. The patient characteristics, stratified by NKp46 expression groups, are summarized in Table 1. Baseline NKp46 expression on NK cells at diagnosis was assessed by flow cytometry in 180 patients with newly diagnosed AML. Two cohorts of patients were merged for this study. The Paoli Calmettes Institute (IPC) prospective cohort included 114 patients with newly diagnosed non-acute promyelocytic leukemia (APL) AML admitted between November 2007 and November 2012, aged 18 to 65 y and treated with conventional 3+7 induction chemotherapy as described previously.[42] The Groupe Ouest Est d'Etude des Leucémies Aiguës et autres Maladies du Sang (GOELAMS) cohort included 66 patients. The median age at induction was 48 y (range: 19–59). All patients were included in the LAM2006IR prospective multicenter randomized trial between November 2007 and April 2012 (NCT00860639). All patients had previously untreated AML with intermediate cytogenetics, as defined by Slovak et al.[43] Patients received conventional 3+7 induction chemotherapy with or without the addition of Gemtuzumab Ozogamicin.[44] Patients with APL AML, patients above 66 y and patients without CR after one or two courses of induction chemotherapy were not eligible for this study. The median age at induction was 47 y (range: 18–65). Out of 180 patients, 22 had favorable cytogenetics (12.2%), 139 had intermediate cytogenetics (77.2%) and 19 had unfavorable cytogenetics (10.6%). Sixty-six (36.7%) patients received allo-SCT in their CR1.

Clinical samples

Fresh total peripheral blood samples (IPC cohort) or peripheral blood mononuclear cells (PBMC) cryopreserved in 90% fetal calf serum / 10% Dimethyl Sulfoxide (DMSO) (GOELAMS cohort) were obtained from randomly selected patients at diagnosis before induction chemotherapy and analyzed by flow cytometry. Fresh total peripheral blood from age-matched (range: 18–65) HV was obtained from the Etablissement Français du Sang (EFS). Samples were stained and analyzed according to the procedure used for the IPC cohort (N = 24). For the GOELAMS validation cohort, handling, conditioning and storing of patients samples were performed by the FILO-theque AML (N° BB-0033–00073), tumor bank of the FILO group, Cochin hospital, Paris.

Flow cytometry

For the IPC cohort, analyses were performed in the Biopathology department of the Paoli Calmettes Institute. A FACS Canto II (BD Biosciences), and FACS Diva Software (BD Biosciences) were used for flow cytometry. For the GOELAMS cohort, analyses were performed in the Immunomonitoring platform of the Paoli Calmettes Institute. A LSR Fortessa (BD Biosciences) was used for flow cytometry. Cells were immunostained with Krome Orange (KOTM)- or allophycocyanin (APC)-conjugated anti-CD45, fluorescein isothiocyanate (FITC)–conjugated or Phycoerythrin-Cyanine 7 (PC7)- or Phycoerythrin-Texas Red-xTM (ECD)-conjugated anti-CD3, and PC7-, APC AF700- or APC–conjugated anti-CD56. Triggering receptor expression NKp46 was measured with Phycoerythrin-Cyanine 5 (PC5)-conjugated monoclonal antibodies. Isotype controls were mouse immunoglobulin G conjugated to PC5. All antibodies used in this study were kindly provided by Beckman-Coulter. For total blood, red blood cells were lysed with BD FACS Lysing solution (BD Biosciences) before data acquisition. The NKp46 mean fluorescence intensity (MFI) ratio (NKp46 MFI / isotype control MFI, referred to as rMFI) was calculated for each patient. Assays were performed blinded to the study end point.

Threshold determination

Thresholds were calculated based on the results of the IPC cohort. Patients were classified into two groups, NKp46^{low} and NKp46^{high}, according to NKp46 rMFI. The dichotomy between NKp46^{low} and NKp46^{high} patients was based on dispersion criteria of the population. Inter-individual variability of NKp46 expression in AML patients (Fig. S1A) was represented on a distribution histogram. The distribution of NKp46 expression was a juxtaposition of two Gaussian distributions (d'Agostino-Pearson normality test and Kernel density estimation). The threshold between these two peaks was NKp46 rMFI = 43.5 (Fig. S1A). Of note, this threshold was above the 90th percentile of HV (Fig. 3).

All the possible thresholds were tested in the range of NKp46 expression for OS and PFS (Fig. S1B and S1C, respectively). The threshold based on dispersion criteria was also the most discriminant threshold for survival analyses. For the rest of the study, patients were classified into two distinct subgroups (NKp46^{high} and NKp46^{low} phenotype) for survival analyses according to this threshold. For samples from the GOELAMS cohort, analyses were performed on a different cytometer. A correction factor was applied for NKp46 rMFI so the cohorts could be merged. The correction factor was the equation of the regression of paired samples (fresh samples analyzed on a CantoII and paired frozen samples analyzed on a LSRII Fortessa).

Statistical analysis

Statistical analyses were performed using Graph Pad Prism (Graph Pad Software, San Diego, CA) and R software (http://www.r-project.org). The limit of significance was set at $p < 0.05$. OS from CR was defined by the time between CR achievement after induction therapy until death from any cause, and PFS as time between CR achievement and relapse or death, whatever occurred first. Patients without an event were censored at the time of their last follow-up. Survival times were estimated by the Kaplan–Meier method and compared using the log-rank test. For OS and PFS stratified by post-remission therapy, hazard ratios for OS and PFS were determined by Cox regression analysis, while treating allo-SCT as a time-dependent covariate. The cumulative incidences of leukemia relapse and death in CR1 were calculated using the Prentice estimator, considering relapse and death in CR1 as mutually competing events. The impact of NKp46 on these cumulative incidences was evaluated with the Gray test.[45] Cox regression analysis was performed to adjust the impact of NKp46 using age (continuous variable), white blood cell count at diagnosis (continuous variable), cytogenetics (low vs. intermediate vs. high) and number of induction course to obtained CR1 (1 vs. 2). Analyses for the main end points were performed on an intention-to-treat basis. The X^2 or Fisher's exact test was used to assess association between variables. The subgroup analysis of patients treated with allo-SCT was defined *post hoc*, and those results therefore have to be considered hypothesis generating.

Authors' disclosures of potential conflicts of interest

Anne-Sophie Chretien: Patents, Royalties, Other Intellectual Property : Inserm Transfert. **Raynier Devilliers**: No relationship to disclose. **Cyril Fauriat**: Patents, Royalties, Other Intellectual Property : Inserm Transfert. **Florence Orlanducci**: No relationship to disclose. **Samia Harbi**: No relationship to disclose. **Aude Le Roy**: No relationship to disclose. **Jérôme Rey**: Consulting or Advisory Role: Novartis. Travel, Accommodations, Expenses : Novartis. **Gaelle Bouvier Borg**: Employment: Immunotech Beckman Coulter. **Emmanuel Gautherot**: Employment: Immunotech Beckman Coulter. **Jean-François Hamel**: No relationship to disclose. **Norbert Ifrah**: No relationship to disclose. **Catherine Lacombe**: No relationship to disclose. **Pascale Cornillet-Lefebvre**: No relationship to disclose. **Jacques Delaunay**: No relationship to disclose. **Antoine Toubert**: No relationship to disclose. **Christine Arnoulet**: Patents, Royalties, Other Intellectual Property : Inserm Transfert. **Norbert Vey**: Consulting or Advisory Role : Novartis, Roche. Travel, Accommodations, Expenses : Amgen, Novartis Patents, Royalties, Other Intellectual Property : Inserm Transfert. **Didier Blaise**: Patents, Royalties, Other Intellectual Property : Inserm Transfert. **Daniel Olive**: Stock or Other Ownership : Imcheck Therapeutics. Research Funding : GSK. Patents, Royalties, Other Intellectual Property : Inserm Transfert, GSK.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Beckman Coulter for their technical advices and for providing the antibodies used in this study. The authors thank the Paoli Calmettes Institute immunomonitoring platform for their valued contributions to this work. The authors thank the Groupe Ouest Est d'Etude des Leucémies Aiguës et autres Maladies du Sang (GOELAMS), the FILOtheque AML (N° BB-0033–00073), and Lamya Haddaoui, for their implication in this study.

Funding

This work has been financially supported by the INCa, the SIRIC (grant INCa-DGOS-INSERM 6038), the GS IBiSA. D.O. team was labeled "Equipe FRM DEQ 201 40329534." D.O. is Senior Scholar of the Institut Universitaire de France. All antibodies used were kindly provided by Beckman-Coulter, Marseille, France. Beckman Coulter and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries. All other trademarks are the property of their respective owners.

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