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To the editor:

No association between the presence of killer-cell immunoglobulin-like receptor genes and susceptibility to childhood ALL

Killer-cell immunoglobulin-like receptors (KIRs) play a pivotal role in immunosurveillance and reduction of relapse after hematopoietic stem cell transplantation in acute leukemia.[1,2] Outside the setting of hematopoietic stem cell transplantation, it was recently reported by Almalte et al that inherited KIR genes might be associated with susceptibility or resistance to childhood acute lymphoblastic leukemia (ALL).[3] They determined 6 activating KIRs (KIR2DS1-5 and KIR3DS1) in a cohort of 145 children with B-cell leukemia (B-ALL) and 30 children with T-cell leukemia (T-ALL) and compared it with a healthy control group of 245 children of French-Canadian ethnicity. Children with more activating KIR genes had a lower risk for ALL.[3] A subsequent study by Babor et al analyzed the presence of stimulatory KIRs in a similar cohort of children with B-ALL (n = 185) and T-ALL (n = 33).[4] In contrast, they did not find a reduction of stimulatory KIRs in childhood ALL patients, although allele frequencies of stimulatory KIRs between the Canadian and the European study did not differ significantly.[4]

Given these observations, we determined KIR genotypes of the 17 known KIR genes of 328 pediatric patients (203 male and 125 female) with ALL (86 B-ALL, 179 common ALL, and 63 T-ALL) treated according to the AIEOP-BFM-ALL 2000 protocol. The majority of patients were of European origin with a frequency being in line with Babor's work.[4] The control group consisted of 339 healthy blood donors with a median age of 25 and self-reported Caucasian ethnicity. We used a DNA-based quantitative real-time polymerase chain reaction–based method according to Vilches et al and Alves et al.[5,6]

Analyzing prognostic factors often reveals different and potentially coincidental results in different analyses, often because of the fact that no adjustment for multiple testing was done. Therefore, we carefully accounted for this problem in calculating our sample size and in interpreting our analyses. Power calculation was based on a 2-sample test for proportions using proportions for the 6 KIR genes of main interest given in Almalte et al.[3] Adjusting for multiple testing of 6 genes, the level of significance was set to .008 to reach a global significance level of .05 (with a power of .8). Therefore, a minimum of 256 patients and 256 controls had to be included, and the end point of all analyses was the presence of ALL (control vs patient). A possible association between occurrence of KIR genes and age, sex, DNA index, or genetics was not observed (data not shown).

Univariate logistic regression did not reveal any significant difference in frequency distribution of KIR alleles and genotypes in children with ALL compared with healthy controls (Table 1). We also analyzed receptor distributions of the 6 activating receptors among the different leukemic risk categories (low, 35%; intermediate, 46%; and high, 19%). No significant difference in receptor combinations could be found between controls and these 3 subgroups (data not shown). Furthermore, KIR genes were assigned to either KIR haplotype A or B. The distribution of these 2 haplotypes among ALL patients and the healthy reference group was 26.8% vs 30.4% for haplotype A and 73.2% vs 69.6% for haplotype B, respectively. These data are in line with published KIR gene frequencies in a Caucasian population.[7] We next determined KIR B-content scores for patients and controls and found similar score distributions between the 2 groups. Furthermore, involvement of the central nervous system was independent of activating KIRs, KIR haplotype, and B-content score. An increasing risk for ALL with an increasing number of activating KIR genes was also excluded ($P = .173$).

Our results are in sharp contrast to the findings of Almalte et al.[3] Diversity of KIR gene expression among different ethnic groups has to be taken into account as an explanation for the discrepancy of results. For instance, an increased KIR A/A genotype was found in Hispanic children with ALL, but not in non-Hispanic children.[8] Moreover, the possibility of coincidental results in statistical analyses might have to be excluded.

In summary, we did not find a significant difference in KIR gene expression between pediatric patients with ALL and healthy controls, and our results do not support the hypothesis that the number of activating KIR genes confers susceptibility or resistance to pediatric ALL. Further research is necessary to elucidate genetic factors involved in the development of ALL in children.

Table 1. Influence of activating KIRs, KIR haplotype, KIR B-content score, and inhibiting KIRs on the risk for the development of ALL

Factor	ALL patients	Controls	OR (95% CI)	P
KIR2DS1				
Yes	112 (34.1%)	127 (37.5%)		
No	216 (65.9%)	212 (62.5%)	1.154 (0.842-1.585)	.372
KIR2DS2				
Yes	172 (52.4%)	180 (53.1%)		
No	156 (47.6%)	159 (46.9%)	1.027 (0.758-1.392)	.865
KIR2DS3				
Yes	107 (32.6%)	93 (27.4%)		
No	221 (67.4%)	246 (72.6%)	0.781 (0.560-1.088)	.143
KIR2DS4				
Yes	310 (94.5%)	318 (93.8%)		
No	18 (5.5%)	21 (6.2%)	0.879 (0.460-1.681)	.698
KIR2DS5				
Yes	103 (31.4%)	103 (30.4%)		
No	225 (68.6%)	236 (69.6%)	0.952 (0.686-1.323)	.776
KIR3DS1				
Yes	122 (37.2%)	126 (37.2%)		
No	206 (62.8%)	213 (62.8%)	0.999 (0.730-1.368)	.993
KIR haplotype				
A	88 (26.8%)	103 (30.4%)		
B	240 (73.2%)	236 (69.6%)	0.8401 (0.600-1.176)	.3103
B-content score				
0	96 (29.3%)	103 (30.4%)		
1	128 (39.0%)	134 (39.5%)		
2	81 (24.7%)	79 (23.3%)		.313
3	21 (6.4%)	19 (5.6%)		
4	2 (0.6%)	4 (1.2%)		
KIR2DL1				
Yes	313 (95.4%)	333 (98.2%)		
No	15 (4.6%)	6 (1.8%)	24.139 (1.018-6.941)	.046
KIR2DL2				
Yes	176 (53.7%)	178 (52.5%)		
No	152 (46.3%)	161 (47.5%)	0.955 (0.703-1.293)	.766
KIR2DL3				
Yes	287 (87.5%)	303 (89.4%)		
No	41 (12.5%)	36 (10.6%)	1.201 (0.746-1.934)	.448
KIR2DL4				
Yes	328 (100%)	339 (100%)		
No	0 (0%)	0 (0%)	1.033 (0.02-52.241)	.987
KIR2DL5				
Yes	183 (55.8%)	176 (51.9%)		
No	145 (44.2%)	163 (48.1%)	0.856 (0.631-1.160)	.316
KIR3DL1				
Yes	312 (95.1%)	323 (95.2%)		
No	16 (4.9%)	16 (4.7%)	0.744 (0.363-1.518)	.416
KIR3DL2				
Yes	328 (100%)	338 (99.7%)		
No	0 (0%)	1 (0.3%)	0.344 (0.014-8.463)	.512
KIR3DL3				
Yes	313 (95.4%)	328 (96.8%)		
No	15 (4.6%)	11 (3.2%)	1.428 (0.645-3.158)	.378
KIR2DP1				
Yes	320 (97.6%)	337 (99.4%)		
No	8 (2.4%)	2 (0.59%)	4.213 (0.888-19.988)	.070
KIR2DP1f1				
Yes	105 (32.01%)	115 (33.9%)		
No	223 (67.99%)	224 (66.08%)	1.090 (0.788-1.505)	.599
KIR2DP1f2				
Yes	305 (93%)	324 (95.5%)		
No	23 (7%)	15 (4.4%)	1.629 (0.833-3.180)	.153

CI, confidence interval; OR, odds ratio.

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To the editor:

Lenalidomide, idelalisib, and rituximab are unacceptably toxic in patients with relapsed/refractory indolent lymphoma

Understanding of the tumor microenvironment has led to the development of novel agents for lymphoma, although few studies have combined these as a therapeutic strategy. We report unacceptable toxicity from such a biological triplet. Patients were treated in a multiarm phase 1 study using different combinations of chemotherapy, immunomodulators, and anti-CD20 monoclonal antibodies in combination with idelalisib (www.clinicaltrials.gov; #NCT01088048). We report on the cohort of patients treated with idelalisib, lenalidomide, and rituximab. The inclusion criteria were relapsed indolent lymphoma, age >18 years, measurable disease, and performance status ≤2. Patients were excluded if they had anticancer therapy within 4 weeks, prior allogeneic stem cell transplant, or active central nervous system involvement, were pregnant or breast-feeding, or had active serious infection, serum creatinine ≥2.0 mg/dL, neutrophils $<1.0 \times 10^9$/L or platelets $<75 \times 10^9$/L, bilirubin >2 mg/dL, aspartate aminotransferase or alanine aminotransferase (ALT) ≥2 times the upper limit of normal, Child-Pugh class B or C hepatic impairment, infection with HIV or hepatitis B or C virus, or prior treatment with idelalisib.

Treatment comprised lenalidomide 5 mg (days 8-21 cycle 1, days 1-21 thereafter), rituximab 375 mg/m^2 day 1, and idelalisib 150 mg twice daily from day 1 (cycle 1, 35 days; subsequent cycles, 28 days). Seven patients enrolled in the initial cohort. The median age was 62 (range, 49-72) years. Five patients had follicular lymphoma (FL), 1 had small lymphocytic lymphoma, and 1 had marginal zone lymphoma. The median number of prior treatments was 1.5 (range, 1-5). None had liver disease or hepatic involvement with lymphoma. Three of the 6 patients (50%) who developed liver function test abnormalities were taking statins at study entry. The first 4 patients developed ALT elevation (2 grade 2 and 2 grade 4). Lenalidomide was held in all patients. Four patients (57%) had elevation in bilirubin (grade 1 in 2 patients, grade 2 in 1 patient, and grade 4 in 1 patient). ALT elevations of the first 4 subjects began on days 22, 25, 30, and 36, at which point patients had 15 to 21 days of concurrent lenalidomide. All therapy was interrupted until resolution of abnormalities. No patients developed renal impairment at the time of development of liver function test abnormalities. Three remaining patients had 1 week of concurrent lenalidomide before discontinuation. These patients continued on idelalisib and rituximab, but 2 developed subsequent grade 3 (days

50 and 98) ALT elevations (Figure 1A). All patients had imaging: 2 showed diffuse hypoechogenicity and 1 borderline fatty hepatomegaly. The median time to resolution of ALT and aspartate aminotransferase was 42 (range, 21-59) and 14 (range, 3-38) days, respectively. Four patients resumed idelalisib alone; patient 2 developed recurrent elevation in ALT on rechallenge. This arm of the study was closed to new patient enrollment. Two patients died due to toxicity and are described below.

Patient 6 (62 years, male, FL) presented on day 90 with dyspnea and chest pain with computed tomography suggestive of pneumonia. Idelalisib was discontinued, and bronchoalveolar lavage isolated *Fusarium* species. Although the total bilirubin increased initially after commencing voriconazole, this trend continued after therapy was switched to liposomal amphotericin B. Quantitative cytomegalovirus and repeat hepatitis testing were negative; potentially hepatotoxic drugs were discontinued.

Liver biopsy on day 112 showed acute cholangitis with mixed portal inflammation and centrilobular cholestasis without viral inclusions, fungal organisms, or lymphoma. Immune dysregulation was suspected and peripheral blood lymphocytes were analyzed on samples collected prior to therapy and day 120. Analysis of T-cell subsets revealed reduced numbers of total T cells, CD4$^+$ and CD8$^+$ T cells, and natural killer cells at day 120, but Foxp3$^+$ regulatory T-cell numbers were unchanged (Figure 1B). Interestingly, both CD4$^+$ and CD8$^+$ T cells showed broad expression of markers consistent with marked activation at day 116 (Figure 1C). To abrogate immunologic dysregulation, the patient was given pulse methylprednisolone on day 120 and continued prednisone at doses of >30 mg/day with transient stabilization of liver function. Mycophenolate mofetil and ursodeoxycholic acid were initiated but ineffective, and the patient died of hepatic failure.

Patient 5 (68 years, female, FL) experienced grade 3 ALT elevation requiring dose interruption during cycle 2, and she achieved complete response to therapy after cycle 3. Idelalisib and rituximab were recommenced from cycle 4. She had reported no significant gastrointestinal adverse events other than grade 1 constipation. During treatment cycle 7, she developed abdominal pain, nausea, vomiting, and hematochezia. Because of suspected idelalisib-induced colitis, high-dose IV corticosteroids were administered with symptomatic improvement. However, prior to discharge, she developed suspected