Q141K polymorphism of ABCG2 protein is associated with poor prognosis in adult acute myeloid leukemia treated with idarubicin-based chemotherapy

Overexpression of multidrug resistance protein ABCG2 has been associated to chemotherapy failure in solid and hematologic tumors.[1-3] More than 40 'synonymous' and 'non-synonymous' single nucleotide polymorphisms (SNPs) of ABCG2 have been identified.[4] Among them, the 421C>A (Q141K), that is also the most common in Caucasian ethnicity (around 10%)[5] has been shown to alter protein function and to modify *in vitro* sensitivity to many anticancer drugs,[6,7] including classical anthracyclines and mitoxantrone. A recent report on the prognostic value of six ABCG2 gene polymorphisms in 184 Chinese acute leukemia patients seems to suggest that the presence of Q141K is associated with worse outcome.[8] The aim of this study was to evaluate the frequency of the Q141K variant in a cohort of Caucasian patients with acute myeloid leukemia (AML) and to assess the impact of this polymorphism on the outcome of a treatment strategy that included idarubicin as the only anthracycline.

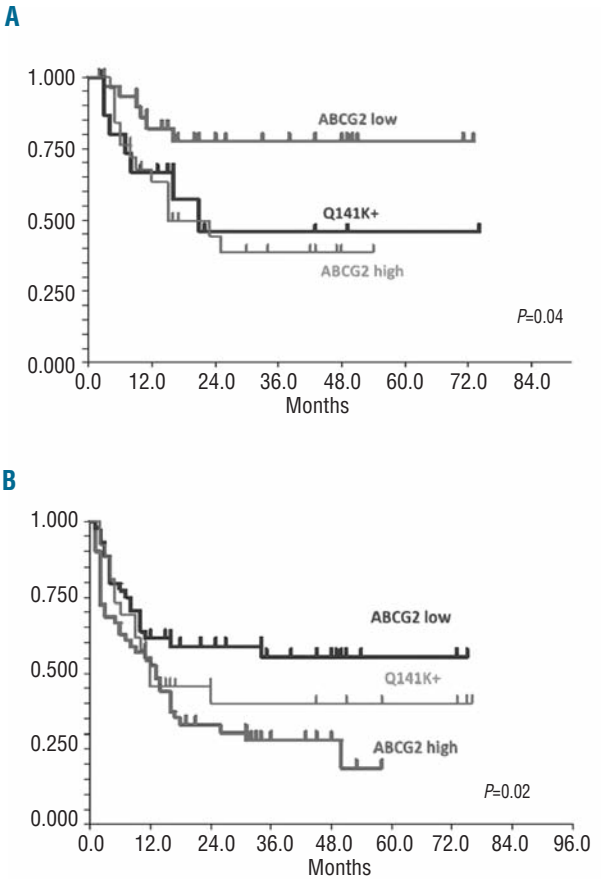

Figure 1. Outcome of patients according to ABCG2 expression and Q141K polymorphism; all *P* values were calculated using the log rank test. (A) Disease-free survival of Q141K polymorphism (n=16) compared to wild-type ABCG2 with high (n=27) or low (n=30) protein expression. (B) Overall survival of Q141K polymorphism (n=26) compared to wild-type ABCG2 with high (n=54) or low (n=45) protein expression.

One hundred and sixty-three consecutive patients with non-promyelocytic AML were included in the study. Leukemic cells were collected during diagnostic procedure after obtaining informed consent. One hundred and twenty-five patients received chemotherapy according to the institutional protocols active at diagnosis, including an induction course with FLAI/FLAIE regimen and at least one consolidation course with high-dose cytarabine, as previously described.[1] Idarubicin was the only anthracycline used through the entire chemotherapy program. ABCG2 expression was studied on blast cells by flow cytometry, as previously described.[1] ABCG2 genotype was analyzed with the TaqMan SNP Q141K assay (Applied Biosystems, C_15854163_70) and the test was performed in duplicate.

Q141K polymorphism was detected in 29 of 163 patients (18%), and 2 patients were homozygous for the mutation. Of the 125 patients receiving chemotherapy, 26 displayed Q141K variant while 99 were ABCG2 wt. Table 1 summarizes the characteristics of patients with or without ABCG2 polymorphism; no significant differences were observed in the clinical or biological features of the two cohorts, also when taking ABCG2 expression into consideration. Seventy-three out of 125 patients (58%) attained complete remission (CR) after induction therapy with FLAI/FLAIE. No impact on CR was demonstrated for ABCG2 overexpression and Q141K polymorphism. Twenty-seven of 73 (37%) patients relapsed after a median time of 26 months. A shorter disease-free survival (DFS) was associated with CD56 aberrant expression, CD34 positivity and ABCG2 overexpression. Again, ABCG2 Q141K polymorphism had no impact on relapse; among $Q141K^+$ patients, 7 of 16 (44%) relapsed while 20 of the 57 patients (35%) with wt ABCG2 who attained CR relapsed; 3-year DFS was 47% [CI: 30-64] in $Q141^+$ cases, and 61% [CI: 47-75] in wt patients (*P*=0.36). However, when patients were stratified in three groups according to ABCG2 expression (low or high) and presence of Q141K polymorphism, a negative impact of Q141K emerged. As shown in Figure 1A, DFS in $Q141K^+$ patients was similar to that of cases with wt ABCG2 but protein overexpression (14 of 27 patients relapsed, 52%; 3-year DFS 44%, CI: 23-65). This was significantly shorter than in patients with

Table 1. Clinical characteristics of the 125 patients treated for AML, divided according to ABCG2 Q141K polymorphism.

	$Q141K^+$ (n = 26)	$Q141K^-$ (n = 99)	*P*
Age: median (range)	56 (20-75)	60 (25-84)	0.36
WBC x10^9/L): median (range)	10.2 (1.4-258.0)	16.0 (0.5-265.0)	0.63
FAB subtype: n. (%)			0.70
M0-M1	7/26 (27)	32/99 (32)	
M2	9/26 (35)	24/99 (24)	
M4-M5	10/26 (38)	43/99 (44)	
Karyotype: n. (%)			0.13
Favorable / Intermediate	19/22 (86)	55/82 (67)	
Unfavorable	3/22 (14)	27/83 (33)	
$CD34^+$: n. (%)	15/26 (58)	59/99 (60)	1.00
$CD56^+$: n. (%)	1/26 (4)	18/99 (18)	0.13
ABCG2			0.95
overexpression: n. (%)	15/26 (58)	54/99 (54%)	
mRNA: mean ± SD	0.96±2.00	1.17±2.76	
protein expression: mean ± SD	0.37±0.28	0.30±0.19	

low ABCG2 expression and no polymorphism (6 of 30 relapses, 20%; 3-year DFS 77%, CI: 61-93) (χ^2=6.6, *P*=0.04). Combination of low protein expression and wt ABCG2 maintained its favorable impact on DFS also in the multivariate analysis (z value = 2.96, *P*=0.003).

Seventy-three of 125 patients (58%) died, with a median overall survival (OS) of 16 months and a 3-year overall survival (OS) of 40% (CI: 30-49). Factors negatively affecting OS were advanced age, CD34 expression and ABCG2 overexpression. Regarding DFS, Q141K polymorphism *per se* did not affect survival, but stratifying patients in the three groups, patients with low ABCG2 and wt gene had a longer OS compared to patients with Q141K ABCG2 or with high ABCG2 expression (55%, CI: 38-72% *vs*. 40%, CI: 20-60% *vs.* 27%, CI: 13-41, respectively) (χ^2 = 7.5, *P*=0.02) (Figure 1B). An advantage for the ABCG2 wt / low expression group was confirmed in multivariate analysis (z value = 1.96, *P*=0.05).

Q141K-ABCG2 is the most frequent polymorphism in Caucasians, and in this study this was detected in 18%. This is slightly higher than the frequency reported in literature.[5,9] Recently, Hampras *et al.*[10] reported on 261 AML patients, mainly of Caucasian ethnicity, treated with a standard anthracycline plus cytarabine regimen. They found that patients with C421A polymorphism had a 2-fold higher risk of death, although this was not statistically significant. In a group of 184 Chinese patients with acute leukemia, C421A polymorphism was associated with worse survival.[6] In our AML patients receiving a therapeutic program that includes idarubicin as the only anthracycline, Q141K is associated with poor outcome, comparable to that of patients over-expressing wild-type ABCG2 protein. These are the first data demonstrating a negative impact of ABCG2 polymorphism in Caucasian patients with AML. Since polymorphism does not seem to influence achievement of CR, the presence of this protein variant could be helpful in decision-making as to the choice of anthracycline to be used in post-induction therapy, and could promote the use of drugs the efficacy of which should be enhanced, such as mitoxantrone.

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References

1. Damiani D, Tiribelli M, Calistri E, Geromin A, Chiarvesio A, Michelutti A, et al. The prognostic value of P-glycoprotein (ABCB) and breast cancer resistance protein (ABCG2) in adults with de novo acute myeloid leukemia with normal karyotype. Haematologica. 2006;91(6):825-8.
2. de Jonge-Peeters SD, Kuipers F, de Vries EG, Vellenga E. ABC transporter expression in hematopoietic stem cells and the role in AML drug resistance. Crit Rev Oncol Hematol. 2007;62(3):214-26.
3. Benderra Z, Faussat AM, Sayada L, Perrot JY, Chaoui D, Marie JP, et al. Breast cancer resistance protein and P-glycoprotein in 149 adult acute myeloid leukemias. Clin Cancer Res. 2004;10(23):7896-902.
4. Iida A, Saito S, Sekine A, Mishima C, Kitamura Y, Kondo K, et al. Catalog of 605 single-nucleotide polymorphisms (SNPs) among 13 genes encoding human ATP-binding cassette transporters: ABCA4, ABCA7, ABCA8, ABCD1, ABCD3, ABCD4, ABCE1, ABCF1, ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8. J Hum Genet. 2002;47(6):285-310.
5. Kim KA, Joo HJ, Park JY. ABCG2 polymorphisms, 34G>A and 421C>A in a Korean population: analysis and a comprehensive comparison with other populations. J Clin Pharm Ther. 2010;35(6): 705-12.
6. Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, et al. C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Mol Cancer Ther. 2002;1(8):611-6.
7. Sparreboom A, Danesi R, Ando Y, Chan J, Figg WD. Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. Drug Resist Updat. 2003;6(2):71-84.
8. Wang F, Liang YJ, Wu XP, Chen LM, To KK, Dai CL, et al. Prognostic value of the multidrug resistance transporter ABCG2 gene polymorphisms in Chinese patients with de novo acute leukaemia. Eur J Cancer. 2011;47(13):1990-9.
9. Zamber CP, Lamba JK, Yasuda K, Farnum J, Thummel K, Schuetz JD, et al. Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. Pharmacogenetics. 2003;13(1):19-28.
10. Hampras SS, Sucheston L, Weiss J, Baer MR, Zirpoli G, Singh PK, et al. Genetic polymorphisms of ATP-binding cassette (ABC) proteins, overall survival and drug toxicity in patients with Acute Myeloid Leukemia. Int J Mol Epidemiol Genet. 2010;1(3):201-7.