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ABCG2 overexpression in patients with acute myeloid leukemia: Impact on stem cell transplantation outcome

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ABGG2 protein overexpression in acute myeloid leukemia (AML) has been associated with poor response to conventional chemotherapy and increased relapse risk. No data are available on the role of allogeneic stem cell transplantation (SCT) in reversing its negative prognostic role. We have reviewed the outcome of 142 patients with high risk AML who underwent allogeneic SCT in complete remission (n = 94) or with active disease (n = 48). Patients with ABCG2 overexpression at AML diagnosis have lower leukemia free survival (LFS) and increased cumulative incidence of relapse (CIR) compared with ABCG2− patients (5-year LFS 50% vs. 65%, P = 0.01; 5-year CIR 46% vs. 27%, P = 0.003). Five-year overall survival was not significantly different between ABCG2+ and ABCG2− patients (39% vs. 51%, P = 0.1). However, if we consider only disease-related deaths, ABCG2 maintains its negative role (64% vs. 78%, P = 0.018). The negative impact of ABCG2 overexpression was higher in patients undergoing SCT in CR compared with patients receiving transplant with active disease. Conditioning regimen did not abrogate the effect of ABCG2 overexpression, as CIR was higher in ABCG2+ patients receiving both myeloablative (44% vs. 22%, P = 0.018) or reduced intensity conditioning (50% vs. 32%, P = 0.03). In conclusion, ABCG2 overexpression at AML diagnosis identifies a subset of patients with poor outcome also after allogeneic SCT, mainly in terms of higher relapse rates. Prospective studies employing conditioning drugs or post-transplant strategies able to target ABCG2 are needed to maximize the curative potential of stem cell transplantation.

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of hematopoietic progenitors, which, as a consequence of various genetic mutations, lose their maturation capacity and acquire proliferative advantage, thus resulting in accumulation of immature, nonfunctional cells in bone marrow and peripheral blood.

Despite in the past three decades significant improvements were made in the knowledge of leukemia pathogenesis, prognostic factors, drugs' availability and in patients care, the overall prognosis of AML remains poor, and estimated 5-year overall survival (OS) after standard dose chemotherapy is 38% [1,2]. In elderly patients efficacy of conventional chemotherapy is even lower, with OS around 10% 5 years after diagnosis [1,3,4]. So, allogeneic stem cell transplantation (SCT) is considered the recommended postinduction therapy [5–7]. The advent of reduced intensity conditioning (RIC) regimens, and the increased availability of alternative donors have widened the number of patients that may benefit from SCT, changing the treatment algorithm of intermediate and high risk patients [8,9]. Unfortunately, relapse still occurs in a consistent part of patients and remains the major cause of treatment failure after allogeneic SCT [10–12]. Therefore, efforts have been focused on the identification of factors that can predict disease recurrence and on strategies to possibly prevent it. Pretreatment cytogenetic alterations and molecular abnormalities, such as FLT3 gene mutations, are associated with increased incidence of relapse [13–15], even if mechanisms underlying this risk remain largely unknown. In the last years, various studies had found that overexpression of multidrug resistance protein ABCG2 confer resistance to many different chemotherapeutic agents [16,17], and that ABCG2 overexpression is associated with a worse prognosis in AML patients [18–20]. No information is available on the impact of ABCG2 in patients undergoing allogeneic SCT for AML.

We have retrospectively evaluated 142 patients who underwent allogeneic SCT for AML, with the aim to clarify the ability of preparative regimens and of the new immune system to abrogate the negative prognosis associated with ABCG2 expression.

Materials and Methods

Patients. We reviewed our database of patients and identified 142 patients who underwent allogeneic SCT for high risk AML between 2001 and 2013 at the Division of Hematology of Udine. Cytogenetic risk group assignment was done according to the 2010 revised MRC criteria [21]. Multidrug resistance associated proteins expression on

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TABLE I. Patients and Transplant Characteristics

	$n = 142$
Age (yrs)	
Median (range)	49 (17–69)
>50	65 (45%)
Sex	
M	67
F	75
Secondary AML	42 (29%)
Cytogenetics	
Favorable	10 (7%)
Intermediate	96 (67%)
Unfavorable	30 (21%)
Missing	6 (4%)
WBC $>30 \times 10^9$/L	51 (36%)
MDR proteins expression	59 (41%)
Late CR	38 (27%)
Disease status at transplant	
CR	94(66%)
No CR	48(34%)
Donor type	
Sibling	64 (45%)
Unrelated	78 (55%)
Compatibility	
Full-matched	81 (57%)
Mis-matched	61 (43%)
Female donor/male recipient	
No	117 (82%)
Yes	25 (18%)
Stem cell source: bone marrow	47 (33%)
Peripheral blood	95 (67%)
Stem cell dose	
$\geq 4 \times 10^6$/kg	70 (49%)
$<4 \times 10^6$/kg	74 (51%)
Conditioning regimen	
Myeloablative	88 (62%)
Reduced intensity	54 (38%)

MDR: multidrug resistance.

blast cells at diagnosis were evaluated by flow cytometry as previously described [19]. Cases with mean fluorescence intensity above the identified cut-off were considered "over-expressing" and referred as "positive" in the text.

All patients received induction chemotherapy according to the protocol in use at the time of diagnosis and at least one consolidation course of chemotherapy with high-dose cytarabine and idarubicin.

Patients' and transplant's characteristics are summarized in Table I. Indications to SCT included high-risk features at diagnosis (i.e. high blast count, unfavorable karyotype, secondary AML), late complete remission (CR) achievement, primary resistance or disease relapse. EBMT score was used to define transplant risk.

Median age was 49 years (range, 17–69 years) and 65/142 (45%) were older than 50 years and 28 patients (20%) were over 60 years. Thirty-one out of 142 (22%) patients had secondary AML, 51 (36%) had WBC count $>30 \times 10^9$/L, 30/142 (21%) had an unfavorable cytogenetics, and 59/142 (41%) overexpressed one or more MDR proteins. Thirty-eight patients (27%) did not achieve CR after induction chemotherapy. Fifty-six (39%) had an high-risk EBMT score (>4).

Sixty-six patients (46%) received stem cells from an HLA identical sibling, 69 (49%) from a matched unrelated donor and 7 (5%) from a haploidentical donor. Twenty-five of 142 (18%) were males with a female donor; stem cell source was bone marrow in 48 patients (34%), peripheral blood in 94 patients (66%). Conditioning regimen was myeloablative in 88/142 (62%) cases, while 54 patients (38%) received a reduced-intensity conditioning (RIC) (Supporting Information Table I). Graft-versus-host disease (GVHD) prophylaxis consisted of a calcineurin inhibitor (cyclosporine or tacrolimus) combined with methotrexate, with or without anti-thymocyte globulin (ATG); rapamycin and micofenolate mofetil were used according to the protocol in use at time of RIC transplant. Supportive care for all patients followed institutional standards.

Definitions and statistical analysis. CR was defined as peripheral blood normalization and absence of bone marrow minimal residual disease by morphological, molecular or immunophenotypic evaluation. Leukemia free survival (LFS) was defined as survival without evidence of disease recurrence or progression from transplant. Overall survival (OS), defined as time from date of transplantation to death, independently of the cause. Nonrelapse mortality (NRM) was defined as death without prior relapse.

Statistical analyses were performed by the NCSS 8 software package (NCSS, LLC, Kaysville, UT; available at: www.ncss) and by EZR package [22] in presence of competing risks. Patient-, disease-, and transplant-related categorical variables were compared by χ^2 test. Survival curves were obtained by Kaplan-Meier method and differences between groups compared by log-rank test. Hazard ratios for LFS and OS were determined by Cox regression analysis [23]. Cumulative incidence of relapse (CIR) was calculated by Gray's method considering NRM as competing risk [24]. Multivariate analysis was performed for factors with statistical significance or borderline significance ($P < 0.1$) using Fine-Gray proportional hazard regression for competing events [25]. All P values are two-sided with type I error fixed at 0.05.

Results

Transplant procedure

Ninety-four out of 142 patients (66%) underwent transplant in first or second CR, at a median of 7 months (range, 3–179 months) from diagnosis. Forty-eight patients (34%) received HSCT with evidence of leukemia, as defined either by morphological and/or cytogenetic/molecular evaluation.

Neutrophil (absolute neutrophil count $>1 \times 10^9$/L without G-CSF) and platelet (platelets $>20 \times 10^9$/L without transfusions) were attained after a median 19 (range, 17–23) and 20 (range, 14–35) days, respectively. Acute GVHD occurred in 70/142 patients (49%), but in only 16/70 (23%) was of grade $\geq$3, without differences between donor type or stem cell source. Chronic GVHD was evaluable in 108/142 patients (76%). Fifty-one (47%) developed cGVHD, that was extensive in 10 (20%). Microbiological documented pre-engraftment infections occurred in 61/142 patients (43%): Gram+ sepsis in 28 patients, Gram− sepsis in 18 patients, pneumonia in 25 (bacterial, n = 21 or fungal, n = 4) patients; CMV reactivation occurred in 23 cases. Post-engraftment infections occurred in 49 patients, and were mainly of viral etiology (30 CMV reactivation, 8 EBV reactivation, 6 HZV infection, and 1 HBV hepatitis). Severe noninfective post-transplant complications included hemorrhagic cystitis (n = 13), congestive heart failure (n = 6), veno-occlusive disease (n = 3), and acute pericarditis (n = 2).

Outcomes: Univariate analysis

Leukemia free survival. At the time of the analysis, 83/142 (58.4%) patients were alive and in CR, with a cumulative 5-year LFS of 56% (95% confidence interval [CI], 47–65%). Factors affecting LFS are listed in Table II. Among transplant related factors, a negative association was observed only with unrelated donor (52%; 95% CI, 38–64%) compared with sibling donor (65%; 95% CI, 53–77%) (P = 0.03), and with the absence of chronic GVHD (53%; 95% CI, 40–66% vs. 84%; 95% CI, 73–95%; P = 0.001). With regard to disease characteristics, the persistence of leukemia at SCT was associated with reduced LFS: 30% (95% CI, 16–46%) versus 72% (95% CI, 67–82%) in patients transplanted in CR ($P < 0.0001$). Unfavorable cytogenetics (32%; 95% CI, 12–51%, vs. 67%; 95% CI, 57–77% in intermediate-risk and 67%; 95% CI, 34–97% in favorable-risk groups; P = 0.01), and ABCG2 overexpression at diagnosis (50%; 95% CI, 37–64% vs. 65%; 95% CI, 53–76%, in negative patients; P = 0.01) were associated with shorter LFS (Fig. 1a).

Cumulative incidence of relapse (CIR) and cumulative incidence of nonrelapse mortality (CIRNM). As shown in Table II, considering transplant factors only chronic GVHD has a protective effect on relapse risk (P = 0.014). However, the ability of chronic GVHD to exert a GVL effect was particularly evident in the ABCG2-negative group (CIR: 10%, 95% CI, 4–31% in presence of cGVHD and 46%, 95% CI, 33–64% in patients without cGVHD, P = 0.001) (Supporting Information Fig. 1). Conversely only a trend in lower relapse rate was observed in ABCG2-positive patients (P = 0.18) (Supporting Information Fig. 1). Nonsignificant risk factors included donor/recipient compatibility, donor type, stem cell source and dose, conditioning

TABLE II. Univariate Analysis of Factors Affecting SCT Outcome

Variable	5-year LFS		5-year OS		5-year CIR		5-year NRM	
	%(95% CI)	*P*	%(95% CI)	*P*	%(95% CI)	*P*	%(95% CI)	*P*
Age								
<50	66(54–78)	0.22	54(41–67)	**0.05**	41(30–52)	0.07	21(13–31)	0.23
≥50	52(10–64)		38(26–50)		27(16–38)		18(9–28)	
Cytogenetics								
Favorable	67(34–97)	**0.01**	50(19–81)	0.13	30(6–59)	**0.02**	30(6–60)	0.20
Intermediate	67(57–77)		51(40–62)		27(19–36)		21(14–30)	
Unfavorable	32(12–51)		33(15–51)		55(34–71)		10(2–24)	
ABCG2								
Positive	50(37–64)	**0.01**	39(23–49)	0.10	46(33–58)	**0.003**	15(7–26)	0.08
Negative	65(53–76)		51(39–63)		27(18–37)		23(14–33)	
Disease status at SCT								
CR	72(67–82)	**<0.0001**	63(53–74)	**<0.0001**	21(13–30)	**0.0002**	18(10–27)	0.70
No CR	30(16–46)		15(5–25)		59(44–71)		23(13–36)	
Donor type								
SIB	65(53–77)	**0.03**	58(43–71)	**0.0004**	30(19–41)	0.10	12(5–22)	**0.03**
URD	52(38–64)		35(42–46)		39(28–49)		26(17–36)	
HLA matching								
Full match	59(48–70)	0.23	50(38–62)	**0.02**	35(24–45)	0.35	15(8–24)	**0.03**
Mismatch	37(22–52)		33(19–47)		42(27–55)		30(17–43)	
Female donor to male recipient								
No	55(46–65)	0.55	44(34–54)	0.46	36(27–45)	0.90	21(14–29)	0.41
Yes	71(54–89)		54(34–74)		28(12–46)		19(5–39)	
Conditioning								
MAC	66(52–80)	0.17	55(44–66)	**0.003**	33(23–43)	0.43	12(6–20)	**0.013**
RIC	51(36–67)		30(16–43)		37(25–51)		32(30–46)	
Stem cell source								
BM	61(47–76)	0.45	52(37–66)	0.39	34(30–55)	0.70	17(8–30)	0.62
PB	57(45–68)		42(31–53)		35(26–45)		22(14–32)	
Stem cell dose								
$<4 \times 10^6$/kg	51(38–64)	0.22	43(30–55)	0.30	43(30–55)	0.21	16(8–27)	0.66
$\geq 4 \times 10^6$/kg	63(50–75)		50(37–63)		30(20–41)		18(10–28)	
Acute GVHD								
No	59(46–72)	0.44	56(43–70)	0.18	32(24–41)	0.42	16(10–24)	**0.003**
Yes	67(56–79)		40(25–51)		25(7–49)		50(23–72)	
Chronic GVHD								
No	53(40–66)	**0.001**	57(43–70)	0.34	41(28–53)	**0.001**	9(3–19)	**0.01**
Yes	84(73–95)		59(44–74)		12(4–24)		23(11–38)	

SCT = stem cell transplantation; LFS = leukemia free survival; OS = overall survival; CIR = cumulative incidence of relapse; NRM = nonrelapse mortality; URD = unrelated donor; SIB = sibling donor; MAC = myeloablative conditioning; RIC = reduced intensity conditioning; BM = bone marrow; PB = peripheral blood; GCVH = graft versus host disease.
Values in bold are statistical significant.

regimens and acute GVHD. Advanced recipient's age has a trend for a higher risk of relapse ($P = 0.07$). Five-year CIR was significantly higher in patients with AML persistence at SCT (59%; 95% CI, 44–71%), unfavorable karyotype (55%; 95% CI, 34–71%), and ABCG2 positivity (46%; 95% CI, 33–58%) (Fig. 1b). The negative impact of ABCG2 overexpression was particularly evident in the cohort of patients transplanted while in CR: 5-year CIR was 32% (95% CI, 19–51%) in ABCG2+ compared with 15% (95% CI, 8%-27%) in ABCG2− patients ($P = 0.02$; Fig. 1c). Conversely, in patients undergoing allogeneic SCT with active AML, difference in CIR between ABCG2+ (67%; 95% CI, 50–88%) and ABCG2− cases (52%; 95% CI, 36–74%), did not reach statistical significance ($P = 0.09$; Fig. 1d). The higher relapse rate associated with high ABCG2 overexpression was not influenced by conditioning regimen: CIR after myeloablative SCT was 44% (95% CI, 28–59%) in ABCG2+ and 22% (95% CI 12–35%) in ABCG2-negative group ($P = 0.018$); after RIC SCT, CIR was 50% (95% CI, 25–71%) in ABCG2+ and 32% (95% CI, 17–31%) in ABCG2− patients, respectively ($P = 0.03$).

ABCG2 expression did not influence NRM: 5-year CINRM was 23% (95% CI, 14–33%) in ABCG2-positive patients, compared with 15% (95% CI, 8%-24%) in negative ones ($P = 0.08$). CINRM was affected by donor type (unrelated 26% vs. sibling 12%, $P = 0.03$), HLA compatibility (mismatched 30%, in vs. matched 15%, $P = 0.03$), conditioning regimen (RIC 32% vs. MAC 12%, $P = 0.013$) and development of acute GVHD (50% vs. 16%, $P = 0.003$) or chronic GHVD (23% vs. 9%, $P = 0.01$).

Overall survival. At the time of this analysis, 61/142 (43%) patients were alive, 41 (29%) died of AML recurrence and 40 (28%) died from NRM. One-year OS was 62% (95% CI, 54–70%) and 46% patients (95% CI, 38–55%) were alive 5 years after SCT. Five-year OS was higher in patients younger than 50 years (54%; 95% CI, 41–67%) compared with elderly ones (38%; 95% CI, 26–50%; $P = 0.05$), in patients receiving SCT while in CR (63%; 95% CI, 53–74% vs. 15%; 95% CI, 5–25%; $P < 0.0001$), in recipient of transplant from siblings (58%; 95% CI, 43–71% vs. 35%; 95% CI, 42–46%; $P = 0.0004$) and from full matched donors (50%; 95% CI, 38–62% vs. 33%; 95% CI, 19–47%; $P = 0.02$) and in patients receiving myeloablative conditioning (55%; 95% CI, 44–66% vs. 30%; 95% CI, 16–4%; $P = 0.003$). Though ABCG2 overexpression did not influence OS, if we consider only disease-related deaths 5-year survival rate was significantly lower in ABCG2+ patients (64%; 95% CI, 50–74%) compared with ABCG2− ones (78%; 95% CI, 66–85%; $P = 0.018$).

Outcomes: Multivariate analysis

Results of multivariate analysis on the different outcomes are summarized in Table III. By Cox proportional hazard regression, age >50 years (HR =2.32; 95% CI, 1.1–5.0%), unfavorable cytogenetics (HR = 2.01; 95% CI, 1.04–3.85%), high ABCG2 expression at

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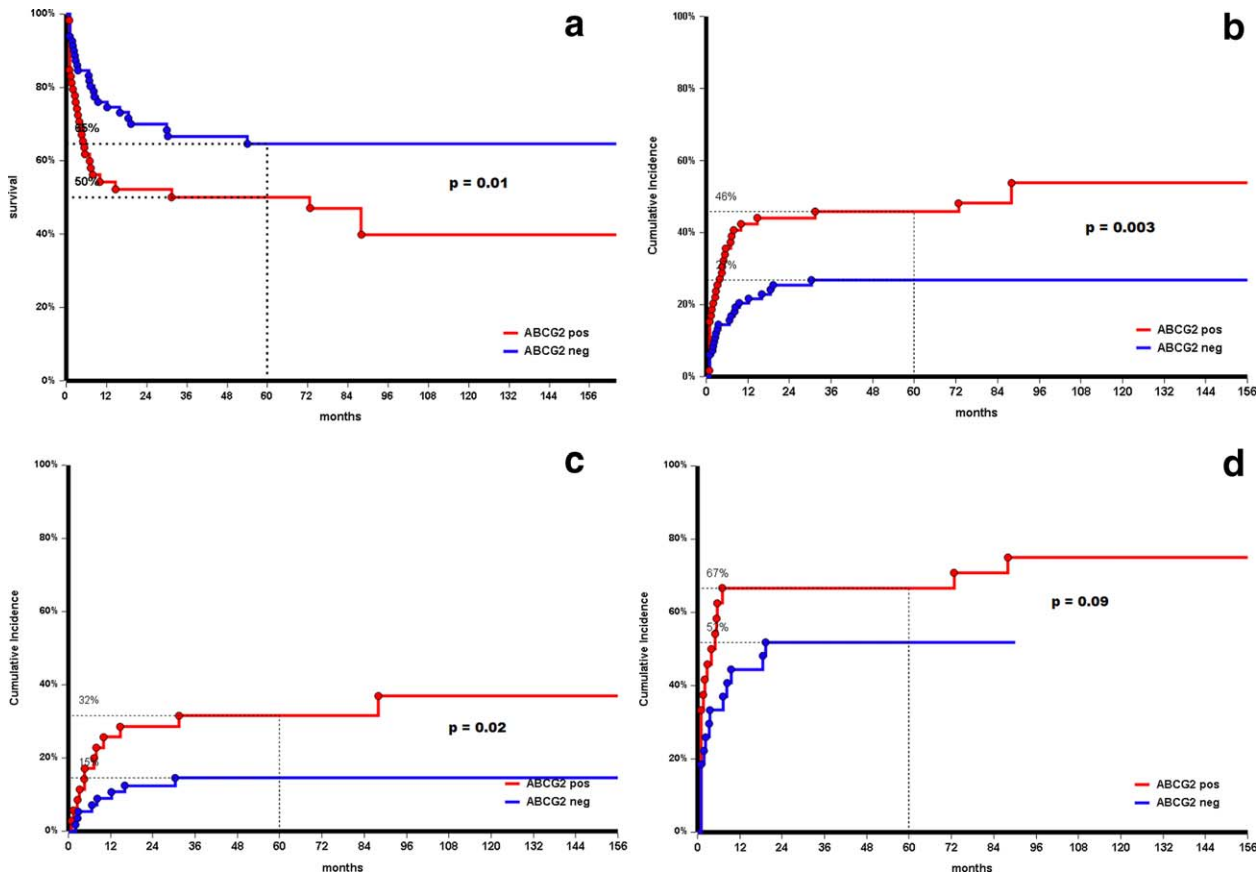

Figure 1. Leukemia free survival (a) and cumulative incidence of relapse in the whole population (b) according to ABCG2 expression. Cumulative incidence of relapse in patients underwent SCT in CR (c) and with active disease (d) according to expression of ABCG2.

diagnosis (HR = 2.32; 95% CI, 1.12–4.76%), and disease persistence at SCT (HR = 3.69; 95% CI, 1.85–7.35%) are independently associated with poor LFS. Conversely, a benefit for LFS was seen in patients developing chronic GVHD (HR = 0.28; 95% CI, 0.13–0.61%).

Advanced age (HR = 2.17; 95% CI, 1.19–3.84%) and no CR at transplant (HR = 4.63; 95% CI, 2.66–8.05%) confirmed their negative impact also on OS. A higher risk of death was observed also in recipients of unrelated donor SCT (HR = 2.42; 95% CI, 1.14–5.15%).

As for LFS, older age (HR = 0.36; 95% CI, 0.16–0.80%), ABCG2 positivity (HR = 0.32; 95% CI, 0.15–0.71%), no CR at transplant (HR = 0.50; 95% CI, 0.33–0.76%) and chronic GVHD (HR = 0.28; 95% CI, 0.12–0.66%) were associated with a higher incidence of relapse. Development of chronic GVHD was the only factor significantly associated with an increased risk of NRM (HR = 1.85; 95% CI, 1.02–3.35%).

Discussion

The ultimate challenge in allogeneic SCT for high-risk AML is to prevent relapse, eradicating the leukemic stem cell population, without increasing procedure-related toxicity. In the past years efforts have been focused on discovering factors affecting disease recurrence after transplant. It is well known that an important role is played by cytogenetic abnormalities present at diagnosis [13,14] and by some molecular markers in the subset of cytogenetically normal AML [15].

In the present work we evaluated factors affecting long-term outcomes in a series of 142 patients receiving allogeneic SCT in complete remission or with active leukemia. Among transplant-related characteristics, only the development of chronic GVHD and the availability of a sibling donor were associated with better OS and LFS,

TABLE III. Multivariate Analysis of Prognostic Factors for LFS, OS, CIR, and NRM

Variable	HR	95% CI	*P*
LFS			
Age >50 yr	2.32	1.10–5.0	**0.02**
Unfavorable cytogenetics	2.01	1.04–3.85	**0.03**
ABCG2 positive	2.32	1.20–4.76	**0.02**
No CR at SCT	3.69	1.85–7.35	**0.0002**
Unrelated donor	1.62	0.81–3.21	0.16
Chronic GVHD	0.28	0.13–0.61	**0.001**
OS			
Age >50 yr	2.17	1.19–3.84	**0.01**
ABCG2 positive	1.23	0.73–2.08	0.43
No CR at SCT	4.63	2.66–8.05	**<0.0001**
Unrelated donor	2.42	1.14–5.15	**0.02**
Full HLA matching	1.05	0.53–2.07	0.88
Myeloablative conditioning	1.04	0.54–1.71	0.89
CIR			
Age >50 yr	2.77	1.25–6.25	**0.01**
Unfavorable cytogenetics	1.84	0.78–4.36	0.16
ABCG2 positive	3.12	1.40–7.14	**0.005**
No CR at SCT	1.99	1.31–3.02	**0.001**
Chronic GVHD	0.28	0.12–0.66	**0.004**
NRM			
ABCG2 positive	1.20	0.39–3.70	0.75
Unrelated donor	1.44	0.41–5.04	0.57
Full HLA matching	0.99	0.17–5.55	0.99
Myeloablative conditioning	2.35	0.73–7.50	0.15
Acute GVHD	1.10	0.17–7.12	0.92
Chronic GVHD	1.85	1.02–3.35	**0.04**

SCT = stem cell transplantation; LFS = leukemia free survival; OS = overall survival; CIR = cumulative incidence of relapse; NRM = nonrelapse mortality; GVHD = graft versus host disease.

respectively. Considering pretransplant factors, AML status and unfavorable cytogenetic remain the stronger predictors, in line with previous literature data [26–28]. The most important novel finding emerging from our data is the negative role of ABCG2 overexpression on SCT outcome. Many authors have associated the overexpression of different members of ABC proteins with resistance to conventional induction chemotherapy in AML[18,19,29] but, till now, no data are available on the role of ABCG2 level at AML diagnosis in patients undergoing allogeneic SCT. We found that patients with high ABCG2 expression have a shorter LFS rate compared with ABCG2-negative ones (50% vs. 65 at 5 years after SCT). The unfavorable impact of ABCG2 was confirmed by the higher rate of CIR in ABCG2-positive patients (46% at 5 years). Of note, the correlation between ABCG2 positivity and higher relapse risk was statistically significantly only in patients who underwent SCT while in CR, as if in patients transplanted with active AML the kinetics of disease growth under immunosuppressive therapy would predominate on the reduced drug sensitivity mediated by ABC transporter proteins. The negative role of ABCG2 is retained in the multivariate model, with a risk of relapse three times higher in ABCG2+ patients. Moreover, ABCG2-mediated drug extrusion is only one of many mechanisms used by leukemic cells to survive to chemotherapy, but our findings suggest that the management of high-risk AML and the efforts to prevent disease relapse should consider the role of ABCG2. Many reports on solid tumors have recently underlined that ABCG2 is implicated not only in inducing drug resistance in the proliferating pool, but confers to blasts the characteristics of a stem cell-like population, surviving after therapy as a "disease reservoir." We hypothesizes that ABCG2 overexpression in the whole leukemic population is associated with higher number of more immature ABCG2+ cells closely correlated with the so-called "side population," that seems to be involved not only in the initiation but also in progression and relapse of acute leukemia, and that may be able to survive after SCT despite conditioning chemotherapy and transplant-related immune control [29–35]. Laboratory studies are currently ongoing to prove this hypothesis.

Prospective studies are needed to investigate the possibility to eradicate the leukemic stem cell (LSC) compartment by modulating conditioning regimens. Many new tyrosine kinase inhibitors are able to inhibit ABCG2 activity or to favor its degradation via lysosome, ultimately reversing LSCs properties [36–43]. Also epigenetic regulation of ABCG2 expression has been recently proposed as a cause of poor response to new chemotherapeutic agents. Bram et al. observed that drug-induced ABCG2 promoter demethylation is responsible for a new form of acquired drug resistance [44]. This observation is of particular importance in the post-transplant setting, since hypomethylating drugs have been used to improve outcome on the basis that hypomethylating agents up-regulate anti-tumor CD8+ T-cell response, limiting at the same time the risk of GVHD by accelerating CD4+ Treg recovery [45–47]. However, Craddock et al. have recently reported that azacytidine (AZA) fails to eradicate the stem/progenitor population in acute leukemia [48]. A possible explanation could be that DNA-demethylation induced by AZA promotes ABCG2 expression, thus increasing bone marrow LSCs reservoir.

In conclusion, our data outline the negative role played by ABCG2 overexpression in AML receiving allogeneic SCT. Although the impact of MDR-related proteins in affecting response to induction chemotherapy and LFS has been recognized for 20 years, to date no significant therapeutic changes have been made. In this era of technological explosion and of new "target drugs," recognition of the negative role of ABCG2 may help in designing post-transplant strategies to maximize the curative potential of SCT for acute leukemia.

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