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Differential expression of *hERG1A* and *hERG1B* genes in pediatric acute lymphoblastic leukemia identifies different prognostic subgroups

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Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood, with 85% of cases being of B-cell lineage (B-cell precursor (BCP)-ALL) and 15% of T-cell lineage (T-ALL).[1] With wider use of intensive chemotherapy, the prognosis of childhood ALL has improved remarkably, and nearly 80% of BCP-ALL[2] patients can currently be cured. The prognosis of children with T-ALL has improved and has been reported to be similar to that for BCP-ALL (no differences in the 5-year event-free survival (EFS) rate).[3] However, long-term survival rates for pediatric T-ALL are still lower than those for BCP-ALL by up to 20%.[3] Systemic toxicity and chemoresistance are nowadays the main shortcomings of standard chemotherapy.[2]

Current interest focuses on identifying new specific molecular targets to be exploited either for risk stratification or for identification of novel, patient-tailored, therapeutic approaches that can improve therapy efficacy and reduce toxicity in pediatric ALL.

We have provided evidence that K^+ channels encoded by the ether-a-gò-gò-related gene 1 (*hERG1*), hERG1 channels, besides exploiting a relevant role in cardiac physiology,[4] are often aberrantly expressed in human cancers including leukemias.[5,6] In pediatric BCP-ALL, hERG1 channels sustain the development of chemoresistance,[7] as they modulate pro-survival signals triggered by the bone marrow microenvironment. In adult acute myeloid leukemias (AML), hERG1 regulates cell motility and transendothelial migration through an interplay with angiogenic signaling pathways. This effectively correlates with the worse prognosis in AML patients displaying high hERG1 expression.[5]

The hERG1 encoding gene shows two main alternative transcripts, *hERG1A* and *hERG1B*. *hERG1B* encodes a protein, hERG1B, with a unique N-terminus that justifies the peculiar biophysical features of hERG1B-sustained currents.[8] The two hERG1 isoforms are expressed at different ratios and differentially contribute to sustain hERG1 currents in the tissues where hERG1 is functionally expressed. For example, whereas hERG1B is expressed at low levels in the human heart,[9] it represents the main hERG1 isoform in tumor cells, such as neuroblastomas and leukemias.[10] This fact makes hERG1B a promising tumor-specific target.[6] To be exploited for diagnostic and therapeutic purposes, the differential expression of *hERG1A* and *hERG1B* transcripts in primary tumors must be well defined. Whereas a high expression of *hERG1B* has been reported in adult primary AML cases,[5] no data on the differential expression of *hERG1A* and *hERG1B* in ALL have been reported so far.

In the present study, we analyzed the expression and prognostic impact of the two *hERG1* encoding genes in two cohorts of pediatric ALL patients, BCP-ALL and T-ALL. In particular, we examined the expression of *hERG1A* and *hERG1B* mRNA by SYBR Green real-time quantitative PCR (Rt-qPCR) in 100 BCP-ALL ($n=94$ children and $n=6$ infants below 1 year of age) and 111 T-ALL patients. Expression values were compared with those obtained in pooled CD19+ B and CD3+ T cells, respectively. All the patients studied were enrolled in the AIEOP LAL 2000-R2006 therapy protocol, whose details are reported in.[11] The clinico-pathological characteristics of the patients, along with the expression of the two *hERG1* transcripts, are shown in Table 1.

In BCP-ALL children (Table 1, upper panel) the *hERG1A* isoform was downregulated (median value = 0.03; 0.01–0.07), whereas *hERG1B* was upregulated (median value = 6.68; 2.48–16.63), compared with normal B cells. Although generally hypo-expressed, *hERG1A* was higher in CALL and pre-B immunophenotype subgroups ($P=0.0326$) and in prednisone poor responder (PPR) patients compared with prednisone good responder (PGR) patients ($P=0.0492$). A marginally statistically significant higher expression was evidenced in BCP-ALL patients with no chromosomal translocations, compared with patients with either the 12;21 or the 4;11 translocation ($P=0.0593$).

In the infant subgroup of BCP-ALL, both *hERG1A* and *hERG1B* transcripts were hypo-expressed (median values: 0.03 and 0.24, respectively; Supplementary Table 1S).

Similarly to BCP-ALL patients, T-ALL patients (Table 1, lower panel) showed an overexpression of the *hERG1B* transcript and a downregulation of *hERG1A*, although the gap between the two isoforms was less evident (median values: 5.11; 2.00–19.98 and 0.76; 0.17–4.08, respectively). A higher expression of *hERG1A* was detected in T-ALL patients with a WBC count $\geqslant 50.000$ compared with patients with a WBC count <50.000 (median value: 1.85 vs 0.23; $P=0.001$) and in PPR compared with PGR patients (median value: 2.13 vs 0.49; $P=0.005$). An indication of overexpression was evidenced also in patients with involvement of the central nervous system (CNS) ($P=0.096$). The *hERG1B* transcript was in general overexpressed, in particular in patients with WBC count <50.000 ($P=0.031$) and, although not significantly, in standard-risk (SR) patients.

Finally, we evaluated the impact of the differential expression of *hERG1A* and *hERG1B* on relapse in the two cohorts of BCP-ALL and T-ALL patients. The optimal cutoff value was determined on the basis of the receiver operator characteristic analysis. In BCP-ALL, a cutoff value with proper sensitivity and specificity was found only for the *hERG1A* transcript. The cumulative relapse rate at 5 years was 32.3% in patients with *hERG1A* <0.03 and 13.4% in patients with *hERG1A* $\geqslant 0.03$ ($P=0.04$) (Figure 1a). After adjusting for risk groups in a multivariate Cox model, this association was not statistically significant (HR of relapse in patients with *hERG1A* <0.03 versus $\geqslant 0.03$:1.88; CI 0.77–4.60; $P=0.166$).

In T-ALL patients, discriminant values of expression were obtained for both *hERG1A* and *hERG1B*. The cumulative relapse rate at 5 years was 37% in patients with *hERG1A* $\geqslant 0.74$ and 22% in patients with *hERG1A* <0.74 ($P=0.020$) (Figure 1b). On multivariate analysis, *hERG1A* lost its statistically significant association with relapse (HR = 1.61 95%CI 0.73–3.54 $P=0.2404$). Patients with *hERG1B* $\geqslant 6.8$ relapsed with higher frequency compared with patients <6.8 (5 years' cumulative incidence of relapse: 38 vs 22%, $P=0.17$). The Cox model after adjusting for classical prognostic factors (immunophenotype, risk group and WBC) identified *hERG1B* as an independent factor of higher risk of relapse (HR 2.6; CI 1.26–5.30, $P=0.009$) (Figure 1c, left panel).

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Table 1. Distribution of clinical and biological features and results of the univariate analysis in children with BCP-ALL and T-ALL

	hERG1A median (1st–3rd quartile)				P	*hERG1B median (1st–3rd quartile)*				P
BCP-ALL										
Sex	F (43)	M (51)				F (43)	M (51)			
	0.03 (0.01–0.04)	0.02 (0.01–0.08)			0.359	8.91 (3.90–24.49)	5.4 (2.39–12.40)			0.1554
Age	1–5 years (57)	6–9 years (22)	10–17 years (15)			1–5 years (57)	6–9 years (22)	10–17 years (15)		
	0.03 (0.01–0.06)	0.03 (0.01–0.07)	0.01 (0.01–0.06)		0.2155	6.95 (3.01–18.00)	6.68 (2.48–16.63)	4.91 (1.41–15.88)		0.9248
IF	Pre pre B (6)	Call (55)	Pre-B (31)	B-lin (2)		Pre pre B (6)	Call (55)	Pre-B (31)	B-lin (2)	
	0.01 (0–0.01)	0.03 (0.01–0.07)	0.03 (0.01–0.04)	0.01,0.02	0.0326	7.07 (1.55–15.88)	8.1 (4.20–22.78)	4.71 (2.10–9.43)	5.81,6.43	0.2968
WBC	<50 000 (67)	>50 000 (27)				<50 000 (67)	>50 000 (27)			
	0.03 (0.01–0.07)	0.02 (0.01–0.06)			0.7052	6.97 (3.88–17.32)	5.22 (1.42–15.88)			0.2643
PDN response	PPR (14)	PGR (79)				PPR (14)	PGR (79)			
	0.05 (0.03–0.14)	0.03 (0.01–0.05)			0.0492	2.25 (1.50–9.78)	6.72 (3.44–17.32)			0.3493
CNS	Positive (1)	Negative (93)				Positive (1)	Negative (93)			
	0.01	0.03 (0.01–0.07)				0.82	6.69 (2.57–16.72)			
MRD	SR (30)	MR (40)	HR (11)			SR (30)	MR (40)	HR (11)		
	0.04 (0.03–0.11)	0.01 (0.01–0.03)	0.01 (0.01–0.03)		0.2127	6.97 (2.48–16.63)	6.43 (4.06–15.88)	3.31 (1.02–16.80)		0.3855
Risk group	SR (27)	MR (43)	HR (24)			SR (27)	MR (43)	HR (24)		
	0.04 (0.03–0.11)	0.02 (0.01–0.04)	0.01 (0.01–0.07)		0.2613	8.05 (3.90–25.43)	6.56 (4.40–16.94)	2.25 (1.26–10.13)		0.2103
Translocation	12;21 (25)	MLL (3)	No (66)			12;21 (25)	MLL (3)	No (66)		
	0.02 (0.01–0.07)	0.00, 0.01, 0.01	0.03 (0.01–0.07)		0.0593	6.3 (2.48–25.43)	0.82, 1.42, 5.22	8.1 (2.73–15.88)		0.1858
T-ALL										
Sex	F (18)	M (93)				F (18)	M (93)			
	0.66 (0.11–1.42)	0.76 (0.20–4.29)			0.2250	2.91 (1.90–14.00)	6.35 (2.35–20.59)			0.2921
Age	1–5 years (35)	6–9 years (31)	10–17 years (45)			1–5 years (35)	6–9 years (31)	10–17 years (45)		
	0.49 (0.20–2.86)	0.71 (0.09–3.70)	1.35 (0.31–4.86)		0.4145	5.11 (2.54.15.94)	6.57 (1.53—23.66)	4.85 (1.94–20.18)		0.9664
IF	Early T (53)	Thym (41)	T mature (15)			Early T (53)	Thym (41)	T mature (15)		
	1.1 (0.25–4.25)	0.6 (0.16–1.19)	1.41 (0.76–15.00)		0.1186	5.11 (1.94–17.06)	4.85 (2.18–19.37)	3.3 (2.67–31.31)		0.5783
WBC	<50 000 (42)	>50 000 (68)				<50 000 (42)	>50 000 (68)			
	0.23 (0.09–0.74)	1.85 (0.49–8.00)			<0.001	12.98 (2.70–23.66)	3.41 (1.92–12.13)			0.0312
PDN response	PPR (37)	PGR (73)				PPR (37)	PGR (73)			
	0.49 (0.13–1.50)	2.13 (0.74–6.60)			0.0045	3.3 (1.90–12.27)	6.83 (2.20–23.66)			0.5758
CNS	Positive (6)	Negative (101)				Positive (6)	Negative (101)			
	6.31 (1.35–9.97)	0.71 (0.17–3.70)			0.0951	16.29 (2.92–31.31)	5.71 (2.35–19.37)			0.4684
MRD	SR (14)	MR (56)	HR (27)			SR (14)	MR (56)	HR (27)		
	1.11 (13.00–1.99)	0.65 (0.21–4.54)	0.93 (0.16–2.86)		0.9691	7.61 (3.14–22.95)	4.86 (1.65–19.32)	3.73 (1.9–16.25)		0.3565
Risk group	SR (13)	MR (48)	HR (50)			SR (13)	MR (48)	HR (50)		
	1.03 (0.13–1.42)	0.49 (0.17–4.06)	1.13 (0.26–4.29)		0.4728	10.11 (3.43–22.95)	5.11 (1.92–19.32)	4.72 (2.42–18)		0.3572

Abbreviations: BCP-ALL, B-cell precusor acute lymphoblastic leukemia; CNS, central nervous system; HR, high risk; MR, medium risk; MRD, minimal residual disease; ND, no data; PDN, prednisone; PGR, prednisone—good responder; PPR, prednisone—poor responder; SR, standard risk; T-ALL, T acute lymphoblastic leukemia; WBC, white blood count. Note: When the number of observations is less than four, the actual values of hERG1A and hERG1B are given. Levels of *hERG1A* and *hERG1B* mRNA expression in pediatric BCP-ALL ($n=94$) and T-ALL ($n=111$) patients in different subgroups measured by SYBR Green RQ-PCR. Levels of the *hERG1A* and *hERG1B* transcripts were normalized to levels of the corresponding transcript in normal CD19+ cells or CD3+ cells. Statistical analyses were performed with R. Values reported in each column are referred to the median value (1st–3rd quartile). The healthy donor specimens used as calibrator in RQ-PCR experiments are a pool of five healthy buffy coats. CD19+ cells or CD3+ cells were sorted after pooling to be used as calibrators in BCP- or T-ALL analyses, respectively. RQ-PCR experiments were always performed in triplicate. BCP-ALL: total median *hERG1A:* 0.03 (0.01–0.08); total median *hERG1B:* 5.11 (1.42–18). T-ALL: total median *hERG1A:* 0.76 (0.17–4.08); total median *hERG1B:* 5.11 (2.00–19.98). BCP-ALL: <85% blasts median *hERG1A:* 0.04 (0.01–0.08) vs >85% blasts median *hERG1A:* 0.02 (0.01–0.06), $P=0.38$; <85% blasts median *hERG1B:* 6.12 (1.64–16.40) vs >85% blasts median *hERG1B:* 5.88 (1.52–17.6), $P=0.27$.

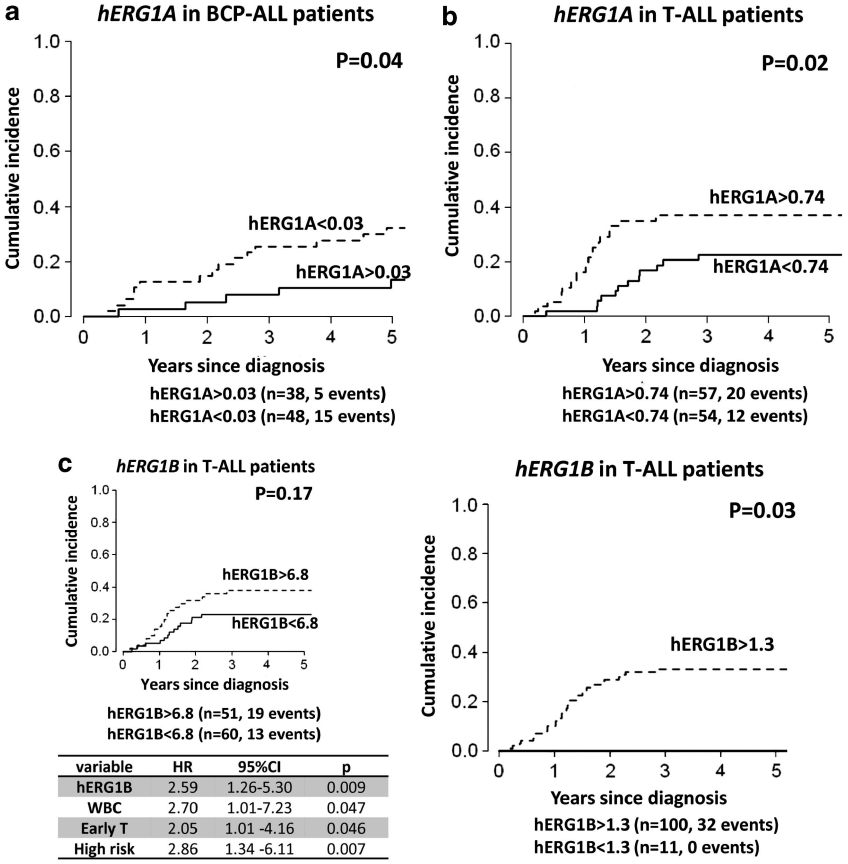
Notably, a lower *hERG1B* cutoff value of 1.3 identified a group of patients (with *hERG1B* < 1.3) with no relapse (Figure 1c, right panel, $P = 0.03$). In a limited set of four patients classified as early T-cell precursor leukemia (ETP-ALL), usually associated with a very high risk of relapse,[12] *hERG1B* expression was always higher than the cutoff value of 1.3 (Supplementary Table 2S).

In the present study we investigated, for the first time, the differential expression of the two main isoforms of hERG1 potassium channels, *hERG1A* and *hERG1B*, in ALL pediatric patients. hERG1 channels exert a relevant role in tumor biology,[6] and their use as prognostic markers in human malignancies is emerging.[5,13] In leukemias, hERG1 channels regulate either cell migration and transendothelial migration (in AML), or chemoresistance (in ALL). We previously reported that AML blasts from adult patients express both *hERG1A* and *hERG1B* transcripts[5] and that several leukemia cell lines preferentially express the *hERG1B* isoform.[10] However, no data regarding the prognostic relevance of the differential expression of the two hERG1 isoforms in leukemia patients have been reported so far. We show here that, in ALL blasts, either B or T lineage, the hERG1 transcript that is overexpressed compared with normal $CD19^+$ B or $CD3^+$ T cells is exclusively *hERG1B*. The genetic mechanisms underlying such overexpression could be related to the GpC islands and consensus sites for transcription factors,[14] which differentiate the *hERG1B* from the *hERG1A* promoter. Moreover, the *hERG1B*-encoded protein, hERG1B, has peculiar biophysical features, which makes hERG1B-sustained currents optimal to allow cell cycle progression in tumor cells.[10]

Notably, the two *hERG1* transcripts may have diagnostic and therapeutic relevance in ALL. In particular, the *hERG1A* transcript, which is generally hypo-expressed in both BCP-ALL and T-ALL, could identify groups of patients with a higher rate of relapse either when deeply downregulated (in BCP-ALL) or when slightly upregulated (in T-ALL).

The most relevant data provided here clearly show that, in the T-ALL cohort, the overexpression of *hERG1B* has a negative impact on outcome. Indeed, *hERG1B* expression displays a hazard ratio comparable to that of other factors used for patients' stratification in pediatric T-ALL. The high expression of *hERG1B* in ETP-ALL, although obtained in very few patients, further reinforces the negative impact of *hERG1B* on T-ALL outcome. In T-ALL no independent prognostic molecular marker, except Notch1 mutation profile,[15] has clinical relevance, and patients' stratification relies on MRD status and the T cell phenotype.[16] Hence, the expression of *hERG1B* isoform could be exploited for future stratification of pediatric T-ALL.

Finally, the *hERG1B* overexpression may have a therapeutic relevance, independently of the ALL immunophenotype, either B or T. In fact, we provided evidence that hERG1 blockers can overcome chemoresistance both *in vitro* and in leukemia mouse models.[7] However, several of the many hERG1 blocking drugs that are available on the market can cause severe cardiotoxicity. Hence, the preferential targeting of the hERG1B isoform could be an approach for overcoming such hindrances.[6] Indeed, we have recently provided evidence that a novel pyrimido-indole compound has a clear antileukemic effect as it preferentially



variable	HR	95%CI	p
hERG1B	2.59	1.26-5.30	0.009
WBC	2.70	1.01-7.23	0.047
Early T	2.05	1.01 -4.16	0.046
High risk	2.86	1.34 -6.11	0.007

Figure 1. Cumulative incidence of relapse in BCP-ALL and T-ALL patients according to the expression of *hERG1A* and *hERG1B*. Cumulative incidence of relapse was estimated by adjusting for competing risks (death) and compared using the Gray test. Statistical analyses were performed with R. (**a**) Cumulative incidence of relapse in BCP-ALL patients according to the expression of *hERG1A*. (**b**) Cumulative incidence of relapse in T-ALL patients according to the expression of *hERG1A*. (**c**) Cumulative incidence of relapse in T-ALL patients relative to the expression of *hERG1B*, according to different cutoffs (6.8 and 1.3) and multivariate analysis.

blocks hERG1B-sustained currents, with no adverse cardiac effect (Gasparoli L, unpublished results). This, or similar drugs, could hence be proposed for a patient's tailored therapeutic approach especially in nonresponsive pediatric T-ALL, such as ETP-ALL, with a high *hERG1B* expression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

SP, BA and VS performed the research and biological assays; PR performed and MGV supervised the statistical analysis and reviewed the manuscript; AA and SP designed the research and wrote the manuscript; GB contributed primary samples and edited the manuscript.

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REFERENCES

1 Redaelli A, Laskin BL, Stephens JM, Botteman MF, Pashos CL. A systematic literature review of the clinical and epidemiological burden of acute lymphoblastic leukaemia (ALL). *Eur J Cancer Care* 2005; **14**: 53–62.
2 Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med* 2006; **354**: 166–178.
3 Goldberg JM, Silverman LB, Levy DE, Dalton VK, Gelber RD, Lehmann L *et al.* Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium experience. *J Clin Oncol* 2003; **21**: 3616–3622.
4 Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. *Nature* 2006; **440**: 463–469.
5 Pillozzi S, Brizzi MF, Bernabei PA, Bartolozzi B, Caporale R, Basile V *et al.* VEGFR-1 (FLT-1), β_1 integrin and hERG K^+ channel form a macromolecular signaling complex in acute myeloid leukemia: role in cell migration and clinical outcome. *Blood* 2007; **110**: 1238–1250.
6 Arcangeli A, Pillozzi S, Becchetti A. Targeting ion channels in leukemias: a new challenge for treatment. *Curr Med Chem* 2012; **19**: 683–696.
7 Pillozzi S, Masselli M, DeLorenzo E, Accordi B, Cilia E, Crociani O *et al.* Chemotherapy resistance in acute lymphoblastic leukemia requires hERG1 channels and is overcome by hERG1 blockers. *Blood* 2011; **117**: 902–914.
8 London B, Trudeau MC, Newton KP, Beyer AK, Copeland NG, Gilbert DJ *et al.* Two isoforms of the mouse ether-a-go-go-related gene coassemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier K+ current. *Circ Res* 1997; **81**: 870–878.
9 Jones EM, Roti Roti EC, Wang J, Delfosse SA, Robertson GA. Cardiac IKr channels minimally comprise hERG 1a and 1b subunits. *J Biol Chem* 2004; **279**: 44690–44694.
10 Crociani O, Guasti L, Balzi M, Becchetti A, Wanke E, Olivotto M *et al.* Cell cycle-dependent expression of HERG1 and HERG1B isoforms in tumor cells. *J Biol Chem* 2003; **278**: 2947–2955.
11 Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grümayer R, Möricke A *et al.* Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. *Blood* 2010; **115**: 3206–3214.
12 Coustan-Smith E, Mullighan CG, Onciu M, Behm FG, Raimondi SC, Pei D *et al.* Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol* 2009; **10**: 147–156.
13 Lastraioli E, Bencini L, Bianchini E, Romoli MR, Crociani O, Giommoni E *et al.* hERG1 channels and Glut-1 as independent prognostic indicators of worse outcome in Stage I and II colorectal cancer: a pilot study. *Transl Oncol* 2012; **5**: 105–112.
14 Luo X, Xiao J, Lin H, Lu Y, Yang B, Wang Z. Genomic structure, transcriptional control, and tissue distribution of HERG1 and KCNQ1 genes. *Am J Physiol Heart Circ Physiol* 2008; **294**: 1371–1380.
15 Tosello V, Ferrando AA. The NOTCH signaling pathway: role in the pathogenesis of T-cell acute lymphoblastic leukemia and implication for therapy. *Ther Adv Hematol* 2013; **4**: 199–210.
16 Chiaretti S, Vitale A, Cazzaniga G, Orlando SM, Silvestri D, Fazi P *et al.* Clinico-biologic features of 5202 acute lymphoblastic leukemia patients enrolled in the Italian AIEOP and GIMEMA Protocols and stratified in age-cohorts. *Haematologica* 2013; **98**: 1702–1710.

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A common alternative splicing signature is associated with *SF3B1* mutations in malignancies from different cell lineages

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The RNA maturation is an important and complex biological process. It requires several small nuclear ribonucleoproteins (snRNPs) that comprise the two forms of spliceosomes. The major form of spliceosome (U2-type) is composed of U1, U2, U4/6 and U5 snRNPs, and catalyzes most splicing events in metazoans.[1]

Mutations of genes, such as *SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*, and to a lesser extent *SF1*, *SF3A1*, *U2AF2* or *PRPF40B*, encoding spliceosome compounds have been found to occur at high frequencies in myelodysplastic syndromes (MDS) and chronic lymphocytic leukemia (CLL).[2–4] Subsequently, *SF3B1* mutations were also found in solid tumors such as endometrial, lung, bladder, pancreatic and breast carcinomas and cutaneous melanomas.[5] We and others also reported that 15–20% of uveal melanoma (UM) carry

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