Mutations of *TP53* gene in adult acute lymphoblastic leukemia at diagnosis do not affect the achievement of hematologic response but correlate with early relapse and very poor survival

In adult acute lymphoblastic leukemia (ALL) the evaluation of clinical and biological conventional risk criteria at diagnosis is important but not sufficient to predict clinical outcome. The impact of *TP53* mutations has been investigated in a limited number of studies and has still not been defined. A study conducted on 98 newly diagnosed unselected adult ALL patients reported that *TP53* mutations were present in 8% of the patients and were associated to a poor response to induction therapy.[1] A more recent investigation described the mutational status of *TP53* gene in a wide cohort of heterogeneously treated childhood and adult ALL, also including mature/MYC positive B-ALL patients. Next generation sequencing (NGS) data revealed the presence of *TP53* mutations in 16% of patients and the incidence increased with age and in the presence of a hypodiploid karyotype. In all cases, *TP53* aberrations were independently associated with short survival.[2,3]

The aim of this study was to evaluate the impact of *TP53* genetic alterations, analyzed by NGS, on the outcome of a cohort of 171 adult Philadelphia-negative ALL [57 T lineage ALL (T-ALL) and 114 B-precursor ALL (Bp-ALL)], enrolled into the NILG-ALL 09/2000 clinical trial (*clinicaltrials.gov identifier: 00358072*).[4] Patients' characteristics are detailed in *Online Supplementary Table S1*). *TP53* mutational status was evaluated on a total of 171 DNA diagnostic samples isolated from mononuclear cells obtained from bone marrow or peripheral blood, containing at least 30% blasts. The experimental design for the amplification of exons 4-11 was defined in collaboration with the ALL working group of the IRONII consortium

Table 1. Clinical /biological characteristics of the patients mutated for *TP53* gene.

Clinical/biological characteristics at diagnosis							*TP53* mutation			Clinical outcome				
Patient ID	Age years/ sex	WBC	Phenotype	Molecular genetics	Risk class	Genomic mutation (NC_000017.10) and aminoacid	Mutation type change	Mutation load	Copy number (%)	CR after 1st induction	Relapse	CR duration therapy	Allo SCT (months)	Survival (months)
BG_4205	31/F	5.7	B-common	Normal	SR-B	g.7577121G>A p.R273C	Missense	79	1	Y	N	136.3+	N	137.5 +
BG_8345	33/F	3.9	B-common	Normal	SR-B	g.7577093 DUP(CGGTCTCT) p.R283Efs*65	Frameshift	63	1	Y	Y	1.8	N	3.7
BG_2873	37/M	11.54	pro-B	Negative*	HR-B	g.7577568C>T p.C238Y	Missense	45	2	Y	Y	4.6	N	11.1
BG_11584	38/F	634	pro-B	KMT2A-AFF1*	HR-B	g.7577094G>C p.R282G	Missense	4	2	Y	Y	1.8	Y§ (with disease)	6.6
BG_9445	46/M	3.08	B-common	Hypodiploid	HR-B	g.7577539G>A p.R248W	Missense	90	1	Y	Y	6.6	Y§ (CR2)	17.2
BG_5702	62/F	4.03	pro-B	Nearly triploid	HR-B	g.7578479G>T p.P151T	Missense	80	2	Y	Y	10.2	N	12.9
BG_4254	63/F	81.5	pro-B	Nearly triploid	HR-B	g.7578406C>T p.R175H	Missense	97	1	Y	Y	3.7	N	7.3
BG_10112	63/M	45	pro-B	KMT2A-AFF1	HR-B	g.7578433G>A p.S166L	Missense	37	2	Y	Y	2.5	N	7.5
BG_11543	63/F	3.31	B-common	Normal	SR-B	g.7577120C>T p.R274H	Missense	22	2	Y	Y	13.8	N	17.4
BG_2097	63/M	5.9	B-common	Complex	HR-B	g.7577139G>A p.R267W	Missense	7	2	Y	Y	5.8	N	8.5
BG_6490	64/M	11.3	pro-B	Nearly triploid	HR-B	g.7577117A>G p.V274A	Missense	90	UKN	Y	Y	14.7	N	18.6
BG_10442¥	44/M	30.5	cortical-T	Hyperdiploid	SR-T	g.7578195 DUP(CACC)/ g.7577597 INS (GGTCAAACCCC) p.P219Gfs*4/ p.C229Gfs*3	Frameshift	36/21	2	Y	Y	5	Y§ (CR2)	16.3
BG_8142¥	45/M	44	cortical-T	Complex	SR-T	g.7578457C> T/g.7578184G>A p.R158H/p.P222L	Missense	80/85	1	Y	Y	5	N	16.2
BG_8646	59/M	3.7	pre-T	Complex	HR-T	g.7577121G>A p.R273C	Missense	5	2	Y	Y	4	N	10.6

*WBC: white blood count (x10⁹/L); CR: complete remission; SCT: stem cell transplantation. Patients defined normal for molecular genetics proved negative for BCR-ABL1, KMT2A-AFF1 and TCF3-PBX1 fusion transcripts and have a normal karyotype. *For these patients only molecular analysis was performed and was negative for BCR-ABL1, KMT2A-AFF1 and TCF3-PBX1 fusion transcripts. §Patients died due to disease persistence or recurrence and not to transplant-related events. ¥Patients BG_10442 and BG_8142 showed two different mutations. Unfortunately, in both cases the alterations were not present in the same amplicon, thus it was not possible to establish if they were on the same allele. For patient BG_10442, we recognized an insertion of 11 nucleotides (g.7577597 INS(GGTCAAACCCC)) and a duplication of 4 nucleotides (g.7578195 DUP(CACC)). The computational analysis proved that, even if the aberrations were on the same allele, the reading frame would not be restored.*

Table 2. Univariate and multivariate analysis for clinical outcome.

	Univariate analysis						Multivariate analysis**			
	Cumulative Incidence of relapse				Overall survival		Cumulative Incidence of relapse (N=150; relapses=95)		Overall survival (N=165; deaths=111)	
Characteristics	CIR 4 years	Gray's test P	SHR (CI 95%)	Fine & Gray test P	OS 4 years	Log-rank test P	SHR (CI 95%)	Fine & Gray test P	HR (CI 95%)	Wald test P
Adjusted age*			1.07 (0.99-1.14)	0.0750		0.0007	0.99 (0.91-1.07)	0.720	1.09 (1.01-1.17)	0.0252
Sex										
Male	69%				33%					
Female	54%	0.0280			40%	0.3068				
WBC, x10^9/L										
0-30	54%	0.0001			48%	<0.0001	1.00		1.00	
> 30-100	69%				23%		1.96 (1.18-3.26)	0.0097	1.98 (1.25-3.15)	0.0039
>100	85%				10%		3.89 (2.10-7.20)	<0.0001	3.14 (1.90-5.18)	<0.0001
Hemoglobin, g/dL										
<10	68%				33%		1.00		1.00	
≥10	58%	0.0554			38%	0.0420	0.84 (0.52-1.35)	0.470	0.76 (0.51-1.13)	0.1747
Platelets, x10^9/L										
<100	66%				33%		1.00		1.00	
≥100	54%	0.0596			44%	0.0453	0.95 (0.55-1.64)	0.850	0.97 (0.59-1.60)	0.9018
Phenotype										
B	61%				39%					
T	67%	0.3994			29%	0.2841				
Hepatomegaly/ splenomegaly										
No	61%				38%					
Yes	64%	0.2820			33%	0.3748				
CNS involvement										
No	60%				38%		1.00		1.00	
Yes	–	<0.0001			10%	0.0040	7.64 (4.08-14.34)	<0.0001	3.38 (1.63-6.99)	0.0010
Cytogenetics/ molecular genetics#										
Normal/not adverse	55%	0.0047			46%	0.0045	1.00		1.00	
Adverse	75%				19%		1.32 (0.71-2.45)	0.380	1.31 (0.77-2.21)	0.3190
Not evaluable	73%				26%		1.56 (0.94-2.59)	0.087	1.31 (0.83-2.05)	0.2430
TP53 copy number										
2	65%	0.4748			34%	0.5567				
1	80%				30%					
3	–				–					
TP53 mutation										
No	60%				38%		1.00		1.00	
Yes	93%	<0.0001			7%	0.0013	5.03 (2.34-10.83)	<0.0001	2.80 (1.45-5.38)	0.0021

*Centered on the median and rescaled (divided by 5 years). **Variables included in the multivariable model: adjusted age, white blood cell (WBC) count, platelets, central nervous system (CNS) involvement, cytogenetics/molecular genetics and TP53 mutation. #Cytogenetics/ molecular genetics. Adverse; t(4;11) or positivity for KMT2A-AFF1, +8, near triploidy, low hypodiploidy, complex, del6q; not adverse: any other alteration not comprised in adverse category; CIR: cumulative incidence of relapse.

co-ordinated by the MLL Munich Leukemia Laboratory (Germany) and Roche Diagnostics (Germany).[5] Deep-sequencing was performed using a GS Junior Platform by Roche Diagnostics (Roche Diagnostics, Mannheim, Germany) following the manufacturer's recommendations and the *TP53* mutations were validated by conventional Sanger methodology. The copy number status of *TP53* was also evaluated by a quantitative PCR (qPCR) method using hTERT as reference gene for 158 patients as described by the manufacturer (Applied Biosystems, Foster City, CA, USA). Sequencing data analysis and SNP analysis were performed as described in the *Online Supplementary Appendix*. More details also on statistical and outcome end point analysis are provided in the *Online Supplementary Appendix*.

The NGS analysis of the *TP53* gene allowed identification of 25 different genetic variations present with an allele burden ranging between 97% and 4%, indicating that these alterations can be present at diagnosis also in a minority of leukemic clones. Nineteen of the 25 mutations identified were located in the exonic region and 6 were intronic. All the intronic and 3 exonic variations were recognized as polymorphic variants in dbSNP and IARC databases (*Online Supplementary Appendix and Tables S2 and S3*).[6] The other 16 exonic variants were detected in exons 5-8 corresponding to the DNA-binding domain which is the hotspot region for *TP53* mutations.[7] These mutations were of different types: 13 were single nucleotide variants (SNV), 2 were duplications (one of 4 and the other of 8 nucleotides) and one was an 11 nucleotides insertion (Table 1). The 13 SNV, identified in 12 patients, were missense mutations. The 3 frameshift

mutations identified in 2 samples were not described in the IARC database[6] and led to the introduction of a premature stop codon (Table 1). All the alterations with a mutation load higher than 25% were validated by Sanger sequencing.

The *TP53* copy number status was investigated by qPCR in patients with available DNA material. The analysis revealed that 10 of 158 (6.3%) patients presented one copy of the *TP53* gene. Five of these 10 patients belong to the cohort bearing also a *TP53* mutation (38.5%), demonstrating a strong correlation between these two genetic alteration (P=0.00049), as previously described.[2] However *TP53* deletion has no impact on clinical outcome. Furthermore, 2 cases (1.3%) showed 3 copies of the *TP53* gene. In 9 patients, qPCR results were also confirmed by SNP analysis. Interestingly, patient BG_4205, affected by p.R273C mutation, showed a *TP53* monosomy at diagnosis revealed by qPCR and SNP analysis and suffered the insurgence of two other different cancers during the clinical follow up. In this patient, Sanger sequencing showed the presence of the p.R273C mutated allele also in a sample from hematologic remission with a similar load of wild-type allele (*Online Supplementary Figure S1*). This finding demonstrated that the alteration was a germline mutation described to be associated with a severe tumor predisposition.[6]

Overall, we detected *TP53* mutations in 14 of 171 ALL patients, corresponding to an 8% incidence. This is similar to data previously published in a cohort of adult ALL, with a comparable median age,[1] but lower than the cohort published by Stangel *et al.*, which was characterized by an older median age and included also mature B-ALL.[2] These aberrations were recognized with a higher frequency in Bp-ALL (9.7%) than in T-ALL (5.3%), as reported in the study by Stengel *et al.*,[2] but not in the study by Chiaretti *et al.*[1] This discrepancy can be partially explained by the unselected and prospective collection of our specimens. Furthermore, we analyzed Bp-ALL and T-ALL, excluding Ph$^+$ ALL, whereas other cohorts also included Ph$^+$ ALL.[1,2]

The univariate analysis indicated a clear relationship with a linear trend between the presence of *TP53* mutations and increasing age (P=0.00032) (*Online Supplementary Figure S2*). Moreover, within the limits of a small number of subjects analyzed, we found a high rate of positivity for *TP53* mutation in the nearly triploid cytogenetic subgroup of patients (P=0.0008) (*data not shown*). No other correlation with clinical features, such as gender, hemoglobin, white blood cell (WBC) count, platelets, percentage of blasts at diagnosis, and clinical risk class, emerged from this analysis, as previously reported.[1,2]

In our cohort of patients, median follow up was 20 months and the maximum follow up reached nearly 15 years. The clinical outcome of *TP53* mutated patients are summarized in Table 1. All patients carrying a *TP53* alteration entered clinical remission after induction therapy, but 93% of them suffered an early relapse (within 15 months from first remission). Indeed, the frequency of relapse in patients mutated for *TP53* was significantly higher than in wild-type ones (P=0.019). These data showed that the presence of a mutated *TP53* does not itself produce a primary resistance to the induction chemotherapy, but rather leads to a greater susceptibility to relapse. This finding differs to reports by Chiaretti *et al.* and we can only speculate that a different treatment intensity (such as those used in pediatric patients) could be the reason for such a discrepancy. Nevertheless, most of the *TP53* mutated patients of our cohort could not benefit from transplantation in first CR as a consolidation

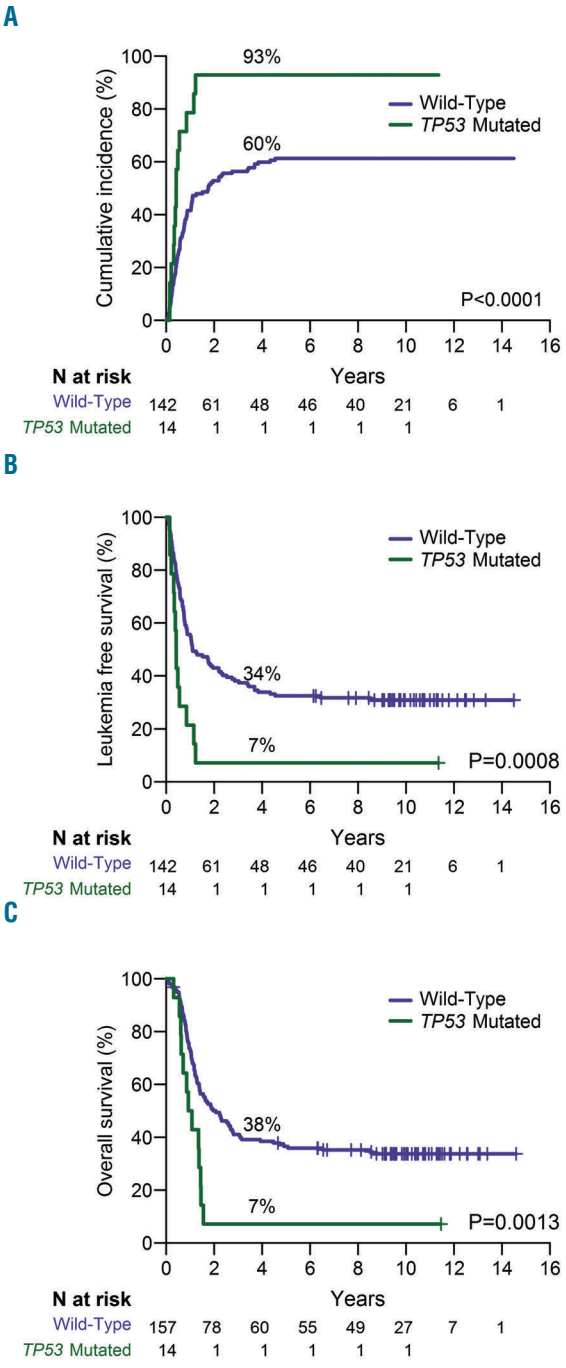

Figure 1. Analysis of the clinical outcome of the *TP53* wild-type *versus* *TP53* mutated patients. (A) Cumulative incidence of relapse. (B) Leukemia-free survival. (C) Overall survival.

therapy because of advanced age (>60 years) or a very early relapse, which did not allow completion of the induction/consolidation phase and the identification of a suitable donor (Table 1).

Globally, the cumulative incidence of relapse (CIR) was 63% (95%CI: 56%-71%) at four years while the leukemia-free survival (LFS) was 31% (95%CI: 25%-40%) at four years and the overall survival (OS) of the entire cohort was 36% (95%CI: 29%-44%) at four years. We observed that the CIR at four years was significantly higher in *TP53* mutated [93% (95%CI: 77%-100%)]

compared to wild-type [60% (95%CI: 52%-69%)] patients (P<0.0001) (Figure 1A). In agreement with this, both the 4-year LFS and OS were dramatically shorter in *TP53* mutated compared to wild-type patients: LFS was 7% (95%CI: 1%-47%) in mutated *versus* 34% (95%CI: 27%-43%) in wild-type patients (P=0.0008); OS was 7% (95%CI: 1%-47%) in mutated *versus* 39% (95%CI: 32%-47%) in wild-type patients (P=0.0013) (Figure 1B and C).

A univariate analysis was conducted to investigate the correlation of the clinical/biological characteristics of the patients and their outcome in terms of CIR and OS: the presence of the *TP53* mutation, age, WBC, central nervous system (CNS) involvement and adverse cytogenetics significantly correlate with a higher CIR and with a lower OS (Table 2). Interestingly, a correlation with minimal residual disease (MRD) status was not possible because, for reasons that are still unclear, in many *TP53* mutated patients, no informative clonal rearrangements of immunoglobulin or T-cell receptor genes could be found following the EuroMRD guidelines.[4,8-10] By multivariate analysis, age, WBC, CNS involvement and the presence of a *TP53* mutation at diagnosis proved to be independently associated with a worse clinical outcome in terms of OS and, except for age, also in terms of CIR (Table 2).

To our knowledge, this study is the first in which a large cohort of adult ALL patients with a long follow up (up to 15 years) enrolled in the same clinical protocol was analyzed for the presence of *TP53* mutations at diagnosis. Since the presence of *TP53* alterations at diagnosis defines a group of patients with a very poor outcome, the definition of the mutational status of this gene must be included in the diagnostic workup of adult ALL. The broad range of mutation load we found in our diagnostic specimens underlines the importance of evaluating samples with a highly sensitive technique, such as NGS methodologies, also at disease presentation.

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References

1. Chiaretti S, Brugnoletti F, Tavolaro S, et al. TP53 mutations are frequent in adult acute lymphoblastic leukemia cases negative for recurrent fusion genes and correlate with poor response to induction therapy. Haematologica. 2013;98(5):e59-61.
2. Stengel A, Schnittger S, Weissmann S, et al. TP53 mutations occur in 15.7% of ALL and are associated with MYC-rearrangement, low hypodiploidy, and a poor prognosis. Blood. 2014;124(2):251-258.
3. Muhlbacher V, Zenger M, Schnittger S, et al. Acute lymphoblastic leukemia with low hypodiploid/near triploid karyotype is a specific clinical entity and exhibits a very high TP53 mutation frequency of 93%. Genes Chromosomes Cancer. 2014;53(6):524-536.
4. Bassan R, Spinelli O, Oldani E, et al. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). Blood. 2009;113(18):4153-4162.
5. Kohlmann A, Martinelli G, Hofmann W-K, et al. The Interlaboratory Robustness of Next-Generation Sequencing (IRON) Study Phase II: Deep-Sequencing Analyses of Hematological Malignancies Performed by an International Network Involving 26 Laboratories. ASH Annual Meeting Abstracts. 2012;120(21):1399.
6. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat. 2002;19(6):607-614.
7. Prokocimer M, Unger R, Rennert HS, Rotter V, Rennert G. Pooled analysis of p53 mutations in hematological malignancies. Hum Mutat. 1998;12(1):4-18.
8. van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia. 2003;17(6):1013-1034.
9. Bruggemann M, Schrauder A, Raff T, et al. Standardized MRD quantification in European ALL trials: proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18-20 September 2008. Leukemia. 2010;24(3):521-535.
10. Pfeifer H, Cazzaniga G, Spinelli O, et al. International Standardization of Minimal Residual Disease Assessment for in Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia (Ph+ALL) Expressing m-BCR-ABL Transcripts: Updated Results of Quality Control Procedures by the EWALL and ESG-MRD-ALL Consortia. ASH Annual Meeting Abstracts. 2011;118(21):2535.