TP53 mutations are frequent in adult acute lymphoblastic leukemia cases negative for recurrent fusion genes and correlate with poor response to induction therapy

Acute lymphoblastic leukemia (ALL) is a disease of either B-cell (80-85%) or T-cell (20-25%) derivation. Several molecular aberrations (i.e. *BCR-ABL1, MLL/AFF1, SIL/TAL1* and *E2A/PBX1*) confer an overall poor outcome.[1,2] However, a proportion of patients do not carry known genetic abnormalities and have a heterogeneous clinical course.

P53 plays a crucial role in cell cycle regulation and apoptosis after DNA damage, and its role in tumorigenesis is well-recognized in solid and hematologic malignancies, particularly acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL), in which its deregulation represents an important predictor of poor outcome.[3-10]

In ALL, *TP53* mutations have been poorly investigated, mainly in children, for whom the incidence is low at diagnosis, increases at relapse, and is associated with poor outcome.[8-10] In adult ALL, the few studies performed include mostly relapsed cases and small cohorts of patients.[11,12]

We evaluated 98 newly diagnosed adult ALL cases to address the incidence and prognostic impact of *TP53* mutations; in 10 cases, paired material collected at relapse was also available.

Sixty-two cases had B-cell ALL (B-ALL) and 36 were T-ALL. Within B-ALL cases, 25 were *BCR-ABL1*, 9 *MLL/AFF1*$^+$ and 4 *E2A/PBX1*$^+$; within T-ALL cases, 7 were *SIL/TAL1*$^+$, 1 *NUP214/RAP*$^+$ and 1 *SET/NUP214*$^+$. The remaining 24 B-ALL and 27 T-ALL cases did not carry the above mentioned fusion genes (defined "negative for recurrent fusion genes").

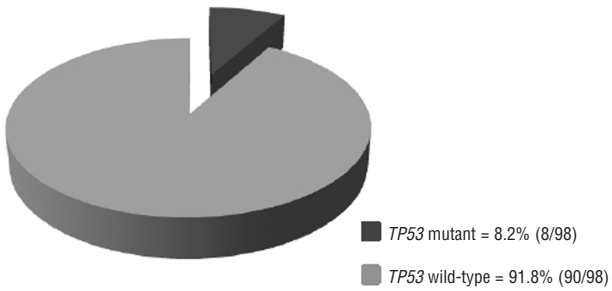
TP53 mutation analysis was performed using the AmpliChip p53 Research Prototype Test (Roche Molecular Systems Inc., Pleasanton, CA, USA).[13] Results were validated by Sanger Sequencing.[14]

Statistical tests were two-sided and $P \leq 0.05$ was considered significant. *P* values are not reported since significance was not reached because of the small number of *TP53* mutated patients. Further details are given in the *Online Supplementary Appendix*.

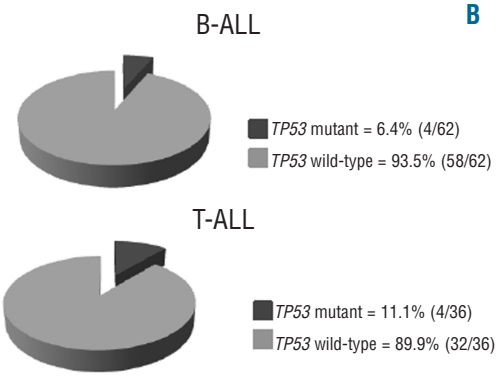
At diagnosis, *TP53* mutations were detected in 8 of 98 patients (8.2%) (Figure 1A), similar to figures reported in AML at diagnosis[5] and in CLL at the time of progression and first line treatment.[6]

The incidence of *TP53* mutated ALL appears slightly higher in adults than in children: in fact, while in childhood B-ALL the reported prevalence is 2%[8] in our cohort of 62 adult patients, 4 had a TP53 mutation (6.4%) (Figure 1B). In pediatric T-ALL,[9] *TP53* mutations were detected in 5% of cases, while in our cohort of 36 adult cases, 4 carried TP53 mutations (11.1%) (Figure 1B).

A



B



C

	TP53 mutant n=8	*TP53* wild-type n=90
Age at diagnosis (median)	30.12	36.42
Gender: M/F	6/2	54/36
White blood cell count x10^9/L (median)	52.03	57.05
Platelet count x10^9/L (median)	52.5	45
Immunophenotype		
B-ALL (n=62)	4 (6.4%)	58 (93.6%)
T-ALL (n=36)	4 (11.1%)	32 (88.9%)
B-ALL molecular aberrations		
MLL/AFF1 (n=9)	0 (0%)	9 (100%)
BCR/ABL (n=25)	1 (4%)	24 (96%)
E2A/PBX1 (n=4)	0 (0%)	4 (100%)
Negative for recurrent fusion genes (n=24)	3 (12.5%)	21 (87.5%)

D

	TP53 mutant (n=7)	TP53 wild-type (n=36)	Fisher's exact test
CR (n=30)	3	27	
Refractory (n=6)	3	3	0.059
Death in induction (n=7)	1	6	

Figure 1. Distribution of *TP53* mutations and clinical features of *TP53* mutated patients. (A) Distribution of *TP53* mutations in the whole cohort (n=98). (B) Distribution of *TP53* mutations in B-ALL (n=62) and in T-ALL (n=36). (C) Clinical and biological features of TP53 mutant and wild-type patients. (D) Correlation between *TP53* mutations and response to induction treatment.

Table 1. Details of *TP53* mutations identified at diagnosis.

Patient	Mutation type[a]	Exon	Codon	Base change	Genomic description[a]	Protein description[a]
20	Missense	7	237_2	T>C	g.7518296A>G	p.M237T
43	Missense	8	283_1	C>T	g.7517816G>A	p.R283C
55	Missense	7	254_1	A>G	g.7518246T>C	p.I254V
60	Missense	8	283_1	C>T	g.7517816G>A	p.R283C
71	Missense	7	248_2	G>A	g.7518263C>T	p.R248Q
106	Missense	7	248_1	C>T	g.7518264G>A	p.R248W
122	Missense	10	357_2	A>T	g.7514682T>A	p.K357M
132	Missense	5	179_1	C>A	g.7519120G>T	p.H179N

[a]*According to IARC TP53 mutation database: IARC TP53 reference NC000017.9*

Notably, all *TP53* mutated T-ALL cases were negative for recurrent fusion genes. Of the 4 B-ALL cases with *TP53* mutations, 3 did not harbor any recurrent fusion gene and one had a BCR-ABL1 rearrangement. Thus, a *TP53* mutation was found only in one of 38 (2.6%) molecularly positive cases. This contrasts with Hof and colleagues[10] who reported a significant association between *TP53* mutations and MLL/AFF1 in a cohort of relapsed children. The differences may be explained by the fact that the analysis was performed at disease recurrence.

TP53 mutations appear, therefore, the most frequent mutation in adult ALL negative for recurrent fusion genes.

All mutations affected the DNA-binding domain and known hotspots (Table 1). All but one were confirmed by Sanger sequencing; the reasons for this single discordance are not clear, but it is likely that the clone harboring the *TP53* mutation was too small to be detected using this technique.

The majority of *TP53* mutated patients were males, tended to be younger and had lower median white blood cell and higher platelet counts (Figure 1C).

Of the 8 *TP53* mutated cases at diagnosis, 4 (50%) failed to achieve complete remission (CR). Since most of the mutations (n=7) occurred in cases negative for recurrent fusion genes, a detailed response to induction chemotherapy was evaluated in this subset, including 51 patients. Forty-three patients were evaluable: 30 achieved a CR, 6 were refractory, and 7 died during induction therapy.

When response to induction was correlated with *TP53* status, 3 of 6 (50%) refractory cases harbored a *TP53* mutation, suggesting a negative impact on CR achievement (P=0.059); one further TP53 mutated patient died during induction therapy (Figure 1D).

Of the 3 *TP53* mutated patients who obtained a CR, 2 relapsed after 8 and 9 months, and one patient is in continuous CR 10 months later. Interestingly, this patient was enrolled in a pediatric-like regimen. Although the numbers are too small to draw definitive conclusions, it is tempting to speculate that more aggressive regimens may overcome *TP53* disruption. A larger series of cases is needed to conclusively document the impact of *TP53* mutations in adult ALL.

Paired genomic material at diagnosis and 1st relapse was available for 10 *TP53* wild-type at diagnosis (7 B-ALL and 3 T-ALL). Two patients acquired the mutation at relapse: one patient had a *BCR-ABL1*$^+$ B-ALL and the other a T-ALL: in one, ultra-deep sequencing of the diagnostic sample confirmed the absence of the mutation. Overall, 10 *TP53* mutations were identified in the current study: 8 at diagnosis and 2 at relapse. These results confirm previous reports[10-12] that *TP53* mutations increase at disease reappearance. The number of mutations at relapse is lower than that reported for childhood ALL.[12] This was probably because of sample selection criteria since in the pediatric series a backtracking of mutated cases was carried out, whereas in our study paired analysis only included *TP53* wild-type cases at diagnosis. Furthermore, we analyzed a small cohort of paired diagnosis-relapse cases.

In conclusion, to the best of our knowledge, this is the largest adult ALL series studied so far and the results indicate that *TP53* screening could be useful in a patient stratification algorithm for adult ALL. Furthermore, they indicate that *TP53* mutations are more frequent in T-ALL than in B-ALL and can be detected in 14% of cases negative for recurrent fusion genes, regardless of lineage derivation. Given the negative influence on response to induction chemotherapy, we suggest that *TP53* status should be investigated at diagnosis particularly in patients negative for recurrent fusion genes, where the genetic-based prognostic stratification is still limited.[15] This is particularly relevant considering that, nowadays, the therapeutic algorithms for adult ALL are progressively being tailored on the basis of specific molecular lesions. Prospective analyses on larger cohorts are warranted.

Sabina Chiaretti,[1] Fulvia Brugnoletti,[1*] Simona Tavolaro,[1] Silvia Bonina,[1] Francesca Paoloni,[2] Marilisa Marinelli,[1] Nancy Patten,[3] Massimiliano Bonifacio,[4] Maria Grazia Kropp,[5] Simona Sica,[6] Anna Guarini,[1] and Robin Foà[1]*

**Equal contribution*

[1]Division of Hematology, Department of Cellular Biotechnologies and Hematology, "Sapienza" University of Rome, Italy; [2]GIMEMA Data Center, GIMEMA Foundation, Rome, Italy; [3]Roche Molecular Systems, Inc., Pleasanton, CA, USA; [4]Department of Medicine, Section of Hematology, University of Verona, Italy; [5]Department of Oncology and Hematology, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, Italy; and [6]Department of Hematology, Catholic University of the Sacred Heart, Rome, Italy

The online version of this article has a Supplementary Appendix.

Funding: supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC), Special Program Molecular Clinical Oncology, 5 x 1000 Milan, Italy, the Ministero dell'Università e Ricerca (MIUR), Fondo per gli Investimenti della Ricerca di Base (FIRB), Rome, Italy, Fondazione Internazionale di Ricerca in Medicina Sperimentale (FIRMS), Torino, Italy, the Fondazione Buzzati-Traverso, Pavia, Italy, and the Compagnia di S. Paolo, Turin, Italy.

Acknowledgments: we are also grateful to Roche Molecular

Systems for providing part of the reagents and the AmpliChip p53 arrays. Finally, we are grateful to Dr. Irene Della Starza for part of the molecular analyses and Dr. Alfonso Piciocchi for providing help for part of the statistical analysis.

Correspondence: rfoa@bce.uniroma1.it
doi:10.3324/haematol.2012.076786

Key words: adult acute lymphoblastic leukemia, TP53 mutations, response to induction chemotherapy.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008;371(9617):1030-43.
2. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. J Clin Oncol. 2011;29(5):532-43.
3. Peller S, Rotter V. TP53 in hematological cancer: low incidence of mutations with significant clinical relevance. Hum Mutat. 2003; 21(3):277-84.
4. Lane DP. Cancer. p53, guardian of the genome. Nature. 1992;358 (6381):15-6.
5. Fenaux P, Preudhomme C, Quiquandon I, Jonveaux P, Laï JL, Vanrumbeke M, et al. Mutations of the P53 gene in acute myeloid leukaemia. Br J Haematol. 1992;80(2):178-83.
6. Zenz T, Eichhorst B, Busch R, Denzel T, Häbe S, Winkler D, et al. TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol. 2010;28(29):4473-9.
7. Wattel E, Preudhomme C, Hecquet B, Vanrumbeke M, Quesnel B, Dervite I, et al. p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood. 1994:84(9):3148-57.
8. Wada M, Bartram CR, Nakamura H, Hachiya M, Chen DL, Borenstein J, et al. Analysis of p53 mutations in a large series of lymphoid hematologic malignancies of childhood. Blood. 1993;82(10): 3163-9.
9. Kawamura M, Ohnishi H, Guo SX, Sheng XM, Minegishi M, Hanada R, et al. Alterations of the p53, p21, p16, p15 and RAS genes in childhood T-cell acute lymphoblastic leukemia. Leuk Res. 1999; 23(2):115-26.
10. Hof J, Krentz S, van Schewick, Körner G, Shalapour S, Rhein P, et al. Mutations and deletions of the TP53 gene predict nonresponse to treatment and poor outcome in first relapse of childhood acute lymphoblastic leukemia. J Clin Oncol. 2011;29(23):3185-93.
11. Tang JL, Tien HF, Lin MT, Chen PJ, Chen YC. Frequent p53 mutation in relapsed acute lymphoblastic leukemia with cytogenetic instability: a longitudinal analysis. Anticancer Res. 1998;18(2B):1273-8.
12. Zhu YM, Foroni L, McQuaker IG, Papaioannou M, Haynes A, Russell HH. Mechanisms of relapse in acute leukaemia: involvement of p53 mutated subclones in disease progression in acute lymphoblastic leukaemia. Br J Cancer. 1999;79(7-8):1151-7.
13. Chiaretti S, Tavolaro S, Marinelli M, Messina M, Del Giudice I, Mauro FR, et al. Evaluation of TP53 mutations with the AmpliChip p53 Research Test in chronic lymphocytic leukemia: correlation with clinical outcome and gene expression profiling. Genes Chromosomes Cancer. 2011;50(4):263-74.
14. Del Giudice I, Mauro FR, De Propris MS, Santangelo S, Marinelli M, Peragine N, et al. White blood cell count at diagnosis and immunoglobulin variable region gene mutations are independent predictors of treatment-free survival in young patients with stage A chronic lymphocytic leukemia. Haematologica. 2011;96(4):626-30.
15. Zhang J, Mullighan CG, Harvey RC, Wu G, Chen X, Edmonson M, et al. Key pathways are frequently mutated in high-risk childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood. 2011;118(11):3080-7.