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

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Funding: this work was partially supported by grants from the Associazione Italiana per la Ricerca contro il Cancro (AIRC-Investigator Grant 2014- code 16105) to AR and by the Associazione Italiana Lotta alla Leucemia (AIL) Bergamo – sezione Paolo Belli.

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doi:10.3324/haematol.2015.137059

SUPPLEMENTARY DATA

Supplementary Methods

SNP analysis on samples with unbalanced SNP distribution along TP53

Nine out of 171 patients were further investigated by a SNP analysis performed by Cytogenetics 2.7M array according to the manufacturer's instructions (Affymetrix, Santa Clara, CA, USA). This test provides whole genome coverage with a density of 2.7 million markers including 400 000 SNPs, with a mean physical distance of 2 kb between SNP. Results were analyzed using the analysis software Affymetrix® Chromosome Analysis Suite (Version 2.0.0.195 (r5758)). Copy number was determined based on the log 2 ratio of the signal intensity.

Sequencing and Sequencing Data Analysis

All data generated by the sequencing procedure were analyzed by GS Run Browser and GS Amplicon Variant Analyzer software provided by Roche Diagnostics using hg19, NC_000017.10, chr17: 7571720-7590863 sequence as reference. Based on the platform detection limits, we considered only variants above 4%, (1) which were input in dbSNP (2) and IARC (3) databases to investigate their biological consequences.

Statistical Analysis

Categorical data and continuous data were presented through absolute frequencies with percentages and median with range respectively. The association between clinical features and presence of *TP53* mutation was tested with χ^2 test or Fisher exact test for categorical variables, while non-parametric Mann-Whitney U test was applied with continuous variables. Cumulative Incidence of Relapse (CIR) was the primary end-point of the study; death without relapse was considered a competing event. In univariate analysis groups were compared using Gray's test, while Fine and Gray's models were applied in multivariate setting. Secondary end-points were Overall Survival (OS) and Leukemia Free Survival (LFS), assessed using Kaplan-Meier method and compared between risk groups with log-rank test. Multivariate analysis was performed with a Cox proportional hazard model and proportional hazard assumption was verified. All end-points were defined according to the EBMT

statistical guidelines (4). Reported p-values were two sided and the conventional 5% significance level was fixed. Statistical analysis was performed using R software (version 3.1.2) (5). The definition of clinical risk was based on known risk factors as previously described (6).

Ethical standards on human sperimentation

Our protocol was approved by the Ethical Committees of all participating centers. Written informed consent was obtained in accordance with the Declaration of Helsinki.

Supplementary Table 1 (S1): Clinical and biological characteristics of the patients

Characteristics	Evaluable	Positive (%)	Median (range)
Age, y	171		34.6 (15.6-64.8)
Male sex, no.	171	97 (57)	
WBC, 10^3/mcL	171		19.2 (0.4-900.0)
Hemoglobin, g/dL	171		10.0 (2.4-16.0)
Platelets, 10^3/mcL	171		48.0 (4.0-420.0)
Peripheral blood blasts, %	169		64 (0-100)
Bone marrow blasts, %	166		90 (30-100)
Hepatomegaly/Splenomegaly, no.	170	90 (53)	
CNS involvement, no.	168	10 (6)	
B or T	171		
T		57 (33)	
B		114 (67)	
Cytogenetics/molecular genetics	171		
Normal/Not adverse		96 (56)	
Adverse		31 (18)	
Not evaluable		44 (26)	
TP53 Mutation, no.	171	14 (8)	
TP53 Copy number	158		
1		10 (6)	
2		146 (93)	
3		2 (1)	
Complete Remission, no.	171	156 (91)	
Relapse, no.	156	100 (64)	
Death, no.	171	116 (68)	

Supplementary table 2 (S2): Intronic Polymorphisms

Intronic SNP	Location (intron)	Genotipic Description (NC_000017.10)	Trascriptional Description (NM000546.5)	Genotype Frequencies in Studied Population
rs1625895	6	g.7578115T>C	c.672+62A>G	TT: 0,035; TC: 0,328; CC: 0,637
rs12947788	7	g.7577427G>A	c.782+72C>T	GG: 0,840; GA: 0,154; AA: 0,006
rs12951053	7	g.7577407A>C	c.782+92T>G	AA: 0,840; AC: 0,154; CC: 0,006
rs1800899	9	g.7576841A>G	c.993+12T>C	AA: 0,970; AG: 0,030; GG: 0,000
rs17880847	10	g.7573897T>A	c.1100+30A>T	TT: 0,977; TA: 0,023; AA: 0,000
rs17881850	10	g.7573057G>A	c.1101-49C>T	GG: 0,977; GA: 0,023; AA: 0,000

Supplementary table 3 (S3): Exonic Polymorphisms

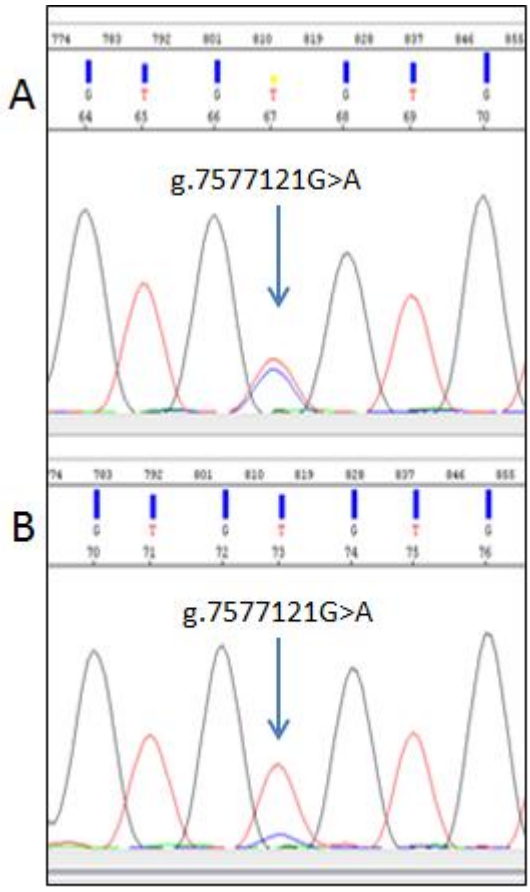
Exonic SNP	Location (exon)	Genotipic description (NC_000017.10)	Trascriptional description (NM_000546.5)	Protein description	Mutation Type	Genotype Frequencies in Studied Population
rs1042522	4	g.7579472G>C	c.215C>G	p.R72P	Missense	GG: 0,088; GC: 0,424; CC: 0,488
rs1800372	6	g.7578210T>C	c.639A>G	p.R213R	Silent	TT: 0,942; TC: 0,059; CC: 0,000
rs373710656	11	g.7572960G>A	c.1149C>T	p.L383L	Silent	GG: 0,988; GA: 0,012; AA: 0,000

All these polymorphisms are described both in dbSNP and in IARC database.

Polymorphisms rs1800372 and rs373710656 are characterized by single nucleotide changes that do not give an aminoacid modification thus not affecting protein functionality. The polymorphism rs1042522 is characterized by a G>C transvertion that causes the substitution of an arginine with a proline in position 72 (p.R72P). This variant is a very well described polymorphism associated with a possible predisposition to cancer development even though with conflicting data (7).

Supplementary Figure 1 (SF1)

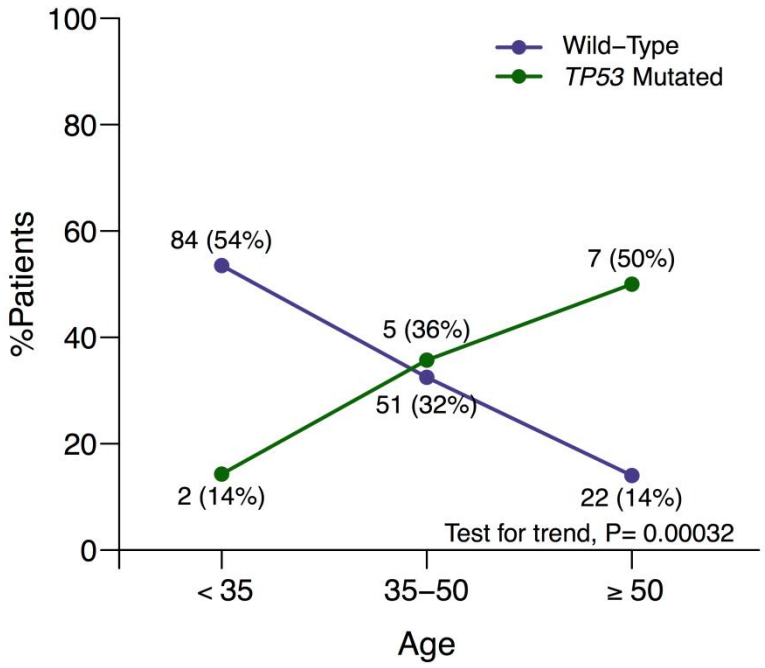
Supplementary Figure (SF1): Analysis of point mutation g.7577121G>A in patient BG_4205 on remission sample (A) and leukemia sample (B)



Panel A: The point mutation g.7577121G>A in the DNA sample of the clinical remission with a mutation load estimated around 50%. This experiment indicated that the mutation was germline.

Panel B: The point mutation g.7577121G>A in the DNA sample of leukemia diagnosis with a mutation load around 80%, as resulted in NGS analysis (graph not shown). The wild type allele was lost, as suggested by SNP analysis.

Supplementary Figure 2 (SF2)



Supplemantary Figure 2 (SF2): The correlation between the presence of *TP53* mutation and the increasing age is characterized by a linear trend. The figure also shows the reciprocal correlation between wild-type status and decreasing age. For the analysis the cohort was split in the following subgroups: teenage and young adult ALL (years<30), adult ALL (years 30-50) and older adult ALL (years>50).

Supplementary Bibliography

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