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ORIGINAL PAPER

# Telomerase reverse transcriptase (*TERT*) A1062T mutation as a prognostic factor in Egyptian patients with acute myeloid leukemia (AML)

**Salah Aref · Mohamed Sabry El-Ghonemy · Tarek Elsayed Abouzeid · Amr Mohamed El-Sabbagh · Mohamed Ali El-Baiomy**

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**Abstract** This study aimed to evaluate the incidence and clinical and prognostic impact of TERT A1062T mutation in AML patients treated at Mansoura Oncology Center. Screening for TERT A1062T mutation in exon 15 of the TERT gene was performed on diagnostic DNA samples from 153 AML patients and 197 healthy subjects as a control group by using sequence-specific primers. TERT A1062T mutation was detected in 18 cases out of 153 patients (11.8 %) and in one out of 197 control group subjects (0.51 %). The achievement of complete remission was significantly higher in AML group with wild type as compared to that in the mutant one (53.3 vs 16.7 %, respectively). In addition, the relapse rate was significantly higher in the mutant patients as compared to those with wild type (62.5 vs 28.2 %, respectively). The AML patients with TERT (A1062T) mutation had shorter overall survival than patients with wild type ($P = 0.001$). In a multivariable analysis, TERT (A1062T) mutational status is independently worse predictor factor ($P = 0.007$) when controlling for cytogenetic status ($P = <0.001$), performance status ($P = <0.001$) and bone marrow blast cells ($P = 0.001$). In conclusion, TERT A1062T mutation is an independent negative prognostic factor in AML patients. Therefore, molecular testing for TERT A1062T mutation in patients with AML is recommended in order to delineate their prognostic status.

**Keywords** AML · TERT A1062T · Mutation · Prognosis

S. Aref (✉) · M. S. El-Ghonemy
Hematology Unit, Clinical Pathology Department, Mansoura Faculty of Medicine, Mansoura, Egypt
e-mail: Salaharef@yahoo.com

T. E. Abouzeid
Medical Hematology Unit, Mansoura Faculty of Medicine, Mansoura, Egypt

A. M. El-Sabbagh
Medical Microbioloy and Immunology, Mansoura Faculty of Medicine, Mansoura, Egypt

M. A. El-Baiomy
Medical Oncology Unit, Mansoura Oncology Center, Mansoura Faculty of Medicine, Mansoura, Egypt

## Introduction

Acute myeloid leukemia (AML) or acute non-lymphoblastic leukemia (ANLL) is a clonal malignant disease of hematopoietic tissue that is characterized by abnormal proliferation of (leukemic) myeloblast cells principally in marrow and impaired production of normal blood cells. AML is a clinically and genetically heterogeneous disease that accounts for 20 and 70 % of acute leukemia in children and adults, respectively [1, 2]. The cytogenetic finding was considered as the cardinal marker for AML risk stratification. The somatic mutations that have been identified in the following genes (e.g., *NPM1*, *FLT3*, *DNMT3A, WT1*) are involved in the pathogenesis of AML and affect the prognosis of these patients [3]. In addition, it was reported that the mutations in telomerase complex genes are associated with AML and occur with a frequency of approximately 3–5 % [3–5].

Telomeres are complex structures capping the ends of all eukaryotic cell chromosomes. In vertebrates, telomeres consist of thousands of double-stranded tandem TTAGGG nucleotide repeats shielded by various proteins that seal the DNA structure [6–8]. The telomerase complex is expressed in highly proliferating cells, and is responsible for maintaining telomeres, which cap the ends of chromosomes and protect genomic DNA from eroding during cell division [7, 9]. Impaired telomerase function can result in extremely

short telomeres, which limit the proliferating capacity of progenitor cells, and can also lead to chromosomal instability, thus predisposing to malignant transformation [10, 11]. Loss of function mutations in telomerase complex genes reduces telomerase activity and can clinically manifest as bone marrow (BM) failure disease, which predispose to AML [5, 8, 12].

The aim of this study was to evaluate the incidence, clinical and prognostic impact of the most common *TERT* mutation A1062T in AML patients treated in Mansoura Oncology Center.

## Subjects and methods

### Patients and treatment protocols

- This study was conducted on two groups: the first group was 153 adult patients (17–65 years), consisted of 75 males and 78 females; diagnosed as AML on the basis of BM and peripheral blood (PB) morphology. The second group was 197 healthy subjects, consisted of 107 males and 90 females as a control group.
- Immunophenotyping (using Coulter Epics XL Flow cytometer PN 42372238 B, Coulter Corporation, Miami, Florida 33196, USA) for confirming diagnosis (Cyt. MPO, CD 13, CD 33, CD 117) primary panel for myeloid lineage (CD14, CD36, CD11b) for M4 and M5, (CD61, glycophorin A) for M6 and (CD41, CD42) for M7.
- The first group of 153 patients had de novo AML (2 M0, 15 M1, 45 M2, 53 M4, 20 M5, 15 M6 and 3 M7).
- All patients gave informed consent for both treatment and genetic analysis. All patients received intensive induction therapy (Cytarabine 100 mg/m$^2$/d for 7 days i.v. continuous infusion and Daunorubicin 90 mg/m$^2$/d for 3 days i.v.) consolidation therapy (Cytarabine 1 gm/m$^2$/12 h on the 1st, 3rd and 5th days with Daunorubicin 45 mg/m$^2$/d for 3 days i.v). The patients who achieved complete remission with poor risk cytogenetic or failed induction or relapsed were prepared of BM transplant.
- All patients were observed for 48 month or until death.

### Cytogenetic and molecular genetic analyses

- Pretreatment blood samples from all patients were studied by chromosome banding analysis to improve the accuracy of cytogenetic diagnosis.
- The specimens were also analyzed by fluorescence in situ hybridization for the presence of t(8;21)(q22;q22) for M2, t(15;17)(q22;q12) for M3 inv(16)(p13q22) for M4e or 11q23 for M5.

### Determination of *TERT* (A1062T) mutation in exon 15 of the *TERT* gene by SSP

DNA was extracted from PB or BM leukocytes, and polymerase chain reaction (PCR) amplification of the *TERT* (A1062T) mutation was performed with approximately 50 ng of genomic DNA, 1X QIAGEN Multiplex PCR Master Mix and 10 pmol of primers designed to flanking intronic regions: F (5′- CAAGGGCGCCACCGGCCCTC TG -3′), R (5′- CAGAGGGCCGGTGGCGCCCTTG -3′). The annealing temperature was 67 °C, and 35 cycles of amplification was performed.

### Statistical analysis

The statistical analysis of data was done by using excel program and SPSS version 16 (statistical package for social science). Qualitative data were described in the form of numbers and percentages. Quantitative data were described in the form of mean (±) standard deviation (SD). Statistical analysis was done by comparison between groups using chi-square test regarding qualitative data, while quantitative nonparametric data comparison was performed using one-way ANOVA and paired sample *t* test. The probability of being by chance (*P* value) was calculated for all parameters (*P* is significant if $<$or $=$ 0.05 at confidence interval 95 %).

## Results

### Prevalence of *TERT* (A1062T) mutation

- Our study was conducted on two groups: the first group was 153 adult AML patients (17–65 years); consisted of 75 males and 78 females. The second group was 197 healthy subjects consisted of 107 males and 90 females as a control group.

Screening for *TERT* (A1062T) mutation in exon 15 of the *TERT* gene was performed on diagnostic DNA samples from 153 AML patients and 197 control healthy subjects by using sequence-specific primers. *TERT* (A1062T) mutation was detected in 18 cases out of 153 patients (11.8 %) and one case out of 197 control group subjects (0.51 %).

### Patient characteristics in relation to *TERT* (A1062T) mutation status

Characteristics of the *TERT* (A1062T) mutant and wild-type cases are shown in Table 1. There was no difference between the two groups as regard sex, French–American–British subtypes, cytogenetic status (favorable or intermediate/adverse), hemoglobin concentration and platelet

**Table 1** Clinical and demographic characteristics of the 153 AML patients

| | Non mutant type | | Mutant type | | *P* value |
|---|---|---|---|---|---|
| | No | % | No | % | |
| FAB type | | | | | |
| M0 (No = 2) | 2 | 1.5 | 0 | 0 | 0.344 |
| M1 (No = 15) | 12 | 8.9 | 3 | 16.7 | |
| M2 (No = 45) | 41 | 30.4 | 4 | 22.2 | |
| M4 (No = 53) | 47 | 34.8 | 6 | 33.3 | |
| M5 (No = 20) | 16 | 11.9 | 4 | 22.2 | |
| M6 (No = 15) | 14 | 10.4 | 1 | 5.6 | |
| M7 (No = 3) | 3 | 2.2 | 0 | 0 | |
| Age | | | | | |
| <40 (No = 101) | 98 | 72.6 | 3 | 16.7 | <0.001 |
| ≥40 (No = 52) | 37 | 27.4 | 15 | 83.3 | |
| Sex | | | | | |
| Female (No = 78) | 72 | 46.7 | 6 | 33.3 | 0.11 |
| male (No = 75) | 63 | 53.3 | 12 | 66.7 | |
| WBC × 109/L | | | | | |
| <50 (No = 58) | 55 | 40.7 | 3 | 16.7 | 0.048 |
| ≥50 (No = 95) | 80 | 59.3 | 15 | 83.3 | |
| % BM blast | | | | | |
| <64 (No = 66) | 61 | 45.2 | 5 | 27.8 | 0.16 |
| ≥64 (No = 87) | 74 | 54.8 | 13 | 72.2 | |
| Extra-medullary disease | | | | | |
| Absent (No = 136) | 131 | 97 | 5 | 27.8 | <0.001 |
| Present (No = 17) | 4 | 4 | 13 | 72.2 | |
| Performance status | | | | | |
| 0 and 1 (No = 136) | 126 | 93.3 | 10 | 55.6 | <0.001 |
| 2 (No = 17) | 9 | 55.6 | 8 | 44.4 | |
| Cytogenitics | | | | | |
| Favorable (No = 71) | 66 | 48.9 | 5 | 27.8 | 0.092 |
| Intermediate/adverse (No = 82) | 69 | 51.1 | 13 | 72.2 | |
| Hemoglobin | | | | | |
| Mean ± SD | 7.6 ± 1.7 | | 8.5 ± 0.16 | | 0.23 |
| Platelets | | | | | |
| Mean ± SD | 51 ± 41.4 | | 43.8 ± 22.7 | | 0.587 |

$P < 0.05$ is significant, $P < 0.001$ is highly significant and $P > 0.05$ is not significant

count. On the other hand, there were statistical differences in age, extra-medullary disease and performance status.

Response to therapy and clinical outcome in *TERT* (A1062T) mutant patients

In univariable analysis, the patients with *TERT* (A1062T) mutation have less tendency to come in remission as compared to those with wild type (16.7 vs 53.3 %, $P = 0.003$) (Table 2). In addition, there was high incidence of relapse (62.5 vs 28.2 %) between *TERT* (A1062T) mutant and wild-type patients, respectively ($P = 0.045$) (Table 3). On the other hand, there was no marked difference between the two group in the rate of remission after the second cycle chemotherapy ($P = 0.12$) (Table 4). As regard the overall survival (OS), the patients with *TERT* (A1062T) mutation had shorter OS than patients with wild type ($P = 0.001$) (Fig. 1).

In a multivariable analysis (Table 5), *TERT* (A1062T) mutational status is independently worse predictor factor ($P = 0.007$) when controlling for cytogenetic status ($P = <0.001$), performance status ($P = <0.001$) and BM blast cells ($P = 0.001$).

## Discussion

In a previous study, the A1062T *TERT* mutation was the most common gene variant among the *TERT*-mutated

**Table 2** Response to therapy in *TERT* (A1062T) mutant and wild-type patients

| | | | Remission after 1st cycle | | Total | *P* |
|---|---|---|---|---|---|---|
| | | | Not in remission | In remission | | |
| *TERT* mutation | No mutation | Count | 63 | 72 | 135 | 0.003 |
| | | % | 46.7 | 53.3 | 100.0 | |
| | Mutation | Count | 15 | 3 | 18 | |
| | | % | 83.3 | 16.7 | 100.0 | |

**Table 3** The rate of relapse in *TERT* A1062T mutant and wild-type patients

| | | | Relapse* | | Total | *P* |
|---|---|---|---|---|---|---|
| | | | No relapse | Relapse | | |
| *TERT* mutation | No mutation | Count | 61 | 24 | 85 | 0.045 |
| | | % | 71.8 | 28.2 | 100 | |
| | Mutation | Count | 3 | 5 | 8 | |
| | | % | 37.5 | 62.5 | 100 | |

* Ninety-three patients out of 153 were evaluated for relapse because the rest of patients died before achieving CR due to disease or treatment related mortality

**Table 4** Response to 2nd cycle chemotherapy in patients who did not achieve remission after 1st cycle

| | | | Remission after 2nd cycle* | | Total | *P* |
|---|---|---|---|---|---|---|
| | | | Not in remission | In remission | | |
| *TERT* mutation | No mutation | Count | 37 | 26 | 63 | 0.12 |
| | | % | 58.7 | 41.3 | 100 | |
| | Mutation | Count | 12 | 3 | 15 | |
| | | % | 80 | 20 | 100 | |

* Seventy-eight patients did not achieve remission after 1st cycle induction, 29 patients of them achieved CR

AML patients, and its allele frequency was three times higher in patients than in controls [9]; however, their prognostic impact is not fully elucidated.

In this study, The A1062T *TERT* mutation was identified in 18 cases out of the 153 young adult AML patients (11.8 %) and one case out of 197 control group subjects (0.51 %). This mutation is highly correlated with AML cases ($P = < 0.001$). Calado et al. [9] reported similar findings. The percentage of cases in the patients group is slightly higher than that reported by Wagner et al. [13] who reported that the frequency of this mutation among AML cases was 3.3 %. The difference could be attributed to environmental factors [14].

Correlation of the demographic and laboratory data in the current studied cohort of AML patients revealed that there was no significant difference between the A1062T *TERT* mutant and wild type as regard sex, BM blast, hemoglobin concentration, platelet count and French–American–British subtypes. Calado et al. [9] reported similar findings.

Our study revealed that there was no significant difference in karyotypes/genotypes character between the mutant and wild-type patients ($P = 0.09$). On the other hand, the patients with *TERT* mutations had a trend toward less favorable karyotypes/genotypes (72.2 vs. 51.1 %) in intermediate and adverse karyotypes/genotypes between the mutant and wild-type patients, respectively. This is in agreement with Wagner et al. [13]. On the other hand, Calado et al. [9] demonstrated that there was a statistically significant association between mutation status and trisomy 8 ($P = 0.02$) and a marginal association with inversion 16 ($P = 0.08$).

In the current study, there was significant difference between the *TERT* mutant and wild-type patients regarding age, extra-medullary disease, performance status and WBC count. On the other hand, Wagner et al. [13] demonstrated

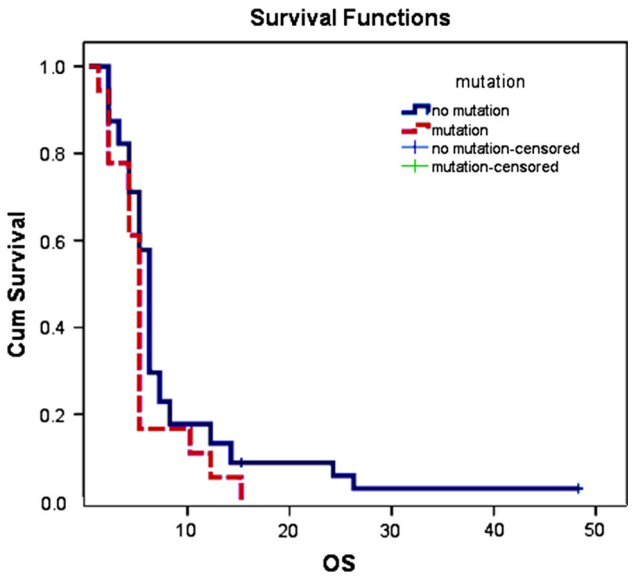


**Fig. 1** Overall survival (OS) in AML patients according to the TERT mutational status ($P = 0.001$)

**Table 5** Multivariate analysis of OS for all patients

| Prognostic factors | Hazard ratio | 95 % confidence interval (CI) | *P* value |
|---|---|---|---|
| Patient age | | | |
| <40 years | 1 | 0.6–1.3 | 0.58 |
| ≥40 years | 0.89 | | |
| TERT mutation | | | |
| No mutation | 1 | 1.3–6.6 | 0.007 |
| Mutation | 2.99 | | |
| Bone marrow blast cells | | | |
| <64 % | 1 | 1.2–2.5 | 0.001 |
| ≥64 % | 1.7 | | |
| Cytogenetic | | | |
| Favorable karyotypes | 1 | 2.8–6.1 | <0.001 |
| Intermediate/adverse karyotypes | 4.2 | | |
| Performance status | | | |
| Score 0 and 1 | 1 | 0.19–0.61 | <0.001 |
| Score 2 | 0.3 | | |
| Extra-medullary disease | | | |
| No extra-medullary disease | 1 | 0.4–2.1 | 0.93 |
| Extra-medullary disease | 1.03 | | |

that the clinical and molecular parameters did not differ from wild-type and mutant patients.

The effect of TERT mutation on the induction of remission rate was evaluated In this study, the AML patients with TERT mutation had lower remission induction rate when compared to those with wild-type patients (16.7 vs. 53.3 %, respectively) ($P = 0.003$). This finding was in agreement with the finding of Calado et al. [9] and Wagner et al. [13] who demonstrated that there was significant difference between the *TERT* mutant and wild-type patients and demonstrated the germ-line origin of mutations in patients from whom remission BM slides (<5 % blast cells) were available for genetic analysis. In addition, a high relapse rate was demonstrated in *TERT* mutant AML patients group as compared to those with wild-type patients (62.5 vs. 28.2 %, $P = 0.045$). On the other hand, there was no marked difference between the two group in the rate of remission after the second cycle chemotherapy ($P = 0.12$).

In univariate analysis, patients with *TERT* mutations had a significantly inferior OS as compared to that in patients with wild type (HR 2.99; 95 % CI 1.3–6.6, $P = 0.007$). In addition, in multivariate analysis, *TERT* mutation was an independent negative prognostic factor for OS. This is in agreement with the finding reported by Wagner et al. [13] who found that mutated patients showed a high rate of treatment related mortality and found that 33 % of the mutated patients died during induction therapy or in CR as compared to 15 % of the wild type patients ($P = 0.07$). In addition, they noted that three of four TERT-mutated patients who received allogeneic stem cell transplantation in first CR died in CR. In the TERT-mutated patients, (93 %) suffered from non-hematological/non-infectious grade 3 or 4 adverse events (mostly hepatic and/or mucosal) as compared to (53 %) in wild-type patients ($P = 0.006$).

The molecular role and mechanisms of TERT mutation in cancer patients have been emerged in several studies [3, 12, 15–18]. They stated that TERT mutation has a role in induction of genomic instability and predispose to cancer development.

In conclusion, *TERT* A1062T mutation is an independent negative prognostic factor in AML patients. Therefore, molecular testing for *TERT* A1062T mutation in patients with AML is recommended in order to delineate their prognostic status.

**Conflict of interest** The authors declare that there is no conflict of interest.

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