Efficacy and safety of decitabine combined with low-dose cytarabine, aclarubicin, and granulocyte colony-stimulating factor compared with standard therapy in acute myeloid leukemia patients with *TP53* mutation

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Tumor suppressor gene *P53* (*TP53*) is a critical tumor suppressor gene. The mutant p53 protein enhances the activity, invasion and metastasis of tumor cells. Acute myeloid leukemia (AML) patients with *TP53* mutations respond poorly to conventional chemotherapy and have poor clinical outcomes. We previously conducted a multi-center phase II clinical trial (No. ChiCTRONC-11001700) in elderly AML patients with decitabine, low-dose cytarabine, aclarubicin, and granulocyte colony-stimulating factor (G-CSF) (DCAG regimen), which showed an overall response rate (ORR) of 82.4% and a complete remission (CR) rate of 64.7%.[1] Given this satisfactory effect of DCAG regimen, the present study has been designed to compare the efficacy and safety of DCAG regimen with standard therapy in AML patients with *TP53* mutations.

Thirty-three patients with *TP53* mutations, diagnosed between September 2011 and July 2018, were enrolled in this study. Among them, 22 cases were confirmed with *TP53* mutations by targeted gene sequencing (the whole panel included 26 genes including *DNMT3A*, *IDH1*, *IDH2*, *ASXL1*, *RUNX1*, *NPM1*, *CEBPA*, *FLT3*, *KIT*, and *TP53*). The other 11 patients were confirmed with *TP53* mutations by Sanger sequencing. Giemsa R-banding method was used to detect the karyotype of metaphase bone marrow cells. Minimal residual disease (MRD) was monitored by flow cytometry. Diagnosis of AML was based on the World Health Organization (WHO) 2016 criteria, which contains subgroups of AML with recurrent cytogenetic abnormalities (AML-RCA), AML with myelodysplasia-related changes (AML-MRC), therapy-related AML (t-AML), AML, not otherwise specified (AML, NOS), myeloid sarcoma and myeloid proliferations associated with Down syndrome. Exclusion criteria were acute promyelocytic leukemia or other malignancies without remission.

This study was performed in accordance with the *Declaration of Helsinki*, and written informed consent was provided by all participants. This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (No. 2015-SRFA-009).

Twenty-one patients received DCAG therapy while 12 patients received standard induction treatment (idarubicin and cytarabine [IA], $n = 10$; fludarabine, high dose cytarabine, and G-CSF [FLAG], $n = 2$). DCAG: decitabine 15 mg/m^2 intravenously over 4 h for 5 consecutive days (day 1–5); cytarabine 10 mg/m^2 hypodermic injection q12h for 7 days (day 3–9); aclarubicin 10 mg/day for 4 days (day 3–6); and G-CSF 300 μg/day from day 0, which was discontinued if white blood cell (WBC) count $>20 \times 10^9$/L. Hydroxyurea was administered as rescue medication to control WBC count to $<5.0 \times 10^9$/L but was

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discontinued at least 24 h before decitabine treatment. IA: idarubicin 10 to 12 mg/m^2 intravenously for three consecutive days (day 1–3) and cytarabine 100 mg/m^2 intravenously for seven consecutive days (day 1–7). FLAG: fludarabine 30 mg/m^2 for 5 days (day 1–5); cytarabine 1 g/m^2 for 5 days (day 1–5); and G-CSF 300 μg/day from day 0 until WBC count $>20 \times 10^9$/L. The three patients who achieved CR after standard induction therapy were given 3 to 4 cycles of high dose cytarabine for consolidation. Among the 12 patients who achieved CR with DCAG, nine were given additional 2 to 4 cycles for consolidation, two received standard dose of cytarabine along with homoharringtonine or idarubicin due to absence of aclarubicin that time, and one was censored (lost to follow-up).

Treatment responses were assessed according to the National Comprehensive Cancer Network (NCCN) clinical practice guidelines of AML (version 3, 2020).[2] ORR included the rates of CR, CR with incomplete bone marrow recovery and partial remission (PR). Treatment-related early death was defined as death within 30 days of diagnosis during induction therapy. Overall survival (OS) was measured from the time of diagnosis to death due to any reason or censored on the last follow-up. The classification of adverse events (AEs) was judged according to the evaluation criteria of common AEs of the National Cancer Institute (CTCAE version 4.0). Neutrocytopenia duration was calculated from the time after absolute neutrophil count $<0.5 \times 10^9$/L to $\geq 0.5 \times 10^9$/L after the end of chemotherapy. Thrombocytopenia duration was defined as the interval between platelet count $<20 \times 10^9$/L and $\geq 20 \times 10^9$/L. The last follow-up was on May 10, 2019. Statistical analyses were performed using SPSS 21.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism 5 (GraphPad software, San Diego, CA, USA).

The median age of all patients was 66 (range: 27–86) years. There were 17 males and 16 females. The study cohort included three cases of AML-RCA, 11 cases of AML-MRC, three cases of t-AML, and 16 cases of AML, NOS. Patients in the DCAG group were significantly older than those in the standard therapy group (median age: 67.0 vs. 51.5 years, $P < 0.001$). The proportions of patients with AML-MRC and poor-risk karyotype in the DCAG group were significantly increased as compared to the other group (47.6% vs. 8.3%, $P = 0.038$; 81.0% vs. 41.7%, $P = 0.019$).

Among all the patients, three suffered treatment-related deaths (one in the DCAG group and two in the standard group), and 30 were available for final evaluation. In the DCAG regimen group, CR rate was 60% (12/20), PR rate was 10% (2/20), ORR was 70% and median OS was 8.7 months. Four patients receiving DCAG with intermediate-risk karyotypes had ORR of 100% (three CR and one PR), with a median OS of 13.8 months. Seventeen patients in the DCAG group had poor karyotype, of which one suffered therapy-related early death. Among the remaining 16 patients, nine achieved CR (56.3%) and one achieved PR (6.3%), with a median OS of 7.8 months. Among 12 patients in the standard treatment group, ten

received IA regimen (two suffered therapy-related early death) and two received FLAG therapy. The total CR rate of the patients in the standard treatment group was 30% (3/10), with an ORR of 30% (3/10), and a median OS of 4.2 months. Six patients in the standard treatment group had intermediate karyotype, of whom two suffered treatment-related early death. Three of the remaining four patients achieved CR (75%) and one developed non-remission (25%). The median OS was 22.1 months. Five patients had poor-risk karyotype, and none of them achieved CR, with the median OS of only 3.0 months.

The overall CR rate, ORR, and median OS [Figure 1A] in the DCAG group were higher than those in the standard treatment group, although the differences were not statistically significant. There were no significant differences in the CR rate, ORR or median OS between the two treatment groups with intermediate karyotypes [Figure 1B]. However, among the patients with poor karyotype, the CR rate, ORR, and median OS of the DCAG group were significantly superior to those of the standard treatment group ($P = 0.045$, 0.035, and 0.006, respectively) [Figure 1C]. Five patients in the two groups underwent a retest of the *TP53* mutation frequency after one course of treatment (the median interval between the two tests was 74 days, which was about 4 weeks after the end of the first cycle of DCAG). Mutations in *TP53* significantly reduced in variant allele frequency (VAF) in both groups [Figure 1D], suggesting that both regimens could effectively clear the *TP53* mutation clone. Nine patients achieved negative MRD (<0.1%) during therapy, of which five were in the DCAG group and four were in the standard treatment group. These patients with *TP53* clearance were verified as MRD-negative by flow cytometry.

The main AEs in the patients were cytopenia and secondary infections. During the treatment, one patient in the DCAG group died of secondary lung infection and respiratory failure during myelosuppression. Two patients in the standard treatment group suffered therapy-related early death. One died of soft tissue infection and septic shock, and the other died of lung infection and respiratory failure during bone marrow suppression. The incidence of infection in both groups showed no significant difference (85.7% vs. 100%, $P = 0.27$). All patients experienced grade 3–4 cytopenia. The median duration of neutrocytopenia in the DCAG group was 14 days (range: 0–28 days), with no significant difference ($P = 0.812$) compared with the standard treatment group (median: 13.5 days; range: 7–25 days). No significant difference ($P = 0.234$) was observed in the median duration of thrombocytopenia between the two groups (median: 12 days; range: 6–30 days vs. median: 17.5 days; range: 6–30 days). The gastrointestinal reactions and liver/kidney dysfunction of patients were grade 1–2 and could be tolerated by supportive treatment.

Decitabine is a specific DNA methyltransferase inhibitor, which can activate silent tumor suppressor genes. The functions of decitabine are not related to the p53 protein-dependent classical cytotoxicity-related effects; consequently, it can also induce differentiation of *TP53* mutant
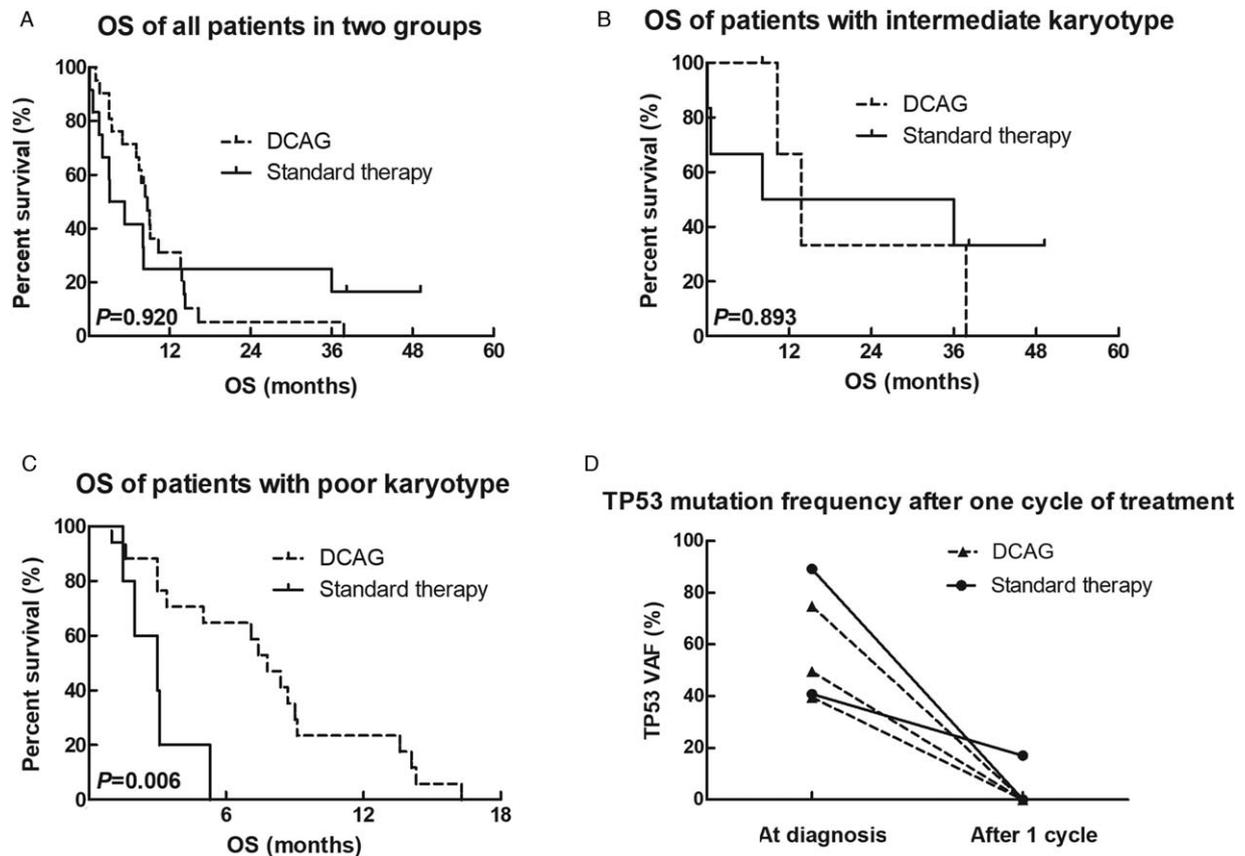
Figure 1: Survival curves of both DCAG group and the standard treatment group stratified by karyotype and the clearance of *TP53* clone after one course of treatment. (A) There was no significant difference in median OS between the DCAG group and the standard treatment group (*P* value 0.920). (B) There was no significant difference in median OS between the two treatment groups with intermediate karyotype (*P* value 0.893). (C) Among the patients with poor karyotype, the median OS of the DCAG group was significantly longer than that of the standard treatment group (*P* value 0.006). (D) A total of five patients in the two groups underwent tests of the TP53 mutation frequency both at diagnosis and after one course of treatment. The VAF of three patients in the DCAG group turned negative; one patient in the standard treatment group turned negative, and the other decreased from 40.83% to 17.01%. DCAG: Decitabine, low-dose cytarabine, aclarubicin, and granulocyte colony-stimulating factor; OS: Overall survival; *TP53*: Tumor suppressor gene P53; VAF: Variant allele frequency.

cells.[3] The inhibition of DNA methyltransferase by decitabine is mainly in the S phase and division phase of the cell cycle, and G-CSF can increase the killing effect of chemotherapy drugs on leukemia cells by promoting the G0/G1 phase leukemia cells to enter the S phase.[4] In addition, decitabine can enhance the cytotoxicity of cytarabine and exhibit additive or synergistic effects on the apoptosis of human leukemia cell lines *in vitro*.[5]

This small-cohort study found that DCAG regimen had relatively better efficacy in patients with *TP53* mutations. These findings need to be validated by prospective studies with a larger cohort of patients and a longer follow-up.

Funding

Conflicts of interest

None.

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