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Research paper

Comparison of anthracyclines used for induction chemotherapy in patients with *FLT3*-ITD-mutated acute myeloid leukemia

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ABSTRACT

This retrospective analysis compared anthracyclines (as part of an induction regimen) in 128 newly diagnosed *FLT3*-ITD-mutated AML patients. Induction regimens comprised high-dose daunorubicin (HD-DN; 90 mg/m^2/d × 3d; n = 44), standard-dose daunorubicin (SD-DN; 45 mg/m^2/d × 3d; n = 51), or idarubicin (IDA; 12 mg/m^2/d × 3d; n = 33) in combination with cytarabine (100–200 mg/m^2/d × 7d). Fifty-three patients showing persistent leukemia on interim bone marrow examination received a second course of induction chemotherapy comprising 2 days of daunorubicin (45 mg/m^2/d) or IDA (8 or 12 mg/m^2/d) in addition to 5 days of cytarabine. Complete remission (CR) rates were 77.3%, 56.9%, and 69.7% for HD-DN, SD-DN, and IDA, respectively (P = 0.101; HD-DN *vs.* SD-DN, P = 0.036; HD-DN *vs.* IDA, P = 0.453; IDA *vs.* SD-DN, P = 0.237). The HD-DN showed higher overall survival (OS) and event-free survival (EFS) than SD-DN and IDA: the differences between HD-DN and SD-DN (P = 0.009 for OS and P = 0.010 for EFS) were statistically significant.

Results of *in vitro* studies using *FLT3*-ITD-mutated cell lines supported these findings. In conclusion, HD-DN improved the CR rate, OS, and EFS of *FLT3*-ITD-mutated AML patients. HD-DN also tended to yield better outcomes than IDA, though the difference was not significant. The superiority of HD-DN over IDA should be confirmed in future studies.

1. Introduction

FMS-like tyrosine kinase 3 (*FLT3*) gene mutations are found in approximately 30% of newly diagnosed acute myeloid leukemia (AML) patients; internal tandem duplication (ITD) mutations in the juxtamembrane domain of *FLT3* account for two-thirds of *FLT3* mutations. Patients with *FLT3*-ITD mutations have a poor prognosis, with shorter remission duration and higher relapse rates than *FLT3* wild-type AML patients. Although several tyrosine kinase inhibitors (TKIs) targeting FLT3 improve clinical outcomes of *FLT3*-ITD-mutated AML, the median survival time of these patients is approximately 1 year. One of the major causes of treatment failure with TKIs targeting FLT3 is the lack of a durable and deep response, which is mainly due to acquired drug resistance [1,2].

There are several randomized studies of the benefits of increased doses of daunorubicin for induction chemotherapy for AML patients. In 2009, the results of E1900, which was conducted to investigate the role of daunorubicin intensification in young AML patients, showed that high-dose daunorubicin (HD-DN; 90 mg/m^2/day for 3 days) was associated with better complete remission (CR) rates and overall survival (OS) than standard-dose daunorubicin (SD-DN; 45 mg/m^2/day for 3 days) [3]. During a similar period, we also conducted a prospective study to compare two different doses of daunorubicin; we concluded that patients receiving HD-DN (90 mg/m^2/day) showed better outcomes than those treated with SD-DN (45 mg/m^2/day): in terms of higher CR rate and longer overall and event-free survivals [4]. Subsequent studies report the benefits of increasing daunorubicin exposure in *FLT3*-ITD-mutated AML patients. Recent long-term follow-up results from the E1900 trial revealed that HD-DN showed superior outcomes to SD-DN with regard to remission and overall and event-free survival in *FLT3*-ITD-mutated AML patients [5]. Subgroup analysis of UK NCRI AML17, which was originally conducted to compare daunorubicin doses of 90 mg/m^2 and 60 mg/m^2, revealed that *FLT3*-ITD-mutated AML patients benefited from 90 mg/m^2 rather than 60 mg/m^2

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daunorubicin (90 mg/m^2 yielded lower relapse rates and longer survival) [6]. In addition, we recently reported a phase 3 trial comparing idarubicin (IDA; 12 mg/m^2) with HD-DN (90 mg/m^2). HD-DN treatment of a subgroup of patients with *FLT3*-ITD mutation resulted in significantly longer survival than treatment with IDA [7].

Here, we performed a retrospective analysis to examine the effect of different types and doses of anthracyclines as a part of induction regimen on the response to induction treatment, relapse, and survival outcome of newly diagnosed *FLT3*-ITD-mutated AML patients.

2. Patients and methods

2.1. Patient population

Adult patients with AML harboring *FLT3*-ITD mutations diagnosed between January 2002 and September 2016 at the Asan Medical Center and who were treated with a standard 7 + 3 induction regimen were included in the analysis. A diagnosis was based on the definition of the World Health Organization classification [8]. The study protocol was approved by the Institutional Review Board of Asan Medical Center in accordance with the 2008 Declaration of Helsinki. Cytogenetic analysis was performed using standard techniques. Before treatment, all patients underwent a heart scan or transthoracic echocardiography to exclude cardiac dysfunction, which is a major contraindication to anthracycline use. Patients with acute promyelocytic leukemia were excluded from the analysis.

2.2. Treatment

All patients received cytarabine of 100 or 200 mg/m^2 daily for 7 consecutive days by continuous intravenous infusion and concomitant anthracycline for 3 days. Induction chemotherapy regimens were classified into three groups according to the type and dose of anthracycline: HD-DN (90 mg/m^2/day), SD-DN (45 mg/m^2/day), and IDA (12 mg/m^2/day). If interim bone marrow (BM) examination, which was performed between Days 14 and 21 of induction, showed residual leukemic blasts, patients received a second course of induction chemotherapy comprising cytarabine (100 or 200 mg/m^2 daily continuous intravenous infusion) for 5 consecutive days plus daunorubicin (45 mg/m^2) or IDA (8 or 12 mg/m^2 daily) for 2 days. Patients who achieved CR usually received either four to six courses of consolidation chemotherapy or allogeneic hematopoietic cell transplantation (HCT). Consolidation chemotherapy regimens included high-dose cytarabine (HDAC, 3 g/m^2 twice a day on Days 1, 3, and 5) or intermediate-dose cytarabine (1 g/m^2 for consecutive 5 days) plus etoposide. The consolidation chemotherapy was administered based on the protocols of two consecutive randomized trials which were performed between 2001 and 2014 [4,7]; all patients enrolled in the former study received HDAC and those who of the latter study received either HDAC or cytarabine plus etoposide, according to the cytogenetic risk. The patients who were not included in the randomized trials received consolidation treatment at the physician's discretion. Allogeneic HCT was performed in first CR patients at the discretion of the attending physician, usually after two courses of consolidation chemotherapy.

2.3. Detection and quantification of FLT3-ITD mutations

FLT3-ITD mutations were identified by polymerase chain reaction (PCR) and capillary electrophoresis. Briefly, genomic DNA was extracted from the BM mononuclear cells and primer sets were designed to detect the *FLT3*-ITD mutation. The sequences of the primers specific to FLT3 primers were as follows: forward, 5′-FAM-AGCA ATT TAG GTA TGA AAG CCA GCTA-3′; reverse, 5′-CTT TCA GCA TTT TGA CGG CAA CC-3′. The PCR products were diluted at a ratio of 1:30 and analyzed by capillary electrophoresis using an ABI 310 genetic analyzer (Biosystems Inc., Foster City, CA, USA) and GeneScan Analysis software. The results were presented as the ratio of *FLT3*-ITD mutant cells to *FLT3* wild-type cells. The lowest detection limit using this protocol was 5% [9].

2.4. Evaluation and statistical analysis

CR was defined according to standard criteria: < 5% blasts in BM aspirates, with hematologic recovery measured in terms of the absolute neutrophil ≥1000/μL and platelet ≥100,000/μL counts in the peripheral blood (PB). Relapse after CR was defined as re-appearance of leukemic blasts in the PB, ≥5% blasts in a BM aspirate not explained by any other cause such as recovering marrow after chemotherapy, or the presence of extramedullary disease. OS and EFS were calculated from the date of diagnosis. An event was defined as induction treatment failure, relapse from CR, or death from any cause. Relapse was evaluated in patients who achieved CR using a cumulative incidence function with respect to competing risks.

The Chi-square test was used to compare categorical variables, and the Mann-Whitney *U* test or Student *t* test was used to compare continuous variables. Multivariate analysis of CR rates was performed using multiple logistic regression analysis. Survival was calculated using the Kaplan-Meier method, and data were compared using the log-rank test (univariate analysis) or Cox regression (multivariate analysis). The cumulative incidence of relapse (CIR) was calculated and compared using the method of Gray [10]. Variables with a *P*-value < 0.1 in univariate analysis were selected for analysis in multivariate models. When allogeneic HCT was included in the Cox regression models, it was included as a time-varying covariate to prevent immortal time bias, which is the result of classifying patient time before HCT and time since HCT [11].

2.5. In vitro cell line studies

In vitro comparison of daunorubicin and IDA using six AML cell lines was conducted for experimental simulation of clinical situations. Three cell lines (MV4-11, MOLM-13, and MOLM-14) harbored *FLT3*-ITD mutations, and three (THP-1, HL-60, and U937) did not. MOLM-13 and MOLM-14 cell lines were purchased from DSMZ (Braunschweig, Germany), and the other cell lines were purchased from ATCC (Manassas, VA, USA). Cell viability was assessed using the luminescence-based CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA), according to the manufacturer's instructions. Cells were treated with two different doses of daunorubicin (50 nM and 100 nM) or IDA (10 nM and 20 nM) (Sigma-Aldrich, St. Louis, MO, USA). These concentrations were chosen from pharmacokinetic studies of daunorubicin *versus* IDA [12]. The peak plasma concentration after intravenous injection of daunorubicin at a dose of 45 mg/m^2 was 227 ± 116 ng/mL (around 500 nM), whereas that of IDA 10 mg/m^2 was 49 ± 15 ng/mL (around 100 nM). A one-tenth concentration of the peak plasma levels achievable from the administration of clinical doses of daunorubicin (45 or 90 mg/m^2) or IDA (10 or 20 mg/m^2) was selected. Cell viability was determined after 24 h. All cell viability experiments were performed six or nine times. For western blot analysis, cells were lysed with lysis buffer after exposure to daunorubicin or IDA for 12 h (Cell Signaling Technology, Beverly, MA, USA). Proteins were detected using antibodies specific for phospho-FLT3 (Tyr591), FLT3, poly(ADP-ribose) polymerase (PARP), cleaved caspase-3 (all from Cell Signaling Technology), and beta-actin (Sigma-Aldrich).

3. Results

3.1. Patient characteristics

Between January 2002 and September 2016, 128 patients were diagnosed with *FLT3*-ITD-mutated AML and received one of three induction regimens: 44 patients received HD-DN, 51 received SD-DN, and 33 received IDA. The median age of the patients in each group was 47.5