Phase I Study of Clofarabine in Adult Patients with Acute Myeloid Leukemia in Japan

Tatsuya Suzuki¹, Takahiro Yamauchi², Kiyoshi Ando³, Tadashi Nagai⁴, Kazuhiko Kakhana⁵, Yasuhiko Miyata⁶, Toshiki Uchida¹, Yasuhiro Tabata⁷ and Michinori Ogura¹,*

¹Nagoya Daini Red Cross Hospital, Nagoya, ²University of Fukui Hospital, Fukui, ³Tokai University Hospital, Isehara, ⁴Jichi Medical University Hospital, Shimotsuke-shi, ⁵Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, ⁶National Hospital Organization Nagoya Medical Center, Nagoya and ⁷Genzyme, Tokyo, Japan

*For reprints and all correspondence: Michinori Ogura, Department of Hematology and Oncology, Nagoya Daini Red Cross Hospital, 2-9 Myoken-cho, Showa-ku, Nagoya 466-8650 Japan. E-mail: mi-ogura@naa.att.ne.jp

Received July 10, 2013; accepted September 13, 2013

Objective: There are limited treatment options for relapsed/refractory acute myeloid leukemia patients or previously untreated elderly (≥60 years) patients with acute myeloid leukemia. In Phase II studies from the USA and Europe, single-agent clofarabine demonstrated activity and acceptable toxicity in elderly patients with previously untreated acute myeloid leukemia. This Phase I, multicenter study assessed the maximum-tolerated dose, safety, pharmacokinetics and efficacy of clofarabine in Japanese adults with acute myeloid leukemia.

Methods: Intravenous clofarabine (20, 30 and 40 mg/m²/day) was administered for 5 days to Japanese adult patients with relapsed or refractory acute myeloid leukemia or elderly patients with newly diagnosed acute myeloid leukemia.

Results: Fourteen patients, median age of 67.5 (59–72) years, were enrolled in this study. Eleven out of 14 patients had relapsed/refractory acute myeloid leukemia. Three patients received clofarabine at 20 mg/m², six at 30 mg/m² and five at 40 mg/m². Frequently reported treatment-related adverse events included thrombocytopenia (100%), anemia (93%), neutropenia (86%), nausea (86%), alanine aminotransferase increase (71%), headache (71%) and febrile neutropenia (57%). Three patients experienced reversible dose-limiting toxicities; two had increased alanine aminotransferase with 30 and 40 mg/m² and one had Grade 3 elevation of serum amylase with 40 mg/m². The maximum-tolerated dose was 30 mg/m²/day. Cmax and exposure area under the curve0–24h increased with increasing dose and were proportional to dose through the tested dose range. Among the 14 assessable patients, four (29%) achieved complete remission and two (14%) complete remission without platelet recovery. The overall remission rate was 43%.

Conclusions: These results demonstrate safety and preliminary, promising activity of clofarabine in Japanese patients with acute myeloid leukemia. Further investigation is warranted.

Key words: clofarabine – myeloid leukemia – acute – clinical trial – Phase I – aged

INTRODUCTION

Although the treatment of adult patients with newly diagnosed acute myeloid leukemia (AML) has improved, there are limited options for those with relapsed or refractory disease, and for elderly patients ≥60 years (1,2). The goal for treatment of patients with AML is to achieve complete remission (CR) because it has been shown to correlate with longer survival and better quality of life (3,4). Regimens including
PATIENTS AND METHODS

Patient Criteria

Eligible patients had a diagnosis of relapsed or refractory AML according to the fourth World Health Organization classification of myeloid neoplasms criteria (12) or were elderly patients (60–74 years of age) with previously untreated AML who were deemed to be unlikely to benefit from standard induction chemotherapy. Additional inclusion criteria included Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2; adequate hepatic [total bilirubin ≤ 1.5 × institutional upper limit of normal (ULN), aspartate aminotransferase (AST), alanine aminotransferase (ALT) ≤ 2.5 × ULN], renal (estimated glomerular filtration rate ≥ 60 ml/min/1.73 m²), pancreatic (serum amylase level ≤ 1.5 ULN, serum lipase level within normal limit) and cardiac [left ventricular fractional shortening on echocardiography (ECHO) ≥ 22% or left ventricular ejection fraction (LVEF) on ECHO or multigated acquisition (MUGA) scan ≥ 40%] functions.

Exclusion criteria included diagnosis of acute promyelocytic leukemia, prior HSCT, prior radiation therapy to the pelvis, uncontrolled infection, severe concurrent disease that was difficult to control by drug therapies and history of serious organ dysfunction. Pregnant and lactating women were also ineligible.

All patients provided written informed consent. This study was conducted in accordance with the principles stipulated by the Declaration of Helsinki and by the standards stipulated by Good Clinical Practice. The Institutional Review Boards at each participating institution approved the study.

TREATMENT PLAN

The study employed a 3 + 3 design (13). Three patients were enrolled in a dose cohort and assessed for DLTs during the first cycle. If none of the patients in a cohort experienced a DLT, three new patients were enrolled in the next dose cohort. If a patient developed a DLT, three new patients were added to that cohort for a total of six patients included in that cohort for tolerability assessment. If no additional patients developed a DLT, three new patients were enrolled in the next dose cohort. If two of the six patients developed a DLT, that dose level was deemed not to be tolerable. By definition, DLTs were any Grade 3 non-hematologic toxicities (except for Grade 3 pyrexia, anorexia, nausea, vomiting, malaise and transient changes in laboratory values related to hepatic function) and severe Grade 4 myelosuppression that persisted until Day 42 from the start of therapy (an absolute neutrophil count (ANC) < 500/mm³ and platelet count < 25 000/mm³ due to myelosuppression in the absence of persistent leukemia cell in bone marrow and peripheral blood). The MTD was defined to be the highest dose at which no more than one of six patients in the cohort developed DLTs during the first cycle.

Patients received once daily clofarabine 20 mg/m² in Cohort 1, 30 mg/m² in Cohort 2 and 40 mg/m² in Cohort 3 as a 1 h intravenous infusion for five consecutive days. Patients received one cycle, but patients with a hematologic remission after one cycle could receive up to a maximum of three cycles. Treatment had to be discontinued if patients did not achieve a CR or a CR without platelet recovery (CRp) after two cycles of treatment with clofarabine.

Patients in CR or CRp who did recover in peripheral blood cell count (ANC ≥ 1000/mm³ and platelet count ≥ 50 000/mm³ without the need for transfusion) from Days 57 to 84 proceeded to the next cycle with a 10 mg/m² dose reduction. Patients who did not show a recovery in peripheral blood cell count by Day 84 were withdrawn from the study. Only one dose reduction was allowed for delayed peripheral blood cell...
count recovery. All patients who developed myelosuppression that met the definition of DLT were withdrawn from the study.

Prophylactic administration of antibacterial drugs, antifungal agents and antiviral agents was recommended, but not mandated.

**Patient Evaluations**

To assess response, bone marrow assessments and peripheral blood smear tests were performed on Day 21 in Cycles 1 and 2. Follow-up continued for 45 days after the last dose. If the results of the bone marrow test were inconclusive for determination of response, the bone marrow tests were repeated every 7–14 days. Efficacy assessments were made by the investigator using the International Working Group criteria for diagnosis and treatment of AML (14).

Patients were hospitalized during the DLT evaluation period. An independent Safety Data Monitoring Committee oversaw the study. Toxicities were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE version 3.0).

**Pharmacokinetic Analyses**

Blood samples for pharmacokinetic assessments were collected from patients before, during and at various time points after drug administration on Days 1–5 of Cycle 1. Urine samples were collected from patients before and after drug administration on Days 1 and 5 of Cycle 1. All blood and urine samples were analyzed by MicroConstants, Inc. (San Diego, USA) for measurement of clofarabine concentrations.

Clofarabine pharmacokinetic parameters were estimated using the times of drug administration and blood collection. All parameters were calculated using industry standard software for pharmacokinetics analyses. The following plasma pharmacokinetic parameters were calculated using non-compartmental methods: area under the curve (AUC<sub>0–24</sub>) from time 0 up to 24 h calculated using the log trapezoidal method, the maximum observed plasma concentration (C<sub>max</sub>) and time to maximum observed concentration (T<sub>max</sub>). The relation of dose exposure with AUC<sub>0–24</sub> and C<sub>max</sub> was assessed using a Scatter plot.

**Statistical Analysis**

This was a Phase I dose-escalation study. The primary objectives of this study were to assess the MTD, safety and pharmacokinetics of clofarabine intravenously administered at 20, 30 and 40 mg/m<sup>2</sup>/day for 5 days to Japanese adult patients with AML.

All patients who received clofarabine for five consecutive days in Cycle 1 at each dose level or patients who experienced a DLT in Cycle 1 were assessed for the development of DLTs to evaluate the tolerability of clofarabine. The full analysis set, which comprised of all patients who received at least one dose of clofarabine, was used for the primary safety and efficacy analyses.

**Results**

**Patient Characteristics**

In total, 14 patients with a median age of 67.5 (range 59–72) years were enrolled at six study sites. Table 1 lists the patient demographics and baseline disease characteristics. Ten patients (71%) had refractory AML and most had an ECOG PS of 0.

**Treatment Exposure**

All enrolled patients (n = 14) received at least one dose of clofarabine: three patients in Cohort 1, six in Cohort 2 and five in Cohort 3. Overall, patients received a median of one (range 1–3) cumulative number of clofarabine cycles. Only two of 14 patients (14%) had a dose reduction or modification. One patient in Cohort 1 had a dose reduction due to neuropathy during the second cycle. A patient in Cohort 2 had the infusion time extended.

**Toxicity and MTD**

All patients experienced at least one adverse event (AE) regardless of the relationship to clofarabine treatment and all experienced at least one AE related to clofarabine treatment. Table 2 lists the treatment-related AEs experienced by at least three patients, according to the maximum NCI-CTCAE grade. The most frequently reported treatment-related hematologic AEs were thrombocytopenia (100%), anemia (93%), neutropenia (86%) and febrile neutropenia (57%). The most common treatment-related non-hematologic AEs were nausea (86%), headache (71%), increased ALT (71%) and increased AST (64%). Overall, the most common treatment-related Grade 4 AEs were thrombocytopenia (93%), neutropenia (86%), anemia (36%) and leukopenia (21%).

There was no major difference in the incidence of AEs or laboratory abnormalities between dose cohorts, with the exception of increases in Grade ≥3 of ALT and AST, which were reported more frequently in Cohort 3 than in the other cohorts (0, 17 and 100% in Cohorts 1, 2 and 3, respectively).

Three out of 14 patients experienced DLTs as assessed by the independent Safety Data Monitoring Committee. These DLTs included increased ALT in one patient in Cohort 2 (Grade 4) and prolongation of increased ALT in one in Cohort 3 (Grade 3) and Grade 3 elevation of serum amylase in one patient in Cohort 3. In this patient, the increase of laboratory test values was transient (reaching its worst level at Day 7 with recovery by Day 14) and occurred in the absence of any clinical symptoms suggestive of pancreatitis. Furthermore, the pattern of amylase isozyme values (for example, at Day 7, 43% for P-amylase and 57% for S-amylase) was not consistent with acute pancreatitis.

One patient in Cohort 2 and the one in Cohort 3 withdrew from the study due to DLTs. All the three patients recovered from their DLTs. Thus, the MTD was determined to be 30 mg/m<sup>2</sup>/day.

One patient experienced a serious (Grade 3) herpes zoster deemed to be possibly related to clofarabine treatment. Once...
diagnosed with this infection, this patient was admitted to the hospital and treated with antiviral medication and after discharge, he recovered from herpes zoster with neuralgia as a sequelae.

There were no deaths during the study period. Six patients died after the study follow-up period, five of these deaths were due to disease progression and were considered not study related. For one death (reported as aggravated pneumonia), the relationship to clofarabine treatment was considered to be unlikely. This patient (a 66-year-old man) experienced drug-related pneumonia during the study period which improved at study completion, 10 days later. The pneumonia was reported to have been controlled 5 days later when the patient began an alternative treatment with aclorubicin hydrochloride, cytarabine and lenograstim. Almost 3 weeks after starting this new treatment, the patient’s condition rapidly deteriorated and he died a week later. While the casual relationship between clofarabine treatment and the aggravated pneumonia that led to death appeared to be unlikely, it could not be ruled out given that no autopsy was performed.

**PHARMACOKINETICS**

After an intravenous infusion of clofarabine at doses of 20, 30 and 40 mg/m², maximum clofarabine concentrations were observed at the end of the infusion in the majority of patients (Table 3). $C_{\text{max}}$ and exposure $AUC_{0–24h}$ increased with increasing dose and were proportional to dose over the 20–40 mg/m² dose range tested (Fig. 1). The pharmacokinetics of clofarabine exhibited low-to-moderate inter-patient variability on both Days 1 and 5.

The elimination half-life ($t_{1/2}$) was not dependent on the dose or duration of treatment and was estimated consistently from each dose level, following either single or multiple dose regimens. Given the relatively short $t_{1/2}$ of clofarabine,
minimal drug accumulation was observed following daily intravenous infusion for 5 days.

**EFFICACY**

All 14 patients were included in the efficacy assessment (Table 4). Four patients (29%) achieved CR, two (14%) achieved CRp and none had PR, leading to an overall remission rate (CR + CRp + PR) of 43%. Table 5 lists the characteristics of responding patients. Two of the three newly diagnosed patients achieved CR and the other achieved CRp. Among the 10 patients with relapse/refractory AML, two (20%) achieved CR and one (10%) achieved CRp.

**DISCUSSION**

The results from this multicenter Phase I study of clofarabine monotherapy show the MTD to be 30 mg/m² administered...
intravenously daily for 5 days, and demonstrate preliminary but encouraging efficacy in 14 patients with AML, including 12 elderly patients. This MTD in Japanese patients is lower than the recommended Phase II doses of 40 mg/m² for adult patients and 52 mg/m² for pediatric patients with acute leukemia reported in two Phase I studies from the USA (13,15). However, this MTD of 30 mg/m² is identical to that used to treat elderly patients with AML in Phase II studies from Europe and the USA (10,11). The safety profile observed in the present study was similar to that reported in the previous studies.

One patient in our study experienced possibly drug-related Grade 3 herpes zoster; he recovered after treatment but withdrew from the study as per protocol. The study protocol recommended, but did not mandate prophylactic antiviral treatment. The use of prophylactic antiviral, antifungal and antibacterial agents in the USA (9,10) and EU (11) studies was as per institutional guidelines. Although cases of herpes zoster were not reported in these studies (10,11), prophylaxis against herpes is recommended for patients treated with T-cell depleting agents such as fludarabine (16), another purine analog, and should also be considered for patients undergoing clofarabine.

In the pharmacokinetic analysis, the C_{max} and AUC_{0–24h} of clofarabine increased dose proportionally over the dose range tested, with low-to-moderate inter-patient variability. The relative short t_{1/2} was independent of dose or treatment duration; there was minimal drug accumulation. The pharmacodynamic analysis indicated that clofarabine did not have a significant impact on blood pressure, heart rate and electrocardiography parameters. There was no evident relationship between clofarabine concentration and the pharmacodynamic end points tested.

In addition to a tolerable safety profile, the results from this study show that clofarabine appears to have promising clinical activity, producing four CRs and two CRp, for an overall remission rate of 43%. This overall efficacy rate is consistent with that reported in the aforementioned studies from Europe (48%) (11) and the USA (46%) (10). The efficacy results obtained with clofarabine monotherapy in the treatment of elderly patients in these different studies appear to be higher than the rates reported in other studies of monotherapies in similar patient populations. For example, complete remission rates for single-agent decitabine ranged from 26% in a large Phase II study to 18% in a randomized Phase III study comparing it with best supportive care or low-dose cytarabine (which together had an 8% CR + CRp rate) (17,18). A sub-group analysis of a randomized study reported an 18% CR rate for azacitidine monotherapy in elderly patients with low bone marrow blast count AML (19). The primary analysis of this randomized trial found a survival benefit for azacitidine compared with the conventional regimens in the treatment of patients with intermediate-2 and high-risk myelodysplastic syndromes (MDS) (20). In another study, low-dose cytarabine treatment was associated with an 18% complete remission rate in patients with AML and high-risk MDS considered to be ineligible for intensive chemotherapy (18, 21).

In summary, this Phase I study showed that clofarabine monotherapy is well tolerated and has preliminary, promising activity in Japanese elderly patients with newly diagnosed AML and adult patients with relapsed/refractory disease. Further clinical study in a larger group of patients is warranted.

Acknowledgements
We are very grateful to the patients and their families who participated in this study. We thank the following colleagues for their contributions to this study: Ryuzo Ohno, MD (medical expert); Shuichi Miyawaki, MD (coordinating investigator), Tomoki Naoe, MD (Safety Data Monitoring Committee), and Keizo Horibe, MD (Safety Data Monitoring Committee). We also thank Monica Nicosia, PhD, and Angela Partisano, PharmD, for assistance in preparing and editing the manuscript.

Funding
This work was supported by Genzyme Corporation (now Sanofi), Cambridge, MA, USA.

Conflict of interest statement
Yasuhiro Tabata is an employee of Genzyme Corporation (now Sanofi).
References