グループ(JALSG)において初発 CML-CP の治癒に向けて国際標準法による CMR の達成率をニロチニブとダサチニブで前方視的第Ⅲ相ランダム化試験にて比較することを目的とした。また、引き続き実施予定の薬剤中止試験への登録可能症例を蓄積することも目的とした。

B. 社会的意義

本試験によってどちらの薬剤がより効率に CMR を達成するのかが明らかになれば、CML 患者さんにとって薬剤選択の際の重要な情報となる。また、引き続き薬剤中止試験を実施する予定であるが、薬剤を中止することが可能になれば、高額な医療費が免除され、患者さんにとって利益が大きいのみでなく、社会的にも医療費の削減となり、社会的利益も大きい。

C. 試験の相とデザイン

初発 CML-CP に対するニロチニブとダサチニブの 18 ヶ月時点までの国際標準法による CMR の累積達成率を前方視的に比較する多施設共同の第Ⅲ相ランダム化比較試験。

D. 対象

16歳以上のECOG Performance Status(PS) 0～2で、肝、腎、心機能に重篤な合併症を有さない初発 CML-CP症例

E. 治療レジメン

対象症例をニロチニブ 300mg, 1 日 2 回投与(bid)群とダサチニブ群 100mg, 1 日 1 回投与(qd)群にランダム化割り付けする。その際、CMR 達成に最も影響する Sokal スコアについて両群で人数分布に偏りが生じないよう、Sokal スコアを層別化因子として用いる。
効果不十分例や不耐容例では、プロトコール治療中止とし、中止後の治療は規定しない。

F. エンドポント

1)プライマリーエンドポイント
ニロチニブ群とダサチニブ群における国際標準法による 18 ヵ月時点までの CMR の累積達成率
評価法：全割付症例を解析対象として Intention to treat 解析を行う。
2)セカンダリーエンドポイント
①両薬剤の安全性
②両薬剤の治療継続性
③両薬剤の治療効果
治療開始後 12, 18, 24, 36 か月時点での細胞遺伝学的効果、分子遺伝学的効果〔MMR, CMR, 2 回連続の CMR(Confirmed CMR)など〕、無増悪生存率(PFS)、無イベント生存率(EFS)、全生存率(OS)、治療開始後 12, 18, 24, 36 か月までの細胞遺伝学的効果、分子遺伝学的効果の累積達成率、European LeukemiaNet(ELN) 2009 の治療効果判定基準に基づく総合的治療効果、細胞遺伝学的、分子遺伝学的レスポンスまでの時間
④両薬剤の Sokal スコア、EUTOS スコア別の治療効果
⑤両薬剤投与時の *BCR-ABL* 遺伝子の点突然変異の出現と変異出現例の治療反応性
3)探索的エンドポイント
① 両薬剤のトラフ濃度と治療効果の相関性
② CML 細胞における網羅的遺伝子発現解析、全ゲノム（あるいは全エクソン）の塩基配列解析などによる異常の有無と治療反応性の関係
③ 正常細胞のゲノム DNA における治療抵抗性の背景となる異常や一塩基多型(Single Nucleotide Polymorphism, SNP)などの有無を全ゲノム（あるいは全エクソン）の塩基配列などの網羅的解析で明らかにする

G. 予定登録症例数と研究期間

1)予定登録症例数：450 例
2)予定登録期間：承認後 2 年半
3)追跡期間：登録後 36 ヶ月（全研究期間 5 年半）
本研究は、引き続き実施する薬剤中止試験の症例を蓄積することも目的としているため、36 ヶ月間を追跡期間とする。

**〔症例数決定の根拠〕**

初発CML-CP を対象としてニロチニブ 300mg, bid、ニロチニブ 400mg, bid とイマチニブ 400mg. qd の有効性をランダム化比較した ENESTnd において観察期間の中央値が 18.5 カ月時点での 18 ヶ月までの CMR の累積達成率はニロチニブ 300mg, bid 群で 21%、ニロチニブ 400mg, bid 群で 18%（イマチニブ 400mg, qd 群で 6%）であった。本試験の結果、ニロチニブ 300mg, bid 群はニロチニブ 400mg, bid 群に治療効果で劣らないことが確認された。同様に、ダサチニブ 100mg, qd とイマチニブ 400mg, qd をランダム化比較した DASISION では観察期間の中央値が 18 カ月時点での 18 ヶ月までの CMR の累積達成率はダサチニブ 100mg, qd 群で 13%(イマチニブ 400mg, qd 群で 7%)

であった。一方、MD Anderson 癌センターにおいて初発 CML-CP に対するニロチニブ 400mg, bid とダサチニブ 100mg, qd の第Ⅱ相試験がそれぞれシングルアームで実施されている。その結果、ニロチニブの試験では 18 ヶ月までの CMR の累積達成率は 21%、ダサチニブの試験では 18 ヶ月時点での CMR の累積達成率は 6%であった。これらの結果から、18 ヶ月までの CMR の累積達成率をニロチニブ 300mg, bid 群で 21%、ダサチニブ 100mg, qd 群で 9.5%と想定し、1 対 1 にランダム化し、「18 ヵ月までの CMR の累積達成率でニロチニブが優ること」を検出率(1-・)90%、・値 5%で stratified CMH (Cochran-Mantel-Haenszel) test を用いて両側検定するのに、1 群あたり 204 例が必要となる。脱落症例を約 10%見込むと、1 群あたり 225 例、両群併せて 450 例となる。

H. 観察・検査項目

本研究における保険適応外検査は日本血液学会が実施する新 TARGET の観察研究 1 のシステムを利用して、その観察スケジュールに基づいて実施する。従って、本研究に参加する施設は、本研究と新 TARGET の観察研究 1 の両方について施設の倫理委員会の承認を受け、両方の試験に症例を登録する必要がある。ただし、新 TARGET の登録は平成 25 年 3 月末で終了予定であり、新 TARGET の終了後は、本試験を独自で遂行する。

1)患者背景

身長、体重、性別、年齢、合併症、既往歴、CML 確定診断日、Sokal スコア、EUTOS スコア、ECOG PS を調査する。

2)治療内容

薬剤名、投与量および休薬期間を調査する。

3)血液検査

ヘモグロビン、白血球数、白血球分画、血小板数、血液生化学検査として AST(GOT)、ALT(GPT)、総ビリルビン(TB)、直接ビリルビン(DB)、アルブミン、アミラーゼ、リパーゼ、クレアチニン、Na, K, Cl, Ca, Mg, P、血糖値を観察・検査スケジュールに従って測定する。

なお、新 TARGET では、白血球分画、TB、DB、アミラーゼ、リパーゼ、Na, K, Cl, Ca, Mg, P、血糖値を検査項目としていないので、本研究では有害事象の捕捉のために、これらの検査も実施し、データを回収する。

4.骨髄検査

CCyR 未達成の患者に対しては、検査スケジュールに従って骨髄サンプルを採取し、G バンド法によって細胞遺伝学的効果の判定を行う。細胞遺伝学的効果が評価できない場合は、末梢血（好中球）を蛍光 *in situ* ハイブリダイゼーション（FISH）法で測定した検査データを代用してもよい。

5)末梢血（好中球）FISH

CCyR 未達成の患者に対して検査スケジュールに従って実施し、細胞遺伝学的効果の判定を行う。末梢血（好中球）を FISH 法にて測定する。CCyR 達成後は不要とする。

6) *BCR-ABL* 遺伝子発現レベル

末梢血サンプルを用い、株式会社ビー・エム・エルにおいて国際標準法であるMMD社のキットを用いて測定する。採血は、新TARGETの検査スケジュールに従って実施する。CMR用の解析はMMR達成症例について実施する。

7) *BCR-ABL* 遺伝子の変異解析

遺伝子変異解析は、ベースライン及び12ヶ月時点以外にPCR値が最低値から5倍以上増加した時点で実施可とする。末梢血サンプルを採取し、株式会社ビー・エム・エルにおいてダイレクトシークエンス法により*BCR-ABL*遺伝子のcodon 225-505における変異を解析する。

8)網羅的な遺伝子発現解析、塩基配列の解析

探索的研究として治療効果と遺伝子異常との関係を検討する。治療前の末梢血より RNA、ゲノム DNA を採取し、網羅的遺伝子発現解析、次世代シークエンサーを用いた全ゲノム（あるいは全エクソン）の塩基配列解析、SNP Array を用いた網羅的ゲノムの構造解析、遺伝子のメチル化領域の網羅的解析を行い、正常細胞をコントロールとし、CML 細胞における異常の有無を解析する。

また、MMR達成の次の採血時の末梢血10mlより得られる正常ゲノムDNAを採取する。プロトコール治療開始後も末梢血中にCML細胞が残存し、正常な血液細胞を採取できない場合には、治療抵抗性あるいは不耐容例ではプロトコール治療を中止する時点、プロトコール治療を継続しても21ヶ月までにMMRを達成できなかった症例については24ヶ月時点で、スワブにより頬粘膜より正常ゲノムDNAを採取する。これらの正常ゲノムDNAを用いて治療抵抗性の背景となる異常や一塩基多型(Single Nucleotide Polymorphism, SNP)などの有無を全ゲノム（あるいは全エクソン）の塩基配列などの網羅的解析で明らかにする。

9)血漿中薬剤トラフ濃度

維持投与量を 1 週間以上継続している患者においてニロチニブ、ダサチニブの血漿中薬剤濃度を測定

する。

10.胸部X線
登録症例全例で治療開始前に実施する。ダサチニブ群では、投薬開始1週間、2週間、1ヶ月後に実施し、胸水貯留、間質性肺炎などの有害事象がないことを確認する。

11.心電図
登録症例全例で、治療開始前、投薬開始1週間、2週間、1ヶ月後に実施し、QTc延長や不整脈の出現などの有害事象がないことを確認する。

12.将来の研究のための検体保存
本研究では、残余検体(RNA、ゲノムDNA)の保存について倫理委員会の承認を得られた施設において登録され、同意が得られている症例については、残余検体を匿名化状態で保存する。同意書には研究参加とは別に残余検体の保存についての確認項目を設ける。
〔残余検体〕
1. RNA
*BCR-ABL*遺伝子発現レベルなどの検査に用いた残りのRNA。
2.ゲノムDNA
探索的研究に用いたCML細胞、正常細胞の残りのゲノムDNA。
〔検体保存場所〕
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保管・管理責任者：麻生範雄

**【研究の進捗状況】**
本試験は、研究の実施組織であるJALSGのプロトコール委員会、運営委員会、検体保存委員会にて承認済みである。
また、現在、研究事務局である近畿大学医学部において倫理委員会の審査中であり、
平成24年4月より開始予定である。

# Ⅳ. 研究成果の刊行に関する一覧表

# 研究成果の刊行に関する一覧表

書籍

| 著者氏名 | 論文タイトル名 | 書籍全体の編集者名 | 書　籍　名 | 出版社名 | 出版地 | 出版年 | ページ |
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# 研究成果の刊行に関する一覧表

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# V. 研究成果の刊行物・別刷

Cancer Science
The official journal of the Japanese Cancer Association JCA

# Phase I trial of gemtuzumab ozogamicin in intensive combination chemotherapy for relapsed or refractory adult acute myeloid leukemia (AML): Japan Adult Leukemia Study Group (JALSG)-AML206 study

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(Received February 15, 2011/Revised March 28, 2011/Accepted April 5, 2011/Accepted manuscript online April 16, 2011/Article first published online May 18, 2011)

**In order to investigate better molecular-target therapy for acute myeloid leukemia (AML), we conducted a phase I trial of a combination of gemtuzumab ozogamicin (GO) with conventional chemotherapy. Between January 2007 and December 2009, a total of 19 adult Japanese patients with relapsed or refractory CD33-positive AML (excluding acute promyelocytic leukemia) were enrolled. All registered patients received a standard dose of cytarabine (Ara-C) (100 mg/m$^2$ × 7 days), combined with either idarubicin (IDR) (10–12 mg/m$^2$ × 3 days) or daunorubicin (DNR) (50 mg/m$^2$ × 3–5 days), and then GO (3–5 mg/m$^2$), which was administered 1 day after the last infusion of IDR (IAG regimen) or DNR (DAG regimen). While doses of both GO and IDR and the administration period of only DNR were increased, the dose-limiting toxicity (DLT) was assessed. Among 19 patients (nine in the IAG regimen, 10 in the DAG regimen), the median age was 59 years (range 33–64), and the relapsed/refractory ratio was 13/6. In the therapy using 3 mg/m$^2$ GO in the IAG or DAG regimen, grade 3/4 leukopenia and neutropenia were observed in all patients, but none had grade 3/4 non-hematological toxicities, except febrile neutropenia. Three patients in the IAG regimen who were administered 5 mg/m$^2$ GO showed DLT. No patients had veno-occlusive disease or sinusoidal obstructive syndrome. In conclusion, 3 mg/m$^2$ GO combined with Ara-C and IDR or DNR can be safely administered, and phase II trials should be conducted to investigate the clinical efficacy of the combination therapy. (*Cancer Sci* 2011; 102: 1358–1365)**

Current standard induction treatment for acute myeloid leukemia (AML) involves drug regimens with two or more agents that include an anthracycline or anthraquinone and cytarabine (Ara-C).[1–6] A recent clinical trial of the Japan Adult Leukemia Study Group (JALSG) for younger adult patients (16–64 years of age) with newly diagnosed AML showed a 77.9% complete remission (CR) rate.[4] Remission rates achieved by us and others range approximately 55–90% in adult patients, depending on the composition of the population treated.[1–6] However, these high CR rates did not always translate into improved outcomes for patients, mainly because approximately 40–50% eventually relapsed. Although there are various clinical trials for patients with relapsed or refractory AML, the probability of a second CR is approximately 50% in younger patients, but the duration of CR is nearly always much shorter than the first CR. No standard chemotherapy regimen provides a high rate and durable CR for patients with relapsed/refractory AML, and all such patients should be considered eligible for clinical trials if available.[7]

Among newer antileukemia agents being examined for the treatment of AML, an antibody to CD33 antigen is one of the most promising drugs. The CD33 antigen is expressed on 80–90% of AML blasts and acts as a target for antibody-mediated destruction. Gemtuzumab ozogamicin (GO) is a recombinant humanized anti-CD33 monoclonal antibody conjugated to calicheamicin (a cytotoxin), which is 1000 times as potent as doxorubicin.[8,9] This conjugated antibody is rapidly internalized and causes subsequent apoptosis.[10] GO was shown to be effective in patients with relapsed AML in nonrandomized studies and gained regulatory approval in the United States (the US Food and Drug Administration [FDA]) for relapsed older patients (older than 60) with AML.[11,12] GO was also approved by the Japanese government in 2005 for use in patients with relapsed/refractory AML, but only for monotherapy based on a phase I/II study for Japanese patients.[13] GO does not cause alopecia or mucositis, even though it causes myelosuppression, an infusional syndrome, and liver damage such as hyperbilirubinemia and/or hepatic transaminitis (or elevation of transaminase). Several studies have indicated that GO combined with conventional chemotherapy would provide a more potent antileukemia effect than GO monotherapy.[14–19] We considered that addition of GO to conventional chemotherapy in induction therapy would improve the clinical outcome of AML patients of all ages. To find the optimal usage of GO in combination with conventional chemotherapy for relapsed or refractory AML, we conducted a phase I study. Here we report the results of this JALSG-AML206 trial in adult patients with relapsed or refractory AML, younger than age 65, in which the dosage of GO, combined with our two types of standard remission induction therapy for de novo AML, were tested.[4]

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This study was registered at UMIN Clinical Trials Registry (http://www.umin.ac.jp/ctr/index-j.htm) as UMIN000001141 and UMIN000001142.


doi: 10.1111/j.1349-7006.2011.01957.x

## Materials and Methods

**Patient eligibility.** Between January 2007 and December 2009, 19 eligible patients with relapsed and refractory AML were enrolled in the present study. The inclusion criteria were as follows: (i) diagnosed as $CD33^+$AML (excluding acute promyelocytic leukemia); (ii) relapsed ≥6 months after the first CR (CR1) or were refractory to initial standard induction therapy; (iii) age: 20–64 years old; (iv) 0–2 by the Eastern Cooperative Oncology Group (ECOG) performance status; (v) no active double cancer; (vi) adequate cardiac, renal and hepatic function with left ventricular ejection fraction ≥50%, creatinine ≤2.0 mg/dL, bilirubin ≤1.5 mg/dL; (vii) no uncontrolled infection; and (viii) no human immunodeficiency virus (HIV) infection. Patients who received more than 500 mg/m$^2$ of daunorubicin (DNR) in a prior therapy were ineligible to DNR-including protocol. Cytogenetic abnormalities were grouped by standard criteria and classified according to the UK Medical Research Council (MRC) classification.[20]

**Study design.** The study was conducted by six designated institutions among JALSG members, and consisted of two parts: idarubicin (IDR), Ara-C plus GO (IAG regimen), and DNR, Ara-C plus GO (DAG regimen). The treatment schedules of both regimens are shown in Figure 1.

*IAG regimen.* The starting doses (level 1) consisted of IDR 10 mg/m$^2$ administered intravenously (d.i.v.) over 30 min daily for three consecutive days (days 1–3), Ara-C 100 mg/m$^2$ as a continuous intravenous infusion (c.i.v.) for seven consecutive days (days 1–7) and GO 3 mg/m$^2$ for 2 h d.i.v. on day 4. While the dose and schedule of Ara-C were fixed, doses of IDR and GO were increased in levels 2 and 3 as shown in Figure 1.

*DAG regimen.* The starting doses (level 1) consisted of DNR 50 mg/m$^2$ administered d.i.v. over 30 min daily for three consecutive days (days 1–3), Ara-C 100 mg/m$^2$ c.i.v. (days 1–7) and GO 3 mg/m$^2$ for 2 h d.i.v. on day 4. While the dose and schedule of Ara-C were fixed, doses of DNR and GO were scheduled to increase in levels 2, 3 and 4 (Fig. 1).

All patients were hospitalized during therapy and received optimal supportive care. For prophylaxis of GO infusion reaction, antihistamines and corticosteroids were given 1 h before the infusion. Granulocytopenic patients were placed in single rooms with conventional isolation or in laminar airflow rooms. Broad-spectrum antibiotics were given for fever higher than 38°C in the presence of granulocytopenia, and were continued until defervescence and recovery of granulocyte counts above $0.5 \times 10^9$/L. Random donor platelet concentrates were administered to maintain a platelet count above $20 \times 10^9$/L. Packed red blood cell (RBC) transfusions were performed to maintain hemoglobin above 7.0 g/dL.

**Response criteria.** Responses were evaluated according to the recommendations of the International Working Group.[21] A CR was defined as disappearance of all clinical and/or radiological evidence of disease with ≤5% marrow blasts, neutrophil (ANC) count $\geq 1 \times 10^9$/L and platelet (PLT) count $\geq 100 \times 10^9$/L. A CR without PLT recovery (CRp) had identical marrow results and ANC recovery as for CR, but with PLT $<100 \times 10^9$/L and $\geq 20 \times 10^9$/L. Partial remission consisted of a peripheral blood recovery as for CR, but with a decrease in marrow blasts of ≥50% compared with baseline before therapy, and not more than 6–25% blasts in the marrow. All other responses were considered failures. After the IAG or DAG treatment, patients received the most appropriate AML therapy determined by their individual physicians.

**Adverse events/toxicities.** During the entire period of induction, blood cell counts were performed daily and liver and renal

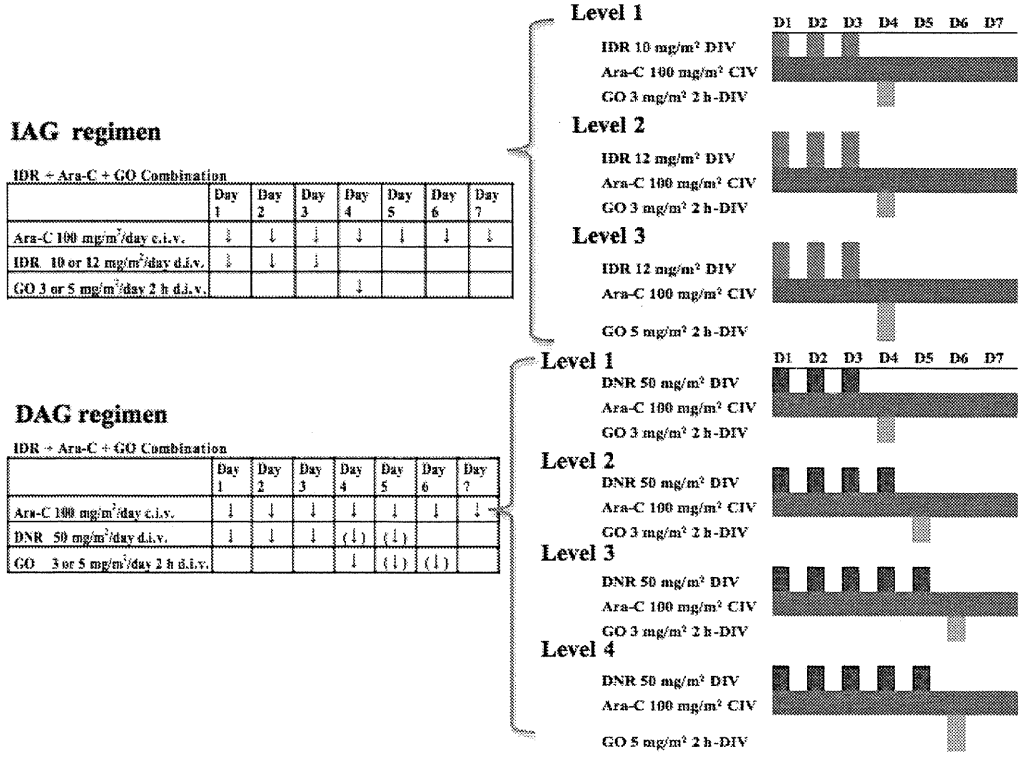


**Fig. 1.** Treatment schedule of the Japan Adult Leukemia Study Group (JALSG)-AML206 study. Ara-C, cytarabine; CIV and c.i.v., continuous intravenous infusion; DIV and d.i.v., drip intravenous infusion; DNR, daunorubicin; GO, gemtuzumab ozogamicin; IDR, idarubicin.

**Table 1. Patient characteristics**

| | Overall ($n$ = 19) | IAG regimen ($n$ = 9) | DAG regimen ($n$ = 10) |
|---|---|---|---|
| Male:Female | 9:10 | 4:5 | 5:5 |
| Age (years)† | 59 (33–64) | 61 (52–64) | 58 (33–62) |
| ≤60 | 10 | 3 | 7 |
| >60 | 9 | 6 | 3 |
| WBC ($\times 10^9$/L)† | 3.0 (1.0–39.2) | 3.7 (2.6–39.2) | 2.05 (1.0–25.3) |
| Blast (%)† | 42.8 (7.9–96.8) | 56.4 (17.3–88.0) | 29.9 (7.9–96.8) |
| CD33 positivity in blast (%)† | 89.4 (39.0–100) | 92.9 (62.8–100) | 80.6 (39.0–96.9) |
| Disease status | | | |
| Relapsed/Refractory | 13/6 | 7/2 | 6/4 |
| FAB type (no. patients) | | | |
| M0 | 1 | | 1 |
| M1 | 3 | 2 | 1 |
| M2 | 8 | 3 | 5 |
| M4 | 6 | 3 | 3 |
| M5 | 1 | 1 | |
| Cytogenetic group (no. patients) | | | |
| Favorable | 2 | 1 | 1 |
| Intermediate | 11 | 6 | 5 |
| Adverse | 6 | 2 | 4 |
| Performance status (no. patients) | | | |
| 0 | 1 | 0 | 1 |
| 1 | 18 | 9 | 9 |

†Median value and range in parentheses. FAB, French-American-British Classification; WBC, white blood cells.

blood tests three times weekly. Electrocardiography (ECG) was also performed once a week.

Hematological and non-hematological toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) ver 3.0, National Institutes of Health.

**Statistical analysis.** The primary objective of the study was to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of GO in combination with standard chemotherapy in Japanese patients. Dose escalation of anthracycline and GO in the IAG or DAG treatment followed a standard 3 + 3 phase I design in which cohorts of three patients at a time were treated at a dose and schedule level. If no DLT was observed, the next cohort was escalated to the next level. If one or two of the first three patients experienced a DLT, up to a total of six patients were enrolled at the same dose level. The next cohort was escalated only if a total of less than two patients presented with a DLT. If three of the first three patients experienced a DLT, the dose-escalation was stopped and the prior dose level was considered the MTD.

All ≥grade 3 drug-related nonhematological toxicities that occurred after treatment were considered DLT, with the exception of nausea and vomiting (if manageable with supportive care), alopecia, drug-related fevers, asymptomatic abnormalities of lactate dehydrogenase, alkaline phosphatase, disturbances of electrolytes and febrile neutropenia (FN) as these are common events in patients with relapsed AML.

Myelosuppression was not considered a DLT except for prolonged bone marrow aplasia longer than 6 weeks (or 42 days). Secondary objectives were to evaluate the efficacy of these treatment regimens.

The study was approved by the Institutional Review Board at each participating institution. Written informed consent was obtained from all patients before registration in accordance with the Declaration of Helsinki. The study was registered at the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (http://www.umin.ac.jp/ctr/) as UMIN000001141 and UMIN000001142.

## Results

**Patient characteristics.** A total of 19 patients with relapsed or refractory $CD33^+$AML were enrolled and evaluated (Table 1). The median age of patients was 59 years (range 33–64), the male/female ratio was 9/10, and the relapsed/refractory ratio was 13/6. The median value of blasts in the bone marrow before treatment was 42.8% (range 7.9–96.8%), and the median expression of CD33 antigen was 89.4% (range 39–100%). Patient characteristics in the IAG and DAG groups were similar, with the exception of age. Patients older than 60 years were more frequently enrolled in the IAG regimen. Among adverse cytogenetic groups, four patients had complex karyotypes (two in each group), one had t(6:9) in the DAG group and one had inv(5)del(7) in the DAG group.

**Safety.** *In the IAG regimen.* Hematological toxicities were commonly observed as expected for re-induction therapy (Table 2). Levels of white blood cells (WBC) at the time of GO administration tended to be lower than $3.0 \times 10^9$/L and those of ANC were $<1.5 \times 10^9$/L. Grade 4 leukopenia and neutropenia

**Table 2. IAG regimen: hematological toxicities**

| | Level 1 ($n$ = 3) (IPt-1/IPt-2/IPt-3) | Level 2 ($n$ = 3) (IPt-4/IPt-5/IPt-6) | Level 3 ($n$ = 3) (IPt-7/IPt-8/IPt-9) |
|---|---|---|---|
| WBC ($\times 10^9$/L) at GO administration | 2.4/1.1/5.4 | 1.3/0.4/2.3 | 0.8/1.2/3.0 |
| WBC (grade 3/4) | 0/3 | 0/3 | 0/3 |
| Days to nadir after GO administration | 4/6/13 | 10/5/10 | 6/5/7 |
| ANC ($\times 10^9$/L) at GO administration | 1.7/1.5/4.4 | 0.5/0/1.0 | 0.3/0.2/0.4 |
| ANC (grade 3/4) | 0/3 | 0/3 | 0/3 |
| Days to nadir after GO administration | 11/6/10 | 7/5/7 | 6/13/7 |
| Days toward ANC recovery | 31/35/26 | 24/34/35 | 42/38/24 |
| PLT ($\times 10^9$/L) at GO administration | 62/64/146 | 24/51/159 | 87/23/44 |
| PLT (grade 3/4) | 3/0 | 2/1 | 2/1 |
| Days to nadir after GO administration | 8/8/14 | 10/10/14 | 11/5/14 |
| PLT transfusion (units) | 90/130/100 | 130/130/50 | 70/220/70 |
| Days toward PLT recovery | 31/NA/NA | NA/43/35 | 25/87/31 |
| Hemoglobin (grade 0/1/2/3/4) | 0/1/2/0/0 | 0/1/2/0/0 | 1/1/1/0/0 |
| RBC transfusion (units) | 4/4/12 | 4/6/2 | 8/16/4 |

ANC, neutrophils; GO, gemtuzumab ozogamicin; NA, data was not available because the next treatment proceeded before platelet recovery due to disease progression; PLT, platelets; RBC, red blood cells; WBC, white blood cells.



doi: 10.1111/j.1349-7006.2011.01957.x

**Table 3. IAG regimen: non-hematological toxicities**

| | Level 1 ($n$ = 3) | Level 2 ($n$ = 3) | Level 3 ($n$ = 3) |
|---|---|---|---|
| Febrile neutropenia (grade 0/3/4) | 0/3/0 | 1/2/0 | 0/2/1 |
| Sepsis (grade 4) | 0 | 0 | 1 |
| Cerebral abscess (grade 4) | 0 | 0 | 1 |
| Hepatic toxicity (grade 0/1/2/3) | 2/0/1/0 | 2/0/1/0 | 1/0/1/1 |
| Nausea and vomiting (grade 0/1/2/3) | 3/0/0/0 | 3/0/0/0 | 1/0/2/0 |
| Diarrhea (grade 0/1/2/3) | 3/0/0/0 | 3/0/0/0 | 2/1/0/0 |
| Edema (grade 0/1/2/3) | 3/0/0/0 | 3/0/0/0 | 2/1/0/0 |
| Skin rash (grade 0/1/2/3) | 3/0/0/0 | 3/0/0/0 | 2/0/1/0 |
| VOD/SOS | 0 | 0 | 0 |

SOS, sinusoidal obstructive syndrome; VOD, veno-occlusive disease.

was observed in all patients. Days to nadir of ANC after GO administration were 5–13 days, and days toward ANC-recovery were 24–42 days. As one patient in level 3 (IPt-7) did not recover from neutropenia for 42 days (6 weeks), we regarded this prolongation of neutropenia as a DLT.

All patients had grade 4 thrombocytopenia and required plenty of PLT transfusion. Some patients took more than 30 days to recover to at least the initial level of PLT. As one patient in level 3 (IPt-8) required 220 units of PLT transfusion and took 87 days for recovery without disease progression, we regarded this prolongation of thrombocytopenia as a DLT.

Among non-hematological toxicities (Table 3), febrile neutropenia (FN) was common and severe. One patient in level 3 (IPt-9), although eventually recovered and attained CR, suffered from grade 4 neutropenia, sepsis and brain abscess. We regarded this FN with an infectious episode as a DLT.

Most non-hematological toxicities other than FN were clinically manageable and none of the patients had grade 4 hepatic toxicity, veno-occlusive disease (VOD) or sinusoidal obstructive syndrome (SOS).

*In the DAG regimen.* Grade 4 leukopenia and neutropenia was observed in all patients (Table 4). All except one patient in level 2 (DPt-6) recovered within 5 weeks. Grade 3/4 of thrombocytopenia was also observed in all patients, and plenty of PLT transfusion was required. The majority of patients recovered from thrombocytopenia within 5 weeks except one patient (DPt-6) who died of central nervous system (CNS) bleeding due to progression of leukemia within 30 days. The patient, DPt-6, was a 60-year-old man who was refractory to initial induction therapy. His leukemic blasts were reduced 47% in his bone marrow (BM) on day 15 of DAG level 2 (10 days after GO) and 4% in his peripheral blood (PB) on day 19. However, the duration of his response was short as his blasts rapidly increased to 85.2% in the BM on day 23 and 57% in PB on day 26. He suffered from disseminated intravascular coagulation (DIC) and eventually CNS bleeding on day 26, although the platelet count was maintained at >40 $\times$ $10^9$/L. Autopsy confirmed that progression of leukemia was the cause of his death without any clinical effect of the chemotherapy.

Among the non-hematological toxicities (Table 5), although FN was common and severe, none of the patients developed fatal infection, or had VOD or SOS. None of the grade 4 non-hematological toxicities developed either. As all patients in level 3 of the IAG regimen had DLT as mentioned above, the safety review board (SRB) recommended that level 4 of the DAG should be cancelled, because 5 mg/m$^2$ GO would be too toxic in combination with chemotherapy. Our previous study[4] indicated that the dose and schedule of DNR of level 3 of the DAG is equally effective and intensive as those of IDR of levels 2 and 3 in the IAG. Therefore, we considered that adding 5 mg/m$^2$ of GO to DNR + Ara-C (level 4 of the DAG) would be as toxic as level 3 of IAG, and accepted the recommendation of the SRB.

**Antileukemic activity.** A CR was achieved in nine of 19 patients and one attained a CRp, making the overall response rate 52.6%. In addition, two patients obtained partial remission, and four patients showed blast clearance, but three patients were resistant to therapy (Table 6). CR/CRp was observed in all levels of IAG and DAG. A CR was obtained in two patients with adverse karyotypes such as t(6:9) and complex. The rate of

**Table 4. DAG regimen: hematological toxicities**

| | Level 1 ($n$ = 3) (DPt-1/DPt-2/DPt-3) | Level 2 ($n$ = 4) (DPt-4/DPt-5/DPt-6/DPt-7) | Level 3 ($n$ = 3) (DPt-8/DPt-9/DPt-10) |
|---|---|---|---|
| WBC ($\times 10^9$/L) at GO administration | 1.1/1.2/0.7 | 2.4/1.7/0.5/0.3 | 0.6/1.7/2.0 |
| WBC (grade 3/4) | 0/3 | 0/4 | 0/3 |
| Days to nadir after GO administration | 7/10/7 | 7/11/3/8 | 3/5/7 |
| ANC ($\times 10^9$/L) at GO administration | 0.4/0.6/0.2 | 0.2/1.3/0.2/0.0 | 0.4/1.0/1.2 |
| ANC (grade 3/4) | 0/3 | 0/4 | 0/3 |
| Days to nadir after GO administration | 7/8/7 | 11/13/5/8 | 7/7/12 |
| Days toward ANC recovery | 26/29/33 | 23/18/NA/28 | 34/24/26 |
| PLT ($\times 10^9$/L) at GO administration | 361/47/122 | 71/199/53/3 | 32/147/193 |
| PLT (grade 3/4) | 2/1 | 3/1 | 3/0 |
| Days to nadir after GO administration | 11/11/17 | 13/13/17 | 14/8/17 |
| PLT transfusion (units) | 150/60/90 | 50/70/110/170 | 170/60/40 |
| Days toward PLT recovery | 22/39/31 | 32/20/NA/26 | 28/29/21 |
| Hemoglobin (grade 0/1/2/3/4) | 0/3/0/0/0 | 2/1/1/0/0 | 0/1/2/0/0 |
| RBC transfusion (units) | 18/0/6 | 0/6/10/10 | 6/6/8 |

ANC, neutrophils; NA, data was not available because of central nervous system bleeding due to disease progression before ANC and PLT recovery; PLT, platelets; RBC, red blood cells; WBC, leukocytes.

**Table 5. DAG regimen: non-hematological toxicities**

| Toxicity | Level 1 ($n$ = 3) | Level 2 ($n$ = 4)† | Level 3 ($n$ = 3) |
|---|---|---|---|
| Febrile neutropenia (grade 0/3/4) | 0/2/1 | 1/3/0 | 1/2/0 |
| Hepatic toxicity (grade 0/1/2/3) | 2/0/1/0 | 3/0/1/0 | 1/1/1/0 |
| Nausea and vomiting (grade 0/1/2/3) | 2/0/1/0 | 4/0/0/0 | 2/0/1/0 |
| Colitis (grade 0/1/2/3) | 2/0/1/0 | 4/0/0/0 | 3/0/0/0 |
| Diarrhea (grade 0/1/2/3) | 3/0/0/0 | 4/0/0/0 | 2/0/1/0 |
| Cardiac (grade 0/1/2/3) | 3/0/0/0 | 3/0/1/0 | 2/0/1/0 |
| VOD/SOS | 0 | 0 | 0 |

†One patient in level 2 died of CNS bleeding due to disease progression. SOS, sinusoidal obstructive syndrome; VOD, veno-occlusive disease.

**Table 6. Response**

| | IAG regimen | | | DAG regimen | | | Overall | |
|---|---|---|---|---|---|---|---|---|
| | Level 1 ($n$ = 3) | Level 2 ($n$ = 3) | Level 3 ($n$ = 3) | Level 1 ($n$ = 3) | Level 2 ($n$ = 4) | Level 3 ($n$ = 3) | ($n$ = 19) | |
| CR | 1 | 2 | 1 | 1 | 3 | 1 | 9 | 52.6% |
| CRp | | | 1 | | | | 1 | |
| PR | | | | 1 | | 1 | 2 | |
| Blast clearance | 1 | | 1 | 1 | | 1 | 4 | |
| Resistant disease | 1 | 1 | | | 1 | | 3 | |

CR, complete remission; CRp, CR without platelet recovery; PR, partial remission.

**Table 7. Response according to patient characteristics**

| Overall response (CR + CRp) | 10/19 (52.6%) |
|---|---|
| Disease status | |
| Relapsed | 8/13 (61.5%) |
| Refractory | 2/6 (33.3%) |
| Cytogenetic group | |
| Favorable | 1/2 (50.0%) |
| Intermediate | 7/11 (53.6%) |
| Adverse | 2/5 (40.0%) |

CR, complete remission; CRp, CR without platelet recovery.

response tended to be higher in relapsed patients (61.4%) than in patients refractory to initial therapy (33.3%) (Table 7).

## Discussion

As Kell *et al.*[19] suggested, the development of antibody-directed chemotherapy with more specificity against leukemic blasts has been one of the goals of cancer treatments for several years. CD33 antigen has emerged as a favored target epitope because it is expressed in over 80–90% of AML blasts.[22] Although unconjugated humanized anti-CD33 monoclonal antibodies has met with little success in relapsed disease, the antigen–antibody complex is rapidly internalized, suggesting that this would be a convenient drug delivery system to leukemia cells. GO is a humanized anti-CD33 monoclonal antibody conjugated to the extremely potent (toxic) antitumor drug calicheamicin. In the final report of a phase II trial in the USA and Europe, 277 patients were treated with standard doses of GO (9 mg/m$^2$, 2 h d.i.v. on days 1 and 15).[23] The response rate of younger patients was 27% (CR, 13%; CRp, 14%). Other clinical trials reported similar results with an approximate response rate of 26% (CR, 13%; CRp, 13%),[8,11,24] and the phase II part of the clinical trials in Japan resulted in a response rate of 30% (CR, 25%; CRp, 5%).[13]

As clinical efficacy of GO monotherapy for patients with relapsed or refractory AML has been limited, clinical studies are required for exploration of the role of GO in combination therapy with conventional chemotherapy. Even though several groups in the USA and Europe have been evaluating the potential of GO already in different situations in the treatment of AML, the optimal usage of GO in combination therapy is still unknown, especially for Japanese patients. For this reason, we conducted the present study, starting from phase I, in order to evaluate the safety of GO-combined therapy.

As the final goal of our study is to investigate whether GO-combined therapy is meaningful for de novo adult AML (younger than age 65 years), we selected standard induction therapies, which are IDR 12 mg/m$^2$ on days 1–3 plus Ara-C 100 mg/m$^2$ on days 1–7, and DNR 50 mg/m$^2$ on days 1–5 plus Ara-C 100 mg/m$^2$ on days 1–7, as partner chemotherapeutic regimens.[4]

In the present study for relapsed or refractory AML, GO was administered on the next day after the final administration of anthracycline (IDR or DNR) with continuing administration of Ara-C.

As expected, grade 3/4 hematological toxicities and febrile neutropenia was observed in most patients, but those toxicities were clinically manageable. None of the patients died of adverse events, although one patient died of disease progression. The DLT (prolongation of neutropenia and thrombocytopenia, and serious infection [i.e. cerebral abscess]) were observed in all patients in level 3 of the IAG regimen (a dose of 5 mg/m$^2$ GO), but none in level 2 of the IAG regimen or level 3 of the DAG regimen. Therefore, the MTD of the IAG regimen was determined as level 2 (i.e. 3 mg/m$^2$ GO, 12 mg/m$^2$ IDR and 100 mg/m$^2$ Ara-C), and that of the DAG regimen as level 3 (i.e. 3 mg/m$^2$ GO, 50 mg/m$^2$ DNR and 100 mg/m$^2$ Ara-C).

Several attempts that combined the approved dosage of GO (9 mg/m$^2$, administered twice) with chemotherapy resulted in excess toxicity such as infection and liver toxicity, including increased risk of VOD/SOS.[25] The Cancer and Leukemia Group B (CALGB) 19902 study indicated that the dose schedule of 9 mg/m$^2$ GO on day 7 and 4.5 mg/m$^2$ GO on day 14 with high-dose Ara-C (3 g/m$^2$ per day for 5 days) caused a high rate of treatment-related death (four of the first seven patients, 57%).[18] In the present study, severe hepatotoxicity or VOD/


doi: 10.1111/j.1349-7006.2011.01957.x

**Table 8. Selected phase II trials of gemtuzumab ozogamicin (GO)-combining therapy for relapsed or refractory adult acute myeloid leukemia (AML)**

| Authors (name of regimen) | Institutes | No. patients | Median age (range) (years) | Combination of drugs | Dose and schedule of GO | % Response (CR/CRp) | Median OS (months) | Grade 3/4 non-hematological toxicity |
|---|---|---|---|---|---|---|---|---|
| Tsimberidou *et al.*[14] 2003 (MFAC) | MDACC | 32 | 53 (18–78) | FLD: 15 mg/m$^2$ i.v. q12 h/day, days 2–4<br>Aa-C: 500 mg/m$^2$ 2 h d.i.v. q12 h/day, days 2–4<br>CSA: 6 mg/kg 2 h d.i.v. + 16 mg/kg c.i.v., days 1, 2 | 4.5 mg/m$^2$ 2 h d.i.v., day 1 | 34 (28/6) | 5.3 | Hyperbilirubinemia (18%), hepatic transaminitis (9%), VOD (3%) |
| Alvarado *et al.*[15] 2003 (MIA) | MDACC | 14 | 61 (34–74) | IDR: 12 mg/m$^2$/day i.v., days 2–4<br>Ara-C: 1.5 g/m$^2$/day, days 2–5 | 6 mg/m$^2$ 2 h d.i.v., days 1, 15 | 42 (21/21) | 2 | Sepsis (71%), liver damage, VOD (14%) |
| Chevallier *et al.*[16] 2008 (MIDAM) | France | 62 | 56 (16–71) | Ara-C: 1.5 g/m$^2$ 2 h d.i.v. q12 h/day, days 1–5<br>MIT: 12 mg/m$^2$/day i.v., days 1–3 | 9 mg/m$^2$ 2 h d.i.v., day 4 | 63 (50/13) | 9.5 | Hyperbilirubinemia (16%), VOD (3%), early toxic death (6%) |
| Fianchi *et al.*[17] 2008 (G-Ara-My) | Italy | 53 | M | G-CSF: 5 μg/kg/day s.c., days 1–8<br>Ara-C: 100 mg/m$^2$/day c.i.v., days 2–8 or 4–8 | 6 mg/m$^2$ 2 h d.i.v., day 9 | 45 (43/2) | 9 | Infection (36%), infusion reaction (5.5%), VOD (2%) |
| Stone *et al.*[18] 2010 (CALGB 19902) | CALGB | 37 | 64 (55–70) | Ara-C: 3 g/m$^2$ 3 h d.i.v./day, days 1–5 | 9 mg/m$^2$ 2 h d.i.v., day 7 | 35 (32/3) | 8.9 | Hepatic transaminitis (29%), hyperbilirubinemia (27%), infection (92%), death of tox (8.1%) |

Ara-C, cytarabine; CALGB, Cancer and Leukemia Group B; c.i.v., continuous venous infusion; CR, complete remission; CRp, CR without platelet recovery; CSA, cyclosporin A; d.i.v., drip venous infusion; FLD, fludarabine; G-CSF, granulocyte colony stimulating factor; IDR, idarubicin; iv, venous infusion; MDACC, MD Anderson Cancer Center; MIT, mitoxantrone; q12 h, every 12 h; OS, overall survival; VOD, veno-occlusive disease.

SOS was not observed in either of the IAG or DAG regimens, because we selected an initial dose of GO at 3 mg/m$^2$.

The MRC group already indicated in the AML15 prelude trial that a combination of 3 mg/m$^2$ but not 6 mg/m$^2$ of GO with intensive chemotherapy was safe and feasible for a multicenter trial in induction and consolidation therapy.[19] Our study confirmed a safe dose of GO as 3 mg/m$^2$, even though the timing of administration was different.

Although the present study was not designed to assess efficacy, it was of note that CR and CRp were achieved in nine (47.4%) and one (5.2%), respectively, out of 19 patients with relapsed or refractory AML. This overall rate of response, 52.6%, was comparable to the results of previous phase II trials for relapsed or refractory AML[14–18] (Table 8).

Clinical efficacy of the combination of GO with IDR + Ara-C (named MIA) was already evaluated by the MD Anderson Cancer Center.[15] Compared with our IAG regimen, the response rate of MIA (42%; CR, 21%; CRp, 21%) was quite similar, but their incidence of severe non-hematological toxicity was higher. Despite the fact that the doses of Ara-C and GO were lower in our IAG regimen, this combination will be feasible as an induction therapy for relapsed or refractory AML.

The MRC AML15 prelude trial[19] investigated safety and efficacy of GO in combination with DNR + Ara-C, in which DNR (50 mg/m$^2$ for 3 days) and Ara-C were combined with 3 mg/m$^2$ GO on day 1. Hematolopoietic recovery was satisfactory, and although two of eight enrolled patients developed grade 3 toxicity, all patients achieved CR and tolerated subsequent chemotherapy. In levels 2 and 3 of our DAG regimen, although the dose of DNR was higher than that of the MRC trial, the recovery from myelosuppression was satisfactory without excess of unexpected non-hematological toxicity.

During this phase I trial of GO in combination with chemotherapy for relapsed or refractory AML, several multicenter trials to investigate the role of GO combination for *de novo* AML have been completed in the USA and Europe. Burnett *et al.*[26] presented the results of the MRC AML15 trial, in which 1113 mostly younger, newly diagnosed patients with AML (except acute promyelocytic leukemia) were randomly assigned to one of three conventional induction therapies with or without 3 mg/m$^2$ GO on day 1. After achieving CR, 978 patients were randomly assigned to GO in combination with chemotherapy in course 3 of the consolidation therapy. The addition of GO was well tolerated with no significant increase in toxicity. Although there was no overall difference in response or survival, a predefined analysis by cytogenetic risk groups showed a significant survival benefit for patients with favorable risk and a trend for those with intermediate risk disease.

A similar study conducted by the Southwest Oncology Group (SWOG) was reported in abstract format.[27] In this SWOG 106 study, 627 patients with untreated AML (age 18–60 years) were randomly assigned to receive induction therapy either with Ara-C (100 mg/m$^2$ × 7 days) + DNR (60 mg/m$^2$ × 3 days) or with Ara-C (100 mg/m$^2$ × 7 days) + DNR (45 mg/m$^2$ × 3 days) + GO (6 mg/m$^2$). An interim analysis showed a CR rate of 66% in the GO-combined arm and 69% in the chemotherapy-alone arm (control arm), ruling out the originally hypothesized increase in CR of 12% by the addition of GO. There was no difference in disease-free survival (DFS) either, and the rate of fatal adverse events was higher in the GO-combined arm compared with the control arm (5.8% *vs* 0.8%). Based on these negative findings of the GO-combined arm, the FDA recommended to withdraw GO from the market in the USA.

However, as Burnett *et al.*[26] suggested, the SWOG 106 study is confounded, as the dose of DNR was lower in patients given GO, which might have masked any benefit of GO. In addition, the induction death rate in the GO arm was similar to what had been reported in other AML induction trials, but the mortality rate of the control arm was unexpectedly low. Nevertheless, in the SWOG study the benefit in the favorable subtype of AML was similarly observed in the MRC study.

Another smaller phase II study reported a high molecular response rate and DFS by GO in combination with high-dose Ara-C for core binding factor (CBF) leukemias.[28]

In conclusion, the present study demonstrated that 3 mg/m$^2$ of GO with IDR + Ara-C or DNR + Ara-C can be administered safely in younger adult patients with relapsed or refractory AML. As three clinical studies of GO-combined chemotherapy for newly diagnosed adult AML have indicated, there are subsets of AML, such as CBF leukemias, that could benefit from the addition of GO to conventional therapy. Intensive induction chemotherapy followed by a modest dose of GO like in our study protocol will be safely provided for salvage therapy regardless of cytogenetic risk groups. Fortunately, GO is still commercially available in Japan, therefore there is a need for confirmatory studies that investigate the efficacy of GO-combined chemotherapy for patients with AML as both initial and salvage therapy.

## Acknowledgments

This work was supported in part by grants from the Ministry of Health, Labour, and Welfare of Japan, and the Nonprofit Organization for Support Japan Adult Study Group (NPO-JALSG). The authors thank the clinicians and leaders of the six institutions who entered their patients into the JALSG-AML206 study and provided the necessary data to make this study possible. The authors also thank Ryuzo Ohno, MD (Aichi Cancer Center, Nagoya, Japan), Kiyohiko Hatake, MD (Cancer Institute Hospital, Tokyo, Japan), and Toshiteru Ohshima, MD (Ibuki Institution, Kawagoe, Japan), for their critical advice as members of the safety review board.

## Disclosure Statement

The authors have no conflict of interest.

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JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2011;41(6)820–824
doi:10.1093/jjco/hyr029
Advance Access Publication 16 March 2011

Short Communication

# Administration Schedule of Daunorubicin for Elderly Patients with Acute Myelogenous Leukemia: A Single-institute Experience

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Received October 21, 2010; accepted February 13, 2011

We evaluated the efficacy of daunorubicin (40 mg/m$^2$/day for 5 days, 200 mg/m$^2$/cycle) combined with standard dose of cytarabine (100 mg/m$^2$/day for 7 days) for acute myelogenous leukemia patients aged 65–74 years as induction therapy. Complete remission (81.3%) was achieved in 13 of 16 patients following the therapeutic program. The median duration of recovering absolute neutolophilic counts over 1000/μl and platelet counts over 100 000/μl were 33 days and 27 days, respectively. None of the patients had any adverse cardiac complications or died during administration of the induction therapy. Patients achieving complete remission received post-remission therapy consisting of two regimens other than induction therapy. The 3-year disease-free and overall survival rates were 36.9 and 50.0%, respectively. Extending the total period of the daunorubicin therapy might be an alternative to increasing the daily dose of daunorubicin in the induction therapy for elderly patients who were candidates for receiving intensified chemotherapy.

*Key words: elderly patients – AML – intensified DNR – induction therapy*



## INTRODUCTION

Advances in the treatment for acute myelogenous leukemia (AML) have been obtained with intensified approaches and development of novel agents. In elderly AML patients who were judged by physicians to be fit for intensive chemotherapy, standard therapy such as '3 + 7' [3 days of daunorubicin (DNR) and 7 days of cytarabine at conventional dose] or intensive investigational therapy are usually employed (1–3). However, the most appropriate chemotherapy for elderly AML patients is still controversial due to both biological disease-related and patient-specific factors (4–6). There is an urgent need to find innovative treatments for elderly AML patients. We previously reported two studies on intensified DNR in induction therapy for adult AML patients younger than 65 years (7,8). To increase the intensity of induction therapy, we administrated DNR (40 mg/m$^2$/day) by expanding the total period of infusion more than 3 days instead of increasing daily dose of DNR in these two studies. On the basis of our institution's experience, we administered DNR (40 mg/m$^2$/day for 5 days, 200 mg/m$^2$/cycle) combined with cytarabine (100 mg/m$^2$/day for 7 days) to previously untreated AML patients aged 65–74 years as induction therapy. Here, we conducted retrospective analysis of the clinical outcome of elderly AML patients treated by extending the total period of DNR combined with cytarabine in induction therapy.

## PATIENTS AND METHODS

### Patients

Between January 2003 and March 2008, 21 untreated AML patients aged 65–74 years, except previously diagnosed myelodysplastic syndrome were admitted to our institution. Of the 21 patients, 4 patients did not undergo the therapeutic program because of their comorbid conditions (cerebral hemorrhage, senile dementia, uncontrolled diabetes mellitus and active double cancer). One patient refused receiving

intensive chemotherapy. Thus, 16 patients (76.2%) were enrolled in the therapeutic program.

### Treatment Protocol

The dose and schedule of induction therapy were as follows: DNR was administered intravenously (IV) at a dose of 40 mg/m$^2$/day for 5 days, and cytarabine was administered continuous intravenously (CIV) at a dose of 100 mg/m$^2$/day for 7 days. When bone marrow (BM) examination revealed sufficient hypoplastic marrow (cellularity < 10 000 cells/μl) and M1 marrow (<5% blasts) at 4 days after initiating therapy, the administration of DNR was discontinued. If complete remission (CR) was not attained by the first cycle of treatment, 40 mg/m$^2$/day of DNR for 3 days and 100 mg/m$^2$/day of cytarabine for 7 days were administrated as the second cycle. The post-remission therapy was administered as follows: one cycle of DNR (40 mg/m$^2$/day IV for 3 days) combined with cytarabine (100 mg/m$^2$/day CIV for 7 days), two cycles of behenoyl cytarabine (170 mg/m$^2$/day IV for 7 days) combined with aclarubicin (17 mg/m$^2$/day IV for 7 days) and two cycles of mitoxantrone (10 mg/m$^2$/day IV for 1 day) combined with etoposide (200 mg/body/day orally for 5 days) and cytarabine (80 mg/m$^2$/day subcutaneous injected for 5 days).

### Response Criteria and Statistical Analysis

CR was defined as the normalization of peripheral blood count and <5% blasts in the BM with normal cellularity. Relapse was defined as the reappearance of leukemic cells in the BM (>5% blasts) and/or reappearance of clinical evidence of the disease. Non-hematologic toxicity was graded according to the National Cancer Institute's Common Toxicity Criteria (version 3.0).The duration of disease-free survival (DFS) of a patient was measured from the first documentation of achievement of CR to the date of either the first incidence of relapse or death, and overall survival (OS) of a patient was measured from the time of initiation of the induction therapy to the death of the patient from any cause. DFS and OS distributions were computed with the Kaplan–Meier product limit estimator. The difference in DFS and OS between subgroups was evaluated by means of the log-rank test.

## RESULTS

### Patient Characteristics

The characteristics of the patients and their response to induction therapy are outlined in Table 1. All patients provided their written informed consent. Their median age was 70 years. Of the 10 patients with a normal karyotype, 7 patients were tested for FMS-like tyrosine kinase 3 (FLT3)-internal tandem duplication (ITD) by a semiquantitative polymerase chain reaction assay; the FLT3-ITD mutation was detected in only one patient. Nucleophosmin (*NPM1*) gene status was not tested in those patients. The median follow-up period for seven patients who were still alive at the date of last contact was 54.9 months.

### Response to Induction Therapy and Survival

CR (81.3%; 95% confidence interval: 54.4–96.0%) was achieved in 13 patients; 4 of the 13 patients received a second cycle of the induction therapy. One patient, who did not achieve CR after the first cycle of the induction therapy, refused further treatment. The median percentage of BM blasts at day 4 of the therapy was 30.4% (range: 2–84.2). Only two patients were administered DNR for 3 days because their BM examination showed sufficient degree of hypoplasia at day 4 of the treatment. With regard to the hematologic changes resulting from the induction therapy, the median durations for recovery of the absolute neutrophil count to over 1000/μl and the platelet count to over 100 000/μl were 33 days (range: 19–37+) and 27 days (range: 21–39+), respectively. The major non-hematologic toxicities (grade: >2) were infection (92%), diarrhea (25%), mucositis (19%) and hepatotoxicity (13%). Granulocyte colony-stimulating factor was administered to four patients, because of complication of the documented infection. None of the patients had any adverse cardiac complications (grade: >2), and the median percentages of the left ventricular ejection fraction, measured by echocardiography, were 68% (range: 61–79) after receiving the induction therapy and 67% (range: 59–80) before initiation of the treatment.

The 13 patients who had achieved CR underwent the post-remission therapy. During this post-remission therapy, three patients relapsed and one patient died of pneumonia. After the post-remission therapy, four patients relapsed. Five patients maintained first CR at the date of this analysis. The 3-year DFS and OS rates were 36.9% (95% CI: 12.5–62.0%) and 50.0% (95% CI, 24.5–71.1%), respectively (Fig. 1A and B). Comparison of the outcomes between the favorable/normal karyotype group and the unfavorable karyotype group was made with regard to DFS and OS rates. There was no significant difference in those groups ($P = 0.749$ in DFS rate, $P = 0.331$ in OS rate) (Fig. 1C and D).

On the other hand, the clinical outcome of five patients who did not undergo the therapeutic program were as follows: three patients were treated with less intensive chemotherapy (DNR, 40 mg/m$^2$/day IV for 1 day, combined with cytarabine); however, they died of leukemia at 2, 6, 24 months after initiating the treatments, respectively. Two patients moved in other hospitals, and we lost their follow-up data.

## DISCUSSION

The current report describes the response of 16 elderly patients to the induction therapy with a combination of DNR (administered for 5 days) and standard dose of cytarabine.

**Table 1.** Characteristics of patients and response to induction therapy

| No. | Age | Sex | PS | FAB | WBC ($\mu l^{-1}$) | BM blasts (%) | Karyotype | FLT3/ ITD | Induction therapy | Total DNR dose in induction therapy ($m^{-2}$) | During induction therapy | | Response |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | G-CSF rescue | Infection (>grade 2) | |
| 1 | 70 | M | 0 | M5 | 39 000 | 40.0 | 46XY | Positive | One cycle | 200 | | FN | CR |
| 2 | 71 | M | 0 | M2 | 24 600 | 94.6 | 46XY | Negative | Two cycles | 320 | yes | Sepsis | CR |
| 3 | 65 | F | 1 | M1 | 29 400 | 82.8 | Complex, +21, −20 | | One cycle | 200 | | FN | CR |
| 4 | 73 | M | 1 | M2 | 42 800 | 30.0 | 46XY | Negative | One cycle | 200 | | FN | CR |
| 5 | 70 | M | 1 | M4 | 30 300 | 30.2 | Complex, del(5) | | One cycle | 200 | | | NR[a] |
| 6 | 72 | M | 3 | M1 | 1000 | 30.4 | 46XY | Negative | One cycle | 200 | | FN | CR |
| 7 | 69 | M | 3 | M2 | 31 000 | 54.4 | 46XY | Negative | Two cycles[b] | 240 | yes | Pneumonia | CR |
| 8 | 69 | M | 1 | M4 | 89 700 | 64.6 | 46XY | Negative | One cycle | 200 | yes | Sepsis | CR |
| 9 | 66 | M[c] | 1 | M1 | 1900 | 24.5 | Complex, −5, +8 | | Two cycles[b] | 240 | | FN | CR |
| 10 | 73 | F | 1 | M2 | 1200 | 37.5 | 46XX | ND | Two cycles | 320 | yes | Sepsis | CR |
| 11 | 72 | M | 0 | M2 | 4000 | 28.2 | t(8;21), −Y | | One cycle | 200 | | FN | CR |
| 12 | 71 | M | 1 | M4 | 6500 | 26.2 | 46XY | ND | Two cycles | 320 | | FN | NR |
| 13 | 69 | F | 2 | M4 | 31 400 | 78.2 | 46XX | Negative | Two cycles | 320 | | FN | NR |
| 14 | 69 | F[c] | 1 | M0 | 600 | 66.6 | 49XX, +8 × 3 | | One cycle | 200 | | Sepsis | CR |
| 15 | 70 | M | 0 | M5 | 14 600 | 63.4 | 46XY | ND | One cycle | 200 | | FN | CR |
| 16 | 65 | F[c] | 1 | M1 | 5700 | 77.2 | Complex, −5 | | One cycle | 200 | | FN | CR[d] |

PS, performance status; FAB, French-American-British classification; WBC, white blood cell counts; BM, bone marrow; ND, not determined; FLT3/ITD, FMS-like tyrosine kinase 3-internal tandem duplication; DNR, daunorubicin; G-CSF, granulocyte colony-stimulating factor; FN, febrile neutropenia; CR, complete remission; NR, no response.
[a]This patient discontinued therapeutic program after receiving the first cycle of the induction therapy.
[b]In these patients, DNR was administrated for 3 days.
[c]These patients were diagnosed with therapy-related AML.
[d]Chromosomal aberration was retained after hematologic CR.

The dose of DNR per day (40 mg/m$^2$) was relatively low; however, the total DNR dose per cycle (200 mg/m$^2$) was higher than the conventional dose per cycle, which is 135 mg/m$^2$ (45 mg/m$^2$/day for 3 days). Even though we administered a relatively high dose of DNR in the induction therapy, the toxicity of this therapy was mostly manageable, with no increase in early mortality. Elderly AML patients have a poor prognosis, attributable to having poorer performance status, unfavorable AML karyotype, comorbid disease, antecedent hematologic disorder, and relatively poor tolerance of cytotoxic agents (9–11). Therefore, the ratio of patients diagnosed to patients included in a therapeutic program is insufficient compared with young adult patients (12). In our study, four patients (19%) did not undergo the therapeutic program due to their comorbid disease and poor PS. Genetic alterations also affect clinical outcome in elderly AML patients. The multidrug resistance gene (*MDR1*) is frequently expressed in leukemic blasts derived from elderly AML patients and associated with lower CR and DFS rates due to resistance to chemotherapeutic agents such as vinca alkaloids and anthracyclines (13). FLT3-ITD is frequently found in elderly AML patients. While FLT3-ITD and *NPM1* gene status were associated with normal karyotype in younger AML patients, one study reported the clinical impact of the two genes in elderly AML patients regardless of normal karyotype (14). Further molecular investigation might relieve finding innovative treatments for elderly AML patients.

Intensified chemotherapeutic approach is expected of possibility benefits for patients who were candidates for receiving intensified chemotherapy. To improve prognosis of elderly AML patients, anthracyclines other than DNR in induction therapy and high-dose cytarabine in post-remission therapy have been examined. Unfortunately, anthracyclines (or anthraqinone) other than DNR have not demonstrated an improvement in OS rates and high-dose cytarabine has been too toxic for those patients (15–17). Recently, the benefits of intensified DNR (90 mg/m$^2$/day for 3 days, 270 mg/m$^2$/cycle) as induction therapy compared with conventional dose DNR (45 mg/m$^2$/day for 3 days) have been assessed in

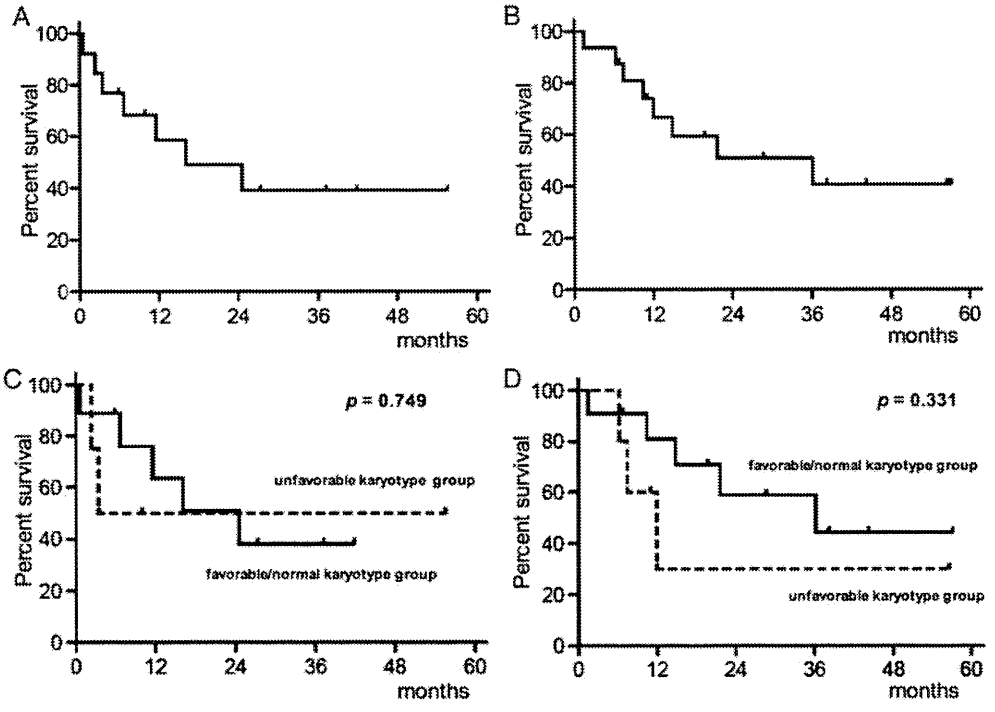


**Figure 1.** Disease-free survival (DFS) curve (A) and overall survival (OS) curve (B). DFS curves (C) and OS curves (D) according to the favorable/normal karyotype group and the unfavorable karyotype group. The median DFS and OS times were 17.2 and 19.9 months, respectively. There was no significant difference in DFS and OS by comparison between the favorable/normal karyotype group and the unfavorable karyotype group.

young adult and elderly patients with AML. High-dose DNR resulted in a higher CR rate and improved OS in AML patients younger than 65 years; however, the benefits of such an intensified chemotherapeutic approach were reduced in patients older than 65 years (18,19). The Japan Adult Leukemia Study Group (JALSG) conducted a randomized phase 3 study of AML patients younger than 65 years, which compared intensified DNR with conventional dose idarubicin (IDR) (12 mg/m$^2$/day for 3 days). DNR was administrated at a dose of 50 mg/m$^2$/day for 5 days (250 mg/m$^2$/cycle) in the study by JALSG, and intensified DNR proved to be equivalent efficacy without much more adverse events compared with conventional dose IDR (20). Further prospective studies might be needed to establish the optimal dose and schedule of DNR in induction therapy for elderly AML patients who are candidates for receiving intensified chemotherapy.

The number of patients was small, more than half of patients were normal karyotype and *MDR1* gene status was not tested; therefore, the results of our study should be interpreted with caution in comparison with other studies. Nevertheless, the CR rate of 81.3% and the 3-year OS rate of 50.0% with this therapeutic program appear high for a group of elderly patients who were candidates for receiving intensified chemotherapy. Main cellular target of DNR is recognized to be DNA topoisomerase II significantly expressed only in dividing cells during selected mitotic phase of cell cycle (21). Expanding the total period of DNR infusion may have an advantage of gain in exposure times for sensitive phase of cell cycle and lead to more anti-tumor activity compared with increasing daily dose of DNR. In addition, extending the total period of the DNR therapy might be an alternative to increasing the daily dose of DNR in induction therapy for selective elderly AML patients.

## Conflict of interest statement

None declared.

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