Table 2 Mean values of observed data, predicted minimum, and predicted maximum values of FK228 in combination with other anticancer agents

Combined drug	Cell line	No. of data point	Observed data[a]	Predicted min[b]	Predicted max[c]	Effect
Cytarabine	HL60	9	0.63	0.12	0.89	Additive
	KU812	10	0.39	0.26	0.78	Additive
	MOLT3	8	0.44	0.23	0.95	Additive
	Raji	12	0.67	0.40	0.75	Additive
Carboplatin	HL60	9	0.73	0.27	0.93	Additive
	KU812	11	0.56	0.35	0.90	Additive
	MOLT3	7	0.65	0.35	0.89	Additive
	Raji	11	0.61	0.34	0.71	Additive
Doxorubicin	HL60	7	0.60	0.39	0.84	Additive
	KU812	12	0.71	0.27	0.85	Additive
	MOLT3	9	0.52	0.32	0.90	Additive
	Raji	10	0.64	0.25	0.91	Additive
Etoposide	HL60	8	0.58	0.15	0.88	Additive
	KU812	10	0.42	0.25	0.83	Additive
	MOLT3	10	0.53	0.37	0.78	Additive
	Raji	10	0.43	0.21	0.83	Additive
4-hydroperoxy	HL60	7	0.74	0.28	0.93	Additive
Cyclophosphamide	KU812	8	0.56	0.30	0.90	Additive
	MOLT3	6	0.61	0.31	0.80	Additive
	Raji	9	0.60	0.43	0.71	Additive
6-mercaptopurine	HL60			ND		
	KU812	6	0.70	0.31	0.89	Additive
	MOLT3	6	0.50	0.19	0.84	Additive
	Raji	7	0.84	0.40	0.84	Additive
Methotrexate	HL60	9	0.93	0.32	0.87	Anatgonism ($P < 0.05$)
	KU812	10	1.13	0.15	0.44	Anatgonism ($P < 0.01$)
	MOLT3	8	0.87	0.19	0.86	additive/anatgonism (NS)
	Raji	11	0.98	0.29	0.76	Anatgonism ($P < 0.01$)
SN-38	HL60	8	0.79	0.24	0.93	Additive
	KU812	9	0.50	0.31	0.82	Additive
	MOLT3	8	0.71	0.39	0.88	Additive
	Raji	9	0.63	0.41	0.72	Additive
Vincristine	HL60	11	0.82	0.22	0.93	Additive
	KU812	10	0.78	0.15	0.77	additive/anatgonism (NS)
	MOLT3	8	0.51	0.18	0.71	Additive
	Raji	11	0.91	0.27	0.75	antagonism ($P < 0.02$)
Imatinib	KU812	8	0.71	0.49	0.83	Additive
	K562	8	0.66	0.50	0.95	Additive
	TCC-S	9	0.82	0.42	0.94	Additive

[a]Mean value of observed data.

[b]Mean value of the predicted minimum values for an additive effect.

[c]Mean value of predicted maximum values for an additive effect.

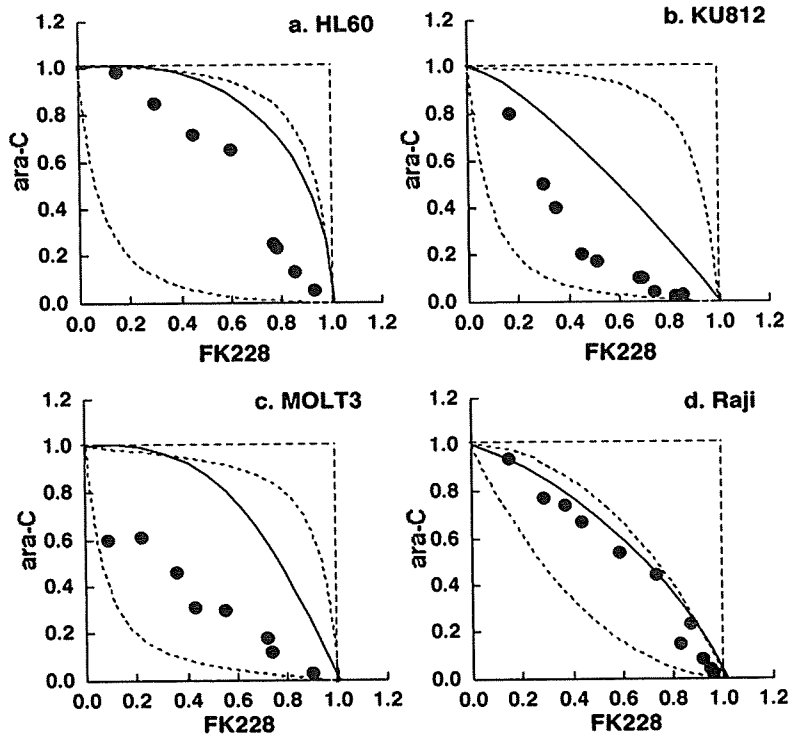

Fig. 5 Isobolograms of simultaneous exposure to FK228 and cytarabine (ara-C) in HL60 (a), KU812 (b), MOLT3 (c) and Raji (d) cells. In all four leukemia cell lines, the data points of the combinations fell within the envelope of additivity, suggesting an additive effect. Each point represents the mean value for 3 independent experiments; the SEs were less than 25% and thus were omitted

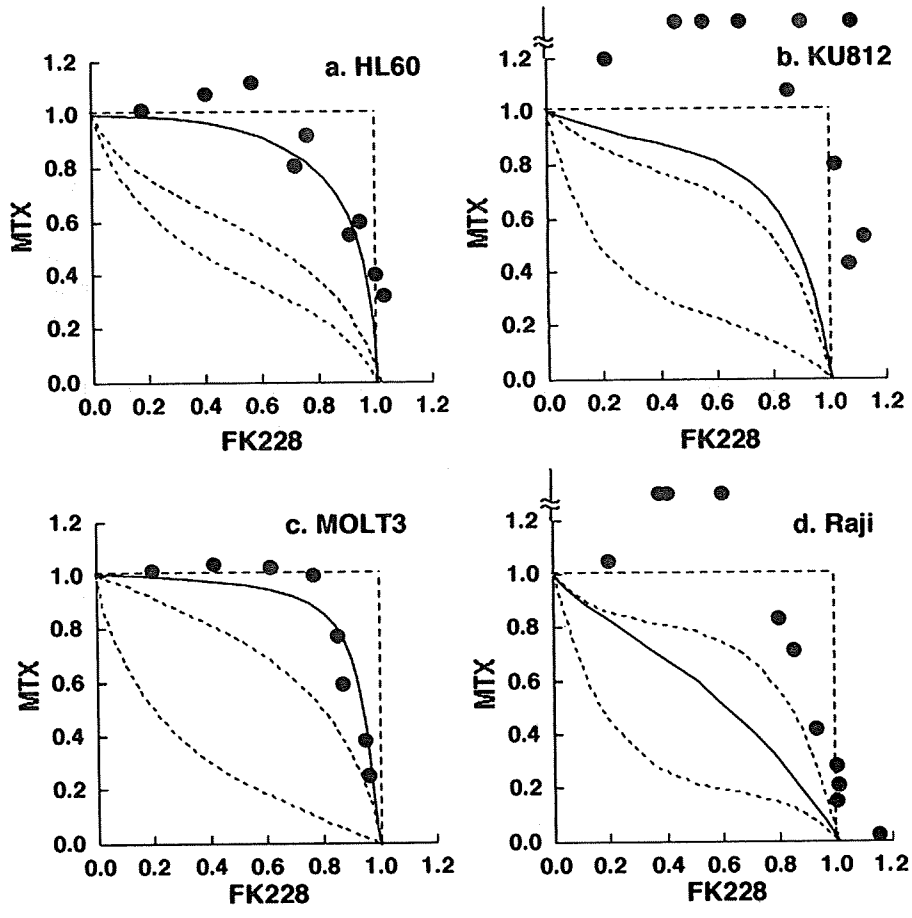

Fig. 6 Isobolograms of simultaneous exposure to FK228 and methotrexate (MTX) in HL60 (a), KU812 (b), MOLT3 (c) and Raji (d) cells. In HL60, KU812 and Raji cell, all or most data points fell in the areas of sub-additivity and protection. In MOLT3 cells, data points fell within the envelope of additivity and in the areas of sub-additivity and protection. Each point represents the mean value for 3 independent experiments; the SEs were less than 30% and thus were omitted

Cytotoxic effects of FK228 in combination with vincristine: In HL60 and MOLT3 cells, all data points fell within the envelope of additivity (isobolograms not shown), indicating that simultaneous exposure to FK228 and vincristine produced an additive effect (Table 2). In KU812 cells, data points fell within the envelope of additivity and in the area of protection. The mean value of the data was larger than that that of the predicted maximum values for an additive effect ($P > 0.05$), indicating an additive/antagonistic effect (Table 2). In Raji cells, all data points fell in the areas of sub-additivity and protection. The mean value of the observed data was larger than that of the predicted maximum additive values ($P < 0.01$), indicating an antagonistic effect of simultaneous exposure to these two agents (Table 2).

Cytotoxic effects of FK228 in combination with imatinib: We studied this combination in three Ph-positive cell lines KU812, K562 and TCC-S. All combined data points fell within the envelope of additivity (isobolograms not shown). The mean values of the data were larger than those of the predicted minimum values and smaller than those of the predicted maximum values, indicating that simultaneous exposure to FK228 and imatinib produced an additive effect (Table 2).

Discussion

FK228 is a histone deacetylase inhibitor that exerts a potent antitumor effect on various cancer cell lines *in vitro* and *in vivo* via modulation of the expression and functions of several cell cycle regulators and apoptosis-related molecules [3]. For example, FK228 causes cyclin D1 down-regulation and p53-independent p21/Waf1 induction, leading to growth arrest in the early G1 phase, while G2 arrest by FK228 is p21/Waf1-independent but associated with significant cytotoxicity [22, 23]. FK228 is also known to deplete the levels of several oncoproteins that are normally stabilized by binding to heat shock proteins in cancer cells. The resulting ability of FK228 to diminish signal transduction via pathways involving Raf-1 and ERK may contribute to the potency and specificity of this agent [24]. We have also found that FK228 inhibits the growth of malignant melanoma cells by suppressing the Raf-ERK pathway through up-regulation of Rap1, a small GTP-binding protein of the Ras family [25]. Recently, it has been reported that FK228-induced apoptosis was caspase-dependent, selectively involving the TNF-receptor initiating caspase 8 and effector caspase 3 [21, 26, 27]. In addition, FK228 increases the cellular responsiveness to IL-6 type cytokines by enhancing the expression of receptor proteins [28]. FK228 has been shown to block hypoxia-stimulated angiogenesis through suppression of HIF-1α activity [29, 30]. These observations suggest that FK228 has both direct and indirect inhibitory effects on tumor cell growth. Considering a variety of mechanisms of action of FK228, it is a reasonable approach to investigate the influence of FK228 on the effects of other anti-cancer agents and identify optimal agents that produce additive or synergistic effects with FK228 for future clinical application.

In this study, we examined the cytotoxic effects of FK228 in combination with commonly used anti-leukemia/lymphoma agents *in vitro*. We found that FK228 produced additive effects with cytarabine, carboplatin, doxorubicin, etoposide, 4-hydroperoxy-cyclophosphamide, 6-mercaptopurine and SN-38 (an active metabolite of irinotecan) in all cell lines studied. The therapeutic or clinical synergy for the combination does not necessarily follow from a discovery of the *in vitro* synergy. The combination with an additive effect has therapeutic advantages when the toxicity is less than additive for normal tissue. In other words, the additivity implies a clinical or therapeutic synergy if two drugs can be combined without unacceptable side effects. Since, however, the dose-limiting toxicity of FK228 and combined agents involves myelosuppression, there must be careful monitoring for myelosuppression during combination treatment.

In contrast, FK228 showed an antagonistic effect with methotrexate in HL60, KU812 and Raji cells and an additive/antagonistic effect in MOLT3 cells. Many data points of these combinations fell in the area of protection, suggesting that the simultaneous administration of FK228 with methotrexate has no cytotoxic advantage over the administration of each agent and thus may be inappropriate. The reasons for the antagonistic effect of this combination are at present unknown. Methotrexate mainly acts on and stops tumor cells at the early S-phase, while vincristine mainly acts on tumor cells at the S-phase and stops cells at the M-phase [31]. As shown in Fig. 2 and the previous publication [21], FK228 causes cell cycle arrest at the G1- and/or G2/M-phases. Actions of FK228 and methotrexate at difference phases of the cell cycle may be a cause of antagonistic or sub-optimal results of this combination. Furthermore, FK228 may interfere with the cytotoxic effects of methotrexate via direct influence on the expression of the molecules essential for the action of this agent. Since cytotoxic effects are often schedule-dependent, sequential exposure to FK228 followed by other agents or the reverse sequence may not show the same effects as simultaneous exposure to these agents. Indeed, pretreatment with FK228 has been reported to sensitized SW-1736 cells to doxorubicin [32]. In our experiments, we observed that sequential exposure to methotrexate followed by FK228 produced an additive effect (data not shown), suggesting that the sequential administration of methotrexate followed by FK228 is the optimal schedule of this combination.

FK228 combined with vincristine showed an additive effect in HL60 and MOLT3 cells, an additive/antagonistic effect in KU812 cells and an antagonistic effect in Raji cells. These results suggest that the cytotoxic effect of the simultaneous administration of FK228 and vincristine is antagonistic and thus sub-optimal. However, our observed data in KU812 and Raji cells were close to the predicted maximum value (Table 2), implying that the simultaneous administration of FK228 and vincristine is not always inappropriate considering clinical convenience and minimum overlapping toxicities.

We demonstrated that FK228 produced an additive effect with imatinib against 3 Ph^+ leukemia cells studied. Although no data are available regarding the combination of FK228 and imatinib, Yu et al. [33] also reported that the combined exposure of K562 and LAMA-84 cells to histone deacetylase inhibitors (SAHA and sodium butyrate) and imatinib showed synergistic effects. The discrepancy may be due to the differences in histone deacetylase inhibitors, experimental design and/or analysis used in each study. The median dose effect analysis that Yu et al. used is generous in giving synergism and antagonism [34, 35]. On the other hand, the isobologram of Steel and Peckham is stricter for synergism and antagonism. As described, an additive effect in the isobologram indicates that the cytotoxic capacity of the combination is generally much superior to that of either agent alone. Therefore, additive cytotoxic combinations have therapeutic advantages when the toxicity is less than additive for normal tissue. Since imatinib shows a minimum toxicity against normal tissues, the combination of FK228 with imatinib should produce clinical benefits.

In conclusion, FK228 has an additive effect with most anticancer drugs such as cytarabine, carboplatin, doxorubicin, etoposide, 4-hydroperoxy-cyclophosphamide, 6-mercaptopurine, SN-38 and imatinib in simultaneous administration, suggesting that FK228 is a promising candidate for combination with these agents. However, the simultaneous administration of FK228 with methotrexate or vincristine seems to be inappropriate. Continued preclinical and clinical studies would provide further insights and assist in the establishment of optimal combinations and schedules of FK228 in clinical application.

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Phase I Trial of FLAGM with High Doses of Cytosine Arabinoside for Relapsed, Refractory Acute Myeloid Leukemia: Study of the Japan Adult Leukemia Study Group (JALSG)

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Abstract

This study was designed to determine the optimal high dose for cytosine arabinoside (ara-C) in combination with fludarabine, granulocyte colony-stimulating factor, and mitoxantrone (FLAGM) in adult patients with relapsed or refractory acute myeloid leukemia. Nine patients were enrolled at increasing dosage levels of ara-C (8, 12, and 16 g/m^2 per dose level). Ara-C and fludarabine were administered once a day at level 1, once or twice a day at level 2, and twice a day at level 3. All patients had grade 4 hematologic toxicity. The most common adverse events were of grade 2 or less, with nausea and vomiting being the most common (6 events), followed by diarrhea (5 events), and rash (5 events). Of the 13 grade 3 nonhematologic toxicities reported, the 2 most common were febrile neutropenia (6 events) and disseminated intravascular coagulation (3 events). No early deaths were observed. FLAGM with high-dose ara-C was considered safe for patients, and the recommended dosage of ara-C in this study was 2 g/m^2 every 12 hours for a total dose of 16 g/m^2.
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Key words: AML; Ara-C; FLAGM therapy; Cytarabine; High-dose ara-C; Phase I study

1. Introduction

Treatment for acute myeloid leukemia (AML) has improved over the years since the addition of cytosine arabinoside (ara-C) to anthracycline therapy, which has enabled 70% to 80% of patients to achieve complete remission (CR). Even patients treated with this combination show long-term survival rates of only approximately 30%, however, and relapses occur in many patients [1,2]. In patients who relapse or have refractory disease, salvage therapy is imperative for long-term survival [3]. One type of salvage therapy is high-dose ara-C. Rudnick and colleagues [4] reported that 1 to 7.5 g/m^2 of ara-C is effective in refractory AML patients. Miyawaki et al [5] conducted a phase II study in which 2 g/m^2 of ara-C administered every 12 hours for a total of 24 g/m^2 was shown to be effective in patients with relapsed and refractory AML.

Arabinosylcytosine 5′-triphosphate (ara-CTP) is a metabolite of ara-C; studies have shown that fludarabine, a purine nucleoside analogue, can augment ara-CTP

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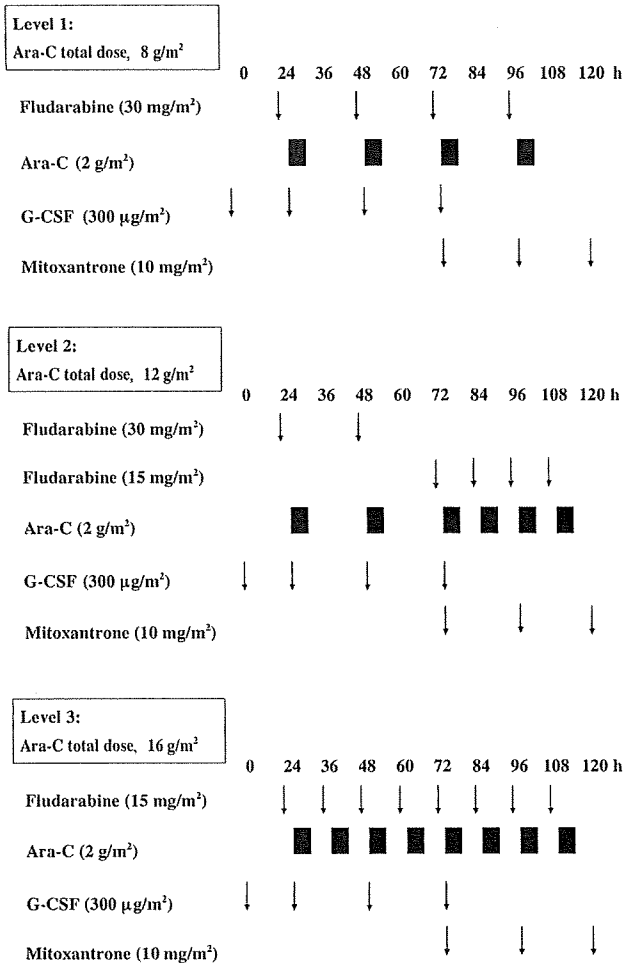

Figure 1. Dosing schedule for the 3 dosage levels. At all dosage levels, granulocyte colony-stimulating factor (G-CSF) was administered in 4 doses every 24 hours, beginning at the start of therapy, and mitoxantrone was administered in 3 doses every 24 hours, beginning 72 hours after the start of therapy. Ara-C (total dose per dosage level: 8, 12, and 16 g/m^2) was administered every 12 or 24 hours, beginning 24 hours after the start of therapy, and fludarabine (30 mg/m^2 once daily or 15 mg/m^2 twice daily) was administered 4 hours before each ara-C dose.

accumulation in leukemic cells [6-9]. This combination of fludarabine and ara-C was studied by Estey et al [10] in patients with newly diagnosed AML or myelodysplastic syndromes, and a CR rate of 53% was achieved. Other investigators also examined this combination therapy in patients with relapsed and refractory AML and achieved similar CR rates (28%-59%) [11-13]. The total-dose range of ara-C administered in these studies was 3 to 10 g/m^2. Because higher doses of ara-C have successfully been used to treat AML patients, the current phase I study was designed to determine the optimal high dose for ara-C in FLAGM, a combination with fludarabine, granulocyte colony-stimulating factor (G-CSF), and mitoxantrone, in patients with recurrent or refractory AML. In a high-dose ara-C regimen, ara-C is generally administered every 12 hours; therefore, fludarabine was administered twice a day. The optimal doses derived from the results of this study will be used in phase II studies.

2. Materials and Methods

The present study was conducted from October 2001 to June 2002 at 8 institutions belonging to the Refractory Leukemia Committee of the Japan Adult Leukemia Study Group (JALSG). Registration of the participants began after consent was obtained by the Ethics Committee or the Institutional Review Board of each institution.

2.1. Study Population

Patients who had recurrent AML (excluding M3 and hybrid leukemia) after a CR or who had failed 2 courses of standard induction therapy were enrolled in the study. M3 was excluded because this disease entity was treated with a specific regimen, and hybrid leukemia was excluded because it was not included in the AML category.

To be eligible, patients were required to meet the following criteria: having had an interval of ≥4 weeks before treatment; having a performance status of 0 to 2; being older than 18 years but younger than 65 years; having a life expectancy of ≥2 months and no major organ dysfunction (hemoglobin ≥9.0 g/dL; platelets ≥20,000 × 10^9/L; leukocytes ≥2000 × 10^9/L; total bilirubin ≤1.5 mg/dL; liver function tests ≤3 times the normal maximum value used by each institution; and serum creatinine ≤1.5 mg/dL); and having an arterial blood oxygen saturation ≥90%. All patients were required to provide written consent at the start of receiving the study medication.

2.2. Study Design and Treatment

As shown in Figure 1, 3 cohorts at 3 ara-C dosage levels (8 g/m^2, 12 g/m^2, and 16 g/m^2) were set in this study, and the administration of ara-C was started at 8 g/m^2 by the dose-escalation method. Each ara-C administration was given as a 3-hour infusion. G-CSF was subcutaneously administered at every dosage level at the start of treatment and was given every 24 hours for a total of 4 doses. Fludarabine (30 mg/m^2 once daily or 15 mg/m^2 twice daily) was administered as a 30-minute infusion 4 hours before the ara-C dose. The total fludarabine dose administered at each level was 120 mg/m^2. Lastly, 10 mg/m^2 mitoxantrone was administered as a 30-minute infusion for a total of 3 doses every 24 hours, beginning 72 hours after the start of therapy.

The sample size for each cohort was set at 3 patients. When the critical toxicity was observed in 1 of the 3 patients, 3 more patients were added to that cohort. When the critical toxicity was not seen in any of the 3 patients or was seen in 1 of the 6 patients, the dose was increased for the next cohort. Finally, when the critical toxicity was encountered in 2 of the 6 patients, the maximum tolerated dose was considered to have been reached in that cohort.

The treatment schedule for this study was derived from that of a phase II study in which 2 g/m^2 of ara-C was administered every 12 hours for a total of 12 doses

Table 1.
Demographic and Baseline Clinical Characteristics*

Dosage Level	Age, y	FAB Classification	Status	Karyotype (MRC Classification)	Duration of CR, mo	WBC, $\times 10^9$/L (blasts, %)	Nucleated Cell Count in BM, $\times 10^9$/L (blasts, %)
1	21	M4	Relapse 1	46,XY,del(12)(p?) (I)	11	9890 (47.0)	3.8 (40.4)
1	37	M2	Relapse 1	46,XY,t(6;9)(p23;q34),47,idem,+13 (I)	4	14,470 (50.0)	12.6 (78.8)
1	33	M2	Relapse 1	46,XY,del(1)(p?),add(3)(q21),add(5)(q22) 46,idem,add(7)(q32),add(9)(p13) 47,idem,+Y (P)	19	3800 (13.5)	19.7 (15.0)
2	57	M5b	Relapse 1	46,XY,t(2;3)(p23;q29) (I)	14	5770 (38.0)	7.5 (94.0)
2	60	M4	Relapse 2	46,XY (I)	18	20,630 (75.0)	7.6 (72.8)
2	29	M2	Relapse 1	46,XX,t(8;21)(q22;q22) (F)	24	2600 (34.0)	NA (30.0)
3	41	M5a	Relapse 1	46,X,add(Y)(p11),del(5)(p?),add(8)(q22) (I)	34	2100 (14.5)	8.9 (94.0)
3	51	M2	Relapse 1	46,XY (I)	16	5400 (0)	91.7 (10.4)
3	55	M4	Relapse 1	46,XY (I)	13	40,100 (80.0)	NA

*FAB indicates French-American-British; MRC, Medical Research Council; CR, complete remission; WBC, white blood cells; BM, bone marrow; I, intermediate; P, poor; F, favorable; NA, not available.

(total dose, 24 g/m^2) [5]. Treatment-related deaths occurred in 5 of 46 patients in that study. Therefore, fludarabine and mitoxantrone were given concurrently in the present study, and 16 g/m^2 was taken to be the maximum administrable dose of ara-C. If none of the 3 patients or 1 of the 6 patients showed critical toxicity at dose level 3, the trial was terminated without further increase in the dose.

2.3. Supportive Care

Inhaled amphotericin B, amphotericin B syrup, and nystatin were administered to neutropenic patients to prevent airway, oral, and esophageal fungal infections. Oral polymyxin B sulfate was administered to limit colonization in the gastrointestinal tract, and the prophylactic use of isoniazid was prescribed to patients who had a history of tuberculosis. Platelets were supplemented as needed to maintain a platelet count ≥20,000 × 10^9/L, and G-CSF was administered within the scope of the protocol guidelines.

2.4. Safety Evaluations and Study End Points

Safety was the primary study end point, and adverse events were graded according to the National Cancer Institute Common Toxicity Criteria. The critical toxicity was decided as follows: (1) grade 3 or higher nonhematologic toxicity (except for nausea and vomiting, loss of appetite, diarrhea, infection, or fever of grade 4); and (2) early death (defined as death occurring within 2 months after the start of treatment). The secondary end points included the type, degree, and frequency of adverse events of grade 1 or 2, and the efficacy of treatment. For the assessment of efficacy, the JALSG criteria were followed [2]. A CR was established when observations of fewer than 5% blasts in normocellular marrow were accompanied by a normal level of peripheral blood neutrophils (>1200 × 10^9/L) and a normal platelet count (>100,000 × 10^9/L). The definition of partial remission was established when a decrease of at least 50% in the percentage of blasts, to between 5% and 25%, was observed in the bone marrow aspirate.

Table 2.
Summary of Adverse Events*

	Dosage Level 1 (n = 3)					Dosage Level 2 (n = 3)					Dosage Level 3 (n = 3)				
	Grade, n					Grade, n					Grade, n				
	1	2	3	4	Total, n (%)	1	2	3	4	Total, n (%)	1	2	3	4	Total, n (%)
Diarrhea	2	0	0	0	2 (67)	0	1	0	0	1 (33)	2	0	0	0	2 (67)
DIC	0	0	1	0	1 (33)	0	0	0	0	0 (0)	0	0	2	0	2 (67)
Fever (allergy)	0	0	0	0	0 (0)	2	0	0	0	2 (67)	0	1	0	0	1 (33)
Hyperglycemia	0	0	0	0	0 (0)	0	0	0	0	0 (0)	0	0	1	0	1 (33)
Nausea/vomiting	3	0	0	0	3 (100)	0	2	0	0	2 (67)	0	1	0	0	1 (33)
Febrile neutropenia	0	0	2	0	2 (67)	0	0	1	0	1 (33)	0	0	3	0	3 (100)
Rash	1	0	0	0	1 (33)	0	2	0	0	2 (67)	1	1	0	0	2 (67)
Sepsis	0	0	1	0	1 (33)	0	0	0	0	0 (0)	0	0	0	0	0 (0)
SGOT elevation	0	0	0	0	0 (0)	0	0	1	0	1 (33)	1	0	0	0	1 (33)
SGPT elevation	1	0	0	0	1 (33)	0	0	1	0	1 (33)	2	0	0	0	2 (67)
Stomatitis	0	0	0	0	0 (0)	1	0	0	0	1 (33)	0	1	0	0	1 (33)

*DIC indicates disseminated intravascular coagulation; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase.

Table 3.
Overall Outcome*

Dosage Level	Early Death, n	Response			WBC Nadir, $\times 10^9$/L	Duration of WBC $<1000 \times 10^9$/L, d	Duration of Plt $>10 \times 10^{13}$/L, d	Death, n (%)
		CR, n	PR, n	Overall, n (%)				
1 (n = 3)	0	1	0	1 (33)	70, 100, 270	15, 36, 43	—, 75, —	3 (100)
2 (n = 3)	0	3	0	3 (100)	50, 150, 160	14, 18, 22	18, 22, 35	1 (33)
3 (n = 3)	0	1	2	3 (100)	100, 100, 110	15, 18, 30	22, 33, 38	0 (0)
Total	0	5	2	7 (78)	Median = 100	Median = 18	Median = 33	4 (44)

*CR indicates complete remission; PR, partial remission; WBC, white blood cells; Plt, platelets.

3. Results

3.1. Demographic and Baseline Characteristics

Nine AML patients were enrolled, and all were eligible for this study. Their demographics and baseline characteristics are shown in Table 1. The median age was 41 years (range, 21-60 years). Eight of the 9 patients were in their first relapse, and 1 patient had a karyotype aberration involving core-binding factor [14].

3.2. Safety

No early deaths occurred within 2 months after the start of treatment. Grade 4 leukopenia ($<1000 \times 10^9$/L) was seen in all patients. The median leukocyte count was 100×10^9/L (range, 50-270 $\times 10^9$/L), and the median period for which the count was $<1000 \times 10^9$/L was 18 days (range, 14-43 days). The leukocyte count and the period over which that count was $<1000 \times 10^9$/L were not related to the ara-C dose.

The most commonly reported adverse events were of grade 2 or less, with nausea and vomiting being the most common (6 events), followed by diarrhea (5 events) and rash (5 events) (Table 2). Of the 13 grade 3 nonhematologic toxicities reported, the most common were febrile neutropenia (6 events) and disseminated intravascular coagulation (DIC) (3 events). Of the 3 DIC events, 2 were considered to be related to AML, and 1 was related to cytomegalovirus infection. In addition, 1 case of grade 3 sepsis was seen. Because DIC is a type of hematologic toxicity and because sepsis is caused by an infection, these adverse events were judged not to fall under the heading of critical toxicities as defined in the present study. Grade 3 hyperglycemia was detected after the administration of steroid drugs, and grade 3 increases in serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase concentrations were seen after the administration of antibiotics. One patient developed hepatotoxicity 1 month following chemotherapy. This patient had experienced hepatotoxicity and skin eruptions caused by the same antibiotics during previous chemotherapy. Accordingly, these events were considered to have had no causal relationship to the FLAGM therapy. Therefore, no critical toxicity attributable to this study was seen in any cohort.

3.3. Response to Dosing Regimens

The overall response rate in the study was 78% (7 cases), and the overall responses are summarized in Table 3. One patient (33%) achieved CR at dose level 1, 3 patients (100%) achieved CR at dose level 2, and 1 patient (33%) achieved a CR at dose level 3. In addition, 2 patients at dose level 3 had a partial response, and 2 patients at dose level 1 showed resistant disease. During the follow-up period, 2 patients who received doses at level 1 died from progressive disease, and 2 patients (1 each from dose levels 1 and 2) died from complications arising from a transplant received after having achieved CR.

4. Discussion

With the goal of improving response rates and long-term survival in patients with AML, treatments with new drugs such as mitoxantrone [11-13] and idarubicin [15-17] have recently been added to FLAG therapy (fludarabine, ara-C, and G-CSF). Because idarubicin is used in Japan as induction therapy for AML, this study developed the FLAGM regimen to determine the optimal dose for high-dose ara-C.

The fludarabine dosage in this study was 30 mg/m^2 administered once a day or 15 mg/m^2 twice daily. This dosing regimen was based on the results of Gandhi et al [18], who determined that both regimens would maximize ara-CTP accumulation in AML blasts. Studies have shown that numerous central nervous system adverse events can occur at ara-C dosages of 3 g/m^2 administered twice daily for 6 days (total dose, 36 g/m^2) [19]. In our previous phase II study of ara-C in which 2 g/m^2 was administered twice daily for 6 days (total dose, 24 g/m^2), we observed 5 deaths that were attributable to the treatment among a total of 46 cases [5]. In the present study, when we considered that fludarabine and mitoxantrone were to be administered concurrently with ara-C, we strictly fixed the maximum dose at 16 g/m^2.

As expected, the main adverse events were hematologic toxicities and febrile neutropenia, but both were manageable with supportive care. Neither reduction of the leukocyte count nor prolongation of the period of leukopenia due to higher ara-C doses was observed. In a study with a dosing regimen similar to that in our study, Hanel et al [13] demonstrated that the median period during which the leukocyte count was $<500 \times 10^9$/L was 21 days (range, 4-51 days), which was similar to the present results (ie, $\leq 1000 \times 10^9$/L

leukocytes for 18 days; range, 14-43 days). In another study of high-dose ara-C in patients with relapsed or refractory AML, the median period during which the leukocyte count was $<1000 \times 10^9$/L was 19 days [5]; this period was also similar to our results. The nonhematologic toxicities were manageable, and no central nervous system toxicity was observed. Koller et al [12] conducted a study in which fludarabine, ara-C, and comparable doses of mitoxantrone were administered concurrently; hyperbilirubinemia was reported in approximately 60% of the patients. In the present study, although 1 case of grade 3 liver failure was reported, no adverse events of a high bilirubin concentration were observed. Clavio et al [11] treated poor-risk AML patients with the same drug combination and the same mitoxantrone doses that we used in our protocol, and they found no patient with hyperbilirubinemia, as was the case in our study. The difference between the protocol of Koller et al and those used in the study of Clavio et al and our study is the dosage of mitoxantrone administered. Given this difference, a low mitoxantrone dose may be closely correlated with an absence of patients with hyperbilirubinemia.

Although the CR rate achieved in this study was 56%, the number of patients was small. In another study of high-dose ara-C therapy, however, the reported CR rate was 45.7%, and the remission rate was 51.4%. Therefore, it is possible that the FLAGM therapy regimen used in this study is more efficacious than the regimen of high-dose ara-C therapy. We conducted a phase I study for the purpose of selecting doses of high-dose ara-C for FLAGM therapy in patients with relapsed or refractory AML. The results of the study showed a high degree of effectiveness at dose levels 2 and 3, and we observed no treatment-related mortality at any dosage level. Therefore, the treatment was considered well tolerated. At the ara-C dose that we presumed to be the maximum administrable dose, 16 g/m^2, we observed no critical toxicity attributable to the study, and we therefore concluded that the recommended dosage for ara-C for phase II clinical trials should be 2 g/m^2 administered twice daily for 4 days, for a total dose of 16 g/m^2.

This regimen is currently being evaluated in phase II studies. However, the safety of this regimen should be continually evaluated in the phase II study because of the relatively small number of patients included in the phase I study.

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Case Report

CD20- and CD56-Positive T-Cell Large Granular Lymphocyte Leukemia in a Human T-Cell Leukemia Virus Type 1 Carrier

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Abstract

A 60-year-old man was diagnosed with asymptomatic T-cell granular lymphocyte (T-LGL) leukemia in September 2006. He was serologically positive for human T-cell leukemia virus type 1 (HTLV-1). However, monoclonal integration of the HTLV-1 genome was not detected in the peripheral blood, suggesting that HTLV-1 did not contribute to the pathogenesis of T-LGL leukemia in the present case. Phenotypically, neoplastic cells of our case were $CD3^+$, $CD4^-$, $CD8^+$, $CD16^-$, $CD56^+$, $CD57^-$, and T-cell receptor (TCR) $\alpha\beta^+$. They also coexpressed CD20 antigen with weak intensity. This represented a unique case of T-LGL leukemia showing a typical clinical and phenotypic features.

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Key words: T-cell granular lymphocyte (T-LGL) leukemia; Human T-cell leukemia virus type 1 (HTLV-1); Adult T-cell leukemia lymphoma (ATLL); CD20 antigen; CD56 antigen

1. Introduction

T-cell large granular lymphocyte (T-LGL) leukemia is characterized by a persistent increase in the number of peripheral blood large granular lymphocytes (LGLs) without any clearly identified causes [1]. Patients with T-LGL leukemia often show recurrent infections secondary to chronic neutropenia, severe anemia sometimes complicated with pure red cell aplasia, and autoimmune diseases such as rheumatoid arthritis [2-5]. On the other hand, substantial numbers of patients with T-LGL leukemia are asymptomatic at presentation [3-5]. Phenotypically, the majority of T-LGL leukemia cells have mature T-cells showing $CD4^-$, $CD8^+$, $CD16^+$, $CD56^-$, $CD57^+$, and T-cell receptor (TCR) $\alpha\beta^+$, though other rare phenotypic variants have also been reported [4-6]. To the contrary, human T-cell leukemia virus type 1 (HTLV-1) has been known to affect mostly mature T-cells of the $CD4^+/CD8^-$ phenotype and cause adult T-cell leukemia lymphoma (ATLL) [7,8]. In this paper, we report an HTLV-1 carrier who was incidentally diagnosed as having asymptomatic $CD4^-$, $CD8^+$, $CD16^-$, $CD56^+$, $CD57^-$, $TCR\alpha\beta^+$, $CD20^+$ T-LGL.

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2. Case Report

A 60-year-old man, who was born in Wakayama, visited our hospital in September 2006 because of mild lymphocytosis persisting for more than 1 year. He showed neither skin eruptions nor lymph node swelling. Hepatosplenomegaly was also absent. Laboratory findings were as follows: hemoglobin, 14.9 g/dL; platelets, 282×10^9/L; white blood cells, 8×10^9/L, with 68% lymphoid cells. Morphologically, the majority of these lymphoid cells were large, with abundant cytoplasm, and contained of fine or sometimes coarse azurophilic granules, which seemed characteristic to large granular lymphocytes (Figure 1). On the other hand, abnormal lymphoid cells with irregular-shaped nuclei suggesting ATLL cells were rarely found (less than 1%). The bone marrow was normocellular with 44% LGL and less than 1% ATLL-like cells. Immunophenotypically, the majority of lymphoid cells in both the peripheral blood and bone marrow were $CD1a^-$, $CD2^+$, $CD3^+$, $CD4^-$, $CD5^+$, $CD7^+$, $CD8^+$, $CD10^-$, $CD16^-$, $CD19^-$, $CD25^-$, $CD56^+$, $CD57^-$, HLA-DR^-, $TCR\alpha\beta^+$, and

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Clinical features and outcome of T-lineage acute lymphoblastic leukemia in adults: A low initial white blood cell count, as well as a high count predict decreased survival rates

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Abstract

Although biological and clinical features differ between B-lineage acute lymphoblastic leukemia (ALL) and T-lineage ALL (T-ALL), there have been few reports that focused on the prognosis for T-ALL in adults, primarily due to its rarity. Here, we studied the long-term outcomes and prognostic factors specific for adult T-ALL by combining patient data from the three prospective trials conducted by the Japan Adult Leukemia Study Group (JALSG). Among 559 patients whose immunophenotypes could be evaluated, 87 (15.6%) were identified as T-ALL. Of them, 66 patients (75.8%) achieved complete remission, and relapse occurred in 41 patients. With a median follow-up for surviving patients of 7.5 years, the probability of overall survival was 35.0% at 5 years. Risk factor analysis revealed that serum albumin levels, initial white blood cell (WBC) counts, and age had independent values for predicting survival. For WBC, not only the high-count group ($50 \times 10^9\,l^{-1}$ or higher), but also the low-count group (less than $3 \times 10^9\,l^{-1}$) showed a significantly lower survival rates than the intermediate-count group ($p = 0.0055$ and 0.0037, respectively). Although our findings need confirmation, these results will be helpful in the identification of prognostically distinct subgroups within adult T-ALL.

Keywords: Acute lymphoblastic leukemia; T-ALL; Survival; Prognostic factor; White blood cell count

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1. Introduction

Recent clinical trials have shown that although 70–90% of adult patients with acute lymphoblastic leukemia (ALL) achieve complete remission (CR), the percentage of long-term survivors is not much improved [1–7]. Despite intensive induction and post-remission chemotherapy, a majority of remitters eventually relapse, and the outcome for relapsed patients is almost exclusively grim. Several factors have been reported as affecting the outcome of the disease, including age, initial white blood cell (WBC) count, time to achieve CR, immunophenotype, and cytogenetics [8,9], most of which were, however, identified from the analysis of the entire cohort of each study. Adult ALL represents a heterogeneous disease, and it is well recognized that biological and clinical features differ between B-lineage ALL and T-lineage ALL (T-ALL) [10–12]. Because T-ALL accounts for only around 20% of adult ALL, commonly used prognostic factors for ALL may not be necessarily applicable to T-ALL. Owing to its rarity, there have been few reports that have studied a large group of adult T-ALL patients. Under these circumstances, we analyzed the data of 87 T-ALL patients that had been entered into previous ALL trials conducted by the Japan Adult Leukemia Study Group (JALSG), investigated clinical features and long-term outcomes, and identified the prognostic factors specific for T-ALL in adults.

2. Patients and methods

2.1. Patients

All patients were subjects of one of the three prospective trials conducted by the JALSG; the ALL90 (1990–1993) [13], ALL93 (1993–1997) [6], and ALL97 studies (1997–2001). For all the trials, newly diagnosed, previously untreated ALL patients were eligible if they were 15 years or older, and showed adequate heart, lung, liver, and renal function. Informed consent was obtained from all participants before their enrollment. Diagnosis of ALL was carried out according to the French–American–British (FAB) classification [14], and confirmed by the Central Review Committee. Patients who were immunophenotyped and met the definition for T-ALL on the basis of the criteria described below were considered for the subsequent analysis.

2.2. Treatment

Details of each treatment schedule are described in Table 1. For the ALL90 study, induction therapy consisted of six drugs; doxorubicin (ADR), vincristine (VCR), cyclophosphamide (CPM), L-asparaginase (L-ASP), prednisolone (PSL), and mitoxantrone (MIT). Patients with CR received four courses of consolidation and maintenance/intensification therapy. For the ALL93 study, instead of omitting MIT for induction, the dose-intensity of ADR was increased by more frequent administration on days 1–3, and 8–10. After completion of three courses of consolidation, patients were randomized to receive early sequential or intermittent intensification during maintenance therapy. For the ALL97 study, induction therapy comprised the five drugs similar to the ALL93 study. After achieving CR, patients received eight courses of consolidation featuring dose-intensified ADR and CPM, and intermediate-dose methotrexate (MTX), followed by maintenance therapy. Central nervous system (CNS) prophylaxis was given by means of intrathecal (IT) injection of MTX, cytarabine (Ara-C) and steroids during both consolidation and intensification courses. Patients with symptomatic or cytological evidence of CNS leukemia received additional IT injections. Prophylactic whole cranial irradiation was given at a total dose of 20–24 Gy to patients either with cytologically diagnosed CNS leukemia or with high initial WBC counts ($100 \times 10^9\,l^{-1}$ or higher for the ALL90/ALL93 studies and $50 \times 10^9\,l^{-1}$ or higher for the ALL97 study).

2.3. Definition

CR was defined as the presence of all of the following: less than 5% of blasts in bone marrow (BM), no leukemic blasts in peripheral blood (PB), recovery of PB values to a neutrophil count of at least $1.5 \times 10^9\,l^{-1}$ and a platelet count of at least $100 \times 10^9\,l^{-1}$, and no evidence of extramedullary leukemia. Patients who failed to attain CR after two courses of induction therapy were regarded as failure cases. Relapse was defined as the presence of at least one of the following; recurrence of more than 10% leukemic cells in BM or of any leukemic cells in PB or extramedullary sites. Performance status was assessed on the basis of criteria from the Eastern Cooperative Oncology Group (ECOG). Surface markers were considered positive when more than 20% of blasts expressed the antigens. The immunophenotype was classified according to criteria from the Cancer and Leukemia Group B (CALGB) [11]. T-lineage ALL was defined as the presence of either (1) CD2 or CD7 positivity combined with positivity of CD1, CD3, CD4, CD5, CD8; or (2) CD5 positivity without CD19 or CD20 positivity. Myeloid antigen positivity was defined as positive expression of either or both of CD13 and CD33.

2.4. Statistical analysis

Kaplan–Meier analysis was used to estimate the probabilities of overall survival (OS) and event-free survival (EFS). OS was defined as the time from the first day of therapy to death or last visit, and EFS as the time from the first day of therapy to induction failure, relapse, death, or last visit. For EFS, patients who failed to achieve CR were categorized as failure cases at time zero. Patients undergoing hematopoietic stem cell transplantation (HSCT) were not censored at the time of transplantation unless indicated. Differences

Table 1
Treatment schedules

ALL90 study	ALL93 study	ALL97 study	
Remission induction			
VCR 1.4 mg m^{-2} IV × 3–4	VCR 1.3 mg m^{-2} IV × 4	VCR 1.3 mg m^{-2} IV × 4	
ADR 25 mg m^{-2} IV × 1–3	ADR 30 mg m^{-2} IV × 6	DNR 45 mg m^{-2} IV × 3	
CPM 600 mg m^{-2} IV × 1–2	CPM 600 mg m^{-2} IV × 1	CPM 1200 mg m^{-2} IV × 1	
PSL 40–60 mg m^{-2} PO × 14	PSL 40 mg m^{-2} PO × 10	PSL 60 mg m^{-2} PO × 14	
L-ASP 5000 IU m^{-2} IV or SC × 0–2	L-ASP 6000 IU m^{-2} IV × 7	L-ASP 3000 IU m^{-2} IV × 6	
MIT 6 mg m^{-2} IV × 1–3			
Consolidation			
ETP 100 mg m^{-2} IV × 5	MIT 6 mg m^{-2} IV × 3	C-1	C-5
BHAC 200 mg m^{-2} IV × 5	ETP 100 mg m^{-2} IV × 5	VCR 1.3 mg m^{-2} IV × 1	Same as C-1 except for substituting
VDS 2 mg m^{-2} IV × 1	Ara-C 100 mg m^{-2} IV × 6	ADR 60 mg m^{-2} IV × 1	DEX 10 mg m^{-2} PO × 3 for PSL
PSL 40 mg m^{-2} PO × 5	IT × 1	CPM 1000 mg m^{-2} IV × 1	
IT × 1		PSL 60 mg m^{-2} PO × 3	
	MTX 600 mg m^{-2} IV × 2	IT × 1	C-6
MIT 7 mg m^{-2} IV × 3	L-ASP 10000 IU m^{-2} IV × 2		Same as C-2 except for substituting
BHAC 200 mg m^{-2} IV × 5	IT × 1	C-2	DEX 10 mg m^{-2} PO × 3 for PSL
IT × 1		MTX 500 mg m^{-2} IV × 1	
	ACR 14 mg m^{-2} IV × 8	VCR 1.3 mg m^{-2} IV × 1	
CPM 800 mg m^{-2} IV × 1	Ara-C 70 mg m^{-2} IV × 7	ADR 45 mg m^{-2} IV × 1	C-7
ACR 50 mg m^{-2} IV × 2	PSL 40 mg m^{-2} PO × 7	PSL 60 mg m^{-2} PO × 3	Same as C-5
VDS 2 mg m^{-2} IV × 1	IT × 1	IT × 1	
PSL 40 mg m^{-2} PO × 5			C-8
IT × 1		C-3	Same as C-4 except for substituting
		Same as C-1	MIT 8 mg m^{-2} IV × 2 for ETP
MTX 400 mg m^{-2} IV × 1			
L-ASP 6000 IU m^{-2} IM or SC × 2		C-4	
		ETP 100 mg m^{-2} IV × 4	
		Ara-C 200 mg m^{-2} IV × 4	
		6MP 60 mg m^{-2} IV × 4	
		PSL 60 mg m^{-2} PO × 4	
		IT × 1	
Intensification			
DNR 30 mg m^{-2} IV × 3	ADR 30 mg m^{-2} IV × 6		
VDS 2 mg m^{-2} IV × 2	VCR 1.3 mg m^{-2} IV × 3		
CPM 700 mg m^{-2} IV × 2	PSL 30 mg m^{-2} PO × 10		
PSL 40–60 mg m^{-2} PO × 14			
IT × 1	MIT 6 mg m^{-2} IV × 3		
	ETP 100 mg m^{-2} IV × 5		
MIT 6 mg m^{-2} IV × 3	Ara-C 100 mg m^{-2} IV × 6		
VDS 2 mg m^{-2} IV × 2	IT × 1		
CPM 700 mg m^{-2} IV × 2			
PSL 40–60 mg m^{-2} PO × 14	MTX 600 mg m^{-2} IV × 2		
IT × 1	L-ASP 10000 IU m^{-2} IV × 2		
	IT × 1		
ADR 20 mg m^{-2} IV × 3			
VDS 2 mg m^{-2} IV × 2	ACR 14 mg m^{-2} IV × 8		
CPM 700 mg m^{-2} IV × 2	Ara-C 70 mg m^{-2} IV × 7		
PSL 40–60 mg m^{-2} PO × 14	PSL 40 mg m^{-2} PO × 7		
IT × 1	IT × 1		
Maintenance			
6MP 60 mg m^{-2} PO daily	6MP 60 mg m^{-2} PO daily	VCR 1.3 mg m^{-2} IV monthly	
MTX 20 mg m^{-2} PO weekly	MTX 20 mg m^{-2} PO weekly	PSL 60 mg m^{-2} PO × 5 monthly	
		6MP 60 mg m^{-2} PO daily	
		MTX 20 mg m^{-2} PO weekly	

Maximum dose of VCR was 2.0 mg/body. For remission induction in the ALL90 study, number of doses for each drug was determined according to the findings of serial bone marrow aspirations. Drugs used for IT injection were MTX 15 mg/body, Ara-C 40 mg/body and PSL 10 mg/body in the ALL90/ALL93 studies, and MTX 15 mg/body and DEX 4 mg/body with or without Ara-C 40 mg/body in the ALL97 study. ALL, acute lymphoblastic leukemia; VCR, vincristine; ADR, doxorubicin; CPM, cyclophosphamide; PSL, prednisolone; L-ASP, L-asparaginase; MIT, mitoxantrone; DNR, daunorubicin; ETP, etoposide; BHAC, behenoyl-ara-C; VDS, vindesine; DEX, dexamethasone; Ara-C, cytarabine; ACR, aclarubicin; MTX, methotrexate; 6MP, 6-mercaptoprine; IV, intravenous; PO, oral; SC, subcutaneous; IM, intramuscular; IT, intrathecal.

between the curves were compared using a log-rank test. For risk factor analysis, a multivariate Cox proportional hazards model was constructed for OS, and a logistic regression model for CR achievement. Variables with p-values of less than 0.10 by log-rank test for OS, and in univariate logistic analysis for CR achievement were included in the respective final multivariate model. A hazard ratio (HR) and an odds ratio (OR) were calculated in conjunction with a 95% confidence interval (CI). Stata Version 8 software (Stata-Corp, College Station, TX, USA) was used for all statistical analyses.

3. Results

3.1. Patients

Among 559 patients whose immunophenotypes were evaluable, 87 (15.6%) were identified as T-ALL. Baseline characteristics of the 87 patients are summarized in Table 2. The median age was 26 years (range, 15–60 years), with 60 males and 27 females. Involvements in CNS, skin, and mediastinum were detected in 7.0, 4.6 and 17.2%, respectively. Of the 60 patients for which cytogenetic information was available, 34 showed abnormal karyotype, including del(5q) in 4, del(6q) in 3, del(9p) in 3, del(11q) in 3, t(11;14) in 2, t(1;12) in 2, trisomy 8 in 2, and del(12p) in 2 patients.

3.2. Response to induction therapy

In total, 66 patients (75.8%) achieved CR after one course of remission induction therapy ($n=59$), or two courses ($n=7$). Of the remaining 21 patients, toxicity-related death during induction therapy occurred in four (4.6%). Their causes of death were sepsis ($n=2$), intracranial hemorrhage ($n=1$), and liver failure ($n=1$). Multivariate analysis indicated two factors were significantly associated with CR achievement. Patients aged 30 or older had a greater risk of induction failure (OR: 5.13, 95% CI: 1.52–17.5, $p=0.009$), as did those whose serum albumin level was lower than 3.5 g dl^{-1} (OR: 6.71, 95% CI: 1.64–27.4, $p=0.008$). Other variables such as initial WBC count, sex, performance status, and any surface markers did not correlate with CR achievement.

Table 2
Presenting characteristics

	$n=87$
Age (years)	26 (15–60)
Sex: male/female	60/27
FAB type: L1/L2	27/60
WBC count ($\times 10^9\,l^{-1}$l)	17.1 (0.3–396)
RBC count ($\times 10^{12}\,l^{-1}$)	3.20 (1.49–6.33)
Platelet count ($\times 10^9\,l^{-1}$)	57 (4–341)
Performance status: 0–1/2–3	66/17
CNS involvement: present/absent	6/80
Skin involvement: present/absent	4/83
Mediastinal involvement: present/absent	15/72
Karyotype: normal/abnormal/NE	26/34/27

Values are presented as median (range) unless indicated. FAB, French–American–British; WBC, white blood cell; RBC, red blood cell; CNS, central nervous system; NE, not evaluable (not carried out or failed).

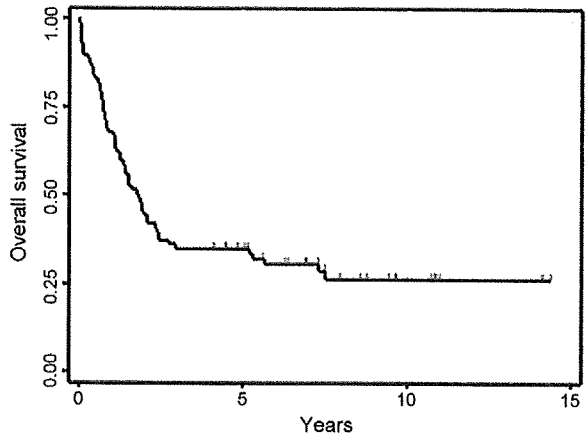

Fig. 1. Kaplan–Meier curve for overall survival. The probability of overall survival was 35.0% at 5 years for the whole population ($n=87$).

3.3. Survival

At the end of observation, 26 patients were alive with a median follow-up of 7.5 years (range, 0.8–14.3 years). Among 66 remitters, relapse occurred in 41 patients. A total of 33 patients underwent allogeneic HSCT, and three underwent autologous HSCT. Disease status at the time of transplantation was first CR for 17, second CR for 7, third CR for 1, non-CR for 9, and unknown for 2.

Fig. 1 shows the survivals for all patients. The probability of OS at 5 years was $35.0 \pm 5.1\%$ for the whole population. As there was no survival difference among the three studies ($p=0.475$), nor between the two studies (data not shown), all patients were grouped for the risk factor analysis. OS according to patient characteristics is presented in Table 3. Univariate analysis showed that their initial WBC counts and serum albumin levels strongly affected survival. As presented in Fig. 2, patients with a WBC count of $50 \times 10^9\,l^{-1}$ or higher had lower survival ($19.2 \pm 7.7\%$ at 5 years). Unexpectedly, the outcome for those with a WBC count lower than $3 \times 10^9\,l^{-1}$ was also worse ($20.0 \pm 10.0\%$ at 5 years) than those with an intermediate count ($48.0 \pm 7.5\%$ at 5 years). Induction failure or disease recurrence occurred in 14 of 15 patients in the low-count group, in 26 of 45 in the intermediate-count group, and in 22 of 27 in the high-count group. These observations resulted in inferior EFS rates for those in the low- and high-count groups to those in the intermediate-count group, too (Fig. 3). Even when patients undergoing HSCT were analyzed as censored cases at the time of transplantation, differences in terms of both OS and

Table 3
Overall survival at 5 years according to patient characteristics

Characteristics	Number of patients	Overall survival (%)	p-Value
All cases	87	35.0 ± 5.1	
Treatment protocol			0.475
ALL90	21	33.3 ± 10.3	
ALL93	26	44.7 ± 9.9	
ALL97	40	30.0 ± 7.2	
Age			0.054
Younger than 30	50	42.0 ± 7.0	
30 or older	37	25.4 ± 7.3	
Sex			0.700
Male	60	31.7 ± 6.0	
Female	27	42.9 ± 9.7	
WBC count			0.003
Lower than $3 \times 10^9\,l^{-1}$	15	20.0 ± 10.0	
3×10^9–$50 \times 10^9\,l^{-1}$	45	48.0 ± 7.5	
$50 \times 10^9\,l^{-1}$ or higher	27	19.2 ± 7.7	
Serum albumin			<0.001
Lower than 3.5 g dl^{-1}	14	7.1 ± 6.9	
3.5 g dl^{-1} or higher	68	39.0 ± 6.0	
Performance status			0.577
0–1	66	37.1 ± 6.0	
2–3	17	29.4 ± 11.1	
CNS involvement			<0.001
Present	6	0.0 ± 0.0	
Absent	80	38.1 ± 5.5	
Skin involvement			<0.001
Present	4	0.0 ± 0.0	
Absent	83	36.7 ± 5.3	
Mediastinal involvement			0.077
Present	15	53.3 ± 12.9	
Absent	72	31.2 ± 5.5	
No. of induction course[a]			0.972
1 Course	59	44.9 ± 6.5	
2 Courses	7	42.9 ± 18.7	

ALL, acute lymphoblastic leukemia; WBC, white blood cell; CNS, central nervous system. Values are presented with standard errors.
[a] Only patients who achieved complete remission are considered.

EFS remained statistically significant (data not shown). Of the 14 patients with serum albumin lower than 3.5 g dl^{-1}, seven failed to obtain CR, and all of the remaining patients with CR had a relapse. The probability of survival for these patients was only 7.1 ± 6.9% at 5 years. An age of 30 or older, and the presence of mediastinal involvement were also associated with a trend in favor of survival. Although the number was small, patients who presented CNS or skin involvement had an extremely poor prognosis, and no long-term survivors existed. We failed to detect a significant effect of sex, performance status, or number of induction courses on survival. Neither did surface markers including CD2, CD3, CD34, or myeloid antigens have any prognostic significance. Based on these results, serum albumin levels, initial WBC counts, age, and mediastinal involvement were subjected to a multivariate analysis. The results are shown in Table 4. Lower albumin levels, too low or too high WBC counts, and older age were identified as independently associated with lower survival.

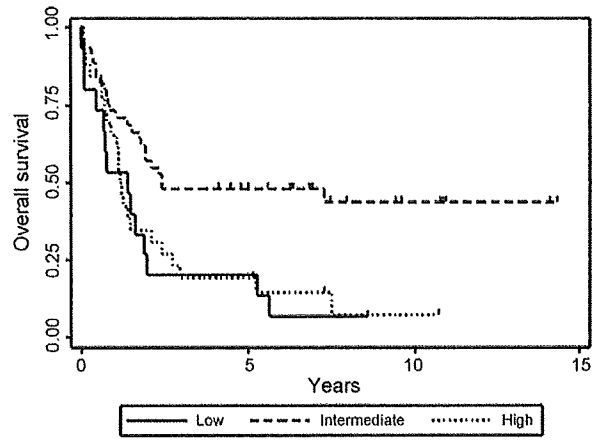

Fig. 2. Kaplan–Meier curves for overall survival according to the initial white blood cell count. The low-count group (less than $3 \times 10^9\,l^{-1}$, $n=15$) as well as the high-count group ($50 \times 10^9\,l^{-1}$ or higher, $n=27$) showed significantly worse overall survival than the intermediate-count group (3–$50 \times 10^9\,l^{-1}$, $n=45$: $p=0.0037$ and 0.0055, respectively).

3.4. Outcome after relapse

A total of 41 remitters had a disease recurrence after a median CR duration of 8.6 months (range, 0.6–79.4 months). The sites of relapse were BM in 28, CNS in 9, concurrent BM and CNS in 2, intraocular area in one, and mamma in one. Among 11 cases whose disease recurred in CNS, none had CNS involvement at presentation. The probabilities of OS for the whole recurred patients were 26.7 ± 7.1% at 1 year, and 11.9 ± 5.4% at 5 years after relapse. The survival curves are shown in Fig. 4. No patients could survive long-term unless they underwent HSCT after relapse.

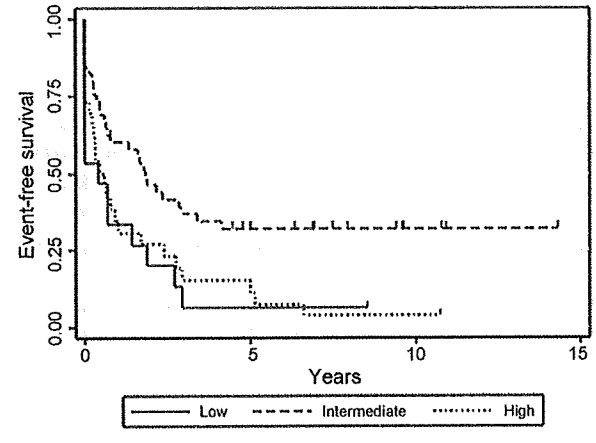

Fig. 3. Kaplan–Meier curves for event-free survival according to the initial white blood cell count. The low-count group (less than $3 \times 10^9\,l^{-1}$, $n=15$) as well as the high-count group ($50 \times 10^9\,l^{-1}$ or higher, $n=27$) showed significantly worse event-free survival than the intermediate-count group (3–$50 \times 10^9\,l^{-1}$, $n=45$: $p=0.0146$ and 0.0221, respectively).

Table 4
Factors associated with overall survival

Univariate analysis *p*-value	Multivariate analysis		
	p-Value	HR (95% CI)	Factor
Serum albumin			
<0.001	0.013	2.25 (1.18–4.28)	Lower than 3.5 g dl^{-1}
		1.00	3.5 g dl^{-1} or higher
WBC count			
0.004	0.018	2.34 (1.16–4.73)	50×10^9 l^{-1} or higher
0.005	0.036	1.91 (1.04–3.53)	Lower than 3×10^9 l^{-1}
		1.00	3×10^9–50×10^9 l^{-1}
Age			
0.057	0.049	1.70 (1.00–2.89)	30 or older
		1.00	Younger than 30
Mediastinal involvement			
0.084	0.212	1.68 (0.74–3.80)	Absent
		1.00	Present

An HR higher than unity indicates worse survival for patients with the factor. HR, hazard ratio; 95% CI, 95% confidence interval; WBC, white blood cell.

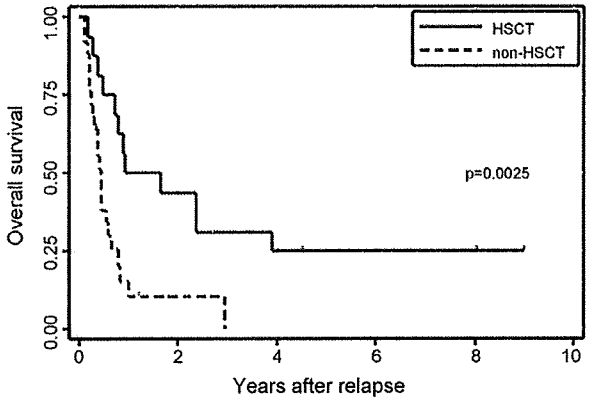

Fig. 4. Kaplan–Meier curves for survival after relapse The 5-year probability of overall survival after relapse was 25.0% for patients who underwent transplantation thereafter ($n = 16$), and 0% for those who did not ($n = 25$).

4. Discussion

A recent meta-analysis [15] showed that patients with high-risk ALL benefit from allogeneic HSCT during first CR, whereas its efficacy is unclear for those with standard-risk ALL, suggesting the importance of prognostic prediction at diagnosis or soon thereafter. Risk stratification for ALL has been traditionally studied without distinguishing T-ALL from B-lineage ALL; however, because they are two distinct clinical entities, determining prognostic factors separately should mean more accuracy. But the relatively small number of patients, especially those with T-ALL, available for a single trial has made such an analytical examination difficult. In this study, through combining data for patients from the three prospective JALSG trials, we studied long-term outcomes and prognostic factors specific for T-ALL.

Given that highly intensive regimens in recent reports have increased survival rates for adult T-ALL up to 40–60% [8–11], the 5-year survival rate of 35% for our patients was somewhat low. As it is suggested that CPM and Ara-C may play an important role in the treatment of T-ALL [8], our results would leave room for improvement through the use of treatment that features intensified administration of such agents. Risk factor analysis identified serum albumin levels, initial WBC counts, and age as having independent values for predicting survival. As reported previously, an initial WBC count had significant influence on survival, but the striking finding was that not only the high-count group (50×10^9 l^{-1} or higher) but also the low-count group (less than 3×10^9 l^{-1}) showed a significantly worse survival rate than the intermediate-count group ($p = 0.0055$ and 0.0037, respectively). This observation is in accordance with the report from the Pediatric Oncology Group (POG) [16]. They described that the subgroup of patients with T-ALL who had an initial WBC count of less than 10×10^9 l^{-1} at diagnosis fared worse than those with a WBC count between 10×10^9 and 50×10^9 l^{-1}. It is an accepted concept that a high WBC count has less influence on the prognosis of T-ALL than of B-lineage ALL [8]. Many investigators have pursued the upper cut-off points to discriminate outcomes, with these cut-off points recently set at 100×10^9 l^{-1} in adults [7,8], which is much higher than for B-lineage ALL. From the results of both POG and our studies, it can be assumed that a poor prognosis for T-ALL patients with a low WBC count may partly offset the prognostic significance of the high WBC count. Possible reasons why a low WBC count affected survival adversely could not be identified from a careful examination of our patient data. A French group showed that the T-cell receptor (TCR) status could stratify T-ALL into four groups, and patients in the immature subset presented a lower WBC count, and had inferior survival mainly due to a lower CR rate [17]. However, for our patients, a low WBC count did not exert any significant effect on CR achievement, but was associated with shorter survival due to a higher rate of relapse. Immunophenotypic maturation stages have also been indicated in correlating with the outcome for T-ALL [16,18–21], although

interactions between such maturation stages and initial WBC counts have not been established. Lack of information for CD1a expression in our dataset enabled us to classify our patients according to the criteria by the European Group for the Immunological Characterization of Leukemias (EGIL) [22]; however, the observations that expression of surface markers including CD3 and CD34 revealed no prognostic relevance seem to show that worse outcomes for patients with low WBC counts cannot be explained by maturation stages. More recently, risk assessment for T-ALL has been investigated based on the genetic characteristics of leukemic cells, including the expression of specific genes such as HOX11 [23,24] and HOX11L2 [25], gene expression profiles using microarray technology [26], and DNA methylation profiles [27]. Also, prognostic significance of minimal residual disease (MRD) during or after treatment has been vigorously studied, and several groups showed clinical utility of MRD quantification by flow cytometry [28,29] or polymerase chain reaction [30–32]. Although such research should be continued, risk assessment according to information commonly available at all hospitals remains important in clinical practice. It should be noted that our study has several limitations, and the results must be interpreted with caution. The limitations include the retrospective nature of the study, and the relatively small number of patients, especially of those in the low WBC count group ($n = 15$). Validations for a larger number of patients will be needed.

In summary, from the analysis of a relatively large cohort of 87 adult patients with T-ALL, serum albumin levels, initial WBC counts, and age were identified as prognostic factors for survival. For WBC counts, not only patients with a high count, but also those with a low count had significantly worse outcomes than patients with an intermediate count. Although our findings need confirmation, these results will be helpful in the identification of prognostically distinct subgroups within adult T-ALL.

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Imatinib Provides Durable Molecular and Cytogenetic Responses in a Practical Setting for Both Newly Diagnosed and Previously Treated Chronic Myelogenous Leukemia: A Study in Nagasaki Prefecture, Japan

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Abstract

To evaluate the efficacy of imatinib in a practical setting, we registered 43 patients with newly diagnosed chronic myelogenous leukemia (CML) (group I) and 56 patients with previously diagnosed CML (group II) at 11 hematology centers in Nagasaki prefecture, Japan, from December 2001 to July 2005 and analyzed the molecular responses. Cytopenia, fluid retention, and skin rash were major adverse events, along with elevation in creatine phosphokinase levels. With a follow-up of approximately 3.5 years, imatinib treatment led to 88.7% overall survival (OS) and 85.2% progression-free survival (PFS) rates for group I, and 79.8% OS and 76.6% PFS rates for group II; the rates were not significantly different despite a lower average imatinib dose in group II. The rates of complete cytogenetic response at 30 months and major molecular response at 24 months were 86.1% and 62.5%, respectively, in group I, and 77.9% and 58.3% in group II; the rates were not significantly different. As has been reported by other groups, these results demonstrate that imatinib treatment can provide excellent clinical and molecular effects for not only newly diagnosed but also previously treated CML patients in practical settings that cover a wider variety of patients than clinical trials.

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Key words: Imatinib; Chronic myelogenous leukemia; Molecular response; Cytogenetic response; New diagnosis; Previous diagnosis

1. Introduction

Treatment for chronic myelogenous leukemia (CML) has changed dramatically since the introduction of imatinib [1]. Imatinib blocks the activity of the specific Philadelphia chromosome (Ph)-derived BCR-ABL fusion protein, which confers a strong growth advantage upon Ph-positive CML cells [1-3]. Reflecting in vitro observations are many reports regarding the clinical effectiveness of imatinib on Ph-positive leukemias [4-8]. For example, the International Randomised Study of Interferon versus STI571 (IRIS) study [4], a phase III clinical trial that compared imatinib treatment and treatment with interferon (IFN) plus cytosine arabinoside for CML patients in the first chronic phase, clearly demonstrated the excellent clinical and cytogenetic/molecular effects of imatinib.

See "Appendix" for affiliations of the members of the Nagasaki CML Study Group.

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