significantly between the two regimens. Signs of hematological toxicity were the major adverse reactions observed following treatment with both regimens. The incidences of neutropenia and alopecia were lower with the weekly regimen. On the basis of these results, Belani concluded that weekly PTX treatment combined with GEM is also useful as first-line chemotherapy for NSCLC.

In conclusion, weekly chemotherapy with PTX and GEM is a tolerable and active regimen for patients with advanced NSCLC previously treated with platinum-containing chemotherapy regimens. It should be recommended as a candidate regimen in planning a phase III clinical study of NSCLC previously treated with platinum-containing chemotherapy, and will ultimately be evaluated in a phase III clinical study.

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PRECLINICAL STUDIES

Cytotoxic effects of histone deacetylase inhibitor FK228 (depsipeptide, formally named FR901228) in combination with conventional anti-leukemia/lymphoma agents against human leukemia/lymphoma cell lines

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Summary FK228 is a novel antitumor depsipeptide that inhibits histone deacetylases and restores the expression of genes aberrantly suppressed in cancer cells. This agent was shown to have broad antitumor activity in preclinical studies, and is currently under phase I/II evaluations. Because of its wide spectrum of actions, it is reasonable to consider the combination with other anticancer drugs in clinical application. We studied the cytotoxic interaction of FK228 in combination with conventional antileukemic agents using human promyelocytic leukemia HL60, Philadelphia chromosome-positive (Ph^+) chronic myelogenous leukemia KU-812, T-cell lymphoblastic leukemia MOLT3 and Burkitt's lymphoma Raji cell lines. For the combination of FK228 and imatinib, Ph^+ leukemia KU812, K562 and TCC-S cell lines were used. The cells were exposed simultaneously to FK228 and other agents for 4 days. Cell growth inhibition was determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. We used the isobologram method of Steel and Peckham to evaluate the cytotoxic interaction at the concentration of drugs that produced 80% cell growth inhibition (IC_{80}). FK228 showed an additive effect with cytarabine, carboplatin, doxorubicin, etoposide, 4-hydroperoxy-cyclophosphamide, 6-mercaptopurine and SN-38 (active metabolite of irinotecan) in all cell lines studied. FK228 with methotrexate and vincristine showed an antagonistic effect in three and one of the four cell lines, respectively. FK228 was additive with imatinib in all three Ph^+ leukemia cells. Our findings suggest that FK228 is a promising candidate for combining with most anticancer agents except for methotrexate and vincristine, which produce suboptimal effects.

Keywords FK228 · Depsipeptide · Histone deacetylase inhibitor · Antagonism · Synergism

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Introduction

Histone acetylation and deacetylation play an important role in the control of gene transcription by affecting the interaction between DNA and histones [1]. Acetylation is linked to activation of gene transcription, whereas deacetylation is associated with transcriptional repression. Both processes are catalyzed by specific enzymes, histone acetyltransferases and histone deacetylases, respectively [2].

Recent advances in molecular oncology suggest that histone deacetylation may play a role in the uncontrolled growth of cancer cells [2]. Histone deacetylase inhibitors have been observed to cause growth arrest, differentiation and apoptosis against a variety of cancer cell lines [3, 4]. Five structural classes of histone deacetylase inhibitors have been identified, including FK228, trichostatin A, MS-27-275, oxamflatin and SAHA [3, 4]. Several agents are currently under clinical investigations.

FK228, formally named FR901228, is a novel antitumor depsipeptide isolated from *Chromobacterium violaceum* [5]. FK228 inhibits histone deacetylases and causes arrest of the cell cycle in the G1 and/or G2-phase, cell differentiation and apoptosis [6]. FK228 effectively suppresses the growth of human tumor cells *in vitro*, i.e., the concentrations of FK228 required for 50% cell growth inhibition of clinical samples of chronic lymphocytic leukemia and a variety of lymphoid cell lines are much lower than those of normal hematopoietic progenitor cells [7, 8]. Small cell lung cancer cell lines resistant to etoposide, irinotecan or cisplatin are not cross-resistant to FK228 [9]. In animal studies, FK228 prolongs the survival time of mice bearing murine and human tumors [10, 11].

Phase I trials of FK228 in patients with refractory neoplasms, lymphomas and leukemias have been reported [12–14]. Three patients with cutaneous T-cell lymphoma had a partial response and one patient with peripheral T-cell lymphoma, unspecified, had a complete response [12]. On the other hand, FK228 shows limited antitumor activity in patients with chronic lymphocytic leukemia and acute myeloid leukemia, while it effectively inhibits histone deacetylase *in vivo* [14]. The dose-limiting toxicities were fatigue, nausea, vomiting, and transient thrombocytopenia and neutropenia. These results suggest that FK228 has some therapeutic effects in the treatment of cancer including lymphoma and leukemias, but, for clinical development, it is important to combine with other anticancer agents. However, cytotoxic effects of FK228 combined with commonly used antitumor agents have not been studied.

In the present study, we investigated the *in vitro* cytotoxic interactions of FK228 in combination with commonly-used anti-leukemia/lymphoma agents against human leukemia/lymphoma cell lines. The results underline the importance of the design of the combination of FK228 with other anticancer agents for clinical therapy.

Materials and methods

Cell lines

The experimental studies of FK228 in combination with standard anti-leukemic agents were conducted with human promyelocytic leukemia HL60, Ph^+ chronic myelogenous leukemia KU-812, T-cell lymphoblastic leukemia MOLT3 and B-cell Burkitt's lymphoma Raji cells. The combination of FK228 with imatinib was studied using Ph^+ leukemia, KU812, K562 and TCC-S cells. Cells were maintained in 75-cm^2 plastic tissue culture flasks containing RPMI1640 medium (Sigma Co., St. Louis, Mo., USA) supplemented with 10% heat-inactivated fetal calf serum (Sigma Co.) and antibiotics.

Drugs

FK228, SN-38 and imatinib were kindly provided by Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan), Yakult Co., Ltd. (Tokyo, Japan) and Novartis Inc. (Basel, Switzerland), respectively. Other anticancer agents used and their sources were: cytarabine (Nihon Shinyaku Co. Ltd., Tokyo), doxorubicin (Meiji Co., Ltd., Tokyo), etoposide (Nihon Kayaku Co., Ltd., Tokyo), 4-hydroperoxy-cyclophosphamide and vincristine (Shionogi Co., Ltd., Tokyo), methotrexate (Lederle Japan, Ltd., Tokyo) and 6-mercaptopurine (Sigma Co.). FK228 and Imatinib were dissolved in dimethyl sulfoxide. SN-38 was dissolved in 0.1 N NaOH. All other drugs were dissolved in RPMI1640. Appropriate concentrations of stock solutions of all drugs were stored at $-80°C$. The drugs were diluted with RPMI1640 before use.

Cell cycle analysis by flow cytometry

Cell cycle analysis was performed using KU812 and MOLT3 cells. Cells were cultured in the presence of either no drug or a variety of concentrations of FK228 for 24, 48 and 72 h. The cells were then stained with propidium iodide in preparation for flow cytometry with the FACScan/CellFIT system (Becton-Dickinson, San Jose, CA). A DNA histogram was obtained by analyzing 25,000 cells with the ModFIT program (Becton-Dickinson) [15].

Cell culture

HL60, KU812, MOLT3 and Raji cells were used for the combination studies. These cells in the logarithmic phase were harvested and resuspended in a final concentration of $0.5–2 \times 10^5$ cells/ml of fresh medium containing 10% fetal calf serum. Cell suspensions (100 μl) were dispensed into individual wells of a 96-well tissue culture plate with a lid (Falcon, Oxnard, CA). Eight plates were prepared for the testing of each drug combination. Each plate had one 8-well control column containing medium alone and one 8-well control column containing cells but no drugs. For each drug or drug combination, 8 wells were used. Cells were incubated in a humidified atmosphere of 95% air/5% CO^2 at 37°C overnight. Drug solutions of FK228 and other drugs at different concentrations were then added (50 μl) to 8 wells containing cell suspensions and the plates were incubated under the same conditions for 4 days. The final concentration of dimethyl sulfoxide in the media was less than 0.1%, and it had no effect on cell growth inhibition in our study.

MTT assay

Viable cell growth was determined by using a modified MTT assay as described previously [16].

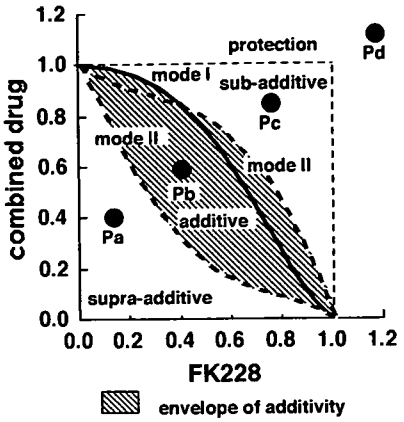

Fig. 1 Schematic representation of an isobologram. Envelope of additivity, surrounded by Mode I (solid line) and Mode II (dotted line) isobologram lines, is constructed from the dose-response curves of FK228 and a combined drug. The concentrations that produce 80% cell growth inhibition are expressed as 1.0 on the ordinate and the abscissa of isobolograms. Combined data points Pa, Pb, Pc and Pd show supra-additive, additive, sub-additive and protective effects, respectively

Isobologram method of Steel and Peckham

Cytotoxic interactions of FK228 with other agents at the point of IC_{80} were evaluated by the isobologram method of Steel and Peckham [17]. The theoretical basis of the isobologram method and the procedure for making isobolograms have been described in detail previously [18, 19].

Based upon the dose-response curves of FK228 and the other agents, three isoeffect curves were constructed (Fig. 1). If the agents were acting additively by independent mechanisms, the combined data points will lay near the Mode I line (hetero-addition). If the agents were acting additively by similar mechanisms, the combined data points will lay near the Mode II lines (iso-addition).

Since it is unknown in advance whether the combined effects of two agents will be hetero-additive, iso-additive or an effect intermediate between these extremes, all possibilities should be considered. Thus, when the data points of the drug combination fell within the area surrounded by three lines (envelope of additivity), the combination was regarded as additive. When the data points fell to the left of the envelope, i.e., the combined effect was caused by lower doses of the two agents than was predicted, we regarded the drug combination as having a supra-additive effect (synergism). When the points fell to the right of the envelope, i.e., the combined effect was caused by higher doses of the two agents than was predicted, but within the square or on the line of the square, we regarded the combination as having a sub-additive effect, i.e., the combination was superior or equal to a single agent but was less than additive. When the data points were outside the square, the combination was regarded as having a protective effect, i.e., the combination was inferior in cytotoxic action to a single agent. Both sub-additive and protective interactions were regarded as antagonism.

Data analysis

When the observed data points for the combination mainly fell in the area of supra-additivity or in the areas of sub-additivity and protection, i.e., the mean value of the observed data was smaller than that of the predicted minimum values or larger than that of the predicted maximum values, the combination was considered to have a synergistic or antagonistic effect, respectively. To determine whether the condition of synergism (or antagonism) truly existed, statistical analysis was performed. The Wilcoxon signed-ranks test was used for comparing the observed data with the predicted minimum (or maximum) values for additive effects, which were closest to the observed data (i.e. the data on the boundary (Mode I or Mode II lines) between the additive area and supra-additive area (or sub-additive and protective areas) [20]. Probability (P) values ≤ 0.05 were considered significant. Combinations with $P > 0.05$ were regarded as indicating additive/synergistic (or additive/antagonistic) effects. All statistical analyses were performed using the Stat View 4.01 software program (Abacus Concepts, Berkeley, CA).

Results

Cytotoxic effects of FK228 and conventional anticancer agents on human leukemia/lymphoma cell lines

The dose-response curves of FK228 for the HL60, MOLT3, Raji, KU812, K562, and TCC-S cells are shown in Fig. 2. The IC_{80} values of a 96-h exposure to FK228 for these cells were

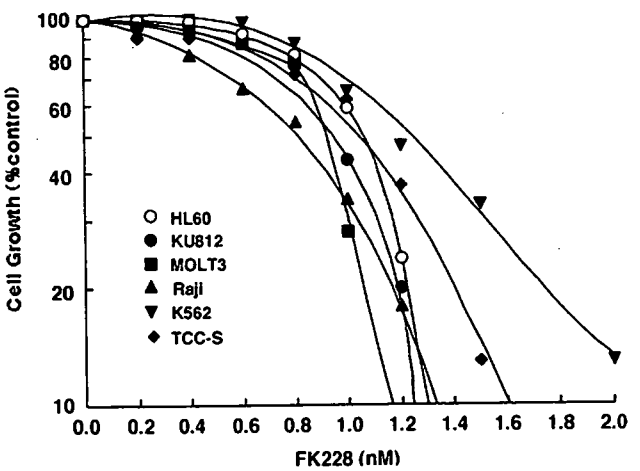

Fig. 2 Dose-response curves for FK228 in HL60, MOLT3, KU812, Raji, K562 and TCC-S cells

3.7 ± 0.2 nM, 3.6 ± 0.3 nM, 3.2 ± 0.2 nM, 3.6 ± 0.3 nM, 5.1 ± 0.2 nM, and 4.2 ± 0.4 nM, respectively (Fig. 2, Table 1), all of which are clinically achievable *in vivo* according to a recent clinical study [12]. The IC_{80} values of a 96-h exposure to other agents for these cells are also shown in Table 1.

Cell cycle analysis of FK228-treated cells

We analyzed the effects of FK228 on the cell cycle profile of leukemia cell lines. Cell cycle arrest at G1 to the early S-phase was observed in KU812 cells at 24 h of culture with FK228 at concentrations of more than 4.5 nM, followed by the induction of apoptosis at 72 h (Fig. 3). FK228, at concentrations of more than 4.5 nM, induced G1 arrest and apoptosis simultaneously after 48 h of culture in MOLT3 cells (data not shown). In contrast, FK228 arrested HL60 and K562 cells in G2/M phase of the cell cycle (data not shown, see Sutheesophon et al. [21] for detail).

Cytotoxic effects of FK228 in combination with other agents

Figure 4 shows the dose-response curves for FK228 in combination with either cytarabine or methotrexate in KU812 cells. Each isobologram was generated based on such dose-response curves.

Cytotoxic effects of FK228 in combination with cytarabine: Figure 5 shows the isobolograms of this combination in HL60, KU812, MOLT3 and Raji cells. In HL60 cells, all combined data points fell within the envelope of additivity (Fig. 5a). The mean value of the data (0.63) was larger than that of the predicted minimum values (0.12) and smaller than that of the predicted maximum values (0.89), indicating that the simultaneous exposure to FK228 and cytarabine produced an additive effect (Table 2). In MOLT3, KU812 and Raji cells, all data points also fell within the envelope of additivity, indicating that the simultaneous exposure to FK228 and cytarabine also produced an additive effect.

Cytotoxic effects of FK228 in combination with either carboplatin, doxorubicin, etoposide, 4-hydroperoxycyclophosphamide or SN-38: In all four cell lines, all or most combined data points fell within the envelope of additivity (isobolograms not shown), indicating that simultaneous exposure to FK228 and these drugs produced an additive effect (Table 2).

Cytotoxic effects of FK228 in combination with 6-mercaptopurine: Because HL60 was resistant to 6-mercaptopurine, we used other three cell lines to study this combination. In MOLT3, KU812 and Raji cells, all or most combined data points fell within the envelope of additivity (isobolograms not shown), indicating that simultaneous exposure to FK228 and 6-mercaptopurine produced an additive effect (Table 2).

Cytotoxic effects of FK228 in combination with methotrexate: Figure 6 shows the isobolograms of this combination in HL60, KU812, MOLT3 and Raji cells. In HL60, KU812 and Raji cells, all or most data points fell in the areas of sub-additivity and protection. The mean values of the observed data (0.93, 1.13 and 0.98, respectively) were larger than those of the predicted maximum additive values (0.87, 0.44 and 0.76, respectively) (Table 2). Statistical analysis showed that the difference was significant ($P < 0.05$, $P < 0.01$ and $P < 0.01$, respectively), indicating an antagonistic effect of simultaneous exposure to these two agents. In MOLT3 cells, data points fell within the envelope of additivity and in the areas of sub-additivity and protection. The mean value of the observed data (0.87) was larger than that of the predicted maximum additive values (0.86), but the difference was not statistically significant ($P > 0.05$), indicating an additive/antagonistic effect of simultaneous exposure to these two agents.

Table 1 The IC_{80} values of FK228 and combined agents for leukemia cell lines[a]

	HL60	MOLT3	Raji	KU812	K562	TCC-S
FK228 (nM)	3.7	3.6	3.2	3.6	5.1	4.2
Cytarabine (nM)	400	160	1400	8900	n.d.	n.d.
Carboplatin (μM)	7.8	5.3	15	100	n.d.	n.d.
Doxorubicin (nM)	92	15	220	25	n.d.	n.d.
Etoposide (μM)	0.44	0.25	1.3	4	n.d.	n.d.
4-Hydroperoxy-cyclophosphamide (μM)	6.2	1.3	1.4	7.4	n.d.	n.d.
6-Mercaptopurine (μM)	10	1.7	4	1.7	n.d.	n.d.
Methotrexate (nM)	25	25	70	18	n.d.	n.d.
SN-38 (nM)	8.3	0.94	2.1	27	n.d.	n.d.
Vincristine (nM)	1	0.67	2.2	1.5	n.d.	n.d.
Imatinib (nM)	n.d.	n.d.	n.d.	97	270	85

[a] Values represent the means of at least three independent experiments.

n.d. = not done.

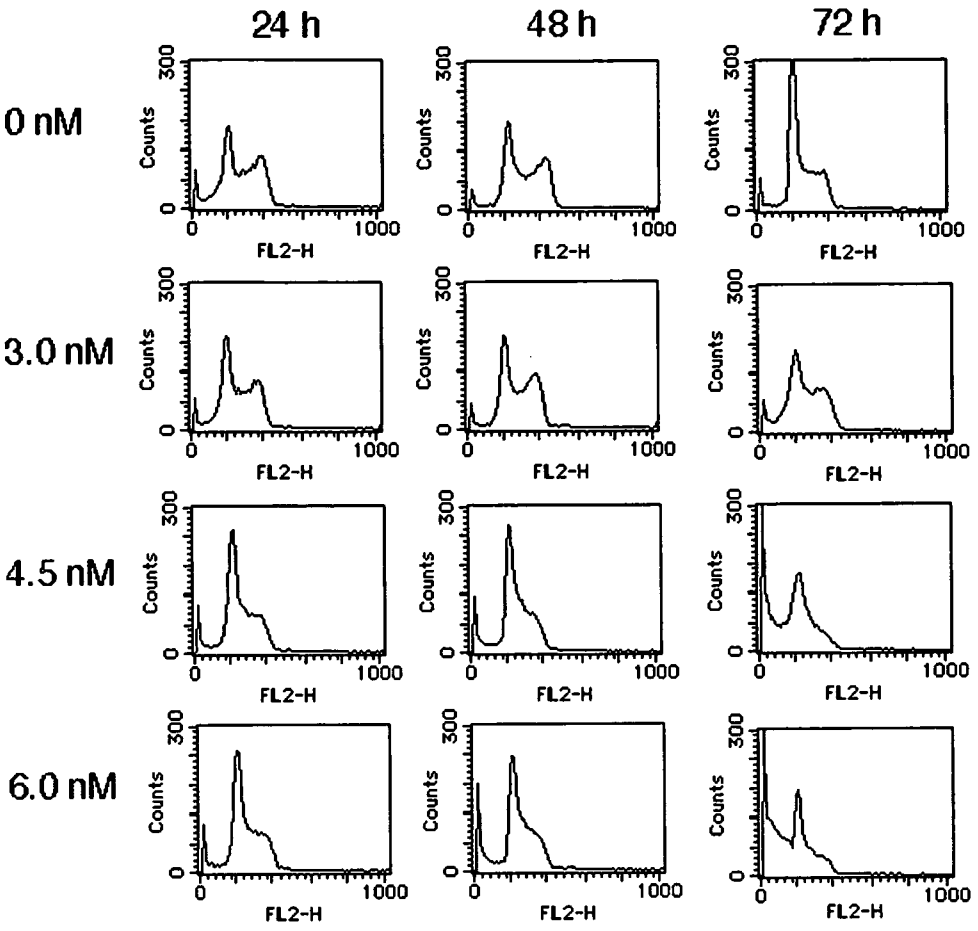

Fig. 3 Cell cycle analysis of KU812 cells treated with FK228. Cells were cultured in the absence or presence of various concentrations of FK228, and DNA histograms were obtained after 24, 48 and 72 h of culture

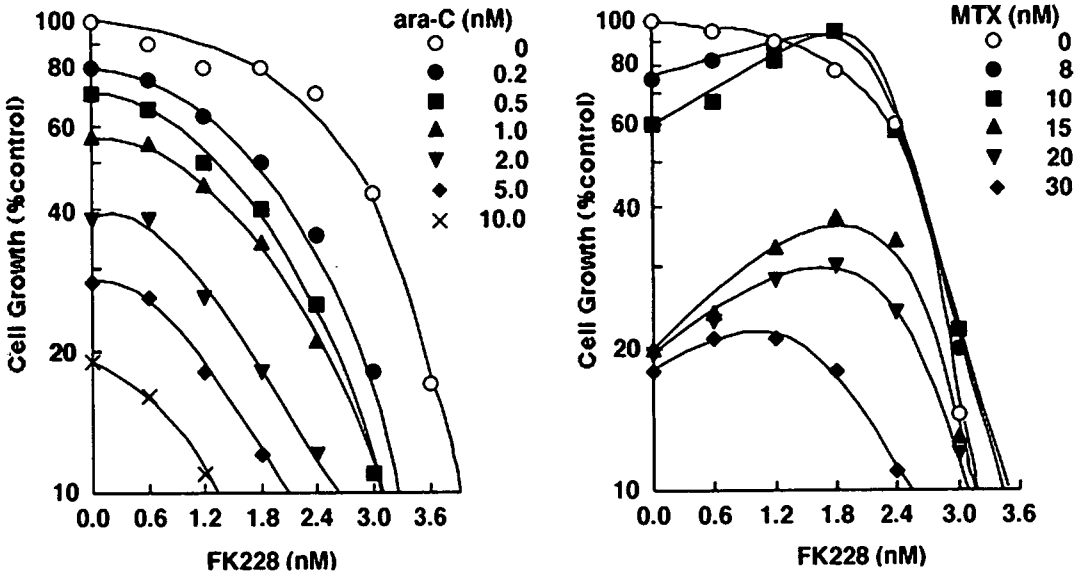

Fig. 4 Dose-response curves for FK228 in combination with cytarabine (ara-C) and methotrexate (MTX) in KU812 cells. Cell growth was measured by the MTT assay after 4 days and was plotted as a percentage of the control (cells not exposed to drugs). FK228 concentrations are shown on the abscissa. Each point represents the mean value for at least 3 independent experiments; the SEs were less than 20% and thus were omitted

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.

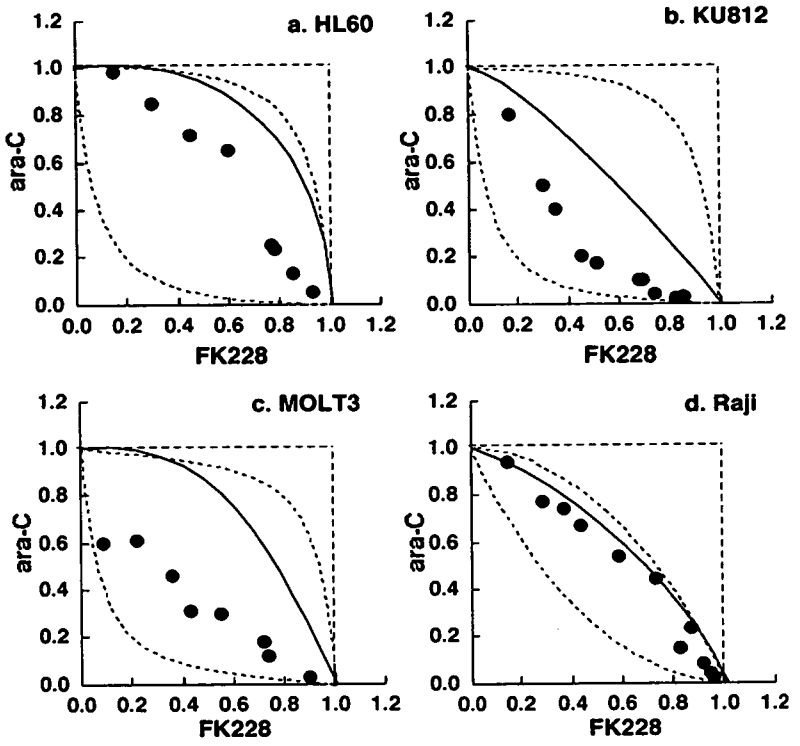

Fig. 5 Isobolograms of simultaneous exposure to FK228 and cytarabine (ara-C) in HL60 (a), KU812 (b), MOLT3 (c) and Raji (d) cells. In all four leukemia cell lines, the data points of the combinations fell within the envelope of additivity, suggesting an additive effect. Each point represents the mean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

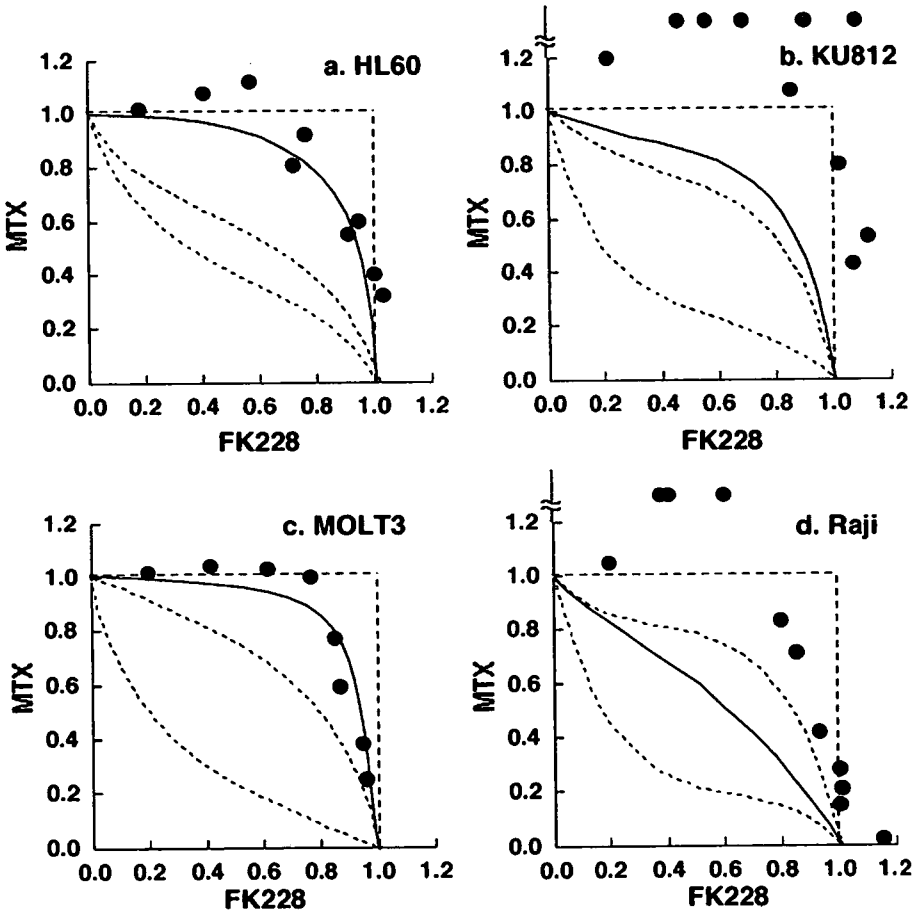

Fig. 6 Isobolograms of simultaneous exposure to FK228 and methotrexate (MTX) in HL60 (a), KU812 (b), MOLT3 (c) and Raji (d) cells. In HL60, KU812 and Raji cell, all or most data points fell in the areas of sub-additivity and protection. In MOLT3 cells, data points fell within the envelope of additivity 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, pre-treatment 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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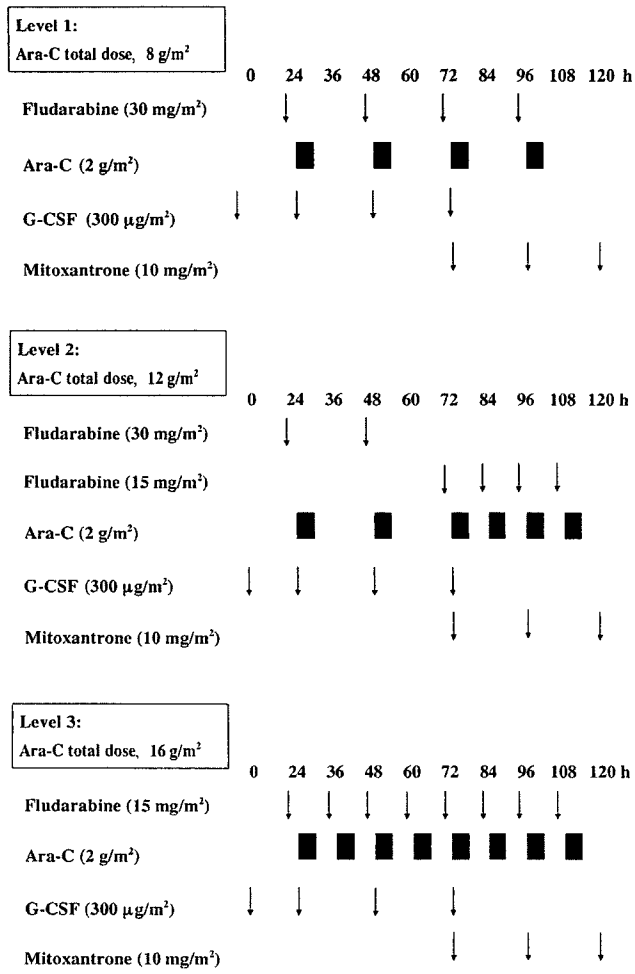

Figure 1. Dosing schedule for the 3 dosage levels. At all dosage levels, granulocyte colony-stimulating factor (G-CSF) was administered in 4 doses every 24 hours, beginning at the start of therapy, and mitoxantrone was administered in 3 doses every 24 hours, beginning 72 hours after the start of therapy. 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