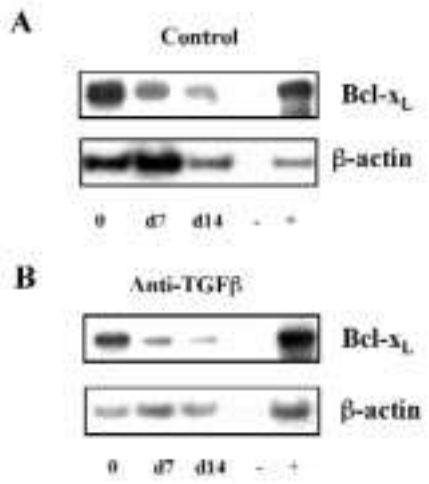

Figure 2. Semiquantitative RT-PCR analysis of Bcl-xL mRNA in one sample of RAEB cells (representative experiment). A) Bcl-xL was expressed at high levels in CD34⁺ cells on day 0, then dramatically decreased in CD41⁺ cells on day 7 and day 14. B) Neutralizing anti-TGF-β1 antibody did not modify this altered pattern of Bcl-xL mRNA expression. Negative and positive controls are indicated with – and +, respectively.

of viable CD41⁺ cells be harvested at day 14 of culture. Bcl-xL mRNA was detected at high levels in all samples of RAEB CD34⁺ cells at day 0, and it appeared progressively down-regulated at days 7 and 14 of culture (Figure 2A).

Moreover, the Bcl-xL expression pattern was similar in control cultures and in cultures containing anti-TGF-β1 neutralizing antibody (Figure 2B). The deregulated expression of Bcl-xL in MDS CD34⁺ cells was not dependent on impaired TGF-β1 autocrine production. In fact, we have previously demonstrated that MDS CD34⁺ cells induced towards megakaryocytic differentiation produce TGF-β1.[1]

In conclusion, our data confirm that Bcl-xL up-regulation plays a fundamental role in normal megakaryocytopoiesis and demonstrate that it depends on the autocrine TGF-β1 produced by differentiating cells. Furthermore, this study highlights that Bcl-xL expression in RAEB CD34⁺ cells undergoing megakaryocytic commitment is completely altered and suggests that it can play a role in their defective differentiation capacity.

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Aggressive natural killer cell leukemia: clinical features and treatment outcome

The clinical features of thirteen patients with very aggressive natural killer cell leukemia (ANKL) were retrospectively analyzed. The common symptoms and signs were fever (69.2%), B symptoms (100%), hepatosplenomegaly (76.9%), disseminated intravascular coagulopathy (76.9%), and hemophagocytosis (62%). Epstein-Barr virus (EBV) *in situ* hybridization was positive in 91%. Clinical response to treatment was observed in 38%, however, the median survival was 47days.

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Aggressive natural killer-cell leukemia (ANKL) is the rarest and least well characterized malignancy and the clinical features have been described only in a few case reports.[1-8] In this study, we conducted a retrospective study to investigate the natural history of cancer and the clinical outcome after treatment.

Between March 1994 and August 2001, 13 patients were diagnosed with ANKL. The diagnostic criteria were based on bone marrow infiltration of malignant cells with typical immunohistochemical features and characteristic clinical features. The cells were negative for sCD3 and positive for cCD3 and CD56. Tumor cells were negative for CD13 and CD33. Immunophenotypes of bone marrow samples were analyzed by flow cytometer using anti-CD2, -CD3, -CD4, -CD7, -CD8, -CD19, -CD16/56, and, anti-TdT antibodies. Immunostaining using CD30 and TIA-1 was performed on a core biopsy of bone marrow. Epstein-Barr virus (EBV) RNA was detected by an *in situ* hybridization (ISH) tech-

Table 2. Treatment outcome and survival duration.

Case No.	Age/Sex	PS	Major involvement	HPS	EBV	BM (FAX,%)	Initial treatment	Response	Response duration	Survival
1	60/F	3	BM, liver	–	–	ND	CVP	–		35 d
2	64/M	4	Skin, CSF	–	+	ND	DHAP	–		12 d
3	60/M	3	BM	+	+	25	DEC#1	+	33 d	50 d
4	63/F	3	BM	+	+	55	CVP	–		15 d
5	28/M	2	BM	–	ND	76	IA	–		40 d
6	42/M	4	BM	–	+	ND	BVP	–		4 d
7	57/F	2	BM, nose	–	+	ND	CHOP#1→ESHAP#1	–		58 d
8	50/F	3	BM	+	+	98	DVPL	+	23 d	47 d
9	27/M	1	BM	+	+	38	DEC#3	–		55 d
10	19/F	1	BM, liver	+	ND	55	DHAP	–		14 d
11	38/M	3	BM, liver/spleen	+	+	16	CVP→CHOP#3→mobilization*	+	103 d	120 d
12	25/M	4	BM, nose, skin	+	+	46	CVP→DEC#3→Autologous PBSCT**	+	161 d	163 d
13	20/F	1	BM	+	+	20	DEC#3→Autologous PBSCT**	+	162 d+	162 d+

*PS: performance status (ECOG), BM: bone marrow, ND: not done, PBSCT: peripheral stem cell transplantation; *death during peripheral stem cell collection; **conditioning with BEAM; CHOP: D1 Cytoxan 750 mg/m² i.v., D1 Doxorubicin 50 mg/m² i.v., D1 Vincristine 1.4 mg/m² (max. 2 mg) i.v., D1-D5 Prednisolone 100 mg/d p.o. q 3wks; BVP: D1-5 Bleomycin 10 mg/m² i.v., D1, D8 Vincristine 2 mg i.v., D1-D5 Prednisolone 60 mg/m² p.o. q 3wks; CVP: D1 Cyclophosphamide 800-1000 mg/m² i.v. ,D1 Vincristine 1.4 mg/m²(max. 2 mg) i.v., D1-D5 Prednisolone 100 mg p.o. q 3wks; DEC: D1 Cytoxan 1250 mg/m² i.v., D1 Doxorubicin 75 mg/m2 i.v., D1 Vincristine 1.4 mg/m² (max.2 mg) i.v., D1-D5 Prednisolone 100 mg/d p.o. with G-CSF q 2wks; ESHAP: D1-D4 Etoposide 40 mg/m² i.v., D1-D4 Methylprednisolone 500 mg i.v., D1-D4 Cisplatin 25 g/m² i.v. q 3wks; DHAP: D1 Cisplatin 100 mg/m² i.v.,D2 Cytarabine 2 g/m² q 12hrs (total 2 doses) i.v., D1-D4 Dexamethasone 40 mg i.v. q 3wks; DVPL: D1-D3 Daunorubicin 50 mg/m² i.v.D1, 8, 15, 22 Vincristine 2 mg i.v., D1-D28 PD 20 mg/m² q 8hrs p.o., D17-D28 L-Asparaginase 6,000 U/m² i.m. IA: D1-D3 Idarubicin 12 mg/m² i.v. D1-D7 Cytarabine 200 mg/m² i.v. BEAM : D-7 Carmustine 300 mg/m² i.v., D-6-D-3 Etoposide 200 mg/m² i.v., D-6-D-3 Cytarabine 200 mg/m² i.v. q 12hrs, D-2 Melphalan 140 mg/m² i.v. day –1.*

nique using FITC-conjugated EBV oligonucleotides complementary to the nuclear RNA portion of the EBER 1 and 2 genes. All of the patients were staged according to the Ann Arbor staging system.

A variety of chemotherapeutic regimens were used (Table 2). For patients with poor liver function, initially regimens not containing anthracyclines, e.g. CVP in two patients and BVP in one, were used. For patients with tolerable liver function or improved liver function after initial chemotherapy, anthracycline-containing regimens were used, which included CHOP in two patients and dose-escalated CHOP in three patients, DVPL in 1 and, IA in two patients. Since January 2001, after diagnosis, 3 cycles of dose-escalated CHOP followed by autologous peripheral blood stem cell transplantation under BEAM conditioning were planned.

Clinical tumor response was defined by improved symptoms with improved laboratory findings or decreased size of measurable lesions. Improved laboratory findings were defined as i) decreased lactate dehydrogenase (normal or 1/2 of baseline) or ii) improved liver function tests (normal or bilirubin < 3 mg/dL if baseline >10 mg/dL) or iii) improved cytopenia (absent in next cycle) and without deterioration of other laboratory findings.

The clinical characteristics of the 13 patients are given in Tables 1 and 2. The most common presenting symptom was fever (69.2%). B symptoms were noted in all patients (100%). Hepatosplenomegaly was commonly observed (76.9%). The majority of the cases (77%) were of high-intermediate and high risk according to the *International prognostic Index*.[2,3] EBV *in situ* hybridization was positive in 10 of 11 (91%). Disseminated intravascular coagulopathy was observed in 10 of 13 patients (76.9%). Hemophagocytosis was common in bone marrow (62%).

A clinical response to treatment was observed in 5 of 13 patients (38%, 95% confidence interval 13 – 63 %). The response was maintained for 23 to 162+ days (Table 2). In three patients (cases #11,12, and 13), high-dose chemotherapy with BEAM

Table 1. Patients' characteristics.

No.	13
Median age (y)	42 (range 19-64)
< 40 y	6 (46.2%)
Male:female	7:6
Performanace status (ECOG)	
0-2	5 (38.5%)
3-4	8 (61.5%)
Initial presentation	
Fever	9 (69.2%)
Dyspnea	2 (15.4%)
Abdominal pain	1 (7.7 %)
Skin lesion	1 (7.7 %)
B symptom (+)	13 (100 %)
LDH > normal	12 (92.3%)
Internal prognostic index (IPI)	
Low-intermediate	3 (23.1%)
High-intermediate	4 (30.8%)
High	6 (46.2%)
Hepatomegaly	10 (76.9%)
Splenomegaly	10 (76.9%)
Peripheral lymphadenopathy	5 (38.5%)
Hemophagocytic syndrome (HPS)	8 (61.5%)
EBV ISH (+) (n=11)	10 (90.9%)
DIC (+)	10 (76.9%)

EBV ISH: Epstein Barr virus in situ hybridization; DIC: disseminated intravascular coagulopathy.

and autologous peripheral blood stem cell transplantation (A-PBSCT) were planned after 3 cycles of chemotherapy. In case #11, A-PBSCT was planned after 3 cycles of CHOP. However, disease progressed during stem cell mobilization and the patient died of the disease. In the other two patients (cases #12 and 13), A-PBSCT under BEAM conditioning was performed without serious complications. However, the bone marrow aspiration and biopsy after A-PBSCT showed remnant malignant cells in the bone marrow of both patients. Case #12 died of disease progression after A-PBSCT. However, a complete response was observed in case #13 after three cycles of the salvage chemotherapy containing etoposide, ifosfamide, and cisplatin. The patient has had a second A-PBSCT using cyclophosphamide plus total body irradiation conditioning.

The median survival of all patients was 47 days (range 4-163+ days). Age under 40 (p=0.05), and receiving anthracycline-containing chemotherapy (p<0.05) were good prognostic factors for survival by univariate analysis.

ANKL corresponds to the cases originally described in Japan as aggressive NK-cell leukemia/lymphoma.[8,10] ANKL occurs predominantly in young to middle-aged patients, who present with fever, B symptoms, hepatosplenomegaly, bone marrow infiltration and variable cutaneous lesions.[1-9] The majority of the cases were of high-intermediate and high risk according to the IPI. In contrast to Jaffe's observation, in our study, ANKL was strongly associated with EBV infection (91%).

There are some limitations to determining the prognostic factors because of the rarity of the disease and also because of very short duration of survival. However, our study suggests that younger age and anthracycline-containing treatment seem to be good prognostic factors. There have been only limited reports on the treatment of ANKL. Several articles indicated that NK-cell leukemia was highly aggressive and refractory to any chemotherapeutic trials.[2,4,5,7,8] In our study, clinical response was observed in only 5 of 13 patients (38%) resulting in an extremely poor survival rate.

New therapeutic strategies need to be investigated to improve the outcome of patients with ANKL.

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