Supplementary Table I. Clinical characteristics of 19 enrolled ANKL patients.

Case no.	Sex	Age of onset (year)	Country	Clinical manifestation	WBC ($\times 10^9$/L)	LDH (U/L)	HLH			DIC	EBV	Remarks
							Time	Primary	Mutant gene			
1	F	15	China	Fever, Hem (skin), S, A, and T	1.52	936	4 months before diagnosis of ANKL	No	No	During chemotherapy	(+)	Presenting with relapsed/ refractory HLH
2	M	14	China	Fever, Hep, S, EM (lung), A, and T	0.83	1961	7 days before diagnosis of ANKL	No	No	No	(+)	Lung involvement with cough and chest pain
3[15]	F	14	China	EM (perineum) and L	2.98	919	No			No	(+)	Perineal mass as main manifestation
4[16]	M	16	China	Fever, S, A, and T	2.2	466	No			No	(-)	No
5[17]	F	7	China	Fever, Hem (skin, nose), Hep, S, A, and T	1.06	1394.2	At the time of diagnosis of ANKL	No	NA	Before chemotherapy	(+)	Misdiagnosed as viral hepatitis
6[18]	M	11	China	Fever, Hem (hematuria), S, A, and T	25.77	1856.5	No			No	NA	Presenting with hemolytic anemia
7[19]	M	1.58	China	Fever, SL, L, Hep, S, and A	13.4	295	No			No	(-)	Rash as main manifestation
8[20]	M	16	China	Fever, L, Hep, and A	Elevated, NA	Elevated, NA	No			No	(+)	No
9[21]	M	16	China	Fever, Hem (skin), and S	16.88	NA	No			Before chemotherapy	(-)	No
10[22]	F	17	America	Fever, S, A, and T	1.9	8944	No			No	(+)	Presenting with cold agglutinin-induced autoimmune hemolytic anemia
11[23]	M	5	Australia	Fever, Hep, S, and T	0.71	NA	Almost 4 months before diagnosis of ANKL	No	No	No	(+)	Presenting with relapsed/ refractory HLH

Abbreviations: A anemia; ANKL aggressive natural killer cell leukemia; DIC disseminated intravascular coagulation; EBV Epstein-Barr virus; EM extramedullary mass; F female; Hem hemorrhage; Hep hepatomegaly; HLH hemophagocytic lymphohistiocytosis; L lymphadenopathy; LDH lactate dehydrogenase; M male; NA not available; S splenomegaly; SL skin lesion; T thrombocytopenia; WBC white blood cell; (+) positive; (-) negative.

Supplementary Table I. Continued.

Case no.	Sex	Age of onset (year)	Country	Clinical manifestation	WBC ($\times 10^9$/L)	LDH (U/L)	HLH			DIC	EBV	Remarks
							Time	Primary	Mutant gene			
12[24]	M	18	China	Fever, L, Hep, S, and T	45.5	NA	No			During chemotherapy	(-)	Co-existent ANKL and acute monocytic leukemia
13[25]	M	13	India	S, A, and T	1.1	NA	No			No	NA	No
14[26]	M	0.75	India	Fever, A, EM (testicle), Hep, S, and proptosis	5.0	NA	No			No	NA	Bone marrow and central nervous system relapse during maintenance treatment
15[27]	F	14	Korea	Fever, L, Hep, S, A, T, and right thigh pain	3.56	589				No	(-)	No
16[28]	M	16	Korea	Fever, Hep, S, and T	2.97	NA	At the time of diagnosis of ANKL	No	NA	No	(+)	No
17[8]	M	1.58	Mexico	Fever, Hep, S, A, and myomatoid swelling of submandibular gland	113.1	NA	No			No	(+)	No
18[3]	M	16	Mexico	Fever, Hep, S, and T	NA	10512	1 month before diagnosis of ANKL	No	NA	Before chemotherapy	(-)	Initial manifestation was similar to dengue fever. During course of disease, community-acquired pneumonia, sepsis, septic shock (with DIC), primary HLH, severe hepatitis, and systemic lupus erythematosus were diagnosed successively until 1 day before death (1 month after onset), and ANKL was finally diagnosed
19[29]	M	9.16	China	Fever, Hem (nose), S, A, and T	2.61	625	No			No	(-)	ANKL misdiagnosed as malignant lymphoma and finally confirmed by bone marrow pathology after death

Abbreviations: A anemia; ANKL aggressive natural killer cell leukemia; DIC disseminated intravascular coagulation; EBV Epstein-Barr virus; EM extramedullary mass; F female; Hem hemorrhage; Hep hepatomegaly; HLH hemophagocytic lymphohistiocytosis; L lymphadenopathy; LDH lactate dehydrogenase; M male; NA not available; S splenomegaly; SL skin lesion; T thrombocytopenia; WBC white blood cell; (+) positive; (-) negative.

Supplementary Table II. Morphological, immunophenotypic, cytogenetic, and molecular biological characteristics of 19 enrolled ANKL patients.

Case no.	Morphology	Abnormal cell immunophenotype	Cytogenetics	Molecular biology	
				Fusion gene	Mutant gene
1	BMS: Unidentified cells accounted for 80%	CD56(+), CD16(-)	44,XX,del(1)(p32),+8,der(12),add(13)(q34),-15,-16,add(17)(q25),del(20)(q12),-21	(-)	PIGA, SETBP1, and SRP72
2	BMS: Unidentified cells accounted for 22.5%	CD56(+), CD16(-)	45,XY,del(9)(q13q22),-21[1]/39,XY,add(5)(q13),-7,del(10)(p11.2),-14,-14,add(15)(q25),-16,-19,-20,-21[1]/46,XY[18]	(-)	(-)
3[15]	BMS: Leukemia cells (morphologically similar to lymphocytes) accounted for 90.5%	CD56(+)	ND	ND	ND
4[16]	BMS: Juvenile cells with irregular morphology accounted for 40%	CD56(+), CD16(-)	ND	ND	ND
5[17]	BMS: Some large granular lymphocytes	CD56(+)	91,XX,del(Xq22-24),add(Xq22),+3X2,+7X2,+8,add(9p24)X2,-11,-12,-14X2,-17X2,-18,+20,+21	ND	ND
6[18]	BMS: The proportion of large granular lymphocytes increased BMB: Lymphocytes morphologically similar to prolymphocytes	I: CD56(+), CD16(-) IHC: CD56(+), TIA1(+)	ND	ND	ND
7[19]	BMS: Abnormal lymphocytes and large lymphocytes	CD56(+)	ND	ND	ND
8[20]	BMS: The proportion of lymphocyte-like cells increased significantly	CD56(+), CD16(+)	ND	ND	ND
9[21]	BMS: ANKL	ND	ND	ND	ND
10[22]	BMS: Large granular lymphocytes with basophilic cytoplasm containing azurophilic granules	CD56(+), CD16(+)	Multiple abnormalities including three copies of an abnormal X with material attached at Xp11.1, an inverted chromosome 3 with breakpoints at 3q21 and 3q26, and additional material attached to 8q13	ND	ND
11[23]	BMS: A population of medium-to-large primitive lymphoid cells, with diffuse chromatin and no cytoplasmic granules BMB: A diffuse interstitial infiltrate of abnormal cells	I: CD56(+) IHC: CD56(+)	46,XY,t(5;15)(p11;q11),add(6)(q23),der(7)t(7;17)(q32;21),der(7)t(7;17)(q22;q21) [cp15]/46,XY del(7)(?q22q43) [cp2]/46,XY[1]	ND	ND

Abbreviations: ANKL aggressive natural killer cell leukemia; BMB bone marrow biopsy; BMS bone marrow smear; I immunophenotyping; IHC immunohistochemistry; ND not done; PBS peripheral blood smear; TB testicular biopsy; (-) negative.

Supplementary Table II. Continued.

Case no.	Morphology	Abnormal cell immunophenotype	Cytogenetics	Molecular biology	
				Fusion gene	Mutant gene
12[24]	BMS: A small number of medium-sized natural killer (NK) cells	CD56(+), CD16(+)	46, XY, der(3)(p26),t(4;10)(q26;p15), -9, +mar (12/20 cells)/46,idem, der (2)(q37) (4/20 cells)/46, idem, -der(2)(q37), +2, der(6)(q27) (4/20 cells)	ND	ND
13[25]	PBS: 94% large granular mononuclear cells	CD56(+), CD16(-)	Normal	ND	ND
14[26]	BMS: MPO-negative malignant cells with unknown classification TB: A round cell tumor with brisk mitotic activity	I: Negative IHC: CD56(+), CD99(+), ALK-1(-)	Trisomy of chromosome 8	BCR/ABL (-), TEL/AML1 (-), and MLL(-)	ND
15[27]	BMS: Mostly immature blasts with variable features (95.4%)	CD56(+)	50,XX,t(1;6)(q21;q23),+6,add(6)(q23),+20,+21[4]/49,idem,-6[2]/46,XX[14]	BCR/ABL (-), TEL/AML1 (-), and MLL(-)	ND
16[28]	BMS: Large granular lymphocytes accounted for up to 11.6% of the total nuclear cell number PBS: Relative lymphocytosis and large granular lymphocytes (60%)	CD56(+)	47,XY,+X[8]/46,XY[12]	ND	ND
17[8]	BMS: L2 morphology blasts (96%)	CD56(+), CD16(-)	ND	ND	ND
18[3]	PBS: 32% blast cells	CD56(+), CD16(+)	ND	ND	ND
19[29]	BMS: Abnormal cells with huge volume accounted for 13%, BMB: ANKL	I: Negative IHC: CD56(+)	46, XY	(-)	ND

Abbreviations: ANKL aggressive natural killer cell leukemia; BMB bone marrow biopsy; BMS bone marrow smear; I immunophenotyping; IHC immunohistochemistry; ND not done; PBS peripheral blood smear; TB testicular biopsy; (-) negative.

Supplementary Table III. Immunophenotype of 19 enrolled ANKL patients.

Case no.	CD2	CD3	cCD3	CD4	CD5	CD7	CD8	CD13	CD16	CD33	CD56	MPO	$TCR_{\gamma\delta}$	$TCR_{\alpha\beta}$	CD94
1	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+
2	+	-	-	-	-	-	-	-	-	+	+	-	-	-	+
3[15]	-	-	-	-	/	/	/	-	/	-	+	-	/	/	/
4[16]	-	-	/	/	/	+	-	/	-	/	+	/	/	/	/
5[17]	+	/	-	/	-	+	/	/	/	/	+	/	-	-	/
6[18]	+	-	/	-	-	-	-	/	-	/	+	/	/	/	/
7[19]	+	-	/	/	/	/	-	/	/	/	+	/	/	/	/
8[20]	+	-	-	-	/	+	-	/	+	/	+	/	/	/	/
10[22]	+	/	/	/	/	/	/	/	+	/	+	/	/	/	/
11[23]	+	/	+	-	-	-	+	/	/	/	+	/	-	-	/
12[24]	+	/	+	/	/	+	/	+	+	+	+	+	/	/	/
13[25]	/	-	+	-	-	/	/	/	-	-	+	/	/	/	/
14[#26]	/	-	/	/	/	/	/	/	/	/	+	-	/	/	/
15[27]	/	/	-	/	/	-	/	-	/	-	+	-	-	-	/
16[28]	/	+	/	/	/	/	+	/	/	/	+	/	/	/	/
17[8]	-	-	-	-	-	-	-	-	-	-	+	-	/	/	/
18[3]	+	-	-	-	-	+	-	-	+	/	+	-	/	/	/
19[*29]	/	+	/	/	/	/	/	/	/	/	+	/	/	/	/

and *: No lymphocytes with immunophenotypic abnormalities detected by bone marrow flow cytometry.
#: Immunohistochemistry of testicular biopsy.
*: Immunohistochemistry of bone marrow biopsy.
+: positive; –: negative; /: not reported or not done.
Abbreviations: ANKL aggressive natural killer cell leukemia.

Supplementary Table IV. Treatment and outcome of 19 enrolled ANKL patients.

Case No.	Chemotherapy regimen and main drugs for ANKL	Allo-HSCT	Outcome	Survival time (day)	Cause of death	Remark
1	VDLP: VCR, IDA, PEG-ASP, and Pred	No	Death	9	Multiple organ failure	She received HLH-2004, COP, and L-DEP regimen for HLH. After the diagnosis of ANKL, she received a VDLP regimen, but tumor lysis syndrome occurred on eighth day of chemotherapy
2	EDCH+P (two cycles): VCR, CPM, Dex, liposome doxorubicin, VP16, and PEG-ASP DDGP: CDDP, Dex, gemcitabine, and PEG-ASP VP16, Dex, and chidamide	Yes	Survival CR	570+		HLH-2004 was given for HLH. The response to chemotherapy was PR
3[15]	CDDP, VCR, ADR, and IFO (two cycles)	No	NR (LTF)	NR (LTF)	NR (LTF)	Size of perineal mass decreased and then increased after first cycle, but with no response after second cycle
4[16]	VDCP, EAVCP, and VDCLP	No	Death	About 105	Multiple organ failure	There was no response to VDCP, EAVCP, or VDCLP regimen. Allo-HSCT was not performed due to severe infection and multiple organ failure
5[17]	Dex and VCR	No	Death	10	Respiratory failure	Other chemotherapeutic drugs were rejected
6[18]	No	No	Death	32	Intracranial hemorrhage	No effective chemotherapy regimen was received
7[19]	No	No	Death	30	NR	Treatment was abandoned
9[21]	No	No	NR (LTF)	NR (LTF)	NR (LTF)	Treatment was abandoned
10[22]	No	No	Death	NR	Intracranial hemorrhage	Clinical condition rapidly deteriorated before any treatment was directed to ANKL
11[23]	No	No	Death	2	Multiple organ failure	HLH-2004 was given for HLH. However, clinical condition rapidly deteriorated before any treatment was directed to ANKL
12[24]	Induction chemotherapy: HAA: HHT, Ara-C, and ACM DOAP×2: DNR, VCR, Ara-C, and Pred Consolidation chemotherapy with several courses of anthracycline and targeting both ALL and AML	No	Survival CR	NR		

HLH-2004 regimen included Dex, VP16, and cyclosporin. COP regimen included CPM, vindesine, and Pred. L-DEP regimen included PEG-ASP, ADR, VP16, and methylprednisolone. Abbreviations: ACM aclacinomycin; ADR adriamycin; ALL acute lymphocytic leukemia; Allo-HSCT allogeneic hematopoietic stem cell transplantation; AML acute myeloid leukemia; ANKL aggressive natural killer cell leukemia; Ara-C cytarabine; CDDP cisplatin; CPM cyclophosphamide; CR complete remission; Dex dexamethasone; DNR daunorubicin; HHT homoharringtonine; IDA idarubicin; IFO ifosfamide; L-ASP L-asparaginase; LTF loss to follow-up; 6-MP 6-mercaptopurine; (HD-) MTX (high-dose) methotrexate; NR not reported; PEG-ASP pegaspargase; PR partial remission; Pred prednisone/prednisolone; 6-TG 6-thioguanine; VCR vincristine; VDS vindesine; VP16 etoposide; + more than.

Supplementary Table IV. Continued.

Case No.	Chemotherapy regimen and main drugs for ANKL	Allo-HSCT	Outcome	Survival time (day)	Cause of death	Remark
13[25]	AIEOP-95 HR ALL regimen: VCR, Pred, DNR, L-ASP, CPM, 6-MP, Ara-C, MTX, Dex, ADR, and 6-TG	No	Survival CR	1716		Allo-HSCT was not considered due to economic constraints
14[26]	Interfant-99 protocol: VCR, Pred, Dex, Ara-C, DNR, L-ASP, 6-MP, MTX, 6-TG, CPM, and VP16	No	NR (LTF)	NR (LTF)	NR (LTF)	Patient had a relapse in bone marrow and cerebrospinal fluid 16 months after diagnosis during maintenance therapy
15[27]	ALL-BFM95: VCR, Pred, Dex, Ara-C, DNR, L-ASP, 6-MP, MTX, 6-TG, CPM, VP16, VDS, IFO, and ADR	No	Survival CR	840		
16[28]	Induction chemotherapy: CCG-106B protocol: Pred, VCR, L-ASP, DNR, and CPM CCG-1882 Protocol: Pred, VCR, L-ASP, and DNR SMILE regimen: Dex, MTX, IFO, L-ASP, VP16, and Pred Consolidation chemotherapy: IFO, VP16, L-ASP, and Pred	Yes	Survival CR	1734		Conditioning regimen contained busulfan, fludarabine, VP-16, and antithymocyte globulin
17[8]	MIED regimen: HD-MTX, IFO, VP16, and Dex SMILE regimen: HD-MTX, IFO, L-ASP, VP16, and Dex	No	Survival PR	NR		Tumor lysis syndrome occurred after blood exchange treatment for severe anemia
18[3]	No	No	Death	1	Gastrointestinal hemorrhage	Clinical condition rapidly deteriorated before any treatment directed to ANKL
19[29]	No	No	Death	9	Intracranial hemorrhage	Clinical condition rapidly deteriorated and led to death before diagnosis of ANKL

HLH-2004 regimen included Dex, VP16, and cyclosporin. COP regimen included CPM, vindesine, and Pred. L-DEP regimen included PEG-ASP, ADR, VP16, and methylprednisolone. Abbreviations: ACM aclacinomycin; ADR adriamycin; ALL acute lymphocytic leukemia; Allo-HSCT allogeneic hematopoietic stem cell transplantation; AML acute myeloid leukemia; ANKL aggressive natural killer cell leukemia; Ara-C cytarabine; CDDP cisplatin; CPM cyclophosphamide; CR complete remission; Dex dexamethasone; DNR daunorubicin; HHT homoharringtonine; IDA idarubicin; IFO ifosfamide; L-ASP L-asparaginase; LTF loss to follow-up; 6-MP 6-mercaptopurine; (HD-) MTX (high-dose) methotrexate; NR not reported; PEG-ASP pegaspargase; PR partial remission; Pred prednisone/prednisolone; 6-TG 6-thioguanine; VCR vincristine; VDS vindesine; VP16 etoposide; + more than.