B-lymphoblastic lymphoma with *TCF3-PBX1* fusion gene

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Supplemental Data

B-lymphoblastic lymphoma with TCF3-PBX1 fusion gene

Running tittle; B-LBL with TCF3-PBX1

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Case 1

A 15-year-old female presented to the hospital with coxalgia. X-ray test revealed the presence of the external bone tumor. Magnetic resonance imaging (MRI) and ^{18}fluorodeoxyglucose positron emission tomography/computed tomography (^{18}FDG-PET/CT) confirmed massive tumor at the femur, ovary, and thorax. (Figure 1 A, B and C). Laboratory test showed moderately increased white blood cell (WBC) counts, increased LDH, C-reacted protein (CRP) and soluble IL-2 receptor level. Blast cells were not observed in peripheral blood. Open biopsy was performed and processed for pathologic examination, which revealed a lymphoma (Figure 1 D). Immunohistochemical staining study revealed positivity for CD10, CD79, Ki67, and TdT. The flow cytometry analysis using tumor tissue revealed CD3(-), CD10(+), CD19(+), CD20(-), HLA-DR(+), CD38(+) in CD45 dull population. Tumor cell was not observed by bone marrow aspirations from the independent bilateral site of the ileum. The patient was diagnosed with B-LBL. Cytogenetic analysis using tumor tissue revealed tumor cell karyotype was 46,XX,add(5)(q11.2),der(19)t(1;19)(q23;p13.3). RT-PCR targeting TCF3-PBX1 was positive. Patients were treated with NHL-BFM95 based chemotherapy and achieved complete remission. However, relapsed at thorax immediately after termination of maintenance therapy. Patients are treating for hematopoietic cell transplantation.

Case 1

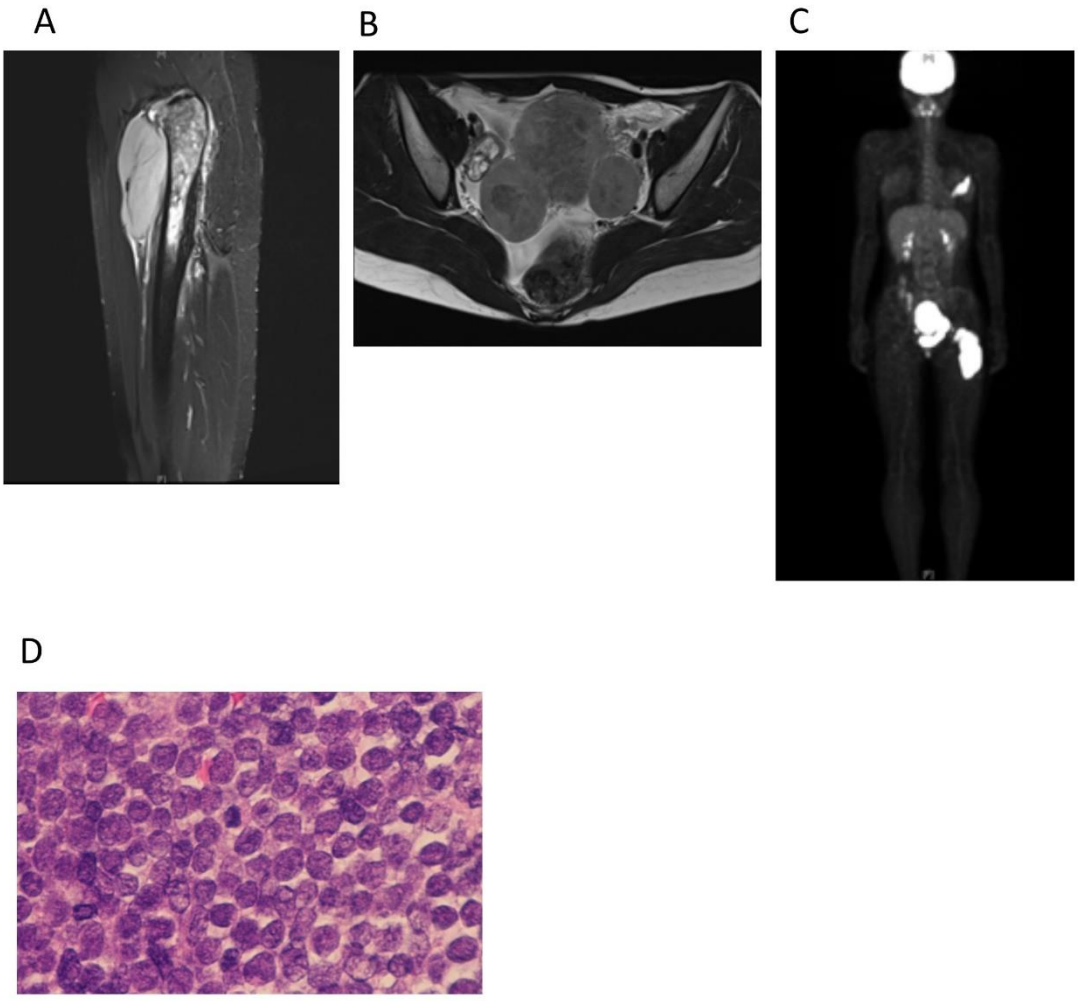


Figure 1.

A: MRI shows massive tumor at proximal femur and external femur. **B**: MRI shows massive tumor at ovary. **C**: ^{18}FDG PET/CT shows hypermetabolic lesions in tumor at femur, external femur, ovary and thorax. **C:** Photomicrograph of biopsy specimen. Hematoxylin-Eosin staining.

Case 1 Laboratory test

Peripheral blood analysis	
WBC	14.7×10^{9}/L (Blast 0 %)
RBC	4.06×10^{12} /L
Hb	10.7 g/dL
Hct	33.1 %
Platelet	3.86×10^{11} /L
Reticulocyte	15.2 ‰

Blood chemical analysis	
TP	7.0 g/dL
BUN	13 mg/dL
Cre	0.44 mg/dL
LDH	3599 U/L
AST	31 U/L
ALT	13 U/L
CRP	3.61 mg/dL
sIL2R	771 U/mL

Case 2

An 11-year-old female presented to the hospital with knee joint pain and fever. X-ray picture suggested the presence of a bone tumor. MRI and ^{18}FDG-PET/CT confirmed massive tumor at distal femur metaphysis and proximal tibia metaphysis (Figure 2 A and B). Laboratory test showed moderately increased WBC counts, increased LDH, CRP, and soluble IL-2 receptor level. Blast cells were not observed in peripheral blood. A biopsy was performed and processed for pathologic examination, which revealed a lymphoma. (Figure 2 C). Bone marrow aspirations from ileum revealed 0.8% of blast cells. Immunohistochemical staining study revealed positivity for CD45(+), CD10(+), CD20(+), BCL2(+). The patient was diagnosed with B-LBL. Cytogenetic analysis using tumor tissue revealed tumor cell karyotype was 47,XX,+i(1)(q10),t(1;19)(q23;p13.3). RT-PCR targeting TCF3-PBX1 was positive. The patient was treated with NHL-BFM95 based chemotherapy and achieved complete remission.

Case 2

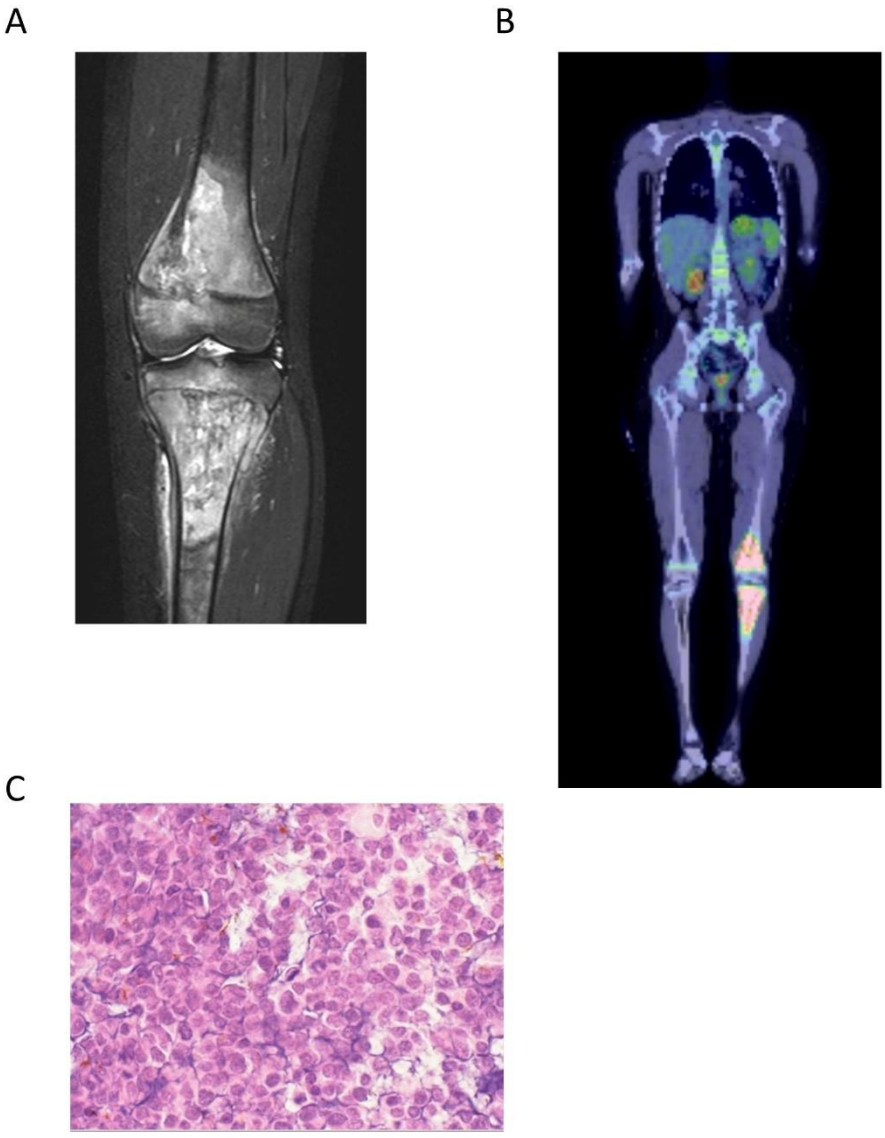


Figure 2

A: MRI shows massive tumor at distal femur metaphysis and proximal tibia metaphysis .

B: ^{18}FDG PET/CT shows hypermetabolic lesions in tumor at proximal tibia. **C:**

Photomicrograph of biopsy specimen. Hematoxylin-Eosin staining.

Case 2 Laboratory test

Peripheral blood analysis	
WBC	10.17×10^{9}/L (Blast 0 %)
RBC	4.98×10^{12} /L
Hb	13.7 g/dL
Hct	39.8 %
Platelet	3.40×10^{11} /L
Reticulocyte	5.7 ‰

Blood chemical analysis	
TP	8.2 g/dL
BUN	10 mg/dL
Cre	0.35 mg/dL
LDH	583 U/L
AST	23 U/L
ALT	12 U/L
CRP	2.02 mg/dL
sIL2R	709 U/mL

Case 3

An 8-year-old male presented to the hospital with facial edema, dyspnea. Superior vena cava syndrome was suspected. X-ray picture revealed the accumulation of pleural effusion and enlargement of cardiothoracic ratio (CTR) dilatation (Figure 3A). CT confirmed mass lesion in right atrium and thorax (Figure 3B). Ultrasound cardiogram showed right atrium is almost occupied with tumor (Figure 3C). ^{18}FDG-PET/CT confirmed massive tumor at right atrium, mediastinum, thorax distal and peritoneal lymph-node (Figure 3D). Laboratory test showed increased WBC counts, increased LDH and CRP. Blast cells were not observed in peripheral blood. A biopsy was performed and processed for pathologic examination, which revealed a lymphoma. (Figure 3E). Bone marrow aspirations from ileum revealed no blast cells. Blast cells were observed in cerebrospinal fluid. Immunohistochemical staining study revealed positivity for CD10(+), CD20(+), CD79a(+), PAX5(+) and TdT(+). The patient was diagnosed with B-LBL. A cytogenic evaluation was not evaluable. RT-PCR targeting TCF3-PBX1 was positive. After termination of NHL BFM95 based chemotherapy, bone marrow relapse occurred. Relapsed tumor cell was positive for TCF3-PBX1, and its karyotype was 46,XY,add(1)(q?21),add(9)(q22), inc. G-banding karyotype analysis was unable to detect t(1;19). Therefore, FISH analysis was performed and confirmed presence of the TCF3-PBX1 fusion gene (Figure 3E). Although hematopoietic cell transplantation was performed, patients died by secondary relapse.

Case 3

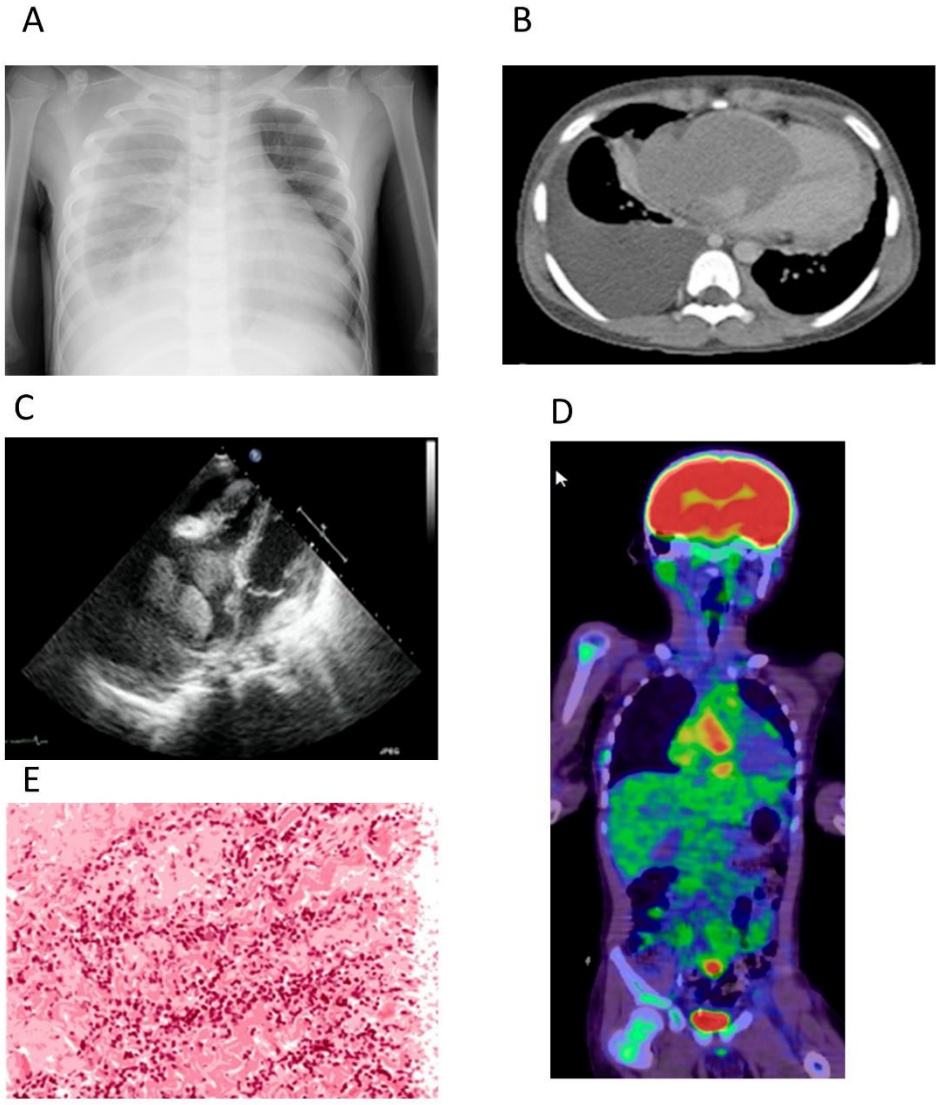


Figure 3

A: Chest X-ray picture shows massive right pleural. **B:** CT shows massive tumor at right atrium, pleural effusion and mass at dorsal thorax. **C**: Ultra sound cardio gram shows right atrium is almost occupied with tumor. **D**: ^{18}FDG PET/CT shows hypermetabolic lesions in mediastinum. **E:** Photomicrograph of biopsy specimen. Hematoxylin-Eosin staining.

Case 3 Laboratory test

Peripheral blood analysis	
WBC	17.98×10^{9}/L (Blast 0 %)
RBC	5.71×10^{12} /L
Hb	13.5 g/dL
Hct	44.2%
Platelet	4.06×10^{11} /L

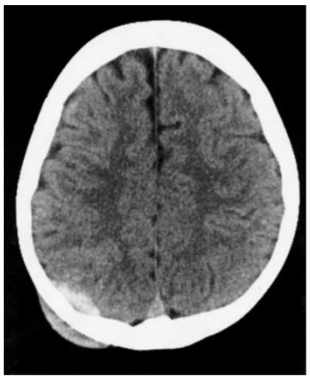
Blood chemical analysis	
TP	6.3 g/dL
BUN	10.3 mg/dL
Cre	0.63 mg/dL
LDH	521 U/L
AST	18 U/L
ALT	18 U/L
CRP	5.4 mg/dL
sIL2R	527 U/mL

Case 4

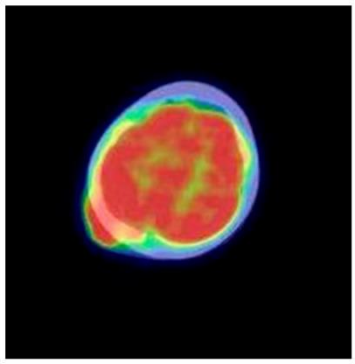
A 9-year-old female presented to the hospital with extracranial mass, low-grade fever, and femur and knee joint pain. MRI and ^{18}FDG-PET/CT confirmed massive tumor at the head. ^{8}FDG-PET/CT positive accumulation was also observed at the jaw, ilium, lumber vertebrate 3 and 4, rib and kidney (Figure 4A and B). Laboratory test showed increased LDH, CRP level. Blast cells were not observed in peripheral blood. Bone marrow aspirations from pelvis revealed 1.2% of blast cells. A biopsy was performed and processed for pathologic examination, which revealed a lymphoma (Figure 4 C). Immunohistochemical staining study revealed positivity for CD20(-/+),CD79a(+) and TdT(+).The patient was diagnosed with B-LBL. G-banding karyotype analysis was unable to analyze. Therefore, FISH analysis was performed and confirmed presence of the TCF3-PBX1 fusion gene (Figure 4D). RT-PCR targeting TCF3-PBX1 from bone marrow revealed positivity. The patient was treated with NHL-BFM95 based chemotherapy and achieved complete remission.

Case 4

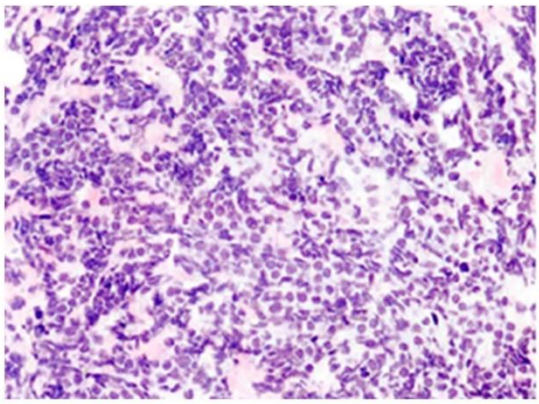
A



B



C



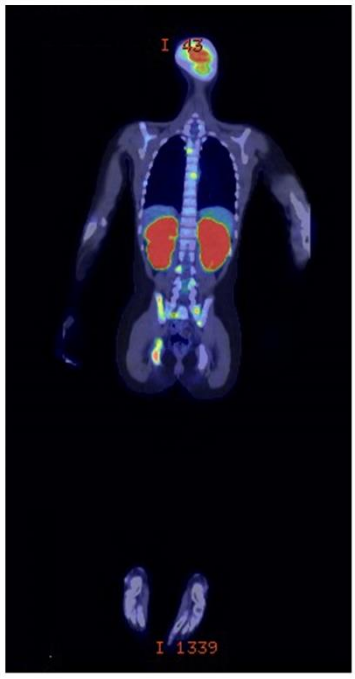


Figure 4

A: CT shows massive tumor outside of skull. **B:** ^{18}FDG PET/CT shows hypermetabolic lesions at outside of skull, jaw, ilium, lumber vertebrate 3 and 4, rib and kidney. **C:** Photomicrograph of biopsy specimen. Hematoxylin-Eosin staining.

Case 4 Laboratory test

Peripheral blood analysis	
WBC	6.31×10^{9}/L (Blast 0 %)
RBC	4.27×10^{12} /L
Hb	12.1 g/dL
Hct	35.4 %
Platelet	4.00×10^{11} /L

Blood chemical analysis	
TP	7.2 g/dL
BUN	12 mg/dL
Cre	0.59 mg/dL
LDH	542 U/L
AST	28 U/L
ALT	17 U/L
CRP	2.5 mg/dL

Case 5

A 13-years-old girl exhibited yellowing of the skin, intermitted abdominal pain, and numbness in both legs for two weeks. She visited emergency room complaining unable to walk, strong abdominal pain, and back pain. Tumorous lesions of the pancreatic head and thoracic cord were pointed out by contrast CT and delivered to our hospital. At the consultation, he had paresis of the lower limbs and jaundice. After hospitalization, dexamethasone administration was started, and emergency radiation of 4 Gy was performed on the thoracic lesion on the day after hospitalization. Then, lower limb paralysis recovered. The biopsy for the pancreatic head was performed. Immunohistochemical staining revealed positivity for CD 10, CD19, and TdT and negativity for CD3, CD20, and MPO. Bone marrow examination showed blast cells were less than 3%. As a result, diagnosis with B-LBL Stage 4 was made. G-banding karyotype analysis was unable to analyze. Therefore, FISH analysis was performed and confirmed presence of the TCF3-PBX1 fusion gene (Figure 5D). Whole transcriptome analysis using biopsy specimen revealed TCF3-PBX1 positivity, and TCF3-PBX fusion mRNA was confirmed by Sanger sequencing. NHL-BFM95 based chemotherapy was performed and maintains remission.

Case 5

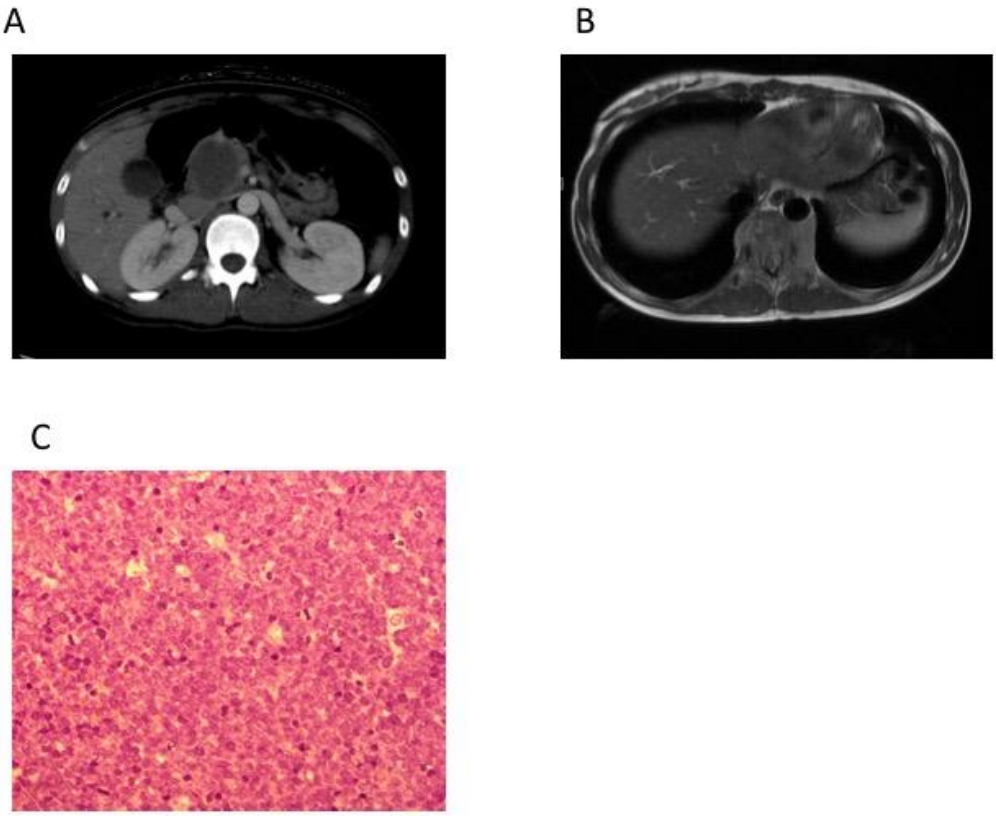


Figure 5

A: CT shows massive tumor at the pancreatic head. **B:** MRI imaging. **C:** Photomicrograph of biopsy specimen. Hematoxylin-Eosin staining.

Case 5 Laboratory test

Peripheral blood analysis

WBC	7.09×10^{9}/L (Blast 0 %)
RBC	419×10^{12} /L
Hb	13.0 g/dL
Hct	40.2 %
Platelet	28.6×10^{11} /L
Reticulocyte	26 ‰

Blood chemical analysis

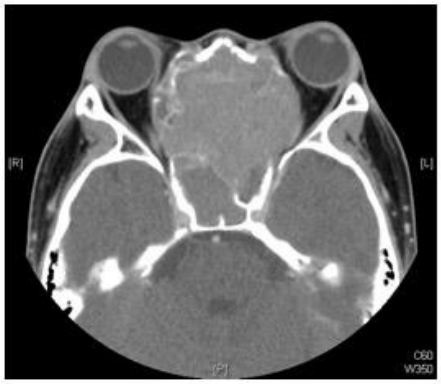
TP	7.4 g/d
BUN	6.9 mg/dL
Cre	0.48 mg/dL
LDH	320 U/L
AST	93 U/L
ALT	195 U/L
CRP	0.81 mg/dL
sIL2R	604 U/mL

Case 6

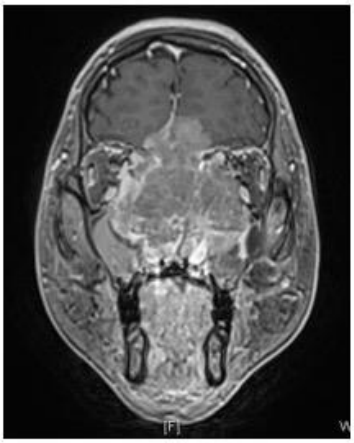
A 13-years-old male complained nasal obstruction and pain in the forehead for 2 months. Then, eyeball protrusion and vision impairment appeared. CT and MRI imaging showed the tumor lesion occupying the skull base from the ethmoid sinus. The endoscopic biopsy was performed. The steroid was administrated after the biopsy. Immunohistochemical staining revealed positivity for CD 19, CD20, and TdT and negativity for CD3 and MPO. Bone marrow examination showed blast cells were less than 3%. Cerebrospinal fluid was negative for blast infiltration. As a result, diagnosis with B-LBL Stage 3 was made. NHL-BFM95 based chemotherapy was performed, and now on therapy.

Case 6

A



B



C

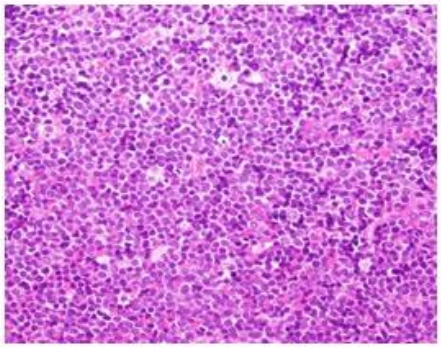


Figure 6

A: CT shows massive tumor lesion occupying the skull base from the ethmoid sinus. **B: :** MRI imaging for coronal section. **C:** Photomicrograph of biopsy specimen. Hematoxylin-Eosin staining.

Case 6 Laboratory test

Peripheral blood analysis	
WBC	1.543×10^{9}/L (Blast 0 %)
RBC	587×10^{12} /L
Hb	16.9 g/dL
Hct	49.9 %
Platelet	29.1×10^{11} /L
Reticulocyte	19 ‰

Blood chemical analysis	
TP	7.4 g/d
BUN	18.5 mg/dL
Cre	0.53 mg/dL
LDH	257 U/L
AST	18 U/L
ALT	49 U/L
CRP	0.03 mg/dL

Case 7

A 14-years-old male noticed a mass at the right palate. Right jaw pain, polyarthralgia, and fever appeared afterward. Then he visited the hospital and treated with prednisolone under the diagnosis of juvenile idiopathic arthritis by a previous doctor. However, gingival swelling of the right lower jaw was seen 4 months after initiation of the therapy. Biopsy of the swelled gingiva was performed and diagnosed as lymphoblastic lymphoma (LBL) (CD10, 19 positive, CD20, κ, λ negative). Gallium scintigraphy showed abnormal accumulation in the right lower jaw and left shoulder joint. Bone marrow examination revealed the percentage of lymphoblasts was 16.5%, and chromosome analysis revealed t(1;19) (q23;p13) positive cells in 4 out of 20 cells. Under the diagnosis of TCF3-PBX1 positive B cell precursor LBL, treatment with TLK-88 protocol which is the ALL treatment protocol of Kyoto Prefectural Medical University was started. The tumor disappeared by the first chemotherapy. Complete remission was also confirmed by the bone marrow examination. The minimal residual cells detected by RT-PCR turned to negative 4 months after the start of treatment. However, during maintenance therapy, back pain and fever appeared. A bone scintigraphy revealed abnormal accumulation in the back of the left rib. Bone marrow examination also revealed 86% of cells were lymphoblast cells. Although we started re-induction therapy, remission had no be achieved. Then, cord blood transplantation was performed under none remission status. However, the blast remained. Although HLA haplo-matched peripheral blood stem cell transplantation was done as a second-round transplantation, the tumor was uncontrollable, and the patient was dead.

Case 7 Laboratory test

Peripheral blood analysis	
WBC	8.420×10^{9}/L
RBC	478×10^{12} /L
Hb	13.8 g/dL
Hct	42.8 %
Platelet	33.8×10^{11} /L

Blood chemical analysis	
TP	7.1 g/d
BUN	6.8 mg/dL
Cre	0.53 mg/dL
LDH	447 U/L
AST	16 U/L
ALT	15 U/L
CRP	12.4 mg/dL