HemaSphere
Powered by EHA

EHA EUROPEAN HEMATOLOGY ASSOCIATION

Case Report
OPEN ACCESS

Lymphoblastic T Cells and Mature NK Cells With the Same TCRγ Rearrangement: A Common Origin?

Anne Roggy[1], Sabeha Biichle[2], Christophe Ferrand[1,2], Florian Renosi[1,2], Fabrice Larosa[3], Eric Deconinck[2,3], Etienne Daguindeau[2,3], Marie-Agnes Collonge Rame[4], Françoise Schillinger[1], Estelle Seilles[1,2], Francine Garnache-Ottou[1,2]

Correspondence: Anne Roggy (anne.roggy@efs.sante.fr).

We report a case of a proliferation of blast cells expressing T antigens, associated with mature NK proliferation, and raises the question of whether the 2 populations identified may derive from a bipotent progenitor maintaining T and NK commitment, or whether they represent a unique NK cell proliferation mature and immature.

A 79-year-old-diabetic male was hospitalized for fever of unknown origin. His blood cell count showed thrombocytopenia (platelets: 66 G/L), no anemia (hemoglobin: 13.7 g/dl), and leukocytosis (white blood cell count: 33.7 G/L) composed of large granular lymphocytes (LGLs: 8.7 G/L) and a population with a blastic morphology (blasts: 17.2 G/L). Neutropenia was also found (polymorphonuclear neutrophils [PNN]: 1.3 G/L). The clinical examination showed changes in the general state, with loss of appetite, weight loss (8 kg lost in 1 mo), dry cough, polyadenopathy. Bone marrow aspiration showed dual infiltration with 50% blasts and 44% LGL.

The blast cells were of intermediate size with a regular nucleus, dispersed nuclear chromatin, multiple variably prominent nucleoli and no cytoplasmic granules (Figure 1). The lymphocytes had LGL-type morphology, with moderate to abundant pale cytoplasm and fine or coarse azurophilic granules (Figure 1B1 and C2).

Flow cytometry analysis performed on the blood revealed 2 different populations. The first represented 34% of blood cells, and expressed immaturity-associated markers ($CD45^{low}$, $CD34^{+}$), T-cell associated antigens (intracytoplasmic (c) CD3, CD7, CD5) and no surface (s) CD3, CD2, CD4, CD8, or CD1a. This population also expressed a low level of myeloid-associated antigens: cCD13 (37%) and CD33 (61%), and a low level of lymphoid B-associated antigen: cCD79a (32%). These cells did not express CD56, CD16, or CD57 (Figure 1A). The second population represented 36% of blood cells, did not express immaturity markers ($CD45^{high}$, $CD34^{-}$), and expressed cCD3, $CD8^{low}$, CD5, CD7, CD56 without expression of other T-cell associated antigens (sCD3, CD4, and CD2) and no expression of other NK-associated antigens (CD16 and CD57). The bone marrow biopsy also showed blast proliferation and LGL infiltration. Puncture of axillary lymph nodes and cerebrospinal fluid (CSF) was performed, and the 2 distinct populations were also found (2% of blasts and 12% of LGL in the CSF). The karyotype performed on bone marrow was complex:

46,XY,del(7)(p14p22),der(7)t(7;21)(p15;q22),der(21)dup(q22q22)t(7;21)(p15;q22)[19]/46,XY[1].nuc ish(RUNX1x4)[95/100].

Based on these results, a diagnosis of T-cell acute lymphoblastic leukemia (T-ALL) and chronic lymphoproliferative disorder of NK cells (CLPD-NK) based on CD56 expression and the absence of membrane CD3 expression was retained.[1]

The molecular analysis performed on peripheral blood and bone marrow cells detected rearrangement of TCRδ and detected 2 clonally rearranged T-cell receptor γ (TCRγ), the main one of 214bp TCRγ (Vγ1.8-Jγ1.1/2.1) and the second of 182 bp (TCRγ Vγ10-Jγ1.1/2.1) (Figure 2A and B). High throughput sequencing on whole blood only detected nonspecific mutations for T/NK-cell neoplasms at low VAF (2%–3%) in *BCL2*, *CIITA*, *MEF2B*, *KMT2A* and in *KMT2D* of 6 variants.

To determine whether blasts and LGL shared the same TCRγ rearrangement profile, we sorted blasts ($CD45^{low}$ $CD34^{+}$), LGL ($CD45^{high}$ $CD3^{-}$ $CD56^{+}$) and T lymphocytes ($CD45^{high}$ $CD3^{+}$ $CD56^{-}$) by FACS. The same TCRγ gene rearrangement (214 bp) was found in blasts and LGL (Figure 2C and D) but not on the T lymphocytes. Another TCRγ gene rearrangement (182 bp) was identified only on T lymphocytes (Figure 2D), and it was very low and probably a canonical rearrangement. No rearrangement of TCR β was found in these populations. Mutations in the signal transducer and activator of transcription 3 gene (STAT3), inducing aberrant STAT3 signaling but was not detected in our case (mutations Y 640D and D661Y).

An acute lymphoid leukemia-type treatment (GRAASPAL group old age) was introduced after 5 days of corticoid therapy. At day 23, after induction therapy, there were still 3% of lymphoblasts in blood. Due to complications of chemotherapy, palliative treatment was introduced, but the percentage of blast

[1]*Laboratoire d'hématologie et d'immunologie, Etablissement Français du Sang de Bourgogne/Franche-Comté, Besançon, France*
[2]*Univ. Bourgogne Franche-Comté, INSERM, EFS BFC, UMR1098, RIGHT. Interactions Greffon-Hôte-Tumeur/Ingénierie Cellulaire et Génique, Besançon, France*
[3]*Service d'hématologie Clinique, Centre Hospitalier Universitaire de Besançon, Besançon, France*
[4]*Service de Génétique Biologique, Centre Hospitalier Universitaire de Besançon, Besançon, France*
Copyright © 2021 the Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the European Hematology Association. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.
HemaSphere (2021) 5:8(e608).
http://dx.doi.org/10.1097/HS9.0000000000000608.
Received: 19 April 2020 / Accepted: 27 May 2021

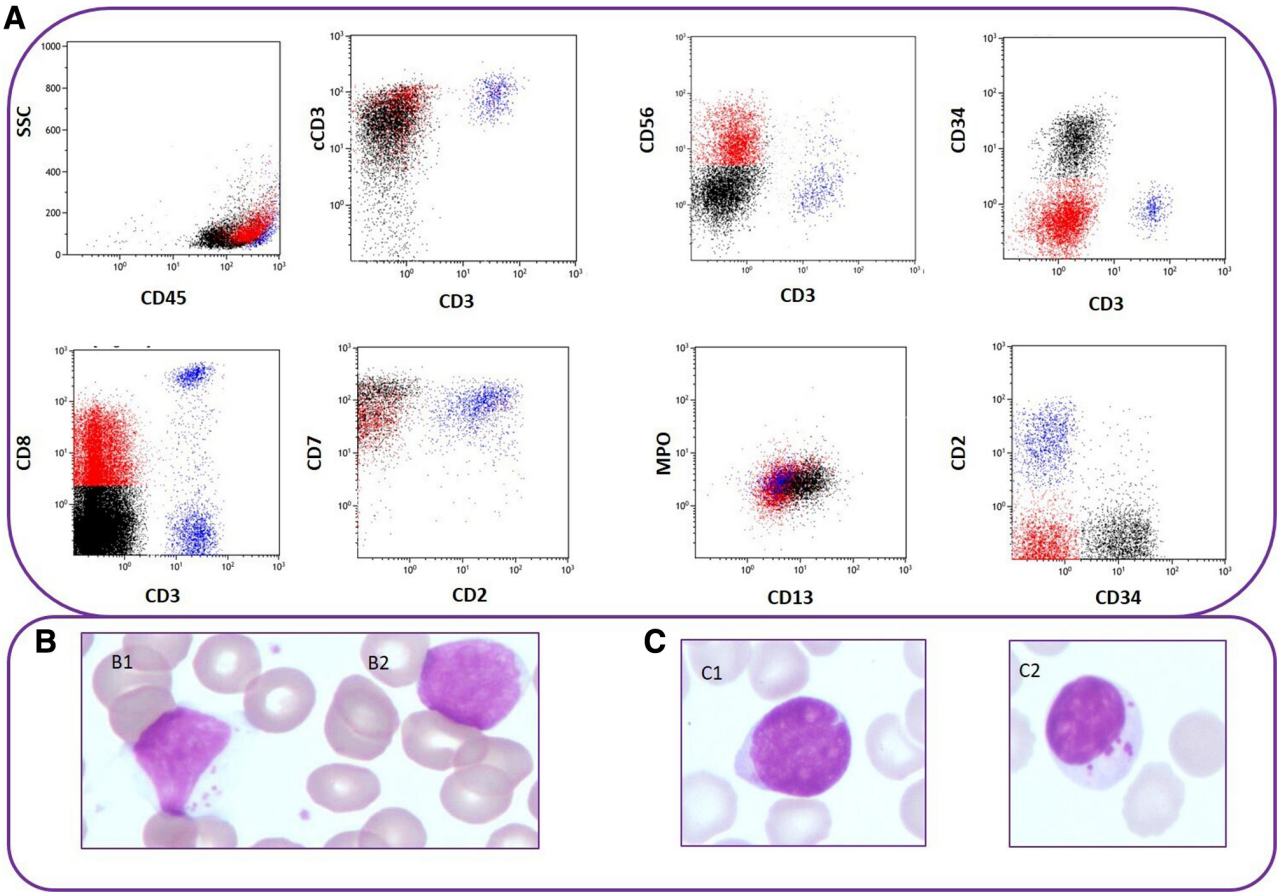

Figure 1. Phenotype and cytologic characterization of the 2 subpopulations. (A), Flow cytometry analysis performed on blood revealed a blast population (black) CD45^{low}, CD34^{+} that expressed T-cell associated antigens (intracytoplasmic cCD3, CD7) but no surface CD3, CD2, CD8 and also expressed a low level of myeloid-associated antigens: CD13. The cells did not express CD56. The second population was CD45^{high}, CD34^{-} (in red), expressed intracytoplasmic cCD3, CD8^{low,} CD7, CD56 without expression of other T-cell associated antigens (sCD3 and CD2). The blue population corresponds to residual T lymphocytes. (B), Blood examination showed blast cells (B2) and large granular lymphocytes (B1). (C), Bone marrow examination showed blast cells (C1) and large granular lymphocytes (C2) CD45$^{PerCp5.5}$, CD3^{PE}, CD2^{FITC}, CD7^{FITC}, CD56$^{FITC\ or\ PE,}$ CD8$^{PE\ or\ FITC}$, MPO^{FITC}, CD13^{PE}, CD34^{APC}. APC = allophycocyanin; FITC = fluorescein isothiocyanate; PE = phycoerythrin; PerCP5.5 = Peridinin-chlorophyll protein 5.5. Beckman Coulter Immunotech, Miami, FL, USA; BD Biosciences, San Jose, CA, USA.

cells increased in the blood, cytopenia worsened, and the patient died 7 months after the start of chemotherapy.

We report an original case of proliferation of T lymphoblasts, associated with NK cell proliferation. These 2 populations have definitively different morphologies and phenotypes, but expressed the same TCRγ rearrangement, indicating that they arose from the same progenitor, or that the more mature population (LGL) arose from the immature one (lymphoblasts).

Normal bipotential T/NK precursors are physiologically found in the thymus[2] that produces predominantly T cells and relatively few NK cells, suggesting that the thymus microenvironment physiologically favors the development of T cells over NK cells. In the thymus, uncommitted CD34^{+} CD1a^{-} precursors can be subdivided into an early CD5^{-} and a later CD5^{+} stage, both having T and NK cell potential.[3] Development through the first pro-T-cell stage is coordinated with migration through distinct thymic environments in relation with environmental inputs, such as ligands for the Notch cell-surface receptors inducing Notch signaling, which initiates the T-cell-lineage program.[4] Non-T-cell factors such as inhibitors of DNA binding (Id)2, SCL (TAL1), C/EBPα, GATA2, and PU.1 are likely to have direct roles in maintaining non-T-cell developmental options.[4] Notably, the cytokine IL-15 and Id2, which negatively regulates the activity of basic helix-loop-helix transcription factors (E2A and HEBalt), both play key roles in NK cell development. Indeed, it was reported that Id2 increased a pool of lineage CD1a^{-} CD5^{+} progenitors, which, in synergy with IL-15, furthered expansion and differentiation into NK cells.[5]

NK-cell development also includes 2 stages maintaining NK, T and DC capacity and, therefore, some tripotential T/NK/DC common progenitors.[6] Unfortunately, these NK progenitors express markers that overlap with those of T-cell ALL, including CD7, CD2, and even CD5 and cytoplasmic CD3, so the distinction between T-ALL and immature NK-cell tumors may be difficult based on phenotype.[6] Moreover, uncommitted progenitors may initiate TCRγ gene rearrangements, even before reaching the thymus and in the same way, immature CD34^{+} CD38^{+} CD1a^{-} thymic subsets can harbor TCRγ rearrangements (Vγ-Jγ1.1/2.1) before the initiation of TCRδ rearrangement,[7] suggesting that a TCRγ rearrangement can be found in T and NK progenitors.

In light of these data, we hypothesize: (1) that the 2 populations identified may derive from a bipotent progenitor maintaining T and NK differentiation capacity, with a pool of still immature cells (identified as T lymphoblasts in our case) and a pool of cells that mature into NK cells under microenvironmental influences or (2) blasts and LGL represent a unique NK cell proliferation with a contingent of immature NK blast cells (blastic morphology, CD34+, CD45^{low,} cCD3^{+} CD56^{-}) and another contingent of more mature cells (LGL morphology, CD34^{-}, CD45^{high}, cCD3^{+}, CD56^{+}) that mature from the blastic population.

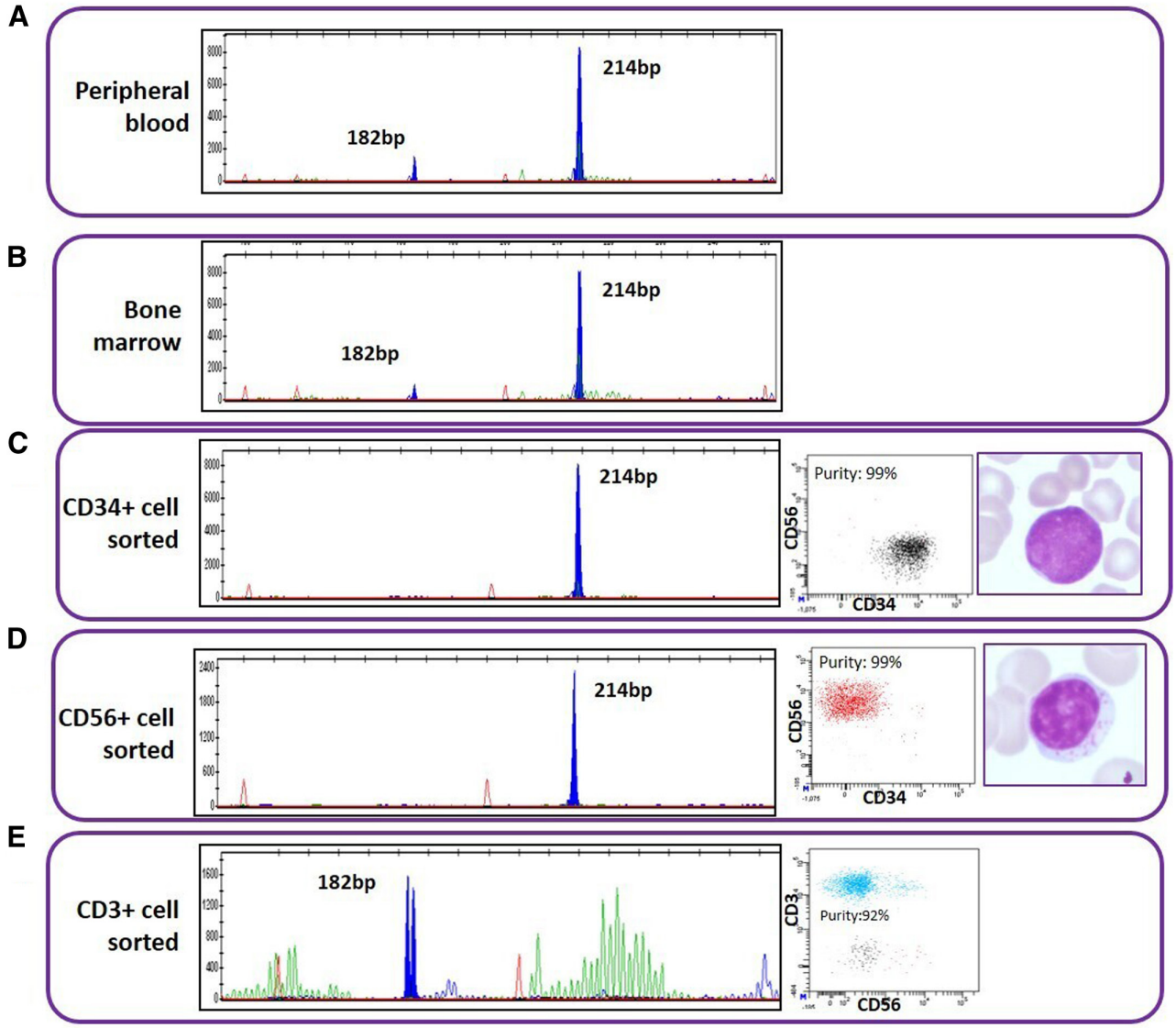

Figure 2. TCR rearrangement performed CD34+, CD56+, and CD3+ sorted cells (peripheral blood or bone marrow cells). The red line is the molecular weight GS400 Rox, the blue line corresponds to the patient profile and green line to the polyclonal rearrangement. (A), Blood: detection of 2 clonally rearranged T-cell receptor γ (TCRγ): the main one of 214 bp, and the second of 182 bp. (B), The same rearranged TCR γ in the bone marrow. (C), In black, the CD34+ sorted cells (CD45^{low} CD34^{+}, purity of 99%) have a monoclonal rearranged TCR γ of 214 bp, as the CD56+ cells. (D), In red: the LGL cells (CD45^{high} CD3^{-} CD56^{++}, purity of 99%). (E), In blue, CD3+ sorted cells (CD45^{high} CD3^{+}, purity of 92%) have the second rearranged T-cell receptor γ (TCRγ) of 182 bp.

Recurrent translocations involving *RUNX1* on the chromosomal band 21q22 are common in patients with acute leukemia. Translocation t(7;21) (p15;q22) has been sometimes described in acute myeloid leukemia (AML) and in one case of ETP-ALL.[8–10] Partner genes on 7p15.2 have been hypothesized twice, to the best of our knowledge, firstly with *EVX1*, in an *RUNX1-EVX1* in-frame fusion protein, detected by RNA-seq in ETP-ALL with normal karyotype.[10] Interestingly, the RHD domain of *RUNX1* and the homeodomain of *EVX1* were conserved. In a second case report of de novo AML, the *HOXA* cluster was suspected to be involved.[8] However, the *HOXA* cluster is only 50 kb away from *EVX1*, and considering the analytical resolution of FISH (around 100–200 kb), an *RUNX1-EVX1* fusion cannot be ruled out in that case.

In our case, overrepresentation of *RUNX1* was detected with 4 signals by FISH, without amplification (as in B-ALL or therapy-related AML with iAMP21 where 5 signals or more are necessary[11–13]).

Differential diagnosis of this case might consider acute transformation of a chronic large granular lymphoproliferation, which was previously described in a case presenting a blastic LGL population,[14] but this was not the case in our patient, in whom the blast population displayed no granulations. Myeloid/natural killer cell precursor AL (MNKL) is an uncommon entity described in the WHO 2008 classification and considered in the WHO 2017 classification as an immature myeloid AL,[12] the low level of myeloid-associated antigens: cCD13 and CD33 is not sufficient to evoke the diagnosis of myeloid acute leukemia. NK cell lymphoblastic leukemia/lymphoma (NK-ALL/BL) is defined by expression of CD56 along with immature T-cell associated markers such as cCD3, CD2, and CD7, with the absence of B lymphoid, myeloid markers, and TCR and immunoglobulin (Ig) receptor rearrangement.[15] In our case, the LGL expressed CD56 but not the blast population, and cells were TCRγ rearranged. Finally, we cannot exclude formally that mature LGL cells are T-LGL cells having lost membrane CD3 (in the relation with an oncogenic abnormality) and not true NK cells; according to this

hypothesis it should be a proliferation of a mature T-LGL cells and a leukemic block of an immature T progenitor, developing an acute-T-cell leukemia.

In conclusion, we describe a case with an excess of lymphoblasts and LGL showing clear cytological and phenotypical distinction, but a common clonal origin. This presentation does not clearly fit with known leukemia/lymphomas already described in the literature or in the WHO classification and highlights that an excess of LGL associated with "T or NK" lymphoblastic proliferation must be considered as potentially clonal, instead of reactive LGL, and might prompt biologists and physicians to diagnose other cases to clarify this type of heterogeneous proliferation.

Acknowledgments

The authors would like to thank Fiona Ecarnot, PhD (EA3920, University of Franche-Comté, Besancon, France) for her help in preparing the manuscript.

Disclosures

The authors have no conflicts of interest to disclose.

References

1. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). *Leukemia*. 1995;9:1783–1786.
2. Sánchez MJ, Muench MO, Roncarolo MG, et al. Identification of a common T/natural killer cell progenitor in human fetal thymus. *J Exp Med*. 1994;180:569–576.
3. Lu M, Tayu R, Ikawa T, et al. The earliest thymic progenitors in adults are restricted to T, NK, and dendritic cell lineage and have a potential to form more diverse TCRbeta chains than fetal progenitors. *J Immunol*. 2005;175:5848–5856.
4. Maillard I, Fang T, Pear WS. Regulation of lymphoid development, differentiation, and function by the Notch pathway. *Annu Rev Immunol*. 2005;23:945–974.
5. Schotte R, Dontje W, Nagasawa M, et al. Synergy between IL-15 and Id2 promotes the expansion of human NK progenitor cells, which can be counteracted by the E protein HEB required to drive T cell development. *J Immunol*. 2010;184:6670–6679.
6. Freud AG, Caligiuri MA. Human natural killer cell development. *Immunol Rev*. 2006;214:56–72.
7. Dik WA, Pike-Overzet K, Weerkamp F, et al. New insights on human T cell development by quantitative T cell receptor gene rearrangement studies and gene expression profiling. *J Exp Med*. 2005;201:1715–1723.
8. Moon Y, Horsman DE, Humphries RK, et al. A novel translocation involving RUNX1 and HOXA gene clusters in a case of acute myeloid leukemia with t(7;21)(p15;q22). *Immune Netw*. 2013;13:222–226.
9. Jeandidier E, Dastugue N, Mugneret F, et al. Abnormalities of the long arm of chromosome 21 in 107 patients with hematopoietic disorders: a collaborative retrospective study of the Groupe Français de Cytogénétique Hématologique. *Cancer Genet Cytogenet*. 2006;166:1–11.
10. Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature*. 2012;481:157–163.
11. Podgornik H, Debeljak M, Zontar D, et al. RUNX1 amplification in lineage conversion of childhood B-cell acute lymphoblastic leukemia to acute myelogenous leukemia. *Cancer Genet Cytogenet*. 2007;178:77–81.
12. Swerdlow SH; World Health Organization; International Agency for Research on Cancer, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Revised 4th ed. International Agency for Research on Cancer; 2017.
13. Andersen MK, Christiansen DH, Pedersen-Bjergaard J. Amplification or duplication of chromosome band 21q22 with multiple copies of the AML1 gene and mutation of the TP53 gene in therapy-related MDS and AML. *Leukemia*. 2005;19:197–200.
14. Ohno Y, Amakawa R, Fukuhara S, et al. Acute transformation of chronic large granular lymphocyte leukemia associated with additional chromosome abnormality. *Cancer*. 1989;64:63–67.
15. Sedick Q, Alotaibi S, Alshieban S, et al. Natural killer cell lymphoblastic leukaemia/lymphoma: case report and review of the recent literature. *Case Rep Oncol*. 2017;10:588–595.