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CASE REPORT

Very rare near-haploid acute lymphoblastic leukemia resistant to immunotherapy and CAR-T therapy in 19-year-old male patient

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Abstract

Near-haploid acute lymphoblastic leukemia is rare subgroup of the disease, which is very important due to very poor prognosis and resistance to treatment including novel monoclonal antibodies and CAR-T therapy.

KEYWORDS

acute lymphoblastic leukemia, blinatumomab, CAR-T therapy, inotuzumab ozogamicin, near-haploid

Marie Jarosova and Michael Doubek contributed equally as senior authors.

1 | INTRODUCTION

Aneuploidy, the gain or loss of whole chromosomes, is recognized as one of the major genomic events in human cancers.[1–3] Aneuploidy is found in ~60% of hematological malignancies.[4] In acute lymphoblastic leukemia (ALL), aneuploidy with a modal number of <40 chromosomes (hypodiploidy) is represented by two subtypes: near-haploidy (24–30 chromosomes) and low hypodiploidy (31–39 chromosomes). Near-haploidy (NH) is genetically characterized by the presence of numerous monosomies and only a few disomies.[5] The diagnosis of such NH ALL is a challenge because, at the time of diagnosis, most blasts can bear a doubled chromosomal content creating a subclone with 48–58 chromosomes (a phenomenon denoted "masked hypodiploidy").[5–7] This doubled clone contains tetrasomies for disomic chromosomes in the original hypodiploid clone, and uniparental disomies with complete loss of heterozygosity (LOH) for chromosomes that were initially monosomic. Chromosomal doubling is believed to occur via endoreduplication (i.e., replication of the genome without subsequent cytokinesis).[8–12] Mistaking masked NH for hyperdiploidy could lead to erroneous risk classification and, hence, risk of treatment failure.[5] The presence of LOH of all chromosomes that are not gained can confirm masked hypodiploidy by using SNP array or NGS analyses.

Thus far, the total number of registered cases in the Mitelman database with near-haploidy is 197 ALL cases.[7] NH ALL cases are immunophenotypically B-cell precursors and morphologically distinguishable blasts of 2 different sizes, where small blasts harbor near-haploidy and larger ones hyperdiploidy. While NH is rare among children (<15 years) with ALL, it has virtually never been reported in adults.[13] The prognosis is generally poor.[6]

2 | CASE REPORT

In our report, we describe a unique case of NH ALL. A 19-year-old male patient was diagnosed with ALL in June 2020. The patient was previously healthy and had no relevant medical history. Initially, the patient presented in the hospital with a 10-day history of weakness, musculoskeletal pain, frequent sweats, and nausea. A peripheral blood cell count revealed severe thrombocytopenia 22×10^9/L, normal hemoglobin level, and a leukocyte count of 6.13×10^9/L containing 31% morphologically unclassifiable blasts. A bone marrow smear showed 93.6% infiltration with myeloperoxidase negative and PAS-positive blasts of two different size (Figure S1), with a common B-ALL immunophenotype (CD10+19+20–34+38-/+DR+66c+304+73+22-/+24+9-/+81+c79a+/--cTdT+/-). Neither extramedullary nor CNS infiltration were observed at the time of diagnosis.

The patient was recruited into the "Blina-CELL" clinical trial (NCT04554485) and treated with a short 7-day run-in phase chemotherapy containing dexamethasone, cyclophosphamide, daunorubicine, and vincristine, plus an induction therapy with one cycle of blinatumomab. Treatment response assessments after the run-in phase and induction showed a refractory disease and resulted in a treatment change. Two cycles of inotuzumab ozogamicin were applied as a salvage treatment without achieving a remission. A fulminant disease progression occurred immediately in October 2020. Autologous anti-CD19 chimeric antigen receptor T cells (CAR-T cells) tisagenlecleucel were manufactured and administered with a corticosteroid bridging therapy and fludarabine and cyclophosphamide as a lymphodepleting regimen. Grade 4 cytokine release syndrome (CRS) and grade 4 immune effector cell-associated neurotoxicity syndrome (ICANS) occurred as a complication of CAR-T. The patient was treated with corticosteroids and tocilizumab. The toxicity grades decreased; however, a response assessment 2 weeks after CAR-T therapy revealed refractory disease again, and the patient died a few days later. A timeline summarizing the patient's treatment and karyotyping results at different time points are depicted in Figure 1A. Although all novel treatment options for ALL were utilized, the patient failed to achieve even a hematologic response.

At the time of diagnosis and subsequently during the disease course, flow cytometry, morphology, cytogenomics, and targeted next-generation sequencing (NGS)[14] analyses were performed on bone marrow cells (Figure 1A). Cytogenomic examinations involved karyotyping, fluorescence *in situ* hybridization (FISH), and arrayCGH/SNPs (methods can be found in Appendix S1).

At diagnosis, cytogenetics revealed hyperdiploid karyotype 49,X,+X,-Y,+8,+21,+21 with the sole tetrasomy of chromosome 21 (Figure 1B). Chromosome 8 trisomy, additional chromosome X, and loss of chromosome Y were confirmed by using FISH. The arrayCGH/SNPs revealed cnLOH for all chromosomes except chromosomes 8 and 21 (Figure 1C). Moreover, this arrayCGH/SNPs showed three regions with biallelic deletion: 7p- including *IKZF1* gene, 9p- involving *CDKN2A/B* and 13q-encompassing *RB1* gene, all considered to be negative prognostic factors in ALL.[15–20] To confirm the presence of only duplicated clone at diagnosis, we performed iFISH analysis using an XL BCR/ABL1 probe (MetaSystems) on the bone marrow smear and revealed 97% of cells showed normal findings of two signals for both *ABL1* and *BCR* genes. This finding confirmed a clone detected by karyotyping with partial duplication of chromosomes 21 and X and with trisomy of chromosome 8 (Figure S2). Chromosome 8 trisomy,

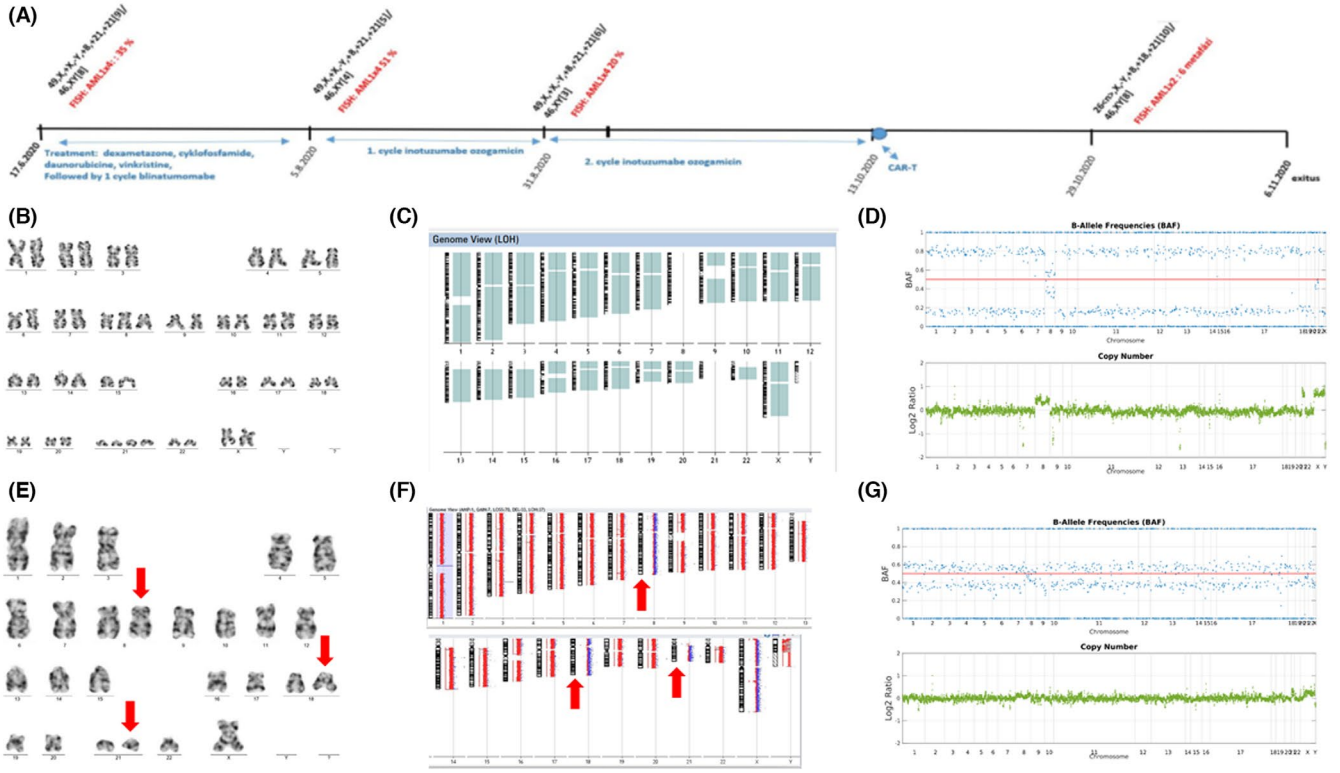

FIGURE 1 Karyotyping, arrayCGH and NGS outputs from different timepoints. (A) Time line depicting therapy interventions and cytogenomics analyses, (B) karyotype 49,X,+X,-Y,+8,+21,+21 from the diagnosis illustrating "masked hypodiploid" clone, (C) arrayCGH/SNPs (Cytogenomics Agilent) plot showing cnLOH of all chromosomes (blue lines along whole chromosome) except of chromosomes 8 and 21, (D) NGS output for the analysis of chromosomal aberrations at the diagnosis (read depth and BAF approach are depicted), (E) Karyotype 26<n>,X,-Y,+8,+18,+21 at the time of relapse after CAR-T treatment confirming NH ALL, (F) arrayCGH/SNP plot at relapse showing losses of chromosomes (red line along the chromosome) and normal disomies of chromosomes 8, 18, and 21 (red arrows), (G) NGS output from follow-up sample

instead of the expected tetrasomy, could lead to cytogenetic misinterpretation of the "masked hypodiploidy." This duplicated clone was present in follow-up samples taken from the patient (Table 1); however, conventional cytogenetics performed after CAR-T therapy revealed near-haploid clone only (Figure 1E). The loss of all chromosomes except for chromosomes 8, 18, and 21 was also confirmed by arryCGH/SNPs (Figure 1F).

Targeted NGS analysis[14] focused on integrative detection of gene and chromosomal aberrations was performed in two consecutive samples collected at the diagnosis and during therapy. Based on this analysis, four gene variants associated with hematologic malignancies were identified in both samples with different variant allele frequency (VAF): *NF1* (NM_000267.3: c.1260+1G>A, VAF 61.3% and 15.7%), *NOTCH2* (NM_024408.3: c.3980A>G, VAF 17.3% and 41.4%), *TYK2* (NM_003331.4: c.211T>C, VAF 16.9% and 36.6%), and *FBXW7* (NM_033632.3, c.45_46insCCT, VAF 80.8% and 59.3%). The splicing *NF1* gene variant was classified as "probably pathogenic."[21] Mutations in *NF1* gene have been associated with Ph-like ALL subtype.[22] The impact of *NOTCH2*, *FBXW7*, and *TYK2* variants was evaluated as well, and they were classified as variants of potential clinical significance. Detailed NGS results for gene variants are attached in Table S1. Moreover, NGS panel revealed an extensive cnLOH affecting all chromosomes except for chromosomes 8 and 21 and in diagnostic sample confirmed array results and shows deletions on chromosomes 7, 9, and 13, Y loss and also gains in chromosomes 8 and 21, and X (Figure 1D). See Table S2.

3 | DISCUSSION

Near-haploid ALL is characterized by genetic alterations disrupting receptor tyrosine kinase signaling, Ras signaling, and the *IKZF3* gene.[13] The study published by Pui et al.[23] confirmed that patients with near-haploidy have activated Ras and phosphatidylinositol 3-kinase (PI3K) signaling. Inhibitors of PI3K and PI3K/mammalian target of rapamycin were demonstrated to inhibit proliferation of both near-haploid and low-hypodiploid cells *ex vivo*.[23] Novel treatment options for B-cell ALL, including

TABLE 1 Patient karyotype, fluorescence in situ hybridization (FISH), and arrayCGH/SNPs at defined timepoints

Timepoint	Sample	Karyotype (ISCN)	FISH	ArrayCGH/SNPs
At diagnosis	BM	49,X,+X,-Y,+8,+21,+21[9]/46,XY[8]	nuc ish(RUNX1x4)[70/200]/(D8Z2,MYC)x3[70/200]/(CEPXx2,CEPYx0)[80/200]	arr[GRCh37] (X)x2,(Y)x0,(1–7)x2 mos hzm,7p12.2(50414022_50462400)x0~1,(8)x3,(9–13)x2 mos hmz,9p21.3(21905380_22002337)x0~1,13q14.2(49047472_49068928)x0~1,(14–17)x2 mos hmz,(18)x(21)x4,(22)x2 mos hmz
After induction I with blinatumomab	BM	49,X,+X,-Y,+8,+21,+21[5]/46,XY[4]	nuc ish(RUNX1x4)[153/300]	
After salvage with inotuzumab ozogamicin	BM	49,X,+X,-Y,+8,+21,+21[6]/46,XY[3]	nuc ish(RUNX1x4)[40/200]/(D8Z2x3)[26/200]	
After CAR-T	BM	26<n>,X,-Y,+8,+18,+21[10]/46,XY[8]	ish 21q22(AML1x4)[1]/21q22(AML1x2)[6]/(D8Z2x2)[8]	arr[GRCh37] (X)x1,(Y)x0,(1–7)x1,7p12.2(50414022_50462400)x0~1,8q23.2q24.13(111675315_124526607)x2 hmz,(9–13)x1,9p21.3(21905380_22002337)x0~1,13q14.2(49047472_49068928)x0~1,(14–17)x1,(19–20)x1,(21)x2,(22)x1

Abbreviation: BM, bone marrow.

monoclonal antibodies and CAR T cells, may also have therapeutic potential in these patients.[24,25] However, our case does not confirm these observations. Considering patient´s refraction to given treatment, the chance for finding new possible drug sensitivities could lay in performing drug response profiling studies. This could be a potential way of finding alternative treatment options, although there is currently lack of experimental evidence in adult patients with ALL.[26]

4 | CONCLUSION

Our results showed high complexity of chromosomal changes in NH ALL and variability of the endoduplication which may not appear on all chromosomes in the haploid set. We demonstrated a great benefit of parallel analysis by arrayCGH/SNP and targeted NGS for the detection of NH clone. From a biological point of view, we speculate that the cell response to CAR-T therapy could lead to the interruption of endoduplication, as the only near-haploid clone was detected after this therapy. From the medical point of view, we have not confirmed the success of targeted therapy in this NH ALL case.

CONFLICTS OF INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTION

T.A., S.H., M.O., F.F., P.S., and. J.M. treated the patient and collected clinical data; E.B., H.J., Z.V., L.B., V.N., E.O., M.S., and S.P., performed cytogenetic, NGS, and molecular analyses; A.B. performed morphologic analysis; M.J. and M.D. treated the patient, performed cytogenetic analyses, and supervised the team's work.

CONSENT

Written informed consent was obtained from the patient parents to publish this report in accordance with the journal's patient consent policy.

DATA AVAILABILITY STATEMENT

All data available on request from the authors.

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REFERENCES

1. Boveri T. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. *J Cell Sci*. 2008;121:1-84.
2. Ben-David U, Amon A. Context is everything: aneuploidy in cancer. *Nat Rev Genet*. 2020;21:44-62.
3. Gordon DJ, Resio B, Pellman D. Causes and consequences of aneuploidy in cancer. *Nat Rev Genet*. 2012;13:189-203.
4. Duijf PGH, Schultz N, Benezra R. Cancer cells preferentially lose small chromosomes. *Int J Cancer*. 2013;132:2316-2326.
5. Safavi S, Paulsson K. Near-haploid and low-hypodiploid acute lymphoblastic leukemia: two distinct subtypes with consistently poor prognosis. *Blood*. 2017;129(4):420-423.
6. Lundin-Ström KL, Ström K, Biloglav A, et al. Parental origin of monosomic chromosomes in near-haploid acute lymphoblastic leukemia. *Blood Cancer J*. 2020;10:51.
7. Mitelman F, Johansson B, Mertens F. Mitelman database of chromosome aberrations and gene fusions in cancer. http://cgap.nci.nih.gov/Chromosomes/Mitelman. Accessed February 22, 2021.
8. Callen DF, Raphael K, Michael PM, Garson OM. Acute lymphoblastic leukemia with a hypodiploid karyotype with less than 40 chromosomes: the basis for division into two subgroups. *Leukemia*. 1989;3(10):749-752.
9. Gibbons B, MacCallum P, Watts E, et al. Nearhaploid acute lymphoblastic leukemia: seven new cases and a review of the literature. *Leukemia*. 1991;5(9):738-743.
10. Oshimura M, Freeman AI, Sandberg AA. Chromosomes and causation of human cancer and leukemia. XXIII. Near-haploidy in acute leukemia. *Cancer*. 1977;40(3):1143-1148.
11. Ma SK, Chan GCF, Wan TSK, et al. Near-haploid common acute lymphoblastic leukaemia of childhood with a second hyperdiploid line: a DNA ploidy and fluorescence in-situ hybridization study. *Br J Haematol*. 1998;103(3):750-755.
12. Misawa S, Oguma N, Testa JR. A case of acute lymphoblastic leukemia with severe hypodiploidy. *Cancer Genet Cytogenet*. 1985;16(2):137-143.
13. Moorman A. The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia. *Blood Rev*. 2012;26:123-135.
14. Navrkalova V, Plevova K, Hynst J, et al. LYNX (LYmphoid NeXt-generation sequencing) panel: a comprehensive capture-based sequencing tool for the analysis of prognostic and predictive markers in lymphoid malignancies. *J Mol Diagnostics*. 2021;23(8):959-974.
15. Zhang W, Kuang P, Liu T. Prognostic significance of CDKN2A/B deletions in acute lymphoblastic leukaemia: a meta-analysis. *Ann Med*. 2019;51(1):28-40.
16. Zhang W, Kuang P, Li H, Wang F, Wang Y. Prognostic significance of IKZF1 deletion in adult B cell acute lymphoblastic leukemia: a meta-analysis. *Ann Hematol*. 2017;96(2):215-225.
17. Stanulla M, Dagdan E, Zaliova M, et al. IKZF1plus defines a new minimal residual disease-dependent very-poor prognostic profile in pediatric B-cell precursor acute lymphoblastic leukemia. *J Clin Oncol*. 2018;36(12):1240-1249.
18. Zaliova M, Stuchly J, Winkowska L, et al. Genomic landscape of pediatric B-other acute lymphoblastic leukemia in a consecutive European cohort. *Haematologica*. 2019;104(7):1396-1406.
19. da Conceição BT, Mansur MB, Blunck CB, Emerenciano M, Pombo-de-Oliveira MS. Characterization of RB1 in pediatric TCF3-PBX1+ acute lymphoblastic leukemia. *Blood*. 2017;130(Supplement 1):3976.
20. Hrabovsky S, Vrzalova Z, Stika J, et al. Genomic landscape of B-other acute lymphoblastic leukemia in an adult retrospective cohort with a focus on BCR-ABL1-like subtype. *Acta Oncol*. 2021;22:1-11.
21. Laycock-van Spyk S, Thomas N, Cooper DN, Upadhyaya M. Neurofibromatosis type 1-associated tumours: their somatic mutational spectrum and pathogenesis. *Hum Genomics*. 2011;5(6):623-690. doi:10.1186/1479-7364-5-6-623
22. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med*. 2014;371(11):1005-1015.
23. Pui HC, Rebora P, Schrappe M, et al. Outcome of children with hypodiploid acute lymphoblastic leukemia: a retrospective multinational study. *J Clin Oncol*. 2019;37(10):770-780.
24. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378:439-448.
25. Gökbuget N, Dombret H, Bonifacio M, et al. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. *Blood*. 2018;131:1522-1531.
26. Frismantas V, Dobay MP, et al. Ex vivo drug response profiling detects recurrent sensitivity patterns in drug-resistant acute lymphoblastic leukemia. *Blood*. 2017;129(11):e26-e37.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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