**LETTER**

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**ACUTE LYMPHOBLASTIC LEUKEMIA**

**UBTF::ATXN7L3** gene fusion defines novel B cell precursor ALL subtype with CDX2 expression and need for intensified treatment

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TO THE EDITOR:

Genomic aberrations—gene fusions in the majority of cases—and corresponding transcriptional regulations define an increasingly complex landscape of molecular subtypes in B cell precursor acute lymphoblastic leukemia (B-CP-ALL) [1]. Up to 15% of patients cannot be allocated to established subtypes, suggesting the presence of unrecognized drivers—especially in adult patients who have been less studied so far.

We performed transcriptome sequencing (RNA-Seq) on \( n = 568 \) adult B-CP-ALL patients prospectively treated according to pediatric-based protocols of the German Multicenter Acute Lymphoblastic Leukemia (GMLA) study group including risk stratification based on minimal residual disease (MRD) and treatment intensification for high-risk patients. To define molecular subtypes, we used our previous integrative analyses [1] to train a machine learning classifier to predict subtype allocation from gene expression profiles of subsequently sequenced samples. Feature selection (LASSO) was used to identify the most informative genes. Underlying genomic aberrations were analyzed (whole-genome sequencing (WGS), whole-exome sequencing (WES); SNP-arrays) to confirm subtype allocation in selected cases. With this approach, we were able to allocate \( n = 535/568 (94\%) \) samples to 15 previously established [1] molecular subtypes (Fig. 1A–D), with confirmation of corresponding genomic alterations in 91% of analyzed cases (Fig. 1D). Unsupervised gene expression analysis of previously unassigned samples revealed a distinct patient subset (\( n = 12 \); Supplementary Fig. S1A) defined by a novel in-frame gene fusion of upstream binding transcription factor \( (UBTF) \) and ataxin-7-like protein 3 \( (ATXN7L3) \) occurring exclusively in this patient cluster (\( n = 12/12 \) vs. \( n = 0/556 \) in remaining cohort; \( p < 1E−10 \); Fig. 1D). Comparison of gene expression profiles revealed that \( UBTF::ATXN7L3 \) rearranged cases in our cohort match to a recently described B-CP-ALL subtype, which so far was identified by increased expression of the homeobox transcription factor CDX2 \( (\text{CDX2 high}) \) ALL [2] (Supplementary Fig. S1B). \( UBTF::ATXN7L3 \) represents an 11.3 kbp in-frame read-through between \( UBTF \) exon 17/21 and a 5′ UTR splice site of \( ATXN7L3 \), with the same sanger sequencing confirmed break point in all samples (Fig. 2A, Supplementary Methods). WGS of 3 samples revealed a 10.08 kb genomic deletion involving \( UBTF \) 3′ exons (18-21) and most of the intergenic region between \( UBTF \) and \( ATXN7L3 \) as underlying mechanism (Fig. 2A, Supplementary Fig. S2). Break-point-specific PCR and Sanger sequencing confirmed presence of the deletion in \( n = 11/11 \) \( UBTF::ATXN7L3 \) patients with available material (Supplementary Fig. S3). The same \( ATXN7L3 \) transcript breakpoint has previously been identified in a patient with diffuse large B cell lymphoma \( (GPATCH8::ATXN7L3) [3] \), suggesting a shared driver function in different B-lymphoid malignancies. Both fusion partners were highly expressed across the entire cohort without significant overlapping expression.

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differences in the novel subtype (Supplementary Fig. S4), suggesting either gain-of-function or a dominant-negative effect of the gene fusion.

Both, UBTF and ATNX7L3 are global epigenetic regulators involved in transcriptional control. UBTF is an essential co-activator of ribosomal RNA expression. Very recently, UBTF has been characterized as novel oncogene in acute myeloid leukemia (AML), where internal tandem duplications define a distinct molecular subtype with poor outcome and highest incidence in early adolescents [4, 5]. WGS and sanger sequencing ruled out...
UBTF internal tandem duplications in UBTF::ATXN7L3 patients (data not shown). ATXN7L3 is a global gene expression co-activator through the SAGA complex. It is essential for activation of the SAGA histone deubiquitinase module (DUBm) through USP22, which is part of the 11-gene signature “Death-from-cancer” [6] defining poor outcomes across entities. The SAGA DUBm competes for ATXN7L3-binding with other deubiquitinases suggesting global changes in gene expression upon imbalances in ATXN7L3-substrate binding [7]. These findings align well with data on other molecular ALL subtypes driven by epigenetic perturbations [8, 9]. Analysis of subtype-specific gene expression by multi-comparison ANOVA revealed 332 genes with differential expression in UBTF::ATXN7L3 ALL when compared to each other subtype (Fig. 1B; Supplementary Tables S1 and 2). These differentially expressed genes included upregulation of 18 cancer-associated genes (COSMIC Cancer gene census, Supplementary Table S3), one of which was CDX2, which has been used to define ‘CDX2-high’ ALL [2] (Fig. 1C). However, few samples from other subtypes also showed increased CDX2 expression levels, limiting its applicability to identify this subtype. UBTF and ANTX7L3 are global epigenetic regulators without described functional interactions with CDX2. CDX2 is expressed in AML [10] and ALL [11], independently of the driver subtype. Conditional Cd knockover expression in hematopoietic progenitors resulted in meyelodysplasia and required acquisition of secondary aberrations for transformation [9, 12], suggesting TADs as a fundamental function during leukemogenesis. Although UBTF::ATXN7L3-specific gene expression showed little overlap with published CDX2 expression models (Supplementary Fig. S5A), we identified a functional module relating CDX2 to HOXA9 and MEIS1 overexpression in UBTF::ATXN7L3 ALL, in line with similar findings in AML [13] (Supplementary Figure S5B,C). HOXA9/MEIS1 are essential co-factors for KMT2A-driven leukemogenesis [9, making it possible that a CDX2-HOXA9/MEIS1 axis exerts a similar function during leukemia promoting role in UBTF::ATXN7L3 ALL. Further oncoproteins related to hematologic malignancies were also upregulated in UBTF::ATXN7L3 patients (Fig. 1C, Supplementary Figure S6), including NTRK3 which might represent a therapeutic target for specific inhibitors (e.g. larotrectinib, entrectinib). To evaluate additional genomic driver aberrations, we performed WES (n = 7) and/or SNP-array analyses (n = 6) showing a described enrichment of chromosome 1q gains [2] (n = 5/7) and heterogeneous single chromosome aberrations. However, no subtype-specific recurrent driver events were identified (Supplementary Fig. S7A), supporting the functional relevance of UBTF::ATXN7L3 as recurrent hallmark of this subtype. UBTF::ATXN7L3 ALL was enriched for pro-B immunophenotypes (n = 5/12, 42% vs. n = 70/530, 13%; p = 0.016) and occurred predominantly in female patients (n = 10/12, 83% vs. 237/534, 44%; p = 0.008) and patients of advanced age (median: 48.5 years vs. 38 years; p = 0.05).

Outcome evaluable UBTF::ATXN7L3 patients (n = 11/12; Fig. 2B) received treatments on pediatric inspired GMALL protocols. Risk stratification identified 6 patients as high-risk due to pro-B immunophenotype (n = 4) or late response (n = 2). One patient died during induction therapy and another patient failed to achieve hematologic CR after consolidation I (overall cytologic CR rate: 82%). Only n = 3/10 patients cleared MRD after consolidation I (cytologic and molecular CR) compared to n = 271/402 (67%; p = 0.019; Fig. 2C) in the remaining cohort. Two out of these three good responders remained in molecular CR after conventional chemotherapy including allogenic stem cell transplantation (HSCT) due to high-risk criteria. One patient relapsed after discontinuation of standard chemotherapy due to poor performance status and achieved a second molecular CR after inotuzumab ozogamizin. Patients with intermediate MRD response (positive MRD < 10^-4 or below quantifiable range, n = 3) experienced molecular relapses on standard therapy, received Blinatumomab followed by HSCT and remained in long-term remission (n = 2/3) or achieved sustained CR on standard therapy (n = 1/3). Among the remaining poor responders (n = 4), one cytologic non-responder achieved MRD-negativity after Blinatumomab, received HSCT and died due to transplant-related complications. Two patients received Blinatumomab, achieved a molecular CR, proceeded to HSCT, and remained in long-term remission. The fourth patient received HSCT without Blinatumomab, relapsed, and achieved intermediate MRD after 2nd HSCT. Together, we observed a median overall survival probability of UBTF::ATXN7L3 patients of 80% (±12%) compared to 73% (±2%; p = 0.07; Fig. 2D) in the remaining cohort, which is comparable to the ongoing GMALL08/2013 study [14]. Yasuda et al [2], reported markedly lower survival rates (POS: 26.7%, (4.8-56.3)) in ‘CDX2-high’ patients treated in historical cohorts without MRD-based risk stratification. Together, these data suggest that UBTF::ATXN7L3 ALL represents a less chemo-sensitive disease subtype, which can be successfully salvaged by current MRD-based concepts incorporating immunotherapies and stem cell transplantation [14].
course of novel molecular subgroups in the context of current treatment strategies.

Yasuda et al [2], described a second novel BCP-ALL subtype defined by IDH1/2 hotspot mutations (1.9% of cohort). We screened RNA-Seq data of all remaining ‘unassigned’ samples of our cohort (n = 22) for the described gene expression signature or IDH1/2 mutations and identified one patient harboring IDH2 p. R140Q, which was confirmed by PCR on gDNA level, contributing to the heterogeneous frequency distribution of molecular subtypes in different BCP-ALL cohorts.

| Patient | Age (years) | Sex | Immuno-phenotype | Initial WBC count (x10^3 / μL) | Initial risk stratification | Cyto logic remission after consolidation I | Minimal residual disease after consolidation I | Therapy course after consolidation I | Outcome |
|---------|-------------|-----|------------------|------------------------------|---------------------------|---------------------------------|---------------------------------|---------------------------------|---------|
| 21ORD12106 | 55 | female | common | 5.050 | CR | negative | standard chemotherapy | molecular CR |
| 139863 | 54 | female | pro-B | 3.400 | HR | CR | negative | standard chemotherapy, discontinued due to poor performance status, relapse, intensification | molecular CR |
| J35028 | 51 | female | common | 5.100 | SR, re-stratified IT (PR after induction | CR | negative | HSCT | molecular CR |
| J32981 | 54 | female | pro-B | 4.000 | HR | not reached | not reached | death in induction cycle II | induction death |
| J29815 | 40 | female | common | 11.300 | SR | CR | Intermediate (p<1x10^4 > 1x10^5) | standard chemotherapy | molecular CR |
| 21ORD11986 | 40 | male | common | 7.000 | SR | CR | Intermediate (p<1x10^5) | standard chemotherapy, molecular relapse, molecular CR after blnematamols, HSCT | molecular CR |
| 21ORD12022 | 55 | female | common | 6.630 | SR | CR | Intermediate (p<1x10^3 > 1x10^4) | standard chemotherapy, molecular relapse, molecular CR after blnematamols, HSCT | molecular CR |
| J2780 | 39 | female | common | 1.800 | SR | failure | positive | molecular CR after blnematamols, HSCT Transplant-related death |
| J29851 | 46 | female | common | 13.810 | SR, re-stratified IT (PR after induction | CR | positive (2x10^7) | molecular CR after blnematamols, HSCT | molecular CR |
| J32993 | 31 | female | pro-B | 3.070 | HR | CR | positive (4x10^7) | molecular CR after blnematamols, HSCT | molecular CR |
| REFUNM34317 | 30 | male | pro-B | 7.320 | HR | CR | positive (3x10^7) | HSCT in molecular failure, relapse, 2nd HSCT, MDR positive = quantitatively | lost to follow-up |

Abbreviations: WBC, white blood cell; SR, standard risk; HR, high risk; CR, complete remission; PR, partial remission; HSCT, hematopoietic stem cell transplantation.
Our data identify **UBTF**:ATXN7L3 resulting from a **17q21.31** variant as novel subgroup defining candidate driver fusion for the recently described **‘CDX2-high ALL’** subtype. Poor MRD response indicates reduced chemosensitivity in these patients. Our data suggest MRD-based treatment intensification using salvage immunotherapies and allo-genic stem cell transplantation as a promising strategy to rescue this high-risk phenotype.

**REFERENCES**

1. Bastian L, Schroeder MP, Eckert C, Schlee C, Tanchez JD, Kämpf S, et al. PAX5 biallelic genomic alterations define a novel subgroup of B-cell precursor acute lymphoblastic leukemia. Leukemia. 2019;33:1895–909.

2. Yasuda T, Sanada M, Kawazu M, Kojima S, Tsuchi S, Ueno H et al. Two novel high-risk adult B-cell acute lymphoblastic leukemia subtypes with high expression of **CDX2** and **IDH1/2** mutations. Blood. 2021;2021011921.

3. Gao Q, Liang W-W, Foltz SM, Mutharasu G, Jayasinghe RG, Cao S, et al. Driver fusions and their implications in the development and treatment of human cancers. Cell Rep. 2018;23:227–e3.

4. Stratmann S, Yones SA, Mayhfoer M, Norgren N, Skattason A, Sun J, et al. Genomic characterization of relapsed acute myeloid leukemia reveals novel putative therapeutic targets. Blood Adv. 2021;5:900–12.

5. Umeda M, Ma J, Huang BJ, Hagiwara K, Westover T, Abdelhamid S et al. Integrated genomic analysis identifies **UBTF** tandem duplications as a recurrent lesion in pediatric acute myeloid leukemia. Blood Cancer Discov 2022: blood-cancdis.CBD-21-0160-A.2021.

6. Glinsky GV, Berezovska O, Glinskii AB. Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. J Clin Invest. 2005;115:1503–21.

7. Atanassov BS, Mohan RD, Lan X, Huang X, Lu Y, Lin K, et al. **ATXN7L3** and **EN2** coordinate activity of multiple H2B deubiquitinases important for cellular proliferation and tumor growth. Mol Cell. 2016;62:2558–71.

8. Huang Y, Moutett B, Warnatz H-J, Risch T, Rietmann F, Frommelt F, et al. The leukemogenic **T3/3-**HLF complex rewrites enhancers driving cellular identity and self-renewal conferring EP300 vulnerability. Cancer Cell. 2019;36:630–44.e9.

9. Ayton PM, Cleary ML. Transformation of myeloid progenitors by **MLL** oncoproteins is dependent on **Hoxa7** and **Hoxa9**. Genes Dev. 2003;17:2298–307.

10. Scholl C, Bansal D, Döhner K, Ewen K, Huntly BJ, Lee BH, et al. The homeobox gene **CDX2** is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis. J Clin Investig. 2007;117:1037–48.

11. Thoene S, Rawat VPS, Heilmeyer B, Hoster E, Metzeler KH, Herold T, et al. The homeobox gene **CDX2** is aberrantly expressed and associated with an inferior prognosis in patients with acute lymphoblastic leukemia. Leukemia. 2009;23:649–55.

12. Tu V, Straube J, Porter AH, Bywater M, Song A, Ling V, et al. Hematopoietic stem and progenitor cell-restricted **CDX2** expression induces transformation to myelodysplasia and acute leukemia. Nat Commun. 2020;11:3021.

13. Rawat VPS, Thoene S, Naidu VM, Arseni N, Heilmeyer B, Metzeler K, et al. Overexpression of **CDX2** perturbs **HOX** gene expression in murine progenitors depending on its N-terminal domain and is closely correlated with deregulated **HOX** gene expression in human acute myeloid leukemia. Blood. 2008;111:309–19.

14. Goebberget N, Steljes M, Viardot A, Nachtkamp K, Steffen B, Schneller F, et al. First results of the risk-adapted, MRD-stratified GMALL trial 08/2013 in 705 adults with newly diagnosed acute lymphoblastic leukemia/lymphoma (ALL/LBL). Blood. 2021;138:362–362.e3.

15. Nicorici D, Satalan M, Edgren H, Kangaspeska S, Murumagi A, Kallioniemi O, et al. FusionCatcher - a tool for finding somatic fusion genes in paired-end RNA-sequencing data. 2014. [https://www.bioniv.org/content/10.1101/011650v1](https://www.bioniv.org/content/10.1101/011650v1).

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**AUTHOR CONTRIBUTIONS**

LB, MBr and CDB designed the study; LB, AMH, TB, SH, JK, and MN processed, analyzed, and interpreted high-throughput sequencing data; MBu performed and analyzed experiments; SF, MW, AF, IN, MS, MPH performed high-throughput sequencing and processed data; LB, AMH, TB, SH, and NG performed statistical analyses; SS, BS, AV, KD, MK, GW, KW, AR, AK, HT, HT, MBr and NG contributed and interpreted data; LB, NG, and MBr supervised the project; LB, AMH, TB and CDB drafted the first version of the manuscript; and all authors revised and approved the final version of the manuscript.

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**ADDITIONAL INFORMATION**

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