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ONCOLOGY: BRIEF REPORT

Molecular features of non-anaplastic peripheral T-cell lymphoma in children and adolescents

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Abstract

Non-anaplasticperipheral T-cell lymphomas (PTCL) are rare tumors in children, adolescents, and young adults (CAYA) with poor prognosis and scarce genetic data. We analyzed lymphoma tissue from 36 patients up to 18 years old with PTCL, not otherwise specified (PTCL-NOS), hepatosplenic T-cell lymphoma, Epstein–Barr virus (EBV)-positive T-lymphoproliferative diseases, subcutaneous panniculitis-like T-cell lymphoma, and other PTCL types. Twenty-three patients (64%) had at least one genetic variant detectable, including *TET2*, *KMT2C*, *PIK3D*, and *DMNT3A*. *TP53* and *RHOA* variants, commonly found in adults, were not identified. Eight of 20 (40%) CAYA PTCL-NOS had no detectable mutations. The genetic findings suggest that CAYA PTCL differ from adult cases.

Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; CAYA, children, adolescents, and young adults; EBV, Epstein–Barr virus; ENKTL, extranodal NK/T-cell lymphoma, nasal type; HSTL, hepatosplenic T-cell lymphoma; PTCL, peripheral T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; T-LBL, T-lymphoblastic lymphoma; T-LPD, T-cell lymphoproliferative disorder; T-NHL, T-cell non-Hodgkin lymphoma; T-PLL, T-cell prolymphocytic leukemia; WES, whole exome sequencing.

KEYWORDS
pediatric lymphoma, PTCL

1 | INTRODUCTION

Non-anaplastic peripheral T-cell lymphoma (PTCL) is a heterogeneous group of tumors rare in children, adolescents, and young adults (CAYA). The most common subtype is PTCL, not otherwise specified (PTCL-NOS), followed by extranodal NK/T-cell lymphoma, nasal type (ENKTL), hepatosplenic T-cell lymphoma (HSTL), and subcutaneous panniculitis-like T-cell lymphoma (SPTCL).[1,2] The few published studies of CAYA PTCL described clinical parameters only and demonstrated that PTCL-NOS, ENKTL, and HSTL had the least favorable outcome among pediatric lymphomas.[1,2] We performed for the first time targeted mutation analyses and described potential therapeutic targets of CAYA PTCL from European and Asian countries.

2 | METHODS

Detailed methods are described in the Supporting Information. Formalin-fixed paraffin-embedded (FFPE) tumor tissues from patients up to 18 years old with PTCL diagnosed between 2001 and 2018 were retrieved from participating institutions. Diagnoses were confirmed by experts in the European Intergroup for Childhood Non-Hodgkin Lymphoma (EICNHL) Pathology Panel and translated into 2017 WHO Classification of Lymphoid Neoplasms[3] similar to our previous studies.[1] Immunohistochemical (IHC) staining and clonality testing were applied to exclude precursor T-cell neoplasms, ALK-positive anaplastic large-cell lymphoma, and non-neoplastic lymphoproliferations as described previously.[1,4] Nonsilent genetic variants were identified by targeted capture sequencing of 19 genes recurrently mutated in adult T-cell non-Hodgkin lymphomas (T-NHLs; Table S1).[5] *HAVCR2* (TIM3) mutations were screened in two SPTCL cases with sufficient tumor material by Sanger sequencing. CD30, PD1, TBX21, and GATA3 protein expression status by IHC was obtained at diagnosis or retrospectively performed and scored visually. Five adult PTCL-NOS, four adult angioimmunoblastic T-cell lymphomas (AITL), and six pediatric T-lymphoblastic lymphomas (T-LBL) were selected from the Kiel Lymph Node Registry for comparison (Table S3).

3 | RESULTS AND DISCUSSION

From the 58 cases of CAYA PTCL collected from participating institutions, 36 (62%) had adequate material for targeted-capture sequencing (Figure 1A), including 20 (56%) PTCL-NOS, four (11%) HSTL, four (11%) Epstein–Barr virus (EBV)-positive T-cell lymphoproliferative disorders (EBV+ T-LPD), three (8%) SPTCL, two (6%) primary cutaneous CD30+ T-LPD, one (3%) primary cutaneous gamma/delta T-cell lymphoma, one (3%) enteropathy-associated T-cell lymphoma (EATL), and one (3%) T-cell prolymphocytic leukemia (T-PLL) (Table 1; Table S2). The EBV+ T-LPD group included one patient with systemic EBV+ T-cell lymphoma of childhood, one with ENKTL, and two with EBV+ T-LPD without further classification.

Twenty-three of 36 (64%) cases of CAYA PTCL had at least one protein changing variant, with 30 unique variants identified (Figure 1B; Table S4). Verification by Sanger sequencing was performed on cases with adequate material (nine of 30 variants, data not shown). Majority were missense mutations (27/30, 90%), the remaining were splice site, splice acceptor, and stop gain mutations (each one of 30, 3%). Variants were labeled as high impact (2/30, 7%), moderate deleterious (4/30, 13%), moderate mixed (17/30, 57%), moderate tolerated (6/30, 20%), or low impact (1/30, 3%) using ENSEMBL predictor (Supporting Methods). The high-impact and moderate-deleterious groups included variants in *TET2* (affecting two of 11 patients with *TET2* variants), *DNMT3A* (2/2), *JAK1* (1/1), and *FLT3* (1/1) (Figure 1). Determination of variant allele frequency is imprecise with our amplification-based assay. Data of adult PTCL and T-LBL control cases are shown in Table S4 and Figure S1.

In CAYA PTCL-NOS, 12/20 cases (60%) had at least one variant detected (Figure 1B). The most recurrently altered gene was *TET2* (6/20, 30%), followed by *KMT2C* (5/20, 25%), *PIK3D* (2/20, 10%), and *DMNT3A* (2/20, 10%). *PHIP*, *JAK1*, *JAK3*, and *SETD2* were mutated in one of 20 cases, respectively (Figure 1; Table S4). Three of six patients had the same *TET2* c.86C>G p.Pro29Arg mutation, which was not reported in adult PTCL before. As germline DNA was not available, the possibility of germline variant cannot be entirely excluded. Eight of 20 (40%) CAYA PTCL-NOS had no detectable somatic mutation by our targeted sequencing panel, which is more frequent compared to adult controls (one of nine cases). We did not identify *RHOA* or *TP53* variants, despite frequently reported in adult PTCL-NOS (15%–30% and 5%–30%, respectively).[6–8] Although determination of mutational load is imprecise using a targeted sequencing approach like in our study, and whole exome sequencing (WES) is required to clarify the full genomic spectrum in these cases, the low number of mutations appears to be compatible with other childhood cancers.[9] The lack of *TP53* variants is also intriguing, as it is most frequently mutated in childhood cancer and found in 4% of pediatric tumors (mainly B-ALL and solid cancers).[9]

Further studies were limited by the rarity of cases, small biopsy size, and lack of paired nontumor tissue. Copy number analysis and gene expression profiling could not be performed, making it difficult to match our findings to data from adult patients.[10,11] Nevertheless, our data suggest that CAYA PTCL-NOS have a molecular signature different from adult cases with overall fewer mutations among analyzed genes and absence of *TP53* alterations, which raise the possibility that CAYA PTCL-NOS belongs to "Group 3 PTCL" described by Watatani et al.[8] The absence of *RHOA* mutations and low level of PD1 expression (see below) suggest that CAYA PTCL-NOS cases have less follicular T-helper cell differentiation compared to adults.

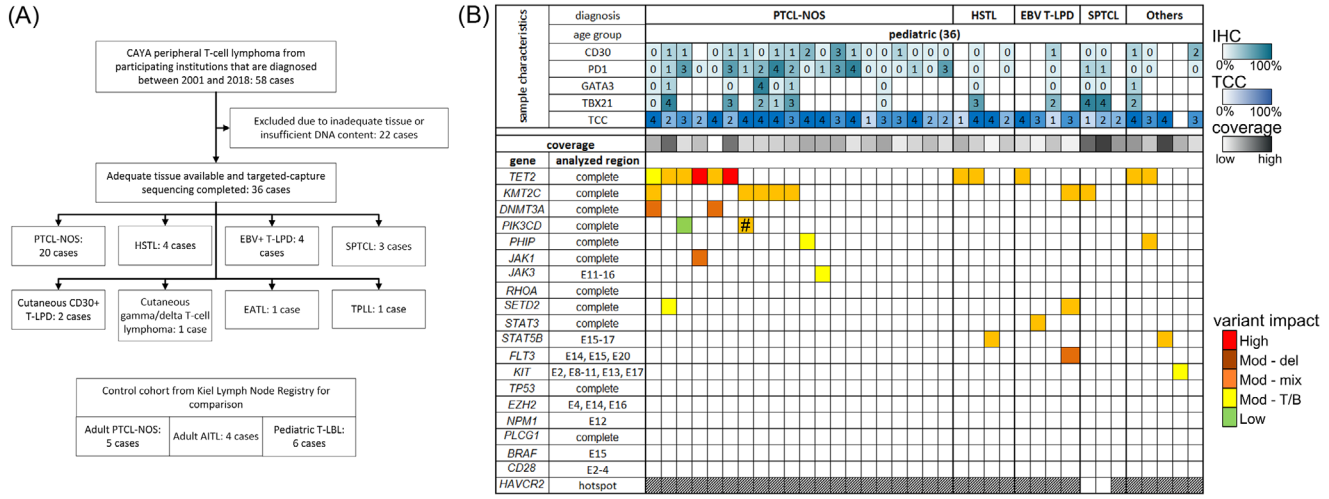

FIGURE 1 Case selection process and mutational pattern of CAYA non-anaplastic peripheral T-cell lymphoma. (A) Flowchart showing the case selection process of the CAYA PTCL cohort. (B) Samples were characterized by the expression pattern of different T-cell markers and by the tumor cell content (TCC; 0: 0%; 1: 1%–25%; 2: 26%–50%; 3: 51%–75%; 4: >75% positive tumor cells). Potential protein changing variants were colored according to the variant effect prediction (red: high; dark orange: moderate-deleterious; orange: moderate-deleterious or tolerated based on different algorithms; yellow: moderate-tolerated; green: low impact). HAVCR2 (TIM3) mutation analysis was performed by Sanger sequencing in two SPTCL samples with available material. HSTL, hepatosplenic T-cell lymphoma; EBV T-LPD, EBV-positive T-cell lymphoproliferative disorder; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; others: two patients with primary cutaneous CD30+ T-LPD, one patient with primary cutaneous gamma/delta T-cell lymphoma, one patient with enteropathy-associated T-cell lymphoma (EATL), and one patient with T-cell prolymphocytic leukemia (T-PLL); E: exon; #: variants were pathogenic or likely pathogenic by CLIN_SIG database

TABLE 1 Number and median age of CAYA patients with non-anaplastic peripheral T-cell lymphoma

Tumor type	Number of patients	Male sex	Median age in years (min–max)
PTCL-NOS	20/36 (56%)	8/20 (40%)	11.5 (1–18)
HSTL	4/36 (11%)	4/4 (100%)	9.0 (3–18)
EBV+ T-LPD	4/36 (11%)	4/4 (100%)	9.0 (4–15)
SPTCL	3/36 (8%)	3/3 (100%)	14.0 (1–16)
Primary cutaneous CD30+ T-LPD	2/36 (6%)	1/2 (33%)	3.0 (2–4)
Primary cutaneous gamma/delta T-cell lymphoma	1/36	0/1	3 (range not applicable)
EATL	1/36	1/1	12 (range not applicable)
T-PLL	1/36	1/1	6 (range not applicable)
Total	36/36 (100%)	22/36 (61%)	10.0 (1–18)

Abbreviations: EATL, enteropathy-associated T-cell lymphoma; EBV, EBV-positive T-cell lymphoproliferative disorders; HSTL, hepatosplenic T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphomas, not otherwise specified; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; T-LPD, T-cell lymphoproliferative disorder; T-PLL, T-cell prolymphocytic lymphoma.

Mutations in other T-NHL cases varied among entities. *TET2*, *KMT2C*, *SETD2*, *STAT5B*, and *FLT3* variants were identified in three of four HSTLs (75%) (Figure 1; Table S4). None had mutations in *DNMT3A*, *PIK3CD*, *STAT3*, or *TP53* that were reported in 10% of adult HSTLs.[12] Two of four (50%) patients with EBV+ T-LPD had variants affecting either *TET2* or *STAT3*. The patient with the *TET2* mutation was diagnosed with a systemic EBV-positive T-cell lymphoma of childhood. The patient with ENKTL carried *STAT3* missense mutation, suggesting that it belongs to the TSIM subtype described recently.[13] Two SPTCL cases had tissue available for *HAVCR2* Sanger sequencing, both had wild-type status. *PHIP* and *TET2* variants were found in the two cases of CD30+ cutaneous T-LPD. Potentially druggable targets such as mutations in *PIK3D*, *KIT*, and *JAK1* were rare.

On IHC, diffuse expression of PD1 (>50% tumor cells) was identified in six of 20 (30%) CAYA PTCL-NOS, similar to adult non-AITL PTCL-NOS cases in the literature (29%)[14] (Figure 1B; Table S2). Only one of 19 CAYA PTCL-NOS sample had CD30 expression in >50% of cells. One patient (PTCL-19) had biopsies at three different timepoints: at first diagnosis (12 years old), first relapse (14 years old), and second relapse (16 years old). No mutation was identified by our panel in all three biopsies, but the amount of PD1-positive cells changed dramatically, from 1%–25% to >75% and then 0%. CD30 staining was negative

in the first and third biopsies, but weakly positive (1%–25% of cells) in the second biopsy. IHC for GATA3 and TBX21 was performed on PTCL-NOS cases with available tissue, three of seven cases (43%) and five of seven cases (71%) had positive GATA3 and TBX21 expressions, respectively. From published studies, 56% and 37% of adult PTCL-NOS cases showed TBX21 and GATA3 expressions, respectively,[10] although direct comparison is difficult due to the few CAYA PTCL-NOS cases with GATA3 and TBX21 data.

In summary, we showed that mutations in CAYA PTCL may harbor potentially druggable mutations, for example, *PIK3D*, *JAK1*, and *KIT*. However, our data suggest that these rare lymphomas require individual workup, and a broader screening strategy for therapy targets (e.g., WES) would be needed, as the number of mutations per tumor appears low. Future collaborations will be required to gain deeper insight into the pathogenic mechanisms of these lymphomas to find new therapeutic approaches. Further studies using WES may also resolve the limitations of our targeted sequencing approach.

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CONFLICT OF INTEREST

Wolfram Klapper reports grants from Roche, Amgen, Takeda, and Regeneron paid to his institution outside the submitted work. The remaining authors have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Rex K. H. Au-Yeung, Julia Richter, and Wolfram Klapper designed the studies. Rex K. H. Au-Yeung, Julia Richter, Ingram Iaccarino, Dmitriy Abramov, Chris M. Bacon, Olga Balagué, Emanuele S. G. d'Amore, Ingrid Simonitsch-Klupp, Konnie Hebeda, Atsuko Nakazawa, Ilske Oschlies, and Wolfram Klapper provided essential biopsy material and generated data. Udo Kontny, Wilhelm Woessmann, and Birgit Burkhardt provided the data and analyzed it. Rex K. H. Au-Yeung, Julia Richter, and Wolfram Klapper wrote the manuscript. All authors agreed with the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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