**Clinical genomic profiling of novel grey zone lymphoma paired lesions with sequential central nervous system involvement in two adolescent patients**

Grey zone lymphoma (GZL), defined as B-cell lymphoma, unclassifiable, with features intermediate between large B-cell lymphoma (LBCL) and classic Hodgkin lymphoma (cHL) (BCL-U-IND) is a rare diagnostic entity. Synchronous GZL, LBCL and cHL occurring simultaneously in the same patient, and sequential GZL, LBCL preceding or following a diagnosis of cHL, are even less common. We identified two adolescent patients, a 17-year-old male (17M, case #1) and 16-year-old female (16F, case #2), who were diagnosed with stage IV nodular sclerosis cHL (NS-cHL) with primary mediastinal location and subsequent central nervous system (CNS) LBCL. Copy-number alterations were assessed using Affymetrix OncoScan® microarray analysis, and targeted next-gener-

Table 1. Clinicopathological summary of sequential grey zone lymphomas.

| Case/age (yr)/sex | Presentation (time after initial diagnosis) | Biopsy site | Diagnosis | Morphology | Immunophenotype | Therapy | Outcome (follow-up period) |
|-------------------|---------------------------------------------|-------------|-----------|------------|-----------------|---------|---------------------------|
| #1/17/M           | Large mediastinal and supraclavicular masses with spleen, liver, abdominal and bone lesions | Bone Marrow | LBCL-like synchronous GZL | Focal sheets of large lymphoma cells with large round nuclei, smooth nuclear contours, vesicular chromatin, and prominent centrally located nucleoli with eosinophilic cytoplasm | Large lymphoma cells: Positive: CD19, CD79a, CD45; Negative: CD3, Cytokeratin, TdT, CD30. | NA | NA |
| #2/16/F           | Large mediastinal mass with cervical LN, multiple supraclavicular bilateral pulmonary and renal nodules | Deep right supraventricular LN | cHL, nodular sclerosis subtype, stage IVB | Characteristic mononucleated Hodgkin and binucleated Reed-Sternberg cells (HRS) in the background of lymphocytes, histiocytes, neutrophils, and eosinophils; the nodules separated by thick collagen band | HRS cells: Positive: CD30, CD15, Pax-5 (weak); Negative: CD45, CD20, CD79a, LMP-1, EBER and EMA | ABVE-PC | Complete remission |
|                   | Solitary right temporal lobe brain lesion (7 months) | Right temporal lobe brain lesion | LBCL-like sequential GZL | Diffuse sheets of large lymphoma cells having open chromatin, prominent centrally located nucleoli and a moderate amount of clear to eosinophilic cytoplasm. | Large lymphoma cells: Positive: CD45, CD20, CD30, PAX-5; Negative: CD79a, EBER, ALK | POG9917 Arm | Alive with no evidence of disease (13.5 months) |

1Outside bone marrow with limited slides reviewed as consultation. ABVE-PC: adriamycin, bleomycin, vincristine sulfate, etoposide phosphate, prednisone, cyclophosphamide; BMT: bone marrow transplant; cHL: classic Hodgkin lymphoma; F: female; GZL: grey zone lymphoma; HRS: Hodgkin and Reed-Sternberg; LBCL: large B-cell lymphoma; LN: lymph node; M: male; MMUD: mismatched unrelated donor; NA: not applicable.
Table 2. Tissue-based cancer microarray and next-generation sequencing analysis of sequential grey zone lymphomas.

| Cytobands     | Size (Mbp) | Array Nomenclature | Interpretation |
|---------------|------------|--------------------|----------------|
| Xp22.13-q24.33| 133.6      |                    |                |
| Yp11.11-q11.23| 26.1       |                    |                |

**Case Report**

**Case #1, 16M, Paired NS-cHL and CNS LBCL Microarray**

**Gene** | **Pos (hg19)** | **RefSeq RNA** | **CDS; Protein; VAF (NS-cHL/LBCL)** | **Interpretation** |
|----------|----------------|----------------|-------------------------------------|--------------------|
| APC      | chr5:11202044  | NM_000038.5    | c.157G>A; p.Gly53Arg; 0.05           | III, CNS LBCL only |
| FAT4     | chr4:12639844  | NM_024582.4    | c.2287T>G; p.Leu761Trp; 0.37         | III, CNS LBCL only |
| NOTCH3   | chr15:2572111  | NM_000435.2    | c.632G>A; p.Arg204Glu; 0.48          | III, CNS LBCL only |
| CREBBP   | chr16:3819311  | NM_004380.2    | c.2921C>A; p.Ahr745Asn; 0.44/0.47    | III, Shared, Reported in GZL |
| APC      | chr5:1120215   | NM_000038.5    | c.3802T>A; p.Ile130Tyr; 0.56/0.45    | III, Shared         |
| OARAI    | chr12:10220647 | NM_032790.3    | c.58G>A; p.Gly20Ser; 0.62/0.29       | III, Shared         |
| SETX     | chr9:15145055  | NM_015465.6    | c.7234A>G; p.Leu2412Val; 0.47/0.28   | III, Shared         |
| SOS1     | chr3:9521525   | NM_006333.3    | c.1098T>G; p.Asp366Glu; 0.44/0.48    | III, Shared         |
| SYNE1    | chr6:15625191  | NM_182861.3    | c.3540C>T; p.Asn1181Lys; 0.47/0.38   | III, Shared         |
| SYNE1    | chr6:15625090  | NM_182861.3    | c.1717C>T; p.Thr572Asn; 0.42/0.51    | III, Shared         |
| SYNE1    | chr6:15625079  | NM_182861.3    | c.1903G>T; p.Arg645Ser; 0.44/0.46    | III, Shared         |
| SYNE1    | chr6:15342686  | NM_182861.3    | c.2509C>T; p.Pro8364Leu; 0.47/0.51   | III, Shared         |

**Case #2, 17T, Paired NS-cHL and CNS LBCL Microarray**

**Gene** | **Pos (hg19)** | **RefSeq RNA** | **CDS; Protein; VAF (NS-cHL/LBCL)** | **Interpretation** |
|----------|----------------|----------------|-------------------------------------|--------------------|
| FAT4     | chr4:12639844  | NM_024582.4    | c.1098T>A; p.Glu366Val; 0.44/0.48   | III, Shared         |
| CBL      | chr11:1191047  | NM_005188.3    | c.1055A>G; p.Asn352Ser; 0.51/0.49    | III, Shared         |
| FGFR1    | chr16:382212   | NM_005435.2    | c.1856C>T; p.Pro619Leu; 0.47/0.37    | III, Shared         |
| ERG      | chr21:1584571  | NM_000058.5    | c.2447G>A; p.Glu815Val; 0.44/0.48   | III, Shared         |

**Case #3, 18F, Paired NS-cHL and CNS LBCL NGS**

**Gene** | **Pos (hg19)** | **RefSeq RNA** | **CDS; Protein; VAF (NS-cHL/LBCL)** | **Interpretation** |
|----------|----------------|----------------|-------------------------------------|--------------------|
| TP53     | chr7:757569   | NM_000564.5    | c.712T>C; p.Cys237Arg; 0.51          | III, Shared, Reported in GZL |

*Detected in both CNS LBCL cases. NS-cHL: classic Hodgkin lymphoma; nodular sclerosis subtype; CNS: central nervous system; LBCL: large B-cell lymphoma; NGS: next-generation sequencing; Pos: genomic coordinate; RefSeq: reference transcript ID; CDS: coding sequence; VAF: variant allele frequency; Mbp: mega basepairs; GZL: grey zone lymphoma; CG-LOH: copy-gain loss of heterozygosity; CNS: CNS LBCL; CN-LOH: copy-neutral loss of heterozygosity.*
Figure 1. Representative pathologic findings of sequential grey zone lymphomas (Case #1). Initial cervical lymph node biopsy shows classic Hodgkin lymphoma (upper panel, A to C). (A) Characteristic Hodgkin and Reed–Sternberg (HRS) cells are present in a polymorphous inflammatory background (hematoxylin and eosin stain [H&E]); the HRS cells are negative for EBER (inset A). (B) The neoplastic HRS cells are positive for CD30 (red, membranous) and weakly positive for PAX-5 (brown, nuclear). (C) They are negative for CD20. (D to F) Lesional brain biopsy shows sequential central nervous system large B-cell lymphoma (lower panel). (D) Diffuse sheets of large lymphoma cells shows centrally located prominent nucleoli (H&E); they are negative for EBER (inset D). (E) The lymphoma cells are diffusely positive for CD30 (red, membranous) with strong nuclear PAX-5 (brown) expression, (F) and express strong and homogeneous CD20.

**Molecular findings.** The microarray and NGS results are summarized in Table 2. In both NS-cHL, near-diploid male or female genomes and no variants of established or potential clinical significance (Tier I/II, Table 2) were detected consistent with “negative” genomic profiles reported in bulk cHL lesions without Reed-Sternberg cell enrichment.6,7 In case #2, a shared 3.0 MB region of copy-neutral loss of heterozygosity (LOH) in chromosome 1p36.11-p35.3 was observed that was most likely germline in origin. Both CNS LBCL harbored complex cytogenomic arrays including 2p16.1 and 9p24.1 gains (detected in both cases, Table 2, denoted by *) and 16p13.3 copy-number abnormalities (case #2 only). LOH of chromosome 6p and gain of chromosome 12p were also observed in both CNS LBCL (Table 2, denoted by *). NGS revealed shared NS-cHL/CNS LBCL variants of uncertain significance (VUS, Tier III) in CREBBP p.T974N (case #1) and RELN p.N352S and KMT2D p.E4694Q (case #2). The sequential CNS LBCL in case #1 harbored addi-
tional Tier III variants including APC p.G53R, FAT1 p.L761W, and NOTCH3 p.R2109Q. The sequential CNS LBCL in case #2 harbored pathogenic (Tier I/II) TFB3 p.C238R and FBXW7 p.R465H missense variants.

In this report, we detailed the clinicopathologic and molecular features of two adolescent patients with sequential GZL involving the CNS. Notably, this is the first report describing CNS involvement as a manifestation of sequential GZL, a finding which expands the clinicopathologic spectrum of this rare pediatric disease. Consistent with previous reports, both patients presented with mediastinal NS-cHL and advanced extranodal disease with similar histopathologic and immunophenotypic findings, and developed GZL in a similar chronologic fashion.4,8 The sequential CNS lesions showed differing morphologic and immunohistochemical profiles with strong and diffuse expression of several B-cell markers and CD30, the latter arguing against an extramediatinal primary mediastinal B-cell lymphoma (PMBCL) diagnosis, and the NS-cHL diagnosis preceded the diagnosis of LBCL temporally establishing the sequential GZL diagnosis. Additionally, the findings of synchronous GZL with subsequent development of sequential GZL in the first patient is also exceptional. Furthermore, unlike previous reports, an early evolution (e.g., second lymphoma diagnosis within 1 year) may not necessarily portend a poor clinical outcome given the favorable clinical responses in our two patients and a relatively long term follow-up in the first.

Recent molecular characterization of GZL supports the classification of two distinct subtypes of GZL: a “thymic” subtype that occurs in the anterior mediastinum and resembles Epstein-Barr virus (EBV)-negative cHL and PMBCL, and a “non-thymic” subtype which occurs outside the thymus and harbors TP53 mutations in a subset of cases.9,10 In our two patients, the CNS location and mutations in TP53 (case #2) and other associated genes (e.g., CREBBP, RELN, and KMT2D) support a “non-thymic” GZL classification. The presence of complex genomic profiles is also consistent with dysregulated TP53 signaling, and both CNS LBCL harbored complex cytogenomic arrays with copy number abnormalities previously reported in GZL11-13 and frequently reported in cHL and PMBCL.14,15 We acknowledge that a thorough investigation of enriched Reed-Sternberg cells from the CHL lesions and specific subsets of lesional cells may yield valuable molecular insights but this was beyond the scope of the current study.

In summary, we present the first report of sequential GZL with CNS involvement in two adolescent patients, and the first clinical genomic profiling of such paired lesions. These lesions showed chromosome aberrations identified in GZLs and NGS mutations associated with non-thymic GZL. These findings expand the clinicopathologic and genomic spectrum of this rare pediatric disease.

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