An aggressive hematolymphoid neoplasm with homozygous *SMARCB1* loss shows response to EZH2 inhibition

SMARCB1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1) protein, also known as integrase interactor 1 (INI1), encoded by the *SMARCB1* gene, is a core subunit of chromatin remodeling complexes, known alternately as SWItch/Sucrose Non-Fermentable (SWI/SNF) or BRG1/BRM associated factor. SMARCB1 functions as a tumor suppressor, and mice that are deficient in the orthologous *Smarcb1* gene develop aggressive neoplasms, including T-cell lymphomas.[1] Aggressive hematopoietic neoplasms with SMARCB[1] loss have been only rarely reported, almost exclusively in the pediatric age group.[2-4] Here we report a case of an adult presenting with an aggressive hematopoietic neoplasm with features of T-lineage differentiation, and with biallelic *SMARCB1* loss, that demonstrated partial response to an enhancer of zeste homolog 2 (EZH2) inhibitor.

A 67-year-old woman, without significant past medical history, presented with pain, swelling and erythema of the right lower extremity, as well as night sweats, and weight loss of approximately 18 kg over the prior five months. She was initially treated with antibiotics for presumptive cellulitis, with no response. Subsequent magnetic resonance imaging of the right lower extremity revealed a 10 x 7.5 x 5.4 cm mass involving nearly all proximal muscles of the leg and knee (Figure 1A). Multifocal smaller lesions with similar characteristics were noted adjacent to the dominant mass. An ^{18}F-FDG positron emission tomography / computed tomography scan demonstrated intense radiotracer uptake in the right lower extremity mass, with a maximum standardized uptake value of 20.4, with an adjacent 1.7 cm mass showing maximum standardized uptake value of 24.2, as well as multiple hypermetabolic cutaneous and subcutaneous lesions (Figure 1B). Admission laboratory values were remarkable for platelet values of 549 x10^9/L (reference range: 150-450 x10^9/L, lactate dehydrogenase 611 U/L (reference range: 100-220 U/L), erythrocyte sedimentation rate >120 mm/hr (reference range: 0-38 mm/hr), C-reactive protein of 124.6 mg/L (reference range: 0.0-5.0 mg/L), and creatinine of 1.3 mg/dL (reference range: 0.5-1.1 mg/dL). Hemoglobin and white blood cell count were normal.

An incisional biopsy of the leg mass showed aggregates of large, pleomorphic, atypical hematolymphoid cells with round to irregular nuclear contours, one to three prominent nucleoli, dispersed chromatin, scant to moderately abundant eosinophilic cytoplasm, and frequent mitotic figures, with interspersed apoptotic bodies and karyorrhectic debris, and extensive zones of tumor necrosis (Figure 2A-C). By immunohistochemistry, the viable cells expressed CD45, and T-lineage / myeloid markers (CD43, CD7, CD33) (Figure 2D-G), but were negative for CD34, terminal deoxy-nucleotidyl transferase, CD3, CD4, CD8, CD5, CD30, ALK1, CD56, CD19, CD20, CD79a, PAX5, CD10, BCL2, BCL6, c-MYC, CD23, Cyclin D1, MUM1, CD138, and Kaposi sarcoma-associated herpesvirus. *In situ* hybridization for Epstein-Barr virus was negative. The Ki67 proliferation index was 50-60% (Figure 2H). By flow cytometry, the neoplastic cells were positive for CD45 (dim) and CD7, while negative for other T- and B-lineage markers (Figure 2J-L). T-cell receptor gene rearrangement studies were positive for clonal rearrangement of *TRG*, and negative for clonal rearrangement of *TRB*. An initial diagnosis of a poorly differentiated hematolymphoid neoplasm with T-lineage differentiation was rendered. Peripheral blood reverse transcriptase PCR, and PCR studies, were negative for human T-lymphotropic virus types 1 and 2, and Epstein-Barr virus, respectively.

Due to extreme frailty, the patient was not deemed a candidate for chemotherapy; she received definitive local radiotherapy at 40 Gy and steroids, achieving an excellent initial response. However, two months later, follow-up imaging detected new hypermetabolic osseous, cutaneous, subcutaneous, and liver lesions (Figure 1C). Biopsy of a subcutaneous lesion showed morphologic and immunophenotypic features similar to those of the initial biopsy, with additional immunohistochemistry, performed only on this subsequent biopsy, showing the neoplastic cells to be positive for CD15, TIA1, and PD-L1, while negative for myeloperoxidase, CD117, CD57, and granzyme B. DNA and RNA-based comprehensive genomic profiling (FoundationOne HEME) was then performed on the initial biopsy, which showed *SMARCB1* alterations with homozygous loss of exons 1-6, as well as rearrangement of Intron 6 with a *BCR*::*SMARCB1* fusion. Molecular analysis was also notable for a low tumor mutational burden (1 mut/Mb), and absence of other potential driver mutations. In keeping with the loss of *SMARCB1*, further immunohistochemistry of the initial biopsy demonstrated absence of INI1 expression in tumor cells (Figure 2 I). In view of the association between SMARCB1 deficiency and the upregulation of EZH2,[5] coupled with the recent FDA approval of the EZH2 inhibitor tazemetostat for treating sarcomas with *SMARCB1* loss, single agent therapy with tazemetostat was initiated at a dose of 800 mg, twice daily, with palliative intent. Within two months of initiating therapy, the patient reported improvement in energy, appetite, and ambulation, as well as resolution of night sweats. Imaging three months

after initiating therapy showed resolution of the liver lesion and some of the skeletal lesions, as well as decreased size and number of cutaneous and subcutaneous nodules in the right lower extremity (Figure 1D). However, a new hypermetabolic 10.8 cm left paravertebral / chest wall mass had developed (Figure 1D), which on biopsy was consistent with persistent disease; immunohistochemistry demonstrated that the tumor cells remained negative for INI1. At this time, given the expression of PD-L1 in the previous biopsy, a trial of pembrolizumab was initiated. However, the patient died five days subsequent to administration of the first dose, in the setting of presumptive sepsis.

SMARCB1 (INI1) is a core component of SWI/SNF complexes. These chromatin remodeling complexes modulate chromatin accessibility, and have key roles in cellular differentiation, including in hematopoiesis.[6,7] SWI/SNF complexes, which are also referred to as BRG1/BRM-associated factor complexes, have a tumor suppressive role, and mutations in genes encoding SWI/SNF complex constituents have been identified in up to 20% of human cancers.[8] Biallelic loss of *SMARCB1* is characteristic of rare solid tumors, often with poor prognosis, including malignant rhabdoid tumors, renal medullary carcinoma, epithelioid sarcoma, and poorly-differentiated pediatric chordoma, and correlates with

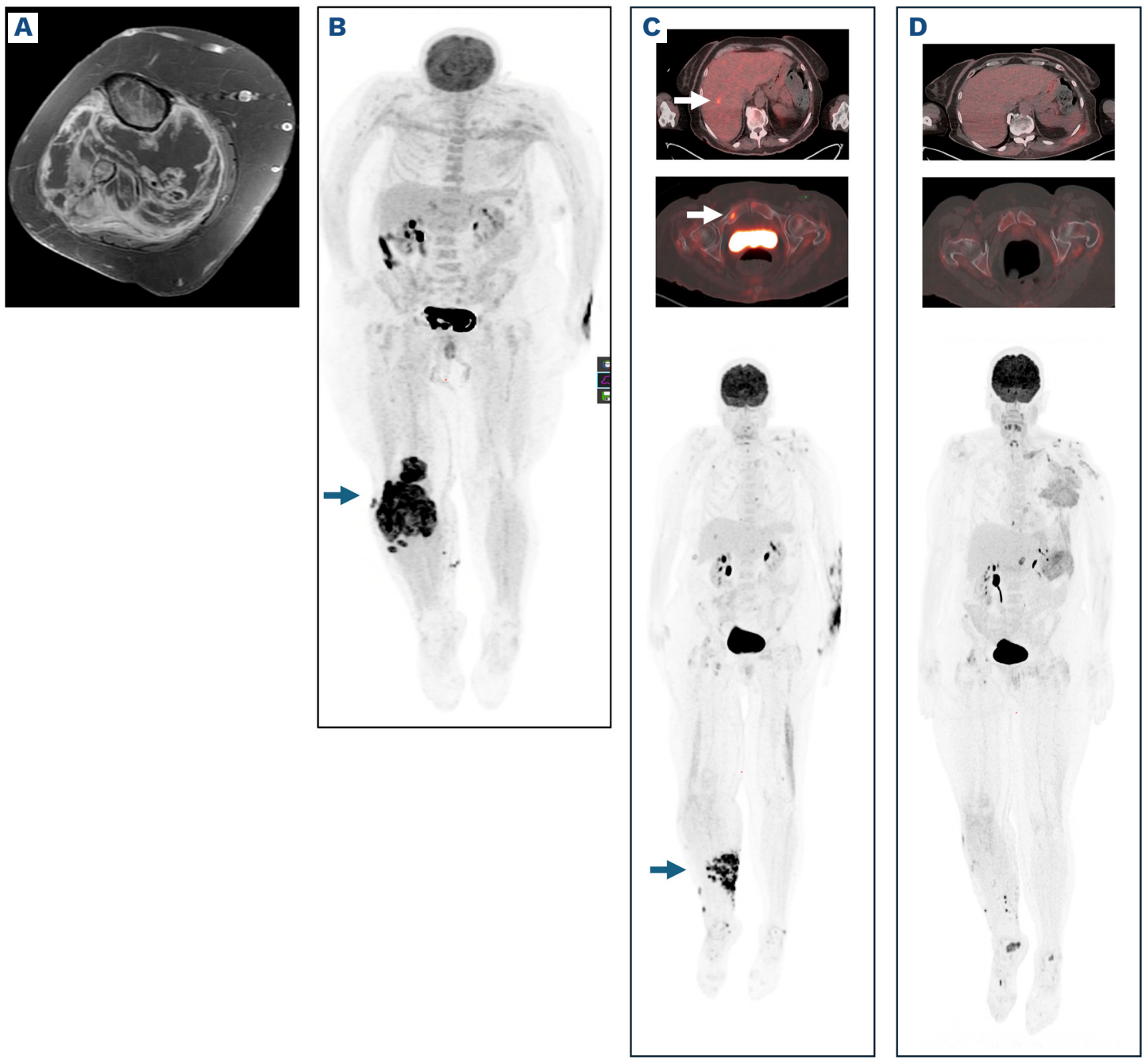

Figure 1. Sequential imaging studies over the course of disease. (A) Magnetic resonance imaging showing a 10 x 7.5 cm T1 iso to hypointense to muscle, heterogeneously short tau inversion recovery hyperintense, peripherally enhancing lesion with thick enhancing internal septations, centered in and involving nearly all the proximal muscles of the leg and knee, consistent with an aggressive necrotic neoplasm. (B) Maximum intensity projection positron emission tomography (PET) image, with an arrow indicating the level of the right lower extremity mass. (C) PET/computed tomography (PET/CT) images following local radiotherapy and steroids, showing decreased prominence of the right lower extremity mass with significantly decreased radiotracer avidity; and development of new hypermetabolic liver and pubic bone lesions (insets, indicated by arrows), and new cutaneous / subcutaneous lesions, most conspicuous in the right lower extremity (indicated by arrow). (D) PET/CT images three months subsequent to initiation of monotherapy with tazemetostat showing interval resolution of liver and pubic bone lesions (insets), significant decrease in extent and degree of right lower extremity cutaneous / subcutaneous lesions, and interval appearance of large hypermetabolic soft tissue lesions along the left paravertebral, chest wall and scapular regions as well as the left upper extremity.

loss of protein expression, as demonstrable by absence of immunohistochemical labeling for INI1.[9,10] Other tumor types can show diminished expression of INI1 (synovial sarcoma), or a mosaic pattern of expression, such as gastrointestinal stromal tumor, ossifying fibromyxoid tumor, and schwannomas associated with schwannomatosis (reviewed by Kohashi *et al.*).[10] These tumors may show various alterations of *SMARCB1*, including germline missense or splice-site mutations (schwannomatosis), or hemizygous deletion (ossifying fibromyxoid tumor).[10]

Amongst hematolymphoid neoplasms, *SMARCB1* deletions are a frequent occurrence in chronic myeloid leukemia (30-80% of cases) and T-cell prolymphocytic leukemia (55% of cases), respectively.[6] Mutations have also been identified in mantle cell lymphoma, and in a small proportion (3%) of cutaneous T-cell lymphoma.[6] While biallelic loss of *SMARCB1* is not commonly associated with lymphoma in humans, loss of *Smarcb1* in mice leads to the development of aggressive T-lineage and rhabdoid tumors, with high penetrance (100%) and rapid onset.[1] Table 1 summarizes the clinical, histopathological, and immunophenotypic features of the present case, in the context of rare previously reported hematolymphoid

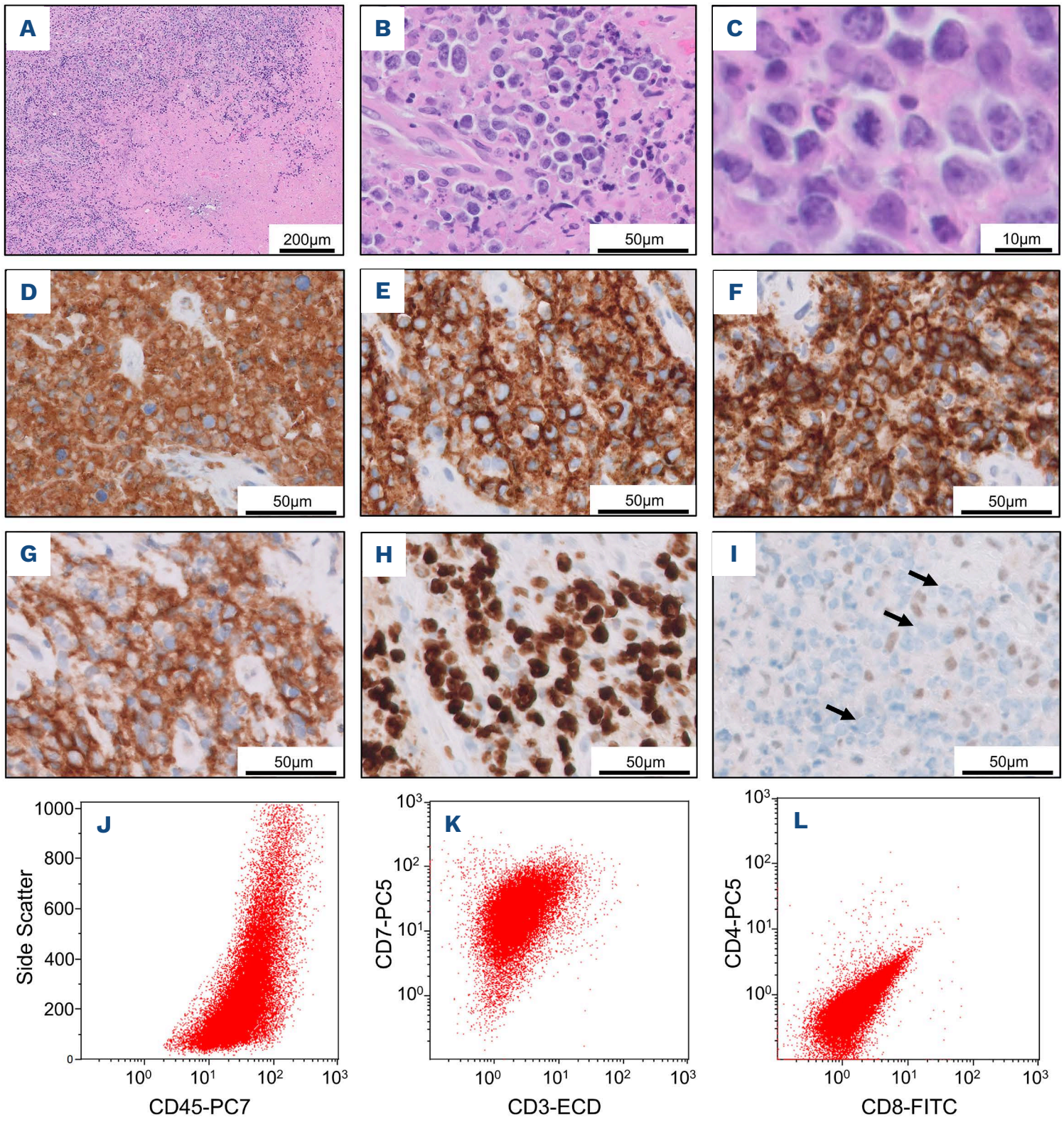

Figure 2. Morphological and immunophenotypic features of the initial right lower extremity mass biopsy. (A-C) Haematoxylin & Eosin stain shows aggregates of large, atypical, cells with interspersed apoptotic debris. (D-I) By immunohistochemistry the atypical cells express CD45 (D), CD43 (E), CD7 (F), CD33 (G), with Ki67 proliferation index of 50-60% (H), and show loss of integrase interactor 1 (INI1) (I); representative neoplastic cells are indicated by arrows, with INI1 expression retained in background stromal cells. (J-L) Flow cytometry shows that the neoplastic cells (red) express CD45 (dim) and CD7, but are negative for surface expression of CD3, CD4, and CD8. (Magnifications: A, X50; B, X400; C, X1000; D-I, X400).

neoplasms with *SMARCB1* loss. Salient characteristics include: aggressive clinical and histopathological features; propensity for occurrence in the pediatric age group; frequent presentation with mediastinal mass; expression of T-lineage markers, with possible co-expression of B-lineage and myeloid markers. Kinnaman and co-workers reported a case of hematopoietic malignancy with biallelic *SMARCB1* loss, which presented in a 14-year-old male, with an upper mediastinal mass, and bilateral cervical and supraclavicular lymphadenopathy; the neoplasm showed aggressive behavior, as well as lineage ambiguity, with expression of NK/T, myeloid, and B-lineage markers by immunohistochemistry.[2]

Table 1. Patient characteristics, clinical, morphological and immunophenotypic features of SMARCB1/INI1 deficient hematolymphoid neoplasms.

Case		Patient characteristics	Clinical presentation	Morphology	Immunophenotype	Clinal course, treatment, and response
1	Present case	67-year-old female	Right lower extremity mass; night sweats; 18 kg weight loss	Large atypical hematolymphoid cells; round to irregular nuclear contours; 1-3 prominent nucleoli, dispersed chromatin, scant to moderately abundant eosinophilic cytoplasm, frequent mitotic figures, zones of tumor necrosis.	Initial biopsy: CD45^{+}, CD43^{+}, CD7^{+}, CD33^{+}, CD34^{-}, TdT^{-}, CD3^{-}, CD4^{-}, CD8^{-}, CD5^{-}, CD30^{-}, ALK1^{-}, CD56^{-}, CD19^{-}, CD20^{-}, CD79a^{-}, PAX5^{-}, CD10^{-}, BCL2^{-}, BCL6^{-}, c-MYC^{-}, CD23^{-}, cyclin D1^{-}, MUM1^{-}, CD138^{-}, KSHV^{-}, INI1^{-} Biopsy on relapse: CD15^{+}, TIA1^{+}, PD-L1^{+}, MPO^{-}, CD117^{-}, CD57^{-}, granzyme B^{-} Biopsy after tazemetostat: CD45^{+}, CD43^{+}, CD7^{+}, CD15^{+}, CD3^{-}, CD5^{-}, CD30^{-}, CD20^{-}, CD117^{-}, INI1^{-}	Local radiotherapy and steroids; on relapse received single agent therapy with tazemetostat (800 mg twice daily) for 5 months, with partial response; pembrolizumab then added (200 mg IV, every 3 weeks); died 5 days after initiation of pembrolizumab.
2	Kinnaman, *et al.* (2020)[2]	14-year-old male with autism spectrum disorder, growth delay	Upper mediastinal mass, bilateral enlarged cervical and supraclavicular lymph nodes	Diffuse atypical mononuclear cells; irregular nuclear contours; vesicular chromatin; scattered large pleomorphic cells, some with multinucleation. Numerous mitotic figures. Focal necrosis.	CD45^{+}, CD2^{+}, CD7^{+}, CD79a^{+}, MPO^{dim+}, CD13^{+}, CD3^{-}, CD34^{-}, TdT^{-}, cytokeratin^{-}, myogenin^{-}	DA-EPOCH, 6 cycles, followed by progression of disease. Salvage therapy with brentuximab vedotin, nivolumab, bendamustine, followed by rapid progression of disease and death.
3	Havens *et al.* (2023)[3]	6-year-old male with distal chromosome 22q11.2 microdeletion syndrome	Back pain and multiple bone lesions	Not specified	CD45^{+}, CD2^{+}, CD3^{+}, CD5^{+}, CD43^{+}, CD4$^{focal\ weak+}$, INI1^{-}	Not specified
4	Havens *et al.* (2023)[3]	11-year-old female	Mediastinal mass	Not specified	CD45^{+}, CD4^{+}, CD7^{+}, CD2^{-}, CD3^{-}, CD30^{focal+}, INI1^{-}	Not specified
5	Havens *et al.* (2023)[3]	9-year-old male	Mediastinal mass, dyspnea, pleural effusion	Cytospin and cell block of pleural effusion with “pleomorphic neoplastic cells”	CD45^{+}, CD43^{+}, CD5^{+}, CD4^{patchy}, CD30^{patchy}, INI1^{-}	Not specified

DA-EPOCH: dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; INI1: integrase interactor 1; IV: intravenous; KSHV: Kaposi sarcoma-associated herpesvirus; MPO: myeloperoxidase; TdT: terminal deoxy-nucleotidyl transferase.

Three subsequently reported cases of INI1-deficient hematolymphoid neoplasms (all pediatric), showed expression of T-lineage markers by immunohistochemistry, without indication of B-lineage or myeloid differentiation.[3] A recent study by Fischer and co-workers found lack of SMARCB1 protein expression in a subset of peripheral T-cell lymphoma, not otherwise specified. These cases were remarkably skewed toward the pediatric, adolescent, and young adult age groups, and genomic profiling of nine of these SMARCB1 negative samples revealed three cases with biallelic *SMARCB1* mutations/deletion.[4]

SMARCB1-deficient neoplasms typically exhibit remarkably stable genomes, in keeping with the current case, which showed a low tumor mutational burden and an absence of other driver mutations.[11] Rather than the accumulation of mutations, it is proposed that the pathogenesis of *SMARCB1*-deficient neoplasms is characterized by wide-ranging epigenetic changes.[4,12] SMARCB1 normally represses EZH2, the catalytic subunit of polycomb repressive complex-2, which effects gene silencing by trimethylation of histone H3 on lysine 27, thereby down-regulating various tumor suppressor genes.[5] With decreased or absent expression of *SMARCB1*, EZH2 is up-regulated, which renders *SMARCB1*-deficient neoplasms potentially responsive to therapy with EZH2 inhibitors.[5] Tazemetostat, an inhibitor of EZH2 methyltransferase activity, has received FDA approval for treatment of epithelioid sarcoma and relapsed/refractory *EZH2* mutation positive follicular lymphoma, and has also shown promising results in the treatment of *SMARCB1* deficient rhabdoid tumors.[13,14] Notably, however, EZH2 inhibitors have limited efficacy as monotherapy.[15] Proposed mechanisms of resistance include acquired mutation of the EZH2 drug binding site, as well as mutations affecting the RB1/E2F axis, effectively decoupling cell cycle control from EZH2-dependent differentiation.[15]

The present case highlights several points of interest. Although seemingly very rare, *SMARCB1*-deficient hematolymphoid neoplasms may be underdiagnosed, and both immunohistochemistry for INI1 and molecular characterization should be considered in hematolymphoid neoplasms with aggressive histological features and lineage ambiguity. Furthermore, incorporation and characterization of *SMARCB1* into routine sequencing panels for hematologic malignancies may be warranted. Of note, the neoplastic cells in this case also showed expression of PD-L1, which may represent an additional therapeutic target for this neoplasm. Finally, the patient's initial response to single agent therapy with tazemetostat suggests that other patients with hematolymphoid neoplasms with *SMARCB1* loss may also benefit from EZH2 inhibitor therapy.

Authors

Iman Sarami,[1] Amy S. Duffield,[2] Suchitra Sundaram,[3] Douglas I. Lin,[4] Christian Salib,[2] Shafinaz Hussein,[2] Siraj El Jamal,[5] Nasrin Ghesani,[6] Busra Cangut[6] and Bruce E. Petersen[2]

[1]Department of Hematopathology, University of Texas MD Anderson Cancer Center, Houston, TX; [2]Department of Pathology, Molecular and Cell Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY; [3]Department of Medicine, Division of Hematology and Medical Oncology, Icahn School of Medicine at Mount Sinai, New York, NY; [4]Foundation Medicine, Cambridge, MA; [5]Department of Pathology and Laboratory Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ and [6]Department of Diagnostic, Molecular, and Interventional Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Correspondence:
B.E. PETERSEN - bruce.petersen@mountsinai.org

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Contributions

IS prepared the manuscript. ASD, CS, SH and SEJ reviewed and edited the manuscript. SS provided clinical information and edited the manuscript. DIL provided interpretation of sequencing data. NG and BC prepared the imaging studies figure and edited the manuscript. BEP supervised the study, prepared the pathology figure, and edited the manuscript.

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Data-sharing statement

Original data are available upon request in accordance with applying data protection rules.

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