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Germline mutations of *SMARCA4* in small cell carcinoma of the ovary, hypercalcemic type and in SMARCA4-deficient undifferentiated uterine sarcoma: clinical features of a single family and comparison of large cohorts

Yamicia D. Connor[1,3], **Diana Miao**[7], **Douglas I. Lin**[5], **Cynthia Hayne**[3,4], **Brooke E. Howitt**[6], **John L. Dalrymple**[1,3], **Kimberly R. DeLeonardis**[2,3], **Michele R. Hacker**[1,3], **Katharine M Esselen**[*,1,3], **Meghan Shea**[*,2,3]

[1.]Department of Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Boston, MA

[2.]Department of Internal Medicine, Division of Medical Oncology, Beth Israel Deaconess Medical Center, Boston, MA

[3.]Harvard Medical School, Boston, MA

[4.]Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA

[5.]Foundation Medicine, Cambridge, MA

[6.]Department of Pathology, Stanford University, Stanford, CA

[7.]Department of Gynecology and Obstetrics, Johns Hopkins Medicine, Baltimore, MD

Abstract

Objective: Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) and SMARCA4-deficient undifferentiated uterine sarcoma (SMARCA4-DUS) are rare and aggressive tumors, primarily affecting pre- and perimenopausal women. Inactivating *SMARCA4* mutations are thought to be the driving molecular events in the majority of these tumors. Here, we report the clinical course of a family with germline *SMARCA4* mutation and compare large cohorts of these rare tumor types.

Methods: We extracted clinico-pathological medical record data for the family with germline *SMARCA4* mutation. Clinico-genomic data from SCCOHT and SMARCA4-DUS cohorts were retrospectively extracted from the archives of a large CLIA-certified reference molecular laboratory.

Results: We identified a single family with an inherited germline *SMARCA4* mutation, in which two different family members developed either SCCOHT or SMARCA4-DUS, both of whom died within one year of diagnosis, despite aggressive surgical, chemotherapy and immunotherapy

Authors for correspondence: Meghan Shea MD, Program in Gynecologic Medical Oncology, Beth Israel Deaconess Medical Center, 330 Brookline Ave, Boston, MA 02215; mshea4@bidmc.harvard.edu.

*These authors contributed equally to this work

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DIL is a full-time employee of Foundation Medicine, Inc, which is a whole subsidiary of Roche.

treatment. Retrospective comparative analysis of large SCCOHT (n=48) and SMARCA4-DUS (n=17) cohorts revealed that SCCOHT patients were younger (median age: 28.5 vs. 49.0) and more likely to have germline *SMARCA4* alterations (37.5% vs. 11.8%) than SMARCA4-DUS patients.

Conclusions: Growing understanding of the role *SMARCA4* plays in the pathogenesis of these rare cancers may inform recommended genetic testing and counseling in families with these tumor types.

1. Introduction

Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is a rare and aggressive form of ovarian cancer, recently described to be oncogenically driven exclusively by somatic and/or germline mutations in *SMARCA4* [1,2]. Inactivating mutations in *SMARCA4 also* have been observed in a rare and aggressive form of uterine sarcoma, termed SMARCA4-deficient undifferentiated uterine sarcoma (SMARCA4-DUS, also known as malignant rhabdoid tumor of the uterus) [3,4]. Both of these rare cancer types affect young women. Due to their rarity and lack of clinical trials, optimal treatment strategies remain uncertain. Thus, interest has grown in further characterizing these *SMARCA4* mutations to guide development of targeted therapies and genetic counseling recommendations for patients and their family members.

SMARCA4 is a member of the mammalian SWI/SNF family of chromatin regulators. Somatic mutations in the SWI/SNF complex (e.g. *ARID1A*, *ARID2*, *PBRM1*, *SMARCA4*, *SMARCB1*) occur in up to 20% of human malignancies [5]. BRG1 (encoded by the *SMARCA4* gene), a protein subunit of the SWI/SNF complex, is the most commonly mutated chromatin remodeling ATPase in cancer [5,6], occurring in lung adenocarcinomas [7], lymphomas [8], and medulloblastomas [9], which are associated with a poor prognosis [10-12]. Loss of function somatic *SMARCA4* mutations have also been described as secondary oncogenic events in microsatellite unstable gynecological carcinomas leading to de-differentiated carcinomas [13].

Germline mutations in *SMARCA4* are best described as an inherited variant in rhabdoid tumor predisposition syndrome (RTPS) type 2 (RTPS2; OMIM 613325) [14]. RTPS is a complex familial disorder with an autosomal dominant pattern of inheritance with variable penetrance predisposing to formation of tumors that develop in the brain, spine, lung, bladder, pelvis, kidney, or ovary of young children or adults [14]. Genetic profiling has demonstrated these mutations in SCCOHT and SMARCA4-DUS, as well as atypical teratoid rhabdoid tumors (AT/RTs), malignant rhabdoid tumors (MRTs), and aggressive SMARCA4-deficient thoracic sarcomas (SMARCA4-DTS) [14,15,17]. Unlike SCCOHT and SMARCA4-DUS, no germline mutations in *SMARCA4* have been observed in SMARCA4-DTS tumors [15,16]. All of these tumors represent aggressive malignant rhabdoid tumors across varying tissue types associated with *SMARCA4* inactivation, though the genetic, histological, and phenotypic relationships is still an active area of research.

In this study, we describe the clinical course of a patient with SCCOHT and her mother with SMARCA4-DUS, previously noted to share a germline mutation in *SMARCA4* [4]. In

addition, we expand upon knowledge regarding known *SMARCA4* variants in SCCOHT and SMARCA4-DUS with analysis of variants within large cohorts of these tumors. A somatic-germline-zygosity bioinformatics mutational algorithm is used to estimate the proportion of *SMARCA4* alleles observed in a germline versus somatic context SCCOHT and SMARCA4-DUS. Understanding inherited mutations associated with *SMARCA4* is critical to providing appropriate genetic counseling and treatment recommendations for SCCOHT and SMARCA4-DUS.

2. Methods

2.1 SCCOHT and SMARCA-4-DUS clinical cases

We extracted clinical and pathologic data for a mother and daughter affected by an inherited germline *SMARCA4* mutation leading to SMARCA4-DUS and SCCOHT, respectively. Both patients were treated at Beth Israel Deaconess Medical Center (Boston, MA). Patient demographics, surgical management, medical management, and pathology were all extracted from medical records. The Beth Israel Deaconess Medical Center approved this study (protocol #2019000116). We obtained verbal permission from patient 2 prior to passing for release of genetic information.

2.2 Immunohistochemistry

Immunohistochemistry was performed on 4μm thick, formalin-fixed, paraffin-embedded tumor sections, using the DAKO linker 48 automated system. The following BRG1 antibody clone, dilution, vendor was used: SMARCA4/BRG1, ERP3912,1:50, Abcam. Appropriate internal positive and negative controls were evaluated.

2.3 Genomic Profiling and Germline mutation algorithm

Approval for retrospectively analyzing genomics of SCCOHT and SMARCA4-DUS cohorts at Foundation Medicine, a CLIA- and CAP-certified reference molecular pathology laboratory, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (Protocol 20152817). A retrospective database search was performed for SCCOHT and SMARCA4-DUS. All samples analyzed were processed by Foundation Medicine as part of the clinical care of these patients, prior to the initiation of this study. DNA and RNA were extracted from formalin-fixed paraffin-embedded tumors with a minimum of 20% tumor cells. Adapter-ligation and hybrid capture next-generation sequencing was performed for all coding exons in 406 cancer related genes in addition to introns from 31 highly mutated genes. The samples were fully sequenced and evaluated for all mutations, base substitutions, insertions, deletions, copy number alterations (amplifications and homozygous deletions), and select gene fusions/rearrangements. SCCOHT cases that did not have a *SMARCA4* mutation were excluded. Nineteen of the forty-eight SCCOHT cases were previously described by Lin DI et al.[17]. Sixteen of the seventeen SMARCA4-DUS cases were also previously published by Lin DI et al.[4]. The SMARCA4-DUS cases were submitted to Foundation Medicine with the diagnosis of uterine sarcoma (n=17) and based on morphology and genomics, they were reclassified to SMARCA4-DUS by 2 board-certified gynecological pathologists.

The SCCOHT and SMARCA4-DUS cases had *SMARCA4* inactivating mutations with no other or only few co-occurring alterations as previously described.

To assess whether a SMARCA4 alteration was potentially germline, a validated somatic germline zygosity algorithm was applied to each genomic variant as previously described[18]. For each sample, variants were detected using the standard FoundationOne analysis algorithm, which aligns unique sequence reads and obtains candidate mutations with associated mutant allele frequencies[19]. The algorithm also creates a genome-wide copy number profile based on coverage and allele frequencies (at over 3500 Single nucleotide polymorphisms (SNPs)) which are segmented and modeled to estimate the overall tumor purity, ploidy, per segment copy number, and minor allele count. To obtain a log-ratio profile of signal intensity, aligned tumor sequence reads are normalized by dividing read depth by that of a process-matched normal control, followed by a GC-content bias correction using Lowess regression. The minor allele frequency profile is obtained from the heterozygous genome-wide SNPs. These constitute the observed data for the statistical model. Given the output of the copy number model, each varian's measured allelic frequency is compared to expected at its local segment to determine whether a variant is predicted somatic, germline, or indeterminant. “Indeterminant” is used to signify mutations that were processed through the algorithm and unable to be classified as germline or somatic. “Unknown” are samples that were unable to be processed through the algorithm given incompatibility of the initial genetic sequence inputs required. These samples were either acquired prior to development of the algorithm or processed according to an alternative protocol. Statistical analysis was performed across the cohort ages using a Mann-Whitney Test using GraphPad Prism (GraphPad Software, San Diego, CA).

3. Results

3.1 SCCOHT patient

Patient 1 was a 31-year-old nulliparous woman diagnosed with stage IIIB SCCOHT (Figure 1a). On presentation, a CT of the abdomen and pelvis showed a large heterogeneous pelvic tumor and moderate ascites but no lymphadenopathy or evidence of distant metastasis (Figure 1b). The patient underwent fertility-sparing cytoreduction, including a left salpingo-oophorectomy, resection of pelvic peritoneal tumor, omentectomy, and pelvic and para-aortic lymph node dissection. There was no visible disease at the conclusion of the case.

Tumor pathology showed a SCCOHT of the left ovary with metastatic disease to the peritoneum. The tumor showed classic features of SCCOHT composed of mitotically active pale spindle and epithelioid cells with irregular nuclei with scant cytoplasm (Figure 1c). The tumor architecture was diverse, characterized by arrangements in cords and islands, large cell morphology, and prominent geographic necrosis. The tumor cells demonstrated loss of BRG1 (Figure 1c). Single gene germline testing of *SMARCA4* identified a deleterious mutation c.1831C>T (p.Gln611*).

Following surgery, she was treated with three cycles of cisplatin/etoposide followed by one cycle of carboplatin/paclitaxel. Interval Positron Emission Tomography/Computed Tomography (PET/CT) scan demonstrated fluorodeoxyglucose avidity in the left pericolic

gutter, presacral region, and deep pelvis and prominent left external iliac lymph nodes (Figure 1b). She underwent a diagnostic laparoscopy notable for multiple peritoneal implants in the pelvis, paracolic gutters and right upper quadrant. Intra-operative biopsies confirmed progressive disease. She was initiated on second-line therapy with two cycles of pembrolizumab and continued to have disease progression. Due to advancing disease, she suffered multiple other complications precluding further treatment, including severe hydronephrosis requiring bilateral percutaneous nephrostomy tubes, pulmonary embolism, and large bowel obstruction. She ultimately died of her disease approximately six months after her initial diagnosis (Supplementary Table 1).

3.2 SMARCA4-DUS Patient

Patient 2 was the 55-year-old mother of patient 1 (Figure 2a). She presented to the emergency department approximately five months following the death of her daughter with two weeks of heavy vaginal bleeding. Exam showed a 3 cm lesion protruding from cervix. Biopsies revealed a malignant neoplasm consistent with a sarcoma.

A CT scan of her chest, abdomen and pelvis showed a large endometrial mass with multifocal areas of deep myometrial invasion, as well as tumor extension to involve the bilateral adnexa (Figure 2b). There was evidence of peritoneal carcinomatosis, but no intrathoracic metastases. The patient underwent an exploratory laparotomy, total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, debulking, lysis of adhesion, and cystoscopy. The tumor involved the ovaries, fallopian tubes, peritoneum, omentum, small and large bowel, diaphragm, and bladder. Lymphovascular invasion was identified. There was no gross residual disease left at the end of the procedure.

Pathology showed diffuse sheets of highly mitotically active malignant cells (Figure 2c) with significant nuclear atypia and mitotically active. There were areas of rhabdoid morphology. The immunophenotype was notable for intact expression of mismatch repair proteins, loss of BRG1 (Figure 2c), and negative PD-L1. She was diagnosed with Stage IVB high grade uterine sarcoma. Postoperative imaging revealed no evidence of residual disease. Germline genetic testing was performed for 23 genes associated with breast and gynecologic cancers including *BRCA1/2, TP53, MMR* genes, and *SMARCA4* based on the patient's personal and family history and identified only the same *SMARCA4* mutation previously identified in the patient's daughter (c.1831C>T (p.Gln611*)).

Patient 2 received four cycles of doxorubicin, ifosfamide, and mesna (AIM) with a 20% dose reduction between cycles 2 and 3 given development of significant thrombocytopenia requiring platelet transfusion. After completion of treatment, a repeat CT of the chest, abdomen, and pelvis was performed and showed no evidence of disease. After three months, the patient re-presented with worsening bloating, severe constipation, abdominal pain, and nausea; imaging showed disease recurrence with multiple omental nodularities, malignant ascites, and pelvic lymphadenopathy (Figure 2b). Prior to initiation of treatment, the patient was admitted to the hospital with severe dyspnea and subsequently developed septic shock with multi-organ failure and succumbed to her disease eight days following her re-presentation, and less than nine months following her initial diagnosis.

3.3 *SMARCA4* Mutation

A germline *SMARCA4* c.1831C>T (p.Gln611*) mutation was identified in both patients. This nonsense mutation likely results in absent or disrupted protein product. This variant was not found in the ExAC database of normal human germline exomic variants [20], supporting a pathogenic effect of this mutation. This variant has not been seen previously in patients with SCCOHT with available genetic sequencing of the *SMARCA4* gene [2,21]. An additional somatic frameshift variant of unknown significance in *SMARCA4* (p.K1602fs*8) was identified in the mother's tumor tissue. This mutation lies near the terminus of the 1647 amino-acid the BRG1 protein (encoded by *SMARCA4* gene). The daughter's genotype at this locus is unknown because somatic testing was not performed on Patient's 1 tumor at the time of her diagnosis and treatment. The SMARCA4-DUS tumor was found to be microsatellite stable with low mutational burden (TMB-low, 2 mutations/megabase), consistent with other previously described SMARCA4-DUS tumors [3,4]. Ten variants of unknown significance were also identified in Patient 2 (Supplementary Table 2). Though some of these missense variants occur in known cancer-related genes (e.g. *JAK2*), none are known to be hotspot mutations associated with tumor suppressor or oncogenic functions based on analysis of more than 40,000 cancer exomes [22,23].

Examination of a three-generation pedigree (Supplementary Figure 1) was completed to assess risk of *SMARCA4*-related disease in the patient's family members. Limited phenotypic or genotypic information was available for the extended family because the mother was raised separately from her siblings and had limited contact with these individuals. Given the autosomal dominant mode of inheritance of *SMARCA4*-related genetic susceptibility to malignancies, the patient's three sisters presumably each have a 50% chance of harboring increased risk for *SMARCA4*-related malignancy. It is also possible that the mutation occurred *de novo* in the mother.

3.4 Germline mutations in SCCOHT and SMARCA4-DUS cohorts

SMARCA4 inactivating genomic alterations were identified in 48 cases of SCCOHT and 17 cases of SMARCA4-DUS. In the SCCOHT cohort of 48 patients, ages ranged from 8 to 56 years, with a median of 28.5 years (Table 1). Germline mutations (Table 2) were predicted in approximately 38% of patients, while 25% were predicted to be somatic and 38% were of indeterminate origin. The median age of patients with germline mutations was 28.0 years (range: 12-45 years), while that of patients predicted to have somatic mutations was 32.0 years (range: 24-56 years; p=0.13). Patients with mutations of indeterminate origin had a median age of 26 years (range: 8-42).

Overall, the 17 SMARCA4-DUS patients were older than SCCOHT patients, with a median age of 49.0 years compared with 28.5 years, respectively. This trend is independent of mutation status (germline, 28.0 vs 47.5 years, p=0.06; somatic, 32.0 vs 53.5 years, p=0.01). Fewer patients in the SMARCA4-DUS cohort were predicted to have germline *SMARCA4* mutations, representing only 11.8% of the population (Table 3). Similar to the results seen in SCCOHT, the patients that were predicted to carry germline mutations versus somatic mutations were slightly younger, with a median age of 47.5 years (range: 40-55 years)

compared to 53.5 years (range 43-70 year; p= 0.53). However, these results are limited by the small sample size and large fraction reported as indeterminate or unknown.

The *SMARCA4* gene is composed of seven highly conserved domains (Figure 3). The majority of the mutations identified are frameshift mutations (Figure 3, **Brown)** followed by nonsense mutations (Figure 3, **Green)**. A much smaller number of mutations are splice mutations (Figure 3, **Yellow)**, missense mutations (Figure 3, **Blue)** and in-frame deletions (Figure 3, **Pink)**. All of these mutations are predicted to lead to loss of function of the SMARCA4 protein.

4. Discussion

This study details the clinical course of a mother-daughter pair with inherited *SMARCA4* germline mutation leading to two aggressive and rare rhabdoid tumors - SCCOHT and SMARCA4-DUS. Despite aggressive treatment with surgery, chemotherapy, and, in the case of the daughter, immunotherapy, they both succumbed to their disease in 6 and 9 months, respectively, highlighting the aggressive nature of these tumors.

These clinical findings are consistent with previous descriptions of SCCOHT and *SMARCA4*-DUS. Prior studies have demonstrated that these tumors are morphologically and genetically related, [3,4] now further established through demonstration of a germline link between the diseases. Both tumors affect relatively young patients, with an average age of diagnosis for SCCOHT of 24 years, with a range described in the literature from 14 months to 71 years [21]. Patients affected by *SMARCA4*-DUS are slightly older (median age: 49 years, range: 32-70 years), though this is younger than the average age of diagnosis for the more common uterine cancers. There is a non-significant difference in age between SCCOHT and SMARCA4-DUS patients with germline versus somatic variants in *SMARCA4,* suggesting that germline mutations may affect patients at slightly younger ages, consistent with previous reports [21]. However, these results are limited by the small sample size and the high percentage of indeterminate or unknown results in each cohort.

Both patients harbored a nonsense mutation in *SMARCA4*, expected to lead to decreased production of functional BRG1 protein. Though this mutation has not been specifically described in the literature in other patients, the vast majority of mutations in the *SMARCA4* gene associated with pathologically-confirmed SCCOHT are frameshift, splice-site, or nonsense mutations [2], which are expected to produce truncated, non-functional protein. There were several mutations in our cohort that overlapped with mutations previously identified in the literature [21] (Supplementary Table 3).

Prognosis is generally poor for both SCCOHT and *SMARCA4*-DUS, with a 5-year survival of 40% for SCCOHT [21]. More recent studies using high-dose, multi-agent chemotherapy in combination with surgery, radiation, and autologous stem cell transplant have led to improved overall survival rates (up to 50% at three years) [24,25]. A review of the 14 cases of *SMARCA4*-DUS described in the literature shows a similarly aggressive course, with most patients dying of the disease within months of diagnosis [3].

Further understanding of the shared genetic features of SCCOHT and *SMARCA4*-DUS and their response to targeted therapy may contribute to genomically-guided clinical decision-making for these challenging cancer types. Recent studies have suggested that *SMARCA4*-deficient ovarian cancers may be selectively sensitive to EZH2 inhibition [26], with possible implications for treatment of *SMARCA4*-DUS as well [3]. Treatment of ten patients with SCCOHT with the EZH2-inhibitor tazemetostat led to clinical partial response in one patient, with duration of response of 32 weeks [27]. Further study of this agent in this clinical context has been halted due to failure to pass stage 2 futility. Additionally pre-clinical data highlighted ponantinib, a tyrosine kinase receptor inhibitor, and palbociclib, a CDK4 inhibitor, as agents with selective activity against SCCOHT [28,29], though this too requires further clinical investigation.

Notably, four patients with SCCOHT have responded to anti-PD-1 monotherapy , which is unexpected for a cancer with low-mutational-burden [30]. The authors additionally describe unusually high PD-L1 expression and T-cell infiltration in this cancer type. This result is especially interesting given the association between loss of *PBRM1* - a member of the SWI/SNF protein family closely related to *SMARCA4* - and sensitivity to anti-PD-1 immune checkpoint therapy in renal cell carcinoma, another generally low-mutational-burden cancer with a prominent immune infiltrate [31].

Reports of families affected by germline *SMARCA4* mutations are rare in the literature [32,33]. Growing understanding of the role of *SMARCA4* in the pathogenesis of rare gynecological cancers, as well as other aggressive rhabdoid tumors, may inform recommended genetic testing and counseling for these families. Given the potential severity of the phenotype and autosomal dominant inheritance pattern, identifying germline *SMARCA4* as a driver of a hereditary cancer syndrome has important implications on treatment and counseling of patients. In some of these cases, patients and their families have opted for prophylactic bilateral salpingo-oophorectomy (BSO), though the decision to proceed with this surgery is a difficult choice given incredibly young average age of onset in this population. The reliability of the medical management recommendations is further limited by insufficient information regarding the penetrance and lifetime cancer risks of carriers of these mutations.

It is critical that all individuals with suspected SCCOHT or SMARCA4-DUS receive genetic counseling. Germline testing should be recommended preferentially for the affected individual to clarify hereditary cancer risk in the family. If a germline mutation is identified, genetic testing should be offered to all at-risk family members, acknowledging the lack of published medical management guidelines. As suggested by previous authors, in the absence of a germline mutation, the tumor should be sequenced to identify possible somatic mutations [32]. Male family members should also be counseled because they could be carriers, putting their female offspring at risk. Further recommendations for male carriers and association with future cancer risk is unavailable; however, the data regarding *SMARCA4* in inherited cancers is rapidly expanding and future recommendations or associations may become available underlying the importance of including both male and female carriers in the genetic counseling when able.

Importantly, there is limited data at this time to make definitive recommendations regarding prophylactic surgery. All recommendations should involve shared decision making between the clinical team and the patient. In unaffected germline mutation carriers who have completed child-bearing, it is reasonable to discuss prophylactic BSO, as well hysterectomy or careful endometrial surveillance given potential risk of developing *SMARCA4*-DUS. Although, the optimal age to make this recommendation is unclear. This data suggests that by the third decade of life, approximately 55% of patients would have developed malignancy, heavily weighted toward SCCOHT. By the fourth decade this number increases to over 80% (Supplementary Figure 2). If a patient is an unaffected carrier over the age of 30, it may be reasonable to pursue prophylactic surgery to reduce the risk of future occurrence of SCCOHT and SMARCA4-DUS. In young women, this decision is far more complex given implications on future fertility, as well as harmful effects to mental and physical health of early menopause. Although, in highly motivated patients, long-term hormonal supplementation may be a reasonable option. In the appropriate clinical scenario, these patients may benefit from oocyte preservation prior to prophylactic surgery. As in other cancers that impact reproductive age women [34], a discussion of fertility goals should be included in the counseling of these patients. Of the two reported cases of prophylactic BSO in germline carriers, both demonstrated retained BRG1 protein expression [32,33]. The interpretation of this result is unclear and highlights that more data is needed to understand the penetrance in germline carriers. However, there is currently no alternative screening modality or biomarker to offer these families. Given the complexity of the counseling and rapidly evolving treatment and diagnosis paradigm, patients with these diagnoses should be referred to high-volume centers that have experience in treatment and genetic counseling of these malignancies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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8.

Highlights

- SCCOHT and SMARCA4-DUS are morphological and genetically related tumors driven by loss of function mutations in SMARCA4
- These tumors affect young women and generally carry a poor prognosis
- Identification of germline mutations in SMARCA4 is critical to providing counseling and treatment to affected individuals

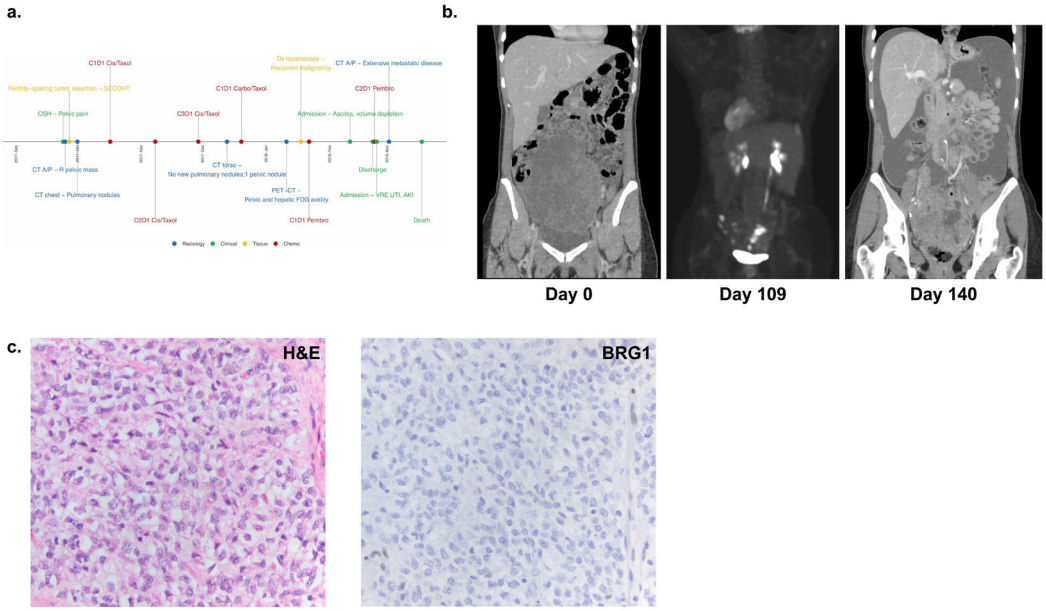

Figure 1: Clinical Summary of Patient 1 diagnosed with SCCOHT.

(a) Timeline of major clinical events (b) Computed tomography (CT) scans at initial presentation (left panel), showing FDG-avid disease in the pelvis and liver following cycle 1 of carboplatin/ paclitaxel (middle panel), and at presentation to the hospital after cycle 2 of pembrolizumab (right panel). (c) Pathological evaluation with H&E staining (left panel) and immunohistochemistry demonstrating loss of BRG1 (right panel).

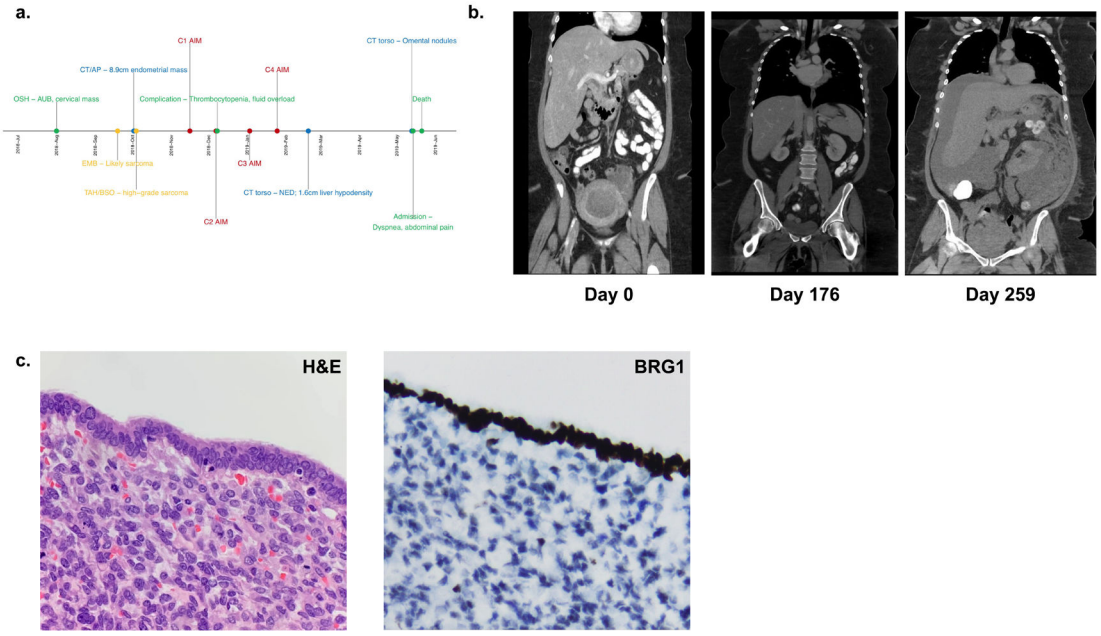

Figure 2: Clinical Summary of Patient 2 diagnosed with SMARCA4-DUS.

(a) Timeline of major clinical events. (b) Computed tomography (CT) scans at initial presentation (left panel), showing no evidence of disease (NED) following completion of 4 cycles of AIM chemotherapy (middle panel), and after re-presentation with diffuse metastatic disease (right panel). (c) Pathological evaluation with H&E staining revealing rhabdoid morphology and sheets of highly mitotically active cells characteristic of SMARCA4-DUS (left panel), with immunohistochemistry demonstrating loss of BRG1 (right panel).

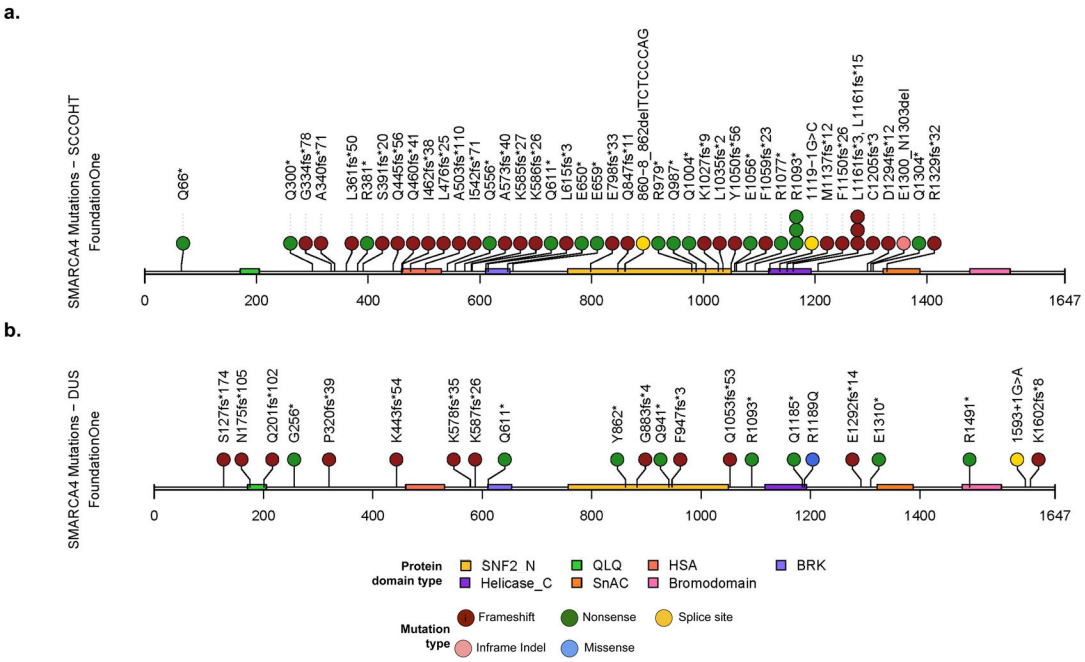

Figure 3: Inactivating SMARCA4 mutations identified in SCCOHT and SMARCA4-DUS.

(a) Schematic of the mutations identified in the SCCOHT cohort and (b) the SMARCA-4 DUS cohorts respectively. Green represents nonsense mutations, brown represents frameshift mutations, yellow represents splice site mutations, blue represents missense mutations and pink represents in-frame deletions. QLQ; HSA, helicase/SANT-associated domain; BRK, brahma and kismet domain; SNF2_N, SNF2 family N-terminal domain; Helicase_C, helicase superfamily C-terminal domain; SnAC, Snf2-ATP coupling, chromatin remodelling complex, Bromodomain.

Table 1:

Age demographics in SCCOHT and SMARCA4-DUS cohorts

	SCCOHT			SMARCA4-DUS		
	# of Cases	**Median**	**Range**	**# of Cases**	**Median**	**Range**
All	48	28.5	8-56	17	49.0	32-70
Germline	37.5% (18/48)	28.0	12-45	11.8% (2/17)	47.5	40-55
Somatic	25% (12/48)	32.0	24-56	23.6% (4/17)	53.5	43-70
Indeterminate	37.5% (18/48)	26.0	8-42	47.1% (8/17)	50.0	33-60
Unknown	n/a	n/a	n/a	17.6% (3/17)	48.0	32-51

Table 2:

SMARCA4 Mutations in SCCOHT

n	Age	*SMARCA4 mutation*	Predicted Origin	Mutation Type	Previously Identified
1	24	p.Arg1077* c.2438+1G>A	Somatic Somatic	Nonsense Splice site	*Yes *Yes
2	24	p.Gln987*	Indeterminate	Nonsense	No
3	23	p.Leu1161fs*3	Germline	Frameshift	No
4	31	Deletion / loss	Somatic	Deletion / loss	No
5	12	p.Gln300* 3215+1G>A	Germline	Nonsense Splice site	No No
6	38	p.Gln445fs*56 p.Asp1294fs*12	Germline	Frameshift Frameshift	No
7	22	p.Glu1300_Asn1303del	Germline	Inframe Indel	Yes
8	8	p.Leu1161fs*15	Indeterminate	Frameshift	No
9	12	p.Leu1161fs*15	Indeterminate	Frameshift	No
10	45	p.Leu361fs*50	Germline	Frameshift	No
11	31	p.Gln460fs*41	Somatic	Frameshift	No
12	21	c.1119-G>C homozygous loss	Germline	Splice site Homozygous loss	Yes
13	42	p.Glu798fs*33	Germline	Frameshift	No
14	29	c.3215+1G>T	Germline	Splice site	No
15	25	c.2859+1G>C	Germline	Splice site	Yes
16	36	p.Ala340fs*71 c.2960_2973+2del16	Indeterminate	Frameshift Splice site	No
17	36	p.Lys586fs*26	Indeterminate	Frameshift	No
18	29	p.Phe1150fs*14	Indeterminate	Frameshift	No
19	27	p.Leu1035fs*2	Indeterminate	Frameshift	No
20	19	p.Lys585fs*27 p.Arg1093*	Germline	Frameshift Nonsense	Yes
21	25	p.Gln847fs*11	Somatic	Frameshift	No
22	40	pLeu615fs*3 p.Gln1004*	Indeterminate	Frameshift Nonsense	No
23	31	p.Glu659*	Indeterminate	Nonsense	No
24	40	p.Gln1304*	Somatic	Nonsense	No

n	Age	*SMARCA4 mutation*	Predicted Origin	Mutation Type	Previously Identified
25	45	p.Lys1027fs*9	Somatic	Frameshift	No
26	17	p.Ile542fs*71	Indeterminate	Frameshift	No
27	25	p.Ala503fs*110	Indeterminate	Frameshift	No
28	27	p.Arg979*	Somatic	Nonsense	Yes
29	15	p.Arg381* p.Ala573fs*40	Germline	Frameshift Frameshift	Yes
30	33	2438+1G>A	Somatic	Splice site	No
31	33	p.Gly334fs*78	Germline	Frameshift	No
32	19	p.Arg1329fs*32	Indeterminate	Frameshift	No
33	21	c.3168+1G>A	Indeterminate	Splice site	No
34	38	p.Glu650*	Indeterminate	Nonsense	No
35	24	p.Glu1056*	Indeterminate	Nonsense	No
36	24	p.Met1137fs*12 c.3952-2A>G	Germline	Frameshift Splice site	No
37	28	p.Leu476fs*25	Indeterminate	Frameshift	No
38	38	p.Tyr1050fs*56 c.3382+2T>G	Germline	Frameshift Splice site	No
39	24	p.Cys1205fs*3	Indeterminate	Frameshift	No
40	33	c.2859+1G>A	Somatic	Splice site	Yes
41	39	p.Arg1093*	Somatic	Nonsense	Yes
42	31	c.860-8_862delTCTCCCAG	Germline	Splice site	No
43	42	p.Arg1093* p.Ser391fs*20	Indeterminate	Nonsense Frameshift	No
44	44	p.Phe1059fs*23	Germline	Frameshift	No
45	28	p.Gln66* c.2859+1G>C	Somatic	Nonsense Splice site	Yes
46	56	Ile462fs*38 p.Gln556*	Somatic	Frameshift Nonsense	No
47	27	c.3168+2T>C	Germline	Splice site	No
48	31	p.Gln611*	Germline	Nonsense	No

Table 3:

SMARCA4 mutations in DUS

n	Age	*SMARCA4 mutation*	Predicted Origin	Mutation Type	Previously Identified
1	48	p.Ser127fs*174 p.Gln941*	Unknown	Frameshift Nonsense	No
2	51	Loss	Unknown	Loss	No
3	63	p.Asn175fs*105 p.Gln1053fs*53	Somatic	Frameshift Frameshift	No
4	52	p.Arg1093*	Indeterminate	Nonsense	Yes
5	43	p.LysK443fs*54 p.Glu1292fs*14	Somatic	Frameshift Frameshift	No
6	51	p.Gln1185*	Indeterminate	Nonsense	No
7	60	p.Arg1491*	Indeterminate	Nonsense	No
8	49	p.Gln201fs*102 p.Phe947fs*3	Indeterminate	Frameshift Frameshift	No
9	33	p.Tyr862*	Indeterminate	Nonsense	No
10	70	c.2505+1G>A	Somatic	Splice site	No
11	52	p.Gly256*	Indeterminate	Nonsense	No
12	44	p.Lys587fs*26	Somatic	Frameshift	No
13	38	p.Gly883fs*4 c.3236C>T	Indeterminate	Frameshift Splice site	No
14	55	p.Gln611* p.Lys1602fs*8	Germline	Nonsense Frameshift	No
15	45	c.1593+1G>A p.Glu1310*	Indeterminate	Splice site Nonsense	No
16	32	p.Lys578fs*35	Unknown	Frameshift	No
17	40	Loss c.3951+2_3951+20>AG	Germline	Loss Splice site	No