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Genetic Basis of SMARCB1 Protein Loss in 22 Sinonasal Carcinomas

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Abstract

SMARCB1-deficient sinonasal carcinoma (SNC) is an aggressive malignancy characterized by INI1 loss mostly due to homozygous *SMARCB1* deletion. With the exception of a few reported cases, these tumors have not been thoroughly studied by massive parallel sequencing (MPS). A retrospective cohort of 22 SMARCB1-deficient SNC were studied by light microscopy, immunohistochemistry, FISH (n=9), targeted exome MPS (n=12) and by FACETS (n=10), a bioinformatics pipeline for copy number/zygosity assessment. SMARCB1-deficient SNC was found in 13 (59%) men and 9 (41%) women. Most common growth patterns were basaloid (59%), occurring mostly in men (77%) and plasmacytoid/eosinophilic/rhabdoid pattern (23%), arising mostly in women (80%). The former group was significantly younger (median age 46 years, range 24–54, vs 79 years, range 66–95, p<0.0001). Clear cell, pseudoglandular, glandular, spindle cell and sarcomatoid features were variably present. SMARCB1-deficient SNC expressed cytokeratin (100%), p63 (72%), neuroendocrine markers (52%), CDX-2 (44%), S-100 (25%), CEA (4/4 cases), Hepatocyte (2/2 cases), and aberrant nuclear β -catenin (1/1 case). *SMARCB1* showed homozygous deletion (68%), hemizygous deletion (16%) or truncating mutations associated with copy neutral-loss of heterozygosity (11%). Co-existing genetic alterations were 22q loss including loss of *NF2* and *CHEK2* (50%), chromosome 7 gain (25%), and *TP53* V157F, *CDKN2A* W110*

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and *CTNNB1* S45F mutations. At 2-years and 5-years, the disease-specific survival and disease-free survival were 70% and 35%, and 13% and 0%, respectively. SMARCB1-deficient SNC is phenotypically and genetically diverse and these distinctions warrant further investigation for their biological and clinical significance.

Keywords

sinonasal SMARCB1-deficient carcinoma; homozygous deletion; next-generation sequencing

INTRODUCTION

SMARCB1 gene, a putative tumor suppressor gene (1) is located at 22q11.2 and is a member of SWItch/sucrose non-fermentable (SWI/SNF) chromatin-remodelling complex. SWI/SNF i.e. human analogue BRG1/BRM-associated factor (BAF) complex is a chromatin-remodelling complex, which by modifying the spatial configuration of the DNA regulates the accessibility to gene transcription factors (2, 3). Somatic *SMARCB1* alterations, typically whole gene deletion, were found in various malignancies including rhabdoid tumors (4), medulloblastoma (5), epithelioid sarcoma (6), medullary renal cell carcinoma (7), cribriform neuroepithelial tumor (8), poorly differentiated chordoma (9) and, more recently, in a subset of aggressive sinonasal carcinomas (SNC) (10–12). SMARCB1-deficient (SNC) was first reported in 2014 (10–12) as an aggressive sinonasal malignancy characterized by SMARCB1 (INI1) protein loss and somatic *SMARCB1* gene deletion. Although most reported cases tend to display undifferentiated morphology reminiscent of sinonasal undifferentiated carcinoma (SNUC), these tumors can be morphologically and immunophenotypically rather heterogeneous (13–15). SMARCB1 protein loss could be explained by homozygous *SMARCB1* gene deletion detected by fluorescence *in situ* hybridization (FISH) in most cases (13). However, the genome of SMARCB1-deficient SNC has not been studied in greater detail and the current knowledge is limited to a few reported cases (10, 16, 17). Here, we performed a detailed phenotypic and molecular characterization of our retrospective cohort of SMARCB1-deficient SNC.

MATERIALS AND METHODS

1. Cases

The study was approved by the Internal Review Board of Memorial Sloan Kettering Cancer Center (MSKCC). Twenty-two cases of primary sinonasal SMARCB1-deficient carcinomas were retrieved from MSKCC pathology archive, including 4 research and 18 clinical cases. All cases were reviewed by at least one pathologist with an interest in head and neck pathology (SD). Four cases were reported before (16) and outcome on 15 patients was included in other study (18).

2. DNA extraction and molecular testing

In 12 cases, targeted exome massive parallel sequencing (MPS) assay MSK-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT™) was performed to evaluate genetic alterations in 279–468 cancer-related genes as previously described (19,

20). DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor sections and from normal tissue. Matched normal FFPE tissue or normal blood was used for DNA extraction in 10 cases, and unmatched pooled normal DNA was used in 2 cases. Copy number aberrations (CNA) were identified by comparing the sequence coverage of targeted regions in a tumor sample relative to a standard diploid normal sample. CNA were expressed as the $\log_2$ transformed tumor/normal ratio and minimum of 2.0-fold change was required to consider gene amplification or deletion (19, 20). Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing (FACETS) analysis for copy number/zygosity assessment was performed in 10 cases with available matched normal DNA as previously described (21). Oncogenicity was determined based on OncoKB annotation in cBioPortal (22).

3. Fluorescence *in situ* hybridization for *SMARCB1* gene

Nine cases were evaluated for *SMARCB1* gene copy number status by fluorescence *in situ* hybridization (FISH) assay using 4µm FFPE tissue sections. In 4 cases, bacterial artificial chromosome (BAC) probes, including telomeric *EWSR1* and 22q11 (control probes) were used to assess the *SMARCB1* gene copy number status. In the presence of both control signals, either telomeric-*EWSR1* or 22q11, two *SMARCB1* copies indicated normal/intact *SMARCB1* gene status, one *SMARCB1* copy indicated hemizygous deletion, and absence of both *SMARCB1* copies indicated homozygous deletion as previously described (12). In 5 cases tri-color FISH was performed as detailed before (23). In one case, the material was insufficient to perform molecular or cytogenetic studies.

4. Immunohistochemistry and *in situ* hybridization

Immunohistochemistry (IHC) was performed on the Ventana Benchmark Ultra platform (Ventana Medical Systems Inc., Tucson, AZ, United States) using a streptavidin-biotin-peroxidase secondary (iView, Ventana) or on a Leica-Bond-3 automated stainer platform (Leica, Buffalo Grove, IL), using a secondary polymeric detection kit (Refine, Leica) and a heat-based antigen retrieval method with a high pH retrieval buffer according to the manufacturer's recommendations. *SMARCB1* protein status was assessed using INI1 antibody (clone 25/BAF47, BD Biosciences, Franklin Lakes, NJ, USA) at 1:200 dilution. The details on other antibodies used for IHC and ISH probes are summarized in Table S1. Positive IHC labelling in >25% cells was considered "positive", 6%–25% "focally positive", <1%–5% "very focally/rare cells positive".

5. Statistical analysis

Statistical analysis was performed using Fisher's exact test for nonparametric variables and Student's *t* test for continuous variables. All tests performed were two-tailed. P values <0.05 were considered significant. Survival analysis was performed using Log rank test.

RESULTS

I. Clinical outcome

Most patients were men (13/22, 59%) presenting at the median age 47.5 years (range 24–95), and were significantly younger than women (9/22, 41%) who presented at the median age of 66 years (range 35–83; *p*=0.033). Clinical characteristics for all patients are

summarized in Table 1. Clinical follow-up was available for 18 patients with the median of 22 months (range 1–199 months). At 2-years, 3-years and 5-years the overall survival (OS) was 66%, 50% and 33%, disease-specific survival (DSS) was 70%, 54% and 35%, and disease-free survival (DFS) was 13%, 13% and 0%, respectively.

II. Pathologic and molecular features

a) Morphology—SMARCB1-deficient SNC were morphologically diverse showing most often basaloid growth pattern (13/22, 59%) reminiscent of undifferentiated or non-keratinizing squamous cell carcinoma with the tumor cells arranged in compact sheets and nests (Figure 1) and was seen in relatively younger patients, median age 46 years (range 24–54) and mostly men (10/13, 77%). The second most common, plasmacytoid/eosinophilic/rhabdoid pattern was found in 5 (23%) patients, who were mostly women (4/5, 80%, $p=0.047$) and significantly older than the former group (median age 79 years, range 66–95, $p<0.0001$). Two of the latter cases showed focal glandular differentiation. Pseudoglandular/eosinophilic and pseudoglandular/spindle cell morphology was seen in the remaining 4 (18%) patients (median age 61, range 47–69). The amount of intervening stroma varied from scanty, which was seen in tumors with basaloid growth pattern to abundant and mucoid in cases with pseudoglandular, glandular and/or spindle cell features. Clear cell features, oncocyctic, sarcomatoid foci and bizarre multinucleated giant cells were also variably present. The rhabdoid cells appearance ranged from subtle with clear cytoplasm and eccentric nucleus to prominent with plasmacytoid appearance. Occasionally, increased amount of eosinophilic material formed large intracytoplasmic inclusions and with peripherally located nuclei provided a characteristic rhabdoid appearance (case SN_25) and some cases showed clear, “empty” cytoplasmic vacuoles (Figure 1).

b) Mutation profile and SMARCB1 gene status—All cases ($n=22$, 100%) tested either by molecular or FISH method showed loss of at least one *SMARCB1* allele. Among the cases with available zygosity status ($n=19$), most (13/19, 68%) showed homozygous *SMARCB1* deletion (Table 2 and Figure 2). In 3 (16%) cases, there was hemizygous *SMARCB1* loss and no other *SMARCB1* mutation, 2 (11%) cases had a truncating mutation, *SMARCB1* X265_splice site or *SMARCB1* Y44* variant associated with copy neutral-loss of heterozygosity (CN-LOH; Figure 3). In one case with hemizygous *SMARCB1* loss tested by FISH, the mutation status of the alternate allele remained unknown (Table 2). By MSK-IMPACT, 21 genes were mutated in 12 cases, with a median 2 mutations per case (range 0–5) excluding CNA. *SMARCB1* was the only gene with recurrent, (likely) oncogenic alterations and these often co-occurred with loss of neighboring genes at 22q (6/12, 50%) including *NF2* and *CHEK2* in all such cases, and variable loss of *MAPK1*, *RAC2*, *CRKL* and/or *EP300* (Table 2). Mutations in 3 other tumor suppressor genes, including a hotspot *CTNNB1* S45F, *TP53* V157F, and *CDKN2A* W110* were detected in 3 (25%) cases. Three (25%) cases showed chromosome 7 gain. Random broad copy alterations included 1q gain and 2q35–36 and 3q26–28 losses. No particular associations between the type of *SMARCB1* mutation, with or without concurrent alterations, and the tumor phenotype or outcome could be identified.

c) Immunophenotype—Immunohistochemical studies and *in situ* hybridization studies results are summarized in Figure 4. All cases were positive for at least one cytokeratin with AE1/AE3 (19/19) and Cam5.2 (9/9) being the most reliable and consistently positive in all tested cases. The remaining 3 cases were positive either for CK7, CK20 and/or BerEP4. About 72% (13/18) cases were positive for p63 and 59% (10/17) for p40. Weak/focal p63/p40 staining was observed in about one third of cases showing non-basaloid morphology. Among myoepithelial markers, S-100 was weakly/focally positive in 25% (5/20) cases, while calponin or smooth muscle actin (SMA) expression were rare. Fifty-two percent (11/21) were positive either for synaptophysin or chromogranin, including 2 cases with strong positive labeling, one of which was initially diagnosed as large cell neuroendocrine carcinoma (SN_26, Figure 4) and both showing predominantly plasmacytoid/eosinophilic/rhabdoid morphology (Figure 1). CDX-2 was expressed in 4/9 (44%) tested cases, CEA in 4/4 and Hepatocyte in 2/2 tested cases including SN_25, which was initially misdiagnosed as primary hepatocellular carcinoma. No case expressed NUT, and no high-risk human papillomavirus (HPV) or Epstein-Barr virus were detected.

DISCUSSION

In the present study, we further expanded the phenotypic spectrum of SMARCB1-deficient SNC and found associations between the tumor morphology and patients characteristics. We provided a detailed molecular characterization of SMARCB1-deficient SNC, identified distinct genetic patterns consistent with SMARCB1 protein loss and revealed co-existing, potentially significant genetic alterations.

After the description of first reported SMARCB1-deficient SNC cases in 2014 which were rather uniformly undifferentiated, multiple following studies demonstrated that these tumors can display a variety of histologies suggesting that SMARCB1-deficient SNC might still be underrecognized and likely more common than it has been currently perceived (13–15, 24). In line with the prior studies, our data further illustrate a wide morphological and immunophenotypic diversity of SMARCB1-deficient SNC. We have also found that the most common, basaloid growth pattern can be associated with relatively younger age and male sex, whereas carcinomas with plasmacytoid/eosinophilic/rhabdoid appearance might be more likely to arise in older women. In addition to the variety of morphologies, including pseudoglandular and glandular appearance reminiscent of high-grade adenocarcinoma, clear tumor cells, spindle cell and sarcomatoid features, it is important to keep in mind that SMARCB1-deficient SNC can occasionally express immunomarkers commonly used to determine the site or organ of origin such as CDX-2 (15) and Hepatocyte. Therefore, caution must be exercised not to interpret poorly differentiated/high-grade CDX-2-positive carcinomas simply as sinonasal intestinal-type adenocarcinoma or as metastatic carcinoma of lower gastrointestinal tract without further INI1 IHC work-up. Similarly, a positive Hepatocyte immunostain should not be misinterpreted as metastatic hepatocellular carcinoma. INI1 IHC should not be either excluded from a diagnostic work-up of high-grade SNC in the presence of a strong and diffuse neuroendocrine marker expression or aberrant nuclear β -catenin immunopositivity.

A very limited number of SMARCB1-deficient SNC subjected to MPS published to date demonstrated *SMARCB1* whole gene deletion in these cases (10, 16, 17). FISH analysis showed homozygous deletion was the most predominant genetic alteration followed by hemizygous deletion of *SMARCB1*. Rarely, *SMARCB1* was intact by FISH (13). We confirmed homozygous *SMARCB1* deletion to be present in the majority of cases. Inactivating *SMARCB1* mutation coupled with CN-LOH could explain INI1 protein loss in a minor subset of cases. However, in some cases, hemizygous *SMARCB1* deletion was the only detected event raising a question if INI1 protein loss in such cases could be partly due to gene rearrangement involving the alternate allele akin to that seen in medullary renal cell carcinomas (23) or due to miRNA-mediated epigenetic silencing of *SMARCB1* protein expression as reported in epithelioid sarcomas (25, 26).

A paucity of co-existing (likely) oncogenic mutations including *CTNNB1*, *TP53* and *CDKN2A* supports the role of deficient *SMARCB1* as a putative driver of malignant transformation in this subset of SNC.

However, a substantial degree of molecular heterogeneity is evident at the genetic level as half of the cases showed concurrent losses of neighboring genes at 22q, including *NF2* and *CHEK2* losses. Recent methylation-based studies on atypical teratoid/rhabdoid tumors (AT/RT) helped substratify these tumors into three distinct, biologically relevant categories; while AT/RT-MYC subset was enriched for focal *SMARCB1* gene deletions, AT/RT-TYR tumors comprised mostly of cases with broad 22q deletions (27). Therefore, larger, more comprehensive studies on SMARCB1-deficient SNC to explore the significance of concurrent, broad genetic losses at 22q would be justified.

Clinically, SMARCB1-deficient SNC has been shown to be aggressive malignancy with frequent recurrences and poor outcome (13, 18). Our cohort, which originates from a single institution, supports the published data and demonstrates the aggressive nature of this sinonasal malignancy. Indeed, the majority of SMARCB1-deficient SNC patients are likely to recur within 2 years and overall, less than one third of patients will survive 5 years.

Limitations of our study are mainly related to the lack of adequate tissues to perform further studies to explore, for instance, additional mechanisms of SMARCB1 protein loss in cases with hemizygous *SMARCB1* deletion. However, we have shown these cancers are phenotypically diverse and less common morphologies such as plasmacytoid/eosinophilic/rhabdoid pattern may be relatively more common in elderly female patients. We have demonstrated that SMARCB1-deficient SNC display heterogeneity at the molecular level and that loss of SMARCB1 protein could be due to truncating mutations associated with CN-LOH in a significant minority of cases. Co-existing genetic alterations including recurrent *NF2* and *CHEK2* losses and chromosome 7 gain can provide rationale for further, larger studies aiming to elucidate the biological significance of distinct molecular findings in SMARCB1-deficient SNC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Morphologic spectrum of SMARCB1-deficient SNC. Basaloid growth pattern and scattered rhabdoid cells were subtle and showed clear cytoplasm with eccentrically placed nucleus (yellow arrow; SN_62, A). Infiltrative growth with involvement of surface epithelium (case SN_23, yellow star, B), and exophytic papillary features were seen (case SN_70, C). SN_26 comprised of sheets (D), trabeculae and cords (E) of oncocytic tumor cells with a striking predominance of rhabdoid cells; they were enlarged and contained deeply eosinophilic or red round cytoplasmic inclusions (yellow arrows, D) and showed diffuse and strong immunolabeling for synaptophysin (case SN_26, upper inset, E) and chromogranin (lower inset, E). SN_25 comprised of sheets of predominantly clear cells interwoven with scanty fibrotic stroma (F) and scattered cells with large eosinophilic cytoplasmic inclusions (yellow

arrow, G) and was strongly positive for hepatocyte (inset, G). SN_24 comprised of solid basaloid sheets of tumor cells (not shown) and pseudoglandular structures filled with basophilic mucoid material (H); discohesive single or small clusters of tumor cells were surrounded by abundant mucoid stroma. Rhabdoid cells are pointed by the yellow arrow (I). Areas with pseudo-glandular appearance (J) alternated with sarcomatoid foci comprised of pleomorphic sarcomatoid tumor cells in SN_72 (K). Large bizarre multinucleated tumor cells were seen in SN_84 (yellow arrow, L).

Figure 2.

SMARCB1 deletion in SNC. Case SN_78 with predominantly pseudoglandular/spindle cell growth (left inset, H&E, A) was immunopositive for CDX-2 (right inset, A). CNA plot depicts a homozygous deletion of *SMARCB1* gene (lower green arrow) and deletion of neighboring genes on 22q including *NF2* (upper green arrow). Y-axis depicts copy number changes expressed as the log_2 transformed tumor/normal ratio according to their genomic positions indicated on the x-axis. Each dot represents one exon. Red dots indicate 2-fold tumor/normal ratio (A). FACETS analysis shows deletion of both *SMARCB1* alleles as indicated by the total integer copy number 0 (black line, y-axis). Red line indicates minor allele. The vertical green line indicates *SMARCB1* genomic position on chromosome 22 (B). Case SN_76 showed oncocytic gland-forming foci (left inset, H&E, C) and diffuse

complete nuclear loss of SMARCB1 protein (right inset, Baf-47, C). CNA plot shows *FUBP1* intragenic deletion (blue arrow), 2q35–36 and 3q26–28 losses and hemizygous *SMARCB1* deletion (green arrow); FACETS indicated the total copy number of 1 (D). Heterozygous gain of chromosome 7 is indicated by total integer copy number 3 and minor allele copy number 1 (blue arrow, D).

Abbreviations: CNA, copy number alterations; FACETS, Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing.

Figure 3. Truncating *SMARCB1* mutations in SNC. In SN_75 oncocytic tumor cells formed cords and trabeculae (left inset, H&E, A). The IGV screenshot depicts *SMARCB1* splice site mutation c.795+2_795+44del which results in a 44bp deletion including the splice site (green arrow) as detected by MSK-IMPACT. Gray bars represent sequence reads which are aligned according to the reference genome at the bottom. Solid black lines represent sequence reads with *SMARCB1* mutation. Nitrogenous bases are color-coded and the corresponding aminoacids are represented by blue rectangular bars. Non-coding sequence is shown as a blue line (A). Loss of nuclear Baf-47 in the tumor cells confirms the loss of normal *SMARCB1* protein (right inset, A). CN-LOH detected by FACETS was consistent with the total *SMARCB1* copy number 2 (black) and minor allele copy number 0 (red).

Heterozygous gain of chromosome 7 is indicated by blue arrow (B). SN_85 showed a basaloid growth pattern (H&E, C) and harbored three oncogenic variants as depicted in IGV screenshots (D): *SMARCB1* Y44* (c.132C>G; upper left), *CTNNB1* S45F (c.134C>T; upper middle) and *CDKN2A* W110* (c.330G>A; upper right). Each mutation was consistent with the respective abnormal protein expression; nuclear loss of Baf-47 (lower left), aberrant nuclear expression of β -catenin (lower middle), and loss of p16 (lower right, D).

Abbreviations: CNA, copy number alterations; FACETS, Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing; IGV, integrated genome viewer; bp, base pairs; CN-LOH, copy neutral-loss of heterozygosity.

Case	SN_23	SN_24	SN_25	SN_26	SN_62	SN_63	SN_70	SN_71	SN_72	SN_73	SN_74	SN_75	SN_76	SN_77	SN_78	SN_79	SN_81	SN_84	SN_85	SN_86	SN_87	SN_88
SWI/SNF PROTEINS	SMARCB1 (Baf-47)																					
	SMARCA4 (BRG-1)																					
	SMARCA2 (BRM)																					
CYTOKERATINS & EPITHELIAL MARKERS	AE1/AE3																					
	34BE12																					
	CK7																					
	CK20																					
	Cam5.2																					
	CK5/6																					
	EMA																					
	BerEP4																					
NEUROENDOCRINE MARKERS	Synaptophysin																					
	Chromogranin																					
	CD56																					
	NSE																					
	INSM1																					
MYOEPIITHELIAL MARKERS	p63																					
	p40																					
	S-100																					
	Calponin																					
	SMA																					
	SOX10																					
	Vimentin																					
	GFAP																					
LINEAGE-RELATED MARKERS	TTF-1																					
	CDX-2																					
	Hepatocyte																					
	HMB45																					
	AR																					
	ER																					
	Mammaglobin																					
MUSCLE MARKERS	Desmin																					
	Myogenin																					
	MSA (HHF-35)																					
OTHER	NUT																					
	mCEA/pCEA																					
	Mucicarmine																					
	p16																					
	CD99																					
	Beta-catenin																					
VIRUS	HPV-ISH																					
	EBV (EBER)-ISH																					

Figure 4.

Immunophenotype of SMARCB1-deficient SNC. Each column represents one case as indicated in the top row. IHC results are color-coded according to legend.

Table 1.

Clinical summary of patients with SMARCB1-deficient sinonasal carcinoma.

	N=22	p value
Sex (n=23)		
Men	13 (59%)	
Age, median (range, years)	47.5 (24–95)	0.033
Women	9 (41%)	
Age, median (range, years) Stage	66 (35–83)	
T stage		
T1	0	
T2-T3	3 (14%)	
T4	18 (82%)	
Unknown	1 (5%)	
N stage		
N0	16 (73%)	
N1-N2	5 (23%)	
Unknown	1 (5%)	
M stage		
M0	19 (86%)	
M1	2 (9%)	
Unknown	1 (5%)	
Clinical stage		
I	0	
II-III	2 (9%)	
IV	19 (86%)	
Unknown	1 (5%)	
Treatment (n=18)		
SxCRT	7 (39%)	
CRT	5 (28%)	
SxRT	2 (11%)	
SxC	1 (6%)	
Sx	1 (6%)	
C	1 (6%)	
Unknown	1 (6%)	
Recurrence/metastasis (n=18)		
All	14 (78%)	
Local	7 (39%)	
Regional	4 (22%)	
Distant	11 (61%)	

Abbreviations: Sx, surgery; C, chemotherapy; RT, radiation therapy.

Table 2.

Genetic characteristics of SMARCB1-deficient SNC.

Test	Case ID	Age/ Sex	Histology	Gene	AA change	cDNA change	Variant class	Zygosity	OncoKB annotation	Broad chromosomal gains	Broad chromosomal losses
MSK-IMPACT	SN_23	54M	Basaloid and plasmacytoid/eosinophilic	<i>SMARCB1</i> <i>CDKN2A</i> <i>INPP4A</i>	n/a A57V Q550_L554del	n/a c.170C>T c.1650_1664del	Del Missense In frame del	n/a n/a n/a	Likely onc Unknown Unknown		
	SN_24	47M	Pseudoglandular/eosinophilic	<i>SMARCB1</i>	n/a	n/a	Del	Homozygous	Likely onc		
	SN_25	54M	Basaloid, clear cell and plasmacytoid/eosinophilic	<i>SMARCB1</i> <i>ETV6</i>	n/a R127W	n/a n/a	Del Missense	Hemizygous Diploid	Likely onc Unknown		
	SN_26	95M	Plasmacytoid/eosinophilic/rhabdoid with trabecular growth pattern	<i>SMARCB1</i> <i>PDGFRA</i> <i>PTPRS</i> <i>ATR</i>	n/a I989T I962V X1913_splice	n/a c.2966T>C n/a c.5739-3delACTT CCTT	Del Missense Missense Splice site	n/a n/a n/a n/a	Likely onc Unknown Unknown Unknown	7	
	SN_62	24M	Basaloid	<i>SMARCB1</i> <i>CHEK2</i> <i>MAPK1</i> <i>NF2</i> <i>TCF3</i> <i>PTPRD</i>	n/a n/a n/a n/a S359F R123K	n/a n/a n/a n/a c.1076C>T c.368G>A	Del Loss Loss Loss Missense Missense	Hemizygous Hemizygous Hemizygous Hemizygous Diploid Diploid	Likely onc Unknown Unknown Unknown Unknown Unknown		
	SN_63	33M	Basaloid	<i>SMARCB1</i> <i>MYCN</i> <i>CHEK2</i> <i>NF2</i>	n/a R383H n/a n/a	n/a c.1148G>A n/a n/a	Del Missense Loss Loss	Homozygous Diploid Hemizygous Hemizygous	Likely onc Unknown Unknown Unknown	1q	
	SN_74	43M	Basaloid	<i>SMARCB1</i> <i>BRCA2</i>	n/a Q1037K	n/a c.3109C>A	Del Missense	Homozygous Diploid	Likely onc Unknown		
	SN_75	66F	Plasmacytoid/eosinophilic/rhabdoid with glandular features	<i>SMARCB1</i> <i>CHEK2</i>	X265_splice n/a	c.795+2_795+44del n/a	Splice site Loss	CN-LOH CN-LOH	Likely onc Unknown	7	2q35-36 3q26-28

Test	Case ID	Age/ Sex	Histology	Gene	AA change	cDNA change	Variant class	Zygosity	OncKB annotation	Broad chromosomal gains	Broad chromosomal losses
	SN_76	79F	Plasmacytoid/ eosinophilic/ rhabdoid with glandular features	<i>CRKL</i>	n/a	n/a	Loss	CN-LOH	Unknown		
				<i>EP300</i>	n/a	n/a	Loss	CN-LOH	Unknown		
				<i>MAPK1</i>	n/a	n/a	Loss	CN-LOH	Unknown		
				<i>NF2</i>	n/a	n/a	Loss	CN-LOH	Unknown		
				<i>RAC2</i>	n/a	n/a	Loss	CN-LOH	Unknown		
				<i>PRKD1</i>	X329 splice	c.986-2A>C	Splice site	Diploid	Unknown		
				<i>MSH2</i>	X314_splice	c.943-1G>A	Splice site	Diploid	Unknown		
				<i>FH</i>	A200V	c.599C>T	Missense	Diploid	Unknown		
				<i>SMARCB1</i>	n/a	n/a	Deletion	Hemizygous	Likely onc	7	
				<i>CHEK2</i>	n/a	n/a	Loss	Hemizygous	Unknown		
	SN_78	53F	Pseudoglandular/ spindle cells	<i>CRKL</i>	n/a	n/a	Loss	Hemizygous	Unknown		
				<i>EP300</i>	n/a	n/a	Loss	Hemizygous	Unknown		
				<i>MAPK1</i>	n/a	n/a	Loss	Hemizygous	Unknown		
				<i>NF2</i>	n/a	n/a	Loss	Hemizygous	Unknown		
				<i>RAC2</i>	n/a	n/a	Loss	Hemizygous	Unknown		
				<i>PREX2</i>	n/a	n/a	Loss	Hemizygous	Unknown		
				<i>FUBP1</i>	A1284V intragenic del of exons 2-18	c.3851C>T n/a	Missense Intragenic del	Diploid n/a	Unknown Unknown		
				<i>SMARCB1</i>	n/a	n/a	Del	Homozygous	Likely onc		
				<i>CHEK2</i>	n/a	n/a	Del	Homozygous	Likely onc		
				<i>CRKL</i>	n/a	n/a	Del	Homozygous	Unknown		
	SN_81	26M	Basaloid with clear cell features	<i>MAPK1</i>	n/a	n/a	Del	Homozygous	Unknown		
				<i>NF2</i>	n/a	n/a	Del	Homozygous	Unknown		
				<i>TP53</i>	V157F	c.469G>T	Missense	Homozygous	Likely onc		
				<i>AR</i>	R841H	c.2522G>A	Missense	Diploid	Likely onc		
				<i>WHSC1</i>	V1287L	c.3859G>T	Missense	Diploid	Unknown		
				<i>SMARCB1</i>	n/a	n/a	Deletion	Homozygous	Likely onc		
				<i>CHEK2</i>	n/a	n/a	Loss	Hemizygous	Unknown		
				<i>CRKL</i>	n/a	n/a	Deletion	Homozygous	Unknown		
				<i>MAPK1</i>	n/a	n/a	Deletion	Homozygous	Unknown		
					n/a	n/a	Deletion	Homozygous	Unknown		

Test	Case ID	Age/ Sex	Histology	Gene	AA change	cDNA change	Variant class	Zygosity	OncoKB annotation	Broad chromosomal gains	Broad chromosomal losses
				<i>NF2</i>	n/a	n/a	Loss	Hemizygous	Unknown		
	SN_85	51F	Basaloid	<i>SMARCB1</i> <i>CTTNB1</i> <i>CDKN2AIP144RF</i> <i>CDKN2AIP16INK4A</i> <i>RAD51D</i>	Y44* S45F G125R W110* R127W	c.132C>G c.134C>T c.373G>A c.330G>A c.379C>T	Nonsense Missense Missense Nonsense Missense	CN-LOH n/a n/a n/a n/a	Likely onc Likely onc Unknown Likely onc Unknown		
FISH	SN_70	35F	Basaloid	<i>SMARCB1</i>	n/a	n/a	Del	Homozygous	n/a	n/a	n/a
	SN_71	76F	Plasmacytoid/ eosinophilic/ rhabdoid					Hemizygous			
	SN_73	53M	Basaloid					Homozygous			
	SN_77	37F	Basaloid					Homozygous			
	SN_79	69M	Pseudoglandular/ spindle cells					Homozygous			
	SN_84	48M	Basaloid with multinucleated giant cells					Homozygous			
	SN_86	71F	Pseudoglandular/ eosinophilic					Homozygous			
	SN_87	46M	Basaloid					Homozygous			
	SN_88	83F	Plasmacytoid/ eosinophilic/ rhabdoid								
n/a	SN_72	53M	Basaloid/spindle with sarcomatoid cells		n/a				n/a		

Abbreviations: SNC, sinonasal carcinoma; AA, aminoacid; del, deletion; onc, oncogenic; CN-LOH, copy neutral-loss of heterozygosity; n/a, not available