IDH2 Mutation Analysis in Undifferentiated and Poorly Differentiated Sinonasal Carcinomas for Diagnosis and Clinical Management

Cristina Riobello, MSc, Alejandro López-Hernández, PhD,* Virginia N. Cabal, BSc,* Rocío García-Marín, PhD,* Laura Suárez-Fernández, BSc,* Paula Sánchez-Fernández, MD,† Blanca Vivanco, MD, PhD,‡ Verónica Blanco, MD, PhD,‡ Fernando López, MD, PhD,† Alessandro Franchi, MD,§ José L. Llorente, MD, PhD,† and Mario A. Hermsen, PhD**

Abstract: A large number of tumor types arise from the mucosa of the sinonasal cavities. Although presenting clinically distinct behavior, due to poorly differentiated histologic features, they can be difficult to classify correctly. Our aim was to investigate whether *IDH2* and *IDH1* mutations may be specific to a subset of undifferentiated and poorly differentiated sinonasal carcinomas. A total of 125 tumor samples of 7 different histologic subtypes were analyzed for *IDH* mutations by sequencing and mutant-specific immunohistochemistry, and the results were correlated to clinical and follow-up data. The highest incidence of *IDH2* mutations occurred in sinonasal undifferentiated carcinoma, with 11/36 (31%) cases affected. However, also, 1/9 neuroendocrine carcinomas, 2/4 high-grade non–intestinal-type adenocarcinomas, and 1/8 poorly differentiated squamous cell carcinomas carried the *IDH2* mutation, whereas 1/48 intestinal-type adenocarcinomas harbored an *IDH1* mutation. Immunohistochemical analysis of mutant IDH1/2 produced a number of false-negative results, but also 1 false-positive tumor was found. Disease-specific survival was more favorable in *IDH2*-mutant versus wild-type cases. Our data suggest that *IDH*-mutant sinonasal cancers, independent of their histologic subtype, may represent a distinct tumor entity with less aggressive clinical behavior. Clinically, patients with these mutations may benefit from specific IDH-guided therapies.

Key Words: sinonasal undifferentiated carcinoma, sinonasal carcinoma, IDH1, IDH2

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From the *Department of Head and Neck Oncology, ISPA; Departments of †Otolaryngology; ‡Pathology, HUCA, Oviedo, Spain; and §Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy.

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Correspondence: Mario A. Hermsen, PhD, Department of Head and Neck Oncology, ISPA, IUOPA, CEBER-ONC, Edf. FINBA, N-1 F49, C/Avenida de Roma s/n, Oviedo 33011, Spain (e-mails: mhermsen@hca.es; mariohermsen@gmail.com).

The sinonasal tract harbors one of the histologically most diverse groups of neoplasms observed in the entire human body.[1] A consensus classification of the different sinonasal malignancies has been established,[2] but some classes still constitute a spectrum of entities, and some tumor types overlap each other. The understanding of the molecular events associated with the development of the different sinonasal neoplasms could improve this classification and identify new therapeutic options for patients. Both are much needed, as the 5-year survival rate of sinonasal cancer patients is still low, ranging between 20% and 70%, depending on the tumor type, despite advances in endoscopic surgery, precision radiotherapy, heavy ion radiotherapy, and histotype-driven induction chemotherapies.[1–4]

To date, a number of sinonasal tumor entities have been identified by genetic characteristics, such as NUT carcinoma,[5] Human papillomavirus (HPV)-related multiphenotypic carcinoma,[6] and SMARCB1-deficient carcinoma.[7] A number of publications have revealed the presence of *IDH2* hotspot mutations in up to 82% of undifferentiated carcinomas (sinonasal undifferentiated carcinomas [SNUCs]).[8–11]

IDH (isocitrate dehydrogenase) is involved in the Krebs cycle and has 3 isoforms; IDH1 is located in the cytoplasm, whereas IDH2 and IDH3 stay in the mitochondria. IDH1 and IDH2 catalyze α-ketoglutarate formation using NAD and NADP, respectively.[12] However, proteins of mutant *IDH1* and *IDH2* lose their normal enzymatic activity and acquire an abnormal activity that increases the level of the (R)-2-hydroxyglutarate (2-HG), which is structurally similar to α-ketoglutarate but cannot replace its function in the cellular metabolism.[8,13] Cells cannot metabolize 2-HG, and its accumulation in the nucleus induces DNA hypermethylation and interruption of lineage-specific progenitor cell differentiation into terminally differentiated cells.[14] Moreover, cells with increased 2-HG levels demonstrate a markedly reduced capacity for homologous DNA repair.[15]

IDH mutations that cause this enzymatic deregulation are restricted to specific, conserved, and functionally important arginine residues: R132 in the IDH1 protein, and R140 and R172 in the IDH2 protein.[16,17] These hotspot *IDH1*/*IDH2*–activating mutations have been identified in

many tumor types. *IDH1* mutations predominate in solid tumors, including gliomas, chondrosarcomas, and cholangiocarcinomas, whereas *IDH2* mutations are more frequent in hematological tumors, such as acute myeloid leukemia and angioimmunoblastic T-cell lymphoma.[18–22] The high frequency of *IDH* mutants in these tumor types makes it an attractive therapeutic target. Small molecules inhibiting mutant isoforms of IDH1 and IDH2 proteins can restore the presence of 2-HG to physiological levels and, to a certain extent, are capable of reversing some of the epigenetic and cell biological consequences.[23–25] Moreover, in SNUC, IDH mutation provides an opportunity to improve clinical management, currently restricted to surgical resection combined with postoperative (chemo)radiotherapy.[1] However, it is still unclear whether *IDH* mutations are exclusive to SNUC and could serve the classification of a specific subset of SNUC. The aim of our study was to identify *IDH1* and *IDH2* mutations and to evaluate the relation to the histologic subtype and to follow-up data in a heterogeneous series of undifferentiated and poorly differentiated sinonasal tumors.

MATERIALS AND METHODS

Tumor Samples

This study concerns 125 cases of 7 different sinonasal subtypes: 36 SNUCs, 9 sinonasal neuroendocrine carcinomas (SNECs), 19 olfactory neuroblastomas (ONBs), 48 intestinal-type sinonasal adenocarcinomas (ITACs), 4 high-grade non–intestinal-type sinonasal adenocarcinomas (HG-non-ITACs), 8 poorly differentiated sinonasal squamous cell carcinomas (PD-SNSCCs), and 1 NUT carcinoma. This cohort includes cases from Hospital Universitario Central de Asturias (HUCA), Oviedo, Spain; from VU Medical Center (VUmc), Amsterdam, The Netherlands; from University Medical Center Utrecht (UMC), Utrecht, The Netherlands; and from Florence, Italy. All experimental protocols were approved by the Institutional Ethics Committee of the Hospital Universitario Central de Asturias and by the Regional CEIC from Principado de Asturias (approval number: 66/15 for project PI15/01629 and 07/16 for project CICPF16008-HERM). All methods were carried out in accordance with the guidelines of the Institutional Ethics Committee of the Hospital Universitario Central de Asturias. Informed consent was obtained from all patients. The clinical characteristics of all 125 sinonasal tumors are summarized in Table 1.

Histologic Classification of Sinonasal Tumors

As the cases were collected over a long period of time and at different institutions, the initial diagnoses were revised by an experienced pathologist (A.F.). All the available slides were reviewed in each case, and tumors were diagnosed strictly applying the diagnostic criteria described in the fourth edition of the World Health Organization (WHO) Classification.[2] Additional immunohistochemical (IHC) stainings were applied when necessary. In particular, carcinomas showing no evidence of squamous or glandular differentiation and poorly differentiated squamous cell carcinomas were stained for cytokeratin (CK) 5/6, p40, p16, synaptophysin, chromogranin, NUT, SMARCB1, and SMARCA4. Those cases positive for p16 were further tested for HPV using

TABLE 1. Clinical Features of All Tumors

	n (%)						
	SNUC (N = 36)	**SNEC (N = 9)**	**HG-Non-ITAC (N = 4)**	**PD-SNSCC (N = 8)**	**ONB (N = 19)**	**ITAC (N = 48)**	**NUT ca (N = 1)**
Age, mean (range) (y)	57 (31-85)	58 (47-77)	40 (29-51)	61 (34-77)	45 (13-69)	68 (49-88)	40
Sex							
Male	19/35 (54)	3/8 (37)	3/4 (75)	6/7 (86)	6/16 (37)	48/48 (100)	0/1 (0)
Female	16/35 (44)	5/8 (62)	1/4 (25)	1/7 (14)	10/16 (62)	0/48 (0)	1/1 (100)
Tumor site							
Maxillary sinus	2/36 (5)	0/8 (0)	0/2 (0)	0/8 (0)	0/16 (0)	0/48 (0)	0/1 (0)
Ethmoid sinus	17/36 (47)	1/8 (12)	2/2 (50)	2/8 (25)	7/16 (44)	48/48 (100)	0/1 (0)
Sphenoid sinus	1/36 (3)	0/8 (0)	0/2 (0)	0/8 (0)	0/16 (0)	0/48 (0)	0/1 (0)
Nasal cavity	15/36 (42)	7/8 (87)	2/2 (50)	6/8 (75)	9/16 (56)	0/48 (0)	1/1 (100)
LNM	1/36 (3)	0/8 (0)	0/2 (0)	0/8 (0)	0/16 (0)	0/48 (0)	0/1 (0)
Stage							
T1+T2	12/35 (34)	4/6 (66)	2/4 (50)	5/7 (71)	7/13 (54)	35/48 (73)	1/1 (100)
T3+T4a+T4b	23/35 (66)	2/6 (33)	2/4 (50)	2/7 (29)	6/13 (46)	13/48 (27)	0/1 (0)
Patient status							
Alive	7/16 (44)	2/4 (50)	1/2 (50)	2/3 (67)	9/9 (100)	24/48 (50)	NA
DOD	7/16 (44)	2/4 (50)	1/2 (50)	1/3 (33)	0/9 (0)	17/48 (35)	NA
DOC	2/16 (12)	0/4 (0)	0/2 (0)	0/3 (0)	0/9 (0)	7/48 (15)	NA
Recurrence	6/18 (33)	4/4 (100)	1/2 (50)	0/2 (0)	5/10 (50)	20/48 (42)	NA
Metastases	8/18 (44)	2/4 (50)	1/2 (50)	2/3 (67)	1/9 (11)	2/48 (6)	NA
Follow-up, mean (range) (mo)	27 (1-97)	42 (11-118)	20 (1-39)	26 (12-46)	48 (0-172)	39 (0-220)	NA
DFS, mean (range) (mo)	24 (0-97)	21 (6-45)	6 (1-11)	26 (12-46)	37 (0-172)	7 (0-66)	NA

DFS indicates disease-free survival; DOC, died of other causes; DOD, died of disease; LNM, lymph node metastasis; NA, not available; NUT ca, NUT carcinoma.

TABLE 2. IHC Characterization of the Sinonasal Tumor Cohort

	SNUC (n = 36) (%)	SNEC (n = 9) (%)	HG-Non-ITAC (n = 4) (%)	PD-SNSCC (n = 8) (%)	ONB (n = 19) (%)	ITAC (n = 48) (%)	NUT ca (n = 1) (%)
Pancytokeratin	95	100	100	100	26	ND	100
CK5/6	0	ND	ND	100	ND	ND	ND
P40	0	ND	ND	100	ND	ND	ND
P16	27	0	0	12.5	0	ND	ND
HPV*	0	ND	ND	0	ND	ND	ND
Synaptophysin/ chromogranin	27	100	50	12.5	100	ND	100
NUT	0	0	0	0	0	0	100
SMARCB1 (INI-1)†	3	0	0	0	0	0	0
SMARCA4 (BRG1)†	0	11	0	0	0	0	0
CK20	ND	ND	100	ND	ND	ND‡	ND
CDX2	ND	ND	100	ND	ND	ND‡	ND

*HPV-DNA-PCR was performed only in cases with diffuse and nuclear p16 staining.
†SMARCB1 and SMARCB4 results reflect the absence of staining.
‡One case carrying IDH1 mutation was found CK20 weakly and CDX2 moderately positive.
ND indicates not done; NUT ca, NUT carcinoma.

DNA-polymerase chain reaction (PCR) (see below). Tumors showing evidence of glandular differentiation were stained for CDX2 and CK20 to assess intestinal differentiation.

As a result of these additional IHC analyses, 1 case originally diagnosed as SNUC was reclassified as NUT carcinoma, showing 100% nuclear and diffuse "speckled" immunopositivity. In addition, 1 SNUC was found to be SMARCB1-deficient and 1 SNEC was SMARCA4-deficient; however, guided by the fourth edition of the WHO Classification,[2] we kept these 2 cases classified as SNUC and SNEC, respectively. All tumors with diffuse and nuclear p16 staining were found to be HPV-negative. The diagnostic IHC results are included in Table 2. Twenty-seven of the 125 cases changed classification after histologic revision by the experienced pathologist (A.F.) aided by IHC stainings, highlighting the difficulty in the diagnosis of sinonasal tumors. Representative photomicrographs of hematoxylin and eosin and diagnostic IHC stainings of the main tumor types are given in Figure 1.

Immunohistochemistry

Tissue microarray (TMA) blocks were prepared from formalin-fixed, paraffin-embedded tumor tissues using the Beecher Tissue Microarray (Beecher Instruments, Silver Spring, MD). TMA blocks contained three 1 mm cores from different areas of each of the tumors. Each block included normal sinonasal mucosa samples as internal control. Tumors not included in the TMAs were stained separately. IHC was performed on an automatic staining workstation (Dako Autostainer Plus; DakoCytomation, Glostrup, Denmark) with antigen retrieval by EnVision FLEX+Mouse (DakoCytomation) for 20 minutes. The following antibodies were applied when necessary for tumor classification: mouse anti-CK clone AE1/AE3, mouse anti-CK20 clone K_s20.8, mouse anti-CDX2 clone DAK-CDX2, mouse anti-synaptophysin clone SY38, mouse anti-chromogranin A clone DAK-A3, mouse anti-CK5/6 clone D5/16 B4, mouse anti-p63 clone DAK-p63 (DAKO, Glostrup, Denmark), mouse anti-p40 clone BC-28, mouse CINtec anti-p16 clone E6H4 (Roche, Mannheim, Germany), rabbit anti-BRG1 clone ab70558 (Abcam, Cambridge, UK), rabbit anti-SMARCB1/BAF47 clone D8M1X, and rabbit anti-NUT clone C52B1 (Cell Signaling Technology, MA). Mutated IDH1 and IDH2 protein expression was determined by IHC with the multispecific antibody mouse anti-IDH1/2 mutant R132/172 clone MsMab-1 at a 1:100 dilution (Millipore, Darmstadt, Germany). Cases were considered positive for IDH staining when either strong or weak granular cytoplasmic staining was present in $>10\%$ of the tumor cells.

HPV-DNA Detection

The quality of the extracted DNA was checked by PCR amplification of β-globin (forward primer 5′-ACACAACTTGTGTGTTCACTAGC-3′ and reverse primer 5′-CAAACTTCATCCACGTTCACC-3′). PCR with MY11/GP6+ primers (site-directed L1 fragment of HPV) was performed to detect a broad spectrum of HPV genotypes (11,18). Briefly, the PCR was performed in 25 µl of reaction mixture containing 1x PCR buffer, 2 mmol/L MgCl2, 50 µmol/L of each deoxynucleoside, 0.5 µmol/L of sense and antisense primers, 10 µl of DNA sample, and 1 U Taq DNA polymerase (Promega Biotech Iberica S.L. Madrid, Spain), by thermal profile of 35 cycles: denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minutes, with an initial denaturation at 94°C for 5 minutes and a final extension at 72°C for 10 minutes. The amplified DNA fragments of ~200 bp were identified by electrophoresis in 1.5% agarose gel with ethidium bromide.

DNA Extraction and Sequencing of *IDH1* and *IDH2*

DNA of sufficient quality for sequencing could be obtained from 26 SNUC, 6 SNEC, 12 ONB, 48 ITAC, 2-HG–non-ITAC, and 2 PD-SNSCC samples. DNA was extracted using the Qiagen tissue extraction kit (Qiagen GmbH, Hilden, Germany).

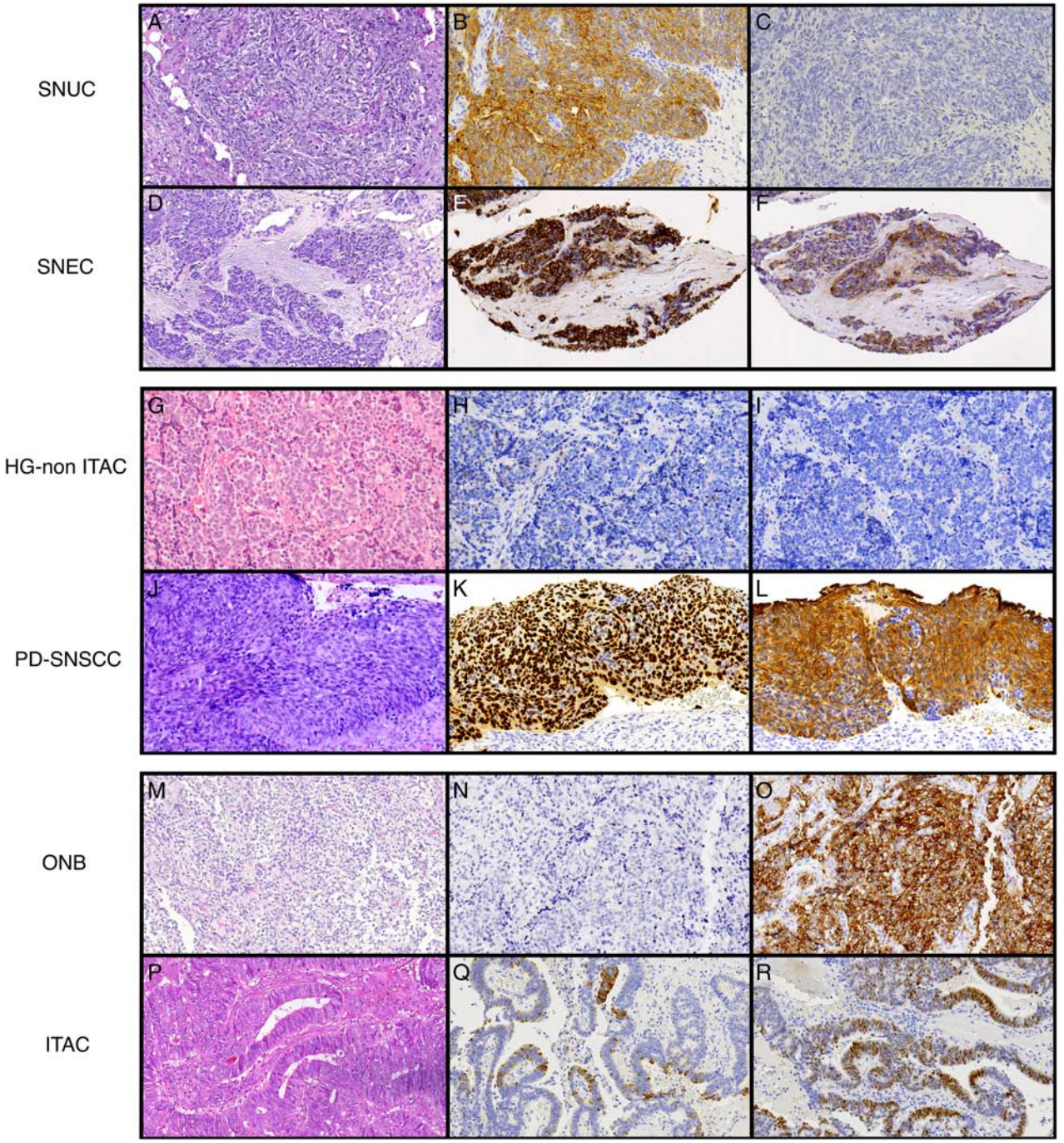

FIGURE 1. Diagnostic hematoxylin and eosin (H&E) and IHC stainings of representative cases from each tumor subtype. SNUC (A: H&E, B: CK AE1/AE3, C: synaptophysin), SNEC (D: H&E, E: CK AE1/AE3, F: synaptophysin), HG-non-ITAC (G: H&E, H: CK20, I: CDX2), PD-SNSCC (J: H&E, K: p63, L: CK5/6), ONB (M: H&E, N: CK AE1/AE3, O: synaptophysin), and ITAC (P: H&E, Q: CK20, R: CDX2) (All images ×200 magnification).

Exon 4 of *IDH1* and *IDH2* was sequenced to investigate the presence of hotspot mutations. The amplification of a 129 bp nucleotide fragment that includes *IDH1* exon 4 was performed with forward primer FW 5′-CGGTCTTCAGAGAAGCCATT-3′ and reverse primer RV 5′-GCAAAATCACATTATTGCCAAC-3′, and a 182 bp nucleotide fragment of *IDH2* exon 4 was performed with forward primer FW 5′-AGCCCATCATCTGCAAAAAC-3′ and reverse primer RV 5′-TGTGGCCTTGTACTGCAGAG-3′, run on a Simpliamp Thermal Cycler VXA24811 (Applied Biosystems, Foster City, CA). The PCR conditions were as follows: [5 min/95°C+(30″ 95°C/45″ 57°C/1 min 72°C)×32

TABLE 3. Results of IDH1/2 Exon 4 Sequencing and IHC Expression of Mutant IDH1/2

	n (%)		
Tumor Type	***IDH* Mutation**	**IDH Immunopositivity**	**Total**
SNUC	9/26 (35)	7/33 (21)	11/36 (31)
SNEC	0/6 (0)	1/7 (14)	1/9 (11)
HG-non-ITAC	1/2 (50)	2/4 (50)	2/4 (50)
PD-SNSCC	1/2 (50)	2/8 (25)	1/8* (12)
ONB	0/12 (0)	0/14 (0)	0/19 (0)
ITAC	1/48 (2)	NA	1/48 (2)
NUT ca	ND	0/1 (0)	0/1 (0)
Total	12/96 (13)	12/67 (18)	16/124 (13)

*One of the 2 *IDH*-mutated cases, as detected by IHC, was found to be wild-type by sequencing and considered false-positive.

NA indicates not available; ND, not done; NUT ca, NUT carcinoma.

cycles+7 min/72°C+4°C]. PCR products were purified with Exo-BAP Mix (EURx Ltd, Gdansk, Poland) and analyzed by Sanger sequencing using the ABI PRISM 3100 and 3730 Genetic Analyzer (Applied Biosystems).

In a parallel study (unpublished data), the 48 ITACs were analyzed by next generation sequencing (NGS) using a panel of 120 cancer-related genes with the SureSelect QXT Target Enrichment Kit for Ilumina Multiplexed Sequencing (Agilent Technologies, Santa Clara, CA). Sequencing pools were then sequenced in a MiSeq system (Ilumina Inc., San Diego, CA). The processing and filtering of raw sequencing data were performed by DREAMgenics (Oviedo, Spain). Those cases showing *IDH1* or *IDH2* hostpot mutations were confirmed by Sanger sequencing and IHC.

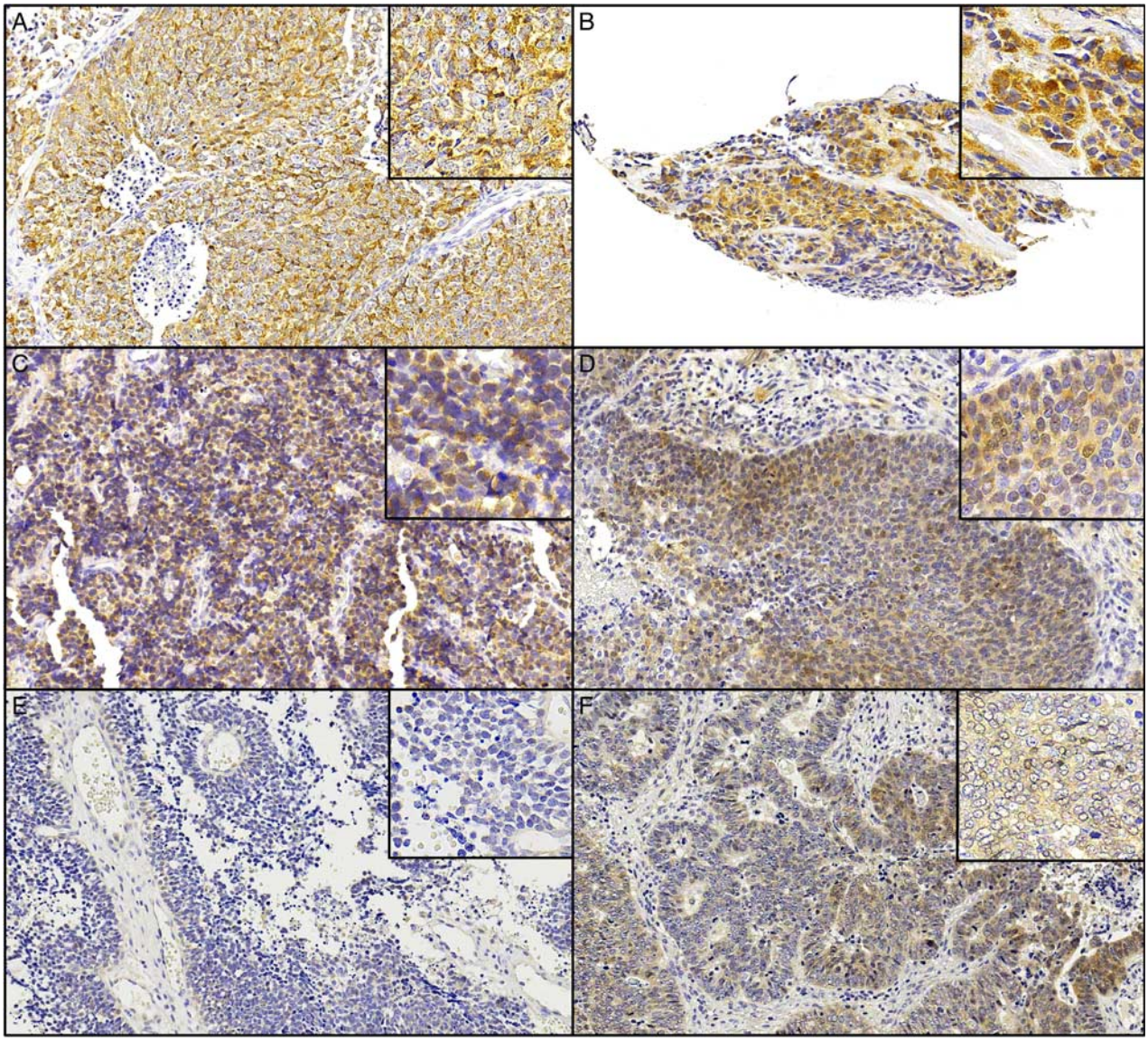

FIGURE 2. IHC analyses of mutated IDH1 and IDH2 by MsMab-1. All subtypes showed positive staining in at least 1 case except ONB. A: SNUC, B: SNEC, C: HG-non ITAC, D: PD-SNSCC, E: ONB; F: ITAC. All images ×200 magnification, insets ×400 magnification.

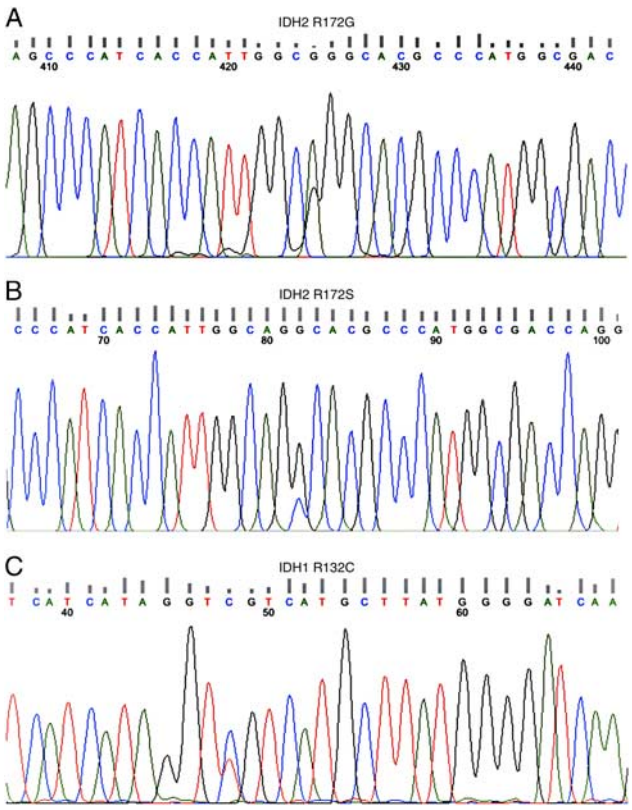

FIGURE 3. Electropherograms showing the 4 different *IDH1* and *IDH2* exon 4 mutations found in the sinonasal cohort. A, *IDH2* R172G. B, *IDH2* R172S. C, *IDH1* R132C.

Statistical Analysis

Possible correlations between IDH status and clinical parameters were statistically analyzed by SPSS 15.0 software for Windows (SPSS, Chicago, IL), using the Pearson χ^2 test, Fisher exact test, and Student *t* test. Kaplan-Meier analysis was performed for estimation of survival, comparing distributions of survival through the logarithmic range test (log-rank test). Values of $P < 0.05$ were considered significant.

RESULTS

Mutation Sequencing

Of the total of 96 sequenced tumors, 12 carried *IDH* mutations. Nine of 26 (35%) SNUCs, 1/2 (50%) HG-non-ITACs, and 1/2 (50%) PD-SNSCCs carried *IDH2* mutations, and 1/48 ITACs had a mutation in *IDH1* (Table 3). No *IDH* mutations were detected in ONB, SNEC, or NUT carcinoma. Among the *IDH2* sequence variants, 7 R172S were identified exclusively in SNUC, while R172G occurred in 4 tumors (2 SNUCs, 1 HG-non-ITAC, and 1 PD-SNSCC). One *IDH1* mutation was R132C, observed in a colonic type ITAC (Fig. 2).

IHC Analysis

Interpretable IHC stainings against IDH-mutant proteins were obtained from 115 tumors (Fig. 3), showing positivity in 7/33 (21%) SNUCs, 1/7 (14%) SNECs, 0/14 ONBs, 1/48 (2%) ITACs, 2/4 (50%) HG-non-ITACs, 2/8 (25%) PD-SNSCCs, and 0/1 (0%) NUT carcinoma (Table 3). Overall, IDH-mutant protein immunopositivity concurred with *IDH* mutation. However, 3 cases showed discrepancies. In 2 SNUCs identified as *IDH* mutants by Sanger sequencing (both R172S), the antibody did not show staining. Conversely, 1 immunopositive PD-SNSCC was found to be wild-type by exon 4 Sanger sequencing of *IDH1* and *IDH2* (Fig. 4). NGS panel sequencing of this case confirmed the Sanger sequencing results, but revealed an *IDH1* nonpathogenic change V178I.

Clinical-Pathologic Features in Relation to IDH Status

The tumor types included in this study carry different survival rates. Figure 5 shows that none of the 19 ONB patients died of disease in the 15 years of follow-up. The worst disease-specific survival was observed for SNEC and HG-non-ITAC.

Analyzing all cases with available follow-up information, we could find no correlation between *IDH2* mutation status (either detected by sequencing or IHC) and sex, age, localization, T-stage, or follow-up. However, when we considered only those tumor subtypes that had shown ≥ 1 cases with *IDH2* mutation (all except ONB, ITAC, and NUT carcinoma), we found that *IDH2*-mutated cases carried a significantly more favorable disease-specific survival than IDH2 wild-type (log-rank 6.303, $P = 0.047$). Furthermore, when we took only SNUC into consideration, *IDH2*-mutated cases appeared to have a better prognosis (log-rank 3.933, $P = 0.061$) (Fig. 5). In addition, mutations occurred more frequently (Fisher exact $\chi 2$, $P = 0.034$) in women than in men, respectively 8/16 (50%) and 3/20 (15%).

DNA Copy Number Profiles

The copy number aberration profiles of 29 SNUCs were available from our previously published study.[26] We compared gains and losses of 8 *IDH2*-mutant cases (by sequencing, IHC, or both) and 21 *IDH* wild-type cases. The result is shown in Figure 6. On a genome-wide level, the *IDH2*-mutant tumors appear to carry fewer deletions than the *IDH2* wild-type cases. Specifically, gains on chromosome arm 1q were found in 7/8 (88%) *IDH2*-mutant SNUCs, contrasting with 6/21 (29%) in the *IDH2* wild-type tumors ($P = 0.004$). In addition, combined loss of 17p and gain of 17q was more frequent in the *IDH2*-mutated cases.

DISCUSSION

Poorly differentiated sinonasal tumors are extremely difficult to classify. Particularly SNUC, first described by Frierson et al 1986,[27] represents a group of tumors without a clear consensus for diagnosis. ONB and SNEC can appear very similar and become mistaken for SNUC.[28,29] The 2017 WHO classification of head and neck tumors has defined SNUC as a highly aggressive tumor of uncertain histogenesis without evidence of squamous or glandular

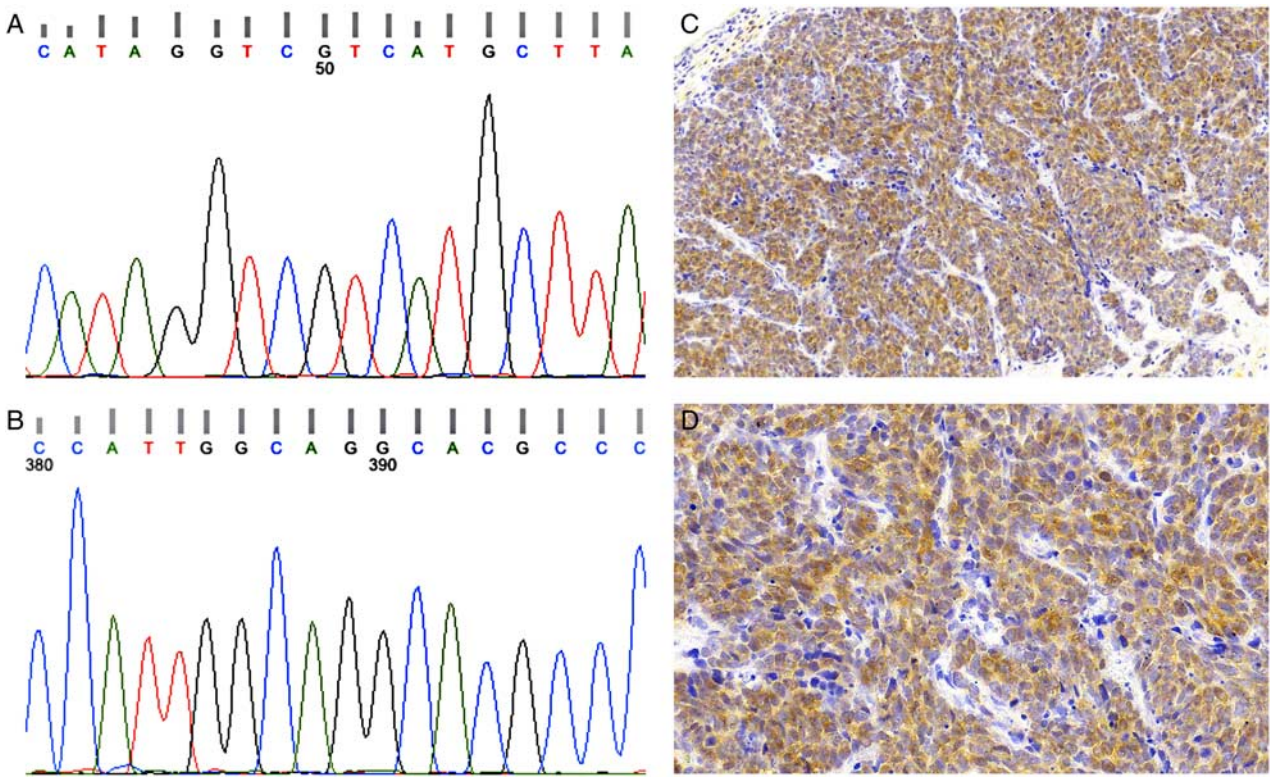

FIGURE 4. Absence of *IDH1* (A) and *IDH2* (B) hotspot mutations of a PD-SNSCC with discordant immunopositivity using (C, ×200 magnification, D, ×400 magnification) the MsMab-1 antibody against *IDH1/2* mutant R132/172.

differentiation, thus representing a category with a diagnosis of exclusion.[2]

An increasing number of tumor types are now being characterized by specific molecular-genetic markers. Recent findings have identified NUT (NUclear protein in Testis) carcinoma, defined by a chromosomal translocation involving the nuclear protein in testis gene (*NUT1*) at 15q14, and SMARCB1 (INI-1)-deficient sinonasal carcinoma, defined by the absence of SMARCB1/INI1 IHC staining, as new tumor entities, although for SMARCB1 (INI-1)-deficient sinonasal carcinoma it remains to be decided whether it constitutes an entity separated from SNUC.[2,5,7] The past 2 years have seen reports of frequent mutations in *IDH2* in up to 82% of SNUCs[8–10]; however, as the total number of tumors studied is still low, it is still not clear whether or not this alteration defines a histologically distinct subset of SNUC with different clinical behavior. In this study, we add 125 sinonasal tumors to the published literature, analyzing *IDH1* and *IDH2* by DNA sequencing and mutant protein expression and correlating the findings to clinicopathologic characteristics and follow-up data.

Our finding of 31% (11/36) *IDH2* mutations (detected either by sequencing or IHC) in SNUC is lower than those in previous studies, which ranged from 49% (26/53)[10] and 55% (6/11),[9] to 82% (14/17).[8] We also sequenced sinonasal tumors with histologies similar to SNUC, and detected *IDH2* mutations in 11% (1/9) SNECs, 50% (2/4) HG-non-ITACs, and 13% (1/8) PD-SNSCCs. ONB, NUT carcinoma, and the 2 cases with the absence of SMARCB1 or SMARCA4 expression were all *IDH* wild-type. Our results on IDH2 mutations in these tumor types additional to SNUC is in line with published data, although the reported mutation frequencies vary greatly. In their 2017 paper, Dogan and colleagues[8,30] found mutations in 25% (1/4) SNECs and, in their 2019 paper, in 83% (5/6). In contrast, Mito and colleagues found no mutations in 8 SNECs. HG-non-ITAC, also named poorly differentiated non-ITAC, has been reported to harbor *IDH2* mutations in 20% (2/10)[8] and in 38% (3/8) of poorly differentiated carcinoma that included tumors with glandular, neuroendocrine, and squamous morphology.[10] The problematical histologic classification of poorly differentiated tumors is probably responsible for the observed differences in mutation frequency of these cases. It may also explain that our present and published series[8,10,31,32] showed absence of *IDH* mutations in ONB, whereas 2 other studies did detect them in a subset of 11% to 17% of ONBs. As the ONB cases of the latter 2 studies expressed higher CK and lower neuroendocrine markers, an explanation is that they were probably misclassified.[11,33]

Our study is the first to present a PD-SNSCC with an *IDH2* mutation. This case showed immunopositivity for CK5/6 and p63 (Figs. 1K, L), confirming the histologic difference with SNUC.[34] Furthermore, we found 1 colonic type ITAC with mutation in *IDH1*. No *IDH1* or *IDH2* mutations have been described before in ITAC.[8,10] It is curious that

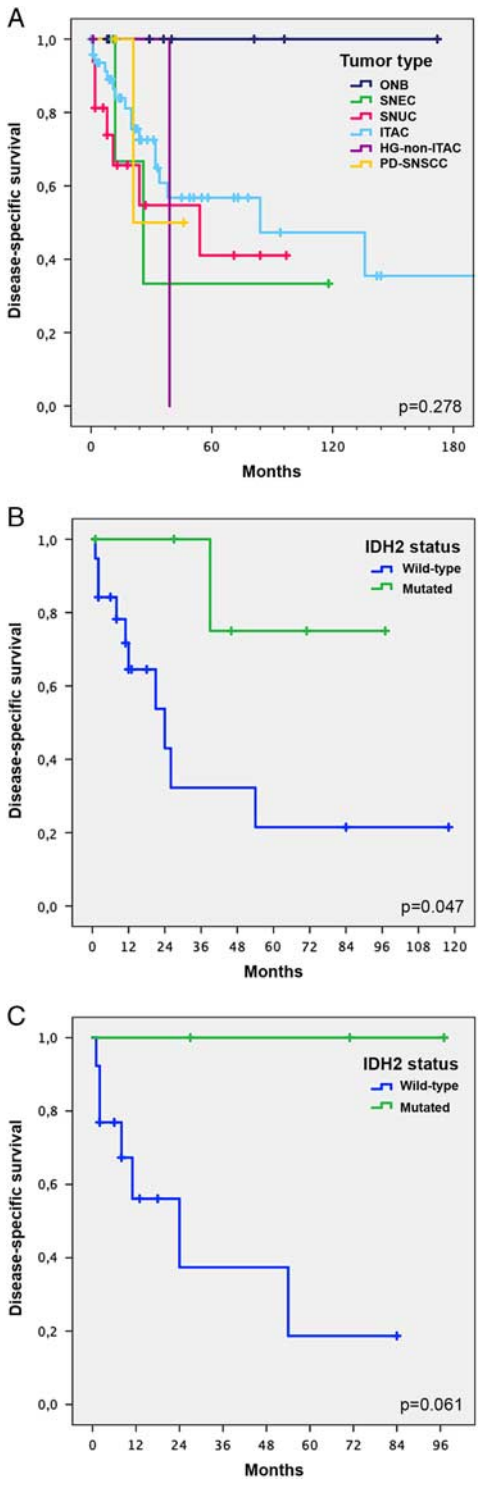

FIGURE 5. Kaplan-Meier survival analysis. A, Disease-specific survival according to sinonasal tumor type using data from 16 SNUCs, 4 SNECs, 9 ONBs, 48 ITACs, 2-HG–non-ITACs, and 3 PD-SNSCCs (no follow-up data were available for the NUT carcinoma case). B, Disease-specific survival of tumor subtypes that had shown ≥1 cases with *IDH2* mutation (SNUC, SNEC, HG-non-ITAC, and PD-SNSCC), according to IDH mutation status; 6 *IDH*-mutated versus 19 *IDH* wild-type. C, Disease-specific survival of SNUC according to *IDH* mutation status; 3 *IDH*-mutated versus 13 *IDH* wild-type.

well-differentiated tumors such as ITAC would carry this mutation, although this particular case demonstrated only few and focal CK20 and moderate CDX2 expression (Figs. 1Q, R), indicating loss of differentiation. Ward et al[35] suggested that *IDH1* mutations produce lower 2-HG levels and thus may produce a less undifferentiated phenotype than *IDH2* mutants.

From the 51 tumors studied both by sequencing and IHC, 4 were not in agreement: 3 cases were IDH-mutant immunonegative, while carrying the mutation, and 1 case was immunopositive in the absence of mutation. The false negatives confirm the lack of sensitivity of the MsMab-1 antibody to detect all IDH2 R172 variants.[10,17] In our series, it failed to stain 2 of 7 cases with R172S. A different anti-mutant-IDH antibody (11C8B1; NewEast Biosciences) consistently stained positive with R172S and R172T, but was ambiguous is IDH1 R132 and did not detect IDH2 R172G,[8] a variant that, in our present study, occurred in 4 tumors. These results indicate that sequencing is the best method to detect all *IDH* mutations. One false immunopositive case was confirmed as wild-type for codon 132 of *IDH1* and codons 140 and 172 of *IDH2* by NGS, but revealed a nonpathogenic variant at codon 178 of *IDH1* (V178I). In cell culture studies, this variant was shown to have normal IDH activity without increasing 2-HG levels Ward et al.[36]

Comparing 8 *IDH2*-mutated versus 21 nonmutated SNUC cases, the most outstanding copy number alterations in the *IDH2*-mutated tumors were gains on chromosome arm 1q and combined loss of 17p and gain of 17q (suggestive of isochromosome 17q), and loss of 22q. Interestingly, exactly these alterations were also found in 7 *IDH2*-mutated SNUC cases by Capper et al[11] Nevertheless, we cannot speculate about candidate genes in such large chromosomal regions. Three epigenetic studies agree that IDH2-mutant SNUC harbor strong global, but also a strong CpG island methylation,[11,30,33] similar to *IDH*-mutant gliomas.[37] Moreover, cluster analysis of methylation patterns grouped *IDH2*-mutated SNUC, SNEC, and HG-non-ITAC together.[30] Analogous to *IDH*-mutant glioma that is now regarded a separate tumor class from *IDH* wild-type glioma,[38,39] the shared genetic characteristics indicate that *IDH*-mutant SNUC and other *IDH*-mutant poorly differentiated sinonasal cancers might be considered as separate distinct tumor entities, despite differences in histology.

With regard to clinical management, our data and previous studies suggest that *IDH*-mutated tumors (particularly SNUCs) have a more favorable outcome, with longer disease-specific survival.[8,30] Important is also the possible therapeutic value of *IDH2* mutation. At present, there are several clinical trials with agents that either directly target IDH, or indirectly the effects of 2-HG accumulation. The FDA recently approved anti-IDH agents IDHIFA (Enasidenib, AG-221) and Tibsovo (Ivosidenib, AG-120) for patients with relapsed or refractory acute myeloid leukemia.[39] Decitabine, a hypomethylating agent that inhibits DNA methyltransferase to reactivate silent genes, has been approved for the treatment of myelodysplastic malignancies.[40] In addition, preclinical studies using *IDH*-mutated primary glioma cell lines have shown

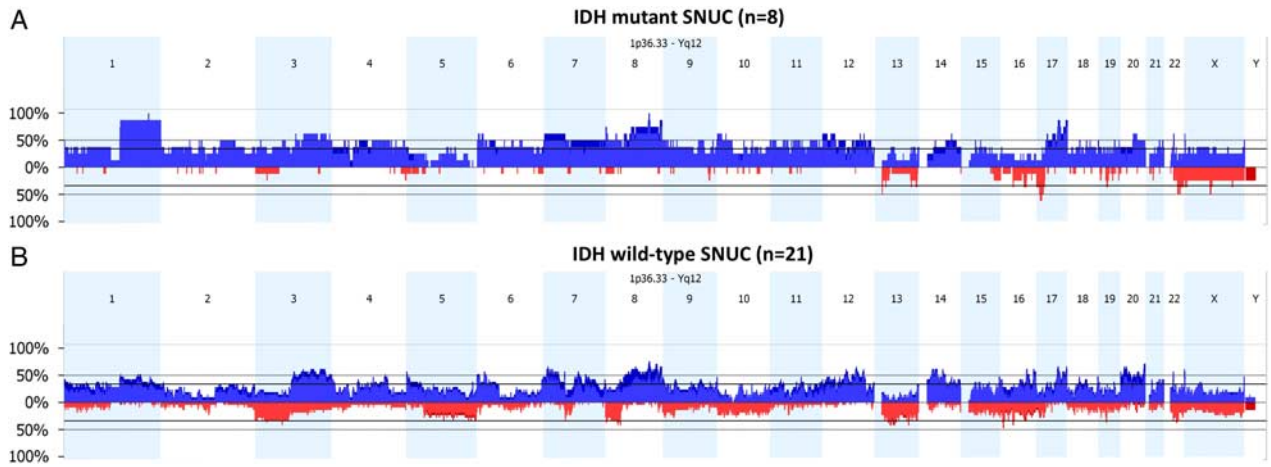

FIGURE 6. Copy number aberration profiles of 8 *IDH* mutant (A) versus 21 *IDH* wild-type SNUC (B). The percentage of cases with copy number gains and losses are given in blue and red, respectively.

sensitivity to PARP inhibitors, probably due to defective homologous recombination caused by *IDH* mutation.[15]

In conclusion, *IDH2* mutations occur frequently in SNUC and in a number of poorly differentiated sinonasal carcinomas. Although more studies are needed, the available genetic data suggest that *IDH*-mutant sinonasal cancers, independent of their histologic subtype, may represent a distinct tumor entity with less aggressive clinical behavior. Clinically, patients with these mutations may benefit from specific IDH-guided therapies.

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