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Original article

KRAS/NRAS variants and copy number alterations prognostically stratify patients with sinonasal melanoma

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Summary
Sino-nasal mucosal melanoma (SN-MM) is an aggressive and rare form of melanoma arising from mucosal melanocytes with pathogenesis unrelated to sun exposure. Conversely to cutaneous melanoma (CM), the molecular bases underling SN-MM development and progression are unclear, and no molecular predictive markers have been identified yet. To better define the molecular landscape of SN-MM, a retrospective series of 37 SN-MMs from 31 patients was analysed for both somatic mutations and cytogenetic alterations. The somatic mutation analysis identified the presence of a driver gene pathogenic variant in 54% of cases. In detail, mutually exclusive *NRAS* mutations were found in 42% of cases, *KRAS* mutations in 6%, and *KIT* mutations in 6% of cases. Remarkably, no *BRAF* mutations were detected. Patients with *NRAS*-mutated/*KRAS*-wild type (wt) melanomas showed better outcome than patients with *NRAS*-wt/*KRAS*-mutated melanomas, which were associated with multiple recurrences at local or regional sites. On the other hand, focusing on genomic alterations, copy number variants (loss of 1p36, loss of 3p/3q) were identified in 19% of SN-MMs, which showed poor overall survival and short disease-free survival with early metastatic dissemination. This work describes a new integrated characterization of both single nucleotide variants and, for the first time, genomic alteration in SN-MM, providing a new insight into molecular bases of these neoplasms and prompting further efforts for personalized clinical protocols according to tumour aggressiveness.

Key words: Sinonasal melanoma, molecular analysis, *KRAS*, *NRAS*, copy number alterations

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Introduction

Mucosal melanoma (MM) is an aggressive and rare form of melanoma arising from mucosal melanocytes with pathogenetic mechanisms unrelated to sun exposure [1]. Head and neck, in particular the oral cavity and the sinonasal tract (SN), represents the most common MM site [2]. SN-MM is the tumour type associated with the worst oncological outcome among paranasal sinuses neoplasms, due to its high tendency to recur and metastasize, regardless of stage at diagnosis [3]. At present, survival rates are dismal, with 5-year overall survival less than 30% [4 5]. Despite technological developments in surgery and improvement of radiation and systemic therapies, no increased survival is achieved [6]. The rarity of this neoplasm, the delay in diagnosis, its heterogeneity in growth pattern

(superficial spreading vs deep infiltration), and different sites of origin (nasal fossae, paranasal sinuses, nasal septum, nasopharynx) have hampered identification of specific prognostic factors [6]. Nevertheless, molecular bases underlying tumour initiation and progression are still unclear and there are not predictive molecular markers to use for specific target therapies. Conversely, molecular genetics of cutaneous melanoma (CM) and uveal melanoma (UM) are better characterized. Indeed, for CM a molecular classification able to identify different clinical subgroups based on mutations targeting *BRAF*, *NRAS,* and *NF1* has been proposed and currently used [7]. Analogously, cytogenetic abnormalities have largely been described in UM and used to stratify patients in different prognostic groups [8]. Chromosome 3 and chromosome 8 status are considered prognostic factors that strongly correlate with clinical outcome. Moreover, oncogenic variants have been identified in *GNA11*, *GNAQ* and *BAP1* genes [9].
To better define the molecular landscape of SN-MM, a retrospective series of 37 MM from 31 patients was analysed for both somatic mutations and cytogenetic alterations.

Materials and methods

Case series

This retrospective study includes 37 SN-MMs from 31 patients who referred to the University Hospital (ASST Sette Laghi) of Varese from 2000 to 2017. Only patients receiving surgical-based treatment with curative intent were included in the study. At least 12 months of follow-up for surviving patients was considered.
In detail, local extension of disease was assessed by means of multiplanar computed tomography (CT) scan and contrast-enhanced magnetic resonance imaging (MRI) in all cases. All patients underwent preoperative nasal endoscopy with multiple biopsies to define tumour histology before treatment. Surgical procedures were performed according to a previously described technique [3]. In detail, for all 31 patients, surgical resection was tailored on preoperative radiological findings with the aim to obtain a radical resection. The surgical procedures performed included an exclusive endoscopic endonasal resection (EER), endoscopic endonasal resection with transnasal craniectomy (ERTC), combined cranio-endoscopic approach (CER), craniofacial resection (CFR). Final histology report was used to define the surgical margin status (infiltrated versus uninvolved).
In adjuvant setting, post-operative radiotherapy (PORT) was delivered in cases with locally advanced stage of disease (stage pT4a and pT4b) and in cases with microscopically-involved surgical (20 out of 31 patients, Supplementary Table I). The irradiation modality included 3D-conformal radiotherapy (3D-CRT) and intensity-modulated radiotherapy (IMRT) for patients treated before the 2012 and carbon ion radiotherapy (CIR) for patients treated since that date. Concurrent cisplatin-based chemo-radiotherapy (POCHTRT) was performed only in selected cases of macroscopic persistence of disease in sites not further amenable for surgery and in case of early systemic dissemination of disease during the post-operative period or during the adjuvant irradiation delivery (3 out of 31 patients, Supplementary Table I).
All cases were re-staged according to the 8th edition of the American Joint Committee on Cancer (AJCC) tumour/node/metastasis (TNM) classification for mucosal melanoma of the head and neck [10]. All patients were followed according to a protocol that included monthly endoscopic examinations and MRI every 4 months during the first year; endoscopic examination and MRI every 2 and 6 months, respectively, during the second year; and both examinations at 6-month intervals thereafter, as previously described [3 5]. A total body PET-CT scan was prescribed once per year in order to rule out systemic dissemination of disease.
All histological slides were reviewed, and, for each case, the following parameters were evaluated: cell morphology, growth pattern, pigmentation, presence of necrosis, vascular invasion, and local infiltration. For each sample, immunohistochemical staining including MelanA, HMB45, S100, and SOX10 were performed.
The study was approved by the Institutional Review Board (Insubria Board of Ethics, approval number 0033025/2015) and performed in accordance with the Helsinki declaration and its later amendments. Informed consent was obtained from all participants.

Gene mutation analysis by MALDI-TOF Mass Spectrometry

Tumour DNA was extracted from three representative 8µm-thick sections obtained from 37 formalin fixed and paraffin embedded (FFPE) samples available for the molecular analyses and neoplastic areas were manually microdissected in order to have at least 50% of tumour cells. DNA was extracted by using Maxwell® DNA FFPE Kit and Maxwell 16 system (Promega, Madison, Wisconsin, USA) according to the manufacturer's protocol. Each sample was quantified using Qubit dsDNA High Sensitivity Assay kit (Invitrogen, Thermo Fisher Scientific, USA). Genotype analysis of four genes, namely *KRAS*, *BRAF*, *NRAS* and *PIK3CA* was performed by MassARRAY® System (Agena Bioscience™, Hamburg, Germany) using Myriapod

Colon kit as recommended by the manufacturer (Diatech Pharmacogenetics, Jesi, Italy). A total of 184 mutations (Supplmentary Table II) were tested in 8 multiplex reactions for each tumour. The spectra were processed using SpectroACQUIRE software (Sequenom) and genotype calls are automatically generated using mathematical algorithms by the MassARRAY Typer 4.0 software (Sequenom). Dossier software (Diatech Pharmacogenetics) was used for automated data analysis, accompanied by visual inspection of extension products.

Targeted next generation sequencing (NGS) analysis

A gene-targeted NGS analysis was performed on a subset of 18 DNA samples, for which a good quality DNA was available, using the Human Actionable Solid Tumor Mutations QIAseq DNA Panel (DHS-101Z, Qiagen, Hilden, Germany) that analyses 22 oncogenes (*BRAF, PDGFRA, EGFR, KRAS, NRAS, KIT, AKT1, ALK, CTNNB1, ERBB3, ESR1, FOXL2, GNA11, GNAQ, IDH1, IDH2, MET, RAF1, RET, ERBB2, PIK3CA, TP53*). A targeted amplicon-based library was constructed as previously described by our group [11 12] according to the manufacturer protocol. Barcoded libraries were pooled together at 8pM and sequenced on an Ion S5 XL System (A27214, Thermo Fisher Scientific) using Ion 530 chip (Thermo Fisher Scientific). Unmapped BAM (uBAM) files were imported into the CLC Genomics Workbench (Qiagen Bioinformatics, Germany, version 12) and mapped on the Human hg19 genome. Sequencing data were analysed using the Biomedical Genomics Analysis plugin and filtered ensuring a coverage of at least 100X and a variant allele frequency (VAF) higher than 5%.

Multiplex Ligation-dependent Probe Amplification Assay specific for Uveal Melanoma abnormalities

Deletions/duplications analysis of regions in chromosomes 1p, 3, 6 and 8 was performed using SALSA MLPA (Multiplex Ligation-dependent Probe Amplification) probemix P027 Uveal Melanoma (MRC-Holland, Amsterdam, Netherlands) according to the manufacturer's protocol. Electrophoresis of the amplified products was performed with a SeqStudio Genetic Analyser and the electropherograms were checked with GeneMapper Software version 6 (Thermo Fisher Scientific). Output data were analysed comparing the samples with three healthy controls using Coffalyser.net MLPA Analysis Software (MRC-Holland). The cut-off values used to evaluate gene/exon imbalances were 0.8 and 1.2 for loss and gain of signal, respectively.

Statistical Analysis

Statistical analysis was performed using Pearson chi2-test for groups comparison, while the Kaplan-Meier method was used to estimate the Overall (OS) and Disease-Free Survival (DFS) survival probability with Greenwood standard errors. Survival was estimated and compared based on log rank test. A multivariate proportional hazard Cox-regression model was implemented for the same endpoints (OS and DFS). Age at diagnosis, as a continuous variable, was analysed using univariate Cox models. Results are shown in term of hazards ratios (HR), 95% confidence intervals (CIs), and p values. All statistical tests were two-tail and p values were considered significant when ≤ 0.05. Pearson chi2-test was performed using GraphPad v.5.0 software (GraphPad Software Inc, San Diego, CA, USA) and survival analyses were performed using IBM SPSS statistical software, version 25 (Chicago, IL, USA).

Results

Clinical and histopathological results

In Table I and Supplementary Table I are reported the clinico-pathological characteristics of the series. After a mean follow-up of 41.1 months (range, 3-206 months; median, 17 months), 6 patients (19.4%) were alive without evidence of disease (NED), 2 (6.4%) were alive with disease (AWD), 22 (71%) died of disease and 1 (3.2%) died from other causes (DOC). Interestingly, independently from treatment administrated, 10 patients out of 31 patients showed a significant longer overall and disease-free survival (median OS 206 months and median DFS of 51 months, respectively) than that observed for the remaining 21 patients (p-value < 0.0001 for both test) and were classified as "long survival". Nine patients developed multiple melanomas which were operated in different surgical sessions: multiple samples were available for molecular analysis for four patients of this subset. In detail, two asynchronous melanomas were available for case#3, case#16 and case#21 and four different melanomas were tested for case#20.

The prevalent histotype recognized was the epithelioid one (25/37, 67.6% Fig. 1A), followed by mixed type consisting of both epithelioid and spindled cells (6/37, 16.2% Fig. 1B) or epithelioid and pleomorphic (6/37, 16.2% Fig. 1C). The pattern of growth was evaluable in 36 out of 37 SN-MMs and the main architectures were diffuse (16/36, 44.4% Fig. 1D) and mixed diffuse and pseudopapillary pattern (16/36, 44.4%); 3 cases showed pseudopapillary pattern of growth alone (8.4%, Fig. 1E) and only 1 case displayed a mixed diffuse and alveolar architecture (2.8%, Fig. 1F). Mel-

anin pigmentation was evaluable in 35/37 SN-MMs: in 10 cases no pigmentation was present (28.6%), 11 cases presented focal pigmentation (rare and interspersed positive cells, 31.4%, Fig. 1G) and 14 SN-MMs showed zonal pigmentation (positive areas, 40%, Fig. 1H). Intra-tumoral necrosis was present in 28/36 SN-MM (77.8%, Fig. 1I) and was significantly associated with poor prognosis (chi^2-test $p = 0.0461$, Tab. I). Finally, the level of tissue invasion was evaluable in 35 SN-MMs showing invasion of sub-epithelial connective tissue in 21 cases (60%, level II of invasion, Fig. 1J), whereas 14 cases showed level III invasion of the deep-tissue (40%, Fig. 1K). Mucosal ulceration was observed in 21/32 SN-MM (65.6%, Fig. 1L).

Gene mutation analyses

Mutation analysis of *KRAS*, *BRAF*, *NRAS* and *PIK-3CA* genes was possible in 36 out of 37 SN-MMs from 30 patients by using MassARRAY® System. Fifteen out of 36 cases (41.7%) showed at least one pathogenic variant within one of the 4 analysed genes, as reported in Supplementary Table I. In detail, in 13 SN-MMs (42%) a pathogenic *NRAS* variant was identified in codon 12 (8 melanomas) or codon 61 (5 cases), while two cases showed *KRAS* mutations (6%). Additionally, 18 out of 36 SN-MMs were also tested with

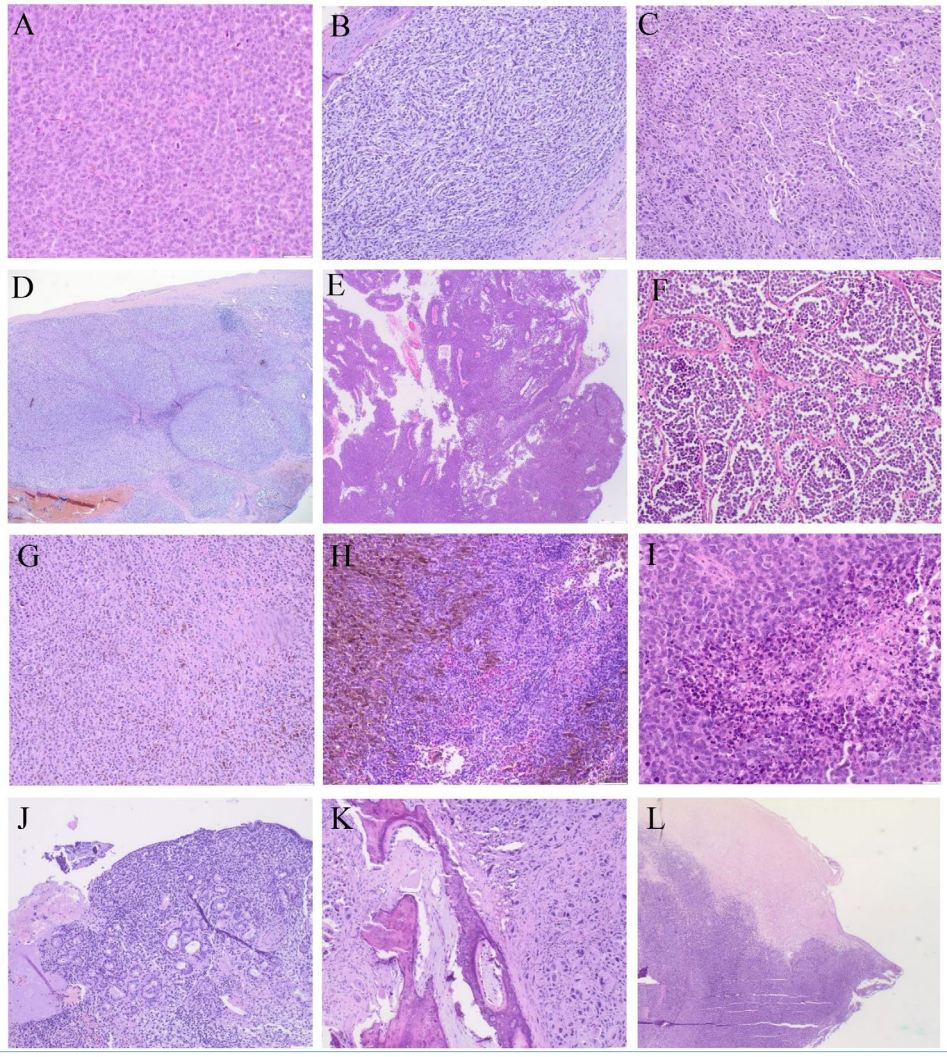

Figure 1. Histopathological features. Cell morphology: examples of epithelioid cells (1A; EE 10X), spindled cells (B; EE 10X), pleomorphic cells (C; EE 10X); growth pattern: diffuse (D; EE 4X), pseudopapillary (E; EE 4X) or alveolar (F; EE 20X); pigmentation type: focal (G; EE 10X) and zonal (H; EE 10X); example of intra-tumoral necrosis (I; EE; 10X); example of level II (J; EE 4X) and III (K; EE 10X) tissue invasion; example of ulcerated SN-MM (L, EE 10X).

NGS approach, which analysed other 18 oncogenes in addition to *KRAS*, *BRAF*, *NRAS* and *PIK3CA*. Synonymous or variants listed in 1000 Genome Project were filtered out. As summarized in Supplementary Table I, all variants identified with MassARRAY® System were confirmed with NGS analysis. Moreover, cases MN#27 and MN#48 showed a pathogenic mutation in *cKIT* gene (p.Asn822Tyr and p.Leu576Pro,

Table I. Clinicopathological features of 37 sino-nasal melanomas from 31 patients.

	All cohort	Long survival	Poor survival	p-value
Number of patients	**31**	**10**	**21**	
Age of onset (mean)	68 years	67.3 years	68.2 years	ns
Patients with multiple melanomas	9	6	3	
Gender				ns
Male	15	4	11	
Female	16	6	10	
Number of melanomas	**37**	**15**	**22**	
Site				
Naso-ethmoid	28	13	15	
Maxillary	6	2	4	
Naso-pharynx	2	0	2	
Mesentery metastasis	1	0	1	
Cell morphology				ns
Epithelioid	25	10	15	
Mixed (epithelioid and spindled/pleomorphic)	12	5	7	
Architecture				ns
Diffuse	16	6	10	
Pseudopapillary	3	2	1	
Mixed (diffuse and alveolar/pseudopapillary)	17	7	10	
Not evaluable	1	0	1	
Pigmentation				ns
Present/Zonal	14	7	7	
Absent/Focal	21	8	13	
Not evaluable	2	0	2	
Level of Infiltration				ns
I	0	0	0	
II	21	10	11	
III	14	5	9	
Not evaluable	2	0	2	
Necrosis				***0.0461***
Present	28	9	19	
Absent	8	6	2	
Not evaluable	1	0	1	
Treatment				ns
Surgery alone	8	1	7	
*Surgery and PORT/POCHTRT**	23	9	14*	
Overall survival				
Median survival (months)	17	206	13	***< 0.0001***
Died	23	4	19	***0.0059***
Alive	8	6	2	
Disease free survival				
Median survival	9	51	5	***< 0.0001***
Relapsed	25	5	20	***0.0075***
Disease free	6	5	1	

Legend: PORT, post-operative radiotherapy; POCHTRT, post-operative chemo-radiotherapy; *plus chemotherapy.

Table II. Cox regression model for Overall Survival and Disease-Free Survival.

Variable	*OS*			*DFS*		
	HR	**HR 95%**	**p-value**	**HR**	**HR 95%**	**p-value**
Age (continue variable)	1.01	0.96 – 1.05	0.65	1.02	0.97 – 1.06	0.37
NRAS (wt vs mutated)	6.40	1.45 – 28.17	**0.01**	2.55	0.82 – 7.92	0.10
1p-3p/3q-8p LOSS (yes vs no)	9.12	2.19 – 37.97	**0.002**	3.58	1.08 – 11.87	**0.03**

Legend: OS overall survival; DFS disease-free survival; HR hazard ratio; wt wild type; vs versus.

respectively). Interestingly, case MN#4A and case MN#22B had two concomitant pathogenic variants in *NRAS* and in *PIK3CA* (p.Arg524Lys) and *CTNNB1* (p.Thr41Ala), respectively. Conversely, *NRAS*, *KRAS* and *KIT* variants were mutually exclusive events. Remarkably, no *BRAF* alterations were observed in any case. The mutation analysis of the four patients who experienced multiple local recurrences (patient 3, 16, 20 and 21) showed different types of *NRAS* variants in different samples of recurrent disease (MN4A-B, MN22A-B, MN30A-D and MN32A-B, respectively). Of note, the four melanomas (MN#30A, MN#30B, MN#30C and MN#30D) developed by patient 20 showed three different *NRAS* variants, namely *NRAS* p.Gly12Arg (MN30A and MN30B), *NRAS* p.Gln61His (MN30C) and *NRAS* p.Gly12Asp (MN30D).

The prognostic impact of the gene mutations found in this case-series was analysed in univariate analysis. *NRAS* and *KRAS* mutations showed an association with prognosis (Fig. 2), whereas *KIT* did not show any

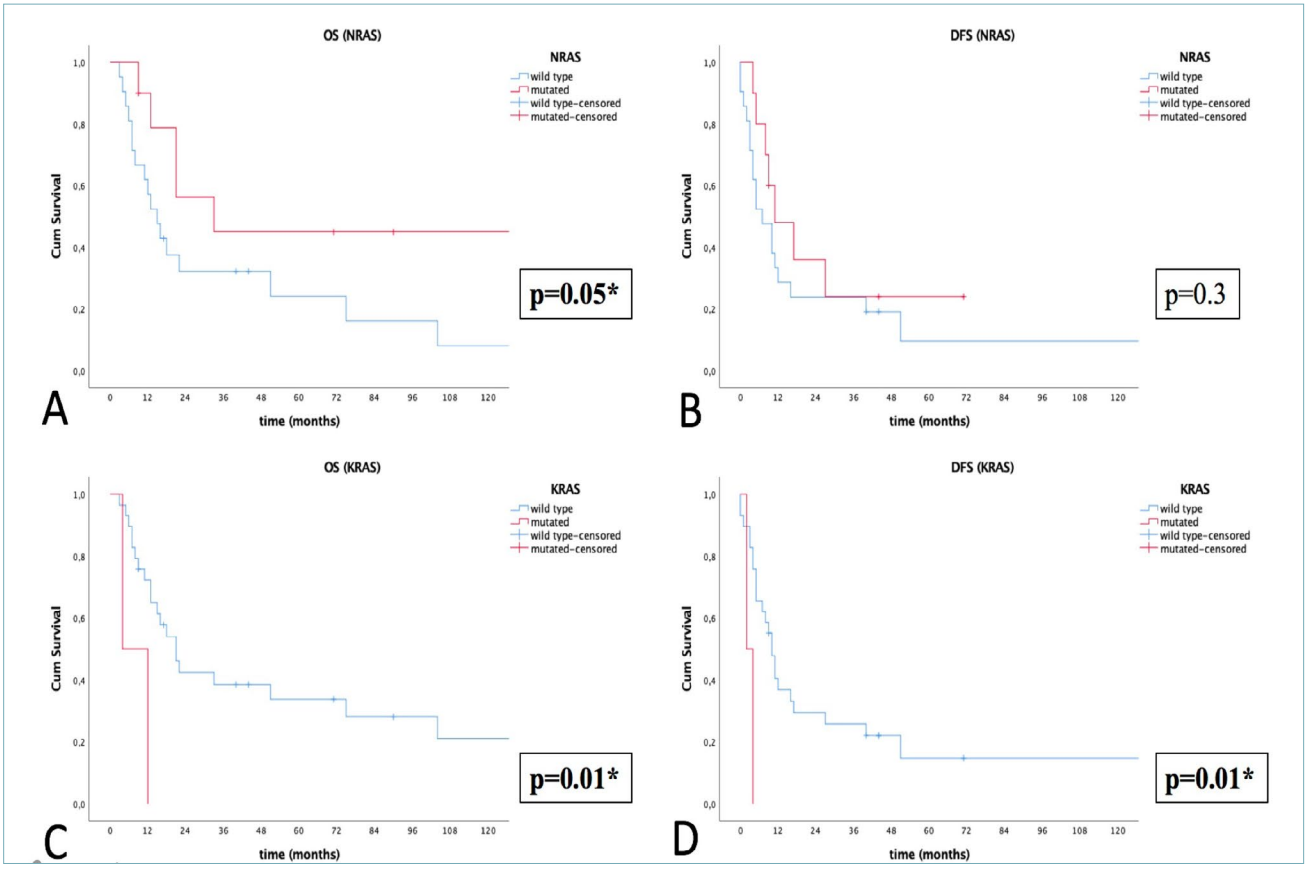

Figure 2. Prognostic impact of gene mutations in 31 patients affected by SN-MM. (A) Overall Survival (OS) analysis comparing *NRAS* mutated cases (red) with *NRAS* wild-type cases (blue, *p-value = 0.05*); (B) Disease-Free Survival (DFS) analysis comparing *NRAS* mutated cases (red) with *NRAS* wild-type cases (blue, *not significant p-value*); (C) Overall Survival (OS) analysis comparing *KRAS* mutated cases (red) with *KRAS* wild-type cases (blue, *p-value = 0.01*); (D) Disease-Free Survival (DFS) analysis comparing *KRAS* mutated cases (red) with *KRAS* wild-type cases (blue, *p-value = 0.01*).

prognostic role in our series. *NRAS* mutated cases had increased OS rates ($p = 0.05$) compared to *wild type* cases, but this difference was not found in terms of DFS ($p = 0.3$). On the other hand, *KRAS* mutations were associated with worst prognosis both in terms of OS ($p = 0.01$) and DFS ($p = 0.01$).

Multiplex Ligation-dependent Probe Amplification Assay for Uveal Melanoma

MLPA assay for the detection of deletions or duplications in chromosomes 1p, 3, 6 and 8 obtained suitable results in 26 cases, with a specific pattern of copy number alteration (CNA) as represented in Figure 3. In details, the most frequently observed alterations

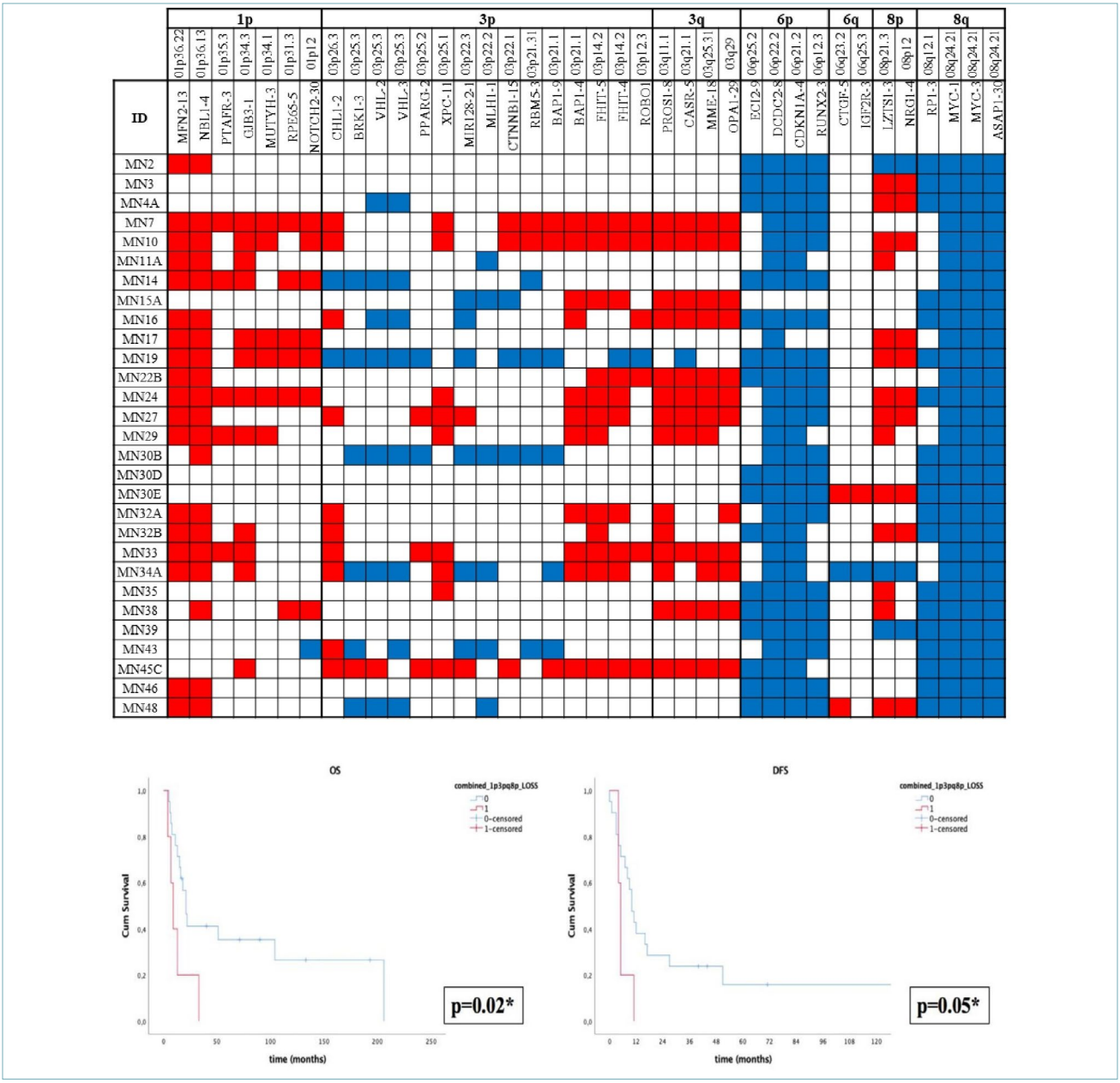

Figure 3. Schematic representation of genomic alteration of 26 SN-MM (upper panel). Each column indicates a specific chromosomic region, each raw indicates a single SN-MM analysed. Red square indicates loss of the chromosomic region; blue square indicates duplication of the chromosomic region; white square indicates no copy number alteration. Prognostic impact of the Copy Number Alteration (CNA) pattern characterized by 1p36 loss, 3p/3q loss and 8p loss in 26 patients affected by SN-MM (lower panels). On the left, Overall Survival (OS) analysis comparing the presence of the CNA pattern (red) with the absence of CNA pattern (blue, *p-value = 0.02*); on the right, Disease-Free Survival (DFS) analysis comparing the CNA pattern (red) with the absence of CNA pattern (blue, *p-value = 0.05*).

were the gain of 6p (25/26 cases, 96.1%) and the gain of 8q (26/26 cases, 100%), which could be considered as a distinctive signature of SN-MM. Loss of 1p36 was observed in 18/26 (69.2%) cases and it was associated with poor prognosis, with statistically significant values both in terms of OS ($p = 0.006$) and DFS ($p = 0.01$). Loss of 3p and/or 3q was observed in 11/26 (42.3%) cases and it was associated with poor prognosis, with statistically significant values both in terms of OS ($p = 0.004$) and DFS ($p = 0.009$). Loss of 8p was observed in 10 (38.4%) cases and it was associated with poor prognosis, with a statistically significant value only in terms of OS ($p = 0.003$) but not for DFS ($p = 0.14$). Gain of 3p and/or 3q was observed in 7/26 (26.9%) cases but no statistically significant associations with prognosis were found in terms of OS and DFS. Globally, the MLPA CNA pattern characterized by 1p36 loss, 3p/3q loss and 8p loss (5/26 cases, 19.2%) was found to be a strong negative prognosticator, with statistically significant worse outcomes both in terms of OS ($p = 0.02$) and DFS ($p = 0.05$), as shown in Figure 3. Finally, a Cox regression model was implemented in order to identify independent prognostic factors in MM (Tab. II). *Wild-type NRAS* status was associated with worse prognosis and increased risk of overall death (HR = 6.40, $p = 0.01$) while the combined loss of 1p, 3p/3q and 8p was associated with and increased risk of overall death (HR = 9.12, $p = 0.002$) and increased of recurrence (HR = 3.58, $p = 0.003$). It is worth noting that *KRAS* mutations, which were previously identified as strong negative prognostic marker in univariate analysis, were not statistically significant in the multivariate setting, indicating that was not an independent prognostic factor.

Discussion

Both basic and drug discoveries research in CM have achieved enormous progress, whereas little is known about the genetic alterations which characterize SN-MM cancerogenesis that may be considered to improve targeted therapies. As a result, patients affected by SN-MM are suffering from limited treatment options and undesirable response rates that lead to ominous prognoses. Data currently available in literature on clinical trials have produced sparse and conflicting results, which seem to support a limited efficacy of therapies currently in use for CM when extended to SN-MM patients. Therefore, extensive analysis of genetic landscape of SN-MM is urgently required to find biomarkers able to predict prognosis and to identify patients who can benefit from target therapy.

In this context, we have retrospectively analyzed a cohort of 31 patients affected by SN-MM in order to describe their clinical course and to investigate their genetic profile. In this study, we investigated genetic alterations that are associated to CM, such as *BRAF, NRAS, c-KIT* variants, together with genomic alterations involved in tumorigenesis of UM such as chromosome 1p36 loss, chromosome 3 monosomy, chromosome 6 aberrations and chromosome 8q gain. Our analysis has shown that SN-MM has a unique pattern of alterations, including both gene variants and copy number alterations.

First of all, the somatic mutation analysis identified a driver gene pathogenic variant in 54% of SN-MMs, a percentage considerably lower compared to that observed by TCGA consortium for CM (more than 80%) [7]. In detail, in our cohort, we found mutually exclusive *NRAS* mutations in 42% of cases, *KRAS* mutations in 6% of cases and *KIT* mutations in 6% of cases. Remarkably, in line with other literature data [13] no *BRAF* mutations, which are involved in the classical molecular profile of CM, were found, strongly argues against the clinical utility of BRAF inhibitors in such group of patients. These results, besides clarifying the molecular pathogenesis of SN-MM, prompt some considerations about therapeutic options for these patients. First, *NRAS* mutations, although considered as undruggable targets until recently in melanoma, are emerging as potential actionable driver mutations using MEK inhibitors target therapy and PI3K-AKT-mTOR inhibitors associated with CDK4/6 inhibitors [14,15]. Analogously, *KRAS* was considered a not actionable marker, but recent discoveries have led to the clinical use of several inhibitors that target the p.Gly-12Cys mutation in non-small cell lung cancer [16,17]. In this series, all *KRAS* variants were p.Gly12Ala. However, due to the small number of the cohort, further studies are required to search for p.Gly12Cys in larger series of SN-MM. Finally, the role of *KIT* as marker for target therapy is well known in GIST and, recently, in metastatic CM [18,19].

Our survival analysis suggests a relevant prognostic role of *NRAS* and *KRAS* variants. In detail, the occurrence of *NRAS* mutations was associated with an improvement in OS (p = 0.05) while *KRAS* mutations was correlated with worst OS (p = 0.01) and DFS (p = 0.01). Despite the dismal overall prognosis of such cancer, the occurrence of *NRAS* or *KRAS* mutation might be used in the future to stratify patients in “long-survivors” (*NRAS*-mutated/*KRAS*-wt), who can experience multiple recurrences at local or regional sites but late systemic dissemination of disease, and “short-survivors” (*NRAS*-wt/*KRAS*-mutated) with early metastatic spread of disease and OS rates less

than 3 years. Although other molecular markers, not investigated in this study, could be identified from genome-wide analysis on lager cohorts, this study highlighted the therapeutic impact of *NRAS*, *KRAS* and *KIT* mutations and the prognostic impact of *NRAS* and *KRAS* mutations, which should be analyzed and incorporated into routine clinical testing for SN-MM in the precision medicine era.

Interestingly, our study identified the occurrence of different genetic alterations during the follow-up time of SN-MM disease in the same patient (patient 20), which can be explained by two hypotheses. One hypothesis can be related to therapy, as it has been reported that radiochemotherapy and/or immunotherapy can switch or select specific tumor clones with different driver mutations in few cases of SN-MMs [20]. Indeed, this patient was exposed to intensity modulated radiotherapy and multiple resections. A second explanation, less likely, is the co-occurrence of multiple primary tumors [21]. However, the cancer site of onset was spatially located always in the same region and no family history of cancer was identified for this patient. Nevertheless, these findings are relevant and provide an important rationale for longitudinal molecular testing of SN-MM, based on the evidence of unforeseen recurrent event of molecular switch in progressing SN-MM.

Finally, focusing on genomic alteration and on chromosomal instability, the analysis of specific copy number alterations in our series of SN-MM was able to reveal some chromosomal abnormalities associated with poor overall prognosis (loss of 1p36, loss of 3p/3q and loss of 8p) and short DFS with early metastatization rates (loss of 1p36 and loss of 3p/3q). Prognostication was improved by considering chromosome 3 loss (both 3p and/or 3q arms) together with 1p36 loss and 8p loss, which was found to be an independent predictor of poor overall outcome and early metastatic dissemination of disease and might represent a signature of chromosomal instability that can add a specific HR value for classification of risk categories. This result is innovative in the field of SN-MM and is in line with findings already described for uveal melanoma, where it has been demonstrated that monosomy of chromosome 3 was associated with increased risk of metastasis [8,9,22,23]. At present, cytogenetic abnormalities observed both in SN-MM and UM do not have direct implications in terms of molecular-based therapeutic approaches. However, multiple genomic aberrations described in our series may represent a step forward in the management of SN-MM. Indeed, genomic instability might help identifying cancers showing a more aggressive behavior and, consequently, patients at high risk of early recurrence or systemic dissemination spread to tailor intensified treatment strategies including radical surgical resection, carbon ion irradiation in case of involved surgical margins or unresectable disease.

Conclusions

This work describes a new integrated characterization of both single nucleotide variants and, for the first time, genomic alteration in SN-MM, providing a new insight into molecular bases of these neoplasms and prompting further efforts for drug discovery and personalized clinical protocols according to tumor aggressiveness.

Conflicts of interest statement

The authors declare no conflict of interest.

Funding

No funds were received for this study.

Authors' contributions

Clinical data collection: MTZ, ADA; Histological assessment: FP, DM, CF, MC; molecular analysis: LL, NS, DF; data interpretation LL, NS, MTZ, FP; writing (original draft): LL, NS, MTZ, FP, DM; writing (review and editing): CF, SLR; supervision: PB, DF, MB, PC, FS, SLR

Ethical consideration

The study was approved by the Institutional Review Board (Insubria Board of Ethics, approval number 0033025/2015) and performed in accordance with the Helsinki declaration and its later amendments. Informed consent was obtained from all participants.

References

1 Yde SS, Sjoegren P, Heje M, et al. Mucosal Melanoma: a Literature Review. Curr Oncol Rep 2018;20(3):28. https://doi.org/10.1007/s11912-018-0675-0 [published Online First: 20180323]

2 Ascierto PA, Accorona R, Botti G, et al. Mucosal melanoma of the head and neck. Crit Rev Oncol Hematol 2017;112:136-52. https://doi.org/10.1016/j.critrevonc.2017.01.019 [published Online First: 20170213]

3 Castelnuovo P, Turri-Zanoni M, Battaglia P, et al. Sinonasal Malignancies of Anterior Skull Base: Histology-driven Treatment Strategies. Otolaryngol Clin North Am 2016;49(1):183-200. https://doi.org/10.1016/j.otc.2015.09.012

4 Turri-Zanoni M, Medicina D, Lombardi D, et al. Sinonasal mucosal melanoma: Molecular profile and therapeutic implications from a series of 32 cases. Head Neck 2013;35(8):1066-77. https://doi.org/10.1002/hed.23079 [published Online First: 20120712]

5 Crippen MM, Kılıç S, Eloy JA. Updates in the management of sinonasal mucosal melanoma. Curr Opin Otolaryngol Head Neck Surg 2018;26(1):52-57. https://doi.org/10.1097/MOO.0000000000000428

6 Na'ara S, Mukherjee A, Billan S, et al. Contemporary Multidisciplinary Management of Sinonasal Mucosal Melanoma. Onco Tar-

gets Ther 2020;13:2289-98. https://doi.org/10.2147/OTT.S182580 [published Online First: 20200316]

7 Network CGA. Genomic Classification of Cutaneous Melanoma. Cell 2015;161(7):1681-96. https://doi.org/10.1016/j.cell.2015.05.044

8 Jager MJ, Shields CL, Cebulla CM, et al. Uveal melanoma. Nat Rev Dis Primers 2020;6(1):24. https://doi.org/10.1038/s41572-020-0158-0 [published Online First: 20200409]

9 Robertson AG, Shih J, Yau C, et al. Integrative Analysis Identifies Four Molecular and Clinical Subsets in Uveal Melanoma. Cancer Cell 2017;32(2):204-20.e15. https://doi.org/10.1016/j.ccell.2017.07.003

10 Amin Mahul B. ESB, Greene Frederick L, Byrd David R, Brookland Robert K. AJCC Cancer Staging Manual2018.

11 Bolzacchini E, Libera L, Church SE, et al. Tumor Antigenicity and a Pre-Existing Adaptive Immune Response in Advanced BRAF Mutant Colorectal Cancers. Cancers (Basel) 2022;14(16) https://doi.org/10.3390/cancers14163951 [published Online First: 20220816]

12 Sahnane N, Libera L, Facchi S, et al. Similarities and differences in gene expression profiles of. Front Oncol 2023;13:1268127. https://doi.org/10.3389/fonc.2023.1268127 [published Online First: 20231003]

13 Nassar KW, Tan AC. The mutational landscape of mucosal melanoma. Semin Cancer Biol 2020;61:139-48. https://doi.org/10.1016/j.semcancer.2019.09.013 [published Online First: 20191023]

14 Broman KK, Dossett LA, Sun J, et al. Update on BRAF and MEK inhibition for treatment of melanoma in metastatic, unresectable, and adjuvant settings. Expert Opin Drug Saf 2019;18(5):381-92. https://doi.org/10.1080/14740338.2019.1607289 [published Online First: 20190424]

15 Ascierto PA, Schadendorf D, Berking C, et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. Lancet Oncol 2013;14(3):249-56. https://doi.org/10.1016/S1470-2045(13)70024-X [published Online First: 20130213]

16 Hong DS, Fakih MG, Strickler JH, et al. KRAS. N Engl J Med 2020;383(13):1207-17. https://doi.org/10.1056/NEJMoa1917239 [published Online First: 20200920]

17 O'Sullivan É, Keogh A, Henderson B, et al. Treatment Strategies for KRAS-Mutated Non-Small-Cell Lung Cancer. Cancers (Basel) 2023;15(6) https://doi.org/10.3390/cancers15061635 [published Online First: 20230307]

18 Hodi FS, Corless CL, Giobbie-Hurder A, et al. Imatinib for melanomas harboring mutationally activated or amplified KIT arising on mucosal, acral, and chronically sun-damaged skin. J Clin Oncol 2013;31(26):3182-90. https://doi.org/10.1200/JCO.2012.47.7836 [published Online First: 20130617]

19 Guo J, Carvajal RD, Dummer R, et al. Efficacy and safety of nilotinib in patients with KIT-mutated metastatic or inoperable melanoma: final results from the global, single-arm, phase II TEAM trial. Ann Oncol 2017;28(6):1380-87. https://doi.org/10.1093/annonc/mdx079

20 Freiberger SN, Turko P, Hüllner M, et al. Who's Driving? Switch of Drivers in Immunotherapy-Treated Progressing Sinonasal Melanoma. Cancers (Basel) 2021;13(11) https://doi.org/10.3390/cancers13112725 [published Online First: 20210531]

21 Ricci C, Altavilla MV, de Biase D, et al. Unveiling the molecular landscape and clinically relevant molecular heterogeneity of mucosal melanoma of the head and neck region. Histopathology 2025. https://doi.org/10.1111/his.15456 [published Online First: 20250415]

22 Damato B, Dopierala JA, Coupland SE. Genotypic profiling of 452 choroidal melanomas with multiplex ligation-dependent probe amplification. Clin Cancer Res 2010;16(24):6083-92. https://doi.org/10.1158/1078-0432.CCR-10-2076 [published Online First: 20101025]

23 Ewens KG, Kanetsky PA, Richards-Yutz J, et al. Genomic profile of 320 uveal melanoma cases: chromosome 8p-loss and metastatic outcome. Invest Ophthalmol Vis Sci 2013;54(8):5721-9. https://doi.org/10.1167/iovs.13-12195 [published Online First: 20130823]