Title:

Targeted Next-generation Sequencing of Japanese Patients with Sinonasal Mucosal Melanomas Identifies Frequent NRAS and CTNNB1 Mutations

Authors:

Nayuta Tsushima, MD [1], Satoshi Kano, MD, PhD [1], Kanako C. Hatanaka, MD, PhD [2], Takayoshi Suzuki, MD, PhD [1], Seijiro Hamada, MD, PhD [1], Hiroshi Idogawa, MD [1] ,Yuji Nakamaru, MD, PhD [1], Masanobu Suzuki, MD, PhD [1], Yutaka Hatanaka, PhD [2], Akihiro Homma, MD, PhD [1].

1. Department of Otolaryngology-Head and Neck Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Hokkaido, Japan
2. Center for Development of Advanced Diagnostics, Institute of Health Science Innovation for Medical Care, Hokkaido University Hospital, Hokkaido, Japan

Corresponding author: Nayuta Tsushima

Department of Otolaryngology-Head and Neck Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Hokkaido, Japan

Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan

Phone: +81-11-707-5958; Fax: +81-11-717-7566;

E-mail address: tsushima-na@huhp.hokudai.ac.jp

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Abstract

Objective

Mucosal melanoma is a rare malignancy; however, the reported incidence rate of mucosal melanoma is higher in Asians than in Caucasians. Sinonasal mucosal melanoma (SNMM) is an aggressive malignancy with a poor prognosis due to distant metastasis. Systemic therapy with BRAF inhibitor and MEK inhibitor is one of the standards of care for cutaneous melanoma patients with BRAF V600 mutations. However, no molecular targeted therapy for patients with mucosal melanoma has been established. Relatively few studies have described the genetic mutations associated with mucosal melanoma because of its low frequency. Furthermore, to the best of our knowledge, the genetic mutations among Japanese patients have not been reported. Therefore, in the current study, we evaluated the genetic and clinicopathological characteristics of patients with SNMM.

Methods

A total of 18 tissue samples obtained from patients with SNMM were analyzed for genetic mutations based on targeted next-generation sequencing to investigate the driver of tumorigenesis and/or candidate genes for predicting clinical outcomes in SNMM. We also performed immunohistochemistry for patients identified with CTNNB1 mutations.

Results

Eight of the 18 (44%) patients had genetic mutations. The most frequent mutation was NRAS (6/18, 33%), followed by CTNNB1 (2/18, 11%) and BRAF (1/18, 5.6%). One patient had both NRAS and CTNNB1 mutations. Clinical outcomes did not differ significantly between those with and without genetic mutations. NRAS mutations were associated with relatively higher T classification and worse survival rates, although the differences were not significant. The nuclear translocation of β-catenin was detected in both tumors with CTNNB1 mutations. The amino acid change in the BRAF mutation was K601R in exon 15. In the current study, no BRAF V600 mutations were detected.

Conclusion

Genetic mutations were not significantly associated with clinical outcomes. However, NRAS mutations may be a prognostic predictor and CTNNB1 mutation may be a treatment effector for immune check inhibitors. A larger prospective study is required to clarify the clinical importance of genetic mutations in patients with SNMM.

Keywords

Sinonasal mucosal melanoma, genetic mutations, NRAS, CTNNB1, BRAF, β-catenin, distant metastasis.

Introduction

Mucosal melanoma is one of the rare malignancies, with an estimated incidence of 0.7 in 100,000 new cases per year [1]. More than 50% of mucosal melanomas arise in the head and neck area, with 66% of these arising in the sinonasal tract [2]. However, the reported incidence rate of mucosal melanoma is higher in Asians than in Caucasians [3]. Sinonasal mucosal melanoma (SNMM) is an aggressive malignancy with a poor prognosis due to local recurrence and distant metastasis, with the 5-year overall survival rate (OS) reported to be 24% [4]. Previous studies reported that the cumulative incidence rate of distant metastasis was 66% [5].

The standard of care for SNMM is surgery and postoperative radiotherapy (PORT) [6]. Adjuvant treatment with immune checkpoint inhibitors (ICI) is also a treatment option. However, the respective indications for PORT and adjuvant systemic therapy have not been established. Recent advances in endoscopic resection have enabled endoscopic surgery to be adopted for malignant sinonasal tumors [7–9]. Patients with mucosal melanomas were reported to tend to be less responsive to ICIs than those with cutaneous melanomas [10].

The etiology of cutaneous melanoma has been widely reported. Past studies reported that 66% of cutaneous melanoma had BRAF mutations, with 80% of these

being BRAF V600E mutations [11]. Systemic therapy with BRAF inhibitor and MEK inhibitor is one of the standards of care for patients with BRAF V600 mutations. Usually, distant metastatic disease is treated by systemic therapy, including molecular targeted agents. However, relatively few studies have described the genetic mutations in mucosal melanoma because of its low frequency. Furthermore, to the best of our knowledge, there have been no reports on genetic mutations among Japanese patients. In the current study, we performed targeted next generation sequencing (NGS) analysis to evaluate the genetic and clinicopathological characteristics of patients with SNMM. We also assessed treatment outcome according to the presence of genetic mutations.

Patients and methods

Patients

This was a retrospective study based on the medical records of patients with SNMM treated at Hokkaido University Hospital between January 2003 and March 2021. The inclusion criteria for this study were as follows: (1) previously untreated sinonasal mucosal melanoma; (2) histologically proven malignant melanoma; (3) curative-intent surgery. We excluded patients whose tissue specimens were inappropriate for NGS.

Twenty-nine patients met the inclusion criteria, among which 8 patients were later

excluded from this study. Therefore, 21 patients were eligible for this study. This study was approved by the Institutional Review Board at Hokkaido University Hospital (No. C-T2020-0152) and was performed in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Treatment strategy

The clinical stage was evaluated by endoscopic nasal examination, contrast-enhanced CT imaging, MRI, and positron emission tomography (PET), where possible. Precontrast CT imaging or MRI was acceptable for patients unable to tolerate the contrast agent. For all patients, the clinical stage was determined before treatment by the departmental cancer board consisting of head and neck surgeons, radiation oncologists, diagnostic radiologists, and medical oncologists. All staging was completed according to the 7th Union for International Cancer Control (UICC) criteria.

Our treatment strategy is shown in Fig. 1. In general, treatment for the primary lesion comprises endoscopic resection and PORT. We performed combined endoscopic and open resection or applied an external approach alone when sufficient resection was difficult to achieve endoscopically. We recommended definitive radiotherapy if patients had unresectable disease or did not want to undergo surgical treatment. We did not

perform neoadjuvant or adjuvant systemic therapy. Patients were usually monitored monthly for recurrence during the first year, every two months during the second year, and every 3 to 6 months thereafter until death. CT and MRI were routinely performed every 3 to 6 months, with PET performed as required.

Sample preparation for NGS assays

Tissue samples were obtained from surgical specimens from the primary tumors. Sections of 10 μm in thickness were cut from formalin-fixed paraffin-embedded tissues. Areas with a high proportion of tumor cells were identified by hematoxylin and eosin staining. If the percentage of tumor cells in the samples were less than 90%, a macro-dissection technique was used to remove the non-tumor region.

DNA was extracted from the samples using a GeneRead DNA FFPE Tissue Kit (QIAGEN, Hilden, NRW, Germany) according to the manufacturer's instructions. RNA was extracted from the samples using a ReliaPrep FFPE Total RNA Miniprep System (Promega, Madison, WI, USA) according to the manufacturer's instructions. The DNA or RNA quantity and quality were measured using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and TapeStation (Agilent, Santa Clara, CA, USA), respectively, according to the manufacturers' instructions.

Next-Generation sequencing

The Ampliseq for Illumina Focus Panel (Illumina, San Diego, CA, USA) was used to assess 52 genes with known relevance to solid tumors (Supplementary Table 1). For library construction, DNA and RNA were amplified using the Ampliseq library PLUS for Illumina (Illumina). After amplification, a MiSeq sequencer (Illumina) was used for the sequence reaction. All of these procedures were carried out according to the respective Illumina methodology and recommendations.

Sequencing data analysis

After sequencing, each FASTQ file was processed using the DNA and RNA amplicon tools on Base Space Sequence HUB (Illumina) for SNV/Indel and fusion calling, respectively. Significant mutations were filtered under the following conditions: (1) a variant allele frequency higher than 10%; (2) more than 10 altered variant read-outs; (3) non-synonymous single nucleotide variants, deletion, or insertion; (4) mutations within the coding region of the genes; and (5) mutations already reported in the Catalogue Of Somatic Mutations In Cancer (COSMIC) database (https://cancer.sanger.ac.uk/cosmic). Functional analysis was performed using the ClinVar

(https://www.ncbi.nlm.nih.gov/clinvar/) database to ascertain the pathogenicity of the variants.

Immunohistochemistry

We performed immunohistochemistry (IHC) for patients identified with CTNNB1 mutations. The tissue sections of 3 μm thickness were dehydrated with xylene and graded alcohol. Antigen retrieval was accomplished by boiling the tissue sections in EnVision FLEX Target Retrieval Solution, High pH (Dako, Carpinteria, CA, USA) for 20 min. After gently cooling, blocking of endogenous peroxidase was achieved by EnVision FLEX Peroxidase-Blocking Reagent (Dako) for 5 min at room temperature. The slides were then incubated with 1:200 Purified Mouse Anti-β-Catenin (BD Biosciences, NJ, USA) for 30 min at room temperature. At the end of incubation, slides were incubated in secondary EnVision FLEX/HRP Detection Reagent (Dako) for 30 min at room temperature. Visualization was performed using EnVision FLEX DAB+ Chromogen (Dako) in EnVision FLEX Substrate Buffer (Dako) for 10 min at room temperature. The sections were counterstained with hematoxylin before dehydration and mounting.

Statistics

All statistical analyses were performed using JMP Pro 17.0.0 statistical software (SAS Institute, Cary, NC, USA). Fisher's exact test, Chi-square test, or Wilcoxon test was used to correlate the mutation status with clinicopathological features such as age, gender, anatomical site, T and N classification, clinical stage, type of surgery, and PORT. The Kaplan-Meier method was used for the calculation of OS, recurrence-free survival rate (RFS), and cumulative incidence of distant metastasis. The time of interest was the period from the first day of the treatment to death or failure. Follow-up data were obtained until June 30, 2023. Differences in probabilities between curves were evaluated by log-rank test. The level of statistical significance was set at $p < 0.05$.

Results

Clinicopathological characteristics

Eighteen of the 21 eligible patients were enrolled in the analysis, with three patients not proceeding to NGS due to the insufficient quality of the extracted DNA. Patient characteristics are shown in Table 1. There were 10 males and 8 females with a median age at diagnosis of 67.5 years. The median follow-up period was 3.2 years (range: 0.6–9.6 years). The number of patients with clinical stage III or IV were 11 and 7,

respectively.

Surgery and radiotherapy

The details of the treatment are shown in Supplementary Table 2. Twelve of the 18 (67%) patients underwent resection by endoscopic approach alone. Postoperative radiotherapy was performed for all 18 patients. The number of patients irradiated with photon or proton beams was 13 and 5, respectively. Until the year 2017, radiotherapy was predominantly performed with photon beams, and from 2018 with proton beams. Basically, postoperative irradiation was administered via photon beam therapy with a total dose of 60 Gy in 30 fractions or proton beam therapy with 30 Gy relative biological effectiveness (RBE) in 5 fractions. In cases of macroscopic residual tumors after surgery or definitive intent, radiotherapy was performed with proton beams at a total dose of 60 Gy RBE in 15 fractions. No patients underwent preoperative therapy, and no elective neck dissection or irradiation was performed in patients without neck metastasis.

Mutation analysis

The flow chart of NGS data analysis is shown in Fig. 2. Twenty-six somatic variants

were retained based on the criteria of present in the COSMIC database. Of these, 18 variants were excluded because of benign or likely benign classification in the ClinVar database. Finally, eight mutations were considered pathogenic.

A Summary of identified mutations is shown in Table 2. A BRAF mutation was detected in one patient (5.6%). The amino acid change was K601R in exon 15. In the current study, no BRAF V600 mutations were not detected. NRAS mutations were identified in six patients (33%). Among the identified NRAS mutations, two were in exon 3 (both were Q61R) and four in exon 2 (G13Y, G12V, G12R, and G12S). CTNNB1 mutations were found in two patients. Their amino acid changes were S45F and S37F in exon 3. One of them also had an NRAS mutation (CTNNB1 S45F in exon 3 and NRAS G12R in exon 2). For RNA analysis, no fusion genes were detected in any of the cases.

Immunohistochemistry

We performed IHC for two patients with CTNNB1 mutations. The nuclear translocation of β-catenin was found in both tumors with CTNNB1 mutations, but not detected in two negative controls (Fig. 3). The CTNNB1 gene encodes the β-catenin protein and the gene mutations are associated with the translocation of β-catenin to the nucleus.

Association of mutations with clinicopathological features

Clinicopathological and NGS findings are summarized in Fig. 4 and Tables 3 and 4. No significant difference was found between patients with and without genetic mutations. NRAS mutations were more frequently found in younger patients.

Association of mutations with survival and distant metastasis

The 2-year OS of patients with or without genetic mutations was 77% and 63%, respectively (P = 0.79, Fig. 5a). Further, no significant differences were found for RFS or cumulative incidence of distant metastasis (P = 0.97, 0.96; Fig. 5b, c). When we divided patients with or without NRAS mutations, no significant differences were found in OS, RFS, or cumulative incidence of distant metastasis (P = 0.90, 0.30, and 0.55, respectively; Fig. 5d–f).

Discussion

In the current study, 8 of the 18 (44%) patients had genetic mutations. The most frequent mutation was NRAS (6/18, 33%), followed by CTNNB1 (2/18, 11%) and

BRAF (1/18, 5.6%). Past studies reported that 16–38% of patients with mucosal melanoma have genetic mutations, with BRAF mutations rarely observed [12–16]. On the other hand, the frequency of BRAF mutations was reported to be 66% in patients with cutaneous melanoma [11].

NRAS mutations were the most frequent in our study. Past articles reported that the frequency of NRAS mutations in cutaneous melanoma was 15–20% [17]. In mucosal melanoma, NRAS mutations were found in 7 of 71 (10%), 2 of 30 (7%), and 8 of 56 (14%) Caucasian patients, but in 4 of 7 (57%) Chinese patients [13,14,16,18]. NRAS mutations may be more frequent in Asian patients with mucosal melanoma than in Caucasians. In our study, NRAS mutations were more common in younger patients (median age 64.5 vs. 71 years, P = 0.04). However, contrary to our findings, other studies on cutaneous and mucosal melanoma did not show an age association with NRAS mutations [17,19]. A separate study on cutaneous and mucosal melanoma even found that patients with NRAS mutations were, on average, older than those without (mean age 56.5 vs. 50.8, P = 0.02) [11]. Consequently, the relationship between patient age and NRAS mutation remains contentious. Additional research is needed to determine whether NRAS mutations impact the age of onset in mucosal melanoma patients. In the current study, two patients had Q61R mutations, while others had G12V,

G12R, G12S, and G13V mutations. These mutations were compatible with reported hotspot mutations [20]. A past study, which included 11 and 20 patients with mucosal and cutaneous melanoma, respectively, reported that NRAS mutations were associated with poor survival outcomes [11]. In the current study, NRAS mutations were associated with relatively higher T classification and worse survival rates, although the differences were not significant. NRAS mutations lead to retaining NRAS in its active state, resulting in aberrant downstream activation of the MAPK pathway through RAF. Activated RAF sequentially activates MEK and ERK, leading to the activation of multiple cytoplasmic and nuclear molecules involved in regulating cell proliferation, differentiation, and survival. No molecular targeted therapy for NRAS mutations has yet been established. However, Binimetinib, a MEK inhibitor, was reported to be associated with prolonged progression-free survival in patients with cutaneous melanoma[21]. Another study on cutaneous melanoma reported that the overall response rate was 46.7% for combination therapy with Naporafenib (a pan-RAF inhibitor) and Trametinib (a MEK inhibitor) [22]. Molecular targeted therapy with a combination of RAF and MEK inhibitors may also be effective in SNMM patients with NRAS mutations because of the activation of RAF and MEK in such patients.

The Wnt/β-catenin pathway comprises a family of proteins that play critical roles

in embryonic development and adult tissue homeostasis [23]. The dysregulation of Wnt/β-catenin signaling often leads to various serious diseases, including malignant tumors [24]. β-catenin is encoded by the CTNNB1 gene, and β-catenin signaling has been implicated in tumorigenesis [25]. When activating mutations of CTNNB1 occur, β-catenin translocates to the nucleus and functions as a transcription factor [26]. In the current study, two patients (11%) had CTNNB1 mutations, and nuclear accumulation of β-catenin was confirmed in both by IHC (Fig.3). A past study reported CTNNB1 mutations in 4 of 67 (6%) patients with mucosal melanoma [27]. CTNNB1 mutations may also contribute to the relatively poorer responses to immunotherapy in advanced-stage mucosal melanoma patients [10]. Past studies reported that tumor-intrinsic β-catenin signaling could inhibit T-cell infiltration [26]. Melanoma gene microarray data have suggested that an activated Wnt/β-catenin signal in the cancer microenvironment can be correlated with a lack of immune cell infiltration [26]. Clinical outcomes in response to ICI tend to be less favorable in Asian patients than in their Caucasian counterparts [28]. A past study reported that differences in the prevalence of mutations affecting Wnt/β-catenin pathway activation may contribute to the unfavorable outcomes observed for Chinese patients with melanoma treated with ICI [10]. CTNNB1 also has hotspot mutations that are known to occur at S33, S37, S45,

T41, D32, and G34 [29]. In our study, S37F and S45F were detected, and nuclear accumulation of β-catenin was reported in association with both mutations in other malignancies [25]. IHC for β-catenin is less expensive, more widely available, and faster than CTNNB1 mutational analysis, and we think that treatment outcomes for ICIs may be estimated with IHC for β-catenin.

This study showed that a BRAF mutation was present in one patient (5.6%). The prevalence of BRAF mutations was less in mucosal melanoma than in its cutaneous counterpart. Past studies reported that BRAF mutation rates were 0–14% in mucosal melanoma [13,19,30,31]. With regard to SNMM, BRAF mutations were reported to be found in 4 of 90 patients (4.4%), and no V600 mutations were detected [32]. BRAF K601R mutations, one of which we detected in this study, are not related to molecular targeted therapy with BRAF and MEK inhibitors. Few patients with SNMM would be regarded as suited to treatment with such molecular targeted therapy due to the low frequency of BRAF V600 mutations.

We previously reported that local disease was controlled by endoscopic surgery and PORT, while distant metastasis was difficult to control [9]. Compared to cutaneous melanoma, mucosal melanoma has fewer BRAF V600 mutations, which are effective therapeutic targets in cutaneous melanoma, and is less responsive to treatment with ICI.

However, the NRAS and CTNNB1 mutations identified in this study may be predictors of the efficacy of such therapies.

We acknowledge several limitations in this study. First, because of the small number of cases in this study, the mutational characteristics associated with mucosal melanoma were not obtained for the general Japanese population. In particular, we need to clarify the relationship between the effects of ICI and genetic mutations through the accumulation of a much larger set of genetic data for Japanese population. Second, the targeted NGS used in this study only examined a limited number of cancer-related genes. Whole exon sequencing and whole genome sequencing for mucosal melanoma are desired for the identification of additional therapeutic targets and predictors of therapeutic response.

Conclusion

In this study, clinical outcomes did not differ significantly between those with and without genetic mutations; however, NRAS mutations may be a prognostic predictor and CTNNB1 mutations may be a treatment effector for ICI. A larger prospective study is required to clarify the clinical importance of genetic mutations in patients with SNMM.

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Author Contributions

Contributor NT was responsible for the organization and coordination of the trial. NT was the chief investigator and responsible for the data analysis. SK, KH, YH, and AH developed the trial design. TS, SH, HI, MS, YN, SK, and AH helped in drafting the manuscript and revised it critically. All authors contributed to the writing of the final manuscript.

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Figure Legends

Table 1. Characteristics of the eligible patients (n = 18)

Table 2. Summary of identified mutations

Table 3. Summary of genetic mutations and clinicopathological features

Table 4. Summary of NRAS mutations and clinicopathological features

Fig. 1. Treatment strategy. Patients underwent an external approach when sufficient resection is difficult to achieve endoscopically. Abbreviations: SNMM, sinonasal mucosal melanoma

Fig. 2. Flow chart of NGS data analysis. Twenty-six somatic variants were retained by the criteria of present in the COSMIC database. Of these, 18 variants were excluded because of benign or likely benign classification in the ClinVar database. Finally, eight

mutations were considered pathogenic.

Fig. 3. The nuclear translocation of β-catenin was found in tumors that had CTNNB1 mutations (a, b), but was not detected in negative controls (c). Original magnification: x 100.

Fig. 4. The summary of clinicopathological and NGS findings. The number of patients with BRAF, NRAS, and CTNNB1 mutations were one, six, and two, respectively. A significant difference was not found between patients with and without any genetic mutations.

Fig. 5. Overall survival, recurrence-free survival, and cumulative incidence of distant metastasis for patients stratified by genetic mutations (a–c) and NRAS mutations (d–f). The 2-year survival rates or cumulative incidence are described in each figure.

Table 1. Characteristics of the eligible patients (n = 18)

Characteristic	
Overall	18
Gender	
Male	10
Female	8
Age, years	
Median (range)	67.5 (49–83)
Follow-up period, years	
Median (range)	3.2 (0.6–9.6)
Subsite	
Nasal cavity	17
Paranasal sinus	1
T classification	
3	11
4	7
N classification	
0	17
1	1
Stage	
III	11
IV	7

Table 2. Summary of the identified mutations

Patient	Sex	age	Anatomical site	Gene	Exon	Nucleotide change	Amino-acid change
1	M	63	Nasal cavity	NRAS	3	182A>G	Q61R
2	F	66	Nasal cavity	NRAS	3	182A>G	Q61R
3	F	49	Nasal cavity	NRAS	2	38G>T	G13V
4	F	72	Nasal cavity	NRAS	2	35G>T	G12V
5	F	67	Nasal cavity	NRAS	2	34G>C	G12R
				CTNNB1	3	134C>T	S45F
6	M	60	Nasal cavity	NRAS	2	34G>A	G12S
7	F	74	Nasal cavity	BRAF	15	1801A>G	K601R
8	m	83	Nasal cavity	CTNNB1	3	110C>T	S37F

Table 3. Summary of genetic mutations and clinicopathological features

	Genetic mutation n (%)		
	Mutation n = 8	Normal n = 10	P value
Median age (range)	66.5 (49-83)	69.5 (59-78)	0.30
Gender male	3 (38)	7 (70)	0.16
Anatomical site			1
Nasal cavity	8 (100)	9 (90)	
Maxillary sinus	0	1 (10)	
T classification			0.63
3	4 (50)	7 (70)	
4	4 (50)	3 (23)	
N classification			1
0	8 (100)	9 (90)	
1	0	1 (10)	
Stage			0.63
III	4 (50)	7 (70)	
IV	4 (50)	3 (30)	
Type of Surgery			0.80
ESS	6 (75)	6 (60)	
Maxillectomy	1 (13)	4 (40)	
Combined	1 (13)	0	
Type of PORT			1
Photon	6 (75)	7 (70)	
Proton	2 (25)	3 (30)	

Table 4. Summary of NRAS mutations and clinicopathological features

	NRAS mutation n (%)		
	Mutation n = 6	Normal n = 12	P value
Median age (range)	64.5 (49-72)	71 (59-83)	0.04
Gender male	2 (33)	8 (80)	0.32
Anatomical site			1
Nasal cavity	6 (100)	11 (92)	
Maxillary sinus	0	1 (8)	
T classification			0.14
3	2 (33)	9 (82)	
4	4 (67)	3 (18)	
N classification			1
0	6 (100)	11 (92)	
1	0	1 (8)	
Stage			0.14
III	2 (33)	9 (82)	
IV	4 (67)	3 (18)	
Type of Surgery			0.89
ESS	4 (75)	8 (73)	
Maxillectomy	1 (13)	4 (27)	
Combined	1 (13)	0	
Type of PORT			1
Photon	4 (67)	79(73)	
Proton	2 (33)	3 (27)	

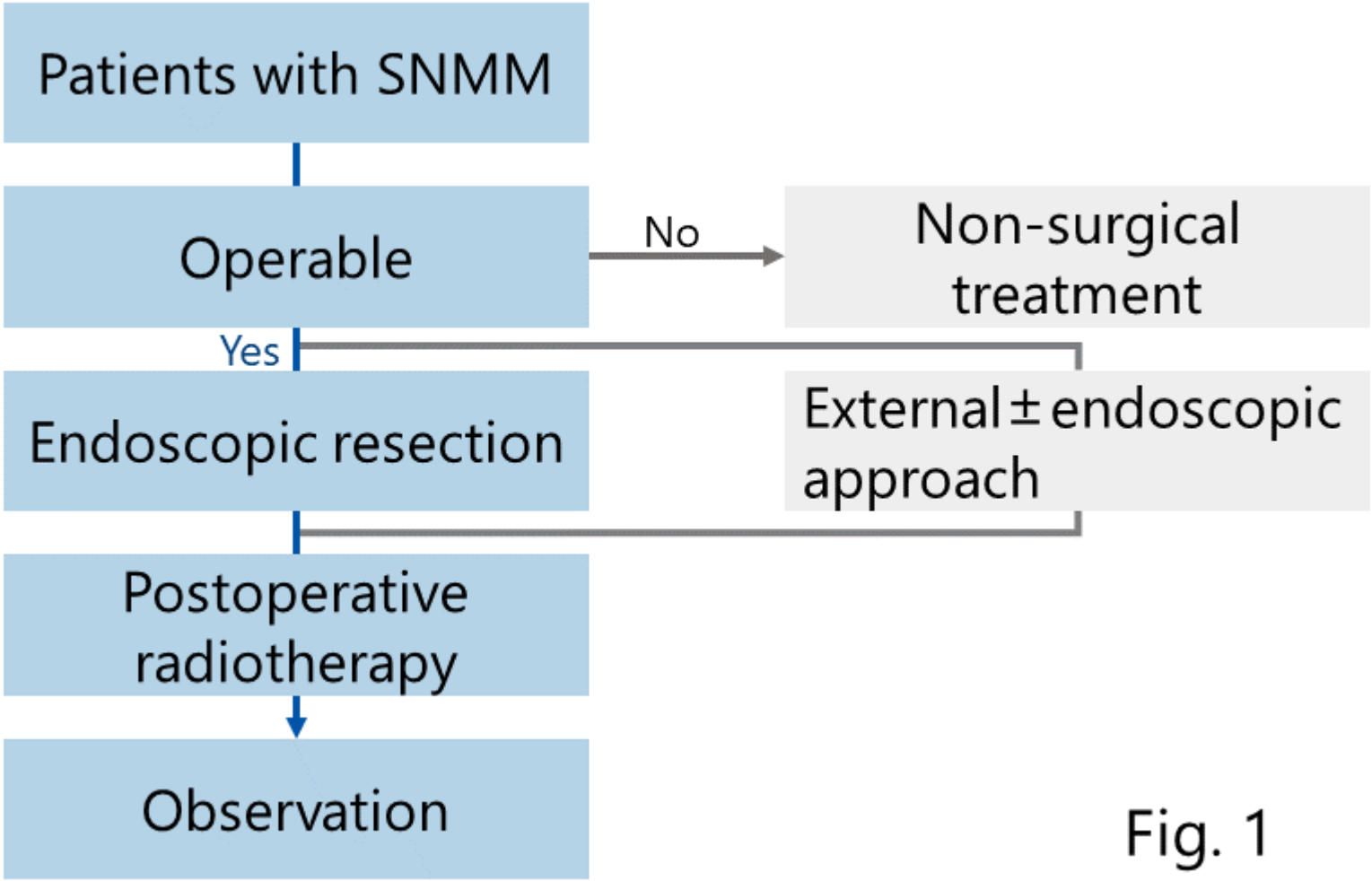

Patients with SNMM
Operable
No
Non-surgical treatment
Yes
Endoscopic resection
External ± endoscopic approach
Postoperative radiotherapy
Observation

Fig. 1

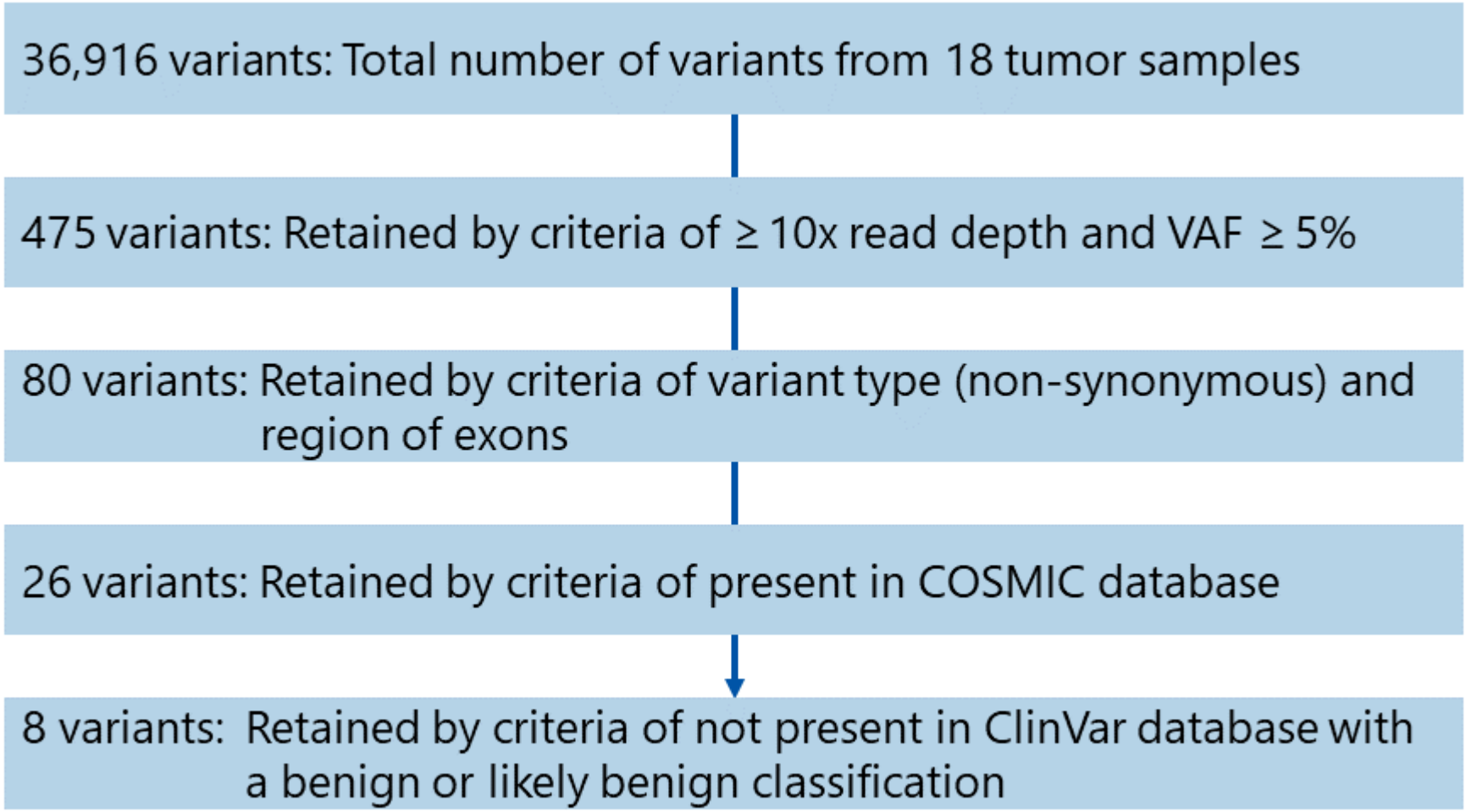

36,916 variants: Total number of variants from 18 tumor samples
475 variants: Retained by criteria of ≥ 10x read depth and VAF ≥ 5%
80 variants: Retained by criteria of variant type (non-synonymous) and region of exons
26 variants: Retained by criteria of present in COSMIC database
8 variants: Retained by criteria of not present in ClinVar database with a benign or likely benign classification

Fig. 2

Fig. 3a

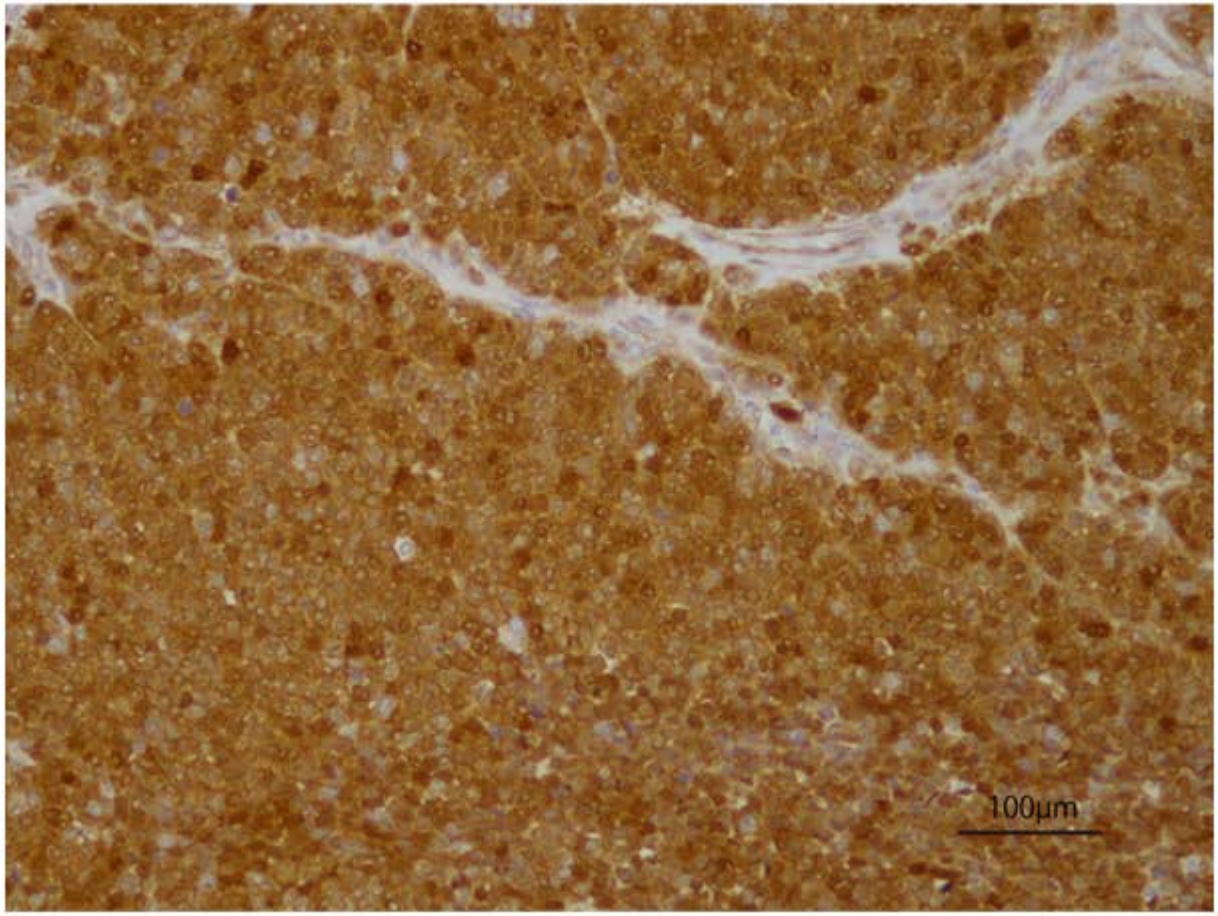


Fig. 3b

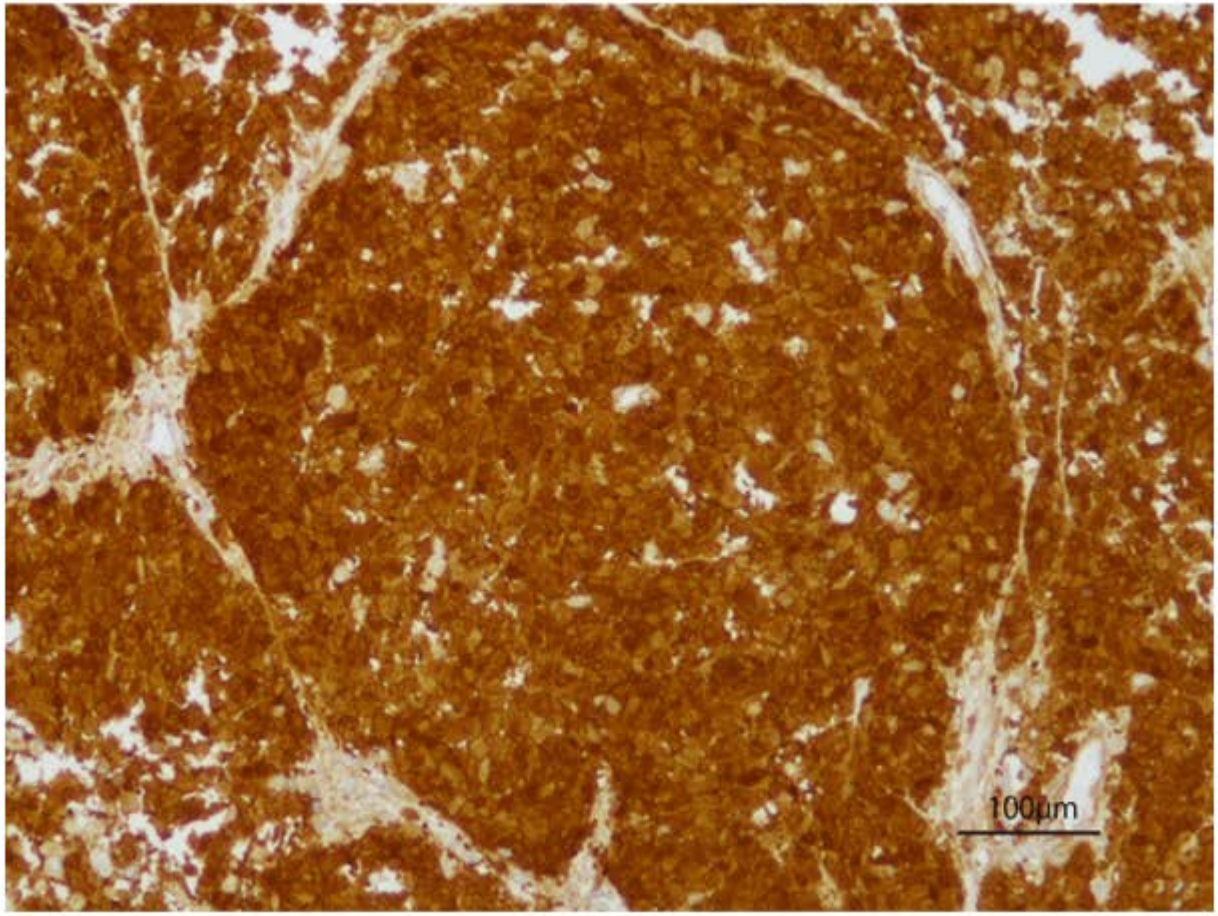
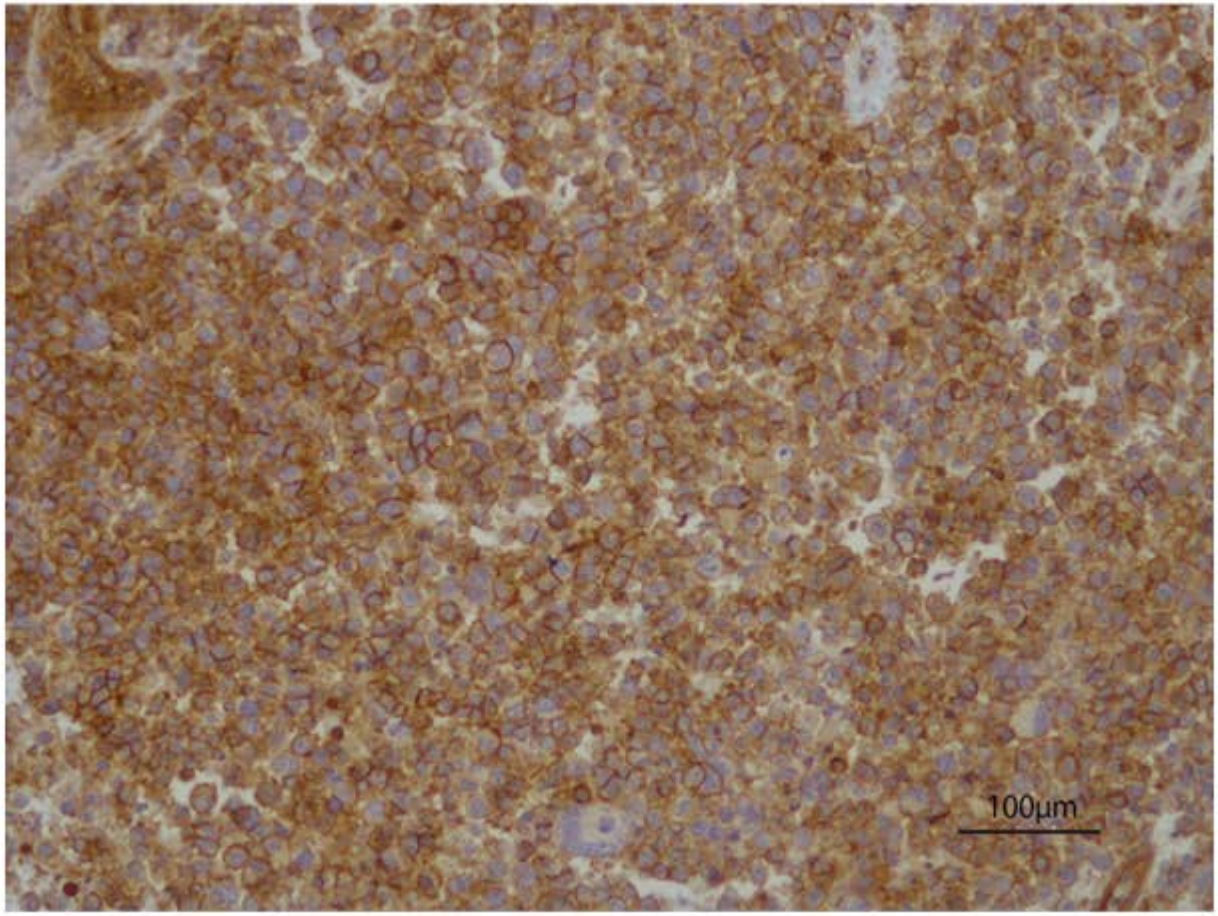


Fig. 3c



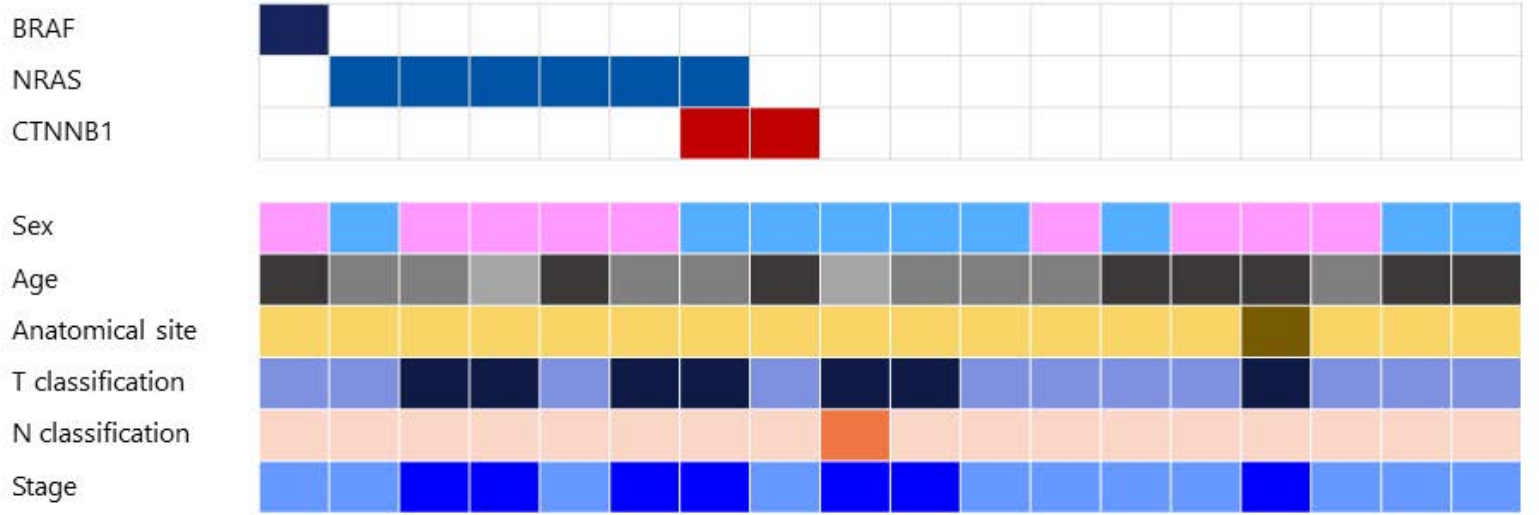

BRAF
NRAS
CTNNB1
Sex
Age
Anatomical site
T classification
N classification
Stage
Age
-29
30-39
40-49
50-59
60-69
70-
Sex
Male
Female
Anatomical site
Nasal cavity
Maxillary sinus
T classification
3
4
N classification
0
1
Stage
III
IV

Fig. 4

Fig. 5a

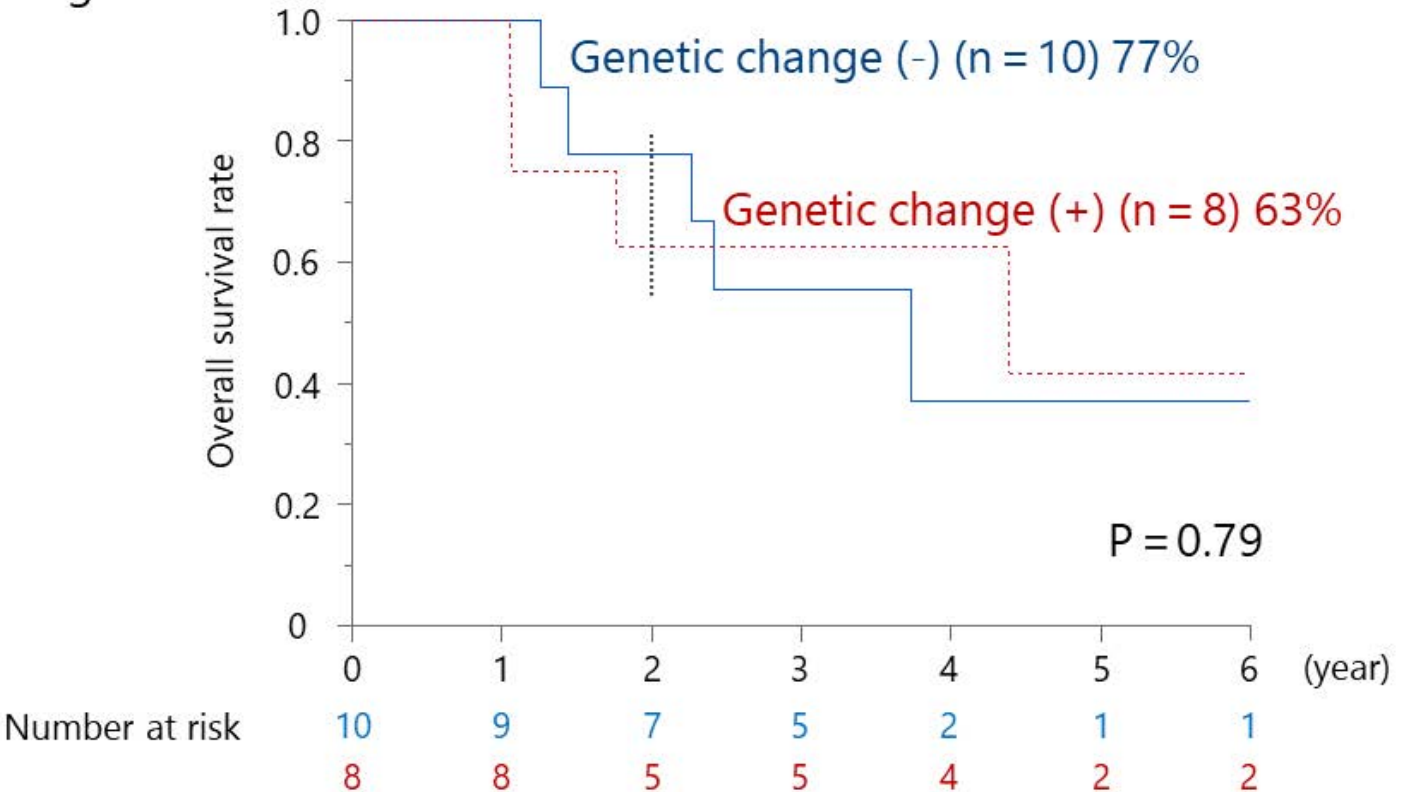

Overall survival rate
1.0
0.8
0.6
0.4
0.2
0
Genetic change (-) (n = 10) 77%
Genetic change (+) (n = 8) 63%
P = 0.79
0
1
2
3
4
5
6
(year)
Number at risk
10
9
7
5
2
1
1
8
8
5
5
4
2
2

Fig. 5b

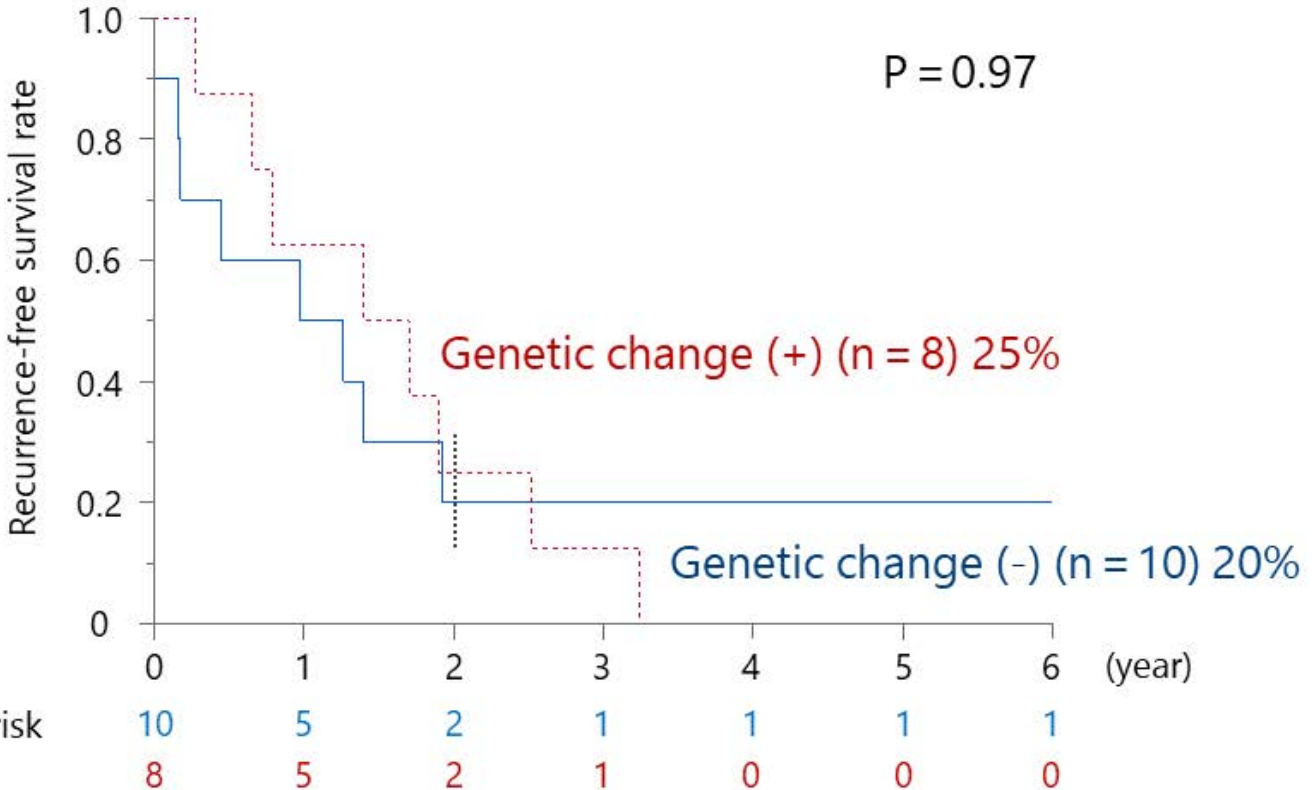

Recurrence-free survival rate
1.0
0.8
0.6
0.4
0.2
0
P = 0.97
Genetic change (+) (n = 8) 25%
Genetic change (-) (n = 10) 20%
0
1
2
3
4
5
6
(year)
Number at risk
10
5
2
1
1
1
1
8
5
2
1
0
0
0

Fig. 5c

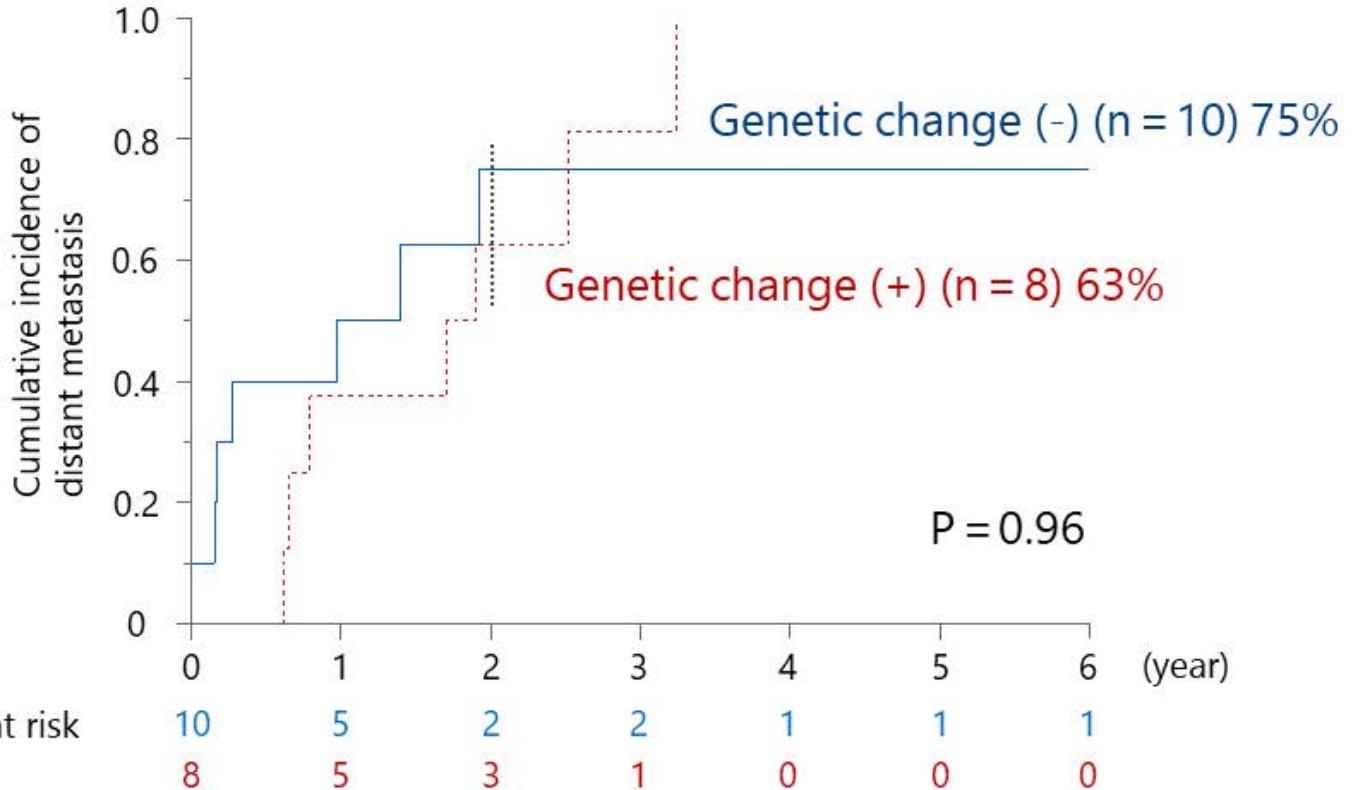

1.0
0.8
0.6
0.4
0.2
0
Cumulative incidence of distant metastasis
Genetic change (-) (n = 10) 75%
Genetic change (+) (n = 8) 63%
P = 0.96
0
1
2
3
4
5
6
(year)
Number at risk
10
5
2
2
1
1
1
8
5
3
1
0
0
0

Fig. 5d

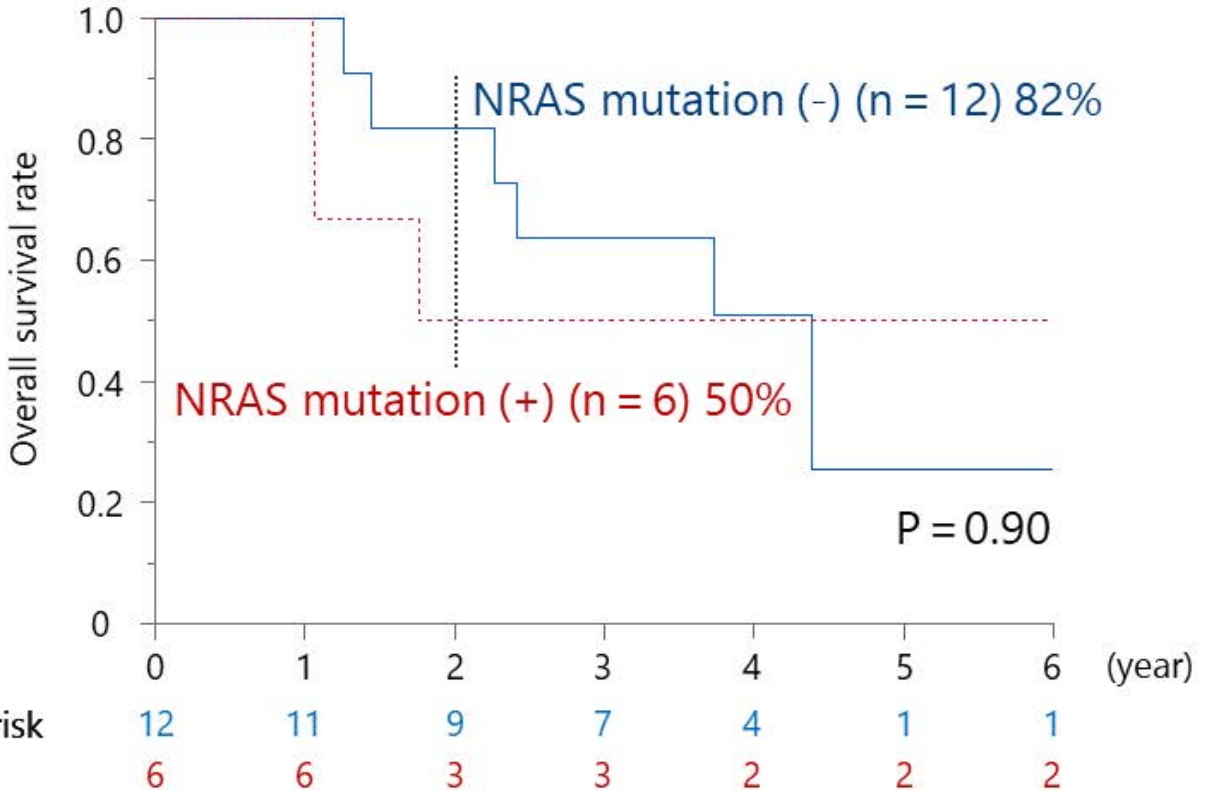

1.0
0.8
0.6
0.4
0.2
0
Overall survival rate
NRAS mutation (-) (n = 12) 82%
NRAS mutation (+) (n = 6) 50%
P = 0.90
0
1
2
3
4
5
6
(year)
Number at risk
12
11
9
7
4
1
1
6
6
3
3
2
2
2

Fig. 5e

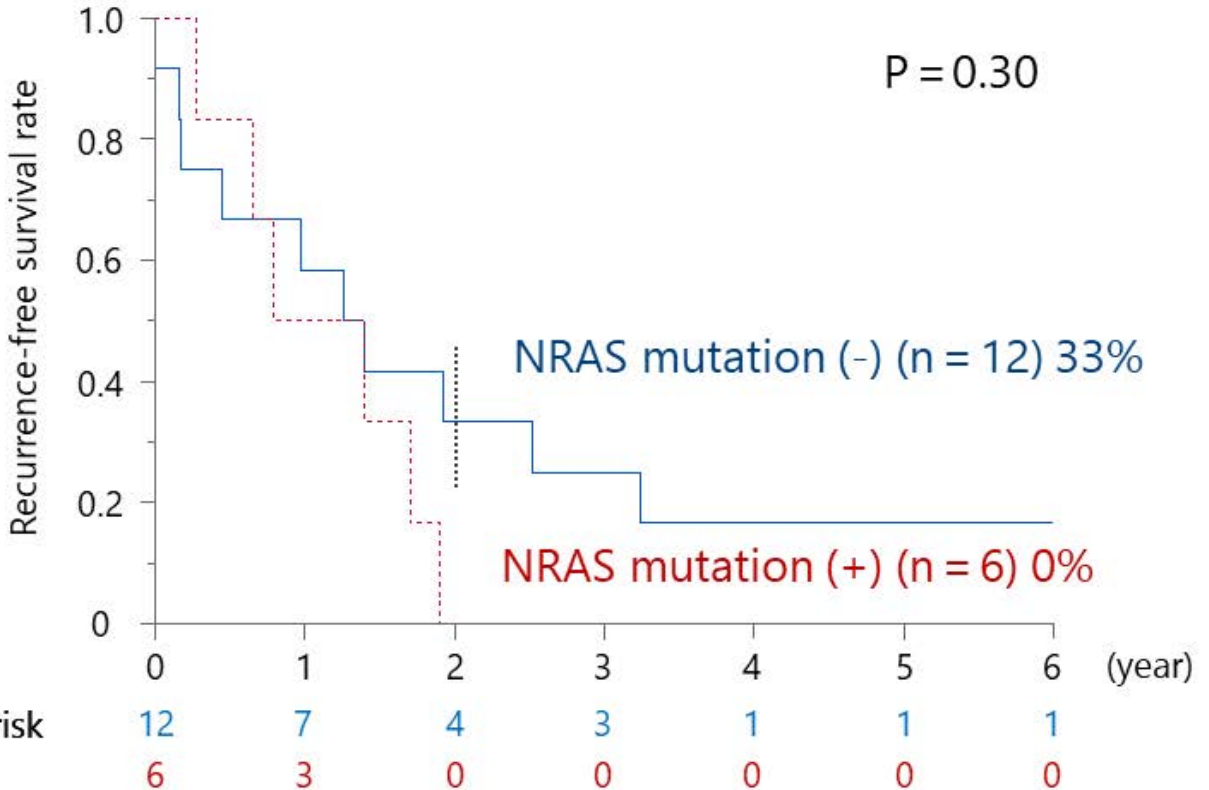
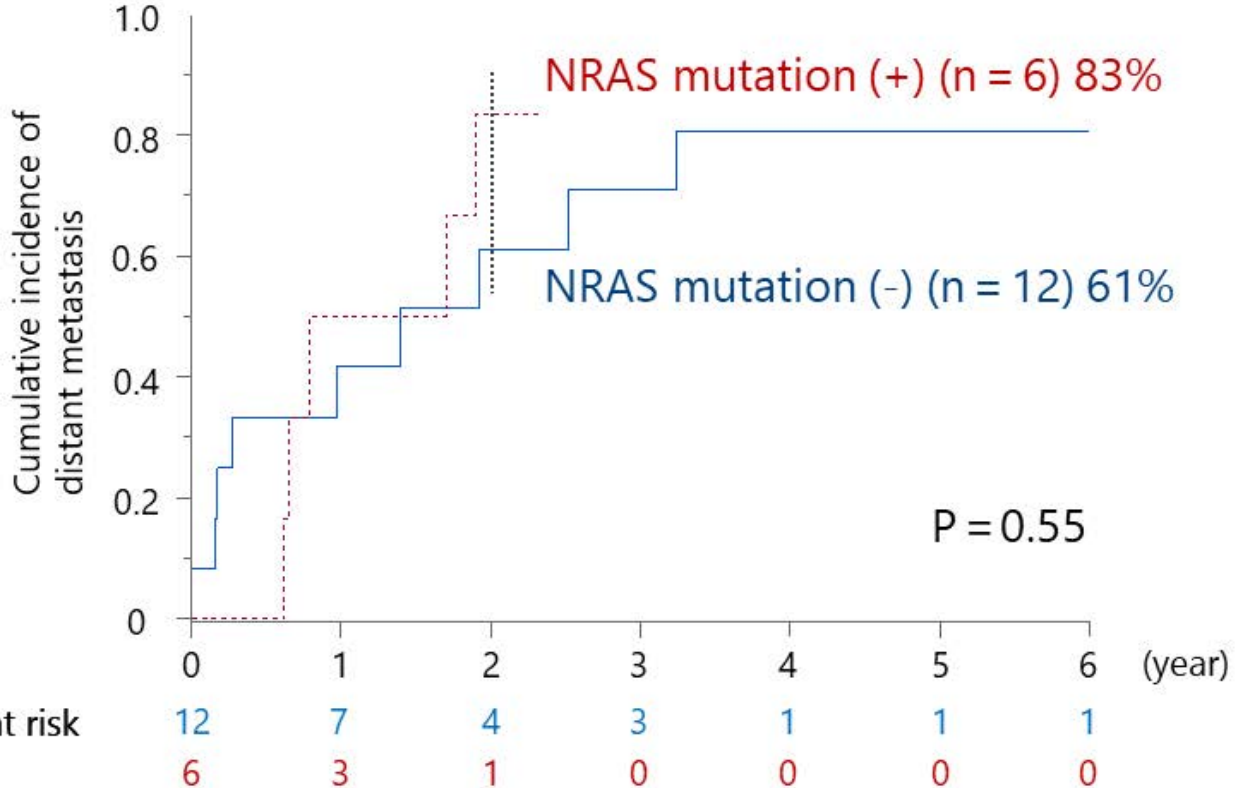

Recurrence-free survival rate
1.0
0.8
0.6
0.4
0.2
0
P = 0.30
NRAS mutation (-) (n = 12) 33%
NRAS mutation (+) (n = 6) 0%
0
1
2
3
4
5
6
(year)
Number at risk
12
7
4
3
1
1
1
6
3
0
0
0
0
0

Fig. 5f


1.0
0.8
0.6
0.4
0.2
0
Cumulative incidence of distant metastasis
NRAS mutation (+) (n = 6) 83%
NRAS mutation (-) (n = 12) 61%
P = 0.55
0
1
2
3
4
5
6
(year)
Number at risk
12
7
4
3
1
1
1
6
3
1
0
0
0
0