The Impact of KRAS Mutation in Patients With Sporadic Nonampullary Duodenal Epithelial Tumors

Hideaki Kinugasa, MD, PhD1, Hiromitsu Kanzaki, MD, PhD1, Takehiro Tanaka, MD, PhD2, Shumpei Yamamoto, MD1, Yasushi Yamasaki, MD, PhD1, Kazuhiro Nousu, MD, PhD1, Kouichi Ichimura, MD, PhD3, Masahiro Nakagawa, MD, PhD4, Toshiharu Mitsuhashi, MD, PhD5 and Hiroyuki Okada, MD, PhD1

INTRODUCTION: The genomic characterization of primary nonampullary duodenal adenocarcinoma indicates a genetic resemblance to gastric and colorectal cancers. However, a correlation between the clinical and molecular characteristics of these cancers has not been established. This study aimed to elucidate the clinicopathological features of sporadic nonampullary duodenal epithelial tumors, including their molecular characteristics and prognostic factors.

METHODS: One hundred forty-eight patients with sporadic nonampullary duodenal epithelial tumors were examined in this study. Patient sex, age, TNM stage, tumor location, treatment methods, histology, KRAS mutation, BRAF mutation, Fusobacterium nucleatum, mucin phenotype, and programmed death-ligand 1 (PD-L1) status were evaluated. KRAS and BRAF mutations, Fusobacterium nucleatum, mucin phenotype, and PD-L1 status were analyzed by direct sequencing, quantitative polymerase chain reaction, and immunochemical staining.

RESULTS: The median follow-up duration was 119.4 months. There were no deaths from duodenal adenoma (the primary disease). Kaplan-Meier analysis for duodenal adenocarcinoma showed a significant effect of TNM stage (P < 0.01). In univariate analysis of primary deaths from duodenal adenocarcinoma, TNM stage II or higher, undifferentiated, KRAS mutations, gastric phenotype, intestinal phenotype, and PD-L1 status were significant factors. In multivariate analysis, TNM stage II or higher (hazard ratio: 1.63 × 10^10, 95% confidence interval: 18.66–6.69 × 10^10) and KRAS mutation (hazard ratio: 3.49, confidence interval: 1.52–7.91) were significant factors.

DISCUSSION: Only KRAS mutation was a significant prognostic factor in primary sporadic nonampullary duodenal adenocarcinoma in cases in which TNM stage was considered.

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A717 and http://links.lww.com/CTG/A718

INTRODUCTION

In recent years, the clarification of gastrointestinal tumor characteristics has been enabled by instrumentation and reagent developments in endoscopy and DNA sequencing (1). In particular, the molecular biological characteristics of colorectal cancer have been determined, and the choice of treatment such as EGFR inhibitors, BRAF inhibitors, angiogenesis inhibitors, and immune checkpoint inhibitors differs in clinical practice depending on the presence or absence of KRAS mutation, BRAF mutation, and microsatellite instability (2). Tumor characteristics also contribute to prognosis, with anatomical location, such as the right or left side of the colon, identified as an important factor in determining treatment selection (3). Sporadic nonampullary duodenal epithelial tumors (SNADETs) have been reported periodically. However, low detection rates compared with gastric and colorectal tumors have hampered efforts to analyze their clinicopathology. The prevalence of SNADETs is extremely low (0.02%–0.5%) (4–6), and primary sporadic nonampullary duodenal adenocarcinoma accounts for only 0.5% of all gastrointestinal malignancies (7). However, SNADET detection rates have improved with the development of endoscopic diagnosis techniques (8). Furthermore, SNADETs...
cases with a poor prognosis are on the rise (9). Consequently, the importance of research into SNADETs clinicopathology has been recognized, and their characteristics are becoming clearer as a result (10–12).

Whole-genome sequencing has revealed new insights into the genes involved in primary sporadic nonampullary duodenal adenocarcinoma (13). The genomic characterization of primary sporadic nonampullary duodenal adenocarcinoma indicates a genetic resemblance to gastric and colorectal cancers (13). However, a correlation between the clinical and molecular characteristics of these cancers has not been elucidated. On the other hand, the anatomical location of the tumor on the oral side of the papilla of Vater (oral side of Vater) (14,15), gastric mucin phenotype, which is one of the mucin phenotypes of the tumor (15,16), and programmed death-ligand 1 (PD-L1) status (17) are currently being investigated as factors potentially contributing to the prognosis of SNADETs. However, because of its rarity, the role of these factors has not been revealed in specific types of SNADETs, including duodenal adenoma and early- to advanced-stage duodenal adenocarcinoma.

In this study, prognostic factors were investigated in 148 patients with SNADETs. The analysis focused not only on tumor location, mucin phenotype, and PD-L1 status but also on factors such as KRAS and BRAF mutations, which are important in colorectal cancer. Furthermore, Fusobacterium nucleatum (Fn) (18), which has attracted research attention regarding its role in the progression of colorectal cancer, was also included in this investigation.

METHODS

Patients
One hundred forty-eight patients with SNADETs, treated at the Okayama University Hospital and Hiroshima City Hospital in Japan from 2006 to 2018, were enrolled in this study. Tissue samples were collected in all cases. Demographic, clinicopathological, and tumor characteristics were investigated and included patient sex, age, TNM stage, tumor location, treatment methods, histology, KRAS mutation, BRAF mutation, Fn, mucin phenotype, and PD-L1 status. The SNADETs were staged in accordance with the Union for International Cancer Control TNM staging system (19). In this study, stages 0 and I were defined as early stage and stages II, III, and IV as advanced stage. The median follow-up duration was 47.7 months (range, 0.2–163 months). Somatic mutations of KRAS and BRAF, Fn, mucin phenotype, and PD-L1 status were examined by direct sequencing, real-time quantitative polymerase chain reaction (qPCR), and immunohistochemistry.

Endoscopic treatment was used for adenomas and duodenal adenocarcinoma (stage 0), surgery for duodenal cancer without distant metastasis (stage II and stage III), and chemotherapy for duodenal adenocarcinoma with distant metastasis (stage IV).

The Institutional Review Boards of Okayama University Hospital and Hiroshima City Hospital approved this study (2103-051/2021-8), which was conducted in accordance with the Declaration of Helsinki. Patients provided either written informed consent to participate or were required to opt out if their data were accessed retrospectively.

DNA extraction
Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were obtained from patients who had been biopsied, or resected by endoscopy or surgery, for SNADETs. All tissue sections were reviewed by expert gastrointestinal pathologists (T.T. and K.I.). Histological examinations confirmed that the samples contained a minimum of 30% tumor cells. DNA was extracted from five 10-μm-thick sections of the FFPE samples using a QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA), according to the manufacturer’s instructions. All DNAs were eluted in a final volume of 50 μL and stored at −30 °C. DNAs extracted from FFPE were quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA).

Direct sequencing analysis
KRAS and BRAF were amplified using polymerase chain reaction (PCR) with forward and reverse primers (see Supplemental Table 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A718). Each 50 μL PCR reaction contained 100 nM of each primer, 1 ng template DNA, and master mix reagent (AmpliTaq Gold 360 PCR Master Mix; Applied Biosystems, Foster City, CA). Amplification conditions consisted of 10 minutes at 94 °C, followed by 40 cycles at 94 °C for 10 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds, in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). The PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. Then, the PCR products were purified before direct sequencing was performed using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) on an ABI Prism 310 genetic analyzer (Applied Biosystems). KRAS mutations in codons 12 and 13 and BRAF mutation in codon 600 were examined according to the raw nucleotide sequencing data in waveform obtained by direct sequencing.

Fn analysis
The amount of Fn DNA in tissues was measured by qPCR. Custom-made primer/probe sets were used to amplify Fn and the reference human gene solute carrier organic anion transporter family number 2A1 (SLCO2A1), as previously described (20). The primer and probe sequences are summarized in Supplemental Table 1 (Supplementary Digital Content 1, http://links.lww.com/CTG/A718). The qPCR was performed in 20 μL reactions containing 30 ng of genomic DNA (2 μL), 1× final concentration Prime time gene expression Master Mix 2.0 (IDT, Coralville, IA) (10 μL), each Prime qPCR assay (FAM/HEX) (1 μL), and deionized distilled water (6 μL). The DNA was amplified and detected with a Roche LightCycler 96 system (Roche, Basel, Switzerland) under the following conditions: 10 minutes at 95 °C, then 45 cycles of 15 seconds at 95 °C and 60 seconds at 60 °C.

All specimens were analyzed in duplicate. To exclude non-specific PCR amplification, we regarded a specimen as Fn positive when both specimens were positive. The amount of Fn DNA in each tissue was calculated by 2−ΔCt, where ΔCt was the difference in the Ct value of Fn and SLCO2A1. The mean of the 2 Ct values for each reaction was used for analysis.

Histopathological examinations and Vienna classification
For histological analysis, SNADETs tissue specimens were routinely fixed with formalin and completely embedded in paraffin. Tissue blocks were thinly sectioned, routinely processed, and stained with hematoxylin and eosin. All SNADETs were histologically graded based on the revised Vienna classification (VCL) system (21). We defined VCL category 3 as low-grade adenoma/
dysplasia, 4.1 as high-grade adenoma/dysplasia, and 4.2 as carcinoma in situ. VCL categories 5.1 and 5.2 were considered as intramucosal carcinoma and submucosal carcinoma or beyond, respectively. We classified VCL categories 3 and 4.1 as adenoma and 4.2 or more as adenosquamous carcinoma. Adenocarcinoma was subdivided into differentiated or undifferentiated types depending on histopathological grading. Two investigators (T.T. and K.I.) assessed histological grade independently, and any disagreements were resolved through consensus.

Immunohistochemical examinations
FFPE tissue blocks were cut into 3-μm-thick tissue sections and subjected to hematoxylin and eosin and immunohistochemical staining. Immunohistochemical staining was performed by the standard avidin-biotin-peroxidase complex method with an automated immunostainer (BenchMark XT; Ventana Medical System, Tucson, AZ). Mucin phenotype of SNADETs was examined using MUC2 (Cep58, monoclonal mouse; Dako, Denmark, UK), MUC5AC (CLH2, monoclonal mouse; Dako), MUC6 (CLH5, monoclonal mouse; Novus Biologicals, Littleton, CO), and CD10 (56C6, monoclonal mouse; Leica Biosystems, Newcastle, UK). The tumor’s cytoplasmic immunoreactivity was judged positive for MUC2, MUC5AC, and MUC6. Luminal membrane immunoreactivity was judged positive for CD10. Immunohistochemical staining for gastric phenotype markers (MUC5AC and MUC6) and intestinal phenotype markers (MUC2 and CD10) were considered positive when distinct staining was observed in >10% of the cancer cells. The SNADETs were classified into 4 subtypes based on mucin immunohistochemistry: (1) gastric phenotype, (2) intestinal phenotype, (3) gastric and intestinal phenotype (mixed phenotype), and (4) not staining (null phenotype) (see Supplementary Figure 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A717).

For PD-L1 staining, sections were retrieved in EDTA buffer (pH 8.0) at 98°C for 20 minutes. A monoclonal antibody was used against the membranous and cytoplasmic domain of PD-L1 (SP263, monoclonal rabbit; Ventana Medical Systems) in immunostaining, and reactivity was evaluated for cancer cells. PD-L1 positivity was defined as a positive cell staining of any intensity on ≥1% of the cell membrane and cytoplasm.

Two investigators (T.T. and K.I.), blinded to the patients’ clinical information, collaboratively assessed the immunohistochemical results as well as the histological analysis and VCL classification.

Statistical analysis
All continuous variables are reported as the median (range), and comparisons were made using the Wilcoxon rank-sum test. All categorical variables are summarized as frequencies (percentages), with Pearson χ² or Fisher exact tests used for examining comparisons. Overall survival (OS) was estimated by the Kaplan-Meier method, and differences were evaluated using the log-rank test. A Cox proportional hazard model was used to assess OS by TNM stage, tumor location, treatment methods, KRAS mutation, BRAF mutation, Fn, mucin phenotype, and PD-L1 status. All statistical tests were 2 sided, and a P value less than 0.05 was considered statistically significant. Statistical analyses were performed using the JMP 14 software program (SAS Institute, Cary, NC).

RESULTS

Patient characteristics
Of the 148 patients with SNADETs, 55 and 93 had duodenal adenomas and duodenal adenosquamous carcinomas, respectively. The median age was 67 years, and there were 97 men and 51 women. Tumors were located on the oral (n = 79) or anal (n = 69) side of the papilla of Vater. Some patients also received endoscopic treatment (n = 71), surgical procedures (n = 59), chemotherapy (n = 9), and other treatments (n = 9; Table 1).

There were no cases of primary mortality from duodenal adenoma. On the other hand, primary mortality from duodenal adenosquamous carcinoma increased with TNM stage, involving 0, 0, 4, 11, and 16 patient deaths from stages 0, I, II, III, and IV, respectively. Kaplan-Meier analysis divided on TNM stage revealed a significant effect on OS, as median survival was 93.1 months in stage II, 42.9 months in stage III, and 9.5 months in stage IV (P < 0.01; Figure 1).

Genetic and epigenetic characteristics
Of the 148 patients with SNADETs, 33 had KRAS mutation, 13 had BRAF mutation, and 29 were Fln positive. Furthermore, a range of patients demonstrated gastric phenotype (n = 41), intestinal phenotype (n = 74), mixed phenotype (n = 24), and null phenotype (n = 9; Table 1).

Comparing the 55 patients with duodenal adenoma with the 93 patients with duodenal adenosquamous carcinoma showed significant differences in anatomical location (P < 0.01), treatment methods (P < 0.01), BRAF mutation (P = 0.01), Fln (P = 0.03), and mucin phenotype (P < 0.01). On the other hand, the 45 patients with early-stage duodenal adenosquamous carcinoma showed significant differences in anatomical location (P = 0.04), treatment methods (P < 0.01), tumor histology (P < 0.01), BRAF mutation (P = 0.01), mucin phenotype (P < 0.01), and PD-L1 status (P < 0.01) compared with the 48 patients with advanced-stage duodenal adenosquamous carcinoma (Table 1).

Duodenal adenocarcinoma
Kaplan-Meier analysis of survival in duodenal adenosquamous carcinoma showed that TNM stage II or higher (P < 0.01), anatomical location (oral side of Vater; P = 0.03), undifferentiated (P < 0.01), KRAS mutation (P < 0.01), gastric phenotype (P = 0.01), intestinal phenotype (P < 0.01), and PD-L1 status (P < 0.01) significantly influenced patient outcomes (Figures 1–3).

In univariate analysis of primary mortality from duodenal adenosquamous carcinoma, TNM stage II or higher (hazard ratio [HR]: 1.8 × 10^-5, 95% confidence interval [CI]: not calculable; P < 0.01), undifferentiated (HR: 3.66, CI: 1.43–8.24; P < 0.01), KRAS mutation (HR: 2.44, CI: 1.14–4.99; P = 0.02), gastric phenotype (HR: 2.45, CI: 1.19–5.30; P = 0.01), intestinal phenotype (HR: 0.15, CI: 0.03–0.43; P < 0.01), and PD-L1 status (HR: 2.84, CI: 1.37–5.77; P < 0.01) were significant factors (Table 2). In multivariate analysis, TNM stage II or higher (HR: 1.63 × 10^-5, CI: 18.66–6.69 × 10^-6; P < 0.01) and KRAS mutation (HR: 3.49, CI: 1.52–7.91; P < 0.01) were found to be significant factors (Table 2).

Characteristics of the patients with KRAS mutation
Patients with (n = 33) and without (n = 115) KRAS mutation exhibited significant differences in sex (P = 0.02), BRAF mutation (P < 0.01), and mucin phenotype (P < 0.01; Table 3). Moreover, of the 55 patients with duodenal adenoma, there were 10 patients with KRAS mutation, but no significant differences were found...
between these patients and the other 45 patients without KRAS mutation. However, among the 93 patients with duodenal adenocarcinoma, there were 23 patients with KRAS mutation, and they were significantly different from the 70 patients without KRAS mutation in terms of sex \((P = 0.01)\), BRAF mutation \((P < 0.01)\), and mucin phenotype \((P = 0.01; \text{Table 3})\).

Figure 1. Kaplan-Meier plots for duodenal adenocarcinoma with TNM stage. There were no cases of primary mortality from duodenal adenocarcinoma in stages 0 and I. Median survival times in stages II, III, and IV were 93.1 months, 42.9 months, and 9.5 months, respectively \((P < 0.01)\). This figure indicates disease specific survival.

### Table 1. Patient and tumor characteristics

| Neoplasia \((n = 148)\) | Adenoma \((n = 55)\) | Adenocarcinoma \((n = 93)\) | \(P\) value | Adenocarcinoma (n = 93) | Stages 0 and I \((n = 45)\) | Stages II, III, and IV \((n = 48)\) | \(P\) value |
|-------------------------|----------------------|-----------------------------|-------------|----------------------|-----------------------------|-----------------------------|-------------|
| Sex (male/female)       | 36/19                | 61/32                       | 0.98        | 28/17                | 33/15                       | 0.50                        |
| Age, median (range)     | 65 (36–83)           | 68 (29–90)                  | 0.33        | 68 (36–84)           | 68 (29–90)                  | 0.54                        |
| VCL (3/4/1.4/2.5/1.5/2) | 52/3/0/0/0            | 0/0/30/8/5                 |             | 0/0/30/8/7           | 0/0/0/48                    | —                           |
| TNM stage (0/II/III/IV) | —                    | 30/15/11/16/21             | <0.01\*     | 30/15/0/0/0          | 0/0/11/16/21                | —                           |
| Location (oral Vater/anal Vater) | 20/35                | 59/34                       | <0.01\*     | 24/21                | 35/13                       | 0.04\*                      |
| Treatment (endoscopy/surgery/chemo/others) | 50/50/00            | 21/54/9/9/9                | <0.01\*     | 21/24/0/0/0          | 0/30/9/9                    | <0.01\*                      |
| Histology (differentiated/undifferentiated) | —                    | 81/12                       | —           | 44/1                 | 37/11                       | <0.01\*                      |
| KRAS, n (%)             | 10 (18.1)            | 23 (24.7)                   | 0.34        | 8 (17.7)             | 15 (31.2)                   | 0.12                        |
| BRAF, n (%)             | 1 (1.8)              | 12 (12.9)                   | 0.01\*      | 2 (4.4)              | 10 (20.8)                   | 0.01\*                      |
| Fusobacterium, n (%)    | 6 (10.9)             | 23 (24.7)                   | 0.03\*      | 11 (24.4)            | 12 (25.0)                   | 0.95                        |
| Mucin phenotype (gastric/intestinal/mix/null) | 2/40/12/1          | 39/34/12/8                  | <0.01\*     | 11/26/8/0           | 28/8/48                     | <0.01\*                      |
| PD-L1 (negative/positive) | —                    | 68/25                       | —           | 41/4                 | 27/21                       | <0.01\*                      |

Chemo, chemotherapy; PD-L1, programmed death-ligand 1; VCL, Vienna classification.

*Statistically significant difference.
DISCUSSION
We analyzed prognostic factors in 148 patients with SNADETs, focusing on TNM stage, the anatomical location of the tumor, KRAS mutation, BRAF mutation, F unveil phenotype, and PD-L1 status. There were no primary deaths from nonampullary duodenal adenoma in this study. KRAS mutation was an independent factor for primary mortality in nonampullary duodenal adenocarcinoma, regardless of TNM stage (stage II or higher). These results indicate that KRAS mutation is a more important prognostic factor than anatomical location (oral side of Vater), gastric phenotype, and PD-L1 status, which have previously been reported as poor prognostic factors in sporadic nonampullary duodenal adenocarcinoma (14–16).

In the present study, anatomical location, mucin phenotype, and PD-L1 status were significant factors influencing OS (Kaplan-Meier analysis), but its effect was not supported in multivariate analysis. These findings demonstrate that careful consideration should be given to a patient’s background information when evaluating mucin phenotype and PD-L1 status as prognostic factors. However, there seems to be some correlation between higher TNM stage and mucin phenotype and PD-L1 status (Table 1), suggesting that early detection may be difficult due to rapid clinical progression. The high prevalence of PD-L1 in more advanced TNM stages suggests that a more personalized treatment strategy, such as immune checkpoint inhibitors, could be possible for advanced-stage duodenal adenocarcinoma.

With regard to the importance of KRAS mutations, there have been no previous reports showing a relationship between KRAS mutation and prognosis. Although some studies have reported subanalyses, all of which might have lacked significance because of the small number of patients included and the distribution of TNM stages (17,22). In our study, KRAS mutation was an independent prognostic factor along with TNM stage (stage II or higher), unlike anatomical location, mucinous phenotype, and PD-L1 status. Moreover, even if stages II, III, and IV were analyzed separately, KRAS mutation remained the only significant factor in stages II and III. Considering the poor prognosis of stage IV, the importance of KRAS mutation as a prognostic factor in stages II and III is even more distinguished (HR: 4.01 × 10^2, CI: 2.70–4.90 × 10^2, P = 0.01; HR: 17.04, CI: 2.01–433.72; P < 0.01). Also, it is very interesting to note from Table 3 that there is a relationship between KRAS and sex or gastric phenotype.
However, KRAS and BRAF could be understood from the perspective of a paradoxical relationship.

The incidence of KRAS mutation in duodenal adenoma, early-stage duodenal adenocarcinoma, and advanced-stage duodenal adenocarcinoma was 18.1%, 17.7%, and 31.2%, respectively, with no significant differences (P = 0.20). There were cases of duodenal adenoma and early-stage duodenal adenocarcinoma with KRAS mutation but without primary mortality. Therefore, KRAS mutation in advanced-stage duodenal adenocarcinoma might have specific implications, and active treatment might be important for preventing advanced-stage neoplasia.

Relationships between the role of KRAS mutation and prognosis have been reported in various carcinomas such as colorectal cancer (23,24) and pancreatic cancer (25). Among the KRAS mutations, mutations in codon 12 and codon 13 are particularly noteworthy. Some basic studies have shown that the basic GTPase activity of G12V is about one-fourth that of G12D and one-tenth that of wild-type KRAS (26,27). Furthermore, Rat-1 cells with G12V mutations have been shown to be significantly more invasive in vitro than clones with G12D mutations or wild-type KRAS (28,29). Based on these results, it is easy to speculate that cells with G12V mutations are more invasive and contribute to a worse prognosis. In our current results, we could not find any significant difference between G12V and other type of KRAS mutation in relation to prognosis (P = 0.20), although median survival time in G12V was shorter than others (11 months and 31.5 months, respectively). To clarify which type of KRAS mutation is particularly associated with prognosis, we will continue to accumulate more cases.

The importance of Fn in colorectal tumors has recently been revealed. We have previously reported the presence of Fn in colorectal adenoma and colorectal cancer (20), and we hypothesized that Fn might play an important role in SNADETs as well. The incidence of Fn in duodenal adenoma, early-stage duodenal adenocarcinoma, and advanced-stage duodenal adenocarcinoma was 10.9%, 24.4%, and 25.0%, respectively (P = 0.10). However, Fn was not found to be a prognostic factor in this study.

Several limitations of the present study should be noted. First, this was a retrospective study, which may not provide the same level of evidence that could be achieved with a prospective study. Furthermore, comparisons based on other demographic factors, such as race, were not possible. It is hoped that international collaborative studies will enable the collection of more cases for investigation. Second, the prognosis shown in Kaplan-Meier analysis requires careful interpretation because sample size of this study is inevitable.
small due to the focus on rare disease. Third, although some previous study demonstrated higher risk of malignant transformation in duodenal adenocarcinoma with CpG island methylator phenotype (30), current study did not evaluate CpG island methylation. Although conventional endoscopic treatment for duodenal adenomas and early-stage duodenal adenocarcinomas may be acceptable, the present findings suggest that treatment strategies for advanced-stage duodenal adenocarcinomas could potentially undergo a major shift. In light of our new findings, it is likely that molecularly targeted therapies, such as KRAS inhibitors, BRAF inhibitors, angiogenesis inhibitors, and immune checkpoint inhibitors, should be validated for treating advanced-stage duodenal adenocarcinoma, similar to the studies of colorectal cancer. If the response of duodenal adenocarcinoma was similar to that of colorectal cancer, angiogenesis inhibitors would be better than EGFR inhibitors for the treatment of duodenal adenocarcinoma with KRAS mutation. KRAS status might also be

| Table 2. Univariate and multivariate analyses of primary mortality from duodenal adenocarcinoma |
| --- |
| | Univariate | Multivariate |
| | HR | P value | HR | P value |
| TNM stage (II, III, IV/0I) | 1.80 x 10^10 (NA) | <0.01a | 1.63 x 10^10 (18.66–6.69 x 10^20) | <0.01a |
| Location (anal Vater/oral Vater) | 0.53 (0.23–1.13) | 0.10 |
| Histology (undifferentiated/differentiated) | 3.66 (1.43–8.24) | <0.01a | 1.41 (0.43–4.24) | 0.54 |
| KRAS (mutation/wild) | 2.44 (1.14–4.99) | 0.02a | 3.49 (1.52–7.91) | <0.01a |
| BRAF (mutation/wild) | 1.71 (0.63–3.91) | 0.26 |
| Fusobacterium (positive/negative) | 1.51 (0.65–3.21) | 0.31 |
| Mucin phenotype (gastric/others) | 2.45 (1.19–5.30) | 0.01a | 0.58 (0.22–1.64) | 0.29 |
| Mucin phenotype (intestinal/others) | 0.15 (0.03–0.43) | <0.01a | 0.24 (0.04–1.05) | 0.05 |
| Mucin phenotype (mix/others) | 0.92 (0.22–2.63) | 0.90 |
| PD-L1 (positive/negative) | 2.76 (0.92–6.74) | 0.06 |

HR, hazard ratio; NA, not available; PD-L1, programmed death-ligand 1.
aStatistically significant difference.

| Table 3. Clinicopathological features focusing on the KRAS gene |
| --- |
| Neoplasia (n = 148) | Adenoma (n = 55) | Adenocarcinoma (n = 93) |
| KRAS negative (n = 115) | KRAS positive (n = 33) | P value | KRAS negative (n = 45) | KRAS positive (n = 10) | P value | KRAS negative (n = 70) | KRAS positive (n = 23) | P value |
| Sex (male/female) | 81/34 | 16/17 | 0.02a | 30/15 | 6/4 | 0.69 | 51/19 | 10/13 | 0.01a |
| Age, median (range) | 67 (29–84) | 68 (46–90) | 0.18 | 65 (36–83) | 67.5 (46–80) | 0.85 | 67 (29–84) | 69 (51–90) | 0.16 |
| Tumor (adenoma/adenocarcinoma) | 45/70 | 10/23 | 0.34 | — | — | — | — | — | — |
| TNM stage (0/I/II/III/IV) | — | — | — | — | — | — | 25/12/8/10/15 | 5/3/6/6 | 0.59 |
| Location (oral Vater/anal Vater) | 57/58 | 22/11 | 0.07 | 14/31 | 6/4 | 0.09 | 43/27 | 16/7 | 0.47 |
| Treatment (endoscopy/surgery/chemo/others) | 57/46/6/6 | 14/13/3/3 | 0.69 | 41/4/0/0 | 9/1/0/0 | 0.91 | 16/42/6/6 | 5/12/3/3 | 0.83 |
| Histology (differentiated/undifferentiated) | — | — | — | — | — | — | 60/10 | 21/2 | 0.47 |
| KRAS, n (%) | — | — | — | — | — | — | — | — | — |
| BRAF, n (%) | 13 (11.3) | 0 (0) | <0.01a | 1 (2.2) | 0 (0) | 0.52 | 12 (17.1) | 0 (0) | <0.01a |
| Fusobacterium, n (%) | 21 (18.2) | 8 (24.2) | 0.45 | 5 (11.1) | 1 (10.0) | 0.91 | 16 (22.8) | 7 (30.4) | 0.47 |
| Mucin phenotype (gastric/intestinal/mix/null) | 24/65/18/8 | 17/9/6/1 | <0.01a | 1/36/7/1 | 1/4/5/0 | 0.06 | 23/29/11/7 | 16/5/1/1 | 0.01a |
| PD-L1 (positive/positive) | — | — | — | — | — | — | 52/18 | 16/7 | 0.66 |
| PD-L1, programmed death-ligand 1. | — | — | — | — | — | — | — | — | — |
aStatistically significant difference.
Sporadic nonampullary duodenal adenocarcinoma had better survival.

**KRAS** mutation of sporadic nonampullary duodenal adenocarcinoma was a significant prognostic factor.

**REFERENCES**

1. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer–associated genes. Nature 2013;499:214–8.

2. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350–6.

3. Petrelli F, Tomasello G, Borgonovo K, et al. Prognostic survival associated with left-sided vs right-sided colon cancer: A systematic review and meta-analysis. JAMA Oncol 2017;3:211–9.

4. Shukla SK, Elias EG. Primary neoplasms of the duodenum. Surg Gynecol Obstet 1976;142:858–60.

5. Hofmng BP, Grayzel DM. Benign tumors of the duodenum. Am J Surg 1945;70:394–400.

6. Darling RC, Welch CE. Tumors of the small intestine. N Engl J Med 1959;260:397–408.

7. Alwmark A, Andersson A, Lasson A. Primary carcinoma of the duodenum. Ann Surg 1980;191:13–8.

8. Goda K, Kikuchi D, Yamamoto Y, et al. Endoscopic diagnosis of superficial non-ampullary duodenal epithelial tumors in Japan: Multicenter case series. Dig Endosc 2014;26(Suppl 2):23–9.

9. Sakae H, Kanzaki H, Nasu J, et al. The characteristics and outcomes of small bowel adenocarcinoma: A multicentre retrospective observational study. Br J Cancer 2017;117:1607–13.

10. Okada K, Fujisaki J, Kasuga A, et al. Sporadic nonampullary duodenal adenocarcinoma in the natural history of duodenal cancer: A study of follow-up surveillance. Am J Gastroenterol 2011;106:357–64.

11. Matsuzaki J, Suzuki H, Shimoda M, et al. Clinical and endoscopic findings to assist the early detection of duodenal adenoma and adenocarcinoma. United European Gastroenterol J 2019;7:250–60.

12. Endo M, Abiko Y, Oana S, et al. Usefulness of endoscopic treatment for duodenal adenoma. Dig Endosc 2016;28:360–5.

13. Yuan W, Zhang Z, Dai B, et al. Whole-exome sequencing of duodenal adenocarcinoma identifies recurrent Wnt/beta-catenin signaling pathway mutations. Cancer 2016;122:1689–96.

14. Niwa A, Kuwano S, Tomita H, et al. The different pathogeneses of sporadic adenoma and adenocarcinoma in non-ampullary lesions of the proximal and distal duodenum. Oncotarget 2017;8:41078–90.

15. Matsuoka K, Kanzaki H, Matsuoka K, et al. The clinicopathological differences of sporadic non-ampullary duodenal epithelial neoplasm depending on tumor location. J Gastroenterol Hepatol 2019;34:1540–4.

16. Toba T, Inoshita N, Kaise M, et al. Clinicopathological features of superficial non-ampullary duodenal epithelial tumor; gastric phenotype of histology correlates to higher malignant potency. J Gastroenterol 2018;53:64–70.

17. Watanabe J, Maitani S, Ito C, et al. Molecular alterations and PD-L1 expression in non-ampullary duodenal adenocarcinoma: Associations among clinicopathological, immunophenotypic and molecular features. Sci Rep 2019;9:10526.

18. Yachida S, Mizutani S, Shiroma H, et al. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. Nat Med 2019;25:968–76.

19. Bertolo L, Massa F, Metovic J, et al. Eighth edition of the UICC classification of malignant tumors: An overview of the changes in the pathological TNM classification criteria—What has changed and why? Virchows Arch 2018;472:519–31.

20. Yamamoto S, Kinugasa H, Hirai M, et al. Heterogeneous distribution of Fusobacterium nucleatum in the progression of colorectal cancer. J Gastroenterol Hepatol 2021;36(7):1869–1876.

21. Schlemper RJ, Riddell RH, Kato Y, et al. The Vienna classification of gastrointestinal epithelial neoplasia. Gut 2006;54:251–5.

22. Matsuba A, Sekine S, Kusima R, et al. Frequent GNAS and KRAS mutations in pyloric gland adenoma of the stomach and duodenum. J Pathol 2013;229:579–87.

23. Andrevey HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: The “RASCAL II” study. Br J Cancer 2001;85:692–6.

24. Bazan V, Migliavacca M, Zanna I, et al. Specific codon 13 K-ras mutations are predictive of clinical outcome in colorectal cancer patients, whereas codon 12 K-ras mutations are associated with mucinous histotype. Ann Oncol 2002;13:1438–46.

25. Ogura T, Yamazaki K, Hara K, et al. Prognostic value of K-ras mutation status and subtypes in endoscopic ultrasound-guided fine-needle aspiration specimens from patients with unresectable pancreatic cancer. J Gastroenterol 2013;48:640–6.

26. John J, Frech M, Wittinghofer A. Biochemical properties of Ha-ras encoded p21 mutants and mechanism of the autophosphorylation reaction. J Biol Chem 1988;263:11792–8.

27. Kaplan PL, Ozanne B. Cellular responsiveness to growth factors correlates with a cell’s ability to express the transformed phenotype. Cell 1986;44:931–8.

28. Al-Mulla F, MacKenzie EM. Differences in vitro invasive capacity induced by differences in Ki-Ras protein mutations. J Pathol 2001;195:459–60.

29. Fu T, Pappou EP, Guzzetta AA, et al. CpG island methylator phenotype-positive tumors in the absence of MLH1 methylation constitute a distinct group of colorectal cancers. Clin Cancer Res 2012;18:4743–52.

30. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: Towards implementation of circulating tumour DNA. Nat Rev Cancer 2017;17:233–8.