Genomic Landscape and Clinical Impact of Homologous Recombination Repair Gene Mutation in Small Bowel Adenocarcinoma

Short title: Clinical impact of HRR gene mutation in SBA

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Financial Support: This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (#22H02828 and #24K11153 to M.O.), grants from the Foundation for Promotion of Cancer Research (to M.O.), and grants from the Mitsubishi Foundation (to M.O.).

ABSTRACT

Background:

Small bowel adenocarcinoma (SBA) is a rare malignancy with a poor prognosis and limited treatment options. Although homologous recombination deficiency has been studied as a biomarker for other cancer types, the clinical and genomic implications of homologous recombination repair (HRR) gene mutations in SBA remain unclear.

Methods:

We retrospectively analyzed the data of 628 patients with advanced or recurrent SBA from a nationwide genomic database. Patients were categorized into HRR mutation and non-HRR mutation groups and compared for their clinical and genomic characteristics including tumor mutational burden (TMB) and microsatellite instability-high (MSI-H) were compared. Treatment efficacy and overall survival (OS) were assessed based on HRR gene mutation status and primary tumor site (duodenal adenocarcinoma [DA] vs. small intestinal carcinoma [SIC]).

Results:

Patients with the HRR mutations had higher frequencies of TMB and MSI-H than those without the mutation ($P < 0.0001$). In DA, HRR gene mutation positivity was associated with improved OS and higher overall response rates (ORR) to platinum-based chemotherapy (OS: not reached vs. 23.5 months, $P = 0.040$; ORR: 33% vs. 19%, $P = 0.046$), whereas no significant associations were observed with SIC.

Conclusion:

HRR gene mutation may be a potential biomarker for platinum-based chemotherapy efficacy in SBA, especially in DA, highlighting the need for site-specific therapies.

Keywords: homologous recombination repair; small bowel adenocarcinoma; genome

INTRODUCTION

Small bowel adenocarcinoma (SBA) is a rare malignancy with a poor prognosis [1], and its incidence is increasing [2]. However, its low incidence poses a challenge for comprehensive genomic profiling (CGP), thereby hindering the development of effective targeted therapies. Consequently, elucidating its genomic characteristics and identifying effective therapeutic targets are clinically significant.

Recent large-scale genomic analyses have revealed a distinct genomic profile for SBA compared to gastric and colorectal adenocarcinomas [3]. Furthermore, small bowel subsites have different clinicopathological and molecular features [4]. Prognostic differences have also been observed, with proximal adenocarcinomas associated with worse outcomes than distal adenocarcinomas [5, 6].

Homologous recombination deficiency (HRD) has been recently considered an important factor influencing prognosis and treatment efficacy across various cancer types [7-9]. Some recent studies have investigated the impact of HRD by examining the incidence of mutations in homologous recombination repair (HRR) gene mutation [10, 11]. However, the role of genomic profiling focusing on HRR gene mutations in relation to primary tumor location and treatment outcomes in SBA remains unclear owing to limited research. This study investigated the clinical significance of HRR mutations in patients with SBA. Specifically, we sought to elucidate the differences in genomic profiles based on HRR gene mutations and assess the impact of HRR gene mutations on treatment efficacy and prognosis.

MATERIALS AND METHODS

Study flow and patient selection

We retrospectively analyzed the clinical and genomic datasets of patients with unresectable or recurrent SBA registered in the Center for Cancer Genomics and Advanced Therapeutics (C-CAT) database between August 2019 and May 2025. Patients were categorized into the HRR mutation and non-HRR mutation groups. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the institutional review board (2111-047) and C-CAT Ethics Committee (CDU2022-012E02).

Data acquisition and genomic annotation

Demographic, clinical, and therapeutic details including age, sex, primary tumor site, Eastern Cooperative Oncology Group Performance Status (ECOG PS), family history of cancer, smoking and alcohol drinking habits, type of CGP test, tissue sampling site, and percentage of platinum-based regimens in first-line chemotherapy were collected. The primary tumor sites were classified into duodenal adenocarcinoma (DA) and small intestinal carcinoma (SIC) based on the terminology used in the C-CAT database, with DA referring to tumors originating in the duodenum, and SIC encompassing those originating in the jejunum and ileum. Genetic analyses focused on the variant frequencies of genes with mutation rates exceeding 5% including mismatch repair (MMR) genes (*MSH6, MLH1, MSH2,* and *PMS2*) and HRR genes (*ARID1A, ATM, ATRX, BAP1, BARD1, BRCA1, BRCA2, BRIP1, CDK12, FANCA, FANCC, MRE11, PALB2, PTEN, RAD51D,* and *RAD51C*). Only variants classified as “oncogenic” or “pathogenic” were included, whereas those with uncertain clinical significance were excluded. Genomic data were annotated using the C-CAT database, which consolidates global resources on gene alterations, therapeutic options, and clinical trials [12].

Genomic profiling methods and quality assurance

Tumor samples were genomically profiled using validated panel assays. Formalin-fixed paraffin-embedded tissue samples were processed using the Foundation One CDx (Foundation Medicine), OncoGuide NCC Oncopanel System (Sysmex Corporation), or GeneMine TOP (Konica Minolta Inc.), whereas liquid biopsies were analyzed using Foundation One Liquid CDx (Foundation Medicine) or Guardant 360 CDx (Guardant Health Incorporated) assays. Quality control checks including nucleic acid concentration, yield, and fragmentation ensured sample integrity before testing. Liquid biopsy testing was employed if the tissue samples did not meet the quality standards.

HRR gene mutation status and its impact on genomic profiling and treatment outcomes

HRR gene mutation positivity was defined as the presence of germline or somatic genetic alterations in the HRR variants. The clinical and genomic characteristics of the HRR mutation and non-HRR mutation groups including TMB and microsatellite instability (MSI) status were compared across all SBA cases and within specific primary tumor sites. Co-occurring and mutually exclusive mutations were analyzed using heat maps to elucidate patterns that highlight the biological significance of specific genomic alterations and to inform potential therapeutic strategies. The treatment efficacy of first-line platinum-based chemotherapy was assessed by evaluating the overall response rate (ORR) and disease control rate (DCR). ORR was defined as the proportion of patients achieving the best overall response (complete or partial response), whereas DCR was defined as the proportion of patients achieving complete response, partial response, or stable disease. Overall survival (OS) defined as the time from treatment initiation to death. Patients with incomplete clinical data or those enrolled in clinical trials were excluded from the survival analyses.

Statistical analysis

Sequential data are expressed as medians and interquartile ranges. Categorical data were compared using the chi-square test or Fisher's exact test. Nonparametric data were compared using the Wilcoxon signed-rank test. Kaplan–Meier curves were generated to estimate OS, and differences between the groups were evaluated using the log-rank test. Median values and 95% confidence intervals were calculated. A two-tailed $P < 0.05$ was considered significant. All computations were performed using the JMP Pro 17 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient characteristics

In total, 628 patients with advanced or recurrent SBA (130 and 498 patients with and without HRR mutations, respectively) were included. CGP was performed for all participants. After excluding cases with insufficient OS data, OS was evaluated in 90 HRR mutation and 374 non-HRR mutation cases. Subsequently, treatment efficacy was analyzed in a subset of patients who received a platinum-based regimen as first-line chemotherapy including 87 HRR mutation and 358 non-HRR mutation patients **(Figure 1)**.

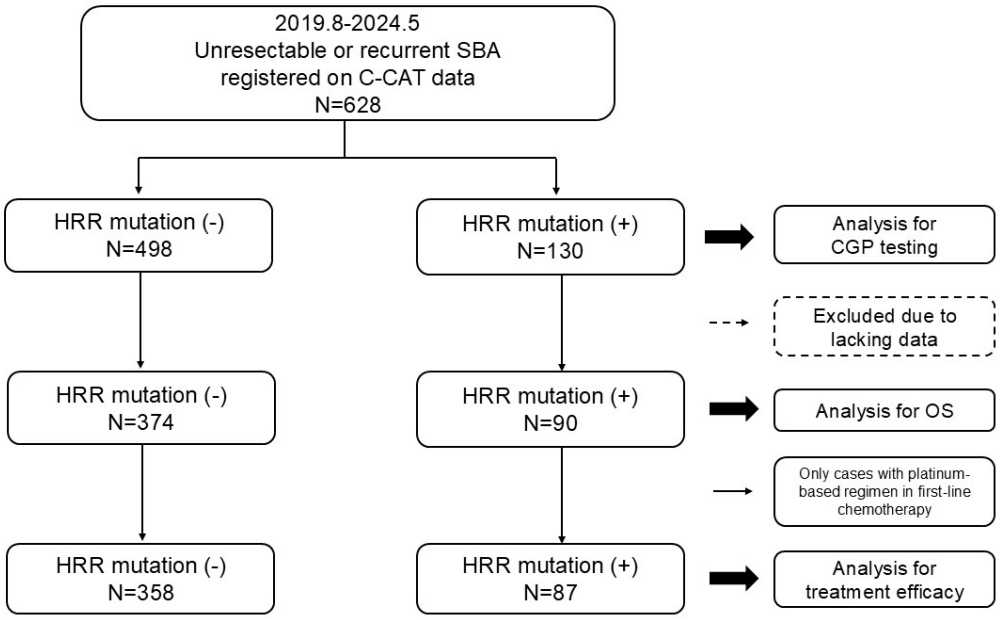

Figure 1. Study flowchart
We identified 628 patients diagnosed with unresectable or recurrent small bowel adenocarcinoma from the Center for Cancer Genomics and Advanced Therapeutics database between August 2019 and May 2024. These patients were categorized into two cohorts based on their homologous recombination repair (HRR) gene mutation status as follows: HRR mutation (n = 130) and non-HRR mutation (n = 498). Comprehensive genomic profiling analysis was performed in both groups. Overall survival (OS) was subsequently analyzed between the two cohorts. Patients enrolled in clinical trials or those with incomplete survival or treatment data were excluded (40 cases in the HRR mutation group and 124 cases in the non-HRR mutation group were excluded from the OS analysis). Consequently, 90 HRR mutation and 374 non-HRR mutation patients were included in OS analysis. We also compared the treatment efficacy of first-line platinum doublet chemotherapy between the two groups. A total of 87 HRR mutation and 358 non-HRR mutation patients were included in this analysis. SBA, small bowel adenocarcinoma; HRR, homologous recombination repair; C-CAT, Center for Cancer Genomics and Advanced Therapeutics; CGP, comprehensive genomic profiling; OS, overall survival; N, number.

Table 1 summarizes the clinical characteristics of patients stratified by HRR gene mutation status. No statistically significant differences in age, sex, primary tumor site, ECOG PS performance status, family history of cancer, smoking and alcohol use, CGP test type, tissue sampling site, and platinum-based first-line regimen were observed between the two groups **(Table 1)**.

	HRR gene mutation (+) n = 498	HRR gene mutation (−) n = 130	*P*-value
Age, years, median (IQR)	65 (56–72)	65 (54–72)	0.56
Sex, n (male/female)	303/195	87/43	0.22
Primary site, n (%)			
DA	262 (53)	70 (54)	0.84
SIC	236 (47)	60 (46)	0.84
ECOG PS 0–1, n (%)	463 (93)	124 (95)	0.43
Family history of cancer n (%)	342 (69)	88 (68)	0.83
Smoking, n (%)	222 (45)	65 (50)	0.28
Alcohol, n (%)	62 (12)	12 (9.2)	0.36
Genomic profiling test, n (%)			0.92
F1CDx	407 (82)	106 (82)	
NOP	42 (8.4)	12 (9.2)	
F1L	36 (7.2)	9 (6.9)	
TOP	10 (2.0)	3 (2.3)	
Guardant 360	3 (0.6)	0	
Tissue sampling area, n (%)			0.39
Primary	377 (82)	103 (85)	
Metastatic	81 (18)	17 (14)	
Unknown	1 (0.2)	1 (0.8)	
Platinum regimen in the first-line chemotherapy, n (%)	358 (72)	87 (67)	0.28

Table 1. Clinical characteristics between homologous recombination repair gene mutation-positive and -negative patients

HRR, homologous recombination repair; IQR, interquartile range; n, number; DA, duodenal adenocarcinoma; SIC, small intestinal carcinoma; ECOG-PS, Eastern Cooperative Oncology Group performance status; F1CDx, FoundationOne CDx; NOP, OncoGuide NCC Oncopanel System; F1L, FoundationOne Liquid CDx; Guardant 360, Guardant 360 CDx; TOP, GenMine TOP.

MSI status and TMB in relation to HRR gene mutation

First, we evaluated MSI status and TMB values according to HRR gene mutation status. The frequency of MSI-high (MSI-H) was significantly higher in HRR gene-mutation-positive patients than in HRR gene-mutation-negative patients (18% vs. 1.2%, $P < 0.0001$) **(Figure 2A)**. Likewise, the median TMB values were significantly elevated in HRR gene mutation-positive cases compared to that in HRR gene mutation-negative cases (5.0 Muts/Mb vs. 3.8 Muts/Mb, $P < 0.0001$) **(Figure 2D)**. Furthermore, the proportion of MSI-H cases was significantly higher in HRR mutation patients than in non-HRR mutation patients in both the DA (20% vs. 0.8%, $P < 0.0001$) and SIC subgroups (15% vs. 1.7%, $P = 0.0001$) **(Figure 2B and C)**. Similarly, the HRR gene mutation-positive patients had significantly higher median TMB values than the HRR gene mutation-negative patients in the DA (4.5 Muts/Mb vs. 3.0 Muts/Mb, $P = 0.0157$) and SIC subgroups (5.0 Muts/Mb vs. 4.0 Muts/Mb, $P = 0.010$) **(Figure 2E and F)**. Thus, HRR gene mutation positivity was associated with a higher frequency of MSI-H and elevated TMB levels compared to HRR gene mutation negativity, regardless of the primary tumor site.

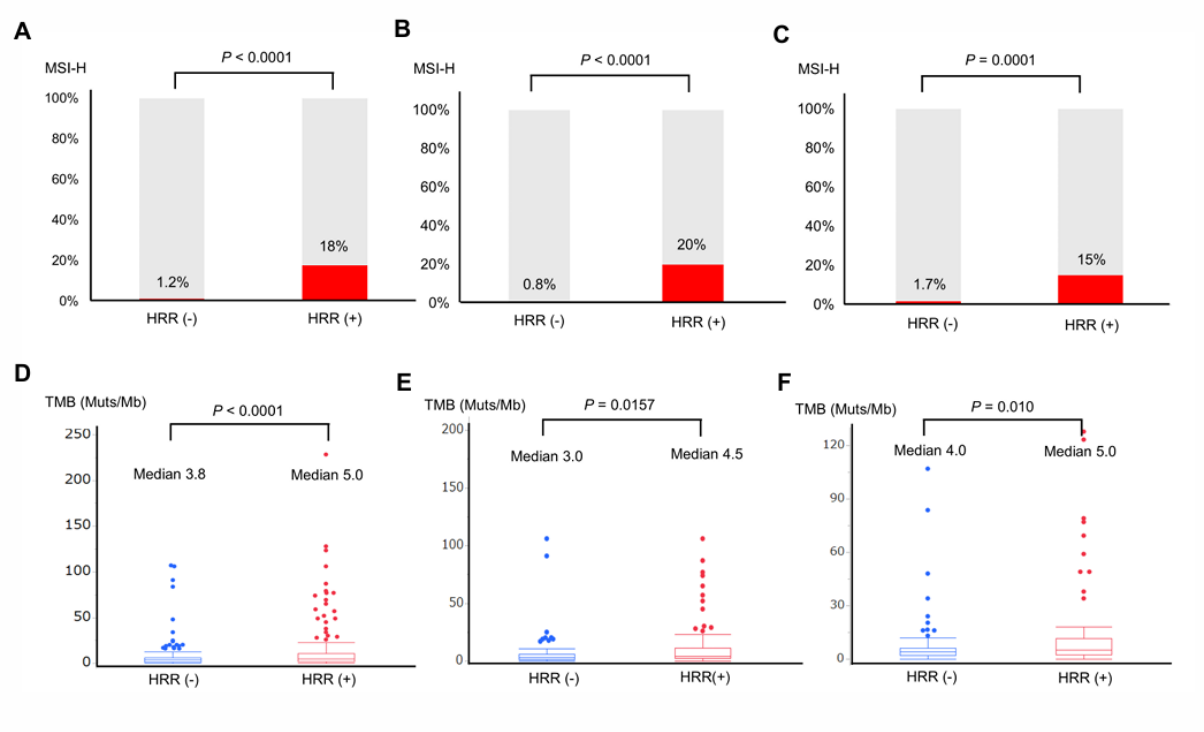

Figure 2. Frequency of microsatellite instability-high and tumor mutation burden values stratified by homologous recombination repair gene mutation status
The frequency of microsatellite instability-high (MSI-H) stratified by homologous recombination repair (HRR) gene mutation status is depicted in the upper panels for (A) all small bowel adenocarcinoma (SBA), (B) duodenal adenocarcinoma (DA), and (C) small intestinal carcinoma (SIC) cases. The proportion of MSI-H cases was significantly higher in HRR mutation cases than in non-HRR mutation cases across all groups. The tumor mutation burden (TMB) values stratified by HRR gene mutation status are shown for (D) all SBA, (E) DA, and (F) SIC cases in the lower panels. Similarly, the HRR gene mutation-positive patients had significantly higher median TMB values than the HRR gene mutation-negative patients across all groups. MSI-H, microsatellite instability high; TMB, tumor mutation burden; Mut, mutation; Mb, megabase; HRR, homologous recombination repair.

Variant frequencies of each gene based on HRR gene mutation status

Next, we analyzed the differences in variant frequencies of genes with a mutation frequency exceeding 5% in the entire cohort including MMR-related genes between HRR gene mutation-positive and HRR gene mutation-negative groups. In the entire SBA cohort, the HRR mutation group had significantly higher frequencies of *PIK3CA, TGFBR2, RNF43, KMT2D*, and all MMR-related genes than the non-HRR mutation group. Conversely, *TP53* and *SMAD4* variants were significantly more frequent in the non-HRR mutation group than in the HRR mutation group **(Figure 3A)**. In the subgroup analyses of the DA and SIC groups, a similar trend was

observed between the two groups **(Figure 3B and C)**. No significant difference in gene frequencies was noted between the two groups across the primary tumor sites.

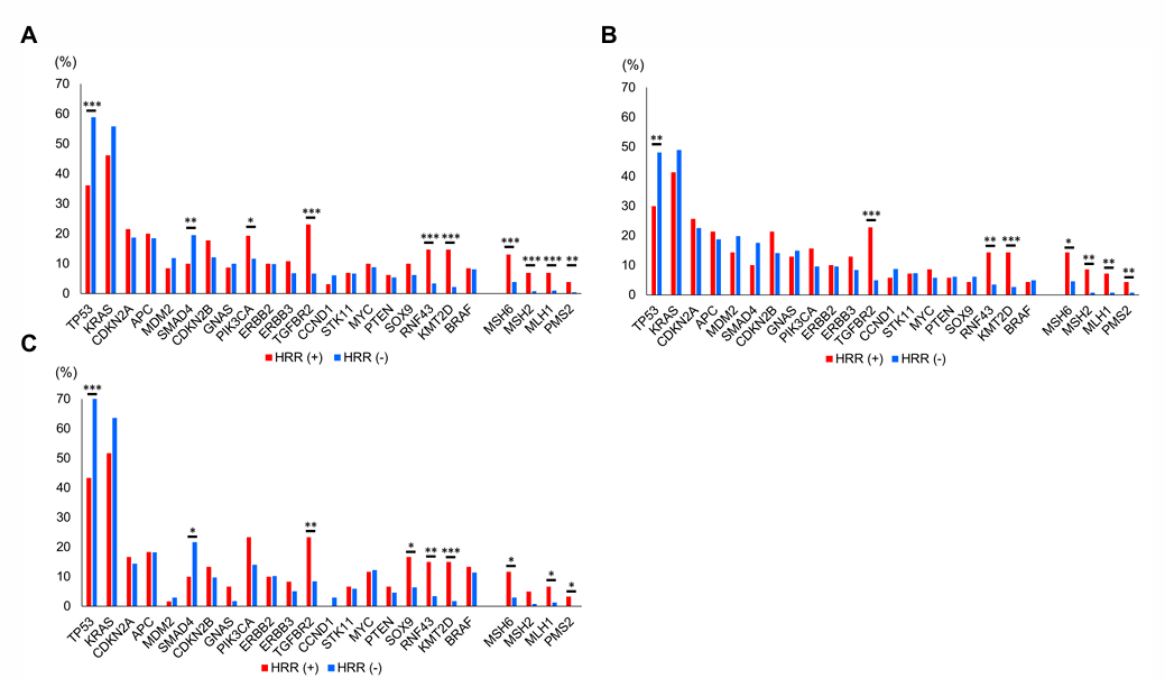

Figure 3. Differences in variant frequencies based on homologous recombination repair gene mutation status
The bar chart illustrates the variant frequencies of individual genes including mismatch repair-related genes (*MSH2*, *MSH6*, *MLH1*, and *PMS2*) in all (A) small bowel adenocarcinoma, (B) duodenal adenocarcinoma, and (C) small intestinal carcinoma cases. The analysis only included genes with mutation frequencies exceeding 5%. Red and blue bars indicate the homologous recombination repair gene mutation-positive and HRR gene mutation-negative groups, respectively. Similar trends in the genomic profiles were observed across these groups. HRR, homologous recombination repair. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Variant frequencies of each gene based on primary site

Subsequently, to evaluate the differences in the genomic profiles between the DA and SIC groups, we analyzed the variant frequencies of genes with mutation frequencies exceeding 5% in the entire cohort, HRR genes, and MMR-related genes. The DA group demonstrated significantly frequencies of *MDM2, GNAS,* and *CCND1* variants than the SIC group. Conversely, *TP53, KRAS, MYC,* and *BRAF* variants were significantly more frequent in the SIC group than in the DA group **(Figure 4A)**. Regarding the variant frequencies of the HRR genes, no significant differences were observed between two groups (21% vs. 20%, $P = 0.84$).

However, among the individual HRR genes, *BRCA1* variants were significantly more frequent in the DA group, whereas *BRCA2* variants were significantly more frequent in the SIC group. No significant differences in the frequencies of MMR-related genes at the primary site were observed between the groups **(Figure 4B)**.

We also analyzed the genomic differences based on primary sites within the HRR mutation group. The DA group had significantly higher frequency of *MDM2* variants than the SIC group (14% vs. 1.7%, $P = 0.011$). Conversely, *SOX9* variants were significantly more frequent in the SIC group than in the DA group (17% vs. 4.3%, $P = 0.037$) **(Figure 4C)**.

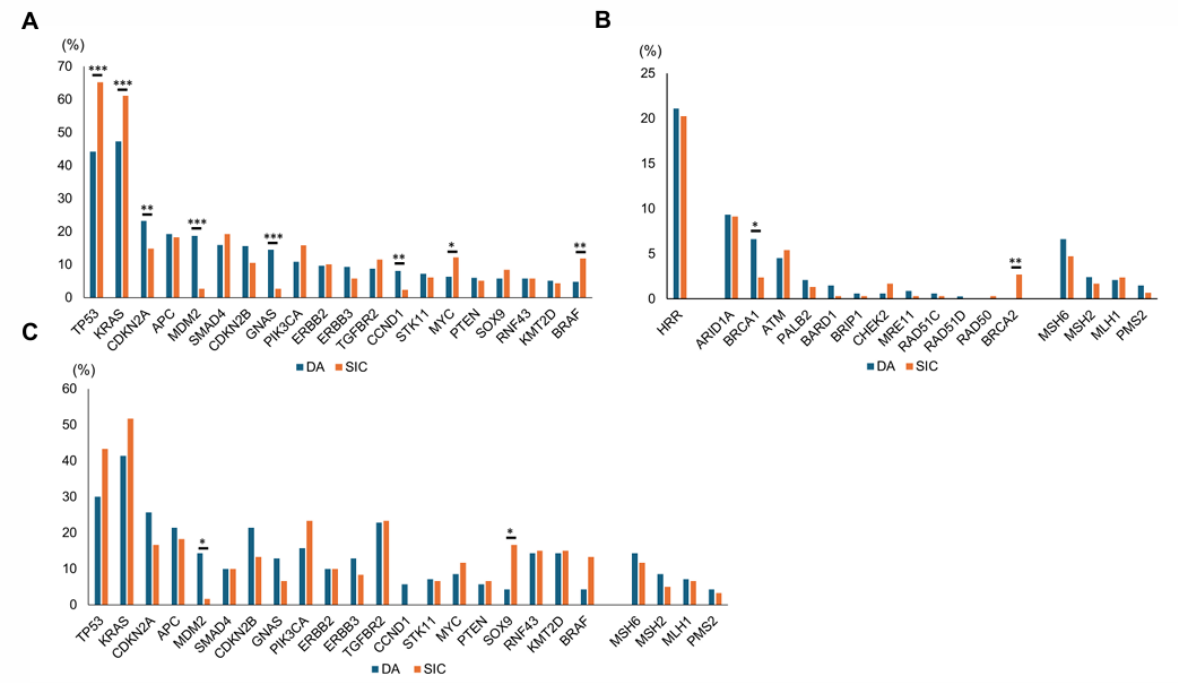

Figure 4. Differences in variant frequencies by primary site
(A) The bar chart depicts the variant frequencies of genes with mutation rates exceeding 5% in total by primary site. Dark blue bars represent the duodenal adenocarcinoma (DA) group, whereas orange bars indicate the small intestinal carcinoma (SIC) group. The SIC group had significantly higher mutation frequencies of *TP53, KRAS, MYC*, and *BRAF* than the DA group. Conversely, variants in *CDKN2A, MDM2, GNAS*, and *CCND1* were more frequent in the DA group than in the SIC group. (B) The bar chart shows the variant frequencies of homologous recombination repair (HRR) genes (*ARID1A, ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, MRE11, PALB2, RAD50, RAD51C*, and *RAD51D*) and mismatch repair (MMR)-related genes (*MSH2, MSH6, MLH1,* and *PMS2*). HRR frequency was calculated as the proportion of cases with mutations in any HRR gene. The frequency of *BRCA1* variants was significantly higher in the DA group, whereas *BRCA2* variants were significantly more frequent in the SIC group. (C) The bar chart illustrates the variant frequencies of genes with mutation rates exceeding 5% in total and MMR-related genes in the HRR gene mutation-positive group, stratified by primary site. *MDM2* variants were significantly more frequent in the DA group than in the SIC group.

By contrast, *SOX9* variants were significantly more frequent in the SIC group than in the DA group. DA, duodenal adenocarcinoma; SIC, small intestinal carcinoma. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Analysis of co-occurring and mutually exclusive gene alteration patterns in SBA

We analyzed the patterns of co-occurrence and mutual exclusivity of gene alterations in SBA. In the entire SBA cohort, HRR-related genes and *PIK3CA* exhibited significant co-occurrence ($P < 0.05$), whereas HRR genes were mutually exclusive with *SMAD4* and *TP53* ($P < 0.01$ and $P < 0.0001$, respectively). Additional co-occurring and mutually exclusive gene pairs were also identified in the SBA cohort **(Figure 5A)**.

The subgroup analyses of the DA and SIC cohorts revealed significant findings. DA cases exhibited more co-occurring mutations than SIC cases, whereas SIC cases exhibited more mutually exclusive mutations than DA cases **(Figure 5B and C)**. Thus, a higher prevalence of co-occurrence in DA may be associated with more aggressive disease progression, whereas a higher prevalence of mutual exclusivity in SIC indicates that treatments targeting specific mutations may be more effective.

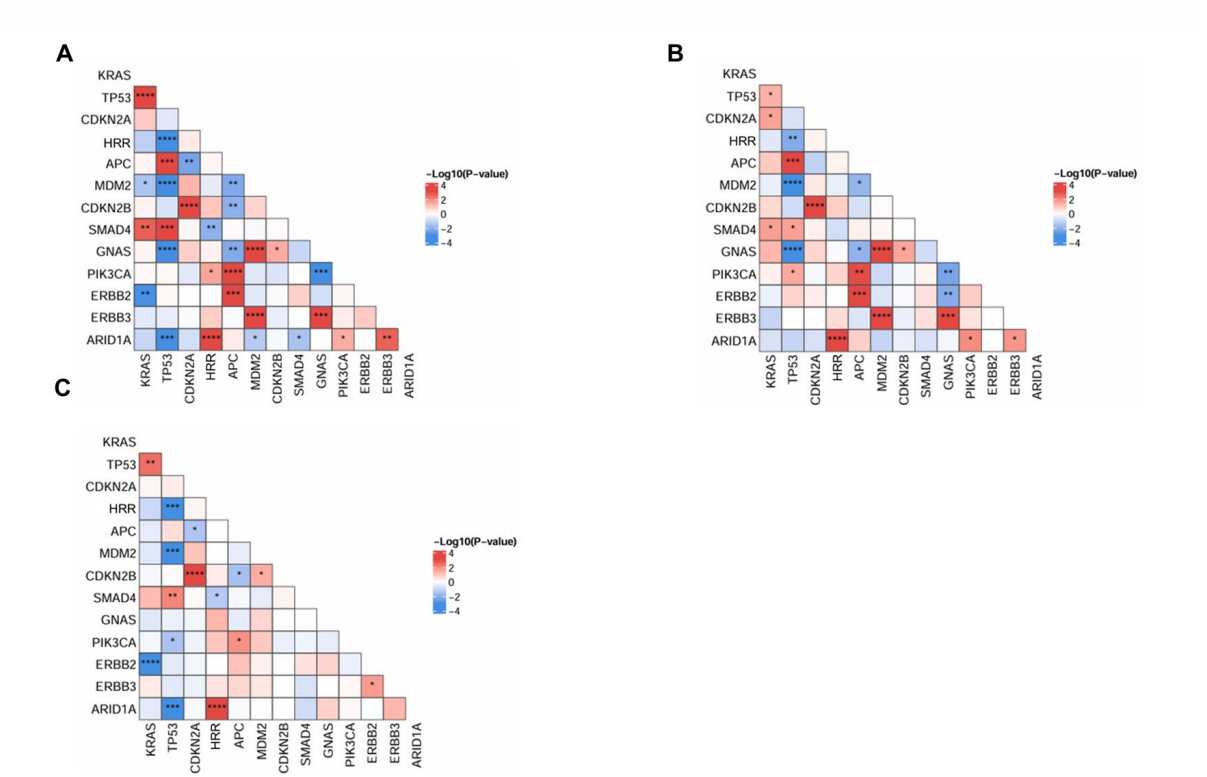

Figure 5. Co-occurrence and mutual exclusivity of gene alteration patterns
Heatmaps illustrate co-occurring and mutually exclusive mutations in (A) all small bowel adenocarcinoma, (B) duodenal adenocarcinoma (DA), and (C) small intestinal carcinoma (SIC) cases. Co-occurring mutations are represented by red squares, whereas mutually exclusive mutations between gene pairs are shown in blue. DA cases exhibited more co-occurring mutations than SIC cases, whereas SIC cases demonstrated more mutually exclusive mutations than DA cases. Color intensity reflects-log10 (*P*-value). *P*-values were calculated using the Fisher's exact test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Analysis of survival and treatment outcomes based on HRR gene mutation status

Finally, we evaluated the clinical significance of HRR mutations on OS and the treatment efficacy of platinum-based chemotherapy in patients with SBA. In the entire SBA cohort, no significant difference in median OS was observed between the HRR gene mutation-positive and HRR gene mutation-negative groups (41.4 months vs. 36.3 months, $P = 0.27$) **(Figure 6A)**. In the DA cohort, the median OS was significantly longer in HRR gene mutation-positive patients than in HRR gene mutation-negative patients (not reached vs. 23.5 months, $P = 0.040$) **(Figure 6B)**. Conversely, no significant difference in median OS was observed between HRR gene mutation-positive and HRR gene mutation-negative patients in the SIC cohort (38.1 months vs. 52.0 months, $P = 0.54$) **(Figure 6C)**.

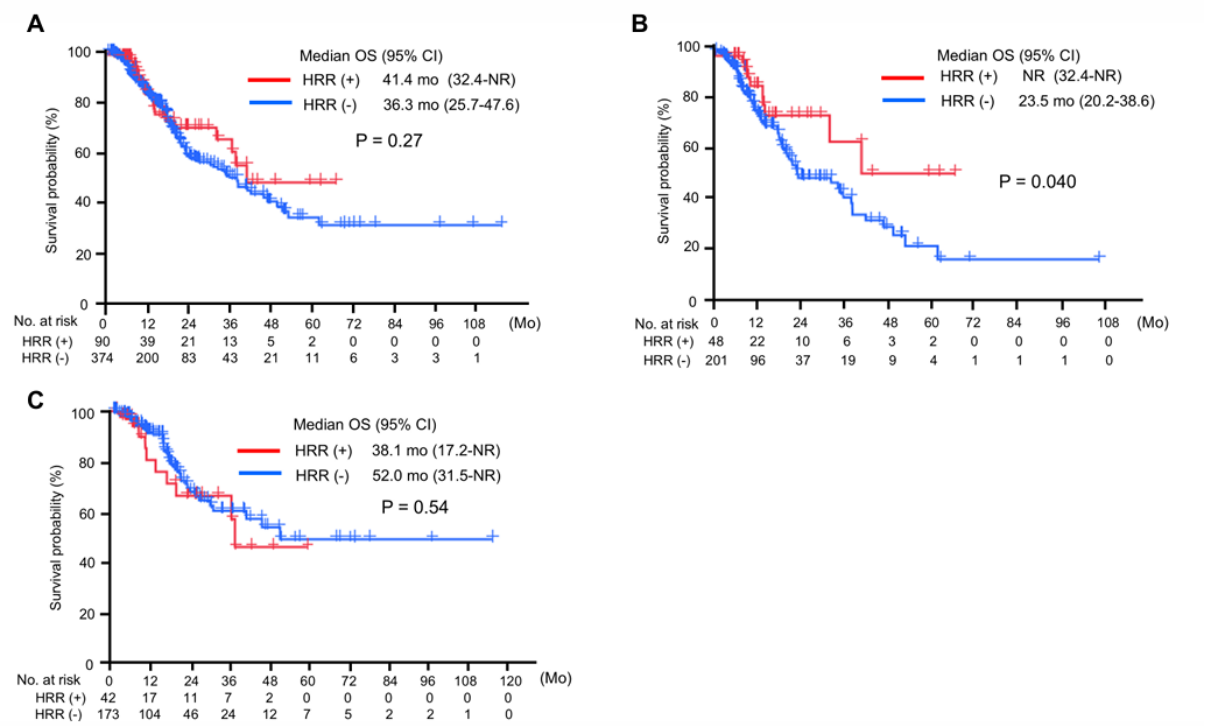

Figure 6. Overall survival according to homologous recombination repair gene mutation status.
(A) Kaplan–Meier curves for overall survival (OS) in the entire small bowel adenocarcinoma cohort. No significant differences in median OS were observed between the homologous recombination repair (HRR) gene mutation-positive and HRR gene mutation-negative groups (41.4 months vs. 36.3 months, $P = 0.27$). (B) Kaplan–Meier curves for OS in the duodenal adenocarcinoma subgroup. The median OS was significantly longer in the HRR gene mutation-positive group than in the HRR gene mutation-negative group (not reached vs. 23.5 months, $P = 0.040$). (C) Kaplan–Meier curves for OS in the small intestinal carcinoma subgroup. No significant differences in median OS were noted between the HRR gene mutation-positive and HRR gene mutation-negative groups (38.1 months vs. 52.0 months, $P = 0.54$). OS, overall survival; HRR, homologous recombination repair; NR, not reached; Mo, months; CI, confidence interval; No, number.

Next, we compared the ORR and DCR between HRR mutation and non-HRR mutation patients in the SBA cohort. In the entire SBA cohort, HRR gene mutation-positive patients had a significantly higher ORR than HRR gene mutation-negative patients (30% vs. 20%, $P = 0.042$). However, DCR was not significantly different between the HRR mutation and non-HRR mutation groups (56% vs. 46%, $P = 0.095$). In the DA cohort, the HRR gene mutation-positive patients demonstrated a significantly higher ORR than the non-HRR mutation patients (33% vs. 19%, $P = 0.046$). Additionally, DCR was significantly higher in the HRR mutation patients than in the non-HRR mutation patients (58% vs. 40%, $P = 0.044$). In the SIC cohort, no significant differences in ORR or DCR were observed between the two groups (ORR: 26% vs. 20%, $P = 0.40$; DCR: 55% vs. 46%, $P = 0.39$, respectively) **(Table 2)**.

	SBA			DA			SIC		
	HRR (+) n=87	HRR (−) n=358	*P*-value	HRR (+) n=45	HRR (−) n=178	*P*-value	HRR (+) n=42	HRR (−) n=180	*P*-value
ORR (%)	30	20	0.042	33	19	0.046	26	20	0.40
DCR (%)	56	46	0.095	58	40	0.044	55	46	0.39

Table 2. Overall response and disease control rates of platinum-based regimens as first-line chemotherapy in homologous recombination repair gene mutation-positive and -negative patients with duodenal adenocarcinoma and small intestine carcinoma.
DA, duodenal adenocarcinoma; SIC, small intestinal carcinoma; n, number; ORR, overall response rate; DCR, disease control rate; HRR, homologous recombination repair.

DISCUSSION

In this study, we investigated the clinical impact of HRR mutations on the genomic profiling, treatment outcomes, and prognosis of patients with SBA using a nationwide genomic profiling database. HRR mutation positivity was associated with a higher frequency of MSI-H and elevated TMB levels across all primary tumor sites. Additionally, we identified differences in genomic profiles based on HRR gene mutation status, variations across primary tumor sites among HRR mutation cases, and site-specific patterns of co-occurring and mutually exclusive mutations involving HRR genes. The impact of HRR mutations on the efficacy of platinum-based chemotherapy and OS varied according to the primary tumor site.

Although the association between HRD and increased TMB levels has been reported across various tumor types [13], to our knowledge, such a relationship has not been previously described in SBA. Notably, SBA has been reported to exhibit a relatively high prevalence of MSI-H status compared to other gastrointestinal malignancies [14]. In this context, our study is novel in demonstrating a significant association between HRD and both MSI-H and increased TMB levels in SBA, regardless of the primary tumor location within the small intestine. These findings may have important therapeutic implications, suggesting a potential role for HRD as a biomarker to guide the development or selection of targeted therapies, including poly adenosine diphosphate–ribose polymerase (PARP) inhibitors and immune checkpoint inhibitors, in this rare cancer type.

The prognostic implications of primary tumor site-specific genomic alterations including HRR genes in SBA remain unclear. DA has a worse prognosis than SIC, potentially because of its higher prevalence of gastric-type features [5, 15]. Our findings revealed novel associations between HRR mutation status, genomic alterations, and the primary tumor site. The frequent co-occurrence of mutations in DA may contribute to its aggressive clinical behavior, whereas the mutual exclusivity observed in SIC highlights distinct genetic

characteristics.

Although the role of HRD as a predictive marker for platinum-based chemotherapy and PARP inhibitors is well established in pancreatic, ovarian, and other cancers[16, 17], its impact in SBA remains understudied. The definition of HRD is inconsistent and complex, making its evaluation challenging [18, 19]. Therefore, in the present study, we assessed HRD using HRR mutations. Our results demonstrated that although the HRR gene mutation status was not associated with treatment efficacy or prognosis in the overall SBA and SIC cohorts, HRR gene mutation positivity in DA was linked to improved efficacy of platinum-based chemotherapy and longer OS. This may be explained by the recommendation to add oxaliplatin in MSI-H colorectal cancer chemotherapy due to the limited efficacy of fluoropyrimidine-based chemotherapy alone [20]. In this study, the difference in the frequency of MSI-H and TMB levels according to HRR gene mutation status tended to be more pronounced in DA cases compared to the overall SBA cohort and SIC cases. Therefore, HRR gene mutations may confer greater benefit from platinum-based combination chemotherapy specifically in DA. Taken together, these findings suggest that HRR gene mutations may serve as simplified surrogate markers for HRD in DA, although further validation is warranted.

Additional genetic mutations may also modulate the efficacy of platinum-based chemotherapy. For example, *MDM2* mutations have been associated with increased sensitivity to platinum-based agents [21], whereas *SOX9* mutations may confer oxaliplatin resistance [22]. *MDM2* mutations were more frequent in the DA group, whereas *SOX9* mutations were predominant in the SIC group. These findings may explain the differential impact of HRR gene mutations on treatment efficacy and prognosis between these subtypes.

SBA is a rare malignancy with limited effective treatment options [23, 24]. However, owing to its rarity, clinical trial opportunities for newly diagnosed patients with SBA are scarce, resulting in a relative lack of diagnostic, predictive, and prognostic biomarkers [25]. In

accordance with colorectal cancer, the additive effects of anti-EGFR and anti-VEGF antibodies in chemotherapy have been investigated [26-28]. Genetic alterations such as *KRAS* and *ERBB2* have been evaluated as potential predictive biomarkers for the efficacy of anti-EGFR antibody therapy [29]. However, to date, no studies have reported on HRD-targeted therapies for small bowel cancer. The findings of this study may contribute to the development of novel targeted therapies. Specifically, our findings indicate that HRR mutations may serve as predictive biomarkers for the efficacy of platinum-based chemotherapy, particularly in DA. In contrast, their predictive value may be limited in SIC, where the high prevalence of mutually exclusive alterations such as *ERBB2* and *KRAS* suggests the presence of distinct molecular pathways.

Several limitations should be acknowledged. First, most patients underwent tissue-based panel testing, which made it difficult to distinguish somatic from germline mutations. Second, the cohort was limited to Japanese patients, which may restrict generalizability. Future prospective studies and external validation cohorts will be necessary to confirm the predictive value of HRR gene mutations in SBA. Lastly, the C-CAT database contains substantial amounts of missing survival and clinical outcome data. Nevertheless, cases with missing data were excluded to ensure the reliability of the results.

In conclusion, HRR gene mutations significantly influenced genomic profiles and treatment outcomes in SBA. HRR mutation positivity, particularly in DA, was associated with better outcomes following platinum-based chemotherapy and improved OS. These findings highlight the potential of HRR mutations as biomarkers for personalized treatment strategies of SBA.

Acknowledgements

We would like to thank Editage (www.editage.jp) for English language editing.

Author contributions

Conceptualization: YK and MO. Data curation: TO, HY, AH, DE, S. Tomida, and S. Toyooka. Formal analysis: YK and MO. Writing — review & editing: YK and MO wrote the manuscript.

Data availability

All the data are available from the corresponding author upon request.

Declaration of interest statement

The authors have no competing interests to declare.

Ethics statement

All individuals registered with the portal provided written informed consent for secondary utilization of their clinical records and CGP outcomes.

REFERENCES

[1] Overman MJ, Hu CY, Kopetz S, Abbruzzese JL, Wolff RA, Chang GJ. A population-based comparison of adenocarcinoma of the large and small intestine: insights into a rare disease. Ann Surg Oncol. 2012;19:1439-45. https://doi.org/10.1245/s10434-011-2173-6.

[2] Bouvier AM, Robaszkiewicz M, Jooste V, Cariou M, Drouillard A, Bouvier V, et al. Trends in incidence of small bowel cancer according to histology: a population-based study. J Gastroenterol. 2020;55:181-8. https://doi.org/10.1007/s00535-019-01636-z.

[3] Schrock AB, Devoe CE, McWilliams R, Sun J, Aparicio T, Stephens PJ, et al. Genomic Profiling of Small-Bowel Adenocarcinoma. JAMA Oncol. 2017;3:1546-53. https://doi.org/10.1001/jamaoncol.2017.1051.

[4] Pandya K, Overman MJ, Gulhati P. Molecular Landscape of Small Bowel Adenocarcinoma. Cancers (Basel). 2022;14. https://doi.org/10.3390/cancers14051287.

[5] Wilhelm A, Galata C, Beutner U, Schmied BM, Warschkow R, Steffen T, et al. Duodenal localization is a negative predictor of survival after small bowel adenocarcinoma resection: A population-based, propensity score-matched analysis. J Surg Oncol. 2018;117:397-408. https://doi.org/10.1002/jso.24877.

[6] Laforest A, Aparicio T, Zaanan A, Silva FP, Didelot A, Desbeaux A, et al. ERBB2 gene as a potential therapeutic target in small bowel adenocarcinoma. Eur J Cancer. 2014;50:1740-6. https://doi.org/10.1016/j.ejca.2014.04.007.

[7] Gu W, Zhuang W, Zhuang M, He M, Li Z. DNA damage response and repair gene mutations are associated with tumor mutational burden and outcomes to platinum-based chemotherapy/immunotherapy in advanced NSCLC patients. Diagn Pathol. 2023;18:119. https://doi.org/10.1186/s13000-023-01401-0.

[8] An J, Oh JH, Oh B, Oh YJ, Ju JS, Kim W, et al. Clinicogenomic characteristics and synthetic lethal implications of germline homologous recombination-deficient hepatocellular carcinoma. Hepatology. 2023;78:452-67. https://doi.org/10.1002/hep.32812.

[9] Cerniglia M, Xiu J, Grothey A, Pishvaian MJ, Baca Y, Hwang JJ, et al. Association of Homologous Recombination-DNA Damage Response Gene Mutations with Immune Biomarkers in Gastroesophageal Cancers. Mol Cancer Ther. 2022;21:227-36. https://doi.org/10.1158/1535-7163.MCT-20-0879.

[10] Ishigaki K, Tokito Y, Takahara N, Nishio H, Endo G, Fukuda K, et al. Association

between homologous recombination deficiency and time to treatment failure to platinum-based chemotherapy for pancreatic cancer by using the C-CAT database. J Gastroenterol. 2025;60:247-256. https://doi.org/10.1007/s00535-024-02173-0.

[11] Ichikawa H, Aizawa M, Kano Y, Hanyu T, Muneoka Y, Hiroi S, et al. Landscape of homologous recombination deficiency in gastric cancer and clinical implications for first-line chemotherapy. Gastric Cancer. 2024;27:1273-86. https://doi.org/10.1007/s10120-024-01542-1.

[12] Kohno T, Kato M, Kohsaka S, Sudo T, Tamai I, Shiraishi Y, et al. C-CAT: The National Datacenter for Cancer Genomic Medicine in Japan. Cancer Discov. 2022;12:2509-15. https://doi.org/10.1158/2159-8290.CD-22-0417.

[13] Jung J, Heo YJ, Park S. High tumor mutational burden predicts favorable response to anti-PD-(L)1 therapy in patients with solid tumor: a real-world pan-tumor analysis. J Immunother Cancer. 2023;11. https://doi.org/10.1136/jitc-2022-006454.

[14] Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357:409-13. https://www.science.org/doi/10.1126/science.aan6733

[15] Hoshimoto A, Tatsuguchi A, Yamada T, Kuriyama S, Hamakubo R, Nishimoto T, et al. Relationship Between Immunophenotypes, Genetic Profiles, and Clinicopathologic Characteristics in Small Bowel Adenocarcinoma. Am J Surg Pathol. 2024;48:127-39. https://doi.org/10.1097/PAS.0000000000002161.

[16] Yadav S, Kasi PM, Bamlet WR, Ho TP, Polley EC, Hu C, et al. Effect of Germline Mutations in Homologous Recombination Repair Genes on Overall Survival of Patients with Pancreatic Adenocarcinoma. Clin Cancer Res. 2020;26:6505-12. https://doi.org/10.1158/1078-0432.CCR-20-1788.

[17] Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res. 2014;20:764-75. https://doi.org/10.1158/1078-0432.CCR-13-2287.

[18] Lotan TL, Kaur HB, Salles DC, Murali S, Schaeffer EM, Lanchbury JS, et al. Homologous recombination deficiency (HRD) score in germline BRCA2- versus ATM-altered prostate cancer. Mod Pathol. 2021;34:1185-93. https://doi.org/10.1038/s41379-020-00731-4.

[19] Weigelt B, Bi R, Kumar R, Blecua P, Mandelker DL, Geyer FC, et al. The Landscape of

Somatic Genetic Alterations in Breast Cancers From ATM Germline Mutation Carriers. J Natl Cancer Inst. 2018;110:1030-4. https://doi.org/10.1093/jnci/djy028.

[20] Cohen R, Taieb J, Fiskum J, Yothers G, Goldberg R, Yoshino T, et al. Microsatellite Instability in Patients With Stage III Colon Cancer Receiving Fluoropyrimidine With or Without Oxaliplatin: An ACCENT Pooled Analysis of 12 Adjuvant Trials. J Clin Oncol. 2021;39:642-51. https://doi.org/10.1200/JCO.20.01600.

[21] Smith G, Ng MT, Shepherd L, Herrington CS, Gourley C, Ferguson MJ, et al. Individuality in FGF1 expression significantly influences platinum resistance and progression-free survival in ovarian cancer. Br J Cancer. 2012;107:1327-36. https://doi.org/10.1038/bjc.2012.410.

[22] Liu Y, Wu H, Luo T, Luo Q, Meng Z, Shi Y, et al. The SOX9-MMS22L Axis Promotes Oxaliplatin Resistance in Colorectal Cancer. Front Mol Biosci. 2021;8:646542. https://doi.org/10.3389/fmolb.2021.646542.

[23] Horimatsu T, Nakayama N, Moriwaki T, Hirashima Y, Fujita M, Asayama M, et al. A phase II study of 5-fluorouracil/L-leucovorin/oxaliplatin (mFOLFOX6) in Japanese patients with metastatic or unresectable small bowel adenocarcinoma. Int J Clin Oncol. 2017;22:905-12. https://doi.org/10.1007/s10147-017-1138-6.

[24] Aparicio T, Henriques J, Manfredi S, Tougeron D, Bouché O, Pezet D, et al. Small bowel adenocarcinoma: Results from a nationwide prospective ARCAD-NADEGE cohort study of 347 patients. Int J Cancer. 2020;147:967-77. https://doi.org/10.1002/ijc.32860.

[25] Raghav K, Overman MJ. Small bowel adenocarcinomas--existing evidence and evolving paradigms. Nat Rev Clin Oncol. 2013;10:534-44. https://doi.org/10.1038/nrclinonc.2013.132.

[26] Gulhati P, Raghav K, Shroff R, Varadhachary G, Javle M, Qiao W, et al. Phase II Study of Panitumumab in RAS Wild-Type Metastatic Adenocarcinoma of Small Bowel or Ampulla of Vater. Oncologist. 2018;23:277-e26. https://doi.org/10.1634/theoncologist.2017-0568.

[27] Overman MJ, Pozadzides J, Kopetz S, Wen S, Abbruzzese JL, Wolff RA, et al. Immunophenotype and molecular characterisation of adenocarcinoma of the small intestine. Br J Cancer. 2010;102:144-50. https://doi.org/10.1038/sj.bjc.6605449.

[28] Legué LM, van Erning FN, Bernards N, Lemmens V, de Hingh I, Creemers GJ. Addition of Bevacizumab to First-Line Palliative Chemotherapy in Patients with Metastatic Small Bowel Adenocarcinoma: A Population-Based Study. Target Oncol. 2019;14:699-705. https://doi.org/10.1007/s11523-019-00681-1.

[29] Rankin A, Klempner SJ, Erlich R, Sun JX, Grothey A, Fakih M, et al. Broad Detection of Alterations Predicted to Confer Lack of Benefit From EGFR Antibodies or Sensitivity to Targeted Therapy in Advanced Colorectal Cancer. Oncologist. 2016;21:1306-14. https://doi.org/10.1634/theoncologist.2016-0148.