

# DOCTORAL THESIS

## Multiomic molecular characterization of the response to combination immunotherapy in MSS/pMMR metastatic colorectal cancer

マイクロサテライト安定/ミスマッチ修復機能保持大腸癌における併用免疫療法への治療効果に関する分子学的特性のマルチオミック解析





September 2024  
(2024年9月)

**Shogo Takei**  
武井 将伍

Department of Gastroenterological Surgery  
Yokohama City University Graduate School of Medicine  
横浜市立大学 大学院医学研究科 医科学専攻  
消化器・腫瘍外科学 Next Generation Oncologist 養成コース

(Doctoral Supervisor: Itaru Endo, Professor)  
(指導教員：遠藤 格 教授)

# Multiomomic molecular characterization of the response to combination immunotherapy in MSS/pMMR metastatic colorectal cancer

Shogo Takei <sup>1,2</sup>, Yosuke Tanaka,<sup>3</sup> Yi-Tzu Lin,<sup>4</sup> Shohei Koyama <sup>4</sup>, Shota Fukuoka,<sup>5</sup> Hiroki Hara,<sup>6</sup> Yoshiaki Nakamura,<sup>1</sup> Yasutoshi Kuboki,<sup>1</sup> Daisuke Kotani,<sup>1</sup> Takashi Kojima,<sup>1</sup> Hideaki Bando,<sup>1</sup> Saori Mishima,<sup>1</sup> Toshihide Ueno,<sup>3</sup> Shinya Kojima,<sup>3</sup> Masashi Wakabayashi,<sup>7</sup> Naoya Sakamoto,<sup>8</sup> Motohiro Kojima,<sup>8</sup> Takeshi Kuwata,<sup>8,9</sup> Takayuki Yoshino,<sup>1</sup> Hiroyoshi Nishikawa <sup>4,10</sup>, Hiroyuki Mano,<sup>3</sup> Itaru Endo,<sup>2</sup> Kohei Shitara <sup>1,10</sup>, Akihito Kawazoe<sup>1</sup>

**To cite:** Takei S, Tanaka Y, Lin Y-T, *et al.* Multiomomic molecular characterization of the response to combination immunotherapy in MSS/pMMR metastatic colorectal cancer. *Journal for ImmunoTherapy of Cancer* 2024;**12**:e008210. doi:10.1136/jitc-2023-008210

► Additional supplemental material is published online only. To view, please visit the journal online (<https://doi.org/10.1136/jitc-2023-008210>).

ST, YT and Y-TL contributed equally.

Accepted 16 January 2024



© Author(s) (or their employer(s)) 2024. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

## Correspondence to

Dr Akihito Kawazoe;  
akawazoe@east.ncc.go.jp

Dr Kohei Shitara;  
kshitara@east.ncc.go.jp

## ABSTRACT

**Background** Immune checkpoint inhibitor (ICI) combinations represent an emerging treatment strategies in cancer. However, their efficacy in microsatellite stable (MSS) or mismatch repair-proficient (pMMR) colorectal cancer (CRC) is variable. Here, a multimomic characterization was performed to identify predictive biomarkers associated with patient response to ICI combinations in MSS/pMMR CRC for the further development of ICI combinations.

**Methods** Whole-exome sequencing, RNA sequencing, and multiplex fluorescence immunohistochemistry of tumors from patients with MSS/pMMR CRC, who received regorafenib plus nivolumab (REGONIVO) or TAS-116 plus nivolumab (TASNIVO) in clinical trials were conducted. Twenty-two and 23 patients without prior ICI from the REGONIVO and TASNIVO trials were included in this study. A biomarker analysis was performed using samples from each of these studies.

**Results** The epithelial-mesenchymal transition pathway and genes related to cancer-associated fibroblasts were upregulated in the REGONIVO responder group, and the G2M checkpoint pathway was upregulated in the TASNIVO responder group. The MYC pathway was upregulated in the REGONIVO non-responder group. Consensus molecular subtype 4 was significantly associated with response ( $p=0.035$ ) and longer progression-free survival ( $p=0.006$ ) in the REGONIVO trial. CD8<sup>+</sup> T cells, regulatory T cells, and M2 macrophages density was significantly higher in the REGONIVO trial responders than in non-responders. Mutations in the *POLE* gene and patient response were significantly associated in the TASNIVO trial; however, the frequencies of other mutations or tumor mutational burden were not significantly different between responders and non-responders in either trial.

**Conclusions** We identified molecular features associated with the response to the REGONIVO and TASNIVO, particularly those related to tumor microenvironmental factors. These findings are likely to contribute to the development of biomarkers to predict treatment

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Immune checkpoint inhibitor combinations are emerging treatment strategy in cancer. However, biomarkers of response in microsatellite stable mismatch repair-proficient (MSS/pMMR) colorectal cancer have not been identified.

## WHAT THIS STUDY ADDS

⇒ We identified molecular features associated with the response to regorafenib plus nivolumab or TAS-116 plus nivolumab combinations. Specifically, activation of genes in the epithelial-mesenchymal transition pathway and consensus molecular subtype 4 enrichment were predictive biomarkers in the REGONIVO trial.

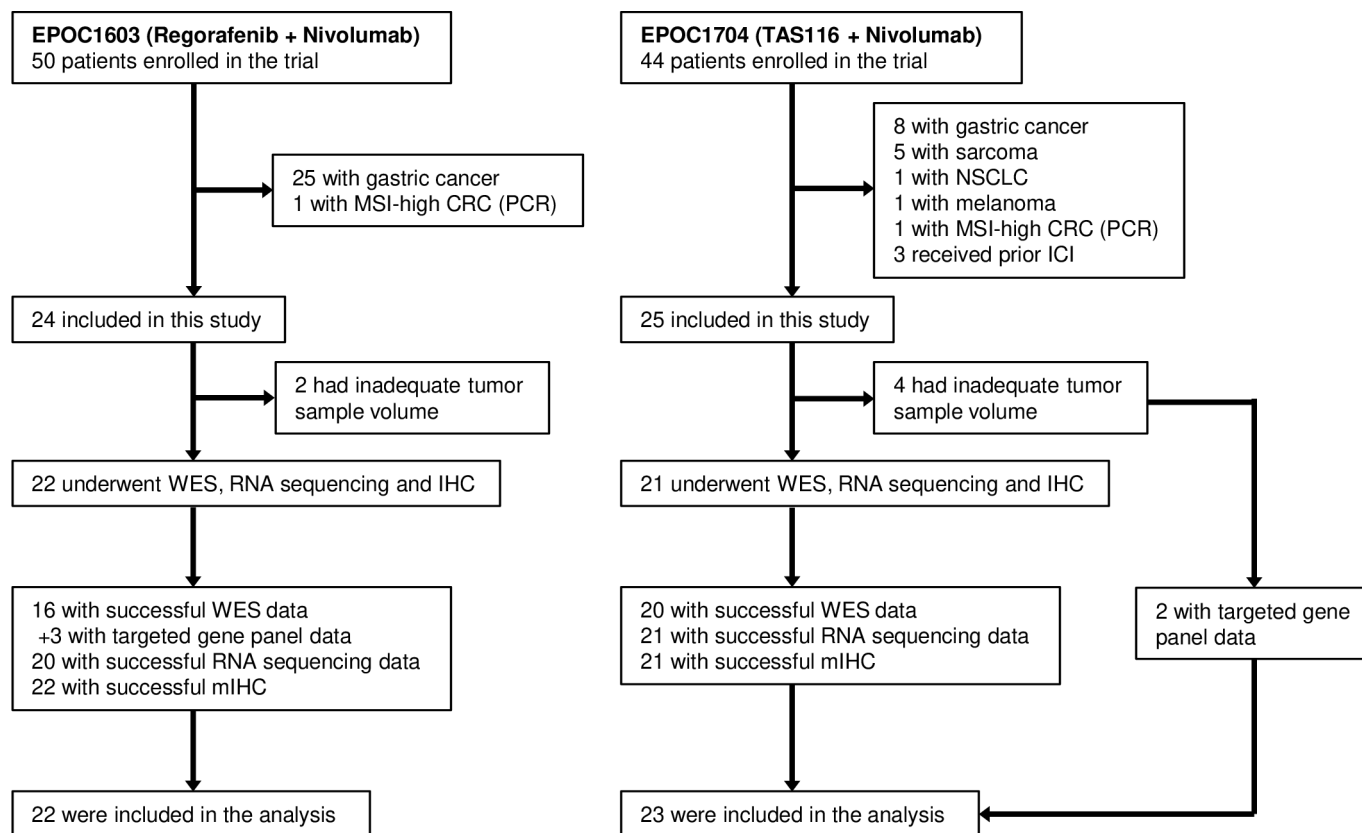
## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our analyses could lead to the further development of biomarkers for MSS/pMMR colorectal cancer and additional combinations of immunotherapies for treatment.

efficacy for MSS/pMMR CRC and future immunotherapy combinations for treatment.

## INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths worldwide.<sup>1</sup> Increasing numbers of immune checkpoint inhibitors (ICIs) have become available as a treatment option for various malignant tumors.<sup>2–7</sup> However, for metastatic CRC, the efficacy of ICIs is limited to patients with microsatellite instability-high or mismatch repair-deficient tumors, and the majority of microsatellite stable (MSS) or mismatch



**Figure 1** Flow diagram of the study. The figure illustrates the process of research sample selection and the number of analyses successfully completed with each method. Only patients with microsatellite stable or mismatch repair-proficient colorectal cancer were included in this study. Patients without WES data due to inadequate sample volume or unsuccessful WES were also included in the analysis if targeted gene panel analysis data were available. CRC, colorectal cancer; ICI, immune checkpoint inhibitors; IHC, immunohistochemistry; mIHC, multiplex fluorescence immunohistochemistry; MSI, microsatellite instability; NSCLC, non-small cell lung cancer; WES, whole-exome sequencing.

repair-proficient (pMMR) CRC tumors do not respond to treatment with individual ICIs.<sup>8–11</sup>

The limited effect of ICIs on MSS and pMMR CRC may be attributed to a low neoantigen load and few tumor-infiltrating lymphocytes, which prevents a robust immune response. Numerous immunosuppressive cells, such as regulatory T (Treg) cells and tumor-associated macrophages, may also infiltrate the tumor microenvironment to prevent antitumor activity.<sup>12–14</sup> To overcome these resistance mechanisms, several immunotherapy combinations have been evaluated for MSS or pMMR CRC<sup>15,16</sup>; however, most have been largely ineffective. Combinations of the MEK inhibitor cobimetinib and the programmed death-ligand 1 (PD-L1) inhibitor atezolizumab as well as the multikinase inhibitor lenvatinib and the programmed cell death protein 1 (PD-1) inhibitor pembrolizumab as salvage therapy have failed to exhibit a survival benefit compared with the standard of care in phase III trials.<sup>15,16</sup> Further development of ICI combinations for MSS or pMMR CRC is necessary.

Previously, we conducted two investigator-initiated trials of ICI combinations of the PD-1 inhibitor nivolumab with drugs expected to activate the immune response, namely, the multikinase inhibitor regorafenib plus nivolumab (REGONIVO) and the HSP90 inhibitor

TAS-116 (pimipitespib) plus nivolumab (TASNIVO) for MSS or pMMR CRC, which demonstrated efficacy in a limited number of these patients.<sup>17,18</sup> These findings highlighted the need to identify biomarkers to identify patients who would benefit from such combinations and to understand the mechanisms through which this efficacy was achieved for the further development of ICI combinations. To identify predictors of response to ICI combinations in patients with MSS or pMMR CRC, we characterized tumors from patients who received REGONIVO or TASNIVO in clinical trials using whole-exome sequencing (WES), RNA sequencing, and multiplex fluorescence immunohistochemistry (mIHC). By applying this multiomics approach, we characterized these tumors at the molecular level and identified molecular features that may contribute to the development of predictive biomarkers and future immunotherapy combinations.

## METHODS

### Patients

The eligibility criteria for this study were as follows: (1) enrollment in a phase Ib trial of REGONIVO (EPOC1603)<sup>17</sup> or a phase Ib trial of TASNIVO (EPOC1704)<sup>18</sup>; (2) patients with MSS or pMMR CRC; and

**Table 1** Patient characteristics

	REGONIVO	TASNIVO
No. of patients	22	23
Age		
Median, years (range)	57.5 (41–77)	61 (32–77)
≥65, n (%)	6 (27.3)	10 (43.5)
Sex, n (%)		
Male	16 (72.7)	12 (52.2)
Female	6 (27.3)	11 (47.8)
ECOG PS, n (%)		
0	22 (100)	19 (82.6)
1	0 (0)	4 (17.4)
Previous treatment regimens, n (%)		
1	0 (0)	1 (4.3)
≥2	22 (100)	22 (95.7)
Primary tumor location, n (%)		
Right side	3 (13.6)	14 (60.9)
Left side	19 (86.4)	9 (39.1)
Metastatic sites, n (%)		
Liver	10 (45.5)	10 (43.5)
Lung	14 (63.6)	15 (65.2)
Lymph node	14 (63.6)	9 (39.1)
Peritoneum	3 (13.6)	11 (47.8)
Number of metastatic organs, n (%)		
1	4 (18.2)	7 (30.4)
≥2	18 (81.8)	16 (69.6)

ECOG, Eastern Cooperative Oncology Group; PS, performance status; REGONIVO, regorafenib plus nivolumab; TASNIVO, TAS116 plus nivolumab.

(3) no ICI therapy prior to trial enrollment. The detailed methods of these trials have been previously reported.<sup>17,18</sup> The study was conducted in accordance with the Declaration of Helsinki. The results are publicly available on the official website of National Cancer Center Hospital East, and the research subjects were provided with an opportunity to decline participation.

### Samples

Tissues were obtained from patients prior to the administration of the investigational treatment and were formalin-fixed and paraffin-embedded (FFPE). The FFPE samples were subjected to WES, RNA sequencing, and mIHC staining. Most of the samples were primary tumors that were surgically resected before patient enrollment, and all tumor samples were collected prior to any ICI combination therapy, with none collected after any immunotherapy; additional details are provided in online supplemental table S1. Peripheral blood mononuclear cells or normal colon tissue were also used as germ line controls.

### WES

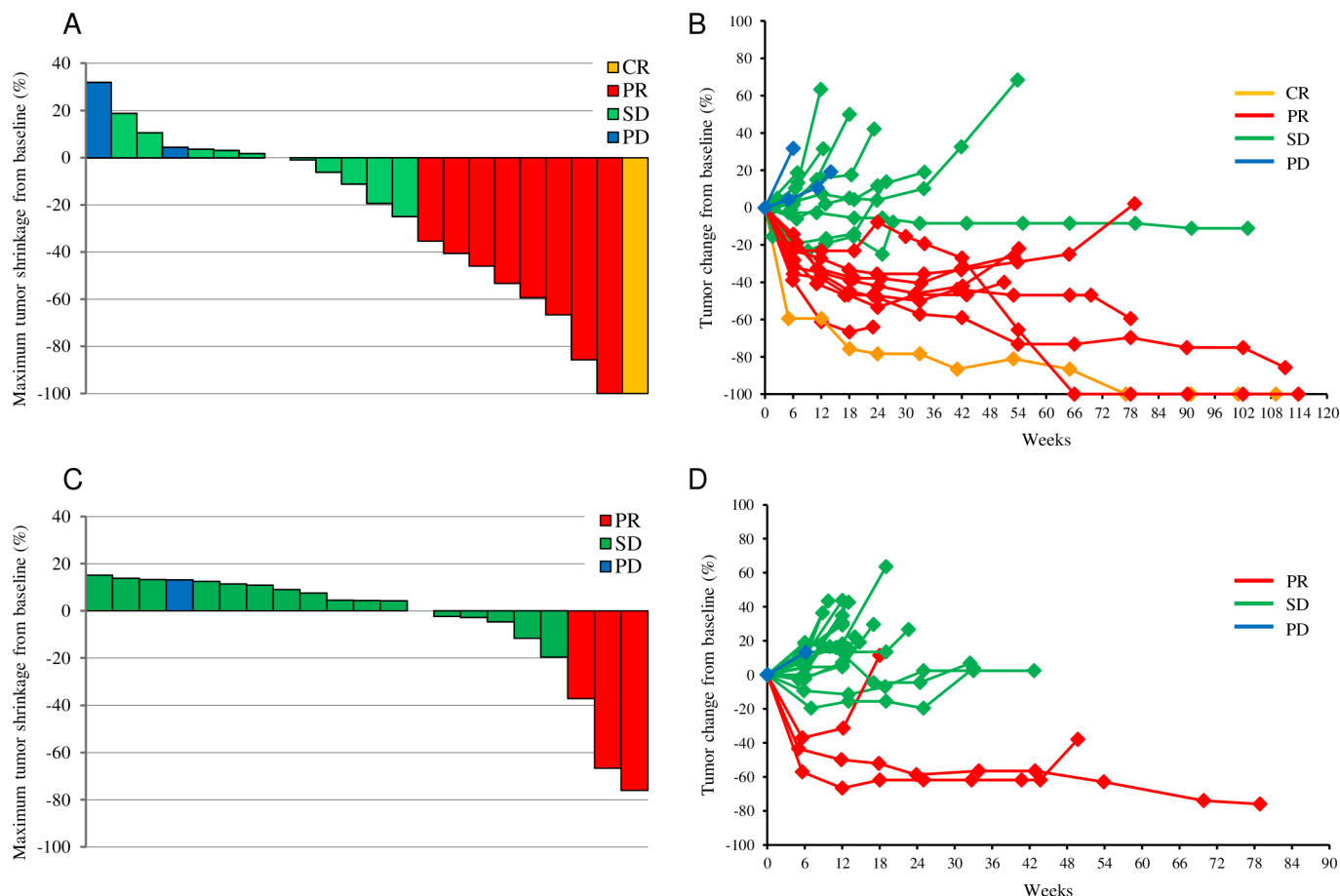
Genomic DNA tissue was extracted from FFPE tissues with the GeneRead DNA FFPE Kit (QIAGEN). DNA was enriched using the Twist Library Preparation Kit (Twist Bioscience). The DNA in the resulting libraries was subjected to next-generation sequencing, and 150 bp was sequenced from both ends on a NovaSeq 6000 (Illumina) to produce paired-end reads. Paired-end sequencing reads with masked nucleotides with quality scores less than 20 were aligned to the hg38 reference genome using BWA-MEM (<http://bio-bwa.sourceforge.net/>) and Bowtie2 (<http://bowtie-bio.sourceforge.net/bowtie2/index.shtml>). Somatic synonymous and non-synonymous mutations were called using our in-house caller and two publicly available mutation callers: Mutect2, as part of the Genome Analysis Toolkit (<https://gatk.broadinstitute.org/hc/en-us>), and VarScan2 (<http://varscan.sourceforge.net/>). Mutations meeting any of the following criteria were discarded: tumor sample variant allele frequency <0.05; mutant read number in the germline control samples of >2; mutations detected in only one strand of the genome; or the variant present in the normal human genome in either the 1,000 Genomes Project data set (<https://www.internationalgenome.org/>) or our in-house database. Gene mutations were annotated using SnpEff (<http://snpeff.sourceforge.net>). Tumor mutational burden (TMB) was defined as the total number of mutations per megabase in the WES bait region. Targeted gene panel analysis data (OncoPrint Cancer Research Panel, Thermo Fisher) were used for complementarity when WES data were not available.

### RNA sequencing

Total RNA was extracted from FFPE tumor samples using the RNeasy FFPE Kit (QIAGEN). Ribosomal RNA depletion was performed using the NEBNext rRNA Depletion Kit (New England Biolabs). RNA integrity was assessed using TapeStation (Agilent Technologies). To exclude the degraded RNA, RNA of sufficient integrity was used for RNA sequencing (RNA-seq) with an NEBNext Ultra Directional RNA Library Prep Kit (New England Biolabs). Prepared RNA libraries were subjected to next-generation sequencing on a NovaSeq 6000 (Illumina) to produce paired-end sequencing reads. For RNA-seq data expression profiling, paired-end reads were aligned to the hg38 human genome and quantified using TopHat2 (<https://github.com/infphilo/tophat>) and Cufflinks (<https://github.com/cole-trapnell-lab/cufflinks>).

Gene Set Enrichment Analysis (GSEA) was performed using GSEA V.4.3.2 (<https://github.com/GSEA-MSigDB>). Genes were ranked based on a log<sub>2</sub>-fold change in expression and gene enrichment scores were calculated based on the rank of the genes and gene sets. Gene sets from the Molecular Signatures Database V.7.2.

Consensus molecular subtypes (CMSs) were evaluated as described previously.<sup>19</sup>



**Figure 2** Efficacy of REGONIVO and TASNIVO treatment in patients included in this study waterfall plot (A) showing the maximum percentage change in tumor size from baseline as measured by Response Evaluation Criteria in Solid Tumors (RECIST) in the REGONIVO trial. Spider plot (B) showing the longitudinal change in RECIST percentage from baseline in the REGONIVO trial. Waterfall plot (C) and spider plot (D) as above but showing data from the TASNIVO trial. CR, complete response; PD, progressive disease; PR, partial response; REGONIVO, regorafenib plus nivolumab; SD, stable disease; TASNIVO, TAS116 plus nivolumab.

### Multiplex immunohistochemistry and PD-L1 staining

FFPE tumor tissue blocks were sliced into 4 mm-thick sections and placed on adhesion microscope slides (Matsunami). The tissue slides were deparaffinized and rehydrated prior to mIHC staining. Antigen retrieval and staining were performed using Opal 7-Color IHC Kits (AKOYA Biosciences) according to the manufacturer's protocol. CD3 (Clone SP7; Abcam), CD8 (Clone C8/144B; Dako), CD206 (Clone CL0387; Invitrogen), CD11b (Clone D6×1N; Cell Signaling Technology), FOXP3 (Clone 236A/E7; Abcam), PDGFR $\alpha$  (Clone D1E1E; Cell Signaling Technology) and Cytokeratin (Clone AE1/AE3; Abcam) staining was examined and images were acquired using the Vectra 3 System (PerkinElmer). Images were exported using inForm Tissue Analysis Software (AKOYA Biosciences). Cell density and expression percentage of specific protein were calculated based on the average of at least three regions of interest (682  $\mu\text{m} \times 510 \mu\text{m}$ /region) using HALO image analysis software (Indica Labs).

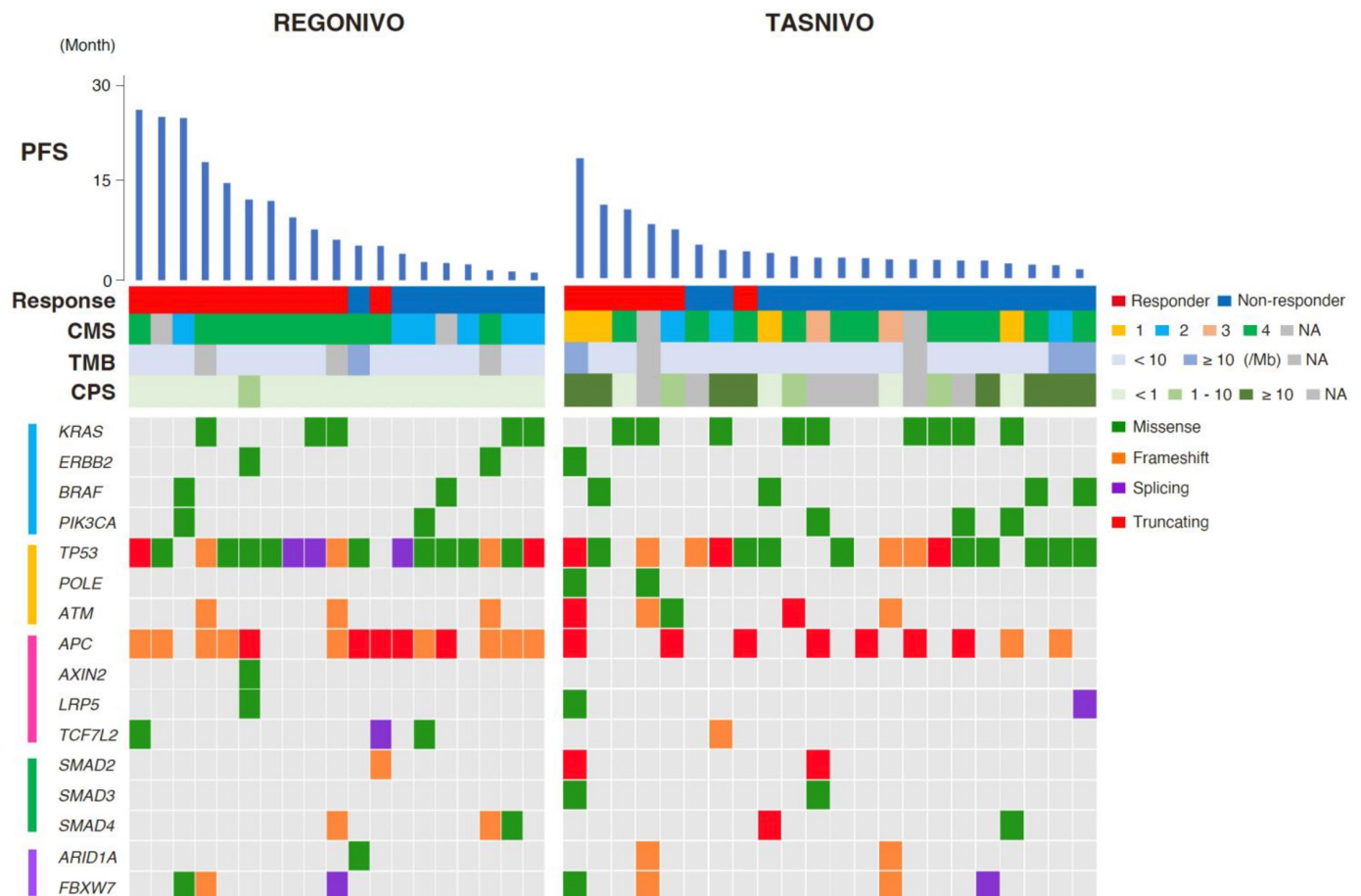
For PD-L1 staining using the anti-PD-L1 28–8 antibody, the combined positive score (CPS) was assessed

by a pathologist (TKu) and defined as the percentage of total tumor cells (including tumor cells, lymphocytes, and macrophages) multiplied by 100 in the REGONIVO trial. In the TASNIVO trial, CPS was measured by the PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies).

### Outcomes and statistics

Patients experiencing a clinical benefit (responders) were defined as those who achieved a complete response (CR), partial response (PR), or stable disease (SD) lasting more than 6 months as evaluated by Response Evaluation Criteria in Solid Tumors V.1.1 criteria. Progression-free survival (PFS) was defined as the time from registration for clinical trials to disease progression or death (for any reason). Overall survival (OS) was defined as the time from registration to death (for any reason). Quantitative data are presented as the median and range. The Mann-Whitney U and  $\chi^2$  tests were used for comparisons between continuous and categorical variables, respectively. PFS and OS were estimated using the Kaplan-Meier method, and HRs and CIs were estimated using a Cox





**Figure 3** Molecular characterization The top section of the figure shows the duration of PFS. The middle section indicates the response status (CR, PR, or SD $\geq$ 6 months), CMS, TMB, and PD-L1 CPS. The bottom section shows the distribution of gene mutations determined by WES or targeted gene panel analysis. CR, complete response; CPS, combined positive score; CMS, consensus molecular subtypes; NA, not available; PFS, progression-free survival; PR, partial response; REGONIVO, regorafenib plus nivolumab; SD $\geq$ 6, stable disease duration of at least 6 months; TASNIVO, TAS116 plus nivolumab; TMB, tumor mutational burden; WES, WES, whole exome sequencing.

proportional hazards model. All statistical analyses were performed using SAS Release V.9.4 (SAS Institute).

## RESULTS

### Patients

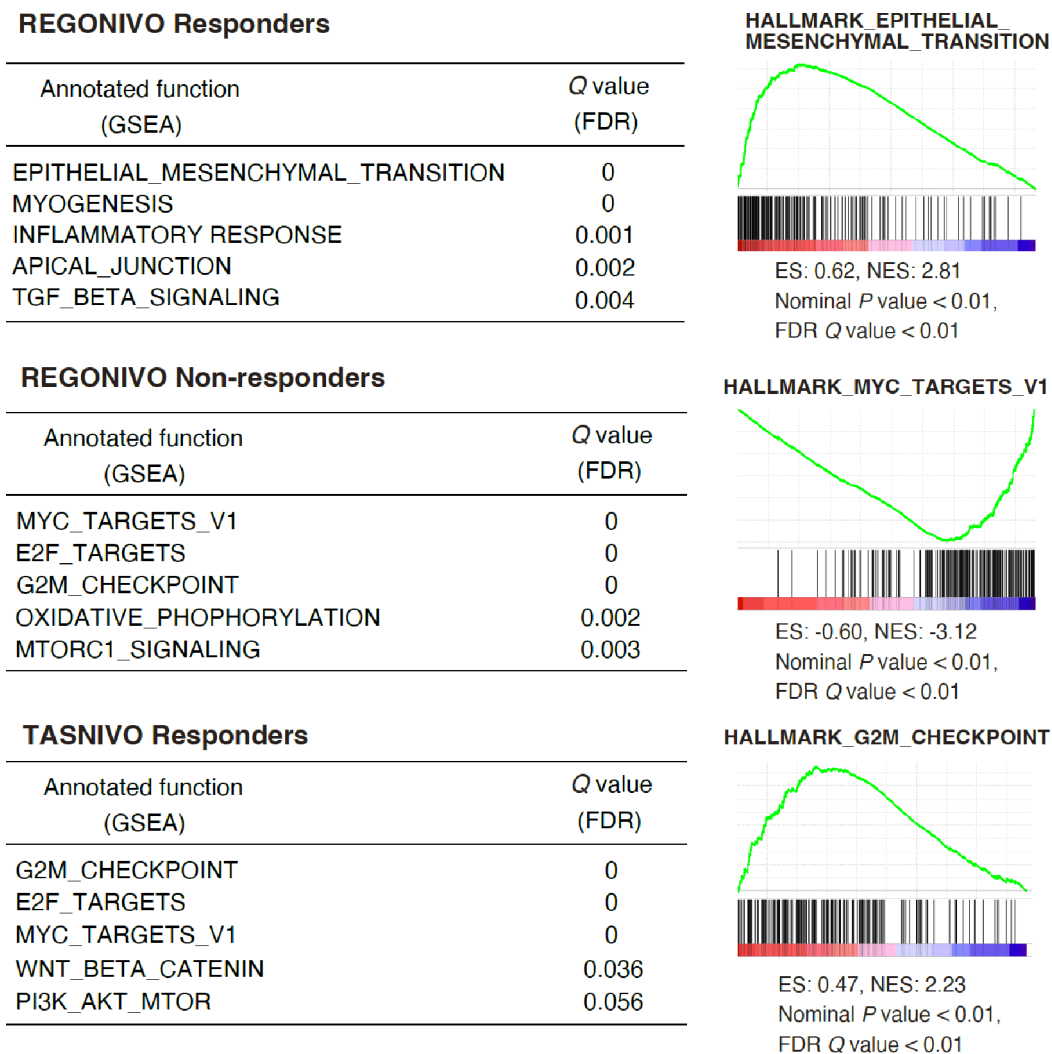
Twenty-four and 25 patients from the REGONIVO and TASNIVO trials, respectively, with MSS or pMMR CRC without prior ICI treatment, met the eligibility criteria for this study (figure 1). We successfully performed RNA-seq and WES and obtained gene panel and mIHC data for 22 and 23 patients in the REGONIVO and TASNIVO trials, respectively (figure 1). Patients with left-sided tumors were observed more frequently in the REGONIVO trial (86.4%), whereas those with right-sided tumors were observed more frequently in the TASNIVO trial (60.9%) (table 1). Ten patients in each of the REGONIVO (45.5%) TASNIVO trials (43.5%) had liver metastasis (table 1). Thirteen of 22 (59%) patients in the REGONIVO trial and 7 of 23 (30%) patients in the TASNIVO trial were classified as responders (CR, PR, and SD $\geq$ 6 months) (figure 2). In this study, all samples used for

WES, RNA-seq, and mIHC staining were obtained from patients prior to ICI therapy and were FFPE.

### Molecular features associated with clinical outcomes

#### Mutational features

We analyzed the differences in the mutational profiles between responders and non-responders in each trial using WES (figure 3 and online supplemental table S2). The mutational landscape of each trial cohort was comparable with that of previous reports.<sup>20</sup> We observed a significant association between *POLE* (DNA polymerase epsilon) mutations and positive response in the TASNIVO trial ( $p=0.015$ ), in which two cases with *POLE* missense mutations achieved PR. One out of two patients harboring *POLE* mutations exhibited an extremely high TMB (78 mutations/Mb). The TMB of the other patient could not be analyzed because no samples were available. The frequencies of the other representative gene mutations in CRC, including *KRAS*, *ERBB2*, *BRAF*, *PIK3CA*, *TP53*, *ATM*, *APC*, *AXIN2*, *LRP5*, *TCF7L2*, *SMAD2/3/4*, *ARID1A*, and *FBXW7*, were not significantly different between responders and non-responders in either trial



**Figure 4** GSEA gene sets enriched in responders and non-responders in the REGONIVO and TASNIVO trial. ES, enrichment score; FDR, false discovery rate; GSEA, Gene Set Enrichment Analyses; NES, normalized enrichment score; REGONIVO, regorafenib plus nivolumab; TASNIVO, TAS116 plus nivolumab; TGF, transforming growth factor.

(figure 3 and online supplemental table S2). TMB was not associated with the response to either of the ICI combinations even after excluding *POLE* cases (online supplemental figure S1).

#### Transcriptomic features

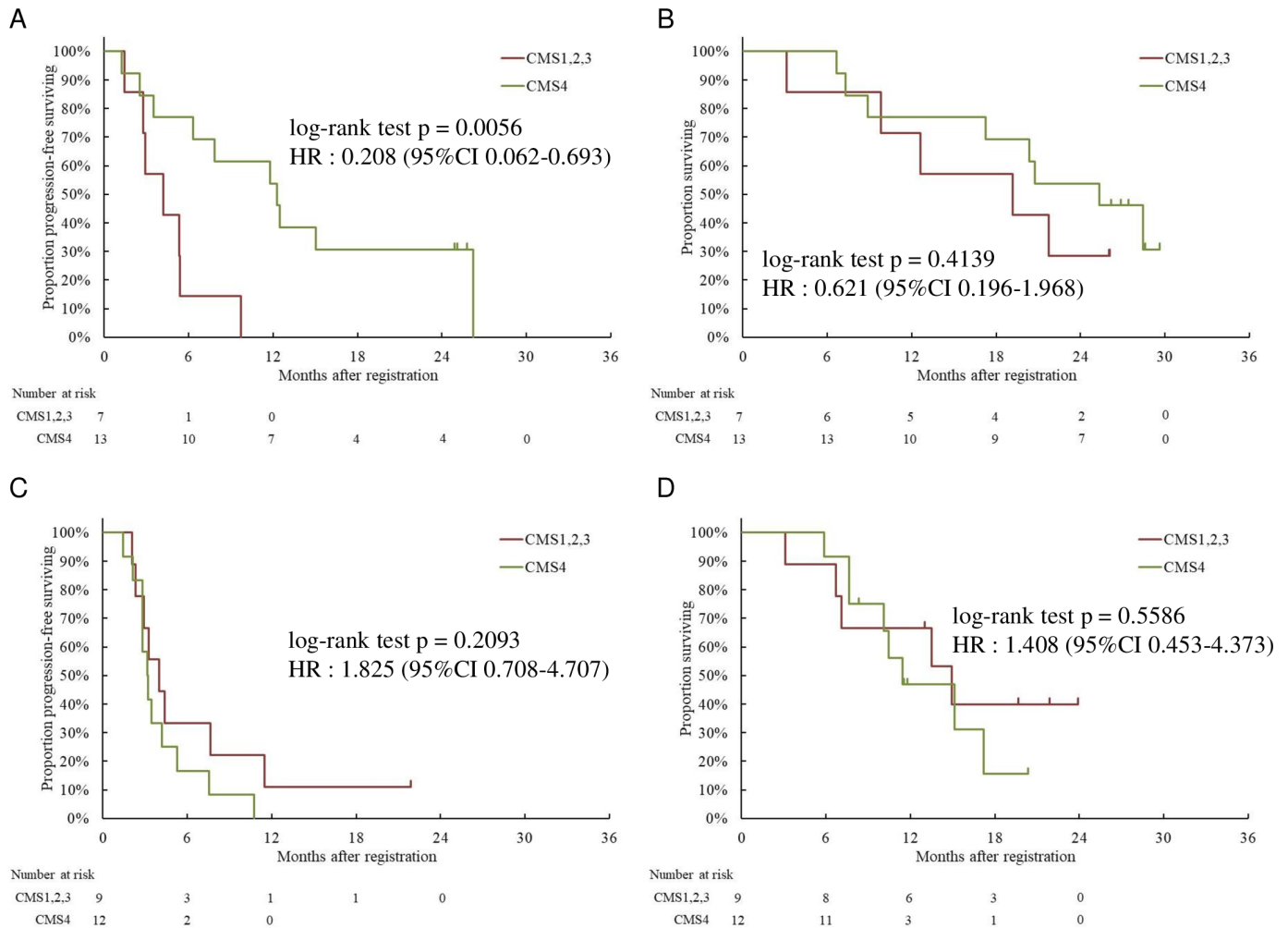
To find the difference in gene expression and upregulated signal between responders and non-responders, we next performed transcriptome analysis and GSEA on the cohorts in both trials. These analyses revealed pathways associated with the response to each combination therapy. Specifically, upregulation of the epithelial-mesenchymal transition (EMT) pathway was observed in the REGONIVO responder group (figure 4). The expression of representative EMT pathway genes, such as *TGFB3*, *VIM* and *FNI*, were upregulated in the responder group (online supplemental figure S2A). Notably, genes related to cancer-associated fibroblasts (CAFs) were also upregulated (online supplemental figure S2B). In addition, genes related to the inflammatory response were upregulated in the REGONIVO responder group,

and we observed a significant upregulation of immune-related genes such as *STAT3* (online supplemental figure S2A). Importantly, we also observed upregulation of the *PDGFRA* gene, a known target of regorafenib, in the REGONIVO responder group (online supplemental figure S2A).

Upregulation of the MYC pathway was observed in the REGONIVO non-responder group (figure 4). Upregulation of genes associated with the G2M checkpoint pathway was observed in the TASNIVO responder group. Additionally, upregulation of PI3K\_AKT\_MTOR pathway genes was observed in the responder group, and *AKT1* and *HRAS* expression were significantly upregulated (online supplemental figure S2C).

#### CMS classification of CRC

Given the results of our transcriptomic analysis, we next sought to elucidate the differences in CMS classification using RNA-seq data. CMS classification was possible in 20 of 22 patients in the REGONIVO trial and 21 of 23 patients in the TASNIVO trial. CMS1, CMS2, CMS3, and



**Figure 5** Survival curves based on the CMS classification Kaplan-Meier plots the PFS (A) and OS (B) of patients in the regorafenib plus nivolumab trial with tumors classified as CMS4, or as other CMS subtypes. PFS (C) and OS (D) of patients in the TAS116 plus nivolumab trial with tumors classified as CMS4 or as other CMS subtypes. CMS, consensus molecular subtypes; OS, overall survival PFS, progression-free survival.

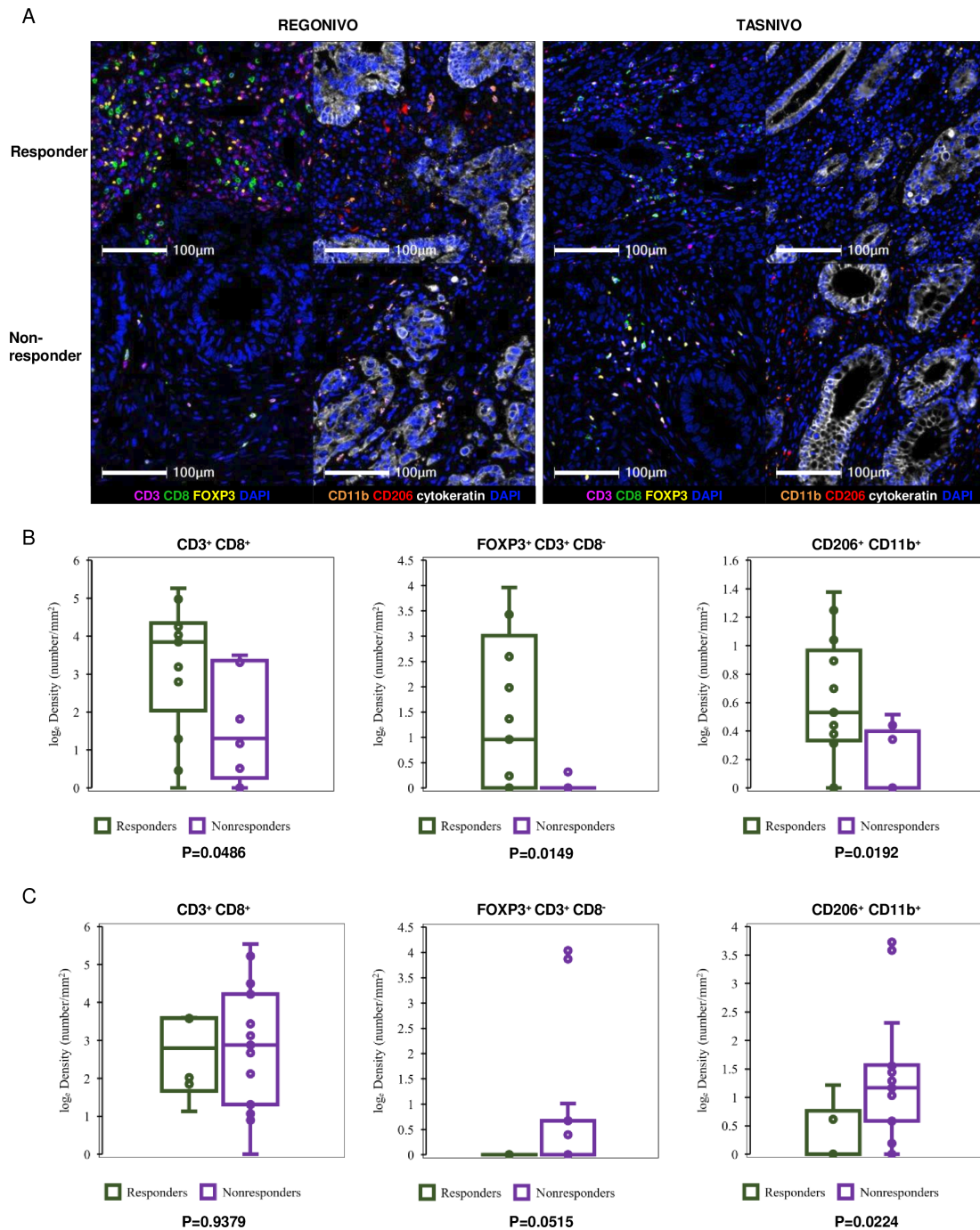
CMS4 were detected in 0, 7 (35%), 0, and 13 (65%) cases in the REGONIVO trial and 4 (19%), 3 (14%), 2 (10%), and 12 (57%) cases in the TASNIVO trial, respectively. In the REGONIVO trial, CMS4 was significantly associated with patient response compared with the other CMS subtypes ( $p=0.035$ ), but CMS4 was not associated with patient response in the TASNIVO trial (online supplemental table S2). Among 13 patients with CMS4 in the REGONIVO trial, one had a CR, six had a PR, and three had SD lasting more than 6 months. Patients with CMS4 in the REGONIVO trial demonstrated a significantly longer PFS (median 12.3 months vs 4.2 months; HR 0.208 (95% CI 0.062 to 0.693);  $p=0.006$ ) and a longer OS (median 25.3 months vs 19.2 months; HR 0.621 (95% CI 0.196 to 1.968);  $p=0.4139$ ) than did those with other CMS subtypes, whereas those with CMS4 in the TASNIVO trial did not (figure 5). In addition, when considering only cases of non-liver metastasis, a significant improvement in PFS was observed in patients with CMS4 in the REGONIVO trial (median 15.0 months vs 4.1 months; HR 0.072 (95% CI 0.006 to 0.808);  $p=0.006$ ), which was

not observed in cases with liver metastasis (online supplemental figure S3).

#### Multiplex fluorescence immunohistochemistry

mIHC of FFPE specimens obtained prior to treatment was performed to compare the tumor immune cell infiltration of responders and non-responders in the REGONIVO and TASNIVO cohorts using image analysis software (figure 6A). In the REGONIVO trial, the density of CD8<sup>+</sup>T cells (CD3<sup>+</sup>CD8<sup>+</sup>), Treg cells (FOXP3<sup>+</sup>CD3<sup>+</sup>CD8<sup>-</sup>), and M2 macrophages (CD206<sup>+</sup>CD11b<sup>+</sup>) in the intratumoral area was significantly higher in responders ( $n=13$ ) than in non-responders ( $n=9$ ) (figure 6B). In contrast, M2 macrophage density was significantly lower in the responders ( $n=6$ ) than in the non-responders ( $n=15$ ) (figure 6C) in the TASNIVO trial. Similar trends were observed when focusing on primary lesions (online supplemental figure S4). In the combined analysis of samples from both trials, higher infiltration of CD8<sup>+</sup> T cells was observed in the CMS4 subtype than in the CMS2 and CMS3 subtypes, and infiltration of Treg cells and M2 macrophages was also





**Figure 6** Multiplex immunohistochemistry analysis of the tumor immune microenvironment. Representative multiplex IHC images of samples from responders and non-responders in the REGONIVO and TASNIVO trials (A). Comparative analysis of tumor-infiltrating immune cells, CD8<sup>+</sup> T cells (CD3<sup>+</sup>CD8<sup>+</sup>), Treg cells (FOXP3<sup>+</sup>CD3<sup>+</sup>CD8<sup>+</sup>), and M2 macrophages (CD206<sup>+</sup>CD11b<sup>+</sup>), was performed by multiplex IHC and HALO image analysis software between responders and non-responders in the REGONIVO (B) and TASNIVO (C) trials. IHC, immunohistochemistry; REGONIVO, regorafenib plus nivolumab; TASNIVO, TAS116 plus nivolumab.

observed; however the differences were not statistically significant (online supplemental figure S5). One patient harboring *POLE* mutations demonstrated a higher-than-average infiltration of CD8<sup>+</sup> T cells with lower infiltration of Treg cells and M2 macrophages. Furthermore, in line with transcriptome analysis, responders (n=7) presented higher PDGFR $\alpha$  expression than non-responders (n=8)

in the REGONIVO trial (online supplemental figure S6A, B).

We also evaluated the association between PD-L1 CPS, which is commonly associated with ICI response, and the proportion of responders, however, there was no significant difference (figure 3 and online supplemental table S2).

## DISCUSSION

To identify predictors of response to ICI combinations in patients with MSS or pMMR CRC, we conducted comprehensive biomarker analyses using WES, RNA-seq, and mIHC on the two investigator-initiated trials combining nivolumab with drugs expected to activate the immune response. We identified molecular features associated with the response to ICI combinations, particularly those related to tumor microenvironmental factors including EMT pathways and CMS4. To our knowledge, this is the first report to establish a multiomic molecular landscape of the response to ICI combinations in MSS or pMMR CRC.

We found that *POLE* mutations were significantly associated with response in the TASNIVO trial and that no specific gene mutations were associated with response in the REGONIVO trial. *POLE* mutations have been reported to be associated with a hypermutation phenotype and response to anti-PD-1 monotherapy in CRC.<sup>21–24</sup> In this study, one patient in the TASNIVO trial with *POLE* mutations had an extremely high TMB and high CD8<sup>+</sup> T-cell infiltration and low Treg cell and M2 macrophage infiltration. Thus, it is highly likely that the response achieved in the two cases with *POLE* mutations identified in the TASNIVO trial was primarily driven by nivolumab. However, aside from *POLE* mutation, no genomic features, including TMB, were identified as predictive markers for the response to ICI combinations in MSS or pMMR CRC in each study.

With respect to the tumor microenvironment, transcriptome analysis of samples from patients in the REGONIVO trial revealed upregulation of the EMT pathway and genes related to CAFs in responders and upregulation of the MYC pathway in non-responders. Furthermore, mIHC results revealed that the density of CD8<sup>+</sup> T cells, Treg cells, and M2 macrophages was significantly higher in responders, which was comparable to the findings presented in a previous report.<sup>25</sup>

Interestingly, patients with CMS4 in the REGONIVO trial were associated with better clinical outcomes, which was not observed in the TASNIVO trial. CMS4 is characterized by “mesenchymal” features, such as upregulation of the EMT and transforming growth factor (TGF)- $\beta$  signaling pathways along with the high expression of genes associated with angiogenesis or extracellular matrix remodeling resulting in a high presence of CAFs,<sup>19 26</sup> which are known to be associated with treatment resistance. Although the tumor immune microenvironment of CMS4 is considered “immune inflamed” with the presence of a higher number of infiltrating CD8<sup>+</sup> T cells compared with CMS2 or CMS3,<sup>19 27</sup> immune suppressive cells, such as Treg cells and M2 macrophages, which are involved in inhibiting cytotoxic T cells and suppressing the immune response,<sup>27</sup> also infiltrated this subtype. It has been reported that Treg cells are recruited via CD70 expressed on CAFs in CRC, and accumulate due to CCL28 in the hypoxic environment caused by abnormal angiogenesis.<sup>28 29</sup> In the tumor microenvironment regorafenib

leads to a decrease in Treg cells with the inhibition of CAF proliferation inducing apoptosis and potent anti-angiogenic effects, which are expected to improve the hypoxic environment.<sup>30 31</sup> It has also been reported that regorafenib inhibits TAM infiltration and M2 macrophage activation by blocking the TIE2 pathway, thereby promoting a persistent M1 phenotype.<sup>30 32 33</sup> Indeed, in preclinical models, regorafenib modified the tumor immune microenvironments decreasing the infiltration of CAFs, Treg cells and M2 macrophages, thus restoring the antitumor activity of PD-1 inhibitors.<sup>34 35</sup> Additionally, it has been reported that PDGFRA, PDGFRB, and KIT, which are targets of regorafenib, are highly expressed in CMS4 CRC and have been proposed as therapeutic targets.<sup>36 37</sup> Indeed, our study found that PDGFRA was highly expressed in the REGONIVO trial responders, which is consistent with these reports. These findings suggest that combining regorafenib and PD-1 inhibitors could be effective for some CRC, specifically for the CMS4 subtype, in which infiltrating CD8<sup>+</sup> T cells are suppressed by immunosuppressive cells.

In contrast, CMS2, which accounted for more than half of the non-responders in the REGONIVO trial, is associated with upregulation of the MYC and WNT signaling pathways, low levels of tumor-infiltrating immune cells, and poor intertumoral immune cell activation.<sup>19 26 27 38</sup> Consistent with our finding that there was no correlation between CMS4 and REGONIVO response or a favorable clinical outcome in patients with liver metastases, preclinical models have indicated that the presence of liver metastases induces apoptosis in antigen-specific activated T cells, resulting in a systemic immunological desert.<sup>39</sup> The development of further ICI combinations may be needed to address certain molecular subtypes and immune microenvironment phenotypes.

Transcriptome analysis showed upregulation of the G2M checkpoint pathway in the TASNIVO trial responders. WEE1, a client protein of HSP90, regulates the G2/M transition in the cell cycle by phosphorylating cyclin-dependent kinase 1.<sup>40 41</sup> The AKT pathway and the MAP kinase cascade may be inhibited by HSP90 blockade.<sup>42 43</sup> Thus, the HSP90 inhibitor TAS-116 may exert antitumor activity in tumors with elevated G2M checkpoint-related genes or high expression of *AKT1* and *HRAS* in the TASNIVO trial. We previously reported that TAS-116 enhanced the antitumor activity of PD-1 inhibitors by reducing Treg cells in vitro and in vivo.<sup>44</sup> However, in the present study, the significant infiltration of M2 macrophages in non-responders suggests that even if Treg cells were eliminated, the immune suppression by M2 macrophages could not be overcome by the HSP90 inhibitor.

This study has several limitations. The primary limitation is that this study was conducted with a limited sample size of patients from early clinical trials, and not all patient data were included in the biomarker analyses due to inconsistent sample availability. For example, only CMS2 and CMS4 were observed among patients in the REGONIVO



trial, probably due to the small number of included patients. Therefore, the presented results should be interpreted as preliminary, and further studies are warranted to validate these findings. Furthermore, because our analysis was performed using pretreatment samples only, a future comparative analysis of pretreatment and post-treatment samples would potentially strengthen our findings regarding the tumor microenvironment.<sup>43</sup>

In conclusion, we identified molecular features, particularly those related to tumor microenvironmental factors, that were associated with the response to REGONIVO and TASNIVO. Of note, CMS classification may correlate with the clinical outcome of REGONIVO in MSS or pMMR CRC. These findings may be helpful for the development of predictive biomarkers for precision medicine applications or new combination immunotherapies.

#### Author affiliations

<sup>1</sup>Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center-Hospital East, Kashiwa, Japan

<sup>2</sup>Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Yokohama, Japan

<sup>3</sup>Division of Cellular Signaling, National Cancer Center Research Institute, Tokyo, Japan

<sup>4</sup>Division of Cancer Immunology, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center-Hospital East, Kashiwa, Japan

<sup>5</sup>Department of Gastroenterology, Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Tokyo, Japan

<sup>6</sup>Department of Gastroenterology, Saitama Cancer Center, Kitaadachi-gun, Japan

<sup>7</sup>BioStatistics Division, Center for Research Administration and Support, National Cancer Center-Hospital East, Kashiwa, Chiba, Japan

<sup>8</sup>Department of Pathology and Clinical Laboratories, National Cancer Center-Hospital East, Kashiwa, Chiba, Japan

<sup>9</sup>Department of Genetic Medicine and Services, National Cancer Center-Hospital East, Kashiwa, Chiba, Japan

<sup>10</sup>Department of Immunology, Nagoya University Graduate School of Medicine, Nagoya, Japan

**Correction notice** This article has been corrected since it was first published. The footnote has been added that ST, YT and Y-TL contributed equally.

**Acknowledgements** We would like to thank the patients and their families, the nurses, and the investigators who participated in this study.

**Contributors** ST, YT, SKoy, KS, and AK designed the study. ST, YT, Y-TL, SKoy, TU, SKoj, MW, NS, MK, and TKu processed the experimental data and performed the analysis. SF, HH, YN, YK, DK, TKo, HB, and SM performed sample preparation. TY, HN, HM, IE, KS, and AK performed conceptualization and supervised the project. ST, YT, Y-TL, and AK wrote the manuscript with input from all coauthors. KS and AK are guarantors of this study. All authors have read, reviewed, and edited the manuscript and approved the final version for submission.

**Funding** EPOC1603 was supported by Bayer HealthCare Pharmaceuticals and Ono Pharmaceuticals. EPOC1704 was supported by Taiho Pharmaceutical and Ono Pharmaceuticals. The funding bodies had no role in the study design; data collection, analysis, or interpretation; or in the writing of the report.

**Competing interests** ST, YT, and Y-TL have nothing to disclose. SKoy has received research funding from Otsuka Pharmaceutical, Shionogi Pharmaceutical and Chugai Pharmaceutical outside the submitted work. SF has nothing to disclose. HH has received honoraria from Bayer, Bristol-Myers Squibb, Chugai, Daiichi-Sankyo, Kyowa Hakko Kirin, Lilly, Merck Biopharma, MSD, Ono, Taiho, Takeda, and Yakult; fees for consulting or advisory roles for Bristol-Myers Squibb, Boehringer Ingelheim and Daiichi-Sankyo; and research grants from ALX Oncology, Amgen, Astellas, AstraZeneca, Bayer, BeiGene, Boehringer Ingelheim, Bristol-Myers Squibb, Chugai, Daiichi-Sankyo, Eisai, Janssen, Merck Biopharma, MSD, Ono Pharmaceutical, and Taiho Pharmaceutical outside the submitted work. YN has received honoraria from Chugai, Merck, and Guardant Health AMEA and research grants from Taiho, Chugai, Guardant Health, Genomedica, Daiichi-Sankyo, Seagen,

and Roche Diagnostics outside the submitted work. YK has nothing to disclose. DK has received personal fees for advisory roles from Takeda and Eisai; honoraria from MSD, Pfizer, Chugai, Merck biopharma, Lilly, Sysmex, Nipponkayaku, Bristol-Myers Squibb, Ono Pharmaceutical, Taiho Pharmaceutical, Takeda, Eisai, and Daiichi-Sankyo; and research funding from Ono Pharmaceutical, MSD, Novartis, Sanofi, Janssen, Pierre Fabre Medicament, Isofol Medical AB, Hutchison MediPharma Limited, and Nippon Serivier outside the submitted work. TKo has received honoraria from MSD, Bristol-Myers Squibb and Ono Pharmaceutical; personal fees for advisory roles from Boehringer Ingelheim, Kyowa Kirin and Taiho Pharmaceutical; and research funding from AstraZeneca, BeiGene, MSD, Amgen, Chugai Pharmaceutical, Taiho Pharmaceutical, Shionogi Pharma, and Amgen Astellas BioPharma outside the submitted work. HB has received honoraria from Ono Pharmaceutical, Taiho Pharmaceutical, and Eli Lilly Japan and research funding from Ono Pharmaceutical outside the submitted work. SM, TU, SKoj, MW, NS, and MK have nothing to disclose. TKu has received honoraria from AstraZeneca, Astellas Pharma, Ono Pharmaceutical, Bristol-Myers Squibb Japan, Daiichi-Sankyo, and Roche Diagnostics; personal fees for consulting or advisory roles from Astellas Pharma; and research funding from Daiichi-Sankyo and Roche Diagnostics outside the submitted work. TY has received research grants from Taiho, Ono, Chugai, Amgen, MSD, Daiichi-Sankyo, Eisai, FALCO biosystems, Genomedica, Molecular Health, Nippon Boehringer Ingelheim, Pfizer, Roche Diagnostics, Sysmex, and Sanofi outside the submitted work and honoraria from Bayer, Chugai, Merck Biopharma, MSD, Ono, and Takeda. HN has received research funding and honoraria from Ono Pharmaceutical, MSD, Bristol-Myers Squibb, and Chugai Pharmaceutical and research funding from Taiho Pharmaceutical, Daiichi-Sankyo, Kyowa Kirin, Zenyaku Kogyo, Oncolys BioPharma, Debiopharma, Asahi-Kasei, Sysmex, Fujifilm, SRL, Astellas Pharmaceutical, Sumitomo Dainippon Pharma, and BD Japan outside the submitted work. HM has nothing to disclose. IE has received research grants from Taiho Pharmaceutical and Ono Pharmaceutical outside the submitted work. AK has received personal fees for advisory roles from Roche and Lilly; honoraria from Roche, Merck Biopharma, Lilly, Bristol-Myers Squibb, Ono Pharmaceutical, Taiho Pharmaceutical, and Daiichi-Sankyo; and research funding from Ono Pharmaceutical, MSD, Sanofi, AstraZeneca, and Eisai outside the submitted work.

**Patient consent for publication** Not applicable.

**Ethics approval** The study was approved by the National Cancer Center Hospital East Certified Review Board (ID: 2022-041). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available upon reasonable request. Data access will be granted upon request to the corresponding author.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See <http://creativecommons.org/licenses/by-nc/4.0/>.

#### ORCID iDs

Shogo Takei <http://orcid.org/0000-0003-0614-8383>

Shohei Koyama <http://orcid.org/0000-0002-6897-9417>

Hiro Yoshi Nishikawa <http://orcid.org/0000-0001-6563-9807>

Kohei Shitara <http://orcid.org/0000-0001-5196-3630>

#### REFERENCES

- Sung H, Ferlay J, Siegel RL, *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021;71:209–49.
- Herbst RS, Giaccone G, de Marinis F, *et al.* Atezolizumab for First-Line Treatment of PD-L1-Selected Patients with NSCLC. *N Engl J Med* 2020;383:1328–39.



- 3 James L, Vanna C-S, Rene G, *et al.* Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* 2015;373:1270–1.
- 4 Bellmunt J, de Wit R, Vaughn DJ, *et al.* Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. *N Engl J Med* 2017;376:1015–26.
- 5 Garon EB, Rizvi NA, Hui R, *et al.* Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. *N Engl J Med* 2015;372:2018–28.
- 6 Kato K, Cho BC, Takahashi M, *et al.* Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol* 2019;20:1506–17.
- 7 Kang Y-K, Boku N, Satoh T, *et al.* Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;390:2461–71.
- 8 Overman MJ, McDermott R, Leach JL, *et al.* Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol* 2017;18:1182–91.
- 9 André T, Shiu K-K, Kim TW, *et al.* Pembrolizumab in Microsatellite Instability-High Advanced Colorectal Cancer. *N Engl J Med* 2020;383:2207–18.
- 10 Le DT, Uram JN, Wang H, *et al.* PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *New Engl J Medicine* 2015;372:2509–20.
- 11 Overman MJ, Lonardi S, Wong KYM, *et al.* Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/ Microsatellite Instability-High Metastatic Colorectal Cancer. *J Clin Oncol* 2018;36:773–9.
- 12 Angelova M, Charoentong P, Hackl H, *et al.* Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumor escape mechanisms and novel targets for immunotherapy. *Genome Biol* 2015;16:64.
- 13 Kang J-C, Chen J-S, Lee C-H, *et al.* Intratumoral macrophage counts correlate with tumor progression in colorectal cancer. *J Surg Oncol* 2010;102:242–8.
- 14 Kim JE, Chun S-M, Hong YS, *et al.* Mutation Burden and I Index for Detection of Microsatellite Instability in Colorectal Cancer by Targeted Next-Generation Sequencing. *The Journal of Molecular Diagnostics* 2019;21:241–50.
- 15 Eng C, Kim TW, Bendell J, *et al.* Atezolizumab with or without cobimetinib versus regorafenib in previously treated metastatic colorectal cancer (IMblaze370): a multicentre, open-label, phase 3, randomised, controlled trial. *Lancet Oncol* 2019;20:849–61.
- 16 MERCK PR. Merck and Eisai provide update on phase 3 trials of KEYTRUDA® (Pembrolizumab) plus LENVIMA® (Lenvatinib) in certain patients with advanced Melanoma (LEAP-003) and metastatic colorectal cancer (LEAP 017). 2023. Available: <https://www.merck.com/news/merck-and-eisai-provide-update-on-phase-3-trials-of-keytruda-pembrolizumab-plus-lenvima-lenvatinib-in-certain-patients-with-advanced-melanoma-leap-003-and-metastatic-colorectal-cancer/>
- 17 Fukuoka S, Hara H, Takahashi N, *et al.* Regorafenib Plus Nivolumab in Patients With Advanced Gastric or Colorectal Cancer: an Open-Label, Dose-Escalation, and Dose-Expansion Phase Ib Trial (REGONIVO, EPOC1603). *JCO* 2020;38:2053–61.
- 18 Kawazoe A, Itahashi K, Yamamoto N, *et al.* TAS-116 plus nivolumab in patients with colorectal cancer and other solid tumors: an, open-label, dose-finding. *Clin Cancer Res* 2021;27.
- 19 Guinney J, Dienstmann R, Wang X, *et al.* The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015;21:1350–6.
- 20 Muzny DM, Bainbridge MN, Chang K, *et al.* Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012;487:330–7.
- 21 Giannakis M, Mu XJ, Shukla SA, *et al.* Genomic Correlates of Immune-Cell Infiltrates in Colorectal Carcinoma. *Cell Rep* 2016;15:857–65.
- 22 Heitzer E, Tomlinson I. Replicative DNA polymerase mutations in cancer. *Curr Opin Genet Dev* 2014;24:107–13.
- 23 Gong J, Wang C, Lee PP, *et al.* Response to PD-1 Blockade in Microsatellite Stable Metastatic Colorectal Cancer Harboring a POLE Mutation. *J Natl Compr Canc Netw* 2017;15:142–7.
- 24 Domingo E, Freeman-Mills L, Rayner E, *et al.* Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. *Lancet Gastroenterol Hepatol* 2016;1:207–16.
- 25 Fakih M, Raghav KPS, Chang DZ, *et al.* Regorafenib plus nivolumab in patients with mismatch repair-proficient/microsatellite stable metastatic colorectal cancer: a single-arm, open-label, multicentre phase 2 study. *EClinicalMedicine* 2023;58:101917.
- 26 Becht E, de Reyniès A, Giraldo NA, *et al.* Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy. *Clin Cancer Res* 2016;22:4057–66.
- 27 Karpinski P, Rossowska J, Sasiadek MM. Immunological landscape of consensus clusters in colorectal cancer. *Oncotarget* 2017;8:105299–311.
- 28 Facciabene A, Peng X, Hagemann IS, *et al.* Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. *Nature* 2011;475:226–30.
- 29 Jacobs J, Deschoolmeester V, Zwaenepoel K, *et al.* Unveiling a CD70-positive subset of cancer-associated fibroblasts marked by pro-migratory activity and thriving regulatory T cell accumulation. *Oncoimmunology* 2018;7:e1440167.
- 30 Abou-Elkacem L, Arns S, Brix G, *et al.* Regorafenib inhibits growth, angiogenesis, and metastasis in a highly aggressive, orthotopic colon cancer model. *Mol Cancer Ther* 2013;12:1322–31.
- 31 Zhang M, Li X, Wu W, *et al.* Regorafenib Induces the Apoptosis of Gastrointestinal Cancer-Associated Fibroblasts by Inhibiting AKT Phosphorylation. *Stem Cells Dev* 2022;31:383–94.
- 32 Zhao P, Wang Y, Kang X, *et al.* Dual-targeting biomimetic delivery for anti-glioma activity via remodeling the tumor microenvironment and directing macrophage-mediated immunotherapy. *Chem Sci* 2018;9:2674–89.
- 33 Chiang I-T, Lee Y-H, Tan Z-L, *et al.* Regorafenib enhances antitumor immune efficacy of anti-PD-L1 immunotherapy on oral squamous cell carcinoma. *Biomed Pharmacother* 2022;147:112661.
- 34 Doleschel D, Hoff S, Koletnik S, *et al.* Regorafenib enhances anti-PD1 immunotherapy efficacy in murine colorectal cancers and their combination prevents tumor regrowth. *J Exp Clin Cancer Res* 2021;40:288.
- 35 Akiyama T, Yasuda T, Uchihara T, *et al.* Stromal Reprogramming through Dual PDGFR $\alpha/\beta$  Blockade Boosts the Efficacy of Anti-PD-1 Immunotherapy in Fibrotic Tumors. *Cancer Res* 2023;83:753–70.
- 36 Ubink I, Elias SG, Moelans CB, *et al.* A Novel Diagnostic Tool for Selecting Patients With Mesenchymal-Type Colon Cancer Reveals Intratumor Subtype Heterogeneity. *J Natl Cancer Inst* 2017;109.
- 37 Peters NA, Constantinides A, Ubink I, *et al.* Consensus molecular subtype 4 (CMS4)-targeted therapy in primary colon cancer: a proof-of-concept study. *Front Oncol* 2022;12:969855.
- 38 Lal N, White BS, Goussous G, *et al.* KRAS Mutation and Consensus Molecular Subtypes 2 and 3 Are Independently Associated with Reduced Immune Infiltration and Reactivity in Colorectal Cancer. *Clin Cancer Res* 2018;24:224–33.
- 39 Yu J, Green MD, Li S, *et al.* Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T cell elimination. *Nat Med* 2021;27:152–64.
- 40 Harvey SL, Charlet A, Haas W, *et al.* Cdk1-dependent regulation of the mitotic inhibitor Wee1. *Cell* 2005;122:407–20.
- 41 Mollapour M, Tsutsumi S, Neckers L. Hsp90 phosphorylation, Wee1 and the cell cycle. *Cell Cycle* 2010;9:2310–6.
- 42 Haarberg HE, Paraiso KHT, Wood E, *et al.* Inhibition of Wee1, AKT, and CDK4 underlies the efficacy of the HSP90 inhibitor XL888 in an in vivo model of NRAS-mutant melanoma. *Mol Cancer Ther* 2013;12:901–12.
- 43 Vaughan CK, Mollapour M, Smith JR, *et al.* Hsp90-Dependent Activation of Protein Kinases Is Regulated by Chaperone-Targeted Dephosphorylation of Cdc37. *Molecular Cell* 2008;31:886–95.
- 44 Tsuge A, Togashi Y, Shitara K, *et al.* A novel regulatory T Cell-Targeted Immunotherapy by targeting their crucial signal by HSP90 inhibitors. 2019:243.

論文目録

I 主論文

Multioomic molecular characterization of the response to combination immunotherapy in  
MSS/pMMR metastatic colorectal cancer

Takei S., Tanaka Y., Lin Y., Koyama S., Fukuoka S., Hara H., Nakamura Y., Kuboki Y., Kotani D.,  
Kojima T., Bando H., Mishima S., Ueno T., Kojima S., Wakabayashi M., Sakamoto N., Kojima M.,  
Kuwata T., Yoshino T., Nishikawa H., Mano H., Endo I., Shitara K., Kawazoe A.

雑誌名 : Journal for ImmunoTherapy of Cancer Vol.12, No.2, e008210, 2024