The Oncologist, 2024, **29**, e213–e223
https://doi.org/10.1093/oncolo/oyad230
Advance access publication 17 August 2023
Original Article

Increased *KRAS* G12C Prevalence, High Tumor Mutational Burden, and Specific Mutational Signatures Are Associated With *MUTYH* Mutations: A Pan-Cancer Analysis

Umut Disel[1,*], Smruthy Sivakumar[2], Tim Pham[3], Zoe Fleischmann[2], R.I. Anu[4], Ethan S. Sokol[2], Razelle Kurzrock[5]

[1]Acıbadem Adana Hospital, Department of Medical Oncology, Adana, Turkey
[2]Foundation Medicine, Inc., Cambridge, MA, USA
[3]Center for Personalized Cancer Therapy, Moores Cancer Center, UCSD, San Diego, CA, USA
[4]Department of Cancer Biology and Therapeutics, Department of Clinical Biochemistry, MVR Cancer Center and Research Institute Chuloor, Calicut, Kerala, India
[5]WIN Consortium for Personalized Cancer Therapy, Medical College of Wisconsin, Milwaukee, WI, USA
[*]Corresponding author: Umut Disel, MD, Adana Acıbadem Hospital, Adana, Turkey. Tel: +903224554791; Email: doctorhope@gmail.com

Abstract

The aim of this study was to determine the pan-cancer landscape of *MUTYH* alterations and the relationship between *MUTYH* mutations and potentially actionable biomarkers such as specific genomic alterations, tumor mutational burden, and mutational signatures. We used a large pan-cancer comprehensive genomic dataset from patients profiled (tissue next generation sequencing) during routine clinical care. Overall, 2.8% of 229 120 solid tumors had *MUTYH* alterations, of which 55% were predicted germline. Thirty tumor types had a 2% or greater *MUTYH* mutation rate. *MUTYH*-altered versus -WT cancers had significantly higher tumor mutational burden and more frequent alterations in *KRAS G12C,* but not in *KRAS* in general; these observations were statistically significant, especially in colorectal cancers. Across cancers, PD-L1 expression levels (immunohistochemistry) were not associated with *MUTYH* alteration status. In silico computation demonstrated that *MUTYH* mutational signatures are associated with higher levels of hydrophobicity (which may reflect higher immunogenicity of neoantigens) relative to several other signature types such as microsatellite instability. Survival of patients with *MUTYH*-altered versus -WT tumors was similar. In conclusion, comprehensive genomic profiling suggests that several features of *MUTYH*-altered cancers may be pharmacologically targetable. Drugs such as sotorasib (targeting *KRAS G12C*) and immune checkpoint inhibitors, targeting the increased mutational load and higher neo-antigen hydrophobicity/immunogenicity merit investigation in *MUTYH*-mutated malignancies.

Key words: *KRAS* G12C; genomic alterations; tumor mutational burden; *MUTYH* mutations.

Implications for Practice

Of 229120 tumors, 2.8% harbored *MUTYH* alterations (~55% germline), which correlated with higher mutational burden, more hydrophobic (possibly immunogenic) mutational signatures, and $KRAS^{G12C}$ mutations. Immunotherapy and $KRAS^{G12C}$ inhibitors warrant investigation in *MUTYH*-mutated cancers.

Introduction

Exposure to carcinogens, including oxidizing agents, alkylating agents, DNA cross-linking agents, and radiation is capable of damaging DNA.[1] The *MUTYH* gene is located on the short arm of chromosome 1 (1p34.1) and is responsible for coding the MYH glycosylase enzyme. This enzyme is part of the DNA base excision repair system, which repairs oxidized DNA damage. 8-Oxoguanine (8-oxoG), a major oxidized form of guanine generated by reactive oxygen species, is highly mutagenic due to its mispairing with adenine.[2] The function of *MUTYH* is in preventing oxidative damage-induced mutations, such as GC>TA transversions. Accumulation of 8-oxoG in DNA causes somatic mutations, eventually leading to cancer and neurodegenerative diseases.[3,4]

MUTYH-associated polyposis (MAP) is an autosomal recessive disorder characterized by colorectal adenomatous polyps that confers a very high risk of colorectal cancer (CRC), predisposition to duodenal adenomas and a modest increase in risk for non-CRC malignancies.[5] Colorectal cancer cells in patients with MAP fail to repair mismatches induced by 8-oxoG and consequently harbor an excess of KRAS c.34G>T transversions and structural abnormalities such as fusions, a widely recognized hallmark of oxidative stress.[6-9] The association between *MUTYH* mutation in patients with MAP and the increased frequency of *KRAS G12C* mutation

Received: 18 December 2022; Accepted: 19 July 2023.

(NM_004985.5(KRAS):c.34G>T (p.Gly12Cys)) has become especially relevant since *KRAS G12C* is now a druggable target.[10] The base excision repair system defect, such as that induced by *MUTYH* alterations, may correlate with increased mutational burden and a mutational signature (COSMIC SBS18 and SBS36) that reflects typical oxidative damage.[11-15]

We encountered a young (38-year-old) woman in clinic with metastatic CRC, a *MUTYH* mutation, and an elevated tumor mutational burden (TMB), who attained a complete response to a regimen that included immune checkpoint blockade. Because of the potential importance of *MUTYH* pathogenic alterations in cancer pathogenesis, and the paucity of published data available about them, we investigated a database of over 200 000 tumor samples to elucidate the *MUTYH* landscape, and its relationship with specific alterations and immune checkpoint blockade biomarkers.

Methods

Comprehensive Genomic Profiling Cohort

This study consisted of a pan-solid tumor cohort comprising 229 120 patients who underwent comprehensive genomic profiling (CGP), as part of routine clinical care. CGP on formalin-fixed, paraffin-embedded (FFPE) tissue biopsy sections was performed using hybrid capture on up to 395 cancer-related genes and select introns from up to 34 genes frequently rearranged in cancer (Foundation Medicine, Inc.). Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization was obtained from the Western Institutional Review Board (Protocol No. 20152817).

Identification of Genomic Alterations and Biomarker Patterns

Different classes of gene alterations, including short variants (base substitutions and indels), copy number alterations, and rearrangement events were identified, as described previously.[16] Variants were classified as known/likely pathogenic or as variants of unknown significance (VUS). For short variants of interest, somatic/germline status for mutations was computationally predicted without matched normal tissue; in the validation testing of 480 tumor-only predictions against matched normal specimens, accuracy was 95% for somatic and 99% for germline predictions.[17] Further examination of biallelic/monoallelic status of *MUTYH* alterations was also performed using the SGZ algorithm, where available.[17] Specifically, cases with a single heterozygous *MUTYH* alteration were classified as monoallelic. Cases with 2 or more *MUTYH* alterations, or a single short variant in *MUTYH* predicted to be under loss of heterozygosity, or a homozygous copy number deletion of *MUTYH* were classified as biallelic. TMB (mutations/megabase [mut/Mb]) was determined on 0.8-1.1 Mb of sequenced DNA.[18] TMB was assessed in 3 bins: <10 mut/Mb, 10-20 mut/Mb, and ≥20 mut/Mb. Microsatellite instability (MSI) status was by examining homopolymer repeat loci.[19] PD-L1 expression was determined by immunohistochemistry (IHC) performed on FFPE tissue sections and scored using DAKO PD-L1 IHC 22C3 pharmDx's tumor proportion scoring (TPS) method. Positive cases were evaluated at 2 thresholds: ≥1% and ≥50%.

Investigation of Mutational Signatures

COSMIC mutational signatures were identified as previously described.[19-22] Signatures were estimated in samples with at least 10 assessable mutations. Signatures were considered dominant if the maximum signature score met the threshold of 0.4. Given our interest in *MUTYH*-altered tumors, we performed a deeper investigation of SBS18 and SBS36, signatures implicated in defective base excision repair, including DNA damage due to reactive oxygen species.

Detecting rare signatures such as SBS18 and SBS36, which are predicted to result in 0.1-1.0 mutations/Mb when present, is challenging from a targeted panel test and may not contribute enough mutations per sample to be detectable. To overcome this, we used a pooled sample approach. Briefly, all variants that were both predicted non-germline and VUS were pooled for this analysis. Comparisons were performed between the mutant group, defined as patients with a germline *MUTYH* alteration (g*MUTYH*), and the wildtype (WT) group, defined as patients with no identified *MUTYH* alteration. Since mutagenic signatures are typically considered to be additive, the difference between the 2 groups was used to understand the additional effect of *MUTYH* mutations. The contributions of the known COSMIC v3.2 signatures were obtained using the method outlined previously.[20] The number and percentage of mutations attributed to each signature were calculated relative to the total number of pooled mutations.

The stability of this pooled approach was evaluated by resampling the cohorts to a jackknife sample size, defined as the mutations from half the number of samples available in the smaller of the 2 cohorts being compared (mutant and wild type). Both the cohorts were resampled without replacement to the jackknife sample size 1000 times, and signature attribution was assessed on each of the resampled pools of mutations. This provides both the median and 95% CI for the contribution of all known COSMIC v3.2 mutation signatures in each cohort.

In Silico Mutagenesis Probabilistic Simulations

We applied an in silico hydrophobicity analysis method to understand the immunogenicity of the increased mutation load as a result of *MUTYH* mutation. Every possible combination of 2 codons, comprising 6 nucleotides, was generated and the Single Base Substitution (SBS) mutational signatures defined by COSMIC v3.2[20] for *MUTYH* (SBS18 and 36), MSI (SBS6, 14, 15, 20, 21, and 26), and APOBEC (SBS2 and 13) were applied to each, separately. The 6-nucleotide length allowed us to account for point mutations, contextually defined by their flanking nucleotides in COSMIC, in all possible reading frames of a codon, which requires at least 5 nucleotides. In total, 4096 di-codons, before and after mutation, were transformed into their matching amino acid pairs whose hydrophobicity was then additively quantified using the Kyte-Doolittle Hydrophobicity Scale[23] and weighted by their frequency on the human exome, starting from the base frequencies in Kazusa's codon usage database[24] and updated at every iteration, and the probability of signature-defined mutagenesis on each 6-nucleotide fragment.

To illustrate this algorithm more concretely, consider the 6-nucleotide sequence TCTGAT which encodes the peptide Ser-Asp that has a Kyte-Doolittle hydrophobicity index of (−0.8) + (−3.5) = (−4.3) AU. The first codon at the second position can be mutated with SBS36's, a *MUTYH* signature,[21] most common mutation TCT > TAT, which has a frequency of occurrence of 0.208 according to data available at[25] resulting in the sequence TATGAT, or the dipeptide Tyr-Asp, which has a hydrophobicity index of (−1.3) + (−3.5) = (−4.8) AU, a

difference of (−0.5) AU over the original. This difference in hydrophobicity is then multiplied by the joint probability of the codons' occurrence and mutation, calculated by multiplying the frequency of the original dicodon in the human coding genome ($0.0152 \times 0.0218 = 3.31 \times 10^{-4}$) and the frequency of TCT > TAT mutation under SBS 36 (0.208), resulting in a joint mutation-dicodon probability of 6.89×10^{-5}. Multiplying the change in hydrophobicity by the joint mutation-dicodon frequency yields a relative hydrophobicity change of $6.89 \times 10^{-5} \times (-0.5) = (-3.45 \times 10^{-5})$. The same calculation was performed for every potential nucleotide substitution in all positions on the 6-nucleotide stretch with 2 flanking nucleotides (2nd through 5th positions, inclusive), on all 4096 combinations of 6 nucleotides. At the end of each round of mutagenesis, the frequency of codons is updated to reflect the outcome of the just-completed round, and the succeeding cycle is started using these updated codon frequencies.

Every iteration of mutagenesis is equivalent to an arbitrary unit (AU) increase in exposure to the signature's mutagen, with multiple iterations corresponding to more frequent exposures. To model the long-term effects of mutagen exposure on proteome hydrophobicity, we performed these simulations for 100 iterations per signature (SBS18, 36, 6,14,15, 20, 21, 26, 2, and 13).[26,27]

Overall Survival Analysis From a TCGA Pan-Cancer Cohort-In-Silico Analysis

To analyze the influence of alterations within the *MUTYH* gene on overall survival (OS) in patients belonging to a pan-cancer cohort, we queried the gene using the query function of cBioPortal web tool. Thirty two studies incorporating solid tumors and hematological malignancies from TCGA Pan Cancer Atlas containing 10 967 samples from 10 953 patients were used for plotting the Kaplan-Meier curve. Criteria for OS analysis, by default settings, include mutations, structural variants/fusions, and copy number alterations. The plot captures data from up to 370 months of survival. The *MUTYH*-altered group includes 169 patients, while the unaltered group has 10 634 patients. The *P*-value is calculated using the log-rank test, and *q*-value using the Benjamini-Hochberg FDR correction procedure.

Statistical Analysis and Software

Enrichment of *MUTYH* alterations in specific patient subpopulations was evaluated using a Fisher's Exact test with false discovery rate (FDR)-based correction for multiple testing. Computation and plotting were carried out using Python 2.7 (Python Software Foundation) and R 3.6.1 (R Foundation for Statistical Computing).

Results

MUTYH Alterations are Observed Across Multiple Solid Tumors

In a pan-solid tumor cohort of 229 120 patients who underwent genomic profiling during routine clinical care, *MUTYH* alterations were detected in 6297 patients (2.8%). These *MUTYH* alterations were frequently predicted to be pathogenic, with 66% of the *MUTYH*-altered samples having at least one pathogenic *MUTYH* alteration (Fig. 1A; Supplementary Table S1). Pathogenic MUTYH alterations were identified in over 2% of the samples in 30 tumor types, including germ cell (3.6%), adrenal gland (3.1%), colorectal tumors (2.7%), and small intestine tumors (2.6%) (Fig. 1A). Across tumor types, a majority of the MUTYH alterations were predicted to be monoallelic (Fig. 1A). Additionally, we used a computational approach to predict the subset with predicted germline MUTYH alterations (gMUTYH).[17] Among the *MUTYH*-altered samples, 55% harbored a predicted germline *MUTYH* alteration (prevalence of 1.5% across all solid tumors). Several tumor types were frequently positive for *gMUTYH*: germ cell (2.3%), salivary gland (2.2%), cholangiocarcinoma (1.9%), adenoid cystic carcinoma (1.9%), small intestine (1.8%), and colorectal cancers (1.8%) (Fig. 1A).

MUTYH alterations frequently comprised of single-nucleotide variants and short indels, with G382D ($n = 2277$) and Y165C ($n = 867$) being the most commonly identified alterations (Fig. 1B). In addition, recurrent splice site variants, such as 892-2A > G ($n = 278$), 1434 + 2C > T ($n = 67$) and 1145-2A > G ($n = 23$) were also frequently observed (Fig. 1B; Supplementary Table S2). Most alterations were missense and short indel alterations; copy number alterations and rearrangements impacting *MUTYH* were relatively rare (Supplementary Table S2).

MUTYH-Altered Samples Exhibit High Tumor Mutational Burden (TMB), But Not PDL1 Overexpression

Further, we examined the association between the presence of *MUTYH* alterations and TMB. *MUTYH* alterations showed a strong association with TMB-High (≥10 mut/Mb) status, and especially with TMB ≥ 20 mut/Mb (Fig. 2; Supplementary Fig. S1; Supplementary Tables S3 and S5) in the pan-cancer cohort, especially in colorectal cancer and adrenal tumors. *MUTYH*-altered cases had a significantly elevated TMB compared to wild-type (WT) samples (Wilcoxon, $P < 10^{-5}$), with increased median (5 vs. 3.8 mutations/megabase, mut/Mb) and mean (14.9 vs. 7.2 mut/Mb) TMB in *MUTYH*-altered cases compared to WT samples. In comparison to all *MUTYH*-altered cases, samples with germline *MUTYH* (*gMUTYH)* alterations had a slightly lower TMB (median = 3.8 mut/Mb; mean = 7.5 mut/Mb). This pattern was consistently observed across multiple tumor types (Fig. 2A, Supplementary Table S3). Of note, among 517 adrenal gland tumors, cases with a *MUTYH* alteration ($n = 18$; 10 *gMUTYH*) had a significantly higher TMB than those that were WT (median 9.2 vs. 2.5 mut/Mb, $P < 10^{-4}$).

High PD-L1 expression and tumor mutational burden (TMB-H, ≥10 mut/Mb) have been widely used as biomarkers to identify patient subpopulations who would benefit from immune checkpoint inhibitors across multiple tumor types.[28-30] Having observed an elevation of TMB in *MUTYH*-altered samples, we then sought to characterize the association between these immune checkpoint blockade biomarkers and *MUTYH* alterations. We did not observe a significant association with PD-L1 expression (Fig. 2B; Fig. 2C; Supplementary Fig. S1) in the limited subset of samples with available PD-L1 (DAKO 22C3) expression (Supplementary Table S4).

MUTYH Alterations Show Specific Genomic Associations in Colorectal Cancers

We examined the prevalence and genomic association of *MUTYH* alterations in 31 624 CRC samples. Overall, *MUTYH* alterations were observed in 2.7% of CRCs, with *gMUTYH* observed in 1.8% of the overall cohort and in

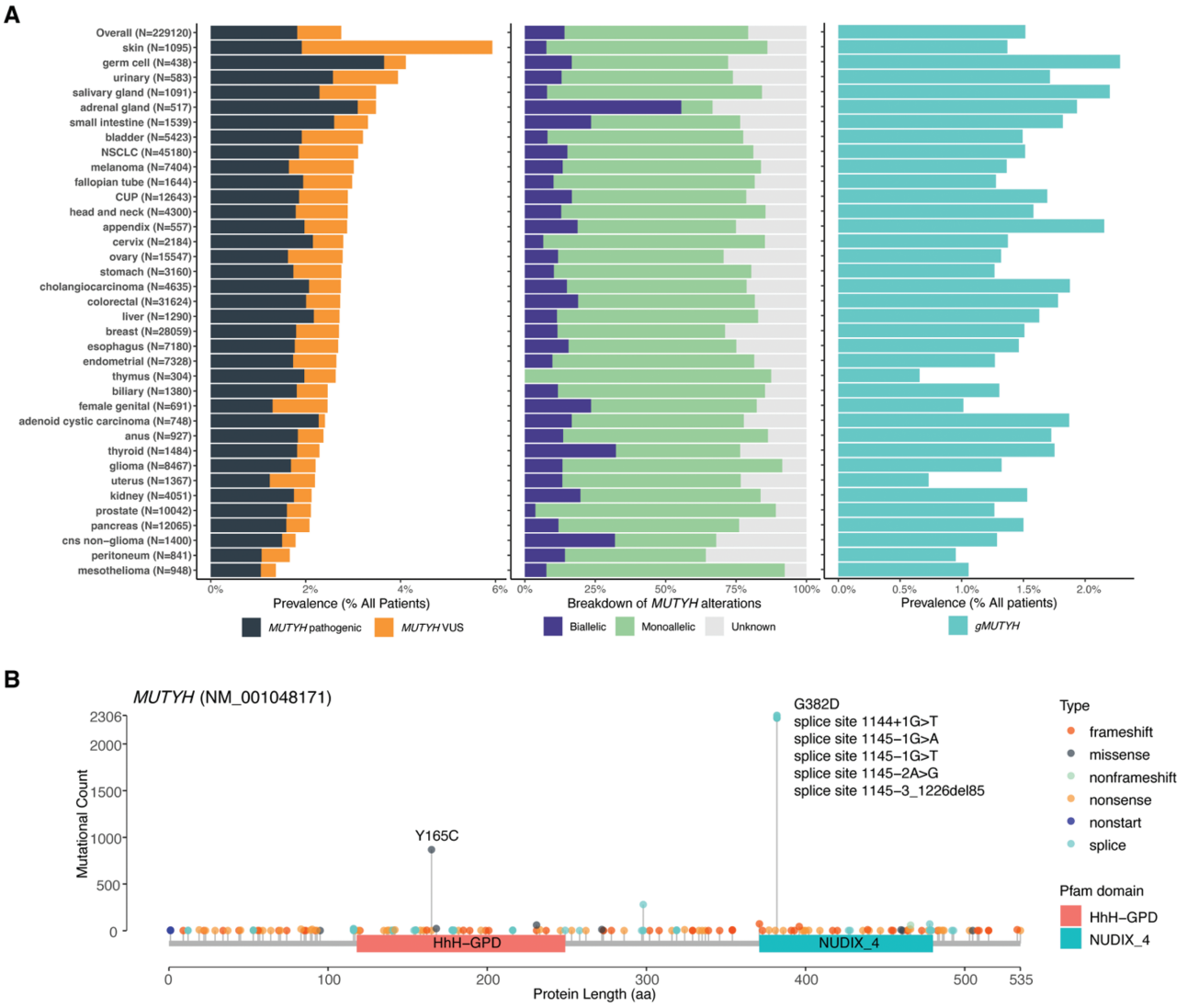

Figure 1. Pan-cancer prevalence of *MUTYH* alterations. (**A**) Bar plots presenting the prevalence of *MUTYH* alterations in a pan-cancer cohort comprising 229 120 cases. The color of the bar corresponds to the type of alteration—prevalence of predicted pathogenic alterations and variants of unknown significance (left), breakdown of monoallelic versus biallelic versus unknown *MUTYH* alterations (middle), as well as prevalence of all predicted germline *MUTYH* alterations (right). (**B**) Lollipop plot of all predicted pathogenic *MUTYH* alterations. Short variants comprising single nucleotide alterations and insertions/deletions identified along the transcript are shown.

65% of all *MUTYH*-altered CRCs (Supplementary Table S1). Similar to the pan-solid tumor cohort, G382D ($n = 382$) and Y165C ($n = 124$) were the most frequently identified *MUTYH* alterations (Supplementary Table S2). An additional 32 recurrent short variant alterations were identified in the cohort, including A371fs*23, R231H, R189C, and splice site alterations such as 892-2A > G, 1434 + 2C > T, and 1145-2A > G (Supplementary Table S2).

MUTYH-altered CRC tumors were more frequently TMB-H (≥10 mut/Mb) compared to *MUTYH*-WT CRC (15.7% vs. 7.1% respectively, $P < 10^{-5}$) (Fig. 3A). Of note, among the 135 TMB-H *MUTYH*-altered CRCs, 85 (63.0%) had a TMB ≥ 20 mut/Mb. Statistical analysis revealed significant association between *MUTYH* and high TMB, especially with TMB ≥ 20 mut/Mb (odds ratio = 3.3, $P < 10^{-5}$; Fig. 3B; Supplementary Table S5). Similarly, *gMUTYH* and pathogenic *MUTYH* alterations were also associated with high TMB (Fig. 3A; Supplementary Fig. S1; Supplementary Table S5). As with the overall pan-solid cohort, we did not observe any association with PD-L1 status in CRC (Fig. 3B; Supplementary Fig. S1; Supplementary Table S4).

Further examination based on MSI status revealed a higher prevalence of *MUTYH* alterations in MSI-H CRC compared to MSS CRC (7.4%, $n = 75/1020$ vs. 2.6%, $n = 756/29\,607$ respectively, $P < 10^{-5}$; Fig. 3C; Supplementary Table S1). The differences were less pronounced for *gMUTYH* (2.4% vs. 1.8%, $P = .18$; Fig. 3C; Supplementary Table S1) and pathogenic *MUTYH* alterations (2.8% vs. 2.0%, $P = .09$; Supplementary Table S1).

MUTYH Alterations Co-Occur With ***KRAS G12C*** Mutations

We next examined any association between *KRAS* alterations with *MUTYH* across different tumor types (Supplementary Table S6). Notably, while *KRAS* alterations overall were not associated with *MUTYH* status, *KRAS G12C* frequency was significantly higher in *MUTYH* tumors (5% in *MUTYH*-altered vs. 3% *MUTYH*-WT samples, $P < 10^{-4}$)

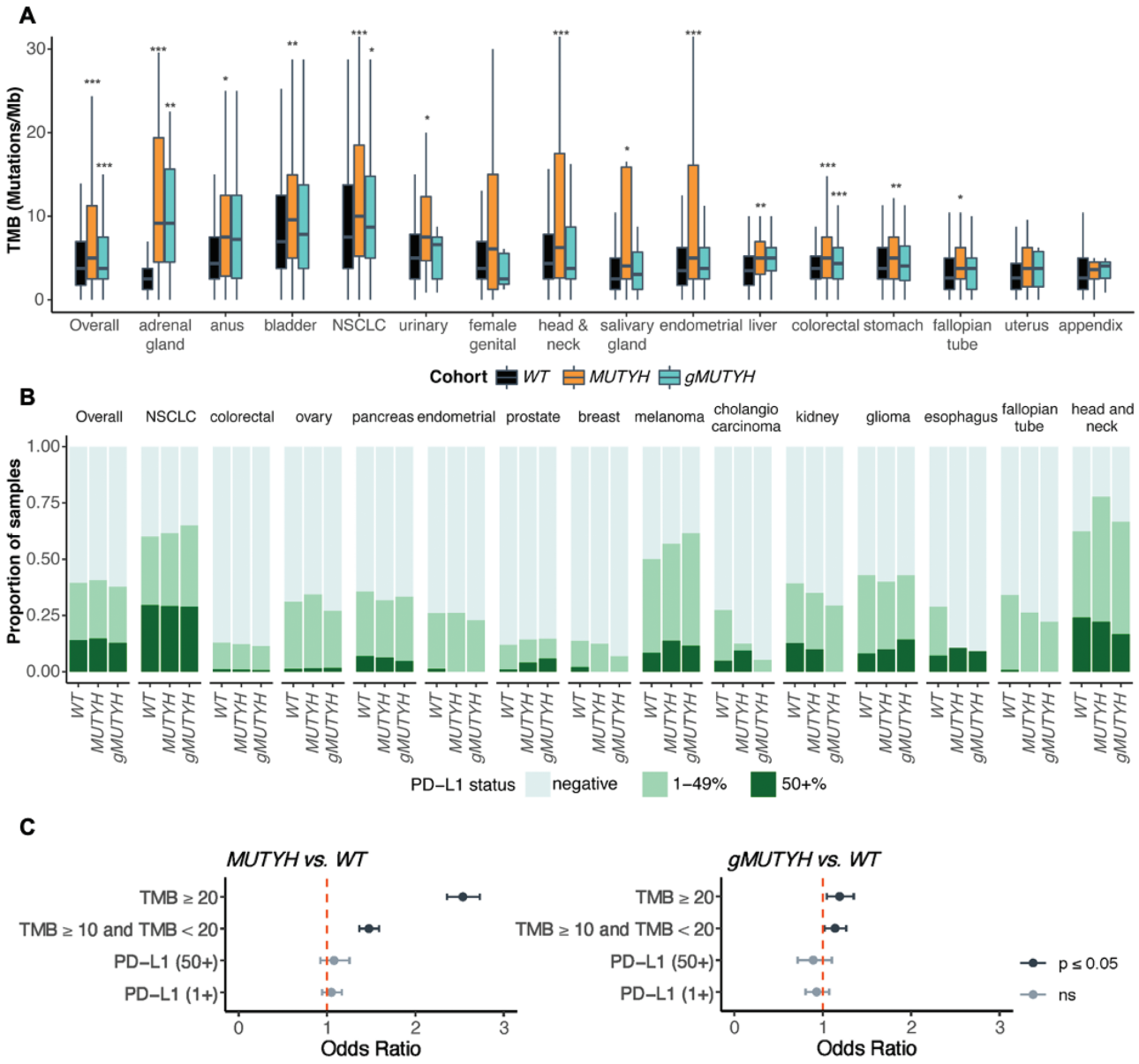

Figure 2. Association of *MUTYH* alterations and immunotherapy-related biomarkers. (**A**) Boxplot of the distribution of TMB across different tumor types based on the *MUTYH* alteration status (*MUTYH*, *gMUTYH*, wild type WT). Comparisons of the TMB distribution was performed against *MUTYH*-WT cases using a Wilcoxon test. Statistically significant differences are displayed (*P*-value thresholds: .0001: ****, .001: ***, .01: **, .05: *). The tumor types are ordered based on the difference in median TMB between *MUTYH*-altered and WT groups; the top 15 tumor types with at least 500 total samples and at least 15 altered samples are shown. **(B)** Breakdown of PD-L1 status (negative, 1%-49%, 50%+) across different tumor types based on the *MUTYH* alteration status. The 15 tumor cohorts with the largest number of samples annotated for PD-L1 status are shown. **(C)** Statistical analysis to test for association between the presence of *MUTYH* alterations and immunotherapy-associated biomarkers using a Fisher's exact test ($P \leq .05$: statistically significant, ns: not significant).

(Supplementary Table S6). In addition to being increased in the pan-solid tumor cohort, *KRAS G12C* was particularly increased in specific cancers such as colorectal cancers (Supplementary Table S6).

Increased transcriptional base mismatches, which develop against oxidative stresses as a result of *MUTYH* deficiency, lead to specific GC > TA transversions. These specific base mismatches can cause increased mutation rates and other co-occurring alterations.[11,12,31-34] To discern if *MUTYH* mutagenic processes were more likely to be associated with particular *KRAS* alterations, we evaluated the spectrum of *KRAS* alterations based on *MUTYH* alteration status.[33,34] In the overall CRC cohort, *KRAS* alterations were observed in nearly 50% of samples, with the prevalence being similar in *MUTYH*-altered samples: 50.2% in *MUTYH*, 56% in *gMUTYH*-altered samples (Supplementary Fig. S2; Supplementary Table S6; Fig. 3B). While the prevalence of *KRAS* was similar across all mutation subgroups, a closer examination of specific *KRAS* alleles revealed strong associations with *KRAS* G12C (Fig. 3B; Supplementary Table S6). *KRAS* G12C alterations were significantly increased in *MUTYH*-altered samples compared to WT (12.5% vs. 3.3% respectively; Odds ratio = 4.5, $P < 10^{-5}$; Fig. 3B; Supplementary Table S6). Of note, this association was stronger in *gMUTYH*-altered CRC (odds ratio = 5.8, $P < 10–^{5}$; Fig. 3B; Supplementary Table S6). The association of *KRAS G12C* was also observed in MSS CRC

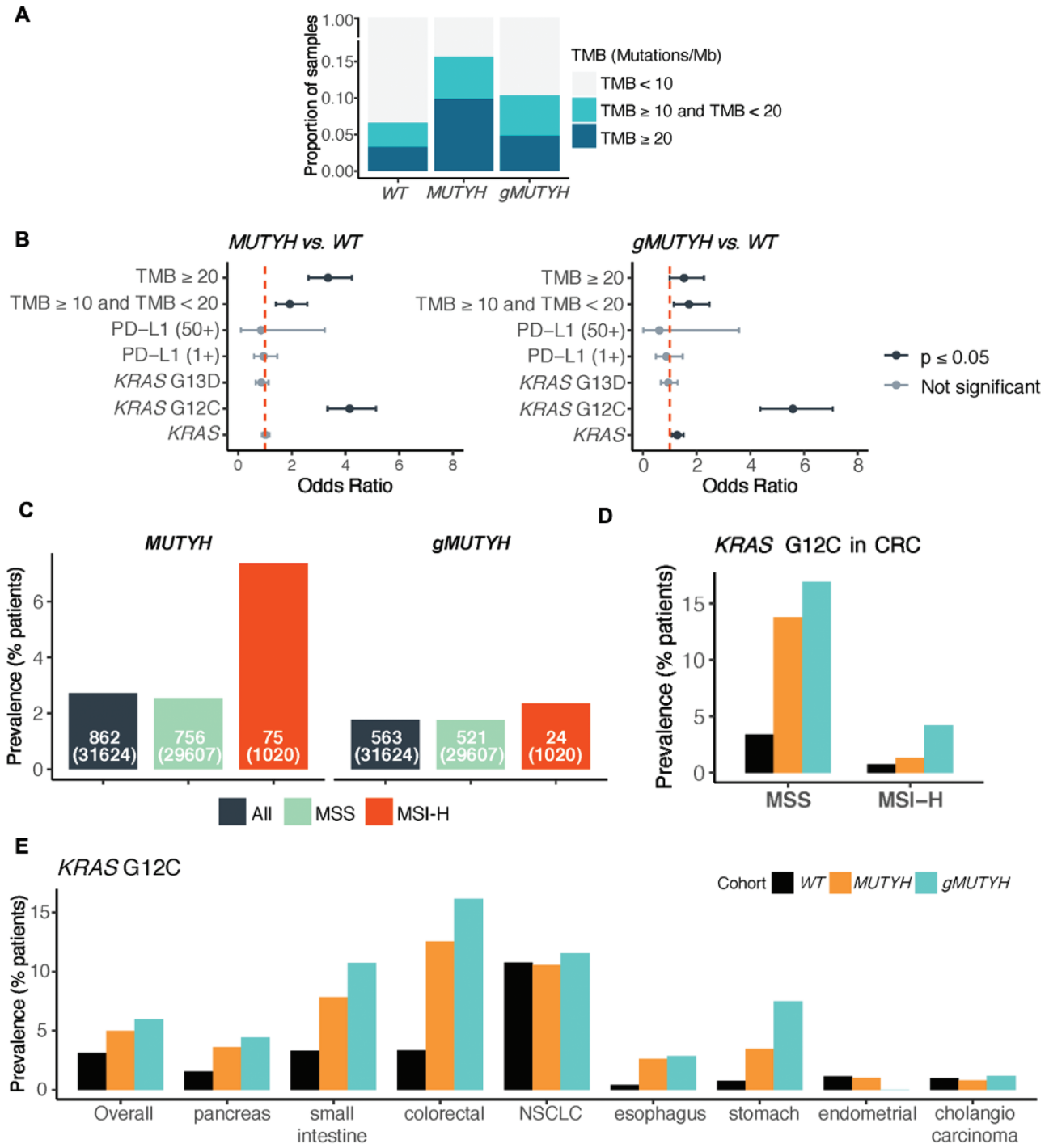

Figure 3. Examination of *MUTYH*-altered colorectal cancers (see also Supplementary Tables S3-S6). **(A)** Distribution of TMB based on the *MUTYH* alteration status (*MUTYH*, *gMUTYH*, wild type WT) in our cohort of colorectal cancer. TMB is assessed in the following bins: <10, 10-20, ≥20 mutations/megabase. **(B)** Statistical analysis to test for association between the presence of *MUTYH* alterations and immunotherapy-associated biomarkers as well as *KRAS* alterations using a Fisher's exact test ($P \leq .05$: statistically significant, ns: not significant). **(C)** Prevalence of *MUTYH* alterations based on microsatellite instability status. **(D)** Prevalence of *KRAS* G12C mutations in *MUTYH*-altered and wildtype cases, in microsatellite stable and instable CRCs. **(E)** Prevalence of *KRAS* G12C mutations in *MUTYH*-altered and wildtype cases, in other tumor types.

(Fig. 3D; Supplementary Table S6), whereas we were underpowered to test these patterns in the MSI-H cohort with only 8 out of the 1020 cases exhibiting *KRAS* G12C alterations.

Beyond CRC, other gastrointestinal tumors (eg, esophagus, stomach, small intestine) also showed the elevated frequency of *KRAS G12C* among *MUTYH*-altered samples in comparison to WT samples (Fig. 3E). In a similar assessment of *KRAS G13D*, we did not observe any association with *MUTYH* status (Supplementary Fig. S1; Supplementary Fig. 2; Supplementary Table S6).

MUTYH Alterations Show Specific Mutational Signatures With Elevated Immunogenicity Potential

MUTYH mutational signatures SBS18 and SBS36 have been implicated in defective base excision repair, including DNA damage due to reactive oxygen species.[33,34] Therefore, we

sought to further characterize these signatures in our cohort of *MUTYH*-altered CRCs. However, targeted panel testing often poses a challenge in detecting these rare signatures. Notwithstanding this limitation, SBS18 and SBS36 combined were more prevalent in *MUTYH*-altered CRCs (prevalence 5.7% *MUTYH* vs. 0.2% WT, $P < 10^{-5}$; 9.9% *gMUTYH* vs. 0.2% WT, $P < 10^{-5}$; Fig. 4A; Supplementary Table S7). This pattern of elevated SBS18 and SBS36 burden was also found in samples with predicted monoallelic (2.5%) and biallelic (22.8%) pathogenic *MUTYH* alterations but not in *MUTYH* wild-type samples (0.2%) (Supplementary Fig. S3). To better assess mutational signature processes based on the *MUTYH* status, we then applied a pooled approach to characterize the contribution of each signature to the observed spectrum of alterations for each cohort (see Methods). Relative to the *MUTYH* WT samples, *MUTYH*-altered samples had a significantly larger number of alterations predicted to result from SBS18 and SBS36 ($P < 10^{-5}$, relative mean compared to *MUTYH* WT: 5.6x and 4.4x mutations, respectively; Fig. 4B). This was consistently observed in a subset of *gMUTYH* samples as well as predicted pathogenic *MUTYH*-altered samples (Fig. 4B; Supplementary Fig. S3).

Hydrophobicity may reflect immunogenicity.[36] Therefore we further evaluated the impact of repeated in silico *MUTYH*-related mutagenesis over the estimated overall hydrophobicity of the coding genome The SBS36 *MUTYH* signature remains among the most hydrophobic signatures; SBS18 *MUTYH* signature exhibited moderate to high hydrophobicity, although not to the extent of SBS36 (Fig. 4C).

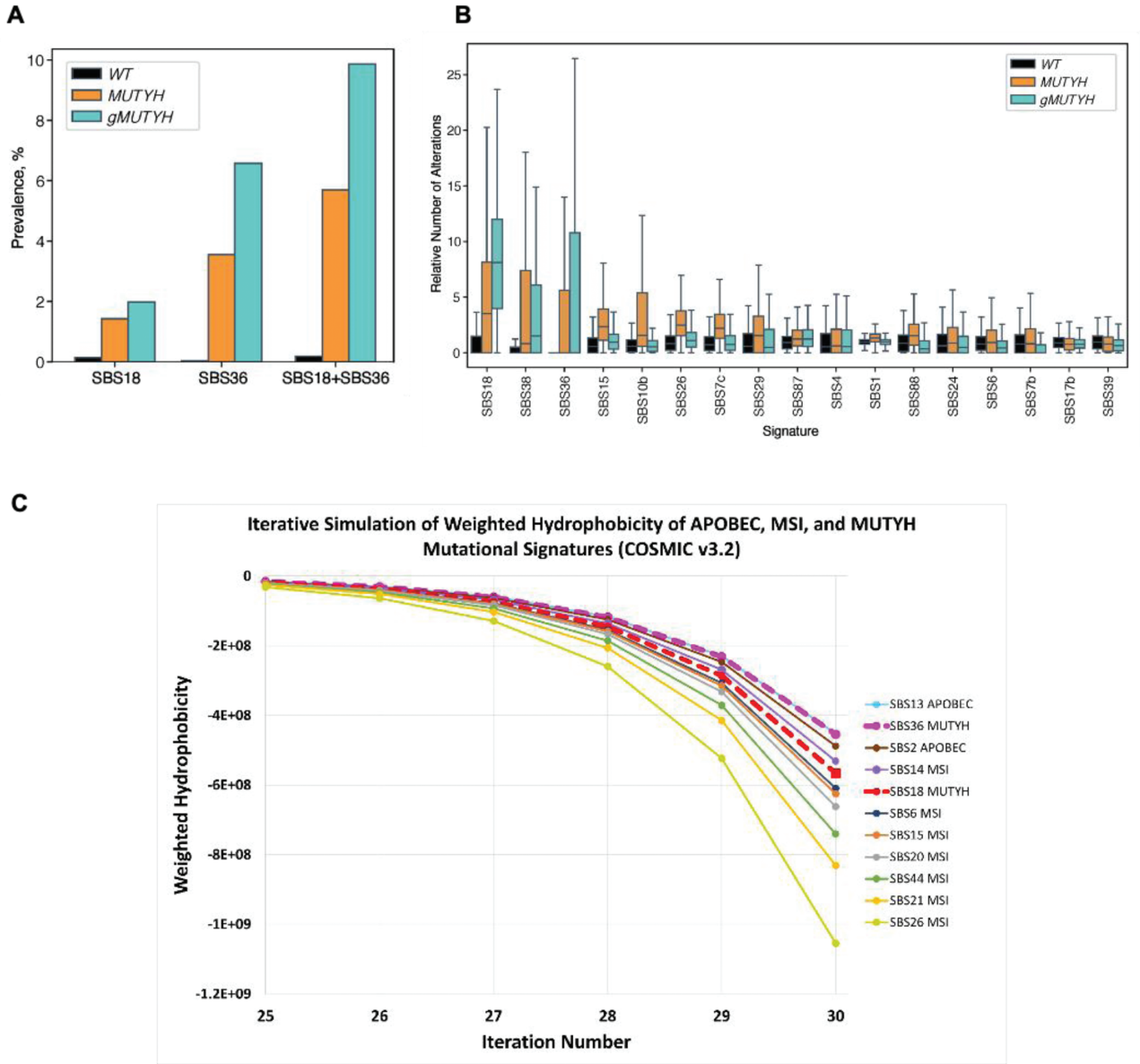

Figure 4. Examination of mutational signatures associated with *MUTYH*. **(A)** Prevalence of mutational signatures SBS 18 and 36 in *MUTYH*-altered and wildtype colorectal cancer cases. **(B)** Relative number of alterations attributed to different mutational signatures based on the *MUTYH*-alteration status in colorectal cancer. **(C)** Iterative simulation of weighted hydrophobicity for MSI (SBS6, 14, 15, 20, 21, 26, and 44), MUTYH (SBS18 and 36), and APOBEC (SBS2 and 13) mutational signatures as performed in Boichard et al.[35].

Survival Analysis Did Not Reveal Differences Based on *MUTYH* Status

Survival analysis revealed no statistically significant difference in overall survival (OS) between patients that were *MUTYH*-altered (n = 169) and those WT (n = 10 634) for MUTYH in a pan-cancer cohort (P = .220) (Fig. 5). The OS in the *MUTYH* altered group is 65.49 months (95% CI, 50.10-107.14) and in the unaltered group is 79.59 months (95% CI, 74.73-84.66). In addition, neither disease-free survivals (P = .890) nor progression-free survival (P = .931) was found to be statistically significant between *MUTYH*-altered and WT groups (data not shown).

Case Presentation

In February 2020, a 38-year-old woman was admitted to the emergency department with acute intestinal obstruction. Primary diversion colostomy was performed on the patient who was suspected of having a mass obstructing the lumen in the sigmoid colon, with 3 cm metastasis in segment 7 of the liver, and suspected of peritoneal involvement. Endoscopic biopsy identified adenocarcinoma, and the patient was initiated on systemic chemotherapy (leucovorin calcium [folinic acid], fluorouracil, and oxaliplatin: FOLFOX-6). Biopsies were obtained from the patient's metastatic sites, particularly the liver, for confirmation prior to initiating therapy. These biopsies provided evidence of tumor presence and confirmed the stage IV disease status. Upfront tumor testing identified a PD-L1 tumor proportion score (TPS) of 80% (Dako 22C3 pharmDxTM IHC) and TMB by F1CDx was 16 mut/Mb (Supplementary Table S8 for complete genomic profile). The patient also exhibited a *CD274* gene rearrangement and a *KRAS G12C* mutation. Importantly, she had a *MUTYH* alteration and an SBS18 mutational signature (consistent with *MUTYH* alteration). Nivolumab 240 mg every 2 weeks schedule was added to the FOLFOX6. After 4 months of chemo-immunotherapy, consolidative radiotherapy was given to the rectum, concomitantly with nivolumab and continued infusion of 5-fluorouracil. Afterward, radiotherapy, curative rectal surgery, and resection of the metastatic focus in the liver were performed. A complete pathological response was detected in the resected surgical specimens. The patient, who received maintenance nivolumab continues to be followed up and treated as no evidence of disease at 24 months after diagnosis. She has a strong family history of CRC affecting 2 first-degree relatives (Informed written consent was obtained from the patient to share the data).

Discussion

To evaluate the prevalence of *MUTYH* mutations, we used a large pan-cancer comprehensive genomic dataset from patients profiled during routine clinical care. Overall, 6297(2.8% of 229 120) solid cancers had *MUTYH* alterations, of which 55% were predicted germline. While the details of *MUTYH*-associated polyposis syndrome are known, the landscape of *MUTYH* mutation in cancers and its association with co-occurring mutations and genomic signatures have not been previously evaluated in a large-scale fashion.

Prior literature identified approximately 1%-2% of the general population with a single (monoallelic) germline *MUTYH* pathogenic variant, with 2 (biallelic) pathogenic variants identified in<1% of individuals diagnosed with CRC.[27,37] We detected *MUTYH*-related pathogenic changes in 6297 (2.8%) patients in the pan-solid tumor cohort (n = 229 120) with 30 tumor types harboring a *MUTYH*

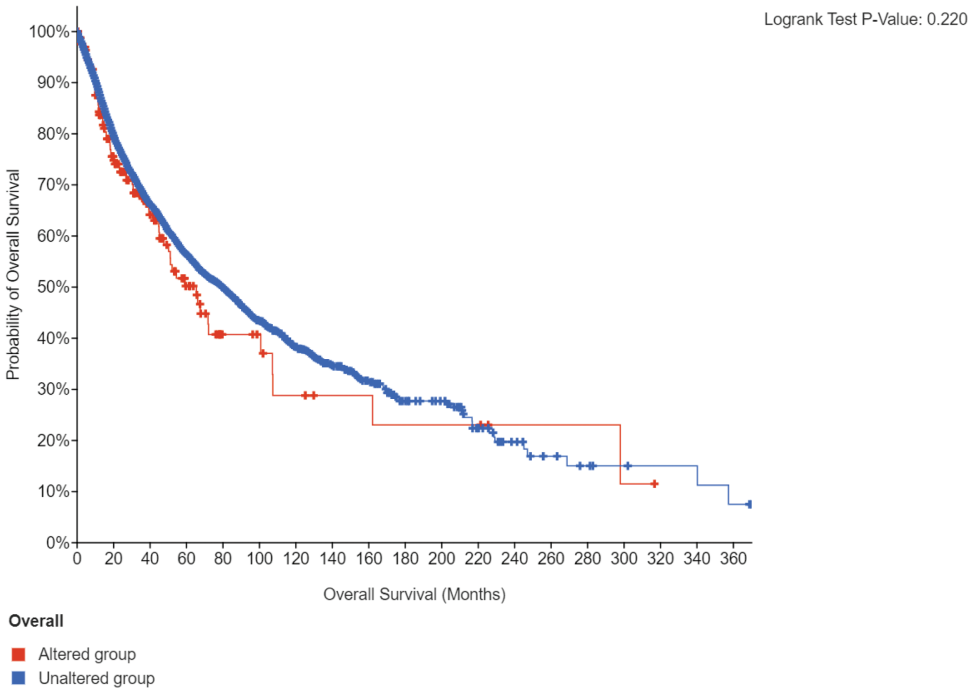

Figure 5. Overall survival analysis comparing patients with altered (n = 169) and unaltered (n = 10634) *MUTYH* variant status from the TCGA pan-cancer dataset within cBioPortal shows no statistically significant relationship between the gene alteration and survival of patients, irrespective of origin of tumor (log-rank test P-value .22).

frequency of 2% or higher, and the highest rate of mutation in germ cell tumors and adrenocortical cancers. Among our *MUTYH*-altered samples, 55% showed a germline *MUTYH* alteration (*gMUTYH*); therefore *gMUTYH* was seen in ~1.5% of all solid tumors. Barreio et al also detected a 1.4% incidence of *gMUTYH* in gnomAD, which is consistent with our findings.[37]

MUTYH-altered cases had a significantly elevated TMB compared to *wildtype* samples, across multiple tumor types in our study. Consistent with our observation, *MUTYH*-altered CRCs are also characterized by increased mutation and neoantigen burden, similar to patients with mismatch repair (MMR) defects. *MUTYH* alterations being associated with elevated mutation rates have been reported most frequently in CRC, but also in gastric, pancreatic, ovarian, and adrenocortical cancers.[3,15,29-31]

Along with the increased mutational burden, PD-L1 IHC expression was also evaluated as one of the markers predicting the immune response.[27] Although Permata et al identified a relation between defects in the base excision repair (BER) system and increased PD-L1 expression,[38] relevant data in the literature is limited. No associations were found between the presence of PD-L1 expression and *MUTYH* alterations in our analysis.

It is well known that increased oxidative stress in the presence of loss of function *MUTYH* mutations leads to increased mutation load and specific GC > TA transversion. Therefore, some specific mutations may co-occur in this scenario. Mutations such as *KRAS G12C* and those in *APC,* and *SMAD* are most common.[11,12,31-34] In our patient set, *KRAS* mutations are observed at similar rates in *MUTYH*-altered compared to *wildtype* tumors; however, the *KRAS G12C* mutation is found at significantly higher rates in *MUTYH*-altered across cancers. This was especially prominent in patients with CRC, with a more pronounced difference in germline *MUTYH* CRC cases. Other gastrointestinal tumors (eg, esophagus, stomach, and small intestine) also showed elevated levels *of KRAS G12C* among *MUTYH*-altered samples in comparison to *WT* samples. Consistent with our observations, Salem et al recently reported that *KRAS G12C*-mutant CRCs have a higher prevalence of *MUTYH* mutations than non-*KRAS G12C*-mutant CRCs (5.77% vs. 2.28%, OR = 2.62, $P = .005$).[31] Other studies also support this observation.[9,11,31-34] The 4-fold increased prevalence of *KRAS G12C* in *MUTYH*-mutated microsatellite stable CRCs is an important finding since *KRAS G12C* is being recognized as a druggable alteration, with the FDA approval of sotorasib in non-small cell lung cancer.[39]

Somatic mutations in cancer genomes are caused by multiple mutational processes, each of which generates a characteristic mutational signature.[21] In our dataset, we examined the mutational signature in cases with *MUTYH* mutations and found COSMIC SBS18 and SBS36 to be elevated in these patients compared to *MUTYH*-WT patients at both a per-specimen level and using a pooled approach to compare cohorts. These 2 signatures reflect DNA damage as a result of defects in the BER system and an increase in oxidative stress.[33,34] Presence of dominant SBS18 and SBS36 mutation signatures in these patients also reveals the role of oxidative stress and *MUTYH* mutations in CRC oncogenesis.[2,15,33,34]

The success of immunotherapy in our index patient suggests that the defect in the BER system may be the result of increased mutational burden and neoantigens, similar to that seen in cancer with mismatch repair gene changes. Furthermore, several authors have indicated that *MUTYH*-altered tumors can also be immune-hot tumors with increased intra-tumor lymphocyte infiltration similar to mismatch repair-defective cases.[11,40,41]

The relationship between higher hydrophobicity and immunogenicity, as well as the association between hydrophobicity and improved outcomes after immunotherapy, has been well-documented in previous studies.[22,26,35,36,42] We found that the *MUTYH* signature is amongst the most hydrophobic of the signatures. Therefore, this observation provides further evidence that cancers harboring the *MUTYH*-altered signature may benefit from immune checkpoint blockade, in the same way seen in our index patient. This concept merits evaluation in prospective clinical trials.

There are studies showing that *MUTYH*-altered CRCs bear molecular and immune infiltrate structures similar to defective mismatch repair CRC cancers.[9,40,41] For this reason, we know that the prognosis is better and survival is longer in patients with mismatch repair CRC cancer. In a study that looked at *MUTYH*-altered versus -sporadic CRC survival, it was reported that the survival of *MUTYH*-altered CRC cases was better.[43] In contrast, we examined the survival of *MUTYH*-altered and -WT patient pan-cancer cohorts in the TCGA database; no survival difference was found. Due to the small number of cases, the CRC cohort could not be examined.

This study has several limitations. First, clinical outcomes and family/pedigree information were not available for the patients in this study. Second, germline testing from the patient's normal tissue was not performed. We therefore had to rely on a computational algorithm for *MUTYH* germline prediction which is detailed described in the method section. Third, it is important to note that tumors with a higher tumor mutational burden may harbor a wider range of mutations, including those affecting genes such as *MUTYH*. Consequently, it becomes imperative to further investigate the immunotherapeutic implications specifically for tumors with *MUTYH* alterations.

In conclusion, in a dataset of 229 120 solid cancers, we found that 2.8% had *MUTYH* alterations, of which 55% were germline. Cancers with *MUTYH* alterations had higher TMB, more frequent alterations in *KRAS G12C* (a potentially actionable *KRAS* mutation) (both observations were especially pronounced in CRCs), but no increase in *KRAS* alterations in general, and a mutational signature with higher levels of hydrophobicity (reflecting immunogenicity) than most microsatellite unstable signatures. PD-L1 expression levels were however not increased. Survival of patients with *MUTYH*-altered versus -WT tumors did not differ. The finding of higher TMB and higher hydrophobicity indicate that patients bearing *MUTYH*-altered cancers may benefit from immune checkpoint blockade (as observed in our index patient), although the lack of association with higher PD-L1 expression indicates that the situation in individual tumors may vary and that defining whether or not checkpoints other than PD-1/PD-L1 are important in *MUTYH*-altered cancers warrants future studies. The role of sotorasib[44] (a *KRAS G12C*-targeted molecule, FDA-approved for lung cancer) also merits further investigation in *MUTYH*-mutated malignancies.

Acknowledgments

We thank Gunes Acar, MD, of Gunes Acar Danismanlik, Istanbul, for providing medical writing assistance.

Funding

Foundation Medicine, Inc. has provided the data to the investigators solely for purposes of the research. Funded in part by NIH grants U01CA217885, U24CA220341, P30CA023100, and U24CA248457 (TP).

Conflict of Interest

Umut Disel: Foundation Medicine as consultant, speaker fees, and/or advisory board. Smruthy Sivakumar: Employee at Foundation Medicine, a wholly owned subsidiary of Roche and a shareholder in Roche. Zoe Fleischmann: Employee at Foundation Medicine, a wholly owned subsidiary of Roche and a shareholder in Roche. Ethan S. Sokol: Employee at Foundation Medicine, a wholly owned subsidiary of Roche and a shareholder in Roche. Razelle Kurzrock: has received research funding from Biological Dynamics, Boehringer Ingelheim, Debiopharm, Foundation Medicine, Genentech, Grifols, Guardant, Incyte, Konica Minolta, Medimmune, Merck Serono, Omniseq, Pfizer, Sequenom, Takeda, and TopAlliance; as well as consultant and/or speaker fees and/or advisory board for Actuate Therapeutics, AstraZeneca, Bicara Therapeutics, Biological Dynamics, Daiichi Sankyo, Inc., EISAI, EOM Pharmaceuticals, Iylon, Merck, NeoGenomics, Neomed, Pfizer, Prosperdtx, Roche, TD2/Volastra, Turning Point Therapeutics, X-Biotech; has an equity interest in CureMatch Inc., CureMetrix, and IDbyDNA; serves on the Board of CureMatch and CureMetrix, and is a co-founder of CureMatch. The other authors indicated no financial relationships.

Author Contributions

Conception/design: U.D., E.S. Acquisition of the data: U.D., S.S., Z.F., A.I., T.P. Data analysis and interpretation: All authors. Manuscript writing: U.S., S.S., T.P., A.I. Final approval of manuscript: All authors. All authors made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

References

1. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature*. 2001;411(6835):366-374. https://doi.org/10.1038/35077232
2. Wallace SS, Murphy DL, Sweasy JB. Base excision repair and cancer. *Cancer Lett*. 2012;327(1-2):73-89. https://doi.org/10.1016/j.canlet.2011.12.038
3. Woods RD, O'Shea VL, Chu A, et al. Structure and stereochemistry of the base excision repair glycosylase MutY reveal a mechanism similar to retaining glycosidases. *Nucleic Acids Res*. 2016;44(2):801-810. https://doi.org/10.1093/nar/gkv1469
4. Beard WA, Batra VK, Wilson SH. DNA polymerase structure-based insight on the mutagenic properties of 8-oxoguanine. *Mutat Res*. 2010;703(1):18-23. https://doi.org/10.1016/j.mrgentox.2010.07.013
5. Al-Tassan N, Chmiel NH, Maynard J, et al. Inherited variants of MYH associated with somatic G:C-->T: a mutations in colorectal tumors. *Nat Genet*. 2002;30(2):227-232. https://doi.org/10.1038/ng828
6. Rashid M, Fischer A, Wilson CH, et al. Adenoma development in familial adenomatous polyposis and MUTYH-associated polyposis: somatic landscape and driver genes. *J Pathol*. 2016;238(1):98-108. https://doi.org/10.1002/path.4643
7. Magrin L, Fanale D, Brando C, et al. MUTYH-associated tumor syndrome: the other face of MAP. *Oncogene*. 2022;41(18):2531-2539. https://doi.org/10.1038/s41388-022-02304-y
8. Madison RW, Hu X, Ramanan V, et al. Clustered 8-oxo-guanine mutations and oncogenic gene fusions in microsatellite-unstable colorectal cancer. *JCO Precis Oncol*. 2022;6:e2100477. https://doi.org/10.1200/PO.21.00477
9. Nielsen M, Morreau H, Vasen HF, Hes FJ. MUTYH-associated polyposis (MAP). *Crit Rev Oncol Hematol*. 2011;79(1):1-16. https://doi.org/10.1016/j.critrevonc.2010.05.011
10. Jones S, Lambert S, Williams GT, et al. Increased frequency of the k-ras G12C mutation in MYH polyposis colorectal adenomas. *Br J Cancer*. 2004;90(8):1591-1593. https://doi.org/10.1038/sj.bjc.6601747
11. Venesio T, Balsamo A, Errichiello E, Ranzani GN, Risio M. Oxidative DNA damage drives carcinogenesis in MUTYH-associated-polyposis by specific mutations of mitochondrial and MAPK genes. *Mod Pathol*. 2013;26(10):1371-1381. https://doi.org/10.1038/modpathol.2013.66
12. Aimé A, Coulet F, Lefevre JH, et al. Somatic c.34G>T KRAS mutation: a new prescreening test for MUTYH-associated polyposis? *Cancer Genet*. 2015;208(7-8):390-395.
13. Pilati C, Shinde J, Alexandrov LB, et al. Mutational signature analysis identifies MUTYH deficiency in colorectal cancers and adrenocortical carcinomas. *J Pathol*. 2017;242(1):10-15. https://doi.org/10.1002/path.4880
14. Landwehr LS, Schreiner J, Appenzeller S, et al. A novel patient-derived cell line of adrenocortical carcinoma shows a pathogenic role of germline MUTYH mutation and high tumour mutational burden. *Eur J Endocrinol*. 2021;184(6):823-835. https://doi.org/10.1530/EJE-20-1423
15. Robinson PS, Thomas LE, Abascal F, et al. Inherited MUTYH mutations cause elevated somatic mutation rates and distinctive mutational signatures in normal human cells. *Nat Commun*. 2022;13(1):3949. https://doi.org/10.1038/s41467-022-31341-0
16. Frampton GM, Fichtenholtz A, Otto GA, et al. 2013. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31(11):1023-1031. https://doi.org/10.1038/nbt.2696
17. Sun JX, He Y, Sanford E, et al. A computational approach to distinguish somatic vs. germline origin of genomic alterations from deep sequencing of cancer specimens without a matched normal. *PLoS Comput Biol*. 2018;14(2):e1005965. https://doi.org/10.1371/journal.pcbi.1005965
18. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. 2017;9(1):34. https://doi.org/10.1186/s13073-017-0424-2
19. Trabucco SE, Gowen K, Maund SL, et al. A novel next-generation sequencing approach to detecting microsatellite instability and pan-tumor characterization of 1000 microsatellite instability-high cases in 67,000 patient samples. *J Mol Diagn*. 2019;21(6):1053-1066. https://doi.org/10.1016/j.jmoldx.2019.06.011
20. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*. 2017;23(6):703-713. https://doi.org/10.1038/nm.4333
21. Alexandrov LB, Kim J, Haradhvala NJ, et al; PCAWG Mutational Signatures Working Group. PCAWG Mutational Signatures

Working Group, Getz G, Rozen SG, Stratton MR; PCAWG Consortium. The repertoire of mutational signatures in human cancer. *Nature*. 2020;578(7793):94-101. https://doi.org/10.1038/s41586-020-1943-3
22. Pham TV, Boichard A, Goodman A, et al. Role of ultraviolet mutational signature versus tumor mutation burden in predicting response to immunotherapy. *Mol Oncol*. 2020;14(8):1680-1694. https://doi.org/10.1002/1878-0261.12748
23. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol*. 1982;157(1):105-132. https://doi.org/10.1016/0022-2836(82)90515-0
24. Nakamura Y, Gojobori T, Ikemura T. Codon usage tabulated from international DNA sequence databases: status for the year 2000. *Nucleic Acids Res*. 2000;28(1):292. https://doi.org/10.1093/nar/28.1.292
25. *COSMIC, the Catalogue Of Somatic Mutations In Cancer*. Mutational Signatures (v3.3 - June 2022). SBS36 · GRCh37 · COSMIC v96 [Cited 17 June 2022]. Available from: https://cancer.sanger.ac.uk/signatures/sbs/sbs36/
26. Boichard A, Tsigelny IF, Kurzrock R. High expression of PD-1 ligands is associated with kataegis mutational signature and APOBEC3 alterations. *Oncoimmunology*. 2017;6(3):e1284719. https://doi.org/10.1080/2162402X.2017.1284719
27. Cleary SP, Cotterchio M, Jenkins MA, et al. Germline MutY human homologue mutations and colorectal cancer: a multisite case-control study. *Gastroenterology*. 2009;136(4):1251-1260. https://doi.org/10.1053/j.gastro.2008.12.050
28. Lagos GG, Izar B, Rizvi NA. Beyond tumor PD-L1: emerging genomic biomarkers for checkpoint inhibitor immunotherapy. *Am Soc Clin Oncol Educ Book*. 2020;40:1-11. https://doi.org/10.1200/EDBK_289967
29. Patel SP, Kurzrock R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. *Mol Cancer Ther*. 2015;14(4):847-856. https://doi.org/10.1158/1535-7163.MCT-14-0983
30. Subbiah V, Solit DB, Chan TA, Kurzrock R. The FDA approval of pembrolizumab for adult and pediatric patients with tumor mutational burden (TMB) ≥10: a decision centered on empowering patients and their physicians. *Ann Oncol*. 2020;31(9):1115-1118. https://doi.org/10.1016/j.annonc.2020.07.002
31. Cook JH, Melloni GEM, Gulhan DC, Park PJ, Haigis KM. The origins and genetic interactions of KRAS mutations are allele- and tissue-specific. *Nat Commun*. 2021;12(1):1808. https://doi.org/10.1038/s41467-021-22125-z
32. Salem ME, El-Refai SM, Sha W, et al. Landscape of *KRAS*^{G12C}, associated genomic alterations, and interrelation with immuno-oncology biomarkers in *KRAS*-mutated cancers. *JCO Precis Oncol*. 2022;6:e2100245. https://doi.org/10.1200/PO.21.00245
33. Georgeson P, Pope BJ, Rosty C, et al. Evaluating the utility of tumour mutational signatures for identifying hereditary colorectal cancer and polyposis syndrome carriers. *Gut*. 2021;70(11):2138-2149. https://doi.org/10.1136/gutjnl-2019-320462
34. Georgeson P, Harrison TA, Pope BJ, et al. Identifying colorectal cancer caused by biallelic MUTYH pathogenic variants using tumor mutational signatures. *Nat Commun*. 2022;13(1):3254. https://doi.org/10.1038/s41467-022-30916-1
35. Boichard A, Pham TV, Yeerna H, et al. APOBEC-related mutagenesis and neo-peptide hydrophobicity: implications for response to immunotherapy. *Oncoimmunology*. 2018;8(3):1550341. https://doi.org/10.1080/2162402X.2018.1550341
36. Chowell D, Krishna S, Becker PD, et al. TCR contact residue hydrophobicity is a hallmark of immunogenic CD8+ T cell epitopes. *Proc Natl Acad Sci USA*. 2015;112(14):E1754-E1762. https://doi.org/10.1073/pnas.1500973112
37. Barreiro RAS, Sabbaga J, Rossi BM, et al. Monoallelic deleterious MUTYH germline variants as a driver for tumorigenesis. *J Pathol*. 2022;256(2):214-222.
38. Permata TBM, Hagiwara Y, Sato H, et al. Base excision repair regulates PD-L1 expression in cancer cells. *Oncogene*. 2019;38(23):4452-4466. https://doi.org/10.1038/s41388-019-0733-6
39. Skoulidis F, Li BT, Dy GK, et al. Sotorasib for lung cancers with KRAS p.G12C mutation. *N Engl J Med*. 2021;384(25):2371-2381. https://doi.org/10.1056/NEJMoa2103695
40. Volkov NM, Yanus GA, Ivantsov AO, et al. Efficacy of immune checkpoint blockade in MUTYH-associated hereditary colorectal cancer. *Invest New Drugs*. 2020;38(3):894-898. https://doi.org/10.1007/s10637-019-00842-z
41. Nielsen M, de Miranda NF, van Puijenbroek M, et al. Colorectal carcinomas in MUTYH-associated polyposis display histopathological similarities to microsatellite unstable carcinomas. *BMC Cancer*. 2009;9:184. https://doi.org/10.1186/1471-2407-9-184
42. Goodman AM, Castro A, Pyke RM, et al. MHC-I genotype and tumor mutational burden predict response to immunotherapy. *Genome Med*. 2020;12(1):45. https://doi.org/10.1186/s13073-020-00743-4
43. Nielsen M, van Steenbergen LN, Jones N, et al. Survival of MUTYH-associated polyposis patients with colorectal cancer and matched control colorectal cancer patients. *J Natl Cancer Inst*. 2010;102(22):1724-1730. https://doi.org/10.1093/jnci/djq370
44. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-sotorasib-kras-g12c-mutated-nsclc