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Genetic diversity of tumors with mismatch repair deficiency influences anti-PD-1 immunotherapy response

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Data and materials availability:

The MSI cell lines are available from T.A.C. under a material transfer agreement with MSKCC. Sequencing files have been deposited into Sequence Read Archive (SRA) (<https://www.ncbi.nlm.nih.gov/sra>) and can be found under the following accession numbers: SAMN09941220, SAMN09941221, SAMN09941222, SAMN09941223, SAMN09941224, SAMN09941225, SAMN09941226, SAMN09941227, SAMN09941228, SAMN09941229, SAMN09941230, SAMN09941231, SAMN09941232, and SAMN09941233. Data reported in this study are tabulated in the main text and supplementary materials.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S15

Tables S1 to S6

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Abstract

Tumors with mismatch repair deficiency (MMR-d) are characterized by sequence alterations in microsatellites and can accumulate thousands of mutations. This high mutational burden renders tumors immunogenic and sensitive to programmed cell death–1 (PD-1) immune checkpoint inhibitors. Yet, despite their tumor immunogenicity, patients with MMR-deficient tumors experience highly variable responses, and roughly half are refractory to treatment. We present experimental and clinical evidence showing that the degree of microsatellite instability (MSI) and resultant mutational load, in part, underlies the variable response to PD-1 blockade immunotherapy in MMR-d human and mouse tumors. The extent of response is particularly associated with the accumulation of insertion-deletion (indel) mutational load. This study provides a rationale for the genome-wide characterization of MSI intensity and mutational load to better profile responses to anti-PD-1 immunotherapy across MMR-deficient human cancers.

Tumor mutational burden has been shown to strongly correlate with clinical response to immunotherapy using checkpoint inhibitors (1–3). Tumors with high microsatellite instability (MSI-H) accumulate substantial numbers of somatic mutations secondary to deficits in DNA mismatch repair (MMR) (4). Recent work has demonstrated a high objective response rate (ORR 53%) to anti-PD-1 (programmed cell death–1) therapy across mismatch repair–deficient (MMR-d) solid tumors (5, 6). These findings have led to the first tissue-agnostic approval for anti-PD-1 therapy across unresectable or metastatic solid tumors with microsatellite instability (MSI) or MMR-d (7). However, MSI tumors include lesions with substantial genomic variation. Moreover, many MMR-d tumors fail to respond to anti-PD-1 therapy, and the proportion that are sensitive display a wide diversity of clinical benefit. What drives this variable response is largely unknown, and a more granular understanding of the mechanistic nature of PD-1 inhibitor sensitivity in MMR-d tumors may help to more precisely inform their use across human cancers. To better characterize the basis for response, we used syngeneic mouse models and interrogated the mutational landscape of MSI-H patients treated with immune checkpoint blockade. Recent work has indicated that inactivation of DNA repair pathways such as MMR results in cumulative neoantigen generation that can promote tumor destruction (8, 9). We explored whether the precise quantification of genomic MSI level—termed MSI intensity—can help elucidate the wide diversity of responses to anti-PD-1 therapy seen in MSI-H tumors. We additionally examined how the degree of MSI genetic diversity influences tumor evolution induced by PD-1 blockade in MMR-d tumors.

Using CRISPR-Cas9 guide RNAs directed against exon 1 of the DNA mismatch repair gene *Msh2*, we knocked out the MSH2 protein in the poorly immunogenic B16F10 mouse melanoma and CT26 mouse colon carcinoma cell lines to generate novel MMR-deficient derivatives (figs. S1 and S2). In parallel with the unedited parental lines, we serially passaged the newly generated MMR-d lines over 4 months to produce variable degrees of MSI. We analyzed the lines from two timepoints (1 and 4 months), hereafter referred to as MSI-intermediate and MSI-high cell lines, respectively (Fig. 1A and fig. S3). This approach enabled us to compare responses to anti-PD-1 immunotherapy in isogenic cell line-derived systems that differed only in MSI intensity levels and resultant mutational loads. Both MSI-intermediate and MSI-high cell lines lack functional MMR protein machinery and possess statistically higher proportions of unstable microsatellites compared to the unedited parental lines (figs. S1 and S2 and Fig. 1C).

We quantified mutational burden (against the parental reference genome), including novel non-synonymous single-nucleotide variations (SNVs) (missense) and coding insertion-deletion (indel) mutations, in MSI-intermediate and MSI-high lines (Fig. 1B and fig. S4). As expected, MSI-high cell lines displayed higher counts of novel non-synonymous SNVs and coding indel mutations as compared to the MSI-intermediate and micro-satellite stable (MSS) parental lines (Fig. 1B). To quantify the precise genomic level of MSI, we used a previously validated algorithm, called MSIsensor, to quantify the number of unstable microsatellites against the reference genome (10). As expected, MSIsensor scores for the high-passage lines (MSI-high) were substantially greater than those of the low-passage lines (MSI-intermediate), and both were higher than those of the parental lines (Fig. 1C). Recent work has indicated that indel mutations can generate a large number of immunogenic neoantigens, potentially driving immunotherapeutic response (11). In our model, preferential expansion of indel mutations over SNVs was observed over time in the MSI-high line, consistent with the pattern of genetic alteration seen in MMR-deficient tumors and the primary mechanism of mutagenesis in these tumors ($p = 0.003$, $p < 0.0001$) (Fig. 1D).

The MSI-intermediate tumors referred to in our murine MMR-d tumor model are distinct from the “MSI-Low” (MSI-L) human tumors previously described (12, 13). Recent evidence suggests that MSI-L tumors are, in fact, MSS with intact MMR machinery and have similar numbers of unstable microsatellites (14–16). However, MSI-intermediate tumors in our model are devoid of functional MMR machinery and do exhibit notable differences in global microsatellite instability. However, they have not had sufficient time to obtain a large number of altered microsatellites.

In our mouse models of microsatellite instability, we found that tumor-bearing mice treated with antibodies against PD-1 displayed a variable response to therapy, depending on their genomic tumor MSI burden (Fig. 1E). In agreement with previous studies, the parental lines were either minimally responsive or nonresponsive to anti-PD-1 therapy compared to isotype control-treated tumors at 24 days after implantation [$p = 0.001$, $p =$ not significant (ns), B16F10 and CT26 tumors, respectively]. The MSI-intermediate lines, despite their “MMR-d/MSI” status, also displayed minimal to no difference in mean tumor volumes, similar to those displayed by the parental lines ($p = 0.01$, $p =$ ns, MSI-intermediate B16F10 and CT26 tumors, respectively). However, in the MSI-high lines, the decrease in mean

tumor volume, although not curative, became markedly more pronounced as compared to the parental (MSS) and MSI-intermediate lines ($p < 0.000001$, $p < 0.0000001$, MSI-high B16F10 and CT26 tumors, respectively) (Fig. 1E). Of note, in the B16F10 line, all three lines treated with isotype control antibodies displayed similar tumor growth kinetics, suggesting that no deficit in tumor fitness was induced by genetic alteration of the lines (fig. S5). In the CT26 line, induction of MMR deficiency results in a slight decrease in tumorigenic take rate and growth, but when implanted into athymic nude mice, these tumors grow efficiently, again suggesting no deficit in nonimmune-related tumor fitness (fig. S6).

We analyzed the tumor-infiltrating lymphocyte populations from pooled tumor samples in treated and untreated groups 24 days after implantation. In the B16F10 line, we observed low T cell infiltration in isotype control-treated mice in all lines (Fig. 2A). By contrast, in the CT26 line, we observed a modest increase in immune infiltration in the MSI-high isotype control-treated tumors compared to isotype control-treated parental and MSI-intermediate tumors, suggesting a slight baseline increase in the immunogenicity of the MSI-high line (Fig. 2A). However, in both lines, we observed a highly pronounced increase in T cell infiltration in the MSI-high tumors after anti-PD-1 therapy compared to parental and MSI-intermediate tumors (Fig. 2A and fig. S7). We examined the relative proportions of CD8⁺ and CD4⁺ T cell populations in treated and untreated groups. In addition to an increase in CD8⁺ T cells in anti-PD-1-treated groups, we also observed an increase in CD4⁺ T cells in anti-PD-1-treated MSI-high tumors in both cell lines (Fig. 2B). CD8⁺ T cell immunofluorescence was also performed and provided orthogonal validation of our flow cytometric findings (Fig. 2, C and D).

To gain an understanding of the tumor evolution of MSI-high tumors, we performed a separate set of experiments to examine the gene expression profiles and genomic characteristics of our B16F10 murine tumors as they underwent treatment with and without anti-PD-1. Unsupervised hierarchical clustering of differentially expressed genes revealed enrichment of immune-related pathways in MSI-high anti-PD-1-treated tumors compared with MSI-high isotype control-treated and parental anti-PD-1-treated tumors. This is in agreement with a more immune-activated microenvironment and a subsequent increase in PD-L1 expression (Fig. 3A and figs. S8 to S10). Additionally, stromal and tumor-associated genes were up-regulated in MSI-high isotype control-treated and parental anti-PD-1-treated tumors as compared to the MSI-high anti-PD-1-treated tumors (fig. S8).

We next analyzed changes in the mutational landscape of B16F10 tumor cells before implantation and after treatment to better understand the nature of immune editing in these tumors as they evolved throughout therapy, quantified against the normal reference genome (mouse splenic DNA). We observed possible immune editing as evidenced by both a loss and a reduction in tumor allele frequency of missense (nonsynonymous SNVs) and indel mutations in anti-PD-1-treated MSI-high tumors from their preimplantation baseline, suggesting that immunoediting of tumor cells containing these mutations drives the variable response to anti-PD-1 therapy seen in MSI-high tumors, which is not or only minimally observed in MSS parental tumors (Fig. 3B and fig. S11). When examining total missense and indel mutations, we observed that the mutational landscape of anti-PD-1-treated MSI-high tumors, compared to the parental and isotype control-treated MSI-high tumors,

becomes markedly more subclonal and displays a narrower distribution of tumor-allelic frequency (Fig. 3C).

Further evidence suggesting immunoediting was observed when we examined only the total mutational (missense and indel mutations) load in post-therapy tumors (Fig. 3D). We observed a greater reduction in missense and indel mutations between isotype control and anti-PD-1 treatment in MSI-high tumors than in parental tumors (Fig. 3D). Similar trends were observed when we performed the same analysis using multiple *n*-peptide-predicted neoantigens (fig. S12). As these peptides are computationally predicted to be expressed on cell surface major histocompatibility complex (MHC) molecules, their specific loss provides further confidence that immunoediting is indeed occurring in our MSI-high anti-PD-1-treated tumors.

Next, we tracked the fate of individual missense and indel mutations that were either (i) present at baseline or (ii) newly generated as a result of MMR deficiency during repeated in vitro passaging (Fig. 3E and fig. S13). As expected, baseline missense and indel mutations (which are presumably largely nonimmunogenic) were minimally edited, whereas MMR deficiency-induced mutations were more preferentially edited following therapy (Fig. 3E). When we considered only mutations not edited after isotype control treatment, MSI-high tumors displayed a larger percentage reduction of indels as opposed to missense mutations after anti-PD-1 therapy, suggesting that indel mutations may have a greater likelihood of generating an immunogenic response over missense mutations in these tumors (Fig. 3F).

Building on the findings established in our murine model, we examined whether similar relationships existed in clinical data between MSI intensity and response to anti-PD-1 therapy in patients. First, we examined baseline immune activity as measured by a previously reported cytolytic (CYT) score across 14 human cancers stratified by genomic MSI intensity level using The Cancer Genome Atlas (TCGA) transcriptome and exome data (Fig. 4A and tables S1 and S2). We observed a general trend toward increased immune infiltration and cytolytic activity in MSI-high tumors compared to MSI low tumors (MSIsensor threshold 3.5) (Fig. 4A and fig. S14). A statistically significant association was observed in cancers frequently detected to be MMR deficient, such as uterine and endometrial carcinomas ($p = 0.005$), stomach adenocarcinomas ($p = 8.68 \times 10^{-9}$), and colorectal carcinomas ($p = 1.07 \times 10^{-9}$).

We next interrogated genomic MSI levels from tumor exomes in a cohort of previously published MMR-proficient and MMR-deficient gastrointestinal tumors before anti-PD-1 therapy with updated clinical data (table S3) (6). As expected, MSIsensor scores were significantly higher in tumors clinically designated as MMR-d, compared to MMR-proficient tumors ($p = 0.002$), but a wide range of MSI intensity levels within these tumors was observed (Fig. 4B). We then focused our analysis specifically on the MMR-d clinically designated tumors in this cohort (Fig. 4, C to G). We observed that clinical responders were associated with higher levels of genomic MSI intensity and, conversely, patients with progressive disease had among the lowest genomic MSI levels in this cohort (Fig. 4C). To better characterize the mutational basis for this association, we compared indel mutational load with genomic MSIsensor score for this cohort and found the two metrics to be

significantly and strongly correlated ($r = 0.8045$, $p = 0.0089$) (Fig. 4D). When performing the same correlative analysis using indel load, we again found that clinical responders were enriched in those patients with the highest tumor indel loads (Fig. 4E). The same associations and correlations did not exist for missense mutational burden and clinical response ($r = 0.3994$, $p = 0.2867$) (Fig. 4, F and G). Together with our murine data, these findings suggest that MSI intensity and resultant exonic indel load may play a critical role in determining anti-PD-1 responsiveness in MSI-H tumors.

Additionally, we analyzed genomic data from previously reported MMR-d colorectal and esophagogastric patient tumors treated with immune checkpoint blockade (table S4) (17, 18). Prior to therapy, these patients underwent targeted sequencing of genomic germline and tumor DNA using MSK-IMPACT, a U.S. Food and Drug Administration (FDA)-authorized next-generation clinical sequencing panel (19). We quantified their precise genomic MSI levels from the available sequencing data using MSIsensor, which has been previously validated for clinical use (20). We examined whether tumor MSI intensity levels within this cohort of MMR-d patients could affect clinical outcome, using survival data. MSIsensor score as a continuous variable was associated with improved survival after immune checkpoint blockade (HR 0.95, log-rank $p = 0.04$). We then observed that patients with an MSIsensor score in the top 80th percentile (defined here as “MMR-d/MSI-High”) were associated with improved survival as compared to patients in the bottom 20th percentile (defined here as “MMR-d/MSI-Intermediate”) (HR = 0.147, log-rank $p = 0.0032$) (Fig. 4H). Notably, these correlations also remained significant when controlling for known biological confounders of tumor histology and total tumor mutational burden (TMB) (table S5). We observed that these correlations also held true when testing multiple thresholds for TMB percentile as well (table S6). These findings may reflect a greater impact of frameshifting indel mutations, associated with increased MSI intensity, over missense mutations in eliciting clinical response (fig. S15). The aim of our analyses here was not to create a model from our clinical data nor establish a specific MSIsensor or indel load threshold for use as a predictive biomarker in the clinical setting, which will require data from large numbers of patients to precisely stratify responders from nonresponders by using these metrics. Nonetheless, these data demonstrate that in multiple independent cohorts of MMR-d human patients treated with checkpoint blockade, a range of relative MSI phenotypes exists and may help identify patients who will derive clinical benefit from immunotherapy.

The human-based and murine evidence presented in this study demonstrates that the genome-wide intensity of microsatellite instability and resultant tumor mutational load influence response to anti-PD-1 immunotherapy and tumor evolution in MMR-deficient tumors. The basis for this response is likely multifactorial and may disproportionately rely on indel mutations over missense mutations to drive clinical outcome. The findings of this study will require validation in larger MSI-H anti-PD-1-treated human cohorts. Additionally, long-term clinical data are needed to assess the durability of clinical responses as selection of nonimmunogenic clones may contribute to eventual disease recurrence. These studies could also elucidate what role tumor progression may have on MSI intensity and mutational load within individual tumors. Our experimental and clinical data suggest that there is substantial diversity within MSI patients and that it may be possible to stratify responders and nonresponders to anti-PD-1 therapy across MMR-deficient cancers by using

the precise next-generation sequencing–based quantification of microsatellite instability intensity

Supplementary Material

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Competing interests: T.A.C., L.G.M., and R.M.S. are inventors on a provisional patent application (62/569,053) submitted by MSKCC that covers use of tumor mutational burden as a predictive biomarker for cancer immunotherapy. T.A.C. is an inventor on a PCT patent application (PCT/US2015/062208) filed by MSKCC, relating to the use of TMB in lung cancer immunotherapy, which has been licensed to Personal Genome Diagnostics, and MSKCC, T.A.C., and L.G.M. receive royalties. T.A.C. is a cofounder of Gritstone Oncology and holds equity in An2H. T.A.C. has served as an advisor for Bristol-Myers Squibb, Illumina, Eisai, and An2H. L.G.M. received consulting fees from Rakuten Aspyrian and speaker fees from Physician Educational Resources. T.A.C. acknowledges grant funding from Bristol-Myers Squibb, AstraZeneca, Illumina, Pfizer, An2H, and Eisai. D.T.L. and L.A.D. are inventors on a patent (WO2016077553A1) held by Johns Hopkins University that covers use of mismatch repair deficiency for diagnosis and therapy. D.T.L. reports honoraria from Merck and research funding from Merck, Bristol-Myers Squibb, and Aduro Biotech. L.A.D. is a paid consultant for Merck, PGDx, and Phoremest; is a member of the board of directors of Personal Genome Diagnostics (PGDx) and Jounce Therapeutics; and holds equity in PapGene, Personal Genome Diagnostics (PGDx), and Phoremest. L.A.D. has participated as a paid consultant for one-time engagements with Caris, Lyndra, Genocoe Biosciences, Illumina, and Cell Design Labs within the past 5 years. J.F.H. has an honorarium from Medscape and Axiom Biotechnologies. N.R. is affiliated with illumina speakers bureau and has research funding from Pfizer and Bristol-Myers Squibb.

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Fig. 1. Generation of microsatellite instability and tumor mutational burden after anti-PD-1 therapy.

(A) Experimental model system for creating MSI-intermediate versus MSI-high tumor cell lines. *Msh2* knockout B16F10 mouse melanoma and CT26 mouse colon cancer cell lines were passaged as illustrated. The unedited parental line was passaged in parallel and served as a control. Blue receptors on cells represent MHC complexes presenting self (black) or neoantigens (colors). (B) Absolute number of novel nonsynonymous single-nucleotide variations (SNVs) and coding region indel mutations observed beyond what was present in the parental unedited line in MSI-intermediate (low-passage) and MSI-high (high-passage) lines. (C) Increased genomic MSI intensity levels in MSI-intermediate and MSI-high cell lines quantified through the use of the MSIsensor algorithm on whole-exome sequencing (150x) data (B16F10 MSI-intermediate line $p = 0.0028$, all other lines $p < 0.0001$). Fisher's exact test was used to compare proportions of unstable microsatellites between the indicated groups and respective parental lines. (D) Increased percentage of novel exonic indel mutations out of total mutations in MSI-high lines as compared to the MSI-intermediate cell lines ($p = 0.003$, $p < 0.0001$). Fisher's exact test was used to compare proportions of novel exonic indels between the indicated groups. (E) In vivo tumor growth kinetics in isotype control antibody-treated and murine anti-PD-1-treated parental, MSI-intermediate, and MSI-high tumor-bearing mice over a 24-day period. B16F10 cell line: $p = 0.001$ (parental), $p = 0.01$ (MSI-intermediate), $p < 0.000001$ (MSI-high); CT26 cell line: $p = ns$ (parental), $p = ns$ (MSI-intermediate), $p < 0.0000001$ (MSI-high). Student's t test was used for the comparison of tumor volume at 24 days after treatment. P value was adjusted by Holm Sidak correction for testing at multiple time points. Data shown as mean $\pm$ SEM, $n = 8$ to 12 mice per experimental arm.

Fig. 2. Immune cell infiltration drives variable responses to anti-PD-1 therapy in MSI-high tumors.

(A) Representative flow cytometric data quantifying CD45⁺CD3⁺ T cell infiltration in pooled tumor samples ($n = 6$ to 7 tumors per arm) resected at day 24 from each experimental arm in isotype control antibody-treated and murine anti-PD-1-treated parental, MSI-intermediate, and MSI-high tumor lines. (B) Percentage tumor infiltration of CD4⁺, CD8⁺, and total CD3⁺ T cell infiltration in isotype control-treated (dark colors) and anti-PD-1-treated (light colors) tumor-bearing mice in all experimental arms ($n = 6$ to 7 tumors per arm). (C) Representative photographs from immunofluorescence (IF) staining of isotype control-treated and murine anti-PD-1-treated parental, MSI-intermediate, and MSI-high tumor lines. Yellow, CD3⁺, tumor nuclei are stained with DAPI (blue). Scale bars, 50 μ m. (D) Mean CD3⁺ T cell counts per high-power field from five representative fields from each experimental arm [shown in (C)], quantified by two trained observers blinded to the treatment and experimental arm. Significance values: ** $p < 0.01$, **** $p < 0.0001$ versus parental isotype control-treated, one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test for comparisons to a single control group (parental isotype treatment).

Fig. 3. Tumor mutational evolution and immunoediting in anti-PD-1-treated MSI tumors. (A) Unsupervised hierarchical clustering of differentially expressed genes demonstrating enrichment of immune-related pathways (cluster 1) in MSI-high anti-PD-1-treated tumors as compared to MSI-high isotype control-treated tumors. Up-regulation of the stromal and tumor-associated genes (cluster 2) seen in MSI-high isotype control-treated tumors as compared to the MSI-high anti-PD-1-treated tumors. (B) Tumor mutational allele frequency (purity-corrected) in isotype control-treated and anti-PD-1-treated parental and MSI-high B1610 tumor cells before implantation (x axis) and post-therapy (y axis). Data points represent mutations (650 $\times$ mean coverage) in isotype control-treated and anti-PD-1-treated parental ($n = 102, 106$ indels; 2291, 2273 SNVs) and MSI-high B16F10 ($n = 233, 176$ indels; 2502, 2442 SNVs) tumor cells before or after therapy. (C) Violin plots depicting frequency distribution (purity-corrected) of missense (nonsynonymous SNVs) and indel mutations in post-therapy B16F10 tumors in the parental and MSI-high lines (p values for significant differences shown as indicated between groups, one-way ANOVA with Tukey's test for pairwise comparisons). (D) Reduction in mutational load (missense and indel counts) in

post-therapy isotype control and anti-PD-1-treated parental and MSI-high tumors ($p < 0.0001$, Fisher's exact test for proportions of lost or gained mutations after antibody treatment between the indicated groups). (E) Changes in tumor allele frequency of individual missense and indel mutations in isotype control and anti-PD-1-treated MSI-high tumors that were either (i) preexisting in the parental line or (ii) generated in vitro through MMR deficiency [$p < 0.0001$; all comparisons are of percentage deletion (proportional loss) after anti-PD-1 therapy of baseline versus MMR-d-induced mutations, Fisher's exact test]. (F) Percentages of MMR-d-induced mutations persisting after isotype control treatment that are deleted (edited) upon addition of anti-PD-1 treatment ($p < 0.0001$, Fisher's exact test to compare the proportions of lost or edited MMR-d-induced mutations after PD-1 blockade between SNV and indel mutational groups).

Fig. 4. Increasing microsatellite instability intensity predicts tumor response to immune checkpoint blockade therapy in human clinical data.

(A) Immune cytolytic (CYT) score across 14 human cancers derived from TCGA sequencing data stratified by MSI-Low (red) and MSI-High (blue) status. MSI designation derived from MSIsensor score using a threshold score of 3.5. All box-and-whisker plots represent the median (solid bars), interquartile range (boxes), and $1.5 \times$ interquartile range (dashed lines); p values shown as indicated between groups, Mann-Whitney U test. (B) MSIsensor scores in clinically designated microsatellite stable colorectal tumors as compared to clinically designated microsatellite unstable colorectal tumors ($p=0.002$, unpaired t test; data shown as mean $\pm$ SEM). (C) MSIsensor scores and clinical response designation within MMR-d/MSI-H patients; each bar represents one patient and is annotated with their known tumor or germline MMR genetic alteration. CR (complete response, green), PR (partial response, blue), SD (stable disease, teal), PD (progressive disease, red). (D) Mutational indel load versus MSIsensor score among MMR-d/MSI-H patients ($r=0.8045$, $p=0.0089$). (E) Tumor

indel load and clinical response designation within MMR-d/MSI-H patients; each bar represents one patient. **(F)** Nonsynonymous SNV (missense) load versus MSI sensor score among MMR-d/MSI-H patients ($r = 0.3994$, $p = 0.2867$). **(G)** Tumor nonsynonymous SNV load and clinical response designation within MMR-d/MSI-H patients; each bar represents one patient. **(H)** Overall survival in immune checkpoint blockade-treated MMR-deficient patients stratified by MMR-d/MSI-Intermediate (blue) designation (bottom 20th percentile of MSI sensor scores) and MMR-d/MSI-High (red) designation (top 80th percentile of MSI sensor scores) (log-rank test $p = 0.0032$, hazard ratio = 0.147, log rank).