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Tumor mutational burden in colorectal cancer: Implications for treatment

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ABSTRACT

Although immune checkpoint inhibitors have revolutionized the treatment of several advanced solid cancers, in colorectal cancer, the transformative benefit of these innovative medicines is currently limited to those with deficient mismatch repair or high microsatellite instability. Tumor mutational burden (TMB) has emerged as a potential predictor of immunotherapy benefit, but the lack of standardization in its assessment and reporting has hindered the introduction of this biomarker in routine clinical practice. Here, we compiled 45 colorectal cancer studies utilizing numerical thresholds for high-TMB. In this group of studies, TMB cut-offs ranged from 6.88 to 41 mut/Mb and were most often set at 10, 17, or 20 mut/Mb. Additionally, we observed divergent TMB definitions and inconsistent disclosure of specific methodological details, which collectively emphasize the substantial lack of harmonization within the field. Ongoing efforts to harmonize TMB assessment will be critical to validate TMB as a predictive marker of immunotherapy response.

1. Introduction

According to the latest GLOBOCAN estimates, colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide (10%) and is the second leading cause (9.4%) of cancer-related deaths (Ferlay et al., 2024; Sung et al., 2021). Although the 5-year survival of CRC is largely stage-dependent—around 90% for stage I and 10% for stage IV—its prognosis remains grim, as patients typically only show symptoms at an advanced stage of the disease (Ogunwobi et al., 2020; Picard et al., 2020). Almost 25% of CRC cases are diagnosed at a metastatic stage, and 50% of patients with localized disease at the time of diagnosis will go on to develop metastasis over time (CRUK, n.d.; Ogunwobi et al., 2020). Although the burden of colorectal cancer has decreased in high-developed countries (Xie et al., 2020), the number of new cases has nearly doubled in individuals younger than 50 years of age, who are often excluded from routine screening initiatives (Done and Fang, 2021). Despite advances in screening, diagnosis, and treatment, CRC remains an important public health challenge, as traditional therapeutic strategies offer a median survival of only 30 months (Fabrizio et al., 2018; Ooki et al., 2021).

Most patients present with unresectable disease, and thus require systemic therapy: current standard-of-care dictates metastatic colorectal cancer (mCRC) should be treated with a combination of chemotherapy and targeted agents, chosen according to molecular testing of the tumor (Ooki et al., 2021). The most frequently used monoclonal antibodies (mAb) target either the epidermal growth factor receptor (EGFR) or the vascular endothelial growth factor (VEGF) pathway (Cervantes et al., 2022). In KRAS or NRAS mutated cancers, anti-VEGF antibodies (*e.g.* bevacizumab) will be the mAB of choice, as anti-EGFR mAb (*e.g.* cetuximab, panitumumab) may only be used to treat non-mutated tumors (Aleksakhina and Imyanitov, 2021). In the second group, the choice between anti-EGFR or anti-VEGF will depend on additional factors, such as the laterality of the primary tumor (Network, 2023). As new genetic markers are uncovered, CRC management has moved even further into an era of personalized medicine, particularly in the chemorefractory setting. For example, tumors with BRAF V600E mutations are highly aggressive but may be treated with a combination of BRAF-inhibitors and anti-EGFR mAb, and although rare, CRCs driven by HER2 amplification often respond to HER2-targeted therapies (Afrăsânie et al., 2019; Aleksakhina and Imyanitov, 2021).

The identification of an immune signature characterized by deficient mismatch repair (dMMR) represents a new hope for CRC research. The MMR pathway is responsible for recognizing and repairing DNA base pair mismatches that occur due to errors in the replication and recombination of DNA (Lizardo et al., 2020; Reilly et al., 2019). Most cases of MMR deficiency are sporadic and caused by epigenetic silencing of MMR

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JHU 2178
Merck Sharp v. Johns Hopkins
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genes, while only a small percentage of CRC patients have actual somatic or germline mutations (Harada and Morlote, 2020). MMR failure results in a phenotype of microsatellite instability (MSI) and is one of the driving mechanisms behind colorectal tumorigenesis (J. Li et al., 2021; Lizardo et al., 2020). These defects lead to a higher frequency of frameshifts and insertion and deletion mutations (indels), which may generate novel peptides within the cell (Reilly et al., 2019; Zheng et al., 2022). If these peptides are displayed by the major histocompatibility complex (MHC) and recognized by the immune system as foreign, they may trigger an immune response, which can be manipulated by immunotherapeutic strategies (Reilly et al., 2019; Zheng et al., 2022).

In the past decade, immunotherapy has revolutionized cancer management. These strategies can be divided into three main groups: cell-based therapies, therapies targeting cytokines, and immune checkpoint inhibitors (ICIs). Checkpoint inhibitors, in particular, have transformed the treatment of many advanced solid cancers, such as melanoma and non-small cell lung cancer (NSCLC) (Borelli et al., 2022; Huyghe et al., 2020). Eight ICIs are currently approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), and three of those—nivolumab, pembrolizumab, and ipilimumab—can be used in dMMR/MSI-H colorectal cancer (European Medicines Agency, 2024, 2023a, 2023b). However, MMR deficiencies in metastatic colorectal cancer are not very common: while microsatellite instability is present in 15% of all CRC cases, this percentage drops to 3–4% in patients with metastatic disease (Pecci et al., 2021).

Some individuals achieve durable and complete remissions (Jardim et al., 2021), but treatment with ICIs is not without risks, which should be carefully considered: although these medicines are reasonably safe, immune-related adverse reactions are common and may be fatal in some cases (Dolladille et al., 2020). In a minority of patients, treatment with ICIs may even cause a paradoxical, accelerated progression of disease, termed hyperprogression (Adashek et al., 2020). Immunotherapy is also incredibly costly; using predictive biomarkers to select cancer patients for ICI therapy is crucial to reduce response variability, increase cost-effectiveness, and prevent unnecessary toxicity.

Currently, MSI status is the only predictive marker of ICI response in colorectal cancer. However, it does not adequately identify all CRC patients who might benefit from treatment with ICIs (Y. Li et al., 2021). In other cancers, measurement of PD-L1 expression via immunohistochemistry is used to predict response to ICIs, but this biomarker has shown limited utility in CRC (Chan et al., 2019). Discovering and validating new biomarkers that can more accurately predict which patients benefit the most from this innovative therapy is an urgent, unmet need.

One highly researched biomarker is tumor mutational burden (TMB), which essentially reflects the number of mutations present in the genome of the cancer cells (Aleksakhina and Imyanitov, 2021). Initial studies in lung cancer suggested a higher mutational burden was associated with improved responses to anti-programmed cell death-1 (PD-1) antibodies (Rizvi et al., 2015), and because MSI is only one of several potential mechanisms contributing to a high tumor mutational load (Aleksakhina and Imyanitov, 2021), stratification according to TMB may identify new subpopulations of CRC patients who could potentially benefit from ICIs. However, the lack of standardization in TMB assessment and the high complexity of the methodology have prevented this biomarker from entering routine clinical practice (McNamara et al., 2020).

In this critical review, we explore the potential of TMB as a predictor of response to ICIs, address the limitations associated with its widespread implementation, and evaluate its utility in metastatic CRC. Additionally, we sought to synthesize current evidence and compiled CRC studies utilizing TMB as a biomarker, allowing for the identification of common patterns in TMB-based stratification in colorectal cancer.

2. The role of checkpoint inhibitors in MSI-H CRC

To prevent excessive immune responses that could harm healthy cells and tissues, the immune system can regulate its own activity through a set of molecules known as inhibitory immune checkpoints, such as anti-PD-1, anti-programmed cell death-ligand 1 (PD-L1), and anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) (Ooki et al., 2021). These immune checkpoints essentially act as brakes for the immune response: following activation through the T-cell receptor (TCR), T-cells require a co-stimulatory signal to become fully functional, which is inhibited by interaction with immune checkpoints (Wang et al., 2016). Some cancer cells exploit this protective mechanism, and by expressing co-inhibitory molecules on the cell surface, are able to escape immune surveillance and survive (Jardim et al., 2021). If these checkpoints are blocked pharmacologically, the body's anti-tumor response can be "reactivated" (Jardim et al., 2021). The discovery of immune checkpoint blockade has transformed the therapeutic landscape of many advanced solid cancers and earned James P. Allison and Tasuku Honjo the Nobel Prize in Physiology or Medicine in 2018 (NobelPrize.org, n.d.). However, despite their success in neoplasms like melanoma and NSCLC, initial studies with checkpoint-blocking mAbs failed to demonstrate a clinical benefit in unselected patients with CRC (Almquist et al., 2020). Indeed, in the first-in-human phase I clinical trial of nivolumab, only 1 out of 14 patients with mCRC experienced (and later maintained) a complete response (Brahmer et al., 2010), and in a larger trial carried out by the same investigators, none of the CRC patients showed objective responses (Topalian et al., 2012).

Based on the hypothesis—and subsequent confirmation—that the one patient who responded to treatment with nivolumab had dMMR (Lipson et al., 2013), Le and colleagues carried out a phase II clinical trial (NCT01876511) where three different cohorts of patients with metastatic cancer received the anti-PD-1 mAb pembrolizumab: cohort A included 11 patients with dMMR colorectal adenocarcinoma, cohort B included 21 patients with proficient mismatch repair (pMMR) colorectal adenocarcinoma, and cohort C included 9 patients with non-colorectal dMMR tumors (Le et al., 2015). Indeed, a deficiency in the MMR pathway was associated with a better response to ICIs: the immune-related overall response rate (ORR) in cohorts A and C was 40% and 71%, respectively, compared to 0% in cohort B (Le et al., 2015). After this initial proof-of-concept trial, the potential for benefit with ICIs in dMMR/MSI-H colorectal cancer was further demonstrated in a series of clinical trials, ultimately resulting in the regulatory approval of three checkpoint inhibitors. The results of these pivotal studies are summarized in Table 1.

The impressive results achieved with pembrolizumab, plus its manageable safety profile and convenient administration protocol, resulted in a tumor-agnostic approval of this ICI by the FDA, the EMA, and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA). Although the different regulatory bodies initially approved pembrolizumab for the second-line treatment of dMMR/MSI-H mCRC, the FDA and EMA now recommend its use in the first-line setting (PMDA, 2020; European Medicines Agency, 2024; U.S. Federal Drug Administration (FDA), 2023).

Nivolumab is currently approved for the treatment of dMMR/MSI-H mCRC in the refractory setting (after prior fluoropyrimidine-based combination chemotherapy), as a single agent (FDA) (U.S. Federal Drug Administration, 2023) or in combination with ipilimumab (FDA, EMA) (European Medicines Agency, 2023b, 2023a). The phase III CheckMate 8HW trial (NCT04008030) is currently studying this ICI combination in the first-line context, and preliminary results have demonstrated improved progression-free survival compared to chemotherapy (Andre et al., 2024; ClinicalTrials.gov, n.d.).

Given the success of ICIs in refractory mCRC and the potential for durable and complete responses, identifying the patients most likely to benefit from these therapies is an urgent priority. However, MSI status leaves out patients who may also benefit from treatment with ICIs (Ritterhouse, 2019). For example, CRCs with pathogenic inactivation of DNA polymerase genes (POLE/POLD1) are clinically and immunologically similar to those with MSI, with a high load of somatic mutations,

Table 1
Summarized results of pivotal clinical studies in dMMR/MSI-H metastatic colorectal cancer.

	CheckMate 142 (n=238)			KEYNOTE-164 (n=124)		KEYNOTE-177 (n=307)	
	Monotherapy Cohort (n=74)	*Combination Cohort (n=119)*	*1st line Cohort (n=45)*	*Cohort A (n=61)*	*Cohort B (n=63)*	*Pembrolizumab (n=153)*	*ChT (n=154)*
% ORR (No.)	31% (23)	65% (77)	69% (31)	33% (20)	33% (21)	44% (67)	33% (51)
% CR (No.)	0% (0)	13% (15)	13% (6)	3% (2)	8% (5)	11% (17)	4% (6)
% PR (No.)	31% (23)	52% (62)	56% (25)	30% (18)	25% (16)	33% (50)	29% (45)
% SD (No.)	38% (28)	21% (25)	16% (7)	18% (11)	24% (15)	21% (32)	42% (65)
% PD (No.)	26% (19)	12% (14)	13% (6)	46% (28)	40% (25)	29% (45)	12% (19)

dMMR, deficient mismatch repair; MSI-H, high microsatellite instability; ChT, chemotherapy; ORR, objective response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

infiltration of CD8+ T-lymphocytes, and increased expression of checkpoint molecules (Domingo et al., 2016; Wang et al., 2018). POLE mutations are often mutually exclusive with dMMR and are present in 1–3% of all cases of CRC (Domingo et al., 2016; Picard et al., 2020). This contributes to the hypothesis that some patients with microsatellite-stable (MSS) cancers may respond positively to ICIs, which has already been observed in MSS POLE-mutant endometrial cancer (Klempner et al., 2020). Indeed, the Phase II AcSé trial (NCT03012581) has shown promising evidence that nivolumab treatment may be effective in MSS tumors with POLE mutations affecting proofreading (Rousseau et al., 2020, 2022). Similarly, variations in AT-rich interaction domain 1 (ARID1A) have been associated with higher mutational burden and strong immune activation (Tokunaga et al., 2020). Somatic mutations in ARID1A are present in 6–9% of patients, but their role in CRC is not yet fully understood (Mehrvarz Sarshekeh et al., 2021; Zhao et al., 2022). As it becomes apparent that MSI status alone cannot explain all instances of high mutational load, TMB has emerged as a potential predictor of ICI efficacy.

3. Tumor Mutational Burden: challenges and opportunities

Pathogenic changes in cellular function and metabolism are at the root of neoplastic transformation. These often result from the accumulation of "driver" mutations, which directly affect cell growth (*e.g.,* RAS, EGFT, TP53) (Chan et al., 2019; Klempner et al., 2020). On the other hand, "passenger" mutations do not affect tumor growth directly but may affect the protein sequence and create tumor-specific peptides. If recognized by T-cells as foreign, these so-called neoantigens can elicit an antitumor immune response (Zheng et al., 2022). A higher mutational load will likely result in a greater number and variety of neoantigens, culminating in increased recognition by T-cells and better responses to immunotherapies; TMB is a proxy for neoantigenicity and may be used as a predictor of response to ICIs (Chan et al., 2019; Klempner et al., 2020). In general, the highest values of TMB tend to be present in tumors caused by defects in DNA repair or induced by environmental exposures, such as UV radiation and cigarette smoke (Aleksakhina and Imyanitov, 2021; Ritterhouse, 2019). ICIs tend to be more effective in cancers that are TMB-high, which explains why researchers initially focused on melanoma and NSCLC, instead of other solid tumors (Y. Li et al., 2021; Stenzinger et al., 2019). However, TMB research has evolved significantly in recent years, leading to its inclusion in trials for an ever-diversifying range of cancers, including CRC (Stenzinger et al., 2019).

3.1. TMB definition and assessment

TMB is officially defined as the number of somatic mutations present in the tumor genome, but it is usually reported as the number of mutations within a megabase (Mb) of genomic material (Y. Li et al., 2021; Ritterhouse, 2019). It was initially determined by whole genome sequencing (WGS) or whole exome sequencing (WES) of formalin-fixed, paraffin-embedded (FFPE) tissue samples (McNamara et al., 2020; Stenzinger et al., 2019). However, there are several limitations to the large-scale clinical application of these methods, which are expensive, time-consuming, and methodologically complex (Y. Li et al., 2021; Normanno, 2017).

Next-generation sequencing (NGS) of targeted gene panels has emerged as a promising alternative and may even be used in blood samples (bTMB) when the amount of tissue obtained is not sufficient for TMB detection (Y. Li et al., 2021; McNamara et al., 2020). Although these targeted panels only cover specific areas of the genome, assays that analyze a minimum of 1 Mb of genomic material have shown statistical concordance with WES (Chalmers et al., 2017; Stenzinger et al., 2019).

Several NGS panels have been approved as companion diagnostics by the FDA: for example, the FoundationOne CDx assay covers 324 genes across 1.1 Mb, and MSK-IMPACT covers 468 genes across 1.2 Mb (U.S. Federal Drug Administration, 2017a, 2017b). These panels use a comprehensive genomic profiling (CGP) approach, where several types of molecular alterations in cancer-related genes (including genomic signatures like MSI) are identified in a single test, some of which are not evaluated in WES (Jardim et al., 2021; Klempner et al., 2020). Because CGP tests cover a wider range of genetic alterations, assay sensitivity is improved, and more patients may be identified as good candidates for ICI therapy (Klempner et al., 2020; Ritterhouse, 2019). Importantly, these same panels have not been approved in the European setting thus far.

3.2. Limitations associated with TMB assessment and interpretation

There are important limitations to consider: the general lack of consensus regarding TMB assessment, reporting, and even definition has hindered the introduction of this biomarker in routine clinical practice (McNamara et al., 2020). While some methods of TMB measurement include all mutations, some only consider non-synonymous mutations, or even only mutations that alter the protein sequence (McNamara et al., 2020). Furthermore, most current versions of TMB do not account for post-translational modifications, which may also contribute to the formation of neoantigens (Chan et al., 2019).

The different approaches used in published studies highlight the need for standardization and harmonization of current TMB estimation and reporting. Friends of Cancer Research (FoCR) and the Quality Assurance Initiative Pathology (QuIP) have partnered to recommend best practices that should minimize variability in TMB assessment in FFPE tissue samples (Stenzinger et al., 2019). To achieve this goal, the two organizations devised a three-step approach: phase I of the project consists of *in silico* analysis of data from The Cancer Genome Atlas (TCGA) (Merino et al., 2020), phase II comprises the analysis of human tumor samples (Vega et al., 2021), and phase III will involve a clinical study of patients who have received ICIs. To date, these recommendations include reporting TMB as mutations/megabase (mut/Mb), validating NGS panels through comparison with standardized reference methodologies (like WES) to minimize bias, using multi-gene panels that analyze a minimum of 1 Mb of genomic material, and testing for actionable driver mutations like EGFR (Stenzinger et al., 2019). While

the different factors contributing to TMB variability are extensively described elsewhere (McNamara et al., 2020; Sung et al., 2022), it is clear that standardization of TMB scoring will be crucial to its implementation into clinical practice.

In addition to methodological limitations, some challenges persist in interpreting the clinical significance and utility of TMB, as some tumors have not responded as well to checkpoint inhibitors as their TMB status predicted (Rousseau et al., 2021; Stenzinger et al., 2019). This discrepancy may stem from the realization that a high neoantigen load by itself is not enough to elicit an adequate immune response; These antigens must be adequately processed and presented via human leukocyte antigen (HLA) molecules at the surface of cancer cells to be recognized as non-self by the immune system. However, only a minority of mutations result in such antigens. Other factors related to the tumor microenvironment, such as the presence of inactivating mutations in the antigen presentation pathway, the downregulation of MHC expression, and the type of checkpoint exploited by the tumor, may also contribute to a poor association between elevated TMB and response to ICIs (Chan et al., 2019; Jardim et al., 2021; Pecci et al., 2021). Nevertheless, existing data shows that responding tumors have generally higher mutation and neoantigen burden than non-responders (Schrock et al., 2019), and panel-based TMB has consistently shown a predictive value for ICI response in different tumor types, including CRC (Jardim et al., 2021).

3.3. A focus on colorectal cancer

Current clinical use of immune checkpoint blockade is mostly restricted to those with high MSI or dMMR (Roth and Das, 2021), but response rates to ICIs are variable, even in this small subgroup of patients: half of all MSI-H CRCs will either possess intrinsic resistance to ICIs or eventually develop acquired resistance (Jenkins et al., 2018; Roth and Das, 2021). There have also been reports of patients with microsatellite stable CRC who have shown durable, complete responses to checkpoint inhibitors (Gomar et al., 2021; Gong et al., 2019; Lutfi et al., 2023). For instance, in a case report published by Gong *et al.,* a 61-year--old male with chemorefractory metastatic rectal adenocarcinoma showed radiographic disease regression after two months of palliative pembrolizumab, and this response was sustained at least for nine months (time of the report). This patient had pMMR and a TMB of 32 mutations per megabase, which was considered high (Gong et al., 2019). A similar outcome was observed in the case of a 50-year-old male with MSS CRC that showed a persistent positive circulating tumor DNA (ctDNA) assay despite no radiological evidence of disease after systemic treatment. Given the suspicion of recurrence, the tumor specimen was evaluated with the FoundationOne assay, which revealed a high TMB of 295 mut/Mb, secondary to POLE mutation. A near-complete response was quickly observed after weekly treatment with 400 mg of pembrolizumab (Lutfi et al., 2023).

The basis of TMB's utility as a predictive biomarker is the ability to stratify patients according to their mutational status: TMB-high or TMB-low. However, the threshold at which a tumor is defined as "TMB-high" has varied across studies, cancer types, assays used, and even pharmacological agents (Klempner et al., 2020; McNamara et al., 2020). In a predetermined exploratory analysis of the KEYNOTE-158 clinical trial (NCT02628067), a high TMB—defined in the study as TMB $\geq$ 10 mutations per megabase of genomic material—was associated with a better response to pembrolizumab regardless of MSI status or PD-L1 expression by the tumor (ClinicalTrials.gov, n.d.; Marabelle et al., 2020). The results of this phase II trial led to the approval of pembrolizumab for advanced solid tumors with TMB-high, defined by the same cut-off, by the FDA (Graham et al., 2021; Marabelle et al., 2020). Importantly, no patients with CRC (or many other frequently diagnosed cancers) were included in this analysis. Although the predictive potential of TMB has been observed in other studies, different thresholds for high-TMB may be more suitable considering that ~10 mutations per megabase corresponds to the average mutation density in CRC (Graham et al., 2021; Zheng et al., 2022).

3.3.1. The current landscape: an overview of TMB methodology in CRC studies

We sought to understand what methods and thresholds for TMB assessment were most often found in the published literature, as well as the rationale behind these choices. To explore common patterns in TMB-based stratification in colorectal cancer, we compiled CRC studies utilizing numerical thresholds for high TMB. We performed a literature search in the Pubmed database for original research articles written in English, using the search strategy ((colorectal cancer OR colon cancer OR colorectal adenocarcinoma OR colon adenocarcinoma) AND (tumor mutational burden OR tumor mutational load OR tumor mutation burden OR tumor mutation load) AND (checkpoint inhibitor OR PD-1 OR PD-L1 OR CTLA-4 OR ipilimumab OR pembrolizumab OR nivolumab)) NOT (Review[Publication Type]). This search yielded 295 results, and the titles and abstracts were screened for relevance. The remaining 95 publications underwent full-text screening: we removed all comments and editorials, animal studies, case reports, and articles where the full text was unavailable. We also excluded articles where the cut-off for high-TMB was entirely data-driven (*e.g.*, median, upper 20%, higher tertile) and no numerical cut-offs were provided. We subsequently reviewed the reference lists of the identified papers to capture any relevant publications we missed in the electronic search. Ultimately, we included 45 original research articles, which were published from 2017 to 2023 and include data from over 120 thousand patients (Table 2). Data collection ended on 25 August 2023.

Most of the 45 original articles included in this review determined TMB through next-generation sequencing of tumor tissue samples. Across these studies, a total of 12 different NGS panels were used. The FoundationOne CDx assay was by far the most frequently used panel, corresponding to over a third of all NGS panels mentioned. In the studies included, information regarding the details of TMB assessment and definition was often unclear or entirely absent, which confirmed the lack of standardization and clarity mentioned by other authors. Even in the cases where all the data elements were provided, the level of detail varied greatly, ranging from simple mentions of *"number of non-synonymous mutations"* to more comprehensive descriptions, such as *"number of somatic synonymous and non-synonymous mutations, stop gain/loss and splicing variants and frameshift and non-frameshift insertions and deletions"*. Despite these disparities, the methodologies themselves were generally similar, and the observed variations may be due to the panel selected. Additionally, only 8 studies (evident in the second column of Table 2) specified whether the analysis was carried out on MSI-H or MSS tumors, critical information which undoubtedly affects the results (Chida et al., 2021; Crisafulli et al., 2022; Klemen et al., 2022; Manca et al., 2023; Salem et al., 2020; Schrock et al., 2019; Valero et al., 2021; Voutsadakis, 2023).

Similar to the findings reported by Li *et al.* (Y. Li et al., 2021), our compilation suggests that the cut-off used to define a "high" TMB is the main source of variation between the different studies. So, we aimed to explore the rationale behind the selection of these thresholds, particularly the most common ones. In the studies included in our review, TMB cut-offs ranged from 6.88 mut/Mb to 41 mut/Mb and were most often set at 10 mut/Mb (29.2%), 17 mut/Mb (12.5%), or 20 mut/Mb (18.8%).

3.3.1.1. Cut-off set at 10 mutations/megabase. Although this rationale was not explicit in most studies, it is reasonable to assume that most thresholds set at 10 mut/Mb were so defined based on the results of the KEYNOTE-158 trial and subsequent FDA approval; This was the case of Moretto et al., for example (Moretto et al., 2022). Budczies and colleagues also suggested 10 mut/Mb as an appropriate cut-off in colorectal adenocarcinoma, as it adequately separated tumors with MSI-H and/or POLE/POLD1 mutations from those with an MSS phenotype. In this

Table 2
Selected original research articles with a numerical threshold for high-TMB in colorectal cancer or multiple cancer types.

Author, Year of Publication (Ref.)	Cancer Type (no. Patients)	ICI Agent	TMB Definition	Methodology	TMB-H Cut-off
(Chida et al., 2021)	MSI-H or dMMR advanced gastrointestinal cancers (45 total; 17 CRC)	Pembrolizumab, nivolumab	Number of nonsynonymous mutations, including indels, per Mb	FFPE tissue specimens: WES or NGS panels (Oncomine Cancer Research, FoundationOne)	10 mut/Mb
(Xiao et al., 2021)	CRC (430 total)	Not applicable	Number of somatic SNVs (synonymous and non-synonymous mutations, stop gain/loss and splicing variants) and indel variants (frameshift and non-frameshift insertions and deletions), per Mb. Non-coding alterations were excluded	FFPE tissue samples: MasterView NGS panel (381 genes)	29 mut/Mb
(Lin et al., 2020)	CRC (110 total)	Anti-PD1/PDL1 monotherapy or in combination with anti-CTLA-4	Number of replacement and indels per Mb in the exon coding region	Data obtained from the MSK-IMPACT cohort (MSK-IMPACT NGS panel) and TCGA database	11 mut/Mb
(Sibilio et al., 2022)	CRC (520 total)	Not applicable	Number of nonsynonymous mutations per 30 Mb (average size of the exome)	Data obtained from the TCGA database: WES (30 Mb)	20 mut/Mb
(Chalmers et al., 2017)	Multiple cancer types (92 439 total, 7758 CRC)	Not applicable	Number of somatic, coding, base substitution and indel mutations (including synonymous alterations), excluding non-coding alterations, per Mb	FFPE clinical cancer specimens: WES and FoundationOne NGS panel (315 genes across 1.1 Mb)	20 mut/Mb
(Parikh et al., 2019)	Tubular gastrointestinal cancers (17 486 total, 12 569 CRC)	Not applicable	Number of somatic base substitutions or indel alterations, excluding known somatic and deleterious mutations, per Mb	FFPE tumor tissue: FoundationOne NGS panel (255 across 0.83 Mb or 343 genes across 1.14 Mb, depending on assay version)	20 mut/Mb
(Fabrizio et al., 2018)	CRC (6 004 total)	Not applicable	Number of synonymous and nonsynonymous mutations, with computational germline status filtering, per Mb	FFPE tumor tissue: FoundationOne NGS panel (exons of 185, 236, 315 or 405 genes and select introns of 19, 28 or 31 genes across 1.11 Mb, depending on assay version)	12 mut/Mb (11.7 mut/Mb)
(Lee et al., 2021)	mCRC (994 total)	Not applicable	Not specified	FFPE tumor tissue (blood when tissue unavailable): FIRST NGS Panel (183 genes), K-MASTER NGS panel (435 genes)	13 mut/Mbp (FIRST panel) 16 mut/Mbp (K-MASTER panel)
(Kim et al., 2021)	Multiple advanced cancer types (501 total, 151 CRC)	Not specified (65 patients received ICIs)	Not specified	FFPE: TruSight Oncology 500 NGS panel (500 genes)	10 mut/Mb
(Quintanilha et al., 2023)	mCRC (320 total)	Pembrolizumab, nivolumab, nivolumab/ipilimumab	Not specified	Tumor specimens: FoundationOne NGS panel (1.1 Mb)	10 mut/Mb
(Chen et al., 2020)	Advanced CRC (180 total)	Durvalumab/tremelimumab	Number of somatic synonymous and nonsynonymous SNVs and indels, excluding germline, clonal hematopoiesis of indeterminate potential (CHIP), driver and resistance variations	Plasma: GuardantOMNI NGS panel (500 genes across 2.15 Mb)	28 vts/Mb
(Zhou et al., 2021)	Multiple cancer types (309 total, 78 CRC)	Nivolumab, atezolizumab, pembrolizumab, ipilimumab, toripalimab	Number of somatic nonsynonymous mutations (including SNVs and indels in the coding regions and splicing sites) in the tumor exome, per Mb	FFPE tumor samples: WES (35 Mb)	10 mut/Mb, top tertile
(Lou et al., 2021)	CRC (17 909 total)	Not applicable	Number of nonsynonymous missense mutations, excluding those previously described as germline alterations	FFPE tumor samples: NGS using the NextSeq platform (592 genes across 1.4 Mb)	17 mut/Mb
(Kitsou et al., 2020)	CRC (453 total)	Not applicable	Not specified	Data obtained from the TCGA database: NGS	34 mut/Mb
(Voutsadakis, 2023)	MSS CRC (2 186 total)	Not applicable	Not specified / May depend on the cohort	Data obtained from the cBioPortal MSK cohorts: MSK-IMPACT NGS panel (341 or 468 genes, depending on assay version) TCGA and DFCI cohorts: WES	10 mut/Mb
(Jung et al., 2023)	Multiple solid cancer types (1 744 total, 547 CRC)	Anti-PD-(L)1, not specified	Number of non-synonymous and synonymous SNVs and indels in the coding region with a variant frequency ≥5%, excluding the variants with an observed allele count ≥10 in any of the GnomAD exome, genome, or 1000 genome databases	FFPE tissue blocks: TruSight Oncology 500 NGS panel	10 mut/Mb (TMB intermediate high) 16 mut/Mb (TMB very high)
(Shimada et al., 2021)	CRC (201 total)	Not applicable	Number of nonsynonymous mutations, per Mb	FFPE samples: NGS panel (1.3 Mb)	27 mut/Mb

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Table 2 (*continued*)

Author, Year of Publication (Ref.)	Cancer Type (no. Patients)	ICI Agent	TMB Definition	Methodology	TMB-H Cut-off
(Moretto et al., 2022)	CRC (9 321 total)	Not applicable	Number of nonsynonymous missense, nonsense, inframe insertion and/or deletion, and frameshift mutations, excluding those previously described as germline alterations in dbSNP151, Genome Aggregation Database, or benign variants identified by Caris geneticists	FFPE tumor samples: Caris MI TumorSeek NGS panel (592 genes), whole transcriptome sequencing	10 mut/Mb
(Xu and Zheng, 2021)	CRC (457 total)	Not applicable	Number of somatic mutations, per Mb	Data obtained from the TCGA database	10 mut/Mb
(R. K. Yang et al., 2020)	Multiple advanced solid cancer types (554 total, 87 CRC)	Ipilimumab, tremelimumab, nivolumab, pembrolizumab, Cemiplimab, atezolizumab, durvalumab	Number of reported somatic mutations (including SNV and indels), excluding intronic or silent mutations, and germline SNPs	FFPE tumor tissue: NGS panel (409 genes across 1.745 Mb)	12 reported mutations (6.88 mut/Mb)
(Zhao et al., 2021)	CRC (382 total)	Not applicable	Number of somatic truncating (nonsense, frameshift indels and splice-site mutations) and non-truncating (missense, in-frame indels) mutations, per Mb	Data obtained from the TCGA database: WES	20 mut/Mb
(Bortolomeazzi et al., 2021)	mCRC (29 total)	Pembrolizumab, nivolumab, nivolumab/ipilimumab	Unclear / Not specified	FFPE tumor blocks: WES	12 mut/Mbp
(Puccini et al., 2019)	CRC (1 126 total)	Not applicable	Number of somatic nonsynonymous missense mutations, excluding those previously described as germline alterations	FFPE tumor samples: NGS using the NextSeq platform (592 genes, across 1.4 Mb)	17 mut/Mb
(Budczies et al., 2018)	Multiple cancer types (5 722 total, CRC not specified)	Not applicable	Number of nonsilent mutations (nonsynonymous mutations in exonic regions and splice sites), per Mb	Data obtained from the cBioPortal and TCGA database	10 mut/Mb
(Pham et al., 2021)	Multiple advanced cancer types (225 total, 29 CRC)	Not specified (55 patients received immunotherapy)	Number of mutations, excluding germline and characterized oncogenic alterations	FFPE tumor samples: FoundationOne NGS panel (182, 236, 315, or 406 genes, depending on the time period, across 1.2 Mb)	20 mut/Mb
(Zaidi et al., 2020)	CRC (2 105 total)	Not applicable	Number of somatic, non-silent, point mutations	FFPE tumor samples: AmpliSeq NGS panel (205 genes)	17 mut/Mb
(Salem et al., 2018)	Gastrointestinal cancers (4 125 total, 1 556 CRC)	Not applicable	Number of somatic nonsynonymous missense mutations, excluding those previously described as germline alterations	FFPE tumor specimens: NGS using the NextSeq platform (592 genes across 1.4 Mb)	17 mut/Mb
(Klemen et al., 2022)	MSI-H or TMB-H mCRC (111 total)	Nivolumab, pembrolizumab, durvalumab	Unclear / Not specified	Tumor tissue and blood: MSK-IMPACT NGS panel (>400 genes)	25 mut/Mb
(Huang et al., 2021)	CRC (27 total)	Not applicable	Number of somatic mutations (including coding SNVs and indels), excluding driver mutations and germline alternations previously described in dbSNP, per Mb	FFPE tissue samples: Cancer Sequencing YS NGS panel (coding exons from 578 genes and 47 selected introns, across 1.25 Mb)	10 mut/Mb
(Goodman et al., 2019)	Multiple cancer types (60 total, 0 CRC)	Multiple agents, not specified	Number of somatic mutations, excluding alterations known to be oncogenic drivers	Tumor samples: FoundationOne NGS panel (182, 236, 315, 327, or 405 genes, depending on the time period, across 1.2 Mb)	20 mut/Mb
(Xue et al., 2021)	CRC (323 total)	Not applicable	Not specified	Data obtained from the TCGA database	10 mut/Mb
(Sicklick et al., 2021)	Multiple advanced cancer types (76 total, 7 CRC)	Not specified	Not specified	Tumor tissue: NGS (236–405 genes) ctDNA: NGS (62 genes)	20 mut/Mb
(Tokunaga et al., 2019)	Appendiceal (224 total) and CRC (4 600 total)	Not applicable	Number of non-synonymous missense mutations, excluding those previously described as germline alterations, per Mb	FFPE samples: Caris NGS panel (592 genes across 1.4 Mb) and NextSeq platform	17 mut/Mb
(Cristescu et al., 2018)	Multiple advanced cancer types (315 total, 5 CRC)	Pembrolizumab	Number of somatic nonsynonymous SNVs in protein coding regions	FFPE pretreatment samples: WES	102.5 mut/exome
(Yarchoan et al., 2019)	Multiple cancer types (9 887 total, CRC not specified)	PD-1/PD-L1 inhibitors	Number of base substitutions, short indels, copy number alterations and gene fusions/rearrangements, excluding oncogenic driver events and germline mutations recorded in the COSMIC, dbSNP and ExAC databases, per Mb	FFPE clinical cancer specimens: FoundationOne NGS panel (up to 405 genes)	10 mut/Mb
(Cho et al., 2021)	Multiple cancer types (588 total, 143 CRC)	Not applicable	Number of clinically relevant classes of genomic alterations (including SNVs, small indels, CNVs and rearrangements/fusions), excluding any variant with an observed allele count ≥10 in any of the GnomAD	FFPE tumor samples: TruSight Oncology 500 NGS panel (>500 genes, 1.28 Mbps), validated against WES (35.9 Mbps)	10 mut/Mb

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Table 2 (*continued*)

Author, Year of Publication (Ref.)	Cancer Type (no. Patients)	ICI Agent	TMB Definition	Methodology	TMB-H Cut-off
			exome, genome, and 1000 genomes databases		
(Hoshino et al., 2022)	Liver-metastatic CRC (24 total)	Not applicable	Number of non-synonymous SNVs and short indels (with allele frequencies of 10% or greater), per Mb	FFPE tumor blocks: Oncomine NGS panel (exon region of 409 genes across 1.2 Mb)	10 mut/Mb
(Salem et al., 2020)	Multiple MSI-H cancer types (1 057 total; 283 CRC)	Not applicable	Number of nonsynonymous missense mutations, excluding those previously described as germline alterations in gnomAD, dbSNP and in house Caris databases	FFPE tumor samples: NGS using the NextSeq platform (592 genes across 1.4 Mb)	17 mut/Mb
(Middha et al., 2017)	Multiple solid cancer types (12 288 total, 1 030 CRC)	Not applicable	Number of reported mutations (somatic mutations, structural variants, and copy number alterations) in all coding regions and select introns, divided by the evaluated genomic area	Tumor samples: MSK-IMPACT NGS panel (341, 410 or 468 genes, depending on assay version)	12 mut/Mb (11.85 mut/Mb)
(Valero et al., 2021)	Multiple MSS solid cancer types (1 678 total, 50 CRC)	Anti-PD(L)1 monotherapy or combination therapy	Number of somatic, nonsynonymous mutations, per Mb	Tumor samples: MSK-IMPACT NGS panel	10 mut/Mb (universal) 8 mut/Mb (8.35 mut/Mb; CRC)
(Crisafulli et al., 2022)	MSS RAS-mutated mCRC (21 total)	Pembrolizumab	Tumor tissue biopsies: Unclear / Not specified ctDNA: Number of somatic synonymous and nonsynonymous SNVs and indels	Tumor tissue biopsies: high-depth WES ctDNA: GuardantOMNI NGS panel (2.145 Mb)	20 mut/Mb
(Zheng, 2022)	Multiple cancer types (1 764 total, CRC not specified)	Not specified	Not specified	NGS panel	13 mut/Mb (high TMB cancer types) 12 mut/Mb (CUP)
(Goodman et al., 2017)	Multiple advanced cancer types (151 total, 5 CRC)	Anti-PD-(L)1, anti-CTLA-4, combination anti-CTLA-4/ anti-PD-(L)1	Number of somatic mutations, excluding those likely or known to be oncogenic drivers and germline polymorphisms, per Mb	FFPE tumor samples: FoundationOne NGS panel (182, 236 or 315 genes depending on the time period, across 1.2 Mb)	20 mut/Mb
(Schrock et al., 2019)	MSI-H mCRC (22 total)	PD-1 or PD-L1 inhibitors (pembrolizumab, nivolumab, nivolumab/ipilimumab, durvalumab/tremelimumab)	Number of synonymous and nonsynonymous mutations	FFPE tissue sections: Foundation Medicine NGS panel (0.8—1.2 Mb region)	37—41 mut/Mb
(Manca et al., 2023)	dMMR/MSI-H mCRC (110 total)	Anti-PD-(L)1 monotherapy, anti-CTLA-4 combinations	Number of mutations per coding area of the genome	Pre-treatment FFPE tumor samples: FoundationOne NGS panel	23 mut/Mb (prognosis) 40 mut/Mb (treatment effects)

TMB, tumor mutational burden; ICI, immune checkpoint inhibitor; TMB-H, high tumor mutational burden; MSI-H, high microsatellite instability; dMMR, mismatch repair deficient; CRC, colorectal cancer; indel, insertion and deletion; Mb, megabase; FFPE, formalin-fixed paraffin-embedded; WES, whole exome sequencing; NGS, next-generation sequencing; mut/Mb, mutations/megabase; SNV, single nucleotide variant; PD-1, programmed cell death-1; PD-L1, programmed cell death-1 ligand; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; TCGA, the cancer genome atlas; mCRC, metastatic colorectal cancer; Mbp, megabase pair; CHIP, clonal hematopoiesis of indeterminate potential; vts/Mb, variants/megabase; GnomAD, genome aggregation database; dbSNP, single nucleotide polymorphism database; ctDNA, circulating tumor DNA; COSMIC, catalogue of somatic mutations in cancer; EXAC, exome aggregation consortium; CNV, copy number variation; MSS, microsatellite-stable; RAS, rat sarcoma virus; CUP, cancer of unknown primary.

study, PD-L1 was also significantly overexpressed in hypermutated tumors as defined by this threshold (Budczies et al., 2018). This was also the cut-off recommended by the FoCR TMB Harmonization Project in a pan-cancer context (Research, 2021).

3.3.1.2. Cut-off set at 17 mutations/megabase. Six out of forty-five studies included in this review categorized tumors as hypermutated when the TMB surpassed the cut-point of 17 mut/Mb. Four of those (three by the same team) based this decision on the fragment analysis of CRC cases and subsequent comparison of TMB and MSI (Puccini et al., 2019; Salem et al., 2020, 2018; Tokunaga et al., 2019). Zaidi and colleagues reached the same conclusion, although by a different method: the group observed that 23 point mutations per sample—equivalent to 17 mut/Mb—was the minimum value between the two distinct peaks observed when plotting all the included CRC samples (Zaidi et al., 2020).

3.3.1.3. Cut-off set at 20 mutations/megabase. In the ARETHUSA trial (NCT03519412), the cut-off for high-TMB was set at 20 mut/Mb, double the TMB required for standard-of-care use of pembrolizumab (ClinicalTrials.gov, n.d.; Crisafulli et al., 2022). This was also the cut-point established by Foundation Medicine (the company behind the FoundationOne CDx assay) and, consequently, Goodman (Goodman et al., 2019, 2017) and Sicklick *et al.* (Sicklick et al., 2021). According to Foundation Medicine, tumors with ≥20 mut/Mb are TMB-high, ≤5 mut/Mb are TMB-low, and the remaining tumors are considered TMB-intermediate. Interestingly, six studies used the FoundationOne panel but did not follow the cut-offs prespecified by the manufacturer: three studies set this cut-point at 10 mut/Mb, one at 12 mut/Mb (Fabrizio et al., 2018), one at 37–41 mut/Mb (Schrock et al., 2019), and one study defined it as 23 or 40 mut/Mb, depending on the desired endpoint (Manca et al., 2023).

Because so many studies either adopted their TMB-H cut-offs from previously published research (42%) or provided no explanation for their choice in threshold (20%), we decided to conduct an overview of reviews on this matter; In addition to the 45 original articles, the same search strategy yielded six scoping reviews, systematic reviews, and meta-analyses. The full texts were screened for relevance, and only two

studied cut-off values for high TMB. The main findings from the included meta-analyses are summarized in Table 3.

In a 2020 meta-analysis of multiple cancer types (Table 3), a higher TMB was associated with reduced mortality and disease progression and higher response rates. These patients were also found to respond better to ICIs rather than to conventional chemotherapy. In this meta-analysis, Yang and colleagues used the Contal and O'Quigley method to estimate the best cut-point for high TMB in several cancers based on the results from studies published until 1 October 2019. Although this cut-off varied across cancer types, a threshold of 12.3 mut/Mb was found to be the best discriminator in colorectal cancer (Z. Yang et al., 2020). This result is consistent with three of the studies we included in this review: Bortolomeazzi *et al.* (12 mut/Mb) defined high TMB based on the lower TMB observed in hypermutated CRC samples in a 2012 study by The Cancer Genome Atlas (Bortolomeazzi et al., 2021), Middha *et al.* (11.85 mut/Mb) calculated the cut-off based on the interquartile range and median TMB (Middha et al., 2017), and Fabrizio *et al.* (11.7 mut/Mb) classified patients based on a Weibull distribution of the TMB values observed within the MSI-H tumors evaluated (Fabrizio et al., 2018). Although the methodologies used to determine the cut-point were different, these authors reached a similar value of ~12 mutations per megabase. In the study by Fabrizio and colleagues, (which sequenced the tumors of over 6000 CRC patients with the FoundationOne assay) not only did this threshold capture 99.7% of MSI-H patients, it also identified 3% of the MSS samples as being TMB-H, increasing the pool of patients who might be eligible for treatment with ICIs by 54% (Fabrizio et al., 2018).

One year later, a systematic review and meta-analysis by Li and colleagues (Table 3) evaluated studies published until 1 May 2021 and identified cut-off choice as one of the key problems of TMB, as it contributed substantially to the heterogeneity of the results (Y. Li et al., 2021). A subgroup analysis based on the TMB cut-off revealed that TMB-H CRC patients respond better to ICIs when the adopted threshold is lower than 28 mut/Mb (Y. Li et al., 2021). Although this meta-analysis did not provide a single numerical cut-off as the optimal threshold for high TMB, it complements the results obtained by Yang and colleagues: 12 mut/Mb is, indeed, lower than 28 mut/Mb.

Most of the included studies adhered to this premise (even if unwittingly), but some studies identified thresholds well above 28 mutations per megabase. For example, in a landmark study of 22 patients with MSI-H mCRC, Schrock and colleagues placed the optimal TMB cut-off somewhere between 37 and 41 mut/Mb—which roughly corresponded to the 35th percentile of MSI-H mCRC cases sorted by TMB in the Foundation Medicine database—after applying log-rank statistics to progression-free survival data (Schrock et al., 2019). Kitsou *et al.* used iCoMut Beta for FireBrowse to categorize tumors into low or high TMB, according to a cut-point of 34 mut/Mb (Kitsou et al., 2020), and Xiao *et al.* set this threshold at 29 mut/Mb based on the optimal Youden index obtained in a Chinese cohort (Xiao et al., 2021). Interestingly, Chen and colleagues found 28 variants per megabase to be the optimal threshold in patients treated with durvalumab plus tremelimumab (Chen et al., 2020) but did not define what a "variant" actually entails.

On the other end of the spectrum, two studies defined TMB-H cut-offs below 10 mutations per megabase, which is seen as the current "standard". In a pan-cancer study, Yang *et al.* determined the optimal cut point for the ICI-treated group through recursive partitioning and tree methods, ultimately reaching a value of 12 reported mutations, or 6.88 mut/Mb (R. K. Yang et al., 2020). An exploratory analysis by Valero and colleagues defined different optimized cut-offs per cancer type according to the Youden method and reached a value of 8.35 mut/Mb for CRC. Importantly, this information is only available in the Supplementary Material (Table 2), and the authors recognize in-text that the cut-off has not been adequately validated and should not be considered definitive (Valero et al., 2021).

This manuscript highlights the inconsistencies prevalent in TMB assessment and reporting, which impact multiple aspects reflected in the data collected in Table 2: we observed divergent TMB definitions (*i.e.,* differing interpretations of which mutations are considered when quantifying TMB), inconsistent disclosure of specific methodological details (*e.g.,* types of samples and testing procedures used) and, of course, a wide range of cut-offs for high TMB. Discrepancies were identified across all these categories, which collectively emphasize the substantial lack of methodological harmony within the field. For example, the absence of a single TMB threshold can confuse clinicians, hindering the decision-making process. Furthermore, the wide variability observed contributes to the idea that these cut-offs are arbitrary and that the evidence is inconsistent and untrustworthy (Prasad and Addeo, 2020; Zheng, 2022). The recommendations proposed by the FoCR Harmonization Project will ultimately help identify the optimal cut-off for each situation (Stenzinger et al., 2019). Until adequate standards for TMB reporting are established, we urge all researchers on this topic to adapt the terminology used and to provide all possible details regarding the methods and rationale employed, to facilitate interdisciplinary dialogue and understanding.

4. Critical view and perspectives

Colorectal cancer is the seventh most mutated neoplasm among the 30 most common cancer types (Zheng et al., 2022), but important mechanisms of hypermutation, like POLE mutation or ARID1A loss of

Table 3
Meta-analyses evaluating optimal TMB-H cut-offs for immunotherapy response.

Title	No. Studies (No. Patients)	Cancer Type	Optimal cut-off for TMB-high	Main Findings	Author, Year of Publication (Ref.)
Clinical significance of tumour mutation burden in immunotherapy across multiple cancer types: an individual meta-analysis	22 (6171)	Multiple cancer types	CRC: 12 mut/Mb (12.3 mut/Mb)	• TMB is associated with better overall survival and a promising predictor of ICI therapy, but the cut-off value differs across cancer types. • The best cut-off value in CRC was 12.3 mut/Mb.	(Z. Yang et al., 2020)
Tumor Mutational Burden Predicting the Efficacy of Immune Checkpoint Inhibitors in Colorectal Cancer: A Systematic Review and Meta-Analysis	13 (5062)	CRC	< 28 mut/Mb	• The TMB-high patient group had longer overall survival and superior ORR compared to the TMB-low patient group. • TMB can be used as a putative predictive biomarker of CRC patients receiving ICIs, but this finding isn't stable enough. • When the cut-off value is < 28 mut/Mb, patients with high TMB respond better to ICIs than when the cut-off is > 28 mut/Mb.	(Y. Li et al., 2021)

TMB-H, high tumor mutational burden; CRC, colorectal cancer; mut/Mb, mutations/megabase; TMB, tumor mutational burden; ICI, immune checkpoint inhibitor; ORR, objective response rate.

function, are not detected by standard-of-care testing (Argilés et al., 2020; Cervantes et al., 2022; Network, 2023). TMB represents a hopeful way forward, but its implementation into the cancer care continuum has faced meaningful technical and methodological challenges. This is not unfamiliar territory: the validation of PD-L1 encountered similar setbacks, which emphasized the need for harmonization in biomarker testing (Chan et al., 2019; Wang et al., 2016).

Importantly, the purpose of this review is not to present TMB as the definitive solution for this puzzle—in fact, the limitations of TMB as a predictive biomarker are highlighted when it is used in isolation (Jardim et al., 2021; Rousseau et al., 2021). Some researchers have suggested the implementation of a cancer immunogram to guide therapeutic decisions: Blank and colleagues postulated that a variety of immune-related aspects, such as the presence of inhibitory checkpoints, infiltration of immune cells, tumor mutational load, lymphocyte count, and MHC expression should all be considered when choosing the appropriate immunotherapeutic agent (Blank et al., 2016). Other emergent subclassifications of CRC are the four consensus molecular subtypes (CMS) and the Immunoscore, which relies on the quantification of different lymphocyte populations in both the tumor core and margin (J. Li et al., 2021; Picard et al., 2020). The potential of TMB as a response biomarker lies in its ability to extend the use of ICIs to additional subpopulations of patients—rather than substituting existing biomarkers, TMB is meant to complement them. For example, in advanced NSCLC, TMB and PD-L1 expression were found to be independent of each other (Hellmann et al., 2018). The standardization of TMB assessment and reporting represents an important opportunity to identify further therapeutic possibilities in CRC.

Although current evidence seems promising, it is not yet sufficient to confidently determine the predictive value of TMB in colorectal cancer. Additional, high-quality information is required before it can be introduced into routine clinical practice. Critical aspects need to be thoroughly considered, such as the evolution of a patient's TMB over time, the need to establish adequate standards for determining the optimal TMB cut-off in each tumor, and the existence of a linear relationship between sample TMB and ICI response. Additionally, ICIs are often used in combination with other treatment modalities, such as radiation or chemotherapy. Certain treatments have been shown to affect a patient's TMB status (Crisafulli et al., 2022; Parikh et al., 2021; Wang et al., 2020), but whether this variation has any clinical significance is also unclear. The utility of TMB and other immune-related biomarkers must likewise be validated in this setting to better guide physicians on how to sequence or combine ICIs with other therapies.

To our knowledge, this is the first comprehensive analysis and critical summary of TMB use in colorectal cancer, specifically. We performed a structured search for original studies utilizing numerical thresholds for high TMB, with no restrictions regarding the year of publication or assessment methodologies used (Yang and colleagues, for example, excluded studies where TMB was calculated from ctDNA or blood (Z. Yang et al., 2020)). Furthermore, we explored the rationale behind the different cut-offs chosen and even consulted different meta-analyses on the subject. Interestingly, only 13 of the 45 studies included in our compilation calculated their own TMB-H threshold. None of these studies ultimately aligned with either of the most common cut-offs, raising an important question: are these thresholds being perpetuated out of scientific convenience rather than rigor? While acknowledging the limitations of a non-systematic review and the reliance on a single database (Pubmed), this manuscript effectively provides an extensive overview of the current landscape of TMB assessment in colorectal cancer. As these methodologies, particularly thresholds, may need to be adapted for different histologies and medicines, this manuscript provides a foundational resource for CRC researchers, successfully filling a gap.

The wide variation observed in the compiled studies highlights the lack of harmonization when it pertains to TMB assessment; Besides the disparate methodological details, different authors may even act according to contrasting schools of thought: while some authors have recommended the definition of a single cut-off for high-TMB (Jardim et al., 2021), some defend that the appropriate threshold should depend on the cancer type (Samstein et al., 2019). The TMB Harmonization Project's work will be critical to further establish and validate TMB as a predictive marker of ICI response and demonstrates the importance of multi-stakeholder involvement. While it is unlikely that TMB alone will comprehensively identify all potential candidates for immunotherapy, it is now clear that as these methods become more robust and the evidence more conclusive, TMB should be included in the diagnostic and staging workup of patients with CRC. Placing tumors on a TMB spectrum will be an important step forward in the sphere of precision oncology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

(PMDA) Pharmaceuticals and Medical Devices Agency, J.P. and M.D.A., 2020. Keytruda Review Report.

Adashek, J.J., Kato, S., Ferrara, R., Lo Russo, G., Kurzrock, R., 2020. Hyperprogression and Immune Checkpoint Inhibitors: hype or progress? Oncologist 25, 94–98. https://doi.org/10.1634/theoncologist.2019-0636.

Afrăsânie, V.A., Marinca, M.V., Alexa-Stratulat, T., Gafton, B., Păduraru, M., Adavidoaiei, A.M., Miron, L., Rusu, C., 2019. KRAS, NRAS, BRAF, HER2 and microsatellite instability in metastatic colorectal cancer-practical implications for the clinician. Radiol. Oncol. 53, 265–274. https://doi.org/10.2478/raon-2019-0033.

Aleksakhina, S.N., Imyanitov, E.N., 2021. Cancer therapy guided by mutation tests: current status and perspectives. Int. J. Mol. Sci. 22, 10931. https://doi.org/10.3390/ijms222010931.

Almquist, D.R., Ahn, D.H., Bekaii-Saab, T.S., 2020. The role of immune checkpoint inhibitors in colorectal adenocarcinoma. BioDrugs 34, 349–362. https://doi.org/10.1007/s40259-020-00420-3.

Andre, T., Elez, E., Van Cutsem, E., Jensen, L.H., Bennouna, J., Mendez, G., Schenker, M., De La Fouchardiere, C., Limon, M.L., Yoshino, T., Li, J., Lenz, H.-J., Manzano Mozo, J.L., Tortora, G., Garcia-Carbonero, R., Cela, E., Yang, Y., Lei, M., Jin, L., Lonardi, S., 2024. Nivolumab (NIVO) plus ipilimumab (IPI) vs chemotherapy (chemo) as first-line (1L) treatment for microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC): First results of the CheckMate 8HW study. LBA768–LBA768 J. Clin. Oncol. 42. https://doi.org/10.1200/JCO.2024.42.3_suppl.LBA768.

Argilés, G., Tabernero, J., Labianca, R., Hochhauser, D., Salazar, R., Iveson, T., Laurent-Puig, P., Quirke, P., Yoshino, T., Taieb, J., Martinelli, E., Arnold, D., ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org, 2020. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann. Oncol. . J. Eur. Soc. Med. Oncol. 31, 1291–1305. https://doi.org/10.1016/j.annonc.2020.06.022.

Blank, C.U., Haanen, J.B., Ribas, A., Schumacher, T.N., 2016. The "cancer immunogram". Sci. (80-.) 352, 658–660. https://doi.org/10.1126/science.aaf2834.

Borelli, B., Antoniotti, C., Carullo, M., Germani, M.M., Conca, V., Masi, G., 2022. Immune-checkpoint inhibitors (ICIs) in metastatic colorectal cancer (mCRC) patients beyond microsatellite instability. Cancers (Basel) 14, 4974. https://doi.org/10.3390/cancers14204974.

Bortolomeazzi, M., Keddar, M.R., Montorsi, L., Acha-Sagredo, A., Benedetti, L., Temelkovski, D., Choi, S., Petrov, N., Todd, K., Wai, P., Kohl, J., Denner, T., Nye, E., Goldstone, R., Ward, S., Wilson, G.A., Al Bakir, M., Swanton, C., John, S., Miles, J., Larijani, B., Kunene, V., Fontana, E., Arkenau, H.T., Parker, P.J., Rodriguez-Justo, M., Shiu, K.K., Spencer, J., Ciccarelli, F.D., 2021. Immunogenomics of colorectal cancer response to checkpoint blockade: analysis of the KEYNOTE 177 trial and validation cohorts. Gastroenterology 161, 1179–1193. https://doi.org/10.1053/j.gastro.2021.06.064.

Brahmer, J.R., Drake, C.G., Wollner, I., Powderly, J.D., Picus, J., Sharfman, W.H., Stankevich, E., Pons, A., Salay, T.M., McMiller, T.L., Gilson, M.M., Wang, C., Selby, M., Taube, J.M., Anders, R., Chen, L., Korman, A.J., Pardoll, D.M., Lowy, I., Topalian, S.L., 2010. Phase I study of single-agent anti–programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J. Clin. Oncol. 28, 3167–3175. https://doi.org/10.1200/JCO.2009.26.7609.

Budczies, J., Seidel, A., Christopoulos, P., Endris, V., Kloor, M., Győrffy, B., Seliger, B., Schirmacher, P., Stenzinger, A., Denkert, C., 2018. Integrated analysis of the

immunological and genetic status in and across cancer types: impact of mutational signatures beyond tumor mutational burden. Oncoimmunology 7. https://doi.org/10.1080/2162402X.2018.1526613.

Cervantes, A., Adam, R., Roselló, S., Arnold, D., Normanno, N., Taïeb, J., Seligmann, J., De Baere, T., Osterlund, P., Yoshino, T., Martinelli, E., 2022. Metastatic colorectal cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. Ann. Oncol. 34, 10–32. https://doi.org/10.1016/j.annonc.2022.10.003.

Chalmers, Z.R., Connelly, C.F., Fabrizio, D., Gay, L., Ali, S.M., Ennis, R., Schrock, A., Campbell, B., Shlien, A., Chmielecki, J., Huang, F., He, Y., Sun, J., Tabori, U., Kennedy, M., Lieber, D.S., Roels, S., White, J., Otto, G.A., Ross, J.S., Garraway, L., Miller, V.A., Stephens, P.J., Frampton, G.M., 2017. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 9, 34. https://doi.org/10.1186/s13073-017-0424-2.

Chan, T.A., Yarchoan, M., Jaffee, E., Swanton, C., Quezada, S.A., Stenzinger, A., Peters, S., 2019. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann. Oncol. 30, 44–56. https://doi.org/10.1093/annonc/mdy495.

Chen, E.X., Jonker, D.J., Loree, J.M., Kennecke, H.F., Berry, S.R., Couture, F., Ahmad, C. E., Goffin, J.R., Kavan, P., Harb, M., Colwell, B., Samimi, S., Samson, B., Abbas, T., Aucoin, N., Aubin, F., Koski, S.L., Wei, A.C., Magoski, N.M., Tu, D., O'Callaghan, C. J., 2020. Effect of combined immune checkpoint inhibition vs best supportive care alone in patients with advanced colorectal cancer: the canadian cancer trials group CO.26 study. JAMA Oncol. 6, 831–838. https://doi.org/10.1001/jamaoncol.2020.0910.

Chida, K., Kawazoe, A., Kawazu, M., Suzuki, T., Nakamura, Y., Nakatsura, T., Kuwata, T., Ueno, T., Kuboki, Y., Kotani, D., Kojima, T., Taniguchi, H., Mano, H., Ikeda, M., Shitara, K., Endo, I., Yoshino, T., 2021. A low tumor mutational burden and PTEN mutations are predictors of a negative response to PD-1 blockade in MSI-H/dMMR gastrointestinal tumors. Clin. Cancer Res. 27, 3714–3724. https://doi.org/10.1158/1078-0432.CCR-21-0401.

Cho, Y.A., Lee, H., Kim, D.G., Kim, H., Ha, S.Y., Choi, Y.La, Jang, K.T., Kim, K.M., 2021. PD-L1 expression is significantly associated with tumor mutation burden and microsatellite instability score. Cancers 13, 1–14. https://doi.org/10.3390/cancers13184659.

ClinicalTrials.gov, n.d. A Study of Nivolumab, Nivolumab Plus Ipilimumab, or Investigator's Choice Chemotherapy for the Treatment of Participants With Deficient Mismatch Repair (dMMR)/Microsatellite Instability High (MSI-H) Metastatic Colorectal Cancer (mCRC) (CheckMate 8HW) (NC [WWW Document]. URL ⟨https://clinicaltrials.gov/study/NCT04008030?term=NCT04008030.&rank=1⟩ (Accessed 29 February 2024b).

ClinicalTrials.gov, n.d. Study of Pembrolizumab (MK-3475) in Participants With Advanced Solid Tumors (MK-3475-158/KEYNOTE-158) [WWW Document]. URL ⟨https://clinicaltrials.gov/study/NCT02628067?term=keynote-158&rank=1⟩.

ClinicalTrials.gov, n.d. Pembrolizumab in MMR-Proficient Metastatic Colorectal Cancer Pharmacologically Primed to Trigger Hypermutation Status (ARETHUSA) (NCT03519412) [WWW Document]. URL ⟨https://clinicaltrials.gov/study/NCT03519412?term=arethusa&rank=1⟩ (Accessed 7 September 2023).

Crisafulli, G., Sartore-Bianchi, A., Lazzari, L., Pietrantonio, F., Amatu, A., Macagno, M., Barault, L., Cassingena, A., Bartolini, A., Luraghi, P., Mauri, G., Battuello, P., Personeni, N., Zampino, M.G., Pessei, V., Vitiello, P.P., Tosi, F., Idotta, L., Morano, F., Valtorta, E., Bonoldi, E., Germano, G., Di Nicolantonio, F., Marsoni, S., Siena, S., Bardelli, A., 2022. Temozolomide Treatment Alters Mismatch Repair and Boosts Mutational Burden in Tumor and Blood of Colorectal Cancer Patients. Cancer Discov. 12, 1656–1675. https://doi.org/10.1158/2159-8290.CD-21-1434.

Cristescu, R., Mogg, R., Ayers, M., Albright, A., Murphy, E., Yearley, J., Sher, X., Liu, X. Q., Lu, H., Nebozhyn, M., Zhang, C., Lunceford, J.K., Joe, A., Cheng, J., Webber, A.L., Ibrahim, N., Plimack, E.R., Ott, P.A., Seiwert, T.Y., Ribas, A., McClanahan, T.K., Tomassini, J.E., Loboda, A., Kaufman, D., 2018. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade–based immunotherapy. Sci. (80-.) 362, 139–148. https://doi.org/10.1126/science.aar3593.

CRUK, C.R.U., n.d. Bowel Cancer Statistics [WWW Document]. Cancer Res. UK. URL ⟨https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer⟩ (accessed 3.1.24).

Dolladille, C., Ederhy, S., Sassier, M., Cautela, J., Thuny, F., Cohen, A.A., Fedrizzi, S., Chrétien, B., Da-Silva, A., Plane, A.-F., Legallois, D., Milliez, P.U., Lelong-Boulouard, V., Alexandre, J., 2020. Immune checkpoint inhibitor rechallenge after immune-related adverse events in patients with cancer. JAMA Oncol. 6, 865. https://doi.org/10.1001/jamaoncol.2020.0726.

Domingo, E., Freeman-Mills, L., Rayner, E., Glaire, M., Briggs, S., Vermeulen, L., Fessler, E., Medema, J.P., Boot, A., Morreau, H., van Wezel, T., Liefers, G.-J., Lothe, R.A., Danielsen, S.A., Sveen, A., Nesbakken, A., Zlobec, I., Lugli, A., Koelzer, V.H., Berger, M.D., Castellví-Bel, S., Muñoz, J., de Bruyn, M., Nijman, H.W., Novelli, M., Lawson, K., Oukrif, D., Frangou, E., Dutton, P., Tejpar, S., Delorenzi, M., Kerr, R., Kerr, D., Tomlinson, I., Church, D.N., 2016. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. Lancet Gastroenterol. Hepatol. 1, 207–216. https://doi.org/10.1016/S2468-1253(16)30014-0.

Done, J.Z., Fang, S.H., 2021. Young-onset colorectal cancer: a review. World J. Gastrointest. Oncol. 13, 856–866. https://doi.org/10.4251/wjgo.v13.i8.856.

European Medicines Agency, 2024. Keytruda: EPAR - Product Information.

European Medicines Agency, 2023a. Yervoy: EPAR - Product Information.

European Medicines Agency, 2023b. Opdivo: EPAR - Product Information.

Fabrizio, D.A., George, T.J., Dunne, R.F., Frampton, G., Sun, J., Gowen, K., Kennedy, M., Greenbowe, J., Schrock, A.B., Hezel, A.F., Ross, J.S., Stephens, P.J., Ali, S.M., Miller, V.A., Fakih, M., Klempner, S.J., 2018. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. J. Gastrointest. Oncol. 9, 610–617. https://doi.org/10.21037/jgo.2018.05.06.

Ferlay, J., Ervik, M., Lam, F., Colombet, M., Mery, L., Piñeros, M., Znaor, A., Soerjomataram, I., Bray, F., 2024. Global Cancer Observatory: Cancer Today [WWW Document]. URL ⟨https://gco.iarc.fr/today⟩ (Accessed 16 February 2024).

Gomar, M., Najafi, M., Aghili, M., Cozzi, S., Jahanbakhshi, A., 2021. Durable complete response to pembrolizumab in microsatellite stable colorectal cancer. DARU, J. Pharm. Sci. 29, 501–506. https://doi.org/10.1007/s40199-021-00404-w.

Gong, J., Robertson, M.D., Kim, E., Fakih, M., Schrock, A.B., Tam, K.W., Burugapalli, B., Monjazeb, A.M., Hendifar, A.E., Hitchins, M., Klempner, S.J., Cho, M., 2019. Efficacy of PD-1 Blockade in Refractory Microsatellite-Stable Colorectal Cancer With High Tumor Mutation Burden. Clin. Colorectal Cancer 18, 307–309. https://doi.org/10.1016/j.clcc.2019.08.001.

Goodman, A.M., Kato, S., Bazhenova, L., Patel, S.P., Frampton, G.M., Miller, V., Stephens, P.J., Daniels, G.A., Kurzrock, R., 2017. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. Mol. Cancer Ther. 16, 2598–2608. https://doi.org/10.1158/1535-7163.MCT-17-0386.

Goodman, A.M., Sokol, E.S., Frampton, G.M., Lippman, S.M., Kurzrock, R., 2019. Microsatellite-Stable Tumors with High Mutational Burden Benefit from Immunotherapy. Cancer Immunol. Res. 7, 1570–1573. https://doi.org/10.1158/2326-6066.CIR-19-0149.

Graham, L.S., Pritchard, C.C., Schweizer, M.T., 2021. Hypermutation, Mismatch Repair Deficiency, and Defining Predictors of Response to Checkpoint Blockade. Clin. Cancer Res. 27, 6662–6665. https://doi.org/10.1158/1078-0432.CCR-21-3031.

Harada, S., Morlote, D., 2020. Molecular Pathology of Colorectal Cancer. Adv. Anat. Pathol. 27, 20–26. https://doi.org/10.1097/PAP.0000000000000247.

Hellmann, M.D., Nathanson, T., Rizvi, H., Creelan, B.C., Sanchez-Vega, F., Ahuja, A., Ni, A., Novik, J.B., Mangarin, L.M.B., Abu-Akeel, M., Liu, C., Sauter, J.L., Rekhtman, N., Chang, E., Callahan, M.K., Chaft, J.E., Voss, M.H., Tenet, M., Li, X.-M., Covello, K., Renninger, A., Vitazka, P., Geese, W.J., Borghaei, H., Rudin, C.M., Antonia, S.J., Swanton, C., Hammerbacher, J., Merghoub, T., McGranahan, N., Snyder, A., Wolchok, J.D., 2018. Genomic Features of Response to Combination Immunotherapy in Patients with Advanced Non-Small-Cell Lung Cancer. Cancer Cell 33, 843–852.e4. https://doi.org/10.1016/j.ccell.2018.03.018.

Hoshino, I., Yokota, H., Iwatate, Y., Mori, Y., Kuwayama, N., Ishige, F., Itami, M., Uno, T., Nakamura, Y., Tatsumi, Y., Shimozato, O., Nagase, H., 2022. Prediction of the differences in tumor mutation burden between primary and metastatic lesions by radiogenomics. Cancer Sci. 113, 229–239. https://doi.org/10.1111/cas.15173.

Huang, J., Liu, H., Zhao, Y., Luo, T., Liu, Jungang, Liu, Junjie, Pan, X., Tang, W., 2021. MicroRNAs Expression Patterns Predict Tumor Mutational Burden in Colorectal Cancer. Front. Oncol. 10, 1–10. https://doi.org/10.3389/fonc.2020.550986.

Huyghe, N., Baldin, P., Van Den Eynde, M., 2020. Immunotherapy with immune checkpoint inhibitors in colorectal cancer: What is the future beyond deficient mismatch-repair tumours? Gastroenterol. Rep. 8, 11–24. https://doi.org/10.1093/gastro/goz061.

Jardim, D.L., Goodman, A., de Melo Gagliato, D., Kurzrock, R., 2021. The challenges of tumor mutational burden as an immunotherapy biomarker. Cancer Cell 39, 154–173. https://doi.org/10.1016/j.ccell.2020.10.001.

Jenkins, R.W., Thummalapalli, R., Carter, J., Cañadas, I., Barbie, D.A., 2018. Molecular and genomic determinants of response to immune checkpoint inhibition in cancer. Annu. Rev. Med. 69, 333–347. https://doi.org/10.1146/annurev-med-060116-022926.

Jung, J., Heo, Y.J., Park, S., 2023. High tumor mutational burden predicts favorable response to anti-PD-(L)1 therapy in patients with solid tumor: a real-world pan-tumor analysis. J. Immunother. Cancer 11, 1–12. https://doi.org/10.1136/jitc-2022-006454.

Kim, H., Hong, J.Y., Lee, J., Park, S.H., Park, J.O., Park, Y.S., Lim, H.Y., Kang, W.K., Kim, K.-M., Kim, S.T., 2021. Clinical sequencing to assess tumor mutational burden as a useful biomarker to immunotherapy in various solid tumors, 175883592199299 Ther. Adv. Med. Oncol. 13. https://doi.org/10.1177/1758835921992992.

Kitsou, M., Ayiomamitis, G.D., Zaravinos, A., 2020. High expression of immune checkpoints is associated with the TIL load, mutation rate and patient survival in colorectal cancer. Int. J. Oncol. 57, 237–248. https://doi.org/10.3892/ijo.2020.5062.

Klemen, N.D., Court, C.M., Fernandes, M.C., Walch, H.S., Chatila, W.K., Saadat, L.V., Maron, S., Crane, C., Shia, J., Cercek, A., Gönen, M., Schultz, N.D., Garcia Aguilar, J., Jarnagin, W.R., D'Angelica, M.I., 2022. Local therapy for oligoprogression or consolidation in high mutational burden stage 4 colorectal cancer treated with PD-1 or PD-L1 blockade. Ann. Surg. Oncol. 29, 8373–8382. https://doi.org/10.1245/s10434-022-12095-x.

Klempner, S.J., Fabrizio, D., Bane, S., Reinhart, M., Peoples, T., Ali, S.M., Sokol, E.S., Frampton, G., Schrock, A.B., Anhorn, R., Reddy, P., 2020. Tumor mutational burden as a predictive biomarker for response to immune checkpoint inhibitors: a review of current evidence. Oncologist 25, e147–e159. https://doi.org/10.1634/theoncologist.2019-0244.

Le, D.T., Uram, J.N., Wang, H., Bartlett, B.R., Kemberling, H., Eyring, A.D., Skora, A.D., Luber, B.S., Azad, N.S., Laheru, D., Biedrzycki, B., Donehower, R.C., Zaheer, A., Fisher, G.A., Crocenzi, T.S., Lee, J.J., Duffy, S.M., Goldberg, R.M., de la Chapelle, A., Koshiji, M., Bhaijee, F., Huebner, T., Hruban, R.H., Wood, L.D., Cuka, N., Pardoll, D. M., Papadopoulos, N., Kinzler, K.W., Zhou, S., Cornish, T.C., Taube, J.M., Anders, R. A., Eshleman, J.R., Vogelstein, B., Diaz, L.A., 2015. PD-1 blockade in tumors with mismatch-repair deficiency. N. Engl. J. Med. 372, 2509–2520. https://doi.org/10.1056/NEJMoa1500596.

Lee, Y., Lee, S., Sung, J.S., Chung, H.J., Lim, A.R., Kim, J.W., Choi, Y.J., Park, K.H., Kim, Y.H., 2021. Clinical application of targeted deep sequencing in metastatic

colorectal cancer patients: actionable genomic alteration in K-MASTER project. Cancer Res. Treat. 53, 123–130. https://doi.org/10.4143/CRT.2020.559.
Li, J., Ma, X., Chakravarti, D., Shalapour, S., DePinho, R.A., 2021. Genetic and biological hallmarks of colorectal cancer. Genes Dev. 38, 787–820. https://doi.org/10.1101/gad.348226.120.
Li, Y., Ma, Y., Wu, Z., Zeng, F., Song, B., Zhang, Y., Li, J., Lui, S., Wu, M., 2021. Tumor mutational burden predicting the efficacy of immune checkpoint inhibitors in colorectal cancer: a systematic review and meta-analysis. Front. Immunol. 12 https://doi.org/10.3389/fimmu.2021.751407.
Lin, A., Zhang, H., Hu, X., Chen, X., Wu, G., Luo, P., Zhang, J., 2020. Age, sex, and specific gene mutations affect the effects of immune checkpoint inhibitors in colorectal cancer. Pharmacol. Res. 159, 105028 https://doi.org/10.1016/j.phrs.2020.105028.
Lipson, E.J., Sharfman, W.H., Drake, C.G., Wollner, I., Taube, J.M., Anders, R.A., Xu, H., Yao, S., Pons, A., Chen, L., Pardoll, D.M., Brahmer, J.R., Topalian, S.L., 2013. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. Clin. Cancer Res. 19, 462–468. https://doi.org/10.1158/1078-0432.CCR-12-2625.
Lizardo, D.Y., Kuang, C., Hao, S., Yu, J., Huang, Y., Zhang, L., 2020. Immunotherapy efficacy on mismatch repair-deficient colorectal cancer: from bench to bedside. Biochim. Biophys. Acta - Rev. Cancer 1874, 188447. https://doi.org/10.1016/j.bbcan.2020.188447.
Lou, E., Xiu, J., Baca, Y., Nelson, A.C., Weinberg, B.A., Beg, M.S., Salem, M.E., Lenz, H.J., Philip, P., El-Deiry, W.S., Korn, W.M., 2021. Expression of immuno-oncologic biomarkers is enriched in colorectal cancers and other solid tumors harboring the a59t variant of kras. Cells 10. https://doi.org/10.3390/cells10061275.
Lutfi, A., Afghan, M.K., Kasi, P.M., 2023. Circulating Tumor DNA response and minimal residual disease assessment in DNA polymerase epsilon-mutated colorectal cancer undergoing immunotherapy. Cureus 2023, 3–7. https://doi.org/10.7759/cureus.43391.
Manca, P., Corti, F., Intini, R., Mazzoli, G., Miceli, R., Germani, M.M., Bergamo, F., Ambrosini, M., Cristarella, E., Cerantola, R., Boccaccio, C., Ricagno, G., Ghelardi, F., Randon, G., Leoncini, G., Milione, M., Fassan, M., Cremolini, C., Lonardi, S., Pietrantonio, F., 2023. Tumour mutational burden as a biomarker in patients with mismatch repair deficient/microsatellite instability-high metastatic colorectal cancer treated with immune checkpoint inhibitors. Eur. J. Cancer 187, 15–24. https://doi.org/10.1016/j.ejca.2023.03.029.
Marabelle, A., Fakih, M., Lopez, J., Shah, M., Shapira-Frommer, R., Nakagawa, K., Chung, H.C., Kindler, H.L., Lopez-Martin, J.A., Miller, W.H., Italiano, A., Kao, S., Piha-Paul, S.A., Delord, J.P., McWilliams, R.R., Fabrizio, D.A., Aurora-Garg, D., Xu, L., Jin, F., Norwood, K., Bang, Y.J., 2020. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol. 21, 1353–1365. https://doi.org/10.1016/S1470-2045(20)30445-9.
McNamara, M.G., Jacobs, T., Lamarca, A., Hubner, R.A., Valle, J.W., Amir, E., 2020. Impact of high tumor mutational burden in solid tumors and challenges for biomarker application. Cancer Treat. Rev. 89, 102084 https://doi.org/10.1016/j.ctrv.2020.102084.
Mehrvarz Sarshekeh, A., Alshenaifi, J., Roszik, J., Manyam, G.C., Advani, S.M., Katkhuda, R., Verma, A., Lam, M., Willis, J., Shen, J.P., Morris, J., Davis, J.S., Loree, J.M., Lee, H.M., Ajani, J.A., Maru, D.M., Overman, M.J., Kopetz, S., 2021. ARID1A mutation may define an immunologically active subgroup in patients with microsatellite stable colorectal cancer. Clin. Cancer Res. 27, 1663–1670. https://doi.org/10.1158/1078-0432.CCR-20-2404.
Merino, D.M., McShane, L.M., Fabrizio, D., Funari, V., Chen, S.J., White, J.R., Wenz, P., Baden, J., Barrett, J.C., Chaudhary, R., Chen, L., Chen, W., Cheng, J.H., Cyanam, D., Dickey, J.S., Gupta, V., Hellmann, M., Helman, E., Li, Y., Maas, J., Papin, A., Patidar, R., Quinn, K.J., Rizvi, N., Tae, H., Ward, C., Xie, M., Zehir, A., Zhao, C., Dietel, M., Stenzinger, A., Stewart, M., Allen, J., 2020. Establishing guidelines to harmonize tumor mutational burden (TMB): In silico assessment of variation in TMB quantification across diagnostic platforms: Phase I of the Friends of Cancer Research TMB Harmonization Project. J. Immunother. Cancer 8 (1), 14. https://doi.org/10.1136/jitc-2019-000147.
Middha, S., Zhang, L., Nafa, K., Jayakumaran, G., Wong, D., Kim, H.R., Sadowska, J., Berger, M.F., Delair, D.F., Shia, J., Stadler, Z., Klimstra, D.S., Ladanyi, M., Zehir, A., Hechtman, J.F., 2017. Reliable pan-cancer microsatellite instability assessment by using targeted next-generation sequencing data. JCO Precis. Oncol. 1–17. https://doi.org/10.1200/po.17.00084.
Moretto, R., Elliott, A., Zhang, J., Arai, H., Germani, M.M., Conca, V., Xiu, J., Stafford, P., Oberley, M., Abraham, J., Spetzler, D., Rossini, D., Antoniotti, C., Marshall, J., Shields, A., Lopes, G., Lonardi, S., Pietrantonio, F., Tomasello, G., Passardi, A., Tamburini, E., Santini, D., Aprile, G., Masi, G., Falcone, A., Lenz, H.J., Korn, M., Cremolini, C., 2022. Homologous recombination deficiency alterations in colorectal cancer: clinical, molecular, and prognostic implications. J. Natl. Cancer Inst. 114, 271–279. https://doi.org/10.1093/jnci/djab169.
Network, N.C.C., 2023. Colon Cancer (version 3.2023 - September 21, 2023).
NobelPrize.org, n.d. The Nobel Prize in Physiology or Medicine 2018 [WWW Document]. Nobel Prize Outreach. URL 〈https://www.nobelprize.org/prizes/medicine/2018/summary/〉 (Accessed 17 June 2023).
Normanno, N., 2017. Tumour Mutational Load: ESMO Biomarker Factsheet [WWW Document]. Oncol. Educ. Libr. Factsheets Biomarkers.. 〈https://oncologypro.esmo.org/education-library/factsheets-on-biomarkers/tumour-mutational-load〉 (Accessed 13 July 2023).
Ogunwobi, O.O., Mahmood, F., Akingboye, A., 2020. Biomarkers in colorectal cancer: current research and future prospects. Int. J. Mol. Sci. 21, 1–20. https://doi.org/10.3390/ijms21155311.
Ooki, A., Shinozaki, E., Yamaguchi, K., 2021. Immunotherapy in colorectal cancer: current and future strategies. J. Anus, Rectum Colon 5, 11–24. https://doi.org/10.23922/jarc.2020-064.
Parikh, A.R., He, Y., Hong, T.S., Corcoran, R.B., Clark, J.W., Ryan, D.P., Zou, L., Ting, D. T., Catenacci, D.V., Chao, J., Fakih, M., Klempner, S.J., Ross, J.S., Frampton, G.M., Miller, V.A., Ali, S.M., Schrock, A.B., 2019. Analysis of DNA damage response gene alterations and tumor mutational burden across 17,486 tubular gastrointestinal carcinomas: implications for therapy. Oncologist 24, 1340–1347. https://doi.org/10.1634/theoncologist.2019-0034.
Parikh, A.R., Szabolcs, A., Allen, J.N., Clark, J.W., Wo, J.Y., Raabe, M., Thel, H., Hoyos, D., Mehta, A., Arshad, S., Lieb, D.J., Drapek, L.C., Blaszkowsky, L.S., Giantonio, B.J., Weekes, C.D., Zhu, A.X., Goyal, L., Nipp, R.D., Dubois, J.S., Van Seventer, E.E., Foreman, B.E., Matlack, L.E., Ly, L., Meurer, J.A., Hacohen, N., Ryan, D.P., Yeap, B.Y., Corcoran, R.B., Greenbaum, B.D., Ting, D.T., Hong, T.S., 2021. Radiation therapy enhances immunotherapy response in microsatellite stable colorectal and pancreatic adenocarcinoma in a phase II trial. Nat. Cancer 2, 1124–1135. https://doi.org/10.1038/s43018-021-00269-7.
Pecci, F., Cantini, L., Bittoni, A., Lenci, E., Lupi, A., Crocetti, S., Giglio, E., Giampieri, R., Berardi, R., 2021. Beyond microsatellite instability: evolving strategies integrating immunotherapy for microsatellite stable colorectal cancer. Curr. Treat. Options Oncol. 22 https://doi.org/10.1007/s11864-021-00870-z.
Pham, T.V., Goodman, A.M., Sivakumar, S., Frampton, G., Kurzrock, R., 2021. Intra-patient stability of tumor mutational burden from tissue biopsies at different time points in advanced cancers. Genome Med 13, 1–9. https://doi.org/10.1186/s13073-021-00979-8.
Picard, E., Verschoor, C.P., Ma, G.W., Pawelec, G., 2020. Relationships between immune landscapes, genetic subtypes and responses to immunotherapy in colorectal cancer. Front. Immunol. 11, 1–19. https://doi.org/10.3389/fimmu.2020.00369.
Prasad, V., Addeo, A., 2020. The FDA approval of pembrolizumab for patients with TMB >10 mut/Mb: was it a wise decision? No. Ann. Oncol. 31, 1112–1114. https://doi.org/10.1016/j.annonc.2020.07.001.
Puccini, A., Lenz, H.-J., Marshall, J.L., Arguello, D., Raghavan, D., Korn, W.M., Weinberg, B.A., Poorman, K., Heeke, A.L., Philip, P.A., Shields, A.F., Goldberg, R.M., Salem, M.E., 2019. Impact of patient age on molecular alterations of left-sided colorectal tumors. Oncologist 24, 319–326. https://doi.org/10.1634/theoncologist.2018-0117.
Quintanilha, J.C.F., Graf, R.P., Fisher, V.A., Oxnard, G.R., Ellis, H., Panarelli, N., Lin, D.I., Li, G., Huang, R.S.P., Ross, J.S., Myer, P.A., Klempner, S.J., 2023. Comparative effectiveness of immune checkpoint inhibitors vs chemotherapy in patients with metastatic colorectal cancer with measures of microsatellite instability, mismatch repair, or tumor mutational burden. JAMA Netw. Open 6, e2252244. https://doi.org/10.1001/jamanetworkopen.2022.52244.
Reilly, N.M., Novara, L., Di Nicolantonio, F., Bardelli, A., 2019. Exploiting DNA repair defects in colorectal cancer. Mol. Oncol. 13, 681–700. https://doi.org/10.1002/1878-0261.12467.
Research, F. of C., 2021. Project Spotlight: Friends TMB Harmonization Project.
Ritterhouse, L.L., 2019. Tumor mutational burden. Cancer Cytopathol. 127, 735–736. https://doi.org/10.1002/cncy.22174.
Rizvi, N.A., Hellmann, M.D., Snyder, A., Kvistborg, P., Makarov, V., Havel, J.J., Lee, W., Yuan, J., Wong, P., Ho, T.S., Miller, M.L., Rekhtman, N., Moreira, A.L., Ibrahim, F., Bruggeman, C., Gasmi, B., Zappasodi, R., Maeda, Y., Sander, C., Garon, E.B., Merghoub, T., Wolchok, J.D., Schumacher, T.N., Chan, T.A., 2015. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Sci. (80-.) 348, 124–128. https://doi.org/10.1126/science.aaa1348.
Roth, M.T., Das, S., 2021. Pembrolizumab in unresectable or metastatic MSI-high colorectal cancer: safety and efficacy. Expert Rev. Anticancer Ther. 21, 229–238. https://doi.org/10.1080/14737140.2021.1851201.
Rousseau, B.-C., Bieche, I., Pasmant, E., Simmet, V., Hamzaoui, N., Masliah-Planchon, J., Pouessel, D., Bruyas, A., Augereau, P., Grob, J.-J., Rolland, F., Saada-Bouzid, E., Cohen, R., Bouche, O., Hoog-Labouret, N., Legrand, F., Simon, C., Lamrani-Ghaouti, A., Chevret, S., Marabelle, A., 2020. 526O High activity of nivolumab in patients with pathogenic exonucleasic domain POLE (edPOLE) mutated Mismatch Repair proficient (MMRp) advanced tumours. Ann. Oncol. 31, S463. https://doi.org/10.1016/j.annonc.2020.08.640.
Rousseau, B., Bieche, I., Pasmant, E., Hamzaoui, N., Leulliot, N., Michon, L., de Reynies, A., Attignon, V., Foote, M.B., Masliah-Planchon, J., Svrcek, M., Cohen, R., Simmet, V., Augereau, P., Malka, D., Hollebecque, A., Pouessel, D., Gomez-Roca, C., Guimbaud, R., Bruyas, A., Guillet, M., Grob, J.-J., Duluc, M., Cousin, S., de la Fouchardiere, C., Flechon, A., Rolland, F., Hiret, S., Saada-Bouzid, E., Bouche, O., Andre, T., Pannier, D., El Hajbi, F., Oudard, S., Tournigand, C., Soria, J.-C., Champiat, S., Gerber, D.G., Stephens, D., Lamendola-Essel, M.F., Maron, S.B., Diplas, B.H., Argiles, G., Krishnan, A.R., Tabone-Eglinger, S., Ferrari, A., Segal, N.H., Cercek, A., Hoog-Labouret, N., Legrand, F., Simon, C., Lamrani-Ghaouti, A., Diaz, L. A., Saintigny, P., Chevret, S., Marabelle, A., 2022. PD-1 blockade in solid tumors with defects in polymerase epsilon. Cancer Discov. 12, 1435–1448. https://doi.org/10.1158/2159-8290.CD-21-0521.
Rousseau, B., Foote, M.B., Maron, S.B., Diplas, B.H., Lu, S., Argilés, G., Cercek, A., Diaz, L.A., 2021. The spectrum of benefit from checkpoint blockade in hypermutated tumors. N. Engl. J. Med. 384, 1168–1170. https://doi.org/10.1056/NEJMc2031965.
Salem, M.E., Bodor, J.N., Puccini, A., Xiu, J., Goldberg, R.M., Grothey, A., Korn, W.M., Shields, A.F., Worrilow, W.M., Kim, E.S., Lenz, H., Marshall, J.L., Hall, M.J., 2020. Relationship between MLH1, PMS2, MSH2 and MSH6 gene-specific alterations and

tumor mutational burden in 1057 microsatellite instability-high solid tumors. Int. J. Cancer 147, 2948–2956. https://doi.org/10.1002/ijc.33115.

Salem, M.E., Puccini, A., Grothey, A., Raghavan, D., Goldberg, R.M., Xiu, J., Korn, W.M., Weinberg, B.A., Hwang, J.J., Shields, A.F., Marshall, J.L., Philip, P.A., Lenz, H.-J., 2018. Landscape of tumor mutation load, mismatch repair deficiency, and PD-L1 expression in a large patient cohort of gastrointestinal cancers. Mol. Cancer Res. 16, 805–812. https://doi.org/10.1158/1541-7786.MCR-17-0735.

Samstein, R.M., Lee, C.-H., Shoushtari, A.N., Hellmann, M.D., Shen, R., Janjigian, Y.Y., Barron, D.A., Zehir, A., Jordan, E.J., Omuro, A., Kaley, T.J., Kendall, S.M., Motzer, R. J., Hakimi, A.A., Voss, M.H., Russo, P., Rosenberg, J., Iyer, G., Bochner, B.H., Bajorin, D.F., Al-Ahmadie, H.A., Chaft, J.E., Rudin, C.M., Riely, G.J., Baxi, S., Ho, A. L., Wong, R.J., Pfister, D.G., Wolchok, J.D., Barker, C.A., Gutin, P.H., Brennan, C.W., Tabar, V., Mellinghoff, I.K., DeAngelis, L.M., Ariyan, C.E., Lee, N., Tap, W.D., Gounder, M.M., D'Angelo, S.P., Saltz, L., Stadler, Z.K., Scher, H.I., Baselga, J., Razavi, P., Klebanoff, C.A., Yaeger, R., Segal, N.H., Ku, G.Y., DeMatteo, R.P., Ladanyi, M., Rizvi, N.A., Berger, M.F., Riaz, N., Solit, D.B., Chan, T.A., Morris, L.G.T., 2019. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat. Genet. 51, 202–206. https://doi.org/10.1038/s41588-018-0312-8.

Schrock, A.B., Ouyang, C., Sandhu, J., Sokol, E., Jin, D., Ross, J.S., Miller, V.A., Lim, D., Amanam, I., Chao, J., Catenacci, D., Cho, M., Braiteh, F., Klempner, S.J., Ali, S.M., Fakih, M., 2019. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. Ann. Oncol. 30, 1096–1103. https://doi.org/10.1093/annonc/mdz134.

Shimada, Y., Okuda, S., Watanabe, Y., Tajima, Y., Nagahashi, M., Ichikawa, H., Nakano, M., Sakata, J., Takii, Y., Kawasaki, T., Homma, K. ichi, Kamori, T., Oki, E., Ling, Y., Takeuchi, S., Wakai, T., 2021. Histopathological characteristics and artificial intelligence for predicting tumor mutational burden-high colorectal cancer. J. Gastroenterol. 56, 547–559. https://doi.org/10.1007/s00535-021-01789-w.

Sibilio, P., Belardinilli, F., Licursi, V., Paci, P., Giannini, G., 2022. An integrative in-silico analysis discloses a novel molecular subset of colorectal cancer possibly eligible for immune checkpoint immunotherapy. Biol. Direct 17, 1–16. https://doi.org/10.1186/s13062-022-00324-y.

Sicklick, J.K., Kato, S., Okamura, R., Patel, H., Nikanjam, M., Fanta, P.T., Hahn, M.E., De, P., Williams, C., Guido, J., Solomon, B.M., McKay, R.R., Krie, A., Boles, S.G., Ross, J.S., Lee, J.J., Leyland-Jones, B., Lippman, S.M., Kurzrock, R., 2021. Molecular profiling of advanced malignancies guides first-line N-of-1 treatments in the I-PREDICT treatment-naïve study. Genome Med 13, 1–14. https://doi.org/10.1186/s13073-021-00969-w.

Stenzinger, A., Allen, J.D., Maas, J., Stewart, M.D., Merino, D.M., Wempe, M.M., Dietel, M., 2019. Tumor mutational burden standardization initiatives: Recommendations for consistent tumor mutational burden assessment in clinical samples to guide immunotherapy treatment decisions. Genes Chromosom. Cancer 58, 578–588. https://doi.org/10.1002/gcc.22733.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca. Cancer J. Clin. 71, 209–249. https://doi.org/10.3322/caac.21660.

Sung, M.T., Wang, Y.H., Li, C.F., 2022. Open the Technical Black Box of Tumor Mutational Burden (TMB): Factors Affecting Harmonization and Standardization of Panel-Based TMB. Int. J. Mol. Sci. 23 https://doi.org/10.3390/ijms23095097.

Tokunaga, R., Xiu, J., Goldberg, R.M., Philip, P.A., Seeber, A., Battaglin, F., Arai, H., Lo, J.H., Naseem, M., Puccini, A., Berger, M.D., Soni, S., Zhang, W., Chen, S., Hwang, J.J., Shields, A.F., Marshall, J.L., Baba, H., Korn, W.M., Lenz, H.-J., 2020. The impact of ARID1A mutation on molecular characteristics in colorectal cancer. Eur. J. Cancer 140, 119–129. https://doi.org/10.1016/j.ejca.2020.09.006.

Tokunaga, R., Xiu, J., Johnston, C., Goldberg, R.M., Philip, P.A., Seeber, A., Naseem, M., Lo, J.H., Arai, H., Battaglin, F., Puccini, A., Berger, M.D., Soni, S., Zhang, W., Hwang, J.J., Shields, A.F., Marshall, J.L., Baba, H., Korn, W.M., Lenz, H.-J., 2019. Molecular profiling of appendiceal adenocarcinoma and comparison with right-sided and left-sided colorectal cancer. Clin. Cancer Res. 25, 3096–3103. https://doi.org/10.1158/1078-0432.CCR-18-3388.

Topalian, S.L., Hodi, F.S., Brahmer, J.R., Gettinger, S.N., Smith, D.C., McDermott, D.F., Powderly, J.D., Carvajal, R.D., Sosman, J.A., Atkins, M.B., Leming, P.D., Spigel, D.R., Antonia, S.J., Horn, L., Drake, C.G., Pardoll, D.M., Chen, L., Sharfman, W.H., Anders, R.A., Taube, J.M., McMiller, T.L., Xu, H., Korman, A.J., Jure-Kunkel, M., Agrawal, S., McDonald, D., Kollia, G.D., Gupta, A., Wigginton, J.M., Sznol, M., 2012. Safety, activity, and immune correlates of anti–PD-1 antibody in cancer. N. Engl. J. Med. 366, 2443–2454. https://doi.org/10.1056/NEJMoa1200690.

U.S. Federal Drug Administration, 2023. Highlights of Prescribing Information - OPDIVO (nivolumab) injection, for intravenous use.

U.S. Federal Drug Administration, 2017a. FDA unveils a streamlined path for the authorization of tumor profiling tests alongside its latest product action [WWW Document]. FDA News Release. URL ⟨https://www.fda.gov/news-events/press-announcements/fda-unveils-streamlined-path-authorization-tumor-profiling-tests-alongside-its-latest-product-action⟩ (Accessed 13 July 2023).

U.S. Federal Drug Administration, 2017b. FDA announces approval, CMS proposes coverage of first breakthrough-designated test to detect extensive number of cancer biomarkers [WWW Document]. FDA News Release. URL ⟨https://www.fda.gov/news-events/press-announcements/fda-announces-approval-cms-proposes-coverage-first-breakthrough-designated-test-detect-extensive⟩ (Accessed 13 July 2023).

U.S. Federal Drug Administration (FDA), 2023. Highlights of Prescribing Information - KEYTRUDA (pembrolizumab) injection, for intravenous use.

Valero, C., Lee, M., Hoen, D., Zehir, A., Berger, M.F., Seshan, V.E., Chan, T.A., Morris, L. G.T., 2021. Response Rates to Anti–PD-1 Immunotherapy in Microsatellite-Stable Solid Tumors With 10 or More Mutations per Megabase. JAMA Oncol. 7, 739. https://doi.org/10.1001/jamaoncol.2020.7684.

Vega, D.M., Yee, L.M., McShane, L.M., Williams, P.M., Chen, L., Vilimas, T., Fabrizio, D., Funari, V., Newberg, J., Bruce, L.K., Chen, S.J., Baden, J., Carl Barrett, J., Beer, P., Butler, M., Cheng, J.H., Conroy, J., Cyanam, D., Eyring, K., Garcia, E., Green, G., Gregersen, V.R., Hellmann, M.D., Keefer, L.A., Lasiter, L., Lazar, A.J., Li, M.C., MacConaill, L.E., Meier, K., Mellert, H., Pabla, S., Pallavajjalla, A., Pestano, G., Salgado, R., Samara, R., Sokol, E.S., Stafford, P., Budczies, J., Stenzinger, A., Tom, W., Valkenburg, K.C., Wang, X.Z., Weigman, V., Xie, M., Xie, Q., Zehir, A., Zhao, C., Zhao, Y., Stewart, M.D., Allen, J., 2021. Aligning tumor mutational burden (TMB) quantification across diagnostic platforms: phase II of the Friends of Cancer Research TMB Harmonization Project. Ann. Oncol. 32, 1626–1636. https://doi.org/10.1016/j.annonc.2021.09.016.

Voutsadakis, I.A., 2023. High tumor mutation burden (TMB) in microsatellite stable (MSS) colorectal cancers: Diverse molecular associations point to variable pathophysiology. Cancer Treat. Res. Commun. 36 https://doi.org/10.1016/j.ctarc.2023.100746.

Wang, C., Gong, J., Tu, T.Y., Lee, P.P., Fakih, M., 2018. Immune profiling of microsatellite instability-high and polymerase ε (POLE)-mutated metastatic colorectal tumors identifies predictors of response to anti-PD-1 therapy. J. Gastrointest. Oncol. 9, 404–415. https://doi.org/10.21037/jgo.2018.01.09.

Wang, X., Teng, F., Kong, L., Yu, J., 2016. PD-L1 expression in human cancers and its association with clinical outcomes. Onco. Targets Ther. 9, 5023–5039. https://doi.org/10.2147/OTT.S105862.

Wang, Y., Wei, B., Gao, J., Cai, X., Xu, L., Zhong, H., Wang, B., Sun, Y., Guo, W., Xu, Q., Gu, Y., 2020. Combination of fruquintinib and anti–PD-1 for the treatment of colorectal cancer. J. Immunol. 205, 2905–2915. https://doi.org/10.4049/jimmunol.2000463.

Xiao, J., Li, W., Huang, Y., Huang, M., Li, S., Zhai, X., Zhao, J., Gao, C., Xie, W., Qin, H., Cai, S., Bai, Y., Lan, P., Zou, Y., 2021. A next-generation sequencing-based strategy combining microsatellite instability and tumor mutation burden for comprehensive molecular diagnosis of advanced colorectal cancer. BMC Cancer 21 (1), 12. https://doi.org/10.1186/s12885-021-07942-1.

Xie, Y.H., Chen, Y.X., Fang, J.Y., 2020. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct. Target. Ther. 5 https://doi.org/10.1038/s41392-020-0116-z.

Xu, L., Zheng, Q., 2021. Identification and validation of a miRNA-related expression signature for tumor mutational burden in colorectal cancer. World J. Surg. Oncol. 19 (1), 12. https://doi.org/10.1186/s12957-021-02137-1.

Xue, W., Wang, Y., Xie, Y., Yang, C., Gong, Z., Guan, C., Wei, C., Zhu, C., Niu, Z., 2021. miRNA-based signature associated with tumor mutational burden in colon adenocarcinoma. Front. Oncol. 11, 1–10. https://doi.org/10.3389/fonc.2021.634841.

Yang, R.K., Qing, Y., Jelloul, F.Z., Routbort, M.J., Wang, P., Shaw, K., Zhang, J., Lee, J., Medeiros, L.J., Kopetz, S., Tetzlaff, M.T., Broaddus, R.R., 2020. Identification of biomarkers of immune checkpoint blockade efficacy in recurrent or refractory solid tumor malignancies. Oncotarget 11, 600–618. https://doi.org/10.18632/oncotarget.27466.

Yang, Z., Wei, S., Deng, Y., Wang, Z., Liu, L., 2020. Clinical significance of tumour mutation burden in immunotherapy across multiple cancer types: an individual meta-analysis. Jpn. J. Clin. Oncol. 50, 1023–1031. https://doi.org/10.1093/jjco/hyaa076.

Yarchoan, M., Albacker, L.A., Hopkins, A.C., Montesion, M., Murugesan, K., Vithayathil, T.T., Zaidi, N., Azad, N.S., Laheru, D.A., Frampton, G.M., Jaffee, E.M., 2019. PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. JCI Insight 4, 1–10. https://doi.org/10.1172/jci.insight.126908.

Zaidi, S.H., Harrison, T.A., Phipps, A.I., Steinfelder, R., Trinh, Q.M., Qu, C., Banbury, B. L., Georgeson, P., Grasso, C.S., Giannakis, M., Adams, J.B., Alwers, E., Amitay, E.L., Barfield, R.T., Berndt, S.I., Borozan, I., Brenner, H., Brezina, S., Buchanan, D.D., Cao, Y., Chan, A.T., Chang-Claude, J., Connolly, C.M., Drew, D.A., Farris, A.B., Figueiredo, J.C., French, A.J., Fuchs, C.S., Garraway, L.A., Gruber, S., Guinter, M.A., Hamilton, S.R., Harlid, S., Heisler, L.E., Hidaka, A., Hopper, J.L., Huang, W.Y., Huyghe, J.R., Jenkins, M.A., Krzyzanowski, P.M., Lemire, M., Lin, Y., Luo, X., Mardis, E.R., McPherson, J.D., Miller, J.K., Moreno, V., Mu, X.J., Nishihara, R., Papadopoulos, N., Pasternack, D., Quist, M.J., Rafikova, A., Reid, E.E.G., Shinbrot, E., Shirts, B.H., Stein, L.D., Teney, C.D., Timms, L., Um, C.Y., Van Guelpen, B., Van Tassel, M., Wang, X., Wheeler, D.A., Yung, C.K., Hsu, L., Ogino, S., Gsur, A., Newcomb, P.A., Gallinger, S., Hoffmeister, M., Campbell, P.T., Thibodeau, S.N., Sun, W., Hudson, T.J., Peters, U., 2020. Landscape of somatic single nucleotide variants and indels in colorectal cancer and impact on survival. Nat. Commun. 11, 1–12. https://doi.org/10.1038/s41467-020-17386-z.

Zhao, H., Sheng, D., Qian, Z., Ye, S., Chen, J., Tang, Z., 2021. Identifying GNG4 might play an important role in colorectal cancer TMB. Cancer Biomark. 32, 435–450. https://doi.org/10.3233/CBM-203009.

Zhao, S., Wu, W., Jiang, Z., Tang, F., Ding, L., Xu, W., Ruan, L., 2022. Roles of ARID1A variations in colorectal cancer: a collaborative review. Mol. Med. 28, 42. https://doi.org/10.1186/s10020-022-00469-6.

Zheng, M., 2022. Tumor mutation burden for predicting immune checkpoint blockade response: the more, the better. J. Immunother. Cancer 10, 1–11. https://doi.org/10.1136/jitc-2021-003087.

Zheng, Y., Fu, Y., Wang, P.P., Ding, Z.Y., 2022. Neoantigen: a promising target for the immunotherapy of colorectal cancer. Dis. Markers 2022. https://doi.org/10.1155/2022/8270305.

Zhou, C., Chen, S., Xu, F., Wei, J., Zhou, X., Wu, Z., Zhao, L., Liu, J., Guo, W., 2021. Estimating tumor mutational burden across multiple cancer types using whole-

exome sequencing, 1437–1437 Ann. Transl. Med. 9. https://doi.org/10.21037/atm-21-4227.

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