Immune Checkpoint Inhibition in Colorectal Cancer: Microsatellite Instability and Beyond

Romain Cohen, Benoît Rousseau, Joana Vidal, Raphaël Colle, Luis A. Diazjr., Thierry André

TARGETED ONCOLOGY - Review

Immune checkpoint inhibition in colorectal cancer: microsatellite instability and beyond

Authors: Romain Cohen[1*], Benoît Rousseau[2*], Joana Vidal[2,3], Raphaël Colle[1], Luis A Jr Diaz[2], Thierry André[1]

Affiliations:

1: Sorbonne Université, medical oncology department, hôpital Saint-Antoine, AP-HP, F-75012, Paris, France

2: Department of Medicine - Solid Tumor Division, Memorial Sloan Kettering Cancer Center, New York, USA

3: Medical Oncology Department, Hospital del Mar-IMIM, CIBERONC Instituto de Salud Carlos III, Barcelona

*Equal contribution

Corresponding Author: Romain Cohen, MD, PhD

ORCID 0000-0001-9602-5162

Hôpital Saint-Antoine, 184 rue du faubourg Saint-Antoine, 75012 Paris, France

Tel: +33 1 49 28 23 36; Fax:+33 1 49 28 34 98; email address: romain.cohen@aphp.fr

ABSTRACT

Immune checkpoints inhibitors (ICI) have been a breakthrough, with unique response and survival patterns compared with chemotherapy, for patients with advanced Mismatch Repair-deficient/Microsatellite instable (dMMR/MSI) colorectal cancer but have shown disappointing results in Mismatch Repair-proficient/Microsatellite stable (pMMR/MSS) colorectal cancer. As up to 50% of patients harboring dMMR/MSI advanced cancers will ultimately progress after PD-1 blockade, biomarkers are needed to predict response/resistance to immunotherapy and select patients for immunomodulating combinations. Patients with pMMR/MSS colorectal cancer present with distinct immune profiles compared to dMMR/MSI tumors, giving evidence of different immune escape mechanisms which could be overcome through individualized immunotherapeutic strategies.

In this review we discuss the latest developments in the field of immunotherapy for dMMR/MSI and pMMR/MSS colorectal cancers and unresolved questions and considerations concerning use of ICI therapies in this population. Future immunomodulation strategies based on biomarker selection (tumor mutational burden, Immunoscore®, mutational profile) are exposed.

KEYPOINTS

Despite dramatic responses to immune checkpoint inhibitors in patients with MSI (microsatellite instability) colorectal cancers (CRC), nearly half of the patients will ultimately progress and no biomarker is currently validated to correctly predict the resistance or benefit derived from immunotherapy.

POLE exonucleasic domain mutations are a major alternative biomarker to select the best candidates for immunotherapy in MSS (microsatellite stable) CRC.

The incorporation of immuno-genomicanalyses into future studies that assess immunomodulation/targeted therapeutic combinations would improve the identification of subsets of patients who would benefit from these treatments as well as provide support to move the field towards individualized immunotherapeutic strategies in CRC.

ABBREVIATIONS

Best supportive care: BSC

Beta-2-microglobulin: B2M

Blood Tumor mutational burden (bTMB)

Colorectal cancer: CRC

Disease-free Survival: DFS

Disease response rate: DCR

Food and Drug Administration: FDA

Hazard Ratio: HR

Immune checkpoint inhibitor: ICI

Immune Response Evaluation Criteria in Solid Tumors criteria: iRECIST

Immune-related Response Evaluation Criteria in Solid Tumors criteria: irRECIST

Insertion/deletion mutations: InDels

Interferon gamma: IFN-γ

Major histocompatibility complex: MHC

Metastatic colorectal cancer: mCRC

Microsatellite instability: MSI

Microsatellite stable: MSS

Mismatch repair: MMR

Mismatch-repair deficiency: dMMR

Mismatch repair-proficient: pMMR

Mutations per megabase: mt/mb

Objective response rate: ORR

Overall survival: OS

Polymerase proofreading-associated polyposis: PPAP

Progression-free survival: PFS

Response Evaluation Criteria in Solid Tumors criteria: RECIST

Tumor mutational burden: TMB

Vascular Endothelial Growth Factor: VEGF

1. Introduction

Immune checkpoint inhibitors (ICI) represent one of the major therapeutic breakthroughs in the history of medical oncology. In this context, metastatic colorectal cancer (mCRC) represent an intriguing entity, with a minority (4 to 5%) of tumors being highly sensitive to these compounds (*i.e.* CRC harboring microsatellite instability (MSI) and/or mismatch-repair deficiency (dMMR) [1]) but a vast majority are cold tumors refractory to immunotherapeutic strategies [2–4]. It has been known for years that MSI/dMMR tumors are highly infiltrated by immune cells [5]. These tumors are characterized by a high tumor mutational burden (TMB) with highly immunogenic neoantigens arising from frameshift mutations [6]. Moreover, they are associated with an upregulation of checkpoint inhibitors that exhaust intratumoral cytotoxic T lymphocytes and consequently protect MSI cancer cells from their hostile immune microenvironment [7,8].

Following the durable complete response of one patient with an MSI/dMMR mCRC treated with anti-PD1 ICI in a phase I study that included various types of unselected solid tumors, research and development of ICI for CRC focused on MSI/dMMR mCRC [9]. The impressive results of several phase II studies demonstrated that immune checkpoint inhibition is a breakthrough therapeutic strategy for dMMR/MSI mCRC [1,10,11]. Pembrolizumab or nivolumab alone or in combination with ipilimumab have been therefore approved by the Food and Drug Administration (FDA) for the treatment of chemoresistant MSI/dMMR mCRC patients. More, the FDA granted accelerated approval to pembrolizumab for advanced MSI/dMMR tumors progressing following prior treatment, in a tissue/site-agnostic fashion [12]. The efficacy of ICI in MSI/dMMR mCRC is now well-established, and trials are currently ongoing to assess ICI efficacy in first line, adjuvant or even neoadjuvant setting.

However, questions remain concerning the role of ICI for the treatment of microsatellite stable (MSS) and mismatch repair-proficient (pMMR) CRC. The combination of ICI with

other anticancer drugs is currently being evaluated in MSS/pMMR mCRC. However, the disappointing results of the phase III IMblaze 370 trial (atezolizumab with or without cobimetinib *versus* regorafenib) raises concerns regarding the testing of therapeutic ICI strategies without a guiding biomarker in these "cold" tumors [13]. It has been shown that TMB could be a biomarker for ICI efficacy in various histologies and some MSS/pMMR CRCs show a high TMB, notably due to mutations within genes coding for DNA polymerase [14,15]. Furthermore, the consensus molecular subtype (CMS) classification, which allows for better comprehension of CRC molecular heterogeneity, might be a tool worth considering for the development of new immunotherapeutic strategies in MSS/pMMR CRC [16].

In this review, we highlight how MSI/dMMR has emerged as a powerful biomarker for ICI efficacy in mCRC. Then we will focus on the currently explored ways to expand the use of ICI in CRC beyond MSI/dMMR and also in adjuvant and neoadjuvant settings.

2. Immune checkpoint blockade in colorectal cancer: from disappointment to microsatellite instability

The evaluation of immune checkpoint inhibitors (ICI) in CRC has been initially disappointing. The results of phase I basket trials including molecularly unselected mCRC patients did not show evidence of clinical activity. Notably, no objective responses were observed amongst the 3, 19 and 18 mCRC patients treated with pembrolizumab, nivolumab (anti PD-1 monoclonal antibodies), or BMS-936559 (anti-PD-L1 monoclonal antibody), respectively [2–4].

2.1. Clinical activity of ICI in patients with CRC MSI/dMMR

The breakthrough of immunotherapy in CRC came from one out of the 14 mCRC patients included in the basket phase I trial CA209-001 (NCT00441337), who experienced a durable complete response with nivolumab. This patient's tumor presented an MSI phenotype [9].

The proof-of-concept phase II study (MK-3475-016, NCT01876511) was designed to assess the efficacy of pembrolizumab in three cohorts of chemoresistant metastatic cancer patients: MSI/dMMR mCRC, MSI/dMMR non-CRC and MSS/pMMR CRC [1]. Preliminary results published in the New England Journal of Medicine in 2015 showed a 40%, 57% and 0% objective response rates (ORR), respectively, in the three pembrolizumab-treated cohorts (N = 11; N = 9; N = 21). This was the first step to recognize MSI/dMMR as a major predictive tissue-agnostic biomarker for the efficacy of ICI [12]. The updated data after 28 MSI/dMMR mCRC patients treated with pembrolizumab confirmed these encouraging results, with 11% of complete responses, 46% of partial responses and only 4% of progressive diseases [17].

Results of immune checkpoint blockade strategy in MSI/dMMR mCRC patients are summarized in Table 1. In the Keynote-164 study, pembrolizumab was evaluated in two distinct cohorts: (i) cohort A, after 2 or more prior lines of therapy including

fluoropyrimidine, oxaliplatin and irinotecan, and; (ii) cohort B, after 1 or more prior line of therapy. In these 2 cohorts, pembrolizumab was associated with disease control rate (DCR) of 51% and 44%, and 1-year progression-free survival (PFS) rate of 34% and 41%, respectively [18–20].

Nivolumab has been also evaluated in the context of MSI/dMMR mCRC. In the CheckMate-142 phase II trial, nivolumab alone (nivolumab 3 mg/kg every 2 weeks) and its combination with ipilimumab (nivolumab 3 mg/kg every 3 weeks + ipilimumab 1 mg/kg every 3weeks for 4 cycles then nivolumab 3 mg/kg every 2 weeks) until disease progression exhibited a 34% and 58% ORR, respectively, with 36% and 12% RECIST1.1 progressive disease at first evaluation, respectively. The 2-year PFS and OS (overall survival) rates were 60% and 74% with the combination of nivolumab and ipilimumab [21,22]. In both cohorts, patients were resistant or intolerant to at least 1 prior line of chemotherapy before inclusion: 54% and 40% of patients, respectively, received 3 lines of systemic treatment or more before ICI. In a third cohort of the CheckMate-142 trial, 45 patients with no prior treatment for MSI/dMMR mCRC were treated with nivolumab 3 mg/kg every 2 weeks plus low-dose ipilimumab every 6 weeks until disease progression. ORR was 77% and only 1 progressive disease was observed; 1-year PFS and OS rates were 77% and 83%, respectively [23].

Durvalumab, an anti-PD-L1 monoclonal antibody, has also been tested in the context of MSI/dMMR mCRC. Amongst 36 MSI/dMMR mCRC patients treated with durvalumab 10 mg/kg IV every 2 weeks (NCT01693562 and NCT02227667.), ORR was 22%, DCR for more than 24 weeks was 47% and the 2-year OS rate 54% [24]. Interestingly, patients were treated for a maximum of 12 months whereas in other trials ICI treatment was maintained for 2 years or until disease progression.

2.2. ICI compared to conventional chemotherapy

Based on the positive results from these clinical trials [1,10–12], pembrolizumab as well as nivolumab alone or in combination with ipilimumab have been granted accelerated approval by the FDA for chemoresistant MSI/dMMR mCRC patients. However, the European Medicine Agency has not yet approved ICI for these subsets of patients.

Several randomized trials are currently evaluating the efficacy of ICI as compared to standard-of-care chemotherapy ± targeted therapy in first or second-line metastatic setting (Table 2). Notably, the KEYNOTE-177 and COMMIT phase III studies might lead to the approval of pembrolizumab and atezolizumab, respectively, in frontline MSI/dMMR mCRC if they meet their primary endpoint. The ongoing randomized phase II trial PRODIGE 54 – SAMCO(NCT03186326) evaluates the efficacy of avelumab in second-line *versus* standard of care treatment (chemotherapy ± targeted therapy). In the randomized phase III CA209-8HW study (NCT04008030), patients are randomized to receive nivolumab alone, nivolumab plus ipilimumab or investigator's choice chemotherapy ± targeted therapy. The main objective is to compare the clinical benefit, as measured by PFS, ORR and OS, achieved by nivolumab in combination with ipilimumab or by nivolumab monotherapy in patients with MSI/dMMR mCRC.

Importantly, 2 phase III trials are currently assessing the efficacy of ICI in adjuvant setting. The Alliance A021502 trial (NCT02912559) is evaluating mFOLFOX6 ± atezolizumab in patients with stage III colon cancer. In the experimental arm, patients receive 6 months of adjuvant mFOLFOX6 concurrently with atezolizumab, followed by 6 additional months of atezolizumab monotherapy. The POLEM trial (EudraCT 2017000370_10) is testing the benefit of adding ICIs after the completion of capecitabine-based adjuvant chemotherapy (3 months of capecitabine plus oxaliplatin or 6 months of capecitabine monotherapy) in MSI/dMMR or *POLE*-mutated stage III CRC. At the end of adjuvant chemotherapy, patients

who are randomized to the investigational arm will receive additional 24 weeks of treatment with avelumab. The primary endpoint is disease-free survival in both trials.

2.3. Biomarkers within the MSI/dMMR population

Potential biomarkers of ICI efficacy amongst MSI/dMMR population are summarized in table 3.

Despite high rates of response and durable clinical benefit with ICI, 10 to 50% of MSI/dMMR mCRC tumors exhibit primary resistance to immunotherapy. However, a significant amount of these refractory tumors are mistakenly diagnosed as MSI/dMMR. In a cohort study of 38 mCRC patients included in ICI clinical trials based on a positive MSI and/or dMMR status as determined by local laboratories, misdiagnosis of MSI or dMMR status was responsible for 3 of 5 cases of primary resistance to ICI [25]. Thus, the first concern when observing immediate tumor progression under ICI is the possibility of a misdiagnosed MSI phenotype that requires confirmatory analysis by immunohistochemistry or molecular assay, the alternate hypothesis being tumor pseudo-progression [25,26].

Preliminary results on potential biomarkers for the efficacy of ICI amongst MSI/dMMR mCRC did not find significant predictive impact neither in *RAS*/*RAF* mutational status, tumor PD-L1 expression, the inherited (*i.e.* Lynch syndrome) nor sporadic origin of MMR deficiency nor the tumor mutational load [1,10,11,27]. Concerning the mechanism underlying MMR deficiency, it is worthy to note that the results remain questionable since the definition of Lynch syndrome-related cancers and sporadic cases is partly equivoque. In the analysis of ICI clinical trials, MMR deficiency origin was determined by investigators based on past medical history collected from clinical records without a systematic molecular approach that might skew the results [1,11,28]. Moreover, it has been recently proven that Lynch-like tumors (MSI tumors without *MLH1* promotor hypermethylation, *BRAF* mutation, or germline

MMR gene mutation) arise from biallelic somatic MMR gene inactivation in approximately 50% of patients [29]. Therefore, further studies are warranted to confirm these findings.

Several potential mechanisms of resistance are under evaluation, such as notably deleterious mutations of *JAK* (found in approximately 10 to 20% of MSI/dMMR tumors), loss of major histocompatibility complex (MHC) molecules or *B2M* (beta-2-microglobulin) loss-of-function mutations (20-30% of MSI CRC) [30–33]. The latter was not found to be associated with ICI resistance in a cohort of 13 *B2M*-mutated MSI/dMMR mCRC with ORR of 85% (11/13). Remarkably, the authors showed that MHC class I expression is variable in *B2M*-mutated MSI CRC [34] but not associated with response to ICI. To note, there is currently no available data on the impact of gut microbiota in MSI/dMMR cancer patients treated with ICI [35,36]. Histopathological characteristics such as the amount of extracellular mucin and PD-L1 expression at the invasive front might be associated with resistance to ICI [37].

The impact of tumor mutational load remains controversial within the MSI/dMMR population. Important recently published works detected a positive correlation between tumor mutational load and the efficacy of ICI amongst MSI/dMMR population [38,39]. However, the sample sizes remain small, with potential tumors misdiagnosed as MSI/dMMR amongst ICI-resistant cases [25]. Moreover, the threshold of tumor mutational load to be considered as predictive biomarker may be different from other tumor types. Larger translational studies are therefore warranted to establish the role of TMB in MSI/dMMR tumors. Finally, intra- and inter-tumoral heterogeneity might negatively influence ICI efficacy on sporadic MSI/dMMR tumors especially in cases of mixed dMMR/pMMR tumors [40,41].

The quality of the neoantigens seems capital as a potential new biomarker, as demonstrated in MSI/dMMR tumors treated by ICI [12]. Even in the context of high TMB and predicted neoantigens, very few effective immune responses directed against tumor associated neoantigens are detected, suggesting that immune response to ICI may rely only on a few

highly immunogenic antigens. These antigens are derived mostly from insertion/deletion mutations (InDels), a hallmark of dMMR tumors [38,42]. Importantly, this phenomenon may be difficult to overcome in MSS/pMMR tumors where InDels are rarely detected .

2.4. Controversies in the clinical management of MSI/dMMR mCRC patients treated with ICI

Pseudo-progression is a noted phenomenon of ICI therapy. Pseudo-progression is an initial radiographic tumor enlargement (consistent with progression per Response Evaluation Criteria in Solid Tumors criteria (RECIST)) followed by measurable tumor regression, sometimes months to years after therapy initiation. The frequency of pseudo-progression in MSI/dMMR population is currently unknown but it may be common in this highly sensitive population. Amongst patients from the CheckMate-142 trial with progression of disease as best response (n=14) and who continued treatment beyond progression (N = 11), those with a reduction (N = 3) or stabilization of target lesions (N = 3) were more likely to survive more than 12 months [21]. Distinguishing pseudo-progression from true disease progression is tricky, and irRECIST (Immune-related RECIST) or iRECIST (immune RECIST) criteria can be helpful [43]. Clinical parameters such as the improvement of performance status, improvement of symptoms or decrease of serum tumor marker levels may help in the clinician's decision to continue the ICI and wait for later radiologic evaluations. To note, the KEYNOTE-016 trial used irRECIST to assess response, meanwhile others such asCheckMate-142 and KEYNOTE-164 used RECIST v.1.1. This difference in radiologic evaluation criteria might explain the difference in ORR across studies [10,11,17,18,20].

Concerning secondary resections of primary tumor or metastasis after ICI treatment, the data are scarce. Some case reports suggested that pre-operative immunotherapy followed by resection of primary tumor and or metastatic sites is associated with high rates of pathological

complete response, despite the presence of residual lesions on radiological exams [44]. This brings to light some issues in the therapeutic management of ICI-treated patients with MSI/dMMR cancer: (i) what is the best therapeutic strategy for long responder patients: ICI continuation, surgcal removal of residual lesions or ICI discontinuation without surgery?, and ; (ii) Would those patients experiencing disease progression confined to a single site benefit from surgery? These issues are currently emerging since the number of ICI-treated MSI/dMMR mCRC patients is growing exponentially.

Another emerging crucial concern is the optimal duration of treatment for patients who respond to ICI. The strategy varies across trials, with a fixed duration of ICI (usually limited to 1 or 2 years of treatment) or the continuation of treatment until progression or unacceptable toxicity, which is a burden for patients and healthcare systems. The question remains unanswered and clinical trials are needed to find the optimal treatment duration.

3. Immunotherapy in colorectal cancer beyond MSI/dMMR

Ongoing clinical trials evaluating therapeutic strategies with ICI for MSS/pMMR CRC patients are displayed in table 4.

3.1. Hypermutability amongst microsatellite stable colorectal cancer: DNA polymerase mutations

The biological substratum of ICI efficacy on MSI/dMMR tumors relies on immunogenic frameshift mutations and high TMB. Several studies have shown that MSI/dMMR CRC could be detected through TMB evaluation with similar accuracy as polymerase chain reaction or immunohistochemistry [45,46]. Moreover, there is a high-TMB population that do not exhibit MSI/dMMR but displays an ultramutated phenotype with significantly higher TMB than MSI/dMMR CRC: all these tumors harbor mutations in DNA polymerase genes (Figure 1) [15,45,47].

In the TCGA cohort (N = 276), 16% of CRC samples (N=44) exhibited hypermutation (defined as a TMB greater than 12 mutations per 10^6 bases). Among these, all MSS tumors (N=7) harbored exonucleasic proofreading domain *POLE*-mutations (DNA epsilon polymerase) and presented the highest TMB. Similarly, in a study by Stadler and colleagues, 31 out of 224 CRC samples were hypermutated, of which 3 MSS *POLE*-mutated tumors presented a TMB 3-fold higher than MSI/dMMR cases [45]. In a study by Vanderwalde and colleagues, 93 out of 1395 CRC (6.7%) samples were hypermutated (TMB high if $\geq$ 17 mutations per megabase (mt/mb)) and 80 (5.7%) were MSI (and only 4 MSI tumors had low TMB) [46].

DNA polymerase enzymes are involved in DNA synthesis and repair during S phase of cell cycle. Delta and epsilon polymerases (*POLD* and *POLE*) are the main DNA polymerases in humans, with a proofreading exonuclease domain activity allowing error correction during

replication. Deleterious mutations in the exonuclease domain of *POLD* and *POLE* genes are responsible for a dramatic genetic instability associated with an ultramutated tumor phenotype. Germline mutations of *POLD* and *POLE* genes are associated with an autosomic dominant familial cancer syndrome favoring notably endometrial and colorectal cancers occurrence (PPAP: polymerase proofreading-associated polyposis). Somatic *POLE* gene mutations may also occur [48,49]. Interestingly, *POLE* mutations have been described in MSI/dMMR cancers, but arise distantly to the exonuclease domain and are therefore considered as passenger mutations due to the high genetic instability of MSI/dMMR tumors. This hypothesis is supported by the higher TMB of *POLE*-mutated MSS CRC compared to *POLE*-mutated MSI CRC tumors [42,50].

Exonucleasic domain *POLE*-mutated CRCs typically arise from left colon and rectum in younger male patients. Not all POLE mutations seem to be driving hypermutagenesis and the high mutation load phenotype seems restricted to specific hotspots. These mutations are observed in 1% of stage II and III tumors and are associated with favorable prognosis. This population is very rare in the metastatic setting, accounting for less than 1% of cases [47,51], and its prognosis and response to conventional treatments are still poorly understood. Considering ICI in this population, the literature is scarce but nevertheless promising with case reports suggesting major clinical activity for *POLE*-mutated CRC patients [52–54]. Several ongoing clinical trials are dedicated to this population (NCT03827044, NCT02715284, NCT03012581).

3.2. Tumor Mutational Burden and efficacy of ICI in CRC

TMB is an exonic genomic measure of non-synonymous mutations in tumor cells. Using algorithms comparing DNA sequences from control tissues to tumor samples, TMB helps to predict the number of somatic mutations within tumors. Therefore, it is an indirect measure of tumoral predicted neoantigens [55]. The usefulness of TMB is based on the concept that when

it is high, the likelihood of observing an effector T cell response directed against a specific tumor antigen is increased. This concept seems to be verified in MSI/dMMR tumors, as both are hypermutated and rich in effector immune cells. A recent study evaluated the predictive value of TMB in MSI/dMMR tumors treated by anti PD-1 [41]. Authors showed that patients with high TMB (> 37 mt/Mb) tumors displayed an ORR of 100% (N = 13) and improved PFS compared with patients with low TMB tumors (N = 9) [39]. While these results are promising, confirmation in prospective larger cohorts is needed before being translated to clinical practice because, as previously mentioned, the impact of the mutation load varies from one study to another.

MSS/pMMR CRC tumors may also display a high TMB but with a low frequency, including exonucleasic domain *POLE*-mutated tumors and other CRCs in which the mechanism of hypermutation remains unclear. A recent 2:1 randomized phase II clinical trial, CCTG CO.26, assessed the combination of tremelimumab, an anti-CTLA-4 and durvalumab, *versus* Best Supportive Care (BSC) in 180 MSS chemoresistant mCRC patients [56]. The trial reached its primary endpoint, with a median OS of 6.6 months for the ICI combination *versus* 4.1 months for BSC (HR 0.72 P = 0.07 for a preplanned threshold <0.1 for statistical significance) but without any improvement in the median PFS which were 1.8 and 1.9 months, respectively. DCR was 22.7% for the experimental arm *versus* 6.6% for BSC. TMB was assessed on cell free DNA (cfDNA) using a 500-gene next generation sequencing panel [57], and, excluding 2 MSI/dMMR patients, the mean TMB was 20.4 ± 16.3 mts/Mb (range: 0.96 - 114.0). Interestingly, 21% of tumors had a high TMB (defined as ≥28 mt/Mb), which was associated with better OS compared to patients with low or intermediate TMB tumors (5.5 month *versus* 3 months respectively, HR 0.34, 90% CI 0.18-0.63). Nevertheless, the pre-specified threshold of 20 mt/Mb was not predictive of benefit either for DCR, PFS or OS.

Genotyping of circulating tumor DNA (ctDNA) allows for a non-invasive quantification and characterization of the tumoral molecular profile and can indicate tumor heterogeneity and changes in TMB in real time. High blood TMB (bTMB) has been recently associated with an increase of response and survival in other tumor types treated with immunotherapy becoming a promising biomarker for ICI [58–60]. However, bTMB shows higher number of mutations per megabase compared to classic TMB assessed in tissue resulting in variable concordance data between tissue and liquid samples. Moreover, in CRC it remains unclear why a benefit is observed in OS but not in other outcomes. Further prospective studies are needed for implementation of bTMB into clinical practice.

The theoretical presence of tumoral neoantigens may not be able to independently predict their immunogenicity. A high number of neoantigens and/or high quality neoantigens are necessary to induce a durable antitumor immune response. A recent study underlined that non-hypermutated MSS CRC have a very low predicted number of neoantigens (45 ± 22) compared to MSI (635 ± 308), or hypermutated MSS CRC (1651 ± 1475) [61]. Notably, the number of predicted neoantigens decreases from early to advanced stage in the context of an active immunoediting, resulting in the elimination of the immunoreactive subclones and selection of immune privileged CRC subclones [62]. Specific translational immunological studies should be performed at latter stage to better understand the immune escape mechanisms in mCRC.

3.3. Immunoscore® and efficacy of ICI in CRC

The immunoscore® is a measure of the tumor-associated inflammatory stroma, assessing pan- / cytotoxic lymphocyte densities ($CD3^+$ and $CD8^+$, respectively) inside the tumor and at the invasion margin [63]. The immunoscore® is a validated independent prognostic factor in early stage CRC to predict disease-free survival. Patients with a low immunoscore®, *i.e.* low infiltration of $CD3^+$ and $CD8^+$ cells, have higher risk of recurrence thereby justifying its use

as a stratification factor in adjuvant clinical trials [64]. In this publication, when stratified into two immunoscore® categories (0-1, and 2-4), 49 MSI tumors (16%) had a low immunoscore® (0-1) and 255 (84%) had intermediate and high immunoscore® (2-4). Patients with intermediate and high immunoscore® had prolonged DFS and OS, irrespective of their microsatellite status (unadjusted HR for high versus low immunoscore® in MSI tumors 0.56, 95%CI 0.34-0.90; P = 0.0150). DFS of patients with immunoscore® high was similar for MSS and MSI tumors (around 80% at 3 years) and DFS of patients with immunoscore® low was similar for MSS and MSI tumor (around 60%).

The usefulness of immunoscore® in advanced disease seems more complex, as it relies on the assessment of the immune contexture of the invasion margin. Indeed, discordances between the primary tumor and the metastases has been observed and only the immunoscore® of metastases remained an independent prognostic factor in multivariate analyses [65]. In other tumors, the lymphocytic infiltration or intratumoral PD-L1 expression is not always associated with a benefit from immunotherapy, which is better predicted by transcriptional signatures where interferon gamma (IFN-γ) response genes are included [66]. The immunoscore® correlation with IFN-γ response remains to be established and, if confirmed, would allow the prospective assessment of ICI according to immunoscore® both in dMMR and pMMR tumors.

3.4. Molecular alterations and immune escape mechanisms in CRC

The international consensus molecular classification of CRC has been proposed, with 4 subtypes described so far: CMS1 (immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal) [16]. Interestingly, the CMS are associated with specific immune and stromal profile [67,68]. The CMS was developed using a set of localized tumors and its value in the metastatic setting remains controversial: (i) spatial CMS heterogeneity has been described, as some tumors have a homogeneous CMS and others have a heterogeneous CMS,

which complicates the allocation of tumors to a specific group and modulate the prognosis [69]; (ii) CMS modifications has been observed within metastatic tumor under active antitumoral treatment such as anti-EGFR agents [70]. Consequently, CMS clinical applicability in the metastatic setting remains limited and even reinforced by the complexity of bioinformatical analyses. Nevertheless, CMS helped to identify at least three main immune escape mechanisms in CRC that are related to distinct molecular pathway alterations. Importantly, there is also an increasing body of evidence showing that the genomic landscape of CRC correlates with specific immune profiles and immune escape mechanisms, which are consistent with the one described in the CMS. Figure 2 proposes an immune classification of CRC based on molecular pathway alterations and the potential treatment strategies to immunomodulate each tumor subtype.

Firstly, tumors with DNA repair impairment, such as MMR deficiency or mutations within the polymerases exonucleasic domain, are prone to accumulate immunogenic mutations. These tumors are immune inflamed and characterized by high infiltration in CD8 cells, a high number of neoantigens, and increased expression of CMH-I and immune checkpoints such as PD-1, PD-L1, CTLA-4, resulting in an immune profile that is highly favorable for ICI activity.

Secondly, CRC with TGF-β pathway dependency presents a strong angiogenesis activation and an immune inflamed profile. The role of TGF-β in colorectal carcinogenesis is complex as it may activate multiple transduction pathways and has a dual anti- and pro-tumoral role from early stage to late stage cancer [71]. Multiple alterations in genes implicated in TGF-β transduction pathways have been described in CRC, such as in the *SMAD* family genes, *BMPR1A* or *TGFb* receptors itself. The impact of these alterations on immune cell recruitment in human CRC remains to be elucidated, but multiple preclinical models have shown that impairment of these genes results in a chronic inflammation favoring colorectal cancer

progression [71]. Even if immune inflamed, the immune contexture of TGF-β dependent tumors is different from DNA repair impaired tumors and is characterized by an imbalance between the pro- and anti-tumoral immune cells with higher Treg, M0 and M2 macrophages and lower number of $CD8^+$ cells. Enrichment in immunosuppressive and complement factors such as high expression of chemokines attracting myeloid cells is also observed, reinforcing the pro-tumoral immune response. Recent preclinical studies suggest that targeting the TGF-β pathway in CRC favors the recruitment of T cells and enhances the Th1 response leading to an increased sensitivity to ICI [72]. Another seductive target is angiogenesis, as Vascular Endothelial Growth Factor (VEGF) pathway inhibitors have shown encouraging results in other tumor types in combination with ICI [73,74]. Indeed, in CRC, anti-VEGF agents were shown to increase CD8+ cells infiltration and decrease Treg in resected metastases, giving a rationale for combination strategies with ICI [75].

Finally, tumors with WNT/β-catenin pathway activation derived from *APC* or *CTNNB1* mutations have been shown to be poorly infiltrated by immune cells through the lack of immune cells trafficking [76] and characterized by: (i) few nonsynonymous mutations; (ii) low intratumoral immune infiltrate with a global decrease in lymphocytes, CD8+ cells, Th1, activated NK, and PD-L1+ mono/macrophages, and; (iii) low CMH-1 and B2M expression, suggesting poor antigen presentation. Preclinical models of CRC suggested that inhibition of WNT/β-catenin pathway could increase the immunological “hotness” of these tumors and generate sensitivity to ICI [77].

Compared to CMS assessment, immuno-genomic profiling could be more easily transposable in clinical practice as it relies on DNA sequencing and/or pathological assessment. This immuno-genomic profiling should probably be repeated overtime during the course of the metastatic disease and after exposure to treatments due to constant immunoediting, which actively modulates the immuno-genomic phenotype [62].

Setting aside the issue of the CMS classification, a recent early phase I trial assessing the combination of nivolumab and regorafenib (anti-VEGF Receptor inhibitor) in MSS mCRC showed encouraging results with an ORR of 33% and a median PFS of 6.3 months warranting further development [78], suggesting that angiogenesis inhibitors and ICI are synergistic in a subset of CRC, possibly the TGFβ/angiogenesis subgroup. Nevertheless, the use of other kinase inhibitors, such as MEK inhibitors, in combination with anti PD-L1 failed to show an improvement compared to regorafenib monotherapy in MSS mCRC tumors, despite showing promising results in phase Ib trials [13].

4. Conclusion and perspectives

ICI has been a major oncologic breakthrough in dMMR/MSI CRC but showed very limited results in low-TMB pMMR/MSS patients, with disappointing results in several clinical trials. Despite dramatic responses to pembrolizumab or nivolumab in patients with dMMR/MSI tumors, nearly half of the patients would finally progress, and no biomarker is currently validated to correctly predict the resistance or the benefit derived from ICI, precluding further clinical development. Potential biomarkers are controversial because they are assessed retrospectively or in *post hoc* translational studies of small cohorts. Two examples are (i) *B2M* mutations thought to be predictive of resistance in early studies and not confirmed in latter studies, and (ii) the discrepancies between mutation load and TMB correlations with clinical outcome, potentially explained by technical limitations and non-consensual bioinformatical analyses. Combination of anti-PD-1/PD-L1 and anti CTLA-4 agents seems to increase the benefit with an acceptable safety profile in selected patients with MSI/dMMR mCRC. Currently, there are still many unresolved questions concerning the clinical management of patients with ICI-treated MSI/dMMR mCRC such as duration of therapy, surgical removal of post-immunotherapy residual lesions, the best predictive factors of efficacy, or the choice of the best treatment scheme approach.

POLE/D exonucleasic domain mutations are an important alternative biomarker to select best candidates for ICI in MSS/pMMR mCRC. *POLE*-mutated tumors seem rare and confirming ICI activity in this subset may be challenging prospectively as the pathogenicity of these mutations remains unclear outside of the described hotspots. TMB is a seductive biomarker to offer ICI to patients with MSI/pMMR CRC with high mutation load. First results seem to indicate a limited benefit on OS but not on other outcomes. However, TMB assessment remains expensive, without a clear threshold and technical/bioinformatical pitfalls, and its predictive value remains to be established both for MSS/pMMR and MSI/dMMR tumors. The

Immunoscore® and IFN-γ response signature are also emerging biomarkers but preclinical/translational studies are still necessary to confirm their value in CRC, especially in pMMR patients before designing prospective clinical trials. Finally, immuno-genomic classification may be useful, especially to separate MSS/pMMR CRC in clinical trials in accordance with immune escape mechanisms. Incorporating immune-genomic profiling in future studies assessing immunomodulation/targeted therapeutic combinations would be of added value to identify subsets of patients benefiting from these treatments and move to individualized immunotherapeutic strategies in CRC.

ACKNOWLEGMENT

We would like to thank Michelle Lamendola-Essel for editing assistance and English editing.

COMPLIANCE WITH ETHICAL STANDARDS

Funding:

No external funding was used in the preparation of this manuscript.

Conflict of interest:

Benoît Rousseau served in a consulting/advisory role for Bayer, Roche, Novartis, Gilead, Servier and has received travel, accommodations, and expenses from Bayer, Servier, Astellas. Joana Vidal has received honoraria for advisory role, speaker or travel grants from Amgen, Hoffman La-Roche, Merck-Serono, Sanofi and Sysmex Inostics. Romain Cohen has received honoraria from Amgen, Sanofi and Servier, and travel fees from Sanofi. Thierry André reported receiving honoraria from Roche, Genentech, Ventana, Sanofi, Bristol-Myers Squibb, MSD Oncology, Servier, Pierre Fabre; consulting or advisory role for Roche, Amgen, Bristol-Myers Squibb, HalioDX; speakers' bureau for Bristol-Myers Squibb and Servier; travel, accommodations, and expenses from Roche, Genentech, Amgen, Bristol-Myers Squibb. Raphaël Colle and Luis A Jr Diaz declare that they have no conflicts of interest that might be relevant to the contents of this manuscript.

BIBLIOGRAPHY

1. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372:2509–20.

2. Patnaik A, Kang SP, Rasco D, Papadopoulos KP, Elassaiss-Schaap J, Beeram M, et al. Phase I Study of Pembrolizumab (MK-3475; Anti–PD-1 Monoclonal Antibody) in Patients with Advanced Solid Tumors. Clin Cancer Res. 2015;21:4286–93.

3. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. N Engl J Med. 2012;366:2443–54.

4. Brahmer JR, Tykodi SS, Chow LQM, Hwu W-J, Topalian SL, Hwu P, et al. Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer. N Engl J Med. 2012;366:2455–65.

5. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology. 2007;50:113–30.

6. Maby P, Tougeron D, Hamieh M, Mlecnik B, Kora H, Bindea G, et al. Correlation between Density of CD8+ T-cell Infiltrate in Microsatellite Unstable Colorectal Cancers and Frameshift Mutations: A Rationale for Personalized Immunotherapy. Cancer Res. 2015;75:3446–55.

7. Marisa L, Svrcek M, Collura A, Becht E, Cervera P, Wanherdrick K, et al. The Balance Between Cytotoxic T-cell Lymphocytes and Immune Checkpoint Expression in the Prognosis of Colon Tumors. J Natl Cancer Inst. 2018;110.

8. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The Vigorous Immune Microenvironment of Microsatellite Instable Colon Cancer Is Balanced by Multiple Counter-Inhibitory Checkpoints. Cancer Discov. 2015;5:43–51.

9. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. 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. 2010;28:3167–75.

10. Overman M, Lonardi S, Wong K, Lenz H, Gelsomino F, Aglietta M, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. J Clin Oncol. 2018;36:773–9.

11. Overman M, McDermott R, Leach J, Lonardi S, Lenz H, Morse M, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncol. 2017;18:1182–91.

12. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357:409–13.

13. Eng C, Kim TW, Bendell J, Argilés G, Tebbutt NC, Di Bartolomeo M, et al. Atezolizumab with or without cobimetinib versus regorafenib in previously treated metastatic colorectal cancer (IMblaze370): a multicentre, open-label, phase 3, randomised, controlled trial. Lancet Oncol. 2019;20:849–61.

14. Samstein RM, Lee C-H, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019;51:202–6.

15. Muzny DM, Bainbridge MN, Chang K, Dinh HH, Drummond JA, Fowler G, et al. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487:330–7.

16. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015;21:1350–6.

17. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. Programmed death-1 blockade in mismatch repair deficient colorectal cancer. J Clin Oncol. 2016;34:suppl; abstr 103.

18. Le DT, Kavan P, Won Kim T, Burge M, Van Cutsem E, Hara H, et al. KEYNOTE-164: Pembrolizumab for patients with advanced microsatellite instability high (MSI-H) colorectal cancer. J Clin Oncol. 2018;36:abstr 3514.

19. Diaz L, Marabelle A, Kim TW, Geva R, Van Cutsem E, Andre T, et al. Efficacy of pembrolizumab in phase 2 KEYNOTE-164 and KEYNOTE-158 studies of microsatellite instability high cancers. Ann Oncol. 2017;28:386P.

20. Le DT, Kim TW, Van Cutsem E, Geva R, Jäger D, Hara H, et al. Phase II Open-Label Study of Pembrolizumab in Treatment-Refractory, Microsatellite Instability–High/Mismatch Repair–Deficient Metastatic Colorectal Cancer: KEYNOTE-164. J Clin Oncol. 2019;JCO.19.02107.

21. Overman MJ, Lonardi S, Wong KYM, Lenz H-J, Gelsomino F, Aglietta M, et al. Nivolumab (NIVO) + low-dose ipilimumab (IPI) in previously treated patients (pts) with microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC): Long-term follow-up. J Clin Oncol. 2019;37:abstr 635.

22. Overman MJ, Bergamo F, McDermott RS, Aglietta M, Chen F, Gelsomino F, et al. Nivolumab in patients with DNA mismatch repair-deficient/microsatellite instability-high (dMMR/MSI-H) metastatic colorectal cancer (mCRC): Long-term survival according to prior line of treatment from CheckMate-142. J Clin Oncol. 2018;36:abstr 554.

23. Lenz H-JJ, Van Cutsem E, Limon ML, Wong KY, Hendlisz A, Aglietta M, et al. LBA18_PRDurable clinical benefit with nivolumab (NIVO) plus low-dose ipilimumab (IPI) as first-line therapy in microsatellite instability-high/mismatch repair deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC). Ann Oncol. 2018;29:mdy424.019.

24. Segal NH, Wainberg ZA, Overman MJ, Ascierto PA, Arkenau H-T, Butler MO, et al. Safety and clinical activity of durvalumab monotherapy in patients with microsatellite instability–high (MSI-H) tumors. J Clin Oncol. 2019;37:abstr 670.

25. Cohen R, Hain E, Buhard O, Guilloux A, Bardier A, Kaci R, et al. Association of Primary Resistance to Immune Checkpoint Inhibitors in Metastatic Colorectal Cancer With Misdiagnosis of Microsatellite Instability or Mismatch Repair Deficiency Status. JAMA Oncol. 2018;

26. Svrcek M, Lascols O, Cohen R, Collura A, Jonchère V, Fléjou J-F, et al. MSI/MMR-deficient tumor diagnosis: Which standard for screening and for diagnosis? Diagnostic modalities for the colon and other sites: Differences between tumors. Bull Cancer (Paris). 2019;106:119–28.

27. O'Neil BH, Wallmark JM, Lorente D, Elez E, Raimbourg J, Gomez-Roca C, et al. Safety and antitumor activity of the anti–PD-1 antibody pembrolizumab in patients with advanced colorectal carcinoma. PLOS ONE. 2017;12:e0189848.

28. Cohen R, Buhard O, Cervera P, Hain E, Dumont S, Bardier A, et al. Clinical and molecular characterisation of hereditary and sporadic metastatic colorectal cancers harbouring microsatellite instability/DNA mismatch repair deficiency. Eur J Cancer. 2017;86:266–74.

29. Salvador MU, Truelson MRF, Mason C, Souders B, LaDuca H, Dougall B, et al. Comprehensive Paired Tumor/Germline Testing for Lynch Syndrome: Bringing Resolution to the Diagnostic Process. J Clin Oncol Off J Am Soc Clin Oncol. 2019;37:647–57.

30. Kopetz S, André T, Overman MJ, Zagonel V, Lonardi S, Aglietta M, et al. Exploratory analysis of Janus kinase 1 (JAK1) loss-of-function (LoF) mutations in patients with DNA mismatch repair-deficient/microsat- ellite instability-high (dMMR/MSI-H) metastatic colorectal cancer (mCRC) treated with nivolumab plus ipilimumab in CheckMate-142. Proc Am Assoc Cancer Res. 2018;59:abstr 2603.

31. Sveen A, Johannessen B, Tengs T, Danielsen SA, Eilertsen IA, Lind GE, et al. Multilevel genomics of colorectal cancers with microsatellite instability-clinical impact of JAK1 mutations and consensus molecular subtype 1. Genome Med. 2017;9:46.

32. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. N Engl J Med. 2016;375:819–29.

33. Shin DS, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Hu-Lieskovan S, Kalbasi A, et al. Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations. Cancer Discov. 2017;7:188–201.

34. Middha S, Yaeger R, Shia J, Stadler ZK, King S, Guercio S, et al. Majority of B2M-Mutant and -Deficient Colorectal Carcinomas Achieve Clinical Benefit From Immune Checkpoint Inhibitor Therapy and Are Microsatellite Instability-High. JCO Precis Oncol. 2019;3.

35. Routy B, Chatelier EL, Derosa L, Duong CPM, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science. 2018;359:91–7.

36. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. Science. 2018;359:97–103.

37. Llosa NJ, Luber B, Siegel N, Awan AH, Oke T, Zhu Q, et al. Immunopathologic Stratification of Colorectal Cancer for Checkpoint Blockade Immunotherapy. Cancer Immunol Res. 2019;canimm.0927.2019.

38. Mandal R, Samstein RM, Lee K-W, Havel JJ, Wang H, Krishna C, et al. Genetic diversity of tumors with mismatch repair deficiency influences anti–PD-1 immunotherapy response. Science. 2019;364:485–91.

39. Schrock AB, Ouyang C, Sandhu J, Sokol E, Jin D, Ross JS, et al. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. Ann Oncol. 2019;30:1096–103.

40. Tachon G, Frouin E, Karayan-Tapon L, Auriault M-L, Godet J, Moulin V, et al. Heterogeneity of mismatch repair defect in colorectal cancer and its implications in clinical practice. Eur J Cancer Oxf Engl 1990. 2018;95:112–6.

41. Kim ST, Cristescu R, Bass AJ, Kim K-M, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med. 2018;1.

42. Campbell BB, Light N, Fabrizio D, Zatzman M, Fuligni F, de Borja R, et al. Comprehensive Analysis of Hypermutation in Human Cancer. Cell. 2017;171:1042-1056.e10.

43. Gerwing M, Herrmann K, Helfen A, Schliemann C, Berdel WE, Eisenblätter M, et al. The beginning of the end for conventional RECIST - novel therapies require novel imaging approaches. Nat Rev Clin Oncol. 2019;

44. Sehdev A, Cramer HM, Ibrahim AAM, Younger AE, O'Neil BH. Pathological complete response with anti-PD-1 therapy in a patient with microsatellite instable high, BRAF mutant metastatic colon cancer: a case report and review of literature. Discov Med. 2016;21:341–7.

45. Stadler ZK, Battaglin F, Middha S, Hechtman JF, Tran C, Cercek A, et al. Reliable Detection of Mismatch Repair Deficiency in Colorectal Cancers Using Mutational Load in Next-Generation Sequencing Panels. J Clin Oncol. 2016;34:2141–7.

46. Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by next- generation sequencing and compared with PD- L1 and tumor mutational burden in 11,348 patients. Cancer Med. 2018;7:746–56.

47. Ahn S-M, Ansari AA, Kim J, Kim D, Chun S-M, Kim J, et al. The somatic POLE P286R mutation defines a unique subclass of colorectal cancer featuring hypermutation, representing a potential genomic biomarker for immunotherapy. Oncotarget. 2016;7:68638–49.

48. Rayner E, Gool IC van, Palles C, Kearsey SE, Bosse T, Tomlinson I, et al. A panoply of errors: polymerase proofreading domain mutations in cancer. Nat Rev Cancer. 2016;16:71–81.

49. Bourdais R, Rousseau B, Pujals A, Boussion H, Joly C, Guillemin A, et al. Polymerase proofreading domain mutations: New opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency. Crit Rev Oncol Hematol. 2017;113:242–8.

50. Domingo E, Freeman-Mills L, Rayner E, Glaire M, Briggs S, Vermeulen L, et al. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. Lancet Gastroenterol Hepatol. 2016;1:207–16.

51. Guerra J, Pinto C, Pinto D, Pinheiro M, Silva R, Peixoto A, et al. POLE somatic mutations in advanced colorectal cancer. Cancer Med. 2017;6:2966–71.

52. Gong J, Wang C, Lee PP, Chu P, Fakih M. Response to PD-1 Blockade in Microsatellite Stable Metastatic Colorectal Cancer Harboring a POLE Mutation. J Natl Compr Canc Netw. 2017;15:142–7.

53. Santin AD, Bellone S, Buza N, Choi J, Schwartz PE, Schlessinger J, et al. Regression of Chemotherapy-Resistant Polymerase ε (POLE) Ultra-Mutated and MSH6 Hyper-Mutated Endometrial Tumors with Nivolumab. Clin Cancer Res Off J Am Assoc Cancer Res. 2016;22:5682–7.

54. Bhangoo MS, Boasberg P, Mehta P, Elvin JA, Ali SM, Wu W, et al. Tumor Mutational Burden Guides Therapy in a Treatment Refractory POLE-Mutant Uterine Carcinosarcoma. The Oncologist. 2018;23:518–23.

55. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348:69–74.

56. Chen EX, Jonker DJ, Loree JM, Kennecke HF, Berry S, Couture F, et al. CCTG CO.26: Updated analysis and impact of plasma-detected microsatellite stability (MSS) and tumor mutation burden (TMB) in a phase II trial of durvalumab (D) plus tremelimumab (T) and best supportive care (BSC) versus BSC alone in patients (pts) with refractory metastatic colorectal carcinoma (rmCRC). J Clin Oncol. 2019;suppl:abstr 3512.

57. Artyomenko A, Sikora M, Lefterova M, Raymond VM, Gavino D, Barbacioru C, et al. Microsatellite instability detection by targeted sequencing of cell-free DNA. Ann Oncol. 2018;29:viii400–41.

58. Wang F, Zhao Q, Wang Y-N, Jin Y, He M-M, Liu Z-X, et al. Evaluation of POLE and POLD1 Mutations as Biomarkers for Immunotherapy Outcomes Across Multiple Cancer Types. JAMA Oncol. 2019;

59. Gandara DR, Paul SM, Kowanetz M, Schleifman E, Zou W, Li Y, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat Med. 2018;24:1441–8.

60. Khagi Y, Goodman AM, Daniels GA, Patel SP, Sacco AG, Randall JM, et al. Hypermutated Circulating Tumor DNA: Correlation with Response to Checkpoint Inhibitor-Based Immunotherapy. Clin Cancer Res Off J Am Assoc Cancer Res. 2017;23:5729–36.

61. Angelova M, Charoentong P, Hackl H, Fischer ML, Snajder R, Krogsdam AM, et al. Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumor escape mechanisms and novel targets for immunotherapy. Genome Biol. 2015;16:64.

62. Angelova M, Mlecnik B, Vasaturo A, Bindea G, Fredriksen T, Lafontaine L, et al. Evolution of Metastases in Space and Time under Immune Selection. Cell. 2018;175:751-765.e16.

63. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome. Science. 2006;313:1960–4.

64. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou F-S, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet Lond Engl. 2018;391:2128–39.

65. Kwak Y, Koh J, Kim D-W, Kang S-B, Kim WH, Lee HS. Immunoscore encompassing CD3+ and CD8+ T cell densities in distant metastasis is a robust prognostic marker for advanced colorectal cancer. Oncotarget. 2016;7:81778–90.

66. Sun C, Mezzadra R, Schumacher TN. Regulation and Function of the PD-L1 Checkpoint. Immunity. 2018;48:434–52.

67. Becht E, Reyniès A de, Giraldo NA, Pilati C, Buttard B, Lacroix L, et al. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy. Clin Cancer Res. 2016;22:4057–66.

68. Karpinski P, Rossowska J, Sasiadek MM. Immunological landscape of consensus clusters in colorectal cancer. Oncotarget. 2017;8:105299–311.

69. Laurent-Puig P, Marisa L, Ayadi M, Blum Y, Balogoun R, Pilati C, et al. 60PDColon cancer molecular subtype intratumoral heterogeneity and its prognostic impact: An extensive molecular analysis of the PETACC-8. Ann Oncol [Internet]. 2018 [cited 2019 Nov 11];29. Available from: https://academic.oup.com/annonc/article/29/suppl_8/mdy269.058/5140786

70. Woolston A, Khan K, Spain G, Barber LJ, Griffiths B, Gonzalez-Exposito R, et al. Genomic and Transcriptomic Determinants of Therapy Resistance and Immune Landscape Evolution during Anti-EGFR Treatment in Colorectal Cancer. Cancer Cell. 2019;36:35-50.e9.

71. Jung B, Staudacher JJ, Beauchamp D. Transforming Growth Factor β Superfamily Signaling in Development of Colorectal Cancer. Gastroenterology. 2017;152:36–52.

72. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, et al. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. Nature. 2018;554:538–43.

73. McDermott DF, Huseni MA, Atkins MB, Motzer RJ, Rini BI, Escudier B, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. Nat Med. 2018;24:749–57.

74. Wallin JJ, Bendell JC, Funke R, Sznol M, Korski K, Jones S, et al. Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. Nat Commun. 2016;7:12624.

75. Manzoni M, Rovati B, Ronzoni M, Loupakis F, Mariucci S, Ricci V, et al. Immunological effects of bevacizumab-based treatment in metastatic colorectal cancer. Oncology. 2010;79:187–96.

76. Grasso CS, Giannakis M, Wells DK, Hamada T, Mu XJ, Quist M, et al. Genetic Mechanisms of Immune Evasion in Colorectal Cancer. Cancer Discov. 2018;8:730–49.

77. Xiao Q, Wu J, Wang W-J, Chen S, Zheng Y, Yu X, et al. DKK2 imparts tumor immunity evasion through β-catenin-independent suppression of cytotoxic immune-cell activation. Nat Med. 2018;24:262–70.

78. Fukuoka S, Hara H, Takahashi N, Kojima T, Kawazoe A, Asayama M, et al. Regorafenib plus nivolumab in patients with advanced gastric (GC) or colorectal cancer (CRC): An open-label, dose-finding, and dose-expansion phase 1b trial (REGONIVO, EPOC1603). J Clin Oncol. 2019;37:2522–2522.

79. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, et al. Immunogenic chemotherapy sensitizes tumors to checkpoint blockade therapy. Immunity. 2016;44:343–54.

80. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. Nat Rev Clin Oncol. 2018;15:325–40.

81. Holubec L, Polivka J, Safanda M, Karas M, Liska V. The Role of Cetuximab in the Induction of Anticancer Immune Response in Colorectal Cancer Treatment. Anticancer Res. 2016;36:4421–6.

82. Pelster MS, Amaria RN. Combined targeted therapy and immunotherapy in melanoma: a review of the impact on the tumor microenvironment and outcomes of early clinical trials. Ther Adv Med Oncol [Internet]. 2019 [cited 2019 Oct 25];11. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6384439/

83. Vanpouille-Box C, Formenti SC, Demaria S. Toward Precision Radiotherapy for Use with Immune Checkpoint Blockers. Clin Cancer Res. 2018;24:259–65.

84. Weintraub K. Take two: Combining immunotherapy with epigenetic drugs to tackle cancer. Nat Med. 2016;22:8–10.

85. Marin-Acevedo JA, Soyano AE, Dholaria B, Knutson KL, Lou Y. Cancer immunotherapy beyond immune checkpoint inhibitors. J Hematol OncolJ Hematol Oncol. 2018;11:1–25.

Table 1: Immune checkpoint inhibitors in MSI/dMMR metastatic colorectal cancer

	Prior systemic treatment	N	CR (%)	PR (%)	SD (%)	PD (%)	NE (%)	1-year PFS rate (%)	1-year OS rate (%)	2-year PFS rate (%)	2-year OS rate (%)	Median follow-up (months)
Keynote-016 [17]												
Pembrolizumab	≥ 1	28	11	46	32	4	7	-	-	-	-	8.7
Keynote-164 [18–20]												
Pembrolizumab, cohort A	≥ 2	61	0	28	23	46	3	34	-	-	-	13.2
Pembrolizumab, cohort B	≥ 1	63	3	29	25	40	3	41	-	-	-	12.6
CheckMate-142 [21–23]												
Nivolumab	≥ 1	74	9	24	31	31	5	44	-	-	-	21
Nivolumab + Ipilimumab	≥ 1	119	6	52	28	12	3	71	85	60	74	25.4
Nivolumab + Ipilimumab	0	45	7	53	24	2	0	77	83	-	-	13.8

***CD-ON*-MEDI4736-1108** [24]								
Durvalumab	≥ 1	36	-	22	38		54	29
NCT02227667 [24]								
Durvalumab	≥ 1	11	-	27	36			30

1 CR: complete response; PR: partial response; SD: stable disease; NE: not evaluable; PFS: progression-free survival; OS: overall survival

Table 2: Randomized trials with immune checkpoint inhibitors for MSI colorectal cancer

Study name	NCT number	Study type	Setting	Disease	Intervention	Status
KEYNOTE-177	NCT02563002	Phase III	First line	Metastatic colorectal cancer	Pembrolizumab (anti-PD1) Standard-of-care chemotherapy (mFOLFOX6 or FOLFIRI alone or in combination with bevacizumab or cetuximab)	Closed to recruitment
COMMIT	NCT02997228	Phase III	First line	Metastatic colorectal cancer	Atezolizumab (anti-PD-L1) Atezolizumab + mFOLFOX6 + bevacizumab mFOLFOX6 + bevacizumab	Recruiting
CA209-8HW	NCT04008030	Phase III		Metastatic colorectal cancer	Nivolumab Nivolumab and ipilimumab	Recruiting

					Investigator's Choice chemotherapy ± targeted therapy	
PRODIGE 54 - SAMCO	NCT03186326	Randomized phase II	Second line	Metastatic colorectal cancer	Avelumab (anti-PD-L1) Standard-of-care chemotherapy (mFOLFOX6 or FOLFIRI alone or in combination with bevacizumab or cetuximab)	Recruiting
Alliance A021502	NCT02912559	Phase III	Adjuvant	Stage III colon cancer	Atezolizumab (anti-PD-L1) + mFOLFOX6 mFOLFOX6	Recruiting
POLEM	NCT03827044	Phase III	Adjuvant	Stage III colon cancer (MSI or POLE-mutated)	Avelumab (anti-PD-L1) + CAPOX 3 months or capecitabine 6 months CAPOX 3 months or capecitabine 6 months	Recruiting

1

Table 3: Emerging biomarkers predicting the activity / resistance to immune checkpoint blockade in patients with MSI/dMMR and MSS/pMMR metastatic colorectal cancer

Population	Biomarker	Outcomes	References
dMMR	High frameshift mutation load	Associated with Response and PFS	[38]
	High TMB	Associated with response and OS	[39]
	MSIsensor score	Associated with response and OS	[38]
	RAS and *BRAF* mutations	Not associated with resistance or benefit	[1,10,11]
	Germline MMR mutations	Not associated with resistance or benefit	[1,10,11]
	JAK mutations	Associated with resistance?	[30]
	HLA class I and *B2M* mutations	Controversial, not associated with outcome	[34]
	Immunoscore®, immune profile	Higher CD8 infiltration associated with better response	
pMMR	High blood TMB	Associated with better survival	[56]
	PD-L1	No benefit associated with PD-L1 expression by tumor cells	[27]
	Immunoscore®	No data	
	CMS classification	No data	

B2M: beta-2-microglobulin; CMS: consensus molecular subtype; dMMR: mismatch repair-deficient; OS: overall survival; PFS: progression-free survival; pMMR: mismatch repair-proficient; TMB: tumor mutation burden

Table 4: Ongoing immunotherapy clinical trials for metastatic colorectal cancer beyond MSI

Clinical trials identifiers	Study phase	Intervention	Target
Combination of ICIs			
NCT03867799	II	Nivolumab + relatlimab	PD1, LAG3
NCT03642067	II	Nivolumab + relatlimab	PD1, LAG3
ICIs and chemotherapy [79]			
NCT03202758	I-II	Durvalumab + tremelimumab + FOLFOX	PDL1, CTLA4
NCT03626922	I	Pembrolizumab + oxaliplatin + pemetrexed	PD1
ICIs and antiangiogenics / vascular disrupting agents [80]			
NCT03647839	II	Nivolumab + BNC105 or nivolumab + BBI608	PD1, STAT3, disrupting agent
NCT04126733	II	Nivolumab + regorafenib	PD1
NCT03396926	II	Pembrolizumab + capecitabine + bevacizumab	PD1
NCT04110093	I-II	Nivolumab + regorafenib	PD1
NCT03946917	I-II	JS001 + regorafenib	PD1
NCT02298959	I	Pembrolizumab + ziv-aflibercept	PD1
ICIs and anti-EGFR agents and/or tyrosine kinase inhibitors [81,82]			
NCT03608046	II	Avelumab + cetuximab + irinotecan	PDL1, EGFR

NCT03442569	II	Nivolumab + ipilimumab + panitumumab	PD1, CTLA4, EGFR
NCT03428126	II	Duvalumab + trametinib	PDL1, MEK
NCT04044430	I-II	Nivolumab + encorafenib + binimetinib	PD1, BRAF, MEK
NCT04017650	I-II	Nivolumab + encorafenib + cetuximab	PD1, BRAF, EGFR
NCT03711058	I-II	Nivolumab + copanlisib	PD1, PI3K
ICIs and radiotherapy [83]			
NCT03101475	II	Radiofrequency ablation or sterotactic body radiotherapy + durvalumab + tremelimumab	PDL1, CTLA4
NCT03927898	II	Stereotactic body radiotherapy + toripalimab	PD1
NCT04030260	II	Radiotherapy + regorafenib + nivolumab +/- irinotecan	PD1
NCT04108481	I-II	Yttrium-90 radioembolization + durvalumab	PDL1
ICIs and DNA methylation agents [84]			
NCT03832621	II	Nivolumab + ipilimumab + temozolomide	PD1, CTLA4, MGMT
Immunotherapies beyond ICIs [85]			
NCT03950154	III	CAPOX + bevacizumab +/- PD-1 monoclonal antibody-activated autologous peripheral blood T-lymphocyte (PD1-T)	T cells

NCT04119830	II	Rintatolimod and pembrolizumab	PD1, immunomodulation
NCT03222089	II	FOLFOXIRI + interleukin-2 + GM-CSF	IL2
NCT03631407	II	Vicriviroc + pembrolizumab	CCR5 receptor, PD1
NCT03206073	I-II	Pexa-Vec Oncolytic Virus + durvalumab + tremelimumab	Antitumor virus, PDL1, CTL4
NCT04068610	I-II	FOLFOX + bevacizumab + durvalumab + oleclumab	PDL1, CD73
NCT02834052	I-II	Poly-ICLC + pembrolizumab	PD1, immunomodulation
NCT03555149	I-II	Atezolizumab + Imprime PGG + bevacizumab, or atezolizumab + isatuximab, or atezolizumab + selicrelumab + bevacizumab, or atezolizumab + idasanutlin	Immunomodulation, CD-38, CD-40, MDM2
NCT04062721	I-II	Radiofrequency ablation + chemotherapy + in situ hydrogel with TLR agonist and GM-CSF	TLR
NCT03507699	I	Liver radiation therapy + nivolumab + ipilimumab + intra-tumoral CMP-001	PD1, PDL1, TLR9
NCT03720678	I	FOLFOX + AB928	Adenosine receptor
NCT03692429	I	FOLFOX + Allogeneic NKG2D-based CYAD-101 Chimeric Antigen Receptor T-cells	NKG2D

NCT03256344	I	Talimogene Laherparepvec + atezolizumab	Antitumor vaccine, PDL1
NCT03866239	I	Obitunuzumab, followed by cibisatamab + atezolizumab + tocilizumab	CD20, PDL1, CEA-positive tumor cells and CD3+ T-cells, IL6-R

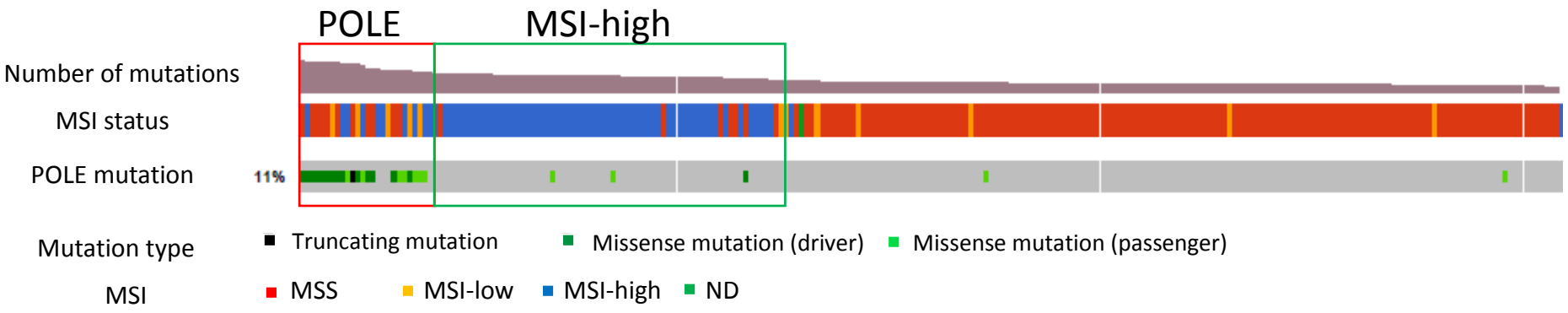
1

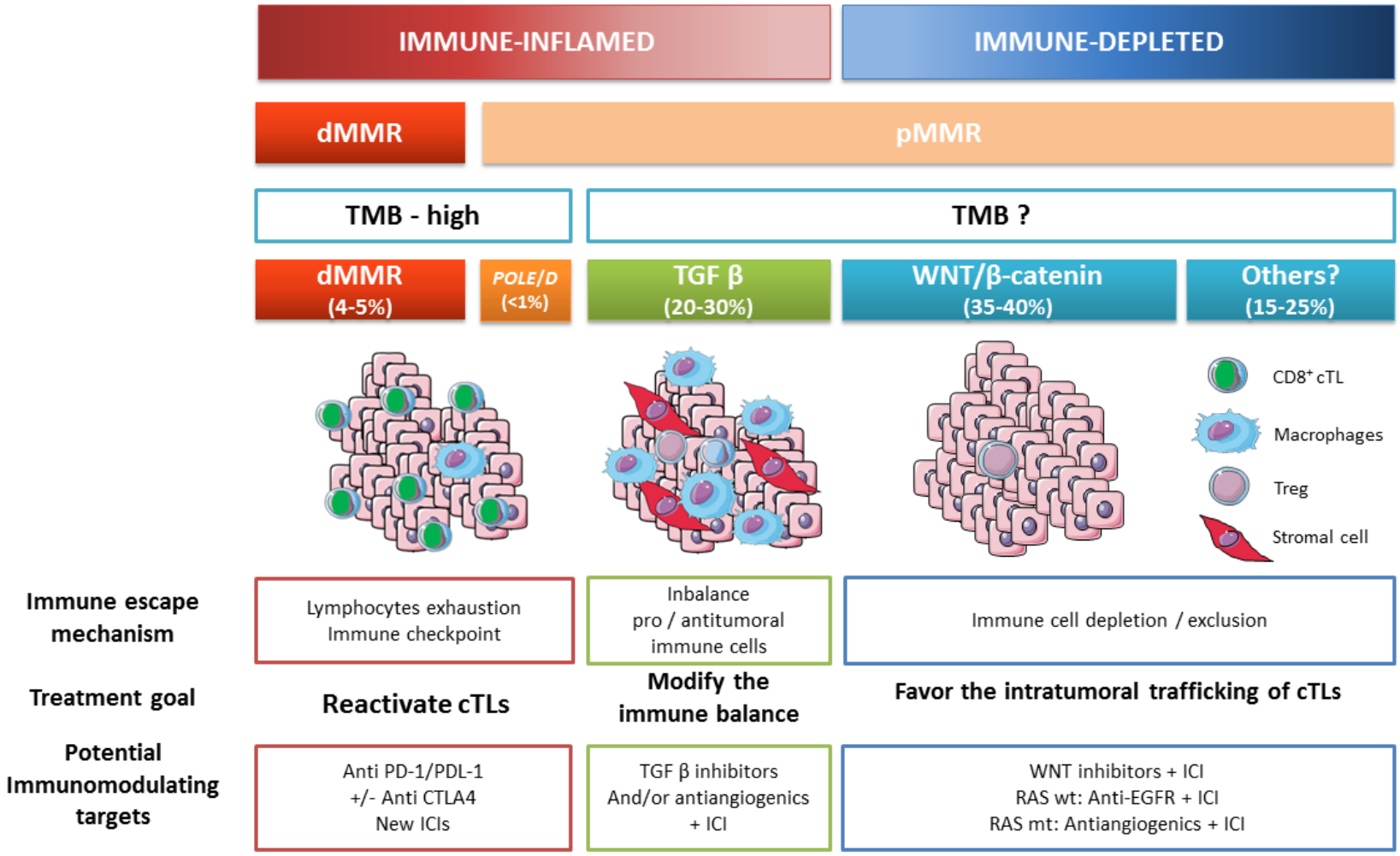
FIGURE CAPTIONS

Figure 1: Association of *Polymerase E* mutations with MSI status and hypermutability in colorectal cancer (data from the The Cancer Genome Atlas)

Figure 2: Immune classification of colorectal cancer subtypes based on molecular pathways alteration

Figure 1: Association of *Polymerase E* mutations with MSI status and hypermutability in colorectal cancer (data from the The Cancer Genome Atlas)




IMMUNE-INFLAMED
IMMUNE-DEPLETED
dMMR
pMMR
TMB - high
TMB ?
dMMR (4-5%)
POLE/D (<1%)
TGF β (20-30%)
WNT/β-catenin (35-40%)
Others? (15-25%)
CD8+ cTL
Macrophages
Treg
Stromal cell
Immune escape mechanism
Lymphocytes exhaustion
Immune checkpoint
Inbalance
pro / antitumoral
immune cells
Immune cell depletion / exclusion
Treatment goal
Reactivate cTLs
Modify the immune balance
Favor the intratumoral trafficking of cTLs
Potential Immunomodulating targets
Anti PD-1/PDL-1
+/- Anti CTLA4
New ICIs
TGF β inhibitors
And/or antiangiogenics
+ ICI
WNT inhibitors + ICI
RAS wt: Anti-EGFR + ICI
RAS mt: Antiangiogenics + ICI