Results and challenges of immune checkpoint inhibitors in colorectal cancer

Sheik Emambux, Gaelle Tachon, Audelaure Junca & David Tougeron

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Results and challenges of immune checkpoint inhibitors in colorectal cancer

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ABSTRACT

Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide and clinical outcome has improved substantially during the last two decades with targeted therapies. The immune system has a major role in cancers, especially the CD8 + T cells specific to tumor antigens. However, tumors can escape immune response by different mechanisms including upregulation of inhibitory immune checkpoint receptors, such as well-known Programmed cell Death protein-1 (PD-1)/Programmed cell Death Ligand 1 (PD-L1) interaction, leading CD8+ T cells to a state of anergy. Immunotherapy, with the so-called immune checkpoint inhibitors (CPIs), has recently been approved in treatment of multiple cancers due to its prolonged disease control and acceptable toxicities. The recent groundbreaking success involving anti-PD-1 CPIs in metastatic CRC with deficient mismatch repair system (dMMR) is promising, with several trials ongoing. Major challenges are ahead in order to determine how, when and for which patients we should use these CPIs in CRC.

Areas covered: This review highlights some promises and challenges concerning personalized immunotherapy in CRC. First results and ongoing breakthrough trials are presented. The crucial role of biomarkers in selecting patient is also discussed.

Expert opinion: As of now, dMMR and POLE mutations (DNA polymerase ε) with ultramutator phenotype are the most powerful predictive biomarkers of CPI efficacy. The most challenging issue is pMMR mCRC and determination of how to convert a ‘nonimmunogenic’ neoplasm into an ‘immunogenic’ neoplasm, a combination of CPIs with radiation or MEK inhibitor probably being the most relevant strategy. Next-generation sequencing (NGS) assays to quantify mutational load could be more reliable predictive biomarkers of CPIs efficacy than PD-L1 expression or immune scores.

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide, with 1.4 million new cases in 2012 and a leading cause of death, with 694,000 deaths in 2012. More precisely, it is the third most common cancer in men (746,000 cases) and the second in women (614,000 cases) worldwide [1]. It is estimated that about 50% of CRCs become metastatic during their evolution. Unfortunately, most patients cannot undergo resection of their metastases because of unresectable disease and/or comorbidities. Clinical outcome has nonetheless improved over the past 20 years. Chemotherapy with biological agent remains the standard of care. Today, median overall survival (OS) for patients with metastatic colorectal cancer (mCRC) is around 30 months for those harboring a RAS wild-type status versus 26 months for those harboring a RAS mutated mCRC [2–4]. Median first-line progression-free survival (PFS) is around 8–11 months, which is more than double compared to 20 years ago [2–4]. Many factors have contributed to improved outcome: efficacy of systemic therapies, new drugs, improved surgical techniques, and supportive care. Two biological agents are primarily used in mCRC, anti-vascular endothelial growth factor [5] and/or anti-epithelial growth factor receptor (anti-EGFR) [6] monoclonal antibodies (mAbs).

The immune system has a major role in cancer, especially as a suppressor of tumor initiation and progression. Immune cells, notably CD8+ T cells, search and destroy pre-cancerous/cancerous cells. However, tumors can escape immune response by different mechanisms including upregulation of the immunoinhibitory molecules like ligands of co-inhibitory immune checkpoint receptors, leading T cells to a state of anergy. Inhibition of the well-known Programmed cell Death protein-1 (PD-1)/Programmed cell Death Ligand 1 (PD-L1) interaction could restore T-cell activity against tumor cells and is currently the subject matter of intense cancer research. Indeed, immunotherapy is a more targeted, potentially less toxic therapy, and the so-called immune checkpoint inhibitors (CPIs) have recently been approved in multiple cancers such as melanoma, lung cancer, and bladder and head and neck tumors [7]. The recent ground-breaking success of immunotherapy [8] with anti-programmed cell death protein-1 (anti-PD-1) CPI, pembrolizumab, in mCRC patient subgroups is promising with several phase III trials ongoing (Table 1). The subset of patients with a clinical response consists in those harboring a tumor microsatellite instability-high (MSI-H) phenotype, also called deficient DNA mismatch repair (dMMR) CRC. This review will...
Immunotherapy combined with other treatment modalities is a major focus on the challenges with personalized antibody-based immunotherapy in CRCs, particularly dMMR CRCs.

1.1. Colorectal cancers and microsatellite instability

1.1.1. Microsatellite instability

Colorectal carcinogenesis is characterized by three major mechanisms, namely chromosomal instability, microsatellite instability (MSI), and CpG island methylator phenotype (CIMP). MSI occurs in approximately 15% of all CRCs [10]. This phenotype is due to a deficit in one or more mismatch repair (MMR) proteins resulting in error accumulation in microsatellite regions. Microsatellites or ‘short-tandem repeats’ are defined as repeats of the same base or sequence of bases, with unit length ranging from one to six bases [11]. They are scattered throughout the coding and noncoding regions of the genome. The core components of MMR machinery are two heterodimers consisting of MSH2/MSH6 and MLH1/PMS2.

Tumors with a defect in any one of these components develop frameshift mutations leading to truncated protein products [11]. dMMR CRCs due to germline mutation in one of the MMR genes account for =2.5% of all CRCs and are defined as Lynch syndrome [12]. Germline deletions in EPCAM gene (epithelial cell adhesion molecule) also cause Lynch syndrome via epigenetic inactivation of MSH2 [13]. Sporadic dMMR CRCs account for the remaining =12.5% and usually arise from epigenetic silencing of the MLH1 promoter, associated with CIMP phenotype and BRAF V600E mutation [10]. dMMR tumors are commonly characterized by a high level of tumor-infiltrating lymphocytes, Crohn-like lymphocytic reaction, poor differentiation, and mucinous components [14]. Moreover, dMMR neoplasms tend to arise from sessile serrated adenomas in the proximal colon [15].

The Association of Molecular Pathology recommends that all new CRC cases be subjected to parallel testing for MSI, BRAF V600E mutation, and immunohistochemistry (IHC) for the four MMR proteins [12]. Other guidelines recommend MSI analysis or MMR IHC only in CRC patients younger than 60 years or with medical history evoking Lynch syndrome [16]. Due to the high efficacy of CPIs in dMMR tumors, this testing will soon become universal in CRCs and probably other tumors. It is worth noting that MSI phenotype has also been observed in many digestive tumors (gastric, small bowel, etc.), and non-digestive tumors (endometrium, ovarian, glioblastoma, etc.) in the context of Lynch syndrome and sporadic cases as well. For MSI testing, the 1997 Bethesda guidelines recommend a reference panel of five microsatellites for testing. MSI-H phenotype is defined as a shift in size of at least two of the five microsatellite loci in tumor relative to normal, or of 30% or more loci when larger panels are tested [17]. For MMR IHC, the four MMR proteins should be tested (MSH2, MSH6, MLH1, and PMS2).

### 1.1.2. Outcome

Concerning clinical outcome, dMMR CRCs represent 15–20% of stage II and III CRCs and are associated with better prognosis than pMMR tumors [18]. Fluoropyrimidine-based chemotherapy is not indicated in the adjuvant setting for

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Table 1. Key immunotherapy trials in metastatic colorectal cancer (adapted from Boland and Ma [9]).

<table>
<thead>
<tr>
<th>Trials</th>
<th>Drugs</th>
<th>Target</th>
<th>Population</th>
<th>Patients</th>
<th>Response rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le et al. [8]</td>
<td>Pembrolizumab</td>
<td>PD-1 Refractory dMMR CRC</td>
<td>25</td>
<td>57%</td>
<td>Sporadic cases: 100% (n = 6/6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>LS cases: 27% (n = 3/11)</td>
</tr>
<tr>
<td>Overman et al. [58]</td>
<td>Nivolumab</td>
<td>PD-1 Refractory dMMR CRC</td>
<td>74</td>
<td>31%</td>
<td>Sporadic cases: 36% (n = 10/36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LS cases: 30% (n = 8/27)</td>
</tr>
<tr>
<td>Andre et al. [63]</td>
<td>Nivolumab + ipilimumab</td>
<td>PD-1 + CTLA-4 Refractory dMMR CRC</td>
<td>27</td>
<td>41%</td>
<td></td>
</tr>
<tr>
<td>Le et al. [8]</td>
<td>Pembrolizumab</td>
<td>PD-1 Refractory pMMR CRC</td>
<td>28</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Andre et al. [63]</td>
<td>Nivolumab + ipilimumab</td>
<td>PD-1 + CTLA-4 Refractory pMMR CRC</td>
<td>20</td>
<td>5%</td>
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</tr>
<tr>
<td>Chung et al. [59]</td>
<td>Tremelimumab</td>
<td>CTLA-4 Refractory CRC</td>
<td>49</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Topalian et al. [60]</td>
<td>Nivolumab</td>
<td>PD-1 Refractory CRC</td>
<td>19</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Brahmer et al. [61]</td>
<td>BMS-936559</td>
<td>PD-L1 Refractory CRC</td>
<td>18</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Bendell et al. [91]</td>
<td>Atezolizumab + bevacizumab</td>
<td>PD-L1 Refractory CRC</td>
<td>14</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Bendell et al. [91]</td>
<td>Atezolizumab + FOLFOX/bev</td>
<td>PD-L1 Metastatic CRC (70% first line)</td>
<td>30</td>
<td>40% (total)</td>
<td>48% (first-line)</td>
</tr>
<tr>
<td>Bendell et al. [92]</td>
<td>Atezolizumab + cobimetinib</td>
<td>PD-L1 MEK Refractory CRC (30% pMMR, 70% unknown)</td>
<td>23</td>
<td>17% (3 pMMR, 1 unknown)</td>
<td></td>
</tr>
</tbody>
</table>
patients with stage II dMMR CRC due to excellent prognosis and 5-FU chemoresistance [19,20]. Concerning oxaliplatin, stage III dMMR CRCs are chemosensitive to oxaliplatin but the data concerning high-risk stage II lack the power to justify a definitive conclusion [10]. In the metastatic setting, dMMR CRCs represent only around 5% of metastatic CRCs and are associated with a poor prognosis [18]. A pooled analysis of four phase III studies in first-line treatment of mCRC (CAIRO, CAIRO2, COIN, and FOCUS) was performed by Venderbosch and colleagues [21], and found that median PFS and OS were significantly worse for patients with dMMR compared with proficient MMR (pMMR) tumors (HR, 1.33; 95% confidence interval [CI], 1.12–1.57 and HR, 1.35; 95% CI, 1.13–1.61, respectively). Recent results presented at the 2017 ASCO meeting confirm that dMMR mCRC are associated with poor prognosis (OS at 17.9 months) and chemoresistance (PFS at 3.9 months), whatever the chemotherapy regimen or targeted therapy used (bevacizumab or anti-EGFR) [22].

1.2. Immune system

1.2.1. Role

The immune system has a major role in cancer, from tumorigenesis to its treatment. Immune cells can act both as suppressors of tumor initiation and progression and as promoters of proliferation, infiltration, and metastasis [23]. In addition, the immune system can influence the efficacy of chemotherapy and biological agents, an example being the anti-EGFR mAbs used in mCRC [24]. Conversely, drugs such as oxaliplatin or radiation can activate the immune system against cancer cells [25].

1.2.2. Tumor microenvironment

The tumor microenvironment contains different types of cells, including innate and adaptive immune cells. The innate cells consist of macrophages, mast cells, neutrophils, dendritic cells, myeloid-derived suppressor cells, and natural killer (NK) cells. Dendritic cells (Figure 1) have a central role in antigen cross-presentation [26]. NK cells play an important role by causing direct cytotoxicity in cancer cells that do not express any major histocompatibility complex (MHC) class I alleles. MHC class I loss is a frequent mechanism of immune escape in cancer and this characteristic renders these cells more prone to NK killing [27]. Adaptive cells T and B lymphocytes interact directly with the tumor or through chemokine and cytokine signaling [23]. The adaptive T cells which have an important role in cancer immunosurveillance include CD8+ cytotoxic T lymphocytes (CTLs), CD4+ Th1 cells, CD4+ Th17 cells, and regulatory T cells (Tregs) [23].

1.2.3. Immunoediting

The ‘three Es of cancer immunoediting’ refer to elimination, equilibrium, and escape. The first ‘elimination’ phase reflects active immunosurveillance, which facilitates tumor eradication and is mostly mediated by tumor-associated antigen-specific lymphocytes and NK cells. As immune cells search and destroy pre-cancerous cells, they select tumor cells displaying decreased tumor immunogenicity. The second ‘equilibrium’ phase refers to the period during which tumor growth is still prevented by the host immune system even though the surviving tumor and its stroma are also shaped by the immune response. The immune cells gradually become unable to eliminate all the cancer cells even though they can prevent expansion and metastasis in the ‘equilibrium phase’. Then comes the third ‘escape' phase, during which dynamic interaction between the immune system and the tumor selects tumor cells leads to the development of clinically apparent tumors [23].

Antigen cross-presentation: Central role of the dendritic cell

Figure 1. Dendritic cells and antigen cross-presentation.


Antigen cross-presentation: Dendritic cells are able to capture exogenous antigens through endocytosis and present them to CD8+ cytotoxic T cells via MHC I.
Tumors use multiple mechanisms to escape immune-mediated rejection. Tumors form a microenvironment that actively suppresses immune response. Immunosuppressive factors such as transforming growth factor beta (TGF-β) are released by the cancer cells in order to prevent destruction of the tumor by CTLs and NK cells. Also, Tregs and cells known as myeloid-derived suppressive cells (MDSC) are recruited, thereby evading lymphocyte-induced death [28]. Moreover, innate immune cells such as macrophages and neutrophils stimulate the process of new vasculature formation, following proinflammatory signals triggered by necrotic cell death. Interleukin-1 (IL-1) is one of these signals that promote angiogenesis and the homing of immune cells [29]. In addition to the immunosuppressive microenvironment, other mechanisms to escape or evade tumor immune response include loss of antigenicity and loss of immunogenicity. Tumors express a variety of antigens with the potential to elicit tumor-specific immune responses. However, to avoid immune-mediated elimination, cancer cells may lose their antigenicity. Loss of antigenicity may be achieved through the acquisition of defects in antigen processing and presentation (e.g. loss of MHC expression or dysregulation of antigen-processing machinery) or immune selection of cancer cells which lack or mutate immunogenic tumor antigens. Moreover, malignant cells can gain additional immunosuppressive properties, such as overexpression of PD-L1 or secretion of suppressive cytokines (e.g. IL-10), which further reduce their immunogenicity.

1.3. Colorectal cancer and the immune microenvironment

1.3.1. Immune response

The role of the immune system in regulating the development of CRC is well established. First of all, immune response has a crucial role in the control of tumoral progression in CRC; a close link between the rate of tumor-infiltrating lymphocytes (TIL) and prognosis has been found [30]. High densities of CD8+ and CD45RO+ cells predict decreased tumor recurrence and improved survival in patients with early-stage disease [31]. Moreover, node-negative CRC exhibits an increasing percentage of CD3+ immunoreactive areas that reduce the risk of metachronous tumors whereas high densities of CD3+ TILs are not associated with the absence of postsurgical metastasis in patients with node-positive CRC, a factor suggesting the importance of immune evasion in CRC [32].

CD4 T-helper cells as well as activated and cytotoxic CD8+ TILs are significantly increased in CRC tumor tissue compared to normal mucosa [33]. Increased activation, cytotoxic activity, and functional reactivity of TILs have been correlated with the presence of functional tumor antigen-reactive T cells in the blood and bone marrow. Earlier tumor stages have shown higher proportions of activated CD8+ TILs, suggesting that early-stage CRC may be recognized and undergo surveillance by the immune system. Several tumoral antigens that could induce an immune response in CRC have been identified. The carcinoembryonic antigen is the most widely studied [34] but its immunogenicity remains low. In dMMR CRC, due to frameshift mutations leading to truncated protein products, several immunogenic neo-antigens are generated and explain the high level of TILs in these tumors [35]. For example, CTLs specific to neo-antigens derived from TGFβRII frameshift mutation have been identified in dMMR CRC harboring this mutation [36].

1.3.2. Immune escape

However, as with other tumors, CRC can escape immune response. Frequent mechanisms in CRC include loss of MHC class I expression, dysregulation of antigen processing machinery, upregulation of the immunoinhibitory molecules, production of immunosuppressive molecules (i.e. indoleamine 2,3-dioxygenase), and recruitment of immunosuppressive cells (i.e. Tregs) [37,38]. All of these data should be considered in the development of immunotherapy strategies in CRC. CRC tumor cells release immunosuppressive factors like TGF-β and immunosuppressive immune cells, such as Tregs and MDSC, which primarily contribute to cancer cell evasion from lymphocyte-induced death. The case of FoxP3+ Tregs is complex and varies across tumor type, but in CRC they inhibit the local inflammatory processes that promote carcinogenesis [39]. A shift in CD4 T cells Th1 to Th2 immune responses (production of immunosuppressive molecules) is frequently observed in CRC microenvironment. An alteration of MHC I expression is observed in more than 70% of the CRCs from the early stages, as well as loss of expression of co-stimulatory molecules. The CRC tumor cells can also escape the immune response by developing mechanisms of resistance to lysis, especially by the loss of membrane expression of Fas and TP53 or caspase genes mutations. Finally, another mechanism in T-cell deletion is the expression of Fas-Ligand (FasL) which is upregulated in colon cancer and induces apoptosis of Fas-expressing T lymphocytes [40].

1.3.3. Nutrition and the immune system

The link between CRC carcinogenesis and obesity/overfeeding/Western-style diet is already well known but the mechanisms involved are only recently in the process of being identified and are partially associated with immune response. These nutritional parameters are associated not only with chronic subclinical inflammation (i.e. proinflammatory cytokines, M1 proinflammatory macrophages, etc.) but also with immunological abnormalities (i.e. decrease of cytotoxic CD8+ T cells, diminished dendritic-cell function, etc.) that promote carcinogenesis [41]. In contrast, nutritional interventions can enhance cancer treatment efficacy through immune-related mechanisms that enhance the adaptive immune response to tumor cells such as an increase in infiltrating cytotoxic CD8+ T lymphocytes and antigen-presenting myeloid cells and a decrease in immunosuppressive Foxp3+ Tregs. Nevertheless, to our knowledge, there is no data concerning the impact of nutritional interventions on CPI efficacy.

1.3.4. Microbiome and the immune system

Recent data suggest a strong link between microbiome and cancer carcinogenesis, especially in CRC. The most relevant mechanism for bacterially driven carcinogenesis is barrier failure, which results in increased microbiota–host interactions enhancing proliferation and DNA alteration [42]. In experimental colorectal carcinogenesis, bacterial translocation was detected at sites of tumor initiation, and eradication
of the bacterial microbiota by antibiotics reduced CRC development [42,43]. Several authors have reported interesting results indicating that colon cancer might be a bacteria-related disease, due mostly to interaction with local/systemic immune responses [42,44,45]. Several mechanisms are involved, including chronic mucosal inflammation, activation of Tregs, and overexpression of pro-inflammatory helper T cell (Th17) [46]. Chemotherapy and radiation could induce immunogenic cell death by promoting the presentation of tumor antigens that might provoke an adaptive immune response. While oxaliplatin, anti-angiogenic agents, and radiation, commonly used in CRC, possess such immunogenic properties, the true magnitude of this effect remains unclear [47]. When combined, FOLFOX and bevacizumab may decrease granulocytic MDSCs and increase Th17 T-cell frequency, rendering a favorable micro-environment for immune CPI treatment [48].

1.3.5. Radiation therapy and the immune system
For years, rare abscopal responses have been described with radiotherapy, and more recently, pre-clinical models suggest that molecules such as PD-1 can prevent abscopal responses [49]. Abscopal effect refers to a tumor regression at a site distant from the primary site of radiotherapy. Local radiation induces immunogenic cell death and systemic immunemediated anti-tumor effects, in particular by promoting recruitment and activation of T cells within the tumor microenvironment [50]. Nevertheless, this phenomenon has rarely been observed in routine clinical practice when radiation therapy is administered alone. With the advent of immune modifiers, however, this effect has been increasingly reported in both preclinical models and patients. The combination of local radiotherapy and immune-modulator can increase local tumor control and cause distant anti-tumor effects (abscopal effects) through diverse mechanisms: increased tumor-antigen release and cross-presentation, enhanced dendritic-cell function, improved T-cell priming, increased trafficking of lymphocytes into the tumor microenvironment, and induction of positive immunomodulatory pathways [51,52].

1.4. Immune checkpoint inhibitors and efficacy in colorectal cancer
1.4.1. Checkpoint inhibitors
Immunotherapeutic approaches have been aimed at either enhancing anti-tumor immune response through strategies such as vaccination in combination with immune stimulatory cytokines or preventing the suppression of a response using so-called CPIs. Hereby, we will review advances in immunotherapy with the CPIs in metastatic CRC (mCRC). Two triggering signals are required to initiate adaptive immune response by T cells: MHC-antigen peptide recognition by the T-cell receptor and co-stimulation via an array of receptors interacting with cognate ligands on antigen-presenting cells (APCs), thereby avoiding T-cell anergy [18]. CD28 is an example of co-stimulatory (positive) molecules and is constitutively expressed on the T-cell surface. It binds to B7.1 (CD80) or B7.2 (CD86), which are expressed on APCs and provide the co-stimulatory signals required for T-cell activation and survival [53]. In contrast, B7 molecules also interact with cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), which is expressed on T cells, to which it transmits an inhibitory signal. In many cancers, ligands of co-inhibitory immune checkpoint receptors are up-regulated in cancer cell and/or tumor micro-environment, leading to T-cell functional exhaustion and unresponsiveness (a state of anergy) and therefore to the loss of tumor growth control (tumor escape). Well-known immune checkpoints include B7/CTLA-4, PD-L1/PD-1, MHC class II/lymphocyte activation gene 3 (LAG-3), galectin-9/T-cell immuno-globulin, and mucin containing protein-3 (TIM-3). These interactions allow negative feedbacks and, using this rationale, several CPIs have been developed for cancer treatment (mAbs blocking co-inhibitory immune checkpoint receptors or their ligands). Through its ligand, Galectin-9, TIM-3 is believed to play a critical role in inhibiting Th1 responses and inducing cell death; an increase in Tim-3+PD-1+CD8+ T cells has been observed in CRC tissue [54]. Another interesting target for immune checkpoint blockade is LAG-3, also known as CD223, which is also overexpressed in CRC. Through its interaction with MHC class II, it has been demonstrated that LAG-3 plays a pivotal role in negative regulation of T-cell proliferation. There are many other known immune checkpoints (OX40, 4-1BB, CD40L, CD27) or that have yet to be discovered.

1.4.2. Efficacy of checkpoint inhibitors
The first successful results with CPIs (anti-PD-1 and anti-CTLA-4) have been observed in patients with advanced melanoma and non-small cell lung cancer (NSCLC) [55–57]. Le DT and colleagues [8] conducted a phase 2 study evaluating the clinical activity of pembrolizumab, an anti-PD-1, in 41 patients with chemoresistant metastatic adenocarcinoma with or without MMR deficiency. The cohort included 11 patients with dMMR mCRC, 9 patients with dMMR non-CRC, and 21 patients with pMMR mCRC. Only 10 patients in the dMMR mCRC cohort and 18 in the pMMR mCRC cohort could be evaluated for objective response. The immune-related objective response rate (ORR) was higher in dMMR mCRC (40% [95% confidence interval (CI) 12–74] versus 0% [95% CI 0–19]) as was the PFS rate at 20 weeks (78% [95% CI 40–97] versus 11% [95% CI 1–35]). Both PFS and OS were not reached in the dMMR mCRC compared to a median PFS of 2.2 months (95% CI, 1.4–2.8) and median OS of 5.0 months (95% CI, 3.0 to not estimable) in the pMMR cohort with a hazard ratio for PFS of 0.10 (p < 0.001), and a hazard ratio for OS of 0.22 (p = 0.05). Furthermore, all patients with sporadic dMMR tumors had an objective response, whereas only 3 of 11 patients (27%) with tumors associated with the Lynch syndrome had a response (p = 0.009). We have no explanation for the difference between sporadic versus Lynch cases but larger cohorts described below do not confirm this finding. Indeed, whatever the mechanism of inactivation of the MMR system, the consequences are the same, i.e. a hypermutated tumor that should be sensitive to immunotherapy. Analysis of whole-exome sequencing showed a mean of 1782 somatic mutations per tumor in the dMMR CRC cohort, as compared with 73 mutations per tumor in the pMMR CRC cohort (p = 0.007).
High numbers of somatic mutations and potential mutation-associated neoantigens were associated with longer PFS ($p = 0.018$). Of note, the expression of CD8 and PD-L1 in tumors was significantly associated neither with PFS nor with OS. Other trials confirm the ineffectiveness of CPIs in pMMR CRC, notably atezolizumab (anti-PD-L1), nivolumab (anti-PD1), and tremelilumab (anti-CTLA-4) [58–61]. Recently, these results have been expanded to evaluate the efficacy of PD-1 blockade in patients with advanced MMR-deficient cancers across 12 different tumor types [62]. Eighty-six consecutive patients were enrolled, including 40 dMMR CRC. Objective radiographic responses were noted in 53% of patients, with 21% achieving complete radiographic response. The objective response rate was similar between CRC (52%) and other cancer subtypes (54%). There was no significant difference in the objective response rate between Lynch and non-Lynch syndrome-associated tumors, 46% versus 59%, respectively ($p = 0.27$). Neither median PFS nor median OS was reached. The PFS and OS were not significantly different in patients with CRC as compared to those with other cancer types. Neither PFS (HR 1.2; 95% CI of 0.582–2.512, $p = 0.61$) nor OS (HR 1.71; 95% CI of 0.697–4.196; $p = 0.24$) was influenced by tumors associated with Lynch syndrome. While 74% of patients experienced an adverse effect, most were low-grade. Endocrine disorders, mostly hypothyroidism, occurred in 21% of patients and were easily managed with thyroid hormone replacement. As expected, functional analysis in a responding patient demonstrated neoantigen-specific T-cell clones that were reactive to mutant neoepitopes found in the tumor. Updated results were presented at the 2017 ESMO meeting (KEYNOTE 158 and KEYNOTE 164) on 61 dMMR CRCs with 2.3 months of PFS and 72% of OS at 12 months.

Nivolumab, another anti-PD-1 mAb, has been tested alone and in combination with ipilimumab, an anti-CTLA-4 mAb, in dMMR mCRC (CHECKMATE142). Eligible patients had progressed during or after, or been intolerant of, at least one previous line of treatment, including fluoropyrimidine and oxaliplatin or irinotecan. Initial results of the nivolumab alone arm were recently published (3 mg/kg nivolumab every 2 weeks) [58]. Of the 74 patients who were enrolled, 54% had previously received three or more treatments. Objective response rate was 31.1% and 69% had a disease control for 12 weeks or longer. Median duration of response was not reached. A 12-month PFS was 50% and 12-month OS was 73%. Response rates were not different according to PD-L1 expression, BRAF or KRAS mutations, and clinical history of Lynch syndrome or not. Drug-related serious adverse events occurred in 12% of patients and included hepatitis, colitis, gastritis, stomatitis, and arthritis. Results of the nivolumab plus ipilimumab arm were reported at ASCO 2017 ($n = 84$) [63]. Objective response rate was 41% and 79% had a disease control for 12 weeks or longer. Drug-related serious adverse events occurred in 18% of patients.

Up until now, there has been no published trial comparing CPIs to chemotherapy in dMMR mCRC. An ongoing clinical trial with pembrolizumab, KEYNOTE 177, is a phase 3 trial comparing pembrolizumab with standard chemotherapy in the first-line setting of dMMR mCRC. Another trial will compare avelumab (anti-PD1) to chemotherapy in the second-line setting in dMMR mCRC (SAMCO trial). To our knowledge, only one trial is ongoing combining CPIs and chemotherapy in dMMR mCRC. In this study, 439 patients with dMMR mCRC who are treatment-naïve will be enrolled and randomized to FOLFOX and bevacizumab, atezolizumab alone (anti-PD-L1), or atezolizumab combined with FOLFOX and bevacizumab (NCT02997228).

To conclude, CPIs have high efficacy in dMMR mCRC with more than half of patients with disease control at 2 years independently of PD-L1 expression or Lynch syndrome history and acceptable safety profile. Pembrolizumab has recently received breakthrough designation by the US Food and Drug Administration (FDA) for use in dMMR tumors, whatever the tumor site. Unfortunately, CPIs are not effective in pMMR mCRC as monotherapy, and rendering these tumors sensitive to immunotherapy remains a major challenge.

### 1.5. Challenges

Even though CPIs have shown major efficacy in dMMR CRCs, there are still many questions which need to be answered, e.g. how to make pMMR CRCs respond to CPIs and how to find predictive biomarkers of efficacy.

#### 1.5.1. Immunoscore

The presence of CD8+ T cells, CD27+CD45RA− effector memory T cells and a Th1 gene signature in CRC is associated with survival [64,65]. Investigation of the CRC primary tumor microenvironment uncovered an association of favorable outcomes with efficient coordination of the intratumoral immune response [65]. The type, density, and location of immune cells had a prognostic value surpassing the UICC-TNM classification. An immunoscore was created by Galon et al. ranging from 0 to 4, based on assessment of CD8+ and CD45RO+ cell densities in the center and in the invasive margins of the tumor. A higher score means that there is a higher density of Th1/cytotoxic memory T lymphocytes both in the center and at the margin of CRC, and this correlates with higher DFS and OS, as well as low risk of relapse and metastasis [66]. Mlecnik and colleagues demonstrated that this immunoscore was more strongly associated with outcome than was dMMR status [67]. The prognostic value of immunoscore has been validated in non-metastatic colon cancers (stages I/II/III) by a large international retrospective study coordinated by the Society for Immunotherapy of Cancer [68]. The challenges right now consist in implementing this score in clinical daily practice, with two clinical trials underway to prospectively validate the prognostic value of this immunoscore (NCT01688232 and NCT02274753). NCT0161688232, a multicentric prospective clinical study, intends to include 400 patients with CRC stage I to IV from 6 French centers, with a follow-up of 3 years for each patient to assess the ‘immunoscore,’ consisting of three markers (CD45RO, CD3, and CD8), and to measure its prognostic value. NCT02274753 is designed to take advantage of NCT01688232 study to extend the investigation beyond the immunoscore, favoring an integrative approach for the identification of prognostic and theranostic
parameter combinations at the time of surgery and during the clinical course. One would hope that this score could help identify high-risk patients in danger of recurrence and needing more aggressive treatment. Nevertheless, up until now, no aggressive therapeutic strategy (only oxaliplatin-based adjuvant) has been validated, even though FOLFIRINOX evaluation is ongoing for stage III T4 and/or N2 (iROCAS trial). In cases of favorable immunoscore in stage III with low risk of recurrence, the question of less aggressive treatment (fluoropyrimidine alone or 3 months of oxaliplatin-based chemotherapy) or no adjuvant treatment at all may arise. This must be prospectively validated taking into account the results of the International Duration Evaluation of Adjuvant chemotherapy (IDEA) trials.

Immunoscore should be evaluated in rectal cancer and there remain unanswered questions concerning whether or not it is possible to implement this score in the metastatic setting. Preliminary data suggest that immunoscore can predict response to chemoradiation in rectal cancer and chemosensitivity in mCRC [69,70]. Immune densities have been quantified in the center and invasive margins of metastases from CRC, and high T- and B-cell score and immunoscore were associated with prolonged median survival of 70.5 months, while low-scoring patients survived only 25.1–38.3 months [70]. It is worth noting that different immune scores have been used in other studies and have not been compared with one another [71]. In fact, immune scores are not routinely used in pathology departments. In addition, the prognostic value of different immunoscores compared to other well-known prognostic markers (T4, vascular invasion, etc.) has not been well determined by a complete multivariate analysis. Sophisticated scoring systems require a complex computerized program and nearly one third of tumors remain unevaluable/unclassifiable. Moreover, Galon’s immunoscore is protected by a license (HalioDx®) and its cost-effectiveness has not been evaluated. More importantly, the predictive value of response to CPIs has yet to be evaluated.

Moreover, recent data suggest that perhaps a simpler immunoscore taking into account only CD3 and/or CD8 T-cell infiltrate is as efficient as a complex immunoscore, like that of Galon’s, in the evaluation of CRC prognosis [71,72]. Emile et al., in the PETACC8 phase III study, evaluated an immunoscore based on linear quantification of CD3+ lymphocyte infiltration in invasive front from the outside to the inside of the tumor (high versus low lymphocyte infiltration). In multivariable analysis, lymphocyte infiltration is significantly associated with OS [71,73]. These results still need to be confirmed in independent series of colon cancer patients and in metastatic patients. Preliminary data suggest that high CD3+ lymphocytes at the tumor front and intratumoral mast cells appear to be prognostic for patients with colorectal liver metastases [74].

Finally, the role of immunoscore has not been tested in predicting response to CPIs, neither in dMMR CRC nor in pMMR CRC. Nevertheless, a trial testing pembrolizumab plus chemotherapy as first-line treatment in pMMR mCRC patients with a high immune infiltrate will be starting soon (POCHI trial).

1.5.2. Biomarkers
1.5.2.1 Prognostic value of PD-1 and PD-L1 expression and predictive value to CPIs response. The heterogeneous cellular expression of PD-1/PD-L1 may account for the selective response of CPIs [60,61]. Moreover, PD-1/PD-L1 expression is usually evaluated on primary tumors having at times been resected many years before the metastatic setting, and inducing spatial and temporal heterogeneity. Carbognin et al. identified 20 trials (1475 patients with advanced melanoma, NSCLC and genitourinary cancer) for a meta-analysis and found that the ORR with CPIs was 34.1% in the PD-L1-positive population and 19.9% in the PD-L1-negative population [75]. Gatalica and colleagues showed that the expression of both PD-1 and PD-L1 in colon cancer (n = 87) inversely correlated with the tumor stage [76]. PD-1+ lymphocytes directly in contact with cancer cells were consistently identified in 50% of colon cancers and strong membranous staining of PD-L1 was consistently expressed in 21%. The simultaneous expression of PD-1 and PD-L1 was found to be 12%. As expected, dMMR tumors (n = 27) exhibited a higher rate of positivity for PD-1+ TILs than pMMR tumors (n = 60) (77% versus 39%, p = 0.002). The proportion of PD-L1+ cancers was also significantly higher in dMMR than in pMMR colon cancers (38% versus 13%, p = 0.02). Both PD-1 and PD-L1 expression significantly decreased with the tumor stage (p = 0.021 and 0.031, respectively).

Dreeser et al. [77] reported that PD-L1 expression was associated with improved survival. A tissue microarray of 1420 specimens of primary CRC and 71 normal colon mucosa specimens was stained with two specific PD-L1 antibody preparations. Surprisingly, strong PD-L1 expression was observed in 37% of pMMR CRCs (n = 1197) and in 29% of dMMR CRCs. Median OS was 32 months for high PD-L1 expression pMMR tumors and 23 months for no or low PD-L1 expression tumors (p = 0.003) but the difference was significant only in univariate analysis. Nevertheless, in other studies, PD-L1 expression was higher in dMMR CRC as compared to pMMR CRC. For example, Gatalica et al. reported that PD-L1 expressions represented 77% of TILs and 38% of tumor cells in dMMR CRC and 39% of TILs and 13% of tumor cells in pMMR CRC [76]. These conflicting results may reflect heterogeneous tumor stages and different immunostaining techniques and antibodies, as well as which cells are analyzed.

In dMMR mCRC, pivotal trials did not demonstrate an association between PD-L1 expression and treatment efficacy [8,78]. In contrast to other tumors, PD-L1 expression in pMMR mCRC is not predictive of CPI efficacy [74]. PD-L1 expression as a biomarker of response in CRC may indeed have a limited role. Nevertheless, in some tumors due to different immunostaining techniques, there are conflicting results concerning the predictive value of PD-L1 expression with regard to CPI efficacy.

Finally, soluble PD-L1 (sPD-L1) can be detected using enzyme-linked immunosorbent assay (ELISA) or sandwich ELISA in the plasma of patients with cancer. sPD-L1 level is higher than in control patients [79], and it decreases in patients with lung cancer responding to chemotherapy [80]. Few data are available.
concerning blood leukocyte subpopulations and responses to CPI. Memory CD8+ blood cells were recently reported to be associated with responses to CPI [81].

1.5.2.2 Microsatellite instability as a biomarker for CRC treatment. dMMR CRC are associated with a lower stage at diagnosis and improved stage-specific prognosis in non-metastatic settings. There are conflicting results concerning prognosis impact of BRAF mutation in dMMR CRC [82,83]. Moreover, fluoropyrimidine-based chemotherapy is not effective in the adjuvant setting for patients with stage II CRC with a dMMR phenotype [19,20]. Nevertheless, stage II and III dMMR CRC remain chemosensitive to oxaliplatin [10]. A trial is ongoing to evaluate the efficacy of CPIs in stage III dMMR CRC, comparing folfox versus folfox plus atezolizumab (NCT02912559).

As previously described, dMMR status strongly predicts the clinical benefit of CPIs. Nevertheless, most but not all dMMR CRCs respond to CPIs. Dudley and colleagues [11] suggested that the ultimate biomarker for immunotherapeutic response was not dMMR or the mutational burden but rather the presence of immunogenic neoepitopes (proteins containing a mutation-associated neoantigen due to dMMR). Further studies are needed to provide validation for the most robust biomarkers, but a close link between dMMR status, mutational burden, and immunogenic neoepitopes has by now been established. We expect CPIs to soon be part of the standard of care for dMMR mCRC. Treating the vast majority of patients with pMMR is another major challenge lying ahead, and efforts should be made to identify other potentially ‘targetable’ subgroups [7], especially in the era of molecular subtypes [84].

1.5.2.3 Mutational load and consensus molecular subtype. Stadler and colleagues [85] hypothesized that a next-generation sequencing (NGS) panel for CRC that identifies RAS/BRAF and other actionable somatic mutations could reliably identify dMMR tumors on the basis of increased mutational load. A custom NGS assay using a 341-gene assay (MSK-IMPACT) was used to determine tumor mutation load and compare with MMR status as determined by routine IHC. All tumors with fewer than 20 mutations were considered as pMMR. Tumors with ≥20 mutations were dMMR (90%) or harbored the P286 hotspot mutation within the exonuclease domain of POLE consistent with an ultramutator phenotype. Among dMMR tumors, the median number of mutations was 50 (range 20–90) compared with 6 (range 0–17) in pMMR/POLE-wild type tumors (p < 0.001). With a mutational load cutoff of ≥20 and <150 for dMMR detection, sensitivity and specificity were both 100%. It is worth noting that POLE mutations contribute to an ultramutated but pMMR phenotype in colorectal tumors that is uniquely distinct from dMMR tumors. Preliminary data suggest that CPIs are also very effective in POLE-mutated CRC, probably at the same magnitude of dMMR tumors [86]. Another study, published by Giannakis et al., performed whole-exome sequencing of 619 CRCs and interpreted the results along with tumor immunity, pathology, and survival data. High neoantigen load, due to specific genomic aberrations, was positively associated with overall lymphocytic infiltration, TILs, and memory T cells. An association with TILs was evident not only in dMMR CRC, but also in pMMR CRC. Finally, as previously demonstrated, high neoantigen load and lymphocyte infiltration are associated with better CRC-specific survival [87].

CRC is classified as four consensus molecular subtype (CMS): CMS1 (dMMR-like), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal) [84]. CMS1 (mostly germline or sporadic dMMR tumors or tumors with mutation in polymerase genes POLE and POLD1) and CMS5 (TGF-β pathway activation and stromal invasion) showed high expression of immune signatures and overexpression of PD-1 ligands [88]. Nevertheless, the predictive value of CMS classification as regards CPI efficacy remains to be demonstrated.

Nevertheless, more studies are needed to clarify unresolved questions. Firstly, it is unknown whether there exist any pMMR/POLE-wild type mCRCs with high mutational load that could be sensitive to CPIs. In addition, we do not know if mutational load is a better predictor of CPI efficacy as compared to dMMR. Moreover, tumor samples included primary and metastatic tumors and it is unclear which sample should be used for future sequencing. Also, it is unclear whether these NGS panels could be implemented in different healthcare settings with vastly different resources [89]. Cost-effectiveness studies are indeed needed in the era of personalized medicine.

1.5.3. Improving the efficacy of CPIs

In untreated melanoma, dual CPIs have been shown to increase responses over monotherapy [90]. Dual checkpoint blockade increased response rates in dMMR mCRC in the CHECKMATE-142 trial [58]. Numerous additional combinations are being studied in pMMR and dMMR mCRC. An ongoing randomized study will formally evaluate durvalumab (PD-L1 inhibitor) and tremelimumab in refractory settings (NCT02870920). Agents that might block suppressive immune factors, such as indoleamine 2,3-dioxygenase (IDO) or LAG-3, are being investigated in phase I trials, combined with PD-L1 or PD-L1 inhibitors. While there are multiple additional immune CPIs and immunomodulatory compounds in development and in phase I, up until now no results concerning mCRCs are available. OX40, 4-1BB, CD70, and CD40 are other costimulatory immune checkpoint molecules capable of stimulating therapeutic immune responses. Henceforward, clinical trials with these molecules are progressing into phase I studies, with or without a combination of PD-1 inhibitors in patients with advanced solid malignancies. It is therefore not unexpected that clinical trials with these inhibitors have been designed to enroll CRC patients.

A major challenge is to render pMMR mCRC sensitive to CPIs. Chemotherapy is known to have detrimental effects on the immune system, but certain chemotherapies such as oxaliplatin as well as radiation therapy can generate antitumor immune response. When combined, FOLFOX and bevacizumab may decrease granulocytic MDSCs and increase pro-inflammatory helper T-cell (Th17) frequency, thereby producing a micro-environment favorable to immune CPI
treatment [91]. Nevertheless, trials evaluating CPIs combined with oxaliplatin and bevacizumab have not yet demonstrated the existence of a significant efficacy signal in pMMR mCRC (NCT01633970 trial) [92]. One major way may consist in identifying ‘dMMR like tumors,’ namely pMMR mCRC with an immune microenvironment like dMMR and possibly high efficacy of CPIs. Recent data suggest that approximately 20% of pMMR mCRCs have high immune infiltration but response to CPIs has yet to be evaluated in this subgroup of CRCs [71,74].

Recent data also suggest that microbiome and antibiotics have an impact on immunotherapy efficacy. Indeed, in melanoma patients treated with CPIs, significant differences have been observed in the diversity and composition of the patient gut microbiome of responders versus non-responders [93]. Antibiotics can inhibit the clinical benefit of CPI in patients with advanced cancer. In mouse model, fecal microbiota transplantation from cancer patients who responded to CPI into germ-free or antibiotic-treated mice improved CPI efficacy. For example, Akkermansia muciniphila restored the efficacy of PD-1 blockade by increasing the recruitment of CCR9+CXCR3 +CD4+ T lymphocytes in the tumor [94]. While there is an association between individual bacterial species and response to CPIs, stronger data are needed to assess the exact role of the intestinal microbiome in modulating this response.

Recently, due to remarkable abscopal effects, there has been great interest in combining CPIs with radiotherapy. Local radiation induces immunogenic cell death in targeted lesions with local as well as systemic immune-mediated anti-tumor effects increased by CPIs. In mice, radiation and CPI have synergistically reduced the local accumulation of tumor-infiltrating MDSCs, which was associated with increased CD8+ T-cell infiltration and priming sites [95]. Park et al. demonstrated that radiotherapy and anti-PD-1 treatment resulted in almost complete regression of the primary tumors treated and a 66% reduction in distant tumors via abscopal responses [49]. In the clinical setting, palliative radiotherapy given concurrently with maintenance CPI treatment in patients with melanoma caused regression of the targeted lesion as well as marked abscopal effects [96,97]. An ongoing phase II study is evaluating the abscopal effects of CPIs after radiation in patients with chemoresistant metastatic pMMR CRC. Preliminary results demonstrate a tolerable safety profile but also a partial response in non-irradiated lesions [98]. All of these results support the hypothesis that radiation can render the tumor sensitive to CPI and that CPI can increase the abscopal anti-tumor effects of radiation.

Certain features in the tumor microenvironment may contribute to a tumor’s ability to escape immune destruction, and in combination with CPIs, this should also be a target [7]. For example, clinical trials are ongoing with CPIs in combination with other immune-modulating therapies like interferon-α, bevacizumab, or CD40 agonist. Other research areas include strategies to enhance tumor cell immunogenicity (targeting other dysfunctional pathways at the same time) and epigenetic therapy. Combinations with MEK inhibitors are also promising. MEK inhibition upregulates interferon-gamma-mediated HLA molecule and PD-L1 expression. Based on this data, a phase I study of MEK inhibition with cobimetinib and PD-L1 inhibition with atezolizumab is ongoing in KRAS-mutated mCRC. Of the 23 mCRC patients enrolled, four (17%) achieved partial response and 6-month OS is 72%, better than might have been expected (NCT01988896 trial) [92]. A phase III study is ongoing, in which patients with refractory mCRC are randomized to cobimetinib and atezolizumab, atezolizumab alone, or regorafenib (NCT02788279).

Finally, some trials are ongoing to test immunotherapy as maintenance treatment. IMPALA is a randomized, international, multicenter, open-label phase III trial that has included 540 patients and evaluated the potent TLR-9 agonist MGN1703, a synthetic DNA-based immunomodulator targeting the innate immune system (NK cells), after standard induction chemotherapy in responder patients. The ongoing MODUL trial is a randomized phase III multicenter trial with biomarker-driven maintenance therapy. After a 4-month FOLFOX plus bevacizumab induction therapy, patients with disease control will be treated by maintenance therapy with 5FU, cetuximab, and vemurafenib in BRAF-mutated tumors or with 5FU, bevacizumab, and atezolizumab in BRAF wild-type tumors (the control arm in both cohorts will be 5FU and bevacizumab).

1.5.4. Immunoprevention of CRC

Immune cells can destroy pre-cancerous cells and prevent cancers [23]. In mouse models of colon polyps, tumor antigen vaccines in combination with aspirin, which can increase tumor-trafficking CD8 T cells, have shown encouraging results in reducing polymp formation [99]. Indeed, vaccines inducing immunity against tumor antigens are under evaluation in CRC.

Immunoprevention may also involve patients with Lynch syndrome. Indeed, pre-cancerous lesions of patients with Lynch syndrome already harbor frameshift mutations which can be targeted by immune system. A vaccine targeting mutations that define Lynch syndrome to prevent the development of cancer is under development [100].

1.6. Conclusion

Immune systems regulate tumor development in CRC and have a high prognostic impact. Before implementation of the immunoscore in clinical daily practice, prospective validation of prognostic value in non-metastatic and mCRCs is necessary along with relevant multivariate analysis as well as other prognostic factors. In addition, any demonstration of predictive value of CPI efficacy remains a challenge. CPIs in mCRC have an important emerging role, especially for dMMR CRC. Based on the breakthrough results of CPIs in dMMR tumors, several trials are ongoing, comparing the different therapeutic options with CPIs. Nevertheless, CPIs alone are to date ineffective in ‘all-comers’ pMMR CRC, even though some therapies seem to be able to convert a ‘non-immunogenic’ neoplasm into an ‘immunogenic’ neoplasm. Indeed, the major challenge remains to find ways to increase the efficacy of CPIs in pMMR CRC. MEK and PD-L1 combination is one of the most promising approaches. Another challenge remains which is the use of unequivocal biomarkers for patient selection, mutational load seeming to be the most relevant approach.
1.7. Expert opinion

The clinical outcome of CRC has improved substantially over the past 20 years. The efficacy of systemic therapies and biomarker-based treatment has been paramount in this positive shift. Progress has been made to more precisely characterize the tumor cells and microenvironment. This should be helpful in the future development of therapeutic strategies. dMMR status and POLE mutations are the most powerful predictive biomarkers for the efficacy of CPI. In dMMR CRC, results have been impressive, with more than half of the patients surviving at 2 years while being resistant to chemotherapy. However, some patients present primary or secondary resistance to CPIs. We do not yet know whether mutational load or other previously described biomarkers will help to identify patients with prolonged treatment response to CPIs in dMMR CRC. Nevertheless, mCRC with dMMR phenotype consists of only approximately 3–5% of the mCRC population. In non-metastatic CRC, dMMR accounts for approximately 12%. An adjuvant trial with an anti-PD-L1 is ongoing, but it is not certain that CPI is effective in this situation, which involves residual tumor cells whose mechanism of resistance is not necessarily the overexpression of immune checkpoints. Nevertheless, adjuvant ipilimumab has been shown to prolong survival in stage III melanoma after curative surgery [101].

The most challenging issue is pMMR mCRC and determination of the ways to convert a ‘nonimmunogenic’ neoplasm into an ‘immunogenic’ neoplasm. CPIs alone will not demonstrate significant efficacy, nor will standard chemotherapy likely to do so. Combination with radiation or MEK inhibitor is probably more relevant. The answer will be known soon, as the results of the phase III comparing cobimetinib and atezolizumab versus atezolizumab alone versus regorafenib in chemotherapy-resistant mCRC will be presented at ASCO 2018.

Another key challenge in the era of personalized immunotherapy is the future use of multigene NGS assays to quantify mutational load, which could be a more reliable predictive biomarker of CPI efficacy compared to PD-L1 expression. Moreover, it is not clear which tumor burden should be assessed, if the primary tumor is sufficient or if metastases should also be taken into consideration. Furthermore, we do not know the optimal timing for this assay prior to CPI treatment. Moreover, this technique is expensive and not available in every laboratory. Cost-effectiveness should be used as an end point in clinical studies as many societies are now facing situations where governing or medical bodies are asked to choose the most appropriate treatment option at a reasonable cost. Identification of the patients who will most benefit from the CPI is the most cost-effective strategy and the FDA recommended that companion diagnostics-based treatment strategy for oncology therapeutics had to be determined. Trials testing both different mutational loads and immunoscores as predictors of response to CPIs in pMMR mCRC are ongoing but the results are not yet available. And there remain many challenges concerning comparison of different tests, prospective validation, standardization and cost. New immunotherapy strategies should also be evaluated in CRC, such as bispecific antibodies or CAR T-cells (chimeric antigen receptor T-cells). Carcinoembryonic antigen T cell bispecific antibody (CEA TCB) is a bispecific antibody used to recognize CEA and CD3 via a novel molecular format that induces T cell-mediated killing of CEA over-expressing tumors while sparing primary cells with low CEA expression. Phase I trials combining CEA TCB and TCI are ongoing in mCRC.

The use of CPIs will create a specific field of internal medicine dedicated to the treatment of side effects affecting the immune system. New protocols should be discussed by the different experts (e.g. dose decrease, management of hypothyroidism or hyperthyroidism). Working collaboratively is the key to a more intelligent approach to this problem in the near future with multidisciplinary teams (gastroenterologist, dermatologist, endocrinologist, etc.). Defining the best criteria for evaluation of CPI efficacy is another important challenge. Radiological assessment should also be more standardized. The new iRECIST criteria defined by Seymour et al. should be used and radiologists should be aware of them [102]. As reported in numerous clinical trials, the benefit of CPIs is usually noted on OS, but not on PFS. So-called pseudo-progression is a distinctive radiological hallmark of immunotherapy and this notion should be further delved into in future clinical trials, in order to evaluate its predictive value of CPI efficacy.

Finally, from an economic or scientific perspective, the duration of treatment should be more precisely specified in future trials as we believe that CPIs should be offered on a global scale and at a reasonable cost for any health-care system. Some preliminary results suggest that 2 or 3 months of treatment is enough to induce durable T-cell activation, especially when combinations of CPIs are used [103].

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Declaration of Interest

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.


