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REVIEW

Role of immune checkpoint inhibitors in the treatment of colorectal cancer: focus on nivolumab

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ABSTRACT

Introduction: Metastatic colorectal cancer (mCRC) is a challenging disease, whose systemic therapy has traditionally been based on a generalized population of patients, with unsatisfactory clinical outcomes. Immunotherapy has been shown to be efficacious in hypermutated tumors, such as those with microsatellite-instability (MSI-H). Nivolumab, and other immune checkpoint inhibitors (ICI), have recently been evaluated in MSI-H mCRC, with remarkable results.

Areas covered: Focused on nivolumab, we aim to present the rationale for the applicability of ICI in MSI-H CRC, and the results of completed phase I/II studies. Ongoing studies, including randomized clinical trials, and perspectives of immunotherapy in clinical scenarios in CRC will be discussed.

Expert opinion: Phase I and II clinical trials provide strong evidence for the use of nivolumab and other ICI in the systemic therapy of MSI-H mCRC. Regulatory approvals are restricted to subsequent lines of therapy, but preliminary results in treatment-naïve patients are encouraging. The findings for advanced disease and in the pilot phase II study in early-stage colon cancer open a new avenue for the applicability of immunotherapy in neoadjuvant and adjuvant settings, which are currently under investigation. With the exception of POLE-mutated patients, there is little evidence for the use of immunotherapy in MSS patients.

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1. Introduction

Colorectal cancer (CRC) is the fourth most common cancer worldwide, responsible for approximately 1,100,000 new cases and 550,000 deaths in 2018 [1]. Despite significant improvements in the systemic therapy for this disease in recent decades [2–5], overall survival (OS) of metastatic colorectal cancer (mCRC) patients is dismal, with a 5-year OS rate of only 13.5% [6].

The recognition of molecular heterogeneity of cancer has been a determinant for therapeutic development recently. Monoclonal antibodies and tyrosine-kinase inhibitors designed to target specific molecular abnormalities have changed the landscape of cancer therapy. Nevertheless, the applicability of genome-guided personalized therapy has been incipient in mCRC. *RAS* and *BRAF* mutations have proven useful to select patients who benefit from anti-epidermal growth factor receptor (EGFR) monoclonal antibodies, but these are resistance markers [7,8]. The subset of patients who derive the greatest benefit from targeted therapy in mCRC remains unknown.

The successful initial studies of immunotherapy in melanoma, non-small cell lung cancer, and renal-cell carcinoma ushered in a new era in cancer therapy [9–13]. In spite of ignorance of specific molecular abnormalities and driver mutations, the use of immune checkpoint inhibitors (ICI) (including the anti-CTLA-4 inhibitors, ipilimumab and tremelimumab, and the anti-PD-1/PD-L1 inhibitors, nivolumab, pembrolizumab, atezolizumab, durvalumab, and avelumab) has demonstrated to be revolutionary, and these have been progressively incorporated in the therapeutic arsenal of many solid and hematologic tumors.

To date, it is unclear how to identify patients who will derive the greatest benefit from ICI. PD-L1 expression and tumor mutational burden (TMB) are emerging predictive biomarkers [14]. The potential correlation of number of non-synonymous mutations and sensitivity to immunotherapy prompted the evaluation of the efficacy of ICI in patients with high-frequency microsatellite instability (MSI-H) [15], with remarkable results [16].

In this review, we intend to discuss the role of nivolumab, an anti-PD-1 inhibitor, and other ICI in the treatment of mCRC, as well as present the ongoing clinical trials and perspectives in the applicability of immunotherapy in this heterogeneous disease.

2. Standard of care

In the United States, it is estimated that 20% of patients with colorectal cancer present with metastatic disease [17]. In addition, approximately 20% of stage II patients and 35% of stage III will present distant metastasis in 5 years, even with the use of adjuvant chemotherapy [18–20]. The mCRC patients with liver-only or lung-only metastasis are potentially curable if the lesions are completely resected or managed with regional therapies [21–25]. Otherwise, the goal of the treatment will

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Article highlights

- Comprehensive molecular characterization of CRC has demonstrated the presence of two main groups of patients, with distinct genomic, epigenomic, and transcriptomic profiles: chromosomal instability (CIN) and microsatellite instability (MSI-H).
- Nivolumab, the most extensively studied immune checkpoint inhibitor (ICI) in metastatic CRC, as well as other ICI, has shown to be efficacious in the MSI-H population, who typically present with high tumor mutational burden (TMB).
- PD-L1 expression and TMB are emerging predictive biomarkers for immunotherapy, but MSI-H is the only biomarker used to select patients for ICI in mCRC.
- Intensive research is currently underway with the intent to identify non-MSI-H patients who may derive benefit from ICI, such as, POLE-mutated patients.
- Promising ongoing clinical trials may extend the use of immunotherapy to adjuvant setting of stage III MSI-H and/or POLE-mutated CRC patients.
- Adoptive T-cell therapies, and strategies to overcome resistance to immunotherapy may benefit a larger population of CRC patients than expected.

be palliative, with the intent to reduce the symptom burden and prolong survival.

The cornerstone of systemic therapy of CRC is the fluoropyrimidines: 5-FU or capecitabine. Infusional 5-FU presents higher efficacy and safety compared to bolus 5-FU [26], and capecitabine has shown higher response rates, but equivalent progression-free survival (PFS) and overall survival (OS) when compared to bolus 5-FU [27,28]. Both the oral and intravenous fluoropyrimidine can be used interchangeably.

Fluoropyrimidines may be used alone in systemic therapy, but they offer higher response rate, PFS and OS, when combined in doublets with oxaliplatin or irinotecan [29,30]. Triplet-regimens, such as FOLFOXIRI, are associated with elevated response rates (65%) and represent alternative regimens [31,32]. They are preferentially used in patients who need higher depth of response, both for the preoperative management of potentially resectable metastatic disease and for decreasing the symptom burden in advanced disease [33]. By contrast, these are associated with higher rates of toxicity.

Anti-angiogenics inhibit mainly the vascular endothelial growth factor (VEGF) pathway, attaching either to the circulating VEGF (bevacizumab, aflibercept) or to the VEGF-receptor (VEGF-R) (ramucirumab) [34]. They improve PFS and OS in advanced disease, both in the first-line and second-line setting [35–38]. Bevacizumab is the prototype of anti-angiogenic therapy, but aflibercept [39] and ramucirumab [40] are also alternatives for second-line therapy in association with doublet-regimens of chemotherapy. There is a benefit in maintaining anti-angiogenic therapy even when there is progressive disease on the previous regimen [37,39,40], regardless of mutational profile, as there are no predictive biomarkers for the use of anti-angiogenic therapy.

Anti-EGFR monoclonal antibodies, such as cetuximab and panitumumab, attach to the extracellular domain of EGFR, and thereby do not have efficacy on the presence of activating mutations in the proteins that form the downstream signaling pathway, such as RAS and RAF proteins [7]. Therefore, these must be used only in RAS and BRAF wild-type patients, who are approximately 35% of mCRC patients. In first-line therapy, when compared to bevacizumab, the use of anti-EGFR monoclonal antibodies is associated with higher response rates, but these present similar PFS and OS, even in expanded RAS analysis [41,42]. However, in a subgroup analysis of CALGB 80405, cetuximab showed superiority over bevacizumab in both response rates and OS in RAS wild-type left-sided tumors [43].

Through comprehensive molecular characterization, it has been demonstrated that right-sided and left-sided colon cancers are molecularly distinct [44,45]. Such molecular heterogeneity based upon sidedness has prognostic and predictive impact. Exploratory analysis of subgroups of patients from randomized clinical trials are strongly suggestive that anti-EGFR monoclonal antibodies are associated with superior OS when compared to bevacizumab in first-line setting of left-sided tumors [46]. These data also suggest that anti-EGFR therapy presents lower efficacy in right-sided tumors. Despite being derived from exploratory analysis, the data prompted modifications in the current guidelines of the treatment of mCRC, which favors that RAS and BRAF-wild type patients with left-sided tumors be preferentially treated with anti-EGFR monoclonal antibodies in first-line therapy. And in the case of the right-sided counterparts, should be submitted to anti-EGFR therapy in case of failure to the first-line of systemic therapy [33,47].

Unselected refractory mCRC patients exposed to fluoropyrimidines, oxaliplatin, irinotecan, anti-angiogenics, and anti-EGFR monoclonal antibodies have especially poor prognosis. Regorafenib [48], a multi-target tyrosine-kinase, and TAS-102 [49] (trifluridine/tipiracil) have prolonged PFS and OS, when compared to placebo, and they have been incorporated into the therapeutic arsenal of mCRC.

Neurotrophic receptor tyrosine kinase (NTRK) fusion seems to be rare in CRC (0.5% of the mCRC patients) [50], but once detected, the use of tropomysin receptor kinase inhibitors must be strongly considered [51]. The FDA recently approved Larotrectinib for patients who are metastatic, for whom surgical resection is likely to result in severe morbidity and who have no satisfactory alternative treatments, or whose cancer has progressed following treatment [52].

The use of ICI has also been recently approved by the FDA in MSI-H mCRC [53–55]. This subset of patients, who constitute approximately 5% of the overall population of mCRC, has pembrolizumab [56], nivolumab [57], and the association of nivolumab and ipilimumab [57,58] as therapeutic options following first-lines of therapy. The efficacy and safety of ICI in mCRC will be discussed in the following sections.

3. Molecular characterization of colorectal cancer

CRC may be understood as a group of diseases characterized by a wide range of genetic and epigenetic abnormalities (Figure 1). From a pathophysiological standpoint, despite being constituted by a marked molecular heterogeneity at phenotype level, CRC stems from key steps at the genetic and epigenetic levels, which results in the formation of two

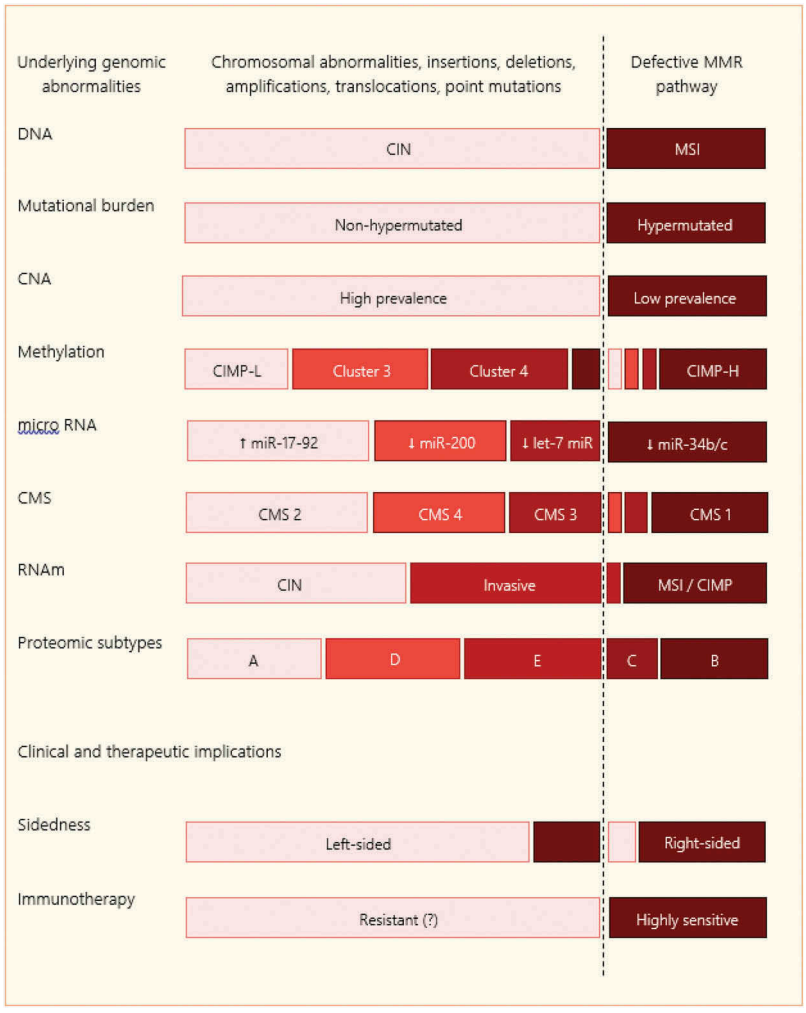

Figure 1. Comprehensive molecular characterization of colorectal cancer.

main molecularly distinct groups of disease. The initial phases of this process of malignant transformation are microsatellite instability (MSI-H) and chromosomal instability (CIN) [59–62].

The molecular underpinning of MSI-H tumors is the loss of function of the DNA MMR genes [62,63]. This defective MMR (dMMR) pathway may result from two mechanisms: point mutations in one of the several genes responsible for the DNA MMR or epigenetic silencing of the promoter regions of the MMR genes by hypermethylation. Regardless of the mechanism, the result will be genomic instability, and thereby a higher number of mutations. Some regions of the genome are particularly vulnerable to mutation, such as regions with homologous repeats of nucleotide sequences, called satellites [5,64] (Figure 2). These are called microsatellites when distributed throughout the genome in repetitive short sequences of nucleotide bases. Microsatellite instability of high frequency (MSI-H) is so named due to the high rate of mutations in the microsatellite regions [63,64]. Therefore, CRCs associated with the dMMR pathway are typically hypermutated. The main genes involved in the MMR pathway are *MLH1, MSH2, MSH6*, and *PMS2*, of which the first two genes are the most commonly affected [59,62,63].

Germline mutations in one of the several MMR genes occur in Lynch syndrome, also called hereditary nonpolyposis CRC (HNPCC) [65]. The other inherited patterns of CRC are not involved with MMR genes. These are involved with APC gene, and form the polyposis syndromes: familial adenomatous polyposis (FAP), MUTYH-associated polyposis (MAP), and the hamartomatous polyposis syndromes (Peutz-Jeghers, juvenile polyposis, Cowden syndrome) [66]. A less understood pattern is familial CRC, estimated to be present in 25% of patients [67]. These patients have family history of CRC, but they do not have an inherited identified genetic mutation, and do not have a pattern consistent with one of the inherited syndromes.

The most common mechanism of dMMR pathway is the hypermethylation of the promoter regions of the MMR genes, mainly MLH1 [62,63]. Promoter regions of the MMR genes have high frequency of CpG islands, which once methylated, result in silencing of the gene expression. Hypermethylation of CpG island methylator phenotype (CIMP-high) is associated with the presence of BRAF V600E mutation and absence of KRAS mutation [65,68].

The second and most common group of CRC presents CIN [59,60] (Figure 1). It is associated with a proficient MMR pathway, also called microsatellite stable or with low frequency of microsatellite instability (MSS or MSI-low, respectively) [62]. At genomic level, CIN is the basis for approximately 85% of the

Figure 2. Schematic representation of satellites, minisatellites, and microsatellites. Repetitive sequences of nucleotide bases throughout the genome are called satellites. According to their length, the sequences may be denominated satellites, minisatellites, and microsatellites, if they have >100 bp, between 10 to 100 bp, and <10 bp, respectively. These repetitive sequences are particularly vulnerable to mutations. The high frequency of mutations in the microsatellites will be called microsatellite instability (MSI-H). They are secondary to the presence of a defective MMR pathway, caused either by germline mutations in the MMR genes or by hypermethylation of the promoter regions of the MMR genes. Such condition results in tumors with high tumor mutational burden (TMB), and thereby a high number of neoantigens, which is the hypothesis to explain the presence of lymphocyte infiltration associated to these tumors, and the higher sensitivity to immune checkpoint inhibitors [5,63,64].

cases of CRC [59,60]. Also called non-hypermutated, CIN associates with another mutational profile compared to MSI-H. *APC, TP53, KRAS, PIK3CA*, and *FBXW7* are the most common genes mutated in CIN, compared to *ACVR2, APC, TGFBR2, BRAF*, and *MSH3* in MSI [59–61].

CIN presents far more copy number alterations compared to MSI-H tumors [59,60]. It is observed arm-level changes, such as gains of 1q, 7p and q, and 12q, and deletions of 18p and q, 17p and q, and 1p. At the gene-level, amplifications at 13q12.13, 20q13.12, and 17q21.1 can be identified, as well as deletions at *SMAD4, APC*, and *PTEN* genes.

It is also observed that altered expression of miRNA correlates with the key steps in the pathogenesis of CRC. Epigenetic silencing of miR-34b/c, components of p53 network, is associated with CIMP-high [69]. miR-200 and let-7 families also seem to be involved in tumor suppressive functions in CRC, as well as in other human cancers [59,70].

From a transcriptional standpoint, recently the presence of four distinct groups of CRC, with specific patterns of gene expression have been recognized [61]. There is a consistent association of genetic and epigenetic abnormalities with certain patterns of gene expression. For example, the majority of hypermutated tumors present a consensus molecular subtype (CMS) 1 pattern, which presents overexpression of proteins involved in DNA damage repair and strong immune activation. Conversely, CMS 2, CMS 3, and CMS 4 display higher chromosomal instability (Figure 1).

It is paramount to understand the molecular characterization of CRC to identify the patients who would derive benefit from immunotherapy. From lessons learned from melanoma and non-small cell lung cancer, it is hypothesized that hypermutated tumors are the most sensitive to ICI [71–73]. The expression of neoantigens would elicit infiltration of T lymphocytes in the tumor microenvironment, which might explain higher sensitivity to immunotherapy. Consistent with this hypothesis, it has been demonstrated that MSI-H tumors, which typically present high tumor mutational burden (i.e. the total number of mutations per coding area of a tumor genome – TMB), are great responders to ICI. The emerging biomarkers and the clinical trials evaluating this hypothesis will be discussed in the following sections.

4. Immune checkpoint inhibition

Regardless of the underlying cause of lymphocyte activation (e.g. infectious, neoplastic or auto-antigens), immune response has auto-regulatory mechanisms that diminish tissue damage and autoimmunity [74]. Upon lymphocyte activation mediated through the ligation of MHC complexes from antigen-presenting cells (APCs) to T-cell receptor (TCR), there is up-regulation of membrane receptors in the so-called 'immunological synapse', where there are interactions of diverse proteins involved in the modulation of immune response, among them membrane receptors that play an inhibitory role over lymphocyte function (Figure 3). Two proteins expressed by T-lymphocytes seem to be especially important: CTLA-4 and PD-1. Biallelic genetic deletion of *Ctla4* results in fatal massive lymphoproliferation in mice at 3 to 4 weeks of age [74–77], and, similarly, genetic loss of *Pdcd1* leads to development of lupus-like autoimmune pathology and autoimmune dilated cardiomyopathy in mice [74,78,79]. These inhibitory proteins of immune cells are called immune checkpoint inhibitors.

Immune evasion is one of the hallmarks of cancer [80]. These mechanisms remain to be fully elucidated, but it was demonstrated that tumor cells and tumor-infiltrating APCs express ligands of CTLA-4 and PD-1, such as B7-1, B7-2, and PD-L1, PD-L2, respectively. B7-1 and B7-2 are usually expressed by APCs, and their ligation to CD28 on T-cells is a co-stimulatory mechanism for lymphocyte activation [81,82]. CTLA-4 has a high affinity to B7-1 and B7-2, and acts as a negative stimulator by competitive inhibition. Similarly, the ligation of PD-L1 and PD-L2 to PD-1 directly regulates TCR signaling to attenuate T-cell signaling [83]. Another inhibitory role played by PD-L1 is through the interaction with B7-1 [84,85]. Expression of PD-1, as well as LAG3 and TIM3, is one of the markers of exhausted T cells [74].

The mechanisms of action of ICI are highly complex and not fully understood. The primary mechanism of CTLA-4 blockade seems to be through direct blockade of the receptor, which allows free ligation of B7-1 and B7-2 to CD28, and thereby unrestrained positive costimulation of T cells [74]. It seems that anti-CTLA-4 does not have a generalized effect on all T cells, but

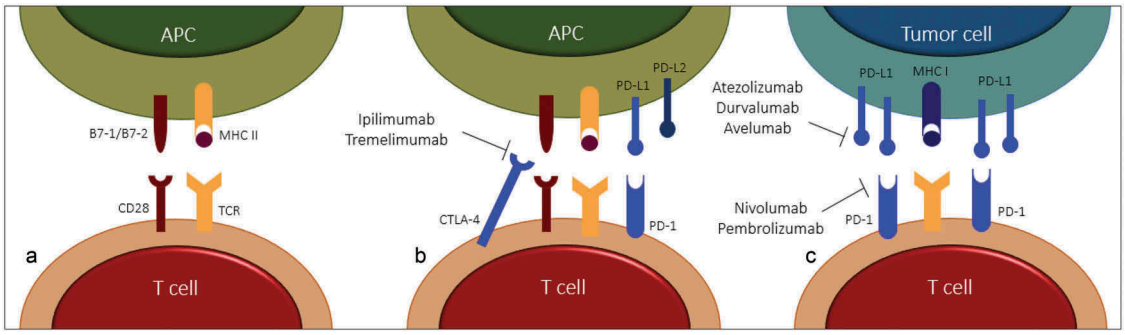

Figure 3. Mechanism of action of the immune checkpoint inhibitors. (a) T-cell activation is mediated through the ligation of MHC complexes from antigen-presenting cells (APCs) to T-cell receptor (TCR), and through co-stimulatory mechanisms, such as the interaction of CD28 on T-cells to B7-1 and B7-2 expressed by APCs [74]. (b) After T-cell activation, it will be up-regulation of membrane receptors in the so-called 'immunological synapse', where there are interactions of diverse proteins involved in the modulation of immune response, among them membrane receptors that play an inhibitory role over T-cell function. Two proteins expressed by T-cells seem to be especially important: CTLA-4 and PD-1. These inhibitory proteins are called immune checkpoint inhibitors. CTLA-4 has a high affinity to B7-1 and B7-2, and acts as a negative stimulator by competitive inhibition [81,82]. Similarly, the ligation of PD-L1 and PD-L2 to PD-1 directly regulates TCR signaling to attenuate T-cell signaling [83]. Another inhibitory role played by PD-L1 is through the interaction with B7-1 [84,85]. The primary mechanism of CTLA-4 blockade seems to be through direct blockade of the receptor, which allows free ligation of B7-1 and B7-2 to CD28, and thereby unrestrained positive costimulation of T cells (e.g. ipilimumab, tremelimumab) [74]. (c) Anti-PD-1 antibodies (e.g. nivolumab, pembrolizumab) increase functional activity of CD8 T cells through direct inhibition of the PD-1 receptor, avoiding the attenuation of TCR signaling [74]. Anti-PD-L1 inhibitors (e.g. atezolizumab, durvalumab, avelumab), besides acting on the PD-1/PD-L1 axis, may also exert a direct immune tumor rejection by antibody-dependent cellular cytotoxicity (ADCC) [88]. Moreover, they inhibit the ligation of PD-L1 to B7-1, which might enhance antitumoral activity [84,85,89].

on a specific expansion of tumor neoantigen-specific CD8 T cells within the tumor microenvironment [86]. The anti-CTLA-4 widely evaluated in clinical trials was ipilimumab [9,87]. Tremelimumab does not have FDA-approved indications.

Anti-PD-1 antibodies (e.g. nivolumab, pembrolizumab) increase functional activity of CD8 T cells through direct inhibition of the PD-1 receptor, avoiding the attenuation of TCR signaling [74]. Anti-PD-L1 inhibitors (e.g. atezolizumab, durvalumab, avelumab), besides acting on the PD-1/PD-L1 axis, may also exert a direct immune tumor rejection by antibody-dependent cellular cytotoxicity (ADCC) [88]. Moreover, they inhibit the ligation of PD-L1 to B7-1, which might enhance antitumoral activity [84,85,89]. However, allowing the interaction of PD-1 to PD-L2, they can weaken autoimmunity [90]. Despite the absence of randomized clinical trials comparing PD-1 inhibitors to PD-L1 counterparts, clinical trials do not suggest differential efficacy and safety among them [57,87,89,91,92].

The observation that up-regulation of additional immune checkpoint molecules is one of the mechanisms that limit the efficacy of ICI (e.g. up-regulation of PD-1 after the use of anti-CTLA-4 antibodies) raised the hypothesis that blockade of both immune checkpoints might enhance therapeutic efficacy. Randomized clinical trials demonstrated that the combination of nivolumab plus ipilimumab added benefit to the treatment of melanoma and renal-cell carcinoma [87,93], and, it is suggestive to be superior to monotherapy in mCRC as well [58]. The mechanistic effects of the blockade of CTLA-4 and PD-1/PD-L1 remain to be clearly understood. It is possible that different antibodies act in different cells (e.g B cells and/or T cells) and in different tissue sites (e.g. lymph nodes and/or tumor) [74].

5. Nivolumab

a. *Mechanism of action*

Nivolumab (BMS-936559) is a fully human IgG4 kappa monoclonal antibody that has a calculated molecular mass of 146 kDa. It binds to the PD-1 receptor and blocks interaction with PD-L1 and PD-L2, avoiding PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response [54].

b. *Pharmacokinetics and pharmacodynamics*

Steady-state concentrations of nivolumab are reached by 12 weeks when administered at 3 mg/kg every 2 weeks. The exposure to nivolumab increases dose proportionally over the dose range of 0.1 to 10 mg/kg administered every 2 weeks. The predicted exposure to nivolumab after a 30-min infusion is comparable to that observed with a 60-min infusion [54]. The time-averaged steady-state exposure and safety profile of nivolumab 480 mg every 4 weeks were compared with 3mg/kg every 2 weeks, and both are equivalent [94,95]. Based on the pharmacokinetic profile, nivolumab is the first ICI to be approved in 4-week-interval administration, 30-min infusion, in the United States, Canada, and European Union [54].

Pharmacokinetics analysis also suggests that age, weight, gender, renal impairment (even severe), and mild hepatic impairment have no clinically important effect on the clearance of nivolumab [54]. This was not studied in populations with severe hepatic impairment (total bilirubin greater than 3 times the upper limit of normal).

c. *Treatment-related adverse events (TRAE)*

Based on the mechanism of action of the PD-1 inhibitors, lymphocyte activation and enhancement of antitumoral activity are expected. Nevertheless, since the PD-1/PD-L1 pathway is a physiological regulatory mechanism that avoids autoimmune response, immune-related adverse events are major safety concerns of these immune checkpoint inhibitors.

PD-1 inhibitors share equivalent safety profiles as a drug class, even when considering pharmacokinetics and pharmacodynamics differences. Pembrolizumab and nivolumab, when used as monotherapy, consistently demonstrate

fatigue (any grade in 10% to 36% of the patients), rash (15% to 23%), pruritus (17% to 21%), and diarrhea (14% to 21%) as the most common adverse events [87,91,92]. Discontinuation of treatment due to grade 3 and 4 adverse events usually occurs in less than 10% of patients. Severe adverse events, with meaningful clinical implications, such as pneumonitis, colitis, and hypophysitis have incidence rates below 3% [87,91,92]. Anti-PD-L1 inhibitors, even with the distinct mechanism of action allowing the interaction of PD-L2 and PD-1 intact, potentially weakening autoimmunity, also present similar safety profiles compared to anti-PD-1 inhibitors [89,96].

In mCRC, fatigue (23%), diarrhea (21%), and pruritus (14%) were the most common any grade TRAE associated to nivolumab monotherapy [57]. Grade 3 or 4 TRAE was reported in 20% of patients, of which increased lipase (8%) and increased amylase (3%) were the only grade 3 or 4 events that occurred in more than one patient. Discontinuation of treatment due to TRAE occurred in 7% of patients, including increased ALT, colitis, duodenal ulcer, acute kidney injury, and stomatitis (one each) [57].

The combination of nivolumab and ipilimumab is usually associated with toxicity rates higher than those observed with each agent alone [87]. However, in CheckMate-142 cohorts, fatigue, diarrhea, and pruritus were also the most frequent TRAE in the combination therapy, with equivalent rates: 18%, 22%, 17%, respectively [58]. TRAE leading to discontinuation of the medication occurred in 13% of patients submitted to the combination. In the majority of patients (71–96%) the adverse events were completely resolved, with the exception of endocrine TRAE (40%), which typically demanded longer time to be controlled [58,97].

d. ***FDA-approved indications***

The first approval of nivolumab was 22 December 2014, granted accelerated approval for melanoma patients with advanced disease, based on CheckMate-037 data [98], which has demonstrated a response rate of 32% in previously treated advanced melanoma patients. Subsequently, through CheckMate-066 publication, it was demonstrated that nivolumab also produced overall survival benefit in previously untreated patients when compared to dacarbazine [92]. Since then, nivolumab received attestation in nine different cancers, both isolated or in combination with ipilimumab (Table 1) [54].

Table 1. FDA-approved indications of nivolumab.

Tumor	Indication
Melanoma	Unresectable or metastatic disease, as a single agent or in combination with ipilimumab. Lymph node involvement or metastatic disease who have undergone complete resection, in the adjuvant setting.
NSCLC	Advanced (metastatic) disease with progression on or after platinum-based chemotherapy.
Renal cell carcinoma	Advanced disease who have received prior antiangiogenic therapy. Intermediate or poor risk, previously untreated advanced disease, in combination with ipilimumab.
Hodgkin lymphoma	Adult patients with classical Hodgkin lymphoma that has relapsed or progressed after: (1) autologous HSCT and brentuximab vedotin, or (2) 3 or more lines of systemic therapy that includes autologous HSCT.
Head and neck cancer	Recurrent or metastatic disease with disease progression on or after a platinum-based therapy.
Urothelial carcinoma	Patients with locally advanced or metastatic disease who: (1) have disease progression during or following platinum-containing chemotherapy, and (2) have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.
Colorectal cancer	Adult and pediatric (12 years and older) patients with MSI-H or dMMR mCRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan, as a single agent or in combination with ipilimumab.
Hepatocellular carcinoma	Patients who have been previously treated with sorafenib.
SCLC	Patients with metastatic disease with progression after platinum-based chemotherapy and at least one other line of therapy.

Abbreviations: NSCLC: non-small cell lung cancer, HSCT: hematopoietic stem cell transplantation, MSI-H: microsatellite-instability, dMMR: deficient mismatch repair, mCRC: metastatic colorectal cancer, SCLC: small cell lung cancer.

6. Completed clinical trials

6.1. Phase I clinical trials with nivolumab

Initial studies evaluating the role of PD-1 inhibition in an unselected population of mCRC patients were intriguing (Table 2). The first human phase I trial of nivolumab in patients with treatment-refractory solid tumors showed that only 1 out of 14 mCRC patients presented objective response [16]. A subsequent trial revealed that none of the 19 mCRC patients had demonstrated sensitivity to the therapy [99], in contrast to melanoma, non-small cell lung cancer, and renal-cell carcinoma patients, who were far more sensitive [16,99]. However, that single responding mCRC patient was the only one who presented with microsatellite-instability, which is known to have more somatic mutations than tumors with proficient DNA MMR pathway [100]. Moreover, the patients who had been demonstrated to be highly sensitive to immune checkpoint inhibition (melanoma, non-small cell lung cancer) are characterized by the high number of somatic mutations [101,102].

6.2. Phase II clinical trials with nivolumab

The largest initiative, to date, was launched by the CheckMate 142 trial, a phase II study designed to evaluate the efficacy of nivolumab monotherapy or nivolumab in combination with other anti-cancer drugs in MSI-H and non-MSI-H mCRC patients (clinicaltrials.gov NCT02060188) (Table 2). Originally, the study had six cohorts (1): nivolumab monotherapy, (2) nivolumab 3mg/kg + ipilimumab 1mg/kg every 3 weeks for 4 doses, followed by nivolumab 3 mg/kg every 2 weeks until progression, (3) nivolumab 3 mg/kg every 2 weeks + ipilimumab 1 mg/kg every 6 weeks for 4 doses, followed by nivolumab 3 mg/kg every 2 weeks until progression, (4) nivolumab 3mg/kg + ipilimumab 1 mg/kg every 6 weeks, combined with cobimetinib dosed orally once daily 21 days on/7 days off, (5) nivolumab + BMS-986016, and (6) nivolumab + daratumumab. The primary endpoint is objective response rate. Secondary

Table 2. Clinical trials with reported results evaluating immune checkpoint inhibitors in metastatic colorectal cancer.

Author	Treatment	n	Lines of therapy	MMR status	ORR (%)	PFS (months)	Disease control ≥12w (%)	12-month PFS (%)	12-month OS (%)	G3-4 toxicity (%)*
Chung et al [118].	Tremelimumab 15 mg/kg every 3 months	47	100% ≥ 1 line	-	0	2.3	-	-	11	19
Brahmer et al [16].	Nivolumab (0.3 mg, 1 mg, 3 mg, and 10 mg/kg) q2w	14	100% ≥ 2 lines	1 MSI-H	7	-	-	-	-	39
				13 MSS						
Topalian et al [99].	Nivolumab (1 mg, 3 mg, and 10 mg/kg) q2w	19	100% ≥ 1 line	-	0	-	-	-	-	14
Brahmer et al [103].	Nivolumab (0.3 mg, 1 mg, 3 mg, and 10 mg/kg) q2w	18	100% ≥ 1 line	-	0	-	-	-	-	9
Bendell et al [111,112].	Atezolizumab 20 mg/kg q3w + Bev	14	≥3 lines	-	8	-14.1	-	-	-	7
	Atezolizumab 14 mg/kg q3w + Bev + mFOLFOX6	30	70% naïve		36					20
Le et al [56].	Pembrolizumab 10 mg/kg q2w	53	100% ≥ 2 lines	10 MSI-H	50	NR	90	-	-	41**
				18 MSS	0	2.4	11			
Hochster et al [104].	Atezolizumab 1200 mg q3w + Bev	10	100% ≥ 1 line	MSI-H	30	NR	90	-	-	40
O'Neil et al [117].	Pembrolizumab 10 mg/kg q2w	23	95% ≥ 2 lines	1 MSI-H	4	1.8	-	4	30	35
				22 MSS						
Tabernero et al [113,114].	Atezolizumab 1200 mg q3w + CEA CD3 TCB (5–160mg) q1w	35	-	11 MSS	12	-	-	-	-	31
Diaz et al [119].	Pembrolizumab 200 mg q3w	61	90% ≥ 2 lines	MSI-H	26	-	51	-	-	-
Overman et al [57].	Nivolumab 3 mg/kg q2w	74	84% ≥ 2 lines	MSI-H	31	14.3	64	50	73	20
Overman et al [58].	Nivolumab 3 mg/kg + Ipilimumab 1 mg/kg once q3w, for 4 doses, followed by Nivolumab 3 mg/kg q2w	119	76% ≥ 2 lines	MSI-H	55	NR	80	71	85	32
Lenz et al [108].	Nivolumab 3 mg/kg q2w + Ipilimumab 1 mg/kg q6w	45	Naive	MSI-H	60	NR	84	77	83	16
Bendell et al [105,116].	Atezolizumab 800 mg q2w + Cobimetinib 60 mg 14/14 or 21/7d on/off	84	100% ≥ 2 lines	2 MSI-H	8	1.9	31	11	43	37
				62 MSS						
Grothey et al [123].	Atezolizumab 800 mg q2w + FP + Bev	297	Naive	-	-	7.2	-	-	-(HR:0.86)	-
	FP + Bev	148				(HR:0.96)				
Chen et al [124].	Durvalumab 1500 mg + Tremelimumab 75 mg q4w	119	100% ≥ 1 line	MSS	1	1.8	22.7	2	17	64**
	BSC alone	61			0	1.9	6.6	2	13	
Eng et al [125].	Atezolizumab 840 mg q2w + Cobimetinib 60 mg 21/7d on/off	183	100% ≥ 2 lines	333 MSS	3	1.9	26	3	39	61
	Atezolizumab monotherapy 1200 mg q3w	90		6 MSI-H	2	1.9	21	0	27	31
	Regorafenib 160 mg 21/7d on/off	90			2	2.0	34	6	37	58

Abbreviations: MMR: mismatch repair, ORR: overall response rate, PFS: progression-free survival, OS: overall survival, G: grade, MSI-H: microsatellite instability, MSS: microsatellite stable, NR: not reached, BSC: best supportive care

*Treatment-related adverse events

** Any adverse event

endpoints are safety, PFS, the association between biomarkers (such as PD-L1 expression), and quality of life. The study design was atypical and consisted of parallel with non-comparator arms. Enrolled patients must have failed at least one previous line of treatment, including a fluoropyrimidine, oxaliplatin, and irinotecan, except in cohort 3. MMR status was assessed locally before screening, but MSI-H was subsequently evaluated by a central laboratory.

The safety and efficacy results of the 74 MSI-H patients enrolled in cohort 1 have been recently reported [57], demonstrating 32% objective response rate in locally determined MSI-H patients (Table 2). The median response time is 2.8 months and the median duration of response has not yet been reached, but 8 out of 23 responding patients have responses lasting 12 months or longer. There was no differential benefit according to the PD-L1 expression: 29% of objective response and 52% of disease control for ≥12 weeks in patients with PD-L1 expression ≥1%; and 28% and 75%, respectively, in those patients with PD-L1 expression <1%. From the 90% of patients with mutation status available, 16% (n: 12) had BRAF V600E mutation, and presented 25% of objective response. In 23 non-MSI-H patients, median PFS was only 1.4 months [106].

The safety profile of nivolumab monotherapy was consistent with that which was reported in other solid tumors [11,13,87]. Increased lipase (8%) and increased amylase (3%) were the most common grade 3 and 4 adverse events. Drug-related serious adverse events occurred in 12% of the patients (adrenal insufficiency, increased ALT levels, colitis, diarrhea, gastritis, stomatitis, acute kidney injury, pain, and arthritis, each one with 1% of frequency). Patient-reported outcome analyses demonstrated clinically meaningful improvements in function (emotional, role, and social), symptoms and global quality of life, with prolonged duration through week 37 or beyond.

The results of cohort 3 of MSI-H patients submitted to the combination of nivolumab plus ipilimumab have also been reported recently [58]. From the 119 patients included, 65 (55%) objective response rate, including 3% with complete response. Median PFS had not been reached, and 83% of patients had durable responses lasting ≥6 months. Increased AST (8%) and increased ALT (7%) were the most common severe adverse events. Considering any-grade treatment-related adverse events (TRAE), diarrhea (22%), fatigue (18%), and pruritus (17%) were the most frequent. TRAE leading to discontinuation of treatment occurred in 13% of patients, with autoimmune hepatitis and acute kidney injury as the only TRAE leading to discontinuation in more than one patient. Equivalent to nivolumab monotherapy, the combination of ICI was also associated with clinically meaningful improvements in patient-reported outcomes, with early improvement (by week 13 or earlier).

An exploratory analysis of the efficacy of nivolumab monotherapy suggested that patients submitted to fewer lines of therapy might derive greater benefit [107]. From the 74 patients evaluated, the 21 who had been submitted to ≤2 lines of therapy experienced a 52% response rate compared to 26% in the group of patients heavily treated. The results of the cohort of treatment-naïve mCRC patients submitted to the combination of nivolumab plus ipilimumab has been recently presented [108], and confirmed the hypothesis of higher therapeutic activity in that population (Table 2).

Based on the findings of the studies aforementioned, NCCN guidelines recently incorporated the indication of nivolumab and the combination of nivolumab plus ipilimumab in the management of MSI-H mCRC patients regardless of therapeutic line [109]. Nevertheless, both monotherapy and combination treatments have been approved by FDA for MSI-H mCRC only with progression following treatment with fluoropyrimidine, oxaliplatin and/or irinotecan [54,55].

A pilot phase II study evaluating the potential role of neoadjuvant ICI in locally advanced colon cancer was recently reported at European Society of Medical Oncology (ESMO) 2018 Meeting [110]. Patients with resectable, early-stage colon cancer, received nivolumab 3mg/kg on D1, D15 plus ipilimumab 1mg/kg on D1, followed by surgery. Primary endpoints were safety and feasibility, and secondary endpoint included efficacy assessed by pathological response criteria. From 14 patients, major pathological responses (<5% viable tumor cells) were observed in all 7 dMMR patients (100%), with 4 patients (57%) presenting complete responses. Four of these dMMR tumors were clinically stage IIIB-IIIC before the start of treatment. No major pathological responses were seen in pMMR tumors [110].

6.3. Phase I clinical trials with other immune checkpoint inhibitors

Atezolizumab has been intensively investigated in mCRC (Table 2). A phase Ib trial aimed to evaluate the safety and efficacy of this PD-L1 inhibitor in two cohorts: refractory and oxaliplatin-naïve patients (NCT01633970) [111,112]. The former cohort was exposed to atezolizumab plus bevacizumab, and all patients had been treated with ≥3 lines of systemic therapy, while the latter cohort had 70% of the patients with no prior systemic therapy, and they were exposed to atezolizumab associated with mFOLFOX6 plus bevacizumab. The objective response rates were discrepant between cohorts: 8% and 36%, respectively [111,112].

In dose-escalation phase Ia and Ib studies involving CEA-positive solid tumors (NCT02324257, NCT02650713), atezolizumab was associated to CEA CD3 TCB, a novel T-cell bispecific antibody targeting CEA on tumor cells and CD3 on T cells [113,114]. Two out of 10 patients (20%) presented objective response to ≥60 mg of the bispecific antibody. One responding patient had MSS status [113,114].

The association of MEK inhibitor with immune checkpoint inhibitors has been hypothesized as a promising therapeutic strategy, based on preclinical evidence that MEK inhibition promotes accumulation and survival of intratumoral tumor-specific T cells, and therefore might have a synergistic activity with immunotherapy [115]. A phase I/Ib study evaluated the association of atezolizumab plus cobimetinib in treatment-refractory metastatic or locally advanced solid tumors [116].

The primary objective of the study was to evaluate the safety and tolerability of the association. The secondary objectives included overall response, duration of response, PFS and OS. The incidence of grade 3–4 TRAE was 44%, with the most common being diarrhea (6%), rash, fatigue, and blood CPK increase (5% each). Of the 150 patients included, 84 had mCRC, of which 7 (8%) showed objective response. From two patients with MSI-H status, one presented objective response, compared with 6 out of 62 (10%) with MSI low/MSS.

Pembrolizumab was evaluated in a phase Ib multicohort trial KEYNOTE-028 trial (NCT02054806). The cohort of patients with advanced PD-L1 positive mCRC patients, including both MSI-H and MSS, has been recently reported [117]. Primary endpoints were safety and overall response rate by investigator review. Secondary endpoints were PFS, OS, and duration of response. With 138 patients screened for tumor PD-L1 expression, 23 were enrolled. The majority of patients had been heavily treated, with 15 (65%) exposed to ≥3 lines of systemic therapy. There was 1 MSI-H patient, whose objective response was the only one registered in the study (4% of overall objective response). This patient also harbored a BRAF V600E mutation and discontinued treatment at 23.2 months due to consent withdrawal, with no evidence of disease progression. The remaining 22 MSS patients, despite being PD-L1 positive, did not present objective response to pembrolizumab.

6.4. Phase II clinical trials with other immune checkpoint inhibitors

The first clinical trial evaluating the role of immune checkpoint inhibitors in mCRC was a single-arm phase II study with tremelimumab, an anti-CTLA-4, reported in 2010 (Table 2) [118]. All 47 patients enrolled had been exposed to fluoropyrimidines, oxaliplatin, and irinotecan previously. Primary endpoint was objective response, and secondary endpoints were safety, duration of response, PFS, and OS. With disappointing results, of the 45 response-evaluable patients, 44 did not reach second dose (43 with progressive disease and 1 discontinuation of therapy). One patient received five doses, and the response duration was 6 months. Diarrhea was the most frequently reported TRAE (36% of any grade), and five patients (11%) presented grade 3 diarrhea.

Based on the findings from phase I clinical trials which raised the hypothesis of a benefit derived from the immune checkpoint inhibition according to the MMR status [16,99], a phase II trial evaluating the activity of pembrolizumab in 41 patients with metastatic solid tumors was initiated [56]. The coprimary endpoints were the immune-related objective response rate and the 20-week immune-related PFS rate. They were divided into three groups: dMMR CRC (n: 10), pMMR CRC (n: 18), and dMMR non-CRC (n: 7). The results confirmed the initial hypothesis: the immune-related objective response rate was 40%, 0%, and 71%; and the 20-week immune-related PFS rate was 78%, 11%, and 67%, respectively. This study was one of the five studies [56,119–122] that led to the accelerated approval of pembrolizumab for adult and pediatric patients with unresectable or metastatic, MSI-H or dMMR, solid tumors that progressed following prior treatment and had no alternative treatment options, or with MSI-H or dMMR colorectal cancer that progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan [53]. It was the first tissue/site-variable approval by FDA. The five studies comprised 149 MSI-H patients, of which 90 had mCRC. Overall response rate was 40%, and 36% in a subgroup of mCRC patients [56,119–122].

Randomized phase II study MODUL (NCT02291289) evaluating the role of chemoimmunotherapy as maintenance therapy has an umbrella design. Patients with treatment-naïve unresectable mCRC receive eight cycles of induction FOLFOX plus bevacizumab followed by maintenance with fluoropyrimidine plus bevacizumab, or experimental treatment in 1 of the 4 cohorts: (1) 5-FU plus cetuximab plus vemurafenib, if BRAF V600E-positive, (2) 5-FU or capecitabine plus bevacizumab plus atezolizumab, if BRAF V600E wild-type, (3) capecitabine plus trastuzumab plus pertuzumab, if HER2-positive, (4) atezolizumab plus cobimetinib, if MSI-H. The primary endpoint was PFS per investigator assessment. The results of cohort 2 have been reported recently [123], and there was no benefit to the addition of maintenance atezolizumab compared to the group of patients treated with only fluoropyrimidines plus bevacizumab. Considering all cohorts, 696 patients were included, and 445 BRAF wild-type (cohort 2) were randomly allocated to fluoropyrimidines plus bevacizumab (148 patients) or the same regimen plus atezolizumab (297 patients). Median PFS was 7.13 months in the experimental arm versus 7.39 months (HR: 0.92, p = 0.480). Median OS was 21.91 months versus 22.05 months, respectively (HR: 0.86, p = 0.283) [123].

A phase II randomized study of durvalumab, an anti-PD-1 antibody, plus tremelimumab and best supportive care (BSC) versus BSC alone in patients with refractory MSS mCRC was recently presented (CCTG CO.26 trial) [124]. Patients were randomized 2:1 to immunotherapy arm versus BSC alone arm, and the primary endpoint was OS. From 180 patients enrolled and with a median follow-up of 15.2 months, the median OS was 6.6 months in the immunotherapy arm versus 4.1 months (HR: 0.72, 90% CI 0.54–0.97, p = 0.07). Median PFS was 1.8 months versus 1.9 months, respectively (HR 1.01, 90% CI 0.76–1.34; p = 0.97). However, disease control rate was superior in the immunotherapy arm: 23% versus 7%, respectively (p = 0.006) [124].

6.5. Phase III clinical trials with other immune checkpoint inhibitors

IMblaze370 is the first completed phase III study evaluating the role of ICI in CRC [125]. The 363 treatment-refractory patients were recruited to one of three arms (2:1:1): (1) atezolizumab (840 mg intravenously every 2 weeks) plus cobimetinib (60 mg orally once daily for days 1–21 of a 28-day cycle), (2) atezolizumab monotherapy (1200 mg intravenously every 3 weeks), or (3) regorafenib (160 mg orally once daily for days 1–21 of a 28-day cycle). The enrollment of MSI-H patients was allowed, but it was capped at 5% of the overall population. The primary endpoint was overall survival.

Immunotherapy arms did not present superiority compared to regorafenib. Median OS was 8.8 months with atezolizumab plus cobimetinib, 7.1 months with atezolizumab, and 8.5 months with regorafenib (HR: 1.00 (95% CI 0.73–1.38, p = 0.99) for the combination versus regorafenib, and HR: 1.19 (95% CI 0.83–1.71,

p = 0.34) for atezolizumab versus regorafenib). Median PFS was 1.9 months, 1.9 months, and 2.0 months, respectively. Objective response rates were 3%, 2%, and 2%, respectively, with no complete responses recorded. Likewise, it was not found statistically significant difference in OS and PFS between atezolizumab arms. Of the 339 patients with MSI status available, 6 were MSI-H (1.7%), of which three were in the combination arm and three in the atezolizumab arm. Three of them (2 and 1, respectively) presented partial responses.

Grade 3–4 adverse events rates were 61% with atezolizumab plus cobimetinib, 31% with atezolizumab, and 58% with regorafenib. Serious adverse events rates were 40%, 17%, and 23%, respectively. The most common grade 3–4 adverse events in the combination arm were diarrhea (10%), increased blood creatine phosphokinase (7%), anemia (6%), and fatigue (4%).

7. Ongoing clinical trials

Due to the promising results in the cohort of treatment-naïve MSI-H patients, it is expected that larger studies will follow to address this issue. Two randomized clinical trials are currently recruiting patients to evaluate the benefit of nivolumab associated to oxaliplatin-based chemotherapy in first-line therapy of an unselected population of mCRC (NCT03414983, NCT03388190) (Table 3).

It is noteworthy the ongoing phase III studies evaluating the potential benefit of ICI in cure rates of stage III MSI-H colon cancer. Atezolizumab and avelumab will be associated to FOLFOX in the adjuvant therapy, with estimated enrollment of 700 and 180 patients, respectively (Table 4).

Table 3. Ongoing clinical trials evaluating nivolumab in colorectal cancer.

Phase	Population	Setting	Arms	Status	NCT number
Randomized trials					
II/III	Metastatic	1st line	Nivolumab + FOLFOX + Bevacizumab FOLFOX + Bevacizumab	Recruiting	NCT03414983
II	Metastatic	1st line	FLOX + Nivolumab FLOX	Recruiting	NCT03388190
II	Liver-limited metastatic	1st line	Preoperative Nivolumab + mFOLFOX6 Nivolumab + MVA-CV301 followed by Nivolumab + FPV-CV301	Recruiting	NCT03547999
I/II	Metastatic MSS + RAS-mutant	Refractory	Binimetinib + Nivolumab Binimetinib + Nivolumab + Ipilimumab	Active, not recruiting	NCT03271047
Phase I/II single-arm trials					
II	Stage I, II, III	Neoadjuvant	Nivolumab + Ipilimumab + Celecoxib	Recruiting	NCT03026140
II	Lynch syndrome	Any	Nivolumab	Recruiting	NCT03631641
II	Metastatic MSS	Refractory	Temozolomide followed by Nivolumab	Recruiting	NCT03879811
II	Metastatic MSS	Refractory	Nivolumab + Ipilimumab + Temozolomide	Not yet recruiting	NCT03832621
II	Metastatic	Refractory	Nivolumab + TAS-102	Completed	NCT02860546
II	Metastatic MSS	Refractory	Nivolumab + Relatlimab	Recruiting	NCT03642067
II	Metastatic	Refractory	Nivolumab + BBI608 or Nivolumab + BNC105	Recruiting	NCT03647839
II	Metastatic MSS	Refractory	Nivolumab + Metformin	Recruiting	NCT03800602
II	Metastatic	Refractory	Nivolumab + Ipilimumab + Radiation therapy	Recruiting	NCT03104439
II	Metastatic	Refractory	Nivolumab + Ipilimumab	Active, not recruiting	NCT03350126
II	Metastatic	Refractory	Nivolumab + Relatlimab	Not yet recruiting	NCT03867799
II	Metastatic	Refractory	Nivolumab + other drugs (Ipilimumab, Cobimetinib, Daratumumab, anti-LAG-3)	Active, not recruiting	NCT02060188
II	Metastatic MSS	Refractory	Nivolumab + Ipilimumab + Panitumumab	Recruiting	NCT03442569
II	Metastatic (Mucinous)	Refractory	Nivolumab + Ipilimumab	Not yet recruiting	NCT03693846
II	Metastatic/Liver lesions	Refractory	Nivolumab + Tadalafil + Vancomycin	Not yet recruiting	NCT03785210
II	Metastatic/Liver lesions	Refractory	Trans-arterial tirapazamine embolization + Nivolumab or Pembrolizumab	Recruiting	NCT03259867
II	Metastatic	Refractory	Nivolumab + ALT-803	Recruiting	NCT03228667
Ib/II	Stage II, III rectal cancer	Neoadjuvant	Chemoradiotherapy followed by Nivolumab	Recruiting	NCT02948348
Ib/II	Metastatic MSS	Refractory	Nivolumab + ONC201	Not yet recruiting	NCT03791398
Ib/II	Metastatic	Refractory	Nivolumab + Guadecitabine	Not yet recruiting	NCT03576963
I/II	Metastatic MSS	1st line or 2nd line	Nivolumab + Ipilimumab + GRT-C901/GRT-R902	Recruiting	NCT03639714
I/II	Metastatic MSS	Refractory	Copanlisib followed by Nivolumab	Recruiting	NCT03711058
I/II	Metastatic	Refractory	Nivolumab + Trametinib ± Ipilimumab	Recruiting	NCT03377361
I/II	Metastatic MSI-H	Refractory	Nivolumab + Copanlisib	Recruiting	NCT03735628
I/II	Metastatic	Refractory	Nivolumab + BMS-813160	Recruiting	NCT03184870
I/II	Metastatic	Refractory	Nivolumab + Varlilumab	Completed	NCT02335918
I/II	Metastatic	Refractory	Nivolumab + Epacadostat	Not yet recruiting	NCT02327078
I/II	Metastatic	Refractory	Nivolumab + NKTR-262 + NKTR-214	Recruiting	NCT03435640
I/II	MSI-H ≤ 18y	Refractory	Nivolumab	Recruiting	NCT02992964
I/II	Metastatic	Refractory	Nivolumab	Not yet recruiting	NCT03169777
I	Metastatic/Liver lesions	1st line	Preoperative Nivolumab or Ipilimumab + VX15/2503	Recruiting	NCT03373188
I	Metastatic	2nd line	Nivolumab or Bevacizumab + Oxaliplatin + S95005	Recruiting	NCT02848443
I	Metastatic/Liver lesions	Any	Nivolumab + Ipilimumab + CMP-001 + Radiosurgery	Recruiting	NCT03507699
I	Metastatic	Refractory	Nivolumab + Regorafenib	Recruiting	NCT03712943
I	Metastatic MSS	Refractory	Nivolumab + Enadenotucirev	Recruiting	NCT02636036
I	Metastatic	Refractory	Nivolumab + TPST-1120	Recruiting	NCT03829436
I	Metastatic	Refractory	FT500 + Nivolumab	Not yet recruiting	NCT03841110

Abbreviations: MSS: microsatellite stable, MSI-H: microsatellite-instability

Table 4. Ongoing phase III clinical trials evaluating immune checkpoint inhibitors in colorectal cancer.

Population	Setting	Arms	Estimated accrual	Status	NCT number
Atezolizumab					
Stage III, MSI-H	Adjuvant	FOLFOX + atezolizumab FOLFOX	700	Recruiting	NCT02912559
Metastatic, MSI-H	1st line	mFOLFOX + bevacizumab + atezolizumab mFOLFOX + bevacizumab	347	Recruiting	NCT02997228
Avelumab					
Stage III, MSI-H and/or POLE-mutant	Adjuvant	5-FU based chemotherapy followed by avelumab 5-FU based chemotherapy	180	Recruiting	NCT03827044
Nivolumab					
Metastatic	1st line	FOLFOX + Bevacizumab + Nivolumab FOLFOX + Bevacizumab	180	Recruiting	NCT03414983

Abbreviations: MSI-H: microsatellite-instability

8. Predictive biomarkers

It is not clear why certain tumors are more sensitive to immunotherapy. Based on the target of anti-PD-1/PD-L1 therapies, it is logical to evaluate the benefit of these monoclonal antibodies according to the expression of PD-L1. Initial data from phase I study on the use of nivolumab in melanoma, NSCLC, renal-cell carcinoma, prostate cancer, and mCRC showed that patients with PD-L1 expression in ≥5% of the tumor cells had higher probability of response: 9 (36%) out of 25 patients, compared to none in the patients deemed PD-L1 negative [14,99]. Corroborating these initial findings, melanoma and NSCLC patients considered PD-L1-positive also presented superior PFS and OS compared to the negative counterparts [12,126,127]. Nevertheless, objective responses and prolonged survival can also be reached in patients with lower expression of PD-L1 or even with no expression. PD-L1-negative advanced melanoma patients had 41% objective response rate when treated with nivolumab, and 55% with the combination of nivolumab plus ipilimumab [127]. They also reached 11.2 months of median PFS when exposed to the combination therapy, and 5.3 months with nivolumab monotherapy. The threshold in the definition of PD-L1 positivity is also unclear. Cutoffs of 1% [128], 5% [127], and 50% [91] have been used in clinical trials, though it is not possible to state which value has greater accuracy. Therefore, the negative predictive value of PD-L1 expression is suboptimal to select patients to PD-1/PD-L1 inhibitors, and it is estimated to be 58% for nivolumab, and 45% for nivolumab plus ipilimumab, based on CheckMate 067 data [14,127].

The most responsive tumors to ICI observed, such as melanoma, NSCLC, and urothelial carcinoma, were those with higher rates of somatic mutations [15,73]. Patients with genomic instability, such as those with germline or somatic abnormalities in DNA MMR pathways, tend to present higher number of nonsynonymous mutations, and thereby high TMB. In the phase II trial evaluating pembrolizumab in cohorts of patients according to MMR status, a mean of 1782 somatic mutations per tumor in dMMR patients was found, compared to 73 per tumor in pMMR patients [56]. The objective response rate to pembrolizumab was 40% versus 0%, respectively, when considered mCRC patients. There is a high concordance rate between MSI-H and TMB. In a study with more than 62,000 tumor samples analyzed, 83% of MSI-H patients had high TMB [129]. However, the converse was not true. Only 16% of the samples with high TMB were classified as MSI-H. Similarly to PD-L1 expression, the cutoff to dichotomize TMB in high and low is also uncertain, with studies using both >100 [130] and ≥178 [131] nonsynonymous mutations as thresholds.

In the face of the cost and potential benefit of ICI, it is imperative to find biomarkers with higher accuracy to better select patients, which is a field of intensive research. Tumor-infiltrating lymphocytes [132], T-cell receptor clonality [133], neoantigen burden [71], immune gene signatures [72], and multiplex immunohistochemistry [133] have also been evaluated as potential biomarkers.

In CRC, both PD-L1 and TMB are not used as biomarkers to select patients for using ICI. As aforementioned, the benefit of nivolumab [57], pembrolizumab [56], and the combination of nivolumab plus ipilimumab [58] were only demonstrated in MSI-H patients. Based on these studies, FDA-approval indications request MSI-H positivity to select patients for ICI in mCRC [53–55].

9. Perspectives

Prompted by the studies of ICI in melanoma, non-small cell lung cancer, renal-cell carcinoma, and MSI-H tumors, intensive investigation is currently underway to address the following questions: (1) Besides CTLA-4 and PD-1, are there other potential therapeutic targets? (2) What are the most accurate biomarkers to predict benefit from immunotherapy? (3) Might apparently resistant tumors, such as MSS, be turned into sensitive tumors? (4) Besides metastatic disease, might localized disease benefit from immunotherapy? (5) Might adoptive T-cell therapies, such as CAR-T cell therapy, be beneficial in mCRC?

Based on the concept of 'immunological synapse' and the evidence that modulation of antitumoral immune response is played by several membrane receptors besides CTLA-4 and PD-1, the identification of new immune checkpoints and therapeutic development targeted to those receptors and pathways is a field of accelerated research. LAG3, TIM3, TIGIT, and VISTA are examples of co-inhibitory molecules that are currently under investigation as potential therapeutic targets [74]. Co-stimulatory receptors are also being evaluated, and may be represented by ICOS, OX40, GITR, 4-1BB, CD40, and CD27 [74].

As mentioned earlier, PD-L1 expression and TMB are emerging biomarkers to predict benefit from immunotherapy. However, PD-L1 does not present consistent findings among tumors, and has a negative predictive value [14,127], while

TMB demands larger biological samples, is associated with higher turnaround time and costs, and does not have well accepted thresholds [134]. In mCRC, MSI-H has limitations in the selection of patients to immunotherapy, since approximately 15% of the treatment-naïve MSI-H patients seem to be resistant to ICI [108]. The association of biomarkers (MSI-H and TMB) may be useful to better select patients, since sensitive patients presented higher median TMB (54 mutations/Mb) compared to resistant ones (29 mutations/Mb) in a cohort of 22 MSI-H mCRC patients treated with PD-1/PD-L1 inhibitors [135]. Beyond its predictive value, TMB may also present prognostic significance in mCRC. Post-hoc exploratory analysis of CALGB 80405 study demonstrated that patients with high TMB (≥8 mutations/Mb) had better overall survival compared with low TMB counterparts (33.8 months versus 28.1 months, HR: 0.73, 95% CI 0.57–0.95) [136]. However, TMB did not predict benefit from neither bevacizumab nor cetuximab. The association of MSI-H with immunoscore may also be potentially useful in the identification of immunotherapy-sensitive patients. MSI-H patients are associated with high immunoscore, but 20% of MSS CRC patients also present immunoscore high, and might be sensitive to immunotherapy [137–139]. Studies searching for predictive biomarkers in ctDNA have an interesting rationale. It seems that ctDNA reflects more accurately the intratumoral and intertumoral heterogeneity and might demonstrate the dynamics of antitumoral immune response with better accuracy [140].

The reasons the majority of cancer patients seem to be resistant to the available ICI are unclear. Insufficient anti-tumor T-cell generation, inadequate anti-tumor T-cell effector function, and impaired formation of T-cell memory are hypotheses that might explain both innate (primary) and acquired (secondary) resistance to ICI [141,142]. Tumor-extrinsic mechanisms may also be involved in the resistance, involving both the non-neoplastic cells in the tumor microenvironment and systemic factors (e.g. gut microbiota) [141–143]. Microbiome signatures have been implicated in the pathogenesis of CRC and other gastrointestinal malignancies, mainly *Fusobacterium* species [144,145]. It has been demonstrated that microbial signatures might also be involved with sensitivity to immunotherapy. *Akkermansia muciniphila, Ruminococcacea* species, *Bifidobacterium longum, Collinsela aerifaciens*, and *Enterococcus faecium* seem to be overrepresented among responder patients [143,146,147]. Antibiotic consumption has been associated with poor response to ICI, and that oral supplementation of probiotics may restore response to immunotherapy [143]. Strategies to overcome these mechanisms of resistance are currently underway, and include fecal transplantation [148].

To date, ICI has been demonstrated beneficial only in patients with advanced disease. The greatest benefit of immunotherapy might be for patients with localized disease, when it might be used both in preoperative and postoperative settings. As aforementioned, atezolizumab and avelumab are currently under investigation in a population of stage III MSI-H colon cancer patients in phase III trials (NCT02912559, NCT 03827044). The pilot trial evaluating neoadjuvant nivolumab plus ipilimumab in localized colon cancer raises the hypothesis of a potential benefit of immunotherapy in the preoperative treatment of borderline resectable liver metastasis, as well as in the neoadjuvant therapy of rectal cancer.

Based on the successful experience of CAR-T cell therapy in hematological malignancies [149,150], preclinical and clinical studies evaluating adoptive T-cell therapies have been progressively conducted in solid tumors. Colorectal cancer has been studied in preclinical models and phase I escalating-dose trials investigating the role of CAR-T cell therapy [151,152], with encouraging results.

Subsets of patients with actionable mutations have been more intensively studied in the last years. Clinical trials evaluating anti-HER2 therapies, such as trastuzumab plus lapatinib [153], and trastuzumab plus pertuzumab [154], have shown remarkable results in treatment-refractory HER2-positive mCRC, comprising approximately 5% of patients [155]. In patients with BRAF V600E mutation, triple targeted therapy with encorafenib plus binimetinib plus cetuximab [156], or with vemurafenib plus irinotecan plus cetuximab [157], has gained more attention after notable results in phase II clinical trials. These targeted therapies, despite not yet being FDA-approved, associated with immunotherapy in MSI-H colorectal cancer, may dramatically change the landscape of personalized therapy of CRC in a near future.

10. Conclusion

Colorectal cancer has proven to be a challenging disease. Systemic therapies have been historically developed to an indistinct population of patients, ignoring the molecular heterogeneity of the disease, and demonstrated to be of limited efficacy, with poor clinical outcomes. Similar to other solid tumors, significant advances in systemic therapy for colorectal cancer have been found when specific molecular abnormalities were taken into account. The use of anti-EGFR monoclonal antibodies based on RAS/BRAF status and sidedness, as well as the clinical trials of BRAF inhibitors in BRAF V600E-mutated patients, are recent examples of the benefit of genome-guided personalized therapy. Natural antitumoral immune response is a genuinely personalized therapy by host organisms. Immune cells recognize discrete neoantigens that would be hardly detectable by diagnostic methods. ICI, despite apparently neglecting specific molecular abnormalities of cancer, in reality intend to boost an already present natural personalized therapy. Phase I and II clinical trials evaluating the ICI in dMMR/MSI-H mCRC patients provide strong evidence of the benefit that nivolumab, pembrolizumab, and the association of nivolumab and ipilimumab can bring to that specific population of hypermutated colorectal cancer patients. Moreover, immunotherapy-resistant patients are being investigated to ascertain if they can derive benefit from novel therapeutic strategies. Work remains to be done, but the intensive patient-centered research on molecular biology of cancer, and the accelerated therapeutic development observed in the last decades provide evidence for an optimistic future for our colorectal cancer patients.

11. Expert opinion

Undoubtedly, immunotherapy has ushered in a new era in cancer therapy. Nevertheless, a meaningful group of patients will not present objective response to the main modality of immunotherapy, the immune checkpoint inhibitors (ICI). However, how to measure the benefit to ICI? The majority of clinical trials have analyzed objective response by RECIST, as well as progression-free survival (PFS) and overall survival (OS) as the main endpoints. ICI can elicit peculiar patterns of response not taken into account in RECIST, which prompted the publication of the immune-related response criteria (irRC) [158]. It is estimated that RECIST underestimates the benefit of immunotherapy in approximately 15% of the patients [158]. It is not clear which are the most appropriate endpoints to address the benefit derived from ICI. Laboratory assays that evaluate cellular immune response may be needed to study the sensitivity to immunotherapy, as well as new statistical methods that consider delayed separation of survival curves seen in clinical trials [159]. Therefore, ICIs have been incorporated into clinical practice in several cancers, but the selection of patients and the estimation of the magnitude of benefit might not be properly evaluated.

In metastatic colorectal cancer (mCRC), the clinical trials conducted so far show strong evidence for the use of ICI in the population of patients enriched with microsatellite-instability (MSI-H) tumors, who comprise approximately 5% of the total population of mCRC patients [59]. The FDA has approved pembrolizumab for palliative treatment of site flexible MSI-H tumors that progressed following prior treatment [53], and nivolumab monotherapy or associated to ipilimumab in MSI-H mCRC patients who failed to standard therapies [54,55]. There is special concern related to the financial impact associated to the applicability of immunotherapy, even in developed countries, but especially in low- and middle-income countries. It is likely that immunotherapy offers benefit to a larger group of patients, and may be used in earlier lines of systemic therapy, which would increase the financial impact on health care. Governments, health authorities, health insurance companies, pharma industry, advocacy groups, and society must foster a deep discussion of such a meaningful topic in order to warrant the sustainability of health systems in the future.

To date, key areas for improvement of immunotherapy in CRC are: (1) Are there microsatellite stable (MSS) patients who are sensitive to immunotherapy? (2) Is it possible to turn immunotherapy-resistant patients into sensitive ones? (3) Are the benefits observed in metastatic disease applicable to localized disease? The clinical trials evaluating unselected population of MSS mCRC patients have been disappointing. Some MSS patients are hypermutated, and thereby potentially sensitive to immunotherapy, such as POLE-mutant patients. Exploratory analysis of those prospective clinical trials, mainly correlating with potential biomarkers in ctDNA, may identify if there is a subgroup of MSS patients who derive benefit from immunotherapy. Both innate (primary) and acquired (secondary) mechanisms are responsible for resistance to immunotherapy. Modulation of gut microbiome through oral supplementation of probiotics or fecal transplantation [143,148], and increasing the exposure of neoantigens through radiation therapy (abscopal effect) [160] are examples of strategies under investigation to overcome resistance [141,142]. Despite having benefit in metastatic disease, irinotecan, anti-EGFR monoclonal antibodies and anti-angiogenics have never been demonstrated to be beneficial in early-stage disease. Pilot clinical trial with neoadjuvant immunotherapy in early-stage colon cancer has been encouraging from a pathological standpoint [110], but it needs longer follow-up, and we must await results from the randomized clinical trials in stage III MSI-H and/or POLE-positive CRC before adopting ICI in adjuvant setting. Such studies raise the hypothesis of the potential benefit of immunotherapy in preoperative therapy of liver metastasis and neoadjuvant therapy of rectal cancer in MSI-H patients.

From a speculative viewpoint about how the field will evolve in the future, it would be interesting to analyze the applicability of immunotherapy in two distinct populations: MSI-H and non-MSI-H. As expected in systemic therapies, the objective response rate of immunotherapy in MSI-H mCRC has been demonstrated to be higher in earlier lines of therapy [57,58,107,108]. It is likely that ICI becomes the standard of care in first-line therapy of MSI-H mCRC in a near future. Given that 7% of 45 MSI-H mCRC patients experienced clinical complete response to combination of ICI [108], and 100% of 7 MSI-H early-stage colon cancer patients had major pathological responses (<5% viable tumor cells), with 4 patients (57%) experiencing pathological complete response [110], immunotherapy may be a potential preoperative therapy in MSI-H colon and rectal cancer. Based on the aforementioned studies, there is a high probability of benefit derived from immunotherapy as adjuvant therapy in stage III MSI-H and/or POLE-mutant patients.

From a molecular standpoint, MSI-H patients comprise a distinct population, with unique genomic, epigenomic and transcriptomic abnormalities. Non-MSI-H CRC patients constitute approximately 85% of the total population of CRC, with molecular heterogeneity [59]. It is possible that a small subgroup, other than POLE-mutant patients, be sensitive to immunotherapy, but it is unlikely that those patients will be identified in the next few years. The recognition of new predictive biomarkers besides PD-L1 expression, TMB and MSI-H will require extensive work and validation through prospective clinical trials. Likewise, it is unlikely that the majority of MSS patients will be initially sensitive to immunotherapy. Strategies to overcome primary resistance are currently underway, and they will demand great efforts to be implemented in clinical practice.

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