Nivolumab in the treatment of microsatellite instability high metastatic colorectal cancer

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Nivolumab is a PD-1 inhibitor approved for the use in treatment of multiple tumor types (such as melanoma, non-small-cell lung cancer, renal cell carcinoma, urothelial cancer and Hodgkin's lymphoma). In July 2017, the US FDA granted accelerated approval of this agent for the treatment of metastatic colorectal cancer patients whose tumor harbors deficient mismatch repair, or microsatellite-instability high and have progressed on conventional chemotherapy. In this review, we will discuss the use, efficacy, and safety of this agent in microsatellite-instability high metastatic colorectal cancer.

First draft submitted: 13 December 2017; Accepted for publication: 2 February 2018; Published online: 23 February 2018

Keywords: colorectal • immunotherapy • microsatellite instability • MSI-H • nivolumab • PD-1

Despite major advances in the understanding of pathophysiology of colorectal cancer (CRC), this disease remains one of the leading causes of cancer deaths in the USA [1]. Prognosis of patients with metastatic colorectal cancer (mCRC) continues to be poor and given limited efficacy of conventional cytotoxic agents, efforts have been made to develop or use alternative treatments such as immunotherapy, alone or in combination with cytotoxic regimens. Among immunotherapeutic agents, pembrolizumab (Keytruda, Merck, NJ, USA) and nivolumab (Opdivo, Bristol-Myers Squibb, NY, USA) have been approved for use in a selected subset of CRC patients whose tumor harbors deficient mismatch repair (dMMR) or high-level microsatellite-instability (MSI-H). In this article, we will review the use of nivolumab, outline the previous and current clinical trials and summarize efficacy and toxicity of this agent in CRC patients.

PD-1 inhibition in CRC

While human cancers demonstrate genetic alterations and express neoantigens which are potentially recognizable by host immune system, the immune response is usually ineffective as tumor cells develop resistance mechanisms. These mechanisms include tolerance induction, T-cell dysfunction, and immunosuppression [2–4]. Expressing ‘immune checkpoints’, such as PD-1 and CTL-4, by tumor cells has been shown to be one of the important mechanisms of evasion from the immune system. In normal conditions, the checkpoints downregulate immune responses after antigen activation [5]. One of the checkpoints extensively studied in human cancers is PD-1. PD-1 is a cell membrane receptor expressed by various immune cells including T cells, B cells, monocytes and natural killer cells [6]. Interaction of PD-1 with its ligands (PD-L1 and PD-L2) results in inhibition of T-cell signaling and downregulation of proinflammatory cytokines [7]. Preclinical models have demonstrated upregulation of PD-1 by tumor cells and by cells in the tumor microenvironment [8]. Over the past few years, inhibition of PD-1 has gained specific attention in the landscape of cancer treatment [9,10]. Targeting PD-1, alone or in combination with other treatment modalities (such as chemotherapy, surgery or radiotherapy) has revolutionized the treatment paradigm in different tumor types (such as melanoma, non-small-cell lung cancer, renal cell carcinoma and Hodgkin's lymphoma) [6]. Nivolumab is a fully humanized monoclonal immunoglobulin G4 (IgG4) anti-PD-1 antibody. By blocking the binding of PD-1 to PD-L1 and PD-L2, this agent promotes T-cell proliferation and cytokine production [11]. Despite encouraging results of the PD-1 blockade in various tumor types, the majority of patients with CRC have not shown benefit from currently approved immunotherapeutic agents [12]. This limitation was evident even from the initial human studies of immune checkpoint inhibitors. The first study of single agent

Table 1. Key nivolumab trials in colorectal cancer patients.

Trial	Year	Agent	Study	Population	Number of patients	Response rate	Disease-control rate	Ref.
Topalian <i>et al.</i>	2012	Nivolumab	Phase Ib	Refractory CRC (predominantly MSS)	19	0%	0%	[5]
Overman <i>et al.</i> (CheckMate 142)	2017	Nivolumab	Phase II	Refractory MSI-H	74	31%	69%	[23]
Andre <i>et al.</i> (CheckMate 142)	2017	Nivolumab + ipilimumab	Phase II	Refractory MSI-H	27	41%	78%	[26]

CRC: Colorectal cancer; MSI-H: Microsatellite-instability high; MSS: Microsatellite stable.

intravenous infusion of nivolumab at varying doses of 0.3–10 mg/kg was reported in 39 patients with advanced solid tumors in 2010 [8]. In this trial, no maximum tolerated dose was identified, and out of 14 patients with refractory CRC, only one patient achieved complete response (CR). This single responder harbored microsatellite-instability (MSI), and long-term follow-up showed a sustained CR for more than 3 years. Further investigation of this patient's biopsy, revealed a high number of T cells and increased expression of PD-L1 in tumor and stroma [13]. Subsequently, in a Phase Ib trial evaluating 296 patients with various advanced solid cancers receiving nivolumab, no objective response was noted in 19 patients with CRC [5].

Prior to these trials, it had been shown that tumors harboring dMMR develop a high level of mutations in repetitive DNA sequences (microsatellites), which results in MSI. Hypermutated tumors were shown to express neoantigens leading to robust T-cell infiltration and improved immune response in tumors [14–16]. Of note, MSI-H occurs in minority of cases with CRC (15% of cases with early CRC and almost 4% of cases with mCRC) [17–19].

Subsequently, two studies of anti-PD-1 inhibitors on MSI-H CRC patients were initiated. The first report was preliminary data from a small Phase II trial of nine CRC patients with dMMR, out of which, seven (78%) patients responded to the anti-PD-1 monoclonal antibody (pembrolizumab) [20]. Further expansion to Phase II trial of pembrolizumab included 28 patients with dMMR and 25 patients with proficient mismatch repair (pMMR) mCRC, which again revealed superior benefit of the PD-1 blockade in dMMR CRC. These patients had response rate (RR) and disease-control rate (DCR – defined as CR, partial response or stable disease) of 50 and 89%, while pMMR CRC demonstrated 0 and 16% of RR and DCR, respectively. While median progression-free survival (PFS) was 2.4 months for pMMR CRC, median PFS was not reached in patients with dMMR [21]. Further evaluation of pembrolizumab in patients with mCRC showed overall response rate (ORR) of 26.2% and DCR of 50.8% at median follow-up of 7.4 months. At the time of the report, all responses were ongoing (KeyNote 164, NCT02460198) [22]. The second study of anti-PD-1 therapy in CRC was initiated using nivolumab (CheckMate 142 trial, NCT02060188). In this single arm Phase II trial on dMMR/MSI-H mCRC, patients received intravenous infusion of nivolumab at the dose of 3 mg/kg every 2 weeks until disease progression, death, unacceptable adverse events, withdrawal of consent or study end. At the median follow-up of 12 months, median duration of response was not reached and among 74 patients, 23 (31.1%) patients achieved objective response and 51 (69%) patients showed disease-control for at least 12 weeks [23]. Despite lack of a control group in this trial, the clinical response was substantial enough such that on 31 July 2017, the US FDA granted accelerated approval of nivolumab (at the dose of 240 mg given every 2 weeks) for heavily pretreated mCRC patients whose tumor harbors dMMR or MSI-H [24]. This Phase II trial has been expanded in CRC and continues to evaluate nivolumab alone or in combination with other anticancer drugs using multiple arms. Most recently, long-term outcomes at median follow-up of 21 months were reported; 12-month PFS and overall survival (OS) rates were 44 and 72%, respectively. ORR and DCR were noted in 25 (34%) and 46 (62%) patients, respectively. Also, with the longer follow-up, the rate of CR increased from 3 to 9% [25].

Some of the key trials of nivolumab in CRC patients are summarized in Table 1.

In the most recent update from CheckMate 142 trial, long-term survival and outcomes by prior standard chemotherapy (i.e., fluoropyrimidine, oxaliplatin, and irinotecan) with nivolumab were also reported. As compared with those who received all three chemotherapies prior to nivolumab, those who had ≤ 2 standard chemotherapies showed more robust responses to nivolumab. This finding supports evaluation of nivolumab combinations in the first-line setting [25].

One important characteristic of treatment with anti-PD-1 in solid tumors (including CRC) is the durability of response. Inducing sustainable response and achieving either stable disease or complete remission after discontinuation of immunotherapy is the final goal in immunotherapy. In the most recent update from CheckMate 142 trial, both response and stable disease were durable as median duration of response was not reached. Of note, 80% of responders showed ongoing responses at the time of analysis and 64% had durable responses lasting ≥ 12 months [25].

The other challenging aspect of treatment with anti-PD-1 is discontinuation in responders. It is still unknown if anti-PD-1 therapy in solid tumors can be stopped following CR or durable stable disease. Le *et al.* observed that after an average time-off therapy of 8.3 months, no recurrence was observed among 11 patients with MSI-H solid tumors who achieved CR and were taken off therapy after 2 years of treatment with pembrolizumab. They also demonstrated similar findings in patients with residual disease who were followed after an average of 7.6 months after discontinuation of therapy [27]. In spite of this study, no definitive conclusion can be made due to small sample size and short duration of follow-up.

Biomarkers predicting response

Tumor mismatch-repair (MMR) deficiency can be evaluated by either direct assessment of the expression of MMR proteins, or by MSI testing (i.e., assessing alterations of microsatellite lengths in DNA). Altered expression of MMR proteins due to mutation or epigenetic silencing results in MSI. The expression of four MMR proteins (MLH1, MSH2, MSH6 and PMS2) in tumor can be evaluated using immunohistochemistry-based test as is recommended in recent guidelines [28]. Tumor MMR deficiency can also be assessed by MSI testing. MSI testing is a PCR assay, and per recommendation from the National Cancer Institute, a panel of generally five microsatellites are evaluated for instability; MSI-H is defined as instability in two or more of the five microsatellites [15]. However, there is ongoing work to optimize MSI testing with alternate markers, as revealed in the study of Buhard [29,30]. Deficient MMR by immunohistochemistry (IHC) has high concordance to MSI-H per PCR and tumors with pMMR usually exhibit low (instability in one microsatellite) or no instability (MSI-L or microsatellite stable). The concordance of MSI and MMR testing in prior studies was reported to be 92% [31,32]. Despite reported concordance in CheckMate 142 trial, some cases assessed as dMMR/MSI-H in local institution could not be confirmed by a central laboratory. The discrepancy appeared to be mainly due to inadequate pretreatment biopsy tissue [23].

In search for predictive markers, the investigators in the CheckMate 142 study evaluated Lynch syndrome (vs sporadic), PD-L1 expression, KRAS and BRAF mutations as potential predictors of response to anti-PD-1 therapy. No difference in response to nivolumab is observed between tumors expressing PD-L1 $\geq 1\%$ and those having that $<1\%$. Therefore, PD-L1 expression level did not seem to be useful in predicting response. Also, KRAS and BRAF mutational status did not appear to correlate with response as clinical benefit was seen across all subgroups of patients with KRAS or BRAF mutations. Similarly, those with or without clinical history of Lynch syndrome had similar response to this therapy as well [23].

Regarding BRAF V600E mutation, it is important to note that cases harboring this mutation (within the group of pMMR) comprise a distinct subset of CRC patients who clearly have a more aggressive disease and lower clinical response to conventional cytotoxic agents. Despite lack of significant interaction between BRAF mutation and dMMR, it has been suggested that poor prognosis of dMMR is driven by BRAF mutation [33]. In CheckMate 142, the RR of patients with BRAF V600E mutation to nivolumab mirrored activity of the broader MSI-H population [34–36].

Combination of nivolumab with other agents

Safety and efficacy of combination of nivolumab and ipilimumab in 119 patients with dMMR/MSI-H mCRC who had progressed on or were intolerant of ≥ 1 prior line of therapy were evaluated in a follow-up study of CheckMate 142 trial. Patients received nivolumab (3 mg/kg) and ipilimumab (1 mg/kg) every 3 weeks for four doses followed by the same dose of nivolumab every 2 weeks until disease progression or discontinuation for other reasons. Primary end point was ORR. ORR and DCR were reported in 65 (55%) and 95 (80%) patients, respectively, and the combination had 32% grade 3–4 treatment-related adverse events [37]. Although the results for combination therapy appear to be moderately superior to the outcome for single anti-PD-1 therapy in the same study, this was not randomized and further data on toxicity and efficacy may be required.

Table 2. Current registered nivolumab trials including colorectal cancer patients.

	Trial identifier	Trial description	Condition	Phase	Intervention
1	NCT03026140	Nivolumab, ipilimumab and COX2-inhibition in early stage colon cancer	Early-stage colon cancer	Phase II	Anti-PD-1 Anti-CTLA-4 COX2-inhibition
2	NCT02948348	Nivolumab following preoperative chemoradiotherapy	Rectal cancer	Phase I/II	Anti-PD-1
3	NCT03085914	Epacadostat in combination with a PD-1 inhibitor and chemotherapy (ECHO-207)	Advanced/metastatic solid tumors (including CRC)	Phase I/II	IDO1 inhibitor Chemotherapy Anti-PD-1 Anti-PD-L1
4	NCT02327078	Epacadostat in combination with nivolumab (ECHO-204)	Advanced/metastatic solid tumors (including CRC)	Phase I	IDO1 inhibitor Anti-PD-1
5	NCT02423954	Nivolumab plus chemotherapy (NivoPlus)	Advanced/metastatic solid tumors (including CRC)	Phase I/II	Chemotherapy Anti-PD-1
6	NCT02335918	Varlilumab and nivolumab	Advanced refractory solid tumors	Phase I/II	Anti-CD-27 Anti-PD-1
7	NCT02636036	Enadenotucirev and nivolumab	Metastatic or advanced epithelial tumors (including CRC)	Phase I	Oncolytic adenovirus Anti-PD-1
8	NCT02060188	Nivolumab and nivolumab in combination with other anticancer drugs (CheckMate 142)	Recurrent and metastatic MSS and MSI-H CRC	Phase II	Anti-PD-1 Anti-CTLA4 Anti-CD-38 MEK inhibitor Anti-LAG-3 antibody
9	NCT03104439	Nivolumab and ipilimumab and radiation therapy	MSS and MSI-H CRC and pancreatic cancer	Phase II	Anti-PD-1 Anti-CTLA-4 Radiotherapy
10	NCT03184870	BMS-813160 in combination with chemotherapy or nivolumab	Advanced CRC and pancreatic cancer	Phase I/II	Dual CCR2/CCR5 chemokine antagonist Anti-PD-1 Chemotherapy
11	NCT03271047	Binimetinib + nivolumab with or without ipilimumab	MSS and RAS-mutant CRC	Phase I/II	MEK inhibitor Anti-PD-1 Anti-CTLA-4
12	NCT03307603	Yttrium-90 radioembolization + nivolumab	Liver + extra-hepatic metastases from CRC	Phase I/II	Yttrium-90 radioembolization Anti-PD-1

CRC: Colorectal cancer; IDO1: Indoleamine 2,3-dioxygenase; MSI-H: Microsatellite-instability high; MSS: Microsatellite stable.

Adverse events

The safety profile of nivolumab in CRC patients resembles that found in clinical studies on other solid tumors [23] and no CRC specific toxicities have been identified. The most common adverse event of monotherapy with this agent in solid tumors has been rash and pruritus (>20% of patients). Other adverse events of all grades, occurring in more than 10% of patients, are infections and peripheral edema. Clinically important adverse reactions (with frequency of <10%) include ventricular arrhythmia, iridocyclitis, infusion-related reactions, peripheral and sensory neuropathy. Most commonly reported laboratory abnormalities are elevated liver enzymes, hyponatremia and hyperkalemia [38].

Future perspective

The clinical benefit of nivolumab in metastatic MSI-H CRC patients is certainly promising. Since MSI-H comprises a small subset (4%) of cases with mCRC, future studies to address the other 95% with microsatellite stable are warranted. Trials on combinations of nivolumab and various anticancer drugs are summarized in Table 2.

Financial & competing interests disclosure

S Kopetz and M Overman have received funding for advisory boards from BMS and Merck. The work discussed in the above manuscript arose, in whole or in part, from direct costs funded by NIH, or from NIH staff, grant number: NIH ROI CA187238. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Nivolumab (at the dose of 240 mg every 2 weeks) can be used for heavily pretreated metastatic colorectal cancer patients whose tumor harbor deficient mismatch repair (MMR) or microsatellite-instability (MSI) high.
- MMR/MSI status is a reliable marker for predicting response to anti-PD-1 treatment. MSI testing (by PCR) and MMR testing (by IHC) are virtually equivalent and reported to have high concordance of 92%.
- In CheckMate 142, clinical benefit of therapy with nivolumab appears to be seen across all subgroups of patients with KRAS or BRAF mutations as well as those with or without clinical history of Lynch syndrome.
- The safety profile of nivolumab in colorectal cancer patients is similar to what has been reported in clinical studies on other solid tumors. The most common adverse event of monotherapy with this agent in solid tumors is dermatologic manifestations such as rash and pruritus.
- It is unclear if anti-PD-1 therapy can be safely stopped following complete response or durable stable disease. Also, there are no data on optimal duration of treatment.
- Although the results for combination therapy appear to be moderately superior to the outcome for single anti-PD-1 therapy in the CheckMate 142 study, this was not randomized and further data on toxicity and efficacy may be required.

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