Current Colorectal Cancer Reports (2018) 14:167–174
https://doi.org/10.1007/s11888-018-0413-x

Overview of Microsatellite Instability and Immune Checkpoint Inhibitors in Colorectal Cancer

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Published online: 28 September 2018

Abstract

Purpose of Review This review examines the pathophysiological features of microsatellite instability (MSI) high colorectal cancer and discusses recent clinical studies of immune checkpoint inhibitors for MSI high colorectal cancer.

Recent Findings Emerging clinical data demonstrated durable clinical activity and safety of PD-1 blockade agents in diverse cancers, and PD-1 blockade agents have led to a paradigm shift in the cancer therapy. Although initial clinical data showed disappointing result of anti-PD-1 therapy in unselected metastatic colorectal cancer, recent data demonstrated promising results with significant anticancer activity of PD-1 blockade in colorectal cancers with microsatellite instability which have highly immunogenic tumor microenvironment.

Summary Anti-PD-1 therapy demonstrated durable clinical activity and safety, and it has changed the landscape of cancer therapy in MSI high colorectal cancer. Further studies with better understanding of tumor microenvironment will improve clinical outcomes of colorectal cancer.

Keywords Microsatellite · PD-1 immunotherapy · Colorectal cancer

Introduction

Despite significant improvement in the survival of the colorectal cancer patients over the past decade with new drugs such as regorafenib and trifluridine/tipiracil, almost all patients with metastatic disease will succumb to the disease, resulting in more than 50,000 deaths yearly [1]. Therefore, new treatment strategies are needed to further improve the outcome of metastatic colorectal cancer patients.

The rapid advances in tumor immunology have improved the understanding of key regulators of T cell response and have led to the development of new immunotherapeutic approach targeting immune checkpoint such as cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and programmed death-1 (PD-1). Several different antibodies blocking PD-1/PD-L1 pathway have been extensively studied in a wide spectrum of malignancies. These efforts are rapidly translating into remarkable success of PD-1 and PD-L1 blockade agents such as atezolizumab, avelumab, durvalumab, nivolumab, and pembrolizumab in diverse cancers. Unfortunately, the remarkable success was not replicated with metastatic colorectal cancer. However, several data suggested that a subset of colorectal cancers with alterations in the mismatch repair (MMR) pathway that causes high levels of microsatellite instability have highly immunogenic tumor microenvironment, and further clinical studies demonstrated significant anticancer activity of PD-1 blockade therapy in this subset of colorectal cancers [2••, 3]. In this paper, we will attempt to concisely summarize clinical data of colorectal cancer with microsatellite instability and their response to immune checkpoint inhibitors.

This article is part of Topical Collection on *Systemic Therapies in Colorectal Cancer*

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Microsatellite Instability

Pathogenesis

Microsatellites are repeat sequences of one to six base pairs (tandem DNA repeats) and are scattered throughout coding and noncoding regions of genome. Microsatellites are prone to DNA replication errors such as frameshift and missense mutations due to slippage of DNA polymerase. Usually, DNA MMR system corrects nucleotide mismatches to maintain genomic stability during DNA replication. Microsatellite instability (MSI) is the DNA replication error caused by a dysfunction of the DNA MMR system, and it is a well-established pathway of tumorigenesis in colorectal cancer [4]. Germline mutations in one of the MMR genes including *MLH1*, *MSH2*, *MSH6*, and *PMS2* can induce hereditary non-polyposis colon cancer (Lynch syndrome) which accounts for 3% of all colorectal cancer [5]. Somatic defects in MMR system mainly through epigenetic inactivation from hypermethylation of the *MLH1* promoter are observed in 15% of colorectal cancers [6]. This subset of colorectal cancers with dysfunction of MMR has distinct clinicopathological features in addition to high mutation burden and microsatellite instability-high (MSI-high). Molecularly, *BRAF* V600E mutation is one of the most frequent genetic alterations, and fewer *APC*, *KRAS*, and *TP53* mutations were observed in colorectal cancer with dysfunction of MMR [6]. MMR-deficient colorectal cancers are associated with mucinous histology, signet-ring cell differentiation, poor differentiation, intra- and peritumoral lymphocytic infiltration, and a Crohn's-like lymphocytic infiltration [7, 8]. Clinically, patients with MSI-high colorectal cancer tend to be younger and to have primary tumor in the right-sided colon [7, 9]. Although mucinous histology, signet-ring cell differentiation, poor differentiation, and right-sided colon are known poor prognostic factors [10–12], and MSI-high colorectal cancers are more likely to have these poor prognostic features, MSI-high colorectal cancers have a relatively favorable clinical outcome and reduced likelihood of metastasis in localized disease [13]. In contrast, the prognostic impact of microsatellite instability is not clear in metastatic advanced colorectal cancer. Some clinical data demonstrated poor clinical outcome of MSI-high metastatic colorectal cancer [14, 15], which may be attributed to strong expression of immune suppressive checkpoint proteins including PD-L1, CTLA-4, LAG-3, and indoleamine 2,3-dioxygenase (IDO) in tumor microenvironment [16•], limited use of EGFR inhibitors [17], and high frequency of poor prognostic *BRAF* mutations [15].

Immunological Feature of MSI-High Colorectal Cancer

Without the functional MMR system to correct DNA replication errors, MMR-deficient tumors harbor greater than 100-fold frameshift and missense mutations compared to MMR-proficient tumors [18]. These new mutations can induce new peptide sequence (neoantigens) which can be recognized as non-self by immune cells and elicit cytotoxic T cell immune response. Several data demonstrated infiltration of abundant immune cells including activated cytotoxic CD8 T cells with high concentration of cytotoxic granules (granzyme B and perforins) and mature dendritic cells with an activated dendritic cell marker (CD208) expression in tumor microenvironment of MSI-high tumors [19–21]. The tumor-infiltrating lymphocytes (TILs) and Crohn's-like lymphoid reaction (peritumoral lymphoid aggregates) are commonly observed in MSI-high tumors, and they are well-known prognostic markers in colorectal cancer [22, 23], suggesting that MSI-high tumors can induce antitumor immunity, and the antitumor immunity may delay cancer progression. However, tumor cells can upregulate immune checkpoints in tumor microenvironment as an antitumor immunity escape mechanism. MMR-deficient colorectal cancer showed highly upregulated expression of multiple immune checkpoints such as PD-1, PD-L1, CTLA-4, lymphocytes activation gene 3 (LAG-3), and IDO in tumor microenvironment in comparison to MSS cancers [16•, 24, 25], supporting the rationale of using immune checkpoint inhibitors in this subset of colorectal cancers.

Clinical Data of Immune Checkpoint Inhibitors

Initial clinical studies of immune checkpoint inhibitors failed to show anticancer activity in unselected colorectal cancer. With better understanding of immunogenic tumor microenvironment of MSI-high tumors, immune checkpoint inhibitor therapy has been evaluated in subgroup of colorectal cancer patients with MMR deficiency in following studies. Table 1 summarizes results of several key trials of immune checkpoint inhibitors in MMR-deficient colorectal cancer.

CTLA-4 Blockade

CTLA-4 is an inhibitory immune checkpoint molecule that plays a critical role in regulating T cell-mediated antitumor immunity. It is expressed on activated T cells and binds to B7 molecules (CD80 and CD86) on antigen-presenting cells to block costimulatory signals which are essential for T cell clonal expansion and initiation of effector functions of cytotoxic T cells. Ipilimumab and tremelimumab are currently used in clinical practice to block CTLA-4. In a phase II study, 47 patients with refractory metastatic colorectal cancer were treated with tremelimumab [26]. The treatment was well tolerated with 19.1% of grade 3/4 toxicities. However, only one partial response (2.2%) was observed, and the disappointing result did not support further investigation of anti-CTLA-4 single agent. However, it is also important to note that patients were not selected based on MMR status in this study.

PD-1/PD-L1 Blockade

PD-1 is one of the negative immune regulators which plays an essential role in suppression of antitumor immunity in local tumor environment. PD-1 expressed on the surface of activated T cells has two ligands, PD-L1 (B7-H1) and PD-L2 (B7-

Table 1 Summary of studies on checkpoint inhibitor and its efficacy on metastatic colorectal cancer

Immunotherapeutic agent	Population	Phase	Number	Response rate	Median OS/PFS (months)	Reference
Tremelimumab	Unselected	II	47	2%	NA	26
Nivolumab	Unselected	I	14	7%	NA	30
Nivolumab	Unselected	I	19	0%	NA	31
Pembrolizumab	Unselected	I	3	0%	NA	32
Pembrolizumab	pMMR/MSS vs	II	21	0%	5.0/2.2	2
	dMMR		11	40%	NR/NR	
Nivolumab	dMMR/MSI-H	II	74	31%	NR/14.3	36
Nivolumab plus ipilimumab	dMMR/MSI-H	II	119	55%	NR/NR	37

dMMR MMR deficient, *pMMR MMR* proficient, *MSI-H* microsatellite instability-high, *MSS* microsatellite stable, *NA* not available, *NR* not reached, *OS* overall survival, *PFS* progression-free survival

DC); PD-L1 is broadly displayed on antigen-presenting cells and tumor cells, and the expression of PD-L1 is upregulated by interferon which is predominately produced by effector T cells [27]. The binding of PD-1 and PD-L1 inhibits T cell proliferation and activation, and induces apoptosis of antigen-specific T cells to prevent collateral tissue damage and autoimmune disease [28]. The PD-1/PD-L1 pathway is hijacked by tumor cells to inhibit antitumor immunity, and various cancer cells have been reported to upregulate PD-L1 to escape immune surveillance and anticancer immunity [29]. With remarkable success of PD-1 blockade therapy in diverse cancers, two PD-1 inhibitors, nivolumab and pembrolizumab, have been extensively studied in colorectal cancer. Similar with the result observed in tremelimumab, both nivoluamab and pembrolizumab failed to show anticancer activity in unselected colorectal cancer. In a phase I study of nivolumab in refractory solid tumors, one durable complete response was reported in 14 patients with unselected metastatic colorectal cancer [30]. In a subsequent large phase I study of nivolumab in advanced solid tumors, no objective response was observed in 19 unselected metastatic colorectal cancer patients [31]. Another PD-1 inhibitor, pembrolizumab, was evaluated in patients with advanced solid tumors, and three colorectal cancer patients were enrolled in the study [32]. All of them showed early disease progression. Similar with previous studies, colorectal cancer patients were not selected based on MMR status in the study.

Recent clinical studies reported that high tumor mutation burden was associated with improved objective response, durable clinical benefit, and progression-free survival in patients receiving immune checkpoint inhibitors in melanoma and non-small cell lung cancer [33, 34, 35•], suggesting a potential predictive role of tumor mutation burden in immune checkpoint inhibitor therapy. Based on these findings, immune checkpoint blockade has been evaluated in MMR-deficient refractory metastatic colorectal cancers which have more somatic mutations than MMR-proficient cancers. In a phase II study, pembrolizumab was administered in 40 patients with MMR-deficient and 18 with MMR-proficient metastatic colorectal cancer [2••, 3]. While 16 patients had objective response (52%) including 5 complete responses in patients with MMR deficiency, no objective response was observed in MMR-proficient cohort. The responses were durable, and median progression-free survival (PFS) and median overall survival (OS) were not reached at a median follow-up of 36 weeks in MMR-deficient colorectal cancers. However, median PFS and OS were only 2.2 months and 5.0 months in MMR-proficient cohort. Observed treatment-related toxicities were low-grade rash/pruritus (24%), asymptomatic pancreatitis (15%), thyroiditis/hypothyroidism/hypophysitis (10%), and pneumonitis (2%). All the toxicities were well-manageable. Based on these data, the US Food Drug Administration (FDA) approved pembolizumab for patients with metastatic, MSI-high, or MMR-deficient solid tumors that progressed following prior treatment and who have no alternative treatment options or with MSI-high or MMR-deficient colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan. High tumor mutation burden of MMR-deficient tumor and the correlation between mutation burden and survival benefit were confirmed in the study. A mean of 1782 somatic mutations per tumor were observed in MMR-deficient tumors as compared with 73 in MMR-proficient tumors, and high somatic mutation loads were associated with prolonged PFS [2••]. The expression of PD-L1 and CD8 in tumor microenvironment was prominent in MMR-deficient tumors when compared to MMR proficient tumors. However, the expression of PD-L1 and CD8 was not significantly associated with PFS or OS in the study [2••].

Another anti-PD-1 inhibitor, nivolumab, was also evaluated in MMR-deficient metastatic colorectal cancers. In a phase

II study, 74 patients with refractory metastatic MSI-high colorectal cancer were received nivolumab [36••]. Objective response and disease control rates were 31% and 69%, respectively. At a median follow-up of 12 months, the median PFS was 14.3 months, and the median OS was not reached. Grade 3/4 drug-related adverse events were reported in 20% including increased lipase (8%) and increased amylase (3%). Five patients (7%) were discontinued treatment due to drug-related toxicities including increased ALT, colitis, duodenal ulcer, acute kidney injury, and stomatitis. PD-L1 expression on tumor cells or immune cells and mutation status (*BRAF* and *KRAS*) were investigated as predictive biomarkers of nivolumab treatment in the study. However, none of them showed any predictive value of nivolumab. In a subsequent study, combination of nivolumab and ipilimumab was evaluated in refractory advanced MSI-high colorectal cancers [37••] based on the fact that ipilimumab primarily acts at the induction (early) phase of antitumor T cell activity, and nivolumab primarily acts at the effector (late) phase in tumor microenvironment. Total of 119 patients with refractory metastatic MSI-high colorectal cancer were treated with four doses of nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg) every 3 weeks followed by nivolumab (3 mg/kg) every 2 weeks. Of 119 patients, 55% achieved an objective response including 4 complete responses (3%), and a disease control rate was 80%. Median time to response was 2.8 months, and 83% of responders had responses lasting ≥ 6 months. Thirty-eight patients (33%) experienced grade 3 (27%) or 4 (5%) treatment-related toxicities such as elevated AST/ALT (11%), elevated lipase (4%), anemia (3%), and colitis (3%), and 16 patients (13%) discontinued the treatment due to drug-related toxicities, which are similar to that reported in other solid cancers [38, 39]. An objective response rate of patients who discontinued treatment because of drug-related toxicities was 63% which was similar with that of the overall population. FDA granted approval to nivolumab single agent and nivolumab in combination with ipilimumab for patients with MSI-high or MMR-deficient metastatic colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan based on these data.

Interestingly, nivolumab plus ipilimumab showed higher response rate (55%) [37••] than nivolumab single agent (31%) [36••], but it was similar with pembrolizumab single agent (52%) [2••], although these studies were not designed for direct comparison. These findings are likely due to the limited number of patients ($n = 40$) in the pembrolizumab study [2••]. Large randomized controlled studies are needed to confirm these findings.

Currently, there are several ongoing clinical studies to evaluate anticancer activity of PD-1/PD-L1 blockade immunotherapy in adjuvant, front-line, refractory treatment setting for MSI-high/MMR-deficient colorectal cancer (Table 2).

Predictive Biomarkers

Various biomarkers and tumor characteristics to predict clinical response have been evaluated for appropriate selection of patients most likely and least likely to benefit from PD-1 blockade. Several predictive biomarkers have been suggested such as intratumoral CD8 T cells [40], tumor mutation burden [34], neoantigen heterogeneity [41], relative lymphocyte count [42], relative eosinophil count [42], LDH [42], absence of metastasis other than soft tissue and lung [42], Epstein-Barr virus infection [43], baseline tumor size [44], ratio of T cell invigoration to tumor burden [45], and PD-L1 expression [40] in several cancers. Among these markers, the correlation between PD-L1 expression in tumor microenvironment and better clinical outcome to PD-L1 inhibitors has been reported in multiple cancers including head and neck squamous cell carcinoma [46], melanoma [47], NSCLC [48], urothelial cancer [49], and gastric/gastroesophageal junction cancer [50]. Especially, PD-L1 expression is routinely used in metastatic NSCLC and gastric/gastroesophageal junction adenocarcinoma as a biomarker for pembrolizumab treatment. The

Table 2 Ongoing phase II and III studies of immune checkpoint inhibitors in MSI-high/MMR-deficient colorectal cancer

Clinical trial number	Regimen	Phase	Study population	Primary endpoint	Number of patients
NCT02563002	Pembrolizumab vs standard chemotherapy	III	First-line MSI-h mCRC	OS/PFS	270
NCT029997228	Atezolizumab vs atezolizumab/FOLFOX/bevacizumab vs FOLFOX/bevacizumab	III	First-line MSI-h mCRC	PFS	347
NCT02912559	Atezolizumab/FOLFOX vs FOLFOX	III	Adjuvant MSI-h stage III colon cancer	DFS	700
NCT03104439	Nivolumab/ipilimumab/radiation	II	MSI-h vs MSS refractory mCRC	DCR	80
NCT02788279	Atezolizumab vs Atezolizumab/cobimetinib vs Regorafenib	III	MSI-h (cap 5% of the study) vs MSS refractory mCRC	OS	360
NCT02060188	Nivolumab vs Nivolumab/ipilimumab vs Nivolumab/anti-LAG-3	II	Refractory MSI-h mCRC	ORR	340

DCR disease control rate, *DFS* disease-free survival, *mCRC* metastatic colorectal cancer, *MSI-h* microsatellite instability-high, *MSS* microsatellite stable, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival predictive value of PD-L1 was also investigated in MSI-high colorectal cancer. However, PD-L1 expression on tumor or immune cells did not correlate with clinical outcome in MSI-high colorectal cancer patients receiving nivolumab [36••]. The discrepancy of predictive value of PD-L1 expression can be explained by multiple complicating factors including the following: (1) significant discordance of PD-L1 expression has been reported between primary tumors and metastatic lesions in several malignancies including melanoma [51], NSCLC [52], and renal cell carcinoma [53]; (2) there are at least 12 different anti-PD-L1 antibodies and several different staining techniques for determination of PD-L1 expression which have different sensitivity [52]; (3) the cutoff value of PD-L1 staining positivity is not clearly defined; and (4) PD-L1 can be induced by aberrant expression of oncogene such as PTEN loss [54] and dysregulation of JAK/STAT pathway [55] without immune cell infiltrate. Further studies with better understanding of tumor microenvironment are needed to overcome the challenge and accurately identify patients who will benefit from PD-1 inhibitors.

Anti-PD-1 Refractory Disease

While several clinical trials confirmed the remarkable and durable anticancer activity of PD-1 blockade in patients with MSI-high colorectal cancer, 20–30% patients does not have any clinical benefit from PD-1 inhibitors, and a significant number of responders develop disease progression, eventually. To improve clinical outcome in patients with anti-PD-1 therapy-resistant tumors, innate and acquired resistance mechanisms of PD-1 inhibitors have been extensively studied although limited data are available in MSI-high colorectal cancer. So far, several resistance mechanisms have been suggested, including (1) constitutive activation of WNT/β-catenin signaling pathway leading to lack of T cell infiltration [56]; (2) loss of PTEN increasing expression of immunosuppressive cytokines and decreasing T cell infiltration [57]; (3) expression of IDO which suppresses effector T cells and activates regulatory T cells [58]; (4) upregulation of genes involving mesenchymal transition, cell adhesion, extracellular matrix remodeling, angiogenesis, and wound healing [59]; (5) loss of function mutation in the genes encoding *Janus kinase 1* (*JAK1*) or *Janus kinase 2* (*JAK2*) resulting in insensitivity to the antiproliferative effects of interferon γ on cancer cells [60]; and (6) mutation in the gene encoding beta 2 microglobulin leading to loss of expression of major histocompatibility complex (MHC) class I [60]. With the improved understanding of resistance mechanisms, several combination approaches of PD-1 blockade agents with other therapeutic modalities are undergoing evaluation to overcome the resistance and improve clinical outcomes, as PD-1 blockade agents are combined with agents that target other immunosuppressive molecules such as CTLA-4, LAG-3, TIM-3, and IDO or combined with CD137 agonist, OX-40 agonist, talimogene laherparepvec, cancer vaccines, adoptive T cell therapies, or radiation therapy.

Gut Microbiota and PD-1 Blockade

Gut microbiota is essential for human health since it protects against pathogens, strengthens gut integrity, harvests energy, and regulates host immunity [61]. In addition, several data suggests that gut microbiota may amplify or mitigate carcinogenesis and responsiveness to immunotherapy as individual microbes or as a microbial community [62]. In colorectal cancer, microbiota including *Fusobacterium*, *Bacteroids*, *Selenomonas*, and *Prevotella* species can influence cancer development, progression, and metastasis [63]. Gut microbiota also influences anticancer activity of immune checkpoint inhibitors [64•, 65•, 66•]. Abnormal composition of gut microbiota induced by antibiotics was associated with primary resistance to PD-1 blockade treatment. While mice with fecal microbiota transplantation from responders demonstrated significant tumor regression after anti-PD-1 therapy, mice transplanted with stool from non-responders failed to show tumor response to anti-PD-1 therapy [64•, 65•, 66•]. Responders have abundant *Akkermansia muciniphila*, *Bifidobacterium longum*, *Collinsella aerofaciens*, *Enterococcus faecium*, and *Ruminococcaceae* species in these studies [64•, 65•, 66•]. Although the precise mechanisms of specific gut bacterial species to enhance antitumor activity of PD-1 blockade remain unknown, gut microbiota may have a significant effect on innate and adaptive immune system since pathogen-associated molecular patterns (PAMPs) from bacteria can trigger immune response by binding to pattern recognition receptors (PRRs) on innate immune cells [67]. These findings suggest new strategies to enhance anticancer activity of immune checkpoint inhibitors by modulation of gut microbiota. However, further studies are needed for better understanding of the relationship between gut microbiota and antitumor immunity in colorectal cancer.

Dosing of Nivolumab and Pembrolizumab

Nivolumab has been tested in multiple trials with doses ranging from 0.1 to 10 mg/kg, and the antitumor activity with respect to objective response rates approached a plateau at 3 mg/kg with no increased benefit at doses of > 3 mg/kg. Based on the data, initial studies used nivolumab 3 mg/kg for MSI-high metastatic colorectal cancer. Recently, population pharmacokinetics analysis and dose/exposure-response analysis data demonstrated that the pharmacokinetic exposure, safety, and efficacy of 240 mg every 2 weeks flat dose were comparable to 3 mg/kg every 2 weeks [68, 69], and FDA approved flat dose nivoluamb (240 mg every 2 weeks) in July 2017. However, approved dose of nivolumab is 3 mg/kg when combined with ipilimumab (1 mg/kg) due to only available dosing data from the CheckMate 142 study [37••].

Similar with nivolumab, initial studies of pembrolizumab used weight-based dosing of 10 mg/kg every 2 weeks [2••, 3]. However, a simulation of three different weight-based pembrolizumab dosing regimens (2 mg/kg every 3 weeks, 10 mg/kg every 2 weeks, and 10 mg/kg every 3 weeks) using population pharmacokinetics model showed that the safety profile, overall response, and survival outcomes were similar across the three different dosing regimens [70]. Furthermore, 200 mg every 3 weeks fixed dosage provides near maximal efficacy and similar exposure distributions with weight based dosing regimens [71]. Based on these data, FDA approved 200 mg every 3 weeks flat dose in September 2017.

Conclusion

The remarkable success of PD-1 blockade immunotherapy has changed the landscape of cancer therapy, and the efficacy and safety of PD-1 blockade have been validated in MSI-high colorectal cancer. Despite the success of PD-1 blockade, however, further studies are needed to improve clinical outcomes of innate and acquired resistant disease to PD-1 inhibitors. In addition, new clinical studies in neoadjuvant or adjuvant setting are eagerly awaited in MSI-high colorectal cancer.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68(1):7–30.
2.•• Le DT UJN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509–20 **The first study to demonstrate anticancer activity of PD-1 blockade immunotherapy in MSI-high colorectal cancer**.
3. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357(6349):409–13.
4. Chung DC, Rustgi AK. DNA mismatch repair and cancer. Gastroenterology. 1995;109(5):1685–99.
5. Yurgelun MB, Kulke MH, Fuchs CS, Allen BA, Uno H, Hornick JL, et al. Cancer susceptibility gene mutations in individuals with colorectal cancer. J Clin Oncol. 2017;35(10):1086–95.
6. Cancer Genome Atlas N. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487(7407):330–7.
7. Buckowitz A, Knaebel HP, Benner A, Blaker H, Gebert J, Kienle P, et al. Microsatellite instability in colorectal cancer is associated with local lymphocyte infiltration and low frequency of distant metastases. Br J Cancer. 2005;92(9):1746–53.
8. Yearsley M, Hampel H, Lehman A, Nakagawa H, de la Chapelle A, Frankel WL. Histologic features distinguish microsatellite-high from microsatellite-low and microsatellite-stable colorectal carcinomas, but do not differentiate germline mutations from methylation of the MLH1 promoter. Hum Pathol. 2006;37(7):831–8.
9. Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. Am J Pathol. 1994;145(1):148–56.
10. Nissan A, Guillem JG, Paty PB, Wong WD, Cohen AM. Signet-ring cell carcinoma of the colon and rectum: a matched control study. Dis Colon Rectum. 1999;42(9):1176–80.
11. Shin US, Yu CS, Kim JH, Kim TW, Lim SB, Yoon SN, et al. Mucinous rectal cancer: effectiveness of preoperative chemoradiotherapy and prognosis. Ann Surg Oncol. 2011;18(8):2232–9.
12. Petrelli F, Tomasello G, Borgonovo K, Ghidini M, Turati L, Dallera P, et al. Prognostic survival associated with left-sided vs right-sided colon cancer: a systematic review and meta-analysis. JAMA Oncol. 2016.
13. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med. 2000;342(2):69–77.
14. Smith CG, Fisher D, Claes B, Maughan TS, Idziaszczyk S, Peuteman G, et al. Somatic profiling of the epidermal growth factor receptor pathway in tumors from patients with advanced colorectal cancer treated with chemotherapy +/− cetuximab. Clin Cancer Res. 2013;19(15):4104–13.
15. Venderbosch S, Nagtegaal ID, Maughan TS, Smith CG, Cheadle JP, Fisher D, et al. Mismatch repair status and BRAF mutation status in metastatic colorectal cancer patients: a pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS studies. Clin Cancer Res. 2014;20(20):5322–30.
16.• Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov. 2015;5(1):43–51 **Demonstrated that MSI high tumor expresses multiple immune checkpoint inhibitors**.
17. Price TJ, Karapetis CS, Joanne Y, Roy A, Padbury R, Maddern G, et al., editors. Outcomes for metastatic colorectal cancer (mCRC) based on microsatellite instability. 2018 Gastrointestinal Cancers Symposium; 2018; Chicago, IL.
18. Eshleman JR, Lang EZ, Bowerfind GK, Parsons R, Vogelstein B, Willson JK, et al. Increased mutation rate at the hprt locus accompanies microsatellite instability in colon cancer. Oncogene. 1995;10(1):33–7.
19. Banerjea A, Ahmed S, Hands RE, Huang F, Han X, Shaw PM, et al. Colorectal cancers with microsatellite instability display mRNA expression signatures characteristic of increased immunogenicity. Mol Cancer. 2004;3:21.
20. Phillips SM, Banerjea A, Feakins R, Li SR, Bustin SA, Dorudi S. Tumour-infiltrating lymphocytes in colorectal cancer with microsatellite instability are activated and cytotoxic. Br J Surg. 2004;91(4):469–75.
21. Bauer K, Michel S, Reuschenbach M, Nelius N, von Knebel Doeberitz M, Kloor M. Dendritic cell and macrophage infiltration

in microsatellite-unstable and microsatellite-stable colorectal cancer. Familial Cancer. 2011;10(3):557–65.
22. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313(5795):1960–4.
23. Rozek LS, Schmit SL, Greenson JK, Tomsho LP, Rennert HS, Rennert G, Gruber SB Tumor-infiltrating lymphocytes, Crohn's-like lymphoid reaction, and survival from colorectal cancer. J Natl Cancer Inst 2016;108(8).
24. Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, et al. Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. Cancer Epidemiol Biomark Prev. 2014;23(12):2965–70.
25. Inaguma S, Lasota J, Wang Z, Felisiak-Golabek A, Ikeda H, Miettinen M. Clinicopathologic profile, immunophenotype, and genotype of CD274 (PD-L1)-positive colorectal carcinomas. Mod Pathol. 2017;30(2):278–85.
26. Chung KY, Gore I, Fong L, Venook A, Beck SB, Dorazio P, et al. Phase II study of the anti-cytotoxic T-lymphocyte-associated antigen 4 monoclonal antibody, tremelimumab, in patients with refractory metastatic colorectal cancer. J Clin Oncol. 2010;28(21):3485–90.
27. Yao S, Chen L. PD-1 as an immune modulatory receptor. Cancer J. 2014;20(4):262–4.
28. Dai S, Jia R, Zhang X, Fang Q, Huang L. The PD-1/PD-Ls pathway and autoimmune diseases. Cell Immunol. 2014;290(1):72–9.
29. Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. Immunity. 2018;48(3):434–52.
30. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28(19):3167–75.
31. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443–54.
32. Patnaik A, Kang SP, Rasco D, Papadopoulos KP, Elassaiss-Schaap J, Beeram M, et al. Phase I study of pembrolizumab (MK-3475; anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. Clin Cancer Res. 2015;21(19):4286–93.
33. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371(23):2189–99.
34. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348(6230):124–8.
35.• Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. J Clin Oncol. 2018;36(7):633–41 **Described the correlation between tumor mutation burden and clinical outcome of PD-1 immunotherapy**.
36.•• Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncol. 2017;18(9):1182–91 **Confirmed anticancer activity of nivolumab in MSI-high colorectal cancer**.
37.•• Overman MJ, Lonardi S, Wong KYM, Lenz HJ, Gelsomino F, Aglietta M, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. J Clin Oncol. 2018;36(8):773–9 **Confirmed anticancer activity of nivolumab plus ipilimumab in MSI-high colorectal cancer**.
38. Motzer RJ, Tannir NM, McDermott DF, Aren Frontera O, Melichar B, Choueiri TK, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N Engl J Med. 2018;378(14):1277–90.
39. Hellmann MD, Rizvi NA, Goldman JW, Gettinger SN, Borghaei H, Brahmer JR, et al. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. Lancet Oncol. 2017;18(1):31–41.
40. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568–71.
41. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016;351(6280):1463–9.
42. Weide B, Martens A, Hassel JC, Berking C, Postow MA, Bisschop K, et al. Baseline biomarkers for outcome of melanoma patients treated with pembrolizumab. Clin Cancer Res. 2016;22(22):5487–96.
43. Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, et al. Abundant PD-L1 expression in Epstein-Barr virus-infected gastric cancers. Oncotarget. 2016;7(22):32925–32.
44. Joseph RW, Elassaiss-Schaap J, Kefford RF, Hwu WJ, Wolchok JD, Joshua AM, Ribas A, Hodi FS, Hamid O, Robert C, Daud A, Dronca R, Hersey P, Weber JS, Patnaik A, de Alwis DP, Perrone A, Zhang J, Kang SP, Ebbinghaus S, Anderson KM, Gangadhar TC Baseline tumor size is an independent prognostic factor for overall survival in patients with melanoma treated with pembrolizumab. Clin Cancer Res 2018.
45. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature. 2017;545(7652):60–5.
46. Chow LQM, Haddad R, Gupta S, Mahipal A, Mehra R, Tahara M, et al. Antitumor activity of pembrolizumab in biomarker-unselected patients with recurrent and/or metastatic head and neck squamous cell carcinoma: results from the phase Ib KEYNOTE-012 expansion cohort. J Clin Oncol. 2016;34(32):3838–45.
47. Daud AI, Wolchok JD, Robert C, Hwu WJ, Weber JS, Ribas A, et al. Programmed death-ligand 1 expression and response to the anti-programmed death 1 antibody pembrolizumab in melanoma. J Clin Oncol. 2016;34(34):4102–9.
48. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015;372(21):2018–28.
49. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet. 2016;387(10031):1909–20.
50. Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 clinical KEYNOTE-059 trial. JAMA Oncol. 2018;4(5):e180013.
51. Madore J, Vilain RE, Menzies AM, Kakavand H, Wilmott JS, Hyman J, et al. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. Pigment Cell Melanoma Res. 2015;28(3):245–53.
52. McLaughlin J, Han G, Schalper KA, Carvajal-Hausdorf D, Pelekanou V, Rehman J, et al. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. JAMA Oncol. 2016;2(1):46–54.

53. Callea M, Albiges L, Gupta M, Cheng SC, Genega EM, Fay AP, et al. Differential expression of PD-L1 between primary and metastatic sites in clear-cell renal cell carcinoma. Cancer Immunol Res. 2015;3(10):1158–64.
54. Song M, Chen D, Lu B, Wang C, Zhang J, Huang L, et al. PTEN loss increases PD-L1 protein expression and affects the correlation between PD-L1 expression and clinical parameters in colorectal cancer. PLoS One. 2013;8(6):e65821.
55. Marzec M, Zhang Q, Goradia A, Raghunath PN, Liu X, Paessler M, et al. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). Proc Natl Acad Sci U S A. 2008;105(52):20852–7.
56. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature. 2015;523(7559):231–5.
57. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. Cancer Discov. 2016;6(2):202–16.
58. Holmgaard RB, Zamarin D, Munn DH, Wolchok JD, Allison JP. Indoleamine 2,3-dioxygenase is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4. J Exp Med. 2013;210(7):1389–402.
59. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. Cell. 2016;165(1):35–44.
60. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med. 2016;375(9):819–29.
61. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474(11):1823–36.
62. Brennan CA, Garrett WS. Gut microbiota, inflammation, and colorectal cancer. Annu Rev Microbiol. 2016;70:395–411.
63. Bullman S, Pedamallu CS, Sicinska E, Clancy TE, Zhang X, Cai D, et al. Analysis of Fusobacterium persistence and antibiotic response in colorectal cancer. Science. 2017;358(6369):1443–8.
64.• Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science. 2018;359(6371):104–8 **Described the associated between gut microbiota and anticancer activity of immune checkpoint inhibitors**.
65.• Routy B, Le Chatelier E, Derosa L, CPM D, Alou MT, Daillere R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science. 2018;359(6371):91–7 **Described the associated between gut microbiota and anticancer activity of immune checkpoint inhibitors**.
66.• Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science. 2018;359(6371):97–103 **Described the associated between gut microbiota and anticancer activity of immune checkpoint inhibitors**.
67. Suresh R, Mosser DM. Pattern recognition receptors in innate immunity, host defense, and immunopathology. Adv Physiol Educ. 2013;37(4):284–91.
68. Zhao X, Suryawanshi S, Hruska M, Feng Y, Wang X, Shen J, et al. Assessment of nivolumab benefit-risk profile of a 240-mg flat dose relative to a 3-mg/kg dosing regimen in patients with advanced tumors. Ann Oncol. 2017;28(8):2002–8.
69. Ahamadi M, Freshwater T, Prohn M, Li CH, de Alwis DP, de Greef R, et al. Model-based characterization of the pharmacokinetics of pembrolizumab: a humanized anti-PD-1 monoclonal antibody in advanced solid tumors. CPT Pharmacometrics Syst Pharmacol. 2017;6(1):49–57.
70. Freshwater T, Kondic A, Ahamadi M, Li CH, de Greef R, de Alwis D, et al. Evaluation of dosing strategy for pembrolizumab for oncology indications. J Immunother Cancer. 2017;5:43.
71. Bajaj G, Wang X, Agrawal S, Gupta M, Roy A, Feng Y. Model-based population pharmacokinetic analysis of nivolumab in patients with solid tumors. CPT Pharmacometrics Syst Pharmacol. 2017;6(1):58–66.