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Immunotherapy of colorectal cancer: Challenges for therapeutic efficacy

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ABSTRACT

A better knowledge of the complex interactions between cancer cells and the immune system has led to novel immunotherapy approaches. Treatment with selective anti-PD1, anti-PD-L1 and/or anti-CTLA-4 monoclonal antibodies (mAbs) has been a revolution in the therapeutic scenario of several cancer types, with the highest clinical efficacy in melanoma and in lung cancer. Colorectal cancer is one of the tumours in which immunotherapy has been shown less effective. Whereas in deficient mismatch repair (MMR) or in highly microsatellite instable (MSI-H) metastatic colorectal cancer there is clear clinical evidence for a therapeutic role of immune checkpoint inhibitors, the vast majority of patients with proficient MMR or with microsatellite stable (MSS) tumours do not benefit from immunotherapy. Defining the molecular mechanisms for immunogenicity in metastatic colorectal cancer is needed in order to develop predictive biomarkers and effective therapeutic combination strategies. A major challenge will be to identify, among the heterogeneous spectrum of this disease, those patients with specific tumour and tumour infiltrating stroma molecular and functional characteristics, that could be effectively treated with immunotherapy. In this review, we discuss the role of immune response in the context of metastatic colorectal cancer. We summarize the available clinical data with the use of anti PD-1/PD-L1 mAbs as single agents or in combination with anti CTLA-4 mAbs in MSI-H patients. Finally, we address the challenges and the potential strategies for rendering the more frequent microsatellite stable (MSS) tumours "immune-competent" and, therefore, amenable for effective immunotherapy interventions.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second type in terms of mortality. Over 1.8 millions of CRC cases and 881.000 CRC-related deaths were estimated to occur in 2018 in the World [1]. Despite significant improvements in CRC treatment, the prognosis of patients with metastatic CRC (mCRC) remains poor, with a median overall survival (OS) of approximately 30 months [2]. For this reason, novel and more effective therapeutic strategies are necessary for metastatic disease.

In the last few years, a better knowledge of the complex interactions between cancer cells and the immune system has led to novel immunotherapy approaches. The development of immune checkpoint inhibitors, such as anti-CTLA-4 monoclonal antibodies (mAbs) (ipilimumab, tremelimumab), anti-PD-1 mAbs (nivolumab, pembrolizumab) and anti-PD-L1 mAbs (atezolizumab, durvalumab, avelumab), has dramatically changed the therapeutic scenario for several types of cancer, including melanoma, lung, head and neck, kidney, bladder, Merckel cell carcinoma and mCRC with high microsatellite instability (MSI-H) [3–21]. However, the use of immune checkpoint inhibitors has demonstrated little or no clinical activity in the majority of patients with mCRC [22].

In this review, we discuss the role of immune response in the context of mCRC. We summarize the available clinical data with the use of anti PD-1/PD-L1 mAbs as single agents or in combination with anti CTLA-4 mAb in MSI-H mCRC patients. Finally, we address the challenges and the potential strategies for rendering the more frequent microsatellite stable (MSS) mCRC tumours "immune-competent" and, therefore, amenable for effective immunotherapy interventions.

Evidence for immune competence in colorectal cancers with microsatellite instability

Microsatellites are short segments of DNA that consist of multiple repetitions of one to ten nucleotide base pairs. During DNA synthesis by DNA polymerase, these sequences are frequently subjected to mutations, like base insertions or deletions, thus leading to frameshift mutations. The mismatch-repair (MMR) system has a major function in

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recognizing and correcting these errors in the microsatellite region, thus preventing genomic alterations [23].

The presence of microsatellite instability, determined by the deficiency of one MMR protein, has been found in approximately 15–20% CRC, with some differences according to tumour stage [24,25]. In fact, the prevalence of deficient mismatch repair (dMMR) is higher in Stage II (20%) and Stage III (12%) as compared to Stage IV (4%) CRC [25–27]. The majority of MSI-H CRC are sporadic tumours and are associated to the inactivation by methylation of MLH1 [28]. However, approximately 3% of dMMR CRC are related to the Lynch Syndrome (LS), which is associated with germ line mutations in the genes involved in MMR (MLH1, MSH2, MSH6 and PMS2) [29]. Interestingly, patients with MSI-H CRC share a characteristic clinical-pathological profile such as the more frequent association with female sex, older age, BRAF activating mutations, peritoneal disease, the presence of mucin-rich and signet ring cancer cells, right sided primary tumours, early stage and poorly differentiated tumours, with an abundance of tumour infiltrating lymphocytes (TILs) and a Crohn-like lymphoid reaction [30,31].

Diagnosis of MSI status can be assessed by various methodologies, like real time polymerase chain reaction (PCR), immunohistochemistry (IHC) and next generation sequencing (NGS), with a good level of concordance [32–35]. dMMR CRC is characterized by a high tumour mutational burden (TMB), as a consequence of a large number of tumour DNA damages, including deletions, insertions and frameshift mutations during cancer cell replication.

Measurement of TMB in the primary tumour and/or in blood samples from melanoma or lung cancer patients treated with immune checkpoint inhibitors has been suggested as a potential biomarker of therapeutic efficacy [36,37]. However, these results are largely from retrospective analyses and are obtained with different NGS-based techniques. In fact, it is still under investigation, in these tumour types, which is the most appropriate cut-off for defining high TMB for therapeutic use. In this respect, the evaluation of TMB has been recently reported in a retrospective, subgroup and hypothesis-generating analysis of tumour samples from CRC patients that received as first line treatment for metastatic disease a 5-FU-based chemotherapy doublet plus bevacizumab or cetuximab [38]. In the 35 patients with MSI-H tumours, the median TMB was 52 mutations/Mb, whereas in the 475 patients with MSS tumours, the median TMB was 6 mutations/Mb. Authors defined high TMB cases those with 8 or more mutations/Mb. According to this seledted cut-off, better OS could be observed in mCRC patients with high TMB, regardless of the first line treatment [38]. These data need to be validated in a prospective fashion in appropriately designed studies in order to define the TMB cut-off for clinical use and if high TMB could be confirmed as a positive prognostic marker in mCRC.

The presence of a high number of tumour-associated neoantigens could favour the identification of cancer cells by the host immune system [39–41]. In this respect, MSI-H CRC are correlated with an increased infiltration of TILs, such as $CD8^+$ cytotoxic lymphocytes, T_h1 activated cells that produces IFNγ, and CD45 RO+ T memory cells, which are also correlated with a better survival as compared to MSS CRC [42–44]. Moreover, the results of the international validation of the consensus immunoscore for the classification of colon cancer have demonstrated that a high immunoscore, based on $CD3^+$ and $CD8^+$ T cell densities within the tumour, as measured by IHC and quantified by digital pathology, is an independent positive prognostic biomarker for the risk of recurrence in Stage I-III CRC [45]. High immunoscores were observed in 45% of cases with MSI-H tumours (138/304) and only in 21% of cases with MSS tumours (273/1275) [45].

Further, a tissue microarray analysis of a series of CRC samples detected high tumour PD-L1 expression in 5% of cases (19/394) and high tumour infiltrating lymphocytes PD-1 expression in 19% of cases (76/392) [46]. dMMR tumours had significantly higher levels of PD-1 and PD-L1 as compared to proficient MMR (pMMR) tumours (18% vs 2% and 50% vs 13%, respectively; $P < 0.001$ for both). In multivariate analysis, both PD-1 and PD-L1 high expression were independent factors associated with better recurrence-free survival, but only in dMMR cases [46].

Molecular classification of colorectal cancer: implications for immune competence

Colorectal cancer is a genetically heterogeneous disease in which several and different molecular pathways could be involved in tumour initiation, growth and progression. According to a comprehensive re-evaluation and comparison of CRC molecular gene expression profiling that has been obtained by the use of different platforms, a Consensus Molecular Subtype (CMS) classification has been developed, that is based on both tumour as well as infiltrating stroma gene expression. According to the CMS classification, four major groups can be identified. CMS1 (MSI Immune, approximately 14% of cases) are hypermutated tumours, generally with MSI-H features, can harbour BRAF mutations and show a robust immune infiltration. CMS2 (Canonical, approximately 37% of cases) are tumours, which are characterized by the activation of the *Wnt* and *Myc* pathways. CMS3 (Metabolic, approximately 13% of the cases) tumours have frequently KRAS mutations and display a deregulation in cancer cell metabolic pathways. CMS4 (Mesenchymal, approximately 23% of the cases) tumours are characterized by transforming growth factor beta (TGFβ) pathway activation, enhanced angiogenesis, stromal activation and inflammatory infiltrate [47].

Interestingly, the four CRC CMSs are characterized by relevant differences in tumour microenviroment (TME). CMS1 and CMS4 are “hot” tumours with an intense immune infiltration, whereas CMS2 and CMS3 are “cold” tumours and lack immune activation. Therefore, the CMS classification represents a useful model to define potential therapeutic options for activating host immune responses, taking in account that different strategies are needed for “hot” and for “cold” CRCs. In this respect, CMS1 CRC has a diffuse immune infiltrate with CD8+ TILs and CD68+ macrophages. However, the concomitant up-regulation of immune checkpoint molecules (CTLA-4, PD-1, PD-L1) may represent a major mechanism of immune evasion in these tumours [48]. Thus, the use of immune checkpoint inhibitors could reverse the immune blockade and could activate an effective antitumor immune response in this CRC subtype. On the other hand, CMS4 tumours are characterized by a different immune infiltration pattern, in which T regulatory cells (T_{reg}), myeloid-derived suppressor cells (MDSCs), monocyte-derived cells and T helper 17 (T_H17) cells play a major role [49]. This ‘inflamed’ subtype, that is generally found in the microenvironment of immune-tolerant malignancies, is characterized by marked up-regulation of immunosuppressive factors, such as TGFβ and CXCL12, and high expression of genes encoding for chemokines that attract myeloid cells, including C-C motif chemokine ligand 2 (CCL2), interleukin 23 (IL-23) and IL-17 [50]. Therefore, the potential immune response in CMS4 tumours is blocked by the activation in the stroma of several mechanisms that favour an inflammatory environment and that, therefore, suppress the immune response against cancer cells. In this respect, TGFβ and other functional activators of epithelial to mesenchymal transition (EMT) and of angiogenesis may be major players for tumour immune evasion. It has been recently demonstrated by using a mouse genetic model of colon cancer development, that cancer progression and metastatic spreading is due to the key role of TGFβ activation in the tumour stroma that specifically inhibits the immune response [51]. In this respect, a therapeutic strategy of combining selective TGFβ inhibitors with immune checkpoint inhibitors is able to re-activate a robust immune response in this mouse colon cancer model [51].

In contrast to CMS1 and CMS4 tumours, in which high levels of immune infiltrates are observed, although with different functional characteristics, CMS2 and CMS3 tumours lack immune activation and, therefore, could be defined as “ immune desert” cancers. Different mechanisms may be responsible for this phenomenon, including

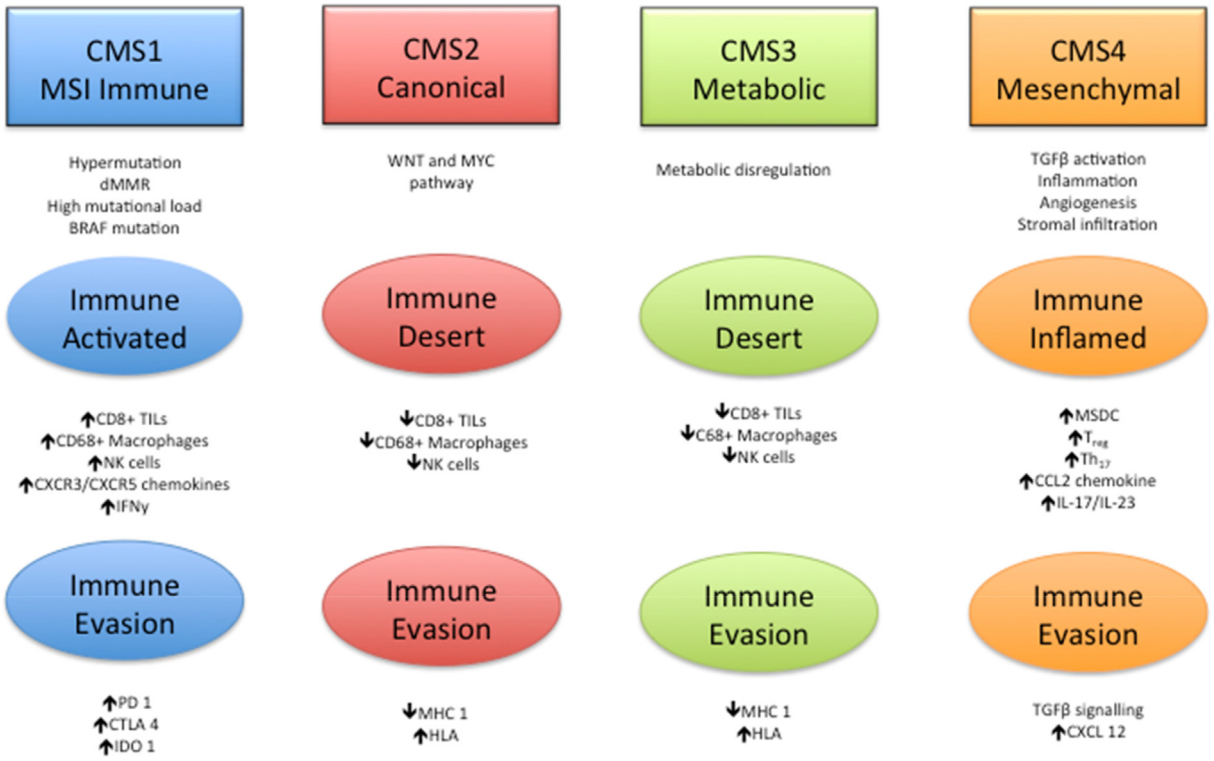

Fig. 1. Consensus molecular classification according to the immune phenotype. CMS1 and CMS4 show activation of the immune system, with different orientation. On the other hand CMS2 and CMS3 are poorly immunogenic and display a little activation of the immune response. dMMR: deficient mismatch repair; Transforming growth factor beta: TGFβ; TILs: tumour infiltrating lymphocytes; NK: natural killer; CXCR3/CCR5: Chemokine (C-X-C motif) receptor 3/C-C chemokine receptor type 5; IFNy: interferon gamma; MSDCs: myeloid suppressor derived cells; CCL2: chemokine (C-C motif) ligand 2; IL-17/IL-23: interleukin 17/interleukin 23; PD1: programmed death protein 1, CTLA4: cytotoxic T-lymphocyte-associated protein 4, IDO1: Indoleamine-pyrrole 2,3-dioxygenase; MHC I: major histocompatibility complex 1; HLA: human leukocyte antigens; CXCL12: Chemokine (C-X-C-motif) ligand 12.

specific oncogenic-driven cancer cell pathways, lack of major histocompatibility complex (MHC) class 1 molecules, up-regulation of non-classical human leukocyte antigens (HLA), that all may concur to cancer immune evasion [52]. For these reasons, novel therapeutic approaches are clearly needed to render immunogenic these tumours and possibly offer effective immunotherapies to CRC patients whose tumours are CMS2 or CMS3 (Fig. 1).

Clinical efficacy of immune checkpoint inhibitors in mCRC patients with microsatellite unstable tumours

Several trials have evaluated the clinical activity of immune checkpoint inhibitors in dMMR mCRC. In the phase II clinical trial KEYNOTE 016, 41 patients with pMMR and dMMR chemorefractory mCRC or with dMMR non-CRCs were treated with the anti-PD1 mAb pembrolizumab at the dose of 10 mg/kg every 3 weeks [22]. In the cohort of patients with dMMR mCRC (10/41), the overall response rate (ORR) was 40% (4/10), with durable clinical responses, whereas ORR was 0% in pMMR mCRC (18/41). Moreover, a high number of somatic mutations were associated with longer progression free survival (PFS). Pembrolizumab therapy was well tolerated: Grade 3–4 treatment related adverse events (TRAEs) occurred in 17% of patients. Updated results of the KEYNOTE 016 trial which included data on 86 patients with dMMR cancers, reported 52% ORR with 12% complete responses (CR) in the cohort of CRC patients. The 2-year progression free survival rate was 59% and the 2-year overall survival rate was 72% [15].

In the CheckMate 142, a nonrandomised phase II clinical trial, a first cohort of 74 patients with chemorefractory dMMR mCRC were treated with the anti PD-1 mAb nivolumab as single agent at the dose of 3 mg/kg, every 2 weeks [10]. The ORR was 31.1%. Disease control longer than 12 weeks was achieved in 69% of patients, with median time to response of 2.8 months. Twelve months PFS was 50.4%, while 12 months OS was 73.4%. Grade 3–4 TRAEs occurred in 20% of patients. These adverse events were mainly asthenia, diarrhoea and pruritus. In a different cohort, 119 patients with pre-treated dMMR mCRC received as induction treatment the combination of nivolumab, 3 mg/kg, plus the anti-CTLA-4 mAb ipilimumab, 1 mg/kg, every 3 weeks, followed by nivolumab, 3 mg/kg, every 2 weeks as maintenance [11]. The combination of anti PD-1 and anti-CTLA-4 immunotherapy displayed an ORR of 55% and a disease control rate at 12 weeks of 80%. Twelve month PFS rate was 71%, while 12 month OS was 85%. Grade 3–4 TRAEs were reported in 32% of patients.

Recently, the promising preliminary results of the combination with nivolumab, 3 mg/kg, every 2 weeks plus low-dose ipilimumab, 1 mg/kg, every 6 weeks in an untreated patient population with MSI-H mCRC have been presented [53]. This study has enrolled 45 patients. The ORR was 60% (27/45), with 7% CRs (3/45). The 12 months PFS and OS rates were 77% and 83%, respectively. Grade 3–4 TRAEs occurred in 16% of patients, with only 7% of patients that had to discontinue therapy for an adverse event. Based on these results, the USA Food and Drug Administration (FDA) has approved pembrolizumab, nivolumab, and nivolumab plus ipilimumab, for the treatment of MSH-I mCRC patients that have progressed after therapy with fluoropyrimidines plus irinotecan or oxaliplatin.

Several phase III clinical trials are currently on-going to evaluate the efficacy of anti-PD1, anti-PD L1 and CTLA-4 mAbs in dMMR mCRC (Table 1). Keynote 177 (NCT02563002) is a phase III randomized trial, which is comparing pembrolizumab versus a standard of care chemotherapy as first line treatment in dMMR mCRC. Further, the COMMITT trial (NCT02997228) is exploring the clinical efficacy of treatment with the anti-PD L1 mAb atezolizumb as single agent or in combination with FOLFOX plus bevacizumab, as compared to FOLFOX

Table 1
Ongoing phase II/III clinical trials evaluating the use of immune-checkpoint inhibitors in microsatellite instable colorectal cancer.

Study name	Agent	Target	Study population	Primary endpoint	Phase	Recruitment status
ATOMIC NCT02912559	FOLFOX/Atezolizumab Vs FOLFOX	PD-L1	Stage III MSH-I CRC	DFS	III	Active, recruiting
POLEM trial (NCT02912559)	Avelumab Vs Observation After adjuvant chemotherapy	PD-L1	Stage III MSH-I, POLE mutant CRC	DFS	III	Active, recruiting
KEYNOTE-177 (NCT02563002)	Pembrolizumab vs Standard therapy	PD-1	First line MSH-I mCRC	PFS OS	III	Active, recruitment completed
COMMIT Trial (NCT02997228)	Atezolizumab Vs FOLFOX/Atezolizumab/Bevacizumab Vs FOLFOX/Bevacizumab	PD-L1	First line MSH-I mCRC	PFS	III	Active, recruiting
NCT02227667	Durvalumab	PD-L1	Pretreated mCRC	ORR	II	Active

MSH-I: High microsatellite instability; mCRC: Metastatic colorectal-cancer; DFS: Disease free survival; PFS: Progression free survival; OS: Overall survival; ORR: Overall response rate.

plus bevacizumab as first line therapy of dMMR mCRC patients. Two trials are currently assessing the use of immune therapy in the adjuvant setting. The ATOMIC trial (NCT02912559) is investigating the combination of FOLFOX plus atezolizumab vs FOLFOX as adjuvant treatment in Stage III MSH-I CRC; while the POLEM trial (NCT02912559) is addressing the role of avelumab as maintenance treatment after 5-FU based adjuvant chemotherapy for Stage III MSI-H or POLE mutant CRC.

Are POLE and POLD1 mutations predictive for immune competence of colorectal cancer?

DNA polymerase epsilon (POLE) and DNA polymerase delta (POLD1) are two key enzymes involved in the process of DNA synthesis and repair, which guarantee the correct replication of the genome during cell cycle [54]. Interestingly, exonuclease domain mutations in POLE or in POLD1 genes determine a specific alteration of the enzyme proof-reading activity, with a deficit in DNA repair; thus, leading to the accumulation of a very high number of mutations, up to ten times higher than MSH-I CRC. This translates in an increased risk of developing several types of cancer, including endometrial, colorectal, gastric, pancreatic, breast and brain tumours [55–57].

POLE mutations have been found in approximately 1–2% in the overall population of pMMR CRCs. However, the frequency of this mutation arises to 5–7% in tumours of patients aged less than 50 years. This rare type of “ultra-mutated” tumour shares similar clinical-pathological features with dMMR CRC, such as high levels of TILs, up-regulation of immune checkpoint molecules, increase in cytotoxic T cell markers and in effector cytokines, suggesting a potentially enhanced immunogenicity. Moreover, Stage II-III CRC patients harbouring POLE mutations showed a reduced risk of recurrence after surgery as compared to pMMR POLE wild type CRC patients [57]. A few clinical data are currently available regarding the potential activity of immune checkpoint inhibitors in mCRC patients whose tumours carry POLE/POLD1 mutations. Of note, durable clinical responses were reported with the use of the anti PD-1 mAb pembrolizumab in a patient with POLE mutated endometrial cancer and in a patient with POLE mutated pMMR CRC [58,59]. Probably in the near future, based on these promising findings, POLE mutations will be tested alongside MMR in order to better define CRC patients that could benefit from immunotherapy.

Is it possible to render MSS tumours immune-competent and amenable for immunotherapy?

The use of immune-checkpoint inhibitors in patients with dMMR mCRC determines significant and durable clinical responses, even in heavily pre-treated patient populations. Nevertheless, this relevant clinical benefit is limited to only a small group of tumours, which represents approximately 4% of mCRC [60,61]. In fact, therapy with anti-PD1 mAbs has no efficacy in pMMR mCRC patients. Novel therapeutic strategies to render these tumours immune-competent are urgently needed.

It has been shown that chemotherapy, molecular targeted therapies and radiotherapy could cause immunogenic cell death (ICD) in cancer cells. This may be due to the release of damage associated molecular patterns (DAMPs), which in turn are recognised by host dendritic cells (DC), that are able to present these antigens to $CD8^+$ cytotoxic lymphocytes and, therefore, to activate these cells against cancer cells [62–65]. These observations have led to the hypothesis that combining treatment with immune checkpoint inhibitors and other anticancer therapies could potentially overcome the primary resistance to immunotherapy of MSS CRC.

The potential role of radiotherapy

The “abscopal effect” is a rare phenomenon described for the first time in 1953. It consists of tumour regression in a site distant from the field of irradiation [66]. This may be due to the reactivation of host immune response against cancer cells. In fact, radiotherapy (RT) can increase the expression of MHC class I on cell membrane, and, thus, can improve antigen presentation by DC with a strong immune activation and subsequent ICD [67,68]. Currently, a few data are available for understanding the role of the combination of immune checkpoint inhibitors and RT for the treatment of CRC. One study has evaluated the combination of AMP224, a PD-1 inhibitor, with stereotactic body radiation therapy (SBRT) directed to liver metastases in patients with mCRC. The treatment was safe and feasible. However, no responses were observed [69]. Another trial investigated the clinical activity of pembrolizumab in combination with RT (cohort 1) or with local ablation (cohort 2) in a pre-treated population of mCRC patients. In the first cohort, one major response (ORR 9%, 1/11) was observed in a metastatic site distant from the irradiation field [70]. Currently, several clinical trials are on-going (Table 2).

The role of chemotherapy and of molecular targeted agents

In mCRC the combination of chemotherapy (5-fluorouracil, irinotecan, oxaliplatin) with molecular targeted agents that inhibit angiogenesis (bevacizumab, aflibercept, ramucirumab) or epidermal growth factor receptor (EGFR), such as cetuximab and panitumumab (in RAS wild type tumours) represents currently the standard of care for

Table 2
Principal clinical trial investigating the combination of immune checkpoint inhibitors and radiotherapy.

Study name	Agent	Target	Study population	Primary endpoint	Phase	Recruitment status
NCT03102047	Chemoradiation/Durvalumab	PD-L1	Stages II-IV MSS CRC	Median Neoadjuvant Rectal Score	II	Active, recruiting
NCT03104439	Nivolumab/Ipilimumab/Radiation	PD-L1 CTLA-4	MSS/MSH-I mCRC	Disease control rate	II	Active, recruiting
NCT03007407	Durvalumab/Tremelimumab/Radiotherapy	PD-L1 CTLA-4	MSS mCRC	ORR	II	Active, recruiting
NCT02888743	Durvalumab/Tremelimumab ± Radiotherapy	PD-L1 CTLA-4	Metastatic to liver MSS CRC	ORR	II	Active, recruiting

MSS: Microsatellite stability; MSH-I: High microsatellite instability; ORR: Overall response rate.

the first two lines of treatment [71–75].

Novel experimental evidence suggests that the anti-tumour activity of chemotherapy is not only due to the direct cytotoxic effects on cancer cells but also potentially to the stimulation of the host immune response [76]. In fact, conventional chemotherapy can augment the immunogenicity of cancer cells by inducing immunogenic cell death and by blocking mechanisms of immune-tolerance [77]. In particular, myeloid-derived suppressor cells (MDSCs) can promote immune escape by inhibiting the activation and proliferation of $CD8^+$ lymphocytes. In preclinical models, 5-fluorouracil treatment determines the apoptosis of MDSCs, favouring the production of IFNγ by $CD8^+$ cells and, therefore, increasing T-cell mediated anti-tumour response [78]. Oxaliplatin can also induce ICD by leading to the release of high mobility box binding protein-1 (HMGB-1) from cancer cells, which is recognised by the toll like receptor 4 (TLR4) and activates T CD8+ cells [77,79].

Vascular endothelial growth factor (VEGF) plays a central role in physiologic angiogenesis and tissue and wound repair processes and is often up-regulated in numerous malignancies in which significantly contributes to tumour-induced neoangiogenesis [80]. Further, VEGF may be involved in regulating immune functions in the TME by several mechanisms, such as by decreasing T-cell migration from lymph nodes to tumour sites; by up-regulating suppressive immune checkpoint molecules (PD-1, PD-L1, CTLA-4, LAG 3); by increasing MCSDs; by inhibiting DC maturation; by down-regulation of MHC I and, consequentially, by reducing the activation of $CD8^+$ cells [81–89]. In this respect, a study has recently evaluated the clinical activity of the combination of the anti-PD-L1 mAb atezolizumab with bevacizumab (cohort A) or the same combination plus modified FOLFOX6 chemotherapy (cohort B) [90]. In cohort A, 14 chemo-refractory mCRC patients, who had received at least two previous lines of chemotherapy, were treated with atezolizumab; 20 mg/kg, plus bevacizumab, 15 mg/kg, every 3 weeks. One patient achieved a partial response (ORR, 1/14, 7%) and 9 patients had stable disease as best clinical response. In cohort B, 23 mCRC patients received as first line of treatment atezolizumab 800 mg, bevacizumab 10 mg/kg and modified FOLFOX6 every 2 weeks. The ORR was 52% (12/23) with a median PFS of 14.1 months and a median duration of response of 11.4 months [91]. The treatment had an acceptable safety profile. Grade 3–4 TRAEs were observed in 67% patients. These preliminary results that suggest a potential synergistic activity of atezolizumab with chemotherapy plus bevacizumab need to be confirmed in appropriately sized, larger, randomized clinical trials. On the other hand, MODUL is a multicentre, randomized clinical trial, in which the best strategy of maintenance treatment in mCRC following a first line induction therapy with FOLFOX plus bevacizumab is being investigated with different therapeutic options. Recently, the results of the cohort of patients, that have been treated the addition of atezolizumab to standard treatment with fluoropyrimidine and bevacizumab after chemotherapy plus bevacizumab have been presented [92]. Unfortunately, no clinical advantage of this triplet maintenance treatment as compared to fluoropyrimidine plus bevacizumab was reported.

Cetuximab is a IgG 1 chimeric mouse-human mAb that inhibits the EGFR. Preclinical data have demonstrated that *in vitro* cetuximab could activate the opsonisation and phagocytosis of human colon cancer cell by DC, and, therefore, may favour the activation of T-cell mediated immune response [89]. Furthermore, cetuximab stimulates NK-mediated cell antibody-dependent cellular cytotoxicity (ADCC) [93]. In a recent phase II clinical trial (AVETUX), 43 mCRC patients with all RAS and BRAF wild type tumours were treated with the anti-PD-L1 mAb avelumab plus cetuximab and FOLFOX6, as first line of therapy. An interim analysis of the AVETUX study on 20 patients has reported a promising ORR of 75% (15/20) with DCR of 95% 19/20), and an acceptable tolerability profile [94]. Furthermore, rechallenge with anti-EGFR mAb treatment in mCRC patients, that have previously obtained a clinical response to anti-EGFR therapy in previous lines of treatment, is a potentially effective strategy, as recently suggested by small size, proof of concept studies [95,96]. In this respect, our research group is currently conducting a large, single arm, multicentre phase II clinical study, which evaluates the combined treatment with cetuximab plus avelumab in pretreated mCRC patients, that obtained a partial or a complete response with an anti-EGFR mAb-based chemotherapy in the first line of treatment. Overall survival (OS) is the primary endpoint of this study (CAVE Colon study, Eudract Number 2017-004392-32).

Table 3 summarizes the clinical trials that are currently on going to evaluate the role of combination strategies with chemotherapy, molecular targeted agents and immune checkpoint inhibitors in mCRC.

The role of MEK inhibition in combination with immune checkpoint inhibitors

MEK is a key signalling molecule in the MAPK signalling pathway. In preclinical models, it has been suggested that MEK inhibition can lead to up-regulation of MHC I and can increase the infiltration of CD8+ lymphocytes into tumours. Moreover, in an *in vivo* mouse model the combination of PD-1 and MEK blockade has determined a more effective inhibition of tumour growth as compared to single agent treatments, indicating a potential synergistic effect [97,98].

In 2016, Bendell presented the initial results of a phase Ib clinical trial, that was evaluating the clinical activity of the combination of atezolizumab, 800 mg every 2 weeks, plus cobimetinib, 60 mg orally, once a day (21 day on/7 day off) in chemo-refractory mCRC patients [99]. Twenty-three patients were treated. The ORR was 17% (4/23). Interestingly, 3 patients with a major response had an MSS tumour, while in the fourth patient with major response the tumour microsatellite status was unknown. Updated results from an expansion cohort of 84 heavily pre-treated patients reported an ORR of 8% (7/84), with durable responses (median duration of response 14.8 months) and median OS of 10 months [100]. On the basis of these promising results, the IMblaze370 (COTEZO) phase III randomized clinical trial was initiated. The study enrolled 363 chemo-refractory mCRC patients, that were randomized (2:1:1) to receive atezolizumab, 1200 mg plus cobimetinib 60 mg (21 day on/7day off schedule); atezolizumb monotherapy at the dose of 840 mg every 2 weeks or regorafenib at standard dose as control, standard arm of treatment. Unfortunately, the IMBlaze370 trial did not met its primary endpoint. The median OS was 8.9 month with atezolizumab plus cobimetinib vs. 8.5 month with regorafenib monotherapy [hazard ratio (HR) 1.00, 95% CI: 0.73, 1.38

Table 3
Selection of ongoing clinical trial investigating the combination of immunotherapy with chemotherapy and target agents.

Study name	Agent	Target	Study population	Primary endpoint	Phase	Recruitment status
CheckMate9X8 (NCT03414983)	FOLFOXBevacizumab/Nivolumab Vs FOLFOX/Bevacizumab	PD-1	First line CRC (MMR not spciefied)	PFS	II/III	Active, recruiting
MEDITREME (NCT03202758)	FOLFOX/Durvalumab/Tremelimumab	PD-1 CTLA- 4	First line CRC (MMR not spciefied)	Safety	Ib/II	Active,recruiting
ElevetiION:CRC 101 (NCT03176264)	FOLFOX/Bevacizumab/PDR001	PD1	First line MSS CRC	DLT ORR	I	Terminated
AVETUX (NCT03174405)	FOLFOX/Cetuximab/Avelumab	PD-L1	First line CRC (MMR not spciefied)	PFS	II	Active, recruitment completed
BACCI (NCT02873195)	Capecitabine Bevacizumab/Atezolizumab vs Capecitabine/Bevacizumab	PD-L1	Pretreated metastatic CRC (MMR not spciefied)	PFS	II	Active, recruitment completed
NCT02860546	TAS-102/Nivolumab	PD-1	Pretreated metastatic MSS CRC	irORR	II	Completed
NCT03396926	Capecitabine/Bevacizumab/Pembrolizumab	PD-1	Pretreated metastatic MSS CRC	ORR	II	Active, recruiting
NCT02848443	TAS 102/Oxaliplatin/Nivolumab ± Bevacizumab	PD-1	Pretreated metastatic MSS CRC	Safety	I	Active, recruiting
CAVE Colon (Eudract 2017-004392-32)	Cetuximab/Avelumab	PD-L1	Pretreated metastatic CRC (MMR not spciefied)	OS	II	Active, recruiting

CRC: Colorectal-cancer; MMR: mismatch repair; PFS: Progression free survival; MSS: Microsatellite stability; DLT: Dose limiting toxicities; ORR: Overall response rate; PFS: Progression free survival; irORR: Immune related overall response rate; OS: Overall survival.

(p = 0.987)]. Median OS was 7.1 month with atezolizumab monotherapy [(HR vs. regorafenib, 1.19 (95% CI: 0.83, 1.71)]. Moreover, there were no differences in terms of PFS and ORR across the treatment arms [101].

The role of combined PD-1 and CEA CD3 TCB blockade

Carcinoembryonic antigen (CEA) is a member of the immunoglobulin supergene family. It is localized on the cell membrane of enterocytes in the colonic mucosa. CEA is over-expressed in the majority of mCRC with levels of expression which could be up to 60 times higher than in normal cells [102,103].

CEA CD3 TCB (RG7802, RO6958688) is a T-cell bispecific antibody (CEA-TCB) that binds simultaneously CEA on tumour cells and CD3 on T cells. Thanks to its 2:1 ratio design, with two domains that bind CEA on tumour cells and one domain which recognizes CD3 on T cells, CEA-TCB can induce T cell migration, activation and proliferation in tumour sites with a potential significant anti-cancer activity [104]. Tabernero has recently presented the first results of a phase I clinical trial evaluating CEA-TCB as monotherapy or in combination with atezolizumab in patients with CEA positive metastatic tumours [105]. In the monotherapy cohort, 31 patients with chemo-refractory mCRC were treated with CEA-TCB at doses of 60 mg or higher. Disease control rate was 45%, with two patients having a partial response (PR, 2/31, 6%) and with 12 patients reporting as best response a stable disease (SD, 12/31, 39%). In the combination arm, 11 patients were treated at doses of CEA-TCB between 80 and 160 mg that were shown to have a clinical activity. Interestingly, in this heavily pre-treated mCRC population, two patients had a partial response (PR, 2/11, 18%) and 7 a stable disease (SD, 7/11, 64%). CEA-TCB treatment displayed a complex safety profile. The most common grade 3 TRAEs, at doses of 40 mg or higher, were diarrhoea, infusion related reactions, pyrexia, with five patients in the single agent treatment arm that experienced a dose limiting toxicity (DLT) and two patients that experienced a DLT in the combination arm. Thus, the safety profile represents a major issue for the future development of this drug.

The role of cancer vaccines

The activation of a selective host immune response against cancer cell antigens could be an effective strategy to boost specific immunotherapy. Several studies have been done to develop cancer vaccines for human CRC. However, little or no clinical efficacy has been observed so far [106].

DCs are able to process tumour-associated antigens (TAA), present them on the cell surface, causing the activation of T-cell and leading a cancer specific immune response. Dendritic cell vaccines can be obtained by isolating DC (autologous DC, ADC) from the host, pulsing them *ex vivo* with TAA, tumour lysate and then after activation, reinfusing them into the patient. The results of a phase II clinical trial, which was aimed to compare autologous tumour lysate DC plus best supportive care versus best supportive care, in pre-treated mCRC patients, were recently published [107]. Despite the fact that ADCs generated a tumour specific immune response, there was no clear benefit in terms of PFS or OS for the experimental treatment as compared with the control arm.

Peptide vaccines are based on combination of one or multiple antigenic epitopes derived from tumour-associated antigens with a vaccine adjuvant. These peptides are recognized and processed by DCs, stimulating a T-cell specific activation against cancer cells. A phase II study evaluated the combination of 5 peptides (RNF43, TOMM34, KOC1, VEGFR1 and VEGFR2) with an oxaliplatin-based chemotherapy, as first line treatment for patients with mCRC, but failed to demonstrate any clinical benefit [108].

OncoVAX is an active specific immunotherapy (ASI) that consists in the administration to patients of autologous irradiated cancer cells, which could combine with the Bacillus Calmette-Guerin (BCG), as immune-adjuvant. In a phase III study, 254 patients with stage II or III resected colon cancer were randomized to receive ASI or not as adjuvant treatment [109]. ASI consisted in 3 weekly vaccinations, starting 28 days after surgery, with a fourth vaccination at 6 months with irradiated tumour cells. Interestingly, for patients with stage II disease, it was observed a 61% reduction of the risk of the recurrence with ASI as compared to surgery alone with a trend toward improvement in OS. Based on these results, a confirmatory, randomized phase III trial is now recruiting patients with stage II colorectal cancer to receive OncoVAX or observation after surgery (ACTIVE trial, NCT02448173).

Oncolytic viruses are genetically modified viruses that could be able to selectively recognize and kill cancer cells; thus, avoiding damages to the host normal tissues. In a preclinical model, AD881, an oncolytic adenovirus, was able *in vitro* to induce ICD in CT26 cancer cells [110]. Similarly, G207 (a multi-mutated herpes simplex virus type I) had a

Table 4
Selection of ongoing trial investigating the combination of immunotherapic agents.

Study name	Agent	Target	Study population	Primary endpoint	Phase	Recruitment status
NCT02448173	Surgery/OncoVAX Vs Surgery	OncoVAX	Stage II CRC (MRR non specified)	DFS	III	Active, recruiting
NCT02981524	GVAX colon vaccine/Pembrolizumab	PD-1	Pretreated metastatic MSS CRC	ORR	II	Active, non recruiting
NCT02959437	Azacitidine/Epacadost/Pembrolizumab	PD-1 IDO	Pretreated metastatic MSS CRC	Safety ORR	I/II	Active non recruiting
NCT02559024	MEDI6469	OX-40	Liver metastasis of CRC (MRR non specified)	Safety Immune score	I	Active, non recruiting
NCT03241173	INCAGN01949, INCAGN01949/Ipilimumab, INCAGN01949/ Ipilimumab,	OX-40	Advanced solid tumour	Safety ORR	I/II	Active, non recruiting
NCT02777710	PEXIDARTINIB/Durvalumab	CSF-1R PD-l1	Pretreted metastatic CRC (MRR non specified)	Safety ORR	I	Active, non recruiting
NCT02503774	Oleclumab ± Durvalumab	CD73 PD-L1	Advanced solid tumour	Safety	I	Active, recruiting
NCT03207867	PDR001/NIR178	A2AR PD-1	Advanced solid tumour (including MSS CRC)	ORR	II	Active, recruiting
NCT03549000	NZV930, NZV930/PDR001, NZV930/NIR178, NZV930/NIR178/PDR001	A2AR CD73 PD-1	Advanced solid tumour	ORR	I	Active, recruiting

CRC: Colorectal-cancer; MMR: mismatch repair; DFS: Disease free survival; MSS: Microsatellite stability; ORR: Overall response rate.

significant cytotoxic activity in a panel of five human colon cancer cells lines [111]. Despite these compelling preclinical results, to date limited evidence is available in patients with mCRC. A phase I/II clinical trial evaluated the safety and the anti-tumour activity of NV1020, a genetically engineered oncolytic herpes simplex virus, in pre-treated chemo-refractory mCRC patients with liver metastasis [112]. Among the twenty-two patients that received the optimal dose treatment, disease control rate was 68% (1 partial response and 14 stable disease as best responses); median time to progression was 6.4 months and median OS was 11.8 months.

The role of IDO inhibitors

Indolamine 2,3-dioxygenase (IDO) is an enzyme that catalyses the conversion of tryptophan in kynurenine. Tryptophan is an essential amino acid for T-lymphocytes functions. Its depletion induces apoptosis and prevents T cell activation. On the other hand, kynurenine can promote T_{reg} activation and has an immunosuppressive effect [113–115].

It was found that high IDO expression was present in nearly 40% of CRC, it correlates with poor prognosis and with liver metastasis [116]. In a mouse model of colon cancer, IDO inhibitions determined a modification in tumour microenvironment by reducing T_{reg} and by increasing the levels of pro-inflammatory cytokines. However, IDO inhibition alone was not able to induce tumour regression, indicating that a combination treatment with immune checkpoint inhibitors could be a better strategy. A few data are available on the use of IDO inhibitors in a clinical setting in mCRC. A phase I study has assessed the tolerability of the IDO inhibitor epacadost in 52 patients with pre-treated solid tumours, including 29 patients with mCRC [117]. Further, a phase I/II clinical trial evaluating the combination of epacadostat with pembrolizumab and the epigenetic agent azacitidine in patients with advanced non small cell lung cancer and MSS mCRC is currently on-going (NCT02959437).

The role of OX40 agonists

OX40 (CD134) is a member of the tumour necrosis factor family of receptors, which is expressed by activated T cell, and acts as a co-stimulatory molecule. Its ligand OX40L is exposed on the cell membrane of antigen-presenting cells, such as Langerhans cells, mast cells, NK cells, epithelial cells, endothelial cells and smooth muscle cells. Interactions between OX40 and OX40L stimulate proliferation, activation and cytokine productions of CD4+ and CD8+ cells, the expansion of antigen-specific memory T cells and suppress the differentiation of $T\text{-}_{reg}$ cells [118]. Interestingly, it has been reported that high levels of expression of OX40 in TILs were founds in 50% of tumours samples and were correlated with a increased overall survival [119].

An *in vivo* study using the Colon 26 cancer model showed that treatment with an OX40-agonist induced depletion of $T\text{-}_{reg}$ and induced tumour-regression [120]. Several phase I/II trials are investigating the safety and activity of OX40 agonists as monotherapy or in combination with immune checkpoint inhibitors in different tumours including mCRC (see Table 4).

The role of macrophage colony-stimulating factor 1 receptor (CSF1R) inhibition

Myeloid-derived cell infiltration in TME could represent a mechanism of tolerance and immune escape. In fact, MSDSs can suppress the maturation of DCs, inhibit the activation and proliferation of T-cells and contribute to invasiveness and metastasis [121].

CSF1R is a receptor that is expressed on monocytoid cells. its activation by CSF favours the differentiation in MSDSs. In preclinical models of colon cancer, CSF1R inhibition suppressed MSDCs and delayed tumour growth [122]. Thus, a phase I study with the combination of a CSF1R inhibitor, pexidartinib, with an anti-PD-L1 antibody, durvalumab, in patients including colorectal and pancreatic cancer, is now under evaluation (NCT02777710).

The role of CD73 inhibition

High levels of adenosine in TME elicit the migration of cancer cells, stimulate angiogenesis, activate stromal remodelling and determine an immunosuppressive microenviroment. In fact, adenosine binds the adenosine A2A receptor (A2AR) and inhibits proliferation and activation of cytotoxic lymphocytes. Moreover, adenosine promotes the infiltration of immunosuppressive cells, such as $T\text{-}_{reg}$ and MDSCs [123]. The enzyme CD73 is expressed on cancer and immune cells and catalyse the conversion of adenosine monophosphate to adenosine. Recently, it has been shown in a mouse model that dual inhibitions of A2AR and CD73 improve anti-cancer immune response [124]. To date, three

clinical trial evaluating the feasibility and activity of immune checkpoint inhibitors with anti-CD73 antibodies (NCT02503774), A2AR antagonists (NCT03207867) and combinations of both (NCT03549000) are on-going.

Conclusions

Immunotherapy of cancer has become a novel effective therapeutic strategy since the role of immune checkpoint inhibitors has been elucidated in activating host immune response. Treatment with selective anti-PD1, anti-PD-L1 and/or anti-CTLA4 mAbs has been a revolution in the therapeutic scenario of several cancer types, with the highest clinical efficacy so far in melanoma and in lung cancer. However, even for these cancers, it is evident that immunotherapy is not effective in all patients and that biomarker selection is needed for the optimization of treatments.

Unfortunately, colorectal cancer is currently one of the tumour types in which immunotherapy has been shown less effective. Whereas in dMMR or MSI-H mCRC there is clear clinical evidence for a therapeutic role of immune checkpoint inhibitors, the majority of mCRC patients with pMMR or MSS tumours do not benefit from immunotherapy. Up to date, although promising preclinical findings and preliminary clinical results, we do not yet have clear evidence for immunotherapy efficacy in these patients.

A better knowledge of the molecular mechanisms that define the immune competence of a mCRC is needed in order to develop predictive biomarkers and effective therapeutic combination strategies such as with chemotherapies or with other molecular targeted agents or with co-stimulatory molecules. A major challenge will be in the next future to identify, among the heterogeneous spectrum of mCRC, those patients with specific tumour and tumour infiltrating stroma molecular and functional characteristics, that could be effectively treated with immunotherapy.

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Conflict of interests

FC has participated to Advisory Boards for Merck KgA, Bayer, Amgen, Roche, Servier, Pfizer, and he has received institutional research grants from Merck KgA, Bayer, Amgen, Roche, Ipsen. The other authors have no interests to disclose.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424. https://doi.org/10.3322/caac.21492.

[2] Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol 2016;27:1386–422. https://doi.org/10.1093/annonc/mdw235.

[3] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711–23. https://doi.org/10.1056/NEJMoa1003466.

[4] Ribas A, Hanson DC, Noe DA, Millham R, Guyot DJ, Bernstein SH, et al. Tremelimumab (CP-675,206), a cytotoxic T lymphocyte associated antigen 4 blocking monoclonal antibody in clinical development for patients with cancer. Oncologist 2007;12:873–83. https://doi.org/10.1634/theoncologist.12-7-873.

[5] Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung cancer. N Engl J Med 2015;373:1627–39. https://doi.org/10.1056/NEJMoa1507643.

[6] Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. N Engl J Med 2015;373:123–35. https://doi.org/10.1056/NEJMoa1504627.

[7] Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob J-J, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 2017;377:1345–56. https://doi.org/10.1056/NEJMoa1709684.

[8] Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015;373:1803–13. https://doi.org/10.1056/NEJMoa1510665.

[9] Kang Y-K, Boku N, Satoh T, Ryu M-H, Chao Y, Kato K, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017;390:2461–71. https://doi.org/10.1016/S0140-6736(17)31827-5.

[10] Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz H-J, 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;18:1182–91. https://doi.org/10.1016/S1470-2045(17)30422-9.

[11] Overman MJ, Lonardi S, Wong KYM, Lenz H-J, Gelsomino F, Aglietta M, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair–deficient/microsatellite instability-high metastatic colorectal cancer. J Clin Oncol 2018;36:773–9. https://doi.org/10.1200/JCO.2017.76.9901.

[12] Cohen EEW, Soulières D, Le Tourneau C, Dinis José, Licitra L, Ahn M-J, et al. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. Lancet 2019;393:156–67. https://doi.org/10.1016/S0140-6736(18)31999-8.

[13] Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. N Engl J Med 2016;375:1823–33. https://doi.org/10.1056/NEJMoa1606774.

[14] Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee J-L, Fong L, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 2017;376:1015–26. https://doi.org/10.1056/NEJMoa1613683.

[15] 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 (New York, NY) 2017;357:409–13. https://doi.org/10.1126/science.aan6733.

[16] Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018;379:2108–21. https://doi.org/10.1056/NEJMoa1809615.

[17] Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017;389:255–65. https://doi.org/10.1016/S0140-6736(16)32517-X.

[18] Barlesi F, Vansteenkiste J, Spigel D, Ishii H, Garassino M, de Marinis F, et al. Avelumab versus docetaxel in patients with platinum-treated advanced non-small-cell lung cancer (JAVELIN Lung 200): an open-label, randomised, phase 3 study. Lancet Oncol 2018;19:1468–79. https://doi.org/10.1016/S1470-2045(18)30673-9.

[19] Choueiri TK, Larkin J, Oya M, Thistlethwaite F, Martignoni M, Nathan P, et al. Preliminary results for avelumab plus axitinib as first-line therapy in patients with advanced clear-cell renal-cell carcinoma (JAVELIN Renal 100): an open-label, dose-finding and dose-expansion, phase 1b trial. Lancet Oncol 2018;19:451–60. https://doi.org/10.1016/S1470-2045(18)30107-4.

[20] D'Angelo SP, Russell J, Lebbé C, Chmielowski B, Gambichler T, Grob J-J, et al. Efficacy and safety of first-line avelumab treatment in patients with stage IV metastatic merkel cell carcinoma: a preplanned interim analysis of a clinical trial. JAMA Oncol 2018;4:e180077. https://doi.org/10.1001/jamaoncol.2018.0077.

[21] Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after chemoradiotherapy in stage III non–small-cell lung cancer. N Engl J Med 2017;377:1919–29. https://doi.org/10.1056/NEJMoa1709937.

[22] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–20. https://doi.org/10.1056/NEJMoa1500596.

[23] Vilar E, Gruber SB. Microsatellite instability in colorectal-cancer, the stable evidence. Nat Rev Clin Oncol 2010;7:153–62. https://doi.org/10.1038/nrclinonc.2009.237.

[24] Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Screening for the lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352:1851–60. https://doi.org/10.1056/NEJMoa043146.

[25] Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 2010;138:2073–2087.e3. https://doi.org/10.1053/j.gastro.2009.12.064.

[26] Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60–00 trial. J Clin Oncol 2010;28:466–74. https://doi.org/10.1200/JCO.2009.23.3452.

[27] Koopman M, Kortman GAM, Mekenkamp L, Ligtenberg MJL, Hoogerbrugge N, Antonini, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. Br J Cancer 2009;100:266–73. https://doi.org/10.1038/sj.bjc.6604867.

[28] Veigl ML, Kasturi L, Olechnowicz J, Ma AH, Lutterbaugh JD, Periyasamy S, et al. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. PNAS 1998;95:8698–702.

[29] Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol Off J Am Soc Clin Oncol 2008;26:5783–8. https://doi.org/10.1200/JCO.2008.17.5950.

[30] Greenson JK, Bonner JD, Ben-Yzhak O, Cohen HI, Miselevich I, Resnick MB, et al. Phenotype of microsatellite unstable colorectal carcinomas: well-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability. Am J Surg Pathol 2003;27:563–70.
[31] Graham D, Appelman HD. Crohn's-like lymphoid reaction and colorectal carcinoma: a potential histologic prognosticator. Mod Pathol 1990;3:332–5.
[32] Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248.
[33] Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J Molecul Diagn JMD 2008;10:293–300. https://doi.org/10.2353/jmoldx.2008.080031.
[34] Lindor NM, Burgart LJ, Leontovich O, Goldberg RM, Cunningham JM, Sargent DJ, et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. JCO 2002;20:1043–8. https://doi.org/10.1200/JCO.2002.20.4.1043.
[35] Stadler ZK, Battaglin F, Middha S, Hechtman JF, Tran C, Cercek A, et al. Reliable detection of mismatch repair deficiency in colorectal cancers using mutational load in next-generation sequencing panels. J Clin Oncol Off J Am Soc Clin Oncol 2016;34:2141–7. https://doi.org/10.1200/JCO.2015.65.1067.
[36] 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;371:2189–99. https://doi.org/10.1056/NEJMoa1406498.
[37] Ready Neal, Hellmann Matthew D, Awad Mark M, Otterson Gregory A, Gutierrez Martin, Gainor Justin F, Borghaei Hossein, Jolivet Jacques, Horn Leora, Mates Mihaela, Brahmer Julie, Rabinowitz Ian, Reddy Pavan S, Chesney Jason, Orcutt James, Spigel David R, Reck Martin, O'Byrne Kenneth John, Paz-Ares Luis, Hu Wenhua, Zerba Kim, Li Xuemei, Lestini Brian, Geese William J, Szustakowski Joseph D, Green George, Chang Han, Ramalingam Suresh S. -Line nivolumab plus ipilimumab in advanced non–small-cell lung cancer (CheckMate 568): outcomes by programmed death ligand 1 and tumor mutational burden as biomarkers. JCO 2019;37(12):992–1000https://ascopubs.org/doi/10.1200/JCO.18.01042https://doi.org/10.1200/JCO.18.01042.
[38] Innocenti Federico, Ou Fang-Shu, Qu Xueping, Zemla Tyler J, Niedzwiecki Donna, Tam Rachel, Mahajan Shilpi, Goldberg Richard M, Bertagnolli Monica M, Blanke Charles D, Sanoff Hanna, Atkins James, Polite Blasé, Venook Alan P, Lenz Heinz-Josef, Kabbarah Omar. Mutational analysis of patients with colorectal cancer in CALGB/SWOG 80405 identifies new roles of microsatellite instability and tumor mutational burden for patient outcome. JCO.18.01798 JCO2019http://ascopubs.org/doi/10.1200/JCO.18.01798https://doi.org/10.1200/JCO.18.01798.
[39] Mouw KW, Goldberg MS, Konstantinopoulos PA, D'Andrea AD. DNA damage and repair biomarkers of immunotherapy response. Cancer Discov 2017. https://doi.org/10.1158/2159-8290.CD-17-0226.
[40] McGranahan N, Furness AJS, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016;351:1463. https://doi.org/10.1126/science.aaf1490.
[41] Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415. https://doi.org/10.1038/nature12477.
[42] Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 2013;39:782–95. https://doi.org/10.1016/j.immuni.2013.10.003.
[43] Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960. https://doi.org/10.1126/science.1129139.
[44] Watanabe T, Wu T-T, Catalano PJ, Ueki T, Satriano R, Haller DG, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med 2001;344:1196–206. https://doi.org/10.1056/NEJM200104193441603.
[45] Pagès F, Mlecnik B, Marliot F, Bindea G, Ou F-S, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet 2018;391:2128–39. https://doi.org/10.1016/S0140-6736(18)30789-X.
[46] Lee Lik Hang, Cavalcanti Marcela S, Segal Neil H, Hechtman Jaclyn F, Weiser Martin R, Smith J Joshua, Garcia-Aguilar Julio, Sadot Eran, Ntiamoah Peter, Markowitz Arnold J, Shike Moshe, Stadler Zsofia K, Vakiani Efsevia, Klimstra David S, Shia Jinru. Patterns and prognostic relevance of PD-1 and PD-L1 expression in colorectal carcinoma. Mod Pathol 2016;29(11):1433–42http://www.nature.com/articles/modpathol2016139https://doi.org/10.1038/modpathol.2016.139.
[47] Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350–6. https://doi.org/10.1038/nm.3967.
[48] Becht E, de Reyniès A, Giraldo NA, Pilati C, Buttard B, Lacroix L, et al. Immune and stromal classification of colorectal cancer is associated with molecular subtypes and relevant for precision immunotherapy. Clin Cancer Res 2016;22:4057–66. https://doi.org/10.1158/1078-0432.CCR-15-2879.
[49] Dienstmann R, Vermeulen L, Guinney J, Kopetz S, Tejpar S, Tabernero J. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. 268–268 Nat Rev Cancer 2017;17. https://doi.org/10.1038/nrc.2017.24.
[50] Angelova M, Charoentong P, Hackl H, Fischer ML, Snajder R, Krogsdam AM, et al. Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumor escape mechanisms and novel targets for immunotherapy. Genome Biol 2015;16. https://doi.org/10.1186/s13059-015-0620-6.
[51] Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, et al. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. Nature 2018;554:538. https://doi.org/10.1038/nature25492.
[52] Roelands J, Kuppen PJK, Vermeulen L, Maccalli C, Decock J, Wang E, et al. Immunogenomic classification of colorectal cancer and therapeutic implications. Int J Mol Sci 2017;18:2229. https://doi.org/10.3390/ijms18102229.
[53] Lenz H-JJ, Cardin D, Dragovich T, Shah U, Atasoy A, Postema R, et al. LBA18_PRDurable clinical benefit with nivolumab (NIVO) plus low-dose ipilimumab (IPI) as first-line therapy in microsatellite instability-high/mismatch repair deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC). Ann Oncol 2018;29. https://doi.org/10.1093/annonc/mdy424.019.
[54] Prindle MJ, Loeb LA. DNA polymerase delta in DNA replication and genome maintenance. Environ Mol Mutagen 2012;53:666–82. https://doi.org/10.1002/em.21745.
[55] Heitzer E, Tomlinson I. Replicative DNA polymerase mutations in cancer. Curr Opin Genet Dev 2014;24:107–13. https://doi.org/10.1016/j.gde.2013.12.005.
[56] Rayner E, van Gool IC, Palles C, Kearsey SE, Bosse T, Tomlinson I, et al. A panoply of errors: polymerase proofreading domain mutations in cancer. Nat Rev Cancer 2016;16:71. https://doi.org/10.1038/nrc.2015.12.
[57] Domingo E, Freeman-Mills L, Rayner E, Glaire M, Briggs S, Vermeulen L, et al. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. Lancet Gastroenterol Hepatol 2016;1:207–16. https://doi.org/10.1016/S2468-1253(16)30014-0.
[58] Mehnert JM, Panda A, Zhong H, Hirshfield K, Damare S, Lane K, et al. Immune activation and response to pembrolizumab in POLE-mutant endometrial cancer. J Clin Investig 2016;126:2334–40. https://doi.org/10.1172/JCI84940.
[59] Gong J, Wang C, Lee PP, Chu P, Fakih M. Response to PD-1 blockade in microsatellite stable metastatic colorectal cancer harboring a POLE mutation. J Natl Compr Canc Netw 2017;15:142–7. https://doi.org/10.6004/jnccn.2017.0016.
[60] Gryfe R, Kim H, Hsieh ETK, 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;342:69–77. https://doi.org/10.1056/NEJM200001133420201.
[61] Kawakami H, Zaanan A, Sinicrope FA. Microsatellite instability testing and its role in the management of colorectal cancer. 30–30 Curr Treat Opt Oncol 2015;16. https://doi.org/10.1007/s11864-015-0348-2.
[62] Bracci L, Schiavoni G, Sistigu A, Belardelli F. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. Cell Death Differ 2014;21:15–25. https://doi.org/10.1038/cdd.2013.67.
[63] Overman MJ, Ernstoff MS, Morse MA. Where we stand with immunotherapy in colorectal cancer: deficient mismatch repair, proficient mismatch repair, and toxicity management. Am Soc Clin Oncol Educ Book 2018;239–247. https://doi.org/10.1200/EDBK_200821.
[64] Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4–dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007;13:1050–9. https://doi.org/10.1038/nm1622.
[65] Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer 2012;12:860–75. https://doi.org/10.1038/nrc3380.
[66] Mole RH. Whole body irradiation—radiobiology or medicine? British J Radiol 1953;26:234–41. https://doi.org/10.1259/0007-1285-26-305-234.
[67] Reynders K, Illidge T, Siva S, Chang JY, De Ruysscher D. The abscopal effect of local radiotherapy: using immunotherapy to make a rare event clinically relevant. Cancer Treat Rev 2015;41:503–10. https://doi.org/10.1016/j.ctrv.2015.03.011.
[68] Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med 2006;203:1259–71. https://doi.org/10.1084/jem.20052494.
[69] Duffy AG, Makarova-Rusher OV, Pratt D, Kleiner DE, Fioravanti S, Walker M, et al. A pilot study of AMP-224, a PD-L2 Fc fusion protein, in combination with stereotactic body radiation therapy (SBRT) in patients with metastatic colorectal cancer. 560–560 JCO 2016;34. https://doi.org/10.1200/jco.2016.34.4_suppl.560.
[70] Segal NH, Kemeny NE, Cercek A, Reidy DL, Raasch PJ, Warren P, et al. Non-randomized phase II study to assess the efficacy of pembrolizumab (Pem) plus radiotherapy (RT) or ablation in mismatch repair proficient (pMMR) metastatic colorectal cancer (mCRC) patients. 3539–3539 JCO 2016;34. https://doi.org/10.1200/JCO.2016.34.15_suppl.3539.
[71] Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–42. https://doi.org/10.1056/NEJMoa032691.
[72] Tabernero J, Yoshino T, Cohn AL, Obermannova R, Bodoky G, Garcia-Carbonero R, et al. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, multicentre, phase 3 study. Lancet Oncol 2015;16:499–508. https://doi.org/10.1016/S1470-2045(15)70127-0.
[73] Van Cutsem E, Tabernero J, Lakomy R, Prenen H, Prausová J, Macarulla T, et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer

previously treated with an oxaliplatin-based regimen. JCO 2012;30:3499–506. https://doi.org/10.1200/JCO.2012.42.8201.
[74] Douillard J-Y, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab–FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 2013;369:1023–34. https://doi.org/10.1056/NEJMoa1305275.
[75] Van Cutsem E, Lenz H-J, Köhne C-H, Heinemann V, Tejpar S, Melezínek I, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J Clin Oncol 2015;33:692–700. https://doi.org/10.1200/JCO.2014.59.4812.
[76] Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell 2015;28:690–714. https://doi.org/10.1016/j.ccell.2015.10.012.
[77] Emens LA, Middleton G. The interplay of immunotherapy and chemotherapy: harnessing potential synergies. Cancer Immunol Res 2015;3:436–43. https://doi.org/10.1158/2326-6066.CIR-15-0064.
[78] Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, Martin F, Apetoh L, Rebe C, Ghiringhelli F. 5-fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res 2010;70(8):3052–61http://cancerres.aacrjournals.org/cgi/doi/10.1158/0008-5472.CAN-09-3690https://doi.org/10.1158/0008-5472.CAN-09-3690.
[79] Tesniere A, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene 2010;29:482–91. https://doi.org/10.1038/onc.2009.356.
[80] Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000;407:249–57. https://doi.org/10.1038/35025220.
[81] Chen DS, Hurwitz H. Combinations of bevacizumab with cancer immunotherapy. Cancer J 2018;24:193–204. https://doi.org/10.1097/PPO.0000000000000327.
[82] Hegde PS, Wallin JJ, Mancao C. Predictive markers of anti-VEGF and emerging role of angiogenesis inhibitors as immunotherapeutics. Semin Cancer Biol 2018;52:117–24. https://doi.org/10.1016/j.semcancer.2017.12.002.
[83] Villadangos JA, Schnorrer P. Intrinsic and cooperative antigen-presenting functions of dendritic-cell subsets in vivo. Nat Rev Immunol 2007;7:543–55. https://doi.org/10.1038/nri2103.
[84] Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP, et al. Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-κB activation in hemopoietic progenitor cells. J Immunol 1998;160:1224.
[85] Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005;307:58–62. https://doi.org/10.1126/science.1104819.
[86] Motz GT, Santoro SP, Wang L-P, Garrabrant T, Lastra RR, Hagemann IS, et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. Nat Med 2014;20:607–15. https://doi.org/10.1038/nm.3541.
[87] Ohm JE. VEGF inhibits T-cell development and may contribute to tumor-induced immune suppression. Blood 2003;101:4878–86. https://doi.org/10.1182/blood-2002-07-1956.
[88] Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet A-L, et al. VEGF-A modulates expression of inhibitory checkpoints on CD8 $^{+}$ T cells in tumors. J Exp Med 2015;212:139–48. https://doi.org/10.1084/jem.20140559.
[89] Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer 2012;12:237–51. https://doi.org/10.1038/nrc3237.
[90] Bendell JC, Powderly JD, Lieu CH, Eckhardt SG, Hurwitz H, Hochster HS, et al. Safety and efficacy of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) and/or FOLFOX in patients (pts) with metastatic colorectal cancer (mCRC). 704–704 JCO 2015;33. https://doi.org/10.1200/jco.2015.33.3_suppl.704.
[91] Wallin J, Pishvaian MJ, Hernandez G, Yadav M, Jhunjhunwala S, Delamarre L, et al. Abstract 2651: clinical activity and immune correlates from a phase Ib study evaluating atezolizumab (anti-PDL1) in combination with FOLFOX and bevacizumab (anti-VEGF) in metastatic colorectal carcinoma. Cancer Res 2016;76:2651. https://doi.org/10.1158/1538-7445.AM2016-2651.
[92] Grothey A, Tabernero J, Arnold D, De Gramont A, Ducreux MP, O'Dwyer PJ, et al. LBA19Fluoropyrimidine (FP) + bevacizumab (BEV) + atezolizumab vs FP/BEV in BRAFwt metastatic colorectal cancer (mCRC): Findings from Cohort 2 of MODUL – a multicentre, randomized trial of biomarker-driven maintenance treatment following first-line induction therapy. Ann Oncol 2018;29. https://doi.org/10.1093/annonc/mdy424.020.
[93] Dechant M, Weisner W, Berger S, Peipp M, Beyer T, Schneider-Merck T, et al. Complement-dependent tumor cell lysis triggered by combinations of epidermal growth factor receptor antibodies. Cancer Res 2008;68:4998–5003. https://doi.org/10.1158/0008-5472.CAN-07-6226.
[94] Stein A, Binder M, Al-Batran S-E, Hinke A, Waberer L, Goekkurt E, et al. Avelumab and cetuximab in combination with FOLFOX in patients with previously untreated metastatic colorectal cancer (MCRC): Results of the safety run-in phase of the phase II AVETUX trial (AIO-KRK-0216). 3561–3561 JCO 2018;36. https://doi.org/10.1200/JCO.2018.36.15_suppl.3561.
[95] Santini D, Vincenzi B, Tonini G, Imperatori M, Garufi C, Masi G, et al. Cetuximab rechallenge in metastatic colorectal cancer patients: how to come away from acquired resistance? Ann Oncol 2012;23:2313–8. https://doi.org/10.1093/annonc/mdr623.
[96] Cremolini C, Rossini D, Dell'Aquila E, Lonardi S, Conca E, Del Re M, Busico A, et al. Rechallenge for patients with RAS and BRAF Wild-type metastatic colorectal cancer with acquired resistance to first-line cetuximab and irinotecan: a phase 2 single-arm clinical trial. JAMA Oncol 2018. https://doi.org/10.1001/jamaoncol.2018.5080.
[97] Ebert PJR, Cheung J, Yang Y, McNamara E, Hong R, Moskalenko M, et al. MAP kinase inhibition promotes T cell and anti-tumor activity in combination with PD-L1 checkpoint blockade. Immunity 2016;44:609–21. https://doi.org/10.1016/j.immuni.2016.01.024.
[98] Liu L, Mayes PA, Eastman S, Shi H, Yadavilli S, Zhang T, et al. The BRAF and MEK inhibitors dabrafenib and trametinib: effects on immune function and in combination with immunomodulatory antibodies targeting PD-1, PD-L1, and CTLA-4. Clin Cancer Res 2015;21:1639–51. https://doi.org/10.1158/1078-0432.CCR-14-2339.
[99] Bendell JC, Kim TW, Goh BC, Wallin J, Oh D-Y, Han S-W, et al. Clinical activity and safety of cobimetinib (cobi) and atezolizumab in colorectal cancer (CRC). 3502–3502 J Clin Oncol 2016;34. https://doi.org/10.1200/JCO.2016.34.15_suppl.3502.
[100] Hellmann MD, Desai J, Solomon BJ, Hernandez G, Das Thakur M, Wongchenko MJ, et al. Phase Ib study of atezolizumab combined with cobimetinib in patients with solid tumors. [in press]. Ann Oncol2019. https://doi.org/10.1093/annonc/mdz113.
[101] Eng C, Kim T, Bendell J, Argiles G, Tebbuit NC, Di Bartolomeo M, et al. Atezolizumab with o without cobimetinib versus regorafenib in previously treated metastatic colorectal cancer (Imblaze370): a multicentre, open-label, phase 3, randomised, controlled trial. [in press]. Lancet Oncol2019. https://doi.org/10.16/S1470-2045(19)30027-0.
[102] Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clin Chem 2001;47:624.
[103] Boucher D, Cournoyer D, Stanners CP, Fuks A. Studies on the control of gene expression of the carcinoembryonic antigen family in human tissue. Cancer Res 1989;49:847.
[104] Bacac M, Fauti T, Sam J, Colombetti S, Weinzierl T, Ouaret D, et al. A novel carcinoembryonic antigen T-cell bispecific antibody (CEA TCB) for the treatment of solid tumors. Clin Cancer Res 2016;22:3286. https://doi.org/10.1158/1078-0432.CCR-15-1696.
[105] Tabernero J, Melero I, Ros W, Argiles G, Marabelle A, Rodriguez-Ruiz ME, et al. Phase Ia and Ib studies of the novel carcinoembryonic antigen (CEA) T-cell bispecific (CEA CD3 TCB) antibody as a single agent and in combination with atezolizumab: Preliminary efficacy and safety in patients with metastatic colorectal cancer (mCRC). 3002–3002 JCO 2017;35. https://doi.org/10.1200/JCO.2017.35.15_suppl.3002.
[106] Berry J, Vreeland T, Trappey A, Hale D, Peace K, Tyler J, Walker A, Brown R, Herbert G, Yi F, Jackson D, Clifton G, Peoples GE. Cancer vaccines in colon and rectal cancer over the last decade: lessons learned and future directions. Exp Rev Clin Immunol 2017;13:235–45. https://doi.org/10.1080/1744666X.2016.1226132.
[107] Caballero-Baños M, Benitez-Ribas D, Tabera J, Varea S, Vilana R, Bianchi L, et al. Phase II randomised trial of autologous tumour lysate dendritic cell plus best supportive care compared with best supportive care in pre-treated advanced colorectal cancer patients. Eur J Cancer 2016;64:167–74. https://doi.org/10.1016/j.ejca.2016.06.008.
[108] Hazama S, Nakamura Y, Tanaka H, Hirakawa K, Tahara K, Shimizu R, et al. A phase II study of five peptides combination with oxaliplatin-based chemotherapy as a first-line therapy for advanced colorectal cancer (FXV study). J Transl Med 2014;12:108. https://doi.org/10.1186/1479-5876-12-108.
[109] Vermorken JB, Claessen AM, van Tinteren H, Gall HE, Ezinga R, Meijer S, et al. Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. Lancet 1999;353:345–50. https://doi.org/10.1016/S0140-6736(98)07186-4.
[110] Yamano T, Kubo S, Fukumoto M, Yano A, Mawatari-Furukawa Y, Okamura H, et al. Whole cell vaccination using immunogenic cell death by an oncolytic adenovirus is effective against a colorectal cancer model. Mol Ther Oncolyt 2016;3:16031. https://doi.org/10.1038/mto.2016.31.
[111] Kooby DA, Carew JF, Halterman MW, Mack JE, Bertino JR, Blumgart LH, et al. Oncolytic viral therapy for human colorectal cancer and liver metastases using a multi-mutated herpes simplex virus type-1 (G207). FASEB J 1999;13:1325–34. https://doi.org/10.1096/fasebj.13.11.1325.
[112] Geevarghese SK, Geller DA, de Haan HA, Hörer M, Knoll AE, Mescheder A, et al. Phase I/II study of oncolytic herpes simplex virus NV1020 in patients with extensively pretreated refractory colorectal cancer metastatic to the liver. Hum Gene Ther 2010;21:1119–28. https://doi.org/10.1089/hum.2010.020.
[113] Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. J Exp Med 1999;189:1363–72.
[114] Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. J Immunol 2010;185:3190. https://doi.org/10.4049/jimmunol.0903670.
[115] Brandacher G. Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. Clin Cancer Res 2006;12:1144–51. https://doi.org/10.1158/1078-0432.CCR-05-1966.
[116] Takamatsu M, Hirata A, Ohtaki H, Hoshi M, Ando T, Ito H, et al. Inhibition of indoleamine 2,3-dioxygenase 1 expression alters immune response in colon tumor microenvironment in mice. Cancer Sci 2015;106:1008–15. https://doi.org/10.1111/cas.12705.
[117] Beatty GL, O'Dwyer PJ, Clark J, Shi JG, Bowman KJ, Scherle P, et al. First-in-human phase 1 study of the oral inhibitor of indoleamine 2,3-dioxygenase-1 epacadostat (INCB024360) in patients with advanced solid malignancies. clincanres.2272.2016 Clin Cancer Res2017. https://doi.org/10.1158/1078-0432.CCR-16-2272.
[118] Croft M, So T, Duan W, Soroosh P. The significance of OX40 and OX40L to T-cell

biology and immune disease. Immunol Rev 2009;229:173–91. https://doi.org/10.1111/j.1600-065X.2009.00766.x.

[119] Petty JK, He K, Corless CL, Vetto JT, Weinberg AD. Survival in human colorectal cancer correlates with expression of the T-cell costimulatory molecule OX-40 (CD134). Am J Surg 2002;183:512–8.

[120] Linch SN, McNamara MJ, Redmond WL. OX40 agonists and combination immunotherapy: putting the pedal to the metal. 34 34 Front Oncol 2015;5. https://doi.org/10.3389/fonc.2015.00034.

[121] Sun H-L, Zhou X, Xue Y-F, Wang K, Shen Y-F, Mao J-J, et al. Increased frequency and clinical significance of myeloid-derived suppressor cells in human colorectal carcinoma. World J Gastroenterol 2012;18:3303–9. https://doi.org/10.3748/wjg.v18.i25.3303.

[122] Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. Cancer Cell 2014;25:846–59. https://doi.org/10.1016/j.ccr.2014.05.016.

[123] Vijayan D, Young A, Teng MWL, Smyth MJ. Targeting immunosuppressive adenosine in cancer. Nat Rev Cancer 2017;17:709. https://doi.org/10.1038/nrc.2017.86.

[124] Young A, Ngiow SF, Barkauskas DS, Sult E, Hay C, Blake SJ, et al. Co-inhibition of CD73 and A2AR adenosine signaling improves anti-tumor immune responses. Cancer Cell 2016;30:391–403. https://doi.org/10.1016/j.ccell.2016.06.02.