**Review**

# Immunotherapy in colon cancer: approaching to the future

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## Abstract

Colon cancer is still one of the most common neoplasias every year, although screening strategies have reduced its incidence. Unfortunately, the median survival in metastatic cases, not candidates to surgery, remains less than 3 years, far away from the expectations. A few years back, new agents were introduced in its armamentarium. Bevacizumab and cetuximab created hopes then. However, systemic options have not evolved as quickly as expected and fluorouracil-based chemotherapy is still the standard. Moreover patients stop responding at some point due to resistance to these agents and disease progression after two lines of treatment entails a short survival. However, some patients are still able to receive further treatment and thus, new agents are urgently needed. The appearance of immunotherapies has opened a hopeful new therapeutic approach. These treatments alter the immune system and although these have shown efficacy in multiple cancers, colon is not one of them. However, emerging data show that there are some patients who may benefit. This article will review published and ongoing clinical trials of immunotherapy in colon cancer.

**Keywords:** Colon cancer, pembrolizumab, microsatellite instability, nivolumab, programmed death 1, programmed death-ligand 1

## INTRODUCTION

Colon cancer is still a very common disease worldwide[1], (although the use of screening strategies has reduced its incidence)[2] and unfortunately, the median survival in metastatic cases, not candidates to surgery, remains still less than 3 years[3].

After the initial emotion with the adoption of new drugs, namely bevacizumab or cetuximab into the armamentarium of metastatic disease, systemic options have remained stationary and fluorouracil-based cytotoxic chemotherapy continues to be the standard. Moreover, patients stop responding to the therapy at some point, due to intrinsic or acquired resistance to these agents[4], and disease progression after two lines of treatment entails a short survival of about 4-6 months with best supportive care only[5].

However, some patients are still able to receive further treatment and the choice of an option will depend on previously used treatments, the patient thoughts and performance status and the tumour biology. In this scenario, strategic trials are relevant to ascertain the best therapeutic sequence but new agents are urgently needed. It is in this context when the appearance of immunotherapies has enabled a hopeful new therapeutic approach.

These novel agents were accepted immediately due to the good results seen with the use of immune checkpoint inhibitor drugs.

It is a fact that several gastrointestinal neoplasias showed positive outcomes but unfortunately colonic tumours are not one of them. However, emerging data show that there are some patients who may benefit from these agents.

This article will discuss the current situation of immunotherapy treatment in colon cancer by reviewing the ongoing and already published trials.

## MOLECULAR ALTERATIONS IN COLON CANCER: A KEY POINT TO KNOW

Colon cancer origins from a collection of genetic modifications, and most of them appear to originate from adenomas which evolve to carcinoma following a pathway of oncogene activation and loss of suppressor genes. *RAS* mutations have been detected in about 60% of adenomas smaller than 1 cm[6].

Wnt/B-catenin route seems to be deregulated due to inactivation of *APC* (a gene that abolish tumours) which is a crucial step but not sufficient to generate cancer and in fact to allow tumor progression other mutations are needed[7].

*RAS* mutations have been documented in more than half of these cancers and BRAF is present in about 5% to 10%[8,9] while Her-2amplifications have been detected in 2%-5% of them[10] but all of those play a role in this neoplasia progression and behaviour.

Genomic instability is a key factor in an early phase of tumorigenesis and this creates a tolerant environment that allows a cell to transform into a neoplastic cell[11]. There are two relevant classes of genomic instability: microsatellite instability or MSI and chromosome instability or CINMSI[12] which is present in 15% of the cases. This is due to a malfunctioning mismatch repair (MMR) system which is caused by *MMR* mutations or hypermethylation of mutL homologue 1 (the MLH1) promoter.

MSI which appears in > 90% of Lynch syndrome colonic tumours[13], stimulates the formation of tumours through promotion of gene alterations in crucial genes, i.e., transforming growth factor-beta (TGF-β) and BAX[13]. In sporadic MSI-positive colon cancers, hypermethylation of MLH1 promoter seems to be present[14] as well.

CIN is another form of genomic instability, which is in fact, the one that causes the majority of colon neoplasias[15] and seems to be produced by errors in the mitosis either in the process or in the apparatus, that will lead to mitotic failures[15].

This pathway shows alterations in chromosome numbers (aneuploidy) or heterozygosity damage and it is the result of faults in chromosomal segregation, DNA damage response, *etc*.

Karyotypic alterations and the collection of crucial genes mutations are needed for cancer to originate and to progress.

Chromosomal translocations, a transfer of genes between chromosomes, can promote neoplasias but some could also be targeted by new therapies. One recurrent translocation involves *TTC28* on chromosome 22 and it is known that when this TTC28 becomes inactive (this have been seen in around 22% of cases), its role as inhibitor of cancer growth is lost.

## MUTATIONAL LOAD

Genomic instability in colon cancer places this tumour on the average group of mutation load[16], however, some cases have a high rate of mutations. These are characterized by alterations in *MMR* genes leading to microsatellite instability high (MSI-H) which is present in only some of colon neoplasias as mentioned above, and its incidence reduces with more advanced stages. As such, it has been reported in 22%, 12% and 3% of stages II, III and IV[17].

Somatic mutations in the exonuclease of polymerase epsilon catalytic subunit (POLE) although not frequent in advanced colon cancers, have been reported in microsatellite stable (MSS) and hypermutated tumours.

These change the function of POLE at DNA replication leading to wrong bases introduction although more needs to be done to know the biological implications[18] of these changes. The key point is that tumours with high load of mutations are important for immunotherapy.

## CONSENSUS MOLECULAR SUBTYPES IN COLON CANCERS

As described, colon neoplasia is a very heterogeneous disease with different molecular mistakes leading to the classification in four molecular subtypes [consensus molecular subtypes (CMS)] with different patient outcomes.

CMS1 is present in 14% of the cases and it shows strong immune activation. Tumours are hypermutated, with *BRAF* mutations, MSI and CpG island methylator phenotype (CIMP)[19].

CMS2, in 37% of the cases is a subtype that shows *APC* mutations and a significant activation of MYC and Wnt signalling[19].

CMS3, in 13%, shows CIMP low, metabolic dysregulation and *KRAS* mutations[19].

And CMS4, in 23%, mesenchymal, with transforming growth factor-β activation, angiogenesis and invasion of the stroma and it has also been correlated with worse overall survival (OS)[19].

Finally a mixed group, which is present in 13% of the cases and represents a transition phenotype or just intratumoral heterogeneity[19].

CMS1 tumours express specific genes to cytotoxic lymphocytes making them potentially susceptible to immune checkpoint inhibition.

CMS4 shows lymphocytic and monocytic markers with an immunosuppressive and inflammatory profile with a significant infiltration of fibroblasts. These tumours will need a treatment targeting monocytes and cytokines.

Finally, CMS2 and 3 show low inflammatory and immune signatures making them "cold" tumours and as such will need an immune stimulus (i.e., radiotherapy, *etc.*) as part of the treatment.

## IMMUNOTHERAPY IN COLON CANCER

### PD-1

Recently, cancer immunotherapy has emerged as a relevant treatment in oncology as significant clinical benefits have been obtained by inhibiting the programmed death 1 (PD-1) receptors or its ligands (PD-L1 or PD-L2) in several types of cancer[20-22].

In fact, the expression of PD-1 on the tumour or T, B and natural killer cells constitutes a predictive marker of benefit to PD-1 inhibitors.

PD-1 exerts an inhibitory activity by joining to PD-L1 and PD-L2 and as such, it blocks apoptosis of the tumour cell and promotes peripheral T-effector cell consumption and transformation to regulatory T (Treg) cells[23].

Following the success in the treatment of other neoplasias, different trials have been carried out in cancers such as colon.

### Immune check-point inhibitors

As already mentioned, those metastatic colon cancers showing MSI-H, are associated with a high mutational burden and immune cell invasion, making them ideal for immune checkpoint inhibitors [Table 1].

Two clinical trials have suggested the activity of these drugs for these tumours, leading to the National Comprehensive Cancer Center Network (NCCN) to recommend nivolumab and pembrolizumab in second and third lines[24].

A phase II clinical trial, KEYNOTE-164 tested pembrolizumab (MK-3475) in second or third line metastatic colon cancers with or without MMR deficiency[25]. Pembrolizumab was given intravenously (iv) at 10 mg/kg every two weeks and patients were divided into 3 groups: colon MMR-deficient ($n$ = 11), colon MMR-proficient ($n$ = 21), MMR-deficient non-colon ($n$ = 9). The primary end-points were the immune-related objective response rate (ORR) and immune-related progression-free survival (PFS) at twenty weeks.

This trial showed ORR of 40% and PFS of 78% for colon MMR-deficient while 0% and 11% for proficient while the OS was not reached for colon MMR-deficient. PFS and OS were reported as 2.2 and 5.0 months for colon MMR-proficient and a post hoc comparison of colon groups MMR-deficient and proficient documented a hazard ratio (HR) for progression or death of 0.10 ($P$ < 0.001), with a HR for death of 0.22 ($P$ = 0.05).

Non-colon cancers MMR-deficient showed similar results to colon MMR-deficient with ORR of 71% and PFS of 67%. This trial documented a longer PFS linked with high somatic mutation ($P$ = 0.02) and longer OS ($P$ = 0.02) and interestingly, the sequencing of the whole-exome reported 1782 somatic mutations in MMR-deficient cases and only 73 in MMR-proficient ($P$ = 0.007).

Authors concluded that this study confirmed that MMR-deficient profile predicts benefit from pembrolizumab.

Most frequent side-effects were manageable with fatigue (32%), rash or pruritus (24%), diarrhea (24%), abdominal pain (24%), constipation (20%), anemia/lymphopenia (20%), headache (17%), arthralgia (17%),

**Table 1. Immunotherapy trials**

| Agents | Trial | Phase | Line | MMR | RR | PFS/OS | References |
|---|---|---|---|---|---|---|---|
| Pembrolizumab | KEYNOTE-164 | II | 2nd-3rd line | Def/prof | In MSI-H colon cancer | | [25] |
| Pembrolizumab | KEYNOTE-158 | II | 2nd or further lines | Def | RR 27.9% 6 m PFS 43% mOS not reached | | [27] |
| Pembrolizumab | KEYNOTE-177 | III | 1st line | Def | Ongoing Not recruiting | | [32] |
| Nivolumab/ipilimumab | CHECKMATE-142 | | 2nd-3rd line | Def/prof | MSI-H show RR 31.1% OS one year 85% | | [28] |
| BMS936559 (MDX 1105) | | I/II | | | 0% | | [34] |
| Atezolizumab/FOLFOX/Bevacizumab | COMMITT (NCT02997228) | III | | Def | Recruiting | | |
| Atezolizumab/adjuvant FOLFOX | NCT02912559 | III | | Def | Recruiting | | [36] |
| Atezolizumab/bevacizumab/FOLFOX | NCT01633970 | Ib | Refractory | | RR 8% with Beva 36% with FOLFOX/Beva | | [37] |
| Atezolizumab | NCT01633970 | Ib | | Def | RR 30% | | [38] |
| Atezolizumab/cobimetinib | | Ib | | Def/prof | RR 17% | | [39] |
| Atezolizumab/cobimetinib combo *vs.* atezolizumab vx regorafenib | IMblaze370 NCT02788279 | III | | | Data to be released | | [37] |
| epacadostat /azacitidine/ pembrolizumab | ECHO-206 NCT03182894 | | | | Active but not recruiting | | |
| Pexidartinib/durvalumab | NCT02777710 | I | | | Recruiting | | |
| Pembrolizumab/cetuximab | NCT02713373 | I/II | | | Recruiting | | [43] |

MMR: malfunctioning mismatch repair; RR: response rate; PFS: progression-free survival; OS: overall survival; MSI-H: microsatellite instability high

pancreatitis (15%) and dyspnea (15%), hypothyroidism/thyroiditis (10%) as the most frequent. Grade 3/4 adverse events described were lymphopenia (20%), anemia (17%), hypoalbuminemia (10%), hyponatremia (7%) and diarrhea (5%).

Pembrolizumab is a humanized IgG4 monoclonal antibody directed against PD-1 receptors which had been tested initially in a phase I study including 32 cases with advanced tumours, three of those with metastatic colon cancer. Unfortunately all these patients showed early progression and discontinued the treatment[26].

KEYNOTE158 is a phase II trial testing pembrolizumab in MSI-H non-colon cancer patients with ≥ 1 previous treatment and the primary end-point was ORR[27]. KEYNOTE164 enrolled 61 MSI-H colon cancers and KEYNOTE158 included 77 patients with MSI-H non- (> 50% with ≥ 2 previous treatments), such as endometrial, other gastrointestinal cancers, mesothelioma and small cell lung among others. Results showed an ORR of 27.9% (95% CI 17.1%-40.8%) in MSI-H colon cancers and 37.7% (95% CI 26.9%-49.4%) in MSI-H non-colon cancers, OS was not reached and at the OS at 6 months was 87% and 73% for MSI-H colon and non-colon respectively. Duration of response was not reached either and all responses were continuing, same as survival and safety.

Other trials have tested the efficacy of nivolumab as monotherapy or in combination such as the CHECKMATE-142 which used nivolumab +/- ipilimumab, as 2nd or 3rd line for stage IV colon cancer regardless MS status and established the ORR as the primary endpoint. The interim findings were discussed at ASCO Annual Meeting 2016[28].

Patients received nivolumab 3 mg/kg iv every 14 days or nivolumab 3 mg/kg + ipilimumab 1 mg/kg iv every 3 weeks x 4 doses, then, patients continued on nivolumab until disease progression.

The study recruited 59 MSI-H patients and 23 non-MSI-H and showed favourable results in those patients dMMR in comparison to pMMR.

Another update in the monotherapy arm was discussed at ASCO Gastrointestinal Cancers Symposium 2017 as the combination arm was still recruiting[29] and the results showed that 74 patients MSI-H who were treated with single-agent nivolumab had ORR of 31.1% and DCR of 68.9%. PFS at one year was 48.9% with OS rate of 73.8%. The OS median had not been reached yet.

Most patients (> 50%) had the treatment after 3 previous lines of chemotherapy and therate of *BRAF* and *KRAS* mutations was 16% and 35%, respectively.

Around 30% had PD-L1 positive tumours and Lynch syndrome was present in around 30%.

ORR was documented without any relation to PD-L1 status or the *BRAF/KRAS* mutational status. Lynch syndrome did not impact either.

This trial showed similar adverse events to those documented in previous immunotherapy trials without any treatment-related deaths and grade 3-4 side-effects were documented in around 20% of the cases. Diarrhoea and fatigue in the monotherapy arm (around 30% each in nivolumab alone) and diarrhea (around 45%) in the combination arm were the most frequently reported side-effects.

These results led to NCCN guidelines (1.2017) to adopt pembrolizumab and nivolumab as potential options for advanced MSI-H colon cancer after the Food and Drug Administration (FDA) approved both for the treatment of metastatic colon cancer MSI-H that has progressed after previous treatment. Earlier, in 2015, pembrolizumab had been granted breakthrough therapy designation for MSI-H colon cancer.

In July 2018 the FDA has approved the combination of nivolumab and ipilimumab for the treatment of metastatic colon cancer MSI-H that has failed in previous treatments.

Confirmatory trials are still ongoing, such as the already mentioned KEYNOTE-164 (NCT02460198) although it is not recruiting and it is expected to be complete in one year.

Nivolumab is another monoclonal antibody directed against PD-1 that was initially assessed in a phase I study of non-haematological cancers[30]. Thirty-nine patients were recruited, 14 of them with metastatic colon cancer and one showed a durable complete response off-treatment. This patient had a dMMR tumour which was considered a predictive factor of benefit, the same as positivity for PD-L1 on the cancer cell surface.

Topalian *et al.*[31] carried out a phase II study of nivolumab in 296 patients with several solid neoplasias and unfortunately no responses were reported on colon cancer patients. KEYNOTE-177 is a phase III study, international, open-label, conceived to assess the efficacy and safety of pembrolizumab in comparison to standard-of-care (SOC) treatment in first-line regardless MMR status in colon cancer(NCT02563002)[32].

Two hundred and seventy cases will be recruited and will receive chemotherapy at the investigator's decision (mFOLFOX6, FOLFIRI either alone or combined with bevacizumab or cetuximab) *vs.* pembrolizumab, although crossover is allowed. The primary endpoint is PFS while OS and ORR will be secondary endpoints and the treatment will be maintained until progressive disease (PD) is documented or significant toxicity, patient/investigator's choice, or 35 cycles are completed (pembrolizumab only).

The trial is active but not recruiting.

MSI-H colon cancers are significantly infiltrated by lymphocytes and show a significant expression of PD-1 and PD-L1[33], and the pembrolizumab inhibits the connection PD-1-PD-L1 and PD-1-PD-L2, allowing

the activation of an antineoplastic immune attack. KEYNOTE-016 is a phase II trial that showed with pembrolizumab an ORR of 40% in MSI-H stage IV colon cancers treatment refractory *vs.* 0% in MMR-proficient.

**Programmed death-ligand 1 inhibition**
BMS936559 (MDX 1105), an anti Programmed death-ligand 1 (PD-L1) monoclonal antibody has been evaluated in a phase I/II trial with > 200 cases, including several non-haematological tumours. In this study, iv anti-PD-L1 antibody was given to patients (escalating doses from 0.3 to 10 mg per kilogram of weight) every 2 weeks in 6-week cycles for up to 16 or until a complete response is confirmed or disease progression whichever was documented first. Eighteen of these patients had colon cancer and the ORR reported was 17% but none was seen in colon cancer patients[34].

Atezolizumab is an anti PD-L1 that blocks PD1 and B7.1 to stimulate T-cell priming and all suppressed immune cells and its anticancer activity as monotherapy has been reported, although the RR in MSS colon cancer is not encouraging[35].

Another study called COMMITT (NCT02997228) will assess atezolizumab in 439 untreated cases with stage IV colon cancer MSI-H/dMMR. They will be randomized to FOLFOX/bevacizumab, atezolizumab monotherapy or atezolizumab/FOLFOX/bevacizumab. The primary endpoint is PFS and other endpoints are OS, ORR, duration of response, DCR and QOL. This study is recruiting patients.

A phase III study will test if the combination of atezolizumab with adjuvant FOLFOX can improve patient disease-free survival *vs.* FOLFOX alone in stage III dMMR or MSI colon cancers (NCT02912559).

By inhibiting PD-1/PD-L1 connection, atezolizumab may activate T cells, thereby, repairing the capacity to find and destroy malignant cells.

Limited results suggest that FOLFOX may increase intratumoral cytotoxic CD8+ T cells that may serve as "immune priming".

Patients with curatively resected stage III colon cancers dMMR will be randomized to modified FOLFOX6 for 6 months (12 cycles) alone or combined with atezolizumab (840 mg iv every two weeks) continued as monotherapy for 6 months (total duration of 12 months). Patients will be stratified by T, N stage and tumour sidedness and the local testing for MSI or MMR proteins is permitted. Atezolizumab should start with cycle 2 or before. This study will be performed by the Alliance for Clinical Trials in Oncology and is planning to recruit around 700 patients[36]. A multicenter phase Ib trial was carried out to test atezolizumab with bevacizumab in 14 refractory metastatic colon cancer and with bevacizumab and FOLFOX in 30 cases oxaliplatin-naive metastatic colon cancer[37].

The dose of atezolizumab was 20 mg/kg every 3 weeks and bevacizumab 15 mg/kg every 3 weeks in the first group and atezolizumab 14 mg/kg every 2 weeks, bevacizumab 10 mg/kg every 2 weeks and FOLFOX at standard doses in the second group.

The study showed an ORR of 8% in combination with bevacizumab and 36% in combination with chemotherapy in patients who had received previous therapies, whereas an ORR of 44% was documented in treatment naïve cases.

Bevacizumab is an anti-VEGF-A antibody with reported clinical activity in metastatic colon cancer. In preclinical studies, it has shown an enhanced T-cell infiltration in tumours. It has been suggested that by

combining atezolizumab and bevacizumab, the anti-tumour immune responses would increase and so would the clinical benefit.

A phase Ib study (NCT01633970) tested combinations with atezolizumab and several chemotherapeutic/ biologic regimens in advanced solid neoplasias (also metastatic colon cancer).

Atezolizumab was given at 1200 mg plus bevacizumab 15 mg/kg both every 3 weeks. Safety was the primary endpoint and efficacy parameters were secondary endpoints.

Ten metastatic colon cancers MSI-H were recruited. The study showed an ORR of 30% (95% CI, 6.7%-65.3%), OS was not reached after 11 months and 40% of patients had a G3/4 adverse event (proteinuria was the most frequent side-effect documented). The disease control rate was 90% and the authors concluded that this treatment did not have any unexpected side-effects[38] and was given with good tolerance.

Active research is still needed for patients with MSI-L or MSS.

## COMBINATION THERAPIES

### MEK inhibitors
In preclinical studies, targeted blocking of MEK will upregulate MHC I on malignant cells, stimulate intraneoplasticT-cell infiltration and stimulates anti-PDL1.

A phase Ib clinical trial of cobimetinib combined with atezolizumab in colon cancer was presented at the 2016 ASCO Annual Meeting[39].

Cobimetinib dose ranged from 20 to 60 mg daily (3 out of 4 weeks) and atezolizumab was given at 800 mg iv every 2 weeks.

Twenty-three colon cancers were included and only one of them was wild type KRAS. The ORR was 17% (4 partial responses and 5 stable diseases), OS at 6 months was 72% and these results led to an extension of the trial.

Three responses lasted long, and are still present at the time of the presentation of these results; one of those was reported in MMR status unknown but three were seen in pMMR and these were not related to the positivity of PD-L1.

A serial biopsy cohort demonstrated upregulation in PD-L1 activity, CD8 T-cell infiltration and expression of MHC I.

The most frequent adverse events were diarrhea (in almost 70%), fatigue (around 50%), rash either acneiform or maculopapular, pruritus and nausea. G3-4 side-effects were seen in 34.8%.

The authors concluded that MSS colon cancer can respond to the combination of cobimetininb and atezolizumab[39].

### Indoleamine 2,3-dioxygenase inhibitors
Different checkpoint inhibitors and immunomodulatory agents have been and continue to be tested in phase I research. Drugs blocking repressing immune factors, for example the enzyme indoleamine 2,3-dioxygenase (IDO) or LAG-3 are evaluated in phase I trials in combination with check point inhibitors. IDO is an intracellular enzyme in charge of degradation of tryptophan whose expression increases by the stimulation

of IFN gamma in several neoplasias and this produces a reduction in immune response through a reduction in the levels of tryptophan in the neoplasia and lymph nodes. Finally this creates immunotolerance through inactivity of T cells and dendritic cells (DCs).

Basic studies have considered that blocking IDO delays tumour progression, stimulates DC vaccines and shows synergism with chemotherapy.

The d-1-methyl-tryptophan (d-1-MT) is an IDO inhibitor which has been chosen for phase I trials.

IDO is upregulated during inflammation and this transforms the microenvironment in immunosuppressive. In colon cancer patients, IDO is linked to reduced CD3+ infiltrating T cells and shorter prognosis[40].

Epacadostat is an IDO inhibitor which has been recently tested in advanced solid tumours. Fifty-two patients received epacadostat with escalating dosages and continued on this drug until progression or significant side-effects was confirmed. Dose-limiting toxicity (DLT) was detected with 300 mg twice daily (pneumonitis post radiotherapy) and with 400 mg twice daily (asthenia). Main toxicities were fatigue, gastrointestinal side effects, abdominal or back pain, cough, and breathlessness. On this treatment, seven patients showed long stable disease lasting ≥ 16 weeks.

Authors concluded that this drug is generally well tolerated and has caused the maximal blockage of IDO if administered at ≥ 100 mg twice daily and threre are some ongoing trials evaluating epacadostat with different immunomodulatory agents[41]. Multiple other trials are ongoing such as ECHO-206 which combines epacadostat with azacitidine and pembrolizumab in cases diagnosed with advanced solid neoplasias (NCT03182894). This trial is active but not recruiting.

**Colony-stimulating factor-1 receptor inhibitors**
The macrophage colony-stimulating factor 1 receptor (CSF-1R) is a target for CSF-1 and seems to be in charge of the macrophages' function. Preclinical studies suggested that blocking CSF-1/CSF-1R shifts monocytes population from promoters to tumour suppressors[42].

Pexidartinib is a CSF-1R inhibitor which is under study combined with durvalumab in a phase I trial which includes colon and pancreatic cancer patients (NCT02777710). This trial is recruiting patients.

**Anti-EGFR agents**
Pembrolizumab is also under study in combination with cetuximab (NCT02713373) as this one has demonstrated in preclinical studies, to stimulate cellular cytotoxicity and to produce EGFR-specific T-cell response and antigen dissemination in head and neck cancers[43]. In metastatic colon cancers treated with different chemotherapies, those who receive anti-EGFR treatments show significant intratumoral T-cell infiltrates. The primary end points for this trial are ORR and PFS[44].

Cetuximab is a chimeric IgG1 anti-EGFR monoclonal antibody. The Phase Ib/II study combining pembrolizumab and cetuximab tested the safety and efficacy of this combination in metastatic colon cancer. Patients with RAS wild type metastatic colon cancer after progression on one previous line of treatment were included and received pembrolizumab every 3 weeks at 200 mg and cetuximab at 400 mg/m$^2$ loading dose, followed by 250 mg/m$^2$ weekly. Nine patients recruited onto the phase Ib did not show any dose limiting toxicities and most frequent side-effects were acneiform rash, xerosis, hypomagnesemia, vomiting and fatigue. Grade 3 and 4 toxicities were not frequent so authors concluded that this combination was well tolerated and currently the phase II is running and will recruit 33 patients to test the efficacy of this regimen

in terms of ORR and PFS at 6 months.

## LYMPHOCYTE-ACTIVATION GENE 3 (CD223)

Cases of MSI-H show shorter prognosis if they are found to be positive for lymphocyte-activation gene 3 (LAG-3)[4]. pMMR liver metastatic colon cancer seems to show higher sensitivity to checkpoint inhibitors than proficient original cancers. LAG3 and PD1 regulate autoimmunity and if blocked at the same time, a stronger anti-tumour immune response is seen. Sometimes this event produces autoimmunity[45]. Zhou *et al.*[46] have reported that blocking LAG3 stimulates tumour-infiltrating T-cell responses of these patients and therefore this could be a new target for liver metastatic colon cancer.

## THERAPEUTIC VACCINES

Cancer vaccination has been used in several cancers to obtain an anti-cancer immune response, remove the cancer and provide maintained surveillance to protect against a recurrence or progression. There are different vaccines used in colon cancer such as autologous, peptide, viral vector and DC [Table 2].

### Autologous vaccines

These use malignant cells taken directly from the patient's cancer. Whole tumour cell vaccines have shown reduced clinical activity as most antigens are present in normal cells and the generated immune response is not specific to cancer cells[47].

A randomized phase III trial with a patient-specific vaccine using autologous cancer cells combined with BCG vaccine[48] was carried out. Patients were randomized to two arms, surgical resection plus the vaccine *vs.* resection only. DFS and OS were not significantly different at 7 years[48] but longer follow-up did show statistically significant benefits in all endpoints including DFS and OS but only in stage II[49].

There are other ways to increase the immunogenicity of autologous vaccines by using autologous tumour cell vaccine modified by a non-lytic, low pathogenic strain of the Newcastle disease virus (NDV).

A phase II trial recruited 23 patients to receive tumour cells incubated with NDV. The results showed a reduction in recurrence rate of 61% compared to 87% in a historical matched control group[50]. A phase III trial randomized patients with stage IV colon or rectal cancer to receive either NDV-infected autologous tumour cell vaccine group or to control group[51] and did not show any differences in OS, however, a subgroup analysis demonstrated a significant improvement in colon cancer compared to rectal cancer. Autologous vaccines have not changed significantly the clinical practice due to low activity, however, some evidence could support them in colon cancer but not in rectal cancer.

### DC vaccines

DCs are powerful antigen presenting cells that play an important role in promoting immune responses[52]. These cells will stimulate naïve T lymphocytes responses and also memory T cells responses and they are key controllers of anticancer responses by releasing co-stimulatory signals and producing cytokines. DC based vaccines have been under investigation for long time[53]. These have been prepared by harvesting DCs from patients, loading them *ex vivo* with neoplastic antigens, tumour RNA or whole malignant cells and once these are activated, the DC vaccine is re-infused into the patient with the aim getting a tumour specific immune response[53].

Some of these vaccines have used CEA as tumour antigen, as this is present in the majority of colon cancers. Early evidence has shown that these vaccines are safe and effective in promoting a specific tumour response[54] but unfortunately there are not phase III trials to support this. A recent phase II study has compared autologous tumour lysate DC vaccines with best supportive care only[55] and reported that this

**Table 2. Trials with therapeutic vaccines**

| Vaccines | Phase | Results | References |
|---|---|---|---|
| Autologous cancer cells combined with BCG +/- resection | III | No differences at 7 years<br>Longer follow up showed benefit in stage II only | [48] |
| Tumour cells incubated with NDV | II | Reduction in recurrence rate of 61% *vs*. 87% in historical | [50] |
| NDV-infected autologous tumour cell vaccine group or control group | III | No differences in OS<br>But significant improvement in colon compared to rectal cancer | [51] |
| Autologous tumour lysate DC vaccines *vs*. BSC | II | No benefits in PFS/OS | [55] |
| DC vaccine based on Wilms' tumour (WT1) class I/II peptides | I | OS prolongation | [56] |
| Five peptides vaccine/chemotherapy | II | No benefits in RR/PFS/OS | [61] |
| Seven peptides vaccine/chemotherapy | | > OS | [62] |
| Fowlpox-CEA(6D)- TRICOM alone and sequentially with vaccinia-CEA(6D)- TRICOM with/without GM-CSF in CEA cancers | I | Safe<br>SD as best response<br>Duration response > 4 m | [64] |
| Chemotherapy/vaccine canarypox virus (ALVAC) expressing CEA and B7-1 (ALVACCEA/B7-1 | II | RR 40%<br>But no significant differences | [65] |
| Genetically engineered oncolytic herpes simplex virus (NV1020)/chemotherapy | I/II | TTP 6.4 m<br>OS 11.8 m<br>OS at 1 year 47.2% | [70] |
| Pexa-Vec oncolytic virus/tremelimumab NCT03206073 | Ib/II | | |

NDV: Newcastle disease virus; OS: overall survival; GM-CSF: granulocyte macrophage colony-stimulating factor; PFS: progression-free survival; RR: response rate; DC: dendritic cell; BSC: best supportive care; TTP: time to progression

vaccine was able to stimulate a tumour specific immune response, but no benefits were seen in terms of PFS (2.7 *vs*. 2.3 months, $P = 0.628$) and OS (6.2 months *vs*. 4.7 months, $P = 0.41$).

A phase I trial assessed a DC vaccine based on Wilms' tumour (WT1) class I/II peptides in colon cancer patients[56] and showed benefit in relation to WT1 expression by immunohistochemistry in tumoral tissue and the immunity continued for two years and this was associated with a survival prolongation.

**Peptide vaccines**
Peptide vaccines can stimulate T cells responses against tumour specific antigens and they could stimulate tumour specific immune response as well[57].

In colon cancer there are several tumour associated antigens that have been used in peptide vaccines. CEA[58], EGFR[59], mucin 1 [60], among others.

These vaccines have not demonstrated any benefits in survival and some research have been performed to create vaccines agains several antigens with longer aminoacids. A phase II trial including 96 metastatic colon cancer patients showed that a peptide vaccine with five peptides is safe if given with chemotherapy[61]. but did not show any advantages in response rate, PFS and OS.

Another trial demonstrated that a 7 peptide vaccine combined with chemotherapy in metastatic colon cancer produces better OS[62].

**Viral vector vaccines**
The pathogenicity of a virus can be used to generate a tumor-specific immune response. Viral vectors can be engineered to express any antigen[63] and these vaccines are very efficient at producing tumour response compared to peptide vaccines. The viruses used are adenoviruses, lentiviruses, poxviruses and retroviruses.

A phase I trial tested sequential vaccinations with fowlpox-CEA(6D)- TRICOM alone and sequentially with

vaccinia-CEA(6D)- TRICOM, with and without granulocyte macrophage colony-stimulating factor (GM-CSF) in patients with CEA-expressing cancers[64]. This study showed that this was safe but had limited efficacy in selecting patients with stable disease and the duration of the response was of at least 4 months. Another trial, phase II, assessed the efficacy of chemotherapy combined with a vaccine with canarypox virus (ALVAC) expressing CEA and B7-1 (ALVACCEA/B7-1)[65].

Although 50% of the patients had anti-CEA-specific T cell responses and 40% of the patients showed objective clinical response, no differences were detected between the two groups.

TroVax is a highly attenuated strain of vaccinia virus encoding the 5T4 protein (an oncofetal antigen and a transmembrane glycoprotein highly expressed in colon cancer) that has become a good target[66]. Small clinical trials have shown that TroVax is active in metastatic colon cancer because it can lead to antibody formation against the 5T4 antigen and the virus as well[66].

**Oncolytic viral therapy**
This technique uses a virus as an anticancer treatment which is able to destroy malignant cells without hurting healthy tissue. The phase III OPTIM trial used a GM-CSF expressing variant of herpes simplex virus 1 (HSV-1) and showed superior OS with an acceptable toxicity profile for unresected melanoma when compared to to subcutaneous GM-CSF[67]. This led to its approval but unfortunately for colon cancer there are not available treatments yet[68]. Pre-clinical research has shown that G207, herpes simplex virus type-1 with multiple mutations, is effective in five different colon cancer cells lines[69].

A multicenter phase I/II trial showed that four doses of a genetically engineered oncolytic herpes simplex virus (NV1020) in liver-dominant metastatic colon cancer disease[70], through hepatic artery infusion, followed by standard chemotherapy was well tolerated and achieved a median TTP of 6.4 months, OS 11.8 months and 47.2% were alive in one year.

Another trial, phase 1b assessed Pexa-Vec, an oncolytic virus used to treat refractory colon cancer patients and showed stable disease in 67%. Unfortunately, this trial could not determine the maximum tolerated dose of Pexa-Vec. Another phase I/II trial is testing Pexa-Vec oncolytic virus in combination with tremelimumab in refractory colon cancer. This trial is still recruiting patients.

**Other check points**
There are other checkpoints targeted in early studies such as LAG3, OX40 and TIM3. The inhibition of OX40 has shown good results in preclinical research[71] and there is currently a trial recruiting patients with metastatic colon cancer to be treated with an anti-OX40 (NCT02559024).

LAG3 is expressed on regulatory T cells and agents blocking LAG3 and PD-1 have shown an improvement in survival in a MC38 colon cancer mouse model[72].

**Adoptive cell therapy**
Adoptive cell therapy uses *ex vivo* expanded tumor-infiltrating lymphocytes (TIL) against tumour antigens and this strategy has shown good responses in melanomas. Most of *KRAS* mutations occur at codon 12 where glycin is changed to aspartic acid, called KRAS G12D and is present in 13% of colon cancers[73]. In 2016 Vaughn *et al.*[73] published a case showing tumour regression with the infusion of cytototoxic T cells against the KRAS G12D.

**Targeted chimeric antigen receptor T cells**
This is an innovative therapy that uses engineered T cells expressing a chimeric molecule formed by the

antigen-recognition domain of an antibody and intracellular signaling domains of T cell receptor which will recognize a tumor antigen, and the T cells will activate and change to cytolytic.

For this therapy one of the key points is the production of antibodies to bind to the tumours but not to healthy tissues, and also to select the antigens. The tumour associated form of MUC1[74] seems to be a relevant antigen and MUC1-CAR-T cell treatment has shown benefits but needs further evaluation[74]. A trial assessing CEA-redirected-CAR-T cells in CEA transgenic mice demonstrated regression of CEA positive cancers as primary treatment and also a good recall response after the second challenge with these tumours[75]. A CEA-T cell receptor therapy assessed in a small study showed objective response in metastatic colon cancer in three patients[76].

A phase I/II study is assessing an anti-MUC1 CAR-T cell therapy in patients with a MUC1+ tumor and includes patients with colon cancer amonth othes (NCT02617134). This trial is recruiting patients at this time.

## CONCLUSION

Immunotherapy is a breakthrough in cancer therapy and several agents are already approved to be used routinely in clinical practice, however, much remains to be done to be able to select the optimal population to achieve maximum responses.

A group of colon cancer patients, those with MSI-H, seems to be the target for immunotherapy as the highest responses to PD-1/PD-L1 inhibition are shown in highly mutated tumours.

Moreover, the identification of tumours significantly infiltrated by cytotoxic T-cells, is a predictive factor for benefit from PD-1/PD-L1 inhibitors.

Although all these data are promising, we need to remind that only 30% of MSI-H patients, show a benefit with immunotherapy and thus research should continue to find other options to maximise the benefit.

Here is where the treatments that stimulate antigen presentation and boost T-cell priming (i.e. chemotherapy, radiation and monoclonal antibodies) can help transform a non-immunogenic tumour to an immunogenic one.

Perhaps combining immunotherapies between them or with other drugs could increase the benefits without increasing the side-effects significantly.

Unfortunately, no definite agent has been found yet for MSS and non-hypermutated tumours. However, MEK inhibitors combined with anti-PD-L1 are under investigation with some positive results so far.

It seems that we are now advancing at good speed in the understanding of immunotherapy and great optimism should remain.

## DECLARATIONS

**Authors' contributions**
The author contributed solely to the article.

**Availability of data and materials**
The data were strictly obtained from medical literature according to the privacy policy and ethics code of our institute.

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The author declared that there are no conflicts of interest.

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Not applicable.

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