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Immunotherapy for colorectal cancer

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ABSTRACT

Progress in understanding complex interactions between cancer cells and the immune system has led to the development of new methods of treatment — immunotherapy, modulating the anti-cancer response of the immune system. For several years, colorectal cancer (CRC) was thought to be a cancer with low immune stimulation potential, but in recent years the favorable prognostic value of lymphocytic infiltrates in the tumor has been noted. Currently it is well known that the stimulation of the immune system by CRC cells is associated with the accumulation of mutations in DNA microsatellites. This phenomenon results from impairment of function of genes (mainly MLH1, MSH2, MSH6 and PMS2) encoding proteins involved in correction of mismatched nucleotides during replication (dMMR), whose phenotypic reflection is microsatellite instability (MSI). It affects about 15–20% of CRC, with clear differences depending on the stage of cancer — about 20% in stage II, 12% in stage III, and only around 4% in stage IV. dMMR/MSI cancers are highly immunogenic through overexpression of tumor antigens and can induce a deep immune response. Cancers with intact repair gene system (pMMR) and stable microsatellites (MSS) show poor immunogenicity, which makes it difficult to induce an anti-tumor immune response. The relationship between impairment of the mismatch repair system and the induction of an anti-cancer immune response justifies the use of checkpoint inhibitors of this response in the treatment of patients with CRC MSI/dMMR. In MSS/pMMR cancers, checkpoint inhibitors used in monotherapy are not effective. However, studies are underway to combine these drugs with other methods of systemic treatment (chemotherapy, EGFR inhibitors, angiogenesis inhibitors, MET inhibitors), as well as radiotherapy.

Key words: colorectal cancer, immunotherapy, microsatellite instability, MSI/dMMR, microsatellite stable colorectal cancers, MSS/pMMR

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the world and the second leading cause of cancer-related deaths. There were 1.9 million new cases of CRC and over 900 000 deaths considered CRC-related in 2020 worldwide [1].

Overall mortality from CRC is slightly decreasing, but survival in advanced disease remains unsatisfactory. Median overall survival (OS) in patients with metastatic CRC does not exceed 3 years [2]. For this reason, new, more effective methods of treatment are constantly being sought.

For decades, chemotherapy based on 5-fluorouracil (5FU) has been the mainstay for CRC patients. At the end of the 20th century, irinotecan and oxaliplatin were introduced, allowing for doubling median OS [3]. Further improvement was achieved in the 2000s with the use of monoclonal antibodies inhibiting proliferation and angiogenesis. The first group includes

cetuximab and panitumumab — antibodies directed against the epidermal growth factor receptor (EGFR), registered for the treatment of CRC patients without mutations in the *KRAS* and *NRAS* genes. Angiogenesis inhibitors include bevacizumab [an antibody that binds to the vascular endothelial growth factor receptor (VEGFR) ligand], aflibercept (a recombinant fusion protein consisting of fragments of the extracellular domains of VEGF receptors 1 and 2), and ramucirumab (an antibody directed against VEGFR) [4]. A slight prolongation of median OS can also be achieved by using regorafenib (a small molecule inhibitor of angiogenesis signaling pathway) or trifluridine/tipiracil (a combination of an anticancer thymidine nucleoside analog and a thymidine phosphorylase inhibitor) in subsequent lines [5, 6]. In recent years, a combined molecularly targeted treatment for CRC patients with the *BRAF*^{V600E} mutation — cetuximab and encorafenib (a small molecule inhibiting BRAF kinase) has also been registered. Improved survival is probably also observed, to some extent, due to the introduction of a multi-specialist approach to the treatment of advanced disease, as well as improved supportive care.

Over the past decade or so, accumulation of knowledge about complex interactions between cancer cells and the immune system has led to the development of new methods of treatment — immunotherapy, which modulates the anticancer response of the immune system. Introduction of immune checkpoint inhibitors, such as monoclonal antibodies against cytotoxic T cell antigen 4 (CTLA-4 — ipilimumab, tremelimumab), programmed death receptor 1 (PD-1 — nivolumab, pembrolizumab, dostarlimab), and its ligand (PD-L1 — atezolizumab, durvalumab, avelumab) has significantly changed the treatment landscape for various cancers, including melanoma, lung, head and neck, kidney, bladder, and Merkel cell carcinoma. Immunotherapy has also been used in the treatment of CRC patients, but so far, its effectiveness has been confirmed only in the group of patients with deficient DNA mismatch repair (dMMR) and the phenotypic reflection of this disorder, e.g. microsatellite instability (MSI). In the remaining population of CRC patients, the value of such treatment has not been demonstrated [7].

The importance of the immune system in colorectal cancer

One of the most important factors modulating the tumor microenvironment, apart from somatic mutations and epigenetic regulation of gene expression, is the interaction of cancer cells with immune cells. The immune system is a set of innate and adaptive regulatory mechanisms that modulate immune activity by promoting tolerance to self-antigens and triggering reactions against foreign antigens, including cancer. As a tumor develops, the ability of the host's immune system to recognize tumor antigens and destroy cancer cells gradually decreases. Cancer cells demonstrate many mechanisms to escape immune surveillance (e.g. secretion of cytokines promoting regulatory T cells and myeloid-derived suppressor cells to inhibit CD4+ and CD8+ cytotoxic T cells, loss of normal MHC class expression, making them invisible to T cells, and finally increasing expression of immune checkpoint proteins — PD-1 or PD-L1, which results in T cells exhaustion). In order to reverse these unfavorable mechanisms, various strategies are used to increase the ability of the immune system to recognize and destroy cancer cells [8, 9]. The above-mentioned immune checkpoint inhibitors are already widely used, and further strategies are still in various phases of clinical trials [10].

For several years, CRC was thought to be a low-level immune-interfering cancer. Recently, however, many studies have reported the favorable prognostic significance of tumor-infiltrating lymphocytes [11]. In addition, a large variation of the immune activity in different CRC molecular subtypes was observed. The CMS1 subtype (immunogenic, approx. 14% of cases) and CMS4 (mesenchymal, approx. 23% of cases) are immunologically active, "hot" tumors, usually with intense lymphocyte infiltration in the histopathology, while the CMS2 subtype (canonical, approx. 37% of cases) and CMS3 (metabolic, about 13% of cases) are "cold" tumors and lack an immunological activity [12]. Currently, the ability of CRC cells to interact with the immune system is associated with the accumulation of unrepaired mutations in DNA microsatellites.

Microsatellites are short stretches of DNA that consist of many repeats of one to ten nucleotide base pairs. During DNA synthesis by DNA polymerase, these sequences often undergo mutations, such as nucleotide insertions or deletions, leading to a shift in the reading frame of the genetic code. The system responsible for repairing such mismatches — MMR, which includes mutator genes, mainly MLH1, MSH2, MSH6, and PMS2 — plays a major role in recognizing and correcting errors in the microsatellite region, thus preventing genomic changes [13]. Mutations in the genes listed above result in accumulation of mismatches and instabilities in microsatellites. According to the European Society for Medical Oncology (ESMO) guidelines, MSI or dMMR testing is recommended in all CRC patients [14]. The polymerase chain reaction (PCR) test, e.g. the Bethesda panel, or next-generation sequencing (NGS) is used to determine microsatellite instability (MSI) or the IHC test to evaluate the expression of MMR proteins. Both methods are costly and require additional sections of tumor tissue in addition to routine

hematoxylin and eosin (H&E) staining [15]. Moreover, the guidelines treat MSI or dMMR determination equally. Due to the limitations of both these methods and the risk of false-positive results, the value of double determination — MSI and dMMR — is more and more often indicated [16]. There are also ongoing tests with use of artificial intelligence and machine learning to determine MSI/dMMR in routine histological preparations, which would be cheaper and faster than molecular diagnostics. However, the clinical use of this technology requires high efficiency and multi-center validation, which has not yet been achieved.

In the literature describing MSI/dMMR testing, there are various classifications of these disorders. Until recently, depending on the percentage of abnormal microsatellite regions detected in individual assays, a distinction was made between cancers with a high (MSI-high, MSI-H) or low degree of instability (MSI-low, MSI-L) or microsatellite stable (MSS) cancers. Clinically, dMMR corresponds to the MSI-H phenotype, while the MSI-L or MSS phenotypes correspond to MMR-proficient tumors. Recently, the following classification has become more common:
— cancers with microsatellite instability (MSI), corresponding to dMMR, also referred to as MSI/dMMR,
— cancers without microsatellite instability — MSS corresponding to pMMR, also referred to as MSS/pMMR.

This approach was introduced by the panel of experts from Bethesda and is also used in the ESMO guidelines [17, 18]. Such nomenclature has been adopted in the present work although when citing clinical trials, the original provisions used in the publications have been retained.

The presence of MSI, determined by a deficiency of one of the proteins of the MMR system, was found in about 15–20% of CRC patients, with distinct differences depending on cancer stage, i.e. approx. 20%, approx. 12%, and only about 4% in stages II, III, and IV, respectively [19]. These differences are explained by the overexpression of cancer antigens in tumors with such a highly mutated phenotype, which is supposed to result in increased immunogenicity of the tumor and induction of a deep host immune response, i.e. better control of the tumor by the immune system. Thus, MSI tumors are not only more frequently observed in the early stages, but also have a better overall prognosis [20]. MSS/pMMR CRC show poor immunogenicity, which makes it difficult to induce an anticancer immune response [21].

The majority of MSI/dMMR CRC are sporadic tumors associated with an epigenetic disorder — hypermethylation of the MLH1 gene promoter, which leads to its transcription silencing and lack of expression of the encoded protein. A higher incidence of sporadic microsatellite instability is associated with older age,

female sex, right-sided location of the primary tumor, high grade of histology, mucinous, medullary, or signet ring cell histology, and the presence of lymphocytic infiltrates. Sporadic MSI-H colorectal cancers show a higher percentage of $BRAF^{V600E}$ mutations (30–40%) compared to other cancers. The presence of the $BRAF^{V600E}$ mutation is a criterion excluding germline disorders of mutator genes and is used as a molecular marker of sporadic MSI cancers [22].

About one-third of dMMR CRC is associated with the presence of germline mutations. In rectal cancer, MSI is less common than in colon cancers — about 5% of cases, but the majority of such cancers (84%) are caused by a germline disorder [23].

Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC) is the most common genetic disorder associated with germline mutations of one of these four mutator genes. The most common mutation is in the *MLH1* or *MSH2* genes (42% and 33%, respectively), and less frequently in the *MSH6* and *PMS2* genes (18% and 7%, respectively). The syndrome is inherited as autosomal dominant and is associated with an increased predisposition to CRC (the risk is 30–73%) and endometrial cancer (30–51%), as well as ovarian, gastric, small intestine, and pancreatic cancer. A rare variant of Lynch syndrome that is associated with hereditary MSI is the germline exon 3 deletion in the *TACSTD1* gene encoding the EpCAM protein. This disorder leads to congenital epigenetic loss of *MSH2* gene function.

Other genetic syndromes associated with increased risk of CRC include:
— Muir-Torre syndrome, associated with a simultaneous germline mutation of the *MSH2* and *MLH1* genes and additionally characterized by the presence of seborrheic skin tumors;
— Turcot syndrome caused by a congenital mutation of the *APC* gene and one of the mutator genes *MLH1* or *PMS2* and associated with familial polyposis with the coexistence of primary brain tumors [24].

Immunotherapy for colorectal cancer

The relationships between disorders of the DNA *mismatch repair system* and the induction of an anticancer immune response justify the use of immune checkpoint inhibitors in the treatment of patients with MSI/dMMR CRC. Currently, there are two such inhibitors targeting the PD-1 receptor (pembrolizumab and nivolumab) and one directed against CTLA-4 (ipilimumab), which have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in the last 5 years for patients with MSI-H/dMMR CRC.

The results of the phase-II study KEYNOTE-016 with pembrolizumab provided the first evidence of immunotherapy effectiveness in patients with metastatic MSI-H/dMMR CRC. The study involved 42 patients: 11 patients with dMMR CRC, 21 patients with pMMR CRC, and 9 patients with dMMR metastatic cancer other than CRC. All patients were heavily pretreated with all standard treatment methods. The objective response rate (ORR) in the group of patients with MSI-H/dMMR CRC was 40% [25]. In a later analysis, including 54 patients, presented at the 2016 American Society of Clinical Oncology (ASCO) Annual Meeting (not published yet), the ORR increased to 50%, and in patients with dMMR cancers other than CRC was even higher (71%). However, in the subgroup of patients with MSS/pMMR CRC, there were no objective responses (0%). Based on the results of this study, together with the results of four other phase Ib and II studies (KEYNOTE-164, KEYNOTE-012, KEYNOTE-028, and KEYNOTE-158), the FDA approved pembrolizumab in 2017 for the treatment of patients with MSI-H/dMMR CRC after failure of conventional chemotherapy. Based on the results of additional cohort analyses from the aforementioned and other trials, this registration was then extended to all dMMR tumor subtypes in patients who had exhausted standard treatment options [26]. It was the first tissue agnostic drug approval of the neoplastic disease.

The aforementioned KEYNOTE-164 phase-II study evaluated the efficacy of pembrolizumab monotherapy after failure of 5-FU-based combination chemotherapy in a subgroup of 124 patients with MSI-H/dMMR CRC. The response rate was 34%, and after 3 years of follow-up, the response to treatment was maintained in 92% of patients [27].

In the randomized phase-III study KEYNOTE-177, pembrolizumab monotherapy was compared to the standard first-line treatment — doublet chemotherapy with the addition of a biological agent (bevacizumab or cetuximab) — in patients with MSI-H/dMMR CRC. The primary endpoints of this study were progression-free survival (PFS) and OS. The use of pembrolizumab was associated with a significant increase in median PFS (16.5 vs. 8.2 months), a reduction in the risk of progression (HR = 0.60; 95% CI 0.45–0.80; p = 0002), a higher ORR (44% vs. 33%), and prolonged median duration of response. After 2 years of follow-up, the response to treatment was maintained in 83% of patients treated with pembrolizumab compared to 35% treated with chemotherapy [28]. Median OS was not reached in the pembrolizumab group compared to 36.7 months for chemotherapy (HR = 0.74; 95% CI 0.53–1.03; p = 0.0359). This result was not statistically significant due to the assumed alpha level > 0.0246, resulting from the planned interim OS analyses and repeated testing [29]. Interpretation of the result was complicated by the fact that 60% of patients treated with chemotherapy received immunotherapy after progression. The rate of grade 3 and 4 adverse events for pembrolizumab was 22% vs. 66% for chemotherapy. A clinically significant improvement in the quality of life of patients receiving immunotherapy has also been demonstrated [30]. However, it should be noted that primary disease progression was more common in the immunotherapy group — 30% versus 12% in the chemotherapy group.

Based on these studies, EMA approved pembrolizumab monotherapy in patients with metastatic MSI-H/dMMR CRC in first-line treatment and after previous fluoropyrimidine-based combination therapy.

The efficacy of nivolumab in patients with metastatic MSI-H/dMMR CRC was confirmed in a phase-II multi-cohort study CheckMate-142, in which nivolumab was used as monotherapy or in combination with ipilimumab in the first or subsequent treatment lines. The first cohort included 74 previously treated patients who received nivolumab monotherapy (3 mg/kg every 2 weeks). The second cohort consisted of 119 treatment-experienced patients who received a combination of ipilimumab (1 mg/kg) and nivolumab (3 mg/kg) every 3 weeks for the first 4 cycles, followed by nivolumab (3 mg/kg) in monotherapy at two-week intervals. The third cohort consisted of 45 patients who received combination immunotherapy as first-line treatment. In patients treated with nivolumab alone, the ORR was 31%. After 12 months of follow-up, one-third of these patients were still progression-free [31]. The 5-year survival rate in this cohort was 46%. Combined immunotherapy resulted in an ORR of 65% in the second cohort, including 13% of complete remissions. Three-quarters of patients had received two or more prior treatment lines. The 5-year PFS and OS rates were 52% and 68%, respectively [32, 33]. Patients from the third cohort achieved similarly favorable results although the follow-up time in this cohort is much shorter. The compilation of these results may indicate that combined immunotherapy is more effective than nivolumab alone, but these two strategies have never been directly compared.

Based on the results of the CheckMate-142 study, EMA approved ipilimumab in combination with nivolumab for the treatment of patients with advanced dMMR/MSI-H CRC after prior fluoropyrimidine-based combination chemotherapy.

Immunotherapy for advanced MSS/PMMR colorectal cancer

More than 80% of advanced CRCs are MSS/pMMR tumors. They do not induce a significant immune response, and checkpoint inhibitors alone are not effective.

However, it is believed that the combination of these drugs with other methods of systemic treatment (chemotherapy, EGFR inhibitors, angiogenesis inhibitors, MET inhibitors) and radiotherapy may be a valuable option [34]. Studies evaluating the value of such combinations are ongoing, but so far, they have not been successful.

A promising strategy might be use of immunotherapy in combination with molecularly targeted treatment in patients with the presence of a molecular target, e.g. with the $BRAF^{V600E}$ or $KRAS^{G12C}$ mutation, and such studies are currently ongoing.

There is some hope for new-generation checkpoint inhibitors that could induce sensitivity to immunotherapy. An example of such a drug is botensilimab, an antibody directed against CTLA4 with a modified fragment crystallizable (FC) region to improve the activation of dendritic cells and NK cells. A phase-Ib study in which 41 patients with metastatic MSS CRC were treated with a combination of botensilimab and balstilimab (an anti-PD1 antibody) showed an ORR of 24%. An interesting observation was a lack of benefit from treatment in patients with liver metastases. This may indicate the important role of the tumor microenvironment in immunotherapy [35].

The future of immunotherapy in the treatment of patients with colorectal cancer

New indications

Apart from the above-mentioned directions of new research, which concern the combination of immunotherapy with other methods in patients with MSS CRC, there are studies assessing the value of immunotherapy in earlier treatment lines, including (neo)adjuvant treatment. Two studies on the use of immunotherapy in the first-line treatment of metastatic disease have already been mentioned (KEYNOTE 177 and ChechMate-142). The 3-arm COMMIT study compares atezolizumab monotherapy with FOLFOX chemotherapy combined with bevacizumab and FOLFOX chemotherapy combined with bevacizumab and atezolizumab in patients with advanced MSI/dMMR CRC. Data from preclinical studies show that chemotherapy containing oxaliplatin in combination with anti-angiogenic treatment increases the anticancer activity of the PD-L1 pathway [36]. Atezolizumab is also combined with standard chemotherapy (12 × FOLFOX) in the adjuvant treatment of patients with stage III MSI CRC. Such a combination aims to increase the activity of intratumoral cytotoxic CD8+ T cells (A021502NCTN) [37].

A small subgroup of patients with rectal cancer demonstrates MSI/dMMR. Early observations from a prospective study of 12 patients with locally advanced (94% of stage III) MSI/dMMR rectal cancer indicate high activity of immunotherapy. The study design was based on the administration of a PD-1 inhibitor, dostarlimab, 500 mg every 3 weeks for 6 months followed by radiochemotherapy (RChT) and surgery. Patients who achieved a complete clinical response defined by magnetic resonance imaging and endoscopic examination after dostarlimab could be actively monitored without RChT and surgery. The first 12 patients included in the study achieved complete clinical remission after 6 months of treatment with dostarlimab. They did not require any additional treatment and were actively monitored. By the time the results were published, the follow-up period ranged from 6 to 25 months. The treatment was well tolerated, and grade 3 and 4 side effects were not reported [38].

The multicenter non-randomized NICHE-2 study in patients with dMMR CRC assessed the effectiveness of neoadjuvant immunotherapy consisting of 1 dose of ipilimumab (1 mg/kg) and 2 doses of nivolumab (3 mg/kg) followed by surgery. The primary endpoint was safety and surgery feasibility after immunotherapy and the 3-year disease-free survival (DFS) rate. During the 2022 ESMO Congress, data on safety and pathomorphological responses to treatment were presented. The study evaluated 112 patients with a primary tumor stage of at least cT3, as assessed on the basis of a CT scan. cT4a or cT4b stage was found in 64% of patients, and N2 disease in 62% of patients. After initial immunotherapy, all patients underwent surgery. In the histopathological examination, 67% of patients achieved pathologic complete response (pCR) and 95% of patients had less than 10% of the residual tumor mass (MPR) [39].

The role of the microbiome

Another interesting area of research is the interaction of the gut microbiome with the immune system. Some studies indicated an association of changes in the gut microbiome with the risk of CRC as well as other cancers. Patients treated with immunotherapy achieve better results if their intestinal flora is not changed by antibiotic therapy [40, 41]. However, the actual impact of the gut microbiome in supporting immunotherapy is still not known.

Other methods of immunotherapy

Immunotherapy methods other than the aforementioned checkpoint inhibitors are also the subject of research. Many types of vaccines — autologous, peptide, and dendritic cell vaccines — have been studied in patients with CRC, but no survival benefits have been obtained compared to standard treatment

or placebo [42, 43]. The results of studies on the combination of vaccines with checkpoint inhibitors have so far been discouraging [44]. There is an ongoing study evaluating talimogene laherparepvec (T-VEC), which is a form of immunotherapy based on a derivative of the herpes simplex virus type 1, designed to replicate in tumor cells and produce granulocyte-macrophage colony-stimulating factor (GM-CSF). The idea is to enhance the immune response against cancer cells. In a study of patients with metastatic MSS CRC, T-VEC is injected into the tumor in combination with intravenous atezolizumab [45].

Therapy with T cells genetically engineered to express a synthetic chimeric antigen receptor (CAR) was very successful in the treatment of patients with refractory hematological malignancies, in particular B-cell acute lymphoblastic leukemia [46]. Different phases of studies are currently ongoing to extend CAR-T indications to solid tumors, including CRC [47].

Conflict of interest

B.R.: received remuneration from Merck, Amgen, BMS, MSD, GSK, Roche, and Servier, unrelated to the article.

M.G.: received remuneration from Merck, Amgen, BMS, MSD, and Servier, unrelated to the article.

M.K.: reports no conflict of interest.

T.K.: received honoraria from Roche, MSD, and BMS, unrelated to the article.

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