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review

Consensus molecular subtypes (CMS) in metastatic colorectal cancer - personalized medicine decision

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Background. Colorectal cancer (CRC) is one of the most common types of cancer in the world. Metastatic disease is still incurable in most of these patients, but the survival rate has improved by treatment with novel systemic chemotherapy and targeted therapy in combination with surgery. New knowledge of its complex heterogeneity in terms of genetics, epigenetics, transcriptomics and microenvironment, including prognostic and clinical characteristics, led to its classification into various molecular subtypes of metastatic CRC, called consensus molecular subtypes (CMS). The CMS classification thus enables the medical oncologists to adjust the treatment from case to case. They can determine which type of systemic chemotherapy or targeted therapy is best suited to a specific patient, what dosages are needed and in what order.

Conclusions. CMS in metastatic CRC are the new tool to include the knowledge of molecular factors, tumour stroma and signalling pathways for personalized, patient-orientated systemic treatment in precision medicine.

Key words: metastatic colorectal cancer; heterogeneity; biomarkers; consensus molecular subtypes; CMS1; CMS2; CMS3; CMS4

Introduction

Colorectal cancer (CRC) is still one of the most common types of cancer and one of the lead causes of cancer- related deaths worldwide, as well as in Slovenia. According to the Cancer Registry of Slovenia, there were 1467 new cases of CRC in 2016, of which 871 men and 596 women.[1] The prognosis of these patients has improved significantly over the last decade because of successful preventive screening programme, improved surgical techniques, radiation therapy and systemic treatment for both early and advanced stages. In Slovenia, the incidence of CRC has been declining in the last few years, mainly due to increased awareness and preventive screening programme called SVIT, which has been implemented in Slovenia in 2009. According to the National Cancer Control Program Slovenia, the incidence of CRC has been declining annually. In the last official report from 2015, there were about 400 cases less from 2010 to 2015 (from 1729 cases in 2010 to 1357 cases in 2015).[2]

Metastatic CRC is still an incurable disease for most of the patients, with most commonly liver, lung or lymph nodes and peritoneal metastases. In the past, 15 years ago, median overall survival (mOS) was approximately 12 months and the 5-year survival rate was 13%. However, the survival rate of these patients has increased, mainly due to the combined treatment of metastases with surgery and systemic therapy.[3-5] Long-term survival or even cure can be attained in 20%–50% of the patients who undergo complete R0 resection of liver or lung metastases, and around 70% 5-year survival of these patients can be achieved.[3,4]

However, in the field of systemic therapy there has been a significant progress with new drugs in the recent years. There are more options of initial systemic chemotherapy, oxaliplatin, irinotecan, and fluoropyrimidines, in combination with targeted therapy with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (cetuximab, panitumumab) in case of *KRAS* wild type tumours or anti-vascular endothelial growth factor (VEGF) inhibitors (monoclonal antibodies bevacizumab, aflibercept, ramucirumab, regorafenib as per oral tyrosine kinase inhibitor).[3-5] The combination of these novel chemotherapy and targeted therapy now extends the mOS up to 40 months.[3-5]

Additionally, testing for new biomarkers enables the usage of new targeted treatment in metastatic CRC patients, such as human epidermal growth factor receptor 2 (HER2/new) amplifications for double HER2 blockade, immunotherapy with anti-programmed cell death protein 1 (PD-1) monoclonal antibodies in high microsatellite instable (MSI) tumours, and neurotrophic tyrosine kinase receptor *(NTRK)* inhibitors in case of *NTRK* gene fusions.[3-5] *BRAF* V600E mutation is associated with poor prognosis under standard treatment of mOS less than 1 year and the responses to targeted therapy of combinations with anti- EGFR, BRAF and MEK inhibitors are promising with longer mOS.[3-5]

Pharmacogenomics' biomarkers such as dihydropyrimidine dehydrogenase, uridine diphosphate glucuronosyltransferase 1A1, excision repair cross complementing rodent repair deficiency complementation group 1, VEGF and thymidylate synthase are also important when planning the treatment and deciding on the type (to choose the alternative systemic therapy), appropriate combination (less toxic) and dosages (to adjust the dose to lower the frequency and grade of the adverse effects) of systemic therapy.[6]

New knowledge about the molecular heterogeneity of CRC, the discovery of biomarkers as predictive factors for disease prognosis and response to systemic treatment, and thus personalized medicine in this field, have also significantly contributed to the prolonged survival rates of patients. Besides gene mutations, tumour stroma and immunity also play a very important role in response to the systemic treatment and the prognosis of the disease.

In 2015, Guinney *et al.* first published the classification of consensus molecular subtypes (CMS), namely MSI immune CMS1, canonical CMS2, metabolic CMS3 and mesenchymal CMS4.[7] The CMS classification includes clinical factors, all pathological and molecular features of the tumour, signalling pathways and immunity. However, it still currently has not translated into regular clinical practice, which could guide the clinicians in their more personalized treatment decisions. At present, the CMSs do not have an impact on clinical decisions, because we do not yet have approved algorithms available for everyday clinical practice

The clinical implications of CMS

Colorectal cancer is genetically and transcriptomically heterogeneous disease. In adjuvant setting for early-stage CRC, there are several gene expression signatures such as ColoPrint, Oncotype DX and others, but they are still not recommended in everyday clinical practice by international guidelines for CRC.[2,3] In metastatic setting, *MSI, RAS* and *BRAF* mutational statuses are routinely tested for prognosis and predictions for systemic treatment. *KRAS* mutational status was the first biomarker in metastatic CRC to predict the response to anti-EGFR inhibitors since 2008. Additionally, mutational status testing in *RAS* gene (*KRAS* and *NRAS* genes) is used in daily clinical practice since 2013. In the past, BRAF mutation was a negative prognostic biomarker for a shorter median OS of 12 months. This was also confirmed in our prospective clinical trial, conducted at the Institute of Oncology Ljubljana between 2010 and 2013, in which we analysed the impact of the molecular biomarkers and histological parameters on survival and response to the first- line systemic therapy of metastatic colorectal cancer patients.[8] Median OS of wild type wt*BRAF* patients was significantly longer than in mutated mt*BRAF* patients, with 59.2 and 27.6 months, respectively, $p = 0.05$.

Today, targeted therapy combining BRAF inhibitors and MEK inhibitors in combination with anti-EGFR inhibitors with mOS of 24 months is approved by FDA, but not by EMA in Europe for the BRAF mutated patients.[2,9] However, metastatic CRC is not a simple disease but rather a heterogeneous one, with different treatment responses and outcomes. Thus, these routinely identified biomarkers provide only some information about tumour biology.

In 2015 Guinney *et al.* in the CRC Subtyping Consortium established four consensus molecular subtypes 1 (CMS1), 2 (CMS2), 3 (CMS3) and 4 (CMS4), based on six independent CRC classification systems.[7] They analysed tumour characteristics of more than 4000 patients, including not only

TABLE 1. Classification of consensus molecular subtypes (CMS). Adopted by Guiney *et al.*[7]

CMS subtype	CMS1 - MSI immune	CMS2 - Canonical	CMS3 – Metabolic	CMS4 - Mesenchymal
Frequency	14%	37%	13%	23%
Characteristics	MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
	BRAF mutation		KRAS mutation	
	Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
	Worse survival after relapse			Worse relapse-free and overall survival

CIMP = CpG island methylator phenotype; MSI = microsatellite instable; SCNA = somatic copy number alterations; TGF-β = transforming growth factor beta

their genetic alterations, but also their immune system, cellular metabolism, epithelium, signalling activation, immune tumour infiltration, tumour microenvironment and angiogenesis. The CMS are characterized and named by their main distinguishing features. CMS1 is denoted as MSI immune, presented in 14% of the cases, hypermutated, microsatellite unstable and with strong immune cell infiltration and activation. CMS2 is canonical, presented in 37% of the cases, with marked WNT and MYC signalling activation. CMS3 is called metabolic, presented in 13% of the cases, with epithelial and evident metabolic dysregulation, with *KRAS* mutations and mixed MSI status, low somatic copy number alterations (SCNA) and CpG island methylator phenotype (CIMP). CMS4 is called mesenchymal, presented in 23% of the cases, with prominent transforming growth factor β activation, stromal infiltration and angiogenesis. The main features of CMS subtypes are presented in Table 1.

The CMS subtypes are not classified only by molecular features, but also by clinical features, with prognosis included in its classification.[10-13] Sidedness of the primary tumour is also included. Right-sided tumours, including cecum, ascending colon or transverse colon are characterized by mucinous, signet ring histology, microsatellite instability, hypermethylation, poor differentiation, higher mutation rates of PI3KCA, KRAS and BRAF. They are more frequent in older patients and female patients. Left-sided tumours, including descending colon, sigmoid colon and rectum are characterized by chromosomal aberrations, 18q loss and 20q gain, aneuploidy, p53 mutation, EGFR and HER2 gain, high VEGF-1 mRNA, cyclooxygenase 2 (COX2), high EGFR ligand epiregulin and amphiregulin expression.[10-13]

However, tumour location inside the intestine is even more important than sidedness.[12,14] Namely, CMS1 is more often present in the proximal colon (the cecum, the ascending colon, the transverse colon), CMS2 in the distal colon (the descending colon, the sigmoid colon) and the rectum, CMS3 in the sigmoid colon and the rectum and CMS4 in the distal colon (the descending colon, the sigmoid colon) and the rectum. Tumours of distal colon and rectum appear unique and tumours of the transverse colon appears distinct from other tumours of the right colon.[14] Because of this tumour heterogeneity of different parts of colon and the differences between tumours of colon and rectum, and also intra- tumour heterogeneity of the primary tumour, Fontana *et al*. highlighted the importance of the careful sampling from biopsies or resected primary tumour for each patient to get the right information about his biomarkers.[12]

Since secondary acquired resistance can develop during specific systemic therapy with anti EGFR inhibitors, because of tumour heterogeneity and clonal selection process, it is important to include circulating tumour DNA analyses in evaluation of effectiveness of systemic therapy. This technique can detect genomic alterations in *RAS* and other genes to help adjust systemic therapy before clinical and radiological progression.[11,15-17]

Two recently published papers explain the impact of CMS subtypes on the survival of metastatic CRC patients and the differences to the response to systemic treatment according to CMS subtypes.[18,19] Patients from two phase III clinical trials, the CALBG/SWOG 80405 and the FIRE-3, were included in this analysis. Both clinical trials assessed the combination of anti-VEGFR inhibitor bevacizumab or anti- EGFR inhibitor cetuksimab with different types of chemotherapy - oxalipl-

atin with 5-FU (FOLFOX) in 75% of the patients in CALGB/SWOG 80405 and irinotecan with 5-FU (FOLFIRI) in all patients in the FIRE- 3.[18-20] Both studies showed that left-sided colorectal cancer responded better to cetuximab-based in combination with irinotecan therapy in case of CMS2 and CMS4 compared to bevacizumab-based therapy, whereas for right-sided tumours this possibility has to be further explored.

Lenz *et al*. have retrospectively analysed the impact of the CMSs on survival of *KRAS* wild type metastatic CRC patients from CALGB/SWOG 80405 clinical study.[18] For the CMS classification, the NanoString panel for the CALBG/SWOG 80405 cohort and the official CMS classifier software were used. Based on the CALGB study results, CMSs are predictive biomarkers for bevacizumab and cetuximab in terms of OS and progression-free survival (PFS). In the CMS2 cohort, patients who received cetuximab had significantly longer OS and slightly improved PFS compared to those who received bevacizumab, although this was not statistically significant. In the CMS1 cohort, patients who received bevacizumab had significantly longer OS and longer PFS compared to the patients who received cetuximab. They concluded that CMS classification is an independent prognostic marker for metastatic CRC patients in the first-line systemic therapy with a combination of chemotherapy with bevacizumab or cetuximab. Patients with CMS1 had the shortest OS and PFS, whereas patients with CMS2 had the longest OS with the lowest risk of death and PFS. They also emphasized the limitations of their analysis to the KRAS wild-type metastatic patients and stated that it was not possible to do a more detailed exploration of the interactions between a specific chemotherapy and targeted therapy. However, in 2019, Aderka *et al*. published a research, studying this topic.[19] The responses of the patients with different CMS subtypes to systemic chemotherapy with oxaliplatin or irinotecan in combination with different targeted therapy, anti- VEGFR inhibitor bevacizumab or anti- EGFR inhibitor cetuximab were analysed. They found that both cytostatics have synergistic effect in combination with cetuximab. Irinotecan upregulates EGFR and promotes the binding of cetuximab and so promotes its antibody-dependent cell-mediated cytotoxicity (ADCC), stimulates the release of IFN-γ and activates dendritic cells, macrophages, T cells and encourages the apoptosis of cancer cells. Furthermore, cetuximab inhibits the tumour's multidrug resistance mechanism for the active metabolite of irinotecan - SN-38 - which accumulates in the cells and thus improves its antitumour effect. The oxaliplatin acts in two ways, as oxaliplatin – DNA adducts and causes DNA oxidative damage. EGRF activation upregulates nucleotide excision repair proteins and base excision repair proteins (*ERCC1)* and in this way neutralises effects of oxaliplatin. The combination of oxaliplatin and anti- EGFR inhibitor cetuximab has a synergistic effect in terms of cetuximab downregulation of *ERCC1* and, which could further improve oxaliplatin activity.[19]

The tumour microenvironment is also an important factor in resistance of CRCs to specific combination of chemotherapy and targeted therapy. The CMS1 and CMS4 tumours have a fibroblast-rich microenvironment.[19] In that case of CMS1 and CMS4 oxaliplatin has an antagonistic action to anti-EGFR inhibitors cetuximab and panitumumab, inducing the release of interleukin 17A from fibroblasts promoting proliferation of cancer stem cells and antagonising the growth suppression and apoptosis of cancer stem cells induced by cetuximab. Activated cancer-associated fibroblasts also secrete transforming growth factor beta (TGF-β) and mediate tumour resistance to anti-EGFR inhibitors by providing an intrinsic EGFR-independent survival pathway to cancer cells. TGF-β also prolongs inhibitory effect on the cetuximab-mediated antibody-dependent cellular cytotoxicity (ADCC), inhibits activation of immune cells, natural killer cells, dendritic cells and macrophages.[19]

In both articles, of Aderka and Lenz, the authors also explained why such differences occur.[18,19] The first significant factor is the previously described synergistic or antagonistic action of the combination of chemotherapy and the biological drug. The second important factor is the sequence of biologicals, bevacizumab and cetuximab, in terms of CMS, which is supported by both studies. If anti VEGFR inhibitor bevacizumab is administrated in first-line systemic treatment, before cetuximab, it reduces the permeability of blood vessels and consequently diffusion and tumour cell binding of cetuximab. The third factor is the half-life of bevacizumab compared to cetuximab, which is also important concerning the sequence of. With a half-life of 21 days, bevacizumab is still active for a period when initiating a second line of cetuximab treatment, reducing the permeability to tumour stroma and the anti-EGFR effect after the first line of bevacizumab. Lastly, in the FIRE-3 study, chemotherapy with only irinotecan hydrochloride (CPT 11) with 5-fluorouracil (5-FU) was used in combination with bevacizumab or cetuksimab; and oxaliplatin with 5-FU was used in 75% in combination with bevacizumab

or cetuximab in CALGB study. Thus, researchers concluded that both studies are complementary and not opposing in terms to relevant conclusions from retrospective analyses.[19]

Based on all clinical and molecular knowledge, the mOS for 16 different combinations of oxaliplatin, irinotecan and targeted therapy in first-line treatment was calculated for each CMS subtype. The most effective first- line combination is oxaliplatin with bevacizumab, irinotecan or oxaliplatin with cetuximab, oxaliplatin with cetuximab and irinotecan with cetuximab, in CMS1, CMS2, CMS3 and CMS4 respectively.[19]

Additionally, Stintzing *et al*. conducted an analysis according to CMS classification in terms of objective responses (OR) and PFS from the FIRE-3 clinical trial, in which the first-line therapy was FOLFIRI (irinotecan plus 5-FU) with bevacizumab or cetuximab in *KRAS* wild-type metastatic CRC patients.[20] The retrospective analysis was carried out for *RAS* wild-type metastatic CRC patients. They confirmed the prognostic role of CMS classification in CMS3 and CMS4 subtypes and the predictive role for a better outcome in CMS4 subtype in *RAS* wild-type patients, treated with FOLFIRI and cetuximab. Significantly higher overall response rate (ORR) were seen in CMS2 subtype in the same regimen. OS of patients with CRC subtype CMS4 was significantly longer in treatment with FOLFIRI cetuximab compared to that with FOLFIRI bevacizumab. In patients with CMS3, OS was in favour of FOLFIRI and cetuximab, OS in CMS1 and CMS2 were comparable and independent of targeted therapy.

Lastly, gut microbiome is probably another important biomarker to consider in future studies in treating metastatic CRC patients.[10,21] Gut microbiomes are associated with CMS1 and CMS2 subtypes. It is known that gut microbiome has an important role in carcinogenesis of CRC, showing initial inflammation and modulation of different signalling pathways. Each part of the colon and rectum is characterized by different strains of bacteria. The most important and studied strains were *Fusobcterium nucleatum*, *Escherichia coli* and *Bacteroides fragilis*. Gut microbiome also varies geographically, seven strains are the most important for carcinogenesis, *B. fragilis*, four oral as *F. nucleatum, Parvimonas micra, Porphyromonas asaccharolytica* and *Prevotella intermedia, Alistipes finegoldii* and *Thermanaerovibrio acidaminovorans*.[21] Bacterial biomarkers have potential to detect CRC, predict clinical outcome and have a prognostic value.[21] Gut microbiome also mediates the response to chemotherapy, especially of irinotecan, oxaliplatin and 5-flurouracil, prescribed in treatment of metastatic CRC. There are several ways like immunomodulation, metabolism regulation, resistance to chemotherapy, microbial translocation, reduced ecological diversity and others. It also plays an important role in effectiveness of immunotherapy with checkpoint inhibitors in terms of to enhance the action of it. It can be also associated with the adverse effects of immunotherapy, especially with immune-related colitis, depending of the presented strains of bacteria in the gut.[21] Therefore; the knowledge about gut microbiome will have clinical implications for CRC prevention, improvement of treatment responses and reduction of the adverse effects.

Conclusions and future directions

Predictive and prognostic biomarkers are important for personalized medicine and treatment of patients with metastatic CRC and therefore enable better optimization and tailoring of treatment. Pharmacogenomics biomarkers will allow us to adjust and determine the optimum effective dose of the drug for each patient. Gut microbiome is another important biomarker predicting the prognosis of disease and the response to the specific systemic therapy.

CMS subtypes, including molecular heterogeneity at different levels of genetics, epigenetics, transcriptomic, clinical features and more important tumour microenvironment will enable us to estimate the prognosis and make precision medicine individualized for each patient.

In the future, it is important to develop algorithms for everyday clinical practice to determine the CMS subtype for each patient individually, based on patient and tumour characteristics. This will result in the most optimal, patient-tailored treatment to maximize the response, prolong survival, minimize the treatment cost and avoid potential unwanted adverse effects of ineffective therapy.

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