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Molecular subtypes of colorectal cancer as a potential prognostic and predictive factor in the selection of the optimal treatment strategy

ABSTRACT
Colorectal cancer is one of the most common cancers and is the third cause of death from malignant tumours. In recent years, a consensus has been developed that distinguishes four subtypes of colon cancer (CMS, consensus molecular subtypes): CMS1 — immunological, CMS2 — canonical, CMS3 — metabolic, and CMS4 — mesenchymal. They differ in terms of clinical course and response to chemotherapy and biological treatment. The practical application of molecular classification can be helpful as a prognostic and predictive factor in the selection of an optimal and individualised strategy for the treatment of individual patients.

Key words: colon cancer, CMS, chemotherapy, molecular-targeted treatment

Colorectal cancer molecular classification

According to GLOBCAN 2018, colorectal cancer is classified as the fifth most common cancer in the world (1.8 million cases per year) and the third as a cause of fatalities when considering malignant tumours (881 thousand cases per year) [1]. In the EUROCARE-5 survey the five-year survival rate (conducted between 1999 and 2007) was reported as 47% in Poland and 57% in the whole of Europe [2]. The observed clinical improvement is likely to be connected with screening tests and more effective therapeutic procedures both in local cancer and generalised disease. For many years, histopathological examination and staging alongside clinical assessment were the only factors that contributed to making therapeutic decisions. Identification of risk factors such as the presence of RAS and BRAF oncogenes or microsatellite instability (MSI) were the foundation for the new definition of colorectal cancer, seen as a heterogenic disease with varied molecular background with diverse course and prognosis [3]. Phenotype variety, reflecting molecular features of colorectal cancer, resulted in the emergence and systemisation of new molecular subtypes [4].

Perez-Villamil et al. suggested a gene signature-based classification favouring four types of colorectal cancer. The low-stroma-like type, which is the most distinct and gives the best prognosis, also shows gene expression that is least related to stroma, on the contrary to the high-stroma-like type. We also differentiate mucinous-like type, which is characterised by more common BRAF gene detection and MSI presence, and finally immunoglobulin-related type, which has greater immunoglobulin expression [5].

Sadanandam et al. submitted six subtypes of colorectal cancer: stem-like subtype, which is the least differentiated with stem cell presence (high expression of Wnt/β-catenin trail) (I); inflammatory subtype with the highest expression of cytokine and interferon encoding genes (II); enterocyte subtype (specific for enterocytes) (III); goblet cell subtype with higher expression of trefoil factor (TFF3) and MUC2 (IV); and two subtypes with features of TA-transit amplifying cells (different cetuximab responsiveness) (V, VI) [6].
As a result of the research of De Sous E Melo, three molecular subtypes were suggested. CCS1-CIN associated with chromosomal instability, CCS-MSI related to microsatellite instability, and CCS3-serrated characterised by a gene profile that is similar to sessile serrated lesions (SSL) [7].

In 2014, as a result of a summary analysis of all research groups led by Sadanandam and De Sousa E Melo, a conclusion was arrived at. Gene profiles and phenotype features of subtype CCS1-CIN (due to the classification) correspond to TA and enterocyte type in Sadanandam order. Subtype CCS1-MSI corresponds to inflammatory and goblet cell subtypes, and stem-like subtype is close to CCS3-serrated type [6]. Further research led to establishing an international expert consortium and unifying six independently existing molecular classifications. Thus, a final point was reached, and four molecular subtypes of colorectal cancer were distinguished: CMS1 — immunological, CMS2 — canonical, CMS3 — metabolic, and CMS4 — mesenchymal [6]. CMS classification comprised around 87% of examined malignant colorectal cancer cases, and the rest (13%) were characterised by a mix of all subtypes. CMS groups are differentiated in terms of gene signature, molecular changes, and clinical presentation. Recent data support the theory claiming that particular subtypes of colorectal cancer are connected with the primary location, sex, histopathological results, and staging at the moment of diagnosis [8].

CMS1 (immunological, MSI)

Subtype CMS1 is relatively rare (barely 14% of all colorectal cancer cases), but its biology is very different from other groups [6]. It is characterised by high immunogenicity and MSI presence, created in the process of mutator genes (MLH1, MSH1, PMS2, and MSH6) inactivation. MSI is a favourable prognostic factor, which might be connected to gross lymphocytic infiltration around the tumour and immunological response activation [9]. In the CMS1 subtype hypermutation might be observed. V600E BRAF, PTEN, and ATM mutations occur frequently. CpG island methylator phenotype (CIMP), causing inactivation of suppressor genes, is also highly characteristic; it is one of the first processes when speaking about molecular processes in carcinogenesis. On clinical presentation CMS1 is seen as a mucinous cancer, which is usually located in the proximal part of the intestines and occurs more frequently in women. It is less likely to recur and constitutes better prognosis, especially when diagnosed at an early stage. Unfortunately, recurrence is related to the aggressive nature of a cancer and short life expectancy. Precursor changes of this subtype are typically serrated polyps [8].

CMS2 (canonical)

CMS2 canonical subtype is diagnosed most commonly (37% of cases). It is characterised by CIN. Typical molecular features of this subtype are high expression of Wnt/β-catenin trail and MYC transcription factor, responsible for cell proliferation and diversion. CMS2 is not associated with hypermutation, and in comparison with CMS1 chromosomal rearrangements and aneuploidy are more frequently observed. Epithelial phenotype with accompanying loss of APC suppressor gene is typical, the same as KRAS gene mutation and TP53 gene inactivation. The most common location of CMS2 subtype cancer is the distal part of the large intestine (59% of cases). It has better prognosis, and the five-year survival rate is the highest out of all types. Longer survival, reaching 35 months, is also observed in recurrence [10].

CMS3 (metabolic subtype)

This occurs in 13% of patients with colorectal cancer. It barely holds cases of CIN (chromosomal instability) and represents the highest occurrence of KRAS gene mutations (68% of cases). In 3–5% of the cases HER2 gene amplification is stated [11]. Metabolic subtype appears with similar prevalence in different parts of large intestine [8]. It has a relatively favourable prognosis, at all stages a total of around 75% of patients live longer than five years [10].

CMS4 (mesenchymal subtype)

This subtype is second in terms of colorectal cancer occurrence (23% of cases). A crucial molecular feature is compliance when speaking about gene signatures of activated stroma. Marked angiogenesis, transforming growth factor β (TGFβ) trail activation, and proteins connected with microinflammation are common [12]. Occurrence is higher in men (55% of cases), mainly in a distal part of the intestine. CMS4 subtype cancer is related to poor prognosis, frequent relapse, and overall five-year survival and five-year survival free from relapse is 62% and 60%, respectively [8].

Clinical significance

Hand in hand with the development of molecular classification, an attempt was made to implement it in clinical practice. The work of Sadanandam et al. revealed a connection between cancer molecular subtypes and possible response to administered treatment [13]. In patients with generalised disease the first-line chemotherapy (due to FOLFIRI [folinic acid, fluorouracil, irinotecan] model) response percentage was
Table 1. Colorectal cancer molecular subtypes based on consensus molecular subtypes (CMS) (based on [8])

<table>
<thead>
<tr>
<th>Subtype</th>
<th>CMS1</th>
<th>CMS2</th>
<th>CMS3</th>
<th>CMS4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additional name</td>
<td>MSI, inflammatory</td>
<td>Canonical</td>
<td>Metabolic</td>
<td>Stromal, mesenchymal</td>
</tr>
<tr>
<td>Basic molecular features</td>
<td>Hypermutations, MSI, strong immunogenic activation</td>
<td>Epithelial, activation of WNT and MYC trails</td>
<td>Epithelial, metabolic regulation disturbed</td>
<td>High TGFβ expression; epithelial invasion and angiogenesis ± WNT</td>
</tr>
<tr>
<td>Incidence</td>
<td>14%</td>
<td>37%</td>
<td>13%</td>
<td>23%</td>
</tr>
<tr>
<td>Gene signature</td>
<td>MSI, high number of mutations, low number of copies</td>
<td>CIN, low-moderate mutation and copy index</td>
<td>CIN, moderate no. of mutations, and low no. of copies</td>
<td>CIN, low no. of mutations, and high no. of copies</td>
</tr>
<tr>
<td>Epigenome features</td>
<td>High methylation</td>
<td>Low methylation</td>
<td>Indirect methylation</td>
<td>Low methylation</td>
</tr>
<tr>
<td>Molecular trails</td>
<td>Inflammatory activation, JAK-STAT, caspases</td>
<td>WNR trail, MYC, EGFR, SRC, VEGF/VEGFR activation; integrins, TGFβ, IGF, IRS2, HNF4α and HER2, and cyclin activation</td>
<td>DNA repairing, glutaminolysis, lipogenesis</td>
<td>Stroma activation, immunosuppression, integrins</td>
</tr>
<tr>
<td>Microenvironment</td>
<td>Low no. of fibroblasts connected to cancer (CAF), highly immunogenic, immunological infiltrations, acquired immunological response</td>
<td>Really low number of CAF; poorly immunogenic; congenital immunological response</td>
<td>Low CAF; highly immunogenic; acquired immunological response</td>
<td>High no. of CAF; inflammatory, acquired immunological response, EMT</td>
</tr>
<tr>
<td>Related mutations</td>
<td>MSH6, RNF43, ATM, TGFβR2, BRAF, PTEN</td>
<td>APC, KRAS, TP53, PIK3CA</td>
<td>APC, KRAS, TP53, PIK3CA</td>
<td>APC, KRAS, TP53, PIK3CA, TOP1, CES2</td>
</tr>
<tr>
<td>Age</td>
<td>69</td>
<td>66</td>
<td>67</td>
<td>64</td>
</tr>
<tr>
<td>Location</td>
<td>Proximal</td>
<td>Distal</td>
<td>Mixed</td>
<td>Distal</td>
</tr>
</tbody>
</table>

MSI — microsatellite instability; TGFβ — transforming growth factor β; CIN — chromosomal instability; EGFR — epidermal growth factor receptor; SRC — sarcoma; VEGF — vascular endothelial growth factor; VEGFR — VEGFR receptor; IGF — insulin-like growth factor; HNF4α — hepatocyte nuclear factor 4α; CAF — carcinoma-associated fibroblasts; EMT — epithelial-mesenchymal transition

Results of research by Okita et al. also point out the connection between colorectal cancer molecular subtype and efficiency of systemic therapy. Over 193 patients presenting generalised disease underwent retrospective analysis and were divided corresponding to types: CMS1 (N = 21), CMS2 (N = 53), CMS3 (N = 69), and CMS4 (N = 50). Then, chemotherapy efficiency was analysed based on irinotecan and oxaliplatin and anti-EGFR therapy in particular molecular subtypes. In the analysed group, longer progression-free survival (PFS) and overall survival (OS) were noted in patients treated with irinotecan as first-line chemotherapy (compared to oxaliplatin therapy). Numbers presented are 12.8 vs. 10.7 months (HR = 0.64; 95% CI 0.49–0.89, p < 0.01) and 46.3 vs. 35.5 months (HR = 0.67; 95% CI 0.44–1.0, p = 0.06). The biggest result was noted in the CMS4 subtype mainly considering time free from progression (HR = 0.31; 95% CI 0.13–0.64), but less considering OS rate (HR = 0.45; 95% CI 0.19–0.99). Therefore, the percentage of objective responses was higher in the group treated with irinotecan (for CMS4 subtype it was 80%). The lowest percentage of positive response was noted in CMS1 subtype. Irinote-
can, topoisomerase 1 inhibitor, is metabolised to an active form using carboxylesterase (CES). Mutations in TOP1 and CES2 genes constitute characteristic gene signature of CMS4 subtype and might be responsible for results of the irinotecan treatment [14]. These observations are proven by other analysis [15]. Genes gathered in the RAS and RAF group were also examined in terms of mutation presence. The biggest percentage of RAS mutations was present in CMS3 subtype and B~RAF~ mutation in CMS1 subtype. Hypermethylation was noted in the majority of the CMS1 group. Patients, who did not carry the RAS mutation (wild type, RAS-wt) and went through anti-EGFR therapy were also examined. The worst results were observed in the CMS1 subtype with a PFS median of 2.4 months (hazard ratio [HR] = 2.50; 95% CI 1.31–4.39, p < 0.01) and overall survival (OS) median of 5.7 month (HR = 4.23; 95% CI 1.83–9.04, p < 0.01). The best results were noted in the CMS2 subtype with a PFS median of 8.0 months (HR = 0.67; 95% CI 0.44–1.01, p = 0.05) and OS median of 26.6 months (HR = 0.49; 95% CI 0.27–0.87, p = 0.02). On multi-causal analysis, status of DNA methylation turned out to be the only predictive factor both for PFS and OS, whereas location of the primary tumour (on the left side of the colon) was predictive for PFS. According to authors, both identification of CMS1 subtype as well as methylation status might be helpful when considering anti-EGFR therapy [14].

Our conclusions correspond with the results presented by Fontan and Sandandam during the ASCO GI conference (2018). In the RAS-wt colon cancer group, objective response for anti-EGFR therapy was vastly differentiated depending on subtype: CMS1 — 20%, CMS2 — 76%, CMS3 — 23%, and CMS4 — 88% [16].

It seems that the connection between molecular subtypes and location of primary tumour and prognosis is of relevance. During the FIRE-3, CRYSTAL post-hoc research it was proven that the location of the primary tumour in the proximal part of the colon is an adverse prognostic factor for PFS and OS [17].

Retrospective data analysis of 728 patients participating in the CALGB/SWOG 80405 trial (comparing bevacizumab and cetuximab combined with first-line chemotherapy in metastatic colon cancer) showed that patients with primary tumour on the left side of the colon have a significantly longer survival rate rather than those with primary tumour on the right side of the colon. OS median for left-side location was 32.9 months in comparison with 19.6 months for right side (p < 0.0001). In patients with KRAS/B~RAF~-wt, treated with cetuximab, the overall survival rate was far more different, in favour of primary tumour located on the left side of the colon (OS median: left-side vs. right-side, 40.3 vs. 18.4 months, p = 0.003). In turn, in a group with B~RAF~ mutation, treated with bevacizumab, results were more favourable for right-side location of primary tumour (OS median: left-side vs. right-side, 12.0 vs. 23.7 months, p = 0.035). Of the left-side tumour cases, the majority were CMS2 and CMS4 subtypes, and of the right-side tumour cases, CMS1 and CMS3 [18].

In research by Sagawa et al. results obtained in a group of patients treated with cetuximab, in terms of survival time free from progression and total survival time, were significantly better for patients with left-sided tumour (PFS: left vs. right, 18.3 vs. 6.8 months, p = 0.0415; OS: 50.6 vs. 10.5 months, p = 0.0004). PFS and OS were also significantly longer in right-sided colon patients treated with bevacizumab: left vs. right, 11.6 vs. 7.3 months (p = 0.1904) and 25.7 vs. 16.2 months (p = 0.0389). Administering cetuximab in a primarily right-sided disease was connected with longer survival rate compared to bevacizumab (PFS: cetuximab vs. bevacizumab, 18.0 vs. 11.6 months, p = 0.1088; OS: 50.6 vs. 25.7 months, p = 0.0354). There were no changes observed in patients with primary tumour located on the right side of the colon [19].

In additional analysis, conducted in the FIRE-3 (AIO KRK-0306) project, which compared the accuracy of cetuximab and bevacizumab combined with first-line chemotherapy, according to the FOLFIRI scheme, the CMS subtype was a relevant prognostic factor in terms of PFS (p < 0.001), OS (p < 0.001), and percentage of objective response (p = 0.023). The connection between overall survival rate and subtype and type of administered treatment was noted. In the CMS4 group this connection was statistically relevant, and the OS median for cetuximab vs. bevacizumab was 41.3 vs. 22.3 months (HR 0.53, p = 0.016). Details are presented in Table 2 [20].

The presence of molecular aims used currently in other types of cancer might be a premise for targeted therapy in particular subtypes of large intestine cancer. Approximately 3% of CMS3 cancer and 5% of CMS4 cancer have high protein expression of HER2 receptor. In these cases, anti-HER2 antibodies or tyrosine kinase inhibitors (TKI), related to the ERBB group, e.g. lapatinib and neratinib, might be active. Attempts to use immunotherapy with checkpoint inhibitors (inter alia pembrolizumab and nivolumab) might be most effective in CMS1, considering the immunogenetics [22].

**Conclusions**

Consensus molecular subtypes might be a helpful classification enabling better understanding of colon cancer’s biology. Subtypes: CMS1, CMS2, CMS3, and CMS4 may have a significant prognostic and predictive quality and might influence the choice of optimal treatment for particular patients (both in local and generalised disease). Most of the accessible research
results concern oxaliplatin, irinotecan, bevacizumab, and cetuximab. An advantage of bevacizumab in CMS3 and cetuximab in CMS4, and CMS2 was noted [17, 23]. It might be connected to proangiogenic factors produced e.g. by tumour macrophages [24]. The influence of complimentary treatment with oxaliplatin at the third stage of disease was noted mainly in CMS1 type, whilst in CMS3 it was almost non-existent [25]. Benefits of irinotecan therapy were mainly related to CMS3 and CMS4 presence (with CMS2 less effective) [13]. Moreover, biologic treatment and chemotherapy might mutually modify each other, e.g. combining oxaliplatin with cetuximab in an environment rich in fibroblasts such as CMS4 might be antagonistic [26]. The above-mentioned differences are eager to be responsible for fluctuating prognosis and therapy observed (depending on primary tumour location). According to research presented by Lenz in 2017, tumours located primarily on the left side were characterised by a significant percentage of right-sided CMS2 occurrence rather than left-sided — 48% vs. 23%, respectively, and lower CMS1 — 9% vs. 37%, respectively [27].

Based on previous research, it transpires that CMS1 (immunological type) might be treated optimally by oxaliplatin, bevacizumab combined therapy, CMS2 (canonical type) — cetuximab combined with oxaliplatin or irinotecan chemotherapy, CMS3 (metablic type) — oxaliplatin with cetuximab and CMS4 (mesenchymal) combined irinotecan chemotherapy with cetuximab [27]. There is less information available in terms of panitumumab therapy rather than cetuximab, but it cannot be excluded that the points previously stated might apply to the entire anti-EGFR group. Improving access to molecular research methods may popularise colorectal cancer classification and make it an efficient and crucial tool for therapeutic decisions.

Currently the main limitation of molecular testing, allowing to assess CMS, is its price, which is between 500 and 1500 euros.

### Table 2. Results of chemotherapy with cetuximab or bevacizumab due to consensus molecular subtypes (CMS) (based on [20])

<table>
<thead>
<tr>
<th></th>
<th>PFS median [months]</th>
<th>OS median [months]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FOLFIRI Cetuximab</td>
<td>FOLFIRI Bevacizumab</td>
</tr>
<tr>
<td>CMS1</td>
<td>33/35</td>
<td>32/35</td>
</tr>
<tr>
<td></td>
<td>(0.2–10.1)</td>
<td>(8.4–32.3)</td>
</tr>
<tr>
<td>CMS2</td>
<td>93/117</td>
<td>64/117</td>
</tr>
<tr>
<td></td>
<td>(9.5–14.9)</td>
<td>(27.5–49.2)</td>
</tr>
<tr>
<td>CMS3</td>
<td>27/30</td>
<td>20/30</td>
</tr>
<tr>
<td></td>
<td>(6.2–8.4)</td>
<td>(NE–41.2)</td>
</tr>
<tr>
<td>CMS4</td>
<td>81/93</td>
<td>56/93</td>
</tr>
<tr>
<td></td>
<td>(6.9–14.1)</td>
<td>(19.2–63.4)</td>
</tr>
</tbody>
</table>

*p — log-rank test; PFS — progression-free survival; OS — overall survival; FOLFIRI folinic acid, fluorouracil, irinotecan; HR — hazard ratio; NE — not examined

### References


