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Fundamentals of personalised medicine in colorectal cancer

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Personalised treatment which is a dynamically developing branch of medicine, is based on individualisation of diagnostic and therapeutic procedures. Its aim is to optimise treatment by increasing therapy effectiveness, while minimising side effects. It is designed both for patients with a diagnosed hereditary cancer syndrome, as well as those with sporadic cancers. In the case of a diagnosed colorectal cancer, personalised treatment requires patient selection based on predictive factors. This involves determination of the genetic status within the epidermal growth factor receptor (EGFR) signalling pathway, including assessment of the cancer tissue genotype with respect to *RAS* gene mutations (*KRAS*, *NRAS*) and *BRAF* gene mutations. In patients who do not respond to anti-EGFR targeted therapy, chemotherapy aimed at vascular endothelial growth factor (VEGF) is introduced. In personalised medicine it is also essential to introduce prophylactic and therapeutic measures, both in carriers of germline mutations, and members of their families who have not been diagnosed with this mutation, but who meet family history and clinical criteria of hereditary cancer syndrome.

Key words: personalised medicine, colorectal cancer, hereditary cancer syndrome, germline mutation, somatic mutation, epidermal growth factor EGFR, *RAS, BRAF*

Introduction

According to the National Cancer Register, the colorectal cancer is the third most common neoplasm diagnosed in Poland in men (after prostate cancer and lung cancer) and second in women (after breast cancer). The incidence is increasing gradually and since 1980 it has increased 4 times in men and 3 times in women [1].

Risk factors affecting development of the colorectal cancer include above all age, low-fibre diet, inflammation of the colon (e.g., ulcerative colitis and Crohn's disease), metabolic disorders (including mainly obesity, hypercholesterolemia, hypertension and diabetes), as well as smoking, polyps within the colon or diagnosis of the same neoplasm in members of the patient's family [2].

Genetic background of the colorectal cancer

The aetiology of colorectal neoplasms is complex. A vast majority, about 65–75% of them, are sporadic (non-hereditary) and in such cases the major risk factor is age. Further 10–15% are familial colorectal cancers. Both in the case of sporadic, and familial colorectal cancers, the basis for their development is complex: genetic ("genetic background" constituted by medium and low penetrance gene variants, which increase susceptibility to environmental carcinogens) along with the environmental exposure to carcinogens (usually shared for families). Variants in medium-penetrance genes confer increased cancer risk as compared to the general population, while variants in low-penetrance genes may modulate individual susceptibility to carcinogens [3].

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The remaining 5–10% of colorectal cancers are associated with hereditary predisposition. Such syndromes are suspected in families where the family history and clinical criteria for diagnosis/suspicion of a hereditary cancer syndrome are met (number of cases, relationship between patients, age of onset, histopathological diagnosis) [4].

Colorectal neoplasms, which develop as a result of hereditary cancer syndromes, may arise both on the basis of polyposis and without the increased number of polyps in the intestine [4, 5].

Hereditary cancer syndromes with polyposis-related colorectal cancer cases in their spectrum include [3]:

1. Adenomatous polyposis:
- *familial adenomatous polyposis* (FAP) – caused by *APC gene mutation,* characterised by autosomal dominant (AD) inheritance, including classic and benign forms of FAP, Turcot syndrome and Gardner syndrome,
- MAP syndrome (*MUTYH-associated polyposis*) – caused by mutations in the *MUTYH gene,* characterised by autosomal recessive (AR) inheritance.
2. Hamartomatous polyposis – dominantly autosomally inherited (AD):
- Peutz-Jeghers syndrome – caused by mutations in the *STK11* gene,
- Cowden syndrome – caused by mutations in the *PTEN* gene,
- hereditary mixed polyposis syndrome – caused by mutations in the *CRAC1* gene,
- juvenile polyposis of the colon – caused by mutations in the *BMPR1A* and *SMAD4* genes.

The only hereditary colorectal cancer syndrome without polyposis is Lynch syndrome (*hereditary non-polyposis colorectal cancer* – HNPCC) – caused mainly by mutations of *MLH1*, *MSH2*, *MSH6*, *PMS2* and *EPCAM* genes [3–5].

Hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome)

HNPCC is diagnosed in approximately 3–4% of colorectal cancer patients. The risk of cancer development in carriers of the syndrome's germline mutation (hereditary mutation present in all cells of the body) increases with age, reaching the lifetime level of 80% in men and 40% in women (average age of onset is 40 years, in contrast to sporadic cancers for which average age of onset is 60–70) [6, 7].

The people diagnosed with Lynch syndrome, apart from colorectal cancer, are also at an increased risk of developing other malignant neoplasms outside the large intestine. These neoplasms belong to the so-called spectrum of Lynch syndrome and include malignant neoplasms of the following organs [8, 9]:

- endometrium (risk of developing the disease 30–51%) and ovary (4–15%) in women,
- stomach (up to 18%) and small intestine (3–5%),
- collecting system of the kidney/ureter/bladder (2–20%),
- bile ducts / gallbladder,
- pancreas (4%),
- central nervous system (typically glioblastoma and astrocytoma),
- prostate (in carriers of the mutation in the *MSH2* gene),
- breast (in carriers of the mutation in the *MLH1* gene).

Carriers of pathogenic variants in the *MLH1* and *MSH2* genes have a significantly higher risk of developing colorectal cancer at an earlier age, as compared to the carriers of pathogenic changes in the *MSH6* and *PMS2* genes. The incidence of endometrial and urinary tract cancer is higher in carriers of the *MSH2* gene mutation [10].

Clinically, the following forms of Lynch syndrome are distinguished [8, 11]:

1. colorectal cancer only,
2. colorectal cancer and other cancers within the spectrum,
3. Torre Muir syndrome – malignant tumours of the colon and other diseases within the spectrum are associated with skin cancers (e.g., spinous cell carcinoma, squamous cell carcinoma, as well as sebaceous cysts and adenomas),
4. Turcot syndrome – coexistence of malignant tumours of the large intestine with primary brain tumours.

Genetic background of Lynch syndrome

The genetic background of Lynch syndrome, inherited autosomally dominantly, involves mutations in mutator genes (*DNA mismatch repair genes* – MMR genes), mainly *MLH1*, *MSH2*, *MSH6* and *PMS2*, as well as changes in the *EPCAM* gene (approximately 1–3% of HNPCC cases). Deletion of the *EPCAM* gene causes hypermethylation of the adjacent *MSH2* gene, which results in its inactivation [6, 10, 11, 12].

The role of mutator genes concerns coding proteins involved in the process of removing mismatched bases within the DNA chain; loss of their function leads to impairment of the process of repair of mismatched bases, thus causing accumulation of mutations within a cell. Loss of MMR gene/protein function is expressed in development of the "mutator phenotype", characterised by microsatellite instability (*MSI*), i.e., increased number of errors occurring at replication of the DNA chain – mainly in repeated sequences called microsatellites. More than 70% of mutations in tumours with high microsatellite instability are identified in the *MLH1*, *MSH2* and *EPCAM* genes [6, 13].

Mutations inactivating the MMR genes lead to the lack of expression of the corresponding MMR protein evidenced by immunohistochemistry test (*immunohistochemistry staining* – IHC). MMR and IHC tests performed in colorectal tumours allow for identification of the microsatellite instability status and are characterised by high sensitivity (approx. 94%) and specificity (approx. 88%) [6].

Approximately 15–20% of sporadic colorectal carcinomas show microsatellite instability and loss of expression of *MLH1*

in tumour tissue, most commonly due to somatic hypermethylation of the *MLH1* gene promoter associated with a BRAF *V600* gene mutation. Therefore, when loss of expression of *MLH1* is present (alone or with loss of expression of *PMS2*), it is necessary to first exclude hypermethylation of the *MLH1* promoter in the tumour or assess the presence of the somatic mutation V600 in the *BRAF* gene. In the case when loss of *MLH1* expression coexists with loss of expression of *MSH2*, *MSH6*, or isolated expression of *PMS2* gene, genetic analysis should be performed for presence of germline mutations in the above genes. The MMR IHC and/or MSI test, followed by the analysis of hypermethylation of the promoter of the *MLH1* gene (in the case of loss of expression of *MLH1* gene), should also be performed in women diagnosed with endometrial cancer, due to the fact that 2–3% endometrial carcinomas belong to the spectrum of tumours in Lynch syndrome [6, 11].

Presence of somatic mutations of the *BRAF* protooncogene in colorectal tumours allows for excluding with high probability the Lynch syndrome, indicating sporadic disease. However, absence of V600 mutation within the *BRAF* does not unequivocally signify diagnosis of Lynch syndrome-related colorectal cancer [5, 6].

In patients who cannot have molecular testing of tumour tissue performed, predictive models are applied which allow estimation of probability of finding a pathogenic variant of a mutator gene (PREMM 5 MODEL). The clinical criteria used to identify people with suspicion of Lynch syndrome are the Amsterdam II criteria and the modified Bethesda criteria [3, 6, 8].

The detailed algorithm of management in patients with diagnosed colorectal cancer, depending on availability of tumour tissue, is described in ESMO recommendations [6].

Amsterdam criteria II (fulfilment of all criteria allows for clinical diagnosis of Lynch syndrome, is an indication for genetic diagnosis of this disorder and an indication for the implementation of preventive recommendations, even in the absence of molecular confirmation of the syndrome):

- at least 3 family members with histopathologically confirmed LS spectrum malignancy,
- cases of colorectal cancer or LS spectrum neoplasms in at least 2 consecutive generations,
- at least one of those suffering from colorectal cancer or LS spectrum cancer is a first degree relative to the others,
- at least one case of colorectal cancer or LS spectrum cancer occurred before the age of 50,
- in the case of colorectal cancers, familial polyposis (FAP) should be excluded,
- verified histopathological diagnosis.

Modified Bethesda criteria (meeting at least one of them is an indication for molecular diagnostics for Lynch syndrome):

- colorectal cancer diagnosed before the age of 50,
- multifocal colorectal cancer regardless of the age of diagnosis (applies to both synchronic and metachronic foci),
- colorectal cancer with high microsatellite instability, diagnosed before the age of 60,
- colorectal cancer in the patient and at least one neoplasm from the LS spectrum in 1st/2nd degree relatives, including at least one onset before 50 years of age,
- colorectal cancer in the patient and at least 2 malignant neoplasms from the LS spectrum among 1st/2nd degree relatives, regardless of age.

Genetic diagnostics in Lynch syndrome

Testing for hereditary mutations is performed on DNA isolated from the patient's somatic cells (lymphocytes, mucosa cells). Due to the complex molecular background (diversity of genes involved in the aetiology of the syndrome) and the multitude of pathogenic changes occurring within them, (nonsense mutations, missense changes) reading frame shift, splicing mutations, as well as large rearrangements, i.e. deletions/duplications or inversions), the genetic diagnostics of Lynch syndrome should include, first of all (due to the significant predominance of point mutations) sequencing (using the method *next generation sequencing* – NGS) of the gene panel of *MLH1, MSH2*, *MSH6*, *PMS2*. If no mutations are detected in the sequencing of the above genes, as well as *EPCAM*, the MLPA method (*multiplex ligation-dependent probe amplification*) should be used to analyse the presence of large rearrangements within the studied genes [3, 14].

Prophylaxis for Lynch Syndrome

Prophylactic care should be applied to people in predisposed families, as diagnosed based on the analysis of the family history and clinical criteria, and to people with diagnosed critical mutation (even if the family history and clinical criteria are not met). It is aimed at early detection of cancer through active supervision of people at increased risk, thus extending their survival time and improving the quality of life. Thanks to the advances in oncogenetics, such supervision may be adapted to the identified genetic change and family history of disease [12].

Further, at-risk patients are advised to avoid carcinogens, including especially smoking, and to observe healthy lifestyle, including maintaining the normal body weight. Detailed rules of preventive treatment are presented in table I.

Familial adenomatous polyposis (FAP)

Hereditary familial adenomatous polyposis syndrome accounts for less than 1% of all cases of malignant colorectal neoplasms, while being the most common cause of polyposis with a known genetic basis. FAP is characterised by autosomal dominant inheritance and it is caused by germline mutations in the APC suppressor gene [6, 7, 11].

Table I. Principles of prophylactic management for patients at risk of HNPCC based on the NCCN, ESMO and NMHN guidelines. [4, 6, 8, 10, 15, 16]

Organ	Study type	Age	Frequency
large intestine	• colonoscopy*1 • in patients with diagnosed cancer – colectomy*2	• MSH1/MSH2 25 years of age • MSH6/PMS2 35 years of age • or 5 years earlier than the earliest disease in the family, if the diagnosis <25 years of age	every 12–24 months
uterine body	• transvaginal ultrasound • biopsy of the uterine body*3 • prophylactic hysterectomy and/or bilateral adnexectomy*4	30–35 years of age	• every 12 months • in any case of atypical vaginal bleeding (beyond the expected menstruation or after the end of menstruation)
ovary	• transvaginal ultrasound • CA-12 marking • prophylactic hysterectomy and/or bilateral adnexectomy*4	30–35 years of age	every 12 months
stomach	• upper GI endoscopy • *Helicobacter pylori* testing should be considered in all mutation carriers	30–35 years of age	every 24–36 months
pancreas	• MRI and/or ultrasound to be considered*5	50 years of age or 5 years earlier than the earliest disease in the family	
urinary tract	no confirmation of the effectiveness of the test due to a too high percentage of false-positive results		
CNS	neurological examination		every 12 months

*1 Indigo carmine chromoendoscopy has been shown to be significantly more effective in people with LS compared to standard colonoscopy. It is recommended to perform the test in reference centres.

*2 It has been shown that there is an increased risk of metachronic colorectal cancer after partial colectomy and that patients' quality of life was similar after partial and total colectomy. Therefore, extended colectomy should be an option for patients with Lynch syndrome undergoing primary surgery for colorectal cancer, especially if the disease occurs at a young age.

*3 Recommended for identification of patients with precancerous endometrial lesions or asymptomatic endometrial cancer.

*4 In the case of mutation carriers who have completed their procreation plans (optimally at 35–40 years of age); after surgery, HRT at the lowest effective dose should be considered.

*5 Recommended for patients with pancreatic cancer who have a 1st degree relative with the same cancer.

The clinical diagnosis of familial adenomatous polyposis is based on the following phenotypes [3, 4, 17]:

1. Classic FAP:
- presence of over 100 adenomatous polyps in the large intestine (polyps may appear as early as in childhood, and from 40 to 50 years of age the risk of cancer development is up to 98%),
- fewer than 100 polyps in the large intestine and at least 1 relative diagnosed with FAP.
2. Attenuated FAP (AFAP):
- fewer than 100 colon polyps before the age of 30 and/or
- a relative with confirmed AFAP, and/or
- more than 100 polyps in the colon over the age of 40.
3. Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS):
- presence of polyps confined to the stomach body and fundus,
- more than 100 (sometimes thousands) polyps in proximal stomach or more than 30 polyps in a 1st degree relative of a person with GAPPS,
- polyps most commonly derived from the fundic glands of the stomach (*fundic gland polyps* – FGPs), some of which may have regions of dysplasia, or a family member of FGPs with dysplasia or gastric adenocarcinoma,
- no polyps in the large intestine and duodenum.

Extraintestinal symptoms of FAP

Hereditary familial adenomatous polyposis in a disease associated with extraintestinal symptoms, and the risk of their occurrence increases with age [3, 18, 17]. They include:

- congenital hypertrophy of the retinal pigment epithelium (CHRPE) – the risk of developing it reaches 70–80%,
- epidermoid tumours – 50%,
- osteomas of the mandible – 50–90%,
- desmoid tumours – 10–15%,
- changes in dentition, including extra teeth – 11–27%,
- polyps located in the higher parts of the digestive tract (the bottom of the stomach and duodenum),
- increased risk of neoplasms, including thyroid gland (papillary carcinoma, risk of 2–3%), stomach, duodenum, brain (usually medulloblastoma, risk <1%), and hepatoma (approximately 1%).

The number of polyps is closely correlated to the risk of developing colorectal cancer and to the location of the

mutation in the *APC* gene. Nonsense mutations (appearance of a stop codon) located between codon 169 and 1600 co-exist with the phenotype of the classical FAP syndrome with hundreds of polyps. Pathogenic changes around codon 1300 cause the appearance of thousands of polyps and correlate with the highest risk of developing colorectal cancer. Mutations present in 5′ direction from codon 160 and 3′ from codon 1600 are associated with a benign form of inherited familial adenomatous polyposis.

The number of polyps has a significant impact on the risk and the age at which a polyp will become malignant. In patients with a molecular confirmation of mutated APC gene and with presence of thousands of polyps, without introducing prophylactic measures, colorectal cancer is diagnosed on average at 28 years of age, while the average age of onset in patients with hundreds of polyps is 44 years, and in people with mild polyposis – about 55 years. Further, mutations in the 3′ region from codon 1400 cause an increased risk of desmoid tumours, while pathogenic changes located 5′ from exon 9 (codons 312–438) do not cause CHRPE (except for single changes in exon 6) [19].

Genetic diagnostics of FAP

In the case of hereditary familial adenomatous polyposis, even 20–25% of the mutations are *de novo*, which means that there is no family burden (the family history and clinical criteria for suspicion/diagnosis of the syndrome are not met). Germline mutations in the *APC* gene are responsible for approximately 90% of classic FAP cases. Molecular diagnostics should include sequencing of this gene (tests can be started with the analysis of the presence of the 4 most common mutations in exon 11, i.e., c.1500T > A (p.Tyr500X), c.3183_3187delACAAA, c.3202_3205delTCAA, c.3927_3931delAAAGA). If no mutation is identified in the patient, large rearrangements within the *APC* gene or the region in which it resides should be analysed. With availability of multi-gene panels, it is also possible to analyse genes related to colon polyposis at the same time, including *MUTYH*, *POLE*, *POLD1*, *NTHL1*, *STK11*, *SMAD4*, *BMPR1A* [3, 19].

If polyposis of the colon is found or a familial mutation is identified, genetic diagnostics should be provided to all members of the family selected based on the phenotype, even before they turn 18 (considering the risk of FAP syndromes onset in early childhood) [17].

Prophylactic management of FAP

Increased surveillance should be provided for all mutation carriers, as well as members of the given family in whom no germline mutation can be identified. The rules of prophylactic treatment for patients at risk of FAP are presented in table II.

Diagnostic options available in the funding programme of the Ministry of Health

In Poland, in accordance with Module II of the National Cancer Control Program the Ministry of Health for 2018–2021,

Table II. Rules of prophylactic management for patients at risk of FAP based on the NCCN, ESMO and NMHN guidelines [4, 6, 10, 15, 16, 17]

Organ	Study type	Age	Frequency
large intestine	• flexible sigmoidoscopy and colonoscopy (in the case of adenomas and depending on age) • preventive colectomy/proctocolectomy at the age of 16–20	from 10–15 years of age	• every 12–24 months, gradually extending the period between tests to 36 months • in patients after colectomy – colonoscopy every 6–12 months (depending on the presence of polyps)
duodenum	• endoscopy of the upper digestive tract (front and side view)	• from 25–30 years of age (according to ESMO) • from 20–25 years of age (according to NCCN) • depending on the family burden	every 1–5 years[*1]
stomach	• endoscopy of the upper digestive tract (front and side view)	from 25–30 years of age	
thyroid	• thyroid ultrasound • palpation	from 25–30 years of age	every 12 months
liver	• marking of blood serum alpha-fetoprotein • abdominal ultrasound • liver palpation	up to 7 years of age	every 3–6 months
desmoid tumours	• CT • MRI		
pancreas	• abdominal ultrasound	depending on family history	
CNS tumours	• physical examination (due to limited data, no indications for imaging tests)		every 12 months

[*1] testing frequency should be based on Spiegelman's guidelines

genetic and preventive diagnostics is available for families with suspected hereditary cancer syndromes with dominant predisposition to development of colorectal cancer, including:

- familial adenomatous polyposis syndrome (FAP),
- Lynch syndrome (HNPCC),
- Peutz-Jeghers syndrome (PJS),
- juvenile polyposis (JPS),
- recessive polyposis syndrome, which is conditioned by mutations in the *MUTYH* gene.

The diagnostics is aimed at identifying the mutation (in the first place) in the sick person, or in the absence of such a possibility (e. g. death, no consent to perform a genetic test) in a 1st degree relative. This allows for the introduction of an optimal scheme of care for the mutation carrier and their family, which (in the long term) increases the survival time of the carrier of the *APC* gene mutation by about 10–12 years and helps to extend the survival time of the carriers of mutation in *MLH1*, *MSH2*, *MSH6*, *PMS2, STK11*, *SMAD4*, *BMPR1A*, *EPCAM* and *MUTYH* genes.

Patients are qualified for the program by a clinical genetics specialist on the basis of family history and clinical data, which take into account the type/location of the neoplasm and the age of disease onset in both the probate and their first- and second-degree relatives, possibly other family members. Module II provides for:

- detection of mutations in genes: *APC*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *STK11*, *SMAD4*, *BMPR1A*, *EPCAM* and *MUTYH* in the carrier (including molecular testing, immunohistochemistry and microsatellite instability assessment),
- evaluation of the expression of mutator genes in colorectal cancers diagnosed before the age of 60,
- regular colonoscopy, gastroscopy, gynaecological ultrasound and serum Ca-125 marking [15].

Personalised treatment for colorectal cancer

Personalised medicine, which is currently used in oncology, is intended both for patients with diagnosed hereditary cancer syndrome and patients with sporadic neoplasms. The concept of "individual" (personalised) treatment requires selection of patients based on molecular predictors (necessary to assess the response to treatment) in order to increase the effectiveness of therapy and minimise exposure to adverse effects [20].

Personalised treatment of colorectal cancer based on molecular characteristics of the tumour tissue, started from confirmation of the fact that downstream abnormalities in genes of the EGFR signalling pathways (epidermal growth factor receptor) contribute to development of this neoplasm. Normal cells divide in response to growth factor signals that interact with cell surface receptors. In the case of an increase in the number of growth factor receptors or their excessive sensitivity, as in the case of colorectal cancer, signals are sent to the inside of the cell leading to its excessive and uncontrolled proliferation. Therefore, for a dozen years now, cetuximab and panitumumab (monoclonal antibodies to bloc EGFR receptor) have been applied in patients with metastatic colorectal cancer. The mechanism of both antibodies is the same as they bind to the extracellular ligand-binding domain, thereby inhibiting the activity of EGFR signalling pathways. These are mainly two intracellular pathways: RAS-RAF-MEK-ERK (responsible for the proliferation of tumour cells) and PI3K-PTEN-AKT (responsible for the survival, growth and invasion capacity of the tumour) [21].

The results of many studies over the past decades have shown that both cetuximab and panitumumab become ineffective in the presence of mutations in genes regulating subsequent steps in intracellular signalling. Therefore, an indispensable part of the targeted treatment in patients with metastases of colorectal cancer is to assess the genotype of the neoplastic tissue in terms of mutations in the *RAS* genes (especially *KRAS* and *NRAS* – mutations present in 30–50% of CRC patients), as well as the *BRAF* gene (mutations occurring in approximately 8–12% of patients). Pioneering observations allowed a conclusion that benefits of treatment with anti-EGFR antibodies concern patients with the correct form of the *KRAS* gene (wild type – WT), while further analyses proved that the presence of mutations in this gene is a negative predictor of response to anti-EGFR therapy. Many analyses carried out so far also indicate that the *KRAS* gene should be assessed along with other biomarkers of the RAS-RAF-MEK-ERK and PI3K-PTEN-AKT pathways, as the expected response to treatment is also determined by the condition of the remaining components of signalling pathways [21, 22].

The success of therapy in patients with metastatic colorectal cancer is also threatened by the risk of mutations in *RAS* genes occurring during the treatment and leading to developing resistance to anti-EGFR drugs. To avoid another tumour tissue biopsy, it is possible to detect development of these mutations by analysing the DNA of the tumour tissue circulating in the patient's blood, but this technique is not routinely applied.

In patients with tumours not responsive to anti-EGFR components-based chemotherapy, another class of drugs can be applied that target vascular endothelial growth factor (VEGF) instead of epidermal growth factor. This factor promotes the growth of blood vessels, including those that supply blood to the neoplastic tissue, and when it's blocked, sufficient blood supply to the tumour is prevented, thus causing its contraction. The most commonly used anti-VEGF drug is the monoclonal antibody bevacizumab. Aflibercept and ramucirumab have also been used [22, 23].

Selection of tissue for biomarker testing is important, and so is enrichment of samples by macrosection to maximise the tumour cell content (>50%) prior to DNA isolation. Primary tumour tissue or liver metastases are recommended for the *RAS*

mutation test. Other sites of metastasis (lymph nodes, lungs) are only considered if no primary tumour specimen or liver metastases are available. In parallel, in each case, the status of the *BRAF* mutation should be assessed for prognostic and, to a lesser extent, predictive assessment [22].

RAS mutations

The presence of mutations within the proto-oncogene family of *RAS* genes (including *KRAS* – about 40%, *NRAS* – 3–8% and *HRAS* – 3–4%) is a negative predictive biomarker for anti-EGFR antibody therapy in patients with metastatic colorectal cancer. This is due to the fact that, despite the inhibition of EGFR activity, EGFR-independent signal transmission takes place via the RAS-RAF-MEK-ERK pathway to the cell nucleus. This leads to increased, uncontrolled proliferation. Therefore (in accordance with the applicable standards) testing for this mutation should be performed in all patients at the time of diagnosis; however, it is mandatory prior to treatment with anti-EGFR monoclonal antibodies (cetuximab and panitumumab). The current standards, approved by ESMO and NCCN, require confirmation of *KRAS* wild-type (no mutation) before initiating cetuximab and panitumumab treatment. Moreover, patients with a confirmed mutation in the *KRAS* gene are not treated with anti-EGFR monoclonal antibodies, because such therapy does not bring any benefits, and additionally it may lead to a shortened survival while causing exposure to numerous side effects [21, 24].

BRAF mutations

Mutations of the *BRAF* proto-oncogene are present in tumour tissue in approximately 8–12% of patients with metastatic colorectal cancer and are excluded from the *KRAS* mutations. More than 90% of these mutations concern codon 600 (V600), where valine is replaced with another amino acid, most often glutamic acid (V600E). Therefore, in accordance with the guidelines of NCCN and ESMO, it is recommended to analyse the *BRAF* mutation in cancers with wild-type *KRAS* before administration of anti-EGFR therapy (currently *BRAF* mutation status is assessed in parallel with the *RAS* mutation status).

A study of patients with metastatic colorectal cancer with identified *BRAF* mutation found that two-thirds of them had the primary tumour on the right side of the colon and associated with an increased risk of metastases to the peritoneum and distant lymph nodes, with a concomitant reduced frequency of lung metastases. Presence of the *BRAF* gene mutation was also shown to be a negative prognostic marker, with a 10.4-month survival in patients with the current mutation, compared to 34.7-month survival in patients with wild-type BRAF tumours. Nearly one-third of tumours with the *BRAF* mutation also showed microsatellite instability (MSI), and the same percentage of MSI tumours contained the *BRAF* mutation [22, 25, 27].

Contrary to the predictive status of *KRAS*, the value of the mutation *BRAF* is still under investigation. It seems that the predictive value of *BRAF* depends on whether patients receive anti-EGFR preparations in first-line treatment (most often chemosensitive tumours) or in the 2nd- or 3rd-line treatment (chemoresistant tumours) [25].

Genetic/dagnostic tests used to assess mutation status of *RAS* and *BRAF* genes

Assessment of the mutational status of the *RAS* and *BRAF* genes in treatment of metastatic colorectal cancer has become a standard of diagnostic procedure in recent years, which supports the selection of the best therapy for a given patient.

Most often, DNA is isolated from previously prepared paraffin blocks. A qualified pathologist, who assesses the percentage of neoplastic cells in such a preparation, plays an important role in choosing the right block. Therefore, close collaboration between pathologists and molecular biologists is essential. Moreover, the technique of producing the block, including the buffers used, are of great importance for the quality of the nucleic acids isolated from them.

A laboratory where genetic tests are performed requires not only appropriate equipment and highly qualified personnel, but it should also participate in international quality control tests to confirm quality of tests performed. In the case of *RAS* and *BRAF* genes, tests are organised, among others, by the European Society of Pathology: Colon External Quality Assessment Scheme.

Currently, commercially available kits are used for routine assessment of the status of the *RAS/BRAF* genes. They allow assessment of the most frequent mutations and the analysis of pathogenic changes in *RAS* should cover at least exons 2, 3 and 4 of the *KRAS* genes (codons 12, 13, 59, 61, 117 and 146), as well as exons 2, 3 and 4 of the *NRAS* gene (codons 12, 13, 59, 61 and 117) [22]. The advantage of ready-made tests over tests created independently by a laboratory involves validation as well as approval/certification for *in vitro* diagnostics (CE-IVD). This, in turn, is related to the high reliability of the obtained results. Currently, there are also several commercial kits approved by the US Food and Drug Administration (*FDA)*. The assays for the *RAS/BRAF* mutations are mainly based on the Real-Time PCR method, assessing more than ten mutations in the *KRAS/NRAS* and mutations in the *BRAF* V600 gene at the same time. There is also commercially available, FDA-approved kit for next generation sequencing, which allows assessment of 56 mutations in *KRAS/NRAS*. The scope of testing variants is constantly updated according to the latest knowledge and recommendations.

PI3K/PTEN/AKT axis

The correct form of the *RAS* gene (in particular *KRAS*) does not guarantee a positive response to anti-EGFR treatment. This means that the therapy in patients with colorectal cancer depends also on other mechanisms, hence the need to analyse other markers. The PI3K/PTEN/AKT pathway is also associated with the KRAS/

BRAF signalling pathway. Mutations in the *PIK3CA* gene (which codes the catalytic subunit p110α of the PI3K enzyme) occur in about 10–20% of colorectal cancers and are associate both with *KRAS* mutations, and tumour microsatellite instability [26]. The mutated form of *PIK3CA* leads to constant signal transmission to AKT pathway, inducing increase and proliferation of tumour cells. In turn, the protein product of the suppressor gene *PTEN* (phosphatase and tensin homolog; a component of the pathway) is responsible for inhibiting the AKT kinase pathway. Loss of PTEN activity (most often caused by gene mutations, its deletion or promoter methylation) leads to hyperactivation of the PIK3/AKT pathway. There were individual cases of coexistence of *PIK3CA* mutation and *PTEN* inactivation. Data on the influence of these disorders on the response to treatment with anti-EGFR preparations are contradictory, which makes it difficult to assess their value as predictors. This is most likely due to the variety of mutations that can appear within *PIK3CA*. The most frequently found and analysed mutations (*hotspots*) are variants present in exons 9 and 20 of the *PIK3CA* gene. Based on the results of experimental and epidemiological studies, it seems that mutations present in exon 20 play a significant role in the treatment, as opposed to mutations occurring in exon 9. At present, the predictive value of the *PIK3CA* gene mutation for anti-EGFR therapy in patients with normal (wild) type *RAS* genes is low and further research is required [21].

Defining therapeutic strategy

The optimal therapeutic strategy for each patient is determined on the basis of a clinical examination, blood count, determination of the parameters of kidney and liver function, measurement of the level of tumour markers, imaging tests (including CT and MRI of the abdominal cavity and chest) and assessment the patient's general clinical condition. The general condition and fitness of the patient are important both prognostic and predictive factors for the introduced chemotherapy (tab. III) [22, 23].

1. First-line treatment

- FOLFIRI (leucovorin + fluorouracil + irinotecan) + cetuximab (in cases of no mutations in *RAS* and *BRAF*),
- FOLFOX (leucovorin + fluorouracil + oxaliplatin) + panitumumab (in cases of no mutations in *RAS* and *BRAF*),
- FOLFIRI + panitumumab (in cases of no mutations in *RAS* and *BRAF*),
- FOLFIRI + bevacizumab (in cases of *RAS* mutation), combined with prior adjuvant chemotherapy including oxaliplatin, and resection of the primary lesion,
- FOLFOXIRI (leucovorin + fluorouracil + oxaliplatin + irinotecan) + bevacizumab (in cases with *BRAF* mutation) and removal of the primary lesion [29],
- fluoropyridine monotherapy in patients who do not tolerate aggressive treatment [22, 23, 27, 28].

2. Second-line treatment

- FOLFOX + bevacizumab (provided that no adjuvant chemotherapy containing oxaliplatin and resection of the primary lesion were applied),
- FOLFIRI + aflibercept (with no irinotecan chemotherapy applied, in cases of no effect of oxaliplatin and fluoropyrimidine chemotherapy and resection of the primary lesion) [22, 23, 27].

Second-line therapy begins with the change of the first-line therapy strategy, primarily because of the failure of the original assumptions. It is usually offered to patients in good general condition, with normal internal organ function and depends on the choice of first-line therapy.

3. Third-line treatment

- cetuximab or panitumumab (in cases of no *RAS* and *BRAF* mutations and no prior anti-EGFR treatment),
- regorafenib (recommended in patients previously treated with fluoropyrimidine, oxaliplatin, irinotecan, for whom treatment with anti-VEGF or anti-EGFR is not considered),
- trifluridine with tipiracil (Lonsurf) with insensitivity to previous systemic therapy based on fluoropyridine, oxaliplatin and irinotecan,
- if microsatellite instability (6–8% of tumours) and resistance to chemotherapy are diagnosed, anti-PD-1 immunotherapy should be considered [22, 23, 27].

Table III. Selection of systemic therapy in accordance with the treatment algorithm for patients with metastatic colorectal cancer (excluding patients with oligometastases) – based on the ESMO recommendations

Treatment objective	Cytoreduction (tumour atrophy)			Disease control (progression control)		
molecular profile	*RAS* wt	*RAS* mt	*BRAF* mt	*RAS* wt	*RAS* mt	*BRAF* mt
first line						
preferred choice	double chemotherapy + EGFR antibody	double chemotherapy + bevacizumab	FOLFOXIRI + bevacizumab	double chemotherapy + bevacizumab or dual chemotherapy + EGFR antibody	double chemotherapy + bevacizumab	FOLFOXIRI +/– bevacizumab
second choice	FOLFOXIRI +/– bevacizumab	FOLFOXIRI + bevacizumab	double chemotherapy + bevacizumab	FP + bevacizumab		double chemotherapy + bevacizumab
third choice	double chemotherapy + bevacizumab	FOLFOXIRI	FOLFOXIRI			

Treatment objective	Cytoreduction (tumour atrophy)			Disease control (progression control)		
observation						
preferred choice	FP + bevacizumab	FP + bevacizumab	FP + bevacizumab	FP + bevacizumab	FP + bevacizumab	FP + bevacizumab
second choice	intermission	intermission	intermission	intermission	intermission	intermission
second line						
preferred choice	double chemotherapy + bevacizumab	double chemotherapy + bevacizumab	double chemotherapy + bevacizumab	dual chemotherapy + bevacizumab or dual chemotherapy + EGFR antibody	double chemotherapy + bevacizumab	double chemotherapy + bevacizumab
second choice	dual chemotherapy + EGFR or FOFIRI antibody + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab
third line						
preferred choice	double chemotherapy + EGFR antibody or irinotecan + cetuximab	regorafenib or trifluridine/ tipiracil	regorafenib or trifluridine/ tipiracil	double chemotherapy + EGFR antibody or irinotecan + cetuximab	regorafenib or trifluridine/ tipiracil	regorafenib or trifluridine/ tipiracil
second choice	monotherapy with EGFR antibodies			monotherapy with EGFR antibodies		
third choice	regorafenib or trifluridine/ tipiracil			regorafenib or trifluridine/ tipiracil		

FP – fluoropyrimidine; mt – mutation; wt – wild type; EGFR antibodies – cetuximab and panitumumab [22]

Conclusion

Year by year, personalised medicine is ever more broadly applied in management of cancer, especially colorectal cancer. Application of targeted therapy based on molecular predictors is aimed at administering treatment which would increase survival time and improve life comfort by minimising adverse effects of the therapy applied. Further, identification of patients with familial cancer predisposition allows introduction of prophylaxis and diagnostic-prophylactic process for all relatives of the patient selected based on the family history. Implementation of such procedures in the case of colorectal cancer and other cancers in its spectrum requires cooperation of a team of specialists, including a clinical geneticist, surgeon, oncologist, pathologist and lab diagnostician.

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