Improving the management of colorectal neoplasms in clinical practice

Leicher, L.W.

CHAPTER 3

Consequences of testing for Mismatch Repair deficiency of colorectal cancer in clinical practice

3

L.W. Leicher*, M.H.A. Lammertink*, S.R. Offerman, H. Morreau, M.M. de Jong, J.W.B. de Groot, H.L. van Westreenen, H.F.A. Vasen, W.H. de Vos tot Nederveen Cappel.

** both authors equally contributed to this manuscript.*

Scandinavian Journal of Gastroenterology, 2017.

ABSTRACT

INTRODUCTION

Mismatch repair deficiency (dMMR) can be found in Lynch syndrome (LS)-associated colorectal carcinoma and in 15% of sporadic colorectal cancer(CRC). Outcome of MMR-deficiency testing is important for surgical decisions as extended colectomy is recommended in young LS-patients with CRC. Moreover, the finding of a dMMR tumour has consequences for the choices of adjuvant chemotherapy as MMR-deficient CRC is resistant to 5-fluorouracil (5-FU) monotherapy. Aims of our study are to evaluate whether MMR-deficiency testing leads to (1) identification of LS, (2) change in surgical treatment and (3) adjustment of systemic therapy in patients with dMMR CRC.

METHODS

We performed a multicentre, retrospective study, in a community hospital and a University Medical Centre. We included all CRC-patients between 2012 and 2016 who were tested for microsatellite instability. We collected clinical data such as gender, age, referral to clinical geneticist, surgical procedure and choice of chemotherapy.

RESULTS

We analysed 225 CRC's. Twenty-four (10,7%) of 225 CRC were MMR-deficient. Of the 24 patients with dMMR CRC, 18 (75%) were referred to the clinical geneticist and in 9 (37%) patients a MMR mutation was identified. In one (4%) of 24 the patients a subtotal colectomy was performed. In 7 (35%) out of 20 MMR deficient patients the chemotherapy regimen was adjusted.

CONCLUSION

The finding of a dMMR CRC had consequences for decisions on chemotherapy in a relative high proportion of patients. We recommend testing in all patients with CRC independent of age at diagnosis, as proper treatment decisions and genetic counselling are very important.

INTRODUCTION

The most common hereditary variant of colorectal cancer worldwide is Lynch syndrome (LS) which accounts for 2-5% of all new CRC cases.[1] In LS patients, the lifetime risk of developing CRC varies between 25 and 75% depending on the underlying gene defect.[2] Other LS-associated tumours are cancer of the endometrium, stomach, hepatobiliary tract, ovaries, urinary tract, and small bowel.[3] LS is characterized by an early age of onset of CRC and a higher risk of developing synchronous and metachronous CRC or LS-associated tumours.[1-3]

In LS, a pathogenic germline mutation in one of the DNA mismatch repair (MMR) genes (*MLH1, MSH2, MSH6* or *PMS2*) causes genomic instability in the tumour, called microsatellite instability (MSI), the hallmark of LS.[4,5] MSI analysis is performed by polymerase chain reaction (PCR) with specific microsatellite markers. Through immunohistochemistry (IHC) the absence of the MMR proteins can be detected with specific antibodies.[6,7] Tumours with MSI or MMR protein expression loss are called MMR-deficient. MSI is also present in 15% of sporadic CRC due to hypermethylation of the *MLH1* promoter.[8,9] In order to differentiate between LS and sporadic tumours a methylation-specific PCR (MSP) is performed. Patients with MMR deficiency without hypermethylation should be referred to the clinical geneticist for mutation analysis of the MMR-genes.

Through identification of LS families, family members that turn out to be mutation carriers are invited to participate in surveillance programs. Long-term surveillance leads to risk reduction of developing CRC by removing adenomas, the detection of CRC at an earlier stage and reduction of mortality associated with CRC.[10] Until recently, the revised Bethesda guidelines were used to identify individuals with CRC that should be tested for MSI.[11,12] Nowadays however, in many countries MSI analysis or IHC is performed in all CRC patients under the age of 70 years. Subsequently, the chance of missing LS in patients with CRC is low and this also turned out to be cost-effective.[13]

The risk of developing CRC during surveillance with intervals of 1-2 years is 6% in 10 years.[14] The majority of these tumours (>85%) are at stage I or II.[15] In LS patients who developed CRC the risk of developing metachronous CRC is reported to be approximately 16% at 10 years follow-up following segmental resection or hemicolectomy, despite close surveillance.[16] The overall life expectancy gain of subtotal colectomy compared to hemicolectomy at ages 27, 47 and 67 was respectively 2.3, 1, and 0.3 years.[17] Therefore, the option of subtotal colectomy should be discussed in young patients (<60 years) who develop CRC while under surveillance. However, in many cases the diagnosis of LS is not known at time of surgery, unless MSI analyses

and immunohistochemical analysis of the MMR-proteins (IHC) are performed on biopsies taken at endoscopic diagnosis.[18,19]

Tumours with MMR deficiency are associated with a better overall survival.[20] Also many studies showed that patients with MSI-high stage II and III CRC do not benefit from adjuvant chemotherapy with 5-fluorouracil (5-FU).[21-25]
The aim of our study is to evaluate all the above described consequences of MSI-analysis or IHC in daily clinical practice. Are patients with MMR-deficient tumours referred to the clinical geneticist and how many LS families are identified? Does MSI status influence surgical treatment and does it influence the decision on the type of adjuvant chemotherapy?

METHODS

Study design

We performed a multicentre retrospective observational study in the Netherlands. Participating hospitals included a large community hospital, Isala Zwolle, and the Leiden University Medical Centre (LUMC). We included patients from April 2012 to January 2016. Our study was approved by the local research ethics committee. Our primary outcomes are referral to the clinical geneticist, changes in type of surgery and changes in the choice of adjuvant chemotherapy.

Patients

We included all patients with a primary CRC who were analysed for MSI or MMR protein expression loss and were discussed both preoperatively and postoperatively in a multidisciplinary team of specialists. MSI analysis or MMR-protein analysis was performed in all consecutive CRC patients who fulfilled the Bethesda criteria.[12] Additionally, a small proportion of patients were tested according to the new Dutch guideline "Hereditary Colorectal Cancer" published in January 2016, recommending MSI analysis or immunohistochemical testing in all patients with CRC <70 years. This guideline was already implemented a few months before publication in the LUMC what explains a small proportion of patients <70 years included.

Patients who were already diagnosed with LS were excluded. Medical reports were retrieved, including the documentation of the multidisciplinary meeting, surgical report, histology report, correspondence of the clinical geneticist and the treatment of the oncologist. Patients variables (sex, age) and tumour variables (tumour localization, results of MSI analysis, IHC staining and hypermethylation) were documented. The consequences of MSI analysis and IHC were checked from the reports of the surgeon, clinical geneticist and oncologist. We analysed the consequences of MMR deficiency on the treatment and referral policy.

Molecular analysis of CRC

Tumour specimen for MSI or IHC analysis could be obtained preoperatively through colonoscopy biopsies and from the surgical resection specimen after surgery.

Microsatellite instability (MSI) analysis Genomic DNA from the tumour and normal tissue was extracted on either fresh, frozen or paraffin-embedded tumour tissue and was sectioned at 4 µm. The tumour percentage of the tissue has to be above 20% for a sensitive test. MSI analysis is a fluorescent assay based on PCR to detect MSI in the tumour cells. Fluorescently labelled primers were used for co-amplification of 7 markers including 5 mononucleotides repeat markers for MSI determination and 2 pentanucleotide repeat markers to detect potential sample mix-ups or contamination.[26] Tumour samples with more than 2 changed markers out of 5 were classified as MSI-high (MSI-H), 1 out of 5 as MSI-Low (MSI-L) and tumours without a changed marker as microsatellite stable (MSS).

Immunohistochemistry (IHC) was performed by staining the MMR-proteins with anti-MLH1, anti-PMS2, anti-MSH2, and anti-MSH6 antibodies. This is performed on formalin-fixed, paraffin-embedded tissues. The expression of MLH1, PMS2, MSH2, and MSH6 was scored as positive (+), negative with a positive internal control (0/+), and doubtfully negative [when both tumour and internal control stain negative (0/0)], and when the internal control was stronger than the positive tumour cell, it was scored as +/++.[12] Immunohistochemistry was only performed in LUMC.

Hypermethylation (MLH1 promoter) In case of MMR deficient tumours either due to expression loss of the MLH1 protein by IHC or MSI, differentiation between LS and sporadic CRC due to methylation of the *MLH1* promoter was performed by using MSP.[27]

Data management

All data was entered and managed in the data management tool of Research Manager. This program provides a protected environment to ensure the safety of the patients' data. The completed data was converted into an Excel document to analyse the outcomes.

RESULTS

Over a period of almost 4 years we performed MSI and/or IHC analyses in 225 colorectal tumours, 108 MSI analyses in Isala and 117 IHC stainings in LUMC. Of all 225 CRC patients, the mean age was 64.5 (± 9.9) years, 140 (62%) patients were male. Of the 117 IHC that were performed, 41 showed expression loss in one or more of the MMR proteins. Most patients showed dual loss of expression of the MLH1 and PMS2

proteins (N=29, 70,7%), followed by MLH1 alone (N=5, 12,19%), MSH6 (N=4, 9,75%) and the combinations of MSH1+MSH6 (N=2, 4,8%) and MLH1+PMS2+MSH6 (N=1, 4,1%). (Table 1) Twenty-eight patients got additional MSP to exclude hypermethylation of the *MLH1* promoter. In 23 of these 28 patients, the expression loss of the

Table 1.
Results of IHC and MSI analysis

	LUMC	Isala	Total
MMR analysis	**117**	**108**	**225**
MMR analysis on biopsies	58	26	86
Immunohistochemistry staining (IHC)	117	-	117
Loss of MMR protein expression	41	-	41
MLH1	5		
MLH1 + PMS2	29		
MLH1 + PMS2 + MSH6	1		
MSH2 + MSH6	2		
MSH6	4		
MLH1 hypermethylation	29	-	29
MSP performed	28		
MLH1 hypermethylation	23		
MSP not performed	13		
MLH1 hypermethylation assumed due to age	6		
MSI analysis	-	108	108
MSI-High	-	12	12
IN TOTAL:			
Suspect for MMR mutation (LS)	12	12	24

MLH1 protein was caused by *MLH1* promoter hypermethylation. In 6 patients with a mean age of 80 years MSP was not performed because of the assumption that hypermethylation caused the MLH1 protein loss. Following additional MSP analysis, a total of 12 patients were suspected for Lynch syndrome. MSI analysis was performed in 108 patients. Twelve patients (11%) had MSI-high tumours. In total 24 patients were suspected for LS and further analysis was indicated.

Referral to clinical geneticist

A total of 18 patients were referred to the clinical geneticist for DNA analysis. Of these 18 patients, 2 patients cancelled their intake appointment. In 6 referred patients with MSI high tumours hypermethylation of the *MLH1* promoter was found. In 10 patients, genomic DNA analysis was performed and 9 MMR mutations were found (*MLH1* (N=2); *MSH2* (N=1); *MSH6* (N=6)) confirming LS in these patients. In the remaining patient mosaicism caused the MMR expression loss. (Table 2)

Table 2.
Consequences for patients suspect for a MMR mutation (LS): genetic counselling (GC) and surgical treatment.

	Total (n=24)	
Genetic counselling (GC)		
Not referred for GC	**6**	
Referred for GC	**18**	
Actual visited clinical geneticist	16	
Appointment cancelled	2	
MMR analysis	**16**	
MSP	6	
MLH1 hypermethylation	6	
DNA analysis	10	
MMR mutation	9	
Mosaicism	1	
MMR mutation	**9**	
MLH1	2	
MSH2	1	
MSH6	6	
Surgical treatment		
Patients <60 years	**4**	
MMR analysis results available before surgery		**8**
< 60 years	2	
Change in type of surgery	**1**	
Subtotal colectomy	1	

Influence on surgical treatment

Overall, 86 (38%) of the total of 225 analysis that were performed were available pre-operatively. Of 24 patients that were suspected for LS, molecular analysis was performed before surgery in 8 (33%). (Table 2) Four patients out of 24 were aged under 60 years of which 2 were analysed preoperatively. In one of them surgical treatment changed because of MMR deficiency. This 42-year-old female patient underwent a subtotal colectomy instead of a hemicolectomy due to MMR deficiency and positive family history. Further analysis showed that she was a carrier of a MSH2-mutation. The other 3 patients <60 years also turned out to be MMR gene carriers.

Influence on chemotherapy

Of the 54 patients with MMR deficient tumors, 20 patients had an indication for adjuvant chemotherapy according to the advice of the multidisciplinary meeting based on national guideline, including 15 patients with a stage III tumours and 5 with a stage IV tumours. In 7 (35%) patients the regimen choice of chemotherapy type was changed by the test results. Oxaliplatin was added to 5-FU monotherapy in two patients (10%). In 5 (25%) patients with a stage III tumour, 5-FU (Capecitabine) monotherapy was refrained because of MMR deficiency (Table 3).

Table 3.
Consequences for chemotherapy for all MMR-deficient tumors

Chemotherapy	N
MMR-deficient tumours	54
Stage	
I	2
II	8
III	31
IV	10
Unknown	3
Indication chemotherapy*	20
Stage	
III	15
IV	5
Change in chemotherapy	7
Refrained from 5-FU monotherapy (all stage III tumours)	5
Added Oxaliplatin to 5-FU monotherapy	2

*Advised by the multidisciplinary team

DISCUSSION

Molecular testing of CRC for MMR-deficiency is important not only for the identification of Lynch syndrome families but also for the decision-making on surgical treatment in patients suspected of LS and decisions on adjuvant chemotherapy in LS-patients and patients with sporadic MMR-deficient CRC. In the present study, we evaluated the outcome of MSI and IHC analyses in 225 patients. We found that 24 patients should have been referred for further analysis. Strictly, these patients were not all suspected for LS. Patients from Isala with MSI high tumours that were not yet tested to rule out hypermethylation were included in this number. This is explained by the fact that during the study period immunohistochemistry to rule out hypermethylation for Isala patients was performed by the clinical geneticist after referral. Therefore, in Isala, they were suspected for LS because the tumours were MSI high and they should have been referred. Currently, immunohistochemistry analysis is performed in Isala as well. Only 4% of all patients selected for MSI analyses or MMR testing were found to have LS which is lower compared with results of a previous study which reported LS in 9.2% of pre-selected patients, using the Bethesda criteria.[28] The lack of an adequate referral procedure may be the explanation that one third of the patients did not receive proper genetic counseling. A systematic discussion of the result of MSI analyses or IHC should be incorporated in the multidisciplinary meeting and it should be decided who will be responsible for referral to a clinical genetic centre. Irons et al suggested a method where genetic counselors are responsible for initiating conversations about counseling which may improve the compliance rates to the referral. In their study, they had a compliance with referral of only 35,7%, with the surgeon being responsible to refer the patient. Other studies showed the compliance with the referral to the clinical geneticist is higher when they themselves are responsible for initiating conversations about further germline testing. Also, further research was suggested to identify possible barriers to visit the clinical geneticist to finally improve compliance with the referral.[29]

According to the current guidelines extended colorectal surgery (subtotal colectomy) is recommended in patients with evidence for LS and age <60 years. In our study only one patient (4%) underwent a subtotal colectomy instead of hemicolectomy based on a suspicion of LS due to MMR deficiency and a young age (42 years) at diagnosis of CRC. After surgery, an MSH2 mutation was identified. This low number is due to the fact that only 4 of 24 patients were under age 60 years. Another explanation is that the majority of MSI analysis and IHC were performed on the resected

specimen (139 of total 225 (61.7%)) instead of the biopsies. In 2011, Parry et al. investigated the risk of developing metachronous CRC in MMR gene mutation carriers. Of 382 study subjects, 332 had a partial resection. A total of 74 of the 332 subjects were diagnosed with metachronous CRC. Cumulative risk of metachronous CRC was 16% (95% CI 10–25%) at 10 years, 41% (95% CI 30–52%) at 20 years and 62% (95% CI 50–77%) at 30 years after segmental colectomy. These risk estimates could help in the decision-making regarding the extent of primary surgical resection.[30] If biopsies with enough tumour tissue are available preoperatively, MMR testing on the biopsies is preferred as the result might influence the surgical treatment and we recommend to discuss these results during the preoperative multidisciplinary meeting. For instance, in young (<60 years of age) patients with MMR protein expression loss and MSI-H tumours (without *MLH1* hypermethylation) with a strongly suspected family history, a subtotal colectomy should be discussed. Nowadays in some hospitals in the Netherlands there is even a possibility to perform fast track DNA analysis to confirm or rule out LS before surgery within only a few weeks. Another advantage of testing on biopsies is that effects of (chemo-) radiation treatment are avoided in case of rectal cancer.

In the literature, there is an increasing amount of evidence that adjuvant chemotherapy with 5-FU in patients with a stage II or III CRC with MMR-defective tumours does not improve the prognosis. A study of 754 CRC patients showed an improvement of survival in patients who received adjuvant chemotherapy with 5-FU only in patients with a MMR-competent tumor. Overall survival of patients with MMR-deficient tumors did not improve with adjuvant 5-FU monotherapy.[31] Another meta-analysis of several randomized clinical trials confirmed this finding.[32] Therefore, MSI/IHC analysis becomes increasingly relevant for the decision making on adjuvant chemotherapy, especially in patients with stage II or III colorectal cancer. In our study, in 7 (35%) of the 20 patients who had an indication for adjuvant chemotherapy, the initial planned treatment with 5-FU monotherapy was changed due to MMR deficiency. The current guideline in most countries is to restrict MSI/IHC-testing to patients with CRC <70 years. Because decisions on chemotherapy are equally important in patient with CRC >70 years, we recommend to test all CRC patients independent of the age of diagnosis. Moreover, also in the metastatic CRC setting MSI/IHC-testing becomes increasingly relevant since treatment with anti-Programmed Death-1 inhibitor immunotherapy provides durable responses and disease control in pre-treated patients with dMMR/MSI-H metastatic CRC.[33]

The strength of the study is that we evaluated the outcome of MSI and IHC-analysis in clinical practice over a relative long period of time in two large hospitals. One of the limitations is the relatively small sample size and the small number of patients with abnormal MSI/IHC. Another limitation is the different techniques of MMR testing between the two hospitals.

In conclusion, MSI and IHC analysis resulted in the identification of a relatively low number of LS patients possible due to the fact that a considerable number of patients were not referred for genetic counselling. In only one patient the analyses had consequences with respect to the type of surgery. In a substantial number of patients, the results of MSI and IHC had consequences for the choice of chemotherapy. For all these reasons, we recommend to perform MSI and/or IHC in all patients with CRC independent of age, if possible the analyses should be performed on biopsies.

REFERENCES

1. Vasen HF. Review article: The lynch syndrome (hereditary nonpolyposis colorectal cancer). Aliment Pharmacol Ther. 2007;26 Suppl 2:113-126.
2. Barrow E, Hill J, Evans DG. Cancer risk in lynch syndrome. Fam Cancer. 2013;12(2):229-240.
3. Aarnio M, Mecklin JP, Aaltonen LA, Nystrom-Lahti M, Jarvinen HJ. Life-time risk of different cancers in hereditary non-polyposis colorectal cancer (HNPCC) syndrome. Int J Cancer. 1995;64(6):430-433.
4. Liu B, Parsons R, Papadopoulos N, et al. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. Nat Med. 1996;2(2):169-174.
5. Peltomaki P, Vasen H. Mutations associated with HNPCC predisposition -- update of ICG-HNPCC/INSiGHT mutation database. Dis Markers. 2004;20(4-5):269-276.
6. Hendriks Y, Franken P, Dierssen JW, et al. Conventional and tissue microarray immunohistochemical expression analysis of mismatch repair in hereditary colorectal tumors. Am J Pathol. 2003;162(2):469-477.
7. de Jong AE, van Puijenbroek M, Hendriks Y, et al. Microsatellite instability, immunohistochemistry, and additional PMS2 staining in suspected hereditary nonpolyposis colorectal cancer. Clin Cancer Res. 2004;10(3):972-980.
8. Bonnet D, Selves J, Toulas C, et al. Simplified identification of lynch syndrome: A prospective, multicenter study. Dig Liver Dis. 2012;44(6):515-522.
9. Niv Y. Microsatellite instability and MLH1 promoter hypermethylation in colorectal cancer. World J Gastroenterol. 2007;13(12):1767-1769.
10. Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology. 2000;118(5):829-834.
11. Rodriguez-Bigas MA, Boland CR, Hamilton SR, et al. A national cancer institute workshop on hereditary nonpolyposis colorectal cancer syndrome: Meeting highlights and bethesda guidelines. J Natl Cancer Inst. 1997;89(23):1758-1762.
12. Umar A, Boland CR, Terdiman JP, et al. Revised bethesda guidelines for hereditary nonpolyposis colorectal cancer (lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96(4):261-268.

13. Sie AS, Mensenkamp AR, Adang EM, Ligtenberg MJ, Hoogerbrugge N. Fourfold increased detection of lynch syndrome by raising age limit for tumour genetic testing from 50 to 70 years is cost-effective. Ann Oncol. 2014;25(10):2001-2007.
14. Vasen HF, Abdirahman M, Brohet R, et al. One to 2-year surveillance intervals reduce risk of colorectal cancer in families with lynch syndrome. Gastroenterology. 2010;138(7):2300-2306.
15. Vasen HF, de Vos tot Nederveen Cappel,W.H. Cancer: Lynch syndrome--how should colorectal cancer be managed? Nat Rev Gastroenterol Hepatol. 2011;8(4):184-186.
16. Vasen HF, Blanco I, Aktan-Collan K, et al. Revised guidelines for the clinical management of lynch syndrome (HNPCC): Recommendations by a group of european experts. Gut. 2013;62(6):812-823.
17. de Vos tot Nederveen Cappel,W.H., Buskens E, van Duijvendijk P, et al. Decision analysis in the surgical treatment of colorectal cancer due to a mismatch repair gene defect. Gut. 2003;52(12):1752-1755.
18. Natarajan N, Watson P, Silva-Lopez E, Lynch HT. Comparison of extended colectomy and limited resection in patients with lynch syndrome. Dis Colon Rectum. 2010;53(1):77-82.
19. Karlitz JJ, Hsieh MC, Liu Y, et al. Population-based lynch syndrome screening by microsatellite instability in patients </=50: Prevalence, testing determinants, and result availability prior to colon surgery. Am J Gastroenterol. 2015;110(7):948-955.
20. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010;138(6):2073-2087.e3.
21. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med. 2003;349(3):247-257.
22. Jover R, Zapater P, Castells A, et al. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. Gut. 2006;55(6):848-855.
23. He EY, Hawkins NJ, Mak G, et al. The impact of mismatch repair status in colorectal cancer on the decision to treat with adjuvant chemotherapy: An australian population-based multicenter study. Oncologist. 2016.
24. Boland CR, Lynch HT. The history of lynch syndrome. Fam Cancer. 2013;12(2):145-157.

25. Diaz LA,Jr, Le DT. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;373(20):1979.
26. Promega Corporation. MSI analysis system, version 1.2: Technical manual. 08/14;TM255:www.promega.com.
27. Herman JG, Umar A, Polyak K, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci U S A. 1998;95(12):6870-6875.
28. Serrano M, Lage P, Belga S, et al. Bethesda criteria for microsatellite instability testing: Impact on the detection of new cases of lynch syndrome. Fam Cancer. 2012;11(4):571-578.
29. Irons RF, Contine KM, Horte JJ et al. Success of referral to genetic counseling after positive lynch syndrome screening test. Int J Colorectal Dis (2017) 32:1345–1348
30. Parry S, Win AK, Parry B, et al, Metachronous colorectal cancer risk for mismatch repair gene mutation carriers – the advantage of more extensive colon surgery, Gut. 2011 July ; 60(7): . doi:10.1136/gut.2010.228056.
31. Jover R, Zapater P, Castells A, et al. The efficacy of adjuvant chemotherapy with 5-fluorouracil in colorectal cancer depends on the mismatch repair status. Eur J Cancer. 2009;45(3):365-373.
32. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 2010;28(20):3219-3226.
33. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch reapair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncl. 2017;7;19. pii: S1470-2045(17)30422-9. doi: 10.1016/S1470-2045(17)30422-9.