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Metastatic Colorectal Cancer Clinical and Genetic Prognostic and Predictive Factors

Abstract of The Doctoral Thesis for obtaining a doctoral degree (*PhD)*

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Sub-sector – Oncology

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The Doctoral Thesis was carried out at Institute of Oncology of Riga Stradiņš University and Clinic of Oncology of Pauls Stradins Clinical University Hospital, Latvia

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The Doctoral Thesis is available in the RSU library and at RSU website: https://www.rsu.lv/en/dissertations

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Abbreviations

5-FU	*5-fluorouracil*
APC	*Adenomatous polyposis coli (gene)*
BPI	*Breakpoint instability index*
BRAF	*B-Raf proto-oncogene, serine-threonine kinase*
CEA	*Carcinoembryonic antigen*
CIN	*Chromosomal instability*
CNV	*Copy number variations*
COSMIC	*Catalogue of Somatic mutations in Cancer*
dMMR	*Mismatch repair deficient*
EGFR	*Epidermal growth factor receptor*
FTD/TPI	*Trifluridine/Tipiracil*
KRAS	*Kirsten ras oncogene homolog*
mCRC	*Metastatic colorectal cancer*
MMR	*Mismatch repair*
mOS	*Median overall survival*
mPFS	*Median progression free survival*
MSI	*Microsatellite instability*
MSI-H	*Microsatellite instability-high*
MSS	*Microsatellite stable*
mut	*mutant*
OS	*Overall survival*
PFS	*Progression free survival*
pMMR	*Mismatch repair proficient*
TNM	*Tumour Node Metastases (staging system)*
wt	*wild type*

Introduction

Colorectal cancer (CRC) is one of the most common types of cancer in both women and men and the third most common type of cancer in all age groups. CRC is the second most common cause of cancer deaths in Europe.

Approximately 1,800,000 individuals are diagnosed with colon and rectal cancer every year worldwide; the third of patients will die of this disease.

CRC is classified in the right sided cancer (cecum, ascending colon, liver flexure, and transverse colon), left sided cancer (splenic flexure, descending colon and sigmoid colon) and rectal cancer. This division is based on different clinical symptoms, tumour biology, treatment and prognosis.

The increasing knowledge in cancer molecular biology shows that colorectal cancer is a heterogeneous group of malignancies with one anatomical localisation in colon. Further research of mCRC genome makes it possible to identify prognostic and predictive factors that can be used in clinical practice. The most commonly described mutations are KRAS, NRAS, BRAF, which are used in selection of anticancer treatment, as well as changes in MMR (miss match repair) resulting in microsatellite instability. All these genetic alterations impact treatment efficacy and prognosis. Next generation sequencing and copy number variation (CNV) analysis reveal multiple chromosomal aberrations and gene mutations developing early in oncogenetic transformation that impact aggressiveness of tumour, resistance to therapy and prognosis. Also, various oncogenetic mechanisms for sporadic and hereditary CRC have been detected.

Surgical treatment (resection of metastases), medical treatment and invasive procedures (chemoembolisation and irradiation) are available in Latvia for treatment of mCRC. It should be noted that the number of targeted treatment medications has been reimbursed from 2018, allowing mCRC patients to live longer with acceptable quality of life. The biggest challenge for medical

oncologists is treating patients who became resistant to received chemotherapy and targeted therapy.

Aim of the study

Both the clinical factors and the chromosomal aberrations can be used as prognostic and predictive factors in patients with metastatic colorectal cancer.

Tasks of the study

1. To assess factors that affect progression-free survival (PFS) and overall survival (OS) in patients with mCRC treated in the Clinic of Oncology, Pauls Stradins Clinical University Hospital.
2. To evaluate the impact of clinical factors (neutropenia and duration of previous treatment) on PFS and OS in patients with refractory mCRC receiving trifluridine/tipiracil (FTD/TPI) treatment.
3. To evaluate the impact of break point instability index (BPI) and *chromothripsis* on PFS and OS.
4. To determine the most frequent deletions in patients with *chromothripsis*

Hypothesis

Prognosis of mCRC is affected by clinical and molecular factors. mCRC cells harbour multiple chromosomal breaks and deletions that occur early in the oncogenesis process and impact efficacy of medical treatment and mCRC prognosis.

Novelty of the study

This is the first copy number variation analysis of metastatic colorectal cancer in Latvia.

Results of mCRC genome analysis provided in the study could be used as prognostic and predictive biomarkers.

Place of work

Patient selection, treatment, follow-up and collection of blood samples was done at Clinic of Oncology, P.Stradins Clinical University hospital.

Tissue sampling from paraffin blocks for further genome analysis was done at Institute of Pathology, Pauls Stradins Clinical University Hospital.

The copy number variation analysis took place at the Institute of Oncology, Rīga Stradiņš University.

Part of the study was performed with the Lonsurf Compassionate Use programme (Expanded Access programme) – 2 patients received treatment in the Clinic of Oncology, Pauls Stradins Clinical University Hospital, while 12 patients were treated at the Oncology Centre of Riga East University Hospital.

Ethical aspects

Ethical permission was granted by Rīga Stradiņš University Ethics Committee (06.10.2011). The study was conducted in accordance with World Medical Association Helsinki Declaration and Good Clinical Practice. Informed consent was obtained from all participants. Written informed consent was obtained from every patient who participated in the Lonsurf Compassionate Use programme (Expanded Access programme).

1. Matherials and methods

The study consists of 3 parts (Figure 1.1):

1) Analysis of patients with unresectable mCRC treated at Clinic of Oncology, Pauls Stradins Clinical University Hospital;

2) Analysis of clinical prognostic and predictive factors in patients with refractory mCRC receiving FTD/TPI;

3) C*hromothripsis* as a predictive factor in patients with mCRC receiving first line chemotherapy .

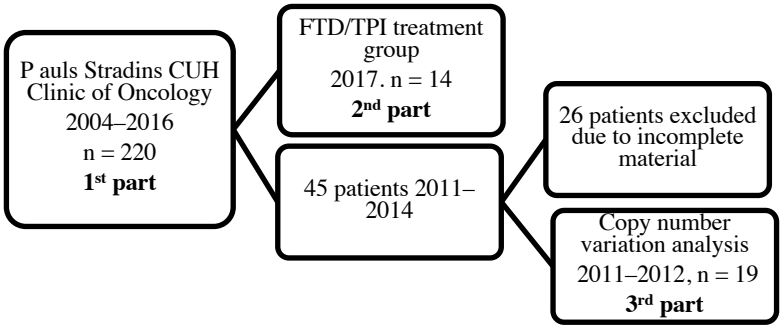

Fig.1.1. **Design of the study**

1.1. Analysis of patients with unresectable mCRC treated at Clinic of Oncology, Pauls Stradins Clinical University Hospital

Study population. A retrospective study of 220 patients with unresectable mCRC (C18-C20) treated at Clinic of Oncology, Pauls Stradins Clinical University Hospital between June 2004 and December 2016. The data were obtained from medical records (out-patient and in-patient clinic records), computer programmes "Ārstu birojs" and "AI-RIS PSKUS" radiological examination server. Data of *exitus letalis* were received from The Centre for Disease Prevention and Control (CDPC) of Latvia.

Inclusion criteria:

1) Patients diagnosed with unresectable mCRC. Patients underwent resection of metastases prior or after palliative chemotherapy were excluded from the study;

2) The patient has received at least 2 cycles of chemotherapy;

3) Medical documentation contains data of received chemotherapy, follow-up, CT-scans, progression. Patients who were lost on follow up were excluded from the study.

Statistical analysis. Data of age, sex, date of diagnosis, stage, first and second line chemotherapy, date of progression confirmed in radiological examination, localisation of metastases and date of cancer related death were obtained. Statistical analysis was performed using MedCalc programme, v.16.4.8 (MedCalc Software, Ostend, Belgium). The overall survival (OS) and progression-free survival (PFS) has been calculated using the Kaplan-Meier method, log-rank test and cox regression model.

1.2. Analysis of clinical prognostic and predictive factors in patients with refractory mCRC receiving FTD/TPI

Study population. Prospective study. A total of 14 mCRC patients who received FTD/TPI chemotherapy in two institutions in Latvia (Clinic of Oncology of Pauls Stradins Clinical University Hospital and Oncology Centre of Riga East University Hospital) between April, 2016 and January, 2017 were analysed. The study was performed with the Lonsurf Compassionate Use programme (Expanded Access programme) and written informed consent was obtained from every patient who participated in the study.

Inclusion criteria:

1. Metastatic cancer of the colorectum;

2. Neutrophil count > 1.500/mm^3, platelet count > 75.000/mm^3, hemoglobin level > 9.0 g/dl;

3. At least 2 previous chemotherapy lines, refractory to or intolerant of fluoropyrimidines, oxaliplatin and irinotecan.

The patients underwent 1–13 months follow-up.

Treatment. FTD/TPI (with each dose consisting of 35 mg/m^2) was administered orally twice daily, for 5 days a week, with 2 days of rest, for 2 weeks, followed by a 14-day rest period, thus completing one cycle. The regimen was repeated every 4 weeks. The dose was recommended as standard in all sites participating in the Lonsurf Compassionate Use programme. Treatment was discontinued upon clinically or radiologically confirmed disease progression.

Statistical analysis. Progression-free survival (PFS) was calculated from the start of FTD/TPI until clinical or radiological progression, and overall survival (OS) was calculated from the start of FTD/TPI until death from any cause or censoring at the last follow-up. The median OS and PFS (mOS and mPFS, respectively) were estimated using the Kaplan-Meier method. The log-rank test was used to calculate any significant differences between the subgroups (patients with vs. without grade 3–4 neutropenia, and duration of previous treatment ≤ vs. > 18 months) by univariate analysis. Significance levels were set at $p < 0.05$. All statistical analyses were performed by MedCalc software, version 16.4.8 (MedCalc Software, Ostend, Belgium).

1.3. *Chromothripsis* as a predictive factor in treatment of metastatic colorectal cancer

Study population. Prospective study. A total of 19 mCRC patients who received chemotherapy at the Clinic of Oncology of Pauls Stradins Clinical

University Hospital between August, 2011 and October, 2012 were selected. Tissue samples were acquired as part of a series of routine diagnostic and pathological analyses at the hospital.

Inclusion criteria:

1. Metastatic cancer of colorectum.
2. Measurable disease regarding RECIST v1.1 criteria.
3. Chemotherapy *naive* patient.
4. ECOG – 0, 1 or 2;
5. Age >18 years.
6. Patient is appropriate for FOLFOX first line treatment.
7. Absolute neutrophil count (ANC) > 1.500/mm^3, platelet count > 100.000/mm^3, bilirubin < 2 times upper limit of normal (ULN), AST or ALT < 5 times of ULN, serum creatinine < 1.5 times of ULN.
8. Signed informed consent.

Exclusion criteria:

1. CNS metastases.
2. Uncontrolled diabetes, infection, cardiovascular disease, wound infections.

Treatment:

1. First line chemotherapy – oxaliplatin containing FOLFOX type therapy (mFOLFOX-6 or FOLFOX4) – every 2 weeks with/without targeted treatment.
2. Duration of chemotherapy – until progression of disease or uncontrolled toxicity, min 4 cycles, optimal 8–12 cycles.
3. CEA before every chemotherapy cycle, CT-scan every 2 months.

4. Follow-up after discontinuation of chemotherapy – CEA once in a month, CT-scan every 2 months until progression.

Genotyping. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) samples with QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Quality was evaluated using the Illumina FFPE QC kit (Illumina, San Diego, CA, USA) by reverse transcription-polymerase chain reaction. DNA was restored with the Illumina DNA restoration kit (Illumina). Microarray analysis was performed using the Infinium HumanOmniExpress-12 v1.0 FFPE BeadChip kit (Illumina). BeadChip was scaned on HiScan (Illumina). Analysis was performed by GenomeStudio software (Illumina) and R version 3.1.2. (https://www.r-project.org/). Copy number variation and breakpoints on the chromosomes were analysed using the DNA copy package (http://bioconductor.org/packages/release/bioc/html/DNAcopy.html).

OS and progression-free survival (PFS) rates were estimated using the Kaplan-Meier method. The log-rank test was used to calculate any significant difference between the subgroups by univariate analysis. Significance levels were set at $p < 0.05$. All statistical analyses were performed using MedCalc software, version 16.4.8 (MedCalc Software, Ostend, Belgium).

2. Results

2.1. Analysis of patients with unresectable mCRC treated at Clinic of Oncology, Pauls Stradins Clinical University Hospital

220 patients who received palliative chemotherapy due to unresectable mCRC at Clinic of Oncology of Pauls Stradins Clinical University Hospital between 2004 and 2016 220 patients were involved in retrospective study.

The study included 120 males (54.5 %) and 100 females (45.5 %) with a median age of 63 (22–78, 95%CI 62–65). 28 patients (12.7 %) were under the age of 50. 106 patients (48.2 %) were diagnosed with mCRC between 2004 and 2011, and 114 patients (51.8 %) – between 2012 and 2016.

143 patients (65 %) were diagnosed with synchronous mCRC (Stage IV), while 77 patients (35 %) were diagnosed with metachronous mCRC (metastases developed > 6 months after curative treatment of primary Stage I–III cancer).

In metachronous mCRC, 46 patients (59.7 %) had Stage III (T2-4N+) cancer, 26 patients (33.8 %) – Stage II (T3-4N0) cancer, 3 patients (3.9 %) – Stage I (T2N0) cancer, and 2 patients (2.6 %) – unknown cancer stage. Median time to metastases was 18 months (6–84 months). In node positive patients (n = 42) median time to metastases was 16.5 months, but in node negative patients (n = 28) – 20 months (HR 0.4917; 95%CI 0.2929–0.8257; p = 0.0073).

187 patients (85 %) had full information about localisation of metastases. 92 patients (41.8 %) had liver only metastases, 16 patients (7.3 %) – lung metastases, 31 patients (14.1 %) – metastases in abdominal lymph nodes or peritoneum, 48 patients (21.8 %) – multiple metastases, while 33 patients (15 %) had no accurate data of metastatic spread of cancer (Fig.2.1).

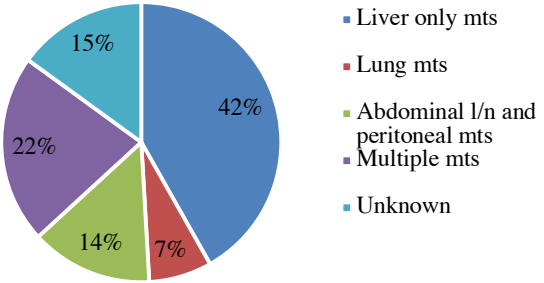

Fig.2.1. **Localisation of metastases**
mts – metastases, l/n – lymph nodes

First line chemotherapy

All patients enrolled in the study received at least two cycles of first line chemotherapy. The most common type of chemotherapy FOLFOX (mFOLFOX6 or FOLFOX4) was received by 127 patients (57.7 %). FOLFOX + monoclonal antibodies – 24 patients (10.9 %), oral Ftorafur and oxaliplatin combination treatment – 29 patients (13.1 %), fluoropyrimidine monotherapy (capecitabine, 5FU, Ftorafur) – 25 patients (11.4 %), irinotecan containing treatment – 15 patients (6.8 %) (Fig.2.2).

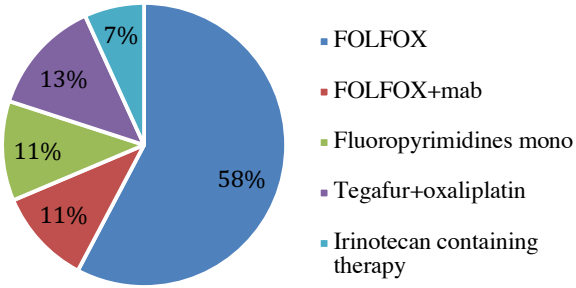

Fig.2.2. **First line chemotherapy**
mab – monoclonal antibodies, mono - monotherapy

In the synchronous mCRC group, FOLFOX type therapy was received by 58.7 % patients, FOLFOX + monoclonal antibodies – 10.5 %, oral Ftorafur and oxaliplatin combination treatment – 11.6 %, fluoropyrimidine monotherapy (capecitabine, 5FU, Ftorafur) – 14.3 %, irinotecan containing treatment – 4 %.

In the metachronous mCRC group, FOLFOX type therapy was received by 55.8 % patients, FOLFOX + monoclonal antibodies – 11.7 %, oral Ftorafur and oxaliplatin combination treatment – 15.6 %, fluoropyrimidine monotherapy (capecitabine, 5FU, Ftorafur) – 5.2 %, irinotecan containing treatment – 11.7 %.

Second line chemotherapy

Second line chemotherapy was received by 125 patients (56.8 %), but 95 patients (43.2 %) discontinued treatment. The most common type chemotherapy FOLFIRI was received by 95 patients (76 %), FOLFIRI + targeted therapy – 11 patients (8.8 %), fluoropyrimidine monotherapy – 16 patients (12.8 %), oxaliplatin containing therapy – 3 patients (2.4 %) (Fig.2.3).

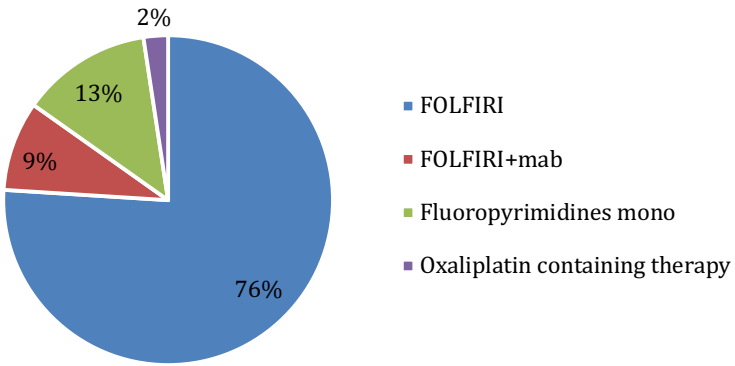

Fig. 2.3. **Second line chemotherapy**
mab – monoclonal antibodies, mono - monotherapy

In the synchronous mCRC group 86 patients received second line chemotherapy (60.1 %). The most common type of chemotherapy was FOLFIRI – 67 patients (77.9 %), FOLFIRI + targeted treatment was received by 7 patients (8.1 %), fluoropyrimidine monotherapy – 9 patients (10.5 %), oxaliplatin containing therapy – 3 patients (3.5 %).

In the metachronous mCRC group 39 patients received second line chemotherapy (50.6 %). The most common chemotherapy type was FOLFIRI – 28 patients (71.8 %), FOLFIRI + targeted treatment was received by 4 patients (10.3 %), fluoropyrimidine monotherapy – 7 patients (17.9 %), oxaliplatin containing therapy – 3 patients (3.5 %).

Survival analysis

The overall survival (OS) and progression-free survival (PFS) of all patients (n = 220) involved in the study is shown in Table 2.1. Median overall survival (mOS) was 17 months, while median progression-free survival (mPFS) was 8 months. 5 year OS was 3.1 %.

Table 2.1

Survival (PFS and OS) in study population (n = 220)

Survival	**All patients**	**Survival**	**All patients**
mOS (months)	17	mPFS (months)	8
1yOS	66.6 %	6mPFS	60.4 %
2yOS	25.2 %	12mPFS	20 %
3yOS	8.6 %	18mPFS	5.70 %
4yOS	4.3 %	24mPFS	1.70 %
5yOS	3.1 %		

mOS – median overall survival; 1yOS – 1 year overall survival; mPFS – median progression free survival; 6mPFS – 6 month progression free survival

No difference was found between synchronous mCRC and metachronous mCRC in terms of survival – mPFS (8 vs. 8 months) and mOS (17 vs. 17 months).

5 years OS was improved in metachronous mCRC (5.4 % vs. 1.2 %), but the result did not reach statistical significance (Table 2.2).

Table 2.2

Survival in patients with synchronous and metachronous mCRC

Survival	**Synchronous mCRC (n = 143)**	**Metachronous mCRC (n = 77)**	**p**	**HR**	**95%CI**
mOS	17 months	17 months	0.90	1.0182	0.7559–1.3714
1yOS (%)	69.10 %	62 %			
2yOS 5yOS	22.80 % 1.2 %	20.30 % 5.4 %			
mPFS	8 months	8 months	0.56	0.9	0.6440–1.2700
6mPFS	60.8 %	59.4 %			
1yPFS	22.1 %	14.90 %			

mOS – median overall survival; 1yOS – 1 year overall survival; mPFS – median progression free survival); 6mPFS – 6 month progression free survival

The impact of first line chemotherapy on survival has been represented in Table 2.3. Increased mPFS (10 months) was detected in patients receiving FOLFOX in combination with targeted therapy, but decreased mPFS (5 months) – in patients receiving irinotecan containing chemotherapy; however, the results did not reach statistical significance. Significant difference in OS was not found in chemotherapy subgroups. In synchronous mCRC improved mOS (24 months) was found in patients receiving FOLFOX with targeted therapy, and decreased (13 months) – in patients receiving fluoropyrimidine monotherapy, but the difference was not statistically significant.

Table 2.3

Impact of type of first line chemotherapy on mPFS and mOS

Group	FOLFOX	FOLFOX + mab	Fluoro-pyrimidine mono	Ftorafur + ox	Iri cont thr	p
mPFS (months)						
All	8	10	8	7	5	0.28
Synchr mCRC	9	10	8	6	6	0.327
Metachr mCRC	8	8	5	7	5	0.617
mOS (months)						
All	18	16	13	18	14	0.415
Synchr mCRC	18	24	13	18	14	0.09
Metachr mCRC	18	8	11	16	11	0.17

mOS – median overall survival; mPFS – median progression free survival; mab – monoclonal antibody; synchr – synchronous; metachr – metachronous; mono – monotherapy; ox – oxaliplatin; Iri – irinotecan; cont – containing; thr – therapy

Out of 125 patients receiving second line chemotherapy, exact data of next progression was known in 114 patients. The mPFS2 in all patients (n = 114) was 6 months, 3 month PFS2 – 74.9 %, but 6 month PFS2 – 40.6 %. Statistically significant difference in PFS was found in metachronous mCRC group. OS was significantly improved in patients receiving more than one line of palliative chemotherapy (Table 2.4). In patients receiving second line chemotherapy, mOS was 20 months compared to mOS of 11 months in patients who discontinued chemotherapy after first progression (HR 0.36; $p < 0.0001$) (Fig.2.4), but 5-year OS was observed in 3.8 % and 0 %, respectively.

Table 2.4

Impact of received chemotherapy lines on survival

Group	More than one chemo line	One chemo line	p	HR	95%CI
Overall survival					
All group mOS 1yOS 2yOS 5yOS	20 months 83 % 36.2 % 3.8 %	11 months 43.5 % 7.8 % 0 %	**< 0.0001**	0.3658	0.2636–0.5075
Synchronous mCRC	20 months	11 months	**0.0017**	0.4414	0.264–0.726
Metachronous mCRC	21 months	11.5 months	**< 0.0001**	0,286	0.184–0.444
Progression free survival					
mPFS2 (n = 114)	6 months	-			
3mPFS2 6mPFS2	74.90 % 40.60 %				

mOS – median overall survival; 1yOS – 1 year overall survival; mPFS2 – median progression free survival; 6mPFS2 – 6 month progression free survival; chemo – chemotherapy

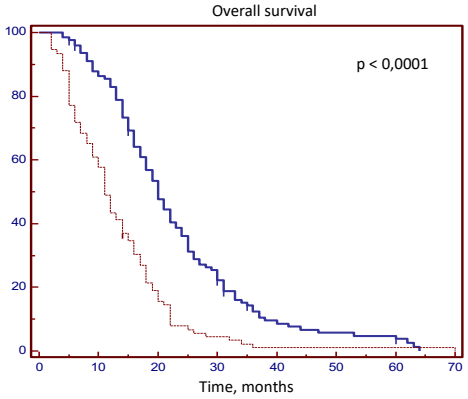

Fig. 2.4. **Impact of received chemotherapy lines on overall survival**
mOS is elevated in patients receiving more than one chemotherapy (blue line) line: 20 months; mOS in patients who received only one line of chemotherapy (red line): 11 months. HR 0.36; 95% KI 0.26–0.51; p < 0.0001

The impact of type of second line chemotherapy on survival is seen in Table 2.5. Decreased mPFS (4 months) was observed in patients who received fluoropyrimidine monotherapy, but statistically significant difference was observed only in metachronous mCRC subgroup. Increased mOS was detected in patients receiving FOLFIRI in combination with targeted treatment: 35 months in main group ($p < 0.0001$), 38 months in synchronous mCRC ($p < 0.0001$) and 30 months in the metachronous mCRC group ($p = 0.03$).

Table 2.5

Impact of type of second line chemotherapy on survival

Group	FOLFIRI	FOLFIRI + mab	Fluoropyrimidines mono	Ox cont chemo	No 2nd line	p
mPFS						
All (months)	5	8	4	10	na	0.49
Synchr mCRC (months)	5	8	7.5	10	na	0.749
Metachr mCRC (months)	6	12	3.5	na	na	**0.042**
mOS						
All (months)	19	35	20	20	11	**< 0.0001**
Synchr mCRC (months)	19	38	19	20	11	**< 0.0001**
Metachr mCRC (months)	21	30	21,5	na	11.5	**0.03**

mOS – median overall survival; mPFS2 – median progression free survival; mab – monoclonal antibodies, chemo – chemotherapy; synchr – synchronous; metachr – metachronous; ox – oxaliplatin; cont – containing

In further analysis, it was detected that younger patients (< 50 years) had better outcome – mOS 30 vs. 16 moths ($p = 0.0002$) and mPFS 9 vs. 8 months ($p = 0.045$). 5 year OS in the < 50 years subgroup reached 18.8 %, compared

with 0.8 % in patients ≥ 50 years old (Table 2.6). Similar results were observed in a much younger group – < 40 years (n = 6): mOS 33 vs. 16 months (p = 0.0109; HR 0.4244; 95% CI 0.2195–0.8207).

Table 2.6

Impact of patient age on survival

Survival	**< 50 years (n = 28)**	**≥ 50 years**	**p**	**HR**	**95%CI**
mOS	30 months	16 months	**0.0002**	0.498	0.3453–0.7184
1yOS 2yOS 5yOS	77.8 % 51.2 % 18.8 %	65 % 21.8 % 0.8 %			
mOS 2004–2011 mOS 2012–2016	20 months 31 months	15 months 17 months	**0.0055** **0.0036**	0.4944 0.4465	0.3008–0.8128 0.2593–0.7688
mPFS	9 months	8 months	**0.045**	0.647	0.4225–0.9901
mPFS 2004–2011 mPFS 2012–2016	7 months 9 months	8 months 7 months	0.1862 0.1643	0.6474 0.6664	0.3398–1.2334 0.3762–1.1807

mOS – median overall survival; 1yOS – 1 year overall survival; mPFS – median progression free survival

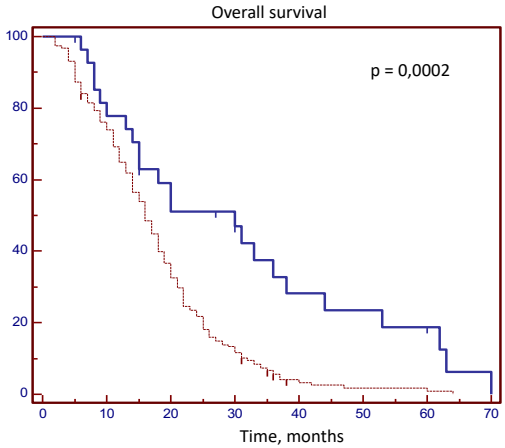

Fig. 2.5. **Impact of age on overall survival**
mOS in patients < 50 years old (blue line): 30 months; mOS on patients ≥ 50 years old (red line): 16 months. HR 0.49; 95% KI 0.34–0.72; p = 0.0002

Analysis of PFS and OS in patients who received treatment in different time intervals (2004–2011 or 2012–2016) revealed no statistically significant difference (Table 2.7). Improved mOS, 1 year OS and 2 year OS was observed in patients who received therapy after 2012, but no statistical significance was achieved (Fig 2.6).

Table 2.7

Impact of year of diagnosis on survival

Survival	**2004–2011 n = 106**	**2012–2016 n = 114**	**p**	**HR**	**95%CI**
mPFS	8 months	8 months	0.95	1.0097	0.7405–1.3769
6mPFS 12mPFS	63.5 % 17.1 %	57.6 % 24 %			
mOS	16 months	18 months	0.1036	1.2679	0.9527–1.6873
1yOS 2yOS 5yOS	63 % 19.8 % 2.9 %	70 % 30.1 % 2.7 %			

mOS – median overall survival; 1yOS – 1 year overall survival; mPFS – median progression free survival; 6mPFS – 6 month progression free survival

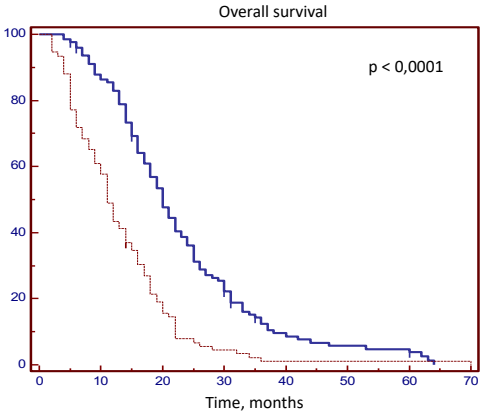

Fig. 2.6. **Impact of year of diagnosis on overall survival**
mOS in patients received treatment in 2004–2011 (blue line): 16 months; mOS in patients received treatment in 2012–2016 (red line): 18 months. HR 1.27; 95% KI 0.95–1.68; p = 0.103

Survival rate was decreased in patients with multiple metastases – mPFS 8 months and mOS 14.5 months (Table 2.8). The largest subgroup (n = 92) was in patients with liver only metastases. In this subgroup, mPFS was 8 months and mOS – 17 months.

Table 2.8

Impact of localisation of metastases on survival

Survival	Liver only mts	Lung mts	l/n or peritoneal mts	Multiple mts	p
mPFS 6mPFS 12mPFS	8 months 59,9 % 19.4 %	11 months 78.1 % 34.7 %	9 months 62.3 % 30 %	8 months 55.5 % 14 %	0.149 4
mOS 1yOS 2yOS 5yOS	17 months 74.8 % 24 % 3.4 %	20 months 73.7 % 26.8 % 0 %	18 months 71 % 38.7 % 0 %	14.5 months 56.2 % 14.6 % 0 %	0.042 4

mOS – median overall survival; 1yOS – 1 year overall survival; mPFS – median progression free survival; 6mPFS – 6 month progression free survival; mts – metastases; l/n – nymph nodes.

2.2 Analysis of clinical prognostic and predictive factors in patients with refractory mCRC receiving FTD/TPI

14 patients received FTD/TPI treatment in the Lonsurf Compassionate Use programme in Latvia between April 2016 and January 2017 in the two participating institutions. All the patients had received 2–4 previous lines of chemotherapy and progressed. The starting dose of FTD/TPI was 35 mg/m^2, and the duration of treatment was 1–9 cycles (median, 5.8 cycles). The clinical characteristics of the participants are listed in Table 2.9.

Table 2.9

FTD/TPI patient clinical characteristics (n = 14)

Characteristics	**No (%)**
Age, years	65 (52–76)
Sex:	
Male	6 (42.8 %)
Female	8 (57.2 %)
ECOG PS	
0	5 (35.7 %)
1	9 (64.3 %)
Primary site	
Colon	9 (64.3 %)
Rectum	5 (35.7 %)
KRAS status	
Wt	1 (7.1 %)
Mut	4 (28.6 %)
unknown	9 (64.3 %)
Metastases	
Synchronous – Stage IV at diagnosis	8 (57.2 %)
Metachronous	6 (42.8 %)
median time to metastases, months	22 months (8–36)
Stage II at diagnosis – 1	
Stage III at diagnosis – 5	
Median time from start of first-line chemotherapy, month (range)	32.2 months (8–90)
> 18 months	9 (64.3 %)
≤ 18 months	5 (35.7 %)
Median number of prior lines (range)	2.7 (2–4)

Continuation of table 2.9

Characteristics	No (%)
Prior chemotherapeutic agents:	
5FU	14 (100 %)
Ftorafur	3 (21.4 %)
Capecitabine	3 (21.4 %)
Oxaliplatin	13 (92.8 %)
Irinotecan	14 (100 %)
Bevacizumab	6 (42.8 %)
Aflibercept	1 (7.1 %)
Cetuximab	1 (7.1 %)

ECOG, Eastern Cooperative Oncology Group; PS, performance status; KRAS, K-ras proto-oncogene; 5-FU, 5-fluorouracil.

One patient had a delay of 1 week due to grade 3 neutropenia; none of the patients required dose reduction, and all patients eventually discontinued FTD/TPI due to disease progression.

4 patients (28.5 %) experienced Grade 3–4 neutropenia during treatment with FTD/TPI, in one patient diarrhea was observed, but in 5 patients (35.7 %) – nausea.

At the median follow-up time of 7.1 months (range, 1–14 months), the mPFS was 5 months (95%CI 4.09–5.90), and the mOS was 7 months (95% CI 5.95–8.04). The 6-month PFS was 35.7 % and the 6-month OS was 57.1 %. All 14 patients progressed on FTD/TPI treatment and 9 deaths were reported.

Increased PFS and OS were reported in patients with grade 3–4 neutropenia: the mPFS was 7 months in patients with neutropenia vs. 5 months in those without neutropenic events (HR 0.24, 95% CI 0.07–0.89; p = 0.033); the mOS was 7 months in patients without neutropenia, whereas in the neutropenic group mOS was not met (HR 0.25, 95% CI 0.06–1.14, p = 0.075) (Fig.2.7).

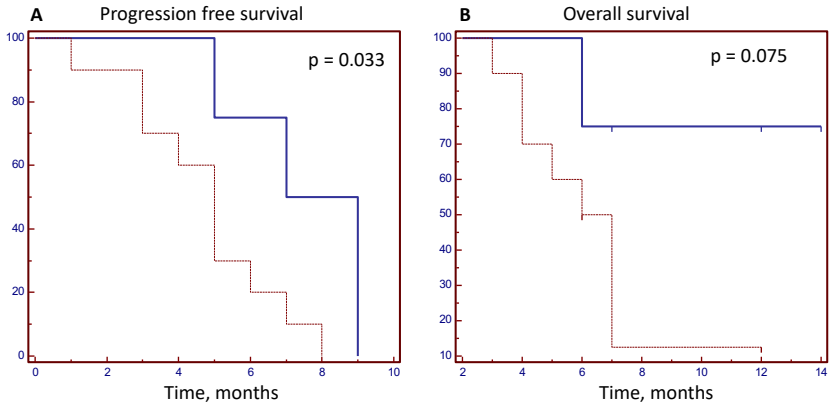

Fig. 2.7. **Grade 3–4 neutropenia as prognostic and predictive factor in patients with refractory mCRC**

(A) The mPFS was 5 months in patients without neutropenic events (red line) vs. 7 months in the neutropenia group (blue line) (HR 0.24, 95%CI 0.07–0.89; p = 0.033. (B) The mOS was 7 months in patients without neutropenia (red line), but in the neutropenic group mOS was not met (blue line) (HR 0.25, 95%CI 0.06–1.14, p = 0.075)

Furthermore, increased PFS and OS were observed in patients with a time of > 18 months from the start of chemotherapy for mCRC, with a mPFS of 7 months vs. 5 months in patients with a shorter (≤ 18 months) previous treatment duration (HR 0.15, 95%CI 0.03–0.83, p = 0.029); the mOS was not met in patients with a time of > 18 months, whereas it was 6 months in the ≤ 18 months patient group (HR 0.23, 95% CI 0.05–1.12, p = 0.069) (Fig 2.8).

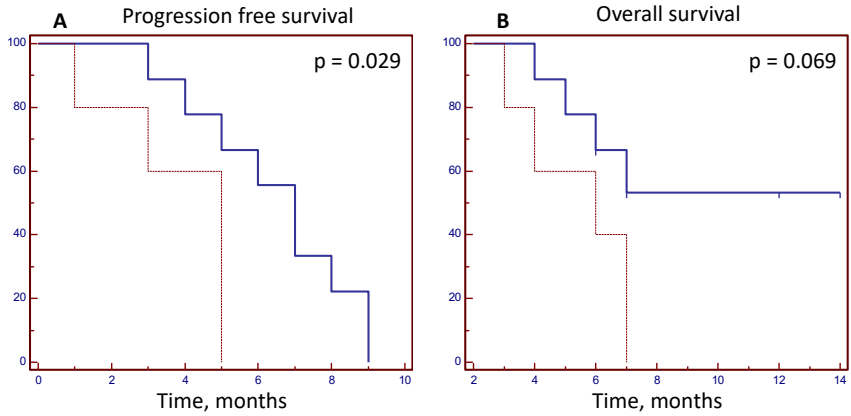

Fig.2.8. **Duration of previous treatment as a prognostic and predictive marker in refractory mCRC**

(A) The mPFS in patients with a time of > 18 months from the start of first-line mCRC treatment (blue line) was 7 months vs. 5 months in those with a time of ≤ 18 months (red line) (HR 0.15, 95% CI 0.03–0.83; p = 0.029). (B) The mOS in patients with a time of ≤ 18 months from the start of first-line mCRC treatment (red line) was 6 months, but the mOS was not met in patients with a time of > 18 months (blue line) (HR 0.23, 95% CI 0.05–1.12, p = 0.069)

2.3. *Chromothripsis* as a predictive factor in treatment of metastatic colorectal cancer

A total of 19 mCRC patients, who received chemotherapy at the Clinic of Oncology of Pauls Stradins Clinical University Hospital (Riga, Latvia) between August, 2011 and October, 2012 were selected. The patients were followed up for 3–48 months (median 25.5 months). The clinical and biological characteristics of the patients are summarised in Table 2.10.

Of the 19 patients, 15 had primary metastatic cancer (stage IV), whereas the 4 remaining patients developed metastases after the treatment of the primary cancer. A total of 10 patients developed metastases only to the liver; 16 patients underwent primary tumour surgery, whereas in 3 patients biopsy alone was

performed. In 7 patients, the carcinoembryonic antigen (CEA) level was ≤ 5.5 ng/ml prior to chemotherapy.

All patients received FOLFOX first-line chemotherapy. After disease progression, 15 patients received irinotecan-containing second-line chemotherapy, 1 patient was rechallenged with FOLFOX, 1 patient received oral fluoropyrimidine therapy with Ftorafur (tegafur) and 2 patients received best supportive care. Only 5 patients (26.3 %) received third-line therapy (irinotecan, oxaliplatin or 5-fluorouracil (5FU)). One patient underwent hepatic surgery for CRC metastases after discontinuation of second-line chemotherapy, 2 patients received salvage transcatheter arterial chemoembolisation of CRC liver metastases by irinotecan-eluting microspheres, and 1 patient received palliative radiotherapy for local rectal cancer.

Table 2.10

Clinical characteristics of patients (n = 19)

Characteristics	**No (%)**
Age, years – mean (range)	63.15 (38–78)
Sex: Male Female	 11 (57.89 %) 8 (42.11 %)
Tumour localisation: Left side (sigmoid colon, rectal cancer) Right side (colon cancer)	 11 (57.89 %) 8 (42.11 %)
Grade Unknown G2 G3	 1 (5.26 %) 13 (68.42 %) 5 (26.32 %)
Metastases: Synchronous Metachronous median time to metastases 12,25 months (9–18) Stage II (n = 3) Stage III (n = 1)	 15 (78.95 %) 4 (21.05 %)
Metastases:: Liver only Other	 10 (52.63 %) 9 (47.37 %)

Continuation of table 2.10

Characteristics	**No (%)**
KRAS status in primary tumour:	
Wild type	10 (52.64 %)
Mutation in 12 codon	7 (36.84 %)
Mutation in 13 codon	1 (5.26 %)
Unknown	1 (5.26 %)
Serum CEA prior to chemotherapy	
CEA ≤ 5.5 ng/ml	7 (36.84 %)
CEA > 5.5 ng/ml	12 (63.16 %)
Level of CEA, ng/ml – mean (range)	232.1 (7.1–959.8)
Median follow up (months) – mean (range)	25.5 (3–48)

The total number of breakpoints per genome in cancer tissue (breakpoint instability index (BPI)) was 368–4009. The highest breakpoint count was seen in chromosome 1 (27–365 breakpoints), followed by chromosome 2 (25–315) and chromosome 6 (22–298), but the lowest density of breaks occurred in chromosome 21 (7–99 breakpoints) (Table 2.11).

In 10 of the tumour samples (52.6 %), multiple chromosomal fragmentations were found, referred to as *chromothripsis*. The most commonly affected chromosomes were chromosomes 1, 2 and 6. The maximal count of chromosomes affected by chromothripsis was 20, which was observed in 1 patient (No.14).

No association of BPI value and *chromothripsis* with cancer localisation (rectal or colon cancer), CEA level, KRAS mutational status in primary cancer and stage (synchronous metastatic disease (stage IV) vs. metachronous metastatic disease) was observed.

Table 2.11

Chromosomes affected by chromothripsis and total breakpoint count per chromosome

Chromosome	Breakpoint, no, range (median)	Chromothripsis > 100 breakpoints, no of patients (%)
Chr.1	27–365 (150.2)	10 (52.6)
Chr.2	25–315 (136.8)	10 (52.6)
Chr.3	21–234 (94.5)	7 (36.8)
Chr.4	10–232 (73.9)	5 (26.3)
Chr.5	20–189 (87.1)	5 (26.3)
Chr.6	22–298 (118.9)	10 (52.6)
Chr.7	16–195 (82.7)	5 (26.3)
Chr.8	16–199 (81.6)	5 (26.3)
Chr.9	11–205 (79.2)	5 (26.3)
Chr.10	21–275 (106.8)	7 (36.8)
Chr.11	34–252 (104.1)	8 (42.1)
Chr.12	18–206 (84.2)	6 (31.6)
Chr.13	14–166 (54.1)	3 (15.8)
Chr.14	12–152 (58.6)	4 (21.1)
Chr.15	3–166 (58.9)	4 (21.1)
Chr.16	9–158 (60.5)	4 (21.1)
Chr.17	10–206 (66.8)	4 (21.1)
Chr.18	10–149 (50.5)	2 (10.5)
Chr.19	7–137 (44.2)	2 (10.5)
Chr.20	9–124 (42.3)	2 (10.5)
Chr.21	7–99 (24.7)	0
Chr.22	4–131 (37)	1 (5.3)

Chr – chromosome

The highest breakpoint count per genome was in patient No.14 – BPI 4009, but the lowest – in patient No.5 – BPI 368.

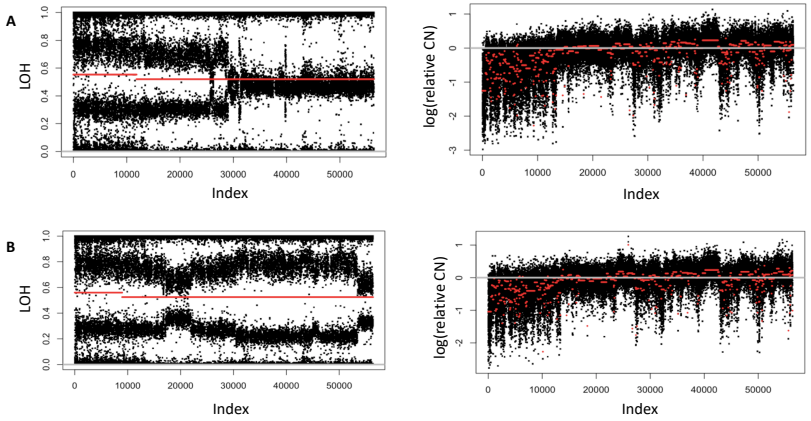

Fig. 2.9. **Copy number variation analysis**
A – Multiple chromosome 1 fragmentation (chromothripsis) (patient No 14), BPI 365. B – Multiple chromosome 1 fragmentation (chromothripsis) (patient No 8), BPI 349

Chromothripsis and survival

PFS and OS were measured for all the patients in study – mPFS was 8 months, 1yPFS – 33.3 %, 2yPFS – 5.6 %, but mOS was 21 months, 1yOS – 78.9 %, 2yOS – 42.1 %, 3yOS – 21.1 %.

A positive correlation between high BPI, *chromothripsis* and increased PFS was observed: mPFS in patients exhibiting *chromothripsis* was 14 months, compared to 8 months in patients without *chromothripsis* (HR 3.43; 95%KI 1.07–10.99; p = 0.03); mPFS for BPI ≥ 1400 was 14 months, compared to 8 months in cases of BPI < 1400 (HR 3.43; p = 0.03) (Fig. 2.10).

Decreased PFS was observed in patients with left-sided metastatic cancer (sigmoid colon and rectal cancer) and elevated CEA level prior to chemotherapy. mPFS in elevated CEA was 8 months, but mPFS in CEA ≤ 5.5 ng/ml: 14 months (HR 0.29; p = 0.03) (Fig.2.11). mPFS in patients diagnosed with metastatic rectal or sigmoid colon cancer was 8 moths, compared to 11.5 months in patients with metastatic colon cancer (HR 0.33; 95%CI 0.11–0.97; p = 0.043).

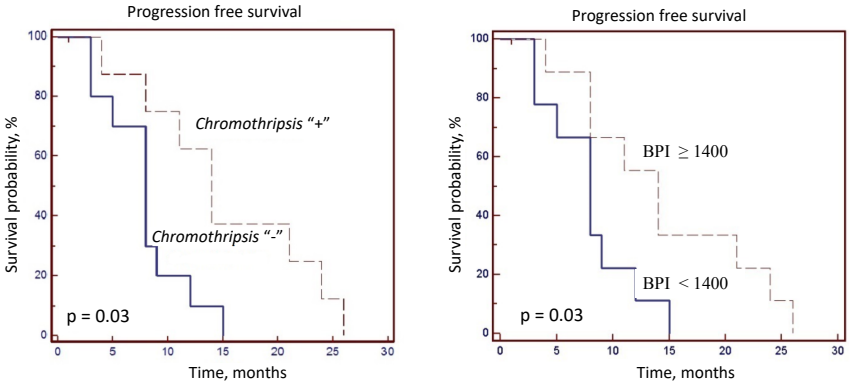

Fig. 2.10. **Impact of BPI and *chromothripsis* on progression free survival**
mPFS in patients without *chromothripsis:* 8 months; mPFS in patients with *chromothripsis*: 14 months; HR 3.43; p = 0.03. mPFS for BPI ≥ 1400: 14 months, mPFS for BPI < 1400: 8 months; HR 3.43; p = 0.03. BPI – breakpoint instability index

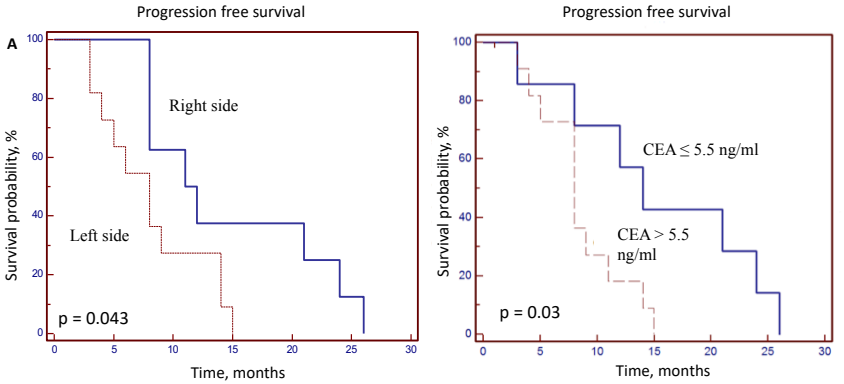

Fig. 2.11. **Impact of clinical factors on progression free survival**
mPFS in patients diagnosed with metastatic rectal or sigmoid colon cancer (left side): 8 moths, mPFS in patients with metastatic colon cancer (right side): 11.5 months; HR 0.33; p = 0.043. mPFS in patients with elevated CEA: 8 months; mPFS in patients with CEA ≤ 5.5 ng/ml: 14 months; HR 0.29, p = 0.03

The effect of BPI and *chromothripsis* on clinical findings was analysed; however, the number of patients in each subgroup was insufficient to establish a statistically significant correlation. In patients with detected *chromothripsis* and

CEA ≤ 5.5 ng/ml (n = 4), a median PFS (mPFS) of 22.5 months was observed; in patients without *chromothripsis* and elevated CEA level (n = 7), an mPFS of 8 months was observed, but these findings did not reach statistical significance.

A statistically significant effect of any clinical or biological factors on overall survival was not observed. mOS in patients with *chromothripsis* was 33 moths, compared to 19 months in patients without *chromothripsis* (HR 1.40, p = 0.52). Identical rates were observed in patients with high BPI. In patients with elevated CEA mOS was 19.5 months, compared to 33 months in patients normal level of CEA (HR 0.81, p = 0.69).

2.4. Deletions in patients with *chromothripsis*

In previous study in 10 tumour samples, it was found multiple chromosomal fragmentations (> 100 breakpoints detected at one chromosome) – *chromothripsis*. In the next study step, deletions only in patients with *chromothripsis* were analysed. Clinical characteristics of 10 patients are shown in Table 2.12. All ten patients received FOLFOX type first line chemotherapy. One patient received liver and peritoneal metastasis cytoreductive surgery. After progression, 8 patients received irinotecan containing second line chemotherapy; two patients underwent best supportive care. Only two patients (20 %) received third line therapy. One patient underwent hepatic surgery for colorectal metastases after discontinuation of second line chemotherapy, one patient – salvage transcatheter arterial chemoembolisation (TACE) of colorectal cancer liver metastases by irinotecan-eluting microspheres. Data on clinical follow up until August 2016 were obtained. mPFS in ten patients was 14 months (range 4–26 months).

Table 2.12

Clinical characteristics of patients, n = 10

Patient, no	**PFS, months**	**Age**	**Tu grade**	**CEA, ng/ml**	**Primary tu**	**Metastases**	***KRAS* status**
21	4	74	2	343.6	rectum	liver, lungs	mt
14	6	74	2	19.5	colon	liver, peritoneum	nk
3	8	38	2	7.1	colon	peritoneum	wt
6	8	68	3	644.4	colon	liver, suprarenes, retroperitoneal l/n	mt
9	11	67	3	959.8	colon	liver	wt
18	14	63	2	10.2	colon	liver	wt
19	14	60	nk	2.4	rectum	peritoneum	wt
20	21	70	2	2.6	colon	liver	mt
24	24	52	3	0.8	colon	peritoneum	wt
8	26	65	2	2.4	colon	peritoneum, solitary liver mts	mt

PFS – progression free survival, CEA-carcinoembryonic antigen before chemotherapy, mts – metastases, nk – not known; tu - tumour

Deleted regions which were overlapping in 5 or more patients (> 50%) were analysed. Overlapping deleted regions in chromosome 1 of 10 patients are observed in Fig. 2.12.

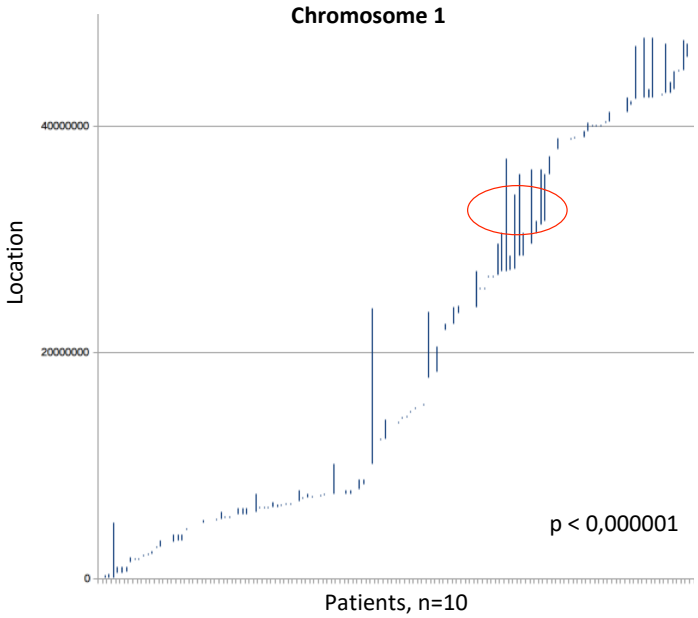

Fig. 2.12. **Chromosome 1 in all patients (n = 10) exhibiting *chromothripsis* with overlapping regions**
Blue line – deleted regions of each patient. Red line – overlapping deletions in the same location

Genes are identified, divided in five groups: 1) genes involved in proliferation or drug resistance, 2) possible tumour suppressor genes, 3) protocadherin/cadherin genes 4) epigenetic regulators and 5) genes of unclear function in oncogenesis. Frequently deleted regions are shown in Table 2.13.

Table 2.13

Frequently deleted genes, n=10

1. Genes involved in cell proliferation/drug resistance		
Gene	**Description, importance of gene**	**COSMIC**
COL11A1	*COL11A1* is overexpressed in recurrent non-small cell lung cancer and promotes cell proliferation, migration, invasion and drug resistance (*Shen et al*., 2016).	1174/41930
MAD2L1	Possible resistance to chemotherapy (*Lopez-Saavedra et al*., 2016).	70/32840
ADAM29	The expression of *ADAM29* and its mutations in different domains significantly influenced proliferation, migration and invasion of breast cancer and colorectal cancer cells (*Ashktobar et al*., 2010).	510/33364
GPM6A	Identification of *GPM6A* and *GPM6B* as potential new human lymphoid leukemia-associated oncogenes (*Charfi et al*., 2014).	167/32746
EPHA7	Downregulation or loss of EphA7 mRNA expression was detected in prostate carcinomas (*Guan et al*., 2009).	616/34447
ADK	Adenosine kinase gene expression was significantly higher in cancer than in normal-appearing tissue, in line with our previous measurements of adenosine kinase enzyme activities in colorectal tumour samples (*Giglioni et al*., 2008).	81/32840
NRG3	This gene is a member of the neuregulin gene family. This gene family encodes ligands for the transmembrane tyrosine kinase receptors *ERBB3* and *ERBB4* – members of the epidermal growth factor receptor family.	458/33190
2. Putative tumour suppressors		
Gene	**Description**	**COSMIC**
ROBO2	Down-regulation of *ROBO2* expression is detected in prostate cancers (*Lopez-Saavedra et al*., 2016).	753/42092
CADM2	Aberrant methylation and loss of *CADM2* tumour suppressor expression is associated with human renal cell carcinoma tumour progression. Low *CADM2* expression predicts high recurrence risk of hepatocellular carcinoma patients after hepatectomy (*He et al*., 2013; *Yang et al*., 2014).	297/41801
FAT4	*FAT4* functions as a tumour suppressor in gastric, breast and colorectal cancer (*Cai et al*., 2015; *Hou et al*., 2016; *Yu et al*., 2015).	1907/33867

Continuation of table 2.13

Gene	Description	COSMIC
TSG1	The grade of *TSG* expression was found to be significantly associated with gastric cancer patient survival. *TSG1* deletion was found in prostatic cancer (*Lee et al*., 2003; *Sun et al*., 2006).	109/32746
CTNNA3	*CTNNA3* is tumour suppressor gene in hepatocellular carcinoma. Loss of expression in found in multiple tumour types (*He et al*., 2016; *Smith et al*., 2006).	504/32927
3. Epigenetic regulators		
Gene	**Description**	**COSMIC**
Mir4275	Short RNA region with unknown function	
MIR1269A	miR-1269 promotes colorectal cancer metastasis. miR-1269a is upregulated in late-stage CRCs. Promotes proliferation in hepatocellular carcinoma through suppression of *FOXO1* [8,10].	
MIR2054	Short RNA region with unknown function	
MIR4643	Short RNA region with unknown function	
MIR4490	Short RNA region with unknown function	
LINCO2549	Unknown function	
PRDM9	With histone methyltransferase activity that catalyses histone H3 lysine 4 trimethylation (*H3K4me3*) during meiotic prophase (*Houle et al*., 2018).	701/32967
4. Unclear/probably unrelated		
Gene	**Description**	**COSMIC**
GABRG1	Membrane protein inhibits neurotransmission by binding benzodiazepine receptors.	403/41616
GABRG2	This gene encodes a gamma-aminobutyric acid (GABA) receptor. GABA is the major inhibitory neurotransmitter in the mammalian brain, where it acts at GABA-A receptors, which are ligand-gated chloride channels. Mutations in this gene have been associated with epilepsy and febrile seizures.	317/41865
COX7B2	COX7B2 (Cytochrome C Oxidase Subunit 7B2) is a Protein Coding gene. Polimorphism of COX7B2 gene is reported in a Cantonese family with nasopharyngeal carcinoma (*Liang et al*., 2004)	46/41646
TECRL	Mutations in this gene result in ventricular tachycardia	247/41578
UGT2B4	Gene polymorphism associated with predisposition to breast cancer and may play a role in the onset of menarche (*Sun et al*., 2011).	283/32747
GLRA3	Glycine receptor coding gene.	204/32748
FAM174A	Membrane protein 157 coding gene.	47/32746

Gene	Description	COSMIC
ST8SIA4	A modulator of the adhesive properties of neural cell adhesion molecule (*NCAM1*)	187/32812
PRR16	Encodes Largen – a molecular regulator of mammalian cell size control. Largen 39ocal important link between mRNA translation, mitochondrial functions, and the control of mammalian cell size (*Yamamoto et al*., 2014).	163/32724
FTMT	Mitochondrial ferritin.	240/32746
KHDRBS2	Signal transduction associated protein	303/32878
EYS	The protein is expressed in the photoreceptor layer of the retina, and the gene is mutated in autosomal recessive retinitis pigmentosa	424/33464
MANEA	Endomannosidase protein in Golgi complex	123/32809
FUT9	The protein encoded by this gene belongs to the glycosyltransferase family. Inactivation of *FUT9* gene is reported in colorectal cancer oncogenesis (*Auslander et al*., 2017).	218/32746
GRIK2	Neurotransmitter receptor in the mammalian brain. Activated in a variety of normal neurophysiologic processes.	518/33002
SSPO	SCO-spondin, involved in placental physiology and development.	
SGCZ	*SGCZ* encodes one of the sarcoglycan complex protein's, that is part of the dystrophin-associated glycoprotein complex (DGC), which bridges the inner cytoskeleton and the extra-cellular matrix	258/32747
CNTN5	Neuronal membrane protein that functions as a cell adhesion molecule.	602/33002
5. Protocadherin/cadherin gene superfamilies		
Gene	**Description**	**COSMIC**
PCDH10	*PCDH10* inhibits the proliferation, invasion and migration ability of pancreatic cancer cells and is frequently downregulated by promoter methylation in pancreatic cancer cells (*Qiu et al*., 2016). The epigenetic silencing of *PCDH10* has been identified as an important tumour suppressor gene with key roles in colorectal carcinogenesis, invasion and metastasis as a frequent and early event (*Zhong et al*., 2017). The loss of *PCDH10* function promotes not only tumour progression but also liver metastasis. The genetic deletion of *PCDH10* represents an adverse prognostic marker for the survival of patients with colorectal cancer (*Jao et al*., 2014).	763/32849

Continuation of table 2.13

Gene	Description	COSMIC
PCDH18	*PCDH18* is frequently inactivated by promoter methylation in colorectal cancer (*Zhou et al*, 2017).	563/32837
CDH18	Mutation and loss of expression of cadherins have been implicated in the progression of some malignant tumours, suggesting that cadherins may also act as tumour/metastasis suppressor genes. *CDH18* may be functionally linked to CRC development (*Venkatachalam et al*., 2011; *Chalmers et al*., 1999).	670/33085
CDH12	Cadherin-12 enhances proliferation in colorectal cancer cells and increases progression. High expression of *CDH12* was associated with tumour invasion depth and predicts poor prognosis. *CDH12* is expected to become a new diagnostic and prognostic marker and a novel target of the treatment of colorectal cancer (*Ma et al*., 2016).	560/32970
CDH10	*CDH10* mutation might inactivate the cell adhesion-related functions and could be a feature of gastric and colorectal cancer with MSI-H (*An et al*., 2015).	799/33400
PCDH15	*PCDH15* is a member of the cadherin superfamily, encode integral membrane proteins that mediate calcium-dependent cell-cell adhesion.	1187/33222

COSMIC – Catalogue of Somatic mutations in Cancer

Eight deleted tumour suppressor genes (*ROBO2, CADM2, FAT4, PCDH10, PCDH18, CDH18, TSG1, CTNNA3*) and four deleted oncogenes (*CDH12, GPM6A, ADAM29, COL11A1*) were identified in more than half of the patients. In 60 % patients, deletion in *COL11A1* was detected. Deletion of *MIR1269, MIR4465, MIR1261* and *MIR4490* in patients with longer time to progression was observed. Four patients (40 %) with PFS over 14 months, presented with *NRG3* deletion (oncogene) that could possibly decrease proliferation of cancer cells via decreasing EGFR activation.

Overlapping deletions ad PFS are seen in Table 2.14.

Table 2.14

Overlapping deletions

Patient number	21	14	6	3	9	18	19	20	24	8
Progression free survival(PFS), months	**4**	**6**	**8**	**8**	**11**	**14**	**14**	**21**	**24**	**26**
ROBO2										
ROBO1										
CADM2 GBE1										
PROS1 NSUN3 DHFRL1 EPHA6										
PRKAA2 C1orf168										
NEGR1										
LRRIQ3 TNNI3K FPGT-TNNI3K										
ELTD1										
COL11A1										
MIR4275										
GABRG1 GABRA2 GABRA4 COX7B2										
ADGRL3										
TECRL										
miR-1269										
UGT2B4										
MAD2										
FAT4										
PCDH10										
PCDH18										
DCLK2 LRBA										
FSTL5										
GALNTL6										
ADAM29										
GPM6A										
CDH18										
CDH12 PRDM9 CDH10										
FAM174A ST8SIA4 SLCO4C1										
PRR16 FTMT										
KHDRBS2										
EYS										
LINC02549										
BAI3										
MiR-4643 LOC101929083										
EPHA7 TSG1 MANEA FUT9										
GRIK2										
mir-4465										
SSPO										
SGCZ										
PCDH15 MTRNR2L5										
CTNNA3										
ADK										
NRG3										
MiR-4490 miR-1261										
CNTN5										

Yellow colour – deletions

3. Discussion

The study consists of three separate parts of metastatic colorectal cancer prognostic and predictive factors. Both clinical factors (age, treatment choice, neutrophil count, etc.) and molecular factors (BPI, *chromothripsis*) and their impact on progression free and overall survival were studied. In the first and second study sections clinical prognostic and predictive factors were analysed, while in the third study section at the center of attention were genetic factors. mCRC is heterogeneous disease with multiple factors affecting treatment outcome and the prognosis. In the last decades with the development of molecular diagnostics, new promising predictive and prognostic factors are increasingly detected.

3.1. Analysis of patients with unresectable mCRC treated at Clinic of Oncology, Pauls Stradins Clinical University Hospital

Standard treatment of unresectable metastatic colorectal cancer is combined chemotherapy and targeted therapy with the aim of prolongation of survival, controlling of symptoms with acceptable quality of life. Analysis of patients received chemotherapy in Clinic of Oncology of Pauls Stradins Clinical University Hospital was done to compare the results with the data about CRC in Latvia, as well as previously published trial results. Data from 220 patients were collected. Patients who did not receive chemotherapy due to poor performance status or *bulky* metastatic disease, as well as patients who underwent resection of metastases were excluded from the recent study. Also, patients with incomplete information about the data of diagnosis, progression or type of treatment were excluded from the study to achieve objective results.

Surgical resection is a curative treatment cornerstone in early and locally advanced colorectal cancer, while in the case of metastatic colorectal cancer

combined chemotherapy is the main treatment method. Median progression free survival (mPFS) in patients receiving first line FOLFOX or FOLFIRI in clinical trials reaches 8 months, but median overall survival (mOS) – 18 months (*Colucci et al.,* 2005; *Tournigand et al.*, 2004). Addition of bevacizumab or anti-EGFR monoclonal antibodies to oxaliplatin containing chemotherapy prolong mPFS to 10 months and mOS – to 24 months (*Bokemeyer et al.*, 2009; *Douillard et al.*, 2010; *Tol et al.,* 2009). Similar to the data from clinical trials, mPFS in patients included in our study was 8 months, but mOS – 17 months. Addition of targeted therapy to standard chemotherapy did not significantly improve either mPFS or mOS, it could be explained by the small number of patients in the subgroups.

The most common type of first line chemotherapy in the study was oxaliplatin containing therapy (FOLFOX), accordingly to ESMO guidelines – it was prescribed to 82 % patients. Regarding the fact that bevacizumab, cetuximab and panitumumab were included in the list of reimbursed medications only in 2018, a small number of our patients (11 %) received the targeted treatment and covered the costs themselves. Irinotecan containing therapy in the first line setting was slightly more frequent (11.7 % vs. 4 %) in metachronous mCRC subgroup, which can be explained with the fact that oxaliplatin containing regiment (FOLFOX) had been received previously in the adjuvant treatment following radical surgery for 43ocalized colorectal cancer (Stage II–III).

Data of the prognostic significance of synchronous and metachronous mCRC have been published. Data from the various studies show conflicting results (*Tournigand et al*., 2004; *Colluci et al*., 2005). *Mekenkamp et al.* published results from CAIRO trial update in 2010 – analysis of 550 patients' clinical factors impact on survival. There was no difference in survival in patients with synchronous or metachronous mCRC (HR 1.05, p = 0.74). Similarly, there were no statistically significant differences in PFS and OS between two subgroups: mOS 17 vs. 17 months (HR 1.02, p = 0.9) and mPFS 8 vs. 8 months (HR 0.9, p = 0.56). Although the study published by *Mekenkamp* showed adverse

prognostic factors in synchronous mCRC such as poor differentiation, right sided primary tumour, poor performance status and elevated LDH, the survival rates where similar. The authors associated this finding with the fact that metachronous mCRC patients could be resistant to chemotherapy they received in adjuvant therapy, resulting in similar survival in both subgroups.

> 80 % of patients progressed on first line chemotherapy are eligible for second line chemotherapy significantly increasing survival. In our retrospective study, 57 % of the patients received second line treatment. The most frequent treatment choice (85 %) was irinotecan containing chemotherapy. We observed that patients who received more than one line of chemotherapy gained statistically significant improvement in survival – mOS 20 vs. 11 months (HR 0.36, $p < 0.0001$); in addition, in patients with metachronous mCRC receiving FOLFIRI plus targeted therapy mOS reached 38 months ($p < 0.0001$). mPFS in patients receiving FOLFIRI in second line was 5 months, these data are similar to the results from clinical trials (4–6 months).

5 % of the patients diagnosed with colorectal cancer in Latvia in 2017 were under the age of 50 according to Latvian Centre for disease prevention and Control (CDPC) data. 12.7 % of the patients in our study were under 50. Similar results from metanalysis of 24 trials involving 20,000 patients receiving first line chemotherapy were published by *Lieu et al.* in 2014 – 15 % of the patients were under 50. The impact of patient's age on PFS and OS was observed: younger patients had 19 % higher risk of cancer related death and 22 % higher risk of progression compared to older patients. The current study data show the opposite results – both mPFS (9 months vs. 8 months, $p = 0.045$) and mOS (30 months vs. 16 months, $p = 0.0002$) are improved in patients under 50 years of age. In addition, 5-year OS in younger patient subgroup reached 18.8 %. These results can be explained by the fact that at this age group the overall performance status is good, chronic diseases are less common and tolerability of chemotherapy is better. As well as tumour biology and genetics may be different in young patients:

hereditary colorectal cancer syndromes are more common than sporadic colorectal cancer arising in classical "adenoma-carcinoma" oncogenesis pathway in this age subgroup.

Liver is the most common localisation of colorectal cancer metastases. In the current study, liver only metastases were observed in 42 % of the patients resulting in mPFS – 8 months and mOS – 17 months. Only in this subgroup 74.8 % of the patients were alive one year after the diagnosis and 3.4 % – 5 years after the diagnosis. Decreased survival was observed in the subgroup with multiple metastases – mOS of 14.5 months and only 56 % of the patients were alive one year after the diagnosis.

Patients treated in two different time periods (2004–2011 and 2012–2016) were compared and no statistically significant difference was found. Analysis of first type period of the study is published in *Acta Chirurgica Latviensis* (*Skuja et al.,* 2012). Interestingly that patients in metachronous mCRC group had better mPFS and mOS, but in the current study, which includes all patients from both time periods, the difference is no longer observed. This can be explained with doubling of study population that makes results much more objective. The overall survival was slightly better in patients treated in 2012–2016, but the results did not meet the statistical significance. This trend in survival improvement could be associated with the growing experience in treatment of mCRC, more frequent use of mFOLFOX6 instead of FOLFOX4, administration of two and more lines of chemotherapy and more careful selection of patients for therapy. It is important to continue analysis of mCRC patients, because of increasing the number of study population and including the patients receiving targeted therapy, it becomes possible to improve treatment results and prognosis.

3.2. Analysis of clinical prognostic and predictive factors in patients with refractory mCRC receiving FTD/TPI

Therapeutic efficacy of trifluridine/tipiracil (FTD/TPI) was initially demonstrated in 169 patient in Phase II trial (*Yoshino et al*., 2012) and confirmed in randomised, double-blind, placebo-controlled RECOURSE trial (*Mayer et al.*, 2015). The study included 800 patients with refractory mCRC who were previously treated with at least two lines of palliative chemotherapy. Patients received FTD/TPI 35 mg/m^2 orally twice daily with best supportive care (BSC) or placebo with BSC. Patients in both treatment groups had similar demographical and clinical data – all patients had an ECOG 0 or 1.49 % of the included patients was KRASwt and 51 % – KRAS mutant. Patients continued the study treatment until the progression or unacceptable toxicity. This trial confirmed the efficacy of FTD/TPI in patients with pretreated mCRC, as treatment with FTD/TPI was associated with a significant improvement in mOS (7.2 vs. 5.2 months; HR 0.69; $p < 0.0001$) and mPFS (HR 0.48; $p < 0.001$) vs. placebo. 1 year OS was nearly twice better in FTD/TPI group – 27.1 % compared to 16.6 % in placebo group.

Patients receiving FTD/TPI achieved longer disease control (44 % compared to 16 %, $p < 0.01$) and maintained longer better performance status and good quality of life. The most common side effects were leucopenia, neutropenia, anemia and thrombocytopenia, mostly asymptomatic. Treatment discontinuation rate was low: 3.6 % in FTD/TPI compared to 1.5 % in placebo group (*Mayer et al.,* 2015; *Yoshino et al.,* 2012).

In total, 2093 patients participated in the Lonsurf Compassionate Use programme in 20 countries (*Salvatore et al.,* 2016), including 14 patients from Latvia.

Similar survival data were observed in the current study, with a mPFS of 5 months and a mOS of 7 months. In the present study, grade 3–4 neutropenia

was the most frequently observed clinically meaningful adverse event, occurring in 28 % of the patients. This adverse event was associated with better treatment outcomes (mPFS of 7 months, HR 0.24 and increased mOS, HR 0.25). Previously reported data demonstrated that, in patients with advanced colorectal cancer, FTD/TPI-induced severe neutropenia was associated with superior survival. Impact of neutropenia on survival was recently reported by *Hamauchi et al.* (2017). 21 % of the patients experienced grade 3 or 4 neutropenia after the first cycle of FTD/TPI. In this subgroup of patients increased survival was observed – mPFS (2 months vs. 4.3 months, HR 0.41, p = 0.002) and mOS (5.7 months vs. 8.0 months, HR 0.59, p = 0.084).

Despite the small number of included patients, it was observed that the time from the start of first-line chemotherapy to the start of FTD/TPI treatment may be a prognostic factor, as the mPFS in patients with a time of > 18 months from the start of mCRC treatment was 7 months (HR 0.15), and the HR for cancer-specific mortality was 0.23 (mOS was not met). Similar results were reported by *Hamauchi et al.* in 2017 – improved mPFS (HR 0.47, p = 0.01) and mOS (HR 0.44, p = 0.01) were observed in patients with a time of > 18 months from the start of mCRC treatment. Also, recently published study results showed better overall survival (HR 1.42, p = 0.043) in patients with longer time from beginning mCRC treatment (> 18 months) (*Moriwaki et al.,* 2019). It may be suggested that patients with a shorter duration of previous treatment lines and rapid progression under previous chemotherapy may benefit less from FTD/TPI treatment. This observation may lead to the hypothesis that more aggressive and chemotherapy-resistant tumours possibly harbour mutations leading to FTD/TPI resistance. Therefore, a variety of previous chemotherapy lines, patient performance status, primary tumour and metastases location may affect the duration of PFS and OS.

There were certain limitations to the present study. Due to the limited number of participants in the Lonsurf Compassionate Use programme, the

research was conducted on a small size of the mCRC population (n = 14). Therefore, to generalise the results for a larger population, the study would require more participants. FTD/TPI has been reimbursed for treatment of refractory mCRC in Latvia since 2019, the number of patients who receive this treatment are growing.

Severe neutropenia may be considered as a surrogate marker for predicting FTD/TPI treatment outcomes. In addition, patients with a time of < 18 months from the start of first-line mCRC treatment to the first FTD/TPI administration have a poor prognosis. However, further studies are required to confirm these findings.

3.3. *Chromothripsis* as a predictive factor in treatment of mCRC

45 patients diagnosed with mCRC, who had signed informed consent, were initially included in the study. Only 19 patients were appropriate for further analysis because of poor quality of cancer tissue – content of cancer cells in the sample was less than 80 % or it was impossible to extract the DNA for further CNV analysis. In tissue samples with a cancer cell less than 80 %, the result would be insignificant because of the impact of normal stromal cell DNA to the main result.

The effect of chromosomal rearrangements and mutations on pathogenesis, prognosis and resistance to treatment are widely described in mCRC studies. The prognostic and predictive role of breakpoint instability index (BPI) and *chromothripsis* remains unclear.

In the present study, correlation between massive DNA fragmentation (chromothripsis) and PFS in mCRC was observed. As opposed to recent studies suggesting chromothripsis to be associated with worse prognosis, we found chromothripsis to be a positive predictive factor for first-line chemotherapy. It may be hypothesised that cancer cells exhibiting radical DNA rearrangements,

such as chromothripsis, are more sensitive to nucleic acid-damaging therapy with 5FU and oxaliplatin. Second hypothetical assumption is – tumours with high BPI could be characterised by genomic instability which, in turn, is related to better prognosis. This hypothesis is mentioned in summary analysis of different cancer types, including two colorectal cancer studies, published by *Luijten* referring to our publication (*Luijten et al.,* 2018). The study data are also mentioned by *van Poppelen* in analysis of *chromothripsis* in uveal melanoma (*van Poppelen et al.,* 2018). The authors of both studies suggest that tumours exhibiting *chromothripsis* are more aggressive and tend to be diagnosed in a late stage, which explains the recently published data of association of *chromothripsis* with decreased survival, assuming that metastases with this genomic alteration could better respond to chemotherapy.

Breakpoint instability index was previously reported in breast cancer study by *Przybytkowski et al.* in 2014; they reported a BPI of 25–300 in breast cancer tissue, with the highest density of breaks in chromosome 17 and the lowest density in chromosome 4. The BPI appeared to be different in different breast cancer molecular subtypes, with the highest breakpoint count in aggressive triple-negative breast cancer. In comparison, in the authors' study on CRC tissue, the highest breakpoint density was found in chromosomes 1 and 2 and the lowest in chromosome 21, but the BPI was significantly higher (368–4009). In addition, 10 tumour samples (52.6 %) exhibited a chromothripsis pattern on $\geq$ 3 chromosomes, which was higher compared to previous reports. The high BPI value and high prevalence of chromothripsis in the current study may be attributed to the fact that all patients had late-stage metastatic disease, which is consistent with the prevalence of chromothripsis reported in high-risk aggressive tumours.

Extensive *chromothripsis* analysis of 38 different cancer types including 2658 patient tissue samples was published earlier this year (*Cortés-Ciriano et al.*, 2020). The total incidence of *chromothripsis* in all types of cancer was 40 %, the

highest rate described so far. Also, *chromothripsis* was detected in 32.7 % of colorectal cancer samples (in 17 out of 52 samples), which, in turn, is less than observed in the current study.

The present study demonstrated that chromothripsis is associated with increased PFS, but not with OS in mCRC. Overall survival is affected by many factors – performance status of a patient, concomitant diseases, type and sequence of chemotherapy and molecular alterations in cancer DNA.

Eight deleted tumour suppressor genes (*ROBO2, CADM2, FAT4, PCDH10, PCDH18, CDH18, TSG1, CTNNA3*) and four deleted oncogenes (*CDH12, GPM6A, ADAM29, COL11A1*) were identified in more than half of the patient population. In 60 % patients' deletion in *COL11A1* was detected. It has been previously reported that *COL11A1* overexpression is associated with proliferation, migration and chemo-resistance to platinum in lung cancer (*Shen et al*., 2016). It can be suggested that deletion of this oncogene could be associated with better survival due to slower progression and migration. Similarly, overexpression of *MAD2L1* is reported in platinum resistant testicular germ cell tumours (*Lopez-Saavedra et al.,* 2016). 50 % of the study patients showed *MAD2L1* deletion, which, in turn, could reduce cell proliferation and disease progression. In 50 % patients' overlapping deletion of*MIR1269A* was seen – overexpression of it promotes metastases and proliferation in hepatocellular carcinoma (*Yang et al*., 2014). In the current study, deletion of *MIR1269*, as well as *MIR4465*, *MIR1261* and *MIR4490*, was observed in patients with longer time to progression. It might be beneficial for mCRC patients to harbour deletions of *MIR* genes because of decreased potential of proliferation and formation of metastases.

Four patients (40 %) with PFS over 14 months presented with *NRG3* deletion that could possibly decrease proliferation of cancer cells via decreasing EGFR activation. This gene encodes ligand of EGFR which activates intracellular signaling cascade and promotes cell proliferation, differentiation

and migration. As a result of deletion of this gene, activation of EGFR is decreased. Although, alterations of *NRG3* gene have been published in relation to psychoneurological diseases (*Kao et al*., 2010; *Sonuga-Barke et al*., 2000), only one point mutation can impact progression free survival of ovarian cancer patients receiving carboplatin chemotherapy (*Huang et al*., 2011).

In previous publication, the authors have reported chromothripsis as a surrogate marker for increased mPFS and possible indicator for better response to oxaliplatin based treatment — chromothripsis was associated with mPFS 14 vs. 8 months ($p = 0.03$). It was observed that patients with multiple overlapping deletions have increased time to progression. There are two patients in the study group who are exceptional. Patient No. 21 presents multiple overlapping deletions but has very short time to progression. This patient was diagnosed with bulky metastases in liver and lungs, discontinued fist line FOLFOX treatment due to poor performance status and toxicity and progressed in 4 months. The second exceptional patient No. 8 had 26-months remission period but presented only one overlapping deletion (*SSPO*, gene with unknown function). Such a long PFS was observed due to the fact that he had undergone resection of primary tumour in colon and resection of metastases in peritoneum and liver (maximum cytoreductive surgery) before the start of palliative chemotherapy. It is known that treatment strategy – surgery, chemotherapy intensity, targeted treatment, as well as patient related factors, such as performance status, comorbidities, burden of metastatic disease and tumour molecular characteristics, impact time to progression and OS in mCRC patients. For further research it is important to have a more homogenous patient group with similar clinical findings and treatment strategy.

Conclusions

1. The most important factors that improve survival are the patient's age < 50 years, the number of chemotherapy lines (more than one line of palliative chemotherapy) and the type of second line treatment. In turn, multiple metastases decrease survival.
2. Clinical factors – Grade 3 and 4 neutropenia and duration of previous treatment > 18 months – improve survival (PFS and OS) in refractory metastatic colorectal cancer patients receiving third line of FTD/TPI.
3. *Chromothripsis* and high BPI increase PFS in patients with mCRC receiving first line FOLFOX chemotherapy, but do not affect OS.
4. The most common deletions in patients with *chromothripsis* are: tumour supressor genes' *ROBO2*, *CADM2*, *FAT4*, *PCDH10*, *PCDH18*, *CDH18*, *TSG1*, *CTNNA3* deletions and oncogenes' *CDH12*, *GPM6A*, *ADAM29*, *COL11A1*, *NRG* deletions.

Practical recommendations

1. In patients with unresectable mCRC, palliative chemotherapy consisting of several lines is a standard treatment that improves progression-free and overall survival.
2. In refractory mCRC patients who are considered of third line FTD/TPI treatment, the duration of previous treatment should be taken into account in order to predict efficacy of treatment. The development of Grade 3 and 4 neutropenia is a positive prognostic and predictive marker in patients receiving current treatment.
3. *Chromothripsis* and high BPI are potential predictive markers in mCRC patients receiving first line FOLFOX therapy. It is important to have a more homogenous and bigger patient group with similar clinical findings and treatment strategy to confirm the clinical relevance of these novel genetic markers.

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