# Treatment of stage IV colorectal cancer: A retrospective cohort study assessing whether failure of first-line treatment indicates failure of second-line treatment

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**Abstract.** Colorectal cancer (CRC) is one of the most frequent malignancies and, despite screening programs, it is often diagnosed at late stages. Although current first- and second-line therapies stratify for KRAS/NRAS/BRAF mutations, microsatellite instability, tumour location and co-morbidities, the therapeutic mainstay for the first- and second-line treatment of the majority of patients consists of 5-fluorouracil (5-FU)-based chemo-immunotherapy. The present study evaluated the responses of patients with stage IV CRC, treated at the University Hospital Krems between January 1, 2015 and December 31, 2021, who received at least two therapy lines (n=49), with the aim of investigating whether the response to first-line therapy could predict the response to second-line therapy. All patients with first-line complete response (CR) had at least stable disease in response to second-line treatment [overall response rate (ORR)=66.6%]. On the other hand, all patients with progressive disease (PD) in response to first-line treatment (n=7) did not respond to second-line therapy (ORR=0%). These findings also translated to overall survival (OS): Patients with first-line CR had a median OS time of 80 months, whereas patients with PD had a median OS time of 12 months (P<0.001). Furthermore, different parameters were analysed for their impact on OS; the results revealed that BRAF alterations were associated with poor prognosis. Other factors (sex, tumor sidedness, KRAS and MSS/MSI status) had in this cohort no significant effect on OS. In conclusion, the present study demonstrated that, with current treatment strategies applying 5-FU-based chemo-immunotherapy as first- and second-line treatment for patients with metastatic CRC, response to first-line therapy may be a strong predictor for the response to second-line therapy and OS. By exchanging the chemotherapeutic combination partner from oxaliplatin to irinotecan or vice versa, plus the additive anti-epidermal growth factor receptor/anti-vascular endothelial growth factor antibody, the negative factor of non-response to first-line therapy could not be overcome by second-line treatment in this study population. These findings must be confirmed in larger studies, but indicate the need for novel treatment options, especially for patients not responding to first-line 5-FU-based chemo-immunotherapy.

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*Abbreviations:* 5-FU, 5-fluorouracil; CAPIRI, capecitabine, irinotecan; CAPOX, capecitabine, oxaliplatin; CR, complete response; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FOLFIRI, folinic acid, 5-FU, irinotecan; FOLFOX, folinic acid, 5-FU, oxaliplatin; FOLFOXFIRI, folinic acid, 5-FU, oxaliplatin, irinotecan; HGMA2, high-mobility group A 2 gene; mAbs, monoclonal antibodies; mCRC, metastatic colorectal cancer; MSI, microsatellite instability; MSS, microsatellite stable; NCCN, National Comprehensive Cancer Network; ORR, overall response rate; OS, overall survival; PARP1, poly [ADP-ribose] polymerase 1; PD, progressive disease; PFS, progression free survival; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; VEGF, vascular endothelial growth factor

*Key words:* stage IV CRC, first-line treatment, second-line treatment, 5-FU-based chemo-immunotherapy, multidrug-resistance, retrospective cohort study

## Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide and the second leading cause of cancer-associated deaths. Unfortunately, 20% of patients show distant metastases at diagnosis and about 35% will develop metastases during the course of the disease (1). In 75-90% of advanced cases, it is not possible to resect all lesions and thus treatment with palliative chemo-immunotherapy is applied (2). Although current first- and second-line therapies consider genetic characteristics, like KRAS/NRAS/BRAF mutations, microsatellite instability (MSI), and other factors, including tumour location, age, and co-morbidities (3), the therapeutic mainstay consists of 5-Fluorouracil (5-FU)-based chemotherapy.

Generally, 5-FU or its prodrug capecitabine is applied with or without irinotecan and/or oxaliplatin, plus targeted

immunotherapy, like the anti-vascular endothelial growth factor (VEGF) antibody bevacizumab or the anti-epidermal growth factor receptor (EGFR) agents cetuximab or panitumumab (4). Anti-EGFR antibodies are less effective in right-sided CRC and not effective in RAS-mutated CRC (5,6).

5-FU is an antimetabolite, which, due to its structural similarity to the ribonucleic acid uracil, gets incorporated into RNA instead of uracil and leads to the inhibition of DNA biosynthesis and cell growth. It also inhibits the thymidylate synthase, an enzyme important for pyrimidine synthesis. 5-FU is often combined with Leucovorin® (folinic acid), a chemo-protectant that blocks the side effects of 5-FU and is used for the potentiation of the 5-FU tumouricidal effects. It increases patients' survival and response rates (7). Oxaliplatin is a platinum compound and causes inhibition of DNA synthesis by crosslinking DNA (8). Irinotecan is an analogue of camptothecin, which is converted to its active metabolite in the body. Irinotecan inhibits topoisomerase 1, causes inhibition of DNA synthesis and DNA double strand breaks, and ultimately leads to cell cycle arrest and cell death (9).

The combination-chemotherapy regimens FOLFOX (folinic acid, 5-FU and oxaliplatin; CAPOX when oral capecitabine is applied) and FOLFIRI (folinic acid, 5-FU and irinotecan; CAPIRI when oral capecitabine is applied) show equal efficacy, however, have a different toxicity profile, which can guide decision-making (10).

The triplet FOLFOXFIRI can be used in certain cases, e.g. for fit patients without co-morbidities or for patients harbouring a BRAF mutation (10).

In mCRC patients, current second-line treatment depends mainly on the prior first-line treatment. Patients, who have received an oxaliplatin-containing doublet combination therapy, are switched to an irinotecan-containing doublet, and vice versa. Again, in second-line treatment, anti-EGFR monoclonal antibodies (mAbs) and bevacizumab can be added to the chemotherapy. Aflibercept is an alternative anti-VEGF agent, which can be applied in second-line therapy (10). Anti-VEGF agents can be effective, although the patient progressed during first-line treatment. Until recently, anti-EGFR antibodies were considered only useful in one line of treatment. New treatment options suggest that reintroduction or continuing use of anti-EGFR therapy can be beneficial, if no KRAS mutations have occurred during the initial treatment (7,11).

However, tumours can be resistant against chemotherapeutic agents, either prior to treatment start ('innate chemoresistance') or during the course of treatment ('acquired chemoresistance'). Often tumours are not only resistant to one drug, but also to other or similar agents ('multidrug-resistance') (7,12). Many mechanisms, such as elevated metabolism, enhanced drug efflux, increased DNA repair capacity, growth factors, or genetic and epigenetic factors are involved in the process of chemoresistance, but this process is yet not fully understood (13).

This study thus aimed to investigate the role of multidrug-resistance in a real-world setting: this study explored, if under current first- and second-line protocols for mCRC, response to first-line therapy can predict response to second-line therapy. The null-hypothesis of this study is that the rates of remissions in second-line chemo-immunotherapy do not differ between patients with CRC stage IV, who had a response in first-line chemo-immunotherapy, and patients, who had no response in first-line chemo-immunotherapy.

## Patients and methods

*Study design*. This study is a retrospective cohort study analysing patients' data from the data management system of the University Hospital Krems, and the Oncology Information System (OIS) of Lower Austria. All participants received treatment for CRC stage IV at the Department of Oncology of the University Hospital Krems.

The study was approved by the Commission for Scientific Integrity and Ethics at the Karl Landsteiner University of Health Sciences in September 2022 (EK No. 1046/2022) and was conducted according to the Declaration of Helsinki. Due to the retrospective nature of this study informed consent was waived as approved by the Commission for Scientific Integrity and Ethics at the Karl Landsteiner University of Health Sciences.

*Study population and statistical analyses*. Between 01.01.2015 and 31.12.2021, 125 patients were diagnosed with advanced CRC and received treatment at the University Hospital Krems. After screening all eligible patients against the predefined inclusion and exclusion criteria, 49 patients could be included in this study. These patients were analysed according to their response to treatment for mCRC.

Inclusion criteria were patients with histologically proven CRC stage IV, who underwent palliative treatment with chemo-immunotherapy consisting of either 5-FU or the 5-FU derivative capecitabine with or without oxaliplatin and/or irinotecan, respectively, combined with the mAbs bevacizumab, cetuximab or panitumumab, between 01.01.2015 and 31.12.2021 at the University Hospital Krems. Exclusion criteria were patients, who were cured by surgery following initial or adjuvant chemo-immunotherapy and not experiencing a recurrence of the disease, other cancer types or stages and patients under the age of 18.

Treatment responses to first- and second-line therapy were confirmed according to the Response Evaluation Criteria in Solid Tumors (RECIST), which comprise complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) based on computer tomography (CT) or magnetic resonance imaging (MRI) re-staging examinations after initiation of chemo-immunotherapy. These criteria were established in 2000 by an international collaboration including the European Organisation for Research and Treatment of Cancer (EORTC), National Cancer Institute of the United States, and the National Cancer Institute of Canada Clinical Trials Group (14), later updated in 2009 (15) and have meanwhile evolved as standard treatment response criteria in the majority of clinical trials for solid tumours (16).

All data were analysed and presented in a pseudonymized form, as every patient received initially a pseudonymized identification number (001, 002…).

The following variables were analysed and used for statistical evaluation: The descriptive parameters age (in years, at begin of treatment), sex (female, male), tumour sidedness (left, right), KRAS status (wild-type, mutant), NRAS status (wild-type, mutant), BRAF status (wild-type, mutant) as well as microsatellite status (MSS, MSI).

Table I. Characteristics of the patient population.

| Characteristic | Value |
|---|---|
| Median age, years (range) | 63 (38-78) |
| Sex, n (%) | |
| Female | 17 (34.7%) |
| Male | 32 (65.3%) |
| Tumour sidedness, n (%) | |
| Left | 34 (69.4%) |
| Right | 15 (30.6%) |
| KRAS mutation, n (%) | |
| Positive | 25 (51.0%) |
| Negative | 24 (49.0%) |
| NRAS mutation, n (%) | |
| Positive | 4 (8.2%) |
| Negative | 38 (77.5%) |
| Not determined | 7 (14.3%) |
| BRAF mutation, n (%) | |
| Positive | 2 (4.1%) |
| Negative | 38 (77.5%) |
| Not determined | 9 (18.4%) |
| Microsatellite status, n (%) | |
| MSI | 4 (8.2%) |
| MSS | 25 (51.0%) |
| Not determined | 20 (40.8%) |

MSI, microsatellite instability; MSS, microsatellite stable.

Table II. Response and survival after first- and second-line therapy (overall cohort).

| Variable | N (%) |
|---|---|
| Response first-line | |
| CR | 6 (12.2) |
| PR | 20 (40.8) |
| SD | 16 (32.7) |
| PD | 7 (14.3) |
| Response second-line | |
| CR | 3 (6.1) |
| PR | 10 (20.4) |
| SD | 12 (24.5) |
| PD | 24 (49.0) |
| Progress after second-line | |
| Yes | 43 (87.8) |
| No | 6 (12.2) |
| Living status at the end of the observation period | |
| Alive | 13 (26.5) |
| Dead | 36 (73.5) |

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Measured outcome parameters included response to first-line treatment (CR, PR, SD, PD), response to second-line treatment (CR, PR, SD, PD), progression free survival of first-line treatment (PFS1), progression free survival of second-line treatment (PFS2) and overall survival (OS).

After analysing the above variables, patients were assigned to different groups based on their remission status upon first-line treatment (CR, PR, SD, PD) to compare their response rates to second-line treatment. Data were analysed according to an intent-to-treat approach. $P<0.05$ was considered to indicate a statistically significant difference. Moreover, Kaplan-Meier curves of progression free and overall survival were plotted and analysed using the log-rank test. This was done for stratification based on therapy-response to first- or second-line therapy or on patient or tumour specific factors. All statistical analyses were done with IBM SPSS Statistics 29.0 and Microsoft Excel for Microsoft 365 (Version 2303).

## Results

*Patient population*. In total, 49 patients were included in this study: 17 females (34.7%) and 32 (65.3%) males. The age span was 38 to 78 years, with a median age at therapy start of 63 years. All patients were treated at the Department of Internal Medicine 2 of the University Hospital Krems for CRC stage IV. The gender distribution was shifted towards male sex in the patient population. Table I shows an overview of the patients' characteristics including gender, age, tumour sidedness as well as mutational and microsatellite status.

Patients' tumour specimens were tested for various genetic mutations and alterations: all tumour samples were tested for KRAS mutations, here, 25 (51.0%) were positive. Only 2 (4.1%) samples displayed a BRAF mutation, one patient with a right-sided primary tumour, the other with left-sided CRC. Moreover, 4 (8.2%) tumour samples had an NRAS mutation and 4 patients (8.2%) harboured MSI. These tests, however, were not performed in all tumour patient samples (Table I), as in the first years of the observation period, these tests have not been routinely established. Two patients harboured two mutations at the same time: one patient had KRAS and NRAS alterations, the other NRAS and BRAF.

Furthermore, the location of the tumour of each patient was evaluated. 34 (69.4%) patients had a primary tumour on the left side of the colon, while only 15 (30.6%) participants were diagnosed with right-sided CRC. Tumors located in the lower parts of the large intestine, the sigmoid colon and rectum, predominated in our study cohort. 10 (66.7%) of the patients with right-sided CRC were women, the other 5 (33.4%) were men. KRAS mutations were nearly equally distributed between these two groups with 18 (52.9%) of 34 patients with a left-sided tumour, and 7 (46.7%) of 15 patients with a right-sided tumour. Considering MSI, 2 of 4 patients had right-sided CRC and the other 2 had left-sided CRC.

*Response*. All included patients received anti-tumour therapy in a first- and second-line setting. Table II shows a general overview of the response and survival of patients after each line of therapy. After first-line therapy 6 patients (12.2%) achieved a complete response, while the majority reached either a partial

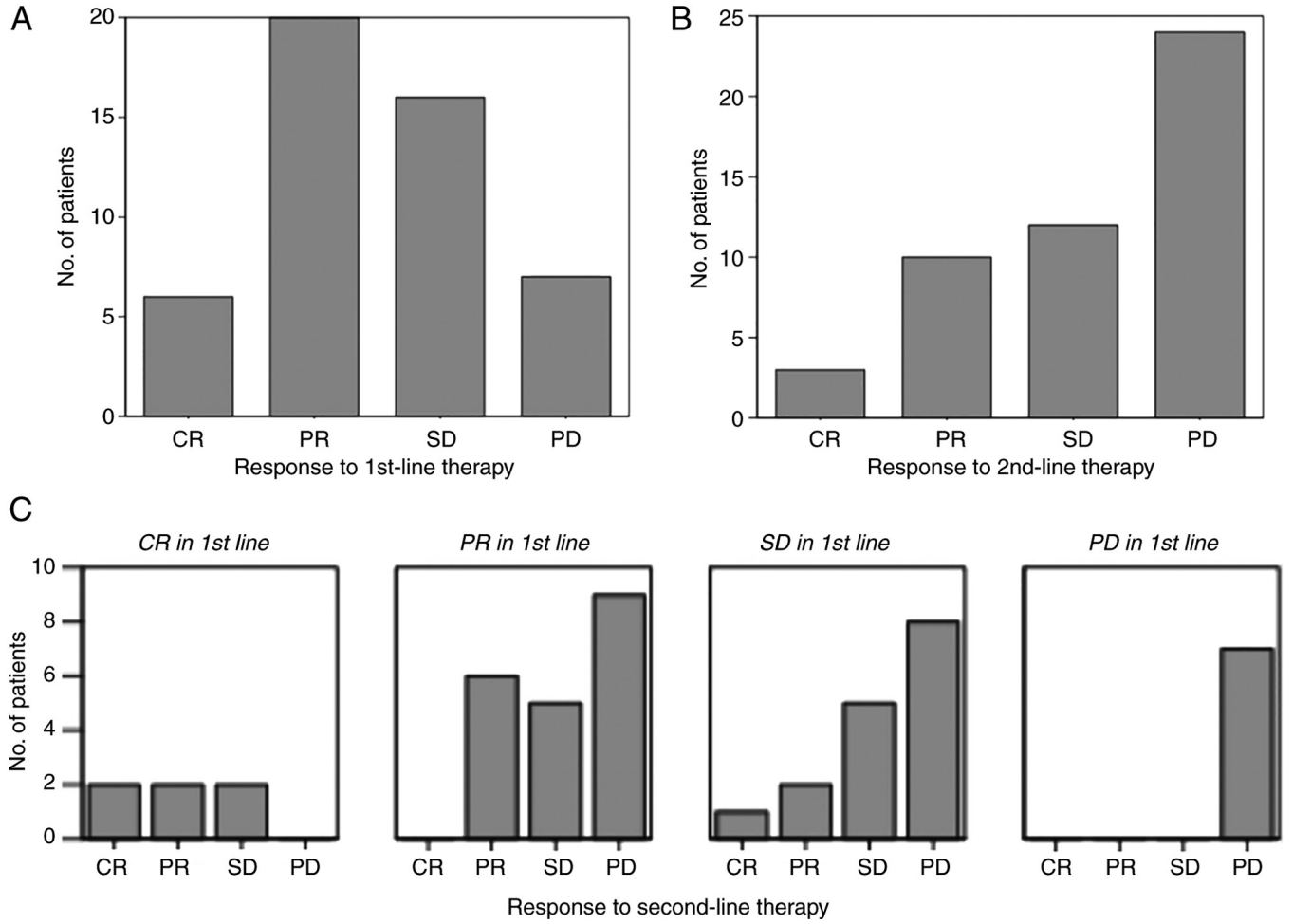


Figure 1. Responses to (A) first- and (B) second-line therapy based on Response Evaluation Criteria in Solid Tumors-criteria. (C) Responses to second-line therapy based on response to first-line therapy. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

response (20; 40.8%) or stable disease (16; 32.7%). Overall response rate (ORR) was thus 53%. Conversely, in 7 (14.3%) patients the disease showed a progressive behaviour despite first-line treatment (Fig. 1A).

In second-line treatment, nearly half of the patients (24; 49%) had progressive disease. 10 (20.4%) participants achieved a partial response and 12 (24.5%) stable disease. Only 3 (6.1%) patients reached a complete response; ORR was 26.5%, as can be seen in Fig. 1B. The 2 patients with a BRAF mutation had progressive disease in both therapy lines.

When grouped by first-line therapy response, Fig. 1C clearly displays that all patients included in this study with a complete response after first-line therapy have achieved at least 'stable disease' in second-line therapy (ORR=66.6%). On the contrary, patients with progressive disease after first-line treatment did also not respond to second-line therapy (ORR=0%).

*Survival analyses*. Next, we performed survival analyses. With regard to progression-free survival, Fig. 2A shows the PFS curves of first-line therapy (PFS1) based on the response to first-line therapy. Patients, who achieved a CR in first-line treatment had the longest PFS1, with a median PFS1 of 16 months [m; 95% confidence interval (CI): 14.9-17.1m] which was statistically significantly longer than for patients with PR (median PFS1=8m, 95% CI: 5.1-10.9; P=0.002) and compared to patients with PD (median PFS1=2m, 95% CI: 0-4.6; P<0.001). Also, patients with a PR in first-line therapy had statistically significantly longer PFS1 than patients with PD (P=0.003). This could also be observed for patients with SD (median PFS1=8m, 95% CI: 2.8-13.2) compared to patients with PD (P=0.001).

We also performed progression-free survival analyses of second-line treatment (PFS2) based on the response to first-line treatment. The Kaplan-Meier curves of Fig. 2B clearly depict, that patients with a CR in first-line treatment also had the longest PFS2 of all included patients (median PFS2=14m, 95% CI: 7.6-20.4). This is highly statistically significant in comparison to patients, who had progressive disease in first-line therapy (median PFS2=2m, 95% CI: 1.1-2.9; P<0.001). Again, patients with a PR in first-line therapy had longer PFS2 (median PFS2=4m, 95% CI: 1.2-6.8) than patients with PD in first-line therapy, although not statistically significant (P=0.143). For patients with SD compared to patients with PD in first-line therapy, PFS2 was also longer and statistically significant (median PFS2=5m, 95% CI: 3.7-6.3; P=0.019).

With regard to overall survival, Fig. 2C displays, that patients with a CR after first-line therapy had the longest OS, with a median OS of 80 months (95% CI: 46-114). On the contrary, patients with PD had the shortest OS, with only median 12 months (95% CI: 6.9-17.1).

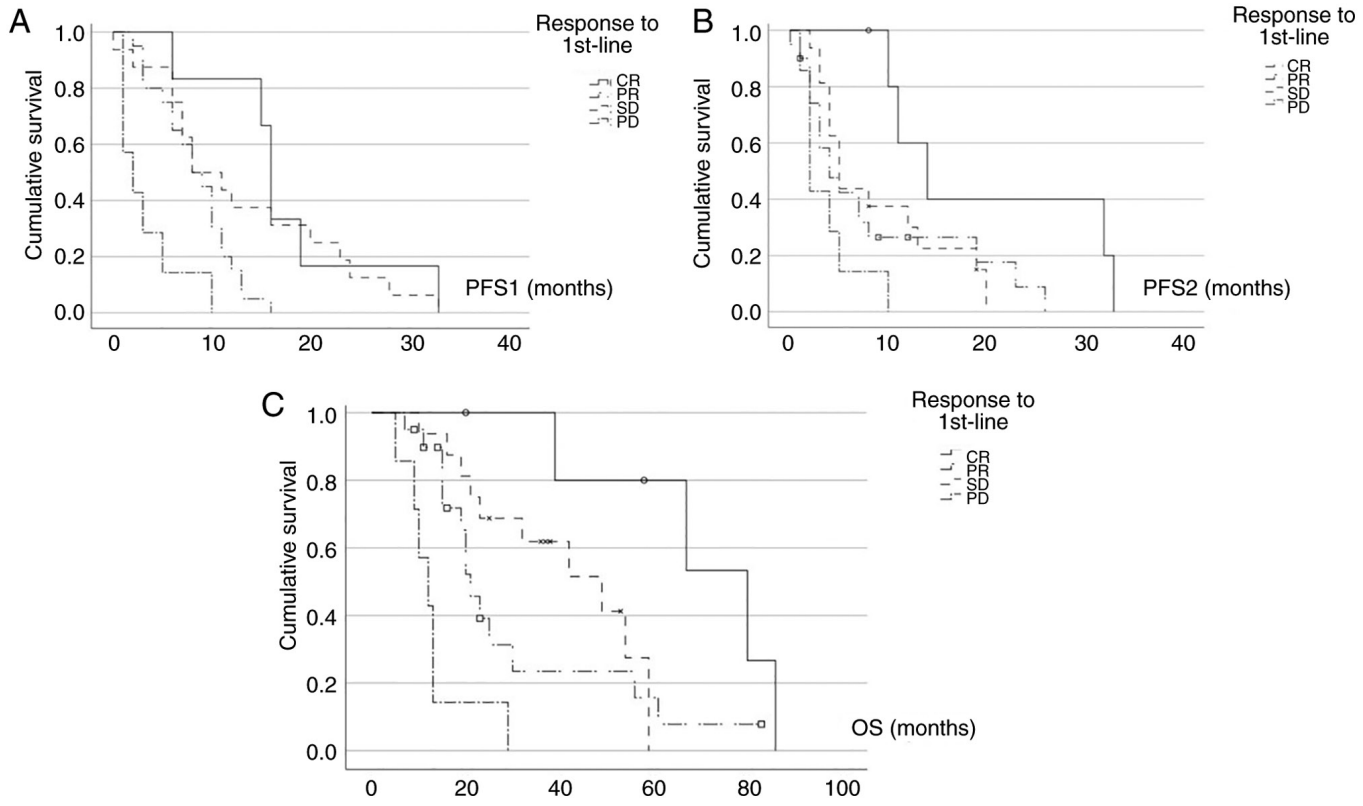


Figure 2. (A) PFS curves of first-line therapy (PFS1) based on the response to first-line therapy. (B) PFS curves of second-line therapy (PFS2) based on the response to first-line therapy. (C) OS curves of patients based on the response to first-line therapy. OS, overall survival; PFS, progression free survival.

Here, response to first-line therapy stratifies the groups clearly with statistically significant differences for patients with CR in first-line compared to patients with PD (P=0.019), patients with PR in first-line (median OS=21m, 95% CI: 17.3-24.7) in comparison to patients with PD (P=0.003) and patients with SD (median OS=49m, 95% CI: 25.6-72.4) compared to patients with PD (P<0.001).

*Survival analyses depending on patient characteristics*. We further looked at survival parameters based on patients and tumour characteristics. Here, stratification was not done based on therapy-response to first- or second-line therapy as in Fig. 2 but on patient or tumour specific factors (Fig. 3). Fig. 3A illustrates that sex had no impact on OS in our study cohort (female sex: median OS=23m, 95% CI: 0-71.2; male sex: median OS=29m, 95% CI: 17.5-40.5; P=0.938). Fig. 3B shows that also tumour sidedness had no statistically significant impact on OS in our patient groups, although a trend towards longer OS could be observed for patients with left sided primary tumours (left side: median OS=30m, 95% CI: 10.4-49.6; right side: median OS=21m, 95% CI: 10.9-31.1; P=0.176).

Furthermore, patients with wild type KRAS (wtKRAS) had a slightly longer OS compared to patients with a KRAS mutation (KRASmut), but again not statistically significant (wtKRAS: median OS=30m, 95% CI: 0-62.2; KRASmut: median OS=23m, 95% CI: 10.7-35.3; P=0.697), which is depicted in Fig. 3C.

Fig. 3D shows that patients with a BRAF mutation (BRAFmut) had significantly lower OS compared to patients without a BRAF mutation (wtBRAF: median OS=32m, 95% CI: 9.7-54.3; BRAFmut: median OS=9m, 95% CI: not calculable, due to low patient number of n=2; P=0.003).

Considering MSI, here again no significant difference could be observed with regards to OS (MSS: median OS=42m, 95%CI: 21.3-62.7; MSI: median OS=23m, 95% CI: 0-58.3; P=0.320; Fig. 3E).

These results clearly reject the Null-hypothesis, that the rates of remission in second-line chemo-immunotherapy do not differ between patients with CRC stage IV, who had a remission in first-line chemo-immunotherapy, and patients, who had no remission in first-line chemo-immunotherapy.

## Discussion

Cancer research has aimed for decades to better understand carcinogenesis, tumour biology and host-tumour interactions in order to find more specific and less toxic therapies. For mCRC, several important biological factors such as tumour sidedness, KRAS/NRAS/BRAF mutations, or microsatellite instability have been described and therapy algorithms were developed in order to optimally treat these patient subgroups.

Nonetheless, for the majority of patients, first- and second line-therapy of mCRC is usually based on combination chemotherapy with a 5-FU backbone, if not for BRAF-mutated or MSI-high tumours. Thus, therapy-resistance could be an important factor, based on the high similarity of first- and second-line treatment. We therefore investigated this factor in a retrospective cohort study and could clearly demonstrate that patients, who do not respond to first-line treatment have little benefit of second-line treatment. In our cohort none of

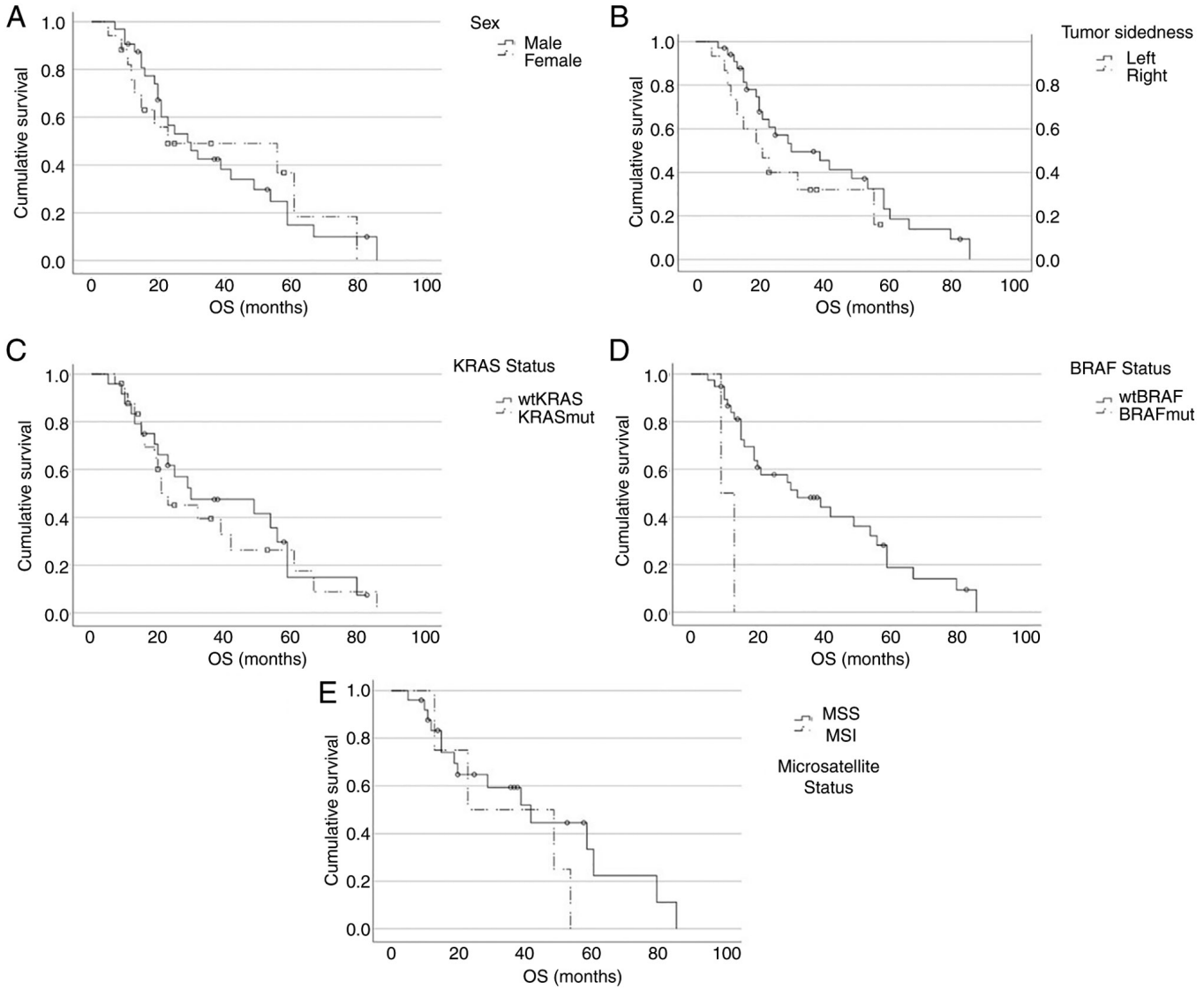


Figure 3. OS based on (A) sex, (B) tumour sidedness, (C) KRAS status, (D) BRAF status and (E) microsatellite status. MSI, microsatellite instability; MSS, microsatellite stable; mut, mutant; OS, overall survival; wt, wild type.

the patients, who had PD to first-line therapy responded to second-line therapy (ORR=0%). This dramatic finding has to be further investigated in larger studies and the true percentage of ORR is for sure not zero; however, it clearly demonstrates the limited benefit of current treatment strategies for these patients and the high need for different therapy approaches.

Unfortunately, clinical data on response to second-line treatment in mCRC patients not responding to first-line treatment is scarce. To the best of our knowledge, we could not find one single randomized clinical trial (RCT) nor prospective analysis addressing specifically this question. There are numerous RCTs evaluating in general the efficacy and toxicity of second-line systemic therapy in mCRC patients, where disease progressed, recurred or did not respond to first-line systemic therapy.

Due to the retrospective nature of this study, mechanistic aspects of therapy-resistance could unfortunately not be investigated, but it seems most likely, that innate chemoresistance plays a major role. Biological factors for chemo- or multidrug-resistance comprise numerous mechanisms, such as elevated metabolism, enhanced drug efflux, increased DNA repair capacity, growth factors, or genetic and epigenetic factors (13,17,18). Antimetabolites, such as 5-FU cause base lesions, which promote replication fork stalling in proliferating cancer cells. Here, resistance can be acquired by e.g. stabilization of these stalled replication forks (19). Another mechanism for 5-FU resistance is enhancement of poly [ADP-ribose] polymerase 1 (PARP1) activity, increasing the base excision repair capacity in cancer cells (20). As PARP-inhibitors are broadly applied in clinical oncology with a favourable toxicity profile, these substances could be interesting candidates for combination strategies in order to overcome chemo-resistance.

For oxaliplatin, which induces intra-strand dinucleotide DNA adducts that have to be repaired again by nucleotide excision repair mechanisms, it could be shown, that upregulation of the high-mobility group A 2 gene (HMGA2) could induce oxaliplatin-resistance (21).

Eventually, for irinotecan, it could be shown, that also its mode of action (trapping the topoisomerase I, leading to replication fork stalling and collapse and cytotoxic double strand

breaks), could be counterfeit by HGMA2, as HGMA2 can inhibit topoisomerase I trapping (17,22).

This preclinical evidence of cellular mechanisms, especially of HGMA2 conveying resistance to two important drugs for chemotherapy of mCRC cells, should underline the high importance of multidrug-resistance.

To date, clinical research data on chemo-resistance in CRC is unfortunately still scarce.

Due to the retrospective nature of this study, also our data has numerous limitations. As mentioned before, in the first years of the observation period, molecular biologic testing for BRAF and MSI was not routinely established and therefore especially data on these patient groups have many missing variables. Thus, the OS curves in Fig. 3 stratify only for patient or tumour characteristics but not in conjunction with first- or second-line therapy responses in these respective subgroups, as these subgroups are too small for robust interpretation. In future studies, special emphasis should be put on these patient groups in order to evaluate the effect of stratified therapies compared to 5-FU based second-line chemo-immunotherapy.

Moreover, retrospective studies always harbour the risk of selection bias and the influence of confounding variables.

In the future, patients with PD to first-line therapy should be tested thoroughly by gaining novel tumour specimens, which have to be comprehensively tested, including for known factors of multidrug-resistance. Ideally, detected alterations should be compared to the original specimen harboured during primary diagnosis in order to differentiate between acquired and innate chemoresistance. These patients should be regarded as 'functional high risk' and thus primarily treated within clinical trials. Based on the comprehensive testing results, targeted therapies or immunotherapies should be preferred to conventional chemotherapies. If the comprehensive profiling lacks specific targets or biomarkers for response to immunotherapy, clinical trials that specifically address chemo-resistance should be performed in this patient group.

For another proportion of these patients, especially frail ones and patients that suffered from severe toxicities of first-line therapy, BSC might be an option in order to avoid further side effects of futile therapies. So far, the current National Comprehensive Cancer Network (NCCN)-guidelines recommend BSC as second line option for patients with PD after first line and no improvement to functional status, or after failure to second-line treatment (23).

On the long run, however, novel and different therapy approaches have to be developed in order to benefit also this precarious patient group. This is most effectively done in randomized clinical trials and thus future studies should focus especially on this patient group, because of their high medical need.

Besides its retrospective nature, one big limitation of this study is its small sample size due to the design as single-centre study. Moreover, not all patients could be included, because they had to receive at least two lines of chemo-immunotherapy to evaluate PFS1, PFS2 and OS. Furthermore, not all patients, which were included in the study, were tested for all genetic markers, thus leading to missing data. Because of the small sample size, also subgroup analyses could not be performed. Future studies should be large enough to do subgroup analyses in order to rule out confounding factors such as co-morbidities, treatment response time, and other clinical characteristics.

In conclusion, this study demonstrates that, with current treatment strategies applying 5-FU-based chemo-immunotherapy in first-as well as second-line treatment of mCRC patients, response to first-line therapy is a strong predictor for response in second-line and OS. By only exchanging the chemotherapeutic combination partner and additive antibody, the negative factor of not responding to first-line therapy, most likely caused by multidrug-resistance, could not be overcome in this study population.

These findings have to be confirmed in larger studies, but raise the need for more basic research in CRC and on multidrug-resistance in order to gain novel treatment options, especially for patients not responding to first-line 5-FU-based chemo-immunotherapy.

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**Availability of data and materials**

The data generated in the present study may be requested from the corresponding author.

**Authors' contributions**

This work is based on the bachelor's thesis of HP, submitted in July 2023, at the Karl Landsteiner University of Health Sciences to acquire the academic degree Bachelor of Health Sciences. JS and MP conceived and designed the study. JS and HP acquired the data. JS and HP confirm the authenticity of all the raw data. JS, HP, GK and MP analysed and interpreted the data. JS and HP drafted the article. JS, HP, GK and MP revised the manuscript critically for important intellectual content. JS, HP, GK and MP read and approved the final version of the manuscript.

**Ethics approval and consent to participate**

The study was approved by the Commission for Scientific Integrity and Ethics at the Karl Landsteiner University of Health Sciences in September 2022 (EK No. 1046/2022) and was conducted according to The Declaration of Helsinki. Due to the retrospective nature of the present study the need for informed consent was waived; this was as approved by the

Commission for Scientific Integrity and Ethics at the Karl Landsteiner University of Health Sciences.

**Patient consent for publication**

Not applicable.

**Competing interests**

JS declares honorarium payments from Abbvie, Amgen, Gilead, Janssen, Kite, Merck, Merck Sharp & Dohme, Miltenyi, Novartis, Pfizer, Roche and Servier as an invited speaker or expert consulting, which are not relevant for this study. MP declares financial support from Roche for research projects, also not relevant for this study. The other authors declare that they have no competing interests.

**References**


1. De Falco V, Napolitano S, Roselló S, Huerta M, Cervantes A, Ciardiello F and Troiani T: How we treat metastatic colorectal cancer. ESMO Open 4 (Suppl 2): e000813, 2019.
2. Tharin Z, Blanc J, Alaoui IC, Bertaut A and Ghiringhelli F: Influence of first line chemotherapy strategy depending on primary tumor location in metastatic colorectal cancer. J Gastrointest Oncol 12: 1509-1517, 2021.
3. Ogura T, Kakuta M, Yatsuoka T, Nishimura Y, Sakamoto H, Yamaguchi K, Tanabe M, Tanaka Y and Akagi K: Clinicopathological characteristics and prognostic impact of colorectal cancers with NRAS mutations. Oncol Rep 32: 50-56, 2014.
4. Network NCC. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Colon Cancer. In. 2022.
5. Tejpar S, Stintzing S, Ciardiello F, Tabernero J, Van Cutsem E, Beier F, Esser R, Lenz HJ and Heinemann V: Prognostic and predictive relevance of primary tumor location in patients with RAS wild-type metastatic colorectal cancer: Retrospective analyses of the CRYSTAL and FIRE-3 trials. JAMA Oncol 3: 194-201, 2017.
6. Antoniotti C, Borelli B, Rossini D, Pietrantonio F, Morano F, Salvatore L, Lonardi S, Marmorino F, Tamberi S, Corallo S, *et al*: AtezoTRIBE: A randomised phase II study of FOLFOXIRI plus bevacizumab alone or in combination with atezolizumab as initial therapy for patients with unresectable metastatic colorectal cancer. BMC Cancer 20: 683, 2020.
7. Vodenkova S, Buchler T, Cervena K, Veskrnova V, Vodicka P and Vymetalkova V: 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. Pharmacol Ther 206: 107447, 2020.
8. Culy CR, Clemett D and Wiseman LR: Oxaliplatin. A review of its pharmacological properties and clinical efficacy in metastatic colorectal cancer and its potential in other malignancies. Drugs 60: 895-924, 2000.
9. Fujita KI, Kubota Y, Ishida H and Sasaki Y: Irinotecan, a key chemotherapeutic drug for metastatic colorectal cancer. World J Gastroenterol 21: 12234-12248, 2015.
10. Cervantes A, Adam R, Roselló S, Arnold D, Normanno N, Taïeb J, Seligmann J, De Baere T, Osterlund P, Yoshino T, *et al*: Metastatic colorectal cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. Ann Oncol 34: 10-32, 2023.
11. Aparicio J, Virgili Manrique AC, Capdevila J, Muñoz Boza F, Galván P, Richart P, Oliveres H, Páez D, Hernando J, Serrano S, *et al*: Randomized phase II trial of FOLFIRI-panitumumab compared with FOLFIRI alone in patients with RAS wild-type circulating tumor DNA metastatic colorectal cancer beyond progression to first-line FOLFOX-panitumumab: the BEYOND study (GEMCAD 17-01). Clin Transl Oncol 24: 2155-2165, 2022.
12. Dariya B, Aliya S, Merchant N, Alam A and Nagaraju GP: Colorectal cancer biology, diagnosis, and therapeutic approaches. Crit Rev Oncog 25: 71-94, 2020.
13. Bukowski K, Kciuk M and Kontek R: Mechanisms of multidrug resistance in cancer chemotherapy. Int J Mol Sci 21: 3233, 2020.
14. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European organization for research and treatment of cancer, national cancer institute of the united states, national cancer institute of Canada. J Natl Cancer Inst 92: 205-216, 2000.
15. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, *et al*: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
16. Tirumani SH, Kim KW, Nishino M, Howard SA, Krajewski KM, Jagannathan JP, Cleary JM, Ramaiya NH and Shinagare AB: Update on the role of imaging in management of metastatic colorectal cancer. Radiographics 34: 1908-1928, 2014.
17. Campos Gudiño R, McManus KJ and Hombach-Klonisch S: Aberrant HMGA2 expression sustains genome instability that promotes metastasis and therapeutic resistance in colorectal cancer. Cancers (Basel) 15: 1735, 2023.
18. Hammond WA, Swaika A and Mody K: Pharmacologic resistance in colorectal cancer: A review. Ther Adv Med Oncol 8: 57-84, 2016.
19. Yu H, Lim HH, Tjokro NO, Sathiyanathan P, Natarajan S, Chew TW, Klonisch T, Goodman SD, Surana U and Dröge P: Chaperoning HMGA2 protein protects stalled replication forks in stem and cancer cells. Cell Rep 6: 684-697, 2014.
20. Summer H, Li O, Bao Q, Zhan L, Peter S, Sathiyanathan P, Henderson D, Klonisch T, Goodman SD and Dröge P: HMGA2 exhibits dRP/AP site cleavage activity and protects cancer cells from DNA-damage-induced cytotoxicity during chemotherapy. Nucleic Acids Res 37: 4371-4384, 2009.
21. Deng X, Kong F, Li S, Jiang H, Dong L, Xu X, Zhang X, Yuan H, Xu Y, Chu Y, *et al*: A KLF4/PiHL/EZH2/HMGA2 regulatory axis and its function in promoting oxaliplatin-resistance of colorectal cancer. Cell Death Dis 12: 485, 2021.
22. Peter S, Yu H, Ivanyi-Nagy R and Dröge P: Cell-based high-throughput compound screening reveals functional interaction between oncofetal HMGA2 and topoisomerase I. Nucleic Acids Res 44: e162, 2016.
23. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Farkas L, *et al*: Colon cancer, version 2.2021, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 19: 329-359, 2021.


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