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REVIEW ARTICLE

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Bevacizumab or standard chemotherapy in previously treated patients with metastatic colorectal cancer — a systematic review

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ABSTRACT

Introduction. The *BRAF V600E* mutation (*BRAF*mt) occurring in the metastatic colorectal cancer (mCRC) patients is associated with poorer prognosis, in comparison to the wild-type variant of the *BRAF* gene (*BRAF*wt). Aim of this work was to assess the clinical efficacy of bevacizumab (BEVA) or standard chemotherapy (ChT) in the 2nd or further lines of treatment in mCRC *BRAF*mt population.

Material and methods. MEDLINE/PubMed, Embase and Cochrane CENTRAL databases were systematically searched. The reference lists of relevant studies were also checked.

Results. 6 eligible trials were identified: MOMA (BEVA ± ChT), allowing for limited overall survival (OS) assessment, WJOG 6210G (BEVA + FOLFIRI), RAISE and 20050181 (FOLFIRI), PICCOLO and Spindler 2013 (irinotecan monotherapy). None of those trials were designed for the treatment evaluation in *BRAF*mt population. Available evidence was restricted to limited analyses in small subgroups (from a few to several dozens of patients), occasionally comprising *RAS* gene mutation (*RAS*mt) as well. Based on the identified studies, the comparison of BEVA ± ChT *vs.* ChT or among different ChTs in *BRAF*mt population was not feasible.

In case of BEVA (MOMA), OS hazard ratio (HR) for *BRAF*mt *vs.* *BRAF*wt was 1.52 (95% CI: 0.79–2.89) with difference in medians equal to 12.1 months (19.2 *vs.* 31.3 months, respectivelly), and *BRAF*mt or *RAS*mt patients had median OS lower by 7.9 months and median progression free survival (PFS) by 3.0 months in WJOG 6210G trial. In case of ChT, median PFS was lower in *BRAF*mt by 12–67% (HRs range: 1.01–5.3), and median OS by 34–73% (HRs range: 1.05–5.00).

Conclusions. Due to limited clinical evidence, assessment of further lines of treatment in *BRAF*mt mCRC patients is uncertain, however existing data consistently suggest lower effectiveness of BEVA ± ChT or ChT in *BRAF*mt, than in *BRAF*wt subgroup. Hopefully, combining anti-EGFR therapies with BRAF/MEK inhibitor is expected to improve prognosis of those patients.

Key words: BRAF, colorectal cancer, systematic review, bevacizumab, chemotherapy

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Introduction

Substitution of valine (Val) with glutamic acid (Glu) in codon 600 (*V600E*) of the proto-oncogenic BRAF kinase gene that is part of the mitogen-activated protein kinase (MAPK or RAS-RAF-MEK-ERK) signalling pathway, is present in 8–12% of metastatic colorectal cancer (mCRC) cases, more often with right-sided primary tumor location [1, 2]. This pathway plays an essential role in the regulation of cell proliferation,

differentiation, survival, and apoptosis, it is also responsible for signal transduction from growth factor receptors, including epidermal growth factor receptor (EGFR) [1–3]. This point mutation leads to constitutive kinase phosphorylation, which drives sustained activation of MAPK signalling pathway. The mechanism of this process has not been fully understood, but it seems that cancers with such a genetic abnormality constitute a distinct phenotypic group [2, 4–6]. The V600E *BRAF* mutation is detected in 40–60% of sporadic cancers with microsatellite instability but almost never in Lynch syndrome (about 1%) and in tumors with *KRAS* and *NRAS* mutations [1, 2, 7]. However, the co-occurrence of *BRAF* aberration and microsatellite instability may be associated with a better prognosis by abolishing the opposing effects of both genetic changes [2, 7, 8]; this mechanism is not fully understood [2]. It is widely accepted that the presence of *BRAF V600E* mutation in patients with colorectal cancer is associated with a poor prognosis at any stage of the disease [1, 2], and the median overall survival may be up to three times lower compared to patients with a wild-type gene variant [9]. *BRAF* mutations other than V600 occur much less frequently and most likely bear no adverse prognostic significance [1, 2].

Most available data in mCRC patients relate to first-line treatment; there are no clear differences in progression-free survival (PFS) when chemotherapy alone is used, however, overall survival is markedly shorter in *BRAF*mt group [2, 10]. Despite the limited scientific evidence, bevacizumab added to FOLFOXIRI chemotherapy is currently the recommended molecularly targeted drug in the first-line treatment of advanced disease [9–14]. However, according to available clinical data [15, 16], a response to anti-EGFR therapy (cetuximab, panitumumab) is unlikely, and the occurrence of the *V600E* mutation is a contraindication to such treatment unless it is combined with anti-BRAF therapy [7, 14]. There is scarce data concerning the clinical efficacy of further treatment lines. The aim of this study was to systematically review the clinical trials assessing bevacizumab or irinotecan- or oxaliplatin-based chemotherapy in second and further treatment lines of mCRC with *BRAF* mutation.

Methods

A systematic search of MEDLINE, Embase and Cochrane CENTRAL databases was conducted on August 5, 2019. The search strategy included all types of studies, i.e. secondary and primary, including both randomized and non-randomized clinical trials, as well as non-controlled ones, assessing the use of bevacizumab or chemotherapy containing irinotecan or oxaliplatin in second or further treatment lines in advanced CRC. Studies assessing clinical efficacy (OS, PFS, objective response rate — ORR) in patients with the *BRAF V600E* gene mutation were included, encompassing comparative assessment between sought interventions in the target population or assessment in relation to patients without the *BRAF* mutation. The defined strategy also allowed to find secondary studies. Detailed information on the search strategy and systematic review is provided in the Supplemental materials (Tab. S1, S2, Fig. S1).

Two-stage publication selection (titles and abstracts analysis followed by full texts analysis) in accordance with the defined PICOS scheme (Tab. S2) as well as the assessment of study quality and risk of bias in the ROB 2.0 [17] and ROBINS-I [18] scales were performed by two independently working researchers (W.S., M.H.) (Tab. S3). Data extraction was carried out in pairs in which one of the persons checked the correctness of the data. Doubts were discussed with the third person (M.K.) until consensus was reached. The above assumptions were pre-determined before the actual review. Presenting the results, the data for *BRAF*mt subgroup were extracted, referring to *BRAF*wt group when possible. In some cases, the necessary calculations were made to present the result for *BRAF*mt *vs. BRAF*wt comparison and based on available data the relative benefit (RB), response rate and a difference in median survival were estimated. The systematic review was carried out in accordance with current Health Technology Assessment (HTA) guidelines of the Agency for Health Technology Assessment and Tariffs (AOTMiT, Agencja Oceny Technologii Medycznych i Taryfikacji) [19].

Results

As a result of the systematic review (Tab. S1), six primary trials (presented in six publications) were found: MOMA [20], WJOG 6210G [21], RAISE [22], 20050181 [23], PICCOLO [24] and Spindler 2013 [25] (Fig. S1). The results of additional analysis of data from the PICCOLO — Seligmann 2016 study were also taken into account [10]. Five of the included studies [20–24] were randomized clinical trials (RCT), but none of them was specifically targeted at the population with *BRAF* mutation — determination of this mutation was not required by inclusion criteria, and the assessment of the significance of *BRAF* mutation was exploratory and included only a subgroup of patients with available material and genotyping results. Furthermore, each study in one arm used intervention not included in the criteria of the presented review — the combination of anti-EGFR or anti-VEGF drug with chemotherapy. In one study [20] only limited assessment of OS was pos-

sible, including the use of BEVA with chemotherapy in the next treatment line after disease progression in the majority of patients. Only the observational study Spindler 2013 [25] was aimed at assessing the impact of *BRAF* mutations. Available results were sufficient only for analysis of clinical efficacy within a small *BRAF*mt subgroups (from several to several dozen patients), sometimes including *RAS*mt [21] and referring them to *BRAF*wt population. The identified studies did not allow for comparative assessment of BEVA with chemotherapy *vs.* CHT or various CHTs within the *BRAF*mt population. No systematic reviews were found assessing the use of the given intervention in further treatment lines. The characteristics of the included trials are presented in Table S4 and the main results are summarized in Table 1. No meta-analyzes of the results were performed due to high clinical heterogeneity.

Bevacizumab (BEVA) + chemotherapy

In the MOMA trial (Cremolini 2019) [20], 232 patients with mCRC were randomized to one of two protocols: 8 cycles of first-line induction therapy with FOLFOXIRI + BEVA, followed by maintenance therapy continued to disease progression — BEVA or BEVA + metronome chemotherapy (capecitabine and cyclophosphamide). Central determination of *BRAF* (exon 15 [*V600E*] assessment with use of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight MassARRAY system or *RAS* gene mutations was performed in 203 patients, and in 20 (10%) patients mutated *BRAF* status was detected. During a median follow-up of 47.8 months, a total of 210 patients progressed and 152 (72%) received next treatment line, of which 91 (60%) were re-treated with BEVA + FOLFOXIRI, and 31 (20%) — BEVA + FOLFIRI/FOLFOX, and 3 (2%) — BEVA + fluorouracil. In total, BEVA was used in 82% of patients receiving the subsequent treatment line. Therefore, overall survival (OS) analysis also included the use of BEVA in the second treatment line, however, it can be assumed that the observation concerned a maximum of approximately 11 *BRAF*mt patients who had progressed and received BEVA again in the next line. In the *BRAF*mt population, the median OS was 19.2 months and was significantly lower than in *RAS*wt and *BRAF*wt patients (N = 36) — 31.3 months (difference of 12.1 months), similarly to *RAS*mt (N = 150) — 24.9 months (difference of 6.4 months). In the whole group, the risk of death at a given time point was higher for *BRAF*mt compared to *BRAF*wt and *RAS*wt, but the difference did not reach the statistical significance threshold: HR = 1.52 (95% CI: 0.79–2.89), P = 0.208 [20] (Fig. 1.).

The evaluation of bevacizumab in further treatment lines was also carried out in a randomized West Japan Oncology Group (WJOG 6210G) study (Shitara 2016) [21], which included patients with mCRC or inoperable, locally advanced CRC, with clinically or radiologically confirmed progression during or up to 3 months after the last dose of first-line chemotherapy with fluoropyrimidine, oxaliplatin and bevacizumab. In addition, it was required to exclude *KRAS* gene mutation (*KRAS*wt) in exon 2 (codon 12 or 13) in the central or local evaluation of paraffin-embedded tumor tissue. The study included 121 patients who were randomized to receive BEVA + FOLFIRI or panitumumab + FOLFIRI. Two patients in each group were excluded from further efficacy analysis due to failure to meet inclusion criteria. After progression, 77.8% of patients received another line of treatment, of which 34.1% received bevacizumab. In addition, 109 patients underwent extended genetic profiling covering *KRAS* and *NRAS* gene mutations — exon 2 (codons 12 and 13), exon 3 (codons 59, 61, 117 and 146) and *BRAF* — exon 15 (codon 600) using next-generation sequencing (NGS) of circulating tumor DNA in serum. *BRAF* gene mutation was detected in 5 (4.6%) patients and *RAS* genes mutations in 14 (12.8%) patients. The results are presented in a way that allows comparison of the combined subgroup with *BRAF* or *RAS* mutation with tumors without mutations in the tested genes (wild-type). Among *BRAF*mt or *RAS*mt patients receiving BEVA + FOLFIRI treatment in the second line (N = 11), the median OS was 8.2 months (95% CI: 6.0–13.7) and was 7.9 months lower compared to wild type subgroups (N = 44) — 16.1 months (95% CI: 12.7–21.1). The median PFS was lower by approximately 3 months in the *BRAF*mt and *RAS*mt groups: 3.7 months (95% CI: 1.8–6.0) *vs*. 6.7 months (95% CI: 5.4–9.4), respectively. The authors also reported that among patients with measurable disease receiving BEVA + FOLFIRI, the objective response rate in the *BRAF*mt or *RAS*mt subgroup was 18.2% and 2.6% in non-mutated patients, respectively. The available data did not allow further calculations, and when interpreting the results it should also be considered that in the BEVA + FOLFIRI group only 3 patients achieved an objective response [21].

Chemotherapy

The assessment of chemotherapy in further treatment lines in patients with mCRC harboring *BRAF* mutation was based on four clinical trials, two of which enabled the evaluation of FOLFIRI scheme: RAISE [22] and 20050181 [23]; and another two irinotecan monotherapy: PICCOLO [24] and Spindler 2013 [25].

The RAISE study evaluated the efficacy and safety of ramucirumab combined with FOLFIRI compared to placebo + FOLFIRI in patients with progression of mCRC during or within 6 months after the last dose of

Table 1. The main results of studies enabling the assessment of clinical efficacy in patients with *BRAF V600E*-mutated mCRC

Study	Sample size	OS	PFS	ORR	RoB
Bevacizumab ± chemotherapy					
MOMA [20]	*BRAF*mt *vs. BRAF*wt and *RAS*wt[1]: 20 *vs.* 36	Median (–39%*): 19.2 *vs.* 31.3 months HR = 1.52 (95% CI: 0.79–2.89); P = 0.208	–	–	Critical
WJOG 6210G [21]	*BRAF*mt or *RAS*mt *vs. BRAF*wt and *RAS*wt[2, 3]: 11 *vs.* 44	Median (–49%*): 8.2 (95% CI: 6.0–13.7) *vs.* 16.1 (95% CI: 12.7–21.1) months	Median (–45%*): 3.7 (95% CI: 1.8–6.0) *vs.* 6.7 (95% CI: 5.4–9.4) months	18.2% *vs.* 2.6%[4]	Critical
FOLFIRI					
RAISE [22]	*BRAF*mt *vs. BRAF*wt and *RAS*wt[5]: 21 *vs.* 143	Median (–73%*): 4.2 *vs.* 15.5 months	Median (–53%*): 2.7 *vs.* 5.7 months	–	Critical
20050181 [23]	*BRAF*mt and *RAS*wt *vs. BRAF*wt and *RAS*wt[6]: 23 *vs.* 190	Median (–63%*): 5.7 *vs.* 15.4 months HR* = 5.00 (95% CI: 3.03–7.69)	Median (–67%*): 1.8 *vs.* 5.5 months HR* = 3.23 (95% CI: 1.96–5.26)	–	Critical
Irinotecan monotherapy					
PICCOLO [24]	*BRAF*mt and *KRAS*wt *vs. BRAF*wt and *RAS*wt and *PIK3CA*wt[7]: 31 *vs.* 163	HR = 1.56 (95% CI: 1.03; 2.37); P = 0.035	–	n/N: 6.5%* (2/31) *vs.* 12.3%* (20/163) RB* = 0.53 (95% CI: 0.13–2.14); P = 0.3688	Critical
PICCOLO — additional analysis [10]	*BRAF*mt *vs. BRAF*wt: 40 *vs.* 419	Median (–34%*): 6.7 (95% CI: 3.9–18.6) *vs.* 10.2 (95% CI: 5.4–18.1) months HR[8] = 1.21 (95% CI: 0.84–1.76); P = 0.31	Median (–12%*): 3.5 (95% CI: 2.6–7.3) *vs.* 4.0 (95% CI: 2.7–8.0) months HR[8] = 1.01 (95% CI: 0.69–1.49); P = 0.93	5.0% *vs.* 8.1% OR[8] = 0.56 (95% CI: 0.13–2.49); P = 0.45	Serious
Spindler 2013 — prospective cohort [25]	*BRAF*mt *vs. BRAF*wt: 8 *vs.* 89	HR* = 3.33 (95% CI: 0.96–11.11)	HR* = 3.57 (95% CI: 0.99–12.50)	0% *vs.* 14% (NS)	Critical
Spindler 2013 — retrospective cohort [25]	*BRAF*mt *vs. BRAF*wt: 8 *vs.* 101	HR* = 1.05 (95% CI: 0.45–2.50)	HR* = 1.79 (95% CI: 0.70–4.55)	0% *vs.* 15% (NS)	Critical
Spindler 2013 — multivariate analysis[9] [25]	–	HR = 4.3 (95% CI: 1.7–10.6); P = 0.002	HR = 5.3 (95% CI: 2.1–13.0); P = 0.0002	–	Critical

OS — overall survival; PFS — progression-free survival; ORR — objective response rate; RoB — risk of bias; HR — hazard ratio; RB — relative benefit; OR — odds ratio; NS — not significant

RoB is the lowest rating among the ROBINS-I scale domains. For almost all included studies, this arises from a critical risk assessment resulting from the presence of interfering factors in the study population (the exception is PICCOLO — additional analysis, where this risk was assessed as serious).

*Estimated based on available data; [1]overall survival (OS) analysis included the use of BEVA in 2nd treatment line (for *BRAF*mt population it can be assumed that the follow-up ultimately involved a maximum of approximately 11 patients who had progressed and re-received BEVA in the next treatment line); [2]in BEVA + FOLFIRI subgroup; [3]including three *BRAF*mt patients, taking into account that in total in *BRAF*mt or *RAS*mt group they accounted for 26%; [4]the total number of patients with objective response in the BEVA + FOLFIRI group was 3; [5]in placebo + FOLFIRI subgroup; [6]in FOLFIRI subgroup; [7]in irinotecan monotherapy subgroup; [8]results adjusted to response to previous treatment, performance status, presence of peritoneal metastases, primary tumor resection, and tumor location; [9]multivariate analysis taking into account age, performance status and *BRAF* and *KRAS* genes status

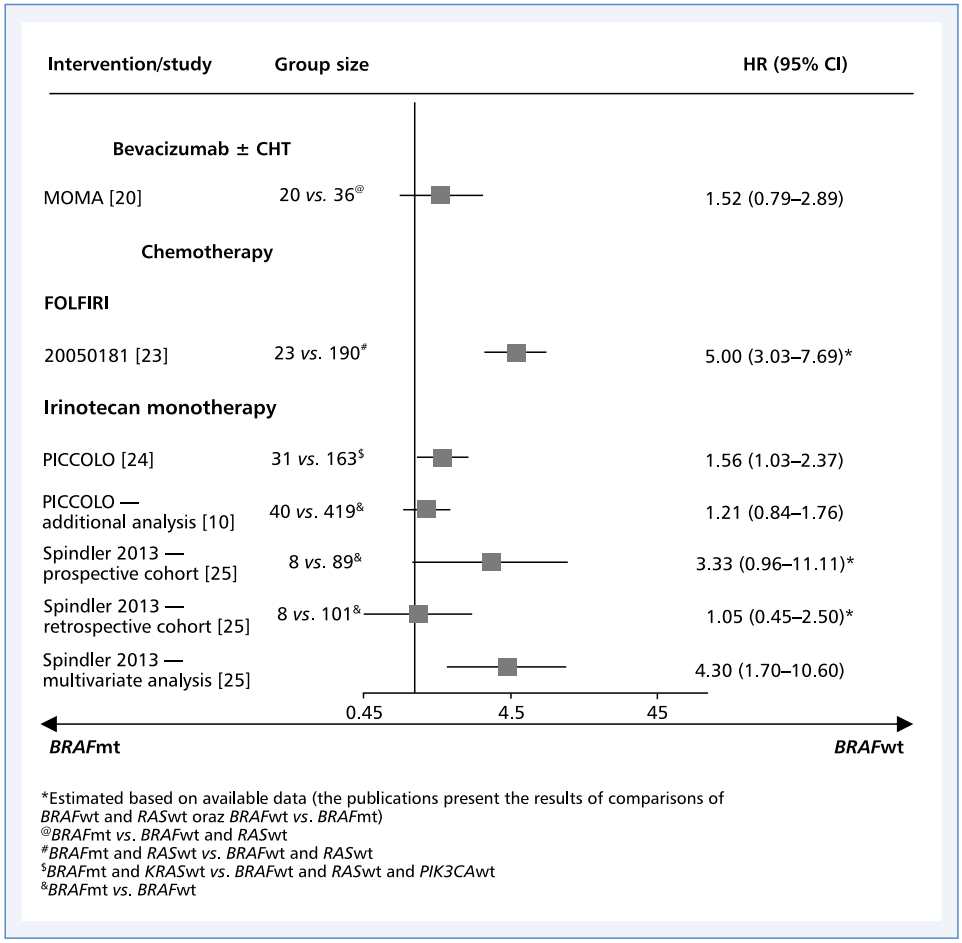

Figure 1. Hazard ratio (HR) for overall survival (OS)

first-line therapy of metastatic disease, including bevacizumab, oxaliplatin and fluoropyrimidine, if they received at least one cycle of therapy [22]. In total, 1,072 patients were included, of which exploratory assessment of the effect of *RAS* and *BRAF* mutations in the tumor tissue on the clinical effectiveness of the intervention was possible in 912 patients, and *BRAF*mt (*V600E*) was detected in 41 (4.5%) patients. Among patients receiving FOLFIRI chemotherapy alone, the median OS (4.2 months) was 11.3 months lower in *BRAF*mt patients (N = 21), compared to *BRAF*wt and *RAS*wt groups (N = 143) — 15.5 months, and 7.3 months compared to *RAS*mt patients (N = 294) — 11.5 months. Similarly, the median PFS was 2.7 months in *BRAF*mt patients compared to 5.7 months in *BRAF*wt and *RAS*wt patients and 4.3 months in *RAS*mt patients [22].

The 20050181 trial was another study enabling the evaluation of the FOLFIRI regimen in further treatment lines in patients with mCRC harboring *BRAF* mutation [23]. A total of 1,186 patients who progressed during or within 6 months after completing the first line FU-containing chemotherapy were randomized to panitumumab + FOLFIRI or FOLFIRI. Of these, 1,014 (85%) patients had assessed *RAS* mutations, and then among 421 *RAS*wt patients, 45 (11%) were found to have *BRAF*mt. A total of 638 (54%) patients had *RAS* or *BRAF* mutations. Extended genetic diagnostics of paraffin-embedded tumor tissue in patients with normal exon 2 of the *KRAS* gene included Sanger sequencing of exon 3 (codons 59/61) and 4 (codons 117/146) of the *KRAS* gene; exon 2 (codons 12/13), 3 (codons 59/61) and 4 (codons 117/146) of the *NRAS* gene and exon 15 (codon 600) of the *BRAF* gene.

The authors of 20050181 study performed an exploratory analysis of clinical efficacy depending on the *BRAF* status. Among patients treated with FOLFIRI, the median OS was lower by 9.7 months in patients with *BRAF*mt and *RAS*wt tumors (N = 23) — 5.7 months, compared to *BRAF*wt and *RAS*wt (N = 190) — 15.4 months: HR = 5.00 (95% CI: 3.03–7.69) (Fig. 1). Similarly, the median PFS in *BRAF*mt and *RAS*wt group was 1.8 months, e.g. 3.7 months lower than in *BRAF*wt and *RAS*wt groups — 5.5 months: HR = 3.23 (95% CI: 1.96–5.26) (Fig. 2). In both cases, the observed diffe-

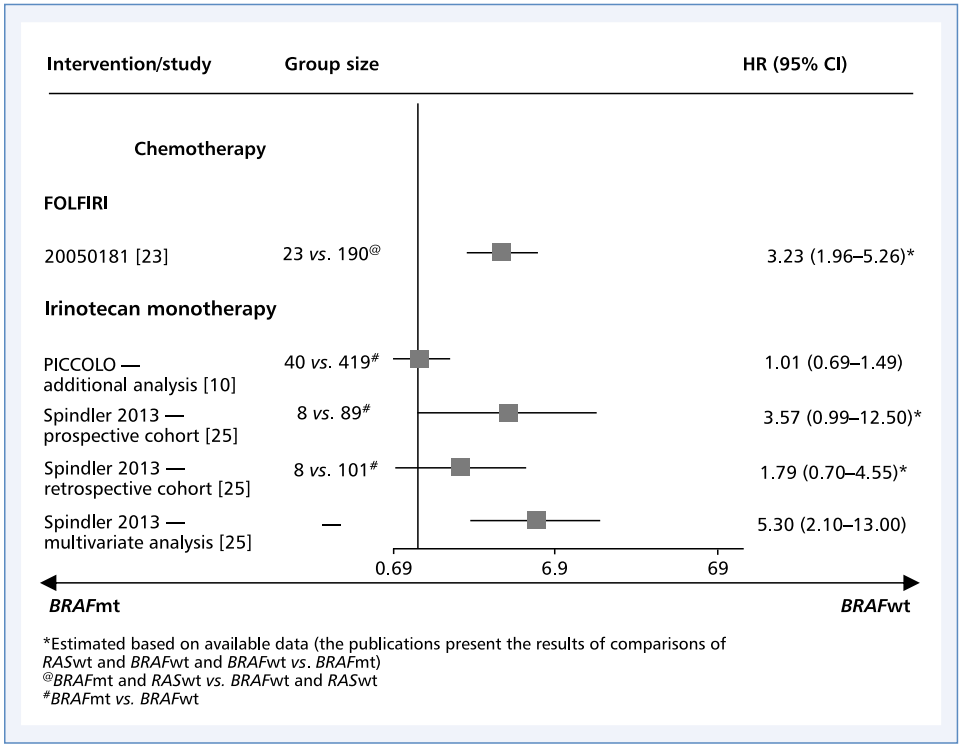

Figure 2. Hazard ratio (HR) for progression-free survival (PFS)

rences depending on the presence of *BRAF* mutations were statistically significant.

Another two trials evaluated irinotecan-based chemotherapy in the further treatment lines of advanced CRC with *BRAF* gene mutation. PICCOLO RCT included patients with inoperable, locally advanced, or metastatic CRC with prior progression during or after fluoropyrimidine-containing chemotherapy. Almost all patients had previously received oxaliplatin. After protocol amending, only patients with wild-type 12, 13 and 16 codons of *KRAS* gene were recruited (no prior anti-EGFR therapy was required). Finally, the analysis included 460 patients randomly assigned to receive panitumumab + irinotecan or irinotecan alone [24]. Further pyrosequencing of available paraffin-embedded tumor tissue was also carried out including codon 146 of *KRAS* gene, codon 12, 13 and 61 of *NRAS* gene, codon 542, 545, 546 and 1047 of *PIK3CA* gene and codon 600 of *BRAF* gene. Mutations in the *BRAF* gene were detected in 68 (14.8%) patients. OS in patients with *BRAF* gene mutation receiving irinotecan alone (N = 31) was significantly shorter compared to the group with wild type of all of the genes listed (*BRAF*wt, *RAS*wt and *PIK3CA*wt) (N = 163): HR = 1.56 (95% CI: 1.03–2.37), P = 0.035. This study also assessed objective response rate (ORR) according to RECIST criteria, which was twice lower in *BRAF*mt patients compared to all-wt: 2 (6.5%) *vs.* 20 (12.3%); RB = 0.53 (95% CI: 0.13–2.14), however with no statistical significance (P = 0.3688) [24].

In addition, Seligmann et al. [10] assessed the effect of *BRAF* mutation on the effectiveness of irinotecan monotherapy based on patients-level data from the PICCOLO study, considering the entire population, regardless of *KRAS* mutation status. The analysis included 459 patients with available results of *BRAF* mutation assessment, among which in 40 patients *V600E* mutation was found. Median OS in *BRAF*mt individuals was 3.5 months lower compared to the *BRAF*wt group: 6.7 *vs.* 10.2 months, but the difference did not reach statistical significance: HR = 1.21 (95% CI: 0.84–1.76). PFS medians were similar: 3.5 *vs.* 4.0 months; HR = 1.01 (95% CI: 0.69–1.49), while ORR probability was lower by 44%, but not reaching statistical significance: 5.0% *vs.* 8.1%, OR = 0.56 (95% CI: 0.13–2.49); P = 0.45.

The last analyzed trial was the non-randomized study — Spindler 2013 [25], evaluating the effect of *KRAS* and *BRAF* gene mutations on the outcomes in mCRC patients receiving irinotecan monotherapy in the second line (in prospective and retrospective cohort). The study included 110 patients in the prospective cohort, of which in 97 patients *BRAF* mutation status of tumor tissue was evaluated, with 8 (7%) positive results; and 111 patients in the retrospective cohort, among whom 109 were genotyped and *BRAF*mt was detected in 8 (8%) subjects. Assessment of mutation in 600 codon of *BRAF* gene was performed with the use of Amplification Refractory Mutation System-Quantitative PCR of DNA isolated from paraffin-embedded tumor tissue.

In the prospective cohort, HR for OS in *BRAF*mt (N = 8) *vs*. *BRAF*wt (N = 89) was 3.33 (95% CI: 0.96–11.11), while in the retrospective cohort — 1.05 (95% CI: 0.45–2.50) in 8 and 101 patients, respectively. Similarly, the risk of progression or death (PFS analysis) was higher in *BRAF*mt patients in both the prospective (HR = 3.57 [95% CI: 0.99–12.50]) and the retrospective cohort (HR = 1.79 [95% CI: 0.70–4.54]), however with no statistical significance. In the multivariate analysis considering age, performance status (PS) and *BRAF* and *KRAS* genes mutational status, the presence of *BRAF* mutations was associated with significantly worse prognosis: HR = 4.3 (95% CI: 1.7–10.6), P = 0.002, for comparison *BRAF*mt *vs. BRAF*wt in OS analysis (Fig. 1) and HR = 5.3 (95% CI: 2.1–13.0), P = 0.0002 in PFS analysis (Fig. 2). No *BRAF*mt patient achieved the objective response compared to 14% of *BRAF*wt patients in the prospective cohort and 15% in the retrospective cohort, but these differences did not reach statistical significance [25].

Risk of bias assessment

Five RCTs were included in the systematic review [20–24], however, the randomization did not refer to the subject of this review: *BRAF* gene mutational status was neither an inclusion criterion nor a stratification factor of randomization, genetic analysis was performed only in part of included patients, and the analysis of *BRAF* mutation impact was exploratory. These studies were not designed to compare interventions that *BRAF*mt patients were randomized to, and one of the trial arms included intervention whose assessment was not the purpose of this review (panitumumab + FOLFIRI [21], ramucirumab + FOLFIRI [22], panitumumab + irinotecan [24]). In one study [20] only limited inference based on OS assessment was possible due to the fact that observation within this endpoint also included BEVA in the subsequent treatment line after disease progression used in the majority of patients. Ultimately, in these RCTs, it was only possible to assess clinical efficacy within one study arm among patients with a known *BRAF* mutation and to refer these results to patients with a wild genotype. Accordingly, it was considered that in the context of presented study it would be appropriate to assess the risk of bias using a scale for non-randomized trials, as it will allow taking into account the baseline differences in demographic and clinical characteristics resulting from the lack of effective randomization. Table S3 presents the result of the risk of bias assessment of all 6 publications included in the systematic review in the ROBINS-I scale. The risk of bias was generally high, and most of the limitations found resulted from the analysis of outcomes only in subgroups distinguished based on *BRAF* gene mutational status, for which many significant data were not presented in publications yet. Considering the construction of the ROBINS-I scale, such a severe limitation in the interfering factors domain translates into a critically high risk in the overall assessment of the likelihood of endpoints reliability, regardless of the result of the assessment in other domains in the scale. An exception was the additional analysis in the PICCOLO study [10], in which the use of appropriate statistical adjustments allowed to partially eliminate the risk of bias associated with the uneven distribution of prognostic factors between groups — therefore the cumulative risk of bias was assessed as high.

Discussion

According to published reports, the *BRAF* mutation is associated with a significantly reduced survival of colorectal cancer patients receiving chemotherapy, both in the early and advanced stages [26]. While some evidence is available on the efficacy of 1st line treatment in advanced disease, there is limited data regarding further treatment. To our knowledge, this is the first published systematic review of available evidence assessing the efficacy of bevacizumab and chemotherapy in 2nd and further treatment lines of advanced colorectal cancer with the *BRAF V600E* mutation.

Although the presented review included predominantly RCTs, the available results only allowed for assessment of clinical efficacy within particular treatment arms, and the comparison did not relate to different interventions in the *BRAF*wt population (presence of *BRAF* mutation or even a requirement for genetic evaluation of this genetic abnormality were not an inclusion criterion in any of the RCTs), but only a reference of the outcomes observed in subjetcs receiving the same intervention with the mutation to those with the wild gene. Therefore, the assessment of the impact of *BRAF* mutation on treatment outcomes had an exploratory nature and was only possible in some patients with available material and genetic tests performed. The analysis of the effect of *BRAF* mutation on the effectiveness of irinotecan monotherapy was a goal of only Spindler 2013 observational study [25]. To WJOG 6210G [21] and PICCOLO [24] (after protocol amendment) trials only patients with non-mutated *KRAS* gene were enrolled. Similarly, in study 20050181 [23], only patients with *KRAS*wt underwent extended genetic diagnostics, including *BRAF* gene assessment. In general, control groups in RCTs included patients with wild genotype, according to both *BRAF* and *RAS* mutations (and additionally *PIK3CA* [24]). Only in one study, the extended genetic profiling was carried out using peripheral blood circulating tumor DNA [21], in others, they were performed using paraffin-embedded

tissue specimens. Patients with metastatic CRC were included in most clinical trials, and only two enrolled patients with inoperable, locally advanced tumors [21, 24].

Severely limited data was found to assess the efficacy of bevacizumab (± CHT) in 2nd and subsequent treatment lines in patients with advanced CRC harboring *BRAF* mutation. In one study (MOMA) with bevacizumab in the 1st treatment line of metastatic disease only limited OS analysis was possible because this observation also included subsequent treatment lines, and most patients received re-treatment with bevacizumab [20]. However, it is estimated that up to app. 11 patients with the *BRAF* mutation were subjected to such analysis. On the other hand, in another study (WJOG 6210G), the assessment included a total of 11 patients with either *BRAF* or *RAS* mutation and it can be assumed that the former one occurred only in about 3 patients [21]. Nevertheless, in both studies, the median OS was consistently reduced in patients with the *BRAF* mutation, by 39% and 49%, respectively [20, 21], and the risk of death was 1.5 times higher [20]; similarly, the median PFS was reduced by 45% [21]. The data on the objective response rates in the bevacizumab group were insufficient, which makes impossible to draw plausible conclusions.

Studies on the efficacy of chemotherapy in further treatment lines (FOLFIRI or irinotecan monotherapy) also had significant limitations but evaluated *BRAF*mt population was greater and included from 16 to 31 (40, taking into account the alternative analysis of PICCOLO study data [10]) patients in particular studies [22–25], a total of 91 patients (100 including [10]). When using FOLFIRI in one study, the median OS was 73% lower in the *BRAF*mt group and PFS by 53% [22], while in the other by 63% and 67%, respectively [23]. The risk of death at a given time point was several times higher if the mutation was present — five times [23], about 1.5 times [10, 24] and more than four times (multivariate analysis [25]), and the differences were statistically significant. Similarly, the risk of death or disease progression was more than three and four times higher ([23] and [25] — multivariate analysis, respectively), although in an alternative estimation of the PICCOLO study results there was no significant difference in PFS (median 12% lower, HR = 1.01 [10]).

Regarding the objective response rate, available data was markedly limited, in one study the incidence of this endpoint was almost twice lower in *BRAF*mt patients (statistically insignificant difference) [10, 24], while in the other study no patient with *BRAF* mutation ORR was reported [25].

Seligmann et al. [10] assessed the effect of *BRAF* mutation on the results of treatment of advanced CRC with standard chemotherapy using patient-level data from RCTs: COIN [27, 28] and FOCUS [29] (oxaliplatin and fluorouracil in the 1st line) and PICCOLO [24] (irinotecan in the 2nd line). The results of this additional analysis regarding the PICCOLO are presented in the main part of this publication. For the 1st line treatment of advanced disease, the authors found that the presence of the *BRAF* mutation is a significant OS prognostic factor (cumulative data for both RCTs: 10.8 *vs.* 16.4 months [HR = 1.49 (95% CI: 1.23–1.80); P < 0.001)], also after matching with respect to baseline characteristics. However, no clear impact of the mutation on PFS and ORR was observed. Survival after progression was also assessed, defined as the time from progression to death among patients with disease progression; when the date of progression was unknown, the date of the last chemotherapy cycle was taken into account. Patients with the *BRAF* mutation had a shorter survival after progression compared to those with the wild-type gene in both 1st line studies (COIN and FOCUS), the results for both clinical trials: 3.2 *vs*. 8.6 months; HR = 1.72 (95% CI: 1.35–2.19), P < 0.001 [10]. It is worth noting that significantly fewer *BRAF*mt patients received subsequent treatment line: 33% *vs*. 51%, P < 0.001; and a significantly higher percentage of *BRAF*mt patients with rapid progression (< 6 months) was observed in both the 1st and 2nd treatment line — 36.5% compared to — 21.9% in non-mutated patients; P < 0.001 [10].

It should be noted that inference based on the collected data is subject to uncertainty, due to the small size of *BRAF*mt population, and on the other hand with methodological limitations of included trials. In addition, the generally high risk of systematic error in the included studies greatly limits conclusions of the analysis. This is mainly due to the nature of the analyzes that were only possible when the included studies were treated as single-arm. It should be noted that the result of the assessment in the other domains of the ROBINS-I scale was better, although this does not change the overall assessment of systematic error risk. The clinical heterogeneity of the trials (especially in terms of interventions used) prevents proper data synthesis and may affect the interpretation and the ability to relate the review results to the target population of metastatic colorectal cancer patients.

Despite the aforementioned numerous limitations, the analysis quite clearly indicates lower effectiveness of evaluated interventions (bevacizumab ± chemotherapy or chemotherapy) in *BRAF*mt patients. In this group, none of the studied therapies were as effective as in *BRAF*wt population. The advantage of this systematic review is the extended search, which was carried out in 3 databases and also included non-randomized studies to comprehensively assess the effectiveness of the examined intervention. However, gray literature not being indexed in medical databases and ongoing research were not included which could

affect the scope of the evidence described. Only full-text articles in Polish or English were selected, and there were few studies, and some of them did not fully answer the clinical question, which is also a limitation of this analysis.

Combination of BRAF inhibitors with other medications may be more effective than monotherapy, which may lead to resistance by secondary activation of the MAPK pathway — this mechanism may be due to increased EGFR and MEK/ERK pathway signalling activity [1, 2, 30, 31]. Therefore, particular hope is given in the combination of BRAF inhibitors with drugs directed against parallel signalling pathways [1, 6]. There are promising recently published results of phase III BEACON study, in which the combination of cetuximab (anti-EGFR drug), encorafenib (BRAF inhibitor) and binimetinib (MEK inhibitor), as well as cetuximab with encorafenib in the 2nd or 3rd line of treatment significantly increased overall survival of patients with *BRAF V600E* mutation compared to standard therapy [32]. Some hope is also raised by a treatment regimen containing dabrafenib (BRAF inhibitor), panitumumab (anti-EGFR) and trametinib (MEK inhibitor), which has shown promising efficacy in patients with the *BRAF V600E* mutation [33]. Both of these regimens are currently recommended in the US NCCN guidelines for the treatment of patients with mCRC with the current *BRAF* mutation in 2nd and further treatment lines [7].

Clinical comment (P.P.)

When colorectal cancer progresses to metastatic disease, the *BRAF V600E* mutation, which is detected in about 10% of patients, becomes a factor that significantly worsens prognosis. The median survival of patients enrolled in current phase III clinical trials is over 30 months, but if the *BRAF* mutation is present, this value is two or three times lower [36].

It has been demonstrated that patients with *BRAF* mutation undergoing chemotherapy receive systemic treatment of subsequent lines less frequently than other patients, which results from the rather dynamic progression often causing symptoms and deterioration of the general condition preventing further cancer treatment. Until recently, this molecular abnormality has not been routinely studied in patients enrolled in clinical trials, so data on the efficacy of various chemotherapy and biological treatment regimens are based largely on retrospective analyses and are inevitably burdened with selection bias. However, these data quite consistently indicate that, apart from adverse effect on prognosis, the *BRAF V600E* mutation is a determinant of ineffectiveness or very little benefit from the use of anti-EGFR antibodies, especially in monotherapy.

For the reasons given above, it is suggested that in patients with this molecular disorder, 1st line systemic treatment should be as intensive as possible (at least doublet or triplet chemotherapy, i.e. FOLFOXIRI regimen) preferably with the addition of bevacizumab.

The data on the value of subsequent line therapies are extremely scarce, but the efficacy of chemotherapy and anti-angiogenic drugs appears to be low, as confirmed by this systematic review.

After successes in melanoma patients, BRAF tyrosine kinase inhibitors appeared to be the natural choice for next-line therapy, especially in combination with MEK inhibitors, but early phase clinical trials were disappointing [33]. Some optimism was brought only by attempts to use triple therapy additionally containing anti-EGFR antibody. The results of the BEACON phase III study dedicated to previously treated patients with *BRAF V600E* mutation have been recently published. Combination of cetuximab with a BRAF inhibitor encorafenib, as well as triple therapy containing an additional MEK inhibitor binimetinib, have been shown to increase overall survival and time to the quality of life deterioration compared to cetuximab combined with irinotecan-based chemotherapy [32, 37].

Another potentially very effective treatment method may be anti-PD1 immunotherapy or a combination of anti-PD1 and anti-CTLA4 because *BRAF V600E* mutation quite often coexists with microsatellite instability, which is a favorable predictor for this treatment [38]. Available data, however, come from phase II non-controlled studies, and patients with *BRAF V600* mutation were a minority [39].

Conflict of interest

The authors have no conflict of interest to declare.

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Appendix

Table S1. Search strategy

#	Query
PubMED	
#2	advanced OR metastatic
#3	colon cancer OR colorectal cancer
#4	(#2 AND #3)
#7	randomized controlled trial[pt]
#8	random allocation[mh]
#9	random*[tiab]
#10	controlled[tiab]
#11	(#7 OR #8 OR #9 OR #10)
#13	BRAF
#16	(bevacizumab OR FOLFOXIRI OR FOLFIRI OR FOLFOX OR oxaliplatin OR irinotecan)
#17	(#4 AND #16)
#18	(#17 AND #11)
#20	(#13 AND #17)
#22	(#18 OR #20)
Cochrane	
#1	advanced OR metastatic in Trials
#2	[mh „colorectal neoplasms"] OR „colon cancer" in Trials
#3	#1 AND #2 in Trials
#4	bevacizumab OR FOLFOXIRI OR FOLFIRI OR FOLFOX OR oxaliplatin OR irinotecan in Trials
#5	#3 AND #4 in Trials
#6	BRAF in Trials
#7	#6 AND #5 in Trials
#8	#7 OR #5 in Trials
Embase	
#1	(advanced:de OR metastatic:de) AND [embase]/lim
#2	(‚colon cancer':de OR ‚colorectal cancer'/exp) AND [embase]/lim
#3	(‚bevacizumab':de OR folfoxiri:de OR ‚folfiri':de OR ‚folfox':de OR ‚oxaliplatin':de OR ‚irinotecan':de) AND [embase]/lim
#4	#1 AND #2
#5	#3 AND #4
#6	[randomized controlled trial]/lim AND [embase]/lim
#7	random*:ab,ti AND [embase]/lim
#8	controlled:ab,ti AND [embase]/lim
#9	randomization:de AND [embase]/lim
#10	#6 OR #7 OR #8 OR #9
#11	#5 AND #10
#12	braf AND [embase]/lim
#13	#5 AND #12
#14	#11 OR #13

Table S2. PICOS scheme

Parameter	Inclusion criteria
Population	Adults with advanced CRC and assessed *BRAF* (V600E) status, progression after first-line treatment of advanced disease
Intervention	Bevacizumab (± CHT) in 2nd or further treatment line due to advanced disease Oxaliplatin- or irinotecan-based chemotherapy regimens in 2nd or further treatment line due to advanced disease
Comparison	As above or none
Outcomes	Overall survival (OS) Progression-free survival (PFS) Objective response rate (ORR) Studies enabling comparative assessment of sought interventions in the *BRAF*mt population or relating their effectiveness to *BRAF*wt patients were included
Study design	Randomized, controlled clinical trials, controlled or non-controlled non-randomized studies, published in full-text in English or Polish Systematic reviews published in English or Polish

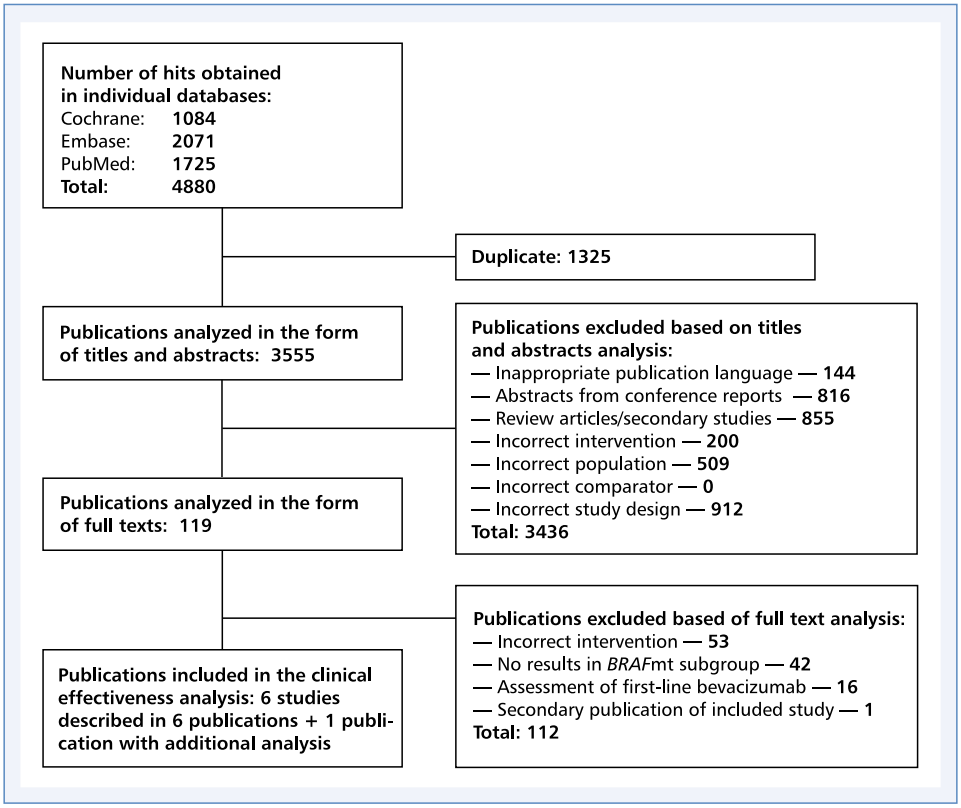

Figure S1. Search results. List of excluded studies at the stage of full texts analysis along with the reasons for exclusion is available on request

Table S3. Assessment of risk of bias in the included studies using the ROBINS-I tool

Study name	Domain	Disturbing factors	Patients selection	Intervention classification	Deviations from planned interventions	Missing data	Outcomes	Selection of described results	Total rating
MOMA [20]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
WJOG 6210G [21]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
	PFS						Serious		
RAISE [22]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
	PFS						Moderate		
20050181 [23]	OS	Critical	Low	Low	Low	Serious	Low	Low	Critical
	PFS						Serious		
PICCOLO [24]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
	PFS						Serious		
	ORR						Serious		
PICCOLO — additional analysis [10]	OS	Serious	Low	Low	Low	Moderate	Low	Low	Serious
	PFS						Serious		
	ORR						Serious		
Spindler 2013 — prospective cohort, multivariate analysis [25]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
	PFS						Serious		
Spindler 2013 — retrospective cohort [25]	OS	Critical	Low	Low	Low	Low	Low	Low	Critical
	PFS						Serious		

The risk of bias on the ROBINS-I scale can be assessed as (in order from lowest to highest): low, moderate, serious and critical, and in the absence of relevant information: unspecified. The total risk error rating is not higher than the lowest among the results in individual domains.

Table S4. Characteristics of included clinical trials

Study name	Study type and location	Intervention	Comparator	Population[a]	Total number/ /number analyzed for mutation/ /*BRAF*mt number	Observation period, endpoints	Funding
MOMA [20]	RCT, non-blinded 16 cancer centers in Italy	After 8 cycles of **FOLFOXIRI** induction therapy (irinotecan 165 mg/m^2 iv + next oxaliplatin 85 mg/m^2 iv + levofolinic acid 200 mg/m^2 + next fluorouracil 3200 mg/m^2 in continuous 48 h infusion) + **BEVA** (5 mg/ kg iv) (q2w) randomized to BEVA (7.5 mg/kg iv) or **BEVA** (7.5 mg/kg iv) + **metronomic chemotherapy** (capecitabine [500 mg 3 × daily] and cyclophosphamide [50 mg daily])	–	mCRC	232/206/20	Median 47.8 months I: PFS II: OS, objective response rate[b], resection rate, safety	GONO Foundation, the ARCO Foundation and F. Hoffmann--La Roche
WJOG 6210G [21]	RCT, non-blinded, multicenter (Japan)	**Panitumumab** (6 mg/kg) + **FOLFIRI** (doses not described) (q2w)	**BEVA** (5 mg/kg) + **FOLFIRI** (doses not described) (q2w)	mCRC or locally advanced inoperable CRC, progression after treatment with fluoropyrimidine, oxaliplatin and bevacizumab, *KRAS*wt in exon 2	117/109/5	15.4 months *vs*. 13.4 months I: OS II: PFS, objective response rate[b], safety, biomarkers analysis	West Japan Oncology Group (WJOG)
RAISE* [22]	RCT, double-blinded (blinded: patients, sites personnel involved, study sponsor), multicenter, international	**Ramucirumab** (8 mg/kg in 60 min) + next **FOLFIRI** (irinotecan 180 mg/m^2 in 90 min iv + next or simultaneously folinic acid 400 mg/m^2 in 120 min iv + next fluorouracil 400 mg/m^2 in 2–4 min bolus iv + next fluorouracil 2400 mg/m^2 in continuous 48 h infusion) (q2w)	**Placebo** (in 60 min) + next **FOLFIRI** (irinotecan 180 mg/m^2 in 90 min iv + next or simultaneously folinic acid 400 mg/m^2 in 120 min iv + next fluorouracil 400 mg/m^2 in 2–4 min bolus iv + next fluorouracil 2400 mg/m^2 in continuous 48 h infusion) (q2w)	mCRC, progression after treatment with fluoropyrimidine, oxaliplatin and bevacizumab	1072/912/41	– I: OS II: PFS, objective response rate[b], disease control, safety, PROs (PROs questionnaires: EORTC QLQ-C30 version 3.0, EQ-5D)	Eli Lilly and Company

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Table S4 cont. Characteristics of included clinical trials

Study name	Study type and location	Intervention	Comparator	Population[a]	Total number/ /number analyzed for mutation/ /*BRAF*mt number	Observation period, endpoints	Funding
20050181* [23]	RCT, non-blinded multicenter, international	**FOLFIRI** (irinotecan 180 mg/m^2 iv + folinic acid 400 mg/m^2 iv or levofolinate 200 mg/m^2 iv + fluorouracil 400 mg/m^2 bolus iv + fluorouracil 2400 mg/m^2 as a continuous infusion over 2 days)	**Panitumumab** (6.0 mg/kg in 60 min + next in 30 min in subsequent infusions) + next **FOLFIRI** (irinotecan 180 mg/m^2 iv + folinic acid 400 mg/m^2 iv or levofolinate 200 mg/m^2 iv + fluorouracil 400 mg/m^2 bolus iv + fluorouracil 2400 mg/m^2 as a continuous infusion over 2 days)	mCRC, progression after fluoropyrimidine-based CTH	1186/421[c]/45	Median 48 wks. I: OS, PFS II: objective response rate[b] and duration, safety, PROs	Amgen Inc
PICCOLO [24]	RCT, non-blinded 60 sites in the UK	**Panitumumab** (9 mg/kg iv) + **irinotecan** (350 mg/m^2; if age > 70 years or performance status 2 — 300 mg/m^2) (q3w)	**Irinotecan** (350 mg/m^2; if age > 70 years or performance status 2 — 300 mg/m^2) (q3w)	mCRC or locally advanced inoperable CRC, progression after fluoropyrimidine-based CTH, no previous anti-EGFR therapy	460/bd./68 (in all $KRAS_{c.12–13,61}$ wt) Additional analysis — irinotecan [10]: 511/459/40	Median of treatment cycles: 4 (range: 0–28) I: OS II: PFS, objective response rate[b], PROs, safety (questionnaire: EORTC QLQ-C30)	Cancer Research UK, Amgen Inc.
Spindler 2013 [25]	Non-RCT, prospective--retrospective, single center (Denmark)	**Irinotecan** (both cohorts: 350 mg/m^2) (q3w)	–	mCRC, 2nd line treatment	Prospective cohort — 110/97/8 Retrospective cohort — 111/109/8	Prospective cohort: median of treatment cycles 4 (1–15); Retrospective cohort: median of treatment cycles 6 (2–15) OS, PFS, objective response rate, safety	Tryg Fonden and the Research Counsil Hospital Lillebaelt

RCT — randomized controlled trial; BEVA — bevacizumab; q2w/q3w — dose administered every 2 and 3 weeks, respectively; PROs — patient-reported outcomes, CTH — chemotherapy

*Detailed data on the research methodology come from the main publications that were not included at the stage of research selection due to the lack of results related to the analysis — RAISE [34], 20050181 [35]

[a]Detailed population data for the subgroups analyzed was not available

[b]Tumor assessment according to the RECIST criteria 1.1

[c]*RAS*wt patients