Exploring the poor outcomes of *BRAF*-mutant advanced colorectal cancer: Analysis from 2530 patients in randomised clinical trials.

Jenny F. Seligmann[1], David Fisher[2], Christopher G. Smith[3], Susan D. Richman[1], Faye Elliott,[1] Sarah Brown[4], Richard Adams[3], Tim Maughan[5], Philip Quirke[1],Jeremy Cheadle[3], Matthew Seymour[1*] and Gary Middleton[6*].

Affiliations

1. Leeds Institute of Cancer and Pathology, University of Leeds, St. James's University Hospital, Beckett St., Leeds, United Kingdom, LS9 7TF.
2. Medical Research Council Clinical Trials Unit, University College London, London, London
3. Institute of Medical Genetics, Institute of Cancer and Genetics, Cardiff University, Cardiff, United Kingdom
4. Leeds Institute of Clinical Trials Research, University of Leeds, Leeds, UK
5. CRUK/MRC Oxford Institute for Radiation Oncology, University of Oxford, Oxford
6. Institute of Immunology and Immunotherapy, University of Birmingham and University Hospital Birmingham, UK

* MTS and GM are joint senior authors

Corresponding Author

Professor Gary Middleton

Institute of Immunology and Immunotherapy, University of Birmingham and University Hospital Birmingham, UK
Tel: 44 7789502237
Email: G.Middleton@bham.ac.uk

Abstract

Commented [JS1]: 250 words

BACKGROUND:

To understand the poor prognosis of *BRAF*-mutant advanced colorectal cancer (aCRC) patients we examined individual data from patients treated with chemotherapy alone in three randomised trials to identify points on the treatment pathway where outcomes differ from *BRAF*-wild-types.

METHODS:

2530 aCRC patients were assessed from three large randomised trials. End-points were progression free survival (PFS), response rate (RR), post progression survival (P-PS) and overall survival (OS). Treatments included first-line oxaliplatin/fluorouracil (OxFU), and second-line irinotecan. Clinicians were unaware of *BRAF*-status

RESULTS

231 patients (9.1%) had *BRAF*-mutant tumours. Compared with wild-type, *BRAF*-mutant patients in COIN treated with first-line OxFU had marginally inferior RR (34.3% vs 47.5%; adjusted OR=0.58,p=0.020), but similar PFS (5.7 vs 6.3 months; adjusted HR=1.14, p=0.26). Following progression on first-line chemotherapy, *BRAF*-mutant patients had markedly shorter P-PS (4.2 vs 9.2 months, adjusted HR=1.69,p<0.001). *BRAF*-mutant status did not confer a disadvantage for patients without progression having planned chemotherapy-free intervals (OS adjusted HR=0.97, p=0.75).

Fewer *BRAF*-mutant patients received second-line treatment (33% *vs* 51%, p<0.001). However, for those who did, *BRAF*-mutation was not associated with inferior second-line outcomes (RR adjusted OR=0.56, p=0.45; PFS adjusted HR=1.01, p=0.93).

Commented [JS2]: Do we want coin 2nd line data in here instead?

CONCLUSIONS

BRAF-mutant aCRC confers a markedly worse prognosis independent of associated clinic-pathological features. Chemotherapy does provide meaningful improvements in outcome throughout treatment lines. Post-progression survival is markedly worse and vigilance is required to ensure the appropriate delivery of treatment after first- line progression. However, *BRAF*-mutant patients may still enjoy treatment breaks when not progressing, and if treated with second-line chemotherapy are no less likely to benefit.

Introduction

Commented [JS3]: 3041 words currently

The V600E activating mutation in *BRAF* (*BRAF*-mutant) is found in the tumours of 8-12% of patients with advanced colorectal cancer (aCRC). These patients represent a distinct population with typical clinico-pathological features.[1-6] *BRAF*-mutant aCRC is consistently associated with poor overall survival (OS) in case series[4,7,8] and randomised controlled trials (RCTs).[9,10]

The underlying mechanism for this poor outcome is unknown. One hypothesis is that *BRAF*-mutant status confers primary resistance to standard chemotherapy or targeted therapies. Retrospective single-centre studies describe inferior outcomes with chemotherapy compared with *BRAF* wild-type (*BRAF*-wt) patients.[4,7,8] However, analysis of a large phase III trial of chemotherapy, FOCUS, found that whilst *BRAF*-mutant status was associated with markedly inferior OS, the *BRAF*-mutant and *BRAF*-wt patients benefited to a similar extent from adding a second drug (oxaliplatin or irinotecan) to 5FU.[11] Similarly, there is no evidence *BRAF*-mutant status lessens the impact of the addition of bevacizumab to chemotherapy.[12,13] For only one class of drug, anti-EGFR antibodies, has *BRAF*-mutant status been reported to confer lack of benefit,[14] but this finding is inconsistent[10,15,16] and, given the modest overall impact of these drugs on survival, does not explain the major survival disadvantage seen in *BRAF*-mutant patients.

Another consistent finding is that *BRAF*-mutant is associated with a greater detriment in OS than in progression-free survival (PFS). In a pooled

analysis of first-line trials, whilst PFS was modestly inferior in *BRAF*-mutant patients (6.2 vs 7.7 months, HR = 1.34 p<0.001), this small difference contrasted with very markedly inferior OS (11.4 vs 17.2 months, HR =1.91 p<0.001).[5] This raises the question whether *BRAF*-mutant status confers tumour biological changes that lead to accelerated decline following progression on therapy, and it is this rather than primary drug resistance that drives the poor prognosis.

To investigate this phenomenon, we examine individual patient data from three RCTs to identify points on the treatment pathway at which *BRAF*-mutant outcomes differ from *BRAF*-wt patients treated with cytotoxic chemotherapy. As cytotoxic agents remain the backbone of contemporary treatment of aCRC this analysis is pertinent to modern oncology treatment. We compare detailed treatment outcomes in two first-line RCTs with oxaliplatin/fluorouracil (OxFU), behaviour during chemotherapy-free intervals and following disease progression. We then report patterns of, and outcomes with second-line therapy. In order to avoid potential interactions of *BRAF* status with anti-EGFR drugs we focus on patients treated in arms that did not include targeted therapies, and at a time when these drugs were not widely available in the UK for post-trial use. Potential confounding factors were prospectively identified, and analyses adjusted accordingly. *BRAF*-status was unknown to clinicians treating patients in each trial.

Patients and Methods:

Patient population and treatment:

Individual patient data were obtained from selected arms of three large randomised trials, to reflect different clinical uses of standard cytotoxic chemotherapy (without targeted therapy) in aCRC (Figure 1).

- FOCUS (ISRCTN 79877428) was a sequencing trial of first-line and planned second-line therapy, and provided a cohort of 430 patients receiving single-agent 5FU ahead of planned second-line irinotecan or oxaliplatin-based therapy, plus a cohort of 357 randomised to first-line doublet (IrFU or OxFU).[17]
- COIN (ISRCTN 27286448) provided a cohort of 1284 patients randomised to first-line oxaliplatin/fluoropyrimidine (OxFp) doublet either continuously (Arm A) or with planned chemotherapy-free intervals (Arm C).[18,19]
- PICCOLO (ISRCTN 93248876) provided a cohort of 511 OxFp-resistant patients treated with second-line irinotecan.[14,20]

Inclusion criteria for FOCUS and COIN were consistent and both patient groups were treated in centres in the UK. Full reports of these studies have been published.[14,17-20] National ethical approval and patient consent was obtained for all aspects of the clinical and translational research. DNA extraction and genotyping for mutations including *BRAF*$_{V600E}$ was performed retrospectively as previously reported.[11,14,16,20]

Statistical analysis

Stata was used (*Release 12 (2011)*, StataCorp. College Station, Texas). Baseline patient characteristics were compared between *BRAF*-wt patients (with or without other MEK/AKT pathway mutations) and *BRAF*-mutant patients using two-tailed T-tests, Wilcoxon rank sum tests (for variables with non-normally distributed frequency distributions) and Pearson Chi-squared tests (for categorical variables).

In addition to OS (time from randomisation to death from any cause), three treatment-related clinical endpoints were used: PFS (time from randomisation to first evidence of progression or death); 12-week RECIST response rate (RR), and disease control rate (DCR).[21] Finally, we compared post-progression survival time (P-PS), defined as time from progression to death in those with a progression event.

The prognostic influence of *BRAF*-mutant status on survival outcomes (PFS, P-PS and OS) for first-line trials (FOCUS and COIN), then the second-line trial (PICCOLO) were analysed using Cox proportional hazards modelling and described using hazard ratios (HRs) and 95% confidence intervals (CIs), adjusted for factors known to be prognostic or likely to interact with *BRAF*-status. In COIN and FOCUS these were: WHO performance status (2 vs 0/1); primary tumour resected (yes vs no); primary tumour location (PTL) (right colon vs other); platelet count (< vs ≥ 400,000 /μl); peritoneal metastases (present vs absent) and mismatch repair (MMR) status. In PICCOLO, adjustment was made for: response to previous therapy; performance status;

peritoneal metastases; primary tumour resected and PTL. As these factors individually interact with prognosis, adjusted values are reported primarily but unadjusted values are provided (Table 2).

Kaplan-Meier (KM) curves were plotted. For response endpoints, odds ratios (ORs) and 95% CIs were estimated from logistic regression models for the effect of *BRAF*-mutant status, adjusted for the markers previously described.

Results:

BRAF association with clinicopathological variables

BRAF-mutant status was available for 787/2135 (36.9%) patients in FOCUS, 1284/1630 (78.8%) in COIN and 459/511 (89.8%) in PICCOLO (Figure 1). The *BRAF*-mutant prevalence was consistent with published values (FOCUS 61/787 [7.8%], COIN 130/1284 [10.1%], PICCOLO 40/459 [8.7%]). *BRAF*-mutant patients were more likely than *BRAF*-wt to be female, have right-sided PTL, have peritoneal metastases and nodal metastases, but less likely to have lung metastases. *BRAF*-mutant tumours were more likely to have dMMR than *BRAF*-wt tumours. 8/2530 (0.3%) patients' tumours had dual mutations in both *BRAF* and *KRAS* (Table 1).

Commented [r4]: Were these MSI also?

BRAF status as a prognostic marker for overall survival

BRAF-mutant status was a significant prognostic marker for OS in both first-line studies (COIN 9.8 vs 16.6 months, unadjusted HR = 1.78 [1.46-2.17], p<0.001; FOCUS 10.9 vs 16.2 months, unadjusted HR=1.55 [1.18-2.04], p=0.030)(Table 2). Combining these data [n=2071] gave a median OS of 10.8 vs 16.4 months (HR=1.49 [1.23-1.80] p<0.001)(Figure 2).

As *BRAF*-mutant status is associated with clinico-pathological characteristics that may interact with survival (Table 1), the impact of these were explored in a univariate, then multivariate analysis in pooled data from COIN and FOCUS. Significant factors predicting poor OS at univariate testing

were *BRAF*-mutant status, poor performance status, high platelet count, right PTL, peritoneal metastases, primary tumour *in-situ* and dMMR status; in multivariate testing, all factors remained significant other than dMMR status (Table 2).

Following adjustment, *BRAF*-mutant status remained a significant prognostic marker in both trials (COIN adjusted HR = 1.51 [1.19-1.91], p<0.001; FOCUS adjusted HR=1.44 [1.04-2.00], p=0.030). However given the demonstrated prognostic effect of clinical factors associated with *BRAF*-mutant status, subsequent analyses are adjusted.

Impact of *BRAF* status on treatment-related endpoints on first-line combination chemotherapy

In contrast to its marked effect on OS, *BRAF*-mutant status had modest or insignificant impact on the first-line PFS and response endpoints.

For patients treated with first-line OxFP in COIN, *BRAF*-mutant patients had an inferior 12-week RR (34.3% vs 47.5%, adjusted OR=0.58 [0.37-0.92], p=0.020); however, the differences in DCR and PFS were not significant (DCR 59.2% vs 72.0%, adjusted OR=0.76 [0.49-1.20], p=0.24; PFS 5.7 vs. 6.3 months, adjusted HR=1.14 [0.91-1.42], p=0.26)(Table 3). There was no evidence of a differential effect of *BRAF* status according to the doublet used (OxFU or OxCap)(data not shown).

Similarly for patients treated with first-line combination chemotherapy in FOCUS, there were no differences in efficacy endpoints in *BRAF*-mutant compared with *BRAF*-wt patients: PFS was 8.2 vs 8.8 months (adjusted HR=1.07 [0.69-1.67], p=0.75); RR was 43.7% vs 43.1% (adjusted OR=1.09 [0.45-2.65], p=0.85); DCR was 68.9% vs 69.9% (adjusted OR=1.01 [0.36-2.84], p=0.97)(Table 3). There was no evidence of a differential effect of *BRAF* status according to regimen used (OxFU or IrFU, p=0.26).

Impact of *BRAF* status on post-progression survival

Following progression on first-line combination chemotherapy, *BRAF*-mutant patients had markedly reduced P-PS compared with *BRAF*-wt in both first-line trials. In COIN PPS was 4.5 months in *BRAF*-mutant compared with 9.6 months in *BRAF*-wt patients (adjusted HR=1.64 [1.26-2.13], p<0.001). Similarly in FOCUS inferior PPS was observed between *BRAF*-mutant and wild-types (3.2 vs 8.1 months; adjusted HR=1.65 [1.03-2.67], p=0.038)(Table 3). Combining this data PPS was inferior in the *BRAF*-mutant compared with the *BRAF*-wt group (4.2 vs 9.2 months, HR=1.62 [1.29-2.04], p<0.001)(Figure 3). These marked differences were independent of first-line treatment received (in COIN, OxFU vs OxCap p=0.57, in FOCUS OxFU vs IrFU p=0.91)(data not shown).

When other prognostic factors were tested in a combined multivariate model, a significant negative effect on P-PS was seen after first-line chemotherapy for peritoneal metastases and dMMR status (peritoneal

metastases HR=1.39, p<0.0001; dMMR HR=1.38, p=0.025). However the negative prognostic impact of peritoneal metastases and dMMR appears limited to the *BRAF*-wt population, and neither factor impacted further on the poor P-PS seen in *BRAF*-mutant patients (interaction p= 0.005 and p=0.05 respectively), showing that it is the *BRAF*-mutation driving the observed poor outcomes (Supplementary Table 1).

Impact of *BRAF* status on salvage therapy

To explore the mechanism for inferior first-line P-PS in *BRAF*-mutant patients, we studied uptake of post-progression therapies and survival outcomes of those who received second-line treatment, compared to those who did not.

In COIN, *BRAF*-mutant patients were less likely to receive second-line therapy after first-line progression (33% vs. 51%, p=0.0002). Similarly, after completion of the FOCUS plan, which for all patients included two drugs (FU and either oxaliplatin or irinotecan, given over 1 or 2 'lines'), 123/401 (30.7%) *BRAF*-wt and 3/29 (10.3%) *BRAF*-mutant patients received subsequent salvage therapy (p=0.020)(data not shown).

The duration of second-line therapy (regimens including FU-based, Ir-based, oxaliplatin-based, cetuximab and bevacizumab) for those who received it, was unaffected by *BRAF*-mutant status (COIN p=0.55, FOCUS p=0.18). The only exception was the subgroup of FOCUS patients

randomised to receive IrFU after progression on FU alone, where *BRAF*-mutant status was associated with shorter treatment duration (p=0.019)(data not shown).

OS was improved in COIN for those who received subsequent second-line chemotherapy compared with those without, regardless of *BRAF* status (*BRAF*-mut 16.1 vs 7.8 months [HR=0.56, p=0.005]; *BRAF*-wt 21.1 vs 11.6 months [HR=0.45, p<0.001]; interaction p=0.66)(Figure 4). However *BRAF*-mutant patients had worse OS whether treated with second line chemotherapy, (HR=1.91[1.36-2.69], p<0.001), or not (HR=1.44 [1.12-1.84], p=0.004), compared with wild-types(data not shown).

Commented [r5]: Is this second line rather than re-challenge?

Commented [r6]: Not sure what additional this is telling us other than those who are alive and fit enough to have further chemo will do better?

Commented [JS7]: Does this make sense?

Impact of chemotherapy-free intervals in *BRAF*-mutant patients

In contrast to the higher death rate after failure of first-line chemotherapy, there was no evidence that *BRAF*-mutant patients fare less well with a planned treatment break when first-line treatment has not yet failed. COIN, which compared continuous or intermittent chemotherapy strategies, found that intermittent chemotherapy was non-inferior for OS (adjusted HR=1.04 [0.98–1.10], p=0.16);[19] in *BRAF*-mutant patients this was also the case (adjusted HR=0.97 [0.80–1.17], p=0.75) (Supplementary Figure 1).

Commented [r8]: Of relevance and interest what is the effect of treatment breaks in those with peritoneal mets? And uptake of second line therapies

Overall in COIN, progression events in patients during chemotherapy breaks led to shorter PFS (adjusted HR=1.27 [1.21–1.33], p<0.001).[19]

Interestingly, however, *BRAF*-mutant patients were the only molecular sub-group not to have a PFS disadvantage with intermittent chemotherapy (*BRAF*-mutant PFS adjusted HR=1.09 [0.91–1.31], p=0.33; *BRAF*-wt PFS adjusted HR=1.29 [1.21–1.37], p<0.001; interaction p=0.14)(Supplementary Figure 1).

Outcomes with single agent chemotherapy

We additionally examined the impact of *BRAF*-status on outcomes with single agent chemotherapy, often utilised in combination with targeted agents. With first-line single-agent 5FU in FOCUS, PFS was similar in *BRAF*-mutant and *BRAF*-wt patients (6.5 vs 6.7 months; adjusted HR=0.96 [0.60-1.52], p=0.30); RR was 17.2% vs 21.7% (adjusted OR=0.54 [0.17,1.72], p=0.30); DCR 48.3% vs 60.6% (adjusted OR=0.72 [0.27-1.94], p=0.52)(Supplementary Table 2).

Commented [r9]: In those with small volume disease or greater frailty?

Following progression on single-agent 5FU, PPS was reduced in the *BRAF*-mutant group (3.5 vs 9.3 months; adjusted HR = 2.19[1.30-3.69],p=0.003) (Supplementary Table 2), again with a lower uptake of second line therapies (39.3% vs 58.4%, p=0.048).

The impact of *BRAF*-status on outcomes for 459 patients treated with second-line Ir were examined in the PICCOLO trial. Whilst OS was shorter for *BRAF*-mutant patients compared with wild-types, the difference did not reach statistical significance: 6.7 vs 10.2 months (adjusted HR=1.21 [0.84-1.76], p=0.31)(Supplementary Table 2 and Supplementary Figure 2).

Similar to first line data efficacy data, and subsequent outcomes with salvage therapy, there were no significant differences in the treatment-related endpoints between *BRAF*-mutant to *BRAF*-wt patients: PFS was 3.5 vs 4.0 months (adjusted HR=1.01 [0.69-1.49], p=0.93); RR was 5.0% vs. 8.1% (adjusted OR=0.56 [0.13-2.49], p=0.45); DCR was 42.5% vs. 47.7% (adjusted OR=0.82[0.41-1.62], p=0.57)(Supplementary Table 2).

In PICCOLO, P-PS was 5.9 months in *BRAF*-mutant patients, 6.5 months in *BRAF*-wt patients (adjusted HR=1.28 [0.81-2.01], p=0.29) (Supplementary Table 2). The only factor predicting shorter P-PS in multivariate testing was the presence of peritoneal metastases (HR=1.34[1.04-1.75], p=0.026)(data not shown).

Commented [r10]: What of the BRAF and MSI impact on this.. or numbers too small

Discussion

This is the largest and most comprehensive clinical series assessing the outcomes of *BRAF*-mutant patients treated with chemotherapy at different points of the aCRC pathway. The poor prognosis of *BRAF*-mutant aCRC compared with wild-types was confirmed. The novel and most striking findings are that this poor outlook is not driven by chemoresistance, and that the point at which outcomes markedly diverge from wild-types is following progression on first-line chemotherapy. Results were consistent between FOCUS and COIN, independent of chemotherapy strategy and other standard prognostic factors.

The poor outcomes advanced *BRAF*-mutant aCRC are well described, but these cancers are associated with specific clinico-pathological features: older age, proximal primary tumour, high grade, deficient MMR, mucinous histology and peritoneal and lymph node metastases,[5-10] most of which interact with prognosis. In a careful multivariate analysis in a large, prospectively gathered cohort, *BRAF* mutation still conferred a worse prognosis and is not simply attributable to associated clinico-pathological features.

We then examined at what points in the aCRC pathway did this poor outcome manifest, and have convincingly demonstrated it is not due to intrinsic chemo-resistance. There was no difference in the adjusted PFS between *BRAF*-mutant and wild-type patients on first line OxFP in COIN and

in any FOCUS strategies. Furthermore, there was no difference in efficacy endpoints in patients treated with second-line irinotecan monotherapy by *BRAF*-mutant status in PICCOLO, or in the relative benefit of second-line therapy after failure on COIN treatment. Thus, chemotherapy throughout the lines of therapy provides equivalent degrees of disease modification irrespective of *BRAF*-status. However the absolute impact is less due to the poor overall outcome; highlighted by the worse overall survival of *BRAF*-patients receiving further chemotherapy in COIN, compared to wild-types. Thus, the equivalent absolute outcome benefits (PFS and DCR) on first-line OxFP are noteworthy.

Other studies suggest that oxaliplatin may be particularly important in *BRAF*-mutant patients. Biomarker analysis from MOSAIC (testing the addition of oxaliplatin to FP in adjuvant CRC) reported that the OS HR for OxFP vs FP alone was 0.55 in the *BRAF*-mutants, and 0.93 in wild-types. The 3 year DFS, 5 year OS and 10 year OS absolute differences for the addition of oxaliplatin were 16.4%, 9.5% and 10.1% respectively compared with only 2.4, 1% and 1.9 in wild type patients. In the TRIBE study (FOLFOXIRI plus bevacizumab vs FOLFIRI plus bevacizumab in the first-line treatment of aCRC), PFS HR for the addition of oxaliplatin to FOLFIRI/Bevacizumab in *BRAF*-mutant patients was 0.54, compared with 0.85 in the *RAS/RAF* wild-types; the ORs for response was 1.82 and 1.17 respectively.

Commented [r11]: Indicate that this is metastatic having just talked about adjuvant?

Commented [JS12]: Can we add HRs/ ORs without p values and CIs here?

BRAF-mutant patients have markedly worse survival after progression on first-line treatment, with important implications for patient management.

Prompt initiation of second-line treatment appears to slightly ameliorate this risk: in COIN *BRAF*-mutant patients without second-line treatment demonstrated rapid decline after first line therapy failure. This finding was independent of poor performance status. However in the first-line trials, fewer *BRAF*-mut patients proceeded to receive second-line chemotherapy. It is important emphasise that treating physicians were unaware of *BRAF*-status therefore this finding is not due to selection bias. Extra vigilance is therefore needed in *BRAF*-mutant patients to detect progression and rapidly institute second-line therapy as appropriate given that such treatment significantly improves overall survival albeit with less absolute benefit than in wild-type patients.

Some may view the observed rapid decline after first-line progression and risk of being unable to deliver second-line treatment as a strong argument for using upfront FOLFOXIRI-based regimens in *BRAF*-mutant patients. Indeed, a potential criticism of the current study is that we did not investigate outcomes with triplet treatment. However the current data remain highly pertinent given that many patients with advanced cancer are not fit enough to receive this treatment in spite of being well enough to potentially benefit from sequential chemotherapy.

Equally importantly for routine practice, we found that whilst *BRAF*-mutant patients are at risk of accelerated decline after progression, this does not mean that they cannot safely enjoy an intermittent strategy including periods off chemotherapy when treatment has not yet failed. Thus such

patients with disease control can be appropriately counselled about the safety of chemotherapy free intervals.

Commented [r13]: Any published data from CAIRO3 re bev cape maintenance?

These data allow the development of two non-mutually exclusive hypotheses to explain the inferior survival of *BRAF*-mutant patients. Firstly these patients may simply have a worse prognosis from initiation of their treatment programme and that equivalent PFS and DCR reflects enhanced relative benefit from first-line chemotherapy, particularly with oxaliplatin, in comparison with wild-type patients. Alternatively the poor survival is driven by mechanisms mediating first-line chemotherapy resistance when superimposed on the *BRAF*-mutational landscape: supported by markedly worse post-progression survival independent of the delivery of second-line treatment, and the lack of PFS and OS deterioration in *BRAF*-mutant patients stable on first-line Ox/FP receiving chemotherapy-free breaks. The molecular basis for these observations requires study.

Disappointing results of *BRAF*-inhibitors as single agents in aCRC[11] and a growing appreciation of molecular complexity of *BRAF*-mut aCRC[12] suggest that targeted approaches may require multi-agent combinations. Early clinical studies report encouraging clinical activity and acceptable toxicity with the combination of a *BRAF*-inhibitor, a MEK inhibitor and an anti-EGFR agent.[27] These regimens are complex and likely to be expensive and will complement rather than replace chemotherapy.

This, the largest and most comprehensive analysis of chemotherapy outcomes in *BRAF*-mutant CRC patients provides new and important information with clinical relevance. In summary, *BRAF*-mutation confers a markedly worse prognosis independent of associated clinic-pathological features. Chemotherapy does provide meaningful improvements in outcome throughout treatment lines. Post-progression survival is markedly worse and vigilance is required to ensure the appropriate delivery of treatment after first-line progression.

Legend to Figures and Tables

Figure 1- Consort diagram of study participants from the FOCUS, COIN and PICCOLO trial

Figure 2 –OS KM curves for *BRAF*-mut vs *BRAF*-wt for first line chemotherapy (FOCUS and COIN, all strategies)

Figure 3 - Post-progression survival KM curves for *BRAF*-mut vs *BRAF*-wt following failure on first-line chemotherapy (COIN and FOCUS)

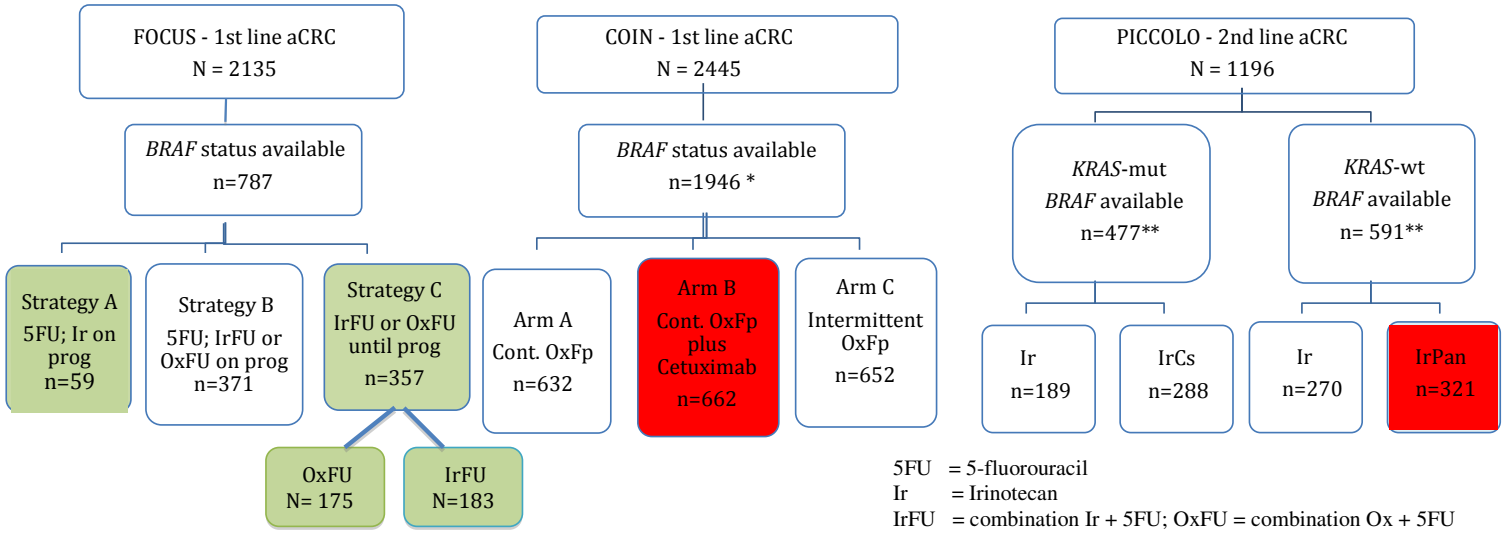
Figure 4 – Overall survival KM curves for second line treatment, vs none in *BRAF*-mutant and wild-type patients

Table 1 – Patient characteristics by *BRAF* status

Table 2 – Estimated crude HRs and 95% CIs for the effect of clinic-pathological factors associated with BRAF on overall survival

Table 3 - Estimated crude HRs and 95% CIs for the effect of *BRAF*-status (mutant vs wild-type) on PFS, P-PS and OS, then estimated crude ORs and 95% CIs for the effect of *BRAF*-status (mutant vs wild-type) on RR and DCR

Figure 1



= Trial arm(s) included in RR, PFS, P-PS and OS analysis

= Trial arm(s) excluded from all analyses

5FU = 5-fluorouracil
Ir = Irinotecan
IrFU = combination Ir + 5FU; OxFU = combination Ox + 5FU
IrCs = combination Ir + Ciclosporin
IrPan = combination Ir + Panitumumab
OxFp = combination of Oxaliplatin + free choice of either 5FU (OxFU) or Capecitabine (OxCap)
Cont. = continuous
Prog = disease progression
* = *BRAF* status in 1284/1630 excluding arm B
** = *BRAF* status in 459/511 excluding IrCs & IrPan

Figure 2

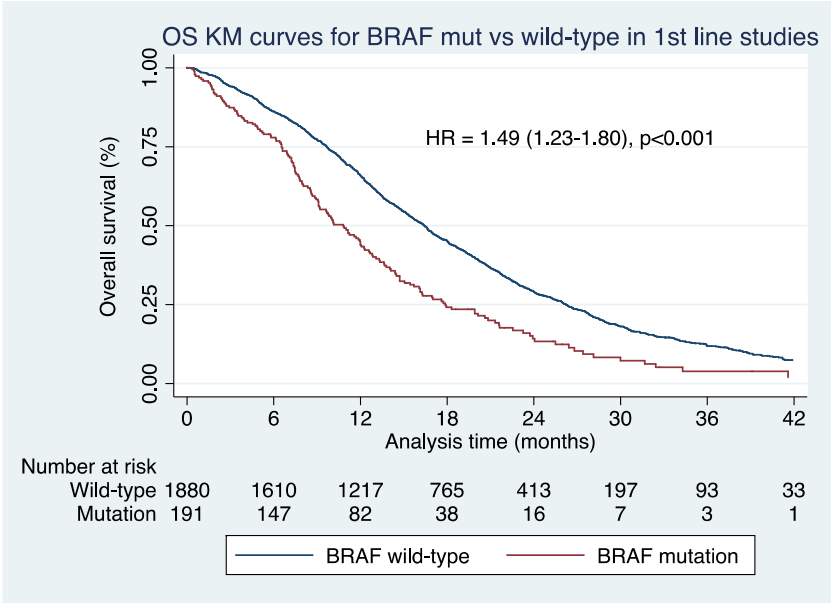


Figure 3

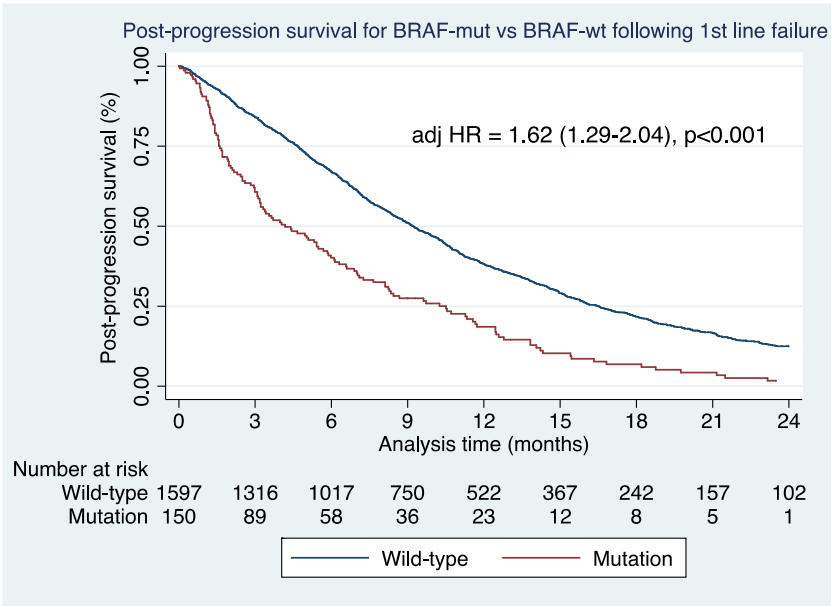


Figure 4

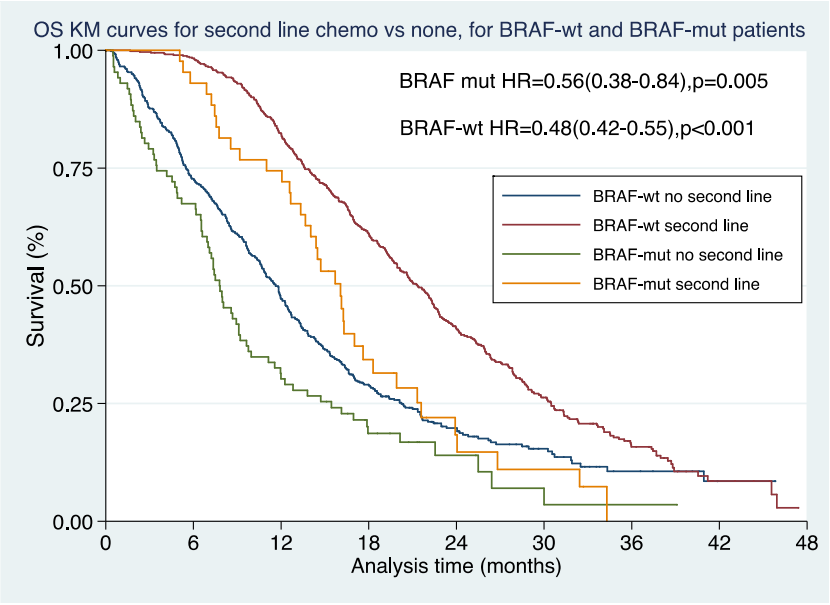

OS KM curves for second line chemo vs none, for BRAF-wt and BRAF-mut patients
BRAF mut HR=0.56(0.38-0.84),p=0.005
BRAF-wt HR=0.48(0.42-0.55),p<0.001
BRAF-wt no second line
BRAF-wt second line
BRAF-mut no second line
BRAF-mut second line
Survival (%)
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
30
36
42
48
Analysis time (months)

Table 1

		1st line study population (FOCUS and COIN) (n=2071)			2nd line study population (PICCOLO) (n=459)			All patients		
		***BRAF*-mut (n=191)**	***BRAF*-wt (n = 1880)**	**p-value****	***BRAF* mut (n = 40)**	***BRAF*-wt (n = 419)**	**p-value****	***BRAF-mut (n=231)***	***BRAF-wt (n=2299)***	**p-value****
Median age (IQR)		63.4 (57-71)	64 (57-69)		63.1 (56-67)	62.7 (56-67)		63.5 (57.0-69.0)	63.4 (57.0-69.4)	
Sex n(%)	**Male**	107 (56.0)	1271 (67.6)	p=0.002	13 (32.5)	295 (70.4)	p<0.001	120 (52.0)	1566 (68.1)	p<0.001
	Female	84 (44.0)	609 (32.4)		27 (67.5)	120 (28.7)		111 (48.0)	729 (31.7)	
	Missing	0 (0.0)	0 (0.0)		0 (0.0)	4 (0.9)		0	4 (0.2)	
WHO PS n(%)	**0-1**	173 (90.6)	1750 (93.1)	p=0.20	39 (97.5)	393 (93.8)	p=0.50*	212 (91.8)	2143 (93.2)	p=0.41
	2	18 (9.4)	130 (6.9)		1 (2.5)	26 (6.2)		19 (8.2)	156 (6.8)	
Resected primary n(%)	**Yes**	131 (68.6)	1326 (70.5)	p=0.91	36 (90.0)	299 (71.3)	p=0.01*	167 (72.3)	1625 (70.7)	p=0.34
	No	50 (26.2)	496 (26.4)		4 (10.0)	118 (28.2)		54 (23.4)	614 (26.7)	
	Missing	10 (5.2)	58 (3.1)		0 (0.0)	2 (0.5)		10 (4.3)	60 (2.6)	
Primary tumour location n(%)	**Right**	111 (58.1)	451 (24.0)	p<0.001	22 (55.0)	126 (30.1)	p=0.001	133 (57.6)	577 (25.1)	p<0.001
	Left	70 (36.7)	1327 (70.6)		17 (42.5)	284 (67.8)		87 (37.6)	1611 (70.1)	
	Missing	10 (5.2)	102 (5.4)		1 (2.5)	9 (2.1)		11 (4.8)	111 (4.8)	
Previous clinical benefit(%)	**Yes**	n/a	n/a	n/a	21 (52.5)	271 (64.7)	p=0.13	21 (52.5)	271 (64.7)	p=0.13
	No	n/a	n/a		12 (30.0)	112 (26.7)		12 (30.0)	112 (26.7)	
	Missing	n/a	n/a		7 (17.5)	36 (8.6)		7 (17.5)	36 (8.6)	
Peritoneal mets n(%)	**Yes**	42 (22.0)	263 (14.0)	p=0.003	16 (40.0)	97 (23.2)	p=0.02	58 (25.1)	360 (15.7)	p=0.001
	No	148 (77.5)	1603 (85.3)		24 (60.0)	311 (74.2)		172 (74.5)	1914 (83.2)	
	Missing	1 (0.5)	14 (0.7)		0	11 (2.6)		1 (0.4)	25 (1.1)	
Lung mets n(%)	**Yes**	45 (23.6)	754 (40.1)	p<0.001	15 (37.5)	246 (58.7)	p=0.006	60 (26.0)	1000 (43.5)	p<0.001
	No	145 (75.9)	1112 (59.2)		25 (62.5)	164 (39.1)		170 (73.6)	1276 (55.5)	
	Missing	1 (0.5)	14 (0.7)		0 (0.0)	9 (2.2)		1 (0.4)	23 (1.0)	
Liver mets n(%)	**Yes**	129 (67.5)	1395 (74.2)	p=0.04	30 (75.0)	305 (72.8)	p=0.89	159 (68.8)	1700 (73.9)	p=0.16
	No	61 (31.9)	471 (25.1)		10 (25.0)	107 (25.5)		71 (30.8)	578 (25.1)	
	Missing	1 (0.5)	14 (0.7)		0 (0.0)	7 (1.7)		1 (0.4)	21 (0.9)	
Nodal mets n(%)	**Yes**	104 (54.5)	811 (43.1)	p=0.003	16 (40.0)	103 (24.6)	p<0.001	120 (52.0)	914 (39.8)	P<0.001
	No	86 (45.0)	1055 (56.1)		24 (60.0)	311 (74.2)		110 (47.6)	1366 (59.4)	
	Missing	1 (0.5)	14 (0.7)		0 (0.0)	5 (1.2)		1 (0.4)	19 (0.8)	
MMR status n(%)	**dMMR**	24 (12.6)	56 (3.0)	p<0.001	2 (5.0)	2 (0.5)	0.03*	26 (11.2)	58 (2.5)	p<0.001
	pMMR	143 (74.9)	1583 (84.2)		2 (5.0)	43 (10.3)		145 (62.8)	1626 (70.7)	
	Missing	24 (12.6)	241 (12.8))		36 (90.0)	374 (89.2)		60 (26.0)	615 (26.8)	
***KRAS* status n(%)**	**WT**	180 (94.2)	993 (52.8)	p<0.001	36 (90.0)	219 (52.3)	p<0.001*	216 (93.5)	1212 (52.7)	p<0.001
	Mut	8 (4.2)	857 (45.6)		0 (0.0)	172 (41.0)		8 (3.5)	1029 (44.8)	
	Missing	3 (1.6)	30 (1.6)		4 (10.0)	28 (6.7)		7 (3.0)	58 (25.2)	

* Fishers exact test
**Missing values excluded from comparisons

Table 2

Prognostic marker	Median survival (IQR)	Comparison	Unadjusted HR (95% CI)	Adjusted HR** (95% CI)
BRAF-mut 191/2071 (9.2%)	10.8 (6.5-17.9)	*BRAF*-mut vs wild-type	n=2071, fail =1667	n=1608, fail = 1305
			1.69(1.44-1.99)p=<0.001	1.47 (1.21-1.78)p<0.001
Poor PS* 304/ 3765 (8.1%)	9.0 (3.6-16.1)	Poor vs good PS	n=3765, fail =3086	n=1608, fail=1305
			1.81 (1.60-2.06), p<0.001	1.39 (1.12-1.73), p=0.003
Plts >400 639/3500 (18.2%)	10.9 (5.9-18.6)	High vs low plts	n=3500,fail =2849	n=1608 ,fail=1305
			1.82 (1.65-1.99), p<0.001	1.57 (1.37-1.81),p<0.001
Primary tumour in situ 1303/ 3762 (34.6%)	12.4 (6.8-20.3)	Primary in situ vs resection	n=3762, fail = 3086	n=1608, fail = 1305
			1.53 (1.42-1.64), p<0.001	1.45 (1.27-1.65),p<0.001
Right PTL 807/ 2982 (27.1%)	12.8 (7.4-21.6)	Right PTL vs left &rectal	n=2982, fail=2445	n=1608, fail = 1305
			1.29 (1.18-1.41),p<0.001	1.17 (1.03-1.32),p=0.017
dMMR 134/ 2558 (5.2%)	12.3 (6.5-21.2)	dMMR vs pMMR	n=2558, fail=2143	n=1608, fail=1305
			1.23 (1.02-1.49),p=0.030	1.17 (0.89-1.53), p=0.25
peritoneal mets 527/ 3717 (14.2%)	11.7 (6.3-19.8)	peritoneal mets vs no peritoneal mets	n=3717, fail=3048	n=1608, fail=1305
			1.46 (1.32-1.61), p<0.001	1.29 (1.10-1.51),p=0.001

*Poor PS is defined by WHO ≥2
**All prognostic markers included in the multivariate analysis

Table 3

Clinical Endpoint	Treatment strategy	Median (IQR) survival (mo)		Unadjusted HR (95% CI)	Adjusted HR** (95% CI)
		***BRAF*-mut**	***BRAF*-wt**		
PFS	1st line OxFU or IrFU (FOCUS)	n=32	n=325	n=357, fail =348	n=274, fail=266
		8.2 (3.6-10.3))	8.8 (5.8-11.9)	1.09(0.76-1.58), p=0.63	1.07(0.69-1.67), p=0.75
	1st line OxFU (COIN)	n=130	n=1154	n=1284,fail =1219	n=1009,fail=955
		5.7 (3.1-8.5)	6.3 (4.9-9.6)	1.20(0.99-1.45),p=0.057	1.14 (0.91-1.42),p=0.26
P-PS	1st line OxFU or IrFU (FOCUS)	n=24	n=281	n=305, fail=268	n=266, fail = 247
		3.2 (1.5-10.7)	8.1 (4.3-15.9)	1.91 (1.25-2.91),p=0.003	1.65 (1.03-2.67),p=0.038
	1st line OxFU (COIN)	n=102	n=970	n=1072, fail=829	n=836, fail=655
		4.5(1.7-10.5)	9.6 (4.7-17.0)	2.00(1.61-2.49),p<0.001	1.64 (1.26-2.13),p<0.001
OS	All FOCUS strategies	n=61	n=726	n=787, fail = 692	n=532, fail = 599
		10.9 (7.7-17.7)	16.2 (9.5-25.2)	1.55 (1.18-2.04),p=0.030	1.44 (1.04-2.00),p=0.030
	1st line OxFU (COIN)	n=130	n=1154	n=1284, fail = 975	n=1009 fail = 773
		9.8 (6.2-17.9)	16.6 (9.7-27.5)	1.78 (1.46-2.17),p<0.001	1.51 (1.19-1.91),p<0.001
Clinical Endpoint	**Treatment strategy**	**RR / DCR (%)**		**Unadjusted OR (95% CI)**	**Adjusted OR** (95% CI)**
		***BRAF*-mut**	***BRAF*-wt**		
RR	1st line OxFU or IrFU (FOCUS)	n=32	n=325	n=357	n=274
		43.7%	43.1%	1.02 (0.49-2.13), p=0.94	1.09 (0.45-2.65),p=0.85
	1st line OxFp (COIN)	n=130	n=1154	n=1284	n=1009
		34.3%	47.5%	0.52 (0.35-0.76),p=0.001	0.58 (0.37-0.92),p=0.020
DCR	1st line OxFU or IrFU (FOCUS)	n=16	n=159	n=357	n=274
		68.9%	69.9%	0.95 (0.43-2.08), p=0.89	1.01 (0.36-2.84), p=0.97
	1st line OxFp (COIN)	n=130	n=1154	n=1284	n=1009
		59.2%	72.0%	0.56 (0.39-0.82),p=0.003	0.76 (0.49-1.20),p=0.24

HRs and ORs are for *BRAF*-mut versus *BRAF*-wt
*excluding arm B
** FOCUS and COIN adjusted for performance status, resection of primary tumour, PTL, baseline platelet count, peritoneal metastases and MSI status. PICCOLO adjusted for performance status, resection of primary tumour, PTL, peritoneal metastases and previous response to therapy.

References

1. Tran B, Kopetz S, Tie J, et al: Impact of *BRAF* mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer 117:4623-32, 2011
2. Clancy C, Burke JP, Kalady MF, et al: *BRAF* mutation is associated with distinct clinicopathological characteristics in colorectal cancer: a systematic review and meta-analysis. Colorectal Dis 15:e711-8, 2013
3. Gonsalves WI, Mahoney MR, Sargent DJ, et al: Patient and tumor characteristics and *BRAF* and *KRAS* mutations in colon cancer, NCCTG/Alliance N0147. J Natl Cancer Inst 106:7, 2014
4. Tie, J, Gibbs P, Lipton L, et al: Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the *BRAF(V600E)* mutation. Int J Cancer 128:2075-84, 2011
5. Venderbosch S, Nagtegaal ID, Maughan TS, et al: Mismatch Repair Status and *BRAF* Mutation Status in Metastatic Colorectal Cancer Patients: A Pooled Analysis of the CAIRO, CAIRO2, COIN, and FOCUS Studies. Clin Cancer Res 20:5322-30, 2014
6. Popovici, V, Budinska E, Tejpar S, et al: Identification of a poor-prognosis *BRAF*-mutant-like population of patients with colon cancer. J Clin Oncol 30:1288-95, 2012
7. Souglakos J, Philips J, Wang R, et al: Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer 101:465-72, 2009
8. Morris, V, Overman MJ, Jiang ZQ, et al: Progression-Free Survival Remains Poor Over Sequential Lines of Systemic Therapy in Patients With *BRAF*-Mutated Colorectal Cancer. Clin Colorectal Cancer, epub 30/07/2014.
9. Tol, J, Nagtegaal ID, Punt CJ, *BRAF* mutation in metastatic colorectal cancer. N Engl J Med 361:98-9, 2009
10. Van Cutsem E, Kohne CH, Lang I, et al: Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor *KRAS* and *BRAF* mutation status. J Clin Oncol 29: 2011-9, 2011
11. Richman SD, Seymour MT, Chambers P, et al: *KRAS* and *BRAF* mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. J Clin Oncol 27:5931-7, 2009
12. Ince WL, Jubb AM, Holden SN, et al: Association of *k-ras, b-raf,* and p53 status with the treatment effect of bevacizumab. J Natl Cancer Inst 97:981-9, 2005
13. Price TJ, Hardingham JE, Lee CK, et al: Impact of *KRAS* and *BRAF* Gene Mutation Status on Outcomes From the Phase III AGITG MAX Trial of Capecitabine Alone or in Combination With Bevacizumab and Mitomycin in Advanced Colorectal Cancer. J Clin Oncol 29:2675-82, 2011
14. Seymour MT, Brown SR, Middleton G, et al: Panitumumab and irinotecan versus irinotecan alone for patients with *KRAS* wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. Lancet Oncol 14:749-59, 2013

15. Douillard JY, Oliner KS, Siena S, et al., Panitumumab-FOLFOX4 treatment and *RAS* mutations in colorectal cancer. N Engl J Med 369:1023-34, 2013
16. Smith CG, Fisher D, Claes B, et al: Somatic profiling of the epidermal growth factor receptor pathway in tumors from patients with advanced colorectal cancer treated with chemotherapy +/- cetuximab. Clin Cancer Res 19:4104-13, 2013
17. Seymour MT, Maughan TS, Ledermann JA, et al: Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. Lancet 370:143-52, 2007
18. Maughan TS, Adams RA, Smith CG, et al: Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet 377:2103-14, 2011
19. Adams RA, Meade AM, Seymour MT, et al: Intermittent versus continuous oxaliplatin and fluoropyrimidine combination chemotherapy for first-line treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet Oncol 12:642-53, 2011
20. Middleton G, Brown S, Lowe C, et al: A randomised phase III trial of the pharmacokinetic bimodulation of irinotecan using oral ciclosporin in advanced colorectal cancer: results of the Panitumumab, Irinotecan & Ciclosporin in Colorectal cancer therapy trial (PICCOLO). Eur J Cancer 49:3507-16, 2013
21. Therasse P, Arbuck SG, Eisenhauer EA, et al: 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: 205-16, 2000
22. Loupakis, F., et al., *FOLFOXIRI plus bevacizumab as first-line treatment in BRAF mutant metastatic colorectal cancer.* Eur J Cancer, 2014. **50**(1): p. 57-63.
23. Loupakis F, Cremolini C, Masi G, et al: Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. N Engl J Med 371: 1609-18, 2014
24. Peeters M, Oliner KS, Price TJ, et al: Updated analysis of *KRAS/NRAS* and *BRAF* mutations in study 20050181 of panitumumab (pamb) plus FOLFIRI for second-line treatment of metastatic colorectal cancer. J Clin Oncol 32:(5s), 2014 suppl; abstr 3568.
25. Kopetz SD, Desai J, Chan E. PLX4032 in metastatic colorectal cancer patients with mutant *BRAF* tumors. J Clin Oncol 28:(15s), 2010; abstr.3534.
26. Prahallad A, Sun C, Huang S, et al: Unresponsiveness of colon cancer to *BRAF*(V600E) inhibition through feedback activation of EGFR. Nature 483:100-3, 2012
27. Atreya C, van Custem E, Bendell JC, et al., Updated efficacy of the *MEK* inhibitor trametinib, *BRAF* inhibitor dabrafenib, and anti-EGFR antibody panitumumab in patients with *BRAF* V600E mutated metastatic colorectal cancer. J Clin Oncol 33(Suppl), 2015 (abstr 103)

1. Andre, T., et al., *Adjuvant Fluorouracil, Leucovorin, and Oxaliplatin in Stage II to III Colon Cancer: Updated 10-Year Survival and Outcomes According to BRAF Mutation and Mismatch Repair Status of the MOSAIC Study.* J Clin Oncol, 2015. **33**(35): p. 4176-87.
2. Cremolini, C., et al., *FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study.* Lancet Oncol, 2015. **16**(13): p. 1306-15.
3. Richman, S.D., et al., *KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial.* J Clin Oncol, 2009. **27**(35): p. 5931-7.
4. Seymour, M.T., et al., *Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial.* Lancet, 2007. **370**(9582): p. 143-52.
5. Tran, B., et al., *Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer.* Cancer, 2011. **117**(20): p. 4623-32.
6. Clancy, C., et al., *BRAF mutation is associated with distinct clinicopathological characteristics in colorectal cancer: a systematic review and meta-analysis.* Colorectal Dis, 2013. **15**(12): p. e711-8.
7. Gonsalves, W.I., et al., *Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147.* J Natl Cancer Inst, 2014. **106**(7).
8. Tie, J., et al., *Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation.* Int J Cancer, 2011. **128**(9): p. 2075-84.
9. Venderbosch, S., et al., *Mismatch Repair Status and BRAF Mutation Status in Metastatic Colorectal Cancer Patients: A Pooled Analysis of the CAIRO, CAIRO2, COIN, and FOCUS Studies.* Clin Cancer Res, 2014. **20**(20): p. 5322-30.
10. Smith, C.G., et al., *Somatic profiling of the epidermal growth factor receptor pathway in tumors from patients with advanced colorectal cancer treated with chemotherapy +/- cetuximab.* Clin Cancer Res, 2013. **19**(15): p. 4104-13.
11. Kopetz, S.D., J.; Chan, E., *PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors.* J Clin Oncol, 2010. **28(Suppl.)**: p. 15s [abstr.3534].
12. Prahallad, A., et al., *Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR.* Nature, 2012. **483**(7387): p. 100-3.