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

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Abstract

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, \( p=0.93 \)).

**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.

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The V600E activating mutation in \textit{BRAF} (\textit{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.\textsuperscript{[1-6]} \textit{BRAF}-mutant aCRC is consistently associated with poor overall survival (OS) in case series\textsuperscript{[4,7,8]} and randomised controlled trials (RCTs).\textsuperscript{[9,10]}

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

Another consistent finding is that \textit{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 \textit{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). 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).

*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 \textit{BRAF}-mutant compared with \textit{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 \textit{BRAF} status according to regimen used (OxFU or IrFU, p=0.26).

\textbf{Impact of \textit{BRAF} status on post-progression survival}

Following progression on first-line combination chemotherapy, \textit{BRAF}-mutant patients had markedly reduced P-PS compared with \textit{BRAF}-wt in both first-line trials. In COIN PPS was 4.5 months in \textit{BRAF}-mutant compared with 9.6 months in \textit{BRAF}-wt patients (adjusted HR=1.64 [1.26-2.13], p<0.001). Similarly in FOCUS inferior PPS was observed between \textit{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 \textit{BRAF}-mutant compared with the \textit{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 \textit{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 \textit{BRAF} status \textit{[BRAF}-mut 16.1 vs 7.8 months [HR=0.56, p=0.005]; \textit{BRAF}-wt 21.1 vs 11.6 months [HR=0.45, p<0.001]; interaction p=0.66](Figure 4). However \textit{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).

**Impact of chemotherapy-free intervals in \textit{BRAF}-mutant patients**

In contrast to the higher death rate after failure of first-line chemotherapy, there was no evidence that \textit{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);\textsuperscript{[19]} in \textit{BRAF}-mutant patients this was also the case (adjusted HR=0.97 [0.80–1.17], p=0.75) (Supplementary Figure 1).

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).\textsuperscript{[19]}

\begin{commented_text}
\textsuperscript{[r5]}: Is this second line rather than re-challenge?
\textsuperscript{[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?
\textsuperscript{[JS7]}: Does this make sense?
\textsuperscript{[r8]}: Of relevance and interest what is the effect of treatment breaks in those with peritoneal mets? And uptake of second line therapies
\end{commented_text}
Interestingly, however, \textit{BRAF}-mutant patients were the only molecular sub-group not to have a PFS disadvantage with intermittent chemotherapy (\textit{BRAF}-mutant PFS adjusted HR=1.09 [0.91–1.31], p=0.33; \textit{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 \textit{BRAF}-status on outcomes with single agent chemotherapy, \textit{often utilised in combination with targeted agents}. With first-line single-agent 5FU in FOCUS, PFS was similar in \textit{BRAF}-mutant and \textit{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).

Following progression on single-agent 5FU, PPS was reduced in the \textit{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 \textit{BRAF}-status on outcomes for 459 patients treated with second-line Ir were examined in the PICCOLO trial. Whilst OS was shorter for \textit{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).

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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.

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

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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.

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\textsuperscript{11} and a growing appreciation of molecular complexity of BRAF-mut aCRC\textsuperscript{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.\textsuperscript{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
FOCUS - 1st line aCRC
N = 2135

BRAF status available
n=787

Strategy A
5FU; Ir on prog
n=59

Strategy B
SFU; IrFU or OxFU on prog
n=371

Strategy C
IrFU or OxFU until prog
n=357

Arm A
Cont. OxFp
n=632

Arm B
Cont. OxFp
plus Cetuximab
n=662

Arm C
Intermittent OxFp
n=652

COIN - 1st line aCRC
N = 2445

BRAF status available
n=1946 *

Strategy A
5FU; Ir on prog
n=59

Arm A
Cont. OxFp
n=632

Arm B
Cont. OxFp
plus Cetuximab
n=662

Arm C
Intermittent OxFp
n=652

PICCOLO - 2nd line aCRC
N = 1196

KRAS-mut
BRAF available
n=477**

Arm A
Ir
n=189

Arm B
IrCs
n=288

Arm C
Ir
n=270

KRAS-wt
BRAF available
n=591**

Arm A
Ir
n=321

IrFU = combination Ir + 5FU
OxFU = combination Ox + 5FU
IrCs = combination Ir + Ciclosporin
IrPan = combination Ir + Panitumumab
OxFp = combination of Oxaliplatin + free choice of either SFU (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

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

= Trial arm(s) excluded from all analyses
Figure 2

OS KM curves for BRAF mut vs wild-type in 1st line studies

HR = 1.49 (1.23-1.80), p<0.001

Number at risk

Wild-type: 1880, 1610, 1217, 765, 413, 197, 93, 33
Mutation: 191, 147, 82, 38, 16, 7, 3, 1

Analysis time (months)

BRAF wild-type
BRAF mutation

Figure 3

Post-progression survival for BRAF-mut vs BRAF-wt following 1st line failure

adj HR = 1.62 (1.29-2.04), p<0.001

Number at risk

Wild-type: 1597, 1316, 1017, 750, 522, 367, 242, 157, 102
Mutation: 150, 89, 58, 36, 23, 12, 8, 5, 1

Analysis time (months)

Wild-type
Mutation
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
<table>
<thead>
<tr>
<th>Table 1</th>
<th>1st line study population (FOCUS and COIN) (n=2071)</th>
<th>2nd line study population (PICCOLO) (n=459)</th>
<th>All patients</th>
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<tbody>
<tr>
<td></td>
<td>BRAF-mut (n=191)</td>
<td>BRAF-wt (n = 1880)</td>
<td>BRAF-mut (n = 40)</td>
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<tr>
<td>Median age (IQR)</td>
<td>63.4 (57.71)</td>
<td>64 (57.49)</td>
<td>63.1 (56.67)</td>
</tr>
<tr>
<td>Sex n(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
<tr>
<td>WHO PS n(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
<tr>
<td>Resected primary n(%)</td>
<td>84 (44.0)</td>
<td>609 (32.4)</td>
<td>13 (32.5)</td>
</tr>
<tr>
<td>Primary tumour location n(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
<tr>
<td>Previous clinical benefit(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
<tr>
<td>Peritoneal mets n(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
<tr>
<td>Lung mets n(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
<tr>
<td>Liver mets n(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
<tr>
<td>Nodal mets n(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
<tr>
<td>MMR status n(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
<tr>
<td>KRAS status n(%)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
<td>Missing 0 (0.0)</td>
</tr>
</tbody>
</table>

*p Fisher’s exact test

**Missing values excluded from comparisons
### Table 2

<table>
<thead>
<tr>
<th>Prognostic marker</th>
<th>Median survival (IQR)</th>
<th>Comparison</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted HR** (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF-mut</td>
<td>10.8 (6.5-17.9)</td>
<td>BRAF-mut vs wild-type</td>
<td>1.68 (1.44-1.99), p=0.001</td>
<td>1.47 (1.21-1.78), p=0.001</td>
</tr>
<tr>
<td>Poor PS</td>
<td>9.0 (3.6-16.1)</td>
<td>Poor vs good PS</td>
<td>1.81 (1.60-2.00), p&lt;0.001</td>
<td>1.39 (1.12-1.73), p=0.003</td>
</tr>
<tr>
<td>Pts &gt;740</td>
<td>10.9 (5.9-16.6)</td>
<td>High vs low Pts</td>
<td>1.82 (1.65-1.99), p&lt;0.001</td>
<td>1.57 (1.37-1.81), p&lt;0.001</td>
</tr>
<tr>
<td>Primary tumour in situ</td>
<td>12.4 (6.8-20.3)</td>
<td>Primary in situ vs resection</td>
<td>1.53 (1.42-1.64), p&lt;0.001</td>
<td>1.45 (1.27-1.65), p&lt;0.001</td>
</tr>
<tr>
<td>dMMR</td>
<td>12.3 (6.5-21.2)</td>
<td>dMMR vs pMMR</td>
<td>1.82 (1.65-1.99), p&lt;0.001</td>
<td>1.57 (1.37-1.81), p&lt;0.001</td>
</tr>
<tr>
<td>peritoneal mets</td>
<td>11.7 (6.3-19.8)</td>
<td>peritoneal mets vs no peritoneal mets</td>
<td>1.46 (1.32-1.61), p&lt;0.001</td>
<td>1.29 (1.10-1.51), p=0.001</td>
</tr>
</tbody>
</table>

*Poor PS is defined by WHO ≥2

**All prognostic markers included in the multivariate analysis

### Table 3

<table>
<thead>
<tr>
<th>Clinical Endpoint</th>
<th>Treatment strategy</th>
<th>Median (IQR) survival (mo)</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted HR** (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS</td>
<td>1st line OxFU or IrFU (FOCUS)</td>
<td>n=32</td>
<td>n=325</td>
<td>n=353, fail =348</td>
</tr>
<tr>
<td></td>
<td>1st line OxFU (FOCUS)</td>
<td>n=130</td>
<td>n=1154</td>
<td>n=124, fail =219</td>
</tr>
<tr>
<td></td>
<td>1st line OxFp (COIN)</td>
<td>n=24</td>
<td>n=281</td>
<td>n=305, fail=268</td>
</tr>
<tr>
<td></td>
<td>1st line OxFU or IrFU (FOCUS)</td>
<td>n=130</td>
<td>n=1154</td>
<td>n=1284, fail =219</td>
</tr>
<tr>
<td></td>
<td>2nd line OxFp (COIN)</td>
<td>n=61</td>
<td>n=720</td>
<td>n=787, fail =692</td>
</tr>
<tr>
<td></td>
<td>3rd line OxFU (COIN)</td>
<td>n=150</td>
<td>n=159</td>
<td>n=157, fail=929</td>
</tr>
<tr>
<td></td>
<td>4rd line OxFp (COIN)</td>
<td>n=32</td>
<td>n=325</td>
<td>n=337, fail=348</td>
</tr>
<tr>
<td></td>
<td>5th line OxFU (FOCUS)</td>
<td>n=130</td>
<td>n=1154</td>
<td>n=1284, fail =219</td>
</tr>
<tr>
<td></td>
<td>6th line OxFp (COIN)</td>
<td>n=150</td>
<td>n=159</td>
<td>n=157, fail=929</td>
</tr>
</tbody>
</table>

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


