White Rose Research Online

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Leeds.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/110632/

Version: Accepted Version

Article:
Seligmann, JF, Fisher, D, Smith, CG et al. (2017) Investigating the poor outcomes of BRAF - mutant advanced colorectal cancer: Analysis from 2530 patients in randomised clinical trials. Annals of Oncology, 28 (3). pp. 562-568. ISSN: 0923-7534

https://doi.org/10.1093/annonc/mdw645

© The Author(s) 2016. Published by Oxford University Press on behalf of the European Society for Medical Oncology. All rights reserved. This is a pre-copyedited, author-produced PDF of an article accepted for publication in Annals of Oncology following peer review. The version of record, 'Seligmann, JF, Fisher, D, Smith, CG et al (2017) Investigating the poor outcomes of BRAF - mutant advanced colorectal cancer: Analysis from 2530 patients in randomised clinical trials. Annals of Oncology, 28 (3). pp. 562-568,' is available online at: https://doi.org/10.1093/annonc/mdw645. Uploaded in accordance with the publisher's self-archiving policy.

Reuse
Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

Annals of Oncology Advance Access published December 19, 2016

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

J.F. Seligmann[1], D. Fisher[2], C.G. Smith[3], S.D. Richman[1], F. Elliott[1], S. Brown[4], R. Adams[3], T. Maughan[5], P. Quirke[1], J. Cheadle[3], M. Seymour[1*] and G. Middleton[6*].

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

Affiliations

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

* MTS and GM are joint senior authors

Word count: 3609

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

© The Author 2016. Published by Oxford University Press on behalf of the European Society for Medical Oncology. All rights reserved. For permissions, please email: journals.permissions@oup.com.

Abstract

Background:

To improve strategies for the treatment 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.

Patients and Methods:

2530 aCRC patients were assessed from three randomised trials. End-points were progression free survival (PFS), response rate (RR), disease control rate (DCR), 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. *BRAF*-mutation conferred significantly worse survival independent of associated clinicopathological factors known to be prognostic. Compared with wild-type, *BRAF*-mutant patients treated with first-line OxFU had similar DCR (59.2% vs 72%; adjusted OR=0.76,p=0.24) and PFS (5.7 vs 6.3 months; adjusted HR=1.14, p=0.26). Following progression on first-line chemotherapy, *BRAF*-mutant patients had a markedly shorter P-PS (4.2 vs 9.2 months, adjusted HR=1.69,p<0.001).

Fewer *BRAF*-mutant patients received second-line treatment (33% *vs* 51%, p<0.001), but *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).

Significant clinical heterogeneity within the *BRAF*-mutant population was observed: a proportion (24.3%) had good first-line PFS and P-PS (both >6 months; OS=24.0 months), however 36.5% progressed rapidly through first-line chemotherapy and thereafter, with OS=4.7 months.

Conclusions

BRAF-mutant aCRC confers a markedly worse prognosis independent of associated clinicopathological features. Chemotherapy provides meaningful improvements in outcome throughout treatment lines. Post-progression survival is markedly worse and vigilance is required to ensure appropriate delivery of treatment after first-line progression.

Key words

Colorectal cancer; *BRAF*-mutant; chemotherapy; prognosis

Key messages

We show that BRAF-mutant aCRC patients derive similar relative benefit from chemotherapy as wild-types. The point at which outcomes differ is following progression on first-line chemotherapy. Significant clinical heterogeneity was observed and efforts should be concentrated on identifying BRAF-mutant

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

patients who benefit from chemotherapy, and alternative strategies tested for those who don't.

INTRODUCTION

The V600E activating mutation in *BRAF* (*BRAF*-mutant) is found in the tumours of 8-12% patients with advanced colorectal cancer (aCRC). *BRAF*-mutant aCRC is consistently associated with poor overall survival (OS) and progression free survival (PFS) in case series[1] and randomised controlled trials (RCTs).[2] In a recent RCT of previously untreated aCRC, median OS was 13.4 months in *BRAF*-mutant patients compared with 37.1 months in *RAS* and *BRAF* wild-types.[3]

The mechanism for the poor prognosis is poorly understood, and it is unclear at what point in the aCRC treatment pathway that *BRAF*-mutant outcomes diverge from wild-types; whilst OS is uniformly poor, less impact is seen with PFS compared with wild-types.[4,5] It has been hypothesised that poor outcomes are secondary to intrinisc chemoresistance but there is a paucity of data describing the outcomes of *BRAF*-mutant aCRC with chemotherapy alone, particularly beyond the first-line. This is particularly important as *BRAF*-mutant patients have questionable benefit from anti-epidermal growth factor receptor (anti-EGFR) therapies[6] and *BRAF*-targeted strategies have yet to make clinical impact in aCRC.[7,8]

Importantly previous publications have not performed careful multivariate analysis. This is critical as *BRAF*-mutant aCRC is associated with clinicopathological features which are themselves negative prognostic factors,[9] including defective mismatch repair (dMMR) status[4,10], right sided primary tumour location (PTL)[11] and a high incidence of peritoneal metastases.[12] The observed poor outcomes may instead be driven by such factors so it is essential to prospectively factor this into analyses of outcomes.

This paper provides detailed analysis of the natural history of *BRAF*-mutant aCRC to give more clarity about prognosis and an evidence base to quantitate the benefits of different chemotherapy strategies throughout the treatment pathway.

In a pre-planned analysis we have examined individual patient data from three RCTs to identify points on the treatment pathway at which *BRAF*-mutant outcomes differ from *BRAF* wild-type patients treated with cytotoxic chemotherapy, to assess the impact of potential confounders and to provide clinicians with detailed information of outcomes with various chemotherapy strategies. We analysed 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. Potential confounding factors were prospectively identified, and analyses adjusted accordingly. *BRAF*-status was unknown to clinicians treating patients in each trial, eliminating potential bias.

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

PATIENTS AND METHODS:

Patient population and treatment:

Individual patient data were obtained from selected arms of three large randomised trials (FOCUS [ISRCTN 79877428],[13] COIN [ISRCTN 27286448],[14,15] PICCOLO [ISRCTN 93248876][16,17] to reflect different clinical uses of standard cytotoxic chemotherapy (without targeted therapy) in aCRC (Supplementary Figure 1).

Inclusion criteria for FOCUS and COIN were consistent. 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.[16,18,19]

Statistical analysis

Stata was used (*Release 12 (2011)*,StataCorp). Baseline patient characteristics were compared between *BRAF*-mutant patients (with or without other MEK/AKT pathway mutations) and *BRAF* wild-type patients using two-tailed T-tests, Wilcoxon rank sum tests and Pearson Chi-squared tests.

Endpoints used were OS (time from randomisation to death from any cause), PFS (time from randomisation to first evidence of progression or death); 12-week RECIST response rate (RR), and disease control rate (DCR).[20] Finally, we compared post-progression survival time (P-PS), defined as time from progression to death in those with a progression event, however when date of progression data was unavailable date of last chemotherapy cycle was used instead.

The prognostic influence of *BRAF*-mutant status on survival outcomes 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: WHO performance status (2 vs 0/1); primary tumour resected (yes vs no); 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.

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.

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

RESULTS

Clinicopathological variables associated with *BRAF*-mutant aCRC

BRAF status was available for 787/2135 (36.9%) patients in FOCUS, 1284/1630 (78.8%) in COIN and 459/511 (89.8%) in PICCOLO (Supplementary 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 wild-types to be female, have right-sided PTL, have peritoneal or nodal metastases, but less likely to have lung metastases. *BRAF*-mutant tumours were more likely to have dMMR than wild-type tumours (12.6% vs 3.0%, p<0.001). 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)(Supplementary Figure 2).

The prognostic impact of clinicopathological characteristics associated with *BRAF*-mutant status that may interact with survival were explored in a univariate and multivariate analysis (Supplementary Table 1). 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)(Table 2). Given the demonstrated prognostic effect of clinicopathological factors associated with *BRAF*-mutant status all subsequent analyses are adjusted.

There was no evidence that *BRAF*-mutant patients had inferior OS 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 in the entire population 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 3).

OS was improved in COIN for those who received subsequent second-line chemotherapy compared with those without, regardless of *BRAF*-status (*BRAF*-mutant 16.1 vs 7.8 months [HR=0.56, p=0.005]; wild-type 21.1 vs 11.6 months [HR=0.48, p<0.001]; interaction p=0.66). 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.

The impact of *BRAF*-status on OS for the 459 patients treated with second-line irinotecan was examined in the PICCOLO trial. Whilst OS was shorter for *BRAF*-mutant patients compared with *BRAF* wild-type, the difference did not

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

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 4).

Impact of *BRAF*-status on chemotherapy outcomes: progression free survival, response rates and disease control rates.

In contrast to its marked effect on OS, *BRAF*-mutant status had modest or insignificant impact on the first-line PFS and response endpoints. Although 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), 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 2).

Similarly for patients treated with first-line combination chemotherapy in FOCUS, there were no differences in efficacy endpoints in *BRAF*-mutant compared with *BRAF* wild-type 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 2). There was no evidence of a differential effect of *BRAF*-status according to regimen used (OxFU or IrFU, p=0.26). With first-line single agent 5FU in this trial (n=430), 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).

We examined the impact of chemotherapy-free intervals on PFS in *BRAF*-mutant patients in COIN. In all patients progression events in patients during chemotherapy breaks led to shorter PFS (adjusted HR=1.27 [1.21–1.33], p<0.001).[19] *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* wild-type PFS adjusted HR=1.29 [1.21–1.37], p<0.001; interaction p=0.14)(Supplementary Figure 3).

For patients treated with second-line single-agent irinotecan in PICOLLO there were no significant differences between *BRAF*-mutant to wild-type patients in PFS (3.5 vs 4.0 months, adjusted HR=1.01 [0.69-1.49], p=0.93), RR (5.0% vs. 8.1%, adjusted OR=0.56 [0.13-2.49], p=0.45)) and DCR (42.5% vs. 47.7% (adjusted OR=0.82[0.41-1.62],p=0.57)(Supplementary Table 2).

Outcomes of *BRAF*-mutant patients treated with anti-EGFR agents in COIN and PICCOLO have been previously reported,[9,18] and discussed in the Supplementary Material.

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 wild-types in both first-line trials. In COIN PPS was 3.2 months in *BRAF*-mutant compared with 8.6

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

months in wild-type patients (adjusted HR=1.72 [1.35-2.19], p<0.001). Similarly in FOCUS inferior P-PS 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 2). Combining this data P-PS was inferior in the *BRAF*-mutant compared with the *BRAF*-wt group (3.2 vs 8.6 months, HR=1.72 [1.35-2.19], p<0.001)(Supplementary Figure 5). These marked differences were independent of first-line treatment received (in COIN, OxFU vs OxCap p=0.53, in FOCUS OxFU vs IrFU p=0.91)(data not shown). Finally, 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),

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* wild-type 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), suggesting that it is the *BRAF*-mutation driving the observed poor outcomes (Supplementary Table 3).

To explore the mechanism for inferior first-line P-PS in *BRAF*-mutant patients, we studied uptake of post-progression therapies. 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* wild-type and 3/29 (10.3%) *BRAF*-mutant patients received subsequent salvage therapy (p=0.020).

We then performed an exploratory analysis to ascertain whether the reduction in P-PS in *BRAF*-mutant patients was due to rapid progression after initial first-line benefit, rapid progression in patients who also rapidly progressed through first-line treatment, or both. Table 3 shows the breakdown of patients by mutational status into 4 groups depending upon duration of first-line PFS and subsequent P-PS: good PFS/ P-PS defined as greater than 6 months, poor PFS/P-PS defined as less than 6 months.

Fewer *BRAF*-mut patients had good first-line PFS and good P-PS compared with wild-type patients (24.3% vs 39.3%, p<0.001). Conversely there was a significantly higher proportion of *BRAF*-mutant patients with very poor outcomes (both less than 6 months first-line PFS and P-PS) compared with wild-type patients (36.5% and 21.9% respectively, p<0.001). Thus, around a third of *BRAF*-mutant patients not only fail to obtain useful benefit from first-line therapy but also rapidly progress thereafter. The difference in median survival of *BRAF*-mutant patients between these two groups is 24.0 months to 4.7 months. There were no significant differences in patient demographics between these two groups; however there was a trend towards lower median

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

age in the poor PFS/P-PS compared with the good PFS/P-PS group (61.9 vs 65.2, p=0.07).

Further difference in treatment patterns were observed: 67.5% of *BRAF* wild-type patients with good initial PFS also had a good P-PS compared with 48.4% of the *BRAF*-mutant patients (p<0.001). Whilst 47.4% of *BRAF* wild-type patients in spite of an initial poor first-line PFS went onto have a greater than 6 months P-PS this was the case in just 26.6% of *BRAF*-mutant patients.

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

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. 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.

Within this dataset the poor outlook is not driven by chemoresistance. We observed no difference in the adjusted PFS or DCR between *BRAF*-mutant and wild-type patients receiving first-line chemotherapy. There was also no difference in adjusted PFS or DCR between *BRAF*-mutant and wild-type patients who received second-line irinotecan monotherapy. Results were consistent between both first-line trials, independent of chemotherapy strategy and other standard prognostic factors. OxFU is a commonly used first-line therapy in aCRC, and was the first-line regimen used in the majority of patients analysed herein, and indeed oxaliplatin may provide particular benefits in *BRAF*-mutant patients [3, 21].

Our analyses suggest instead that the point at which outcomes markedly diverge between *BRAF*-mutant and wild-types is following progression on or after benefit from first-line chemotherapy. Further investigation suggested that the observed significantly worse P-PS compared with wild-types may be due to the combined impact of two distinct patterns. Firstly *BRAF*-mutant patients were more likely to rapidly progress through first-line therapy and then subsequently rapidly deteriorate, either too unfit to receive subsequent treatment or progressing through that therapy. Secondly, *BRAF*-mutant patients with an initial good outcome on first-line chemotherapy were more likely to rapidly progress thereafter. Whilst around two-thirds of wild-type patients with good outcomes on initial therapy subsequently survive more than 6 months after progression on first-line chemotherapy this fell to half of *BRAF*-mutant patients.

Although, the study is limited by relative small numbers of *BRAF*-mutant patients compared to wild-types and findings should be interpreted with caution particularly sub-group analyses, the data suggests that a significant proportion of *BRAF*-mutant patients can obtain meaningful benefit from chemotherapy. Thus uniform nihilism about the impact of chemotherapy in deflecting the natural history of *BRAF*-mutant aCRC is unjustified. Furthermore, *BRAF*-mutant patients with disease control can be appropriately counselled about the safety of chemotherapy free intervals even though caution is required in the interpretation of this sub-set analysis. However, post-progression survival after first-line progression is clearly worse in *BRAF*-mutant patients and fewer receive second-line therapy. It is important to emphasise that treating physicians were unaware of *BRAF*-status, so this latter finding is not due to selection bias. Thus, we suggest that extra vigilance is required when treating *BRAF*-mutant patients, to promptly detect initial progression and then rapidly institute second-line therapy in the knowledge that this has the capacity to significantly improve survival.

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

A third of *BRAF*-mutant patients rapidly progress on and then after first-line therapy with no obvious benefit from chemotherapy. These patients drive much of the observed poor outcomes of *BRAF*-mutant aCRC and such aggressive clinical behaviour is what clinicians often have in mind when thinking about *BRAF*-mutant aCRC patients. A biomarker is required to identify such patients who might benefit from an alternative therapeutic strategy, such as targeted therapy. The combination of a *BRAF*-inhibitor dabrafenib, a MEK inhibitor trametinib and an anti-EGFR agent panitumumab demonstrated an unconfirmed response rate of 30%.[22]

Recent transcriptional analyses have sub-divided *BRAF*-mutant aCRC into 2 sub-types with widely differing biology.[23] BM1 tumours constitute one-third of *BRAF*-mutant cancers and are characterised by enrichment of a *KRAS* signature and sensitivity to *BRAF* and *MEK* inhibition. The other two-thirds of *BRAF*-mutant aCRC, the BM2 sub-type, are characterised by accelerated G2/M phase with low ATM with sensitivity to cdk1 inhibition. These checkpoint abnormalities could contribute to chemosensitivity by preventing DNA damage being repaired prior to mitosis. Thus, application of these signatures to these two clinically divergent groups of *BRAF*-mutant patients appears warranted. Furthermore MMR testing should be encouraged in *BRAF*-mutant aCRC patients and entry into RCT testing immunotherapy agents where available.

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 clinicopathological features. However, in some patients chemotherapy does provide meaningful improvements in outcome throughout treatment lines and translational efforts need to be made to identify them and those who appear to derive no benefit from chemotherapy. Post-progression survival is worse in *BRAF*-mutant patients and vigilance is required to ensure the appropriate delivery of treatment after first-line progression.

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

ACKNOWLEDGEMENTS

We are deeply indebted to the patients who participated in this study and to their families and carers. We would like to thank all the staff, past and present, at the Medical Research Council Clinical Trials Unit and the University of Leeds Clinical Trials Unit. We are grateful to staff at all the centres that contributed to the FOCUS, COIN and PICCOLO trials, including clinical management of patients, data collection, and contribution of tumour blocks to each trial biobank.

FUNDING

This work was supported by Cancer Research UK and Yorkshire Cancer Research (no grant numbers apply)

COMPETING INTERESTS

No authors have declared no conflicts of interest

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

REFERENCES

1. 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* 2014; 13(3):164-71
2. Tol J, Nagtegaal ID, Punt CJ,et al. *BRAF* mutation in metastatic colorectal cancer. *N Engl J Med* 2009;361:98-9
3. Cremolini C, Loupakis F, Antoniotti 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):1306-15
4. 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* 2014; 20:5322-30
5. 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* 2011; 29:2675-82
6. Rowland A, Dias MM, Wiese MD, et al. Meta-analysis of *BRAF* mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. *B J Cancer* 2015;112(12):1888-94
7. Kopetz SD, Desai J, Chan E, et al. PLX4032 in metastatic colorectal cancer patients with mutant *BRAF* tumors*. J Clin Oncol* 28:(15s), 2010; abstr.3534.
8. Corcoran RB, Atreya CE, Falchook GS, et al. Combined *BRAF* and MEK inhibition with dabrafenib and trametinib in *BRAF* V600-mutant colorectal cancer. *J Clin Oncol* 2015;33(34):4023-31
9. 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* 2013;15:e711-8
10. 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* 2011;117(20): 4623-32
11. Loupakis F, Yang D, Yau L, et al. Primary tumour location as a prognostic factor in metastatic colorectal cancer. *J Natl Cancer Inst* 2015; 107(3)
12. Franko J, Shi Q, Goldman CD, et al. Treatment of colorectal peritoneal carcinomatosis with systemic chemotherapy: a pooled analysis of north central cancer treatment group phase III trials N9741 and N9841. *J Clin Oncol*. 2011;30(3): 263-7
13. 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* 2007;370:143-52
14. Maughan TS, Adams RA, Smith CG, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011;377:2103-14
15. 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* 2011;12:642-53
16. 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* 2013;14:749-59
17. 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* 2013;49:3507-16
18. 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* 2009;27:5931-7
19. 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* 2013;19:4104-13
20. 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* 2000;92: 205-16
21. Andre T, de Gramont A, Vernery D, et al. Adjuvant Fluoruracil, 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
22. Corcoran RB, Andre T, Yoshino T et al.,Efficacy and circulating tumour DNA analysis of the BRAF inhibitor dabrafanib, MEK inhibitor trametinib and anti-EGFR antibody panitumumab in patients with BRAF V600E-mutated metastatic colorectal cancer. *Annals Oncol* 2016;34(Suppl),(abstr 455))
23. Barras D, Missiaglia E, Wirapati P, et al, BRAF V600E mutant colorectal cancer subtypes based on gene expression. *Clin Cancer Res*. DOI 10.1158/1078-0432.CCR-16-0140

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

Table 1 – Patients characteristics by *BRAF* status

		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 n(%)	**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 missing from comparison*

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

Table 2 – 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 for first-line combination chemotherapy.

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=119	n=1082	n=1072, fail=829	n=836, fail=655
		3.2(1.3-9.6)	8.6(3.8-16.1)	2.03(1.66-2.48),p<0.001	1.72 (1.35-2.19),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.*

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016

Table 3: Breakdown of all patients by PFS and P-PS duration, then proportion of patients in each PFS/P-PS grouping by mutation status.

Grouping by PFS/ P-PS*	All pts (n=2052)** + median survival (IQR)	*BRAF*-mut (n=189) + median survival (IQR)	*BRAF*-wt (n=1862) +median survival	*p-value for proportion by BRAF*	*RAS*-mut (n=890) +median survival	*RAS/RAF*-wt (n=982) + median survival
Good PFS/Good P-PS	779 (38.0%)	46 (24.3%)	733 (39.3%)	p<0.001	314 (35.3)	424 (54.4%)
	25.8 (19.8-34.0)	24.0 (20.6-31.7)	25.9 (19.7-34.1)		24.0 (19.3-31.6)	27.1 (20.1-37.4)
Good PFS/ Poor P-PS	402 (19.5%)	49 (25.9%)	352 (18.9%)	p=0.02	173 (19.4)	180 (18.3%)
	12.6 (9.8-15.7)	11.6 (8.7-14.0)	12.8 (9.8-16.2)		12.6 (9.8-15.1)	13.2 (10.2-18.1)
Poor PFS/ Good P-PS	393 (19.2%)	25 (13.2%)	368 (19.8%)	p=0.03	170 (19.1)	199 (20.2%)
	16.3 (12.3-22.2)	14.4 (11.2-17.7)	16.5 (12.4-22.6)		16.5 (12.4-22.2)	16.4 (12.3-23.6)
Poor PFS/ Poor P-PS	478 (23.3%)	69 (36.5%)	409 (21.9%)	p<0.001	233 (26.2)	179 (18.2%)
	4.9 (2.7-7.3)	4.7 (2.3-7.0)	5.0 (2.9-7.4)		4.9 (2.9-7.2)	5.2 (2.7-7.7)

*Good PFS or P-PS is >6 months; Poor PFS or P-PS is <6mths

***9 pts had BRAF and RAS mut*

Downloaded from http://annonc.oxfordjournals.org/ at University of Birmingham on December 20, 2016