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Original Research

Clinical and exploratory biomarker findings from the MODUL trial (Cohorts 1, 3 and 4) of biomarker-driven maintenance therapy for metastatic colorectal cancer

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Vemurafenib

Abstract ***Purpose:*** MODUL is an adaptable, signal-seeking trial of biomarker-driven maintenance therapy following first-line induction treatment in patients with metastatic colorectal cancer (mCRC). We report findings from Cohorts 1 ($BRAF^{mut}$), 3 (human epidermal growth factor 2 [HER2]+) and 4 (HER2−/high microsatellite instability, HER2−/microsatellite stable [MSS]/$BRAF^{wt}$ or HER2−/MSS/$BRAF^{mut}$/RAS^{mut}).
Methods: Patients with unresectable, previously untreated mCRC without disease progression following standard induction treatment (5-fluorouracil/leucovorin [5-FU/LV] plus oxaliplatin plus bevacizumab) were randomly assigned to control (fluoropyrimidine plus bevacizumab) or

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cohort-specific experimental maintenance therapy (Cohort 1: vemurafenib plus cetuximab plus 5-FU/LV; Cohort 3: capecitabine plus trastuzumab plus pertuzumab; Cohort 4: cobimetinib plus atezolizumab). The primary efficacy end-point was progression-free survival (PFS).
Results: Cohorts 1, 3 and 4 did not reach target sample size because of early study closure. In Cohort 1 ($n = 60$), PFS did not differ between treatment arms (hazard ratio, 0.95; 95% confidence intervals 0.50–1.82; $P = 0.872$). However, Cohort 1 exploratory biomarker data showed preferential selection for mitogen-activated protein kinase (MAPK) pathway mutations (mainly *KRAS, NRAS, MAP2K1* or *BRAF*) in the experimental arm but not the control arm. In Cohort 3 ($n = 5$), PFS ranged from 3.6 to 14.7 months versus 4.0 to 5.4 months in the experimental and control arms, respectively. In Cohort 4 ($n = 99$), PFS was shorter in the experimental arm (hazard ratio, 1.44; 95% confidence intervals 0.90–2.29; P = 0.128).
Conclusions: Vemurafenib plus cetuximab plus 5-FU/LV warrants further investigation as first-line maintenance treatment for $BRAF^{mut}$ mCRC. MAPK-pathway emergent genomic alterations may offer novel therapeutic opportunities in $BRAF^{mut}$ mCRC. Cobimetinib plus atezolizumab had an unfavourable benefit:risk ratio in HER2–/MSS/$BRAF^{wt}$ mCRC. New strategies are required to increase the susceptibility of MSS mCRC to immunotherapy.
Trial registration: ClinicalTrials.gov: NCT02291289.

1. Introduction

Approximately 8–9% of colorectal cancers are characterised by the presence of *BRAF* mutations ($BRAF^{mut}$) [1–3], most commonly V600E substitutions, which lead to constitutive activation of mitogen-activated protein kinase (MAPK) signalling [4]. The presence of $BRAF^{mut}$ in metastatic colorectal cancer (mCRC) is a negative prognostic marker and is associated with a poor response to standard chemotherapy [1–3]. Current first-line treatment options for patients with $BRAF^{mut}$ mCRC include oxaliplatin- or irinotecan-based chemotherapy with or without bevacizumab [5]. Single-agent BRAF inhibitors have limited efficacy in $BRAF^{mut}$ mCRC [6–8]. Preclinical studies indicate that this may be a result of rapid reactivation of the MAPK pathway via the epidermal growth factor receptor (EGFR) [9,10], suggesting that combination therapy may be required for these tumours. It has since been demonstrated that BRAF inhibitors have markedly improved anti-tumour efficacy in patients with $BRAF^{mut}$ mCRC when combined with an EGFR inhibitor [11–13]. The combination of BRAF inhibitor encorafenib plus cetuximab has recently been recognised as a new standard of care for previously treated patients with $BRAF^{V600E}$ mCRC [14]. However, acquired resistance has been observed in patients with $BRAF^{mut}$ mCRC treated with BRAF inhibitor combinations, with reported evolution of MAPK signalling activation mutations in individual patients [12,15].

MODUL is an adaptable, signal-seeking trial which employed a biomarker-based approach to select novel regimens for maintenance therapy following standard first-line induction treatment in patients with mCRC [16]. In MODUL, patients with unresectable, previously untreated mCRC received standard induction treatment [5-fluorouracil/leucovorin (5-FU/LV) plus oxaliplatin (FOLFOX) plus bevacizumab] followed by randomisation to experimental or control maintenance treatment in one of four biomarker-driven cohorts. We report the efficacy, safety and exploratory biomarker findings from Cohort 1 of the MODUL trial, in which patients with $BRAF^{mut}$ mCRC received maintenance therapy with vemurafenib plus cetuximab plus 5-FU/LV (experimental arm) or standard maintenance therapy with a fluoropyrimidine plus bevacizumab (control arm). We also briefly report the findings from Cohort 3, which evaluated maintenance treatment with capecitabine plus trastuzumab and pertuzumab in patients with human epidermal growth factor receptor 2-positive (HER2+) mCRC and Cohort 4, which evaluated maintenance treatment with cobimetinib plus atezolizumab in patients with HER2–/high microsatellite instability [MSI-H], HER2–/microsatellite stable (MSS)/*BRAF* wildtype ($BRAF^{wt}$) or HER2–/MSS/$BRAF^{mut}$/RAS^{mut} mCRC. Findings from Cohort 2 with $BRAF^{wt}$ mCRC are reported separately [17].

2. Patients and methods

2.1. Study design

MODUL was a randomised, multicentre, active-controlled, open-label, parallel-group clinical trial of biomarker-driven maintenance treatment for first-line mCRC conducted in Europe, Asia, Africa and South America (Fig. 1a; ClinicalTrials.gov: NCT02291289). Eligible patients received standard induction treatment over approximately 4 months. Within 3 weeks of

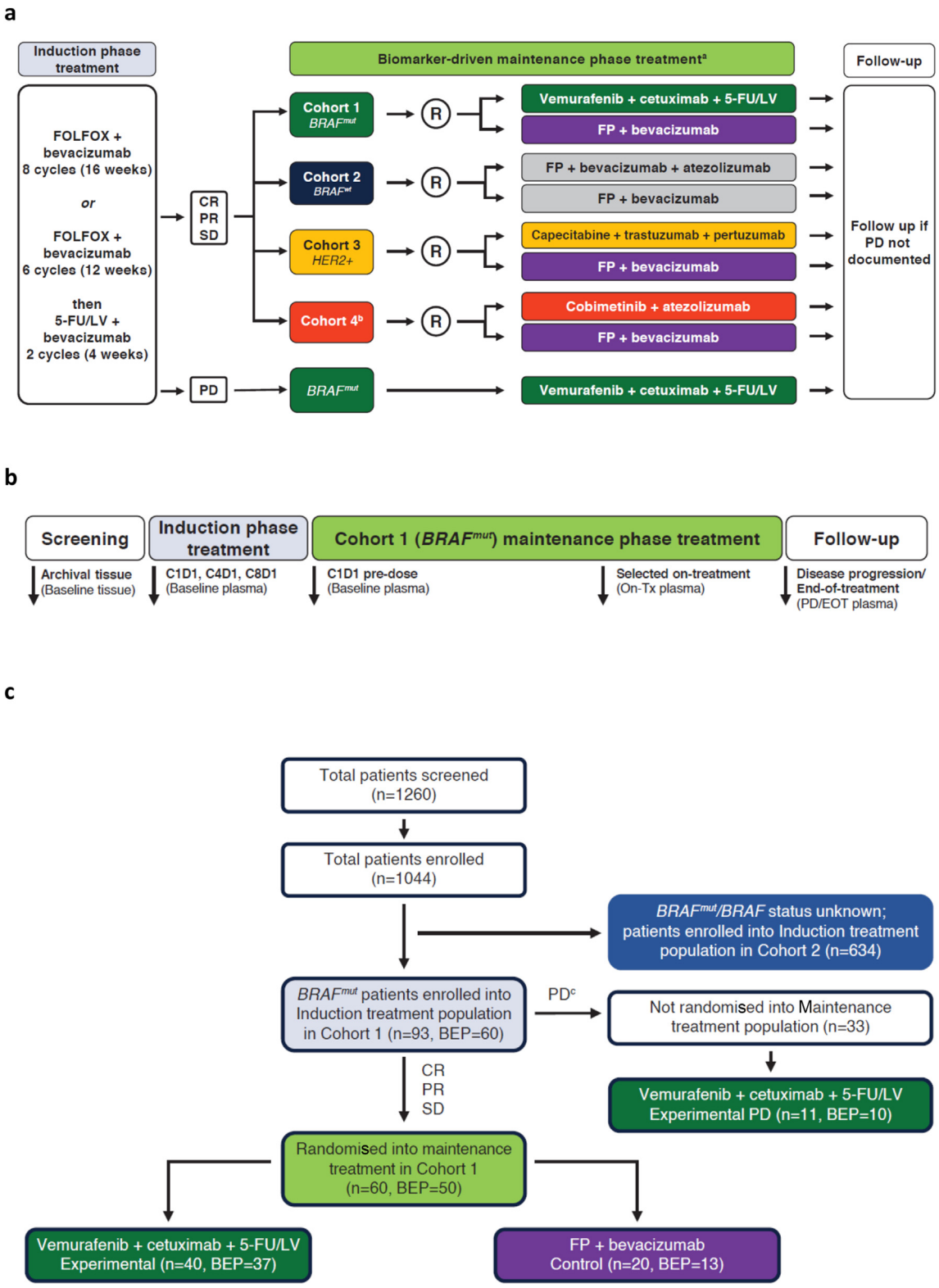

Fig. 1. **(a)** MODUL study design, **(b)** Cohort 1 biomarker sample collection and **(c)** Cohort 1 patient disposition and biomarker evaluable population. 5-FU/LV, 5-fluorouracil/leucovorin; BEP, biomarker evaluable population; CR, complete response; CXDX, cycle x day x; FOLFOX, 5-fluorouracil/leucovorin + oxaliplatin; FP, fluoropyrimidine; HER2+, human epidermal growth factor receptor 2-positive; PD, progressive disease; PR, partial response; R, randomisation; SD, stable disease. [a]Stratification factors: Cohort 1—geographic region (Europe, Americas, Africa or Asia), induction treatment response (CR or PR versus SD) and *BRAF*^{mut} (V600E variant); Cohort 3—induction treatment response (CR or PR versus SD) and HER2 immunohistochemistry score (0, 1+ or 2+ versus 3+); Cohort 4—geographic region (Europe versus rest of the world), induction treatment response (CR or PR versus SD), microsatellite stability (MSI-

completing induction treatment, patients who had not progressed and were unresectable were assigned to one of four maintenance treatment cohorts based on the biomarker profile of their primary tumour: Cohort 1 ($BRAF^{mut}$); Cohort 2 ($BRAF^{wt}$); Cohort 3 (HER2+); Cohort 4 (HER2−/MSI-H, HER2−/MSS/$BRAF^{wt}$ or HER2−/MSS/$BRAF^{mut}$/RAS^{mut}). Patients were then randomised (2:1 ratio) to either experimental or control maintenance treatment within their assigned cohort by an independent interactive voice- or web-based response system using a dynamic randomisation algorithm and stratified by cohort-specific factors (see Fig. 1a for details). Findings for Cohorts 1, 3 and 4 are described herein. Cohort 2 is reported separately [17].

An independent data monitoring committee was responsible for study conduct and for the regular review of safety and survival data. Based on a recommendation from the independent data monitoring committee following a review of safety data, accrual into Cohort 4 was suspended on 12th February 2018 due to an unfavourable benefit-risk evaluation. Overall study enrolment was also suspended at that time and remained permanently closed to further enrolment. Consequently, Cohorts 1, 3 and 4 did not reach their target sample size.

All study procedures were in accordance with the International Conference on Harmonisation E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki or local laws and regulations. All patients provided written informed consent to participate in the study. The study protocol, informed consent forms and relevant supporting information were approved by the Institutional Review Board/Ethics Committee before the study was initiated. The study protocol is available at: https://clinicaltrials.gov/ProvidedDocs/89/NCT02291289/Prot_000.pdf.

2.2. Patients

The study population included patients 18 years of age or older with histologically confirmed, measurable, unresectable mCRC who had received no prior chemotherapy for metastatic disease and had an Eastern Cooperative Oncology Group (ECOG) performance status of $\leq$2. Biomarker status of the primary tumour was determined by next-generation sequencing at a sponsor-approved laboratory or Foundation Medicine to guide maintenance treatment cohort assignment. The following biomarkers were considered: HER2 (HER2+ defined as gene copy number $\geq$5 by central HER2 fluorescence in situ hybridisation assay); microsatellite stability (MSS or MSI-H); *BRAF* mutation status ($BRAF^{mut}$ or $BRAF^{wt}$); *KRAS* and *NRAS* mutation status.

2.3. Procedures

First-line induction treatment was specified to be either eight 2-week cycles of FOLFOX plus bevacizumab or six 2-week cycles of FOLFOX plus bevacizumab, followed by two 2-week cycles of 5-FU/LV plus bevacizumab (Fig. 1a). For maintenance therapy, patients randomised to the control arms received a fluoropyrimidine plus bevacizumab in 2- or 3-week treatment cycles depending on the fluoropyrimidine used (5-FU 1600–2400 mg/m^2 46-h intravenous (IV) infusion and LV 400 mg/m^2 2-h IV infusion plus bevacizumab 5 mg/kg 15–30-min IV infusion every 2 weeks or capecitabine 1000 mg/m^2 twice daily orally on days 1–14 every 21 days plus bevacizumab 5 mg/kg 15–30-min IV infusion every 2 weeks). For Cohort 1, patients randomised to the experimental arm received vemurafenib (960 mg twice daily orally) plus cetuximab (500 mg/m^2 IV infusion every 2 weeks) plus 5-FU/LV (5-FU 1600–2400 mg/m^2 46-h IV infusion and LV 400 mg/m^2 2-h IV infusion every 2 weeks). Patients whose primary tumour was $BRAF^{mut}$ with an induction treatment response of progressive disease (PD) were not qualified for randomisation but eligible for second-line treatment with the Cohort 1 experimental treatment regimen (Fig. 1a). No formal study objectives were planned for these early progressors. They were, therefore, excluded from the primary efficacy analysis, but included in the exploratory genomic analysis of Cohort 1. For Cohort 3, patients randomised to the experimental arm received capecitabine (1000 mg/m^2 twice daily orally on days 1–14 every 21 days) plus trastuzumab (8 mg/kg loading dose IV infusion then 6 mg/kg every 3 weeks) plus pertuzumab (840 mg loading dose IV infusion then 420 mg every 3 weeks). For Cohort 4, patients randomised to the experimental arm received cobimetinib (60 mg once daily orally on days 1–21 every 28 days) plus atezolizumab (840 mg IV infusion every 2 weeks). Patients in the experimental arm of Cohort 4 alone were allowed to continue maintenance treatment after a first tumour assessment showing progression as long as they had evidence of clinical benefit and had no signs or symptoms of PD.

2.4. Outcomes

The primary efficacy end-point was progression-free survival (PFS) per Response Evaluation Criteria in Solid Tumours (RECIST; version v1.1) within each cohort. Secondary efficacy end-points were overall

H versus MSS) and RAS status (wildtype *KRAS* and *NRAS* versus mutant *KRAS* and/or *NRAS*). [b]HER2−/MSI-H, HER2−/MSS/$BRAF^{wt}$ or HER2−/MSS/$BRAF^{mut}$/RAS^{mut}. [c]Eleven patients completed induction treatment but were not randomised and 24 patients discontinued all treatment during the induction treatment phase. Main reasons for not being randomised into the Maintenance treatment population were disease progression, physician decision and adverse events.

survival (OS); overall response rate (ORR); disease control rate (DCR); time to treatment response; duration of response and change in ECOG performance status. Definitions for efficacy end-points are provided in Table A.1.

Treatment-emergent adverse events (TEAEs) were summarised by grade, relationship to treatment, seriousness and fatal outcome for the maintenance treatment phase, defined as the date of first maintenance treatment administration until 30 days from the last day of maintenance treatment. TEAEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). TEAEs of special interest for vemurafenib and bevacizumab were identified using a standardised MedDRA query-based search methodology.

2.5. Tissue and circulating tumour DNA sample collection

In Cohort 1, ten formalin-fixed paraffin-embedded section slides were obtained from archival primary tumour samples from initial diagnosis, and 2.5–10 ml of plasma per biomarker evaluable patient were collected per time-point (Fig. 1b). Baseline samples were defined as any archival tissue sample and any plasma sample collected prior to maintenance treatment. Baseline plasma samples were collected mostly on day 1 of cycle 1 (C1D1) of the induction treatment phase or, if not evaluable, during cycle 4 or cycle 8 of induction treatment or pre-dose C1D1 of the maintenance treatment phase. Acquired resistance was assessed in plasma samples collected at selected time-points during maintenance treatment, at PD, or at the last available plasma sample if PD was not documented, defined as end of treatment (EOT).

2.6. Exploratory genomic analysis

For Cohort 1 patients, an exploratory genomic analysis was performed on archival tissue and selected plasma samples. The genomic landscape was compared at baseline and post-maintenance therapy using plasma samples collected on treatment, at PD or EOT. Samples were submitted to a Clinical Laboratory Improvement Amendments-certified, New York State-accredited and College of American Pathologists-accredited laboratory (Foundation Medicine) for next-generation sequencing-based genomic profiling of extracted DNA from tissue and plasma using FoundationOne CDx (F1CDx; Foundation Medicine) [18] and FoundationOne Liquid CDx (F1LCDx; Foundation Medicine) [19], respectively. Genomic DNA was extracted from tissue by digestion in a proteinase K buffer for 12–24 h followed by purification with the Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Promega) and circulating cell-free DNA (cfDNA) extracted from plasma by King-Fisher™ Flex Magnetic Particle Processor (ThermoFisher Scientific). Targeted high-throughput hybridisation-based capture technology is used for the detection of substitutions, insertion and deletion alterations, and copy number alterations in 324 (F1CDx) or 311 (F1LCDx) genes and select gene rearrangements, as well as genomic signatures, including microsatellite instability and tumour mutational burden. Tumour mutational burden was calculated as the number of somatic base substitutions and short insertions and deletions identified from coding regions within the FoundationOne panel (filtering out known or likely oncogenic driver mutations to reduce bias). Variant allele frequency (VAF) is the percent of total reads at a given allele that indicate the presence of a given variant. Only variants which are known or likely impactful were considered; variants of unknown significance were

Table 1
Baseline characteristics at randomisation: Cohort 1 (first-line *BRAF*^{mut} patients).

	Vemurafenib + cetuximab + 5-FU/LV (n = 40)	Fluoropyrimidine + bevacizumab (n = 20)
Median age, years (range)	61 (35–77)	59 (35–78)
Sex, *n* (%)		
Male	16 (40.0)	10 (50.0)
Female	24 (60.0)	10 (50.0)
Geographic location, *n* (%)		
Europe	36 (90.0)	19 (95.0)
Americas	2 (5.0)	0
Asia	2 (5.0)	1 (5.0)
Response at end of induction treatment, *n* (%)		
CR/PR	25 (62.5)	14 (70.0)
SD	15 (37.5)	5 (25.0)
Other	0	1 (2.5)[a]
ECOG performance status, *n* (%)		
0	21 (52.5)	11 (55.0)
1	19 (47.5)	9 (45.0)
Cancer type, *n* (%)		
Colon	38 (95.0)	19 (95.0)
Rectal	2 (5.0)	1 (5.0)
Sites of metastatic disease, *n* (%)		
Liver	24 (60.0)	16 (80.0)
Lung	16 (40.0)	4 (20.0)
Initial diagnosis, *n* (%)	n = 40	n = 19
Synchronous	37 (92.5)	19 (100)
Metachronous	3 (7.5)	0
Baseline biomarker status		
RAS mutation status, *n* (%)		
Wildtype	40 (100.0)	19 (95.0)
Missing	0	1 (5.0)
Microsatellite stability status, *n* (%)		
MSI-H	3 (7.5)	1 (5.0)
MSS	36 (90.0)	17 (85.0)
Missing	1 (2.5)	2 (10.0)
HER2 overexpression, *n* (%)		
HER2-negative	12 (30.0)	8 (40.0)
Missing	28 (70.0)	12 (60.0)

5-FU/LV, 5-fluorouracil/leucovorin; CR, complete response; ECOG, Eastern Cooperative Oncology Group; MSI-H, high microsatellite instability; MSS, microsatellite stable; PR, partial response; SD, stable disease.

[a] One patient with progressive disease at the end of induction therapy was randomised and treated in the control arm.

removed from analysis. Variant impact was assessed by standard Foundation Medicine criteria [19]. Known short variants and copy number alterations were those known to be recurrent somatic, while likely impactful short variants were those that disrupt tumour suppressor genes or are in known hotspot regions. Known rearrangements were those involving known fusion partners, or other known functional events, while likely impactful rearrangements were those that disrupt tumour suppressor genes or other likely functional events.

2.7. Statistical analysis

Sample size for each cohort was calculated based on assumptions of the primary study end-point (PFS) within the cohort population (Table A.2), and a primary analysis was planned once the target number of PFS events had been reached. However, the study was closed to further enrolment before Cohorts 1, 3 and 4 had reached their planned sample size. The primary analyses, rather than being event-driven as originally planned, were based instead on data collected until the clinical cut-off date of 31st May 2019.

For Cohorts 1 and 4, the primary population for the analysis of efficacy was the maintenance phase population, defined as all randomised patients. All formal statistical tests for the primary end-point (PFS) were two-sided and performed with an alpha of 5%. All other reported *P*-values are considered descriptive only. Time-to-event end-points were compared between experimental and control arms using an unstratified log-rank test and estimated for each arm using the Kaplan–Meier product-limit method. The Brookmeyer–Crowley method was used to compute 95% confidence intervals (CIs). An unadjusted Cox regression model was used to estimate hazard ratios and corresponding 95% CIs. ORR and DCR were summarised and presented with 95% Clopper–Pearson CIs. For Cohort 1, a sensitivity analysis of PFS without censoring for patients who underwent colorectal surgery with palliative or curative intent during maintenance therapy was performed. PFS and OS analyses were also repeated for predefined subgroups. For Cohort 3, analyses were limited to individual patient data due to low patient numbers.

For the analysis of genomic data in Cohort 1, gene set enrichment analysis was performed using R (version 4.1.1.) with the Molecular Signatures Database (MsigDB; version 7.4) Reactome collection gene set [20] using Fisher's Exact Test on genes mutated or not in each arm. *P*-values were two-sided and adjusted using the Benjamini-Hochberg method to account for multiple hypothesis testing.

3. Data availability

Qualified researchers may request access to individual patient-level data through the clinical study data request platform (https://vivli.org). Further details on Roche's criteria for eligible studies are available here (https://vivli.org/members/ourmembers). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see: https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm.

Table 2
Overview of efficacy outcomes: Cohort 1 (first-line $BRAF^{mut}$ patients).

End-point	Vemurafenib + cetuximab + 5-FU/LV (n = 40)		Fluoropyrimidine + bevacizumab (n = 20)
Median duration of follow-up, months (range)	16.4 (3.0–41.2)		16.8 (4.1–35.9)
Median progression-free survival, months (95% CI)	10.0 (7.7–12.6)		11.6 (3.6–15.7)
Hazard ratio (95% CI)		0.95 (0.50–1.82)	
Log-rank test *P*-value[a]		0.872	
Median overall survival, months (95% CI)	24.0 (16.0–40.5)		21.3 (7.9–27.0)
Hazard ratio (95% CI)		0.69 (0.34–1.38)	
Log-rank test *P*-value[a]		0.287	
Overall response rate, n (%)	20 (50.0)		5 (25.0)
(95% CI)	(33.8–66.2)		(8.7–49.1)
Chi-squared test *P*-value (two-sided)		0.064	
Disease control rate, n (%)	36 (90.0)		15 (75.0)
(95% CI)	(76.3–97.2)		(50.9–91.3)
Chi-squared test *P*-value (two-sided)		0.125	
Median duration of response, months (95% CI)	11.5 (7.7–21.5)		8.7 (5.4–19.0)
Log-rank test *P*-value[a]		0.421	
Median time to response, months (range)	3.9 (1.2–29.7)		5.6 (1.4–8.0)
ECOG performance status from baseline to end of maintenance treatment phase, *n* (%)			
Improved	4 (10.0)		1 (5.0)
Improved or stayed the same	30 (75.0)		17 (85.0)

5-FU/LV, 5-fluorouracil/leucovorin; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group.
[a] *P*-value (two-sided) obtained from an unstratified log-rank test.

4. Results

4.1. Patients

Cohort 1 ($BRAF^{mut}$): Of 1260 screened patients across the MODUL study cohorts, 1044 patients were enrolled between 17th April 2015 and 5th July 2019. A total of 93 patients (8.9%) had $BRAF^{mut}$ tumours and received induction treatment in Cohort 1. Of these, 60 patients were randomised to receive maintenance treatment (vemurafenib plus cetuximab plus 5-FU/LV, $n = 40$; fluoropyrimidine plus bevacizumab, $n = 20$; Fig. 1c). Baseline characteristics of patients in Cohort 1 are shown in Table 1. With the exception of a higher rate of lung metastases in the experimental arm (40.0% versus 20.0%), there were no clinically relevant imbalances between the experimental and control arms in terms of geographic location, age, sex, cancer type, initial diagnosis, ECOG performance status or responses at the end of induction treatment. Tumour biomarker status at the time of randomisation to maintenance treatment was also well balanced between treatment arms. Over 90% of patients had MSS tumours and 100% had RAS^{wt} tumours in both arms.

Cohort 3 (HER2+): Five patients completed induction treatment and were randomised to maintenance treatment (capecitabine plus trastuzumab plus pertuzumab, $n = 3$; fluoropyrimidine plus bevacizumab, $n = 2$). There were 3 women and 2 men with ages ranging from 37 to 56 years.

Cohort 4 (HER2−/MSI-H, HER2−/MSS/$BRAF^{wt}$, or HER2−/MSS/$BRAF^{mut}$/RAS^{mut}): 99 patients completed induction treatment and were randomised to maintenance treatment (cobimetinib plus atezolizumab, $n = 65$; fluoropyrimidine plus bevacizumab $n = 34$). Baseline characteristics of patients in Cohort 4 are shown in Table A.3.

4.2. Efficacy

Cohort 1 ($BRAF^{mut}$): The median duration of maintenance treatment was 36 weeks (range, 2−180) in the experimental arm and 21 weeks (range, 6−99) in the control arm. At an overall median follow-up of 16.4 months (range, 3.0−41.2), median PFS was not improved in the experimental arm versus control arm (hazard ratio, 0.95; 95% CI 0.50−1.82; $P = 0.872$), although median OS appeared to be numerically (but not statistically) longer in the experimental arm (hazard ratio, 0.69; 95% CI 0.34−1.38; $P = 0.287$; Figure A1). ORR and DCR were numerically higher in the experimental arm, although other secondary end-points were similar in the experimental versus control arms (Table 2). Preplanned PFS and OS subgroup analyses were limited by low patient numbers (data not shown). PFS was also not improved in a sensitivity analysis without censoring for colorectal cancer surgery (hazard ratio, 0.79; 95% CI 0.44−1.41; $P = 0.432$).

Cohort 3 (HER2+): PFS ranged from 3.6 to 14.7 months (censored observation) in the experimental arm ($n = 3$) compared with 4.0 and 5.4 months in the control arm ($n = 2$). Apart from one censored observation in the experimental arm, all primary end-point events were PD.

Cohort 4 (HER2−/MSI-H, HER2−/MSS/$BRAF^{wt}$ or HER2−/MSS/$BRAF^{mut}$/RAS^{mut}): After an overall median follow-up of 15.0 months (range, 0.6−20.9), the experimental arm was associated with a shorter PFS (hazard ratio, 1.44; 95% CI 0.90−2.29; $P = 0.128$) and OS (hazard ratio, 1.35, 95% CI: 0.67−2.73; $P = 0.406$) than the control arm (Table A.4).

4.3. Safety

Cohort 1 ($BRAF^{mut}$): Almost all patients experienced at least one TEAE: 40 (100.0%) patients in the experimental arm and 17 (94.4%) patients in the control arm (Table A.5). The most common all-grade TEAEs occurring in >10% of patients were as would be expected in this setting: patients receiving vemurafenib plus cetuximab plus 5-FU/LV experienced higher rates of arthralgia, nausea, diarrhoea, rash and other skin toxicities than those receiving fluoropyrimidine plus bevacizumab. More than half (60.0%) of experimental arm patients experienced grade ≥3 adverse events versus 27.8% of control arm patients. Five patients (12.5%) in the experimental arm and one patient (5.6%) in the control arm experienced grade 4 adverse events. The proportion of patients experiencing serious adverse events (SAEs) or treatment-related SAEs was numerically higher in the experimental arm (37.5%, related SAEs: 12.5%) versus control arm (27.8%, related SAEs: 11.1%). At the primary analysis cut-off date, no patients had died during the maintenance treatment phase. The proportion of patients discontinuing any study drug due to an adverse event was similar in both arms (experimental: 10.0%, control: 11.1%). TEAEs of special interest were identified in 1 patient for vemurafenib (grade ≥3 photosensitivity) and 5 patients for bevacizumab (experimental: grade ≥3 post-procedural haemorrhage, $n = 1$; grade ≥3 hypertension, $n = 1$; grade ≥3 pulmonary embolism; $n = 1$; control: abdominal wall abscess, $n = 1$; implant site thrombosis, $n = 1$). Laboratory parameters, vital signs and electrocardiogram results were consistent with the known effects of study treatments.

Cohort 3 (HER2+): None of the patients experienced an SAE or adverse event leading to study drug withdrawal during maintenance treatment.

Cohort 4 (HER2−/MSI-H, HER2−/MSS/$BRAF^{wt}$, or HER2−/MSS/$BRAF^{mut}$/RAS^{mut}): A higher proportion of patients in the experimental arm versus control arm experienced any TEAE (98.4% versus 88.2%), grade ≥3

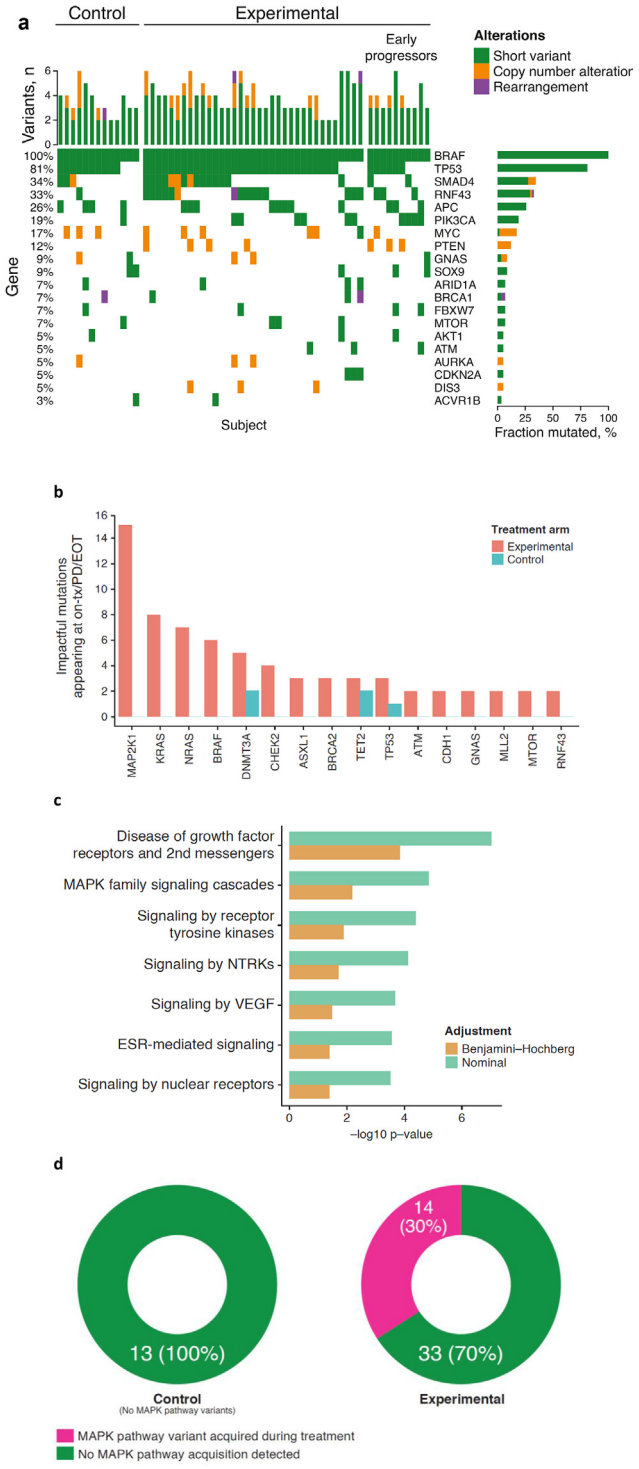

Fig. 2. Acquired mutations in the MAPK pathway genes are the most prevalent in Cohort 1 patients treated with a BRAF inhibitor-based combination (vemurafenib plus cetuximab plus 5-fluorouracil/leucovorin). Impactful somatic variants (known and likely) detected in the biomarker evaluable population of Cohort 1 before and during treatment. **(a)** Baseline tumour variant status in the 20 genes most frequently mutated in Cohort 1. Variants were identified from FoundationOneCDx testing of tissue biopsy samples taken at the start of the study. Short variants are substitutions or short insertion/deletions, copy number variants are whole-gene deletions or amplifications of at least n = 4, and rearrangements are all other variants. Note: 2 patients in the experimental arm did not have tissue FoundationOneCDx (F1CDx) data and, therefore, are absent from this analysis. **(b)** Counts of impactful somatic variants appearing post-treatment of the maintenance phase, as detected by FoundationOne Liquid CDx testing, compared to archival tissue or plasma collected prior to maintenance phase treatment. Genes shown are those that were observed to be the most frequently mutated. **(c)** Gene set enrichment analysis testing of acquired variant trends. All known/likely impactful variants acquired in the study were tested for significant association with the gene sets of the Reactome collection [20]. Nominal and Benjamini-Hochberg adjusted two-sided p-values are shown for enrichment tests. Gene sets shown are those with adjusted *P*-values <0.1. **(d)** Depiction of acquired MAPK pathway mutations post-maintenance phase treatment in the control and experimental arms. Note: Cohort 1 experimental maintenance therapy was vemurafenib plus cetuximab plus 5-fluorouracil/leucovorin, and control maintenance therapy was fluoropyrimidine plus bevacizumab. EOT, end of treatment; MAPK, mitogen-activated protein kinase; on-Tx, on treatment; PD, progressive disease.

TEAEs (76.6% versus 17.6%), SAEs (40.6% versus 8.8%) and TEAEs with fatal outcome (4.7% versus 0%). Key grade ≥3 TEAEs with a higher incidence in the experimental arm versus control arm were increased blood creatine phosphokinase (23.4% versus 0%), diarrhoea (9.4% versus 0%) and neutropenia (9.4% versus 0%) (Table A.6).

4.4. Cohort 1 ($BRAF^{mut}$): exploratory genomic analysis

The biomarker evaluable population in Cohort 1 included 50 patients randomised to maintenance treatment (vemurafenib plus cetuximab plus 5-FU/LV, $n = 37$; fluoropyrimidine plus bevacizumab, $n = 13$) plus 10 early progressors during induction treatment who received vemurafenib plus cetuximab plus 5-FU/LV as second-line therapy (Fig. 1c). Next-generation sequencing (tissue and plasma samples) was performed in all 60 patients.

At baseline, all patients had $BRAF^{V600E}$ mutations and most had *TP53* mutations (Fig. 2a). In general, patients enrolled into the control and experimental arms had similar genomic landscapes at baseline. Variants detected in tissue versus plasma at C1D1 of induction treatment were mostly overlapping (purple boxes in Figure A2). Where discrepancies were observed (red or blue boxes in Figure A2), the fraction of variants seen only in tissue (15%) or only in plasma (13%) were generally balanced.

Impactful variants, detected in plasma cfDNA, that were acquired between baseline (tissue or pre-induction or pre-maintenance plasma) and any point after initiation of maintenance treatment (including clinical progression and EOT plasma) were counted for each arm and listed (Fig. 2b). The experimental arm showed more acquired mutations per patient (mean 6.3) than the control arm (mean 5.0). Importantly, the most prevalent

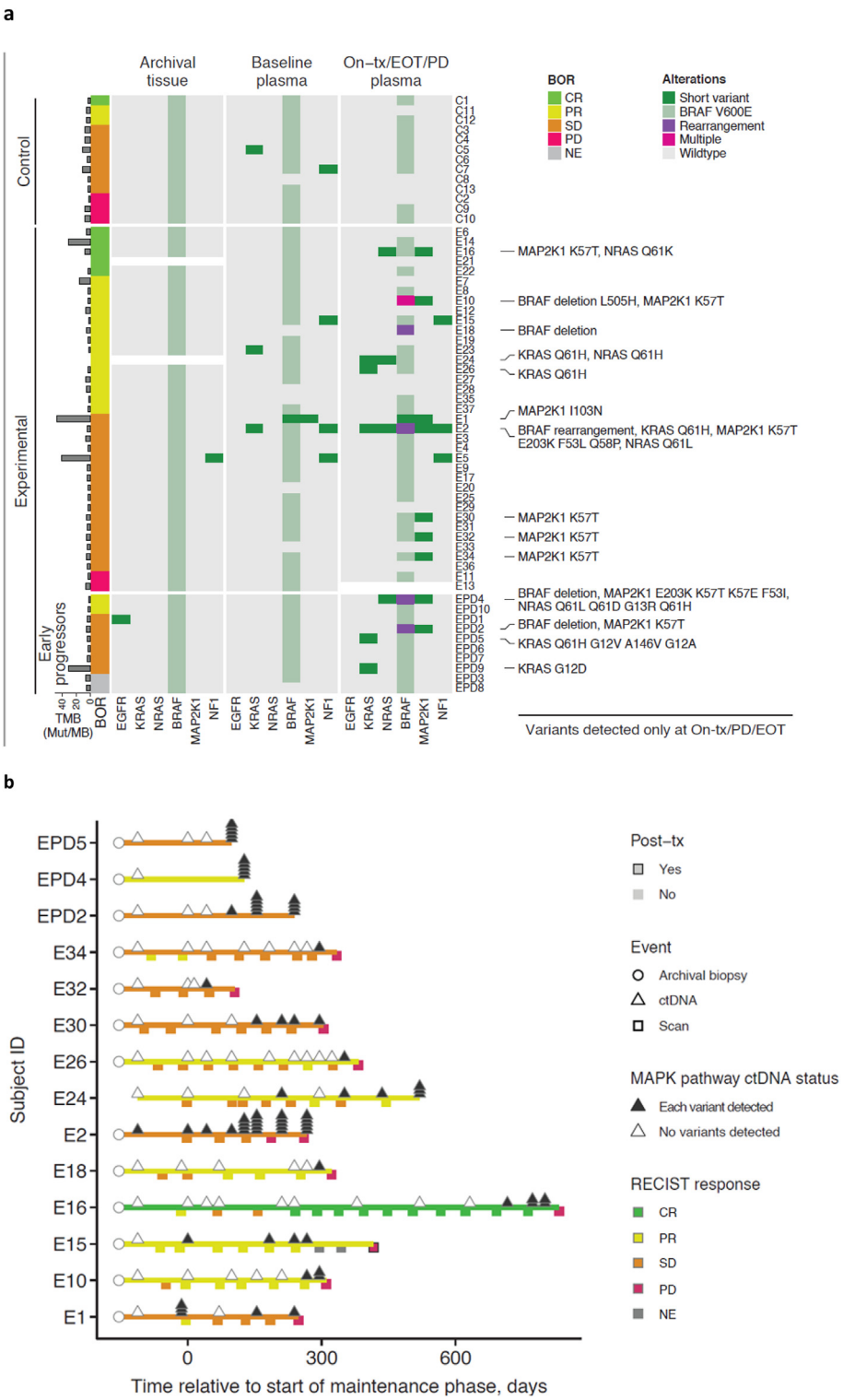

Fig. 3. **(a)** Alterations in MAPK pathway genes were selectively enriched in patients following BRAF inhibitor-based combination therapy (experimental: vemurafenib plus cetuximab plus 5-fluorouracil/leucovorin) but not anti-VEGF-based therapy (control: fluoropyrimidine plus bevacizumab) in $BRAF^{mut}$ metastatic colorectal cancer. Impactful MAPK pathway variants detected in patients at three phases: in tumour tissue biopsies at or before the start of the study (Archival); in plasma collected before or at C1D1 of maintenance treatment (Baseline); or in plasma post-maintenance phase treatment (On-Tx/EOT/PD). White cells indicate that there was no sample available for testing. Variants labelled are those appearing only in the final phase. Tumour mutational burden (TMB) was calculated from variants observed in baseline tumour samples. BOR is RECIST-confirmed. *BRAF* variants are indicated in light green if V600E, or dark green if any other *BRAF* variant was detected. **(b)** Early detection of molecular progression is depicted across patients who acquired MAPK pathway mutations post-maintenance treatment ($n = 14$). Impactful MAPK pathway variants and RECIST-confirmed response over time

acquired mutations were in genes primarily in the MAPK pathway. *MAP2K1* was the most prevalent, with 15 variants observed in the experimental arm only. These data suggest that, with vemurafenib plus cetuximab plus 5-FU/LV maintenance treatment, there was preferential selection for mutations in the MAPK pathway. The Reactome gene set collection was queried for significant over-representation of variants in each arm (Fig. 2c). MAPK pathway was a top hit, enriched specifically in the experimental arm. Overall, no control arm patients versus 14/47 (30%) experimental arm patients exhibited acquired MAPK pathway mutations (Fig. 2d).

Potential drivers of resistance to vemurafenib plus cetuximab plus 5-FU/LV (i.e. genomic alterations found in cfDNA post-maintenance treatment) mainly involved effectors of the MAPK pathway, namely MAP2K1, KRAS, NRAS or BRAF (Fig. 3a; Figure A3). Alterations including *MAP2K1* (K57T/E, F53I, E203K, I103M, Q58P), *KRAS* (Q61H and G12D), *NRAS* (Q61K/L/D/H and G13R) and *BRAF* (deletions, rearrangement, L597Q, L505H) were observed post-maintenance treatment in the experimental arm but not the control arm. Notably, activating mutations in *MAP2K1*, *KRAS*, *NRAS* and *BRAF* were rare in plasma collected before the initiation of experimental maintenance treatment. Such an exception was seen in patient E1 (Figure A.4), where MAPK pathway gene mutations emerged during the induction phase, then were suppressed early (stable disease) during maintenance therapy with vemurafenib plus cetuximab plus 5-FU/LV. Nevertheless, consistent with the acquired resistance seen in other patients, a newly acquired gain-of-function variant of *MAP2K1* (I103N) emerged and preceded RECIST-defined PD. In patient E5, an *NF1*-mutated lesion of unknown MAPK biology was detected in the baseline sample during the induction phase which stayed stable through the maintenance phase (Figure A3). This *NF1* mutation may be a passenger mutation with no impact on efficacy or dependency on the MAPK pathway, or it could be a mode of innate resistance in this patient who showed no clear response to the maintenance therapy. Of note, 2 control arm patients (C5 and C7) had a MAPK pathway mutation in their baseline plasma samples that was no longer detected during maintenance treatment (Fig. 3a; Figure A3); however, as mentioned above, no control patients exhibited an emergence of acquired MAPK pathway mutations during treatment with fluoropyrimidine plus bevacizumab.

Longitudinal testing of cfDNA (Fig. 3b; Figure A.5) showed that patients treated with vemurafenib plus cetuximab plus 5-FU/LV displayed molecular progression, i.e. detection of an acquired resistance mutation in the MAPK pathway in circulating tumour DNA (ctDNA) before radiographic confirmation of PD. Median time from MAPK pathway variant detection to tumour progression ($n = 10$) was 6 months (quartiles 1.6, 9.5). For example, in patient E2, despite the presence of an oncogenic *KRAS* (G12V) variant with 3.55% VAF at baseline with *KRAS* codon 12 mutations suggested as a major resistance mechanism to anti-EGFR treatment [21], stable disease was achieved with vemurafenib plus cetuximab plus 5-FU/LV before disease progression defined by RECIST at 187 days post-maintenance treatment. In accordance with the observed disease control, the *KRAS* variant was undetected in 3 plasma samples tested for ctDNA during the maintenance therapy before resurfacing with 0.124% VAF around the time of disease progression (Figure A.5a). However, between 100 and ~150 days post-maintenance treatment, multiple *MAP2K1* variants (K57T/E203K, F53L) and an additional *KRAS* (Q61H) variant were simultaneously acquired and represent an example of strong molecular progression prior to radiographic confirmation of PD. Similarly in patient E26, a partial response was achieved with vemurafenib plus cetuximab plus 5-FU/LV maintenance therapy; however, *KRAS* (Q61H) variant emerged prior to PD with a parallel increase in lesion diameter in two different lesions (Figure A.5b). In patient E32, while all non-MAPK-related genes displayed a decrease in allele frequency in the plasma during disease stabilisation, the gain-of-function *MAP2K1* (K57T) variant emerged and was detected a couple of months prior to PD (Figure A.5c).

It was observed that the patients with $BRAF^{mut}$ tumours who progressed early on induction treatment seemed to benefit from second-line therapy with vemurafenib plus cetuximab plus 5-FU/LV. Despite their early PD status, disease control (stable disease, $n = 7$; partial response; $n = 2$) was achieved in 9 out of the 11 $BRAF^{mut}$ early progressor patients following second-line treatment with vemurafenib plus cetuximab plus 5-FU/LV (early progressors biomarker evaluable population; $n = 10$ out of 11 shown in Fig. 3a). In a

in patients with at least one such variant acquired in the maintenance phase. Subject timelines are coloured according to confirmed best overall response. Patients were considered MAPK pathway ctDNA status negative (white triangle) at a time-point if no variants were detected in the *EGFR*, *KRAS*, *NRAS*, *BRAF*, *MAP2K1* or *NF1* genes, and positive (black triangle) at a time-point if any such variant was detected. The number of stacked black triangles represents the number of variants detected at a particular time-point. BOR, best overall response; CR, complete response; ctDNA, circulating tumour DNA; EOT, end of treatment; on-Tx, on treatment; PD, progressive disease; Post-tx, post-treatment; PR, partial response; RECIST, response evaluation criteria in solid tumour; SD, stable disease; TMB, tumour mutational burden.

similar fashion to patients in the first-line experimental arm, *BRAF*^{mut} early progressor patients (4 out of 10) acquired multiple gain-of-function MAPK pathway gene mutations following treatment with vemurafenib plus cetuximab plus 5-FU/LV (Fig. 3a; Figure A3; Figure A.5bc).

5. Discussion

In Cohort 1 of the MODUL trial, patients with *BRAF*^{mut} primary colorectal tumours received experimental maintenance treatment with a BRAF–EGFR inhibitor doublet in combination with 5-FU/LV, a regimen designed to overcome the adaptive MAPK feedback loop that drives resistance to BRAF blockade alone [9,10]. Findings from this cohort revealed no meaningful difference in PFS, the primary study end-point, between treatment arms, although evaluations of OS and tumour response end-points were more favourable for the experimental regimen. The difference in median OS, in particular, suggested the possibility of clinical benefit (experimental: 24.0 months versus control: 21.3 months; $P = 0.287$), although the results were underpowered and descriptive only. The lack of a consistent efficacy signal in Cohort 1 differs from other studies of BRAF–EGFR inhibitor combinations as second- or later-line therapy in *BRAF*^{mut} mCRC populations [11–14]. These studies demonstrated improved outcomes with BRAF–EGFR inhibitor combinations, although it should be noted that the control regimens were EGFR-based combinations in all studies rather bevacizumab-based therapy as in MODUL. It is also relevant that the median PFS (~10 months) for both treatment arms in Cohort 1 was higher than protocol estimates (experimental: 7 months; control: 4.9 months) and slightly higher than the median OS in the BEACON trial (9.3 months) [14]. This disparity may be explained in part by the effect of induction therapy. Over one-third of patients receiving induction treatment did not go on to randomisation to maintenance therapy because disease progression occurred or may have occurred, suggesting that the group of patients proceeding to maintenance treatment were a population enriched for a better prognosis.

Despite initial disease control with targeted therapies in patients with cancer, relapse often occurs due to acquired mutations assumed to arise as an adaptation to selective pressure [22]. Our biomarker data from Cohort 1 supports that acquired resistance to BRAF inhibitor-based combination therapy in *BRAF*^{mut} mCRC involves the emergence of new activating mutations in the MAPK signalling pathway, an observation that is consistent with the genomic findings from the BEACON trial following BRAF plus EGFR inhibition [23]. While acquired mutations in EGFR were reported in response to EGFR-directed monotherapy [24,25], no *EGFR* mutations were acquired in response to combination treatment with an EGFR and BRAF inhibitor in MODUL. This observation highlights the selective pressure associated with targeting the downstream BRAF target in patients with *BRAF*^{mut} mCRC and supports that MAPK reactivation is downstream of EGFR following treatment with both EGFR and BRAF inhibitors. Further, within the Cohort 1 experimental treatment arm only, mutations in MAPK pathway genes emerged, often concurrently, following a period of disease control and prior to disease progression. We observed a median time-frame of 6 months from the detection of MAPK pathway gene mutations by cfDNA testing to disease progression identified by a scan. These observations suggest that ctDNA monitoring may be used as a surveillance tool to detect the emergence of resistance to targeted therapy and inform clinical decisions prior to disease progression. The time-frame between a molecular progression and RECIST-defined progression in this study may have been affected by the genomic assay limit of detection. ctDNA monitoring assays are constantly evolving, and future studies might uncover a longer period from emergence of molecular resistance to relapse identified by RECIST tumour assessment.

In Cohort 1, the safety results for vemurafenib plus cetuximab plus 5-FU/LV were consistent with the known safety profiles of BRAF and EGFR inhibitors. As would be expected, the rate of adverse events, including SAEs, was higher with the triplet experimental regimen than the doublet control regimen, although the rate of treatment-related SAEs was lower than reported with first-line encorafenib plus cetuximab plus mFOLFOX6 (13% versus 26%) but similar to encorafenib plus cetuximab plus FOLFIRI (13% versus 13%) in the recent BREAKWATER study in patients with *BRAF*^{wt} mCRC [26]. Treatment exposure was higher in the experimental arm but the proportion of patients in each arm who discontinued any study drug due to an adverse event was similar. Overall, adverse events observed during maintenance therapy appeared manageable and were consistent with the known safety profiles of the study treatments with no new safety signals identified.

In Cohort 4, which was comprised mainly of patients with HER2–/MSS/*BRAF*^{wt} tumours, MAPK kinase inhibition with cobimetinib combined with the immune checkpoint inhibitor atezolizumab did not improve PFS but increased toxicity. Our findings are consistent with the IMblaze370 study in which atezolizumab-based therapy did not improve OS in patients with previously treated MSS mCRC [27], suggesting that other strategies are required to increase the susceptibility of MSS mCRC to immunotherapy.

We acknowledge several strengths and limitations of our study. MODUL is the largest randomised umbrella

maintenance trial in the first-line mCRC setting reported to date. The study design included a common control regimen for all maintenance cohorts, which permitted comparisons between experimental treatments and also mitigated recruitment issues. However, the study was closed to enrolment prematurely which means that Cohorts 1, 3 and 4 did not reach their target sample size. Interpretation of the study results must be tempered by the limitations of small sample size. The MODUL trial, as a whole and in particular the part reported here, did not show any therapeutic improvement that could have changed the standard treatment of patients. However, this study has allowed in-depth translational studies that generate hypotheses for future work. Furthermore, the progressive segmentation of colon cancer into a lot of 'rare colon cancers' with, e.g., the arrival of anti-KRAS molecules, shows that this type of platform trial remains relevant.

Biomarker-driven maintenance therapy after first-line induction treatment in patients with mCRC is feasible. MODUL supports further investigation of vemurafenib plus cetuximab plus 5-FU/LV as first-line maintenance treatment for $BRAF^{mut}$ mCRC in an adequately powered study. The development of more effective targeted combinations in $BRAF^{mut}$ mCRC is still needed with the MAPK pathway as a critical target. In patients with HER2−/MSS/$BRAF^{wt}$ tumours, cobimetinib plus atezolizumab had an unfavourable benefit:risk ratio compared to the control regimen (fluoropyrimidine plus bevacizumab). Other strategies are required to increase the susceptibility of MSS mCRC to immunotherapy.

Author contributions

All authors: Conceptualization; Data curation; Formal analysis; Roles/Writing - original draft; Writing - review & editing.

All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interests

MD declares personal financial interest in the form of scientific consultancy roles for Roche, Genentech, Amgen, Bayer, AstraZeneca, BeiGene, Daiichi Sankyo, Merck Serono, Servier, Pierre Fabre, Terumo, and Sirtex, and has research grants that have been paid to his institution by Bayer, Roche, and Keocyt.

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Appendix A. Supplementary data

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