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**A randomised phase 2 study of intermittent versus continuous dosing of dabrafenib plus trametinib in patients with *BRAF*<sup>V600</sup> mutant advanced melanoma (INTERIM)**

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## Highlights

- Intermittent dosing has been proposed as a mechanism to enhance duration of benefit for patients with metastatic melanoma receiving oral BRAF + MEK inhibitors
- In the INTERIM trial, intermittent dosing of dabrafenib + trametinib was inferior to standard continuous dosing for all efficacy end-points
- Three exploratory melanoma trials from the UK, USA and Spain now all consistently demonstrate lack of patient benefit from different intermittent dosing schedules
- Measurement of *BRAF*<sup>V600E</sup> ctDNA using ddPCR had prognostic but not predictive value

## Abstract

### Background

BRAF+MEK inhibitors extend life expectancy of patients with *BRAF*<sup>V600</sup> mutant advanced melanoma. Acquired resistance limits duration of benefit, but preclinical and case studies suggest intermittent dosing could overcome this limitation. INTERIM was a phase 2 trial evaluating an intermittent dosing regimen.

### Methods

Patients with *BRAF*<sup>V600</sup> mutant advanced melanoma due to start dabrafenib+trametinib were randomised to receive either continuous (CONT), or intermittent (INT; dabrafenib d1-21, trametinib d1-14 every 28 days) dosing. A composite primary endpoint included progression-free survival (PFS) and quality of life (QoL). Secondary endpoints included response rate (ORR), overall survival (OS) and adverse events (AEs). Mutant *BRAF*<sup>V600E</sup> ctDNA was measured by droplet digital PCR (ddPCR), using mutant allele frequency of >1% as the detection threshold.

### Results

79 patients (39 INT, 40 CONT) were recruited; median age 67 years, 65% AJCC (7<sup>th</sup> ed) stage IV M1c, 29% had brain metastases. With 19 months median follow-up, INT was inferior in all efficacy measures: median PFS 8.5 vs 10.7mo (HR 1.39, 95%CI 0.79-2.45, p=0.255); median OS 18.1mo vs not reached (HR 1.69, 95%CI 0.87-3.28, p=0.121), ORR 57% vs 77%. INT patients experienced fewer treatment-related AEs (76% vs 88%), but more grade  $\geq 3$  AEs (53% vs 42%). QoL favoured CONT. Detection of *BRAF*<sup>V600E</sup> ctDNA prior to treatment correlated with worse OS (HR 2.55, 95%CI 1.25-5.21, p=0.01) in both arms. A change to undetected during treatment did not significantly predict better OS.

### Conclusion

INTERIM findings are consistent with other recent clinical trials reporting that intermittent dosing does not improve efficacy of BRAF+MEK inhibitors.

## **Background**

Just under 50% of all melanomas harbour a *BRAF*<sup>V600</sup> gene mutation. BRAF+MEK inhibitors have been shown to extend life of patients with *BRAF*<sup>V600</sup> mutant advanced melanoma compared with standard of care in several international registration clinical trials<sup>1-3</sup>. Median life expectancy of treated patients is now approaching 3 years. However, most patients progress despite treatment due to acquired resistance.

Continuous daily dosing of oral kinase inhibitors may promote clonal expansion of drug resistant cells and intermittent drug dosing was proposed as a strategy to delay the onset of disease progression<sup>4,5</sup>. In a mouse model, vemurafenib-resistant tumour cells were shown to become drug-dependent<sup>6</sup>. Resistant cells suffered a fitness deficit in the absence of drug and mice survived twice as long (200 days compared with 100 days) on an intermittent dosing schedule of vemurafenib (4 weeks on 2 weeks off) compared with continuous daily dosing. Clinical case reports also suggested that interrupted dosing of BRAF inhibitors can reverse resistance to the drugs<sup>7</sup> and improve tolerability<sup>8</sup>.

Three randomised phase 2 trials have been conducted to test whether pre-planned intermittent dosing of oral BRAF targeted agents may provide a means of sustaining patients on treatment for longer. The first 2 studies, one conducted in the USA<sup>9</sup>, the other in Spain<sup>10</sup>, have published their findings and both failed to demonstrate a benefit with intermittent dosing. The UK INTERIM study is the third study to test this concept.

## **Methods**

### **Patients**

Patients aged  $\geq 18$  years with AJCC (7<sup>th</sup> edition) stage III unresectable or stage IV *BRAF*<sup>V600</sup> mutant advanced melanoma and Eastern Cooperative Oncology Group performance status (ECOG PS) 0-2 due to start dabrafenib+trametinib were eligible to take part in the INTERIM trial. Other key entry criteria were measurable disease prior to randomisation, life expectancy  $\geq 12$  weeks,

adequate bone marrow and liver function, and no prior BRAF or MEK inhibitors; presence of brain metastases and prior immunotherapy were allowed. The INTERIM trial protocol (ISRCTN18183156) was approved by Cambridge South Research Ethics Committee (ref: 17/EE/0340) and was performed in accordance with the Declaration of Helsinki and the European Clinical Trials Directives 2001/20/EC. All patients provided written informed consent. Grant funding was provided by the NIHR Research for Patient Benefit Programme. The funder had no role in the study design, data collection, analysis, interpretation, or writing of the report.

### **Treatment**

Consenting patients were randomised 1:1 using the minimisation with random element method to receive standard doses of dabrafenib (150mg bid) and trametinib (2mg od) either continuously (CONT; dabrafenib+trametinib d1–28) or intermittently (INT; dabrafenib d1-21 + trametinib d1–14 on a 28-day cycle). Stratification factors were ECOG PS, AJCC stage (IIIC/IVM1a/IVM1b/IVM1c), presence or absence of brain metastases and baseline blood lactate dehydrogenase (LDH) level ( $\leq$  upper limit of normal range (ULN); between  $>$ ULN and  $\leq 2 \times$  ULN;  $>2 \times$  ULN). Patients were allowed to continue on treatment beyond radiological disease progression if they were deriving clinical benefit, or if progressive disease was resectable.

### **Study procedures**

Patients were clinically assessed every 2 weeks during the initial 2 cycles, then prior to the beginning of every cycle until inoperable disease progression, then 3-monthly for a minimum of 9 months follow-up from their randomisation date. Adverse events (AEs) were recorded using NCI CTCAE version 4.03 for up to 30 days after the last dose of protocol treatment and resolution of all  $\geq$  grade 2 AEs. Quality of life (QoL) was assessed using the EORTC QLQ-C30 and EQ5D-L questionnaires every 12 weeks before disease progression and every 3 months with a minimum of 9 months after progression since the date of randomisation. Imaging was performed at baseline, 6 weeks after starting treatment and then every 8 weeks until disease progression to assess the objective response rate (ORR) using RECIST V1.1. Research blood samples

to measure plasma *BRAF*<sup>V600E</sup>ctDNA were collected from all patients on cycle 1 d1 (prior to starting treatment) and 15, cycle 2 d1 and 15, cycle 3 d1 and then at the start of alternate cycles until disease progression, as well as on disease progression.

### **Plasma ctDNA assay**

DNA was extracted from plasma using the Qiagen Qiasymphony SP instrument, QIASymphony DSP Circulating DNA kit and quantified using the Qubit fluorometer 4.0 and the Qubit™ dsDNA HS kit. The Droplet Digital PCR (ddPCR) *BRAF*<sup>V600E</sup> test was used to detect a sequence variant that was present at a very low frequency in a pool of wild-type backgrounds with the QX200™ Droplet Digital™ PCR System. Following PCR, each droplet was analysed or read in a flow cytometer to determine the fraction of PCR-positive droplets in the original sample. Data were then analysed using Poisson statistics to determine the target DNA template concentration in the original sample.

### **Study endpoints and statistical analysis**

A composite primary endpoint of this feasibility study was designed to 1) measure clinical efficacy by comparing progression-free survival (PFS) in the 2 arms; 2) evaluate patient QoL and 3) assess recruitment rate, as well as treatment compliance with intermittent dosing. Secondary endpoints were objective response rate (ORR), overall survival (OS) and toxicity. An exploratory analysis of *BRAF*<sup>V600E</sup>ctDNA was undertaken to explore its prognostic and predictive utility, measured by ddPCR, using mutant allele frequency of >1% as the detection threshold.

Patient experience of taking part in the trial was explored in a qualitative substudy, which has been fully published elsewhere<sup>11</sup>.

For PFS, the intended sample size was 100 patients (50 patients in each arm), to be recruited in 30 months with a minimum of 9 months follow-up to have a total of 80 PFS events, from up to 20 UK sites. The sample size was compatible with an anticipated hazard ratio (HR) for PFS of 0.75 in favour of the intermittent

arm, which would provide some evidence of the clinical efficacy of the intermittent arm. More details on the sample size calculation are provided in the supplementary materials.

Recruitment rate and compliance in the intermittent arm were of interest in case of planning a future phase III trial. The aim was to demonstrate recruitment of 2 patients per site per month when all sites were up and running, Compliance was defined as the percentage of patients completing the allocated treatment at 6 months from the date of randomisation and was compared in both arms.

Efficacy and safety analyses were based on the intention-to-treat (ITT) principle and patients who had at least one dose of trial treatment. The log-rank test was applied to compare PFS and OS between arms, and ORR was compared using the Chi-squared test. The subscales of EORTC QLQ-C30 and EQ-5D-5L questionnaires were derived according to standard-scoring manuals. Analyses of changes from baseline over time and differences between the 2 arms for subscales were carried out with repeated measures using ANCOVA, adjusting for baseline level, time, treatment and interaction of time and treatment. Kaplan–Meier plots were generated for PFS and OS. Odds ratios (ORs) were estimated using logistic regression models and HRs with Cox regression models.

## **Results**

### **Patients and treatment delivery**

From December 2017 to February 2020, 79 patients were recruited at 19 UK sites at an average recruitment rate of 3 patients per month across all sites; 39 patients were randomised to the intermittent [INT] schedule arm and 40 to the continuous [CONT] schedule arm (Consort diagram is provided in Supplementary **Figure S1**). The study closed to recruitment prematurely in March 2020 due to the onset of the COVID-19 pandemic and follow up was stopped in September 2021 with a median follow up of 19 months

Patient demographics and baseline characteristics were well balanced (**Table 1**). Median patient age was 67 (range 34-85) years, 88% were ECOG PS 0-1,

65% were AJCC stage 4M1c, 29% had brain metastases and 46% had LDH > ULN. One patient in the INT arm was found to be ineligible after randomisation and did not receive any trial treatment. 24 (63%) patients in the INT arm and 31 (78%) patients in the CONT arm completed at least 6 cycles of treatment. Four patients in the INT arm switched to continuous dosing during the study period, no patients in the CONT arm switched to intermittent dosing regimen.

The median treatment duration was 8.5 (interquartile range [IQR] 14.8) months in the INT arm and 11.3 (IQR 9.6) months in the CONT arm.

	INT (n=39)	CONT (n=40)	All (n=79)
<b>Age (years)</b>			
Median (range)	69 (34-85)	62 (38-78)	67 (34-85)
<b>Gender</b>			
Female	22 (56%)	21 (52%)	43 (54%)
Male	17 (44%)	19 (48%)	36 (46%)
<b>ECOG performance status</b>			
0	19 (49%)	19 (48%)	38 (48%)
1	15 (38%)	17 (42%)	32 (40%)
2	5 (13%)	4 (10%)	9 (11%)
<b>AJCC (7<sup>th</sup> ed) stage</b>			
IIIC	2 (5%)	1 (2%)	3 (4%)
IVM1a	5 (13%)	6 (15%)	11 (14%)
IVM1b	6 (15%)	8 (20%)	14 (18%)
IVM1c	26 (67%)	25 (62%)	51 (65%)
<b>Presence of brain metastases</b>			
No	29 (74%)	27 (68%)	56 (71%)
Yes	10 (26%)	13 (32%)	23 (29%)
<b>LDH relative to ULN</b>			
≤ ULN	21 (54%)	22 (55%)	43 (54%)
> ULN and ≤ 2 x ULN	13 (33%)	14 (35%)	27 (34%)
> 2 x ULN	5 (13%)	4 (10%)	9 (11%)
<b>Tissue BRAF<sup>V600E</sup> status<sup>†</sup></b>			
Confirmed	29 (74%)	26 (65%)	55 (70%)
Unconfirmed	10 (26%)	14 (35%)	24 (30%)
<b>Plasma ctDNA Positive for BRAF<sup>V600E</sup></b>			
Confirmed	19 (59%)	21 (62%)	40 (61%)
Unconfirmed	13 (41%)	13 (38%)	26 (39%)
<b>ctDNA BRAF<sup>V600E</sup> mutant allele frequency &gt;1%</b>			
Detected	15 (47%)	12 (35%)	27 (41%)
Not detected	17 (53%)	22 (65%)	39 (59%)
<b>Previous systemic therapy</b>			
CPI <sup>†*</sup> (1st line metastatic)	13 (33%)	17 (42%)	30 (38%)
CPI (adjuvant)	4 (10%)	7 (18%)	11 (14%)

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	INT (n=39)	CONT (n=40)	All (n=79)
CPI (unknown)	1 (3%)	1 (2%)	2 (3%)
Avastin (adjuvant)	1 (3%)	0 (0%)	1 (1%)

All patients had tumour BRAF<sup>V600</sup> mutation confirmed prior to study entry; only a proportion had a confirmed specific tissue BRAF<sup>V600E</sup> status is coded avariants confirmed if the histologically or cytologically confirmed melanoma BRAF mutation is V600E at screening.

\* CPI = antiPD1+/-antiCTLA4 checkpoint inhibitor; no patients received adjuvant BRAF targeted therapy

**Table 1.** Patient baseline characteristics

### Efficacy

With a median follow-up of 19 months, 48 patients (25 INT, 23 CONT) had progressed and 35 died (21 INT, 15 CONT). Median PFS was 8.5 months (95% CI 5.5-18) in the INT arm and 10.7 months (95% CI 5.8-not reached) in the CONT arm, HR 1.39 (95% CI 0.79–2.45, p = 0.255) (**Figure 1A**). Median OS was 18 months (95% CI 10.8 to not reached) in the INT arm and not reached in the CONT arm, HR 1.69 (95% CI 0.87-3.28, p = 0.121) (**Figure 1B**).

Twenty-two patients (13 CONT and 9 INT) who had at least one dose of trial treatment had brain metastases. In this study, prior treatment of brain metastases was not a requirement, but presence/absence of brain metastases was a stratification factor. As expected, patients with brain metastases had poorer OS compared with those who did not (HR 2.1, 95% CI 1.1-4.1). The numbers of patients with brain metastases were small, but the impact of the different treatment schedules was similar to what we observed in the whole population. There was no evidence to suggest that intermittent dosing impacted outcomes of patients with brain metastases differently compared with those without brain metastases.

Seventy-two patients (37 INT, 35 CONT) were evaluable for response using RECIST V1.1 criteria, assessed by local investigators. ORR was higher in the CONT arm (77% versus 57%, p = 0.069). Complete and partial responses were 23% and 54% in the CONT arm and 16% and 41% in the INT arm. The median time to treatment failure (time from starting treatment to discontinuation for any

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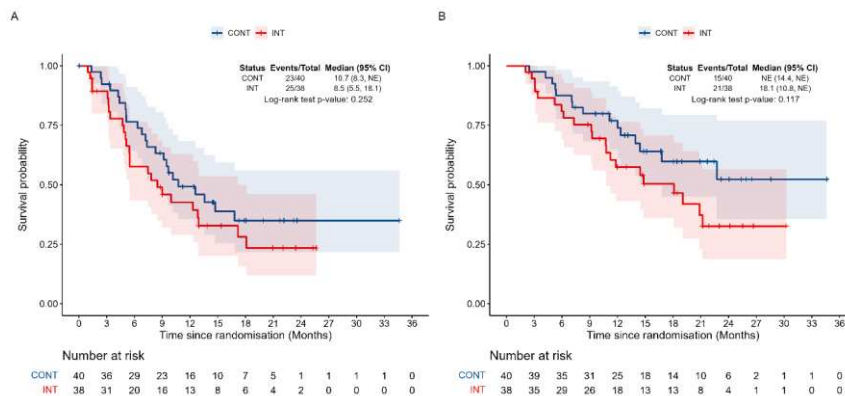
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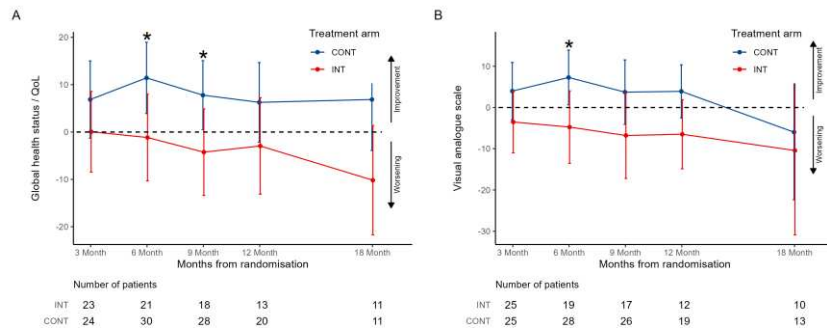
reason) was 7.9 months in the INT arm and 10.7 months in the CONT arm (HR 1.26, 95% CI 0.82-2.32,  $p = 0.26$ ).



**Figure 1.** Kaplan-Meier curves of (A) progression-free survival and (B) overall survival for patients who have received at least one dose of treatment on the intermittent (INT) or continuous (CONT) dosing schedule.

### Quality of life

Seventy-two patients completed baseline EORTC QLQ-C30 questionnaires (34 INT, 38 CONT). The number of patients who completed questionnaires decreased over time, as expected (**Figure 2A**). The mean QLQ-C30 global health status was consistently higher for the CONT arm compared with the INT arm throughout the study period. At month 6, the change in global health status compared to baseline was +11.44 for CONT and -1.16 for INT (95% CI 0.73-24.5,  $p = 0.038$ ). The favourable QoL with CONT vs INT was also evident using the EQ-5D-5L visual analogue scale (+7.29 for CONT and -4.75 for INT at 6 months) (**Figure 2B**).



**Figure 2.** Least-squares mean change from baseline of EORTC QLQ-C30 global health status (**A**) and EQ-5D-5L visual analogue scale (**B**). Asterisk indicates a statistical difference between the treatment arms of  $p < 0.05$ .

### Safety and toxicity

78 (38 INT and 40 CONT) patients were evaluated for safety. The proportion of patients with any AE was slightly higher in the CONT arm (92% versus 87%), but patients in the INT arm reported more grade  $\geq 3$  AEs (53% versus 42%) (**Table 2**). The CONT arm experienced more treatment-related AEs (TRAEs) (88.5% versus 76.3% any grade; 48% versus 37% grade  $\geq 3$ ), but more patients in the INT arm experienced SAEs (45.7% versus 38.7%). A detailed breakdown of AEs is provided in Supplementary **Table S1**.

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	INT (n=38)	CONT (n=40)
Any AE	33 (87%)	37 (92%)
AE grade $\geq 3$	20 (53%)	17 (42%)
Treatment-related AE	29 (76%)	35 (88%)
<u>Treatment-related AE grade <math>\geq 3</math></u>	<u>14 (37%)</u>	<u>19 (48%)</u>
AE attributed to dabrafenib	29 (76%)	35 (88%)
AE attributed to trametinib	26 (68%)	33 (82%)
AE related to underlying disease	18 (47%)	24 (60%)
Any SAE	17 (45%)	15 (38%)
Any Hospitalization	21 (55%)	19 (48%)

**Table 2.** Summary of adverse events (AEs) in patients who received at least one dose of trial treatment

Fifty-nine (76%) patients (28 INT, 31 CONT) had a treatment interruption during the first 6 cycles, AEs were the most common reason (23 INT, 27 CONT) for

dose interruption, patient compliance being the second most common reason (10 INT, 13 CONT). The most common reason for stopping treatment early was disease progression (15 INT, 13 CONT) and lack of clinical benefit (6 INT, 4 CONT). Seven patients stopped treatment due to unacceptable toxicity (5 INT, 2 CONT).

There was no significant difference in the number of patients who achieved at least 80% of planned dose intensity for both dabrafenib (28 INT, 26 CONT,  $p=0.41$ ) and trametinib (32 INT, 30 CONT,  $p=0.31$ ).

### ctDNA analysis

Baseline, pre-treatment plasma samples were available for 66 patients to undertake *BRAF*<sup>V600E</sup>ctDNA analysis. Droplet digital PCR identified *BRAF*<sup>V600E</sup>ctDNA in 40/66 (61%) samples. The median mutant allele frequency was 7.8 (range 0.02-67.3). Using mutant allele frequency >1% as the cut-off, *BRAF*<sup>V600E</sup>ctDNA was considered as detectable in 27 (41%) patients at baseline. ctDNA detection correlated with higher disease burden, as determined by plasma LDH level >ULN ( $p<0.001$ ) (**Table 3**).

Baseline *BRAF*<sup>V600E</sup>ctDNA detection did not predict for ORR, but there was an apparent correlation with PFS, which was much more robust for OS (**Figure 3**). Patients with detected *BRAF*<sup>V600E</sup>ctDNA had a worse survival outcome with HR ranging from 2.55 (95% CI 1.25-5.21,  $p$ -value=0.01) in an unadjusted model to 2.91 (95% CI 1.25-6.75,  $p$ -value=0.013) in a model incrementally adjusted for treatment arm, and stratification factors (**Table 3**). Worse outcomes were also seen when stratifying for serum LDH levels above and below normal range (Supplementary **Figure S3** and **Table S3**). The data suggested that disease burden (as determined by LDH level) was likely to be a stronger predictor of survival outcomes rather than BRAF/MEKi dosing schedule.

There was no interaction between baseline *BRAF*<sup>V600E</sup>ctDNA detection status and treatment arm for PFS or OS. When inspecting the data summarized in Figures 3B and 3D, it appears that patients with detectable ctDNA at baseline

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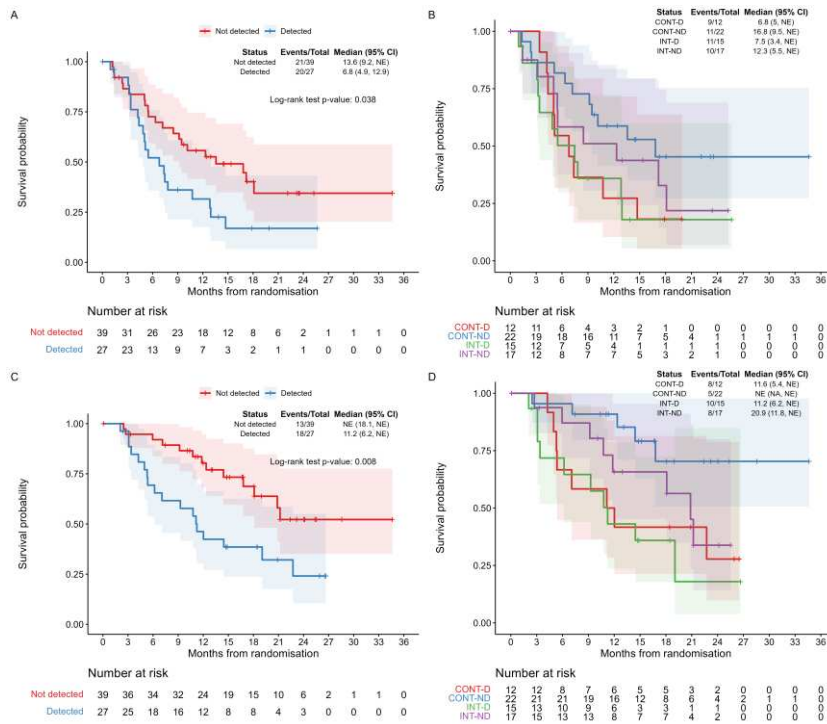
had lower PFS and OS probability regardless of their treatment arm. This was confirmed in a re-analysis where the p-value for interaction between baseline ctDNA detection status and treatment arm was 0.125 and 0.285 for PFS and OS.

	Detected (N=27)	Not detected (N=39)	P-value
<b>ECOG PS</b>			
0	10 (37.0%)	20 (51.3%)	0.373
1-2	17 (63.0%)	19 (48.7%)	
<b>AJCC stage</b>			
4M1c	22 (81.5%)	22 (56.4%)	0.0631
Others	5 (18.5%)	17 (43.6%)	
<b>Brain metastases</b>			
No	20 (74.1%)	26 (66.7%)	0.71
Yes	7 (25.9%)	13 (33.3%)	
<b>LDH</b>			
≤ ULN	6 (22.2%)	30 (76.9%)	<0.001
> ULN	21 (77.8%)	9 (23.1%)	
<b>ORR at Week 16</b>	11 (57.9%*)	16 (53.3%**)	0.754
<b>Relapse<sup>†</sup></b>			
Unadjusted	1.91 (1.03, 3.54)	reference	0.041
Model 1	1.82 (0.97, 3.40)	reference	0.061
Model 2	2.01 (0.99, 4.09)	reference	0.053
<b>Death<sup>†</sup></b>			
Unadjusted	2.55 (1.25, 5.21)	reference	0.010
Model 1	2.43 (1.19, 4.98)	reference	0.015
Model 2	2.91 (1.25, 6.75)	reference	0.013

\* assessed for 19 patients; \*\* assessed for 30 patients.

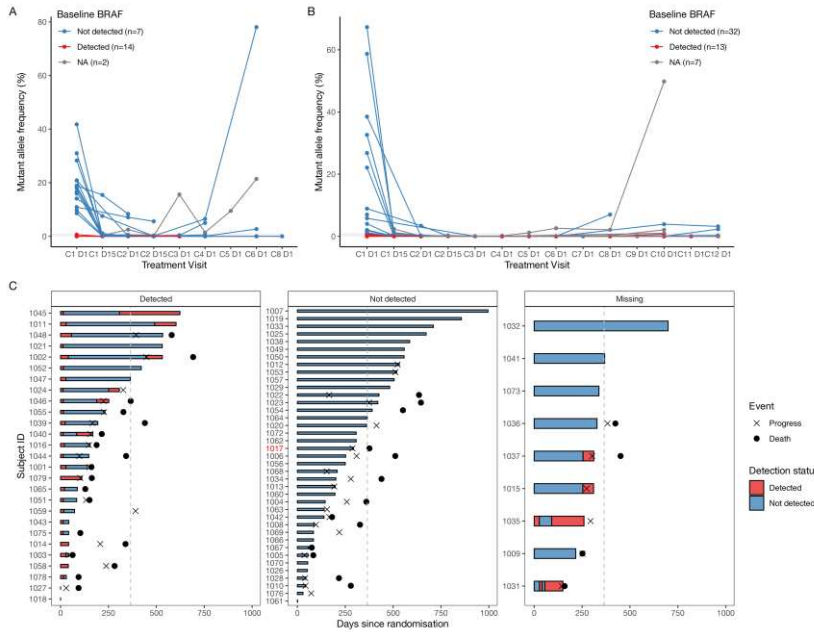
<sup>†</sup> HR for relapse and death were determined by Cox model, with not detected as reference. Model 1 adjusted for treatment arm; Model 2 adjusted for Model 1 + ECOG PS, AJCC stage and presence of brain metastases. LDH was not included due to the co-linearity with ctDNA.

**Table 3.** Correlation of baseline plasma *BRAF*<sup>V600E</sup> ctDNA detection status with patient characteristics and treatment outcome



**Figure 3.** Progression-free survival according to baseline *BRAF*<sup>V600E</sup>ctDNA detection status (A) and treatment arm plus *BRAF*<sup>V600E</sup>ctDNA detection status (B); Overall survival according to baseline *BRAF*<sup>V600E</sup>ctDNA detection status (C) and treatment arm plus *BRAF*<sup>V600E</sup>ctDNA detection status (D)

Longitudinal measurement of ctDNA mutant allele frequency identified step drops within the first cycle of treatment for patients with detectable ctDNA at baseline irrespective of treatment arm (**Figure 4A**). Measurement of ctDNA every 2 weeks for the first 8 weeks of treatment in both arms confirmed there was no evidence of a rebound in ctDNA levels associated with intermittent dosing (Supplementary **Table S4**). A change from detectable to undetectable ctDNA did not influence treatment outcome (Supplementary **Table S5**). Disease progression was associated with emergence of ctDNA detection in only 3 patients with baseline detectable ctDNA and 1 patient with baseline undetectable ctDNA (**Figure 4C**). There was no clear pattern in ctDNA dynamic changes with PFS or OS.



**Figure 4.** *BRAF*<sup>V600E</sup>ctDNA mutant allele frequency measured at different time points for (A) patients who died within 1 year of starting treatment and (B) patients who were alive beyond 1 year of starting treatment (red = detected; blue = not detected). Swimmer plots (C) summarising changes of plasma *BRAF*<sup>V600E</sup>ctDNA detection status since starting treatment. Grey dashed line indicates 1 year since randomisation. No ctDNA was detected for patient 1017 at baseline, but became detectable on disease progression.

## Discussion

The UK INTERIM study did not meet its primary endpoint of PFS improvement with intermittent dosing of dabrafenib+trametinib. All main study end-points including PFS, OS, ORR and QoL favoured standard continuous dosing. Although the frequency of adverse events was higher with continuous dosing, the seriousness and grading of adverse events were worse with intermittent dosing.

Two other studies evaluating different intermittent dosing schedules of BRAF+MEKi have demonstrated similar inferior outcomes compared with standard continuous dosing. The first USA SWOG study<sup>9</sup> was designed so that

patients received 8 weeks continuous dosing with dabrafenib+trametinib and only those patients who were progression-free were randomized to either continuous or intermittent dosing of both drugs on a 3 weeks off, 5 weeks on schedule. 206 patients were recruited between 2014 and 2019 and 206 went on to be randomised. Post-randomisation PFS was greater with continuous treatment (median PFS 9.0 vs 5.5 months, HR 1.36, p=0.06). Overall survival of 29.2 months was identical in both arms. The second, smaller study (N=70) conducted by the Spanish Melanoma Group<sup>10</sup> evaluated a different BRAF+MEKi combination, vemurafenib+cobimetinib and again initiated treatment with a continuous dosing run-in, this time lasting 12 weeks. Thereafter, the intermittent schedule comprised vemurafenib 4 weeks on, 2 weeks off and cobimetinib 3 weeks on 3 weeks off. Median PFS was 16.2 months vs 6.9 months (p=0.079), favouring continuous dosing. No statistically significant differences were observed in OS.

INTERIM is the first study to test intermittent dosing from the point of initiating BRAF+MEKi. However, this strategy neither conferred advantage, nor disadvantage compared with a continuous dose run-in, with PFS favouring standard scheduling and no significant difference on OS. Furthermore, the safety profile as measured by frequency of grade >3 treatment-related AEs was remarkably consistent across all 3 trials: 48% CONT and 37% INT in the INTERIM study; 43% CONT and 34% INT in the US study; 43% CONT and 40% INT in the Spanish study. The choice of the intermittent dosing schedule tested in INTERIM took into account both pharmacokinetic and pharmacodynamic considerations, as well as anticipated acceptability to patients and physicians. Dabrafenib has a 5.2 hour half-life, but generates several active metabolites which persist, while trametinib has a mean terminal half-life of 5.3 days. Taken together, the preclinical data suggested that a minimum of 1 week break from BRAF inhibitor and 2 weeks break from MEK inhibitor would be required to relieve inhibition of the MAP kinase pathway. Even so, consistent with previous investigators<sup>9,10</sup>, we have been unable to replicate the beneficial effects of intermittent BRAFi dosing seen in mice, with no clear biological explanation for this failure. The preclinical data was limited to a single animal model, but supported by retrospective patient data and case

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reports. Whether additional preclinical modelling would have obviated the need to undertake multiple prospective clinical trials, which are both time and resource consuming, is an unknown question.

Data interpretation from the INTERIM study might be considered to be hampered somewhat because the intended sample size of 100 patients was not reached. The anticipated recruitment rate was not met largely because of the changing melanoma treatment landscape, and BRAF targeted therapy was replaced by immune checkpoint inhibitors as first line therapy for metastatic disease for most patients during the course of this trial<sup>12</sup>. Recruitment to treatment de-escalation trials is challenging, needing both investigators and patients to be in equipoise<sup>13</sup>. Our qualitative substudy exploring patient experience of being offered and treated in the INTERIM trial<sup>11</sup> found that, despite verbal and written information being given, some still struggled to understand the goal of treatment *per se*, as well as the meaning of randomisation. Challenges with recruitment led us to create a Youtube video with the help of patient representatives aimed at helping potential participants make a better informed decision more confidently (<https://www.youtube.com/watch?v=ulxyNH-8aGc>). Even so, the decision to close the study early was taken as the first wave of the COVID-19 pandemic impacted clinical practice and ability to maintain clinical trial recruitment in the UK. Despite limited sample size, there is no suggestion from analysing the INTERIM patient cohort of 78 patients (excluding 1 patient with no trial treatment given) that the outcomes would have been different should our larger target recruitment have been met.

Our exploratory analysis of *BRAF*<sup>V600</sup>ctDNA using ddPCR demonstrated that, in those patients with detectable ctDNA at baseline, levels fell rapidly during the first cycle of treatment and levels remained low during the first 8 weeks of treatment in both arms, with no evidence of any rebound effect in the intermittent arm while patients were off treatment. ctDNA monitoring during treatment gave no other clues regarding any differences in biological behaviour of the 2 arms and is likely to reflect the limitations of ddPCR as a predictive tool for measuring ctDNA with clinical application, at least in the context of

metastatic melanoma. Technologies utilising wider, patient-specific panels<sup>14</sup> or measuring DNA methylation<sup>15</sup> which are in development might be more informative. Time-based intermittent dosing did not yield differences in ctDNA dynamics in our small population. This leaves open the possibility that biologically-driven intermittent dosing, for example according to changes in ctDNA, might still have merit. The UK DYNAMIC clinical trial (ISRCTN14643179) is using changes in detection of *BRAF*<sup>V600</sup>ctDNA by ddPCR measured intermittently during BRAF targeted treatment to direct decisions whether to continue or interrupt dosing. This trial is likely to be the ultimate test of whether intermittent dosing of BRAF-targeted therapy has any clinical relevance.

In most cases, progression in our patient cohort was not associated with reappearance of *BRAF*<sup>V600</sup>ctDNA. Secondary resistance is typically characterised by emergence of other new mutations and/or gene amplifications, which would require to be detected using broader sequencing technologies<sup>16</sup>. On the other hand, we have confirmed, as have others previously<sup>17</sup>, that detection of *BRAF*<sup>V600</sup>ctDNA pre-treatment is a strong poor prognostic indicator, being associated with shorter patient survival, irrespective of treatment arm. Even so, the strong correlation with high disease burden as measured by a readily available, simple and cheap LDH blood test, means adoption of ctDNA as a routine test remains undefined, until such time as a clinically meaningful application can be demonstrated.

## **Conclusion**

In summary, 3 randomised trials testing different intermittent BRAF+MEKi intermittent dosing schedules have now failed to show any patient benefit, so continuous dosing regimens remain standard of care.

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### **Author contributions**

PC, MM, AG & RM conceived the clinical trial; AD, JW & NW provided statistical support; JN, RB, MP, AR, AF, SD, BE, JC, CHB, NS, MB, EB, MG, MH, LP, SK, AW, AG & PC recruited the patients; GB, LD & EB undertook the ctDNA analyses; ST led study coordination; PC & AD led manuscript writing; all authors contributed to data interpretation and approved the final manuscript.

### **Declaration of competing interest**

~~Dr. Middleton reports grants from Roche, grants from AstraZeneca, grants from GSK, other from Novartis, grants and other from Immunocore, other from BMS, other from Pfizer, other from Merck/MSD, other from Regeneron, other from BiolineRx, other from Replimune, grants from GRAIL, outside the submitted work. The authors have declared no conflicts of interest associated with the submitted work.~~

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## References

1. Robert C, Grob JJ, Stroyakovskiy D, et al. Five-Year Outcomes with Dabrafenib plus Trametinib in Metastatic Melanoma. *N Engl J Med*. 2019;381(7):626-636. doi:10.1056/NEJMoa1904059
2. Ascierto PA, Dréno B, Larkin J, et al. 5-Year Outcomes with Cobimetinib plus Vemurafenib in BRAFV600 Mutation-Positive Advanced Melanoma: Extended Follow-up of the coBRIM Study. *Clin Cancer Res*. 2021;27(19):5225-5235. doi:10.1158/1078-0432.CCR-21-0809
3. Dummer R, Flaherty KT, Robert C, et al. COLUMBUS 5-Year Update: A Randomized, Open-Label, Phase III Trial of Encorafenib Plus Binimetinib Versus Vemurafenib or Encorafenib in Patients With BRAF V600-Mutant Melanoma [published correction appears in *J Clin Oncol*. 2023 Apr 20;41(12):2301]. *J Clin Oncol*. 2022;40(36):4178-4188. doi:10.1200/JCO.21.02659
4. Poulidakos PI, Persaud Y, Janakiraman M, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature*. 2011;480(7377):387-390. Published 2011 Nov 23. doi:10.1038/nature10662
5. Alcalá AM, Flaherty KT. BRAF inhibitors for the treatment of metastatic melanoma: clinical trials and mechanisms of resistance. *Clin Cancer Res*. 2012;18(1):33-39. doi:10.1158/1078-0432.CCR-11-0997
6. Das Thakur M, Salangsang F, Landman AS, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature*. 2013;494(7436):251-255. doi:10.1038/nature11814

7. Seghers AC, Wilgenhof S, Lebbé C, Neyns B. Successful rechallenge in two patients with BRAF-V600-mutant melanoma who experienced previous progression during treatment with a selective BRAF inhibitor. *Melanoma Res.* 2012;22(6):466-472. doi:10.1097/CMR.0b013e3283541541
8. Dooley AJ, Gupta A, Bhattacharyya M, Middleton MR. Intermittent dosing with vemurafenib in BRAF V600E-mutant melanoma: review of a case series. *Ther Adv Med Oncol.* 2014;6(6):262-266. doi:10.1177/1758834014548187
9. Algazi AP, Othus M, Daud AI, et al. Continuous versus intermittent BRAF and MEK inhibition in patients with BRAF-mutated melanoma: a randomized phase 2 trial. *Nat Med.* 2020;26(10):1564-1568. doi:10.1038/s41591-020-1060-8
10. Gonzalez-Cao M, Mayo de Las Casas C, Oramas J, et al. Intermittent BRAF inhibition in advanced BRAF mutated melanoma results of a phase II randomized trial. *Nat Commun.* 2021;12(1):7008. Published 2021 Dec 1. doi:10.1038/s41467-021-26572-6
11. Nwolise C, Corrie P, Fitzpatrick R, et al. Burden of cancer trial participation: A qualitative sub-study of the INTERIM feasibility RCT. *Chronic Illn.* 2023;19(1):81-94. doi:10.1177/17423953211060253
12. Michielin O, Atkins MB, Koon HB, Dummer R, Ascierto PA. Evolving impact of long-term survival results on metastatic melanoma treatment. *J Immunother Cancer.* 2020;8(2):e000948. doi:10.1136/jitc-2020-000948
13. Piccart MJ, Hilbers FS, Bliss JM, et al. Road Map to Safe and Well-Designed De-escalation Trials of Systemic Adjuvant Therapy for Solid Tumors. *J Clin Oncol.* 2020;38(34):4120-4129. doi:10.1200/JCO.20.01382
14. Wan JCM, Heider K, Gale D, et al. ctDNA monitoring using patient-specific sequencing and integration of variant reads. *Sci Transl Med.* 2020;12(548):eaaz8084. doi:10.1126/scitranslmed.aaz8084
15. Aleotti V, Catoni C, Poggiana C, Rosato A, Facchinetti A, Scaini MC. Methylation Markers in Cutaneous Melanoma: Unravelling the Potential Utility of Their Tracking by Liquid Biopsy. *Cancers (Basel).* 2021;13(24):6217. doi:10.3390/cancers13246217
16. Proietti I, Skroza N, Bernardini N, et al. Mechanisms of Acquired BRAF Inhibitor Resistance in Melanoma: A Systematic Review. *Cancers (Basel).* 2020;12(10):2801. Published 2020 Sep 29. doi:10.3390/cancers12102801
17. Lee JH, Long GV, Boyd S, et al. Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma. *Ann Oncol.* 2017;28(5):1130-1136. doi:10.1093/annonc/mdx026

## **A randomised phase 2 study of intermittent versus continuous dosing of dabrafenib plus trametinib in patients with *BRAF*<sup>V600</sup> mutant advanced melanoma (INTERIM)**

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Dabrafenib; trametinib; BRAF; metastatic melanoma; intermittent dosing; continuous dosing; scheduling; circulating tumour DNA

Clinical trial registration: EudraCT 2016-005228-27; ISRCTN 18183156

## **Abstract**

### **Background**

BRAF+MEK inhibitors extend life expectancy of patients with *BRAF*<sup>V600</sup> mutant advanced melanoma. Acquired resistance limits duration of benefit, but preclinical and case studies suggest intermittent dosing could overcome this limitation. INTERIM was a phase 2 trial evaluating an intermittent dosing regimen.

### **Methods**

Patients with *BRAF*<sup>V600</sup> mutant advanced melanoma due to start dabrafenib+trametinib were randomised to receive either continuous (CONT), or intermittent (INT; dabrafenib d1-21, trametinib d1-14 every 28 days) dosing. A composite primary endpoint included progression-free survival (PFS) and quality of life (QoL). Secondary endpoints included response rate (ORR), overall survival (OS) and adverse events (AEs). Mutant *BRAF*<sup>V600E</sup> ctDNA was measured by droplet digital PCR (ddPCR), using mutant allele frequency of >1% as the detection threshold.

### **Results**

79 patients (39 INT, 40 CONT) were recruited; median age 67 years, 65% AJCC (7<sup>th</sup> ed) stage IV M1c, 29% had brain metastases. With 19 months median follow-up, INT was inferior in all efficacy measures: median PFS 8.5 vs 10.7mo (HR 1.39, 95%CI 0.79-2.45, p=0.255); median OS 18.1mo vs not reached (HR 1.69, 95%CI 0.87-3.28, p=0.121), ORR 57% vs 77%. INT patients experienced fewer treatment-related AEs (76% vs 88%), but more grade  $\geq 3$  AEs (53% vs 42%). QoL favoured CONT. Detection of *BRAF*<sup>V600E</sup> ctDNA prior to treatment correlated with worse OS (HR 2.55, 95%CI 1.25-5.21, p=0.01) in both arms. A change to undetected during treatment did not significantly predict better OS.

### **Conclusion**

INTERIM findings are consistent with other recent clinical trials reporting that intermittent dosing does not improve efficacy of BRAF+MEK inhibitors.

## **Background**

Just under 50% of all melanomas harbour a *BRAF*<sup>V600</sup> gene mutation. BRAF+MEK inhibitors have been shown to extend life of patients with *BRAF*<sup>V600</sup> mutant advanced melanoma compared with standard of care in several international registration clinical trials<sup>1-3</sup>. Median life expectancy of treated patients is now approaching 3 years. However, most patients progress despite treatment due to acquired resistance.

Continuous daily dosing of oral kinase inhibitors may promote clonal expansion of drug resistant cells and intermittent drug dosing was proposed as a strategy to delay the onset of disease progression<sup>4,5</sup>. In a mouse model, vemurafenib-resistant tumour cells were shown to become drug-dependent<sup>6</sup>. Resistant cells suffered a fitness deficit in the absence of drug and mice survived twice as long (200 days compared with 100 days) on an intermittent dosing schedule of vemurafenib (4 weeks on 2 weeks off) compared with continuous daily dosing. Clinical case reports also suggested that interrupted dosing of BRAF inhibitors can reverse resistance to the drugs<sup>7</sup> and improve tolerability<sup>8</sup>.

Three randomised phase 2 trials have been conducted to test whether pre-planned intermittent dosing of oral BRAF targeted agents may provide a means of sustaining patients on treatment for longer. The first 2 studies, one conducted in the USA<sup>9</sup>, the other in Spain<sup>10</sup>, have published their findings and both failed to demonstrate a benefit with intermittent dosing. The UK INTERIM study is the third study to test this concept.

## **Methods**

### **Patients**

Patients aged  $\geq 18$  years with AJCC (7<sup>th</sup> edition) stage III unresectable or stage IV *BRAF*<sup>V600</sup> mutant advanced melanoma and Eastern Cooperative Oncology Group performance status (ECOG PS) 0-2 due to start dabrafenib+trametinib were eligible to take part in the INTERIM trial. Other key entry criteria were measurable disease prior to randomisation, life expectancy  $\geq 12$  weeks,

adequate bone marrow and liver function, and no prior BRAF or MEK inhibitors; presence of brain metastases and prior immunotherapy were allowed. The INTERIM trial protocol (ISRCTN18183156) was approved by Cambridge South Research Ethics Committee (ref: 17/EE/0340) and was performed in accordance with the Declaration of Helsinki and the European Clinical Trials Directives 2001/20/EC. All patients provided written informed consent. Grant funding was provided by the NIHR Research for Patient Benefit Programme. The funder had no role in the study design, data collection, analysis, interpretation, or writing of the report.

### **Treatment**

Consenting patients were randomised 1:1 using the minimisation with random element method to receive standard doses of dabrafenib (150mg bid) and trametinib (2mg od) either continuously (CONT; dabrafenib+trametinib d1–28) or intermittently (INT; dabrafenib d1-21 + trametinib d1–14 on a 28-day cycle). Stratification factors were ECOG PS, AJCC stage (IIIC/IVM1a/IVM1b/IVM1c), presence or absence of brain metastases and baseline blood lactate dehydrogenase (LDH) level ( $\leq$  upper limit of normal range (ULN); between  $>$ ULN and  $\leq 2 \times$  ULN;  $>2 \times$  ULN). Patients were allowed to continue on treatment beyond radiological disease progression if they were deriving clinical benefit, or if progressive disease was resectable.

### **Study procedures**

Patients were clinically assessed every 2 weeks during the initial 2 cycles, then prior to the beginning of every cycle until inoperable disease progression, then 3-monthly for a minimum of 9 months follow-up from their randomisation date. Adverse events (AEs) were recorded using NCI CTCAE version 4.03 for up to 30 days after the last dose of protocol treatment and resolution of all  $\geq$  grade 2 AEs. Quality of life (QoL) was assessed using the EORTC QLQ-C30 and EQ5D-L questionnaires every 12 weeks before disease progression and every 3 months with a minimum of 9 months after progression since the date of randomisation. Imaging was performed at baseline, 6 weeks after starting treatment and then every 8 weeks until disease progression to assess the objective response rate (ORR) using RECIST V1.1. Research blood samples

to measure plasma *BRAF*<sup>V600E</sup>ctDNA were collected from all patients on cycle 1 d1 (prior to starting treatment) and 15, cycle 2 d1 and 15, cycle 3 d1 and then at the start of alternate cycles until disease progression, as well as on disease progression.

### **Plasma ctDNA assay**

DNA was extracted from plasma using the Qiagen Qiasymphony SP instrument, QIASymphony DSP Circulating DNA kit and quantified using the Qubit fluorometer 4.0 and the Qubit™ dsDNA HS kit. The Droplet Digital PCR (ddPCR) *BRAF*<sup>V600E</sup> test was used to detect a sequence variant that was present at a very low frequency in a pool of wild-type backgrounds with the QX200™ Droplet Digital™ PCR System. Following PCR, each droplet was analysed or read in a flow cytometer to determine the fraction of PCR-positive droplets in the original sample. Data were then analysed using Poisson statistics to determine the target DNA template concentration in the original sample.

### **Study endpoints and statistical analysis**

A composite primary endpoint of this feasibility study was designed to 1) measure clinical efficacy by comparing progression-free survival (PFS) in the 2 arms; 2) evaluate patient QoL and 3) assess recruitment rate, as well as treatment compliance with intermittent dosing. Secondary endpoints were objective response rate (ORR), overall survival (OS) and toxicity. An exploratory analysis of *BRAF*<sup>V600E</sup>ctDNA was undertaken to explore its prognostic and predictive utility, measured by ddPCR, using mutant allele frequency of >1% as the detection threshold.

Patient experience of taking part in the trial was explored in a qualitative substudy, which has been fully published elsewhere<sup>11</sup>.

For PFS, the intended sample size was 100 patients (50 patients in each arm), to be recruited in 30 months with a minimum of 9 months follow-up to have a total of 80 PFS events, from up to 20 UK sites. The sample size was compatible with an anticipated hazard ratio (HR) for PFS of 0.75 in favour of the intermittent

arm, which would provide some evidence of the clinical efficacy of the intermittent arm. More details on the sample size calculation are provided in the supplementary materials.

Recruitment rate and compliance in the intermittent arm were of interest in case of planning a future phase III trial. The aim was to demonstrate recruitment of 2 patients per site per month when all sites were up and running. Compliance was defined as the percentage of patients completing the allocated treatment at 6 months from the date of randomisation and was compared in both arms.

Efficacy and safety analyses were based on the intention-to-treat (ITT) principle and patients who had at least one dose of trial treatment. The log-rank test was applied to compare PFS and OS between arms, and ORR was compared using the Chi-squared test. The subscales of EORTC QLQ-C30 and EQ-5D-5L questionnaires were derived according to standard-scoring manuals. Analyses of changes from baseline over time and differences between the 2 arms for subscales were carried out with repeated measures using ANCOVA, adjusting for baseline level, time, treatment and interaction of time and treatment. Kaplan–Meier plots were generated for PFS and OS. Odds ratios (ORs) were estimated using logistic regression models and HRs with Cox regression models.

## **Results**

### **Patients and treatment delivery**

From December 2017 to February 2020, 79 patients were recruited at 19 UK sites at an average recruitment rate of 3 patients per month across all sites; 39 patients were randomised to the intermittent [INT] schedule arm and 40 to the continuous [CONT] schedule arm (Consort diagram is provided in Supplementary **Figure S1**). The study closed to recruitment prematurely in March 2020 due to the onset of the COVID-19 pandemic and follow up was stopped in September 2021 with a median follow up of 19 months

Patient demographics and baseline characteristics were well balanced (**Table 1**). Median patient age was 67 (range 34-85) years, 88% were ECOG PS 0-1,

65% were AJCC stage 4M1c, 29% had brain metastases and 46% had LDH > ULN. One patient in the INT arm was found to be ineligible after randomisation and did not receive any trial treatment. 24 (63%) patients in the INT arm and 31 (78%) patients in the CONT arm completed at least 6 cycles of treatment. Four patients in the INT arm switched to continuous dosing during the study period, no patients in the CONT arm switched to intermittent dosing regimen.

The median treatment duration was 8.5 (interquartile range [IQR] 14.8) months in the INT arm and 11.3 (IQR 9.6) months in the CONT arm.

	INT (n=39)	CONT (n=40)	All (n=79)
<b>Age (years)</b>			
Median (range)	69 (34-85)	62 (38-78)	67 (34-85)
<b>Gender</b>			
Female	22 (56%)	21 (52%)	43 (54%)
Male	17 (44%)	19 (48%)	36 (46%)
<b>ECOG performance status</b>			
0	19 (49%)	19 (48%)	38 (48%)
1	15 (38%)	17 (42%)	32 (40%)
2	5 (13%)	4 (10%)	9 (11%)
<b>AJCC (7<sup>th</sup> ed) stage</b>			
IIIC	2 (5%)	1 (2%)	3 (4%)
IVM1a	5 (13%)	6 (15%)	11 (14%)
IVM1b	6 (15%)	8 (20%)	14 (18%)
IVM1c	26 (67%)	25 (62%)	51 (65%)
<b>Presence of brain metastases</b>			
No	29 (74%)	27 (68%)	56 (71%)
Yes	10 (26%)	13 (32%)	23 (29%)
<b>LDH relative to ULN</b>			
≤ ULN	21 (54%)	22 (55%)	43 (54%)
> ULN and ≤ 2 x ULN	13 (33%)	14 (35%)	27 (34%)
> 2 x ULN	5 (13%)	4 (10%)	9 (11%)
<b>Tissue BRAF<sup>V600E</sup> status *</b>			
Confirmed	29 (74%)	26 (65%)	55 (70%)
Unconfirmed	10 (26%)	14 (35%)	24 (30%)
<b>Plasma ctDNA Positive for BRAF<sup>V600E</sup></b>			
Confirmed	19 (59%)	21 (62%)	40 (61%)
Unconfirmed	13 (41%)	13 (38%)	26 (39%)
<b>ctDNA BRAF<sup>V600E</sup> mutant allele frequency &gt;1%</b>			
Detected	15 (47%)	12 (35%)	27 (41%)
Not detected	17 (53%)	22 (65%)	39 (59%)
<b>Previous systemic therapy</b>			
CPI <sup>†</sup> (1st line metastatic)	13 (33%)	17 (42%)	30 (38%)
CPI (adjuvant)	4 (10%)	7 (18%)	11 (14%)

	INT (n=39)	CONT (n=40)	All (n=79)
CPI (unknown)	1 (3%)	1 (2%)	2 (3%)
Avastin (adjuvant)	1 (3%)	0 (0%)	1 (1%)

\* All patients had tumour BRAF<sup>V600</sup> mutation confirmed prior to study entry; only a proportion had a confirmed specific BRAF<sup>V600E</sup> variant confirmed

† CPI = antiPD1+/-antiCTLA4 checkpoint inhibitor; no patients received adjuvant BRAF targeted therapy

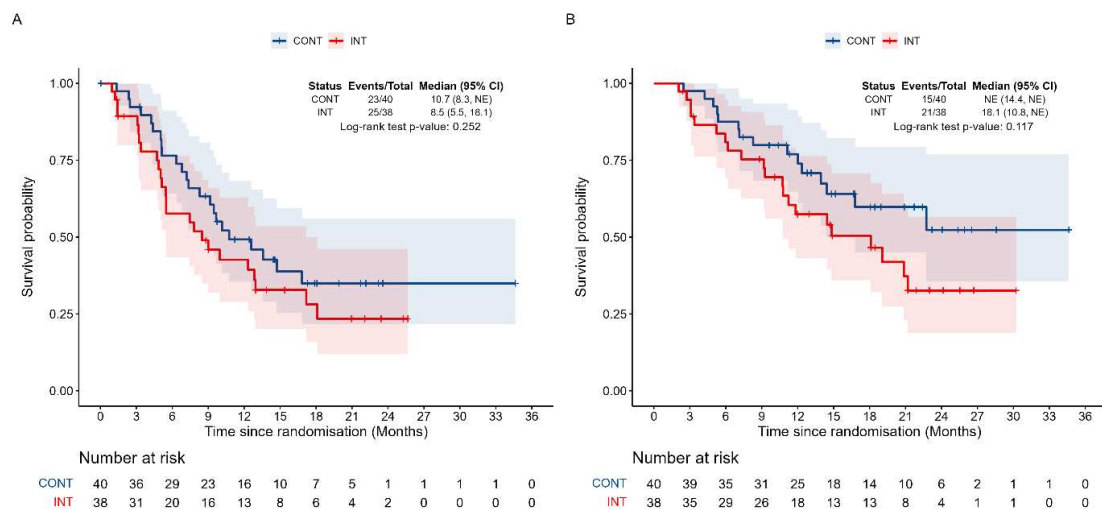
**Table 1.** Patient baseline characteristics

## Efficacy

With a median follow-up of 19 months, 48 patients (25 INT, 23 CONT) had progressed and 35 died (21 INT, 15 CONT). Median PFS was 8.5 months (95% CI 5.5-18) in the INT arm and 10.7 months (95% CI 5.8-not reached) in the CONT arm, HR 1.39 (95% CI 0.79–2.45,  $p = 0.255$ ) (**Figure 1A**). Median OS was 18 months (95% CI 10.8 to not reached) in the INT arm and not reached in the CONT arm, HR 1.69 (95% CI 0.87-3.28,  $p = 0.121$ ) (**Figure 1B**).

Twenty-two patients (13 CONT and 9 INT) who had at least one dose of trial treatment had brain metastases. In this study, prior treatment of brain metastases was not a requirement, but presence/absence of brain metastases was a stratification factor. As expected, patients with brain metastases had poorer OS compared with those who did not (HR 2.1, 95% CI 1.1-4.1). The numbers of patients with brain metastases were small, but the impact of the different treatment schedules was similar to what we observed in the whole population. There was no evidence to suggest that intermittent dosing impacted outcomes of patients with brain metastases differently compared with those without brain metastases.

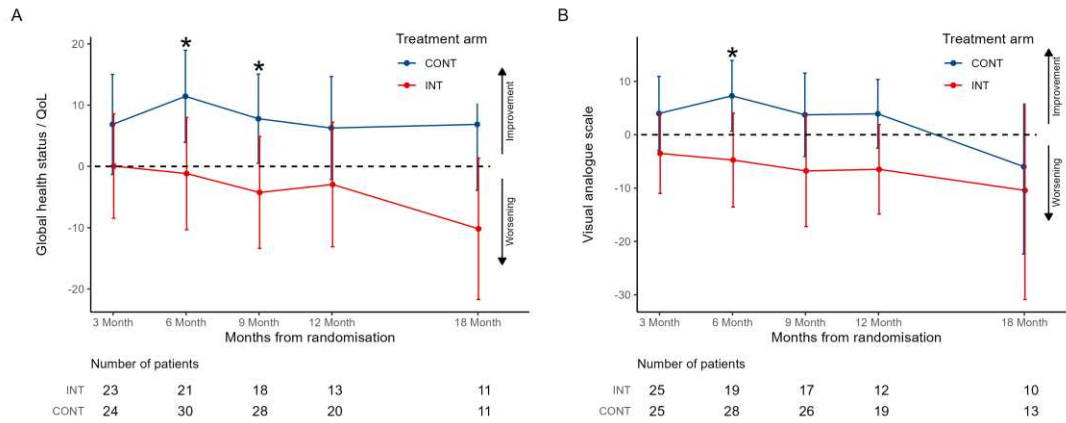
Seventy-two patients (37 INT, 35 CONT) were evaluable for response using RECIST V1.1 criteria, assessed by local investigators. ORR was higher in the CONT arm (77% versus 57%,  $p = 0.069$ ). Complete and partial responses were 23% and 54% in the CONT arm and 16% and 41% in the INT arm. The median time to treatment failure (time from starting treatment to discontinuation for any reason) was 7.9 months in the INT arm and 10.7 months in the CONT arm (HR 1.26, 95% CI 0.82-2.32,  $p = 0.26$ ).



**Figure 1.** Kaplan-Meier curves of (A) progression-free survival and (B) overall survival for patients who have received at least one dose of treatment on the intermittent (INT) or continuous (CONT) dosing schedule.

### Quality of life

Seventy-two patients completed baseline EORTC QLQ-C30 questionnaires (34 INT, 38 CONT). The number of patients who completed questionnaires decreased over time, as expected (**Figure 2A**). The mean QLQ-C30 global health status was consistently higher for the CONT arm compared with the INT arm throughout the study period. At month 6, the change in global health status compared to baseline was +11.44 for CONT and -1.16 for INT (95% CI 0.73-24.5,  $p = 0.038$ ). The favourable QoL with CONT vs INT was also evident using the EQ-5D-5L visual analogue scale (+7.29 for CONT and -4.75 for INT at 6 months) (**Figure 2B**).



**Figure 2.** Least-squares mean change from baseline of EORTC QLQ-C30 global health status (**A**) and EQ-5D-5L visual analogue scale (**B**). Asterisk indicates a statistical difference between the treatment arms of  $p < 0.05$ .

### Safety and toxicity

78 (38 INT and 40 CONT) patients were evaluated for safety. The proportion of patients with any AE was slightly higher in the CONT arm (92% versus 87%), but patients in the INT arm reported more grade  $\geq 3$  AEs (53% versus 42%) (**Table 2**). The CONT arm experienced more treatment-related AEs (TRAEs) (88% versus 76% any grade; 48% versus 37% grade  $\geq 3$ ), but more patients in the INT arm experienced SAEs (45% versus 38%). A detailed breakdown of AEs is provided in Supplementary **Table S1**.

	INT (n=38)	CONT (n=40)
Any AE	33 (87%)	37 (92%)
AE grade $\geq 3$	20 (53%)	17 (42%)
Treatment-related AE	29 (76%)	35 (88%)
Treatment-related AE grade $\geq 3$	14 (37%)	19 (48%)
AE attributed to dabrafenib	29 (76%)	35 (88%)
AE attributed to trametinib	26 (68%)	33 (82%)
AE related to underlying disease	18 (47%)	24 (60%)
Any SAE	17 (45%)	15 (38%)
Any Hospitalization	21 (55%)	19 (48%)

**Table 2.** Summary of adverse events (AEs) in patients who received at least one dose of trial treatment

Fifty-nine (76%) patients (28 INT, 31 CONT) had a treatment interruption during the first 6 cycles, AEs were the most common reason (23 INT, 27 CONT) for

dose interruption, patient compliance being the second most common reason (10 INT, 13 CONT). The most common reason for stopping treatment early was disease progression (15 INT, 13 CONT) and lack of clinical benefit (6 INT, 4 CONT). Seven patients stopped treatment due to unacceptable toxicity (5 INT, 2 CONT).

There was no significant difference in the number of patients who achieved at least 80% of planned dose intensity for both dabrafenib (28 INT, 26 CONT,  $p=0.41$ ) and trametinib (32 INT, 30 CONT,  $p=0.31$ ).

### **ctDNA analysis**

Baseline, pre-treatment plasma samples were available for 66 patients to undertake *BRAF*<sup>V600E</sup>ctDNA analysis. Droplet digital PCR identified *BRAF*<sup>V600E</sup>ctDNA in 40/66 (61%) samples. The median mutant allele frequency was 7.8 (range 0.02-67.3). Using mutant allele frequency >1% as the cut-off, *BRAF*<sup>V600E</sup>ctDNA was considered as detectable in 27 (41%) patients at baseline. ctDNA detection correlated with higher disease burden, as determined by plasma LDH level >ULN ( $p<0.001$ ) (**Table 3**).

Baseline *BRAF*<sup>V600E</sup>ctDNA detection did not predict for ORR, but there was an apparent correlation with PFS, which was much more robust for OS (**Figure 3**). Patients with detected *BRAF*<sup>V600E</sup>ctDNA had a worse survival outcome with HR ranging from 2.55 (95% CI 1.25-5.21,  $p$ -value=0.01) in an unadjusted model to 2.91 (95% CI 1.25-6.75,  $p$ -value=0.013) in a model incrementally adjusted for treatment arm, and stratification factors (**Table 3**). Worse outcomes were also seen when stratifying for serum LDH levels above and below normal range (Supplementary **Figure S3** and **Table S3**). The data suggested that disease burden (as determined by LDH level) was likely to be a stronger predictor of survival outcomes rather than BRAF/MEKi dosing schedule.

There was no interaction between baseline *BRAF*<sup>V600E</sup>ctDNA detection status and treatment arm for PFS or OS. When inspecting the data summarized in Figures 3B and 3D, it appears that patients with detectable ctDNA at baseline

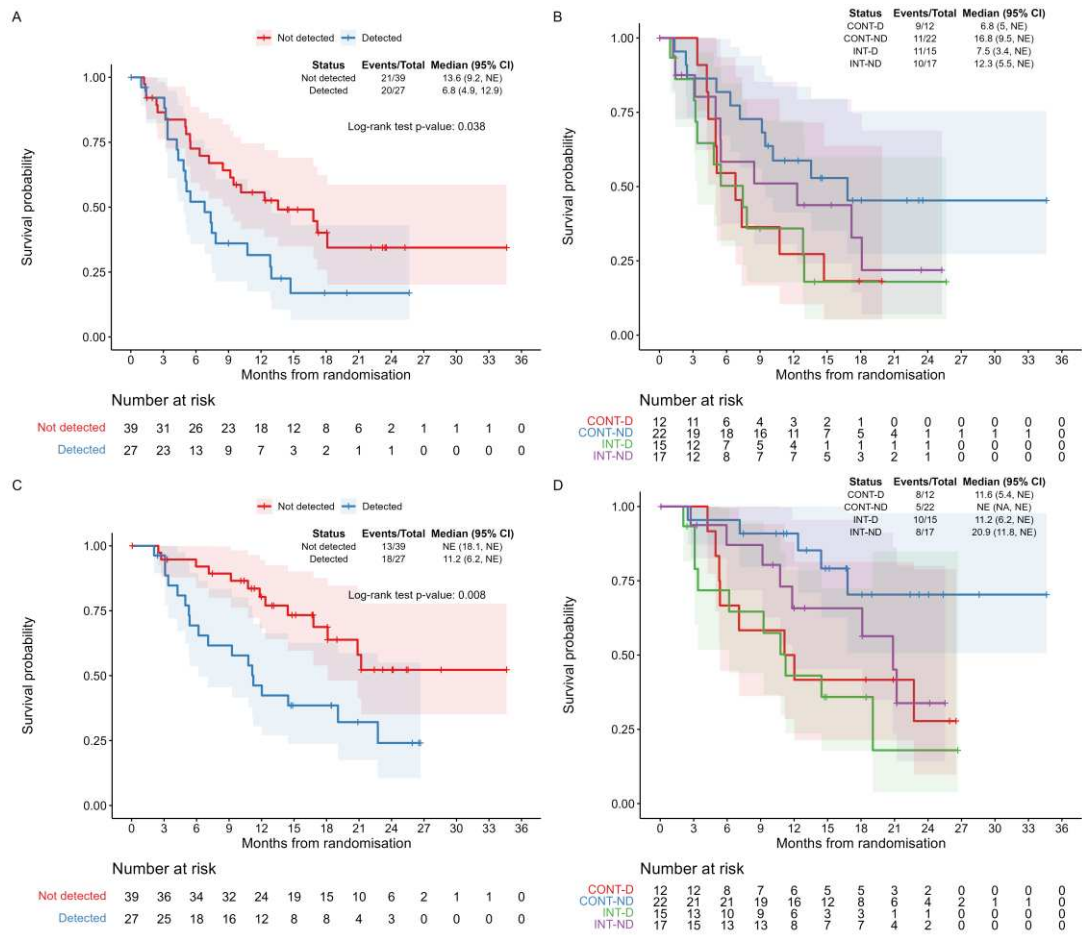
had lower PFS and OS probability regardless of their treatment arm. This was confirmed in a re-analysis where the p-value for interaction between baseline ctDNA detection status and treatment arm was 0.125 and 0.285 for PFS and OS.

	<b>Detected (N=27)</b>	<b>Not detected (N=39)</b>	<b>P-value</b>
<b>ECOG PS</b>			
0	10 (37.0%)	20 (51.3%)	0.373
1-2	17 (63.0%)	19 (48.7%)	
<b>AJCC stage</b>			
4M1c	22 (81.5%)	22 (56.4%)	0.0631
Others	5 (18.5%)	17 (43.6%)	
<b>Brain metastases</b>			
No	20 (74.1%)	26 (66.7%)	0.71
Yes	7 (25.9%)	13 (33.3%)	
<b>LDH</b>			
≤ ULN	6 (22.2%)	30 (76.9%)	<0.001
> ULN	21 (77.8%)	9 (23.1%)	
<b>ORR at Week 16</b>	11 (57.9%*)	16 (53.3%**)	0.754
<b>Relapse<sup>†</sup></b>			
Unadjusted	1.91 (1.03, 3.54)	reference	0.041
Model 1	1.82 (0.97, 3.40)	reference	0.061
Model 2	2.01 (0.99, 4.09)	reference	0.053
<b>Death<sup>†</sup></b>			
Unadjusted	2.55 (1.25, 5.21)	reference	0.010
Model 1	2.43 (1.19, 4.98)	reference	0.015
Model 2	2.91 (1.25, 6.75)	reference	0.013

\* assessed for 19 patients; \*\* assessed for 30 patients.

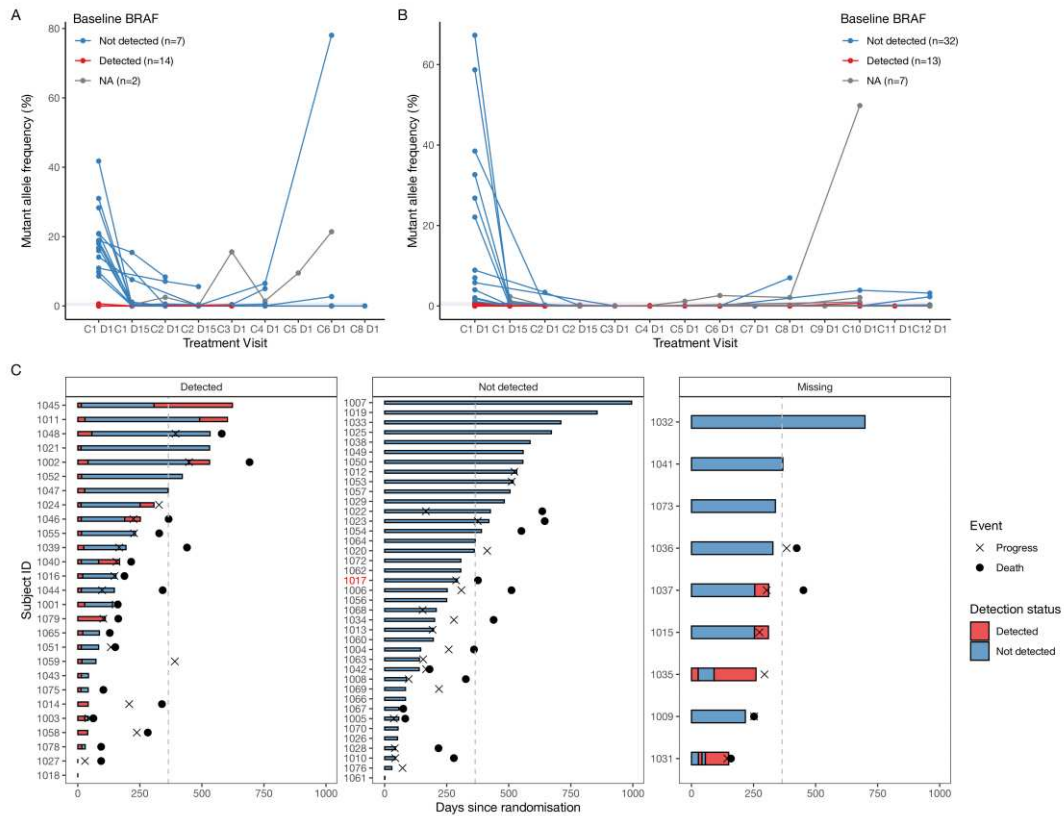
<sup>†</sup> HR for relapse and death were determined by Cox model, with not detected as reference. Model 1 adjusted for treatment arm; Model 2 adjusted for Model 1 + ECOG PS, AJCC stage and presence of brain metastases. LDH was not included due to the co-linearity with ctDNA.

**Table 3.** Correlation of baseline plasma *BRAF*<sup>V600E</sup> ctDNA detection status with patient characteristics and treatment outcome



**Figure 3.** Progression-free survival according to baseline  $BRAF^{V600E}$ ctDNA detection status (A) and treatment arm plus  $BRAF^{V600E}$ ctDNA detection status (B); Overall survival according to baseline  $BRAF^{V600E}$ ctDNA detection status (C) and treatment arm plus  $BRAF^{V600E}$ ctDNA detection status (D)

Longitudinal measurement of ctDNA mutant allele frequency identified step drops within the first cycle of treatment for patients with detectable ctDNA at baseline irrespective of treatment arm (**Figure 4A**). Measurement of ctDNA every 2 weeks for the first 8 weeks of treatment in both arms confirmed there was no evidence of a rebound in ctDNA levels associated with intermittent dosing (Supplementary **Table S4**). A change from detectable to undetectable ctDNA did not influence treatment outcome (Supplementary **Table S5**). Disease progression was associated with emergence of ctDNA detection in only 3 patients with baseline detectable ctDNA and 1 patient with baseline undetectable ctDNA (**Figure 4C**). There was no clear pattern in ctDNA dynamic changes with PFS or OS.



**Figure 4.** *BRAF*<sup>V600E</sup>ctDNA mutant allele frequency measured at different time points for (A) patients who died within 1 year of starting treatment and (B) patients who were alive beyond 1 year of starting treatment (red = detected; blue = not detected). Swimmer plots (C) summarising changes of plasma *BRAF*<sup>V600E</sup>ctDNA detection status since starting treatment. Grey dashed line indicates 1 year since randomisation. No ctDNA was detected for patient 1017 at baseline, but became detectable on disease progression.

## Discussion

The UK INTERIM study did not meet its primary endpoint of PFS improvement with intermittent dosing of dabrafenib+trametinib. All main study end-points including PFS, OS, ORR and QoL favoured standard continuous dosing. Although the frequency of adverse events was higher with continuous dosing, the seriousness and grading of adverse events were worse with intermittent dosing.

Two other studies evaluating different intermittent dosing schedules of BRAF+MEKi have demonstrated similar inferior outcomes compared with standard continuous dosing. The first USA SWOG study<sup>9</sup> was designed so that

patients received 8 weeks continuous dosing with dabrafenib+trametinib and only those patients who were progression-free were randomized to either continuous or intermittent dosing of both drugs on a 3 weeks off, 5 weeks on schedule. 206 patients were recruited between 2014 and 2019 and 206 went on to be randomised. Post-randomisation PFS was greater with continuous treatment (median PFS 9.0 vs 5.5 months, HR 1.36, p=0.06). Overall survival of 29.2 months was identical in both arms. The second, smaller study (N=70) conducted by the Spanish Melanoma Group<sup>10</sup> evaluated a different BRAF+MEKi combination, vemurafenib+cobimetinib and again initiated treatment with a continuous dosing run-in, this time lasting 12 weeks. Thereafter, the intermittent schedule comprised vemurafenib 4 weeks on, 2 weeks off and cobimetinib 3 weeks on 3 weeks off. Median PFS was 16.2 months vs 6.9 months (p=0.079), favouring continuous dosing. No statistically significant differences were observed in OS.

INTERIM is the first study to test intermittent dosing from the point of initiating BRAF+MEKi. However, this strategy neither conferred advantage, nor disadvantage compared with a continuous dose run-in, with PFS favouring standard scheduling and no significant difference on OS. Furthermore, the safety profile as measured by frequency of grade  $\geq 3$  treatment-related AEs was remarkably consistent across all 3 trials: 48% CONT and 37% INT in the INTERIM study; 43% CONT and 34% INT in the US study; 43% CONT and 40% INT in the Spanish study. The choice of the intermittent dosing schedule tested in INTERIM took into account both pharmacokinetic and pharmacodynamic considerations, as well as anticipated acceptability to patients and physicians. Dabrafenib has a 5.2 hour half-life, but generates several active metabolites which persist, while trametinib has a mean terminal half-life of 5.3 days. Taken together, the preclinical data suggested that a minimum of 1 week break from BRAF inhibitor and 2 weeks break from MEK inhibitor would be required to relieve inhibition of the MAP kinase pathway. Even so, consistent with previous investigators<sup>9,10</sup>, we have been unable to replicate the beneficial effects of intermittent BRAFi dosing seen in mice, with no clear biological explanation for this failure. The preclinical data was limited to a single animal model, but supported by retrospective patient data and case

reports. Whether additional preclinical modelling would have obviated the need to undertake multiple prospective clinical trials, which are both time and resource consuming, is an unknown question.

Data interpretation from the INTERIM study might be considered to be hampered somewhat because the intended sample size of 100 patients was not reached. The anticipated recruitment rate was not met largely because of the changing melanoma treatment landscape, and BRAF targeted therapy was replaced by immune checkpoint inhibitors as first line therapy for metastatic disease for most patients during the course of this trial<sup>12</sup>. Recruitment to treatment de-escalation trials is challenging, needing both investigators and patients to be in equipoise<sup>13</sup>. Our qualitative substudy exploring patient experience of being offered and treated in the INTERIM trial<sup>11</sup> found that, despite verbal and written information being given, some still struggled to understand the goal of treatment *per se*, as well as the meaning of randomisation. Challenges with recruitment led us to create a Youtube video with the help of patient representatives aimed at helping potential participants make a better informed decision more confidently (<https://www.youtube.com/watch?v=ulxyNH-8aGc>). Even so, the decision to close the study early was taken as the first wave of the COVID-19 pandemic impacted clinical practice and ability to maintain clinical trial recruitment in the UK. Despite limited sample size, there is no suggestion from analysing the INTERIM patient cohort of 78 patients (excluding 1 patient with no trial treatment given) that the outcomes would have been different should our larger target recruitment have been met.

Our exploratory analysis of *BRAF*<sup>V600</sup>ctDNA using ddPCR demonstrated that, in those patients with detectable ctDNA at baseline, levels fell rapidly during the first cycle of treatment and levels remained low during the first 8 weeks of treatment in both arms, with no evidence of any rebound effect in the intermittent arm while patients were off treatment. ctDNA monitoring during treatment gave no other clues regarding any differences in biological behaviour of the 2 arms and is likely to reflect the limitations of ddPCR as a predictive tool for measuring ctDNA with clinical application, at least in the context of

metastatic melanoma. Technologies utilising wider, patient-specific panels<sup>14</sup> or measuring DNA methylation<sup>15</sup> which are in development might be more informative. Time-based intermittent dosing did not yield differences in ctDNA dynamics in our small population. This leaves open the possibility that biologically-driven intermittent dosing, for example according to changes in ctDNA, might still have merit. The UK DYNAMIC clinical trial (ISRCTN14643179) is using changes in detection of *BRAF*<sup>V600</sup>ctDNA by ddPCR measured intermittently during BRAF targeted treatment to direct decisions whether to continue or interrupt dosing. This trial is likely to be the ultimate test of whether intermittent dosing of BRAF-targeted therapy has any clinical relevance.

In most cases, progression in our patient cohort was not associated with reappearance of *BRAF*<sup>V600</sup>ctDNA. Secondary resistance is typically characterised by emergence of other new mutations and/or gene amplifications, which would require to be detected using broader sequencing technologies<sup>16</sup>. On the other hand, we have confirmed, as have others previously<sup>17</sup>, that detection of *BRAF*<sup>V600</sup>ctDNA pre-treatment is a strong poor prognostic indicator, being associated with shorter patient survival, irrespective of treatment arm. Even so, the strong correlation with high disease burden as measured by a readily available, simple and cheap LDH blood test, means adoption of ctDNA as a routine test remains undefined, until such time as a clinically meaningful application can be demonstrated.

## **Conclusion**

In summary, 3 randomised trials testing different intermittent BRAF+MEKi intermittent dosing schedules have now failed to show any patient benefit, so continuous dosing regimens remain standard of care.

## **Acknowledgements**

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1215-20008). The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care. We thank all the site research staff for their help in recruiting and supporting the patients who took part in this trial, as well as the patients themselves. Thanks to the UK Melanoma Focus charity for filming the YouTube video, to Simon Rodwell for acting as executive producer and writing the script, to Mrs Doreen Milne for contributing to script writing and participating in its creation, to Mrs Claire Mather for project managing and appearing in the film, and to Mrs Debra Blackstone for appearing in the film.

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### **Author contributions**

PC, MM, AG & RM conceived the clinical trial; AD, JW & NW provided statistical support; JN, RB, MP, AR, AF, SD, BE, JC, CHB, NS, MB, EB, MG, MH, LP, SK, AW, AG & PC recruited the patients; GB, LD & EB undertook the ctDNA analyses; ST led study coordination; PC & AD led manuscript writing; all authors contributed to data interpretation and approved the final manuscript.

### **Declaration of competing interest**

The authors have declared no conflicts of interest associated with the submitted work.

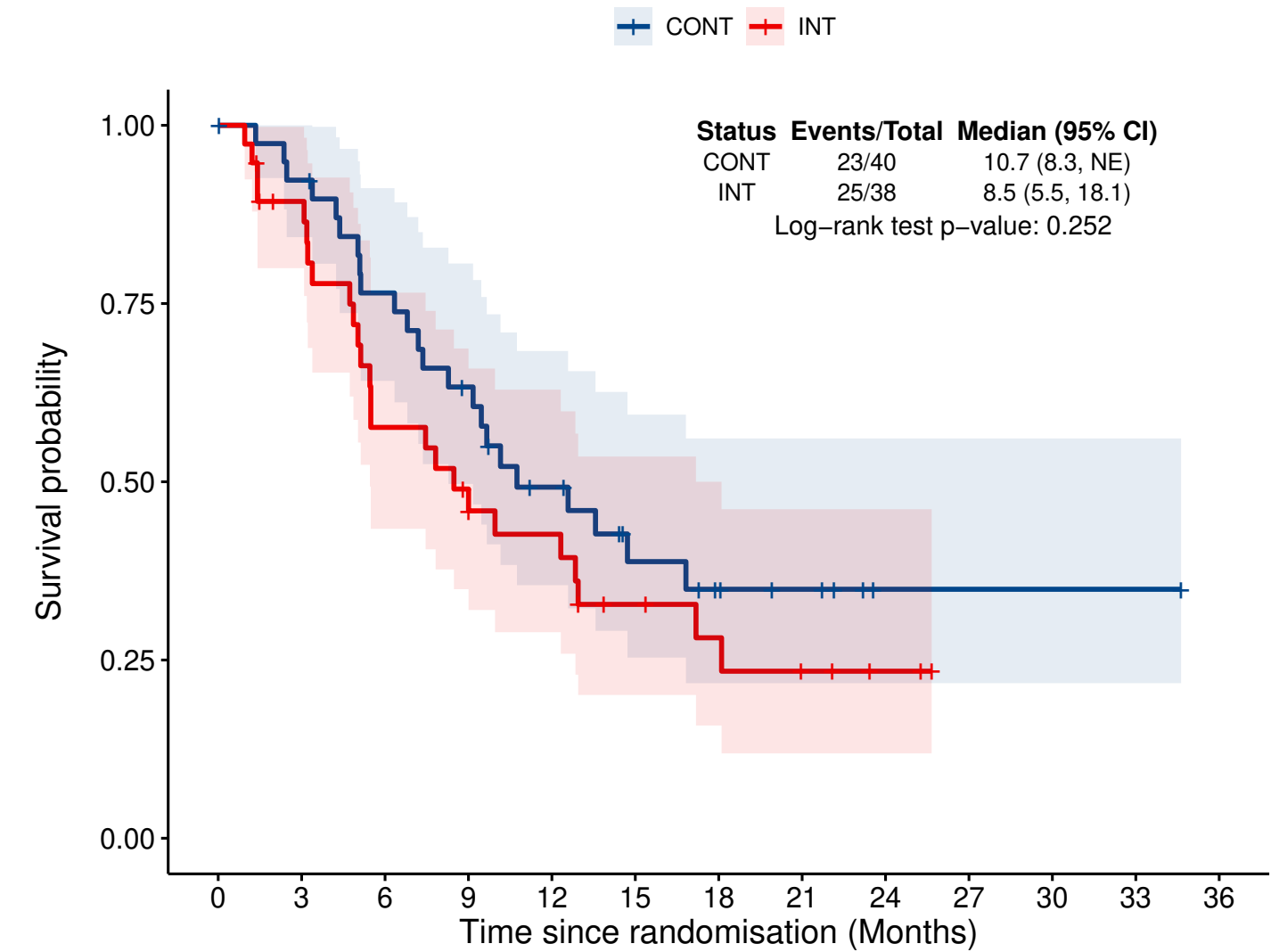
## References

1. Robert C, Grob JJ, Stroyakovskiy D, et al. Five-Year Outcomes with Dabrafenib plus Trametinib in Metastatic Melanoma. *N Engl J Med*. 2019;381(7):626-636. doi:10.1056/NEJMoa1904059
2. Ascierto PA, Dréno B, Larkin J, et al. 5-Year Outcomes with Cobimetinib plus Vemurafenib in BRAFV600 Mutation-Positive Advanced Melanoma: Extended Follow-up of the coBRIM Study. *Clin Cancer Res*. 2021;27(19):5225-5235. doi:10.1158/1078-0432.CCR-21-0809
3. Dummer R, Flaherty KT, Robert C, et al. COLUMBUS 5-Year Update: A Randomized, Open-Label, Phase III Trial of Encorafenib Plus Binimetinib Versus Vemurafenib or Encorafenib in Patients With BRAF V600-Mutant Melanoma [published correction appears in *J Clin Oncol*. 2023 Apr 20;41(12):2301]. *J Clin Oncol*. 2022;40(36):4178-4188. doi:10.1200/JCO.21.02659
4. Poulidakos PI, Persaud Y, Janakiraman M, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature*. 2011;480(7377):387-390. Published 2011 Nov 23. doi:10.1038/nature10662
5. Alcalá AM, Flaherty KT. BRAF inhibitors for the treatment of metastatic melanoma: clinical trials and mechanisms of resistance. *Clin Cancer Res*. 2012;18(1):33-39. doi:10.1158/1078-0432.CCR-11-0997
6. Das Thakur M, Salangsang F, Landman AS, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature*. 2013;494(7436):251-255. doi:10.1038/nature11814
7. Seghers AC, Wilgenhof S, Lebbé C, Neyns B. Successful rechallenge in two patients with BRAF-V600-mutant melanoma who experienced previous progression during treatment with a selective BRAF inhibitor. *Melanoma Res*. 2012;22(6):466-472. doi:10.1097/CMR.0b013e3283541541
8. Dooley AJ, Gupta A, Bhattacharyya M, Middleton MR. Intermittent dosing with vemurafenib in BRAF V600E-mutant melanoma: review of a case series. *Ther Adv Med Oncol*. 2014;6(6):262-266. doi:10.1177/1758834014548187
9. Algazi AP, Othus M, Daud AI, et al. Continuous versus intermittent BRAF and MEK inhibition in patients with BRAF-mutated melanoma: a randomized phase 2 trial. *Nat Med*. 2020;26(10):1564-1568. doi:10.1038/s41591-020-1060-8
10. Gonzalez-Cao M, Mayo de Las Casas C, Oramas J, et al. Intermittent BRAF inhibition in advanced BRAF mutated melanoma results of a phase II

randomized trial. *Nat Commun.* 2021;12(1):7008. Published 2021 Dec 1.  
doi:10.1038/s41467-021-26572-6

11. Nwolise C, Corrie P, Fitzpatrick R, et al. Burden of cancer trial participation: A qualitative sub-study of the INTERIM feasibility RCT. *Chronic Illn.* 2023;19(1):81-94. doi:10.1177/17423953211060253
12. Michielin O, Atkins MB, Koon HB, Dummer R, Ascierto PA. Evolving impact of long-term survival results on metastatic melanoma treatment. *J Immunother Cancer.* 2020;8(2):e000948. doi:10.1136/jitc-2020-000948
13. Piccart MJ, Hilbers FS, Bliss JM, et al. Road Map to Safe and Well-Designed De-escalation Trials of Systemic Adjuvant Therapy for Solid Tumors. *J Clin Oncol.* 2020;38(34):4120-4129. doi:10.1200/JCO.20.01382
14. Wan JCM, Heider K, Gale D, et al. ctDNA monitoring using patient-specific sequencing and integration of variant reads. *Sci Transl Med.* 2020;12(548):eaaz8084. doi:10.1126/scitranslmed.aaz8084
15. Aleotti V, Catoni C, Poggiana C, Rosato A, Facchinetti A, Scaini MC. Methylation Markers in Cutaneous Melanoma: Unravelling the Potential Utility of Their Tracking by Liquid Biopsy. *Cancers (Basel).* 2021;13(24):6217. doi:10.3390/cancers13246217
16. Proietti I, Skroza N, Bernardini N, et al. Mechanisms of Acquired BRAF Inhibitor Resistance in Melanoma: A Systematic Review. *Cancers (Basel).* 2020;12(10):2801. Published 2020 Sep 29. doi:10.3390/cancers12102801
17. Lee JH, Long GV, Boyd S, et al. Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma. *Ann Oncol.* 2017;28(5):1130-1136. doi:10.1093/annonc/mdx026

Figure 1

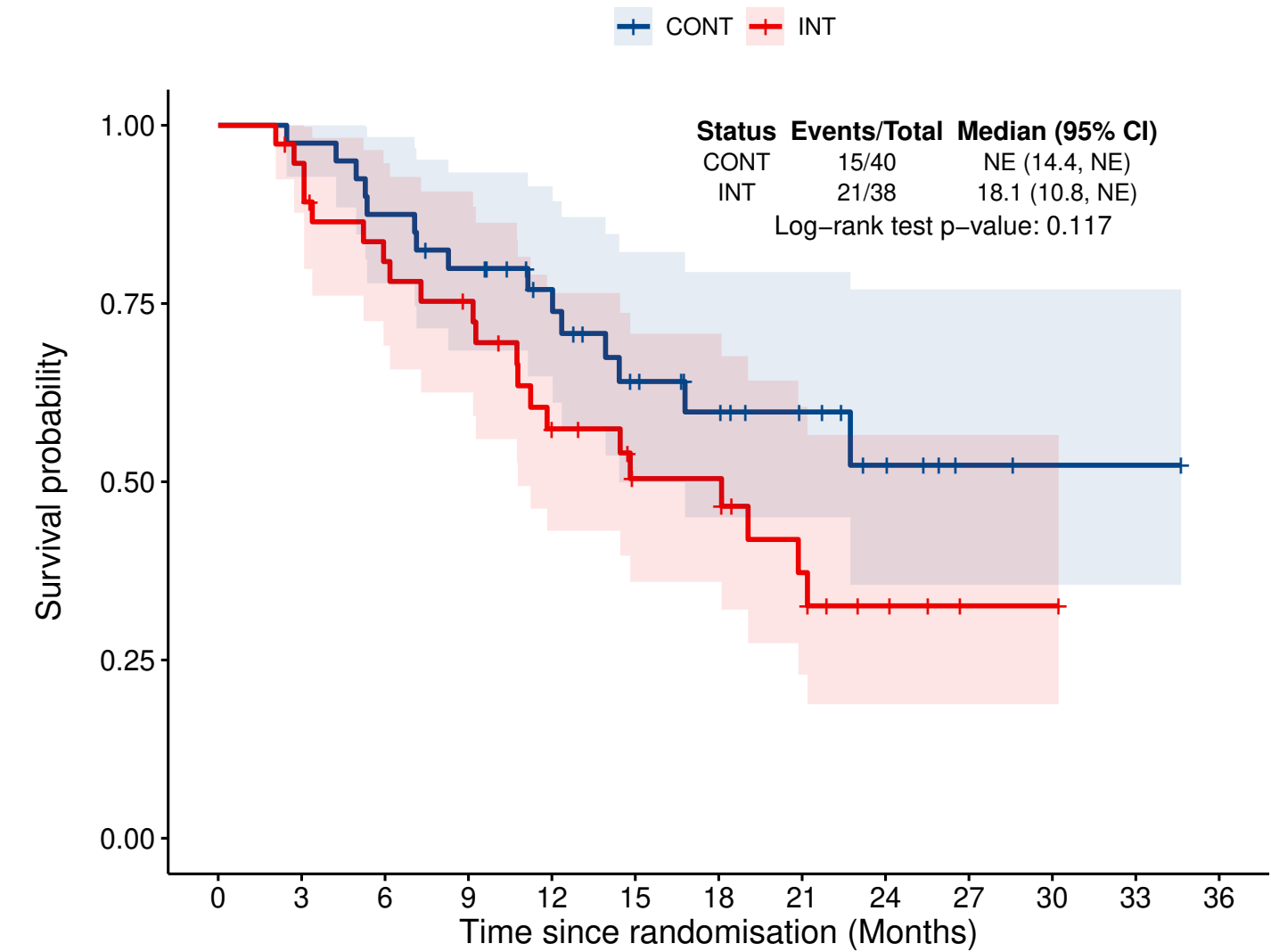


Number at risk

CONT	40	36	29	23	16	10	7	5	1	1	1	1	0
INT	38	31	20	16	13	8	6	4	2	0	0	0	0

B

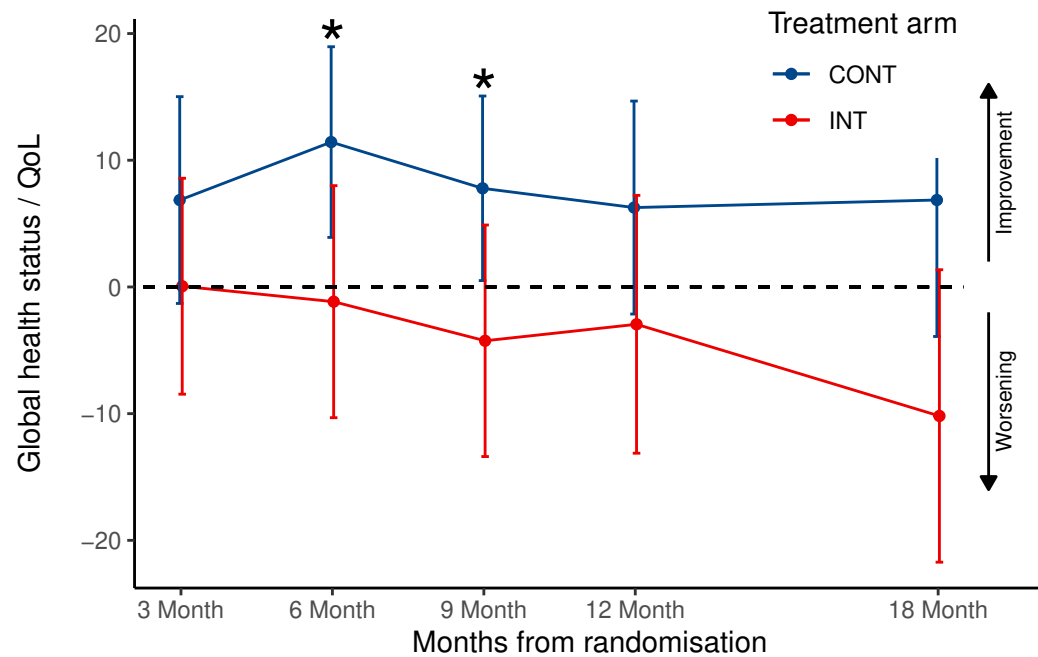
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Number at risk

CONT	40	39	35	31	25	18	14	10	6	2	1	1	0
INT	38	35	29	26	18	13	13	8	4	1	1	0	0

Figure 2  
A

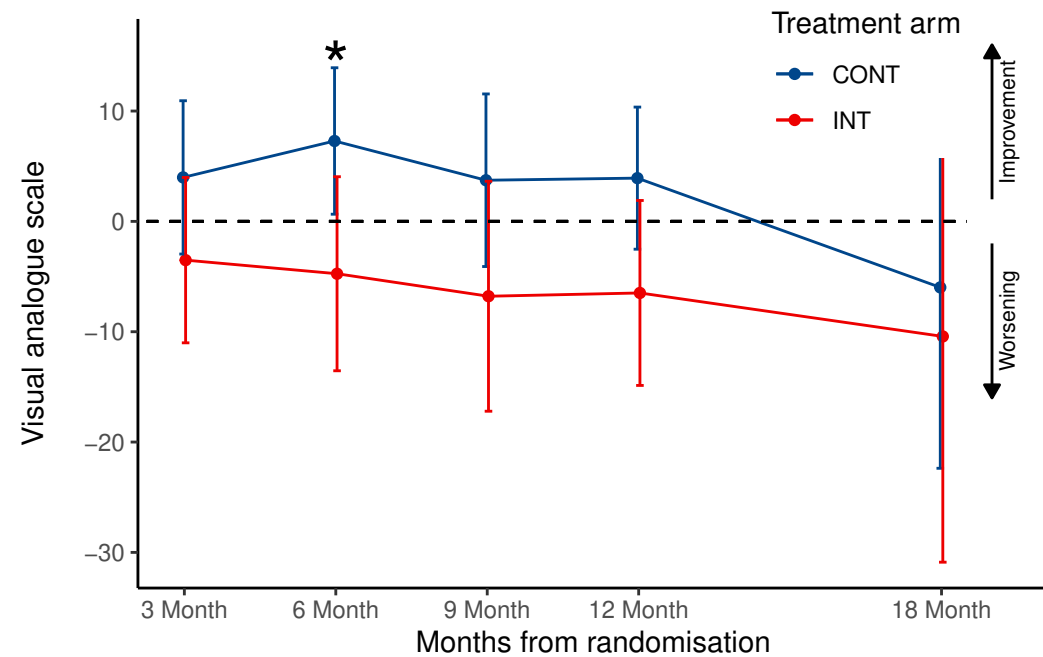


Number of patients

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CONT	24	30	28	20	11

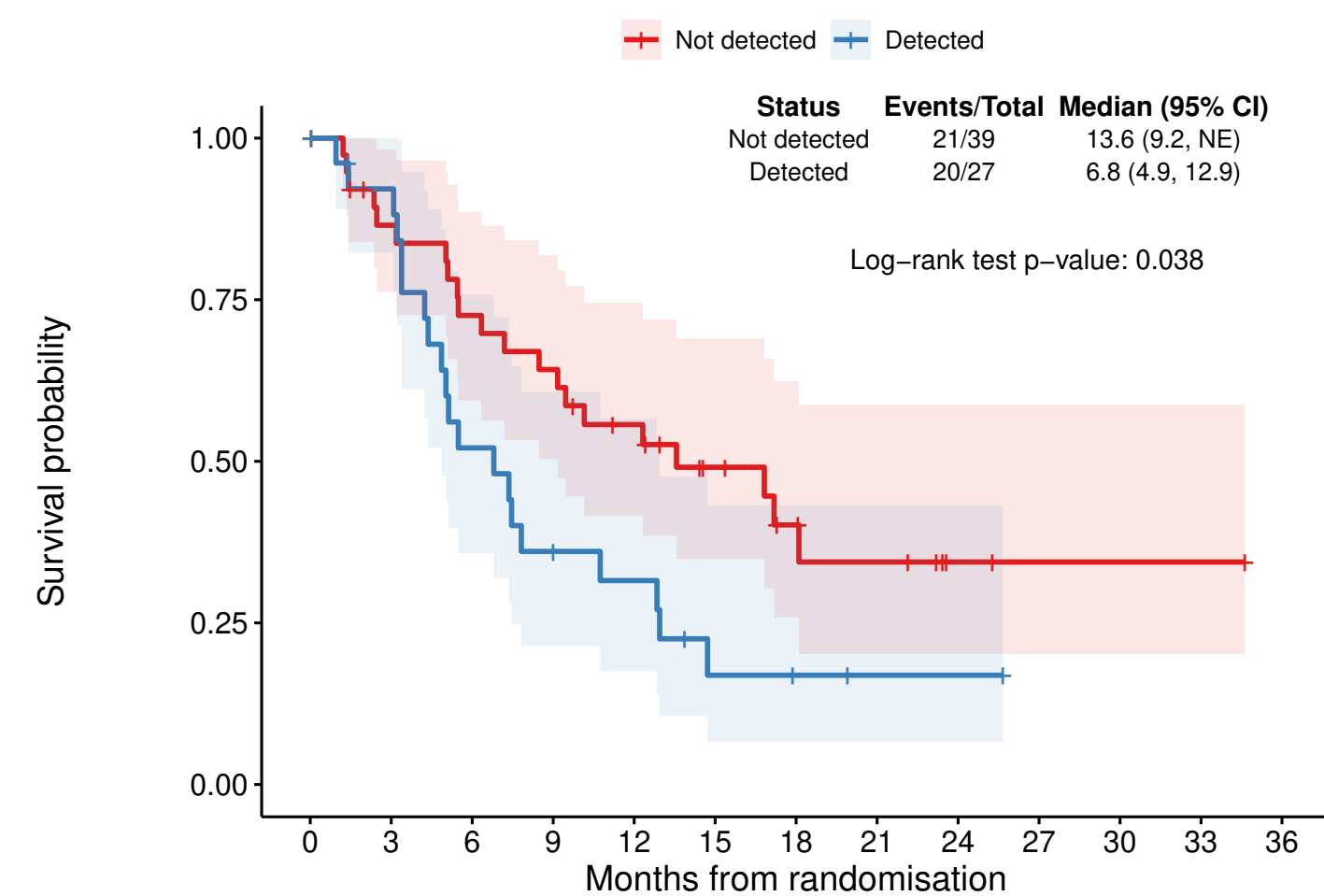
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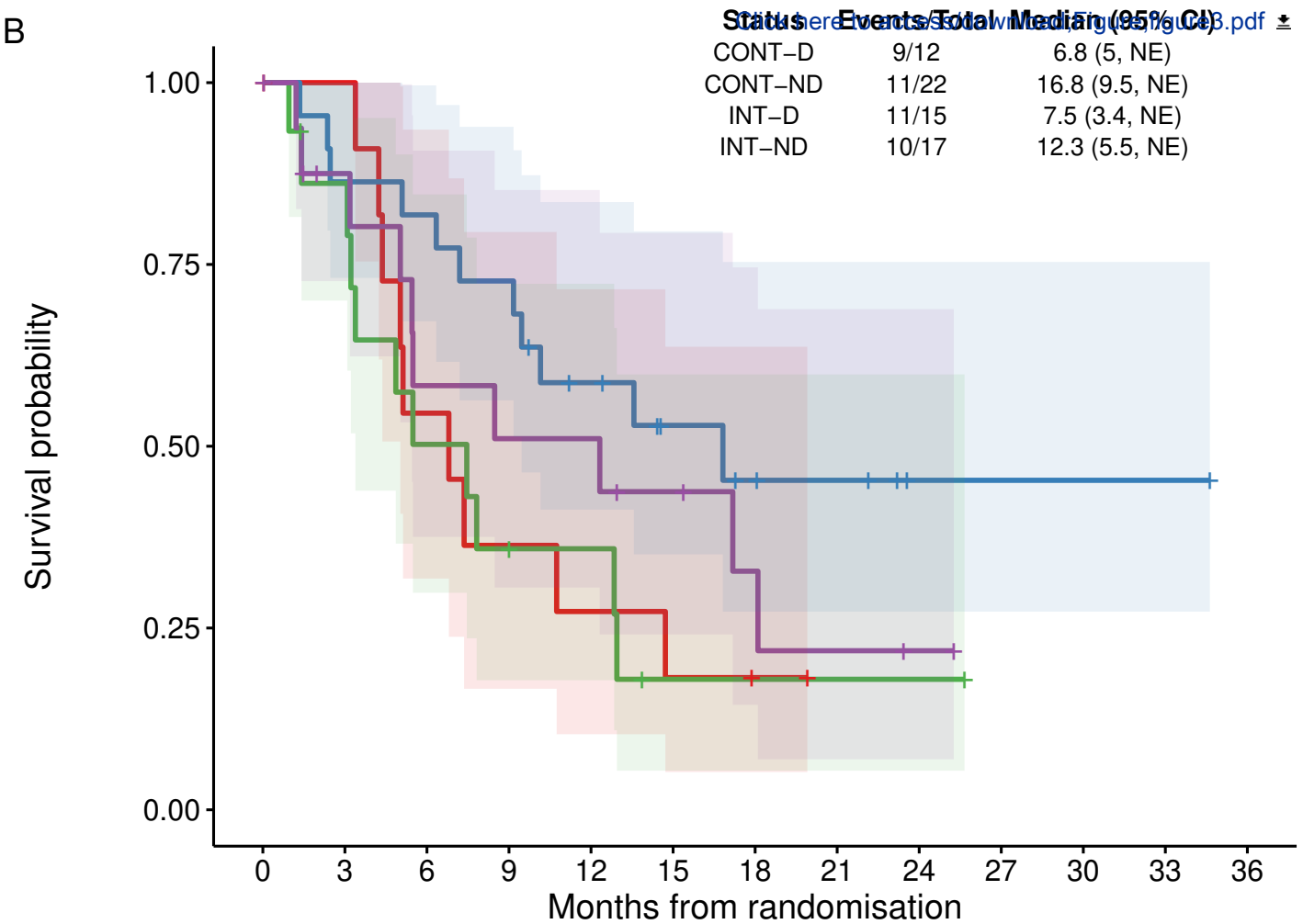
Number of patients

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CONT	25	28	26	19	13



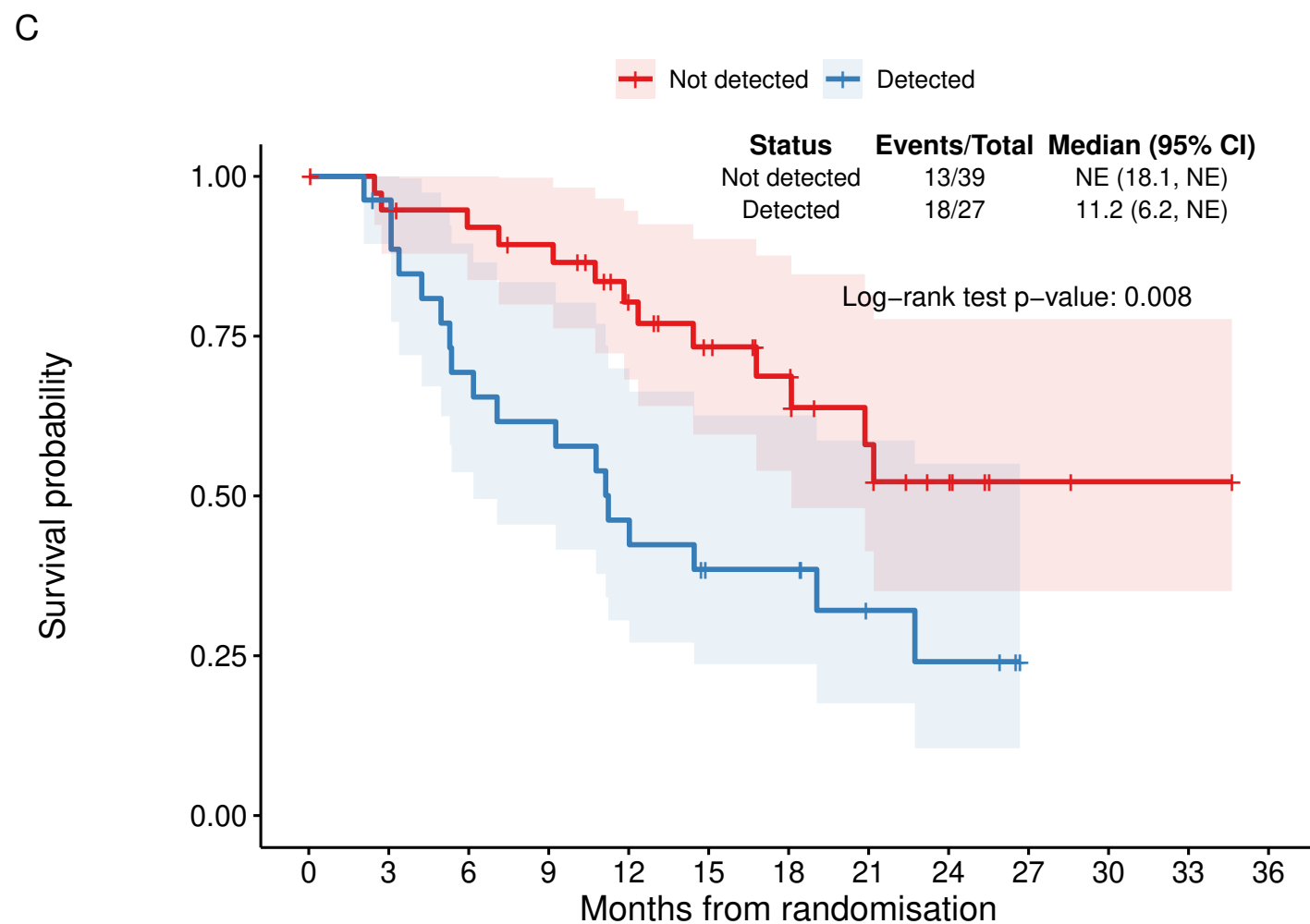
Number at risk

Not detected	39	31	26	23	18	12	8	6	2	1	1	1	0
Detected	27	23	13	9	7	3	2	1	1	0	0	0	0



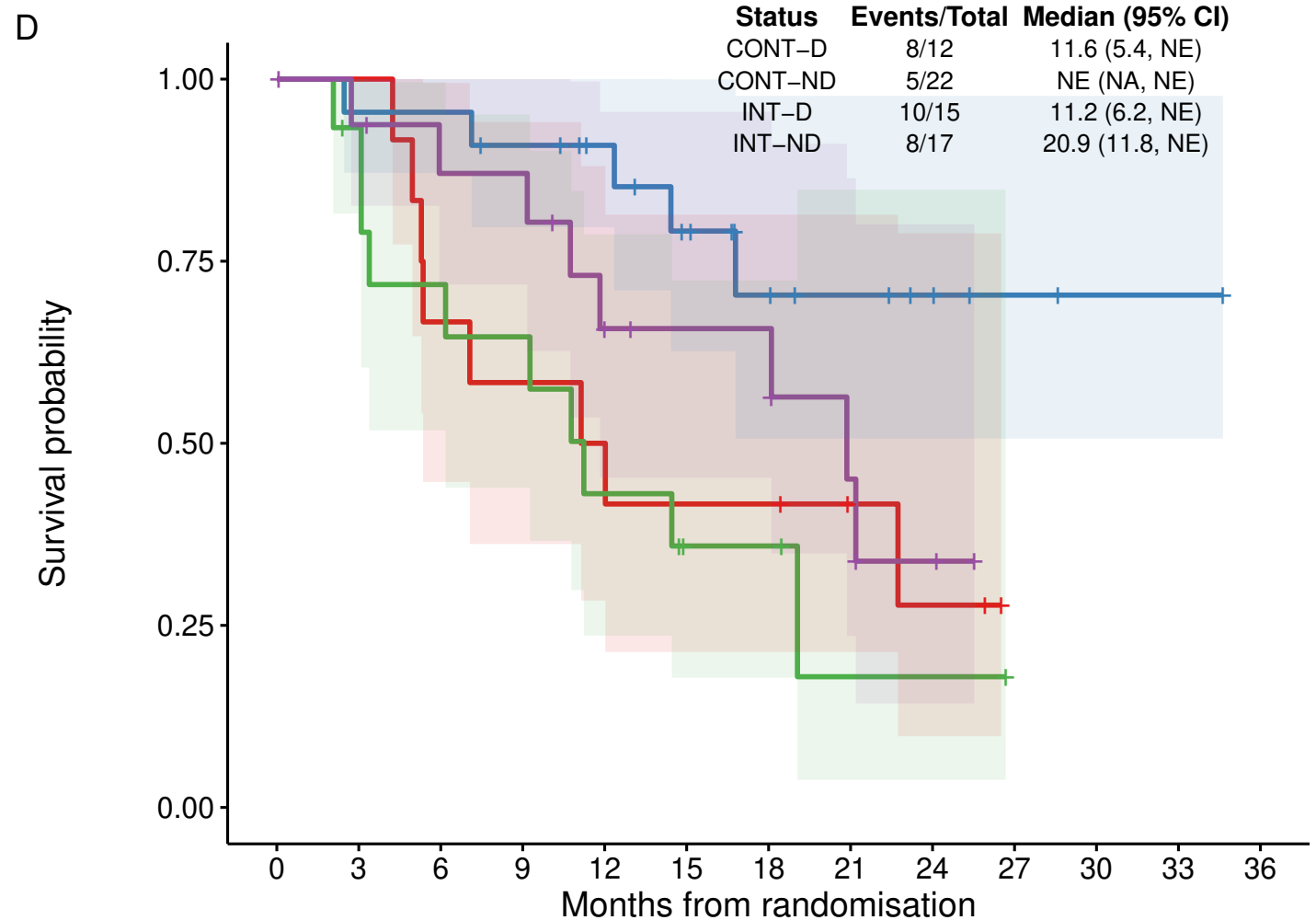
Number at risk

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CONT-ND	22	19	18	16	11	7	5	4	1	1	1	1	0
INT-D	15	12	7	5	4	1	1	1	1	0	0	0	0
INT-ND	17	12	8	7	7	5	3	2	1	0	0	0	0



Number at risk

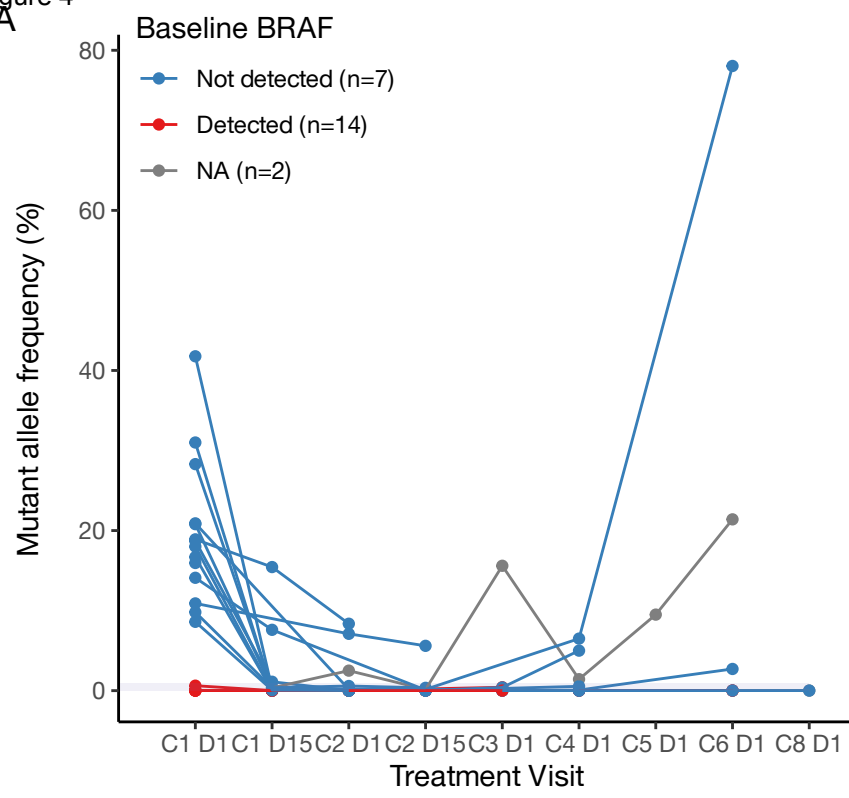
Not detected	39	36	34	32	24	19	15	10	6	2	1	1	0
Detected	27	25	18	16	12	8	8	4	3	0	0	0	0



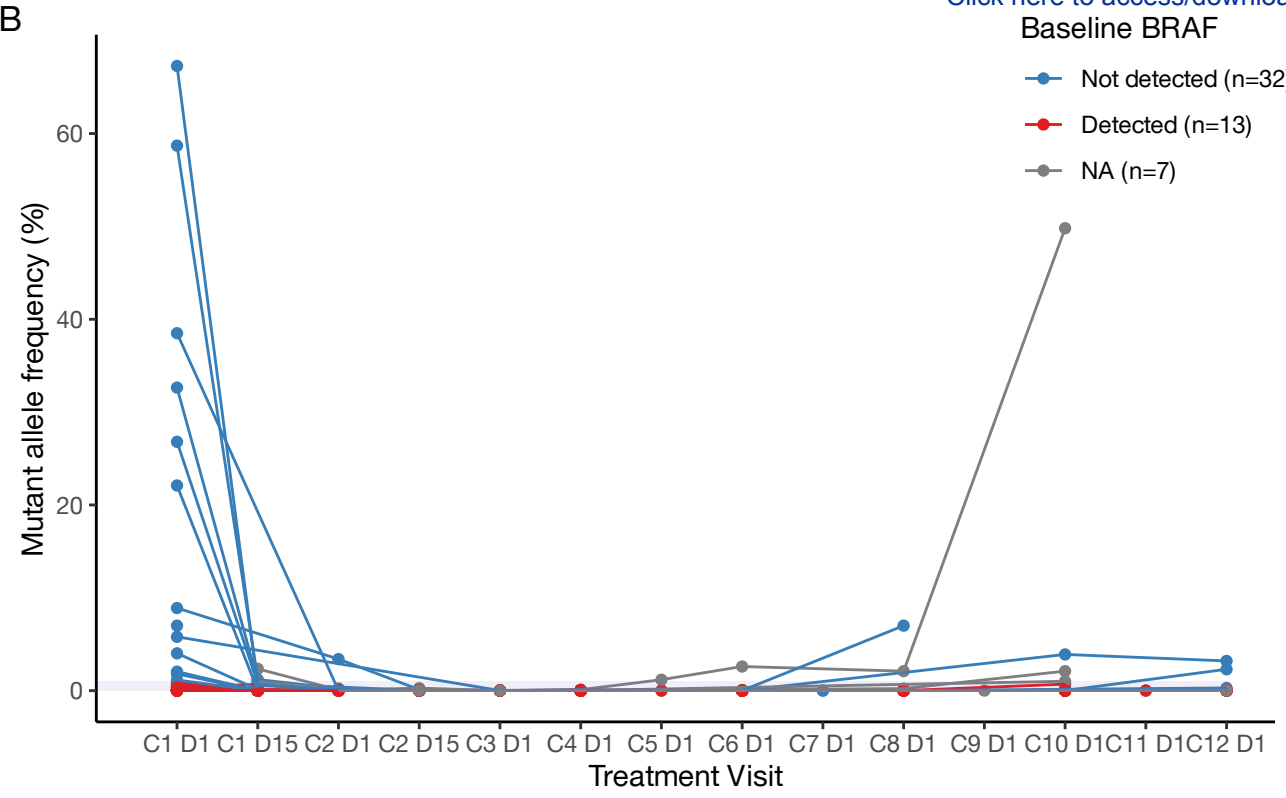
Number at risk

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CONT-ND	22	21	21	19	16	12	8	6	4	2	1	1	0
INT-D	15	13	10	9	6	3	3	1	1	0	0	0	0
INT-ND	17	15	13	13	8	7	7	4	2	0	0	0	0

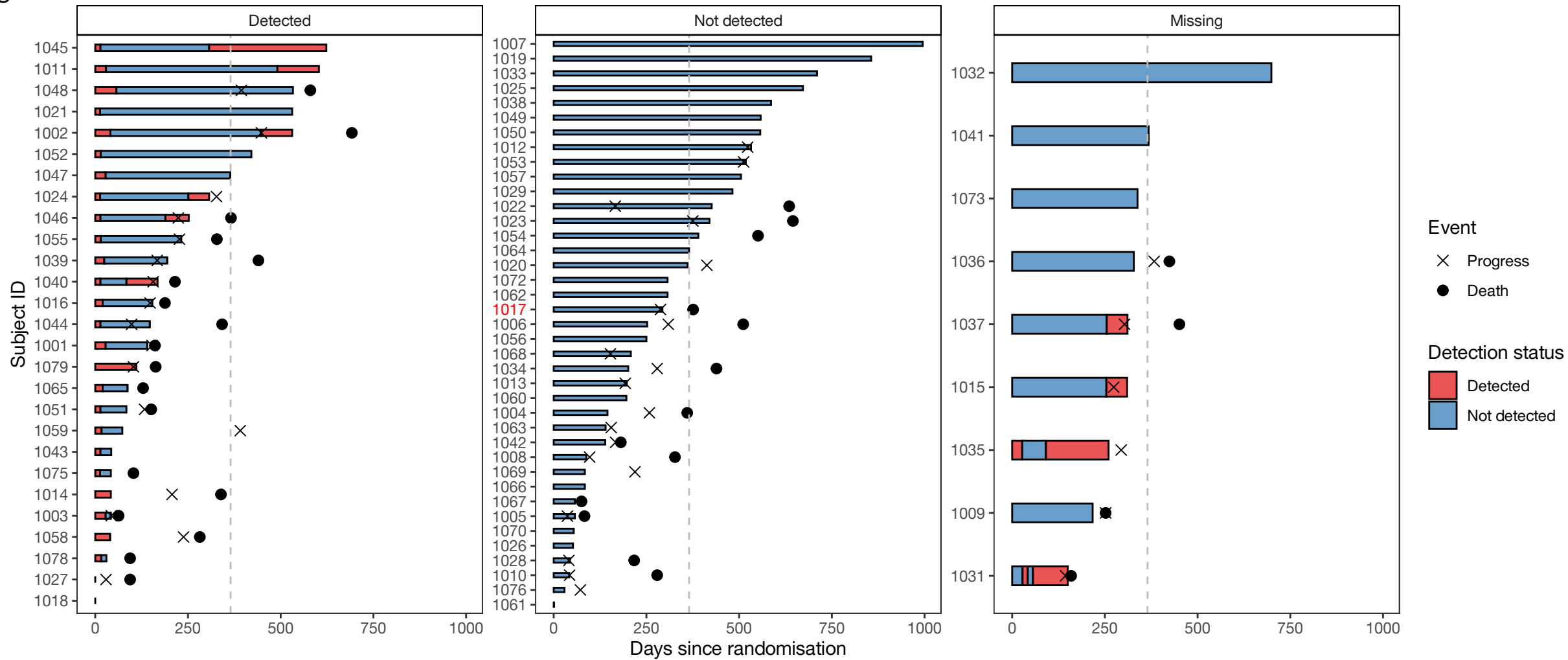
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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

**Author contributions**

PC, MM, AG & RM conceived the clinical trial; AD, JW & NW provided statistical support; JN, RB, MP, AR, AF, SD, BE, JC, CHB, NS, MB, EB, MG, MH, LP, SK, AW, AG & PC recruited the patients; GB, LD & EB undertook the ctDNA analyses; ST led study coordination; PC & AD led manuscript writing; all authors contributed to data interpretation and approved the final manuscript.