Neoadjuvant plus adjuvant dabrafenib and trametinib versus standard of care in patients with high-risk, surgically resectable melanoma: a single-centre, open-label, randomised, phase 2 trial

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Summary

Background Dual BRAF and MEK inhibition produces a response in a large number of patients with stage IV *BRAF*-mutant melanoma. The existing standard of care for patients with clinical stage III melanoma is upfront surgery and consideration for adjuvant therapy, which is insufficient to cure most patients. Neoadjuvant targeted therapy with BRAF and MEK inhibitors (such as dabrafenib and trametinib) might provide clinical benefit in this high-risk p opulation.

Methods We undertook this single-centre, open-label, randomised phase 2 trial at the University of Texas MD Anderson Cancer Center (Houston, TX, USA). Eligible participants were adult patients (aged ≥18 years) with histologically or cytologically confirmed surgically resectable clinical stage III or oligometastatic stage IV $BRAF^{V600E}$ or $BRAF^{V600K}$ (ie, Val600Glu or Val600Lys)-mutated melanoma. Eligible patients had to have an Eastern Cooperative Oncology Group performance status of 0 or 1, a life expectancy of more than 3 years, and no previous exposure to BRAF or MEK inhibitors. Exclusion criteria included metastases to bone, brain, or other sites where complete surgical excision was in doubt. We randomly assigned patients (1:2) to either upfront surgery and consideration for adjuvant therapy (standard of care group) or neoadjuvant plus adjuvant dabrafenib and trametinib (8 weeks of neoadjuvant oral dabrafenib 150 mg twice per day and oral trametinib 2 mg per day followed by surgery, then up to 44 weeks of adjuvant dabrafenib plus trametinib starting 1 week after surgery for a total of 52 weeks of treatment). Randomisation was not masked and was implemented by the clinical trial conduct website maintained by the trial centre. Patients were stratified by disease stage. The primary endpoint was investigator-assessed event-free survival (ie, patients who were alive without disease progression) at 12 months in the intent-to-treat population. This trial is registered at ClinicalTrials.gov, number NCT02231775.

Findings Between Oct 23, 2014, and April 13, 2016, we randomly assigned seven patients to standard of care, and 14 to neoadjuvant plus adjuvant dabrafenib and trametinib. The trial was stopped early after a prespecified interim safety analysis that occurred after a quarter of the participants had been accrued revealed significantly longer event-free survival with neoadjuvant plus adjuvant dabrafenib and trametinib than with standard of care. After a median follow-up of 18·6 months (IQR 14·6–23·1), significantly more patients receiving neoadjuvant plus adjuvant dabrafenib and trametinib were alive without disease progression than those receiving standard of care (ten [71%] of 14 patients *vs* none of seven in the standard of care group; median event-free survival was 19·7 months [16·2–not estimable] *vs* 2·9 months [95% CI 1·7–not estimable]; hazard ratio 0·016, 95% CI 0·00012–0·14, p<0·0001). Neoadjuvant plus adjuvant dabrafenib and trametinib were well tolerated with no occurrence of grade 4 adverse events or treatment-related deaths. The most common adverse events in the neoadjuvant plus adjuvant dabrafenib and trametinib group were expected grade 1–2 toxicities including chills (12 patients [92%]), headache (12 [92%]), and pyrexia (ten [77%]). The most common grade 3 adverse event was diarrhoea (two patients [15%]).

Interpretation Neoadjuvant plus adjuvant dabrafenib and trametinib significantly improved event-free survival versus standard of care in patients with high-risk, surgically resectable, clinical stage III–IV melanoma. Although the trial finished early, limiting generalisability of the results, the findings provide proof-of-concept and support the rationale for further investigation of neoadjuvant approaches in this disease. This trial is currently continuing accrual as a single-arm study of neoadjuvant plus adjuvant dabrafenib and trametinib.

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Research in context

Evidence before this study

The existing standard of care for patients with clinical stage III melanoma is upfront surgery, and consideration for adjuvant therapy, which is insufficient to cure most patients. Combined BRAF and MEK inhibition is known to be effective in treating metastatic melanoma, especially in patients with a low disease burden at the time of treatment initiation. Patients with surgically resectable clinical stage III and oligometastatic melanoma are a high-risk population, and less than 50% are still alive 5 years after diagnosis. We searched PubMed with the terms "neoadjuvant therapy", "melanoma", and "clinical trial" for for English-language studies published from 1990 up to Jan 1, 2014. We identified five single-arm neoadjuvant trials, including one of neoadjuvant temozolomide, one of high dose interferon-alfa 2b, and three of biochemotherapy in patients with melanoma. There were no prospective assessments of neoadjuvant BRAF or MEK inhibitor therapy, and no direct, prospective comparison of neoadjuvant treatments with standard of care.

Added value of this study

To our knowledge, this study is the first randomised trial of neoadjuvant therapy versus standard of care in patients with high risk, resectable *BRAF*^{V600E} or *BRAF*^{V600K} (ie, Val600Glu or Val600Lys)-mutant melanoma. The results of the study show that patients who were given neoadjuvant plus adjuvant dabrafenib and trametinib had substantially longer event-free survival than those who received standard of care, and support the safety of this treatment approach.

Implications of all the available evidence

Findings from this study provide a strong rationale for continued exploration of neoadjuvant plus adjuvant therapy in high-risk, *BRAF*-mutant, resectable melanoma. Understanding predictors of a pathological complete response to neoadjuvant therapy and its association with long-term outcomes will help to develop new strategies to further improve outcomes and overcome treatment resistance.

Introduction

Upfront surgical management is the current standard of care for patients with clinical stage III melanoma.[1,2] According to the 7th edition of the melanoma American Joint Committee on Cancer staging manual,[2] only 30–50% of patients with stage IIIB or IIIC melanoma survive for 5 years after diagnosis. Adjuvant medical therapy is discussed with patients with stage III disease after surgery. However, at the the time of this trial, use of available adjuvant therapies such as interferon alfa and high-dose ipilimumab was heterogenous.

The first adjuvant therapy approved for melanoma was interferon-alfa2b, which achieved significant but mild improvements in disease-free survival (hazard ratio [HR] 0·83, 95% CI 0·78–0·87) and overall survival (0·91, 0·85–0·97) compared with patients undergoing observation or treated with another adjuvant therapy, as determined by a 2013 meta-analysis[3] of more than 10 000 patients with stage II–III cutaneous melanoma. More recently, adjuvant treatment with ipilimumab improved 5-year event-free survival (HR 0·76, 95% CI 0·58–0·88), distant metastasis-free survival (0·76, 0·64–0·92), and overall survival (0·72, 0·58–0·88) versus placebo in the EORTC 18071 phase 3 study.[4] Although these results were impressive, 54% of patients treated with ipilimumab had grade 3 or 4 adverse events and there were five ipilimumab-related deaths. The choice of placebo as the control group in this trial highlights the continued dilemma and absence of consensus regarding adjuvant therapy for patients with melanoma—better treatments need to be developed for this high-risk population.

Neoadjuvant chemotherapy is the standard of care for many types of cancer, including breast, gastric, oesophageal, and rectal cancers.[1] Potential benefits of neoadjuvant therapy include reduced morbidity of definitive surgery, objective assessment of treatment response, and collection of biospecimens for translational research. Neoadjuvant therapy for melanoma has been restricted historically, mainly because of the low activity of chemotherapy in this disease. All prospective neoadjuvant trials in melanoma reported so far have been non-randomised single-arm studies, and thus have not compared clinical benefit in a neoadjuvant group versus a standard of care group.[5–7]

Combination targeted therapy with dabrafenib and trametinib is approved for patients with stage IV melanoma with a *BRAF*^{V600} mutation, which is present in around 50% of cutaneous melanomas.[8] A pooled analysis[9] of data from 617 patients treated in randomised trials showed that 67% of patients with stage IV *BRAF*^{V600E} or *BRAF*^{V600K} (ie, Val600Glu or Val600Lys) melanoma treated with dabrafenib and trametinib achieved an overall response and 91% achieved disease control, with a well-characterised and manageable toxicity profile.[10] Clinical trials assessing dabrafenib and trametinib as adjuvant therapy for patients with stage III melanoma were pending results at the time of this trial. We postulated that dabrafenib and trametinib would be safe and effective in the neoadjuvant setting. Therefore, we did a randomised phase 2 trial to assess event-free survival with neoadjuvant and adjuvant dabrafenib and trametinib compared with standard of care in patients with surgically resectable, clinical stage III and oligometastatic stage IV *BRAF*^{V600E} or *BRAF*^{V600K} melanoma.

Methods

Study design and participants

We undertook a single-centre, open-label, randomised phase 2 trial at the University of Texas MD Anderson

Cancer Center (Houston, TX, USA). Eligible participants were patients aged at least 18 years with histologically or cytologically confirmed locally advanced clinical stage III or oligometastatic stage IV *BRAF*^{V600E} or *BRAF*^{V600K}-mutated melanoma and a life expectancy of more than 3 years. Clinical stage III disease was defined as at least one palpable lymph node metastasis measuring 1·5 cm in short axis or at least one 1·0 cm in-transit metastasis, consistent with the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[11]

Patients with oligometastatic stage IV melanoma were eligible if there were less than four sites of metastases, excluding bone, CNS, and sites where a complete surgical resection was not feasible. Only patients with surgically resectable disease—as determined by multidisciplinary consensus of surgical oncologists, medical oncologists, and radiologists—were eligible. All patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 with normal organ function, bone marrow function, and coagulation status. Key exclusion criteria included previous exposure to BRAF or MEK inhibitors, current use of cancer therapy or investigational anti-cancer drugs, current use of anticoagulants, major surgery within the previous 3 weeks, or active concurrent second cancer within the previous 2 years (excluding non-melanoma skin cancers such as basal cell carcinomas or squamous cell carcinomas). Patients with history of central serous retinopathy or retinal vein occlusion, active gastrointestinal disease interfering with oral drug absorption, QTc interval longer than 480 msec, or New York Heart Association class II, III, or IV heart failure were prohibited from study entry. Patients who had previously received systemic therapy for melanoma who progressed with biopsy-proven clinical stage III or oligometastatic stage IV disease were allowed to be included after a washout period of 28 days. All patients were required to give written informed consent before commencing screening. The trial was done in accordance with the Declaration of Helsinki and the guidelines for Good Clinical Practice, and was reviewed and approved by the University of Texas MD Anderson Cancer Center Institutional Review Board.

Randomisation and masking

We randomly assigned patients (1:2) to either upfront surgery followed by consideration of standard adjuvant therapy (standard of care group), or neoadjuvant (before surgery) plus adjuvant (after surgery) dabrafenib and trametinib. Treatment group stratification was done on the basis of disease stage (IIIB, IIIC, IV, or M1A *vs* IVM1B or IVM1C) using Pocock-Simon minimisation.[12] Participants were assigned a unique number at the time of enrolment. Randomisation was implemented by the Clinical Trial Conduct website maintained by the Department of Biostatistics at the University of Texas MD Anderson Cancer Center. Enrolment and randomisation were done by the trial's designated research nurse. Because of the nature of the treatments involved, no masking of assignments was attempted, and patients, investigators, and data analysts and assessors were aware of treatment assignment.

Procedures

Patients assigned to the standard of care group underwent definitive surgery done in accordance with National Comprehensive Cancer Network and local practice guidelines, including completion lymphadenectomy of involved nodal basin(s) or complete metastectomy in patients with stage IV disease.[1] Patients were offered standard of care adjuvant therapy including interferon-alfa2b,[3] pegylated interferon-alfa2b,[13] ipilimumab,[4] biochemotherapy with a five-drug regimen of interferon alpha, infusional interleukin-2, cisplatin, vinblastine, and dacarbazine,[14] or observation, in keeping with the available adjuvant treatments available at the time of this trial. Adjuvant radiotherapy was not allowed.

Patients in the neoadjuvant plus adjuvant dabrafenib and trametinib group received 8 weeks of neoadjuvant daily dabrafenib and trametinib treatment. Restaging was done at 8 weeks and patients with continued resectable disease proceeded to surgery. Dabrafenib and trametinib were stopped 48 h before surgery and then resumed within 1 week after surgery. Patients received up to 44 weeks of adjuvant dabrafenib and trametinib (52 weeks total treatment; appendix p 2). The starting dose of dabrafenib was 150 mg by mouth twice per day and for trametinib was 2 mg by mouth per day. Up to two dose reductions (to dabrafenib 100 mg twice per day with trametinib 1·5 mg per day, and to dabrafenib 75 mg twice per day with trametinib 1 mg per day) were allowed for a third occurrence of a grade 2 intolerable toxicity or a first occurrence of grade 3 or worse toxicity. Treatment was discontinued in the event of a grade 4 drug-related toxicity. Laboratory tests, including complete blood count, electrolytes, and kidney and hepatic function, were done monthly. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0[15] throughout the study on a monthly basis at mandatory clinic visits. Adverse event monitoring was done by the investigator from the date of first study drug dose to the last dose.

See Online for appendix

Imaging (PET–CT or CT of the chest, abdomen, and pelvis, and MRI or CT of the brain) was obtained at baseline for all patients and after 8 weeks of neoadjuvant dabrafenib and trametinib in the neoadjuvant plus adjuvant treatment group. All patients underwent physical examinations and imaging every 12 weeks after surgery for a planned follow-up of 2 years. Radiological assessments to classify best response to therapy as complete or partial response, stable disease, or progressive disease were done for the patients undergoing 8 weeks of neoadjuvant therapy according to

RECIST 1.1 criteria.[11] All patients in the neoadjuvant and adjuvant dabrafenib and trametinib group underwent baseline electrocardiogram (ECG), echocardiogram, and ophthalmic assessments. ECG and echocardiogram were repeated every 12 weeks and ophthalmic assessments were repeated only as needed for a visual complaint. All patients had scans every 12 weeks in the adjuvant setting to assess disease progression.

Patients in the neoadjuvant and adjuvant dabrafenib and trametinib group underwent a tumour biopsy and blood collection at baseline, and at weeks 3 and 5 of treatment (appendix p 3). Tumour samples were obtained as core, punch, or excisional biopsies taken by treating clinicians or an interventional radiologist. Samples were immediately snap-frozen or formalin-fixed and paraffin-embedded before analysis. RNA or DNA extraction, whole-exome sequencing, T-cell receptor sequencing, transcriptome-wide gene expression microarrays, and immunohistochemistry were undertaken as previously described[16,17] (details in appendix p 13). Week 8 surgical samples were analysed by our dermatopathology group for pathological responses. Pathological complete response was defined as an absence of residual viable malignant cells on haematoxylin and eosin staining. Pathological partial response was defined as less than 50% viable tumour cells or more than 50% fibrosis.[18] No pathological response was defined as more than 50% viable tumour cells at surgical resection. Patients were removed from protocol if they were no longer found to receive clinical benefit (documented disease progression) or had unacceptable toxicity from study medications. Patients in both groups were deemed to have completed the study after being followed up every 3 months for 1 year after surgery.

Outcomes

The primary endpoint of this study was event-free survival at 12 months, assessed by the treating investigators. Secondary endpoints were safety, overall survival, the proportion of patients who achieved a radiographic and pathological complete response at the time of surgery in the patients randomly assigned to neoadjuvant and adjuvant dabrafenib and trametinib, and biomarkers predictive of treatment response in neoadjuvant-treated patients. Distant metastasis-free survival (defined as the time from randomisation to development of documented distant metastatic disease outside the locoregional site of the primary tumour or lymph node metastasis) was a post-hoc exploratory endpoint. Event-free survival was defined as the time from randomisation to recurrence event (local or distant disease development, or death). Overall survival was defined as the time from randomisation to death. Patients who remained alive were censored at their last contact date for overall survival. Patients who remained alive without disease progression (event-free survival) or distant metastasis (distant metastasis-free survival) were censored at their last date of clinical follow-up for progression. Pathological complete responses in the neoadjuvant and adjuvant therapy group were determined as the percentage of patients with absence of any viable melanoma in the examined surgical specimen. Safety in the neoadjuvant plus adjuvant dabrafenib and trametinib group was measured as the frequency and severity of adverse events by clinical history and physical examination, vital signs, and laboratory findings.

Statistical analysis

On the basis of observations from previous studies showing that 50% of patients were alive without disease progression (event-free survival) at 12 months,[2,19] we estimated that 84 patients (28 assigned to standard of care and 56 to neoadjuvant and adjuvant dabrafenib and trametinib) would provide 80% power to detect an increase in the proportion of patients alive without progression at 12 months from 50% to 70% with a 5% type-1 error. A single planned interim analysis was scheduled for when half the expected event-free survival events (32 of 63 events) had occurred, which would have used an O'Brien-Fleming stopping boundary. The primary endpoint was assessed by the log-rank test. Cox proportional hazards regression analysis was used to assess the association between event-free survival, overall survival, and covariates.

The MD Anderson data safety monitoring board monitored results in ten-patient increments. Because in this trial an interim analysis led to early trial closure and a low number of events, Firth's penalised maximum likelihood method was used to account for the low number of events and estimate HRs and 95% CIs.[20] Estimation of the HR would refer only to the degree of separation between two curves, and as such has no requirement for attainment of any particular survival fraction in either curve. Safety parameters were compared between groups using Fisher's exact tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. Associations between the probability of achieving a pathological complete response and clinical and disease covariates were assessed by logistic regression.

All randomly assigned patients (ie, the intent-to-treat population) were assessable for the primary endpoint of event-free survival in addition to the endpoints of safety, distant metastasis-free survival, and overall survival. Only patients who underwent surgery were considered for analyses of overall survival, event-free survival, and distant metastasis-free survival after surgery. Only patients assigned to neoadjuvant plus adjuvant dabrafenib and trametinib who were treated and underwent surgery were assessed for pathological complete response and included in analyses of this endpoint. The population studied for radiographic response were those patients assigned to neoadjuvant plus adjuvant dabrafenib and trametinib who were treated and restaged. Restaging was done at prespecified

time points (ie, after 8 weeks of neoadjuvant therapy). Additional landmark analyses of event-free survival, overall survival, and distant metastasis-free survival were done starting at the date of surgery to assess radiographic and pathological responses at the time of surgery. All statistical analyses were done with R version 3.4.0.[21] This trial is registered at ClinicalTrials.gov, number NCT02231775, and is currently continuing accrual as a single-arm study of neoadjuvant plus adjuvant dabrafenib and trametinib.

Role of the funding source

The funder of the study supplied the drugs used in this study but had no role in study design, study execution, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

Results

Between Oct 23, 2014, and April 13, 2016, we screened 46 patients and enrolled and randomly assigned 21 eligible patients to treatment: seven to standard of care and 14 to neoadjuvant plus adjuvant dabrafenib and trametinib (figure 1). The demographic and baseline characteristics (including disease stage and tumour burden) were generally well balanced across the two treatment groups, although patients in the standard of care group were younger, were more likely to have had previous surgery, and had more superficial spreading melanomas, whereas more patients in the neoadjuvant plus adjuvant dabrafenib and trametinib group had nodular melanomas (table 1).

All seven patients in the standard of care group underwent surgery and were evaluable for disease recurrence. Six patients who were offered observation versus standard of care adjuvant therapy (including interferon) chose close observation post-operatively, and one patient chose to receive adjuvant therapy (the five-drug biochemotherapy regimen). One patient assigned to neoadjuvant plus adjuvant dabrafenib and trametinib withdrew consent before initiating therapy and therefore was not assessable for imaging or pathological responses. All remaining 13 patients received neoadjuvant therapy, but one withdrew consent before surgery because of an excellent partial response on imaging to neoadjuvant treatment; thus, 12 patients in this group underwent surgery and were evaluable for pathological complete response. No data for the two patients who did not have surgery in this group were included after the date they withdrew consent. All patients were assessable for the primary endpoint.

After an interim analysis of the first 21 randomised patients done on April 12, 2016, after a median follow-up of 7·1 months (IQR3·4–11·0), the MD Anderson data safety monitoring board determined that it was no longer ethical to continue enrolment into the standard of care group and the trial was closed to new patient entry. At the time of this interim safety analysis, seven event-free survival events had occurred: five in patients assigned to standard of care and two in those assigned to neoadjuvant plus adjuvant dabrafenib and trametinib. The reported p value (p<0·0001) did not cross the O'Brien-Fleming stopping boundary for seven events, which would have required a p value of less than $3·4\times10^{-9}$. However, we undertook a predictive probability analysis in which future trial outcomes were simulated many times. The predicted probability that neoadjuvant plus adjuvant dabrafenib and trametinib would be superior to standard of care was 0·991, necessitating closure of the standard of care group.

At a median follow-up of 18·6 months (IQR 14·6–23·1), significantly more patients receiving neoadjuvant plus

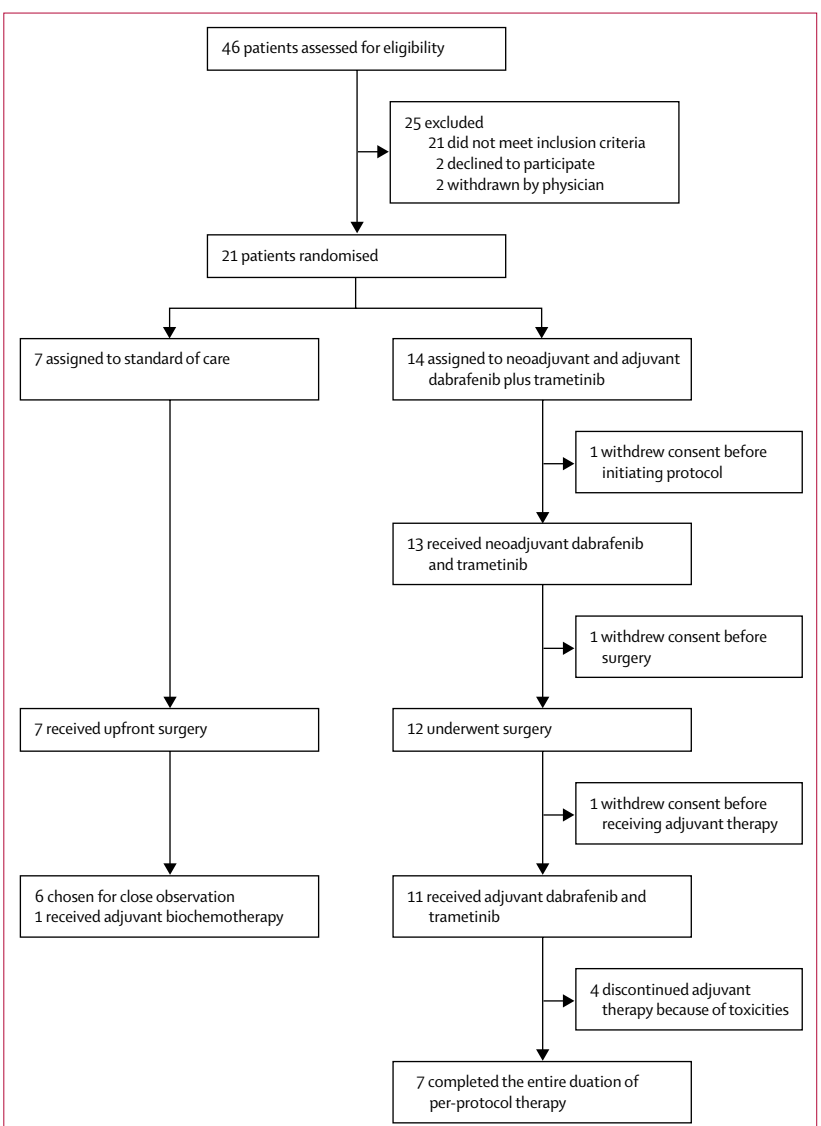

***Figure 1*: Trial profile**

adjuvant dabrafenib and trametinib were alive without disease progression (event-free survival) than those receiving than standard of care (ten [71%] of 14 *vs* zero of seven; HR 0·016 [95% CI 0·00012–0·14], p<0·0001; figure 2A). Median event-free survival was 2·9 months (95% CI 1·7–not estimable) in the standard of care group and 19·7 months (16·2–not estimable) in the neoadjuvant plus adjuvant dabrafenib and trametinib group. Four of the seven patients assigned to standard of care and three of the 14 patients assigned to neoadjuvant plus adjuvant dabrafenib and trametinib had a distant metastasis-free survival event. Neoadjuvant and adjuvant dabrafenib and trametinib was also associated with longer distant metastasis-free survival than standard of care (median not reached [16·2–not estimable] *vs* 7·7 months [2·3–not estimable]; HR 0·024, 95% CI 0·00017–0·28, p<0·001; figure 2B). Two of the seven patients assigned to standard of care and one of the 14 patients assigned to neoadjuvant plus adjuvant dabrafenib and trametinib died, all from disease progression. No between-group differences in overall survival were detected (figure 2C), and median overall survival was not reached in either group.

	Standard of care (n=7)	Neoadjuvant plus adjuvant dabrafenib and trametinib (n=14)
Age, years	44 (38–47)	59 (48–66)
Sex		
Male	4 (57%)	9 (64%)
Female	3 (43%)	5 (36%)
ECOG performance status*		
0	6 (86%)	13 (93%)
1	1 (14%)	1 (7%)
Clinical stage†		
IIIB	3 (43%)	2 (14%)
IIIC	3 (43%)	10 (71%)
IV	1 (14%)	2 (14%)
Primary tumour type		
Superficial spreading	4 (57%)	1 (7%)
Nodular	0	4 (29%)
Acral lentiginous	1 (14%)	0
Unspecified	1 (14%)	5 (36%)
Unknown primary	1 (14%)	4 (29%)
Primary tumour ulceration status		
Ulcerated	3 (43%)	7 (50%)
Not ulcerated	2 (29%)	1 (7%)
Unknown	1 (14%)	2 (14%)
NA (unknown primary)	1 (14%)	4 (29%)
Previous SLNB		
Yes—positive	1 (14%)	1 (7%)
Yes—negative	4 (57%)	1 (7%)
No	0	4 (29%)
Stage III at diagnosis	2 (29%)	8 (57%)
Lactate dehydrogenase status		
Raised	1 (14%)	1 (7%)
Not raised	6 (86%)	13 (93%)
BRAF mutation type		
V600E	7 (100%)	11 (79%)
V600K	0	3 (21%)
Previous therapy		
Treatment naive	1 (14%)	6 (43%)
Previous surgery	6 (86%)	8 (57%)
Sum of lesion diameters, mm	44 (33–108)	62 (32–98)

Data are median (IQR) or n (%). NA=not applicable. SLNB=sentinel lymph node biopsy. *Eastern Cooperative Oncology Group (ECOG) status: 0=asymptomatic, 1=symptomatic and ambulatory. †As defined by American Joint Commission on Cancer, 7th edition.

Table 1: **Baseline characteristics of the intention-to-treat population**

In the group receiving neoadjuvant plus adjuvant dabrafenib and trametinib, 11 (85%) of 13 patients achieved an overall radiographic response after neoadjuvant therapy (two complete responses, nine partial responses, and two stable disease), and all 13 patients (100%) achieved radiographic disease control (figure 3A). There were no radiographic response data for the standard of care group since they had upfront surgery and thus no intervention in the neoadjuvant setting for radiographic assessment.

Seven (58%) of 12 patients in the neoadjuvant plus adjuvant dabrafenib and trametinib group who underwent surgery achieved a pathological complete response and two (17%) of 12 achieved a pathological partial response. Three patients (25%) had more than 50% viable tumour in their surgical specimens at week 8, denoting a pathological non-response (figure 3B). In the neoadjuvant plus adjuvant dabrafenib and trametinib group, there were no differences in event-free survival or distant metastasis-free survival after surgery by radiographic response category (appendix pp 4, 12). Neither of the two patients with a complete response, three of the eight patients with a partial response, and one of the two patients with stable disease had an event-free survival event. Neither of the two patients with a complete response, two of the eight patients with a partial response, and one of the two patients with stable disease had a distant metastasis-free survival event. Median follow-up after surgery for all 19 patients who underwent surgery was 17·4 months (IQR 12·8–22·1). Event-free survival after surgery also did not differ significantly by pathological response (appendix pp 5, 12). However, patients with a pathological complete response did have significantly longer distant metastasis-free survival after surgery than patients who did not achieve a pathological complete response after neoadjuvant dabrafenib and trametinib (figure 3C, appendix p 12). There was no correlation between RECIST responses and pathological responses (figure 3). Of seven patients who achieved a pathological complete response, two had a complete response, four had a radiographic partial response, and one had stable disease.

At a median follow-up of 17·3 months (IQR 12·5–23·7) from the date of surgery, only one patient with a

pathological complete response in the neoadjuvant plus adjuvant dabrafenib and trametinib group had relapsed, with a small-volume locoregional recurrence (appendix p 6). Three patients with a non-pathological complete response relapsed, each with brain metastases and two with additional visceral metastases (appendix p 6).

Molecular and immune profilings were done to identify the mechanisms and predictors of achieving a pathological complete response in the neoadjuvant plus adjuvant dabrafenib and trametinib group. Increased expression of phospho-ERK (pERK) in tumour tissue taken just before initiation of dabrafenib and trametinib was frequent in patients with a non-pathological complete response compared with those achieving a complete response (appendix p 7). Using a composite pERK plus viability score, patients achieving a pathological complete response had significantly lower baseline pERK positivity or non-viable melanoma within sampled tissue than patients who did not (figure 4A). Patients who achieved a pathological complete response had little to no detectable pERK expression or viable tumour remaining as early as the 3-week, on-treatment biopsy (appendix p 7). Whole-exome sequencing revealed no significant difference in total mutation load or global somatic copy-number alteration burden at baseline between patients who did and who did not achieve a pathological complete response (appendix p 8). By contrast with tumours that had a pathological complete response, those without a pathological complete response frequently harboured melanoma-associated genetic aberrations known to confer resistance to combination dabrafenib and trametinib in the metastatic setting, including mutations in *PTEN*, activating mutations in *KIT* and *MAP2K1*, and compound aberrations within individual tumours (figure 4B).[19,22,23]

Immunohistochemical profiling of the immune infiltrate in baseline tumours revealed no quantitative differences in CD8 concentrations by the pathological response achieved (appendix p 9). However, expression of *TIM-3* and *LAG-3* was significantly increased on CD8+ PD-1 T cells within baseline tumours that did not achieve a pathological complete response compared with tumours that did (figure 4C). T-cell receptor sequencing showed that tumours from patients with a pathological complete response showed little to no remodelling of the T-cell repertoire with neoadjuvant therapy with most of the T-cell clones shared between baseline and surgery (appendix p 9). However, greater variation in the T-cell repertoire between baseline and surgery occurred in patients who did not achieve a pathological complete response (appendix p 9). Accordingly, a higher abundance of the most dominant T-cell clones occurred in patients with a pathological complete response than in those without a pathological complete response at all timepoints (figure 4D)—this finding could help to distinguish between patients with and without a pathological complete response when considering

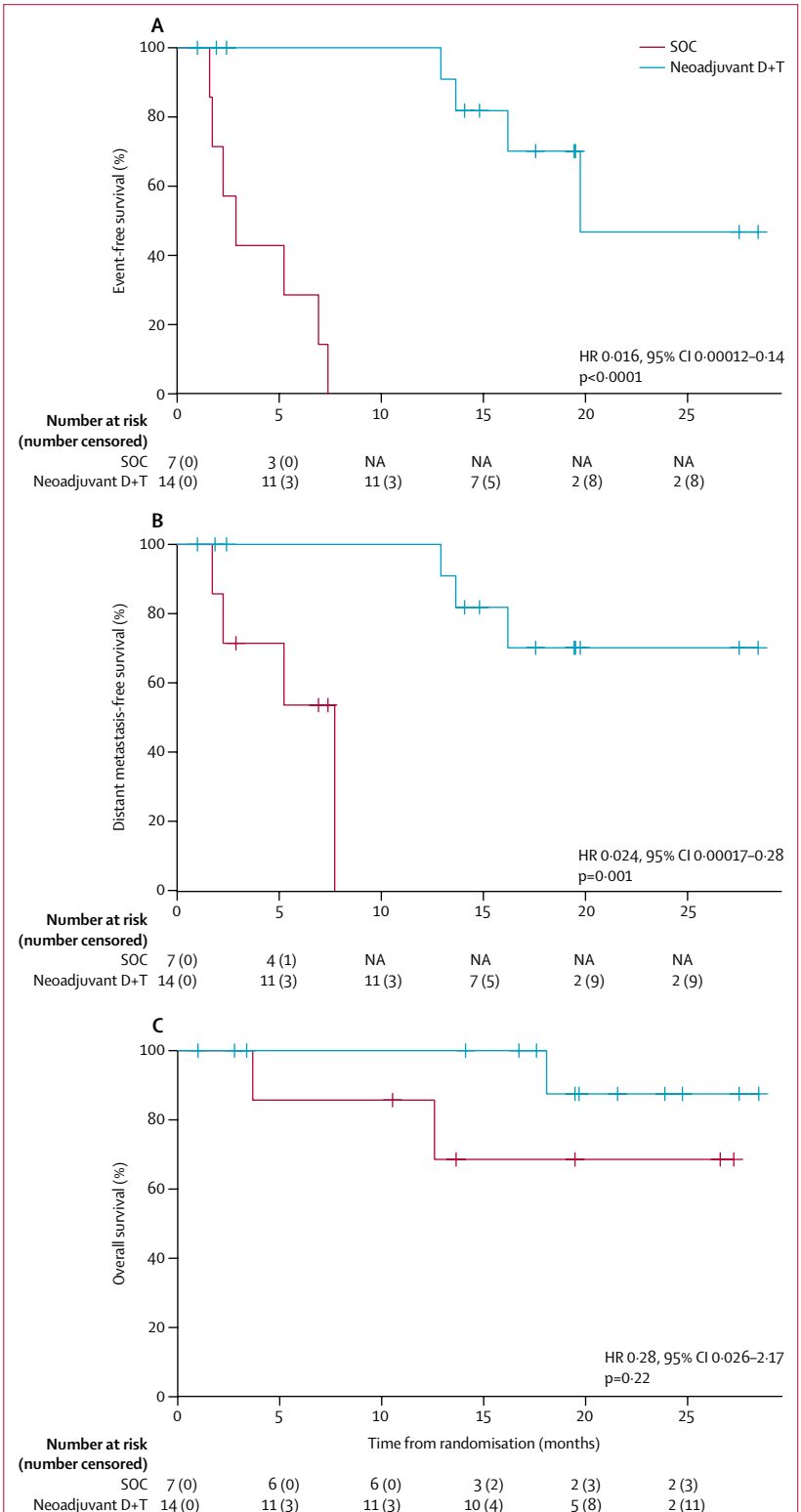

***Figure 2*: Time-to-event outcomes**
Kaplan-Meier curves for (A) event-free survival, (B) distant metastasis-free survival, and (C) overall survival. Tick marks denote censored patients. SOC=standard of care. D+T=dabrafenib and trametinib. HR=hazard ratio. NA=not applicable.

combinations up to the 20 most dominant T-cell clones (appendix pp 10–11). These data suggest that tumour-reactive T-cell clones might pre-exist within the tumours of patients achieving a pathological complete response with activation during the *MAPK* inhibitor-mediated response. However, T cells present in tumours of patients not achieving a pathological complete response might be dysfunctional, as shown through the high coexpression of checkpoint molecules (figure 4C). Transcriptional profiling of baseline and early on-treatment tumour samples showed strong upregulation of cytotoxic CD8+ T-cell genes (*CD8A, CD3E, PFR1,* and *GZMA*) from baseline to early on-treatment in melanomas with subsequent pathological complete response , which did not occur in tumours from patients without a pathological complete response (figure 4E).

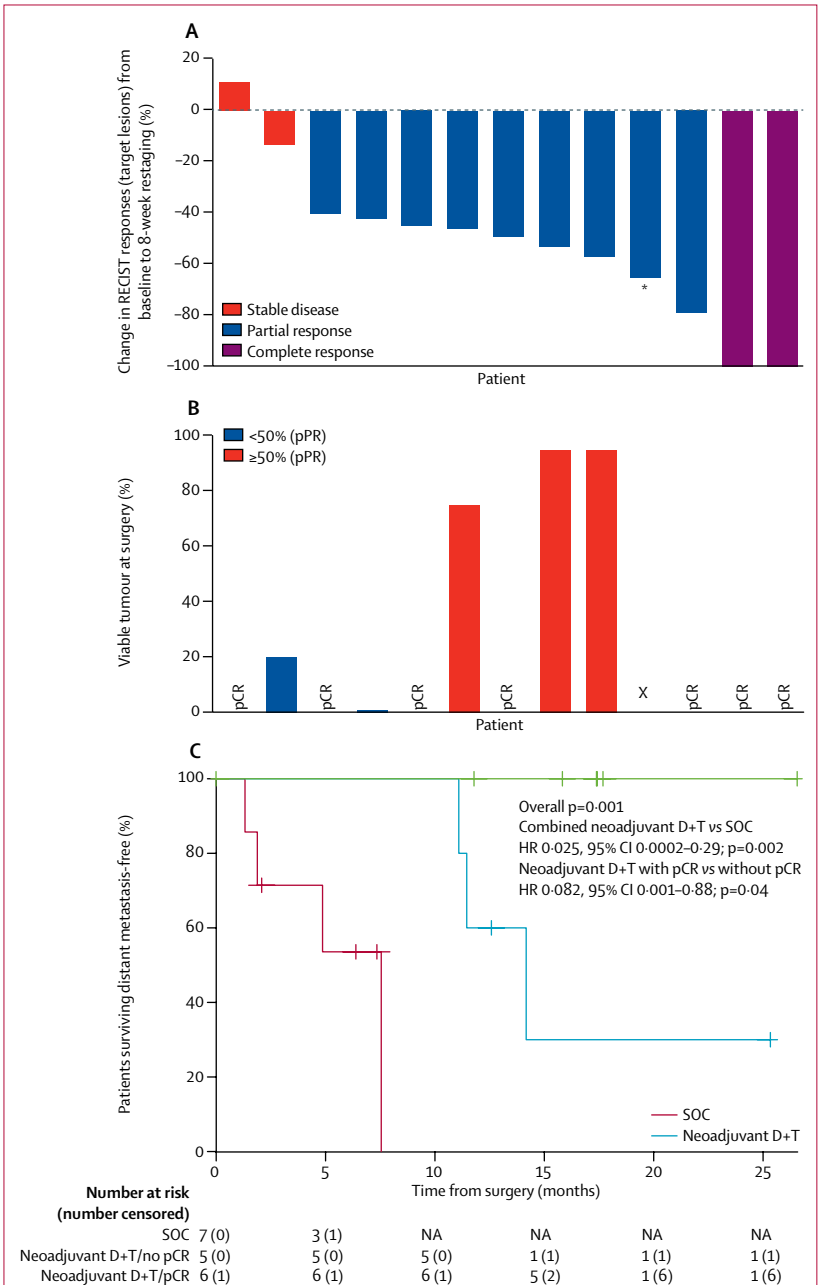

***Figure 3*: Radiographic and pathological responses to neoadjuvant dabrafenib and trametinib**
(A) Response Evaluation Criteria in Solid Tumors (RECIST) responses for the 13 patients in the neoadjuvant dabrafenib and trametinib group assessable for a radiographic response. (B) Matching pathological response data for each patient (X denotes the patient who refused surgery at the end of neoadjuvant therapy). (C) Distant metastasis-free survival since surgery by pathological responses. pPR= pathological partial response. pNR=pathological non-response. pCR=pathological complete response. HR=hazard ratio. SOC=standard of care. D+T=dabrafenib and trametinib. NA=not applicable. *Did not undergo surgery.

Patients who received neoadjuvant plus adjuvant dabrafenib and trametinib had expected grade 1–2 toxicities similar to previous reports of patients with stage IV disease treated with dabrafenib and trametinib in the metastatic setting[10] (table 2); the most common of these were chills (12 patients [92%]), headache (12 [92%]), and pyrexia (ten [77%]), all thought to be treatment related. Serious adverse events (all grade 3) thought to be treatment-related included diarrhoea (two [15%]), and pyrexia, dehydration, rash, post-operative wound infection, atrial fibrillation, and syncope (each in one [8%] of 13 patients). There were no unexpected post-operative toxicities attributed to dabrafenib and trametinib neoadjuvant therapy. Additionally, surgery was not technically more challenging for patients who underwent neoadjuvant therapy compared with surgery for those treated with standard of care. No treatment-related deaths or grade 4 adverse events occurred. Dabrafenib and trametinib were temporarily stopped in 12 (92%) of 13 patients during neoadjuvant treatment to mitigate fever episodes (for a median duration of 5 days [IQR 3·25–11·25]). Five (38%) of 13 patients required at least one dose reduction during neoadjuvant treatment for toxicity mitigation. Toxicities did not delay surgical resection for any neoadjuvant-treated patients. There were no unexpected perioperative complications or impaired wound healing attributable to neoadjuvant therapy. One patient in the neoadjuvant and adjuvant group decided not to proceed with adjuvant dabrafenib and trametinib postoperatively because of intolerable grade 2 toxicities (fatigue, fever, and anorexia) in the neoadjuvant setting. Of the 11 patients who proceeded with adjuvant dabrafenib and trametinib, four (36%) discontinued treatment during the adjuvant phase due to adverse events despite dose reductions. Seven (64%) of 11 patients completed the planned 44 weeks of adjuvant therapy.

Discussion

The results of this study showed that neoadjuvant plus adjuvant dabrafenib and trametinib can produce substantially longer event-free survival in patients with surgically resectable, high-risk, $BRAF^{V600E}$ or $BRAF^{V600K}$-mutated melanoma, compared with the standard of care upfront surgery and consideration of adjuvant therapy. Furthermore, our results showed that

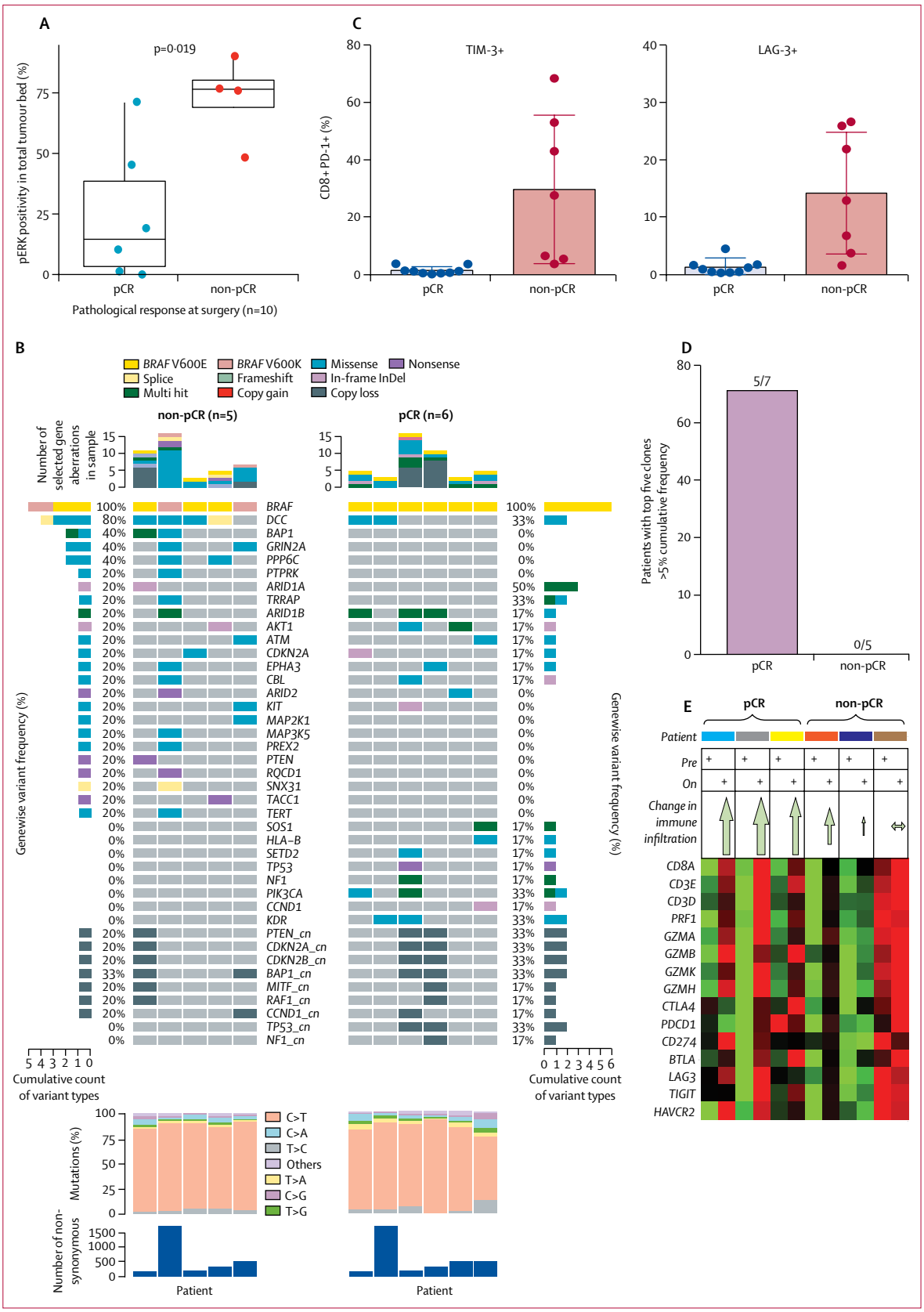

Figure 4: **Molecular and immune correlates of responses to neoadjuvant and adjuvant dabrafenib and trametinib**

(A) Composite score of pERK immunostaining in tumour bed at baseline (non-viable tumour=0%). (B) Whole-exome sequencing genomic analysis of baseline tumours. (C) CD8+ PD-1+ cells with positive expression of TIM-3 or LAG-3 in pre-treatment and on-treatment tumours. (D) Frequency with which the five most dominant T-cell clones were present at a cumulative frequency of >5% of the total T-cell repertoire in patients' tumours based on the pathological response status. (E) Relative expression heatmap of cytolytic CD8+ T-cell marker genes in patient-matched tumours before (pre) and after 3-week (on) neoadjuvant and adjuvant dabrafenib and trametinib treatment; expression levels are high (red), intermediate (black), and low (green). Arrow size represents the magnitude, and arrow orientation represents the direction (up or no change), of the difference in immune infiltration between the pre and on treatment timepoints. pERK=phospho-ERK. pCR=pathological complete response. Indel=insertion/deletion variant.

this approach was tolerable and feasible, since all patients in the neoadjuvant plus adjuvant dabrafenib and trametinib remained resectable after neoadjuvant treatment with no increase in post-operative complications. To our knowledge, this is the first prospective randomised study to compare clinical outcomes with neoadjuvant plus adjuvant dabrafenib and trametinib versus standard of care in this high-risk population. The magnitude of the improvement in event-free survival with neoadjuvant plus adjuvant dabrafenib and trametinib was large and clinically significant, which necessitated early closure of the standard of care group. A large number of patients receiving neoadjuvant dabrafenib and trametinib achieved a pathological complete response, which was associated with improved distant metastasis-free survival. A comparative analysis of molecular and immune features associated with pathological complete response identified significant differences between those with and without a complete pathological response that suggested rational strategies to further improve outcomes such as addition of ERK inhibition for maximal MAPK pathway inhibition or addition of PD-1, LAG-3, or TIM-3 blockade to mitigate exhaustion of T cells in tumours of patients not achieving a pathological complete response. Taken together, these results highlight the potential for neoadjuvant treatment approaches in patients with high-risk, surgically resectable melanoma.

	Grade 1–2	Grade 3
Constitutional		
Pyrexia*	10 (77%)	1 (8%)
Chills	12 (92%)	0
Fatigue	11 (85%)	0
Headache	12 (92%)	0
Anorexia	5 (38%)	0
Dehydration	1 (8%)	1 (8%)
Gastrointestinal		
Nausea	9 (69%)	0
Diarrhoea	9 (69%)	2 (15%)
AST or ALT increases	3 (23%)	0
Musculoskeletal		
Arthralgia	3 (23%)	0
Myalgia	5 (38%)	0
Cutaneous		
Rash	4 (31%)	1 (8%)
Postoperative		
Wound infection	4 (31%)	1 (8%)
Seroma	3 (23%)	0
Deep-vein thrombosis	1 (8%)	0
Cardiovascular		
Hypertension	7 (54%)	0
Atrial fibrillation	0	1 (8%)
Syncope	0	1 (8%)

Data are n (%) in 13 patients. Events were classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. No deaths or grade 4 events occurred in this group during the study. AST=aspartate aminotransferase. ALT=alanine aminotransferase. *Defined as fever >38·5°C.

Table 2: **Adverse events thought to be treatment-related in the neoadjuvant plus adjuvant dabrafenib and trametinib group**

Treatment with dabrafenib and trametinib in patients with unresectable stage IV melanoma is associated with a high number of patients achieving a response, but the duration of responses is typically short (median around 1 year).[9,10] However, the approximate 15% of patients who achieve a radiographic complete response have high rates of long-term progression-free survival (≥2 years).[9] The presence of a small tumour burden (≤3 sites of disease) predicts a higher likelihood of achieving a complete response in patients with stage IV disease treated with dabrafenib and trametinib.[9] Similar associations have also been reported with the US Food and Drug Agency-approved BRAF and MEK inhibitor combination vemurafenib plus cobimetinib.[24] Thus, it is possible that with targeted therapy, higher numbers of patients could achieve a complete response at earlier stages of disease, and consequently gain more durable benefit. Consistent with this hypothesis, in our cohort of patients with surgically resectable metastases treated with neoadjuvant dabrafenib and trametinib, a large proportion achieved a pathological complete response (58%), and so far none of these patients has developed distant metastatic disease. Although the power of our study to assess the clinical significance of pathological complete response was reduced because of the early study closure, the correlation with improved distant metastasis-free survival was promising and is consistent with results from studies of other cancer types.[25] The ability to predict long-term benefit after short-term neoadjuvant therapy could lead to strategies to optimise adjuvant therapy in individual patients, and potentially a new endpoint to assess novel agents in this disease. The three patients who had disease progression after neoadjuvant plus adjuvant dabrafenib and trametinib relapsed initially with brain metastases, consistent with the high risk of CNS metastasis in patients with *BRAF*^{V600} mutations.[26] Whether neoadjuvant plus adjuvant dabrafenib and trametinib might be inducing a resistant disease phenotype predisposed to development of CNS metastases, or if this high-risk patient population is inherently prone to development of CNS disease spread, remains unknown. Additional patients need to be treated to further characterise incidence of CNS disease development after neoadjuvant plus adjuvant treatment.

This study was not powered to detect a benefit in overall survival and its early closure further restricted power to detect significant differences in survival between the treatment groups. However, to design studies in melanoma that can detect significant differences in overall survival is challenging, because of

rapid evolution and success of subsequent lines of therapy. Further follow-up of this study and additional, larger cohorts of patients will be needed to assess the effect of neoadjuvant plus adjuvant dabrafenib and trametinib on overall survival, and to ascertain whether treatment in this setting is more effective than deferring treatment until the development of unresectable metastatic disease.

Although not formal study objectives, no differences were noted in ease of surgery, the frequency of post-operative complications, or the length of hospital stay in the patients receiving neoadjuvant dabrafenib and trametinib therapy compared with those who had surgery alone. Such outcomes are important to assess further in future trials. The toxicities reported with neoadjuvant plus adjuvant dabrafenib and trametinib were similar to those reported with this regimen in the metastatic setting.[10] Almost all patients required a treatment break during the course of neoadjuvant therapy to mitigate toxicities, but all patients were able to proceed with surgery within the designated timeframe. However, four (36%) of 11 patients did not complete the entire 44-week duration of adjuvant dabrafenib and trametinib. Whether the high frequency of treatment discontinuation was due to cumulative toxicity or waning patient motivation is unclear. Although our cohort size was small, discontinuation was similar to that in larger adjuvant targeted-therapy trials such as COMBI-AD (NCT01682083) and BRIM-8 (NCT01667419).

The rapid rate of tumour recurrence in the standard of care group was surprising, but consistent with this high-risk patient population.[2,19] Only one patient in this group chose to pursue adjuvant therapy; thus the group was largely an observation group. The decision to exclude adjuvant radiotherapy in this trial was made mainly to avoid bias if it was only offered to patients assigned to standard care. It is possible that the outcomes of the standard care group might have been improved if more patients had decided to receive adjuvant therapy. Because of the trial's timing, adjuvant ipilimumab therapy was only available as standard of care for one patient who chose not to pursue this treatment because of concerns about toxicity.[4] Adjuvant interferon therapy was not used, but this is typical and consistent with the use of placebo in the control group in the EORTC 18071 trial.[4] Patients in future randomised trials will benefit from standardisation of adjuvant therapy after up-front surgery, which will probably include new options such as nivolumab. Additionally, the use of adjuvant radiotherapy is an important area to standardise in future trials. Apart from age, there were no notable differences in the baseline characteristics of the two groups of patients. However, differences between the groups in ECOG performance status and lactate dehydrogenase concentrations could have affected the outcomes of this study. We have re-opened this study as a single-arm trial of neoadjuvant plus adjuvant dabrafenib and trametinib to further characterise the activity and safety of this treament regimen. Results of this larger cohort (total enrolment of 69 patients) will be analysed to assess these and other clinical predictors of treatment outcome.

The design of our study did not allow for determination of the relative contribution of the neoadjuvant and the adjuvant therapy to the improved outcomes recorded for patients in the neoadjuvant and adjuvant group. This too could be determined in future studies. However, such evidence has not yet been obtained in studies of breast cancer, a disease in which neoadjuvant therapy is used routinely. Although our results do not prove that neoadjuvant therapy was crucial to achieve the benefit in event-free survival, we did record improved clinical outcomes in patients in the neoadjuvant and adjuvant therapy group who achieved a pathological complete response versus those who did not. The fact that three (60%) of five patients without a pathological complete response went on to develop distant metastatic disease compared with none of the patients who achieved a pathological complete response is striking. This result, which needs to be confirmed in larger cohorts of patients and in independent studies, suggests that neoadjuvant therapy might confer clinical benefit by providing an early surrogate marker for long-term outcomes that could be used to optimise and personalise adjuvant therapy for this patient population. This surrogate endpoint could also become an important measure with which to assess new therapies.

Although the early closure of our study limited the sample size, our translational studies provided some notable insights. Molecular characterisation showed that lower pERK concentrations or lower pre-treatment tumour cell viability were predictive of a pathological complete response with neoadjuvant dabrafenib and trametinib, and persistence of pERK during neoadjuvant treatment correlated with an absence of pathological complete response. The low baseline pERK expression was not unexpected since we and other investigator groups have noted that pERK concentrations are highly variable, even in patients with $BRAF^{V600}$ mutations, including in the melanoma TCGA analysis.[8] The number of patients in figure 4 was based on tissue availability for analysis. In all cases, sample availability was limiting and thus not all analyses could be done on a sample from every patient at each timepoint. Depending on the priority or temporal order in which samples were used for the numerous scientific analyses, samples became used up at different times. Low pERK expression might be an informative biomarker and should be assessed in future studies. Furthermore, pERK was still expressed in tumours that did not achieve a pathological complete response. This finding is consistent with results from previous studies showing a direct correlation between the degree of MAPK pathway inhibition and the extent of tumour shrinkage achieved with response to BRAF inhibitors, and the

frequent reactivation of MAPK pathway signalling in acquired resistance to BRAF or MEK inhibitor therapy in patients with stage IV disease.[27,28] Whole-exome sequencing of baseline tumours frequently identified several molecular events known to be associated with targeted therapy resistance in the examined patients without a pathological complete response, but not in those who achieved a pathological complete response.[19,22,23] Because of the early trial closure, we were able to study only a few tumours from patients without a pathological complete response. However, even within our small cohort, we noted a diversity of longitudinal mutation patterns across baseline and surgical samples. Further mutational analysis, including assessments of tumours collected after neoadjuvant treatment, will be addressed as we continue to enrol patients on our re-designed single-arm study of neoadjuvant dabrafenib and trametinib. Together, these findings support the rationale to maximise inhibition of MAPK pathway activity upfront, perhaps through the addition of ERK inhibitors, as a strategy to increase the number of patients achieving a pathological complete response and thus improve clinical outcomes.

Our group and others have previously shown[29,30] that the benefit of BRAF inhibitor-based targeted therapy partly correlates with the induction of an effective anti-tumour immune response manifested through increases in T-cell infiltration and cytotoxicity. Results of the present trial are in line with these previous findings, with increased expression of markers of T-cell infiltration and cytotoxicity such as CD3, CD8, and granzyme in tumour tissue following neoadjuvant dabrafenib and trametinib therapy. Furthermore, results from previous studies examining the T-cell repertoire during BRAF-targeted therapy showed an increase in clonality, as well as an increased frequency of pre-existing clones, in the tumours of patients responding best to this form of therapy.[31] In the neoadjuvant setting, increased clonality followed neoadjuvant dabrafenib and trametinib in all patients, and there was less T-cell remodelling in patients with a pathological complete response than in those who did not achieve such a response, suggesting that the T-cell clones contributing to the anti-tumour response were already present before dabrafenib and trametinib and might already have been responding. This finding contrasts with the lower cumulative frequency of the most abundant T-cell clones in patients without a pathological complete response, potentially because of the high expression of markers of T-cell dysfunction such as PD-1, TIM-3, and LAG-3—a profile suggestive of profound T-cell exhaustion, which might prevent T-cell responses and expansion. Studies of additional patients treated in the neoadjuvant or adjuvant setting will be necessary to determine the extent to which the immunomodulatory effects of MAPK inhibitors contribute to clinical benefit in this setting. Furthermore, such studies will help to elucidate the interactions between T-cell population dynamics and functional state relevant to BRAF inhibitor-based response, and determine which—if any—T-cell metrics might be viable biomarkers for clinical use. Together, these findings suggest that adding checkpoint-inhibitor immunotherapy could augment responses to neoadjuvant dabrafenib and trametinib by overcoming some of the resistance mechanisms at play in patients who do not achieve a pathological complete response to treatment.

In summary, in our randomised trial, patients with surgically resectable oligometastatic melanoma had significantly longer event-free survival with neoadjuvant plus adjuvant dabrafenib and trametinib treatment than did patients who received standard of care. The large difference in event-free survival necessitated early closure of the trial, which potentially limited the generalisability of the results, but they provided important proof-of-concept and data for future studies. Our study was hypothesis-generating, and the clinical and translational results strongly support the rationale for further assessment of neoadjuvant therapy in patients with high-risk, surgically resectable melanoma.

Contributors

RNA, MIR, JEG, MAD, and JAW designed the study. RB developed the statistical analysis plan. RNA, MIR, ICG, JC, W-JH, HAT, SPP, JEL, JEG, PH, AD, MKW, RR, AL, MAD, and JAW recruited and treated patients. RNA, PAP, MTT, AR, MCA, CNS, VG, RB, LS, RM, CWH, LZ, HZ, ZAC, AL, EMB, SR, PS, JA, PAF, SEW, MAD, JAW, KW, and JLM analysed and interpreted data. All authors developed and approved the manuscript.

Declaration of interests

RNA reports grants from Merck, Bristol-Myers Squibb, and Array Biopharma, all outside the submitted work. MTT reports personal fees from Myriad Genetics, Seattle Genetics, and Galderma, all outside the submitted work. MCA reports grants from Pfizer Australia, and non-financial support from Merck and Bristol-Myers Squibb Australia, outside the submitted work. W-JH reports research grants from Merck, Bristol-Myers Squibb, MedImmune, GlaxoSmithKline, and has served on an advisory board for Merck, all outside the submitted work. HAT reports personal fees from Novartis, grants from Merck and Celgene, and grants and personal fees from BMS and Genentech, all outside the submitted work. JEG reports advisory board participation with Merck and Castle Biosciences. CNS and VG report patents for gut microbiome pending. RB reports grants from NIH. AL reports personal fees from BMS, Novartis, Merck, and Genentech/Roche; personal fees and non-financial support from ArcherDX and Beta-Cat; grants and non-financial support from Medimmune/Astra Zeneca and Sanofi; and grants, personal fees, and non-financial support from Janssen, all outside the submitted work. MKW reports personal fees from Merck and EMD Serono, outside the submitted work. PS reports consultant or advisor fees from Bristol-Myers Squibb, GlaxoSmithKline, AstraZeneca, Amgen, Jounce, Kite Pharma, Neon, Evelo, EMD Serono, and Astellas, during the conduct of the study; stock from Jounce, Kite Pharma, Evelo, Constellation, and Neon outside the submitted work; and has a patent licensed to Jounce for a novel immunotherapy outside the submitted work. MAD reports personal fees from Novartis, BMS, and Vaccinex; grants from Astra Zeneca and Merck; and grants and personal fees from Roche/Genentech and Sanofi Aventis, all outside the submitted work. JAW has received compensation for a speaker's bureau and honoraria from Dava Oncology, Bristol-Myers Squibb, and Illumina, and has served on advisory committees for GlaxoSmithKline, Roche/Genentech, Novartis, and Astra Zeneca. All other authors declare no competing interests.

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