Neoadjuvant dabrafenib combined with trametinib for resectable, stage IIIB–C, *BRAF*^{V600} mutation-positive melanoma (NeoCombi): a single-arm, open-label, single-centre, phase 2 trial

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Summary

Background Adjuvant dabrafenib plus trametinib therapy improves relapse-free survival in patients with resected stage III melanoma. We aimed to ascertain the proportion of patients who would have a pathological response and a response according to Response Evaluation Criteria in Solid Tumors (RECIST) after neoadjuvant dabrafenib plus trametinib therapy for resectable clinical stage III melanoma.

Methods NeoCombi was a single-arm, open-label, single-centre, phase 2 study done at Melanoma Institute Australia (Sydney, NSW, Australia). Eligible patients were adults (aged ≥18 years) with histologically confirmed, resectable, RECIST-measurable, clinical stage IIIB–C (American Joint Committee on Cancer [AJCC] 7th edition), *BRAF*^{V600}-mutant melanoma, and had an Eastern Cooperative Oncology Group performance status of 1 or lower. Patients received 150 mg dabrafenib orally, twice daily, plus 2 mg trametinib orally, once daily, for 52 weeks (12 weeks of neoadjuvant therapy before complete resection of the pre-therapy tumour bed, and 40 weeks of adjuvant therapy thereafter). CT and PET scans were done at baseline and before resection. The primary outcomes were the proportion of patients achieving a complete pathological response and the proportion of patients achieving a response according to RECIST at week 12, analysed as per protocol. This trial is registered with ClinicalTrials.gov, NCT01972347, and follow-up of patients is ongoing.

Findings Between Aug 20, 2014, and April 19, 2017, 40 patients were screened, of whom 35 eligible patients were enrolled, received neoadjuvant dabrafenib plus trametinib, and underwent resection. At the data cutoff (Sept 24, 2018), median follow-up was 27 months (IQR 21–36). At resection, 30 (86%) patients achieved a RECIST response; 16 (46%; 95% CI 29–63) had a complete response and 14 (40%; 24–58) had a partial response. Five patients (14%; 95% CI 5–30) had stable disease, and no patients progressed. After resection and pathological evaluation, all 35 patients achieved a pathological response, of whom 17 (49%; 95% CI 31–66) patients had a complete pathological response and 18 (51%; 95% CI 34–69) had a non-complete pathological response. Treatment-related serious adverse events occurred in six (17%) of 35 patients and grade 3–4 adverse events occurred in ten (29%) patients. No treatment-related deaths were reported.

Interpretation Neoadjuvant dabrafenib plus trametinib therapy could be considered in the management of RECIST-measurable resectable stage III melanoma as it led to a high proportion of patients achieving a complete response according to RECIST and a high proportion of patients achieving a complete pathological response, with no progression during neoadjuvant therapy.

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Introduction

In the past decade, there have been major advances in the treatment of melanoma with drugs targeting the MAP kinase pathway or inhibiting immune checkpoints.[1] Specifically, the combination of the BRAF inhibitor, dabrafenib, and MEK inhibitor, trametinib, improves overall survival of patients with *BRAF*^{V600} mutation-positive unresectable stage III or stage IV melanoma, with nearly 70% of patients achieving a response according to the Response Evaluation Criteria in Solid Tumors (RECIST), and reduction in tumour size occurring in 95% of patients in phase 3, randomised clinical trials.[2–4] Additionally, 12 months of adjuvant therapy with dabrafenib plus trametinib has been shown to improve relapse-free survival and overall survival in patients with resected, stage III melanoma.[5]

Research in context

Evidence before this study

We searched Medline and Embase from database inception to Aug 26, 2018, using melanoma as a MeSH heading, and search terms dabrafenib and trametinib plus their trade names. We limited our search to randomised controlled trials or searched with the additional keyword "neoadjuvant". Four phase 3 trials in patients with stage III or IV unresectable melanoma were identified, which reported higher proportions of patients achieving responses and improved progression-free and overall survival in patients treated with combined BRAF and MEK inhibitors than with single therapies. A clinical trial of dabrafenib and trametinib combination therapy after resection of stage III melanoma also showed improved relapse-free survival compared with placebo (n=870). In one small prospective trial in which 12 patients received neoadjuvant dabrafenib plus trametinib and seven patients underwent surgery followed by standard of care, seven (58%) patients who received neoadjuvant combined dabrafenib and trametinib therapy achieved a complete pathological response, with improved relapse-free survival. Given the small size of the two neoadjuvant studies, uncertainty remains as to the clinical benefit of neoadjuvant therapy with dabrafenib and trametinib, and the association of complete pathological response with relapse-free survival.

Added value of this study

This phase 2 trial reports the proportion of patients with resectable stage III melanoma achieving a pathological response following neoadjuvant dabrafenib plus trametinib therapy. We show that operability was improved in nearly half of patients who had a complete pathological response (and was unchanged in the remainder), and the neoadjuvant therapy was well tolerated. Despite achieving a complete pathological response, patients relapsed, which is in contrast to the results observed with neoadjuvant anti-programmed cell death 1 (PD-1) immunotherapy.

Implications of all the available evidence

Dabrafenib combined with trametinib seems to be an effective neoadjuvant therapy for patients with stage III resectable melanoma; however, in contrast to neoadjuvant anti-PD-1 immunotherapy, patients with complete a pathological response still have a high risk of relapse.

Surgical resection is the standard treatment for clinically localised melanoma, and frequently results in cure for patients with early-stage disease, with 10-year survival rates of 95% for patients with stage I melanoma and 84% for those with stage II melanoma.[6] However, patients with clinically palpable regional lymph node or in-transit metastases (stage IIIB–D, American Joint Committee on Cancer [AJCC] 8th edition) have a high risk of relapse and the proportion of these patients dying at 5 years ranges from 17% (stage IIIB) to 68% (stage IIID) because of the presence of micrometastatic melanoma that is undetectable at the time of initial presentation.[6] Therapeutic lymph node dissection remains a recommended standard treatment for patients with palpable lymph nodal metastasis, but it is associated with substantial morbidity, including lymphoedema and pain, which can result in impaired emotional and social functioning, global quality of life, and fatigue,[7,8] and does not prevent distant relapse in many patients with stage IIIC and IIID melanoma.

Neoadjuvant therapy is a standard approach for many cancers, providing improved resectability, improved survival, and prognostic information that could guide subsequent adjuvant therapy. However, the role of neoadjuvant dabrafenib combined with trametinib to decrease tumour load, improve resectability, and prevent relapse and death in patients with resectable stage III melanoma is uncertain. A small study of patients randomly assigned to undergo standard upfront therapeutic lymph node dissection (n=7) versus neoadjuvant dabrafenib combined with trametinib followed by therapeutic lymph node dissection at 8 weeks (n=12) closed prematurely because of a significant improvement in overall survival in patients who received neoadjuvant dabrafenib and trametinib. This study did not have sufficient numbers of patients to adequately investigate the association between a complete pathological response and relapse-free survival in those who received neoadjuvant dabrafenib plus trametinib.[9]

We aimed to ascertain the proportion of patients who would have a pathological response following 12 weeks of neoadjuvant dabrafenib plus trametinib therapy for RECIST-measurable, resectable, clinically apparent, stage III melanoma, including the proportion of patients with a pathologcial complete response. We correlated the proportion of patients achieving a pathological response with those achieving a RECIST response, metabolic response, relapse-free survival, and translational outcomes.

Methods

Study design and participants

This single-arm, open-label, single-centre, phase 2 study was done at Melanoma Institute Australia (Sydney, NSW, Australia), in patients aged 18 years or older with an Eastern Cooperative Oncology Group (ECOG) performance status score of 0–1, histologically confirmed, resectable (complete lymph node dissection or excision of in-transit disease) clinically apparent, stage IIIB–C (AJCC 7th edition), *BRAF*^{V600} mutation-positive melanoma that was measurable as per RECIST version 1.1 guidelines. Eligible patients were required to be naive to previous systemic drug therapy, but were allowed to have had previous surgery for melanoma and, if relevant, definitive radiotherapy to the primary melanoma site

(see appendix p 1 for complete inclusion and exclusion criteria). Haematological, hepatic, and renal laboratory tests were done to assess eligibility in terms of adequate organ function at screening, and women of childbearing age had to have a negative serum pregnancy test before enrolment. Ineligible patients were those with HIV, a history of glucose-6-phosphate dehydrogenase deficiency, hypersensitivity to dimethyl sulfoxide or chemically related drugs, previous malignancy within 3 years (except for patients who had been disease-free for 3 years with life expectancy >5 years or those with completely resected non-melanoma skin cancer or successfully treated in-situ carcinoma), cardiovascular disease (as defined in the protocol; appendix p 1), history or risk of retinal vein occlusion or central serous retinopathy, pregnancy, breastfeeding, or any serious or unstable pre-existing medical conditions that could interfere with patient safety or compliance. The study protocol was approved by the Human Research Ethics Committee. The study was done in accordance with both the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. All participants provided written, informed consent. There were no protocol amendments affecting trial recruitment or conduct. There was one protocol deviation, approved by the Human Research Ethics Committee (incidental low-grade thyroid cancer at screening, completely resected, and one patient enrolled).

Procedures

Patients received 150 mg dabrafenib orally twice daily and 2 mg trametinib orally once daily for 52 weeks (12 weeks of neoadjuvant therapy and 40 weeks of adjuvant therapy). Biopsy samples of lymph node or in-transit melanoma were taken at screening (before treatment or at baseline) and at day 3–7 (early during treatment), followed by complete resection of the pre-therapy tumour bed at week 12, including complete lymph node dissection for those with nodal involvement. Pathological evaluation of all biopsy samples was done according to recommendations of the International Neoadjuvant Melanoma Consortium.[10] Blood was collected at screening, day 3–7, week 2, then every 4 weeks up to 12 weeks, every 12 weeks up to 36 months, and at relapse. CT scans (of the neck, chest, abdomen, and pelvis), MRI or CT scans of the brain, and PET/CT scans were done at screening and just before resection at week 12. Radiological response was ascertained according to RECIST, version 1.1.[11] Metabolic response was ascertained according to European Organisation for Research and Treatment of Cancer (EORTC) criteria.[12] Ultrasound of the pre-therapy tumour bed was done every 4 weeks during the first 12 weeks of therapy. Following complete resection of the pre-therapy tumour bed at week 12, CT monitoring and brain imaging (CT or MRI) was continued every 12 weeks up to 2 years, every 6 months up to 5 years, and then yearly. Patients were followed up for melanoma recurrence until the first recurrence was observed, and thereafter patients were followed up for survival only. Therapy was discontinued during the 52-week treatment period following disease progression, death, unacceptable toxicity, withdrawal of consent, or if recommended by the treating clinician. Dose reductions and delays were allowed in response to adverse events (appendix p 3). Adverse events were graded by the investigator throughout the study by use of the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0), from the first study dose until 30 days after discontinuation of study treatment. Ease of surgical resection was assessed by the surgeon at screening and after resection of the pre-therapy tumour bed, using a questionnaire (appendix p 4).

Multiplex immunofluorescent staining was done on melanoma samples to assess the expression of oncogenic signalling proteins (eg, pERK and pAKT), proliferation (Ki67), SOX10 expression in melanoma cells, and immune cell infiltrate biomarkers (CD8, PDL1, and FOXP3), as previously described.[13,14] Circulating tumour DNA (ctDNA) extraction and analysis were done as previously described.[15]

See Online for appendix

Outcomes

The primary outcomes were the proportion of patients with a pathological response (either a complete pathological response [defined as no evidence of melanoma in the resected tumour bed] or a partial pathological response [defined as a reduction in the proportion of viable melanoma tissue] after 12 weeks of neoadjuvant dabrafenib plus trametinib therapy) and the proportion of patients with a RECIST response (complete or partial response) after 12 weeks of neoadjuvant dabrafenib plus trametinib therapy. Secondary outcomes were adverse events, including surgical adverse events, the proportion of patients with pyrexia, relapse-free survival, and overall survival. Other secondary outcomes include associations of tumour and blood biomarkers with response and survival outcomes, host immune response, and blood and serum changes associated with pyrexia. Relapse-free survival was calculated from the first dose of study treatment until earliest relapse or death. Overall survival was calculated from the first dose of study treatment until death. Patients who neither relapsed nor died by the data cutoff date were censored at their last tumour assessment. EORTC metabolic response and ease of surgical resection of the tumour bed were prespecified exploratory outcomes.

Statistical analysis

The primary outcomes (pathological responses and RECIST clinical responses) were summarised with frequencies and proportions with the two-sided 95% Clopper–Pearson exact CIs. The study required 35 evaluable patients at 90% power to decide whether the treatment was unsuccessful (primary outcome of complete pathological response achieved in ≤10% of

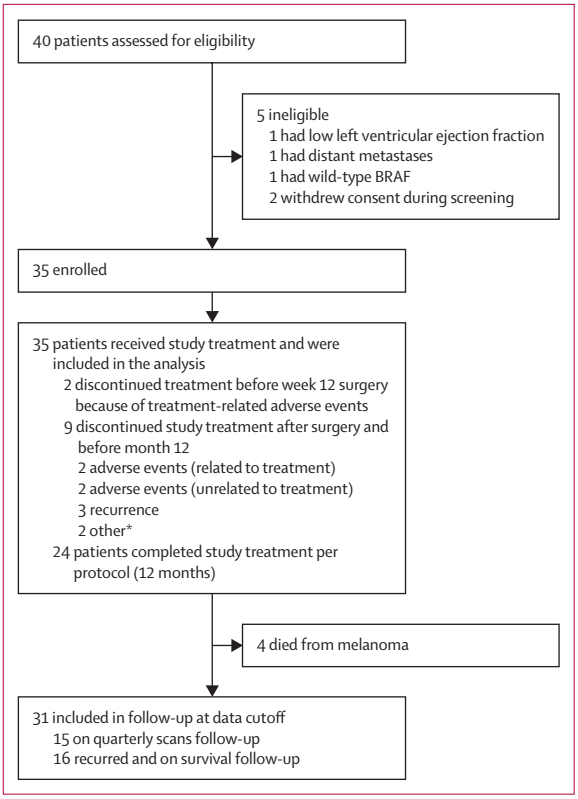

Figure 1: **Trial profile**
*One patient was incarcerated and one started adjuvant radiotherapy.

patients) or successful (primary outcome of complete pathological response achieved in ≥30% of patients). The target error rate was set to 0·05. All analyses were done in all study participants. For secondary outcomes of relapse-free survival and overall survival, survival curves were estimated with the Kaplan–Meier product limit method overall and by pathological response. Given the sample size limitations, only univariate analyses were done. Median and corresponding two-sided 95% CIs were also computed for all survival outcomes. Survival at 12 months and 24 months with 95% CIs were estimated with the Kaplan–Meier method. Safety data (secondary outcome) were summarised by the number and proportions of patients affected by adverse events. Statistical analyses of multiplex immunohistochemistry (secondary outcome of tumour biomarkers associated with response and outcome) were done with Mann-Whitney tests to assess differences in expression between response groups, and Wilcoxon signed ranks tests for patient matched pairs for longitudinal analysis were done with SPSS (version 22). Associations between proportion of patients achieving a complete pathological response and pre-selected tissue markers, including the identification of optimal cutoff values, were done through receiver-operating characteristic (ROC) curve analysis.[16] Associations between ctDNA and either baseline clinicopathological factors or week 12 outcomes were done using Fisher's exact test. Post-hoc outcomes included distant metastasis-free survival and concordance of the pathological, RECIST and EORTC metabolic response measures, after 12 weeks of neoadjuvant dabrafenib and trametinib. Statistical analyses were done with Prism (version 7.02), R (version 3.4.1), SAS (version 9.3), and SPSS (version 22).

This trial is registered with the ClinicalTrials.gov, NCT01972347, and follow-up of patients is ongoing.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. GVL and RFK designed the study. Melanoma Institute Australia maintained the study database. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 20, 2014, and April 19, 2017, 40 patients were screened, five were ineligible, and 35 patients were enrolled (figure 1). The median age of the patients was 56 years (IQR 46–64), 15 (43%) patients were female, 34 (97%) were positive for the *BRAF*^{V600E} mutation, one (3%) had the *BRAF*^{V600K} mutation, 13 (37%) had elevated lactate dehydrogenase, and 29 (83%) had stage IIIC resectable melanoma according to the AJCC 7th edition, of whom 15 (54%) required an inguinal or ilioinguinal dissection (table 1).

At the data cutoff (Sept 24, 2018) the median follow-up was 27 months (IQR 21–36). All 35 (100%) patients had no evidence of clinical or radiological progression at the time of resection of the pre-therapy tumour bed, and 15 (43%) remained in the study (figure 1). All 35 patients achieved a pathological response after resection and pathological evaluation, of whom 17 (49%; 95% CI 31–66) patients had a complete pathological response and 18 (51%; 95% CI 34–69) had a non-complete pathological response. 30 (86%) of 35 patients achieved a RECIST response; 16 (46%; 95% CI 29–63) had a complete response and 14 (40%; 24–58) had a partial response. For the exploratory endpoint of metabolic response, 18 (51%; 95% CI 34–69) patients had a metabolic complete response as assessed by ^{18}F-fluorodeoxyglucose (FDG) PET scan, whereas 17 (49%; 95% CI 31–66) had a non-complete metabolic response (table 2). All three response measures were concordant in 21 (60%) patients; complete responses were concordant in 11 patients and non-complete responses were concordant in ten patients (appendix p 9). In a post-hoc analysis, there was no association between patients who had a complete pathological response and any baseline clinicopathological features, including age, sex, *BRAF* genotype, AJCC stage, ECOG performance status, baseline lactate dehydrogenase (LDH) concentration, and disease site (appendix pp 5–6).

	Total population (n=35)
Median age at enrolment, years (IQR)	56·0 (46·0–64·0)
Sex	
Female	15 (43%)
Male	20 (57%)
BRAF genotype	
$BRAF^{V600E}$	34 (97%)
$BRAF^{V600K}$	1 (3%)
ECOG performance status	
0	32 (91%)
1	3 (9%)
Elevated serum lactate dehydrogenase	13 (37%)
AJCC clinical stage (7th edition)	
IIIB	6 (17%)
IIIC in-transit metastasis only	7 (20%)
IIIC lymph node positive or negative in-transit metastasis	22 (63%)
Disease site	
In-transit metastasis only	7 (20%)
In-transit plus lymph node metastasis	4 (11%)
Lymph node metastasis only*	24 (69%)
Disease region†	
Lymph node	
Neck	3 (11%)
Neck or axilla	4 (14%)
Axilla	5 (18%)
Inguinal or ilioinguinal	15 (54%)
Inguinal, ilioinguinal, or other	1 (4%)
In-transit metastasis	
Head and neck	1 (9%)
Trunk	5 (45%)
Upper limb	3 (27%)
Lower limb	2 (18%)

Data are n (%), unless otherwise indicated. ECOG=Eastern Cooperative Oncology Group. AJCC=American Joint Committee on Cancer. *One patient had a concurrent primary melanoma and lymph node metastasis at baseline. †Some patients are in multiple categories if there are several disease sites (eg, lymph node plus in-transit metastases).

Table 1: **Baseline characteristics**

	Study population (n=35)
Pathological response	
Complete	17 (49%; 95% CI 31–66)
Non-complete	18 (51%; 95% CI 34–69)
RECIST response	
Complete response	16 (46%; 95% CI 29–63)
Partial response	14 (40%; 95% CI 24–58)
Stable disease	5 (14%; 95% CI 5–30)
Metabolic response	
Complete	18 (51%; 95% CI 34–69)
Non-complete	17 (49% 95% CI 31–61)

Data are n (%; 95% CI). RECIST=Response Evaluation Criteria in Solid Tumors.

Table 2: **Patients with pathological, RECIST, and metabolic responses at 12 weeks**

At the data cutoff, 20 (57%) of the 35 patients had recurred. No patient progressed during 12 weeks of neoadjuvant therapy; eight (40%) of 20 recurred after resection within the first year (week 12–52), four of whom were on dabrafenib and trametinib therapy and four of whom had stopped treatment because of toxicity, and 12 (60%) of 20 patients recurred after 52 weeks. No baseline clinical factors were associated with relapse at data cutoff (appendix p 6). The first site of recurrence was distant in nine (45%) of 20 patients and locoregional in 11 (55%) patients (three in-transit, eight in regional lymph nodes; appendix p 6).

Median relapse-free survival for the whole group of 35 patients was 23·3 months (95% CI 17·7 to not reached), with a 1-year landmark relapse-free survival of 77·1% (64·4 to 92·4) and a landmark 2-year relapse-free survival of 43·4% (28·6 to 65·7; table 3, figure 2A). Recurrences and relapse free-survival outcomes in patients who did versus did not achieve a pathological complete response are presented in table 3 and figures 2B and 2C.

Four patients had died from melanoma at data cutoff; three had a non-complete pathological response (these patients died 15, 24, and 30 months after the start of study therapy), and one had a complete pathological response (died 21 months after the start of study therapy). The median overall survival in this cohort was not reached (95% CI not reached), and the 1-year overall survival was 100% (100–100) and 2-year overall survival was 93·8% (85·9–100; appendix p 9).

Surgical complications of any grade occurred in 22 (63%) patients, nine (26%) of which were grade 3 (appendix p 7) and most of which occurred in patients who had inguinal or ilioinguinal dissections (appendix p 7). Complications included postoperative infection requiring intravenous antibiotics (nine [26%]), seroma (ten [29%]), bleeding (two [6%]), new or worse lymphoedema (nine [26%]), and deep vein thrombosis or pulmonary embolus (two [6%]).

Ease of surgical resection of the pre-therapy tumour bed (a prespecified exploratory endpoint) was assessed as improved after 12 weeks of neoadjuvant treatment compared with at screening in 16 (46%) of 35 patients and unchanged in 19 (54%) patients.

Multiplex immunohistochemistry was used to assess the expression of phosphorylated oncogenic signalling proteins (pERK and pAKT), Ki67, and immune infiltrative markers (CD8, PD-L1, and FOXP3) at baseline, early during treatment (day 3–7), and in melanoma biopsy samples taken at week 12 for associations with pathological response and melanoma recurrence (appendix p 7). Patients who had a complete pathological response had a significantly higher proportion of Ki67-positive (20·50% [IQR 15·30–28·18] *vs* 12·35% [1·55–13·70]; p=0·057) and PD-L1-positive and SOX10-positive (332·90 cells per mm² [179·90–682·80] *vs* 0·61 cells per mm² [0–5·70];

	Total population (n=35)	Patients with a complete pathological response (n=17)	Patients with a non-complete pathological response (n=18)
Number of events	20	8	12
Median, months (95% CI)	23·3 (17·7 to not reached)	30·6 (20·1 to not reached)	18·0 (14·6 to not reached)
1-year relapse-free survival (95% CI)	77·1% (64·4 to 92·4)	82·4% (66·1 to 100)	72·2% (54·2 to 96·2)
2-year relapse-free survival (95% CI)	43·4% (28·6 to 65·7)	63·3% (43·7 to 91·7)	24·4% (9·7 to 61·8)

Table 3: **Relapse-free survival from start of dabrafenib plus trametinib treatment**

p=0·008) melanoma cells at baseline, and a higher density of intratumoural CD8-positive T cells (93·70 cells per mm² [14·63–1676·00] *vs* 2·20 cells per mm² [0–21·52]; p=0·043) in baseline samples than patients who had a non-complete pathological response (figure 3A–C). ROC curve analysis was used to identify the optimal cutoff values of these markers to distinguish patients with a complete pathological response from those with a non-complete pathological response (figure 3D). Of the three biomarkers, PD-L1-positive melanoma cell density was the most predictive of complete pathological response, with an optimal cutoff of 134 PD-L1-positive melanoma cells per mm² (n=14, area under the curve [AUC] 0·88, sensitivity 100%, specificity 83·3%, p=0·0201); followed by CD8-positive intratumoural density, with a cutoff of 62 cells per mm² (n=14, AUC 0·88, sensitivity 100%, specificity 83·3%, p=0·0201). Ki67 expression in melanoma cells was not significantly predictive of complete pathological response in the ROC curve analysis (p=0·0518). No other markers were significantly associated with pathological response or disease recurrence in baseline melanoma biopsy samples (data not shown). The proportion of Ki67-positive melanoma cells decreased from baseline to early during treatment, but the change was only significant in patients who had a complete pathological response (figure 3E). There were no other significant changes from baseline to early during treatment for the remaining biomarkers, including pERK, pAKT, CD8, PDL1, FOXP3, as a whole group, or within patients who had a complete pathological response and those who had a non-complete pathological response (data not shown).

Toxicities related to dabrafenib plus trametinib were consistent with previous reports in patients with stage III melanoma,[5] although a high proportion of patients had pyrexia (28 [80%]), with three (9%) grade 3 and one (3%) grade 4, and one (3%) patient had reversible posterior leukoencephalopathy syndrome (table 4). Grade 3–4 adverse events occurred in ten (29%) patients (table 4). Serious adverse events occurred in 18 (51%) patients, six (17%) of whom had treatment-related serious adverse events (three had pyrexia, two had syncope, and one had acute kidney injury). No patients had dose reductions, but four (11%) patients discontinued dabrafenib plus trametinib because of toxicity, including two during the neoadjuvant period: one because of diabetes insipidus and associated acute renal failure, one because of recurrent pyrexia, one because of neuropathy, and one because of reversible posterior leukoencephalopathy. A further four (11%) patients discontinued dabrafenib plus trametinib during the adjuvant phase (week 12–52) without recurrence or toxicity; one patient was diagnosed with tuberculosis, one was treated with radiotherapy, one developed complicated cellulitis, and one was incarcerated (figure 1). No treatment-related deaths occurred.

At data cutoff, 14 (40%) of 35 patients had recurred at a distant metastatic site, including eight (23%) in the brain. Median distant metastasis-free survival (post-hoc analysis) was 30·6 months (95% CI 23·2 to not reached) in the overall patient population (appendix pp 7, 10), 38·0 months (23·2–not reached) in patients who had a complete pathological response, and 27·7 months (19·4–not reached) in those who had a non-complete pathological response (p=0·58; appendix p 7, 11). Details of the treatments given to these 14 patients after their first distant recurrence are in the appendix (p 6).

ctDNA was detectable in 14 (40%) of 35 patients (13 patients with the $BRAF^{V600E}$ mutation) at baseline (median 73 copies per mL plasma, IQR 23–153; range 4–415), and correlated with tumour burden as per RECIST (appendix p 8). All 14 patients with detectable ctDNA at baseline became undetectable before surgical resection; with seven (50%) of 14 patients having undetectable ctDNA by first blood draw early during treatment (day 3–7). The remaining patients had undetectable ctDNA by week 2 (n=3), week 8 (n=3), and week 12 (n=1).

We observed no association between ctDNA detectability at baseline and all three outcome measures at week 12, including RECIST response, pathological response, and metabolic response (appendix p 8). Furthermore, we found no association between baseline ctDNA and recurrence-free survival (exploratory outcome); ten (48%) of 21 patients with an undetectable ctDNA and eight (57%) of 14 patients with detectable ctDNA developed disease recurrence. The median time to recurrence in patients with undetectable ctDNA at baseline was 23·7 months (IQR 17·8–not reached), compared with 19·7 months (7·6–48·9) in patients with detectable ctDNA at baseline (hazard ratio [HR] 0·56 [95% CI 0·22–1·41], p=0·19). Three of four patients with detectable ctDNA at baseline who did not have disease recurrence had rapid clearance of their ctDNA within 7 days (zeroconversion by erythrocyte density test blood draw), with the remaining patient undergoing zeroconversion by week 2.

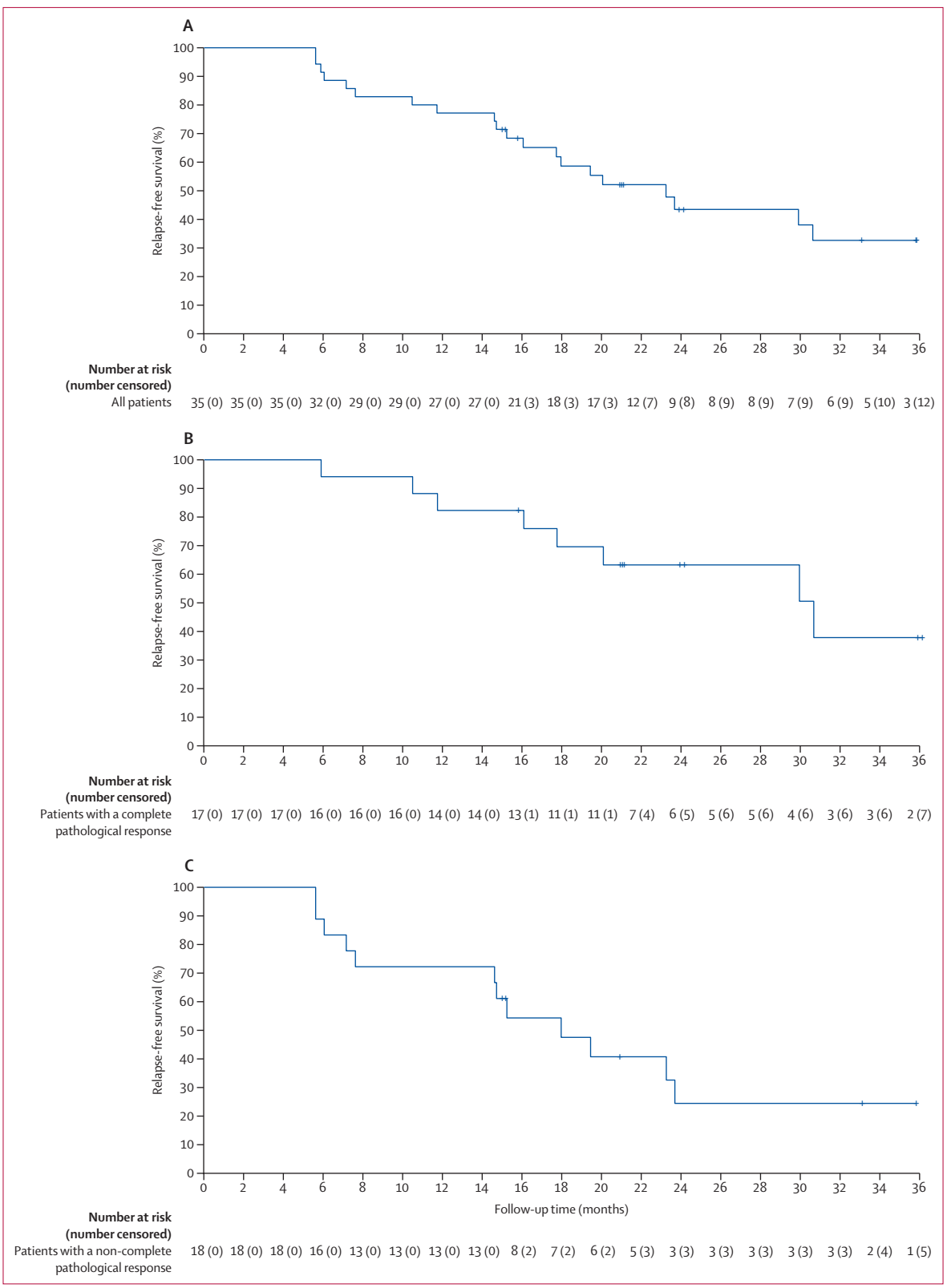

Figure 2: **Relapse-free survival**
(A) Relapse-free survival from the start of dabrafenib plus trametinib treatment in all patients. (B) Relapse-free survival from the start of dabrafenib plus trametinib treatment in patients who had a complete pathological response. (C) Relapse-free survival from start of dabrafenib plus trametinib treatment in patients who had a non-complete pathological response.

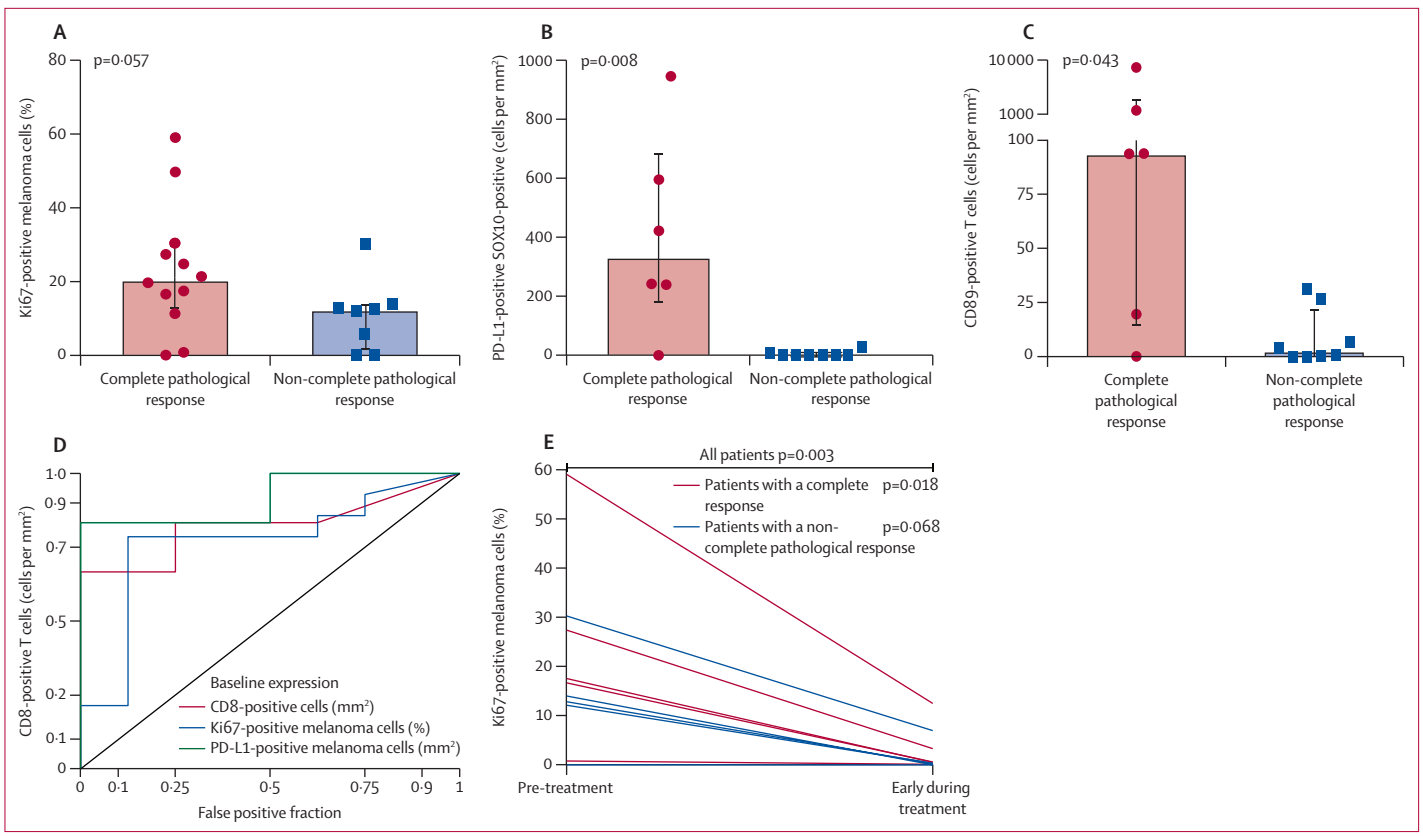

Figure 3: **Quantitative multiplex immunohistochemistry of biopsy samples taken at baseline and early during treatment**
(A) Comparison of baseline Ki67-positive melanoma cells in patients with a complete pathological response versus those with a non-complete pathological response. (B) Comparison of baseline PD-L1-positive SOX10-positive melanoma cells in patients with a complete pathological response versus those with a non-complete pathological response. (C) Comparison of baseline CD8-positive T cells in patients with a complete pathological response versus those with a non-complete pathological response. (D) Receiver-operating characteristic (ROC) curve analysis comparing baseline CD8-positive T cells, Ki67-positive melanoma cells, and PD-L1-positive melanoma cells in patients with a complete pathological response versus those with a non-complete pathological response. (E) Expression of Ki67-positive melanoma cells in patients with a complete pathological response versus those with a non-complete pathological response with matched biopsy samples taken at baseline and early during treatment. Error bars represent IQR and plotted points represent patients.

ctDNA was measured at the time of disease recurrence in 18 (90%) of 20 patients, with ctDNA detectable in eight (44%) of 18 patients, either at the time of disease recurrence (in five patients) or 3–12 months before disease recurrence (in three patients).

Discussion

This study is, to the best of our knowledge, the largest phase 2 trial of neoadjuvant therapy in patients with clinically apparent stage III resectable melanoma, showing that 12 weeks of neoadjuvant dabrafenib plus trametinib resulted in a complete pathological response in 49% of patients. No patients progressed before surgery, drug toxicity was similar to that observed in the adjuvant and metastatic settings, and surgical complications were similar to historical data.[7,8] These results suggest that neoadjuvant dabrafenib combined with trametinib is safe, feasible, and reduces tumour burden in patients with stage III resectable melanoma.

In this study, concordance of pathological response and imaging (CT or PET) response was modest, and no baseline clinical factors were associated with achievement of a complete pathological response, suggesting that achievement of a complete pathological response might be more dependent on biological rather than clinical factors. In support of this view, tissue-based analysis identified a higher proliferative proportion (Ki67-positive melanoma cells) in baseline melanoma biopsy samples of patients who had a complete pathological response, and evidence of a pre-existing immune response with CD8-positive T-cell infiltration and melanoma PD-L1 expression. These results are consistent with previous correlations of early immune infiltrates in melanoma deposits responsive to these drugs,[17,18] as well as recent biomarker investigations from the dabrafenib plus trametinib adjuvant trial,[5] which showed that tumours with a higher baseline tumour interferon signature had a better prognosis and a longer recurrence-free survival with adjuvant therapy.[19] Translational studies of the melanoma tissue and tumour bed from this trial are ongoing.

Surgical resection of the tumour bed was considered easier after neoadjuvant treatment than at screening in nearly half of patients based on a simple questionnaire,

and the International Neoadjuvant Melanoma Consortium is currently establishing an objective and more detailed assessment tool to assess this important endpoint for future studies. Despite the high proportion of patients with a complete pathological response (49%), 57% of all patients recurred regardless of whether or not a complete pathological response was achieved, and relapse-free survival in the overall patient population was 23 months, with recurrences occurring both locally and distantly in similar proportions. Although the study was not powered to detect survival differences between patients with or without a complete pathological response, median relapse-free survival was 30·6 months in those with a complete pathological response compared with 18·0 months in those without, and nearly half of patients with a complete pathological response had recurred at data cutoff. This finding is in contrast to observations with neoadjuvant immunotherapy, where a pathological complete response is strongly associated with absence of relapse, although a higher proportion of grade 3–4 toxicities was observed with neoadjuvant dabrafenib plus trametinib in our study than with BRAF-targeted therapy.[20–22] A limitation of this study was that it was done in a single centre, as a single-arm, phase 2 study, and was not powered to detect a difference in survival based on pathological response. Pooled analyses will be required to explore the association of pathological response and complete pathological response with relapse-free survival further, and the International Neoadjuvant Melanoma Consortium has been established to unify trial design to enable cross-trial comparisons.[10]

Unlike immunotherapy, in which preclinical models and early clinical data suggest greater efficacy with neoadjuvant immunotherapy[20,23] than with adjuvant immunotherapy, targeted therapy might not result in superior survival when given in the neoadjuvant setting compared with the adjuvant setting; patients in this study had lower 1-year relapse-free survival (77%) and 2-year relapse-free survival (43%) than did those in the COMBI-AD trial with stage IIIC melanoma (1-year relapse-free survival 86% and 2-year relapse-free survival 61%) or macrometastases (1-year relapse-free survival 87% and 2-year relapse-free survival 67%).[5,24] Nevertheless, this outcome might be influenced by the exclusion (ie, screening failure) of patients with very early recurrence after surgery in the adjuvant trial, and the fact that patients in this neoadjuvant study might have had poorer prognostic features (eg, 37% had elevated baseline LDH). Even if neoadjuvant targeted therapy is not superior to adjuvant therapy with regard to efficacy, it has several other advantages; pathological response provided some prognostic information, and surgery was deemed easier in almost half of patients in this study. Furthermore, the safety and efficacy of neoadjuvant dabrafenib and trametinib suggest it would be a good platform for further combinations, such as with anti-PD-1 antibodies (NCT02858921). As with previous studies, pyrexia was common,[2–5] and we have previously published the pharmacokinetic and cytokine profiles of patients from this study, showing no apparent associations of pyrexia and exposure to drugs or metabolites, although pyrexia was associated with elevations in interleukin-1β and interleukin-6.[25] Some uncommon events were observed in patients on this study, such as a single case of neuropathy, posterior reversible encephalopathy syndrome, and diabetes insipidus, which we attributed to the drugs because we could not exclude other causes. Neuropathies and posterior reversible encephalopathy syndrome have been reported with these drugs previously, but not diabetes insipidus. Given the small numbers of

	Grade 1–2	Grade 3	Grade 4
Any	35 (100%)	9 (26%)	1 (3%)
Pyrexia	24 (69%)	3 (9%)	1 (3%)
Fatigue	27 (77%)	..	..
Chills	24 (69%)	..	..
Nausea	21 (60%)	..	..
Vomiting	14 (40%)	..	..
Diarrhoea	12 (34%)	..	..
Stomach pain	7 (20%)	..	..
Dry mouth	6 (17%)	..	..
Abdominal pain	4 (11%)	..	..
Oral mucositis	4 (11%)	..	..
Headache	26 (74%)	1 (3%)	..
Dizziness	5 (14%)	1 (3%)	..
Reversible posterior leukoencephalopathy syndrome	..	1 (3%)	..
Myalgia	18 (51%)	..	..
Arthralgia	17 (49%)	..	..
Pain in arms and legs	..	1 (3%)	..
Hyperhidrosis	13 (37%)	..	..
Other skin and subcutaneous tissue disorders	10 (29%)	..	..
Maculopapular rash	6 (17%)	..	..
Hot flashes	9 (26%)	..	..
Flushing	4 (11%)	..	..
Hypertension	..	1 (3%)	..
Increased alanine aminotransferase	4 (11%)	1 (3%)	..
Increased aspartate aminotransferase	5 (14%)	..	..
Increased gamma-glutamyl transferase	..	1 (3%)	..
Other hepatobiliary disorders	..	1 (3%)	..
Left ventricular systolic dysfunction	..	1 (3%)	..
Acute kidney injury	..	1 (3%)	..
Syncope	..	2 (6%)	..
Increased C-reactive protein	..	1 (3%)	..
Seizure	..	1 (3%)	..

No treatment-related deaths occurred. For grade 1 or 2 adverse events, only those occurring in 10% or more patients are reported. All grade 3 and 4 events are reported.

Table 4: **Treatment-related adverse events**

individual different toxicities involved, we cannot know whether they were more or less prevalent than in the adjuvant or metastatic settings.

Similar to the COMBI-AD trial,[5] in which for the higher risk stage IIIC and macrometastasis cohorts, most recurrences occurred 12 months after discontinuing dabrafenib and trametinib, in this study most patients recurred after discontinuing treatment. Although 20 (57%) patients in this study recurred, only four patients recurred while on treatment. Longer durations of adjuvant therapy might be required for some cohorts of patients.

This trial included very high-risk stage III patients, all with RECIST-measurable disease at baseline and 37% with elevated LDH. Despite these characteristics, measurement of ctDNA did not have high clinical utility: only 40% of patients had measurable circulating tumour DNA (ctDNA) at baseline, similar to the number with high baseline LDH (neither predicted complete pathological response); baseline ctDNA did not predict whether or not ctDNA could be detected at relapse; and ctDNA was detectable at relapse in only eight of 18 patients measured. However, all patients with detectable ctDNA who did not have disease recurrence had early clearance of their ctDNA with neoadjuvant treatment.

In conclusion, the results of this phase 2 study show that a high proportion of patients had a complete pathological response with neoadjuvant dabrafenib plus trametinib therapy. Neoadjuvant dabrafenib combined with trametinib was tolerable, with no relapses during the 12-week neoadjuvant period, and all patients were able to undergo surgery. This treatment could therefore be a feasible approach in a subset of patients in whom neoadjuvant anti-PD-1-based therapy might not be suitable.

Contributors

GVL contributed to study design, protocol development, patient enrolment, data collection, data interpretation, and wrote the manuscript and coordinated all author reviews of the manuscript. RPMS contributed to data collection, data interpretation, and review of the manuscript. SL did the statistical analyses, generated figures, and reviewed the manuscript. OEN contributed to data collection, data interpretation, and review of the manuscript. KFS contributed to data collection, data interpretation, and review of the manuscript. MG contributed to protocol development, data collection, and review of the manuscript. AG contributed to patient enrolment and review of manuscript. JHL contributed to data collection (ctDNA), statistical analysis, data interpretation, and review of the manuscript. HL contributed to generation of figures, data collection (tissue analysis), statistical analysis, data interpretation, and review of the manuscript. PMF contributed to data collection (tissue analysis) and review of the manuscript. JSW contributed to generation of figures, data collection (tissue analysis), statistical analysis, data interpretation, and review of the manuscript. JFT contributed to data collection, data interpretation, and review of the manuscript. RFK contributed to study design, data interpretation, and review of the manuscript. SC contributed to data collection, data interpretation, and review of the manuscript. JRS contributed to data collection and review of the manuscript. LE contributed to data collection (PET scans), data interpretation, and review of the manuscript. HR contributed to data analysis (ctDNA), data interpretation, and review of the manuscript. AJS contributed to data collection, data interpretation, and review of the manuscript. RAS contributed to data collection, data analysis, data interpretation, and review of the manuscript. AMM contributed to patient enrolment, data collection, data interpretation, and drafting and review of the manuscript.

Declaration of interests

GVL is a consultant advisor to Aduro, Amgen, Array, Bristol-Myers Squibb, Merck Sharp Dohme, Novartis, Pierre-Fabre, Oncosec, and Roche. JFT has received honoraria for participation in advisory boards for GlaxoSmithKline, Bristol-Myers Squibb, Merck Sharp Dohme, and Provectus. All other authors declare no competing interests.

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