Title

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Journal

Nature Medicine, 29(9)

ISSN

1078-8956

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Publication Date

2023-09-01

DOI

10.1038/s41591-023-02498-y

Peer reviewed

nature medicine

Article

https://doi.org/10.1038/s41591-023-02498-y

Ipilimumab with or without nivolumab in PD-1 or PD-L1 blockade refractory metastatic melanoma: a randomized phase 2 trial

Received: 23 February 2023

Accepted: 12 July 2023

Published online: 17 August 2023

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In this randomized phase 2 trial, blockade of cytotoxic T-lymphocyte protein 4 (CTLA-4) with continuation of programmed death protein 1 (PD-1) blockade in patients with metastatic melanoma who had received front-line anti-PD-1 or therapy against programmed cell death 1 ligand 1 and whose tumors progressed was tested in comparison with CTLA-4 blockade alone. Ninety-two eligible patients were randomly assigned in a 3:1 ratio to receive the combination of ipilimumab and nivolumab, or ipilimumab alone. The primary endpoint was progression-free survival. Secondary endpoints included the difference in CD8 T cell infiltrate among responding and nonresponding tumors, objective response rate, overall survival and toxicity. The combination of nivolumab and ipilimumab resulted in a statistically significant improvement in progression-free survival over ipilimumab (hazard ratio = 0.63, 90% confidence interval (CI) = 0.41–0.97, one-sided $P = 0.04$). Objective response rates were 28% (90% CI = 19–38%) and 9% (90% CI = 2–25%), respectively (one-sided $P = 0.05$). Grade 3 or higher treatment-related adverse events occurred in 57% and 35% of patients, respectively, which is consistent with the known toxicity profile of these regimens. The change in intratumoral CD8 T cell density observed in the present analysis did not reach statistical significance to support the formal hypothesis tested as a secondary endpoint. In conclusion, primary resistance to PD-1 blockade therapy can be reversed in some patients with the combination of CTLA-4 and PD-1 blockade. Clinicaltrials.gov identifier: NCT03033576.

At the time of study design, treatment with programmed death protein 1 (PD-1) blocking antibodies was the most widely used standard-of-care front-line therapy for patients with metastatic melanoma[1–4]. The optimal therapeutic approach for patients who do not respond to initial single-agent anti-PD-1 treatment is unclear. We reasoned that we could potentially reverse resistance by addressing the main mechanism of lack of response to PD-1 blockade, that is, the absence of preexisting intratumor T cell infiltrates[5,6]. It is possible that an immune checkpoint such as cytotoxic T-lymphocyte protein 4 (CTLA-4), which inhibits T cell proliferation at the time of T cell activation, limits the ability of antitumor T cells to infiltrate cancer lesions[7,8]. CTLA-4 blockade with therapeutic antibodies allows T cells to expand, circulate and infiltrate tumors, as demonstrated in mouse models[9–11]. Similarly, in humans, CTLA-4 blocking antibodies induce cell proliferation in lymphoid organs, diversify the peripheral immune response and increase intratumor T cell infiltration[12–16]. On reaching tumors, T cells can still become negatively regulated by the reactive expression of programmed cell death 1 ligand 1 (PD-L1) on tumors and other cells of the immune tumor microenvironment[17], arguing that there may be a benefit for combined CTLA-4 and PD-1 blockade therapy, over CTLA-4 blockade alone, to reverse primary

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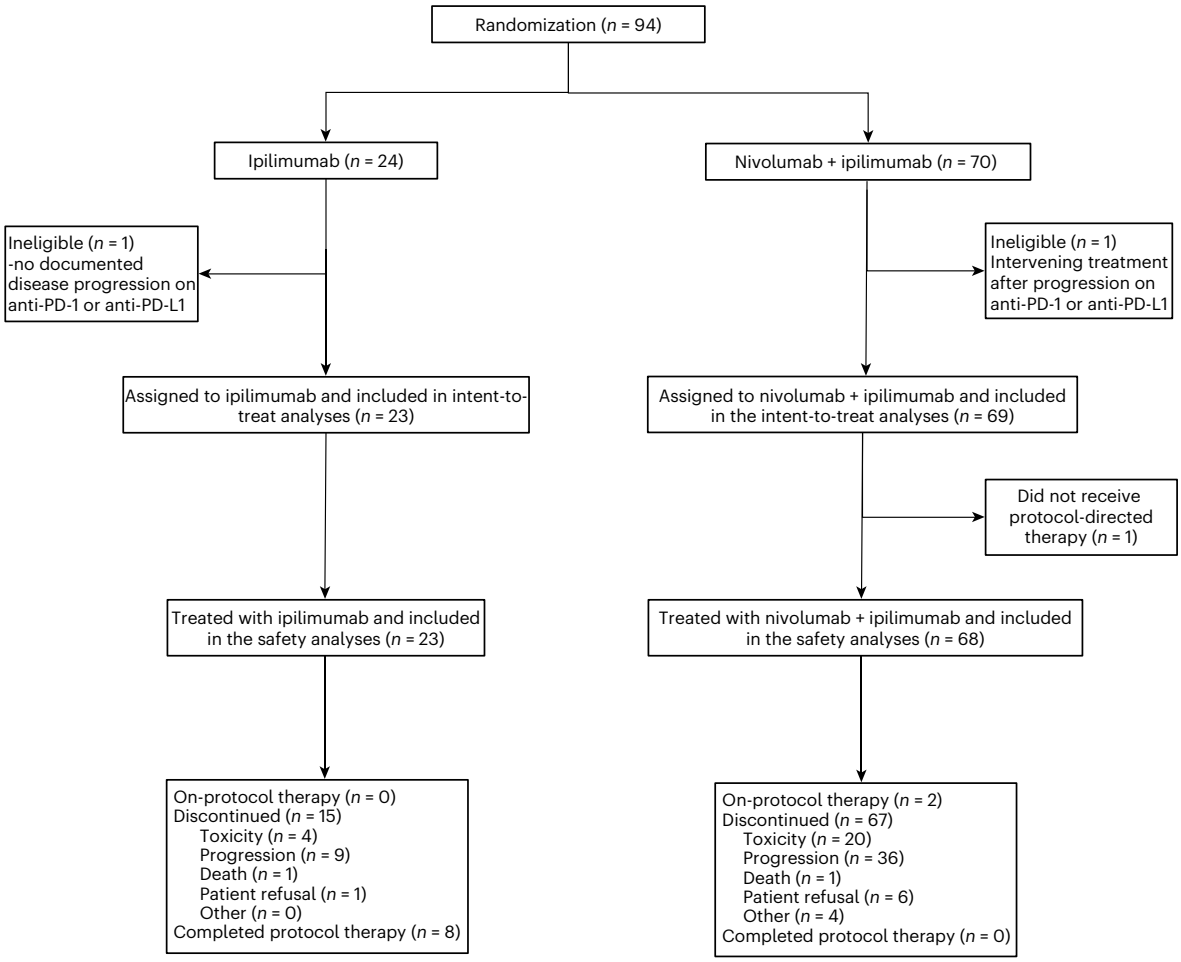

Fig. 1 | CONSORT diagram. The diagram includes patient enrollment, randomization and follow-up. All eligible patients who were randomized were included in the intention-to-treat population. The safety population included all patients who received at least one dose of treatment that they were randomly assigned to.

resistance to anti-PD-1. The concept of combined CTLA-4 and PD-1 blockade to reverse resistance to PD-1 blockade alone is supported by three published clinical trials, one retrospective[18] and two prospective[19,20].

The Southwest Oncology Group (SWOG) Cancer Research Network clinical trial S1616 is a randomized phase 2 study to address the scientific question whether CTLA-4 blockade, alone or in combination with continued PD-1 blockade, could reverse resistance to previous anti-PD-1 sequentially or concomitantly. All patients had advanced melanoma with primary resistance to anti-PD-1 or anti-PD-L1 inhibitors, defined as tumors having no objective clinical response (complete or partial response (PR)) to the prior use of anti-PD-1 or anti-PD-L1 blocking agents without intervening therapy for advanced disease, or with recurrence while on adjuvant anti-PD-1 therapy. The clinical trial was designed to test the hypothesis that combination nivolumab plus ipilimumab is superior to single-agent ipilimumab in terms of progression-free survival (PFS) in this anti-PD-1 or anti-PD-L1-experienced population, with the analysis of changes in intratumor infiltration by CD8 T cells as a secondary endpoint. A 3:1 randomization was used to power a secondary objective to evaluate changes in CD8 T cell infiltration of biopsies of patients in the combination group, requiring a larger number of patients receiving the combination. We anticipated that the benefit of the combination would manifest itself through improved PFS mediated by increasing intratumor CD8 T cell infiltration on releasing the CTLA-4 immune checkpoint with continued PD-1 blockade therapy[6–8,10]. This clinical trial was open at 39 academic sites across the United States (US). Detailed experimental methods are provided in the Methods and in the Reporting Summary.

Results

Study population

Between 17 July 2017 and 15 July 2020, 94 patients were registered into this non-blinded, randomized study. Of these, 92 met the eligibility criteria (two were found to be ineligible after registration and were excluded from analyses); 91 received the study therapy, with one patient in the combination arm refusing the therapy as randomized due to a new-onset diagnosis of diabetes; 68 patients received nivolumab plus ipilimumab and 23 received ipilimumab alone (Fig. 1). The primary endpoint analysis was performed once the protocol-specified anticipated number of 78 events had occurred, with data lock of 9 March 2022, at a time when the median follow-up among patients last known to be alive and progression-free was 28 months (range: 4–40 months). This data lock date was used for the PFS analysis because it was event-driven and conducted at the specified event timing according to the protocol design. All other analyses used the final data lock date of 3 November 2022, when the median follow-up among patients last known to be alive was 36 months (range: 4–55 months), to provide the most accurate and recent assessment of disease outcomes and toxicities. The randomized groups were generally well balanced (Table 1), including related to the time from ending previous anti-PD-1 or anti-PD-L1 therapy and starting on the S1616 protocol treatment (Supplementary Table 1). All eligible patients had received previous anti-PD-1 therapy without intervening therapy, with 10% in the combination group and 13% in the single-agent group having received anti-PD-1 therapy in the adjuvant setting.

https://doi.org/10.1038/s41591-023-02498-y

Table 1 | Patient and disease characteristics

Characteristic	Ipilimumab (*n*=23)		Nivolumab+ pilimumab (*n*=69)		*P*
Age, years	69 (40–91)		64 (34–90)		0.55
Age					
<65 years	9	39%	35	51%	0.47
≥65 years	14	61%	34	49%	
Sex					
Male	15	65%	46	67%	1
Female	8	35%	23	33%	
Ethnicity					
White	22	96%	63	91%	1
Black	0	0%	1	1%	
Asian	1	4%	3	4%	
Unknown	0	0%	2	3%	
Performance status					
0	15	65%	45	65%	0.85
1	6	26%	20	29%	
2	2	9%	4	6%	
LDH at baseline					
Elevated LDH	6	26%	9	13%	0.18
Normal LDH	5	22%	28	41%	
LDH not done	12	52%	32	46%	
AJCC melanoma classification					
Stage III	6	26%	12	17%	0.37
Stage IV	17	74%	57	83%	
Adjuvant therapy					
No previous adjuvant therapy	17	74%	58	84%	0.22
Adjuvant PD-1	3	13%	7	10%	
Adjuvant *BRAF* or *MEK*	0	0%	2	3%	
Other adjuvant therapy	3	13%	2	3%	
Previous metastatic therapy					
Adjuvant therapy only	1	4%	6	9%	0.51
Anti-PD-1 only	20	87%	54	78%	
BRAF or *MEK* followed by PD-1	1	4%	1	1%	
Other anti-PD-1 combination	1	4%	8	12%	
Duration of previous anti-PD-1 or PD-L1 therapy					
<6 months	15	65%	44	64%	1
≥6 months	8	35%	25	36%	
Brain and central nervous system involvement at baseline					
Yes	2	9%	5	7%	1
No	21	91%	64	93%	

Patient characteristics among randomized patients. The median (range) and *n* (%) are reported. Two-sided *P* values obtained using a Wilcoxon test (quantitative covariates) and Fisher exact test (categorical covariates). AJCC, American Joint Committee on Cancer; LDH, lactate dehydrogenase.

Efficacy

PFS was significantly longer with combination therapy versus ipilimumab therapy alone (hazard ratio (HR) = 0.63, 90% CI = 0.41–0.97, $P = 0.04$, prespecified one-sided $\alpha = 0.1$; Fig. 2a). The 6-month PFS estimates were 34% (90% CI = 25–43%) and 13% (4–27%) for the combination therapy versus ipilimumab-alone groups, respectively. The PFS benefit of the combination therapy was consistent when analyzing different subgroups, although subgroups with fewer than ten individuals did not provide reliable data (Fig. 2b). S1616 was not powered to detect differences in overall survivor (OS), and survival data were collected as a secondary endpoint; there was no significant difference between the two groups as of the last data lock of 3 November 2022 (HR = 0.83, 90% CI = 0.50–1.39, $P = 0.28$, Extended Data Fig. 1).

Objective response rate (ORR) (defined as a complete response (CR) or PR to therapy as per RECIST v.1.1), also reported based on an analysis of data that were complete as of 3 November 2022 (ref. 21), was 28% (90% CI = 19–38%) in the combination therapy group and 9% (90% CI = 2–25%) in the ipilimumab-alone group ($P = 0.05$, one-sided Fisher exact test). Because this was not the primary endpoint, no threshold for significance was prespecified and *P* values should be interpreted qualitatively. Eight patients (12%) receiving combination therapy achieved a CR and 11 (16%) a PR. No patients on ipilimumab alone achieved a CR, and two (9%) achieved a PR (Fig. 3a,b and Supplementary Table 2). The best response according to change in the sum of target lesions for combination therapy and ipilimumab alone can be seen in Fig. 3a,b, respectively. Among patients with response to therapy, the two patients receiving ipilimumab alone had ongoing responses of 16+ and 33+ months, respectively, while 9 of 19 (47%) patients in the nivolumab plus ipilimumab group continued in response over a range of 6–37+ months. Median duration of response in the nivolumab plus ipilimumab group was 40.9 months (90% CI = 8 to not reached) (Fig. 3c), whereas it could not be estimated for the patients in the ipilimumab-alone group due to the small sample size.

Toxicity

In the nivolumab plus ipilimumab group, 34 patients (50%) experienced a maximum of grade 3 treatment-related adverse events, four patients (6%) experienced a grade 4 adverse event and one patient (1%) experienced a grade 5 adverse event (disseminated intravascular coagulation) (Table 2). In the combination arm, 20 patients (29%) discontinued protocol therapy due to toxicity. In the ipilimumab-alone group, five patients (22%) experienced a maximum of grade 3 treatment-related adverse events, two patients (9%) experienced grade 4 adverse events and one patient (4%) experienced a grade 5 adverse event (colonic perforation). In the ipilimumab-alone arm, four patients (17%) discontinued therapy due to toxicity. In both groups, the most frequent grade 3 or higher adverse event was diarrhea. There was no significant difference between the groups for any individual grade 3 or higher adverse event, but the total grade 3 or higher adverse events were numerically higher with the combination (57%) compared to single-agent ipilimumab (35%) ($P = 0.09$).

Changes in CD8 T cell density

Changes in CD8 T cell density were evaluated by comparing biopsies collected before therapy (baseline, $n = 75$ patients) and approximately 4 weeks after commencing therapy (on-treatment, $n = 53$ patients) from 81 patients; this resulted in a total of 39 paired biopsies after excluding samples that could not be analyzed because of absence of tumor cells because the antitumor immune response had already happened or due to insufficient tumor cells in the tissue obtained (Extended Data Fig. 2a–c and Supplementary Table 3). Biopsies were reviewed to annotate the tumor bed and periphery to quantitate $CD8^+$ T cells within the tumor region and along the invasive margin (Extended Data Fig. 3a,b). CD8 T cell density was similar across baseline biopsies between patients who did and did not respond to combination therapy ($P = 0.58$). Following the protocol definition of the assessment of the secondary endpoint, analyzing the change in density of CD8 T cells between baseline and on-therapy biopsies from patients in the combination therapy group, there was no significant difference (two-sided $\alpha = 0.05$) in the change

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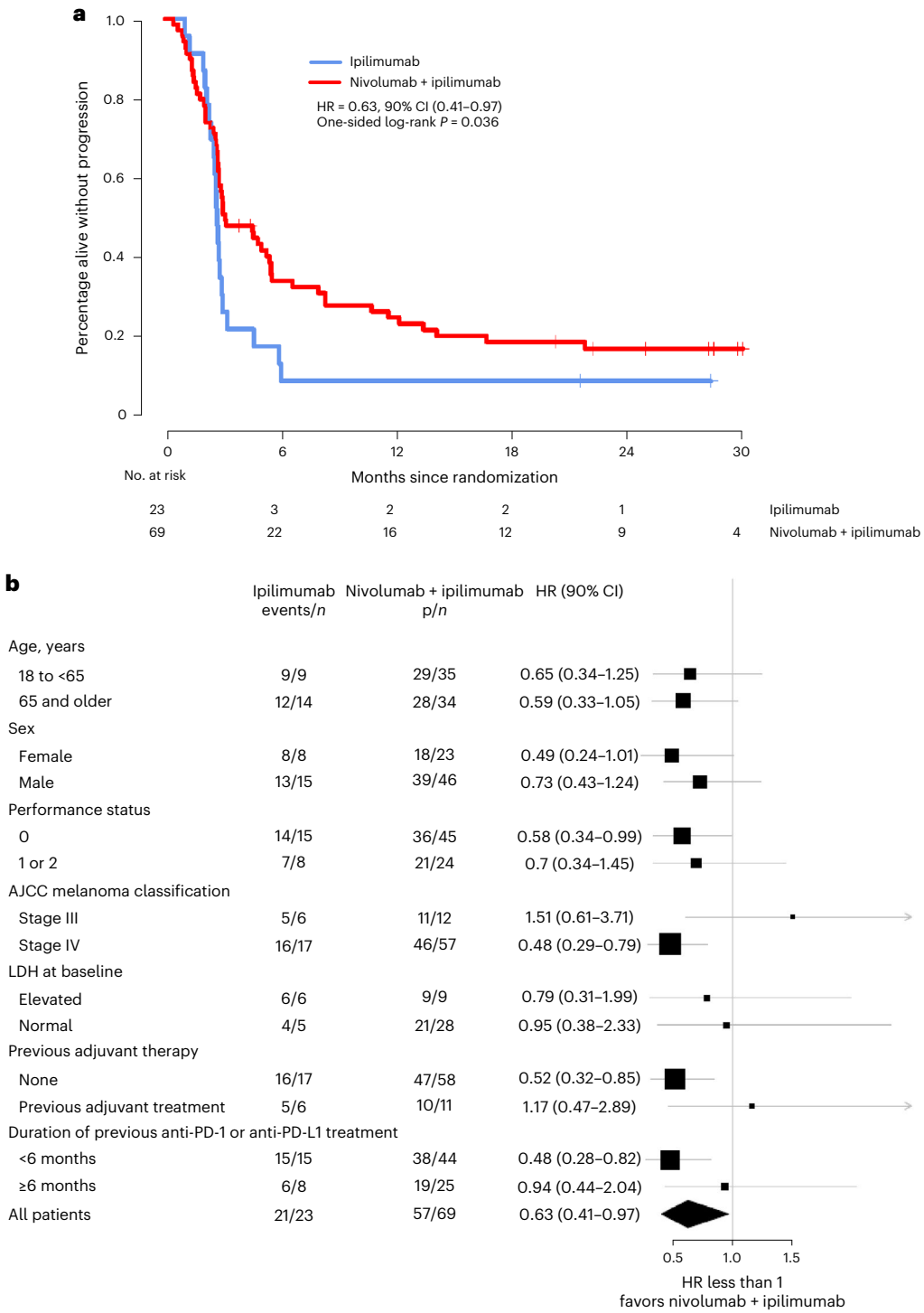

Fig. 2 | Analysis of PFS. a, Kaplan–Meier estimates of PFS as assessed by local investigators. The 6-month PFS estimates were 34% (90% CI = 25–43%) and 13% (4–27%) for the combination therapy versus ipilimumab-alone groups, respectively. **b**, Forest plot for PFS according to subgroups. The HR and 90% CI from a Cox regression model are reported and represented by the solid black squares and error bars; no adjustment was made for multiple comparisons. AJCC, American Joint Committee on Cancer; LDH, lactate dehydrogenase.

in CD8 T cell density between patients who did and did not respond to combination therapy ($P = 0.77$; Fig. 4a and Extended Data Fig. 3c). Pathological features consistent with the presence of regressed melanoma (defined as tumor necrosis, areas of tumor regression with fibrosis and absence of melanoma cells, or melanosis, which is a pathological description of macrophages that have engulfed pigmented melanoma cells after tumor regression, according to the analysis of samples after neoadjuvant immunotherapy[22]) were observed only in on-therapy biopsies from patients with a clinical CR or PR to combination therapy (11 of 14 patients with CR or PR had features of pathological regression present, compared to 0 of 18 patients who did not have CR or PR; Fisher exact test, $P = 2.8 \times 10^{-6}$). The tumor areas with features of pathological regression in the on-therapy biopsies had low CD8 T cell infiltration and absence of melanoma cells (Fig. 4b).

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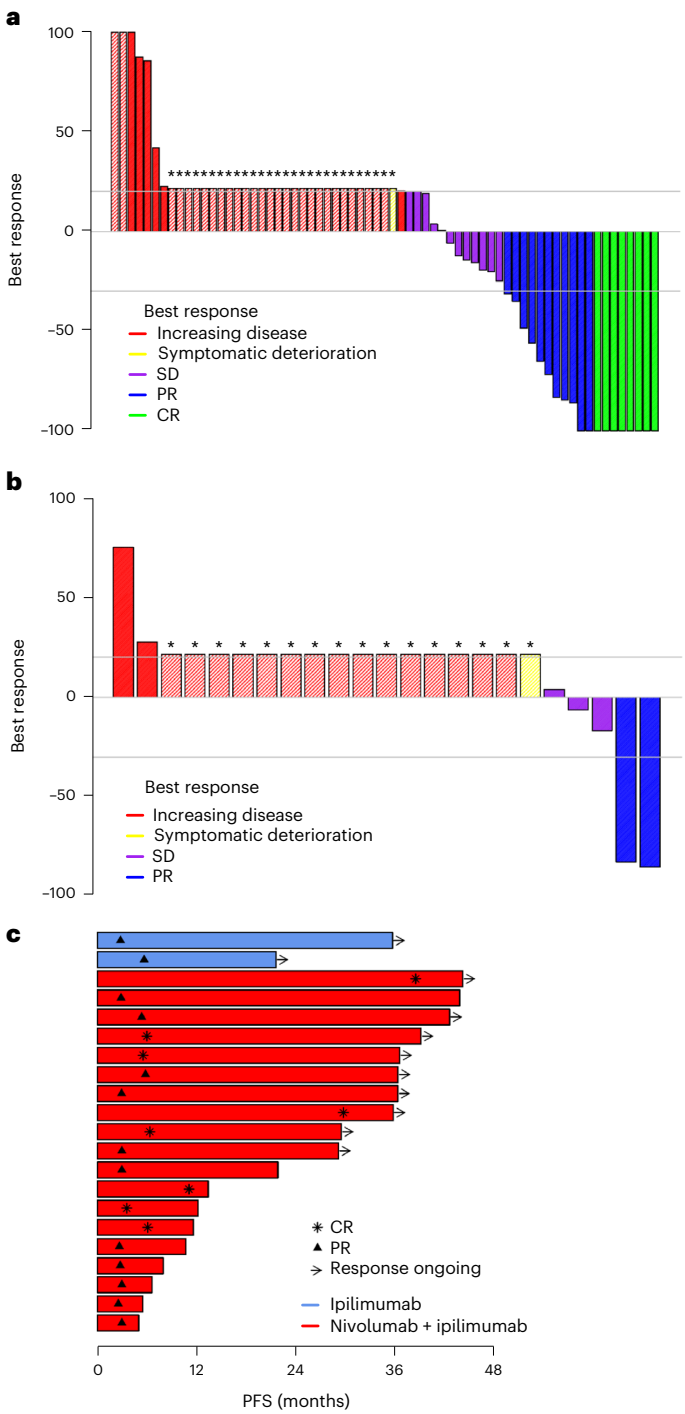

Fig. 3 | Depth of response and response duration. a,**b**, Waterfall plots of overall response in the nivolumab and ipilimumab group (**a**) and the ipilimumab-only group (**b**). The plots show the change in RECIST target lesions. The lines indicate the threshold for objective response (≥30% decrease) or disease progression (>20% increase). Asterisks and hatched bars denote patients whose best response was progression due to new lesions ($n = 23$ with nivolumab and ipilimumab, and $n = 14$ with ipilimumab) or clear worsening of nonmeasurable disease ($n = 2$ with nivolumab and ipilimumab) without assessable RECIST changes, symptomatic deterioration ($n = 1$ with nivolumab and ipilimumab, and $n = 1$ with ipilimumab) or death due to disease ($n = 2$ with nivolumab and ipilimumab, and $n = 1$ with ipilimumab). Two patients on the combination group with changes in RECIST measurements greater than 100% over baseline are denoted on the far left with hatched bars capped at 100%. Two patients on the combination group did not have complete follow-up disease assessment data and were not included. **c**, Swimmer's plot of the course of patients with an objective response to therapy. The bars represent PFS from the time of registration. Patients with arrows are alive and progression-free.

Table 2 | Grade 3 or higher treatment-related toxicities in at least 4% of patients on either arm

Event	Ipilimumab (n=23)	Nivolumab+ipilimumab (n=68)
Diarrhea	3 (13%)	9 (13%)
AST increased	2[a] (7%)	5 (7%)
ALT increased	2[a] (7%)	5 (7%)
Rash	1 (4%)	4 (6%)
Fatigue	1 (4%)	4 (6%)
Anemia	0 (0%)	4 (6%)
Hypotension	0 (0%)	4 (6%)
Hyponatremia	1 (4%)	4[a] (6%)
Pruritus	0 (0%)	3 (4%)
Vomiting	0 (0%)	3 (4%)
Endocrine disorders (other)	0 (0%)	3 (4%)
Alkaline phosphatase increased	1 (4%)	2 (3%)
Colitis	0 (0%)	3[a] (4%)
Hypokalemia	0 (0%)	3[a] (4%)
Adrenal insufficiency	1 (4%)	3[a] (4%)
Atrial fibrillation	1 (4%)	1 (1%)
Bilirubin increased	1 (4%)	0 (0%)
Hypophosphatemia	1 (4%)	0 (0%)
Hyperglycemia	1[a] (4%)	0 (0%)
Colonic perforation	1 (4%)	0 (0%)

All adverse events reported were assessed as possibly, probably or definitely related to the study treatment. To be included in the safety analysis, patients must have received at least one dose of protocol therapy. Adverse event severity was scored using the National Cancer Institute Common Terminology Criteria for Adverse Events v.4.0. One patient in the ipilimumab arm had a grade 5 adverse event (death) due to colonic perforation. One patient in the nivolumab plus ipilimumab arm had a grade 5 adverse event (death) due to disseminated intravascular coagulation. ALT, alanine transaminase; AST, aspartate aminotransferase. [a]One each of these events was a grade 4 adverse event.

Discussion

In this US cooperative group, prospective and randomized clinical trial in patients with metastatic melanoma with primary resistance to anti-PD-1-based therapy, clinical data support the hypothesis that outcomes are improved when introducing CTLA-4 blockade therapy with continuation of anti-PD-1 therapy after progression versus switching to CTLA-4 blockade therapy alone. However, the combination of nivolumab and ipilimumab is associated with a higher rate of grade 3 or higher adverse events, which was consistent with the adverse events previously described for this combination versus single-agent ipilimumab therapy[3]. The analysis of baseline and on-therapy changes in CD8 T cell infiltration confirmed that CTLA-4 blockade could result in increases in CD8 T cell infiltration in some cases with primary resistance to PD-1 blockade[5]; however, the changes in CD8 T cell infiltration observed in the present analysis did not reach statistical significance and thus did not support the formal hypothesis tested as a secondary endpoint of this clinical trial. In some cases, the biopsies showed features of melanoma regression; in those areas the density of CD8 infiltration decreased, suggesting that the biopsy was taken after the peak of the CD8 infiltration induced by blocking CTLA-4 (Extended Data Fig. 4)[6–8,10]. By adding CTLA-4 blockade to continued PD-1 blockade therapy, we hypothesized that T cells (enriched for tumor antigen-specific T cells) would infiltrate metastatic lesions and be able to induce their specific antitumor cytotoxic response by releasing these two major immune checkpoints (Extended Data Fig. 5).

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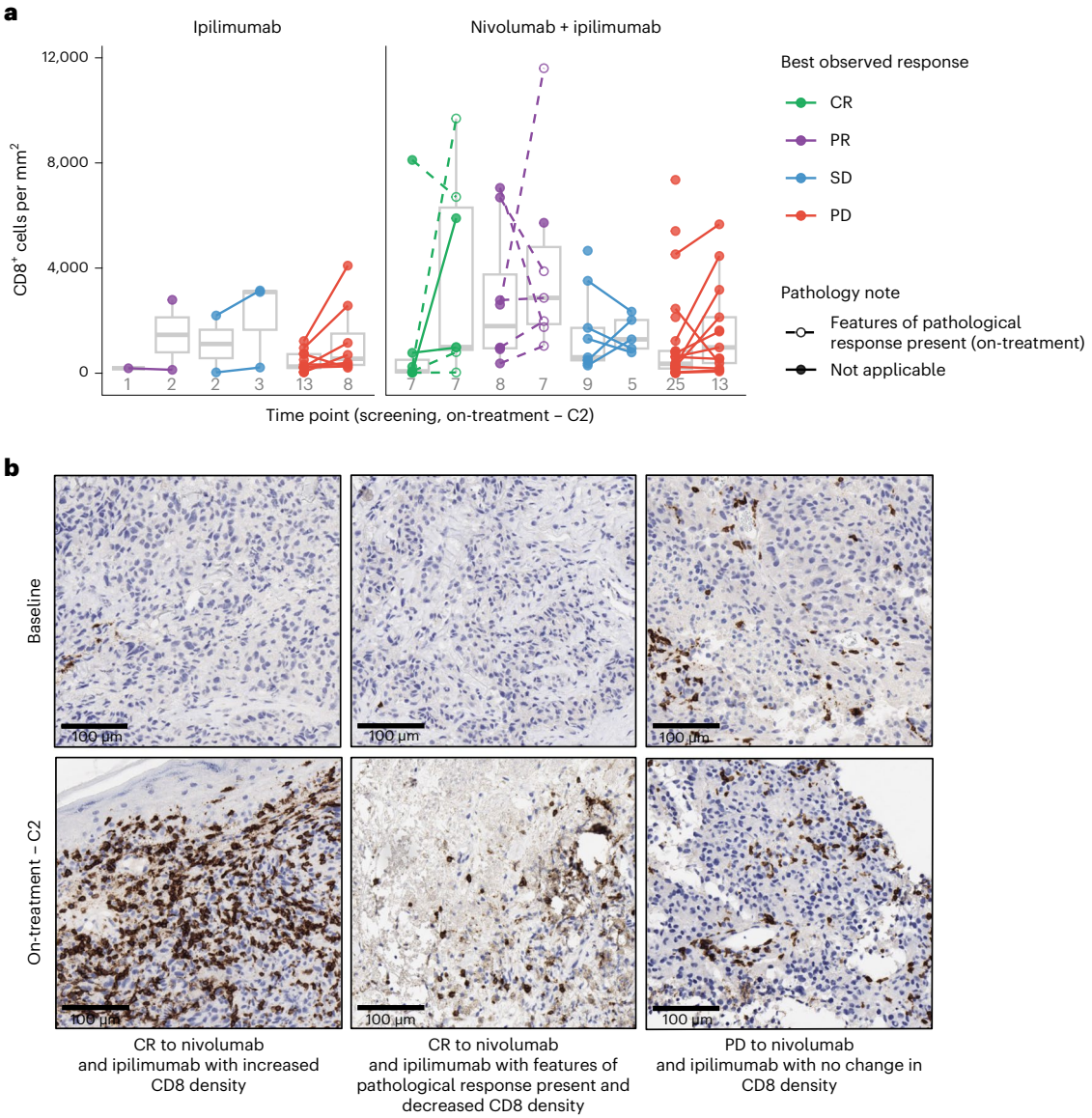

Fig. 4 | CD8 cell quantitation in biopsy specimens. a, Density of $CD8^+$ cells detected within the annotated tumor. Paired baseline on-treatment biopsies are indicated by the points connected by lines. Dashed lines indicate patients with observed features of pathological response present in the on-treatment biopsy. The number of patients in each group is indicated by the number along the *x* axis (ipilimumab, $n = 2$ PR, $n = 3$ stable disease (SD), $n = 13$ progressive disease (PD); nivolumab and ipilimumab, $n = 8$ CR, $n = 9$ PR, $n = 9$ SD, $n = 27$ PD). The box plots indicate the median (middle line), 25th and 75th percentiles (box), and 5th and 95th percentiles (whiskers). $CD8^+$ cell density was compared across biopsies using a Wilcoxon rank-sum test for two-group comparisons and Wilcoxon signed-rank tests for paired comparisons. **b**, Representative images of biopsies. Biopsy of a patient with response to nivolumab and ipilimumab with increased CD8 density (left); biopsy from a patient with response to nivolumab and ipilimumab and detection of features of pathological regression in the on-treatment biopsy with decrease in CD8 density in that area (middle); and biopsy from a patient with PD after nivolumab and ipilimumab with no change in CD8 cell density (right).

In the pivotal CheckMate 067 trial, which tested the combination of nivolumab and ipilimumab in the first-line setting in patients with metastatic melanoma, this combination had an absolute increase of 14% in ORR compared to nivolumab alone, but also had a 38% absolute increase in grade 3 or 4 toxicities. Furthermore, no statistically significant OS benefit has been documented between the combination of nivolumab and ipilimumab over nivolumab alone because studies such as the CheckMate 067 clinical trial were not designed to directly compare outcomes of these two groups[23]. Therefore, our study indirectly suggests that it may be reasonable to offer single-agent anti-PD-1 therapy as first-line treatment, limiting the toxicity of the combination with anti-CTLA-4 to patients who progress on single-agent therapy. Additionally, patients with *BRAF* wild-type melanoma have not had highly efficacious second-line therapies since anti-PD-1 therapy became standard in the first-line setting.

We believe that this study has implications for other cancers because it demonstrates an efficacy benefit with continued PD-1 or PD-L1 blockade therapy beyond progression compared to switching to a new agent altogether. As such, this study serves as a proof of concept across tumor types and provides justification for investigators and drug developers to design studies in the second-line or later that include continued PD-1 blockade therapy even when the patient may have already progressed on PD-1 blockade therapy in a previous line. However, each combination will need to be tested individually because there is a potential that the combination of CTLA-4 and PD-1 blockade has synergistic effects that may not be evident with other combinations of PD-1 blocking agents. Our data agree with three previously reported studies in patients with advanced melanoma resistant to anti-PD-1 or anti-PD-L1. One study included 19 patients who showed

similar response rates when they received nivolumab and ipilimumab or single-agent ipilimumab, and circulating $CD4^+$ T cells were increased with higher polyfunctionality and higher interferon-γ production among patients in both groups who achieved disease control[20]. Two other studies, one a prospective single-arm study of 70 patients treated with ipilimumab 1 mg kg^{-1} and the anti-PD-1 agent pembrolizumab[19], the other a retrospective study analyzing 355 patients[18], showed that anti-CTLA-4 combined with anti-PD-1 resulted in response rates of 29% and 31%, both in a similar range to the 28% response rate in this study. In the retrospective study, the ipilimumab-alone group had an ORR of 13% based on 162 patients, which was similar to the ORR of 9% among the 23 patients treated with ipilimumab alone in S1616. The present study is the only one of these studies to show benefit in an appropriately powered randomized controlled study; however, as a phase 2 study, the CIs around the clinically meaningful and statistically significant HR of 0.64 are wide. While on its own the present study cannot confirm the therapeutic benefit of combination of nivolumab and ipilimumab, its consistency with the data from previous research provides robust confirmation of the previously indicated benefit.

There are several limitations to the S1616 study. First, with 92 randomized patients, the study was not as large as other practice-changing studies. This was due to necessity because the combination of nivolumab and ipilimumab was frequently used as first-line therapy by melanoma clinicians in the US, and doctors and patients needed to be willing to randomize to ipilimumab therapy alone in second-line therapy when the combination of nivolumab and ipilimumab could be obtained outside the clinical trial. Although a PFS benefit was seen despite the small sample size, the small size made it difficult to perform meaningful subset analyses.

Second, due to the relatively long period between study conception and completion (approximately 6 years), certain standards and definitions changed during the course of the study, notably the definition of primary resistance and available first-line therapies. Our study defined primary resistance to PD-1 blockade as melanoma that did not progress on anti-PD-1 therapy at any point after receiving that therapy, as long as there was no previous response and no intercurrent therapy. A white paper published in 2020 defines primary progression slightly differently and requires disease progression within 12 weeks after the last PD-1 dose[24]. Additionally, since the completion of enrollment in our study, a new combination therapy in the first-line setting has been approved by the U.S. Food and Drug Administration. The combination of nivolumab and the lymphocyte activation gene 3 protein (LAG3) blocking antibody relatlimab was demonstrated to have improved PFS compared to nivolumab alone[25], leading some to adopt this as a new first-line therapy for metastatic melanoma. While patients in our study were eligible even if they had received a combination of PD-1 and another therapy (as long as it was not a CTLA-4 inhibitor) before enrollment, in practice very few patients received first-line combination therapy and none of these patients received relatlimab as part of their combination therapy. As such, the utility of combining nivolumab and ipilimumab after progression on the combination of nivolumab and relatlimab is unknown. While patients were eligible regardless of *BRAF* mutation status, no intervening BRAF plus MEK inhibitor-targeted therapy was allowed between progression on anti-PD-1 therapy and enrollment on S1616. At the time the study was developed, no optimal sequencing of BRAF plus MEK inhibitor therapy and immunotherapy had been determined. On S1616, only one patient with a *BRAF* mutation received BRAF plus MEK inhibitor-targeted therapy before receiving any anti-PD-1 therapy, while others did not receive BRAF inhibitor therapy at all before enrolling on the trial. Since S1616 completed enrollment, the DREAMseq clinical trial demonstrated that BRAF plus MEK inhibitor therapy is less efficacious when given before immunotherapy in metastatic melanoma[26]. However, an additional limitation of our study is that the total number of patients with *BRAF* mutations is unknown because of lack of data collected prospectively.

Randomization was performed in an admittedly unusual 3:1 fashion to ensure adequate power for the main translational objective (the first secondary objective), which was designed to assess differences in CD8 T cell infiltration between biopsies of patients with response or no response to therapy in the combination therapy group. This was due to the translational hypothesis that primary anti-PD-1 resistance could be reversed by adding anti-CTLA-4 therapy to continued anti-PD-1 therapy, as evidenced by increases in infiltrating CD8 T cells. This secondary objective was considered sufficiently important to randomize the trial in a manner in which this objective could be tested. To do this, we needed to ensure that there would be enough patients in the combination therapy group whose tumors both responded or did not respond to allow for the comparison to be meaningful. While unbalanced randomization is less common, the reliability of the design and the analysis of the primary endpoint is unaffected. Unbalanced randomization is usually not done because it is less efficient, requiring a larger sample size to maintain adequate statistical power. As such, the study required a larger population of patients (by approximately 15%) than would have been necessary under 1:1 randomization. Despite the relatively large number of biopsies prospectively accrued and analyzed from patients enrolled in S1616, there was heterogeneity in the CD8 density assessment and we could not assure having the same lesion being biopsied twice, all of which contributed to some cases having evidence of decreased CD8 infiltration due to the melanoma already having regressed and being cleared by a previous antitumor immune response.

In summary, S1616 demonstrates that combined therapy with nivolumab and ipilimumab yields superior PFS and ORR compared to single-agent ipilimumab in patients with advanced melanoma with primary resistance to anti-PD-1 or anti-PD-L1 therapy. On the basis of these results, the combination of nivolumab and ipilimumab should be considered the preferred regimen over ipilimumab alone to treat patients with advanced melanoma not responding to previous anti-PD-1, although patients and physicians should consider the corresponding increase in toxicity[27,28].

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-023-02498-y.

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Methods

Patients

Patients were eligible if they were at least 18 years old with pathologically confirmed melanoma that was either stage IV or unresectable stage III. Patients with mucosal or cutaneous melanoma were eligible, but patients with uveal melanoma were excluded. Patients must have had previous treatment with anti-PD-1 or anti-PD-L1 agents and had documented disease progression either while on these agents or after stopping therapy with these agents without intervening therapy. Patients must not have achieved a confirmed PR or CR to the anti-PD-1 or PD-L1 agents before progression, thereby excluding patients with acquired resistance to anti-PD-1 (refs. 24,29). Patients were not allowed to have had previous treatment with ipilimumab or other CTLA-4 antibodies. Patients must have had measurable disease using RECIST v.1.1 (ref. 21). However, if the only measurable disease was cutaneous or subcutaneous, lesions must have been at least 10 mm in the greatest dimension and able to be serially recorded using calipers and photographs. Patients may not have had active central nervous system metastases unless they were adequately treated and symptom-free without requiring steroids for 14 days before registration, and must have had a Zubrod performance status of 0–2, as well as adequate hepatic, renal and hematological function. Patients were not eligible if they had a history of immune-related pneumonitis or colitis requiring steroid treatment. Patients had to be willing to undergo serial biopsies and submit tissue and blood for the translational medicine objectives. Sex or gender was determined by self-report. Patients were enrolled regardless of sex or gender. Characteristics of the study population, including sex and age, are displayed in Table 1.

Trial design and treatments

In this phase 2 randomized study, patients were randomly assigned using a 3:1 ratio to receive combination therapy with nivolumab 1 mg kg^{-1} and ipilimumab 3 mg kg^{-1} every 3 weeks for four cycles followed by nivolumab 480 mg every 4 weeks for up to 2 years, or to ipilimumab 3 mg kg^{-1} every 3 weeks for four cycles. In the combination group, nivolumab was administered intravenously over 30 min on day 1 of each cycle and ipilimumab was administered intravenously over 90 min, starting 30 min after the end of the nivolumab infusion on day 1 of the first four cycles. In the ipilimumab-alone group, ipilimumab was administered intravenously over 90 min on day 1 of the first four cycles only. Tissue and blood biopsies were collected on or before day 1 of the protocol treatment and on days 28–35 of the protocol treatment. If available, archival tissue before previous anti-PD-1 therapy was also collected. Treatment in the ipilimumab group was until progression of disease, until development of unacceptable toxicities or until the completion of four cycles of treatment, whichever was first. Treatment in the nivolumab and ipilimumab group was until PD, development of unacceptable toxicities or until 2 years of treatment with nivolumab, whichever was first. Treatment beyond initial progression as defined by investigators using RECIST v.1.1 was permitted if the investigators assessed that the patient was clinically benefiting from the treatment. Dose reductions were not permitted and dose delays because of toxicity were allowed up to 12 weeks and resumption of dose was generally allowed when toxicity resolved to grade 1 or lower.

Endpoints and assessments

The primary endpoint was PFS assessed according to RECIST v.1.1 (ref. 21) by investigator review and defined as the time between the date of randomization until the earliest date of documented disease progression or the date of death from any cause, whichever occurred first. A single interim analysis was performed on a data lock at 6 March 2020, after 43 progression or death events had occurred. The HR, using ipilimumab as the reference group, was 0.65 (90% CI = 0.37–1.14). The protocol called for early termination if the HR favored the ipilimumab group so the protocol met the criteria to continue. Secondary endpoints included change in CD8 T cell infiltrate between on-study biopsy samples of patients who responded to combination therapy. Additional secondary endpoints included ORR, OS and the toxicity profile of patients in each treatment group. Tumor response, according to RECIST v.1.1, was assessed by the treating investigator every 12 weeks until disease progression. ORR was defined as a CR or PR to therapy according to RECIST v.1.1 (ref. 21). Two secondary endpoints involved assessing the marginal prognostic value of baseline CD8 T cell density, and the change in CD8 density in on-therapy biopsies. Adverse events were assessed continuously throughout the trial and for up to 30 days after completion of the trial using the National Cancer Institute Common Terminology Criteria for Adverse Events v.5.0.

Analysis of CD8 T cell infiltration

Biopsies were processed at each site using standard clinical pathology processing to obtain formalin-fixed paraffin-embedded tissues. The Translational Pathology Core Laboratory at the University of California, Los Angeles performed histological sectioning, hematoxylin and eosin (H&E) or immunohistochemical (IHC) staining, and digital imaging of stained slides. Three sections (5 µm) were cut from each block and consecutive slides were stained for mouse antihuman S-100 (1:400 dilution for melanoma cells, catalog no. 330M, Cell Marque), mouse antihuman CD8 (1:100 dilution for cytotoxic T cells, catalog no. M7103, Agilent Technologies) and H&E (for histological assessment). Stained slides were imaged at ×40 magnification for analysis. IHC and H&E slide images were assessed using QuPath v.0.3.0. S-100, CD8 and H&E images were sequentially assessed by two dermatopathologists (P.S. and L.F.K.) for manual annotation of two types of regions: (1) 'tumor' regions identified by the presence of tumor cells that displayed melanoma cytology or morphology with nuclear and cytoplasmic S-100 protein expression; and (2) 'periphery' regions denoted by invasive lymphocyte patterns and cells with CD8 expression along the edges of the 'tumor' regions. While reviewing each biopsy, the dermatopathologist noted observations related to immune-mediated pathological response patterns or visible limitations to annotating tumor regions, including tumor necrosis, regression, melanosis, dense immune infiltrate or pigmentation. 'Tumor' and 'boundary' regions were annotated on CD8-stained slides and $CD8^+$ cells were identified and quantified within each region using QuPath's positive cell detection functionality (Supplementary Table 3 and Extended Data Fig. 3a,b). $CD8^+$ cell density was summarized by the 'number of $CD8^+$ cells per mm^2 of tissue'. If there were multiple biopsies performed at a given time point, the sum of $CD8^+$ cells across all biopsies was normalized by the sum of the area (in mm^2) of the annotated regions across all biopsies.

Trial oversight

Approval for the trial was obtained through the Cancer Therapy Evaluation Program (CTEP) central institutional review board. Additionally, each investigator received approval from their respective institutional review board. The trial was conducted in accordance with the International Council for Harmonisation Good Clinical Practice guidelines and all patients provided written informed consent before participation. An independent data monitoring committee provided oversight to assess the efficacy and safety of nivolumab and ipilimumab in the trial. The trial was designed by the SWOG Melanoma Committee in conjunction with the National Cancer Institute (NCI) and CTEP and was registered with ClinicalTrials.gov (registration: NCT03033576). Data were collected by SWOG and analyzed in collaboration with the authors. The authors vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. All authors contributed to drafting the manuscript, provided critical review and gave final approval to submit the manuscript for publication.

Randomization

Patients were randomized using a 3:1 ratio to receive combination therapy with nivolumab and ipilimumab, or to ipilimumab alone.

Randomization did not include any stratification factors. Randomization was completed by sites through the SWOG rando-node dynamic balancing algorithm implemented through the NCI's OPEN registration platform.

Statistical analysis

The full details of the design are provided in Section 11 of the protocol document. The statistical design assumed exponential PFS with a median of 3 months in the ipilimumab group (null hypothesis). The study was powered to detect a change in median PFS to 6 months in the combination therapy group (corresponding to an HR of 0.50). A total of 84 patients (63 randomized to the combination group and 21 to the ipilimumab group) with 78 events (across both groups) provided 89% power for a one-sided $\alpha = 10\%$ using a log-rank test. A single, prespecified interim futility analysis was scheduled at 41 events, with a plan to stop early for futility if the estimated HR favored the ipilimumab group. Unequal randomization was necessary to power the secondary objective, comparing $CD8^+$ expression in patients who responded compared to patients who did not respond in the combination therapy group. Fifty-six patients (corresponding to 90% compliance in tissue submission) provided 80% power to detect a mean difference of 0.875 s.d. in $CD8^+$ expression between patients with and without response in the combination therapy group at the two-sided $\alpha = 0.05$ level.

All analyses were conducted at the SWOG statistical center using intent-to-treat analyses among all eligible randomized patients. The Kaplan–Meier method was used to estimate survival outcomes; log-rank tests were used to evaluate associations with the outcomes. Fisher exact and Wilcoxon rank-sum tests were used to assess differences in categorical and quantitative variables across groups. Data were provided to SWOG from individual sites through iMedidata Rave, then imported from Rave into the SWOG SQL database and then exported from the SQL database using SAS (v.9.4). Clinical analyses were completed in R (v.4.0.3) and SAS (v.9.4). $CD8^+$ cell density was compared across biopsies using a Wilcoxon rank-sum test for two-group comparisons and a Wilcoxon signed-rank test for paired comparisons. $CD8^+$ cell density metrics were analyzed in R (v.4.2.0) and summarized using the tidyverse R package (v.1.3.2).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data and code to reproduce the analyses presented in this article are available upon request from SWOG in accordance with SWOG's data sharing policy and process: https://www.swog.org/sites/default/files/docs/2019-12/Policy43_0.pdf. The protocol (including the statistical analysis plan in Section 11 of the protocol) and informed consent are found in the Supplementary Information.

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Acknowledgements

We thank the patients and their caregivers, the support of the patient advocates S. Guild and V. Guild, and the support of Bristol Myers Squibb in providing investigational agents for the study. The study was funded by Southwest Oncology Group (SWOG) National Institutes of Health (NIH) and NCI grant nos. LS1616_R01LDRGAPP01, U10CA180888, U10CA180819, U10CA180821 and U10CA18068. Biopsies and their analyses were supported by the Stand Up to Cancer (SU2C) Catalyst-Bristol Myers Squibb-American Association for Cancer Research grant no. CT06-17. A.R. is funded by NIH and NCI grant nos. P01 CA244118 and R35 CA197633, and the Parker Institute for Cancer Immunotherapy, the Ressler Family Fund and support from K. and D. Schultz, T. and D. Jones, K. and J. Witemyre, T. Stutz and J. Isaacson. K.M.C. was supported by the Cancer Research Institute Postdoctoral Fellowship Program, the V Foundation Gil Nickel Melanoma Research Fellowship and the Parker Institute for Cancer Immunotherapy and V Foundation Bridge Fellowship. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author contributions

A.V., S.L.B., E.S., S.P.P., K.F.G., J.M., M.C.W. and A.R. designed, initiated and oversaw the conduct of the clinical trial. A.V., K.L.K., N.I.K., F.C., J.A.S., A.I., A.I.V., T-G.T., B.C., D.C.P., Y.C., K.M., C.B., C.A.D., D.B.J., Z.E., S.C., S.H-L., S.P.P. and A.R. enrolled, treated and cared for the patients on the clinical study protocol. A.V., S.L.B., J.M., M.C.W. and A.R. conducted the clinical data analyses. K.M.C., E.M., C.R.G., I.B-C., A.V-C. and I.P.G. processed, banked and analyzed the biopsies. P.O.S. and L.F.K. performed the dermatopathological analyses of biopsies. K.M.C., P.O.S., L.F.K. and M.C.W. interpreted the biopsy analyses. A.V., S.L.B., K.M.C., M.C.W. and A.R. wrote the first draft of the manuscript. All authors proofread and approved the final manuscript.

Competing interests

A.V. declares employment by Caris Life Sciences and consults with the George Clinical, West Clinic; he sits on the advisory boards and steering committees of Bristol Myers Squibb, Genentech and Mirati Therapeutics. He has received research funding from SWOG, Stand Up 2 Cancer, Bristol Myers Squibb and American Association for Cancer Research. K.L.K. reports clinical trial funding through the institution from Merck. N.I.K. sits on the advisory board for Bristol Myers Squibb, Regeneron, Merck, Iovance Biotherapeutics, Genzyme, Novartis, Nektar, Castle Biosciences, Instil Bio and the National Comprehensive Cancer Network (via Pfizer); he is a member of the study steering committees of Bristol Myers Squibb, Nektar, Regeneron and Replimune; he is a member of the data safety monitoring boards of Astra-Zeneca and Incyte; he holds common stock stock in Bellicum Pharmaceuticals, Asensus Surgical and Amarin Corporation; and he has received research funding (to the institute) from Bristol Myers Squibb, Merck, Novartis, GSK, HUYA Bioscience International, Amgen, Regeneron, Celgene, Replimmune and Modulation Therapeutics. K.M.C. reports being a shareholder in Geneoscopy and has received consulting fees from Geneoscopy, PACT Pharma, Tango Therapeutics, Flagship Labs 81 and the Rare Cancer Research Foundation. P.S. received research funding, consulted for and served on the advisory board for Castle Biosciences. F.C. reports that his institution receives research funding for clinical trials. A portion of that funding comes from trials sponsored by Amgen and Replimune and helps cover her salary. J.A.S. reports consultation for Apixagen Consultation, Iovance and Necktor and up-to-date royalties. A.I. discloses research funding to her institution from Checkmate Pharmaceuticals, Dynavax Technologies, GSK/S. Cannon, Immunocore, Merck and Neon Therapeutics/S. Cannon. A.I.V. had an investigator-initiated study supported by Bristol Myers Squibb, but it closed 2 years ago and understands that it does not qualify as a competing interest. T.-G.T. reports institutional funding from Bristol Myears Squibb, Merck, Roche, Pfizer, Novartis, Regeneron and Astra-Zeneca. B.C. reports clinical trial support paid to his institution from SWOG, Bristol Myers Squibb, Macrogenics, Merck, Karyopharm, Infinity, Advenchen, Idera, Xencor, Compugen, Iovance, PACT Pharma, RAPT Therapeutics, Immunocore, IDEAYA, Ascentage, Novartis, Atreca, Replimmune, Instil Bio, Adagene, TriSalus and Xilio; he reports payment for lectures for Sanofi Genzyme and sits on the advisory boards of Instil Bio, Nektar, Delcath, Novartis, Genentech, IDEAYA, OncoSec, Iovance and Deciphera. Y.C. reports research funding from BMS RELATIVITY-098

to his institution, personal financial interest in Bristol Myers Squibb as speaker on melanoma and is a member of the advisory board; he has spoken about melanoma on behalf of Pfizer. D.B.J. has served on the advisory boards or as a consultant for Bristol Myears Squibb, Catalyst Biopharma, Iovance, Jansen, Mallinckrodt, Merck, Mosaic ImmunoEngineering, Novartis, Oncosec, Pfizer, Targovax and Teiko, and has received research funding from Bristol Myears Squibb and Incyte. Z.E. reports serving on the advisory boards of Array BioPharma, Pfizer, OncoSec, Regeneron, Genentech, Novartis and Natera; she has received research funding from Novartis, Pfizer and Boehringer Ingelheim. S.C. reports serving on the advisory boards of Bristol Myers Squibb, Novartis, Pfizer and Regeneron. S.H-L. reports consulting for Amgen, Genmab, Xencor, Regeneron, Nektar, Astellas, Bristol Myers Squibb and Merck; she reports support from Amgen and Merck, and has undertaken contracted research for Pfizer, Plexxikon, Genentech, Neon Therapeutics, Nektar, Astellas, F Star, Xencor, Merck, Vedanta, Kite Pharma, Boehringer Ingelheim, OncoC4, Dragonfly, Bristol Myers Squibb and BioAlta. S.P.P. reports the following relevant competing interests: clinical trial support to her institution from Bristol Myers Squibb; advisory board membership of Cardinal Health, Castle Biosciences and Delcath; serving as a consultant for Foghorn Therapeutics (clinical trial support to her institution); providing clinical trial support for Ideaya (via her institution); sitting on the data safety monitoring board of Immunocore; sitting on the advisory board of Immatics; providing clinical trial support via her institution to InxMed, Lyvgen Biopharma and Novartis; serving as consultant on the advisory board of Pfizer; providing clinical trial support to Provectus Biopharmaceuticals; providing clinical trial support via her institution to Reata Pharmaceuticals and sitting on the data safety monitoring board; she has sat on the advisory board of Replimmune and the scientific advisory board of TriSalus Life Sciences; and she has provided clinical trial support (via her institution) to Seagen and Syntrix Bio, and has consulted for Advance Knowledge in Healthcare. K.F.G. is an employee and stockholder of Merck Sharp & Dohme, a subsidiary of Merck & Co. A.R. has received honoraria from consulting with Amgen, Bristol Myers Squibb and Merck; is or has been a member of the scientific advisory board and holds stock in Advaxis, Appia, Apricity, Arcus, Compugen, CytomX, Highlight, ImaginAb, ImmPact, ImmuneSensor, Inspirna, Isoplexis, Kite-Gilead, Lutris, MapKure, Merus, PACT, Pluto, RAPT Therapeutics, Synthekine and Tango; and has received research funding from Agilent and from Bristol Myers Squibb through SU2C, and patent royalties from Arsenal Bio. J.M., L.F.K., D.C.P., K.M., C.L.B., C.A.D., E.M., C.R.G., I.B.C., A.V-C., I.P.G., E.S., S.L.B. and M.C.W. declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-023-02498-y.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-023-02498-y.

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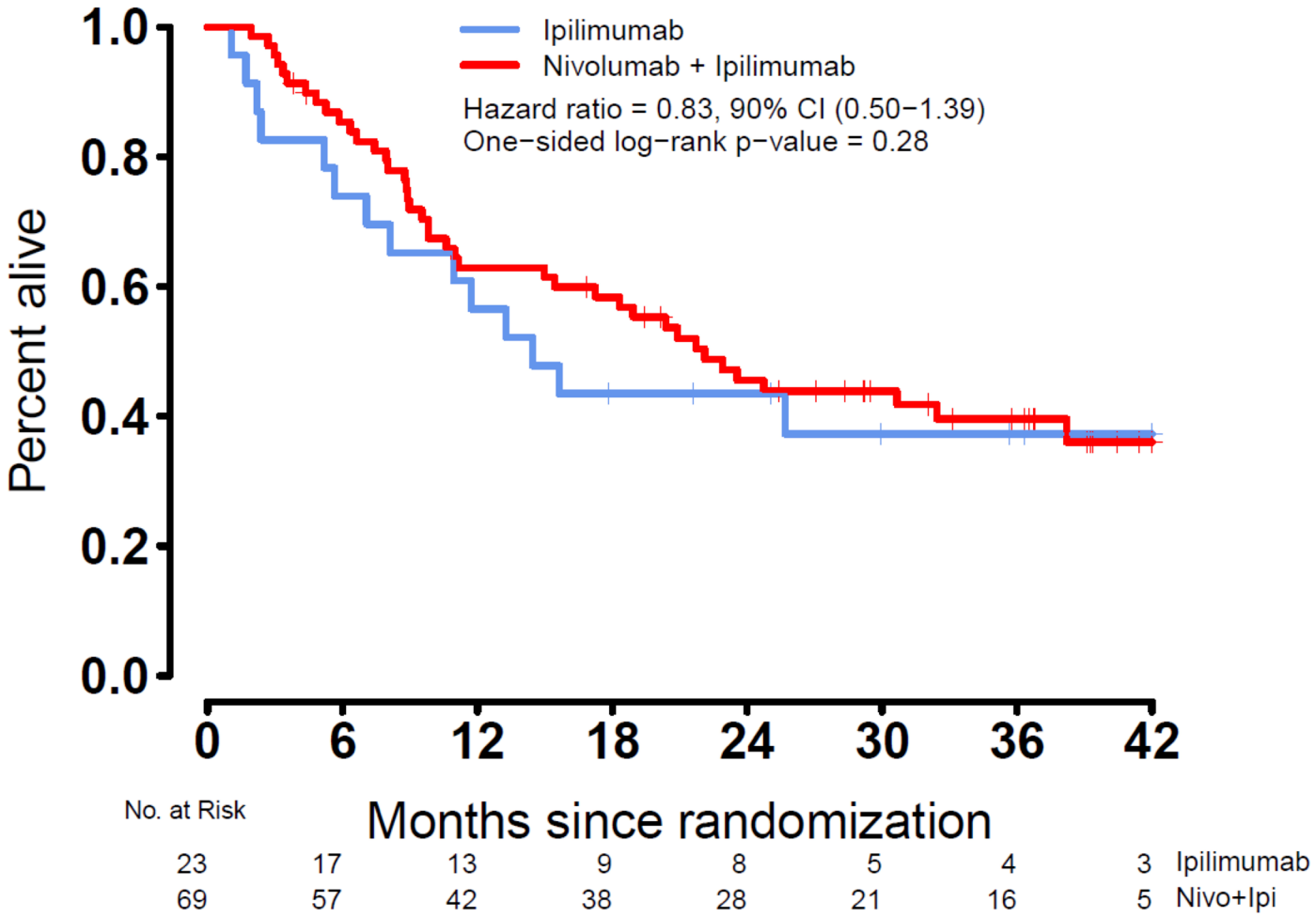
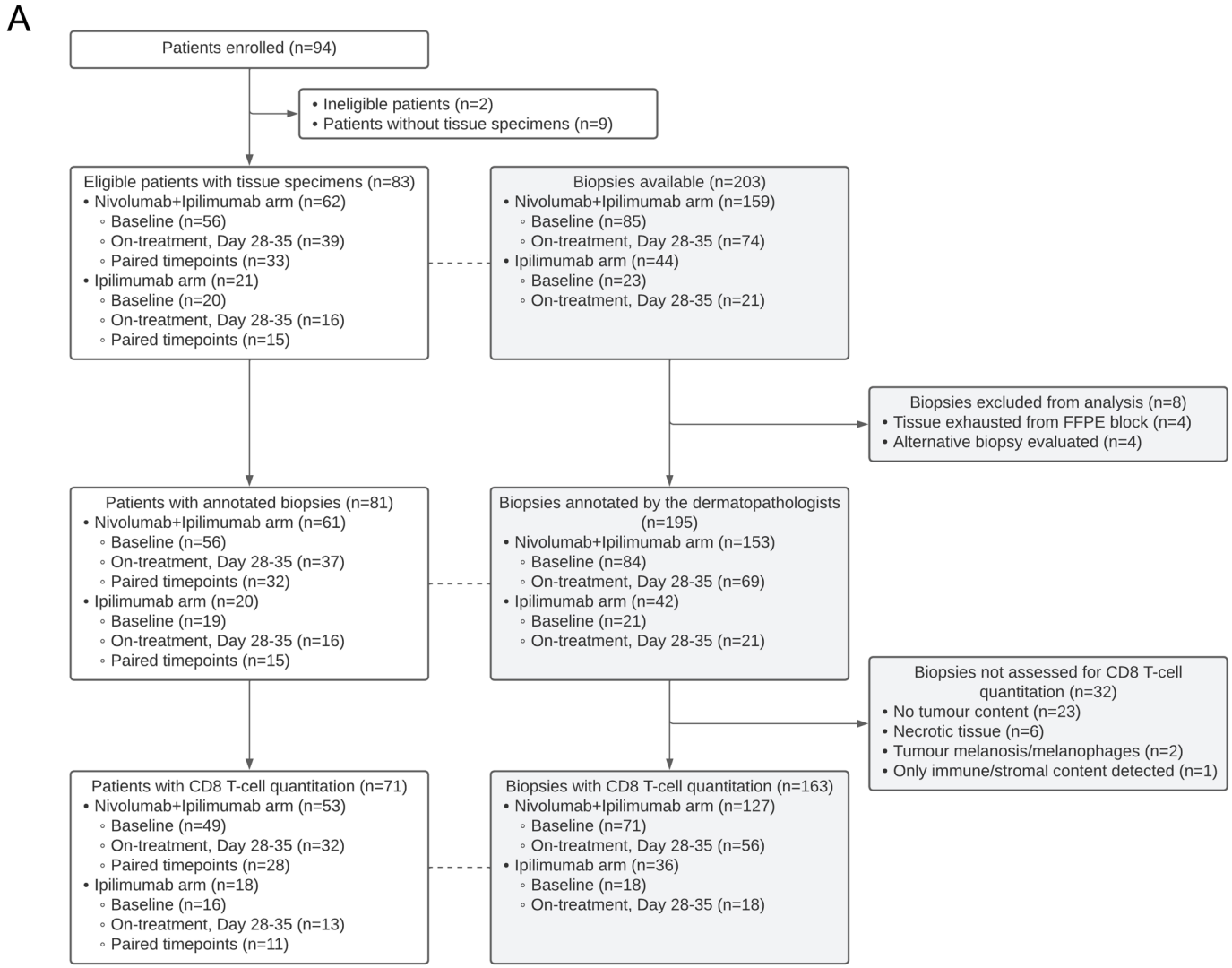
Peer review information *Nature Medicine* thanks Reinhard Dummer, Michael Postow and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Ulrike Harjes, in collaboration with the *Nature Medicine* team.

https://doi.org/10.1038/s41591-023-02498-y



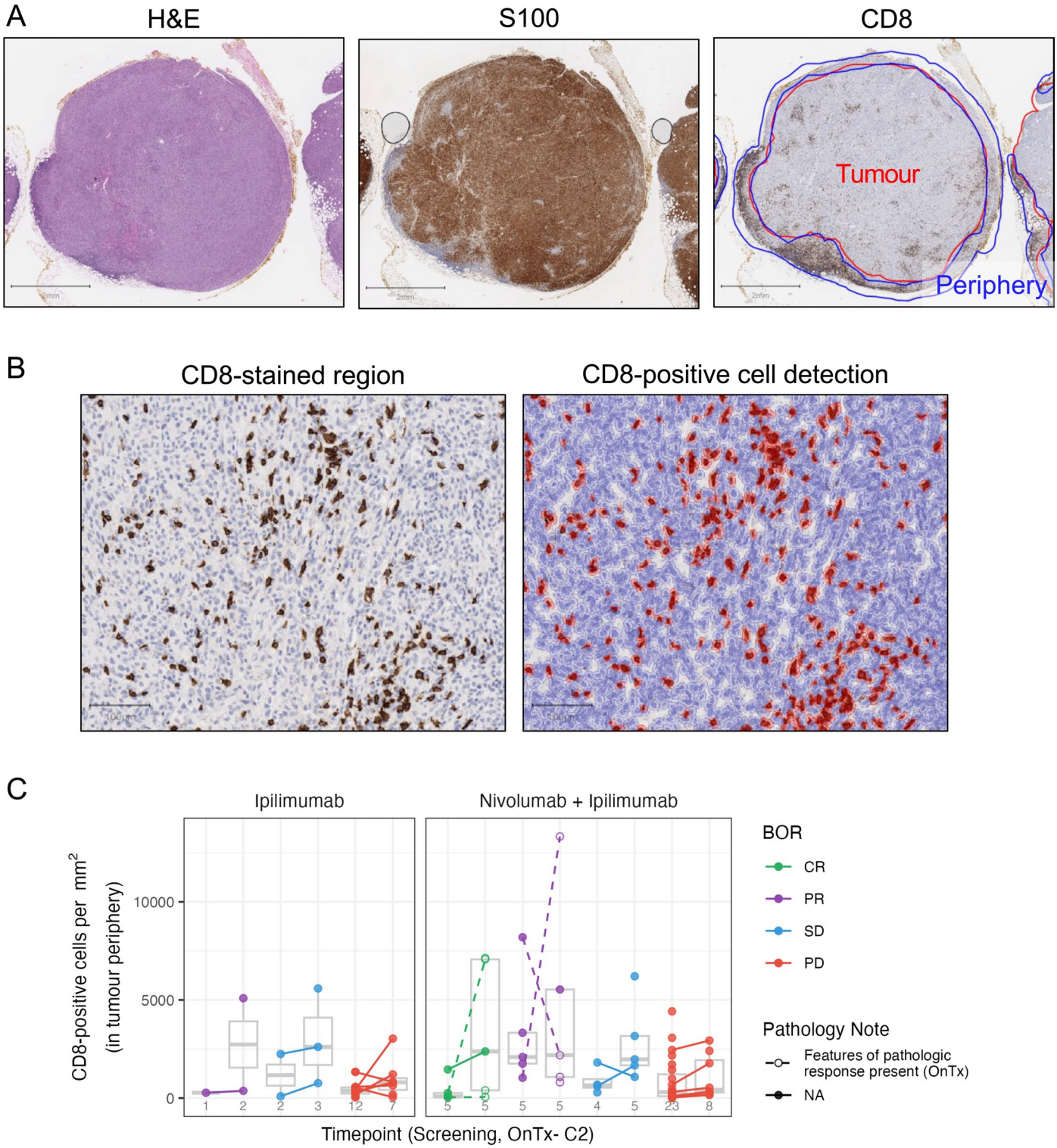
Extended Data Fig. 1 | Kaplan-Meier plot of overall survival. The 12-month OS estimates were 63% (90% CI: 52%–72%) and 57% (38%–71%) for the combination therapy versus ipilimumab alone groups, respectively.

https://doi.org/10.1038/s41591-023-02498-y



Extended Data Fig. 2 | Disposition of tumour biopsies assessed for CD8-positive cell quantitation. a) CONSORT diagram of tumour biopsies assessed for CD8-positive cell quantitation. **b)** Alluvial plot depicting the patient demographics (x-axis) for each patient (alluvium) that was included in the final analysis for CD8 T-cell quantitation. Demographics include trial arm, best overall response (BOR, also shown by fill colour), whether pathologic features consistent with melanoma regression were observed on-therapy, and which sample timepoints were included in analysis (either screening or on-therapy biopsy only, or paired). **c)** Alluvial plot depicting patient demographics and pathologic annotation (x-axis) for each biopsy (alluvium) that was annotated by the dermatopathologists. Fill colour indicates whether the biopsy was included in the final analysis for CD8 T cell quantitation. Biopsies are annotated for the trial arm, BOR, and whether features consistent with pathologic regression of melanoma were noted in the on-therapy biopsies for the corresponding patient, as well as the timepoint the biopsy was taken, and the manual notes for the biopsy from the dermatopathologists.

https://doi.org/10.1038/s41591-023-02498-y



Extended Data Fig. 3 | Semi-automated workflow for CD8-positive cell quantitation in tumour biopsies. a) Representative images for manual annotation of tumour and tumour periphery regions. **b)** Screenshot of cell segmentation, identifying CD8-positive cells (red) and CD8-negative cells (blue), for CD8 cell quantitation. Scale bars in each panel are 100um. **c)** Density of CD8 positive cells detected within the annotated tumour periphery. Paired baseline and on-treatment biopsies are indicated by points connected by lines. Dotted lines indicate patients with pathological features of regressed melanoma in the on-treatment biopsy. The number of patients shown in each group is indicated by the number along the x-axis (Ipilimumab arm, N = 2 partial response [PR], N = 3 stable disease [SD], N = 13 progressive disease [PD]; nivolumab and ipilimumab arm, N = 7 complete response [CR], N = 8 PR, N = 7SD, N = 25 PD). Box plots indicate the median (middle line), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers).

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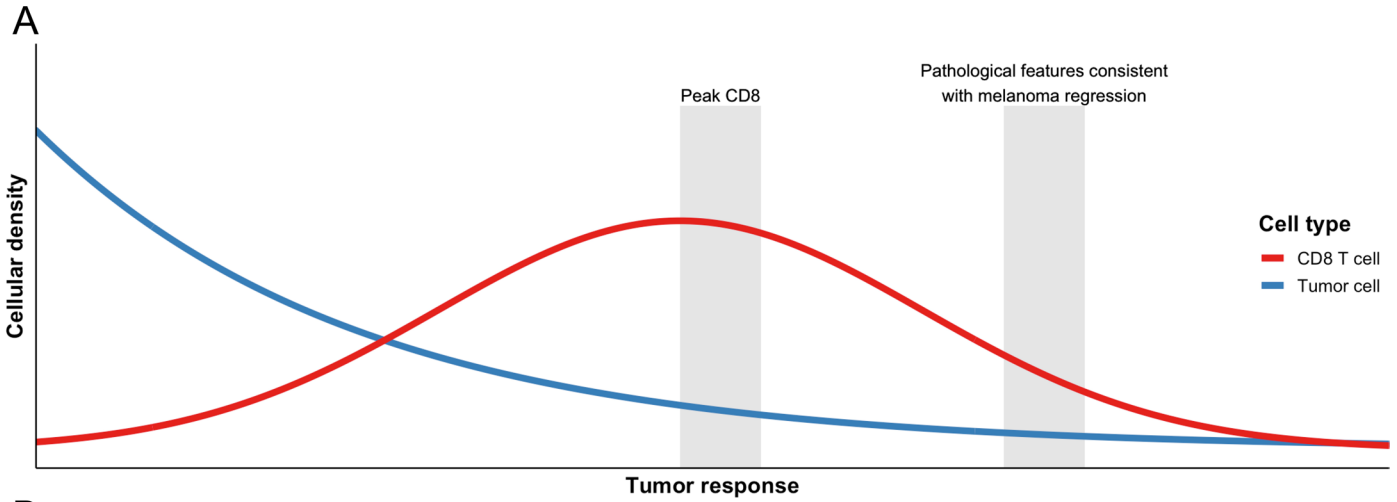
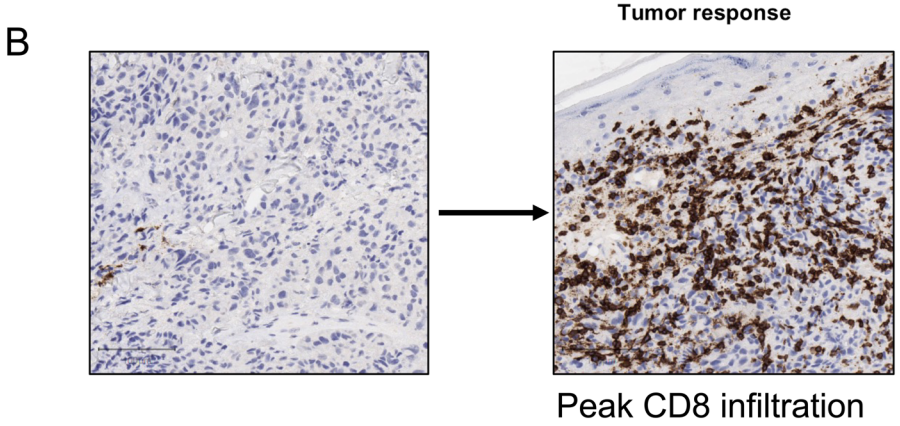
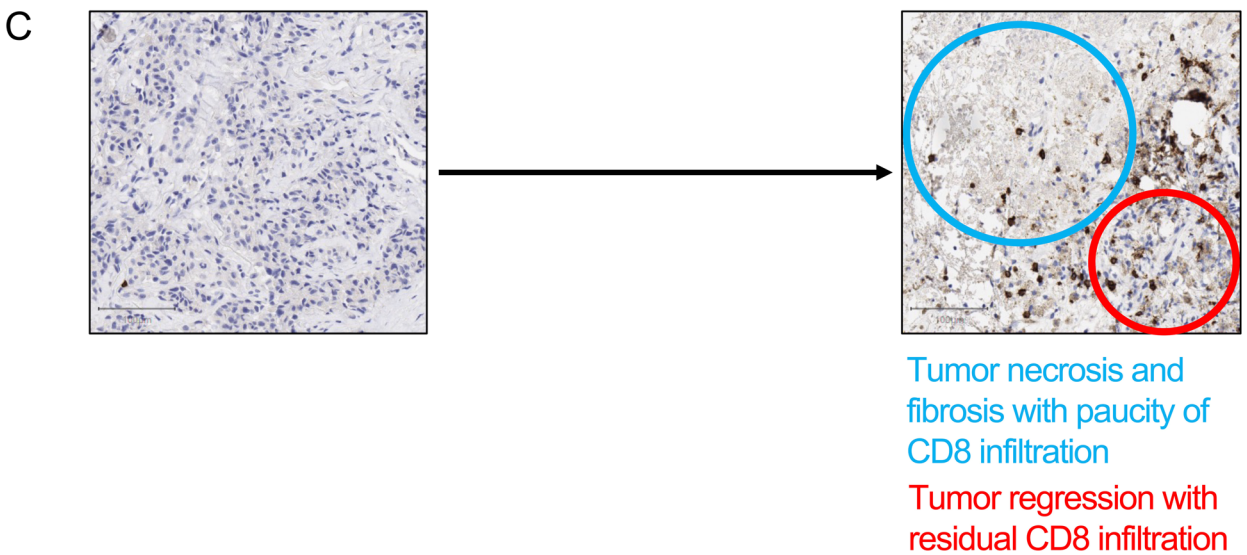
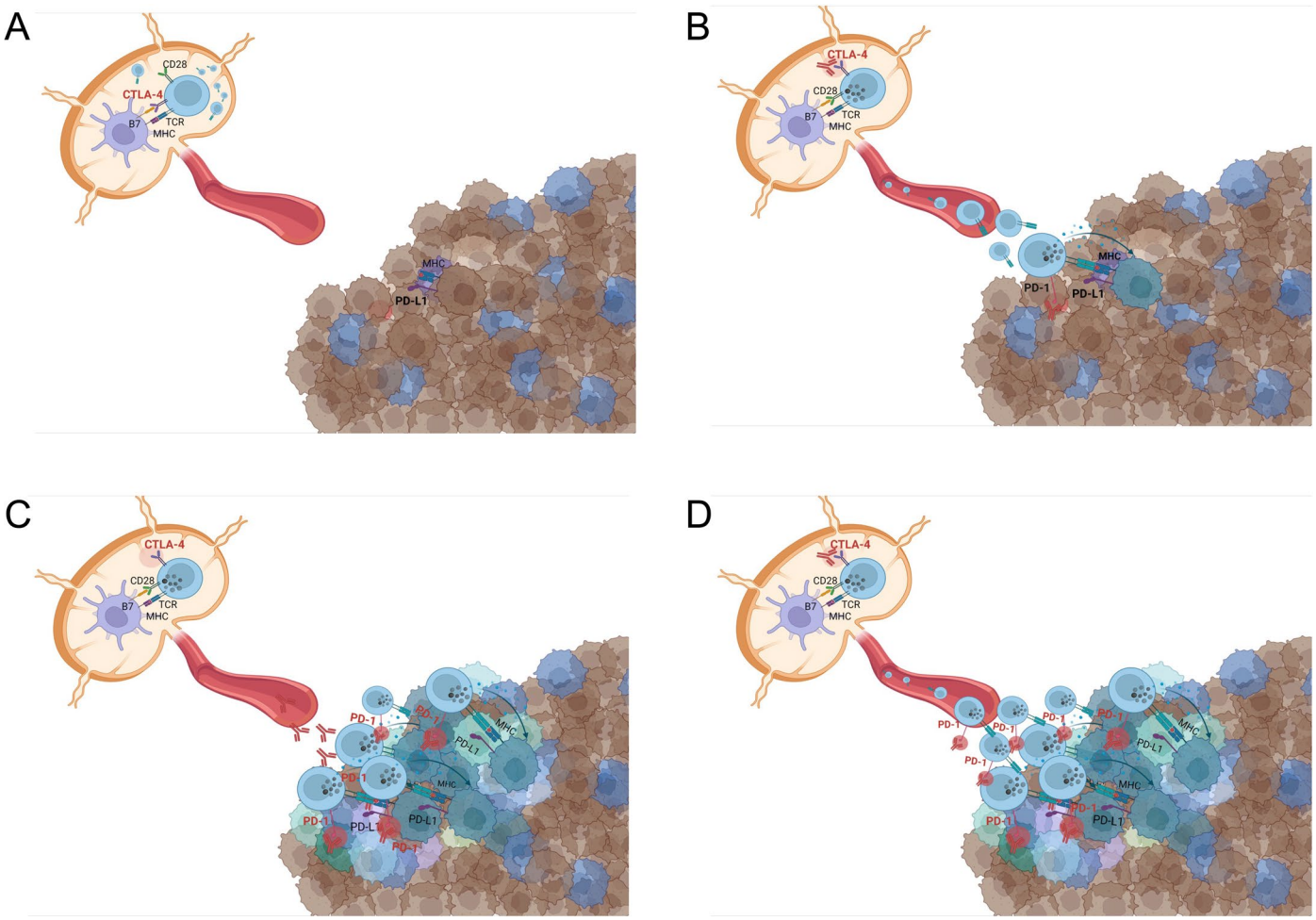






Extended Data Fig. 4 | Modeling of the timing of CD8 infiltration and the pathological assessment of areas of melanoma regression. a) Proposed model for the timing of the anti-tumour CD8 T-cell response (numbers are not based upon real data), captured by on-therapy tumour biopsies collected day 28–35 following the start of combination therapy. The red line indicates the changes of CD8 T cell density with respect to the change in tumour cell density (blue line). The grey regions demonstrate the timing captured across on-therapy biopsies from patients who respond to nivolumab plus ipilimumab, where some biopsies captured the peak or maximum CD8 T cell density. Others were collected following the regression of the majority of the tumour, where few CD8 T cells and few melanoma cells remaining, consistent with pathological features of melanoma regression (for example tumour necrosis, fibrosis, presence of melanophages). **b)** Example of patient-matched screening and on-therapy biopsies. Biopsies collected from a patient who had a complete response to nivolumab plus ipilimumab were stained for CD8 T cells using immunohistochemistry (IHC). The on-therapy biopsy contained residual tumour cells in addition to very high CD8 T cell infiltration. **c)** Example of patient-matched screening and on-therapy biopsies, stained for CD8 T-cells using IHC, where the biopsies of a patient with complete response to nivolumab plus ipilimumab showed pathological features consistent with melanoma regression. Indicated are areas of tumour necrosis and fibrosis (blue) and tumour regression with residual CD8 T cell infiltration (red).

https://doi.org/10.1038/s41591-023-02498-y



Extended Data Fig. 5 | Model for the mechanism of reversal of tumour resistance to anti-PD-1 with the addition of anti-CTLA-4. a) Response to PD-1 blockade therapy is mediated by the intratumour pre-existence of tumour-specific CD8 T cells that are negatively regulated by the reactive expression of PD-L1 by cancer cells. Administering an anti-PD-1/L1 therapy results in tumour regression. **b)** Tumours that are resistant to PD-1 blockade therapy are enriched for low intratumour infiltration by CD8 T cells. **c)** Releasing the CTLA-4 checkpoint promotes and the trafficking of T cells to the tumour. **d)** To obtain the maximum density of CD8 T cells in the tumour, and corresponding clinical responses, it requires concurrent release of both the CTLA-4 and PD-1 checkpoints.

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Last updated by author(s): Jun 29, 2023

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Clinical data from sites reported through iMedidate Rave; transferred to the SWOG SQL database; exported for analysis in R (v4.0.3) and SAS (version 9.4). CD8-positive cell density was quantified from 5um slides stained by immunohistochemistry for CD8 protein and imaged at 40X. Images were imported into Qupath (v0.3.0) and manually annotated by dermatopathologists, merging regions annotated as either Tumor or Other, indicating the tumor periphery regions. CD8-positive cells were quantified for each image and region, using the following command: createSelectAllObject(true); runPlugin('qupath.imagej.detect.cells.PositiveCellDetection', '{"detectionImageBrightfield": "Hematoxylin OD", "requestedPixelSizeMicrons": 0.5, "backgroundRadiusMicrons": 8.0, "medianRadiusMicrons": 0.0, "sigmaMicrons": 1.5, "minAreaMicrons": 10.0, "maxAreaMicrons": 400.0, "threshold": 0.1, "maxBackground": 2.0, "watershedPostProcess": true, "excludeDAB": false, "cellExpansionMicrons": 5.0, "includeNuclei": true, "smoothBoundaries": true, "makeMeasurements": true, "thresholdCompartment": "Cell: DAB OD mean", "thresholdPositive1": 0.2, "thresholdPositive2": 0.4, "thresholdPositive3": 0.6000000000000001, "singleThreshold": true}'); Detections were exported using the following command: path = buildFilePath(PROJECT_BASE_DIR, 'detections'); mkdirs(path); saveDetectionMeasurements(path)
Data analysis	R (v4.0.3 and v4.2.0), including tidyverse R package (v1.3.2) and SAS (v 9.4) were used.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data and code to reproduce the analyses presented here are available upon request from SWOG following SWOG's data sharing policy and process: https://www.swog.org/sites/default/files/docs/2019-12/Policy43_0.pdf.

The protocol (including statistical analysis plan in Section 11 of the protocol) and informed consent are Supplementary Materials.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Patients were screened and enrolled on this study irrespective of their sex/gender. Any data regarding a patient's sex and gender was collected by the clinical trial groups at each site. Sex- or gender-based subgroup analysis are reported in a Forest plot in Figure 2b.
Population characteristics	Provided in Table 1.
Recruitment	Patients were recruited across 39 clinical investigational sites. Given that this was an NCI-funded US cooperative group trial, the sites were limited to the United States of America. There were no biases introduced and patients were screened on a first-come first-serve basis based on meeting the protocol inclusion-exclusion criteria. No protocol waivers were allowed on this study.
Ethics oversight	The trial was conducted in accordance with the principles of the Declaration of Helsinki. The trial protocol and statistical analysis plan were designed in a collaboration between the SWOG and CTEP investigators. The NCI Central IRB and the IRBs from each of the 39 clinical sites enrolling patients approved the study. The SWOG independent Data Safety Monitoring Committee Oversaw its Conduct.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined such that this study had 89% power for the primary outcome (progression-free survival measured from randomization) for a log-rank test with one-sided type I error of 9%. Then based on the considerations below, this study used a design in which 63 patients were to be randomized to receive combination therapy ipilimumab and nivolumab and 21 patients were to be randomized to receive single therapy ipilimumab. Assuming an ineligibility rate of 10% the total accrual goal was 94 patients. Based on Hodi et al., the median PFS for patients randomized to ipilimumab alone was assumed to be 3 months. The study targeted an improvement in PFS for patients randomized to combination therapy such that the median increases to 6 months (alternative corresponds to a hazard ratio of 0.5). We assumed exponential survival, 24 months of accrual, 12 additional months of follow-up after the last patient is randomized, and that the final analysis will be conducted at the one-sided alpha = 10% level when we reached 100% information under the alternative (corresponding to 78 events across both arms). One interim analysis for futility was planned after approximately 50% information under the null hypothesis (41 events across both arms). If the hazard ratio favored the control arm (HR >1), the study would be stop for futility. Using simulations (10,000 replications), this design has overall type 1 error of 9.0% and power of 89%. Unbalanced randomization was necessary for evaluating the first secondary objective of comparing T cell infiltration between patients who have an objective response and patients who do not have an objective response to combination therapy. A 3:1 randomization was necessary to ensure adequate sample size for evaluating this endpoint. Assuming a response rate of 25% and 90% compliance in tissue and biopsy submission, we anticipated accruing 14 eligible objective responders and 43 eligible objective non-responders in the combination arm. Using a two-sample t-test and controlling the type I error at the two-sided 0.05 level, this design has 80% power to detect a mean difference in CD8+

expression of 0.875 standard deviations.

Data exclusions	Ninety-four patients were enrolled in this study. Two patients did not meet pre-established study eligibility criteria as described in Section 5 of the clinical trial protocol and were excluded from the analysis: one patient did not have documented disease progression on prior anti-PD1/L1 therapy; and one patient had intervening treatment after progression on anti-PD1/L1 therapy and prior to study enrollment. All further analyses were intent-to-treat and no eligible patients were excluded.
Replication	N/A. Replication of findings could not be performed, as this was a clinical trial that required SWOG, NCI, and NCI Central Institutional Review Board approval. Here, we are reporting pre-specified analyses of a phase 2 clinical trial. Replication of this study would require another clinical trial with SWOG, NCI, and NCI Central IRB approvals.
Randomization	Dynamic balancing without stratification factors was used to allocate patients 3:1 to the combination therapy vs ipilimumab alone arms. Randomization was completed by sites through the SWOG rando-node dynamic balancing algorithm implemented through NCI's OPEN registration platform.
Blinding	Study was not blinded. Per standard with many oncology studies, because no placebo was given, blinding was not possible since the treatments compared are different.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Antibodies used for treatment of patients within the clinical trial are ipilimumab (Yervoy(R)) and nivolumab (Opdivo(R)), provided by BMS through NCI/CTEP. Antibodies used for immunohistochemistry and analysis include mouse anti-human CD8 (Agilent, M7103, 1-100) and mouse anti-human S100 (Cell Marque, Cat#: 330M, 1-400). Normal mouse IgG (Santa Cruz, Cat#: sc2025, 1-200) was used as a negative control in the absence of primary antibody in both detection systems.
Validation	Clinical grade antibodies used for immunohistochemistry were performed by the Translational Pathology Core Laboratory, a part of the CAP/CLIA certified University of California, Los Angeles according to manufacturer specifications and clinical validation performed for clinical testing within the laboratory.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT03033576
Study protocol	The study protocol, "A Phase II Randomized Study of Nivolumab (NSC-748726) with Ipilimumab (NSC-732442) or Ipilimumab Alone in Advanced Melanoma Patients Refractory to an Anti-PD1 or Anti-PD-L1 Agent" is provided in the Supplemental Information Files.
Data collection	Data was collected at individual sites throughout the United States between July 2017 and July 2020. Sites were responsible for entering and uploading data for participants enrolled on the trial. Each site followed their site-specific rules for data collection and submission, following the timelines provided in Section 14 of the protocol. Data was submitted online through the iMedidata Rave platform, which is uploaded daily into the SWOG SQL database.
Outcomes	Objectives and endpoints were pre-defined in the protocol prior to trial activation. Progression-free survival was measured from the date of randomization to the date of first documentation of progression or symptomatic deterioration, or death due to any cause. Patients last known to be alive without report of progression were censored at the date of last contact. Overall survival was measured from the date of randomization to the date of death from any cause, with patients last known to be alive censored at the date of last contact. RECIST v1.1 criteria were used for tumor response assessment. NCI CTCAE version 5.0 was used to collect adverse event data.