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Pretreatment and on-treatment ctDNA and tissue biomarkers predict recurrence in patients with stage IIIB–D/IV melanoma treated with adjuvant immunotherapy: CheckMate 915

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ABSTRACT

Purpose CheckMate 915 (NCT03068455) compared adjuvant nivolumab monotherapy versus combination nivolumab+ipilimumab in patients with resected stage III/IV melanoma. This exploratory analysis was performed to identify biomarkers that correlate with benefit from adjuvant immunotherapy.

Patients and methods 1,844 patients received nivolumab 480 mg every 4 weeks or nivolumab 240 mg every 2 weeks with ipilimumab 1 mg/kg every 6 weeks. Tumor and peripheral biomarkers were evaluated, including tumor-informed circulating tumor DNA (ctDNA) at postresection baseline and on-treatment, for their association with recurrence-free survival and distant metastases-free survival.

Results Biomarker analyses were conducted in 60–96% of the intention-to-treat population. ctDNA positivity at baseline (seen in 16.2% of patients) and on-treatment was associated with higher risk of recurrence than ctDNA negativity (HR, 1.97; 95% CI, 1.57 to 2.46), with a high specificity (87%) and modest sensitivity (39%). ctDNA status, tumor mutational burden (TMB) status (TMB < or ≥350 mutations/tumor) and interferon gamma-RNA signature score (< or ≥median) evaluated together, as well as ctDNA status with tumor CD8 or cell programmed death ligand 1 expression, were more predictive of survival than ctDNA alone. Tumor bulk RNA-seq expression patterns identified gene expression at baseline associated with recurrence.

Conclusions This study represents the largest assessment of ctDNA and other baseline tumor and peripheral biomarkers for predicting recurrence-free survival in patients with resected melanoma receiving adjuvant immunotherapy. ctDNA alone and in combination with more established biomarkers predicted recurrence-free and distant metastasis-free survival and has potential utility for assessing and monitoring the risk of recurrence in patients with resected melanoma treated with immunotherapy in the adjuvant setting.

Trial registration number NCT03068455.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Although there are well-recognized tumor biomarkers associated with immunotherapy efficacy in metastatic melanoma, predictive biomarkers in the adjuvant setting of immunotherapy are not as well researched. Recently, circulating tumor DNA (ctDNA) has emerged as a promising biomarker associated with more advanced disease and recurrence in the adjuvant treatment setting.

WHAT THIS STUDY ADDS

⇒ To the best of our knowledge, our study represents the largest assessment of ctDNA and its correlation with other biomarkers in a prospective clinical trial (CheckMate 915) of 1,844 patients receiving adjuvant immunotherapy (nivolumab or nivolumab+ipilimumab) following melanoma resection. We showed that baseline ctDNA predicts disease recurrence, and that prediction was enhanced when combined with longitudinal ctDNA analysis or established tissue biomarkers at baseline.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These results may support the development of a tool to predict melanoma recurrence risk using baseline clinical factors, ctDNA, and tissue biomarkers, aiding clinicians in patient risk stratification and discussions on the risk–benefit profile of adjuvant therapy.

INTRODUCTION

Immunotherapy, such as the programmed death-1 (PD-1) inhibitor nivolumab and the cytotoxic T lymphocyte antigen-4 inhibitor ipilimumab, has improved clinical outcomes in patients with melanoma, including in the adjuvant and neoadjuvant settings for advanced disease.[1–11] Although there are well-recognized tumor biomarkers associated

with efficacy in metastatic disease—for example, tumor programmed death ligand 1 (PD-L1) expression and high tumor mutational burden (TMB)[12]—fewer studies have evaluated predictive biomarkers in the adjuvant setting of immunotherapy (eg, in the IMMUNED[9 13] and CheckMate 238[1 14] studies) or targeted therapy (eg, in the COMBI-AD study,[15] which has published results from a comprehensive biomarker analysis[16]).

Recently, there has been interest in circulating tumor DNA (ctDNA) as a promising biomarker associated with more advanced disease and recurrence in the adjuvant treatment setting. Detection of ctDNA can be used as a tool to measure molecular residual disease post resection, as well as response to therapy over time or disease progression during surveillance.[17–21] ctDNA positivity at baseline after surgery has been associated with a greater risk of relapse and poor outcomes in patients with resected melanoma.[17–19]

Here, we report the results of broad biomarker analyses from a large study on immunotherapy for resected stage IIIB–D/IV melanoma (CheckMate 915). These analyses form the largest study to date of longitudinal biomarkers, particularly of ctDNA, in high-risk resectable melanoma; we evaluated tumor-informed ctDNA at postresection baseline and on-treatment, with the aim of determining the contribution of ctDNA alone or with other biomarkers in predicting disease recurrence, thus helping to inform and expand on composite biomarker analyses to predict the recurrence of disease on therapy.

METHODS

Study design and patients

ctDNA and biomarkers associated with antitumor immunity were assessed for associations with recurrence in patients with stage III/IV melanoma treated with adjuvant nivolumab or nivolumab+ipilimumab from CheckMate 915 (NCT03068455). The CheckMate 915 study design has been published previously (online supplemental figure S1).[22] Briefly, patients with resected stage IIIB–D or IV melanoma and no evidence of residual disease on radiographical assessment who were aged 12 years or older and had an Eastern Cooperative Oncology Group performance status of 0 or 1 were randomized 1:1 to receive nivolumab monotherapy 480 mg once every 4 weeks or nivolumab 240 mg every 2 weeks+ipilimumab 1 mg/kg every 6 weeks. The dual primary endpoints were recurrence-free survival (RFS) for nivolumab+ipilimumab versus nivolumab in all randomized patients and in patients with PD-L1 expression on <1% of tumor cells; distant metastasis-free survival (DMFS) was assessed as an exploratory endpoint. RFS was defined as the time from the randomization date to the date of first recurrence (local, regional, or distant), development of new primary melanoma (including melanoma in situ), or death from any cause, whichever occurred first. DMFS was assessed in all patients with stage III disease and was defined as the time from the randomization date to the date of first distant metastasis or death from any cause, whichever occurred first.[22] Additional details are provided in the study protocol (online supplemental file 1).

ctDNA analysis

The ctDNA-evaluable cohort included patients with whole exome sequencing (WES) data available for tumor and matched-normal samples, which were necessary for the successful design of a patient-specific panel (16–200 variants) to evaluate the presence of ctDNA in patient plasma samples, where ctDNA results were both obtained and evaluable after quality control. ctDNA was measured using a tumor-informed molecular residual disease assay, Personalized Cancer Monitoring™ (Invitae Corporation, San Francisco, California, USA), as previously described.[23] Briefly, patient-specific, tumor-informed variant panels identified by WES were used to detect ctDNA in plasma samples. See online supplemental methods for further information.

Biomarker analysis

The percentage of CD8+ T cells in the tumor microenvironment (TME), TMB, the percentage of tumor cells expressing PD-L1, and interferon gamma (IFNγ)-RNA signature scores were evaluated in baseline specimens, collected prior to initiation of adjuvant treatment. The percentage of CD8+ T cells in the TME was measured by immunohistochemistry using a CD8-specific monoclonal antibody (clone C8/144B, cat #M710301-2, Agilent Technologies).[14 24] TMB was measured by WES,[14 24] with high TMB (high tertile) defined as ≥350 mutations/tumor and low TMB (low and medium tertile) defined as <350 mutations/tumor. The percentage of tumor cells expressing PD-L1 was measured using the PD-L1 immunohistochemistry 28–8 pharmDx assay (Dako, Agilent Technologies, Santa Clara, California, USA).[22 24] Gene expression was measured using RNA-seq (see online supplemental methods). Gene expression profiling through RNA-seq was used to evaluate a composite IFNγ-RNA signature score based on the expression of ten genes (*HLA-DRA*, *CXCL9*, *GZMA*, *PRF1*, *CCR5*, *IFNγ*, *CXCL10*, *IDO1*, *STAT1*, and *CXCL11*).[14 25] Extended RNA expression analyses interrogated hallmark[26 27] and TME functional gene sets.[22 26 27]

Statistical analysis

A Cox proportional hazards regression model was used to evaluate associations between biomarkers and disease recurrence in biomarker-evaluated patients in the nivolumab monotherapy, nivolumab+ipilimumab, and pooled treatment arms. Baseline and on-treatment ctDNA status was assessed as a categorical variable as detected or not detected. PD-L1 expression, CD8+ T cell levels, TMB, circulating cytokine levels, and IFNγ-RNA signature score were assessed both as continuous and categorical variables. RFS and DMFS were estimated using the Kaplan-Meier product-limit method, and HRs were estimated using a Cox proportional hazards regression model. See

online supplemental methods for details on the association of individual genes, peripheral cells, and serum factors and gene set enrichment analysis with RFS.

RESULTS

Baseline characteristics, efficacy, and biomarker prevalence

Of the CheckMate 915 intention-to-treat (ITT) population (n=1,844), the number of evaluable patients for each of the biomarkers by treatment arm is reported in online supplemental table S1. Cohort attrition to arrive at the ctDNA-evaluable population (n=1,127) is described in online supplemental figure S2.

No meaningful differences in baseline characteristics were observed between the ctDNA-evaluable and overall treated populations (online supplemental table S2).

24-month RFS in the pooled ctDNA-evaluable population was 62% (95% CI, 59% to 65%), similar to that in the overall treated population, with overlapping CIs (64%; 95% CI, 62% to 66%). In the ctDNA-evaluable population, ctDNA positivity at baseline was found in 16.2% of patients overall (183/1,127; 95% CI, 14% to 18%; table 1). The prevalence of ctDNA positivity was similar across subgroups defined by baseline clinical characteristics and biomarker status, except for a higher prevalence in patients with stage IIID melanoma than in patients with stage IIIB, IIIC, or IV disease (table 1). Despite the low number of patients with stage IIID disease, the trend appeared statistically significant ($p=6.4 \times 10^{-6}$ for the test of equal proportions). There were overlapping CIs for baseline positivity rates by PD-L1, *BRAF*^{V600} mutation status, TMB, and time from resection surgery to ctDNA plasma collection.

Table 1 Prevalence of pretreatment ctDNA at baseline

Baseline characteristic	ctDNA(+)/ctDNA-evaluable, n/N	ctDNA positivity % (95% CI)
Total ctDNA-evaluable population	183/1,127	16 (14 to 18)
Disease stage		
IIIB	35/333	11 (8 to 14)
IIIC	110/596	18 (15 to 22)
IIID	13/32	41 (24 to 59)
IV	25/166	15 (10 to 22)
ECOG PS		
0	166/1,054	16 (14 to 18)
1	17/73	23 (15 to 35)
LDH		
≤ULN	162/1,001	16 (14 to 19)
>ULN	18/113	16 (10 to 24)
BRAF$^{V600E/K}$ status		
Mutant	87/483	18 (15 to 22)
Wild type	96/644	15 (12 to 18)
PD-L1 expression on tumor cells*		
<1%	71/416	17 (14 to 21)
≥1%	108/680	16 (13 to 19)
TMB status†		
High	75/430	17 (14 to 21)
Low	108/695	16 (13 to 18)
Time from surgery to randomization		
<9 weeks	85/496	17 (14 to 21)
≥9 weeks	98/631	16 (13 to 19)

*Assessed using the 28–8 pharmDx assay.
†TMB high (high tertile), ≥350 mutations/tumor; TMB low (low and medium tertile), <350 mutations/tumor.
ctDNA, circulating tumor DNA; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; PD-L1, programmed death ligand 1; TMB, tumor mutational burden; ULN, upper limit of normal.

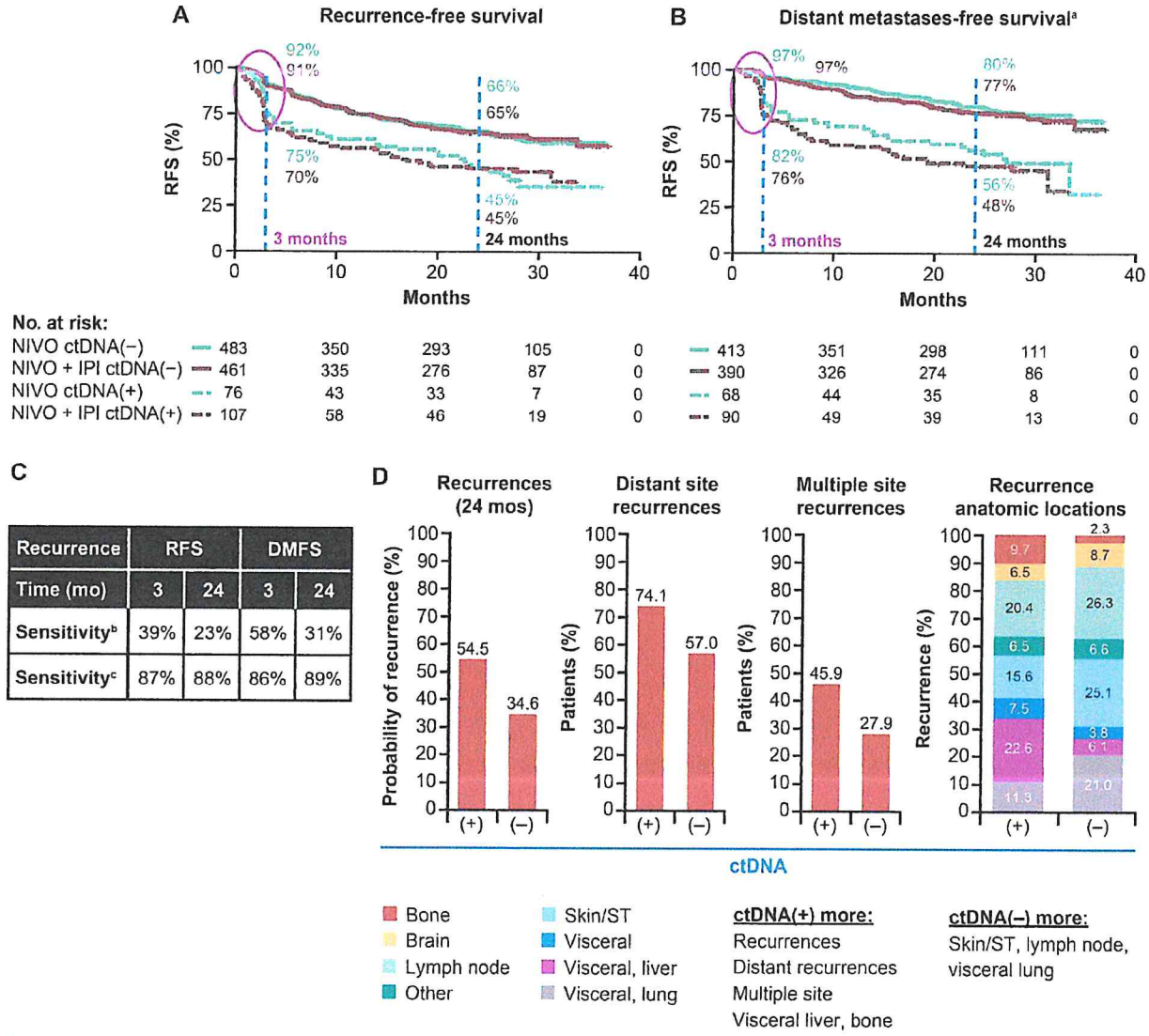
Association of baseline ctDNA status with recurrence in the nivolumab and nivolumab+ipilimumab treatment arms

ctDNA positivity at baseline was associated with shorter RFS and DMFS compared with baseline ctDNA negativity in both the nivolumab and nivolumab+ipilimumab treatment arms (figure 1A,B). The greatest differences in rates of recurrence between baseline ctDNA(+) versus ctDNA(−) were noted for early recurrences (within 3 months) with the recurrence rates being similar following 3 months of study.

There was no significant difference in RFS or DMFS between the nivolumab and nivolumab+ipilimumab treatment arms in the baseline ctDNA(+) and ctDNA(−) subgroups. No significant interaction was observed between baseline ctDNA status and treatment arms (HR, 1.00; 95% CI, 0.63 to 1.57; p=0.995 from the Cox proportional hazard model with interaction term ctDNA status treatment). In addition, the RFS rates for the two treatment arms were similar.[22] Therefore, data from the nivolumab and nivolumab+ipilimumab treatment arms were pooled for subsequent analyses to increase the number of samples in each subgroup and the power of subsequent analyses.

In the overall population, ctDNA positivity at baseline was associated with shorter RFS (HR, 1.97; 95% CI, 1.57 to 2.46) and DMFS (HR, 2.86; 95% CI, 1.90 to 4.30) than baseline ctDNA negativity across treatment arms. The sensitivity and specificity of ctDNA at predicting recurrence were examined at 3 months and 24 months on treatment (figure 1C). Levels of sensitivity were modest, whereas levels of specificity were high. Sensitivity was numerically greater at 3 months than at 24 months (RFS sensitivity, 39% vs 23%; DMFS sensitivity, 58% vs 31%).

Investigations of associations between ctDNA positivity and first recurrences found that ctDNA positivity was associated with higher risks of recurrence, distant recurrence, recurrence at multiple sites, and visceral liver ($p<1 \times 10^{-7}$) and bone (p=0.0002) recurrences than ctDNA negativity



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Recurrence	RFS		DMFS	
Time (mo)	3	24	3	24
Sensitivity[b]	39%	23%	58%	31%
Sensitivity[c]	87%	88%	86%	89%

Figure 1 Association of baseline ctDNA positivity with (A) RFS and (B) DMFS; (C) Sensitivity and specificity for predicting recurrence; (D) Association of baseline ctDNA positivity with first recurrence in the 1,127-patient ctDNA-evaluable subgroup. For recurrences, data from the first recorded 1,138 recurrences in 343 patients in the ctDNA-evaluable cohort with recurrence were used. The pink circles in figures A and B denote early recurrences and highlight the differences in recurrence rates between the baseline ctDNA groups. [a]In patients with stage III disease. [b,c]Sensitivity and specificity for predicting RFS and DMFS at 3 and 24 months are represented. To calculate 3-month RFS sensitivity/specificity, the patients with recurrence after ≤3 months were marked as positives and the remaining patients were marked as negatives. The fraction of ctDNA(+) patients among the positives provided the sensitivity of baseline ctDNA and the fraction of ctDNA(−) patients among the negatives provided the specificity of baseline ctDNA in predicting 3-month recurrence. The sensitivity and specificity for RFS at 24 months, DMFS at 3 months, and DMFS at 24 months were determined in a similar manner. ctDNA, circulating tumor DNA; DMFS, distant metastasis-free survival; IPI, ipilimumab; mo, months; NIVO, nivolumab; RFS, recurrence-free survival; ST, soft tissue.

(figure 1D). Patients who were ctDNA(−) at baseline had a greater numerical rate of skin/soft tissue (p=0.01) and visceral lung (p=0.006) recurrences than the baseline ctDNA(+) population.

Association of longitudinal ctDNA status with RFS in the pooled ctDNA-evaluable population

Due to early recurrences (≤week 13) in the baseline ctDNA(+) subgroup, baseline ctDNA positivity was associated with an increased risk of recurrence at any time after treatment relative to ctDNA negativity (HR, 1.97; 95% CI, 1.57 to 2.46; figure 2Ai). However, the association between baseline ctDNA positivity and risk of recurrences occurring after week 13 decreased (landmark starting time at week 13; HR, 1.49; 95% CI, 1.12 to 1.99) and was not associated with recurrences occurring after week 29 (HR, 1.36; 95% CI, 0.95 to 1.94), owing to the relatively similar risk of recurrence in the baseline ctDNA(+) and ctDNA(−) subgroups from week 13 onwards. The lack of significant association between baseline ctDNA status and RFS in landmark analysis indicates that the prognostic importance of ctDNA may evolve over time on therapy, and that baseline ctDNA status has a reduced association with RFS at later times on therapy.

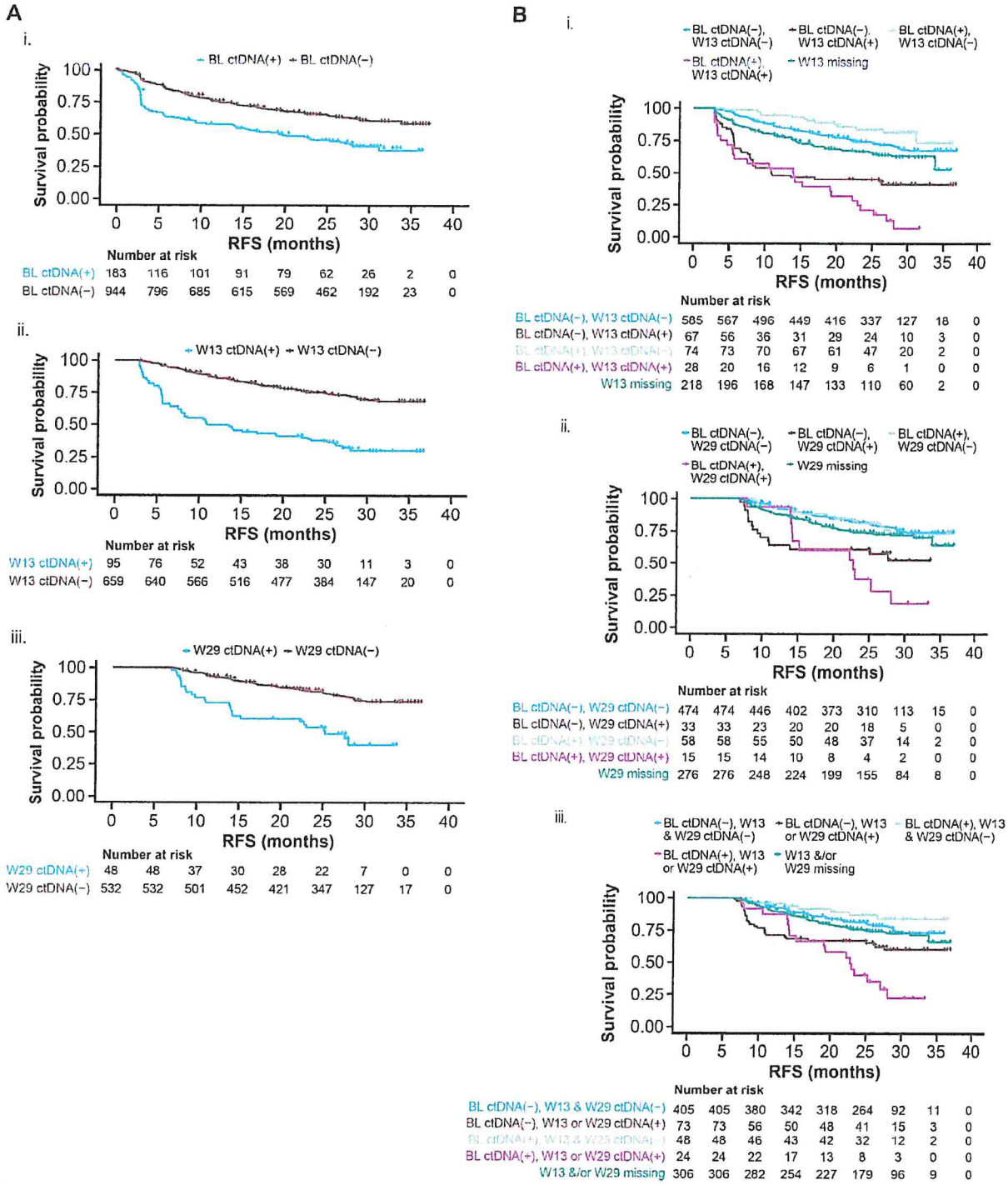

Figure 2 (A) Landmark analysis of RFS at any time by baseline ctDNA status (i) and week 13 (ii) and week 29 (iii) by on-treatment ctDNA status. (B) Association of combined baseline and on-treatment ctDNA status with RFS. BL, baseline; ctDNA, circulating tumor DNA; RFS, recurrence-free survival; W, week.

However, assessment of on-treatment ctDNA status increased the prediction of recurrence. Of the 1,127 patients with baseline samples evaluable for ctDNA, 860 (76%) patients had ctDNA results available at ≥1 on-treatment time point (online supplemental figure S3). On-treatment ctDNA positivity was associated with an increased risk of recurrence at landmark time points at week 13 and 29, respectively, compared with on-treatment ctDNA negativity (figure 2Aii–iii). On-treatment ctDNA status at week 13 and 29 was associated with RFS (week 13, HR, 3.81; 95% CI, 2.86 to 5.07; week 29, HR, 3.20; 95% CI, 2.09 to 4.90), suggesting that ctDNA could serve as a monitoring biomarker accounting for treatment effects or disease progression. In predicting 3-month future recurrence, the week 13 ctDNA sensitivity/specificity was 0.46/0.91 and the week 29 ctDNA sensitivity/specificity was 0.34/0.93.

Longitudinal assessment of ctDNA status further improved associations with RFS (figure 2Bi–iii and online supplemental table S3). Patients were grouped as to whether they zero converted (ie, were ctDNA(+) at baseline and were ctDNA(−) at week 13 and 29), were persistently positive (ie, were ctDNA(+) at baseline and at week 13 or 29), or became positive (ie, were ctDNA(−) at baseline and ctDNA(+) at week 13 or 29). Patients who were persistently positive (median RFS, 22.8 months; 95% CI, 14.2 to 27.1) or became positive (median RFS, not reached (NR); 95% CI, 26.3 to NR), had the greatest risk of recurrence (figure 2B and online supplemental table 3), showing the utility of this peripheral biomarker to predict disease recurrence. Patients who zero converted had the lowest risk of recurrence (median RFS, NR; figure 2B), showing the ability of longitudinal ctDNA assessments to monitor the effect of immunotherapy on the risk of recurrence. The probability of RFS at 24 months was 21.4% (95% CI, 10.5% to 43.6%) in patients who were persistently positive at baseline and week 13, and 83.2% (95% CI, 75.0% to 92.4%) in patients who zero converted at week 13. Thus, although baseline ctDNA status was predictive of outcome on treatment, combined baseline and on-treatment ctDNA assessments may enable peripheral assessment of successful therapy or disease progression during the course of treatment.

Baseline and longitudinal ctDNA and association with tissue biomarkers

To understand relationships between ctDNA status and baseline tumor biomarkers associated with response to immuno-oncology (I-O) drugs and antitumor immunity, biomarker levels were examined in the baseline and on-treatment ctDNA(−) and ctDNA(+) subgroups. Patients who were ctDNA(−) at baseline exhibited higher levels of tumor CD8+ T cell infiltration (figure 3Ai), and similarly, higher IFNγ-RNA signature scores (figure 3Aii) when compared with patients who were ctDNA(+), suggesting baseline ctDNA may be associated with patient antitumor immunity prior to therapy.

Likewise, ctDNA negativity at week 13 was associated with higher baseline CD8+ T cell infiltration, a higher percentage of PD-L1–expressing tumor cells, higher TMB, and higher IFNγ-RNA signature score (figure 3Bi–iv) than patients who were ctDNA(+) at week 13.

Patients who zero converted at week 13 (ie, ctDNA(+) at baseline and ctDNA(−) at week 13) had numerically higher median levels of baseline tumor biomarkers than patients who were persistently positive (ie, ctDNA(+) at both time points; figure 3Bi–iv). Patients who became positive at week 13 (ie, ctDNA(−) at baseline and ctDNA(+) at week 13) had lower median baseline I-O–associated biomarkers (CD8+ T cell infiltration, IFNγ-RNA signature score, and TMB) than patients who were persistently negative. ctDNA negativity at week 29 was associated with numerically higher baseline I-O–associated biomarkers than ctDNA positivity at week 29, except for a lower percentage of PD-L1–expressing tumor cells (figure 3Ci–iv). Overall, baseline tumor I-O biomarkers were less associated with ctDNA status at week 29 than at baseline and week 13 (figure 3B,C), potentially due to the smaller sample size of the week 29 ctDNA-evaluable population or the greater temporal separation between week 29 and baseline tumor biomarkers.

Association of tissue I-O biomarkers with RFS in the nivolumab and nivolumab+ipilimumab treatment arms

Overall, the proportion of patients in the ITT population with biomarker-evaluable samples at baseline was 60–96% (online supplemental table S1). Evaluation of biomarkers as categorical variables showed associations with prolonged RFS for high versus low CD8+ T cell infiltration, PD-L1 expression on tumor cells, TMB, and IFNγ-RNA signature scores in both the nivolumab and nivolumab+ipilimumab treatment arms (figure 4). Associations between biomarkers and RFS were similar across individual and pooled treatment arms, with overlapping CIs. Associations between biomarkers and RFS in each treatment arm remained apparent across tumor cell PD-L1 expression cutoffs of 1%, 5%, and 10%, as well as low, medium, and high tertiles of CD8+ T cell infiltration, TMB, and IFNγ-RNA signature score (online supplemental figure S4).

Association of gene signatures and baseline serum factors with RFS in the nivolumab versus nivolumab+ipilimumab treatment arms

Associations between RFS and tumor gene expression were evaluated in the nivolumab and nivolumab+ipilimumab treatment arms and by primary versus metastatic biopsy site using 50 hallmark[27] and 29 TME functional gene sets identified by Bagaev *et al*[26] to interrogate cellular processes involved in the TME biology, including those interrogating tumor intrinsic, immune, and stromal components (online supplemental figure S5A,B). Enriched expression of hallmark *Myc*-associated genes, as well as gene sets associated with intrinsic tumor gene expression and reduced antitumor immunity (eg, Hallmark Oxidative Phosphorylation) was associated with poor RFS in both treatment arms. The trend was more prominent in the nivolumab arm than in the nivolumab+ipilimumab arm—suggesting, as previously reported,[24 28] that the addition of ipilimumab to nivolumab therapy can enhance responses in patients with lower levels of antitumor immunity.

T-cell and immune-related gene expression signatures were associated with prolonged RFS in both the nivolumab and nivolumab+ipilimumab treatment arms, including both type I-IFN and IFNγ responses, as well as antigen presentation pathway gene expression. Type I-IFN and IFNγ-response gene set enrichment was more highly associated with improved RFS in the nivolumab arm than in the nivolumab+ipilimumab arm, once again showing a greater requirement for baseline tumor inflammation for the efficacy of nivolumab monotherapy relative to the combination with ipilimumab.

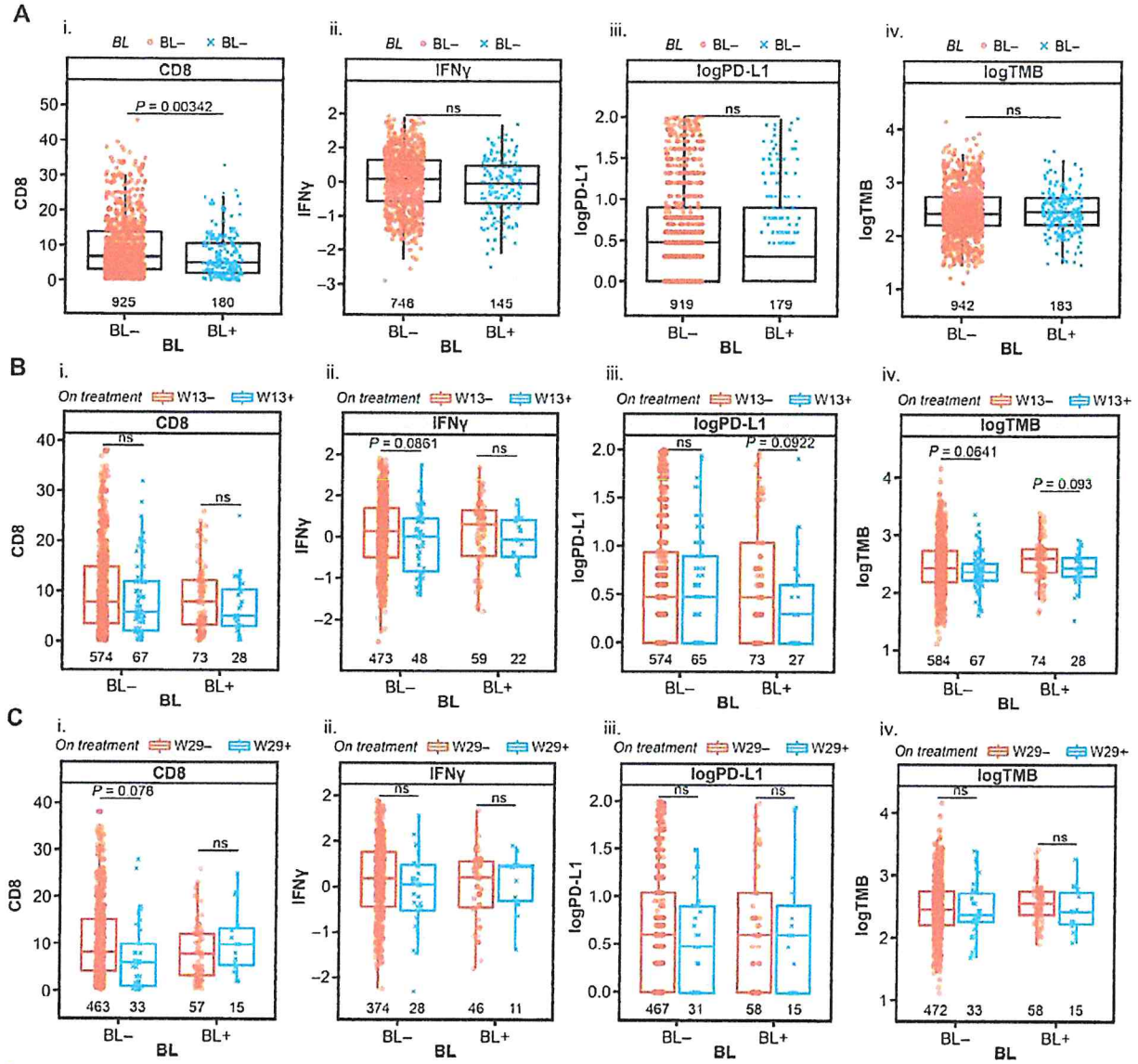

Figure 3 Association of ctDNA status at (A) baseline, (B) baseline and week 13, and (C) baseline and week 29 with baseline; (i) CD8+ T cell infiltration, (ii) IFNγ-RNA signature score, (iii) tumor cell PD-L1 expression, and (iv) TMB. Nominal p values were estimated by the Wilcoxon test, not adjusted for multiplicity. Comparisons that were non-significant and not trending towards significance (p>0.1) were labeled as ns. BL, baseline; ctDNA, circulating tumor DNA; IFNγ, interferon gamma; ns, not significant; PD-L1, programmed death ligand 1; TMB, tumor mutational burden; W, week.

In general, high expression of broad immune gene signatures was associated with longer RFS in both treatment arms, and higher expression of tumor proliferative gene signatures was associated with shorter RFS. When specifically looking at interactions or differences between treatments, the nivolumab+ipilimumab treatment arm showed longer RFS in the presence of higher expression of the tumor proliferation gene expression. The nivolumab+ipilimumab treatment arm had better responses than the nivolumab treatment arm, with a high level of ultraviolet response genes, mammalian target of rapamycin/Akt signaling, fatty acid metabolism, unfolded protein response, and protein secretion. The nivolumab treatment arm showed better responses in cases with high IFNα and IFNγ, and B cells expressing major histocompatibility complex-I and II. With respect to the activity of nivolumab in biopsies from primary versus metastatic-site tumors, gene expression was largely similar in the impact on RFS with a greater impact of tumor proliferation gene expression on shorter RFS in metastatic biopsies than primary biopsies. The impact of tumor proliferative gene expression was greater in primary sites than in metastatic sites, suggesting gene signatures have a marked impact on influencing outcomes of nivolumab.

Baseline levels of 49 serum factors were also investigated to identify factors differentially associated with RFS. A total of 33 serum factors had quantifiable data for evaluation, shown in online supplemental table S4. Higher interleukin (IL)-8 and serum β2 microglobulin (B2M) were significantly associated with worse RFS in the nivolumab arm. Higher serum B2M was also significantly associated with worse RFS in the nivolumab+ipilimumab arm (online

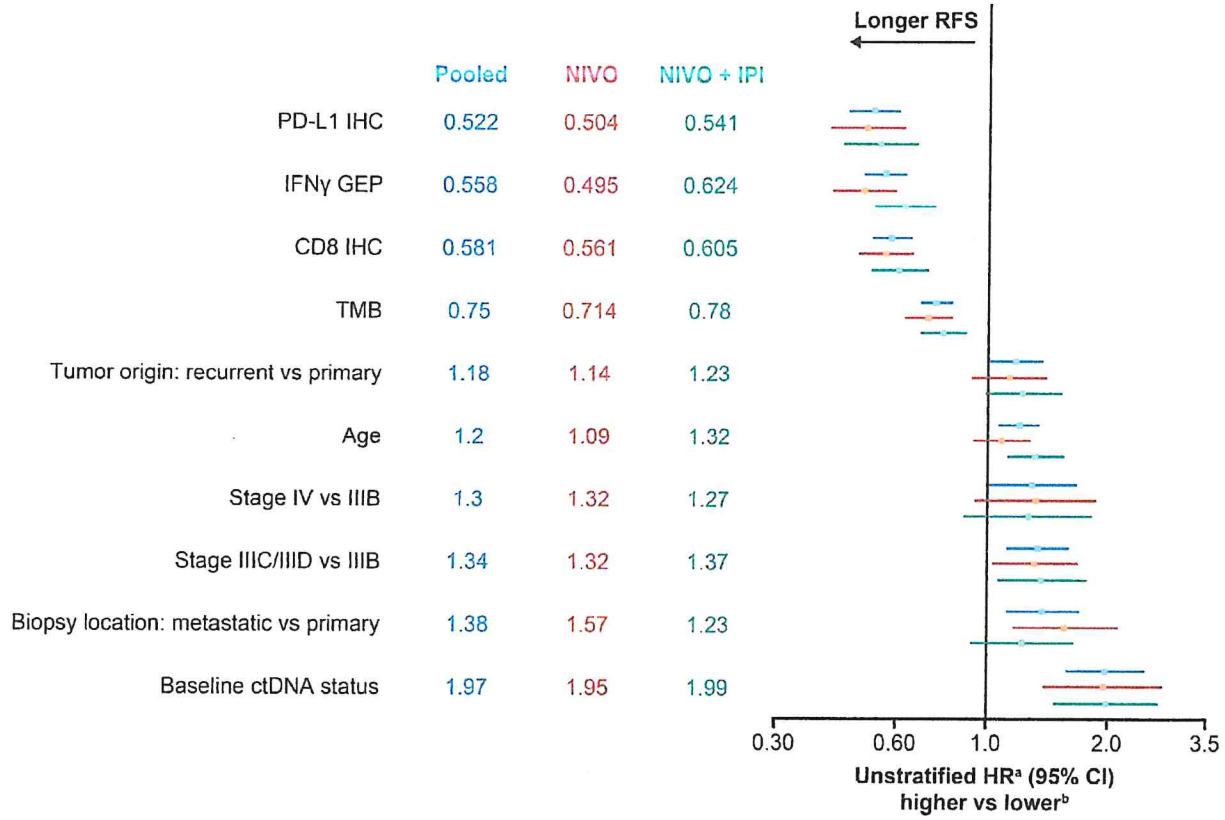

Figure 4 Forest plot showing associations with prolonged RFS for biomarkers, patient and tumor characteristics in the nivolumab and nivolumab+ipilimumab, and combined treatment arms. [a]HR is a higher level of biomarker over lower level of biomarker from the Cox proportional hazards model, which comprises three covariates: the treatment arm, the continuous biomarker, and their interaction. [b]Higher versus lower refers to evaluating biomarkers as continuous variables; higher level of the biomarker is the 75th percentile value of the biomarker in the overall population, and lower level of the biomarker is the 25th percentile value of the biomarker in the overall population. ctDNA, circulating tumor DNA; GEP, gene expression profile; IFNγ, interferon gamma; IHC, immunohistochemistry; IPI, ipilimumab; NIVO, nivolumab; PD-L1, programmed death ligand 1; RFS, recurrence-free survival; TMB, tumor mutational burden.

supplemental figures S6 and S7). A higher absolute neutrophil count was associated with poorer RFS overall.

Association of RFS with combined I-O biomarkers and ctDNA status

To determine the potential utility of tumor biomarkers combined with baseline ctDNA in predicting recurrence, a multivariable analysis was performed. Evaluation of associations between RFS with baseline ctDNA status, TMB, and IFNγ-RNA signature score, alone and in combination, found that the combination of all three markers offered improved predictive value for RFS versus individual biomarkers (figure 5A). Stratification of patients by combined biomarker status showed that the greatest RFS benefit was observed in patients who were ctDNA(−) and had high IFNγ-RNA signature score and high TMB at baseline. Patients who were ctDNA(−) with low TMB and low IFNγ-RNA signature score had worse RFS (24-month RFS probability, 48.6%; 95% CI, 42.9% to 55.1%) than patients who were ctDNA(−) with high TMB and high IFNγ-RNA signature score (24-month RFS probability, 86.5%; 95% CI, 81.2% to 92.1%), suggesting that TMB and IFNγ-RNA signature score may help to identify patients in the ctDNA(−) group who are more likely to recur. RFS did not differ between the nivolumab and nivolumab+ipilimumab treatment arms across patient subgroups stratified by combined biomarker status (data not shown).

Time-dependent analyses were performed to further explore the relationships between the risk of recurrence and baseline ctDNA status or tumor factors in the pooled ctDNA cohort (figure 5B). The specificity and sensitivity of baseline ctDNA to differentiate patients with and without recurrence varied over time (figure 5Bi), with baseline ctDNA status having greater predictive value for early recurrence (≤3 months) than for recurrence at later time points. This finding was also apparent on plotting area under the curve over time for baseline ctDNA status (figure 5Bii). Despite being assessed only at baseline for the resected tumor, other baseline tumor biomarkers were associated with relatively stable prediction of recurrence across the time points, although the predictive value of each biomarker was variable, with IFNγ-RNA signature score having the highest predictive value and PD-L1 status the lowest predictive value (figure 5Biii). Combinations of biomarkers showed greater predictive value than individual biomarkers, with the combination of ctDNA status,

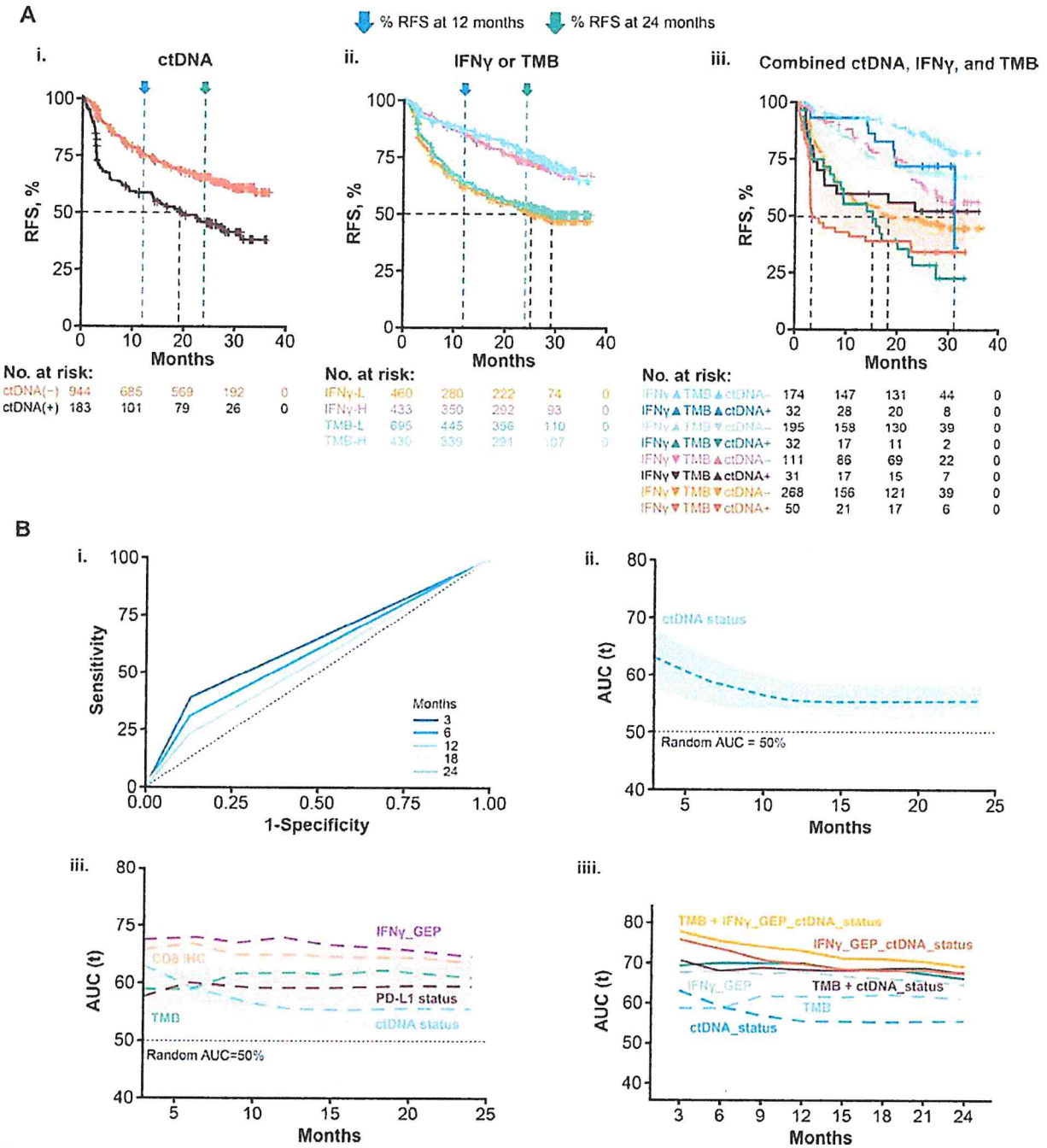

Figure 5 (A) Association of (i) ctDNA status, (ii) TMB and IFNγ-RNA signature score, and (iii) combined ctDNA status, TMB, and IFNγ-RNA signature score with RFS in the pooled nivolumab and nivolumab+ipilimumab treatment arms. (B) Time-dependent association of ctDNA status and tumor factors with recurrence in patients treated with nivolumab+ipilimumab. (i) Receiver operating characteristic curve for baseline ctDNA status for prediction of recurrence across varying time points. (ii) Area under the curve (AUC) for predictive value of ctDNA status over time. (iii) AUC for predictive value of individual baseline biomarkers, with each curve representing an individual biomarker and its predictive capacity for RFS with time. (iv) AUC for predictive value of baseline biomarker combinations. [a]IFNγ-RNA signature score:[25] samples were considered high if the signature score was ≥ median. [b]TMB stratified by tertiles for visualization purposes: TMB low (lower two tertiles), <350 mutations; TMB high (upper tertile), ≥350 mutations. ctDNA, circulating tumor DNA; GEP, gene expression profile; H, high; IFNγ-RNA signature, interferon gamma RNA signature; IHC, immunohistochemistry; L, low; PD-L1, programmed death ligand 1; RFS, recurrence-free survival; t, time; TMB, tumor mutational burden.

IFNγ-RNA signature score, and TMB status showing the highest predictive value for early recurrence and recurrence at later time points (figure 5Biv). The combination of ctDNA status with CD8 or tumor cell PD-L1 expression showed similar trends for improved predictive value over individual biomarkers (data not shown).

Further investigation of associations between RFS and biomarker combinations comprising TMB, tumor cell

PD-L1 expression, and IFNγ-RNA signature score found that high TMB trended with prolonged RFS in both high and low IFNγ-RNA signature score and tumor cell PD-L1 expression subgroups (online supplemental figure S8A,B). No notable differences between treatment arms were observed, with the exception of prolonged RFS with nivolumab+ipilimumab versus nivolumab in patients with low IFNγ-RNA signature score and high TMB.

Additional clinical and translational factors that significantly improved associations with RFS when combined with the baseline ctDNA status were evaluated using analysis of variance, to determine whether the addition of these factors improved the model fitness over ctDNA alone (online supplemental table S5). In addition to TMB and tumor CD8+ T cell infiltration, several clinical factors including tumor ulceration with lymph node involvement, Breslow thickness, and the number of lymph nodes involved were associated with improvements in predicting RFS. Other factors, including *BRAF*^{V600} status, treatment arm, resected tumor origin (primary or recurrent), metastatic stage, and American Joint Committee on Cancer disease stage did not enhance the predictive power of baseline ctDNA status for RFS.

DISCUSSION

A major strength of our study is that, to our knowledge, it represents the largest assessment of ctDNA and ctDNA correlations with other biomarkers in a prospective clinical trial of 1,844 patients receiving adjuvant I-O therapy following melanoma resection. Moreover, we used a patient-specific, tumor-informed approach for ctDNA detection, followed by parallel analyses of other tumor and peripheral biomarkers, and finally, combined analyses of ctDNA and tumor biomarkers.

ctDNA positivity at baseline was seen in 16.2% of patients and was associated with a higher risk of recurrence than ctDNA negativity, with modest sensitivity but high specificity, consistent with trends previously reported in melanoma using *BRAF*-targeted and *NRAS*-targeted analyses in 150 patients.[17]

Longitudinal analysis of ctDNA status (evaluated at week 13 and 29) enhanced the predictive value over baseline ctDNA status alone. Patients who zero converted had the lowest risk of recurrence; conversely, those who were persistently positive had the highest risk of recurrence. Importantly, change in ctDNA status between baseline and on-treatment assessments appeared to be associated with differences in risk of recurrence. Based on these results, longitudinal ctDNA status may indicate a patient's response to therapy and could be useful for guiding treatment decisions.

ctDNA has been used in other solid tumors to guide the use of adjuvant therapy, including in colorectal cancer and urothelial carcinoma.[29 30] The association between ctDNA status and survival in patients with melanoma has been previously reported.[18 20 31] Tan *et al* and Eroglu *et al* found that in the adjuvant setting, ctDNA status post resection identifies patients at risk of relapse.[18 32] Tan *et al* assessed *BRAF* and *NRAS* mutant ctDNA in a cohort of 99 patients post resection, 68 of whom had baseline assessments.[18] As seen in our studies, Tan *et al* found an increased rate of ctDNA positivity at baseline in patients with later-stage disease.[18] Eroglu *et al* used a tumor-informed, personalized approach similar to this study and found 5/29 patients (17%) with stage III disease at baseline with positive ctDNA post resection (similar to the 16.2% seen in our study). Similar to the results in our study, Eroglu *et al* found baseline ctDNA(+) patients showed an increase in distant recurrences during adjuvant treatment with immunotherapy.[32]

Although the prevalence of baseline, postresection ctDNA positivity in our study was not high (16.2%), the ctDNA-evaluable population was sufficiently large to allow informative statistical analysis of the ctDNA(+) subgroup. The prevalence of ctDNA positivity was comparable with other studies in the adjuvant melanoma setting, where the reported prevalence of ctDNA positivity post resection is 17–37%.[18 20 32]

The use of longitudinal ctDNA testing to monitor treatment response over time has also been reported in patients with advanced melanoma treated with immune checkpoint inhibitors, as well as *BRAF* inhibitors.[32–35] While only two on-treatment time points were assessed in our longitudinal analysis, significant changes in RFS outcomes were found, including in patients who shifted from ctDNA(−) to ctDNA(+) and vice versa. These results suggest the effect of I-O therapy (and thus possible disease evolution) can be monitored peripherally in patients and may lead to ctDNA being used to guide treatment cessation, safe de-escalation or, if needed, intensification including adding other therapies in combination. Additional studies evaluating a larger number of on-treatment time points would further enhance the findings in our study for close monitoring of patients with a blood-based biomarker only.

Assessment of various biomarkers that are predictive of response to I-O therapy in advanced melanoma (including PD-L1 expression on tumor cells, tumor CD8+ T cell levels, TMB, and IFNγ-RNA signature score) identified associations with RFS, supporting the potential utility of these markers in the adjuvant melanoma setting.[14 24 36–39] In the CheckMate 238 study, we reported similar results to this report, identifying that a higher percentage of CD8+ cells, a higher percentage of tumor cells expressing PD-L1, higher TMB, and higher IFNγ-RNA signature scores were associated with longer RFS in patients with resected stage III/IV melanoma in patients treated with nivolumab or ipilimumab.[14] Those results are corroborated in this larger current study.

Studies have suggested that evaluating biomarkers in combination, rather than individually, may be more clinically useful for predicting survival, including overall survival, progression-free survival, and RFS.[14 24] This is in line with the results of our study, which showed that ctDNA, TMB, and IFNγ-RNA signature scores evaluated

together, as well as the combination of ctDNA status with CD8 or tumor cell PD-L1 expression, were more predictive of survival than ctDNA alone, with the greatest RFS benefit observed in patients who were ctDNA(−) and had high IFNγ-RNA signature score and high TMB at baseline. Furthermore, combinations of biomarkers can provide information on the underlying biology of the response to adjuvant I-O therapy in patients with melanoma. Results from this study suggest that patients with an elevated inflammatory immune response are likely to respond better to immune checkpoint inhibition, which can further enhance that antitumor response.

In addition, our study identified several gene signatures and cytokines associated with differential RFS benefit in patients with melanoma treated with adjuvant nivolumab versus nivolumab+ipilimumab.

Overexpression of the oncogene *Myc* has been proposed to mediate immunotherapy resistance in melanoma by inhibition of IFNγ response.[40 41] In our study, increased expression of *Myc*-associated genes was associated with poor RFS in both the nivolumab and nivolumab+ipilimumab treatment arms, while enhanced expression of T-cell-related and other broad immune-related gene sets was associated with improved RFS in both treatment arms, including multiple cell types and interferon pathways.

Increased risk of recurrence was also observed in patients with high serum IL-8 levels in the nivolumab arm and high serum B2M levels in the nivolumab+ipilimumab arm, respectively. While serum B2M levels are used clinically for the prognosis of hematologic malignancies, as higher serum B2M levels are often associated with a larger tumor burden and more aggressive disease,[42] we are unaware of an association between baseline serum B2M levels and solid tumor prognosis. It is well established that high baseline levels of IL-8 are associated with inferior immunotherapy treatment outcomes in solid tumors, including melanoma.[43] While it is known that IL-8 mediates protumorigenic neutrophil migration and influx into the tumor,[44] our gene expression analyses showed that higher levels of both myeloid-derived suppressor cells and myeloid-derived suppressor cell trafficking were associated with better RFS in both treatment arms. This may be indicative of generalized immune cell infiltration or proliferation in the tumor. Our findings may also be related to a disconnect between the tumor gene expression and peripheral IL-8 levels.

Patients with high immune-cell infiltration, particularly CD8+ T cells in the TME, have been reported to have the best clinical outcomes in melanoma.[45] In an exploratory analysis of clinical and translational factors in patients with advanced melanoma, expression of genes indicative of broad immune cell types and inflammation was found in patients who did not incur early progression and primary resistance on immunotherapy.[46] The current work extends these findings to the adjuvant setting where the resected tumor immune contexture predicts immunotherapy response despite tumor resection.

Time-dependent analysis of the association of ctDNA positivity alone and in combination with other I-O biomarkers found that baseline ctDNA status was more predictive of early recurrence (occurring within 3 months) than later recurrence (at 24 months), while other baseline biomarkers evaluated appeared to have relatively stable predictive value for prediction of recurrence risk over time. To our knowledge, this is the first time-dependent analysis of associations of a broad range of baseline tumor biomarkers with outcomes of immunotherapy. Furthermore, while associations of baseline tumor biomarkers with recurrence remained relatively stable over time, the predictive level of baseline ctDNA diminished over time. This may be because ctDNA is a marker of ongoing metastases or because the tumor biomarkers are reflective of the patient's systemic antitumor immunity.

The finding that baseline ctDNA positivity was most strongly associated with early recurrences (by week 13) is noteworthy. Owen *et al* reported that of their patients with melanoma who relapsed on adjuvant anti-PD-1 therapy, the majority relapsed within the first 4 months on treatment, highlighting the clinical relevance of early time points.[47] Having biomarkers to identify patients who may relapse early is of great importance. While tumor biomarkers have been shown to be associated with disease recurrence in the adjuvant setting,[14] the finding that this may also be the case for peripheral biomarkers such as ctDNA and serum factors brings us closer to being able to predict which patients are at high risk of recurrence.

One major limitation of our study is that while peripheral ctDNA longitudinal analyses show increased association with treatment outcomes, our tumor-based and cytokine analyses were only assessed at baseline. Tumor analyses on recurrence would provide valuable information regarding changes in the TME or TMB status that are associated with failed treatment. Another limitation is that ctDNA status prior to resection was not conducted; ctDNA levels and potential mutational burden prior to resection may portend the results of therapy post resection. Studies in the neoadjuvant setting[11] are an optimal setting to collect biomarker data before and after resection to refine biomarkers associated with therapy outcomes.

In conclusion, this is the largest analysis of ctDNA and tissue biomarkers for predicting RFS in patients with resected melanoma receiving adjuvant immunotherapy. Baseline ctDNA predicted recurrence, and prediction was enhanced when combined with longitudinal ctDNA analysis or with established tissue biomarkers at baseline. A risk prediction tool using baseline clinical factors as well as ctDNA and tissue biomarkers would help clinicians risk stratify patients and discuss the risks and benefits of adjuvant therapy.

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REFERENCES

1 Weber J, Mandala M, Del Vecchio M, *et al*. Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma. *N Engl J Med* 2017;377:1824–35.
2 Eggermont AMM, Chiarion-Sileni V, Grob J-J, *et al*. Prolonged Survival in Stage III Melanoma with Ipilimumab Adjuvant Therapy. *N Engl J Med* 2016;375:1845–55.
3 Eggermont AMM, Blank CU, Mandalà M, *et al*. Adjuvant pembrolizumab versus placebo in resected stage III melanoma (EORTC 1325-MG/KEYNOTE-054): distant metastasis-free survival results from a double-blind, randomised, controlled, phase 3 trial. *Lancet Oncol* 2021;22:643–54.
4 Ascierto PA, Del Vecchio M, Mandalá M, *et al*. Adjuvant nivolumab versus ipilimumab in resected stage IIIB-C and stage IV melanoma (CheckMate 238): 4-year results from a multicentre, double-blind, randomised, controlled, phase 3 trial. *Lancet Oncol* 2020;21:1465–77.
5 Ascierto PA, Del Vecchio M, Mackiewicz A, *et al*. Overall survival at 5 years of follow-up in a phase III trial comparing ipilimumab 10 mg/kg with 3 mg/kg in patients with advanced melanoma. *J Immunother Cancer* 2020;8:e000391.
6 Larkin J, Chiarion-Sileni V, Gonzalez R, *et al*. Five-Year Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med* 2019;381:1535–46.
7 Larkin J, Chiarion-Sileni V, Gonzalez R, *et al*. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* 2015;373:23–34.
8 Khushalani NI, Vassallo M, Goldberg JD, *et al*. Phase II clinical and immune correlate study of adjuvant nivolumab plus ipilimumab for high-risk resected melanoma. *J Immunother Cancer* 2022;10:e005684.
9 Livingstone E, Zimmer L, Hassel JC, *et al*. Adjuvant nivolumab plus ipilimumab or nivolumab alone versus placebo in patients with resected stage IV melanoma with no evidence of disease (IMMUNED): final results of a randomised, double-blind, phase 2 trial. *Lancet* 2022;400:1117–29.
10 Wolchok JD, Chiarion-Sileni V, Gonzalez R, *et al*. Long-Term Outcomes With Nivolumab Plus Ipilimumab or Nivolumab Alone Versus Ipilimumab in Patients With Advanced Melanoma. *JCO* 2022;40:127–37.
11 Blank CU, Lucas MW, Scolyer RA, *et al*. Neoadjuvant Nivolumab and Ipilimumab in Resectable Stage III Melanoma. *N Engl J Med* 2024;391:1696–708.
12 Li H, van der Merwe PA, Sivakumar S. Biomarkers of response to PD-1 pathway blockade. *Br J Cancer* 2022;126:1663–75.
13 Zimmer L, Livingstone E, Hassel JC, *et al*. Adjuvant nivolumab plus ipilimumab or nivolumab monotherapy versus placebo in patients with resected stage IV melanoma with no evidence of disease (IMMUNED): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2020;395:1558–68.
14 Larkin J, Del Vecchio M, Mandalá M, *et al*. Adjuvant Nivolumab versus Ipilimumab in Resected Stage III/IV Melanoma: 5-Year Efficacy and Biomarker Results from CheckMate 238. *Clin Cancer Res* 2023;29:3352–61.
15 Long GV, Hauschild A, Santinami M, *et al*. Final Results for Adjuvant Dabrafenib plus Trametinib in Stage III Melanoma. *N Engl J Med* 2024;391:1709–20.
16 Dummer R, Brase JC, Garrett J, *et al*. Adjuvant dabrafenib plus trametinib versus placebo in patients with resected, $BRAF^{V600}$-mutant, stage III melanoma (COMBI-AD): exploratory biomarker analyses from a randomised, phase 3 trial. *Lancet Oncol* 2020;21:358–72.
17 Lee RJ, Gremel G, Marshall A, *et al*. Circulating tumor DNA predicts survival in patients with resected high-risk stage II/III melanoma. *Ann Oncol* 2018;29:490–6.
18 Tan L, Sandhu S, Lee RJ, *et al*. Prediction and monitoring of relapse in stage III melanoma using circulating tumor DNA. *Ann Oncol* 2019;30:804–14.
19 Geoffrois L, Harlé A, Sahki N, *et al*. Personalized follow-up of circulating DNA in resected stage III/IV melanoma: PERCIMEL multicentric prospective study protocol. *BMC Cancer* 2023;23:554.
20 Lee JH, Saw RP, Thompson JF, *et al*. Pre-operative ctDNA predicts survival in high-risk stage III cutaneous melanoma patients. *Ann Oncol* 2019;30:815–22.
21 Chan WY, Lee JH, Stewart A, *et al*. Circulating tumour DNA dynamics predict recurrence in stage III melanoma patients receiving neoadjuvant immunotherapy. *J Exp Clin Cancer Res* 2024;43:238.
22 Weber JS, Schadendorf D, Del Vecchio M, *et al*. Adjuvant Therapy of Nivolumab Combined With Ipilimumab Versus Nivolumab Alone in Patients With Resected Stage IIIB-D or Stage IV Melanoma (CheckMate 915). *J Clin Oncol* 2023;41:517–27.
23 Zhao J, Reuther J, Scozzaro K, *et al*. Personalized Cancer Monitoring Assay for the Detection of ctDNA in Patients with Solid Tumors. *Mol Diagn Ther* 2023;27:753–68.
24 Hodi FS, Wolchok JD, Schadendorf D, *et al*. TMB and Inflammatory Gene Expression Associated with Clinical Outcomes following Immunotherapy in Advanced Melanoma. *Cancer Immunol Res* 2021;9:1202–13.
25 Ayers M, Lunceford J, Nebozhyn M, *et al*. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest* 2017;127:2930–40.
26 Bagaev A, Kotlov N, Nomie K, *et al*. Conserved pan-cancer microenvironment subtypes predict response to immunotherapy. *Cancer Cell* 2021;39:845–65.
27 Liberzon A, Birger C, Thorvaldsdóttir H, *et al*. The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* 2015;1:417–25.
28 Gide TN, Quek C, Menzies AM, *et al*. Distinct Immune Cell Populations Define Response to Anti-PD-1 Monotherapy and Anti-PD-1/Anti-CTLA-4 Combined Therapy. *Cancer Cell* 2019;35:238–55.
29 Tie J, Cohen JD, Lahouel K, *et al*. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. *N Engl J Med* 2022;386:2261–72.
30 Powles T, Assaf ZJ, Davarpanah N, *et al*. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. *Nature* 2021;595:432–7.
31 Wan JCM, Heider K, Gale D, *et al*. ctDNA monitoring using patient-specific sequencing and integration of variant reads. *Sci Transl Med* 2020;12:eaaz8084.
32 Eroglu Z, Krinshpun S, Kalashnikova E, *et al*. Circulating tumor DNA-based molecular residual disease detection for treatment monitoring in advanced melanoma patients. *Cancer* 2023;129:1723–34.
33 Lee JH, Menzies AM, Carlino MS, *et al*. Longitudinal Monitoring of ctDNA in Patients with Melanoma and Brain Metastases Treated with Immune Checkpoint Inhibitors. *Clin Cancer Res* 2020;26:4064–71.
34 Syeda MM, Wiggins JM, Corless BC, *et al*. Circulating tumour DNA in patients with advanced melanoma treated with dabrafenib or dabrafenib plus trametinib: a clinical validation study. *Lancet Oncol* 2021;22:370–80.
35 Lee JH, Long GV, Boyd S, *et al*. Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma. *Ann Oncol* 2017;28:1130–6.
36 Eckardt J, Schroeder C, Martus P, *et al*. TMB and BRAF mutation status are independent predictive factors in high-risk melanoma patients with adjuvant anti-PD-1 therapy. *J Cancer Res Clin Oncol* 2023;149:833–40.
37 Cao J, Yang X, Chen S, *et al*. The predictive efficacy of tumor mutation burden in immunotherapy across multiple cancer types: A meta-analysis and bioinformatics analysis. *Transl Oncol* 2022;20:101375.
38 Reijers ILM, Rao D, Versluis JM, *et al*. IFN-γ signature enables selection of neoadjuvant treatment in patients with stage III melanoma. *J Exp Med* 2023;220:e20221952.
39 Long GV, Kirkwood JMM, Hoeller C, *et al*. Association of biomarkers (BMs) with efficacy of adjuvant nivolumab (NIVO) vs placebo (PBO) in patients with resected stage IIB/C melanoma (CA209-76K). *JCO* 2023;41:9504.
40 Li J, Dong T, Wu Z, *et al*. The effects of MYC on tumor immunity and immunotherapy. *Cell Death Discov* 2023;9:103.
41 Markovits E, Harush O, Baruch EN, *et al*. MYC Induces Immunotherapy and IFNγ Resistance Through Downregulation of JAK2. *Cancer Immunol Res* 2023;11:909–24.
42 Yoo C, Yoon DH, Suh C. Serum beta-2 microglobulin in malignant lymphomas: an old but powerful prognostic factor. *Blood Res* 2014;49:148–53.

43 Zou D, Song A, Yong W. Prognostic role of IL-8 in cancer patients treated with immune checkpoint inhibitors: a system review and meta-analysis. *Front Oncol* 2023;13:1176574.
44 Fousek K, Horn LA, Palena C. Interleukin-8: A chemokine at the intersection of cancer plasticity, angiogenesis, and immune suppression. *Pharmacol Ther* 2021;219:107692.
45 Salerno EP, Olson WC, McSkimming C, *et al*. T cells in the human metastatic melanoma microenvironment express site-specific homing receptors and retention integrins. *Int J Cancer* 2014;134:563–74.
46 Long G, Tang T, Apfel A, *et al*. 608 predicting primary resistance and exploring mechanisms in patients with advanced melanoma treated with immune checkpoint inhibitors (ICIs). *J Immunother Cancer* 2022;10.
47 Owen CN, Shoushtari AN, Chauhan D, *et al*. Management of early melanoma recurrence despite adjuvant anti-PD-1 antibody therapy☆. *Ann Oncol* 2020;31:1075–82.

ORIGINAL ARTICLE

Adjuvant Cemiplimab or Placebo in High-Risk Cutaneous Squamous-Cell Carcinoma

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*A list of the investigators in the C-POST trial is provided in the Supplementary Appendix, available at NEJM.org.

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CME

ABSTRACT

BACKGROUND

Patients who have cutaneous squamous-cell carcinoma with high-risk features are at risk for recurrence after definitive local therapy. The benefit of systemic adjuvant therapy options has not been well established in clinical trials.

METHODS

In a phase 3, randomized trial, we enrolled patients with local or regional cutaneous squamous-cell carcinoma, after surgical resection and postoperative radiotherapy, at high risk for recurrence owing to nodal features (extracapsular extension with largest node ≥20 mm in diameter or at least three involved nodes) or nonnodal features (in-transit metastases, T4 lesion [with bone invasion], perineural invasion, or locally recurrent tumor with ≥1 additional risk feature). Patients were assigned in a 1:1 ratio to receive adjuvant cemiplimab (350 mg) or placebo, administered intravenously every 3 weeks for 12 weeks, followed by a dose increase to 700 mg administered every 6 weeks for up to 36 weeks (≤48 weeks total). The primary end point was disease-free survival. Secondary end points included freedom from locoregional recurrence, freedom from distant recurrence, and safety.

RESULTS

A total of 415 patients were assigned to cemiplimab (209) or placebo (206). The median follow-up was 24 months. Cemiplimab was superior to placebo with respect to disease-free survival (24 vs. 65 events; hazard ratio for disease recurrence or death, 0.32; 95% confidence interval [CI], 0.20 to 0.51; P<0.001). The estimated 24-month disease-free survival was 87.1% (95% CI, 80.3 to 91.6) with cemiplimab and 64.1% (95% CI, 55.9 to 71.1) with placebo. Cemiplimab led to lower risks of locoregional recurrence (9 events, vs. 40 with placebo; hazard ratio, 0.20; 95% CI, 0.09 to 0.40) and distant recurrence (10 vs. 26 events; hazard ratio, 0.35; 95% CI, 0.17 to 0.72). Adverse events of grade 3 or higher occurred in 23.9% of the patients who received cemiplimab and in 14.2% of those who received placebo; discontinuation due to adverse events occurred in 9.8% and 1.5%, respectively.

CONCLUSIONS

Adjuvant cemiplimab therapy led to longer disease-free survival than placebo among patients at high risk for recurrence of cutaneous squamous-cell carcinoma. (Funded by Regeneron Pharmaceuticals and Sanofi; C-POST ClinicalTrials.gov number, NCT03969004.)

Cutaneous squamous-cell carcinoma is the second most common skin cancer, with an estimated annual incidence of 2.4 million cases worldwide.[1] Surgery with curative intent is the centerpiece of the clinical management of cutaneous squamous-cell carcinoma, with cure in approximately 95% of patients.[2-5] However, a subset of patients with cutaneous squamous-cell carcinoma have disease recurrence, either locoregional or distant, after undergoing surgery and receiving adjuvant radiotherapy.[6] A randomized, phase 3 trial (Postoperative Skin Trial/Trans-Tasman Radiation Oncology Group [POST/TROG] 05.01) showed no additional benefit of carboplatin administered concurrently with adjuvant radiotherapy, as compared with radiotherapy alone, in patients at elevated risk for recurrence of cutaneous squamous-cell carcinoma.[6] However, that trial helped to identify patient subpopulations at the highest risk for recurrence.[6,7]

Cemiplimab, a programmed death 1 (PD-1)–targeting antibody, is approved for the treatment of locally advanced (i.e., not suitable for resection) or metastatic cutaneous squamous-cell carcinoma, with a response occurring in 47% of patients and an estimated median duration of response of 41 months (range, 2 to 55).[8,9] The C-POST trial is a phase 3, randomized trial comparing adjuvant cemiplimab with placebo in patients at high risk for recurrence of cutaneous squamous-cell carcinoma after surgery and postoperative radiotherapy. We report here the results of the primary analysis, which was conducted after more than approximately half the events expected for the final analysis of disease-free survival had occurred.

METHODS

PATIENTS

We recruited patients from 107 sites across 16 countries. Eligible patients were 18 years of age or older with local or regional cutaneous squamous-cell carcinoma and had completed both curative-intent surgery, with macroscopic gross resection of all disease, and postoperative radiotherapy (or concurrent chemoradiotherapy) at a biologically equivalent dose of at least 50 Gy within 2 to 10 weeks before randomization. Eligible patients had high-risk nodal or nonnodal features (or both) (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). High-risk nodal disease was defined as extracapsular extension with at least one node measuring at least 20 mm in diameter or as at least three involved nodes regardless of extracapsular extension. High-risk nonnodal disease was defined as any of the following: in-transit metastases, radiologic or clinical evidence of perineural invasion of named nerves, T4 primary tumor (with bone invasion), or local recurrence with at least one other adverse feature (nodal stage ≥N2b, ≥T3 lesion [diameter, >4.0 cm], or poorly differentiated histologic characteristics with recurrent lesion measuring ≥20 mm in diameter).

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Patients were excluded if they had concurrent cancer (other than localized cutaneous squamous-cell carcinoma and certain low-risk diagnoses that were permitted according to the protocol), had received a solid-organ or stem-cell transplant previously, had clinically significant autoimmune disease, or had received any previous immunotherapy for cutaneous squamous-cell carcinoma. The full list of inclusion and exclusion criteria is provided in the protocol, available at NEJM.org.

TRIAL DESIGN AND TREATMENT

C-POST is an ongoing, international, randomized, phase 3 trial (Fig. S2). In part 1 of the trial (double-blind design; hypothesis-testing portion), patients were randomly assigned in a 1:1 ratio to receive either cemiplimab or placebo. In the original protocol, the regimen involved administration "every 3 weeks only," with cemiplimab at a dose of 350 mg or placebo administered intravenously every 3 weeks. In protocol amendment 2 (June 29, 2021), the regimen was revised to "start at a frequency of every 3 weeks, with a switch to every 6 weeks." Under this regimen, cemiplimab at a dose of 350 mg was administered intravenously every 3 weeks for 12 weeks, followed by cemiplimab at a dose of 700 mg administered intravenously every 6 weeks for an additional 36 weeks, or placebo was administered intravenously every 3 weeks for 12 weeks, followed by placebo administered intravenously every 6 weeks for an additional 36 weeks. The planned duration for the trial regimen was up to 48 weeks or until disease recurrence, the occurrence of unacceptable toxic effects, or withdrawal of consent, whichever was earlier. In part 2 of the trial (unblinded design), patients in the placebo group who had disease recurrence or those in the cemiplimab group who had disease recurrence that

occurred at least 3 months after the completion of part 1 had the option to receive subsequent cemiplimab.

Randomization was performed according to a central randomization scheme provided by an interactive Web-response system. Randomization was stratified according to tumor location (head and neck vs. non–head and neck), geographic region (North America vs. Australia or New Zealand vs. the rest of the world), high-risk category (nodal vs. nonnodal), Eastern Cooperative Oncology Group performance-status score (0 vs. 1; on a scale from 0 to 5, with higher scores indicating greater disability), and history of chronic lymphocytic leukemia (yes vs. no).

TRIAL OVERSIGHT

The trial was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines of the International Council for Harmonisation, and all applicable regulatory requirements. The protocol was approved by the relevant institutional review boards or ethics committees at each site. All the patients provided written informed consent. An independent data monitoring committee evaluated data approximately every 6 months and provided oversight of the trial.

The trial was sponsored by Regeneron Pharmaceuticals and Sanofi and was designed by employees of Regeneron Pharmaceuticals in collaboration with the Trans-Tasman Radiation Oncology Group, with the first author as the lead investigator. Data were collected by the trial investigators, analyzed by statisticians employed by Regeneron Pharmaceuticals, and interpreted by the authors. Medical writing and editorial assistance with an earlier version of the manuscript was provided, in accordance with Good Publication Practice Guidelines, by a medical writer who was employed by Regeneron Pharmaceuticals. Sanofi approved the trial design but was not involved in data collection or analysis, the preparation of the manuscript, or the decision to submit the manuscript for publication. The authors were responsible for all content and editorial decisions. The authors vouch for the completeness and accuracy of the data and for the fidelity of the trial to the protocol.

END POINTS AND ASSESSMENTS

The primary end point was disease-free survival, defined as the time from randomization to the first documented disease recurrence (locoregional or distant) or death due to any cause. Secondary end points included freedom from locoregional recurrence, freedom from distant recurrence, overall survival, second primary cutaneous squamous-cell carcinoma tumors, and safety. Second primary tumors were defined as new cutaneous squamous-cell carcinoma lesions arising on the skin that could be managed by local therapy as part of routine clinical practice. Protocol-specified exploratory end points included patterns of recurrence, patient-reported outcomes, and correlations between efficacy and tumor expression of programmed death ligand 1 (PD-L1). The trial end points are described in the protocol.

Patient-reported outcomes were evaluated with multiple instruments, including the European Organization for Research and Treatment of Cancer Quality-of-Life Questionnaire–Core 30 (EORTC QLQ-C30), a 30-item questionnaire consisting of five functional scales, nine symptom scales or items, and a global health status–quality of life scale. Scales are linearly transformed into scores ranging from 0 to 100, with higher scores for the functional and general health status–quality of life scales indicating better functional status and quality of life, respectively, and lower scores for the symptom scales indicating lower severity of symptoms. A change of at least 10 points in any scale is considered to be clinically meaningful.[10] Assessment of tumor PD-L1 expression (≥1% vs. <1% of tumor cells expressing PD-L1) according to the tumor proportion score as determined by means of immunohistochemical testing was performed as previously described.[11] Additional details of the EORTC QLQ-C30 instrument and scoring and the PD-L1 expression analyses are provided in the Supplementary Methods section in the Supplementary Appendix.

During the treatment period, radiologic assessments were performed at screening and at the end of each 12-week cycle. In the follow-up period, clinical and radiologic assessments were performed every 4 months for the first 2 years and every 6 months thereafter.

Safety was monitored at each visit, with assessment of adverse events that occurred during the treatment period. Adverse events were graded according to the Common Terminology Criteria for Adverse Events, version 5.0, of the National Cancer Institute. Immune-related adverse events were classified according to a list defined by Re-

generon Pharmaceuticals. Adverse events of special interest were infusion-related reactions of grade 2 or higher and immune-related adverse events of grade 3 or higher. Causality was assessed by the investigator.

STATISTICAL ANALYSIS

We planned for a total enrollment of 412 patients. On the basis of literature review and analysis of the POST/TROG 05.01 trial, a 3-year disease-free survival of 55% in the placebo group, with a hazard ratio of 0.6 for the comparison of cemiplimab with placebo, was assumed (see the protocol). We calculated that 165 events of disease recurrence or death (disease-free survival analysis) with three interim analyses (at approximately 83, 107, and 132 events) would provide the trial with 90% power to detect a significant between-group difference in disease-free survival at a two-sided alpha of 0.05. Prespecified interim analyses used the Lan–DeMets O'Brien–Fleming spending function to control for the type I error. This report presents data from the first interim analysis of the fully enrolled trial (data-cutoff date, October 4, 2024). Because this analysis crossed the prespecified efficacy threshold for disease-free survival, it became the primary analysis.

Efficacy analyses were conducted according to the randomized assignment (intention-to-treat approach). For the primary efficacy analysis of disease-free survival, hypothesis testing between the two groups was performed with the use of a stratified log-rank test. Hypothesis testing was not performed for secondary end points. For time-to-event analyses, hazard ratios and 95% confidence intervals were estimated with the use of a stratified Cox regression model. The stratification factors for log-rank tests and Cox regression models were tumor location (head and neck vs. non–head and neck) and geographic region (North America vs. Australia or New Zealand vs. the rest of the world). Subgroup analyses of disease-free survival estimated the between-group treatment effect and nominal 95% confidence interval in prespecified subgroups. Safety analyses were based on whether the patient received cemiplimab or placebo and were conducted in all the patients who received any cemiplimab or placebo.

Prespecified analyses of patient-reported outcomes included descriptive analyses and overall changes from baseline across treatment cycles, which were analyzed with the use of a mixed-effects model for repeated measures. These analyses were conducted in patients who had a baseline score and at least one postbaseline score for the patient-reported outcome. All the data were analyzed with the use of SAS software, version 9.4 (SAS Institute).

RESULTS

PATIENTS

From June 2019 through August 2024, a total of 526 patients at high risk for recurrence of cutaneous squamous-cell carcinoma underwent screening, and 415 were randomly assigned to receive adjuvant therapy with cemiplimab (209 patients) or placebo (206) (Fig. S3). Among all the patients, the median age was 71 years (range, 33 to 95), 83.9% were men, and 82.7% had primary cutaneous squamous-cell carcinoma of the head and neck (Table 1 and Table S1). The high-risk nodal disease category included 242 patients (58.3%). The most common high-risk criterion was extracapsular extension in at least one node measuring at least 20 mm in diameter (in 48.4% of the patients). Overall, the demographic and disease characteristics of the patients at baseline were well balanced between the two trial groups and were generally representative of patients with high-risk cutaneous squamous-cell carcinoma (Table S2).

Among the 409 patients who underwent randomization and received at least one dose of cemiplimab or placebo, the median duration of exposure was 47.9 weeks (range, 3 to 52) in the cemiplimab group and 47.7 weeks (range, 3 to 51) in the placebo group. Overall, 44 of 205 patients receiving cemiplimab (21.5%) and 61 of 204 patients receiving placebo (29.9%) discontinued the regimen during part 1 of the trial. In the cemiplimab group, the most common reasons for discontinuation were adverse events (in 19 patients), disease relapse (in 10), and patient decision to withdraw (in 9); in the placebo group, the most common reasons were disease relapse (in 50) and patient decision to withdraw (in 5).

Overall, the median potential follow-up from randomization to the data-cutoff date was 24 months (range, 2 to 64). As of the data-cutoff date, 69 patients (16.9%; 36 in the cemiplimab group and 33 in the placebo group) were still receiving cemiplimab or placebo in part 1 of the trial.

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

Characteristic	Cemiplimab (N=209)	Placebo (N=206)
Age		
Median (range) — yr	71 (33–87)	70.5 (36–95)
≥65 yr — no. (%)	153 (73.2)	141 (68.4)
Male sex — no. (%)	174 (83.3)	174 (84.5)
Race — no. (%)†		
Asian	5 (2.4)	8 (3.9)
White	189 (90.4)	189 (91.7)
Other	1 (0.5)	1 (0.5)
Unknown or not reported	14 (6.7)	8 (3.9)
Geographic region — no. (%)		
North America	37 (17.7)	31 (15.0)
Australia or New Zealand	90 (43.1)	90 (43.7)
Rest of the world	82 (39.2)	85 (41.3)
ECOG performance-status score — no. (%)‡		
0	133 (63.6)	131 (63.6)
1	76 (36.4)	75 (36.4)
Anatomical region of resected high-risk tumor — no. (%)		
Head and neck	166 (79.4)	177 (85.9)
Non–head and neck	43 (20.6)	29 (14.1)
High-risk category — no. (%)§		
Nodal	125 (59.8)	117 (56.8)
Nonnodal	84 (40.2)	89 (43.2)
High-risk criteria — no. (%)¶		
Nodal disease with extracapsular extension and ≥1 lymph node ≥20 mm in diameter	105 (50.2)	96 (46.6)
Nodal disease with ≥3 nodes positive on surgical pathology report, regardless of extracapsular extension	33 (15.8)	37 (18.0)
In-transit metastases	20 (9.6)	21 (10.2)
T4 lesion	17 (8.1)	16 (7.8)
Perineural invasion	32 (15.3)	32 (15.5)
Recurrent high-risk cutaneous squamous-cell carcinoma with ≥1 additional feature	55 (26.3)	50 (24.3)
≥N2b disease associated with the recurrent lesion	17 (8.1)	13 (6.3)
≥T3 lesion	37 (17.7)	29 (14.1)
Poorly differentiated histologic findings and diameter of recurrent lesion ≥20 mm	16 (7.7)	13 (6.3)
PD-L1 tumor proportion score — no. (%)		
≥1%	155 (74.2)	154 (74.8)
<1%	42 (20.1)	43 (20.9)
Indeterminate	12 (5.7)	9 (4.4)

* Percentages may not total 100 because of rounding. PD-L1 denotes programmed death ligand 1.
† Race was reported by the patient.
‡ Eastern Cooperative Oncology Group (ECOG) performance-status scores are on a scale from 0 to 5, with higher scores indicating greater disability.
§ Patients with both nodal and nonnodal disease were classified as having nodal disease.
¶ The total for the high-risk criteria column is more than 100% because tumors could have more than one high-risk criterion. In the cemiplimab group, 13 patients had both nodal features (nodal disease with extracapsular extension and at least one lymph node measuring ≥20 mm in the greatest dimension, plus nodal disease with at least three positive nodes on the surgical pathology report, regardless of extracapsular extension) and 16 patients in the placebo group had both nodal features. A T4 lesion indicates disease with bone invasion, and a T3 lesion a tumor diameter of more than 4.0 cm. Perineural invasion was defined as clinical or radiologic involvement of named nerves.

EFFICACY

A significant improvement in disease-free survival was seen in the cemiplimab group as compared with the placebo group (24 vs. 65 events; hazard ratio for disease recurrence or death, 0.32; 95% confidence interval [CI], 0.20 to 0.51; P<0.001) (Fig. 1 and Table S3). The Kaplan–Meier curves for the analysis of disease-free survival separated early and remained so for the duration of follow-up. The estimated disease-free survival at 24 months was 87.1% (95% CI, 80.3 to 91.6) in the cemiplimab group and 64.1% (95% CI, 55.9 to 71.1) in the placebo group.

With regard to the 65 events of disease recurrence or death in the placebo group, 61 patients had disease recurrence and 4 died without disease recurrence; with regard to the 24 events in the cemiplimab group, 18 patients had disease recurrence and 6 died without disease recurrence. The disease-free survival benefit with cemiplimab in relevant subgroups is shown in Figure 2.

Cemiplimab treatment prolonged freedom from both locoregional and distant recurrences as compared with placebo (Fig. 3 and Table S4). The estimated percentage of patients free from locoregional recurrence at 24 months was 94.6% (95% CI, 89.1 to 97.3) in the cemiplimab group and 76.7% (95% CI, 69.1 to 82.6) in the placebo group. Locoregional recurrence occurred in 9 patients in the cemiplimab group and in 40 in the placebo group (hazard ratio, 0.20; 95% CI, 0.09 to 0.40). The estimated percentage of patients free from distant recurrence at 24 months was 94.3% (95% CI, 89.0 to 97.1) in the cemiplimab group and 83.8% (95% CI, 76.3 to 89.0) in the placebo group. Distant recurrence occurred in 10 patients in the cemiplimab group and in 26 in the placebo group (hazard ratio, 0.35; 95% CI, 0.17 to 0.72). Patterns of disease recurrence are provided in Table S5.

Exploratory analyses of disease-free survival according to the two dose regimens that were used in this trial appeared to favor cemiplimab over placebo (Table S6). Additional exploratory analyses showed that the disease-free survival benefit with cemiplimab as compared with placebo appeared to be maintained regardless of tumoral PD-L1 status (among 85 patients with a PD-L1 tumor proportion score of <1%: 8 vs. 16 events [hazard ratio, 0.32; 95% CI, 0.12 to 0.86]; among 309 patients with PD-L1 tumor proportion score of ≥1%: 14 vs. 45 events [hazard ratio, 0.28; 95% CI, 0.15 to 0.52]) (Fig. S4).

Among patients with recurrent disease, the most common subsequent intervention was cemiplimab (Table S7). Among 46 patients who

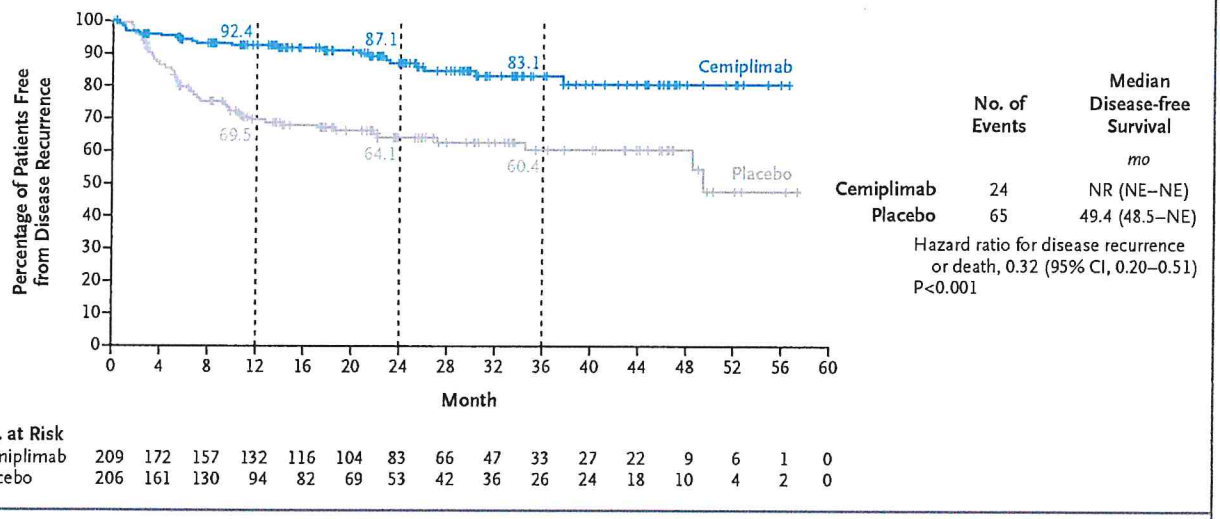

Figure 1. Disease-free Survival.

Analyses of disease-free survival were based on the Kaplan–Meier method, with stratification according to high-risk tumor (head and neck vs. non–head and neck) and geographic region (North America vs. Australia or New Zealand vs. the rest of the world). The threshold for significance was set to 0.00455 on the basis of the O'Brien–Fleming alpha spending function. The P value was based on a stratified proportional-hazards model. Second primary cutaneous squamous-cell carcinoma tumors were not included as events in the primary end-point analysis of disease-free survival. Tick marks indicate censored data. NE denotes could not be evaluated, and NR not reached.

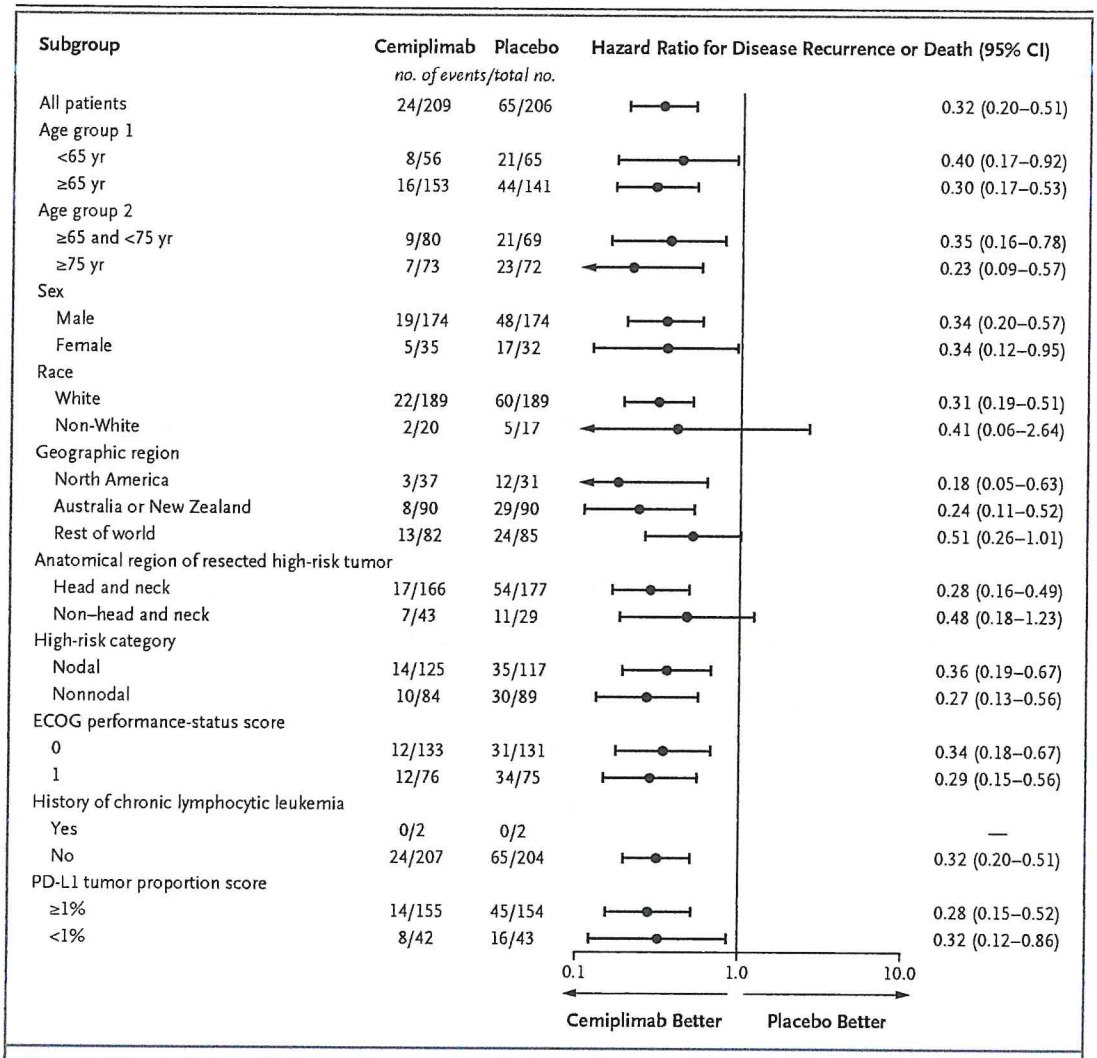

Figure 2. Disease-free Survival According to Prespecified Subgroups.

Race was reported by the patient. Eastern Cooperative Oncology Group (ECOG) performance-status scores are assessed on a scale from 0 to 5, with higher scores indicating greater disability. Arrows indicate that the confidence interval extends outside the graphed area. PD-L1 denotes programmed death ligand 1.

had been originally randomly assigned to the placebo group and received cemiplimab for recurrent disease in part 2 of the trial, 20 (43%) had an objective radiographic response (Table S8).

A total of 25 deaths had occurred as of the data-cutoff date: 12 deaths (4 in the cemiplimab group and 8 in the placebo group) were due to disease progression and 13 deaths (8 in the cemiplimab group and 5 in the placebo group) were due to other causes (Table S9). Overall survival at 2 years was 94.8% (95% CI, 89.6 to 97.4) in the cemiplimab group and 92.3% (95% CI, 86.5 to 95.7) in the placebo group. The hazard ratio for death was 0.86 (95% CI, 0.39 to 1.90), with 51 patients in the placebo group receiving cemiplimab after recurrence (Fig. S5). At a subsequent data-cutoff date of April 7, 2025, there were 33 deaths (15 in the cemiplimab group and 18 in the placebo group), with a hazard ratio of 0.78 (95% CI, 0.39 to 1.56).

SAFETY

Adverse events due to any cause during the treatment period occurred in 91.2% of the patients who received cemiplimab and in 89.2% of those who received placebo (Table 2 and Table S10). The most common adverse events with cemiplimab as compared with placebo were fatigue (in

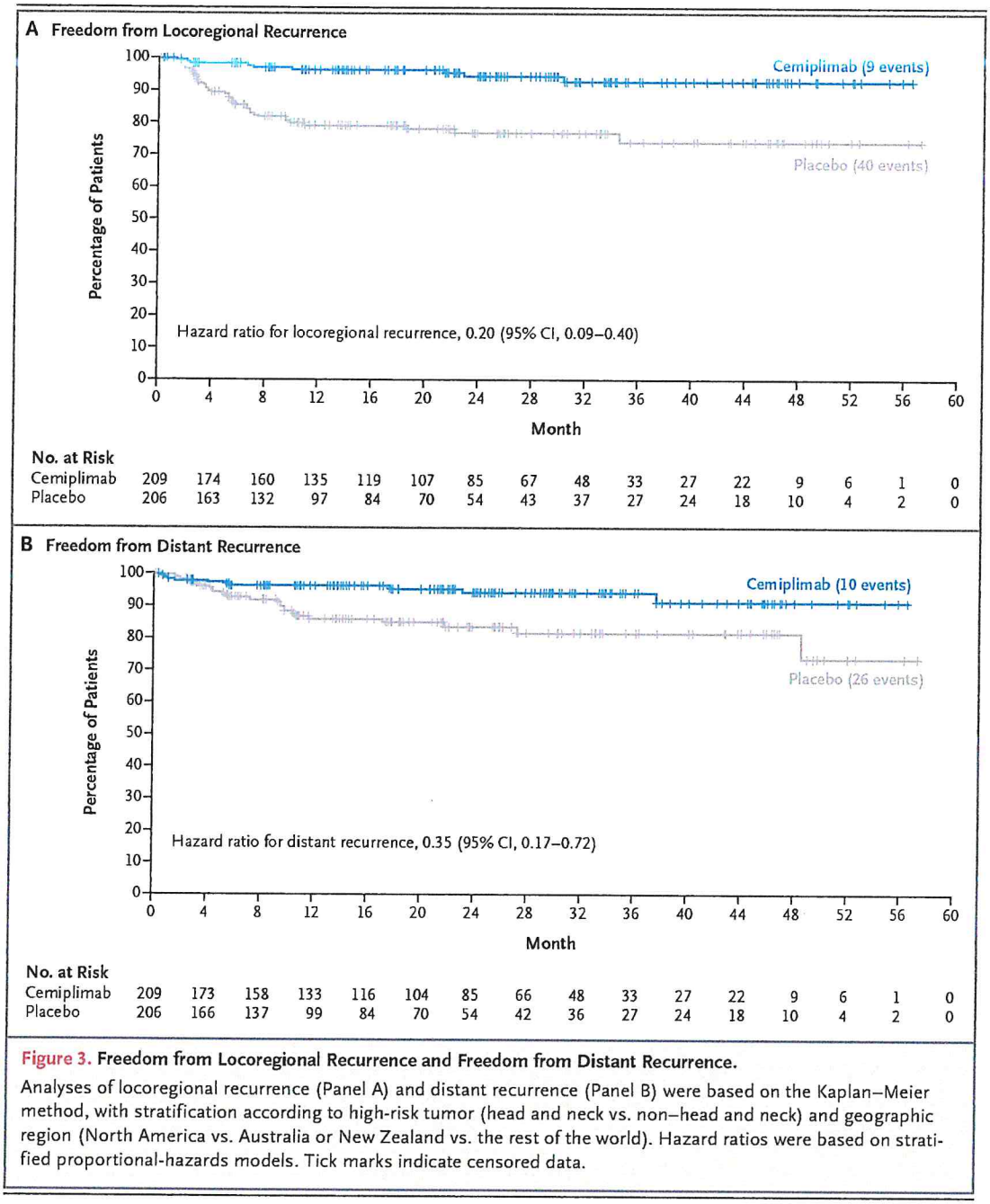

Figure 3. Freedom from Locoregional Recurrence and Freedom from Distant Recurrence.

Analyses of locoregional recurrence (Panel A) and distant recurrence (Panel B) were based on the Kaplan–Meier method, with stratification according to high-risk tumor (head and neck vs. non–head and neck) and geographic region (North America vs. Australia or New Zealand vs. the rest of the world). Hazard ratios were based on stratified proportional-hazards models. Tick marks indicate censored data.

22.0% vs. 21.6% of the patients), pruritus (in 16.1% vs. 12.3%), rash (in 16.1% vs. 8.8%), and diarrhea (in 15.6% vs. 18.6%). Adverse events of grade 3 or higher that were due to any cause occurred in 23.9% of the patients who received cemiplimab and in 14.2% of those who received placebo. Adverse events of grade 3 or higher that were considered by the investigator to be related to treatment occurred in 9.8% of patients in the cemiplimab group (Table S11). Adverse events, regardless of attribution, that led to death occurred in two patients in each group (Table 2). One death due to myositis in a patient receiving cemiplimab was considered by the investigator to be related to treatment.

Cemiplimab or placebo was discontinued owing to adverse events in 9.8% and 1.5% of the patients, respectively. Immune-related adverse

events occurred in 22.9% of the patients who received cemiplimab (with events of grade ≥3 in 7.3%) and in 6.4% of those who received placebo (with no events of grade ≥3). No new immune-related adverse events were observed.

PATIENT-REPORTED OUTCOMES

More than 88% of the patients in each group completed the EORTC QLQ-C30 at baseline and through all the cycles. The overall change from baseline in EORTC QLQ-C30 global health status–quality of life scores across all time points during the treatment period indicated no meaningful between-group difference (difference in the least-squares mean change, −0.94 points [95% CI, −3.65 to 1.77]; clinically meaningful change, ≥10 points) (Table S12 and Fig. S6). Maintenance of the global health status–quality of life scores appeared to be sustained after the treatment period.

DISCUSSION

In this primary analysis of the phase 3 C-POST trial, disease-free survival was significantly longer with adjuvant cemiplimab therapy than with placebo among patients at high risk for recurrence of cutaneous squamous-cell carcinoma after definitive local therapy. The risk of disease recurrence or death was 68% lower with cemiplimab than with placebo, with an estimated 24-month disease-free survival of 87% in the cemiplimab group and 64% in the placebo group.

Most recurrences were observed in the first year after surgical resection and the completion of adjuvant radiotherapy — findings that are consistent with the natural history of cutaneous squamous-cell carcinoma to recur rapidly.[6,12] The Kaplan–Meier curves for disease-free survival diverged early, with continued separation through-

Table 2. Adverse Events during Treatment Period, According to Grade.*

Event	Cemiplimab (N=205)		Placebo (N=204)	
	Any Grade	Grade ≥3	Any Grade	Grade ≥3
	number of patients with event (percent)			
Any adverse event	187 (91.2)	49 (23.9)	182 (89.2)	29 (14.2)
Serious adverse event	36 (17.6)	31 (15.1)	19 (9.3)	14 (6.9)
Adverse event leading to discontinuation of cemiplimab or placebo	20 (9.8)	16 (7.8)	3 (1.5)	2 (1.0)
Adverse event leading to death†	2 (1.0)	2 (1.0)	2 (1.0)	2 (1.0)
Adverse events in ≥10% of the patients in either group‡				
Fatigue	45 (22.0)	1 (0.5)	44 (21.6)	0
Pruritus	33 (16.1)	1 (0.5)	25 (12.3)	0
Rash	33 (16.1)	1 (0.5)	18 (8.8)	0
Diarrhea	32 (15.6)	3 (1.5)	38 (18.6)	0
Arthralgia	26 (12.7)	0	25 (12.3)	0
Hypothyroidism	24 (11.7)	1 (0.5)	6 (2.9)	0
Maculopapular rash	23 (11.2)	0	12 (5.9)	0
Bowen's disease	16 (7.8)	1 (0.5)	21 (10.3)	2 (1.0)

* Shown are adverse events that developed or worsened during the treatment period and any adverse events that were considered by the investigator to be related to cemiplimab or placebo that occurred during the posttreatment period but before part 2 of the trial (subsequent cemiplimab treatment).

† One death due to pneumonia was considered by the investigator to be unrelated to cemiplimab, and one death due to myositis was considered by the investigator to be related to cemiplimab. One death due to pneumonia and one death due to new primary malignant lung neoplasm were both considered by the investigator to be unrelated to placebo.

‡ Patients were counted only once according to the worst grade for multiple occurrences within a preferred term.

out the follow-up period, thus indicating a rapid and sustained clinical benefit with cemiplimab.

Analyses of patterns of recurrence showed that locoregional recurrences were more common than distant recurrences, a finding that was consistent with the results of the POST/TROG 05.01 trial.[6] Locoregional recurrences are important medical events for patients with cutaneous squamous-cell carcinoma because they are associated with considerable risks of disease and death.[13,14] In this trial, the risk of locoregional recurrence was 80% lower with adjuvant cemiplimab therapy than with placebo, and the risk of distant recurrence was 65% lower with cemiplimab than with placebo.

The safety profile of cemiplimab in the context of adjuvant therapy was consistent with the known safety profile of cemiplimab monotherapy in the context of advanced or metastatic disease. Discontinuation of the regimen due to adverse events occurred in 9.8% of the patients in the cemiplimab group. One death was considered by the investigator to be related to cemiplimab treatment. The global health status–quality of life score appeared to be maintained during treatment with adjuvant cemiplimab.

At the time of this primary analysis of disease-free survival, 25 deaths had been observed. A convincing benefit with regard to overall survival has not been observed, although follow-up in this trial is ongoing. Most patients who had recurrent disease were treated subsequently with cemiplimab, which suggests that disease recurrences in this trial were usually considered by the treating physicians to be advanced cutaneous squamous-cell carcinoma. The availability of effective systemic therapy for recurrent disease may have an effect on the magnitude of any overall survival benefit from adjuvant therapy, as has been observed in melanoma.[15,16] Individualized decision making is recommended with regard to whether to treat a patient with high-risk cutaneous squamous-cell carcinoma in the context of adjuvant therapy or to wait until disease recurrence occurs before the initiation of immunotherapy; the circumstances and preferences of the patient should be taken into consideration. Because anti–PD-1 therapy provides durable responses in less than 50% of patients in the context of advanced cutaneous squamous-cell carcinoma,[8,9,17,18] the ability of adjuvant cemiplimab to reduce the risk of recurrence of cutaneous squamous-cell carcinoma is clinically meaningful for patients at high risk for recurrence.

A limitation of the C-POST trial is that it was not designed to formally investigate differences in efficacy and safety between the two dose regimens in the trial. However, this trial provides randomized data regarding the standard administration regimen (every 3 weeks only) and a regimen with an extended administration interval (start at every 3 weeks, with a switch to every 6 weeks). The trial results indicated that both regimens prolonged disease-free survival and had similar safety profiles.

Findings from the POST/TROG 05.01 trial[6] were key determinants in defining the high-risk criteria and categories for the C-POST trial. The current trial was successful in defining a population of patients at high risk for recurrence of cutaneous squamous-cell carcinoma as evidenced by an estimated 3-year disease-free survival of approximately 60% in the placebo group, which closely matched the predicted disease-free survival that was based on data from the POST/TROG 05.01 trial. The results reported here will help to inform future updates to consensus staging criteria, as well as aid in the identification of patients in the clinic who are at the highest risk for disease recurrence.

Another phase 3 trial of adjuvant anti–PD-1 therapy in high-risk cutaneous squamous-cell carcinoma was the KEYNOTE-630 trial of adjuvant pembrolizumab (ClinicalTrials.gov number, NCT03833167). That trial was stopped for futility after a prespecified analysis, according to a press release from the sponsor.[19] Publication of the results of the KEYNOTE-630 trial is awaited and may provide information about why the trial did not show an improvement with pembrolizumab with regard to the primary end point.

In this trial, adjuvant cemiplimab therapy led to a large benefit, as compared with placebo, with regard to disease-free survival among patients at high risk for recurrence of cutaneous squamous-cell carcinoma.

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REFERENCES

1. Guo A, Liu X, Li H, Cheng W, Song Y, Raijmakers N. The global, regional, national burden of cutaneous squamous cell carcinoma (1990–2019) and predictions to 2035. Eur J Cancer Care (Engl) 2023; 2023(1) (https://onlinelibrary.wiley.com/doi/10.1155/2023/5484597).
2. Chong CY, Goh MS, Porceddu SV, Rischin D, Lim AM. The current treatment landscape of cutaneous squamous cell carcinoma. Am J Clin Dermatol 2023; 24:25-40.
3. National Comprehensive Cancer Network. NCCN guidelines — squamous cell skin cancer, version 2.2025. 2025 (https://www.nccn.org/professionals/physician_gls/pdf/squamous.pdf).
4. Stratigos AJ, Garbe C, Dessinioti C, et al. European consensus-based interdisciplinary guideline for invasive cutaneous squamous cell carcinoma. 2. Treatment — update 2023. Eur J Cancer 2023;193: 113252.
5. van Lee CB, Roorda BM, Wakkee M, et al. Recurrence rates of cutaneous squamous cell carcinoma of the head and neck after Mohs micrographic surgery vs. standard excision: a retrospective cohort study. Br J Dermatol 2019;181:338-43.
6. Porceddu SV, Bressel M, Poulsen MG, et al. Postoperative concurrent chemoradiotherapy versus postoperative radiotherapy in high-risk cutaneous squamous cell carcinoma of the head and neck: the randomized phase III TROG 05.01 trial. J Clin Oncol 2018;36:1275-83.
7. Connolly E, Bressel M, Liu HY, Rischin D, Porceddu S. Prognostic subgroups for disease-free survival in cutaneous squamous cell carcinoma of the head and neck: a secondary analysis of the TROG 05.01 trial. Proc ESTRO 2025;E25-1045 (https://user-swndwmf.cld.bz/ESTRO-2025-Abstract-Book/1676/). abstract.
8. Hughes BGM, Guminski A, Bowyer S,

et al. A phase 2 open-label study of cemiplimab in patients with advanced cutaneous squamous cell carcinoma (EMPOWER-CSCC-1): final long-term analysis of groups 1, 2, and 3, and primary analysis of fixed-dose treatment group 6. J Am Acad Dermatol 2025;92:68-77.

9. Migden MR, Rischin D, Schmults CD, et al. PD-1 blockade with cemiplimab in advanced cutaneous squamous-cell carcinoma. N Engl J Med 2018;379:341-51.

10. Osoba D, Rodrigues G, Myles J, Zee B, Pater J. Interpreting the significance of changes in health-related quality-of-life scores. J Clin Oncol 1998;16:139-44.

11. Tewari KS, Monk BJ, Vergote I, et al. Survival with cemiplimab in recurrent cervical cancer. N Engl J Med 2022;386:544-55.

12. Granger EE, Ran NA, Groover MK, et al. Most cutaneous squamous cell carcinoma recurrences occur in the first 3 years after diagnosis: a multicenter retrospective cohort study. J Am Acad Dermatol 2024;91:957-60.

13. Eigentler TK, Leiter U, Häfner H-M, Garbe C, Röcken M, Breuninger H. Survival of patients with cutaneous squamous cell carcinoma: results of a prospective cohort study. J Invest Dermatol 2017;137:2309-15.

14. Schmults CD, Karia PS, Carter JB, Han J, Qureshi AA. Factors predictive of recurrence and death from cutaneous squamous cell carcinoma: a 10-year, single-institution cohort study. JAMA Dermatol 2013;149:541-7.

15. Eggermont AM, Kicinski M, Blank CU, et al. Seven-year analysis of adjuvant pembrolizumab versus placebo in stage III melanoma in the EORTC1325 / KEYNOTE-054 trial. Eur J Cancer 2024;211:114327.

16. Long GV, Hauschild A, Santinami M, et al. Final results for adjuvant dabrafenib plus trametinib in stage III melanoma. N Engl J Med 2024;391:1709-20.

17. Hughes BGM, Munoz-Couselo E, Mortier L, et al. Pembrolizumab for locally advanced and recurrent/metastatic cutaneous squamous cell carcinoma (KEYNOTE-629 study): an open-label, nonrandomized, multicenter, phase II trial. Ann Oncol 2021;32:1276-85.

18. Migden MR, Khushalani NI, Chang ALS, et al. Cemiplimab in locally advanced cutaneous squamous cell carcinoma: results from an open-label, phase 2, single-arm trial. Lancet Oncol 2020;21:294-305.

19. Merck provides update on phase 3 KEYNOTE-867 and KEYNOTE-630 trials. August 29, 2024 (https://www.merck.com/news/merck-provides-update-on-phase-3-keynote-867-and-keynote-630-trials/).

RP1 Combined With Nivolumab in Advanced Anti–PD-1–Failed Melanoma (IGNYTE)

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ABSTRACT

PURPOSE Effective treatment options for melanoma after immune checkpoint blockade failure are limited. RP1 (vusolimogene oderparepvec) is a herpes simplex virus type 1–based oncolytic immunotherapy, here evaluated in combination with nivolumab in anti–PD-1–failed melanoma.

METHODS Patients had advanced melanoma that had confirmed progression on anti–PD-1 (≥8 weeks, last prior treatment). RP1 was administered intratumorally (≤8 doses, ≤10 mL/dose; additional doses allowed) with nivolumab (≤2 years). The objective response rate (ORR) was assessed by independent central review using Response Evaluation Criteria in Solid Tumors version 1.1.

RESULTS Of 140 patients enrolled, 48.6% had stage IVM1b/c/d disease, 65.7% had primary anti–PD-1 resistance, 56.4% were PD-L1 negative, and 46.4% received prior anti–PD-1 and anti–cytotoxic T-lymphocyte antigen-4 therapy (43.6% in combination and 2.9% sequentially). Confirmed ORR (95% CI) was 32.9% (95% CI, 25.2% to 41.3%; 15.0% complete response). Responses occurred with similar frequency, depth, duration, and kinetics for injected and noninjected, including visceral lesions. The median (95% CI) duration of response was 33.7 (95% CI, 14.1 to not reached) months. Overall survival rates (95% CI) at 1 and 2 years were 75.3% (95% CI, 66.9% to 81.9%) and 63.3% (95% CI, 53.6% to 71.5%), respectively. Biomarker analysis demonstrated broad immune activation associated with response, including increased $CD8^+$ T-cell infiltration and PD-L1 expression. Treatment-related adverse event rates were 77.1% grade 1/2, 9.3% grade 3, 3.6% grade 4, and no grade 5 events.

CONCLUSION RP1 combined with nivolumab provided deep and durable systemic responses in patients with anti–PD-1–failed melanoma, including those with poor prognostic factors. The safety profile was favorable, with mostly grade 1/2 adverse events.

ACCOMPANYING CONTENT

- Appendix
- Data Sharing Statement
- Data Supplement
- Protocol

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INTRODUCTION

Immune checkpoint inhibitors (ICIs) have improved outcomes for patients with unresectable or metastatic melanoma.[1-3] However, primary resistance to anti–PD-1 therapy occurs in approximately 30%-50% of patients, with an additional 25% of patients developing secondary resistance.[4-8] Outcomes after progression on anti–PD-1 therapy remain poor, with a median overall survival (OS) of approximately 1 year in real-world clinical practice.[9,10] Subsequent treatment options are limited by patients' medical conditions, suboptimal efficacy, and/or toxicity.[11-15] Tumor-infiltrating lymphocyte therapy (lifileucel) is the only US Food and Drug Administration (FDA)–approved therapy for melanoma in patients previously treated with anti–PD-1 therapy[16,17] and provided an objective response rate (ORR) of 31.4%.[18] However, nearly all patients experienced grade 3/4 treatment-emergent adverse events,[18,19] and the treatment-related mortality rate was 7.5%.[17] Nivolumab plus ipilimumab is an alternative option, although the combination is also associated with high toxicity with grade ≥3 adverse events in 57% of patients.[20,21] BRAF/MEK

CONTEXT

Key Objective

This trial sought to determine the safety and efficacy of RP1 (vusolimogene oderparepvec) in combination with nivolumab in patients with advanced anti–PD-1–failed melanoma.

Knowledge Generated

RP1 combined with nivolumab demonstrated an objective response rate of 32.9% by RECIST 1.1, with reductions seen in both injected and noninjected lesions and a median duration of response of 33.7 months, with a 2-year survival of 63.3% in patients with advanced melanoma that had confirmed progression while being treated with an anti–PD-1–containing regimen. Treatment-related adverse events were primarily grade 1/2 with no treatment-related deaths.

Relevance *(G. McArthur)*

The RP1 oncolytic immunotherapy combined with nivolumab provides safe and effective activity in melanoma progressing on anti–PD-1–based therapy. If randomized trials demonstrate significant activity, this would be a new standard approach for these patients with high unmet need.*

*Relevance section written by *JCO* Associate Editor Grant McArthur, PhD, MBBS.

inhibition is an option for the approximately 50% of patients with *BRAF*-mutant melanoma, but responses may not be durable and treatment can diminish the response to subsequent immunotherapy[15,22,23]; additionally, grade ≥3 treatment-related adverse events (TRAEs) occur in up to 60% of patients on combination therapy.[22,23] Therefore, there is an unmet need for effective and less toxic therapies that provide clinically meaningful benefit for patients with anti–PD-1–progressed melanoma.

RP1 (vusolimogene oderparepvec) is a next-generation, replication-selective, oncolytic immunotherapy that is administered intratumorally. RP1 is engineered from a new clinical strain of herpes simplex virus type 1 (HSV-1) that was selected for its enhanced oncolytic activity. RP1 expresses granulocyte-macrophage colony-stimulating factor (GM-CSF) and the fusogenic gibbon ape leukemia virus glycoprotein with the R sequence deleted (GALV-GP-R$^-$) to enhance direct oncolytic activity, promote immunogenic cell death, and increase the overall systemic antitumor effect.[24] Preclinical data demonstrated that RP1 provides antitumor activity in both injected and noninjected tumors, which is enhanced through the inclusion of GALV-GP-R$^-$ and further increased in combination with anti–PD-1 therapy.[24,25] RP1 was designed to provide enhanced clinical efficacy compared with talimogene laherparepvec (T-VEC), the first FDA-approved oncolytic immunotherapy,[26] in particular through the multimodal activity of GALV-GP-R$^-$ to increase both direct tumor killing and the immunogenicity of tumor cell death. T-VEC, which is also an HSV-based oncolytic immunotherapy, received approval in melanoma based on data from anti–PD-1–naïve patients,[27] but subsequent studies in patients with melanoma that progressed on anti–PD-1 therapy demonstrated only limited activity outside of disease that had progressed on or after adjuvant anti–PD-1 therapy.[28,29]

IGNYTE is a phase I/II clinical trial evaluating the safety and efficacy of RP1 as monotherapy or in combination with nivolumab in patients with advanced tumors. Here, we report data from the registrational phase II cohort of patients with advanced melanoma with confirmed progression while being treated with an anti–PD-1–containing regimen.

METHODS

Patients

Patients age ≥18 years with unresectable stage IIIB-IV cutaneous melanoma were eligible if they had confirmed progression while being treated for ≥8 weeks with anti–PD-1 alone or combined with another anticancer therapy, including in the adjuvant setting, as their last prior therapy. At least one measurable tumor per RECIST 1.1 and injectable lesions comprising ≥1 cm in the longest diameter were required. Key exclusion criteria included prior oncolytic therapy, current antiviral therapy, or a history of serious complications from ICI therapy.

Study Design and Treatment

The registration-intended cohort assessed RP1 combined with nivolumab in patients with advanced melanoma and confirmed progression while being treated with a prior anti–PD-1–containing regimen. Patients received an initial intratumoral dose of RP1 (1×10^6 plaque-forming units [PFU]/mL; each dose ≤10 mL), followed by up to seven additional doses at 1×10^7 PFU/mL every 2 weeks. Nivolumab (240 mg once every 2 weeks) was initiated with the second dose of RP1 for up to eight cycles and then continued at 480 mg once every 4 weeks for up to an additional 21 cycles. Nivolumab was given within a ± 2-day window of each dose

TABLE 1. Baseline Characteristics

Characteristic	All Patients (N = 140)
Age, years, median (range)	62.0 (21.0-91.0)
≥65	57 (40.7)
Male sex	95 (67.9)
White race	103 (73.6)
Stage	
IIIB/IIIC/IVM1a	72 (51.4)
IVM1b/c/d	68 (48.6)
BRAF status	
Wild-type *BRAF*	87 (62.1)
Mutant *BRAF*	53 (37.9)
LDH level	
LDH ≤ULN	92 (65.7)
LDH >ULN	47 (33.6)
LDH ≥2 × ULN	9 (6.4)
LDH unknown	1 (0.7)
PD-L1 tumor expression	
Positive (≥1%)	44 (31.4)
Negative (<1%)	79 (56.4)
Undetermined or missing	17 (12.1)
Prior therapy	
Anti–PD-1	
Anti–PD-1 only as adjuvant therapy	36 (25.7)
Anti–PD-1 other than as adjuvant therapy	104 (74.3)
Anti–CTLA-4	
Anti–PD-1 combined with anti–CTLA-4	61 (43.6)
Anti–PD-1 treated with anti–CTLA-4 sequentially	4 (2.9)
Wild-type *BRAF* and received combination anti–PD-1 and anti–CTLA-4	37 (26.4)
Received BRAF/MEK therapy	17 (12.1)
Other disease characteristics	
Primary resistance to prior anti–PD-1[a]	92 (65.7)
Secondary resistance to prior anti–PD-1[b,c]	48 (34.3)

NOTE. Data are presented as No. (%) unless otherwise indicated.
Abbreviations: CTLA-4, cytotoxic T-lymphocyte antigen 4; LDH, lactate dehydrogenase; ULN, upper limit of normal.
[a]Primary resistance was defined as progression within 6 months of starting the immediate prior course of anti–PD-1 therapy.[30]
[b]Secondary resistance was defined as progression after 6 months of starting the immediate prior course of anti–PD-1 therapy.[30]
[c]Includes one patient with unknown resistance status.

of RP1. RP1 was directly injected into superficial lesions and/or into deeper lesions using appropriate imaging guidance. Multiple lesions, including both superficial and deep lesions (up to the 10-mL maximum allowable dose), could be injected on each treatment day and different lesions could be injected on different treatment occasions. Additional doses of RP1 beyond eight could be given if clinically indicated (Data Supplement and Fig S1). This study was registered with EudraCT (number 2016-004548-12) before enrollment of the first patient and subsequently registered with ClinicalTrials.gov (identifier: NCT03767348).

End Points

The primary end point of the registration-intended cohort was ORR using a modified RECIST 1.1 (mRECIST) by blinded independent central review (BICR). The key modification of mRECIST was that progression needed to be confirmed by further tumor increase to allow for the potential of pseudoprogression. ORR was also analyzed using RECIST 1.1 by BICR to allow better comparison with other clinical trials. Secondary end points included duration of response (DOR), complete response (CR) rate, and progression-free survival (PFS), each by BICR, and 1- and 2-year OS. Responses of injected and noninjected lesions, biomarker analysis of tumor biopsies, and clinical subgroup analyses were exploratory and are detailed in the Data Supplement. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0.

Study Oversight

The study protocol was approved by institutional review boards or independent ethics committees at each participating site and conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and in compliance with Good Clinical Practice. All patients provided written informed consent before enrollment. The investigators collected data, which were analyzed by statisticians employed by the study sponsor.

Statistical Analysis

A sample size of 125 patients was estimated to provide >97% power to reject the null hypothesis of an ORR <15% (given a two-sided 5% or one-sided 2.5% alpha), which would not be considered clinically relevant. The sample size was also considered to provide sufficient characterization of the safety profile. For the primary efficacy analysis of ORR, the point estimate and the two-sided 95% Clopper-Pearson exact CI were computed. The DOR, PFS, and OS were estimated using Kaplan-Meier methodology. Exploratory analyses and safety data were summarized descriptively.

RESULTS

Patient Baseline Clinical Characteristics

A total of 140 patients with anti–PD-1–failed melanoma were enrolled and treated with RP1 combined with nivolumab (Data Supplement, Fig S2). The cutoff date for the primary data analysis reported here was March 8, 2024, when all patients had the potential for at least 12 months of follow-up. The median (range) age was 62.0 (21.0-91.0) years, 67.9% of patients were male, and 73.6% were White

and Across Subgroups by Blinded Independent Central Review Using RECIST 1.1

Anti–PD-1		Stage		Anti–PD-1 Resistance		Anti–PD-1 Adjuvant		*BRAF*		PD-L1 Expression[a]	
-	With Anti–CTLA-4 (n = 65)	IIIB/IIIC/ IVM1a (n = 72)	IVM1b/c/d (n = 68)	Primary (n = 92)	Secondary (n = 48)[c]	Yes (n = 36)	No (n = 104)	Mut (n = 53)	WT (n = 87)	Pos. (n = 44)	Neg. (n = 79)
	5 (7.7)	17 (23.6)	4 (5.9)	16 (17.4)	5 (10.4)	11 (30.6)	10 (9.6)	8 (15.1)	13 (14.9)	11 (25.0)	10 (12.7)
	12 (18.5)	12 (16.7)	13 (19.1)	16 (17.4)	9 (18.8)	5 (13.9)	20 (19.2)	10 (18.9)	15 (17.2)	12 (27.3)	9 (11.4)
	15 (23.1)	17 (23.6)	14 (20.6)	15 (16.3)	16 (33.3)	8 (22.2)	23 (22.1)	17 (32.1)	14 (16.1)	7 (15.9)	19 (24.1)
	26 (40.0)	25 (34.7)	29 (42.6)	39 (42.4)	15 (31.3)	11 (30.6)	43 (41.3)	15 (28.3)	39 (44.8)	11 (25.0)	36 (45.6)
	7 (10.8)	1 (1.4)	8 (11.8)	6 (6.5)	3 (6.3)	1 (2.8)	8 (7.7)	3 (5.7)	6 (6.9)	3 (6.8)	5 (6.3)
)	26.2 (16.0 to 38.5)	40.3 (28.9 to 52.5)	25.0 (15.3 to 37.0)	34.8 (25.1 to 45.4)	29.2 (17.0 to 44.1)	44.4 (27.9 to 61.9)	28.8 (20.4 to 38.6)	34.0 (21.5 to 48.3)	32.2 (22.6 to 43.1)	52.3 (36.7 to 67.5)	24.1 (15.1 to 35.0)

ponse; CR, complete response; CTLA-4, cytotoxic T-lymphocyte antigen 4; Mut, mutant; NE, not evaluable; Neg, negative; ORR, objective response rate; PD, PR, partial response; SD, stable disease; WT, wild-type.

wn PD-L1 expression status.

-PD-1 as monotherapy (ORR 40.9%, CR rate 22.7%). Nine patients received anti–PD-1 in combination with other therapeutic agents.

resistance status.

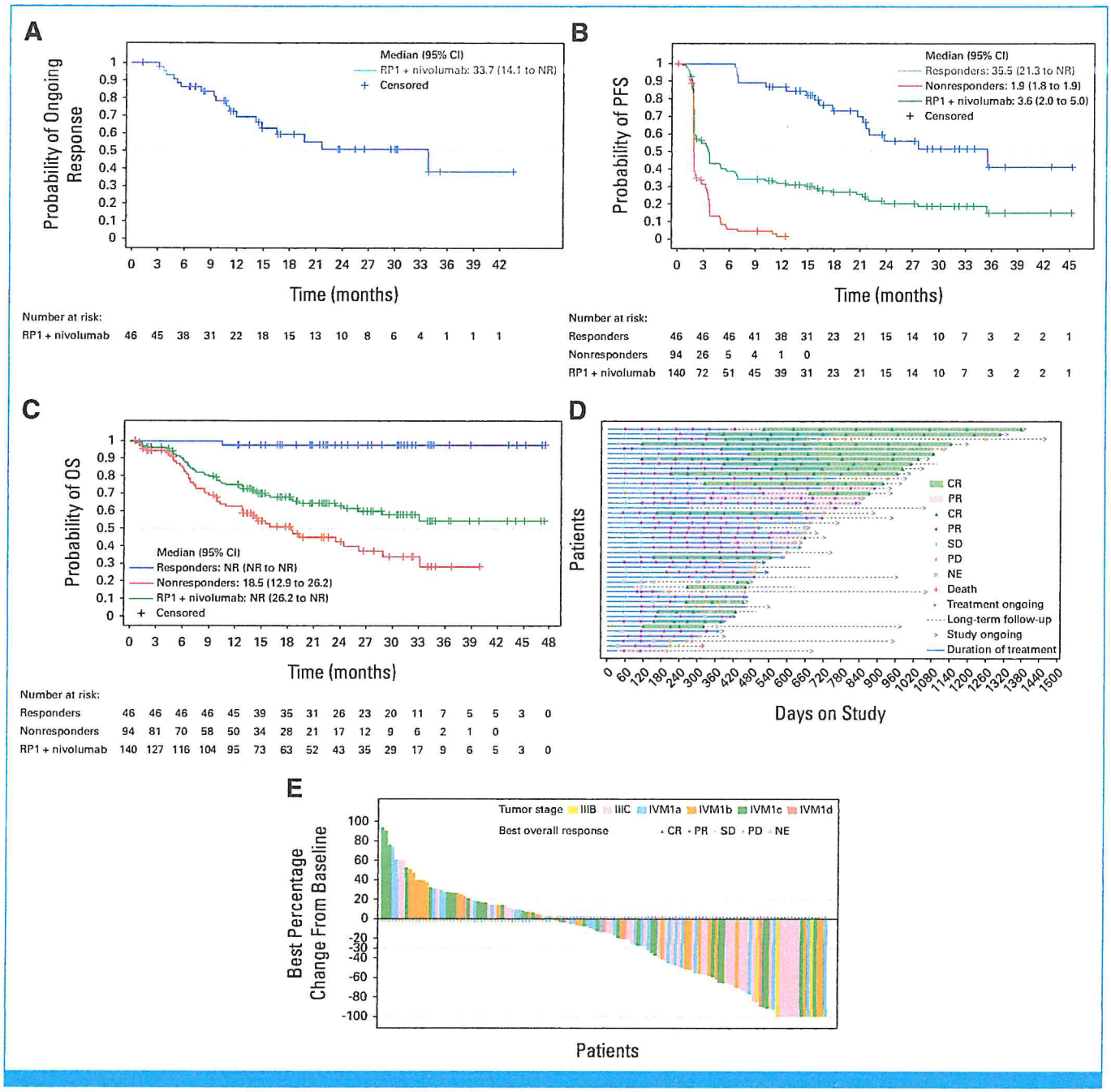

FIG 1. Duration of response and survival outcomes for RP1 combined with nivolumab. (A) Duration of response by RECIST 1.1. (B) PFS for all patients and according to response subgroup (responders and nonresponders) by RECIST 1.1. (C) OS for all patients and according to response subgroup (responders and nonresponders). (D) Clinical course for all responding patients. (E) Percentage change from baseline in target lesions for all patients measured by BICR. Patients who did not have target lesions measured at baseline and/or post-baseline are not included in the figure. BICR, blinded independent central review; CR, complete response; NE, not evaluable; NR, not reached; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

(Table 1). Primary resistance (defined as progression within 6 months of starting the prior course of anti–PD-1 therapy[30]) had occurred in 65.7% of patients and secondary resistance (progression after 6 months of prior anti–PD-1 therapy[30]) in 34.3% of patients. The median (range) duration of the immediate prior anti–PD-1 therapy was 4.6 (1.0-55.2) months. Overall, 46.4% of patients received prior anti–PD-1 therapy either in combination (43.6%) or sequentially (2.9%) with anti–cytotoxic T-lymphocyte antigen 4 (CTLA-4) therapy, 48.6% of patients had stage IVM1b/c/d disease, 37.9% had *BRAF*-mutant tumors, 33.6% had a lactate dehydrogenase (LDH) level greater than the upper limit of normal (ULN), and 56.4% had PD-L1–negative (<1%) tumors. A summary of the RP1 volume administered in the initial course is detailed in the Data Supplement (Table S1).

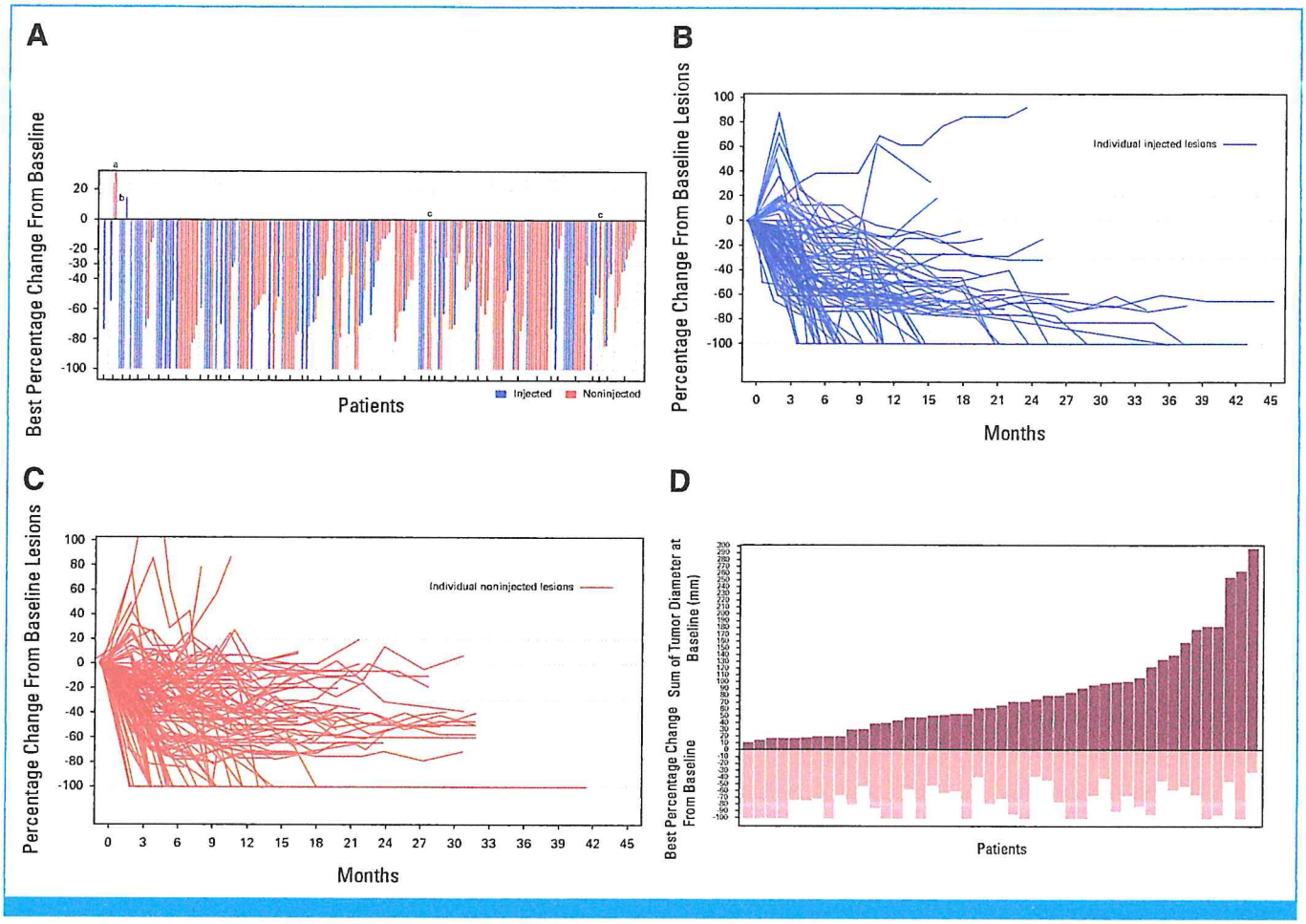

FIG 2. Tumor reduction among responding patients by RECIST 1.1. (A) Best percentage change in individual injected and noninjected lesions from baseline, (B) change in size of individual injected lesions over time, (C) change in size of individual noninjected lesions over time, and (D) overall baseline tumor burden and best reduction in tumor burden. (A) [a]Patient had a CR as a radical resection of all three lesions on the skin of the left foot confirmed full regression; [b]the sum of diameters of four target lesions met the criteria for a PR; and [c]the patient only had noninjected lesions measured. (A and D), One patient was not included because lesions were not measurable by BICR. (B and C) All measurable lesions were measured by BICR for each patient with a best response of confirmed CR or PR by RECIST 1.1. (D) Total tumor burden was plotted above the line for each patient (maroon bars) with percent reduction in tumor burden plotted below the line (pink bars). BICR, blinded independent central review; CR, complete response; PR, partial response.

Clinical Efficacy

The median (range) follow-up at the primary analysis was 15.5 (0.5-47.6) months, with all patients having the potential to be followed for at least 12 months. The median (range) time to response was 4.5 (1.7-21.9) months. Among all patients (N = 140), the ORR (95% CI) was 33.6% (95% CI, 25.8% to 42.0%) by mRECIST (the primary end point) and 32.9% (95% CI, 25.2% to 41.3%) by RECIST 1.1, both according to BICR. The additional end points and analyses are reported here using RECIST 1.1 to allow better comparison with other clinical trials. By RECIST 1.1, the CR rate (95% CI) was 15.0% (95% CI, 9.5% to 22.0%; Table 2), and the median (95% CI) DOR was 33.7 (14.1 to not reached [NR]) months (Fig 1A) with 69.5% of responses ongoing at 1 year after response initiation. The median (95% CI) PFS for all patients was 3.6 (95% CI, 2.0-5.0) months, with 35.5 (95% CI, 21.3 to NR) months for responders and 1.9 (95% CI, 1.8-1.9) months for nonresponders (Fig 1B). The 1- and 2-year OS rates (95% CI) were 75.3% (95% CI, 66.9% to 81.9%) and 63.3% (95% CI, 53.6% to 71.5%), respectively, with the median OS NR. The median (95% CI) OS was NR (95% CI, NR to NR) for responders and 18.5 (95% CI, 12.9-26.2) months for nonresponders (Fig 1C). The overall clinical course for responders is shown in Figure 1D. A waterfall plot showing the overall reduction in target tumor burden is shown in Figure 1E.

Responses in Injected and Noninjected Lesions

Responses occurred among both injected and noninjected lesions (Data Supplement, Fig S3) with a similar frequency, depth, durability, and kinetics, including noninjected lesions in visceral organs (Figs 2A-2C). Among RECIST 1.1 responders, there was a ≥30% reduction in 93.6% (73/78) of injected lesions and 79.0% (94/119) of noninjected lesions

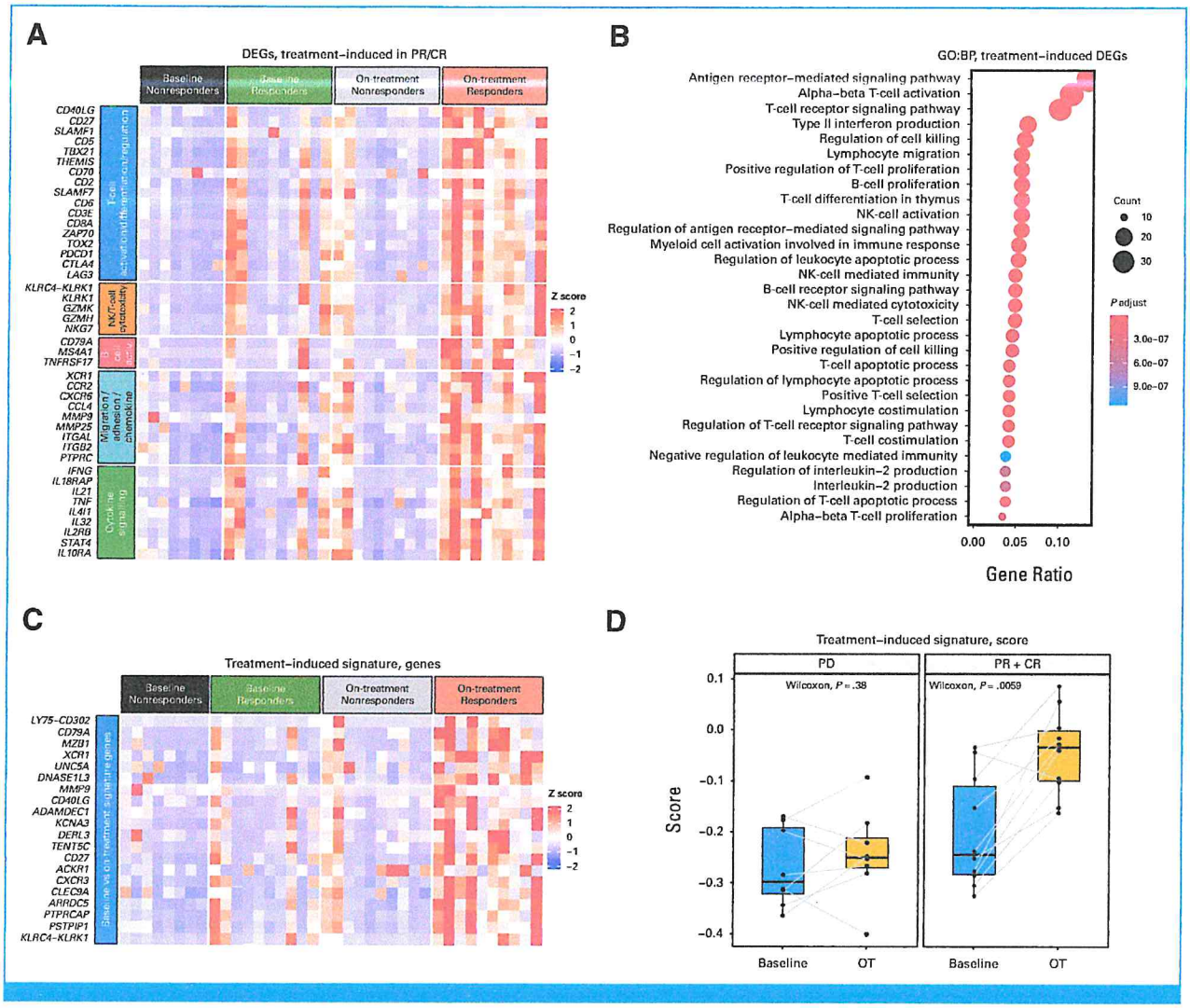

FIG 3. RNA Sequencing–Based Biomarker Analysis. (A) Heatmap demonstrating relative gene expression of selected DEGs between baseline and on-treatment (day 43) tumor samples in responders, representing diverse immune activation, including T, B, and NK cells, cell adhesion, and cytokine/chemokine signaling; each column represents an individual patient. (B) GO:BP terms enriched with genes differentially upregulated between baseline and on-treatment samples in responders. (C) Heatmap demonstrating relative gene expression of treatment-induced signature genes (P adj <.05, $\log_2$FC >1.5). (D) Treatment-induced signature gene score in pretreatment versus on-treatment samples in groups by response. activ, activation; CR, complete response; DEG, differentially expressed gene; GO:BP, Gene Ontology: Biological Processes; NK, natural killer; OT, on-treatment; PD, progressive disease; PR, partial response.

(Figs 2A-2C and Data Supplement, Table S2). Pseudoprogression (initial tumor increase before response) was frequently seen in both the injected and noninjected lesions of responding patients (Figs 2B and 2C). Of the 52 noninjected visceral organ lesions in responding patients (including in the lung and liver), 96.2% showed any reduction from baseline, with 65.4% reduced by ≥30% (Data Supplement, Table S3). Responses were also observed irrespective of injection route, with a 29.8% ORR following only superficial injection, a 40.9% ORR for only deep/visceral injections, and a 42.9% ORR for patients receiving both superficial and deep/visceral injections (Data Supplement, Table S4). Response was also independent of baseline tumor burden (Fig 2D).

Subgroup Analyses

The ORR by BICR using RECIST 1.1 was also assessed across clinical subgroups (Table 2). The ORR (95% CI) was 26.2% (95% CI, 16.0% to 38.5%) in patients having prior anti–PD-1 and anti–CTLA-4 therapy, 34.8% (95% CI, 25.1% to 45.4%) for patients with primary anti–PD-1 resistance, and 29.2% (95% CI, 17.0% to 44.1%) for those with secondary anti–PD-1 resistance. The ORR (95% CI) was 44.4% (95% CI, 27.9% to 61.9%) for patients who received anti–PD-1 in the adjuvant setting and 28.8% (95% CI, 20.4% to 38.6%) for patients who received nonadjuvant anti–PD-1. The ORR (95% CI) was 25.0% (95% CI, 15.3% to 37.0%) for stage IVM1b/c/d disease and 34.0% (95% CI, 21.5% to 48.3%) for *BRAF*-mutant

TABLE 3. Treatment-Related Adverse Events

Event	All Patients (N = 140)	
	Any Grade, No. (%)	Grade ≥3, No. (%)
Any TRAEs	126 (90.0)	18 (12.9)
TRAEs occurring in ≥5% of patients		
Fatigue	46 (32.9)	1 (0.7)
Chills	45 (32.1)	0
Pyrexia	43 (30.7)	0
Nausea	31 (22.1)	0
Influenza-like illness	25 (17.9)	0
Injection-site pain	21 (15.0)	0
Diarrhea	20 (14.3)	1 (0.7)
Vomiting	19 (13.6)	0
Headache	18 (12.9)	0
Pruritus	18 (12.9)	0
Asthenia	14 (10.0)	1 (0.7)
Arthralgia	10 (7.1)	1 (0.7)
Decreased appetite	9 (6.4)	1 (0.7)
Myalgia	9 (6.4)	0
Cough	8 (5.7)	0
Rash	8 (5.7)	0
Injection-site reaction	7 (5.0)	0
Vitiligo	7 (5.0)	0

NOTE. TRAEs were graded according to CTCAE version 5.0.
Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; TRAE, treatment-related adverse event.

tumors versus 32.2% (95% CI, 22.6% to 43.1%) for *BRAF* wild-type tumors. The ORR was 25.5% (95% CI, 13.9% to 40.3%) for patients with LDH levels >ULN versus 35.9% (95% CI, 26.1% to 46.5%) for LDH levels ≤ULN and 24.1% (95% CI, 15.1% to 35.0%) for patients with PD-L1–negative tumors versus 52.3% (95% CI, 36.7% to 67.5%) for those with PD-L1–positive tumors. Similar subgroup response rates were observed according to BICR using mRECIST (Data Supplement, Table S5).

Biomarkers

Tumors were biopsied at screening or on day 1 and day 43. Immunohistochemistry for available biopsies demonstrated increased $CD8^+$ T-cell infiltration with a higher $CD8^+$ T-cell score in 37 of 78 (47.4%) samples and increased PD-L1 expression in 39 of 68 (57.4%) samples at day 43 relative to baseline (Data Supplement, Fig S4). Differential gene expression between 10 responders (five CR and five partial response) and nine nonresponders (best response of progressive disease) was analyzed using RNA sequencing. A total of 313 genes were significantly upregulated in responders (adjusted $P<.05$, logFC >1) versus only five genes in nonresponders compared with their respective baseline. These differentially expressed genes (DEGs) included a wide range of genes encompassing T-cell, B-cell, and natural killer–cell function, as well as chemokine and cytokine signaling. The 313 upregulated DEGs in responders were not significantly changed in nonresponders (Fig 3A). Gene set enrichment analysis further confirmed the diversity of the immune pathways involved (Fig 3B). We then generated an unbiased signature using the DEGs from responders with the highest fold change (log_2FC >1.5, excluding T-cell receptor and immunoglobulin genes; Fig 3C); the expression of genes was more commonly differentially represented in responders compared with baseline (Fig 3D). The treatment-induced gene signature among responders suggests that the induction of a diverse, antiviral-type immune signature may contribute to responsiveness to RP1 therapy. These data further support the immune-mediated mechanism of action of RP1, with the increased expression of immune-related genes being associated with response to treatment, a pattern not observed in nonresponding patients.

Safety

Overall, 90.0% (126/140) of patients experienced at least one TRAE of any grade and 12.9% (18/140) experienced a grade 3/4 TRAE (3.6% grade 4; Table 3 and Data Supplement, Table S6). The most common all-grade TRAEs occurring in >20% of patients were fatigue (32.9%), chills (32.1%), pyrexia (30.7%), and nausea (22.1%). Hypophysitis and rash maculopapular were the only grade 3 TRAEs to occur in more than one patient (n = 2 each). In total, there were five grade 4 TRAEs (cytokine release syndrome, hepatic cytolysis, lipase increased, myocarditis, and splenic rupture [n = 1 each]). No treatment-related deaths were observed.

DISCUSSION

The introduction of ICIs has greatly improved outcomes for patients with advanced melanoma.[1-3] However, many patients experience disease progression on anti–PD-1 therapy given as monotherapy or in combination with other ICIs.[4,5,31] The prognosis after progression remains poor, with a median OS of approximately 1 year,[9,10] and available treatment options have significant limitations. Lifileucel, the only FDA-approved therapy after anti–PD-1 therapy,[16] requires strict patient selection criteria[32] and is associated with significant toxicity.[18,19] Additionally, the efficacy of combined anti–PD-1 and anti–CTLA-4 therapy is limited in this setting, and 57% of patients with anti–PD-1/PD-L1–refractory melanoma given this combination experience grade ≥3 TRAEs.[21] In this study, RP1 combined with nivolumab provided clinically meaningful rates and durability of response when evaluated in the context of historical data for patients with advanced melanoma that progressed on treatment with anti–PD-1 alone or combined with anti–CTLA-4. Approximately one in three patients had a confirmed objective response by RECIST 1.1 (32.9%) and 15.0% had a CR. Although CR rates were notably higher among patients with stage III–IVM1a disease (23.6%) versus stage IVM1b–d disease (5.9%), this is not surprising given that CRs with approved therapies are uncommon among patients

with more advanced disease, even in the first-line setting. The median DOR was 33.7 months, and more than 50% of responses were maintained at 2 years. The median OS was NR. Clinically meaningful rates of response were also observed across all subgroups analyzed, including those with poorer prognoses. This included an ORR of at least 25% in patients with melanoma that progressed on both prior anti–PD-1 and anti–CTLA-4 therapy and in stage IVM1b/c/d, primary resistant, and PD-L1–negative disease. Responses of injected and noninjected, including distant and visceral, lesions were seen with similar frequency, depth, duration, and kinetics, demonstrating a durable, systemic benefit.

The IGNYTE clinical trial was a single-arm study, and the results should therefore be contextualized with historical data for patients with melanoma that progressed while being treated with anti–PD-1 therapy. Previous studies have shown that only 6% to 7% of patients are expected to respond to continued anti–PD-1 monotherapy after confirmed progression on an anti–PD-1–containing regimen.[33] An ORR of 32.9% with 69.5% of responses ongoing for at least 1 year demonstrates a clear benefit of RP1 combined with nivolumab compared with expectations for nivolumab alone. RP1 combined with nivolumab also demonstrated a favorable safety profile, with generally transient grade 1/2 TRAEs consistent with systemic immune activation. There was a low incidence of grade 3/4 TRAEs (12.9%) with 3.6% being grade 4. The safety profile of RP1 combined with nivolumab generally overlapped with that of nivolumab monotherapy[3,7] and demonstrated no evidence of additive toxicity. Hence, the safety profile of RP1 combined with nivolumab compares favorably with lifileucel or the combination of anti–PD-1 and anti–CTLA-4 that may be used following anti–PD-1 therapy.[19-21,32]

RP1 is unique and distinct from previous oncolytic immunotherapies. RP1 was constructed from a new clinical isolate of HSV-1 selected for its enhanced ability to kill a range of human tumor cell lines[24] and encodes the transgenes for GM-CSF and GALV-GP-R$^-$. Expression of GALV-GP-R$^-$ is unique to RP1 and enhances tumor killing by causing cell-to-cell fusion, the primary effect of which is to greatly increase immunogenic cell death and thereby enhance systemic immune activation (shown in preclinical models),[24] which is further enhanced when combined with immune checkpoint blockade.[24] Consistent with preclinical data and the intended mechanism of action of RP1, biomarker data from patients in the IGNYTE study demonstrated broad-spectrum immune activation after treatment with RP1 combined with nivolumab in responding patients, which was not seen in nonresponders. This supports the hypothesis that the induction of an immune-inflamed tumor microenvironment by RP1 is critical for achieving the responses observed.

The key limitation of this study is the single-arm study design. The rationale for conducting this phase II study as a single-arm trial was based on ethical and practicality considerations, stemming from the lack of a well-established standard of care in this patient population to serve as a control arm. Based on the results of this phase II study, a randomized phase III confirmatory study evaluating RP1 combined with nivolumab versus treatment of physician's choice in patients with unresectable or metastatic melanoma that has progressed on anti–PD-1 and anti–CLTA-4 therapy is underway and enrolling (IGNYTE-3; NCT06264180).

In conclusion, RP1 combined with nivolumab demonstrated clinically meaningful and durable responses with a favorable safety profile in patients with advanced melanoma that progressed while being treated with an anti–PD-1–containing regimen.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Larkin J, Chiarion-Sileni V, Gonzalez R, et al: Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 381:1535-1546, 2019
2. Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al: Long-term outcomes with nivolumab plus ipilimumab or nivolumab alone versus ipilimumab in patients with advanced melanoma. J Clin Oncol 40: 127-137, 2022
3. Robert C, Schachter J, Long GV, et al: Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med 372:2521-2532, 2015
4. Lim SY, Shklovskaya E, Lee JH, et al: The molecular and functional landscape of resistance to immune checkpoint blockade in melanoma. Nat Commun 14:1516, 2023
5. Ribas A, Hamid O, Daud A, et al: Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. JAMA 315:1600-1609, 2016
6. Nowicki TS, Hu-Lieskovan S, Ribas A: Mechanisms of resistance to PD-1 and PD-L1 blockade. Cancer J 24:47-53, 2018
7. Robert C, Long GV, Brady B, et al: Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 372:320-330, 2015
8. Shui IM, Scherrer E, Frederickson A, et al: Resistance to anti-PD1 therapies in patients with advanced melanoma: Systematic literature review and application of the Society for Immunotherapy of Cancer Immunotherapy Resistance Taskforce anti-PD1 resistance definitions. Melanoma Res 32:393-404, 2022
9. Patrinely JR Jr., Baker LX, Davis EJ, et al: Outcomes after progression of disease with anti-PD-1/PD-L1 therapy for patients with advanced melanoma. Cancer 126:3448-3455, 2020
10. Nordstrom BL, Hamilton M, Collins JM, et al: Treatment patterns and outcomes following disease progression on anti-PD-1 therapies for advanced melanoma. Future Oncol 18:1343-1355, 2022
11. Hodi FS, Chesney J, Pavlick AC, et al: Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. Lancet Oncol 17:1558-1568, 2016
12. Larkin J, Chiarion-Sileni V, Gonzalez R, et al: Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 373:23-34, 2015
13. Tawbi HA, Schadendorf D, Lipson EJ, et al: Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. N Engl J Med 386:24-34, 2022
14. Long GV, Stephen Hodi F, Lipson EJ, et al: Overall survival and response with nivolumab and relatlimab in advanced melanoma. NEJM Evid 2:EVIDoa2200239, 2023
15. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Melanoma: Cutaneous, 2025. https://www.nccn.org
16. FDA approves first cellular therapy to treat patients with unresectable or metastatic melanoma. News release. 2024. http://tinyurl.com/5n7cuszk
17. AMTAGVI (lifileucel). Prescribing Information. Philadelphia, PA, Iovance Biotherapeutics, 2024
18. Chesney J, Lewis KD, Kluger H, et al: Efficacy and safety of lifileucel, a one-time autologous tumor-infiltrating lymphocyte (TIL) cell therapy, in patients with advanced melanoma after progression on immune checkpoint inhibitors and targeted therapies: Pooled analysis of consecutive cohorts of the C-144-01 study. J Immunother Cancer 10:e005755, 2022
19. Sarnaik AA, Hamid O, Khushalani NI, et al: Lifileucel, a tumor-infiltrating lymphocyte therapy, in metastatic melanoma. J Clin Oncol 39:2656-2666, 2021
20. Pires da Silva I, Ahmed T, Reijers ILM, et al: Ipilimumab alone or ipilimumab plus anti-PD-1 therapy in patients with metastatic melanoma resistant to anti-PD-(L)1 monotherapy: A multicentre, retrospective, cohort study. Lancet Oncol 22:836-847, 2021

21. VanderWalde A, Bellasea SL, Kendra KL, et al: Ipilimumab with or without nivolumab in PD-1 or PD-L1 blockade refractory metastatic melanoma: A randomized phase 2 trial. Nat Med 29: 2278-2285, 2023
22. Atkins MB, Lee SJ, Chmielowski B, et al: Combination dabrafenib and trametinib versus combination nivolumab and ipilimumab for patients with advanced BRAF-mutant melanoma: The DREAMseq trial-ECOG-ACRIN EA6134. J Clin Oncol 41:186-197, 2023
23. Ascierto PA, Mandalà M, Ferrucci PF, et al: Sequencing of ipilimumab plus nivolumab and encorafenib plus binimetinib for untreated BRAF-mutated metastatic melanoma (SECOMBIT): A randomized, three-arm, open-label phase II trial. J Clin Oncol 41:212-221, 2023
24. Thomas S, Kuncheria L, Roulstone V, et al: Development of a new fusion-enhanced oncolytic immunotherapy platform based on herpes simplex virus type 1. J Immunother Cancer 7:214, 2019
25. Khushalani NI, Harrington KJ, Melcher A, et al: Breaking the barriers in cancer care: The next generation of herpes simplex virus-based oncolytic immunotherapies for cancer treatment. Mol Ther Oncolytics 31:100729, 2023
26. Ferrucci PF, Pala L, Conforti F, et al: Talimogene laherparepvec (T-VEC): An intralesional cancer immunotherapy for advanced melanoma. Cancers (Basel) 13:1383, 2021
27. Andtbacka RHI, Collichio F, Harrington KJ, et al: Final analyses of OPTiM: A randomized phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III-IV melanoma. J Immunother Cancer 7:145, 2019
28. Gastman B, Robert C, Gogas H, et al: Primary analysis of a phase 2, open-label, multicenter trial of talimogene laherparepvec (T-VEC) plus pembrolizumab (pembro) for the treatment (Tx) of patients (pts) with advanced melanoma (MEL) who progressed on prior anti–PD-1 therapy: MASTERKEY-115. J Clin Oncol 40:9518, 2022
29. Hu-Lieskovan S, Moon J, Hyngstrom J, et al: Abstract 3275. Combination of talimogene laherparepvec (T-VEC) with pembrolizumab (pembro) in advanced melanoma patients following progression on a prior PD-1 inhibitor: SWOG S1607. Cancer Res 83:3275, 2023
30. Kluger HM, Tawbi HA, Ascierto ML, et al: Defining tumor resistance to PD-1 pathway blockade: Recommendations from the first meeting of the SITC Immunotherapy Resistance Taskforce. J Immunother Cancer 8:e000398, 2020
31. Beaver JA, Hazarika M, Mulkey F, et al: Patients with melanoma treated with an anti-PD-1 antibody beyond RECIST progression: A US Food and Drug Administration pooled analysis. Lancet Oncol 19:229-239, 2018
32. Warner AB, Hamid O, Komanduri K, et al: Expert consensus guidelines on management and best practices for tumor-infiltrating lymphocyte cell therapy. J Immunother Cancer 12:e008735, 2024
33. Ribas A, Kirkwood JM, Flaherty KT: Anti-PD-1 antibody treatment for melanoma. Lancet Oncol 19:e219, 2018

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No other potential conflicts of interest were reported.

APPENDIX

TABLE A1. List of Participating Investigators and Study Sites

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