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Phase 1/1b study of pembrolizumab plus vorinostat in advanced/metastatic non-small cell lung cancer

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Abstract

Purpose: Histone deacetylase inhibitors (HDACi) enhance tumor immunogenicity through several mechanisms and may improve response to immune checkpoint inhibitors (ICIs). In a phase 1/1b trial, we tested the oral HDACi vorinostat combined with the programmed cell death protein 1 inhibitor pembrolizumab in advanced/metastatic non-small cell lung cancer (NSCLC).

Experimental Design: Patients received intravenous pembrolizumab (200 mg every 3 weeks) plus oral vorinostat (200 or 400 mg/day). Primary endpoint was safety/tolerability. Secondary endpoints included response rate, progression-free survival, disease control rate (DCR), and overall survival. Tumor gene expression changes, T-cell density, and myeloid cell levels were studied in serial tissue specimens.

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Results: Thirty-three patients were treated (13 in phase 1, 20 in phase 1b). In phase 1, both ICI-naïve and ICI-pretreated patients were enrolled to determine dose-limiting toxicities (DLTs). No DLTs were observed, and the recommended phase 2 dose was pembrolizumab 200 mg and vorinostat 400 mg. Any-grade adverse events were mainly fatigue (33%) and nausea/vomiting (27%). Of 6 ICI-naïve and 24 ICI-pretreated patients evaluable for response, 4 (13%) had partial response (2 confirmed, 1 unconfirmed with subsequent prolonged stable disease [SD], 1 unconfirmed with subsequent progressive disease [PD]), 16 (53%) had SD, and 10 (33%) had PD for a DCR of 67%. In the ICI-pretreated cohort, 3 patients (1 confirmed, 2 unconfirmed) had partial response and 10 had SD. Pretreatment CD8⁺ T-cell presence in tumor stromal regions was associated with treatment benefit.

Conclusions: Pembrolizumab plus vorinostat was well tolerated and demonstrated preliminary anti-tumor activity despite progression on prior ICI treatment.

INTRODUCTION

Treatment strategies for patients with advanced/metastatic non-small cell lung cancer (NSCLC) are changing. This is mainly due to the development of immune checkpoint inhibitors (ICIs) such as anti-programmed cell death protein 1 (anti-PD-1) and anti-programmed cell death ligand 1 (anti-PD-L1) therapies. These include nivolumab, atezolizumab, and pembrolizumab, which initially all received US Food and Drug Administration approval for previously treated NSCLC patients. As a single agent, pembrolizumab has been approved for patients previously treated with a platinum-based doublet, as well as untreated patients with advanced/metastatic non-squamous NSCLC without actionable mutations and a PD-L1 tumor proportion score (TPS) of ≥1% (1). As a combination therapy in the first-line setting, pembrolizumab and platinum-doublet chemotherapy have been associated with superior rates of median progression-free survival (mPFS) and median overall survival (mOS) compared with platinum-doublet chemotherapy alone for both non-squamous and squamous cell NSCLC, regardless of PD-L1 status (2, 3). The triple combination resulted in increased toxicity rates compared with chemotherapy alone or pembrolizumab alone (2, 3). Docetaxel with or without ramucirumab represents the next-line standard of care treatment (4). Despite these advancements, improving treatment options for NSCLC patients with progression after ICI therapy represents an area of need.

An immune-poor tumor microenvironment characterized by absence or paucity of T cells is associated with resistance to ICI treatment (5, 6). Consequently, treatment modalities with potential to increase number and/or functionality of T cells in tumors may provide benefit to patients refractory to ICI treatment. There is wide interest in epigenetic modulation of the tumor microenvironment to enhance response to immunotherapeutics (7–10). The immunostimulatory activity of histone deacetylase inhibitors (HDACi) has been appreciated for some time and tested in preclinical studies combining HDACi with adoptive T-cell transfer or immune-stimulating antibodies (11, 12). The underlying mechanisms described include HDACi-mediated upregulation of MHC expression and T-cell functionality (11). More recently, preclinical studies have documented the ability of HDACi alone or in combination with other epigenetic agents to enhance response to ICI (13–16). Two studies have suggested that inhibitory effects of HDACi on myeloid-derived suppressor cells

(MDSC) may underlie the ability of the HDACi entinostat to synergize with ICI (13, 14). In our preclinical studies, we found that HDACi induced expression of T-cell chemokines such as *Cxcl9* and *Cxcl10* in lung cancer cells, macrophages, and T cells (16). HDACi co-treatment markedly augmented the response to PD-1 blockade in mouse lung cancer models in part by increasing T-cell trafficking to tumors and augmenting T-cell functionality (16). Studies by Topper et al showed that pan-HDACi combined with hypomethylating agents also induced upregulation of T-cell chemokine *Ccl5* and synergy with ICI (15). Finally, inhibitors of enhancer of zeste homologue 2 (EZH2) have also shown an ability to augment expression of T-cell chemokines *Cxcl9* and *Cxcl10* and enhance response to ICI (17). Collectively, epigenetic agents and HDACi in particular have been shown to act through multiple mechanisms, including the upregulation of expression of MHC, tumor antigens, and T-cell chemokines, stimulatory effects on T cells, and the inhibition of suppressive cell types such as MDSC. To date, however, we are not aware of published reports where HDACi combined with ICI has been tested in cancer patients. In this study, we provide our phase 1/1b trial results of the ICI pembrolizumab in combination with the pan-HDACi vorinostat in patients with advanced/metastatic NSCLC. Specifically, we hypothesized that patients with resistance to prior ICI may benefit from the combination of an ICI and an HDACi.

MATERIALS AND METHODS

Patients

Eligible patients had histologically confirmed advanced or metastatic NSCLC with progression. Patients in phase 1 may or may not have received prior therapy with an ICI, while patients in phase 1b were required to have received prior ICI therapy. Eligible patients also had an Eastern Cooperative Oncology Group performance status of 0 to 1, adequate organ function, and measurable disease according to Response Evaluation Criteria in Solid Tumor (RECIST) guidelines version 1.1. Exclusion criteria included untreated, progressive, and symptomatic brain metastases; active uncontrolled autoimmune disorders; and chronic systemic steroid use equivalent to 10 mg of prednisone. ICI-relapsed patients were defined as those who had achieved stable disease (SD) or better for at least 3 months of prior ICI treatment, and ICI-refractory patients were those who experienced disease progression within 3 months of prior ICI treatment. Patients were enrolled regardless of their PD-L1 status. The study protocol and all amendments were approved by the central institutional review board. All patients willingly provided written informed consent before enrollment. The trial was conducted in accordance with the provisions of the Declaration of Helsinki, Good Clinical Practice guidelines (as defined by the International Conference on Harmonization), and applicable regulatory requirements.

Trial design and treatment

This was a single-center, open-label dose escalation (phase 1 [n = 12]) followed by dose expansion (phase 1b [n = 18]) trial (ClinicalTrials.gov Identifier: NCT02638090). In phase 1, a modified continuous reassessment method (18) was used to evaluate a fixed dose of intravenous pembrolizumab at 200 mg every 3 weeks plus 2 dose levels of oral vorinostat at 200 mg (dose level 1 [DL1]) and 400 mg (dose level 2 [DL2]) given daily (and a back-up dose level of 100 mg daily). Cycles were 21 days long. The primary endpoints of phase 1/1b

were to identify the maximum tolerated dose and to establish the recommended phase 2 dose. Secondary endpoints were response rate, progression-free survival (PFS), and overall survival (OS), which were determined by performing computed tomography scans every 6 weeks in accordance with RECIST guidelines version 1.1. The DCR was the sum of the complete response, partial response (PR), and SD. Safety was assessed in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.

A dose-limiting toxicity (DLT) was defined as any grade 4 immune-related adverse event (irAE) or any grade 3 irAE, excluding colitis or pneumonitis, that did not downgrade to grade 2 within 3 days after onset of the event despite optimal medical management, including systemic corticosteroid administration. A DLT was also defined as grade 2 pneumonitis that did not resolve to grade 1 within 3 days of the initiation of maximal supportive care, grade 3 colitis or non-infectious pneumonitis—irrespective of duration, liver transaminase elevation $> 8 \times$ upper limit of normal, or total bilirubin $> 5 \times$ upper limit of normal. Other grade 3 irAEs defined as DLTs included those that did not downgrade to grade 1 or baseline within 14 days.

Furthermore, a DLT was defined as any grade 3 non-irAE, with several exceptions. These were grade 3 fatigue that lasted ≥ 7 days, grade 3 asymptomatic endocrine disorder that was medically managed, concurrent vitiligo, alopecia of any adverse event (AE) grade, a grade 3 infusion-related reaction that resolved within 6 hours with appropriate clinical management, grade 3 or 4 neutropenia without fever or systemic infection that improved by at least 1 grade within 3 days, grade 3 or 4 lymphopenia, grade 3 thrombocytopenia that was not associated with clinically significant bleeding and that required medical intervention and improved by at least 1 grade within 3 days, and an isolated asymptomatic grade 3 electrolyte that was reversed with appropriate maximal medical intervention within 3 days.

Dose reductions or modifications of pembrolizumab were not permitted. Dose reduction to the next lowest dose level of vorinostat was permitted at the discretion of the treating physician. Treatment was discontinued if a dose delay was required beyond 12 weeks. Treatment continued until progression, intolerance, or withdrawal. Treatment beyond progression was allowed at the discretion of the treating physician or study principal investigator. A fresh biopsy was required for all patients at baseline and during treatment (pembrolizumab cycle 1, day 15–21 [C1D15–21]).

Statistical analyses

Descriptive statistics were used to summarize safety, response, and demographic data, including number of patients, frequency counts, percentages, mean, median, and standard deviation. The Kaplan-Meier method with log-rank test was used to generate survival curves for PFS and OS. The 95% confidence interval (95% CI) of median survival time was estimated as the time at the intersection of the horizontal line of 50% survival rate with the lower and upper limit of survival function (19). When the upper limit of survival function was always above 50% (i.e., no intersection), the upper limit was reported as “not estimable.” Median follow-up time was calculated using reverse Kaplan-Meier method, which treats censor as event to estimate the median follow-up time (20). Time-to-event for PFS was defined as the length of time from initial on-treatment date to date of progression if

a patient progressed; date of death, in the absence of disease progression; or last date known alive if a patient was censored. Time-to-event for OS was defined as the length of time from initial treatment date to date of death or last date known alive if a patient was censored. The Mann-Whitney test was used to analyze biomarker data (e.g., MDSCs in blood or CD33⁺ and CD8⁺ cells in tumors).

PD-L1 expression

Immunohistochemistry was performed on pretreatment tissue specimens to assess PD-L1 expression using the Dako 22C3 antibody (Agilent, Santa Clara, CA, USA). The TPS was used to measure results, as previously described (21) with cut points of < 1%, 1% to 49%, and 50%. Insufficient tissue and unknown PD-L1 status were included in the < 1% cut point category.

Correlative analyses

Correlative studies were performed on 27 DL2 patients who were enrolled in both phase 1 and 1b. Peripheral blood studies were performed and tumor biopsies were obtained before treatment at screening and during treatment at C1D15–21 during treatment with the goal of identifying early potential biomarkers of response to the combination treatment. In addition, the on-treatment biopsy and blood collection timeline was based on early changes in tumor T-cell density reported in prior melanoma studies and preclinical studies showing decrease in systemic MDSCs 1 week after ICI plus HDACi treatment (5, 13). Patients who achieved SD or PR for a period of ≥ 24 weeks (i.e., DCR at 24 weeks) were characterized as having received clinical benefit (≥ 24 weeks), whereas patients who had progressive disease (PD) during the same time period (< 24 weeks) were characterized as having received no clinical benefit.

To determine MDSC levels before and after treatment initiation, freshly isolated peripheral blood mononuclear cells were used. Flow cytometry was used to distinguish between granulocytic (CD14⁺CD11b⁺CD33⁺) and monocytic (HLA-DR⁺Lin⁺CD14⁺CD33⁺) populations of MDSCs. Gene expression changes associated with treatment were evaluated by using RNA-sequencing technology. Immunohistochemistry was performed to determine presence of CD8⁺ T cells and CD33⁺ myeloid cell types in tumor stroma and tumor beds. Details of the methodology used for the correlative analyses are described in Supplementary Methods.

RESULTS

Thirty-three patients were enrolled between March 2016 and September 2017. One patient was replaced during DL1 because of incorrect vorinostat dosing. We enrolled 20 patients during phase 1b; 2 patients withdrew from treatment during phase 1b before undergoing the first scan, leaving 9 who were ICI refractory and 9 ICI relapsed. The median number of lines of prior therapy was 2 (range, 1–5) among all patients who were previously treated with ICI (n = 26). The median follow-up time was 18.2 months for OS and 15.3 months for PFS. Patient characteristics and demographics are summarized in Table 1.

Safety

All 33 patients received at least 1 dose of medication and were therefore evaluated for toxicity (Table 2). Seventy-three percent of patients experienced a treatment-related adverse event (TRAE); 15% were ICI-naïve and 58% were ICI-pretreated. No DLTs were observed or treatment-related deaths occurred. The recommended phase 2 dose was declared at 200 mg of intravenous pembrolizumab every 3 weeks plus 400 mg oral vorinostat daily. The most common AEs were fatigue (33% of patients), nausea (27% of patients), and vomiting (27% of patients) (Table 2). The most common any grade irAEs were hypothyroidism (15% of patients), alanine aminotransferase increased (3% of patients), arthralgia (3% of patients), aspartate aminotransferase increased (3% of patients), colitis (3% of patients), diarrhea (3% of patients), and myalgia (3% of patients). Of 33 patients, 7 patients (~21%) had grade 3 or higher AEs: 0 from phase I (DL1), 1 of 9 patients (~11%) from phase 1 (DL2), and 6 of 20 patients (~30%) from phase 1b. Although they did not meet protocol-mandated dose modification criteria, dose reductions were made in 17 patients (52%) under the discretion of the treating physician or study principal investigator. All TRAEs leading to vorinostat dose reduction were grade 1 or 2, with the most common being nausea and/or anorexia (n = 5), fatigue (n = 4), and elevated creatinine (n = 3). The median number of cycles for dose reduction was 4 (range, 2 to 9). Two patients (6%) discontinued treatment during cycle 1 because of myalgia, and 1 patient discontinued because of colitis, which resolved with steroids.

Efficacy: Treatment naïve

Six of the patients enrolled in phase 1 were treatment naïve and were evaluated for efficacy. The median PD-L1 TPS was 5% (range, 0%–100%). We observed 1 confirmed PR (PD-L1 TPS = 50%), 4 cases of SD (2 were at < 1% and 2 were 1%–49%), and 1 case of PD (PD-L1, < 1%) (Figure 1A-C). The disease control rate (DCR), mPFS, and mOS were 83%, 7.5 (95% CI, 2-not estimable) months, and 16.0 (98% CI, 3.8-not estimable) months, respectively.

Efficacy: ICI-pretreated

When patients from phase 1 and 1b were merged, there were a total of 24 patients who were previously treated with ICI and evaluable for efficacy. Median time to progression on prior ICI treatment was 10 months (range, 7–52 months) for relapsed patients and 2 months (range, 1–3 months) for refractory patients. Median PD-L1 status was 0% (range, 0%–95%) for ICI-refractory NSCLC patients (n = 13) and 50% (range, 0%–97%) for ICI-relapsed NSCLC patients (n = 11) (Figure 1A). One patient with ICI-refractory NSCLC had a confirmed PR with a duration of 12 months (Figure 1A-C). Two patients, 1 ICI refractory and 1 ICI relapsed, had unconfirmed PR (Figure 1A-C): one with subsequent prolonged SD and one with subsequent PD. Of the 24 patients, there were 3 PR (1 confirmed), 11 (46%) cases of SD and 10 (42%) cases of PD, for a DCR of 58%. Furthermore, the DCR was not substantially different between patients with relapsed (6/11, 54%) and refractory (8/13, 61%) status. In the 30 evaluable patients, the best objective response rate was 13.3% and the confirmed response rate was 6.6%. Outcomes and treatment durations were not associated with PD-L1 expression (Figure 1A and B). For those 10 patients with PD, median PD-L1

status was 0% (range, 0%–95%; 60% at < 1% PD-L1 TPS, 10% at 1%–49% PD-L1 TPS, and 30% at 50% PD-L1 TPS); 5 were ICI refractory and 5 were ICI relapsed. Among the 11 patients with SD, median PD-L1 status was 10% (range, 0%–97%; 18.2% at < 1% PD-L1 TPS, 36.4% at 1%–49% PD-L1 TPS, at 45.5% at 50% PD-L1 TPS); 5 were ICI relapsed and 6 were ICI refractory. Pseudo-progression was not observed in any group (Figure 1C).

For the ICI-relapsed NSCLC group, mPFS and mOS were 4.6 (95% CI, 1.5-not estimable) months and 7.3 (95% CI, 5.4-not estimable) months, respectively. For the ICI-refractory NSCLC group, mPFS and mOS were 2.8 (95% CI, 1.8-not estimable) months and 6.8 (95% CI, 3.3-not estimable) months, respectively (Supplementary Figure S1A and B). There was no significant difference with regard to survival curves for PFS and OS between the ICI-relapsed and ICI-refractory patients ($P = 0.75$ and $P = 0.54$, respectively). HR of PFS and OS was 1.16 (95% CI, 0.50–2.71) and 1.31 (95% CI, 0.55–3.15), respectively (the ICI-relapsed NSCLC group was used as the reference) (Supplementary Figure S1A and B). mPFS and mOS by PD-L1 status are described in Table 3. In patients with 50% PD-L1 TPS, the HR for OS was found to be 1.75. While this seems unexpected, the HR was not statistically significant due to small sample size as the 95% CI for the 1.75 HR was 0.68–4.54. In addition to the small sample size, the PD-L1 stratification subgroups were not evenly balanced for features such as histology, gender, and ICI-pretreated status (Supplementary Table S1).

Peripheral blood MDSCs

MDSCs comprise a heterogeneous population that has been implicated in immunosuppression during various stages of cancer progression (22). High MDSC levels are associated with reduced response to immunotherapy and poor survival among patients with melanoma (23–26). In preclinical studies, HDACi treatment combined with ICI was reported to induce systemic MDSC depletion, leading to enhancement of T-cell responses by checkpoint blockade (13, 14). Correlative studies were performed on 27 DL2 patients (200 mg of pembrolizumab plus 400 mg vorinostat) who were enrolled in phase 1 and 1b. Clinical benefit for correlative studies was defined as SD or PR for a period of 24 weeks (i.e., DCR at 24 weeks). Freshly isolated peripheral blood mononuclear cells were used to phenotypically determine MDSC levels in patients before and after initiation of treatment. The granulocytic and monocytic populations were distinguished as $CD14^+CD11b^+CD33^+$ and $HLA-DR^+Lin^-CD14^+CD33^+$ cells, respectively (Supplementary Figure S2). Flow cytometry showed that MDSC levels were significantly more elevated in NSCLC patients than in healthy donors (Figure 2A and B). We observed a substantially greater percentage of increase in the granulocytic polymorphonuclear neutrophil subset than in the monocytic MDSC subset (Figure 2A and B). However, no difference in pretreatment levels of either MDSC subset was found between patients who had clinical benefit (i.e., SD or PR for a period of 24 weeks) and those who did not (Figure 2A and B). Similar results were obtained when patients with prior ICI treatment were separately evaluated (Supplementary Figure S3A and C). A clear association of MDSC levels in ICI-naïve patients could not be made because of limited patient numbers (Supplementary Figure S3B and D). In addition, there was no significant change in MDSC levels after treatment between patients who received benefit and those who were refractory (Figure 2C and D). Collectively, these data

indicate an increase in MDSC levels among NSCLC patients; however, our results suggest that baseline levels are not associated with patient benefit or affected by pembrolizumab plus vorinostat treatment.

T-cell and myeloid cell presence in tumor biopsies

The presence of CD8⁺ T cells in tumors is strongly associated with response to PD-1 blockade (5, 6). Pretreatment screening and early on-treatment biopsies were utilized in accordance with previously described criteria to determine baseline and early on-treatment CD8⁺ T-cell levels (27). To assess the potential role of myeloid cells, we stained tumor biopsies with the pan-myeloid marker CD33. On-treatment biopsies were collected on C1D15–21 during treatment to help identify early potential markers of patient response to treatment. Density of both cell types was scored using a 0 to 3 scale, and presence of these cells was separately defined in tumor beds and stroma. We focused on patients who were previously treated with ICIs (enrolled in either phase 1 or phase 1b), from whom 19 screening biopsies were obtained. Among these ICI-pretreated patients, the levels of CD8⁺ T cells in tumor beds were relatively low and were not associated with benefit (Figure 3A, $P > 0.05$). In contrast, increased presence of stromal CD8⁺ T-cell levels was found in patients who benefited from treatment (Figure 3B, $P = 0.0062$). Additionally, on-treatment biopsies also showed elevated presence of CD8⁺ T cells in the stroma ($P = 0.044$) of benefiting patients, but this difference was not seen in the tumor beds (Figure 3C and D). Specific examples of stromal versus CD8⁺ T-cell tumor distribution are shown in Supplementary Figure S4. Increases in CD8⁺ T cells during treatment were evident in only 2 patients, both of whom benefited from treatment. Unlike T cells, no significant difference in CD33⁺ myeloid cell presence was detected between tumor beds and stroma in these patient cohorts (Supplementary Figure S5). Our results suggest that pembrolizumab plus vorinostat treatment benefit may be associated with levels of stromal CD8⁺ T cells.

Gene expression changes in tumor biopsies

A transition toward a more immunogenic tumor microenvironment is associated with ICI responses in melanoma (5, 6). This is also made evident by increased expression of immune-function genes, such as genes regulated by IFN- γ (6). We determined potential changes in gene expression after pembrolizumab plus vorinostat treatment. Fresh frozen tumor biopsies collected during screening and treatment were used to extract RNA for RNA sequencing (Supplementary Methods). Phase 1 biopsies were processed earlier than phase 1b biopsies and were separately analyzed to avoid batch effects. In phase 1 patients, a substantial number of genes were upregulated after treatment, including IFN- γ and HDACi target genes, such as *GBP2*, *GBP5*, and *Cxcl9* (Supplementary Figure S6A). In contrast, upregulation of these or other candidate genes in phase 1b patients was not seen (Supplementary Figure S6B), even when patients who benefited from treatment were separately assessed (Supplementary Figure S6C). Because phase 1 patients included ICI-naïve patients, we hypothesized that there may be a difference in induction of target genes between ICI-naïve and ICI-pretreated patients. Indeed, we found evidence of strong upregulation of *GBP2* and *GBP5* in the naïve patients (Supplementary Figure S6D). Although these studies are constrained by a small sample size, they suggest potential

differences in how treatment may impact gene expression in ICI-naïve versus ICI-pretreated patients.

DISCUSSION

Given that the majority of patients with NSCLC will undergo ICI treatment in the first-line setting, a DCR of 58% in an ICI-pretreated patient population can be considered clinically significant. No new toxicities were uncovered for either drug, and irAEs were consistent with those previously reported (1, 2, 28–30) (Vorinostat Package Insert). All TRAEs associated with vorinostat dose reductions were grade 1/2. Although our study included a limited sample size, our data are comparable to previous combination studies in ICI-pretreated NSCLC patients. At ASCO 2018, Garon et al (31) reported an overall response rate of 5% (95% CI, 1.2%–12.6%), an mPFS of 1.8 months (95% CI, 1.6–2.5), and an mOS of 8.4 months (95% CI, 6.2–10.4) for 78 ICI-pretreated NSCLC patients who were treated with durvalumab plus tremelimumab (CTLA-4 inhibitor). In their study, DCR at 24 weeks was 21.8% (overall DCR was not indicated), whereas we showed a DCR at 24 weeks of 31.8% in the 22 ICI-treated patients at DL2. No significant differences were observed between the relapsed and refractory groups. At ESMO 2018, Leal et al reported the results of the combination of sitravatinib (a multi-kinase inhibitor) plus nivolumab in patients with ICI-pretreated NSCLC, noting 7/25 patients with PR (4 confirmed, 3 unconfirmed) (32). Hellmann et al (33) presented updated results of ENCORE601, the phase 2 trial of pembrolizumab plus the HDACi entinostat in advanced/metastatic NSCLC, at the World Congress on Lung Cancer 2018 (Toronto, Canada). In the ICI-pretreated group (n = 72), the overall response rate was 10% (95% CI, 4%–19%), 50% of patients achieved SD, and mPFS was 2.6 months (95% CI, 2.1–4.1). Response was irrespective of PD-L1 status. The DCR in their study was 60% versus a DCR of 58% in our trial. Grade 3/4-related irAEs were experienced by 9.2% of patients, 30.3% experienced other grade 3/4-related AEs, and 14% discontinued the study drug because of TRAEs. In the present study, the DCR in ICI refractory patients was 61% and 54% in relapsed patients, suggesting that this treatment regimen may be similarly effective in both patient cohorts.

As additional combination treatments in ICI-pretreated patients are tested, a clearer picture should emerge on how ICI combined with HDACi, with DCRs of 58% and 60% in our and the above-mentioned study, compare to other combination treatment modalities. While the combination of pembrolizumab plus chemotherapy is now a standard of care for first-line patients with stage IV NSCLC, the use of single-agent pembrolizumab has remained a standard for patients with high PD-L1 expression (2). In addition, the US Food and Drug Administration recently approved pembrolizumab in the first-line setting for patients at or above the 1% cut point for PD-L1 expression (34). Thus we believe it will be more widely utilized as single-agent therapy, and strategies to improve upon its single-agent activity without additional toxicity are of significant interest. Of special importance, we believe, is the identification of potential biomarkers of response to anti-PD-1 and HDACi (discussed below), which may help select patients with high likelihood of receiving benefit from this combination.

It has been suggested that tumor-infiltrating lymphocytes (TILs) can be present in stroma and/or tumor beds with distinct association with ICI treatment efficacy. To this end, immune-excluded tumors may represent a major subset with primary resistance to ICI in which T cells are trapped in stroma and excluded from tumor beds (6, 35, 36). Nonetheless, the precise role of stromal TILs in benefit to ICI remains to be determined. Biopsies from ICI-relapsed and ICI-refractory patients showed low levels of CD8⁺ T cells in tumor beds but enrichment in the stroma of a subset of patients. Stromal CD8⁺ T-cell presence was significantly associated with patient benefit (i.e., SD or PR for a period of ≥ 24 weeks), suggesting that presence of an HDACi may sensitize this otherwise resistant cohort to PD-1 inhibition. The ideal association of stromal T cells would be with the response rate, which we hope to address in our ongoing phase 2 trial (see below). We hypothesize that the combination treatment may trigger CD8⁺ T-cell migration from stroma to the tumor bed and that this is associated with benefit from the combination therapy. However, an increase in tumor bed CD8⁺ T cells was observed in only 2 of the 7 responsive patients studied (ICI-pretreated cohort with 24-week DCR) who had baseline stromal T-cell scores of 2 and 3, while an increase after treatment was not seen in stromal T-cell density in benefiting patients. This low rate may be a consequence of the early collection of biopsies during the treatment (i.e., 15–21 days after initiation), as significant T-cell trafficking may not have occurred at this time point. Biopsy collection at a later time point (e.g., cycle 3 day 1) in ICI-pretreated patients could show more robust changes in TIL localization as well as gene expression changes known to be associated with response to ICI. Our ongoing phase 2 studies in ICI-naïve patients will help test the hypothesis that the immune-excluded tumor subset with stromal TIL is more responsive to combined pembrolizumab plus vorinostat than the pembrolizumab alone cohort and also test whether the combination treatment modality triggers T-cell migration into tumor beds. As such, elevated stromal CD8⁺ T-cell density may prove to be a useful biomarker to select patients for this combination treatment.

While tumor PD-L1 expression is positively associated with response to ICI (37), outcomes and treatment benefit in this study were not associated with PD-L1 expression, suggesting that pembrolizumab plus vorinostat combination may benefit patients regardless of PD-L1 status. However, the relatively small sample size and insufficient tumor biopsy tissue to determine PD-L1 TPS in 9 patients enrolled in this trial precludes a clear determination of association of PD-L1 expression with treatment benefit. Despite being significantly elevated at baseline, peripheral MDSC levels were not found to be associated with benefit from treatment. Although we did not observe a significant decrease in MDSC levels at the C1D15–21 treatment time point, it is possible that longer term treatment is required for targeting MDSCs. However, a recent study of HDACi entinostat in metastatic estrogen receptor-positive breast cancer patients showed a decrease in MDSC on day 15 after treatment initiation (38). Further studies from ongoing clinical trials will help determine whether MDSC targeting function of distinct HDACi is different.

Our ongoing randomized phase 2 study is examining pembrolizumab +/- vorinostat in ICI-naïve advanced/metastatic NSCLC patients. These investigations can help further define associations between patient response and PD-L1 expression and MDSC levels. Phase 2 studies can also help determine the association between tumor TIL phenotype and patient response. In light of findings in the present study, it will be especially interesting to

determine whether pembrolizumab plus vorinostat treatment confers superior benefit in the immune-excluded (i.e., tumors with stromal T cells) patient cohort than pembrolizumab alone. In conclusion, vorinostat (400 mg taken orally daily) plus pembrolizumab (200 mg given intravenously every 3 weeks) was well tolerated. The combination demonstrates preliminary anti-tumor activity despite prior ICI progression and an interesting correlation between stromal CD8⁺ T-cell presence and patient benefit. Our ongoing randomized phase 2 study, which opened for enrollment in parallel with the phase Ib study, will determine whether pembrolizumab plus vorinostat improves RR and PFS compared with pembrolizumab alone in ICI-naïve advanced/metastatic NSCLC patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TRANSLATIONAL RELEVANCE

A host of clinical trials are underway to improve immune checkpoint inhibitor (ICI) response rates in advanced/metastatic non-small cell lung cancer. Many studies, including our own, have shown that epigenetic agents such as histone deacetylase inhibitors (HDACi) can help generate a tumor microenvironment that is more favorable to T-cell-dependent therapies. Here, we tested the HDACi vorinostat with the programmed cell death protein 1 inhibitor pembrolizumab in ICI-naïve and previously treated ICI-refractory patients. This combination was well tolerated with a disease control rate of 67%. In ICI-pretreated patients, there were 3 partial responses as best responses. Patients with a 24-week disease control rate had significantly elevated T-cell presence in tumor stromal regions. These studies lay the groundwork for additional trials to assess the impact of epigenetic agents on ICI response and the discovery of biomarkers associated with benefit from this treatment.

Figure 1.

(A) Waterfall plot of best response, defined as percent change from baseline sum of target lesion diameters. ** Those with unknown PD-L1 status (i.e., due to insufficient tissue) were categorized as “PD-L1 < 1%.” (B) Swimmer plot demonstrating response rates of evaluable patients. Progression of disease #2 was defined as an investigator decision to discontinue therapy due to progression after initial treatment beyond progression. Censored is defined as patient death or lost to follow-up without evidence of progression. (C) Spider plot showing change in target lesions over time for ICI-naïve (gray), ICI-relapsed (pink), and ICI-refractory (orange) patients. Efficacy analyses included all patients who underwent at least one on-treatment computed tomography scan. Tumor responses were assessed by investigators in accordance to Response Evaluation Criteria for Solid Tumors version 1.1.

Abbreviations: ICI, immune checkpoint inhibitor; PD, progressive disease; PD-L1, programmed death ligand 1.

Figure 2.

Percentage of granulocytic PMNs and MDSCs in total peripheral blood mononuclear cells of normal healthy donors and NSCLC trial participants. **(A and B)** PMN (CD14⁻CD11b⁺CD33⁺) and monocytic (HLA-DR⁻Lin⁻CD14⁺CD33⁺) populations of MDSCs were determined by flow cytometry in healthy donors (n = 3) and in screening blood draws of patients who received clinical benefit (≥ 24 weeks; n = 10) and those with progressive disease (< 24 weeks; n = 16). Significance of differences in MDSC levels was determined by using the Mann-Whitney test; *P* values are shown for indicated comparisons. Changes in PMN and monocytic MDSC levels were determined in individual patients from treatment initiation until cycle 1 day 15–21 **(C and D)**. Difference in MDSC level change is shown separately for patients who received clinical benefit (≥ 24 weeks; n = 10) and for those with progressive disease (< 24 weeks; n = 16).

Abbreviations: MDSC, myeloid-derived suppressor cell; PMN, polymorphonuclear neutrophil; ns, not significant (*P* > 0.05).

Figure 3.

CD8 staining of patient biopsies. Density of CD8⁺ cells was scored using a 0 to 3 scale, and presence was separately defined for tumor beds and stroma. (**A and B**) Difference in CD8⁺ scores between screening biopsies is shown for patients who received clinical benefit (< 24 weeks; n = 7) and those with progressive disease (< 24 weeks; n = 12) in tumor (**A**) and stroma (**B**). Significant differences were determined with the Mann-Whitney test. (**C and D**) Difference in CD8⁺ scores between on-treatment C1D15 biopsies is shown for patients who received clinical benefit (< 24 weeks; n = 6) and those with progressive disease (< 24 weeks; n = 9) in tumor (**C**) and stroma (**D**). Significant differences were determined with the Mann-Whitney test. *P* values are shown for indicated comparisons.

Abbreviations: C1D15, Cycle 1 day 15 to 21 treatment; ns, not significant (*P* > 0.05).

Table 1.

Baseline characteristics

		Phase I	Phase Ib	Total
No. of Patients		13	20	33
Age, Median (Range), years		66 (47–82)	68 (38–80)	68 (38–82)
ECOG PS	0	1 (8%)	1 (5%)	2 (6%)
	1	12 (92%)	19 (95%)	31 (94%)
Gender	Male	9 (69%)	13 (65%)	22 (67%)
	Female	4 (31%)	7 (35%)	11 (33%)
Smoking Status	Never	1 (8%)	2 (10%)	3 (9%)
	Former/Current	12 (92%)	19 (90%)	30 (91%)
Prior Lines of Therapy, Median (Range)		2 (1–5)	2 (1–4)	2 (1–5)
Histology	Adenocarcinoma	10 (76.9%)	15 (75.0%)	25 (75.8%)
	Adenosquamous Carcinoma	0 (0.0%)	1 (5.0%)	1 (3.0%)
	Squamous Cell Carcinoma	3 (23.1%)	4 (20.0%)	7 (21.2%)
Prior Chemotherapy		11 (85%)	16 (80%)	27 (82%)
Immunotherapy Status	Naive	7 (54%)	0 (0%)	7 (21%)
	Refractory	4 (31%)	10 (50%)	14 (42%)
	Relapsed	2 (15%)	10 (50%)	12 (36%)
PD-L1 TPS	< 1%	2 (15%)	4 (20%)	6 (18%)
	1%–49%	2 (15%)	5 (25%)	7 (21%)
	> 50%	4 (31%)	5 (25%)	9 (27%)
	QNS/Unknown	5 (38%)	6 (30%)	11 (33%)
Mutation Status	None/Other/Unknown	9 (69%)	17 (85%)	26 (79%)
	KRAS (codon 12 or 61)	3 (23%)	1 (5%)	4 (12%)
	EGFR (L858R)	1 (8%)	1 (5%)	2 (6%)
	BRAF (V600E)	0 (0%)	1 (5%)	1 (3%)
	ALK	0 (0%)	0 (0%)	0 (0%)
	ROS1	0 (0%)	0 (0%)	0 (0%)

Abbreviations: ALK, anaplastic lymphoma kinase; BRAF, proto-oncogene B-Raf; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog; QNS, quantity not sufficient; PD-L1, programmed cell death ligand 1; ROS1, c-ros oncogene 1; TPS, tumor proportion score.

Table 2.

Treatment-related adverse events

Arm	Adverse Event Detail	Grade 1	Grade 2	Grade 3	Grade 4	Total
Phase I Dose Level 1 (N = 4)	Anorexia	0	1 (25%)	0	0	1 (25%)
	Dysgeusia	1 (25%)	0	0	0	1 (25%)
	Weight loss	1 (25%)	0	0	0	1 (25%)
	Total	2	1	0	0	3
Phase I Dose Level 2 (N = 9)	Fatigue	3 (33%)	1 (11%)	0	0	4 (44%)
	Hypothyroidism	0	2 (22%)	0	0	2 (22%)
	Diarrhea	2 (22%)	0	0	0	2 (22%)
	Myalgia	0	0	1 (11%)	0	1 (11%)
	Creatinine increased	0	1 (11%)	0	0	1 (11%)
	Dysgeusia	0	1 (11%)	0	0	1 (11%)
	Peripheral sensory neuropathy	0	1 (11%)	0	0	1 (11%)
	Platelet count decreased	0	1 (11%)	0	0	1 (11%)
	Anorexia	1 (11%)	0	0	0	1 (11%)
	Anemia	1 (11%)	0	0	0	1 (11%)
	Cough	1 (11%)	0	0	0	1 (11%)
	Total	8	7	1	0	16
Phase Ib Expansion (N = 20)	Adverse Event Detail	Grade 1	Grade 2	Grade 3	Grade 4	Total
	Vomiting	6 (30%)	2 (10%)	1 (5%)	0	9 (45%)
	Nausea	5 (25%)	4 (20%)	0	0	9 (45%)
	Fatigue	2 (10%)	5 (25%)	0	0	7 (35%)
	Platelet count decreased	5 (25%)	2 (10%)	0	0	7 (35%)
	Anemia	1 (5%)	3 (15%)	2 (10%)	0	6 (30%)
	Anorexia	4 (20%)	2 (10%)	0	0	6 (30%)
	ALT increased	4 (20%)	0	0	1 (5%)	5 (25%)
	Dysgeusia	5 (25%)	0	0	0	5 (25%)
	AST increased	3 (15%)	0	0	1 (5%)	4 (20%)
	Diarrhea	1 (5%)	2 (10%)	1 (5%)	0	4 (20%)
	Creatinine increased	2 (10%)	2 (10%)	0	0	4 (20%)
	Hypothyroidism	1 (5%)	2 (10%)	0	0	3 (15%)
	Alopecia	2 (10%)	1 (5%)	0	0	3 (15%)
	Weight loss	0	2 (10%)	0	0	2 (10%)
	Constipation	1 (5%)	1 (5%)	0	0	2 (10%)
	Neuralgia	1 (5%)	1 (5%)	0	0	2 (10%)
	Colitis	0	0	1 (5%)	0	1 (5%)
	Thromboembolic event	0	0	1 (5%)	0	1 (5%)
	Total	43	29	6	2	80

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase

Reported are adverse events that were attributed as possibly, probably, or definitely related to study treatment, occurring in ≥ 10% of patients (or Grade 3). There were no Grade 5 adverse events. Percentages of events are shown for patients enrolled in individual study arms.

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Table 3:

Median overall survival and progression-free survival by PD-L1 status in ICI pretreated patients

PD-L1 Status		mOS, mo (95% CI)	mOS Hazard Ratio (95% CI)	mPFS, mo	mPFS Hazard Ratio (95% CI)	No.	No. of Refractory Patients	No. of Relapsed Patients
PD-L1	50%	5.1 (3.6-not estimable)	1.75 (0.68, 4.54)	2.8 (2.0-not estimable)	1.05 (0.41, 2.67)	9	2	6
PD-L1	1%–49%	10.6 (6.7-not estimable)	0.61 (0.18, 1.99)	6.7 (4.5-not estimable)	0.56 (0.17, 1.78)	7	2	3
*PD-L1 < 1%		7.4 (3.3-not estimable)		1.8 (1.0-not estimable)		16	10	3

Abbreviations: 95% CI, 95% confidence interval; mOS, median overall survival; mPFS, median progression-free survival; PD-L1, programmed cell death ligand 1.

* Reference group for hazard ratio.