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ORIGINAL RESEARCH

Phase Ib study of eprenetapopt (APR-246) in combination with pembrolizumab in patients with advanced or metastatic solid tumors☆

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Background: We conducted a phase I, multicenter, open-label, dose-finding, and expansion study to determine the safety and preliminary efficacy of eprenetapopt (APR-246) combined with pembrolizumab in patients with advanced/metastatic solid tumors (ClinicalTrials.gov NCT04383938).

Patients and methods: For dose-finding, requirements were non-central nervous system primary solid tumor, intolerant to/progressed after $\geq$1 line of treatment, and eligible for pembrolizumab; for expansion: (i) gastric/gastroesophageal junction tumor, intolerant to/progressed after first-line treatment, and no prior anti-programmed cell death receptor-1 (PD-1)/programmed death-ligand 1 (PD-L1) therapy; (ii) bladder/urothelial tumor, intolerant to/progressed after first-line cisplatin-based chemotherapy, and no prior anti-PD-1/PD-L1 therapy; (iii) non-small-cell lung cancer (NSCLC) with previous anti-PD-1/PD-L1 therapy. Patients received eprenetapopt 4.5 g/day intravenously (IV) on days 1-4 with pembrolizumab 200 mg IV on day 3 in each 21-day cycle. Primary endpoints were dose-limiting toxicity (DLT), adverse events (AEs), and recommended phase II dose (RP2D) of eprenetapopt.

Results: Forty patients were enrolled (median age 66 years; range 27-85) and 37 received eprenetapopt plus pembrolizumab. No DLTs were reported and the RP2D for eprenetapopt in combination was 4.5 g/day IV on days 1-4. The most common eprenetapopt-related AEs were dizziness (35.1%), nausea (32.4%), and vomiting (29.7%). AEs leading to eprenetapopt discontinuation occurred in 2/37 patients (5.4%). In efficacy-assessable patients ($n = 29$), one achieved complete response (urothelial cancer), two achieved partial responses (NSCLC, urothelial cancer), and six patients had stable disease.

Conclusions: The eprenetapopt plus pembrolizumab combination was well tolerated with an acceptable safety profile and showed clinical activity in patients with solid tumors.

Key words: clinical trial, eprenetapopt, pembrolizumab, solid tumors, p53

INTRODUCTION

The tumor suppressor protein p53 is a transcription factor that maintains genome stability by responding to stressors and mediating cell cycle arrest, apoptosis, and cellular senescence, and plays a role in the regulation of cellular metabolism.[1] Additionally, the p53 pathway has been implicated in antitumor immunity, including antigen presentation and T-cell activation,[2] suggesting a potential role for p53 stabilization in altering the tumor microenvironment and enhancing the targeting of tumor cells by the immune system.[3]

Eprenetapopt (APR-246) is a first-in-class, small-molecule p53 reactivator. It is a pro-drug that is spontaneously converted to the active moiety methylene quinuclidinone (MQ), which binds to wildtype and mutant p53 and stabilizes the folded and transcriptionally active conformation of the protein[4-6]; MQ also increases oxidative stress.[6,7] Eprenetapopt monotherapy was well tolerated and induced p53-dependent biologic effects in tumor cells in patients with hematologic

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malignancies and prostate cancer.[8] In clinical studies enrolling patients with hematologic malignancies, eprenetapopt was safe and showed clinical activity in combination with azacitidine.[9,10] Pembrolizumab is an anti-programmed death receptor-1 (PD-1) monoclonal antibody that enhances T-cell immune responses and is indicated for use across multiple solid tumor types.[11] Preclinical studies in mice utilizing p53-intact melanoma models have shown that mice overexpressing wildtype p53 showed enhanced T-cell-dependent tumor control with anti-PD-1 immunotherapy.[12] A similar effect was seen in p53-normal mice with coadministration of eprenetapopt, possibly due to a boosting effect resulting from biophysical stabilization of wildtype p53. Eprenetapopt treatment in combination with immune checkpoint blockade induced a pro-inflammatory tumor microenvironment by reprograming the myeloid cells that facilitated the infiltration and function of antitumor T cells. Furthermore, in melanoma and colorectal cancer mouse models with wildtype p53, there was reduced tumor growth with the combination of eprenetapopt and anti-PD-1 antibodies compared with monotherapy; improved survival was also seen in the melanoma model, and these effects were both p53 and T-cell dependent.[12] The antitumor activity observed in these preclinical models provided a rationale for testing this combination in the clinical setting, particularly in patients who were refractory to or progressed after immuno-oncology (IO) therapy.

Therefore, we conducted a phase I dose-finding and expansion study to determine the safety and preliminary efficacy of eprenetapopt in combination with pembrolizumab in patients with solid tumor malignancies in which IO therapy has established efficacy.

PATIENTS AND METHODS

Study design

This was a multicenter, open-label, dose-finding, and expansion study of eprenetapopt (APR-246) in combination with pembrolizumab in advanced or metastatic solid tumors, conducted at nine academic research hospitals in the United States (ClinicalTrials.gov number, NCT04383938). The primary objectives were to evaluate safety and tolerability of the combination regimen and determine the maximum tolerated dose (MTD) for eprenetapopt in this combination. Secondary objectives included determining preliminary efficacy signals. Screening/baseline evaluations were carried out within 28 days of study treatment initiation. The trial was conducted according to principles of the Declaration of Helsinki, Good Clinical Practice, and applicable regulatory requirements. The protocol, consent procedures, and any amendments were approved by relevant institutional review boards or ethics committees. All patients provided written informed consent before study participation.

Dose-finding and expansion

The dose-finding portion followed a standard 3 + 3 dose de-escalation design, with each cohort enrolling three to six patients. Dose-limiting toxicity (DLT) was assessed after three patients had been enrolled in a dose-finding cohort and the last enrolled patient had completed the 3-week safety assessment period (i.e. one cycle of combination regimen). DLTs were classified and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 and defined as follows: any of the protocol-defined hematological or nonhematological toxicities (described in the following text) considered to be at least possibly related to eprenetapopt occurring during the 3-week safety assessment period after the start of study drug combination administration; failure to administer $\geq$75% of the planned dosage of eprenetapopt as a result of treatment-related toxicity during cycle 1 unless related to reversible central nervous system (CNS) effects previously described; discontinuation of treatment due to treatment-related toxicity; or a >4-week delay in starting cycle 2 because of a treatment-related toxicity, even if the toxicity did not meet DLT criteria. Hematological toxicity was defined as: grade 4 neutropenia for $\geq$7 days; grade 3 or grade 4 febrile neutropenia [grade 3: absolute neutrophil count $<$ 1000/mm^3 with a single temperature of >38.3°C (101°F) or a sustained temperature of $\geq$38°C (100.4°F) for >1 h; grade 4: absolute neutrophil count $<$ 1000/mm^3 with a single temperature of >38.3°C (101°F) or a sustained temperature of $\geq$38°C (100.4°F) for >1 h, with life-threatening consequences and urgent intervention indicated]; or thrombocytopenia <25 000/mm^3 associated with bleeding and/or that requires platelet transfusion. Other nonhematologic toxicities were defined as: any other grade 4 or a grade 5 toxicity; grade 3 toxicities lasting >3 days (excluding nausea, vomiting, fatigue, and diarrhea controlled by medical intervention within 72 h and grade 3 rash in the absence of desquamation, no mucosal involvement, did not require steroids, and resolved to grade 1 by the next scheduled dose of pembrolizumab); grade 3 hypertension not controlled by medication; grade 3 or above gastrointestinal perforation; grade 3 or above wound dehiscence requiring medical or surgical intervention; any-grade thromboembolic event; or any grade 3 nonhematologic laboratory value if medical intervention was required to treat the patient or the abnormality led to hospitalization.

The recommended phase II dose (RP2D) of eprenetapopt was defined as the dose at which less than two of six patients in a dose cohort experienced a DLT during the 3-week safety assessment period after administration of eprenetapopt in combination with pembrolizumab.

The expansion portion was initiated once the RP2D had been determined and comprised three cohorts: gastric/gastroesophageal junction (GEJ) cancer [anti-PD-1/anti-programmed death-ligand 1 (PD-L1)-naïve], bladder/urothelial cancer (anti-PD-1/PD-L1-naïve), and non-small-cell lung cancer (NSCLC; prior anti-PD-1/PD-L1 therapy required).

Patients

Key inclusion criteria were known *TP53* mutation status from recent or archival sample (presence of *TP53* mutation

was not required); age ≥ 18 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0-2; projected life expectancy of ≥12 weeks; and histologically and/or cytologically confirmed solid tumor malignancy. In the dose-finding cohort, patients were eligible if they had an advanced non-CNS primary tumor and were unable to receive, were intolerant to, or had progressed after ≥1 line of treatment, and if pembrolizumab-based therapy was considered appropriate by the investigator. For the expansion cohort, advanced tumors in the following subgroups were eligible: (i) patients with gastric or GEJ tumors who were unable to receive, were intolerant to, or had progressed after first-line treatment and had not received prior anti-PD-1/PD-L1 therapy; (ii) patients with bladder/urothelial tumors who were unable to receive, were intolerant to, or had progressed after first-line treatment with cisplatin-based chemotherapy and had not received prior anti-PD-1/PD-L1 therapy; (iii) patients with NSCLC who had been previously treated with anti-PD-1/PD-L1 therapy. For expansion, measurable disease meeting the following criteria was required: at least one lesion ≥10 mm in the longest diameter for a non-lymph node or ≥15 mm in the short-axis diameter for a lymph node that was serially measurable according to RECIST version 1.1.[13] For both dose-finding and expansion, patients with clinically stable metastatic CNS tumors were eligible with medical monitor approval (CNS imaging was not required in the absence of clinical suspicion).

Key exclusion criteria included concomitant malignancies or previous malignancies with a <1-year disease-free interval at the time of consent [adequately treated basal/squamous cell carcinoma of the skin and carcinoma in situ (e.g. cervix), and advanced prostate cancer were permitted]; an autoimmune condition requiring ≥10 mg prednisone (or equivalent corticosteroid) daily, or any other systemic immunosuppressive treatment within 28 days of first dose of study therapy; or any investigational product within 14 days or five half-lives before study treatment initiation, whichever was shortest.

Treatment

Given the minimal overlap of adverse events (AEs) between eprenetapopt and pembrolizumab, and the absence of presumed drug−drug interactions based on disparate metabolism, the initial starting dose of eprenetapopt was 4.5 g/day. In the initial dose-finding cohort, patients received eprenetapopt 4.5 g/day intravenously (IV) on days 1-4 as a 6-h infusion with pembrolizumab 200 mg IV on day 3 as a 30-min infusion (before the eprenetapopt infusion) in each 21-day cycle. The eprenetapopt fixed dose of 4.5 g was administered in a two-step infusion: (i) loading dose of 1.5 g for the first 45 min (± 2 min); (ii) maintenance dose of 3 g over 5 h 15 min (± 30 min). The eprenetapopt dose could be reduced or treatment interrupted if a patient developed AEs. Details of the planned eprenetapopt dose de-escalation (if required) for the dose-finding portion are provided in the following section. Treatment could be administered on an outpatient basis. Patients remained on study treatment until the end of the trial while deriving clinical benefit, unless there was unacceptable toxicity, progression, death, or patient withdrawal. Patients could remain on therapy after progression if continuing to derive clinical benefit in the opinion of the investigator.

Planned dose de-escalation

The initial cohort of patients was to enroll up to a maximum of six patients. A patient that discontinued therapy during cycle 1 without DLT was considered assessable for the purpose of safety only if at least 75% of scheduled doses of eprenetapopt were administered in the first cycle. At the first dose level of 4.5 g/day of eprenetapopt, if ≤1 patient out of 3 experienced DLT, 3 additional patients were to be enrolled. If ≤1 patient out of 6 experienced DLT, the dose level (4.5 g/day of eprenetapopt) would be deemed the RP2D for that cohort. If ≥2 patients out of the total 3-6 patients in the cohort experienced DLT, the study was to continue enrollment at dose level −1 (4.0 g/day of eprenetapopt). If ≤1 patient out of 6 experienced DLT at this dose level, the dose level (4.0 g/day of eprenetapopt) would be deemed the RP2D for that cohort. If ≥2 patients out of the total 3-6 patients at that dose level experienced DLT, the study would continue enrollment at dose level −2 (3.5 g/day of eprenetapopt). If ≤1 patient out of 6 experienced DLT at that dose level, the dose level (3.5 g/day of eprenetapopt) would be deemed the RP2D for that cohort. If ≥2 patients out of the total 3-6 patients at that dose level experienced DLT, the trial was to be halted and the data review team would consider potential future dosing modifications. No dose reductions in pembrolizumab were planned.

Concomitant medication

Patients were not permitted to receive any other concurrent anticancer therapy, including investigational anticancer agents, while on study treatment. Patients could continue their baseline medication(s) as long as they were not prohibited. Prohibited mediations included systemic immunosuppressive treatment (e.g. prednisone ≥10 mg/day or equivalent corticosteroid), live vaccines, and investigational antitumor products. Palliative and supportive care (e.g. anti-emetics, bisphosphonates) for disease-related symptoms could be utilized according to institutional practices. AEs were treated as clinically indicated. All concomitant medications should have been recorded in the electronic case report form.

If a patient developed an acute infusion reaction (grade ≥2), the infusion was to be interrupted until the reaction resolved to grade ≤1. Premedication (e.g. systemic corticosteroids) could be used as required.

Study assessments

The primary endpoints were DLTs, frequency of treatment-emergent AEs, and serious AEs (SAEs) related to eprenetapopt in combination with pembrolizumab, and the RP2D of eprenetapopt. Safety assessments included AEs, vital signs,

laboratory data, electrocardiogram, and physical examination. AEs were coded using the Medical Dictionary for Regulatory Activities and severity was graded using National Cancer Institute Common Terminology Criteria for Adverse Events v5.0.

Secondary endpoints included the overall response rate (ORR) and clinical benefit rate (CBR). ORR was defined as the proportion of patients with best overall response (BOR) of complete response (CR) or partial response (PR) by RECIST 1.1, measured from treatment start date until date of death from any cause. Patients lost to follow-up and those alive at the date of data cut-off were censored at the last known alive date. CBR was defined as the proportion of patients who had BOR of CR, PR, or durable stable disease (SD; $\geq$23 weeks). Radiological disease assessment was conducted every 9 weeks ($\pm$3 days) after initiating study treatment, before initiation of each odd treatment cycle starting at week 9, then every 6 weeks through the first year and then every 9 weeks, thereafter. Tumor assessments were carried out by investigators based on RECIST 1.1. Patients who responded and discontinued study treatment for reasons other than progressive disease had response assessments every 2 months until disease progression or death.

Statistical analysis

The safety population included all patients receiving at least one dose of eprenetapopt. The efficacy-assessable population included all patients who completed at least one treatment cycle of eprenetapopt plus pembrolizumab and who had at least one post-treatment clinical response assessment. Patients who failed to complete one treatment cycle were included if they showed clear evidence of clinically significant disease progression.

Data outputs are descriptive in nature and formal statistical analyses were not conducted.

The planned sample size was up to 18 patients in the dose-finding portion and up to 100 patients in expansion. For the expansion cohorts, previously reported ORRs with pembrolizumab in patients who had relapsed after or were refractory to previous chemotherapies of 21.1% for urothelial cancer[14] and 22.7% for PD-L1-positive gastric/GEJ adenocarcinoma[15] were considered. The ORR for patients with advanced NSCLC who had previously been treated with anti-PD-1/PD-L1 therapy was expected to be negligible ($\leq$ 20%). Thus, the expected response rate to the combination therapy across indications was $\sim$20%-30%. In order to increase the estimate precision, at least 20 assessable patients were to be included in each of the three cohorts. If the sample size was 20 patients, at least two responders were needed to be over the 95% confidence interval (CI) lower boundary for a 20% ORR (95% CI 5.7% to 43.7%).

RESULTS

Patients

Patients were enrolled from 10 August 2020 to 27 September 2021. The cut-off date for this analysis was 16 February 2022. Median duration of follow-up was 373 days (95% CIs not evaluable). Demographic and disease characteristics for all patients enrolled are shown in Table 1. The median age was 66 years (range 27-85 years) and 33 patients (82.5%) had tumors with *TP53* mutations. Patient disposition is shown in Figure 1. Overall, 37 patients were treated with eprenetapopt plus pembrolizumab (6 in the dose-finding cohort and 31 in the expansion cohort) and at the cut-off date 2 patients remained on treatment. The median number of treatment cycles completed was 2 (range 1-13).

Table 1. Baseline demographic and clinical characteristics

Characteristic	All patients enrolled (N = 40)
Age in years, median (range)	66 (27-85)
Sex, *n* (%)	
Female	17 (42.5)
Male	23 (57.5)
ECOG PS, *n* (%)	
0	4 (10.0)
1	33 (82.5)
2	3 (7.5)
Race, *n* (%)	
White	31 (77.5)
Black	4 (10.0)
Asian	3 (7.5)
Not reported	2 (5.0)
Diagnosis, *n* (%)	
NSCLC	22 (55)
Gastric/GEJ	10 (25)
Bladder/urothelial	5 (12.5)
Other (prostate and colon)	3 (7.5)
Number of prior therapies, median (range)	
NSCLC	5 (1-8)
Gastric/GEJ	4 (1-11)
Bladder/urothelial	1 (0-2)
Other (prostate and colon)	8 (1-14)
TP53 mutation present, *n* (%)[a]	33 (82.5)
Type of *TP53* mutation, *n* (%)[b]	
Missense	20 (50)
Frameshift	6 (15)
Nonsense	1 (2)
Splice site	4 (10)
Multiple types	2 (5)
Mutation or copy number alteration in other genes, *n* (%)[c]	34 (85)
PD-L1 expression, *n* (%)	
Known	24 (60)
Positive[d]	16 (40)
Prior IO treatment in NSCLC, *n* (%)[e]	
Anti-PD-1	18 (82)
Anti-PD-L1	11 (50)
Anti-CTLA-4	3 (14)

CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ECOG PS, Eastern Cooperative Oncology Group performance status; GEJ, gastroesophageal junction; IO, immuno-oncology; NSCLC, non-small-cell lung cancer; PD-1, programmed death receptor 1; PD-L1, programmed death-ligand 1.
[a]Results from local testing on tumor tissue or blood-circulating free DNA.
[b]More than one *TP53* mutation of the same type may be present.
[c]At least one non-variant of uncertain significance mutation, copy number gain, or copy number loss reported in a gene other than *TP53* by local testing on tumor tissue or blood-circulating free DNA.
[d]Tumor proportion score or combined positive score $\geq$ 1.
[e]Denominator is 22 (1 patient with NSCLC in the dose-finding cohort and 21 with NSCLC in the expansion cohort).

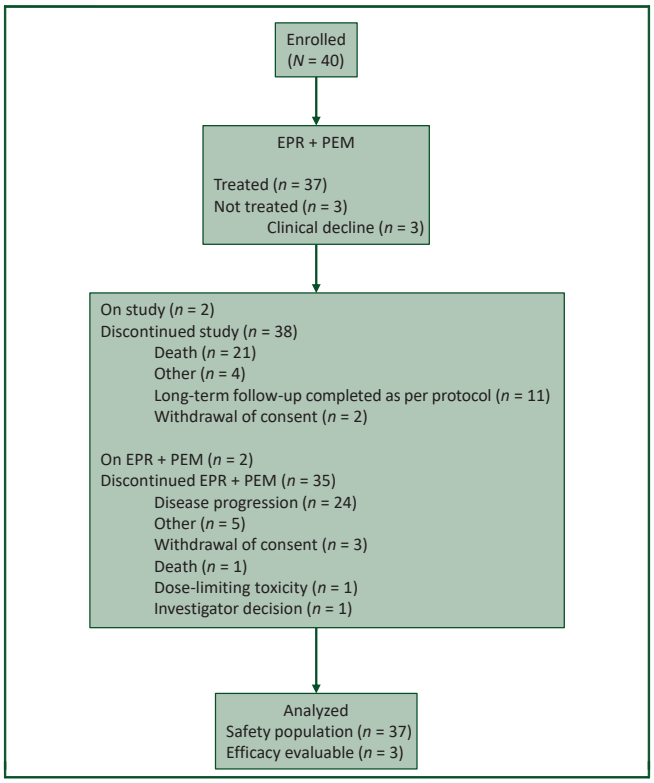

Figure 1. Patient disposition.
EPR, eprenetapopt; PEM, pembrolizumab.

Safety

No DLTs were reported in the initial dose-finding cohort ($n = 6$); therefore, the MTD was not defined and the RP2D for the expansion phase was determined to be eprenetapopt 4.5 g/day IV on days 1-4 in combination with pembrolizumab.

Overall findings in the safety population ($n = 37$) are summarized in Table 2. The most common AEs (>10% of patients) and corresponding all-grade eprenetapopt-related AEs are shown in Table 3. Grade ≥3 AEs occurred in 16 patients (43.2%; Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2022.100573). The only eprenetapopt-related grade ≥3 AE occurring in more than one patient was grade 3 dizziness ($n = 2$, 5.4%). SAEs are summarized in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2022.100573.

Immune-related AEs (irAEs) were reported in three patients (8.1%), all of whom were enrolled in the bladder/urothelial cancer cohort. The majority of irAEs in these three patients were grades 1 and 2; grade 3 irAEs included myalgia ($n = 1$, 2.7%), arthralgia ($n = 1$, 2.7%), and abdominal pain ($n = 1$, 2.7%). There were no grade 4 or 5 irAEs.

AEs leading to eprenetapopt interruption occurred in 11 patients (29.7%), with dizziness ($n = 3$, 8.1%) and hypotension ($n = 2$, 5.4%) occurring in more than one patient. AEs leading to eprenetapopt dose reduction occurred in three patients (8.1%): nausea ($n = 2$, 5.4%), dizziness ($n = 1$, 2.7%), and confusional state ($n = 1$, 2.7%). AEs leading to permanent discontinuation of eprenetapopt therapy occurred in two patients (5.4%) and all were non-serious; one patient experienced dyspnea (grade 2), vertigo (grade 2), and muscular weakness (grade 1) and the other experienced dyspnea, fatigue, and maculopapular rash (all grade 3).

Three patients experienced a fatal AE; all were assessed as not related to study treatment (disease progression, $n = 2$ and hemoptysis, $n = 1$). From the first dose, 30- and 60-day mortality was 0% and 13.5% ($n = 5$), respectively.

Table 2. Overall safety summary

Event	Safety population ($n = 37$), n (%)
Any AE	34 (91.9)
Grade ≥3	16 (43.2)
Any eprenetapopt-related AE	28 (75.7)
Grade ≥3	8 (21.6)
Any SAE	15 (40.5)
Treatment-related SAEs	4 (10.8)
AEs leading to dose interruption of eprenetapopt	11 (29.7)
AEs leading to dose reduction of eprenetapopt	3 (8.1)
AEs leading to discontinuation of eprenetapopt	2 (5.4)
AEs leading to death	3 (8.1)

AE, adverse event; SAE, serious adverse event.

Clinical activity

In the efficacy-assessable population ($n = 29$), the ORR was 10.3% ($n = 3$), with one patient achieving a CR (urothelial cancer) and two achieving a PR (NSCLC and urothelial cancer). The CBR was 13.8% ($n = 4$), comprising the CR, two

Table 3. Most common all-grade AEs (>10% of patients) and corresponding all-grade eprenetapopt-related AEs

AE	Safety population ($n = 37$)	
	All grade all cause, n (%)	All-grade-related, n (%)
Dizziness	15 (40.5)	13 (35.1)
Nausea	14 (37.8)	12 (32.4)
Vomiting	12 (32.4)	11 (29.7)
Decreased appetite	11 (29.7)	5 (13.5)
Constipation	10 (27.0)	3 (8.1)
Fatigue	10 (27.0)	7 (18.9)
Dyspnea	9 (24.3)	2 (5.4)
Abdominal pain	8 (21.6)	2 (5.4)
Anemia	8 (21.6)	3 (8.1)
Diarrhea	7 (18.9)	6 (16.2)
Alanine aminotransferase increased	6 (16.2)	2 (5.4)
Tremor	5 (13.5)	4 (10.8)
Hyponatremia	5 (13.5)	0
Headache	4 (10.8)	4 (10.8)
Hyperglycemia	4 (10.8)	0
Pyrexia	4 (10.8)	3 (8.1)
Aspartate aminotransferase increased	4 (10.8)	1 (2.7)
Blood alkaline phosphatase increased	4 (10.8)	0
Back pain	4 (10.8)	0
Muscular weakness	4 (10.8)	2 (5.4)
Hypotension	4 (10.8)	1 (2.7)
Confusional state	3 (8.1)	3 (8.1)

AE, adverse event.

Table 4. Summary of responses in patients with clinical benefit (CR + PR + durable SD[a])

Patient	Tumor type	Age, sex	*TP53* status	Other baseline mutations	PD-L1 expression	Prior treatment	BOR	DOR	Response trajectory
1	High-grade urothelial bladder cancer, locally advanced	75 years, male	Mutant p.G244C c.730G>T 47.69% Tier 2 PCS	*ERBB2* (Tier 2 PCS), *TERT* (Tier 2 PCS), *ERBB2* amplification, 12 VUS, MSI-low	Unknown	Neoadjuvant platinum-based CT followed by radical cystectomy (ypT2, pN2, cM0); 3 months later had increased retroperitoneal, mediastinal, and left supraclavicular adenopathy	CR	172 days (censored at last follow-up)	First response assessment at 9 weeks showed resolution of lymphadenopathy
2	Squamous NSCLC	85 years, male	Mutant Splice site c.97-1G>A	*CDKN2A* copy loss, *CDKN2B* copy loss, *CUL4A* amplification, *IRS2* amplification, *MTAP* copy loss, MYC amplification, TMB = 11/Mb, MSI-stable	Result = 0	Carboplatin/ paclitaxel/RT and progression on atezolizumab	PR	Durable SD of 266 days, then achieved PR with a duration of 45 days (ongoing, censored at data cut-off)	First response assessment at 9 weeks showed reduction in target lesions of 26.7% from baseline. Durable SD by RECIST, confirmed at 15 and 21 weeks. At ~48 weeks, patient achieved PR with 30.4% reduction of target lesions from baseline
3	Metastatic urothelial carcinoma	71 years, female	Wildtype	TERT (Tier 2 PCS), VUS in *ARID1A* and *DOT1L*, MSI-stable	Unknown	No prior treatment or RT, unable to receive platinum-based therapy	PR	64 days (ongoing, censored at data cut-off)	PR on first restaging scan (>30% shrinkage of target lesions). Slight increase in target lesion on subsequent scan but majority of disease under control
4	Squamous NSCLC	55 years, male	Mutant Splice site SNV 1.9% p.R306* 1.3% p.V173L 0.2% p.H214R 0.1% p.R248W 1.4% p.R175H 0.8% (+1 VUS)	6 VUS, TMB = 61.13/Mb, MSI-high not detected	Negative	Wedge resection of lobes, carboplatin + nab-paclitaxel ×2, nab-paclitaxel, PD on nivolumab, docetaxel and RT	SD ≥ 23 weeks	204 days	First response assessment at 9 weeks showed reduction in total measurable disease of 8.2% from baseline. SD by RECIST, confirmed at 15, 21, and 27 weeks. PD noted at 33-week response assessment

Asterisk denotes a nonsense mutation.

BOR, best overall response; CR, complete response; CT, chemotherapy; DOR, duration of response; Mb, megabase; MSI, microsatellite instability; NSCLC, non-small-cell lung cancer; PCS, potential clinical significance; PD, progressive disease; PD-L1, programmed death-ligand 1; PEM, pembrolizumab; PR, partial response; RT, radiotherapy; SD, stable disease; SNV, single-nucleotide variant; TMB, tumor mutational burden; VUS, variant of uncertain significance.

[a]Durable SD defined as ≥ 23 weeks.

PRs, and one patient (NSCLC) who achieved durable SD (≥23 weeks); responses are summarized in Table 4. Five patients achieved SD of ≥5 weeks (one in dose escalation and four in dose expansion). The patient with urothelial bladder cancer achieving PR and one patient with NSCLC achieving SD ≥5 weeks had wildtype *TP53*; the remaining patients had mutant *TP53*.

DISCUSSION

In this dose-finding and expansion study, the combination of eprenetapopt and pembrolizumab was well tolerated and had an acceptable safety profile in patients with solid tumors. There were no DLTs in the dose-finding cohort. The most common all-grade eprenetapopt-related AEs were dizziness (35.1%), nausea (32.4%), and vomiting (29.7%). The only eprenetapopt-related grade ≥3 AE occurring in more than one patient was dizziness, a known side-effect of eprenetapopt, which occurred in two patients (both grade 3 AEs). Two patients discontinued eprenetapopt due to non-serious AEs of dyspnea ($n = 2$, grades 2 and 3), fatigue ($n = 1$, grade 3), maculopapular rash ($n = 1$, grade 3), vertigo ($n = 1$, grade 2), and muscular weakness ($n = 1$, grade 1). AEs were manageable with standard-of-care measures and administration in the outpatient clinic was feasible.

Preclinical studies with eprenetapopt have demonstrated remarkable efficacy in augmenting tumor control in combination with immune checkpoint blockade.[12] Some of these T-cell-facilitating effects of eprenetapopt are mediated by p53-dependent regulation of the nuclear factor kappa B pathway in the tumor-associated macrophages, which induces T-cell-promoting cytokines such as interferon-γ and interleukin-12 and inhibits T-cell-suppressing metabolites such as indolamine-2,3-dioxygenase and arginine.[16] Boosting the p53 pathway with eprenetapopt treatment thus reprograms the tumor microenvironment to facilitate T-cell infiltration, thereby reinvigorating antitumor T-cell responses mediated by anti-PD-1 therapy; yet, some other effects of eprenetapopt are p53 independent and mediated by cell autonomous increase of antigenicity of tumors via induction of endoplasmic reticulum stress and oxidative stress.[17] Thus, eprenetapopt can promote antitumor activity of immune checkpoint inhibitors such as pembrolizumab by facilitating T-cell infiltration, tumor recognition, and killing of tumor cells.

The preliminary clinical activity of one CR in a patient with locally advanced high-grade urothelial cancer and two PRs (one patient with squamous NSCLC who had prior IO therapy and one patient with metastatic urothelial carcinoma) is encouraging. Furthermore, clinical benefit was observed in six patients with SD, one of whom had durable SD of ≥23 weeks. The patient achieving CR, one patient achieving PR, and the patient achieving durable SD had tumors harboring mutant *TP53*, which is associated with poor prognosis in many solid tumors including lung and bladder cancer.[18]

To our knowledge, this is the first clinical trial evaluating the combination of a p53 reactivator with IO therapy and demonstrates that the combination of eprenetapopt and pembrolizumab is well tolerated, with clinical activity in heavily pre-treated patients with solid tumors. The limitations of the study are those inherent to an early dose-finding study, and the sample size and objectives were not designed to permit a formal assessment of efficacy. Another potential weakness is that, though a drug—drug interaction between eprenetapopt and pembrolizumab is very unlikely based on the known pharmacokinetic (PK) properties of eprenetapopt and pembrolizumab, formal PK studies of either entity in this combination are not available at the time of publication. Additionally, the IO-naïve gastric/GEJ and bladder cancer cohorts enrolled a limited number of patients before the closing of enrollment due to changes in standard practice to incorporate immunotherapy in first-line treatment. Given the promising findings of this trial, randomized studies to further evaluate this combination are warranted in patients with both wildtype and mutant *TP53* tumors to better characterize the antitumor activity and determine tumor subsets likely to respond. In addition, exploration of less-intensive dosing regimens and more convenient formulations, such as the oral mutant p53 reactivator APR-548, is warranted in an effort to improve patient convenience and increase dose exposure.

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