Pharmacological ascorbate improves the response to platinum-based chemotherapy in advanced stage non-small cell lung cancer

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ABSTRACT

Purpose: Platinum-based chemotherapy with or without immunotherapy is the mainstay of treatment for advanced stage non-small cell lung cancer (NSCLC) lacking a molecular driver alteration. Pre-clinical studies have reported that pharmacological ascorbate (P-AscH-) enhances NSCLC response to platinum-based therapy. We conducted a phase II clinical trial combining P-AscH- with carboplatin-paclitaxel chemotherapy.

Experimental design: Chemotherapy naïve advanced stage NSCLC patients received 75 g ascorbate twice per week intravenously with carboplatin and paclitaxel every three weeks for four cycles. The primary endpoint was to improve tumor response per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 compared to the historical control of 20%. The trial was conducted as an optimal Simon two-stage design. Blood samples were collected for exploratory analyses.

Results: The study enrolled 38 patients and met its primary endpoint with an objective response rate of 34.2% (p = 0.03). All were confirmed partial responses (cPR). Median progression-free and overall survival were 5.7 months and 12.8 months, respectively. Treatment-related adverse events (TRAE) included one grade 5 (neutropenic fever) and five grade 4 events (cytopenias). Cytokine and chemokine data suggest that the combination elicits an immune response. Immunophenotyping of peripheral blood mononuclear cells demonstrated an increase in effector CD8 T-cells in patients with a progression-free survival (PFS) ≥ 6 months.

Conclusions: The addition of P-AscH- to platinum-based chemotherapy improved tumor response in advanced stage NSCLC. P-AscH- appears to alter the host immune response and needs further investigation as a potential adjuvant to immunotherapy.

1. Introduction

Lung cancer is the leading cause of cancer-related death in the United States, accounting for more deaths annually than breast, prostate, and colorectal cancers combined. Non-small cell lung cancer (NSCLC) comprises 85% of new lung cancer cases. Unfortunately, most patients...
are diagnosed with advanced disease [1]. Immunotherapy with or without a platinum-based doublet chemotherapy is considered a standard first-line treatment for most patients with advanced stage NSCLC lacking a molecular driver alteration [2-5]. The benefit of anti-programmed death-1 (PD-1) or programmed death ligand 1 (PD-L1) immunotherapy in advanced stage NSCLC correlates with PD-L1 expression on the tumor. Platinum-based chemotherapy therefore is the mainstay of treatment or the backbone of combination therapies for NSCLC patients lacking PD-L1 expression [3-5]. Unfortunately, these patients often have poor outcomes, leaving a significant need to develop more effective and safe therapies.

Pharmacological ascorbate (P-AscH), intravenous infusions resulting in millimolar concentration of ascorbate in plasma) is a potential adjunct to chemotherapy [6-8]. P-AscH is selectively toxic to NSCLC and relatively innocuous to non-malignant cells [9]. P-AscH’s selective cancer cell toxicity is due to fundamental differences in oxidative metabolism; more specifically, iron metabolism between malignant and non-malignant cells [9,10]. Malignant cells have increased steady-state levels of reactive oxygen species (e.g., superoxide (O₂⁻), hydrogen peroxide (H₂O₂)), which can react with iron-containing proteins (e.g., iron-sulfur cluster proteins and ferritin) to release redox active iron. In the presence of P-AscH, redox active ferric iron is reduced to ferrous iron, leading to the formation of H₂O₂. H₂O₂ can be directly toxic to cells by oxidizing critical biomolecules or reacting with Fe²⁺ to produce hydroxyl radicals (HO•) via Fenton chemistry. HO• can damage proteins, lipids, and DNA, leading to platinum-chemotherapy sensitization. Non-malignant cells have lower endogenous levels of redox active iron, H₂O₂, and O₂⁻, leading to less H₂O₂ generated following P-AscH treatment.

Pre-clinical in vitro and in vivo studies have shown that P-AscH sensitizes a variety of solid tumors to chemotherapy and radiation [9]. In early phase clinical trials P-AscH has been evaluated with concurrent temozolamide and radiation in patients with glioblastoma, with gemcitabine in locally advanced stage pancreatic cancer, and with carboplatin-paclitaxel in ovarian cancers. No dose-limiting toxicities were observed in these trials [11-13]. Treatment-related adverse events (TRAE) were similar to the expected toxicity profile associated with standard anti-cancer therapies alone. Moreover, in these early phase, single arm studies subject outcomes were superior to historical controls.

Based on its ability to enhance the cytotoxic effects of chemotherapy and the safety profile, P-AscH is a promising adjunct therapy to platinum chemotherapy in patients with advanced NSCLC. This phase II trial explored the efficacy and safety of the combination of P-AscH with carboplatin and paclitaxel in chemotherapy-naive patients with advanced-stage NSCLC (NCT02420314).

2. Materials and methods

2.1. Patient selection

This open-label, single-arm, non-randomized phase II study, enrolled patients aged 18 years or older with pathologically-confirmed recurrent or stage IV NSCLC who had not received prior systemic therapy for advanced stage disease. Additional criteria for recruitment included an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2, measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, and adequate hematologic, hepatic, and renal function. Patients with a known alteration in EGFR or ALK were allowed to enroll after progression on an approved tyrosine kinase inhibitor. In Patients with treated brain metastases were also allowed to participate. Exclusion criteria included the presence of active hemoptysis, leptomeningeal disease, or glucose-6-phosphate-dehydrogenase deficiency. In December 2016, the protocol was modified to allow patients with PD-L1 tumor proportion score (TPS) ≥50% to enroll in the trial after progression on immunotherapy.

2.2. Treatment

Eligible participants were planned to receive 75 g ascorbate intravenously twice per week for 12 weeks in combination with carboplatin (AUC 6) and paclitaxel (200 mg/m²) every three weeks for 4 cycles. Chemotherapy dose modifications were allowed for toxicity as per established standards. Ascorbate infusions were administered at a fixed dose. After completing four cycles (C) of chemotherapy, patients without progressive disease could receive maintenance or consolidation therapy at the discretion of the treating provider.

2.3. Study objectives

The primary objective of the study was tumor response rate per RECIST v1.1. Tumor assessments were performed after chemotherapy cycles two and four. Participants who achieved partial or complete response underwent confirmatory tumor assessment >4 weeks following the initial imaging demonstrating the response. Patients with a partial response (PR) or stable disease (SD) were followed for disease progression (DP) and survival as per standard of care unless they withdrew consent. Secondary endpoints included progression-free survival (PFS), progression-free survival 2 (PFS2), overall survival (OS), and safety. All participants who received P-AscH per protocol were included in the safety analysis. Adverse events (AEs) were graded per Common Terminology Criteria for Adverse Events (CTCAE) v4.03. After a protocol modification, patient-reported outcome measures were added as an exploratory endpoint and assessed using various Functional Assessment of Cancer Therapy (FACT) scales and subcales.

2.4. Biomarkers analysis

PD-L1 expression was evaluated by immunohistochemistry with 22C3 (Dako, Carpinteria, CA) or E1L3N (Dako Autostainer Link 48) assays. Somatic alterations including substitutions, small deletions and insertions, copy number changes, and microsatellite instability was performed using Ampliseq-based next generation sequencing (NGS) with a 213 gene custom panel on a NextSeq 550 (Illumina Inc., San Diego, CA). Evaluation for gene rearrangements was performed by testing fusion transcripts using the Comprehensive Thyroid Lung FusionPlex Panel (Invitae Corp., San Francisco, CA) on a MiSeq (Illumina, Inc.). All testing was performed at the Clinical Laboratory Improvement Amendments-certified molecular pathology laboratory at the University of Iowa.

Blood and serum samples were collected at baseline, before each chemotherapy cycle, and at follow-up (C4d21 ± 7d) for exploratory analysis. Laboratory studies included pre- and post-therapy serum iron, ferritin, transferrin, transferrin saturation, and total iron binding capacity (TIBC). Redox-active serum markers, including 4-hydroxy-2-nonenal (4HNE) modified proteins, 3-nitrotyrosine (3NT), protein carbonyl, cytokines, and chemokines were also evaluated pre- and post-therapy. Plasma ascorbate levels were measured once during each chemotherapy cycle within 15 min of ascorbate infusions. Serum 4HNE, 3NT, and protein carbonyl assays were performed as previously described [14].

The levels of 38 cytokines and chemokines were determined using the LEGENDplex Human Cytokine Panel 2, LEGENDplex Human Th Cytokine Panel, and LEGENDplex Human Proinflammatory Chemokine Panel 2 (Biolegend, San Diego, CA). Serum samples were thawed and diluted 2-fold with assay buffer supplied by BioLegend. The multiplex cytokine assays were then performed in filter plates per manufacturer’s protocol. The samples were transferred to flat-bottomed 96-well plates and cytokine levels were determined using a Cytek Aurora flow cytometer (Cytek Biosciences, Fremont, CA).

For immunophenotyping, fresh whole blood samples were lysed using 1X Vitalyte (CytoMedical Design Group, St. Paul, MN). Cells were stained for extracellular cell surface markers using antibodies for CD3
(OKT3; BioLegend), CD4 (RPA-T4; Thermo Fisher, Pittsburgh, PA), CD8 (RPA-T8; Thermo Fisher), CCR7 (G043H7; BioLegend), CD45RA (HI100; Thermo Fisher), CD38 (HIT2; Thermo Fisher), HLA-DR (L243; BioLegend), CD127 (A019D5; BioLegend), CD25 (BC96; Thermo Fisher), CCR4 (L291H4; BioLegend), CD19 (HB19; Thermo Fisher), CD20 (2H7; Thermo Fisher), CD14 (61D3; Thermo Fisher), and CD16 (3G8; BioLegend) for 30 min at 4 °C. All cells were fixed with fix/lyse solution (eBioscience, San Diego, CA) for 10 min at room temperature. Cells were run on an LSRFortessa (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (BD Biosciences). Immune cell populations were gated as follows: CD4 T cells (CD3+CD4+), CD8 T cells (CD3+CD8+), activated effector CD4 T cells (CD3+CD4+CCR7+CD45RA+CD38+HLA-DR+), activated effector CD8 T cells (CD3+CD8+CCR7+CD45RA+CD38+HLA-DR+), classical monocytes (CD3+CD19+CD20+HLA-DR+CD14+CD16+), intermediate monocytes (CD3+CD19+CD20+HLA-DR+CD14+CD16+), non-classical monocytes (CD3+CD19+CD20+HLA-DR’CD14−CD16’), regulatory T cells (CD3+CD4+CD25+CCR4+).

2.5. Statistical methods

The primary objective of this phase II trial was to evaluate the antitumor activity of P-AscH combined with platinum-chemotherapy by testing the null statistical hypothesis that the best overall response rate is <20% [15,16] versus the alternative that it is greater. The best overall response was defined as a confirmed complete or partial response. The trial was conducted as an optimal Simon’s two-stage design with 80% power to detect a response rate of 40% with one-sided testing performed at the 10% level of significance. Sample size calculations required 17 patients to be enrolled in the first stage of the study with termination if three or fewer responded. Otherwise, an additional 20 patients were to be evaluated in the second stage. If 11 or more of the total 37 patients responded, the treatment would be deemed worthy of further investigation.

The primary statistical analysis focused on the best overall response rate estimated as a binomial proportion along with a one-sided 90% confidence interval computed with the methods of Koyama & Chen [17]. Secondary analyses focused on safety, duration of response (DOR), PFS, OS, and quality of life (QoL). TRAEs were summarized by type and grade, reporting the most severe grade per patient. DOR was defined as time from first documentation of response to date of DP. PFS was defined as time from study treatment initiation to date of first documentation of DP or death due to any cause. Among participants who progressed and subsequently initiated second-line therapy, PFS2 was defined as time from study treatment initiation to date of first documentation of DP after second-line therapy or death due to any cause. Participants were censored at date of last radiographic assessment for DP. OS was defined as time from study treatment initiation to death due to any cause. Participants who were still alive were censored at the last date they were known to be alive. Survival probabilities were estimated and plotted using Kaplan-Meier. Estimates along with 95% pointwise confidence intervals (CI) are reported. Changes in participant-reported QoL scores were calculated from baseline to each subsequent (cycle and day) assessment time point (CD21, CD31, CD41, CD415). Mean estimates and 95% CI for QoL changes were derived using linear mixed effects regression models to account for the longitudinally-correlated nature of repeated QoL assessments at unequal intervals between visits with a spatial power correlation structure. Correlative analyses evaluated the effect of laboratory pretreatment and fold change values with PFS using Cox regression models. Fold change values were included as time-dependent covariates. All statistical testing of secondary endpoints was two-sided and assessed for significance at the 5% level.

2.6. Ethics and oversight

The protocol was submitted to the U.S. Food and Drug Administration under Investigational New Drug 105715 and registered to ClinicalTrials.gov prior to enrollment of the first patient (NCT02420314). Approval was obtained from The University of Iowa Institutional Review Board (Biomedical IRB01; IRB 21211713). Informed written consent was obtained from each participant prior to receiving protocol-based therapy. The trial was conducted according to the Belmont Report, the United States Common Rule (45CFR46), and the International Council on Harmonisation—Good Clinical Practice as adopted by U.S. Federal law. All investigators were GCP trained. The University of Iowa Holden Comprehensive Cancer Center Data and Safety Monitoring Committee (DSMC) reviewed all data for compliance to protocol and participant safety. Safety and annual reports regarding this trial were submitted as required (21CFR[312.23],[312.32]).

3. Results

Between April 2015 and November 2020, 54 participants consented and 40 were enrolled in the study. This period included a temporary recruitment pause for the prespecified interim futility analysis. Data cut-off was performed on August 31, 2021. Two participants were deemed unenrollable by the study investigators and University of Iowa DSMC (withdrawal of consent [n = 1], chemotherapy dose violation [n = 1]). All participants were included in safety analysis and 38 evaluable participants were included in the statistical analyses of the primary and secondary endpoints (Supplementary Fig. 1).

Demographics and baseline characteristics of participants are shown in Table 1. The median age was 63 years. Thirteen percent (n = 5) of participants had an ECOG PS of 2, 94.7% had metastatic disease including 31.6% with treated brain metastases. Due to insufficient tumor samples, PD-L1 status could not be assessed for seven participants. PD-L1 TPS of <1% represented 61.3% (19/31) of participants while 9.7% had PD-L1 TPS ≥50%. Of the nine participants who enrolled in the study after May 2018 (coinciding with the release of KeyNote189 trial data), six had PD-L1 TPS <1%, two received and progressed on immunotherapy before participation in the study, and one did not have sufficient tissue for PD-L1 evaluation. Tumor samples from 33 participants were evaluated for somatic alterations in cancer-related genes. Molecular data from six participants who received testing through commercial assays as part of their standard of care (Foundation One CDx® [n = 3]; Neogenomics® [n = 2]; and CLARIS® [n = 1]) was included in the analysis of exploratory outcomes. Tumor samples from the remaining participants (n = 27) were tested at the University of Iowa. Since different platforms were used for testing, the number of genes and exonal coverage evaluated differed based on the testing site (Table 1).

Thirteen of 38 (34.2%) participants achieved PR per RECISTv1.1, all were confirmed partial responses (cPR). Median duration of response was six months with a range of 2.8–57.2 months. One participant continues to have ongoing PR. Nineteen participants (50.0%) had SD while five (13.2%) developed DP as their best response. One patient died before an initial response assessment. Depth of response and change in the sum of target disease are shown in Fig. 1A in relation to participant and disease characteristics. Fig. 1B shows participants’ disease status over time.

Median PFS was 5.7 months (95% CI: 4.3–7.6; Fig. 2A). PFS in relation to PD-L1 TPS is shown in Fig. 2B. Median PFS2 (n = 27) for patients receiving second-line therapy was 10.7 months (95% CI: 6.7–13.7; Fig. 2C). Median OS was 12.6 months (95% CI: 8.7–21.7; Fig. 2D). PFS2 and OS with respect to PD-L1 expression are shown in Supplementary Fig. 2.

Patients with ECOG PS 2 (n = 5) did poorly, with none having an objective response. Median PFS and OS for these patients were 3.2 and 5.4 months, respectively (Table 2). PD-L1 TPS was 1–49% for 2 participants, <1% for two participants, and tissue was not available for a remaining patient. Prevalence of STK11 and KEAP/NFE2L2 mutations was 1/3 (33%) and 1/3 (33%) in participants with ECOG PS 2 compared
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ECOG PS 0

PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

ECOG, Eastern Cooperative Group; AJCC, American Joint Committee on Cancer; NFE2L2, nuclear factor erythroid 2-related factor 2; KEAP1, Keap1 (Kelch-like ECH-associated protein 1); STK11, serine/threonine kinase 11; KRAS, Kirsten rat sarcoma viral oncogene homolog 1; P-AscH, platelet ascorbate and oxidative-stress phenotype.

Table 1
Patient demographics and baseline characteristics.

| Variable | Level | n = 38a |
|----------|-------|---------|
| Age      | Median (Min-Max) | 63 (45.74) |
| Gender   | F     | 13 (34.2%) |
|          | M     | 25 (65.8%) |
| Race     | Asian | 2 (5.3%) |
|          | White | 36 (94.7%) |
| Ethnicity| Hispanic or Latino | 1 (2.6%) |
|          | Non-Hispanic | 37 (97.4%) |
| Histology| Adenocarcinoma | 25 (65.8%) |
|          | Squamous Cell Carcinoma | 13 (34.2%) |
| ECOG Performance Status | 0–1 | 33 (86.8%) |
|          | 2     | 5 (13.2%) |
| Smoking Status | Active | 20 (52.6%) |
|          | Former | 14 (36.8%) |
|          | Never | 4 (10.5%) |
| Liver Metastasis | Yes | 4 (10.5%) |
|          | No    | 34 (92.1%) |
| Brain Metastasis | Yes | 12 (31.6%) |
|          | No    | 26 (68.4%) |
| Bone Metastasis | Yes | 12 (31.6%) |
|          | No    | 26 (68.4%) |
| Highest Stage (AJCC 8th) | N3 | 2 (5.3%) |
|          | M1a-b | 19 (50.0%) |
|          | M1c   | 17 (44.7%) |
|          | M1d   | 2 (5.3%) |
| PD-L1 TPS (n = 31) | <1% | 19 (61.3%) |
|          | 1–49% | 9 (29.0%) |
|          | ≥50% | 3 (9.7%) |
| KRAS (n = 33) | Positive | 29 (87.9%) |
| KEAP1 (n = 28) | Positive | 16 (57.1%) |
| NFE2L2 (n = 28) | Positive | 14 (50.0%) |
| Received Immunotherapy prior to enrollment | No | 35 (92.1%) |
| Protocol Defined Cycles Completed | Yes | 3 (7.9%) |
|          | 1     | 2 (5.3%) |
|          | 2     | 2 (5.3%) |
|          | 3     | 2 (5.3%) |
|          | 4     | 2 (5.3%) |
| Received 2nd Line Therapy | No | 11 (28.9%) |
|          | Yes | 27 (71.1%) |
| Type of 2nd Line Therapy | Chemomunotherapy | 4 (14.8%) |
|          | Chemotherapy | 5 (18.5%) |
|          | Immunotherapy | 18 (66.7%) |

ECOG, Eastern Cooperative Group; AJCC, American Joint Committee on Cancer; PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

a Number of patients assessed for PD-L1 TPS, KRAS, STK11, KEAP1, NFE2L2 status varies.

The combination of P-AscH with platinum-based chemotherapy was significantly improved tumor response rate compared to historical control. Response rates in patients with advanced stage NSCLC treated with carboplatin-paclitaxel chemotherapy in large randomized trials has varied between 15% and 25% [3,16–22]. Though the addition of P-AscH improved subject outcomes (34% response rate), response rates in recent trials evaluating the combination of immunotherapy with platinum-based doublet chemotherapy ranged from 37.1% to 59.9% [2–5]. These trials enrolled subjects with an ECOG performance status of 0–1 and utilized different platinum-based regimens. In these trials, pemtrexed was prescribed for non-squamous NSCLC whereas either paclitaxel or nab-paclitaxel was combined with the platinum agent for squamous NSCLC except for IMpower150. In IMpower150, the chemotherapy regimen was similar to our study (i.e. carboplatin-paclitaxel)
with atezolizumab; this combination yielded a response rate of 40.6% with mPFS of 6.9 months. The approval of immunotherapy during study enrollment period influenced patient recruitment; the majority of participants in this trial had PD-L1 TPS <1% or enrolled after progression on first-line immunotherapy. In addition, this study included patients with ECOG PS 2, who are typically excluded from clinical trials. Despite these poor prognostic factors, median PFS and OS were numerically greater in this study than in NSCLC patients receiving standard of care chemotherapy in recently reported studies [2, 4, 5]. Moreover, median PFS for patients with PD-L1 TPS <1% in our study was numerically similar to patients receiving immunotherapy in the KeyNote189, KeyNote407 and 9LA studies [2, 4, 5]. The adverse event profile was similar to those expected from a platinum-containing chemotherapy doublet. Overall, this study suggests that the combination of P-AscH and chemotherapy is both effective and safe for the treatment of advanced NSCLC. However, larger and randomized studies are required to confirm these findings.

We analyzed the relationship between somatic alterations in KEAP1, NFE2L2, and STK11 with participant outcomes as these genes are generally associated with poor clinical outcomes. In addition, these genes play an important role in cellular redox-biology and metabolism. We sought to determine whether an increase in oxidative stress generated by P-AscH could overcome the enabling effect of KEAP1 and NFE2L2 mutations in NSCLC. In our study, three of 28 subjects had KEAP1 or NFE2L2 alterations and a poor outcome (Fig. 1B). While not powered to detect a difference in survival, our data suggest the addition of P-AscH to carboplatin-paclitaxel does not provide an additional benefit. However, two of three subjects with STK11 mutations achieved a PR and had a longer PFS than the median (Table 2, Fig. 1A).

Redox active metals such as iron have been suggested to be selectively elevated in cancer cells representing a therapeutic target for the development of novel therapeutics [23]. P-AscH’s selective toxicity to lung cancer cells depends upon redox active iron pools within the cell [9, 24]. Our group previously demonstrated that resected NSCLC tissue has increased steady-state level of superoxide \( \mathbf{O}_2^- \) and redox-active labile iron compared to adjacent normal lung tissue. Oxidation of ascorbate produces \( \mathbf{H}_2\mathbf{O}_2 \) that reacts with the increased labile iron in cancer cells to mediate Fenton chemistry and cause oxidative damage to cellular macromolecules (i.e., DNA, protein, lipids). Furthermore, \( \mathbf{H}_2\mathbf{O}_2 \) produced from ascorbate oxidation selectively increases cancer cell redox-active iron, partially by disrupting iron-sulfur clusters, further exacerbating the differences in labile iron pool available for oxidation reactions that mediate ascorbate toxicity.

The role of systemic iron in pharmacological ascorbate mediated anti-tumor activity is currently unclear. At natural, dietary plasma levels (<200 μM), ascorbate stimulates iron absorption via ferroportin...
Fig. 2. Kaplan-Meier analysis of progression-free survival per RECIST version 1.1 A) progression-free survival by PD-L1 TPS. B) Progression-free survival. C) and overall survival (D). CI, confidence interval; PD-L1, programmed death ligand 1.

Table 2
Demographics and disease characteristics in relation to outcomes.

| Variable                      | Level      | N  | ORR (%) | PFS (95% CI) | PFS2 (95% CI) | OS (95% CI) |
|-------------------------------|------------|----|---------|--------------|---------------|-------------|
| Age                           | ≤65        | 27 | 10 (37.0) | 6.0 (4.3-8.7) | 11.1 (7.0-15.3) | 15.5 (9.0-39.0) |
|                               | >65        | 11 | 3 (27.3)  | 5.5 (3.0-6.4)  | 7.3 (2.1-13.6)  | 7.5 (2.7-21.7)  |
| Gender                        | Female     | 16 | 6 (46.2)  | 5.7 (2.8-7.9)  | 12.4 (6.2-NR)   | 21.7 (6.4-NR)   |
|                               | Male       | 25 | 7 (28.0)  | 5.7 (3.6-8.5)  | 10.0 (6.6-15.1) | 11.8 (8.7-17.4) |
| Histology                     | Adenocarcinoma | 25 | 9 (36.0)  | 7.8 (5.2-8.9)  | 12.9 (7.0-40.6) | 21.7 (11.6-39.0) |
|                               | Squamous Cell Carcinoma | 13 | 4 (30.8)  | 4.4 (2.8-5.5)  | 7.4 (6.2-11.1)  | 8.7 (6.3-13.1)  |
| ECOG performance status       | 0-1        | 23 | 13 (59.4) | 6.4 (4.9-7.9)  | 11.1 (8.0-15.1) | 13.9 (10.5-20.6) |
|                               | 2          | 5  | 0 (0)     | 3.2 (1.9-3.6)  | 6.0 (5.2-7.0)   | 5.4 (3.2-34.3)  |
| Smoking status                | Active     | 20 | 4 (20.0)  | 4.0 (2.8-6.4)  | 8.0 (5.4-10.7)  | 11.5 (5.4-17.4) |
|                               | Former     | 14 | 6 (42.9)  | 7.4 (4.4-9.5)  | 13.7 (6.2-NR)   | 34.3 (6.4-NR)   |
|                               | Never      | 4  | 3 (75.0)  | 5.6 (4.6-9.5)  | 8.9 (6.6-15.1)  | 14.6 (7.1-NR)   |
| Liver metastasis              | No         | 34 | 12 (35.3) | 5.9 (4.4-7.9)  | 11.1 (7.4-13.7) | 12.8 (8.7-21.7) |
|                               | Yes        | 4  | 1 (25.0)  | 1.6 (1.2-6.8)  | 6.6 (2.1-NR)    | 20.3 (2.7-NR)   |
| Brain metastasis              | No         | 26 | 9 (36.0)  | 6.2 (4.6-7.9)  | 11.7 (7.4-15.1) | 13.5 (9.0-34.3) |
|                               | Yes        | 12 | 4 (33.3)  | 3.3 (1.2-8.5)  | 6.2 (2.1-8.1)   | 9.0 (3.2-29.8)  |
| Bone metastasis               | No         | 26 | 12 (46.2) | 6.2 (3.5-7.9)  | 11.7 (6.7-15.1) | 14.7 (9.0-39.0) |
|                               | Yes        | 12 | 1 (8.3)   | 5.2 (2.5-8.7)  | 7.4 (5.4-15.3)  | 10.2 (5.4-21.7) |
| Highest stage (AJCC 8th)      | N3         | 2  | 0 (0)     | 4.0 (3.5-4.4)  | 8.9 (6.7-11.1)  | 10.8 (9.0-12.5) |
|                               | M1a-b      | 19 | 11 (57.9) | 7.0 (5.5-8.9)  | 13.6 (8.0-15.3) | 21.4 (10.5-39.0) |
|                               | M1c        | 17 | 2 (11.8)  | 3.6 (1.9-6.8)  | 7.2 (5.4-42.1)  | 8.7 (5.4-29.8)  |
|                               | M1d or M1e | 19 | 12 (63)   | 6.4 (4.9-8.7)  | 13.7 (6.6-40.6) | 13.1 (6.4-36.9) |
| PD-L1 TPS                     | <1         | 19 | 7 (36.8)  | 6.4 (4.9-8.7)  | 13.7 (6.6-40.6) | 13.1 (6.4-36.9) |
|                               | 1-49       | 9  | 1 (11.1)  | 3.6 (1.3-6.8)  | 7.4 (5.4-11.1)  | 11.8 (5.4-21.7) |
|                               | ≥50        | 3  | 3 (100)   | 9.5 (7.8-NR)   | NR             | NR           |
| KRAS                          | Positive   | 9  | 3 (33.3)  | 7.9 (1.9-10.4) | 10.0 (5.2-15.1) | 34.3 (5.4-NR)  |
|                               | Negative   | 24 | 9 (37.5)  | 5.7 (4.4-7.0)  | 11.1 (6.6-15.3) | 12.8 (7.1-21.7) |
| STK11                         | Positive   | 3  | 2 (66.7)  | 8.9 (3.6-9.5)  | 10.2 (5.4-15.1) | 11.6 (5.4-NR)  |
|                               | Negative   | 28 | 10 (35.7) | 5.9 (4.6-7.0)  | 10.9 (6.6-15.3) | 15.5 (7.5-34.3) |
| KEAP1 or NFE2L2               | Positive   | 3  | 1 (33.3)  | 3.2 (1.3-5.5)  | 6.2 (5.2-6.6)   | 6.3 (5.4-7.5)   |
|                               | Negative   | 25 | 10 (40.0) | 6.4 (5.2-7.8)  | 12.9 (8.0-40.6) | 21.7 (11.6-39.0) |

PFS, progression-free survival; PFS2, progression-free survival 2; OS, overall survival; ECOG, Eastern Cooperative Group; PD-L1, programmed death ligand 1; TPS, tumor proportion score.

a Median (95% CI).
b Patients who received second-line treatment (n = 27).
expression [25] and is capable of altering serum iron markers. To evaluate the effect of P-AscH on circulating iron markers, we observed changes in serum iron, ferritin and transferrin saturation during relative baseline values. Increased baseline transferrin and total iron binding capacity (TIBC) were associated with decreased risk of progression (Table 3) and this may just reflect the overall intensity of inflammation in the body. Inflammatory states (e.g. metastatic tumors), in general, are associated with a lower level of transferrin and TIBC. In contrast, subjects with low tumor burden may have less systemic inflammation with minimal or no effect on transferrin levels, which may explain this finding. Further investigation is needed to better understand the relationship between P-AscH-related changes in serum iron markers and its influence on malignant cells' redox active-iron pools.

Both IL-6 and CXCL8 are associated with an increase in myeloid-derived suppressor and regulatory T cells in the tumor microenvironment, tumor-cell proliferation, tumor invasiveness, decreased antigen presentation, limited response to IC1, and poor prognosis [26, 27]. Pretreatment changes in serum iron, ferritin and CXCL8 expression were associated with poor outcome (Table 3), which may reflect participants' disease burden as opposed to an effect of the therapeutic interventions. We did observe changes in other cytokines and chemokines. However, dissecting P-AscH-related changes and their significance is challenging (Supplementary Fig. 3). Immunophenotyping of PBMCs was performed in a limited number of participants to assess the impact of P-AscH and/or chemotherapy on the host immune response. Interestingly, participants with longer PFS demonstrated a higher fold increase in circulating activated effector CD8 T cells (Supplementary Fig. 5, Supplementary Table 3), although it is unclear whether this was a direct effect of therapy or an indirect effect from a change in disease burden. Emerging data from an ongoing phase II trial in patients with stage III NSCLC trial that combines P-AscH with ionizing radiation and carboplatin-paclitaxel (NCT02905591) suggests that P-AscH may have direct immunomodulatory effects (unpublished results). We continue to investigate the relevance of these alterations in the distribution of various immune cell populations within the peripheral blood during treatment in the ongoing study.

Finally, preclinical studies have shown that P-AscH stimulates dendritic cells to secrete IL-12, drive differentiation of naïve CD4 T cells into Th1 cells, and increases CD8 effector T cells [28, 29]. P-AscH enhances the expression of human endogenous retroviruses (HERVs) via demethylation, improves immune recognition of tumor cells, increases intratumoral infiltration of CD8 T cells, increases the cytotoxicity of immune cells, and acts synergistically with anti-PD1/PDL-1 treatment in lymphoma, breast, melanoma and colorectal cancer models [30, 31]. These anti-cancer immunomodulatory effects of P-AscH are tumor-agnostic and need further exploration in pre-clinical studies and early phase clinical trials combining P-AscH with immunotherapy + chemotherapy. The encouraging findings of this study and emerging data regarding the immunomodulatory role of P-AscH are promising and warrant further investigation.

5. Conclusions

Addition of P-AscH to platinum-doublet chemotherapy in advanced stage NSCLC significantly improved tumor response rate compared to historical controls. P-AscH affects serum iron biomarkers; however, this did not correlate with clinical outcome. P-AscH or the combination of P-AscH with chemotherapy elicited an immune response. These findings are intriguing and need further investigation in future studies.

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Declaration of competing interest

M Furqan acts as a institutional principal investigator/sub-investigator at AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Merck, Novartis, Pfizer, Roche, Genmab, Elicio Therapeutics, Mirati, Amgen, Replimune, Checkmate Pharmaceuticals, Gilead, GSK, Tesaro and Abbvie. On advisory board for Abbvie, Jazz Pharma, Mirati, Astra Zeneca. DR Spitz and BG Allen received research support from Galera and serve as consultant for Galera Therapeutics. All authors report no

Table 3

| Covariate | Pretreatment Only | Fold Change from Pretreatment |
|-----------|-------------------|-------------------------------|
|           | Hazard Ratio | 95% CI | p-value | Units | Hazard Ratio | 95% CI | p-value |
| CXCL8     | 0.05          | 1.82   | 1.20   | 2.74 | <.01 | 0.20 | 1.01 | 0.85 | 1.21 | 0.08 |
| CXCL10    | 0.09          | 1.39   | 0.96   | 2.02 | <.08 | 0.41 | 0.77 | 0.55 | 1.08 | 0.13 |
| CCL11     | 0.03          | 0.83   | 0.58   | 1.19 | 0.31 | 0.25 | 1.11 | 0.82 | 1.50 | 0.49 |
| CCL17     | 0.30          | 1.50   | 0.93   | 2.43 | 0.10 | 0.56 | 1.15 | 0.79 | 1.66 | 0.47 |
| CCL2      | 0.16          | 0.99   | 0.67   | 1.47 | 0.98 | 0.25 | 1.00 | 0.86 | 1.16 | 0.96 |
| CCL5      | 6.29          | 1.25   | 0.85   | 1.84 | 0.26 | 0.42 | 0.81 | 0.59 | 1.13 | 0.21 |
| CCL6      | 0.06          | 1.34   | 0.85   | 2.12 | 0.21 | 0.28 | 1.11 | 0.74 | 1.69 | 0.61 |
| CCL9      | 0.02          | 0.15   | 0.80   | 1.66 | 0.45 | 0.52 | 0.97 | 0.66 | 1.42 | 0.88 |
| CCL15     | 0.15          | 0.80   | 0.55   | 1.16 | 0.24 | 0.63 | 1.00 | 0.62 | 1.60 | 1.00 |
| CCL20     | 0.02          | 0.14   | 0.96   | 2.24 | 0.08 | 2.68 | 1.33 | 0.88 | 2.01 | 0.18 |
| CCL1     | 0.04          | 1.30   | 0.86   | 1.96 | 0.22 | 0.30 | 1.10 | 0.94 | 1.29 | 0.22 |
| CCL11    | 0.05          | 1.25   | 0.85   | 1.84 | 0.26 | 0.80 | 1.23 | 0.94 | 1.98 | 0.40 |
| IL-6      | 0.18          | 2.15   | 1.29   | 3.60 | <.01 | 0.50 | 1.14 | 0.91 | 1.43 | 0.24 |
| IL-22     | 0.01          | 1.08   | 0.76   | 1.53 | 0.68 | 2.29 | 1.31 | 0.40 | 4.25 | 0.65 |
| IL-12     | 0.91          | 1.06   | 0.74   | 1.51 | 0.76 | 2.27 | 1.07 | 0.34 | 3.83 | 0.91 |
| Ferritin  | 324.01        | 1.51   | 0.99   | 2.29 | 0.05 | 1.71 | 1.00 | 0.81 | 1.24 | 1.00 |
| Iron      | 36.40         | 0.90   | 0.61   | 1.34 | 0.61 | 1.18 | 1.10 | 0.70 | 1.72 | 0.68 |
| Iron saturation | 6.61 | 0.96   | 0.65 | 1.41 | 0.83 | 1.01 | 1.05 | 0.70 | 1.56 | 0.83 |
| Total iron binding capacity | 64.00 | 0.65 | 0.42 | 0.99 | 0.04 | 0.21 | 1.11 | 0.78 | 1.57 | 0.56 |
| Transferin| 44.69         | 0.64   | 0.42   | 0.99 | 0.05 | 0.21 | 1.10 | 0.78 | 1.57 | 0.58 |
| Carboxyl  | 1364.90       | 1.24   | 0.86   | 1.80 | 0.25 | 0.25 | 2.00 | 1.18 | 3.38 | 0.81 |
| 4HNE      | 0.07          | 0.71   | 0.47   | 1.06 | 0.29 | 0.37 | 0.96 | 0.60 | 1.55 | 0.88 |
| 3NT       | 17.32         | 1.02   | 0.70   | 1.49 | 0.92 | 1.83 | 0.99 | 0.75 | 1.30 | 0.94 |

CI, confidence interval; 4HNE, 4-hydroxy-2-nonenal (4HNE) modified proteins; 3NT, 3-nitrotyrosine.

a Units reflect 1 SD of pre-treatment values.
b Units reflect 1 SD of C3D1 values.
conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2022.102318.

References

[1] National cancer Institute surveillance epidemiology and end results program, Cancer Stat Facts: Lung and Bronchus Cancer [Available from: https://seer.cancer.gov/statfacts/html/lungh.html.

[2] L. Paz-Ares, T.E. Cisleeanu, M. Cobo, M. Schenker, B. Zuzarwski, J. Menezes, et al., First-line nivolumab plus ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (CheckMate 9LA): an international, randomized, open-label, phase 3 trial, Lancet Oncol. 22 (2) (2021) 198–211.

[3] M.A. Socinski, R.M. Jotte, F. Capuzzo, F. Orlandi, D. Stroyakovsky, N. Nomgami, et al., Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC, N. Engl. J. Med. 378 (24) (2018) 2288–2301.

[4] L. Paz-Ares, A. Luft, D. Vicente, A. Tafreshi, M. Gumus, J. Mazieres, et al., Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer, N. Engl. J. Med. 379 (21) (2018) 2040–2051.

[5] L. Gandhi, D. Rodriguez-Abreu, S. Gadgeel, E. Esteban, E. Felipe, F. De Angelis, et al., Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer, N. Engl. J. Med. 378 (22) (2018) 2078–2092.

[6] Q. Chen, M.G. Espey, A.Y. Sun, C. Pooput, K.L. Kirk, M.C. Krishna, et al., Pharmacologic ascorbic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice, Proc. Natl. Acad. Sci. U. S. A 105 (32) (2008) 11105–11109.

[7] Q. Chen, M.G. Espey, A.Y. Sun, J.H. Lee, M.C. Krishna, E. Shacter, et al., Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo, Proc. Natl. Acad. Sci. U. S. A. 104 (21) (2007) 8749–8754.

[8] Q. Chen, M.G. Espey, M.C. Krishna, J.B. Mitchell, C.P. Corpe, G.R. Buetner, et al., Pharmacologic ascorbic acid concentrations selectively kill cancer cells in vivo as a pro-drug to deliver hydrogen peroxide to tissues, Proc. Natl. Acad. Sci. U. S. A 102 (38) (2005) 13604–13609.

[9] J.D. Schoenfeld, Z.A. Silbernall, K.A. Mapuskar, B.A. Wagner, K.L. Cramer-Morales, M. Furqan, et al., O2− and H2O2-mediated disruption of Fe metabolism causes the differential susceptibility of NSCLC and GBM cancer cells to pharmacological ascorbate, Cancer Cell 31 (4) (2017) 487–500, e8.

[10] K.A. Mapuskar, K.H. Filippo, J.D. Schoenfeld, D.P. Riley, S. Strack, T.A. Hejleh, et al., Mitochondrial superoxide increases age-associated susceptibility of human dermal fibroblasts to radiation and chemotherapy, Cancer Res. 77 (18) (2017) 5054–5067.

[11] B.G. Allen, K.L. Bodeker, M.C. Smith, V. Monga, S. Sandhur, R. Hohi, et al., First-in-Human phase I clinical trial of pharmacologic ascorbate combined with radiation and temozolomide for newly diagnosed glioblastoma, Clin. Cancer Res.: Off. J. Am. Assoc. Canc. Res. 25 (22) (2019) 6590–6597.

[12] Y. Ma, J. Chapman, M. Levine, K. Polireddy, J. Drisko, Q. Chen, High-dose parenteral ascorbate enhanced chemosensitivity of ovarian cancer and reduced toxicity of chemotherapy, Sci. Transl. Med. 6 (222) (2014), 222ra18.

[13] J.L. Welsh, H.A. Wagner, T.J. van’t Erve, P.S. Zehr, D.J. Berg, T.R. Halfdanarson, et al., Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): results from a phase I clinical trial, Cancer Chemother. Pharmacol. 71 (3) (2013) 765–775.

[14] A. Zahra, M.A. Fath, E. Opat, K.A. Mapuskar, S.K. Bhatia, D.C. Ma, et al., Consuming a ketogenic diet while receiving radiation and chemotherapy for locally advanced lung cancer and pancreatic cancer: the university of iowa experience of two phase I clinical trials, Radiat. Res. 187 (6) (2017) 743–754.

[15] A. Sandler, R. Gray, M.C. Perry, J. Brahmer, J.H. Schiller, A. Dowlati, et al., Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer, N. Engl. J. Med. 355 (24) (2006) 2542–2550.

[16] J.H. Schiller, D. Harrington, C.P. Belani, C. Langor, A. Sandler, J. Krook, et al., Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer, N. Engl. J. Med. 346 (2) (2002) 92–98.

[17] T. Koyama, H. Chen, Proper inference from Simon’s two-stage designs, Stat. Med. 27 (16) (2008) 3145–3154.

[18] C.G. Moerter, T.R. Fleming, E.T. Creagan, J. Rubin, M.J. O’Connell, M.M. Ames, High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy, A randomized double-blind comparison, N. Engl. J. Med. 312 (3) (1985) 137–141.

[19] E.T. Creagan, C.G. Moerter, J.R. O’Fallon, A.J. Schutt, M.J. O’Connell, J. Rubin, et al., Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial, N. Engl. J. Med. 301 (13) (1979) 687–690.

[20] M.A. Socinski, I. Bondaredo, N.A. Karaseva, A.M. Makhson, I. Vynnychenko, I. Okamoto, et al., Weekly nab-paclitaxel in combination with carboplatin versus solvent-based paclitaxel plus carboplatin as first-line therapy in patients with advanced non-small-cell lung cancer: final results of a phase III trial, J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. 30 (17) (2012) 2055–2062.

[21] C.P. Belani, J.S. Lee, M.A. Sociinski, F. Robert, D. Waterhouse, K. Rowland, et al., Randomized phase III trial comparing cisplatin-etoposide to carboplatin-paclitaxel in advanced or metastatic non-small-cell lung cancer, Ann. Oncol.: Off. J. European Soc. Med. Oncol.: ESMO 16 (7) (2005) 1069–1075.

[22] R. Rosell, U. Gatzemeier, D.C. Bettiicher, U. Keppler, H.N. Mcha, R. Pirkner, et al., Phase III randomized trial comparing paclitaxel/carboplatin with paclitaxel/ etoposide in patients with advanced non-small-cell lung cancer: a cooperative multinational trial, Ann. Oncol.: Off. J. European Soc. Med. Oncol.: ESMO 15 (10) (2002) 1539–1549.

[23] D.S. Kalinowski, C. Stefani, S. Toyokuni, T. Ganz, G.J. Anderson, N. V. Subramaniam, et al., Redox cycling metals: pedaling their roles in metabolism and their use in the development of novel therapeutics, Biochim. Biophys. Acta 1863 (4) (2016) 727–748.

[24] L. Zhou, L. Zhang, S. Wang, B. Zhao, H. Lv, P. Shang, Labile iron affects pharmacological ascorbate-induced toxicity in osteosarcoma cell lines, Free Radic. Res. 54 (6) (2020) 385–396.

[25] N. Scheers, A.S. Sandberg, Iron transport through ferroportin is induced by intracellular ascorbate and involves IRP2 and HIF2alpha, Nutrients 6 (1) (2014) 249–260.

[26] Z. Baksouy, T.K. Choueiri, IL-8 and cancer prognosis on immunotherapy, Nat. Med. 26 (5) (2020) 650–651.

[27] D.E. Johnson, R.A. O’Keefe, J.R. Grandis, Targeting the IL-6/JAK/STAT3 signalling axis in cancer, Nat. Rev. Clin. Oncol. 15 (4) (2018) 234–246.

[28] Y.J. Jeong, S.W. Hong, J.H. Kim, D.H. Jin, J.S. Kang, W.J. Lee, et al., Vitamin C-treated murine bone marrow-derived dendritic cells preferentially drive naïve T cells into Th1 cells by increased IL-12 secretions, Gland. Immunol. 266 (2) (2011) 192–199.

[29] Y.J. Jeong, J.H. Kim, J.M. Hong, J.H. Kim, D.H. Jin, J.S. Kang, W.J. Lee, et al., Vitamin C treatment of mouse bone marrow-derived dendritic cells effectively drive naive T cells into Th1 cells by increased IL-12 secretions, Gland. Immunol. 266 (2) (2011) 192–199.

[30] A.M. Magri, G. Germano, A. Lorenzato, S. Lamba, R. Chila, M. Montone, et al., High-dose vitamin C enhances cancer immunotherapy, Sci. Transl. Med. 12 (532) (2020).