

Safety, pharmacokinetics, and pharmacodynamics of oral omaveloxolone (RTA 408), a synthetic triterpenoid, in a first-in-human trial of patients with advanced solid tumors

Ben C Creelan¹
 Dmitry I Gabrilovich²
 Jhanelle E Gray¹
 Charles C Williams¹
 Tawee Tanvetyanon¹
 Eric B Haura¹
 Jeffrey S Weber³
 Geoffrey T Gibney⁴
 Joseph Markowitz⁵
 Joel W Proksch⁶
 Scott A Reisman⁶
 Mark D McKee⁷
 Melanie P Chin⁶
 Colin J Meyer⁶
 Scott J Antonia¹

¹Department of Thoracic Oncology, H. Lee Moffitt Cancer Center and Research Institute, Magnolia Drive, Tampa, FL, USA; ²The Wistar Institute, Philadelphia, PA, USA; ³Laura and Isaac Perlmutter Cancer Center, New York, NY, USA; ⁴Department of Medicine, Georgetown-Lombardi Comprehensive Cancer Center, Washington, DC, USA; ⁵Department of Cutaneous Oncology, H. Lee Moffitt Cancer Center and Research Institute, Magnolia Drive, Tampa, FL, USA; ⁶Reata Pharmaceuticals, Inc., Irving, TX, USA; ⁷AbbVie, Inc., North Chicago, IL, USA

Correspondence: Ben C Creelan
 H. Lee Moffitt Cancer Center, 12902
 Magnolia Drive, Tampa, FL 33612, USA
 Tel +1 813 745 3050
 Fax +1 813 745 3027
 Email ben.creelan@moffitt.org

Background: Omaveloxolone is a semisynthetic oleanane triterpenoid that potently activates Nrf2 with subsequent antioxidant function. We conducted a first-in-human Phase I clinical trial (NCT02029729) with the primary objectives to determine the appropriate dose for Phase II studies, characterize pharmacokinetic and pharmacodynamic parameters, and assess antitumor activity.

Methods: Omaveloxolone was administered orally once daily continuously in a 28-day cycle for patients with stage 4 relapsed/refractory melanoma or non-small cell lung cancer. An accelerated titration design was employed until a grade 2-related adverse event (AE) occurred. A standard 3+3 dose escalation was employed. Single-dose and steady-state plasma pharmacokinetics of the drug were characterized. Downstream Nrf2 activation was assessed in peripheral blood mononuclear cells by quantification of target gene mRNA expression.

Results: Omaveloxolone was tested at four dose levels up to 15 mg given orally once daily. No dose-limiting toxicities were detected, and the maximum tolerated dose was not determined. All drug-related AEs were either grade 1 or 2 in severity, and none required clinical action. The most common drug-related AEs were elevated alkaline phosphatase (18%) and anemia (18%). No drug interruptions or reductions were required. Omaveloxolone was rapidly absorbed and exhibited proportional increases in exposure across dose levels. With some exceptions, an overall trend toward time-dependent and dose-dependent activation of Nrf2 antioxidant genes was observed. No confirmed radiologic responses were seen, although one lung cancer subject did have stable disease exceeding 1 year.

Conclusions: Omaveloxolone has favorable tolerability at biologically active doses, although this trial had a small sample size which limits definitive conclusions. These findings support further investigation of omaveloxolone in cancer.

Keywords: antioxidant inflammation modulator, nitrotyrosine, nitric oxide synthase, melanoma, non-small cell lung cancer, immuno-oncology, myeloid-derived suppressor cells, bardoxolone methyl

Background

Despite progress in T-cell immunotherapy, antigen-specific tolerance caused by MDSCs remains a problematic mechanism of tumor cell resistance.¹ MDSCs facilitate peripheral immunosuppression via multiple mechanisms, including production of ROS thereby suppressing CD8⁺ T-cell cytolytic function.^{2,3} Synthetic triterpenoids are a subclass of antioxidant inflammation modulators, derived from oleananes found

in medicinal plants. This class of compounds reduces the production of reactive oxygen and nitrogen species and also inhibits MDSC-mediated suppression of effector T-cells.⁴ Triterpenoids bind at the specific cysteine residue cys151 on Kelch-like ECH-associated protein 1, thereby releasing the transcription factor Nrf2 into the nucleus.^{5,6} Activation of Nrf2 causes expression of downstream antioxidant genes in a coordinated cellular pathway, thereby protecting cells against oxidative and electrophilic stress. Induction of Nrf2 also has anti-inflammatory effects via inhibition of ROS-mediated inflammatory signaling pathways.⁷

Omaveloxolone (RTA 408) is a semisynthetic triterpenoid developed through lead optimization to suppress interferon- γ -mediated nitric oxide signaling in macrophages, even at nanomolar concentrations.⁸ The drug suppresses reactive oxygen and NT production in spleen-derived MDSCs and tumor xenografts.⁸ At higher concentrations, omaveloxolone selectively inhibits tumor cell growth by blockade of inhibitor of nuclear factor kappa-B kinase subunit.⁸ Similarly, omaveloxolone can promote apoptosis of established murine cancer xenografts.⁹ Administration of systemic omaveloxolone also protects hematopoietic cell function in lethally irradiated mice.¹⁰ Furthermore, topical application of omaveloxolone causes dose-proportional induction of Nrf2-target genes in the skin of rats, and in punch biopsies in human volunteers.^{11–13} Along these lines, pretreatment with omaveloxolone also protects retinal pigment epithelial cells from oxidative destruction via activation of Nrf2.¹⁴ Based on in vitro and animal studies, omaveloxolone exhibited high protein binding with extensive tissue distribution, making it a suitable potential oncologic agent.

Based on robust preclinical antitumor activity of omaveloxolone and related analogs, we sought to test the drug in human subjects.⁸ Therefore, we conducted a first-in-human Phase I trial, with the primary objective to characterize the safety and pharmacokinetics of omaveloxolone as an oral capsule. Our hypothesis was that omaveloxolone would be safe and well tolerated in human subjects.

Methods

Study design

This was an open-label, single-center, Phase I study using a modified 3+3 dose-escalation design (NCT02029729). During dose escalation, the dose of omaveloxolone was to be doubled until drug-related toxicity of grade ≥ 2 was observed. Dose escalations from this point would be limited to $\leq 50\%$ of the previous dose (if grade ≤ 2) or 33% in the event of grade ≥ 3 toxicities.

Omaveloxolone was administered starting on day 1, on a continuous once-daily oral schedule with a cycle of 28 days. For this first-in-human study, the recommended starting dose in humans of 2.5 mg once daily was chosen based on the available preclinical pharmacokinetic data, the concentrations observed to modulate Nrf2 in vitro, and on the no observed adverse effect levels in rodent and monkey species from toxicity studies.

This trial was conducted at the H. Lee Moffitt Cancer Center (Tampa, FL, USA), after approval by institutional review board, Liberty IRB, federal wide registration #008679, study #13.11.0015. It was performed in accordance with the Declaration of Helsinki and the *ICH-GCP* guidelines. Informed consent was obtained from all patients. The primary objectives were to characterize the safety and tolerability of a once-daily oral administration, determine the recommended Phase II dose, pharmacokinetics, pharmacodynamics, and preliminary clinical activity of omaveloxolone.

Eligibility

Patients aged 18 years or older, with histologically confirmed stage 4 NSCLC or melanoma who had experienced disease progression after more than one standard therapy were eligible. Other inclusion criteria included: BNP < 100 pg/mL, toxicity from prior therapy resolved to grade ≤ 1 , an Eastern Cooperative Oncology group performance status ≤ 2 , life expectancy ≥ 12 weeks, and adequate organ function. Significant exclusion criteria included: QTc interval on electrocardiogram (ECG) at screening of > 450 mseconds for males and > 460 mseconds for females. Based on preliminary in vitro data, patients taking substrates for p-glycoprotein, organic anion transporting polypeptide 1B3 (OATP1B3), organic anion transporter 1 (OAT1), CYP2C8, or CYP3A4 were also excluded. Patients with stable, treated brain metastases were eligible.

Definitions of DLT and MTD

Dose-limiting toxicity (DLT) was based on toxicities observed in the first cycle and assessed by the investigator as possibly related to omaveloxolone. A DLT was defined as any hepatobiliary disorders grade ≥ 2 , or any grade ≥ 3 non-hepatobiliary toxicity of any duration. Grade 3 nausea or vomiting were considered as DLTs only if they occurred despite optimal medical management. Total bilirubin, aspartate aminotransferase, and alanine aminotransferase were considered DLTs if $> 8 \times$ upper limit of normal (ULN), $> 5 \times$ ULN for over 2 weeks, or $> 3 \times$ ULN with symptoms. The MTD was defined as the highest dose at

which ≤ 1 of six DLTs (<33%) patients experienced DLT at that dose level.

Safety and efficacy

Clinical and laboratory assessments were conducted at baseline and weekly thereafter. Safety assessments included medical history, physical examination, ECG, hematology, urinalysis, and biochemistry including BNP. QT interval was corrected using Fridericia formula. Adverse events (AEs) were graded using CTCAE version 4.0. Tumor assessments were performed every two cycles using RECIST guideline version 1.1.¹⁵ Patients were followed up for survival and toxicity for at least 30 days after drug discontinuation.

Pharmacokinetics

Plasma levels of omaveloxolone were determined from blood samples collected on day 1: pre-dose, 1, 2, 3, 4, 8, and 24 hours after dose administration; and on day 28: pre-dose, 1, 2, 3, 4, 6, 8, 10, and 24 hours after dose administration. Plasma levels were then collected on day 28 of every cycle thereafter. Plasma was separated and stored at -70°C until analysis. Pharmacokinetic samples were analyzed using a validated liquid chromatography-tandem mass spectrometry quantitation method by Abbvie, Inc. (North Chicago, IL, USA). Standard pharmacokinetic parameters were determined using a noncompartmental method with WinNonlinTM software version 6.3 (Pharsight Corp., Cary, NC, USA). Nominal sample collection times were used in the analysis. Parameters included the C_{\max} , T_{\max} , and $t^{1/2}$. The area under the plasma concentration-time curve from time zero to the time (t) of the last measurable concentration (AUC_{0-t}) was determined using the linear log trapezoidal rule. The apparent terminal half-life was obtained as $0.693/\lambda_z$, where λ_z represents the terminal elimination rate constant. One patient in the 2.5 mg cohort was inadvertently dosed prior to their 24-hour collection and this sample was excluded. One sample tube in the 10 mg cohort was received broken and could not be analyzed.

Nrf2 pathway alteration biomarkers

PBMCs were isolated from blood collected at each timepoint. Day 28 collections were missing for the 15 mg cohort due to patient refusing blood draw (1), and due to missed visit (1). mRNA was quantified using Quantigene Plex 2.0 technology according to manufacturer's protocol (Affymetrix Inc., Santa Clara, CA, USA) and as previously described.¹³ Probe sets were designed against the human genome for analysis of Nrf2 target genes; a modified version of Panel 11834 (Affymetrix)

was used. Genes were normalized to the mean expression of housekeeping genes RPL13A (60S ribosomal protein L13a) and peptidyl-prolyl *cis-trans* isomerase B.

Peripheral blood cells were quantified by automated cell counter using flow cytometry for white blood cell differential (Sysmex, XN9000).

Validation of research antibodies

Slides were stained using a Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, AZ, USA) as per manufacturer's protocol with proprietary reagents. Slides were deparaffinized on the automated system with EZ Prep solution. Levels of inducible isoform of nitric oxide synthase (iNOS) and NT protein in tumor sections were determined by immunohistochemistry (IHC) using polyclonal rabbit anti-NOS2 (Abcam, Cambridge, MA, USA; ab3523) and mouse monoclonal anti-NT (Millipore, Temecula, CA, USA; clone 2A8.2).

For iNOS, optimization was performed with Protease 1 (Ventana) enzymatic retrieval method, using mouse epithelial lung tissue as a positive control. A rabbit primary antibody for iNOS (ab3523; Abcam) was incubated (1:200) in antibody diluent (Dako, Carpinteria, CA, USA) for 32 minutes. The Ventana OmniMap anti-rabbit secondary antibody was incubated for 16 minutes, followed by detection (Ventana ChromoMap kit), and slides were then counterstained with hematoxylin. Slides were then dehydrated and coverslipped as per normal laboratory protocol.

For NT, heat-induced antigen retrieval method was used (Ribo-CC, #760-107; Ventana). Optimization was performed using breast epithelial tumor specimens as a positive control. The mouse monoclonal antibody that reacts to NT (#MAB5404; Millipore) was incubated (1:400) in antibody diluent (Dako) for 60 minutes, followed by anti-mouse secondary antibody (Ventana) for 8 minutes.

Analysis of tumor biopsies

Tumor biopsies were not required as part of the study. However, serial archival tumor samples, collected before and during omaveloxolone treatment, of two patients with metastatic melanoma were available. Tissues were collected and preserved in formalin. Suitable areas were selected by a pathologist and sectioned into $5\ \mu\text{m}$ sections placed on slides. Levels of iNOS and NT protein in tumor sections were determined by IHC using polyclonal rabbit anti-NOS2 (Abcam; ab3523) and mouse monoclonal anti-NT (Millipore; clone 2A8.2). IHC was performed with Dako Link 48 autostainer after high pH heat-induced epitope retrieval digestion.

IHC was performed with appropriate positive and negative controls. Slides at 4× magnification were assessed by an independent pathologist blinded to collection time, using a qualitative intensity scale of negative, weak, moderate, or strong cytoplasmic staining. A quantitative scoring method was not possible due to scant tumor material.

From those individuals with sufficient available archival tumor tissue, gene mutational analysis was carried out on DNA extracted from archival tumor samples using PyroMark® PCR or Sequenom MassARRAY OncoCarta® platform according to the manufacturer's protocol.

Statistical analysis

Categorical variables were compared using Fisher's exact test, and Student's *t*-test was used for continuous variables. Where appropriate, changes between timepoints, QTc, and BNP measurements over time were assessed using Wilcoxon signed-rank test. Median survival was estimated by Kaplan–Meier method and CIs were reported. Statistical analysis was performed using Prism 6.0 (La Jolla, CA, USA) and SPSS 17.0 (SPSS Inc., Chicago IL, USA) software. Nrf2 target gene data were analyzed with Sigmaplot 12.0 (Systat, Inc., San Jose, CA, USA).

Results

Patient characteristics

Of 19 patients screened at a single institution between 2014 and 2015, 11 were eligible and treated. All patients had received several prior therapies and had progressive, measurable disease (Table 1). All patients had significant disease burden with multiple metastases. All melanoma patients had received prior anti CTLA-4 and anti-PD-1 therapies.

Treatment and dose escalation

Dose escalation proceeded through four dose levels up to 15 mg. No DLTs were observed, and the MTD was not determined. A decision was reached to stop the 15 mg dose level in two subjects due to activation of a subsequent dose-escalation trial evaluating omaveloxolone in combination with checkpoint inhibitor therapy; NCT02259231. Subjects were required to complete a mandatory DLT evaluation period of ≥28 days and all were included in the safety analysis.

Safety and tolerability

Subjects received a mean of 87 days of continuous oral drug exposure. Omaveloxolone was well tolerated up to 15 mg, and all drug-related AEs were mild or moderate in severity (Table 2). The most common drug-related AEs

Table 1 Patient characteristics

Characteristic	Total (n=11)
Age, years	
Median (range)	59.0 (43–78)
Gender, n (%)	
Female	4 (36)
Race, n (%)	
Caucasian	10 (91)
Black	1 (9)
Ethnicity, n (%)	
Non-Hispanic	11 (100)
Cancer type, n (%)	
Lung, adenocarcinoma	5 (45)
Lung, squamous uveal	3 (27)
Melanoma	3 (27)
AJCC stage, n (%)	
IV	11 (100)
BMI, mg/m ²	
Mean (SD)	27.7 (4.2)
Albumin, g/dL	
Mean (SD)	4.1 (0.5)
Prior PD-1 or PD-L1 mAb, n (%)	
Yes	6 (55)
No	5 (45)
Cohort, mg; n (%)	
2.5	3 (27)
5	3 (27)
10	3 (27)
15*	2 (18)
Prior lines of systemic therapy	
Median, range	4 (2–7)
Prior radiation, n (%)	
Yes	8 (73)
No	3 (27)
Prior brain metastases, n (%)	
Yes	5 (45)
No	6 (55)

Notes: All percentages are rounded and therefore may not exactly sum to one. Stage is assessed at the date of treatment. *Dose level completed in two subjects.

Abbreviations: AJCC, American Joint Committee on Cancer; BMI, body mass index; mAb, monoclonal antibody; PD-1, programmed cell death protein 1.

were elevated alkaline phosphatase and anemia. The most common AE regardless of causality was pain (54%) and anemia (45%) (Table 3). No drug-related AEs required any clinical action by the investigators. There was no detectable difference in the toxicity profile between the assessed dose levels. No drug interruptions were required. One subject with squamous lung cancer at the 15 mg dose level had emergence of dyspnea with multifocal new nodules identified on CT of the thorax. However, subsequent imaging revealed that this was progression of lung cancer, with enlarging mediastinal and hilar lymph nodes.

No QTc prolongation on serial ECGs was detected during the trial (Figure 1A). In contrast to the previously tested agent RTA 402 with a similar triterpenoid-derived scaffold,¹⁶ no

Table 2 Omaveloxolone attributable AEs

Dose, mg	2.5	5	10	15	Total	
Grade	1	2	1	2	1	2
Symptom						
Chronic joint pain	1	-	-	-	-	-
Nausea or vomiting	1	-	-	-	-	-
Vomiting	1	-	-	-	-	-
Papular rash, arm and leg	1	-	-	-	-	-
Chronic leg swelling	1	-	-	-	-	-
Fatigue	1	-	-	-	-	-
Laboratory parameter						
Anemia mean	2	-	-	-	-	-
LDH increased	1	-	-	-	-	-
ALT increased	-	-	-	1	-	-
AST increased	-	-	1	-	-	-
Alkaline phosphatase increased	-	-	1	1	-	-

Notes: No grade 3 or 4 drug-attributable adverse events were observed. Includes all possible, probable, or definite attributions.

Abbreviations: AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

Table 3 All AEs, regardless of attribution

Grade	1	2	3	4	Total
Symptom					
Pain (all types)	2	2	2	-	6
Cough	1	1	-	-	2
Dyspnea	-	-	2	-	2
Pneumonia	-	-	2	-	2
Vomiting	1	1	-	-	2
Chronic leg swelling	1	-	-	-	1
Constipation	1	-	-	-	1
Dehydration	1	-	-	-	1
Depression	-	1	-	-	1
Dermatitis, scalp	1	-	-	-	1
Fall	1	-	-	-	1
Fever	1	-	-	-	1
Flu-like symptoms	1	-	-	-	1
Laryngeal hemorrhage	1	-	-	-	1
Nausea or vomiting	1	-	-	-	1
Papular rash, arm and leg	1	-	-	-	1
Pericardial effusion	-	1	-	-	1
Pulmonary hypertension	-	1	-	-	1
Wheezing	1	-	-	-	1
Laboratory parameter					
Anemia mean	3	1	1	-	5
Alkaline phosphatase increased	2	-	-	-	2
Hypercalcemia	-	1	1	-	2
ALT increased	-	1	-	-	1
AST increased	-	-	1	-	1
BUN increased	1	-	-	-	1
Cholesterol increased	1	-	-	-	1
Hypoglycemia	1	-	-	-	1
Hypokalemia	-	1	-	-	1
Hyponatremia	1	-	-	-	1
LDH increased	1	-	-	-	1
Leukocytosis	1	-	-	-	1
Phosphorous increased	1	-	-	-	1
Triglycerides increased	1	-	-	-	1

Abbreviations: AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; LDH, lactate dehydrogenase.

increase in plasma BNP over normal levels (<300 pg/mL) was detected (Figure 1B).

Pharmacokinetics

Pharmacokinetic parameters of omaveloxolone were estimated for all dose cohorts and are summarized in Table 4. Under fasting conditions, omaveloxolone was rapidly absorbed after oral administration, with mean T_{max} of 2–4 hours across dose levels. On day 1, systemic exposures were comparable between the 2.5- and 5-mg dose groups, and higher in the 10- and 15-mg dose groups (Figure 2A). Proportional increases in drug exposure (AUC_{0-t} and C_{max}) were observed across the dose range studied (Figure 2B and C). Terminal elimination half-life ranged from 9 hours on day 1 to 20 hours on day 28, with no apparent dose-dependent trends. Because of the limited number of samples collected over a relatively short interval, the reported values were considered estimates.

Additional samples were collected from three patients who continued dosing beyond the initial 28-day cycle, with collections occurring pre-dose and 2 and 4 hours after administration when possible. Generally, the omaveloxolone concentrations observed in these patients on day 28 of the subsequent cycles were similar to the time-matched samples observed on day 28 during the first cycle.

Pharmacodynamics

An increase in select Nrf2 target gene expression was observed during the course of treatment, across dose levels (Figure 3A). The interpretation was limited by higher than expected variation among samples in the assay. Likewise, a dose-dependent increase in mRNA expression of select Nrf2 anti-oxidant genes was observed across dose levels (Figure 3B).

Exploratory IHC was assessed from baseline and on-treatment tumor biopsies from two melanoma patients. In a patient from the 10 mg dose level, reductions in expression of NT and iNOS were observed compared to baseline (Figure 4A). This observation was consistent with prior studies, which demonstrated a decrease in reactive nitration in response to treatment with bardoxolone methyl.⁴ However, a patient from the 5 mg dose level had equivocal change in iNOS and NT (Figure 4B). Both of her biopsies were from bone metastases and contained limited tumor material.

In addition, an apparent decrease in monocytic lineage cells was observed during the course of omaveloxolone treatment compared to other cellular compartments (Figure 5). Unfortunately, sufficient PBMCs for more detailed flow cytometry analysis were not collected. Therefore, the reproducibility or significance of this finding remains unclear.

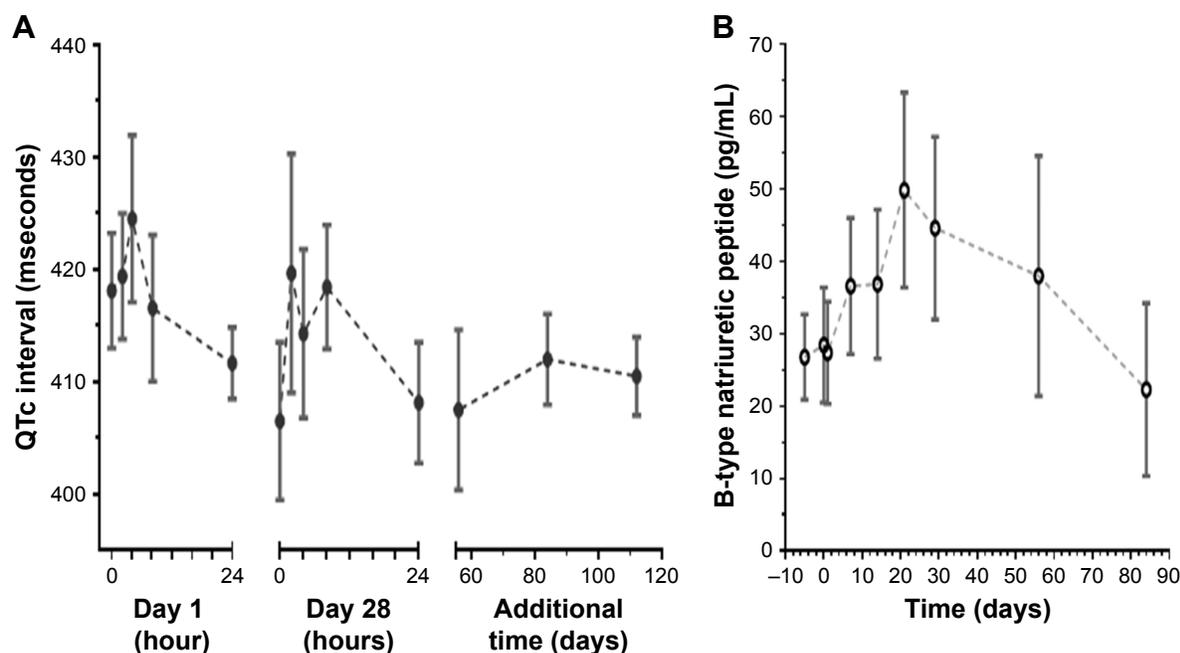


Figure 1 Safety parameters over time.

Notes: (A) QTc by Frederica's formula during omaveloxolone treatment are shown. Levels did not exceed normal range (<440 msec). (B) Overall plasma B-type natriuretic peptide levels during omaveloxolone treatment. Levels did not exceed normal range (<300 pg/mL). Values for all dose levels (n=11) are shown. Time 0 represents prior to dose. Circles represent mean with standard error.

Specifically, we could not determine whether these were monocytes or possibly monocytic suppressor cells. It is conceivable that this was a treatment effect or alternatively due to the effect of metastatic cancer.

Survival outcomes

Median progression-free survival was 1.5 months (95% CI 1.0–1.9), and all subjects experienced progression (Figure 6A).

Table 4 Omaveloxolone pharmacokinetics

Dose, mg	2.5		5		10		15	
Day	1	28	1	28	1	28	1	28
Evaluable (n)	3	3	3	3	3	3*	2**	n/a
AUC _{0-t} (hours ng/mL)								
Mean	27.6	44.8	31.6	63.7	114.4	188.3	183.5	–
SD	32.7	51.5	7.9	17.0	141.7	176.7	27.6	–
T _{max} (hours)								
Mean	3.3	2.9	2.3	2.5	2.0	3.0	3.5	–
SD	2.1	1	0.6	1.2	1.5	–	2.8	–
C _{max} (ng/mL)								
Mean	4.6	4.5	3.9	5.6	15.7	13.4	16.6	–
SD	7.8	4.6	2.1	1.8	34.5	9.7	6.9	–
T _{1/2} (hours)								
Mean	10.2	21.2	8.9	19.7	9.2	19.8	8.7	–
SD	2.4	20.1	1.3	8.1	0.6	4.1	3.7	–

Notes: Averages represent geometric mean. *One tube received broken at day 28. **Dose level completed in two subjects.

Abbreviations: AUC_{0-t}, area under curve from time 0 to last; n/a, no samples available.

Consistent with the poor prognosis of the patients enrolled, median overall survival was 5.8 months (95% CI 2.6–8.9), after nine of 11 events and 27 months of median follow-up. All deaths were attributable to progressive cancer. No confirmed radiologic responses were observed. One subject with lung cancer had stable disease lasting more than 12 months (Figure 6B). Although her target lesion measurements did decrease, the reports consistently included the caveat that the two target lesions were difficult to measure due to lung atelectasis.

Discussion

In this first-in-human dose-escalation Phase I study, we demonstrate the safety of omaveloxolone in patients with advanced solid tumors within a small sample size of patients. Omaveloxolone was tolerated at doses observed to augment Nrf2 signaling in peripheral cells and showed fairly dose-proportional pharmacokinetics. The most common drug-related toxicities included grade 1 anemia and alkaline phosphatase elevation. Because the MTD of oral omaveloxolone was not reached, it is conceivable that the omaveloxolone dose could be further increased. Nonetheless, the suggestion of target modulation at relatively low doses indicated that the 5–10 mg dose was sufficient to serve as a starting point for dose escalation in subsequent combination

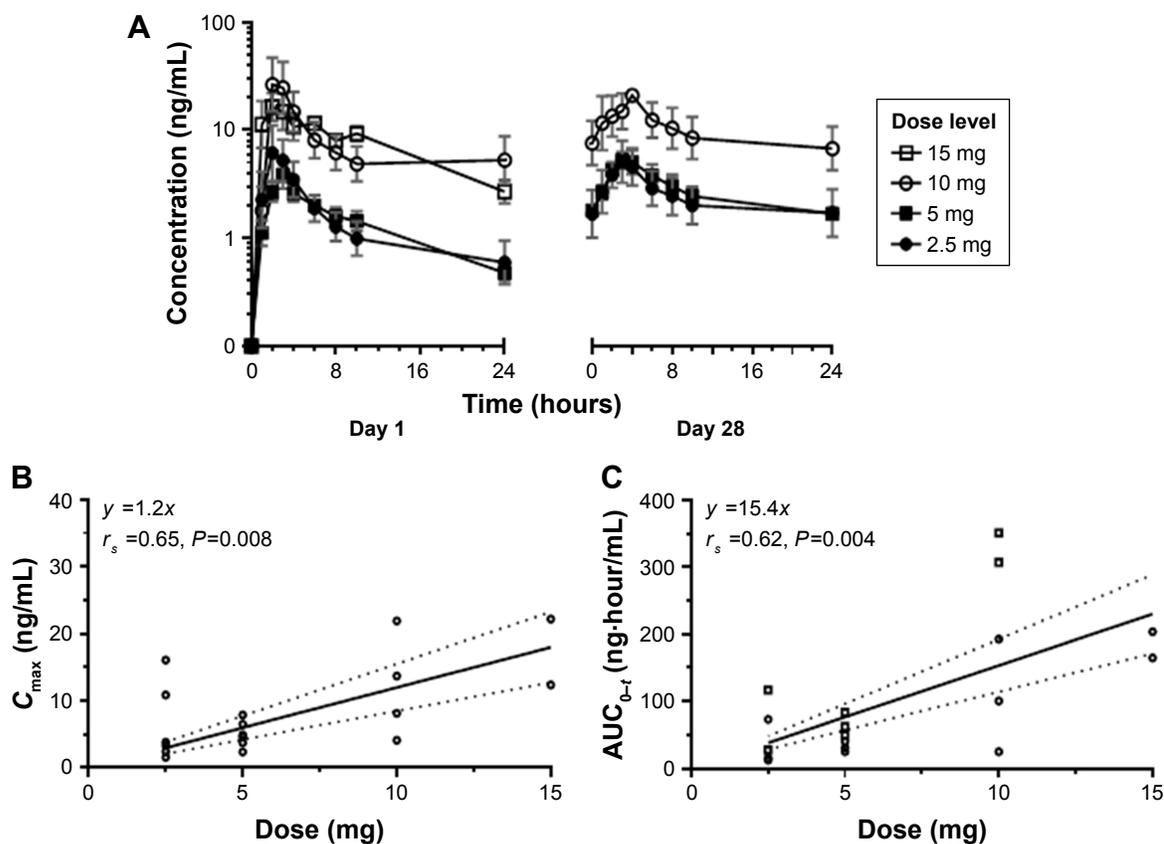


Figure 2 Pharmacokinetic assessment of omeveloxolone.

Notes: (A) Drug concentration over time at days 1 and 28. Day 28 collections were missing for 15 mg cohort. (B) Maximum drug concentration in plasma. (C) Area under the plasma concentration–time curve was plotted against dose using linear regression for both day 1 and 28. Dotted lines represent 90% confidence interval. r_s , Spearman's rho correlation coefficient with two-sided significance.

trials. Currently, omeveloxolone is being further studied in a dose-escalation trial in combination with nivolumab or ipilimumab for solid tumors, which started at the 5 mg dose level.¹⁷

This Phase I trial was characterized by important limitations. Unfortunately, optional tumor biopsies were not performed for most patients, and thus, we have no consistent evidence showing modulation of antioxidant activity within the tumor. Due to the initiation of a competing combination trial for solid tumors, the dose escalation was concluded at 15 mg, before a definitive escalation to MTD could be reached.¹⁷ Therefore, all statements regarding safety must be moderated by the small sample size of this trial. Although this trial had a small sample size, no adverse safety signal was observed. Another limitation is that most subjects progressed within several months of treatment, and mean drug exposure was only 85 days. Therefore, no conclusive assessment of long-term toxicity was possible. This inevitable disease progression was not unexpected, since all subjects had progressive and refractory cancer at

baseline. Omeveloxolone is also being further explored in non-cancer populations in Phase II trials for mitochondrial disorders (Table 5).^{18,19}

Omeveloxolone is a second-generation analog of bardoxolone methyl, whose development was temporarily halted by a higher-than-expected number of diastolic heart failure events in a Phase III registration trial for advanced CKD.²⁰ It was subsequently determined that doses of bardoxolone methyl at 20 mg/day promoted fluid retention in a subset of CKD patients with preexisting risk factors, including prior hospitalization for heart failure and elevated BNP at baseline. Moreover, a cumulative increase in BNP within 4 weeks of treatment with bardoxolone methyl was observed in advanced CKD patients. This phenomenon was likely due to suppression of basal endothelin-1 secretion and endothelin receptor A expression, whereby acute modulation of this pathway in advanced CKD patients' population likely promoted acute sodium retention which translated into frank fluid overload in susceptible patients.¹⁶ Importantly, no cumulative increase in BNP was observed with omeveloxolone during this trial

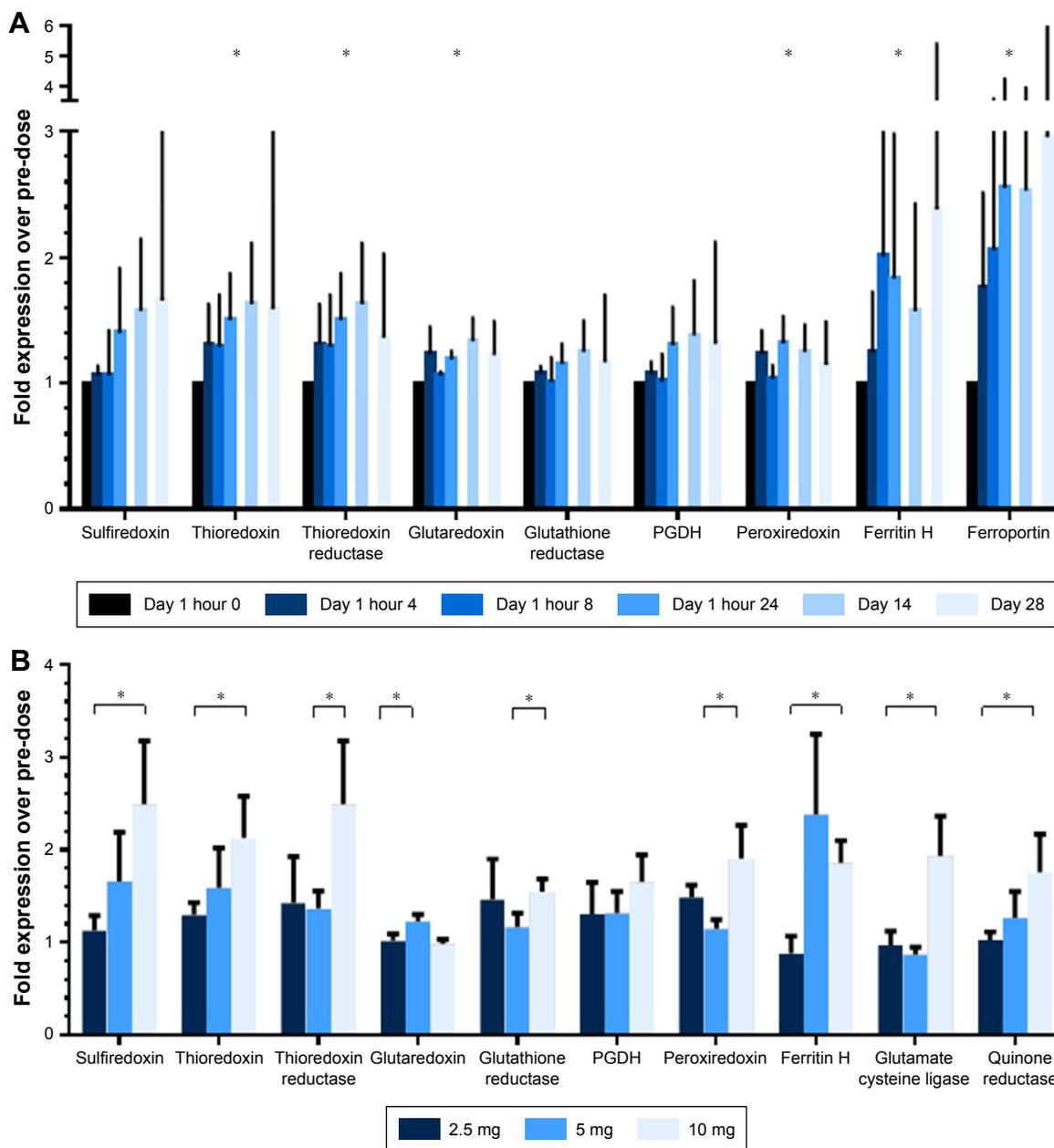


Figure 3 Effect of continuous oral omaveloxolone upon Nrf2 target genes.

Notes: mRNA expression was normalized to two housekeeping genes and then pre-dose level. **(A)** Expression over timepoints, 5 mg cohort example (n=3). Bars represent mean with standard error. * $P < 0.05$ for Wilcoxon signed-rank test of whether post-dose timepoints exceeded normalized pre-dose level of 1. **(B)** Expression by dose level for samples collected at day 28, n=3 for each cohort. Bars represent mean with standard error. * $P < 0.05$ for one-sided t-test for comparison between indicated groups.

Abbreviations: Ferritin H, ferritin heavy subunit; mRNA, messenger RNA; Nrf2, nuclear factor erythroid 2-related factor 2; PGDH, prostaglandin dehydrogenase 1.

(Figure 1B). Moreover, in a subsequent Phase II trial for pulmonary arterial hypertension, doses of bardoxolone methyl as high as 10 mg/day improved exercise performance compared to placebo without evidence of fluid retention.²¹ Therefore, it is possible that the MTD of triterpenoid analogs may exceed the dose required to demonstrate biologic activity.

Although pharmacodynamic activity was seen, no objective responses were observed in our trial. In contrast,

clinical activity was observed in one lymphoma patient and one thyroid cancer patient in a trial of bardoxolone methyl.²² This discordance may be related to the histology of cancers in our trial limited to melanoma and lung cancer only.^{23,24} MDSCs are implicated in tumor cell resistance to adoptive T-cell therapies, and inhibition of MDSC oxidative pathways may restore anti-tumor activity.^{25,26} Peripheral MDSCs are associated with failure to respond to dual checkpoint

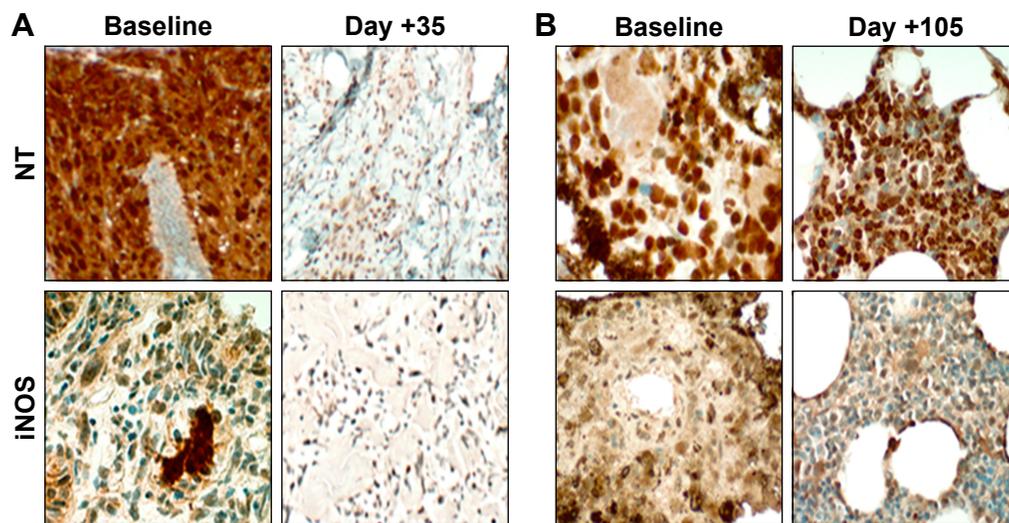


Figure 4 Representative images of NT and iNOS from limited on-treatment tumor biopsies for two patients with metastatic melanoma.

Notes: (A) Punch skin biopsy at day +35 from patient in 10 mg cohort showed overall decrease in expression of nitration markers compared to baseline. (B) Bone metastasis biopsy at day +105 from patient in 5 mg cohort showed little difference in NT or iNOS expression compared to baseline. Archival bone samples were difficult to evaluate due to prior decalcification and scant tumor. Serial biopsies for additional patients were not available. Original magnification 4x.

Abbreviations: iNOS, inducible isoform of nitric oxide synthase; NT, nitrotyrosine.

blockade against CTLA-4 and PD-1 in advanced melanoma and were associated with failure to respond to nivolumab monotherapy.^{27,28} In addition, the MDSCs of non-responders to ipilimumab may produce more nitric oxide than those of responders.²⁹ Moreover, cytokine-directed abrogation of iNOS from cultured ex vivo MDSCs was sufficient to restore T-cell proliferation and inhibit tumor cell growth in vitro.³⁰ Therefore, it is conceivable that omaveloxolone may be

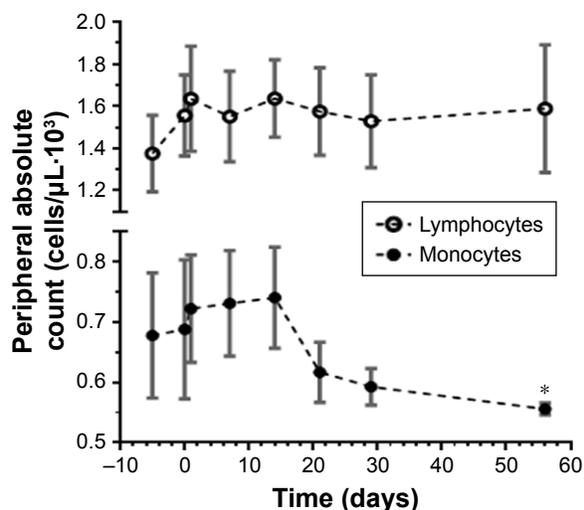


Figure 5 Decrease in peripheral blood monocytic cells over time.

Notes: Absolute peripheral cell counts by automated differential during omaveloxolone treatment for all dose levels (n=11) are shown. Time 0 represents first day of dosing. Circles represent mean with standard error. *Denotes P=0.05 for one-sided t-test compared to baseline.

a favorable combination partner for immune checkpoint blockade of solid tumors.

Alternative strategies to relieve MDSC-mediated tumor immunosuppression include blockade of colony-stimulating factor-1 (CSF-1R) and interleukin-34 signaling via its receptor, CSF-1R. For example, the selective CSF-1R inhibitor BLZ945 reduced the activity of MDSCs and slowed tumor progression in a murine neuroblastoma model and is being tested in Phase I/II trials.³¹ Similarly, neutralizing antibodies to chemokine receptors required for MDSC recruitment, such as CCR2 (C-C chemokine receptor type 2) and CXCR4 (C-X-C chemokine receptor type 4), are being evaluated for solid tumors.³² By promoting major histocompatibility complex II expression on myeloid cells, CD40 agonists can also enhance antigen presentation and subsequent anti-tumor responses.^{33,34} Likewise, antagonism of vascularization via angiotensin receptor (tyrosine kinase with immunoglobulin-like and EGF-like domains 2) or vascular endothelial growth factor receptor inhibitors may induce differentiation of MDSCs into immunocompetent myeloid cells and reduce tumor growth.^{35,36} Thus, semisynthetic triterpenoids are only one of many expanding drug classes intended to abrogate MDSC-associated immunosuppression. Evidence indicates that omaveloxolone induction of Nrf2 target genes can potentially improve muscle function, oxidative phosphorylation, and antioxidant capacity in patients with mitochondrial myopathies.³⁷ Therefore, this Phase I trial represents a first step toward defining the safety of a new drug with potential in multiple disease types.³⁸

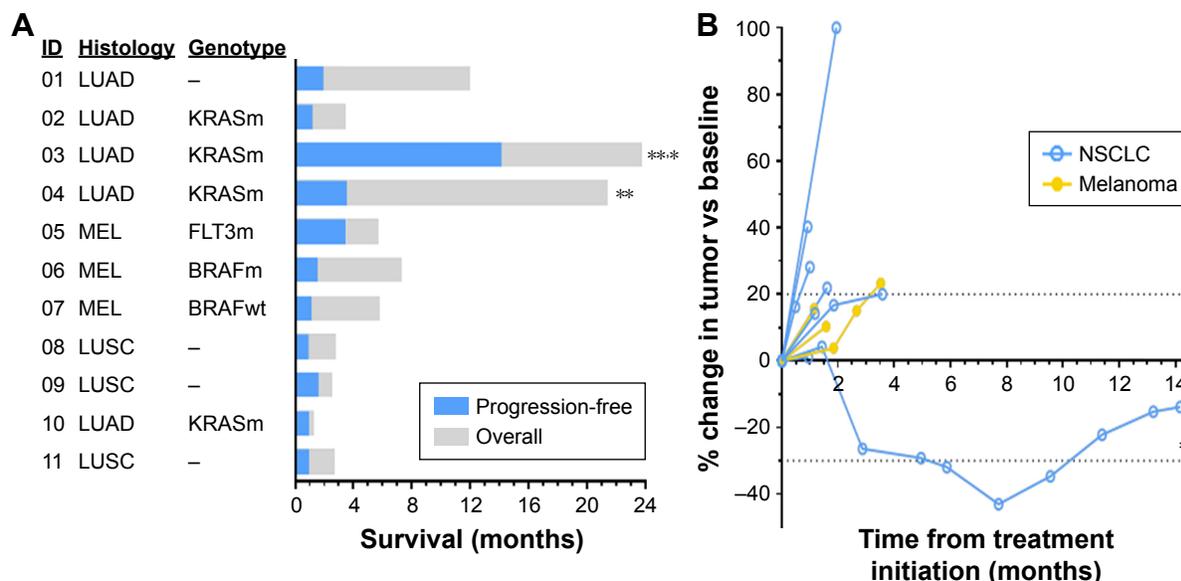


Figure 6 Patient outcome after treatment. **Notes:** (A) Swimmers plot of survival for omaveloxolone-treated subjects according to modified RECIST. Numbers indicate dose level, in mg. (B) Spider plot showing change in target lesions by modified RECIST. *Target lesion noted as difficult to measure due to atelectasis. **Still alive during last assessment. **Abbreviations:** LUSC, squamous lung cancer; LUAD, lung adenocarcinoma; MEL, melanoma; NSCLC, non-small cell lung cancer; RECIST, response evaluation criteria in solid tumor.

Conclusion

Our findings suggest that omaveloxolone is well tolerated by patients with advanced solid tumors, at doses which may activate the Nrf2 pathway in the PBMC compartment. The safety and pharmacology profile from this small trial supports the continued clinical investigation of omaveloxolone for humans.

Abbreviations

AEs, adverse events; AUC_{0-t} , area under the plasma concentration–time curve from time zero to the time (t) of the last measurable concentration; BNP, B-type natriuretic peptide; C_{max} , maximum concentration; CCR2, C–C chemokine receptor type 2; CD, cluster of differentiation; CKD, chronic kidney disease; CSF-1R, colony-stimulating factor-1 receptor; CTCAE, common toxicity criteria for

adverse events; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CXCR4, C-X-C chemokine receptor type 4; DLT, dose-limiting toxicity; ECG, electrocardiogram; ECOG, Eastern Cooperative Oncology group; *ICH-GCP*, International Conference on Harmonization Good Clinical Practice; IHC, immunohistochemistry; iNOS, inducible isoform of nitric oxide synthase; MDSCs, myeloid-derived suppressor cells; mRNA, messenger RNA; MTD, maximum tolerated dose; Nrf2, nuclear factor erythroid 2-related factor 2; NT, nitrotyrosine; PBMCs, peripheral blood mononuclear cells; PD-1, programmed cell death protein 1; RECIST, response evaluation criteria in solid tumor; ROS, reactive oxygen species; RPL13A, 60S ribosomal protein L13a; r_s , Spearman’s rho correlation coefficient; $t^{1/2}$, terminal elimination half-life; T_{max} , time of maximum concentration; ULN, upper limit of normal.

Table 5 Current clinical investigation of omaveloxolone

Trial	Indication	Size	Form	Phase	Start date	NCT#
MOTOR	Treatment of mitochondrial myopathy	56	Oral	2	4–2015	02255422
MOXle	Treatment of Friedrich’s ataxia	56	Oral	2	1–2015	02255435
PRIMROSE	Radiation dermatitis in adjuvant breast cancer	187	Lotion	2	6–2014	02142959
REVEAL*	Treatment of metastatic melanoma	84	Oral	1/2	10–2014	02259231
GUARD	Prevention of post-operative corneal endothelial loss	304	Suspension	2	5–2014	02128113

Note: *Combination trial of omaveloxolone with nivolumab or ipilimumab. **Abbreviation:** NCT, national clinical trials identifier number.

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Availability of data and material

The data that support the findings of this study are not publically available due to a third party restriction and confidentiality disclosure agreement with Reata Pharmaceuticals, Inc.

Ethics approval and consent to participate

All participants provided written informed consent. The study was approved by Liberty IRB; IRB00008679.

Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors MPC, SAR, JWP, and CJM are employed by, and have a financial interest in, Reata Pharmaceuticals. DIG has served as a paid consultant with Reata Pharmaceuticals. MDM is employed by, and has a financial interest in, AbbVie, Inc. The author BCC has served as a consultant/advisory role for AbbVie, Inc in 2017. The authors report no other conflicts of interest in this work.

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