

SPONSOR: Houston Methodist Cancer Center & Houston Methodist Research Institute

TITLE: Phase Ib Trial of L-NMMA in Combination with Pembrolizumab in Patients with Melanoma, Non-Small Cell Lung Cancer, Head and Neck Squamous Cell Carcinoma, Classical Hodgkin Lymphoma, Urothelial Carcinoma, Cervical Cancer, Esophageal Cancer, Gastric Cancer, Hepatocellular Carcinoma, Merkel Cell Carcinoma, Primary Mediastinal Large B-cell Lymphoma, Renal Cell Carcinoma, Small Cell Lung Cancer, Microsatellite Instability-High/Mismatch Repair Deficient Cancer, or for the Treatment of Adult Patients with Unresectable or Metastatic Tumor Mutational Burden-High Solid Tumors

SPONSOR-INVESTIGATOR/

PRINCIPAL INVESTIGATOR: Eric Bernicker, M.D.
Houston Methodist Cancer Center
Houston Methodist Hospital System
6565 Fannin Street
Houston, TX 77030

MEDICAL MONITOR: Eric Bernicker, M.D.
Office: 713-441-9823
Email: bernicker@houstonmethodist.org

IND NUMBER: 135888

IRB NUMBER: Pro00017492

VERSION: 9.0

DATE: February 26, 2021

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List of Abbreviations

Abbreviation	Definition
ALK	Anaplastic lymphoma kinase
ALT	Alanine transaminase
ANC	Absolute neutrophil count
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
AE	Adverse event
β-HCG	Beta-human chorionic gonadotropin
cHL	Classical Hodgkin lymphoma
CI	Confidence interval
CR	Complete response
CRC	Colorectal cancer
CrCl	Creatinine clearance
CRF	Case report form
CRM	Continual Reassessment Method
CTCAE	Common Terminology Criteria for Adverse Events
CT	Computed tomography
CTLA-4	Cytotoxic T-lymphocyte-associated antigen-4
dMMR	Mismatch repair deficient
DLT	Dose-limiting toxicity
DoR	Duration of Response
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
EOT	End of treatment
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
HIV	Human immunodeficiency virus
HNSCC	Head and neck squamous cell carcinoma
HR	Hazard ratio
HSCT	Hematopoietic stem cell transplantation
Ig	Immunoglobulin
IL-2	Interleukin-2
iNOS	Inducible nitric oxide synthase
INR	International normalized ratio
IRB	Institutional review board
IV	Intravenous
KN	KEYNOTE
L-NMMA	NG-monomethyl-L-arginine
MDSC	Myeloid-derived suppressor cell
MSI-H	Microsatellite instability-high
MTD	Maximum tolerated dose

Abbreviation	Definition
MUGA	Multigated acquisition
NCI	National Cancer Institute
NO	Nitric oxide
NOS	Nitric oxide synthase
NSCLC	Non-small cell lung cancer
ORR	Objective response rate
OS	Overall survival
PD	Progressive disease
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1
PD-L2	Programmed death-ligand 2
PDX	Patient-derived xenograft
PFS	Progression-free survival
PK	Pharmacokinetics
PR	Partial response
PT	Prothrombin time
Q2W	Every 2 weeks
Q3W	Every 3 weeks
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Recommended Phase II dose
SAE	Serious adverse event
SD	Stable disease
TIL	Tumor-infiltrating lymphocyte
TIME	Tumor immune microenvironment
TNBC	Triple negative breast cancer
TPS	Tumor proportion score
Tregs	Regulatory T-cells
ULN	Upper limit of normal

1.0 BACKGROUND & RATIONALE

1.1 Background

1.1.1 Programmed Death-1 Targeting for Cancer Immunotherapy

Immune checkpoints are negative regulators of the immune system that are crucial for maintaining self-tolerance, preventing autoimmunity, and protecting tissues from immune collateral damage. Tumors are able to hijack immune checkpoint pathways, thus restraining the ability of the immune system to mount an effective antitumor response. Blockade of immune checkpoints such as cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed death-1 (PD-1) has proved to be a promising approach for inducing antitumor immunity.

Upregulation of programmed death-ligand 1 (PD-L1) and its ligation to PD-1 on antigen-specific CD8⁺ T-cells (termed adaptive immune resistance) represents a major mechanism by which cancer tissues limit the host immune response.^{1,2} Under healthy conditions, PD-1, expressed on the cell surface of activated T-cells, functions to downmodulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to CD28 and CTLA-4 that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2).

The mechanism by which PD-1 downmodulates T-cell responses is similar to but distinct from that of CTLA-4, as both molecules regulate an overlapping set of signaling proteins.³ PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4⁺ and CD8⁺ T-cells, B-cells, regulatory T-cells (Tregs), and natural killer cells.⁴ PD-1 expression has also been shown during thymic development on CD4⁺CD8⁻ (double negative) T-cells as well as subsets of macrophages and dendritic cells.⁵ The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues and various tumors. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue and chronic inflammatory environments.⁶ PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers have been demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma.⁷ This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered an attractive target for therapeutic intervention.

1.1.2 Pembrolizumab

Pembrolizumab (MK-3475; trade name Keytruda) is a potent and highly selective humanized monoclonal antibody of the IgG4/kappa isotype designed to directly block the interaction

between PD-1 and its ligands, PD-L1 and PD-L2. Antibody-mediated PD-1 blockade with pembrolizumab and other similar agents reinvigorates the immune system, allowing for cancer cell targeting and destruction. Pembrolizumab is one of a number of closely related therapies dubbed immune checkpoint blockade and is approved for the treatment of melanoma, non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), classical Hodgkin lymphoma (cHL), urothelial carcinoma, cervical cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, merkel cell carcinoma, primary mediastinal large B-cell lymphoma, renal cell carcinoma, small cell lung cancer, microsatellite instability-high (MSI-H)/mismatch repair deficient (dMMR) cancer and for the treatment of adult patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥ 10 mutations/megabase (mut/Mb)] solid tumors.

1.1.2.1 Pembrolizumab and Melanoma

Melanoma is the deadliest form of skin cancer. Although it comprises less than 5% of skin cancer cases, melanoma accounts for the great majority of skin cancer-related deaths. The CTLA-4 antibody ipilimumab is standard of care for patients with advanced melanoma. Patients with melanoma that progresses on or after ipilimumab and BRAF or mitogen-activated protein kinase kinase inhibitor-based therapy if disease is BRAF^{V600} mutated have few therapeutic options.

Pembrolizumab has been approved by the United States Food and Drug Administration (FDA) for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. FDA approval was based on the results of KEYNOTE (KN)001 (NCT01295827) and KN002 (NCT01704287). KN001 was an open-label, first-in-human Phase Ib trial of pembrolizumab in subjects with progressive locally advanced or metastatic carcinomas. The melanoma cohort included subjects with advanced disease previously treated with ipilimumab or ipilimumab in combination with a BRAF inhibitor (for *BRAF* mutation carriers).⁷ After mandatory biopsy, subjects were treated with one of three doses of pembrolizumab for 12 weeks; responders continued on treatment until disease progression. Among 173 subjects treated with the recommended 2-mg/kg pembrolizumab dose, the overall response rate was 24%, with a duration of response (DoR) of 1.4 to 8.5 months.

KN002 was a partially blinded, randomized Phase II trial designed to evaluate 2 doses of pembrolizumab versus a chemotherapy control arm in subjects with ipilimumab-refractory metastatic melanoma.⁸ Subjects (n = 540) were randomized to receive pembrolizumab 2 mg/kg every 3 weeks (Q3W; n = 180), pembrolizumab 10 mg/kg Q3W (n = 181), or chemotherapy (according to current clinical practice; n = 179). Subjects assigned to the control chemotherapy arm could cross over to the experimental pembrolizumab arm once progression was confirmed (approximately \geq Week 12). Based on 410 progression-free survival (PFS) events, PFS was improved in the pembrolizumab 2 mg/kg (hazard ratio [HR]: 0.57; 95% confidence interval [CI]: 0.45–0.73; $P < 0.0001$) and pembrolizumab 10 mg/kg groups (HR: 0.50; 95% CI: 0.39–0.64; $P < 0.0001$) compared with the chemotherapy group. The 6-month PFS rate was 34%

(95% CI: 27–41) in the pembrolizumab 2 mg/kg group, 38% (95% CI: 31–45) in the pembrolizumab 10 mg/kg group, and 16% (95% CI: 10–22) in the chemotherapy group. Grade 3–4 treatment-related adverse events (AEs) occurred in 20 (11%) subjects in the pembrolizumab 2 mg/kg group, 25 (14%) subjects in the pembrolizumab 10 mg/kg group, and 45 (26%) subjects in the chemotherapy group. The most common Grade 3–4 treatment-related AE in the pembrolizumab groups was fatigue (2 [1%] of 178 subjects in the 2 mg/kg group and 1 [<1%] of 179 subjects in the 10 mg/kg group compared with 8 [5%] of 171 subjects in the chemotherapy group). Other Grade 3–4 treatment-related AEs included generalized edema and myalgia (each in 2 [1%] subjects) in the pembrolizumab 2 mg/kg group; hypopituitarism, colitis, diarrhea, decreased appetite, hyponatremia, and pneumonitis (each in 2 [1%] subjects) in the pembrolizumab 10 mg/kg group; and anemia (9 [5%] subjects), fatigue (8 [5%] subjects), neutropenia (6 [4%] subjects), and leucopenia (6 [4%] subjects) in the chemotherapy group.

Results of the KN006 trial (NCT01866319) led to the expanded indication of pembrolizumab for the frontline treatment of patients with unresectable or metastatic melanoma. KN006 was a randomized, controlled Phase III trial of pembrolizumab versus ipilimumab in subjects with unresectable stage III or IV advanced melanoma who had received no more than one prior systemic therapy.⁹ Subjects (n = 834) were randomized to receive pembrolizumab 10 mg/kg Q3W, pembrolizumab 10 mg/kg every 2 weeks (Q2W), or four cycles of ipilimumab 3 mg/kg Q3W. The estimated 6-month PFS rates were 47.3% and 46.4% for pembrolizumab Q2W and Q3W, respectively, compared with 26.5% for ipilimumab (HR: 0.58; 95% CI: 0.46–0.72 and 0.47–0.72, respectively; P < 0.001 for both pembrolizumab regimens vs. ipilimumab). Estimated 12-month survival rates were 74.1% for pembrolizumab Q2W, 68.4% for pembrolizumab Q3W, and 58.2% for ipilimumab (HR for pembrolizumab Q2W: 0.63; 95% CI: 0.47–0.83; P = 0.0005; HR for pembrolizumab Q3W: 0.69; 95% CI: 0.52–0.90; P = 0.0036). Response rates were higher for pembrolizumab Q2W and Q3W compared with ipilimumab (33.7% and 32.9% vs. 11.9%; both P < 0.001). Response rate was similar between the two pembrolizumab regimens. Rates of Grade 3–5 treatment-related AEs were lower in the pembrolizumab Q2W and Q3W groups than in the ipilimumab group (13.3% and 10.1% vs. 19.9%).

1.1.2.2 Pembrolizumab and NSCLC

Lung cancer is the leading cause of cancer death in the United States. Lung cancer is expected to account for an estimated 222,500 new cases (116,990 in men and 105,510 in women) and 155,870 deaths (84,590 in men and 71,280 in women) in 2017. NSCLC accounts for over 85% of all lung cancer cases. Most patients with NSCLC are diagnosed at an advanced stage and have a poor prognosis, with a 5-year survival rate of <5%. Despite the development of new chemotherapeutic and molecularly targeted agents, outcomes remain poor. Platinum-based chemotherapy is the standard of care for the initial management of advanced and metastatic NSCLC. Fifteen to 30% of NSCLC patients have chemorefractory disease.¹⁰ Furthermore, patients who respond well initially to chemotherapy will ultimately develop resistance.¹¹ Treatment options for patients with refractory/relapsed NSCLC are limited.

Pembrolizumab has been approved for the treatment of patients with metastatic PD-L1-positive (tumor proportion score [TPS] $\geq 1\%$) NSCLC whose disease has progressed on or after platinum-containing chemotherapy or targeted therapy against anaplastic lymphoma kinase (ALK) or epidermal growth factor receptor (EGFR), if appropriate. FDA approval was based on the results from KN001 and KN010 (NCT01905657). In KN001, subjects with metastatic NSCLC ($n = 550$) were treated with various pembrolizumab regimens (2 mg/kg Q2W, 10 mg/kg Q2W or Q3W).¹² The objective response rate (ORR) in the efficacy population, which comprised 61 subjects with PD-L1 strongly positive tumors, was 41% (95% CI: 28.6–54.3); all were partial responses (PRs). At the time of the analysis, responses were ongoing in 21 of 25 (85%) subjects, with 11 (44%) subjects having a DoR of ≥ 6 months. KN010 was a randomized, adaptively designed Phase II/III trial of pembrolizumab at 2 dose levels versus docetaxel in subjects with NSCLC with PD-L1 positive tumors who experienced disease progression after platinum-containing systemic therapy.¹³ Subjects were randomized according to their TPS: TPS $\geq 50\%$, strongly positive and TPS = 1% to 49%, weakly positive. In the total population, median overall survival (OS) was significantly longer for the pembrolizumab 2 mg/kg and 10 mg/kg groups compared with the docetaxel group (10.4 and 12.7 months vs. 8.5 months, respectively; $P = 0.0008$ and $P < 0.0001$, respectively). Median PFS was not significantly different between the pembrolizumab 2 mg/kg and 10 mg/kg groups and the docetaxel group (3.9 and 4.0 months vs. 4.0 months, respectively; $P = 0.07$ and $P = 0.004$, respectively). Among subjects with strongly PD-L1 positive NSCLC, median OS was significantly longer with pembrolizumab 2 mg/kg and pembrolizumab 10 mg/kg than with docetaxel (14.9 and 17.3 months vs. 8.2 months, respectively; $P = 0.0002$ and $P < 0.0001$, respectively). For this population, median PFS was also significantly longer in the pembrolizumab 2 mg/kg and 10 mg/kg groups compared with the docetaxel group (5.0 and 5.2 months vs. 4.1 months, respectively; $P = 0.0001$ and $P < 0.0001$, respectively).

Pembrolizumab has also been approved for the frontline treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression (TPS $\geq 50\%$ as determined by an FDA-approved test) and no *ALK* or *EGFR* genomic tumor aberrations. Approval was based on the results from the open-label, Phase III KN024 trial (NCT02142738). Subjects ($n = 305$) were randomly assigned to receive either pembrolizumab (200 mg Q3W) or investigator's choice of platinum-based chemotherapy.¹⁴ Crossover from the chemotherapy group to the pembrolizumab group was permitted in the event of disease progression. Median PFS was significantly longer in the pembrolizumab group than in the chemotherapy group (10.3 months [95% CI: 6.7 to not reached] vs. 6.0 months [95% CI: 4.2–6.2]; $P < 0.001$). The estimated 6-month OS rate was 80.2% in the pembrolizumab group versus 72.4% in the chemotherapy group (HR for death: 0.60; 95% CI: 0.41–0.89; $P = 0.005$). ORR was higher (44.8% vs. 27.8%) and median DoR was longer (not reached [range: 1.9+ to 14.5+ months] vs. 6.3 months [range: 2.1+ to 12.6+]) in the pembrolizumab group than in the chemotherapy group. Frequency of treatment-related AEs was lower in the pembrolizumab group than in the chemotherapy group (73.4% vs. 90.0%). The most common treatment-related AEs were diarrhea (in 14.3% of subjects), fatigue (10.4%), and pyrexia (10.4%) in the pembrolizumab group and anemia (44.0%), nausea (43.3%), and fatigue (28.7%) in the chemotherapy group. Grade 3–5 treatment-related AEs occurred in twice as many subjects in the chemotherapy group as in the pembrolizumab group (53.3% vs. 26.6%). Grade 3–5 treatment-related AEs that occurred in

four or more subjects were diarrhea (in 3.9% of subjects) and pneumonitis (2.6%) in the pembrolizumab group and anemia (19.3%), neutropenia (13.3%), decreased platelet count (6.0%), thrombocytopenia (5.3%), decreased neutrophil count (4.0%), fatigue (3.3%), and decreased appetite (2.7%) in the chemotherapy group. Treatment-related serious adverse events (SAEs) occurred in a similar percentage of subjects in the pembrolizumab and chemotherapy groups (21.4% and 20.7%, respectively). Discontinuation of treatment because of treatment-related AEs occurred in 7.1% of subjects in the pembrolizumab group and 10.7% of subjects in the chemotherapy group. Treatment-related AEs that led to death occurred in one subject in the pembrolizumab group (sudden death of unknown cause) and three subjects in the chemotherapy group (one death each due to pulmonary sepsis, pulmonary alveolar hemorrhage, and unknown cause).

1.1.2.3 Pembrolizumab and HNSCC

Head and neck cancer is expected to account for an estimated 59,340 new cancer cases and 13,000 deaths in the United States in 2017. Approximately 90% of head and neck cancers are squamous cell carcinomas. Most patients with HNSCC present with locoregionally advanced disease, and more than 50% have recurrence within 3 years.¹⁵⁻¹⁷ Platinum-based chemotherapy is generally used for the treatment of advanced-stage HNSCC; however, treatment efficacy is limited by intrinsic and acquired resistance.^{18,19} An estimated 10–30% of newly diagnosed patients and more than 70% of relapsed patients are resistant to platinum-based chemotherapy.^{19,20} Patients with HNSCC who have cancer progression within 6 months after platinum-based chemotherapy for primary or recurrent disease have a median survival of 6 months or less.²¹

Pembrolizumab has shown great promise in the treatment of HNSCC and has been approved for the treatment of patients with recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy. FDA approval was based on results from the multicenter, open-label, nonrandomized Phase Ib KN012 trial (NCT01848834).²² KN012 evaluated the safety of pembrolizumab in subjects with recurrent or metastatic HNSCC (n = 192). Efficacy was evaluated in 174 of these subjects who had disease progression on or after platinum-containing chemotherapy administered for recurrent or metastatic HNSCC or following platinum-containing chemotherapy administered as part of induction, concurrent, or adjuvant therapy. The median number of prior lines of therapy administered for the treatment of HNSCC was two. Subjects received pembrolizumab at a dose of 10 mg/kg Q2W (n = 53) or a 200-mg fixed dose Q3W (n = 121) until unacceptable toxicity or disease progression. Subjects without disease progression were treated for up to 24 months. The ORR was 16% (95% CI: 11–22). The median DoR had not been reached at the time of analysis. The range for DoR was 2.4 months to 27.7 months (response ongoing). Among the 28 responding subjects, 23 (82%) had responses of 6 months or longer. The ORR and DoR were similar irrespective of dosage regimen. SAEs occurred in 45% of subjects. The most frequent SAEs (reported in at least 2% of subjects) were pneumonia, dyspnea, confusional state, vomiting, pleural effusion, and respiratory failure. The incidence of AEs, including SAEs, was similar between dosage regimens (10 mg/kg Q2W or 200 mg Q3W). The most common AEs (reported in at least 20% of patients) were fatigue (46%), decreased appetite (22%), and dyspnea (20%).

Preliminary results from KN055 have also demonstrated the significant clinical activity of pembrolizumab in subjects with heavily pretreated recurrent/metastatic HNSCC. KN055 (NCT02255097) is an ongoing Phase II trial of single-agent pembrolizumab in subjects (n = 171) with recurrent or metastatic HNSCC who have failed platinum and cetuximab (EGFR inhibitor).²³ Subjects with recurrent or metastatic HNSCC received pembrolizumab at 200 mg Q3W. The median number of prior therapies was 2, and nearly half of subjects (42%) had received ≥ 3 prior systemic therapies. After a median follow-up of 7 months, 54% of subjects experienced a decrease in target lesion size. Median time to response was 2 months, and 75% of responses continued at the time of analysis. Median PFS was 2.1 months and the 6-month PFS rate was 24%. Median OS was 8 months (95% CI: 8–11), with a 6-month OS rate of 65%. The ORR was 17% (95% CI: 9–28) in subjects with PD-L1-positive tumors (n = 76; expression in stroma or $\geq 1\%$ of tumor cells by immunohistochemistry). In the PD-L1-negative group (n = 13), the ORR was 8% (95% CI: 0.2–36). Sixty percent of subjects experienced a treatment-related AE of any grade, which included fatigue (15%), hypothyroidism (8%), diarrhea (6%), decreased appetite (5%), nausea (5%), alanine transaminase (ALT) increase (5%), and rash (5%). Grade 3–5 AEs occurred in 12% of subjects and included anemia, ALT increase, alkaline phosphatase increase, and hepatitis (in 1% of subjects each). There was 1 treatment-related death from pneumonitis and 3 subjects discontinued treatment due to AEs.

Pembrolizumab is currently being evaluated in two Phase III studies for the treatment of head and neck cancer: KN040 (NCT02252042; A Phase III Randomized Trial of MK-3475 [Pembrolizumab] Versus Standard Treatment in Subjects With Recurrent or Metastatic Head and Neck Cancer) and KN048 (NCT02358031; A Phase III Clinical Trial of Pembrolizumab [MK-3475] in First Line Treatment of Recurrent/Metastatic Head and Neck Squamous Cell Carcinoma).

1.1.2.4 Pembrolizumab and cHL

cHL accounts for 94% of all Hodgkin lymphoma cases. Frontline therapy is curative in a high percentage of patients. However, treatment failure occurs in approximately 10% of patients with limited-stage disease.²⁴ In advanced-stage disease, up to 10% of patients will not achieve complete remission and 20–30% of responders eventually relapse after treatment.²⁵ Second-line chemotherapy followed by high-dose therapy (HDT) and hematopoietic stem cell transplantation (HSCT) is the standard treatment for patients with refractory/relapsed cHL. Unfortunately, approximately 50% of patients experience relapse or disease progression after HSCT.²⁶ Patients who relapse after HSCT or who are not eligible for HDT are usually treated with targeted agents including the CD30-directed antibody-drug conjugate brentuximab vedotin. cHL that is refractory to or relapses after HSCT or brentuximab vedotin represents a significant unmet medical need with very limited treatment options.

Pembrolizumab has been approved for the treatment of patients with cHL who are refractory to treatment or who have relapsed after three or more lines of therapy. Pembrolizumab is the only anti-PD-1 therapy approved for the treatment of cHL regardless of prior HSCT or brentuximab vedotin therapy. FDA approval was based on data from the multicohort, open-

label, Phase Ib KN013 trial (NCT01953692) and the multicenter, nonrandomized, open-label, Phase II KN087 trial (NCT02453594).²⁷⁻²⁹ In the KN013 trial, subjects with relapsed or refractory cHL (n = 31) received pembrolizumab 10 mg/kg Q2W until disease progression. Fifty percent of subjects had more than 4 lines of prior therapy, and 71% of subjects had relapsed after HSCT. The overall response rate was 65% (90% CI: 48–79), with complete response (CR) and PR rates of 16% and 48%, respectively. Most responses (70%) lasted longer than 24 weeks (range: 0.14+ to 74+ weeks), with a median follow-up of 17 months. The PFS rate was 69% at 24 weeks and 46% at 52 weeks. Overall, 68% of subjects experienced one or more AEs that were deemed related to the study treatment. The most common treatment-related AEs were hypothyroidism (16%), diarrhea (16%), nausea (13%), and pneumonitis (10%). Five subjects (16%) experienced Grade 3 treatment-related AEs, which included colitis, increased ALT and aspartate transaminase (AST) levels, nephrotic syndrome, joint swelling, back pain, and axillary pain. There were no treatment-related Grade 4 AEs or deaths.

In the KN087 trial, subjects with relapsed or refractory cHL (n = 210) received pembrolizumab 200 mg Q3W until unacceptable toxicity or documented disease progression, or for up to 24 months in subjects who did not progress. The median number of prior lines of therapy administered for the treatment of cHL was 4 (range: 1 to 12). Fifty-eight percent of subjects were refractory to the last prior therapy, including 35% with primary refractory disease and 14% whose disease was chemorefractory to all prior regimens. Additionally, 61% of subjects had undergone prior HSCT, 83% had received prior brentuximab, and 36% had prior radiation therapy. The ORR was 69% (95% CI: 62–75), with CR and PR rates of 22% and 47%, respectively. The median follow-up time was 9.4 months. Among the 145 responding subjects, the median DoR was 11.1 months (range: 0.0+ to 11.1 months). Five percent of subjects discontinued treatment due to AEs, and 26% of subjects experienced treatment interruption due to AEs. The most common AEs included fatigue (26%), pyrexia (24%), cough (24%), musculoskeletal pain (21%), diarrhea (20%), and rash (20%). SAEs occurred in 16% of subjects. The most frequent SAEs ($\geq 1\%$) included pneumonia, pneumonitis, pyrexia, dyspnea, graft-versus-host disease (GVHD), and herpes zoster. Two subjects died from causes other than disease progression; one from GVHD after subsequent allogeneic HSCT and one from septic shock.

Pembrolizumab is currently being evaluated in a Phase III study for the treatment of relapsed/refractory cHL: KN204 (NCT02684292; Phase III, Randomized, Open-label, Clinical Trial to Compare Pembrolizumab With Brentuximab Vedotin in Subjects With Relapsed or Refractory cHL).

1.1.2.5 Pembrolizumab and Urothelial Carcinoma

Urothelial carcinomas, malignant tumors arising from the urothelial epithelium, can involve the lower (bladder and urethra) or upper (renal pelvis and ureter) urinary tract. Bladder tumors account for 90–95% of urothelial carcinomas, whereas upper urinary tract urothelial carcinomas are relatively rare, accounting for 5% of urothelial carcinomas.^{30,31} Most urothelial carcinomas are localized to the urinary tract, but 25% present with or develop locally advanced or metastatic disease.³² Cisplatin-based chemotherapy is the cornerstone of

advanced/metastatic urothelial carcinoma treatment. Although urothelial carcinoma is chemosensitive, responses are short-lived and most patients relapse within a median of 12 months. Patients with advanced/metastatic urothelial carcinoma that progresses after platinum-based chemotherapy have a poor prognosis and limited treatment options.

Pembrolizumab was granted FDA accelerated approval for the treatment of patients with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy. The FDA granted regular approval to pembrolizumab for patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.

The accelerated approval for the first-line indication was based on data from the single-arm, open-label, Phase II KN052 trial (NCT02335424).²⁸ Subjects (n = 370) with locally advanced or metastatic urothelial carcinoma who were not eligible for cisplatin-containing chemotherapy received pembrolizumab 200 mg Q3W. With a median follow-up time of 7.8 months, the ORR was 28.6% (95% CI: 24–34), with CR and PR rates of 7% and 22%, respectively. The median DoR was not reached (range: 1.4+ to 17.8+ months).

The regular approval for the second-line indication was based on data from KN045 (NCT02256436). KN045 was an open-label, multicenter, randomized, active-controlled Phase III trial of pembrolizumab versus paclitaxel, docetaxel, or vinflunine in subjects (n = 542) with advanced urothelial cancer that recurred or progressed after platinum-based chemotherapy.³³ Subjects were randomized to receive pembrolizumab 200 mg Q3W (n = 272) or investigator's choice of chemotherapy with paclitaxel 175 mg/m² (n = 84), docetaxel 75 mg/m² (n = 84), or vinflunine 320 mg/m² (n = 87) Q3W. Treatment continued until unacceptable toxicity or disease progression. Significant improvements in OS and ORR were demonstrated for the pembrolizumab arm compared with the chemotherapy arm. Median OS was 10.3 months (95% CI: 8.0–11.8) in the pembrolizumab group compared with 7.4 months (95% CI: 6.1–8.3) in the chemotherapy group (HR for death: 0.73; 95% CI: 0.59–0.91; P = 0.002). ORR was 21.1% (95% CI: 16.4–26.5) in the pembrolizumab group and 11.4% (95% CI: 7.9–15.8) in the chemotherapy group (P = 0.001). Median time to response was 2.1 months in both groups. Median DoR was not reached in the pembrolizumab group (range: 1.6+ to 15.6+ months) and was 4.3 months (range: 1.4+ to 15.4+) in the chemotherapy group. PFS was not significantly different between the pembrolizumab and chemotherapy groups (HR for death or disease progression: 0.98; 95% CI: 0.81–1.19; P = 0.42). Fewer treatment-related AEs of any grade were reported in the pembrolizumab group than in the chemotherapy group (60.9% vs. 90.2%); fewer Grade 3–5 AEs were also observed in the pembrolizumab group than in the chemotherapy group (15.0% vs. 49.4%).

1.1.2.6 Pembrolizumab and MSI-H/dMMR Cancer

MSI-H is a tumor phenotype associated with somatic or germline inactivating alterations in DNA mismatch repair genes. dMMR leads to an accumulation of genetic mutations, especially in repetitive coding or non-coding DNA sequences (microsatellites), that contributes to

carcinogenesis.³⁴ Mutation rates in tumor cells with dMMR are 100–1000-fold as compared with normal cells.^{35,36} The MSI-H phenotype is mainly found in colorectal cancer (CRC), endometrial cancer, and gastric cancer. Less commonly, the phenotype can also occur in breast, prostate, bladder, and thyroid cancers. Given the high mutation load and increased potential neoantigen expression of MSI-H tumors, immune checkpoint immunotherapies are being actively investigated as a treatment strategy. In a Phase II trial (NCT01876511), pembrolizumab demonstrated promising efficacy in the treatment of MSI-H/dMMR tumors. Subjects with dMMR CRC, MMR proficient CRC, and dMMR non-CRC received pembrolizumab 10 mg/kg Q2W.³⁷ The ORR and disease control rate was 0% (0/25) and 16% in the MMR proficient CRC cohort, 62% (8/13) and 92% in the dMMR CRC cohort, and 60% (6/10) and 70% in the non-CRC cohort, respectively. Mutation associated-neoantigens and mutational load correlated with improved PFS.

On May 23, 2017, the FDA granted accelerated approval to pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic, MSI-H/dMMR solid tumors that have progressed after prior treatment and who have no satisfactory alternative treatment options, as well as for patients with MSI-H/dMMR CRC following progression on a fluoropyrimidine, oxaliplatin, and irinotecan. The approval was based on data from 149 subjects with MSI-H/dMMR cancers enrolled across 5 single-arm clinical trials (KN016 [n = 58], KN164 [n = 61], KN012 [n = 6], KN028 [n = 5], and KN158 [n = 19]; see Table 1).²⁸ Ninety subjects had CRC and the remaining 59 subjects had 1 of 14 other tumor types. Pembrolizumab was administered at 200 mg Q3W or 10 mg/kg Q2W until disease progression, unacceptable toxicity, or a maximum of 24 months. The ORR was 39.6% (95% CI: 31.7–47.9), with CR and PR rates of 7.4% and 32.2%, respectively. The ORR was 36% in subjects with CRC and 46% in subjects with other tumor types. The median DoR was not yet reached (range: 1.6+ months to 22.7+ months). Among subjects who responded to pembrolizumab, 78% had responses that lasted for at least 6 months.

1.1.2.7 Pembrolizumab and Cervical Cancer

The Food and Drug Administration (FDA) granted accelerated approval to Keytruda (pembrolizumab) for the treatment of patients with advanced, PD-L1–positive cervical cancer with disease progression on or after chemotherapy. The agency based its decision on data from the global, open-label, nonrandomized, multicohort, multicenter phase 2 KEYNOTE-158 trial – an ongoing study designed to evaluate the efficacy of Keytruda in more than 1,300 adults who have advanced solid tumors that progressed on chemotherapy. Data for the approval included 98 patients with recurrent or metastatic cervical cancer enrolled in a single cohort of the study. Patients in the study intravenously received Keytruda at a dose of 200 mg every three weeks until unacceptable toxicity or documented disease progression.

At a median follow-up of 11.7 months, the overall response rate (ORR) was 14.3 percent in 77 patients, which included a complete response rate of 2.6 percent and a partial response rate of 11.7 percent among the cervical cancer cohort. In total, 10 patients (12 percent) experienced grade 3/4 treatment-related side effects. The recommended pembrolizumab dose for treatment of cervical cancer is 200 mg every 3 weeks.

1.1.2.8 Pembrolizumab and Esophageal Cancer

The Food and Drug Administration approved pembrolizumab for patients with recurrent, locally advanced or metastatic, squamous cell carcinoma of the esophagus (ESCC) whose tumors express PD-L1 (Combined Positive Score [CPS] ≥ 10), as determined by an FDA-approved test, with disease progression after one or more prior lines of systemic therapy. FDA also approved a new use for the PD-L1 IHC 22C3 pharmDx kit as a companion diagnostic device for selecting patients for the above indication. Efficacy was investigated in two clinical trials, KEYNOTE-181 (NCT02564263) and KEYNOTE-180 (NCT02559687). KEYNOTE-181 was a randomized, open-label, active-controlled trial that enrolled 628 patients with recurrent locally advanced or metastatic esophageal cancer who progressed on or after one prior line of systemic treatment for advanced or metastatic disease. Patients were randomized (1:1) to receive either KEYTRUDA 200 mg intravenously (IV) every 3 weeks or the investigator's choice of the following regimens: paclitaxel 80-100 mg/m² IV on days 1, 8, and 15 of every 4-week cycle; docetaxel 75 mg/m² IV every 3 weeks; or irinotecan 180 mg/m² IV every 2 weeks (control arm). Randomization was stratified by geographic region and histologic subtype (squamous versus adenocarcinoma). PD-L1 status was determined using the PD-L1 IHC 22C3 pharmDx kit. The primary efficacy outcome measure of KEYNOTE-181 was overall survival (OS) in patients with ESCC, patients with tumors expressing PD-L1 CPS ≥ 10 , and all randomized patients. Additional efficacy outcome measures were progression-free survival (PFS), overall response rate (ORR), and response duration. The hazard ratio for OS in patients with ESCC whose tumors expressed PD-L1 CPS ≥ 10 was 0.64 (95% CI: 0.46, 0.90). Median OS was 10.3 months (95% CI: 7.0, 13.5) and 6.7 months (95% CI: 4.8, 8.6) in the pembrolizumab and control arms, respectively.

KEYNOTE-180 was a single arm, open-label trial that enrolled 121 patients with locally advanced or metastatic esophageal cancer who progressed on or after at least 2 prior systemic treatments for advanced disease. With the exception of the number of prior lines of treatment, the eligibility criteria were similar to and the dosage regimen identical to KEYNOTE-181.

The major efficacy outcome measures of KEYNOTE-180 were ORR and response duration. In the 35 patients with ESCC expressing PD-L1 CPS ≥ 10 , ORR was 20% (95% CI: 8, 37) and response durations ranged from 4.2 to 25.1+ months, with 71% (5 patients) having responses of 6 months or longer and 57% (3 patients) having responses of 12 months or longer.

The recommended pembrolizumab dose for esophageal cancer is 200 mg every 3 weeks.

1.1.2.9 Pembrolizumab and Gastric Cancer

The U.S. Food and Drug Administration (FDA) has granted approval for pembrolizumab for the treatment of patients with recurrent, locally advanced or metastatic, with disease progression on or after two or more systemic therapies, including fluoropyrimidine- and platinum-containing chemotherapy and, if appropriate, HER2/neu-targeted therapy, and whose tumors express programmed death-ligand 1 (PD-L1), as determined by an FDA-approved test. Approval was based on demonstration of durable overall response rate (ORR) in a multicenter,

open-label, multicohort trial (KEYNOTE-059/Cohort 1) that enrolled 259 patients with locally advanced or metastatic gastric or GEJ adenocarcinoma. Among the 55% (n = 143) of patients whose tumors expressed PD-L1 based on a combined positive score ≥ 1 and either were microsatellite stable or had undetermined microsatellite instability or mismatch repair status, the confirmed ORR as determined by blinded independent central review was 13.3% (95% CI, 8.2–20.0); 1.4% had complete responses. Response durations ranged from 2.8+ to 19.4+ months; 11 patients (58%) had response durations of 6 months or longer, and 5 patients (26%) had response durations of 12 months or longer. The recommended pembrolizumab dose for gastric cancer is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression.

1.1.2.9.1 Pembrolizumab and Hepatocellular Carcinoma

The Food and Drug Administration has granted approval to pembrolizumab for patients with hepatocellular carcinoma (HCC) who have been previously treated with sorafenib. Approval was based on KEYNOTE 224 (NCT02702414), a single-arm, multicenter trial enrolling 104 patients with hepatocellular carcinoma. Patients were required to have disease progression on or after sorafenib or were intolerant to sorafenib, have measurable disease, and Child-Pugh Class A liver impairment. Twenty-one percent of the patients enrolled were HBV seropositive, 25% were HCV seropositive, and 9 patients (9%) were seropositive for both HBV and HCV. Patients with active autoimmune disease, more than one etiology of hepatitis, medical conditions requiring immunosuppression, or clinical evidence of ascites by physical exam were ineligible. Patients received pembrolizumab 200 mg as an intravenous infusion every three week until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression. The major efficacy outcome measure was confirmed overall response rate, as assessed by independent central review (ICR) according to RECIST 1.1 (modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ). The confirmed ICR-assessed overall response rate was 17% (95% CI: 11, 26), with one complete response and 17 partial responses. Response durations ranged from 3.1 to 16.7 months; 89% of responders had response durations of 6 months or longer and 56% had response durations of 12 months or longer. The recommended pembrolizumab dose for hepatocellular carcinoma is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks.

1.1.2.9.2 Pembrolizumab and Merkel Cell Carcinoma

The Food and Drug Administration has granted approval to pembrolizumab for adult and pediatric patients with recurrent locally advanced or metastatic Merkel cell carcinoma (MCC). Approval was based on Cancer Immunotherapy Trials Network protocol 9 (CITN-09), also known as KEYNOTE-017 (NCT02267603), a multicenter, non-randomized, open-label trial that enrolled 50 patients with recurrent locally advanced or metastatic MCC who had not received prior systemic therapy for their advanced disease. Patients received pembrolizumab 2 mg/kg every 3 weeks. The major efficacy outcome measures were overall response rate (ORR) and response duration assessed by blinded independent central review per RECIST 1.1.

The ORR was 56% (95% CI: 41, 70) with a complete response rate of 24%. The median response duration was not reached. Among the 28 patients with responses, 96% had response durations of greater than 6 months and 54% had response durations of greater than 12 months. The recommended pembrolizumab dose for MCC is 200 mg administered as a 30-minute intravenous infusion every 3 weeks for adults; 2 mg/kg (to a maximum of 200 mg) administered as a 30-minute intravenous infusion every 3 weeks for patients less than 18 years of age (pediatric patients).

1.1.2.9.3 Pembrolizumab and Renal Cell Carcinoma

The Food and Drug Administration approved pembrolizumab plus axitinib for the first-line treatment of patients with advanced renal cell carcinoma (RCC). Approval was based on KEYNOTE-426 (NCT02853331), a randomized, multicenter, open-label trial conducted in 861 patients who had not received systemic therapy for advanced RCC. Patients were enrolled regardless of PD-L1 tumor expression status and were randomly allocated to receive either pembrolizumab 200 mg intravenously every 3 weeks in combination with axitinib 5 mg orally twice daily, or sunitinib 50 mg orally once daily for 4 weeks and then off treatment for 2 weeks. Treatment continued until confirmed disease progression or unacceptable toxicity. Pembrolizumab was received for maximum of 24 months. The main efficacy measures were overall survival (OS) and progression-free survival (PFS), assessed by blinded independent central review (RECIST 1.1.) The trial demonstrated a statistically significant improvement in OS in a pre-specified interim analysis for patients on the pembrolizumab plus axitinib arm (HR 0.53; 95% CI: 0.38, 0.74; $p < 0.0001$). With deaths reported in 18% of patients, the median OS was not reached in either arm. The 12-month OS rate was 90% in the pembrolizumab plus axitinib arm and 78% for those treated with sunitinib. The trial also demonstrated a PFS improvement for patients receiving pembrolizumab plus axitinib (HR 0.69; 95% CI: 0.57, 0.84; $p = 0.0001$). Median PFS was 15.1 and 11.1 months for those receiving pembrolizumab plus axitinib vs. sunitinib, respectively. The recommended pembrolizumab dose for this indication is 200 mg every 3 weeks with axitinib 5 mg orally twice daily.

1.1.2.9.4 Pembrolizumab and Small Cell Lung Cancer

The Food and Drug Administration has granted approval to pembrolizumab for patients with metastatic small cell lung cancer (SCLC) with disease progression on or after platinum-based chemotherapy and at least one other prior line of therapy.

Efficacy was investigated in 83 patients with SCLC who had disease progression on or after two or more prior lines of therapy enrolled in one of two multicenter, multi-cohort, non-randomized, open label trials: KEYNOTE-158 (NCT02628067) Cohort G or KEYNOTE-028 (NCT02054806) Cohort C1. Patients received either pembrolizumab 200 mg intravenously every 3 weeks ($n=64$) or 10 mg/kg intravenously every 2 weeks ($n=19$). Treatment continued until documented disease progression, unacceptable toxicity, or a maximum of 24 months.

The main efficacy outcome measures were overall response rate (ORR) and duration of response (modified RECIST v1.1) assessed by blinded independent central review. The ORR was 19% (95% CI: 11, 29); the complete response rate was 2%. Responses were durable for 6 months or longer in 94%, 12 months or longer in 63%, and 18 months or longer in 56% of the 16 responding patients.

The recommended pembrolizumab dose for SCLC is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression.

1.1.2.9.5 Pembrolizumab and Primary Mediastinal Large B-cell Lymphoma

The Food and Drug Administration has granted approval to pembrolizumab for the treatment of adult and pediatric patients with refractory primary mediastinal large B-cell lymphoma (PMBCL), or who have relapsed after two or more prior lines of therapy. Approval was based on data from 53 patients with relapsed or refractory PMBCL enrolled in a multicenter, open-label, single-arm trial, KEYNOTE-170 (NCT02576990). Patients were treated with pembrolizumab 200 mg intravenously every 3 weeks until unacceptable toxicity or documented disease progression, or for up to 24 months for patients who did not progress. The overall response rate was 45% (95% CI: 32, 60), including 11% complete responses and 34% partial responses. The median duration of response was not reached within the follow-up period (median 9.7 months). The median time to first objective response was 2.8 months; pembrolizumab is not recommended for treatment of patients with PMBCL who require urgent cytoreductive therapy.

The recommended pembrolizumab dose for treatment of adults with PMBCL is 200 mg every 3 weeks. The recommended dose in pediatric patients is 2 mg/kg (up to a maximum of 200 mg) every three weeks.

1.1.2.10 Pembrolizumab and TMB-H solid tumors

Efficacy of pembrolizumab was investigated in a prospectively-planned retrospective analysis of 10 cohorts of patients with various previously treated unresectable or metastatic TMB-H solid tumors enrolled in a multicenter, non-randomized, open-label trial, KEYNOTE-158 (NCT02628067). Patients received pembrolizumab 200 mg intravenously every 3 weeks until unacceptable toxicity or documented disease progression. The main efficacy outcome measures were overall response rate (ORR) and duration of response (DoR) in patients who have received at least one dose of pembrolizumab as assessed by blinded independent central review according to RECIST v1.1, modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ. A total of 102 patients (13%) had tumors identified as TMB-H, defined as TMB ≥ 10 mut/Mb. The ORR for these patients was 29% (95% CI: 21,39), with a 4% complete response rate and 25% partial response rate. The median DoR was not reached, with 57% of patients having response durations ≥ 12 months and 50% of patients having response durations ≥ 24 months. Adverse reactions occurring in patients with TMB-H cancer enrolled in KEYNOTE-158 were similar to those occurring in patients with other solid tumors who received pembrolizumab as a single agent.

The most common adverse reactions to pembrolizumab are fatigue, musculoskeletal pain, decreased appetite, pruritus, diarrhea, nausea, rash, pyrexia, cough, dyspnea, constipation, pain, and abdominal pain. Pembrolizumab is associated with immune-mediated side effects, including pneumonitis, colitis, hepatitis, endocrinopathies, nephritis, and skin adverse reactions.

The recommended pembrolizumab dosage regimen for TMB-H solid tumors is 200 mg every 3 weeks or 400 mg every 6 weeks for adults. The FDA also approved the FoundationOneCDx assay (Foundation Medicine, Inc.) as a companion diagnostic for pembrolizumab.

Table 1. MSI-H/dMMR Trials²⁸

1.1.3 Tumor Immune Microenvironment and Immune Checkpoint Inhibitor Response

Immune checkpoint inhibitors such as the anti-PD-1 antibody pembrolizumab have shown great promise for cancer treatment. However, a majority of patients do not respond to this type of treatment. The tumor immune microenvironment (TIME) has emerged as a critical determinant of immune checkpoint inhibitor response, with responses correlated with an immunologically active T-cell-inflamed microenvironment.³⁸ Accumulation of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and Tregs and insufficient accumulation of effector T-cells and other tumor-infiltrating lymphocytes (TILs) in the TIME have been implicated in the resistance to immune checkpoint therapy. Under healthy conditions, MDSCs and Tregs play critical roles in immune homeostasis. However,

Study	Design and Patient Population	Number of patients	MSI-H/dMMR testing	Dose	Prior therapy
KEYNOTE-016 NCT01876511	<ul style="list-style-type: none"> prospective, investigator-initiated 6 sites patients with CRC and other tumors 	28 CRC 30 non-CRC	local PCR or IHC	10 mg/kg every 2 weeks	<ul style="list-style-type: none"> CRC: ≥ 2 prior regimens Non-CRC: ≥1 prior regimen
KEYNOTE-164 NCT02460198	<ul style="list-style-type: none"> prospective international multi-center CRC 	61	local PCR or IHC	200 mg every 3 weeks	Prior fluoropyrimidine, oxaliplatin, and irinotecan +/- anti-VEGF/EGFR mAb
KEYNOTE-012 NCT01848834	<ul style="list-style-type: none"> retrospectively identified patients with PD-L1-positive gastric, bladder, or triple-negative breast cancer 	6	central PCR	10 mg/kg every 2 weeks	≥1 prior regimen
KEYNOTE-028 NCT02054806	<ul style="list-style-type: none"> retrospectively identified patients with PD-L1-positive esophageal, biliary, breast, endometrial, or CRC 	5	central PCR	10 mg/kg every 2 weeks	≥1 prior regimen
KEYNOTE-158 NCT02628067	<ul style="list-style-type: none"> prospective international multi-center enrollment of patients with MSI-H/dMMR non-CRC retrospectively identified patients who were enrolled in specific rare tumor non-CRC cohorts 	19	local PCR or IHC (central PCR for patients in rare tumor non-CRC cohorts)	200 mg every 3 weeks	≥1 prior regimen
Total		149			

CRC = colorectal cancer

PCR = polymerase chain reaction

IHC = immunohistochemistry

tumor-induced recruitment and activation of Tregs and MDSCs is an important mechanism of tumor-mediated immunosuppression, leading to impaired efficacy of cancer immunotherapy. *In vivo* studies have shown that elevated MDSCs cause resistance to anti-PD-1 immune checkpoint therapy, whereas MDSC suppression eradicates metastatic mouse cancers resistant to anti-PD-1 immune checkpoint therapy.³⁹ Comparison of *in vivo* anti-PD-1-sensitive and anti-PD-1-resistant tumors has suggested that intratumoral Tregs might be responsible for limiting anti-PD-1 therapeutic efficacy.⁴⁰ Tregs and MDSCs in the TIME represent a significant obstacle to immune checkpoint blockade therapies via suppression of immune checkpoint blockade-mediated antitumor T-cell responses. Immune checkpoint blockade has been shown to be most effective in so-called “inflamed” tumors populated with tumor-specific CD8⁺ TILs.⁴¹ Agents capable of increasing the number and activity of T-cells or TILs in the tumor microenvironment may enhance the response to anti-PD-1 therapy.

1.1.4 Nitric Oxide Synthase and the TIME

We and others have shown that nitric oxide synthase (NOS) expression in the TIME is a powerful driver of tumor-mediated immunosuppression.⁴²⁻⁴⁴ Therefore, TIME-targeting with NOS inhibitors may offer a novel combinatorial approach to enhance anti-PD-1 therapeutic efficacy. Nitric oxide (NO) is a bioactive molecule that exhibits pleiotropic effects within cancer cells and tumors, with concentration-dependent protumor and antitumor effects. NO is produced by three different NOS isoforms: neuronal NOS, inducible NOS (iNOS), and endothelial NOS. The role of iNOS in carcinogenesis, tumor progression, tumor survival, and tumor aggressiveness is well recognized. Increased iNOS expression has been found in various cancers including melanoma, NSCLC, HNSCC, and lymphoma and is associated with poor survival.⁴⁵⁻⁵³ iNOS inhibition has been shown to inhibit melanoma growth *in vivo* and to extend the survival of tumor-bearing mice.⁵⁴ Furthermore, iNOS inhibition enhanced cisplatin-mediated growth inhibition in cisplatin-sensitive and cisplatin-resistant melanoma cell lines *in vitro* and a mouse xenograft model *in vivo*.⁵⁴ NOS inhibition with the pan-NOS inhibitor L-NAME (NG-nitro-L-arginine methyl ester) resulted in a 23% increase in median OS, 33% reduction in tumor volume doubling rate, and 29% reduction in total lung tumor burden in a mouse model of *Kras* mutation-positive NSCLC.⁵⁵ iNOS inhibition has been shown to decrease invasion and enhance the antitumor effect of cisplatin *in vitro* in HNSCC cells.^{56,57} Intratumoral NO and NO-derived molecules induce T-cell dysfunction by negatively impacting T-cell signaling, activation, and migration⁵⁸ and prevent intratumoral infiltration of tumor-specific T-cells.⁵⁹ iNOS is a known effector of MDSC-mediated immune suppression.⁶⁰ In syngeneic mouse melanoma models, iNOS inhibition blocked the intratumoral accumulation of MDSCs, leading to increased recruitment of tumor-infiltrating CD8⁺ T-cells.^{61,62} Furthermore, iNOS inhibition was found to drive macrophages from the immunosuppressive M2 phenotype towards the antitumor M1 phenotype.⁶³ Taken together, these data provide solid evidence of the potential of NOS inhibition to dramatically reshape the TIME to improve the efficacy of anti-PD-1 therapy.

1.1.5 NG-Monomethyl-L-Arginine Preclinical and Clinical Trial Data

We have demonstrated the potent antitumor activity of the pan-NOS inhibitor NG-monomethyl-L-arginine (L-NMMA) against triple negative breast cancer (TNBC) *in vitro* and *in vivo*.⁴² L-NMMA was found to decrease cell proliferation, migration, and mammosphere formation in TNBC cell lines and significantly reduce tumor growth, lung metastases, tumor initiation, and self-renewal in TNBC patient-derived xenografts (PDXs).⁴² Furthermore, L-NMMA in combination with docetaxel significantly inhibited *in vivo* tumor growth compared with docetaxel alone in TNBC xenograft models.⁴² Based on these findings, we have an ongoing Phase Ib clinical trial with proprietary L-NMMA and docetaxel in subjects with relapsed/refractory TNBC (NCT02834403).

The safety of L-NMMA has been well established, and the anticipated SAEs are few, known, and documented.⁶⁴ TRIUMPH was a randomized, controlled Phase III trial of L-NMMA in subjects with acute myocardial infarction and cardiogenic shock. The study was conducted at 130 centers in 8 countries in North America and Europe. Inclusion required all of the following: (1) myocardial infarction, confirmed by ischemic symptoms for at least 30 minutes with elevated cardiac markers and/or ST-segment elevation or left bundle-branch block; (2) patency (<70% stenosis) of the infarct artery, either occurring spontaneously and confirmed at angiography or after percutaneous revascularization; (3) refractory cardiogenic shock of less than 24 hours' duration, confirmed by peripheral signs of tissue hypoperfusion and systolic blood pressure less than 100 mmHg despite vasopressor therapy (dopamine ≥ 7 μ g/kg per minute or norepinephrine or epinephrine ≥ 0.15 μ g/kg per minute) continuing longer than 1 hour after infarct artery patency; (4) clinical or hemodynamic evidence of elevated left ventricular filling pressures; and (5) left ventricular ejection fraction of less than 40%. Hemodynamics and requirement for vasopressor treatment were reconfirmed after randomization just prior to study drug administration; subjects with resolving shock were excluded. Between January 2005 and August 2006, 398 subjects met the study inclusion criteria and were enrolled. All baseline characteristics were well balanced between the treatment groups. More than one quarter of the population was older than 75 years; the majority were male and of white race. More than half of the subjects had hypertension and one third had diabetes; 84 (21%) had a history of heart failure and almost a third of those had advanced heart failure symptoms in the 6 weeks prior to enrollment. A quarter of the subjects had baseline creatinine levels of 1.7 mg/dL (150 μ mol/L) or higher. The median supported blood pressure just prior to study drug administration was 88/52 mmHg. Most subjects were supported with a single vasopressor at the time of study drug administration. The majority of subjects presented with anterior, ST-segment elevation myocardial infarction with left anterior descending infarct artery location. Percutaneous coronary intervention was performed in nearly all subjects to achieve the requirement for less than 70% infarct artery stenosis before study entry. L-NMMA had no effect on mortality in subjects with myocardial infarction complicated by refractory cardiogenic shock. Importantly, L-NMMA was well tolerated with few AEs other than transient (minutes) reversible hypertension. Hemodynamic outcomes and SAEs are shown in Tables 2 and 3, respectively. Subjects who received L-NMMA had a greater increase in systolic blood pressure at 2 hours compared with subjects who received placebo (12.0 mmHg vs. 7.0 mmHg; $P < 0.001$). There were a total of 274 SAEs reported (130 in the placebo group and 144 in the L-NMMA group). No significant differences in safety were noted.

Table 2. Hemodynamic Outcomes

Hemodynamic Outcomes	L-NMMA	Placebo	P Value
2-hour change in systolic blood pressure, median, mmHg	12.0 (2.5 to 23.0)	7.0 (-2.0 to 17.0)	0.001
2-hour change in diastolic blood pressure, median, mmHg	5.0 (-4.0 to 11.0)	1.0 (-5.0 to 8.0)	0.16
7-day change in creatinine, mg/dL	0.01 (-0.20 to 0.19)	-0.15 (-0.32 to 0.16)	0.16

Table 3. Serious Adverse Events

	L-NMMA (n = 206)	Placebo (n = 190)
Cardiovascular, n (%)		
Worsening heart failure	56 (27.2)	46 (24.3)
Arrhythmias	10 (4.9)	6 (3.2)
Myocardial infarction	7 (3.4)	6 (3.2)
Thromboembolism	1 (0.5)	2 (1.1)
Worsening shock	1 (0.5)	2 (1.1)
Valve disorders	0	1 (0.5)
Other cardiovascular	2 (1.0)	5 (2.6)
Infectious, n (%)	18 (8.7)	15 (7.9)
Respiratory, n (%)	7 (3.4)	10 (5.3)
Neurological, n (%)	7 (3.4)	9 (4.7)

Renal, n (%)	7 (3.4)	4 (2.1)
Coagulopathy, n (%)	8 (3.9)	2 (1.1)
Gastrointestinal, n (%)	5 (2.4)	4 (2.1)
Other events, n (%)	16 (7.8)	7 (3.7)

The ability of L-NMMA to overcome the hypotensive effects of interleukin-2 (IL-2) was evaluated in subjects with metastatic renal cell carcinoma. Bolus injections of L-NMMA at 3, 6, and 12 mg/kg were shown to be safe when administered to subjects with metastatic renal cell carcinoma prior to infusion of IL-2 (n = 3 per cohort).⁶⁵ L-NMMA was not associated with any hematologic, liver, or renal abnormalities. Doses of 3 and 6 mg/kg did not induce clinically apparent side effects and blood pressure remained unchanged. A transient increase in systolic blood pressure up to 25 mmHg in the absence of any other clinical symptoms was observed with the 12 mg/kg dose; however, this increase in blood pressure normalized rapidly (less than 5 min) on stopping infusion.

1.2 Rationale

1.2.1 Rationale for the Trial and Selected Subject Population

Pembrolizumab has demonstrated significant clinical activity in patients with melanoma, NSCLC, HNSCC, cHL, urothelial carcinoma, cervical cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, merkel cell carcinoma, primary mediastinal large B-cell lymphoma, renal cell carcinoma, small cell lung cancer, MSI-H/dMMR cancer, and for the treatment of adult and pediatric patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥ 10 mutations/megabase (mut/Mb)] solid tumors, as determined by an FDA-approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options.. However, a significant proportion of these patients do not respond to or progress after anti-PD-1 therapy, highlighting the need for novel strategies to optimize the efficacy of this therapy. The TIME has emerged as a critical determinant of immune checkpoint inhibitor response, with responses correlated with an immunologically active T-cell-inflamed microenvironment.³⁸ We and others have shown that NOS induces immune dysfunction in the tumor microenvironment.⁴²⁻⁴⁴ Therefore, we hypothesize that NOS inhibition, by targeting the immunosuppressive tumor microenvironment, will reverse resistance to PD-1 blockade and enhance response rates to pembrolizumab in patients with melanoma, NSCLC, HNSCC, cHL, urothelial carcinoma, cervical cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, merkel cell carcinoma, primary mediastinal large B-cell lymphoma, renal cell carcinoma, small cell lung cancer, MSI-H/dMMR cancer or patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥ 10 mutations/megabase (mut/Mb)] solid tumors. We will increase the number of subjects enrolled to 20 subjects to allow for Screen-Fails/withdrawals/deaths. The protocol study population will remain at 12 evaluable subjects.

1.2.2 Rationale for Dose Selection

All subjects will be treated with the recommended dose of pembrolizumab.

L-NMMA will be initially administered at a dose of 15.0 mg/kg. L-NMMA dose will de-escalate (12.5 mg/kg) or escalate (20.0 mg/kg) based on the occurrence of dose-limiting toxicities (DLTs). We previously demonstrated that L-NMMA at a dose of 200 mg/kg was effective in reducing tumor volume in TNBC PDX mouse models.⁴² The 200 mg/kg dose of L-NMMA translates into a 10–15 mg/kg human equivalent dose.⁶⁶ We are currently conducting a Phase Ib/II trial evaluating the safety and efficacy of proprietary L-NMMA at doses ranging from 7.5–20.0 mg/kg in combination with docetaxel in subjects with relapsed/refractory TNBC (NCT02834403).

2.0 OBJECTIVES

2.1 Primary Objective

- To assess the maximum tolerated dose (MTD) of the L-NMMA and pembrolizumab combination in subjects with melanoma, NSCLC, HNSCC, cHL, urothelial carcinoma, cervical cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, merkel cell carcinoma, primary mediastinal large B-cell lymphoma, renal cell carcinoma, small cell lung cancer, MSI-H/dMMR cancer or for the treatment of adult patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥ 10 mutations/megabase (mut/Mb)] solid tumors.

2.2 Secondary Objectives

- To describe the DLTs and other toxicities associated with the L-NMMA and pembrolizumab combination in subjects with melanoma, NSCLC, HNSCC, cHL, urothelial carcinoma, cervical cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, merkel cell carcinoma, primary mediastinal large B-cell lymphoma, renal cell carcinoma, small cell lung cancer, MSI-H/dMMR cancer, or metastatic tumor mutational burden-high (TMB-H) [≥ 10 mutations/megabase (mut/Mb)] solid tumors, as assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.03 (see Section 11.1).
- To determine the recommended Phase II dose (RP2D) of the L-NMMA and pembrolizumab combination.
- To evaluate the antitumor activity of the L-NMMA and pembrolizumab combination, as assessed by the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 (see Sections 7.1.2.6.1 and 11.2).
- To study the plasma pharmacokinetics (PK) and pharmacodynamics of the L-NMMA and pembrolizumab combination.

3.0 ENDPOINTS

3.1 Primary Endpoint

- The primary endpoint will be the identification of the MTD of the L-NMMA and pembrolizumab combination. DLT will be defined as any treatment-related death or any \geq Grade 3 AE (NCI CTCAE v4.03) unless there is clear alternative evidence that the AE was not caused by the trial treatment (see Section 8.0 — Statistical Considerations for a detailed list of DLTs). The DLT assessment window will be defined as the duration required to complete one full cycle of treatment with the L-NMMA and pembrolizumab combination. If treatment is delayed, the timelines for dose escalation and MTD determinations will be adjusted to allow for the completion of the DLT assessment window. Subjects will be considered evaluable for the purpose of establishing dose escalation decisions and the MTD if they satisfy one of the following criteria: receive at least one complete cycle of the L-NMMA and pembrolizumab combination and complete trial assessments without a DLT within the DLT assessment window or experience a DLT within the DLT assessment window and are withdrawn from the trial.

3.2 Secondary Endpoints

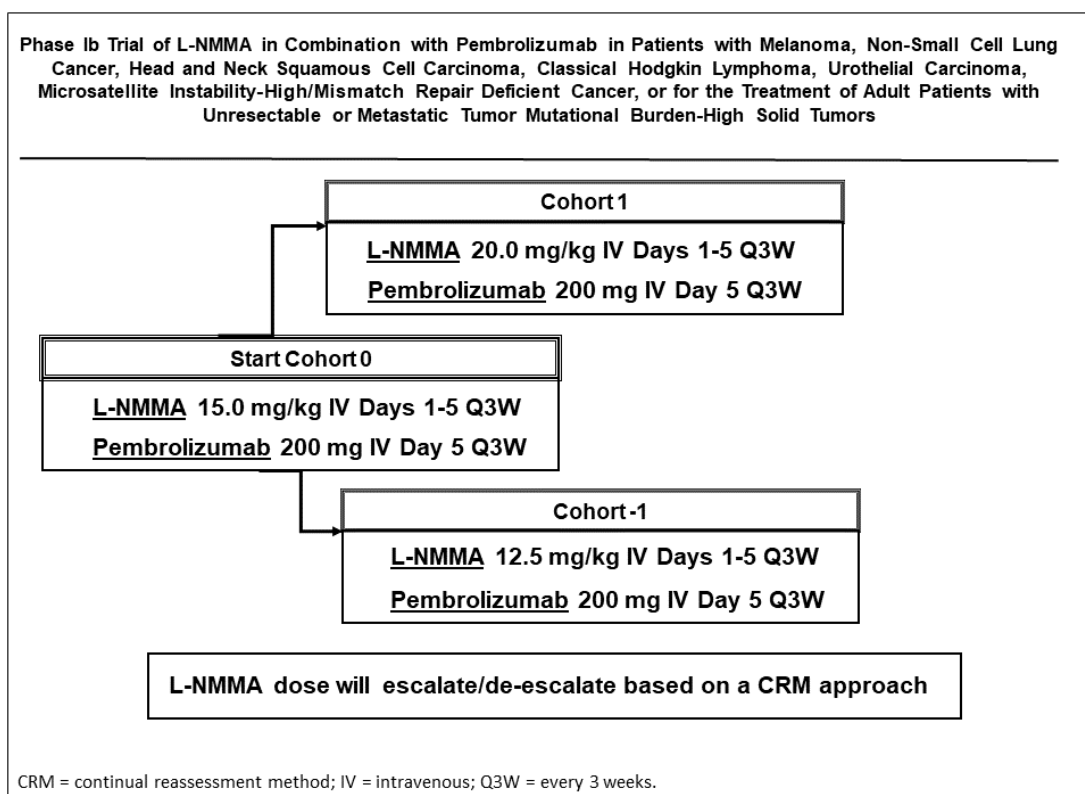
- Secondary endpoints will include evaluation of the A) DLTs and other toxicities (as assessed by the NCI CTCAE v4.03), B) RP2D, C) antitumor activity (as assessed by the RECIST 1.1), and D) plasma PK and pharmacodynamics of the L-NMMA and pembrolizumab combination.

4.0 TRIAL DESIGN

4.1 Description of Trial Design

This is a Phase Ib trial assessing the safety of the pan-NOS inhibitor L-NMMA in combination with pembrolizumab in subjects with melanoma, NSCLC, HNSCC, cHL, urothelial carcinoma, cervical cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, merkel cell carcinoma, primary mediastinal large B-cell lymphoma, renal cell carcinoma, small cell lung cancer, MSI-H/dMMR cancer, or for the treatment of adult patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥ 10 mutations/megabase (mut/Mb)] solid tumors. This trial will determine the MTD, DLTs and other toxicities, and RP2D of the L-NMMA and pembrolizumab combination and will utilize a standard Bayesian model averaging continual reassessment method (CRM) approach to determine the appropriate dose of L-NMMA (see Section 8.0 — Statistical Considerations). A cohort size of 2 will be used.

Figure 1. Study Diagram



5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Female or male subjects ≥ 18 years of age with melanoma, NSCLC, HNSCC, cHL, urothelial carcinoma, cervical cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, merkel cell carcinoma, primary mediastinal large B-cell lymphoma, renal cell carcinoma, small cell lung cancer, MSI-H/dMMR cancer or unresectable or metastatic tumor mutational burden-high (TMB-H) [≥ 10 mutations/megabase (mut/Mb)] solid tumors will be eligible for the trial.

5.1.2 Subject Inclusion Criteria

Subjects must meet all the following inclusion criteria in order to be eligible for participation in this trial:

- Female or male aged ≥ 18 years on the day of informed consent signing;
- Has histologically confirmed metastatic melanoma that is treatment naïve or has relapsed after or is refractory to ipilimumab or BRAF inhibitor (if *BRAF* mutation positive),
OR
histologically confirmed metastatic NSCLC that has high PD-L1 expression (TPS $\geq 50\%$) with no *EGFR* or *ALK* genomic tumor aberrations or histologically confirmed metastatic NSCLC that is PD-L1 positive (TPS $\geq 1\%$) and has progressed on or after platinum containing therapy (subjects with NSCLC harboring *EGFR/ALK* genomic aberrations must have received an FDA-approved targeted therapy for these aberrations)
OR
histologically confirmed HNSCC that has relapsed after or is refractory to platinum-containing chemotherapy,
OR
histologically confirmed cHL that has relapsed after three or more lines of therapy or is refractory to treatment
OR
histologically confirmed locally advanced or metastatic urothelial carcinoma that is not eligible for platinum-containing chemotherapy or that has relapsed after or is refractory to platinum-containing chemotherapy
OR
histologically confirmed advanced, PD-L1–positive cervical cancer with disease progression on or after chemotherapy that is not eligible for platinum-containing chemotherapy or that has relapsed after or is refractory to platinum-containing chemotherapy
OR
histologically confirmed recurrent, locally advanced or metastatic, squamous cell carcinoma of the esophagus (ESCC) whose tumors express PD-L1 (Combined Positive Score [CPS] ≥ 10), as determined by an FDA-approved test, with disease progression after one or more prior lines of systemic therapy.
OR
histologically confirmed recurrent, locally advanced or metastatic Gastric Cancer , with disease progression on or after two or more systemic therapies, including fluoropyrimidine- and platinum-containing chemotherapy and, if appropriate, HER2/neu-targeted therapy, and whose tumors express programmed death-ligand 1 (PD-L1), as determined by an FDA-approved test.

OR
histologically confirmed hepatocellular carcinoma (HCC) who have been previously treated with sorafenib. recurrent locally advanced or metastatic MCC who had not received prior systemic therapy for their advanced disease. OR

histologically confirmed recurrent, locally advanced or metastatic Merkel Cell Carcinoma (MCC) who had not received prior systemic therapy for their advanced disease.

OR

histologically confirmed advanced renal cell carcinoma (RCC) first-line treatment.

OR

histologically confirmed metastatic small cell lung cancer (SCLC) with disease progression on or after platinum-based chemotherapy and at least one other prior line of therapy.

OR

histologically confirmed refractory primary mediastinal large B-cell lymphoma (PMBCL), who have relapsed after two or more prior lines of therapy.

OR

MSI-H or dMMR unresectable or metastatic cancer that has relapsed after prior treatment and has no satisfactory alternative treatment options;

OR

adult patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥ 10 mutations/megabase (mut/Mb)] solid tumors;

- Measurable disease based on RECIST 1.1;
- Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 (see Section 11.3);
- Life expectancy ≥ 6 months;
- Adequate organ function as defined in Table 4. All screening labs should be performed within 28 days of treatment initiation.

Table 4

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	$\geq 1,500/\text{mm}^3$
Platelets	$\geq 100,000/\text{mm}^3$
Hemoglobin	≥ 9 g/dL (transfusion permitted)
Renal	
Serum creatinine OR Measured or calculated ^a creatinine clearance (CrCl) (glomerular filtration rate can be used in place of creatinine or CrCl)	≤ 1.5 X upper limit of normal (ULN) OR ≥ 60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN OR Direct bilirubin \leq ULN for subjects with total bilirubin levels > 1.5 ULN
ALT and AST	≤ 2.5 X ULN OR ≤ 5 X ULN for subjects with liver metastases
Albumin	≥ 2.5 mg/dL
Coagulation	
International normalized ratio (INR) or prothrombin time (PT)	≤ 1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated partial thromboplastin time (aPTT)	≤ 1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

^a CrCl should be calculated per institutional standard.

- Cardiac ejection fraction of $\geq 45\%$;
- Female subjects of childbearing potential should have a negative serum pregnancy (β -human chorionic gonadotropin [β -HCG]) within 7 days prior to receiving the first dose of trial treatment and should not be lactating;
- Female subjects of childbearing potential must be willing to use an adequate method of contraception as outlined in Section 5.5.2 — Contraception for the course of the trial through 120 days after the last dose of trial treatment;
- Male subjects of childbearing potential must be willing to use an adequate method of contraception as outlined in Section 5.5.2 — Contraception for the course of the trial through 120 days after the last dose of trial treatment;
- Willing and able to provide written informed consent/assent for the trial.

5.1.3 Subject Exclusion Criteria

Subjects meeting any of the following exclusion criteria must be excluded from participating in the trial:

- History of poorly controlled hypertension (defined as systolic blood pressure > 150 mmHg). Subjects with medication-controlled hypertension are allowed provided they have been on their current medication for at least 4 weeks prior to Cycle 1, Day 1;
- History of New York Heart Association class III or greater cardiac disease (see Section 11.4);
- History of myocardial infarction, stroke, ventricular arrhythmia, or symptomatic conduction abnormality within the past 12 months;
- History of congenital QT prolongation;
- Absolute corrected QT interval of > 480 msec in the presence of potassium > 4.0 mEq/L and magnesium > 1.8 mg/dL;
- Currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of the trial treatment;
- Concurrent use of medications that interact with nitrate/nitrites (see Section 11.5);
- Concurrent use of any complementary or alternative medicines;
- Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment;
- Known history of active tuberculosis (Bacillus Tuberculosis);
- Hypersensitivity to L-NMMA, pembrolizumab, or any of their excipients;
- Has had a prior anticancer monoclonal antibody within 4 weeks prior to study Day 1 or who has not recovered (i.e., \leq Grade 1 or at baseline) from AEs due to agents administered more than 4 weeks earlier;
- Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 4 weeks prior to study Day 1 or who has not recovered (i.e., \leq Grade 1 or at baseline) from AEs due to a previously administered agent.

- Note: Subjects with \leq Grade 2 neuropathy are an exception to this criterion and may qualify for the trial.
 - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting the trial treatment.
- Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
 - Has known active central nervous system metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 4 weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability;
 - Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e., with use of disease-modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment;
 - History of non-infectious pneumonitis that required steroids or current pneumonitis;
 - Has an active infection requiring systemic therapy;
 - History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator;
 - Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial;
 - Is pregnant or breastfeeding, or expecting to conceive a child within the projected duration of the trial, starting with the prescreening or screening visit through 120 days after the last dose of trial treatment;
 - Received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent;
 - Known history of human immunodeficiency virus (HIV) (HIV 1/2 antibodies);
 - Has known active hepatitis B (e.g., HBsAg reactive) or hepatitis C (e.g., HCV RNA [qualitative] is detected);
 - Received a live vaccine within 30 days of planned start of the trial treatment.
Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines and are not allowed;
 - Unwilling or unable to comply with the trial protocol.

5.2 Trial Treatment

The treatment to be used in this trial is outlined below in Table 5.

Table 5. Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period
L-NMMA	15.0 mg/kg (starting dose)	Q3W	IV infusion	Days 1–5 of each 3-week cycle
Pembrolizumab	200 mg	Q3W	IV infusion	Day 5 of each 3-week cycle
Q3W = every 3 weeks; IV = intravenous				

L-NMMA and pembrolizumab will be administered for 6 cycles. Cycle length will be 21 days. L-NMMA will be administered as a 2-hour intravenous (IV) infusion on Days 1–5 at each cycle. The dose levels of L-NMMA are as follows: Dose Level -1, 12.5 mg/kg; Dose Level 0 (starting dose), 15.0 mg/kg; and Dose Level 1, 20.0 mg/kg. L-NMMA dose will escalate/de-escalate based on the occurrence of DLTs (see Section 8.0 — Statistical Considerations). Pembrolizumab at a fixed dose of 200 mg will be IV infused over 30 minutes on Day 5 at each cycle. Pembrolizumab will be administered 1 hour (\pm 10 minutes) after L-NMMA infusion on Day 5 at each cycle. Subjects will receive the trial treatment past 3 weeks only if they have tolerated the trial treatment without a DLT and their disease has not progressed. Subjects without disease progression after 6 cycles of L-NMMA and pembrolizumab will continue pembrolizumab until disease progression or unacceptable AEs. Subjects will be followed for a maximum of 3 months after the final treatment dose.

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for the selection of doses to be used in this trial is provided in Section 1.2.2 — Rationale for Dose Selection.

5.2.1.2 Dose Modification

Pembrolizumab:

AEs (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These AEs may occur shortly after the first dose or several months after the last treatment dose.

Withhold pembrolizumab for any of the following:

- Grade 2 pneumonitis

- Grade 2 or 3 colitis
- Grade 3 or 4 endocrinopathies
- Grade 4 hematological toxicity in subjects with cHL
- Grade 2 nephritis
- AST or ALT > 3 and up to 5 X ULN or total bilirubin > 1.5 and up to 3 X ULN
- Any other severe or Grade 3 treatment-related AE

Resume pembrolizumab in subjects whose AEs recover to Grade 0–1.

Permanently discontinue pembrolizumab for any of the following:

- Any life-threatening AE (excluding endocrinopathies controlled with hormone replacement therapy or hematological toxicity in subjects with cHL)
- Grade 3 or 4 pneumonitis or recurrent pneumonitis of Grade 2 severity
- Grade 3 or 4 nephritis
- AST or ALT > 5 X ULN or total bilirubin > 3 X ULN
 - For subjects with liver metastasis who begin treatment with Grade 2 ALT or AST, if ALT or AST increases by $\geq 50\%$ relative to baseline and lasts for at least 1 week
- Grade 3 or 4 infusion-related reactions
- Inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks
- Persistent Grade 2 or 3 AEs (excluding endocrinopathies controlled with hormone replacement therapy) that do not recover to Grade 0–1 within 12 weeks after the last dose of pembrolizumab
- Any severe or Grade 3 treatment-related AE that recurs

L-NMMA:

Blood pressure will be monitored during L-NMMA infusions. At each L-NMMA infusion, blood pressure will be measured before infusion, 1 hour (± 15 minutes) after infusion start, and at infusion completion. Automatic blood pressure readings will be performed unless manual reading is needed. If systolic blood pressure is >180 mmHg at the 1-hour (± 15 minutes) check, stop the infusion and recheck blood pressure after 15–30 minutes. If blood pressure is not <160 mmHg, recheck blood pressure after another 15–30 minutes. If blood pressure is still not <160 mmHg, the subject is to be discontinued from the trial. The infusion can be resumed in subjects whose blood pressure lowers to <160 mmHg at the first or second recheck. If systolic blood pressure again increases beyond 180 mmHg, the infusion should be stopped and the subject is to be discontinued from the trial. If systolic blood pressure is >180 mmHg at infusion completion, recheck blood pressure after 15–30 minutes. If blood pressure is not <160 mmHg, recheck blood pressure after another 15–30 minutes. If blood pressure is still not <160 mmHg, the subject is to be discontinued from the trial.

Subjects whose treatment is interrupted or permanently discontinued due to an AE including abnormal laboratory value must be followed at least once a week for 4 weeks and subsequently at 4-week intervals until resolution of the event to Grade 1 or better. Dose interruptions should be reported on the appropriate Dosage Administration case report form (CRF). Any AE during Cycle 1 that delays administration of Cycle 2 by more than 7 days will be considered a DLT (see Section 8.0 — Statistical Considerations). The maximum time allowed for toxicity-related treatment interruption for all subsequent cycles is 3 weeks from the intended dosing day. If interruption is > 3 weeks, the subject must be discontinued from the trial treatment. However, the subject will continue to be followed for toxicity.

5.2.2 Timing of Dose Administration

Trial treatment will be administered on an outpatient basis.

L-NMMA will be administered as a 2-hour IV infusion on Days 1–5 at each cycle. Pembrolizumab will be administered 1 hour (\pm 10 minutes) after L-NMMA infusion on Day 5 at each cycle. Pembrolizumab 200 mg will be administered as a 30-minute IV infusion.

5.2.3 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator, and subject will know the treatment administered.

5.3 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required.

5.3.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the CRF including all prescription, over-the-counter, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

Subjects with medication-controlled hypertension will continue to take their current blood pressure medication.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs.

5.3.2 Prohibited Concomitant Medications/Therapies

Subjects are prohibited from receiving the following during the length of the trial:

- Antineoplastic systemic chemotherapy, hormone therapy, or targeted/biological therapy;
- Immunotherapy not specified in this protocol;
- Investigational treatment other than the combination of L-NMMA and pembrolizumab (i.e., marketed and approved but investigational in combination with L-NMMA);
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, bacillus Calmette-Guérin, and typhoid vaccine;
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an AE of suspected immunologic etiology;
- Concurrent use of medications that interact with nitrate/nitrites (see Section 10.5);
- Concurrent use of any complementary or alternative medicines.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial.

Subjects may receive other medications that the investigator deems to be medically necessary.

There are no prohibited therapies during the post-treatment follow-up phase.

5.4 Rescue Medications & Supportive Care

5.4.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator.

L-NMMA:

Subjects with medication-controlled hypertension will continue to take their current blood pressure medication. Subjects not currently taking blood pressure medication will be premedicated with the calcium channel blocker amlodipine to control any L-NMMA-mediated transient increase in blood pressure. Amlodipine will be administered as 10 mg orally once daily for 6 days at each cycle. Amlodipine administration will start the day before the first dose of L-NMMA. Amlodipine dose may be adjusted by the treating physician if needed. Amlodipine dose will be held if systolic blood pressure remains below 100 mmHg.

Pembrolizumab:

Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined below. For each disorder, attempts should be made to rule out other causes

such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Immune-Mediated Pneumonitis

Pembrolizumab can cause immune-mediated pneumonitis, including fatal cases. Monitor subjects for signs and symptoms of pneumonitis.

≥ **Grade 2 pneumonitis:** Administer corticosteroids at an initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper.

Immune-Mediated Colitis

Pembrolizumab can cause immune-mediated colitis. Monitor subjects for signs and symptoms of colitis.

≥ **Grade 2 colitis:** Administer corticosteroids at an initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper.

Immune-Mediated Hepatitis

Pembrolizumab can cause immune-mediated hepatitis. Monitor subjects for changes in liver function.

Grade 2 hepatitis: Administer corticosteroids at an initial dose of 0.5 to 1 mg/kg/day prednisone or equivalent followed by a taper.

≥ **Grade 3 hepatitis:** Administer corticosteroids at an initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper.

Immune-Mediated Endocrinopathies

Hypophysitis

Pembrolizumab can cause hypophysitis. Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency). Administer corticosteroids and hormone replacement as clinically indicated.

Thyroid Disorders

Pembrolizumab can cause thyroid disorders, including hyperthyroidism, hypothyroidism and thyroiditis. Monitor subjects for changes in thyroid function and for clinical signs and symptoms of thyroid disorders. Administer replacement hormones for hypothyroidism and manage hyperthyroidism with thionamides and beta-blockers as appropriate.

Type 1 Diabetes Mellitus

Pembrolizumab can cause type 1 diabetes mellitus, including diabetic ketoacidosis. Monitor patients for hyperglycemia or other signs and symptoms of diabetes. Administer insulin for type 1 diabetes.

Immune-Mediated Nephritis and Renal Dysfunction

Pembrolizumab can cause immune-mediated nephritis. Monitor patients for changes in renal function.

≥ **Grade 2:** Administer corticosteroids at an initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper.

Other Immune-Mediated Adverse Reactions

Pembrolizumab can cause other clinically important immune-mediated adverse reactions. For suspected immune-mediated adverse reactions, ensure adequate evaluation to confirm etiology or exclude other causes. Based on the severity of the adverse reaction, withhold pembrolizumab and administer corticosteroids. Upon improvement to Grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month. Based on limited data from clinical studies in subjects whose immune-related adverse reactions could not be controlled with corticosteroid use, administration of other systemic immunosuppressants can be considered.

5.5 Diet/Activity/Other Considerations

5.5.1 Diet

Subjects should be on a low nitrite/nitrate diet, including avoidance of processed meats such as hot dogs, ham, bacon, and sausage, 1 week before study treatment start and for the duration of the trial. Subjects should also keep a food diary the week before and the week of the L-NMMA infusion during Cycles 1 and 2.

5.5.2 Contraception

Pembrolizumab may have adverse effects on a fetus *in utero*. It is not known if pembrolizumab has transient adverse effects on the composition of sperm. It is not known whether L-NMMA can cause fetal harm when administered to a pregnant woman.

For this trial, male subjects will be considered to be of nonreproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of nonreproductive potential if they are either:

- (1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age, a high follicle-stimulating hormone [FSH] level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

- (2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy, or bilateral tubal ligation/occlusion at least 6 weeks prior to screening;

OR

(3) have a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving trial treatment and for 120 days after the last dose of trial treatment by complying with one of the following:

(1) practice abstinence[†] from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[†]:

Single method (one of the following is acceptable):

- intrauterine device
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

[†]Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

Subjects should be informed that the trial treatment may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the trial. In order to participate in the trial, subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of trial treatment initiation throughout the trial period up to 120 days after the last dose of trial treatment. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the trial.

5.5.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on the trial treatment, the subject will immediately be removed from the trial. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor without delay and within 24 hours

to the Sponsor if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and followed as described above and in Section 7.2.2.

5.5.4 Use in Nursing Women

It is unknown whether pembrolizumab and L-NMMA are excreted in human milk. Since many drugs are excreted in human milk and because of the potential for serious adverse reactions in the nursing infant, subjects who are breastfeeding are not eligible for enrollment.

5.6 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.4 — Other Procedures.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent
- Confirmed radiographic disease progression
- Any AE during Cycle 1 that delays administration of Cycle 2 by more than 7 days or dose interruption that exceeds 21 days in subsequent cycles
- Unacceptable adverse experiences
- Any \geq Grade 4 AE
- Severe (Grade 3) or life-threatening (Grade 4) immune-mediated complication
- Intercurrent illness that prevents further treatment administration
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Death
- Administrative reasons
- Subjects whose blood pressure is >180 during or after L-NMMA infusion and does not return to <160 mmHg after 2 rechecks spaced 15–30 minutes apart

The end of treatment (EOT) and follow-up visit procedures are listed in Section 6.0 (Trial Flow Chart) and 7.1.4.2 (Visit Requirements). At EOT, each subject will be followed for 30 days for AE monitoring (SAEs will be collected for 90 days after EOT as described in Section 7.2.3.1).

Subjects who discontinue for reasons other than progressive disease (PD) will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent, or becoming lost to follow-up.

6.0 TRIAL FLOW CHART

Table 6. Trial Flow Chart

Trial Period:			Treatment Cycles: L-NMMA + Pembrolizumab						EOT	Continued Pembrolizumab ^u
Treatment Cycle:	Screening ^a	Baseline ^b	1	2	3	4	5	6		
Scheduling Window (Days):	-28 to -7	-7 to -1	± 5	± 5	± 5	± 5	± 5	± 5		± 5
Informed Consent	X									
Inclusion/Exclusion Criteria	X									
Demographics	X									
Medical History	X									
Physical Exam ^c	X	X	X	X	X	X	X	X	X	
ECOG Performance Status	X	X	X	X	X	X	X	X	X	
12-Lead ECG ^d	X		X	X	X	X	X	X	X	
MUGA Scan or ECHO ^e	X									
Serum Pregnancy Test (β-HCG) ^f	X									
PT/INR and aPTT ^g	X									
Hematology ^h	X	X	X	X	X	X	X	X	X	X
Clinical Chemistry ⁱ	X	X	X	X	X	X	X	X	X	X
Sedimentation Rate ^j		X		X		X		X		X
T3, FT4, and TSH ^k		X								
Viral Testing ^l	X									
Urinalysis ^m		X								
CT Scan ⁿ	X			X		X		X	X	X
Brain CT or MRI ^o		X			X			X		X

Trial Period:			Treatment Cycles: L-NMMA + Pembrolizumab						EOT	Continued Pembrolizumab^u
Treatment Cycle:	Screening ^a	Baseline ^b	1	2	3	4	5	6		
Scheduling Window (Days):	-28 to -7	-7 to -1	± 5	± 5	± 5	± 5	± 5	± 5		± 5
L-NMMA Administration ^p			X	X	X	X	X	X		
Pembrolizumab Administration ^p			X	X	X	X	X	X		X
PK/Pharmacodynamics Blood Collection ^q			X	X						
Blood Collection for Future Research ^r		X		X					X	
Tissue Collection ^s		X								
AEs and SAEs ^t			X	X	X	X	X	X	X	

AE = adverse event; ALT = alanine transaminase; aPTT = activated partial thromboplastin time; AST = aspartate transaminase; β -hCG = beta-human chorionic gonadotropin; BUN = blood urea nitrogen; CBC = complete blood count; CT = computed tomography; CyTOF = mass cytometry; DLT = dose-limiting toxicity; ECG = electrocardiogram; ECHO = echocardiogram; ECOG = Eastern Cooperative Oncology Group; EOT = end of treatment; FT4 = free thyroxine; HIV = human immunodeficiency virus; INR = international normalized ratio; IV = intravenous; LDH = lactate dehydrogenase; MRI = magnetic resonance imaging; MUGA = multigated acquisition; PK = pharmacokinetics; PT = prothrombin time; RP2D = recommended Phase II dose; SAE = serious adverse event; T3 = triiodothyronine; TSH = thyroid-stimulating hormone; WBC = white blood cell.

A window of ± 5 days is allowed for trial visits and assessments/procedures (except as otherwise specified).
EOT is defined as 30 ± 5 days after the last dose of trial treatment.

- Within 28 days prior to Cycle 1, Day 1.
- Within 7 days prior to Cycle 1, Day 1.
- Physical exam will be performed at screening/baseline, before every cycle of L-NMMA and pembrolizumab, and at EOT. The baseline symptom-directed medical history and physical examination are not required if the screening medical history and physical examination were conducted within 7 days prior to Cycle 1, Day 1. Physical exam will include vital sign (blood pressure, oral temperature, pulse, and respiratory rate) measurements. Weight will also be measured at each physical exam. Height will be measured at screening only.
- A 12-lead ECG will be performed at screening, before every cycle of L-NMMA and pembrolizumab, at EOT, and when clinically indicated.
- MUGA scan or ECHO will be performed at screening and when clinically indicated. The same method (MUGA scan or ECHO) must be used throughout the duration of the trial.

- f. For women of childbearing potential, the results of a serum β -hCG pregnancy test must be negative within 7 days prior to the administration of the first dose of trial treatment. If the screening serum β -hCG pregnancy test is performed more than 7 days before dosing, it must be repeated at baseline, with results known to be negative prior to the administration of the first dose of trial treatment. Serum β -hCG pregnancy testing is to be performed as clinically indicated.
- g. Coagulation parameters (INR/PT/aPTT) will be tested at screening.
- h. A blood sample for CBC with platelet count and differential WBC count will be obtained at screening/baseline, before every cycle of L-NMMA and pembrolizumab, at EOT, and when clinically indicated.
- i. A blood sample for clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, calcium, chloride, BUN, creatinine, total protein, total bilirubin, alkaline phosphatase, ALT, and AST) and evaluation of magnesium, uric acid, and LDH will be obtained at screening/baseline, before every cycle of L-NMMA and pembrolizumab, at EOT, and when clinically indicated. An extra tube of blood will be collected for CyTOF analysis at baseline, after Cycle 2 and at EOT.
- j. For subjects with classical Hodgkin lymphoma, sedimentation rate testing will be performed at baseline and Cycles 2, 4, and 6 of L-NMMA and pembrolizumab.
- k. TSH, FT4, and total T3 testing will be performed at baseline and when clinically indicated.
- l. Hepatitis panel and HIV testing will be performed at screening.
- m. Urinalysis (blood, glucose, protein, specific gravity) will be performed at baseline and when clinically indicated.
- n. CT scans of the chest, abdomen, and pelvis and/or other sites as appropriate (e.g., head and neck, upper extremities, lower extremities) will be performed at screening, after every 2 cycles of L-NMMA and pembrolizumab and/or at the discretion of the treating physician, and 3 months after the last dose of trial treatment.
- o. For subjects with previously treated stable brain metastases, brain CT or MRI will be performed at baseline and every 2 months (\pm 7 days) as per standard of care.
- p. L-NMMA and pembrolizumab will be administered for six 21-day cycles. The dose levels of L-NMMA are as follows: Dose Level -1, 12.5 mg/kg; Dose Level 0 (starting dose), 15.0 mg/kg; and Dose Level 1, 20.0 mg/kg. L-NMMA dose will escalate/de-escalate based on the occurrence of DLTs (see Section 8.0 — Statistical Considerations). L-NMMA will be administered as a 2-hour IV infusion on Days 1–5 at each cycle. Pembrolizumab will be administered at a fixed dose of 200 mg. Pembrolizumab will be IV infused over 30 minutes on Day 5 (1 hour \pm 10 minutes after L-NMMA infusion) at each cycle. Blood pressure will be monitored during L-NMMA infusions. At each L-NMMA infusion, blood pressure will be measured before infusion, 1 hour (\pm 15 minutes) after infusion start, and at infusion completion. Automatic blood pressure readings will be performed unless manual reading is needed. If systolic blood pressure is >180 mmHg at the 1-hour (\pm 15 minutes) check, stop the infusion and recheck blood pressure after 15–30 minutes. If blood pressure is not <160 mmHg, recheck blood pressure after another 15–30 minutes. If blood pressure is still not <160 mmHg, the subject is to be discontinued from the trial. The infusion can be resumed in subjects whose blood pressure lowers to <160 mmHg at the first or second recheck. If systolic blood pressure again increases beyond 180 mmHg, the infusion should be stopped and the subject is to be discontinued from the trial. If systolic blood pressure is >180 mmHg at infusion completion, recheck blood pressure after 15–30 minutes. If blood pressure is not <160 mmHg, recheck blood pressure after another 15–30 minutes. If blood pressure is still not <160 mmHg, the subject is to be discontinued from the trial (see Section 5.2.1.2).
- q. Blood samples for assessment of plasma PK and pharmacodynamics will be collected predose (10–30 minutes before L-NMMA infusion) on Days 1, 2, and 5 of Cycles 1 and 2. One 5-mL sample will be collected for PK assessment and one 5-mL sample will be collected for pharmacodynamics assessment. **NOTE:** For PK/pharmacodynamics assessment, subjects should be fasted (nothing to eat or drink except water) for 12 hours prior to each collection time point. Subjects should also keep a food diary the week before and the week of the L-NMMA infusion during Cycles 1 and 2.
- r. Blood samples will be collected for future research at baseline, after Cycle 2, and 3 months after the last dose of trial treatment. Blood (20–30 cc) will be collected into standard vacutainer tubes and processed for plasma and viable cryopreserved peripheral blood mononuclear cells for future analyses.
- s. Tissue collection at baseline for archival use.
- t. AEs and SAEs will be captured from the time of informed consent signing up to and including 30 days after the last treatment dose. Trial treatment-related SAEs occurring beyond 90 days after the last dose of trial treatment and any subject death should also be reported.
- u. Patients without disease progression after 6 cycles of L-NMMA and pembrolizumab will continue pembrolizumab until disease progression or unacceptable AEs. CT scans of the chest, abdomen, and pelvis and/or other sites as appropriate (e.g., head and neck, upper extremities, lower extremities) will be performed every 3 cycles of pembrolizumab and/or at the discretion of the treating physician. For subjects with previously treated stable brain metastases, brain CT or MRI will be performed every 2 months (\pm 7 days) as per standard of care. Blood samples for CBC with platelet count and differential WBC count, clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, calcium, chloride, BUN, creatinine, total protein, total bilirubin, alkaline phosphatase, ALT, and AST), and evaluation of magnesium, uric acid, and LDH will be obtained at every cycle of pembrolizumab. For subjects with classical Hodgkin lymphoma, sedimentation rate testing will be performed at every other cycle.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart — Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the investigator for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, hepatitis C, etc.) and thus, local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The investigator or qualified designee must obtain documented consent from each potential subject prior to participating in a clinical trial.

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form, and any written information provided to the subject must receive the institutional review board (IRB) approval in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

The informed consent will adhere to IRB requirements, applicable laws and regulations, and Sponsor requirements.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial. Written informed consent is required before performing any trial-specific tests or procedures. Signing of the informed consent form can occur outside the 28-day screening period. All screening evaluations must be completed and reviewed to confirm that subjects meet all eligibility criteria before trial entry. Results of standard-of-care tests or examinations performed prior to obtaining informed consent and

within 28 days prior to trial entry (except where otherwise specified) may be used for screening assessments rather than repeating such tests. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

7.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the investigator. Details regarding the disease for which the subject has enrolled in this trial will be recorded separately and not listed as medical history.

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this trial will be recorded separately and not listed as a prior medication.

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.5.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation, and surgeries.

7.1.1.5.3 Subsequent Anticancer Therapy Status

The investigator or qualified designee will review all new antineoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anticancer therapy within 30 days after the last dose of trial treatment, the 30 day safety follow-up visit must occur before the first dose of the new therapy.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 AE Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. AEs will be graded and recorded throughout the trial and during the follow-up period according to the NCI CTCAE v4.03 (see Section 11.1). Toxicities will be characterized in terms of seriousness, causality, toxicity grading, and action taken with regard to the trial treatment.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Physical Exam

The investigator or qualified designee will perform a complete physical exam at screening/baseline, before every cycle of L-NMMA and pembrolizumab, and at EOT.

7.1.2.3 Vital Signs

The investigator or qualified designee will take vital signs at each physical exam. Vital signs should include temperature, pulse, respiratory rate, and blood pressure. Blood pressure will also be monitored during L-NMMA infusions as described in Section 5.2.1.2 – Dose Modification. Weight will also be measured at each physical exam. Height will be measured at screening only.

7.1.2.4 ECOG Performance Status

ECOG performance status will be assessed at screening/baseline, before every cycle of L-NMMA and pembrolizumab, and at EOT.

7.1.2.5 Electrocardiogram/Multigated Acquisition Scan/Echocardiogram

A 12-lead electrocardiogram will be performed at screening, before every cycle of L-NMMA and pembrolizumab, at EOT, and when clinically indicated. Multigated acquisition (MUGA) scan or echocardiogram (ECHO) will be performed at screening and when clinically indicated. The same method (MUGA scan or ECHO) must be used throughout the duration of the trial.

7.1.2.6 Tumor Imaging and Disease Assessment

Computed tomography (CT) scans of the chest, abdomen, and pelvis and/or other sites as appropriate (e.g., head and neck, upper extremities, lower extremities) will be performed at screening, after every 2 cycles of L-NMMA and pembrolizumab and/or at the discretion of the treating physician and 3 months after the last dose of trial treatment. Disease status will be assessed using the RECIST 1.1.

For subjects with previously treated stable brain metastases, brain CT or magnetic resonance imaging (MRI) will be performed at baseline and every 2 months (± 7 days) as per standard of care.

7.1.2.6.1 RECIST 1.1

The RECIST 1.1 will be used to assess treatment response. Only subjects with measurable disease will be entered into the study. Measurable disease is defined as tumor lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 10 mm on CT, bone metastases with soft tissue masses measuring ≥ 10 mm on CT (CT scan slice thickness no greater than 5 mm), palpable masses measured as ≥ 10 mm with calipers, and clearly delineated lung lesions surrounded by lung parenchyma measured as ≥ 20 mm on chest radiographs. Non-measurable lesions include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of the skin or lung, and abdominal masses/abdominal organomegaly identified by physical exam that are not measurable by reproducible imaging techniques. All measurable lesions (up to 5 measurable lesions [2/organ]) representative of all involved organs should be identified as target lesions and recorded and measured at baseline. A sum of the longest diameter for all target lesions will be calculated and reported as the baseline sum longest diameter.

- CR

Disappearance of all target lesions.

- PR

At least a 30% decrease in the sum of the longest diameter of target lesions, using the baseline sum longest diameter as a reference.

- Stable disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progressive disease (PD), using the smallest sum longest diameter since treatment start as a reference.

- PD

At least a $\geq 20\%$ increase in the sum of the longest dimensions of the target lesions taking as a reference the smallest sum of the longest dimensions recorded since the treatment started, or the appearance of one or more new lesions. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

- Clinical PD

Subjects who in the opinion of the treating principal investigator have clinical evidence of PD may be classified as having PD.

7.1.2.7 PK/Pharmacodynamics Assessment

Blood samples for plasma PK and pharmacodynamics assessment will be collected predose (10-30 minutes before L-NMMA infusion) on Days 1, 2, and 5 of Cycles 1 and 2. One 5-mL

sample will be collected for PK assessment and one 5-mL sample will be collected for pharmacodynamics assessment. **NOTE:** For PK/pharmacodynamics assessment, subjects should be fasted (nothing to eat or drink except water) for 12 hours prior to each collection time point. Subjects should also keep a food diary the week before and the week of the L-NMMA infusion during Cycles 1 and 2.

7.1.2.8 Blood Sample Collection, Storage, and Use for Future Research

Blood samples will be collected for future research at baseline, after Cycle 2 of L-NMMA and pembrolizumab, and 3 months after the last dose of trial treatment. Blood (20–30 cc) will be collected into standard vacutainer tubes and processed for plasma and viable cryopreserved peripheral blood mononuclear cells for future analyses. The samples will be stored at the Houston Methodist Research Institute Biorepository Core.

7.1.3 Laboratory Procedures/Assessments

7.1.3.1 Laboratory Tests

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below.

Laboratory Safety Evaluations (Hematology, Chemistry, and Urinalysis):

Coagulation parameters (INR/PT/aPTT) will be tested at screening. Hepatitis panel and HIV testing will be performed at screening. Thyroid-stimulating hormone, free thyroxine, and total triiodothyronine testing and urinalysis (blood, glucose, protein, specific gravity) will be performed at baseline and when clinically indicated. A blood sample for complete blood count with platelet count and differential white blood cell count will be obtained at screening/baseline, before every cycle of L-NMMA and pembrolizumab, at EOT, and when clinically indicated. A blood sample for clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, calcium, chloride, blood urea nitrogen, creatinine, total protein, total bilirubin, alkaline phosphatase, ALT, and AST) and evaluation of magnesium, uric acid, and lactate dehydrogenase will be obtained at screening/baseline, before every cycle of L-NMMA and pembrolizumab, at EOT, and when clinically indicated. For subjects with cHL, sedimentation rate testing will be performed at baseline and Cycles 2, 4, and 6 of L-NMMA and pembrolizumab. For women of childbearing potential, the results of a serum β -hCG pregnancy test must be negative at screening. If the screening serum β -hCG pregnancy test is performed more than 7 days before the first dose of trial treatment, it must be repeated at baseline, with results known to be negative prior to the administration of the first treatment dose. β -hCG pregnancy testing is to be repeated when clinically indicated. Laboratory tests may be done more frequently if medically indicated.

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 7.

Table 7. Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hemoglobin	Albumin	Blood	Serum β -hCG†
Platelet count	Alkaline phosphatase	Glucose	PT (INR)
White blood cell count (total and differential)	ALT	Protein	aPTT
ANC	AST	Specific gravity	Total triiodothyronine
Sedimentation rate*	Lactate dehydrogenase	Microscopic exam (<i>If abnormal results are noted</i>)	Free thyroxine
	Carbon dioxide		Thyroid-stimulating hormone
	Creatinine		Hepatitis panel
	Uric acid		HIV test
	Calcium		
	Chloride		
	Glucose		
	Potassium		
	Sodium		
	Magnesium		
	Total bilirubin		
	Total protein		
	Blood urea nitrogen		
†Perform on women of childbearing potential only.			
*Perform on subjects with classical Hodgkin lymphoma only.			

7.1.4 Continuation of Pembrolizumab Treatment

Subjects without disease progression after 6 cycles of L-NMMA and pembrolizumab will continue pembrolizumab until disease progression or unacceptable AEs. CT scans of the chest, abdomen, and pelvis and/or other sites as appropriate (e.g., head and neck, upper extremities, lower extremities) will be performed every 3 cycles of pembrolizumab and/or at the discretion of the treating physician. For subjects with previously treated stable brain metastases, brain CT or MRI will be performed every 2 months (± 7 days) as per standard of care. Blood samples for CBC with platelet count and differential WBC count, clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, calcium, chloride, BUN, creatinine, total protein, total bilirubin, alkaline phosphatase, ALT, and AST), and evaluation of magnesium, uric acid, and LDH will be obtained at every cycle of pembrolizumab. For subjects with cHL, sedimentation rate testing will be performed at every other cycle.

7.1.5 Other Procedures

7.1.5.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any AEs which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 — Assessing and Recording Adverse Events.

7.1.5.2 Visit Requirements

Visit requirements are outlined in Section 6.0 — Trial Flow Chart. Specific procedure-related details are provided above in Section 7.0 — Trial Procedures.

7.1.5.2.1 Post-Treatment Visits

7.1.5.2.1.1 Safety Follow-Up Visit

The mandatory safety follow-up visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anticancer treatment, whichever comes first. All AEs that occur prior to the safety follow-up visit should be recorded. Subjects with an AE of > Grade 1 will be followed until the resolution of the AE to Grade 0–1 or the beginning of a new antineoplastic therapy, whichever occurs first. SAEs that occur within 90 days of EOT or before initiation of a new anticancer treatment should also be followed and recorded.

7.1.5.2.1.2 Survival Follow-Up

Patients who continue to receive pembrolizumab will be followed up for survival status 30 days after the last dose of pembrolizumab.

7.2 Assessing and Recording AEs

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (e.g., abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the trial treatment is also an AE. Progression of the cancer under study is not considered an AE.

From the time of informed consent signing through 30 days following cessation of treatment, all AEs must be reported by the investigator. AE reporting will be performed according to 21 CFR 312.32. AEs will be recorded at each examination on the Adverse Event CRFs. AEs (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the subject's CRF. The reporting timeframe for AEs meeting any serious criteria is described in section 7.2.3.1. The investigator will make every attempt to follow all subjects with non-SAEs for outcome.

AEs will not be collected for subjects during the prescreening period as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a

blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

The occurrence of AEs should be sought by non-directive questioning of the subject at each study visit. AEs may also be detected when they are volunteered by the subject during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each AE should be evaluated to determine:

1. Severity grade (CTCAE Grade 1–4)
2. Duration (start and end dates or if continuing at the safety follow-up visit)
3. Relationship to the study treatment (reasonable possibility that AE is related: no, yes)
4. Action taken with respect to trial treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, hospitalized, unknown, not applicable)
5. Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown)
7. Whether it is serious, where a SAE is defined as in Section 7.2.3.1.

All AEs should be treated appropriately. Such treatment may include changes in trial treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an AE is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the trial treatment, the interventions required to treat it, and the outcome.

7.2.2 Reporting of Pregnancy and Lactation

Although pregnancy and lactation are not considered AEs, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur from the time of informed consent signing through 120 days following cessation of treatment or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage, and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported. Such events must be reported within 24 hours to the Sponsor (hmccsaereports@houstonmethodist.org; FAX 713-790-5106).

7.2.3 Immediate Reporting of AEs

7.2.3.1 SAEs

A SAE is any untoward medical occurrence that at any dose:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization, unless hospitalization is for:
 - routine treatment or monitoring of the studied indication and not associated with any deterioration in condition
 - elective or preplanned treatment for a preexisting condition that is unrelated to the indication under study and has not worsened since the start of trial treatment
 - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
 - social reasons and respite care in the absence of any deterioration in the subject's general condition;
- Is a congenital anomaly/birth defect;
- Is another important medical event. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the subject, require medical or surgical intervention to prevent one of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent.

From the time of informed consent form signing through 90 days following cessation of treatment or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any SAE or follow up to a SAE, including death due to any cause other than progression of the cancer under study, whether or not related to the trial treatment, must be reported within 24 hours to the Sponsor (hmccsaereports@houstonmethodist.org; FAX 713-790-5106).

Additionally, any SAE considered by an investigator who is a qualified physician to be related to the trial treatment that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor.

All subjects with SAEs must be followed up for outcome.

8.0 STATISTICAL CONSIDERATIONS

This is a Phase Ib trial investigating the use of L-NMMA in combination with pembrolizumab in subjects with melanoma, NSCLC, HNSCC, cHL, urothelial carcinoma, cervical cancer, esophageal cancer, gastric cancer, hepatocellular carcinoma, merkel cell carcinoma, primary mediastinal large B-cell lymphoma, renal cell carcinoma, small cell lung cancer, MSI-H/dMMR cancer or for the treatment of adult patients with unresectable or metastatic tumor

mutational burden-high (TMB-H) [≥ 10 mutations/megabase (mut/Mb)] solid tumors. The study is designed to determine the DLTs and MTD of the L-NMMA plus pembrolizumab combination. DLT will be defined as any treatment-related death or any \geq Grade 3 AE (NCI CTCAE v4.03) unless there is clear alternative evidence that the AE was not caused by the trial treatment. DLT will also include any AE during Cycle 1 that delays administration of Cycle 2 by more than 7 days:

- Grade 4 neutropenia ($ANC < 500/mm^3$) lasting ≥ 7 days
- Grade 3 febrile neutropenia lasting > 24 hours (single temperature of $> 38.3^\circ C$ or a sustained temperature of $> 38^\circ C$ for > 1 hour (non-axillary) with $ANC < 1000/mm^3$)
- Grade 4 febrile neutropenia of any duration
- Grade 4 thrombocytopenia (platelets $< 25,000/mm^3$) or Grade 3 thrombocytopenia with significant bleeding
- Hematological toxicities can be managed for a period that will not exceed more than 7 days. In the event that the hematological toxicity persists for more than the allowed time, it will be considered a DLT
- Grade 3–4 elevation in ALT or AST associated with a Grade 2 elevation in bilirubin that is at least possibly related to study drug (Hy's Law)
- Any \geq Grade 3 electrolyte abnormality considered clinically significant or which lasts ≥ 72 hours
- Grade 3 infusion-related reactions that recur despite appropriate medical management
- Any other treatment-related \geq Grade 3 non-hematological AE except hyperlipidemia in subjects not receiving maximum medical management or electrolyte abnormalities that may be managed with supplements

The study will utilize the sequential CRM for two-dimensional dose finding method of Yuan and Yin,⁶⁷ a method based entirely on a Bayesian decision framework. The trial is designed to investigate the combination at one dose level of pembrolizumab (200 mg IV Q3W) and 3 dose levels of L-NMMA (12.5, 15.0, and 20.0 mg/kg). The starting dose will be pembrolizumab at 200 mg IV Q3W and L-NMMA at 15.0 mg/kg. The trial will be comprised of 12 subjects and will utilize a standard Bayesian model averaging CRM approach to determine the appropriate dose of L-NMMA. A cohort size of 2 will be used.

The study was designed and will be conducted using Bayesian model averaging CRM software developed by the Biostatistics Department of the University of Texas M. D. Anderson Cancer Center (<https://biostatistics.mdanderson.org/SoftwareDownload/>).^{68,69} The software assumes the probability of toxicity at dose i (π_i) is modeled as $\pi_i = p_i \times \exp(\alpha)$ where p_i is a constant and α is distributed a priori as a normal random variable with mean 0 and variance 2. The software requires the investigator to specify the prior median probability of toxicity at each of the 3 doses of L-NMMA under consideration (s_i , $i = 1, 2, 3$). The CRM model assumes that toxicity is a monotonic increasing function with dose and thus, the s_i values may not decrease as i increases. The values p_i in the probability model are selected so that $E[p_i \times \exp(\alpha)] = s_i$. After the first cohort, each successive cohort is given the dose whose posterior probability of toxicity given the data collected thus far is closest to the target toxicity (the software does not allow an untried dose to be skipped). Thus as information accumulates, the model is continually updated *posteriorly* and decisions are made on the *posterior* distribution. As a result, no data is lost and

there is the assurance that the decision to increase or decrease the dose level is consistently reassessed as more information becomes available. For a dose level to be chosen as the MTD, at least 6 subjects must have received said dose.

Table 8. L-NMMA Dose Escalation

Dose Level	L-NMMA (mg/kg)
-1	12.5
0	15.0
1	20.0

The target toxicity probability will be 0.25 to 0.30 and a maximum of 12 subjects will be treated. The first cohort of subjects will be treated at dose level 0. As an extra measure of safety, the trial will be stopped early if the lowest dose level is unacceptably toxic, formally if

$$P_r\{p_{-1} \times e^{\alpha} > 0.25 | data\} > 0.80.$$

The Bayesian model averaging CRM will be implemented using the following three toxicity probability sets: 1) $(p_{-1}, p_0, p_1) = (0.05, 0.10, 0.15)$; 2) $(p_{-1}, p_0, p_1) = (0.15, 0.275, 0.40)$; and 3) $(p_{-1}, p_0, p_1) = (0.40, 0.50, 0.60)$. Operating characteristics of the design under five dose-toxicity scenarios are tabulated below.

Table 9. Phase Ib Design Operating Characteristics (optimal decisions are given in boldface type)

Scenario	Study Parameter	Dose Level			None
		-1	0	1	
1	Prob[toxicity]	0.05	0.10	0.15	0.03
	% Selected	0.03	0.18	0.77	
	# treated	1.0	3.3	7.5	
2	Prob[toxicity]	0.10	0.175	0.25	0.10
	% Selected	0.13	0.32	0.46	
	# treated	2.1	3.9	5.2	

3	Prob[toxicity]	0.15	0.25	0.35	0.20
	% Selected	0.24	0.34	0.22	
	# treated	2.9	4.0	3.5	
4	Prob[toxicity]	0.20	0.30	0.40	0.29
	% Selected	0.30	0.27	0.13	
	# treated	3.2	3.8	2.7	
5	Prob[toxicity]	0.40	0.50	0.60	0.75
	% Selected	0.17	0.05	0.01	
	# treated	2.8	2.6	0.8	

Under Scenario 5 in which all dose levels are too toxic, the estimated probability of finding no acceptable dose is 0.75 with the trial design choosing a dose with 40% toxicity 17% of the time. Scenario 1 represents a setting in which none of the doses is too toxic. For this scenario, the design would chose the highest dose as the MTD 77% of the time.

Data management. CRFs will be designed and utilized to capture all patient data. An electronic database will be designed to store patient CRFs. Data quality control will be performed regularly by the research coordinator to ensure timely, accurate, and complete patient data collection as well as protocol compliance. Queries will be generated and resolved prior to the generation of interim and final summary reports.

9.0 ETHICAL CONSIDERATIONS

Compliance with laws and regulations. This study will be conducted in full conformance with the International Conference on Harmonisation E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with U.S. FDA regulations and applicable local, state, and federal laws.

IRB. The protocol and informed consent form will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

Informed Consent. The consent form will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Confidentiality. The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient identification number. Patient medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the informed consent form signed by the patient, unless permitted or required by law. Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA and other national and local health authorities, and the IRB.

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11.0 APPENDICES

11.1 Common Terminology Criteria for Adverse Events v4.03

The descriptions and grading scales found in the revised NCI CTCAE v4.03 will be utilized for AE reporting. (<http://evs.nci.nih.gov/ftp1/CTCAE/About.html>)

11.2 RECIST 1.1

RECIST version 1.1 will be used in this study for assessment of tumor response. While either CT or magnetic resonance imaging may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this trial.

Eisenhauer, EA, et al. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.

11.3 ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
Oken MM, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-55.	

11.4 New York Heart Association Functional Classifications

Class	Description
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on physical activity without discomfort. Symptoms of cardiac insufficiency or of angina syndrome may be present at rest. If any physical activity is undertaken, discomfort is increased.

11.5 Nitrate/Nitrite Drug Interactions

Drug Class	Medications
Phosphodiesterase inhibitors	sildenafil, tadalafil, vardenafil, avanafil
Nitrates	isosorbide dinitrate, isosorbide mononitrate, nitroglycerin,
Nitrites	amyl nitrite, sodium nitrite
General anesthetics	Nitrous oxide