Investigational Drug Substance(s)	Durvalumab (MEDI4736) Ascorbate
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Assessing the Immunomodulatory Effects of Pharmacologic Ascorbate with Durvalumab (MEDI4736) in Non-Small Cell Lung Cancer: A window of opportunity trial

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Durvalumab by AstraZeneca Pharmaceuticals

Revision History

Summary of Changes

Affected Sections	Summary of Revisions Made	Rationale
Table 5.2.3	Added option to use DSW or Sterile water for mixing and included Recommended Infusion Times for Pharmacological Ascorbate mixed with D5W	This modification allows for the use of D5W in place of sterile water if sterile water is not available.

PROTOCOL SYNOPSIS

Clinical Protocol

Study Title: Assessing the Immunomodulatory Effects of Pharmacologic Ascorbate with Durvalumab (MEDI 4736) in Non-Small Cell Lung Cancer: A window of opportunity trial
Protocol Number: 202303162
Clinical Phase: Phase Ib – Window of Opportunity trial
Study Duration: 3 years (2 years of enrollment and 1-year minimum follow up)
Investigational Product(s) and Reference Therapy: Durvalumab concentrate for solution for infusion will be supplied in glass vials containing 500 mg durvalumab at a concentration of 50 mg/mL. Ascor 500® will be purchased from McGuff Pharmaceutical and available in sterile single-use 50 mL vials containing 25 g of ascorbic acid (500 mg/mL).

Research Hypothesis

The pre-clinical and clinical data demonstrated that pharmacological ascorbate (P-AscH-) enhances the activation of CD8+ T cells, which are critical for mounting an anti-tumor immune response. In addition, P-AscH- may act additively or synergistically with anti-PD1/PDL-1 therapies for the treatment of non-small cell lung cancer (NSCLC). Therefore, we hypothesized that P-AscH- along with durvalumab will mount or potentiate an anti-tumor immune response, primarily in the form of increase in CD8+ T cells in the tumor microenvironment.

To assess the effect of P-AscH-, we propose a descriptive, randomized, 3-arm, window of opportunity trial to enroll 36 patients (12 per arm) with newly diagnosed stage I (cT1b-T2a (1.1- 4 cm)N0M0)), resectable NSCLC. Patients will receive neoadjuvant therapy as below.

Arm A: Durvalumab 1500 mg q 3 wks. X 2 cycles as monotherapy Arm B: Combination of pharmacological ascorbate 75 grams intravenously three times a week x 4 wks. and Durvalumab 1500 mg q 3 wks. x 2 cycles Arm C: Serve as control and patients will not receive any neoadjuvant therapy before surgery
Objectives: **Primary Objective:** Determine if P-AscH- can elicit an immune response in the NSCLC tumor-microenvironments evaluated in the surgically resected specimens of patients after receiving neoadjuvant therapy. **Secondary Objectives**: 1) Assess safety and tolerability of the combination of P-AscH- and Durvalumab in the neoadjuvant setting (Arm B), 2) Estimate the pathologic complete response (pCR) rate, 3) Estimate the major pathologic response (MPR) rate, 4) Estimate event-free survival 5) Estimate overall survival **Exploratory Objectives:** 1) Determine other immunomodulatory effects of the combination of P-AscH- and durvalumab compared to durvalumab alone 2) Determine systemic immunomodulatory effects of the combination of P-AscH- and durvalumab compared to durvalumab alone; 3) Correlate circulating tumor (ct)DNA with time to clinical progression as assessed by RECIST v1.1
Study Design: This is a descriptive, proof of concept, open-label, randomized, 3-arm, window of opportunity trial to evaluate the immunomodulatory role of pharmacological ascorbate with Durvalumab. Translational and clinical findings of this study can then be applied more broadly to design and address clinically oriented questions in various NSCLC treatment settings. We plan to enroll 36 patients (12 per arm) with newly diagnosed stage I (cT1-T2a (1.1-4 cm)), resectable NSCLC. Patients will receive neoadjuvant therapy as below. Arm A: Two cycles of Durvalumab 1500 mg q 3 wks, as monotherapy Arm B: Combination of pharmacological ascorbate 75 grams intravenously three times a week x 4 wks plus Durvalumab 1500 mg q 3 wks for two cycles Arm C: Serve as control and patients will not receive any neoadjuvant therapy before going to surgery

Number of Centers: 1 – University of Iowa Hospitals and Clinics (UIHC) – Holden Comprehensive Cancer Center (HCCC)

Number of Patients: 36

Study Population: Patients with Non-Small cell lung cancer (NSCLC)

Inclusion Criteria:

- Histologically or cytologically confirmed diagnosis of non-small cell lung cancer(NSCLC)
- Clinically stage I (T1-4 cm) N0 M0 per American Joint Committee on Cancer 8thedition
- Surgically resectable
- Eastern Cooperative Oncology Group (ECOG) performance score ≤ 1
- ANC ≥ 1500/ul, Platelets ≥ 100,000/ul, Hemoglobin ≥ 9 g/dL,
- Total bilirubin within normal limit, AST & ALT ≤ 2.5 x ULN,
- Creatinine ≤ 1.5 x ULN or Creatinine Clearance ≥ 50mL/min/1.7m^2 if Cr ≥ 1.5 mg/dl
- Adequate lung function to undergo surgery
- Patients weighing > 30 Kg

Exclusion Criteria:

- Concurrent enrolment in another interventional clinical study
- Active or prior documented autoimmune or inflammatory disorders (including inflammatory bowel disease [e.g., colitis or Crohn's disease], systemic lupus erythematosus, Sarcoidosis syndrome, or Wegener syndrome [granulomatosis with polyangitis, Graves' disease, rheumatoid arthritis, hypophysitis, uveitis,etc.]).
- Known to have nodal or distant metastases
- History of active primary immunodeficiency
- History of allogenic organ transplantation.
- Known active hepatitis B or C infection
- Known to have tested positive for human immunodeficiency virus (HIV)
- Current/prior use of immunosuppressive medication within 14 days ofdurvalumab.
- History of prior anti PD-1, PD-L1 and CTLA-4 therapy in the last 2 years

Investigational Product(s), Dose and Mode of Administration:

Arm A: Durvalumab 1500 mg q 3 weeks x 2 intravenously

Arm B: Durvalumab 1500 mg q 3 weeks x 2 intravenously with Ascorbate infusions 75 g three times a week x 4 weeks starting on cycle 1 day 1 of durvalumab Arm C: No investigational products. Patients will undergo surgery after randomization without waiting.
Study Assessments and Criteria for Evaluation: Fresh resected tumor tissue will be evaluated for qualitative and quantitative characterization of immune cell infiltrate in tissue microenvironment to assess primary endpoint of CD8 T cells across different study arms. **Safety Assessments:** Included assessment of dose-limiting toxicity rate for patients in arm B in the first 3-6 patients, and overall tolerability of neoadjuvant therapies across different arms of the study. **Efficacy Assessments:** Treatment effectiveness will be assessed based on qualitative and quantitative differences in immune infiltration in the resection samples across study arms, more specifically CD8+ T cells. Additionally, pathologic response from the neoadjuvant therapy will be assessed and compared across arms. Event-free and overall survival will also be assessed and compared across study arms.
Statistical Methods and Data Analysis: An analysis of variance will be used to evaluate immunomodulatory effects in resected specimens as measured by CD8+ T cells, CD4+ T Cells, T-regs, tissue-resident macrophages, and other immune cell phenotypes between treatment arms. Mixed effects regression models will be used to estimate within and between treatment arm differences in the peripheral blood mononuclear cells, cytokines and chemokines. Random effects will be included to account for the longitudinally correlated nature of repeat measurements within a patient. Cox regression will be used to evaluate the association between circulating tumor DNA and time to clinical progression as defined by per RECIST v1.1.

Sample Size Determination:

The primary objective of this descriptive, window of opportunity trial is to obtain preliminary evidence of the immunomodulatory effect of pharmacological ascorbate as assessed by differences in CD8+ T cells at resection between the Durvalumab and Durvalumab + P-AscH-treatment arms (Arm A and B). Patients will be randomly assigned using a 1:1:1 allocation ratio. The planned sample size of 10 patients per treatment arm ensures 80% power to detect a 1.2 SD mean difference in CD8+ T cells between treatment arms using a two-sample t-test. Power calculations are based on a two-sided 10% significance level. A total of 12 patients per treatment arm (36 total) will be randomized to ensure at least 10 patients per treatment arm have evaluable specimens.

As this is the first time that Durvalumab has been administered preoperatively in NSCLC with P-AscH-, there will be a run-in phase for Arm B, where patients will be observed for DLT. An initial DLT assessment will be made when 3 patients have been randomized to Arm B and are evaluable for DLT. If at most 1 out of 3 patients experience DLT, an additional cohort of 3 patients will be evaluated for DLT. If at most 1 out of 6 patients experience DLT, the combination will be deemed safe. There will be no dose-escalation.

SCHEDULE OF STUDY ASSESSMENTS

Procedures		Screening Day -42 to -1	Day -7 to 1	Visit 1, Cycle 1 Day 1	Week 2	Week 3	Week 4 Cycle 2 Day 1	Week 5-9 (D29-63)	30 days **from surgery +/- 1 wk.**	90 days from surgery +/- 2 wks (or early treatment discontinuation)	Q 3 months during 1st year starting at M6 from surgery +/- 4 wks	Q 6 months during 2nd and 3rd year from surgery	Protocol Section
Informed consent		X											12.3
Demographics		X											
Medical history		X											
Concomitant medication		X		X			X		X	X	X		7.2
Randomization [a]			X										
Study intervention	Durvalumab (Arm A & B)[k]			X			X						
	Ascorbate (Arm B)			X	X	X	X						
	Surgery[l]							X					
Physical exam, vitals and ECOG		X		X			X		X	X	X		10.1, 10.2
Height		X											10.1.2
Weight		X		X			X		X	X			10.1.2
CBC/diff [c]		X		X			X	X	X	X	X		Table 6
CMP and creatinine clearance		X		X	X	X	X	X	X	X	X		Table 7
TSH and reflex free T4[g]		X		X					X	X	X		
Amylase, Lipase, GGT, LDH, Magnesium				X									
Pregnancy test [d]		X											
ECG [e]		X											10.1.1
Urinalysis[f]		X											
Adverse event review and evaluation		X		X			X		X	X			
Radiologic/Imaging assessment (RECIST)[h]		X								X	X	X	
Lung function test: spirometry and DLCO[i]		X											
Tissue procurement (fresh tissue from resected specimen								X					

Procedures	Screening Day -42 to -1	Day -7 to 1	Visit 1, Cycle 1 Day 1	Week 2	Week 3	Week 4 Cycle 2 Day 1	Week 5-9 (D29-63)	30 days **from surgery +/- 1 wk.**	90 days from surgery +/- 2 wks	Q 3 months during 1st year starting at M6 from surgery +/- 4 wks until 1 year	Q 6 months during 2nd and 3rd year of surgery +/- 6 wks	Protocol Section
PBMC, cytokines, and chemokines [L]			X			x	Xm	x	x	x		
Blood for Iron markers[j,L]			X			x		x	x			
Blood for ct-DNA			x				Xm	x	x	x		
Banking: Archival tissue (biopsy and resection sample)									x			
Banking: Whole Blood, Plasma, Serum for miRNA, proteomics etc.			x			x	Xm		x	x		
Follow-up for progression or survival											X	

Note: All assessments on treatment days are to be performed prior to infusion, unless otherwise indicated. No day "0" or week "0"

[a] Subjects must begin therapy within 7 days of randomization

[b] Clinical Chemistries per Table 7 . Clinical chemistry (including LFT monitoring) may be performed more frequently if clinically indicated. If screening clinical chemistry assessments are performed within 7 days prior to Day 1 (first infusion day), they do not need to be repeated at Day 1. Creatinine clearance only need to be calculated at screening and on C1D1 by Cockroft-gault formula.

[c] CBC/diff per Table 6. Hematology may be performed more frequently if clinically indicated. If screening hematology assessments are performed within 7 days prior to Day 1 (first infusion day), they do not need to be repeated at Day 1.

[d] For women of childbearing potential. Serum pregnancy test performed at screening and urine pregnancy tests performed thereafter. Pregnancy test may occur on Day 1, but results must be available and reviewed by the treating physician or Investigator prior to commencing an infusion.

[e] Resting 12 lead Triplicate ECGs (5 minutes apart) at screening and as clinically indicated throughout the study. In case of clinically significant ECG abnormalities, including a QTcF value >470 ms, 2 additional 12-lead ECGs should be obtained over a brief period (eg, 30 minutes) to confirm the finding

[f] Urinalysis should be performed at baseline (screening) then as clinically indicated

[g] Only required for Arms A and B. If TSH was measured within 14 days prior to C1Day 1 (first infusion day), it does not need to be repeated. Free T4 will only be measured if TSH is abnormal

h RECIST assessments will be performed on images from CT (Computed Tomography) Chest preferably with IV contrast (or CT portion of the PET/CT). CT of the abdomen is not required but preferable. Baseline assessments should be performed no more than 42 days before C1D1. If an unscheduled assessment was performed and the patient has not progressed, every attempt should be made to perform the subsequent assessments at their next scheduled visit. MRI brain assessment is only required during screening as clinically indicated; For brain imaging the window will be up to -120 days from C1D1 if indicated (CT brain can be an alternative if MRI is not possible when indicated).

i lung function may have been obtained upto -120-days from C1D1. If laboratory or imaging procedures were performed prior to signing consent, these can be used for screening purposes with consent of the patient. However, all screening laboratory and imaging results must have been obtained within 42 days of randomization except brain imaging and lung function for which window is -120 to C1D1.

[j] Iron panel (Ferritin, Iron Sat, total Iron and TIBC) will be added to labs already drawn for that day.

[k] ±3 days window for durvalumab unless held for toxicity
[L] Patient in Arm C will have PBMC, cytokines, chemokines, and iron markers collected only once prior to surgery. Patients in Arm C do not need to wait for week 5 to have surgery as they will not be getting any neoadjuvant therapy.
[m] Prior to Surgery

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ABBREVIATIONS AND SPECIAL TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
ADA	Anti-drug antibody
ADCC	Antibody-dependent cell-mediated cytotoxicity
AE	Adverse event
AESI	Adverse event of special interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APC	Antigen-presenting cells
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
CDC	Complement-dependent cytotoxicity
CI	Confidence interval
CL	Clearance
C_{max}	Peak concentration

Abbreviation or special term	Explanation
$C_{max,ss}$	Peak concentration at steady state
C_{min}	Trough concentration
$C_{min,ss}$	Trough concentration at steady state
CNS	Central nervous system
CR	Complete response
CT	Computed tomography
CTLA-4	Cytotoxic T-lymphocyte-associated antigen-4
DC	Disease control
DCR	Disease control rate
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
DoR	Duration of response
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	Disodium edetate dihydrate
Fc	Fragment crystallizable
FFPE	Formalin fixed paraffin embedded
FSH	Follicle-stimulating hormone
FTIH	First-time-in-human
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GLP	Good Laboratory Practice
HCl	Hydrochloride
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Conference on Harmonization

Abbreviation or special term	Explanation
ICI	Immune checkpoint inhibitors
IEC	Independent Ethics Committee
IFN	Interferon
IGF	Insulin-like growth factor
IgG1	Immunoglobulin G1
IgG2	Immunoglobulin G2
IGSF	Immunoglobulin superfamily
IHC	Immunohistochemistry
IL	Interleukin
imAE	Immune-mediated adverse event
IRB	Institutional Review Board
IV	Intravenous(ly)
Mab	Monoclonal antibody
MDSC	Myeloid-derived suppressor cells
MedDRA	Medical Dictionary for Regulatory Activities
miRNA	Micro ribonucleic acid
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MTD	Maximum tolerated dose
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NK	Natural killer
NOAEL	No-observed-adverse-effect level
NSCLC	Non-small cell lung cancer
OR	Objective response
ORR	Objective response rate
OS	Overall survival

Abbreviation or special term	Explanation
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PD-1	Programmed cell death 1
PD-L1	Programmed cell death ligand 1
PD-L2	Programmed cell death ligand 2
PFS	Progression-free survival
PK	Pharmacokinetic(s)
P-AscH-	Pharmacologic ascorbate
PR	Partial response
PRO	Patient-reported outcome
PVC	Polyvinyl chloride
Q2W	Every 2 weeks
Q3M	Every 3 months
Q3W	Every 3 weeks
Q3W	Every 3 weeks
Q12W	Every 12 weeks
QoL	Quality of life
QTc	Time between the start of the Q wave and the end of the T wave corrected for heart rate
QTcF	QT interval on ECG corrected using the Frederica's formula
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic acid
SAE	Serious adverse event
SD	Stable disease
SID	Subject identification
sPD-L1	Soluble programmed cell death ligand 1
SUSAR	Suspected unexpected serious adverse reaction

Abbreviation or special term	Explanation
$t_{½}$	Half life
TEAE	Treatment-emergent adverse event
tHTN	Transient Hypertension
TIL	Tumor infiltrating lymphocyte
T_{max}	Time to peak concentration
$T_{max,ss}$	Time to peak concentration at steady state
TNF-α	Tumor necrosis factor alpha
TSH	Thyroid stimulating hormone
ULN	Upper limit of normal
USA	United States of America
WFI	Water for injection
WHO	World Health Organization

1. INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in the US. Annually, it accounts for more deaths than breast, colon, and prostate cancers combined. Nearly 85% of new cases of lung cancer are non-small cell lung cancers (NSCLC). Recently, the addition of immune checkpoint inhibitors (ICI) in the neoadjuvant or adjuvant setting has been shown to improve outcomes in patients with surgically resectable NSCLC. For example, ICI are recommended following chemotherapy in the adjuvant setting for patients with stage IIA (T > 4 cm) to IIIA resected NSCLC and programmed death-ligand 1 (PD-L1) expression of ≥1% based on the IMpower010 study (Felip E et al 2021). On the other hand, in the Keynote-091 study, adjuvant ICI following chemotherapy improved disease-free-survival for all patients, regardless of PD-L1 expression (Paz-Ares L et al. 2022). Similarly, the Checkmate816 study showed that the combination of ICI and chemotherapy in the neoadjuvant setting is safe and increases rates of pathologic complete response (pCR) and major pathologic response (Forde PM et al. 2022). Although the benefit was observed across all levels of PD-L1 expression, it was more evident for patients with higher tumor PD-L1 expression. While these findings are encouraging, these studies did not include early-stage NSCLC, a growing patient population due to the adoption of lung cancer screening guidelines, which now account for 40% of new NSCLC diagnoses.

Stage I NSCLC

The 8th edition of AJCC cancer staging defined T1a as tumors ≤ 1 cm in greatest dimension. T1b tumors are > 1 cm but ≤ 2 cm. T1c tumors are > 2 cm but ≤ 3 cm. T2 tumors were classified as T2a if > 3 cm but ≤ 4 cm and T2b if > 4 cm but ≤ 5 cm.

The recurrence rate for stage I NSCLC after surgery varies from 20 to 30% based on tumor size. Five-year survival for resected NSCLC patients with tumor size of 2.1-3 cm (pT1c) is 81% and for 3-4 cm (pT2a) is 73%.

5-year survival outcomes for pathologically staged disease based upon this classification were as follows:
pT1a tumors 0.1-1 cm: 91% (Stage IA1)
pT1a tumors 1.1-2 cm: 86% (Stage IA2)
PT1b tumors 2.1-3 cm: 81% (Stage IA3)
pT2a tumors 3.1-4 cm: 73% (Stage IB)

5-year survival outcomes for clinically staged disease based upon this classification were as follows:

cT1a tumors 0.1-1cm: 92%
cT1b tumors 1.1-2cm: 83%
cT1c tumors 2.1-3cm: 76%

cT2a tumors 3.1-4cm: 68%

Patterns of recurrence for early-stage NSCLC:

Patterns of survival and recurrence after surgical treatment of early-stage NSCLC was evaluated in 1,018 patients treated on the ACOSOG Z0030 (Su S et al. 2014). In this study, 578 patients had cT1 tumors and 440 patients had cT2 tumors. The local recurrence-free survival at 5 years was 95% for T1 tumors and 91% for T2 tumors. In contrast, distant disease-free survival was only 75.3%. This underscores that the risk of death in patients with surgically resected stage 1 disease is primarily due to distant, metastatic recurrence.

Ways to improve outcomes:

The addition of perioperative chemotherapy has not shown a clinical benefit and may be detrimental for this patient population given associated long-term side effects. Immunotherapy with or without an immunomodulatory agent could potentially improve the outcomes in this subset of patients with NSCLC. Trials are underway to evaluate efficacy of anti-PD(L)-1 therapies in very early-stage NSCLC (NCT04317534, NCT03833154, NCT03794544, NCT03924869, NCT03634241).

Window of opportunity trials:

In addition, it is an optimal clinical setting to perform window of opportunity trials to evaluate pharmacodynamics of new anti-cancer or immunomodulatory agents. Pharmacological doses of ascorbate have shown promising anti-cancer effects in prior studies (Schoenfeld JD et al 2017, Welsh JL et al. 2013, Alexander MS et al. 2018, Allen BG et al 2019, Allen BG et al. 2019, Furqan M et al. 2019, Ma Y et al. 2014). Recent studies in animal models performed by Magri et al. and Luchtel et al. have shown that pharmacological ascorbate acts synergistically with immune system and control cancer growth (Luchtel RA et al 2020, Magri A et al. 2020).

1.1 Disease background

1.1.1 Immunotherapies

It is increasingly understood that cancers are recognised by the immune system, and under some circumstances, the immune system may control or even eliminate tumours (Dunn et al.2004).

PD-L1 is part of a complex system of receptors and ligands that are involved in controlling T -cell activation. The PD-1 receptor (cluster of differentiation [CD]279) is expressed on the surface of activated T cells (Keir et al. 2008). It has 2 known ligands: PD-L1 (B7H1; CD274) and programmed death -ligand 2 (PD-L2) (B7-DC; CD273). PD-1 and PD-L1/PD-L2 belong to

a family of immune checkpoint proteins that act as co-inhibitory factors, which can halt or limit the development of T-cell response. When PD-L1 binds to PD1, an inhibitory signal is transmitted into the T cell, which reduces cytokine production and suppresses T -cell proliferation. Tumour cells exploit this immune checkpoint pathway as a mechanism to evade detection and inhibit immune response.

PDL1 is constitutively expressed by B cells, dendritic cells, and macrophages. Importantly, PDL1 is commonly overexpressed on tumour cells or on nontransformed cells in the tumour microenvironment. PDL1 expressed on the tumour cells binds to PD1 receptors on the activated T cells, leading to the inhibition of cytotoxic T cells. These deactivated T cells remain inhibited in the tumour microenvironment. The PD1/PDL1 pathway represents an adaptive immune resistance mechanism that is exerted by tumour cells in response to endogenous antitumour activity.

The inhibitory mechanism described above is co-opted by tumours that express PD-L1 as a way of evading immune detection and elimination. The binding of an anti-PD-L1 agent to the PD-L1 receptor inhibits the interaction of PD-L1 with the PD-1 and CD80 receptors expressed on immune cells. This activity overcomes PD-L1-mediated inhibition of antitumour immunity. While functional blockade of PD-L1 results in T-cell reactivation, this mechanism of action is different from direct agonism of a stimulatory receptor such as CD28.

PD-L1 is expressed in a broad range of cancers. Based on these findings, an anti-PD-L1 antibody could be used therapeutically to enhance antitumour immune responses in participants with cancer. Results of pre-clinical and clinical studies of monoclonal antibodies (mAbs) targeting the PD-L1/PD-1 pathway have shown evidence of clinical activity and a manageable safety profile, supporting the hypothesis that an anti-PD-L1 antibody could be used to therapeutically enhance antitumour immune response in cancer participants (Brahmer et al. 2012, Iwai et al. 2002, Okudaira et al. 2009, Topalian et al. 2012, Zhang et al. 2008) with responses that tend to be more pronounced in participants with tumours that express PD-L1 (Powles et al. 2014, Rizvi et al. 2015, Segal et al. 2015). In addition, high mutational burden (eg, in bladder carcinoma) may contribute to the responses seen with immune therapy.

In contrast, CTLA-4 is constitutively expressed by regulatory T cells and upregulated on activated T cells. CTLA-4 delivers a negative regulatory signal to T cells upon binding of CD80 (B7.1) or CD86 (B7.2) ligands on antigen-presenting cells. Blockade of CTLA-4 binding to CD80/86 by antiCTLA-4 antibodies results in markedly enhanced T-cell activation and antitumour activity in animal models, including killing of established- murine solid tumours and induction of protective antitumour immunity. Therefore, it is expected that treatment with an anti-CTLA-4 antibody will lead to increased activation of the human immune system, increasing antitumour activity in participants with solid tumours. Pre-clinical data have now been added to a wealth of clinical data showing that blockade of negative regulatory signals to T cells such as CTLA-4 and PD-L1 has promising clinical activity. Ipilimumab was first granted United States (US) FDA approval for the treatment of metastatic melanoma and is currently

under investigation for several other malignancies. Nivolumab and pembrolizumab, 2 anti-PD-1 agents, and atezolizumab, an antiPD-L1 agent, have been granted approvals by agencies for the treatment of several- malignancies including metastatic melanoma, squamous and non-squamous cell non-small-cell lung cancer, squamous cell carcinoma of the head and neck, and urothelial carcinoma. In addition, there are data from agents in the anti-PD-1/PD-L1 class showing clinical activity in a wide range of tumour types.

1.2 PD1-PDL-1 blockade in NSCLC

One of the hallmarks of cancer is immune evasion, in which the immune system does not mount an effective antitumor response. Programmed cell death 1 (PD-1) is a negative costimulatory receptor expressed primarily on the surface of activated or exhausted T cells. The binding of PD-1 to its ligand PD-L1 can inhibit a cytotoxic T-cell response. Tumors can co- opt this pathway to escape T-cell-induced antitumor activity.

Drugs blocking the interaction between PD-1 on T cells and PD-L1 on tumor cells or other cells in the tumor microenvironment has shown great efficacy and improved patient's outcome. For example, several Anti-PD(L)-1 drugs have been approved in NSCLC in various clinical settings:

- Treatment naïve metastatic NSCLC irrespective of PD-L1 tumor proportion score (TPS): Pembrolizumab, Nivolumab, Atezolizumab with other agents
- Metastatic NSCLC in treatment naïve setting with PD-L1 TPS ≥ 1%: Pembrolizumab
- Metastatic NSCLC after failure of platinum-based therapy: Nivolumab, Atezolizumab, Pembrolizumab (for TPS ≥1%)
- Unresectable locally advanced NSCLC without progression after definitive chemo-radiation: Durvalumab
- Resectable stage IIA-IIA NSCLC in neoadjuvant setting: Nivolumab
- Resectable stage II-IIIA NSCLC in adjuvant setting: Atezolizumab (TPS ≥1%)

1.3 Durvalumab background/non-clinical and clinical experience

Durvalumab is a human mAb of the immunoglobulin G 1 kappa subclass that blocks the interaction of PD-L1 (but not PD-L2) with PD-1 on T cells and CD80 (B7.1) on immune cells. It is being developed by AstraZeneca for use in the treatment of cancer. The proposed mechanism of action for durvalumab is interference in the interaction of PD-L1 with PD-1 and CD80 (B7.1). Blockade of PD-L1/PD-1 and PD-L1/CD80 interactions releases the inhibition of

immune responses, including those that may result in tumour elimination. In vitro studies demonstrate that durvalumab antagonises the inhibitory effect of PD-L1 on primary human T cells resulting in the restored proliferation of interferon-γ (IFN-γ) (Stewart et al. 2015). In vivo studies have shown that durvalumab inhibits tumour growth in xenograft models via a Tcell--dependent mechanism (Stewart et al. 2015). Based on these data, durvalumab is expected to stimulate the participant's antitumour immune response by binding to PDL1 and shifting the balance toward an antitumour response. Durvalumab has been engineered to reduce antibody--dependent cellular cytotoxicity and complement-dependent cytotoxicity.

To date, durvalumab has been given to more than 14000 participants as part of ongoing studies (Investigator Brochure edition 18, Nov 2022) either as monotherapy or in combination with other anticancer agents. Details on the safety profile of durvalumab monotherapy are summarised in Section 1.8.1 . Refer to the current durvalumab IB for a complete summary of preclinical and clinical information including safety, efficacy, and PK.

Clinical studies conducted to date on the durvalumab program (including combination studies with other molecules) are conducted in accordance with the study protocols, ICH guidelines, GCP principles, the Declaration of Helsinki, and applicable laws and regulations.

Durvalumab is FDA approved in the following settings

• Stage III NSCLC after completion of Chemo-radiation.

Weight of ≥ 30 kg: 10 mg/kg every 2 weeks or 1,500 mg every 4 weeks

Weight < 30 kg: 10 mg/kg every 2 weeks

• Extensive stage small cell lung cancer (SCLC):

Weight ≥ 30 kg: With etoposide and either carboplatin or cisplatin, 1,500 mg every 3 weeks, and then 1,500 mg every 4 weeks as a single agent.

Weight < 30 kg: With etoposide and either carboplatin or cisplatin, 20 mg/kg every 3 weeks, and then 10 mg/kg every 2 weeks as a single agent

• Locally advanced biliary tract cancer (BTC):

Weight of ≥ 30 kg: 1,500 mg every 3 weeks in combination with Cisplatin/Gemcitabine x 4 cycles and then 1,500 mg every 4 weeks as a single agent

Weight < 30 kg: 20 mg/kg every 3 weeks in combination with chemotherapy, and then 20 mg/kg every 4 weeks as a single agent

1.4 Durvalumab in NSCLC in neo-adjuvant setting and in Stage I-III resectable NSCLC

NeoCOAST (NCT03794544) is a global, randomized phase 2 study of the anti-PD-L1 monoclonal antibody (mAb) durvalumab (D) alone or combined with the anti-CD73 mAb oleclumab (O), the anti-NKG2A mAb monalizumab (M), or the anti-STAT3 antisense oligonucleotide danvatirsen (Da) as neoadjuvant therapy (Cascone T et al. 2022). Patients with previously untreated, cytologically/histologically documented, resectable, Stage I [>2 cm]-IIIA NSCLC and ECOG PS 0-1 were randomized 1:1:1:1 (stratified by lymph node involvement) to receive durvalumab 1500 mg IV alone Q4W or combined with oleclumab 3000 mg IV Q2W, monalizumab 750 mg IV Q2W, or danvatirsen 200 mg IV QW, for one 28- day cycle, followed by surgery. The primary endpoint was investigator-assessed major pathological response (MPR; ≤10% residual viable tumor cells at tumor site and nodes, at surgery). Secondary endpoints were pathological complete response (pCR; no viable tumor cells), safety and tolerability, feasibility of surgery, PK and immunogenicity. Exploratory endpoints included tumor and microbiome biomarkers and blood mRNA signatures.

From March 2019 to Sept 2020, 84 patients were randomized and 83 received D (n=26), D+O (n=21), D+M (n=20), or D + Da (n=16). MPR occurred in 11.1% (95% CI, 2.4-29.2), 19.0% (95% CI, 5.4-41.9), 30.0% (95% CI, 11.9-54.3), and 31.3% (95% CI, 11.0-58.7%), respectively. pCR
occurred in 3.7% (95% CI, 0.1-19.0), 9.5% (95% CI, 1.2-30.4), 10.0% (95% CI, 1.2-31.7%) and 12.5% (95% CI, 1.6-38.3), respectively. Rates of treatment-related AEs were 34.6% with D, 57.1% with D+O, 50.0% with D+M, and 43.8% with D + Da (grade ≥3 in 0%, 4.8%, 0% and 6.3%, respectively). Most patients (76/83; 91.6%) completed surgery with no significant delay; of the 7-patients unable to, 5 had progressive or stage IV disease. Across all arms, MPR was more common in patients with baseline tumor PD-L1 expression ≥1% vs those with <1%. With D, CD73 expression was correlated with greater residual viable tumor cells at surgery; however, with D+O, high CD73 (≥10% tumor cells) was associated with reduced viable tumor cells. Transcriptome analysis of post-treatment and baseline blood samples showed upregulation of genes involved in B cell activation and antigen presentation in patients with MPR in the D+O arm, and upregulation of genes associated with Tregs in patients with MPR in the D+M arm.

1.5 Pharmacological Ascorbate

There has been growing interest in improving the immunogenicity of NSCLC to enhance the efficacy of ICI. Pharmacologic ascorbate (P-AscH-), defined as the intravenous (IV) dosing of

ascorbate (vitamin C) to achieve millimolar concentrations in plasma, is a promising adjunct to ICI. In a lymphoma mouse model, P-AscH- has been shown to synergize with anti-PD-1 therapy to inhibit tumor growth via recruitment of macrophages and CD8+ T-cells into the tumor, enhancement of granzyme-B production by cytotoxic cells, and increased interleukin-12 production by antigen-presenting cells (Luchetel RA. et al. 2020). Similarly, in a mouse model of pancreatic, breast, and colorectal cancers, P-AscH- acted synergistically with ICI directed against cytotoxic-T-lymphocyte-associated protein-4 (CTLA-4) and PD-1 to reduce tumor burden via infiltration and activation of the T lymphocytes in the tumor microenvironment (Figure 1) (Magri A et al. 2020).

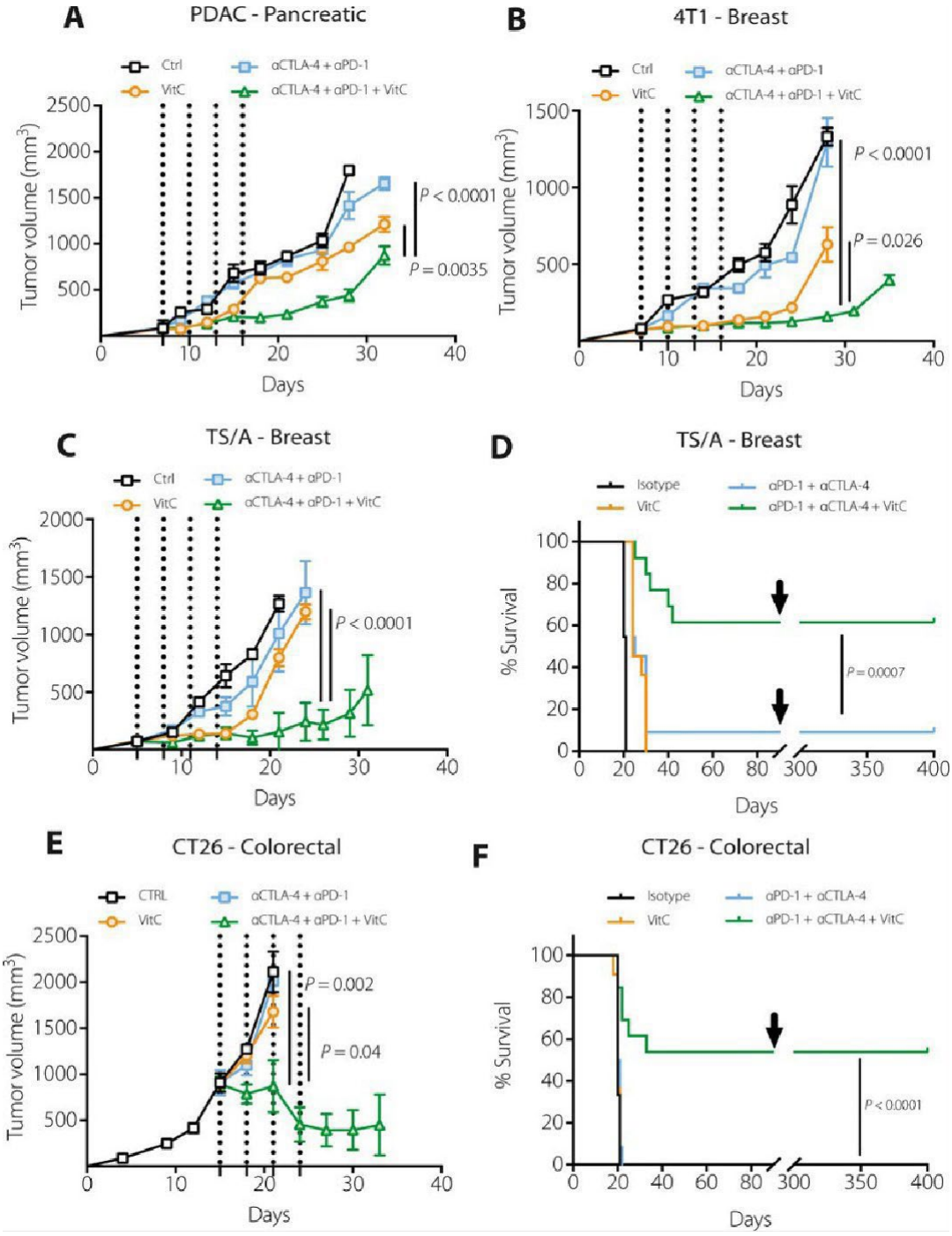


Figure 1: (**A**) PDAC pancreatic cancer cells were injected subcutaneously (500,000 cells) into syngeneic mice that were treated with Vit C, ICI, or their combination. (**B**) 4T1 breast cancer cells were injected orthotopically (100,000 cells, 50% Matrigel) in syngeneic mice that were treated with Vit C, ICI, or their combination. (**C**) TS/A breast cancer cells were injected orthotopically (100,000 cells, 50% Matrigel) in syngeneic mice that were treated with Vit C, ICI, or their combination. (**D**) Tumor relapse-free survival of mice

treated with Vit C, ICI, or their combination and followed for over a year. Two independent experiments performed on a total of n = 13 mice are shown in survival curves. The vertical black arrows indicate the time point at which mice were rechallenged with live tumor cells. (**E**) CT26 colorectal cancer cells were injected subcutaneously (500,000 cells) in syngeneic mice. (**F**) Tumor relapse-free survival of mice treated with Vit C, ICI, or their combination and followed up to a year. Two independent experiments performed on a total of n = 13 mice are shown in survival curves. The vertical black arrow indicates the time point at which mice were rechallenged with live tumor cells. Vit C (4 g/kg) was administered intraperitoneally 5 days per week starting when tumors reached a volume around 100 mm^3 in the TS/A, 4T1, and PDAC models. Vit C treatment started when tumor volume was around 800 to 1000 mm^3 in the CT26 model. Anti–CTLA-4 (200 μg per mouse) and anti–PD-1 (250 μg per mouse) were given at the time points indicated by the dashed vertical lines in the graphs. In combinatorial treatments, Vit C was administered starting with the first cycle of immunotherapy. Every experimental group was composed of at least five mice. Every experiment was performed twice except for those shown in (A) and (B). Data and error bars indicate mean ± SEM. Statistical analysis used one-way ANOVA for tumor growth comparison at the indicated time points and log-rank test (Mantel-Cox) for survival analysis. (Magri A et al. 2020)

In another ongoing trial (NCT 03508726), patients with locally advanced sarcoma receiving neoadjuvant radiation with P-AscH-. Peripheral blood was analyzed to assess changes in immune cell populations prior to treatment, at week 5, and at the end of treatment. Patients treated with P-AscH- have shown a great degree of increase in activated effector CD8+ T-cells and a slight decrease in regulatory T-cells compared to control patient population. Resected tumor specimens were analyzed for necrosis and immune cell infiltrates qualitatively and quantitatively. Findings were compared to patients who received neoadjuvant radiation. The differences in immune cell population in peripheral blood and tumor samples from patients receiving ascorbate with radiation (n=15) vs radiation alone (n=6) is shown in figure 2.

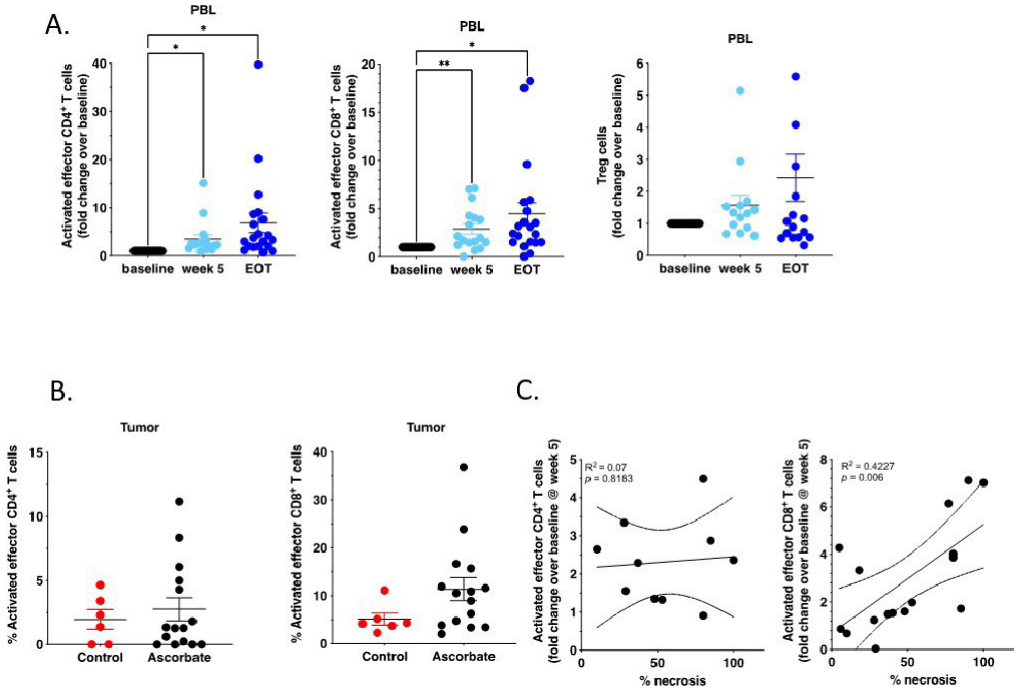


Figure 2. The differences in immune cell population in peripheral blood (A), in resected tumor samples (B) and necrosis (C) from patients receiving ascorbate with radiation (n=15) vs radiation alone (n=6, control).

1.6 Pharmacological Ascorbate in NSCLC pre-clinical and Clinical data

To further explore the relationship between P-AscH- and standard of care therapies in NSCLC, our group performed studies in immunocompetent mice. C57BL/6 mice with subcutaneous Lewis lung carcinoma tumors were treated with chemotherapy (carboplatin, paclitaxel) and anti-PD-1 ICI with or without high-dose intraperitoneal P-AscH-. While chemotherapy and anti-PD-1 therapy were effective at inhibiting tumor growth, the addition of P-AscH- improved the anti-tumor effect in these mice (Figure 3).

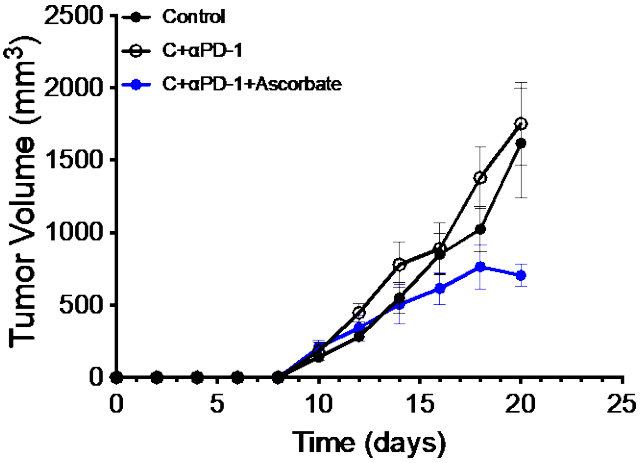


Figure 3: C57BL/6 mice were injected with Lewis Lung cancer cells subcutaneously in the flank. C = chemotherapy (carboplatin + paclitaxel); Ascorbate 4gm/Kg intraperitoneally daily from day 10-20

In an ongoing trial (XACT lung trial - NCT 02905591), patients with unresectable stage III NSCLC are receiving definitive chemo-radiation with P-AscH-. So far, 30 patients have been enrolled. Peripheral blood of these patients is being analyzed in real time to assess changes in immune cell populations prior to treatment, twice during treatment, and 4 weeks post-treatment. Patients treated with P-AscH- have shown a significant increase in activated effector CD8+ T-cells and a reduction in regulatory T-cells at the end of treatment (n=24) compared to the control patient population (figure 4) receiving standard of care chemo-radiation alone (n=15).

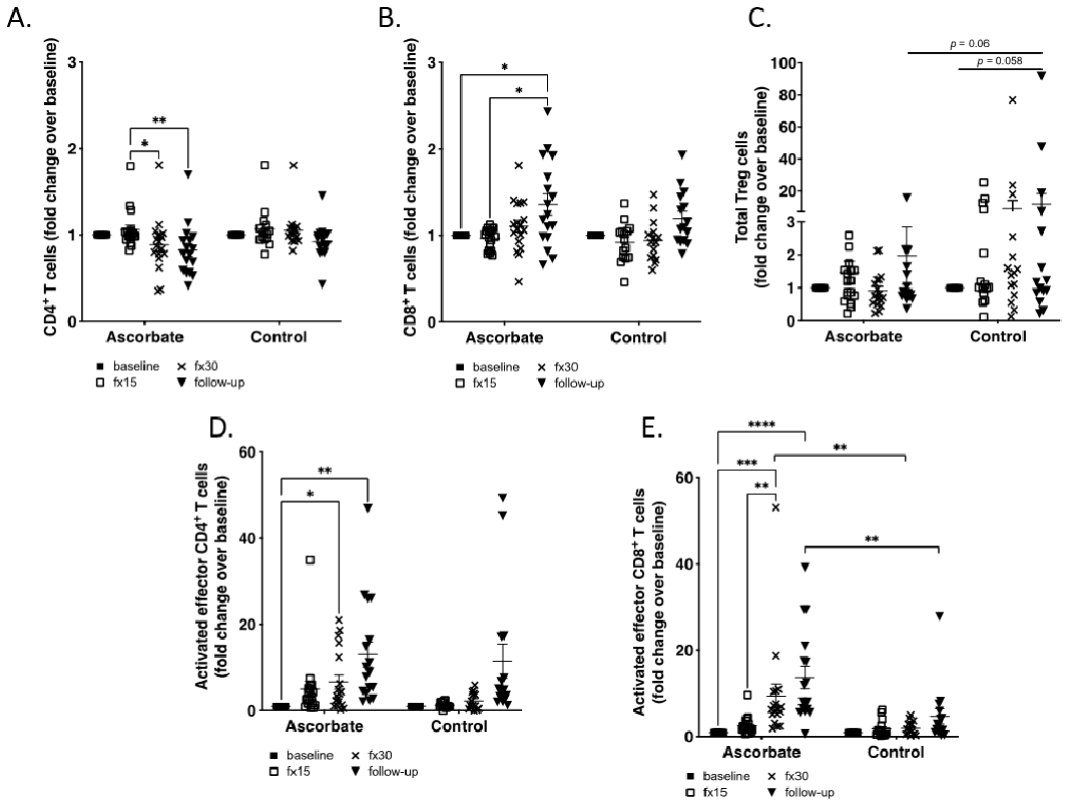


Figure 4: Unpublished data from an ongoing phase II study XACT-LUNG (NCT02905591) Analysis of peripheral blood mononuclear cells from patients receiving definitive chemoradiation with pharmacological ascorbate (P-AscH-) for unresectable stage III NSCLC in an ongoing phase II (n=24). . Control patients were enrolled in a companion study receiving definitive chemo-radiation without ascorbate (n=15).

1.7 Research hypothesis

1.7.1 Ways to improve outcomes in Stage I NSCLC:

The addition of perioperative chemotherapy has not shown a clinical benefit and may be detrimental for this patient population given associated long-term side effects. Immunotherapy with or without an immunomodulatory agent could potentially improve the outcomes in this subset of patients with NSCLC. Trials are underway to evaluate efficacy of anti-PD(L)-1 therapies in very early-stage NSCLC (NCT04317534, NCT03833154, NCT03794544, NCT03924869, NCT03634241).

1.7.2 Window of opportunity trials:

In addition, it is an optimal clinical setting to perform window of opportunity trials to evaluate pharmacodynamics of new anti-cancer or immunomodulatory agents. Pharmacological doses of ascorbate have shown promising anti-cancer effects in prior studies (Schoenfeld JD et al 2017, Welsh JL et al. 2013, Alexander MS et al. 2018, Allen BG et al 2019, Allen BG et al. 2019, Furqan M et al. 2019, Ma Y et al. 2014). Recent studies in animal models performed by Magri et al. and Luchtel et al. have shown that pharmacological ascorbate acts synergistically with immune system and control cancer growth (Luchtel RA et al 2020, Magri A et al 2020).

1.7.3 Hypothesis:

The pre-clinical and clinical data has shown that pharmacological ascorbate (P-AscH-) enhances the activation of CD8+ T cells, which are critical for mounting an anti-tumor immune response. In addition, P-AscH- may act additively or synergistically with anti-PD1/PDL-1 therapies for the treatment of NSCLC. Therefore, we hypothesized that P-AscH- along with durvalumab will mount or potentiate an anti-tumor immune response primarily in the form of increase in CD8 T cells in the tumor microenvironment.

To assess the effect of P-AscH-, we propose a descriptive, randomized, 3-arm, window of opportunity trial to enroll 36 patients (12 per arm) with newly diagnosed stage I (cT1-4cmN0M0), resectable NSCLC. Patients will receive neoadjuvant therapy as below.

Arm A: Durvalumab 1500 mg q 3 wks. X 2 cycles as monotherapy
Arm B: Combination of pharmacological ascorbate 75 grams intravenously three times a week x 4 wks. and Durvalumab 1500 mg q 3 wks. x 2 cycles
Arm C: Serve as control and patients will not receive any neoadjuvant therapy before surgery

Translational and clinical findings of this study can then be applied more broadly to design and address clinically oriented questions in various NSCLC treatment settings

1.8 Rationale for conducting this study

Anti-PD(L)-1 therapy has the potential to improve outcomes in patients with very early-stage NSCLC. Both atezolizumab and pembrolizumab have been shown to improve disease free-survival in patients with resected stage II-IIIA NSCLC. Studies are underway to evaluate the efficacy of these drugs including Durvalumab in stage I NSCLC (NCT04317534, NCT03833154,

NCT03794544, NCT03924869, and NCT03634241) alone or in combination with other drugs to improve patients' outcomes.

Pharmacological ascorbate in combination with anti-PD (L)-1 therapies and enhance T cell infiltration in the tumor microenvironment. Pharmacological ascorbate works synergistically with anti-PD (L)-1 therapies. This decreases the growth of breast, colorectal, melanoma, pancreatic, renal cell, lymphoma, and lung cancer in animal models. Compared to untreated controls, high dose ascorbate alone, or anti PD-L1 alone, the combination showed the highest levels of CD4+ and CD8+ infiltration in the tumors, in addition to elevated IFN-γ and IFN-γ-induced CXCL9, CXCL10 and CXCL11. In addition, clinical data from our ongoing trial (XACT-Lung) showed an increase in CD8 cells in the peripheral blood of patients receiving pharmacological ascorbate with standard chemo-radiation compared to control patient population.

The above pre-clinical and clinical data is very promising; however, it does not inform the pharmacodynamics of ascorbate with anti-PD (L)1 therapy in humans. Obtaining an adequate amount of pre- and post-treatment tumor tissue in patients with advanced stage NSCLC is very challenging due to the nature of the disease. In addition, patients with advanced disease routinely receive chemotherapy with immunotherapy which may muddle studying the effects of P-AscH-. In addition, chemotherapy can also cause suppression of immune system. Therefore, a window of opportunity trial is the best to characterize the effects of P-AscH- in patients with NSCLC.

1.8.1 Durvalumab monotherapy dose rationale

The PK of durvalumab has been well characterised in participants with solid tumours across the doses ranging from 0.1 to 10 mg/kg q2w, 15 mg/kg q3w and 20 mg/kg q4w in participants enrolled in the 5 Phase 3 studies (D419QC00001, D1491C00004, D419AC00001, D4191C00001 and D4193C00002), 3 Phase 2 studies (D4191C00003, D4193C00001 and D4193C00003), 1 Phase 1/2 study (CDONMEDI47361108 [Study 1108]) and 1 Phase 1 study (D4190C00002 [Japan Study 02]), following administration by IV infusion. All studies included male and female participants aged 18 years and older with advanced solid tumours. Overall, the peak and trough concentrations of durvalumab were in a similar range across studies at the same dosing regimen.

PK/Pharmacodynamic data

Based on available PK/pharmacodynamic data from ongoing Study 1108 with doses ranging from 0.1 to 10 mg/kg q2w, 15 mg/kg q3w, or 20 mg/kg q4w durvalumab exhibited nonlinear (dose-dependent) PK consistent with target-mediated drug disposition. The PK approached linearity at ≥ 3 mg/kg q2w, suggesting near complete target saturation (membrane-bound and

sPD-L1), and further shows that the durvalumab dosing frequency can be adapted to a particular regimen given the linearity seen at doses higher than 3 mg/kg. The expected half-life with doses ≥ 3 mg/kg q2w is approximately 21 days. A dose-dependent suppression in peripheral sPD-L1 was observed over the dose range studied, consistent with engagement of durvalumab with PD-L1. A low level of immunogenicity has been observed. No participants have experienced immune-complex disease following exposure to durvalumab. (For further information on immunogenicity, please see the current durvalumab IB).

Pharmacokinetic data from the D419QC00001 study evaluating the 1500 mg q3w regimen revealed the geometric mean peak concentration was 13% higher than that at 20 mg/kg and 149% higher than at 10 mg/kg following the first dose; the geometric mean steady state end of infusion concentration (trough) with repeated dosing measured at Week 12 (Day 84), was 108% higher than at 20 mg/kg q4w and 75% higher than at 10 mg/kg q2w, due to the higher equivalent dose over the same dosing period. The relative increase in dose intensity (1500 mg q3w) was selected for this study to ensure that a similar or higher PK exposure compared to 10 mg/kg q2w could be maintained throughout the dosing interval. These data confirm that the target exposure associated with clinical benefit previously established with 10 mg/kg q2w was maintained with 1500 mg q3w.

Given the similar predicted PK profile (AUC, C_{max} and trough) and the observation that both weight-based and fixed-dose regimens maintain complete sPD-L1 suppression at the achieved trough concentrations, and with available clinical data, the 20 mg/kg every 4 weeks,
1500 mg q3w and 10 mg/kg q2w regimens are expected to have similar efficacy and safety profiles

Currently approved dosing regimens include a weight-based regimen of 10 mg/kg administered q2w (urothelial cancer, non-small cell lung cancer) and a fixed dose regimen (1500 mg) administered q3w in combination with chemotherapy followed by q4w as monotherapy (extensive stage small cell lung cancer) and as monotherapy q4w (unresecatable stage III non-

small cell lung cancer). Refer to the package insert (or label) for the current approved dosing regimens (schedule and indication) for your specific country, as applicable.

Clinical data

Refer to the current durvalumab IB for a complete summary of clinical information including safety, efficacy, and PK at the 1500 mg q 3w regimen.

1.8.2 Rationale for fixed dosing

A population PK model was developed for durvalumab using monotherapy data Study 1108 (N = 292; doses = 0.1 to 10 mg/kg q2w or 15 mg/kg q3w; solid tumours). Population PK analysis indicated only minor impact of body weight on the PK of durvalumab (coefficient of ≤ 0.5). The impact of body weight-based (10 mg/kg q2w) and fixed dosing (750 mg q2w) of durvalumab was evaluated by comparing predicted steady state PK concentrations (5^{th}, median and 95^{th} percentiles) using the population PK model. A fixed dose of 750 mg was selected to approximate 10 mg/kg (based on median body weight of ~75 kg). A total of 1000 participants were simulated using body weight distribution of 40 to 120 kg. Simulation results demonstrate that body weight-based and fixed dosing regimens yield similar median steady state PK concentrations with slightly less overall between-participant variability with fixed dosing regimen.

Similar findings have been reported by others (Ng et al. 2006, Wang et al. 2009, Zhang et al. 2012). Wang and colleagues investigated 12 mAbs and found that fixed and body size- based dosing perform similarly, with fixed dosing being better for 7 of 12 antibodies (Wang et al. 2009). In addition, they investigated 18 therapeutic proteins and peptides and showed that fixed dosing performed better for 12 of 18 in terms of reducing the between-participant variability in PK/pharmacodynamic parameters (Zhang et al. 2012).

A fixed dosing approach is preferred by the prescribing community due to ease of use and reduced dosing errors. Given the expectation of similar PK exposure and variability, AstraZeneca considered it feasible to switch to fixed dosing regimens.

A fixed dose of 1500 mg durvalumab administered q3wis to be used for all participants with a body weight greater than 30 kgin combination with a fixed dose of 75 mg tremelimumab (equivalent to 1 mg/kg) q4w. Currently, the use of a fixed dose of 1500 mg durvalumab, administered both in combination with chemotherapy and as monotherapy, is approved for treatment of extensive stage small cell lung cancer; additionally the 1500 mg fixed dose is approved in some regions as monotherapy for unresectable stage III non-small cell lung cancer. Refer to the package insert (or label) for the current approved dosing regimens for your specific country, as applicable.

1.9 Benefit/risk and ethical assessment

Please see the current version of the IB for a detailed summary of the monotherapy data including AEs, serious adverse events (SAEs), and Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 to 5 events reported across the durvalumab program.

1.9.1 Potential benefits

1.9.1.1 Durvalumab

Neoadjuvant administration of anti-PD(L)-1 therapies were evaluated in the following trials:

Studies	Phase	Stage	Drug	Cycles	N	Primary endpoint	ORR (%)	MPR (%)	pCR (10%)	Grade ≥3 TRAE (%)	TR-SAE (%)
Forde et al 2018	I	I-IIIA	Nivolumab	2	21	Safety and feasibility	10	45	10	4.7	4.7
Lee JM 2021 LCMC3	II	Ib-IIIB	Atezolizumab	2	181	MPR	7	20	7	5	NR
Cascone T etl 2021 NEOSTAR	II	I-IIIA	Nivolumab vs Nivo+ Ipi	3	44	MPR	22 vs 19	24 vs 50	10 vs 38	13 vs 10	13 vs 8.6
Gao S et al 2020	IB	IA-IIIB	Sintilimab	2	40	Safety	20	40	16	10	5
NEOMUN	II	II-IIIA	Pembrolizumab	2	15	Safety	28	27	13	26	
Wislez M et al 2022 IONESCO[i]	II	IB-IIIA	Durvalumab	3	46	R0 resection	8	18	7	0	0
Cascone T et al 2022 NEOCOAST	II	IA-IIIA	Durvalumab	1	24	MPR		11.1	3.7	0	NR

As mentioned in above table, 1-3 cycles of anti-PD(L)1 therapies demonstrated 11-30% MPR and 4-16% complete pathologic response. Long term data is not available from these trials yet,

but this could translate into decreased risk of recurrence. Addition of P-AscH- may add to further improvement in the outcome.

1.9.2 Pharmacological Ascorbate

Ascorbate may improve and increase the immune infiltrate in the tumor microenvironment and increase the odds of major and/or complete pathologic response rate.

1.9.3 Overall risks

Monoclonal antibodies directed against immune checkpoint proteins, such as PD-L1 as well as those directed against PD-1, aim to boost endogenous immune responses directed against tumour cells. By stimulating the immune system, however, there is the potential for adverse effects on normal tissues.

Most adverse drug reactions seen with the immune checkpoint inhibitor class of agents are thought to be due to the effects of inflammatory cells on specific tissues. These risks are generally events with a potential inflammatory or immune mediated mechanism, and which may require more frequent monitoring and/or unique interventions such as immunosuppressants and/or endocrine therapy. These immune mediated effects can occur in nearly any organ system, and are most commonly seen as gastrointestinal AEs such as colitis and diarrhea, pneumonitis/interstitial lung disease (ILD), hepatic AEs such as hepatitis and liver enzyme elevations, skin events such as rash and dermatitis and endocrinopathies including hypo- and hyper-thyroidism

1.9.3.1 Durvalumab

Risks with durvalumab include, but are not limited to, diarrhea/colitis, pneumonitis/interstitial lung disease (ILD), endocrinopathies (ie, events of hypophysitis/hypopituitarism, adrenal insufficiency, hyper- and hypothyroidism, type I diabetes mellitus (which may present with diabetic ketoacidosis), and diabetes insipidus), hepatitis/increases in transaminases, nephritis/increases in creatinine, rash/dermatitis (including pemphigoid, myocarditis, myositis/polymyositis, immune thrombocytopenia, infusion-related reactions, hypersensitivity reactions, pancreatitis, encephalitis, serious infections, subcutaneous injection site reaction, and other rare or less frequent inflammatory events including neuromuscular toxicities (eg, Guillain-Barré syndrome, myasthenia gravis).

For information on all identified and potential risks with durvalumab please always refer to the current version of the durvalumab IB.

In monotherapy clinical studies, AEs at an incidence of ≥ 20% include events such as fatigue and decreased appetite. Approximately 10% of participants discontinued the drug due to an AE.

1.9.3.2 Pharmacological Ascorbate

Can cause diarrhea, dry mouth, nausea, vomiting, facial flushing, sweating, dizziness, headaches, chills and kidney stones. The risk of these AEs is < 10%.

The following is all Ascorbate-Related Adverse Events Occurring in All Patients on recent trial "Pharmacological ascorbate improves the response to platinum-based chemotherapy in advanced stage non-small cell lung cancer".

Toxicity	Any Grade	Grade 1	Grade 2	Grade 3
Any Toxicity	32 (80.0%)	9 (22.5%)	10 (25.0%)	13 (32.5%)
Hypertension	15 (37.5%)	1 (2.5%)	3 (7.5%)	11 (27.5%)
Dry mouth	14 (35.0%)	14 (35.0%)	0 (0%)	0 (0%)
Nausea	10 (25.0%)	7 (17.5%)	2 (5.0%)	1 (2.5%)
Hyponatremia	6 (15.0%)	6 (15.0%)	0 (0%)	0 (0%)
Arthralgia/Myalgia	3 (7.5%)	2 (5.0%)	1 (2.5%)	0 (0%)
Dizziness	3 (7.5%)	2 (5.0%)	1 (2.5%)	0 (0%)
Headache	3 (7.5%)	3 (7.5%)	0 (0%)	0 (0%)
Anorexia/Dysgeusia	2 (5.0%)	2 (5.0%)	0 (0%)	0 (0%)
Chills	2 (5.0%)	2 (5.0%)	0 (0%)	0 (0%)
Edema limbs	2 (5.0%)	0 (0%)	1 (2.5%)	1 (2.5%)
Hypocalcemia	2 (5.0%)	1 (2.5%)	1 (2.5%)	0 (0%)
Hypokalemia	2 (5.0%)	1 (2.5%)	1 (2.5%)	0 (0%)
Hypomagnesemia	2 (5.0%)	1 (2.5%)	1 (2.5%)	0 (0%)
Hypotension	2 (5.0%)	0 (0%)	2 (5.0%)	0 (0%)
Injection site reaction	2 (5.0%)	2 (5.0%)	0 (0%)	0 (0%)
Sensory neuropathy	2 (5.0%)	1 (2.5%)	1 (2.5%)	0 (0%)
Anemia	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Atrial fibrillation	1 (2.5%)	0 (0%)	0 (0%)	1 (2.5%)
Blurred vision	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Bronchopulmonary hemorrhage	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Cognitive disturbance	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Dehydration	1 (2.5%)	0 (0%)	1 (2.5%)	0 (0%)
Diarrhea	1 (2.5%)	0 (0%)	0 (0%)	1 (2.5%)
Dyspnea	1 (2.5%)	0 (0%)	1 (2.5%)	0 (0%)
Fatigue	1 (2.5%)	0 (0%)	0 (0%)	1 (2.5%)
Flushing	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Hiccups	1 (2.5%)	0 (0%)	1 (2.5%)	0 (0%)
Hypernatremia	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Increase in AST/ALT	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Infusion-related reaction	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Kidney injury	1 (2.5%)	0 (0%)	1 (2.5%)	0 (0%)
Oral dysesthesia	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Rash	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)
Sinus tachycardia	1 (2.5%)	0 (0%)	1 (2.5%)	0 (0%)
Sore throat	1 (2.5%)	0 (0%)	1 (2.5%)	0 (0%)

Toxicity	Any Grade	Grade 1	Grade 2	Grade 3
Tinnitus	1 (2.5%)	1 (2.5%)	0 (0%)	0 (0%)

1.9.3.3 Overall benefit-risk

Overall, due to the limited duration of therapy (4-weeks of neoadjuvant therapy), we expect the risk of potential adverse events to be lower. However, the combination may increase the rate of immune mediated AEs causing a potential delay in surgery or interval growth of the tumor. On occasions, these toxicities may also preclude surgery if subject health significantly declines. On the other hand, if the combination therapy is well tolerated and improved tumor immunogenicity or potentiate the immune response to durvalumab then it may improve long term outcomes. Additionally, this regimen can be exploited in many different settings of treatment in patients with NSCLC.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives and Endpoints

OBJECTIVES	ENDPOINTS
Primary	
Determine if P-AscH- and durvalumab can potentiate or enhance an immune response in the NSCLC tumor-microenvironment compared to durvalumab alone. This will be evaluated in the surgically resected specimens of patients after receiving neoadjuvant therapy.	CD8+ T cells infiltrate
Secondary	
1) Assess safety and tolerability of the combination of P-AscH- and Durvalumab in the neoadjuvant setting (Arm:2) 2) Estimate the pathologic complete response (pCR) rate 3) Estimate the major pathologic response (MPR) rate	1) Incidence of DLTs and adverse events (AEs) per CTCAE v5 2) The proportion of patients with a pathologic complete response, defined as no viable tumor.

OBJECTIVES	ENDPOINTS
4) Estimate event-free survival 5) Estimate overall survival	3) The proportion of patients with a major pathologic response, defined as residual viable tumor of 10% or less. 4) Time from randomization to progression of disease that precludes surgery, local or distant recurrence, or death due to any cause. 5) Time from randomization to death due to any cause.
Exploratory	
1) Determine other immunomodulatory effects of the combination of P-AscH- and durvalumab compared to durvalumab alone 2) Determine systemic immunomodulatory effects of the combination of P-AscH- and durvalumab compared to durvalumab alone. 3) Correlate circulating tumor(ct)DNA with time to clinical progression.	1) CD4+, T-regs, tissue-resident macrophages, and other immune cell phenotypes 2) Pre/Post changes in PBMC, cytokines and chemokines 3) ct-DNA will be compared to time to clinical progression as defined per RECIST v1.1.

3. STUDY DESIGN

3.1 Overview of study design

This is a descriptive, proof of concept, open-label, randomized, 3-arm, phase Ib run-in, window of opportunity trial to evaluate the immunomodulatory role of pharmacological ascorbate with durvalumab. Translational and clinical findings of this study can then be applied more broadly to design and address clinically oriented questions in various NSCLC treatment settings.

We plan to enroll 36 patients (12 per arm) with newly diagnosed stage I (cT1.1-4cmN0M0), resectable NSCLC.

Patients will receive neoadjuvant therapy as below.
Arm A: Two cycles of Durvalumab 1500 mg q 3 wks, as monotherapy
Arm B: Combination of pharmacological ascorbate 75 grams intravenously three times a week x 4 wks plus Durvalumab 1500 mg q 3 wks for two cycles
Arm C: Serve as control and patients will not receive any neoadjuvant therapy before going to surgery

Surgical resection will be performed during weeks 5-9 from the day of randomization. After being randomized, patients in the control arm will undergo surgical resection as per the standard of care. Surgical specimens (post-treatment resection specimens) will be analyzed for immune cell infiltrate and PD-L1 expression as described in the study's objectives and endpoints sections. Blood and serum samples will be obtained at multiple time points for the assessment of exploratory objectives. Following surgical resection, patients will receive standard-of-care treatments if/when indicated or followed for toxicities up to 90 days, and for clinical outcomes.

No dose-escalation or de-escalation is planned. Safety and tolerability of Arm B will be determined in this phase Ib run-in window of opportunity trial. The primary objective is to assess CD8 T cells in resected specimens across study arms. Given the exploratory nature and small planned sample size, a formal interim analysis of pharmacological ascorbate's immunomodulatory effect is not feasible. However, patient surgically resected samples will be analyzed in real-time for the study's primary endpoint.

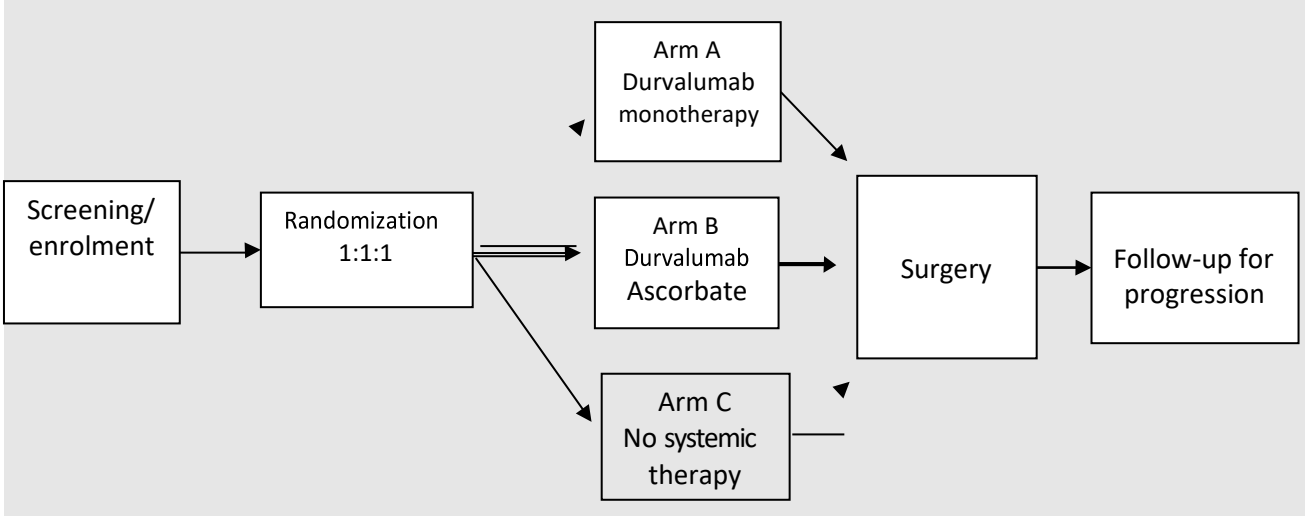
The first 3-6 subjects in Arm B will be assessed for dose-limiting toxicities. For DLT see section 6.3

If DLT rate is

Number of Patients with DLT in Arm B	**Decision Rule**
≤ 1 out of 3 subjects	Randomize *three* more patients
≤ 1 out of 6	Trial may continue as planned. The combination of P-AscH- and durvalumab is tolerable.

3.2 Study schema

Figure 5. ***Study Flow Chart***



Arm A: Two cycles of Durvalumab 1500 mg q 3 wks, as monotherapy

Arm B: Combination of pharmacological ascorbate 75 grams intravenously three times a week x 4 wks plus Durvalumab 1500 mg q 3 wks for two cycles

Arm C: Serve as control and patients will not receive any neoadjuvant therapy before going to surgery. Once randomize, they can go to surgery without any delays

After surgery, patients will be followed for 30 days & 90-days for safety evaluation. Patient will then be followed for surveillance until 3 years for progression, death or study termination whichever occurs earlier.

3.3 Rational for study design

Three-Arm Design:

Arm A: Neo-adjuvant Durvalumab: The findings of this arm will help segregate the effects durvalumab monotherapy with that of a combination of P-AscH- and durvalumab (Arm B). Patients will receive Durvalumab 1500 mg q 3 wks. x 2 cycles.

Arm B: Neo-adjuvant Durvalumab and P-AscH-: The findings on this patient population, will help segregate the effects of the combination of Durvalumab and P-AscH- with that of a Durvalumab (Arm A).

Arm C: No neo-adjuvant therapy. In this arm, patients will directly undergo surgery after randomization, and their findings will serve as a control for the comparison.

Study Population:

The rationale for the study population and the duration of treatment: Randomized patients in arms A & B will receive 4 weeks of neoadjuvant therapy. Two cycles of Durvalumab at 1500 mg dose, 3 weeks apart (Arm A & B) and 4 weeks of intravenous ascorbate infusions three times a week in Arm B along with Durvalumab. We believe 4 weeks of therapy is important as changes in circulating lymphocytes were most evident during weeks 4-6 in our previous & ongoing studies (Figure 3). Changes in peripheral blood correlate well with the tumor microenvironment as evident from the study in Sarcoma patients (results not published). This short therapy duration will be enough to analyze the immunomodulatory effects P-AscH- with durvalumab without compromising patient outcomes or causing any surgery delay. Data from two large national databases i.e., NCDB and VA (Veterans Affairs) Health System, indicated that the median time to surgery for an early-stage NSCLC from the date of diagnosis to surgery was 8 and 10 weeks, respectively (Samson P et al. 2015, Heiden BT et al. 2021) [24, 25] and this delay did not affect outcomes adversely. In addition, patients at the University of Iowa must wait an average of 6 weeks from the diagnosis to the surgery due to operating room scheduling. Therefore, we believe that neoadjuvant therapy in this trial will not delay the definitive resection significantly or compromise the patient's outcome.

This trial will enroll patients with very early-stage NSCLC irrespective of their PD-L1 expression (T1.1-4 cm, N0M0). PD-L1 is not an ideal biomarker and exhibits intra-tumoral spatial heterogeneity. In addition, patient biopsy sample may not be adequate enough to perform PD-L1 immunohistochemistry or be representative of tumor PD-L1. Prior studies e.g., Forde et al have demonstrated that patients with PD-L1 negative NSCLC, did respond to anti-PD1/PDL1 therapies in the neoadjuvant setting. In addition, we are interested in evaluating the effect of ascorbate and durvalumab in PD-L1 negative NSCLC as well. We hope to mitigate the disease heterogeneity created by various PD-L1 expressions, by randomization of patients into different study arms. The development of new combination therapies for the PD-L1 negative patient population is an unmet need.

3.4 Study oversight for safety evaluation

Study will be considered completed at its earliest, once all the subjects completed a 90-day follow up after the surgery as primary endpoint can be evaluated and all the relevant safety data documentation would have been captured. However, we plan to keep the study open until all patients complete 3-years of follow up from the surgery.

Study can also be terminated for safety reasons based on decision of Holden Comprehensive Cancer Center (HCCC) Data Safety and Monitoring Committee (DSMC) or at the discretion of the principal investigator if there is a good reason.

4. PATIENT SELECTION

Individuals of all races and ethnic groups are eligible for this trial. There is no bias towards age, gender or race in the clinical trial outlined. This trial is open to the accrual *for patients* who meet the inclusion/exclusion criteria outlined. *E*very effort to seek out and include women and minorities, the subject population is expected to be like patients with early-stage NSCLC patients seen at University of Iowa Hospitals and Clinics.

4.1 Inclusion criteria

For inclusion in the study patients must fulfill all of the following criteria:

1. Capable of giving signed informed consent which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

2. Age $\geq$ 18 years at time of study entry regardless of gender or ethnic/racial background.

3. Histologically or cytologically confirmed non-small cell lung cancer (any histology)

4. Clinical stage I with tumor size >1 cm to 4 cm (either T1b or T1c or T2a and N0 M0) according to American Joint Committee on Cancer 8th edition

5. Surgically resectable with adequate lung functions to undergo surgery as determined by thoracic surgeon.

6. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1

7. Body weight >30 kg

8. Adequate normal organ and marrow function as defined below:

   - Hemoglobin ≥8.0 g/dL
   - Absolute neutrophil count (ANC) $\geq 1.0 \times 10^9$ /L
   - Platelet count $\geq 75 \times 10^9$/L
   - Serum bilirubin ≤1.5 x institutional upper limit of normal (ULN). This will not apply to patients with confirmed Gilbert's syndrome (persistent or recurrent hyperbilirubinemia that is predominantly unconjugated in the absence of hemolysis or hepatic pathology), who will be allowed only in consultation with their physician.>>
   - AST (SGOT)/ALT (SGPT) ≤2.5 x institutional upper limit ofnormal.
   - Measured creatinine clearance (CL) ≥50 mL/min or Calculated creatinine CL≥50 mL/min by the Cockcroft-Gault formula (Cockcroft and Gault 1976) or by 24-hour urine collection for determination of creatinine clearance:

     Males:

     $$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}}$$

     Females:

     $$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age}) \times 0.85}{72 \times \text{serum creatinine (mg/dL)}}$$

9. Patient is willing and able to comply with the protocol for the duration of the study including undergoing treatment and scheduled visits and examinations including follow up.

10. Must have a life expectancy of at least 12 weeks

11. Female subjects of childbearing potential and non-sterilized male subjects who intend to be sexually active during the study must agree to use a highly effective method of contraception from the time of screening, throughout the total duration

of the drug treatment, and during the 90-day post-drug washout period.

12. Evidence of post-menopausal status or negative urinary or serum pregnancy test for female pre-menopausal patients. Women will be considered post-menopausal if they have been amenorrheic for 12 months without an alternative medical cause. The following age-specific requirements apply: Women 1 year ago, had chemotherapy- induced menopause with last menses >1 year ago, or underwent surgical sterilization (bilateral oophorectomy, bilateral salpingectomy or hysterectomy).

4.2 Exclusion criteria

Patients should not enter the study if any of the following exclusion criteria are fulfilled:

1. Participation in another clinical study with an investigational product during the last 4 weeks prior to randomization

2. Concurrent enrolment in another clinical study, unless it is an observational (non-interventional) clinical study or during the follow-up period of an interventionalstudy

3. Any unresolved toxicity NCI CTCAE Grade ≥2 from previous anticancer therapy with the exception of alopecia, vitiligo, clinically non-significant labs values (e.g., lymphopenia), and the laboratory values defined in the inclusion criteria

    a. Patients with Grade ≥2 neuropathy will be evaluated on a case-by-case basis after consultation with the Study PI

    b. Patients with irreversible toxicity not reasonably expected to be exacerbated by treatment with durvalumab may be included only after consultation with the Study PI.

4. Any concurrent chemotherapy, IP, biologic, or hormonal therapy for cancertreatment. Concurrent use of hormonal therapy for non-cancer-related conditions (e.g., hormone replacement therapy) is acceptable.

5. Radiotherapy treatment to more than 30% of the bone marrow or with a wide fieldof radiation within 4 weeks of the first dose of study drug

6. Major surgical procedure (as defined by the Investigator) within 28 days prior to the first dose of IP.

7. History of allogenic organ transplantation.

8. Active or prior documented autoimmune or inflammatory disorders (including

inflammatory bowel disease [e.g., colitis or Crohn's disease], diverticulitis [with the exception of diverticulosis], systemic lupus erythematosus, Sarcoidosis syndrome, or Wegener syndrome [granulomatosis with polyangiitis, Graves' disease, rheumatoid arthritis, hypophysitis, uveitis, etc]). The following are exceptions to this criterion:

a. Patients with vitiligo or alopecia

9. Patients with hypothyroidism (e.g., following Hashimoto syndrome) clinically stable on hormone replacement. Thyroid functions do not need to be normal for eligibility.
   a. Any chronic skin condition that does not require systemic therapy

   b. Patients without active disease in the last 2 years may be included but only after consultation with the Study PI

   c. Patients with celiac disease controlled by diet alone

   d. Patients with type 2 DM (insulin dependent or independent) are allowed.

10. Uncontrolled intercurrent illness, including but not limited to, ongoing or active infection requiring intravenous antibiotics, symptomatic congestive heart failure, uncontrolled hypertension, unstable angina pectoris, symptomatic active cardiac arrhythmia, interstitial lung disease, serious chronic gastrointestinal conditions associated with diarrhea, or psychiatric illness/social situations that would limit compliance with study requirement, substantially increase risk of incurring AEs or compromise the ability of the patient to give writteninformed consent

11. Active another invasive primary malignancy whose management can be affected by participating in the clinical trial. Patients with additional synchronous or metachronous lung cancer (other than the index NSCLC) whose management would not be affected by participating in the trial will be eligible. Patients with grounds glass or suspicious lung lesions can also be included who are planned to undergo serial imaging follow up for these suspicious lesions. Discussion in the tumor board and with the study PI is required for these cases.

12. Known to have nodal or distant metastases.

13. Mean QT interval corrected for heart rate using Fridericia's formula (QTcF) ≥470 ms

14. History of active primary immunodeficiency

15. Known history of active hepatitis infection, positive hepatitis C virus (HCV) antibody, hepatitis B virus (HBV) surface antigen (HBsAg) or HBV core antibody (anti-HBc), at screening. Participants with a past or resolved HBV infection (defined as the presence of antiHBc and absence of HBsAg) are eligible. Participants positive for HCV antibody are eligible only if polymerase chain reaction is negative for HCV RNA
16. Known to have tested positive for human immunodeficiency virus (HIV) (positive HIV 1/2 antibodies) or active tuberculosis infection (clinical evaluation that may include clinical history, physical examination and radiographic findings, or tuberculosis testing in line with local practice).
17. Current or prior use of immunosuppressive medication within 14 days before the first dose of durvalumab. The following are exceptions to this criterion:
    a. Intranasal, inhaled, topical steroids, or local steroid injections (e.g., intra articular injection)
    b. Systemic corticosteroids at physiologic doses not to exceed 10 mg/day of prednisone or its equivalent
    c. Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication)
18. Receipt of live attenuated vaccine within 30 days prior to the first dose of IP. Note: Patients, if enrolled, should not receive live vaccine while receiving IP and up to 30 days after the last dose of IP.
19. Participant that is pregnant or breastfeeding.
20. Known allergy or hypersensitivity to any of the study drugs or any of the study drug excipients.
21. Prior randomization or treatment in a previous durvalumab clinical study regardless of treatment Arm assignment.
22. Prior anti PD-1, PD-L1 and CTLA-4 therapy in the last 2 years.
    a. Experienced a toxicity that led to permanent discontinuation of prior immunotherapy.

b. All AEs while receiving prior immunotherapy must have completely resolved or resolved to baseline prior to screening for this study.

c. Experienced a ≥Grade 3 immune related AE or an immune related neurologic or ocular AE of any grade while receiving prior immunotherapy. NOTE: Patients with endocrine AE of ≤Grade 2 are permitted to enroll if they are stably maintained on appropriate replacement therapy and are asymptomatic.

d. Required the use of additional immunosuppression other than corticosteroids for the management of an AE, experienced recurrence of an AE if re-challenged, and currently require maintenance doses of > 10 mg prednisone or equivalent per day.

23. Judgment by the investigator that the patient is unsuitable to participate in the study and the patient is unlikely to comply with study procedures, restrictions and requirements.

24. Known allergy or hypersensitivity to IP or any excipient.

25. Clinically significant active infection requiring systemic therapy

Procedures for withdrawal of incorrectly enrolled patients are presented in Section 4.3

4.3 Withdrawal of patients from study treatment and/or study

It is important to discriminate between discontinuation of investigational product only and withdrawal from study (investigational product **and** follow up).

Permanent discontinuation of durvalumab or Ascorbate

Discontinuation of study treatment, for any reason, does not impact the patient's participation in the study. A patient who decides to discontinue IP will always be asked about the reason(s) for discontinuation and the presence of any AE. The patient should continue attending subsequent study visits, and data collection should continue according to the study protocol. If the patient does not agree to continue in-person study visits, a modified follow-up must be arranged to ensure the collection of endpoints and safety information. This follow-up could be a telephone contact with the patient, a contact with a relative or treating physician, or information from medical records. The approach taken should be recorded in the medical records. A patient that agrees to modified follow-up is not considered to have withdrawn consent or to have withdrawn from the study.

Patients who are permanently discontinued from further receipt of IP, regardless of the reason, will be identified as having permanently discontinued treatment. These patients will enter follow-up.

Patients who permanently discontinue drug for reasons other than progression, can undergo imaging studies indicated and data will be captured to evaluate disease progression or death (whichever comes first).

All patients will be followed for survival until the end of the study.

Lost to follow-up

Patients will be considered lost to follow-up only if no contact has been established by the time the study is completed, such that there is insufficient information to determine the patient's status at that time. Patients who refuse to continue participation in the study, including telephone contact, should be documented as "withdrawal of consent" rather than "lost to follow-up." Investigators should document attempts to re-establish contact with missing patients throughout the study period. If contact with a missing patient is re-established, the patient should not be considered lost to follow-up and evaluations should resume according to the protocol.

In order to support key end points of PFS and OS analyses, the survival status of all patients in the full analysis and the safety analysis sets should be re-checked, this includes those patients who withdrew consent or are classified as "lost to follow up."

- Lost to Follow up – site personnel should check hospital records, the patients' current physician, and a publicly available death registry (if available) to obtain a current survival status. (The applicable CRF modules will be updated.)

- In the event that the patient has actively withdrawn consent to the processing of their personal data, the survival status of the patient can be obtained by site personnel from publicly available death registries (if available) where it is possible to do so under applicable local laws to obtain a current survival status. (The applicable CRF modules will be updated.)

Withdrawal of consent

Patients are free to withdraw from the study at any time (IP and assessments) without prejudice to further treatment.

Patients who withdraw consent for further participation in the study will not receive any further IP or further study observation, with the exception of follow-up for survival, which will continue until the end of the study unless the patient has expressly withdrawn their consent to survival follow-up. Note that the patient may be offered additional tests or tapering of treatment to withdraw safely.

A patient who withdraws consent will always be asked about the reason(s) for withdrawal and the presence of any AE. The Investigator will follow up AEs outside of the clinical study.

If a patient withdraws consent, they will be specifically asked if they are withdrawing consent to:

- All further participation in the study including any further follow up (eg, survival contact telephone calls)
- Withdrawal to the use of any samples

4.4 Replacement of patients

Patients will be replaced, if incorrectly enrolled/randomized, could not undergo surgery, or received significantly less neoadjuvant therapy than planned (For A & B: At least 1 dose of Durvalumab is required, For Arm B: at least 60% of ascorbate doses are required to have an impact on immune system)

5. INVESTIGATIONAL PRODUCT(S)

5.1 Durvalumab

5.1.1 Formulation/packaging

Durvalumab will be supplied by AstraZeneca as a concentrate for solution for infusion. Durvalumab 500 mg will be supplied in a vial containing 50 mg/mL durvalumab, 26 mM histidine/histidine hydrochloride, 275 mM trehalose dihydrate, and 0.02% weight/volume (w/v) polysorbate 80; the solution has a pH of 6.0 and density of 1.054 g/mL. The label-claim volume for durvalumab is 10 mL.

5.1.2 Durvalumab doses and treatment regimens

5.1.3 Study drug preparation

Patients in the durvalumab monotherapy (Arm A) treatment group will receive 1500 mg durvalumab via IV infusion Q3W for up to 2 doses/cycles unless there is unacceptable toxicity, withdrawal of consent, or another discontinuation criterion is met. (If a patient's weight fallsto 30 kg or below (≤30 kg) the patient should receive weight-based dosing equivalent to 20 mg/kg of durvalumab Q3W until the weight improves to >30 kg, at which point the patient should start

receiving the fixed dosing of durvalumab 1500 mg Q3W).

Durvalumab + P-AscH- combination therapy arm (Arm B)

Patients will receive 1500 mg durvalumab via IV infusion q3w for up to two doses/cycles or until progression or other treatment discontinuation criteria are met.

5.1.4 Storage of Investigational Products

Durvalumab vials are stored at 2°C to 8°C (36°F to 46°F) and must not be frozen. Investigational product must be kept in original packaging until time of preparation to prevent prolonged light exposure.

The dose of durvalumab for administration must be prepared by the pharmacy staff members delegated by the Investigator (or an appropriate designee trained in study drug preparation), using aseptic technique in compliance with local regulations and site requirements. Total time from needle puncture of the durvalumab vial to the start of administration must not exceed:

- 24 hours at 2°C to 8°C (36°F to 46°F)
- 4 hours at room temperature.

If the final product is stored at both refrigerated and ambient temperatures, the total time must not exceed 24 hours, otherwise a new dose must be prepared from new vials (that is, the individual storage time limits are not additive). Durvalumab does not contain preservatives; any unused portion of the vial must be discarded immediately after use.

Table 1 Investigational Product Storage

Investigational Product		Storage Temperature	Duration
Durvalumab	Vial (500 mg/vial)	2°C to 8°C (36°F to 46°F)	Within the individually assigned expiry date on the label
	Prepared IV bag (needle puncture of vial to start of administration)	Room temperature	4 hours
		2°C to 8°C (36°F to 46°F)	24 hours

5.1.4.1 Durvalumab Infusion Preparation and Administration

Preparation

Durvalumab is a sterile, clear to opalescent, colorless to slightly yellow solution, free from visible particles.

Each vial selected for dose preparation should be inspected. If there are any defects noted with the Investigational Product, the investigator and site monitor should be notified immediately.

The use of elastomeric pumps and pneumatic tube transport with durvalumab have not been studied, please contact AstraZeneca before using.

A dose of 1500 mg (for patients >30 kg in weight) will be prepared using an IV bag containing 0.9% sodium chloride for injection or 5% dextrose for injection. Add 30 mL (i.e. 1500 mg) of durvalumab to the IV bag. The IV bag size should be selected such that the final concentration is within 1 to 15 mg/mL. Mix the bag by gently inverting to ensure homogeneity of the dose in the bag.

If patient weight falls to ≤30 kg, weight-based dosing at 20 mg/kg will be prepared using an IV bag size selected such that the final concentration is within 1 to 15 mg/mL

Administration

Durvalumab will be administered prior to Ascorbate infusions for patients randomized in Arm B.

Durvalumab infusions are to be administered through an IV administration set with a 0.2 or 0.22 μm filter; acceptable configurations include an IV set containing an in-line filter or the attachment of a separate filter to the distal end of the IV tubing.

The durvalumab infusion time is 1 hour ± 10 minutes; however, if there are interruptions, the total allowed time must not exceed 8 hours with the infusion bag maintained at room temperature, otherwise a new dose must be prepared from new vials.

Do not co-administer other drugs through the same infusion line.

The IV line will be flushed with a volume equal to the IV line volume, according to local practices to ensure the full dose is administered. Infusion time does not include the final flush time.

5.1.5 Duration of treatment and criteria for treatment through progression and for retreatment

All treatment will be administered beginning on Cycle 1 Day 1 for up to 2 doses for durvalumab monotherapy Arm A and combination Arm B or until progression, unacceptable toxicity, withdrawal of consent, or another discontinuation criterion is met.

After 2 doses of durvalumab for patients in Arm A and 4 weeks of therapy in Arm B, patients will undergo surgical resection during week 5-9. After surgery, patients may receive FDA approved therapies or treatment per NCCN (National Comprehensive Cancer Network) guideline based on pathologic findings if their tumor found to be greater than 4 cm or positive for lymph nodes. Otherwise, they will be followed according to the protocol.

Patients who develop significant toxicities requiring dose delay or discontinuation will not receive the 2nd cycle/dose of durvalumab. Toxicities will be managed per standard guidelines as in Appendix 1. Effort will be made for this patient to undergo surgery as soon as feasible considering it is safe. Rechallenge/re-treatment with durvalumab is not allowed if patient develops significant toxicities.

Patients who develop interim progression during neo-adjuvant therapy phase will be treated as standard of care as decided by treating physician/team.

Crossover within the study will not be permitted.

Patients who AstraZeneca and the Investigator determine may not continue treatment after disease recurrence or progression will be followed up for survival or until study closure.

5.1.6 Monitoring of dose administration

Patients will be monitored before, during and after the infusion as per institutional standards.

In the event of a ≤Grade 2 infusion-related reaction, the infusion rate of study drug may be decreased by 50% or interrupted until resolution of the event (up to 4 hours) and re-initiated at 50% of the initial rate until completion of the infusion. For patients with a ≤Grade 2 infusion related reaction, subsequent infusions may be administered at 50% of the initial rate. Acetaminophen and/or an antihistamine (e.g., diphenhydramine) or equivalent medications per institutional standard may be administered at the discretion of the investigator. If the infusion related reaction is Grade 3 or higher in severity, study drug will be discontinued. Standard infusion time is 1 hour ± 10 minutes; however, if there are interruptions, the total allowed time must not exceed 8 hours with the infusion bag maintained at room temperature, otherwise a new dose must be prepared from new vials. For management of patients who experience an infusion reaction, please refer to the toxicity and management guidelines in Appendix 1. As with any antibody, allergic reactions to dose administration are possible. Appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis. The study site must have immediate access to emergency resuscitation teams and equipment in addition to the ability to admit patients to an intensive care unit if necessary.

5.1.7 Accountability, Dispensing and Disposition of unused investigational study drug

The site will account for all investigational study drug accountability, dispensing and destruction of unused drug per institutional workflow & standards.

5.2 Ascor ® (Ascorbic Acid)

5.2.1 Formulation/packaging/storage

IND 137968 allows ascorbate to be obtained through McGuff Pharmaceuticals. Their product

Ascor 500® is available in sterile single-use 50 mL vials containing 25 g of ascorbic acid (500 mg/mL). Unopened vials of are stable until the expiration date indicated on the package when stored between 2° – 8°C (36°–46°F). Protect from light and store in the carton until time of use.

5.2.2 Doses and treatment regimens

Patients will be administered 75 g of Ascorbate three times a week during week 1 through 4 – i.e., up to 12 infusions. Minimum of two doses should be given/week if C1 is started in the middle of week, or due to inclement weather or holidays. Ascorbate infusions can be given any time of the week, either daily sequentially or with some gap depending upon feasible for the patient.

Ascorbate is considered a CYP4503A4 inducer especially in men (Rolf PG 2005) [26] Therefore, close monitoring of subjects who may be concomitantly receiving CYP3A4 substrates with narrow therapeutic indexes for toxicities is required. A table of CYP3A4 substrates, inhibitors, and inducers is provided in the Appendix. Ascorbate may decrease plasma/blood concentrations of substrates (Appendix 2). Ascorbate may increase CYP3A4 induction (Appendix 2).

5.2.3 Preparation and administration

Infusions (Arm B only) will be administered at a dose of 75 g over 120 minutes (+/- 10min) if mixed in sterile water or 150 minutes (+/- 10min) if mixed in D5W
Recommended infusions time is 120 minutes (150 minutes if mixed in D5W), however due to interruptions e.g., infusion reaction, it can be given over a longer duration after consulting with treating physician. Changes in infusion rates should be recorded along with reason.

Ascorbate dose (500 mg / mL)	**Total volume***	**Osmolarity (mOsm/L)§**	**Infusion Time**
Recommended Infusion Times for Ascorbic Acid mixed with Sterile Water			
75g (150 mL)	1000 mL	851.7	120 minutes +/- 10 min
Recommended Infusion Times for Ascorbic Acid mixed with D5W			
75g (150 mL)	1500 ml	817	150 minutes +/- 10 min

† Provided by Drug Information Center at the University of Iowa, October 2024

* Sterile water or D5W for injection only.

§ Theoretical calculations provided by Drug Information Center; targeted osmolarity range is 500 – 900 mOsm/L

5.2.4 Monitoring of dose administration

Patients will be monitored for blood pressure at the start and end ofinfusion. Patient's BP need to be below 150/90 to receive the infusion. If there is increase in BP during or after infusion to 170/100 mm Hg, then infusion may be paused temporarily, or patient can be given an antihypertensive. After infusion, patient can be discharged if BP is < 160/100 mmHg.

In the event of a ≤Grade 2 infusion-related reaction, the infusion rate may be decreased by 25-50% or interrupted until resolution of the event of study drug may be decreased by 50% or interrupted until resolution of the event (up to 4 hours) and re-initiated at 50% of the initial rate until completion of the infusion. If the infusion related reaction is Grade 3 or higher in severity, study drug will be discontinued. Standard infusion time is 2 hours (150 minutes if mixed in D5W), however if there are interruptions, the total allowed time must not exceed 8 hours at room temperature (otherwise requires new infusion preparation). For management of patients who experience an infusion reaction, please refer to the toxicity and management guidelines in Appendix 1

Durvalumab will be administered prior to P-AscH- infusions for patients in Arm B.

5.2.5 Accountability, dispensation and disposition of Ascorbate:

The site will account for all investigational study drug accountability, dispensing and destruction of unused drug per institutional workflow & standards.

5.2.6 Toxicities and Cautions:

Diarrhea, nausea and/or vomiting, kidney stones, dry mouth/thirst, headache, abdominal pain, fatigue, facial flushing, sweating, weakness, injection site irritation, and faint/dizzy (after rapid infusion), electrolyte abnormalities and transient hypertension (tHTN) have been reported in the literature. Besides tHTN, all of these toxicities occur in < 10% of patients.

For overlapping toxicities both drugs can be held per investigator discretion or one after the other if of the agent is more likely to have caused the adverse event.

Unreliable laboratory tests

Finger-stick glucometers report high-dose ascorbate erroneously as glucose levels. Patient who require insulin dosing based on finger-stick glucose levels will be informed about the risk.. Urinary dipstick tests are also affected. The FDA-approved prescribed information also notes that laboratory tests based on oxidative/reductive reactions may also be affected.

Ferritin & iron levels

Intravenous ascorbate is likely related to an elevation in ferritin and iron levels. Subjects who have experienced these elevations have remained asymptomatic. It is possible that chronic increased ferritin and iron levels may affect other organs. Treating physicians will

evaluate any necessary work-up based on changes in Ferritin and Iron levels.

6. TREATMENT PLAN

6.1 Patient enrollment & randomization

The study will enroll patients that are seen at the University of Iowa Hospitals and Clinics (UIHC). The adult patients with stage I (according to American Joint Committee on Cancer Edition 8th), surgically resectable NSCLC will be approached to participate in the trial.

Recruitment plan: New patients with very-early-stage NSCLC, who are scheduled to be seen either at the thoracic surgery clinics, pulmonary oncology clinics, and/or thoracic medical oncology clinics will be identified by the clinical team or study team including physicians, research coordinators and clinic coordinators after reviewing their records. Member of the study team will present the study to potential patients to evaluate their interest. Patients may be identified through tumor board review or can be referred from other facilities.

If interested, the patient will be consented to and complete the screening work-up to determine eligibility.

Once informed consent is signed:

- Document consent in accordance with institutional policies and good clinical practice. Note time of consent.
- Register subject in OnCore clinical trials management system
- Upload consent and consent documentation into OnCore
- Complete post-consent screening procedures
- Research team physician or principal investigator must review and sign off on eligibility
- Upload scanned copy of completed and signed eligibility checklist in Oncore along with all source documentation supporting eligibility requirements

Once eligibility is confirmed, the patient will be randomized to one of the three-study arms. Permuted block randomization with a 1:1:1 allocation ratio will be used. Study is open-label and study team, treating staff or patients will not be blinded about intervention.

Arm A: Durvalumab monotherapy
Arm B: Durvalumab + Ascorbate
Arm C: No neo-adjuvant therapy (control arm)

Subjects must begin therapy within 7 days of randomization.

6.2 Dosage and administration

Protocol should not supersede standard of care procedures for patient safety.

Arm A: Durvalumab 1500 mg intravenous (IV) infusion every 3 weeks for 2 cycles.

Arm B: Durvalumab 1500 mg IV infusion every 3 weeks for 2 cycles + Ascorbate IV infusions 75 g three times a week for 4 weeks starting from cycle 1 day 1

Arm C: No neoadjuvant therapy. Patients will undergo surgery without waiting until week 5 and the date depends on OR/scheduling logistics and must be within 9 weeks of randomization.

Durvalumab 1500 mg will be administered as 60-minute intravenous (IV) infusion (±10 min) every 3 weeks for 2 cycles to patients in Arm A and B.

Ascorbate Infusions (Arm B only) will be administered at a dose of 75 gm over 120 minutes (+/-10min) if mixed in sterile water and 150 minutes (+/- 10 min) if mixed with D5W. Recommended infusions time is 120 minutes (150 minutes if mixed with D5W), however due to interruptions e.g., infusion reaction, it can be given over a longer duration after consulting with treating physician. Changes in infusion rates should be recorded along with reason.

Order of Administration: Only applies to Arm B. Durvalumab will be administered prior to P-AscH- infusions.

Surgical resection will be performed during weeks 5-9 from the day of randomization (Arms A and B). After being randomized, patients in Arm C will undergo surgical resection as per the standard of care. Surgical specimens (post-treatment resection specimens) will be analyzed for immune cell infiltrate and PD-L1 expression as described in the study's objectives and endpoints sections. Blood and serum samples will be obtained at multiple time points for exploratory objectives. Following surgical resection, patients will receive standard-of-care treatments if/when indicated and followed for toxicities up to 90 days. If patient started on a treatment before 90 days, then toxicity follow up will be prior to start of the treatment. Subsequently they will enter long-term follow up.

Once 3 and 6 patients are randomized to Arm B, DLT assessment will be performed (See section 6.3) . Additional patients will be randomized to make sure there are 3-6 DLT evaluable subjects in Arm B to evaluate interim safety (See section: 11)

No dose-escalation or de-escalation is planned. Safety and tolerability of Arm B will be determined in this phase Ib-run-in window of opportunity trial.

The primary objective is to assess CD8 T cells in resected specimens across study arms. Given the exploratory nature and small planned sample size, a formal interim analysis of pharmacological ascorbate's immunomodulatory effect is not feasible. However, patient surgically resected samples will be analyzed in real-time for the study's primary endpoint

6.3 Definition of DLT

Dose-limiting toxicities (DLTs) will be evaluated for 1st six DLT evaluable subjects in Arm B only. The period for evaluating DLTs will be 21-days from the time of first administration of durvalumab. Patients who do not remain on the study up to this time for reasons other than DLT will be replaced with another patient. Grading of DLTs will follow the guidelines provided in the Common Terminology Criteria for Adverse Events (CTCAE) version 5

Rule for the DLTs will be as below:

If DLT rate is

Number of Patients with DLT in Arm B	Decision Rule
≤ 1 out of 3 evaluable subjects	Randomize *three* more patients
≤ 1 out of evaluable 6	Trial may continue as planned. The combination of P-AscH- and durvalumab is tolerable.

A DLT is defined as the occurrence of an adverse event (AE) that is **at least possibly related to investigational regimen (IR – combination of durvalumab and ascorbate)**, with two exceptions: any grade of vitiligo or alopecia will not qualify as a DLT. AEs that are **at least possibly related to durvalumab- and/or Ascorbate** shall be assessed as DLTs if they meet any of the following criteria:

Any Death

Hematologic toxicity:

- Grade ≥3 neutropenia complicated by fever >38.3°C
- Grade 4 neutropenia (lasting more than 7 days)
- Grade ≥3 thrombocytopenia with significant bleeding
- Grade 4 thrombocytopenia (regardless of duration)

- Grade 4 anemia (regardless of duration)

Non-hematologic toxicity:

- Any Grade 4 non-immune-mediated AE
- Any Grade 4 immune-mediated AE, excluding endocrinopathies
- Any Grade 3 non-immune mediated AE that does not resolve to ≤Grade 1 orbaseline within 30 days with optimal medical management
- Any Grade 3 immune-mediated AE – excluding diarrhea/colitis, pneumonitis, hepatitis, rash, neurotoxicity, myocarditis, myositis/polymyositis, endocrinopathies and nephritis – that does not resolve to ≤Grade 1 or baseline within 30 days after onset of the event despite optimal medical management including systemic corticosteroids
- Grade 3 diarrhea or colitis that does not resolve to ≤Grade 1 within 14 days [*both* immune- *and* non-immune-mediated indicated here; the same is the case if not specified in remaining bullet points below]
- Grade 3 noninfectious pneumonitis
- Grade 2 noninfectious pneumonitis that does not resolve to ≤Grade 1 within 14 days of the initiation of maximal supportive care
- **Aspartate** aminotransferase (AST) or **alanine** aminotransferase (ALT) ≥3×ULN with concurrent increase in total bilirubin (TBL) ≥2×ULN without evidence of cholestasis or alternative explanations (e.g., viral hepatitis, disease progression in the liver; i.e., "Hy's Law")
- ALT or AST >8×ULN or TBL>5×ULN
- Grade 3 immune-mediated rash that does not resolve to ≤Grade 1 or baseline within30 days
- Grade 2 rash covering > 30% BSA that does not resolve to ≤Grade 1 or baseline within30 days
- Any grade of immune-mediated rash with bullous formation
- Grade 3 immune-mediated neurotoxicity (excluding Guillain-Barre and myasthenia gravis) that does not resolve to ≤Grade 1 within 30 days
- Grade 2 or 3 immune-mediated peripheral neuromotor syndrome (such as Guillain-Barre and myasthenia gravis) that does not resolve to ≤Grade 1 within 30 days or that exhibits signs of respiratory insufficiency or autonomic instability
- Grade 3 immune-mediated myocarditis
- Any symptomatic immune-mediated myocarditis that does not become asymptomatic within 3 days of initiating optimal medical management including systemic corticosteroids
- Grade 2 or 3 immune-mediated myositis/polymyositis that does not resolve to Grade ≤1 within 30 days of initiating optimal medical management including systemic corticosteroids or that exhibits signs of respiratory insufficiency regardless of optimal medical management
- Immune-mediated increase in creatinine >3×ULN, or >3×baseline for patients witha baseline creatinine elevated above ULN

Any treatment-related toxicities that first occurred during the DLT period must be followed for resolution to determine if the event qualifies as a DLT as specified in the DLT criteria above.

DLTs will be counted based on the number of subjects with DLT, not the absolute number of DLTs. No single subject can trigger more than one DLT event.

Abnormal laboratory values or diagnostic test results constitute AEs only if they induce clinical signs or symptoms or require treatment or further diagnostic tests. Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertionof central line) should not be recorded as an AE.

Immune-related AEs are defined as AEs of an immune nature (i.e., inflammatory) in the absence of a clear alternative etiology. In the absence of a clinically significant abnormality, repeat laboratory testing will be conducted to confirm significant laboratory findings prior to designation as a DLT.

6.4 Toxicity management guidelines

6.4.1 Durvalumab

Guidelines for the management of immune-mediated reactions, infusion-related reactions, and non-immune-mediated reactions for durvalumab are provided in the durvalumab Toxicity Management Guidelines (TMGs). Please see section Appendix 1

Patients should be thoroughly evaluated, and appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the imAE. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an imAE diagnosis. In the absence of a clear alternative etiology, events should be considered potentially immune related.

If durvalumab is held due to treatment-related toxicity, ascorbate may continue as scheduled. If attribution of toxicity is unclear, then any therapy can be withheld and once attribution to an agent is determined by the investigator, other therapy may resume per study calendar.

There will be no dose modifications for durvalumab. Adverse events will be managed as described in detail in Appendix 1 “Management of Durvalumab-Specific Adverse Events”.

If a dose of durvalumab needed to be withheld for a durvalumab related toxicity (Grade ≥ 3 or persistent G2), further dosing with durvalumab will not be performed or it will not be

rechallenged as there are only 2 scheduled doses of Durvalumab in this study. This is to avoid delays in definitive therapy i.e., surgical resection.

These guidelines in Appendix 1 have been prepared to help the Investigator exercise his/her clinical judgment in treating these types of toxicities. These guidelines apply to AEs considered causally related to durvalumab monotherapy by the reporting investigator

Dose reductions are not permitted. All toxicities will be graded according to NCI CTCAE v5.

6.4.2 Ascor (Ascorbic Acid)

Ascorbate infusions can cause dry mouth, nausea and tHTN. Most toxicities are reversible and non-overlapping with durvalumab. Optimal medical management is advised to control symptoms, especially blood pressure. For uncontrolled toxicities ascorbate infusions can be interrupted/held or missed until toxicities can be resolved or controlled.

There will be no dose modification for Ascorbate, but it can be delayed depending on the toxicities. If Grate 3-4 ascorbate related toxicities occur, then it will not be administered until the toxicity is resolved to G1 or if a new baseline is achieved. Ascorbate does not need to be withheld for electrolyte or lab only abnormalities if electrolyte issue can be corrected concurrently.

There are 3 planned doses of ascorbate/week. If a patient can't receive all the three doses due to Ascorbate related toxicities during that week, then the missed infusions will not be made up in the following weeks.

If ascorbate can't be administered due to related-toxicities, doses will not be made up to avoid delays in the surgery.

7. RESTRICTIONS DURING THE STUDY AND CONCOMITANT TREATMENT(S)

7.1 Restrictions during the study

The following restrictions apply while the patient is receiving study treatment and for the specified times before and after:

Female patient of child-bearing potential

- Female patients of childbearing potential who are not totally sexuallyabstinent (i.e., refraining from heterosexual intercourse during the entire period of risk

associated with study interventions) and intend to be sexually active with a non sterilized male partner must use at least 1 **highly** effective method of contraception (Table 2) They should have been stable on their chosen method of birth control for a minimum of 3 months before entering the study and continue to use it throughout the total duration of the drug treatment and the drug washout period (90 days after the last dose of durvalumab). Non-sterilized male partners of a female patient of childbearing potential must use male condom plus spermicide throughout this period. Cessation of birth control after this point should be discussed with a responsible physician. Periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of birth control. Female patients should also refrain from breastfeeding throughout this period.

Male patients with a female partner of childbearing potential

- Non-sterilized male patients who are not abstinent and intend to be sexually active with a female partner of childbearing potential must use a male condom plus spermicide from the time of screening throughout the total duration of the drug treatment and the drug washout period (90 days after the last dose of durvalumab monotherapy). However, periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception. Male patients should refrain from sperm donation throughout this period. Vasectomized males are considered fertile and should still use a male condom plus spermicide as indicated above during the clinical study.

- Even if the female partner is pregnant, male participants should still use a condom plus spermicide (where approved), as indicated above during the clinical study, if there is a concern about damaging the developing fetus from drug in ejaculate.

- Female partners (of childbearing potential) of male patients must also use a highly effective method of contraception throughout this period (Table 2).

Females of childbearing potential are defined as those who are not surgically sterile (ie, bilateral salpingectomy, bilateral oophorectomy, or complete hysterectomy) or post- menopausal.

Women will be considered post-menopausal if they have been amenorrheic for 12 months without an alternative medical cause. The following age-specific requirements apply:

- Women <50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatments and if they have luteinizing hormone and follicle-stimulating hormone levels in the post-menopausal range for the institution.

- Women ≥50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatments, had radiation-induced menopause with last menses >1 year ago, had chemotherapy-induced menopause with last menses >1 year ago.

Highly effective methods of contraception, defined as one that results in a low failure rate (ie, less than 1% per year) when used consistently and correctly are described in Table 2. Note that some contraception methods are not considered highly effective (e.g. male or female condom with or without spermicide; female cap, diaphragm, or sponge with or without spermicide; non-copper containing intrauterine device; progestogen-only oral hormonal contraceptive pills where inhibition of ovulation is not the primary mode of action [excluding Cerazette/desogestrel which is considered highly effective]; and triphasic combined oral contraceptive pills).

Table 2 Highly Effective Methods of Contraception (<1% Failure Rate)

• Non hormonal Methods	• Hormonal Methods
• Total sexual abstinence (evaluate in relation to the duration of the clinical study and the preferred and usual lifestyle choice of the participant) • Vasectomized sexual partner (with participant assurance that partner received post-vasectomy confirmation of azoospermia) • Tubal occlusion • Intrauterine device (provided coils are copper-banded)	• Implants: Etonogestrel-releasing implants (e.g. Implanon® or Norplant®) • Intravaginal devices: Ethinylestradiol/etonogestrel-releasing intravaginal devices (e.g. NuvaRing®) • Injection: Medroxyprogesterone injection (e.g. Depo-Provera®)[a] • Combined Pill: Normal and low dose combined oral contraceptive pill • Patch: Norelgestromin/ethinylestradiol-releasing transdermal system (e.g. Ortho Evra®) • Mini pill: Progesterone based oral contraceptive pill usingdesogestrel: Cerazette® is currently the only highly effective progesterone-based • Levonorgestrel-releasing intrauterine system (e.g., Mirena®)[a]

[a] Hormonal methods not prone to drug-drug interactions.

Blood donation

Patients should not donate blood while participating in this study or for at least 90 days following the last infusion of durvalumab or 90 days after receipt of the final dose of durvalumab.

7.2 Concomitant treatment(s)

Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded in the medical record.

Throughout the study, investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care. All prescription and nonprescription concomitant medication should be reconciled, on an ongoing basis at enrollment, as well as changes in such concomitant medication, and any new concomitant

medication taken while the subject is on study, should be recorded in the medical record.

Fluid intake. Subjects will be encouraged to maintain adequate hydration to decrease the risk of nephrolithiasis from pharmacological ascorbate. Those unable to maintain oral hydration should be considered for supplemental IV hydration per institutional care guidelines.

For diabetics, be aware high-dose ascorbate can provide false results for finger-stick glucose readings. This effect is dose dependent but can last up to 6h post-infusion. Serum/plasma tests are not affected (Vasudevan S et al 2014) [27]

Allowed Concomitant Medications and Vaccinations

All treatments 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. Please see Appendix 2 for reference about concomitant therapy with ceralasertib. Following therapies are allowed as needed, - Prophylactic or therapeutic anticoagulation therapy - Inactivated influenza vaccinations, inactivated shingles vaccine and pneumonia vaccine. Other inactivated vaccines may be administered as needed (e.g., COVID-19). - Mineralocorticoids (e.g., fludrocortisone) - Use of short course of systemic corticosteroids for COPD or asthma exacerbation and contrast allergy is allowed. Inhaled corticosteroids, topical or intra-articular use are also allowed as these are not considered systemic steroids. - Physiologic dose of corticosteroids administered for orthostatic hypotension or adrenocortical insufficiency

7.2.1 Permitted concomitant medications

Table 3 Supportive Medications

Supportive medication/class of drug:	Usage:
Concomitant medications or treatments (e.g., acetaminophen or diphenhydramine) deemed necessary to provide adequate prophylactic or supportive care, except for those medications identified as "prohibited," as listed below in Table 4	To be administered as prescribed by the Investigator

Best supportive care (including antibiotics, nutritional support, correction of metabolic disorders, optimal symptom control, and pain management [including palliative radiotherapy to non-target lesions, etc.])	Should be used, when necessary, for all patients
Inactivated viruses, such as those in the influenza vaccine or COVID	Permitted

7.2.2 Excluded concomitant medications

Table 4 Prohibited Concomitant Medications

Prohibited medication/class of drug:	Usage:
Any investigational anticancer therapy other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
mAbs against CTLA-4, PD-1, or PD-L1 other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
Any concurrent chemotherapy, radiotherapy, immunotherapy, or biologic or hormonal therapy for cancer treatment other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment. (Concurrent use of hormones for non-cancer-related conditions [e.g., insulin for diabetes and hormone replacement therapy] is acceptable.)
Immunosuppressive medications including, but not limited to, systemic corticosteroids at doses exceeding 10 mg/day of prednisone or equivalent, methotrexate, azathioprine, and tumor necrosis factor-α blockers	Should not be given concomitantly or used for premedication prior to the immunotherapy infusion. The following are allowed exceptions: • Use of immunosuppressive medications for the management of IP-related AEs, • Short-term premedication for infusion-related reaction is allowed. • Use in patients with contrast allergies. • In addition, use of inhaled, topical, and intranasal corticosteroids is permitted. A temporary period of steroids will be allowed if clinically indicated and considered to be essential for the management of non-immunotherapy relatedevents experienced by the patient (e.g., chronic obstructive pulmonary disease, radiation, nausea, etc.).

Prohibited medication/class of drug:	Usage:
EGFR TKIs	Should not be given concomitantly. Should be used with caution in the 90 days post last dose of durvalumab. Increased incidences of pneumonitis (with third generation EGFR TKIs) and increased incidence of transaminase increases (with 1st generation EGFR TKIs) has been reported when durvalumab has been given concomitantly.
Live attenuated vaccines	Should not be given through 30 days after the last dose of IP (including SoC)
Herbal and natural remedies which may have immune-modulating effects	Should not be given concomitantly unless agreed by the sponsor

Abbreviations: AE Adverse event; CTLA-4 Cytotoxic T-lymphocyte-associated antigen-4; EGFR Epidermal growth factor receptor; mAb Monoclonal antibody; PD-1 Programmed cell death 1; PD-L1 Programmed cell death ligand 1; TKI Tyrosine kinase inhibitor; TL Target lesion.

Unreliable laboratory tests

Finger-stick glucometers report high-dose ascorbate erroneously as glucose levels. For this reason, individuals relying on finger-stick glucose levels for their health will only be enrolled after discussion with principal investigator. Urinary dipstick tests are also affected. The FDA-approved prescribed information also notes that laboratory tests based on oxidative/reductive reactions may also be affected.

Ferritin & iron levels

Intravenous ascorbate is likely related to an elevation in ferritin and iron levels. Subjects who have experienced these elevations have remained asymptomatic. It is possible that chronic increased ferritin and iron levels may affect other organs. Treating physicians will evaluate any necessary work-up based on changes/chronicity of the change in Ferritin and Iron levels.

8. STUDY PROCEDURES

8.1 Schedule of study procedures

Before study entry, throughout the study, and following study drug discontinuation, various clinical and diagnostic laboratory evaluations are outlined. The purpose of obtaining these

detailed measurements is to ensure adequate safety and tolerability assessments. Clinical evaluations and laboratory studies may be repeated more frequently if clinically indicated. The Schedules of Assessments during the screening and treatment period is provided following the Protocol Synopsis.

–

Screening procedures will be performed up to 42 days before Day 1, unless otherwise specified. All patients must first read, understand, and sign the ICF before any study-specific screening procedures are performed. After signing the ICF, completing all screening procedures, and being deemed eligible for entry, patients will be enrolled in the study. Procedures that are performed prior to the signing of the ICF and are considered standard of care may be used as screening assessments if they fall within the 42-day screening window.

The following procedures will be performed during the Screening Visit:

- Informed Consent
- Medical history and demographics
- Complete physical exam
- ECOG Performance Status
- Vitals signs, weight and height
- 12-lead ECG
- Collecting tumor biopsy results including PD-L1 result (if performed)
- Review of prior/concomitant medications
- Imaging by CT Chest or PET/CT (screening window is -42 days from randomization)
- Review of eligibility criteria
- Clinical laboratory tests for:
    - CBC (complete blood count with differential)
    - Clinical chemistry including liver function test
    - TSH with reflex fT4
    - Creatinine Clearance
    - Serum pregnancy test (for women of childbearing potential only)
    - Hepatitis serologies (Hep C antibody, HBV S Ab, HBV S Ag, Core IgG & IgM) if there is a history of hepatitis in the past
    - Urinalysis
    - Lung function test (PFT) – Spirometry and DLCO (window is -120

days). After randomization, study intervention needs to begin within 7 days.

8.1.1 Treatment phase

Arm A and B:

Screening procedures performed within 7 days of Cycle 1 Day 1 (C1D1) do not need to be repeated on C1D1.

Durvalumab: for subjects randomized to Arm A & B - Durvalumab 1500 mg on C1D1and C2D1

Ascorbate infusions: for subjects in Arm B only- Ascorbate infusions 3 times a week x 4 weeks. Ascorbate infusion may begin on C1D1 or after. Minimum 2 infusion per week and can be consecutive days if C1D1 is in the middle of the week or later part of the week.

Blood Draws:

Only for patients in Arm A and B
CBC/diff: C1D1 (-7 days) & C2D1 (-3 days)
CMP: C1D1 (-7 days) then weekly (± 3 days) until week 4
TSH and reflex fT4: C1D1 (-7 days)
Amylase, lipase, GGT, LDH, magnesium: C1D1
Iron panel (Ferritin, Iron Sat, total Iron and TIBC) – C1D1 & C2D1
Cytokine and chemokines panel: C1D1 & C2D1
PBMC: C1D1 & C2D1
Ct-DNA (C1Day1 prior to treatment)
Research samples for banking

Arm C:

Perform only 1-time between randomization to prior to surgery

Iron panel (Ferritin, Iron Sat, total Iron and TIBC)
Cytokine and chemokines panel
PBMC
Ct-DNA
Research samples for banking

AE assessment and attribution (Arm A, B and C):
During the whole treatment phase until 90 days post surgery.

Surgery (Arm A, B and C): Definitive resection to be performed between week 5-9. Effort will be made to perform surgery closer to week 5 to decrease heterogeneity in tumor tissue assessment for

patients in Arm A and B. Patients in Arm C may undergo surgery prior to week 5 as per scheduling.

Need to document the following:

Date of Surgery

Tumor location: RUL, RML, RLL, LUL, LLL

Approach: VATS vs Open Thoracotomy or both

Resection Type: Pneumonectomy, lobectomy, Segmentectomy or wedge

Hilar node/s sampling. Document number and level

Mediastinal node/s sampling: Document number and level

Post-op complications including: A.fib, prolong air-leak (> 48 hr.), infections and other

Pathological assessment – including tumor size, histology, percent necrosis, PD-L1, grade, predominant histological pattern.

Representative tumor sample will be sent to University of Iowa Immunology Core lab for analysis or characterization of immune infiltrate.

30-day follow up post-surgery (±1 week**):**

History, Physical exam, ECOG, vitals, weight

Con medications, adverse events

CBC/diff
CMP
TSH with reflex fT4 (Arm A&B)
Cytokines, chemokines collection
PBMCs collection
Iron Markers
ct-DNA

Follow up assessment (90 days from surgery ±2 weeks)/Safety follow up:

Subjects must return for follow-up with medical oncology or surgical oncology providers including physician assistants:

History, Physical exam, ECOG, vitals, weight
Con medications, adverse events

CT Chest (with IV contrast preferred)

CT abdomen not required, but preferred

CBC/diff, CMP

TSH with reflex fT4 (Arm A & B)
Cytokines, chemokines, PBMCs (only up to 6 months of the surgery) Iron Markers
Ct-DNA
Research samples for banking

End of study treatment or assessments:

Perform the following assessments as in 90-day from surgery when a criterion for removal is met:

For early termination, end-of-study visit should be scheduled within seven calendar days of post termination. If the subject has a recent (±1 week) visit or lab work that supplies the required observation, that can be used in lieu of an additional visit.

Perform a CT chest with contrast if not contraindicated if last CT chest was 3 months ago

Barring subject's withdrawal from all research activities subject will be followed for severe adverse event (if within 90 days of last ascorbate/durvalumab dose), and for progression and overall survival by chart review of electronic health records (EHR) – and if followed elsewhere then records may be requested.

Long-term Follow up:

Follow up assessment (6 months to 1 year after surgery):

Follow-up with either medical oncology or surgical oncology providers including physician assistants at 6, 9 and 12 months(±4 weeks), from the surgery with the following:

History, Physical exam, ECOG, vitals, medications,
CT Chest (with IV contrast preferred)
CT abdomen not required, but preferred
CBC/diff, CMP
TSH with reflex fT4 (only for patients in Arm A& B)
PBMCs,Cytokines, chemokines,

Ct-DNA
Research samples for banking

Follow up assessment (18 months to 3 years)

Follow-up with medical oncology or surgical oncology providers including physician assistants every 6 months during year 2 and 3 (at 18, 24, 30 and 36 months) (± 6 weeks) from surgery with the following:

CT Chest (with IV contrast preferred)
CT abdomen not required, but preferred

Survival Follow up:

Once disease relapse is documented, subjects will enter a survival follow up period every 3 months (±4 wks) for the first year, then every 6 months (±6 wks) for the second and third year for up to 3 years from the time of surgery. Follow up may be accomplished via clinic visit, phone call, or other avenues as appropriate.

Survival Data During Database Locks:

To ensure current and complete survival data is available at the time of database locks, updated survival status may be requested during the study. For example, updated survival status may be requested prior to, but not limited to, a database review, at final analysis. Participants may be contacted for their survival status (excluding participants that have a previously recorded death event in the Oncore/EHR).

8.2 Biospecimen studies and procedures:

See Table 5

PD-L1 on tumor biopsy specimen: If subjects have prior PD-L1 analyses available results using any approved assay will be captured in the study database. If this is not tested, then PD-L1 testing will be performed on diagnostic sample as a research test via Dako 22C3 and Ventana SP263 assay.

PD-L1 & Genetic Sequencing on tumor tissue:
PD-L1 test (via Dako 22C3 and Ventana SP263 assay) and next generation sequencing (NGS) result will be captured in study data base including alterations in the following genes, EGFR, ALK, ROS1, MET, RET, NTRK, KRAS, STK11, KEAP1, NFE2L2, PTEN, PIK3CA and other relevant genes. Testing will be performed on formalin fixed and embedded in paraffin tumor tissue per institutional standards. Tumor mutation burden (TMB) result will be captured if available.

Resected Tumor tissue:
A part of the fresh tumor from the resected specimen will be tested for immune infiltrates in tumor microenvironment including T cells, CD4, CD8, T regulatory cells, MDSCs and macrophages. Tissue will be sent to Immunology core for these analyses.

Differently type of flowcytometry panels will be performed for characterization of immune cell population and its function as described below.

1: The 40-color panel explores that major immune cell populations. It provides an in-depth analysis of the activation, differentiation, and chemokine receptor expression of major immune cell subsets including T cells, B cells, dendritic cells, natural killer cells, and monocytes.

2: The 31-color T cell steady-state panel defines the steady-state phenotype of the subsets of CD4, CD8, and gamma-delta T cells. This panels includes markers to distinguish the main subsets (naïve, memory) of T cells and can determine differentiation status, activation status, and identifies CD4 T helper cell subsets

3: The 23-color T cell panel explores the effector functions of the major T cell subsets. T cells are stimulated with PMA/Ionomycin for 5 hours prior to staining. This panel includes functional markers that comprehensively assess a wide range of different T cell responses.

Additional coregulatory markers on T cells will be evaluated. Panels may be customized based on results from prior patients' samples and availability of antibodies (Appendix 4).

Whole Blood for Flow cytometry and characterization of PBMCs (Peripheral blood mononuclear cells):
Whole blood specimens will be collected prior to C1D1 treatment, prior to C2D1, prior to surgery, 30-day after surgery, at 3, 6, 9, and 12 months after surgery. This analysis will be completed at the Immunology core lab.

PMBCs will be analyzed by 40-color panel or a customized panel for characterization and functionality of circulating immune cells (Appendix 4).

Plasma for Cytokines & Chemokines:
Plasma specimens will be collected prior to C1D1 treatment, prior to C2D1, prior to surgery, 30-day after surgery, at 3, 6, 9, and 12 months after surgery. This will be used for assessment of cytokines, chemokines, ROS and iron marker testing.

Plasma for Iron-related markers:

Plasma specimens will be collected prior to C1D1 treatment, prior to C2D1, prior to surgery, 30-day after surgery, at 3, 6, 9, and 12 months after surgery. This will be used for assessment of Ferritin, Iron, TIBC, Iron saturation.

Whole blood and plasma for ctDNA:

Below correlative biospecimens will be collected and stored at the Holden Comprehensive Cancer Center Bio-repository core and tissue procurement core or at an appropriate facility within the institution. Testing will be performed based on available funding and future research questions.

Archival Tumor Tissue of SurgicalSpecimens
Formalin-fixed, paraffin-embedded archival tumor tissue block of surgical specimens for biomarker evaluation may be requested, if available. Tissue may also be evaluated forwhole exome sequencing, and/or DNA methylation analyses. Any residual nucleic acids remaining after analyses will be banked for future research.

Plasma Collection for miRNA:
Plasma specimens will be collected and stored prior to C1D1 treatment, prior to C2D1, prior to surgery, 30-day after surgery, at 3, 6, 9, and 12 months after surgery.

miRNA may be isolated from plasma samples and analyzed with appropriate platforms such as (but not limited to) quantitative PCR, digital PCR, or next-generation sequencing.

Serum for Proteomics Serum:
Will be collected prior to C1D1 treatment, prior to C2D1, prior to surgery, 30-day after surgery, at 3, 6, 9, and 12 months after surgery. Serum will be submitted at comparable time points for those on Arm C (control arm).

Blood Collection for gene expression/RNA
Whole blood specimens will be collected prior to C1D1 treatment, prior to C2D1, prior to surgery, 30-day after surgery, at 3, 6, 9, and 12 months after surgery. RNA may be isolated for gene expression profiling using techniques such as (but not limited to) RNA-Seq.

Banking of Leftover Biospecimens
Leftover samples that were collected for study specific correlative research will be banked for future research on all subjects who participate in the trial, and is described in the informed consent document. Samples will be banked indefinitely at the Holden Comprehensive Cancer Center Bio- repository core for future unspecified research.

Confidentiality of Biospecimens Samples will be identified by a subject's study number assigned at the time of registration/randomization to the trial. Any material issued to collaborating researchers will be anonymized and only identified by the subject's study number.

All the above studies and their results will not be used to determine patient care and therefore do not required testing in compliance with Clinical Laboratory Improvement Amendments (CLIA) of 1988. In addition, these analyses will not be performed in real-time or at a certain time therefore results can't be shared with treating providers, patient or their families.

Table 5 Volume of Blood Draw

Visit – time points	Sample type	Purpose	Sample form/tube	Transfer to and testing location
From randomization - prior to treatment on C1D1	Whole blood	T cells, CD4, CD8 etc. PBMCs, cytokines and chemokines	2 x 8 ml CPT	Immunology Core Lab – Holden Comprehensive Cancer Center (HCCC)
	Whole blood	Banking	10 ml EDTA, PAXgene RNA tube	HCCC BioMer
	Plasma	Banking	10 ml EDTA	HCCC BioMer
	Serum	Banking	7.5 ml SST top	HCCC BioMer
C2D1 (Prior to treatment (Only for Arm A & B)	Whole blood	T cells, CD4, CD8 etc. PBMCs, cytokines and chemokines	2 x 8 ml CPT	Immunology Core Lab
	Whole blood	Banking	10 ml EDTA, PAXgene tube	HCCC BioMer
	Plasma	Banking	10 ml EDTA	HCCC BioMer
	Serum	Banking	7.5 ml SST	HCCC BioMer
Prior to surgery	Whole blood	T cells, CD4, CD8 etc. PBMCs, cytokines and chemokines	2 x 8 ml CPT	Immunology Core Lab
	Whole blood	ctDNA	2 x Streck tube (8.5 mL)	HCCC BioMer
	Whole blood	Banking	10 ml EDTA, Paxgene tube	HCCC BioMer
	Plasma	Banking	10 ml EDTA	HCCC BioMer
	Serum	Banking	7.5 ml SST	HCCC BioMer
At surgery	Fresh tumor tissue			Immunology Core Lab
At 3, 6, 9 & 12 months from surgery	Archival tissue *	ctDNA	20 x 5um slides	HCCC BioMer
	Archival tissue *	PD-L1 and NGS	Institutional standards	UIHC Pathology lab
	Whole blood	T cells, CD4, CD8 etc. PBMCs, cytokines and chemokines	2 x 8 ml CPT	Immunology Core Lab

(*Archival tissue testing is performed only once)				
	Whole blood	ctDNA	2 x Streck tube (8.5 mL)	HCCC BioMer
	Whole blood	Banking	10 ml EDTA, Paxgene tube	HCCC BioMer
	Plasma	Banking	10 ml EDTA	HCCC BioMer
	Serum	Banking	7.5 ml SST	HCCC BioMer

9. DISEASE EVALUATION AND METHODS

Efficacy endpoints for the durvalumab (Arm A) or combination of pharmacological ascorbate and durvalumab (Arm B) are secondary endpoints in this study and include the followings:

Pathologic Response:

Complete pathologic response (pCR): Defined as no viable tumor tissue in the resected specimen.

Major pathologic response (MPR): Defined as ≤10% residual viable tumor in resected lung and lymph node tissue

A qualified pathologist will examine the resected specimen for the pathologic response according to institutional policy/guidelines.

Recurrence and Event-Free Survival:

In some circumstances it may be difficult to distinguish disease recurrence and relapse from inflammatory findings. It is recommended that a repeat disease assessment be performed if needed to confirm disease relapse or pathologic confirmation prior to taking a subject off study in the absence of clinically significant deterioration. The disease assessment should be performed no sooner than 4 weeks after the criteria for relapse is first met. During this interim period, subjects may continue to be followed per protocol unless subject condition is rapidly deteriorating or unstable.

Of note, clinically significant deterioration is considered to be a rapid tumor progression that necessitates treatment with anticancer therapy other than durvalumab or with symptomatic progression that requires urgent medical intervention (e.g., central nervous system metastasis, respiratory failure due to tumor compression, spinal cord compression)

Recurrence

Disease assessment will be performed locally by the investigator/designee (e.g., radiologist).

This is defined as the first day when the RECIST version 1.1 criteria for disease recurrence are met. The first date when recurrence was observed is considered regardless of the assessment method. Therefore, recurrence will be declared for any lesion when:

□ Only imaging was performed, and progression confirmed.
□ Only pathology was done, and malignancy confirmed (in solitary or in doubtful lesions, cutaneous, subcutaneous or lymph node lesions);
□ Both pathology and imaging were done, and progression/malignancy confirmed. Inthis case, whatever examination came first, its date is considered the date ofrecurrence.
□ If pseudo progression is suspected, relapse will be confirmed by either a biopsy or repeat radiographic scans.

Event Free Survival (EFS)

Recurrence of disease can be a loco-regional recurrence, a distant (metastatic) recurrence or a second primary. Recurrent NSCLC and second malignancies will be considered events. Event Free Survival (EFS) is calculated as the time from randomization to either the date of disease recurrence or the date of death (whatever the cause). The date of first documented disease recurrence (if applicable) will be used as the date of event. Patients alive with no evidence of disease recurrence at the time of their last visit are censored at the time of the last examination.

Overall Survival (OS)
Overall survival is defined by the date of randomization to date of death from any cause.

Imaging Modalities

The same imaging technique should be used in a participant throughout the study to optimize the reproducibility of the assessment of existing and new tumor burden and improve the accuracy of the response assessment based on imaging.

Tumor imaging is strongly preferred to be acquired by CT. CT scan of chest can be sufficient preferably with IV contrast. CT of the abdomen with IV contrast is also preferrable but not required.

Brain imaging is recommended at screening when clinically indicated. Magnetic resonance imaging is preferred; however, CT imaging will be acceptable, if MRI is medically contraindicated.

10. ASSESSMENT OF SAFETY

10.1 Clinical laboratory tests

Blood and urine samples for determination of clinical chemistry, hematology, and urinalysis will be taken at the times indicated in the assessment schedules and as clinically indicated

Clinical laboratory safety tests, including serum pregnancy tests, will be performed through CLIA certified laboratory according to local standard procedures of the University of Iowa Hospitals and Clinics.

Additional safety samples may be collected if clinically indicated at the discretion of the treating provider.

The laboratory variables to be measured are presented in Table 7 (clinical chemistry), Table 6 (hematology), and Table 8 (urinalysis).

The following laboratory variables will be measured:

Table 6 Hematology

Hematology Laboratory Tests

CBC/diff [a,b]

Note: For coagulation parameters, activated partial thromboplastin time and international normalized ratio are to be assessed at baseline on Day 0 (unless all screening laboratory haematology assessments are performed within 3 days prior to Day 0), and as clinically indicated.

[a] Differential can be recorded as absolute counts or as percentages. Absolute counts will be calculated if entered as percentage. Total white cell count therefore has to be provided.

[b] If screening CBC/diff is performed within 7 days prior to C1D1, it does not need to be repeated atC1D1.

Table 7 Clinical Chemistry

Clinical Chemistry Laboratory Tests

CMP [a,f]
Amylase[c]
Gamma glutamyl transferase (GGT)[c]
Lactate dehydrogenase (LDH)[c]
Lipase[c]
Magnesium[c]
Creatinine Clearance[b]

[a] If total bilirubin is ≥2 × upper limit of normal (and no evidence of Gilbert's syndrome) then fractionate into direct and indirect bilirubin.

[b] Creatinine clearance at baseline and C1D1 only

[c] Gamma glutamyl transferase, LDH, amylase, lipase and magnesium testing are only to be performed at C1D1 and if clinically indicated.

[d] Creatinine Clearance will be calculated using Cockcroft-Gault (using actual body weight).

[e] If TSH is measured within 14 days prior to C1Day 1 (first infusion day), it does not need to be repeated at baseline. Free T4 will only be measured if TSH is abnormal or if there is a clinical suspicion of an AE related to the endocrine system

[f] If screening CMP assessment is performed within 7 days prior to C1D1, it does not need to be repeated at C1D1.

Table 8 Urinalysis (UA) Tests[a]

Bilirubin	pH
Blood	Protein
Glucose	Specific gravity
Bilirubin	pH
Ketones	Color and Appearance

[a] UA at screening only. Microscopy if clinically indicated and should be used as appropriate to investigate white blood cells and use the high-power field for red blood cells

If a patient shows an AST or ALT ≥3xULN together with total bilirubin ≥2xULN, refer to Appendix 1. Further instructions on cases of increases in liver biochemistry and evaluation of Hy's Law. These cases should be reported as SAEs if, after evaluation, they meet the criteria for a Hy's law case or if any of the individual liver test parameters fulfill any of the SAE criteria.

Any clinically significant abnormal laboratory values should be repeated as clinically indicated and recorded on the eCRF. Situations in which laboratory safety results should be reported as AEs are described in Section 10.5.

All patients with Grade 3 or 4 laboratory values at the time of completion or discontinuation from IP must have further tests performed until the laboratory values have returned to Grade 1 or 2, unless these values are not likely to improve because of the underlying disease.

10.1.1 Physical examinations

Physical examinations will be performed according to the assessment schedules (see the SoAs). Full physical examinations will include assessments of the head and neck, respiratory, cardiovascular, GI, musculoskeletal, neurological, dermatological, hematologic/lymphatic. Height will be measured at screening or at baseline only. Targeted physical examinations are to be utilized by the Investigator on the basis of clinical observations and symptomatology. Situations in which physical examination results should be reported as AEs are described in Section 10.5.

Resting12-lead ECGs will be recorded at screening and as clinically indicated throughout the study.

In case of clinically significant ECG abnormalities, including a QTcF value >470 ms, 2 additional 12-lead ECGs should be obtained over a brief period (eg, 30 minutes) to confirm the finding.

Situations in which ECG results should be reported as AEs are described in Section 10.5

At Screening, a ECG will be obtained on which QTcF must be <470 ms.

10.1.2 Vital signs

Vital signs (blood pressure [BP], pulse, temperature, and respiration rate) will be evaluated according to the SoAs. Body weight is also recorded at screening, on C1D1, C2D1, at 30 day and 90 day follow up after surgery.

Patients will be monitored during infusions per institutional standard.

10.2 ECOG performance status

ECOG performance status will be assessed at the times specified in the assessment schedules (see the SoAs) based on the following:

0. Fully active; able to carry out all usual activities without restrictions

1. Restricted in strenuous activity, but ambulatory and able to carry out light work or work of a sedentary nature (e.g., light housework or office work)

2. Ambulatory and capable of self-care, but unable to carry out any work activities; up and about more than 50% of waking hours.

3. Capable of only limited self-care; confined to bed or chair more than 50% of waking hours

4. Completely disabled; unable to carry out any self-care and totally confined to bed or chair

5. Dead

Any significant change from baseline or screening must be reported as an AE.

10.3 Safety parameters

10.3.1 Definition of adverse events

The International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP) E6(R1) defines an AE as:

Any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE includes but is not limited to any clinically significant worsening of a patient's pre-existing condition. An abnormal laboratory finding (including ECG finding) that requires

an action or intervention by the investigator, or a finding judged by the investigator to represent a change beyond the range of normal physiologic fluctuation, should be reported as an AE.

Adverse events may be treatment emergent (i.e., occurring after initial receipt of investigational product) or nontreatment emergent. A nontreatment-emergent AE is any new sign or symptom, disease, or other untoward medical event that begins after written informed consent has been obtained but before the patient has received investigational product.

Elective treatment or surgery or preplanned treatment or surgery (that was scheduled prior to the patient being enrolled into the study) for a documented pre-existing condition, that did not worsen from baseline, is not considered an AE (serious or nonserious). An untoward medical event occurring during the prescheduled elective procedure or routinely scheduled treatment should be recorded as an AE or SAE.

The term AE is used to include both serious and non-serious AEs.

Disease progression should not be recorded as an AE, unless it is attributable to the study regimen by the site investigator.

10.3.2 Definition of serious adverse events

A serious adverse event is an AE occurring during any study phase (i.e., screening, run-in, treatment, wash-out, follow-up), at any dose of the study drugs that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect in offspring of the patient
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above.
    - Medical or scientific judgment should be exercised in deciding whether expedited reporting is appropriate in this situation. Examples of medically important events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalizations; or development of drug dependency or drug abuse.

- Adverse Events (AEs) for **malignant tumors** reported during a study should generally be assessed as **Serious** AEs. If no other seriousness criteria apply, the 'Important Medical Event' criterion should be used. In certain situations, however, medical judgement on an individual event basis should be applied to clarify that the malignant tumor event should be assessed and reported as a **non-Serious AE**. For example, if the tumor is included as medical history and progression occurs during the study, but the progression does not change treatment and/or prognosis of the malignant tumor, the AE may not fulfil the attributes for being assessed as Serious, although reporting of the progression of the malignant tumor as an AE is valid and should occur. Also, some types of malignant tumors, which do not spread remotely after a routine treatment that does not require hospitalization, may be assessed as Non-Serious; examples include Stage 1 basal cell carcinoma and Stage 1A1 cervical cancer removed via cone biopsy.

- The above instruction applies only when the malignant tumor event in question is a new malignant tumor (i.e., it is *not* the tumor for which entry into the study is a criterion and that is being treated by the IP under study and is not the development of new or progression of existing metastasis to the tumor under study). Malignant tumors that – as part of normal, if rare, progression – undergo transformation (e.g., Richter's transformation of B cell chronic lymphocytic leukaemia into diffuse large B cell lymphoma) should not be considered a new malignant tumor.

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to AstraZeneca.

10.3.3 Definition of adverse events of special interest (AESI)

An adverse event of special interest (AESI) is one of scientific and medical interest specific to understanding of the Investigational Product and may require close monitoring. An AESI may be serious or non-serious.

If the Investigator has any questions in regard to an event being an imAE, the Investigator should promptly contact the Study Physician.

AESIs observed with durvalumab include:

- Diarrhea / Colitis and intestinal perforation
- Pneumonitis / ILD
- hepatitis / transaminase increases
- Endocrinopathies (i.e. events of hypophysitis/hypopituitarism, thyroiditis, adrenal insufficiency, hyper- and hypothyroidism and type I diabetes mellitus)
- Rash / Dermatitis
- Nephritis / Blood creatinine increases
- Pancreatitis / serum lipase and amylase increases
- Myocarditis
- Myositis / Polymyositis

Other inflammatory responses that are rare/less frequent with a potential immune-mediated etiology include, but are not limited to:

- Pericarditis
- Neuromuscular toxicities (such as Guillain-Barre syndrome and myasthenia gravis)
- Sarcoidosis
- Uveitis
- Other events involving the eye and skin
- Haematological events
- Rheumatological events
- Vasculitis
- Non-infectious meningitis
- Non-infectious encephalitis.

It is possible that events with an inflammatory or immune mediated mechanism could occur in nearly all organs.

In addition, infusion-related reactions and hypersensitivity/anaphylactic reactions with a different underlying pharmacological etiology are also considered AESIs.

Further information on these risks (e.g., presenting symptoms) can be found in the current version of the durvalumab Investigator's Brochure. More specific guidelines for their evaluation and treatment are described in detail in the Dosing Modification and Toxicity Management Guidelines (please see Appendix 1). These guidelines have been prepared by the Sponsor to assist the Investigator in the exercise of his/her clinical judgment in treating these types of toxicities. These guidelines apply to AEs considered causally related to the study drug/study regimen by the reporting investigator.

If new or worsening pulmonary symptoms (e.g., dyspnea) or radiological abnormality suggestive of pneumonitis/interstitial lung disease is observed, toxicity management as described in detail in the Dosing Modification and Toxicity Management Guidelines (see Appendix 1) will be applied.

10.4 Assessment of safety parameters

10.4.1 Assessment of severity

Assessment of severity is one of the responsibilities of the investigator in the evaluation of AEs and SAEs. Severity will be graded according to the NCI CTCAE v5.

It is important to distinguish between serious criteria and severity of an AE. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 10.XXX. A Grade 3 AE need not necessarily be considered an SAE. For example, a Grade 3 headache that persists for several hours may not meet the regulatory definition of an SAE and would be considered a nonserious event, whereas a Grade 2 seizure resulting in a hospital admission would be considered an SAE.

10.5 Recording of adverse events and serious adverse events

AEs and SAEs will be collected from the time of the patient signing the informed consent form until the follow-up period is completed (90 days after the last dose of durvalumab or ascorbate or surgery whichever is later). If a new treatment started then this follow up should be until before then.

During the course of the study, all AEs and SAEs should be proactively followed up for each patient for as long as the event is ongoing. Every effort should be made to obtain a resolution for all events, even if the events continue after the patient has discontinued study drug or the study has completed.

Any AEs that are unresolved to grade 1 or came to a new baseline at the patient's last study visit will be followed by the Investigator for as long as medically indicated, but without further recording in the subject study binder or Oncore CTMS.

AstraZeneca retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

The following variables will be collected for each AE:

In addition, the following variables will be collected for SAEs as applicable:

- AE (verbatim)
- The date when the AE started and stopped
- The maximum CTCAE grade reported
- Changes in CTCAE grade
- Whether the AE is serious or not
- Investigator causality rating against the IPs (yes or no)
- Action taken with regard to IPs
- Administration of treatment for the AE
- Outcome

In addition, the following variables will be collected for SAEs:

- Date the AE met criteria for SAE
- Date the Investigator became aware of the SAE
- Seriousness criteria fulfilled
- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death

- Whether an autopsy was performed
- Causality assessment in relation to study procedure(s)
- Causality assessment in relation to other medication, as explained in Section 10.7
- Description of the SAE

The grading scales found in the NCI CTCAE v5 will be utilized for all events with an assigned CTCAE grading. For those events without assigned CTCAE grades, the recommendation in the CTCAE criteria that converts mild, moderate, and severe events into CTCAE grades should be used. A copy of the CTCAE v5 can be downloaded from the Cancer Therapy Evaluation Program website (http://ctep.cancer.gov).

10.5.1 Study recording period and follow-up for adverse events and serious adverse events

If a patient discontinues from treatment for reasons other than disease progression, and therefore continues to follow on study, then AE or SAEs must be captured until 90 days from surgery unless a new baseline is achieved or toxicities resolve to G1 prior to 90 days.

The investigator or treating provider is responsible for following all SAEs until resolution, until the patient returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation.

10.6 Causality collection

The Investigator will assess causal relationship between the IPs and each AE and answer “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?”

For SAEs causal relationship will also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure, the causal relationship is implied as “yes.”

AEs that are definite, probable, or possibly related to IP will be categorized as yes, and those that not related or unlikely will be categorized as no.

10.7 Relationship to protocol procedures

The Investigator is also required to provide an assessment of the relationship of SAEs to protocol procedures on the SAE report form. This includes both non-treatment–emergent (i.e., SAEs that occur prior to the administration of IP) and treatment-emergent SAEs. A protocol-

related SAE may occur as a result of a procedure or intervention required during the study (e.g., blood collection). The following guidelines should be used by Investigators to assess the relationship of SAEs to the protocol:

- Protocol related: The event occurred due to a procedure or intervention that was described in the protocol for which there is no alternative etiology present in the patient's medical record.

- Not protocol related: The event is related to an etiology other than the procedure or intervention that was described in the protocol. The alternative etiology must be documented in the study patient's medical record. I.e related to primary disease or other medical problems/medications related or a new medical problem.

10.8 Adverse events based on signs and symptoms

All AEs spontaneously reported by the patient or reported in response to the open question from the study personnel: "Have you had any health problems since the previous visit/you were last asked?" or revealed by observation will be collected and recorded.

When collecting AEs, the recording of diagnoses is preferred, when possible, to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

10.9 Adverse events based on examinations and tests

Deterioration as compared to baseline in protocol-mandated laboratory values and vital signs should therefore only be reported as AEs if they fulfill any of the SAE criteria or are the reason for discontinuation of treatment with the IPs.

If deterioration in a laboratory value or vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result or vital sign will be considered as additional information. Whenever possible, the reporting Investigator should use the clinical rather than the laboratory term (e.g., anemia versus low hemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AEs.

Deterioration of a laboratory value that is unequivocally due to disease progression should not be reported as an AE/SAE.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

10.10 Hy's Law

Cases where a patient shows elevations in liver biochemistry may require further evaluation and occurrences of AST or ALT ≥3 × ULN together with total bilirubin ≥2 × ULN may need to be reported as SAEs. Please refer to the Toxicity Management Guidelines, for further instruction on cases of increases in liver biochemistry and evaluation of Hy's law.

10.11 Disease progression

Disease progression can be considered as a worsening of a patient's condition attributable to the disease for which the IP is being studied. It may be an increase in the severity of the disease under study and/or increases in the symptoms of the disease. The development of new or progression of existing metastasis to the primary cancer under study should be considered as disease progression and not an AE. Events that are unequivocally due to disease progression should not be reported as an AE during the study.

10.12 New cancers

The development of a new cancer should be regarded as an SAE. New primary cancers are those that are not the primary reason for the administration of the IP and have been identified after the patient's inclusion in this study.

10.13 Deaths

All deaths that occur during the study treatment period, or within the protocol-defined follow-up period after the administration of the last dose of study drug, must be reported as follows:

- Death clearly resulting from disease progression should be reported to the Study Monitor/Physician at the next monitoring visit and should be documented inthe <<specify where this information will be recorded e.g. eCRF, CRF, etc)>> in the Statement of Death page. It should not be reported as an SAE.
- Where death is not due (or not clearly due) to progression of the disease under study, the AE causing the death must be reported to the Study Monitor/Physician as an SAE within 24 hours. It should also be documented in the Statement of Death page in<<specify where this information will be recorded e.g. eCRF, CRF, etc)>>
- The report should contain a comment regarding the co involvement of PD, if appropriate, and should assign main and contributory causes of death.
- Deaths with an unknown cause should always be reported as an SAE. It should also be documented in the Statement of Death page in<<specify where this information will be recorded e.g. eCRF, CRF, etc)>>
- A post mortem may be helpful in the assessment of the cause of death, and if performed, a copy of the post-mortem results should be forwarded to AstraZeneca Patient Safety or its representative within the timeframes specified in Section10.5.xxx

Deaths occurring after the protocol defined safety follow up period after the administration of the last dose of study drug should be documented in the Statement of Death page. If the death occurred as a result of an event that started after the defined safety follow up period and the event is considered to be due to a late onset toxicity to study drug, then it should also be reported as an SAE.

AstraZeneca retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

10.14 Follow-up of unresolved adverse events

Any AEs that are unresolved at the patient's last visit study visit (90 days from surgery) are followed up by the investigator for as long as medically indicated, but without further recording in the patient's study binder or Oncore.

AstraZeneca/ retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

10.15 Post-study events

After the patient has been permanently withdrawn from the study, there is no obligation for the investigator to actively report information on new AE or SAEs occurring in former study patients after the 90-day safety follow-up period for patients treated with durvalumab. However, if an investigator learns of any SAEs, including death, at any time after the patient has been permanently withdrawn from study, and he/she considers there is a reasonable possibility that the event is related to study treatment, the investigator should notify the study sponsor and AstraZeneca/MedImmune at AEmailboxclinicaltrialTCS@astrazeneca.com

10.16 Reporting of serious adverse events to the IRB and/or the Regulatory Authority

Section 5.6 of the Investigator Brochure (IB) serves as the Reference Safety Information (RSI) for this trial.

This study is monitored by internal oversight specialists at the University of Iowa. The Data and Safety Monitoring Plan of the Holden Comprehensive Cancer Center provides standard operating procedures to monitor all investigator-initiated trials by the Data and Safety Monitoring Committee (DSMC).

A copy of the detailed data and safety monitoring plan for this study is on file with the Data and Safety Monitoring Committee of the Holden Comprehensive Cancer Center and the IRB

of record; an abbreviated copy is provided (Appendix 3). This study has been assigned as a risk level 4 as it utilizes an investigator-sponsored IND.

Serious Adverse Event Reporting to DSMC

For any experience or condition meeting the definition of a serious adverse event (SAE; 21CFR312.32), from the day of consent and continue through the 90-day follow-up period after durvalumab or ascorbate (which-ever occurs later) is discontinued.

Investigators or designee must report to the DSMC any SAE, regardless of attribution to study drug. SAEs must be reported via an OnCore SAE Report ≤ 1 business day of learning of the event.

FDA REPORTING REQUIREMENTS [MOHEMMED MILHEM, IND SPONSOR for ASCORBATE)

HCCC CRS Regulatory specialist(s) has been designated to manage the Investigational New Drug Application (IND) associated with this protocol on behalf of the sponsor-investigator and IND sponsor.

Adverse events meeting the criteria of all three i.e., serious, unexpected, and attributed (possible, probable, or definite) to ascorbate or durvalumab or both must be reported by the sponsor or the sponsor's appointed designee to the FDA:

- Serious adverse events only [21 CFR 312.32]
- Attributable to ascorbate or durvalumab or both with a causal relationship as described [21 CFR 312.32 (c)(1)(i)]
- Unexpected, as defined by the FDA: "…not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended."

The *principal investigator and sponsor-investigator* has final determination in attributing the investigational drug (durvalumab or ascorbate) to the serious adverse event for purposes of this reporting.

For SAEs that are life threatening or result in death report to FDA must be7 calendars days or less after the investigator's initial receipt of the information

For all other SAEs report to FDA must be 15 calendar days or less.

SAEs should be reported on MedWatch Form 3500A or similarform.
MedWatch SAE forms should be sent to the FDA at: MEDWATCH 5600 Fishers Lane Rockville, MD 20852-9787 Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

Copies will be submitted to AstraZeneca and McGuff at the time of submission to FDA

IND Safety Reports Unrelated to this Trial:

AstraZeneca and McGuff Pharmaceuticals will provide HCCC – CRS Regulatory specialist and sponsor-investigator with IND safety reports from external studies that involve the study drug(s) per their guidelines. These safety reports will be reviewed by the sponsor-investigator for relatedness to this study intervention and determine if revisions are needed to the protocol or consent. Same applies to the new Investigator Brochure.

Based on the sponsor-investigator's review, applicable changes will be made to the protocol and informed consent document (if required).

HCCC-CRS regulatory specialist(s) will be responsible for submitting these safety reports to their respective IRBs, per institutional policies.

Institutional Review Board reporting requirements

Adverse events that meet criteria of both serious and attributed (possible, probable, or definite) to the study agent. Thus:

- Serious adverse events only

- Attributable to ascorbate or durvalumab or both Report

to the IRB via HawkIRB within 10 business days ofevent

If any SAE occurs in the course of the study, then Investigators or other site personnel inform the appropriate Sponsor representatives within one day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative works with the Investigator to ensure that all the necessary information is provided to the database **within 1 calendar day** of initial receipt for

fatal and life-threatening events **and within 5 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform Sponsor representatives of any follow-up information on a previously reported SAE within one calendar day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

(US) Sponsor and other site personnel must inform the FDA, via a MedWatch/AdEERs form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32. It is the responsibility of the Sponsor to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines

Sponsor's representative's contact information for study SAE reporting is

Laura Dallas,
Holden Comprehensive Cancer Center,
Regulatory IIT Specialist, Clinical Research services

10.17 Reporting of serious adverse events to AstraZeneca

SAEs related to the Investigational Product (IP) must be provided to Company in an ongoing basis as individual case reports.

SAEs unrelated to the IP must be provided to the Company as individual case reports as a quarterly line listing

At the end of the Study a final unblinded summary line listing of all SAEs must be provided to the Company to enable reconciliation of safety information held by Company for its product(s).

Send SAE reports (individual case reports and line listings) and accompanying cover page to Company (TCS) via Email: AEmailboxclinicaltrialTCS@astrazeneca.com

SAEs that do not require expedited reporting to the Regulatory Authority/IRB/IEC still need to be reported to Company

Suspected Unexpected Serious Adverse Reactions (SUSARs) must be reported to Company at the same time these events are notified to the FDA.

10.17.1 Reporting of deaths to AstraZeneca

All deaths must be recorded and reported as outlined in Section 10.5. In addition, all SAEs resulting in death or death of unknown cause must be reported to AstraZeneca via AEMailboxClinicalTrialTCS@astrazeneca.com within 7 calendar days of awareness or sooner when required (See Section 10.5) if occurring within 90 days of IP administration.

10.18 Other events requiring reporting

10.18.1 Overdose

An overdose is defined as a patient receiving a dose of durvalumab in excess of that specified in the Investigator's Brochure, unless otherwise specified in this protocol.

Any overdose of a study patient with durvalumab, with or without associated AEs/SAEs, is required to be reported within 24 hours of knowledge of the event to the sponsor-investigator. The sponsor-investigator (or designee) must report these to AstraZeneca Patient Safety or designee using the designated Safety e-mailbox (see Section 10.5 for contact information) within 7 calendar days or sooner when required (see Section 10.5). If the overdose results in an AE, the AE must also be recorded as an AE (see Section 10.5). Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be recorded and reported as an SAE (see Section 10.5). There is currently no specific treatment in the event of an overdose of durvalumab.

The investigator will use clinical judgment to treat any overdose.

10.18.2 Hepatic function abnormality

Hepatic function abnormality that fulfills the biochemical criteria of a potential Hy's Law case in a study patient, with or without associated clinical manifestations, is required to be reported as "hepatic function abnormal" ***within 24 hours of knowledge of the event*** to the sponsor-investigator. The Sponsor-investigator (or designee) must report these events to AstraZeneca Patient Safety using the designated Safety e-mailbox (see Section 10.5 for contact information) within 7 calendar days or sooner when required, unless a definitive underlying diagnosis for the abnormality (e.g., cholelithiasis or bile duct obstruction) that is unrelated to investigational product has been confirmed. The criteria for a potential Hy's Law case is Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) ≥3x Upper Limit of Normal (ULN) together with Total Bilirubin (TBL) ≥2xULN at any point during the study following the start of study medication irrespective of an increase in Alkaline Phosphatase(ALP).

- If the definitive underlying diagnosis for the abnormality has been established and is unrelated to investigational product, the decision to continue dosing of the study patient will be based on the clinical judgment of the investigator.

- If no definitive underlying diagnosis for the abnormality is established, dosing of the study patient must be interrupted immediately. Follow-up investigations and inquiries must be initiated by the investigational site without delay.

Each reported event of hepatic function abnormality will be followed by the investigator and evaluated by the sponsor and AstraZeneca.

10.18.3 Pregnancy

10.18.4 Maternal exposure

If a patient becomes pregnant during the course of the study, the IPs should be discontinued immediately.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the IP under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented even if the patient was discontinued from the study.

If any pregnancy occurs in the course of the study, then the Investigator or other site personnel should inform the sponsor within 1 day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The sponsor will work with the Investigator to ensure that all relevant information is provided within 1 to 5 calendar days. The Sponsor must report to AstraZeneca Patient Safety using the designated Safety e-mailbox (see Section 10.5 for contact information) within 7 calendar days or sooner when required (see Section 10.5), for pregnancies with SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

10.18.5 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 180 days after the last dose of durvalumab + any drug combination therapy or 90 days after the last dose of durvalumab monotherapy, whichever is the longer time period.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 180 days after the last dose of durvalumab + any drug combination therapy or 90 days after the last dose of durvalumab

monotherapy, whichever is the longer time period should, if possible, be followed up and documented.

Where a report of pregnancy is received, prior to obtaining information about the pregnancy, the Investigator must obtain the consent of the patient's partner. Therefore, the local study team should adopt the generic ICF template in line with local procedures and submit it to the relevant Ethics Committees (ECs)/Institutional Review Boards (IRBs) prior to use.

10.19 Medication error

For the purposes of this clinical study a medication error is an unintended failure or mistake in the treatment process for an AstraZeneca study drug that either causes harm to the patient or has the potential to cause harm to the patient.

A medication error is not lack of efficacy of the drug, but rather a human or process related failure while the drug is in control of the study site staff or patient.

Medication error includes situations where an error

- Occurred
- Was identified and intercepted before the patient received the drug
- Did not occur, but circumstances were recognized that could have led to an error

Examples of events to be reported in clinical studies as medication errors:

- Drug name confusion
- Dispensing error e.g. medication prepared incorrectly, even if it was not actually given to the patient
- Drug not administered as indicated, for example, wrong route or wrong site of administration
- Drug not taken as indicated e.g. tablet dissolved in water when it should be taken as a solid tablet
- Drug not stored as instructed e.g. kept in the fridge when it should be at room temperature
- Wrong patient received the medication (excluding IVRS/IWRS errors)
- Wrong drug administered to patient (excluding IVRS/IWRS errors)

Examples of events that **do not** require reporting as medication errors in clinical studies:

- Errors related to or resulting from IVRS/IWRS - including those that lead to one of the above listed events that would otherwise have been a medication error
- Patient accidentally missed drug dose(s) e.g. forgot to take medication
- Accidental overdose (will be captured as an overdose)
- Patient failed to return unused medication or empty packaging

- Errors related to background and rescue medication, or standard of care medication in open label studies, even if an AZ product

Medication errors are not regarded as AEs but AEs may occur as a consequence of the medication error.

If a medication error occurs in the course of the study, then the Investigator or other site personnel informs Sponsor within 1 day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

The Sponsor-investigator works with the Investigators to ensure that all relevant information is completed within 1 or 5 calendar days. The Sponsor-investigator (or designee) must report to AstraZeneca Patient Safety using the designated Safety e-mailbox (see Section 10.5 forcontact information) within 7 calendar days or sooner when required (see Section 10.5) if there is an SAE associated with the medication error and within 30 days for all other medicationerrors.

11. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

11.1 Sample Size Determination

The primary objective of this descriptive, window of opportunity trial is to obtain preliminary evidence of the immunomodulatory effect of pharmacological ascorbate as assessed by differences in CD8+ T cells at resection between the Durvalumab and Durvalumab + P-AscH- treatment arms. Patients will be randomly assigned using a 1:1:1 allocation ratio. The planned sample size of 10 patients per treatment arm ensures 80% power to detect a 1.2 SD mean difference in CD8+ T cells between treatment arms using a two-sample t-test. Power calculations are based on a two-sided 10% significance level. A total of 12 patients per treatment arm (36 total) will be randomized to ensure at least 10 patients per treatment arm have evaluable specimens. Given the intent is to obtain preliminary evidence of the immunomodulatory effect of pharmacological ascorbate to inform subsequent studies, results will not be adjusted for multiplicity.

As this is the first time that Durvalumab has been administered preoperatively in NSCLC with P-AscH-, there will be a run-in phase for Arm B, where patients will be observed for DLT. An initial DLT assessment will be made when 3 patients have been randomized to Arm B and are evaluable for DLT. If at most 1 out of 3 patients experience DLT, an additional cohort of 3 patients will be evaluated for DLT. If at most 1 out of 6 patients experience DLT, the combination will be deemed safe. There will be no dose-escalation.

11.2 Populations For Analyses

The intent-to-treat (ITT) population is defined as all randomized patients, irrespective of their compliance to the planned course of treatment. The ITT population is the analysis population for efficacy endpoints.

The safety population includes all patients who received at least one dose of study medication. The safety population is the analysis population for safety endpoints.

11.3 Statistical Analyses

11.3.1 General Approach

In general, continuous variables will be summarized using descriptive statistics such as mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized by count and proportions. Results will be reported separately by treatment arm.

Safety analyses

The incidence of treatment-emergent adverse events will be summarized by system organ class and/or preferred term, type of adverse event, severity (based on NCI CTCAE v5.0 grades), relation to study treatment and treatment arm using the safety population. The most severe grade per patient will be reported. Adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent adverse events will be presented in tabular form.

[10, 28] [29][8][6] Analysis of the Primary Endpoint(s)

The primary efficacy endpoint is CD8+ T cells. An analysis of variance will be used to compare CD8+ T cells in the resected specimen between treatment arms. Graphical plots of the estimated mean and associated 90% confidence intervals will be produced, and p-values for treatment Arm comparisons will be reported.

11.3.2 Analysis of the Secondary Endpoint(s)

Pathologic Complete Response (pCR) Rate: The pCR rate will be defined as the proportion of patients with a pathologic complete response. A pCR is defined as no viable tumor tissue in the resected specimen. All patients that do not meet the criteria for pCR by the analysis cutoff date will be considered non-responders. Binomial proportions and 90% confidence intervals will be reported for each treatment arm.

Major Pathologic Response (MPR) Rate: The MPR rate will be defined as the proportion of patients with a major pathologic response. MPR is defined as ≤10% residual viable tumor in the resected specimen. All patients that do not meet the criteria for MPR by the analysis cutoff date will be considered non-responders. Binomial proportions and 90% confidence intervals will be reported for each treatment arm.

Event-Free Survival (EFS): EFS will be defined as the time from randomization to progression of disease that precludes surgery, local or distance recurrence, or death due to any cause. Patients without events of interest will be censored at last disease evaluation. Cumulative EFS will be descriptively summarized over time with the method of Kaplan-Meier. Median and landmark estimates along with 90% confidence intervals will be reported for each treatment arm.

Overall Survival (OS): OS will be defined as the time from randomization to death due to any cause. Patients still alive will be censored at last follow-up. Cumulative OS will be descriptively summarized over time with the Kaplan-Meier method. Median and landmark estimates along with 90% confidence intervals will be reported for each treatment arm.

11.3.3 Exploratory analyses

An analysis of variance will be used to evaluate other immunomodulatory effects in resected specimens as measured by CD4+ T cells, T-regs, tissue-resident macrophages, and other immune cell phenotypes. To evaluate systemic immunomodulatory effects, mixed effects regression models will be utilized to estimate within and between treatment arm differences in peripheral blood mononuclear cells, cytokines and chemokines. Random effects will be included to account for the longitudinally correlated nature of repeated measurements within a patient. Graphical plots of the estimated mean and associated 90% confidence intervals will be produced. Cox regression will be used to evaluate the association between circulating tumor DNA and time to clinical progression as defined per RECIST v1.1.

11.3.4 Interim analyses

Given the exploratory nature and small planned sample size, a formal interim analysis of pharmacological ascorbate's immunomodulatory effect is not planned. However, patient samples will be assessed in real-time for the study's primary endpoint and descriptive reports of interim data will be included in the annual study process report.

12. ETHICAL AND REGULATORY REQUIREMENTS

12.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, and applicable regulatory requirements Patient data protection.

12.2 Ethics and regulatory review

The final study protocol and the final version of the informed consent form must be approved in writing by an IRB. The site investigator (or designee) is responsible for informing the IRB of any amendment to the protocol in accordance with institutional requirements. In addition, the IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB as local regulations require. Progress reports and notifications of adverse events will be provided to the IRB according to local regulations and guidelines.

12.3 Informed consent

The site investigator and study team will ensure the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects must also be notified they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study. A copy of the signed informed consent form must be given to the subject. The original signed document will be filed in the subject study records.

12.4 Safety Oversight

Safety oversight will be under the direction of a Holden Comprehensive Cancer Center Data and Safety Monitoring Committee (DSMC) composed of individuals with the appropriate expertise per the Data and Safety Monitoring Plan in Appendix 3. The Clinical Research Safety Officer (CRSO) of the DSMC will be the primary interface with the study's PI and research team to collect and assemble all required materials for review by the Committee.

13.2: Staff Training:

Study team and personnel will be trained about the protocol per HCCC regulatory usual workflow with an oversight by the sponsor-investigator**.**

Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor-investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov.

All results of primary and secondary objectives must be posted to CT.gov within a year of completion. The sponsor-investigator has delegated responsibility to protocol review and monitoring committee's (PRMC) & Informatics teams in the HCCC CRS.

Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and study site contact information

13. DATA MANAGEMENT

Generally, clinical data will be electronically captured in the EDC system Advarra EDC and correlative results will be captured in the Oncore system or other secure database(s)/files with sponsor-investigator and statistician.

If procedures on the study calendar are performed for standard of care, at minimum, that data will be captured in the source document. Select standard of care data will also be captured in the EDC system, according to study-specific objectives. The completed dataset is the sole property of the sponsor-investigator's institution. It should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from the sponsor-investigator.

Confidentiality

There is a slight risk of loss of confidentiality of subject information. All records identifying the
subjects will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Information collected will be maintained on secure, password protected electronic systems/files. Paper files that contain personal

information will be kept in locked and secure locations only accessible to the study site personnel. All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor-investigator, representatives of the Institutional Review Board (IRB), regulatory agencies including FDAor pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The study site will permit access to such records.

If/when the results of the study are published, the subject's identity will remain confidential.

Record Retention To enable evaluations and/or audits from Health Authorities the CRS staff will keep records, including the identity of all subjects (sufficient information to link records, e.g., hospital records), all original signed informed consent forms, and records of drug disposition etc. All source documents are to remain in the subject's file and retained per CRS regulatory policy.

14. INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS

14.1 Identity of investigational product(s)

Table 9 List of Investigational Agent

List of Investigational Products for This Study		
Investigational product	**Dosage form and strength**	**Manufacturer**
Durvalumab	*50 mg/mL concentrate for solution for infusion*	AstraZeneca
Ascorbate	25 g / 50 mL	McGuff Pharmaceutical

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Appendix 1 Dosing Modification and Toxicity Management Guidelines (TMGs) for Durvalumab (version October 2022)

Adverse Events	Severity Grade of the Event	Dose Modifications	Toxicity Management
Pneumonitis/Interstitial Lung Disease (ILD)	**Any Grade** Refer to NCI CTCAE v 5.0	**General Guidance**	**For Any Grade**
			− Patients should be thoroughly evaluated to rule out any alternative etiology with similar clinical presentation (e.g., infection, progressive disease). − Monitor patients for signs (e.g., tachypnoea) and symptoms of pneumonitis or ILD (new onset or worsening shortness of breath or cough). Evaluate patients with imaging and pulmonary function tests, including other diagnostic procedures as described below. − Suspected pneumonitis should be confirmed with radiographic imaging and other infectious and disease-related etiologies excluded and managed as described below.
			− Initial work-up may include clinical evaluation, monitoring of oxygenation via pulse oximetry (resting and exertion), laboratory work-up (including clinically relevant culture specimens to rule out infection), and high- resolution computedtomography (CT) scan. −Consider Pulmonary and Infectious Diseases consults.

	Grade 1	No dose modifications required. However, consider holding study drug/study regimen dose as clinically appropriate and during diagnostic work-up for other etiologies.	**For Grade 1**: – Monitor and closely follow up in 2 to 4 days for clinical symptoms, pulse oximetry (resting and exertion), and laboratory work-up, and then as clinically indicated.
	Grade 2	Hold study drug/study regimen dose until Grade 2 resolution to Grade ≤1. – If toxicity improves to Grade ≤1, then the decision to reinitiate study drug/study regimen will be based upon treating physician's clinical judgment and after completion of steroid taper (≤10 mg prednisone or equivalent).	**For Grade2:** – Monitor symptoms daily and consider hospitalization, as clinically indicated. – Consider Pulmonary and Infectious DiseasesConsults. – Promptly start systemic steroids (e.g., prednisone 1 to 2 mg/kg/day PO or IVequivalent).

			– Consider HRCT or chest CT with contrast, repeat imaging study as clinically indicated – If no improvement within 2 to 3 days, additional workup should be considered and prompt treatment with IV methylprednisolone 2 to 4 mg/kg/day started. – If no improvement within 2 to 3 days despite IV methylprednisolone at 2 to 4 mg/kg/day, promptly start

			immunosuppressive therapy. such as tumor necrosis factor (TNF) inhibitors (e.g., infliximab at 5 mg/kg IV once, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. – Consider, discussing with Clinical Study Lead.
	Grade 3 or 4	Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** – Hospitalize the patient – Promptly initiate empiric IV methylprednisolone 1 to 4 mg/kg/day or equivalent. – Obtain Pulmonary and Infectious Diseases Consults; consider discussing with Clinical Study Lead, as needed. – Consider starting anti-infective therapy if infection is still a consideration on the basis of other diagnostic testing despite negative culture results – Supportive care (e.g., oxygen). – If no improvement within 2 days, additional workup should be considered and prompt treatment with additional immunosuppressive therapy such

			as TNF inhibitors (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). Caution: rule out sepsis and refer to infliximab label for general guidance before using infliximab.
Diarrhea/Colitis	**Any Grade**	**General Guidance**	**For Any Grade**
			– Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression,other medications, or infections), including testing for *Clostridium difficile* toxin, etc. – Monitor for symptoms that may be related to diarrhea/enterocolitis (abdominal pain, cramping, or changes in bowel habits such as increased frequency over baselineor blood in stool) or related to bowel perforation (such as sepsis, peritoneal signs, and ileus). – Consider further evaluation with imaging study with contrast. – Consult a gastrointestinal (GI) specialist for consideration of further workup. – WHEN SYMPTOMS OR EVALUATION INDICATE AN INTESTINAL PERFORATION IS SUSPECTED, CONSULT A

			SURGEON EXPERIENCED IN ABDOMINAL SURGERY IMMEDIATELY WITHOUT ANY DELAY. – **PERMANENTLY DISCONTINUE STUDY DRUG FOR ANY GRADE OF INTESTINAL PERFORATION.** – Steroids should be considered in the absence of clear alternativeetiology, even for low-grade events, in order to prevent potential progression to higher grade events, including intestinal perforation. – Use analgesics carefully; they can mask symptoms of perforation and peritonitis.
	Grade 1	No dose modifications.	**For Grade 1** – Monitor closely for worsening symptoms. – Consider symptomatic treatment, including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), loperamide, and other supportive care measures. – If symptoms persist, consider checking lactoferrin; if positive, treat as Grade 2 below. If negative andno infection, continue Grade 1 management.

	Grade 2	Hold study drug/study regimen until resolution to Grade ≤1 – If toxicity improves to Grade ≤1, then study drug/study regimen can be resumed after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 2** – Consider symptomatic treatment, including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), and loperamide and/or budesonide. –Consider further evaluation with imaging study with contrast. –Consider consult of a gastrointestinal (GI) specialist for consideration of further workup. –Promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. – If no improvement within 3 days despite therapy with 1 to 2 mg/kg IV methylprednisolone, reconsult GI specialist and, if indicated, promptly start additional immunosuppressant agent such as infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines. **Caution: it is important to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab.** – **If perforation is suspected, consult a surgeon experienced in abdominal surgery immediately without any delay.**

			− Consider, as necessary, discussing with Clinical Study Lead if no resolution to Grade ≤1 in 3 to 4 days.
	Grade 3 or 4	**Grade 3** −For patients treated with durvalumab monotherapy, hold study drug/study regimen until resolution to Grade ≤1; study drug/study regimen can be resumed after completion of steroid taper (<10 mg prednisone per day, or equivalent). **Permanently discontinue** durvalumab for 1) Grade 3 diarrhea/colitis or 2) Any grade of intestinal perforation	**For Grade 3 or 4** – Urgent GI consult and imaging and/or colonoscopy asappropriate. – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. – Monitor stool frequency and volume and maintainhydration. – If still no improvement within 2 days, continue steroids and promptly add further immunosuppressants. (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution**: Ensure GI consult to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab. – **If perforation is suspected,consult a surgeon experienced in abdominal surgery immediately without any delay.**

		Grade 4 Permanently discontinue study drug/study regimen.	
Hepatitis	**Any Grade**	**General Guidance**	**For Any Grade**
Infliximab should not be used for management of immune-related hepatitis.	ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN	– No dose modifications – If it worsens, then consider holding therapy.	– Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., viral hepatitis, disease progression, concomitant medications). – Monitor and evaluate transaminases (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]) and total bilirubin. –Continue transaminase and total bilirubin monitoring per protocol.
	ALT or AST > 3 ≤ 5 x ULN or total bilirubin > 1.5 ≤ 3 x ULN		

		– Hold study drug/study regimen dose until ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN. Resume study drug/study regimen after completion of steroid taper (<10 mg prednisone or equivalent). – Permanently discontinue study drug/study regimen for any case meetingHy's law laboratory criteria (AST or ALT >3 × ULNAND bilirubin ≥2 × ULN without initial findings of cholestasis (i.e., elevated ALP) and in the absence of any alternative cause.	– Regular and frequent checking of transaminases and total bilirubin (e.g., every 1 to 2 days) until transaminases and total bilirubin elevations improve or resolve. – If no resolution to ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN in 1 to 2 days, consider discussing with Clinical Study Lead, as needed. – If event is persistent (>2 to 3 days) or worsens, promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent.
	ALT or AST > 5- ≤ 10 x ULN	– Hold study drug/study regimen. Resume study drug/study regimen if elevations downgrade to ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN after completion ofsteroid taper (<10 mgprednisone, or equivalent).	– Promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent. – Perform Hepatology Consult, abdominal workup, and imaging as appropriate. – If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, promptly start treatment with an additional immunosuppressant. (e.g., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss with Clinical Study Lead if mycophenolate is not

			available. **Infliximab should NOT be used.**
	Concurrent ALT or AST > 3 x ULN and total bilirubin > 2 x ULN ALT or AST > 10 x ULN OR total bilirubin > 3 x ULN	Permanently discontinue study drug/study regimen.	Promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent. − If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, promptly start treatment with an additional immunosuppressant.(e.g., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss with Clinical Study Lead if mycophenolate is not available. **Infliximab should NOT be used.** − Perform Hepatology Consult, abdominal workup
Hepatitis **(Elevated transaminases and total bilirubin)** ***Infliximab should not be used for management of immune-related hepatitis.***	**Any Elevations of AST, ALT, or T. Bili as Described Below**	**General Guidance**	**For Any ElevationsDescribed** – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., viral hepatitis, disease progression, concomitant medications, worsening of liver cirrhosis [e.g., portal vein thrombosis]). – Monitor and evaluate AST, ALT, ALP, and T. Bili. – For hepatitis B (HBV) + patients: evaluate quantitative HBV viral load, quantitative Hepatitis B surface antigen (HBsAg), or Hepatitis B envelope antigen (HBeAg).

THIS shaded area is guidance *only* for management of "Hepatitis (elevated LFTs)" in HCC patients (or secondary tumour involvement of the liver with abnormal baseline values [BLV])			− For hepatitis C (HCV) + patients: evaluate quantitative HCV viralload. − Consider consulting Hepatology or Infectious Diseases specialists regarding changing or starting antiviral HBV medications if HBV viral load is >2000 IU/ml. − Consider consulting Hepatology or Infectious Diseases specialists regarding changing or starting antiviral HCV medications if HCV viral load has increased by ≥2-fold. − For HCV+ with Hepatitis B core antibody (HBcAb)+: Evaluate for both HBV and HCV as above.
See instructions at bottom of shaded area if transaminase rise is not isolated but (at any time) occurs in setting of either **increasing bilirubin or signs of DILI/liver decompensation**	Isolated AST or ALT>ULN and ≤2.5×BLV,	No dose modifications. − If ALT/AST elevations represents significant worsening based on investigator assessment, then treat as described for elevations in the row below. − For all transaminase elevations, see instructions at bottom of shaded area if transaminase rise is not isolated but (at any time) occurs in setting of either **increasing bilirubin or signs of DILI/liver decompensation**	
	ALT or AST > 2.5- ≤ 5X BLV and ≤ 20xULN	− Hold study drug/study regimen dose until resolution to AST or ALT ≤2.5×BLV. − If toxicity worsens, then treat as described for elevations in the rows below. If toxicity improves to AST or ALT ≤2.5×BLV, resume studydrug/study regimen after co of steroid taper (<10 mg prednisone,or equivalent). completion	− Regular and frequent checking of Transaminases and total bilirubin (e.g., every 1 to 3 days) until elevations of these are improving or resolved. − Recommend consult hepatologist; consider abdominal ultrasound, including Doppler assessment of liver perfusion. − Consider, as necessary, discussing with Clinical Study Lead.

		If event is persistent (>2 to 3 days) or worsens, and investigator suspects toxicity to be an imAE, start prednisone 1 to 2 mg/kg/day PO or IV equivalent. − If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day of prednisone PO or IV equivalent, consider additional workup. If still no improvement within 2 to 3 days despite 2mg/kg/day of IV methylprednisolone, consider additional abdominal workup (including liver biopsy) and imaging (i.e., liver ultrasound), and consider starting additional immunosuppressants. (e.g., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss Clinical Study Lead if mycophenolate mofetil is not available. **Infliximab should NOT be used.**
ALT or AST >5-7X BLV and ≤ 20X ULN OR concurrent 2.5-5X BLV and ≤ 20XULN AND total bilirubin > 1.5 - < 2 x ULN	Withhold durvalumab – Resume study drug/study regimen if elevations downgrade to AST or ALT ≤2.5×BLV and after completion of steroid taper (<10 mg prednisone, or equivalent). − Permanently discontinue study drug/study regimen if the elevations do not downgrade to AST or ALT ≤2.5×BLV within 14 days	Regular and frequent checking of LFTs (e.g., every 1-2 days) until elevations of these are improving or resolved. − Consult hepatologist (unless investigator is hepatologist); obtain abdominal ultrasound, including Doppler assessment of liver perfusion; and consider liver biopsy. − If investigator suspects toxicity to be immunemediated, promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent. − If no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, obtain liver biopsy (if it has not been done already) and promptly start treatment with an additional immunosuppressant. (e.g.,., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with a hepatologist or relevant practice guidelines).Discuss with Study Clinical Lead if mycophenolate is not available. **Infliximab should NOT be used.**

	ALT or AST > 7 X BLV OR > 20 ULN whichever occurs first OR bilirubin > 3ULN	Permanently discontinue study drug/study regimen.	**Same as above** **(Except recommend obtaining liver biopsy early)**
Nephritis and/or renal dysfunction	**Any Grade**	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, infections, recent IV contrast, medications, fluid status). − Consider Consulting anephrologist. −Consider imaging studies to rule out any alternative etiology − Monitor for signs and symptoms that may be related to changes in renal function (e.g., routine urinalysis, elevated serum BUN and creatinine, decreased creatinine clearance, electrolyte imbalance, decreased urine output, or proteinuria). Follow urine protein/creatinine ratio every 3-7 days

	Grade 1	No dose modifications.	**For Grade 1** – Monitor serum creatinine weekly and any accompanyingsymptoms. – If creatinine returns to baseline, resume regular monitoring per study protocol. – If creatinine worsens, depending on the severity, treat as Grade 2, 3, or 4. − Consider hydration, electrolyte replacement, and diuretics, as clinically indicated. − Consider nephrologist consult if not resolved within – 14 days, or earlier as clinically indicated
	Grade 2	Hold study drug/study regimen until resolution to Grade ≤1 or baseline. • If toxicity improves to Grade ≤1 or baseline, then resume study drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 2** – Consider including hydration, electrolyte replacement, and diuretics as clinicallyindicated – Follow urine protein/creatinine ratio every 3-7 days – Carefully monitor serum creatinine as clinically warranted. – Consult nephrologist and consider renal biopsy if clinicallyindicated. – Start prednisone 0.5 – 1 mg/kg/day if other causes are ruled out – If event is persistent beyond 5 days or worsens, increase to prednisone up to 2 mg/kg/day PO or IV equivalent. – If event is not responsive within 5 days or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, consider additional workup. When event returns to baseline, resume study drug/study

			regimen and routine serum creatinine monitoring per study protocol.
	Grade 3 or 4	Permanently discontinue study drug/study regimen	**For Grade 3 or 4** Carefully monitor serum creatinine daily. − Follow urine protein/creatinine ratio every 3-7 days − Consult nephrologist and consider renal biopsy if clinically indicated. − Promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. − If event is not responsive within 3 to 5 days of steroids or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, consider additional workup and prompt treatment with an immunosuppressant
Rash or Dermatitis **(Including Pemphigoid)**	**Any Grade**	**General Guidance**	**For Any Grade** Patients should be thoroughly evaluated to rule out any alternative etiology. − Monitor for signs and symptoms of dermatitis (rash and pruritus). **HOLD STUDY DRUG IF GRADE 3 PEMPHIGOID OR SEVERE CUTANEOUS ADVERSE REACTION (SCAR)1 IS SUSPECTED.** **−PERMANENTLY DISCONTINUE STUDY DRUG IF SCAR OR GRADE 3 PEMPIGOID IS CONFIRMED.**

	Grade 1	No dose modifications.	**For Grade 1** – Consider symptomatic treatment, including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy (e.g., emollient, lotion, or institutional standard).
	Grade 2	For persistent(>1 week) Grade 2 events, hold scheduled study drug/study regimen until resolution to Grade ≤1 or baseline. –If toxicity improves to Grade ≤1 or baseline, then resume drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 2** – Consider dermatology consult and skin biopsy, as indicated. – Consider symptomatic treatment, including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy – Consider moderate-strength topical steroid. – If no improvement of rash/skin lesions occurs within 1 week or is worsening despite symptomatic treatment and/or use of moderate strength topical steroid, consider discussing with Clinical Study Lead, as needed, and promptly start systemic steroids such as prednisone 1 to 2 mg/kg/day PO or IV equivalent.
	Grade 3	**For Grade 3** – Hold study drug/study regimen until resolution to Grade ≤1 or baseline. – If toxicity improves to Grade ≤1 or baseline, then resume drug/study regimen after completion	**For Grade 3** Reconsult a dermatologist. Consider skin biopsy(preferably more than 1) as clinically feasible. – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day orequivalent. –Considerhospitalization.

		of steroid taper (<10mg mg prednisone, or equivalent).	
	Grade 4	**For Grade 4** Permanently discontinue study drug/study regimen.	**For Grade 4** – Reconsult a dermatologist. Consider skin biopsy (preferably more than 1) as clinically feasible. – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. – Consider hospitalization. – Monitor the extent of rash [Rule of Nines]. Consider, as necessary, discussing with Clinical Study Lead.
Endocrinopathy (e.g., hyperthyroidism, thyroiditis, hypothyroidism, type 1 diabetes mellitus)	**Any Grade**	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression including brain metastases, or infections). –Consider consulting an endocrinologist for endocrine events. – Consider discussing with Clinical Study Lead, as needed. – Monitor patients for signs and symptoms of endocrinopathies. (Non-specific symptoms include headache, fatigue, behaviour changes, mental status changes, photophobia, visual field cuts, vertigo, abdominal pain, unusual

		bowel habits, polydipsia, polyuria, hypotension, and weakness.) – Depending on the suspected endocrinopathy, monitor and evaluate thyroid function tests: thyroid stimulating hormone (TSH), free T3 and free T4 and other relevant endocrine and related labs (e.g., blood glucose and ketone levels, hemoglobin A1c (HgA1c)). If a patient experiences an AE that is thought to be possibly of autoimmune nature (e.g., thyroiditis, pancreatitis, hypophysitis, or diabetesinsipidus), the investigator should send ablood sample for appropriate autoimmune antibody testing. –Investigators should ask subjects with endocrinopathies who may require prolonged or continued hormonal replacement, to consult their primary care physicians or endocrinologists about further monitoring and treatment after completion of the study.

Grade 1	No dose modifications.	**For Grade 1** – Monitor patient with appropriate endocrine function tests. – For suspected hypophysitis/hypopituitarism, consider consulting an endocrinologist to guideassessment of early morning adrenocorticotropin hormone (ACTH), cortisol, TSH and freeT4; also consider gonadotropins, sex hormones, and prolactin levels, as well as cosyntropin stimulation test (though it may not be useful in diagnosing early secondary adrenal insufficiency). –If TSH < 0.5 × LLN, or TSH >2 × ULN, or consistently out of range in 2 subsequent measurements, include free T4 at subsequent cycles as clinically indicated and consider consultation of an endocrinologist.
Grade 2, 3, or 4	– For Grade 2-4 endocrinopathies other than hypothyroidism and type 1diabetes mellitus (T1DM), consider holding study drug/study regimen dose until acute symptoms resolve. – Study drug/study regimen can be resumed once patient stabilizes and	**For Grade 2, 3, or 4** Consult endocrinologist to guide evaluation of endocrine function and, as indicated by suspected endocrinopathy and as clinically indicated, consider pituitary scan. – For all patients with abnormal endocrine work up, except those with isolated hypothyroidism or T1DM, and as guided by an endocrinologist, consider short-term corticosteroids (e.g., 1 to 2 mg/kg/day methylprednisolone or IV equivalent) and prompt initiation of treatment with relevant hormone replacement.

		after completion of steroid taper (<10 mg prednisone, or equivalent). − Patients with endocrinopathies who may require prolonged or continued steroid replacement (e.g., adrenal insufficiency) can be retreated with study drug/study regimen if the patient is clinically stable as perclinical judgement.	− Isolated hypothyroidism may be treated with replacement therapy, without study drug/study regimen interruption, and without corticosteroids. − Isolated T1DM may be treated with appropriate diabetic therapy, and without corticosteroids. **Only hold study drug/study regimen in setting of hyperglycemia when diagnostic workup is positive for diabetic ketoacidosis.** − For patients with normal endocrine workup (laboratory assessment or magnetic resonance imaging (MRI) scans), repeat laboratory assessments/MRI as clinicallyindicated.
Amylase/Lipase increased	**Any Grade**	**General Guidance**	**For Any Grade** Patients should be thoroughly evaluated to rule out any alternative etiology (e.g. disease progression, viral infection, concomitant medications, substance abuse). − For modest asymptomatic elevations in serum amylase and lipase, corticosteroid treatment is not indicated as long as there are no other signs or symptoms of pancreatic inflammation. − Assess for signs/symptoms of pancreatitis − Consider appropriate diagnostic testing (e.g., abdominal CT with contrast, MRCP if clinical suspicion of pancreatitis and no radiologic evidence on CT) − If isolated elevation ofenzymes without evidence of pancreatitis, continue immunotherapy. Consider other causes of elevated amylase/lipase – If evidence of pancreatitis, manage according to pancreatitis recommendations
	Grade 1	No dose modifications.	
	Grade 2, 3, or 4	**For Grade 2, 3, or 4** In consultation with relevant gastroenterology specialist consider continuing study drug/study regimen if no clinical/radiologic evidence of pancreatitis ± improvement in amylase/lipase.	

Acute Pancreatitis	**Any Grade**	**General Guidance**	**For Any Grade** – Patients should be thoroughly evaluated to rule out any alternative etiology. – Consider Gastroenterology referral
	Grade 2	**Consider holding study drug/regimen**	**Grade 2** – Consider IV hydration – Consider Gastroenterology referral
	Grade 3, or 4	**For Grade 3** Hold study drug/study regimen until resolution of elevated enzymes and no radiologic findings If no elevation in enzymes or return to baseline values, then resume study drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent). **For Grade 4** Permanently discontinue study drug/study regimen.	**For Grade 3, or 4** – Promptly start systemic steroids prednisone 1 to 2 mg/kg/day PO or IV equivalent. – IV hydration
Nervous System Disorders			

Aseptic Meningitis	**Any Grade**	**General Guidance**	**For Any Grade**
		– Symptoms may include headache, photophobia, and neck stiffness, nausea/ vomiting which may resemble an infectious meningitis. – Patients may be febrile. – Mental status should be normal Permanently discontinue study treatment	Consider neurology consult Consider MRI brain with and without contrast with pituitary protocol and a lumbar puncture for diagnosis. Exclude bacterial and viral infections. (ie HSV) Consider IV acyclovir until polymerase chain reactions are available Consider hospitalization. – Once infection has been ruled out promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent.

Encephalitis	**Any Grade**	**General Guidance** – Symptoms may include Confusion, altered behaviour, headaches, seizures, short-term memory loss, depressed level of consciousness, focal weakness, and speech abnormality.	**For Any Grade** – Consider neurology consult – Consider testing including MRI of the brain with and without contrast, lumbar puncture, electroencephalogram (EEG) to evaluate for subclinical seizures, ESR, CRP, antineutrophil cytoplasmic antibody (ANCA) (if vasculitic process suspected), thyroid panel including TPO and thyroglobulin and additional autoantibodies to rule out paraneoplastic disorders. – Exclude bacterial and viral infections. (i.e. HSV)Consider IV acyclovir until polymerase chain reactions are available.
	Grade 2	**For Grade 2** Permanently discontinue study drug/study regimen.	**For Grade 2** – Consider, as necessary, discussing with the Clinical Study Lead. – Once infection has been ruled out methylprednisolone 1–2 mg/kg/day – For progressive symptoms or if oligoclonal bands are present consider methylprednisolone 1 g IV daily for 3–5 days plus IVIG or plasmapheresis
	Grade 3 or 4	**For Grade 3 or 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** – Consider, as necessary, discussing with Clinical Study Lead. – Consider hospitalization.

			– Once infection is ruled out, start methylprednisolone 1 g IV daily for 3–5 days for progressive symptoms consider adding IVIG or plasmapheresis
Transverse Myelitis	**Any Grade**	**General Guidance** Permanently discontinue Immunotherapy – Consider MRI of the spine and brain Once imaging is complete, consider lumbar puncture Consider testing to rule out additional etiologies: B12, HIV, rapid plasma reagin (RPR), ANA, anti-Ro/La antibodies, aquaporin-4 IgG, myelin oligodendrocyte glycoprotein (MOG) IgG, paraneoplastic panel for anti-Hu and anti-CRMP5/CV2	**For Any Grade** – Consider neurology consult – Inpatient care – Consider prompt initiation of high methylprednisolone pulse dosing – Strongly consider IVIG or plasmapheresis
Peripheral neuropathy	**Any Grade**	**General Guidance**	**For Any Grade** Patients should be evaluated to rule out any alternative etiology for neuropathy (e.g., disease progression, infections, metabolic syndromes or medications). It should be noted that the diagnosis of immune-mediated peripheral neuromotor syndromes can be particularly challenging in patients with underlying cancer, due to the multiple potential confounding effects of cancer (and its treatments) throughout the neuraxis. Given the importance of prompt and accurate diagnosis, it is essential to have a low threshold to obtain a neurological consult. – Neurophysiologic diagnostic testing (e.g., electromyogram and nerve conduction investigations are routinely indicated upon suspicion of such conditions and

			may be best facilitated by means of a neurology consultation.
	Grade 1	No dose modifications.	**For Grade 1** Consider discussing with the Clinical Study Lead, as needed. Monitor symptoms for interference with ADLS, gait difficulties, imbalance, or autonomic dysfunction
	Grade 2	Hold study drug/study regimen dose until resolution to Grade ≤1.	**For Grade 2** Consult a neurologist. Consider EMG/NCS Consider discussing with the Clinical Study Lead, as needed. Observation for additional symptoms or consider initiating prednisone 0.5–1 mg/kg orally If progression, initiate methylprednisolone 2–4 mg/kg/day and treat as GBS Sensory neuropathy/neuropathic pain may be managed by appropriate medications (e.g., gabapentin or duloxetine).

	Grade 3 or 4	**For Grade 3 or 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** Consider discussing with Clinical Study Lead, as needed. Recommend hospitalization. Monitor symptoms and consult a neurologist. **Treat per Guillain-Barré Syndrome recommendations**
Guillain-Barré Syndrome (GBS)		General Guidance	Recommend hospitalization Obtain neurology consult Obtain MRI of spine to rule out compression lesion Obtain lumbar puncture Antibody tests for GBS variants Pulmonary function tests Obtain electromyography (EMG) and nerve conduction studies Frequently monitor pulmonary function tests and neurologic evaluations Monitor for concurrent autonomic dysfunction Initiate medication as needed for neuropathic pain
	Grade 2-4	Grade 2-4 Permanently discontinue	**Start IVIG or plasmapheresis in addition to methylprednisolone 1**

			gram daily for 5 days, then taper over 4 weeks.
Myasthenia gravis		General Guidance	Obtain neurology consult Recommend hospitalization Obtain pulmonary function tests Obtain labs: ESR, CRP, creatine phosphokinase (CPK), aldolase and anti-striational antibodies Consider cardiac exam, ECG, troponin, transthoracic echocardiogram for possible concomitant myocarditis Obtain electromyography EMG) and nerve conduction studies Consider MRI of brain/spine to rule out CNS involvement by disease Avoid medications that might exacerbate MG (e.g. beta blockers, some antibiotics, IV magnesium)
	Grade 2	Permanently discontinue	− Consider pyridostigmine 30mg three times daily and gradually increase based on symptoms (max dose 120mg four times daily) − Consider starting low dose prednisone 20mg daily and increase every 3-5 days. (Target dose 1mg/kg/day. Max dose 100mg daily)
	Grade 3-4	Permanently discontinue	Start methylprednisolone 1-2mg/kg/day. Taper steroids based on symptom improvement Start plasmapheresis or IVIG

			Consider rituximab if refractory to plasmapheresis or IVIG Frequent PFT assessments Daily neurologic evaluations
Myocarditis	**Any Grade**	**General Guidance** Discontinue drug permanently if biopsy- proven immune-mediated myocarditis.	**For Any Grade** Initial work-up should include clinical evaluation, B- type natriuretic peptide (BNP), cardiac enzymes, electrocardiogram (ECG), echocardiogram (ECHO), monitoring of oxygenation via pulse oximetry (resting and exertion), and additional laboratory work-up as indicated. Spiral CT or cardiac MRI can complement ECHO to assess wall motion abnormalities when needed. Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections) The prompt diagnosis of immune-mediated myocarditis is important, particularly in patients with baseline cardiopulmonary disease and reduced cardiac function. Consider discussing with the Clinical Study Lead, as needed. Monitor patients for signs andsymptoms of myocarditis (new onset or worsening chest pain, arrhythmia, shortness of breath, peripheral edema). As some symptoms can overlap with lung toxicities, simultaneously evaluate for and rule out pulmonary toxicity aswell

			as other causes (e.g., pulmonary embolism, congestive heart failure, malignant pericardial effusion). Consult a cardiologist early, to promptly assess whether and when to complete a cardiac biopsy, including any other diagnostic procedures as indicated. Spiral CT or cardiac MRI can complement ECHO to assess wall motion abnormalities when needed.
	Grade 2, 3 or 4	–If Grade 2-4, permanently discontinue study drug/study regimen.	**For Grade 2-4** Monitor symptoms daily, hospitalize. Consider cardiology consultation and a prompt start of high-dose/pulse corticosteroid therapy Supportive care (e.g., oxygen). If no improvement consider additional immunosuppressive therapy such as TNF inhibitors (e.g., infliximab), IVIG or plasmapheresis or other therapies depending on the clinical condition of the patient, based on the discretion of the treating specialist consultant r or relevant practice guidelines. **Caution:It is important to rule out sepsis and**

			refer to infliximab label for general guidance before using infliximab. Infliximab is contraindicated for patients who have heart failure.
Myositis/ Polymyositis	**Any Grade**	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections).
			− Monitor patients for signs and symptoms of poly/myositis. Typically, muscle weakness/pain occurs in proximal muscles including upper arms, thighs, shoulders, hips, neck and back,and; also difficulty breathing and/or trouble swallowing can occur and progress rapidly. Increased general feelings of tiredness and fatigue may occur, and there can be new onset falling, difficulty getting up from a fall, and trouble climbing stairs, standing up from a seated position, and/or reaching up. − If poly/myositis is suspected, a Neurology consultation should be obtained early, with prompt guidance on diagnostic procedures. Myocarditis may co-occur with poly/myositis; refer to guidance under Myocarditis. Given breathing complications, refer to guidance under Pneumonitis/ILD. Given possibility of an existent (but previously unknown) autoimmune disorder, consider Rheumatology consultation.

		−Consider, as necessary, discussing with the Clinical Study Lead. − Initial work-up should include clinical evaluation, creatine kinase, aldolase, lactate dehydrogenase (LDH), blood urea nitrogen (BUN)/creatinine, erythrocyte sedimentation rate or C-reactive protein (CRP) level, urine myoglobin, and additional laboratory work-up as indicated, including a number of possible rheumatological/antibody tests (i.e., consider whether a rheumatologist consultation is indicated and could guide need for rheumatoid factor, antinuclear antibody, anti-smooth muscle, antisynthetase [such as anti-Jo-1], and/or signal- recognition particle antibodies). Confirmatory testing may include electromyography, nerve conduction studies, MRI of the muscles, and/or a muscle biopsy. Consider Barium swallow for evaluation of dysphagia or dysphonia.
Grade 1	− No dose modifications.	**For Grade 1** Monitor and closely follow up in 2 to 4 days for clinical symptoms and initiate evaluation as clinically indicated. Consider Neurology consult.

Grade 2	Hold study drug/study regimen dose until resolution to Grade ≤1. Permanently discontinue study drug/study regimen if it does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency.	**For Grade 2** Monitor symptoms daily and consider hospitalization. − Consider Rheumatology or Neurology consult. − Consider, as necessary, discussing with the Clinical Study Lead. − If clinical course is rapidly progressive (particularly if difficulty breathing and/or trouble swallowing),promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant − If clinical course is *not* rapidly progressive, start systemic steroids (e.g., prednisone 1 to mg/kg/day PO or IV equivalent); if no improvement within 2 to 3 days, continue additional work up and start treatment with IV methylprednisolone 2 to 4 mg/kg/day − If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 3 days, consider additional immunosuppressive therapy such as TNF inhibitors(e.g., infliximab), IVIG or plasmapheresis, or other therapies based on the discretion of the treating specialist consultant or relevant practice guideline **Caution: It is important to rule out sepsis and refer to infliximab label for general guidancebefore using infliximab. rule out sepsis and refer to infliximab label for general guidance before using infliximab.**

Grade 3	**For Grade 3** Hold study drug/study regimen dose until resolution to Grade ≤1. Permanently discontinue study drug/study regimen if Grade 3 imAE does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency.	**For Grade 3** Monitor symptoms closely; recommend hospitalization. Consider Rheumatology and/or Neurology consult Consider discussing with the Clinical Study Lead, as needed. Promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant. If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 2 to 3 days, consider starting another immunosuppressive therapy such as a TNF inhibitor (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.** Consider whether patient may require IV IG, plasmapheresis.

	Grade 4	**For Grade 4** Permanently discontinue study drug/study regimen.	**Grade 4** Monitor symptoms closely; recommend hospitalization. Consider Rheumatology and/or Neurology consult Consider discussing with the Clinical Study Lead, as needed. Promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant. If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 2 to 3 days, consider starting another immunosuppressive therapy such as a TNF inhibitor (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.**

1 SCAR terms include Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN), Erythema Multiforme, Acute Generalized Exanthematous Pustulosis, Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) and Drug-induced hypersensitivity syndrome.

Infusion-Related Reactions

Severity Grade of the Event (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	Dose Modifications	Toxicity Management
Any Grade	General Guidance	**For Any Grade** – Manage per institutional standard at the discretion of investigator. – Monitor patients for signs and symptoms of infusion-related reactions (e.g., fever and/or shaking chills, flushing and/or itching, alterations in heart rate and blood pressure, dyspnea or chest discomfort, or skin rashes) and anaphylaxis (e.g., generalized urticaria, angioedema, wheezing, hypotension, or tachycardia).
Grade 1 or 2	**For Grade 1** The infusion rate of study drug/study regimen may be decreased by 50% or temporarily interrupted until resolution of the event. **For Grade 2** – The infusion rate of study drug/study regimen may be decreased 50% or temporarily interrupted until resolution of the event. – Subsequent infusions may be given at 50% of the initial infusion rate.	**For Grade 1 or 2** – Acetaminophen and/or antihistamines may be administered per institutional standard at the discretion of the investigator. – Consider premedication per institutional standard or study protocol prior to subsequent doses. – Steroids should not be used for routine premedication of Grade ≤2 infusion reactions.
Grade 3 or 4	**For Grade 3 or 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** – Manage severe infusion-related reactions per institutional standard, appropriate clinical practice guidelines, and society guidelines.

Other–Immune-Mediated Reactions

Severity Grade of the Event (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	Dose Modifications	Toxicity Management
Any Grade	Note: It is possible that events with an inflammatory or immune mediated mechanism could occur in nearly all organs, some of them are not noted specifically in these guidelines (e.g. immune thrombocytopenia, haemolytic anaemia, uveitis, vasculitis).	– Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections). – The Clinical Study Lead may be contacted for immune-mediated reactions not listed in the "specific immune-mediated reactions" section – Consultation with relevant specialist – Treat accordingly, as per institutional standard.
Grade 1	No dose modifications.	Monitor as clinically indicated
Grade 2	– Hold study drug/study regimen until resolution to ≤Grade 1 or baseline. – If toxicity worsens, then treat as Grade 3 or Grade 4. – Study drug/study regimen can be resumed once event stabilizes to Grade ≤1 after completion of steroid taper. – Consider whether study drug/study regimen should be permanently discontinued in Grade 2 events with high likelihood for morbidity and/or mortality when they do not rapidly improve to Grade <1 upon treatment with systemic steroids and following full taper	**For Grade 2, 3, or 4** Treat accordingly, as per institutional standard, appropriate clinical practice guidelines, and society guidelines. (See page 4).
Grade 3	Hold study drug/study regimen	
Grade 4	Permanently discontinue study drug/study regimen	

Note: As applicable, for early phase studies, the following sentence may be added: "Any event greater than or equal to Grade 2, please discuss with Clinical Study Lead."

Non-Immune-Mediated Reactions

Severity Grade of the Event (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	Dose Modifications	Toxicity Management
Any Grade	Note: Dose modifications are not required for AEs not deemed to be related to study treatment (i.e., events due to underlying disease) or for laboratory abnormalities not deemed to be clinically significant.	Treat accordingly, as per institutional standard.
Grade 1	No dose modifications.	Treat accordingly, as per institutional standard.
Grade 2-3	Hold study drug/study regimen until resolution to ≤Grade 1 or baseline.	Treat accordingly, as per institutional standard.
Grade 4	Discontinue study drug/study regimen (Note: For Grade 4 labs, decision to discontinue should be based on accompanying clinical signs/symptoms, the Investigator's clinical judgment, and consultation with the Sponsor.).	Treat accordingly, as per institutional standard.

Note: As applicable, for early phase studies, the following sentence may be added: "Any event greater than or equal to Grade 2, please discuss with Clinical Study Lead."

Appendix 2 CYP3A4 Interaction Table

CYP3A4 INTERACTION TABLE
(Ascorbate may decrease the plasma/blood concentrations of substrates)

Drugs with ~~strikeout~~ text are antiretroviral drugs that are not allowed for use on this study.

* 3A4 substrate & inhibitor; # 3A4 substrate & inducer

abiraterone	acetaminophen	ado-trastuzumab	alfentanil
alfuzosin	aliskiren	alitretinoin	almotriptan
alprazolam	ambrisentan	amiodarone*	amitriptyline
amlodipine	amprenavir	apixaban	aprepitant*
aripiprazole	armodafinil#	artemether	asenapine
astemizole	atazanavir*	atorvastatin	avanafil
axitinib	beclomethasone	bedaquiline	benzphetamine
bexarotene	bisoprolol	boceprevir	bortezomib
bosentan#	bosutinib	brentuximab	bromazepam
bromocriptine	budesonide	buprenorphine	buspirone
busulfan	cabazitaxel	cabozantinib	caffeine
canagliflozin	carbamazepine#	cevimeline	chlordiazepoxide
chloroquine	chlorpheniramine	ciclesonide	cilostazol
cinacalcet	cisapride	citalopram	clarithromycin*
clindamycin	clobazam	clomipramine	clonazepam
clopidogrel	clorazepate	clozapine	cobicistat*
cocaine	codeine	colchicine	conivaptan*
crizotinib	cyclobenzaprine	cyclophosphamide	cyclosporine*

CYP3A4 INTERACTION TABLE

(Ascorbate may decrease the plasma/blood concentrations of substrates)

Drugs with ~~strikeout~~ text are antiretroviral drugs that are not allowed for use on this study.

* 3A4 substrate & inhibitor; # 3A4 substrate & inducer

dantrolene	dapsone	darifenacin	~~darunavir~~
dasatinib	delavirdine*	desogestrel	dantrolene
dexamethasone#	dexlansoprazole	dextromethorphan	diazepam
diclofenac	dienogest	dihydroergotamine	diltiazem*
disopyramide	docetaxel	dofetilide	dolasetron
domperidone	donepezil	doxorubicin	dronedarone
droperidol	dutasteride	efavirenz*#	eletriptan
elvitegravir	enzalutamide	eplerenone	ergoloids
ergonovine	ergotamine	erlotinib	erythromycin*
escitalopram	esomeprazole	estazolam	estradiol
estradiol valerate	estrogens	eszopiclone	ethinyl estradiol
ethosuximide	etonogestrel	etoposide	etravirine
everolimus	exemestane	felbamate	felodipine
fentanyl	fesoterodine	fexofenadine	finasteride
fingolimod	flunisolide	flurazepam	flutamide
fluticasone	~~fosamprenavir~~*	fosaprepitant	fulvestrant
galantamine	gefitinib	granisetron	guanfacine
haloperidol*	hydrocodone	hydrocortisone	ifosfamide
iloperidone	imatinib*	imipramine	indacaterol

CYP3A4 INTERACTION TABLE

(Ascorbate may decrease the plasma/blood concentrations of substrates)

Drugs with ~~strikeout~~ text are antiretroviral drugs that are not allowed for use on this study.

* 3A4 substrate & inhibitor; # 3A4 substrate & inducer

~~indinavir~~*	irinotecan	isosorbide dinitrate	isosorbide mononitrate
isradipine	itraconazole*	ivacaftor	ixabepilone
ketamine	ketoconazole*	lansoprazole	lapatinib
lercanidipine	letrozole	levonorgestrel	lidocaine*
linagliptin	lomitapide	loperamide	~~lopinavir~~
loratadine	losartan	lovastatin	lumefantrine
lurasidone	~~maraviroc~~	marijuana	medroxyprogesterone
mefloquine	meloxicam	mestranol	methadone
methylergonovine	methylprednisolone	miconazole*	midazolam
mifepristone	mirabegron	mirtazapine	modafinil
mometasone	montelukast	nateglinide	nefazodone*
~~nelfinavir~~*	~~nevirapine~~#	nicardipine*	nifedipine
nilotinib	nimodipine	nisoldipine	nitrendipine
norethindrone	norgestrel	nortriptyline	omeprazole
ondansetron	ospemifene	oxybutynin	oxycodone
paclitaxel	paliperidone	palonosetron	pantoprazole
paricalcitol	paroxetine	pazopanib	perampanel
perphenazine	pimozide	pioglitazone	pomalidomide

CYP3A4 INTERACTION TABLE

(Ascorbate may decrease the plasma/blood concentrations of substrates)

Drugs with ~~strikeout~~ text are antiretroviral drugs that are not allowed for use on this study.

* 3A4 substrate & inhibitor; # 3A4 substrate & inducer

ponatinib	prasugrel	prednisolone	prednisone
primaquine	progesterone/ progestins	propafenone	propranolol
quazepam	quetiapine	quinidine*	quinine
rabeprazole	ramelteon	ranolazine	regorafenib
repaglinide	rifabutin#	~~rilpivirine~~	risperidone
~~ritonavir~~*	rivaroxaban	roflumilast	romidepsin
ruxolitinib	salmeterol	~~saquinavir~~*	saxagliptin
selegiline	sertraline	sibutramine	sildenafil
silodosin	simvastatin	sirolimus	sitagliptin
solifenacin	sorafenib	sufentanil	sunitinib
tacrolimus	tadalafil	tamoxifen	tamsulosin
~~telaprevir~~	telithromycin*	temsirolimus	teniposide
terfenadine	testosterone	tetracycline*	theophylline
tiagabine	ticagrelor	ticlopidine	tinidazole
~~tipranavir~~	tofacitinib	tolterodine	tolvaptan
topotecan	toremifene	tramadol	trazodone
triazolam	trimethoprim	trimetrexate	trimipramine
ulipristal	vandetanib	vardenafil	vemurafenib

CYP3A4 INTERACTION TABLE

(Ascorbate may decrease the plasma/blood concentrations of substrates)

Drugs with ~~strikeout~~ text are antiretroviral drugs that are not allowed for use on this study.

* 3A4 substrate & inhibitor; # 3A4 substrate & inducer

venlafaxine	verapamil*	vilazodone	vinblastine
vincristine	vinorelbine	vismodegib	voriconazole
warfarin	zaleplon	zileuton	ziprasidone
zolpidem	zonisamide	zopiclone	

Appendix 3 Data and Safety Monitoring Plan

Type of Clinical Trial:

X Investigator-Initiated

Pilot study — **X Phase I**

Phase I/II — Phase II

Phase III — Compassionate- use

X Interventional Treatment — Interventional Non-Treatment

Non-Interventional

Study risk-level:

Level 1—low risk of morbidity or death, * <1% of death or any adverse event

Level 2—risk of death* <1% or any adverse event 1% – 5%

Level 3—risk of death* 1% – 5% or grade 4 – 5 SAE 1% – 5%

X Level 4—risk of death* >5% or grade 4 – 5 SAE >5%

Drugs being used on a "compassionate" basis

* Risk of death" refers specifically to 100-day treatment-related mortality

Reporting and Monitoring Requirements:

All institutional investigator-initiated trials (IITs), regardless of assigned risk level are subject to routine DSMC monitoring activities which may include but are not limited to review of signed consent documents, eligibility and adverse event reporting.

All institutional IITs have the following reporting requirements as part of their DSMP:

- Register all subjects in HCCC's Clinical Trial Management System, OnCore
- Document Adverse Events
- Document protocol deviations

• Provide an annual progress report to the DSMC via OnCore data export

Risk Level 4

Interventional treatment trials involving investigational agents or devices with a risk of death*

(>5% or grade 4 – 5 SAE >5%), e.g. all investigator initiated INDs, most Phase I/II trials, gene

therapy, gene manipulation or viral vector systems high-risk clinical procedures if performed

solely for research purposes. The use of a new chemical or drug for which there is limited or no

available safety data in humans.

Study Safety Review

An independent study monitor and/or the DSMC Chair (or designee), will review study data (provided

by the PI/available in OnCore) and communicate with the PI at least biannually. A copy of this

communication will be forwarded to the DSMC and PRMC Chairs.

Additional Reporting Requirements:

- A scanned copy of the completed eligibility checklist, with screening source documentation and PI

signature, will be attached in OnCore for ongoing review by DSMC staff.

- Serious adverse events will be entered directly into an OnCore SAE report by theresearch team.

OnCore will send an automatic notification to the DSMC Chair/acting Chair and staff for review.

- The DSMC utilizes a risk-based monitoring approach. The trial's research records will be

monitored at minimum twice per year. Monitoring may be done more frequently depending on the

protocol, risks to subjects, reported serious/adverse events, patient population and accrual rate.

Records for a minimum of 25% of subjects will be monitored for the entire study.

Monitoring will involve the following:

- review eligibility of patients accrued to the study,
- check for the presence of a signed informed consent,
- determine compliance with protocol's study plan,
- determine whether SAEs are being appropriately reported to internal and external regulatory

agencies,

- compare accuracy of data in the research record with the primary source documents,
- review investigational drug processing and documentation,
- assess cumulative AE/SAE reports for trends and compare to study stopping rules.

Routine Adverse Event Reporting

For non-serious Adverse Events, documentation must begin from the first day of study treatment and typically continue through the 30 day follow-up period after treatment is discontinued or the specified time point as defined in the protocol.

Collected information should be recorded in the electronic/Case Report Forms (eCRF/CRF) for that subject. A description of the event, its severity or toxicity grade (according to NCI's Common

Toxicity Criteria (CTCAE)), onset and resolved dates (if applicable), and the relationship to the study drug should be included. Documentation should occur in real time. The principal investigator has final responsibility for determining the attribution of the event as it is related to the study drug.

Serious Adverse Event Reporting

For any experience or condition that meets the definition of a serious adverse event (SAE), recording of the event must begin after signing of the informed consent and continue through the 30 day follow-up period after treatment is discontinued.

Investigators must report to the DSMC any serious adverse events (SAE), whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64). SAEs must be reported via an OnCore SAE Report within 24 hours of learning of the event.

An adverse event is considered **serious** if it results in ANY of the following outcomes:

1. Death

2. A life-threatening adverse event

3. An adverse event that results in inpatient hospitalization OR prolongation of existing hospitalizationfor ≥ 24 hours

4. A persistent or significant incapacity or substantial disruption of the ability toconduct normal life functions

5. A congenital anomaly/birth defect.

6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

FDA Reporting Requirements (for Sponsor-Investigators)

It is the responsibility of the IND sponsor-investigator to comply with IND safety reporting as set forth in the Code of Federal Regulations, Section 312.32. This responsibility includes providing an annual IND report to the FDA.

All IND safety reports must be submitted on Form 3500A and be accompanied by Form 1571. The type of report (initial or follow-up) should be checked in the respective boxes on Forms 3500A and 1571.

See Instructions for completing Form 3500A. Please note all instance of UIHC, location, and faculty / staff should be redacted from supporting documentation and the 3500A.

The submission must be identified as:

- “IND safety report” for 15-day reports, or

- "7-day IND safety report" for unexpected fatal or life-threatening suspectedadverse reaction reports, or

- "Follow-up IND safety report" for follow-up information.

For detailed explanation of the above definitions, requirements, and procedures related to IND application safety reports and the responsibilities of IND applications sponsors with regard to such reporting, refer to Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE Studies (PDF -227KB)

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)

- Protocol description (and number, if assigned)

- Description of event, severity, treatment, and outcome, if known (grading the eventper CTCAE)

- Supportive laboratory results and diagnostics

- Sponsor-Investigator's assessment of the relationship of the adverse event toeach investigational product and suspect medication

Data Monitoring and Management

Subject Registration

All studies that undergo PRMC review and/or utilize HCCC Clinical Research Services (CRS) resources are required to register subjects in OnCore. Each subject registration includes the following:

- The subject's IRB approved (version date) consent form and the date of theirconsent.

- Date of eligibility and eligibility status (eligible, not eligible)

- On study date and subject's disease site (and histology if applicable)

- On treatment date (if applicable)

All subject registration information is expected to be entered into OnCore within **2 (two) business days** after the subject's study visit.

Subject Data

For HCCC investigator initiated trials, research staff are responsible for entering subject study data (data collection) into OnCore electronic case report forms (eCRFs). These eCRFs must be approved by the PI and statistician prior to study activation to ensure sufficient and necessary data acquisition. All information entered into eCRFs will be traceable to the source documents which are generally maintained in the subject's file.

eCRF data entry needs to be timely and should be entered into OnCore as soon as possible but no later than **14 (fourteen) business days** after the subject's visit, including adverse events, tumor measurements, administration of study medication, concomitant medications, labs, and vitals. Physical exam assessments must be entered no later than **14 (fourteen) business days** following completion of the physician's clinic note in the medical record.

Timely data entry facilitates remote monitoring of data, allows the data to progress appropriately through the data cleaning process, and helps prevent a backlog of data queries.

Forms Monitoring

OnCore eCRF data are monitored on a routine basis (dependent on accrual) to ensure all data are entered completely, accurately, and within time requirements outlined above. The assigned DSMC monitor will coordinate and complete the data monitoring review. When the time comes to monitor a study (based on patient accrual and assigned risk level of trial) the monitor arranges for a selection of cases to be reviewed from among the subjects registered in OnCore. As part of the forms monitoring process, the assigned monitor will issue queries via OnCore (linked to the eCRF) to resolve missing, incomplete, and/or incorrect information. A member of the research team is expected to respond to these monitoring queries within **14 (fourteen) business days**.

The monitoring process can often identify misunderstandings or deficiencies in the written, research protocol requirements earlier in the study process and thereby improve data quality and reduce rework

Final Reports

A summary of each subject's data record is continually available to the PI, research staff, and DSMC from OnCore's Biostat Console. The availability of this information is a valuable tool for the preparation of final reports and manuscripts as well as ongoing deficiency reports.

Appendix 4 Flowcytometry Panels

40-color Full Immune Profile			
The 40-color panel explores that major immune cell populations. It provides an in-depth analysis of the activation, differentiation, and chemokine receptor expression of major immune cell subsets including T cells, B cells, dendritic cells, natural killer cells, and monocytes.			
Specificity	**Fluorochrome**	**Clone**	**Purpose**
CD45RA	BUV395	5H9	T cell and DC differentiation
CD16	BUV496	3G8	Monocyte, NK cell, and DC differentiation
CCR5	BUV563	2D7/CCR5	Chemokine Receptor; monocyte, DC, T cell, and B cell differentiation
CD314	BUV615	1D11	NK Cell Differentiation
CD39	BUV661	TU66	B cell, Treg, and monocyte differentiation
CD56	BUV737	NCAM16.2	Pan NK cell, gamma-dealta T cell
CD8	BUV805	SK1	CD8 T, NK, NKT-Like cells
CCR7	BV421	G043H7	T cell differentiation
CD123	Super Bright 436	6H6	Plasmacytoid DC
CD11c	Pacific Blue	3.9	DC differentiation
IgD	BV480	IA6-2	B cell differentiation
CD3	BV510	SK7	Pan T cell, NKT-like cells
IgM	BV570	MHM-88	B cell differentiation
CD20	APC-Fire 750	2H7	B cells
IgG	BV605	G18-145	B cell differentiation
CD28	BV650	CD28.2	T cell and NK cell differentiation
CCR6	BV711	G034E3	Chemokine Receptor; T cell and B cell differentiation
CXCR5	BV750	RF8B2	Chemokine Receptor; T cell differentiation
PD-1	BV785	EH12.2H7	T cell inhibitory receptor
CD141	BB515	1A4	DC Differentiation
CD57	FITC	HNK-1	NK and CD8 T cell immune senescence

CD14	NovaFluor Blue 585	MEM-15	Monocyte differentiation
CD45	PerCP	2D1	Leukocytes
CD2	PerCP-Cy5.5	TS1/8	NK cell differentiation
TCRγδ	PerCP-eFluor 710	B1.1	Pan gamma-delta T cells
CD4	eFluor YG584	SK3	CD4 T and NKT-like cells
CD159c	PE	REA205	NK Cell differentiation
CD337	PE-Dazzle 594	P30-15	NK Cell Differentiation
CD24	PE-AF610	SN3	B cell differentiation
CD95	PE-Cy5	DX2	T cell and B cell differentiation
CD25	PE-AF700	CD25-3G10	Regulatory T cells
CXCR3	PE-Cy7	G025HZ	Chemokine receptor; DC, T cell, and B cell differentation
HLA-DR	PE-Fire 810	L243	T cell and monocyte activation, NK cell lineage discriminatiuon, DC lineage
CD159a	APC	REA110	NK, NKT-like, and gamma-delta T cell activation/differentiation
CD1c	AF647	L161	DC , NKT-like cells
CD19	NovaFluor Red 700	HIB19	B cells
CD127	APC-R700	HIL-7R-M21	Cytokine recetpor, T cell and NK cell differentiation
CD27	NovaFluor Blue 610	O323	T and B cell differentiation
CD38	APC-Fire 810	HIT2	Monocyte, DC, T cell, and B cell activation/differentiation
Viability	Live/Dead Blue	N/A	Viability

31-color T cell Steady-state

The 31-color T cell steady-state panel defines the steady-state phenotype of the subsets of CD4, CD8, and gamma-delta T cells. This panels includes markers to distinguish the main subsets (naïve, memory) of T cells and can determine differentiation status, activation status, and identifies CD4 T helper cell subsets

Specificity	**Fluorochrome**	**Clone**	**Purpose**
CD45RA	BUV395	5H9	Subset differentiation
CD11a	BUV496	HI111	Activation/Antigen Experience
CD49d	BUV563	L25	Activation/Antigen Experience
CCR4	BUV615	1G1	Differentiation/Trafficking

CD73	BUV661	AD2	Subset differentiation
CD56	BUV737	NCAM16.2	Activation
CD8	BUV805	SK1	Lineage
CD226	BV421	11A8	Function
CD27	Pacific Blue	M-T271	Subset differentiation
CD3	BV510	SK7	Lineage
CD25	BV605	BC96	Subset differentiation
CCR7	BV650	G043H7	Subset differentiation
CCR6	BV711	G034E3	Differentiation/Trafficking
CD28	BV750	CD28.2	Subset differentiation
TIM-3	BB515	7D3	Co-inhibitory
CD45	AF532	HI30	Lineage
CD4	NovaFluor Blue 610-70s	SK3	Lineage
CD45RO	PerCP	UCHL1	Subset differentiation
CXCR5	BB700	RF8B2	Differentiation/Trafficking
PD-1	PE	NAT105	Co-inhibitory
CD38	Spark YG 581	S17015F	Activation
CXCR3	PE-Dazzle 594	G025H7	Differentiation/Trafficking
CD95	PE-Fire 640	DX2	Subset differentiation
TCRγδ	PE-Fire 700	B1.1	Lineage
CD39	PE-Fire 810	A1	Activation
Foxp3	AF660	PCH101	Subset differentiation
KLRG1	APC	SA231A2	Subset differentiation
CD127	AF700	A019D5	Subset differentation
Granzyme B	APC-Fire 750	QA16A02	Function
HLA-DR	APC-Fire 810	L243	Activation
Viability	Live/Dead Blue	N/A	Viability
* For tissue samples, the following antibodies are substituted in: CD103 BB700 and CD69 Spark YG581. LAG-3 and CTLA-4 are also included.			

23-color Panel

The 23-color T cell panel explores the effector functions of the major T cell subsets. T cells are stimulated with PMA/Ionomycin for 5 hours prior to staining. This panel includes functional markers that comprehensively asses a wide-range of different T cell responses.

Specificity	**Fluorochrome**	**Clone**	**Purpose**
CD45RA	BUV395	5H9	Subset differentiation
IL-4	BUV737	MP4-25D2	Funtion
CD8	BUV805	SK1	Lineage
IL-10	BV421	JES3-9D7	Function
CD154	BV480	TRAP1	Co-stimulatory
CD3	BV510	SK7	Lineage
CD25	BV605	BC96	Subset differentiation
CCR7	BV650	G043H7	Subset differentiation
TNF	BV750	MAb11	Function
CD4	FITC	SK3	Lineage
CD45	AF532	HI30	Lineage
IFNγ	PerCP	4S.B3	Function
IL-22	BB700	MH22B2	Funtion
CD137	BB790	4B4-1	Co-stimulatory
IL-2	PE	MQ1-17H12	Function
IL-8	PE-CF594	G265-8	Function
TCRγδ	PE-Fire 700	B1.1	Lineage
IL-17A	PE-Cy7	BL168	Function
IL-13	APC	JES10-5A2	Function
IL-21	AF647	3A3-N2	Function
CCL4	R718	MP4-25D2	Function
Granzyme B	APC-Fire 750	QA16A02	Function
Viability	Live/Dead Blue	N/A	Viability