



CLINICAL STUDY PROTOCOL: MODULE 1

NUC-3373 in combination with pembrolizumab for the treatment of patients with advanced solid tumours

IMPs	NUC-3373 & pembrolizumab
Protocol Number: Module	NuTide:303: Module 1
Protocol Version, Date	Version 2.2, 15 January 2024
Development Phase	Ib/II
IND Number	135275
EudraCT Number	2022-000722-14
NCT Number	NCT05714553
Sponsor:	NuCana plc 3 Lochside Way Edinburgh, EH12 9DT, UK Telephone: +44 (0)131 357 1111 Email: Nutide303@nucana.com

Approval:

	Dr Elisabeth Oelmann, MD PhD	Date
	SVP, Medical & Clinical Development	
Medical Monitor:	Elisabeth Oelmann NuCana plc 3 Lochside Way, Edinburgh, EH12 9DT, UK Tel: +44 (0) 131 357 1118	

CONFIDENTIALITY STATEMENT

This confidential document is the property of NuCana plc. No unpublished information contained herein may be disclosed without prior written approval from NuCana. Access to this document must be restricted to relevant parties.



PRINCIPAL INVESTIGATOR AGREEMENT AND SIGNATURE

NUC-3373 in combination with pembrolizumab for the treatment of patients with advanced solid tumours

Protocol Number: Module	NuTide:303: Module 1
Protocol Version, Date	Version 2.2, 15 January 2024
IMPs	NUC-3373 & pembrolizumab
Development Phase	Ib/II
Sponsor	NuCana plc
IND Number	135275
EudraCT Number	2022-000722-14
NCT Number	NCT05714553

This study will be conducted in compliance with the clinical study protocol (and amendments), International Council for Harmonisation guidelines for current Good Clinical Practice (ICH-GCP) and applicable regulatory requirements. Compliance with ICH-GCP standards provides assurance that the rights, safety, and wellbeing of study patients are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

Principal Investigator's signature

Date (dd-mmm-yyyy)

Principal Investigator's name (printed)

TABLE OF CONTENTS

LIST OF TABLES	4
MODULE 1 SYNOPSIS	5
SUMMARY SCHEDULE OF EVENTS (MODULE 1)	12
ABBREVIATIONS	15
1 INTRODUCTION AND STUDY RATIONALE.....	17
1.1 Immunotherapy in Solid Tumours.....	17
1.2 Pembrolizumab.....	17
1.3 Module Rationale	18
2 STUDY OBJECTIVES AND ENDPOINTS	20
2.1 Primary Objective.....	20
2.2 Secondary Objectives	20
2.3 Exploratory Objectives	20
2.4 Primary Endpoints	20
2.5 Secondary Endpoints	20
2.6 Exploratory Endpoints.....	20
3 INVESTIGATIONAL PLAN	21
3.1 Overall Study Design.....	21
3.2 Rationale for the Doses Selected	21
3.3 Study Treatment	23
3.3.1 Patient Evaluability and Dose Escalation Decisions	24
3.3.2 Definition of Dose-Limiting Toxicity	25
4 PATIENT SELECTION	26
4.1 Inclusion Criteria	26
4.2 Exclusion Criteria	27
5 STUDY ASSESSMENTS AND PROCEDURES.....	29
5.1 Screening Assessments.....	29
5.2 Evaluations to be Performed During the Study	30
5.2.1 Each Cycle, Day 1	30
5.2.2 Cycle 1 ONLY.....	31
5.2.3 Cycle 2 ONLY.....	32
5.2.4 Each cycle, Day 8	32
5.2.5 Each cycle, Day 15	32
5.2.6 Every 8 weeks (± 7 days) from Cycle 1 Day 1 until disease progression, initiation of a new treatment or death	33
5.2.7 End of Treatment Visit	33
5.2.8 Follow-Up.....	34
5.2.9 Patient Evaluability and Replacement	34
6 SAMPLES FOR LABORATORY ANALYSIS.....	35
6.1 Pharmacokinetics.....	35
6.2 Tumour Tissue Sample and Pharmacodynamics	35
7 INVESTIGATIONAL MEDICINAL PRODUCTS	36
7.1 Treatment Administration.....	36
7.1.1 Dose-validation Phase	36

7.1.2	Dose-expansion Phase	36
7.2	Interaction with Other Drugs	37
8	MANAGEMENT OF TOXICITY	38
8.1	Criteria for Continuation of Treatment	38
8.2	Dose Modifications	39
8.2.1	NUC-3373	39
8.2.2	Pembrolizumab	41
9	OTHER TREATMENTS (NON-IMP)	52
9.1	Prohibited Therapies	52
9.2	Therapies to be Used with Caution	53
10	TUMOUR RESPONSE ASSESSMENTS	54
11	STATISTICAL CONSIDERATIONS	56
11.1	Sample size and Statistical Analyses	56
11.2	Missing, Unused and Spurious Data	56
11.3	Analysis Populations	56
11.3.1	Safety Population	56
11.3.2	Full Analysis Set	57
11.3.3	Evaluable for Response Population	57
11.4	Statistical Methods	57
11.4.1	Patient Disposition	57
11.4.2	Demographics and Baseline Characteristics	57
11.4.3	Concomitant Medications	57
11.4.4	Extent of Exposure	58
11.4.5	Safety Analysis	58
11.4.6	Efficacy Analysis	60
11.4.7	Exploratory Analyses	62
11.5	Primary and Interim Analyses	62
11.6	Changes to the Planned Statistical Methods	62
12	REFERENCES	63

LIST OF TABLES

Table 1	Anticipated dose levels to be evaluated	23
Table 2	Dose escalation guidance rules	24
Table 3	Dose modification guidelines	38
Table 4	NUC-3373 dose modifications for common toxicities	40
Table 5	Management of pembrolizumab-related toxicities	42
Table 6	Management of pembrolizumab-related infusion reactions	51

MODULE 1 SYNOPSIS

Study Title	A Phase Ib/II open label, multi-arm, parallel cohort dose finding and expansion study to assess the safety, pharmacokinetics and efficacy of NUC-3373, a nucleotide analogue, given in combination with other agents in patients with advanced solid tumours
Module Title	NUC-3373 in combination with pembrolizumab for the treatment of patients with advanced solid tumours
Protocol Number	NuTide:303
IND Number	135275
EudraCT Number	2022-000722-14
NCT Number	NCT05714553
Phase	Ib/II
Objectives	Primary Objectives <u>Phase Ib: Dose-validation phase</u> To determine the tolerability and the overall safety profile of NUC-3373 + leucovorin in combination with pembrolizumab. <u>Phase II: Dose-expansion phase</u> To evaluate the preliminary anti-tumour activity of NUC-3373 + LV in combination with pembrolizumab. Secondary Objectives • To evaluate the anti-tumour activity of NUC-3373 + LV in combination with pembrolizumab (Phase Ib) • To further evaluate the safety profile of NUC-3373 + LV in combination with pembrolizumab (Phase Ib and II) Exploratory Objectives • To evaluate potential biomarkers of immune activation in the tumour microenvironment (TME) that may correlate with the activity of NUC-3373 in combination with pembrolizumab (Phase Ib and II)
Study Design	Multi-centre, open-label, multi-arm, parallel cohort, dose validation and expansion design. The study is modular in design, allowing evaluation of the safety, efficacy and pharmacokinetics (PK) of NUC-3373 in combination with other agents for the treatment of patients with different tumour types. The study includes a number of modules, each evaluating a different NUC-3373 combination. Each module of this study consists of a dose-validation phase (Phase Ib) and a dose-expansion phase (Phase II).

	This module (Module 1) will assess NUC-3373 + LV in combination with pembrolizumab for the treatment of patients with advanced/metastatic solid tumours who have progressed on ≤ 2 prior therapies for metastatic disease, that may have included 1 prior immunotherapy-containing regimen (either monotherapy or in combination with chemotherapy) or who have not progressed but where addition of NUC-3373 + LV to standard pembrolizumab monotherapy may be appropriate (e.g., patients who could not tolerate post-IO standard of care therapy). Patients with tumour types for which pembrolizumab is approved and may be considered a suitable treatment option (e.g., melanoma, classical Hodgkin lymphoma, non-small cell lung cancer [NSCLC], renal cell carcinoma [RCC], urothelial carcinoma, head and neck squamous cell carcinoma [HNSCC], cutaneous squamous cell carcinoma (cSCC), oesophageal carcinoma, MSI high colorectal cancer [CRC], gastric cancer, triple negative breast cancer [TNBC], and endometrial carcinoma) may be included. Phase Ib: In Phase Ib (dose-validation), up to 12 evaluable patients will be enrolled to determine the recommended dose for NUC-3373 + LV in combination with pembrolizumab. An initial cohort of 3 patients will receive NUC-3373 at a dose of 1875 mg/m² in combination with LV and pembrolizumab. If this dose is tolerated (<i>i.e.</i>, no DLTs in the first 3 patients), a further 3 patients will be enrolled at the same dose level. Following this, the cohort may be expanded up to a total of 12 patients. If 1875 mg/m² NUC-3373 is not tolerated (<i>i.e.</i>, DLTs are observed in at least 1 of 3 patients), a modified 3+3 dose-escalation design will be initiated with a lower starting dose of NUC-3373. The first cohort of 3 patients will receive NUC-3373 at a dose of 1125 mg/m² in combination with LV and pembrolizumab. The exact number of patients will depend upon the number of dose-escalation steps required to determine a recommended dose. Phase II: Phase II (dose expansion) will further evaluate the combination in exploratory, signal-seeking cohorts of approximately 20-40 patients in 2-4 selected tumour types that have shown encouraging signs of activity (clinical or translational) during Phase Ib. For tumours with indications of efficacy during Phase II (defined as ≥ 1 confirmed complete response [CR], partial response [PR] or stable disease [SD] ≥ 8 weeks with evidence of tumour shrinkage), the protocol may be expanded to a powered analysis of that tumour type.
Study Centres	This module will be conducted at approximately 2-15 sites in the United Kingdom and/or United States.

Endpoints	<u>Primary Endpoints</u> <u>Phase Ib: Dose-validation phase</u> - Safety and tolerability, as assessed by treatment-emergent adverse events (TEAEs), clinically-significant laboratory changes, and changes in physical exam, vital signs and serial electrocardiograms (ECGs) <u>Phase II: Dose-expansion phase</u> - Objective response rate (ORR), defined as the percentage of patients achieving a confirmed complete or partial response to treatment, based on Immune Response Evaluation Criteria in Solid Tumours (iRECIST) criteria <u>Secondary Endpoints</u> Efficacy (Phase Ib and II) Objective disease assessment by radiographic imaging will be performed every 8 weeks and analysed using RECIST v1.1 and iRECIST criteria. Anti-tumour activity will be assessed based on: - Maximum percentage change from baseline in tumour size - ORR (Phase Ib only) - Disease Control Rate (DCR) - Duration of Response (DoR) - Duration of Stable Disease (DoSD) - Progression-Free Survival (PFS) - Overall Survival (OS) Safety (Phase II) - TEAEs (per Common Terminology Criteria for Adverse Events [CTCAE] v5.0) - Clinically significant laboratory changes (per CTCAE v5.0) - Changes in physical exam, vital signs and serial ECGs <u>Exploratory Endpoints</u> Expression of tumour markers
Study Population	Patients with a confirmed diagnosis of a solid tumour that is radiologically measurable, with evidence of locally advanced/unresectable or metastatic disease, for which pembrolizumab treatment would be appropriate; Eastern Cooperative Oncology Group (ECOG) performance status 0-1; adequate haematologic, renal and hepatic function.
Study Treatment	NUC-3373 will be administered at a starting dose of 1875 mg/m² in combination with LV at a dose of 400 mg/m² and pembrolizumab at a dose of 200 mg. If this dose is tolerated, the cohort will be expanded. If this dose is not tolerated, a modified 3+3 dose escalation design will be initiated with a lower NUC-3373 starting dose of 1125 mg/m² in combination with LV at a dose of 400 mg/m² and pembrolizumab at a dose of 200 mg. NUC-3373

	and LV will be administered on Days 1, 8 and 15 and pembrolizumab will be administered on Day 1 of 21-day cycles.
Sample Size	It is anticipated that approximately 6-12 patients will be enrolled in Phase Ib and 20-40 patients will be enrolled in Phase II in this module.
Inclusion Criteria	1. Provision of written informed consent. 2. Confirmed diagnosis of a solid tumour, with evidence of locally advanced/unresectable or metastatic disease, for which pembrolizumab treatment would be appropriate (<i>e.g.</i>, melanoma, classical Hodgkin lymphoma, NSCLC, RCC, urothelial carcinoma, HNSCC, cSCC, oesophageal carcinoma, MSI high CRC, gastric cancer, TNBC, and endometrial carcinoma). 3. Must have progressed on ≤ 2 prior lines of therapy for advanced/metastatic disease, that may have included 1 prior line of an immunotherapy-containing regimen (either monotherapy or in combination with chemotherapy). Patients who have not progressed but where addition of NUC-3373 + LV to standard pembrolizumab monotherapy may be appropriate are also eligible (<i>e.g.</i>, patients who could not tolerate post-IO standard of care therapy). 4. Patient must be willing to undergo a new tumour biopsy at Screening and during therapy on the study. Biopsies are mandatory for patient inclusion, except where taking a biopsy would be associated with unacceptable clinical risk due to the location of the disease. A prior (archival) biopsy that is less than 6 months old (from the date of C1D1) may be substituted for a fresh tumour biopsy at Screening. 5. Age ≥ 18 years. 6. Minimum life expectancy of ≥ 12 weeks. 7. ECOG performance status 0 or 1. 8. Measurable disease as defined by RECIST v1.1. 9. Adequate bone marrow function as defined by absolute neutrophil count (ANC) $\geq 1.5 \times 10^9/L$, platelet count $\geq 100 \times 10^9/L$ (with no evidence of bleeding), and haemoglobin ≥ 9 g/dL. 10. Adequate liver function, as defined by serum total bilirubin $\leq 1.5 \times$ upper limit of normal (ULN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 2.5 \times$ ULN (or $\leq 5 \times$ ULN if liver metastases are present). 11. Adequate renal function assessed as serum creatinine $< 1.5 \times$ ULN and glomerular filtration rate ≥ 50 mL/min (calculated by the Cockcroft-Gault method). 12. Serum albumin ≥ 3 g/dL. 13. Ability to comply with protocol requirements. 14. Female patients of child-bearing potential must have a negative pregnancy test within 7 days prior to the first study drug administration. This criterion does not apply to patients who have had a previous hysterectomy or bilateral oophorectomy. Male patients and female patients of child-bearing potential must agree to practice true abstinence or to use two forms of contraception, one of which must be highly effective. These forms of contraception must be used from the time of

	signing consent, throughout the treatment period, and for 6 months following the last dose of any study medication. Oral or injectable contraceptive agents cannot be the sole method of contraception. 15. Patients must have been advised to take measures to avoid or minimize exposure to UV light for the duration of study participation and for a period of 4 weeks following the last dose of study medication.
Exclusion Criteria:	- History of hypersensitivity or current contra-indications to 5-FU, FUDR, capecitabine (refer to latest package inserts), or the components of the NUC-3373 drug product formulation (super refined polysorbate 80 [SRP80], dimethylacetamide [DMA]). - Prior history of hypersensitivity or current contra-indication to immunotherapy with checkpoint inhibitors. - Any history of hypersensitivity or current contra-indication to the components of pembrolizumab (L-histidine, polysorbate 80, sucrose, sodium hydroxide, hydrochloric acid). - Any prior toxicity attributed to checkpoint inhibitors that resulted in discontinuation of therapy. - Patients previously exposed to checkpoint inhibitors who are not adequately treated for skin rash or have no replacement therapy for endocrinopathies. - Residual toxicities from prior chemotherapy, immunotherapy or radiotherapy which have not regressed to Grade ≤ 1 severity (CTCAE v5.0), except for alopecia, peripheral neuropathy and ototoxicity (which are excluded if $\geq$ Grade 3). - Known neutralising antibodies against checkpoint inhibitors. - Chemotherapy, hormonal therapy, radiotherapy (other than a short cycle of palliative radiotherapy, e.g., for bone pain*), immunotherapy, biological agents, or exposure to another investigational agent within 21 days (or four times the half-life for molecular targeted agents, whichever is shorter) of first administration of study treatment: - For nitrosoureas and mitomycin C within 6 weeks of first administration of NUC-3373 - Corticosteroid treatment is allowed during screening but should be weaned to a dose of 10 mg prednisolone (or steroid equivalent) by Cycle 1 Day 1 <i>* Palliative radiotherapy during participation in the study is permitted, but should not be concurrent with study treatment and recovery should be allowed to prevent overlapping toxicity. It should not include a target lesion.</i> - Patients who have received >2 prior lines of therapy or who have received >1 prior line of an immunotherapy-containing regimen for advanced/metastatic disease. - Systemic steroid therapy or any immunosuppressive therapy (≥ 10 mg/day prednisone or equivalent). - Congenital or acquired immunodeficiency (e.g., serious active infection with HIV). - Patients with a history of drug induced pneumonitis or current pneumonitis.

	13. Symptomatic central nervous system or leptomeningeal metastases. 14. Symptomatic ascites, ascites currently requiring drainage procedures or ascites requiring drainage over the 3 months prior to date of first dose of study drug. 15. Active autoimmune disease or a documented history of autoimmune disease, including ulcerative colitis and Crohn's disease or any condition that requires systemic steroids. 16. Uncontrolled concurrent cancer other than the indication under investigation. Patients with a concurrent cancer whose natural history or treatment does not have the potential to interfere with safety or efficacy assessment are eligible. 17. Presence of an active bacterial or viral infection (including SARS-CoV-2, Herpes Zoster or chicken pox), or known active hepatitis B or C. 18. Presence of any uncontrolled concurrent serious illness, medical condition or other medical history, including laboratory results, which, in the Investigator's opinion, would be likely to interfere with the patient's ability to participate in the study or with the interpretation of the results, including any of the following: a. Congestive heart failure (New York Heart Association Class III or Class IV) b. Clinically significant coronary heart disease or myocardial infarction within 6 months of the first dose of study medication or high risk of uncontrolled arrhythmia c. Unstable or poorly controlled angina pectoris d. Complete left bundle branch, bifascicular block or other clinically significant abnormal ECG finding e. QTc interval >470 milliseconds f. History of or current risk factor for torsade de pointes (<i>e.g.</i>, heart failure, hypokalaemia, or a family history of long QT syndrome) g. History of severe skin reactions h. History of severe ocular disorders i. Interstitial pneumonitis or pulmonary fibrosis 19. Any condition (<i>e.g.</i>, known or suspected poor compliance, psychological instability, geographical location, <i>etc.</i>) that, in the judgment of the Investigator, may affect the patient's ability to sign the informed consent and undergo study procedures. 20. Currently pregnant, lactating or breastfeeding. 21. Required concomitant use of drugs known to prolong QT/QTc interval. 22. Required concomitant use of strong CYP3A4 inducers or strong CYP3A4 inhibitors. The use of strong CYP3A4 inducers within 2 weeks of first receipt of study drug or the use of strong CYP3A4 inhibitors within 1 week of first receipt of study drug is also excluded. 23. Use of live attenuated vaccines against infectious diseases (<i>e.g.</i>, measles mumps rubella [MMR combined vaccines], Rotavirus, Chickenpox, yellow fever) within 4 weeks of initiation of study treatment. Live vaccinations are prohibited throughout the study and for 90 days after the last dose of study treatment.
--	---

	24. Known DPD or TYMP mutations associated with toxicity to fluoropyrimidines. 25. Full-dose anti-coagulation treatment is prohibited. Use of warfarin and other types of long-acting anti-coagulants (such as phenprocoumon and anti-Xa inhibitors with a half-life of >12 hours) is prohibited within 4 weeks of the first dose of study treatment. Patients requiring low dose anti-coagulant treatment should switch to low molecular weight heparin or anti-Xa inhibitors with a half-life of ≤ 12 hours.
Study Duration Per Patient	Patients may continue to receive treatment in the absence of radiological disease progression or unacceptable toxicity that is not ameliorated by optimal medical or non-medical supportive or prophylactic care, or withdrawal of consent. All patients will be followed up until withdrawal of consent, lost to follow-up, death, or the overall end of study, whichever is earliest.
Sample size and statistical methodology	This is an exploratory study focused on tolerability, translational data, and signal-seeking activities; therefore, the Phase Ib and Phase II components are not powered. In Phase Ib, up to 12 evaluable patients will be enrolled to determine the tolerability of NUC-3373 + LV in combination with pembrolizumab. In Phase II, approximately 20-40 patients will be enrolled to exploratory, signal-seeking cohorts. Overall response rate, DCR, and PFS will be assessed as indicators of efficacy. In tumours where efficacy is indicated (defined as ≥ 1 confirmed CR, PR or SD >8 weeks with evidence of tumour shrinkage), the protocol might be expanded to a powered analysis of that tumour type. The statistical considerations for the expansion will be addressed in both a protocol amendment and the statistical analysis plan (SAP).

SUMMARY SCHEDULE OF EVENTS (MODULE 1)

Study Assessments ¹	Screening / Baseline	Cycle 1*			Additional Cycles*			End of Treatment	Follow-up Visits	
	D -28 to 0	D1	D8 (±1)	D15 (±1)	D1	D8 (±1)	D15 (±1)	30 days post last dose (+7d)	Q8 weeks (±7d)	Q12 weeks (±14d)
Informed consent	X									
Inclusion/exclusion criteria	X									
Demographic data	X									
Previous medical history	X									
Concomitant medication	X	X	X	X	X	X	X	X		
Full physical examination ²	X	X ³			X ^{1,2}			X		
Directed physical examination ²					X ^{1,2}					
Urinalysis	X	X ³			X ¹			X		
ECOG performance status	X	X ³			X ¹			X		
Vital signs ⁴	X	X	X	X	X	X	X	X		
Weight	X	X			X			X		
Pre-dose ECG ⁵	X	X			X			X		
Post-dose ECG ⁶		X								
Pregnancy test ⁷	X	X ⁷			X			X		
FBC and chemistry ^{1,8}	X	X ³	X	X	X	X	X	X		
Coagulation profile ¹	X	X ³	X	X	X	X	X	X		
Tumour markers ⁹	X	X ³			X ¹			X		
Thyroid function ¹⁰	X				X ¹⁰			X		
Radiologic tumour assessment ¹¹	X	Assessed every 8 weeks from the start of Cycle 1							X ¹²	
Collect prior scan reports ¹³	Assessed during study participation									
Pembrolizumab administration		X			X					
NUC-3373 administration		X	X	X	X	X	X			
LV administration		X	X	X	X	X	X			
AEs/SAEs ¹⁴	X	X	X	X	X	X	X	X		
Tumour biopsy ¹⁵	X					X ¹⁵				
Biopsy PK blood sample ¹⁶						X ¹⁶				
Follow-up										X ¹⁷

* All cycles are 21 days in duration

- 1 Assessments scheduled on days of dosing should be done prior to administration of all Investigational Medicinal Products (IMPs), unless otherwise specified. Lab assessments, physical examination, and ECOG status may be performed up to 72 hours prior to IMP administration.
- 2 In Phase Ib, a full physical assessment should be completed at C1D1, C2D1 and at End of Treatment. From C3D1 onwards, a directed physical assessment (directed by clinical signs and symptoms) will be completed (in place of the full physical assessment), but only if clinically indicated. In Phase II a full physical assessment should be completed at C1D1 and End of Treatment only. In all other cycles a directed physical assessment will be completed (in place of the full physical assessment), but only if clinically indicated.
- 3 Does not need to be repeated if Screening assessment was performed within 72 hours of C1D1.
- 4 Vital signs include respiration rate, pulse, temperature and blood pressure. Height should be recorded at baseline only. Weight should be recorded at baseline, Day 1 of every cycle and at end of study visit. If a patient's weight increases or decreases by $\geq 10\%$ during the course of the study, the dose of 'NUC-3373 for infusion' should be recalculated.
- 5 **Pre-dose ECGs:** Standard 12-lead ECG measurements will be performed prior to administration of all IMPs on Day 1 of each cycle. All 12-lead ECG measurements should be reviewed by the Investigator or qualified designee for safety and quality.
- 6 **Post-dose ECGs:** Additional standard 12-lead ECG measurements must be taken post administration of NUC-3373 (end of administration ± 5 minutes) during Cycle 1 only at the C1D1 visit. The ECGs should be reviewed by the Investigator or qualified designee for safety and quality.
- 7 Serum pregnancy assessment to be performed within 7 days of C1D1. Required only in women of childbearing potential.
- 8 Clinical chemistry (including hepatic panel) and haematology will be conducted weekly throughout the study. In the event of neutropenia ($ANC < 0.5 \times 10^9/L$), thrombocytopaenia (platelet count $< 50 \times 10^9/L$), or $\geq$ Grade 2 clinical chemistry toxicity, these assessments will be conducted more frequently as clinically indicated until toxicity resolves to $\leq$ Grade 1.
- 9 Collect a pre-dose blood sample for evaluation of standard tumour markers (only for those patients who have tumours with correlative serological markers, *e.g.*, carcinoembryonic antigen [CEA]).
- 10 Thyroid function includes free T4 and TSH. Thyroid function testing should be performed during Screening, on Day 1 every 4 cycles (*i.e.*, Day 1 of Cycles 5, 9, 13, *etc*) and at the End of Treatment visit.
- 11 Computed tomography (CT)/magnetic resonance imaging (MRI) disease assessments will be performed at Screening (within 28 days prior to the first dose of IMP) and every 8 weeks (± 7 days) from C1D1. Additional tests may be requested at the Investigator's discretion.

CRs, PRs and PD must be confirmed by repeated measurement (as per iRECIST criteria). The confirmatory scans should be performed at least 28 days and not more than 42 days after initial assessment of response for CRs and PRs and at least 28 days and not more than 56 days after initial assessment for PD.

If a patient is found to have a response at the confirmatory scan which has improved from PR to CR, then the improved response must also be confirmed within a further 28- to 42-day window.

The same modality should be used throughout.

- 12** Patients withdrawing from study treatment with no radiological evidence of disease progression will remain in the study and receive scans every 8 weeks (± 7 days) from C1D1 until disease progression, initiation of a new anti-cancer treatment or death in order to determine duration of overall response and PFS. Patients who stop treatment following an unconfirmed response should also still have a confirmatory scan within the pre-specified 28- to 42-day window, if the scan can take place prior to the patient starting any subsequent anti-cancer therapies.
- 13** Collection of prior scan reports for assessment of previous tumour measurements can be performed any time during patient participation in the study or during follow-up. This will allow understanding of the trajectory of tumour growth prior to the patient entering this study.
- 14** All adverse events (AEs) occurring from the time of informed consent up to and including 30 days after the last dose of study drug has been administered must be reported in detail on the AE case report form (CRF).

Note: Serious adverse event (SAE) reporting is required until 30-days post the last dose of study medication.

- 15** Tissue samples are mandatory and should be collected as follows:
- A fresh tissue biopsy should be obtained during the Screening period within 14 days of the first dose of study treatment on C1D1. An archival tissue sample taken within 6 months of the first dose of study treatment will be acceptable if a fresh tumour biopsy is not possible.
 - A fresh tissue biopsy should be obtained on C2D8 or on any treatment day after C2D8. It is preferred that this biopsy is obtained within 6 hours, but no later than 24 hours, after the end of NUC-3373 infusion. If this is not possible, it may be obtained at any time after C2D8.

The fresh tissue specimen should not be taken from a lesion to be designated a target lesion for the purposes of RECIST assessment during the study. The mandatory biopsy requirement may be removed if the Sponsor deems that sufficient samples have been collected. All study sites will be notified in writing if this decision is made.

- 16** A blood sample for PK measurements will be taken at the same time as obtaining the on-treatment biopsy (see footnote 15) if the biopsy is taken within 6 hours, but no later than 24 hours, after the end of NUC-3373 infusion on C2D8 or on any treatment day after C2D8.
- 17** All patients who no longer attend clinic every 8 weeks for any reason will be followed up for survival and information regarding subsequent anti-cancer medications prescribed every 12 weeks (± 14 days) from the time of the last scheduled study visit until withdrawal of consent, lost to follow-up, death, or the overall end of study, whichever is earliest. A total of three attempts should be made before the patient is considered as lost to follow-up. Patients in follow-up who have not experienced disease progression should continue to attend the clinic for planned radiologic scans; however, other follow-up data can be collected remotely.

Collection of follow-up data can be performed via a telephone call with the patient where possible. Where not possible, follow-up with the patient's next of kin or physician should be performed, along with review of medical notes or national registries/databases if needed. The data collected and the follow up schedule remain as per the schedule of events. Disease progression and survival will be censored at the end of study date.

ABBREVIATIONS

5-FU	5-fluorouracil
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
aPTT	Activated partial thromboplastin time
ASCO	American Society for Clinical Oncology
AST	Aspartate aminotransferase
CEA	Carcinoembryonic antigen
CR	Complete response
CRC	Colorectal cancer
CRF	Case report form
cSCC	Cutaneous squamous cell carcinoma
CSR	Clinical study report
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
CYP	Cytochrome P450
DAMPs	Damage associated molecular patterns
DCR	Disease control rate
DMA	Dimethylacetamide
DoR	Duration of response
DoSD	Duration of stable disease
DPD	Dihydropyrimidine dehydrogenase
dUrd	Deoxyuridine
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EFR	Evaluable for response
ER	Endoplasmic reticulum
EudraCT	European Union Drug Regulating Authorities Clinical Trials Database
FAS	Full analysis set
FBAL	α -fluoro- β -alanine
FUDR	Floxuridine (5-fluorodeoxyuridine)
FUDR-MP /FdUMP	Fluorodeoxyuridine-monophosphate
FUTP	Fluorouridine triphosphate
G-CSF	Granulocyte colony stimulating factor
HIV	Human Immunodeficiency Virus
HMGB1	High-mobility-group box 1
HNSCC	Head and neck squamous cell carcinoma
IB	Investigator's Brochure
ICD	Immunogenic cell death
ICH-GCP	International Council for Harmonisation - Good Clinical Practice
ICI	Immune checkpoint inhibitor
iCPD	Confirmed progressive disease (iRECIST criteria)
IMP	Investigational medicinal product
IND	Investigational New Drug
INR	International normalised ratio
iRECIST	Immune-related Response Evaluation Criteria in Solid Tumours

IO	Immuno-oncology
iUPD	Unconfirmed progressive disease (iRECIST criteria)
IV	Intravenous(ly)
LDH	Lactate dehydrogenase
LV	Leucovorin
MDSCs	Myeloid-derived suppressor cells
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NE	Not evaluable
NK	Natural killer (cell)
NSCLC	Non-small cell lung cancer
ORR	Objective Response Rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death ligand 1
PFS	Progression-Free Survival
PK	Pharmacokinetics
PR	Partial Response
PT	Prothrombin time
Q3W	Every 3 weeks
QT/QTc	QT interval / Corrected QT interval
RBC	Red blood cells
RCC	Renal cell carcinoma
RECIST	Response Evaluation Criteria in Solid Tumours (version 1.1)
SAE	Serious adverse event
SAP	Statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome-related coronavirus 2
SD	Stable Disease
SOC	System Organ Class
SRC	Safety Review Committee
SRP80	Super refined polysorbate 80
TEAE	Treatment-emergent adverse event
TIGIT	T-cell immunoreceptor with Ig and ITIM domains
TILs	Tumour infiltrating lymphocytes
TME	Tumour microenvironment
TNBC	Triple negative breast cancer
TYMP	Thymidine phosphorylase (gene)
ULN	Upper limit of normal
WBC	White blood cells
WHO DD	World Health Organisation Drug Dictionary

1 INTRODUCTION AND STUDY RATIONALE

This section presents information that is specific to the combination under study in Module 1. Refer to Section 1 of the core protocol for a general overview of NUC-3373 and the key limitations associated with 5-FU.

1.1 Immunotherapy in Solid Tumours

Immune checkpoint inhibitors (ICIs) represent a breakthrough in the treatment of a variety of cancers, leading to improvements in survival, even in some patients with advanced cancers ([Camelliti et al, 2020](#)). Accordingly, anti-programmed cell death protein 1 (PD-1) and anti-programmed cell death ligand 1 (PD-L1) antibodies have become some of the most widely prescribed ICI anti-cancer therapies and are now used as single agents or in combination with other therapies as first- or second-line treatment for around 50 cancer types ([Robert, 2020](#)).

However, many patients demonstrate intrinsic or acquired resistance to ICI monotherapies, limiting their clinical benefit ([Kim et al, 2018](#)). A complex interplay between tumour cells, the host immune system and the TME underlies the anti-cancer immune response ([Puccini et al, 2020](#)). It has been reported that ICIs are more active in ‘hot’ tumours that are characterised by a high density of tumour infiltrating lymphocytes (TILs), an inflammatory cytokine-rich microenvironment and a T cell-inflamed gene expression profile ([Cristescu et al, 2018](#)). On the other hand, ‘cold’ tumours are characterised by high levels of myeloid-derived suppressor cells (MDSCs) and regulatory suppressive T cells in the microenvironment, leading to a lack of TILs in the tumour. Recent research efforts have focused on strategies to turn immunologically ‘cold’ tumours ‘hot’.

The induction of immunogenic cell death (ICD), which is characterised by release of damage-associated molecular patterns (DAMPs), is known to propagate anti-tumoral immunity. DAMPs act as signals that attract immune cells in the tumour microenvironment and activate them ([Krysko et al, 2012](#); [Solari et al, 2020](#)). Thus, ICD-inducing agents are being studied for their ability to turn ‘cold’ tumours ‘hot’ with the aim of augmenting therapeutic efficacy through sensitisation of tumours to anti-PD-1/PD-L1 therapies. Many chemotherapy agents have been shown to promote the release of DAMPs and cause ICD ([Wang et al, 2018](#)). Indeed, several clinical studies have reported that pre-treatment of patients with cancer with ICD-inducing anthracyclines sensitises tumours to ICIs targeting PD-1 and PD-L1 interaction ([Kepp et al, 2019](#)). 5-FU treatment has been shown to promote DAMPs release and increase the frequency of TILs as well as deplete circulating MDSCs. Furthermore, a positive association has been reported between TILs and clinical outcomes in patients with CRC treated with 5-FU-based therapy ([Wang et al, 2018](#)).

1.2 Pembrolizumab

PD-1 is a transmembrane receptor and a checkpoint that regulates the immune response through binding with its ligands PD-L1 and PD-L2, resulting in the transduction of negative signals to T-cells ([Keir et al, 2008](#)). PD-1 is not present on resting lymphoid cells, but is expressed on activated CD4⁺ and CD8⁺ T cells, B cells, natural killer (NK) cells, macrophages and dendritic cells. Sustained expression of PD-1 contributes to an exhausted effector T cell phenotype; thus, the interaction of PD-1 on effector T cells with PD-L1 on cancer cells enables tumors to evade anti-cancer immunity ([Kwok et al, 2016](#)). Based on this, blockade of PD-1 is an important immunotherapeutic strategy for cancer treatment.

Pembrolizumab (Keytruda[®]) is a humanised monoclonal anti-PD1 antibody that has been extensively investigated in numerous cancers. In a series of KEYNOTE studies, pembrolizumab demonstrated anti-tumor activity and a favorable safety profile in patients with

multiple solid tumor types and is currently approved in more than 80 countries for the treatment of one or more advanced malignancies (Shah *et al*, 2021). For example, pembrolizumab demonstrated ORRs of 21-34% in patients with melanoma refractory to targeted therapy and ORRs of 19–25% in patients with refractory NSCLC (Kwok *et al*, 2016). Although pembrolizumab offers a promising treatment option for some patients, these ORRs highlight the fact that many patients do not respond.

The reasons for lack of response or resistance to ICIs such as pembrolizumab are being investigated in a multitude of clinical and nonclinical studies. Indeed, the Society for Immunotherapy of Cancer set up an immunotherapy resistance taskforce to generate consensus clinical definitions for resistance to PD-1/PD-L1 inhibitors (Kluger *et al*, 2020). It is widely accepted that tumour cell PD-L1 expression may be a valid response predictor. In addition, it has been shown that tumours with high gene mutation burdens had better responses to pembrolizumab (Kwok *et al*, 2016).

As described in Section 1.1, ICIs are thought to be more active in immunologically ‘hot’ tumours and a growing body of research suggests that ICD-inducing agents may increase the therapeutic efficacy of anti-PD-1/PD-L1 therapies by priming an anti-tumour immune response. A recent indirect comparison of clinical outcomes between immunotherapy plus chemotherapy and immunotherapy alone in patients with NSCLC found that combination treatment was superior to immunotherapy alone in terms of PFS (HR 0.54, 0.35–0.82) in patients with PD-L1 $\geq 50\%$. However, the PFS benefit did not translate into an OS benefit (HR 0.75, 95% CI 0.51–1.10) (Liang *et al*, 2019). In another indirect comparison, it was found that pembrolizumab plus chemotherapy was superior to pembrolizumab alone in terms of PFS and OS in patients with NSCLC with PD-L1 $\geq 50\%$ (Kim *et al*, 2019).

Given the potential ability for chemotherapies to augment the clinical activity of ICIs, combination therapy strategies could offer a promising treatment option for a wide population of patients with advanced solid tumours.

1.3 Module Rationale

Patients with advanced/metastatic solid tumours represent a high unmet medical need due to lack of response after failure of standard/available therapies and a poor prognosis. These tumour types require novel treatment approaches to overcome cancer resistance mechanisms, which may lead to improved clinical outcomes.

Although ICIs such as pembrolizumab have altered the treatment landscape for many cancers, a large proportion of patients do not respond, or develop resistance, to these agents. Similarly, many patients develop resistance to the chemotherapy agents that represent the standard of care for a variety of tumour indications. However, emerging evidence suggests that the activity of conventional chemotherapy is not limited to tumor cell toxicity, but also involves restoration of immunosurveillance through induction of ICD. Based on this, novel treatment combinations of ICIs with chemotherapy agents warrant further investigation in clinical studies.

NUC-3373, a novel anti-cancer ProTide, is a pre-activated and protected form of the nucleotide analogue floxuridine (FUDR[®]), specifically designed to overcome the key shortcomings that limit the clinical utility of fluoropyrimidines. Refer to Section 1 of the Core Protocol for further information on NUC-3373.

Data from nonclinical studies suggest that NUC-3373 may have the potential to modulate the TME and prime an anti-tumour immune response.

In CRC cell lines, NUC-3373 was shown to cause endoplasmic reticulum (ER) stress and promote the release of DAMPs, including high-mobility-group box 1 (HMGB1), calreticulin and ATP. DAMPs are known to enhance cancer cell recognition by the immune system,

promoting ICD. Co-culture experiments with CRC and NK cell lines revealed that NUC-3373 induced DAMPs activate a NK response as evidenced by increased degranulation and cytokine production. Furthermore, NUC-3373 induced DAMPs reduced the inhibitory signal T-cell immunoreceptor with Ig and ITIM domains (TIGIT), suggesting a mechanism for reducing NK exhaustion.

In additional co-culture experiments, NUC-3373 treatment caused an increase in the surface expression of PD-L1 on both peripheral blood mononuclear cells (PBMCs) and CRC or NSCLC cells, indicating that NUC-3373 may enhance the anti-cancer potential of ICIs targeting the PD-1/PD-L1 axis. In support of this, CRC and NSCLC cells pre-treated with NUC-3373 and co-cultured with PBMCs displayed a reduction in cell viability compared to monocultured CRC or NSCLC cells. Furthermore, NUC-3373 was shown to promote immune cell-mediated ICD in CRC and NSCLC cells co-cultured with PBMCs, with NUC-3373 potentiating the activity of nivolumab in CRC cells and of pembrolizumab in NSCLC cells.

In the clinic, NUC-3373 has shown a more favourable safety profile compared to 5-FU. This is due to differences in mechanism of action and metabolism, which results in decreased generation of toxic metabolites such as fluorouridine triphosphate (FUTP) and α -fluoro- β -alanine (FBAL) and a lower incidence and reduced severity of fluoropyrimidine-related toxicities such as neutropenia, diarrhoea and hand-foot syndrome compared to 5-FU. The lack of haematopoietic toxicity observed in NUC-3373 clinical studies to date may be beneficial when combining NUC-3373 with ICIs through minimisation of chemotherapy-induced immunosuppression, which could hamper the immune activating effects of ICIs.

Based on the nonclinical and clinical data generated to date, NUC-3373 is an attractive combination partner for ICIs.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objective

Phase Ib: Dose-validation phase

To determine the tolerability and the overall safety profile of NUC-3373 + LV in combination with pembrolizumab.

Phase II: Dose-expansion phase

To evaluate the preliminary anti-tumour activity of NUC-3373 + LV in combination with pembrolizumab.

2.2 Secondary Objectives

- To evaluate the anti-tumour activity of NUC-3373 + LV in combination with pembrolizumab (Phase Ib)
- To further evaluate the safety profile of NUC-3373 + LV in combination with pembrolizumab (Phase Ib and II)

2.3 Exploratory Objectives

- To evaluate potential biomarkers of immune activation in the TME that may correlate with the activity of NUC-3373 in combination with pembrolizumab (Phase Ib and II)

2.4 Primary Endpoints

Phase Ib: Dose-validation phase

- Safety and tolerability, as assessed by TEAEs, clinically-significant laboratory changes, and changes in physical exam, vital signs and serial ECGs

Phase II: Dose-expansion phase

- ORR, defined as the percentage of patients achieving a confirmed complete or partial response to treatment, based on iRECIST criteria

2.5 Secondary Endpoints

Efficacy (Phase Ib and II)

Objective disease assessment by radiographic imaging will be performed every 8 weeks and analysed using RECIST v1.1 criteria. Anti-tumour activity will be assessed on the basis of:

- Maximum percentage change from baseline in tumour size
- ORR (Phase Ib only)
- DCR
- DoR
- DoSD
- PFS
- OS

Safety (Phase II)

- TEAEs (per CTCAE v5.0)
- Clinically-significant laboratory changes (per CTCAE v5.0)
- Changes in physical exam, vital signs and serial ECGs

2.6 Exploratory Endpoints

Expression of tumour markers

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design

This module (Module 1) describes the study design for NUC-3373 + LV in combination with pembrolizumab for the treatment of patients with advanced/metastatic solid tumours who have progressed on ≤ 2 prior therapies for metastatic disease, that may have included 1 prior immunotherapy-containing regimen (either monotherapy or in combination with chemotherapy) or who have not progressed but where addition of NUC-3373 + LV to standard pembrolizumab monotherapy may be appropriate (*e.g.*, patients who could not tolerate post-IO standard of care therapy).

Patients with tumour types for which pembrolizumab is approved and may be considered a suitable treatment option (*e.g.*, melanoma, classical Hodgkin lymphoma, NSCLC, RCC, urothelial carcinoma, HNSCC, cSCC, oesophageal carcinoma, MSI high CRC, gastric cancer, TNBC, and endometrial carcinoma) may be included.

In Phase Ib (dose-validation), up to 12 evaluable patients will be enrolled to determine the recommended dose for NUC-3373 + LV in combination with pembrolizumab. An initial cohort of 3 patients will receive NUC-3373 at a dose of 1875 mg/m² in combination with LV and pembrolizumab.

If this dose is tolerated (*i.e.*, no DLTs in the first 3 patients), a further 3 patients will be enrolled at the same dose level. Following this, the cohort may be expanded up to a total of 12 patients.

If 1875 mg/m² NUC-3373 is not tolerated (*i.e.*, DLTs are observed in at least 1 of 3 patients), a modified 3+3 dose-escalation design will be initiated at a lower starting dose of NUC-3373. The first cohort of 3 patients will receive NUC-3373 at a new starting dose of 1125 mg/m² in combination with LV and pembrolizumab.

The exact number of patients will depend upon the number of dose escalation steps required to determine a recommended dose. Dose escalation will follow the guidelines provided in [Section 3.3.1](#).

Phase II (dose expansion) will further evaluate the combination in exploratory, signal-seeking cohorts of approximately 20-40 patients in 2-4 selected tumour types that have shown encouraging signs of activity (clinical or translational) during Phase Ib. For tumours with indications of efficacy during Phase II (defined as ≥ 1 confirmed CR, PR or SD ≥ 8 weeks with evidence of tumour shrinkage), the protocol may be expanded to a powered analysis of that tumour type.

3.2 Rationale for the Doses Selected

NUC-3373 will be administered at an initial starting dose of 1875 mg/m² in combination with LV at 400 mg/m² on Days 1, 8 and 15 of 21-day cycles. Pembrolizumab will be administered at a dose of 200 mg on Day 1 of 21-day cycles.

The proposed dose and schedule for NUC-3373 are derived predominantly from clinical data in the NuTide:301 and NuTide:302 studies, supported by non-clinical data.

The safety of NUC-3373 as a single agent (without the addition of LV) at doses ranging from 125 to 3250 mg/m² administered on Days 1, 8, 15 and 22 of 28-day cycles has been studied in the NuTide:301 study where 2500 mg/m² weekly was identified as the maximum tolerated dose (MTD; based on DLTs of Grade 3 transient hypotension and Grade 2 headache in the

3250 mg/m² cohort). NUC-3373 was well-tolerated, showing a favourable safety profile when compared to historical data for 5-FU and capecitabine.

NUC-3373 in combination with LV has been evaluated in Part 1 of the ongoing NuTide:302 study in patients with advanced CRC, where it was found that LV does not affect the safety or pharmacokinetic (PK) profile of NUC-3373. Based on this, it was determined that NUC-3373 should be administered in combination with the standard dose of LV (400 mg/m² weekly).

In Part 2 of the NuTide:302 study, NUC-3373 has been assessed in combination with LV and either oxaliplatin (NUFOX) or irinotecan (NUFIRI). The MTD of NUC-3373 in the NUFOX regimen was determined to be 1875 mg/m² based on DLTs of Grade 3 fatigue in 2 patients and Grade 3 ALT increased/Grade 2 bilirubin increased in 1 patient. The MTD of NUC-3373 in the NUFIRI regimen was determined to be 1500 mg/m² based on DLTs of Grade 3 fatigue, Grade 3 colitis and Grade 3 ALT/ALP increased in 1 patient each.

Across the NuTide:301 (n=59) and NuTide:302 (n=84) studies to date, the incidence of common chemotherapy-associated toxicities, such as haematological, hepatic, and gastrointestinal toxicity, was lower when compared with historical clinical data for 5-FU and no significant toxicities were reported in these categories for NUC-3373. Based on this favourable safety profile, the NUC-3373 starting dose was selected at the highest combination MTD (1875 mg/m²). This strategy is further supported by the fact that the mechanism of chemotherapy-induced toxicities is different compared to PD-1 inhibitor-induced toxicities and that chemotherapies have typically been given at their standard doses in the Keynote studies in combination with pembrolizumab.

Selecting a dose below the single-agent MTD is anticipated to minimise the potential for any overlapping toxicities when NUC-3373 is combined with pembrolizumab. However, if the 1875 mg/m² starting dose is not tolerated, a modified 3+3 dose escalation design will be initiated at a lower NUC-3373 dose of 1125 mg/m².

Lower doses of NUC-3373 (≤ 2000 mg/m²) can be infused over a shorter period of time and in a lower infusion volume compared to the single-agent MTD, thus providing a more convenient regimen for both patients and clinicians. In the NuTide:301 and NuTide:302 studies, NUC-3373 at doses of 1125 mg/m² and higher has shown promising signals of anti-cancer activity, with patients who have exhausted all standard treatment options showing prolonged periods of disease stabilisation. In addition, clinical PK data have shown high levels of the active anti-cancer metabolite (fluorodeoxyuridine-monophosphate; FUDR-MP) in the PBMCs of patients treated with NUC-3373 at doses of 1500 mg/m² and higher. The selected starting doses are therefore anticipated to optimise the benefit/risk ratio.

The dose and schedule for pembrolizumab (200 mg Q3W) was selected based on its packaging leaflet.

3.3 Study Treatment

Patients who have signed the informed consent and are eligible will be enrolled to receive treatment with NUC-3373 + LV in combination with pembrolizumab. Patients may continue to receive study treatment until documentation of radiological disease progression or unacceptable toxicity. Patients should not discontinue treatment based on progressive disease per RECIST v1.1 unless clinical deterioration or increase in tumour markers is observed. iRECIST should always be considered and a confirmation of progression scan is required at no less than 4 weeks and no more than 8 weeks after unconfirmed progression (iUPD) preferably at the next scheduled visit after first indication of iUPD, unless unequivocal disease progression has been diagnosed (based on clinical deterioration and or tumour marker rise).

Initially, a cohort of 3 evaluable patients will receive NUC3373 at 1875 mg/m² plus LV at 400 mg/m² on Days 1, 8 and 15 in combination with pembrolizumab at 200 mg on Day 1 of 21-day cycles. There will be a 9-day observation period between the treatment of each patient in this first cohort. Depending on the findings from the first 3 patients, the safety observation period will be relaxed to 48 hours for the next 3 patients. Following this, staggering of subsequent patients may be further relaxed depending on emerging safety data.

All patients will be monitored closely for DLTs during Cycle 1.

If treatment at this dose (1875 mg/m² NUC-3373) is tolerable (*i.e.*, no DLTs are observed in the first 3 patients), a further 3 patients will be enrolled at the same dose level. Following this, the cohort may be expanded up to a total of 12 patients.

If 1875 mg/m² NUC-3373 is not tolerated (*i.e.*, DLTs are observed in ≥ 1 of 3 patients), a 3+3 dose-escalation design will be initiated at a lower starting dose of NUC-3373 (1125 mg/m²; Dose Level -2) in combination with LV (400 mg/m²) and pembrolizumab (200 mg) and dose-escalation to Dose Level -1 may proceed as described in [Table 1](#).

Further dose levels will be based on emerging data and the recommendation of the SRC.

If cohorts at additional doses are opened, these will utilise a modified 3+3 design in which a fourth patient may be added to each initial 3-patient cohort to account for a patient that may become unevaluable for DLT evaluation for any reason other than safety. Once a minimum of 3 patients in a given dose cohort have completed the DLT evaluation period (Cycle 1), all available safety and PK data will be evaluated to establish the tolerability of NUC-3373 administered in combination with pembrolizumab.

Table 1 Anticipated dose levels to be evaluated

Dose Level*	NUC-3373 dose (mg/m ²) Days 1, 8 and 15
Starting Dose Level 1	1875
-1	1500
-2	1125
*Additional dose levels may be explored if necessary	

3.3.1 Patient Evaluability and Dose Escalation Decisions

To be evaluable for DLT, a patient must have received NUC-3373 + LV and pembrolizumab, have completed the relevant safety evaluation requirements (Cycle 1; 21 days), and must have:

- Either received 75% of the specified dose of each agent during the DLT evaluation period
or
- experienced a DLT in the evaluation period

In the event that dose escalation is initiated, decisions will be made according to the guidance rules in Table 2 when 3 patients in a dosing cohort have completed the DLT evaluation period or experienced a DLT prior to completing the initial DLT evaluation period cycle.

Dose escalation decisions will occur based on review of safety information obtained during the DLT period, including AEs (regardless of causality), laboratory safety data, vital signs and ECG data.

Emerging safety data for patients who continue treatment beyond Cycle 1 will also be reviewed and study treatment dosing may be adjusted based on this data.

Table 2 Dose escalation guidance rules

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3 patients	Dose is tolerable
≥1 out of 3 patients	Expand cohort, if needed, to include 6 patients. • If only 1 out of 6 has DLT, dose is tolerable • If ≥2 have DLTs, dose is not tolerable and lower dose cohort should be explored
≥2 out of 3-6 patients	This dose level will be declared a non-tolerated dose and a lower dose cohort should be explored

In the case of a tolerable dose, the SRC will decide whether to explore a higher dose level or whether this dose is the recommended dose for the combination.

Patients who withdraw from study treatment prior to the end of Cycle 1 due to a DLT will not be replaced. Patients who withdraw for other reasons may be replaced.

The starting dose of NUC-3373 has been chosen based on previous clinical experience. In the event of the occurrence of new or unexpected toxicities, dose modification of NUC-3373 should be considered first, before delaying or discontinuing pembrolizumab.

Protocol-specific guidelines for study treatment dosage and administration are provided in detail in [Section 7.1](#) of this module. Guidelines for protocol-specific dose modifications and discontinuations of NUC-3373 are provided in [Section 8](#) of this module. Dose modifications for pembrolizumab should be in line with the package insert / local standard procedures (see also [Section 8.2.2](#)), unless safety concerns emerge regarding dosing as determined by the SRC.

3.3.2 Definition of Dose-Limiting Toxicity

DLT is defined as any of the following occurring during the first treatment cycle that are not due to the protocol disease indication or to a known concurrent medical condition and are judged as clinically significant and related to study treatment.

For dose escalation, DLTs are defined as follows
Any Grade 4 AEs will be considered DLTs, with the exception of:
Neutropenia lasting <72 hours that is not associated with fever or other clinical symptoms
Lymphopenia
Electrolyte abnormalities that are not associated with clinical sequelae and are corrected with appropriate management or supplementation within 72 hours of the onset
Any Grade 3 AEs will be considered as DLTs, with the exception of:
Nausea and vomiting persisting for <2 days after optimal anti-emetic therapy
Thrombocytopenia without significant bleeding (including platelet transfusions)
Diarrhoea persisting for <2 days after optimal anti-diarrhoeal treatment
Hypertension persisting <7 days after treatment
Infection or fever in the absence of neutropenia persisting <5 days
Rash or photosensitivity persisting <7 days after treatment
Fatigue persisting <7 days
Liver function test elevations (AST/ALT) persisting <7 days after treatment with corticosteroids
Immune-related adverse events persisting <7 days after treatment with corticosteroids
Alopecia
The following Grade 2 AEs will be adjudicated as DLTs:
Total bilirubin CTCAE v5 $\geq$ Grade 2 with simultaneous ALT/AST CTCAE v5 $\geq$ Grade 2
Pneumonitis persisting >7 days despite treatment with corticosteroids
Eye pain or reduction of visual acuity that does not respond to topical therapy and does not improve to Grade 1 severity within 2 weeks of the initiation of topical therapy OR requires systemic treatment
Other clinically significant toxicities (cardiotoxicity, neurotoxicity) including a single event or multiple occurrences of the same event that lead to a dosing delay of >7 days in Cycle 1

4 PATIENT SELECTION

4.1 Inclusion Criteria

To be enrolled in this module, patients must meet *all* of the following criteria during the Screening period:

1. Provision of written informed consent.
2. Confirmed diagnosis of a solid tumour, with evidence of locally advanced/unresectable or metastatic disease, for which pembrolizumab treatment would be appropriate (*e.g.*, melanoma, classical Hodgkin lymphoma, NSCLC, RCC, urothelial carcinoma, HNSCC, cSCC, oesophageal carcinoma, MSI high CRC, gastric cancer, TNBC, and endometrial carcinoma).
3. Must have progressed on ≤ 2 prior lines of therapy for advanced/metastatic disease, that may have included 1 prior line of an immunotherapy-containing regimen (either monotherapy or in combination with chemotherapy). Patients who have not progressed but where addition of NUC-3373 + LV to standard pembrolizumab monotherapy may be appropriate are also eligible (*e.g.*, patients who could not tolerate post-IO standard of care therapy).
4. Patient must be willing to undergo a new tumour biopsy at Screening and during therapy on the study. Biopsies are mandatory for patient inclusion, except where taking a biopsy would be associated with unacceptable clinical risk due to the location of the disease. A prior (archival) biopsy that is less than 6 months old (from the date of C1D1) may be substituted for a fresh tumour biopsy at Screening.
5. Age ≥ 18 years.
6. Minimum life expectancy of ≥ 12 weeks.
7. ECOG performance status 0 or 1.
8. Measurable disease as defined by RECIST v1.1.
9. Adequate bone marrow function as defined by ANC $\geq 1.5 \times 10^9/L$, platelet count $\geq 100 \times 10^9/L$ (with no evidence of bleeding), and haemoglobin ≥ 9 g/dL.
10. Adequate liver function, as defined by serum total bilirubin $\leq 1.5 \times ULN$, AST and ALT $\leq 2.5 \times ULN$ (or $\leq 5 \times ULN$ if liver metastases are present).
11. Adequate renal function assessed as serum creatinine $< 1.5 \times ULN$ and glomerular filtration rate ≥ 50 mL/min (calculated by the Cockcroft-Gault method).
12. Serum albumin ≥ 3 g/dL.
13. Ability to comply with protocol requirements.
14. Female patients of child-bearing potential must have a negative pregnancy test within 7 days prior to the first study drug administration. This criterion does not apply to patients who have had a previous hysterectomy or bilateral oophorectomy. Male patients and female patients of child-bearing potential must agree to practice true abstinence or to use two forms of contraception, one of which must be highly effective. These forms of contraception must be used from the time of signing consent, throughout the treatment period, and for 6 months following the last dose of any study medication. Oral or injectable contraceptive agents cannot be the sole method of contraception.
15. Patients must have been advised to take measures to avoid or minimize exposure to UV light for the duration of study participation and for a period of 4 weeks following the last dose of study medication.

4.2 Exclusion Criteria

Patients who meet *any* of the following criteria at Screening will be excluded from the study:

1. History of hypersensitivity or current contra-indications to 5-FU, FUDR, capecitabine (refer to latest package inserts), or the components of the NUC-3373 drug product formulation (SRP80, DMA).
 2. Prior history of hypersensitivity or current contra-indication to immunotherapy with checkpoint inhibitors.
 3. Any history of hypersensitivity or current contra-indication to the components of pembrolizumab (L-histidine, polysorbate 80, sucrose, sodium hydroxide, hydrochloric acid).
 4. Any prior toxicity attributed to checkpoint inhibitors that resulted in discontinuation of therapy.
 5. Patients previously exposed to checkpoint inhibitors who are not adequately treated for skin rash or have no replacement therapy for endocrinopathies.
 6. Residual toxicities from prior chemotherapy, immunotherapy or radiotherapy which have not regressed to Grade ≤ 1 severity (CTCAE v5.0), except for alopecia, peripheral neuropathy and ototoxicity (which are excluded if $\geq$ Grade 3).
 7. Known neutralising antibodies against checkpoint inhibitors.
 8. Chemotherapy, hormonal therapy, radiotherapy (other than a short cycle of palliative radiotherapy, *e.g.*, for bone pain*), immunotherapy, biological agents, or exposure to another investigational agent within 21 days (or four times the half-life for molecular targeted agents, whichever is shorter) of first administration of study treatment:
 - a. For nitrosoureas and mitomycin C within 6 weeks of first administration of NUC-3373
 - b. Corticosteroid treatment is allowed during screening but should be weaned to a dose of 10 mg prednisolone (or steroid equivalent) by Cycle 1 Day 1
- * Palliative radiotherapy during participation in the study is permitted, but should not be concurrent with study treatment and recovery should be allowed to prevent overlapping toxicity. It should not include a target lesion.*
9. Patients who have received >2 prior lines of therapy or who have received >1 prior line of an immunotherapy-containing regimen for advanced/metastatic disease.
 10. Systemic steroid therapy or any immunosuppressive therapy (≥ 10 mg/day prednisone or equivalent).
 11. Congenital or acquired immunodeficiency (*e.g.*, serious active infection with HIV).
 12. Patients with a history of drug induced pneumonitis or current pneumonitis.
 13. Symptomatic central nervous system or leptomeningeal metastases.
 14. Symptomatic ascites, ascites currently requiring drainage procedures or ascites requiring drainage over the 3 months prior to date of first dose of study drug.
 15. Active autoimmune disease or a documented history of autoimmune disease, including ulcerative colitis and Crohn's disease or any condition that requires systemic steroids.
 16. Uncontrolled concurrent cancer other than the indication under investigation. Patients with a concurrent cancer whose natural history or treatment does not have the potential to interfere with safety or efficacy assessment are eligible.

17. Presence of an active bacterial or viral infection (including SARS-CoV-2, Herpes Zoster or chicken pox), or known active hepatitis B or C.
18. Presence of any uncontrolled concurrent serious illness, medical condition or other medical history, including laboratory results, which, in the Investigator's opinion, would be likely to interfere with the patient's ability to participate in the study or with the interpretation of the results, including any of the following:
 - a. Congestive heart failure (New York Heart Association Class III or Class IV)
 - b. Clinically significant coronary heart disease or myocardial infarction within 6 months of the first dose of study medication or high risk of uncontrolled arrhythmia
 - c. Unstable or poorly controlled angina pectoris
 - d. Complete left bundle branch, bifascicular block or other clinically significant abnormal ECG finding
 - e. QTc interval >470 milliseconds
 - f. History of or current risk factor for torsade de pointes (*e.g.*, heart failure, hypokalaemia, or a family history of long QT syndrome)
 - g. History of severe skin reactions
 - h. History of severe ocular disorders
 - i. Interstitial pneumonitis or pulmonary fibrosis
19. Any condition (*e.g.*, known or suspected poor compliance, psychological instability, geographical location, *etc.*) that, in the judgment of the Investigator, may affect the patient's ability to sign the informed consent and undergo study procedures.
20. Currently pregnant, lactating or breastfeeding.
21. Required concomitant use of drugs known to prolong QT/QTc interval.
22. Required concomitant use of strong CYP3A4 inducers or strong CYP3A4 inhibitors. The use of strong CYP3A4 inducers within 2 weeks of first receipt of study drug or the use of strong CYP3A4 inhibitors within 1 week of first receipt of study drug is also excluded.
23. Use of live attenuated vaccines against infectious diseases (*e.g.*, measles mumps rubella [MMR combined vaccines], Rotavirus, Chickenpox, yellow fever) within 4 weeks of initiation of study treatment. Live vaccinations are prohibited throughout the study and for 90 days after the last dose of study treatment.
24. Known DPD or TYMP mutations associated with toxicity to fluoropyrimidines.
25. Full-dose anti-coagulation treatment is prohibited. Use of warfarin and other types of long-acting anti-coagulants (such as phenprocoumon and anti-Xa inhibitors with a half-life of >12 hours) is prohibited within 4 weeks of the first dose of study treatment. Patients requiring low dose anti-coagulant treatment should switch to low molecular weight heparin or anti-Xa inhibitors with a half-life of ≤12 hours.

5 STUDY ASSESSMENTS AND PROCEDURES

5.1 Screening Assessments

Screening assessments of consented patients will comprise the following:

- Provision of written informed consent
- Eligibility confirmation
- Recording of demographic data
- Assessment of medical and surgical history
- Recording of concomitant medication
- Full physical examination, including vital signs. Full physical examination includes ears, eyes, nose, head, throat, neck, lungs, heart, abdomen, extremities, skin, musculoskeletal, nervous system, and lymph nodes. Vital signs include measurement of pulse rate, respiratory rate, blood pressure and temperature, after the patient has been seated or in the supine position for 5 minutes
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- ECOG performance status
- Height and weight
- Baseline assessment of symptoms
- 12-lead ECG
 - The 12-lead ECGs should be reviewed by the Investigator or a qualified designee for safety and quality
 - The QTc interval should be calculated using the Fridericia formula. Management of QT/QTc prolongation should be performed in accordance with institutional standard of care at the discretion of the treating physician
 - Digital and certified paper copies should be retained at site and stored as part of the study documents in the event they need to be used in a future analysis
- Blood samples drawn for:
 - Pregnancy testing: For women of childbearing potential serum pregnancy must be performed within 7 days of first dose
 - Haematology: white blood cell (WBC) count, differential WBC count, red blood cell (RBC) count, haemoglobin, haematocrit and platelets
 - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, phosphate, total protein, albumin, adjusted calcium, total bilirubin, direct bilirubin, indirect bilirubin, bicarbonate, chloride, uric acid, alkaline phosphatase (ALP), AST, ALT and lactate dehydrogenase (LDH)
 - Coagulation parameters: prothrombin time/international normalised ratio (PT/INR) and activated partial thromboplastin time (aPTT)
 - Tumour markers (only for those patients who have tumours with correlative serological markers, *e.g.*, CEA)
- Thyroid function test: free T4 and TSH

- Tumour imaging (CT or MRI) – performed within 28 days of C1D1. A recent previously obtained image may be used if obtained within 28 days of C1D1 and images collected meet protocol requirements. Biopsied lesions should not be designated as target lesions for the purposes of RECIST
- A fresh tumour tissue biopsy is mandatory, except if associated with unacceptable clinical risk, and should be taken within 14 days of C1D1. If a fresh biopsy is not possible, a prior (archival) biopsy that is less than 6 months old will be acceptable. A further biopsy will be obtained on or after C2D8
- Advise the patient to take measures to avoid or minimise exposure to UV light for the duration of study participation and for a period of 4 weeks following the last dose of study medication
- Patient registration

5.2 Evaluations to be Performed During the Study

During the treatment period, the assessments detailed in the sections below and summarised in the [Summary Schedule of Events](#) will be performed.

Collection of prior scan reports for assessment of previous tumour measurements can be performed any time during patient participation in the study or during follow-up. This will allow understanding of the trajectory of tumour growth prior to the patient entering this study.

5.2.1 Each Cycle, Day 1

All procedures to be completed prior to dosing:

- Recording of new or changes to concomitant medication.
- Physical examination
 - Full physical examination (including neurological examination) to be performed on C1D1 and C2D1 only in Phase Ib and on C1D1 only in Phase II. Full physical examination includes ears, eyes, nose, head, throat, neck, lungs, heart, abdomen, extremities, skin, musculoskeletal, nervous system, and lymph nodes
 - Directed physical examination (directed by clinical signs and symptoms, including neurological examination), only if clinically indicated, to be performed from Cycle 3 onwards in Phase Ib and from Cycle 2 onwards in Phase II
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- ECOG performance status
- Vital signs, including measurement of pulse rate, respiratory rate, blood pressure and temperature, after the patient has been seated or in the supine position for 5 minutes
- Weight

- **Pre-dose ECGs:** 12-lead ECGs should be performed prior to infusion of all IMPs. *Note: on C1D1 the 12-lead ECG should also be performed post-infusion of NUC-3373 (Section 5.2.2)*
 - The ECGs should be reviewed by the Investigator or a qualified designee for safety and quality
 - The QTc interval should be calculated using the Fridericia formula. Management of QT/QTc prolongation should be performed in accordance with institutional standard of care at the discretion of the treating physician
 - Digital and certified paper copies should be retained at site and stored as part of the study documents in the event they need to be used in a future analysis
- Urine pregnancy test for women of child-bearing potential. If any urine test result is positive, patient dosing will be postponed until the result is confirmed by a serum pregnancy test. Any patient with a positive serum test will not be allowed to receive any study treatment
- Blood samples drawn for:
 - Haematology: WBC count, differential WBC count, RBC, haemoglobin, haematocrit and platelets
 - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, direct bilirubin, indirect bilirubin, ALP, AST, ALT and LDH
 - Coagulation parameters: PT/INR and aPTT
 - Tumour markers where applicable (e.g., CEA)
- Thyroid function test: free T4 and TSH. Testing is to be performed **every 4 cycles** (i.e., Day 1 of Cycles 5, 9, 13, etc)
- IV administration of study drug:
 - Pembrolizumab + LV + NUC-3373
- AE recording and causality assessment

5.2.2 Cycle 1 ONLY

- **Post-dose ECGs:** In addition to the pre-dose ECGs performed on Day 1 of each cycle, 12-lead ECGs should also be performed post-infusion of NUC-3373 on C1D1 only
 - The ECGs should be reviewed by the Investigator or a qualified designee for safety and quality
 - The QTc interval should be calculated using the Fridericia formula. Management of QT/QTc prolongation should be performed in accordance with institutional standard of care at the discretion of the treating physician
 - Digital and certified paper copies should be retained at site and stored as part of the study documents in the event they need to be used in a future analysis

5.2.3 Cycle 2 ONLY

- A fresh on-treatment tumour tissue biopsy is mandatory and should be taken on C2D8 or on any treatment day after C2D8. It is preferred that this biopsy is obtained within 6 hours, but no later than 24 hours, after the end of NUC-3373 infusion. If this is not possible, it may be obtained at any time after C2D8
- A blood sample for PK measurements should be taken at the same time as performing the on-treatment biopsy if the biopsy is taken within 6 hours, but no later than 24 hours, after the end of NUC-3373 infusion on C2D8 or any treatment day after C2D8

5.2.4 Each cycle, Day 8

All procedures to be completed prior to dosing:

- Recording of new or changes to concomitant medications
- Vital signs, including measurement of pulse rate, respiratory rate, blood pressure and temperature, after the patient has been seated or in the supine position for 5 minutes
- Blood samples drawn for:
 - Haematology: WBC count, differential WBC count, RBC, haemoglobin, haematocrit and platelets
 - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, direct bilirubin, indirect bilirubin, ALP, AST, ALT and LDH
 - Coagulation parameters: PT/INR and aPTT
- IV administration of study drug:
 - LV + NUC-3373
- AE recording and causality assessment

5.2.5 Each cycle, Day 15

All procedures to be completed prior to dosing:

- Recording of new or changes to concomitant medications
- Vital signs, including measurement of pulse rate, respiratory rate, blood pressure and temperature, after the patient has been seated or in the supine position for 5 minutes
- Blood samples drawn for:
 - Haematology: WBC count, differential WBC count, RBC, haemoglobin, haematocrit and platelets
 - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, direct bilirubin, indirect bilirubin, ALP, AST, ALT and LDH
 - Coagulation parameters: PT/INR and aPTT

- IV administration of study drug:
 - LV + NUC-3373
- AE recording and causality assessment

5.2.6 Every 8 weeks (± 7 days) from Cycle 1 Day 1 until disease progression, initiation of a new treatment or death

- Tumour imaging (CT or MRI)
 - CRs, PRs and PD must be confirmed by repeated measurement. The confirmatory scans should be performed at least 28 days and not more than 42 days after initial assessment of response for CRs and PRs and at least 28 days and not more than 56 days after initial assessment for PD
 - If a patient is found to have a response at the confirmatory scan which has improved from PR to CR, then the improved response must also be confirmed within a further 28- to 42-day window
 - Additional tests may be requested at the Investigator's discretion. The same modality should be used throughout

5.2.7 End of Treatment Visit

The assessments to be performed on discontinuation due to radiological disease progression or early treatment discontinuation for other reasons (*e.g.*, withdrawal of consent) are summarised below. The End of Treatment visit should occur within a minimum of 30 days to a maximum of 37 days following the last administration of any study medication. During the End of Treatment visit, the following items will be assessed:

- Recording of new or changes to concomitant medication
- Full physical examination, including vital signs. Full physical examination includes ears, eyes, nose, head, throat, neck, lungs, heart, abdomen, extremities, skin, musculoskeletal, nervous system, and lymph nodes. Vital signs include measurement of pulse rate, respiratory rate, blood pressure and temperature, after the patient has been seated or in the supine position for 5 minutes, and temperature
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- ECOG performance status
- Weight
- 12-lead ECG
 - The 12-lead ECGs should be reviewed by the Investigator or a qualified designee for safety and quality
 - The QTc interval should be calculated using the Fridericia formula. Management of QT/QTc prolongation should be performed in accordance with institutional standard of care at the discretion of the treating physician
 - Digital and certified paper copies should be retained at site and stored as part of the study documents in the event they need to be used in a future analysis

- Blood samples drawn for:
 - Pregnancy testing for women of child-bearing potential
 - Haematology: WBC count, differential WBC count, RBC, haemoglobin, haematocrit and platelets
 - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, direct bilirubin, indirect bilirubin, ALP, AST, ALT and LDH
 - Coagulation parameters: PT/INR and aPTT
 - Tumour markers where applicable (*e.g.*, CEA)
- Thyroid function test: free T4 and TSH
- AE recording and causality assessment

5.2.8 Follow-Up

Patients who stop treatment with no evidence of radiological disease progression as defined by iRECIST criteria will continue to receive scans at regular intervals (every 8 weeks [± 7 days] from C1D1) until disease progression, initiation of a new treatment, or death (whichever comes first) in order to determine duration of overall response and PFS.

All patients who no longer attend clinic every 8 weeks for any reason will be followed up for survival and information regarding subsequent anti-cancer medications prescribed every 12 weeks (± 14 days) from the time of the last scheduled study visit until withdrawal of consent, lost to follow-up, death, or the overall end of study, whichever is earliest. A total of three attempts should be made before the patient is considered as lost to follow-up. Patients in follow-up who have not experienced disease progression should continue to attend the clinic for planned radiologic scans; however, other follow-up data can be collected remotely.

5.2.9 Patient Evaluability and Replacement

All patients who receive any study treatment are considered evaluable for safety analysis.

All patients with measurable disease who undergo at least 2 cycles of treatment, receive at least 75% of planned treatment in that 2-cycle period, and undergo a post-treatment objective disease assessment, will be considered evaluable for response assessment.

6 SAMPLES FOR LABORATORY ANALYSIS

6.1 Pharmacokinetics

One blood sample should be collected at the same time as performing the on-treatment tumour biopsy (see [Section 6.2](#)) if the biopsy is taken within 6 hours, but no later than 24 hours, after the end of NUC-3373 infusion on C2D8 or any treatment day after C2D8.

PK analytes measured may include, but are not limited to:

- NUC-3373, 5-FU, FBAL, dUrd in plasma

Standard PK parameters for each compound of interest will then be derived from the measured plasma concentrations. Please refer to the PK and Translational Laboratory Manual for details regarding PK sample collection, processing and shipping.

6.2 Tumour Tissue Sample and Pharmacodynamics

Tumour samples will be collected to explore the relationship between the pharmacodynamics of NUC-3373, safety, and clinical activity.

Tissue samples are mandatory and should be collected as follows:

- A fresh tissue biopsy should be obtained during the Screening period within 14 days of the first dose of study treatment on C1D1. An archival tissue sample taken within 6 months of the first dose of study treatment will be acceptable if a fresh tumour biopsy is not possible.
- A fresh tissue biopsy should be obtained on C2D8 or on any treatment day after C2D8. It is preferred that this biopsy is obtained within 6 hours, but no later than 24 hours, after the end of NUC-3373 infusion. If this is not possible, it may be obtained at any time after C2D8.

The fresh tissue biopsy should not be taken from a lesion to be designated a target lesion for the purposes of RECIST assessment during the study.

A blood sample for PK measurements should be taken at the same time as performing the on-treatment tumour biopsy if the biopsy is taken within 6 hours, but no later than 24 hours, after the end of NUC-3373 infusion on C2D8 or any treatment day after C2D8.

The mandatory biopsy requirement may be removed if the Sponsor deems that sufficient samples have been collected. All study sites will be notified in writing if this decision is made.

For sampling procedures, storage conditions and shipment instructions, please refer to the accompanying PK and Translational Laboratory Manual.

7 INVESTIGATIONAL MEDICINAL PRODUCTS

7.1 Treatment Administration

7.1.1 Dose-validation Phase

The starting dose for NUC-3373 will be 1875 mg/m². An initial cohort of 3 evaluable patients will receive 1875 mg/m² NUC-3373 in combination with 400 mg/m² LV on Days 1, 8, and 15, along with pembrolizumab at a dose of 200 mg on Day 1 of 21-day cycles. There will be a 9-day observation period between the treatment of each patient in this first cohort. All patients will be monitored for DLTs during Cycle 1

During Phase Ib, regular meetings will be held to review and monitor safety and tolerability of NUC-3373 + LV in combination with pembrolizumab to confirm the safety of the starting dose level and next steps for including further patients at this or a lower dose level. Safety data reviewed will be cumulative, including later AEs (after Cycle 1), which will be considered at subsequent SRC meetings for all ongoing patients. The dose decision may be amended depending on emerging data and any decisions will be formally minuted, with the minutes distributed to all SRC members.

In the initial cohort, study treatment will be administered in 21-day cycles as follows:

1. Pembrolizumab IV over 30 minutes at a dose of 200 mg on Day 1.
2. LV 400 mg/m² IV (or equivalent levo-leucovorin [200 mg/m²]) over 120 minutes prior to each NUC-3373 infusion on Days 1, 8 and 15.
3. NUC-3373 IV over 150 minutes at an initial dose of 1875 mg/m² on Days 1, 8 and 15.

There should be no changes to the treatment schedule outlined above. However, in the event of AEs occurring during infusion of any of the study drugs, a dosing delay of 1 day (maximum) may be permitted for subsequent study drugs not yet infused, after discussion with the Medical Monitor.

If the 1875 mg/m² dose of NUC-3373 is not tolerated and dose-escalation is initiated, an initial cohort will receive study treatment in 21-day cycles as follows:

1. Pembrolizumab IV over 30 minutes at a dose of 200 mg on Day 1.
2. LV 400 mg/m² IV (or equivalent levo-leucovorin [200 mg/m²]) over 120 minutes prior to each NUC-3373 infusion on Days 1, 8 and 15.
3. NUC-3373 IV over 120 minutes at an initial dose of 1125 mg/m² on Days 1, 8 and 15.

Once a tolerable dose has been established, enrolment will continue until a total of up to 12 patients have been recruited. Any dose adjustments will be determined during the study by the Medical Monitor and the SRC.

Please refer to the current Pharmacy Manual for further information on NUC-3373 preparation and administration.

7.1.2 Dose-expansion Phase

Following completion of the dose-validation phase, expansion cohorts of approximately 20-40 patients in 2-4 selected tumour types may be initiated at the selected dose level.

7.2 Interaction with Other Drugs

Patients receiving NUC-3373 should be carefully managed for potential drug interactions according to local practice. Refer to [Section 9](#) for information regarding the use of prohibited therapies and therapies to be used with caution.

Interactions between pembrolizumab and any concomitant medications should be checked with the respective package inserts in accordance with local practice. Local practice guidelines should be followed for the management of any potential pembrolizumab/concomitant medication interactions.

8 MANAGEMENT OF TOXICITY

Adverse events may be managed by dose delays and/or dose reductions of NUC-3373, according to the clinical situation.

Guidelines for modification of NUC-3373 and LV dosing for haematological and non-haematological toxicities are given in [Table 3](#). The dose of NUC-3373 should initially be reduced to the next lower dose level. In the event of immune-related AEs, any potentially required dose modifications for NUC-3373 should be discussed on a case-by-case basis with the medical monitor.

NUC-3373 dose reductions may be temporary, in which case the next cycle can revert to the starting dose of the previous cycle, or permanent, which would apply to all subsequent cycles. Over the whole dosing period, each patient may have a maximum of 2 permanent dose reductions, after which treatment with NUC-3373 will be discontinued and the patient will be removed from study.

Dose modifications should always start with dose reductions for NUC-3373, before deciding if dosing delays for pembrolizumab are required.

Table 3 Dose modification guidelines

NUC-3373 dose (mg/m ²)	Leucovorin dose (mg/m ²)
1875	400 ¹
1500	400 ¹
1125	400 ¹
750	400 ¹
¹ Leucovorin dose should be maintained but may be reduced in accordance with label	

Treatment between cycles can be delayed for up to 21 days for patients to meet the re-treatment criteria before starting their next cycle. Patients who do not meet these requirements after this additional time will not be allowed to receive further cycles of NUC-3373 and will be withdrawn from the study unless the patient is receiving clinical benefit in the opinion of the Investigator.

In patients receiving clinical benefit, AEs attributed to pembrolizumab may be managed by dose delays or discontinuation in accordance with standard clinical practice. Treatment with NUC-3373 must be continued for the patient to remain on treatment for the purposes of this protocol.

Dose modifications for pembrolizumab should be in line with the package insert / local standard procedures (see also [Section 8.2.2](#)).

Dose reductions for pembrolizumab are not permitted. As outlined in [Section 8.2.2](#), there will be no dose reductions of pembrolizumab; however, dosing may be delayed for toxicity as described in [Table 5](#).

8.1 Criteria for Continuation of Treatment

Patients must meet all of the following criteria prior to receiving a dose of study treatment:

- ANC (growth factor support permitted after Cycle 1)
 - $\geq 1.5 \times 10^9/\text{L}$ for full treatment dose

- $\geq 1.0 \times 10^9/\text{L}$ for reduced treatment dose (see [Table 4](#))
- Platelet count $\geq 75 \times 10^9/\text{L}$ (platelet transfusion permitted after Cycle 1)
- No evidence of disease progression (based on radiographic assessment)
- Recovery from all clinically significant toxicities to $\leq$ Grade 2 or to baseline grade present at study entry

If a patient fails to meet any of these criteria, administration of study drug should be held until all re-treatment criteria are met. The study drugs can be withheld for a maximum of 21 days; if the delay is >21 days, the patient must be withdrawn from treatment, unless the patient is receiving a clinical benefit.

8.2 Dose Modifications

8.2.1 NUC-3373

Treatment between cycles can be delayed for up to 21 days for patients to meet the re-treatment criteria before starting their next cycle. Patients who fail to meet these requirements after this additional time will not be allowed to receive further cycles of therapy within the study unless they are receiving clinical benefit in the opinion of the Investigator. If a patient experiences multiple toxicities, dose adjustments of NUC-3373 will be based on the most severe toxicity.

Any patient who experiences one or more recurrent, clinically significant toxicity after the initial permanent dose reduction may have one further dose reduction. Patients who continue to experience clinically significant toxicity despite two permanent dose reductions will be discontinued from study treatment. A patient receiving clinical benefit will not require discontinuation and treatment guidelines for such patient will be decided in discussion with the Investigator, Medical Monitor and SRC based on all data available for NUC-3373 at the time.

General guidance for dose modifications of NUC-3373 in response to common toxicities is provided in Table 4.

If the planned dose(s) on a particular visit cannot be administered during that planned visit for any reason and needs to be delayed, the drug(s) may subsequently be administered within the next 24 hours. If the delay needs to be longer than 24 hours, then the planned dose(s) should be skipped and treatment should be delayed until the next planned dose.

Table 4 NUC-3373 dose modifications for common toxicities

Toxicity Grade	During a cycle of therapy	At the start of subsequent cycles of therapy ^a
Neutropenia		
Grade 1 (LLN – 1500/mm ³)	Maintain dose level	Maintain dose level
Grade 2 ($<1500 - 1000/\text{mm}^3$)	Reduce by 1 dose level for the next administration	1 episode: Maintain starting dose used in previous cycle ≥ 2 episodes: Maintain reduced dose level
Grade 3 ($<1000 - 500/\text{mm}^3$)	Omit dose until resolved to $\leq$ Grade 2, then reduce by 1 dose level	Maintain reduced dose level
Grade 4 ($<500/\text{mm}^3$)	Omit dose until resolved to $\leq$ Grade 2, then reduce by 2 dose levels	Maintain reduced dose level
Neutropenic fever		
All	Omit dose until resolved (no fever and $\leq$ Grade 2 neutropenia), then reduce by 2 dose levels	
Other haematologic toxicities		
All	Dose modifications for leukopaenia or thrombocytopaenia during a cycle of therapy and at the start of subsequent cycles of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above.	
Diarrhoea		
Grade 1	Continue dosing, depending on clinical judgement	Maintain dose level
Grade 2	Omit dose until resolved to baseline, then reduce by 1 dose level	1 episode: Maintain starting dose used in previous cycle ≥ 2 episodes: Maintain reduced dose level
Grade 3	Omit dose until resolved to baseline, then reduce by 1 dose level	Maintain reduced dose level
Grade 4	Omit dose until resolved to baseline, then reduce by 2 dose levels	Maintain reduced dose level
Cardiotoxicity		
All	In case of severe cardiotoxicity, discontinue NUC-3373 treatment.	
Other non-haematologic toxicities ^b		
Grade 1	Maintain dose level	Maintain dose level
Grade 2	Reduce by 1 dose level for the next administration	1 episode: Maintain starting dose used in previous cycle ≥ 2 episodes: Maintain reduced dose level

Toxicity Grade	During a cycle of therapy	At the start of subsequent cycles of therapy ^a
Grade 3	Omit dose until resolved to $\leq$ Grade 2, then reduce by 1 dose level	Maintain reduced dose level
Grade 4	Omit dose until resolved to $\leq$ Grade 2, then reduce by 2 dose levels	Maintain reduced dose level
^a Relative to the starting dose used in the previous cycle ^b Excludes alopecia, anorexia, asthenia		

8.2.2 Pembrolizumab

The dosing of pembrolizumab may be delayed based on safety events observed.

Dose reductions of pembrolizumab are not allowed.

If pembrolizumab-related AEs are observed, dose delays should be performed as per the Prescribing Information or standard local practice. Guidelines for the management of select immune-related toxicities are provided in Table 5. Please also refer to the latest ASCO guidelines ([Schneider et al, 2021](#)).

The following general instructions apply:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening immune-related AEs, an IV corticosteroid should be initiated first followed by an oral steroid. Other immunosuppressive treatment should be initiated if the AEs cannot be controlled by corticosteroids.

For a complete overview of pembrolizumab-related AEs, refer to the Prescribing Information.

Table 5 Management of pembrolizumab-related toxicities

Immune-related AEs	Toxicity grade or condition	CTCAE v5.0 description / ASCO guidelines	Action taken with pembrolizumab	Management with corticosteroid and/or other therapies*	Monitor and follow-up
Pneumonitis	Grade 1	Asymptomatic; clinical or diagnostic observations only	Withhold or proceed with close monitoring If held, resume with radiographic evidence of improvement or resolution. If no improvement, treat as Grade 2.	N/A	• Monitor patients weekly with history and physical exam, pulse oximetry • May offer chest imaging if uncertain diagnosis and/or to follow progress • Repeat chest imaging in 3-4 weeks or sooner if patient becomes symptomatic
	Grade 2	Symptomatic; medical intervention indicated; limiting instrumental ADL	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor patients for signs and symptoms of pneumonitis • Evaluate patients with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3	Severe symptoms; limiting self-care ADL; oxygen indicated	Permanently discontinue		
	Grade 4	Life-threatening respiratory death compromise; urgent intervention indicated (e.g., tracheotomy or intubation)			
Diarrhoea/Colitis	Grade 1	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Continue treatment or withhold and then resume if toxicity does not exceed Grade 1 or resolves	• May offer supportive care with medications such as loperamide if infection has been ruled out in patients with diarrhoea only and not colitis-related symptoms as a temporary measure	• Monitor for dehydration and recommend dietary changes • Monitor closely by phone or electronic medical system for symptom changes by clinical providers every 3 days or more frequently if needed until stabilisation

Immune-related AEs	Toxicity grade or condition	CTCAE v5.0 description / ASCO guidelines	Action taken with pembrolizumab	Management with corticosteroid and/or other therapies*	Monitor and follow-up
					• May obtain gastroenterology consult for prolonged Grade 1 cases and consider endoscopy with biopsy
	Grade 2	Increase of 4-6 stools per day over baseline; moderate increase in ostomy output compared to baseline; limiting instrumental ADL/Abdominal pain; mucus or blood in stool	Withhold until recovery to ≤Grade 1	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper, unless diarrhoea is transient	• Monitor patients for signs and symptoms of enterocolitis (<i>i.e.</i>, diarrhoea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (<i>i.e.</i>, peritoneal signs and ileus)
	Grade 3	Increase of ≥7 stools per day over baseline; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self-care ADL/Severe abdominal pain; peritoneal signs	Diarrhoea/colitis: As for Grade 2, but see additions in ASCO guidelines Recurrent Grade 3 colitis: Permanently discontinue		• Patients with ≥Grade 2 diarrhoea suspecting colitis should consider GI consultation and perform endoscopy to rule out colitis • Consider additional anti-inflammatory therapy according to ASCO guidelines
	Grade 4	Life-threatening consequences; urgent intervention indicated	Permanently discontinue and follow additional steps according to ASCO guidelines		• Patients with diarrhoea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
Immune-related skin adverse reactions	Caution should be used when considering the use of pembrolizumab in a patient who has previously experienced a severe or life-threatening skin adverse reaction on prior treatment with other immune-stimulatory anti-cancer agents.				
An example for treatment and monitoring is given for RASH. For further details and other skin conditions, please refer to the	Grade 1	Rash covering <10% BSA, which may or may not be associated with symptoms of pruritus or tenderness.	Continue pembrolizumab	Treat with topical emollients and/or mild-moderate potency topical corticosteroids.	• Patients should be monitored for suspected severe skin reactions and other causes should be excluded

Immune-related AEs	Toxicity grade or condition	CTCAE v5.0 description / ASCO guidelines	Action taken with pembrolizumab	Management with corticosteroid and/or other therapies*	Monitor and follow-up
SmPC for Pembrolizumab and to the ASCO guidelines.				Counsel patients to avoid skin irritants.	• Cases of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) have been reported in patients receiving pembrolizumab • For suspected SJS or TEN, pembrolizumab should be withheld and the patient should be referred to a specialised unit for assessment and treatment. For Grade 4 skin toxicities: Monitor closely for progression to SCAR. Consider alternative antineoplastic therapy over resuming pembrolizumab if the skin irAE does not resolve to # G1. If ICPis are the patient's only option, consider restarting once these side effects have resolved to a G1 level with close dermatology follow-up
	Grade 2	Rash covering 10%-30% BSA with or without symptoms (eg, pruritus, burning, and tightness); limiting instrumental ADL; rash covering 30% BSA with or without mild symptoms.	Consider holding Pembrolizumab and monitor weekly for improvement. Consider initiating prednisone (or equivalent) at dosing 0.5-1 mg/kg, tapering over 4 weeks. In patients with pruritis without rash, consider topical anti-itch remedies (eg, refrigerated menthol and pramoxine).	If skin toxicity is not improved after 4 weeks, then regrade toxicity as Grade 3. In addition, treat with topical emollients, oral anti-histamines, and medium-to-high potency topical corticosteroids. Consider initiating prednisone (or equivalent) at dosing 0.5-1 mg/kg, tapering over 4 weeks. In patients with pruritis without rash, consider topical anti-itch remedies (eg, refrigerated menthol and pramoxine).	
	Grade 3	Rash covering >30% BSA with moderate or severe symptoms; limiting self-care ADL.	Withhold Pembrolizumab until recovery to Grade $\leq$ 1 and consult with dermatology. If SJS or TEN is confirmed, pembrolizumab should be permanently discontinued.	Treat with topical emollients, oral antihistamines, and high-potency topical corticosteroids. Consider phototherapy to treat severe pruritus. Initiate oral prednisone or equivalent (1 mg/kg/d) tapering over at least 4 weeks. Once downgraded to # G1 and prednisone (or equivalent) below 10 mg/d, clinicians may consider resuming ICPi therapy with close monitoring and follow-up with dermatology in certain cases such as psoriasis. In patients with pruritis without rash, may treat with gabapentin, pregabalin, aprepitant, or dupilumab.	

Immune-related AEs	Toxicity grade or condition	CTCAE v5.0 description / ASCO guidelines	Action taken with pembrolizumab	Management with corticosteroid and/or other therapies*	Monitor and follow-up
	Grade 4	Severe consequences requiring hospitalization or urgent intervention indicated or life-threatening consequences.	Immediately hold pembrolizumab Consider permanent discontinuation	May admit patient immediately with direct oncology involvement and with an urgent consult by dermatology. Systemic steroids: IV methylprednisolone (or equivalent) dosed at 1-2 mg/kg with slow tapering when the toxicity resolves.	
AST/ALT elevation and/or increased bilirubin (Any signs of hepatitis)	Grade 1	Asymptomatic AST or ALT >ULN to 3.0 x ULN and/or Total bilirubin >ULN to 1.5 x ULN	Continue treatment with close monitoring	Manage with supportive care for symptom control	- Patients should be monitored for changes in liver function (at the start of treatment, periodically during treatment and as indicated based on clinical evaluation) and for symptoms of hepatitis, and other causes excluded - For Grade 2 and above: - Monitor with liver function tests (every 3 days until liver enzyme value returns to baseline or is stable) - Consult hepatologist
	Grade 2	Asymptomatic AST or ALT >3.0 to ≤5.0 x ULN and/or Total bilirubin >1.5 to ≤3.0 x ULN	Withhold	Administer corticosteroids (initial dose of 0.5 to 1 mg/kg/day prednisone or equivalent) if no improvement is observed within 3-5 days, followed by taper. If symptoms do NOT improve to ≤Grade 1, consider adding mycophenolate mofetil. May resume treatment if recovery to ≤Grade 1 on prednisone ≤10 mg/day.	
	Grade 3	AST or ALT 5.0 to 20.0 x ULN and/or Total bilirubin 3.0 to 10.0 x ULN Or, symptomatic liver dysfunction; fibrosis by biopsy; compensated cirrhosis; and reactivation of chronic hepatitis	Permanently discontinue if symptomatic liver dysfunction is present or based on severity of liver enzyme elevations	Immediately administer corticosteroids (initial dose of 1 to 2 mg/kg/day prednisone or equivalent) followed by taper within 4-6 weeks	
	Grade 4	AST or ALT >20.0 x ULN and/or		Follow Grade 3 recommendations according to	

Immune-related AEs	Toxicity grade or condition	CTCAE v5.0 description / ASCO guidelines	Action taken with pembrolizumab	Management with corticosteroid and/or other therapies*	Monitor and follow-up
		Total bilirubin >10 x ULN Or, decompensated liver function (e.g., ascites, coagulopathy, encephalopathy, and coma)		the list above and ASCO guidelines. Immediately administer 2 mg/kg/day prednisone	
Diabetes	All patients should be monitored for hyperglycaemia or other signs and symptoms of diabetes				
	Grade 1	Asymptomatic or mild symptoms; T2DM with fasting glucose >ULN to 160 mg/dL (>ULN to 8.9 mmol/L). No evidence of CIADM such as ketoacidosis or laboratory evidence of pancreatic autoimmunity	Continue treatment with close clinical follow-up and laboratory evaluation	• Refer to PCP for additional management or: • May initiate oral therapy for those with new-onset T2DM or intensify medical therapy for those with worsening T2DM • Administer insulin for patients with T1DM	• Urgent endocrine consultation for any patient with new-onset CIADM • Referral to emergency department or hospital admission if unable to initiate therapy, urgent outpatient specialist evaluation is not available, developing ketoacidosis, or other concern for CIADM • Endocrine consultation for all patients • Admit for inpatient management of DKA, volume and electrolyte resuscitation, and insulin initiation
	Grade 2	Moderate symptoms, able to perform ADL; T2DM with fasting glucose >160 to 250 mg/dL (>8.9 to 13.9 mmol/L). No ketoacidosis or metabolic derangements but other evidence of CIADM at any glucose level	Withhold until glucose control is obtained	• Initiate insulin for CIADM (or as default therapy if there is any question about the diagnosis)	
	Grade 3-4	Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL. Grade 3: >250 to 500 mg/dL (>13.9 to 27.8 mmol/L) Grade 4: >500 mg/dL (>27.8 mmol/L).	Withhold until glucose control is obtained with reduction of toxicity to ≤Grade 1	• Insulin therapy appropriate for all patients	

Immune-related AEs	Toxicity grade or condition	CTCAE v5.0 description / ASCO guidelines	Action taken with pembrolizumab	Management with corticosteroid and/or other therapies*	Monitor and follow-up
		Ketoacidosis or other metabolic abnormality			
Hypophysitis	Grade 1	Asymptomatic or mild symptoms	Withhold until symptoms stabilised under hormone replacement therapy	• Administer hydrocortisone hormonal replacement therapy and consider initiating other hormonal replacements as clinically indicated	• Endocrine consultation
	Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age- appropriate instrumental ADL	Withhold until symptoms stabilised under hormone replacement therapy	• Administer prednisolone 1mg/kg/day as oral pulse therapy if clinically indicated and continue hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency) and exclude other causes • Hospitalise if clinically indicated • Endocrine consultation • Pituitary function and hormone levels should be monitored to ensure appropriate hormone replacement
	Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; limiting self-care ADL	Withhold (or discontinue for Grades 3 or 4 adrenal insufficiency or symptomatic hypophysitis) until symptoms stabilised under hormone replacement therapy or permanently discontinue if clinically indicated ¹	• Hospitalise or make an emergency department referral for: IV stress dose steroids: Hydrocortisone 50-100 mg Q 6-8 hours initial dosing Oral pulse dose therapy with Prednisone 1-2 mg/kg daily (or equivalent) tapered over at least 1-2 weeks to physiologic Taper stress dose corticosteroids down to oral maintenance doses over 5-7 days Maintenance therapy as in Grade 1	
	Grade 4	Life-threatening consequences; urgent intervention indicated	Continuation of pembrolizumab may be considered after corticosteroid taper, if needed		

Immune-related AEs	Toxicity grade or condition	CTCAE v5.0 description / ASCO guidelines	Action taken with pembrolizumab	Management with corticosteroid and/or other therapies*	Monitor and follow-up
Hyperthyroidism (may be managed symptomatically)	Grade 1	Asymptomatic or mild symptoms	Continue	• Monitor TSH and T4 • For $\geq$Grade 2: • Monitor TSH/T4 • Treat with non-selective beta-blockers (e.g., propranolol) or thionamides as appropriate, hydration and supportive care	• Patients should be monitored for changes in thyroid function (at the start of treatment, periodically during treatment and as indicated based on clinical evaluation) and clinical signs and symptoms of thyroid disorders • Endocrine consultation
	Grade 2	Symptomatic; thyroid suppression therapy indicated; limiting instrumental ADL	Continue		
	Grade 3	Severe symptoms limiting self-care ADL; hospitalization indicated	Withhold until symptoms return to Grade $\leq$ 1 or permanently discontinue ¹		
	Grade 4	Life-threatening consequences; urgent intervention indicated			
Hypothyroidism	Grade 1	Asymptomatic TSH >4.5 and <10 mIU/L	Continue	• Monitor TSH	• Patients should be monitored for changes in thyroid function (at the start of treatment, periodically during treatment and as indicated based on clinical evaluation) and clinical signs and symptoms of thyroid disorders • Monitor for signs and symptoms of thyroid disorders • Consider endocrine consultation
	Grade 2	Symptomatic; thyroid replacement indicated; limiting instrumental ADL	Continue or withhold until symptoms return to baseline	• Initiate thyroid replacement hormones (e.g., levothyroxine or liothyronine) per standard of care	
	Grade 3	Severe symptoms; limiting self-care ADL; hospitalization indicated	Withhold until symptoms return to baseline with appropriate supplementation		
	Grade 4	Life-threatening consequences; urgent intervention indicated			
For patients with Grade 3 or Grade 4 endocrinopathies in general, that improve to Grade 2 or lower and are controlled with hormone replacement, if indicated, continuation of pembrolizumab may be considered after corticosteroid taper, if needed. Otherwise, treatment should be discontinued.					
Nephritis and renal dysfunction (creatinine increased)	Grade 1	1.5-2.0 x baseline; increase of >0.3 mg/dL	Withhold pending consideration of potential alternative aetiologies and baseline renal function	Monitor creatinine	• Nephrology consultation • Monitor changes of renal function • Ensure adequate evaluation to confirm
	Grade 2	>1.5 - 3.0 x baseline; >1.5 - 3.0 x ULN	Withhold, based on severity of creatinine elevations	• Administer corticosteroids (prednisone 1 to 2 mg/kg/day	

Immune-related AEs	Toxicity grade or condition	CTCAE v5.0 description / ASCO guidelines	Action taken with pembrolizumab	Management with corticosteroid and/or other therapies*	Monitor and follow-up
	Grade 3	>3.0 x baseline; >3.0-6.0 x ULN	Permanently discontinue	or equivalent) followed by taper if improved to ≤Grade 1. Taper steroids over at least 4 weeks	aetiology and/or exclude other causes
	Grade 4	>6.0 x ULN			
Myocarditis	Grade 1	-	Withhold	• Based on severity of AE consider steroid treatment	• Ensure adequate evaluation to confirm aetiology and/or exclude other causes
	Grade 2	Symptoms with moderate activity or exertion	Withhold treatment Pembrolizumab may be restarted within 12 weeks after last dose of KEYTRUDA if the adverse reaction recovers to Grade ≤1 and corticosteroid dose has been reduced to ≤10 mg prednisone or equivalent per day	Administer high dose corticosteroids	• Cardiology consultation
	Grade 3	Severe with symptoms at rest or with minimal activity or exertion; intervention indicated; new onset of symptoms	Permanently discontinue		• Management of cardiac symptoms according to ACC/AHA guidelines and with guidance from cardiology
	Grade 4	Severe with symptoms at rest or with minimal activity or exertion; intervention indicated; new onset of symptoms			
All other immune-related AEs	Grade 2 (intolerable/persistent)	See CTCAE v5.0 for each AE of interest	Withhold		• Ensure adequate evaluation to confirm etiology or exclude other cause
	Grade 3		Withhold or discontinue based on the type of event. Events that require discontinuation include but are not limited to: Guillain-		

Immune-related AEs	Toxicity grade or condition	CTCAE v5.0 description / ASCO guidelines	Action taken with pembrolizumab	Management with corticosteroid and/or other therapies*	Monitor and follow-up
	Grade 4 or recurrent grade 3		Barré Syndrome, encephalitis Permanently discontinue	Based on severity of AE administer corticosteroids For Grades 2 or 3: Pembrolizumab may be restarted within 12 weeks after last dose of KEYTRUDA if the adverse reaction recovers to Grade ≤1 and corticosteroid dose has been reduced to ≤10 mg prednisone or equivalent per day	
Abbreviations: ACC=American College of Cardiology; ADL=activities of daily living; AE=adverse event; AHA=American Heart Association; ALT=alanine aminotransferase; AST=aspartate aminotransferase; CIADM=checkpoint inhibitor-associated diabetes mellitus; CTCAE=Common Terminology Criteria for Adverse Events; DKA=diabetic ketoacidosis; GI=gastrointestinal; IV=intravenous; PCP=primary care practitioner; T1DM= Type 1 diabetes mellitus; TSH=thyroid stimulating hormone; ULN=upper limit of normal. Source: KEYNOTE-590 protocol (available from: https://clinicaltrials.gov/ct2/show/NCT03189719) * Please refer to the latest ASCO guidelines (Schneider <i>et al</i>, 2021) for further information. NOTES: - Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. - For patients with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).					

Pembrolizumab may cause severe or life-threatening infusion reactions, including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after infusion and generally resolve completely within 24 hours of infusion completion. In case of pembrolizumab-related infusion reactions, the guidelines provided in Table 6 should be followed.

Table 6 Management of pembrolizumab-related infusion reactions

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., anti-histamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hours	Stop infusion. Additional appropriate medical therapy may include, but is not limited to: • IV fluids • Anti-histamines • NSAIDs • Acetaminophen • Narcotics Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise, dosing will be held until symptoms resolve and the patient should be premedicated for the next scheduled dose. Patients who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study treatment.	Patient may be premedicated 1.5 hours (± 30 minutes) prior to infusion of pembrolizumab with: • Diphenhydramine 50 mg PO (or equivalent dose of antihistamine) • Acetaminophen 500 to 1000 mg PO (or equivalent dose of analgesic)
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Stop infusion. Additional appropriate medical therapy may include, but is not limited to: • Epinephrine** • IV fluids • Anti-histamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Patient is permanently discontinued from further study treatment.	No subsequent dosing
Abbreviations: IV=intravenous; NCI CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events; NSAID=nonsteroidal anti-inflammatory drug; PO=per os (orally). Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, refer to the CTCAE v4.0 at http://ctep.cancer.gov		

9 OTHER TREATMENTS (NON-IMP)

9.1 Prohibited Therapies

Except as included in the specific modules and dosing regimens of this study, use of the following therapies is prohibited (at any dose and at any time) during the study:

- Other cytotoxic chemotherapy
- Radiotherapy, excluding a short cycle of palliative radiotherapy
- Immunotherapy (with the exception of pembrolizumab to be administered in Module 1), including immunosuppressive therapy
- Systemic steroid therapy or any immunosuppressive therapy (≥ 10 mg/day prednisone or equivalent)
- Radioimmunotherapy
- Hormone therapy (other than contraceptives, hormone-replacement therapy, anti-hormonal treatment for patients with prostate cancer, or megestrol acetate)
- Other biologic agents intended for the treatment of cancer under investigation (other than haematopoietic growth factors, which are allowed if clinically indicated and used in accordance with instructions provided in the package inserts. Concomitant dosing with study treatments should be avoided)
- Any therapies intended for the treatment of cancer under investigation, whether approved by local regulatory authorities or investigational, with the exception of treatments for patients with a concurrent well-controlled cancer (as per Exclusion Criterion 16)
- Drugs that are known to prolong the QTc interval (refer to Appendix 4 of the core protocol)
- Strong inducers of CYP3A4, including but not limited to phenytoin, phenobarbital, carbamazepine, rifampin, rifabutin or St. John's wort
- Strong inhibitors of CYP3A4, including but not limited to ketoconazole, clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, or atazanavir
- Live vaccines, including live attenuated vaccines (must also be avoided for 90 days after last dose of study medication)
- Diuretics and laxatives should be avoided in patients experiencing treatment-related diarrhoea and cholinergic reactions
- Warfarin and other long-acting anticoagulants with a half-life of >12 hours (prohibited from 4 weeks prior to the first dose of study treatment until after the last dose of study treatment)
- Herbal preparations/medications, including but not limited to St. John's Wort, kava, ephedra (ma huang), ginkgo biloba, dehydroepiandrosterone, yohimbe, saw palmetto, and ginseng (prohibited from the time that patients enter the Screening period until the last dose of study treatment). Patients should stop using these medications 7 days prior to the first dose of study treatment (3 weeks for St John's Wort)

9.2 Therapies to be Used with Caution

For patients in all modules of the study, warfarin and other types of long-acting anti-coagulants (such as phenprocoumon and anti-Xa inhibitors with a half-life of >12 hours) are prohibited from 4 weeks prior to the first dose of study treatment until after the last dose of study treatment. Patients requiring low dose anti-coagulant treatment should switch to low molecular weight heparin or anti-Xa inhibitors with a half-life of ≤ 12 hours.

In vitro studies have shown the potential for NUC-3373 drug-drug interaction with concomitant medications that are metabolised by CYP3A4 and CYP2C8. The clinical impact of these *in vitro* observations is not known and the use of concomitant medications that are cleared by these CYP enzymes is permitted with caution in patients receiving NUC-3373.

10 TUMOUR RESPONSE ASSESSMENTS

The primary efficacy endpoint (ORR) will be assessed using iRECIST criteria (Appendix 2 of the core protocol) and the secondary efficacy response endpoints will be assessed using RECIST v1.1 criteria (Appendix 1 of the core protocol). Patients may continue to receive study treatment until documentation of radiological disease progression or unacceptable toxicity. Patients should not discontinue treatment based on progressive disease per RECIST 1.1 unless clinical deterioration or increase in tumour markers is observed. iRECIST should always be considered.

Patients must have at least one lesion that can be accurately assessed at baseline by CT or MRI or by callipers and which is suitable for repeated assessment in order to be eligible for this study. All known or suspected disease sites must be assessed at baseline by either CT or MRI scan or by callipers as appropriate. Additional anatomical areas should be assessed by CT or MRI in case of suspicion of presence of metastases based on signs, symptoms, biochemical results and/or as standard of care imaging of patients (*i.e.*, non-study related). The same measurement method used at baseline must be used to follow lesions throughout the study. Disease status must be assessed according to iRECIST (primary endpoint) or RECIST v1.1 (secondary endpoints) criteria, with target and non-target lesions identified, measured and followed throughout the study (Appendix 1 and Appendix 2 of the core protocol). Biopsied lesions should not be designated as target lesions for the purposes of RECIST and a target lesion should not be biopsied during the study unless medically necessary.

Whenever possible, the same qualified physician will interpret results to reduce variability. Radiographic images will be maintained at the study centre and Investigator's assessments will be filed in the patient's source documents, photographs documenting any caliper measurements of visible lesions will also be maintained in patients source documents.

Tumour measurements and disease response assessments are to be performed every 8 weeks (± 7 days) from Cycle 1 Day 1. If the patient stops study treatment for reasons other than confirmed progressive disease per iRECIST, tumour measurements and disease response assessments should continue every 8 weeks (± 7 days) from Cycle 1 Day 1 until progressive disease is confirmed.

Tumour measurements and disease response assessments should be performed any time disease progression is suspected.

CRs and PRs must be confirmed by repeated measurement at least 28 days and not more than 42 days after initial documentation. Patients who stop treatment following an unconfirmed response should also still have a confirmatory scan, if the scan can take place prior to the patient starting any subsequent anti-cancer therapies. If a patient is found to have a response at the confirmatory scan which has improved from PR to CR, then the improved response must also be confirmed within the pre-specified 28- to 42-day window, if the scan can take place prior to the patient starting any subsequent anti-cancer therapy.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval of not less than 7 weeks from baseline.

Assessment of disease progression using iRECIST criteria requires confirmation of PD. Patients should be treated and followed-up post-initial progression (termed unconfirmed PD or iUPD) until subsequent disease progression (termed confirmed PD or iCPD). Note: patients who have unequivocal rapid tumour progression and/or symptomatic progression in the opinion of the Investigator will not be eligible to continue to receive treatment. As per iRECIST, such patients

are not required to continue to the confirmatory scan and the overall visit response should be assessed as iUPD.

In this study a confirmation of progression scan is required at no less than 4 weeks and no more than 8 weeks after the first overall visit response of progressive disease. Confirmed progression is defined as:

- Continued increase in tumour burden (from iUPD) where RECIST 1.1 definitions of progression had been met (from nadir) in target, non-target disease or new lesions
 - Progression in target disease worsens with an increase of at least 5 mm in the absolute value of the sum
 - Continued unequivocal progression in non-target disease with an increase in tumour burden
 - Increase in size of previously identified new lesion (s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions.
- RECIST 1.1 criteria are met in lesion types (target or non-target or new lesions) where progression was not previously identified, including the appearance of additional new lesions.

If progression is not confirmed, then the overall visit response should be assessed as SD/PR or CR and the patient should continue scheduled RECIST CT/MRI scans. If progression is confirmed the overall visit response should be assessed as PD.

11 STATISTICAL CONSIDERATIONS

All statistical analyses will be performed using SAS[®], Version 9.3 or higher.

Unless specified otherwise, all data from each study part (Phase Ib and Phase II) will be presented separately in the tables, figures and listings.

The study database will be locked prior to the primary analyses for inclusion in the clinical study report (CSR). However, interim data will be made available to the SRC to assist with the identification of toxicities and enable dose modification decisions whilst the study is ongoing.

11.1 Sample size and Statistical Analyses

This is an exploratory study focused on tolerability, translational data, and signal-seeking activities; therefore, the Phase Ib and Phase II components are not powered.

In Phase Ib, up to approximately 12 evaluable patients in total will be enrolled to determine the tolerability of NUC-3373 in combination with pembrolizumab.

In Phase II, approximately 20-40 patients will be enrolled to exploratory, signal-seeking cohorts. ORR, DCR, and PFS will be assessed as indicators of efficacy. In tumours where efficacy is indicated (defined as ≥ 1 confirmed CR, PR or SD > 8 weeks with evidence of tumour shrinkage), the protocol might be expanded to a powered analysis of that tumour type. The statistical considerations for the expansion will be addressed in both a protocol amendment and the SAP.

11.2 Missing, Unused and Spurious Data

In general, missing data will remain missing and will not be included in data summaries. Exceptions are described below.

Missing baseline data (safety data)

If a baseline value is not available and a screening value is available for the same parameter, then the last screening value will be used as baseline. This value will also be used for calculations of changes from baseline. Unless otherwise defined, baseline will be defined as C1D1.

11.3 Analysis Populations

The analysis populations defined for this study are detailed below. In addition, and as deemed warranted by the data, efficacy may also be assessed in sub-populations as defined by the Sponsor. Subgroup analyses may assess consistency of treatment effect across potential or expected prognostic factors or biomarkers. Analyses will not be performed if there are too few events available for a meaningful analysis of a particular subgroup.

11.3.1 Safety Population

For each phase of the study, the Safety Population includes all patients in that phase of the study who received any study treatment and will be the primary analysis set for the assessment of safety and tolerability in the study.

For the purpose of data summaries, patients will be included in the Safety Population according to the treatment cohort and dose level initially received, regardless of any subsequent dose adjustments.

11.3.2 Full Analysis Set

For each phase of the study, the Full Analysis Set (FAS) is based on the intention-to-treat principles and includes all patients who received at least one dose of study treatment. Patients will be included in the FAS based on the dose level initially received, regardless of any subsequent dose adjustments.

The FAS will be used to assess PFS, OS and PD markers.

11.3.3 Evaluable for Response Population

The Evaluable for Response (EFR) population is defined as a subset of the FAS with measurable disease at baseline and who have undergone at least two cycles of treatment, received at least 75% of planned treatment over the two cycles, and undergone a post-treatment objective disease assessment.

The EFR population will be the primary analysis set used to assess tumour size, ORR, DCR, DoR and DoSD in all study parts.

11.4 Statistical Methods

11.4.1 Patient Disposition

The disposition of patients will be summarised presenting the number of patients enrolled, the number of patients treated, the number of patients for whom the study drug was discontinued with the reasons for discontinuation, and the number of patients who discontinued participation in the study.

The number and percentage of patients included in each analysis population (as defined in [Section 11.3](#)) will be presented.

Patients with major protocol deviations or other significant deviations as defined in the SAP will be listed and summarised by dose cohort and overall.

11.4.2 Demographics and Baseline Characteristics

Demographic and baseline disease characteristic data will be presented based on the FAS.

Baseline demographic and background data, including, but not limited to age, gender, weight, height, race, ethnicity and ECOG status will be listed and summarised using appropriate descriptive statistics.

Baseline disease characteristics including, but not limited to, primary diagnosis, primary tumour location and disease status at baseline will also be listed and summarised using appropriate descriptive statistics.

Relevant medical history and prior treatment for relevant indication, including, but not limited to systemic therapies, radiation and surgeries, will be listed and summarised using appropriate descriptive statistics. Full details will be provided in the SAP.

11.4.3 Concomitant Medications

Concomitant medication, coded using World Health Organisation Drug Dictionary (WHO DD) by WHO DD anatomical, therapeutic, chemical class and preferred term, will be listed. Summaries may be produced for concomitant medications of specific interest, as determined by the study team physician, for example use of haematopoietic growth factors and transfusions.

11.4.4 Extent of Exposure

Descriptive statistics for patients treated, including the number of infusions received, total dose given, and infusions delayed or missed, will be presented.

To understand the impact of dose reductions on the intended dosing regimen, dose intensity will also be listed and summarised. Dose intensity will be defined in the SAP.

11.4.5 Safety Analysis

Safety analyses in addition to those described in the following subsections may be determined at any time without prejudice in order to most clearly enumerate rates of toxicities and to define further the safety profile of NUC-3373 + LV and pembrolizumab.

11.4.5.1 Adverse Events

AEs will be considered treatment-emergent (TEAE) if they start on or after the time of the first dose of study treatment and up to 30 days after the last dose of study treatment. AEs will be summarised by Medical Dictionary for Regulatory Activities (MedDRA)TM System Organ Class (SOC) and preferred term, the latest version of MedDRA will always be used. The severity of AEs will also be summarised by CTCAE v5.0 (or higher) grade. Non-treatment-emergent AEs will be included in the patient listings and flagged as such but will not be included in the summary tables. Where an AE date is partial or missing, and it is unclear whether the AE is treatment-emergent, the AE will be assumed to be treatment-emergent.

The following summaries will be produced by dose cohort for all TEAEs:

- An overview table of the incidence of TEAEs, Grade 3+ TEAEs, SAEs, treatment-related TEAEs, TEAEs leading to treatment discontinuation and TEAEs leading to death, by dose and overall for each combination
- Summary of TEAEs by SOC and preferred term: Both the number and percentage of patients in each category (patient-level summary) and the number of episodes (episode-level summary)
- Summary of treatment-related TEAEs by SOC and preferred term: Both the number and percentage of patients in each category (patient-level summary) and the number of episodes (episode-level summary)
- Summary of TEAEs occurring in at least 10% of patients, sorted in descending order of frequency (*i.e.*, most frequent event shown first). The order of frequency will be determined by the most frequent preferred term across all cohorts.
- Summary of CTCAE Grade 3 and above TEAEs by preferred term
- Summary of SAEs by preferred term
- Summary of SAEs sorted in descending order of frequency

Additionally, the following will be listed:

- AEs with outcome of death along with the date of onset, study day, dose at onset, treatment status at onset (pre-treatment, ongoing or post-treatment) and Investigator's assessment of severity and relationship to study drug

- All SAEs along with the date of onset, study day, dose at onset, treatment status at onset (pre-treatment, ongoing or post-treatment), date of resolution (if AE is resolved), Investigator's assessment of severity and relationship to study drug(s)
- AEs leading to discontinuation of study medication, listed along with the date of onset, study day, dose at onset, treatment status at onset (pre-treatment, ongoing or post-treatment) and Investigator's assessment of severity and relationship to study drug

If an AE is reported more than once during the study period, the greatest severity and the worst-case attribution will be presented in summary tables. Any AEs commencing >30 days after discontinuation of study treatment will not be included in the tabulations of AE data

11.4.5.2 Laboratory Parameters

Clinical laboratory data (actual and change from baseline) for continuous parameters at each scheduled assessment will be summarised using descriptive statistics including number of observations, mean, standard deviation, interquartile range (for overall only), minimum, median and maximum values. For categorical laboratory assessments, shift from baseline will be summarised using frequency and proportion at each scheduled assessment time.

Additionally, data may be displayed graphically. Full details will be provided in the SAP.

All clinical laboratory data for individual patients will be listed.

11.4.5.3 Vital Signs

All data will be listed and will be summarised for each scheduled assessment using descriptive statistics including number of observations, mean, standard deviation, interquartile range (for overall only), minimum, median and maximum values.

11.4.5.4 ECOG Performance Status

ECOG performance status will be listed. The ECOG Performance Scale is provided in Appendix 3 of the core protocol.

11.4.5.5 Physical Examination

Full physical examination includes ears, eyes, nose, head, throat, neck, lungs, heart, abdomen, extremities, skin, musculoskeletal, nervous system, and lymph nodes.

Listings will be provided for physical examination parameters.

11.4.5.6 ECGs

ECG parameters will be described at each timepoint. The site will be required to review ECGs as a safety check. This will be done immediately by a qualified Investigator or cardiologist at the study site. ECG assessments may be retained for review centrally, where results will be provided to the study site and retained as source data.

All ECG data will be listed. In addition, ECG parameters including, but not limited to QT and QTc, will be summarised by visit summarising both absolute values and the change from baseline.

11.4.6 Efficacy Analysis

11.4.6.1 Change from Baseline in Tumour Size

The percentage change from baseline in tumour size at 8-week intervals ($\% \Delta TS_{\text{timepoint}}$) will be defined as follows:

- Baseline tumour size (TS_{baseline}): sum of longest diameters of target lesions at baseline
- On-study TS ($TS_{\text{timepoint}}$): sum of longest diameters of target lesions at a post-treatment disease assessment timepoint

$$\% \Delta TS_{\text{timepoint}} = \frac{TS_{\text{timepoint}} - TS_{\text{baseline}}}{TS_{\text{baseline}}} \times 100$$

The best percentage change from baseline in tumour size ($\% \Delta TS_{\text{best}}$) across all timepoints will be calculated and presented using waterfall plots.

The $\% \Delta TS_{\text{best}}$ will be defined as follows:

- Baseline tumour size (TS_{baseline}): sum of longest diameters of target lesions at baseline
- Best TS (TS_{best}): smallest sum of longest diameters of target lesions observed at any timepoint, regardless of whether the assessment was scheduled or unscheduled, after first dose and prior to disease progression

$$\% \Delta TS_{\text{best}} = \frac{TS_{\text{best}} - TS_{\text{baseline}}}{TS_{\text{baseline}}} \times 100$$

If a patient with measurable disease has no evaluable post-dose target lesion data then they will be excluded from the waterfall plot of $\% \Delta TS_{\text{best}}$.

Tumour size ($\% \Delta TS_{\text{Wk8}}$ and $\% \Delta TS_{\text{best}}$) will be presented graphically using waterfall plots for presenting each patient's percentage change in tumour size as a separate bar with the bars ordered from the largest increase to the largest decrease. Reference lines at the +20% and -30% change in tumour size levels will be added to the plots, which correspond with the definitions of disease progression and PR, respectively.

11.4.6.2 Objective Response Rate

ORR is defined as the number of patients achieving a confirmed response (CR or PR) in accordance with RECIST v1.1 (for secondary efficacy assessment in Phase Ib; see Appendix 1 of the core protocol) or iRECIST criteria (for primary efficacy assessment in Phase II; see Appendix 2 of the core protocol). The number and percentage of patients in each RECIST response category (CR, PR, SD, progressive disease or not evaluable [NE]), as well as the ORR will be presented.

All data will be summarised and listed.

11.4.6.3 Disease Control Rate

DCR is defined as the number of patients achieving a confirmed response (CR and PR) or SD as a best overall response.

All data will be summarised and listed.

11.4.6.4 Duration of Response

DoR is defined as the time, in days, from the time measurement criteria are first met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). Only responses that were later confirmed, will be considered when calculating the DoR. For patients who were lost to follow-up without progression or reached the time point of analysis without a known record of death or progression, the DoR will be censored at the date of last tumour assessment. DoR will be assessed in the subset of the EFR population categorised as responders for the assessment of ORR.

DoR will be listed and may be summarised if there are sufficient responders.

11.4.6.5 Duration of Stable Disease

SD is defined for the subset of the EFR population categorised as having neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progressive disease. Duration of SD is defined as the time, in days, from the first dose of study treatment (C1D1) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). For patients who were lost to follow-up without progression or reached the time point of analysis without a known record of death or progression, the duration of SD will be censored at the date of last tumour assessment.

Duration of SD will be listed and may be summarised if there are sufficient responders.

11.4.6.6 Progression-Free Survival

PFS is defined as the time from first dose of study treatment (C1D1) until the date of objective disease progression or death (by any cause in the absence of disease progression) regardless of whether the patient withdraws from study therapy prior to progression. Patients who start another anti-cancer therapy prior to progression will be censored at the date of the last available iRECIST assessment.

Patients who have not experienced disease progression or died at the time of analysis will be censored at the time of the latest date of assessment from their last evaluable iRECIST assessment.

The PFS time will always be derived based on the scan/assessment dates rather than visit dates and the following rules will be applied:

- Date of disease progression will be determined based on the earliest of the dates of the component that triggered the disease progression, *i.e.*, if both the target lesions and the non-target lesions indicate disease progression but were scanned on different days, the earlier of the 2 dates would be applied
- When censoring a patient for PFS the patient will be censored at the latest of the dates contributing to a particular overall visit assessment

PFS will be presented using all available data up until the earlier of progression and starting subsequent therapy, or last evaluable iRECIST assessment in the absence of progression.

The number of overall censored patients, number of patients with events and Kaplan-Meier estimates median time will be provided. In addition, the results will be presented graphically in Kaplan-Meier plots.

11.4.6.7 Overall Survival

OS is defined as the time from Screening to the time of death due to any cause. For patients who are alive at the time of analysis, or are permanently lost to follow-up, duration of OS will be censored at the date at which they were last known to be alive.

The date at which the patient is last known to be alive is defined as the latest date of: (i) last site visit; (ii) last date at which the patient had a radiographic scan; and (iii) last date at which the patient, the study investigator, their other physicians, or a family member confirmed that the patient was alive.

The number of overall censored patients, number of patients with events and Kaplan-Meier estimates with 95% confidence intervals will be presented.

11.4.7 Exploratory Analyses

Expression of immune- and response-related markers such as (but not limited to) PD-L1, CD163, CD68 may be analysed from patients in the FAS. The SAP will specify candidate biomarkers, where known, as well as the exploratory nature of the biomarker studies to be conducted and exploration of possible relationships, if evident, of relationships between PK exposure, PD effects, safety and clinical activity.

11.5 Primary and Interim Analyses

The analysis of safety will take place when 3 enrolled patients have completed one treatment cycle or have withdrawn from treatment. At least 3 evaluable patients are required for the analysis to allow the SRC to assess whether the dose is safe to move forward with the Phase Ib expansion and Phase II. The efficacy analysis of % change in tumour size will take place when up to approximately 12 patients have undergone at least 2 imaging assessments for evaluation of percentage change in tumour size by RECIST v1.1 or have withdrawn from treatment. There will be no formal requirements for a database lock to support these ongoing reviews. Upon review of the safety, tolerability and efficacy data from Phase Ib by the SRC, a decision will be made on which indications may be focused on for the Phase II portion of the study and whether an amendment will be submitted to formalise the indications and statistics to move into Phase II.

11.6 Changes to the Planned Statistical Methods

Any changes to the planned statistical methods from those specified in the final SAP will be documented in the CSR.

12 REFERENCES

- Camelliti S *et al.* Mechanisms of hyperprogressive disease after immune checkpoint inhibitor therapy: what we (don't) know. *J Exp & Clin Cancer Res* 2020; 39: 236.
- Cristescu R *et al.* Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* 2018; 362:eaar3593.
- Keir M *et al.* PD1 and its ligands in tolerance and immunity. *Ann Rev Immunol* 2008; 26:677-704.
- Kepp, O *et al.* Clinical evidence that immunogenic cell death sensitized to PD-1/PD-L1 blockade. *Oncoimmunology* 2019; 8:e1637188.
- Kim TK *et al.* Defining and understanding adaptive resistance in cancer immunotherapy. *Trends in Immunology* 2018; 39:624–31.
- Kim R *et al.* First-line pembrolizumab versus pembrolizumab plus chemotherapy versus chemotherapy alone in non-small-cell lung cancer: a systematic review and network meta-analysis. *Clin Lung Cancer* 2019; 20:331–338.
- Kluger HM *et al.* Defining tumor resistance to PD-1 pathway blockade: recommendations from the first meeting of the SITC Immunotherapy Resistance Taskforce. *Journal for ImmunoTherapy of Cancer* 2020; 8:e000398.
- Krysko DV *et al.* Immunogenic cell death and DAMPs in cancer therapy. *Nat Rev Cancer* 2012; 12:860-875.
- Kwok G *et al.* Pembrolizumab (Keytruda). *Hum Vaccin Immunother* 2016; 12:2777-2789.
- Liang H *et al.* PD-(L)1 inhibitors vs. chemotherapy vs. their combination in front-line treatment for NSCLC: an indirect comparison. *Int J Cancer* 2019; 145:3011–3021.
- Puccini A *et al.* Overcoming resistance to anti-PD1 and anti-PD-L1 treatment in gastrointestinal malignancies. *J Immunother Cancer* 2020; 8:e000404.
- Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. *Nature Communications* 2020; 11:3801.
- Shah MA *et al.* KEYNOTE-975 study design: a Phase III study of definitive chemoradiotherapy plus pembrolizumab in patients with esophageal carcinoma. *Future Oncol* 2021; 17:1143-1153.
- Solari JIG *et al.* Damage-associated molecular patterns (DAMPs) related to immunogenic cell death are differentially triggered by clinically relevant chemotherapeutics in lung adenocarcinoma cells. *BMC Cancer* 2020; 20:474.
- Wang Y-J *et al.* Immunogenic effects of chemotherapy-induced tumor cell death. *Genes and Diseases* 2018; 5:194-203.