CLINICAL STUDY PROTOCOL

A Phase Ib/II open label study to assess the safety and pharmacokinetics of NUC-3373, a nucleotide analogue, given in combination with standard agents used in colorectal cancer treatment

IMP	NUC-3373
Protocol Number	NuTide:302
Protocol Version, Date	Version 4.2, 10 August 2022
Development Phase	Ib/II
IND Number	135275
EudraCT Number	2017-002062-53
NCT Number	NCT03428958
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PRINCIPAL INVESTIGATOR AGREEMENT AND SIGNATURE

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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)

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PROTOCOL SYNOPSIS

Study Title	**A Phase Ib/II open label study to assess the safety and pharmacokinetics of NUC-3373, a nucleotide analogue, given in combination with standard agents used in colorectal cancer treatment**
Protocol Number	NuTide:302
IND Number	135275
EudraCT Number	2017-002062-53
NCT Number	NCT03428958
Phase	Ib/II
Objectives	This study consists of two phases (Phase Ib and Phase II) and includes three study parts (Parts 1, 2 and 3) with several treatment arms within each part. Phase Ib includes Part 1 (Arms 1a-1d) and Part 2 (Arms 2a-2d) and Phase II includes Part 3 (Arms 3a-3g). **Primary Objectives** **Phase Ib** • To identify a recommended dose and schedule for NUC-3373 when combined with other agents commonly used in treatment of advanced colorectal cancer (CRC), including: o leucovorin (LV) o oxaliplatin o oxaliplatin and vascular endothelial growth factor (VEGF) pathway inhibitors (bevacizumab) o oxaliplatin and epidermal growth factor receptor (EGFR) inhibitors (cetuximab or panitumumab) o irinotecan o irinotecan and VEGF pathway inhibitors (bevacizumab) o irinotecan and EGFR inhibitors (cetuximab or panitumumab) **Phase II** To explore the anti-cancer activity of NUC-3373 when combined with other agents commonly used in the treatment of advanced CRC using Response Evaluation in Solid Tumours (RECIST) v1.1 criteria. **Secondary Objectives** • To assess the safety and tolerability of each NUC-3373-containing regimen (Phase Ib and Phase II) • To assess the pharmacokinetics (PK) of NUC-3373, oxaliplatin, irinotecan and their metabolites in each NUC-3373-containing regimen (Phase Ib and Phase II) • To make a preliminary assessment of the anti-tumour activity of NUC-3373 alone and in each NUC-3373-containing regimen (Phase Ib only) • To conduct a within-patient and a between-patient analysis of the effect of LV when added to NUC-3373 on intracellular pharmacodynamic

	(PD) parameters and haematological parameters and use this information to determine if the addition of LV to NUC-3373-containing regimens is required for clinical activity (Part 1 Arm 1a and Arm 1b only) **Exploratory Objectives** • To explore predictive biomarkers of biological activity and possible relationships between PK and PD effects (Phase Ib and Phase II) • Depending on emerging data, to potentially conduct a within-patient analysis of the effect of combining NUC-3373 with irinotecan on the plasma PK parameters of both NUC-3373 and irinotecan (Part 2 Arm 2b sub-study only)
Study Design	This is a three-part, open label, Phase Ib/II study of NUC-3373 administered by intravenous (IV) infusion (weekly [Q1W] or fortnightly [Q2W]), either as monotherapy or as part of various combinations with agents commonly used to treat patients with advanced CRC (LV, oxaliplatin, irinotecan, bevacizumab, cetuximab, and panitumumab). Phase Ib includes Part 1 (Arms 1a-1d) and Part 2 (Arms 2a-2d) and Phase II includes Part 3 (Arms 3a-3g). Patients may continue treatment until radiological disease progression or unacceptable toxicity despite optimal medical management or dose or schedule modification, 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 has been reached (defined in Section 3.6), whichever occurs first. **Part 1** Part 1 determined that NUC-3373 should be administered with LV (Arms 1a, 1b and 1c) and will further assess the safety and tolerability of NUC-3373 + LV on a Q1W schedule (Arm 1d). **Part 2** Part 2 will assess the safety and tolerability of different doses of Q1W NUC-3373 + LV when administered in combination with either oxaliplatin (NUFOX) or irinotecan (NUFIRI). **Part 3** Part 3 will assess the safety and efficacy of Q1W NUFOX and NUFIRI regimens administered in combination with bevacizumab (Q2W arms may also be opened). NUFOX and NUFIRI regimens may also be administered in combination with cetuximab or panitumumab, depending on emerging data. In addition, Q1W NUC-3373 + LV in combination with bevacizumab may be assessed in patients qualifying for maintenance therapy. Different patient populations will be included in the study as follows:

Patient Population	Criteria	Study Phase/Part
≥3rd-line patients	Received ≥2 prior lines of therapy for locally advanced or metastatic disease, one must include a fluoropyrimidine plus oxaliplatin, the other must include a fluoropyrimidine plus irinotecan.	Phase Ib Part 1 Part 2 (dose escalation)
Combination chemotherapy ineligible patients	Considered unable to receive combination chemotherapy for locally advanced or metastatic disease and may have received 1 prior line of fluoropyrimidine-containing therapy.	Phase Ib Part 1
2nd-/3rd-line patients	Received at least 1, but no more than 2, prior lines of fluoropyrimidine-containing therapy combined with oxaliplatin and/or irinotecan for locally advanced or metastatic disease. 3rd-line patients enrolled to Arms 2c and 2d should have received prior bevacizumab treatment, unless ineligible or unless bevacizumab was not standard of care according to relevant region-specific treatment recommendations.	Phase Ib Part 2 (expansion)
Rapid progressors (on prior fluoropyrimidine therapy)	Received ≤2 prior lines of fluoropyrimidine-containing therapy combined with oxaliplatin and/or irinotecan for locally advanced or metastatic disease and progressed ≤3 months of starting the last fluoropyrimidine-containing regimen.	To be determined. Cohorts of rapid progressors may be opened depending on emerging data.
2nd-line patients	Received 1 prior line of fluoropyrimidine-containing therapy combined with oxaliplatin and/or irinotecan for locally advanced or metastatic disease.	Phase II Part 3
Maintenance patients	Received ≥12 weeks of 1st-line standard of care therapy for locally advanced or metastatic disease, achieved at least stable disease and are eligible for maintenance therapy.	Phase II Part 3
Previous treatment with triplet chemotherapy-based regimens is permitted (*i.e.,* FOLFOXIRI), as long as any toxicities from the previous regimen still allow repeated treatment with oxaliplatin or irinotecan.		

If positive efficacy signals are observed in any of the patient populations included in Parts 1, 2 or 3 of the study, expansion cohorts may be opened to further assess efficacy in approximately 20 patients per cohort.

A Q2W schedule may be explored at any time, depending on emergent safety, efficacy and PK data.

Part 1

Part 1 determined that NUC-3373 should be administered with LV (Arms 1a, 1b and 1c) and will further assess the safety and tolerability of NUC-3373 + LV (Arm 1d) in patients with advanced or metastatic CRC. Initially, ≥3rd-line patients were randomised via block randomisation to two parallel arms, each receiving NUC-3373 Q2W either with LV (Arm 1a) or without LV (Arm 1b). Upon completion of the Q2W dose administration arms, a third arm of ≥3rd-line patients was enrolled to a Q1W administration schedule of NUC-3373 (1500 and 2500 mg/m^2) plus LV (Arm 1c). Arms 1a, 1b, and 1c are now complete. Following this, approximately 10 combination chemotherapy ineligible patients will be enrolled to a Q1W administration schedule of NUC-3373 plus LV (Arm 1d).

In Arm 1a, patients received a single dose of NUC-3373 at 1500 mg/m^2. This visit was considered Cycle 0, Day 1 (C0D1) for the purposes of the study visits. The initial dose was followed by a 2-week washout period, during which safety and PK/PD were monitored. The next dose of NUC-3373 was given in combination with LV at 400 mg/m^2. This visit was considered Cycle 1, Day 1 (C1D1) for the purposes of the study visits. All subsequent doses of NUC-3373 were given Q2W in combination with LV in 28-day cycles. In Arm 1b, patients received a single dose of NUC-3373 at 1500 mg/m^2 in combination with LV at 400 mg/m^2. This visit was considered C0D1 for the purposes of the study visits. The initial combination dose was followed by a 2-week washout period during which safety and PK/PD were monitored. The next dose of NUC-3373 was given without any LV. This visit was considered C1D1 for the purposes of the study visits. All subsequent doses of NUC-3373 were given Q2W without LV in 28-day cycles.

In Arm 1c, patients received NUC-3373 Q1W at either 1500 mg/m^2 or 2500 mg/m^2 in combination with LV Q1W at 400 mg/m^2. NUC-3373 and LV were administered on Days 1, 8, 15 and 22 of 28-day cycles.

The dose of NUC-3373 selected from Arm 1c for further exploration was 2500 mg/m^2 in combination with LV at 400 mg/m^2.

In Arm 1d, a cohort of approximately 10 combination chemotherapy ineligible patients will receive NUC-3373 at 2500 mg/m^2 in combination with LV at 400 mg/m^2. NUC-3373 and LV will be administered on Days 1, 8, 15 and 22 of 28-day cycles.

All patients in Part 1 (Arms 1a, 1b, and 1c) were monitored for dose-limiting toxicities (DLTs) up to Day 28 of Cycle 1.

If the dose of NUC-3373 was not tolerable or was sub-optimal, alternate dose levels as described in the table below may have been explored.

Anticipated dose levels to be assessed in Part 1

Dose Level*	≥3rd-line patients	
	NUC-3373 dose (mg/m^2) Days 1 and 15 *Arms 1a and 1b*	**NUC-3373 dose (mg/m^2) Days 1, 8, 15 and 22** *Arm 1c*
-1	1000	1250
1	1500	1500

2	2000	1750
3	2500	2000
4	3000	2250

*Additional dose levels could be explored if necessary

If cohorts at additional doses were opened, these were to utilise a modified 3+3 design in which a fourth patient may have been added to each initial 3-patient cohort to account for a patient that became unevaluable for DLT evaluation for any reason other than safety. There was to be a 7-day observation period between treatment of the first patient and treatment of subsequent patients in a cohort. Once a minimum of 3 patients in each of Arms 1a, 1b and 1c had completed the 28-day DLT evaluation period all available safety data, PK and PD data were evaluated to establish the tolerability of NUC-3373 ± LV administered in each dose administration schedule.

In addition to the assessment of safety, tolerability, PK and PD, a radiologic assessment of disease will be performed at 8-week intervals and evaluated using Response Evaluation Criteria in Solid Tumours (RECIST) v1.1 to gain a preliminary assessment of clinical activity.

Part 2

Data from Part 1 of the study showed that LV did not impact the safety or PK of NUC-3373; therefore, the decision was made that LV should be administered at 400 mg/m^2 on each day of NUC-3373 administration in all parts of the study.

Part 2 of the study consists of a dose escalation phase using a modified 3 + 3 design, which may be followed by an expansion phase. The dose escalation phase will assess the safety and tolerability of different doses of NUC-3373 + LV when administered in combination with either oxaliplatin (NUFOX) or irinotecan (NUFIRI) in a Q1W schedule in ≥3rd-line patients. Oxaliplatin and irinotecan will be given at standard doses and schedules. In the expansion phase, cohorts of 2nd-/3rd-line patients may be enrolled to assess Q1W schedules of the NUFOX and NUFIRI regimens selected in the dose escalation phase.

The dose escalation group of patients will receive NUC-3373 + LV to assess the safety and tolerability of different doses of NUC-3373 + LV administered in combination with oxaliplatin (Arm 2a) or irinotecan (Arm 2b).

In Arm 2a, ≥3rd-line patients will receive NUC-3373 + LV on Days 1, 8, 15 and 22 and oxaliplatin on Days 1 and 15 of 28-day cycles.

In Arm 2b, ≥3rd-line patients will receive NUC-3373 + LV on Days 1, 8, 15 and 22 and irinotecan on Days 1 and 15 of 28-day cycles.

The starting dose of NUC-3373 will be 1500 mg/m^2 and the dose levels to be evaluated are described in the table below. Based on a clinical study of NUC-3373 (NuTide:301, EudraCT 2015-002250-13, NCT02723240), alternate and/or additional dose levels may be considered and implemented upon recommendation of the Data Safety Monitoring Committee (DSMC).

Anticipated dose levels to be assessed in Part 2 – Q1W cohorts

Dose Level*	NUC-3373 dose (mg/m^2) Days 1, 8, 15 and 22 *Arm 2a and Arm 2b*	Oxaliplatin dose (mg/m^2)** *Arm 2a*	Irinotecan dose (mg/m^2)** *Arm 2b*
-1	1250	85	180
1	1500	85	180
2	1750	85	180
3	2000	85	180
4	2250	85	180

* Additional dose levels may be explored if necessary
** Alternative dose levels of oxaliplatin and irinotecan may be explored based on PK analyses and safety observations

The decision on which arm to enrol a patient to, Arm 2a or Arm 2b, will be made by the treating physician and will take into account the patient's clinical condition and any therapies that have previously been administered for treatment of CRC.

Patients will be enrolled into cohorts following a modified 3+3 design, in which a fourth patient may be enrolled in each initial 3-patient cohort as described above. The initial patient in each cohort will complete a 7-day observation period before subsequent patients are enrolled. All patients in Arms 2a & 2b will be monitored for DLTs up to Day 28 of Cycle 1. Dose escalation or de-escalation decisions will be based on DSMC review once three patients have completed the first 28-day cycle.

The recommended dose of NUC-3373 + LV in combination with oxaliplatin (Arm 2a) or irinotecan (Arm 2b) will be determined separately, and may be different.

Once the dose escalation groups have been completed, the DSMC will review the available safety, clinical activity and PK/PD data to determine the recommended dose of NUC-3373 + LV when given in combination with either oxaliplatin or irinotecan. If safety concerns arise, additional cohorts assessing alternative doses of oxaliplatin and irinotecan may be opened with agreement from the DSMC.

Expansion cohorts may be enrolled to Part 2 to further assess the safety, tolerability and preliminary efficacy of the Q1W NUFOX and NUFIRI regimens, using the dose of NUC-3373 selected in the dose escalation phase. NUFOX and NUFIRI regimens will be administered as described in the table below.

Arm	Treatment	Number of patients
2c	NUC-3373 + LV Q1W, oxaliplatin Q2W (NUFOX)	~10 2nd-/3rd-line patients
2d	NUC-3373 + LV Q1W, irinotecan Q2W (NUFIRI)	~10 2nd-/3rd-line patients

Part 3

Following determination of the recommended NUFOX and NUFIRI regimens in the Part 2 dose escalation phase, Part 3 will open. The Part 2 expansion phase may open in parallel.

In Part 3, the safety and efficacy of the NUFOX and NUFIRI regimens in combination with bevacizumab will be evaluated. Q1W bevacizumab-containing arms will be opened first and will be evaluated in 2nd-line patients. Following this, Q2W arms and/or cetuximab combination arms may be opened, depending on emerging data. In addition, the safety and efficacy of NUC-3373 + LV in combination with bevacizumab may be evaluated in patients qualifying for maintenance therapy.

The treatment for each arm is described in the table below. The choice of arm to which an eligible patient should be enrolled will be at the Investigator's discretion, taking into account the patient's clinical condition and therapies that have previously been administered.

Dose groups to be assessed in Part 3

Arm	Treatment	Number of patients
3a	NUFOX (NUC-3373 + LV Q1W) + bevacizumab	~10 2nd-line patients
3b	NUFOX (NUC-3373 + LV Q2W) + bevacizumab	~10 2nd-line patients
3c	NUFIRI (NUC-3373 + LV Q1W) + bevacizumab	~10 2nd-line patients
3d	NUFIRI (NUC-3373 + LV Q2W) + bevacizumab	~10 2nd-line patients
3e	NUC-3373 + LV Q1W + bevacizumab	~10 maintenance patients
3f	NUFOX (NUC-3373 + LV Q1W or Q2W) + cetuximab	~10 2nd-line patients
3g	NUFIRI (NUC-3373 + LV Q1W or Q2W) + cetuximab	~10 2nd-line patients

Bevacizumab and cetuximab should be used in accordance with standard local practice.

Panitumumab combination arms may also be opened (~10 2nd-line patients), depending on emerging data.

Cohorts of rapid progressors may also be opened (~10 patients), depending on emerging data.

PK Sub-study

A PK sub-study may be conducted during Part 2 if emerging data suggest it would be beneficial to evaluate the PK relationship between NUC-3373, LV and irinotecan. If conducted, an additional 8 patients would be enrolled in the NUFIRI Q1W cohort (Arm 2b). In Cycle 1 of the sub-study, irinotecan and LV will be administered concurrently on Day 1. On Day 8, NUC-3373 and LV

<table>
<tr><td></td><td>will be administered sequentially. On Day 15, 4 patients (Group A) will first be administered irinotecan concurrently with LV, followed by NUC-3373. The remaining 4 patients (Group B) will first be administered NUC-3373, followed by irinotecan concurrently with LV. On Day 22, NUC-3373 and LV will be administered sequentially. On Cycle 2 Day 1 (29 days after the start of treatment), the infusion sequence will be reversed in a cross-over fashion whereby patients in Group A will first be administered NUC-3373, followed by irinotecan concurrently with LV and patients in Group B will first be administered irinotecan concurrently with LV, followed by NUC-3373.
<table>
<tr><th></th><th>C1D1</th><th>C1D8</th><th>C1D15</th><th>C1D22</th><th>C2D1</th></tr>
<tr><td>Group A</td><td>Irinotecan + LV</td><td>LV ⇒ NUC-3373</td><td>Irinotecan + LV ⇒ NUC-3373</td><td>LV ⇒ NUC-3373</td><td>NUC-3373 ⇒ Irinotecan + LV</td></tr>
<tr><td>Group B</td><td>Irinotecan + LV</td><td>LV ⇒ NUC-3373</td><td>NUC-3373 ⇒ Irinotecan + LV</td><td>LV > NUC-3373</td><td>Irinotecan + LV ⇒ NUC-3373</td></tr>
</table>
Plasma samples will be collected for PK analysis on Cycle 1 Day 1, Cycle 1 Day 8, Cycle 1 Day 15, Cycle 1 Day 22 and Cycle 2 Day 1. In addition, Holter ECG assessments will be performed on Cycle 1 Day 8 and Cycle 1 Day 22 during infusion and up to 24 hours post-infusion. Following this, patients will continue on the treatment schedule outlined for Arm 2b.</td></tr>
<tr><td>Study Centres</td><td>This study will be conducted at approximately 15 sites in the United States, United Kingdom and European Union.</td></tr>
<tr><td>Endpoints</td><td>Primary Endpoints
Phase Ib
To identify a recommended dose and schedule for NUC-3373 when combined with LV and other agents routinely used in the treatment of patients with advanced CRC.
Phase 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:
• Percentage change from baseline in tumour size
• Objective Response Rate (ORR)
• Disease Control Rate (DCR)
• Duration of Response (DoR)
• Duration of Stable Disease (DoSD)
• Progression-Free Survival (PFS)
• Overall Survival (OS) rate at 6 and 12 months</td></tr>
</table>

Secondary Endpoints

Safety (Phase Ib and Phase II)

- Treatment-emergent adverse events (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 electrocardiograms (ECGs)

Pharmacokinetics (Phase Ib and Phase II)

The PK of NUC-3373, oxaliplatin, irinotecan and their metabolites will be assessed, including:

- Concentration at end of NUC-3373 infusion (C_{inf})
- Maximum concentration (C_{max})
- Area under the curve (AUC)
- Half-life ($t_{1/2}$)
- Volume of distribution (V_d)
- Clearance (CL)

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

- In plasma: NUC-3373, oxaliplatin, irinotecan and their metabolites
- In peripheral blood mononuclear cells (PBMCs): NUC-3373, fluorodeoxyuridine monophosphate (FUDR-MP), FBAL, 5-fluorouridine triphosphate (5-FUTP), deoxythymidine monophosphate (dTMP), deoxyuridine monophosphate (dUMP) (Part 1 only)

Efficacy (Phase Ib)

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:

- Percentage change from baseline in tumour size
- ORR
- DCR
- DoR
- DoSD
- PFS
- OS rate at 6 and 12 months

Exploratory Endpoints (Phase Ib and Phase II)

- Pre-treatment and on-treatment measurements of PD markers in PBMCs (Part 1 only)
- Exploration of predictive biomarkers such as gene expression and/or genetic alternations in blood and tumour (*e.g.,* genetic alterations in genes involved in PK exposure, PD effects, safety and efficacy)
- DNA/RNA and protein analysis of pre- and on-treatment tumour samples, including but not limited to KRAS and BRAF
- Potential sub-study to evaluate the PK relationship between NUC-3373, LV and irinotecan (Part 2 Arm 2b sub-study only)

  The PK of NUC-3373, LV and irinotecan will be assessed, including $C_{inf,}$ $C_{max,}$ AUC, $t_{1/2,}$ $V_{d,}$ CL

	The analytes measured in plasma will include, but are not limited to: o NUC-3373, 5-FU, and FBAL o LV and 5-methyl-THF o Irinotecan, SN-38, SN-38 glucuronide, APC and NPC
Study Population	Patients with histologically or cytologically confirmed CRC that is locally advanced or metastatic and radiologically-measurable; Eastern Cooperative Oncology Group (ECOG) performance status 0-1; adequate haematologic, renal and hepatic function.
Study Treatment	The sections below provide suggested administration times and order for each of the combination agents; however, each administration in Parts 1, 2 and 3 of the study may be modified based on emerging safety and PK data (in line with local guidelines/relevant Prescribing Information) with agreement from the DSMC. It is suggested that NUC-3373 at 1500 mg/m^2 is infused over 120 minutes. The infusion duration for lower and higher doses of NUC-3373 should be adjusted relative to the 1500 mg/m^2 dose (*e.g.,* a 25% increase in dose requires an approximately 25% increase in infusion duration). **Phase Ib** **Part 1 (≥3rd-line and combination chemotherapy ineligible patients)** **Arm 1a** (≥3rd-line patients) NUC-3373 was administered by IV infusion at 1500 mg/m^2 over 120 minutes followed by a 14-day washout period. Thereafter, on Days 1 and 15 of 28-day cycles, LV was administered by IV infusion at 400 mg/m^2 over 120 minutes, followed by NUC-3373 administration by IV infusion at the cohort-prescribed dose level. **Arm 1b** (≥3rd-line patients) LV was administered at 400 mg/m^2 by IV infusion over 120 minutes, followed by NUC-3373 infusion at 1500 mg/m^2 over 120 minutes. LV was not administered again in Arm 1b. After a 14-day washout period, NUC-3373 was administered on Days 1 and 15 of 28-day cycles by IV infusion at the cohort-prescribed dose level. **Arm 1c** (≥3rd-line patients) On Days 1, 8, 15 and 22 of 28-day cycles, LV was administered by IV infusion at 400 mg/m^2 over 120 minutes, followed by NUC-3373 administration by IV infusion at the cohort-prescribed dose level. **Arm 1d** (combination chemotherapy ineligible patients) On Days 1, 8, 15 and 22 of 28-day cycles, LV will be administered by IV infusion at 400 mg/m^2 over 120 minutes, followed by NUC-3373 administration by IV infusion at 2500 mg/m^2 over 200 minutes. **Part 2 Dose Escalation (≥3rd-line patients)** In the NUFOX and NUFIRI dose escalation cohorts, patients will receive NUC-3373 + LV Q1W combined with the standard dose and schedule of oxaliplatin and irinotecan.

For patients experiencing toxicity related to NUC-3373 in the NUFOX and NUFIRI cohorts, initially the NUC-3373 dose modification guidelines in Section 9 should be followed. The doses of oxaliplatin and irinotecan may be adjusted (through agreement with the DSMC) based on, but not limited to, safety and PK data generated in patients receiving treatment with NUFOX and NUFIRI regimens. If oxaliplatin or irinotecan-related adverse events are observed, dose de-escalation should be performed as per the respective Prescribing Information or standard local practice, or alternate doses may be explored, with approval from the DSMC. The modified dose of oxaliplatin or irinotecan (in combination with NUC-3373) will be assessed in approximately 6 evaluable patients in each arm.

The order of administration of each of the agents in Part 2 may be modified with approval from the DSMC, so that NUC-3373 + LV is infused first followed by either oxaliplatin (Arms 2a and 2c) or irinotecan (Arms 2b and 2d). Alternate infusion parameters may be implemented with approval from the DSMC.

Arm 2a

NUC-3373 + LV will be administered Q1W and oxaliplatin will be administered Q2W in 28-day cycles, as outlined in the table below.

Infusion order	**Infusion duration**	**D1**	**D8**	**D15**	**D22**
LV (400 mg/m^2)[a]	120 mins[a]	X	X	X	X
Oxaliplatin (85 mg/m^2)[a]		X		X	
NUC-3373 (cohort prescribed dose level)	120 mins[b]	X	X	X	X

[a]LV and oxaliplatin to be administered concurrently

[b]Suggested duration, refer to Pharmacy Manual

Arm 2b

NUC-3373 + LV will be administered Q1W and irinotecan will be administered Q2W in 28-day cycles, as outlined in the table below.

Infusion order[a]	**Infusion duration**	**D1**	**D8**	**D15**	**D22**
LV (400 mg/m^2)[b]	120 mins[c]	X	X	X	X
Irinotecan (180 mg/m^2)[b]		X		X	
NUC-3373 (cohort prescribed dose level)	120 mins[d]	X	X	X	X

[a]The infusion order may change as a result of emerging PK or safety data (refer to the PK Manual for further information)

[b]LV and irinotecan to be administered concurrently

[c]The infusion duration for irinotecan may be changed to 90 mins based on emerging data

[d]Suggested duration, refer to Pharmacy Manual

Part 2 Dose Expansion (2nd-/3rd-line patients)

Following completion of Arms 2a and 2b, expansion cohorts may be initiated in 2nd-/3rd-line patients at the selected dose levels established in the dose escalation phase. The NUFOX and NUFIRI regimens will be administered in separate arms of the study (Arm 2c for NUFOX and Arm 2d for NUFIRI).

Arm 2c

NUC-3373 + LV will be administered Q1W and oxaliplatin will be administered Q2W in 28-day cycles, as outlined in the table below.

Infusion order	Infusion duration	D1	D8	D15	D22
LV (400 mg/m^2)[a]	120 mins[a]	X	X	X	X
Oxaliplatin (85 mg/m^2)[a,b]		X		X	
NUC-3373 (dose selected in Arm 2a)	120 mins[c]	X	X	X	X

[a]LV and oxaliplatin to be administered concurrently
[b]The oxaliplatin dose may be reduced based on observations in Arm 2a
[c]Suggested duration, refer to Pharmacy Manual

Arm 2d

NUC-3373 + LV will be administered Q1W and irinotecan will be administered Q2W in 28-day cycles, as outlined in the table below.

Infusion order[a]	Infusion duration	D1	D8	D15	D22
LV (400 mg/m^2)[b]	120 mins[c]	X	X	X	X
Irinotecan (180 mg/m^2)[b]		X		X	
NUC-3373 (dose selected in Arm 2b)	120 mins[d]	X	X	X	X

[a]The infusion order may change as a result of emerging PK or safety data (refer to the PK Manual for further information)
[b]LV and irinotecan to be administered concurrently
[c]The infusion duration for irinotecan may be changed to 90 mins based on emerging data
[d]Suggested duration, refer to Pharmacy Manual

Phase II

Part 3 (2nd-line patients and maintenance patients)

In Part 3, 2nd-line patients will receive the NUFOX and NUFIRI regimens selected in Part 2 in combination with bevacizumab. Q1W bevacizumab-containing arms will be opened first. Following this, Q2W and/or cetuximab combination arms may be opened, depending on emerging data. In addition, patients qualifying for maintenance therapy may receive NUC-3373 + LV in combination with bevacizumab.

	Arm 3a (2nd-line patients) NUC-3373, LV and oxaliplatin at dose levels used in Arm 2a will be combined with bevacizumab. NUC-3373 and LV will be administered Q1W and oxaliplatin and bevacizumab will be administered Q2W. **Arm 3b** (2nd-line patients) NUC-3373, LV and oxaliplatin at dose levels used in Arm 2a will be combined with bevacizumab. NUC-3373, LV, oxaliplatin and bevacizumab will be administered Q2W. **Arm 3c** (2nd-line patients) NUC-3373, LV and irinotecan at dose levels used in Arm 2b will be combined with bevacizumab. NUC-3373 and LV will be administered Q1W and irinotecan and bevacizumab will be administered Q2W. **Arm 3d** (2nd-line patients) NUC-3373, LV and irinotecan at dose levels used in Arm 2b will be combined with bevacizumab. NUC-3373, LV, irinotecan and bevacizumab will be administered Q2W. **Arm 3e** (Maintenance patients) NUC-3373 (2500 mg/m^2 Q1W) + LV (400 mg/m^2 Q1W) will be combined with bevacizumab (5 mg/kg Q2W). **Arm 3f** (2nd-line patients) NUC-3373, LV and oxaliplatin at dose levels used in Arm 2a may be administered in subsequent cetuximab cohorts. NUC-3373 + LV may be administered Q1W or Q2W, oxaliplatin will be administered Q2W and cetuximab will be administered Q1W. **Arm 3g** (2nd-line patients) NUC-3373, LV and irinotecan at dose levels used in Arm 2b may be administered in subsequent cetuximab cohorts. NUC-3373 + LV may be administered Q1W or Q2W, irinotecan will be administered Q2W and cetuximab will be administered Q1W. In all bevacizumab-containing arms, NUC-3373 dose adjustments may occur depending on emerging data. Panitumumab combination arms may also be opened (~10 2nd-line patients), depending on emerging data. Cohorts of rapid progressors may also be opened (~10 patients), depending on emerging data. In all parts of the study, patients may continue on study until documentation of radiological disease progression or unacceptable toxicity. Guidelines for dose modifications and discontinuations of NUC-3373 are provided; dose and schedule modifications for the other agents (LV, oxaliplatin, irinotecan, bevacizumab, cetuximab, panitumumab) will be in accordance with their respective Prescribing Information or standard local practice, unless safety concerns emerge regarding dosing as determined by the DSMC.

PK sub-study (Arm 2b)

Patients participating in the PK sub-study will receive NUC-3373, LV and irinotecan, as outlined in the table below. Following this, patients will continue on the treatment schedule outlined for Arm 2b.

Infusion order	Infusion duration	C1D1	C1D8	C1D15[c]	C1D22	C2D1[c] (29 days after start of treatment)
LV[a]	120 mins[a]	X	X	X	X	X
Irinotecan[a]	90 mins[a]	X		X		X
NUC-3373	120 mins[b]		X	X	X	X

[a]LV and irinotecan to be administered concurrently
[b]Suggested duration, refer to Pharmacy Manual
[c]Patients will be alternately assigned to two groups (Group A and Group B) for treatment on Cycle 1 Day 15 and Cycle 2 Day 1 (29 days after start of treatment) and will receive the study treatments in an order that has been pre-specified for each group (refer to Section 7.2.1 for details).
Patients enrolled in the PK sub-study will be considered evaluable for PK endpoints if they have received planned study treatment and had PK assessments up to at least C1D15. If patients discontinue study treatment or require dose modifications before C1D15, they will be considered non-evaluable and will be replaced.

Sample Size

Part 1: Up to 50 patients
Part 2: Up to 65 patients
Part 3: Up to 100 patients

Total: Up to 215 patients.

Inclusion Criteria

All patients

1. Provision of written informed consent
2. Have histological confirmation of CRC with evidence of locally advanced/unresectable or metastatic disease
3. Age ≥18 years
4. Life expectancy of ≥12 weeks
5. ECOG Performance status 0 or 1
6. Measurable disease as defined by RECIST v1.1
7. Known RAS and BRAF status. Patients with wild-type KRAS tumours who are to be enrolled to a cohort that does not contain an EGFR pathway inhibitor (Arms 2a, 2b, 2c, 2d, 3a, 3b, 3c, 3d and 3e) must have received prior treatment with an EGFR inhibitor, unless this was not standard of care according to relevant region-specific treatment recommendations. Patients with BRAF V600E mutant tumours should have received prior treatment with encorafenib in combination with an EGFR inhibitor, unless this was not standard of care according to relevant region-specific treatment recommendations
8. Adequate bone marrow function as defined by: absolute neutrophil count $\geq 1.5\times10^9$/L, platelet count $\geq 100\times10^9$/L (with no evidence of bleeding), and haemoglobin ≥9 g/dL

	9. Adequate liver function, as defined by: serum total bilirubin ≤1.5×upper limit of normal (ULN), aspartate aminotransferase and alanine aminotransferase ≤2.5×ULN (or ≤5×ULN if liver metastases are present) 10. Adequate renal function assessed as serum creatinine <1.5×ULN or glomerular filtration rate ≥50 mL/min. This criterion does not apply to the combination chemotherapy ineligible patient subgroup (Arm 1d); please refer to specific criteria for this subgroup below 11. Serum albumin ≥3 g/dL 12. For the cohort in which the patient will participate, there are no contra-indications to receiving the approved partner combination drugs 13. Ability to comply with protocol requirements 14. Female patients of child-bearing potential must have a negative serum 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 (defined in Section 10.3.1) or to use two highly effective forms of contraception, one of which must be a barrier method. 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 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 16. For patients receiving oxaliplatin: Male patients must have been offered advice on and/or sought counselling for conservation of sperm prior to the first dose of study medication In addition to meeting the criteria above for all patients, the following criteria must be met for each patient population: **≥3rd-line patients** 1. Must have received at least two prior lines of therapy for locally advanced or metastatic CRC, including one fluoropyrimidine plus oxaliplatin containing regimen and one fluoropyrimidine plus irinotecan containing regimen. Previous treatment with standard of care chemotherapy regimens in combination with molecular targeted therapies (*e.g.,* therapies including VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Patients who have received FOLFOXIRI-based regimens in 1st- and/or 2nd-line settings may also be included 2. Patients in Part 2 of the study who are to receive NUFOX regimens should be suitable for re-challenge with an oxaliplatin-based regimen 3. Patients in Part 2 of the study who are to receive NUFIRI regimens should be suitable for re-challenge with an irinotecan-based regimen

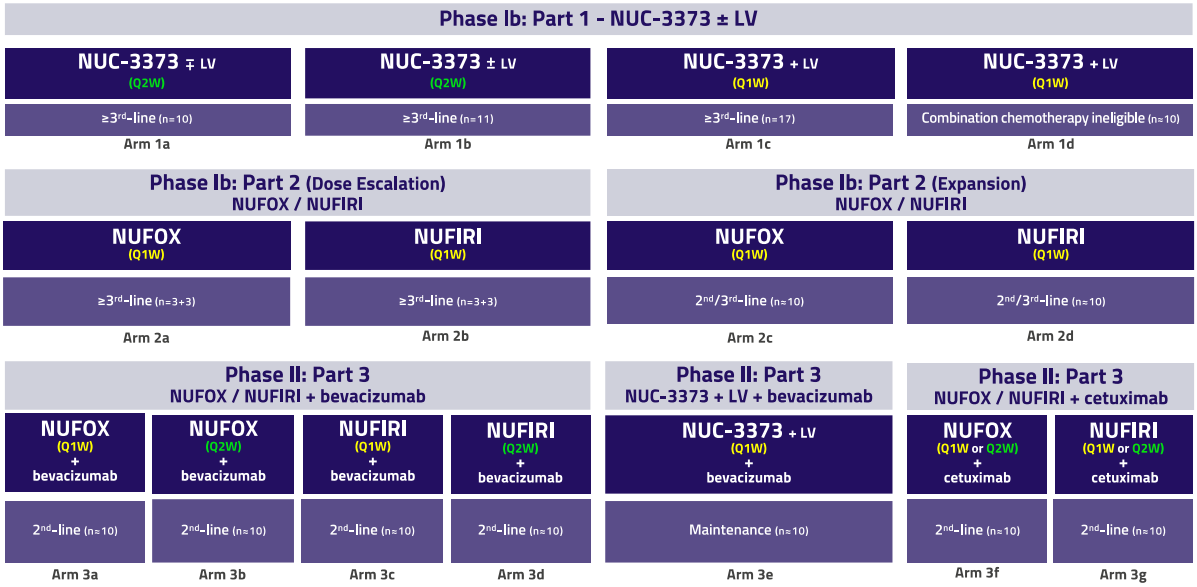
	2nd-/3rd-line patients 1. Must have received at least one, but no more than two, prior lines of fluoropyrimidine-containing therapy in combination with oxaliplatin and/or irinotecan for locally advanced or metastatic CRC. Previous treatment with standard of care chemotherapy regimens in combination with molecular targeted therapies (*e.g.,* therapies including VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Patients who have received FOLFOXIRI-based regimens in 1st- and/or 2nd-line settings may also be included. 3rd-line patients enrolled to Arms 2c and 2d must have received prior bevacizumab treatment, unless ineligible or unless bevacizumab was not standard of care according to relevant region-specific treatment recommendations 2. Patients in Part 2 of the study who are to receive NUFOX regimens should be suitable for re-challenge with an oxaliplatin-based regimen 3. Patients in Part 2 of the study who are to receive NUFIRI regimens should be suitable for re-challenge with an irinotecan-based regimen **Combination chemotherapy ineligible patients** 1. Patients may have received one prior line of fluoropyrimidine-containing therapy for locally advanced or metastatic CRC 2. Ineligible to receive combination therapy for locally advanced or metastatic CRC, as defined by the presence of one or more of the following criteria: a. Dependency for daily activities due to comorbidities (different to deterioration due to cancer) b. Previous history of three or more of the following comorbidities (controlled or uncontrolled by treatments): i. Congestive heart failure ii. Other chronic cardiovascular disease iii. Chronic obstructive pulmonary disease iv. Cerebrovascular disease v. Peripheral neuropathy vi. Chronic renal failure vii. Arterial hypertension viii. Diabetes mellitus ix. Systemic vasculitis x. Severe arthritis c. At least one of the following geriatric features: i. Age >75 years ii. Faecal or urinary incontinence iii. Spontaneous bone fractures iv. Mild or moderate dementia v. Frequent falls 3. Creatinine clearance of >30 mL/min **Rapid progressors** 1. Must have received no more than two prior lines of fluoropyrimidine-containing therapy in combination with oxaliplatin and/or irinotecan for locally advanced or metastatic CRC. Previous treatment with standard of

	care chemotherapy regimens in combination with molecular targeted therapies (*e.g*., therapies including VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Patients who have received FOLFOXIRI-based regimens in 1st- and/or 2nd-line settings may also be included 2. Must have had tumour progression ≤3 months of starting the last fluoropyrimidine-containing regimen 3. Patients who are to receive NUFOX regimens should be suitable for re-challenge with an oxaliplatin-based regimen 4. Patients who are to receive NUFIRI regimens should be suitable for re-challenge with an irinotecan-based regimen **2nd-line patients** 1. Must have received one prior line of fluoropyrimidine-containing therapy in combination with oxaliplatin and/or irinotecan for locally advanced or metastatic CRC. Previous treatment with standard of care chemotherapy regimens in combination with molecular targeted therapies (*e.g.,* therapies including VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Previous treatment with triplet chemotherapy-based regimens is allowed (*i.e.,* FOLFOXIRI) **Maintenance patients** 1. Must have received at least 12 weeks of 1st-line standard of care therapy for locally advanced or metastatic CRC and achieved at least stable disease 2. Eligible for maintenance therapy
Exclusion Criteria:	**All patients** 1. Prior history of hypersensitivity or current contra-indications to 5-FU or capecitabine 2. Prior history of hypersensitivity or current contra-indication to any of the combination agents required for the study arm to which the patient is assigned 3. History of allergic reactions attributed to components of the NUC-3373 drug product formulation (Kolliphor ELP, super refined polysorbate 80, dimethylacetamide [DMA]) 4. Symptomatic central nervous system or leptomeningeal metastases 5. Symptomatic ascites, ascites currently requiring drainage procedures or ascites requiring drainage over the prior 3 months 6. Chemotherapy, radiotherapy (other than a short cycle of palliative radiotherapy [*e.g.,* for bone pain*), immunotherapy, or exposure to another investigational agent within 28 days (or five times the half-life for a biological or molecular targeted agent or three times the half-life for an immunotherapy agent) of first administration of NUC-3373: a. For nitrosoureas and mitomycin C within 6 weeks of first administration of NUC-3373 b. For hormone or biological therapy within 14 days of first administration of NUC-3373 c. Corticosteroid treatment is allowed if not more than stable daily dosing of 10 mg prednisolone (or steroid equivalent)

	** Palliative radiotherapy during participation in the study is permitted, but should not include a target lesion* 7. Residual toxicities from prior chemotherapy or radiotherapy which have not regressed to Grade ≤1 severity (CTCAE v5.0), except for alopecia. In cohorts not containing oxaliplatin, residual Grade 2 neuropathy is allowed 8. Patients who have a history of another malignancy diagnosed within the past 5 years, with the exception of adequately treated non-melanoma skin cancer curatively treated carcinoma *in situ* of the cervix, surgically excised or potentially curatively treated ductal carcinoma *in situ* of the breast, or low-grade prostate cancer or patients after prostatectomy not requiring treatment. Patients with previous invasive cancers are eligible if treatment was completed more than 3 years prior to initiating the current study treatment and there is no evidence of recurrence 9. Presence of an active bacterial or viral infection (including SARS-CoV-2, Herpes Zoster or chicken pox), known Human Immunodeficiency Virus positive or known active hepatitis B or C 10. 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. Myocardial infarction within 6 months of the first dose of study medication c. Unstable or poorly controlled angina pectoris d. Complete left bundle branch, bifascicular block or other clinically significant abnormal ECG finding e. History of or current risk factor for torsade de pointes (*e.g.,* heart failure, hypokalaemia, or a family history of long QT syndrome) f. History of severe skin reactions g. History of severe ocular disorders h. Interstitial pneumonitis or pulmonary fibrosis 11. 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 12. Currently pregnant, lactating or breastfeeding 13. QTc interval >450 milliseconds for males and >470 milliseconds for females 14. Required concomitant use of drugs known to prolong QT/QTc interval 15. Required concomitant use of strong CYP3A4 inducers or strong CYP3A4 inhibitors, or use of strong CYP3A4 inducers within 2 weeks of first receipt of study drug or use of strong CYP3A4 inhibitors within 1 week of first receipt of study drug (refer to Section 10.3.2) 16. For patients receiving irinotecan: Use of strong UGT1A1 inhibitors within 1 week of first receipt of study drug (refer to Section 10.3.3) 17. Has received a live vaccination within four weeks of first planned dose of study medication

	18. Known DPD or TYMP mutations associated with toxicity to fluoropyrimidines 19. 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 anti-coagulant treatment should switch to low molecular weight heparin or anti-Xa inhibitors with a half-life of ≤12 hours **Patients receiving bevacizumab** 1. Patients with a history of haemoptysis (1/2 teaspoon or more of red blood) 2. Wound healing complications or surgery within 28 days of starting bevacizumab (wound healing must have been fully completed before starting bevacizumab) 3. Severe chronic wounds, ulcers or bone fracture 4. Arterial thromboembolic events or haemorrhage within 6 months prior to study entry (except for tumour bleeding surgically treated by tumour resection) 5. Bleeding diatheses or coagulopathy 6. Receiving full-dose anti-coagulation treatment. Patients who have been on stable doses for at least 6 months and have tolerated prior bevacizumab treatment are eligible 7. Uncontrolled hypertension 8. Clinically significant coronary heart disease or myocardial infarction within the last 12 months or high risk of uncontrolled arrhythmia 9. Severe proteinuria (nephrotic syndrome) 10. Acute or subacute ileus, chronic inflammatory bowel disease or chronic diarrhoea 11. Any contraindication present in the bevacizumab Prescribing Information **Patients receiving cetuximab or panitumumab** 1. Clinically significant coronary heart disease or myocardial infarction within the last 12 months or high risk of uncontrolled arrhythmia 2. Acute or subacute ileus, chronic inflammatory bowel disease or chronic diarrhoea 3. Hypomagnesaemia or hypokalaemia not controlled by oral therapy 4. Any contraindication present in the cetuximab or panitumumab Prescribing Information **Patients participating in the PK sub-study (Arm 2b)** 1. Patients with the UGT1A1 *28/*28 genotype (homozygous poor UGT1A1 metabolisers)
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 has been reached (defined in Section 3.6), whichever occurs first.

OVERALL STUDY DESIGN



NUFOX (Q1W) = NUC-3373 Q1W, LV Q1W and Ox Q2W
NUFOX (Q2W) = NUC-3373 Q2W, LV Q2W and Ox Q2W
NUFIRI (Q1W) = NUC-3373 Q1W, LV Q1W and Iri Q2W
NUFIRI (Q2W) = NUC-3373 Q2W, LV Q2W and Iri Q2W

If efficacy signals are observed in any of the patient populations, expansion cohorts of approximately 20 patients may be opened

In Part 3, Q1W NUFOX + bevacizumab and Q1W NUFIRI + bevacizumab cohorts (Arms 3a and 3c) will be opened first; the other arms may subsequently be opened, depending on emerging data

Cohorts of rapid progressors may also be opened (~10 patients), depending on emerging data

Abbreviations: Iri=irinotecan; LV=leucovorin; NUFIRI=NUC-3373 + LV + irinotecan; NUFOX=NUC-3373 + LV + oxaliplatin; Ox=oxaliplatin; Q1W=weekly; Q2W=fortnightly.

SUMMARY SCHEDULE OF EVENTS: ARM 1a AND ARM 1b

Study Assessments[1]	Screening/ Baseline	Cycle 0*	Cycle 1**						Additional Cycles**		End of Treatment	Follow-up Visits[16]
		D1	D1	D2	D3	D8	D15 (±1)	D16	D1 (±3)	D15 (±1)	30 days post last dose (+7 days)	Q8 wks (±7 days)
Informed consent (including pregnancy counselling)	X											
Inclusion/exclusion criteria	X											
Demographic data	X											
Previous medical history	X											
Concomitant medication	X	X	X				X		X	X	X	
Physical examination (including neurological)	X	X	X				X		X	X	X	
Urinalysis	X	X	X						X		X	
ECOG performance status	X	X	X						X		X	
Vital signs (including weight)[2]	X	X	X				X		X	X	X	
ECG[3]	X	X[3]	X[3]				X[3]		X[3]	X[3]	X	
Pregnancy test[4]	X	X	X						X		X	
FBC and chemistry[5]	X	X	X				X		X	X	X	
Coagulation profile	X	X	X								X	
Tumour markers[6]	X	X	X						X		X	
Radiologic tumour assessment (CT / MRI)[7]	X								*Assessed every 8 weeks from the start of Cycle 1*			X[8]
Randomise to Arm 1a or 1b		X										
NUC-3373 administration (all patients)		X	X				X		X	X		
Leucovorin administration[9]		X	X				X		X	X		
AEs/SAEs[10]		X	X	X	X		X	X	X	X	X	X[10]
PK blood sample[11]		X	X	X	X		X	X				
PD blood sample[12]		X	X				X					
PGx blood sample[13]	X								X[13]			
Archived sample[14]	X											
Tumour biopsy[15]	X					X						

* Cycle 0 duration is assumed to be 14 days including the washout period, subject to the patient completing the cycle and subsequently commencing Cycle 1

** Cycle 1 and all additional cycles thereafter are assumed to be 28 days in duration (excluding any allowances for visit window) subject to the patient completing the cycle in full

1 Assessments scheduled on days of dosing should be done prior to administration of all Investigational Medicinal Products (IMPs), unless otherwise specified. Lab assessments may be performed up to 72 hours prior to IMP administration.

2 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 ≥10% during the study, the dose of 'NUC-3373 for infusion' should be recalculated.

3 Standard 12-lead ECG measurements will be performed prior to administration of all IMPs, at the indicated visits.

Note: Additional ECG measurements must be taken at 30-60 minutes post administration of all IMPs at the C0D1, C1D1, C1D15, C3D1, and C3D15 visits.

All 12-lead ECG measurements should be performed in triplicate (keeping the leads in place and the patient supine during readings) and reviewed by the Investigator or qualified designee for safety and quality.

4 Serum pregnancy assessment to be performed within 7 days of C0D1. Required only in women of childbearing potential.

5 Clinical chemistry (including hepatic panel) and haematology will be conducted every 2 weeks throughout the study. In the event of neutropaenia (absolute neutrophil count [ANC] less than $0.5x10^9$/L), thrombocytopaenia (platelet count less than $50x10^9$/L), or ≥Grade 2 clinical chemistry toxicity, these assessments will be conducted more frequently as clinically indicated until toxicity resolves to ≤Grade 1.

6 Collect a pre-dose blood sample for evaluation of carcinoembryonic antigen (CEA).

7 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 Cycle 1 Day 1. Additional tests may be requested at the Investigator's discretion. The same modality should be used throughout.

8 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 treatment for CRC or death in order to determine duration of overall response and progression-free survival (PFS).

9 Leucovorin is administered with all doses beginning at C1D1 in Arm 1a and once at C0D1 in Arm 1b.

10 After informed consent has been obtained, but prior to initiation of study drug, only adverse events (AEs) and serious adverse events (SAEs) caused by a protocol-mandated intervention will be reported. Thereafter, all AEs occurring 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: SAE reporting is required until 30-days post the last dose of study medication.

11 Blood samples for PK measurements, based on a 2-hour and a 4-hour infusion duration of NUC-3373, will be drawn on C0D1, C1D1 (Samples 1-12) and C1D15 (Samples 1-11) as follows:

Sample Number	Sample Collection Time (NUC-3373 2-hour infusion)	Sample Collection Time (NUC-3373 4-hour infusion)	Sample Collection Window
PRIOR TO NUC-3373 INFUSION			
1	Pre-dose		Up to 30 minutes prior to starting infusion
DURING NUC-3373 INFUSION			
2	T0 + 30 minutes	T0 + 60 minutes	+/- 5 minutes
3	T0 + 60 minutes	T0 + 120 minutes	
4	T0 + 120 minutes	T0 + 240 minutes	
AFTER NUC-3373 INFUSION			
5	T1 + 15 minutes		+/- 5 minutes
6	T1 + 30 minutes		
7	T1 + 60 minutes		
8	T1 + 90 minutes		
9	T1 + 120 minutes		+/- 15 minutes
10	T1 + 240 minutes		+ 4 hours / - 15 minutes
11	T1 + 24 hours		+ 4 hours
12*	T1 + 48 hours		

- T0 = Start of NUC-3373 infusion
- T1 = End of NUC-3373 infusion
- Samples 2, 3, and 4 are triggered by the start time of the infusion and assume a 2-hour or a 4-hour infusion duration. Collection times will be modified in alignment with infusion duration changes
- Samples 5-12 are timed from the end of the infusion

** Sample 12 is optional for C1D1. Sample 12 will not be taken for C1D15.*

12 Blood samples for PD measurements will be drawn Pre-dose (sample to be drawn within 30 minutes prior to the first dose) and at 30 minutes (±5 mins) following the end of the NUC-3373 infusion, (*i.e.,* T1+30 mins, where T1 = end of NUC-3373 infusion).

Sample Number	Sample Time	Sample Collection Window
PRIOR TO NUC-3373 INFUSION		
1	Pre-dose	Up to 30 minutes prior to starting infusion
AFTER NUC-3373 INFUSION		
2	T1 + 30 minutes	+/- 5 minutes

13 Blood samples (optional) for mRNA analyses (*e.g.,* PAX gene) will be drawn at baseline or pre-dose on C0D1 and again at C3D1.

14 Original diagnostic block will be recalled (where available).

15 Fresh biopsy (optional) will be performed at Screening (maximum 14-days before the first dose) and on treatment on C1D8 or C1D15 at 3-6 hours after study treatment, in consenting patients.

16 During the COVID-19 pandemic, collection of follow-up data can be performed via a telephone call with the patient where possible (*e.g.*, for data regarding concomitant medications, AEs, and quality of life [QoL]). The data collected and the follow-up schedule remain as per the schedule of events. Patients in follow-up who have not experienced radiological disease progression should continue to attend the clinic for planned radiologic scans; however, other follow-up data can be collected via telephone to limit patient visits.

SUMMARY SCHEDULE OF EVENTS: ARM 1c and ARM 1d

Study Assessments[1]	Screening/ Baseline	Cycle 1*							Additional Cycles*		End of Treatment	Follow-up Visits[18]
	Days -28 to 1	D1	D2	D3	D8 (±1)	D15 (±1)	D16	D22 (±1)	D1 (±3)	D8, 15, 22 (±1)	30 days post last dose (+7 days)	Q8 wks (±7 days)
Informed consent (including pregnancy counselling)	X											
Inclusion/exclusion criteria	X											
Demographic data	X											
Previous medical history	X											
Concomitant medication	X	X			X	X		X	X	X	X	
Physical examination (including neurological)	X	X			X	X		X	X	X	X	
Urinalysis	X	X							X		X	
ECOG performance status	X	X							X		X	
Vital signs (including weight)[2]	X	X			X	X		X	X	X	X	
Height[2]	X											
ECG[3]	X	X[3]			X[3]	X[3]		X[3]	X[3]	X[3]	X	
Pregnancy test[4]	X	X							X		X	
FBC and chemistry[5]	X	X			X	X		X	X	X	X	
RAS/BRAF status testing	X[6]											
Coagulation profile	X	X							X		X	
Tumour markers[7]	X	X							X		X	
Radiologic tumour assessment (CT / MRI)[8]	X								*Assessed every 8 weeks from the start of Cycle 1*			X[9]
NUC-3373 administration (all patients)		X			X	X		X	X	X		
Leucovorin administration[10]		X			X	X		X	X	X		
AEs/SAEs[11]		X	X	X	X	X	X	X	X	X	X	X[11]
PK blood sample[12]		X	X	X		X	X					
PK urine sample[13]		X	X	X		X	X					
PGx blood sample[14]	X[14]								X[14]			
Archived sample[15]	X											
Tumour biopsy[16]	X				X	X						
Survival[17]	*Assessed every 3 months from the start of Cycle 1*											

* Cycle 1 and all additional cycles thereafter are assumed to be 28 days in duration (excluding any allowances for visit window) subject to the patient completing the cycle in full

1. Assessments scheduled on days of dosing should be done prior to administration of all IMPs, unless otherwise specified. Lab assessments may be performed up to 72 hours prior to IMP administration.
2. 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 ≥10% during the study, the dose of 'NUC-3373 for infusion' should be recalculated.
3. Standard 12-lead ECG measurements will be performed prior to administration of all IMPs, at the indicated visits.

   Note: Additional ECG measurements must be taken at 30-60 minutes post administration of all IMPs at the C1D1, C1D15, C3D1, and C3D15 visits.

   All 12-lead ECG measurements should be performed in triplicate (keeping the leads in place and the patient supine during readings) and reviewed by the Investigator or qualified designee for safety and quality.
4. Serum pregnancy assessment to be performed within 7 days of C1D1. Perform only in women of childbearing potential.
5. Clinical chemistry (including hepatic panel) and haematology will be conducted every week throughout the study. In the event of neutropaenia (ANC less than $0.5x10^9$/L), thrombocytopaenia (platelet count less than $50x10^9$/L), or ≥Grade 2 clinical chemistry toxicity, these assessments will be conducted more frequently as clinically indicated until toxicity resolves to ≤Grade 1.
6. If RAS/BRAF mutation status is unknown, perform genetic testing.
7. Collect a pre-dose blood sample for evaluation of CEA.
8. CT/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 Cycle 1 Day 1. Additional tests may be requested at the Investigator's discretion. The same modality should be used throughout.
9. 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 treatment for CRC or death in order to determine duration of overall response and PFS.
10. Leucovorin is administered with all doses beginning at C1D1.
11. After informed consent has been obtained, but prior to initiation of study drug, only AEs and serious adverse events caused by a protocol-mandated intervention will be reported. Thereafter, all AEs occurring up to and including 30 days after the last dose of study drug has been administered must be reported in detail on the AE CRF.

    Note: SAE reporting is required until 30-days post the last dose of study medication.
12. Blood samples for PK measurements, based on a 2-hour and a 4-hour infusion duration of NUC-3373, will be drawn on C1D1 (Samples 1-12) and C1D15 (Samples 1-11) as follows:

Sample Number	Sample Collection Time (NUC-3373 2-hour infusion)	Sample Collection Time (NUC-3373 4-hour infusion)	Sample Collection Window
PRIOR TO NUC-3373 INFUSION			
1	Pre-dose		Up to 30 minutes prior to starting infusion
DURING NUC-3373 INFUSION			
2	T0 + 30 minutes	T0 + 60 minutes	+/- 5 minutes
3	T0 + 60 minutes	T0 + 120 minutes	
4	T0 + 120 minutes	T0 + 240 minutes	
AFTER NUC-3373 INFUSION			
5	T1 + 15 minutes		+/- 5 minutes
6	T1 + 30 minutes		
7	T1 + 60 minutes		
8	T1 + 90 minutes		
9	T1 + 120 minutes		+/- 15 minutes
10	T1 + 240 minutes		+ 4 hours / - 15 minutes
11	T1 + 24 hours		+ 4 hours
12*	T1 + 48 hours		

- Sample 1, pre-dose should be collected prior to infusion of any IMPs
- T0 = Start of NUC-3373 infusion
- T1 = End of NUC-3373 infusion
- Samples 2, 3, and 4 are triggered by the start time of the infusion and assume a 2-hour or a 4-hour infusion duration. Collection times will be modified in alignment with infusion duration changes
- Samples 5-12 are timed from the end of the infusion

** Sample 12 is optional for C1D1. Sample 12 will not be taken for C1D15.*

13. Urine samples for PK analyses will be collected in Arm 1d only. Samples will be collected 0-8 hours and 8-24 hours from the start of NUC-3373 infusion. For patients who provide optional consent, urine collection will continue for 48 hours on C1D1 only.

14. Blood samples (optional) for mRNA analyses (*e.g.,* PAX gene) will be drawn at baseline or pre-dose on C1D1 and again at C3D1.

15. Original diagnostic block will be recalled (where available).

16. Fresh biopsy (optional) will be performed at Screening (maximum 14-days before the first dose) and on treatment on C1D8 or C1D15 at 3-6 hours after study treatment, in consenting patients. If this is not possible, it may be obtained at any time after C1D15 with agreement from the Medical Monitor.

17. All patients will be followed up until withdrawal of consent, lost to follow-up, death, or the overall end of study has been reached (defined in Section 3.6), whichever occurs first. Survival follow-up may be performed via telephone call.

18. During the COVID-19 pandemic, collection of follow-up data can be performed via a telephone call with the patient where possible (*e.g.*, for data regarding concomitant medications, AEs, and QoL). The data collected and the follow-up schedule remain as per the schedule of events. Patients in follow-up who have not experienced radiological disease progression should continue to attend the clinic for planned radiologic scans; however, other follow-up data can be collected via telephone to limit patient visits.

SUMMARY SCHEDULE OF EVENTS: PART 2 (ALL ARMS)

Study Assessments[1]	Screening	Cycle 1*							Additional Cycles*		End of Treatment	Follow-up Visits[19]
	Days -28 to 1	D1	D2	D3	D8 (±1)	D15 (±1)	D16	D22 (±1)	D1 (±3)	D8,15, 22 (±1)	30-days post last dose (+7 days)	Q8 wks (±7 days)
Informed consent (including pregnancy counselling)	X											
Inclusion/exclusion criteria	X											
Demographic data	X											
Previous medical history	X											
Concomitant medication	X	X			X	X		X	X	X	X	
Physical examination (including neurological)	X	X			X	X		X	X	X	X	
Urinalysis	X	X							X		X	
ECOG performance status	X	X							X		X	
Vital signs (including weight)[2]	X	X			X	X		X	X	X	X	
Height[2]	X											
ECG[3]	X	X[3]			X[3]	X[3]		X[3]	X[3]	X[3]	X	
Pregnancy test[4]	X	X							X		X	
FBC and chemistry[5]	X	X			X	X		X	X	X	X	
RAS/BRAF status testing	X[6]											
Coagulation profile	X	X							X		X	
Tumour markers[7]	X	X							X		X	
Radiologic tumour assessment (CT / MRI)[8]	X								*Assessed every 8 weeks from the start of Cycle 1*			X[9]
NUC-3373 (+LV) administration		X			X	X		X	X	X		
Combination agent administration based on arm [10]		X				X			X	X[11]		
AEs/SAEs[12]		X	X	X	X	X	X	X	X	X	X	X[12]
PK blood sample(s)[13]		X	X	X		X	X					
PGx blood sample[14]	X[14]								X[14]			
Archived sample[15]	X											
Tumour biopsy[16]	X				X	*X*						
Survival[17]	*Assessed every 3 months from the start of Cycle 1*											

* Cycle 1 and all additional cycles thereafter are assumed to be 28 days in duration (excluding any allowances for visit window) subject to the patient completing the cycle in full

1. Assessments scheduled on days of dosing should be done prior to administration of all IMPs, unless otherwise specified. Lab assessments may be performed up to 72 hours prior to IMP administration.
2. 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 ≥10% during the study, the dose of 'NUC-3373 for infusion' should be recalculated.
3. Standard 12-lead ECG measurements will be performed prior to administration of all IMPs, at the indicated visits.

   Note: Additional ECG measurements must be taken at 30-60 minutes post administration of all IMPs at the C1D1, C1D15, C3D1, and C3D15 visits.

   All 12-lead ECG measurements should be performed in triplicate (keeping the leads in place and the patient supine during readings) and reviewed by the Investigator or qualified designee for safety and quality.
4. Serum pregnancy assessment to be performed within 7 days of C1D1. Perform only in women of childbearing potential.
5. Clinical chemistry (including hepatic panel) and haematology will be conducted every week throughout the study. In the event of neutropaenia (ANC less than $0.5x10^9$/L), thrombocytopaenia (platelet count less than 50×10^9/L), or ≥Grade 2 clinical chemistry toxicity, these assessments will be conducted more frequently as clinically indicated until toxicity resolves to ≤Grade 1.
6. If RAS/BRAF mutation status is unknown, perform genetic testing.
7. Collect a pre-dose blood sample for evaluation of CEA.
8. CT/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 Cycle 1 Day 1. Additional tests may be requested at the Investigator's discretion. The same modality should be used throughout.
9. 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 treatment for CRC, or death in order to determine duration of overall response and PFS.
10. IV administration of combination agents as follows:

Arms	Cycle 1 Day 1 and beyond
Arm 2a & Arm 2c	Days 1, 8, 15, 22: NUC-3373 + LV Days 1 & 15: oxaliplatin
Arm 2b & Arm 2d	Days 1, 8, 15, 22: NUC-3373 + LV Days 1 & 15: irinotecan

11. Combination agent administration on Day 15 of additional cycles only (Q2W schedule).

12. After informed consent has been obtained, but prior to initiation of study drug, only AEs and serious adverse events caused by a protocol-mandated intervention will be reported. Thereafter, all AEs occurring up to and including 30 days after the last dose of study drug has been administered must be reported in detail on the AE CRF.

Note: SAE reporting is required until 30 days post the last dose of study medication.

13. Blood samples for PK measurements, based on a 2-hour and a 4-hour infusion duration of NUC-3373, will be drawn on C1D1 (Samples 1-12) and C1D15 (Samples 1-11) as follows:

Sample Number	Sample Collection Time (NUC-3373 2-hour infusion)	Sample Collection Time (NUC-3373 4-hour infusion)	Sample Collection Window
PRIOR TO NUC-3373 INFUSION			
1	Pre-dose		Up to 30 minutes prior to starting infusion
DURING NUC-3373 INFUSION			
2	T0 + 30 minutes	T0 + 60 minutes	+/- 5 minutes
3	T0 + 60 minutes	T0 + 120 minutes	
4	T0 + 120 minutes	T0 + 240 minutes	
AFTER NUC-3373 INFUSION			
5	T1 + 15 minutes		+/- 5 minutes
6	T1 + 30 minutes		
7	T1 + 60 minutes		
8	T1 + 90 minutes		
9	T1 + 120 minutes		+/- 15 minutes
10	T1 + 240 minutes		+ 4 hours / - 15 minutes
11	T1 + 24 hours		+ 4 hours
12*	T1 + 48 hours		

- Sample 1, pre-dose should be collected prior to infusion of any IMPs
- T0 = Start of NUC-3373 infusion
- T1 = End of NUC-3373 infusion
- Samples 2, 3, and 4 are triggered by the start time of the infusion and assume a 2-hour or a 4-hour infusion duration. Collection times will be modified in alignment with infusion duration changes
- Samples 5-12 are timed from the end of the infusion

  ** Sample 12 is optional for C1D1. Sample 12 will not be taken for C1D15.*

14. Blood samples (optional) for mRNA analyses (*e.g.,* PAX gene) will be drawn at baseline or pre-dose on C1D1 and again at C3D1.

15. Original diagnostic block will be recalled (where available).

16. Fresh biopsy (optional) will be performed at Screening (maximum 14 days before the first dose) and on treatment on C1D8 or C1D15 at 3-6 hours after study treatment, in consenting patients. If this is not possible, it may be obtained at any time after C1D15 with agreement from the Medical Monitor.

17. All patients will be followed up until withdrawal of consent, lost to follow-up, death, or the overall end of study has been reached (defined in Section 3.6), whichever occurs first. Survival follow-up may be performed via telephone call.

18. During the COVID-19 pandemic, collection of follow-up data can be performed via a telephone call with the patient where possible (*e.g.*, for data regarding concomitant medications, AEs, and QoL). The data collected and the follow-up schedule remain as per the schedule of events. Patients in follow-up who have not experienced radiological disease progression should continue to attend the clinic for planned radiologic scans; however, other follow-up data can be collected via telephone to limit patient visits.

SUMMARY SCHEDULE OF EVENTS: PART 3

Study Assessments[1]	Screening	Cycle 1*							Additional Cycles*		End of Treatment	Follow-up Visits[18]
	Days -28 to 1	D1	D2	D3	D8 (±1)**	D15 (±1)	D16	D22 (±1)**	D1 (±3)	D8,15, 22 (±1)**	30-days post last dose (+7 days)	Q8 wks (±7 days)
Informed consent (including pregnancy counselling)	X											
Inclusion/ exclusion criteria	X											
Demographic data	X											
Previous medical history	X											
Concomitant medication	X	X			X	X		X	X	X	X	
Physical examination (including neurological)	X	X			X	X		X	X	X	X	
Urinalysis	X	X							X		X	
ECOG performance status	X	X							X		X	
Vital signs (including weight)[2]	X	X			X	X		X	X	X	X	
Height[2]	X											
ECG[3]	X	X[3]			X[3]	X[3]		X[3]	X[3]	X[3]	X	
Pregnancy test[4]	X	X							X		X	
FBC and chemistry[5]	X	X			X	X		X	X	X	X	
RAS/BRAF status testing	X[6]											
Coagulation profile	X	X							X		X	
Tumour markers[7]	X	X							X		X	
Radiologic tumour assessment (CT/MRI)[8]	X								*Assessed every 8 weeks from the start of Cycle 1*			X[9]
NUC-3373 (+LV) administration		X			X**	X		X**	X	X**		
Combination agent administration[10]		X			X***	X		X***	X	X***		
AEs/SAEs[11]		X	X	X	X	X	X	X	X	X	X	X[11]
PK blood sample(s)[12]		X	X	X		X	X					
PK urine sample (Arm 3e)[13]		X	X	X		X	X					
PGx blood sample[14]	X[14]								X[14]			
Archived sample[15]	X											
Tumour biopsy[16]	X				X	X						
Survival[17]	*Assessed every 3 months from the start of Cycle 1*											

* Cycle 1 and all additional cycles thereafter are assumed to be 28 days in duration (excluding any allowances for visit window) subject to the patient completing the cycle in full

** Day 8 and Day 22 will only be completed in the Q1W NUC-3373 + LV cohorts (Arms 3a, 3c, 3e, 3f and 3g)

*** Day 8 and Day 22 will only be completed in arms containing cetuximab (Arms 3f and 3g), depending on the chosen schedule

1. Assessments scheduled on days of dosing should be done prior to administration of all IMPs, unless otherwise specified. Lab assessments may be performed up to 72 hours prior to IMP administration.
2. 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 ≥10% during the study, the dose of 'NUC-3373 for infusion' should be recalculated.
3. Standard 12-lead ECG measurements will be performed prior to administration of all IMPs, at the indicated visits.

   Note: Additional ECG measurements must be taken at 30-60 minutes post administration of all IMPs at the C1D1, C1D15, C2D1, C2D15, C3D1, and C3D15 visits.

   All 12-lead ECG measurements should be performed in triplicate (keeping the leads in place and the patient supine during readings) and reviewed by the Investigator or qualified designee for safety and quality
4. Serum pregnancy assessment to be performed within 7 days of C1D1. Perform only in women of childbearing potential.
5. Clinical chemistry (including hepatic panel) and haematology will be conducted every week for patients on Q1W schedules and every 2 weeks for patients on Q2W schedules throughout the study. In the event of neutropaenia (ANC less than $0.5x10^9$/L), thrombocytopaenia (platelet count less than $50x10^9$/L), or ≥Grade 2 clinical chemistry toxicity, these assessments will be conducted more frequently as clinically indicated until toxicity resolves to ≤Grade 1.
6. If RAS/BRAF mutation status is unknown, perform genetic testing.
7. Collect a pre-dose blood sample for evaluation of CEA.
8. CT/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 Cycle 1 Day 1. Additional tests may be requested at the Investigator's discretion. The same modality should be used throughout.
9. 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 treatment for CRC, or death in order to determine duration of overall response and PFS.
10. IV administration of combination agents as follows:

Arm	Cycle 1 Day 1 and beyond
Arm 3a	NUFOX + bevacizumab Days 1, 8, 15, 22: NUC-3373 + LV Days 1 & 15: oxaliplatin
Arm 3b	NUFOX + bevacizumab Days 1 & 15: NUC-3373 + LV + oxaliplatin

Arm 3c	NUFIRI + bevacizumab Days 1, 8, 15, 22: NUC-3373 + LV Days 1 & 15: irinotecan
Arm 3d	NUFIRI + bevacizumab Days 1 & 15: NUC-3373 + LV + irinotecan
Arm 3e	NUC-3373 + LV + bevacizumab Days 1, 8, 15, 22: NUC-3373 + LV
Arm 3f	NUFOX + cetuximab Q1W or Q2W
Arm 3g	NUFIRI + cetuximab Q1W or Q2W
Bevacizumab and cetuximab should be administered in accordance with standard local practice.	

11. After informed consent has been obtained, but prior to initiation of study drug, only AEs and serious adverse events caused by a protocol-mandated intervention will be reported. Thereafter, all AEs occurring up to and including 30 days after the last dose of study drug has been administered must be reported in detail on the AE CRF.

Note: SAE reporting is required until 30-days post the last dose of study medication.

12. Blood samples for PK measurements, based on a 2-hour and a 4-hour infusion duration, will be drawn on C1D1 (Samples 1-12) and C1D15 (Samples 1-11) as follows as follows:

Sample Number	Sample Collection Time (NUC-3373 2-hour infusion)	Sample Collection Time (NUC-3373 4-hour infusion)	Sample Collection Window
PRIOR TO NUC-3373 INFUSION			
1	Pre-dose		Up to 30 minutes prior to starting infusion
DURING NUC-3373 INFUSION			
2	T0 + 30 minutes	T0 + 60 minutes	+/- 5 minutes
3	T0 + 60 minutes	T0 + 120 minutes	
4	T0 + 120 minutes	T0 + 240 minutes	
AFTER NUC-3373 INFUSION			
5	T1 + 15 minutes		+/- 5 minutes
6	T1 + 30 minutes		
7	T1 + 60 minutes		
8	T1 + 90 minutes		
9	T1 + 120 minutes		+/- 15 minutes
10	T1 + 240 minutes		+ 4 hours / - 15 minutes
11	T1 + 24 hours		+ 4 hours
12*	T1 + 48 hours		

- Sample 1, pre-dose should be collected prior to infusion of any IMPs

- T0 = Start of NUC-3373 infusion
- T1 = End of NUC-3373 infusion
- Samples 2, 3, and 4 are triggered by the start time of the infusion and assume a 2-hour or a 4-hour infusion duration. Collection times will be modified in alignment with infusion duration changes
- Sample 6-12 are timed from the end of the infusion

  ** Sample 12 is optional for C1D1. Sample 12 will not be taken for C1D15.*

13. Urine samples for PK analyses will be collected in Arm 3e only. Samples will be collected 0-8 hours and 8-24 hours from the start of NUC-3373 infusion. For patients who provide optional consent, urine collection will continue for 48 hours on C1D1 only.

14. Blood samples (optional) for mRNA analyses (*e.g.,* PAX gene) will be drawn at baseline or pre-dose on C1D1 and again at C3D1.

15. Original diagnostic block will be recalled (where available).

16. Fresh biopsy (optional) will be performed at Screening (maximum 14 days before the first dose) and on treatment on C1D8 or C1D15 at 3-6 hours after study treatment, in consenting patients. If this is not possible, it may be obtained at any time after C1D15 with agreement from the Medical Monitor.

17. All patients will be followed up until withdrawal of consent, lost to follow-up, death, or the overall end of study has been reached (defined in Section 3.6), whichever occurs first. Survival follow-up may be performed via telephone call.

18. During the COVID-19 pandemic, collection of follow-up data can be performed via a telephone call with the patient where possible (*e.g.*, for data regarding concomitant medications, AEs, and QoL). The data collected and the follow-up schedule remain as per the schedule of events. Patients in follow-up who have not experienced radiological disease progression should continue to attend the clinic for planned radiologic scans; however, other follow-up data can be collected via telephone to limit patient visits.

SUMMARY SCHEDULE OF EVENTS: PK SUB-STUDY (PART 2 ARM 2B; CYCLE 1 AND CYCLE 2 DAYS 1 AND 2)

Study Assessments[1]	**Screening**	**Cycle 1**								**Cycle 2**	
	Days -28 to 1	**D1**	**D2**	**D8**	**D9**	**D15**	**D16**	**D22**	**D23**	**D1**	**D2**
Informed consent (including pregnancy counselling)	X										
Inclusion/exclusion criteria	X										
Demographic data	X										
Previous medical history	X										
Concomitant medication	X	X		X		X		X		X	
Physical examination (including neurological)	X	X		X		X		X		X	
Urinalysis	X	X								X	
ECOG performance status	X	X								X	
Vital signs (including weight)[2]	X	X		X		X		X		X	
Height[2]	X										
Safety ECG[3]	X	X[3]		X[3]		X[3]		X[3]		X[3]	
Holter ECG[4]				**X**[4]	**X**[4]			**X**[4]	**X**[4]		
Pregnancy test[5]	X	X								X	
FBC and chemistry[6]	X	X		X		X		X		X	
RAS/BRAF status testing	X[7]										
Coagulation profile	X	X								X	
Tumour markers[8]	X	X								X	
Radiologic tumour assessment (CT / MRI)[9]	X										
Genotyping blood sample[10]	**X**										
NUC-3373 administration[11]				**X**		**X**		**X**		**X**	
LV administration[11]		**X**		**X**		**X**		**X**		**X**	
Irinotecan administration[11]		**X**				**X**				**X**	
AEs/SAEs[12]		X	X	X	X	X	X	X	X	X	X
PK blood sample[13]		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
PGx blood sample[14]	X										
Archived sample[15]	X										
Tumour biopsy[16]	X			X		*X*					

The rows in bold font are the only rows that are different from the main Part 2 Summary Schedule of Events, and only apply to Cycle 1 and Day 1/2 of Cycle 2. Following this, patients in the PK sub-study will continue with the main schedule for Part 2 (Arm 2b).

1. Assessments scheduled on days of dosing should be done prior to administration of all IMPs, unless otherwise specified. Lab assessments may be performed up to 72 hours prior to IMP administration.

2. 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 ≥10% during the study, the dose of 'NUC-3373 for infusion' should be recalculated.

3. Standard 12-lead ECG measurements will be performed prior to administration of all IMPs, at the indicated visits. **Note:** Additional ECG measurements must be taken at 30-60 minutes post administration of all IMPs at the C1D1, C1D15, C3D1, and C3D15 visits.

All 12-lead ECG measurements should be performed in triplicate (keeping the leads in place and the patient supine during readings) and reviewed by the Investigator or qualified designee for safety and quality.

4. Holter ECG measurements will be performed on C1D8 and C1D22, with continuous readings taken over 24 hours, starting from 30 minutes prior to the start of infusion (see footnote 13). Patients must be supine for 30 minutes prior to the start of infusion and for at least 5 minutes prior to, and during, the other timepoints where PK draws are taken (as outlined in footnote 13).

All Holter ECG data should be transmitted for blinded central review.

5. Serum pregnancy assessment to be performed within 7 days of C1D1. Perform only in women of childbearing potential.

6. Clinical chemistry (including hepatic panel) and haematology will be conducted every week throughout the study. In the event of neutropaenia (ANC less than $0.5x10^9$/L), thrombocytopaenia (platelet count less than $50x10^9$/L), or ≥Grade 2 clinical chemistry toxicity, these assessments will be conducted more frequently as clinically indicated until toxicity resolves to ≤Grade 1.

7. If RAS/BRAF mutation status is unknown, perform genetic testing.

8. Collect a pre-dose blood sample for evaluation of CEA.

9. CT/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 Cycle 1 Day 1. Additional tests may be requested at the Investigator's discretion. The same modality should be used throughout.

10. Genotyping to be performed for each patient using genomic DNA from white blood cells isolated from a 3 mL sample of whole blood taken during screening. The genomic DNA will be analysed using pharmacogenomic protocols specific for individual genotypes for drug metabolising enzymes and drug transporters, *e.g.,* TATA box polymorphism of the UGT1A1 promoter region and NR1I2-rs10934498 polymorphism, prior to admission to the sub-study. Genotyping is not required for patients who have a known UGT1A1 status from prior testing.

11. LV is administered on Days 1, 8, 15 & 22 of Cycle 1 and Day 1 of Cycle 2 (29 days after start of treatment); irinotecan is administered on Days 1 & 15 of Cycle 1 and Day 1 of Cycle 2 (29 days after start of treatment); NUC-3373 is administered on Days 8, 15 & 22 of Cycle 1 and Day 1 of Cycle 2 (29 days after start of treatment). Patients will be alternately assigned to two groups (Group A and Group B) for treatment on Cycle 1 Day 15 and Cycle 2 Day 1 and will receive the study treatments in an order that has been pre-specified for each group (refer to Section 7.2.1 for details). Following this, patients in the PK sub-study will continue to receive study treatment as outlined for Arm 2b. Patients enrolled in the PK sub-study will be considered evaluable for PK endpoints if they have received planned study treatment and had PK assessments up to at least C1D15. If patients discontinue study treatment or require dose modifications before C1D15, they will be considered non-evaluable and will be replaced.

12. After informed consent has been obtained, but prior to initiation of study drug, only AEs and serious adverse events caused by a protocol-mandated intervention will be reported. Thereafter, all AEs occurring up to and including 30 days after the last dose of study drug has been administered must be reported in detail on the AE CRF.

Note: SAE reporting is required until 30 days post the last dose of study medication.

13. PK sub-study blood samples for analysis of irinotecan, NUC-3373 and their metabolites will be drawn for patients assigned to Group A and Group B as follows (sample collection times may be adjusted based on emerging data):

Group A

			Timepoints (min post-first infusion)									
		Pre-dose*	60 mins (+/- 5 mins)	120 mins (+/- 5 mins)	150 mins (+/- 5 mins)	180 mins (+/- 5 mins)	250 mins (+/- 5 mins)	280 mins (+/- 5 mins)	300 mins (+/- 5 mins)	360 mins (+/- 15 mins)	480 mins (+/- 15 mins)	24 hours (+/- 2 hours)
C1D1	Infusion		Irinotecan + LV									
	PK Draw	x	x	x		x	x		x	x	x	x
C1D8	Infusion		LV		NUC-3373							
	PK Draw	x				x	x	x	x	x	x	x
C1D15	Infusion		Irinotecan + LV		NUC-3373							
	PK Draw	x	x	x		x	x	x	x	x	x	x
C1D22	Infusion		LV		NUC-3373							
	PK Draw	x				x	x	x	x	x	x	x
C2D1	Infusion		NUC-3373		Irinotecan + LV							
	PK Draw	x	x	x	x	x	x		x	x	x	x

*Pre-dose = up to 30 minutes prior to the administration of irinotecan + LV (C1D1, C1D15) or NUC-3373 (C1D8, C1D22, C2D1)

Group B

			Timepoints (min post-first infusion)									
		Pre-dose*	60 mins (+/- 5 mins)	120 mins (+/- 5 mins)	150 mins (+/- 5 mins)	180 mins (+/- 5 mins)	250 mins (+/- 5 mins)	280 mins (+/- 5 mins)	300 mins (+/- 5 mins)	360 mins (+/- 15 mins)	480 mins (+/- 15 mins)	24 hours (+/- 2 hours)
C1D1	Infusion		Irinotecan + LV									
	PK Draw	x	x	x		x	x		x	x	x	x
C1D8	Infusion		LV		NUC-3373							
	PK Draw	x				x	x	x	x	x	x	x
C1D15	Infusion		NUC-3373		Irinotecan + LV							
	PK Draw	x	x	x	x	x	x		x	x	x	x
C1D22	Infusion		LV		NUC-3373							
	PK Draw	x				x	x	x	x	x	x	x
C2D1	Infusion		Irinotecan + LV		NUC-3373							
	PK Draw	x	x	x		x	x	x	x	x	x	x

*Pre-dose = up to 30 minutes prior to the administration of irinotecan + LV (C1D1, C2D1) or NUC-3373 (C1D8, C1D15, C1D22)

The exact time each PK sample is taken must be recorded.

Note that for C1D8 and C1D22 when the patient is wearing the Holter monitor, the patient must be supine for 30 minutes prior to the start of infusion and for at least 5 minutes prior to, and during, the other timepoints when PK draws are taken (see footnote 4).

14. Blood samples (optional) for mRNA analyses (*e.g.,* PAX gene) will be drawn at baseline or pre-dose on C1D1 and again at C3D1.

15. Original diagnostic block will be recalled (where available).

16. Fresh biopsy (optional) will be performed at Screening (maximum 14 days before the first dose) and on treatment on C1D8 or C1D15 at 3-6 hours after study treatment, in consenting patients. If this is not possible, it may be obtained at any time after C1D15 with agreement from the Medical Monitor.

ABBREVIATIONS

5-FU	5-fluorouracil
5-FUTP	5-fluorouridine triphosphate
5-methyl-THF	5-methyltetrahydofolate
ADR	Adverse drug reaction
AE	Adverse event
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
APC	Irinotecan metabolite (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin)
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the curve
AUC_{0-24}	Area under the plasma concentration-time curve from time zero to 24 hours
BRAF	Proto-oncogene protein B-raf gene
BSA	Body surface area
CAPIRI	Capecitabine + irinotecan
CAPOX	Capecitabine + oxaliplatin
CEA	Carcinoembryonic antigen
CFR	Code of Federal Regulations
C_{inf}	Concentration at the end of infusion
CL	Apparent clearance
C_{max}	Maximum plasma concentration; peak plasma concentration
COVID-19	Coronavirus Disease 2019
CR	Complete response
CRC	Colorectal cancer
CRF	Case report form
CRO	Clinical Research Organisation
CSR	Clinical study report
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CYP3A4	Cytochrome P450 3A4
DCR	Disease control rate
DLT	Dose-limiting toxicity
DMA	Dimethylacetamide
DNA	Deoxyribonucleic acid
DoR	Duration of response
DoSD	Duration of stable disease
DPD	Dihydropyrimidine dehydrogenase
DSMC	Data Safety Monitoring Committee
DSUR	Development safety update report
dTMP	Deoxythymidine monophosphate
dUMP	Deoxyuridine monophosphate
EC_{50}	Half-maximal effective concentration
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EFR	Evaluable for response

EGFR	Epidermal growth factor receptor
EMA	European Medicines Agency
EoT	End of treatment
EudraCT	European Clinical Trials Database
FAS	Full analysis set
FBAL	α-fluoro-β-alanine
FDA	(US) Food and Drug Administration
FOLFIRI	5-FU + LV + irinotecan
FOLFOX	5-FU + LV + oxaliplatin
FOLFOXIRI	5-FU + LV + oxaliplatin + irinotecan
FUDR	Floxuridine; (5-fluorodeoxyuridine)
FUDR-MP	Fluorodeoxyuridine-monophosphate
G-CSF	Granulocyte colony stimulating factor
IB	Investigator's Brochure
ICF	Informed consent form
ICH-GCP	International Council for Harmonisation - Good Clinical Practice
IMP	Investigational medicinal product
IND	Investigational New Drug
INR	International normalised ratio
IRB/EC	Institutional review board/ ethics committee
IV	Intravenous(ly)
KRAS	Gene that codes for the KRAS protein
L	Litre
LDH	Lactate dehydrogenase
LV	Leucovorin
m^2	$Meter^2$
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram
µM	Micromolar
Ml	Millilitre
mM	Millimolar
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NE	Not evaluable
NPC	Irinotecan metabolite (7-ethyl-10-(4-amino-1-piperidino)-carbonyloxycamptothecin)
NUFIRI	NUC-3373 + LV + irinotecan
NUFOX	NUC-3373 + LV + oxaliplatin
OPRT	Orotate Phosphoribosyl Transferase
ORR	Objective Response Rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD	Progressive Disease or Pharmacodynamics
PFS	Progression-Free Survival
PGx	Pharmacogenomics
PK	Pharmacokinetics
PR	Partial Response
PT	Prothrombin time
Q1W	Weekly

Q2W	Fortnightly
QoL	Quality of life
QT/QTc	QT interval / Corrected QT interval
RECIST	Response Evaluation Criteria In Solid Tumours (version 1.1)
RBC	Red blood cell
RNA	Ribonucleic acid
RP2D	Recommended Phase II dose
RSI	Reference Safety Information
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Stable Disease
SmPC	Summary of Product Characteristics
SN-38	Irinotecan metabolite (7-ethyl-10-hydroxycamptothecin)
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{1/2}$	Terminal half-life
TK	Thymidine kinase
TP	Thymidine phosphorylase
TS	Thymidylate synthase
TEAE	Treatment-emergent adverse event
UGT1A1	UDP glucuronosyltransferase 1A1
ULN	Upper limit of normal
V_d	Volume of distribution
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WBC	White blood cell
WHO DD	World Health Organisation Drug Dictionary

1. INTRODUCTION AND STUDY RATIONALE

1.1. Treatment Options for Recurrent Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer in males and the second most common in females, accounting for 10% of all cancers. The incidence worldwide is approximately 1.8 million new cases per year with a 5-year survival rate of 10% for patients diagnosed with metastatic disease (De Falco *et al*, 2020). In the United States, there are approximately 1.5 million people living with CRC and approximately 148,000 new cases of CRC and 53,000 deaths due to the disease are expected in 2020. Worldwide, there were an estimated 881,000 deaths due to CRC in 2018, and the global burden is expected to increase to more than 2.2 million new cases and 1.1 million deaths annually by 2030.

Most systemic therapies for CRC include the pyrimidine analogue 5-fluorouracil (5-FU), either as monotherapy or in combination with another chemotherapeutic, typically oxaliplatin or irinotecan. Both alone and in combination, 5-FU is the single most effective compound against these tumours. Nevertheless, despite being the standard of care, the effectiveness of 5-FU as a single-agent is modest.

1.2. 5-FU in Colorectal Cancer

1.2.1 Adjuvant Treatment

5-FU has been the mainstay of adjuvant treatment for high-risk Stage II or Stage III colon cancer for several decades. Adjuvant chemotherapy with a 5-FU-based regimen has been shown to improve overall survival (OS) by 7% (5% for those with Stage II and 10% with Stage III cancer; Ragnhammar *et al*, 2001). In the adjuvant setting, 5-FU is given with leucovorin (LV) or combined with LV and oxaliplatin (FOLFOX). The oral 5-FU prodrug capecitabine (Xeloda) can be used instead of 5-FU, either as monotherapy or in combination with oxaliplatin (CAPOX).

1.2.2 Treatment for Metastatic Disease

5-FU is an essential component of chemotherapy for CRC in the metastatic setting, as part of FOLFOX (5-FU, LV and oxaliplatin), FOLFIRI (5-FU, LV and irinotecan), FOLFOXIRI (5-FU, LV, oxaliplatin and irinotecan), CAPOX (capecitabine and oxaliplatin) or CAPIRI (capecitabine and irinotecan). The use of 5-FU in these combinations, and the addition of targeted therapies, such as a vascular endothelial growth factor (VEGF) inhibitor, vascular endothelial growth factor receptor (VEGFR) inhibitor or an epidermal growth factor receptor (EGFR) inhibitor, has seen the median survival from CRC increase to 24 months (compared to 12 months with 5-FU alone) (Ikoma *et al*, 2017).

The standard of care for 1st-line metastatic patients is either a FOLFOX-based or FOLFIRI-based regimen, usually in combination with the VEGF inhibitor bevacizumab. Following progression, most patients receive 2nd-line FOLFIRI-based or FOLFOX-based regimens, with or without bevacizumab. A Phase III study investigating the sequence of FOLFOX and FOLFIRI regimens as 1st- and 2nd-line therapy for patients with advanced CRC demonstrated no significant difference in median OS between the FOLFIRI followed by FOLFOX6 (20.4 months) and FOLFOX6 followed by FOLFIRI (21.5 months) regimens (Lee & Sun, 2016).

In the 2nd-line, anti-VEGF therapy with bevacizumab was shown to improve outcomes when combined with FOLFOX compared to FOLFOX alone, with significant differences in median OS (12.9 months vs 10.8 months), median progression-free survival (PFS) (7.3 months vs 4.7 months), and objective response rate (ORR) (22.7% vs 8.6%) (Giantonio *et al*, 2007). Furthermore, it has been shown that continuation of bevacizumab in combination with chemotherapy in 2nd-line treatment resulted in improved OS when compared to 2nd-line chemotherapy alone (median OS: 11.2 months vs 9.8 months) (Bennouna *et al*, 2013).

1.3. Limitations of 5-FU Treatment

First introduced in 1957, 5-FU is still widely used for the treatment of many cancers, including colorectal, breast, stomach, head and neck, and pancreatic cancers. Approximately 500,000 patients in North America receive 5-FU each year. Two other drugs have been approved that generate the same active anti-cancer metabolite as 5-FU: fluorodeoxyuridine/floxuridine, marketed as FUDR®, and capecitabine, marketed as Xeloda®. Like 5-FU, both of these drugs are pro-drugs which must be converted to an active anti-cancer metabolite, fluorodeoxyuridine-monophosphate (FUDR-MP) inside the cancer cell in order to exert their primary cytotoxic activity. Capecitabine is a derivative of 5-FU containing a chemical modification of the nucleoside ring, which allows oral dosing.

FUDR-MP binds to and inhibits thymidylate synthase (TS), a critical enzyme in *de novo* nucleotide synthesis and cell survival (Longley *et al,* 2003). TS is required to convert uridine, specifically deoxyuridine monophosphate, or dUMP, to thymidine, specifically deoxythymidine monophosphate, or dTMP, one of the four nucleotides that comprise DNA. The inhibition of TS results in an imbalance in the ratio of the nucleotides dUMP and dTMP, disrupting DNA synthesis and repair, which ultimately leads to cancer cell death. Due to multiple limitations, 5-FU is not efficiently converted to the active anti-cancer metabolite, FUDR-MP, and is often co-administered with LV in an attempt to enhance its binding to TS and subsequent anti-cancer activity.

Key cancer resistance mechanisms impacting breakdown and activation of 5-FU have been associated with a poor prognosis to treatment (Longley *et al,* 2003; Longley & Johnston, 2005).

1.3.1 Susceptibility to Breakdown

More than 85% of administered 5-FU is degraded by the enzyme dihydropyrimidine dehydrogenase (DPD) in the liver, therefore most of the drug is catabolised before it has an opportunity to enter cancer cells, become activated and exert any therapeutic effect (Diasio & Harris, 1989). DPD is also present in other tissues, such as the gastrointestinal tract (Pizzorno *et al,* 2003), and in tumour cells, resulting in additional 5-FU catabolism.

In an analysis of more than 60 human cancer cell lines, a highly significant inverse correlation between DPD expression and activity and response to 5-FU was demonstrated (Beck *et al,* 1994; Scherf *et al,* 2000). The overexpression of DPD in a number of different cancer cells has been shown to confer resistance to 5-FU (Takebe *et al,* 2001). Furthermore, high levels of DPD were associated with a poorer response to 5-FU in tumour xenograft studies (Ishikawa *et al,* 1999).

In patients, DPD activity has been found to be elevated in hepatocellular carcinomas that are inherently resistant to fluoropyrimidine-based therapy (Jiang *et al,* 1997) and DPD levels were found to predict resistance to 5-FU in patients with CRC (Diasio *et al,* 1999; Etienne *et al,* 1995). High transcription of DPD mRNA in colorectal tumours has been shown to correlate with resistance to 5-FU, potentially caused by more DPD-mediated catabolism of 5-FU in these

tumours (Salonga *et al,* 2000). Moreover, a significantly shorter OS (4.9 vs 13.1 months) has been observed among patients with CRC receiving 5-FU treatment whose DPD levels were high compared to patients with low DPD expression (Salonga *et al,* 2000). The catabolism of 5-FU by DPD is subject to marked circadian variations, leading to increased breakdown of 5-FU at certain times throughout the day with consequent reductions in 5-FU efficacy during these times. Clinical protocols have attempted to overcome these challenges by altering the 5-FU infusion rate on a circadian schedule, necessitating an increased infusion rate in the early morning (Pizzorno *et al,* 2003).

In addition to the reduced efficacy, 5-FU catabolism by DPD results in the generation of toxic by products, such as α-fluoro-β-alanine (FBAL), which has been associated with off-target toxicity including hand foot syndrome, which is also known as chemotherapy-induced acral erythema or palmar plantar erythrodysesthesia. This well-established cutaneous toxicity occurs in 25-75% of patients treated with 5-FU (Meta Analysis Group in Cancer, 1998; Hansen *et al,* 1996; Chiara *et al*, 1997; Chau *et al,* 2005; Kwakman *et al*, 2020) and is characterised by erythema, dysesthesia, pain, cracking, and desquamation on the palms of the hands and the soles of the feet (Nikolaou *et al,* 2016). Hand foot syndrome can be debilitating and significantly affects quality of life, often necessitating 5-FU dose modifications or treatment discontinuation. Additional off-target toxicities associated with 5-FU by-products (predominantly FBAL and its breakdown products fluoroacetate and F-citrate) include cardiotoxicity and neurotoxicity, both of which are known to be dose limiting (Yamashita *et al,* 2004; Jensen & Sorensen, 2006; Deboever *et al,* 2013). Moreover, there is evidence indicating that these 5-FU catabolites interfere with the anti-cancer efficacy of 5-FU (Spector *et al*, 1995). Therefore, circumventing DPD-mediated degradation is anticipated to significantly improve the safety and efficacy of fluoropyrimidines.

Although high levels of DPD have a clear impact on efficacy and safety, 5-FU and related agents are also associated with risk of severe or fatal toxicities in patients who are DPD-deficient. Partial DPD deficiency is carried in an estimated 2-5% of the Caucasian and Asian populations, and in 8% of African-Americans. These patients are unable to effectively breakdown 5-FU through the catabolic DPD pathway, leading to prolonged exposure to toxic levels of 5-FU metabolites with resultant adverse events (AEs), including mucositis, granulocytopaenia, neuropathy, and even death (Baek *et al,* 2006). Techniques are available for the detection of this autosomal dominant condition (Mattison *et al,* 2004; Innocenti, 2014; Henricks *et al*, 2018) and testing is becoming more widely used in clinical practice.

5-FU is also phosphorylated to 5-fluorouridine triphosphate (5-FUTP), which is known to be partially responsible for 5-FU-associated off-target toxicity through incorporation into RNA in healthy cells (Brutcher *et al*, 2018). Indeed, 5-FUTP is thought to be the primary mediator underlying diarrhoea, a key dose-limiting toxicity (DLT), in patients treated with 5-FU (Adjei, 1999). Fluoropyrimidines are also susceptible to enzymatic hydrolysis by thymidine phosphorylase (TP), which is commonly overexpressed in tumours (Longley *et al,* 2003; Bronckaers *et al*, 2009) or introduced by mycoplasma infection (Huang *et al*, 2001) and interferes with chemo-sensitivity (Jetté *et al*, 2008).

Overall, the by-products FBAL and 5-FUTP produced during metabolism of 5-FU are associated with significant off-target toxicities that limit the clinical utility of 5-FU.

1.3.2 Requirement of Activation

5-FU needs to be processed by a series of enzymes in cancer cells to generate the primary active anti-cancer metabolite, FUDR-MP, which binds to and inhibits TS. The inhibition of TS results in an imbalance in the ratio of the nucleotides dUMP and dTMP, disrupting DNA synthesis and repair, which ultimately leads to cancer cell death (Longley *et al,* 2003).

The levels of two key enzymes in the 5-FU activation pathway have been shown to correlate with treatment efficacy. Orotate phosphoribosyltransferase (OPRT) converts 5-FU to fluorouridine monophosphate, an intermediate in the formation of FUDR-MP. Low levels of OPRT in tumour cells is associated with resistance to 5-FU (Tsutani *et al*, 2008). In patients with CRC receiving 5-FU based chemotherapy, low levels of OPRT is associated with significantly worse OS rates (Komori *et al,* 2013). In addition, TP converts 5-FU to FUDR, which is then further processed to FUDR-MP by thymidine kinase (TK). It has been shown that TK deficiency in human cancer cells diminishes the activity of 5-FU (Vande Voorde *et al,* 2011). It has been observed that expression of TP correlates with response to 5-FU therapy (Panczyk, 2014). Higher TP levels theoretically should correlate to greater sensitivity to 5-FU due to increase in the concentration of FUDR-MP. However, studies have shown mixed results regarding response to 5-FU-based chemotherapy and level of TP expression. Low TP expression in one study correlated with improved OS in patients with metastatic CRC treated with adjuvant 5-FU (Soong *et al*, 2008; Salonga *et al*, 2000). A more recent study in patients with mCRC demonstrated longer time to progression with high TP expression (Lindskog *et al*, 2014). Further studies are needed to establish a definitive link between TP expression and 5-FU sensitivity.

1.3.3 Dosing Administration Challenges

5-FU is associated with significant dosing administration challenges due to poor pharmacokinetic (PK) properties. The plasma half-life ($t_{1/2}$) of 5-FU is very short at 8 to 14 minutes; consequently, prolonged infusion times over 46 hours are required to allow uptake and activation of the pro-drug to generate therapeutic levels of FUDR-MP, whilst also attempting to minimise maximum concentration (C_{max}) levels of toxic by-products. This dosing regimen and the substantial off-target toxicities are burdensome for providers, inconveniences patients, and contributes additional costs to the healthcare system.

1.4. NUC-3373

NUC-3373 is specifically designed to overcome the key resistance mechanisms that limit the clinical utility of 5-FU, thereby potentially improving the efficacy and safety profile, whilst also reducing the dosing administration burdens associated with 5-FU (McGuigan *et al,* 2011; Vande Voorde *et al*, 2011). NUC-3373 generates the same active intracellular anti-cancer metabolite, FUDR-MP, as 5-FU, but at significantly higher concentrations.

NUC-3373 is a pre-activated and protected form of FUDR-MP. The addition of the protective phosphoramidate group changes the structural characteristics of NUC-3373 such that it is not a substrate for, and is therefore resistant to enzymatic breakdown by, DPD. This confers the advantage of reduced exposure to by-products and associated off-target toxicities, such as hand foot syndrome.

In addition, NUC-3373 generates much lower levels of FUTP compared to 5-FU, and the incidence of Grade 3 or higher toxicities associated with off-target incorporation of this molecule into the RNA of normal cells has been low in clinical studies to date.

The chemical structure also alters the lipophilicity of the molecule, enabling NUC-3373 to enter cancer cells without the need for nucleobase transporters.

Finally, as NUC-3373 contains the active anti-cancer metabolite, there is no need for enzymatic conversion to FUDR and subsequent phosphorylation, thus overcoming major rate limiting cancer resistance pathways associated with 5-FU.

Together, these unique properties are expected to result in enhanced drug systemic exposure and a reduction in the release of toxic by-products. Once inside the cancer cell, the protective group is cleaved off with release of significantly higher levels of the active anti-cancer metabolite FUDR-MP. This results in enhanced interaction with, and inhibition of, the target enzyme TS, driving an imbalance in the dUMP:dTMP ratio with subsequent disruption of DNA synthesis and repair and, ultimately, cancer cell death.

Refer to the NUC-3373 IB for current information on mechanisms of action as well as the latest non clinical and clinical data.

1.4.1 Non Clinical Data

NUC-3373 has consistently demonstrated greater activity than 5-FU across a range of non-clinical studies.

- *In vitro* cytotoxic activity of NUC-3373 and 5-FU was examined across a range of human tumour cell lines, including the colorectal cell lines HT29, SW620 and Colo205. The half-maximal effective concentration (EC_{50}) data from the majority of cell lines tested demonstrated that NUC-3373 has up to 330-times greater activity than 5-FU.
- The ability of NUC-3373, FUDR and 5-FU to generate the active intracellular metabolite, FUDR-MP, was examined in HT29 human colorectal cells. NUC-3373 and 5-FU were added to HT29 cells at a concentration of 50 µM for 24 hours. FUDR-MP was quantified using ultra-performance liquid chromatography coupled to tandem mass spectrometry. NUC-3373 generated 366-times the amount of intracellular FUDR-MP compared with 5-FU.
- Over half of CRC cases are infected by *mycoplasma* (Huang *et al*, 2001). *Mycoplasma*-encoded TP reduces the activity of several chemotherapeutic agents (Liekens *et al*, 2009), including 5-FU. In cancer cell lines, *mycoplasma* infection decreases 5-FU activity by up to 100-times (Jetté *et el*, 2008). Non clinical studies have shown that NUC-3373 is resistant to TP-mediated degradation, whereas FUDR is significantly broken down, and that in human glioblastoma U87 cells chronically infected by *mycoplasma hyorhinis*, FUDR markedly lost its cytotoxic activity 429-fold, whereas NUC-3373 activity was unaffected (Vande Voorde *et al*, 2011).
- An *in vitro* study investigating the effect of DPD activity on the intracellular concentrations of NUC-3373 was conducted in cell lysate from pooled CRC cell lines (SW620, HCT116 and HT29). NUC-3373 was not susceptible to DPD-mediated degradation unlike 5-FU, which was rapidly catabolised by DPD.
- The cytotoxicity of NUC-3373 and FUDR were compared in CEM wild type and TK-deficient CEM human leukaemia cells (McGuigan *et al*, 2011). NUC-3373 activity was largely independent of TK with less than a 5-fold decrease in EC_{50} values in TK-deficient cells, whereas FUDR loses activity was decreased by more than 136-fold.

In xenograft studies examining the effect of NUC-3373 and 5-FU on the growth of HT29 human colorectal tumour cell line, NUC-3373 showed significant reduction in tumour volume compared to 5-FU. NUC-3373 showed a statistically significant increase in tumour doubling time when compared with the control group, whereas 5-FU did not.

A formal good laboratory practice repeat dose study has been conducted to assess the toxicity and toxicokinetic profile of NUC-3373 in the Beagle dog. NUC-3373 was administered intravenously (IV) to dogs at 1, 4 and 8 mg/kg/day once daily for 5 days/week for 4 consecutive weeks. Doses greater than 4 mg/kg/day induced weight loss as the most significant side effect as well as histopathological changes in testes, epididymides and gall bladder. These findings showed full reversibility after a 2-week treatment-free period, except for testes and epididymides that were recovering but were thought to take longer to fully recover.

Based on the results of this study, the highest non-severely toxic dose was considered to be 8 mg/kg/day. These results compare favourably with the reported toxicity of 5-FU in Beagle dogs following single bolus IV administration (Sayre *et al*, 2012)

More details on the toxicology, pharmacology and toxicokinetics can be found in the NUC-3373 Investigator's Brochure (IB).

1.5. Clinical Data

NuTide:301 was a first-in-human dose escalation study in patients with advanced solid tumours (EudraCT 2015-002250-13, NCT02723240) which enrolled patients across three investigative sites in the UK. The study had a two-part dose escalation and expansion design which evaluated safety, PK, pharmacodynamic (PD) and anti-tumour activity, in addition to establishing the maximum tolerated dose (MTD), recommended Phase II dose (RP2D) and schedule of single-agent IV NUC-3373.

Enrolment has completed and the final results are being collated and analysed. In Part 1, NUC-3373 was administered on a weekly (Q1W) schedule on Days 1, 8, 15 and 22 of a 28-day cycle. In Part 2, NUC-3373 was administered as a fortnightly (Q2W) infusion on Days 1 and 15 of a 28-day cycle.

A total of 59 patients received NUC-3373 in the study. The dosing schedule is summarised in Table 1.

All patients received NUC-3373 by IV infusion over time periods ranging from 30 minutes to 4 hours. Considering that NUC-3373 is 5-times the equimolar weight of 5-FU, the starting dose of 125 mg/m^2 NUC-3373 equates to approximately 25 mg/m^2 5-FU; thus, micro-doses of NUC-3373 were given in the early stages of the study.

This heavily pre-treated patient population (median of 3 prior lines of chemotherapy; range 0-11 lines) had a variety of primary tumour types, with CRC representing approximately half of the cases.

Promising signs of activity have been observed, with at least 10 patients staying on study treatment for at least 4 months and 3 of these patients achieving prolonged stable disease (SD) lasting more than nine months. A 70-year-old male patient with CRC had received 6 prior lines of therapy, including 5 prior lines of 5-FU-based therapy (progressed within 2 months on 3rd-line treatment with CapOx, progressed within 8 months on 4th-line treatment with FOLFIRI, progressed within 3 months on 5th-line treatment with trifluridine-tipiracil). This patient received 1500 mg/m^2 NUC-3373 Q1W and achieved SD for 9 months before choosing to suspend treatment to go on vacation.

A 60-year-old female patient with cholangiocarcinoma who had progressed within 6 months of her 1st-line of therapy (gemcitabine in combination with cisplatin) achieved SD for 11 months during treatment with 1125 mg/m^2 NUC-3373 Q1W.

A 55-year-old male patient with basal cell carcinoma had received 2 prior lines of therapy and had progressed within 3 months of the most previous treatment (paclitaxel in combination with carboplatin). This patient received NUC-3373 at 1500 mg/m^2 every 2 weeks and achieved SD for 10 months.

Table 1 NuTide:301 dose escalation, treatment schedule, and enrolment

Group	Dosing Schedule	Number of Patients
Cohort 1	NUC-3373 IV infusion **125 mg/m^2** *On days 1, 8, 15, 22 of a 28-day cycle*	3
Cohort 2	NUC-3373 IV infusion **250 mg/m^2** *On days 1, 8, 15, 22 of a 28-day cycle*	6
Cohort 3	NUC-3373 IV infusion **500 mg/m^2** *On days 1, 8, 15, 22 of a 28-day cycle*	8
Cohort 4	NUC-3373 IV infusion **750 mg/m^2** *On days 1, 8, 15, 22 of a 28-day cycle*	4
Cohort 5a	NUC-3373 IV infusion **1125 mg/m^2** *On days 1, 8, 15, 22 of a 28-day cycle*	3
Cohort 6a	NUC-3373 IV infusion **1500 mg/m^2** *On days 1, 8, 15, 22 of a 28-day cycle*	3
Cohort 5b	NUC-3373 IV infusion **1500 mg/m^2** *On days 1 and 15 of a 28-day cycle*	4
Cohort 6b	NUC-3373 IV infusion **1875 mg/m^2** *On days 1 and 15 of a 28-day cycle*	6
Cohort 7a	NUC-3373 IV infusion **1875 mg/m^2** *On days 1, 8, 15, 22 of a 28-day cycle*	6
Cohort 7b	NUC-3373 IV infusion **2500 mg/m^2** *On days 1 and 15 of a 28-day cycle*	6
Cohort 8a	NUC-3373 IV infusion **2500 mg/m^2** *On Days 1, 8, 15, 22 of a 28-day cycle*	6
Cohort 9a	NUC-3373 IV infusion **3250 mg/m^2** *On Days 1, 8, 15, 22 of a 28-day cycle*	**4**
TOTAL		**59**

NUC-3373 was well tolerated at doses up to and including 2500 mg/m^2. The most common treatment-emergent adverse events (TEAEs) considered related to NUC-3373 were fatigue (46%), nausea (36%), diarrhoea (31%), infusion reactions (most commonly described as a feeling of flushing; 29%), transaminases increased (19%), vomiting (15%), and anaemia (15%). The majority of events were Grade 1/2, with Grade 3 treatment-related events reported in only 7 patients (4 patients with transaminases increased and 1 patient each with fatigue, shingles and hypotension). None of the reported events appeared to be dose-related.

Twenty-nine serious adverse events (SAEs) were reported in 22 patients. Most of these SAEs resolved and only two were considered by the Investigator to be unexpected. Six of the SAEs were judged by the Investigator to be definitely, probably or possibly related to NUC-3373 (chest pain, transaminases increased, infusion reaction, shingles, PRES and pyrexia).

Four patients experienced DLTs; 1 out of 8 patients in the 500 mg/m^2 Q1W cohort, 1 out of 6 patients in the 1875 mg/m^2 Q1W cohort and 2 out of 4 patients in the 3250 mg/m^2 Q1W cohort. In the 500 mg/m^2 and 1875 mg/m^2 Q1W cohort, DLTs of Grade 3 transaminases increased were reported. In the 3250 mg/m^2 Q1W cohort, DLTs of Grade 2 headache and Grade 3 transient hypotension (decreased blood pressure for <5 minutes) were reported. Based on this, the MTD for single-agent NUC-3373 was determined to be 2500 mg/m^2 Q1W.

1.6. Pharmacokinetics

Plasma and intracellular (PBMC) PK analyses have been conducted on 45 patients recruited to the NuTide:301 (range: 125 to 3250 mg/m^2) and NuTide:302 (1500 mg/m^2) studies. The PK profile of NUC-3373 compares favourably to that of 5-FU.

1.6.1 Plasma Levels

A fully validated bioanalytical liquid chromatography mass spectrometry method was used to calculate PK parameters for NUC-3373 and its metabolites, FUDR and FBAL, over a dose range of 500-2500 mg/m^2. A dose-proportional increase in the NUC-3373 AUC was observed over the dose range. Over the range studied, NUC-3373 had a mean elimination half-life of 5-10 hours (vs 8-14 minutes for 5-FU). The volume of distribution and the clearance of NUC-3373 at the 1500 mg/m^2 dose (n=20) was 198 L and 28.5 L/h, respectively.

The mean C_{max} and AUC values for NUC-3373 at the MTD (2500 mg/m^2) were found to be 41.7 µg/mL and 222.2 µg•h/mL. C_{max} and AUC values for FUDR and FBAL were 0.3 µg/mL and 1.3 µg•h/mL and 3.4 µg/mL and 34.1 µg•h/mL, respectively. No accumulation was observed for NUC-3373 or its circulating metabolites (FUDR and FBAL) between Day 1 and Day 15. ~~M~~.

1.6.2 Intracellular Levels

A dose-proportional increase in the intracellular AUC for the active anti-cancer metabolite FUDR-MP was observed over a dose range of 125-2500 mg/m^2 in PBMCs. The C_{max} and T_{max} of intracellular FUDR-MP was influenced by the time of infusion. In the 1500 mg/m^2 cohort, the mean intracellular C_{max} and AUC_{0-24} of FUDR-MP were 11.7 pmol/million cells and 95.6 pmol/million cells/h, respectively. In the 2500 mg/m^2 cohort, the mean intracellular C_{max} and AUC_{0-24} of FUDR-MP were 23.0 pmol/million cells and 135.5 pmol/million cells/h, respectively. The half-life of intracellular FUDR-MP was calculated to be 10-14 hours. A positive linear relationship was observed between intracellular FUDR-MP and dUMP levels. The toxic metabolites FBAL and FUTP were undetectable intracellularly at the dose levels tested.~~I~~.

1.7. Study Rationale

NUC-3373 is a pre-activated (monophosphorylated) and protected form of the active anti-cancer agent FUDR-MP. The addition of the phosphoramidate group to the FUDR-MP protects NUC-3373 against inactivation by enzymatic breakdown, resulting in greater stability, and reduces the release of potentially toxic metabolites.

NUC-3373 is lipophilic and can enter cancer cells without the need for nucleobase transporters. It is synthesised as the monophosphate and, as such, has already by-passed the activation steps

dependent on the enzymes OPRT and TK. NUC-3373 is resistant to degradation by DPD and to intracellular metabolic breakdown by TP. As a result, NUC-3373 is anticipated to rapidly generate high concentrations of the active metabolite FUDR-MP intracellularly and efficiently inhibit TS to confer therapeutic benefits.

NUC-3373 has shown the ability to generate high levels of FUDR-MP and markedly inhibit tumour cell growth in non clinical studies. In particular, the degree of tumour regression with NUC-3373 is greater than with 5-FU in human CRC xenograft models. Toxicology results compare favourably with the reported toxicity of 5-FU in Beagle dogs following single bolus IV administration (Sayre *et al,* 2012). The first-in-human Phase I clinical study, NuTide:301, has confirmed the potential for NUC-3373 to generate high intracellular levels of the active agent FUDR-MP. NUC-3373 has been well-tolerated in 59 patients at doses up to and including 2500 mg/m^2 and has shown encouraging signs of anti-cancer activity.

Together these data demonstrate the potential for NUC-3373 to overcome the known resistance mechanisms associated with 5-FU and potentially to offer a more effective treatment option for patients with CRC.

The addition of LV potentiates the anti-cancer activity of infusional 5-FU by prolonging the intracellular half-life and increasing intracellular levels of FUDR-MP, as well as stabilising the complex it makes with TS. Because it improves the clinical efficacy of 5-FU, LV has become an integral part of infusional 5-FU based regimens.

Additionally, 5-FU is commonly used in combination with oxaliplatin (comprising the FOLFOX regimen) and irinotecan (comprising the FOLFIRI regimen); these regimens are often combined with agents that target the VEGF (*e.g.,* bevacizumab) and EGFR (*e.g.,* cetuximab) pathways. In order to maximise clinical benefit for patients, it is important to study NUC-3373 in combination with these agents and regimens that are routinely used in, and considered standard of care for, patients with advanced CRC. Thus, the goal for Part 1 of this study is to evaluate the safety and benefit of combining LV with NUC-3373. Parts 2 and 3 of this study will then be undertaken to establish safe and effective regimens of NUC-3373 combinations with the long-term goal of improving upon the activity of 5-FU and establishing more effective treatments for patients with CRC.

Patients who are considered ineligible for combination chemotherapy regimens and patients who have rapidly progressed on prior fluoropyrimidine-based therapies also have limited treatment options. Combination chemotherapy ineligible patients are generally frail with a poorer performance status and, therefore, require more tolerable therapies. Given the favourable risk/benefit profile observed with NUC-3373, these patients could be good candidates for treatment with NUC-3373 + LV. Rapid progressors are unlikely to receive benefit from treatment with further lines of 5-FU based regimens. On the other hand, owing to its advantageous PK profile and ability to generate much higher levels of the active anti-cancer metabolite, NUC-3373 may overcome 5-FU resistance mechanisms. Indeed, NUC-3373 has shown anti-cancer activity in patients who have relapsed on, or are refractory to, prior treatment with a fluoropyrimidine, thus offering a potential treatment option for these patients.

The current standard of care for the 1st-line and 2nd-line treatment of patients with advanced or metastatic CRC is FOLFOX, FOLFIRI or FOLFOXIRI in combination with VEGF inhibitors or EGFR inhibitors. During the patient's treatment journey, components of the 1st-line regimen (*e.g.,* oxaliplatin or irinotecan) will be either paused and re-instated, or switched to introduce a new combination agent, for the 2nd-line regimen. Some patients may receive maintenance therapy with a fluoropyrimidine ± bevacizumab. Data in the 2nd-line setting show that following irinotecan-based 1st-line treatment (*e.g.,* FOLFIRI), FOLFOX is generally the best

option, and the combination of FOLFOX plus bevacizumab appears to be superior to FOLFOX alone. Alternatively, following FOLFOX failure in the 1st-line, irinotecan and FOLFIRI are the most appropriate options for 2nd-line treatment. Interim data from Part 1 of NuTide:302 show that NUC-3373 has promising anti-tumour activity, including tumour shrinkage and encouraging rates of prolonged disease stabilisation beyond 3 months (11/38 patients) in heavily pre-treated patients (3-11 prior lines of treatment for metastatic disease) in whom disease stabilisation is difficult to achieve. Furthermore, NUC-3373 has a favourable tolerability profile compared to either 5-FU or capecitabine (Table 2) and offers an attractive administration schedule with a short (2-4 hours) Q1W or Q2W infusion, instead of the prolonged infusion (46 hours) required for 5-FU. These data suggest that NUC-3373 might be an attractive option to replace 5-FU in the current standard of care FOLFOX and FOLFIRI regimens in the 2nd-line setting. Addition of targeted agents to these combinations offers an additional potential advantage for tumour control in this setting, justifying the evaluation of NUFOX and NUFIRI regimens in combination with bevacizumab or cetuximab in NuTide:302.

Table 2 NUC-3373 safety profile (Part 1 of NuTide:302)

	NUC-3373 (n=38)		5-FU IV (n=143)		5-FU Bolus (n=593)		Capecitabine (n=596)	
	All Grades (%)	G3 or G4 (%)	All Grades (%)	G3 or G4 (%)	All Grades (%)	G3 or G4 (%)	All Grades (%)	G3 or G4 (%)
Neutropenia	**0**	**0**	**48**	**13**	**46**	**21**	**13**	**3**
Anemia	18	5	91	2	79	2	80	3
Diarrhea	**32**	**0**	**45**	**6**	**61**	**12**	**55**	**15**
Nausea	45	5	55	4	51	4	43	4
Vomiting	42	0	32	3	30	5	27	5
Mucositis/Stomatitis	**11**	**0**	**29**	**3**	**62**	**15**	**25**	**3**
Hand-foot syndrome	**0**	**0**	**13**	**1**	**6**	**1**	**54**	**17**
Dermatitis	11	0	20	0	26	1	27	1
Fatigue/lethargy	47	5	NR	NR	46	4	42	4
Elevated bilirubin	11	5	36	11	17	6	48	23
	Heavily pre-treated patients NUC-3373/LV q1w or q2w		First-line patients 5-FU/LV infusional days 1&2, q2w		First-line patients 5-FU/LV bolus days 1-5, q4w		First-line patients Capecitabine BID, 2wks on, 1wk off	

Berlin *et al* (2021). ESMO poster 475, 16-21 Sep 20210

Depending on response to combination therapies in the 1st-line metastatic setting in CRC, maintenance therapies offer patients a break from intensive combination treatments while maintaining tumour control. The goal of maintenance therapy is to maximise quality of life (QoL) by minimising toxicities and delivering superior efficacy in order to delay the initiation of a new intensive combination regimen. Based on the favourable safety and tolerability profile observed to date, and the more convenient administration schedule, NUC-3373 may offer a good maintenance therapy option for patients. Bevacizumab as single agent or in combination with a fluoropyrimidine has shown good efficacy in this setting (single agent activity of disease stabilisation between 5-10 months, depending on the study; Ma *et al*, 2019); thus, the combination of NUC-3373 and bevacizumab could provide an additional option for delaying the initiation of 2nd-line therapy.

2. STUDY OBJECTIVES AND ENDPOINTS

This study consists of two phases (Phase Ib and Phase II) and includes three study parts (Parts 1, 2 and 3) with several treatment arms within each part. Phase Ib includes Part 1 (Arms 1a-1d) and Part 2 (Arms 2a-2d) and Phase II includes Part 3 (Arms 3a-3g).

2.1. Primary Objectives

Phase Ib

The primary objective is to identify a recommended dose and schedule for NUC-3373 when combined with other agents commonly used in treatment of advanced CRC, including:

- LV
- oxaliplatin
- oxaliplatin and VEGF pathway inhibitors (bevacizumab)
- oxaliplatin and EGFR inhibitors (cetuximab or panitumumab)
- irinotecan
- irinotecan and VEGF pathway inhibitors (bevacizumab)
- irinotecan and EGFR inhibitors (cetuximab or panitumumab)

Phase II

To explore the anti-cancer activity of NUC-3373 when combined with other agents commonly used in the treatment of advanced CRC using Response Evaluation in Solid Tumours (RECIST) v1.1 criteria.

2.2. Secondary Objectives

The secondary objectives are:

- To assess the safety and tolerability of each NUC-3373-containing regimen (Phase Ib and Phase II)
- To assess the PK of NUC-3373, oxaliplatin, irinotecan and their metabolites in each NUC-3373-containig regimen (Phase Ib and Phase II)
- To make a preliminary assessment of the anti-tumour activity of NUC-3373 alone and in each NUC-3373-containig regimen (Phase Ib only)
- To conduct a within-patient and between-patient analysis of the effect of LV when added to NUC-3373 on intracellular PD parameters and haematological parameters and use this information to determine if the addition of LV to NUC-3373-containing regimens is required for clinical activity (Part 1 Arm 1a and Arm 1b only)

2.3. Exploratory Objectives

- To explore predictive biomarkers of biological activity and possible relationships between PK and PD effects (Phase Ib and Phase II)
- Depending on emerging data, to potentially conduct a within-patient analysis of the effect of combining NUC-3373 with irinotecan on the plasma PK parameters of both NUC-3373 and irinotecan (Part 2 Arm 2b sub-study only)

2.4. Primary Endpoints

Phase Ib

To identify a recommended dose and schedule for NUC-3373 when combined with LV and other agents routinely used in the treatment of patients with advanced CRC

Phase 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:

- Percentage change from baseline in tumour size
- Objective Response Rate (ORR)
- Disease Control Rate (DCR)
- Duration of Response (DoR)
- Duration of Stable Disease (DoSD)
- Progression-Free Survival (PFS)
- Overall Survival (OS) rate at 6 and 12 months

2.5. Secondary Endpoints

2.5.1 **Safety** (Phase Ib and 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 electrocardiograms (ECGs)

2.5.2 **Pharmacokinetics** (Phase Ib and Phase II)

The PK of NUC-3373, oxaliplatin, irinotecan and their metabolites will be assessed, including:

- Concentration at end of NUC-3373 infusion (C_{inf})
- Maximum concentration (C_{max})
- AUC
- $t_{1/2}$
- Volume of distribution (V_d)
- Clearance (CL)

The analytes measured will include, but are not limited to:

- In plasma: NUC-3373, oxaliplatin, irinotecan and their metabolites
- In peripheral blood mononuclear cells (PBMCs): NUC-3373, FUDR-MP, FBAL, 5-FUTP, dTMP, dUMP (Part 1 only)

2.5.3 **Efficacy Endpoints** (Phase Ib)

Objective disease assessment by radiographic imaging will be performed every 8 weeks and analysed using Response Evaluation Criteria in Solid Tumours (RECIST) v1.1 criteria (Appendix 1). Anti-tumour activity will be assessed on the basis of:

- Percentage change from baseline in tumour size
- ORR
- DCR
- DoR
- DoSD
- PFS
- OS rate at 6 and 12 months

2.6. **Exploratory Endpoints** (Phase Ib and Phase II)

- Pre-treatment and on-treatment measurements of PD markers in PMBCs (Part 1 only)
- Exploration of predictive biomarkers such as gene expression and/or genetic alternations in blood and tumour (e.g., genetic alterations in genes involved in PK exposure, PD effects, safety and efficacy)
- DNA/RNA and protein analysis of pre- and on-treatment tumour samples, including but not limited to KRAS and BRAF
- Potential sub-study to evaluate the PK relationship between NUC-3373, LV and irinotecan (Part 2 Arm 2b sub-study only)

  The PK of NUC-3373, LV and irinotecan will be assessed, including $C_{inf,}$ $C_{max,}$ AUC, $t_{1/2,}$ $V_{d,}$ CL

  The analytes measured in plasma will include, but are not limited to:
  - NUC-3373, 5-FU, and FBAL
  - LV and 5-methyl-THF
  - Irinotecan, SN-38, SN-38 glucuronide, APC and NPC

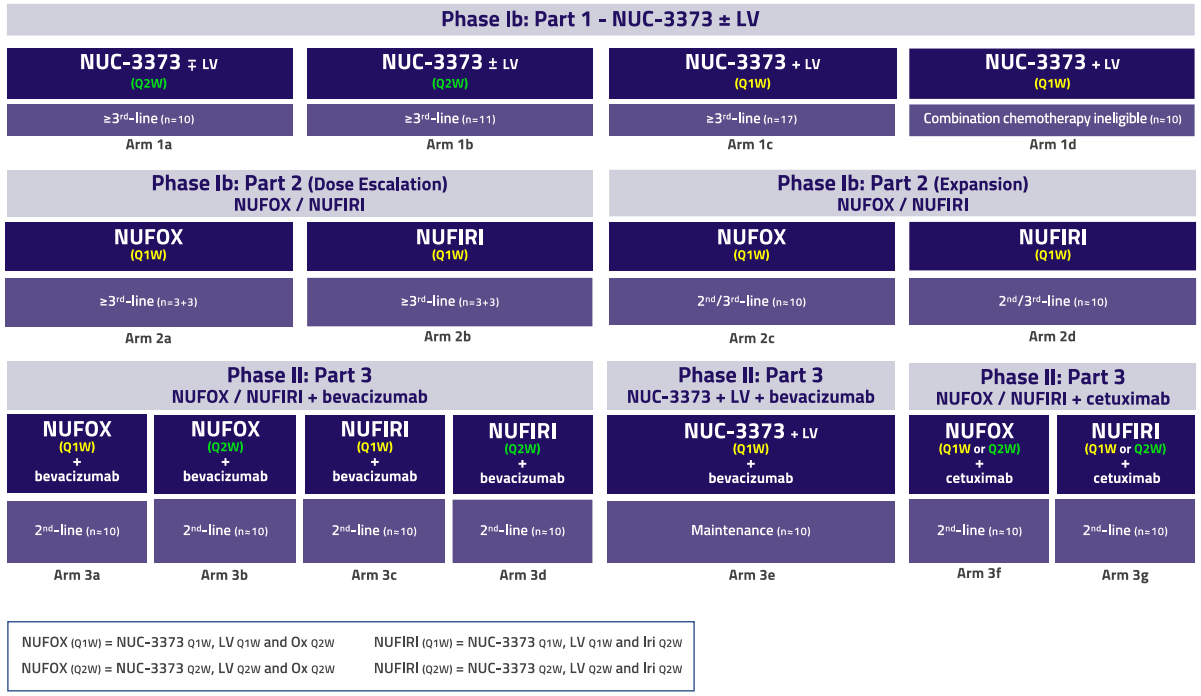
3. INVESTIGATIONAL PLAN

3.1. Overall Study Design

This is a three-part, Phase Ib/II study of NUC-3373 administered by IV infusion (Q1W or Q2W), either as monotherapy or as part of various combinations with LV, oxaliplatin, irinotecan, VEGF inhibitors and EGFR inhibitors. (Figure 1). Phase Ib includes Part 1 (Arms 1a-1d) and Part 2 (Arms 2a-2d) and Phase II includes Part 3 (Arms 3a-3g). The opening and timing of study arms will depend on emerging data.

For VEGF inhibitors, the preferred treatment option is Avastin; however, biosimilars may be used after discussion with the Sponsor. For EGFR inhibitors, the preferred treatment options are Erbitux (cetuximab) or Vectibix (panitumumab); however, if biosimilars become available, their use may be discussed with the Sponsor.

Patients may continue treatment until radiological disease progression or unacceptable toxicity despite optimal medical management or dose or schedule modification, 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 has been reached (defined in Section 3.6), whichever occurs first.



Abbreviations: Iri=irinotecan; LV=leucovorin; NUFIRI=NUC-3373 + LV + irinotecan; NUFOX=NUC-3373 + LV + oxaliplatin; Ox=oxaliplatin; Q1W=weekly; Q2W=fortnightly

Figure 1 NuTide:302 study schema

Part 1 determined that NUC-3373 should be administered with LV (Arms 1a, 1b and 1c) and will further assess the safety and tolerability of NUC-3373 + LV on a Q1W schedule (Arm 1d).

Part 2 will assess the safety and tolerability of different doses of Q1W NUC-3373 + LV when administered in combination with either oxaliplatin (NUFOX) or irinotecan (NUFIRI).

Part 3 will assess the safety and efficacy of Q1W NUFOX and NUFIRI regimens administered in combination with bevacizumab (Q2W arms may also be opened). NUFOX and NUFIRI regimens may also be administered in combination with cetuximab or panitumumab, depending on emerging data. In addition, Q1W NUC-3373 + LV in combination with bevacizumab may be assessed in patients qualifying for maintenance therapy.

A Q2W schedule may be explored at any time, depending on emergent safety, efficacy and PK data.

It is anticipated that approximately 215 patients with advanced or metastatic radiologically-measurable CRC may be required in this study. Additional patients may be enrolled in all parts of the study to replace patients who withdraw prior to completing Cycle 0 (where relevant) and the initial 28-day safety evaluation period in Cycle 1. Additionally, if the Data Safety Monitoring Committee (DSMC) determine that an increased number of patients are required to evaluate alternate doses within a study part, enrolment in a cohort may be expanded. If positive efficacy signals are observed in any of the patient populations included in Parts 1, 2 or 3 of the study, expansion cohorts may be opened to further assess efficacy in approximately 20 patients per cohort.

The patient populations outlined in Table 3 will be included in the study.

Table 3 Patient populations

Patient Population	Criteria	Study Phase/Part
≥3rd-line patients	Received ≥2 prior lines of therapy for locally advanced or metastatic disease, one must include a fluoropyrimidine plus oxaliplatin, the other must include a fluoropyrimidine plus irinotecan.	Phase Ib Part 1 Part 2 (dose escalation)
Combination chemotherapy ineligible patients	Considered unable to receive combination chemotherapy for locally advanced or metastatic disease and may have received 1 prior line of fluoropyrimidine-containing therapy.	Phase Ib Part 1
2nd-/3rd-line patients	Received at least 1, but no more than 2, prior lines of fluoropyrimidine-containing therapy combined with oxaliplatin and/or irinotecan for locally advanced or metastatic disease. 3rd-line patients enrolled to Arms 2c and 2d should have received prior bevacizumab treatment, unless ineligible or unless bevacizumab was not standard of care according to relevant region-specific treatment recommendations.	Phase Ib Part 2 (expansion)
Rapid progressors (on prior fluoropyrimidine therapy)	Received ≤2 prior lines of fluoropyrimidine-containing therapy combined with oxaliplatin and/or irinotecan for locally advanced or metastatic disease and progressed ≤3 months of starting the last fluoropyrimidine-containing regimen.	To be determined. Cohorts of rapid progressors may be opened

		depending on emerging data
2nd-line patients	Received 1 prior line of fluoropyrimidine-containing therapy combined with oxaliplatin and/or irinotecan for locally advanced or metastatic disease.	Phase II Part 3
Maintenance patients	Received ≥12 weeks of 1st-line standard of care therapy for locally advanced or metastatic disease, achieved at least stable disease and are eligible for maintenance therapy.	Phase II Part 3
Previous treatment with triplet chemotherapy-based regimens is permitted (*i.e.,* FOLFOXIRI), as long as any toxicities from the previous regimen still allow repeated treatment with oxaliplatin or irinotecan.		

3.1.1 Part 1

Part 1 determined that NUC-3373 should be administered with LV (Arms 1a, 1b and 1c) and will further assess the safety and tolerability of NUC-3373 + LV (Arm 1d) in patients with advanced or metastatic CRC (Figure 2). Initially, ≥3rd-line patients were randomised via block randomisation to two parallel arms, each receiving NUC-3373 Q2W either with LV (Arm 1a) or without LV (Arm 1b). Upon completion of the Q2W dose administration arms, a third arm of ≥3rd-line patients was enrolled to a Q1W administration schedule of NUC-3373 plus LV (Arm 1c). Arms 1a, 1b, and 1c are now complete. Following this, approximately 10 combination chemotherapy ineligible patients will be enrolled to a Q1W administration schedule of NUC-3373 plus LV (Arm 1d).

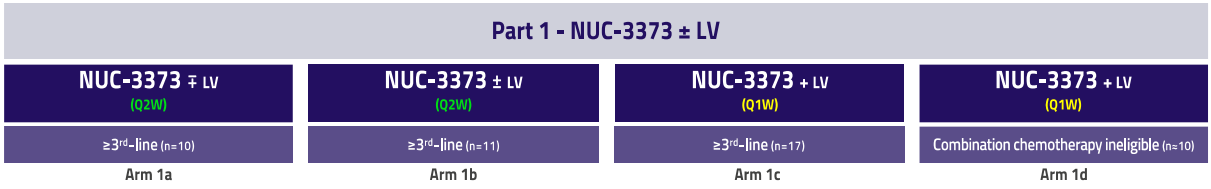


Figure 2 NuTide:302 Part 1 study schema

In Arm 1a, an initial arm of up to 6 evaluable patients were to receive a single dose of NUC-3373 at 1500 mg/m^2. This visit was considered Cycle 0, Day 1 (C0D1) for the purposes of the study visits. The initial dose was followed by a 2-week washout period, during which safety and PK/PD were monitored. The next dose of NUC-3373 was given in combination with LV at 400 mg/m^2. This visit was considered Cycle 1, Day 1 (C1D1) for the purposes of the study visits. All subsequent doses of NUC-3373 were given Q2W in combination with LV in 28-day cycles. A total of 10 patients were finally enrolled in Arm 1a to obtain 6 evaluable patients.

In Arm 1b, an initial arm of up to 6 evaluable patients were to receive a single dose of NUC-3373 at 1500 mg/m^2 in combination with LV at 400 mg/m^2. This visit was considered C0D1 for the purposes of the study visits. The initial combination dose was followed by a 2-week washout period during which safety and PK/PD were monitored. The next dose of NUC-3373 was given without any LV. This visit was considered C1D1 for the purposes of the study visits. All subsequent doses of NUC-3373 were given Q2W without LV in 28-day cycles. A total of 11 patients were finally enrolled in Arm 1b to obtain 6 evaluable patients.

In Arm 1c, two initial cohorts of up to 12 evaluable patients each were to receive NUC-3373 Q1W at either 1500 mg/m^2 or 2500 mg/m^2 in combination with LV Q1W at 400 mg/m^2. NUC-3373 and LV were administered on Days 1, 8, 15 and 22 of 28-day cycles. A total of 17 patients were finally enrolled in Arm 1c to obtain 6 evaluable patients per cohort.

The dose of NUC-3373 selected from Arm 1c for further exploration was 2500 mg/m^2 in combination with LV at 400 mg/m^2.

In Arm 1d, a cohort of approximately 10 combination chemotherapy ineligible patients will receive NUC-3373 at 2500 mg/m^2 in combination with LV at 400 mg/m^2. NUC-3373 and LV will be administered on Days 1, 8, 15 and 22 of 28-day cycles.

All patients in Part 1 (Arms 1a, 1b and 1c) were monitored for DLTs up to Day 28 of Cycle 1.

If the dose of NUC-3373 was not tolerable or was sub-optimal, alternate dose levels (as described in Table 6) may have been explored. If cohorts at additional doses were opened, these were to utilise a modified 3+3 design in which a fourth patient may have been added to each initial 3-patient cohort to account for a patient that became unevaluable for DLT evaluation for any reason other than safety. There was to be a 7-day observation period between treatment of the first patient and treatment of subsequent patients in a cohort.

Once a minimum of 3 patients in each of Arms 1a, 1b and 1c had completed the 28-day DLT evaluation period all available safety, PK and PD data were evaluated to establish the tolerability of NUC-3373 ± LV administered in each dose administration schedule.

Patients who completed the DLT period may have been switched from a Q1W to a Q2W schedule if they experienced drug-related toxicity (other than fatigue) that would otherwise have led to treatment discontinuation. In addition, patients who completed the DLT period may have moved from a Q2W to a Q1W schedule. The decision to switch schedules was to be discussed with and approved by the Sponsor on an individual patient basis.

In addition to the assessment of safety, tolerability, PK and PD, a radiologic assessment of disease will be performed at 8-week intervals and evaluated using RECIST v1.1 to gain a preliminary assessment of clinical activity.

3.1.2 Part 2

Findings from Part 1 of the study showed that LV did not impact the safety or PK of NUC-3373; therefore, the decision was made that LV should be administered at 400 mg/m^2 on each day of NUC-3373 administration in all parts of the study.

Part 2 of the study consists of a dose escalation phase using a modified 3 + 3 design, which may be followed by an expansion phase (Figure 3). The dose escalation phase will assess the safety and tolerability of different doses of NUC-3373 + LV when administered in combination with either oxaliplatin (NUFOX) or irinotecan (NUFIRI) in a Q1W schedule in ≥3rd-line patients. Oxaliplatin and irinotecan will be given at standard doses and schedules. In the expansion phase, cohorts of 2nd-/3rd-line patients may be enrolled to assess Q1W schedules of the NUFOX and NUFIRI regimens selected in the dose escalation phase.

Figure 3 NuTide:302 Part 2 study schema

The dose escalation group of patients will receive NUC-3373 + LV to assess the safety and tolerability of different doses of NUC-3373 + LV administered in combination with oxaliplatin (Arm 2a) or irinotecan (Arm 2b).

In Arm 2a, ≥3rd-line patients will receive NUC-3373 + LV on Days 1, 8, 15 and 22 and oxaliplatin on Days 1 and 15 of 28-day cycles.

In Arm 2b, ≥3rd-line patients will receive NUC-3373 + LV on Days 1, 8, 15 and 22 and irinotecan on Days 1 and 15 of 28-day cycles.

The starting dose of NUC-3373 will be 1500 mg/m^2 and the dose levels to be evaluated are described in Table 7 and Table 8. Based on a clinical study of NUC-3373 (NuTide:301, EudraCT 2015-002250-13, NCT02723240), alternate and/or additional dose levels may be considered and implemented upon recommendation of the DSMC.

The decision on which arm to enrol a patient to, Arm 2a or Arm 2b, will be made by the treating physician and will take into account the patient's clinical condition and any therapies that have previously been administered for treatment of CRC.

Patients will be enrolled into cohorts following a modified 3+3 design, in which a fourth patient may be enrolled in each initial 3-patient cohort as described above. The initial patient in each cohort will complete a 7-day observation period before subsequent patients are enrolled. All patients Arms 2a & 2b will be monitored for DLTs up to day 28 of Cycle 1. Dose escalation or de-escalation decisions will be based on DSMC review once three patients have completed the first 28-day cycle.

The recommended dose of NUC-3373 + LV in combination with oxaliplatin (Arm 2a) or irinotecan (Arm 2b) will be determined separately, and may be different.

Patients who have completed the DLT period may be switched from a Q1W to a Q2W schedule if they experience drug-related toxicity (other than fatigue) that would otherwise lead to treatment discontinuation. In addition, patients who have completed the DLT period may move from a Q2W to a Q1W schedule. The decision to switch schedules must be discussed with and approved by the Sponsor on an individual patient basis.

Once the dose escalation groups have been completed, the DSMC will review the available safety, clinical activity and PK data to determine the recommended dose of NUC-3373 + LV when given in combination with oxaliplatin or irinotecan. If safety concerns arise, additional cohorts assessing alternative doses of oxaliplatin and irinotecan may be opened with agreement from the DSMC.

The MTD for NUFIRI has been established in Arm 2b and was determined to be 1500 mg/m^2 NUC-3373 + 400 mg/m^2 LV + 180 mg/m^2 irinotecan.

Expansion cohorts may be enrolled to Part 2 to further assess the safety, tolerability and preliminary efficacy of the Q1W NUFOX and NUFIRI regimens, using the dose of NUC-3373 selected in the dose escalation phase. NUFOX and NUFIRI regimens will be administered as described in Table 4.

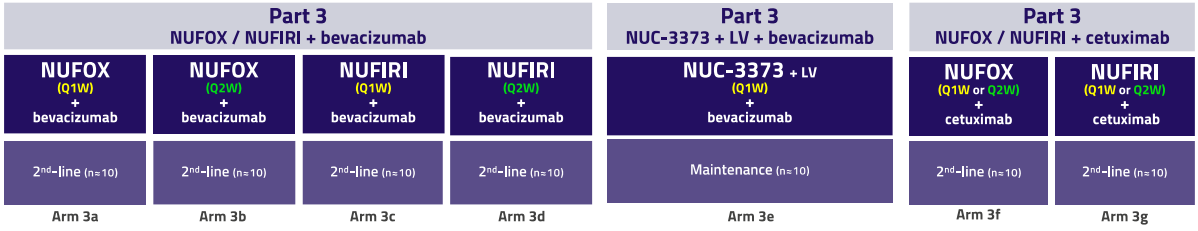
Table 4 Part 2 expansion phase treatment arms

Arm	Treatment	Number of patients
2c	NUC-3373 + LV Q1W, oxaliplatin Q2W (NUFOX)	~10 2nd-/3rd-line patients
2d	NUC-3373 + LV Q1W, irinotecan Q2W (NUFIRI)	~10 2nd-/3rd-line patients

3.1.3 Part 3

Following determination of the recommended NUFOX and NUFIRI regimens in the Part 2 dose escalation phase, Part 3 will open. The Part 2 expansion phase may open in parallel.

In Part 3, 2nd-line patients will receive the NUFOX and NUFIRI regimens selected in Part 2 in combination with bevacizumab (Figure 4). Q1W bevacizumab-containing arms will be opened first. Following this, Q2W arms and/or cetuximab combination arms may be opened, depending on emerging data. In addition, patients qualifying for maintenance therapy may receive NUC-3373 + LV in combination with bevacizumab.



In Part 3, Q1W NUFOX + bevacizumab and Q1W NUFIRI + bevacizumab cohorts (Arms 3a and 3c) will be opened first; the other arms may subsequently be opened, depending on emerging data

Figure 4 NuTide:302 Part 3 study schema

The treatment for each arm is described in Table 5. The choice of arm to which an eligible patient should be enrolled will be at the Investigator's discretion, taking into account the patient's clinical condition and therapies that have previously been administered.

Table 5 Part 3 treatment arms

Arm	Treatment	Number of patients
3a	NUFOX (NUC-3373 + LV Q1W) + bevacizumab	~10 2nd-line patients
3b	NUFOX (NUC-3373 + LV Q2W) + bevacizumab	~10 2nd-line patients
3c	NUFIRI (NUC-3373 + LV Q1W) + bevacizumab	~10 2nd-line patients
3d	NUFIRI (NUC-3373 + LV Q2W) + bevacizumab	~10 2nd-line patients
3e	NUC-3373 + LV Q1W + bevacizumab	~10 maintenance patients
3f	NUFOX (NUC-3373 + LV Q1W or Q2W) + cetuximab	~10 2nd-line patients
3g	NUFIRI (NUC-3373 + LV Q1W or Q2W) + cetuximab	~10 2nd-line patients

Bevacizumab and cetuximab should be used in accordance with standard local practice.

Panitumumab combination arms may also be opened (~10 2nd-line patients), depending on emerging data.

Cohorts of rapid progressors may also be opened (~10 patients), depending on emerging data.

3.1.4 PK Sub-study

A PK sub-study may be conducted during Part 2 if emerging data suggest it would be beneficial to evaluate the PK relationship between NUC-3373, LV and irinotecan. If conducted, an additional 8 patients would be enrolled in the NUFIRI Q1W cohort (Arm 2b). Inclusion into this sub-study will also involve individual patient genotyping for drug metabolising enzymes and drug transporters to predict irinotecan-related toxicities. Genotyping will be performed for each patient using genomic DNA from white blood cells isolated from whole blood samples taken during screening. The genomic DNA will be analysed using pharmacogenomic protocols specific for individual genotypes for drug metabolising enzymes and drug transporters, *e.g.,* TATA box polymorphism of the UGT1A1 promoter region and NR1I2-rs10934498 polymorphism, prior to admission to this sub-study. Genotyping is not required for patients who have a known UGT1A1 status from prior testing.

In Cycle 1 of the sub-study, irinotecan and LV will be administered concurrently on Day 1. On Day 8, NUC-3373 and LV will be administered sequentially. On Day 15, 4 patients (Group A) will first be administered irinotecan concurrently with LV, followed by NUC-3373. The remaining 4 patients (Group B) will first be administered NUC-3373, followed by irinotecan concurrently with LV. On Day 22, NUC-3373 and LV will be administered sequentially. On Cycle 2 Day 1, the infusion sequence will be reversed in a cross-over fashion whereby patients in Group A will first be administered NUC-3373, followed by irinotecan concurrently with LV and patients in Group B will first be administered irinotecan concurrently with LV, followed by NUC-3373. Patients will be alternately assigned to Group A or Group B. The doses of NUC-3373 and irinotecan used in the sub-study will be the recommended doses determined in Arm 2b.

	C1D1	C1D8	C1D15	C1D22	C2D1
Group A	Irinotecan + LV	LV ⇒ NUC-3373	Irinotecan + LV ⇒ NUC-3373	LV ⇒ NUC-3373	NUC-3373 ⇒ Irinotecan + LV
Group B	Irinotecan + LV	LV ⇒ NUC-3373	NUC-3373 ⇒ Irinotecan + LV	LV > NUC-3373	Irinotecan + LV ⇒ NUC-3373

Blood samples will be collected for plasma PK analysis on Cycle 1 Day 1, Cycle 1 Day 8, Cycle 1 Day 15, Cycle 1 Day 22, and Cycle 2 Day 1. In addition, Holter ECG assessments will be performed on Cycle 1 Day 8 and Cycle 1 Day 22 during infusion and up to 24 hours post-infusion.

Patients enrolled in the PK sub-study will be considered evaluable for PK endpoints if they have received planned study treatment and had PK assessments up to at least C1D15. If patients discontinue study treatment or require dose modifications before C1D15, they will be considered non-evaluable and will be replaced. Following this, patients will continue on the treatment schedule outlined for Arm 2b.

3.2. Rationale for the NUC-3373 Dose Selected

The safety of NUC-3373 as a single agent at doses ranging from 125 to 3250 mg/m^2 administered on Days 1, 8, 15 and 22 of a 28-day schedule has been studied in the NuTide:301 study as described in Section 1.5. This study also included a Q2W schedule and doses of 1500 to 2500 mg/m^2 administered on Days 1 and 15 of a 28-day schedule have been evaluated. Enrolment to this study has completed and the final results are being collated and analysed. Four patients experienced DLTs. In the 500 mg/m^2 Q1W and 1875 mg/m^2 Q1W cohorts, DLTs

of Grade 3 transaminases increased were reported. In the 3250 mg/m^2 Q1W cohort, two DLTs were reported, one DLT of Grade 2 headache and one DLT of Grade 3 transient hypotension (drop in blood pressure for <5 minutes). Based on these findings, the MTD for single-agent NUC-3373 was determined to be 2500 mg/m^2 Q1W. The DSMC for the NuTide:301 study has determined that NUC-3373 as evaluated to date is safe and well-tolerated in patients with advanced solid malignancies. Based on safety data from the NuTide:301 study and because the 1500 mg/m^2 dose has been shown to be safe when administered on Days 1 and 15 (Q2W) and when administered more frequently on Days 1, 8, 15 and 22 (Q1W), this dose was selected for all arms of Part 1.

For Part 2, NUC-3373 + LV will be given in combination with oxaliplatin and irinotecan at doses standardly used in the FOLFOX and FOLFIRI regimens. The initial dose of NUC-3373 to be administered on a Q1W basis with oxaliplatin or irinotecan in Arm 2a and Arm 2b was determined on the basis of safety data collected in Part 1 (see Section 3.4) and additional data collected in NuTide:301.

For Part 3, the NUC-3373 dose + LV with oxaliplatin selected as the NUFOX regimen and the NUC-3373 dose + LV with irinotecan selected as the NUFIRI regimen in Part 2 will be administered in combination with bevacizumab or cetuximab. For patients in the maintenance cohort, the NUC-3373 dose + LV selected in Part 1 (2500 mg/m^2) will be administered in combination with bevacizumab.

3.3. Study Treatment

This is a three-part, multi-cohort combination chemotherapy study. Study treatments given in each arm are described below. Patients may continue to receive NUC-3373 and the combination agent(s) until documentation of radiological disease progression using RECIST v1.1 or unacceptable toxicity. Treatment cycles are 28 days in duration.

Protocol-specific guidelines for NUC-3373 dosage and administration are provided in detail in Section 8.3. Guidelines for protocol-specific dose modifications and discontinuations of NUC-3373 are provided in Section 9. For patients experiencing toxicity related to NUC-3373 in NUFOX and NUFIRI cohorts, initially the NUC-3373 dose modification guidelines in Section 9 should be followed. Schedule modifications for the other agents (LV, oxaliplatin, irinotecan, VEGF pathway inhibitors, EGFR inhibitors) will be in accordance with their Respective Prescribing Information or standard local practice, unless safety concerns emerge regarding dosing as determined by the DSMC.

The sections below provide suggested administration times and order for each of the combination agents; however, each administration in Parts 1, 2 and 3 of the study may be modified based on emerging safety and PK data (in line with local guidelines/relevant Prescribing Information) with agreement from the DSMC.

It is suggested that NUC-3373 at 1500 mg/m^2 is infused over 120 minutes. The infusion duration for lower and higher doses of NUC-3373 should be adjusted relative to the 1500 mg/m^2 dose (*e.g.,* a 25% increase in dose requires an approximately 25% increase in infusion duration).

3.3.1 Part 1 (≥3rd-line and combination chemotherapy ineligible patients)

3.3.1.1 Arm 1a (≥3rd-line patients)

NUC-3373 was administered by IV infusion at 1500 mg/m^2 over 120 minutes followed by a 14-day washout period. Thereafter, on Days 1 and 15 of 28-day cycles, LV was administered by IV infusion at 400 mg/m^2 over 120 minutes, followed by NUC-3373 administration by IV infusion at the cohort-prescribed dose level.

3.3.1.2 Arm 1b (≥3rd-line patients)

On Day 1, LV was administered by IV infusion at 400 mg/m^2 over 120 minutes, followed by NUC-3373 infusion at 1500 mg/m^2 over 120 minutes. LV was not administered again in Arm 1b. After a 14-day washout period, NUC-3373 was administered on Days 1 and 15 of 28-day cycles by IV infusion at the cohort-prescribed dose level.

3.3.1.3 Arm 1c (≥3rd-line patients)

On Days 1, 8, 15 and 22 of 28-day cycles, LV was administered by IV infusion at 400 mg/m^2 over 120 minutes, followed by NUC-3373 administration by IV infusion at the cohort-prescribed dose level.

3.3.1.4 Arm 1d (combination chemotherapy ineligible patients)

On Days 1, 8, 15 and 22 of 28-day cycles, LV will be administered by IV infusion at 400 mg/m^2 over 120 minutes, followed by NUC-3373 administration by IV infusion at 2500 mg/m^2 over 200 minutes.

3.3.1.5 NUC-3373 Dosing for Part 1

It was anticipated that 1500 mg/m^2 would be the starting dose for all arms in Part 1 and is noted as Dose Level 1 in Table 6. Other dose levels as described in Table 6 may have been evaluated if recommended by the DSMC.

If cohorts at additional dose levels were opened, these were to utilise a modified 3+3 design in which a fourth patient may have been added to each initial 3-patient cohort to account for a patient that became unevaluable for DLT evaluation for any reason other than safety. There was to be a 7-day observation period between treatment of the first patient and treatment of subsequent patients in a cohort.

Table 6 Anticipated dose levels of NUC-3373 to be evaluated in Part 1

	≥3rd-line patients	
Dose Level*	**NUC-3373 dose (mg/m^2) Days 1 and 15** ***Arms 1a and 1b***	**NUC-3373 dose (mg/m^2) Days 1, 8, 15 and 22** ***Arm 1c***
-1	1000	1250
1	1500	1500
2	2000	1750
3	2500	2000
4	3000	2250

* Additional dose levels could be explored if necessary

3.3.2 Part 2

The order of administration of each of the agents in Part 2 may be modified with approval from the DSMC, so that NUC-3373 + LV is infused first followed by either oxaliplatin (Arms 2a and 2c) or irinotecan (Arms 2b and 2d). Alternate infusion parameters may be implemented with approval from the DSMC.

3.3.2.1 Dose Escalation (≥3rd-line patients)

In the NUFOX and NUFIRI dose escalation cohorts, patients will receive NUC-3373 + LV Q1W combined with the standard dose and schedule of oxaliplatin and irinotecan.

Arm 2a

NUC-3373 + LV will be administered Q1W and oxaliplatin will be administered Q2W in 28-day cycles, as outlined in Table 7.

Table 7 Study treatment in Arm 2a

Infusion order	Infusion duration	D1	D8	D15	D22
LV (400 mg/m^2)[a]	120 mins[a]	X	X	X	X
Oxaliplatin (85 mg/m^2)[a]		X		X	
NUC-3373 (cohort prescribed dose level)	120 mins[b]	X	X	X	X
[a]LV and oxaliplatin to be administered concurrently [b]Suggested duration, refer to Pharmacy Manual					

It is anticipated that 1500 mg/m^2 will be the starting dose in the first dose administration cohort for Arm 2a and is noted as 'Dose Level 1' in Table 8. The starting dose will not be higher than the MTD established in Part 1c. Based on a clinical study of NUC-3373 (NuTide:301, EudraCT 2015-002250-13, NCT02723240), alternate and/or additional dose levels may be considered and implemented upon recommendation of the DSMC.

Table 8 Anticipated dose levels of agents to be evaluated in Arm 2a

Dose Level*	NUC-3373 (mg/m^2) Days 1, 8, 15 and 22	Leucovorin (mg/m^2) Days 1, 8, 15 and 22	Oxaliplatin (mg/m^2)** Days 1 and 15
-1	1250	400	85
1	1500	400	85
2	1750	400	85
3	2000	400	85
4	2250	400	85

* Additional dose levels may be explored if necessary
** Alternative dose levels of oxaliplatin may be explored based on PK analyses and safety observations

Arm 2b

NUC-3373 + LV will be administered Q1W and irinotecan will be administered Q2W in 28-day cycles, as outlined in Table 9.

Table 9 Study treatment in Arm 2b

Infusion order[a]	Infusion duration	D1	D8	D15	D22
LV (400 mg/m^2)[b]	120 mins[c]	X	X	X	X
Irinotecan (180 mg/m^2)[b]		X		X	
NUC-3373 (cohort prescribed dose level)	120 mins[d]	X	X	X	X

[a]The infusion order may change as a result of emerging PK or safety data (refer to the PK Manual for further information)
[b]LV and irinotecan to be administered concurrently
[c]The infusion duration for irinotecan may be changed to 90 mins based on emerging data
[d]Suggested duration, refer to Pharmacy Manual

It is anticipated that 1500 mg/m^2 will be the starting dose in the first dose administration cohort for Arm 2b and is noted as Dose Level 1 in Table 10. The starting dose will not be higher than the MTD established in Part 1c. Based on a clinical study of NUC-3373 (NuTide:301, EudraCT 2015-002250-13, NCT02723240), alternate and/or additional dose levels may be considered and implemented upon recommendation of the DSMC.

Table 10 Anticipated dose levels of NUC-3373 to be evaluated in Arm 2b

Dose Level*	NUC-3373 (mg/m^2) Days 1, 8, 15 and 22	Leucovorin (mg/m^2) Days 1, 8, 15 and 22	Irinotecan (mg/m^2)** Days 1 and 15
-1	1250	400	180
1	1500	400	180
2	1750	400	180
3	2000	400	180
4	2250	400	180

* Additional dose levels may be explored if necessary
** Alternative dose levels of irinotecan may be explored based on PK analyses and safety observations

The MTD for NUFIRI has been established in Arm 2b and was determined to be 1500 mg/m^2 NUC-3373 + 400 mg/m^2 LV + 180 mg/m^2 irinotecan.

3.3.2.2 Dose Expansion (2nd-/3rd-line patients)

Following completion of Arms 2a and 2b, expansion cohorts may be initiated at the selected dose levels established in the dose escalation phase. The NUFOX and NUFIRI regimens will be administered in separate arms of the study (Arm 2c for NUFOX and Arm 2d for NUFIRI).

Arm 2c

NUC-3373 + LV will be administered Q1W and oxaliplatin will be administered Q2W in 28-day cycles, as outlined in Table 11.

Table 11 Study treatment in Arm 2c

Infusion order	Infusion duration	D1	D8	D15	D22
LV (400 mg/m^2)[a]	120 mins[a]	X	X	X	X
Oxaliplatin (85 mg/m^2)[a,b]		X		X	
NUC-3373 (dose selected in Arm 2a)	120 mins[c]	X	X	X	X

[a]LV and oxaliplatin to be administered concurrently
[b]The oxaliplatin dose may be reduced based on observations in Arm 2a
[c]Suggested duration, refer to Pharmacy Manual

Arm 2d

NUC-3373 + LV will be administered Q1W and irinotecan will be administered Q2W in 28-day cycles, as outlined in Table 12.

Table 12 Study treatment in Arm 2d

Infusion order[a]	Infusion duration	D1	D8	D15	D22
LV (400 mg/m^2)[b]	120 mins[c]	X	X	X	X
Irinotecan (180 mg/m^2)[b]		X		X	
NUC-3373 (1500 mg/m^2)	120 mins[d]	X	X	X	X

[a]The infusion order may change as a result of emerging PK or safety data (refer to the PK/PD Manual for further information)
[b]LV and irinotecan to be administered concurrently
[c]The infusion duration for irinotecan may be changed to 90 mins based on emerging data
[d]Suggested duration, refer to pharmacy manual

For patients experiencing toxicity related to NUC-3373 in the NUFOX and NUFIRI cohorts, initially the NUC-3373 dose modification guidelines in Section 9 should be followed. The doses of oxaliplatin and irinotecan may be adjusted (through agreement with the DSMC) based on, but not limited to, safety and PK data generated in patients receiving treatment with NUFOX and NUFIRI regimens. If oxaliplatin or irinotecan-related AEs are observed, dose de-escalation should be performed as per the respective Prescribing Information or standard local practice. The modified dose of oxaliplatin or irinotecan (in combination with NUC-3373) will be assessed in approximately 6 evaluable patients in each arm.

3.3.3 Part 3 (2nd-line patients and maintenance patients)

In Part 3, 2nd-line patients will receive the NUFOX and NUFIRI regimens selected in Part 2 in combination with bevacizumab. Q1W bevacizumab-containing arms will be opened first. Following this, Q2W arms and/or cetuximab combination arms may be opened, depending on emerging data. In addition, patients qualifying for maintenance therapy may receive NUC-3373 in combination with bevacizumab.

Refer to Section 8.3.3 for detailed guidance on administration of each study drug.

3.3.3.1 Arm 3a (2nd-line patients)

NUC-3373, LV and oxaliplatin at dose levels used in Arm 2a will be combined with bevacizumab. NUC-3373 and LV will be administered Q1W and oxaliplatin and bevacizumab will be administered Q2W.

3.3.3.2 Arm 3b (2nd-line patients)

NUC-3373, LV and oxaliplatin at dose levels used in Arm 2a will be combined with bevacizumab. NUC-3373, LV, oxaliplatin and bevacizumab will be administered Q2W.

3.3.3.3 Arm 3c (2nd-line patients)

NUC-3373, LV and irinotecan at dose levels used in Arm 2b will be combined with bevacizumab. NUC-3373 and LV will be administered Q1W and irinotecan and bevacizumab will be administered Q2W.

3.3.3.4 Arm 3d (2nd-line patients)

NUC-3373, LV and irinotecan at dose levels used in Arm 2b will be combined with bevacizumab. NUC-3373, LV, irinotecan and bevacizumab will be administered Q2W.

3.3.3.5 Arm 3e (Maintenance patients)

NUC-3373 (2500 mg/m^2 Q1W) + LV (400 mg/m^2 Q1W) will be combined with bevacizumab and bevacizumab (5 mg/kg Q2W).

Arm 3f (2nd-line patients)

NUC-3373, LV and oxaliplatin at dose levels used in Arm 2a may be administered in subsequent cetuximab cohorts. NUC-3373 + LV may be administered Q1W or Q2W, oxaliplatin will be administered Q2W and cetuximab will be administered Q1W

3.3.3.6 Arm 3g (2nd-line patients)

NUC-3373, LV and irinotecan at dose levels used in Arm 2b may be administered in subsequent cetuximab cohorts. NUC-3373 + LV may be administered Q1W or Q2W, irinotecan will be administered Q2W and cetuximab will be administered Q1W.

In all bevacizumab-containing arms, NUC-3373 dose adjustments may occur depending on emerging data.

Panitumumab combination arms may also be opened (~10 2nd-line patients), depending on emerging data.

Cohorts of rapid progressors may also be opened (~10 patients), depending on emerging data.

3.3.4 PK sub-study

Patients participating in the PK sub-study will receive NUC-3373, LV and irinotecan, as outlined in Table 13.

Table 13 Study treatment in PK sub-study

Infusion order	Infusion duration	C1D1	C1D8	C1D15[c]	C1D22	C2D1[c] (29 days after start of treatment)
LV[a]	120 mins[a]	X	X	X	X	X
Irinotecan[a,]	90 mins[a]	X		X		X
NUC-3373	120 mins[b]		X	X	X	X

[a]LV and irinotecan to be administered concurrently
[b]Suggested duration, refer to Pharmacy Manual
[c]Patients will be alternately assigned to two groups (Group A and Group B) for treatment on Cycle 1 Day 15 and Cycle 2 Day 1 (29 days after the start of treatment) and will receive the study treatments in an order that has been pre-specified for each group (refer to Section 7.2.1 for details)
Patients enrolled in the PK sub-study will be considered evaluable for PK endpoints if they have received planned study treatment and had PK assessments up to at least C1D15. If patients discontinue study treatment or require dose modifications before C1D15, they will be considered non-evaluable and will be replaced.

Following this, patients will continue on the treatment schedule outlined for Arm 2b.

3.4. Dose Escalation Guidelines

In those arms in which dose escalation is considered (Arm 1a, Arm 1b, Arm 1c, Arm 2a, and Arm 2b), dose escalation decisions will be made according to the dose escalation guidance rules in Table 14 when 3 patients in a dosing cohort have completed the 28-day DLT evaluation period or been discontinued due to documentation of a DLT prior to completing the initial 28-day DLT evaluation period cycle. To be evaluable for DLT, a patient must have received NUC-3373 and any assigned combination agent(s) in accordance with the arm to which the patient is assigned, have completed the 28-day safety evaluation requirements, and must have

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

Dose escalation decisions will occur based on review of available safety information obtained during the 28-day 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.

Table 14 Dose escalation guidance rules

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3 patients (0 out of 4 in modified 3+3)	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 being established, either alone or in combination, the DSMC will decide whether to explore a higher dose level or whether this dose is the recommended dose of NUC-3373 for the combination.

At least 6 patients are required for the dose to be declared as the MTD; therefore, additional patients may need to be enrolled.

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.

3.4.1 Definition of Dose-Limiting Toxicity

DLT is defined as any of the following occurring during the first 28-day treatment cycle that are not due to CRC or to a known concurrent medical condition and are judged as clinically significant and related to study treatment.

- Grade 4 neutropaenia (absolute neutrophil count [ANC] $<0.5 \times 10^9$/L) that persists for more than 7 days

- Febrile neutropaenia (ANC <1000/mm^3 with a single temperature of >38.3°C [101°F] or a sustained temperature of ≥38°C [100.4°F] for >1 hour)
- Grade 3 thrombocytopaenia (platelets <50.0x10^9/L) with bleeding complications requiring platelet transfusions or that persists for more than 7 days
- Grade 4 thrombocytopaenia
- Grade 4 anaemia unexplained by underlying disease
- Grade 5 haematological toxicity
- Grade 4 diarrhoea or vomiting that persists despite optimal medical management
- Grade 3 or greater aspartate aminotransferase (AST)/alanine aminotransferase (ALT) elevation (exceptions may be made for transient [<7 days] Grade 3 elevations of AST/ALT in the presence of known liver metastases and without evidence of other hepatic injury, if agreed by the treating physician and the DSMC)
- Grade 2 or greater AST/ALT elevation and Grade 2 or greater bilirubin elevation (exceptions may be made for transient [<7 days] elevations of AST/ALT and bilirubin in the presence of known liver metastases without evidence of other hepatic injury, if agreed upon by the treating physician and the DSMC)
- Any prolonged toxicity that does not resolve to ≤ Grade 1 or baseline within 21 days of dose interruption
- Any other non-haematological toxicity Grade ≥3 except for:
  - Alopecia
  - Grade 3 nausea, vomiting, or diarrhoea events that resolve within 72 hours with anti-emetic or anti-diarrhoeal therapy

3.5 Duration of Patient Participation

Patients may continue to receive NUC-3373 in accordance with the dosing regimen (schedule and specific combination) to which they were assigned until the occurrence of radiological disease progression by RECIST v1.1 or unmanageable drug-related AEs despite optimal medical management or dose or schedule modification. Patients may also decline treatment at any time for any reason, or they may meet any of the other reasons for treatment withdrawal defined in Section 6.1. Reasons for treatment discontinuation must be captured in the patient medical record and on the Treatment Discontinuation page of the case report form (CRF).

Should a patient discontinue treatment without radiological evidence of disease progression, the patient should continue to undergo tumour assessment every 8 weeks from Cycle 1 Day 1 until such time as progression can be documented, a new treatment is initiated, or death.

All patients will be followed up until withdrawal of consent, lost to follow-up, death, or the overall end of study has been reached (defined in Section 3.6), whichever occurs first.

3.6 Study Completion

The study will be considered complete when all evaluable patients in opened arms have exhibited radiological disease progression or 12 months have elapsed since the final patient was enrolled (whichever occurs first, unless the study is terminated early on the recommendation of the DSMC or decision of the Sponsor), and it is determined that no further arms should be opened based on emerging data.

4 PATIENT SELECTION

4.1 Inclusion Criteria

To be enrolled in this study, patients must meet the following criteria during the Screening period:

All patients

1. Provision of written informed consent
2. Have histological confirmation of CRC with evidence of locally advanced/unresectable or metastatic disease
3. Age ≥18 years
4. Life expectancy of ≥12 weeks
5. Eastern Cooperative Oncology Group (ECOG) Performance status 0 or 1
6. Measurable disease as defined by RECIST v1.1
7. Known RAS and BRAF status. Patients with wild-type KRAS tumours who are to be enrolled to a cohort that does not contain an EGFR pathway inhibitor (Arms 2a, 2b, 2c, 2d, 3a, 3b, 3c, 3d and 3e) must have received prior treatment with an EGFR inhibitor, unless this was not standard of care according to relevant region-specific treatment recommendations. Patients with BRAF V600E mutant tumours should have received prior treatment with encorafenib in combination with an EGFR inhibitor, unless this was not standard of care according to relevant region-specific treatment recommendations
8. Adequate bone marrow function as defined by: ANC $\geq 1.5\times10^9$/L, platelet count $\geq 100\times10^9$/L (with no evidence of bleeding), and haemoglobin ≥9 g/dL
9. Adequate liver function, as defined by: serum total bilirubin ≤1.5×upper limit of normal (ULN), AST and ALT ≤2.5×ULN (or ≤5×ULN if liver metastases are present)
10. Adequate renal function assessed as serum creatinine <1.5×ULN or glomerular filtration rate ≥50 mL/min. This criterion does not apply to the combination chemotherapy ineligible patient subgroup (Arm 1d); please refer to specific criteria for this subgroup below
11. Serum albumin ≥3 g/dL
12. For the cohort in which the patient will participate, there are no contra-indications to receiving the approved partner combination drugs
13. Ability to comply with protocol requirements
14. Female patients of child-bearing potential must have a negative serum 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 (defined in Section 10.3.1) or to use two highly effective forms of contraception, one of which must be a barrier method. 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 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
16. For patients receiving oxaliplatin: Male patients must have been offered advice on and/or sought counselling for conservation of sperm prior to the first dose of study medication

In addition to meeting the criteria above for all patients, the following criteria must be met for each patient population:

≥3rd-line patients

1. Must have received at least two prior lines of therapy for locally advanced or metastatic CRC, including one fluoropyrimidine plus oxaliplatin containing regimen and one fluoropyrimidine plus irinotecan containing regimen. Previous treatment with standard of care chemotherapy regimens in combination with molecular targeted therapies (*e.g.,* therapies including VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Patients who have received FOLFOXIRI-based regimens in 1st- and/or 2nd-line settings may also be included
2. Patients in Part 2 of the study who are to receive NUFOX regimens should be suitable for re-challenge with an oxaliplatin-based regimen
3. Patients in Part 2 of the study who are to receive NUFIRI regimens should be suitable for re-challenge with an irinotecan-based regimen

2nd-/3rd-line patients

1. Must have received at least one, but no more of two, prior lines of fluoropyrimidine-containing therapy in combination with oxaliplatin and/or irinotecan for locally advanced or metastatic CRC. Previous treatment with standard of care chemotherapy regimens in combination with molecular targeted therapies (*e.g.,* therapies including VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Patients who have received FOLFOXIRI-based regimens in 1st- and/or 2nd-line settings may also be included. 3rd-line patients enrolled to Arms 2c and 2d must have received prior bevacizumab treatment, unless ineligible or unless bevacizumab was not standard of care according to relevant region-specific treatment recommendations
2. Patients in Part 2 of the study who are to receive NUFOX regimens should be suitable for re-challenge with an oxaliplatin-based regimen
3. Patients in Part 2 of the study who are to receive NUFIRI regimens should be suitable for re-challenge with an irinotecan-based regimen

Combination chemotherapy ineligible patients

1. Patients may have received one prior line of fluoropyrimidine-containing therapy for locally advanced or metastatic CRC
2. Ineligible to receive combination therapy for locally advanced or metastatic CRC, as defined by the presence of one or more of the following criteria:
    a. Dependency for daily activities due to comorbidities (different to deterioration due to cancer)
    b. Previous history of three or more of the following comorbidities (controlled or uncontrolled by treatments):

i. Congestive heart failure
ii. Other chronic cardiovascular disease
iii. Chronic obstructive pulmonary disease
iv. Cerebrovascular disease
v. Peripheral neuropathy
vi. Chronic renal failure
vii. Arterial hypertension
viii. Diabetes mellitus
ix. Systemic vasculitis
x. Severe arthritis

c. At least one of the following geriatric features:

i. Age >75 years
ii. Faecal or urinary incontinence
iii. Spontaneous bone fractures
iv. Mild or moderate dementia
v. Frequent falls

3. Creatinine clearance of >30 mL/min

Rapid progressors

1. Must have received no more than two prior lines of fluoropyrimidine-containing therapy in combination with oxaliplatin and/or irinotecan for locally advanced or metastatic CRC. Previous treatment with standard of care chemotherapy regimens in combination with molecular targeted therapies (e.g., therapies including VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Patients who have received FOLFOXIRI- based regimens in 1st- and/or 2nd-line settings may also be included
2. Must have had tumour progression ≤3 months of starting the last fluoropyrimidine-containing regimen
3. Patients who are to receive NUFOX regimens should be suitable for re-challenge with an oxaliplatin-based regimen
4. Patients who are to receive NUFIRI regimens should be suitable for re-challenge with an irinotecan-based regimen

2nd-line patients

1. Must have received one prior line of fluoropyrimidine-containing therapy in combination with oxaliplatin and/or irinotecan for locally advanced or metastatic CRC. Previous treatment with standard of care chemotherapy regimens in combination with molecular targeted therapies (*e.g.,* therapies including VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Previous treatment with triplet chemotherapy-based regimens is allowed (*i.e.,* FOLFOXIRI)

Maintenance patients

1. Must have received at least 12 weeks of 1st-line standard of care therapy for locally advanced or metastatic CRC and achieved at least stable disease
2. Eligible for maintenance therapy

4.2 Exclusion Criteria

Potential patients who meet any of the following criteria at Screening will be excluded from the study:

All patients

1. Prior history of hypersensitivity or current contra-indications to 5-FU or capecitabine
2. Prior history of hypersensitivity or current contra-indication to any of the combination agents required for the study arm to which the patient is assigned
3. History of allergic reactions attributed to components of the NUC-3373 drug product formulation (Kolliphor ELP, super refined polysorbate 80, dimethylacetamide [DMA])
4. Symptomatic central nervous system or leptomeningeal metastases
5. Symptomatic ascites, ascites currently requiring drainage procedures or ascites requiring drainage over the prior 3 months
6. Chemotherapy, radiotherapy (other than a short cycle of palliative radiotherapy [*e.g.,* for bone pain]*), immunotherapy or exposure to another investigational agent within 28 days (or five times the half-life for a biological or molecular targeted agent or three times the half-life for an immunotherapy agent) of first administration of NUC-3373:
   - For nitrosoureas and mitomycin C, within 6 weeks of first administration of NUC-3373
   - For hormone or biological therapy, within 14 days of first administration of NUC-3373
   - Corticosteroid treatment is allowed if not more than stable daily dosing of 10 mg prednisolone (or steroid equivalent)

   ** Palliative radiotherapy during participation in the study is permitted, but should not include a target lesion*
7. Residual toxicities from prior chemotherapy or radiotherapy which have not regressed to Grade ≤1 severity (CTCAE v5.0), except for alopecia. In cohorts not containing oxaliplatin, residual Grade 2 neuropathy is allowed
8. Patients who have a history of another malignancy diagnosed within the past 5 years, with the exception of adequately treated non-melanoma skin cancer, curatively treated carcinoma *in situ* of the cervix, surgically excised or potentially curatively treated ductal carcinoma *in situ* of the breast, or low-grade prostate cancer or patients after prostatectomy not requiring treatment. Patients with previous invasive cancers are eligible if treatment was completed more than 3 years prior to initiating the current study treatment and there is no evidence of recurrence
9. Presence of an active bacterial or viral infection (including SARS-CoV-2, Herpes Zoster or chicken pox), known Human Immunodeficiency Virus positive or known active hepatitis B or C
10. 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. Myocardial infarction within 6 months of the first dose of study medication
c. Unstable or poorly controlled angina pectoris
d. Complete left bundle branch, bifascicular block or other clinically significant abnormal ECG finding
e. History of or current risk factor for torsades de pointes (*e.g.,* heart failure, hypokalaemia, or a family history of long QT syndrome)
f. History of severe skin reactions
g. History of severe ocular disorders
h. Interstitial pneumonitis or pulmonary fibrosis

11. 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
12. Currently pregnant, lactating or breastfeeding
13. QTc interval >450 milliseconds for males and >470 milliseconds for females
14. Required concomitant use of drugs known to prolong QT/QTc interval
15. Required concomitant use of strong CYP3A4 inducers or strong CYP3A4 inhibitors, or use of strong CYP3A4 inducers within 2 weeks of first receipt of study drug or use of strong CYP3A4 inhibitors within 1 week of first receipt of study drug (refer to Section 10.3.2)
16. For patients receiving irinotecan: Use of strong UGT1A1 inhibitors within 1 week of first receipt of study drug (refer to Section 10.3.3)
17. Has received a live vaccination within four weeks of first planned dose of study medication
18. Known DPD or TYMP mutations associated with toxicity to fluoropyrimidines
19. 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 anti-coagulant treatment should switch to low molecular weight heparin or anti-Xa inhibitors with a half-life of ≤12 hours

Patients receiving bevacizumab

1. Patients with a history of haemoptysis (1/2 teaspoon or more of red blood)
2. Wound healing complications or surgery within 28 days of starting bevacizumab (wound healing must have been fully completed before starting bevacizumab)
3. Severe chronic wounds, ulcers or bone fracture
4. Arterial thromboembolic events or haemorrhage within 6 months prior to study entry (except for tumour bleeding surgically treated by tumour resection)
5. Bleeding diatheses or coagulopathy
6. Receiving full-dose anti-coagulation treatment. Patients who have been on stable doses for at least 6 months and have tolerated prior bevacizumab treatment are eligible
7. Uncontrolled hypertension
8. Clinically significant coronary heart disease or myocardial infarction within the last 12 months or high risk of uncontrolled arrhythmia

9. Severe proteinuria (nephrotic syndrome)
10. Acute or subacute ileus, chronic inflammatory bowel disease or chronic diarrhoea
11. Any contraindication present in the bevacizumab Prescribing Information

Patients receiving cetuximab or panitumumab

1. Clinically significant coronary heart disease or myocardial infarction within the last 12 months or high risk of uncontrolled arrhythmia
2. Acute or subacute ileus, chronic inflammatory bowel disease or chronic diarrhea
3. Hypomagnesaemia or hypokalaemia not controlled by oral therapy
4. Any contraindication present in the cetuximab or panitumumab Prescribing Information

Patients participating in the PK sub-study (Arm 2b)

1. Patients with the UGT1A1 *28/*28 genotype (homozygous poor UGT1A1 metabolisers)

4.3 Waivers to Entry Criteria

Waivers will **not** be granted for a patient who does not satisfy the eligibility criteria. Investigators who are unsure whether the patient satisfies all the entry criteria and wish to clarify matters of clinical discretion must contact the Medical Monitor who will consult the Sponsor before responding to the enquiry.

5 STUDY ASSESSMENTS AND PROCEDURES

Data from all procedures and assessments must be recorded in the patient's medical record for extraction into the CRF. Refer to the Summary Schedule of Events for further details.

5.1 Informed Consent

Potential patients will be given the current approved version of the study information sheet and informed consent form (ICF). They will also receive clear verbal information about the study detailing no less than: the nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be explained that they will be free to withdraw from the study at any time, for any reason, without prejudice to future care, and with no obligation to give a reason for withdrawal. They will have at least 24 hours to consider the information provided and the opportunity to question the Investigator, their GP or other independent parties before deciding whether to participate.

The Investigator or designee who obtains consent must be suitably qualified and experienced. All designees must be authorised by the Investigator to obtain consent. The Investigator is responsible for ensuring that the study consent procedures comply with International Council for Harmonisation Good Clinical Practice (ICH-GCP) and any other additional local regulatory requirements. Informed consent discussions and outcomes must be well documented in the medical record. The Investigator must be satisfied that the patient has made an informed decision before taking consent. The patient and the Investigator or authorised designee who obtains consent must personally sign and date the current approved version of the ICF in each other's presence.

A copy of the study information and signed ICF will be given to the patient. The original signed form will be retained at the study site, with copies held in both the medical record and Investigator Site File. Written informed consent for participation in the study must be obtained *prior to* performing any study-specific screening tests or evaluations.

5.1.1 Informed Consent for Tumour Tissue Collection

This study will evaluate biomarkers to potentially identify patients that are likely to respond to NUC-3373 or patients who may be resistant to NUC-3373. An optional fresh biopsy of tumour tissue is requested during screening and on treatment at either C1D8 or C1D15 for participation in this study. If this is not possible, it may be obtained at any time after C1D15 with agreement from the Medical Monitor. Patients who do not have easily accessible tumour for biopsy should not be put at undue risk for sample collection. Biopsied lesions should not be designated as target lesions for the purposes of RECIST v1.1. In addition, patients will be requested to consent to providing archival tumour tissue from the time of diagnosis, if available.

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

5.2 Patient Registration and Screening Procedures

All screening activities must be performed within 28 days of randomisation or first protocol-prescribed treatment. A Screening Log must be kept of all patients considered for the study (*i.e.,* all those that are included for screening and any that are subsequently excluded). The reason for exclusion must be recorded on this form. A copy of the Screening Log must be retained on site and provided to the Clinical Research Organisation (CRO) upon request, but without patient identifiers.

5.3 Screening Assessments

Screening assessments of consented patients will comprise the following:

- Provision of written informed consent
- Eligibility confirmation, including histological diagnosis of mCRC, with evidence of disease recurrence
- Recording of demographic data
- Assessment of medical and surgical history, including prior therapy for CRC
- Recording of concomitant medication
- Routine physical examination (including neurological exam), including vital signs. 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
- Height and weight
- Baseline assessment of symptoms
- ECOG performance status
- 12-lead ECG
    - The 12-lead ECGs should be performed in triplicate (keeping the leads in place and patient supine during readings) and reviewed by the Investigator or a qualified designee for safety and quality
    - The QTc interval should be calculated for each ECG using the Fridericia formula and averaged. 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:
    - Haematology: white blood cell (WBC) count, differential WBC count, red blood cell count (RBC), haemoglobin, haematocrit and platelets
    - Coagulation parameters*:* prothrombin time/international normalised ratio (PT/INR) and activated partial thromboplastin time (aPTT)
    - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, phosphate, total protein, albumin, adjusted calcium, total bilirubin, bicarbonate, chloride, uric acid, alkaline phosphatase, AST, ALT and lactate dehydrogenase (LDH)
    - Pregnancy testing: For women of childbearing potential serum pregnancy must be performed within 7 days of first dose
    - RAS/BRAF mutation status testing, if unknown
    - Tumour markers (carcinoembryonic antigen [CEA])

  - **PK sub-study only:** genotyping for drug metabolising enzymes and drug transporters. Genotyping is not required for patients who have a known UGT1A1 status from prior testing.

- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- Tumour imaging (computed tomography [CT] or magnetic resonance imaging [MRI] of thorax, abdomen and pelvis) – performed within 28 days prior to first dose of IMP. A recent previously obtained image may be used if obtained within 28 days prior to first dose of IMP and images collected meet protocol requirements. Biopsied lesions should not be designated as target lesions for the purposes of RECIST v1.1
- 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
- For patients receiving oxaliplatin: Male patients must have been advised on and/or sought counselling for conservation of sperm prior to the first dose of study medication
- Patient registration
- Obtain an archival tumour tissue block, if available
- For consenting patients with an accessible tumour that is not a target lesion for the purposes of the study, obtain a fresh tumour specimen. Patients should not be put at undue risk for collection of tumour samples. For sampling procedures, storage conditions, and shipment instructions, see the Laboratory Manual
- Blood samples (optional) for mRNA analyses (*e.g.,* PAX gene) – Refer to Section 7.4 for more details

5.4 Re-Screening Patients who Fail Screening

If a patient does not meet the inclusion/exclusion criteria upon first assessment, the patient can be re-screened within 14 days of screen failure confirmation being determined. Patients who fail at re-screening are ineligible and may not be re-screened.

All Screening assessments that result in a patient failing the initial screening process must be repeated and confirmed as meeting the inclusion criteria for the study in advance of C0D1 (Arms 1a and 1b) or C1D1 of all other parts of the study. During the re-screening process, any Screening assessment that will be greater than 28 days from the date taken to C0D1 (Arms 1a and 1b) or C1D1 of all other parts of the study must also be repeated.

5.5 Evaluations to be Performed during the Study

Patients participating in the PK sub-study in Part 2 will have the assessments detailed in Section 5.5.9 during Cycle 1 and on Days 1 and 2 of Cycle 2. Following this, patients in the sub-study will continue with the normal assessments described for patients in Arm 2b (Sections 5.5.2 to 5.5.8).

5.5.1 Cycle 0 (Arm 1a and Arm 1b)

All procedures to be completed prior to dosing except treatment and collection of on-treatment and post-treatment blood samples.

- Recording of new or changes to concomitant medication
- Routine physical examination (including neurological examination), including vital signs. 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
- Weight
- ECOG performance status
- 12-lead ECGs should be performed prior to infusion of all IMPs
    - At Cycle 0 Day 1 the 12-lead ECGs should also be performed at 30-60 minutes post-infusion of NUC-3373
    - The ECGs should be performed in triplicate (keeping the leads in place and patient supine during readings) and reviewed by the Investigator or a qualified designee for safety and quality
    - The QTc interval should be calculated for each ECG using the Fridericia formula and averaged. 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:
    - Haematology: WBC count, differential WBC count, RBC, haemoglobin, haematocrit and platelets
    - Coagulation parameters*:* PT/INR and aPTT
    - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, alkaline phosphatase, AST, ALT and LDH
    - Tumour markers (CEA)
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- 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
- IV administration of NUC-3373 ± LV:
    - Arm 1a: NUC-3373 only
    - Arm1b: LV + NUC-3373
- AE recording and causality assessment

- Blood samples for PK measurements - Refer to Section 7.2 for more details
- **Part 1 only**: Blood samples for PD measurements - Refer to Section 7.3 for more details
- Blood samples (optional) for mRNA analyses (*e.g.,* PAX gene) if not taken at Baseline/Screening – Refer to Section 7.4 for more details

5.5.2 Each cycle, Day 1

All procedures to be completed prior to dosing except study drug administration and applicable PK samples.

- Recording of new or changes to concomitant medication.
- Routine physical examination (including neurological examination), including vital signs. 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
- Weight
- ECOG performance status
- 12-lead ECGs should be performed prior to infusion of all IMPs
    - At Cycle 1 Day 1, Cycle 2 Day 1 (Part 3 only) and Cycle 3 Day 1 the 12-lead ECGs should also be performed at 30-60 minutes post-infusion of NUC-3373
    - The ECGs should be performed in triplicate (keeping the leads in place and patient supine during readings) and reviewed by the Investigator or a qualified designee for safety and quality
    - The QTc interval should be calculated for each ECG using the Fridericia formula and averaged. 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:
    - Haematology: WBC count, differential WBC count, RBC, haemoglobin, haematocrit and platelets
    - Coagulation parameters*:* PT/INR and aPTT*
        * *Do not repeat for patients enrolled into Arms 1a and 1b*
    - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, alkaline phosphatase, AST, ALT and LDH
    - Tumour markers (CEA)
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable

- 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
- IV administration of study drug:
  - Arm 1a: NUC-3373 + LV
  - Arm 1b: NUC-3373 alone
  - Arm 1c: NUC-3373 + LV
  - Arm 1d: NUC-3373 + LV

  - Arm 2a: NUC-3373 + LV + oxaliplatin
  - Arm 2b: NUC-3373 + LV + irinotecan
  - Arm 2c: NUC-3373 + LV + oxaliplatin
  - Arm 2d: NUC-3373 + LV + irinotecan

  - Arm 3a: NUC-3373 + LV + oxaliplatin + bevacizumab
  - Arm 3b: NUC-3373 + LV + oxaliplatin + bevacizumab
  - Arm 3c: NUC-3373 + LV + irinotecan + bevacizumab
  - Arm 3d: NUC-3373 + LV + irinotecan + bevacizumab
  - Arm 3e: NUC-3373 + LV + bevacizumab
  - Arm 3f: NUC-3373 + LV + oxaliplatin + cetuximab
  - Arm 3g: NUC-3373 + LV + irinotecan + cetuximab
- AE recording and causality assessment.

5.5.3 Cycle 1 ONLY – Days 1, 2 & 3, and Days 15 & 16

- Blood samples for PK measurements - Refer to Section 7.2 for more details
- **Arms 1d and 3e only:** Urine samples for PK measurements - Refer to Section 7.2 for more details
  - 0-8 and 8-24* hour or 48-hour urine collection starting from the beginning of NUC-3373 infusion (to be collected in 3-6 patients in each arm). For patients who provide optional consent, urine collection will continue for 48 hours on C1D1 only.

  *Patient to be provided with sample collection kit so they can continue to collect urine samples at home. The samples will then be brought back when the patient attends for the Day 2 or Day 16 (24 hours) or Day 3 (48 hours) blood samples. For logistical reasons (*e.g.,* if the patient lives far away) it may be necessary for the patient to stay overnight.
- **Part 1 only:** Blood samples for PD measurements **(Days 1 and 15 only)** - Refer to Section 7.3 for more details

- Blood samples (optional) for mRNA analyses (*e.g.,* PAX gene) if not taken at Baseline/Screening – at **Day 1 only in Cycle 1 (Cycle 0 Day 1 in Arms 1a and 1b)** - Refer to Section 7.4 for more details

5.5.4 Cycle 1 ONLY – Day 8 (or Day 15)

- For consenting patients with an accessible tumour that is not a target lesion for the purposes of the study, obtain an optional fresh tumour specimen 3-6 hours after study drug treatment. Patients should not be put at undue risk for collection of tumour samples. For sampling procedures, storage conditions, and shipment instructions, see the Laboratory Manual

5.5.5 Cycle 3 – Days 1 & 15 (C3D1 & C3D15; all cohorts)

- Blood samples (optional) for mRNA analyses (*e.g.,* PAX gene) – at **Day 1 only in Cycle 3** - Refer to Section 7.4 for more details

5.5.6 Each cycle, Day 8 (Q1W dosing cohorts only)

All procedures to be completed prior to dosing:

- Recording of new or changes to concomitant medications
- Routine physical examination (including neurological examination), including vital signs. 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
- 12-lead ECGs will be performed prior to infusion of all IMPs at all relevant visits
    - The ECGs should be performed in triplicate (keeping the leads in place and patient supine during readings) and reviewed by the Investigator or a qualified designee for safety and quality
    - The QTc interval should be calculated for each ECG using the Fridericia formula and averaged. 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:
    - 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, alkaline phosphatase, AST, ALT and LDH
- IV administration of study drug:
    - Arm 1c: NUC-3373 + LV
    - Arm 1d: NUC-3373 + LV

- Arm 2a: NUC-3373 + LV
- Arm 2b: NUC-3373 + LV
- Arm 2c: NUC-3373 + LV
- Arm 2d: NUC-3373 + LV

- Arm 3a: NUC-3373 + LV
- Arm 3c: NUC-3373 + LV
- Arm 3e: NUC-3373 + LV
- Arm 3f: NUC-3373 + LV + cetuximab (if administered Q1W)
- Arm 3g: NUC-3373 + LV + cetuximab (if administered Q1W)

- AE recording and causality assessment

5.5.7 Each cycle, Day 15 (all cohorts)

All procedures to be completed prior to dosing except study drug administration and applicable PK samples.

- Recording of new or changes to concomitant medications
- Routine physical examination (including neurological examination), including vital signs. 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
- 12-lead ECGs should be performed prior to infusion of all IMPs at all visits.
  - At Cycle 1 Day 15, Cycle 2 Day 15 (Part 3 only) and Cycle 3 Day 15 the 12-lead ECGs should be performed at 30-60 minutes post-infusion of NUC-3373.
  - The ECGs should be performed in triplicate (keeping the leads in place and patient supine during readings) and reviewed by the Investigator or a qualified designee for safety and quality
  - The QTc interval should be calculated for each ECG using the Fridericia formula and averaged. 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
  - **Note:** Section 5.5.6 above, Cycle 3 Day 15 requires triplicate ECG measurements at 30-60 minute post dose, in addition to pre-infusion ECG measurements
- 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, alkaline phosphatase, AST, ALT and LDH

- IV administration of study drug:
  - Arm 1a: NUC-3373 + LV
  - Arm 1b: NUC-3373 alone
  - Arm 1c: NUC-3373 + LV
  - Arm 1d: NUC-3373 + LV

  - Arm 2a: NUC-3373 + LV + oxaliplatin
  - Arm 2b: NUC-3373 + LV + irinotecan
  - Arm 2c: NUC-3373 + LV + oxaliplatin
  - Arm 2d: NUC-3373 + LV + irinotecan

  - Arm 3a: NUC-3373 + LV + oxaliplatin + bevacizumab
  - Arm 3b: NUC-3373 + LV + oxaliplatin + bevacizumab
  - Arm 3c: NUC-3373 + LV + irinotecan + bevacizumab
  - Arm 3d: NUC-3373 + LV + irinotecan + bevacizumab
  - Arm 3e: NUC-3373 + LV + bevacizumab
  - Arm 3f: NUC-3373 + LV + oxaliplatin + cetuximab
  - Arm 3g: NUC-3373 + LV + irinotecan + cetuximab
- AE recording and causality assessment
- Blood samples for PK measurements (Cycle 1 only) - Refer to Section 7.2 for more details
- **Part 1 only**: Blood samples for PD measurements (Cycle 1 only) - Refer to Section 7.3 for more details

5.5.8 Each cycle, Day 22 (Q1W cohorts only)

All procedures to be completed prior to dosing with study drug:

- Recording of new or changes to concomitant medications
- Routine physical examination (including neurological examination), including vital signs. 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
- 12-lead ECGs should be performed prior to infusion of all IMPs at all relevant visits
  - The ECGs should be performed in triplicate (keeping the leads in place and patient supine during readings) and reviewed by the Investigator or a qualified designee for safety and quality

  - The QTc interval should be calculated for each ECG using the Fridericia formula and averaged. 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:
  - 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, alkaline phosphatase, AST, ALT and LDH
- IV administration of study drug:
  - Arm 1c: NUC-3373 + LV
  - Arm 1d: NUC-3373 + LV

  - Arm 2a: NUC-3373 + LV
  - Arm 2b: NUC-3373 + LV
  - Arm 2c: NUC-3373 + LV
  - Arm 2d: NUC-3373 + LV

  - Arm 3a: NUC-3373 + LV
  - Arm 3c: NUC-3373 + LV
  - Arm 3e: NUC-3373 + LV
  - Arm 3f: NUC-3373 + LV + cetuximab (if administered Q1W)
  - Arm 3g: NUC-3373 + LV + cetuximab (if administered Q1W)
- AE recording and causality assessment

5.5.9 PK sub-study (Part 2, Arm 2b): Cycle 1 and Cycle 2 D1/D2 ONLY

Patients participating in the PK sub-study in Part 2 will have the assessments detailed in this section during Cycle 1 and on Days 1 and 2 of Cycle 2. Following this, patients in the sub-study will continue with the normal assessments described for patients in Arm 2b (Sections 5.5.2 to 5.5.8).

- Recording of new or changes to concomitant medications (C1D1, C1D8, C1D15, C1D22 and C2D1)
- Routine physical examination (including neurological examination), including vital signs. 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 (C1D1, C1D8, C1D15, C1D22 and C2D1)
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable (C1D1 and C2D1)
- 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 (C1D1 and C2D1)
- ECOG performance status (C1D1 and C2D1)
- Standard 12-lead ECGs should be performed prior to infusion of all IMPs on C1D1, C1D8, C1D15, C1D22 and C2D1. 12-lead ECGs should also be performed at 30-60 minutes post-administration of all IMPs on C1D1 and C1D15
    - The ECGs should be performed in triplicate (keeping the leads in place and patient supine during readings) and reviewed by the Investigator or a qualified designee for safety and quality
    - The QTc interval should be calculated for each ECG using the Fridericia formula and averaged. 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
- Holter ECGs should be performed on C1D8 and C1D22, with continuous readings taken over 24 hours, starting from 30 minutes prior to the start of infusion
    - Patients must remain supine for 30 minutes prior to the start of infusion and for at least 5 minutes before, and during, the other PK draw timepoints (see Section 7.2.1)
    - The ECG data should be transmitted for blinded central review
- Blood samples drawn for:
    - Haematology: WBC count, differential WBC count, RBC, haemoglobin, haematocrit and platelets (C1D1, C1D8, C1D15, C1D22 and C2D1)
    - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted

calcium, total bilirubin, alkaline phosphatase, AST, ALT and LDH (C1D1, C1D8, C1D15, C1D22 and C2D1)

- Coagulation parameters: PT/INR and aPTT (C1D1 and C2D1)
- Tumour markers (CEA) (C1D1 and C2D1)

- IV administration of study treatment as follows:
  - Cycle 1 Day 1: LV + irinotecan
  - Cycle 1 Day 8: LV + NUC-3373
  - Cycle 1 Day 15: LV + irinotecan + NUC-3373*
  - Cycle 1 Day 22: LV + NUC-3373
  - Cycle 2 Day 1: LV + NUC-3373 + irinotecan*

  *Patients will be alternately assigned to two groups (Group A and Group B) for treatment on Cycle 1 Day 15 and Cycle 2 Day 1 and will receive the study treatments in an order that has been pre-specified for each group (refer to Section 7.2.1 for details).
- Blood samples for PK measurements will be collected as follows:
  - Cycle 1 Day 1 & Day 2
  - Cycle 1 Day 8 & Day 9
  - Cycle 1 Day 15 & Day 16
  - Cycle 1 Day 22 & Day 23
  - Cycle 2 Day 1 & Day 2
  - Note that for C1D8 and C1D22 when the patient is wearing the Holter monitor, the patient must be supine for 30 minutes prior to the start of infusion and for at least 5 minutes prior to, and during, the other timepoints when PK draws are taken

The exact time that each PK sample is taken must be recorded

Refer to Section 7.2.1 for more details.

- AE recording and causality assessment (C1D1, C1D2, C1D8, C1D9, C1D15, C1D16, C1D22, C1D23, C2D1 and C2D2)
- For consenting patients with an accessible tumour that is not a target lesion for the purposes of the study, obtain an optional fresh tumour specimen 3-6 hours after study drug treatment on C1D8 or C1D15. Patients should not be put at undue risk for collection of tumour samples. For sampling procedures, storage conditions, and shipment instructions, see the Laboratory Manual

5.5.10 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 of thorax, abdomen and pelvis)

5.5.11 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 (EoT) 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 EoT visit, the following items will be assessed:

- Recording of new or changes to concomitant medication
- Routine physical examination (including neurological examination), including vital signs. 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
- Weight
- ECOG performance status
- 12-lead ECG
    - The 12-lead ECGs should be performed in triplicate (keeping the leads in place and patient supine during readings) and reviewed by the Investigator or a qualified designee for safety and quality
    - The QTc interval should be calculated for each ECG using the Fridericia formula and averaged. 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:
    - Haematology: WBC count, differential WBC count, RBC, haemoglobin, haematocrit and platelets
    - Coagulation parameters*:* PT/INR and aPTT
    - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, alkaline phosphatase, AST, ALT and LDH
    - Pregnancy testing for women of child-bearing potential
    - Tumour markers CEA
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- AE recording and causality assessment

5.5.12 Long-Term Follow-Up

Patients who stop treatment with no evidence of radiological disease progression as defined by RECIST v1.1 criteria will continue to receive scans at regular intervals (every 8 weeks [±7 days] from C1D1) until disease progression, initiation of a new treatment for CRC, or death (whichever comes first) to determine duration of overall response and PFS.

All patients will be followed up until withdrawal of consent, lost to follow-up, death, or the overall end of study has been reached (defined in Section 3.6), whichever occurs first.

During the COVID-19 pandemic, collection of follow-up data can be performed via a telephone call with the patient where possible (*e.g.,* for data regarding concomitant medications, AEs, and QoL). The data collected and the follow-up schedule remain as per the schedule of events. Patients in follow-up who have not experienced radiological disease progression should continue to attend the clinic for planned radiologic scans; however, other follow-up data can be collected via telephone to limit patient visits.

6 PATIENT WITHDRAWAL

6.1 End of Treatment

Patients may continue on study until one of the following occurs:

- Progressive Disease as defined by RECIST v1.1 criteria. Patients should not discontinue NUC-3373 due to clinical signs of progressive disease until it has been confirmed by RECIST v1.1
- Unmanageable toxicity defined as an AE that is considered by the Investigator to warrant permanent discontinuation of NUC-3373 including the following:
    - AE resulting in a dosing delay of more than 21 days in starting the next cycle unless the patient is receiving clinical benefit
    - Clinically-significant treatment-related AE that recurs despite dose reduction in two consecutive cycles. Patients may continue to receive treatment if the Principal Investigator and Medical Monitor agree that the patient is receiving a clinical benefit and the toxicity is manageable, reversible or transient
- Lack of further clinical benefit or unfavourable risk/benefit profile as judged by the Investigator
- Inter-current illness that prevents further administration of NUC-3373
- Patient withdraws consent from further treatment or for further data collection
    - If the patient withdraws consent for further treatment, follow-up visits should continue
    - If the patient withdraws consent for further treatment and data collection, then no additional study visits or data collection should occur
- Patient requires use of a prohibited concomitant medication or therapy
- Pregnancy
- Changes in the patient's condition, which in the opinion of the Investigator, make the patient unsuitable for any further treatment under the protocol; note that at the Investigator's discretion, the patient may discontinue treatment with an agent in the prescribed combination, other than NUC-3373, without requiring discontinuation from the study
- Patient non-compliance
- Lost to follow-up
- Patient withdrawal of consent
- Sponsor request

All study procedures outlined for the EoT visit are to be completed 30 days (+7 days) after the last dose of study drug. The primary reason for study drug discontinuation is to be recorded in the CRF.

6.2 Consent Withdrawal

Patients will be informed that they have the right to withdraw from the study at any time for any reason without prejudice to their medical care.

Consent withdrawal means that a patient has expressed a wish to withdraw from the study altogether. Under these circumstances, the site personnel should document all relevant discussions in the patient notes and mark all future CRF pages as not applicable. Under these conditions, Investigators are still responsible to follow-up any SAEs until resolution.

6.3 Patient Evaluability and Replacement

Enrolment in the dose escalation arms will continue until there are at least 3 (or 6 in the case that an arm or dosing cohort is expanded on the basis of an earlier DLT) patients evaluable for DLT in each arm or dose cohort within an arm.

All patients who receive at least one dose of NUC-3373 are considered evaluable for safety analysis.

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

Patients enrolled in the PK sub-study will be considered evaluable for PK endpoints if they have received planned study treatment and had PK assessments up to at least C1D15. If patients discontinue study treatment or require dose modifications before C1D15, they will be considered non-evaluable and will be replaced.

7 SAMPLES FOR LABORATORY ANALYSIS

7.1 Clinical Laboratory Tests

All clinical laboratory testing will be performed at local sites. A pregnancy test will be performed in women of child-bearing potential at the Screening visit. Laboratory tests may be performed either on the day of a treatment visit (prior to study drug administration) or during the three days prior to a treatment visit.

7.2 Pharmacokinetics

The PK schedule is designed to explore the relationship between the pharmacokinetics of NUC-3373, safety, pharmacodynamics and clinical activity. The full set of mandatory PK samples must be collected from a minimum of four patients per arm.

Blood samples will be collected at the following visits:

- C0D1, C0D2 and C0D3* (Arms *1a and 1b only)*
- Parts 1, 2 and 3: C1D1, C1D2 and C1D3*
- Parts 1, 2 and 3: C1D15 and C1D16

** Optional 48-hour PK sample*

PK endpoints will include assessments of, but are not limited to:

- In plasma: NUC-3373, 5-FU, 5-FUDR, FBAL
- **Part 1 only**: In PBMCs: NUC-3373, FUDR-MP, FBAL, 5-FUTP, dTMP, and dUMP

The PK sampling schedule, based on an NUC-3373 infusion duration of 2 hours and 4 hours, is summarised in Table 15.

Collection times for the ‘During NUC-3373 Infusion’ PK samples (Samples 2-4) should be adjusted proportionally to the NUC-3373 dose and infusion duration, where Sample 2 is collected a quarter of the way through the infusion, Sample 3 halfway through the infusion and Sample 4 at the end of the infusion.

Table 15 PK sampling schedule (Main study)

Sample Number	Sample Collection Time (NUC-3373 2-hour infusion)	Sample Collection Time (NUC-3373 4-hour infusion)	Sample Collection Window
PRIOR TO NUC-3373 INFUSION			
1	Pre-dose		Up to 30 minutes prior to starting infusion
DURING NUC-3373 INFUSION			
2	T0 + 30 minutes	T0 + 60 minutes	+/- 5 minutes
3	T0 + 60 minutes	T0 + 120 minutes	
4	T0 + 120 minutes	T0 + 240 minutes	
AFTER NUC-3373 INFUSION			
5	T1 + 15 minutes		+/- 5 minutes
6	T1 + 30 minutes		
7	T1 + 60 minutes		
8	T1 + 90 minutes		
9	T1 + 120 minutes		+/- 15 minutes
10	T1 + 240 minutes		+ 4 hours / - 15 minutes
11	T1 + 24 hours		+ 4 hours
12*	T1 + 48 hours		

- T_0 = Start of NUC-3373 infusion
- T_1 = End of NUC-3373 infusion
- Sample 1, Pre-dose should be collected prior to infusion of all IMPs
- Samples 2, 3, and 4 are triggered by the start time of the infusion and assume a 2-hour or a 4-hour infusion duration. Collection times will be modified in alignment with infusion duration changes

** Sample 12 = Optional 48-hour PK sample*

Urine samples will be collected in Arms 1d and 3e only at the following visits:

- C1D1, C1D2 and C1D3*
- C1D15 and C1D16

** Optional 48-hour urine PK sample*

All urine passed over the first 24 hours after the administration of NUC-3373 on C1D1 and C1D15 will be collected on each PK study day. Urine from hour 0 to 8 will be collected in a separate container to urine collected between hour 8 and 24. Patients who consent to the 48-hour PK time point will be asked to collect their urine between hour 24 and 48.

Patients are to be provided with a sample collection kit so they can continue to collect urine samples at home. The samples will then be brought back when the patient attends for the Day 2 or Day 16 (24 hours) or Day 3 (48 hours) blood samples. For logistical reasons (*e.g.,* if the patient lives far away) it may be necessary for the patient to stay overnight.

Standard PK parameters for each compound of interest will then be derived from the measured plasma, PBMC (Part 1 only), and urine concentrations. The PK samples will be processed and analysed at a central laboratory. Please refer to the PK Laboratory Manual for details regarding PK sample collection, processing and shipping.

7.2.1 PK Sub-study

A PK sub-study may be conducted during Part 2 if emerging data suggest it would be beneficial to evaluate the PK relationship between NUC-3373, LV and irinotecan (Figure 5). If conducted, an additional 8 patients would be enrolled in the NUFIRI Q1W cohort (Arm 2b). Inclusion into this sub-study will also involve individual patient genotyping for drug metabolising enzymes and drug transporters to predict irinotecan-related toxicities. Genotyping will be performed for each patient using genomic DNA from white blood cells isolated from whole blood samples taken during screening. The genomic DNA will be analysed using pharmacogenomic protocols specific for individual genotypes for drug metabolising enzymes and drug transporters, *e.g.,* TATA box polymorphism of the UGT1A1 promoter region and NR1I2-rs10934498 polymorphism, prior to admission to this sub-study (Deyme *et al,* 2021). Genotyping is not required for patients who have a known UGT1A1 status from prior testing.

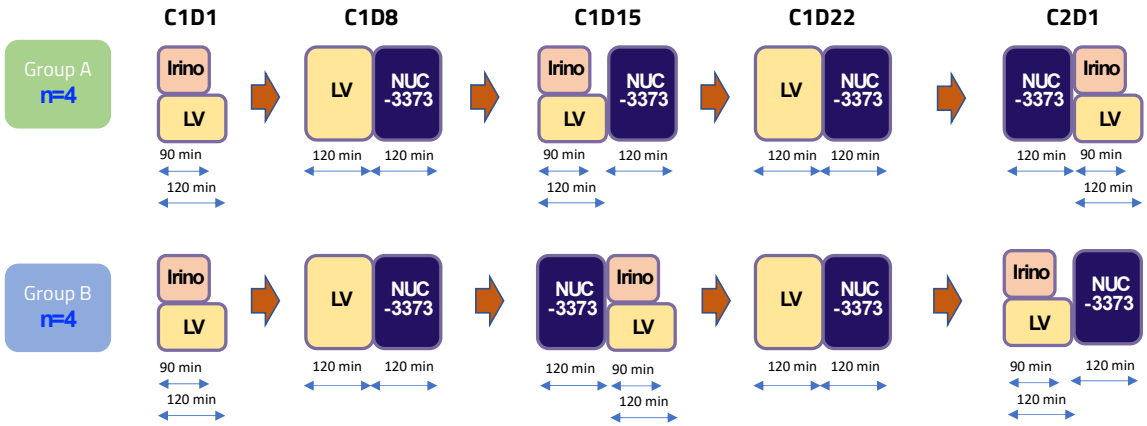


Figure 5 PK sub-study schema

In Cycle 1 of the sub-study, irinotecan and LV will be administered concurrently on Day 1. On Day 8, NUC-3373 and LV will be administered sequentially. On Day 15, 4 patients (Group A) will first be administered irinotecan concurrently with LV, followed by NUC-3373. Prior to the start of NUC-3373 infusion on Day 15 the 2-hour post-start of irinotecan/LV infusion PK sample should be taken, which will also serve as the pre-infusion sample for NUC-3373 PK for Group A. The remaining 4 patients (Group B) will first be administered NUC-3373, followed by irinotecan concurrently with LV. On Day 15, the 2-hour post-start of NUC-3373 infusion PK sample should be taken, which will also serve as the pre-infusion sample for irinotecan PK for Group B.

On Day 22, NUC-3373 and LV will be administered sequentially. On Cycle 2 Day 1, the infusion sequence will be reversed in a cross-over fashion whereby patients in Group A will first be administered NUC-3373, followed by irinotecan concurrently with LV and patients in Group B will be first be administered irinotecan concurrently with LV, followed by NUC-3373. Prior to the start of irinotecan/LV infusion on Cycle 2 Day 1 the 2-hour post-start of NUC-3373 PK sample should be taken, which will also serve as the pre-infusion sample for irinotecan PK for Group A. Prior to the start of NUC-3373 infusion on Cycle 2 Day 1 the 2-hour post-start of irinotecan/LV PK sample should be taken, which will also serve as the pre-infusion sample for NUC-3373 PK for Group B. Patients will be alternately assigned to Group A or Group B.

Patients enrolled in the PK sub-study will be considered evaluable for PK endpoints if they have received planned study treatment and had PK assessments up to at least C1D15. If patients discontinue study treatment or require dose modifications before C1D15, they will be considered non-evaluable and will be replaced.

Holter ECG measurements will be performed on C1D8 and C1D22 for patients in the PK sub-study, with continuous readings taken over 24 hours, starting from 30 minutes prior to the start of infusion. Patients must be supine for 30 minutes prior to the start of infusion and for at least 5 minutes prior to, and during, the other timepoints where PK draws are taken (as outlined in Figure 6). All Holter ECG data should be transmitted for blinded central review.

Plasma samples will be collected for PK analysis on Cycle 1 Day 1, Cycle 1 Day 8, Cycle 1 Day 15, Cycle 1 Day 22, and Cycle 2 Day 1, as detailed in **Figure 6**.

The exact time that each PK sample is taken must be recorded.

Following this, patients will continue on the treatment schedule outlined for Arm 2b.

Group A

			Timepoints (min post-first infusion)									
		Pre-dose*	60 mins (+/- 5 mins)	120 mins (+/- 5 mins)	150 mins (+/- 5 mins)	180 mins (+/- 5 mins)	250 mins (+/- 5 mins)	280 mins (+/- 5 mins)	300 mins (+/- 5 mins)	360 mins (+/- 15 mins)	480 mins (+/- 15 mins)	24 hours (+/- 2 hours)
C1D1	Infusion		Irinotecan + LV									
	PK Draw	X	X	X		X	X		X	X	X	X
C1D8	Infusion		LV		NUC-3373							
	PK Draw	X				X	X	X	X	X	X	X
C1D15	Infusion		Irinotecan + LV		NUC-3373							
	PK Draw	X	X	X		X	X	X	X	X	X	X
C1D22	Infusion		LV		NUC-3373							
	PK Draw	X				X	X	X	X	X	X	X
C2D1	Infusion		NUC-3373		Irinotecan + LV							
	PK Draw	X	X	X	X	X	X		X	X	X	X

*Pre-dose = up to 30 minutes prior to the administration of irinotecan + LV (C1D1, C1D15) or NUC-3373 (C1D8, C1D22, C2D1)

Group B

			Timepoints (min post-first infusion)									
		Pre-dose*	60 mins (+/- 5 mins)	120 mins (+/- 5 mins)	150 mins (+/- 5 mins)	180 mins (+/- 5 mins)	250 mins (+/- 5 mins)	280 mins (+/- 5 mins)	300 mins (+/- 5 mins)	360 mins (+/- 15 mins)	480 mins (+/- 15 mins)	24 hours (+/- 2 hours)
C1D1	Infusion		Irinotecan + LV									
	PK Draw	X	X	X		X	X		X	X	X	X
C1D8	Infusion		LV		NUC-3373							
	PK Draw	X				X	X	X	X	X	X	X
C1D15	Infusion		NUC-3373		Irinotecan + LV							
	PK Draw	X	X	X	X	X	X		X	X	X	X
C1D22	Infusion		LV		NUC-3373							
	PK Draw	X				X	X	X	X	X	X	X
C2D1	Infusion		Irinotecan + LV		NUC-3373							
	PK Draw	X	X	X		X	X	X	X	X	X	X

*Pre-dose = up to 30 minutes prior to the administration of irinotecan + LV (C1D1, C2D1) or NUC-3373 (C1D8, C1D15, C1D22)

Figure 6 PK sub-study blood sampling schema

PK endpoints will include plasma assessments of (but are not limited to):

- NUC-3373, FUDR, NUC-3373 alanine phosphate intermediate
- Irinotecan, SN-38, SN-38 glucuronide, APC and NPC

Standard PK parameters for each compound of interest will then be derived from the measured plasma concentrations. The PK samples will be processed and analysed at a central laboratory. Please refer to the PK Laboratory Manual for details regarding PK sample collection, processing and shipping.

7.3 Pharmacodynamics (Part 1 only)

The PD schedule is designed to explore the relationship between NUC-3373 pharmacodynamics and clinical activity.

Blood samples will be collected at the following visits:

- C0D1 (Arms 1a and 1b only)
- C1D1 and C1D15

PD endpoints will include assessments of:

- **Part 1 only**: In PBMCs: TS - total, bound and unbound

The PD sampling schedule is summarised in Table 16.

Table 16 PD sampling schedule (Part 1 only)

Sample Number	Sample Time	Sample Collection Window
PRIOR TO NUC-3373 INFUSION		
1	Pre-dose	Up to 30 minutes prior to starting infusion
AFTER NUC-3373 INFUSION		
2	T1 + 30 minutes	+/- 5 minutes

- Sample 1, Pre-dose should be collected prior to infusion of all IMPs
- T_1 = End of NUC-3373 infusion

7.4 Exploratory Biomarkers

Blood samples (optional) will be collected for mRNA analyses (*e.g.,* PAX gene) at baseline or C1D1 pre-dose and at C3D1.

7.5 Tumour Tissue Sample Submission

Tissue specimens will be collected from patients enrolled in the study for exploration of pharmacodynamic biomarkers of biological activity and possible relationships between PK exposure, PD effects, safety and efficacy. An archival specimen will be collected from consenting patients for whom an archival tissue exists; the specimen may be collected at any time during the study. An optional fresh tissue specimen also will be collected during the screening period and on treatment on C1D8 or C1D15 3-6 hours after study drug infusion in consenting patients. The fresh tissue specimen should not be taken from a lesion to be designated a target lesion for the purposes of the study. Specific instructions on the collection and shipment of tissue samples will be provided in the Tumour Tissue Laboratory Manual.

7.6 Labelling and Confidentiality of Biological Samples

All biological samples (including blood, PBMC and tumour tissue) sent to analytical laboratories will be labelled with the study code, patient study number and date/time taken. Samples labels must not contain any unique patient identifiers.

7.7 Withdrawal of Consent for Biological Sample Collection or Retention

A patient may withdraw consent to provide samples or allow their samples to be used for research at any time without giving a reason. The Investigator must ensure that their wishes are recorded in the medical record and CRF. The clinical research associate should be informed accordingly. The Investigator should discuss with patients the valuable use of samples that have already been provided and under circumstances where these samples have already been processed and anonymised, it would not be possible to destroy such samples.

8 INVESTIGATIONAL MEDICINAL PRODUCTS

8.1 NUC-3373

8.1.1 NUC-3373 Description

The Drug Product is called 'NUC-3373 for infusion' and is presented as a single-dose sterile liquid formulation for IV injection of NUC-3373 in a clear glass vial.

8.1.2 NUC-3373 Supplies and Study Drug Packaging

'NUC-3373 for infusion' will be supplied to the pharmacy of the investigative clinical site in 10 mL clear glass vials. The vials are packaged in a labelled cardboard outer carton.

NUC-3373 saline-based formulation is prepared by withdrawing the required volume of 'NUC-3373 for infusion' from the vial and adding it directly to the saline infusion bag. Please refer to the dose preparation guidance in the current version of the Pharmacy Manual and the Administration Guidelines for further information on the preparation of NUC-3373 saline-based formulations.

8.1.3 Handling and Storage of NUC-3373

'NUC-3373 for infusion' must be stored in an appropriately secure investigational pharmacy at all times until dispensed for administration to patients on protocol. 'NUC-3373 for infusion' must be stored between 2-8°C (36-46°F) in a temperature-monitored refrigerated unit. Only adequately trained pharmacy staff are permitted to handle 'NUC-3373 for infusion'. The study medication should not be removed from the pharmacy except for the purposes of dispensing to the patient for this protocol. Study drug that has been quarantined for any reason must not be dispensed or administered to patients.

If 'NUC-3373 for infusion' contacts the skin or the mucous membranes, it should be washed immediately and thoroughly. Please refer to the guidance in the current version of the Pharmacy Manual for further information on the preparation of NUC-3373 saline-based formulations.

8.1.4 Preparation of NUC-3373

As with other cytotoxic substances, applicable local procedures should be used in the preparation and administration of 'NUC-3373 for infusion'. Please refer to the administration guidance in the current version of the Pharmacy Manual and Administration Guidelines for further information on the preparation of NUC-3373 saline-based formulation.

Due to known issues regarding DMA and compatibility with polycarbonate, do not use polycarbonate syringes or polycarbonate filter needles to withdraw 'NUC-3373 for infusion'.

8.1.5 NUC-3373 Administration

'NUC-3373 for infusion' will be administered to each patient based on body surface area (BSA; calculated form patient's height and weight) at baseline and/or height at baseline and weight at Day 1 of each cycle based on local standard operating practises. If a patient's weight increases or decreases by ≥10% during the course of the study, the dose of 'NUC-3373 for infusion' should be re-calculated. The Dubois & Dubois BSA calculation is the preferred method, however other standard calculations can also be used. Sites should document the method used in the electronic case report form (eCRF).

Dubois & Dubois BSA calculation:

$$BSA\ (m^2) = 0.007184 \times Height\ (cm)^{0.725} \times Weight\ (kg)^{0.425}$$

The duration of infusion of NUC-3373 saline-based formulations should align with the guidance provided in Table 17. Should a decision on dose modification be taken that increases or reduces the dose of 'NUC-3373 for infusion' administered to the patient, the duration of the infusion should also be checked and adjusted in accordance with the guidance provided.

It is suggested that NUC-3373 at 1500 mg/m^2 is infused over 120 minutes. The infusion duration for lower and higher doses of NUC-3373 should be adjusted relative to the 1500 mg/m^2 dose (*e.g.,* a 25% increase in dose requires an approximately 25% increase in infusion duration), as shown in Table 17.

Table 17 NUC-3373 infusion duration guidance

Dose of NUC-3373	Minimum Infusion Duration*
1125 mg/m^2 (-25%)	90 minutes
1500 mg/m^2	120 minutes
1875 mg/m^2 (+25%)	150 minutes
2000 mg/m^2 (+33%)	160 minutes
2250 mg/m^2 (+50%)	180 minutes
2500 mg/m^2 (+67%)	200 minutes

* - 5 to +15 minute window will be permitted on all infusion durations

8.2 Other IMPs (Agents to be used in Combination with NUC-3373)

All of the agents to be used in combination with NUC-3373 in the different arms of this study are commercially available and should be sourced locally by the Investigative sites. Descriptive information for these agents can be found in the package inserts. These agents should be stored according to the manufacturer's instructions. Further information can be found in the pharmacy manual.

For VEGF inhibitors, the preferred treatment option is Avastin; however, biosimilars may be used after discussion with the Sponsor. For EGFR inhibitors, the preferred treatment options are Erbitux (cetuximab) or Vectibix (panitumumab); however, if biosimilars become available, their use may be discussed with the Sponsor.

8.3 Administration of NUC-3373 and other IMPs

The sections below provide suggested administration times and order for each of the combination agents; however, each administration in Parts 1, 2, and 3 of the study may be modified based on emerging safety and PK data (in line with local guidelines/relevant Prescribing Information) with agreement from the DSMC.

8.3.1 Part 1

A total of 21 eligible and consenting ≥3rd-line patients were randomised to either Arm 1a or Arm 1b and a further 17 patients were enrolled to each of two dosing cohorts in Arm 1c. Arms 1a, 1b, and 1c are now complete. Following this, approximately 10 combination chemotherapy ineligible patients will be enrolled to a Q1W administration schedule of NUC-3373 plus LV (Arm 1d). Dose adjustments or dose delays are to be implemented within

or between cycles based on drug-related toxicities. The dose modification scheme to be employed is detailed in Section 9 of this protocol.

Arm 1a: SINGLE DOSE NUC-3373 / LV + NUC-3373 COMBINATION

At C0D1:

- NUC-3373 IV over 120 minutes at an initial dose of 1500 mg/m^2

NUC-3373 monotherapy was given once followed by a 2-week washout period.

At C1D1 and beyond:

- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to each NUC-3373 infusion on Days 1 and 15 of each 28-day treatment cycle
- NUC-3373 IV over 120 minutes at an initial dose of 1500 mg/m^2 on Days 1 and 15 of each 28-day treatment cycle

Arm 1b: SINGLE DOSE NUC-3373 + LV / NUC-3373 MONOTHERAPY

At C0D1:

- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion
- NUC-3373 IV over 120 minutes at an initial dose of 1500 mg/m^2

NUC-3373 + LV was given once followed by a 2-week washout period.

At C1D1 and beyond:

- NUC-3373 IV over 120 minutes at an initial dose of 1500 mg/m^2 on Days 1 and 15 of each 28-day treatment cycle

Arm 1c: NUC-3373 + LV COMBINATION

At C1D1 and beyond:

- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to each NUC-3373 infusion on Days 1, 8, 15 and 22 of each 28-day treatment cycle
- NUC-3373 by IV over 120 minutes at an initial dose of 1500 mg/m^2 on Days 1, 8, 15 and 22 of each 28-day treatment cycle

Starting doses for subsequent cohorts will be determined during the study by the DSMC.

Arm 1d: NUC-3373 + LV (combination chemotherapy ineligible patients)

At C1D1 and beyond:

- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to each NUC-3373 infusion on Days 1, 8, 15 and 22 of each 28-day treatment cycle
- NUC-3373 IV over 200 minutes at a dose of 2500 mg/m^2 on Days 1, 8, 15 and 22 of each 28-day treatment cycle

8.3.2 Part 2

Eligible consenting patients in Part 2 will receive one of the combinations listed below, as determined by the Investigator and number of patients enrolled per cohort. Patients will continue to receive NUC-3373 + the combination drug(s) until the occurrence of radiological disease progression using RECIST v1.1 or unmanageable drug-related AEs despite dose modification. Guidelines for dose modifications and discontinuations due to AEs will be provided.

The order of administration of each of the agents in Part 2 may be modified with approval from the DSMC, so that NUC-3373 + LV is infused first followed by either oxaliplatin (Arms 2a and 2c) or irinotecan (Arms 2b and 2d). Alternate infusion parameters may be implemented with approval from the DSMC.

Arm 2a: NUFOX Dose Escalation Q1W (≥3rd-line patients)

C1D1 and beyond:

- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion
- Oxaliplatin 85 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion
- NUC-3373 IV over 120 minutes at the combination dose as determined at the DSMC meeting for Part 1

NUC-3373 + LV is given on Days 1, 8, 15 and 22 in each 28-day treatment cycle (Q1W).

Oxaliplatin is given on Days 1 and 15 in each 28-day treatment cycle (Q2W), as per the Prescribing Information.

Arm 2b: NUFIRI Dose Escalation Q1W (≥3rd-line patients)

C1D1 and beyond:

- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion
- Irinotecan 180 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion
- NUC-3373 IV over 120 minutes at the combination dose as determined at the DSMC meeting for Part 1

NUC-3373 + LV is given on Days 1, 8, 15 and 22 in each 28-day treatment cycle (Q1W).

Irinotecan is given on Days 1 and 15 in each 28-day treatment cycle (Q2W), as per the Prescribing Information.

The MTD for NUFIRI has been established in Arm 2b and was determined to be 1500 mg/m^2 NUC-3373 + 400 mg/m^2 LV + 180 mg/m^2 irinotecan.

PK sub-study (Arm 2b): NUFIRI Q1W

C1D1:

- LV (400 mg/m^2 IV; or equivalent levo-leucovorin) over 120 minutes concurrently with irinotecan (180 mg/m^2 IV) over 90 minutes

C1D8:

- LV (400 mg/m^2 IV; or equivalent levo-leucovorin) over 120 minutes
- NUC-3373 (1500 mg/m^2 IV) over 120 minutes

C1D15 (Arm A):

- LV (400 mg/m^2 IV; or equivalent levo-leucovorin) over 120 minutes concurrently with irinotecan (180 mg/m^2 IV) over 90 minutes
- NUC-3373 (1500 mg/m^2 IV) over 120 minutes

C1D15 (Arm B):

- NUC-3373 (1500 mg/m^2 IV) over 120 minutes
- LV (400 mg/m^2 IV; or equivalent levo-leucovorin) over 120 minutes concurrently with irinotecan (180 mg/m^2 IV) over 90 minutes

C1D22:

- LV (400 mg/m^2 IV; or equivalent levo-leucovorin) over 120 minutes
- NUC-3373 (1500 mg/m^2 IV) over 120 minutes

C2D1 (Arm A):

- NUC-3373 (1500 mg/m^2 IV) over 120 minutes
- LV (400 mg/m^2 IV; or equivalent levo-leucovorin) over 120 minutes concurrently with irinotecan (180 mg/m^2 IV) over 90 minutes

C2D1 (Arm B):

- LV (400 mg/m^2 IV; or equivalent levo-leucovorin) over 120 minutes concurrently with irinotecan (180 mg/m^2 IV) over 90 minutes
- NUC-3373 (1500 mg/m^2 IV) over 120 minutes

Arm 2c: NUFOX Dose Expansion Q1W (2nd-/3rd-line patients)

C1D1 and beyond:

- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion (concurrently with oxaliplatin)
- Oxaliplatin 85 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion (concurrently with LV)
- NUC-3373 IV at the combination dose as determined at the DSMC meeting for Part 2 dose escalation

NUC-3373 + LV is given on Days 1, 8, 15 and 22 in each 28-day treatment cycle (Q1W).

Oxaliplatin is given on Days 1 and 15 in each 28-day treatment cycle (Q2W), as per the Prescribing Information.

Arm 2d: NUFIRI Dose Expansion Q1W (2nd-/3rd-line patients)

C1D1 and beyond:

- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion (concurrently with irinotecan)
- Irinotecan 180 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion (concurrently with LV)
- NUC-3373 1500 mg/m^2 IV over 120 minutes

NUC-3373 + LV is given on Days 1, 8, 15 and 22 in each 28-day treatment cycle (Q1W).

Irinotecan is given on Days 1 and 15 in each 28-day treatment cycle (Q2W), as per the Prescribing Information.

Guidance on combination agent infusion time and order has been provided above; however, in all instance's combination agents may be administered in accordance with local and national guidelines for FOLFOX and FOLFIRI regimens.

For patients experiencing toxicity related to NUC-3373 in the NUFOX and NUFIRI cohorts, initially the NUC-3373 dose modification guidelines in Section 9 should be followed. The doses of oxaliplatin and irinotecan may be adjusted (through agreement with the DSMC) based on, but not limited to, safety and PK data generated in patients receiving treatment with NUFOX and NUFIRI regimens. If oxaliplatin or irinotecan-related AEs are observed, dose de-escalation should be performed as per the respective Prescribing Information or standard local practice. The modified dose of oxaliplatin or irinotecan (in combination with NUC-3373) will be assessed in approximately 6 evaluable patients in each arm.

8.3.3 Part 3

In Part 3, 2nd-line patients will receive the NUFOX and NUFIRI regimens selected in Part 2 in combination with bevacizumab. Q1W bevacizumab-containing cohorts will be opened first. Following this, Q2W arms and/or cetuximab combination cohorts may be opened, depending on emerging data. In addition, patients qualifying for maintenance therapy may receive NUC-3373 + LV in combination with bevacizumab.

Eligible consenting patients in Part 3 will receive one of the combinations listed below, as determined by the Investigator and number of patients enrolled per cohort. Patients will continue to receive treatment until the occurrence of radiological disease progression using RECIST v1.1 or unmanageable drug-related AEs despite dose modification. Guidelines for dose modifications and discontinuations due to AEs will be provided.

Alternate infusion parameters may be implemented with approval from the DSMC.

Arm 3a: NUFOX + bevacizumab Q1W (2nd-line patients)

C1D1 and beyond:

- Bevacizumab 5 mg/kg:
  - 90 minutes for the first dose
  - 60 minutes for the second dose (if first dose is tolerated)
  - 30 minutes for subsequent doses (if second dose is tolerated)
- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion (concurrently with oxaliplatin)

- Oxaliplatin 85 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion (concurrently with LV)
- NUC-3373 IV at the combination dose and infusion duration determined at the DSMC meeting for Part 2 dose escalation

NUC-3373 and LV are given on Days 1, 8, 15 and 22 in each 28-day treatment cycle (Q1W).

Oxaliplatin and bevacizumab are given on Days 1 and 15 in each 28-day treatment cycle (Q2W), as per the respective Prescribing Information.

Arm 3b: NUFOX + bevacizumab Q2W (2nd-line patients)

C1D1 and beyond:

- Bevacizumab 5 mg/kg:
  - 90 minutes for the first dose
  - 60 minutes for the second dose (if first dose is tolerated)
  - 30 minutes for subsequent doses (if second dose is tolerated)
- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion (concurrently with oxaliplatin)
- Oxaliplatin 85 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion (concurrently with LV)
- NUC-3373 IV at the combination dose and infusion duration determined at the DSMC meeting for Part 2 dose escalation

NUC-3373, LV, oxaliplatin and bevacizumab are given on Days 1 and 15 in each 28-day treatment cycle (Q2W).

Arm 3c: NUFIRI + bevacizumab Q1W (2nd-line patients)

C1D1 and beyond:

- Bevacizumab 5 mg/kg:
  - 90 minutes for the first dose
  - 60 minutes for the second dose (if first dose is tolerated)
  - 30 minutes for subsequent doses (if second dose is tolerated)
- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion (concurrently with irinotecan)
- Irinotecan 180 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion (concurrently with LV)
- NUC-3373 1500 mg/m^2 IV over 120 minutes

NUC-3373 and LV are given on Days 1, 8, 15 and 22 in each 28-day treatment cycle (Q1W).

Irinotecan and bevacizumab are given on Days 1 and 15 in each 28-day treatment cycle (Q2W), as per the respective Prescribing Information.

Arm 3d: NUFIRI + bevacizumab Q2W (2nd-line patients)

C1D1 and beyond:

- Bevacizumab 5 mg/kg:
  - 90 minutes for the first dose
  - 60 minutes for the second dose (if first dose is tolerated)
  - 30 minutes for subsequent doses (if second dose is tolerated)
- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion (concurrently with irinotecan)
- Irinotecan 180 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion (concurrently with LV)
- NUC-3373 1500 mg/m^2 IV over 120 minutes

NUC-3373, LV, irinotecan and bevacizumab are given on Days 1 and 15 in each 28-day treatment cycle (Q2W).

Arm 3e: NUC-3373 + bevacizumab Q1W (Maintenance patients)

C1D1 and beyond:

- Bevacizumab 5 mg/kg:
  - 90 minutes for the first dose
  - 60 minutes for the second dose (if first dose is tolerated)
  - 30 minutes for subsequent doses (if second dose is tolerated)
- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion
- NUC-3373 2500 mg/m^2 IV over 200 minutes

NUC-3373 and LV are given on Days 1, 8, 15 and 22 in each 28-day treatment cycle (Q1W).

Bevacizumab is given on Days 1 and 15 in each 28-day treatment cycle (Q2W), as per the Prescribing Information.

Arm 3f: NUFOX + cetuximab Q1W or Q2W (2nd-line patients)

C1D1 and beyond:

- Cetuximab infusion to be completed 1 hour prior to NUFOX infusions:
  - First dose: 400 mg/m^2 IV over 120 minutes
  - Subsequent doses: 250 mg/m^2 IV over 60 minutes
- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion (concurrently with oxaliplatin)
- Oxaliplatin 85 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion (concurrently with LV)
- NUC-3373 IV at the combination dose and infusion duration determined at the DSMC meeting for Part 2 dose escalation

The NUC-3373 + LV administration schedule may be Q1W or Q2W, depending on emerging data.

Oxaliplatin is given on Days 1 and 15 in each 28-day treatment cycle (Q2W) and cetuximab is given on Days 1, 8, 15 and 22 in each 28-day treatment cycle (Q1W), as per the respective Prescribing Information.

Arm 3g: NUFIRI + cetuximab Q1W or Q2W (2nd-line patients)

C1D1 and beyond:

- Cetuximab infusion to be completed 1 hour prior to NUFIRI infusions:
  - First dose: 400 mg/m^2 IV over 120 minutes
  - Subsequent doses: 250 mg/m^2 IV over 60 minutes
- LV 400 mg/m^2 IV (or equivalent levo-leucovorin) over 120 minutes prior to NUC-3373 infusion (concurrently with irinotecan)
- Irinotecan 180 mg/m^2 IV over 120 minutes prior to NUC-3373 infusion (concurrently with LV)
- NUC-3373 1500 mg/m^2 IV over 120 minutes

The NUC-3373 + LV administration schedule may be Q1W or Q2W, depending on emerging data.

Irinotecan is given on Days 1 and 15 in each 28-day treatment cycle (Q2W) and cetuximab is given on Days 1, 8, 15 and 22 in each 28-day treatment cycle (Q1W), as per the respective Prescribing Information.

In all bevacizumab-containing arms, NUC-3373 dose adjustments may occur depending on emerging data.

Guidance on combination agent infusion time has been provided above; however, in all instance's combination agents may be administered in accordance with local and national guidelines.

Panitumumab combination arms may also be opened (~10 2nd-line patients), depending on emerging data.

Cohorts of rapid progressors may also be opened (~10 patients), depending on emerging data.

8.4 NUC-3373 Drug Destruction

Used vials of NUC-3373 should be destroyed in accordance with local procedures and documented in the drug accountability and drug destruction log. A copy of the disposal certificates should be kept in the study file.

8.5 NUC-3373 Study Drug Accountability

The US Food and Drug Administration (FDA) and other applicable regulatory authorities require accounting of all study drug received by each study centre. Records of drug disposition required include the date received by the centre, date administered, quantity administered, and the patient to whom study drug was administered. The Investigator is responsible for the accountability of all used and unused study drug containers and unused study drug. Each study centre is to use a study drug accountability log to document study drug disposition. All items on this form are to be completed in full.

The Investigator identification number and patient initials (as allowed by local regulations) and identification number are to be recorded on each study drug accountability log. Each time study personnel dispense study drug for a patient, he or she is to record the date dispensed, amount

of study drug dispensed, Lot number, and the dispenser's initials. Study personnel are to monitor the inventory of clinical supplies and maintain a count of all used and unused study drug. The Sponsor's designated site monitor will review study drug accountability records and remaining drug supplies during routine monitoring visits.

8.6 Management of Overdose of NUC-3373

The initial dose of NUC-3373 intended for use on this protocol is 1500 mg/m^2. In the Phase I, first-in-human study, the highest dose studied at the time of writing this protocol has been 3250 mg/m^2. Should a substantial overdose occur, there is no known antidote.

In the event of a substantial overdose of any of the agents used in the prescribed combinations (LV, oxaliplatin, irinotecan, bevacizumab, cetuximab, panitumumab), the management should follow guidance in the Prescribing Information or standard local practice.

Any patient who inadvertently receives a dose of any agent in this study higher than intended should be monitored closely, managed with appropriate supportive care, including transfusion and haematopoietic growth factors as needed, until recovery. Such overdoses should be recorded as follows:

1. If an overdose occurs in the course of the study, site personnel must inform the Investigator and monitor immediately upon discovery of the event. An overdose will be recorded on the treatment CRF page and any associated AEs/SAEs will be recorded as the AE diagnosis/symptoms on the relevant AE/SAE page in the CRF. An overdose with no associated symptoms is only reported on the treatment CRF.
2. All overdoses should be tracked as a violation.

9 DOSE MODIFICATIONS

Adverse events may be managed by dose delays and/or dose reductions according to the clinical situation. For patients experiencing toxicity related to NUC-3373 in NUFOX and NUFIRI cohorts, initially the NUC-3373 dose modification guidelines should be followed.

Note that for patients in the PK sub-study, any dose modifications required prior to C1D15 will make the patient non-evaluable for the sub-study analysis. Such patients will be replaced.

Advice on how to modify NUC-3373 dosing for haematological and non-haematological toxicities in Part 1 is given in Table 18 and Table 19 and in Part 2 and Part 3 is given in Table 20.

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.

Treatment between cycles can be delayed for up to 21 days in order 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 the case of the agents given in combination with NUC-3373 and in patients receiving clinical benefit, AEs attributed to the combination agents may be managed by dose adjustment, holds or discontinuation of the agent in accordance with standard clinical practice. For example, a patient assigned to the NUFOX regimen who is receiving clinical benefit and who experiences neuropathy that is judged as related to the oxaliplatin may undergo an oxaliplatin treatment-free interval at the discretion of the Investigator. However, treatment with NUC-3373 must be continued for the patient to remain on treatment for the purposes of this protocol.

Table 18 Dose reduction guidelines for Part 1, Arms 1a and 1b

Reduction	NUC-3373 Days 1 and 15 every 28 days	LV Day 1 and 15
1	75% of starting dose[1]	100% of starting dose[2]
2	50% of starting dose[1]	100% of starting dose[2]

[1] Two dose reductions allowed
[2] LV dose should be maintained but may be reduced at the discretion of the Investigator, with confirmation from the Medical Monitor and Sponsor

Table 19 Dose reduction guidelines for Part 1, Arm 1c and 1d

Reduction	NUC-3373 Days 1, 8, 15 and 22 every 28 days	LV Days 1, 8, 15 and 22 every 28 days
1	75% of starting dose[1]	100% of starting dose[2]
2	50% of starting dose[1]	100% of starting dose[2]

[1] Two dose reductions allowed

[2] LV dose should be maintained but may be reduced at the discretion of the Investigator, with confirmation from the Medical Monitor and Sponsor

Table 20 Dose reduction guidelines for Part 2 and Part 3

Reduction	NUC-3373	LV	Combination agent
1	75% of Starting Dose[1]	100% of starting dose[2]	Per Prescribing Information[3]
2	50% of Starting Dose[1]	100% of starting dose[2]	Per Prescribing Information[3]

[1] Two dose reductions allowed. Minimum 25% reduction per reduction

[2] LV dose should be maintained but may be reduced in accordance with label

[3] Doses of combination agents should be reduced in accordance with Prescribing Information or standard local practice

9.1 Criteria for Continuation of Treatment

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

- ANC $\geq 1.5\times10^9$/L (growth factor support permitted after Cycle 1)
- Platelet count $\geq 75\times10^9$/L without platelet transfusion (platelet transfusion permitted after Cycle 1)
- No evidence of disease progression (based on radiographic assessment)
- Recovery from all clinically significant toxicities to ≤Grade 2 or to baseline grade present at study entry

Administration of study drug should be held until all of the re-treatment criteria are met. Any missed dose(s) should not be made up if treatment delay exceeds 24 hours. If treatment delay is <24 hours, subsequent scheduled dose(s) may be administered. If a DLT occurs during a cycle, doses of study drug will be withheld for the remainder of the cycle and subsequent cycles will be administered at the previously tested next lower dose level.

9.2 Dose Modifications (Dose Delays and Dose Reductions)

Treatment between cycles can be delayed for up to 21 days in order for patients to meet the re-treatment criteria before starting their next cycle unless receiving clinical benefit in the opinion of the Investigator. 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 will be based on the most severe toxicity. A patient who experiences a toxicity meeting the definition of DLT (during any cycle) but whose toxicity recovers within 21 days may be dose reduced to the next lower dose level. If a patient experiences a sub-DLT toxicity, they may be dose reduced at the Investigators discretion. Any patient who experiences one or more recurrent, clinically significant toxicities after the initial dose reduction may have one further dose reduction. Patients who continue to experience clinically significant toxicity despite two dose reductions will either be discontinued from study treatment or may be switched to a Q2W schedule, if the timecourse of the toxicity indicates that a different schedule may improve tolerability. 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 Sponsor based on all data currently available for NUC-3373 at the time.

Patients who have completed the DLT period may be switched from a Q1W to a Q2W schedule if they experience drug-related toxicity (other than fatigue) that would otherwise lead to treatment discontinuation. In addition, patients who have completed the DLT period may move from a Q2W to a Q1W schedule. The decision to switch schedules must be discussed with and approved by the Sponsor on an individual patient basis.

General guidance for dose modifications of NUC-3373 is provided in Table 21.

Table 21 Dose modifications for Cycle 2 onwards and for non-DLT toxicities within Cycle 1

Toxicity Grade	Recommended immediate action with NUC-3373	Recommendation for dose modification of NUC-3373 at start of a new cycle
Mucositis		
Grade 2	No immediate action	Continue current dose level with appropriate mouth care.
Grade 3 or 4	Hold	If resolved to ≤Grade 2 within 14 days reduce by 1 dose level. If mucositis has not resolved to ≤Grade 2 within 14 days, permanently stop treatment.
Febrile neutropaenia		
Grade 3 or 4	Hold	If neutropaenia resolved to ≤Grade 1, reduce by 1 dose level. If not resolved to ≤Grade 1 within 14 days, permanently stop treatment.
Neutropaenia		
Grade 2	Hold only if starting a new cycle [delay of max 14 days allowed until ANC≥1.5 x 10^9/L]. If within a cycle, no immediate action required.	If study drug held at the start of new cycle, continue current dose level once neutropaenia resolved to ≤Grade 1 (ANC≥1.5 x 10^9/L).
Grade 3 or 4	Hold, consider growth factor support.	If held **at the start of new cycle** continue at the next lower dose level if neutropaenia resolved to ≤Grade 1 within 14 days. If study drug held **within a cycle**, if neutropaenia resolved ≤Grade 2 within 14 days, continue at next lower dose level. If neutropaenia has not resolved to ≤Grade 2 within 14 days, permanently stop treatment.
Thrombocytopaenia		
Grade 2	Hold if starting or within a cycle [delay of max 14 days allowed until platelets ≤ Grade 1]	If held **at the start or within cycle** continue at the next lower dose level if thrombocytopaenia resolved to ≤Grade 1 within 14 days. If thrombocytopaenia has not resolved to ≤Grade 1 within 14 days, permanently stop treatment.

Toxicity Grade	Recommended immediate action with NUC-3373	Recommendation for dose modification of NUC-3373 at start of a new cycle
Grade 3 or 4	Hold and follow treatment guidelines	When thrombocytopaenia resolved to ≤Grade 1, continue current study at the next lower dose level. If thrombocytopaenia has not resolved to ≤Grade 1 within 14 days, permanently stop treatment.
Diarrhoea		
Grade 2	Hold if starting or within a cycle [delay of max 14 days allowed until platelets ≤Grade 1]	Refer to Appendix 4
Grade 3 or 4	Hold and follow treatment guidelines	Refer to Appendix 4

9.3 Suggested Management of Toxicity

9.3.1 Nausea and Vomiting

- Consider anti-emetic medications, *e.g.*, granisetron, dexamethasone, cyclizine
- Maintain adequate hydration, including use of intravenous fluids if indicated
- Supplement electrolytes, particularly potassium and magnesium, to recommended levels
- Discontinue any concomitant medications that could contribute to nausea and vomiting
- Rule out other potential aetiologies (*e.g.,* gastrointestinal tract obstruction)
- Consider prophylactic anti-emetic medications per ASCO guidelines for chemotherapy regimens of moderate emetic risk prior to next scheduled treatment

9.3.2 Diarrhoea

- All available anti-diarrhoeal medications, including loperamide and opium preparations *etc*, should be considered for treatment
- Maintain adequate hydration, including use of intravenous fluids if indicated
- Supplement electrolytes, particularly potassium and magnesium, to recommended levels
- Avoid oral supplementation of electrolytes since diarrhoea could be exacerbated in some cases
- Rule out other potential causes, including infectious aetiologies
- Discontinue any concomitant medications that could exacerbate diarrhoea
- Avoid the use of diuretics and laxatives
- Refer to Appendix 4 for further guidance

9.3.3 Mucositis

- Encourage good oral hygiene, including cleaning teeth after each meal and at bedtime, using soft-bristled toothbrush
- Rinse mouth using chlorhexidine (or similar) mouthwash after brushing teeth, after mealtimes and in the evenings
- Careful dental flossing once daily; avoid visits to dental hygienist during treatment
- Dentures cleaned after each meal and soaked overnight in cleaning solution
- Avoidance of spicy, rough or crunchy foods
- Manage pain from mucositis with benzydamine mouthwash (Difflam), systemic analgesics such as dispersible paracetamol or aspirin
- For ulcers, local analgesia including Mucaine equivalent 10 mL qds, lidocaine 1% gel, topical Bonjela up to every 3 hours, buccal hydrocortisone (2.5 mg) tablets 4x daily or orabase ointments
- Sucralfate suspension 5 mL qds rinsed around the mouth
- For patients with Grade 3/4 mucositis, use low dose opiates; consider rinsing with Gelcair oral gel (15 mL sachets)

9.3.4 Palmar-Plantar Erythrodysesthaesia (hand-foot syndrome)

- For Grade 1 or above, consider starting pyridoxine 50 mg tds

9.3.5 Dermatologic Toxicity

Severe acneiform rash can occur with exposure to an EGFR inhibitor. Recommended dose modifications for severe (CTCAE v5.0 Grade 3 or 4) acneiform rash are specified in Table 22.

Table 22 Dose modification guidelines for severe acneiform rash

Occurrence	Action with EGFR inhibitor	Outcome	Action
First	Delay treatment 1-2 weeks	Improvement	Continue at standard dose
		No improvement	Discontinue
Second	Delay treatment 1-2 weeks	Improvement	Continue at 80% of the dose
		No improvement	Discontinue
Third	Delay treatment 1-2 weeks	Improvement	Continue at 60% of the dose
		No improvement	Discontinue
Fourth	Discontinue		
In general, patients should be treated as per local standards of care for EGFR inhibitor-related rash. However, the following guidance should also be considered:			

Application of a skin moisturiser and a broad-spectrum sunscreen of SPF ≥30 when going outside is recommended in all patients, since the rash is accompanied by dry skin and may be aggravated by sun exposure.

Prevention of acneiform rash caused by EGFR inhibitors should include topical corticosteroids (e.g., hydrocortisone 2.5%, alclometasone) and oral antibiotics (e.g., minocycline, doxycycline, or antibiotics covering skin flora) twice daily for at least the first 6 weeks.

For occurrence of rash while on therapy, a dermatologist should be consulted. Potential treatment options include:

In patients who have already developed Grade 1/2 rash: topical corticosteroids (e.g., hydrocortisone 2.5%, alclometasone) and oral antibiotics (e.g., minocycline, doxycycline, or antibiotics covering skin flora) twice daily for at least 4 weeks is recommended.

In cases where secondary infections are suspected: a bacterial swab culture should be performed in order to institute oral antibiotic therapy based on sensitivities.

9.3.6 Infusion Reactions

Infusion reactions may occur with any of the study drugs. Infusion reactions should be managed as follows:

- Reduce infusion rate by 50% in patients experiencing a mild or moderate (Grade 1 or 2) infusion reaction for the duration of that infusion
- Terminate the infusion in patients experiencing severe infusion reactions. Depending on the severity and/or persistence of the reaction, permanently discontinue the combination agent.

9.3.7 Ocular Toxicity

Patients presenting with signs or symptoms suggestive of eye disorders such as acute or worsening eye inflammation, lacrimation, light sensitivity, blurred vision, eye pain and/or red eye should be referred promptly to an ophthalmology specialist for evaluation.

9.3.8 Fatigue

Fatigue is an expected AE for patients with heavily pre-treated advanced cancer who will be enrolled to this study. As clinical benefit is most likely to be realised only with continued treatment, best efforts that include supportive care and carefully considered dose reductions of all or some of the agents within a treatment regimen can be considered. Changes in dose for any agent should be discussed with the medical monitor prior to implementation.

9.3.9 Combination Therapies

Patients receiving treatment with combination therapies in Parts 2 and 3 of the study should be managed as outlined in the sections below.

9.3.9.1 Oxaliplatin

Serious and fatal hypersensitivity reactions, including anaphylaxis, can occur with oxaliplatin within minutes of administration and during any cycle of treatment. Oxaliplatin should be discontinued in patients who experience severe hypersensitivity reactions which are not manageable with dose reductions, infusion time adjustments and prophylactic treatment with steroids and histamine receptor antagonists.

For patients experiencing oxaliplatin-related toxicities in NUFOX cohorts, the dose of oxaliplatin may be adjusted (through agreement with the DSMC) based on safety events.

If oxaliplatin-related AEs are observed, dose de-escalation should be performed as per the Prescribing Information or standard local practice.

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

9.3.9.2 Irinotecan

Early and late diarrhoea can occur with irinotecan. Early diarrhoea may be accompanied by cholinergic symptoms, which may be prevented or ameliorated by atropine. Late diarrhoea can be life-threatening and should be treated promptly with loperamide. Patients with diarrhoea must be monitored and given fluid and electrolytes as needed. Antibiotic therapy should be initiated if patients develop ileus, fever or severe neutropaenia.

For patients experiencing irinotecan-related toxicities in the NUFIRI cohorts, the dose of irinotecan may be adjusted (through agreement with the DSMC) based on safety events.

If irinotecan-related AEs are observed, dose de-escalation should be performed as per the Prescribing Information or standard local practice.

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

9.3.9.3 Bevacizumab

Infusion reactions reported across clinical studies and post-marketing experience include hypertension, hypertensive crises associated with neurologic signs and symptoms, wheezing, oxygen desaturation, Grade 3 hypersensitivity, chest pain, headaches, rigors, and diaphoresis. Discontinue bevacizumab in patients who develop a severe infusion reaction and administer appropriate medical therapy (*e.g.,* epinephrine, corticosteroids, intravenous antihistamines, bronchodilators and/or oxygen).

The incidence of severe hypertension is increased in patients receiving bevacizumab as compared to patients receiving chemotherapy alone. Across clinical studies, the incidence of Grade 3-4 hypertension ranged from 5% to 18%. Monitor blood pressure every two to three weeks during treatment with bevacizumab. Treat with appropriate anti-hypertensive therapy and monitor blood pressure regularly.

Bevacizumab treatment must be discontinued in patients with gastrointestinal perforations or wound healing complications requiring medical intervention.

For patients undergoing elective surgery, bevacizumab must be withheld for at least 28 days prior to the surgery and must not be administered for at least 28 days following surgery and until the wound is fully healed.

Bevacizumab should be discontinued in patients with Grade 3-4 haemorrhage.

If bevacizumab-related AEs are observed, dose de-escalation should be performed as per the Prescribing Information or standard local practice.

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

9.3.9.4 EGFR inhibitors

EGFR inhibitors can cause serious and fatal infusion reactions and must be discontinued in patients who experience serious infusion reactions.

Cardiopulmonary arrest or sudden death have occurred in patients receiving EGFR inhibitors with radiation therapy or with platinum-based therapy and fluorouracil and extra caution needs to be applied, especially in patients with pre-existing heart conditions. Patients receiving EGFR inhibitors must be monitored for serum electrolytes, including magnesium, potassium, and calcium, during and after administration.

If EGFR inhibitor-related AEs are observed, dose de-escalation should be performed as per the Prescribing Information or standard local practice.

For a complete overview of EGFR inhibitor-related AEs, refer to the cetuximab or panitumumab Prescribing Information.

9.5 Schedule Modification

For patients having received treatment for at least 4 cycles at a NUC-3373 dose level subsequently determined to be below the recommended dose, the Investigator may, after discussion with the Medical Monitor and Sponsor, consider an increase in the dose of NUC-3373. If the dose level is increased and found to be not as well tolerated as the previous lower dose level, the NUC-3373 dose level may be reduced back to the previous level and not considered one of the maximum of two dose reductions allowed per patient. Best efforts should be given to maintaining a patient receiving clinical benefit on study.

10 OTHER TREATMENTS (NON-IMP)

All prescription and non-prescription medications and therapies, including pharmacologic doses of vitamins, herbal medicines or other non-traditional medicines, taken from 30 days prior to the first dose of NUC-3373 through the EoT Visit must be recorded in the CRF. All prior anti-cancer therapies from initial diagnosis up until enrolment must be recorded in the CRF.

10.1 Support Medication

Patients may receive prophylactic medical treatment at the Investigator's discretion to prevent AEs common to the administration of anti-cancer agents including, but not restricted to, anaphylaxis, nausea and vomiting. All support medications must be recorded in the CRF.

Support medications should be administered according to the Prescribing Information or standard local practice for the relevant combination agents. The following support medications are recommended: anti-emetics (palonosetron, granisetron, aprepitant, dexamethasone, metoclopramide, domperidone, cyclizine); anti-histamines; atropine (*e.g.,* for irinotecan-containing regimens); sunscreen of ≥SPF 30; topical corticosteroids (*e.g.,* hydrocortisone 2.5%, alclometasone); and oral antibiotics (*e.g.,* minocycline, doxycycline, or antibiotics covering skin flora) for cetuximab-containing regimens.

10.2 Haematopoietic Growth Factor Support

The prophylactic use of haematopoietic growth factors (*e.g.,* G-CSF) is not permitted in the first cycle in patients being evaluated for DLT. However, the Investigator may include haematopoietic growth factor support according to local protocols and as prophylaxis after any febrile neutropaenic episode in order to enable the patient to continue on study after completion of Cycle 1. All haematopoietic growth factors used from 30 days prior to date of consent until 30 days after administration of last dose of NUC-3373 must be recorded in the CRF. Any blood and platelet transfusions should also be recorded in the CRF.

10.3 Concomitant Medications

May be given as medically indicated unless prohibited as outlined in Section 10.3.2. All concomitant medications used from 30 days prior to date of consent until 30 days after administration of last administration of NUC-3373 must be recorded in the CRF.

10.3.1 Contraception Methods

As defined below, male patients and female patients of child-bearing potential must agree to practice true abstinence or to use two highly effective forms of contraception, one of which must be a barrier method.

- True abstinence is defined as refraining from sexual intercourse. True abstinence is only acceptable if this practice is in line with the patient's preference and usual lifestyle. Periodic abstinence (*e.g.,* calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to the investigational drug, and withdrawal are not acceptable methods of contraception.
- Surgical sterilisation (males)
- Intrauterine device or intrauterine system
- Oral contraception plus a barrier method

- Double-barrier method (*e.g.,* male condom or a diaphragm plus a vaginal spermicidal cream)

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. Female patients of childbearing potential must have a negative pregnancy test within seven days prior to the first study drug administration. Female patients are considered to be of child-bearing potential unless they are post-menopausal (12 months of amenorrhea) or surgically sterile.

10.3.2 Prohibited Therapy

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

- Other cytotoxic chemotherapy
- Radiotherapy, excluding a short cycle of palliative radiotherapy (*e.g.,* for bone pain)
- Immunotherapy including immunosuppressive therapy
- Radioimmunotherapy
- Hormone therapy (other than contraceptives, hormone-replacement therapy, or megestrol acetate)
- Other biologic agents intended for the treatment of CRC (other than haematopoietic growth factors, which are allowed if clinically indicated and used in accordance with instructions provided in the package inserts)
- Any therapies intended for the treatment of CRC, whether approved by local regulatory authorities or investigational
- Drugs that are known to prolong QTc interval (refer to Appendix 3)
- 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 (must also be avoided for six weeks after last dose of study medication)
- Diuretics and laxatives should be avoided in patients experiencing treatment-related diarrhoea and cholinergic reactions
- Patients in bevacizumab-containing arms should not receive full-dose anti-coagulation therapy, with the exception of patients who have been on stable doses for at least 6 months and have tolerated prior bevacizumab treatment

10.3.3 Therapy to be Used with Caution

The following warnings and precautions regarding concomitant medications apply to irinotecan containing dose combinations:

- **Strong inhibitors of UGT1A1**, including but not limited to ketoconazole, indinavir, itraconazole, lopinavir, nelfinavir, ritonavir, saquinavir, atazanavir, or gemfibrozil, should not be administered with irinotecan. Strong inhibitors of UGT1A1 should be discontinued at least 1 week prior to starting irinotecan. Do not administer strong

inhibitors of UGT1A1 with irinotecan unless there are no other therapeutic alternatives. *Please contact the Medical Monitor before concomitant administration of strong UGT1A1 inhibitors and irinotecan*

- Interaction between irinotecan and neuromuscular blocking agents cannot be ruled out. Irinotecan has anti-cholinesterase activity, which may prolong the neuromuscular blocking effects of suxamethonium and the neuromuscular blockade of non-depolarising drugs may be antagonised. Caution should be used when using neuromuscular blocking agents with irinotecan

For patients in all parts and arms 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) is prohibited within 4 weeks of the first dose of study treatment. Patients requiring anti-coagulant treatment should switch to low molecular weight heparin or anti-Xa inhibitors with a half-life of ≤12 hours.

Refer to the NUC-3373 IB for current information on potential drug interactions.

10.3.4 COVID-19 Vaccinations

A risk assessment performed in accordance with MHRA guidance 'Managing clinical trials during COVID-19' (MHRA, 2021) concluded that a COVID-19 vaccine given to a study patient is considered a simple concomitant medication.

No action is required in relation to COVID-19 vaccination timing with respect to administration of NUC-3373 and the other combination agents used in this study.

11 TUMOUR RESPONSE ASSESSMENTS

11.1 Tumour Measurements and Assessment of Disease Response

Patients must have at least one lesion that can be accurately assessed at baseline by CT or MRI 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. The same radiological method used at baseline must be used to follow lesions throughout the study. Disease status must be assessed according to RECIST v1.1 criteria, with target and non-target lesions identified, measured and followed throughout the study (Appendix 1). Biopsied lesions should not be designated as target lesions for the purposes of RECIST v1.1 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.

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 radiologically-confirmed progressive disease, tumour measurements and disease response assessments should continue every 8 weeks (±7 days) from Cycle 1 Day 1 until progressive disease is radiologically confirmed.

Tumour measurements and disease response assessments should be performed any time disease progression is suspected. Patients should not discontinue treatment because of clinical signs of progression until it has been radiologically confirmed by radiologic assessment.

Complete responses (CRs) and partial responses (PRs) must be confirmed by repeated images at least 4 weeks after initial documentation.

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 after the start of study treatment.

12 SAFETY REPORTING

This study evaluates NUC-3373 in combination with a variety of agents commonly used in treatment of advanced CRC, including LV, oxaliplatin, irinotecan, bevacizumab, cetuximab, and panitumumab. In considering safety assessments and reporting, it is anticipated that some safety findings may be attributable to a specific agent in a combination regimen, while others may not be attributable to a specific agent. In this section, the term "study drug(s)" refers to any of the agents administered to the patient in the arm of the study to which he/she was enrolled.

12.1 Definitions

The Investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of relationship to study drug(s) or clinical significance.

An **AE** is defined as any untoward medical occurrence in a patient enrolled into this study regardless of its causal relationship to study drug(s). Patients or their legally authorised representatives will be instructed to contact the Investigator or Sub-Investigator at any time after signing the ICF if any symptoms develop.

A **TEAE** is defined as any event not present before exposure to study drug(s) or any event already present that worsens in either intensity or frequency after exposure to study drug(s).

A **SAE** is defined as any event that:

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a significant medical event in the Investigator's judgment (*e.g.,* may jeopardise the patient or may require medical/surgical intervention to prevent one of the outcomes listed above)

Disease progression and disease-related death will <u>not</u> be considered an AE or SAE.

An **adverse drug reaction (ADR)** is an AE which is considered to be causally related to any dose of study drug(s). This means that a causal relationship between study drug(s) and the AE is at least a reasonable possibility, *i.e.,* the relationship cannot be ruled out.

An **unexpected drug reaction** is an ADR, the nature or severity of which is not consistent with applicable product information. For NUC-3373, please refer to the IB Reference Safety Information (RSI). For the other agents used in combination with NUC-3373, please refer to the applicable Summary of Product Characteristics (SmPC), which serve as their RSI.

A **suspected unexpected serious adverse reaction (SUSAR)** is a serious ADR, the nature or severity of which is not consistent with the applicable product information (*e.g.,* IB for an unapproved IMP).

An important medical event that may not result in death, be life-threatening, or require hospitalisation may be considered an SAE when, based upon appropriate medical judgment, it may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic

bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalisation, or the development of drug dependency or drug abuse. Please consult Section 12.5 for the specific mechanism by which SAEs are to be reported.

12.2 Adverse Event Reporting

At every study visit, patients will be asked nondirective questions to elicit any medically-related changes in their well-being. They will also be asked if they have been hospitalised, had any accidents, used any new medications, or changed concomitant medication regimens (both prescription and over-the-counter medications). In addition to patient observations, AEs will be documented from any data collected on the CRF or other documents that are relevant to patient safety. Any allergic reaction to the agents administered as study drug treatment must be reported as an AE.

After informed consent has been obtained, but prior to initiation of study drug, only AEs and SAEs caused by a protocol-mandated intervention will be reported. Thereafter, all AEs occurring up to and including 30 days after the last dose of study drug has been administered must be reported in detail on the AE CRF. Disease progression in the medical opinion of the physician and/or disease-related morbidity and mortality as a study endpoint will not be considered an AE or SAE but should be captured on the Death CRF. Information to be collected for each AE includes onset date, type of event, aetiology, Investigator-specified assessment of severity and relationship to study drug, seriousness, any required treatment or evaluations, outcome and date of resolution. AEs resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed for 30 days after the patient's last dose or until resolution, whichever comes first.

Pre-existing conditions (present before the start of the AE collection period) are considered concurrent medical conditions and should not be recorded as AEs. However, if the patient experiences a worsening or complication of such a concurrent condition, the worsening or complication should be recorded as an AE. Pre-existing AEs that worsen should be followed until 30 days after the patient's last dose or resolution to the grade or level present at study entry. Investigators should ensure that the AE term recorded captures the change in the condition (*e.g.,* "worsening of [condition]").

Insufficient clinical response, efficacy, or pharmacological action should NOT be recorded as an AE. The Investigator must make the distinction between exacerbation of pre-existing illness and lack of therapeutic efficacy. Progressive disease is NOT an AE; however, some sequelae of progressive disease (*i.e.,* pain, neurologic impairment) may be reported as AEs (generally not related to study drug(s)).

Abnormal laboratory values or test results constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, require therapy or further diagnosis beyond repeat testing for confirmation, or (if not associated with clinical signs or symptoms) remain at levels consistent with severe abnormalities despite appropriate medical intervention. It is requested that when reporting AEs for which potentially redundant CTCAE terms exist, the Investigator uses the more clinically-oriented terminology (for example, 'anaemia' is preferable to 'haemoglobin decreased').

It is also requested that in the setting of an allergic reaction or suspected allergic reaction considered by the Investigator to be related to study drug(s), the Investigator reports both the specific symptoms associated with the reaction (*i.e.,* 'urticaria', 'dyspnoea') and also report the

appropriate term indicating the allergic reaction ('allergic reaction' or 'anaphylaxis' if appropriate [Immune System Disorders; CTCAE v5.0]).

12.3 Assessment of Causality to Study Drug(s)

The Investigator's assessment of an AE's relationship to study drug(s) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

The relationship of an AE to study drug(s) in this study should be classified using the following guidelines:

Definitely Related: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

Probably Related: There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

Possibly Related: There is some evidence to suggest a causal relationship (*e.g.,* because the event occurs within a reasonable time after administration of the study drug). However, the influence of other factors may have contributed to the event (*e.g.,* the patient's clinical condition, other concomitant treatments).

Unlikely Related: There is little evidence to suggest a causal relationship (*e.g.,* the event did not occur within a reasonable time after administration of the study drug). There is another reasonable explanation for the event (*e.g.,* the patient's clinical condition, other concomitant treatments).

Not Related: There is no evidence of any causal relationship. N.B. An alternative cause for the AE should be given.

12.4 Assessment of Severity

The severity of each AE is to be assessed by the Investigator according to CTCAE, v5.0. If the AE is not included in the CTCAE, then the Investigator should determine the intensity of the AE according to the criteria described in Table 23.

Table 23 Intensity of adverse events not included in CTCAE

Intensity	Criteria
Mild (Grade 1)	AE that disappears or is easily tolerated on continuation of study drug
Moderate (Grade 2)	AE is sufficiently discomforting to cause interference with usual work activities
Severe (Grade 3)	AE that is incapacitating, with inability to work or perform daily activities
Life-Threatening (Grade 4)	AE that is *potentially* life-threatening*
Death (Grade 5)	Death related to AE

* If a life-threatening (Grade 4) AE is *immediately* life-threatening, the event is by definition serious and is to be reported as described in Sections 12.6 and 12.7.

12.5 SAE Reporting

Any AE considered serious by the Investigator or sub-Investigator or that meets the seriousness criteria and that occurs from first administration of study drug(s) through 30 days after last administration of a study drug must be reported to the Sponsor within 24 hours from the time study site personnel first learn about the event. The SAE report should be entered directly in the EDC. If the EDC is unavailable. The SAE report may be completed and emailed or faxed using the contact details in Table 24.

Table 24 Pharmacovigilance contact details

Email	Nucana@primevigilance.com

If the patient is hospitalised because of or during the course of an SAE, then a copy of the hospital discharge summary (if available) should be provided in an SAE follow-up report in the EDC or, if needed, using the contact details listed in Table 24 as soon as it becomes available.

NuCana will notify appropriate regulatory authorities of any unexpected, fatal or life-threatening experience that is determined to be related to the use of the study drug(s). Refer to Section 12.7 for more details.

The Investigator or sub-Investigator is responsible for informing the relevant institutional review board/ethics committee (IRB/EC). Copies of SAE correspondence with all Investigators or sub-Investigators, governing authorities, ethics committees, and the Sponsor (or sponsor designee) must be submitted for filing.

A patient experiencing one or more SAEs will receive treatment and follow-up evaluations by the Investigator or sub-Investigator or will be referred to another appropriate physician for treatment and follow-up. All SAEs must be followed to resolution or, if resolution is unlikely, to stabilisation.

Study endpoints in patients with cancer, including CRC, include disease-related mortality and morbidity; as study endpoints, these will not be reported as expedited Investigational New Drug (IND) safety reports, unless there is a serious and unexpected event with evidence of a causal relationship between study drug(s) and the event. As appropriate and based on the frequency of occurrence, SAEs in the study will be reported to the relevant regulatory authorities at an appropriate interval, such as inclusion in the development safety update report.

The following SAEs will not be reported individually in an expedited manner because they are anticipated to occur in the CRC study population receiving standard of care treatment at some frequency independent of study drug exposure:

- Progression of disease
- Death as a consequence of the underlying malignancy

12.6 Expedited Reporting of SAEs

The following SAE reporting requirements apply regardless of the Investigator's assessment of the causality or expectedness of the SAE. If an SAE occurs that requires reporting, an SAE Report Form should be completed in the EDC and communicated within 24 hours of Investigator awareness using the contact details provided in Table 24.

If the SAE has not been reported within the specified timeframe, a reason for the delay must be provided when sending the SAE Report Form. SAEs that are fatal or life-threatening must be notified immediately. For all SAEs, the Investigator is obliged to pursue and provide all required information in accordance with the timelines provided above.

12.7 SUSAR Reporting

All SUSARs must be reported to the responsible Regulatory Authorities and IRBs/ECs within the required timelines:

- Fatal or life threatening SUSARs will be reported within 7 days of receipt. Any additional information will be reported within 8 days of sending the first report.
- All other SUSARs will be reported within 15 days of receipt.

In addition, other safety issues qualify for expedited reporting where they might materially alter the current risk assessment of NUC-3373 or be sufficient to change NUC-3373 administration or the overall conduct of the study.

The Sponsor will notify appropriate regulatory authorities of any fatal or life-threatening experience that is determined to be related to the use of the study drug(s) (expedited report) as soon as possible but no later than 7 calendar days after the initial receipt of the information. Initial notification will be followed by a written report within 15 calendar days.

For unexpected events associated with the use of the study drug(s) which are not fatal or life threatening, the Sponsor will notify the regulatory authorities as soon as possible and no later than 15 days of the initial receipt of information.

The Investigator is responsible for informing the IRB/EC. Copies of SAE correspondence with all Investigators or Sub-Investigators, regulatory authorities, IRBs/ECs and the Sponsor must be submitted for filing.

12.8 Terms and Grading of Adverse Events and Toxicities

All AEs and toxicities must be graded according to CTCAE v5.0.

12.9 Pregnancy

A woman who becomes pregnant during the course of the study should be withdrawn from study treatment immediately. Pregnancy occurring in a patient or partner during the study require expedited reporting. A Pregnancy Notification Report should be completed and submitted to the Sponsor using the contact information provided in Table 24 and within the same timelines as an SAE. All reported pregnancies should be followed and the outcome reported using the Pregnancy Follow-up Report. If the outcome of the pregnancy meets any of the criteria for seriousness, it must also be reported as an SAE.

Examples of pregnancy outcomes that are SAEs include reports of:

- Congenital anomalies or developmental delay, in the foetus or the child
- Foetal death and spontaneous abortion
- Suspected adverse reactions in the neonate that are classified as serious

12.10 Events Exempt from being Reported as AE/SAEs

12.10.1 Progression of Underlying Disease

Disease progression and resultant death will be captured on the CRF. AEs including hospitalisation that are clearly consistent with disease progression will not be reported as individual AE/SAEs. Clinical symptoms of disease progression will only be reported as AEs if the symptom cannot be determined as exclusively due to progression of the underlying malignancy or does not fit the expected pattern of progression for the disease under study.

Every effort should be made to document the objective progression of underlying malignancy. In some cases, the determination of clinical progression may be based on symptomatic deterioration. For example, progression may be evident from clinical symptoms but is not supported by tumour measurements. Or the disease progression is so evident that the Investigator may elect not to perform further disease assessments.

12.10.2 Death on Study Attributed to Malignancy

Death due to disease under study is to be recorded on the Death CRF form, provided that the death is not unexpected and no causal relationship is suspected. The Investigator must clearly state whether the death was expected or unexpected and whether a causal relationship to NUC-3373 or other protocol treatment intervention is suspected.

12.10.3 Elective Admissions and Supportive Care

Elective admissions to hospital for patient convenience or for planned procedures or investigations or treatment as specified in this protocol and standard supportive care are not SAEs and do not require SAE reporting.

12.11 Informing Investigators of New Safety Information

The sponsor will ensure that all Investigators are informed in a timely manner of new safety information regarding NUC-3373 that becomes available. Investigators are responsible for briefing their study team as appropriate.

12.12 Reference Safety Information (RSI) for Assessment of Expectedness

12.12.1 NUC-3373

The Investigator Brochure (IB) supplied by NuCana for NUC-3373 contains the NUC-3373 RSI for this study. Only the IB version with current regulatory and IRB/EC approval for use in the study will be used to assess SAE reports to identify SUSARs.

- **Significant Changes to the RSI**: If patient safety or the risk/benefit assessment has changed or new expected reactions have been added, then approval of the updated IB by applicable Regulatory Authorities and IRBs/ECs will be sought. If new expected reactions have been added to the IB or events have been down-graded to ‘expected’, a justification for the changes will be included in the amendment request. Changes to the IB that impact on patient safety or alter the risk/benefit assessment may require changes to study documentation, such as the ICF. The sponsor will identify any required changes and ensure ICF revisions are made and approved by applicable Regulatory Authorities and IRBs/ECs, and patients re-consented as applicable. Significant updates to the IB shall be attached to the development safety update report (DSUR) (once approved by applicable Regulatory Authorities and IRBs/ECs); however, the IB in effect at the start of the DSUR reporting period serves at the RSI during the reporting period.

- **Non-Significant Changes to the IB**: If changes to the IB are minor and do not include new/removed expected reactions, do not impact on patient safety or alter the benefit/risk assessment, then sites will not receive the updated IB until the end of the DSUR reporting period.

If the non-significant updated IB *is* to be implemented in the new DSUR reporting period, then Regulatory Authority and IRB/EC should be informed of the intention to implement the updated IB after the DSUR reporting period ends. The updated IB will be attached to the DSUR. If new expected reactions have been added to the IB or events have downgraded to 'expected', then the updated IB must receive approval before it is implemented. The IB will be sent to the study sites with a covering letter documenting the changes. This will be circulated after the DSUR has been submitted at the start of the new DSUR reporting period.

12.11.2 Other Agents to be Used in Combination with NUC-3373

The SmPC of each individual agent intended to be administered in conjunction with NUC-3373 in this study will serve as their own RSI. Representative SmPCs for each agent are provided in the NUC-3373 IB (Section 8.1) for ease of reference.

12.13 Data Safety Monitoring Committee (DSMC)

The DSMC will review ongoing and cumulative safety, dosing intensity, PK, PD and clinical activity data from each cohort. In all cohorts, the DSMC will determine if the dose levels/schedules are safe and tolerable and if an arm should be expanded, if other dose levels/schedules should be explored, or if the cohort should be stopped.

The DSMC will review all data in accordance with the European Medicines Agency (EMA) guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products (EMEA/CHMP/SWP/28367/07 Rev. 1, EMA 01 Feb 2018).

Members of the DSMC will include (at a minimum):

- Chief Investigators from US and Europe
- Medical Monitors
- Sponsor's Medical Director
- Sponsor's study management staff

13 STATISTICAL CONSIDERATIONS

A Statistical Analysis Plan (SAP) will be finalised before database lock and conduct of the final analysis is undertaken. Only the main features of the planned statistical analysis are included below.

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

Unless specified otherwise, all data from each study part will be presented separately in the tables, figures and listings. Furthermore, for Parts 2 and 3, data for each cohort will be presented separately.

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 DSMC to assist with the identification of DLTs and enable dose escalation decisions whilst the study is ongoing.

13.1 Sample Sizes

The study is not formally powered, but has been designed to estimate the safety and tolerability of NUC-3373 when given with the combination agents described in Section 3.3. For the dose escalation phases, a modified 3+3 design has been selected to enable an assessment of safety and tolerability, whilst limiting the number of patients exposed to experimental treatment and procedures.

13.2 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 C0D1 for Part 1 Arm 1a & 1b, or C1D1 for all other cohorts.

13.3 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 will 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 sub-group.

13.3.1 Safety Population

For each part of the study, the Safety Population includes all patients in that part of the study who received at least 1 dose of NUC-3373 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.

13.3.2 Full Analysis Set

For all study parts and cohorts, the Full Analysis Set (FAS) is based on the intention-to-treat principles and includes all patients who received at least one dose of NUC-3373. Patients will be included in the FAS based on the dose of NUC-3373 initially received, regardless of any subsequent dose adjustments.

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

13.3.3 Evaluable for Response Set

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

13.3.4 PK Analysis Set

The PK Population contains all patients who received NUC-3373 as per protocol (*i.e.,* intended dose) and provided at least one usable PK profile. All PK data will be analysed according to treatment received.

This population will comprise all data from patients who received study treatment as per protocol (*i.e.,* intended dose) and did not violate or deviate from the protocol and planned dosing regimen in ways that would significantly affect the PK analyses (for example skipping doses, or taking reduced doses or taking concomitant medications with the potential to cause a drug-drug interaction) during the PK sampling period. Patients who did deviate from the planned dosing regimen may still provide some data for inclusion in the PK set if they have at least one usable PK profile. The population and decisions regarding which profiles are usable will be defined by the study team, pharmacokineticist and statistician prior to any analyses being performed.

13.4 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 13.3) 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 study.

13.5 Statistical Methods

13.5.1 Demographics and Baseline Data

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 CRC, 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.

13.5.2 Concomitant Medications

Concomitant medications, 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.

13.5.3 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.

13.5.4 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 and/or any of the proposed combinations.

13.5.4.1 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)™, Version 18.1 (or higher), System Organ Class (SOC) and preferred term. 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, TEAEs leading to treatment interruption, 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
- 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 TEAEs leading to NUC-3373 interruptions by SOC and preferred term
- Summary of TEAEs leading to treatment discontinuation by SOC and preferred term for each study treatment
- Summary of TEAEs by SOC, preferred term and maximum severity
- Summary of TEAEs by SOC, preferred term and worst-case relationship attribution
- Summary of SAEs by SOC and preferred term
- Summary of SAEs by preferred term sorted in descending order of frequency

Additionally, the following will be listed:

- All AEs, listed with 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.
- 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 SAE 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
- AEs leading to treatment interruptions, 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

13.5.4.2 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.

13.5.4.3 Vital Signs

Actual values at baseline and each scheduled visit and change from baseline at each post-baseline scheduled visit of vital signs (including pulse, respiration, systolic and diastolic blood pressure, oral temperature, and weight) will be summarised with descriptive statistics by dose cohort and time point. All data will also be listed.

13.5.4.4 ECOG Performance Status

ECOG performance status will be summarised and listed. The ECOG Performance Scale is provided in Appendix 2.

13.5.4.5 Physical Examination

Listings will be provided for physical examination parameters.

13.5.4.6 ECGs

13.5.4.6.1 Safety 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.

The triplicate values at each timepoint for a patient will be averaged, and the average value will be used in the summaries. Actual values at baseline and each scheduled visit and change from baseline at each post-baseline scheduled visit in ECG endpoints will be summarised with descriptive statistics by dose cohort and time point. Additionally, abnormal results will be summarised with frequencies and percentages by clinical significance, treatment group, and time point. All ECG data will be displayed in a data listing.

13.5.4.6.2 Holter ECGs (PK sub-study)

Holter ECG monitoring will be implemented for patients in the PK sub-study (Arm 2b) to collect QT/QTc data to allow robust analysis of ECG parameters. Holter monitoring will be performed on C1D8 and C1D22, when patients receive NUC-3373 + LV only.

ECGs will be centrally evaluated. The analysis of ECG parameters for QT/QTc evaluation will be outlined in a separate QT SAP.

13.5.4.7 Pharmacokinetics

The PK of single and multiple-dose NUC-3373 will be assessed, including:

- C_{max}
- AUC
- $t_{1/2}$
- V_d
- CL

PK of the following will be measured, but are not limited to:

- In plasma: NUC-3373, 5-FU, 5-FUDR, FBAL
- **Part 1 only**: In PBMCs: NUC-3373, FUDR-MP, FBAL, 5-FUTP, dTMP and dUMP

All above PK parameters will be analysed in the PK population.

13.5.4.8 Exploratory Endpoints

Genomic, transcriptomic and proteomic biomarkers of biological activity will be analysed in a pre-treatment tumour sample and pre- and on-treatment blood and PBMC (Part 1 only) samples from patients in the intention-to-treat population. 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.

13.5.5 Efficacy Analysis

The primary efficacy endpoints and methods of analysis are defined below:

13.5.5.1 Change from Baseline in Tumour Size

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

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

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

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

The $\%\Delta TS_{best}$ 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

$$\%\Delta TS_{best} = \frac{\mathrm{TS}_{best} - \mathrm{TS}_{baseline}}{\mathrm{TS}_{baseline}} \times 100$$

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

Tumour size ($\%\Delta TS_{Wk8}$ and $\%\Delta TS_{best}$) 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.

13.5.5.2 Objective Response Rate (ORR)

ORR is defined as the number of patients achieving a confirmed response (CR or PR), defined in accordance with RECIST v1.1 (see Appendix 1). The number and percentage of patients in each RECIST v1.1 response category (CR, PR, SD, progressive disease or not evaluable [NE]), as well as the ORR will be presented.

All data will be listed.

13.5.5.3 Disease Control Rate (DCR)

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

DCR may be summarised if there are a number of patients achieving SD or response.

13.5.5.4 Duration of Response (DoR)

DoR is defined for the subset of the EFR population categorised as responders for the assessment of ORR. 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 listed, and may be summarised if there are sufficient responders.

13.5.5.5 Duration of Stable Disease (SD)

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 time measurement criteria are first met for SD 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.

13.5.5.6 Progression-Free Survival (PFS)

PFS is defined as the time from first dose of study treatment 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 RECIST v1.1 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 RECIST v1.1 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 RECIST v1.1 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.

13.5.5.7 Overall Survival (OS)

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.

13.5.6 Pharmacodynamic Endpoints (Part 1 only)

Baseline and post-dose PBMC marker data will be analysed using a paired t test. Full details will be provided in the SAP.

Boxplots for the change from baseline over time in neutrophils, platelets, and lymphocytes will also be produced.

Full details of all planned analyses of PD endpoints will be provided in the SAP.

13.5.7 Primary and Interim Analyses

The analysis of safety for a cohort will take place when all of the enrolled patients have completed one treatment cycle or have withdrawn from treatment. At least 3 evaluable patients are required for the analysis. The efficacy analysis of % change in tumour size will take place when all patients in a given cohort have undergone at least 2 imaging assessments for evaluation of percentage change in tumour size by RECIST v1.1 or have withdrawn from treatment. To enable the DSMC to make dose escalation decisions, safety and tolerability data will be reviewed on an ongoing basis. There will be no formal requirements for a database lock to support these ongoing reviews. Upon review of the safety and tolerability data from each cohort by the DSMC, additional patients may be added to a cohort to further evaluate the safety profile of the combination.

13.5.8 Changes to the Planned Statistical Methods

Planned statistical analyses will be documented in the final SAP before database lock. Any changes to the planned statistical methods will be documented in the CSR.

14 PATIENT DATA HANDLING AND CONFIDENTIALITY

14.1 Case Report Forms

As part of the responsibilities assumed by participating in the study, the Investigator or Sub-Investigator agrees to maintain adequate case histories for the patients enrolled as part of the research under this protocol. The Investigator agrees to maintain accurate CRFs and source documentation as part of the case histories. These source documents include laboratory reports and MRI or CT scans.

An eCRF will be used, please refer to the eCRF Completion Guidelines for further information. The Investigator must review each completed eCRF in a timely manner. The Investigator will be required to review and electronically sign and date the CRFs once the patient's data is complete.

14.2 Monitoring of the Study

Monitoring and auditing procedures developed by the Sponsor or designee will be followed in order to comply with ICH-GCP guidelines. Before a study centre can enter a patient into the study, a representative of the Sponsor or designee will visit the study centre to:

- Determine the adequacy of the facilities
- Discuss with the Investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of the Sponsor or its representatives. This will be documented in a Clinical Study Agreement between the Sponsor and the Investigator

During the study, a monitor from the Sponsor or appointed CRO will have regular contacts with the study centre, for the following:

- Provide information and support to the Investigators
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the source documents and eCRFs, and that drug accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the eCRFs with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient (*e.g.* clinic charts)
- Record and report any protocol deviations not previously sent to the Sponsor
- Confirm AEs and SAEs have been properly documented in the eCRFs and confirm any SAEs have been forwarded to the Sponsor or designee, and those SAEs that met criteria for reporting have been forwarded to the IRB/EC

The monitor will be available between visits if the Investigator or other staff needs information or guidance.

Note: during the COVID-19 pandemic, remote visits may replace on-site visits for as short a period of time as possible.

14.3 Patient Confidentiality

Personal data recorded on all documents will be regarded as highly confidential. To preserve each patient's anonymity, only their patient study number and date of birth (or other identified as appropriate to country regulations and agreed with the Sponsor) will be recorded on the eCRFs.

The Investigator site must maintain the patient's anonymity in all communications and reports related to the research. The Investigator site team must keep a separate log of enrolled patients' personal identification details as necessary to enable them to be tracked. These documents must be retained securely, in strict confidence. They form part of the Investigator Site File and are not to be released externally.

15 ETHICAL AND REGULATORY CONSIDERATIONS

15.1 Good Clinical Practice Compliance

The Sponsor, Investigators and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with ICH-GCP, US 21 Code of Federal Regulations (CFR) 11, 21 CFR 50, 21 CFR 54, 21 CFR 56, 21 CFR 312 and all other applicable regulations.

15.2 Institutional Review Boards/ Ethics Committees

The applicable IRBs/ECs will review all appropriate study documentation to safeguard the rights, safety, and well being of the patients.

The final study protocol and ICF must be approved in writing by the applicable IRBs/ECs for each site. Written IRB/EC approval must be received by the Sponsor or appointed CRO before a study centre can enrol any patients into the study. In addition, the IRB/EC must approve all advertising used to recruit patients for the study.

In the US, the protocol (and associated documents, including amendments) must be re-approved by the IRB/ECs annually, as local regulations require. Progress reports will be provided to the IRB/EC according to local regulations and guidelines.

15.3 Regulatory Authority Approval

Authorisation to conduct the study will be obtained from the applicable Regulatory Authorities prior to initiating the study in each participating country.

15.4 Protocol Amendments

All protocol amendments (and amendments to related study documentation) will be approved by the applicable IRBs/ECs and Regulatory Authorities prior to implementation.

15.5 Protocol Violations and Deviations

The Investigator, or designee, must document and explain in the patient's source documentation any deviation from the approved protocol.

A deviation from the protocol is an unintended and/or unanticipated departure from the procedures and/or processes approved by the Sponsor and the IRB/EC and agreed to by the Investigator or Sub-Investigator. Deviations usually have an impact on individual patients or a small group of patients and do not involve inclusion/ or primary endpoint criteria. Deviations will be tracked by the CRO along with the corrective and preventative actions by responsible party.

A protocol violation occurs when there is nonadherence to the protocol that results in a significant, additional risk to the patient, when the patient or Investigator has failed to adhere to significant protocol requirements (*e.g.,* inclusion/ criteria and the patient was enrolled without prior Sponsor approval), or when there is nonadherence to the FDA or other applicable ICH-GCP guidelines.

The clinical monitor will document protocol violations and deviations throughout the course of monitoring visits. The monitor will notify the Investigators during a visit and in writing of all violations and deviations. The IRB/EC should be notified of all protocol violations and deviations in a timely manner.

15.6 Serious Breaches

A serious breach is defined as a breach of ICH-GCP or the study protocol which is likely to effect to a significant degree the:

- Safety or physical or mental integrity of the patients of the study; *or*
- Scientific value of the study

Investigators will notify the CRO within one working day if any serious breach of ICH-GCP or the protocol is suspected. Upon confirmation of a serious breach, the CRO will notify the applicable Regulatory Authorities. Typically, serious breach notifications should be made within seven days of the CRO becoming aware; however, this timeline may differ as specified by applicable local regulatory requirements.

15.7 Study Reporting Requirements

The Investigator agrees to submit progress reports to their IRB/EC as appropriate. The Investigator also agrees to provide the Sponsor with an adequate report shortly after completion of their participation in the study.

15.8 Financial disclosure

The Investigators and Sub-Investigators are required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the Investigator and Sub-Investigators must provide the sponsor with a commitment to update this information promptly if any relevant changes occur during the investigation and for one year after study completion.

Neither the sponsor nor the CRO is financially responsible for further testing/treatment of any medical condition which may be detected during the screening process. In addition, in the absence of specific arrangements, neither the sponsor nor the CRO is financially responsible for further treatment of the patient's disease.

15.9 Investigator Documentation

Before beginning the study, each investigative site will have all applicable essential documents available, in accordance with ICH-GCP section 8.2.

15.10 Study Records Retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of NUC-3373 clinical development. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the Investigator or Sub-Investigator or institution as to when these documents no longer need to be retained.

If the Investigator becomes unable for any reason to continue to retain study records for the required period, the sponsor should be prospectively notified. The study records must be transferred to a designee acceptable to the sponsor, such as another Investigator, another institution, or to an independent third party arranged by the sponsor. The Investigator must obtain written permission from the sponsor before disposing of any records, even if retention requirements have been met. Retention and storage of central laboratory records supporting PK

endpoints and the disposition of samples donated via the study must also comply with applicable legislation.

15.11 Audit and Regulatory Inspection

The Investigator, Sub-Investigators, and institutions involved in the study will permit study-related monitoring, audits, IRB/EC review, and regulatory inspection(s) by providing direct access to all study records. In the event of an audit, the Investigator or Sub-Investigator agrees to allow the sponsor representatives of the sponsor, the FDA, or other regulatory agency access to all study records.

The Investigator should promptly notify the sponsor and the appointed CRO of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the sponsor.

16 PUBLICATION POLICY

An ICH E3-compliant CSR will be generated based on the final data listings of this study. The final CSR will be submitted to Regulatory Authorities and IRBs/ECs in accordance with the stipulated timelines.

16.1 Communication of Results by Sponsor

The sponsor shall publicly disclose study results through posting on ClinicalTrials.gov, the European Clinical Trials Database (EudraCT) and any other applicable public registries in accordance with local laws and regulations.

Final study results may be submitted to ClinicalTrials.gov within one year of the primary completion date, which is defined as 'the date that the final patient was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical study concluded according to the pre-specified protocol or was terminated'. Final study results may be posted to EudraCT within one year of the end of study.

16.2 Publication by Investigators

Investigators may not publish or disclose results until the study is completed. In addition, Investigators shall acknowledge that, due to the limited patient population in its treatment group, the data generated from its individual participation in the study and evaluation of its individual results, may not be sufficient from which to draw any meaningful scientific conclusion.

The sponsor will provide authorship rights to Investigators in order of greatest contribution of evaluable patients to the study. Publication of study results may also be described in the agreement between the sponsor and each Institution. In addition, the Sponsor may form a publication committee to evaluate and give final approval of publication submission.

The proposed publication (manuscript, abstract or poster) or presentation will be provided to the sponsor by the Investigator for review and comment at least 60 days prior to the planned submission. The Investigator understands and agrees that participation in the study may involve a commitment to publish the study results in a cooperative publication with other Investigators. No publication of confidential information shall be made without the sponsor's prior written approval.

The Investigator agrees, upon sponsor's request, to delete any confidential information that may impact intellectual property protection from the proposed publication. Investigators will comply with recognised ethical publications and authorship standards, including Section II of the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (http://www.icmje.org/icmje-recommendations.pdf).

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18 APPENDICES

APPENDIX 1. RESPONSE EVALUATION CRITERIA IN SOLID TUMOURS (RECIST) VERSION 1.1

The following paragraphs are a quick reference to the RECIST criteria (v1.1). The complete criteria are available at http://www.eortc.be/RECIST and are included in the published RECIST document:

Eisenhauer *et al*. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; 45: 228–247.

Measurability of Tumour Lesions at Baseline – Definitions

Measurable disease – the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions – *tumour lesions* that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with chest x-ray, and as ≥10 mm with CT scan or clinical examination [using callipers]. Bone lesions are considered measurable only if assessed by CT scan and have an identifiable soft tissue component that meets these requirements (soft tissue component ≥10 mm by CT scan). *Malignant lymph nodes* must be ≥15 mm in the short axis to be considered measurable; only the short axis will be measured and followed. All tumour measurements must be recorded in millimetres (or decimal fractions of centimetres) by use of a ruler or callipers. Tumour lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

Non-measurable lesions – all other lesions (or sites of disease), including small lesions are considered non-measurable disease. Bone lesions without a measurable soft tissue component, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, lymphangitic involvement of lung or skin and abdominal masses followed by clinical examination are all non-measurable. Nodes that have a short axis <10 mm at baseline are considered non-pathological and should not be recorded or followed.

Target lesions – when more than one measurable tumour lesion or malignant lymph node is present at baseline all lesions up to *a maximum of 5 lesions total* (and a maximum of *2 lesions per organ*) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to *reproducible repeated measurements*. Note that pathological nodes must meet the criterion of a short axis of ≥15 mm by CT scan and only the *short* axis of these nodes will contribute to the baseline sum.

At baseline, the sum of the target lesions (longest diameter of tumour lesions plus short axis of lymph nodes: overall maximum of 5) is to be calculated and recorded.

Non-target lesions – all non-measurable lesions (or sites of disease) including pathological nodes (those with short axis ≥10 mm but <15 mm), plus any measurable lesions over and above those listed as target lesions are considered *non-target lesions*. Measurements are not required but these lesions should be noted at baseline and should be followed as “present” or “absent”.

All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

Methods of Measurements
The same method of assessment and the same technique should be used to characterise each identified and reported lesion at baseline and during follow-up. Assessments should be identified on a calendar schedule and should not be affected by delays in therapy, which may be treatment arm dependent. While on study, all target lesions recorded at baseline should have their actual measurements recorded on the CRF at each subsequent evaluation, even when very small (*e.g.,* 2 mm). If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned. For lesions which fragment/split add together the longest diameters of the fragmented portions; for lesions which coalesce, measure the maximal longest diameter for the "merged lesion".

Clinical lesions – clinical lesions will only be considered measurable when they are superficial and ≥10 mm as assessed using callipers (*e.g.,* skin nodules). For the case of skin lesions, documentation by colour photography including a ruler to estimate the size of the lesion is recommended. If feasible, imaging is preferred.

Chest X-ray – chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions ≥20 mm on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI – CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.,* for body scans). While PET scans are not considered adequate to measure lesions, PET-CT scans may be used providing that the measures are obtained from the CT scan and the CT scan is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast).

Ultrasound – ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT should be obtained.

Endoscopy, Laparoscopy – the utilisation of these techniques for objective tumour evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in studies where recurrence following complete response or surgical resection is an endpoint.

Tumour markers – tumour markers alone cannot be used to assess objective tumour response. If markers are initially above the upper normal limit, however, they must normalise for a patient to be considered in complete response.

Cytology, Histology – these techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumour types such as germ cell tumours, where known residual benign tumours can remain). When effusions are known to be a potential adverse effect of treatment (*e.g.* with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumour has met criteria for response or stable disease is advised to differentiate between response or stable disease and progressive disease.

Tumour Response Evaluation

All patients will have their BEST RESPONSE from the start of study treatment until the end of treatment classified as outlined below. Complete or PRs may be claimed only if the criteria for each are met at a subsequent time point at least 4 weeks later. Refer to the Appendix 1, Tables 1 and 2 below.

Complete Response (CR) – disappearance of all *target* and *non-target* lesions and normalisation of tumour markers. Pathological lymph nodes must have short axis measures <10 mm (Note: continue to record the measurement even if <10 mm and considered CR). Tumour markers must have normalised. Residual lesions (other than nodes <10 mm) thought to be non-malignant should be further investigated (by cytology or PET scans) before CR can be accepted.

Partial Response (PR) – at least a 30% decrease in the sum of measures (longest diameter for tumour lesions and short axis measure for nodes) of target lesions, taking as reference the baseline sum of diameters. Non-target lesions must be non-PD.

Stable Disease (SD) – neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the smallest sum of diameters on study.

Progressive Disease (PD) – at least a 20% increase in the sum of diameters of measured lesions taking as references the smallest sum of diameters recorded on study (including baseline) AND an absolute increase of ≥5 mm. Appearance of new lesions will also constitute PD (including lesions in previously unassessed areas). In exceptional circumstances, unequivocal progression of non-target disease may be accepted as evidence of disease progression, where the overall tumour burden has increased sufficiently to merit discontinuation of treatment, for example where the tumour burden appears to have increased by at least 73% in volume (which is the increase in volume when all dimensions of a single lesion increase by 20%). Modest increases in the size of one or more non-target lesions are NOT considered unequivocal progression. If the evidence of PD is equivocal (target or non-target), treatment may continue until the next assessment, but on further documentation, the earlier date must be used.

Appendix 1. Table 1. Integration of Target, Non-Target and New Lesions into Response Assessment

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response for this Category Also Requires
Patients with target lesions ± non-target lesions				
CR	CR	No	CR	Normalisation of tumour markers All tumour nodes <10 mm Documented at least once ≥4 weeks from baseline
CR	Non-CR/Non-PD	No	PR	Documented at least once ≥4 weeks from baseline
CR	Not all evaluated	No	PR	
PR	Non-PD/ not all evaluated	No	PR	
SD	Non-PD/ not all evaluated	No	SD	
Not all evaluated	Non-PD	No	NE	
PD	Any	Any	PD	
Any	PD	Any	PD	
Any	Any	Yes	PD	
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression (or evidence of unequivocal disease progression) at that time should be reported as "*symptomatic deterioration"*. This is a reason for stopping therapy, but is NOT objective PD. Every effort should be made to document the objective progression even after discontinuation of treatment.				

Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point at least 4 weeks later. The best overall response can be interpreted from Appendix 1 Table 2 on the next page.

Appendix 1. Table 2. Response Assessment after Subsequent Scan

Response: First Time Point	Subsequent Time Point	BEST Overall Response	Also Requires
CR	CR	CR	Normalisation of tumour markers. All tumour nodes <10 mm.
CR	PR	SD, PD or PR (see comment*)	
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD	
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD	
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE	
PR	CR	PR	
PR	PR	PR	
PR	SD	SD	
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD	
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE	
NE	NE	NE	
* may consider PR providing initial "CR" likely PR on subsequent review – then original CR should be corrected. Recurrence of lesion after true CR is PD.			

Frequency of Tumour Re-Evaluation

Tumours should be assessed at the end of every 2nd cycle.

Date of Progression

This is defined as the first day when the RECIST (v1.1) criteria for PD are met.

Reporting of Tumour Response

All patients included in the study must be assessed for response to treatment, even if there is a major protocol treatment deviation or if they are ineligible, or not followed/re-evaluated. Each patient will be assigned one of the following categories: complete response, partial response, stable disease, progressive disease, early death from malignant disease, early death from toxicity, early death from other cause or unknown (not assessable, insufficient data).

'Early death' is defined as any death occurring before the first per protocol time point of tumour re-evaluation. The responsible Investigator will decide if the cause of death is malignant disease, toxicity or other cause. Patients for whom response is not confirmed will be classified as "unknown", unless they meet the criteria for stable disease (or the criteria for partial response in case of an unconfirmed complete response). Patients' response will also be classified as "unknown" if insufficient data were collected to allow evaluation per these criteria.

APPENDIX 2. ECOG PERFORMANCE SCALE

Activity Performance Description	Score
Fully active, able to carry out all on all pre-disease performance without restriction	**0**
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, *e.g.* light housework, office work	**1**
Ambulatory and capable of all self-care, but unable to carry out any work activities Up and about more than 50% of waking hours	**2**
Capable of only limited self-care Confined to bed or chair more than 50% of waking hours	**3**
Completely disabled Cannot carry out any self-care Totally confined to bed or chair	**4**
Dead	**5**

APPENDIX 3. CONCOMITANT MEDICATIONS THAT MAY PROLONG QTc INTERVAL

Medications with a known risk of torsade de pointes (*i.e.,* significant evidence they cause QT prolongation and are associated with a risk of causing torsade de pointes) are listed below.

Appendix 3. Table 1: Drugs known to prolong QT/QTc interval

Generic Name	Brand Names (Partial List)
Aclarubicin (only on non-US market)	Aclacin®, Aclacinomycine®, Aclacinon®, Aclaplastin®, Jaclacin®
Amiodarone	Cordarone®, Pacerone®, Nexterone®
Anagrelide	Agrylin®, Xagrid®
Arsenic trioxide	Trisenox®
Astemizole (removed from market)	Hismanal®
Azithromycin	Zithromax®, Zmax®
Bepridil	Vascor®
Cesium chloride	Energy catalyst
Chloroquine	Aralen®
Chlorpromazine	Thorazine®, Largactil®, Megaphen®
Chlorprothixene (only on non-US market)	Truxal®
Cilostazol	Pletal®
Ciprofloxacin	Cipro®, Cipro-XR®, Neofloxin®
Cisapride (removed from market)	Propulsid®
Citalopram	Celexa®, Cipramil®
Clarithromycin	Biaxin®, Prevpac®
Cocaine	Cocaine
Disopyramide	Norpace®
Dofetilide	Tikosyn®
Domperidone (only on non-US market)	Motilium®, Motillium®, Motinorm Costi®, Nomit®
Donepezil	Aricept®
Dronedarone	Multaq®
Droperidol	Inapsine®, Droleptan®, Dridol®, Xomolix®
Erythromycin	E.E.S.®, Robimycin®, EMycin®, Erymax®, Ery-Tab®, Eryc Ranbaxy®, Erypar®, Eryped®, Erythrocin Stearate Filmtab®, Erythrocot®, E-Base®, Erythroped®, Ilosone®, MY-E®, Pediamycin®, Zineryt®, Abboticin®, Abboticin-ES®, Erycin®, PCE Dispertab®, Stiemycine®, Acnasol®, Tiloryth®
Escitalopram	Cipralex®, Lexapro®, Nexito®, Anxiset-E® (India), Exodus® (Brazil), Esto® (Israel), Seroplex®, Elicea®, Lexamil®, Lexam®, Entact® (Greece), Losita® (Bangladesh), Reposil® (Chile), Animaxen® (Colombia), Esitalo® (Australia), Lexamil® (South Africa)
Flecainide	Tambocor®, Almarytm®, Apocard®, Ecrinal®, Flécaine®
Fluconazole	Diflucan®, Trican®
Gatifloxacin (removed from US market)	Tequin®

Generic Name	Brand Names (Partial List)
Grepafloxacin (removed from US market)	Raxar®
Halofantrine (only on non-US market)	Halfan®
Haloperidol	Haldol® (US & UK), Aloperidin®, Bioperidolo®, Brotopon®, Dozic®, Duraperidol® (Germany), Einalon S®, Eukystol®, Halosten®, Keselan®, Linton®, Peluces®, Serenace®, Serenase®, Sigaperidol®
Hydoquinidine (Dihydroquinidine) (only on non-US market)	Serecor®
Hydroxychloroquine	Plaquenil®, Quineprox®
Ibogaine (only on non-US market)	None
Ibutilide	Corvert®
Levofloxacin	Levaquin®, Tavanic®
Levomepromazine (only on non-US market)	Nosinan®, Nozinan®, Levoprome®
Levomethadyl acetate (removed from US market)	Orlaam®
Levosulpiride (only on non-US market)	Lesuride®, Levazeo®, Enliva® (with rabeprazole)
Meglumine antimoniate (only on non-US market)	Glucantime®
Mesoridazine (removed from US market)	Serentil®
Methadone	Dolophine®, Symoron®, Amidone®, Methadose®, Physeptone®, Heptadon®
Mobocertinib	Exkivity®
Moxifloxacin	Avelox®, Avalox®, Avelon®
Nifekalant (only on non-US market)	Shinbit®
Ondansetron	Zofran®, Anset®, Ondemet®, Zuplenz®, Emetron®, Ondavell®, Emeset®, Ondisolv®, Setronax®
Oxaliplatin*	Eloxatin®
Papaverine HCl (intra-coronary)	None
Pentamidine	Pentam®
Pimozide	Orap®
Probucol (removed from market)	Lorelco®
Procainamide	Pronestyl®, Procan®
Propofol	Diprivan®, Propoven®
Quinidine	Quinaglute®, Duraquin®, Quinact®, Quinidex®, Cin-Quin®, Quinora®
Roxithromycin (only on non-US market)	Rulide®, Xthrocin®, Roxl-150®, Roxo®, Surlid®, Rulide®, Biaxsig®, Roxar®, Roximycinv®, Roxomycin®, Rulid®, Tirabicin®, Coroxin®
Sertindole (only on non-US market)	Serdolect®, Serlect®
Sevoflurane	Ulane®, Sojourn®
Sotalol	Betapace®, Sotalex®, Sotacor®

Generic Name	Brand Names (Partial List)
Sparfloxacin (removed from US market)	Zagam®
Sulpiride (only on non-US market)	Dogmatil®, Dolmatil®, Eglonyl®, Espiride®, Modal®, Sulpor®
Sultopride (only on non-US market)	Barnetil®, Barnotil®, Topral®
Terfenadine (removed from US market)	Seldane®
Terlipressin (only on non-US market)	Teripress®, Glypressin®, Terlipin®, Remestyp®, Tresil®, Teriss® and others
Terodiline (only on non-US market)	Micturin®, Mictrol® (not bethanechol)
Thioridazine	Mellaril®, Novoridazine®, Thioril®
Vandetanib	Caprelsa®
* Except as included in the specific arms and dosing regimens of this study.	

Note: Medicines on this list are reviewed on an ongoing basis to assure that the available evidence supports their continued placement on this list. The list changes regularly and we recommend checking the website at crediblemeds.org for the most up-to-date information. There may be many additional brand names that are not listed on this table.

If clinically relevant or urgent medical intervention is required with a drug known to prolong the QT/QTc interval, study treatment must be paused and options for re-starting the study drugs should be discussed with the Medical Monitor.

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Woosley, RL and Romero, KA, www.Crediblemeds.org, QT drugs List, [Accessed August 2022], AZCERT, Inc. 1822 Innovation Park Dr., Oro Valley, AZ 85755, USA.

See more at: https://www.crediblemeds.org/#sthash.vzyRSgay.dpuf

APPENDIX 4. MANAGEMENT GUIDE FOR DIARRHOEA

Patients receiving 5-FU are at risk for developing diarrhoea. This side effect often leads to delay in treatment, dose reduction or discontinuation of treatment. There is a small but significant mortality associated with chemotherapy-induced diarrhoea (CID), especially when it occurs concomitantly with mucositis and neutropaenia.

Classification

Chemotherapy-induced diarrhoea is graded using the NCI criteria (Appendix 4 Table 1) which grades diarrhoea on a scale of 0 (normal) to 4 (severe) according to the number of loose stools/days, presence of nocturnal stools, incontinence, cramping and blood in stools.

Appendix 4 Table 1. NCI criteria for assessment of chemotherapy-induced diarrhoea

Criteria	GRADE				
	0	1	2	3	4
Number of stools per day	Normal	2–3	4-6	7-9	>10
Symptom	-	-	Nocturnal stools	Incontinence	Bloody stool
	-	-	Moderate cramping	Severe cramping	Need for parenteral fluid

Management

CID can be a serious complication and requires prompt assessment. The steps in this assessment are as follows:

1. Rule out other or concomitant causes of diarrhoea

Other causes of diarrhoea must be ruled out. These include medications (e.g. stool softeners, laxatives, antacids, etc), infection by *C. difficile* or *Candida* species, partial bowel obstruction, malabsorption, faecal impaction, acute radiation reaction and surgery (short bowel syndrome). Diets high in fibre or lactose may aggravate diarrhoea.

2. Dietary modifications during diarrohea

Mild diarrhoea may be managed with diet to decrease the frequency of stools. Patients should be advised to increase intake of clear fluids (*e.g.,* water, sports drinks, broth, gelatin, clear juices, decaffeinated tea, caffeine-free soft drinks). A BRAT (banana, rice, apples, toast) diet can be helpful.

3. Medications

- **Loperamide**
  Loperamide is indicated for Grade 1 diarrhoea that persists for more than 12-24 hours or for moderate diarrhoea (Grade 2). The standard dose of loperamide is 4 mg followed by 2 mg every 4 hours or after each unformed stool (maximum dose 16 mg/day). This dose may be increased in patients with mild to moderate diarrhoea (Grade 1 or 2) that

- persists for more than 24 hours. The dose is 4 mg to start, followed by 2 mg every 2 hours (or 4 mg every 4 hours at night to allow sleep). Loperamide should be continued for 12 hours following resolution of the diarrhoea and re-establishment of a normal diet.

  High-dose loperamide (4 mg followed by 2 mg every 2 hours) is also recommended at the onset of *any* diarrhoea in patients receiving irinotecan chemotherapy.

- **Atrophine-diphenoxylate**
  At the discretion of the treating physician, it may be useful to add atropine-diphenoxylate 1 to 2 tablets, every 6-8 hours, to loperamide therapy for Grade 1 or 2 diarrhoea. It should not be expected that this would be sufficient for the management of Grade 3 or 4 diarrhoea.

- **Octreotide**
  For Grades 1 and 2 diarrhoea lasting more than 24 hours despite high-dose loperamide + atrophine-diphenoxylate, octreotide 100-150 mcg SC TID may be considered. For Grades 3 and 4 diarrhoea, octreotide 150 mcg SC TID is indicated; these patients usually require hospitalisation. If there is no improvement in the diarrhoea after 24 hours, the dose of octreotide should be increased to 300-500 mcg SC TID. The duration of octreotide therapy should be individualised, but can be discontinued 24 hours after the end of diarrhoea and re-establishment of a normal diet. Alternatively, octreotide may be administered via a syringe driver with infusion over 24 hours of 600 mcg octreotide. If not controlled each day, increase the dose to 1200 mcg, then 1800 mcg, then 2400 mcg, but not beyond this daily dose. If diarrhoea is controlled, then octreotide can be de-escalated.

- **Antibiotics**

  In the presence of concomitant neutropaenia (granulocytes $<1\times10^6$/L), antibiotics (*e.g.,* ciprofloxacin 500 mg BID) should be considered until resolution of diarrhoea and recovery of the granulocyte counts. In addition, oral antibiotics (minocycline, doxycycline, or antibiotics covering skin flora) should be given to prevent or treat