# CLINICAL STUDY PROTOCOL

## A randomised, open-label, Phase II, dose/schedule optimisation study of NUC-3373/leucovorin/irinotecan plus bevacizumab (NUFIRI-bev) versus 5-FU/leucovorin/irinotecan plus bevacizumab (FOLFIRI-bev) for the treatment of patients with previously treated unresectable metastatic colorectal cancer

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| **IMP** | NUC-3373 |
| **Protocol Number** | NuTide:323 |
| **Protocol Version, Date** | Version 2.1, 06 Feb 2024 |
| **Development Phase** | II |
| **IND Number** | 135275 |
| **EudraCT Number** | 2022-001459-17 |
| **ClinicalTrials.gov ID** | NCT05678257 |
| **Sponsor:** | **NuCana plc**<br>3 Lochside Way<br>Edinburgh, EH12 9DT, UK<br>Tel: +44 (0)131 357 1111<br>Email: NuTide323@nucana.com |
| **Approval:** | _____ _____<br>[redacted] Date<br>SVP, Clinical & Medical Development |
| **Medical Monitor:** | [redacted]<br>NuCana plc<br>3 Lochside Way, Edinburgh, EH12 9DT, UK<br>Tel: +44 (0) 131 357 1118 |

# PRINCIPAL INVESTIGATOR AGREEMENT AND SIGNATURE

## A randomised, open-label, Phase II, dose/schedule optimisation study of NUC-3373/leucovorin/irinotecan plus bevacizumab (NUFIRI-bev) versus 5-FU/leucovorin/irinotecan plus bevacizumab (FOLFIRI-bev) for the treatment of patients with previously treated unresectable metastatic colorectal cancer

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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 randomised, open-label, Phase II, dose/schedule optimisation study of NUC-3373/leucovorin/irinotecan plus bevacizumab (NUFIRI-bev) versus 5-FU/leucovorin/irinotecan plus bevacizumab (FOLFIRI-bev) for the treatment of patients with previously treated unresectable metastatic colorectal cancer |
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| **IND** | 135275 |
| **EudraCT** | 2022-001459-17 |
| **ClinicalTrials.gov ID** | NCT05678257 |
| **Phase** | II |
| **Objectives** | **Primary Objectives**<br>• To compare progression free- survival (PFS) of NUC-3373 in combination with leucovorin (LV), irinotecan and bevacizumab (NUFIRI-bev) with 5-fluorouracil (5-FU) in combination with LV, irinotecan and bevacizumab (FOLFIRI-bev)<br>• To determine the optimal NUFIRI-bev dosing schedule<br>**Secondary Objectives**<br>• To compare the efficacy of NUFIRI-bev to FOLFIRI-bev in terms of:<br>o Objective response rate (ORR)<br>o Duration of response (DoR)<br>o Disease control rate (DCR)<br>o Maximum percentage change in tumour size<br>o Overall survival (OS)<br>• To assess the safety and tolerability of NUFIRI-bev compared to FOLFIRI-bev<br>• To assess the pharmacokinetics (PK) of NUFIRI-bev<br>**Exploratory Objective**<br>To determine if there are tumour cell characteristics that may further elucidate the mechanisms through which the clinical activity of NUC-3373 is achieved (archival tissue, where available). |
| **Study Design** | Randomised, open-label, dose/schedule optimisation study comparing NUFIRI-bev to FOLFIRI-bev for the treatment of patients with unresectable metastatic colorectal cancer (CRC).<br>Patients will be randomised 1:1:1 to either NUFIRI-bev based on a weekly (Q1W) NUC-3373 schedule, NUFIRI-bev based on an alternate weekly (Q2W) NUC-3373 schedule, or FOLFIRI-bev (Q2W). Randomisation will be stratified by RAS status (wild-type vs KRAS mutant vs NRAS mutant), prior bevacizumab treatment (yes vs no) and duration of prior line of therapy (<6 months vs ≥6 months). |
| **Study Centres** | This study will be conducted at approximately 61 sites globally. |

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| **Endpoints** | **Primary Endpoint**<br>• PFS, according to Response Evaluation Criteria in Solid Tumours (RECIST) v1.1, defined as the time from randomisation to the first observation of objective tumour progression or death from any cause<br>**Secondary Endpoints**<br>**Efficacy**<br>• ORR, defined as the percentage of patients achieving a complete or partial response to treatment<br>• DoR, defined as the time from initial clinical response (partial response [PR] or complete response [CR]) to the first observation of tumour progression or death from any cause<br>• DCR, defined as the percentage of patients demonstrating a best overall response (BOR) of CR, PR or stable disease (SD)<br>• Maximum percentage change from baseline in tumour size according to RECIST v1.1<br>• OS, defined as the time from randomisation to the time of death from any cause<br>**Safety**<br>Safety and tolerability will be assessed by evaluation of:<br>• Treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs; per Common Terminology Criteria for Adverse Events [CTCAE] v5.0)<br>• Deaths due to TEAEs<br>• Treatment modifications due to TEAEs<br>• Clinically-significant laboratory changes (per CTCAE v5.0)<br>• Electrocardiograms (ECGs)<br>**Pharmacokinetics**<br>The PK of the NUFIRI-bev regimen will be assessed, including:<br>• Concentration at end of infusion ($C_{inf}$)<br>• Maximum concentration ($C_{max}$)<br>• Area under the plasma concentration-time curve (AUC)<br>• Half-life ($t_{1/2}$)<br>• Volume of distribution ($V_d$)<br>• Clearance (CL)<br>The analytes measured in plasma will include, but are not limited to:<br>• NUC-3373, CPF-1027 α-fluoro-β-alanine (FBAL), irinotecan, SN-38, SN-38G, APC<br>**Exploratory Endpoint**<br>• Population or tumour characteristic subtypes that may determine benefit to NUC-3373 treatment will be analysed |

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| **Study Population** | Patients with histologically or cytologically confirmed unresectable colorectal adenocarcinoma that is metastatic and measurable. Patients must have previously received ≥2 months of a 1st-line fluoropyrimidine and oxaliplatin-containing regimen or have relapsed within 6 months of completing a fluoropyrimidine and oxaliplatin-containing neoadjuvant/adjuvant therapy. Patients who started on a fluoropyrimidine and oxaliplatin-containing regimen in any setting but must discontinue the oxaliplatin due to toxicity or allergy (and are now unable to receive oxaliplatin) are considered eligible regardless of the number of cycles of oxaliplatin they received.<br><br>Patients must also be BRAF V600E wild-type and have an Eastern Cooperative Oncology Group (ECOG) performance status 0-1, along with adequate haematologic, renal and hepatic function. Patients with MSI-H or dMMR are not eligible. |
| **Study Treatment** | Patients will be randomised 1:1:1 to receive either:<br>• NUFIRI-bev on a Q1W NUC-3373 schedule (Arm A)<br>• NUFIRI-bev on a Q2W NUC-3373 schedule (Arm B)<br>• FOLFIRI-bev on a Q2W schedule (Arm C)<br><br>In Arm A, bevacizumab will be administered at 5 mg/kg and irinotecan will be administered at 180 mg/m$^2$, both by intravenous (IV) infusion, on Days 1 and 15. LV will be administered at 400 mg/m$^2$ by IV infusion on Days 1, 8, 15 and 22. Following completion of infusions on Days 1, 8, 15 and 22, NUC-3373 will be administered by IV infusion at 1500 mg/m$^2$.<br><br>In Arm B, bevacizumab will be administered at 5 mg/kg, irinotecan will be administered at 180 mg/m$^2$, and LV will be administered at 400 mg/m$^2$, all by IV infusion, on Days 1 and 15. Following completion of infusions on Days 1 and 15, NUC-3373 will be administered by IV infusion at 1500 mg/m$^2$.<br><br>In Arm C, bevacizumab will be administered at 5 mg/kg, irinotecan will be administered at 180 mg/m$^2$, and LV will be administered at 400 mg/m$^2$, all by IV infusion, on Days 1 and 15. Following completion of infusions on Days 1 and 15, 5-FU will be administered by IV bolus at 400 mg/m$^2$ followed by 2,400 mg/m$^2$ by continuous IV infusion over 46 hours.<br><br>Bevacizumab may be substituted with any approved biosimilar consistent with institutional practice. The specific agent administered will be recorded. Thus, bevacizumab in this protocol refers to either bevacizumab or a licensed biosimilar. |
| **Inclusion Criteria** | 1. Provision of written informed consent.<br>2. Histological or cytological confirmation of colorectal adenocarcinoma (excluding appendiceal and anal canal cancers, as well as signet-ring cell carcinoma) that is unresectable and metastatic.<br>3. Measurable disease (as defined by RECIST v1.1).<br>4. Received ≥2 months of a first-line fluoropyrimidine and oxaliplatin-containing regimen for metastatic disease or relapsed within 6 months of completing a fluoropyrimidine and oxaliplatin-containing neoadjuvant/adjuvant therapy. Previous treatment with standard of care chemotherapy regimens in combination with molecular targeted therapies (*e.g.,* VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Previous treatment with maintenance |

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| | therapy (*e.g.,* capecitabine) is also allowed. Patients who started on a fluoropyrimidine and oxaliplatin-containing regimen in any setting but must discontinue the oxaliplatin due to toxicity or allergy (and are now unable to receive oxaliplatin) are considered eligible regardless of the number of cycles of oxaliplatin they received.<br>5. Known RAS and BRAF status. Patients with wild-type RAS tumours must have received prior treatment with an EGFR inhibitor, unless this was not standard of care according to relevant region-specific treatment recommendations.<br>6. Known UGT1A1 status, or patient consents to UGT1A1 status testing if unknown.<br>7. Known DPD activity status, or patient consents to DPD status testing if unknown. See exclusion criterion 1.<br>8. Age ≥18 years.<br>9. Minimum life expectancy of ≥12 weeks.<br>10. ECOG Performance status 0 or 1.<br>11. Adequate bone marrow function as defined by: absolute neutrophil count (ANC) $\geq 1.5\times10^9$/L, platelet count $\geq 100\times10^9$/L, and haemoglobin ≥9 g/dL. Patients with benign neutropenia may be discussed on a case-by-case basis with the medical monitor.<br>12. Adequate liver function, as defined by: serum total bilirubin ≤1.5×upper limit of normal (ULN; this threshold does not apply to patients with Gilbert's syndrome, who should be discussed with the Medical Monitor), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5×ULN (or ≤5×ULN if liver metastases are present).<br>13. Adequate renal function assessed as serum creatinine <1.5×ULN and glomerular filtration rate ≥50 mL/min (calculated by the Cockcroft-Gault method).<br>14. Serum albumin ≥3 g/dL.<br>15. Ability to comply with protocol requirements.<br>16. Female patients of child-bearing potential must have a negative pregnancy test within 7 days prior to the first study drug administration. This criterion does not apply to patients who have had a previous hysterectomy or bilateral oophorectomy. Male patients and female patients of child-bearing potential must agree to practice true abstinence or to use two forms of contraception, one of which must be highly effective. These forms of contraception must be used from the time of signing consent, throughout the treatment period, and for 6 months following the last dose of any study medication. Oral or injectable contraceptive agents cannot be the sole method of contraception.<br>17. Patients must have been advised to take measures to avoid or minimize exposure of the skin and eyes to UV light, including avoiding sunbathing and solarium use, for the duration of study participation and for a period of 4 weeks following the last dose of study medication. |
| **Exclusion Criteria** | 1. History of hypersensitivity or current contra-indications to 5-FU, FUDR, or capecitabine. This includes patients with genotypic or phenotypic (blood uracil level ≥150 ng/mL) evidence of complete DPD deficiency or TYMP mutations associated with toxicity to |

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| | fluoropyrimidines. Patients who tolerated prior 5-FU at a reduced dose level may be enrolled and treated at that same dose.<br>2. History of hypersensitivity or current contra-indication to any of the combination agents required for the study.<br>3. History of allergic reactions attributed to components of the NUC-3373 drug product formulation (super refined polysorbate 80 [SRP80], dimethylacetamide [DMA]).<br>4. History of hypersensitivity to Chinese Hamster Ovary (CHO) cell products or other recombinant human or humanised antibodies.<br>5. History of or known central nervous system or leptomeningeal metastases.<br>6. Symptomatic ascites, ascites currently requiring drainage procedures or ascites requiring drainage over the prior 3 months.<br>7. Mutant BRAF V600E status.<br>8. MSI high or dMMR.<br>9. Prior treatment with irinotecan.<br>10. Chemotherapy, hormonal therapy, radiotherapy (other than a short cycle of palliative radiotherapy [*e.g.,* for bone pain]*), immunotherapy, biological agents, or exposure to another investigational agent within 21 days (or four times the half-life for molecular targeted agents, whichever is shorter) of first administration of study treatment:<br>a. For nitrosoureas and mitomycin C within 6 weeks of first administration of study treatment<br>b. Continuous dosing of ≥10 mg prednisolone (or steroid equivalent) is not allowed during the study. Corticosteroid treatment is allowed during screening but should be weaned to a dose of ≤10 mg prednisolone (or steroid equivalent) by Cycle 1 Day 1<br>** Palliative radiotherapy during participation in the study is permitted, but should not be concurrent with study treatment and recovery should be allowed to prevent overlapping toxicity (refer to Section 10.4). It should not include a target lesion.*<br>11. Residual toxicities from prior chemotherapy or radiotherapy which have not regressed to Grade ≤1 severity (CTCAE v5.0), except for alopecia and residual Grade 2 neuropathy.<br>12. History of other malignancies, except adequately treated non-melanoma skin cancer, curatively treated *in situ* cancer 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. Patients with previous invasive cancers are eligible if treatment was completed >3 years prior to initiating the current study treatment, and the patient has had no evidence or recurrence since then.<br>13. Presence of an active bacterial or viral infection (including SARS-CoV-2, Herpes Zoster, Varicella Zoster or chickenpox), known Human Immunodeficiency Virus (HIV) positive or known active hepatitis B or C.<br>14. 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 the following: |

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| | a. Congestive heart failure (New York Heart Association Class III or Class IV)<br>b. Clinically significant coronary heart disease or myocardial infarction within 6 months of the first dose of study medication or high risk of uncontrolled arrhythmia<br>c. Unstable or poorly controlled angina pectoris<br>d. Complete left bundle branch, fascicular block or other clinically significant abnormal ECG finding<br>e. QTc interval >470 milliseconds<br>f. History of or current risk factor for torsade de pointes (*e.g.,* heart failure, hypokalaemia, or a family history of long QT syndrome)<br>g. History of severe skin reactions (except for skin reactions that are a consequence of recent anti-cancer treatment, including chemotherapies not currently under investigation in this study [*e.g.,* oxaliplatin] or molecular targeted therapies such as EGFR inhibitors)<br>h. History of severe ocular disorders<br>i. Interstitial pneumonitis or pulmonary fibrosis<br>15. 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 provide informed consent and undergo study procedures.<br>16. Patients with a history of haemoptysis (1/2 teaspoon or more of red blood) within 6 months prior to enrolment.<br>17. Wound healing complications or surgery within 28 days of starting bevacizumab (wound healing must have been fully completed before starting bevacizumab). Investigators may allow patients to initiate treatment with the other study drugs (*i.e.,* NUC-3373/5-FU, LV and irinotecan) on C1D1 but withhold bevacizumab for at least 15 days, but no longer than 28 days, to allow completion of wound healing in patients who would otherwise be eligible for the study, in line with standard local practice and after discussion with the Medical Monitor. Patients who have not received bevacizumab by C2D1 must be replaced.<br>18. Unhealed wound, active gastric or duodenal ulcer, or bone fracture.<br>19. Serious thromboembolic event in the 6 months before inclusion (*e.g.,* transitory ischemic stroke, stroke, subarachnoid haemorrhage). Patients with non-serious thromboembolic events (*e.g.,* non-symptomatic pulmonary embolism or peripheral deep vein thrombosis treated with anticoagulants) may be enrolled after discussion with the Medical Monitor.<br>20. Patients with a history of haemorrhage within 6 months prior to enrolment.<br>21. Known inherited or acquired bleeding disorders.<br>22. Red blood cell (RBC) transfusion dependence, defined as requiring more than 2 units of packed RBC transfusions during the 4-week period prior to screening.<br>23. Uncontrolled hypertension.<br>24. Severe proteinuria or nephrotic syndrome (≥Grade 3 [urine dipstick +4 or ≥3.5 g/day]).<br>25. Acute intestinal obstruction or sub-obstruction, history of inflammatory intestinal disease (including colitis or Crohn's disease) |

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| | or extended resection of the small intestine. Presence of a colic prosthesis.<br>26. History of abdominal fistulas, trachea-oesophageal fistulas, any other Grade 4 gastrointestinal perforations, non-gastrointestinal fistulas, or intra-abdominal abscesses 6 months prior to screening.<br>27. Currently pregnant, lactating or breastfeeding.<br>28. Required concomitant use of brivudine, sorivudine and analogues.<br>29. Required concomitant use of St John's Wort.<br>30. Required concomitant use of drugs known to prolong QT/QTc interval.<br>31. Required concomitant use of strong CYP3A4 inducers or strong CYP3A4 inhibitors. The use of strong CYP3A4 inducers within 2 weeks of first receipt of study drug or the use of strong CYP3A4 inhibitors within 1 week of first receipt of study drug is also excluded.<br>32. Use of strong UGT1A1 inhibitors within 1 week of first receipt of study drug.<br>33. Received a live vaccination within four weeks of first planned dose of study medication.<br>34. **Germany only:** Patients who have been placed in an institution by court or official order. |
| **Study Duration Per Patient** | Patients may continue to receive treatment in the absence of disease progression or unacceptable toxicity that is not ameliorated by optimal medical or non-medical supportive or prophylactic care, or withdrawal of consent. Average time on study is estimated at approximately 7-11 months from start of screening to last protocol visit, based on the median PFS of patients with unresectable metastatic disease.<br><br>All patients will be followed up until withdrawal of consent, lost to follow-up, death, or the overall end of study, whichever is earliest. |
| **Sample Size and Statistical Analysis** | <u>Sample Size</u><br><br>In total, 171 patients will be randomised on a 1:1:1 basis (57 patients per arm) to either Q1W NUFIRI-bev, Q2W NUFIRI-bev or FOLFIRI-bev (Q2W).<br><br>The principal statistical objective of this study is to estimate the likely efficacy of the two NUFIRI arms as compared to the FOLFIRI control arm to support decision making regarding the further development of NUFIRI. Median PFS is expected to be 7 months on the FOLFIRI control arm and at least 9.9 months on each of the two NUFIRI arms. Assuming a non-linear recruitment profile over the planned 13-month accrual period ($\eta$=2; Carroll, 2009) and with a minimum of 17 months follow-up post-accrual, a total of 139 PFS events are expected across the three randomised arms. With this amount of information, this study will provide an 80% probability of correctly concluding superiority when NUFIRI is truly better than FOLFIRI in terms of PFS and, similarly, an 80% probability of correctly concluding non-superiority when NUFIRI is truly the same as FOLFIRI in terms of PFS. The smallest observed improvement in median PFS for either NUFIRI arm relative to FOLFIRI to conclude NUFIRI is truly better than FOLFIRI is 1.3 months.<br><br>Further, an evaluation of efficacy may be performed 3 months after the last patient has been randomised. At this time a total of 70 PFS events are expected across the three randomised arms. With this amount of information, the smallest observed improvement in median PFS for either |

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| | NUFIRI arm relative to FOLFIRI to conclude NUFIRI is truly better than FOLFIRI is 1.9 months.<br><u>Statistical Methods</u><br>Patient demographics and clinical characteristics will be summarised using descriptive statistics.<br>*Efficacy Analysis Population*<br>Efficacy endpoints will be assessed in the Full Analysis Set (FAS), defined as all randomised patients. A supportive analysis of efficacy endpoint data will be performed in the modified FAS being defined as the subset of the FAS who had at least one dose of randomised study treatment and at least one follow-up assessment.<br>*Safety Analysis Population*<br>Safety will be assessed in the Safety Set (SS), defined as all randomised patients who receive at least one dose (or partial dose) of study treatment. Patients will be analysed by the treatment received, based on their first dose of randomised study drug.<br>*Efficacy Analysis*<br>PFS time (defined as the time from randomisation to the first progression or death) will be analysed via Cox regression modelling stratified for the randomisation stratification factors (RAS status, first-line treatment, and duration of prior line of therapy) and including a fixed effect term for randomised treatment. Patients free from progression and death will be censored at their last follow-up visit. The hazard ratio will be estimated for each NUFIRI arm versus the FOLFIRI control arm, along with the associated confidence interval (CI) and 2-sided p value. The data will also be displayed using Kaplan-Meier curves and median PFS times will be estimated.<br>The analysis of secondary efficacy endpoints will be detailed in the Statistical Analysis Plan.<br>*Safety Analysis*<br>Adverse events (AEs) will be classified using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) classification system. The severity will be graded according to CTCAE v5.0 whenever possible. All AEs reported from the first dose of study drug until 30 days after the last dose of study drug will be considered as treatment-emergent AEs (TEAEs) and will be summarised descriptively by treatment, and by the frequency of patients experiencing TEAEs corresponding to body systems and MedDRA preferred term. Patients with multiple occurrences of events will only be counted once at the maximum severity/grade for each Preferred Term, System Organ Class (SOC), and overall. Any AEs with missing severity will be classified as severe. Deaths that occur within 90 days after the last dose of study drug are defined as on-study deaths.<br>Scheduled physical examination, vital signs, and haematology and chemistry laboratory data will be summarised by treatment and by cycle. The laboratory results will be graded according to the CTCAE v5.0 severity grade. The frequencies of the worst severity grade observed will be displayed by study medication. Shift tables will be provided to examine the distribution of laboratory toxicities. For parameters for which a CTCAE v5.0 scale does not exist, the frequency of patients with values below, within, and above the normal ranges will be summarised by treatment. |

# OVERALL STUDY DESIGN

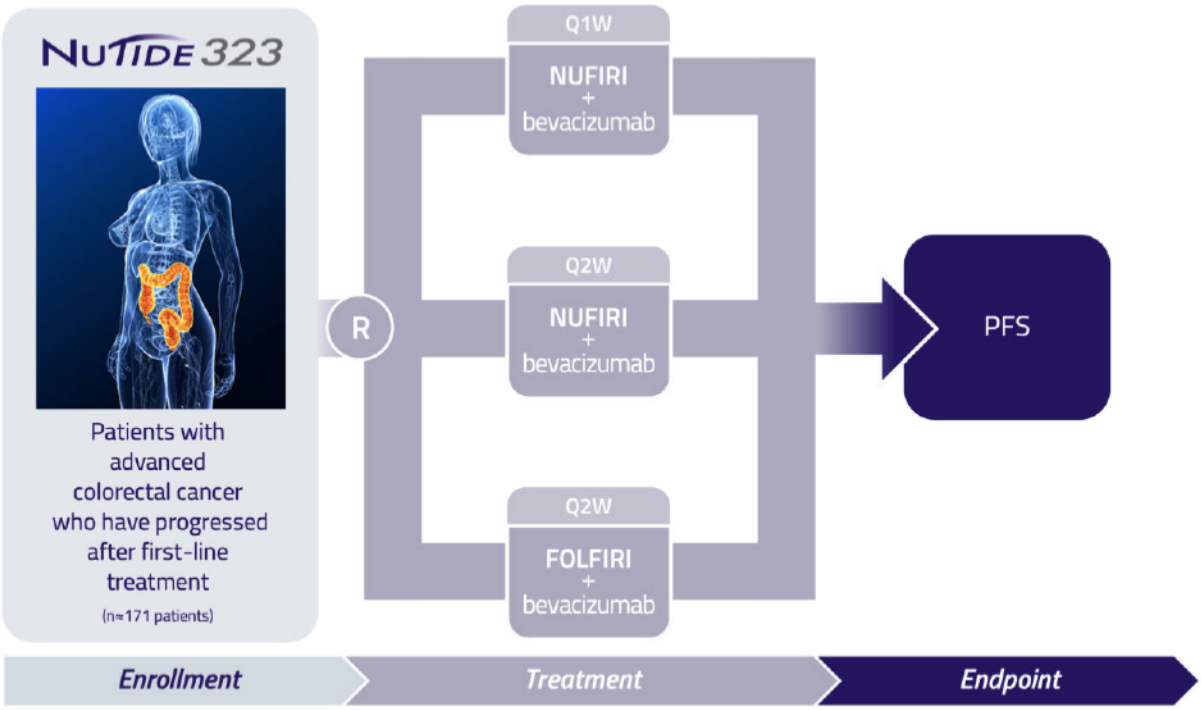


**Figure 1 NuTide:323 study schema**

Abbreviations: FOLFIRI=5-FU + LV + irinotecan; NUFIRI=NUC-3373 + LV + irinotecan; PFS=progression-free survival; Q1W=weekly; Q2W=alternate weekly

# SUMMARY SCHEDULE OF EVENTS

| Study Assessments[1] | Screening/ Baseline | Cycle 1 | | | | Additional Cycles | | | | End of Treatment | Follow-up (France only) | Follow-up | Follow-up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days -28 to 0 | D1 | D8* (±2 days) | D15 (±2 days) | D22* (±2 days) | D1 (±2 days) | D8* (±2 days) | D15 (±2 days) | D22* (±2 days) | 30 days post last dose (+7 days) | Q4 weeks (±3 days) | Q8 weeks (±7 days) | Q12 weeks (±14 days) |
| Informed consent | X | | | | | | | | | | | | |
| Eligibility criteria | X | | | | | | | | | | | | |
| Demographic data | X | | | | | | | | | | | | |
| Medical history[2] | X | | | | | | | | | | | | |
| Concomitant medications | X | X | X | X | X | X | X | X | X | X | | | |
| Full physical examination[3] | X | X[5] | | | | X | | | | X | | | |
| Directed physical examination[3] | | | | | | X[3] | | | | | | | |
| Urinalysis[4] | X | X[5] | | X | | X | | X | | X | | | |
| ECOG status | X | X[5] | | | | X | | | | X | | | |
| Vital signs[6] | X | X | X | X | X | X | X | X | X | X | | | |
| ECG (pre-dose)[7] | X | X | | X | | X | | X[7] | | X | | | |
| ECG (post-dose)[8] | | X | | X | | X[8] | | X[8] | | | | | |
| Pregnancy test[9] | X | X | | | | X | | | | X | X[9] | | |
| FBC and chemistry[10] | X | X[5] | X | X | X | X | X | X | X | X | | | |
| Coagulation profile | X | X | | | | X | | | | X | | | |
| Tumour markers[11] | X | | | | | X | | | | X | | | |
| DPD status testing[12] | X | | | | | | | | | | | | |
| BRAF/ KRAS/ NRAS/ MSI/ MMR status testing[13] | X | | | | | | | | | | | | |
| UGT1A1 status testing[14] | X | | | | | | | | | | | | |
| Radiologic tumour assessment (CT/MRI)[15] | X | *Every 8 weeks (±7 days) from C1D1 until disease progression* | | | | | | | | | | X[16] | |

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| Randomisation | | X[17] | | | | | | | | | | | |
| NUC-3373 + LV administration (Q1W) | | X | X | X | X | X | X | X | X | | | | |
| NUC-3373 + LV administration (Q2W) | | X | | X | | X | | X | | | | | |
| 5-FU + LV administration | | X | | X | | X | | X | | | | | |
| Irinotecan administration | | X | | X | | X | | X | | | | | |
| Bevacizumab administration | | X | | X | | X | | X | | | | | |
| AEs/SAEs[18] | X | X | X | X | X | X | X | X | X | X | | | |
| PK blood sample[19] | | | | | | X[19] | | | | | | | |
| Archived sample[20] | X | | | | | | | | | | | | |
| Dental examination[21] | X | | | | | | | | | | | | |
| Follow-up | | *Ongoing* | | | | | | | | | | | X[22] |

* **Day 8 and Day 22 visits are only to be performed for patients receiving Q1W NUC-3373 (Arm A)**

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

2 Includes recording information on the sidedness of the patient's CRC (left-sided vs right-sided).

3 A full physical assessment should be completed at Cycle 1 Day 1, Cycle 2 Day 1 and at End of Treatment. From Cycle 3 Day 1 onwards, a directed physical assessment will be completed (in place of the full physical assessment), but only if clinically indicated.

4 Urinalysis testing for proteinuria should be performed prior to each bevacizumab administration.

5 Does not need to be repeated if Screening assessment was performed within 72 hours of C1D1.

6 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 course of the study, the dose of study treatments should be recalculated.

7 **Pre-dose ECGs:** Standard 12-lead ECG measurements will be performed prior to administration of all IMPs, at the indicated visits (Day 1 of each cycle) for patients in all treatment arms.

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. The timing between the triplicate ECGs is recommended to be approximately 1 minute.

8 **Post-dose ECGs:** Additional standard 12-lead ECG measurements must be taken post administration of all IMPs on C1D1, C1D15, C2D1 and C2D15 (within 10 minutes of the end of infusion) for patients in the **NUFIRI treatment arms only** (Arms A and B).

9 Serum pregnancy assessment to be performed within 7 days of C1D1. Required only in women of childbearing potential. **In France only:** Pregnancy testing must continue every 4 weeks for 6 months after the last dose of study treatment. This post-study treatment pregnancy testing must not be performed with a home test; however, in order to avoid frequent hospital visits, it may be performed at an external laboratory that is local to the patient, with oversight by the study site. If this occurs, the study site must notify the Sponsor. All instances of positive pregnancy tests must be notified by the external laboratory to the study site, Sponsor and the patient's treating physician.

10 Clinical chemistry (including hepatic panel) and haematology will be conducted weekly in the Q1W treatment arm and alternate weekly in the Q2W treatment arms throughout the study. In the event of neutropenia (ANC $<0.5\times10^9$/L), thrombocytopenia (platelet count $<50\times10^9$/L), or ≥Grade 2 clinical chemistry toxicity, these assessments will be conducted more frequently as clinically indicated until toxicity resolves to ≤Grade 1.

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

12 If not already known, DPD status should be tested during screening and performed according to local and national guidelines and standard practice, either by genotyping or phenotypic testing using blood uracil levels. Patients with blood uracil ≥150 ng/mL are considered to be DPD deficient and are excluded from participation. Patients with blood uracil levels of ≥16 ng/mL to <150 ng/mL are considered to be partially DPD deficient. The 5-FU dose must be adapted for partially DPD deficient patients in countries where this is standard practice as per national and/or local guidelines (refer to Section 9.2.1).

13 If KRAS/NRAS/BRAF/MSI/MMR status is not known, perform genetic testing and obtain results prior to dosing on C1D1.

14 If UGT1A1 status is not known, perform genetic testing. Results do not need to be obtained prior to dosing. If a patient's mutational status is known prior to dosing and they have a mutation that may affect their ability to metabolise irinotecan, an initial dose reduction of irinotecan may be implemented as per the irinotecan SmPC/Prescribing Information and site standard of care. This must be discussed on a case-by-case basis with the medical monitor.

15 Computed tomography (CT) / magnetic resonance imaging (MRI) disease assessments will be performed at Screening (within 28 days prior to randomisation) and every 8 weeks (±7 days) from C1D1.

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

16 Patients discontinuing 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 subsequent line of therapy, or death in order to determine duration of overall response and PFS.

17 Randomisation takes place up to 3 working days prior to administration of study treatment on C1D1.

18 All adverse events (AEs) occurring from the time of informed consent up to and including 30 days after the last dose of study drug has been administered must be reported in detail on the AE case report form (CRF).

**Note**: Investigators must report all SAEs that they become aware of irrespective of the end of study treatment or the end of study, unless the patient has initiated a new therapy after which only SARs must be reported.

19 Collection of blood samples for PK analysis will be performed for patients in the NUFIRI treatment arms only (Arms A and B).

A total of 4 blood samples will be collected on **C2D1** only:

| PK timepoint | Description |
|---|---|
| Pre-dose | Prior to administration of NUC-3373 |
| End of infusion | Within 5 minutes before end of NUC-3373 infusion |
| 2-4 hours post-infusion | 2-4 hours after end of NUC-3373 infusion |
| 6-24 hours post-infusion | 6-24 hours after end of NUC-3373 infusion |

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

20 Original diagnostic or other representative FFPE block containing tumour will be recalled (where available).

21 A dental examination and appropriate preventative dentistry should be considered prior to starting treatment with bevacizumab. In patients who have previously received or are receiving IV bisphosphonates, invasive dental procedures should be avoided if possible.

22 All patients, including those who discontinue study treatment, will be followed up for disease progression, initiation of new treatments and survival every 12 weeks (±14 days) from C1D1 until withdrawal of consent, lost to follow-up, death, or the overall end of study, whichever is earliest. A total of three attempts should be made before the patient is considered as lost to follow-up. Patients in follow-up who have not experienced disease progression should continue to attend the clinic for planned radiologic scans; however, other follow-up data can be collected remotely.

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

# ABBREVIATIONS

| | |
|---|---|
| 5-FU | 5-fluorouracil |
| ADR | Adverse drug reaction |
| AE | Adverse event |
| ALT | Alanine aminotransferase |
| ANC | Absolute neutrophil count |
| APC | 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin (irinotecan metabolite) |
| aPTT | Activated partial thromboplastin time |
| ASCO | American Society for Clinical Oncology |
| AST | Aspartate aminotransferase |
| AUC | Area under the plasma concentration-time curve |
| BOR | Best overall response |
| BRAF | v-Raf murine sarcoma viral oncogene homolog B |
| BSA | Body surface area |
| CEA | Carcinoembryonic antigen |
| CFR | Code of Federal Regulations |
| CHO | Chinese Hamster Ovary (cells) |
| CI | Confidence interval |
| $C_{inf}$ | 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 |
| CYP | Cytochrome P450 |
| DCR | Disease control rate |
| DLT | Dose-limiting toxicity |
| DMA | Dimethylacetamide |
| dMMR | Deficient mismatch repair |
| DoR | Duration of response |
| DoSD | Duration of stable disease |
| DPD | Dihydropyrimidine dehydrogenase |
| DSUR | Development safety update report |
| dTMP | Deoxythymidine monophosphate |
| dUMP | Deoxyuridine monophosphate |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| eCRF | Electronic Case Report Form |
| EDC | Electronic data capture |
| EGFR | Epidermal growth factor receptor |
| EudraCT | European Union Drug Regulating Authorities Clinical Trials Database |
| FAS | Full analysis set |

| | |
|---|---|
| FBAL | α-fluoro-β-alanine |
| FDA | (US) Food and Drug Administration |
| FFPE | Formalin-fixed paraffin embedded |
| FOLFIRI | 5-FU + LV + irinotecan |
| FOLFOX | 5-FU + LV + oxaliplatin |
| FOLFOXIRI | 5-FU + LV + oxaliplatin + irinotecan |
| FUDR | Floxuridine (5-fluorodeoxyuridine) |
| FUDR-MP /FdUMP | Fluorodeoxyuridine-monophosphate |
| FUDR-TP /FdUTP | Fluorodeoxyuridine-triphosphate |
| FUTP | Fluorouridine triphosphate |
| G-CSF | Granulocyte colony stimulating factor |
| HIV | Human Immunodeficiency Virus |
| 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) |
| IxRS | Interactive voice- or web-based response system |
| KRAS | Kirsten rat sarcoma virus |
| LDH | Lactate dehydrogenase |
| LV | Leucovorin |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mFAS | Modified full analysis set |
| MHRA | Medicines and Healthcare Regulatory Agency (UK) |
| MRI | Magnetic resonance imaging |
| MSI-H | Microsatellite instability high |
| MTD | Maximum tolerated dose |
| NE | Not evaluable |
| NRAS | Neuroblastoma RAS viral oncogene homolog |
| 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 |
| PFS | Progression-Free Survival |
| PK | Pharmacokinetics |
| PR | Partial Response |
| PT | Prothrombin time |
| Q1W | Weekly |
| Q2W | Alternate weekly (fortnightly) |
| QT/QTc | QT interval / Corrected QT interval |
| RBC | Red blood cells |
| RECIST | Response Evaluation Criteria in Solid Tumours (version 1.1) |

| | |
|---|---|
| RP2D | Recommended Phase II dose |
| RSI | Reference Safety Information |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| SARS-CoV-2 | Severe acute respiratory syndrome-related coronavirus 2 |
| SD | Stable Disease |
| SmPC | Summary of Product Characteristics |
| SN-38 | 7-ethyl-10-hydroxycamptothecin (irinotecan active metabolite) |
| SN-38G | SN-38 glucuronide (irinotecan inactive metabolite) |
| SOC | System Organ Class |
| SRP80 | Super refined polysorbate 80 |
| SS | Safety set |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| $t_{1/2}$ | Terminal half-life |
| TAF | Tenofovir alafenamide fumarate |
| TEAE | Treatment-emergent adverse event |
| TK | Thymidine kinase |
| TP | Thymidine phosphorylase |
| TS | Thymidylate synthase |
| TYMP | Thymidine phosphorylase (gene) |
| UGT1A1 | UDP glucuronosyltransferase 1A1 |
| ULN | Upper limit of normal |
| $V_d$ | Volume of distribution |
| VEGF | Vascular endothelial growth factor |
| WBC | White blood cells |
| WHO DD | World Health Organisation Drug Dictionary |

# 1. INTRODUCTION AND STUDY RATIONALE

Nucleoside analogs are a key component of standard chemotherapy regimens for a number of solid tumours and haematological malignancies. However, these agents are associated with limited efficacy and significant toxicity due to inherent or acquired cancer resistance mechanisms, inefficient activation pathways, and poor pharmacokinetic profiles.

NuCana is utilising phosphoramidate chemistry to develop a first-in-class range of anti-cancer agents called ProTides that are specifically designed to address the limitations associated with conventional nucleoside analogs. ProTides are synthesised by the addition of a phosphate group onto a nucleoside analog scaffold, thereby creating a pre-activated nucleotide analog. The phosphate is protected by a phosphoramidate motif (consisting of an aryl, an ester, and an amino acid), which stabilises the active compound and protects it against breakdown. As a result, ProTides do not require complex activation pathways, they generate higher intracellular levels of active anti-cancer metabolites, and they are resistant to degradation by the enzymes that breakdown the parent nucleoside analogs. This not only improves the PK profile but also reduces the generation of toxic metabolites and is anticipated to result in greater anti-cancer activity compared to conventional nucleoside analogs. The phosphoramidate approach has been well validated in the anti-viral field with the development of sofosbuvir (Sovaldi®) and tenofovir alafenamide fumarate (TAF).

NUC-3373 is a pre-activated and protected form of the nucleotide analog floxuridine (FUDR®), specifically designed to overcome the key shortcomings that limit the clinical utility of fluoropyrimidines. The overall aim is to improve the efficacy and safety profile, whilst also reducing the dosing administration burdens, associated with 5-fluorouracil (5-FU).

## 1.1. Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer globally, accounting for 10% of all cancers. The incidence worldwide is approximately 1.9 million new cases per year with a 5-year survival rate of 14% for patients diagnosed with Stage 4 disease (Sung *et al*, 2021; ACS, 2022). In the United States, there are approximately 1.5 million people living with CRC and approximately 155,000 new cases of CRC are diagnosed annually. The global burden is expected to increase by 60% to approximately 3.1 million new cases annually by 2040.

The majority of CRC occurs in the rectum and sigmoid colon, while a smaller proportion occurs in the caecum and ascending colon. Approximately 20% of patients have metastatic disease at the time of diagnosis, frequently in the liver, and approximately 35% of patients develop metastases after a curative intent treatment (De Falco *et al*, 2020).

## 1.2. Treatment of Colorectal Cancer

Surgery is the mainstay of curative treatment (5-year survival rates of 20–45% reported after the surgical resection of R0 resectable colorectal liver metastases); however, the management of CRC almost always involves a multi-modal approach. In selected patients, a combination of chemotherapy and surgery can achieve long-term survival and maintained quality of life (Schmoll *et al*, 2012). Systemic chemotherapy continues to be the cornerstone of treatment for patients with advanced or metastatic CRC (Lee & Sun, 2016).

Most systemic therapies for CRC include the pyrimidine analog 5-FU, either as monotherapy or in combination with another chemotherapeutic, typically oxaliplatin or irinotecan. Nevertheless, despite being the standard of care, the effectiveness of 5-FU as a single-agent is modest.

### 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.

### 1.2.2 Treatment for Metastatic Disease

The choice of 1st-line treatment for advanced or metastatic CRC is influenced by the clinical presentation and tumour biology (*e.g.,* sites of metastases; dynamics of progression; present or imminent symptoms; prognostic molecular or biochemical markers, such as BRAF mutation), as well as patient-related factors (*e.g.,* comorbidity and potential to undergo secondary resection) and drug-related factors (availability of targeted drugs; predictive markers, such as RAS mutation) (Schmoll *et al*, 2012). Systemic therapy may include a fluoropyrimidine-based chemotherapy backbone paired with a biologic agent (anti-vascular endothelial growth factor [VEGF] or anti-epidermal growth factor receptor [EGFR] antibodies). The anti-VEGF monoclonal antibody bevacizumab was the first biologic approved for metastatic CRC and benefits all patients with this disease (Hurwitz *et al*, 2004). Currently, either anti-VEGF (bevacizumab) or anti-EGFR (cetuximab or panitumumab) agents can be used in 1st-line treatment for patients with left-sided RAS and RAF wildtype metastatic CRC (Heinemann *et al*, 2014; Venook *et al*, 2017). Generally, right-sided CRCs or RAS mutant CRCs do not benefit from anti-EGFR agents in the 1st-line metastatic setting (Venook *et al*, 2016; Arnold *et al*, 2017); therefore, these patients typically receive a fluoropyrimidine-based chemotherapy regimen in combination with bevacizumab. Patients with BRAF-V600E mutant CRC have a poorer prognosis (Roth *et al*, 2010) as the tumours are aggressive and do not respond well to systemic therapy. For these patients, 1st-line triplet chemotherapy (*i.e.,* FOLFOXIRI) with bevacizumab is recommended and, more recently, combinations with BRAF inhibitors and anti-EGFR agents have been approved as 2nd-line and beyond options for this patient population (Kopetz, 2017; Van Cutsem *et al*, 2019; Kopetz *et al*, 2019). Although immunotherapies such as nivolumab and pembrolizumab have been approved for metastatic CRC, only 4-5% of patients have dMMR or MSI-H tumours; thus, the majority of patients are not eligible for immunotherapy.

The current global standard of care for patients with metastatic CRC is a 5-FU-containing therapy such as FOLFOX (5-FU + LV + oxaliplatin), FOLFIRI (5-FU + LV + irinotecan) or FOLFOXIRI (5-FU + LV + oxaliplatin + irinotecan). The most commonly administered standard of care regimen for 1st-line metastatic patients is a FOLFOX-based regimen, usually in combination with bevacizumab or an anti-EGFR antibody. Following progression, most patients receive 2nd-line FOLFIRI-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 setting, 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).

Given the global burden of CRC and the fact that the majority of patients present with locally advanced or metastatic disease requiring systemic therapy, a fluoropyrimidine-based regimen in combination with biologic agents, most frequently bevacizumab, is likely to remain the most commonly administered standard of care regimen for the 2nd-line treatment of patients with metastatic CRC.

### 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.

The anti-cancer activity of 5-FU and its other forms, FUDR and capecitabine (Xeloda®), is largely attributed to the active anti-cancer metabolite, fluorodeoxyuridine-monophosphate (FUDR-MP; FdUMP), which 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 delivered as a continuous infusion of up to 46 hours.

Key limitations impacting the breakdown, metabolism and activation of 5-FU have been associated with poor prognosis to treatment (Longley *et al,* 2003; Longley & Johnston, 2005). In addition, the generation of catabolites and metabolites by 5-FU causes dose-limiting toxicities (DLTs) and impacts patient safety.

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 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, cardiotoxicity and neurotoxicity. Therefore, circumventing DPD-mediated degradation is anticipated to significantly improve the safety and efficacy of fluoropyrimidines.

During metabolism, 5-FU is also phosphorylated to fluorouridine triphosphate (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, FUTP is thought to be the primary mediator underlying diarrhoea, mucositis and neutropaenia, which are key DLTs in patients treated with 5-FU (Adjei, 1999).

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

As 5-FU is a pro-drug, it needs to be processed by a series of enzymes in cancer cells to generate the primary active anti-cancer metabolite, FUDR-MP. 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 addition, thymidine phosphorylase (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 also been observed that expression of TP correlates with response to 5-FU therapy (Panczyk, 2014).

5-FU is associated with significant dosing administration challenges due to poor pharmacokinetic (PK) properties. The plasma half-life 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 and to shift the metabolite profile from FUTP to FUDR-MP in order to limit toxicities. 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 shortcomings that limit the clinical utility of 5-FU, thereby improving the efficacy and safety profile, and 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 FBAL and associated off-target toxicities, such as hand foot syndrome.

In addition, NUC-3373 generates much lower levels of FUTP compared to 5-FU, thus the incidence of Grade 3 or higher toxicities associated with off-target incorporation of this molecule into the RNA of normal cells (*e.g.,* neutropaenia, diarrhoea, mucositis) 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 (McGuigan *et al*, 2011).

Finally, as NUC-3373 directly delivers the active anti-cancer metabolite intracellularly, there is no need for enzymatic conversion to FUDR and subsequent phosphorylation to FUDR-MP, thus overcoming major rate limiting 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.

Please refer to the current NUC-3373 Investigator's Brochure (IB) for further details.

### 1.4.1 Non-Clinical Data

In non-clinical studies, NUC-3373 has shown the ability to inhibit cell growth across a range of human tumour cell lines. In a panel of 9 different human CRC cell lines, the cytotoxic activity of NUC-3373 was independent of the basal TS level. Furthermore, thymidine supplementation rescued NUC-3373-induced cell death, confirming that NUC-3373 targets the de novo pathway of dTMP synthesis. In addition, potent tumour regression effects were observed in human CRC mouse xenograft models, with NUC-3373 showing greater tumour inhibition than 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). It was demonstrated that NUC-3373 is not susceptible to DPD-mediated degradation unlike 5-FU, which is rapidly catabolised by DPD.

In a series of *in vitro* experiments in CRC cell lines, it was found that NUC-3373 generates substantially higher levels of FUDR-MP and is a more potent inhibitor of TS than 5-FU. Additionally, the area under the curve (AUC) for dUMP was shown to be up to 162-times higher following NUC-3373 treatment compared to 5-FU treatment, suggesting a more pronounced effect on the dUMP:dTTP ratio. NUC-3373's ability to generate high intracellular levels of FUDR-MP resulted in the generation of fluorodeoxyuridine-triphosphate (FUDR-TP; also referred to as FdUTP), which was shown to be incorporated into the DNA of CRC cells. This effect was not observed with 5-FU, indicating that NUC-3373 is not only a more potent TS inhibitor, but is also a more efficient DNA-damaging agent compared to 5-FU. Furthermore, unlike 5-FU, NUC-3373 did not generate the toxic metabolite FUTP in these cell lines.

More details on the toxicology, pharmacology and toxicokinetics can be found in the NUC-3373 IB.

## 1.5. Clinical Data

### 1.5.1. NuTide:301

NuTide:301 was a first-in-human dose escalation study of NUC-3373 monotherapy in patients with advanced solid tumours (EudraCT 2015-002250-13; NCT02723240) conducted across three sites in the UK (Spiliopoulou *et al*, 2021). This was a two-part dose escalation and expansion study designed to evaluate safety, PK, pharmacodynamic, and anti-tumour activity, in addition to establishing the maximum tolerated dose (MTD) and recommended Phase II dose (RP2D) and schedule of single-agent intravenous (IV) NUC-3373.

In Part 1, NUC-3373 was administered on a weekly (Q1W) schedule on Days 1, 8, 15 and 22 of 28-day cycles. In Part 2, NUC-3373 was administered on an alternate weekly (Q2W) schedule on Days 1 and 15 of 28-day cycles. A total of 59 patients with metastatic cancer, who had exhausted all other available treatment options, received NUC-3373 in this study, with 43 patients receiving NUC-3373 on a Q1W schedule at doses ranging from 125 mg/m$^2$ to

3,250 mg/m$^2$ and 16 patients receiving NUC-3373 on a Q2W schedule at doses ranging from 1,500 mg/m$^2$ to 2,500 mg/m$^2$. This heavily pre-treated patient population (median of 3 prior lines of chemotherapy; range: 0-11) had a variety of primary tumour types.

NUC-3373 was well tolerated and, based on DLTs of Grade 2 headache and Grade 3 transient hypotension, the MTD of single-agent NUC-3373 was determined to be 2500 mg/m$^2$ weekly. The most common treatment-related Grade 1-2 adverse events (AEs) reported were fatigue (51%), nausea (37%), infusion reactions (36%), diarrhoea (32%), vomiting (17%), and anaemia (15%). The most common treatment-related Grade 3 AEs were ALT increased, AST increased, and transaminases increased (each in 3% of patients). No Grade 4 treatment-related AEs were reported.

Evidence of durable anti-cancer activity was observed, with 10 patients staying on study treatment for at least 4 months and three of these patients achieving prolonged stable disease of more than nine months. Many of these patients had received prior fluoropyridines as standard of care.

### 1.5.2. NuTide:302

NuTide:302 is an ongoing Phase Ib/II study of NUC-3373 in combination with standard agents used for the treatment of patients with advanced/metastatic CRC (Berlin *et al*, 2021) (EudraCT 2017-002062-53; NCT03428958). In this three-part study, the primary objective is to identify a recommended dose and schedule for NUC-3373 when administered in combinations with LV, oxaliplatin, irinotecan, and bevacizumab.

In Part 1, the safety and tolerability of NUC-3373 when co-administered with LV was assessed in Q1W and Q2W cohorts. The PK and safety profile of NUC-3373 was not affected by co-administration with LV; therefore, it was decided to administer NUC-3373 in combination with the standard dose of LV (400 mg/m$^2$) in subsequent parts of the study. In Part 2, the safety and tolerability of different doses of NUC-3373 when administered as part of Q1W NUFOX and NUFIRI regimens in ≥3$^{rd}$-line patients was assessed. Dose escalation has completed and the Q1W MTDs have been determined. In Part 3, 2$^{nd}$-line patients are receiving the selected NUFOX and NUFIRI regimens on a Q1W schedule in combination with bevacizumab.

To date, 38 patients have been treated in Part 1, with 21 patients treated on a Q2W schedule and 17 patients treated on a Q1W schedule. The most commonly reported treatment-related AEs in Part 1 were nausea (42%), fatigue (40%), vomiting (34%), and diarrhoea (29%). Grade 3 or 4 treatment-related AEs occurred in no more than 2 patients each, with the most common being nausea (5%), alanine aminotransferase (ALT) increased (5%), and anaemia (5%). Importantly, *in vitro* and *ex vivo* data to date strongly suggest that NUC-3373 does not generate FUTP, a primary cause of 5-FU toxicity and a dose-limiting factor (Adjei *et al*, 1999; Brutcher *et al*, 2018). FUTP has not been detected in CRC cell lines treated with NUC-3373 nor from the peripheral blood mononuclear cells (PBMCs) of patients treated with NUC-3373 in the NuTide:301 study. Accordingly, no NUC-3373 treated patients in Part 1 of NuTide:302 experienced Grade 3 or 4 toxicities associated with FUTP (diarrhoea, neutropaenia and mucositis).

Patients in Part 1 of this study were heavily pre-treated (median of 4 prior lines; range 2-13) and had exhausted all other treatment options. Despite this, encouraging efficacy signals have been observed in Part 1 with 11 patients achieving stable disease of at least 3 months. Most of these patients achieved periods of PFS that matched or exceeded those achieved on previous regimens. In addition, notable tumour shrinkage has been observed, including in a patient who was refractory to all prior fluoropyrimidine-containing regimens.

In Part 2, NUC-3373 was generally well-tolerated when administered in the NUFOX and NUFIRI regimens. In the NUFOX cohorts, patients received 400 mg/m$^2$ LV plus 85 mg/m$^2$ oxaliplatin plus NUC-3373 at 1,500 mg/m$^2$, 1,875 mg/m$^2$, or 2,250 mg/m$^2$. In the NUC-3373 2,250 mg/m$^2$ dose group, a total of 3 patients experienced DLTs. Two patients had Grade 3 fatigue and one patient had raised liver function tests potentially meeting the criteria for Hy's law (ALT/AST >3 × upper limit of normal [ULN] and bilirubin >2 × ULN). The NUC-3373 1,875 mg/m$^2$ dose group was expanded and no DLTs were reported; thus, the MTD and RP2D for the NUFOX regimen was determined to be 1,875 mg/m$^2$ NUC-3373 Q1W + 400 mg/m$^2$ LV Q1W + 85 mg/m$^2$ oxaliplatin Q2W.

In the NUFIRI cohorts, patients received 400 mg/m$^2$ LV plus irinotecan plus NUC-3373 as follows: NUC-3373 at 1,500 mg/m$^2$ + irinotecan at 120 mg/m$^2$, NUC-3373 at 1,500 mg/m$^2$ + irinotecan at 150 mg/m$^2$, NUC-3373 at 1,500 mg/m$^2$ + irinotecan at 180 mg/m$^2$, and NUC-3373 at 1,875 mg/m$^2$ + irinotecan at 180 mg/m$^2$. In the NUC-3373 1,875 mg/m$^2$ + irinotecan 180 mg/m$^2$ dose group, a total of 2 patients experienced DLTs. One patient had Grade 3 fatigue and one patient had Grade 3 colitis. As a result, the NUC-3373 1,500 mg/m$^2$ + irinotecan 180 mg/m$^2$ dose group was expanded and one patient experienced a DLT of Grade 3 ALT and ALP increased. Based on this, the MTD and RP2D for the NUFIRI regimen was determined to be 1500 mg/m$^2$ NUC-3373 Q1W + 400 mg/m$^2$ LV Q1W + 180 mg/m$^2$ irinotecan Q2W.

In the NUFIRI cohorts, the most commonly reported treatment-related TEAEs were nausea (43%), fatigue (30%), diarrhoea (26%), vomiting (26%), anaemia (26%), ALT increased (22%), and anorexia (22%). The majority of these events were Grade 1 or 2. The most commonly reported Grade 3 treatment-related TEAEs were fatigue (13%) and ALT increased (9%). No Grade 4 treatment-related TEAEs have been reported.

Data obtained from 8 patients treated in the NUFIRI-bev cohort in Part 3 to date (cut-off 17 Jan 2023) show that the regimen has been well tolerated. Patients received treatment with the RP2D of 1500 mg/m$^2$ NUC-3373 Q1W + 400 mg/m$^2$ LV Q1W + 180 mg/m$^2$ irinotecan Q2W in combination with 5 mg/kg bevacizumab Q2W. All 8 patients completed Cycle 1 without TEAEs that would meet DLT criteria in a dose-escalation design. The most commonly reported treatment-related TEAEs have been diarrhoea, fatigue, ALT increased and AST increased in 2 patients each. Two Grade 3 treatment-related TEAEs have been reported, ALT increased and pancreatitis. There have been no Grade 4 treatment-related TEAEs to date.

### 1.6. Pharmacokinetics

Plasma and intracellular PK analyses have been conducted on 45 patients from the NuTide:301 (range: 125 to 2500 mg/m$^2$) and NuTide:302 (1500 mg/m$^2$) studies. The PK profile of NUC-3373 compares favourably to that of 5-FU.

A dose-proportional increase in the NUC-3373 AUC was observed in plasma. Over the dose range studied, NUC-3373 had a mean plasma elimination half-life of 5-10 hours (vs 8-14 minutes for 5-FU). No accumulation was observed for NUC-3373 or its circulating metabolites (FUDR and FBAL) between Day 1 and Day 15.

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 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.

Please refer to the current NUC-3373 IB for further details.

## 1.7. Study Rationale

NUC-3373 is a pre-activated and protected form of the active anti-cancer agent FUDR that is being developed to replace fluoropyrimidines, such as 5-FU. Due to multiple limitations, 5-FU is not efficiently converted to the active anti-cancer metabolite FUDR-MP, is associated with dose-limiting toxicities due to the generation of specific metabolites and has a challenging administration schedule due to its poor PK profile. NUC-3373 is a targeted TS inhibitor designed to overcome the key shortcomings that limit the clinical utility of 5-FU.

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 FOLFOX failure in the 1st-line, irinotecan and FOLFIRI are the most appropriate options for 2nd-line treatment.

In non-clinical studies, NUC-3373 has shown the ability to inhibit cell growth across a range of human tumour cell lines, including CRC. In addition, significant tumour inhibition effects were observed in human CRC mouse xenograft models, with NUC-3373 showing greater tumour inhibition than 5-FU. A series of *in vitro* experiments provided proof of concept that NUC-3373 generates substantially higher levels of FUDR-MP and is a more potent inhibitor of TS than 5-FU, while generating much lower levels of toxic metabolites.

The non-clinical findings have been substantiated in patients treated with NUC-3373 monotherapy in the NuTide:301 clinical study and with NUC-3373 ± LV (Part 1), NUFOX and NUFIRI (Part 2) in the NuTide:302 clinical study. Data from these studies have confirmed the potential for NUC-3373 to generate high intracellular levels of FUDR-MP. Furthermore, NUC-3373 has been well-tolerated, with a lower incidence and reduced severity of fluoropyrimidine-related toxicities such as neutropaenia, diarrhoea and hand-foot syndrome compared to historical data for 5-FU. Encouraging signals of anti-cancer activity have been observed in heavily pre-treated patients, with tumour shrinkages as well as periods of PFS longer than those achieved on previous regimens. Owing to improved PK properties, NUC-3373 also offers a more convenient administration schedule (2-4 hours vs 46 hours for 5-FU).

Together these data demonstrate the potential for NUC-3373 to overcome the key limitations associated with 5-FU and suggest that NUC-3373 might be an attractive option to replace 5-FU in the current standard of care FOLFIRI regimen in the 2nd-line setting for unresectable metastatic CRC.

# 2. STUDY OBJECTIVES AND ENDPOINTS

## 2.1. Primary Objectives

- To compare PFS of NUC-3373 in combination with LV, irinotecan and bevacizumab (NUFIRI-bev) with 5-FU in combination with LV, irinotecan and bevacizumab (FOLFIRI-bev)
- To determine the optimal NUFIRI-bev dosing schedule

## 2.2. Secondary Objectives

- To compare the efficacy of NUFIRI-bev to FOLFIRI-bev in terms of:
  - Objective response rate (ORR)
  - Duration of response (DoR)
  - Disease control rate (DCR)
  - Maximum percentage change in tumour size
  - Overall survival (OS)
- To assess the safety and tolerability of NUFIRI-bev compared to FOLFIRI-bev
- To assess the PK of NUFIRI-bev

## 2.3. Exploratory Objective

To determine if there are tumour cell characteristics that may further elucidate the mechanisms through which the clinical activity of NUC-3373 is achieved (archival tissue, where available).

## 2.4. Primary Endpoint

- PFS, according to Response Evaluation Criteria in Solid Tumours (RECIST) v1.1, defined as the time from randomisation to the first observation of objective tumour progression or death from any cause

## 2.5. Secondary Endpoints

### 2.5.1 Efficacy

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

- ORR, defined as the percentage of patients achieving a complete or partial response to treatment
- DoR, defined as the time from initial clinical response (partial response [PR] or complete response [CR]) to the first observation of tumour progression or death from any cause
- DCR, defined as the percentage of patients demonstrating a best overall response (BOR) of CR, PR or stable disease (SD)
- Maximum percentage change from baseline in tumour size according to RECIST v1.1
- OS, defined as the time from randomisation to the time of death from any cause

### 2.5.2 Safety

Safety and tolerability will be assessed by evaluation of:

- TEAEs and serious adverse events (SAEs; per Common Terminology Criteria for Adverse Events [CTCAE] v5.0)
- Deaths due to TEAEs
- Treatment modifications due to TEAEs
- Clinically-significant laboratory changes (per CTCAE v5.0)
- Electrocardiograms (ECGs)

### 2.5.3 Pharmacokinetics

The PK of the NUFIRI-bev regimen will be assessed, including:

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

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

- NUC-3373, CPF-1027, FBAL, irinotecan, SN-38, SN-38G, APC

## 2.6. Exploratory Endpoint

- Population or tumour characteristic subtypes that may determine benefit to NUC-3373 treatment will be analysed

# 3. INVESTIGATIONAL PLAN

## 3.1. Overall Study Design

NuTide:323 is a randomised, open-label, dose/schedule optimisation study of NUFIRI-bev versus FOLFIRI-bev for the treatment of patients with unresectable metastatic CRC. Two NUFIRI-bev dosing schedules will be assessed, with a Q1W NUC-3373 + LV schedule and a Q2W NUC-3373 + LV schedule.

The overall aim of the study is to estimate the likely efficacy of the two NUFIRI arms as compared to the FOLFIRI control arm to support decision making regarding the further development of NUFIRI. Determination of the optimal NUFIRI dosing schedule will be based on a comparison of efficacy, safety, and PK between the two NUFIRI treatment arms.

A total of 171 patients will be randomised 1:1:1 (57 patients per arm) to either NUFIRI-bev Q1W, NUFIRI-bev Q2W, or FOLFIRI-bev Q2W. Randomisation will be stratified by RAS status (wild-type vs KRAS mutant vs NRAS mutant), prior bevacizumab treatment (yes vs no) and duration of prior line of therapy (<6 months vs ≥6 months).

Bevacizumab may be substituted with any approved biosimilar consistent with institutional practice. The specific agent administered will be recorded. Thus, bevacizumab in this protocol refers to either bevacizumab or a licensed biosimilar.

Patients may continue to receive treatment in the absence of disease progression or unacceptable toxicity that is not ameliorated by optimal medical or non-medical supportive or prophylactic care. 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 3.5), whichever occurs first.

The primary efficacy analysis will be performed when a total of 139 PFS events have occurred. An evaluation of efficacy may also be performed when a total of 70 PFS events have occurred.

The study will be considered complete once the final patient has completed their End of Treatment visit.

## 3.2. Rationale for the Doses Selected

### 3.2.1. NUFIRI Regimen

Patients in both NUFIRI arms will receive NUC-3373 at a dose of 1500 mg/m$^2$ + LV at a dose of 400 mg/m$^2$, on either a Q1W schedule (Arm A) or a Q2W schedule (Arm B). The irinotecan and bevacizumab components of the regimen will be administered at the standard doses and schedules.

The safety of NUC-3373 as a single agent (without LV) at doses ranging from 125 to 3250 mg/m$^2$ administered on Days 1, 8, 15 and 22 of a 28-day schedule (Q1W) has been studied in the NuTide:301 study. 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. 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.

In the NuTide:302 study of NUC-3373 in combination with standard agents for the treatment of patients with metastatic CRC, patients in Part 2 received NUFIRI on a Q1W NUC-3373 schedule in dose-escalation cohorts. Patients received 400 mg/m$^2$ LV plus irinotecan plus NUC-3373 as follows: NUC-3373 at 1,500 mg/m$^2$ + irinotecan at 120 mg/m$^2$, NUC-3373 at 1,500 mg/m$^2$ + irinotecan at 150 mg/m$^2$, NUC-3373 at 1,500 mg/m$^2$ + irinotecan at 180 mg/m$^2$, and NUC-3373 at 1,875 mg/m$^2$ + irinotecan at 180 mg/m$^2$.

In the NUFIRI cohorts, 2 patients experienced DLTs at the highest dose level tested (NUC-3373 1,875 mg/m$^2$ + irinotecan 180 mg/m$^2$). One patient had Grade 3 fatigue and one patient had Grade 3 colitis. As a result, the NUC-3373 1,500 mg/m$^2$ + irinotecan 180 mg/m$^2$ dose group was expanded and one patient experienced a DLT of Grade 3 ALT and ALP increased. Based on this, the RP2D for the NUFIRI regimen was determined to be 1500 mg/m$^2$ NUC-3373 Q1W + 400 mg/m$^2$ LV Q1W + 180 mg/m$^2$ irinotecan Q2W.

In Part 3 of NuTide:302, the selected NUFIRI regimen is being administered on a Q1W NUC-3373 schedule in combination with bevacizumab (5 mg/kg Q2W) for the 2$^{nd}$-line treatment of patients with advanced CRC. Data obtained from 8 patients treated in the NUFIRI-bev cohort to date (cut-off 17 Jan 2023) show that the regimen has been well tolerated. All 8 patients completed Cycle 1 without TEAEs that would meet DLT criteria in a dose escalation design. The most commonly reported treatment-related TEAEs have been diarrhoea, fatigue, ALT increased and AST increased in 2 patients each. Two Grade 3 treatment-related TEAEs have been reported, ALT increased and pancreatitis. There have been no Grade 4 treatment-related TEAEs. Based on the TEAE profile observed to date, there does not appear to be any overlapping toxicity when bevacizumab is added to the NUFIRI regimen.

The selected NUC-3373 dose for this study is the MTD and RP2D determined in the Q1W NUFIRI dose escalation cohorts in Part 2 of NuTide:302. The NUFIRI and NUFIRI-bev regimens have only been administered on a Q1W NUC-3373 schedule to date; therefore, one of the objectives of this study is to determine the optimal dosing schedule for the NUFIRI-bev regimen. It is anticipated that a Q2W NUC-3373 schedule would not only be well-tolerated, but would also ease the treatment burden on the patient and align with the standard FOLFIRI-bev dosing schedule.

### 3.2.2. FOLFIRI Regimen

In the comparator arm (Arm C), patients will receive the standard FOLFIRI-bev regimen established for the treatment of patients with metastatic CRC.

## 3.3. Study Treatment

This is a three-arm, combination therapy study in which study treatment will be administered in 28-day cycles.

Patients will be randomised 1:1:1 to receive either:

- NUFIRI-bev on a Q1W NUC-3373 schedule (Arm A)
- NUFIRI-bev on a Q2W NUC-3373 schedule (Arm B)
- FOLFIRI-bev on a Q2W schedule (Arm C)

The randomisation process is detailed in Section 8.5.

Protocol-specific guidelines for study treatment dosage and administration are provided in detail in Section 8.3. Guidelines for protocol-specific dose modifications and discontinuations are provided in Section 9. For patients experiencing toxicity related to NUC-3373 in the NUFIRI arms, initially the NUC-3373 dose modification guidelines in Section 9 should be followed. Dose modifications for the other agents (LV, irinotecan, bevacizumab, 5-FU) will be in accordance with their respective SmPCs/Prescribing Information and standard local practice, and guidelines are provided in Section 9.

Bevacizumab may be substituted with any approved biosimilar consistent with institutional practice. The specific agent administered will be recorded.

### 3.3.1 Arm A (NUFIRI Q1W)

In Arm A, NUC-3373 + LV will be administered Q1W and irinotecan and bevacizumab will be administered Q2W in 28-day cycles, as outlined in Table 1.

**Table 1 Study treatment in Arm A**

| Infusion order[a] | Infusion duration | D1 | D8 | D15 | D22 |
|---|---|---|---|---|---|
| Bevacizumab (5 mg/kg) | 30 minutes[b] | X | | X | |
| LV (400 mg/m$^2$)[c] | 120 minutes | X | X | X | X |
| Irinotecan (180 mg/m$^2$)[c] | 90 minutes | X | | X | |
| NUC-3373 (1500 mg/m$^2$) | 120 minutes | X | X | X | X |

[a]The infusion order and/or durations may be updated based on emerging data from the NuTide:302 study.
[b]The first dose of bevacizumab should be administered over 90 minutes. If this is well-tolerated, the second dose should be administered over 60 minutes. If this is well-tolerated, all subsequent doses should be administered over 30 minutes. **Note:** patients who have previously tolerated bevacizumab infusions well may receive all infusions over 30 minutes from the first dose.
[c]LV and irinotecan to be administered concurrently on Days 1 and 15.

### 3.3.2 Arm B (NUFIRI Q2W)

In Arm B, NUC-3373, LV, irinotecan and bevacizumab will be administered Q2W in 28-day cycles, as outlined in Table 2.

**Table 2 Study treatment in Arm B**

| Infusion order[a] | Infusion duration | D1 | D8 | D15 | D22 |
|---|---|---|---|---|---|
| Bevacizumab (5 mg/kg) | 30 minutes[b] | X | | X | |
| LV (400 mg/m$^2$)[c] | 120 minutes | X | | X | |
| Irinotecan (180 mg/m$^2$)[c] | 90 minutes | X | | X | |
| NUC-3373 (1500 mg/m$^2$) | 120 minutes | X | | X | |

[a]The infusion order and/or durations may be updated based on emerging data from the NuTide:302 study.
[b]The first dose of bevacizumab should be administered over 90 minutes. If this is well-tolerated, the second dose should be administered over 60 minutes. If this is well-tolerated, all subsequent doses should be administered over 30 minutes. **Note:** patients who have previously tolerated bevacizumab infusions well may receive all infusions over 30 minutes from the first dose.
[c]LV and irinotecan to be administered concurrently.

### 3.3.3 Arm C (FOLFIRI Q2W)

In Arm C, 5-FU, LV, irinotecan and bevacizumab will be administered Q2W in 28-day cycles, as outlined in Table 3.

**Table 3 Study treatment in Arm C**

| Infusion order | Infusion duration | D1 | D8 | D15 | D22 |
|---|---|---|---|---|---|
| Bevacizumab (5 mg/kg) | 30 minutes[a] | X | | X | |
| LV (400 mg/m$^2$)[b] | 120 minutes | X | | X | |
| Irinotecan (180 mg/m$^2$)[b] | 90 minutes | X | | X | |
| 5-FU bolus (400 mg/m$^2$) | Bolus | X | | X | |
| 5-FU infusion (2400 mg/m$^2$) | 46 hours | X | | X | |

[a]The first dose of bevacizumab should be administered over 90 minutes. If this is well-tolerated, the second dose should be administered over 60 minutes. If this is well-tolerated, all subsequent doses should be administered over 30 minutes. **Note:** patients who have previously tolerated bevacizumab infusions well may receive all infusions over 30 minutes from the first dose.
[b]LV and irinotecan to be administered concurrently.

## 3.4 Duration of Patient Participation

Patients may continue to receive study treatment until the occurrence of radiological disease progression by RECIST v1.1 or unacceptable toxicity 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, they should continue to undergo tumour assessment every 8 weeks (±7 days) from Cycle 1 Day 1 until such time as progression can be documented, a new treatment is initiated, or death.

Following discontinuation of study treatment, patients will receive treatment in accordance with local standard of care.

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 3.5), whichever occurs first.

## 3.5 Study Completion

The study will be considered complete once the final patient has completed their End of Treatment visit.

Following discontinuation of study treatment, patients will receive treatment in accordance with local standard of care.

Patients who are still receiving benefit from study treatment in the event of early termination of the study may continue NUC-3373 at the discretion of the Investigator and in accordance with local regulations until disease progression or until the drug becomes commercially available and accessible locally. A patient will be eligible to receive NUC-3373 after study completion if the following conditions are met:

- There is evidence of continued clinical benefit for the patient and the patient consents to continue with treatment
- There are no appropriate alternative treatment options available to the patient or a change in treatment would pose a risk to the patient
- The patient and his/her doctor comply with and satisfy any legal or regulatory requirements for continuation of NUC-3373

A patient will not be eligible to receive NUC-3373 after study completion if any of the following conditions are met:

- The Sponsor has discontinued development of NUC-3373 or data suggest that NUC-3373 is not effective for metastatic CRC
- The Sponsor has reasonable safety concerns regarding NUC-3373 as treatment for metastatic CRC
- Provision of NUC-3373 is not permitted under the laws and regulations of the patient's country

# 4 PATIENT SELECTION

## 4.1 Inclusion Criteria

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

1. Provision of written informed consent.
2. Histological or cytological confirmation of colorectal adenocarcinoma (excluding appendiceal and anal canal cancers, as well as signet-ring cell carcinoma) that is unresectable and metastatic.
3. Measurable disease (as defined by RECIST v1.1).
4. Received ≥2 months of a first-line fluoropyrimidine and oxaliplatin-containing regimen for metastatic disease or relapsed within 6 months of completing a fluoropyrimidine and oxaliplatin-containing neoadjuvant/adjuvant therapy. Previous treatment with standard of care chemotherapy regimens in combination with molecular targeted therapies (*e.g.,* VEGF and EGFR pathway inhibitors and immuno-oncology agents) is permitted. Previous treatment with maintenance therapy (*e.g.,* capecitabine) is also allowed. Patients who started on a fluoropyrimidine and oxaliplatin-containing regimen in any setting but must discontinue the oxaliplatin due to toxicity or allergy (and are now unable to receive oxaliplatin) are considered eligible regardless of the number of cycles of oxaliplatin they received.
5. Known RAS and BRAF status. Patients with wild-type RAS tumours must have received prior treatment with an EGFR inhibitor, unless this was not standard of care according to relevant region-specific treatment recommendations.
6. Known UGT1A1 status, or patient consents to UGT1A1 status testing if unknown.
7. Known DPD activity status, or patient consents to DPD status testing if unknown. See exclusion criterion 1.
8. Age ≥18 years.
9. Minimum life expectancy of ≥12 weeks.
10. Eastern Cooperative Oncology Group (ECOG) Performance status 0 or 1.
11. Adequate bone marrow function as defined by: absolute neutrophil count (ANC) ≥1.5 × $10^9$/L, platelet count ≥100 × $10^9$/L, and haemoglobin ≥9 g/dL. Patients with benign neutropenia may be discussed on a case-by-case basis with the medical monitor.
12. Adequate liver function, as defined by: serum total bilirubin ≤1.5 × ULN (this threshold does not apply to patients with Gilbert's syndrome, who should be discussed with the Medical Monitor), AST and ALT ≤2.5 × ULN (or ≤5 × ULN if liver metastases are present).
13. Adequate renal function assessed as serum creatinine <1.5 × ULN and glomerular filtration rate ≥50 mL/min (calculated by the Cockcroft-Gault method).
14. Serum albumin ≥3 g/dL.
15. Ability to comply with protocol requirements.

16. 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 forms of contraception, one of which must be highly effective. These forms of contraception must be used from the time of signing consent, throughout the treatment period, and for 6 months following the last dose of any study medication. Oral or injectable contraceptive agents cannot be the sole method of contraception
17. Patients must have been advised to take measures to avoid or minimize exposure of the skin and eyes to UV light, including avoiding sunbathing and solarium use, for the duration of study participation and for a period of 4 weeks following the last dose of study medication

## 4.2 Exclusion Criteria

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

1. History of hypersensitivity or current contra-indications to 5-FU, FUDR or capecitabine. This includes patients with genotypic or phenotypic (blood uracil level ≥150 ng/mL) evidence of complete DPD deficiency or TYMP mutations associated with toxicity to fluoropyrimidines. Patients who tolerated prior 5-FU at a reduced dose level may be enrolled and treated at that same dose.
2. History of hypersensitivity or current contra-indication to any of the combination agents required for the study.
3. History of allergic reactions attributed to components of the NUC-3373 drug product formulation (super refined polysorbate 80 [SRP80], dimethylacetamide [DMA]).
4. History of hypersensitivity to Chinese Hamster Ovary (CHO) cell products or other recombinant human or humanised antibodies.
5. History of or known central nervous system or leptomeningeal metastases.
6. Symptomatic ascites, ascites currently requiring drainage procedures or ascites requiring drainage over the prior 3 months.
7. Mutant BRAF V600E status.
8. MSI high or dMMR.
9. Prior treatment with irinotecan.
10. Chemotherapy, hormonal therapy, radiotherapy (other than a short cycle of palliative radiotherapy [*e.g.,* for bone pain]*), immunotherapy, biological agents, or exposure to another investigational agent within 21 days (or four times the half-life for molecular targeted agents, whichever is shorter) of first administration of study treatment:
    a. For nitrosoureas and mitomycin C within 6 weeks of first administration of study treatment
    b. Continuous dosing of ≥10 mg prednisolone (or steroid equivalent) is not allowed during the study. Corticosteroid treatment is allowed during screening but should be weaned to a dose of ≤10 mg prednisolone (or steroid equivalent) by Cycle 1 Day 1

** Palliative radiotherapy during participation in the study is permitted, but should not be concurrent with study treatment and recovery should be allowed to prevent overlapping toxicity (refer to Section 10.4). It should not include a target lesion.*

11. Residual toxicities from prior chemotherapy or radiotherapy which have not regressed to Grade ≤1 severity (CTCAE v5.0), except for alopecia and residual Grade 2 neuropathy.
12. History of other malignancies, except adequately treated non-melanoma skin cancer, curatively treated in situ cancer 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. Patients with previous invasive cancers are eligible if treatment was completed >3 years prior to initiating the current study treatment, and the patient has had no evidence or recurrence since then.
13. Presence of an active bacterial or viral infection (including SARS-CoV-2, Herpes Zoster, Varicella Zoster or chickenpox), known Human Immunodeficiency Virus (HIV) positive or known active hepatitis B or C.
14. 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 the following:
    a. Congestive heart failure (New York Heart Association Class III or Class IV)
    b. Clinically significant coronary heart disease or myocardial infarction within 6 months of the first dose of study medication or high risk of uncontrolled arrhythmia
    c. Unstable or poorly controlled angina pectoris
    d. Complete left bundle branch, fascicular block or other clinically significant abnormal ECG finding
    e. QTc interval >470 milliseconds
    f. History of or current risk factor for torsade de pointes (*e.g.,* heart failure, hypokalaemia, or a family history of long QT syndrome)
    g. History of severe skin reactions (except for skin reactions that are a consequence of recent anti-cancer treatment, including chemotherapies not currently under investigation in this study [*e.g.,* oxaliplatin] or molecular targeted therapies such as EGFR inhibitors)
    h. History of severe ocular disorders
    i. Interstitial pneumonitis or pulmonary fibrosis
15. 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 provide informed consent and undergo study procedures.
16. Patients with a history of haemoptysis (1/2 teaspoon or more of red blood) within 6 months prior to enrolment.

17. Wound healing complications or surgery within 28 days of starting bevacizumab (wound healing must have been fully completed before starting bevacizumab). Investigators may allow patients to initiate treatment with the other study drugs (*i.e.,* NUC-3373/5-FU, LV and irinotecan) on C1D1 but withhold bevacizumab for at least 15 days, but no longer than 28 days, to allow completion of wound healing in patients who would otherwise be eligible for the study, in line with standard local practice and after discussion with the Medical Monitor. Patients who have not received bevacizumab by C2D1 must be replaced.
18. Unhealed wound, active gastric or duodenal ulcer, or bone fracture.
19. Serious thromboembolic event in the 6 months before inclusion (*e.g.,* transitory ischemic stroke, stroke, subarachnoid haemorrhage). Patients with non-serious thromboembolic events (*e.g.,* non-symptomatic pulmonary embolism or peripheral deep vein thrombosis treated with anticoagulants) may be enrolled after discussion with the Medical Monitor.
20. Patients with a history of haemorrhage within 6 months prior to enrolment.
21. Known inherited or acquired bleeding disorders.
22. Red blood cell (RBC) transfusion dependence, defined as requiring more than 2 units of packed RBC transfusions during the 4-week period prior to screening.
23. Uncontrolled hypertension.
24. Severe proteinuria or nephrotic syndrome (≥Grade 3 [urine dipstick +4 or ≥3.5 g/day]).
25. Acute intestinal obstruction or sub-obstruction, history of inflammatory intestinal disease (including colitis or Crohn's disease) or extended resection of the small intestine. Presence of a colic prosthesis.
26. History of abdominal fistulas, trachea-oesophageal fistulas, any other Grade 4 gastrointestinal perforations, non-gastrointestinal fistulas, or intra-abdominal abscesses 6 months prior to screening.
27. Currently pregnant, lactating or breastfeeding.
28. Required concomitant use of brivudine, sorivudine and analogues.
29. Required concomitant use of St John's Wort.
30. Required concomitant use of drugs known to prolong QT/QTc interval.
31. Required concomitant use of strong CYP3A4 inducers or strong CYP3A4 inhibitors. The use of strong CYP3A4 inducers within 2 weeks of first receipt of study drug or the use of strong CYP3A4 inhibitors within 1 week of first receipt of study drug is also excluded.
32. Use of strong UGT1A1 inhibitors within 1 week of first receipt of study drug.
33. Received a live vaccination within four weeks of first planned dose of study medication.
34. **Germany only:** Patients who have been placed in an institution by court or official order.

**Germany only:**

During Screening, all patients will be interviewed concerning any potential relationship to the Investigator, the medical staff of the study team, the Coordinating Investigator, or the Sponsor.

Patients with a relationship/dependency who may receive potential benefit from the study should not be excluded; however, the patient cannot be enrolled by the person they are dependent on.

### 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 CRO 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. 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. 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. 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.

Standard of care assessments that were completed prior to informed consent but are within the screening window may be used for screening assessments and do not have to be repeated. Imaging completed prior to informed consent for routine clinical practice purposes is acceptable provided it was performed as per RECIST v1.1 criteria and within 28 days of randomisation. All protocol-required assessments that are not deemed standard of care should not be performed prior to written informed consent.

### 5.3 Screening Assessments

Screening assessments of consented patients will comprise the following:

- Provision of written informed consent
- Eligibility confirmation, including histological or cytological diagnosis of CRC that is unresectable and metastatic
- Recording of demographic data
- Assessment of medical and surgical history, including prior therapy for CRC as well as recording information on the sidedness of the patient's CRC (left-sided vs right-sided).
- Recording of concomitant medication
- Full physical examination, including vital signs. Full physical examination includes ears, eyes, nose, head, throat, neck, lungs, heart, abdomen, extremities, skin, musculoskeletal, nervous system, and lymph nodes. Vital signs include measurement of pulse rate, respiratory rate, blood pressure and temperature, after the patient has been seated or in the supine position for 5 minutes
- Height and weight
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- 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 timing between the triplicate ECGs is recommended to be approximately 1 minute
    - 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/or certified paper copies should be retained at site and stored as part of the study documents in the event they need to be used in a future analysis
- Blood samples drawn for:
    - Pregnancy testing: For women of childbearing potential a serum pregnancy test must be performed within 7 days of the first dose of study treatment
    - Haematology: white blood cell (WBC) count, differential WBC count, RBC count, haemoglobin, haematocrit and platelets
    - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, phosphate, total protein, albumin, adjusted calcium, total bilirubin, direct bilirubin, indirect bilirubin, bicarbonate, chloride, uric acid, alkaline phosphatase, AST, ALT and lactate dehydrogenase (LDH)
    - Coagulation parameters: prothrombin time/international normalised ratio (PT/INR) and activated partial thromboplastin time (aPTT)

  - Tumour markers (carcinoembryonic antigen [CEA])
  - DPD status testing, if not known. Performed by either genotyping or phenotypic testing of blood uracil levels. Patients with blood uracil ≥150 ng/mL are considered to be DPD deficient and are excluded from participation. Patients with blood uracil levels of ≥16 ng/mL to <150 ng/mL are considered to be partially DPD deficient. The 5-FU dose must be adapted for partially DPD deficient patients in countries where this is standard practice as per national and/or local guidelines (refer to Section 9.2.1)
  - BRAF/KRAS/NRAS/MSI/MMR status testing, if not known. Results must be obtained prior to the patient receiving the first dose of study treatment
  - UGT1A1 status testing, if not known. Results do not need to be obtained prior to the patient receiving the first dose of study treatment. If a patient's mutational status is known prior to dosing and they have a mutation that may affect their ability to metabolise irinotecan, an initial dose reduction of irinotecan may be implemented as per the irinotecan SmPC/Prescribing Information and site standard of care. This must be discussed on a case-by-case basis with the medical monitor

- Tumour imaging (computed tomography [CT] or magnetic resonance imaging [MRI] of thorax, abdomen and pelvis) – performed within 28 days prior to randomisation. A recent previously obtained image may be used if obtained within 28 days prior to randomisation and images collected meet protocol requirements.
- AE recording and causality assessment
- Obtain an archival tumour tissue block, if available
- 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
- Advise the patient to consider cryo-conservation of their sperm or eggs prior to treatment (refer to Section 9.3.9)
- A dental examination and appropriate preventative dentistry should be considered prior to starting treatment with bevacizumab. In patients who have previously received or are receiving IV bisphosphonates, invasive dental procedures should be avoided if possible
- Patient registration

## 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 reassessed as needed during the 28-day screening period without being considered a screen failure.

If a patient does not meet the inclusion/exclusion criteria by the end of the 28-day screening period, they are considered a screen failure but may be re-screened once. These patients will be reconsented. Patients who fail at re-screening are ineligible and may not be enrolled.

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 C1D1.

During the re-screening process, any Screening assessment that will be greater than 28 days from the date taken to C1D1 must also be repeated.

## 5.5 Evaluations to be Performed during the Study

### 5.5.1 Each cycle, Day 1 (± 2 days; All arms)

All procedures to be completed prior to dosing.

- Recording of new or changes to concomitant medication.
- Physical examination
  - Full physical examination (including neurological examination) to be performed on C1D1 and C2D1 only. Full physical examination includes ears, eyes, nose, head, throat, neck, lungs, heart, abdomen, extremities, skin, musculoskeletal, nervous system, and lymph nodes
  - Directed physical examination (directed by clinical signs and symptoms, including neurological examination), only if clinically indicated, to be performed from Cycle 3 onwards
- Weight
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- ECOG performance status
- Vital signs, including measurement of pulse rate, respiratory rate, blood pressure and temperature, after the patient has been seated or in the supine position for 5 minutes
- **Pre-dose ECGs:** 12-lead ECGs should be performed prior to infusion of all investigational medicinal products (IMPs) for patients in all treatment arms
- **Post-dose ECGs:** 12-lead ECGs should be performed post infusion of all IMPs (within 10 minutes of the end of infusion) on **C1D1 and C2D1** for patients in the NUFIRI treatment arms only (Arms A and B)
  - 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 timing between the triplicate ECGs is recommended to be approximately 1 minute
  - 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/or certified paper copies should be retained at site and stored as part of the study documents in the event they need to be used in a future analysis
- Urine pregnancy test for women of child-bearing potential. If any urine test result is positive, patient dosing will be postponed until the result is confirmed by a serum pregnancy test. Any patient with a positive serum test will not be allowed to receive any study treatment

- Blood samples drawn for:
  - Haematology: WBC count, differential WBC count, RBC count, haemoglobin, haematocrit and platelets
  - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase, AST, ALT and LDH
  - Coagulation parameters*:* PT/INR and aPTT
  - **From Cycle 2 Day 1 onwards:** Tumour markers (CEA)
- Randomisation (can be performed 3 working days prior to C1D1)
- IV administration of study drug:
  - Arm A: Bevacizumab + LV + irinotecan + NUC-3373
  - Arm B: Bevacizumab + LV + irinotecan + NUC-3373
  - Arm C: Bevacizumab + LV + irinotecan + 5-FU
- AE recording and causality assessment
- Blood samples for PK measurements on **C2D1 only** - Refer to Section 7.2 for more details

### 5.5.2 Each cycle, Day 8 (± 2 days; Arm A only)

All procedures to be completed prior to dosing:

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

### 5.5.3 Each cycle, Day 15 (± 2 days; All arms)

All procedures to be completed prior to dosing:

- Recording of new or changes to concomitant medications
- Vital signs, including measurement of pulse rate, respiratory rate, blood pressure and temperature, after the patient has been seated or in the supine position for 5 minutes
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- **Pre-dose ECGs:** 12-lead ECGs should be performed prior to infusion of all investigational medicinal products (IMPs) on **C1D15** and **C2D15** for patients in all treatment arms.
- **Post-dose ECGs:** 12-lead ECGs should be performed post infusion of all IMPs (within 10 minutes of the end of infusion) on **C1D15 and C2D15** for patients in the NUFIRI treatment arms only (Arms A and B)
    - 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 timing between the triplicate ECGs is recommended to be approximately 1 minute
    - 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/or 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 count, haemoglobin, haematocrit and platelets
    - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase, AST, ALT and LDH
- IV administration of study drug:
    - Arm A: Bevacizumab + LV + irinotecan + NUC-3373
    - Arm B: Bevacizumab + LV + irinotecan + NUC-3373
    - Arm C: Bevacizumab + LV + irinotecan + 5-FU
- AE recording and causality assessment

### 5.5.4 Each cycle, Day 22 (± 2 days; Arm A only)

All procedures to be completed prior to dosing:

- Recording of new or changes to concomitant medications

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

### 5.5.5 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)
  - Additional tests may be requested at the Investigator's discretion. The same modality should be used throughout

### 5.5.6 End of Treatment Visit (30 days [+7 days) after last dose)

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

- Recording of new or changes to concomitant medication
- Full physical examination, including vital signs. Full physical examination includes ears, eyes, nose, head, throat, neck, lungs, heart, abdomen, extremities, skin, musculoskeletal, nervous system, and lymph nodes. Vital signs include measurement of pulse rate, respiratory rate, blood pressure and temperature, after the patient has been seated or in the supine position for 5 minutes, and temperature
- Weight
- Urinalysis: pH, specific gravity, ketones, leukocytes, protein, glucose, bilirubin, urobilirubin and occult blood. Dipstick testing is acceptable
- 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 timing between the triplicate ECGs is recommended to be approximately 1 minute

  - 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/or certified paper copies should be retained at site and stored as part of the study documents in the event they need to be used in a future analysis
- Blood samples drawn for:
  - Pregnancy testing for women of child-bearing potential
  - Haematology: WBC count, differential WBC count, RBC count, haemoglobin, haematocrit and platelets
  - Chemistry: sodium, potassium, magnesium, urea, creatinine, glucose, bicarbonate, chloride, uric acid, phosphate, total protein, albumin, adjusted calcium, total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase, AST, ALT and LDH
  - Coagulation parameters: PT/INR and aPTT
  - Tumour markers CEA
- AE recording and causality assessment

### 5.5.7 Long-Term Follow-Up

**In France only:** Pregnancy testing must continue in women of childbearing potential every 4 weeks for 6 months after the last dose of study treatment. This post-study treatment pregnancy testing must not be performed with a home test; however, in order to avoid frequent hospital visits, it may be performed at an external laboratory that is local to the patient, with oversight by the study site. If this occurs, the study site must notify the Sponsor. All instances of positive pregnancy tests must be notified by the external laboratory to the study site, Sponsor and the patient's treating physician.

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 every 12 weeks (±14 days) from C1D1 until withdrawal of consent, lost to follow-up, death, or the overall end of study has been reached (defined in Section 3.5), whichever occurs first. Patients in follow-up who have not experienced disease progression should continue to attend the clinic for planned radiologic scans; however, other follow-up data can be collected remotely.

# 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 study treatment 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 study treatment including the following:
    - AE resulting in a dosing delay of more than 28 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
- Recurrent radiologically confirmed pneumonitis, which does not respond to steroids
- Radiologically confirmed posterior reversible encephalopathy syndrome (PRES)
- Lack of further clinical benefit or unfavourable risk/benefit profile as judged by the Investigator
- Inter-current illness that prevents further administration of study treatment
- 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, 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
- Patient non-compliance
- Lost to follow-up
- Patient withdrawal of consent
- Sponsor request

All study procedures outlined for the End of Treatment 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 Follow-up After Treatment Discontinuation

Patients who have documented disease progression defined by RECIST v1.1 criteria while receiving study treatment will discontinue treatment but will enter the follow-up period. Treatment should not be discontinued because of raised tumour marker levels or other clinical signs of progressive disease until disease status has been assessed by objective measures and disease progression has been determined by RECIST v1.1 criteria.

Patients who stop treatment with no evidence of disease progression will enter the follow-up period and should attend clinic at least every 8 weeks (±7 days) for follow-up scans, and information regarding subsequent anti-cancer medications prescribed. This follow-up should continue until disease progression per RECIST v1.1, initiation of a new anti-cancer medication, or death. Patients who no longer attend clinic every 8 weeks for any reason should continue to be followed for progression status (unless progression has already been documented), for details on any new anti-cancer treatments, and for survival status every 12 weeks (±14 days). If a patient becomes uncontactable, then the Investigator should follow up on their status by other means (such as GP contact, next of kin contact, hospital note review, or national registries/databases), in line with patient consent. A total of three attempts should be made before the patient is considered as lost to follow-up.

## 6.3 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.

Patients may decide to discontinue from further study treatment or from further participation in the study, as described below.

**Decision to discontinue study treatment**

If a patient decides to discontinue any further study treatment, it should be confirmed with them whether they agree to complete the follow-up visits.

**Decision to discontinue study participation**

Due to the importance of collection of PFS and survival data, if a patient decides to discontinue study participation, it should be confirmed with them whether they agree for the Investigator to follow up on their status by other means (such as GP contact, next of kin contact, review of medical notes or national registries/databases) or if they truly wish to withdraw so that no further data is collected on their status. Confirmation of this will be recorded in source data at site.

If a patient withdraws consent for further treatment and participation in the study, then no additional study visits or data collection should occur. 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.

# 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 PK of the NUFIRI-bev regimen and safety, pharmacodynamics and clinical activity.

Blood samples will be collected from patients in the NUFIRI treatment arms (Arms A and B) on C2D1 only at the following timepoints:

| PK timepoint | Description |
|---|---|
| Pre-dose | Prior to administration of NUC-3373 |
| End of infusion | Within 5 minutes before end of NUC-3373 infusion |
| 2-4 hours post-infusion | 2-4 hours after end of NUC-3373 infusion |
| 6-24 hours post-infusion | 6-24 hours after end of NUC-3373 infusion |

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

PK analyses will include plasma assessments of, but not limited to:

- NUC-3373, CPF-1027, FBAL, irinotecan, SN-38, SN-38G, APC

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 Tumour Tissue Sample Submission

An archival specimen will be collected from consenting patients for whom an archival tissue exists during Screening for potential correlation of tumour characteristics and efficacy.

Specific instructions on the collection and shipment of tissue samples will be provided in the Tumour Tissue Laboratory Manual.

## 7.4 Labelling and Confidentiality of Biological Samples

All biological samples (including blood 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.

## 7.5 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 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 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 CRF.

**Dubois & Dubois BSA calculation:**

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

## 8.2 Other IMPs

All of the agents to be used in this study (irinotecan, bevacizumab, 5-FU and LV) are commercially available and should be sourced locally by the Investigative sites where possible; however, they may be provided centrally should this be deemed necessary by the Sponsor.

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.

Refer to the SmPCs/Prescribing Information of these agents for the management of patients, particularly concerning contraindications, special warnings and precautions, posology adaptation in case of toxicity, monitoring, and medications that are contraindicated or that must be used with caution. Copies of these SmPCs/Prescribing Information will be provided to each site.

Bevacizumab may be substituted with any approved biosimilar consistent with institutional practice. The specific agent administered will be recorded.

## 8.3 Administration of NUC-3373 and Other Agents

The sections below provide administration times and order for each of the combination agents in each arm of the study. 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.

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

### 8.3.1 Arm A

In Arm A, bevacizumab will be administered at a dose of 5 mg/kg and irinotecan will be administered at a dose of 180 mg/m$^2$, both by IV infusion, on Days 1 and 15 (Q2W). LV will be administered at a dose of 400 mg/m$^2$ by IV infusion on Days 1, 8, 15 and 22 (Q1W). Following completion of infusions on Days 1 and 15 (bevacizumab, irinotecan and LV) and Days 8 and 22 (LV), NUC-3373 will be administered by IV infusion at a dose of 1500 mg/m$^2$ (Q1W).

Study treatment will be administered in 28-day cycles as follows:

1. Bevacizumab 5 mg/kg on Days 1 and 15:
    - 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)

    **Note:** patients who have previously tolerated bevacizumab infusions well may receive all infusions over 30 minutes from the first dose.
2. LV 400 mg/m$^2$ over 120 minutes on Days 1, 8, 15, and 22
3. Irinotecan 180 mg/m$^2$ over 90 minutes (concurrently with the LV infusion) on Days 1 and 15
4. NUC-3373 1500 mg/m$^2$ over 120 minutes on Days 1, 8, 15, and 22

**Note:** the infusion order and/or durations may be updated based on emerging data from the NuTide:302 study.

### 8.3.2 Arm B

In Arm B, bevacizumab will be administered at a dose of 5 mg/kg, irinotecan will be administered at a dose of 180 mg/m$^2$, and LV will be administered at a dose of 400 mg/m$^2$, all by IV infusion, on Days 1 and 15 (Q2W). Following completion of infusions on Days 1 and 15, NUC-3373 will be administered by IV infusion at a dose of 1500 mg/m$^2$ (Q2W).

Study treatment will be administered in 28-day cycles as follows:

1. Bevacizumab 5 mg/kg on Days 1 and 15:
    - 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)

    **Note:** patients who have previously tolerated bevacizumab infusions well may receive all infusions over 30 minutes from the first dose.
2. LV 400 mg/m$^2$ over 120 minutes on Days 1 and 15
3. Irinotecan 180 mg/m$^2$ over 90 minutes (concurrently with the LV infusion) on Days 1 and 15
4. NUC-3373 1500 mg/m$^2$ over 120 minutes on Days 1 and 15

**Note:** the infusion order and/or durations may be updated based on emerging data from the NuTide:302 study.

### 8.3.3 Arm C

In Arm C, bevacizumab will be administered at a dose of 5 mg/kg, irinotecan will be administered at a dose of 180 mg/m$^2$, and LV will be administered at a dose of 400 mg/m$^2$, all by IV infusion, on Days 1 and 15 (Q2W). Following completion of infusions on Days 1 and 15, 5-FU will be administered by IV bolus at 400 mg/m$^2$ followed by continuous IV infusion at 2,400 mg/m$^2$ over 46 hours (Q2W).

Study treatment will be administered in 28-day cycles as follows:

1. Bevacizumab 5 mg/kg on Days 1 and 15:
    - 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)

    **Note:** patients who have previously tolerated bevacizumab infusions well may receive all infusions over 30 minutes from the first dose.
2. LV 400 mg/m$^2$ over 120 minutes on Days 1 and 15
3. Irinotecan 180 mg/m$^2$ over 90 minutes (concurrently with the LV infusion) on Days 1 and 15
4. 5-FU 400 mg/m$^2$ bolus on Days 1 and 15
5. 5-FU 2400 mg/m$^2$ infusion over 46 hours on Days 1 and 15

### 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 Randomisation

Patients may be randomised up to 3 working days prior to C1D1 using an independent interactive voice- or web-based response system (IxRS). At this time, the Investigator will enter into the IxRS their site information, RAS status (wild-type vs KRAS mutant vs NRAS mutant), prior bevacizumab treatment (yes vs no), and duration of prior line of therapy (<6 months vs ≥6 months). The IxRS will then indicate to which treatment arm the patient has been randomised and the study site will obtain the patient's identification number from the IxRS.

The randomisation will be in a 1:1:1 ratio to receive treatment in Arm A (NUFIRI-bev Q1W), Arm B (NUFIRI-bev Q2W), or Arm C (FOLFIRI-bev Q2W). Randomisation will be stratified by the following 3 factors:

- RAS status (wild-type vs KRAS mutant vs NRAS mutant)
- Prior bevacizumab treatment (yes vs no)
- Duration of prior line of therapy (<6 months vs ≥6 months)

### 8.6 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.

### 8.7 Management of Overdose

The dose of NUC-3373 intended for use on this protocol is 1500 mg/m$^2$. In the Phase I (NuTide:301), first-in-human study, the MTD was 2500 mg/m$^2$ weekly and doses of NUC-3373 should not exceed this dose level. 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, irinotecan, bevacizumab, 5-FU), management should follow guidance in the SmPC/Prescribing Information and 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.

### 8.8 Interaction with Other Drugs

Patients receiving NUC-3373 should be carefully managed for potential drug interactions according to local practice. Refer to Section 10 for information regarding the use of prohibited therapies and therapies to be used with caution.

Interactions between the protocol specified combination agents (LV, irinotecan, bevacizumab) or 5-FU and any concomitant medications should be checked with the respective SmPCs/Prescribing Information in accordance with local practice. Local practice guidelines should be followed for the management of any potential study treatment/concomitant medication interactions.

# 9 MANAGEMENT OF TOXICITY

Adverse events may be managed by dose delays and/or dose reductions according to the clinical situation.

Guidelines for modification of study treatment dosing for haematological and non-haematological toxicities are given in Table 4. Please refer to Section 9.2.1 for dose modification guidelines for patients with partial DPD deficiency.

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, patients in the NUFIRI arms may have a maximum of 2 permanent dose reductions of NUC-3373, after which treatment will be discontinued and the patient will be removed from study. Patients may have 2 permanent dose reductions of irinotecan but can remain on the study as long as they are still receiving treatment with NUC-3373.

**Table 4 Dose reduction guidelines**

| Reduction | NUC-3373[1] | LV[2] | Irinotecan | 5-FU | | Bevacizumab[3] |
|---|---|---|---|---|---|---|
| | | | | Bolus | Infusion | |
| Full dose | 1500 mg/m$^2$ | 400 mg/m$^2$ | 180 mg/m$^2$ | 400 mg/m$^2$ | 2400 mg/m$^2$ | 5 mg/kg |
| -1 | 1125 mg/m$^2$ | 100% | 150 mg/m$^2$ | 200 mg/m$^2$ | 2000 mg/m$^2$ | 100% |
| -2 | 840 mg/m$^2$ | 100% | 120 mg/m$^2$ | 0 mg/m$^2$ | 2000 mg/m$^2$ | 100% |
| -3 | N/A | 100% | N/A | 0 mg/m$^2$ | 1600 mg/m$^2$ | 100% |

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

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

[3]Bevacizumab dose reductions are not permitted. Only dose delays or discontinuations should be considered.

Treatment between cycles can be delayed for up to 28 days for patients to meet the re-treatment criteria before starting their next cycle. Patients who do not meet these requirements after this additional time will not be allowed to receive further cycles of treatment and will be withdrawn from the study, unless the patient is receiving clinical benefit in the opinion of the Investigator.

In patients receiving clinical benefit, AEs attributed to the combination agents may be managed by dose adjustment, holds or discontinuation of the agent in accordance with standard clinical practice. However, treatment with NUC-3373 or 5-FU must be continued for the patient to remain on treatment for the purposes of this protocol.

## 9.1 Criteria for Continuation of Treatment

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

- ANC (growth factor support permitted)
  - $\geq 1.5 \times 10^9$/L for full treatment dose
  - $\geq 1.0 \times 10^9$/L for reduced treatment dose (see Table 5)
- Platelet count $\geq 75 \times 10^9$/L (platelet transfusion permitted)
- 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

If a patient fails to meet any of these criteria, administration of study drug should be held until all of the re-treatment criteria are met. The study drugs can be withheld for a maximum of 28 days; if the delay is >28 days, the patient must be withdrawn from treatment, unless the patient is receiving a clinical benefit.

## 9.2 Dose Modifications

Treatment between cycles can be delayed for up to 28 days for patients to meet the re-treatment criteria before starting their next cycle. Patients who fail to meet these requirements after this additional time will not be allowed to receive further cycles of therapy within the study, unless they are receiving clinical benefit in the opinion of the Investigator. If a patient experiences multiple toxicities, dose adjustments will be based on the most severe toxicity.

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

General guidance for dose modifications of NUC-3373, irinotecan and 5-FU is provided in Table 5. At the discretion of the Investigator, the doses of both drugs (NUC-3373/irinotecan or 5-FU/irinotecan) may be modified or the dose of the drug suspected to be the main underlying cause of the toxicity may be reduced. The dose of LV should be maintained.

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

If a patient's UGT1A1 mutational status is known prior to dosing and they have a mutation that may affect their ability to metabolise irinotecan, an initial dose reduction of irinotecan may be implemented as per the irinotecan SmPC/Prescribing Information and site standard of care. This must be discussed on a case-by-case basis with the medical monitor.

Bevacizumab dose reductions are not permitted; however, dosing may be delayed or discontinued to manage toxicities that are suspected to be related to bevacizumab (see Section 9.3.10). Patients who discontinue bevacizumab can remain on the study as long as they are still receiving treatment with NUC-3373 or 5-FU.

Refer to the SmPCs/Prescribing Information of LV, irinotecan, bevacizumab and 5-FU for the management of patients, particularly concerning contraindications, special warnings and precautions, posology adaptation in case of toxicity, monitoring, and medications that are contraindicated or that must be used with caution. Copies of these SmPCs/Prescribing Information will be provided to each site.

**Table 5 Dose modifications for NUC-3373, irinotecan and 5-FU**

| Toxicity Grade | During a cycle of therapy | At the start of subsequent cycles of therapy[a] |
|---|---|---|
| **Neutropenia** | | |
| Grade 1 (LLN – 1500/mm$^3$) | Maintain dose level | Maintain dose level |
| Grade 2 (<1500 – 1000/mm$^3$) | Reduce by 1 dose level for the next administration | 1 episode: Maintain starting dose used in previous cycle<br>≥2 episodes: Maintain reduced dose level |
| Grade 3 (<1000 – 500/mm$^3$) | Omit dose until resolved to ≤Grade 2, then reduce by 1 dose level | Maintain reduced dose level |
| Grade 4 (<500/mm$^3$) | Omit dose until resolved to ≤Grade 2, then reduce by 2 dose levels | Maintain reduced dose level |
| **Neutropenic fever** | | |
| All | Omit dose until resolved (no fever and ≤Grade 2 neutropenia), then reduce by 2 dose levels | |
| **Other haematologic toxicities** | | |
| All | Dose modifications for leukopenia or thrombocytopenia during a cycle of therapy and at the start of subsequent cycles of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above. | |
| **Diarrhoea** | | |
| Grade 1 | Continue dosing, depending on clinical judgement | Maintain dose level |
| Grade 2 | Omit dose until resolved to baseline, then reduce by 1 dose level | 1 episode: Maintain starting dose used in previous cycle<br>≥2 episodes: Maintain reduced dose level |
| Grade 3 | Omit dose until resolved to baseline, then reduce by 1 dose level | Maintain reduced dose level |
| Grade 4 | Omit dose until resolved to baseline, then reduce by 2 dose levels | Maintain reduced dose level |
| **Cardiotoxicity** | | |
| All | In case of severe cardiotoxicity, discontinue 5-FU or NUC-3373 treatment. | |
| **Other non-haematologic toxicities[b]** | | |
| Grade 1 | Maintain dose level | Maintain dose level |
| Grade 2 | Reduce by 1 dose level for the next administration | 1 episode: Maintain starting dose used in previous cycle<br>≥2 episodes: Maintain reduced dose level |

| Toxicity Grade | During a cycle of therapy | At the start of subsequent cycles of therapy[a] |
|---|---|---|
| Grade 3 | Omit dose until resolved to ≤Grade 2, then reduce by 1 dose level | Maintain reduced dose level |
| Grade 4 | Omit dose until resolved to ≤Grade 2, then reduce by 2 dose levels | Maintain reduced dose level |
| Note: | For mucositis/stomatitis decrease only 5-FU or NUC-3373, not irinotecan | |
| [a]Relative to the starting dose used in the previous cycle<br>[b]Excludes alopecia, anorexia, asthenia | | |

### 9.2.1 Dose Adaptations for Patients with Partial DPD Deficiency

The Prescribing Information and guidelines regarding the treatment of patients with DPD deficiency differ between countries. As such, the intention of the following subsections is to allow individual countries to manage patients in line with local guidelines.

For patients who enter the study with a reduced 5-FU dose level due to prior toxicity, any further dose reductions should be discussed on a case-by-case basis with the Medical Monitor.

#### 9.2.1.1 France

It is essential to be vigilant for patients whose blood uracil level is above the limit of normal (16 ng/mL), especially when this level is high, although below 150 ng/mL.

Patients in Arm C who have partial DPD deficiency by phenotypic testing (blood uracil level of ≥16 ng/mL to <150 ng/mL) must have the initial 5-FU dose adapted as follows:

- 25% dose reduction for blood uracil ≥16 ng/mL to <50 ng/mL
- 50% dose reduction for blood uracil ≥50 ng/mL to <100 ng/mL
- 75% dose reduction for blood uracil ≥100 ng/mL to <150 ng/mL

These dose reduction guidelines apply to both the 5-FU bolus and the infusion. The decision on initial dose adaptations should also consider other risk factors, such as the dose regimen and the age and general condition of the patient.

Following the initial starting dose adaptation, the 5-FU dose potentially can be re-adjusted towards the standard full dose from Cycle 2 onwards at the discretion of the Investigator based on patient tolerability of the lower dose and overall health status (HAS guidelines, 2018; Laures *et al*, 2022).

Guidelines for modification of 5-FU dosing for toxicities in patients who are already receiving a reduced starting dose of 5-FU due to partial DPD deficiency are given in Table 6. 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.

**Table 6 5-FU dose reduction guidelines in patients with partial DPD deficiency**

| Reduction[a] | 5-FU | |
|---|---|---|
| | **Bolus** | **Infusion** |
| **Starting dose reduction: 25%** | | |
| Starting dose | 300 mg/m$^2$ | 1800 mg/m$^2$ |
| -1 | 150 mg/m$^2$ | 1500 mg/m$^2$ |
| -2 | 0 mg/m$^2$ | 1500 mg/m$^2$ |
| -3 | 0 mg/m$^2$ | 1200 mg/m$^2$ |
| **Starting dose reduction: 50%** | | |
| Starting dose | 200 mg/m$^2$ | 1200 mg/m$^2$ |
| -1 | 100 mg/m$^2$ | 1000 mg/m$^2$ |
| -2 | 0 mg/m$^2$ | 1000 mg/m$^2$ |
| -3 | 0 mg/m$^2$ | 670 mg/m$^2$ |
| **Starting dose reduction: 75%** | | |
| Starting dose | 100 mg/m$^2$ | 600 mg/m$^2$ |
| -1 | 50 mg/m$^2$ | 500 mg/m$^2$ |
| -2 | 0 mg/m$^2$ | 500 mg/m$^2$ |
| -3 | 0 mg/m$^2$ | 350 mg/m$^2$ |
| [a] Reduction from standard full dose: 400 mg/m$^2$ bolus followed by 2400 mg/m$^2$ infusion. | | |

### 9.2.1.2 Other Countries

Please refer to the 5-FU SmPC/Prescribing Information and local guidelines for information on the management of patients with DPD deficiency. The 5-FU dose must be adapted for partially DPD deficient patients based on phenotyping and/or genotyping in countries where this is standard practice as per national and/or local guidelines.

Patients with complete or near complete DPD deficiency are at increased risk for acute early-onset toxicity and severe, life-threatening, or fatal adverse reactions caused by 5-FU (*e.g.,* mucositis, diarrhoea, neutropenia, and neurotoxicity).

Patients with partial DPD deficiency may also have increased risk of severe, life-threatening, or fatal adverse reactions caused by 5-FU.

Local standard procedures should be followed for patients with suspected DPD deficiency, based on observations of acute early-onset or unusually severe toxicity to 5-FU.

## 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 IV 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 IV 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 Infusion Reactions

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.

### 9.3.6 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.

### 9.3.7 Fatigue

Fatigue is an expected AE for patients with 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.

### 9.3.8 Cardiotoxicity

Cardiotoxicity has been associated with fluoropyrimidine therapy, including myocardial infarction, angina, arrhythmias, myocarditis, cardiogenic shock, sudden death, stress cardiomyopathy (takotsubo syndrome) and electrocardiographic changes (including very rare cases of QT prolongation). Prior history of coronary artery disease may be a risk factor for some cardiac adverse reactions. Care should therefore be exercised in treating patients who experienced chest pain during courses of treatment, or patients with a history of heart disease. Careful consideration should be given to re-administration of 5-FU or NUC-3373 after a documented cardiovascular reaction (arrhythmia, angina, ST segment changes) as there is a risk of sudden death.

In case of severe cardiotoxicity, treatment with 5-FU or NUC-3373 should be discontinued.

### 9.3.9 Fertility

In accordance with the 5-FU and bevacizumab package inserts, genetic consultation is recommended if the patient wishes to have children after ending treatment. Since treatment with 5-FU may cause irreversible infertility in males and bevacizumab may cause adverse effects on fertility in females, it is recommended that patients who wish to become parents in the future ask for advice regarding cryo-conservation of their sperm or eggs prior to treatment.

### 9.3.10 Combination Therapies

For an overview of AEs and toxicities related to each of the combination agents or 5-FU, refer to the relevant SmPC/Prescribing Information.

Toxicities that are suspected to be related to bevacizumab should be managed through dosing delays or discontinuation. Bevacizumab treatment must be permanently discontinued in patients who develop any of the following:

- Gastrointestinal perforation
- Necrotising fasciitis
- Posterior Reversible Encephalopathy Syndrome "PRES"
- Nephrotic syndrome
- Arterial thromboembolic reactions
- Life-threatening (Grade 4) thromboembolic reactions, including pulmonary embolism (NCI-CTCAE v3). Patients with thromboembolic reactions ≤Grade 3 need to be closely monitored (NCI-CTCAE v3)
- Grade 3 or 4 bleeding during bevacizumab therapy (NCI-CTCAE v3)

# 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 study treatment through the End of Treatment 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 SmPCs/Prescribing Information and standard local practice for the relevant combination agents. The following support medications are recommended: anti-emetics (palonosetron, granisetron, aprepitant, dexamethasone, metoclopramide, cyclizine); anti-histamines; atropine; sunscreen of ≥SPF 30; and topical corticosteroids (*e.g.,* hydrocortisone 2.5%, alclometasone).

## 10.2 Haematopoietic Growth Factor Support

The Investigator may prescribe haematopoietic growth factor support (*e.g.,* G-CSF) according to local protocols and as prophylaxis after any febrile neutropenic episode to enable the patient to continue on study. Concomitant treatment with haematopoietic growth factor support and study treatment should be avoided. All haematopoietic growth factors used from 30 days prior to date of consent until 30 days after administration of last dose of study treatment 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 forms of contraception, one of which must be highly effective. Female patients are considered to be of child-bearing potential following menarche and until becoming post-menopausal (12 months of amenorrhea) unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

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.

Highly effective forms of contraception include:

- Bilateral tubal occlusion
- Vasectomised partner

- Intrauterine device or intrauterine system
- Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Intravaginal
    - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Injectable
    - Implantable

Acceptable forms of contraception that are not considered highly effective include:

- Progestogen-only hormonal contraception, where inhibition of ovulation is not the primary mode of action
- Male or female condom with or without spermicide
- Cap, diaphragm or sponge with spermicide

Two forms of contraception (one of which must be highly effective) 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.

### 10.3.2 Prohibited Therapy

Except as included in the specific arms of this study, use of the following therapies is prohibited (at any dose) at any time 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, anti-hormonal therapy for patients with prostate cancer, 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. Concomitant dosing with study treatments should be avoided)
- 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
- Brivudine, sorivudine and analogues
- Live vaccines (must also be avoided for six months after last dose of study medication)

### 10.3.3 Therapies to be Used with Caution

The following warnings and precautions regarding concomitant medications apply:

- **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*
- *In vitro* studies have shown the potential for NUC-3373 drug-drug interaction with concomitant medications that are metabolised by CYP3A4 and CYP2C8. The clinical impact of these *in vitro* observations is not known and the use of concomitant medications that are cleared by these CYP enzymes is permitted with caution in patients receiving NUC-3373.
- 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
- Patients receiving warfarin or other types of long-acting anti-coagulants (such as phenprocoumon and anti-Xa inhibitors with a half-life of >12 hours) must be regularly monitored for INR, which should be checked weekly until a stable warfarin dose is established. Following this, INR should be checked pre-dose on Day 1 of each cycle and the value must be within the normal range before administering study treatment.

  However, if possible, patients 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. For combination agents, please refer to the respective SmPC/Prescribing Information.

### 10.3.4 COVID-19 Vaccinations

A risk assessment performed in accordance with the UK Medicines and Healthcare products Regulatory Agency (MHRA) guidance 'Managing clinical trials during COVID-19' (MHRA, 2021) concluded that there is no specific scientific contraindication and 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.

### 10.4 Concomitant Procedures

Palliative radiotherapy during participation in the study is permitted, but it should not be concurrent with study treatment. Chemotherapy should be stopped at least 7 days before the start of radiotherapy and can be restarted after all toxicities have resolved or returned to baseline, but not earlier than 7 days after completing radiotherapy. If the radiation field is very small or for any other reasons, exceptions to this rule can be discussed on a case-by-case basis with the Medical Monitor.

Additional caution must be applied with regards to gastrointestinal perforations/fistulae for patients receiving pelvic radiotherapy.

Treatment delays for palliative radiotherapy are considered separately from treatment modifications for adverse events.

Patients should not receive treatment with bevacizumab until any wounds are fully healed, *e.g.,* for at least 28 days before or after surgery.

All concomitant procedures performed 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.

# 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. Additional anatomical areas should be assessed by CT or MRI in case of suspicion of presence of metastases based on signs, symptoms, biochemical results and/or as standard of care imaging of patients (*i.e.,* non-study related).

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 C1D1. 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 C1D1 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 confirmed by radiologic assessment.

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 NUFIRI-bev and FOLFIRI-bev combination regimens. 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 not be considered an AE or SAE.

A significant 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.

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, please refer to the applicable SmPC/Prescribing Information, which serve as their RSI. Representative SmPCs for each agent are provided in the NUC-3373 IB (Section 8.1) for ease of reference.

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).

## 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.

AEs and SAEs will be reported from the point that informed consent has been obtained onwards. 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:

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

**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).

**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 7.

**Table 7 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 the point of informed consent must be reported to the Sponsor within 24 hours from the time study site personnel first learn about the event. Investigators must report all SAEs that they become aware of irrespective of the end of study treatment or the end of study, unless the patient has initiated a new therapy after which only SARs must be reported. The SAE report should be entered directly in the electronic data capture (EDC) system. If the EDC is unavailable, the SAE report may be completed and emailed using the contact details in Table 8.

**Table 8 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 8 as soon as it becomes available.

The Sponsor 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 8.

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 entered directly in the EDC system. If the EDC is unavailable, the pregnancy notification report may be completed and emailed using the contact details in Table 8 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 expected 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 Abnormal Laboratory Test Result Values

The Investigator will identify and record clinically significant laboratory test results. Not every laboratory test result abnormality qualifies as an AE; however, a laboratory test result must be reported as an AE if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (*e.g.,* dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention (*e.g.,* potassium supplementation for hypokalaemia) or a change in concomitant therapy
- Is clinically significant in the Investigator's judgement

It is the Investigator's responsibility to review all laboratory test result findings. Medical and scientific judgement should be exercised in deciding whether an isolated laboratory test result abnormality should be classified as an AE.

If a clinically significant laboratory test result abnormality is a sign of a disease or syndrome (*e.g.,* alkaline phosphatase and bilirubin 5 × ULN associated with cholestasis), the diagnosis (*i.e.,* cholestasis) should be recorded only on the AE CRF.

If a clinically significant laboratory test result abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the AE CRF, along with a descriptor indicating whether the test result is above or below the normal range (*e.g.,* "elevated potassium," as opposed to "abnormal potassium"). If the laboratory test result abnormality can be characterised by a precise clinical term per standard definitions, the clinical term should be recorded as the AE. For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as "hyperkalaemia."

Cases of potential drug-induced liver injury (DILI) that include an elevated ALT or AST in combination with either an elevated bilirubin or INR must be recorded (FDA, 2009).

Treatment interruption/discontinuation should be considered if any of the following criteria are met:

- Treatment-emergent ALT or AST >8 × ULN
- Treatment-emergent ALT or AST >5 × ULN for more than 2 weeks
- Treatment-emergent ALT or AST >3 × ULN **with** total bilirubin >2 × ULN or INR >1.5

- Treatment-emergent ALT or AST >3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

Hy's Law cases, according to FDA guidance on DILI (FDA, 2009), should be considered if the criteria above are met and additionally the following findings are reported:

- No initial finding of cholestasis (elevated serum ALP)
- No other reason can be found to explain the increases in ALT/AST and bilirubin or INR, such as viral hepatitis A, B, or C; pre-existing or acute liver disease; biliary duct obstruction; or another drug capable of causing the observed injury

For patients meeting Hy's law criteria due to drug-related toxicity, consider discontinuation of treatment or, if the patient is deriving benefit in the opinion of Investigator, consider holding treatment until values are <3 × ULN for AST and/or ALT and <2 × ULN for total bilirubin, then restart treatment at a reduced dose (as per Table 4). Laboratory values must be closely monitored and study treatment must be discontinued if patients meets criteria for a second time despite dose reduction.

### 12.12 Abnormal Vital Sign Values

The Investigator will identify and record clinically significant vital sign results.

Not every vital sign abnormality qualifies as an AE; however, a vital sign result must be reported as an AE if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (*e.g.,* dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention or a change in concomitant therapy
- Is clinically significant in the Investigator's judgement

It is the Investigator's responsibility to review all vital sign result findings. Medical and scientific judgement should be exercised in deciding whether an isolated vital sign value abnormality should be classified as an AE.

If a clinically significant vital sign value abnormality is a sign of a disease or syndrome (*e.g.,* high blood pressure), the diagnosis (*i.e.,* hypertension) should be recorded only on the AE CRF.

### 12.13 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.

### 12.14 Reference Safety Information for Assessment of Expectedness

#### 12.14.1 NUC-3373

The IB supplied by the Sponsor 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.

### 12.14.2 Other Agents to be Used in Combination with NUC-3373

The respective SmPC/Prescribing Information of each combination agent administered in conjunction with NUC-3373 in this study will serve as their own RSI. Representative SmPCs of these agents are provided in the NUC-3373 IB (Section 8.1) for ease of reference.

# 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.

The study database will be locked prior to the primary analyses for inclusion in the clinical study report (CSR).

## 13.1 Sample Size

In total, 171 patients will be randomised on a 1:1:1 basis (57 patients per arm) to either Q1W NUFIRI-bev, Q2W NUFIRI-bev or FOLFIRI-bev (Q2W).

The principal statistical objective of this study is to estimate the likely efficacy of the two NUFIRI arms as compared to the FOLFIRI control arm to support decision making regarding the further development of NUFIRI. Median PFS is expected to be 7 months on the FOLFIRI control arm and at least 9.9 months on each of the two NUFIRI arms. Assuming a non-linear recruitment profile over the planned 13-month accrual period ($\eta$=2; Carroll, 2009) and with a minimum of 17 months follow-up post-accrual, a total of 139 PFS events are expected across the three randomised arms. With this amount of information, this study will provide an 80% probability of correctly concluding superiority when NUFIRI is truly better than FOLFIRI in terms of PFS and, similarly, an 80% probability of correctly concluding non-superiority when NUFIRI is truly the same as FOLFIRI in terms of PFS. The smallest observed improvement in median PFS for either NUFIRI arm relative to FOLFIRI to conclude NUFIRI is truly better than FOLFIRI is 1.3 months.

Further, an evaluation of efficacy may be performed 3 months after the last patient has been randomised. At this time a total of 70 PFS events are expected across the three randomised arms. With this amount of information, the smallest observed improvement in median PFS for either NUFIRI arm relative to FOLFIRI to conclude NUFIRI is truly better than FOLFIRI is 1.9 months.

## 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

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

Missing tumour assessment data

Patients who withdraw consent to follow-up may do so prior to having experienced progressive disease. The impact of such patients on the efficacy analysis will be explored via multiple imputation and tipping point analyses.

Patients who have missing tumour assessment data leading to missing objective response data will be included in the ORR analysis as non-responders.

## 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

Safety will be assessed in the Safety Set (SS), defined as all randomised patients who receive at least one dose (or partial dose) of study treatment. Patients will be analysed by the treatment received, based on their first dose of randomised study drug.

### 13.3.2 Full Analysis Set

The Full Analysis Set (FAS) is based on the intention-to-treat principles and includes all randomised patients.

The FAS will be the principal population used to assess efficacy endpoints. Patients will be analysed based on randomised treatment.

### 13.3.3 Modified Full Analysis Set

The Modified Full Analysis Set (mFAS) is defined as a subset of the FAS who had at least one dose of randomised study treatment and at least one follow-up assessment.

The mFAS will be used to perform a supportive analysis of efficacy endpoint data. Patients will be analysed based on randomised treatment.

### 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 screened, the number of patients randomised, the number of patients treated, and the number of patients included in each study population.

In addition, the number and percentage of patients who complete the study and who discontinue the study early, including a breakdown of the primary reasons for study discontinuation, will be presented for the FAS population. Similarly, patients who complete or prematurely

discontinue randomised treatment will be summarised, including a breakdown of the primary reasons for discontinuation.

Patients with important protocol deviations or other significant deviations as defined in the SAP will be listed and summarised.

## 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 Efficacy Analysis

The efficacy endpoints and methods of analysis are defined below.

#### 13.5.4.1 Primary Efficacy Analysis

The primary efficacy endpoint is PFS, defined as the time from randomisation until the date of objective disease progression or death (by any cause in the absence of disease 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 assessment dates contributing to a particular overall visit assessment

PFS will be analysed via Cox regression modelling stratified for the randomisation stratification factors (RAS status, first-line treatment, and duration of prior line of therapy) and including a fixed effect term for randomised treatment. The hazard ratio will be estimated for each NUFIRI arm versus the FOLFIRI control arm, along with the associated confidence interval (CI) and 2-sided p-value. The data will also be displayed using Kaplan-Meier curves and median PFS times will be estimated.

#### 13.5.4.2 Secondary Efficacy Analysis

##### 13.5.4.2.1 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

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

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. Spider plots will also be presented.

#### 13.5.4.2.2 Objective Response Rate

ORR is defined as the number of patients achieving a 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 overall response category (CR, PR, SD, progressive disease or not evaluable [NE]), as well as the ORR will be presented. All data will be listed.

#### 13.5.4.2.3 Disease Control Rate

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

#### 13.5.4.2.4 Duration of Response

DoR is defined for the subset of the FAS population categorised as responders for the assessment of ORR. DoR is defined as the time, in months, from the time measurement criteria are first met for CR or PR (whichever is first recorded) until the first date that 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 DoR will be censored at the date of last tumour assessment. DoR will be summarised and listed.

#### 13.5.4.2.5 Duration of Stable Disease

SD is defined for the subset of the FAS 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 months, from the time measurement criteria are first met for SD until the first date that 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 summarised and listed.

#### 13.5.4.2.6 Overall Survival

OS is defined as the time from randomisation 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.

### 13.5.5 Safety Analysis

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

#### 13.5.5.1 Adverse Events

AEs will be considered treatment-emergent (TEAE) if they start on or after the time of the first dose of study treatment and up to 30 days after the last dose of study treatment. AEs will be summarised by Medical Dictionary for Regulatory Activities (MedDRA)™ System Organ Class (SOC) and preferred term, the latest version of MedDRA will always be used. The severity of AEs will also be summarised by CTCAE v5.0 (or higher), grade. Non-treatment-emergent AEs will be included in the patient listings and flagged as such but will not be included in the summary tables. Where an AE date is partial or missing, and it is unclear whether the AE is treatment-emergent, the AE will be assumed to be treatment-emergent. Any AEs with missing severity will be classified as severe. Deaths that occur within 90 days after the last dose of study drug are defined as on-study deaths.

The following summaries will be produced:

- 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, for each treatment group
- 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 by all grades and Grade 3-4 and 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 treatment 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 Hy's law cases
- Summary of SAEs by SOC and preferred term
- Summary of SAEs by preferred term sorted in descending order of frequency
- Summary of TEAEs leading to death by SOC and preferred term

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), Investigator's assessment of severity and relationship to study drug, and outcome.
- 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 randomised treatment, 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.

#### 13.5.5.2 Laboratory Parameters

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

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

All clinical laboratory data for individual patients will be listed.

#### 13.5.5.3 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.

#### 13.5.5.4 ECOG Performance Status

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

#### 13.5.5.5 Physical Examination

Listings will be provided for physical examination parameters.

#### 13.5.5.6 ECGs

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

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. Additionally, abnormal results will be summarised by treatment group at each time point in terms of frequency counts and percentages. All ECG data will be displayed in a data listing.

### 13.5.6 Pharmacokinetics Analysis

The PK of the NUFIRI-bev regimen 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, FBAL, CPF-1027, irinotecan, SN-38, SN-38G, APC

All PK parameters will be analysed in the PK population.

### 13.5.7 Exploratory Endpoints

Potential biomarkers of biological activity will be analysed in archival tumour samples from patients in the FAS 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, between biomarkers and clinical activity.

### 13.5.8 Primary and Interim Analyses

The primary efficacy analysis will take place after a total of 139 PFS events have occurred.

An evaluation of efficacy may also be performed after a total of 70 PFS events have occurred.

The study will be considered complete when the final patient has completed their End of Treatment visit.

### 13.5.9 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 electronic CRF (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:** if required due to country or site-level health/infection control related restrictions (*e.g.,* 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 Risk Management Considerations

This study complies with the European Medicines Agency guideline on strategies to identify and mitigate risks for first-in-human and early clinical studies with IMPs (EMEA/CHMP/SWP/28367/07 Rev. 1, EMA 01 Feb 2018).

This study will enrol patients with advanced colorectal cancer who have experienced disease progression on prior anti-cancer therapies. Patients will be randomised to receive the standard of care FOLFIRI-bev regimen or the NUFIRI-bev regimen. The only difference between the regimens is the substitution of the 5-FU component in FOLFIRI with the NUC-3373 component in NUFIRI. The sites that have been selected for participation in this study have experience in treating the patient population with the standard of care regimens, including FOLFIRI-bev, and in conducting Phase II clinical studies.

As described in Section 3.2, the dose of NUC-3373 has been appropriately determined from the available nonclinical and clinical data. The NUFIRI treatment combination has been evaluated in dose-escalation cohorts in the NuTide:302 clinical study, and the recommended dose was selected for further investigation in the NuTide:323 clinical study. This study includes two NUFIRI schedules, weekly and alternate weekly, to allow further optimisation of the dose schedule.

To ensure patient safety, dose modification criteria and suggested management procedures for drug-related toxicities are provided for each of the study drugs in Section 9.

ECGs are being routinely monitored throughout the study for any acute cardiac effects (see Summary Schedule of Events).

Patient safety will be monitored closely on an ongoing basis through recording and reporting of AEs, routine laboratory tests, and physical examination/vital signs monitoring. Procedures for safety reporting are clearly outlined in Section 12 and post-study follow-up of patients is described in Section 3.4.

### 15.5 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.

### 15.6 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.

### 15.7 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 and rights of a patient or the reliability and robustness of the data generated in the clinical 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.

### 15.8 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.

### 15.9 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.

### 15.10 Investigator Documentation

Before beginning the study, each investigative site will have all applicable essential documents available, in accordance with ICH-GCP section 8.2.

### 15.11 Study Records Retention

Essential documents should be retained until at least 10 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 10 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.

### 15.12 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 all 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 individual centres, the data generated from their individual centres 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 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<br>All tumour nodes <10 mm<br>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. | | | | |

**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, or not evaluable.

# 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<br>Up and about more than 50% of waking hours | **2** |
| | |
| Capable of only limited self-care<br>Confined to bed or chair more than 50% of waking hours | **3** |
| | |
| Completely disabled<br>Cannot carry out any self-care<br>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 US 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 US 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®, 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® |
| Hydroquinidine (Dihydroquinidine) (only on non-US market) | Serecor® |
| Hydroxychloroquine | Plaquenil®, Quineprox® |
| Ibogaine (only on non-US market) | None |
| Ibutilide | Corvert® |
| Levofloxacin | Levaquin®, Tavanic® |
| Levomepromazine (Methotrimeprazine) (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 US market) | Lorelco® |
| Procainamide | Pronestyl®, Procan® |
| Propofol | Diprivan®, Propoven® |
| Quinidine | Quinaglute®, Duraquin®, Quinact®, Quinidex®, Cin-Quin®, Quinora® |
| Quizartinib | Vanflyta® |
| 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® |

| Generic Name | Brand Names (Partial List) |
|---|---|
| Sotalol | Betapace®, Sotalex®, Sotacor®, Sotalol-AF |
| 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® |
| Terodiline (only on non-US market) | Micturin®, Mictrol® |
| Thioridazine | Mellaril®, Novoridazine®, Thioril® |
| Vandetanib | Caprelsa® |

**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.

**Disclaimer and Waiver:** The information presented is intended solely for the purpose of providing general information about health-related matters. It is not intended for any other purpose, including but not limited to medical advice and/or treatment, nor is it intended to substitute for the users' relationships with their own health care providers. To that extent, by use of this website and the information it contains, the user affirms the understanding of the purpose and releases AZCERT, Inc. from any claims arising out of his/her use of the website and its lists. The absence of drugs from these lists should not be considered an indication that they are free of risk of QT prolongation or torsades de pointes. Many medicines have not been tested for this risk in patients, especially those with congenital long QT syndrome.

Woosley, RL and Romero, KA, www.Crediblemeds.org, QT drugs List, [Accessed February 2024], 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 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 EGFR inhibitor-related rash.

Avoid the use of diuretics, laxatives and other agents know to exacerbate diarrhoea.