Running title: NUC-1031/cisplatin in advanced biliary cancer

Title: A phase Ib study of NUC-1031 in combination with cisplatin for the first-line treatment of patients with advanced biliary tract cancer (ABC-08)

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Abstract:

Background: Cisplatin/gemcitabine is standard 1st-line treatment for patients with advanced biliary tract cancer (ABC). NUC-1031 (phosphoramidate transformation of gemcitabine) is designed to enhance efficacy by maximising intra-tumoural active metabolites.

Methods: Patients with untreated ABC, ECOG-PS 0-1 received NUC-1031 (625 or 725mg/m^2) and cisplatin (25mg/m^2) on days 1 and 8, every 21 days. Objectives: safety and maximum tolerated dose (primary); and ORR, pharmacokinetics, PFS and OS (secondary).

Results: Twenty-one patients (median age 61 years, *n*=13 male; 17 cholangiocarcinoma, 2 ampullary and 2 gallbladder cancer) received NUC-1031 625mg/m^2 (*n*=8 and expansion *n*=7; median 6 cycles) or 725mg/m^2 (*n*=6; median 7.5 cycles). Treatment was well tolerated; most common treatment-emergent grade 3-4 adverse-events occurring in >1 patient with 625mg/m^2 NUC-1031 were increased GGT: 40%, ALT: 20%, bilirubin: 13%, neutropenia: 27%, decreased WCC: 20%, thrombocytopenia: 13%, nausea: 13%, diarrhea: 13%, fatigue: 13%, and thrombus: 20% and with 725mg/m^2, increased GGT: 67% and fatigue: 33%. NUC-1031 725mg/m^2 was selected as the recommended dose with cisplatin in ABC. ORR: 33% (1 CR, 6 PRs), DCR 76%, median PFS 7.2 months (95%-CI 4.3-10.1), median OS 9.6 months (95%-CI 6.7-13.1). The median plasma AUC_{0-24} and C_{max} estimates were highest for NUC-1031 (218-324 μg•h/mL and 309-889 μg/mL, respectively) and lowest for dFdC (0.47-1.56 μg•h/mL and 0.284-0.522 μg/mL, respectively). **Conclusions:** This is the first study reporting on the combination of NUC-1031 with cisplatin in ABC and demonstrated a favourable safety profile; 725mg/m^2 NUC-1031 in combination with cisplatin is undergoing phase III trial evaluation in ABC.

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Background:

Standard of care first-line systemic treatment for patients with advanced biliary tract cancer (ABC) is the cisplatin/gemcitabine combination.[1] The median overall survival (OS) for patients receiving this combination is approximately one year[1]; new therapeutic options are required.

The activity of gemcitabine is limited by inherent and acquired cancer cell resistance mechanisms associated with transport, activation and breakdown.[2] Through the application of ProTide technology, a new agent, NUC-1031, has been designed to overcome the key resistance mechanisms associated with gemcitabine. NUC-1031 is a phosphoramidate transformation of gemcitabine and, like gemcitabine, the cytotoxic effect on cancer cells is largely attributed to the generation of the triphosphate form of the nucleotide analogue (di fluoro-deoxycytidine triphosphate; dFdCTP).[3]

NUC-1031 is designed specifically to generate and maintain higher concentrations of dFdCTP inside the tumour cell compared to gemcitabine. The phosphoramidate moiety enables NUC 1031 to enter the cancer cell, independent of the presence of nucleoside transporters. Once NUC-1031 has entered the cell, the protective group is cleaved off and releases an activated, mono-phosphorylated form of gemcitabine (dFdCMP). The delivery of dFdCMP obviates the need for the activating enzyme, deoxycytidine kinase (dCK), which drives the rate limiting phosphorylation of gemcitabine. dFdCMP is rapidly converted to di fluoro deoxycytidine diphosphate and then the key anti-cancer metabolite, dFdCTP. Moreover, NUC-1031 is not subject to breakdown by cytidine deaminase (CDA).[3] As a result of overcoming all three key resistance mechanisms, NUC-1031 achieves much higher levels of the active anti-cancer metabolite, dFdCTP, than gemcitabine.[3] This mechanism of action has been illustrated in previous publications on NUC-1031.[4, 5]

The results of a phase I dose escalation study of NUC-1031 monotherapy in 68 patients with advanced solid tumours who had progressed on standard therapy concluded that it was well tolerated with the most common grade 3-4 adverse events (AEs) being neutropenia, lymphopenia and fatigue.[4] It also demonstrated clinically-significant anti-tumour activity in patients with prior gemcitabine exposure, including patients refractory to prior gemcitabine treatment (patients who developed progression while on gemcitabine)[4]; in 49 patients with an evaluable response (patients who had completed at least two cycles of NUC-1031 and had at least one follow-up radiographic assessment to measure changes in tumour size), there was a 78% disease control rate reported, with 33 patients having stable disease (SD) and 5 achieving a partial response (PR) (patients with primary cancers of the cervix, lung, fallopian tube, pancreas and unknown primary achieved PRs).[4]

In this study[4], NUC-1031 was detected in plasma up to 24 hours from the end of infusion (estimated $t_{1/2}$ was 8.3 hours). For the analytes analysed, the median plasma AUC_{0-t} and C_{max} values on day 1 were highest for NUC-1031 (269 µM/h and 710 µM, respectively). They were intermediate for 2′,2′-difluorodeoxyuridine (dFdU) (76.0 µM/h and 5.11 µM, respectively), and lowest for di-fluoro-deoxycytidine (dFdC) (2.92 µM/h and 1.82 µM, respectively). The intracellular concentrations of the active anti-cancer moiety dFdCTP remained high throughout the 24 hour pharmacokinetic (PK) sampling period. Urine samples were analysed from 46 patients and 21.7 and 27.3% of the NUC-1031 was excreted via the urine as dFdU over the 24 hours after the dose on days 1 and 15, respectively. In total, less than 1% of the dose was excreted as either NUC-1031 or dFdC.[4]

The recommended phase II dose (RP2D) of NUC-1031 as a single agent was reported as 825mg/m^2 on days 1, 8 and 15 of a 28-day cycle.[4] This study included 7 patients with ABC, all of which had received prior cisplatin/gemcitabine treatment; of these, 6 patients had

response-evaluable disease; the best response to therapy in 5 of these patients was SD (3 had target lesion size reduction (percentage not stated in publication) and 1 had progressive disease (PD).[4]

The aim of this phase Ib, multi-centre, open-label study was to assess the safety of NUC-1031 in combination with cisplatin in patients with ABC (ABC-08), and to define the RP2D, in addition to evaluating its anti-tumour activity, including PK analyses.

Patients and Methods:

Study design

This was a single-arm, open-label, multi-centre, phase Ib dose-escalation study conducted under the auspices of the National Cancer Research Institute Upper Gastrointestinal Clinical studies group (hepatobiliary subgroup) to assess the safety and to determine the RP2D of NUC-1031 in combination with cisplatin in patients with locally advanced/metastatic biliary tract cancer (all centres were high volume, receiving tertiary referrals, with multidisciplinary pathological input).

All patients provided written informed consent prior to any study-related procedures. The study was performed in accordance with Good Clinical Practice guidelines and the principles of the 1964 Declaration of Helsinki and subsequent revisions (North West Liverpool Central Research Ethics Committees reference 15/NW/0160).

The number of patients per cohort during the dose-escalation phase was determined according to a '3+3' classical design. The starting dose level: 625mg/m^2 of NUC-1031 was administered via a central venous catheter (CVC) following 25mg/m^2 cisplatin on days 1 and 8 of a 21-day cycle. The starting dose was selected as approximately 75% of the recommended monotherapy dose of NUC-1031 (825mg/m^2)[4], with a plan to explore up to 4 dose levels (625mg/m^2; 725mg/m^2; 825mg/m^2; 925mg/m^2) in combination with a fixed dose of cisplatin.

The primary objectives were determination of safety and maximum tolerated dose (MTD) of NUC-1031 in combination with cisplatin in patients with ABC. Secondary objectives included assessment of progression-free survival (PFS), OS, objective response rate (ORR) and PK profile of NUC-1031.

Dose-limiting toxicities (DLTs) were determined by clinical and laboratory toxicity assessments performed (day 1 and day 8) during the first 21-day treatment cycle, and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v 4.03.

The following were considered DLTs if at least possibly related to either NUC-1031 or cisplatin: grade 4 neutropenia >7 days; febrile neutropenia defined as a disorder characterised by an absolute neutrophil count <1000/mm^3 and a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of ≥38 degrees C (100.4 degrees F) for more than one hour; grade 4 thrombocytopenia; ≥grade 3 nausea and vomiting despite optimal supportive medication; ≥grade 3 laboratory abnormality or toxicity; delay of >21 days to start cycle 2 treatment due to treatment-related toxicity; and any isolated or recurrent (e.g. cardiac, renal, neurologic) toxicity that was judged by the investigator and Trial Management Group (TMG) to be a DLT. Following a protocol amendment, ≥grade 3 alanine aminotransferase (ALT) or aspartate aminotransferase (AST) were only considered a DLT if they did not resolve to ≤grade 2 within 7 days (as not considered clinically significant), and >grade 3 laboratory abnormalities were only considered a DLT if deemed to be clinically significant by the investigator and TMG.

Patient eligibility

Patients aged ≥18 years with a life expectancy >3 months with histologically/cytologically verified, non-resectable or recurrent/metastatic cholangiocarcinoma (intrahepatic, hilar, distal bile duct), gallbladder cancer or ampulla of Vater carcinoma (biliary subtype) (radiological diagnosis was allowed for recurrent disease if previously histologically/cytologically verified), who had received no prior systemic therapy for ABC, were eligible for inclusion in this study. Prior adjuvant treatment was allowed if completed greater than 6 months prior to

enrolment. Other inclusion criteria included an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0-1, adequate renal, haematological and liver function and adequate biliary drainage, with no evidence of ongoing infection. See supplementary material 1 for full eligibility criteria.

Dose escalation design

A period of at least 48 hours was mandated before recruiting the second and subsequent patients to a single dose cohort. Patients who did not receive both day 1 and day 8 of treatment during cycle 1 for reasons other than toxicity were not considered evaluable for DLT assessments and were replaced. Dose escalation followed a '3+3' design until the MTD was determined, defined as the highest dose level for which ≤1 of 6 (or < 33%) patients experience a DLT.

A Computed Tomography (CT) scan of the thorax, abdomen and pelvis was performed within 28 days prior to registration and every 12 weeks (±7 days) on study (assessed using Response Evaluation Criteria in Solid Tumours (RECIST) v1.1).[6] Response was determined by experienced radiologist review at individual recruiting sites (investigator review). If clinically indicated, magnetic resonance imaging (MRI) was permitted. It was recommended that the imaging method used for assessment at baseline was used at all subsequent time points, if clinically appropriate. Treatment continued until intolerable toxicity or progressive disease or until withdrawal of consent for other reasons. Patients could continue to receive NUC-1031 alone at the investigator's discretion, if cisplatin had to be discontinued for reasons of intolerable toxicity.

Dose modifications and delays

Treatment interruptions up to 21 days were allowed for patients to meet re-treatment criteria before commencing their next cycle. Dose modifications adhered to those from the ABC-02 phase III randomised trial of cisplatin/gemcitabine versus gemcitabine for the first-line treatment of patients with ABC.[1] See supplementary material 2 for details.

Pharmacokinetic analysis

Samples for PK analysis were taken on day 1 only, at baseline (prior to chemotherapy administration), 30 minutes, 60 minutes and 240 minutes following CVC flush at the completion of NUC-1031 administration. Blood (6 mL) was collected using heparinised blood collection tubes spiked with tetrahydrouridine (25 μg/mL) in order to inhibit CDA activity. Plasma samples were assayed for NUC-1031, dFdC, and dFdU using a previously described liquid chromatography-tandem mass spectrometry method.[4] Pharmacokinetic parameters were estimated for ABC-08 using PK samples and a PK model developed with clinical study data from the phase I dose-escalation monotherapy study to assess the safety, efficacy, and PK of NUC-1031 in patients with advanced solid tumours.[4]

Statistical analysis

Adverse events were summarised by maximum toxicity grade and causality assessment for each initial dose level of NUC-1031 and cisplatin.

Efficacy was determined in all patients with measurable disease who received ≥1 cycle of NUC-1031 with cisplatin and who had at least one follow-up radiographic assessment. Objective response was defined as a participant who achieved a best response of PR or complete response (CR) on study treatment. Objective response rates were calculated as the percent of evaluable participants who achieved CR or PR, and confidence intervals (CIs) were constructed. Progression-free survival was defined as the time from registration until

the date of radiological or clinical disease progression or death (from any cause in the absence of progression), regardless of whether the subject withdrew from therapy or received another anti-cancer therapy prior to progression. The OS was calculated from the date of registration to date of death. The date of registration as time of origin for PFS and OS calculation was protocol-defined. Overall survival and PFS were analysed using Kaplan-Meier curves. No formal statistical analyses were planned or performed on safety, PK or efficacy data.

Results:

Patient characteristics

A total of 21 patients with ABC were enrolled at 5 centres in the United Kingdom and received cycle 1 day 1 of treatment between 2 February 2016 and 14 March 2018. There was a 12 month period of patient follow-up from the date of the last patient registered onto the study. The median age was 61 years (range 47-78) and 13 (62%) were male. Seven and 14 patients had an ECOG PS of 0 and 1, respectively. The primary tumour site was hilar cholangiocarcinoma in 7; intrahepatic and distal bile duct cholangiocarcinoma: 5 each; gallbladder cancer and ampulla of Vater carcinoma: 2 each. Seventeen and 4 patients had metastatic and locally advanced disease respectively (Table 1). Eight patients had recurrent disease, with two patients having received previous adjuvant chemotherapy (capecitabine or cisplatin/gemcitabine), completed >6 months from enrolment. No prior radiotherapy was recorded.

Dose determination

Eight patients were treated at the starting dose of 625g/m^2 NUC-1031 in combination with cisplatin 25mg/m^2 on day 1 and 8 of a 21-day schedule. Two patients were not evaluable for DLTs due to omission of day 8 of treatment and so were replaced (one patient with hilar cholangiocarcinoma had disease-related cholangitis requiring endoscopic retrograde cholangiopancreatography and was unable to re-start treatment within 21 days; and the other had grade 2 thrombocytopenia on cycle 1 day 8 (a subsequent protocol amendment was enacted to align with ABC-02[1] permitting treatment on day 8 with NUC-1031 at a 25% dose reduction, with no dose reduction required for cisplatin in the presence of grade 2 thrombocytopenia and/or grade 3 neutropenia).

One of the first three evaluable patients in the 625mg/m^2 cohort had a drug-related rise in AST (grade 3) during cycle 1, which returned to ≤ grade 2 within 7 days. At that time, the decision of the TMG was to expand the 625mg/m^2 cohort to 6 patients, as a ≤ to grade 3 AST/ALT was initially classified as a DLT. An additional patient in the expanded cohort had a rise in ALT (grade 3) during cycle 1, which also returned to ≤ grade 2 within 7 days.

The TMG noted that both the AST and ALT rise returned to ≤ grade 2 within 7 days and recommended a protocol amendment to clarify that patients who had a ≤ grade 3 ALT or AST (drug-related) which returns to ≤ grade 2 within 7 days would not be classified as a DLT, as they would not be considered clinically significant.[7] The TMG decision was made to escalate to 725mg/m^2 NUC-1031 in combination with cisplatin 25mg/m^2, day 1 and 8 of a 21-day schedule.

One patient in the 725mg/m^2 cohort developed a drug-related grade 3 gamma-glutamyl transferase (GGT) rise during cycle 1. This was considered non-clinically significant by the TMG. This dose cohort was expanded by an additional three patients as per the existing protocol version, therefore six patients in total were included in the 725mg/m^2 NUC-1031 cohort.

A protocol amendment was subsequently instituted to clarify the wording in the relevant criteria within the DLT definition to "Greater than or equal to grade 3 NUC-1031-related or chemotherapy combination AE or laboratory abnormality that is deemed clinically significant by the investigator".

Following protocol clarifications, it was concluded by the TMG that no DLTs were experienced by the first 14 patients enrolled (8 and 6 patients in the 625mg/m^2 and 725mg/m^2 NUC-1031 dose cohorts, respectively). On review of the available safety and PK data, it was

determined that there was no discernible difference in terms of the safety or PK between the two dose cohorts of NUC-1031 (625mg/m^2 and 725mg/m^2) in combination with cisplatin. Acknowledging that the RP2D for NUC-1031 monotherapy was 825mg/m^2,[4] the decision was made not to escalate the dose of NUC-1031 in combination with cisplatin further and to expand the 625mg/m^2 NUC-1031 dose cohort by 6 additional patients.

No MTD was therefore defined. Seven patients were enrolled in the expanded 625mg/m^2 cohort (1 patient was replaced as they only received cycle 1 day 1 of treatment, due to disease-related clinical deterioration). There were no DLTs in this expanded dose cohort.

On review of the complete safety, efficacy and PK data, it was determined that there was no difference in safety or PK between the two cohorts. Initially, the 625mg/m^2 NUC-1031 dose was being considered as the dose for phase III evaluation with cisplatin. However, as the data matured, there was an indication that patients in the 725mg/m^2 cohort maintained dose intensity and remained on treatment for longer than patients in the 625 mg/m^2 cohort. At this time-point, based on the available data from the monotherapy study[4] and the current study, the TMG decided that the NUC-1031 725mg/m^2 dose should be selected as the dose (without further cohort expansion) to be given in combination with cisplatin 25mg/m^2, on days 1 and 8 of a 21-day schedule in patients with ABC for phase III evaluation in the first-line setting, additionally allowing greater scope for dose reduction, if required.

Safety and tolerability

The most common treatment-emergent grade 2-4 adverse events occurring in ≥1 patient enrolled in ABC-08 are depicted in Table 2. The most common treatment-emergent grade 3-4 AEs occurring in more than 1 patient in the 625mg/m^2 NUC-1031 cohort were increased GGT (*n*=6; 40%), neutropenia (*n*=4; 27%), increased ALT (*n*=3; 20%), decreased white

blood cells (WBC) (*n*=3; 20%), nausea (*n*=2; 13%), diarrhea (*n*=2; 13%), fatigue (*n*=2; 13%), increased bilirubin (*n*=2; 13%), thrombocytopenia (*n*=2; 13%), and thrombus (*n*=3; 20%) (Table 2).

The most common treatment-emergent grade 3-4 AEs occurring in more than 1 patient in the 725mg/m^2 NUC-1031 cohort were increased GGT (*n*=4; 67%) and fatigue (*n*=2; 33%) (Table 2).

There were 2 patients (13%) and 1 patient (7%) who experienced treatment-emergent grade 4 increased GGT and grade 4 neutropenia in the 625mg/m^2 NUC-1031 cohort, respectively. There were no treatment-emergent grade 4 AEs recorded in the 725mg/m^2 NUC-1031 cohort. The most relevant treatment-emergent grade 1 adverse events occurring in ≥4 patients enrolled in ABC-08 are described in Supplementary Table 1. The impression that there may be fewer overall toxicities in the 725 mg/m^2 NUC-1031 cohort may be a reflection of the fewer patient numbers.

Patients discontinued treatment due to adverse events (*n*=9), intercurrent illness (*n*=1), investigator decision (*n*=2), patient decision (*n*=1), progressive disease (*n*=6) and death (*n*=2). The detailed reasons for treatment discontinuation are described in Table 3.

Efficacy

The median number (range) of received cycles was 6 (1-12) for the cohort that received 625mg/m^2 NUC-1031 (n=15) and 7.5 (1-14) for the 725mg/m^2 NUC-1031 dose (n=6) in combination with cisplatin (day 1 and 8 of a 21-day schedule).

For the 625mg/m^2 NUC-1031 cohort, the median (range) cumulative relative dose was 78.8% (45.3-102.6) for NUC-1031 and 84.1% (43.1-104.6) for cisplatin. For the 725mg/m^2 NUC-1031 cohort, the median (range) cumulative relative dose was 73.7% (55.3-98.1) for NUC-

1031 and 68% (56.5-103.5) for cisplatin. Details on dosing of NUC-1031 and cisplatin received by individual patients enrolled in ABC-08 are provided in Supplementary Table 2, including individual cumulative percentages.

There were 4 patients alive at the end of study follow up; primary site: hilar cholangiocarcinoma (n=2), intrahepatic cholangiocarcinoma and distal bile duct (both n=1), with a median follow up time of 20.6 months (range 16.2-36.0).

In the intention-to-treat (ITT) population (n=21), the overall ORR was 33% (7/21) (1 CR in the NUC-1031 625mg/m^2 cohort and 4 and 2 PRs in the NUC-1031 625mg/m^2 and 725mg/m^2 cohorts respectively (2 patients who had PRs did not have subsequent scans, as treatment was discontinued due to biliary obstruction and deteriorating liver function due to pre-existing underlying cirrhosis (confirmation of PR was not mandated per protocol)); 5/15 (33%) and 2/6 (33%) in the NUC-1031 625mg/m^2 and 725mg/m^2 cohorts respectively. Eight patients had stable disease as best response and 1 patient did not have measurable disease at baseline (non-evaluable), 3 had PD and 2 did not have a second CT scan to assess response (clinical deterioration following biliary obstruction and secondary to co-morbidities respectively). For the efficacy evaluable cohort, two patients did not receive ≥1 cycle of NUC-1031 with cisplatin due to cholangitis and grade 3 GGT (considered non-clinically significant, but before protocol amendment); switched to standard of care cisplatin/gemcitabine. Tumour control (PR, CR or SD) was achieved in 16 of 21 patients (76%) who received NUC-1031 and cisplatin. Of note, the two patients included with ampulla of Vater carcinoma had a CR and SD as best response and a median OS of 10.7 and 17.2 months, respectively. The two patients who received prior adjuvant treatment (capecitabine or cisplatin/gemcitabine) had SD as best response and a median OS of 17.2 and 21.1 months, respectively.

In the efficacy evaluable population (*n*=16), the overall ORR was 44% (7/16) (Figure 1).

The median time between registration and cycle 1 day 1 of treatment on ABC-08 was 2 days. The median PFS (radiological) was 5.7 months (95% CI 3.3-10) in the NUC-1031 625mg/m^2 cohort and 8.6 months (95% CI 2.6-not estimable) in the 725mg/m^2 NUC-1031 cohort. The overall median PFS was 7.2 months (95% CI 4.3-10.1).

The median OS was 9.6 months (95% CI 4.7-10.7) in the NUC-1031 625mg/m^2 cohort and 8.6 months (95% CI 6.7-not estimable) in the NUC-1031 725mg/m^2 cohort. The overall median OS was 9.6 months (95% CI 6.7-13.1). Summary details of overall survival based on ORR, primary tumour site and NUC-1031 dose (mg/m^2) (efficacy evaluable population) are provided in Table 4.

Treatment beyond ABC-08

One patient (recurrent ampulla of Vater carcinoma) was deemed to have surgically resectable liver disease (following CT, MRI and 18F-fluorodeoxyglucose positron emission tomography imaging) after receiving 4 cycles of NUC-1031 625mg/m^2 in combination with cisplatin in the advanced setting, and had surgery and then went on to receive 6 cycles of adjuvant gemcitabine. This patient developed liver and lung recurrence approximately 11 months post resection and was not fit for further systemic treatment.

Further treatment lines in the advanced setting were given to 5 patients; 2 patients received cisplatin/gemcitabine, 1 received oxaliplatin/5-fluorouracil/folinic acid, 1 irinotecan/capecitabine/trastuzumab (patient with advanced gallbladder cancer; human epidermal growth factor receptor 2 (HER2) positive) and 1 Selective internal radiation therapy (SIRT); SIRT was delivered immediately following discontinuation on ABC-08 (due

to grade I asymptomatic pneumonitis). Treatments given post ABC-08 followed previously published literature.[8-11]

Only 3 patients had molecular testing in ABC-08 in addition to the patient described above who had HER2 positivity; 1 had no targetable mutation (distal bile duct cholangiocarcinoma; survival 8.6 months), 1 had a BReast CAncer gene 1 genomic alteration (BRCA1), Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2) and type 2 topoisomerase alpha (TOP2A) amplifications (hilar cholangiocarcinoma; survival 16.3 months) and 1 had mutations in Epidermal growth factor receptor (EGFR), Serine/threonine kinase 11 (STK11), Kirsten ras oncogene homologue (KRAS), cyclin-dependent kinase inhibitor 2A (CDKN2A) and tumour suppressor TP53 (distal bile duct cholangiocarcinoma; survival 21.2 months).

Pharmacokinetics

Pharmacokinetic analysis was available for 17 patients; 12 for the 625mg/m^2 NUC-1031 cohort and 5 from the 725mg/m^2 NUC-1031 cohort (2 patients did not have PK samples taken and samples were misplaced for 2 patients).

Plasma PK of NUC-1031, dFdC and dFdU

The highest plasma exposures were seen for NUC-1031; median plasma NUC-1031 AUC_{0-24} and C_{max} estimates ranged from 218-324 μg•h/mL and 309-889 μg/mL, respectively. The lowest overall plasma exposures were observed for dFdC; median plasma dFdC AUC_{0-24} and C_{max} estimates ranged from 0.47-1.56 μg•h/mL and 0.284-0.522 μg/mL, respectively. The median plasma dFdU AUC_{0-24} and C_{max} estimates ranged from 44.9-71.0 μg•h/mL and 2.65-3.56 μg/mL, respectively. The median terminal half-life for NUC-1031 (estimated from the $T_{1/2,\lambda}$) and dFdU (estimated from the $T_{1/2,\beta}$) was 2.0-3.3 hours and 5.0-13.6 hours, respectively.

Comparison of plasma AUC_{0-24} and C_{max} between NUC-1031 doses

AUC_{0-24} values for NUC-1031 were similar with increasing dose, with median values of 261 and 282 µg•h/mL for the 625mg/m^2 and 725mg/m^2 doses, respectively. C_{max} values for NUC-1031 were also similar with increasing dose, with median values of 552 and 685 µg/mL for the 625mg/m^2 and 725mg/m^2 doses, respectively.

AUC_{0-24} values for dFdC, with increasing dose, were (median values) 0.863 and 1.17 µg•h/mL for the 625mg/m^2 and 725mg/m^2 doses, respectively. C_{max} values for dFdC were similar, with median values of 0.432 and 0.423 µg/mL for the 625mg/m^2 and 725mg/m^2 doses, respectively.

AUC_{0-24} values for dFdU, with increasing dose, were (median values) 48.0 and 71.0 µg•h/mL for the 625mg/m^2 and 725mg/m^2 doses, respectively. C_{max} values for dFdU were similar, with median values of 2.82 and 3.56 µg/mL for the 625mg/m^2 and 725mg/m^2 doses, respectively (Table 5). There was no formal correlation between PKs and toxicity. On review of individual day 1 PK parameters for two patients who received NUC-1031 625mg/m^2 or 725mg/m^2 in combination with cisplatin (without any treatment omissions), the C_{max} values and AUC_{0-24} were 793 µg/mL and 458 µg•h/mL, and 646 µg/mL and 282 µg•h/mL, respectively. In two patients who received NUC-1031 625mg/m^2 or 725mg/m^2 in combination with cisplatin (with one omission within one cycle (day 1 or day 8)), the C_{max} values and AUC_{0-24} were 700 µg/mL and 220 µg•h/mL, and 497 µg/mL and 473 µg•h/mL, respectively, demonstrating variability and no observable trend.

Assessment of PK interaction with cisplatin

In the ABC-08 expansion cohort, the median AUC_{0-24} and C_{max} values for NUC-1031 were found to be 218 µg•h/mL (range 169-263 µg•h/mL) and 309 µg/mL (range 225-400 µg/mL), respectively. In the ABC-08 expansion cohort (NUC-1031 625mg/m^2), the AUC_{0-24} and C_{max} values for dFdU were found to be 49.1 µg•h/mL (range 38.1-58.5 µg•h/mL) and 2.85 µg/mL (range 2.38-3.19 µg/mL), respectively.

Discussion:

This phase Ib study of NUC-1031 combined with cisplatin for the first-line treatment of patients with ABC demonstrated that the combination had a favourable safety profile and achieved good tumour control.

Treatment-emergent grade 3-4 AEs were not unexpected and were similar to those previously reported for gemcitabine in this disease group, including fatigue, hematological toxicity and altered liver function enzymes.[1,12]

The ORR (ITT) was 33% and efficacy evaluable ORR was 44%, compared to 26.1% reported in the ABC-02 study for the cisplatin/gemcitabine combination.[1] Tumour control and OS in ABC-08 were 76% and 9.6 months (with an upper 95% CI of 13.1 months) respectively, similar to the cisplatin/gemcitabine combination in ABC-02: 81.4% and 11.7 months.[1] Despite the potential biological heterogeneity of ABC primary tumour sites,[13] responses seen in ABC-08 were distributed across all five BTC subtypes (intrahepatic, hilar, and distal bile duct cholangiocarcinoma, gallbladder cancer and ampulla of Vater carcinoma).

The median number of cycles received in ABC-08 was 6 and 7.5 in the NUC-1031 625mg/m^2 and 725g/m^2 dose groups respectively, resembling the median duration of cisplatin/gemcitabine combination treatment in ABC-02; 7 cycles (21 weeks).[1] Approximately 19% went on to receive second-line treatment in ABC-08, which approximates with previously reported literature (25%),[14] and four patients were still alive at the end of follow-up, each having survived >16 months (all with a diagnosis of cholangiocarcinoma), which reflects conditional probability of survival or landmark survival in patients with ABC; the longer a patient survives, the greater the chance that they will survive another year, and is potentially influenced by receipt of combination therapy and

anatomic ABC primary site, with those with intrahepatic cholangiocarcinoma and cholangiocarcinoma non-specified having superior landmark survival to those with a gallbladder cancer diagnosis in a recently reported study.[15]

NUC-1031 achieved higher concentrations than its metabolites dFdU and dFdC in this study. AUC_{0-24} values for NUC-1031, dFdU and dFdC were not similar with increasing dose (625mg/m^2 to 725mg/m^2). C_{max} values for NUC-1031, dFdU and dFdC were similar with increasing dose. The C_{max} of dFdU was around 50-fold lower (2.82 and 3.56 µg/mL in 625mg/m^2 and 725mg/m^2, respectively) compared to reported levels for gemcitabine (121 µg/mL),[7] further supporting NUC-1031 is resistant to degradation by CDA. It has previously been reported that the estimated plasma $t_{1/2}$ of NUC-1031 was 8.3 hours, in comparison to the shorter reported plasma $t_{1/2}$ of gemcitabine (up to 94 minutes), potentially allowing tumour cells to have a more prolonged exposure to dFdCTP (and so enhancing its activity).[4] In this current study, the terminal half-life for NUC-1031 was 2.0-3.3 hours (still greater than gemcitabine), and may be a reflection that no PK samples were taken at 6 and 24 hours, as were analysed in the monotherapy study,[4] which would have allowed a more accurate comparison.

In general, the inter-patient variability predicted from the PK model generated in the NUC-1031 monotherapy study[4] was found to be in the region of 40% for NUC-1031. Results for ABC-08 showed that there was an approximate 26% decrease in AUC_{0-24} and a 36% increase in C_{max} for NUC-1031 following combination with cisplatin, when compared to historical monotherapy data[4] and so the combination with cisplatin did not seem to alter the PK profile of NUC-1031.

In conclusion, this is the first study reporting on the combination of NUC-1031 with cisplatin in ABC. A dose of 725mg/m^2 of NUC-1031 is recommended in combination with cisplatin

25mg/m^2 on a day 1 and 8 schedule every 3 weeks in patients with ABC. This regimen is currently being compared with cisplatin plus gemcitabine in the NuTide:121 study (NCT04163900), a global phase III randomised study in patients with ABC.[16]

Additional information:

Ethics approval and consent to participate:

Prior to taking part in this study, all patients signed an informed consent approved by North West - Liverpool Central Research Ethics Committee (reference 15/NW/0160). The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

Consent for publication:

This manuscript does not contain any individual patient's data or identifiable information.

Data availability:

The data included in this study are available on reasonable request.

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Conflicts of interest:

MMN has received research grant support from Servier, Ipsen and NuCana plc. She has received travel and accommodation support from Bayer, Ipsen and Novartis and speaker honoraria from Pfizer, Ipsen, NuCana plc and Mylan. She has served on advisory boards for Celgene, Ipsen, Sirtex and Baxalta.

JB has received honoraria from Merck Serono, Roche, Sanofi, and Bayer.

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OF has no conflicts of interest to declare.

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AP has no conflicts of interest to declare.

WDR has no conflicts of interest to declare.

SB has no conflicts of interest to declare.

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JWV reports Consulting or Advisory role for Ipsen, Novartis, AstraZeneca, Merck, Delcath Systems, Agios, Pfizer, PCI Biotech, Incyte, Keocyt, QED, Pieris Pharmaceuticals, Genoscience Pharma, Mundipharma EDO; Honoraria from Ipsen; and Speakers' Bureau for Novartis, Ipsen, NuCana plc and Imaging Equipment Limited.

Authors's contributions:

Conception and design: MMN, JWV. Acquisition of data: MMN, JB, DP, OF, HW, SB, TRJE, JWV. Lead pharmacist: AP. Analysis of data: WDR. Pharmacokinetic design and analysis: GG, EG. Interpretation of data: All authors. Writing, review and/or revision of manuscript: All authors.

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Figure legend:

Figure 1

Waterfall plot of best response to therapy in ABC-08.

Sixteen patients with measurable disease were assessed for efficacy (received at least one cycle of NUC-1031 with cisplatin, and had at least one follow-up radiographic assessment). In the efficacy evaluable population, the overall objective response rate was 44%.**RECIST**: Response evaluation criteria in solid tumours, **IHC**: Intrahepatic cholangiocarcinoma, **DBD**: Distal bile duct cholangiocarcinoma, **AMP**: Ampulla of Vater carcinoma, **GBC**: Gallbladder cancer.

Table 1: Baseline demographics and clinical characteristics of patients enrolled in ABC-08

Characteristic	***NUC-1031 625mg/m^2 (*n*=15)**	***NUC-1031 725mg/m^2 (*n*=6)**
Age (years)		
Median (range)	61 (55-78)	59.5 (47-71)
Gender		
Male	10	3
Female	5	3
ECOG PS		
0	4	3
1	11	3
Primary tumour site		
Intrahepatic	5	0
Hilar	3	4
Distal bile duct	3	2
Gallbladder cancer	2	0
Ampulla of Vater carcinoma	2	0
Disease status		
Locally advanced	3	1
Metastatic	12	5
Recurrent disease		
No	11	2
Yes	4	4

*In combination with cisplatin 25mg/m^2 on day 1 and 8 (21-day schedule). **ECOG PS**: Eastern Cooperative Oncology Group Performance Status.

Table 2: The most common treatment-emergent grade 2-4 adverse events occurring in ≥1 patient enrolled in ABC-08

Adverse event	***Grade**	****NUC-1031 625mg/m^2 (*n=15*)** *n* **(%)**	****NUC-1031 725mg/m^2 (*n=6*)** *n* **(%)**
GGT increase	2	3 (20)	1 (17)
	3	4 (27)	4 (67)
	4	2 (13)	0 (0)
Neutropenia	2	1 (7)	0 (0)
	3	3 (20)	1 (17)
	4	1 (7)	0 (0)
ALT increase	2	1 (7)	1 (17)
	3	3 (20)	0 (0)
	4	0 (0)	0 (0)
WCC decrease	2	3 (20)	1 (17)
	3	3 (20)	0 (0)
	4	0 (0)	0 (0)
Nausea	2	3 (20)	4 (67)
	3	2 (13)	0 (0)
	4	0 (0)	0 (0)
Vomiting	2	2 (13)	1 (17)
	3	1 (7)	1 (17)
	4	0 (0)	0 (0)
Diarrhoea	2	0 (0)	0 (0)
	3	2 (13)	1 (17)
	4	0 (0)	0 (0)
Fatigue	2	5 (33)	3 (50)

	3	2 (13)	2 (13)
	4	0 (0)	0 (0)
Hyperbilirubinemia	2	0 (0)	0 (0)
	3	2 (13)	0 (0)
	4	0 (0)	0 (0)
Thrombocytopenia	2	4 (27)	3 (50)
	3	2 (13)	0 (0)
	4	0 (0)	0 (0)
Thrombus	2	0 (0)	0 (0)
	3	3 (20)	0 (0)
	4	0 (0)	0 (0)
***Hb decrease**	2	4 (27)	1 (17)
***Mg decrease**	2	0 (0)	1 (17)
***Alk phos increase**	2	3 (20)	1 (17)
***AST increase**	2	1 (7)	1 (17)
***Abdominal pain**	2	1 (7)	0 (0)
***Anorexia**	2	2 (13)	0 (0)
***Dyspnea**	2	0 (0)	1 (17)
***Infection**	2	0 (0)	1 (17)
***Infusion reaction**	2	1 (7)	0 (0)
***Neutropenia**	2	1 (7)	0 (0)
***Urinary tract infection**	2	1 (7)	0 (0)

*According to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03. **In combination with cisplatin 25mg/m^2 on day 1 and 8 (21-day schedule). In total, there were 15 and 6 patients recruited to the NUC-1031 625mg/m^2 and 725mg/m^2 cohorts respectively. ***No Grade 3-4 adverse events. **GGT**: gamma-glutamyl

transferase, **ALT**: alanine aminotransferase, **WCC**: White cell count, **Hb**: haemoglobin, **Mg**: magnesium, **Alk phos**: alkaline phosphatase, **AST**: aspartate aminotransferase.

Table 3: Reasons for treatment discontinuation in ABC-08 (*n*=21)

Reason for treatment discontinuation	**Patients (*n*)**
Adverse events	
$Cholangitis	1
$$Biliary obstruction	2
*Small bowel perforation secondary to adhesions	1
Perforated diverticulitis	1
**Grade 3 GGT	1
***Brain metastases (clinical deterioration)	1
****Grade 2 vomiting and haematemesis secondary to cisplatin	1
*****Radiological pneumonitis (Grade 1)	1
£Intercurrent illness (pre-existing cirrhosis)	1
Investigator decision ((i) surgically resectable disease following ABC-08 combination treatment and (ii) inability to re-start treatment within 21 days due to biliary obstruction)	2
Patient decision (holiday)	1
Progressive disease	6
Death (underlying disease)	2

$Cholangitis related to biliary stent; unrelated to disease progression or treatment; patient not neutropenic, $$Biliary obstruction; unrelated to disease progression or treatment; patients not neutropenic, *In a patient with recurrent distal bile duct cholangiocarcinoma, **Non-clinically significant, but patient decided to commence cisplatin/gemcitabine locally, ***Patient complained of right arm paraesthesia following 1 cycle of treatment on ABC-08, which on questioning was present prior to therapy commencement (brain metastases were identified on Magnetic Resonance Imaging). The decision was made to proceed with treatment, given that patient was relatively asymptomatic and patient went on to achieve a partial response on body imaging, ****Deemed related to cisplatin by treating investigator on assessment and review of relevant Summary of Product Characteristics, *****Asymptomatic on a background of long-standing fibrosis noted in lower lobes of lungs (radiological findings

attributed to NUC-1031/cisplatin). [£]Patient discontinued treatment due to decline in liver function (ascites accumulation) deemed un-related to treatment; a partial response was noted on imaging, **GGT**: gamma-glutamyl transferase.

Table 4: Summary details of overall survival based on objective response rate, primary tumour site and NUC-1031 dose (mg/m^2) (efficacy evaluable population)

Patient	**Change in tumour volume (%)**	**Primary tumour site**	***NUC-1031 dose (mg/m^2)**	**Overall survival (days)**	**Treatment post ABC-08**	**Status at end of trial follow-up**
A	-100	AMP	625	325	No	Dead
B	-54	DBD	625	**784**	Cisplatin/gemcitabine	Dead
C	-51	IHC	625	**1095**	SIRT	Alive
D	-50	Hilar	725	**609**	No	Alive
E	-46	IHC	625	219	No	Dead
F	-43	GBC	625	264	No	Dead
G	-39	Hilar	725	205	No	Dead
H	-28.3	DBD	625	196	No	Dead
I	-26	IHC	625	305	No	Dead
J	-21	GBC	625	291	Irinotecan/capecitabine/ trastuzumab	Dead
K	0	Hilar	725	495	No	Alive
L	+6	Hilar	625	400	No	Dead
M	+10	AMP	625	524	Surgical resection + adjuvant gemcitabine	Dead
N	+12	DBD	725	245	No	Dead
O	+21	IHC	625	307	Oxaliplatin/5-fluorouracil/folinic acid	Dead
P	+60	Hilar	625	143	No	Dead

Change in tumour volume (%) based on Response Evaluation Criteria In Solid Tumours version 1.1 (Efficacy evaluable population: 16 patients with measurable disease who received at least one cycle of NUC-1031 with cisplatin, and had at least one follow-up radiographic

assessment) (an additional patient did not have measurable disease (best response based on non-target lesion was non-complete response/non-progressive disease), 2 did not receive any treatment on ABC-08 beyond cycle 1, day 1 due to cholangitis and raised gamma-glutamyl transferase (surviving 151 and 644 days respectively) and 2 did not have follow-up imaging beyond baseline having died at 45 and 109 days respectively), **AMP**: Ampulla of Vater carcinoma, **DBD**: Distal bile duct cholangiocarcinoma, **IHC**: Intrahepatic cholangiocarcinoma, **GBC**: Gallbladder cancer, **SIRT**: Selective internal radiation therapy. *In combination with cisplatin 25mg/m^2 on day 1 and 8 (21-day schedule). Four patients lived for >16 months.

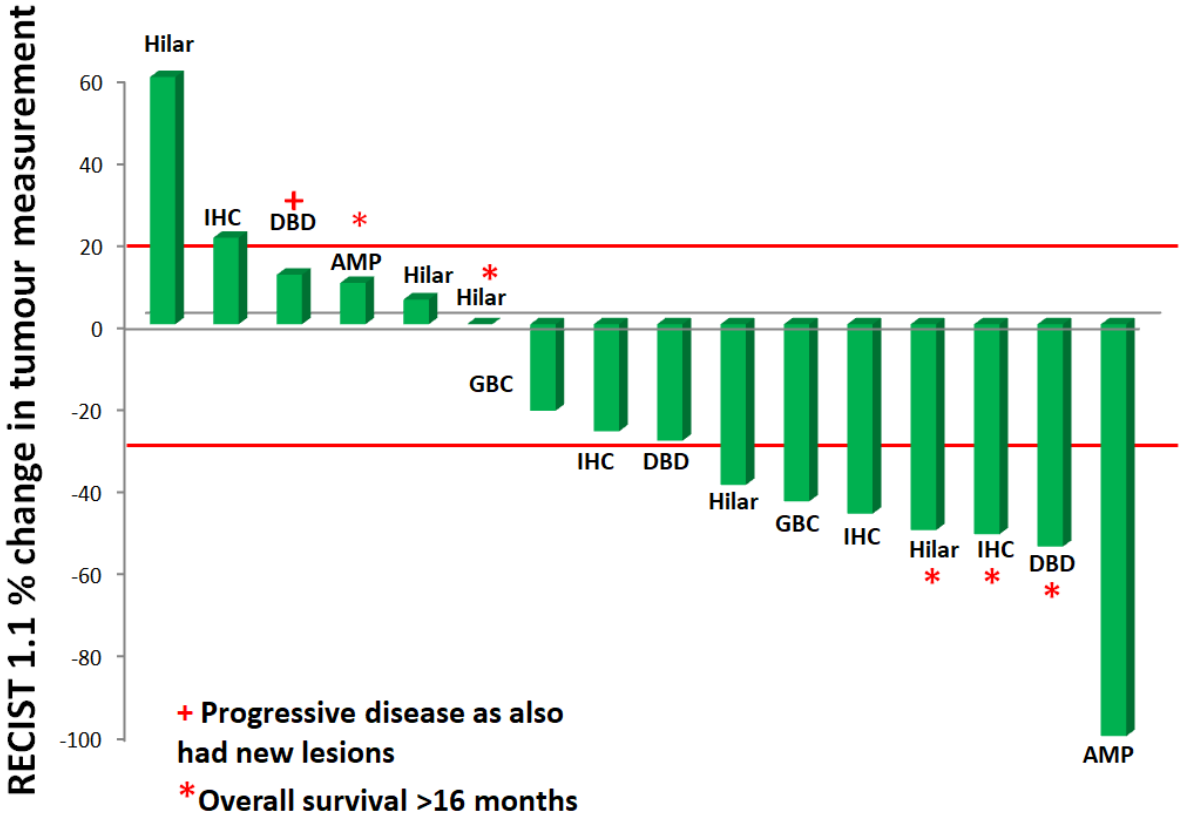
Table 5: Summary statistics (Median [90% confidence intervals]) for individual, model-derived plasma pharmacokinetic parameters by dose (mg/m^2) for participants enrolled in ABC-08

Parameter	**NUC-1031 625mg/m^2 (*n*=12)**	**NUC-1031 725mg/m^2 (*n*=5)**
NUC-1031		
C_{max} (μg/mL)	552 [244-1020]	685 [527-897]
AUC_{0-24} (μg•h/mL)	261 [176-588]	282 [265-573]
$AUC_{0-\infty}$ (μg•h/mL)	261 [176-588]	282 [265-573]
$T_{1/2,\alpha}$ (h)	0.0250 [0.0126-0.0521]	0.0254 [0.0218-0.0424]
$T_{1/2,\beta}$ (h)	0.147 [0.103-0.682]	0.155 [0.130-0.521]
$T_{1/2,\lambda}$ (h)	2.38 [1.15-3.86]	3.07 [2.74-5.66]
Vss (L)	4.28 [2.69-7.81]	4.19 [3.23-4.71]
CL (L/h)	4.46 [2.08-6.62]	4.37 [2.31-4.92]
dFdC		
C_{max} (μg/mL)	0.432 [0.165-0.588]	0.423 [0.363-0.499]
AUC_{0-24} (μg•h/mL)	0.863 [0.358-2.07]	1.17 [1.03-1.69]
$AUC_{0-\infty}$ (μg•h/mL)	0.869 [0.358-2.14]	1.18 [1.04-1.72]
$T_{1/2,\alpha}$ (h)	0.0771 [0.0567-0.117]	0.0920 [0.0756-0.103]
$T_{1/2,\beta}$ (h)	1.52 [0.634-2.90]	1.98 [1.52-2.13]
$T_{1/2,\lambda}$ (h)	1310 [1300-1350]	1320 [1320-1330]
Vss (L)	2820 [2590-3790]	3000 [2760-3100]
CL (L/h)	602 [208-1560]	475 [339-577]
dFdU		
C_{max} (μg/mL)	2.82 [2.21-3.58]	3.56 [3.05-4.32]
AUC_{0-24} (μg•h/mL)	48.0 [39.2-68.9]	71 [52.1-78.2]
$AUC_{0-\infty}$ (μg•h/mL)	63.8 [43.9-110]	98.6 [63.0-141]
$T_{1/2,\beta}$ (h)[a]	7.57 [4.00-19.3]	10.0 [5.39-14.7]

Vss (L)	66.6 [43.4-161]	67.2 [60.4-82.9]
CL (L/h)	6.37 [4.13-9.22]	5.41 [3.61-8.30]

[a]Note that the half-life estimate for dFdU is listed under $T_{1/2,\beta}$, as there is only one applicable half-life for that analyte.

AUC_{0-24}: area under the plasma concentration-time curve from time 0 to last measurable time, **$AUC_{0-\infty}$**: area under the plasma concentration-time curve from time 0 to infinity, **$t_{1/2}$**: half-life, **Vss**: volume of distribution at steady-state, **CL**: clearance, **dFdC**: di-fluoro-deoxycytidine, **dFdU**: 2′,2′-difluorodeoxyuridine.


RECIST 1.1 % change in tumour measurement
60
40
20
0
-20
-40
-60
-80
-100
Hilar
IHC
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*
Hilar
GBC
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Hilar
GBC
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Hilar
*
IHC
*
DBD
*
AMP
+ Progressive disease as also had new lesions
*Overall survival >16 months

Figure 1:

Supplementary Table 1: The most relevant treatment-emergent grade 1 adverse events occurring in ≥4 patients enrolled in ABC-08

Adverse event	***Grade**	****NUC-1031 625mg/m^2 (*n=15*)** **n (%)**	****NUC-1031 725mg/m^2 (*n=6*)** **n (%)**
AST increase	1	8 (53)	2 (33)
Hb decrease	1	7 (47)	3 (50)
ALT increase	1	6 (40)	3 (50)
Anorexia	1	6 (40)	3 (50)
Nausea	1	6 (40)	1 (17)
Thrombocytopenia	1	6 (40)	1 (17)
Fatigue	1	5 (33)	0 (0)
Dysgeusia	1	4 (27)	3 (50)
Alk phos increase	1	4 (27)	2 (33)
Vomiting	1	4 (27)	0 (0)

*According to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03. **In combination with cisplatin 25mg/m^2 on day 1 and 8 (21-day schedule). In total, there were 15 and 6 patients recruited to the NUC-1031 625mg/m^2 and 725mg/m^2 cohorts respectively. **AST**: aspartate aminotransferase, **Hb**: haemoglobin, **ALT**: alanine aminotransferase, **Alk phos**: alkaline phosphatase.

Supplementary Table 2: Details on dosing of NUC-1031 and cisplatin received by individual patients enrolled in ABC-08

Patient number	***Dose of NUC-1031 (mg/m^2) in combination with cisplatin 25mg/m^2**	**Cycle of last dosing**	**Day of last dosing within a cycle**	**Day of last dosing (measured from day 1 of treatment)**	**NUC-1031 cumulative relative dose (%)**	**Cisplatin cumulative relative dose (%)**
1	625	1	1	1	100	100
2	625	11	1	253	50.9	43.1
3	625	2	1	36	45.3	50.6
4	625	4	8	71	100	100
5	625	8	8	162	98.7	98.7
6	625	12	8	288	78.8	84.1
7	625	4	8	71	63.1	75.7
8	625	4	8	71	100	100
9	725	4	1	64	98.1	100
10	725	6	1	134	56.3	56.5
11	725	1	1	1	97.7	100
12	725	9	8	231	55.3	69.0
13	725	14	8	295	76.3	67.0
14	725	9	8	232	71.1	65.7
15	625	8	1	161	76.3	78.5
16	625	11	1	239	78.4	80.8
17	625	6	1	141	72.2	73.7
18	625	11	1	259	68.5	76.1
19	625	1	1	1	100	100
20	625	3	8	50	100	96.0

21	625	6	1	126	88.0	89.9

*Administered day 1 and 8 every 21 days.

Supplementary material 1: Full eligibility criteria for ABC-08

Patients were eligible for the trial if all the inclusion criteria were met and none of the exclusion criteria applied.

Inclusion criteria:

1. Histologically/cytologically verified, non-resectable or recurrent/metastatic cholangiocarcinoma, gallbladder or ampullary carcinoma. (Radiological diagnosis allowed for recurrent disease if previously histologically/cytologically verified).

2. No prior systemic therapy allowed for advanced biliary cancer. Prior low dose chemotherapy used with or without radiotherapy in the adjuvant setting is allowed if completed >6 months from enrolment. Recent palliative radiation (within 28 days prior to consent) is allowed if candidate has measurable disease outside radiation field.

3. ECOG performance status 0-1.

4. Age ≥18 years and life expectancy >3 months.

5. Adequate renal function with serum urea and serum creatinine <1.5 times upper limit of normal (ULN) and creatinine clearance ≥30ml/min.

6. Adequate haematological function: Hb ≥10g/dl, white blood cell count ≥3.0 x 10^9/L, absolute neutrophil count ≥1.5 x 10^9/L, platelet count ≥100 x 10^9/L.

7. Adequate liver function: total bilirubin <30 μmol/L and alkaline phosphatase, along with aspartate aminotransferase and alanine aminotransferase ≤5 x ULN.

8. Adequate biliary drainage, with no evidence of ongoing infection.

9. Women of child bearing age MUST have a negative pregnancy test prior to study entry AND be using a highly effective contraception method (combined or progestogen-only hormonal contraception, intrauterine device, intrauterine hormone-releasing system, vasectomised partner* or sexual abstinence**) which must be continued for 6 months after the end of study treatment, unless childbearing potential has been terminated by surgery/radical radiotherapy or infertility due to bilateral tubal occlusion.

10. Male subjects must either have had a successful vasectomy (confirmed azoospermia) or they and their female partner meet the criteria above (not of childbearing potential or practicing adequate contraception [e.g. combined or progestogen-only hormonal contraception, intrauterine device, intrauterine hormone-releasing system, sexual abstinence**] throughout the study period and for 6 months after the end of study treatment).

11. Patients must not have a history of other malignant diseases (within the previous 5 years and there must be no evidence of recurrence), other than adequately treated non-melanotic skin cancer or insitu carcinoma of the uterine cervix.

12. Patients must have given written informed consent.

* The vasectomised partner must have received medical assessment confirming surgical success.

** Sexual abstinence in line with the preferred and usual lifestyle of the subject.

Exclusion criteria:

1. History of allergic reactions attributed to previous gemcitabine or cisplatin treatment.

2. Documented history of allergic reactions attributed to any of the excipients used in the formulation (Kolliphor ELP; Tween 80; DMA).

3. Previous treatment with NUC-1031.

4. Incomplete recovery from previous therapy (surgery/adjuvant therapy/radiotherapy) or unresolved biliary tree obstruction.

5. Any evidence of severe or uncontrolled systemic diseases which, in the view of the investigator, makes it undesirable for the patient to participate in the trial.

6. Evidence of significant clinical disorder or laboratory finding which, in the opinion of the investigator makes it undesirable for the patient to participate in the trial.

7. Any patient with a medical or psychiatric condition that impairs their ability to give informed consent.

8. Any other serious uncontrolled medical conditions.

9. Clinical evidence of metastatic disease to the brain.

10. Any pregnant or lactating woman.

11. Pre-existing hearing impairment.

Supplementary material 2: Criteria for treatment of patients enrolled onto ABC-08

See supplementary material 1 for Cycle 1 Day 1 inclusion criterion number 6: required blood values.

Participants must meet ALL of the following criteria prior to receiving cisplatin and NUC-1031 on commencement of SUBSEQUENT cycles (day 1). Labs may be assessed within 24 hours of the first scheduled dose.

· Absolute neutrophil count ≥ 1.0 X 10^9/L [use of short-acting growth GCSF is permitted after cycle 1 but must be stopped in advance of the start of a cycle to allow stabilisation of neutrophil count (at least 5 days for short-acting growth factors) but should only be used as per American Society of Clinical Oncology guidelines].

· Haemoglobin ≥80 g/L (transfusions will be permitted).

· Platelet count ≥100 X 10^9/L (WITHOUT platelet transfusions).

· Absence of dose-limiting toxicity (DLT) (If patient had a DLT (during cycle 1) and has recovered, then retreatment should be at next lower dose level).

· No evidence of disease progression (based on clinical or radiographic assessment).

· Recovery from all clinically significant toxicities to ≤Grade 2 or to baseline Grade present at study entry.

If above criteria are not met for Day 1 of a cycle, delay by one week and re-check lab parameters (treatment will be discontinued in patients with progressive disease). If delay is >21 days the patient will be withdrawn from treatment.

Participants should be dosed on Day 8 of a cycle in accordance with the below:

Treatment Day 8 Counts (any Cycle)				
Absolute Neutrophils (x10^9/L)		Platelets (x10^9/L)	NUC-1031 Dose Management	Cisplatin Dose Management
≥1.0	and/or	≥100	No dose change	No dose change
0.5-<1.0	and/or	50-<100	75% dose	No dose change
<0.5	and/or	<50	Delay x 1 week*	Delay x 1 week

The dose of NUC-1031 will be re-escalated to full dose upon recovery of haematological toxicity despite a previous day 8 dose reduction, in order to maintain the dose-intensity of therapy.

If there is a 2 week deferral of Day 8 of a cycle, then omit Day 8 of that cycle, and proceed with the next Cycle Day 1, if criteria for Day 1 are met. See Table below for guidance (Cycle 3 detailed as an example):

No deferral **(Treatment given)**	Cycle 3 Day 1 Yes	Cycle 3 Day 8 Yes	-	-	**As per protocol**
One week deferral **(Treatment given)**	Cycle 3 Day 1 Yes	Cycle 3 Day 8 No	Cycle 3 Day 8 Yes	-	**Cycle 3 Day 8 is given one week late**
Two week deferral **(Treatment given)**	Cycle 3 Day 1 Yes	Cycle 3 Day 8 No	Cycle 3 Day 8 No	Cycle 4 Day 1 Yes	**Cycle 3 Day 8 is omitted altogether and next cycle starts**

Patients must also meet the following criteria prior to receiving cisplatin and NUC-1031 on day 8 of all cycles:

· Recovery from all clinically significant toxicities to ≤Grade 2 or to baseline Grade present at study entry.

· No evidence of disease progression (based on clinical or radiographic assessment).

If a DLT occurs during cycle 1, doses of study drug will be withheld for the remainder of the cycle and subsequent cycles will be administered at the previously tested next lower dose level.

Intra-subject dose delay, dose reduction, or dose escalation

Treatment between cycles can be delayed for up to 21 days in order for patients to meet the retreatment criteria before starting their next cycle. The delay is calculated from the time the next subsequent cycle was due to start. Patients who fail to meet these requirements after this additional time will not be allowed to receive further cycles of therapy and will be withdrawn from study treatment unless otherwise agreed by the Trial Management Group (TMG). In addition, a treatment delay of more than 21 days in starting the next cycle for reasons other than toxicity will result in the patient being removed from study treatment unless otherwise agreed by the TMG. If a patient experiences multiple toxicities, dose adjustments will be based on the most severe toxicity. A patient who experiences a clinically significant causally-related toxicity meeting the definition of DLT during any cycle (beyond cycle 1), but whose toxicity recovers within 21 days will be dose reduced to the previously tested next lower dose level at investigator's discretion.

Any patient who experiences one or more recurrent clinically significant toxicities after the initial dose reduction may have one further dose reduction (If the dose is at 500mg/m^2, further dose reduction levels suggested are 375mg/m^2 and 275mg/m^2). Participants who continue to experience clinically significant toxicity despite two dose reductions will require consultation with the TMG, to determine if further dose reductions are clinically appropriate.

Further drug dose modifications

Toxicity	Grade or Value	NUC-1031	Cisplatin
Anaphylaxis (Acute hypersensitivity reactions)	Severe reactions defined as hypotension requiring treatment, dyspnoea requiring bronchodilators, angioedema or generalised urticaria.	Immediately discontinue NUC-1031 and do NOT re-challenge. Also discontinue cisplatin.	Immediately discontinue treatment and do NOT re-challenge. If allergy due to platinum, patient can continue study on NUC-1031 alone at the discretion of the TMG.
Other grade 3 non-haematological toxicity*	Grade ≥3	Withhold treatment. If resolved to baseline or ≤Grade 2 in <21 days, consider restarting at same dose as was given prior to drug being withheld, if toxicity can be managed with supportive medication.	Withhold treatment. If resolved to baseline or ≤Grade 2 in <21 days, consider restarting at same dose as was given prior to drug being withheld if toxicity can be managed with supportive medication.
Creatinine Clearance	Calculated Glomerular filtration rate (GFR) is <30mL/min	None.	Hold treatment until GFR is verified by Cr51-EDTA or 99mTc-DTPA test. If Cr51-EDTA or 99mTc-DTPA test clearance is <30 mL/min discontinue cisplatin.
Ototoxicity	Grade ≥3	None.	Immediately discontinue treatment and do NOT re-challenge.
Pneumonitis	Radiologically confirmed	Immediately discontinue NUC-1031 and do NOT re-challenge.	Immediately discontinue treatment and do NOT re-challenge.
Posterior	Radiologically	Immediately discontinue NUC-1031	Immediately

reversible encephalopathy syndrome (PRES)	confirmed	and do NOT re-challenge.	discontinue treatment and do NOT re-challenge.

*excluding grade ≥3 toxicities which following case causality assessment is not in the category of 'Certain', 'Probable' or 'Possible' and as such is not related to NUC-1031 and/or cisplatin, or which are not considered a clinically-significant toxicity.