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REVIEW ARTICLE

NUC-1031, use of ProTide technology to circumvent gemcitabine resistance: current status in clinical trials

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Abstract

Background Resistance to gemcitabine chemotherapy is common in patients with pancreatic ductal adenocarcinoma (PDAC), biliary tract cancer (BTC) and ovarian cancers (OC), conferring poor survival. Use of ProTide technology led to the development of a 'partially-activated' monophosphorylated gemcitabine compound, termed NUC-1031. NUC-1031 enters cancer cells independent of the human equilibrative nucleoside transporter, does not require deoxycytidine kinase-mediated activation and resists cytidine deaminase-mediated breakdown into toxic by-products.

Current findings The phase I PRO-001 trial recruited 68 patients with advanced solid tumours; of the 49 patients that had response-evaluable disease, 5 (10%) had a partial response (PR) and 33 (67%) had stable disease (SD). Subsequently, the PRO-002 study assessed the safety and efficacy of NUC-1031 combined with carboplatin for patients with OC ($n=25$); preliminary data from this study reported one (4%) unconfirmed complete response (CR), 8 (35%) PRs and 13 (57%) patients with SD, the final outcome data are awaited. The ABC-08 trial for advanced BTC assessed safety and efficacy of NUC-1031 combined with cisplatin; 14 patients were recruited with a 50% objective response rate in the intention to treat population at interim analysis. ACELARATE, the phase III trial in first-line advanced PDAC comparing NUC-1031 to gemcitabine monotherapy, recruited 200 patients but has been paused for futility analysis.

Conclusion Early studies demonstrate NUC-1031 is well tolerated with favourable pharmacokinetic profiles. NUC-1031 use in PDAC remains unclear, but encouraging results of disease control in BTC and OC has prompted phase II and III trial development. NuTide 121, is a phase III trial comparing cisplatin-NUC 1031 combination to the standard of care cisplatin-gemcitabine and recruitment is ongoing. Recruiting trials and mature data from existing studies will help inform on the impact of NUC-1031 on patient survival over standard gemcitabine.

Keywords Acelarin · NUC-1031 · Gemcitabine resistance · Phase I trial · Ovarian cancer · Biliary tract cancer · Pancreatic ductal adenocarcinoma

Introduction

Patients with ovarian cancer (OC), biliary tract cancers (BTC) and pancreatic ductal adenocarcinoma (PDAC) tend to present at an advanced stage and the aim of systemic anti-cancer treatment is to control disease and not cure [1, 2]. These cancers are associated with a poor prognosis with 5-year survival for advanced stage OC, BTC and PDAC in the United Kingdom (UK) being less than 13%, 15% and 3%, respectively [3]. To date, immunotherapy has failed to

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demonstrate significant therapeutic benefit in these cancers [4–6], and targeted therapies are effective only in a subset of these patients [7–11]. Chemotherapy therefore remains the therapeutic backbone for these cancers, whilst novel therapeutic approaches are sought. Limitations to chemotherapy use include off-target toxicity and the presence or emergence of cellular resistance mechanisms.

Gemcitabine is a well-established nucleoside analogue chemotherapeutic agent administered as monotherapy or in combination with other agents to treat patients with OC, BTC and PDAC amongst other tumour types [1, 2, 12, 13] as summarised in Table 1. Therapeutic outcomes using gemcitabine can be limited by innate or acquired drug resistance mechanisms employed by cancer cells [14]. Delivery of gemcitabine to tumour cells is dependent upon their uptake across the plasma membrane via nucleoside transporter proteins [15]. Human equilibrative and human concentrative nucleoside transporter 1 (hENT1 and hCNT1, respectively) are the two predominant transporter proteins required for gemcitabine entry into the cells. Reduced cell surface membrane expression of hENT1 and hCNT1 limits gemcitabine delivery to the tumour cells, thus preventing any cytotoxic action [16]. Intracellularly, gemcitabine undergoes sequential phosphorylation to difluorodeoxycytidine monophosphate (dFdCMP), difluorodeoxycytidine diphosphate (dFdCDP) and difluorodeoxycytidine triphosphate (dFdTCP). It is the final dFdTCP metabolite which induces cell cycle arrest and cell death by substituting for the cytosine-based nucleoside deoxycytidine during deoxyribonucleic acid (DNA) replication. Phosphorylation of gemcitabine into dFdCMP has been shown to be the rate-limiting step and is mediated by the enzyme deoxycytidine kinase (dCK) [17] (see Fig. 1). Gemcitabine-resistant cancer cells demonstrate lower levels of dCK, thus preventing conversion of gemcitabine to the active compounds. Prior to phosphorylation and activation, gemcitabine is susceptible to enzymatic breakdown by cytidine deaminase (CDA). Resistant cancer cells can have higher levels of this enzyme, thereby depleting intracellular levels of gemcitabine available for activation [18].

In vitro studies using metastatic PDAC cell lines have shown that with continuous exposure to gemcitabine, resistant drug populations emerge and can proliferate despite exposure to maximal gemcitabine dosing [19]. This suggests a possible natural selection favouring cancer cells with an innate gemcitabine resistance to continue to proliferate. Another hypothesis is that cancer cells acquire gemcitabine resistance over time, which may explain the limited therapeutic response in patients [20]. Targeting the mechanisms through which cancer cells develop gemcitabine resistance is clearly required, leading to the development of NUC-1031, a gemcitabine-based ProTide [21].

ProTide technology has been used successfully in the production of a number of anti-viral drugs [22] and describes

Table 1 A summary of current clinical use of gemcitabine (standard of care and investigational) and NUC-1031 (investigational) in patients with ovarian, biliary tract cancer and pancreatic ductal adenocarcinoma

	Gemcitabine		NUC-1031
Ovarian	Adjuvant: • Not used in SOC therapy. Platinum and taxane-based chemotherapies mainstay in Stage Ia and Ib disease only [59]	Palliative • Current use as second line and beyond therapy typically in combination with platinum and/or taxane chemotherapy [60]	Palliative: • PRO-002 study: Phase I trial-carboplatin and NUC-1031 combination [26] • PRO-105 study: Phase II trial-NUC-1031 in platinum-resistant ovarian cancer [30]
Biliary tract cancer	Adjuvant: • Phase III trial comparing combination cisplatin and gemcitabine to SOC oral capecitabine (ACTICCA-1) [49, 50]	Palliative: • SOC cisplatin and gemcitabine [2] • SOC single agent gemcitabine (poor ECOG PS) [2]	Palliative: • AB C-08 study: Phase I trial-cisplatin and NUC-1031 combination (first-line) [33] • NuTide 121: Phase III trial comparing cisplatin and NUC-1031 combination to cisplatin and gemcitabine (first-line) [34]
Pancreatic ductal adenocarcinoma	Adjuvant: • SOC combination gemcitabine and capecitabine [52] • SOC gemcitabine single agent (poor ECOG PS) [51]	Palliative: • SOC nab-paclitaxel and gemcitabine combination [1] • SOC gemcitabine and capecitabine combination [37] • SOC gemcitabine single agent (poor PS) [12]	Palliative: • ACELARATE: Phase III trial comparing NUC-1031 monotherapy to gemcitabine monotherapy (first-line) [38]

SOC Standard of care, *ECOG PS* Eastern Cooperative Oncology Group performance status. Poor performance status defined as ECOG PS 2 or greater

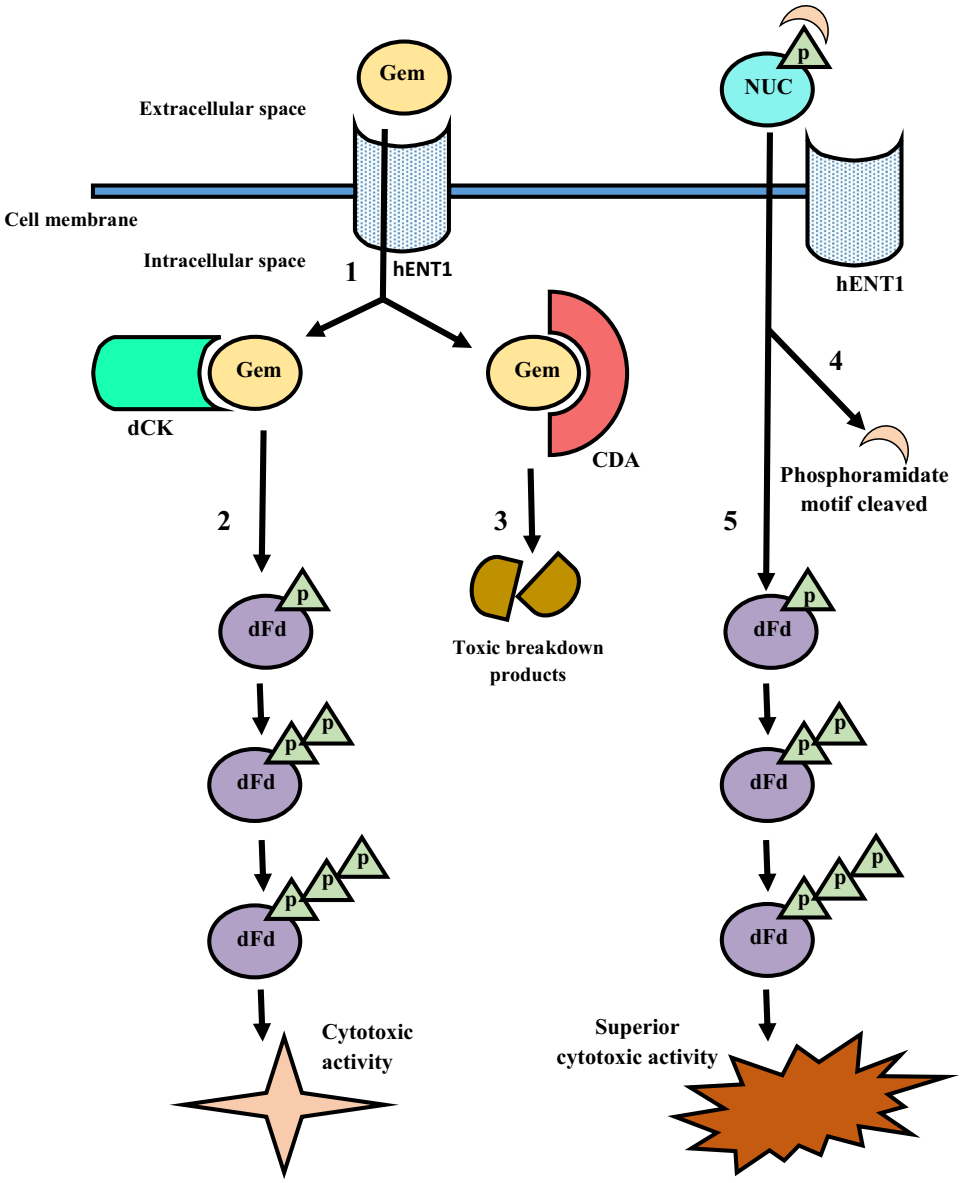

Fig. 1 Pictogram comparing gemcitabine to NUC-1031 for intracellular cytotoxic activity. **1.** hENT1 transporter-mediated entry of gemcitabine into the cancer cell. **2.** dCK-mediated initial phosphorylation of gemcitabine into dFd monophosphate and subsequent phosphorylation to diphosphate and triphosphate compounds. **3.** CDA-mediated breakdown of gemcitabine into toxic inactive breakdown products. NUC-1031 (NUC) depicted with the pre-phosphorylated motif and protective phosphoramidate motif entering the cancer cell independent of hENT1. **4.** Protective phosphoramidate motif cleaved by intracellular esterases to reveal dFd monophosphate compound **5.** thus avoiding the rate-limiting dCK activation and CDA-mediated breakdown. This may allow greater intracellular drug delivery and thus superior cytotoxic action. *Gem* gemcitabine; *hENT1* human equilibrative nucleoside transporter 1; *dCK* deoxycytidine kinase; *CDA* cytidine deaminase; *dFd* difluorodeoxycytidine; *p* phosphate compound

a process of synthetic phosphoramidate chemistry, whereby protective aryl, ester and amino acid groups are added to a pre-phosphorylated nucleotide [21]. These are then cleaved by intracellular esterases releasing the active nucleotide into the cell. NUC-1031 is a novel class of chemotherapy where this technology has been applied to dFdCMP (the monophosphorylated compound of gemcitabine). Through in vitro studies it was demonstrated that NUC-1031 can overcome the gemcitabine resistance pathways as depicted in Fig. 1. NUC-1031 has been shown to enter tumour cells independent of hENT1 transporter proteins, enabling its increased intracellular delivery. Once within the cell, the protective motif is cleaved from NUC-1031, unmasking the dFdCMP compound, bypassing the rate-limiting step of dCK-mediated monophosphorylation. One in vitro study treated pancreatic and ovarian cancer cell lines with gemcitabine and NUC-1031 for 2–24 h at the half maximal effective concentration (EC_{50}) dose [23]. Exogenous deoxycytidine was added as substrate for dCK to compete directly with gemcitabine. Introduction of deoxycytidine induced significant resistance in cell lines treated with gemcitabine with an EC_{50} nine times greater when compared to control cells. In contrast, NUC-1031 treated cancer cells had an EC_{50} three times greater than control cells, suggesting NUC-1031 may require a small amount of dCK for maximal activity but is not the rate-limiting step for efficacy [23].

Furthermore, NUC-1031 is resistant to CDA-mediated breakdown compared to gemcitabine [21]. The net effect is higher intracellular concentrations and longer half-life and therefore superior cytotoxicity of the active drug. In-vivo studies using well established nude mouse models with human pancreatic tumour xenografts demonstrated superior

NUC-1031 efficacy by significantly reducing tumour volume compared to gemcitabine and control compounds. Of note, significantly reduced tumour growth was observed with NUC-1031 in the gemcitabine-resistant BxPC-3 human pancreatic cancer cell line when compared to gemcitabine and control [21]. The experimental promise of NUC-1031 in vitro and in animal studies paved the way for use in clinical trials, which are summarised in Table 2. Here we report the current status and research directions using NUC-1031 in clinical trials from the first PRO-001 Phase I study and subsequent disease group specific trials in OC, BTC and PDAC.

NUC-1031 in patients with advanced solid tumours: PRO-001 trial

This was a 'first in human' phase I study investigating the use of NUC-1031 in patients with recurrent solid tumours who had previously received a mean of 3 chemotherapy regimens [24]. The primary objective of this dose-escalation and expansion trial was to establish the safety, toxicity and recommended phase II dose (RP2D) of NUC-1031. Secondary objectives were to determine its pharmacokinetic profile and preliminary anti-tumour activity (See Table 2). The trial recruited 68 patients; 46 female and 22 male, of which 94% had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. The cohort comprised 19 different primary cancer types; the most frequent were ovarian ($n=10$), pancreas ($n=9$), biliary ($n=7$) and colorectal ($n=7$). Thirty-four (50%) of the patients recruited had previously received gemcitabine-based chemotherapy.

A number of dosing schedules ranging from 500 to 1000 mg/m^2 were evaluated. NUC-1031 was administered on days 1, 8 and 15 of a 28-day cycle; the dose of 825 mg/m^2 was selected for expansion ($n=16$), achieving the study's primary objective. Pharmacokinetic analyses demonstrated NUC-1031 had a longer half-life than has been observed with gemcitabine, with a plasma $t_{1/2}$ of 9.7 h. Rapid NUC-1031 uptake was also observed intracellularly in peripheral blood mononuclear cells (PBMCs). The C_{max} was 764 pmol/10^6 cells/h with a T_{max} of 20 min and successfully converted to the active moiety dFdCTP with a C_{max} of 727.5 pmol/million cells at the 500 mg/m^2 equivalent dose after 30 min post end of infusion (EOI). The pharmacokinetic analyses performed in PRO-001 used PBMCs as a surrogate marker of cancer cells to measure cytotoxic activity. The expression and activity of hENT1, dCK and CDA may differ in PBMCs and therefore may not be indicative of NUC-1031 drug delivery, therapeutic response and resistance mechanisms seen within cancer cells. Importantly, the safety and tolerability of NUC-1031 was comparable to that of gemcitabine with side effects and serious adverse events (SAEs) similar to those reported in the existing literature for gemcitabine [2, 12, 25]. Of the 68 patients, 44 developed SAEs, the most common being infection, fatigue and elevated transaminases. There were 27 grade III/IV SAEs reported with alanine aminotransferase (ALT) increase, fatigue, thrombocytopenia and neutropenia being the most common.

Forty-nine patients received more than 2 cycles of treatment and had paired radiological imaging to assess efficacy, evaluated using Response Evaluation Criteria for Solid Tumours (RECIST) v1.1. In this cohort ($n=49$) the median progression free survival (PFS) was 4 months (range 1–25 months). A best radiological outcome of partial response (PR) was observed in 5 (10%) patients, 33 patients (67%) demonstrated stable disease (SD), of which 12 (24%) patients had SD of at least 6 months duration, and 11 (23%) patients developed disease progression. Seventeen of the 68 patients (25%) completed a 6-cycle course, 14 (21%) of whom continued therapy beyond cycle six. Interestingly, there were 16 (24%) patients who had previously progressed on gemcitabine-based therapy; in this subset of patients, 1(6%) PR and 9 (56%) patients with SD were observed.

Within the subgroup of the 10 patients with OC, all were evaluable for efficacy with one PR, 8 had SD and only one patient progressed on treatment. Given that 90% of patients in this subgroup had favourable tumour control, the PRO-002 study was commenced to assess NUC-1031 in combination with carboplatin in patients with advanced OC [26].

Clinical trials with NUC-1031 in OC: PRO:002 and PRO:105

The annual incidence of sporadic OC in the UK is approximately 7500, with nearly 70% presenting at an advanced stage with peritoneal and distant metastases [27, 28]. The 5-year survival remains less than 25% for patients with advanced disease, with prognosis being governed by factors such as extent of residual disease following de-bulking surgery and sensitivity to platinum-based therapies, which form the mainstay of treatment [28]. A phase III trial in patients with relapsed OC has shown that carboplatin combined with gemcitabine results in a better PFS compared to single agent carboplatin [29]. Given this finding, along with the encouraging early signals for NUC-1031 shown in the PRO-001 study, the PRO-002 trial was designed to investigate the combination of NUC-1031 with carboplatin in patients with advanced, relapsed platinum-sensitive or resistant OC [26]. The primary objective of this trial was to determine the RP2D of NUC-1031 when given in combination with carboplatin, and the secondary objectives were to explore the safety profile and tolerability, pharmacokinetics and treatment responses (See Table 2).

Table 2 A summary of oncological clinical trials, to date, investigating the use of NUC-1031 in patients with various solid tumours

Trial details and Clinical trials.gov reference number	Recruitment status	Key inclusion criteria planned recruitment (*n*)	Primary objectives	Secondary objectives
NUC-1031 in patients with advanced solid tumours [24] PRO-001, *Phase I* *Single Centre: Wellcome Trust CRF, Imperial, London, United Kingdom* NCT01621854	Complete Oct 2012–June 2015	• All solid organ tumours (68)	• RP2D • Safety profile	• PK • Preliminary anti-tumour activity
Safety and efficacy study of NUC-1031 and carboplatin combination to treat patients with recurrent ovarian cancer [26] PRO-002, *Phase I* *Single Centre: Hammersmith Hospital, London, United Kingdom* NCT02303912	Complete Nov 2014–Jan 2017	• Recurrent ovarian cancer (25)	• RP2D of NUC-1031 and carboplatin combination	• ORR • PFS • PK • Clinical Benefit Rate (CR, PR, SD more than 12 weeks) • Safety profile
ABC 08: Phase Ib trial of Acelarin in combination with cisplatin in patients with locally advanced /metastatic biliary tract cancers [33] *Phase Ib* *National multicentre (United Kingdom)* NCT02351765	Complete Jan 2016–Mar 2019	• BTC (21)	• Safety profile of NUC-1031 in combination with cisplatin • Maximum Tolerated Dose	• ORR • PFS • OS • PK
Acelarin first-line randomised pancreatic cancer study [38] ACELARATE, *Phase III* *National multicentre (United Kingdom)* NCT03610100	On-hold [43] Dec 2015–Aug 2019	• PDAC (200)	• OS comparing NUC-1031 to gemcitabine monotherapy	Comparing NUC-1031 to gemcitabine monotherapy: • PFS • Radiological response and disease control rate • Quality of life questionnaires • Safety profile
NUC-1031 in patients with platinum-resistant ovarian cancer [30] *PRO-105, Phase II* *International multicentre (United States and United Kingdom)* NCT03146663	No longer recruiting Sept 2017–Dec 2019	• Platinum-resistant ovarian cancer (64)	• ORR (CR or PR)	• Change from baseline tumour size • PFS • OS • Safety profile

Table 2 (continued)

Trial details and Clinical trials.gov reference number	Recruitment status	Key inclusion criteria planned recruitment (*n*)	Primary objectives	Secondary objectives
A phase III open-label, multicentre, randomised study comparing NUC-1031 plus cisplatin to gemcitabine plus cisplatin in patients with previously untreated locally advanced or metastatic biliary tract cancer [35] NuTide: 121, *Phase III* *International multicentre (Canada, United States and United Kingdom)* NCT04163900	Recruiting	• BTC (828)	• OS • ORR	• PFS • Duration of response • Survival rates at 12 and 18 months • Safety profile • PK • Quality of life questionnaires

https://clinicaltrials.gov/. Accessed 10/04/2020

PDAC Pancreatic ductal adenocarcinoma, *BTC* biliary tract cancer, *RP2D* recommended phase II dose, *PK* pharmacokinetics, *ORR* objective response rate, *OS* overall survival, *PFS* progression free survival, *CR* complete response, *PR* partial response and *SD* stable disease

A total of 25 patients were recruited into PRO-002; all patients had prior exposure to platinum-based therapy and 10 had also received gemcitabine previously. Four dosing cohorts were assessed whereby NUC-1031 was given in combination with carboplatin (AUC 4 or 5) and was given in three weekly cycles with NUC-1031 (500, 625 and 750 mg/m^2) infusions given on day 1 and day 8 [26]. Preliminary results, presented at the European Society for Medical Oncology (ESMO) 2017 Congress, report one (4%) unconfirmed complete response (CR), 8 (35%) PRs to therapy and 13 (57%) patients with SD at least twelve weeks post commencement of treatment. Publication of final mature data are awaited [26].

Due to encouraging results from PRO-001 and the preliminary results from PRO-002 [24, 26], especially in the platinum-resistant population, the phase II PRO-105 trial was developed [30] (See Table 2). The phase II PRO-105 trial recruited patients with platinum-resistant OC, i.e. those whose cancers had progressed within 6 months of completing platinum-based chemotherapy. Eligible patients had received three or more previous lines of chemotherapy and patients were randomised to receive single agent NUC-1031 at 500 mg/m^2 or 750 mg/m^2 on days 1, 8 and 15 of a 28 day cycle with a primary aim to evaluate the objective response rate (ORR) for each dosage arm. Secondary objectives are to measure change in tumour size from baseline, duration of response, PFS and overall survival (OS), safety profile and pharmacokinetics in relation to clinical activity. This study enrolled 45 patients with platinum-resistant OC and all disease responses were assessed with confirmatory scans. Mature data from this study are awaited [31].

Clinical trials with NUC-1031 in BTC: ABC 08 and NuTide 121

Similar to OC, patients with BTC typically present at an advanced stage, and although 1 year survival rates have improved over the last three decades, the overall mortality remains high [32]. Since the ABC-02 clinical trial, cisplatin and gemcitabine combination is the first line established treatment for patients with inoperable and metastatic BTC [2] (see Table 1). The ABC-02 trial concluded a median OS benefit for patients with advanced BTC of 11.7 months using combination cisplatin and gemcitabine compared to 8.1 months with single agent gemcitabine. Combination chemotherapy in ABC-02 achieved a response rate of 26.1% and tumour control (complete or PR or SD) in 131 of 161 patients (81.4%).

In the PRO-001 study, using single agent NUC-1031, five of the six (83%) patients with BTC demonstrated SD on radiological imaging following completion of at least 8 weeks (2 cycles) of therapy. Gemcitabine is fundamental to the current

first-line palliative BTC chemotherapy regimen, and as such the phase I ABC-08 trial was devised to assess the safety and tolerability of NUC-1031 when given in combination with cisplatin in patients with advanced BTC (See Table 2) [33]. The interim results of this trial reported on 14 treatment-naïve patients with locally advanced or metastatic BTC of ECOG performance status 0–1. Similar to the PRO-002 study, NUC-1031 was given alongside the platinum compound in three weekly cycles, with NUC-1031 at doses of 625 mg/m^2 or 725 mg/m^2 and cisplatin at 25 mg/m^2 being administered on days 1 and 8. The aim of the ABC-08 trial was to determine the safety and the RP2D of NUC-1031 in combination with cisplatin, with secondary objectives to measure the ORR, PFS and OS and to undertake pharmacokinetic analyses.

Eleven patients completed more than one cycle of NUC-1031 (6 patients in the 625 mg/m^2 cohort and 5 in the 725 mg/m^2 cohort). The median follow up was 44 weeks (range 16–131 weeks) and the ORR in the intention to treat population was 50%. Grade III SAEs of neutropenia, fatigue, pyrexia and transaminitis were most common. Sustained high levels of the cytotoxic dFdCTP metabolite were observed in PBMCs with a $t_{1/2}$ of 22 h, in keeping with pharmacokinetic analyses of the PRO-002 trial [26]. In the interim analysis, there were no differences in response rate or pharmacokinetics between the two doses of NUC-1031, thus the 725 mg/m^2 dose of NUC-1031 was selected as the RP2D in combination with cisplatin in patients with advanced BTC for phase III evaluation in the first-line advanced setting, additionally allowing greater scope for dose reduction, if required [34].

Given the promising signals of ABC-08, the phase III NuTide 121 trial is recruiting globally using NUC-1031 in combination with cisplatin, given on days 1 and 8 in a 21 day schedule in patients newly diagnosed with advanced inoperable BTC (See Table 2) [34, 35]. This trial aims to recruit over 800 patients and randomise them to first-line combination chemotherapy with gemcitabine at 1000 mg/m^2 in the control arm or NUC-1031 at a dose of 725 mg/m^2 in the experimental arm, given in combination with cisplatin at 25 mg/m^2. The study was opened globally in the last quarter of 2019. The primary objective of this trial is to measure OS and ORR, with secondary objectives including measurement of PFS, duration of response, survival rates at 12 and 18 months, safety and pharmacokinetic profiles and patient-reported quality of life [35].

Clinical trial with NUC-1031 in PDAC: ACELARATE

A standard of care first-line palliative treatment for eligible patients with advanced PDAC is combination folinic acid, 5-fluorouracil, irinotecan and oxaliplatin (FOLFIRINOX) [36]. This triple-drug combination has resulted in a median OS of 11.1 months versus 6.8 months and an ORR of 31.6% versus 9.4%, when compared to single agent gemcitabine in patients with advanced PDAC in the first-line setting [36]. However, FOLFIRINOX is only suitable for patients with adequate physiological reserve of ECOG performance status 0–1 and with little other significant co-morbidities. As such, gemcitabine continues to have an important role as monotherapy [12] and in combination with nab-paclitaxel [1] or capecitabine [37] for patients with advanced PDAC, where more intense regimens are unsuitable (see Table 1). ACELARATE is a phase III randomised-controlled trial comparing single agent gemcitabine to NUC-1031 for metastatic PDAC in patients of ECOG performance status 0–2 for whom monotherapy is indicated, due to fitness issues or potential contra-indications to receiving other regimens [38]. The primary objective is to compare OS between the two treatment arms, with secondary objectives to compare PFS, radiological response, disease control rate, safety profiles and patient quality of life measures (See Table 2).

The trial planned to enrol 328 patients globally and to date 200 patients have been recruited. Recruitment to ACELARATE is currently on-hold to allow data to mature following a futility analysis as announced via a press release in August 2019 [33]. Data will be allowed to mature and biomarker analyses are pending to determine which subgroups derive the greatest benefit.

Discussion

Phase I trials involving NUC-1031 have reported promising results with adequate tolerability profiles and encouraging treatment responses primarily in patients with OC and BTC [24, 26, 33]. Both PRO-001 and PRO-002 studies selected patients with advanced solid tumours who had exhausted therapy options, of which many had received previous gemcitabine therapy [24, 26]. Achieving PR and SD in this therapeutically challenging cohort of patients would suggest that NUC-1031 delivers cytotoxic effects in the context of prior gemcitabine resistance possibly due to NUC-1031 circumventing the fundamental resistance mechanisms that cancers can develop to gemcitabine. NUC-1031 may also have superior cytotoxic capabilities to gemcitabine, given the significantly greater half-life reported in current trials [24, 26, 33], ranging from 8 to 24 h, compared to a maximum of 80 min with gemcitabine in other studies [39]. Less CDA-mediated enzymatic breakdown of NUC-1031 and greater delivery of the drug across the cell would also enable higher intracellular and durable levels of dFdCTP for cytotoxic activity.

Genome sequencing of cancer cells has been utilised to identify genes involved in drug resistance and sensitivity to chemotherapeutic agents [40, 41]. To further investigate the

cytotoxicity of NUC-1031 versus gemcitabine, NUC-1031 has been studied using CRISPR/Cas9 genome sequencing technology and it was found that low dCK expression in the tumour biopsies from patients treated with gemcitabine correlates with a poor prognosis, but the level of dCK expression was non-prognostic in tumour biopsies from patients treated with NUC-1031 in the PRO-001 study [42]. This may suggest cytotoxic activity with NUC-1031 occurs irrespective of degree of dCK expression, and this may help to explain therapeutic responses observed with NUC-1031 in selected patients who had previously progressed on gemcitabine.

Therapeutic responses currently reported with NUC-1031 are from patients within the phase I trials and the current understanding of efficacy has been deduced from a small number of patients with advanced disease. Recruitment to ACELARATE, the phase III trial comparing NUC-1031 to gemcitabine in the first-line setting in patients with advanced PDAC has been held to allow data to mature following a futility analysis [43]. Interestingly, in the original PRO-001 study, only 2 of the 9 patients with advanced PDAC had efficacy evaluable disease, where one patient progressed and the other had SD. The majority, 7 of 9 (78%) did not receive more than 2 doses of NUC-1031 and this highlights one of the greatest hurdles in treating patients with advanced PDAC in particular; disease related complications such as inadequate biliary drainage and resultant infections, secondary diabetes and pancreatic exocrine insufficiency leading to malnutrition are extremely common in these patients, in addition to rapid deterioration of performance status, and this often leads to the halting administration of further chemotherapy [44, 45]. Results of the interim analysis of ACELARATE may highlight challenges exclusive to advanced PDAC treatment, but whether superiority of efficacy exists remains to be seen. It may be possible that a subgroup that derives specific benefit can be identified. It may also be possible that some cancer cells develop other mechanisms of resistance that can potentially overcome the cytotoxic properties of NUC-1031.

It is widely accepted that tumours contain a heterogeneous population of cells, therefore resistance may develop. Cancer stem cells are hypothesised to be a clonal subset of cells which are slow growing, have an invasive phenotype and are crucially resistant to most chemoradiotherapies [46]. Jia et al. report that embryonic cell signalling pathways, namely wnt, notch and hedgehog, are reactivated in gemcitabine-resistant cells, leading to an epithelial to mesenchymal transformation towards resistant cancer stem cells [47]. Whether NUC-1031 targets these more resistant cancer cells remains to be seen and effective novel compounds will need to address this phenomenon. The tumour microenvironment has also been implicated in chemotherapy resistance by providing a cellular barrier to drug delivery to cancer cells, as well as producing anti-apoptosis inhibitory signals, preventing cancer cell death [48]. Translational analysis from the ACELARATE clinical trial may answer some of these unknowns and will be important in gaining a greater understanding of its potential application in PDAC.

The current standard of care uses of gemcitabine and experimental use of NUC-1031 in clinical trials in a number of disease groups are summarised in Table 1. Gemcitabine in combination with cisplatin is currently being investigated as an adjuvant therapy for BTC [49, 50], whilst in PDAC, the European Study group for Pancreatic Cancer (ESPAC) phase III [51] and IV [52] trials have established gemcitabine or gemcitabine and capecitabine combination in fitter patients, as standard of care adjuvant therapy, where modified FOLFIRINOX is not an appropriate treatment option for patients [53]. The investigational use of NUC-1031 is currently confined to the advanced cancer setting, and it remains to be seen if better survival outcomes are achieved in larger prospective patient cohorts, highlighting the importance of ongoing trials. If encouraging results are seen in these studies, then there may be the opportunity to consider prospective trials with NUC-1031 in the adjuvant setting for BTC, at least [34].

In current practice, there are established indications for tyrosine kinase inhibitors, immunotherapies and mutation-targeted therapies as cancer treatments [54]. Whilst some of these therapies, such as immunotherapy in metastatic melanoma, have revolutionised the outlook for many patients [55], outcomes following these therapies in OC, BTC and PDAC have been poor [4–6]. In cancers without potentially targetable mutations [11, 56–58], chemotherapy, to date, remains the best treatment option but it is limited by off-target toxicity and treatment-resistance. There is thus an unmet need in these disease groups to improve existing therapeutic options. Use of the ProTide technology to create NUC-1031 offers the potential to avoid known resistance mechanisms that are associated with traditional gemcitabine therapy. The optimal schedules and combinations are yet to be confirmed.

Despite the current challenges, NUC-1031 has shown potential in overcoming the common resistance mechanisms that hinder the cytotoxicity of gemcitabine. Crucially, phase I trials have demonstrated the drug to be well tolerated with few grade III and IV toxicities. Mature data from ongoing trials and prospective exploration of NUC-1031 as a direct comparator to gemcitabine will be informative.

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Compliance with ethical standards

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