Anti-tumour activity of a first-in-class agent NUC-1031 in patients with advanced cancer: results of a phase I study

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BACKGROUND: Gemcitabine is used to treat a wide range of tumours, but its efficacy is limited by cancer cell resistance mechanisms. NUC-1031, a phosphoramidate modification of gemcitabine, is the first anti-cancer ProTide to enter the clinic and is designed to overcome these key resistance mechanisms.

METHODS: Sixty-eight patients with advanced solid tumours who had relapsed after treatment with standard therapy were recruited to a dose escalation study to determine the recommended Phase II dose (RP2D) and assess the safety of NUC-1031. Pharmacokinetics and anti-tumour activity was also assessed.

RESULTS: Sixty-eight patients received treatment, 50% of whom had prior exposure to gemcitabine. NUC-1031 was well tolerated with the most common Grade 3/4 adverse events of neutropaenia, lymphopaenia and fatigue occurring in 13 patients each (19%). In 49 response-evaluable patients, 5 (10%) achieved a partial response and 33 (67%) had stable disease, resulting in a 78% disease control rate. Cmax levels of the active intracellular metabolite, dFdCTP, were 217-times greater than those reported for equimolar doses of gemcitabine, with minimal toxic metabolite accumulation. The RP2D was determined as 825 mg/m² on days 1, 8 and 15 of a 28-day cycle.

CONCLUSIONS: NUC-1031 was well tolerated and demonstrated clinically significant anti-tumour activity, even in patients with prior gemcitabine exposure and in cancers not traditionally perceived as gemcitabine-responsive.

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However, NUC-1031 (Supplementary Fig. 1) is the first anti-cancer ProTide to enter the clinic. The purpose of this first-in-human study was to assess its safety and anti-tumour activity in patients with advanced solid tumours and define the recommended Phase II dose (RP2D). In addition, pharmacokinetic (PK) analyses were conducted to provide mechanistic proof-of-concept for this first-in-class compound.

**PATIENTS AND METHODS**

**Patient eligibility**

Patients ≥18 years of age with a diagnosis of cancer refractory (or not amenable) to standard therapy were eligible for the study. Other inclusion criteria included a life expectancy of at least 12 weeks, Eastern Cooperative Oncology Group (ECOG) performance status 15 of 0–2 and the following haematological and biochemical parameters: adequate bone marrow (leukocytes ≥ 3 x 10⁹/L, neutrophils ≥ 1.5 x 10⁹/L, platelets ≥ 100 x 10⁹/L, haemoglobin ≥ 9 g/dL), liver function (total bilirubin ≤ 1.5 x upper limit of normal [ULN]), aspartate amino transferase (AST)/alanine amino transferase (ALT) ≤ 2.5 x ULN (or ≤ 5 x ULN if liver metastases) and renal function (serum creatinine ≤ 1.5 x ULN).

All patients provided written informed consent. The study was performed in accordance with Good Clinical Practice guidelines and the principles of the 1964 Declaration of Helsinki and subsequent revisions.

Study design

This was a two-part, open-label, Phase I, dose escalation and expansion study. In Part 1 (dose-escalation), two administration schedules of NUC-1031 were assessed over a 28-day cycle: Schedule A—weekly (days 1, 8 and 15), and Schedule B—twice weekly (days 1, 5, 8, 12, 15 and 19). Six patients were recruited to Schedule B Cohort 3 and received NUC-1031 at 375 mg/m² twice weekly; however, it became apparent that drug administration on Schedule B was logistically challenging for both the patients and the trial site, and further study of Schedule B was discontinued. Thereafter, NUC-1031 was only administered on days 1, 8 and 15 every 28 days. Patients received treatment for six cycles unless there was evidence of clinical or radiological progression, unacceptable toxicity or if they declined further treatment. Patients could receive NUC-1031 beyond Cycle 6 on a compassionate basis. Assessment of toxicity was used to establish the RP2D for the Part 2 expansion cohort.

The primary endpoints were safety and determination of RP2D. Safety was assessed by adverse event profile and changes from baseline in vital signs, clinical laboratory parameters and electrocardiogram (ECG). Secondary endpoints included PK and evaluation of anti-tumour activity. A dose-limiting toxicity (DLT) was defined as one or more events, judged related to NUC-1031, occurring through to the last scheduled day of Cycle 1. These included: Grade ≥3 toxicity (except nausea/vomiting/diarrhoea in the absence of effective therapy), Grade ≥3 nausea/vomiting/diarrhoea that occurs despite standard medical treatment, Grade 4
neutropaenia or thrombocytopenia, febrile neutropaenia, or inability to begin next dose of treatment within 14 days of scheduled dosing due to unresolved toxicity relating to NUC-1031. The study protocol was approved by the Medicines and Healthcare products Regulatory Agency, the West London Research Ethics Committee 12/LO/1100 and local review boards. The study was registered on clinicaltrials.gov (NCT01621854).

Patient evaluation
All patients receiving at least one dose of NUC-1031 were evaluable for toxicity. Only patients who had measurable disease and had received at least two cycles of treatment were considered response-evaluable. Patients who did not complete the first cycle of treatment for reasons other than DLTs were not evaluable for the maximum tolerated dose (MTD) and were replaced. Adverse event data were collected from the start of study drug administration until 30 days after the last dose was administered and graded using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.02. Laboratory safety assessments and vital signs were performed on dosing days from baseline to end of study visit.

Tumours were assessed by computed tomography (CT) or magnetic resonance imaging (MRI) scan after Cycles 2, 4 and 6 and compared with those conducted at study entry using Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria. Efficacy was determined in all patients with measurable disease at baseline and who completed at least two cycles of NUC-1031 and had at least one follow-up radiographic assessment to measure changes in tumour size. The best overall response was defined as best response on at least one time point; results were displayed in a waterfall plot. Progression-free survival (PFS) was measured from first administration of NUC-1031 to disease progression or death (whichever occurred first). Duration of response was calculated from date of first response until RECIST or symptomatic disease progression.

Dose modifications and delays
Treatment interruptions of up to 14 days were permitted in order for participants to meet the re-treatment criteria before starting their next cycle.

Pharmacokinetic analysis
Blood samples were collected for PK analysis during Cycle 1 on days 1 and 15 pre-dose and at numerous time points after the end of infusion (0.217, 0.3, 0.467, 0.633, 1, 1.5, 2, 4, 6 and 24 h). Blood (6 mL) was collected using heparinised blood collection tubes spiked with tetrahydroxuridine (25 μg/mL) to inhibit CDA activity. Plasma was separated by centrifugation and peripheral blood mononuclear cells (PBMCs) were separated using Lymphoprep density gradient (STEMCELL Technologies UK Ltd). PBMCs were extracted and assayed for dFdCMP, dFdCDP and dFdCTP using a previously described liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Plasma and urine samples were assayed for NUC-1031, dFdC and dFdU. (See Supplementary Methods for detailed protocol.)

Statistical analysis
Sample size calculations were based on a Fleming design. No formal statistical analyses were planned or performed on safety, PK or efficacy data.

RESULTS
Patient characteristics
A total of 68 patients with advanced solid malignancies were enrolled into the study between 5 October 2012 and 24 June 2015 (Fig. 2) and were evaluable for safety analysis. Forty-six were female and 22 were male, patients had a mean age of 56 years (range 20–83 years; Table 1). Overall, there were 19 primary cancer types, the most frequent being ovarian (n = 10, 15%), pancreas (n = 9, 13%), biliary (n = 7, 10%) and colorectal (n = 7, 10%). Patients

![Fig. 2 CONSORT flow chart. Sixty-eight patients with advanced solid malignancies were enrolled into the study and were safety-evaluable. In Schedule A, patients received doses of 500 mg/m², 625 mg/m², 675 mg/m², 725 mg/m², 750 mg/m², 825 mg/m², 900 mg/m² and 1000 mg/m² (all given once-weekly). In Schedule B, patients received a dose of 375 mg/m² twice weekly. The most common reasons for withdrawal were progressive disease (25 patients) and Investigator/patient choice (15 patients). Overall, 14 patients completed the study (received ≥6 cycles)
The dose levels studied, the number of patients treated and AEs are summarised in Table 2. During schedule A, the following doses of NUC-1031 were administered: 500 mg/m², 625 mg/m², 675 mg/m², 725 mg/m², 750 mg/m², 825 mg/m², 900 mg/m² and 1000 mg/m² (all given once-weekly, for 3 weeks out of every 4-week cycle). The dose of 825 mg/m² (schedule A) was selected for the expansion (Part 2) of the study, and 12 patients were recruited at this dose, in addition to the four who received this dose in Part 1.

The dose intensity (DI; the proportion of the scheduled NUC-1031 dose that was successfully administered to the patient within the first 8 weeks of study) was measured for each cohort and the results are shown in Supplementary Table 1. In dose cohorts up to and including 825 mg/m² it was possible, on average, to administer >70% of the calculated total dose but the percentage fell as the dose increased above 825 mg/m². At 825 mg/m², 58% of patients achieved 100% DI and 75% of patients achieved ≥75% DI. When the actual DI was at or close to the targeted DI there was a high proportion of disease control as shown in Supplementary Table 2.

Safety and tolerability
NUC-1031 was administered as a short (10–15 min) intravenous infusion via central venous access devices. Forty-four of the 68 patients experienced 94 treatment-emergent serious adverse events (SAEs; Table 2), 27 of which were Grade 3/4 and judged to have a definite, probable or possible relationship to NUC-1031. Six (22%) of the Grade 3/4 related SAEs to be reported by more than one patient were increased alanine amino transferase (n = 4), pyrexia (n = 3), thrombocytopenia (n = 3), hypoxia (n = 2), lung infection (n = 2) and neutropenia (n = 2). Fatigue and transaminitis (56 patients each, 82.4%) were the most frequently reported adverse events, irrespective of grade or relationship. Neutropenia, lymphopaenia and fatigue were the most frequently reported Grade 3/4 adverse events (13 patients each, 19.1%) (Supplementary Table 3).

Six patients died during the study, events that were judged to be unrelated or unlikely to be related to NUC-1031. Of note, 6 (12.3%) patients had pulmonary embolism, 5 of which were incidental radiological findings. Of the sixty-eight patients, 17 (25%) received a complete six-cycle course of study treatment. Fourteen patients opted to continue on NUC-1031 beyond Cycle 6, and received up to 13 additional treatment cycles (Fig. 2).

Pharmacokinetics
Sixty-six patients had evaluable PK samples. As this was a Phase I study with ascending dose schedules and variability in the plasma concentration–time profiles there was some imprecision in AUCC0–inf and t1/2 estimates. The most robust comparisons can be made using AUCC0–21 and Cmax. Nevertheless, after administration, NUC-1031 was detected in the plasma up to 24 h from End Of Infusion (EOI) with an estimated t1/2 of 8.3 h, which is considerably longer than the reported t1/2 of gemcitabine (2.3–80 min) and the reported Cmax of gemcitabine (0.18–1.6 g/L).

Among the analytes evaluated, median plasma AUCC0–21 and Cmax estimates on day 1 were highest for NUC-1031 (269 µM/h and 710 µM, respectively), intermediate for dFdU (76.0 µM/h and 5.11 µM, respectively) and lowest for dFdC (2.92 µM/h and 1.82 µM, respectively).

Of note, there were no trends for increased exposures on Cycle 1, day 15 indicating that systematic accumulation of NUC-1031, dFdC or dFdU concentrations does not occur with either once- or twice-weekly dosing of NUC-1031. Plasma PK data are summarised in Supplementary Table 4. Dose proportionality was only observed...
for NUC-1031 on Cycle 1, day 1. The lack of dose proportionality is not unexpected given that the study was not designed to show dose proportionality and most of the dose levels contained less than 10 patients.

Intracellular concentrations of the active anti-cancer moiety dFdCTP remained high throughout the 24-h PK sampling window (Fig. 3). Mean intracellular dFdCTP AUC<sub>0-t</sub> and C<sub>max</sub> estimates in patients PBMCs on day 1 normalised to a dose of 500 mg/m<sup>2</sup> were 151,413 µM/h and 26,910 µM, respectively (Supplementary Table 5).

Analysis of urine samples from 46 patients demonstrated that 21.7 and 27.3% of the NUC-1031 was excreted via the urine as dFdU over the 24 h after the dose on days 1 and 15, respectively. Overall, less than 1% of the dose was excreted as either NUC-1031 or dFdC, suggesting that the ProTide is stable during plasma transport to the tumour cells.

**Table 2.** Summary of adverse event grades and types

| NUC-1031 dose (mg/m<sup>2</sup>) | 500 (4) | 625 (3) | 675 (3) | 725 (6) | 2 x 375<sup>a</sup> (6) | 750 (8) | 825 (16) | 900 (15) | 1000 (7) |
|---------------------------------|--------|--------|--------|--------|------------------------|--------|--------|--------|--------|
| n in dose cohort (N = 68)       | 6 (100)| 6 (100)| 6 (100)| 6 (100)| 8 (100)                | 16 (100)| 15 (100)| 15 (100)| 7 (100) |
| SAEs                            | 2 (50) | 2 (66.7)| 1 (33.3)| 6 (100)| 4 (67)               | 6 (75) | 8 (50) | 10 (66.6)| 5 (71.4)|
| AEs (all Grades)                | 4 (100)| 3 (100)| 3 (100)| 6 (100)| 6 (100)                | 8 (100) | 16 (100)| 15 (100)| 7 (100) |
| AE(s) (Grade 3/4)               | 3 (75) | 2 (66.7)| 2 (66.7)| 6 (100)| 6 (100)                | 8 (100) | 14 (87.5)| 12 (80.0)| 7 (100) |
| Withdrawn due to AE             | 1 (25) | 1 (33.3)| 0       | 1 (16.7)| 1 (16.7)              | 1 (12.5)| 6 (37.5)| 3 (20.0)| 1 (14.3)|
| Chemistry AE<sup>b</sup> Grade 3| 1 (25) | 2 (66.7)| 1 (33.3)| 3 (50)  | 3 (50)                 | 5 (62.5)| 7 (43.8)| 8 (53.3)| 4 (57.1) |
| Chemistry AE<sup>c</sup> Grade 4| 0       | 1 (33.3)| 0       | 0       | 0                     | 0       | 0       | 0       | 0       |
| Haematological AE<sup>d</sup> Grade 3| 1 (25) | 1 (33.3)| 0       | 4 (66.7)| 4 (66.7)              | 6 (75) | 5 (31.3)| 8 (53.3)| 3 (42.9)|
| Haematological AE<sup>e</sup> Grade 4| 0       | 0       | 0       | 0       | 2 (33.3)              | 2 (25) | 1 (6.3)| 0       | 1 (14.3)|

<sup>a</sup>375 mg/m<sup>2</sup> was administrated twice-weekly in 6 patients (Schedule B)

<sup>b</sup>Chemistry AEs included: ALT increased, hypoaalbuminaemia, blood bilirubin increased, hypokalaemia, hyponatraemia, hypophosphataemia, blood urea increased, hypomagnesaemia, blood creatinine increased, hyperglycaemia, blood albumin decreased, AST increased, blood alkaline phosphatase increased, blood glucose increased, blood phosphorous decreased, hypocalcaemia, hyperkalaemia

<sup>c</sup>Haematological AEs included: neutropaenia, thrombocytopaenia, white blood cell count decreased, platelet count decreased, neutrophil count decreased, anaemia, lymphocyte count decreased, leukopaenia

Efficacy

Of the 68 patients participating in the study, 19 (8 males:11 females; average age 56 years; range 35–73) were not evaluable for efficacy of NUC-1031; two patients died of causes unrelated to study drug, four patients developed progressive disease before completing two cycles, and the remainder withdrew from the study for a variety of reasons (see Fig. 2). The average number of treatment cycles with NUC-1031 in these non-evaluable patients was 0.8 cycles (range 0.3–1.6 cycles).

The 49 patients who received ≥2 cycles of NUC-1031 and had a scan for assessment of efficacy received an average of 4.8 cycles (range 2–19 cycles) with a median PFS of 4.0 months (range 1–25 months). Eleven patients had progressive disease and the best overall responses in the evaluable patients were 5 partial responses (PRs) (10%) and 33 stable diseases (SDs) (67%; Table 3). Of the 33 SDs, 12 (24%) were of at least 6 months duration. Five
patients with primary cancers of the cervix, lung, fallopian tube, pancreas and unknown primary achieved PRs (Table 3; Fig. 4). Two of these five patients had subsequent scans confirming their partial response. Of 16 patients who had previously progressed on or following a gemcitabine-containing regimen, one patient achieved a PR and nine patients had SD.

**DISCUSSION**

We report results from the first-in-human study of NUC-1031 belonging to a new class of anti-cancer agents called ProTides that are designed to improve the efficacy and safety profile of conventional nucleoside analogues. NUC-1031, a chemical modification of gemcitabine, is the first anti-cancer ProTide to enter the clinic. In this Phase I setting of heavily pre-treated patients with advanced solid tumours, NUC-1031 achieved good disease control with an acceptable safety profile. There were no unexpected adverse events and the most common adverse reactions were similar to those observed with gemcitabine and included reversible myelosuppression, gastrointestinal disturbances, fatigue and elevations in liver function enzymes. At or below the RP2D of 825 mg/m², NUC-1031 could be administered at high dose intensity, which corresponded with a more favourable clinical outcome. Ten percent of evaluable patients achieved responses of PR, and SD was observed in a further 67%, resulting in an overall Disease Control Rate of 78%. Responses were durable, the median PFS was 4 months (censored). Notably, responses were observed amongst patients whose tumours had progressed during or following prior gemcitabine therapy. This is in line with pre-clinical data that...
demonstrated NUC-1031 has activity in the context of gemcitabine resistance.

NUC-1031 has a more favourable PK profile than gemcitabine. PK data confirmed higher intracellular concentrations of the active anti-cancer metabolite dFdCTP (Supplementary Table 5), with 217- and 139-fold-higher respective C\textsubscript{max} and AUC\textsubscript{0-\textinfty}, than have been reported with gemcitabine at equimolar doses.\textsuperscript{18–21,23,24} Whilst these results cannot be directly translated from PBMCs to tumour cells they do suggest that, following NUC-1031 administration, tumour cells are exposed to considerably higher levels of the active metabolite dFdCTP than have been observed following gemcitabine. Furthermore, it was noted that NUC-1031 administration resulted in prolonged high intracellular concentrations of dFdCTP throughout the subsequent 24-h period than are observed at the C\textsubscript{max} 2-h time point after gemcitabine administration. The estimated NUC-1031 plasma t\textsubscript{1/2} of 8.3 h compared favourably with the (shorter) reported gemcitabine plasma t\textsubscript{1/2} of 2.3–80 min and confirms the insensitivity of NUC-1031 to CDA-mediated plasma deamination.\textsuperscript{18–21} The longer half-life of NUC-1031 therefore could enable tumour cells to receive a more prolonged exposure to dFdCTP which may enhance its activity, paralleling reports that the anti-tumour activity of gemcitabine is improved using longer infusion times (up to 24 h).\textsuperscript{25} Furthermore, following exposure to NUC-1031, there were considerably lower plasma and intracellular levels of the metabolite dFdU than have been reported with gemcitabine, which may explain the acceptable toxicity profile observed with NUC-1031.

Maintaining the dose intensity is an important factor in achieving optimum benefit for any anti-cancer agent.\textsuperscript{26,27} Patients who received NUC-1031 at a dose at or below 825 mg/m\textsuperscript{2} were generally able to maintain treatment intensity (i.e. receive subsequent infusions when planned); doses of 900 mg/m\textsuperscript{2} and above required more substantial dose optimisation and scheduling due to cumulative fatigue and myelosuppression. Notably, four of the five partial responses occurred in patients who received a doses at or below 825 mg/m\textsuperscript{2}. A limitation of the study was the inability to fully assess PFS because the initial study design did not include any post-treatment follow-up and was censored at 6 months. Thus, the PFS data reported here might underestimate survival.

In conclusion, NUC-1031 is the first of a group of protected nucleotide analogues designed to counter the key cancer resistance mechanisms associated with gemcitabine therapy. NUC-1031 achieved clinical activity across multiple tumour types, even in cancers that are not traditionally perceived as gemcitabine-responsive. This, along with observed activity in patients who were previously exposed to gemcitabine, indicates a broad spectrum of activity for NUC-1031 which is likely to be attributable to its ability to bypass cellular resistance mechanisms. NUC-1031 was well tolerated with no unexpected adverse events and an acceptable safety profile that allowed prolonged administration for many months (up to 20 months in one patient). Although the dose of 825 mg/m\textsuperscript{2} once-weekly for 3 weeks in a 4-weekly cycle was selected for further evaluation as monotherapy, it was noted that doses of 500 mg/m\textsuperscript{2} and higher also showed evidence of clinical activity and warrant further study. Clinical studies of NUC-1031, either as monotherapy or in combination with other agents, are currently ongoing in patients with ovarian, biliary and pancreatic cancers.

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AUTHOR CONTRIBUTIONS
Conception and design: S.P.B., A.R., H.G., H.W., N.H., E.G. Acquisition of data: S.P.B., I.R., P.S., D’O.S., M.P., L.S., A.S., N.B., A.R., M.E.-B. Analysis and interpretation of data: S.P.B., A.S., E.G. Writing, review and/or revision of the manuscript: S.P.B., I.R., P.S., D’O.S., M.P., L.S., A.S., N.B., H.G., M.E.-B., H.W., R.L., N.H., E.G.

ADDITIONAL INFORMATION
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Competing interests: For the duration of this study, E.G. was employed by Queen Mary’s University, London, and received research funding from NuCana plc to conduct the pharmacokinetic assessments described in this manuscript. Following this study, I.R. received research funding from NuCana plc. The other authors declare no competing interests.

Consent for publication: This manuscript does not contain any individual patient’s data or identifiable information

Ethics approval and consent to participate: Prior to participation in the study, all patients signed an informed consent document approved by the West London Research Ethics Committee (reference 12/LO/1100). The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

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Availability of data and materials: The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES
1. Sebastiani, V. et al. Immunohistochemical and genetic evaluation of deoxy-cytidine kinase in pancreatic cancer: relationship to molecular mechanisms of gemcitabine resistance and survival. *Clin. Cancer Res.*, 12, 2492–2497 (2006).
2. Mini, E., Nobilli, S., Caciagli, B., Landini, I. & Mazzei, T. Cellular pharmacology of gemcitabine. *Ann. Oncol. Suppl* 5, v7–v12 (2006).
3. Conway, T. et al. FOLIRINOX versus gemcitabine for metastatic pancreatic cancer. *N. Engl. J. Med.*, 364, 1817–1825 (2011).
4. Kim, M. P. & Gallick, G. E. Gemcitabine resistance in pancreatic cancer: picking the key players. *Clin. Cancer Res.*, 14, 1284–1285 (2008).
5. de Sousa Cavalcante, L. & Monteiro, G. Gemcitabine: metabolism and molecular mechanisms of action, sensitivity and chemoresistance in pancreatic cancer. *Eur. J. Pharmacol.*, 741, 8–16 (2014).
6. Spratlin, J. et al. The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. *Clin. Cancer Res.*, 10, 6956–6961 (2004).
7. Bergman, A. M., Pinedo, H. M. & Peters, G. J. Determinants of resistance to 2,2’-difluorodeoxycytidine (gemcitabine). *Drug Resist. Updat.* 5, 19–33 (2002).
8. Pereira, S., Fernandes, P. A. & Ramos, M. J. Mechanism for ribonucleotide reductase inactivation by the anticancer drug gemcitabine. *J. Comput. Chem.*, 25, 1286–1294 (2004).
9. Poplin, E. et al. Randomized, multicenter, phase II study of CO-101 versus gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma: including a prospective evaluation of the role of FENIT in gemcitabine or CO-101 sensitivity. *J. Clin. Oncol.*, 31, 4453–4461 (2013).
10. Favre, S. J. et al. Phase I dose escalation and pharmacokinetic evaluation of two different schedules of LY2334737, an oral gemcitabine prodrug, in patients with advanced solid tumours. *Invest. New Drugs* 33, 1206–1216 (2015).
11. Venugopal, B. et al. A first-in-human phase I and pharmacokinetic study of CP-4126 (CD-101), a nucleoside analogue, in patients with advanced solid tumours. *Cancer Chemother. Pharmacol.*, 76, 785–792 (2015).
12. McGuigan, C., Shackleton, J. M., Tollerfeld, S. M. & Riley, P. A. Synthesis and evaluation of some novel phosphate and phosphinate derivatives of araA. Studies on the mechanism of action of phosphate triesters. *Nucleic Acids Res.*, 17, 10171–10177 (1989).
13. Saif, M. W., Lee, Y. & Kim, R. Harnessing gemcitabine metabolism: a step towards personalized medicine for pancreatic cancer. *Ther. Adv. Med. Oncol.* 4, 341–346 (2012).
14. Slusarczyk, M. H. et al. Application of ProTide technology to gemcitabine: a successful approach to overcome the key cancer resistance mechanisms leads to a new agent (NUC-1031) in clinical development. *J. Med. Chem.* 57, 1531–1542 (2014).
15. Oken, M. et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am. J. Clin. Oncol.* 5, 649–655 (1982).
16. Veltkamp, S. A. et al. Quantitative analysis of gemcitabine triphosphate in human peripheral blood mononuclear cells using weak anion-exchange liquid chromatography couple with tandem mass spectrometry. *J. Mass Spec.* 41, 1633–1642 (2006).
17. Fleming TR. One sample multiple testing procedure for phase II clinical trials. *Biometrics.* 38, 143–151 (1982).
18. Abbruzzese, J. L. et al. A phase I clinical, plasma, and cellular pharmacology study of gemcitabine. *J. Clin. Oncol.* 9, 491–498 (1991).
19. Grunewald, R. et al. Gemcitabine in leukemia: a phase I clinical, plasma, and cellular pharmacology study. *J. Clin. Oncol.* 10, 406–413 (1992).
20. Cattel, L. et al. Pharmacokinetic evaluation of gemcitabine and 2',2'-difluorodeoxycytidine-5'-triphosphate after prolonged infusion in patients affected by different solid tumors. *Ann. Oncol.* 17(Suppl 5), v142–v147 (2006).
21. Peters, G. J. et al. Clinical phase I and pharmacology study of gemcitabine (2',2'-difluorodeoxycytidine) administered in a two-weekly schedule. *J. Chemother.* 19, 212–221 (2007).
22. Eli Lilly and Company. Prescribing Information. 2014. [http://pi.lilly.com/us/gemzar.pdf](http://pi.lilly.com/us/gemzar.pdf). Accessed: 09 March 2018.
23. Grunewald, R. et al. Pharmacologically directed design of the dose rate and schedule of 2',2'-difluorodeoxycytidine (Gemcitabine) administration in leukemia. *Cancer Res.* 50, 6823–6826 (1990).
24. Rizzuto, I., Ghazaly, E. & Peters, G. J. Pharmacological factors affecting accumulation of gemcitabine’s active metabolite, gemcitabine triphosphate. *Pharmaco-genomics* 18, 911–925 (2017).
25. Veltkamp, S. A., Beijnen, J. H. & Schellens, J. H. Prolonged versus standard gemcitabine infusion: translation of molecular pharmacology to new treatment strategy. *Oncologist* 13, 261–276 (2008).
26. Hryniuk, W., Frei, E. & Wright, F. A. A single scale for comparing dose-intensity of all chemotherapy regimens in breast cancer: summation dose-intensity. *J. Clin. Oncol.* 16, 3137–3147 (1998).
27. Lyman, G. H., Dale, D. C. & Crawford, J. Incidence and predictors of low dose-intensity in adjuvant breast cancer chemotherapy: a nationwide study of community practices. *J. Clin. Oncol.* 21, 4524–4531 (2003).

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