NUC-1031 Causes incorporation of fluorinated deoxycytidine into DNA, inducing persistent damage in biliary tract cancer cells

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BACKGROUND

- Gemcitabine remains the backbone of therapy for a broad range of tumors including biliary tract, pancreatic, ovarian, non-small cell lung, bladder and breast cancers
- Gemcitabine activity is dependent on conversion to the active anti-cancer metabolite, dFdCTP which disrupts DNA synthesis[1-3]
- Three key cancer resistance mechanisms have been associated with poor survival in patients receiving gemcitabine
  - Poor uptake
  - Low activation
  - Increased degradation

NUC-1031: The first anti-cancer ProTide

- ProTide transformation of gemcitabine
- Overcomes key gemcitabine resistance mechanisms[4]
  - Cellular uptake independent of nucleoside transporters (hENT1)
  - Activation independent of deoxycytidine kinase (dCK)
  - Protected from breakdown by cytidine deaminase (CDA)
- In comparison to gemcitabine, NUC-1031 has[5]:
  - Greater plasma stability ($T_{1/2}$ 8.3 hours vs 1.5 hours)
  - Increased intracellular levels of active anti-cancer metabolite dFdCTP (217x)
- NUC-1031 in combination with cisplatin is currently being investigated in a Phase III clinical study for the treatment of patients with advanced biliary tract cancer (NuTide:121)[6]

Aim

Investigate the intracellular activation of NUC-1031 and subsequent incorporation of active metabolites into DNA in biliary tract cancer (BTC) cells

METHODS

- Human intrahepatic cholangiocarcinoma HuCCT1 cells were treated with IC_{50} (1 µM) and sub-IC_{50} (0.5 µM) doses of NUC-1031 for 24 hours
- Cells were sampled at 24-hour intervals in drug-free media, over a time course of 96 hours post NUC-1031 exposure
- Intracellular levels of dFdCTP were measured by mass spectrometry (LC-MS/MS)
- Incorporation of dFdCTP into DNA was quantified by relative dFdC:dG signal (indicative of activated NUC-1031), measured by LC-MS/MS
- Cell cycle analysis was performed using propidium iodide (PI) staining and flow cytometry
- DNA double strand breaks were assessed by histone H2AX phosphorylation (γH2AX) using flow cytometry

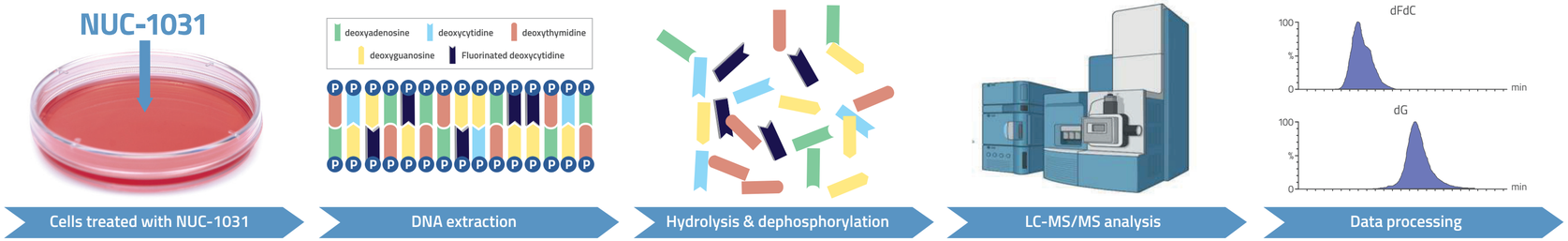


Figure.1 Extracted DNA from cells was hydrolyzed and dephosphorylated to nucleosides. dFdC incorporation into DNA was expressed as a ratio of dFdC:dG as measured by LC-MS/MS

RESULTS

NUC-1031 is converted to the active triphosphate metabolite (dFdCTP)

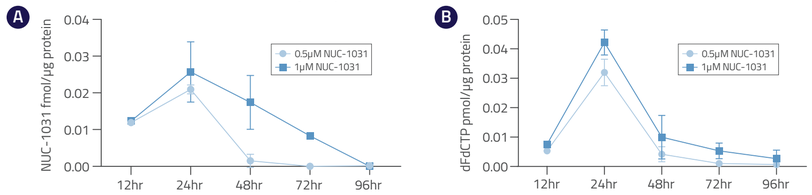


Figure 2: (A) Time course for intracellular NUC-1031 and (B) dFdCTP levels in response to NUC-1031 (n=3)

- NUC-1031 is converted to active metabolite (dFdCTP) with peak levels observed at 24 hours, before media replacement
- Cellular uptake of NUC-1031 and intracellular conversion to dFdCTP increases with dose

NUC-1031 results in sustained incorporation of dFdCTP into DNA

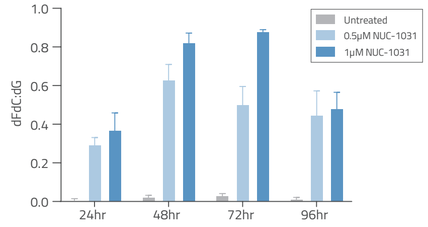
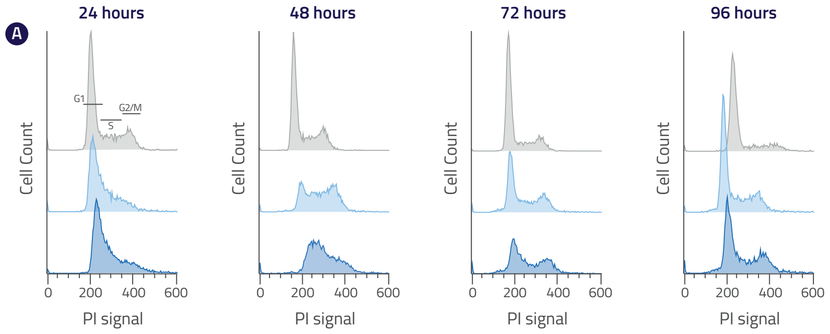


Figure 3: Time course of dFdCTP incorporation into DNA with increasing doses of NUC-1031, given as a ratio of DNA-derived dFdC to the endogenous deoxyguanosine (dG) base pairing (n=3)

- dFdCTP is incorporated into DNA from intracellular pools in a dose-dependent manner over time
- Prolonged effects observed, with increased incorporation at higher dose between 48-72 hours post-exposure
- Similar incorporation between 0.5 and 1µM doses up to 48 hours may suggest a saturation of available sites for fluorinated deoxycytidine incorporation at cell cycle replication forks[7]

NUC-1031 arrests cells in S phase of cell cycle



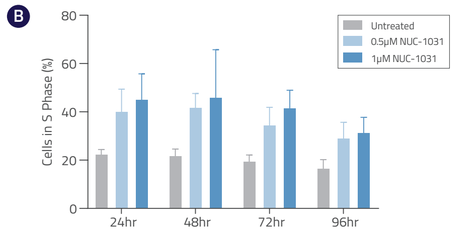
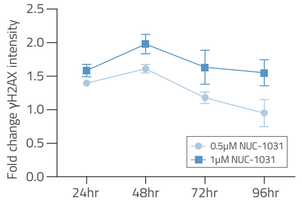


Figure 4: (A) Representative histograms of cell cycle phases over time (B) Percentage of S phase of cells treated with NUC-1031 compared to untreated controls (n=3)

- NUC-1031 increases the proportion of HuCCT1 cells in S phase
- S phase stalling peaks at 48 hours post-treatment, followed by recovery over 96 hours

NUC-1031 elicits a prolonged DNA damage response to double-strand breaks



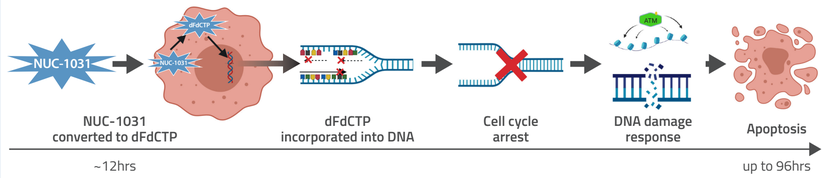
- γH2AX formation occurs in a dose-dependent manner
- Repair foci remain above endogenous levels up to 96 hours post-exposure to NUC-1031
- NUC-1031 may dampen double-stranded DNA damage repair
- Double-strand break response with S phase arrest suggests replication fork collapse in cells after 24 hours[6]

Figure 5: DNA damage response to double-strand breaks in cells treated with NUC-1031, represented by γH2AX signal, normalized to untreated control (n=2)

CONCLUSION

- NUC-1031 is a cytotoxic agent that causes cancer cell death
- NUC-1031 is converted to the active metabolite (dFdCTP) which is incorporated into DNA, leading to cell cycle arrest in a dose-dependent manner over 96 hours
- Cell cycle arrest, induced by NUC-1031, is associated with a DNA damage response
- NUC-1031 induces a persistence of double-strand breaks
- Future studies will investigate NUC-1031 in combination with platinum agents, utilizing fluorinated deoxycytidine (dFdC) detection to investigate synergistic cytotoxic interactions

NUC-1031 Induces Cell Cycle Arrest and DNA Damage Leading to Cell Death



ABBREVIATIONS: BTC: biliary tract cancer dCK: deoxycytidine kinase dFdCTP: difluorodeoxycytidine triphosphate dFdC: difluorodeoxycytidine dG: deoxyguanosine DNA: deoxyribonucleic acid γH2AX: phosphorylated histone H2AX hENT1: human nucleoside transporter 1 LC-MS/MS: liquid chromatography mass spectrometry PI: propidium iodide

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