# NUC-3373: A novel pyrimidine nucleotide analogue that overcomes all the main cancer drug resistance mechanisms that limit patient survival

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## BACKGROUND

### NUC-3373

- Novel pyrimidine NucleoTide Analogue
- Preactivated form of the anti-cancer metabolite FdUMP
- ProTide: utilising innovative phosphoramidate chemistry
- Overcomes all the main mechanisms associated with 5-FU, FUDR and capecitabine resistance

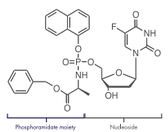



*Figure 1. NUC-3373 structural formula.*

### 5-FU Resistance Mechanisms

**Poor drug activation**

- Low Thymidine Kinase (TK)
  - TK required for 5-FU activation into active agent FdUMP
  - TK predominantly expressed during S-phase of cell cycle[1]
- High Thymidine Phosphorylase (TP)
  - High levels of TP are associated with adverse survival outcome in colorectal cancer patients receiving 5-FU[2]

**Extensive drug degradation**

- High Dihydropyrimidine Dehydrogenase (DPD)
  - 80% of 5-FU degraded by DPD
  - Elevated levels of DPD associated with lower overall survival in colorectal cancer patients receiving capecitabine[3]

**Limited cellular uptake**

- Low human Equilibrative Nucleoside Transporter 1 (hENT1)
  - Nucleobase and nucleoside transporters required for 5-FU / FUDR cellular uptake

**Weak target inhibition**

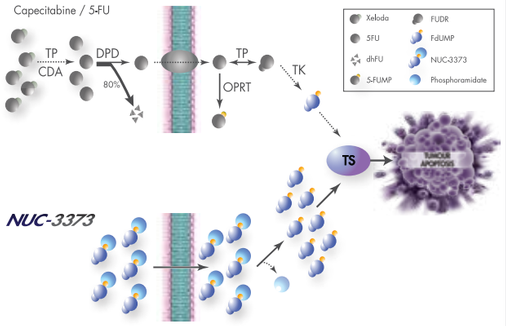
- High Thymidylate Synthase (TS), a target of 5-FU
  - Elevated levels of TS are associated with poor survival in colorectal cancer
  - High TS is a predictive factor for poor response to 5-FU[4]

***Mycoplasma* infection**

- Approximately 55% of colorectal cancers are infected by *Mycoplasma*[5]
- In cancer cell lines, *Mycoplasma* infection decreases 5-FU activity by up to 100x[6]

## MODE OF ACTION / METABOLISM

### NUC-3373 overcomes all the main 5-FU resistance pathways




Top: 5-FU, FUDR and capecitabine require active uptake and multi-step metabolism for conversion into the active anti-cancer agent FdUMP.

Bottom: NUC-3373 is a preactivated form of FdUMP bearing a phosphoramidate moiety (ProTide). NUC-3373 enters the cell independent of nucleoside transporters where the moiety is cleaved to release high levels of active FdUMP intracellularly which directly inhibits Thymidylate Synthase (TS) and induces apoptosis.

## METHODS

### Cytotoxicity

- Establish $EC_{50}$ values in multiple cancer cells including 5-FU resistant lines
- Measure efficacy in resistance conditions using $TK^-$ and $hENT1^-$ cells
- Compare activity in *Mycoplasma* infected cancer cells

### Metabolism

- Quantify intracellular levels of the active moiety FdUMP in colorectal cancer cells
- Evaluate the effect of Dihydropyrimidine Dehydrogenase (DPD) on drug metabolism

### Xenograft

- Validate activity *in vivo* utilising human colorectal cancer cells (HT29)

### Toxicology

- Conduct toxicology studies to establish safety profile

## RESULTS

### NUC-3373 is a potent anti-cancer agent

- NUC-3373 demonstrates up to 330x greater activity than 5-FU across a broad range of 5-FU sensitive and resistant cancer cell lines

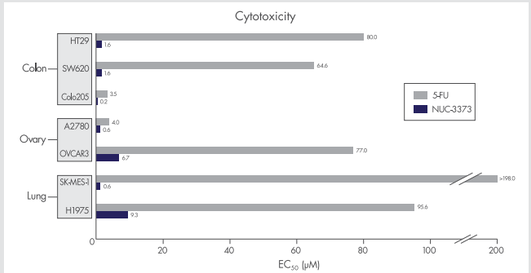



*Figure 2. Comparative anti-proliferative effect of NUC-3373 and 5-FU in colorectal, ovarian and lung cancer cell lines. $EC_{50}$ values standardised for molecular weight.*

### NUC-3373 overcomes all the main cancer resistance mechanisms

- Thymidine Kinase (TK)
  - FUDR activity is significantly impaired in TK deficient cancer cells whilst that of NUC-3373 is unchanged
- Thymidine Phosphorylase (TP)
  - NUC-3373 is resistant to TP degradation

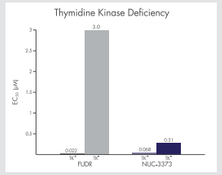



*Figure 3. NUC-3373 activity independent of TK while FUDR loses activity in TK-deficient CEM human leukaemia cancer cell line.*

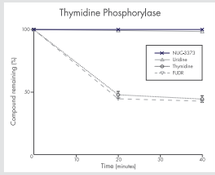



*Figure 4. TP purified from human erythrocytes was incubated with NUC-3373 and FUDR. NUC-3373 is resistant to TP degradation while FUDR is significantly broken down by TP.*

- Nucleoside Transporter (hENT1)
  - FUDR activity is reduced by 63x in hENT1-deficient cancer cells (CEM) while NUC-3373 maintains effective anti-proliferative activity
  - The cellular uptake of NUC-3373 is independent of nucleoside transporters

### NUC-3373 is resistant to DPD degradation

- 5-FU levels are significantly elevated in the presence of a DPD inhibitor (gimeracil) while NUC-3373 levels remain unaffected

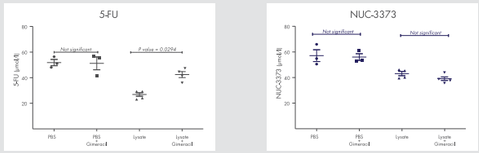



*Figure 5. NUC-3373 and 5-FU levels in combined SW620, HCT-116 and HT29 colorectal cancer cell lysates +/- gimeracil (DPD inhibitor).*

### NUC-3373 achieves high intracellular levels of active agent FdUMP

- FdUMP is the main active anti-cancer metabolite of 5-FU / FUDR
- NUC-3373 generates FdUMP intracellular levels 363x higher than 5-FU
- FdUMP levels generated by NUC-3373 remain high in all the key cancer resistance-like conditions (overexpression of TS, DPD, TP, OPRT, CDA)

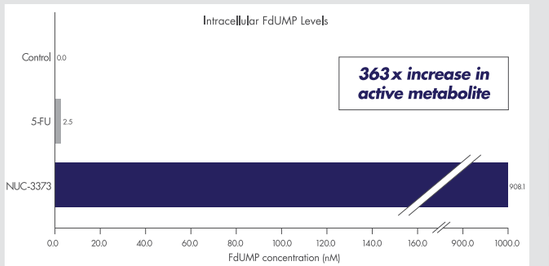
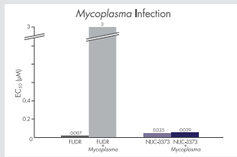



*Figure 6. Intracellular levels of FdUMP generated by NUC-3373 and 5-FU in human colorectal cancer cell line (HT29).*

### NUC-3373 activity is unaffected by *Mycoplasma* infection




- Unlike FUDR, NUC-3373 maintains activity in the presence of *Mycoplasma*

*Figure 7. Anti-proliferative effect of NUC-3373 and FUDR in L1210 leukaemic cancer cell line chronically infected by Mycoplasma hyorhinis.*

### NUC-3373 demonstrates superior inhibition of tumour growth *in vivo*

- NUC-3373 achieves significantly greater reduction in tumour weight and volume than 5-FU in HT29 colorectal xenografts
- NUC-3373 significantly prolongs tumour volume doubling time compared to control (vehicle)

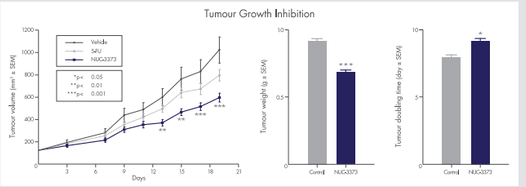



*Figure 8. NUC-3373 and 5-FU effect on HT29 human colorectal cancer xenografts. Control = vehicle.*

### NUC-3373 demonstrates a favourable toxicology profile

- In formal toxicology studies NUC-3373 is significantly better tolerated than 5-FU
- The main toxicities associated with 5-FU after a single dose are not observed with NUC-3373 after single or repeat dosing using equivalent or higher levels of drug
- NUC-3373 is rapidly distributed into tissues following IV bolus administration
- Plasma AUC ratios show low conversion of NUC-3373 into FUDR and dhFU (metabolite : NUC-3373 of 0.01 and 0.03 respectively)

*Table 1. AUC and $C_{max}$ of NUC-3373, FUDR and dhFU in plasma.*

| Analyte | Mean $AUC_{(0-t)}$ | Mean $C_{max}$ |
|---|---|---|
| NUC-3373 | 1745.0 ng.h/ml | 5295.0 ng/ml |
| dhFU | 38.5 ng.h/ml | 38.6 ng/ml |
| FUDR | 18.7 ng.h/ml | 24.1 ng/ml |

*Day 28 values dosed at 8mg/kg/day for 5 consecutive days*

## CONCLUSIONS

- NUC-3373 is a novel pyrimidine nucleotide analogue that overcomes all the main cancer resistance mechanisms associated with 5-FU, FUDR and capecitabine.
- Significantly greater anti-proliferative activity observed with NUC-3373 than 5-FU across a broad range of sensitive and resistant cancer cells including *Mycoplasma* infected cells.
- NUC-3373 generates 363x higher intracellular levels of the active agent, FdUMP, than 5-FU.
- NUC-3373 significantly decreases tumour weight and volume compared to 5-FU *in vivo* and is significantly better tolerated in toxicology studies.
- First In Human Phase I study of NUC-3373 will commence in Q4 2015.
- Results demonstrate that NUC-3373 has the potential to replace 5-FU as the standard of care for colorectal cancer and other solid tumours.

AACR-NCI-EORTC



*References: [1] Huang et al., 1994 [2] Takebayashi et al., 1996 [3] Koopman et al., 2009 [4] Peters et al., 1995 [5] Huang et al., 2001 [6] Jette et al., 2008*