First In Human Phase I study of NUC-3373, a nucleotide analogue designed to overcome fluoropyrimidine drug resistance mechanisms

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BACKGROUND

NUC-3373: Designed to overcome 5-FU resistance mechanisms

A pyrimidine NucleoTide Analogue
A phosphoramidate of FUDR-MP - the active metabolite of 5-FU

5-FU resistance mechanisms:

Thymidine Kinase (TK)
- Required for 5-FU activation to FUDR-MP

Thymidine Phosphorylase
- Degrades FUDR to 5-FU
- High levels associated with poor survival in colorectal cancer[1]

hENT1
- Required for FUDR cellular uptake

Dihydropyrimidine Dehydrogenase (DPD)
- Degrades 80% of 5-FU
- High levels associated with poor patient outcome[2]

Thymidylate Synthase (TS)
- Target of 5-FU
- High levels predict poor patient response[3]

MODE OF ACTION / METABOLISM

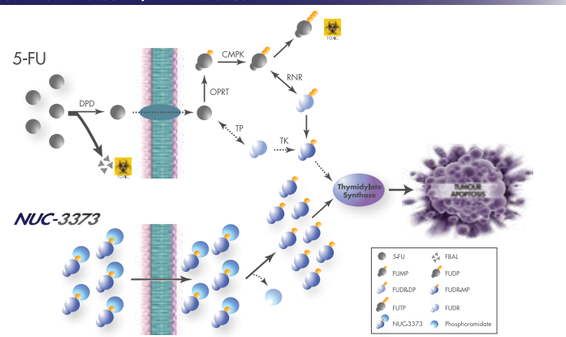

Figure 1. 5-FU and NUC-3373 metabolisms.

Top: 5-FU, FUDR and capecitabine require active uptake and multi-step metabolism for conversion into the active anti-cancer agent FUDR-MP. 5-FU generates toxic metabolites FUTP (intracellular) and FBAL (plasma).

Bottom: NUC-3373 is a phosphoramidate of the potent anti-cancer agent FUDR-MP. NUC-3373 enters cells independent of nucleoside transporters where the protective groups of the phosphoramidate are cleaved off to release high concentrations of FUDR-MP intracellularly which directly inhibits Thymidylate Synthase 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

Metabolism
- Evaluate the effect of DPD on drug metabolism
- Quantify intracellular levels of the active moiety FUDR-MP in colorectal cancer cells

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

Toxicology
- Conduct toxicology studies to establish safety profile

RESULTS

NUC-3373 is a potent anti-cancer agent

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

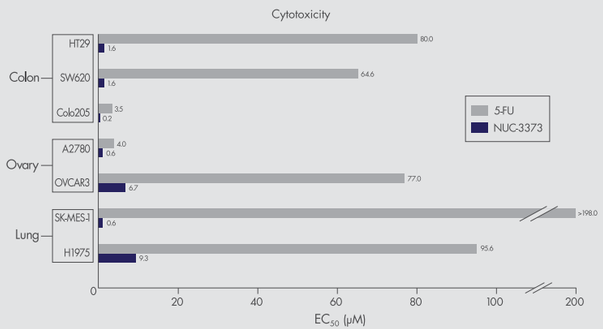

Figure 2. Comparative anti-proliferative effect of NUC-3373 and 5-FU in colorectal, ovarian and lung cancer cell lines. EC_{50} values standardised for molecular weight.

NUC-3373 overcomes all the main cancer resistance mechanisms

- Thymidine Kinase (TK)
  - NUC-3373 retained activity in TK deficient cancer cells
- Thymidine Phosphorylase (TP)
  - NUC-3373 is resistant to TP degradation

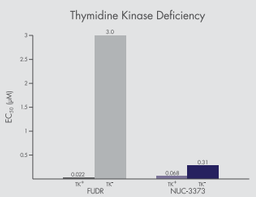

Figure 3. NUC-3373 activity independent of TK while FUDR loses activity in TK-deficient CEM human leukaemia cancer cell line.

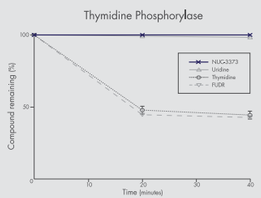

Figure 4. NUC-3373 is not degraded by TP while FUDR is significantly degraded. TP purified from human erythrocytes was incubated with NUC-3373 and FUDR.

- Nucleoside Transporter (hENT1)
  - NUC-3373 maintained effective cytotoxic activity in hENT1-deficient human leukaemia CEM cancer cells whilst that of FUDR is reduced by 63x
  - NUC-3373 cellular uptake is independent of nucleoside transporters

NUC-3373 is resistant to DPD degradation

- In the absence of DPD inhibitor (gimeracil) NUC-3373 levels remain unaffected while 5-FU levels are significantly decreased (p=0.0294)

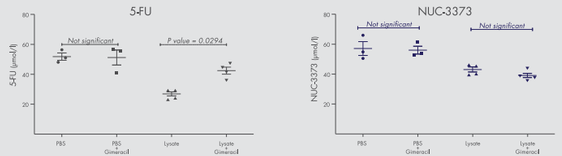

Figure 5. NUC-3373 and 5-FU levels in mixed colorectal SW620, HCT-116 and HT29 cancer cell lysates +/- DPD inhibitor.

NUC-3373 achieves high intracellular levels of active agent FUDR-MP

- FUDR-MP levels generated by NUC-3373 remain high in all the key cancer resistance-like conditions (overexpression of TS, DPD, TP, OPRT, CDA)

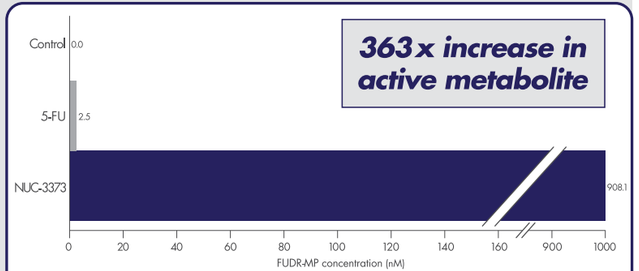

Figure 6. Intracellular FUDR-MP levels achieved by NUC-3373 and 5-FU in human colorectal HT29 cancer cell line.

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 human colorectal HT29 xenografts
- NUC-3373 significantly extends tumour volume doubling time compared to control

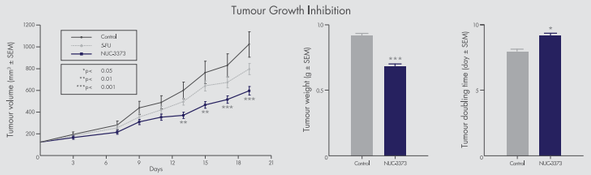

Figure 7. NUC-3373 and 5-FU effect on human colorectal HT29 cancer xenograft.

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 administering equimolar or higher concentrations 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" ratio of 0.01 and 0.03, respectively)

Table 1. $AUC_{0\text{-}t}$ and C_{max} of NUC-3373, FUDR and dhFU in plasma.

Analyte	Mean $AUC_{(0\text{-}t)}$	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 8 mg/kg/day for 5 consecutive days

PHASE I STUDY

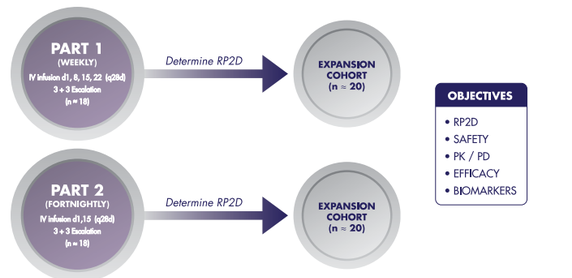

Figure 8. Phase I study design and objectives.

CONCLUSION

- NUC-3373 is a novel pyrimidine nucleotide analogue that overcomes all the main cancer resistance mechanisms associated with 5-FU, FUDR and capecitabine.
- NUC-3373 possesses significantly greater activity than 5-FU across a broad range of human cancer cells that are sensitive and resistant to 5-FU.
- NUC-3373 generates 363-fold higher intracellular levels of the active agent, FUDR-MP, than 5-FU *in vitro*.
- NUC-3373 significantly decreases tumour weight and volume compared to 5-FU *in vivo* and is significantly better tolerated in toxicology studies.
- Results demonstrate that NUC-3373 has the potential to replace 5-FU as the standard of care for colorectal and other solid tumours.
- First In Human Phase I study of NUC-3373 is actively enrolling patients.

References: (1) Takebayashi *et al.*,1996 (2) Koopman *et al.*,2009 (3) Peters *et al.*,1995

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