# Inhibition of thymidylate synthase by the ProTide NUC-3373

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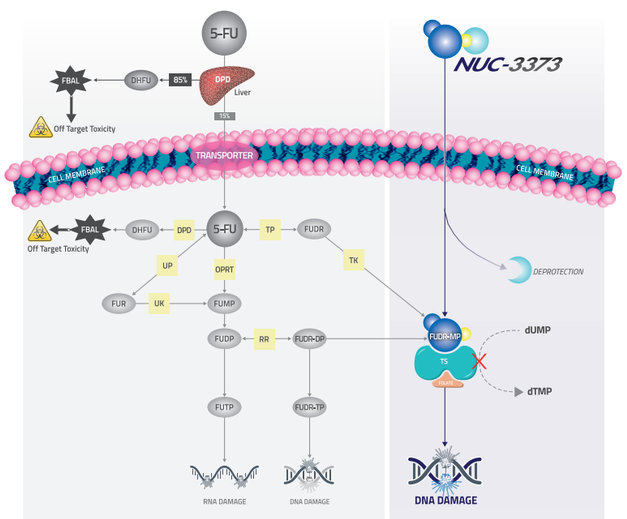
## Background

- 5-fluorouracil (5-FU) is a key anti-cancer agent used across a broad range of tumors
- Fluorodeoxyuridine-monophosphate (FUDR-MP or FdUMP), the active anti-cancer metabolite of 5-FU, causes cell death via inhibition of thymidylate synthase (TS)[1]
  - Prevents the conversion of dUMP to dTMP
- Poor response to 5-FU is a consequence of:
  - Short plasma half-life (8-14 minutes)[2] necessitating prolonged administration (>46 hours)
    - Over 85% of 5-FU is broken down by DPD[3]
    - Production of catabolites such as FBAL (implicated in hand-foot syndrome)
  - Alterations in transport mechanisms
    - Decreased uptake of 5-FU via membrane transporters
    - Increased efflux by ATP-binding cassette transporters[4]
  - Complex activation
    - Alterations to enzymes including thymidine phosphorylase (TP) and thymidine kinase (TK) confer resistance to 5-FU

### NUC-3373 bypasses the key cancer resistance pathways associated with 5-FU




### NUC-3373: A targeted inhibitor of TS

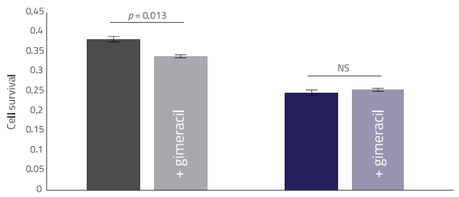
- ProTide transformation of FUDR-MP, the active anti-cancer metabolite of 5-FU
- Designed to overcome the key 5-FU resistance mechanisms[5,6]
  - Protected from breakdown by DPD
  - Cellular uptake independent of membrane transporters
  - FUDR-MP generation independent of intracellular enzymatic activation
- NUC-3373 generates significantly higher levels of FUDR-MP compared to 5-FU[7]
- Currently being investigated in clinical studies:
  - NuTide:301 - Phase Ib dose-finding study in solid tumors
  - NuTide:302 - Phase Ib combination study in colorectal cancer (CRC)

## Methods

- *In vitro* investigations:
  - Cell survival assessed by sulforhodamine B assay in CRC cell lines (HT29 and HCT116)
  - TS expression assessed in HCT116 whole cell lysates by Western blot and quantified by LiCor Odyssey
  - TS cellular localization in HCT116 cells identified by immunocytochemistry and selected for assessment by systematic uniform randomized sampling of 800 cells per treatment
- NuTide:302 Study:
  - A three-part, Phase Ib study in patients with locally advanced or metastatic CRC who have relapsed after ≥2 prior lines of 5-FU-containing therapies
  - Pharmacokinetic analyses via liquid chromatography-mass spectrometry:
    - Plasma: NUC-3373, FUDR and FBAL
    - PBMC: FUDR-MP and dUMP

## Results

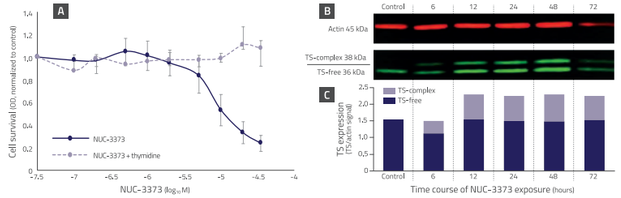
### NUC-3373 is not a substrate for DPD catabolism




*The effect of DPD inhibition on HT29 cells exposed to 20 μM 5-FU and NUC-3373. Cell survival normalized to control.*

- Despite low intracellular DPD expression, pharmacological inhibition still increased the sensitivity of cells to 5-FU
- DPD inhibition had no effect on the sensitivity of cells to NUC-3373

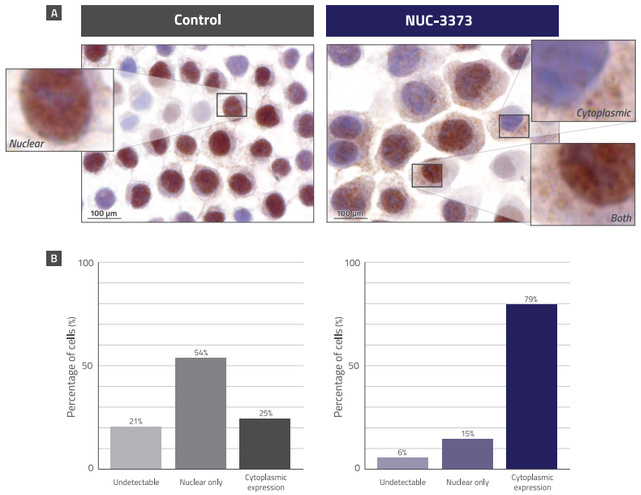
### NUC-3373 targets the *de novo* pathway of dTMP synthesis by forming long-lasting TS ternary complexes




**A:** *The effect of 10 μg/mL thymidine supplementation in HCT116 cells exposed to NUC-3373.*
**B:** *Western blot of TS-ternary complex and TS-free protein expression following exposure to 10 μM NUC-3373.*
**C:** *Quantified TS-ternary complex and TS-free protein expression.*

- Exogenous thymidine rescues cells from NUC-3373-induced death, confirming that dTMP is essential for cell survival
- NUC-3373 forms TS-ternary complexes that were detected for at least 72 hours

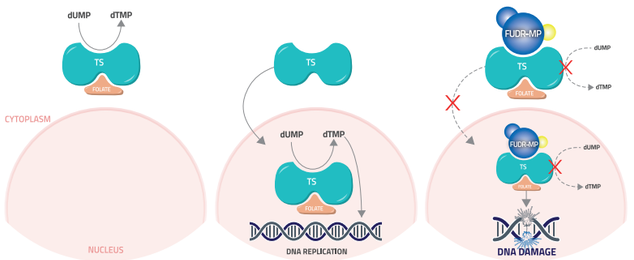
### NUC-3373 exposure is associated with increased cytoplasmic TS expression




**A.** *Cellular localization of TS after 24 hours exposure to 10 μM NUC-3373 in HCT116 cells.*
**B.** *Proportion of cells by TS localization.*

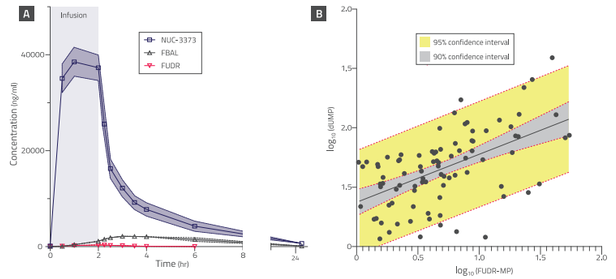
- NUC-3373 increases cell diameter
- Cellular localization of TS is predominantly nuclear in control cells
- NUC-3373 exposure results in a higher proportion of cells expressing cytoplasmic TS

### Cellular localization of dTMP synthesis




**G1-phase -** *de novo dTMP synthesis takes place in the cytoplasm.*
**S-phase -** *TS translocates to the nucleus where de novo dTMP synthesis is required for DNA replication.*
**+FUDR-MP -** *causes G1 arrest[8] regardless of location and an increase in the nuclear dUMP: dTMP ratio, resulting in uracil misincorporation into DNA[9].*

### NUC-3373 demonstrates a favorable PK profile in clinical study NuTide:302 (n=20)




**A:** *Plasma NUC-3373, FUDR and FBAL over time (95% CI).*
**B:** *The relationship between intracellular FUDR-MP and dUMP.*

| | NUC-3373 | FUDR | FBAL |
|---|---|---|---|
| $C_{max}$ (μg/mL) | 43.2 | 0.4 | 2.4 |
| $AUC_{(0-t)}$ (μg·h/mL) | 165.9 | 1.0 | 25.4 |
| $T_{1/2}$ (h) | 5.7 | 1.2 | 5.1 |

*1500mg/m² over 2 hours; mean values reported*

- Elimination half-life $t_{1/2}\beta$ was 5.7 hours (range 3.9 - 10.8 hours; estimated 3-24 hours)
- Low inter-patient variability for all parameters (co-efficient of variation 22-51%)
- Volume of distribution was high indicating extensive tissue absorption (171.6 L)
- Positive linear relationship between intracellular FUDR-MP and dUMP
  - GLP assay optimization for intracellular dTMP ongoing
- Plasma FBAL low and not clinically significant
  - No hand-foot syndrome observed

## Conclusion

### NUC-3373 is a potent inhibitor of TS activity

- *In vitro* data demonstrate:
  - NUC-3373 activity is not impacted by DPD
  - NUC-3373 inhibits TS activity and forms long-lasting TS ternary complexes, preventing cells from converting dUMP to dTMP
- NuTide:302 study demonstrates favorable PK profile:
  - Long half-life
  - Extensive tissue distribution
  - FBAL generation was low (no hand-foot syndrome observed)
  - Intracellular levels of FUDR-MP are associated with increases in dUMP
- NUC-3373 ongoing clinical studies:
  - NuTide:301 - dose-finding study in solid tumors
  - NuTide:302 - combination study in CRC

**ABBREVIATIONS:** 5-FU: 5-fluorouracil FUDR-MP/FdUMP: fluorodeoxyuridine monophosphate TS: thymidylate synthase dUMP: deoxyuridine monophosphate dTMP: deoxythymidine monophosphate DPD: dihydropyrimidine dehydrogenase FBAL: alpha-fluoro-beta-alanine DHFU: dihydrofluorouracil UP: uridine phosphorylase FUR: fluorouridine UK: uridine kinase OPRT: orotate phosphoribosyltransferase TP: thymidine phosphorylase FUDR: fluorodeoxyuridine FUMP: fluorouridine monophosphate FUDP: fluorouridine diphosphate FUTP: fluorouridine triphosphate RR: ribonucleotide reductase TK: thymidine kinase FUDR-DP: fluorodeoxyuridine diphosphate FUDR-TP: fluorodeoxyuridine triphosphate CRC: colorectal cancer $IC_{50}$ half-maximal inhibitory concentration PBMC: peripheral blood mononuclear cell AE: adverse event AUC: area under the curve $C_{max}$: maximal concentration ATP: adenosine triphosphate GLP: good laboratory practice CI: confidence interval

**REFERENCES:** 1. Longley DB, *et al.* 2003 *Nat Rev Cancer*; 3:330-338 2. Heggie GD, *et al.* 1987 *Cancer Research*; 47:2203-2206 3. Diasio RB & Harris BE. 1999 *Clin Pharmacokinet*; 16:215-237 4. Nambaru PK, *et al.* 2011 *DMD*; 39:132-139 5. McGuigan C, *et al.* 2011 *J Med Chem*; 27:7247-7258 6. Vande Voorde J, *et al.* 2011 *Biochem Pharmacol*; 82:441-452 7. Ghazaly E, *et al.* 2017 *Ann Oncol*; 25; Suppl_5:128 8. Tokunaga E, 2000 *Eur J Cancer*; 15:1998-2006 9. MacFarlane AJ, *et al.* 2011 *J Biol Chem*; 286:44015-44023
Data cut-off 7th October 2019, Data cleaning ongoing