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NUC-3373 induces a cytoplasmic translocation of thymidylate synthase in colorectal cancer cell lines

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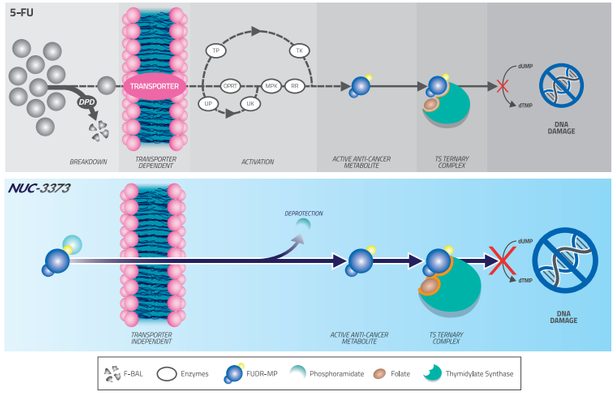
Background

- 5-fluorouracil (5-FU) is a key anti-cancer drug used across a broad range of tumors
- Fluorodeoxyuridine-monophosphate (FUDR-MP), the anti-cancer metabolite of 5-FU, causes cell death by:
  - Inhibiting thymidylate synthase (TS)
  - Reducing the pool of deoxythymidine monophosphate (dTMP)
- Poor response to 5-FU is a consequence of:
  - Over 85% of 5-FU broken down by dihydropyrimidine dehydrogenase (DPD)[1]
  - Limited dosing due to side effects caused by the accumulation of toxic metabolites[2]
  - Key cancer resistance mechanisms:
    - Cellular uptake dependent on nucleoside transporters[3]
    - Complex enzymatic activation to yield active anti-cancer metabolite FUDR-MP[3]
  - Short plasma half-life (8-14 minutes) results in prolonged administration times (>46hours)

NUC-3373 bypasses the key cancer resistance pathways of 5-FU



NUC-3373: A targeted inhibitor of TS

- ProTide transformation of 5-FU
- Designed to overcome the key 5-FU resistance mechanisms[4,5]
  - Protected from breakdown by DPD
  - Cellular uptake independent of nucleoside transporters
  - FUDR-MP generation independent of intracellular enzymatic activation
- 366x higher intracellular levels of FUDR-MP than 5-FU *in vitro*[6]
- Significantly greater anti-cancer activity *in vivo* compared to 5-FU
- Favorable toxicology profile compared to 5-FU

Methods

- Nine colorectal cancer (CRC) cell lines investigated
- IC_{50} values determined by sulforhodamine B assay
- Two cell lines selected for further characterization based on their sensitivity to NUC-3373
  - HCT116 (sensitive)
  - SW480 (less sensitive)
- TS protein expression measured by Western blot (whole cell lysates)
- TS cellular localization investigated by immunocytochemistry (imaged by light and fluorescence microscopy)
- Approximately 800 cells under each condition were assessed

Results

Sensitivity to NUC-3373 is not dependent on basal TS protein level

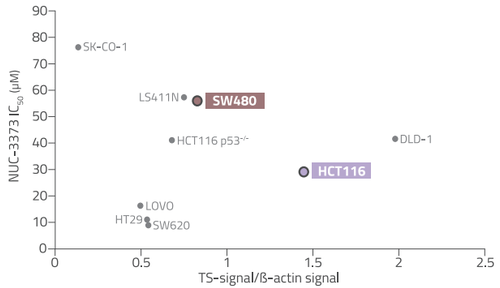


Figure 1. Thymidylate synthase expression vs NUC-3373 IC_{50} values in a panel of nine colorectal cancer cell lines

- Sensitivity to NUC-3373 is not determined by basal TS protein expression
- Cell lines were chosen to represent CRC tumor sensitivity to NUC-3373

CRC cell line characterization

	HCT116	SW480
Sensitivity to fluoropyrimidines	Sensitive[7]	Less Sensitive[7]
NUC-3373 IC_{50} (µM)	29	56
Relative doubling time	Fast	Slow
Basal TS expression	Higher	Lower
MMR status	MSI	MSS

NUC-3373 targets the *de novo* pathway of dTMP synthesis

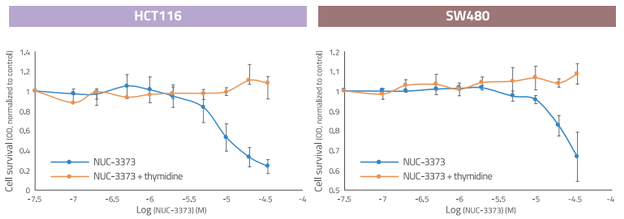


Figure 2. Effect of thymidine supplementation of cells on NUC-3373-induced death

- Thymidine supplementation rescues cells from NUC-3373-induced death

NUC-3373 forms TS ternary complexes in CRC cells

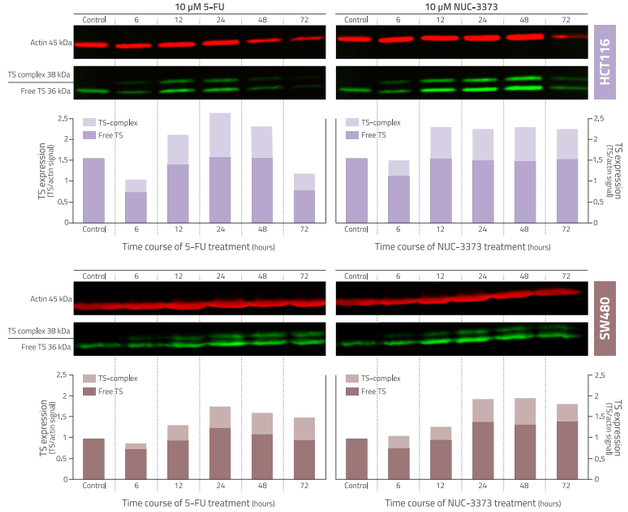


Figure 3. TS protein expression in response to 10 µM 5-FU or NUC-3373 treatment over time

- NUC-3373 induces formation of TS ternary complexes that are sustained for at least 72 hours in both cell lines
- In HCT116 cells, NUC-3373-induced complexes are more durable than those formed by 5-FU

NUC-3373 causes nuclear to cytoplasmic translocation of TS in HCT116 cells

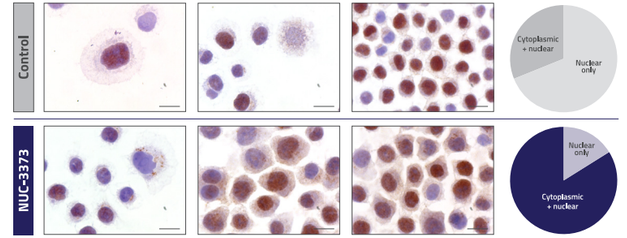


Figure 4. Cellular localization of TS in cytospinned cells and quantitative assessment of cytoplasmic TS (scale bar = 100 µm)

- Cellular localization of TS is predominantly nuclear in untreated control cells
- NUC-3373 increases TS protein expression and induces a translocation into cytoplasm

Cytoplasmic TS is not targeted for lysosomal degradation

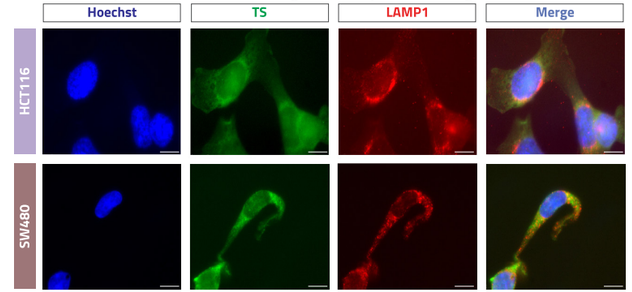


Figure 5. TS and LAMP1 expression in HCT116 and SW480 cells treated with 10 µM NUC-3373 for 24 hours (scale bar = 10 µm)

- In NUC-3373-treated cells, cytoplasmic TS does not co-localize with LAMP1, indicating that it is not targeted for lysosomal degradation

Conclusion

- NUC-3373 targets the *de novo* pathway of dTMP synthesis
- NUC-3373 cytotoxicity is not dependent on basal TS expression
- NUC-3373 causes formation of TS complexes in HCT116 cells which are sustained longer than those induced by 5-FU
- NUC-3373 causes a translocation of TS to the cytoplasm
- **NUC-3373 is a potent inhibitor of TS**

Abbreviations: 5-FU: 5-fluorouracil FUDR-MP: Fluorodeoxyuridine-monophosphate TS: Thymidylate synthase dTMP: deoxythymidine monophosphate DPD: Dihydrogenpyrimidine dehydrogenase CRC: Colorectal cancer IC_{50}: Half maximal inhibitory concentration MMR: Mismatch repair MSI: Microsatellite instable MSS: Microsatellite stable OD: Optical Density LAMP1: Lysosomal associated membrane protein 1

References: 1. Diasio RB & Harris BE. 1999 *Clin Pharmacokinet*, 16: 215–237 2. Miura K. *et al.* 2010 *Cancers*, 2:1717–1730 3. Longley DB. *et al.* 2003 *Nat Rev Cancer*, 3:330–338 4. McGuigan C. *et al.* 2011 *J Med Chem*, 27:7247–7258 5. Vande Voorde J. *et al.* 2011 *Biochem Pharmacol*, 82:441–452 6. Ghazaly E. *et al.* 2017 *Ann Oncol*, 25; Suppl_5:128 7. Mhaidat NM. *et al.* 2014. *Oncol Lett*. 8(2):699–794