Abstract Number: 2081
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NUC-3373 induces endoplasmic reticulum stress in colorectal cancer cells

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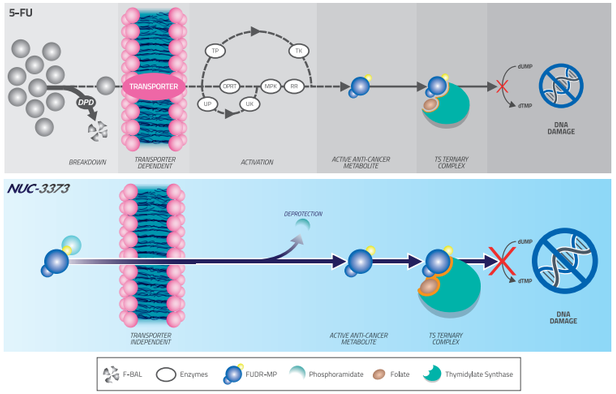
Background

- 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)
- BiP protein expression measured by Western blot (whole cell lysates)
- *CHOP* gene expression assessed by RT-qPCR
- Ultrastructural changes assessed by TEM in sections from glutaraldehyde fixed cells

Unbiased stereology was used to determine changes in ER structure

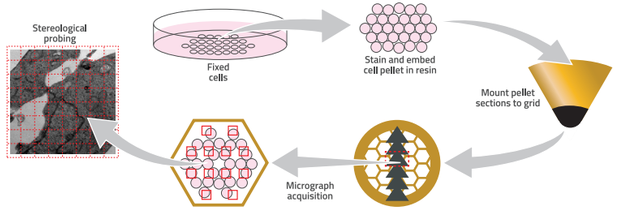


Figure 1. TEM sample preparation and micrograph acquisition

- The volume density (Vv) refers to the volume of the organelle within the reference space, *i.e. the volume of ER in the volume of cytoplasm*

  Vv (ER, Cyt) = Σ points on ER/Σ points on cytoplasm
- The surface density (Sv) refers to the surface area of ER membrane within the reference space, *i.e. the surface area of ER in the volume of cytoplasm*

  Sv (ER, Cyt) = Σ line intersects with ER/Σ points on cytoplasm

Results

NUC-3373 activates the unfolded protein response

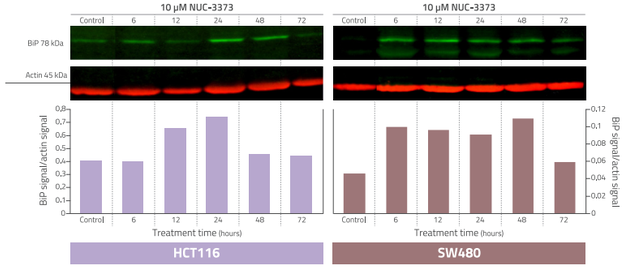


Figure 2. Representative Western blots and quantification of BiP protein expression in HCT116 and SW480 cells

- NUC-3373 induces BiP expression, indicating that the unfolded protein response is activated

NUC-3373 induces prolonged and unmitigated ER stress

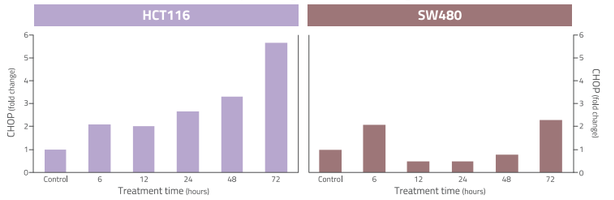


Figure 3. Fold-change in CHOP gene expression in response to NUC-3373 treatment

- NUC-3373 upregulates *CHOP* gene expression in HCT116 cells, indicating prolonged and unmitigated ER stress
- In contrast to HCT116 cells, NUC-3373 has a less pronounced effect on *CHOP* in SW480 cells

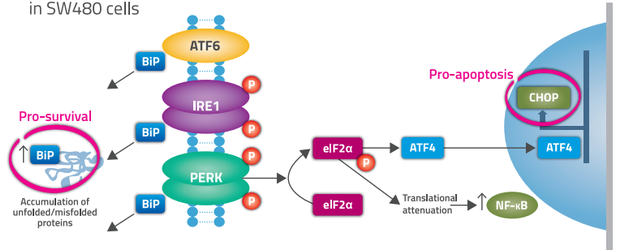


Figure 4. The unfolded protein response: survival vs. apoptosis

NUC-3373 induces ultrastructural changes in ER

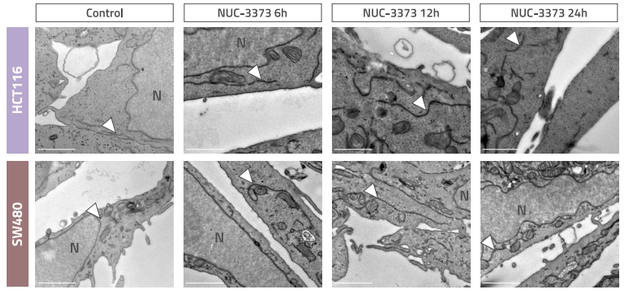
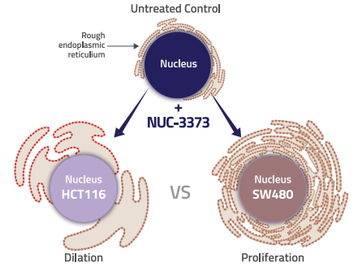


Figure 5. Representative TEM micrographs of cells before and after treatment with 10 μM NUC-3373 (scale bar = 1 μm) white triangles pointing to ER

Figure 6. Quantification of ultrastructural changes to ER in response to 10 μm NUC-3373



- NUC-3373-induced stress is evident by an increased volume density of ER in HCT116 and SW480 cells
- In HCT116 cells, increased volume density is due to **dilation** of the ER lumen
- In SW480 cells, increased volume density is due to **proliferation** of the ER membrane

Figure 7. NUC-3373 induces differential stress response mechanisms in HCT116 and SW480 cell lines

Conclusion

- In addition to being a potent inhibitor of TS, NUC-3373 can also induce cancer cell death by triggering the ER stress response
- Differential ER stress responses were observed in HCT116 and SW480 CRC cell lines
- The on-going NuTide:302 clinical study is investigating NUC-3373 in combination with other anti-cancer agents

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 BiP: Immunoglobulin binding protein CHOP: CCAAT-enhancer-binding protein homologous protein RT-qPCR: Quantitative reverse transcription polymerase chain reaction TEM: Transmission Electron Microscopy ER: Endoplasmic Reticulum Vv: Volume Density Sv: Surface Density Cyt: Cytosplasm

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