NUC-3373 has a more targeted DNA mode of action than 5-FU

INTRODUCTION

5-FU: The cornerstone of treatment for CRC despite limitations

- Rapid degradation by DPD¹

NUC-3373: A targeted inhibitor of TS

- Designed to overcome the key 5-FU resistance mechanisms

Aim

Investigate a potential DNA-mediated mode of action for NUC-3373

Hypothesis

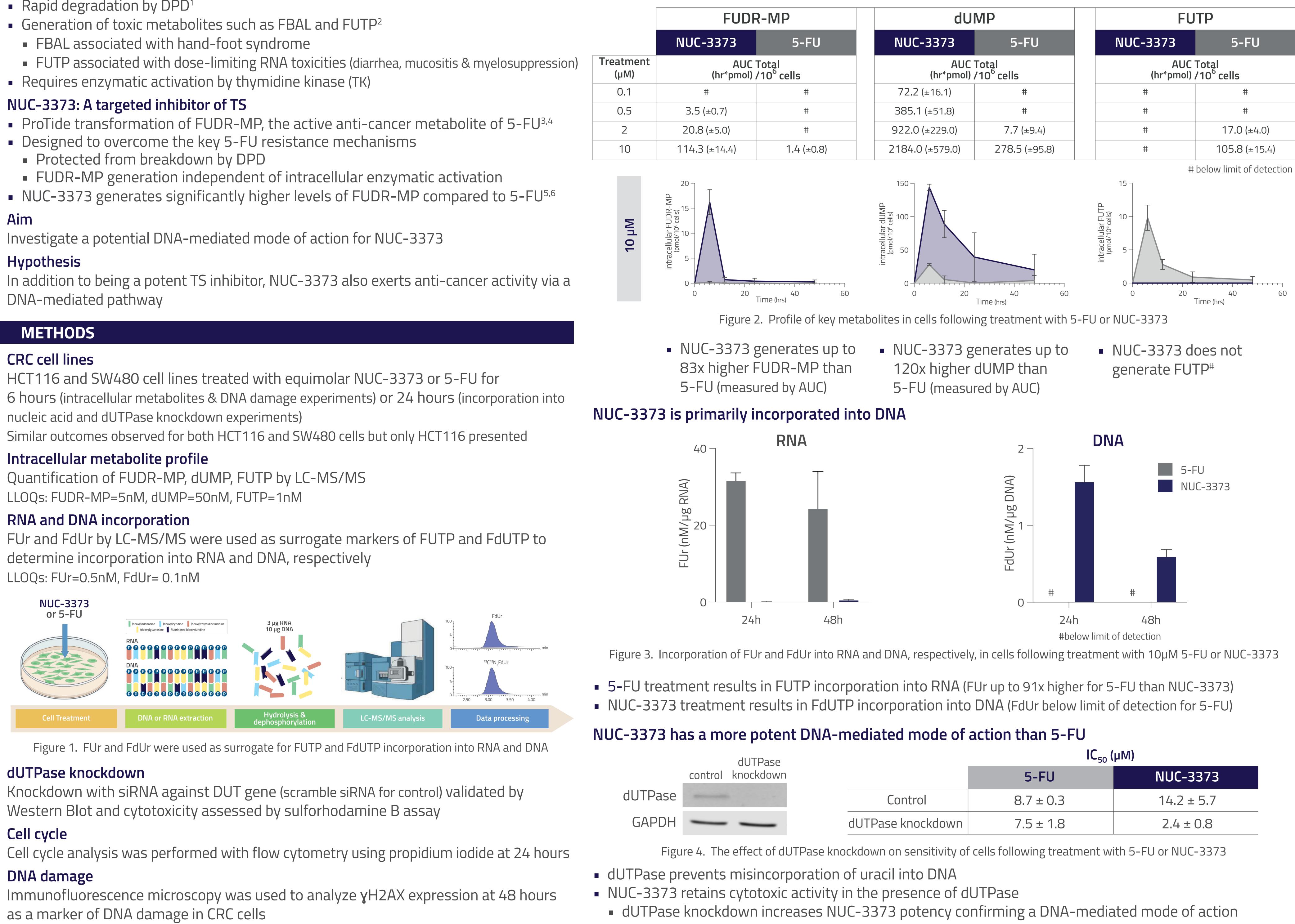
DNA-mediated pathway

CRC cell lines

nucleic acid and dUTPase knockdown experiments)

Quantification of FUDR-MP, dUMP, FUTP by LC-MS/MS LLOQs: FUDR-MP=5nM, dUMP=50nM, FUTP=1nM

determine incorporation into RNA and DNA, respectively LLOQs: FUr=0.5nM, FdUr= 0.1nM



dUTPase knockdown

Western Blot and cytotoxicity assessed by sulforhodamine B assay

Cell cycle

DNA damage

as a marker of DNA damage in CRC cells

REFERENCES: 1. Diasio RB & Harris BE 1999 Oncology; 13(7 Suppl 3): 17-21 2. Brutcher E et al., 2018 Clin J Oncol Nurs; 22(6): 627-634 3. Vande Voorde J et al., 2017 Ann Oncol; Suppl _5128 6. Derissen EJ et al., 2016 Clin Pharmacol; 81: 949-957 4. McGuigan C et al., 2018 Clin J Oncol Nurs; 22(6): 627-634 3. Vande Voorde J et al., 2011 Med Chem; 27: 7247-7258 5. Ghazaly E et al., 2017 Ann Oncol; Suppl _5128 6. Derissen EJ et al., 2016 Clin Pharmacol; 81: 949-957 OPRT: orotate phosphoribosyl, transferase, RNA: ribonucleic acid, siRNA: short interfering RNA, TK: thymidine kinase, TP: thymidine phosphorylase, TS: thymidylate synthase

RESULTS



2) NuCana plc, Edinburgh, UK

IC ₅₀ (μM)	
5-FU	NUC-3373
8.7 ± 0.3	14.2 ± 5.7
7.5 ± 1.8	2.4 ± 0.8

NUC-3373 induces S-phase arrest and DNA damage

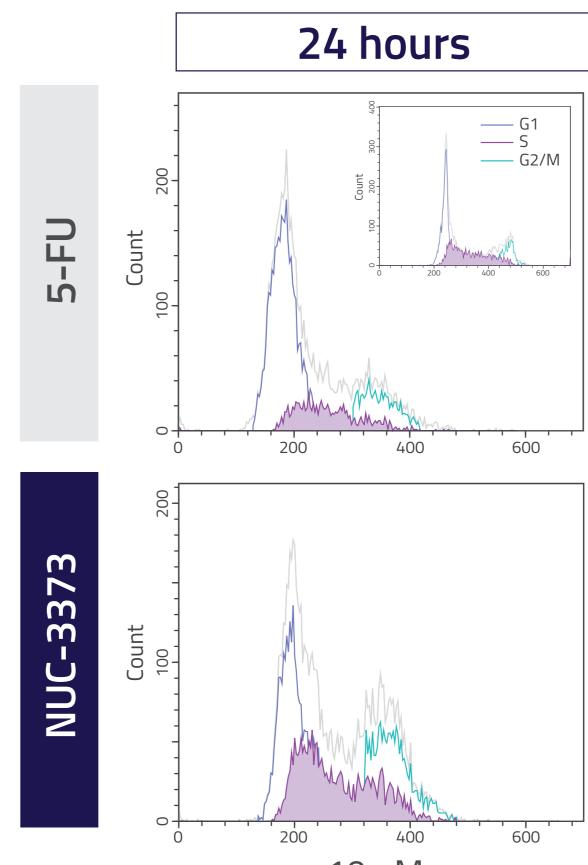


Figure 6. Cell cycle analysis and expression of yH2AX in cells following treatment with 5-FU or NUC-3373 (insets represent untreated cells) • NUC-3373 induces S-phase arrest and causes more DNA damage than 5-FU confirming a DNA-mediated mode of action

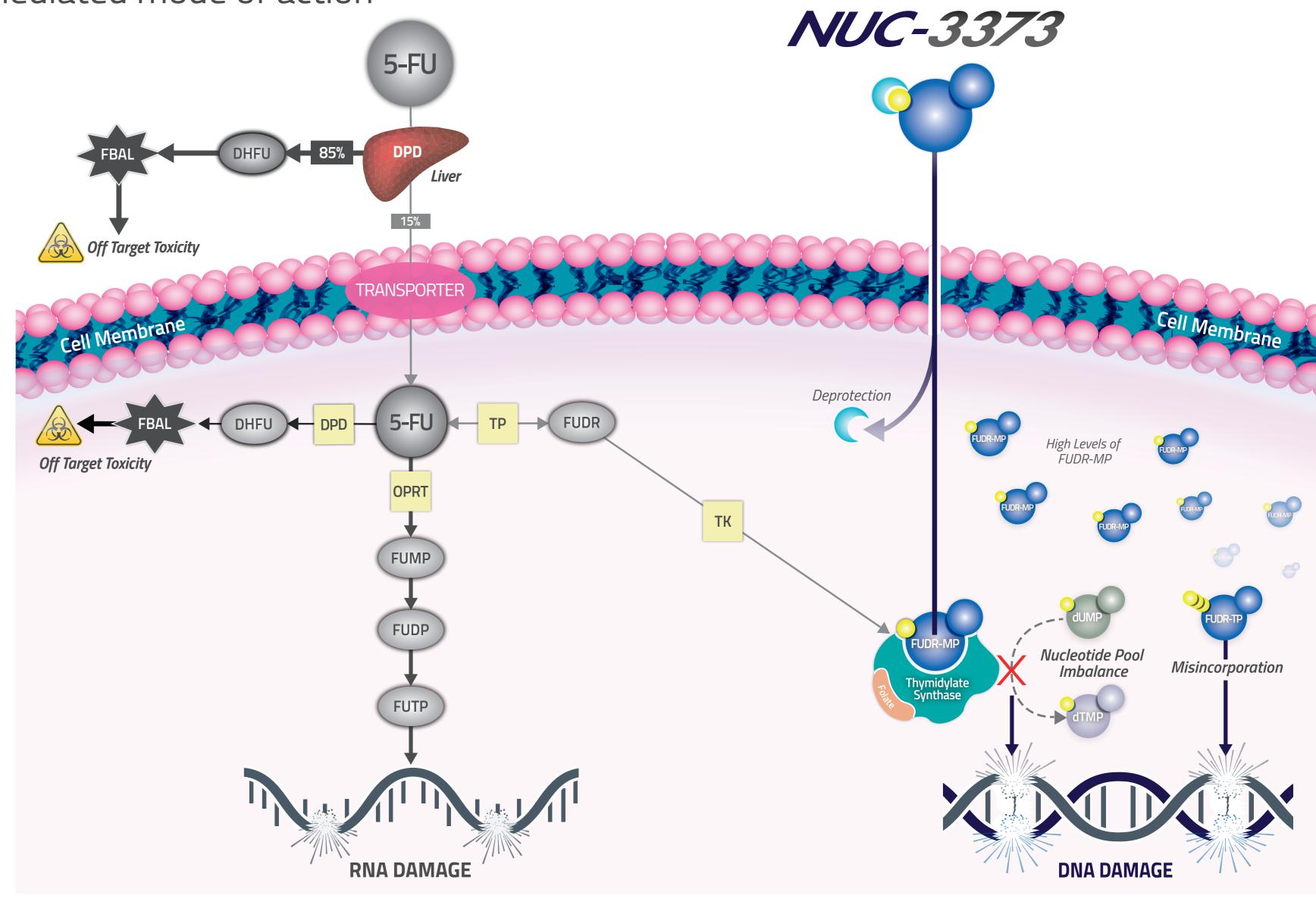


Figure 7. NUC-3373 & 5-FU mode of action and metabolism comparison

CONCLUSION

In addition to being a potent TS inhibitor, NUC-3373 has a cytotoxic DNA-mediated mode of action Compared to 5-FU, NUC-3373:

- Results in greater accumulation of dUMP
- Does not generate detectable levels of FUTP, associated with dose limiting toxicities
- Results in DNA incorporation and induces greater DNA damage

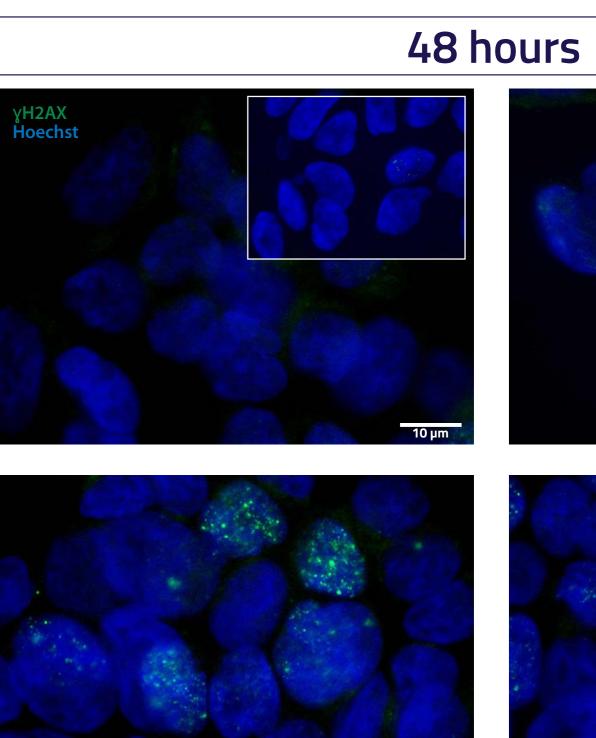
(NuTide:302 Phase lb/II; NCT03428958)

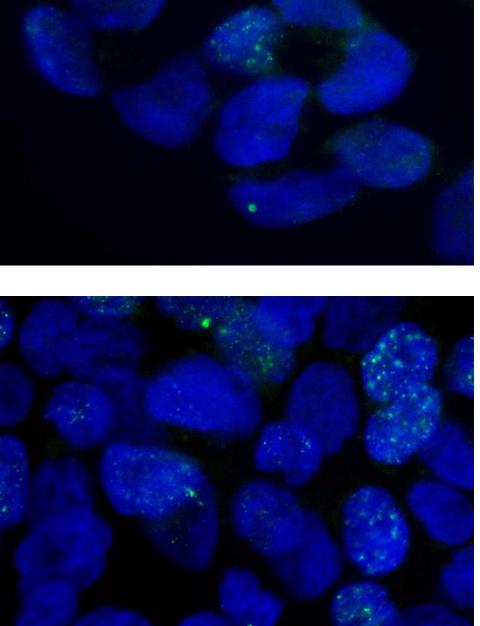
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0.5 µM

10 µM

- Generates significantly higher intracellular levels of FUDR-MP
- NUC-3373 is currently being investigated in patients with advanced CRC