NUC-3373 potentiates immune-mediated cytotoxicity of CRC Cells

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Abstract Number: 1113

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BACKGROUND

NUC-3373 is a potent TS inhibitor with a DNA-mediated mode of action

- ProTide transformation of FUDR-MP[1,2], the active anti-cancer metabolite of 5-FU
- Designed to overcome the key 5-FU resistance mechanisms
  - Protected from breakdown by DPD
  - FUDR-MP generation independent of intracellular enzymatic activation
- NUC-3373 generates significantly higher levels of FUDR-MP compared to 5-FU[3,4]
- NUC-3373 is currently being investigated in patients with advanced CRC (NuTide:302 Phase Ib/II; NCT03428958)
- Interaction between cancer cells and immune cells in the microenvironment is important for anticancer activity

NUC-3373 induces an anti-tumor inflammatory response by:

- Inducing ER stress via accumulation of TS ternary complexes[5]
- Releasing DAMPs (CRT, HSP70, ATP & HMGB1)
- Activating the innate immune system (NK cell response)[6]

Aim:

To confirm that NUC-3373 enhances immunogenic cell death (ICD) via the adaptive immune system and that it can potentiate the effects of PD-1/PD-L1 (e.g. nivolumab)

Hypotheses:

- NUC-3373-induced DAMPs can also promote activation of the adaptive immune system leading to ICD
- NUC-3373 potentiates the effects of PD-1 inhibitor nivolumab

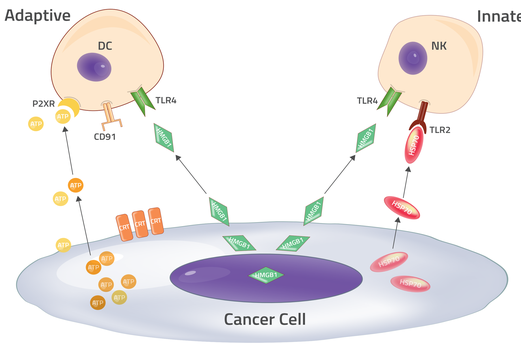

Figure 1. DAMPs release from CRC cells treated with NUC-3373

METHODS

Cell culture

CRC cell lines HCT116 (MSI) and SW480 (MSS) were treated with sub-IC_{50} doses of NUC-3373 for 24 hours prior to coculture with PBMCs (IC_{50}: HCT116 = 25 µM, SW480 = 50 µM) An anti-CD3/28 monoclonal antibody (mAb)-stimulated PBMCs model was used to test T-cell mediated cell killing modulated by NUC-3373 ± nivolumab (10 µg/ml)

Pro-inflammatory cytokine profile

qPCR was used to assess the gene expression of pro-inflammatory cytokines

PD-L1 expression

PD-L1 surface expression on CRC cells and PBMCs was determined by flow cytometry

Cytotoxicity

Cell viability was measured by automated cytometry analysis (Celigo) and sulforhodamine B assay

RESULTS

NUC-3373 induces pro-inflammatory cytokines

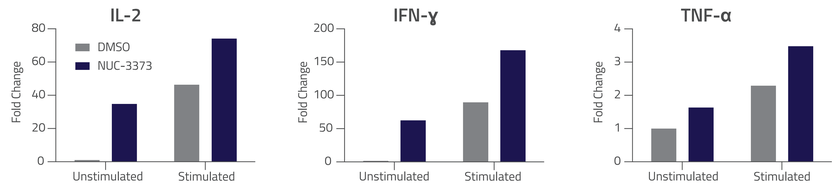

Figure 2. Representative graphs showing effect of 10 µM NUC-3373 (± PBMC stimulation in coculture) on gene expression over 24 hours

- NUC-3373 increases gene expression of pro-inflammatory cytokines
  - Effect potentiated by PBMC stimulation

NUC-3373 induces PD-L1 expression on CRC and immune cells

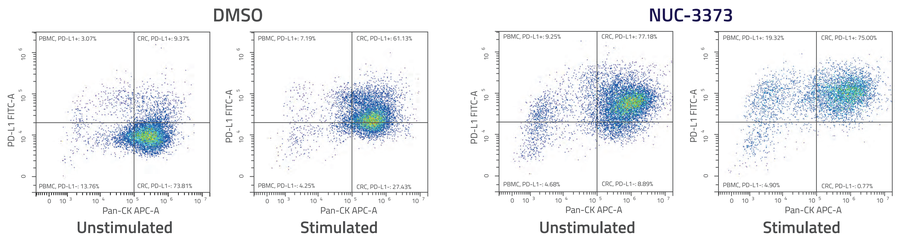

Figure 3a. PD-L1 surface expression on HCT116 CRC cells pre-treated with 10 µM NUC-3373 and cocultured with PBMCs after 48 hours

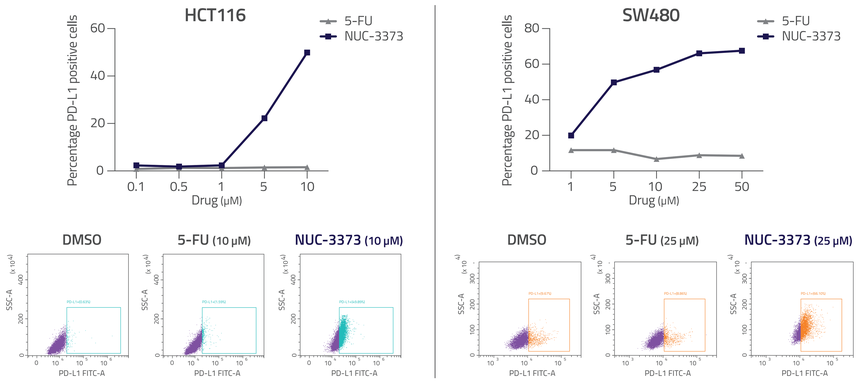

Figure 3b. PD-L1 surface expression in HCT116 (MSI) and SW480 (MSS) CRC cells treated with 5-FU or NUC-3373

- NUC-3373 increased PD-L1 surface expression on CRC cells and PBMCs
  - Effect observed regardless of PBMC stimulation
- NUC-3373 increased PD-L1 surface expression on CRC cells in the absence of PBMCs
  - Independent of MSI status
- 5-FU had no effect on PD-L1 surface expression (equimolar doses)
- These data suggest that PD-L1 induction is NUC-3373 mediated

NUC-3373 enhances immunogenic cell death

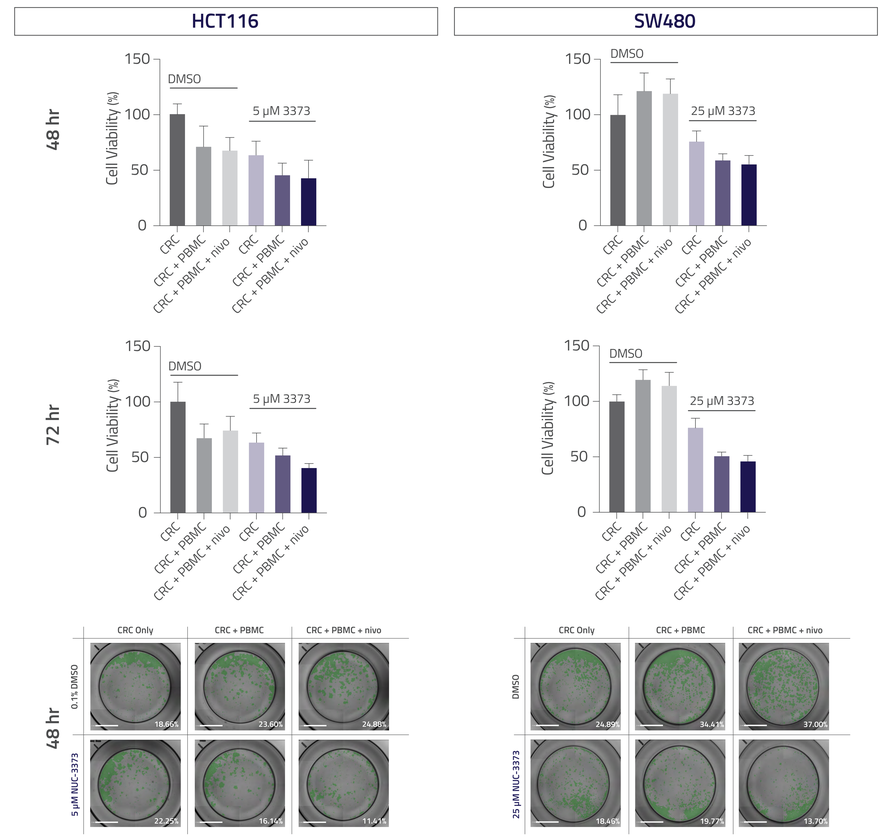

Figure 4. Cell viability of HCT116 (MSI) and SW480 (MSS) CRC cells pre-treated with NUC-3373 and cultured with PBMCs

- NUC-3373 pre-treatment of CRC cells:
  - Enhances ICD caused by PBMCs regardless of MSI status
  - Potentiates the effect of nivolumab

CONCLUSION

- **NUC-3373 induces DAMPs & pro-inflammatory cytokines**
  - **Promoting an anti-cancer immunological environment**
- **NUC-3373 induces PD-L1 expression on CRC & immune cells**
- **NUC-3373 evokes immunogenic cell death & potentiates the effect of nivolumab**

NUC-3373 is an attractive combination partner for PD-1/PD-L1 inhibitors

REFERENCES: 1. Vande Voorde J *et al.*, 2011 *Biochem Pharmacol*; 82: 441-452 2. McGuigan C *et al.*, 2017 *Med Chem*; 27: 7247-7258 3. Ghazaly E *et al.*, 2017 Ann Oncol; Suppl_5128 4. Derissen EJ *et al.*, 2016 *Clin Pharmacol*; 81: 949-957 5. McKissock FG *et al.*, 2020 *Cancer Res*; 80 (Suppl_16): 1848 6. Read OJ *et al.*, 2021 *Cancer Res*; 81 (Suppl_13): 1655

ABBREVIATIONS: 5-FU: 5-fluorouracil, APC-A: Allophycocyanin, ATP: Adenosine triphosphate, CD91: α2-macroglobulin receptor, CRT: Calreticulin, CRC: Colorectal cancer, DAMPs: Damage associated molecular patterns, DPD: Dihydropyrimidine dehydrogenase, DMSO: Dimethyl sulfoxide, DNA: Deoxyribonucleic Acid, DC: Dendritic cell, ER: Endoplasmic reticulum, FITC-A: Fluorescein isothiocyanate, FUDR-MP: Fluorodeoxyuridine monophosphate, HMGB1: High mobility group box 1, HSP-70: Heat shock protein 70, ICD: Immunogenic cell death, IFN-γ: Interferon gamma, IL-2: Interleukin 2, MSI: Microsatellite instability, MSS: Microsatellite stabile, NK: Natural killer, P2XR: P2X receptor, Pan-CK: Pan cytokeratin, PBMC: Peripheral blood mononuclear cells, PD-1: Programmed cell death receptor 1, PD-L1: Programmed death ligand 1, qPCR: Quantitative polymerase chain reaction, SSC-A: Side scatter, TLR: Toll-like receptor, TNF-α: Tumor necrosis factor alpha, TS: Thymidylate synthase