NUC-3373 Induced DAMPs release in CRC cells promotes natural killer cell activation

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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) preventing the conversion of dUMP to dTMP[1]
- Clinical efficacy of 5-FU is limited by
  - Short plasma half-life (8-14 minutes)[1,2] necessitating prolonged administration (46 hours)
  - Over 85% broken down by dihydropyrimidine dehydrogenase (DPD)[3]
  - Production of toxic catabolites such as FBAL (implicated in hand-foot syndrome)
  - Complex enzymatic activation, including thymidine phosphorylase (TP) and thymidine kinase (TK), conferring resistance

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
  - Protected from breakdown by DPD
  - FUDR-MP generation independent of intracellular enzymatic activation
- NUC-3373 generates high levels of FUDR-MP compared to 5-FU in patient PBMCs (31 µM vs 0.1 µM)[4]
- Currently being investigated in clinical studies
  - NuTide:301 - Phase 1 dose-finding study in advanced solid tumors
  - NuTide:302 - Phase 1b combination study in advanced colorectal cancer (CRC)

DAMPs and the immune microenvironment

- Interaction between cancer cells and immune cells in the microenvironment is key for determining patient prognosis and outcome
- Some chemotherapeutics have increased efficacy in immunocompetent over immunocompromised environments due to release of damage associated molecular patterns (DAMPs) from dying/injured cells[5]
- In addition to activation and maturation of dendritic cells, DAMPs can also activate NK cells, a key component of innate immunity[6]
- NUC-3373 induces ER stress and the release of immunogenic DAMPs from CRC cells[7]
  - CRT, ATP, and HMGB1
  - DAMP release occurred at a low dose of NUC-3373 (10 µM)

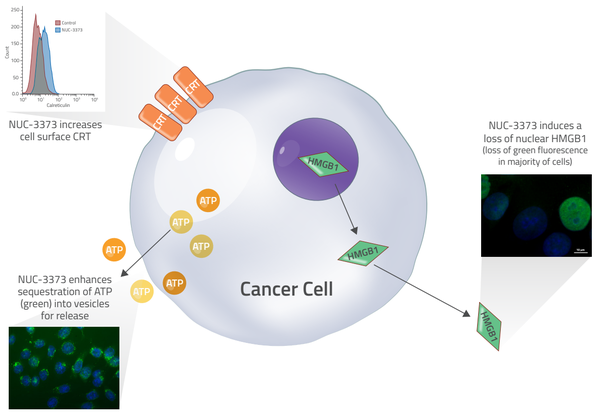


Figure 1: NUC-3373 causes release of DAMPs from stressed cancer cells

Aim

Investigate the effect of NUC-3373 on innate immunity utilizing a co-culture system

Hypothesis

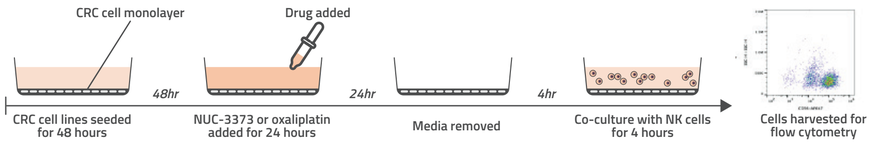
NUC-3373 induced DAMP release activates NK cells contributing to an anti-tumor immune response

METHODS

Characteristics of human CRC cell lines

	HCT116	HT29	SW480
Approximate doubling time (hr)	20	24	38
Basal TS expression	High	Low	Med
Microsatellite status	MSI	MSS	MSS
Concentration NUC-3373 (µM)	10	15	40

CRC and NK cell co-culture system



- NK-92 MI cell line used as a model of NK cells[8]
- Negative Control: DMSO (equivalent dilution) was used as a vehicle control for each cell line
- Positive control: PMA/ionomycin for NK activation
- DAMPs inducer: Oxaliplatin (10 µM)
- For surface Hsp70 experiment there was no co-culture step

Markers

Marker	Description
Hsp70	Stress marker on CRC cells
CD56 (NCAM)	Distinguishes NK cells from CRC cells
LAMP1 (CD107a)	Degranulation marker indicating release of cytolytic vesicles from NK cells
IFN-γ	Indicates NK cell activation
TIGIT	Immunoinhibitory checkpoint molecule, causes NK cell exhaustion

RESULTS

NUC-3373 causes Hsp70 surface exposure on CRC cells indicative of a stress response

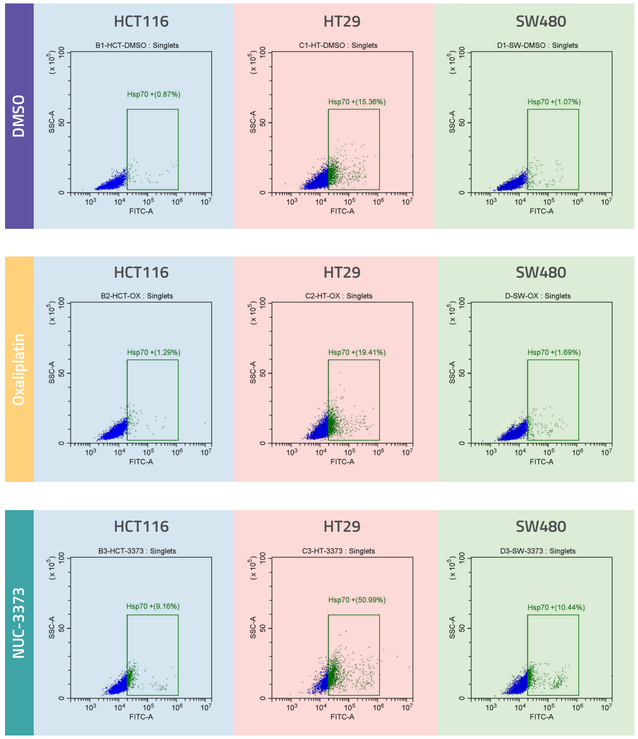


Figure 2: Proportion of CRC cells positive for surface Hsp70 expression

- NUC-3373 caused an increase in cells expressing Hsp70 on the plasma membrane
  - Hsp70 translocates to the cell membrane in stressed cancer cells
  - Hsp70 induces NK activation via interaction with Toll-like receptor 2 (TLR2)

NUC-3373 increases NK activation resulting in degranulation and cytokine production

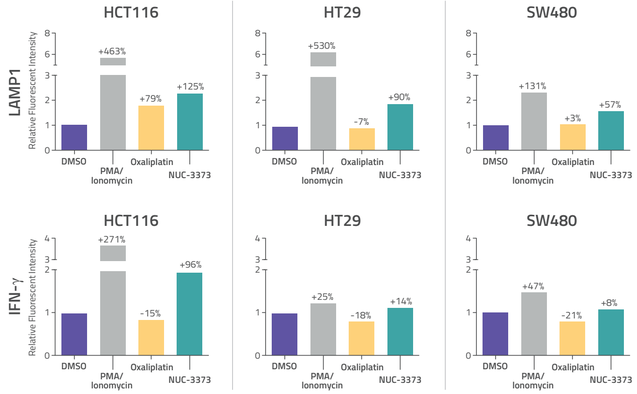


Figure 3: NK cell expression of LAMP1 and IFN-γ following co-culture

- NUC-3373 causes increased surface LAMP1 expression indicating NK cells are being activated for cytotoxic activity
- After activation, NK cells upregulate cytokines such as IFN-γ stimulating an anti-tumor response
  - NUC-3373 caused an increase in intracellular staining of IFN-γ
  - Response was most pronounced in HCT116 cells

NUC-3373 reduces expression of immune checkpoint molecule TIGIT on NK cells

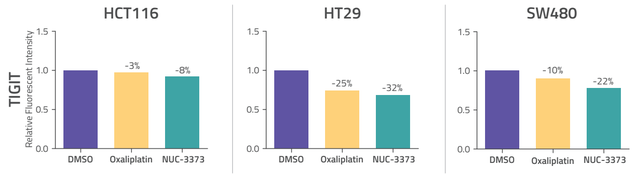


Figure 4: Surface expression of TIGIT on NK cells

- NUC-3373 and oxaliplatin cause a reduction in TIGIT expression on the surface of NK cells
- Reduction in TIGIT may be a consequence of DAMP release from stressed cancer cells
- NUC-3373 induced DAMPs may reduce NK cell exhaustion

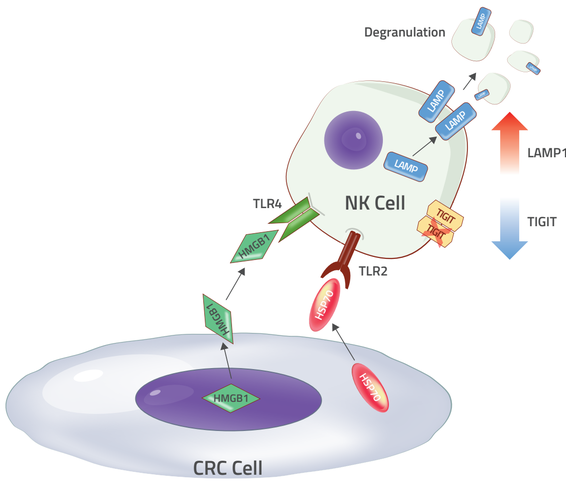


Figure 5: NUC-3373 potentiates an innate anti-tumor immune response in NK cells through upregulation of degranulation and cytokine production and downregulation of inhibitory checkpoint TIGIT

CONCLUSION

- NUC-3373 is a targeted TS inhibitor resulting in DNA damage and cancer cell death
- NUC-3373 also induces ER stress that promotes DAMP release
- NUC-3373 induced DAMPs activate a natural killer response
  - Increased degranulation and cytokine production
  - Output observed in CRC cell lines with different MSI/MSS and basal TS expression
- NUC-3373 induced DAMPs may restore NK cell-mediated immune responses by reducing inhibitory signals
- NUC-3373 has the potential to evoke immunogenic cell death and may enhance the clinical utility of immunotherapy agents

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ABBREVIATIONS: 5-FU: 5-Fluorouracil ATP: Adenosine triphosphate CD: Cluster of differentiation CRC: Colorectal carcinoma CRT: Calreticulin DAMPs: Damage associated molecular patterns DMSO: dimethyl sulfoxide DPD: Dihydropyrimidine dehydrogenase dTMP: Deoxythymidine monophosphate dUMP: Deoxyuridine monophosphate ER: Endoplasmic reticulum FBAL: Fluoro-beta-alanine FUDR-MP/FdUMP: Fluorodeoxyuridine monophosphate HMGB1: High mobility group box 1 hr: hour Hsp70: Heat shock protein 70 IFN-g: Interferon gamma LAMP1: Lysosomal membrane associated protein 1 MSI: Microsatellite instability MSS: Microsatellite stable NCAM: Neural cell adhesion molecule NK: Natural killer PMA: phorbol myristyl acetate TIGIT: T cell immunoreceptor with Ig and ITIM domains TK: thymidine kinase TLR2/4: Toll-like receptor 2/4 TP: thymidine phosphorylase TS: Thymidylate synthase