NUC-1031 causes release of DAMPs and upregulates PD-L1 expression in lung cancer cells

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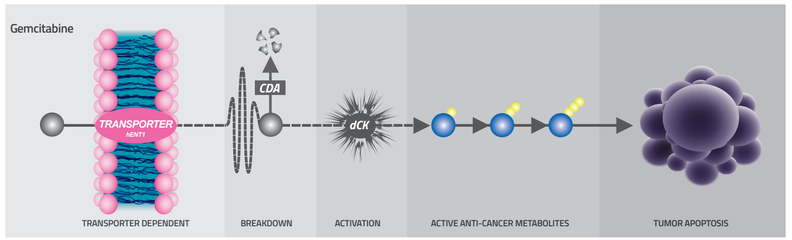
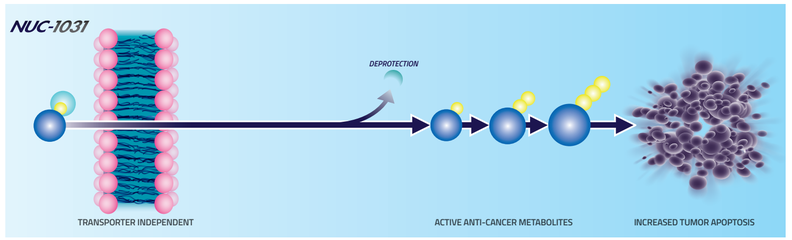
Background

- Gemcitabine remains the backbone of therapy for the treatment of a broad range of tumors including: biliary tract, pancreatic, ovarian, non-small cell lung, bladder and breast cancers
- Gemcitabine activity is dependent on conversion to the active anti-cancer metabolite, dFdCTP, which disrupts DNA synthesis[1-3]
- Three key cancer resistance mechanisms have been associated with a poor survival outcome in patients receiving gemcitabine

NUC-1031 bypasses the resistance mechanisms associated with gemcitabine





NUC-1031: The first-anti-cancer ProTide

- ProTide transformation of gemcitabine
- Overcomes the key gemcitabine resistance mechanisms[4]
  - Cellular uptake independent of nucleoside transporters (hENT1)
  - Activation independent of deoxycytidine kinase (dCK)
  - Protected from breakdown by cytidine deaminase (CDA)
- In comparison to gemcitabine, NUC-1031 has[5]
  - Greater plasma stability ($T_{1/2}$ 8.3 hours vs 1.5 hours)
  - Increased intracellular levels of active anti-cancer metabolite dFdCTP (217x)
  - Reduced toxic metabolites

Scientific Rationale

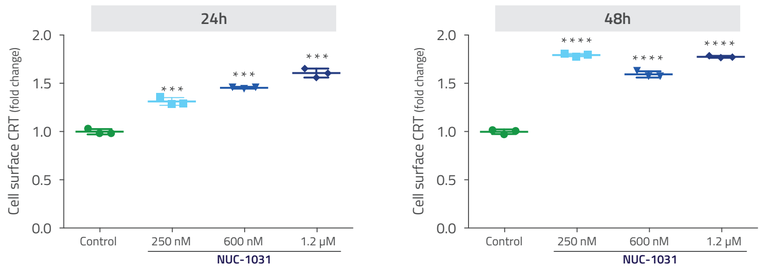
- In addition to causing DNA damage, we hypothesize that NUC-1031 can stimulate release of damage-associated molecular patterns (DAMPs) and promote an immune anti-tumor response resulting in immunogenic cell death (ICD)
- To investigate this we assessed:
  - Exposure of cell surface calreticulin (CRT)
  - Vesicular packaging of adenosine triphosphate (ATP)
- We also investigated the effect of NUC-1031 on PD-L1 expression

Methods

- Human non-small cell lung cancer cells (A549), were treated with NUC-1031 (IC_{50} : 600 nM)
- Cell surface expression of CRT and PD-L1 were assessed by flow cytometry
- Intracellular ATP was labeled using quinacrine

Results

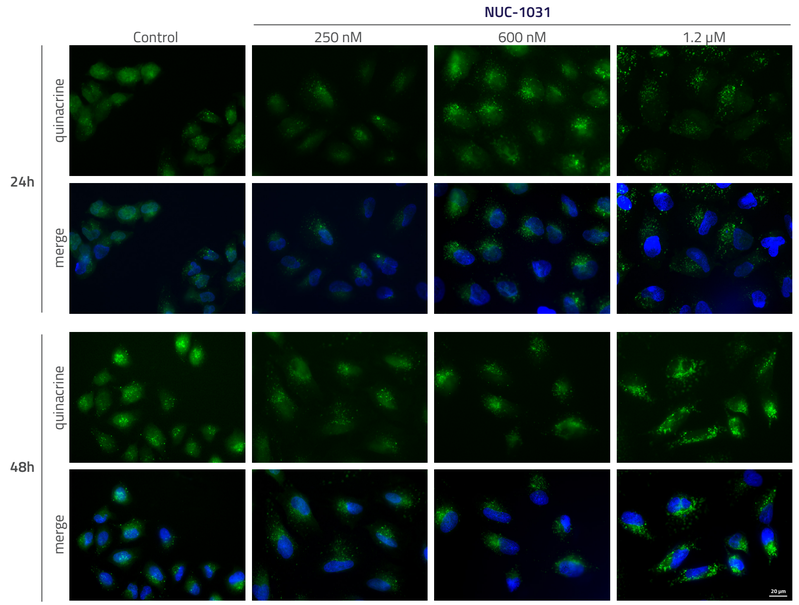
NUC-1031 increases cell surface expression of CRT



***p <0.001; ****p <0.0001 compared to control (unpaired t-test)

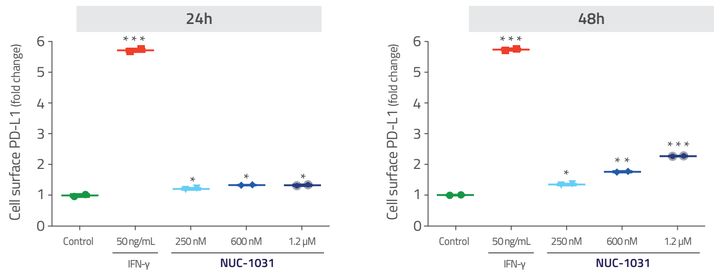
- NUC-1031 promoted the translocation of CRT to the plasma membrane
- Increase of CRT on cell surface is dose-dependent at 24h
- A prolonged response was evident at 48h

NUC-1031 enhances the sequestration of ATP into vesicles for extracellular release



- NUC-1031 increases cell size (arrested in S-phase[6])
- NUC-1031 causes cells to display less intracellular diffuse staining and an increase in punctate staining at 24h, which is further evident at 48h
- This suggests that ATP is being packaged into vesicles for extracellular release[7]

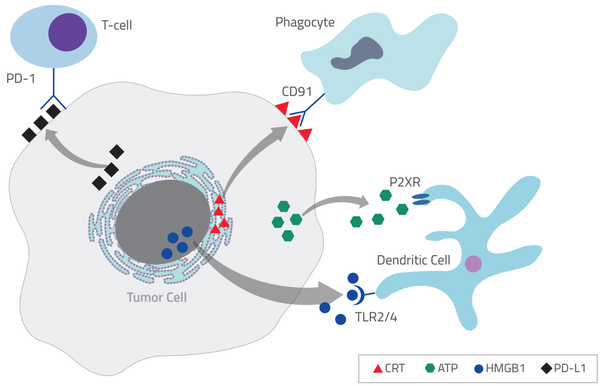
Relocalization of PD-L1 on cell surface is modest in NUC-1031 treated cells



IFN-γ positive control of PD-L1 induction (JAK/STAT pathway)
*p <0.05; **p <0.01; ***p <0.001 compared to control (unpaired t-test)

- NUC-1031 causes a modest increase in cell surface PD-L1 at 24h and 48h without a significant increase in mRNA expression

Cell stress influences immune cells in tumor microenvironment



ICD-associated DAMPs

- Translocation of CRT to the plasma membrane
- Secreted ATP is recognized as a localization signal that attracts immune cells to tumor microenvironment
- Extracellular HMGB1 has a paracrine role, promoting the processing and presentation of tumor antigens by dendritic cells

PD-L1

- Surface expression of PD-L1 increases

Conclusion

- NUC-1031 causes DNA damage resulting in cancer cell death
- In addition, cell injury caused by NUC-1031 is associated with release of DAMPs, which promote immunogenic cell death and acts as a pro-apoptotic signal
- Associated with this, is a modest increase in cell surface PD-L1, which may act as a pro-survival signal
- NUC-1031's direct cytotoxicity may be enhanced by targeting the PD-1/PD-L1 axis, shifting the balance further towards immune-mediated cell death

REFERENCES: 1. Huang *et al. Cancer Research* 1991; 51, 6110–6117 2. Cappella *et al. International Journal of Cancer* 2001; 93, 401–408 3. de Sousa Cavalcante *et al. European Journal of Pharmacology* 2014; 741, 8–16 4. Slusarczyk *et al. J Med Chem* 2014; 57:1531–1542 5. Bladgen *et al. Br J Cancer* 2018;119:815–822 6. Sarr *et al. Sci Rep.* 2019;9(1):7643 7. Martins *et al., Cell Death and Differentiation* (2014) 21, 79–91

ABBREVIATIONS: ATP: adenosine triphosphate CD91: alpha 2-macroglobulin receptor CDA: cytidine deaminase CRT: calreticulin DAMPs: damage associated molecular patterns dCK: deoxycytidine kinase dFdCDP: 2',2'-difluorodeoxycytidine 5'-diphosphate dFdCMP: 2',2'-difluorodeoxycytidine 5'-monophosphate dFdCTP: 2',2'-difluorodeoxycytidine 5'-triphosphate dFdU: 2',2'-difluorodeoxyuridine HMGB1: high mobility group box 1 ICD: immunogenic cell death IFN-γ: interferon gamma P2XR: P2X receptor PD-1: programmed cell death protein 1 PD-L1: programmed cell death ligand 1 $T_{1/2}$: half-life TLR2/4: toll-like receptor 2/4