NUC-3373 induces ER stress and the release of DAMPs in colorectal cancer cells

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Background
- 5-fluorouracil (5-FU) remains the cornerstone of treatment for patients with a broad range of tumors
- The active anti-cancer metabolite of 5-FU is FUDR-MP (5- FU nucleotide)
- FUDR-MP bypasses the resistance mechanisms associated with 5-FU
- NUC-3373 is a targeted inhibitor of TS
- NUC-3373 induces ER stress and the release of DAMPs
- NUC-3373 bypasses the resistance mechanisms associated with 5-FU

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Methods
- Human CRC cells (n=11) were treated with 10µM NUC-3373 (log10 M)
- BiP and TS free and ternary complex protein expression were measured by Western blot (adult cell line)
- TS was knocked down using TMS-targeting siRNA
- Cells were supplemented with 10µg/ml thymidine to prevent tDMP depletion and subsequent DNA damage
- Calreticulin (CRT) was assessed by flow cytometry and fluorescence microscopy (24 h exposure)
- Nuclear high mobility group box protein 1 (HMGB1) was assessed by fluorescence microscopy (1 h exposure)

Results
- NUC-3373 is a targeted TS inhibitor
- Thymidine-rescues cells from NUC-3373-induced death
- Supplementing nucleotide pool with exogenous thymidine counteracts the effects of TS inhibition
- Maintains pool of dTTP allowing DNA replication and repair to continue
- Confirming that NUC-3373 targets the de novo pathway of dTMP synthesis

Discussion
- NUC-3373 causes rapid formation of TS ternary complexes, which correlate strongly with BiP upregulation (R²=0.97)
- TS knockdown studies confirmed that TS ternary complex formation is necessary for the induction of ER stress
- Immunoglobulin-binding protein (BiP) was used as a marker of unfolded protein response (UPR) activation in CRC cells
- Nuclear high mobility group box protein 1 (HMGB1) was assessed by fluorescence microscopy (24 h & 48 h exposure)
- Calreticulin (CRT) was assessed by flow cytometry and fluorescence microscopy (24 h exposure)
- BiP and TS (free and ternary complex) protein expression were measured by Western blot (whole cell lysates)

Conclusion
- NUC-3373 is a targeted TS inhibitor resulting in DNA damage and cancer cell death
- NUC-3373 decreases expression of BiP and HMGB1
- Under resting conditions, CRT is normally resident in the lumen of the rough ER, which is continuous with the nuclear envelope
- NUC-3373 causes CRT translocation from the ER to the cell surface
- NUC-3373 has the potential to evoke immunogenic cell death and may enhance the clinical utility of immunotherapy agents

References:
- Blagden et al. Mol Cancer Ther 2011; 27: 7247-7258