From bench to bedside: Using ProTide chemistry to transform 3'-deoxyadenosine into the novel anti-cancer agent NUC-7738

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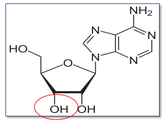
BACKGROUND

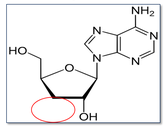
3'-deoxyadenosine (3'-dA)

- 3'-deoxyadenosine (3'-dA; cordycepin) is an adenosine derivative isolated from Cordyceps sinensis[1]
- 3'-deoxyadenosine triphosphate (3'-dATP), the active anti-cancer metabolite of 3'-dA, causes cell death by inhibiting DNA and RNA synthesis, inducing apoptosis and activating AMPK [2,3]
- 3'-dA has not been successful in clinical studies due to cancer resistance mechanisms including:
  - Rapid enzymatic degradation by adenosine deaminase (ADA)
  - Cellular up take dependent on nucleoside transporters (hENT1)
  - Rate limiting phosphorylation by adenosine kinase (AK) to generate active metabolite (3'-dATP)

Adenosine

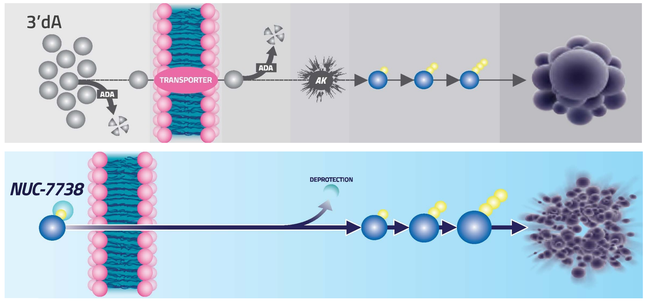


3'-deoxyadenosine



ProTides: Nucleotide Analogues

- A new class of anti-cancer agents
- Transformative phosphoramidate chemistry
- Increase intracellular levels of anti-cancer metabolites
- Broad clinical utility



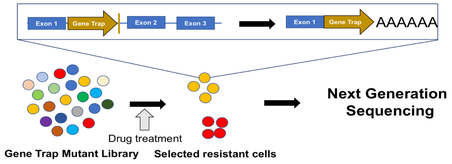
NUC-7738: A phosphoramidate transformation of 3'-dA

- Designed to overcome the cancer resistance mechanisms associated with the uptake, activation and breakdown of 3'-dA

METHOD

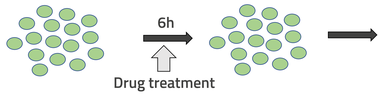
Haploid Genetic Drug Screen

A haploid leukaemia-derived cell line, HAP1, was mutagenized using retroviral gene trapping and the mutant pool was selected for clones resistant to 3'-dA and sequenced by NGS[4]. CRISPR/Cas9 knock out combined with pharmacokinetic determination of the IC50 enabled validation of the top hits.



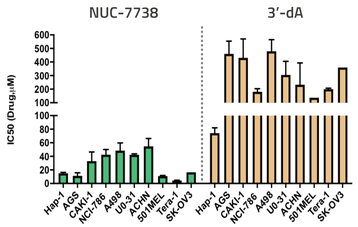
RNA Sequencing

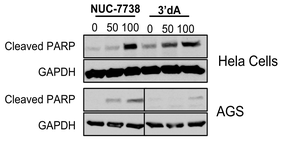
HAP1 cells were treated with 3'-dA or NUC-7738 for 6h before total RNA isolated. RNA deep sequencing-based transcriptomic analysis and gene set enrichment were used to identify the signalling pathways most affected and to define possible mechanisms of action.



RESULTS

NUC-7738, a ProTide version of 3'-dA, has cytotoxic activity

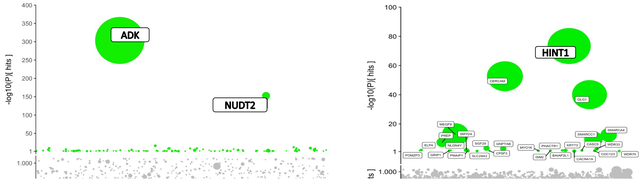




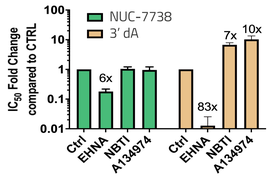
NUC-7738 has greater potency in killing cancer cells than 3'-dA. NUC-7738 demonstrated up to 185x greater anti-cancer potency than 3'-dA across a variety of cancer cells

NUC-7738 and 3'-dA induce apoptosis. Gastric or cervical cancer cells were treated with NUC-7738 or 3'-dA for 24 hours, cleaved PARP was detected by western blotting, indicating activation of apoptosis *in vitro*.

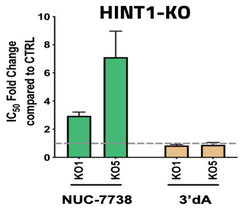
NUC-7738 is resistant to ADA breakdown, its cellular uptake is independent of hENT1 transport channels and its activity bypasses the intracellular activating enzyme

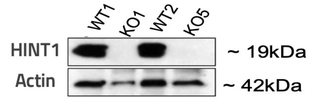


Genome-wide haploid genetic screen identifies genes that confer resistance to 3'-dA and NUC-7738. Gene trap experiments showed that genes encoding the intracellular activating enzyme AK and the hENT1 transporter were amongst the highest enriched genes for 3'-dA while no enrichments were found for these genes in NUC-7738 treated cells. HINT1 (Histidine triad nucleotide-binding protein 1), a phosphoramidase known to be involved in purine metabolism and known to activate NUC-7738 was strongly enriched as expected in all three screens for NUC-7738.



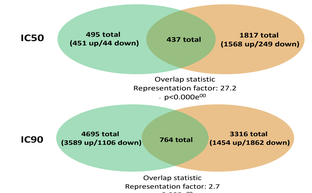
Chemical perturbation of known enzymes involved in processing of 3'-dA. *In vitro* inhibition assays showed that unlike 3'-dA, NUC-7738 is resistant to ADA breakdown, is not reliant on hENT1 transport for its cellular uptake, and is independent of ADK for its activity. HAP1 cells were treated with NUC-7738 and 3'-dA in the presence or absence of the ADA antagonist EHNA, the hENT1 antagonist NBTI, or the ADK inhibitor A134974.



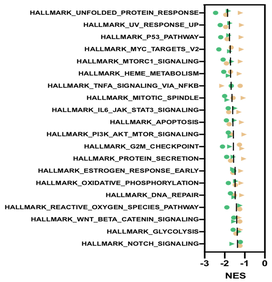


Validation of top hit from genome wide haploid screen. Depletion of HINT1 by CRISPR/Cas9 in HAP1 cells markedly reduced sensitivity to NUC-7738 but not 3'-dA, indicating specificity.

RNAseq of 3'-dA and NUC-7738 treated HAP1 cells

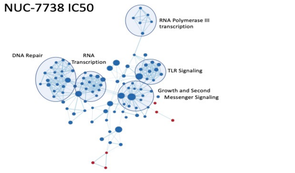


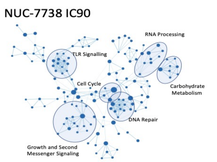
Venn diagram summarizing the number of differentially expressed genes (padj <0.05 and -2>FC>2) in 3'-dA and NUC-7738 treated samples



Both NUC-7738 and 3'-dA down-regulate genes involved in cell survival. Summary of Top 20 enriched pathways for Hallmark set enrichment analysis of hits for NUC-7738 and 3'-dA treated cells. Green and orange indicate NUC-7738 and 3'-dA, respectively. Circles and triangles indicate dosing at IC_{50} and IC_{90}, respectively.

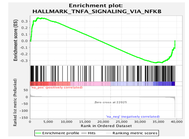
Core target genes underpin the activity of both 3'-dA and NUC-7738. Expression heatmap of 91 genes commonly differentially expressed in all 4 tested conditions

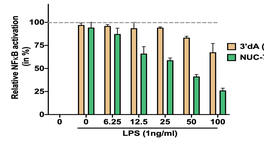




Gene set enrichment analysis (GSEA) and network mapping using Cytoscape. Genes ranked according to their p-value and GSEA performed on REACTOME gene sets. Gene overlaps between different pathways are shown. Nodes are GSEA enriched pathways while Edges represent overlapping shared genes between two pathways.

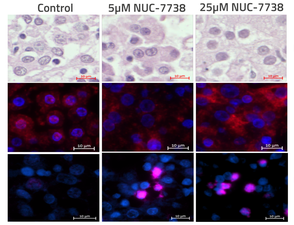
NUC-7738 and 3'-dA affect NFkB pathway *in vitro, ex vivo* and *in vivo*





NFκB signalling cascade was one of the most strongly enriched pathways. Representative enrichment plots for NFkB pathway after transcriptional profiling of NUC-7738 and 3'-dA treated cells.

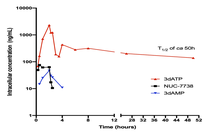
Reduced NFκB activity after NUC-7738 or 3'-dA treatment. NFkB activity was measured using the SEAP reporter gene assay in THP-1 cells. NFkB activity was induced by LPS in the presence or absence of NUC-7738 or 3'-dA.



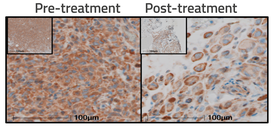
Reduction in the nuclear translocation of NFkB p65 and induction of apoptosis in *ex vivo* model. *Ex vivo* renal cell carcinoma (RCC) tissue treated with NUC-7738 for 24 hours. Immunofluorescence shows a reduction in the nuclear localisation of NFkB, increase in cleaved-caspase-3 and numbers of shrunken pyknotic nuclei.

NUC-7738 is successfully metabolised to 3'-dATP in patients

NuTide:701 is a first-in-human dose-escalation and expansion study of NUC-7738 to which 21 patients with advanced cancers have been enrolled so far (Jan 2021). NUC-7738 has been administered at escalating intravenous weekly doses ranging from 14-900 mg/m^2. The study is open to all patients with advanced solid tumors or lymphoma.



Intracellular levels of 3'-dATP, 3'-dAMP and NUC-7738 in PBMCs from 7 patients treated with NUC-7738 at 400-900 mg/m^2 showing prolonged *in vivo* release of high levels of 3'-dATP.



Redistribution of NFkB to the cell periphery in post-treatment tissue samples. IHC staining for RelA subunit of NFkB (brown) of tissue samples from melanoma patient in NuTide:701 study showing shift from nuclear to cytoplasmic location.

CONCLUSIONS

- NUC-7738 is designed to overcome the key resistance mechanisms of 3'-dA
- NUC-7738 generates high and prolonged intracellular levels of the active anti-cancer metabolite, 3'-dATP
- NUC-7738 is activated by HINT-1, which is ubiquitously expressed in cancer cells (our data and protein atlas)
- NUC-7738 causes cell death by activation of apoptotic pathways as well as through inhibition of NFkB nuclear translocation
- NuTide:701 is an ongoing Phase I study that will establish the RP2D and assess safety in patients with advanced solid tumors

REFERENCES: 1.Tuli, H. S. et al. Life Sci. 93, 863–869 (2013), 2 Kondrashov, A. et al. RNA 18, 2236–2250 (2012) 3. Hawley, S. A. et al. Cell Chem Biol 27, 214–222.e4 (2020) 4. Bürckstümmer, T. et al. Nat Methods 10, 965–971 (2013) ACKNOWLEDGEMENT: This work was funded by NuCana plc.