NUC-7738 promotes alternative polyadenylation site usage and reduces glutaminase GAC isoform

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Abstract Number: 277 **Email:** mustafaelshani@nucana.com

BACKGROUND

Glutamine metabolism in cancer

- Metabolic dysregulation, such as the Warburg effect, is hallmark of cancer and allows tumor cells to sustain high rates of proliferation in unfavorable conditions, including hypoxia[1]
- In addition to glucose, cancer cells rely on glutamine as a major source of energy that feeds into the tricarboxylic acid (TCA) cycle[2]
- Glutaminase-1 (GLS1), the rate limiting enzyme that converts glutamine to glutamate, is frequently upregulated in cancer[3]

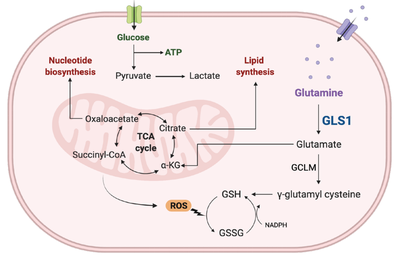

Figure 1. Glycolysis, glutamine metabolism and the TCA cycle

GLS1 isoforms

- GLS1 has two isoforms, generated through alternative polyadenylation and splicing: glutaminase C (GAC) and kidney glutaminase (KGA)[4]
- The presence of GAC favors more metabolically active cell growth, and the GAC:KGA ratio negatively correlates with patient survival[5]
- GAC is highly expressed in kidney, lung and pancreatic cancers[5-7]

NUC-7738: ProTide transformation of 3'-dA (cordycepin)

- Resists breakdown by adenosine deaminase (ADA)
- Generates high intracellular levels of the active anti-cancer metabolite (3'-dATP)
- 3'-dATP is associated with alternative polyadenylation site usage changes[8]
- Induces changes in genes involved in key cellular processes including metabolism, apoptosis, cell differentiation[9-12]
- Currently being investigated as monotherapy and in combination with pembrolizumab in the Phase 1/2 clinical study NuTide:701 (NCT03829254) in patients with advanced solid tumors

Aim

- To investigate the effect of NUC-7738 on GLS1 isoforms in kidney and pancreatic cancer cell lines and *ex vivo* kidney cancer tissue

METHODS

Cell culture: Human renal (786-O and CAKI-1) and pancreatic (MiaPaCa-2 and PANC-1) cancer cell lines were treated with 0.1% DMSO (vehicle control), 20 μM NUC-7738 (renal), 7.5 or 75 μM NUC-7738 (pancreatic) for 6 to 96 hours (doses based on IC_{50} values at 96 hours). Cells were cultured under normoxic and hypoxic conditions (0.5% O_2).

Intracellular metabolites: Cellular 2'&3'-dATP combined levels were determined by LC-MS (LLOQ: 10 nM). 3'-dATP is a structural isomer of endogenous 2'-dATP and cannot be resolved by LC-MS, 2'&3'-dATP values are reported as a sum of both isomers.

GLS1 RNA transcripts: RNA was extracted from adherent cells and GAC and KGA mRNA transcripts were assessed by quantitative RT-PCR using isoform specific primers; GAC exon 14-15 junction and KGA exon 16-17 (normalized to ACTB).

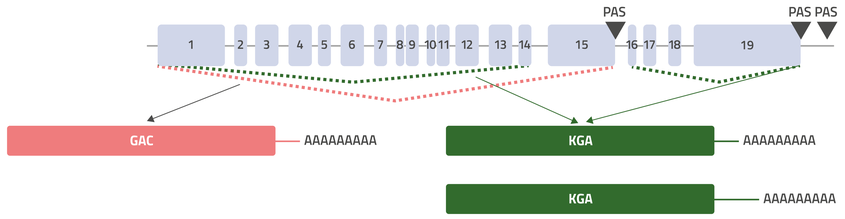

Figure 2. Pre-mRNA and GLS1 transcript isoforms: The canonical isoform, KGA, is generated through exon 1-14 and 16-19; the shorter isoform, GAC, is spliced with an alternate exon and 3'-untranslated region (exon 1-15)[5].

GAC & KGA isoform protein expression: Optimization of GAC-specific antibody (Proteintech, 66265-1-IG) and KGA-specific antibody (Proteintech, 20170-1-AP) was performed to determine the linear range concentration. Whole cell protein lysates were probed with GAC- and KGA-specific antibodies and analyzed by automated JESS Western blot.

***Ex vivo* patient tumor samples:** Kidney cancer tissue (1 cm^3) was collected from patients undergoing total nephrectomy. Sections (250 μm) were treated with 0.1% DMSO or 50 μM NUC-7738 for 24 hours. Immunohistochemistry was performed on paraffin-embedded sections using GAC-specific antibody.

RESULTS

NUC-7738 rapidly generates 3'-dATP

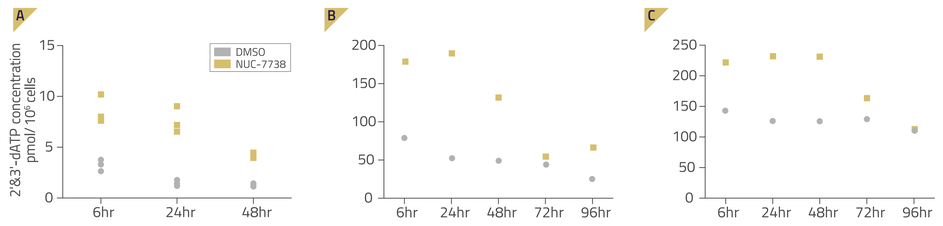

Figure 3. 2' and 3'-dATP levels in A) 786-O renal cancer cells (n=3), B) MiaPaCa-2 pancreatic cancer cells (n=1) C) PANC-1 pancreatic cancer cells (n=1). Each data point represents one biological replicate.

- NUC-7738 is rapidly converted to its active metabolite in 786-O, MiaPaCa-2 and PANC-1 cell lines with C_{max} ranging from of 8 to 200 pmol/10^6 cells

NUC-7738 reduces GAC isoform

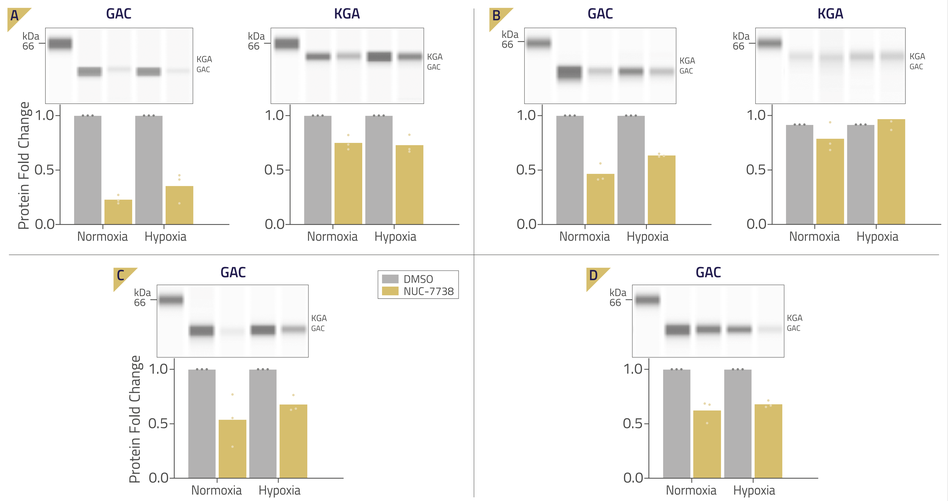

Figure 5. GAC and KGA protein levels in renal cancer cells A) 786-O and B) CAKI-1 and in pancreatic cancer cells C) MiaPaCa-2 and D) PANC-1, treated with DMSO or NUC-7738 for 48 hours under normoxic and hypoxic conditions. Each data point represents an independent biological replicate (n=3).

- NUC-7738 causes approximately 80% reduction of GAC protein in both renal cancer cell lines under normoxic and hypoxic conditions
- NUC-7738 causes approximately 25% reduction of KGA protein in 786-O cells but no change in CAKI-1 cells
- NUC-7738 causes approximately 40% reduction of GAC protein in PANC-1 and MiaPaCa-2 pancreatic cancer cell lines, under both normoxic and hypoxic conditions
- KGA isoform was not detectable in pancreatic cancer cells

NUC-7738 reduces GAC mRNA levels

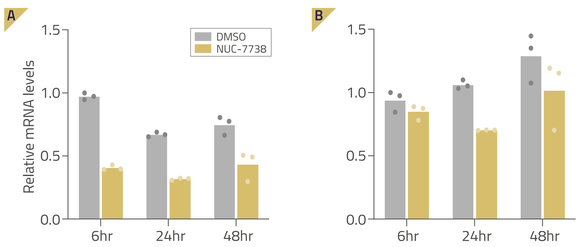

Figure 4. Relative mRNA levels of A) GAC and B) KGA mRNA transcripts in CAKI-1 renal cancer cells. Each data point represents independent biological replicates (n=3).

- NUC-7738 reduced GAC mRNA by >50% in CAKI-1 renal cancer cells for at least 48 hours
- There was approximately 30% decrease in KGA mRNA at 24 hours but no change at other time points

NUC-7738 reduces GAC isoform in *ex vivo* kidney cancer tissue

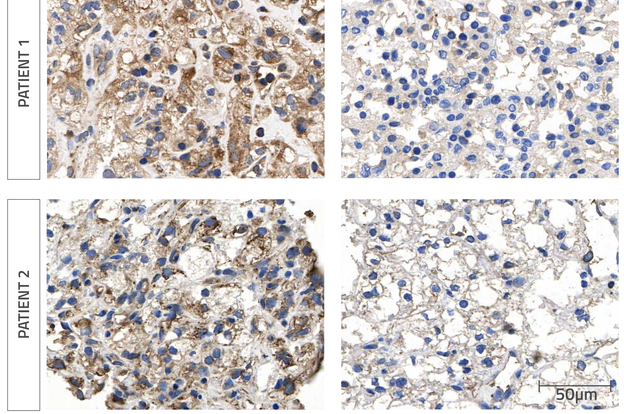

Figure 6. Brown staining represents GAC protein isoform in *ex vivo* kidney cancer tissue (nuclei stained blue). Shown are 2 two adjacent slices from individual patients.

- NUC-7738 reduced GAC isoform levels in *ex vivo* kidney cancer tissue, with levels becoming barely detectable within 24 hours

CONCLUSION

- NUC-7738 generates sustained intracellular levels of active metabolite 3'-dATP
- NUC-7738 reduces the mRNA and protein levels of GAC and to a lesser extent KGA, decreasing the GAC:KGA ratio
- NUC-7738 reduces GAC isoform in *ex vivo* kidney cancer tissue
- NUC-7738 generated 3'-dATP may promote alternative polyadenylation site usage, which reduces glutaminase GAC isoform
- NUC-7738 may be an effective anti-cancer treatment for glutamine-dependent cancers by interfering with cellular metabolism

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ABBREVIATIONS: TCA: tricarboxylic acid GLS1: glutaminase-1 GAC: glutaminase C KGA: kidney glutaminase 3'-dA: 3'-deoxyadenosine 3'-dATP: 3'-deoxyadenosine triphosphate ADA: adenosine deaminase RNA: ribonucleic acid DMSO: dimethyl sulfoxide IC_{50} half maximal inhibitory concentration 2'-dATP: 2'-deoxyadenosine triphosphate LC-MS: liquid chromatography mass spectrometry LLOQ: lower limit of quantification mRNA: messenger ribonucleic acid ACTB: beta-actin ATP: adenosine triphosphate α-KG: alpha-ketoglutarate GCLM: glutamate cysteine ligase modifier subunit ROS: reactive oxygen species GSH: glutathione GSSG: glutathione disulfide NADPH: nicotinamide-adenine dinucleotide phosphate PAS: polyadenylation site