NUC-7738 Regulates β-catenin Signalling Resulting in Reduced Proliferation and Self-Renewal of AML Cells

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BACKGROUND

Acute Myeloid Leukaemia (AML)

- Heterogeneous clonal hematopoietic disease characterised by immature myeloid cell proliferation and bone marrow failure[1]
- Arises from recurrent genetic alterations within hematopoietic stem and progenitor cells (HSPCs), and may result in the generation of leukaemic stem cells (LSCs)[2]

β-catenin signalling in AML

- Involved in the development and maintenance of AML[3,4]
- β-catenin activation associated with chemotherapy-resistance and poor prognosis[5]
- β-catenin regulates the transcription of c-Myc and CD44
  - c-Myc overactivation has been shown to induce AML in mice[6]
  - CD44 overexpression is associated with chemotherapy-resistance[7]
- Constitutive activation of PI3K/Akt pathway is associated with decreased overall survival[8,9]
  - PI3K/Akt/β-catenin axis may promote enrichment of $CD34^+CD38^-CD123^+$ LSC-like cells[10]

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

- Generates high intracellular levels of the active anti-cancer metabolite (3'-dATP)
- Avoids breakdown by adenosine deaminase (ADA)
- Up to 185x greater anti-cancer activity than 3'-dA across a range of human cancer cell lines
- Currently being investigated in Phase 1 / 2 clinical study NuTide:701 (NCT03829254)

Aim & Hypothesis: Investigate the effect of NUC-7738 on β-catenin signalling in AML cells. NUC-7738 inhibits β-catenin signalling resulting in reduced proliferation and self-renewal of AML cells.

METHODS

Cell culture: Cell lines below were treated with 0.1% DMSO (vehicle control; CTRL) or NUC-7738 for 48 or 72 hrs.

Cell line	Disease	FAB Classification	Genetic Signature
OCI-AML3	AML	M4: myelomonocytic leukaemia	NPM1 mutated, HOX overexpression, DNMT3A R882C
HL-60	AML	M2: myeloblastic leukaemia with maturation	CDKN2A mutated, NRAS mutated, TP53 deleted
U937	Histiocytic lymphoma	M5-like: monocytic leukaemia	MLLT10-PICALM: t(10;11)(p13;q14)
KG1a	AML	M0: early myeloblasts	FGFR1OP2-FGFR1, NRAS mutated, TP53 mutated

Flow Cytometry: Annexin-V/DAPI staining was performed to differentiate live, early apoptotic, late apoptotic and dead cells. Immunophenotyping was performed for the cluster of differentiation (CD) markers, CD34, CD38 and CD123.

Western blot analysis: Immunoblotting was performed using antibodies for β-catenin, c-Myc, CD44, PI3-Kinase p110α, p-Akt (Ser473) and p-GSK3β (Ser9). Signals were normalised to total protein.

Immunofluorescence: Immunofluorescence was performed on formalin fixed paraffin embedded (FFPE) cell pellet sections using the Leica Bond RX autostainer to determine localisation of β-catenin.

Colony Forming Cell (CFC) Assay: MethoCult Enriched media was used to perform the colony forming assay. Colonies were analysed following 14 days using QuPath v0.3.2.

Statistical Analysis: Unpaired Student's t-test was used to derive significant p values. Graphs represent mean ± SD.

RESULTS

NUC-7738 causes apoptosis and reduces viability of AML cells

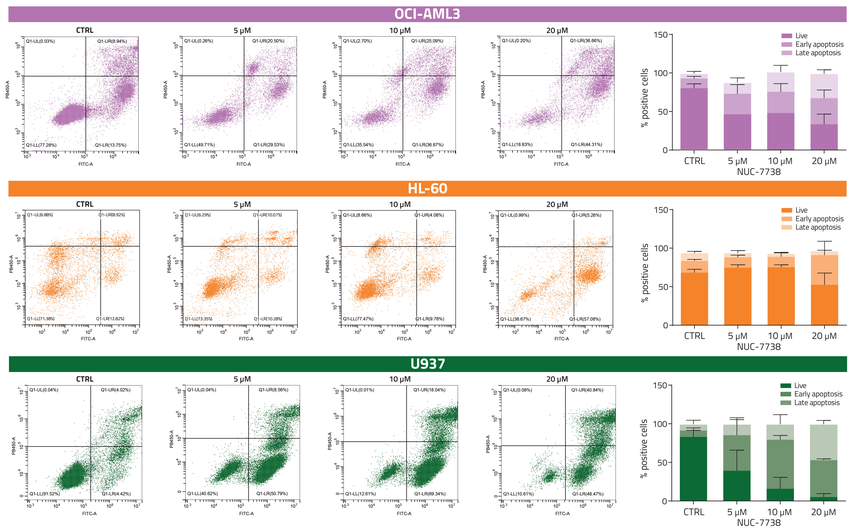
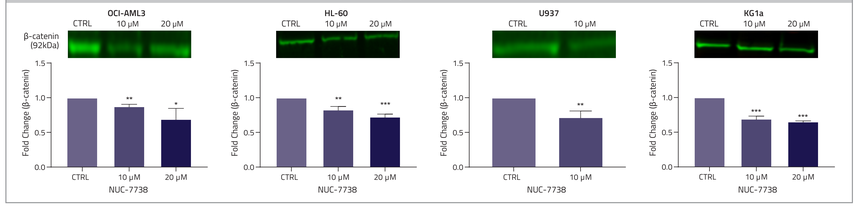


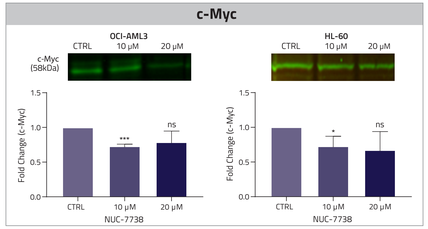
Figure 1. The effect of NUC-7738 on cell viability

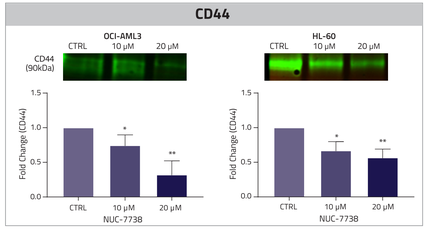
RESULTS (cont)

- NUC-7738 demonstrated dose-dependent cytotoxicity in U937 and OCI-AML3 cells
- Highest proportion of cell death observed at 20 μM in all cell lines (U937, average live cell population at 20 μM = 5.6 %, OCI-AML3, average live cell population 20 μM = 33.9 % and HL-60 average live cell population 20 μM = 53.0 %)

NUC-7738 inhibits β-catenin signalling







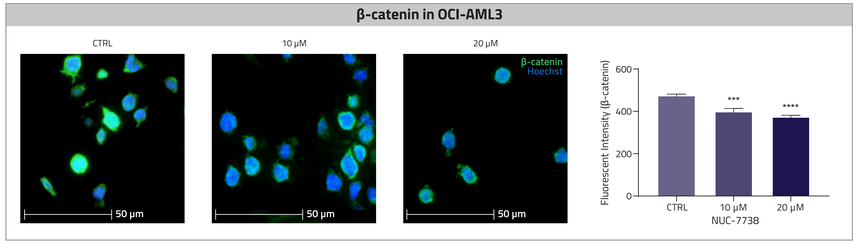


Figure 2. Effect of NUC-7738 treatment on protein expression of β-catenin, c-Myc and CD44, and localisation of β-catenin

- NUC-7738 inhibits β-catenin in multiple AML cell lines
- NUC-7738 reduces expression of c-Myc and CD44
- NUC-7738 reduces nuclear β-catenin expression, indicative of β-catenin signalling inhibition

NUC-7738 reduces the self-renewal of AML cells

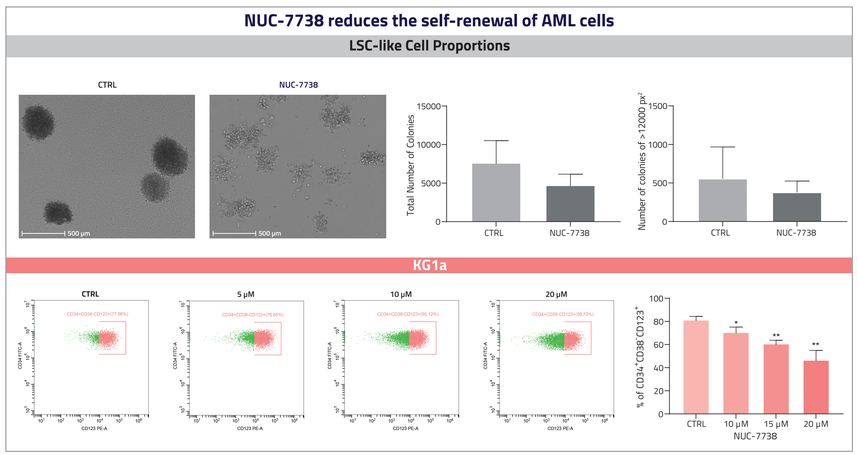


Figure 3. Effect of NUC-7738 on colony formation in HL-60 cells (A) and LSC-like cell proportion (B) in KG1a cells

- NUC-7738 reduces the number and size of leukaemic colonies
- NUC-7738 reduces the percentage of LSC-like ($CD34^+CD38^-CD123^+$) cells

NUC-7738 regulates β-catenin through PI3K/Akt/GSK3β signalling

Figure 4. Effect of NUC-7738 on PI3K/Akt/GSK3β signalling

- NUC-7738 significantly reduces the protein levels of PI3K-p110α, p-Akt (Ser473) and p-GSK3β (Ser9) in OCI-AML3, HL-60 and U937 cells

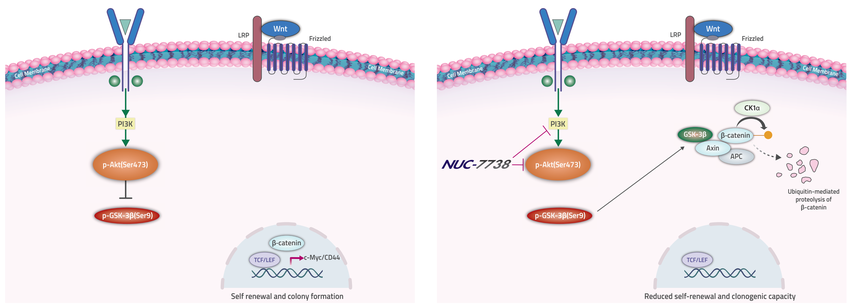


Figure 5. Effect of NUC-7738 on β-catenin signalling pathway in AML

- Constitutive activation of PI3K/Akt pathway inhibits GSK3β-mediated degradation of β-catenin
  - Activated β-catenin increases the transcription of c-Myc and CD44, promoting chemotherapy-resistant LSCs
- NUC-7738 inhibits PI3K/Akt pathway leading to
  - GSK3β-mediated degradation of β-catenin
  - Suppression of clonogenicity and LSC-like cells

Conclusions

- **NUC-7738 induces apoptosis in genetically and morphologically distinct AML cell lines suggesting broad therapeutic potential**
- **NUC-7738 inhibits β-catenin signalling through suppression of PI3K/Akt/GSK3β axis resulting in a reduction in chemotherapy-resistant LSC-like cells**
- **These findings, combined with the anti-cancer activity and favourable safety profile in the clinic, provide support for evaluating NUC-7738 for the treatment of patients with AML**

ABBREVIATIONS: 3'-dA: 3'-deoxyadenosine 3'-dATP: 3'-deoxyadenosine triphosphate ADA: adenosine deaminase AML: acute myeloid leukaemia APC: adenomatous polyposis coli CDKN2A: Cyclin Dependent Kinase Inhibitor 2A CK1α: casein kinase 1α c-Myc: cellular Myc DNMT3A: DNA Methyltransferase 3 A FGFR1: fibroblast growth factor receptor 1 FGFR1OP2: FGFR1 Oncogene Partner 2 GSK3β: glycogen synthase kinase 3 beta HOX: homeobox HSPCs: hematopoietic stem and progenitor cells LEF: lymphoid enhancer-binding factor LRP: low density lipoprotein receptor-related protein LSCs: leukaemic stem cells MLLT10: histone Lysine Methyltransferase DOT1L Cofactor NPM1: nucleophosmin 1 NRAS: NRAS Proto-Oncogene, GTPase PI3K-110α: phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha PICALM: phosphatidylinositol Binding Clathrin Assembly Protein TCF: T-cell factor TP53: tumour protein p53 Wnt: wingless/integrated

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