NUC-7738 causes reduction of soluble and exosome-associated PD-L1 in melanoma cell lines and patients

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INTRODUCTION

- Unmet medical need for patients with metastatic melanoma after progression on immune checkpoint inhibitors (ICIs) and BRAF/MEK inhibitors

Tumor microenvironment (TME)

- In the TME, programmed cell death protein 1 (PD-1) and PD-ligand 1 (PD-L1) interaction can disrupt T-cell activation, allowing tumors to evade the immune system[1]
- ICIs exert anti-cancer effects by modulating the interaction between tumor and immune cells and have become a standard treatment option for multiple types of cancer[2]

Secreted PD-L1 proteoforms

- PD-L1 protein has key secreted forms, including soluble (sPD-L1) and exosomal (exoPD-L1) variants, in part controlled by alternative polyadenylation[3]
- High sPD-L1 concentrations are associated with more advanced stage, poor prognosis, and resistance to ICIs[4,5]
- High exoPD-L1 concentrations in plasma are reported to contribute to T-cell dysfunction[6]

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)
- Induces changes in genes involved in key cellular processes such as metabolism, apoptosis, cell differentiation[7-10]
- Currently being investigated in combination with pembrolizumab in Phase I/II clinical study NuTide:701 (NCT03829254) in patients with advanced solid tumors

Aim:

Investigate the effect of NUC-7738 on secreted forms of PD-L1 in melanoma cells in culture and in patient samples.

METHODS

Cell culture: Human malignant melanoma cells (A375) were treated with 0.1% DMSO (vehicle control) or 10 µM NUC-7738 for 6-96 hours (NUC-7738 dose based on IC_{50} values at 96 hours).

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.

Cell surface PD-L1: Expression on melanoma cells was measured by flow cytometry.

sPD-L1 RNA transcript: RNA extracted from adherent cells was assessed for sPD-L1 mRNA (normalized to ACTB) by RT-qPCR using primers targeting exon 4 and intron 4 junction (ex4-int4).

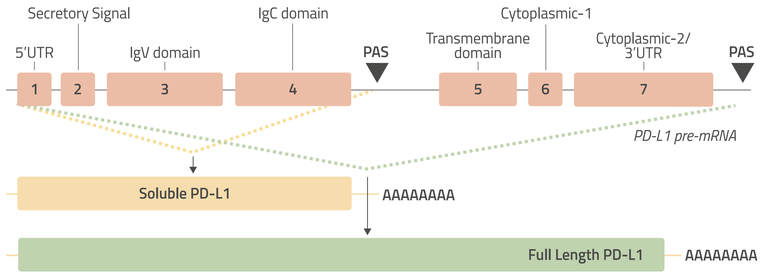


Figure 1. The PD-L1 pre-mRNA includes polyadenylation sites (PAS) in intron 4 and intron 7. The soluble PD-L1 isoform arises from polyadenylation initiated at the PAS between exon 4 and exon 5 of the pre-mRNA and therefore lacks the transmembrane and cytoplasmic domains. mRNAs for membrane bound isoforms of PD-L1 (which do contain the transmembrane and cytoplasmic domains) arise following polyadenylation initiated at the PAS in intron 7.

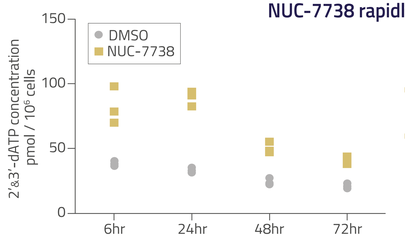
sPD-L1 and exoPD-L1 protein isolation: sPD-L1 and exoPD-L1 protein was extracted from media supernatant after treating cells with either DMSO or NUC-7738. Exosomes were isolated from cell culture supernatant and plasma samples from patients using ExoQuick® ULTRA EV Isolation System kit.

Evaluation of sPD-L1 and exoPD-L1 protein levels: sPD-L1 protein concentration was measured by sandwich ELISA. exoPD-L1 protein levels were assessed by Western blot analysis normalized to total protein. CD81 and CD63 were used as exosome markers.

Patient Plasma Samples: Patients were dosed with NUC-7738 on days 1 and 8 of a 14-day cycle. Paired samples (3 samples from 4 patients) were taken pre-C1D1, 24h post-C1D1 and pre-C2D1. exoPD-L1 levels analyzed by JESS Western blot and normalized to CD81.

RESULTS

NUC-7738 rapidly generates 3′-dATP



- NUC-7738 is rapidly converted to its active metabolite, 3′-dATP
- The C_{max} of 3′-dATP was 97.8 pmol /10^6 cells at 24 hours and decreased by approximately 50% by 72 hours

Figure 2. 2′ & 3′-dATP levels in A375 cells treated with DMSO or NUC-7738. Each data point represents an independent biological replicate (n=3).

NUC-7738 does not alter cell surface PD-L1 expression

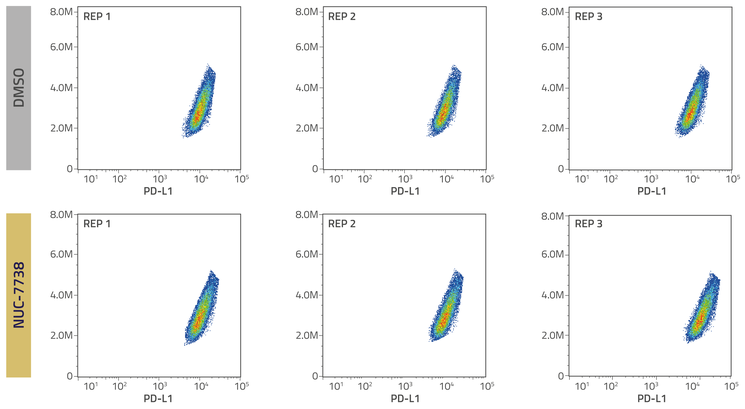


Figure 3. Cell surface PD-L1 expression in A375 cells treated with DMSO or NUC-7738 for 72 hours. Each box point represents an independent biological replicate (n=3).

- NUC-7738 treatment did not alter cell surface PD-L1 expression in A375 melanoma cells

NUC-7738 reduces sPD-L1 mRNA and protein expression

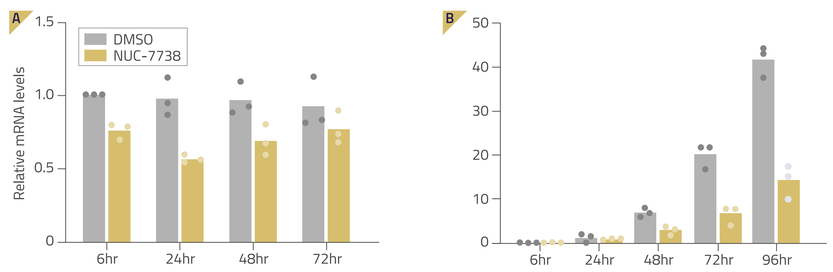


Figure 4. A) sPD-L1 mRNA expression and B) sPD-L1 protein concentration in culture supernatant, in A375 cells treated with DMSO or NUC-7738. Each data point represents an independent biological replicate (n=3).

- NUC-7738 reduced sPD-L1 mRNA expression by approximately 20-40% for up to 48 hours
- NUC-7738 caused a time dependent decrease in sPD-L1 protein in culture supernatant by approximately 60% at 96 hours

NUC-7738 reduces exoPD-L1 levels in cell supernatant

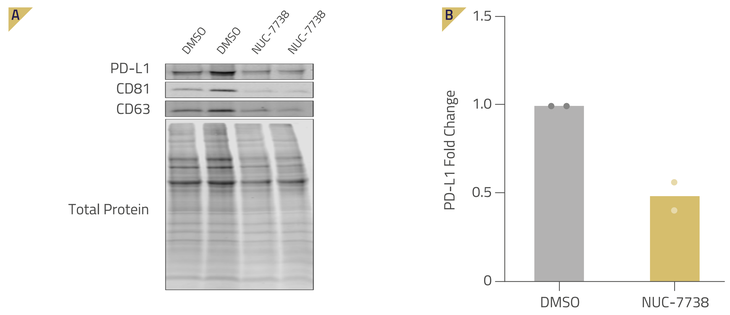


Figure 5. A) PD-L1, CD81, CD63 and total protein expression and B) PD-L1 expression normalized to total protein, from exosomes isolated from A375 cells treated with DMSO or NUC-7738 for 72 hours. Each data point represents an independent biological replicate (n=2).

- NUC-7738 reduces exoPD-L1, CD81 and CD63 exosome markers in A375 supernatant

NUC-7738 reduces exoPD-L1 levels in patient plasma

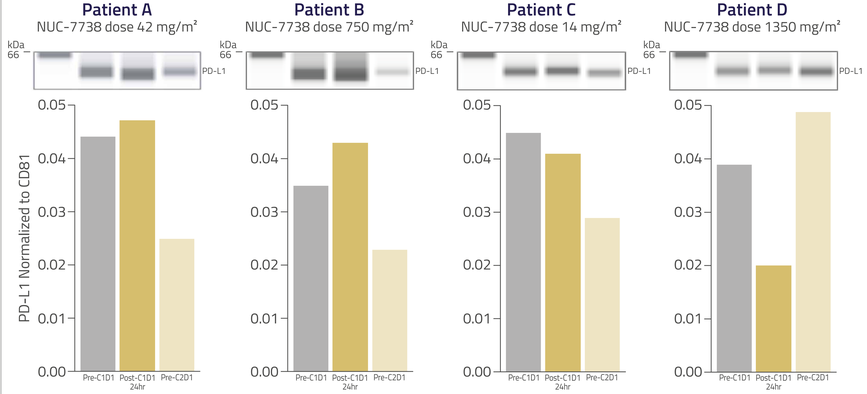


Figure 6. PD-L1 levels in exosomes isolated from plasma of NUC-7738 treated patients.

- Preliminary data indicates that NUC-7738 reduces exoPD-L1 in plasma
- In three out of four patients this reduction occurred after 2 doses of NUC-7738 (administered on days 1 & 8 of 14 day cycle)

CONCLUSION

- NUC-7738 reduces sPD-L1 and exoPD-L1 but not cell surface PD-L1 in melanoma cells
- NUC-7738 reduces plasma exoPD-L1 in patients
- NUC-7738 has the potential to act as an immune sensitizer by reducing secreted forms of PD-L1 and may enhance the clinical utility of anti-PD-(L)1 agents
- NUC-7738 is currently being investigated as a monotherapy and in combination with pembrolizumab in patients with metastatic melanoma (NuTide:701 study)

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ABBREVIATIONS: 2′-dATP: 3′-deoxyadenosine triphosphate 3′-dA: 3′-deoxyadenosine 3′-dATP: 3′-deoxyadenosine triphosphate ACTB: beta-actin ADA: adenosine deaminase BRAF: v-raf murine sarcoma viral oncogene homolog B1 C1D1: cycle 1 day 1 C2D1: cycle 2 day 1 CD63: cluster of differentiation 63 CD81: cluster of differentiation 81 C_{max}: maximal concentration DMSO: dimethyl sulfoxide ELISA: enzyme-linked immunosorbent assay exoPD-L1: exosomal PD-L1 IC_{50}: half maximal inhibitory concentration ICI: immune checkpoint inhibitor LC-MS: liquid chromatography mass spectrometry MEK: mitogen-activated protein kinase kinase mRNA: messenger ribonucleic acid PAS polyadenylation site PD-1: programmed cell death protein 1 PD-L1: programmed death-ligand 1 RNA: ribonucleic acid RT-qPCR: reverse transcription quantitative real-time polymerase chain reaction sPD-L1: soluble programmed death-ligand 1 TME: tumor microenvironment