NUC-7738 in combination with pembrolizumab in patients with metastatic melanoma: Phase 2 results from the NuTide:701 study

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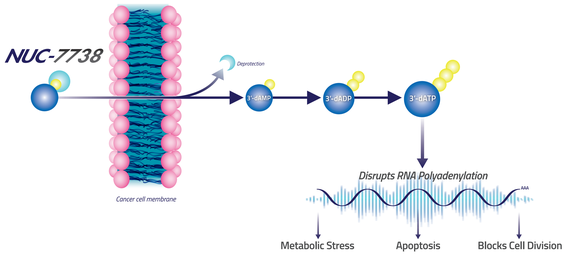
BACKGROUND

Metastatic Melanoma & Immune Checkpoint Inhibitors

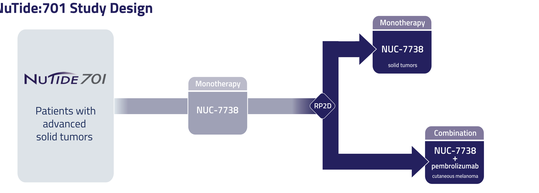
- Patients with metastatic melanoma have limited treatment options after progression on immune checkpoint inhibitors (ICIs) and BRAF/MEK inhibitors
  - Particular unmet need for patients who are primary refractory to ICIs
- Resistance to ICIs may be associated with secreted forms of PD-L1, which are partially controlled by alternative polyadenylation[1]



NUC-7738: ProTide transformation of 3'-dA

- Generates high intracellular levels of active anti-cancer metabolite (3'-dATP)
- 3'-dATP induces transcriptional changes in genes involved in key cellular processes including metabolism, apoptosis, cell differentiation[2-5]
- 3'-dATP is associated with changes in polyadenylation
- Reduces secreted forms of PD-L1 and may enhance the clinical utility of PD-(L)1 agents[6]

NuTide:701 Study Design



Methods

Cell cultures: Human melanoma cells (SK-MEL28) treated with DMSO or NUC-7738 (IC_{50} levels). RNA extracted 24 h post-treatment.

Patient paired tumor biopsies: Patients received either NUC-7738 monotherapy (ranging from 900-1350 mg/m² on days 1 & 8 of a 14-day cycle) or NUC-7738 (1125 mg/m² IV infusion over 2 h on days 1, 8, & 15 of a 21-day cycle) in combination with pembrolizumab (200 mg IV over 30 min prior to NUC-7738 on day 1). Biopsies collected pre- and post-drug infusion (≤ 6 h post infusion).

PBMCs samples: NuTide:701 patient (2000 mg/m² NUC-7738) and healthy volunteers (treated *in vitro* with NUC-7738).

Intracellular metabolites: NUC-7738 and 3'-dATP levels determined by LC-MS (Biopsy tissue: NUC-7738 & 3'-dATP LLOQ = 5 ng/g; PBMCs: NUC-7738 LLOQ = 0.5 nM, 3'-dATP LLOQ = 20 nM).

PolyATail size: Determined with tailfindr R package.

Gene expression mapping and counting: Sequencing reads aligned to transcriptomic reference. Gene-level expression quantification performed with Salmon long-read counting mode *-ont*. Differential gene expression performed using R package edgeR with *-exactTest* option for samples without biological replicates. Heatmaps generated using hierarchical clustering to group genes based on expression similarity.

JESS Western blot: RAB27A and MITF protein levels immunoprobed using capillary Western blots.

Immunofluorescence: Paraffin embedded sections including antibodies specific to RAB27A, MITF, CD8 and PD-1.

RESULTS

NUC-7738 generates 3'-dATP in patients' biopsies

Concentration (ng/g)

Patient biopsies	NUC-7738	3'-dATP
Cutaneous Melanoma	51	376
Endometrial	20	22
Mesothelioma	<LLOQ*	72

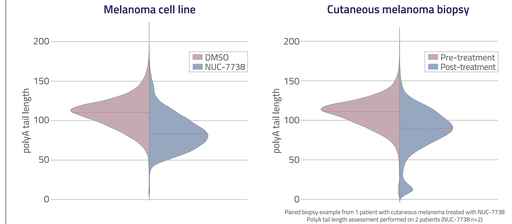
*LLOQ (NUC-7738) = 5 ng/g

NUC-7738 generates equivalent levels in patient PBMCs vs *in vitro* PBMCs

C_{max} (pmol/10^4 cells)

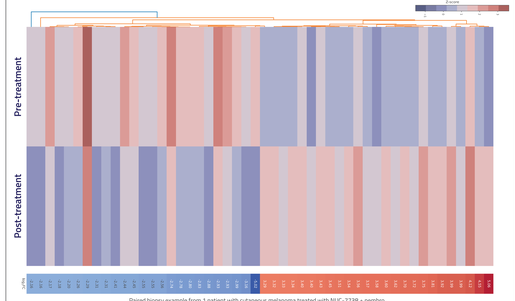
PBMCs	NUC-7738	3'-dATP
From patient (2000 mg/m²)	2	36
From volunteer *in vitro* (10 µM)	2	55
From volunteer *in vitro* (50 µM)	9	181

NUC-7738 causes marked effects on polyadenylation & transcription



Paired biopsy example from 1 patient with cutaneous melanoma treated with NUC-7738
PolyA tail length assessment performed on 2 patients (NUC-7738 n=2)

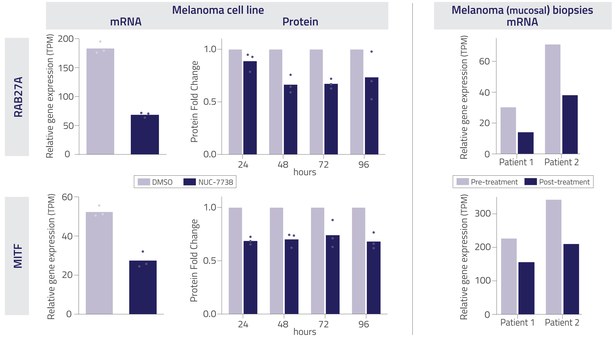
- NUC-7738 caused a shortening of polyA tails (blue vs red) in both a tumor biopsy from a patient and a melanoma cell line



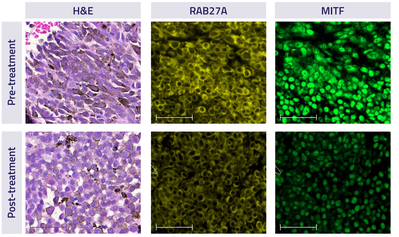
Paired biopsy example from 1 patient with cutaneous melanoma treated with NUC-7738 + pembro
mRNA levels performed on 4 patients (NUC-7738 n=2; NUC-7738 + pembro n=2)

- NUC-7738 ± pembrolizumab causes major changes in mRNA expression in patients' biopsies

NUC-7738 reduces pro-tumorigenic factors RAB27A & MITF



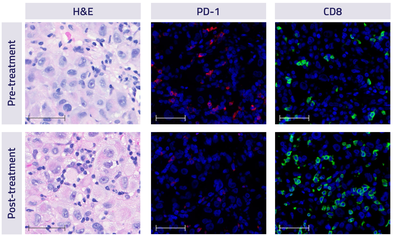
- NUC-7738 reduces RAB27A and MITF mRNA in patients' biopsies, similar to that observed in melanoma cell lines[6]



Paired biopsy example from 1 patient with cutaneous melanoma treated with NUC-7738
Full immuno panel performed on 7 patients (NUC-7738 n=5; NUC-7738 + pembro n=2)

- H&E sections show similar histologically pigmented melanoma (lymphocyte poor)
- RAB27A and MITF protein levels reflect changes observed in mRNA after NUC-7738 treatment

NUC-7738 modulates tumor morphology & immune cell infiltration



Paired biopsy example from 1 patient with cutaneous melanoma treated with NUC-7738 + pembro

- NUC-7738 + pembrolizumab results in morphological cell changes (more prominent nucleoli and cytoplasmic vacuolation) and lymphoid infiltration (H&E sections of inflamed tumor)
- NUC-7738 + pembrolizumab results in a decreased number of PD-1 expressing lymphocytes (red)
- NUC-7738 + pembrolizumab results in an increased number of $CD8^+$ T-cells (green)

Phase 1 (completed)

NUC-7738 monotherapy dose finding study in previously treated patients with advanced solid tumors

- MTD established: 1350 mg/m²

Phase 2 (ongoing)

- Monotherapy (completed): Intra-patient dose refinement (900-1350 mg/m² NUC-7738) in previously treated patients with advanced solid tumors. Starting dose established: 1125 mg/m²
- Combination therapy (ongoing): 1125 mg/m² NUC-7738* + 200 mg pembrolizumab in patients with advanced cutaneous melanoma previously treated with anti-PD-1 therapy

*escalation to 1350 mg/m² NUC-7738 permitted

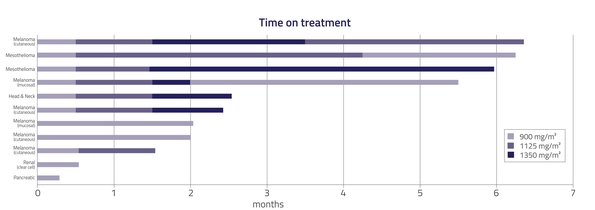
Phase 2: NUC-7738 monotherapy (completed)

Treatment related AEs (n=11)

Preferred term	All Grades	Grade 3
Nausea	5 (46%)	0
Anemia	5 (46%)	1 (9%)
Vomiting	4 (36%)	0
Fatigue	4 (36%)	1 (9%)
Diarrhea	3 (27%)	0
Constipation	2 (18%)	0
Blood creatinine increased	2 (18%)	0
Flushing	2 (18%)	0
Infusion related reaction	2 (18%)	0

Most frequent (≥ 10% population) NUC-7738 related AEs

- No G4 TRAEs
- 1 patient with treatment related SAE; G3 acute kidney injury
- No discontinuations due to AEs



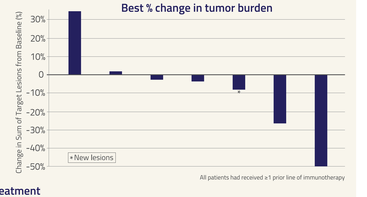
Phase 2: NUC-7738 + pembrolizumab (ongoing)

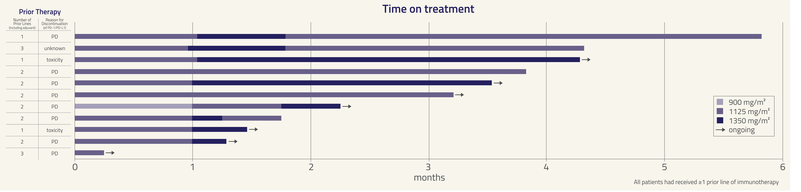
Treatment related AEs (n=11)

Preferred term	All Grades	Grade 3/4
Nausea	7 (64%)	0
ALT increased	4 (36%)	1 (9%)
Diarrhea	4 (36%)	1 (9%)
Vomiting	4 (36%)	1 (9%)
Anemia	3 (27%)	0
Fatigue	3 (27%)	0
AST increased	2 (18%)	1 (9%)
Blood magnesium decreased	2 (18%)	0
Blood potassium decreased	2 (18%)	0

Most frequent (≥ 10% population) NUC-7738 + pembro related AEs

- 2 patients with G3 TRAEs; abdominal pain & diarrhea (1); vomiting (1)
- 1 patient with G4 TRAEs; ALT increased & AST increased
- 3 patients with treatment related SAEs; G3 diarrhea (1), G4 ALT & AST increased (1), G3 vomiting (1)
- 1 patient discontinued due to AE





CONCLUSION

- NUC-7738 anti-cancer metabolite, 3'-dATP, concentrations in patient PBMCs are comparable to those achieved *in vitro*
- NUC-7738 induced molecular changes observed in patient biopsies are similar to those identified *in vitro*
- NUC-7738 ± pembrolizumab has a tolerable safety profile
- NUC-7738 ± pembrolizumab demonstrates encouraging signs of anti-tumor activity in patients with advanced melanoma who have received prior immunotherapies

REFERENCES: 1. Mahoney, *et al* 2019, *Cancer Immunol Immunother*; 68(3): 421-432 2. Kudoy *et al* 2019, Mol Cancer Ther, 18(12 _Supp):C122 3. Schwenzer *et al* 2021, *Clin Cancer Res*, 27(2):6500-6513 4. Shahid *et al* 2021, *Mol Cancer Ther*, 20(12 _Supp): P026 5. Shahid *et al* 2022, *PLoS ONE*, 17(12):e0278209 6. Elshani *et al* 2023, *Cancer Res* (2023) 83 (7_Supplement): 5962.

ABBREVIATIONS: 2'-dATP: 2'-deoxyadenosine triphosphate 3'-dA: 3'-deoxyadenosine 3'-dADP: 3'-deoxyadenosine diphosphate 3'-dAMP: 3'-deoxyadenosine monophosphate 3'-dATP: 3'-deoxyadenosine triphosphate AE: adverse event ALT: alanine aminotransferase AST: aspartate aminotransferase BRAF: v-raf murine sarcoma viral oncogene homolog B1 CD8: cluster of differentiation 8 C_{max}: maximal concentration DMSO: dimethyl sulfoxide H&E: hematoxylin & eosin IC_{50}: half maximal inhibitory concentration ICI: immune checkpoint inhibitor *IV:* intravenous LC-MS: liquid chromatography mass spectrometry LLOQ: lower limit of quantification MITF: microphthalmia-associated transcription factor MEK: mitogen-activated protein kinase kinase PBMC: peripheral blood mononuclear cell PD: progressive disease per RECISTv1.1 PD-1: programmed cell death protein 1 PD-L1: programmed death-ligand 1 PK: pharmacokinetics RAB27A: ras-related protein Rab-27A RNA: ribonucleic acid TPM: transcripts per million TRAE: treatment related adverse event SAE: serious adverse event

Data cleaning ongoing: data cut-off date 19 September 2023