NUCANA

Corporate Presentation

August 2025

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This presentation contains "forward-looking" statements within the meaning of the Private Securities Litigation Reform Act of 1995 that are based on the beliefs and assumptions and on information currently available to management of NuCana plc (the "Company"). All statements other than statements of historical fact contained in this presentation are forward-looking statements. Forward-looking statements include information concerning the company's planned and ongoing preclinical and clinical studies for the Company's product candidates and the potential advantages of those product candidates, including NUC-3373 and NUC-7738; the initiation, enrollment, timing, progress, release of data from and results of the Company's planned and ongoing clinical studies; the utility of prior preclinical and clinical data in determining future clinical results; the timing or likelihood of regulatory filings and approvals for any of its product candidates; the Company's intellectual property; the amount and sufficiency of the Company's cash and cash equivalents to achieve its projected milestones and to fund its planned operations into Q2 2025; and estimates regarding the Company's expenses, future revenues and future capital requirements. In some cases, you can identify forward-looking statements by terminology such as "may," "will," "should," "expects," "plans," "anticipates," "believes," "estimates," "predicts," "potential" or "continue" or the negative of these terms or other comparable terminology.

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Harnessing the Power of Phosphoramidate Chemistry

Transforming Nucleoside Analogs into **ProTides**

Nucleoside Analogs: Cornerstones of Cancer & Viral Treatments

16 FDA Approved Anti-Cancer Nucleoside Analogs

Including:

Vidaza® Azacitidine

5-FU Fluorouracil

Xeloda® capecitabine

GEMZAR® gemcitabine

DACOGEN® decitabine

FUDR® floxuridine

Fludara® FLUDARABINE

Clolar® clofarabine

22 FDA Approved Anti-Viral Nucleoside Analogs

Including:

Zovirax® (Acyclovir)

Viread® tenofovir disoproxil fumarate

VALTREX® VALACYCLOVIR HCl

Copegus® Ribavirinum

RETROVIR® Zidovudin AZT®

EPIVIR® (lamivudine)

ZERIT® (stavudine)

ZIAGEN® (abacavir)

Limitations of Nucleoside Analogs

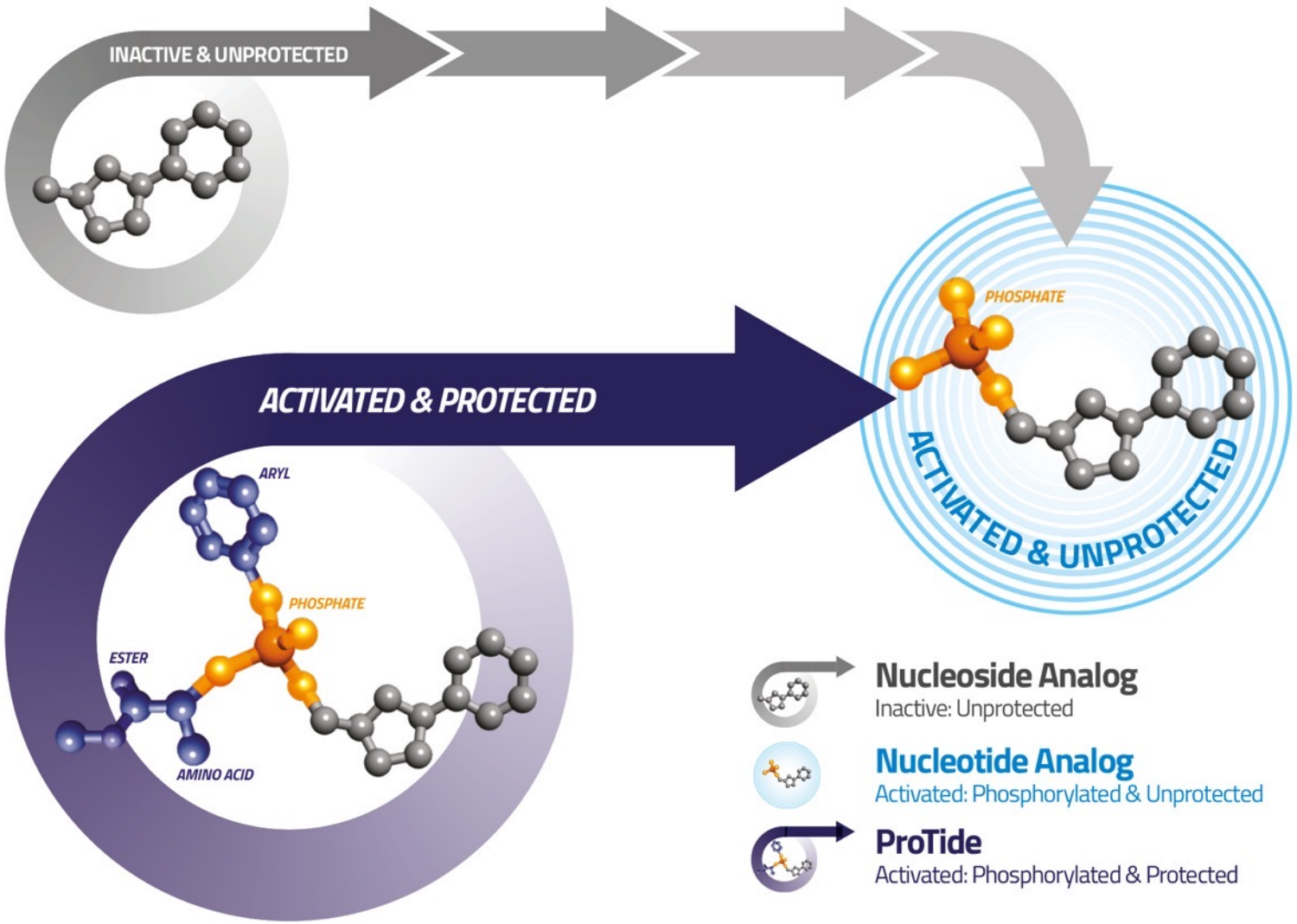
Breakdown & Toxic Byproducts
Off-target toxicity

Uptake
Dependent on transporters to enter cells

Activation
Inefficient generation of active metabolites

Administration Challenges
Poor PK leads to sub-optimal dosing

Transforming Nucleoside Analogs into ProTides



ProTides: A New Era In Anti-Virals

SOVALDI® SOFOSBUVIR
Hepatitis C

- Transformed novel nucleoside analog
- Highly effective treatment for chronic Hepatitis C infection
- Sales: **$71 billion**[1]

TAF tenofovir alafenamide
HIV & Hepatitis B

- Transformed nucleoside analog: Viread® (tenofovir disoproxil fumarate)
- More effective & safer treatment for HIV & HBV than Viread®
- Sales: **$112 billion**[2]

Veklury® remdesivir
COVID-19

- Transformed novel nucleoside analog
- Treatment for COVID-19
- Sales: **$16 billion**[3]

[1] Sovaldi + Harvoni + Epclusa + Vosevi cumulative sales through December 31, 2024
[2] Genvoya + Descovy + Odefsey + Biktarvy + Symtuza + Vemlidy cumulative sales through December 31, 2024
[3] Veklury cumulative sales through December 31, 2024

ProTides: A New Era in Oncology

NUC-7738

- Transformed novel nucleoside analog: 3'-dA
- Profoundly impacts gene expression in cancer cells
- Targets the tumor microenvironment

NUC-3373

- Transformed nucleoside analog: FUDR
- Targeted Thymidylate Synthase Inhibitor
- Induces DNA damage

Key Expected Milestones: 2025

	INDICATION	COMBINATION	PHASE	MILESTONE
NUC-7738 NuTide:701 Study	Melanoma	pembrolizumab	Phase 2	Initiate Study Expansion Announce Expansion Data Obtain FDA Feedback on Registration Strategy
NUC-3373 NuTide:303 Study	Solid Tumors	pembrolizumab	Phase 1b/2	Announce Data

Multiple Inflection Points in 2025

Nasdaq NCNA

Cash & Cash Equivalents (pro forma)
March 31, 2025
~$16.5 million[#]

Cash Runway
into
Q4 2026

Important Data Readouts
in
2025

[#]Based on exchange rate of £1.00 to $1.29 and reported cash of £4.0 million as of March 31, 2025, plus pro-forma gross proceeds from the May 2025 financing of £8.8 million

NUC-7738

Unlocking the Potential of Immunotherapy

Significant progress, however only 15-20% of patients achieve long-term remission

Numerous Tumor Microenvironment characteristics reduce the effectiveness of PD-(L)1 inhibitors

Expression of immune checkpoint molecules

Secretion of cytokines to suppress immune cell function

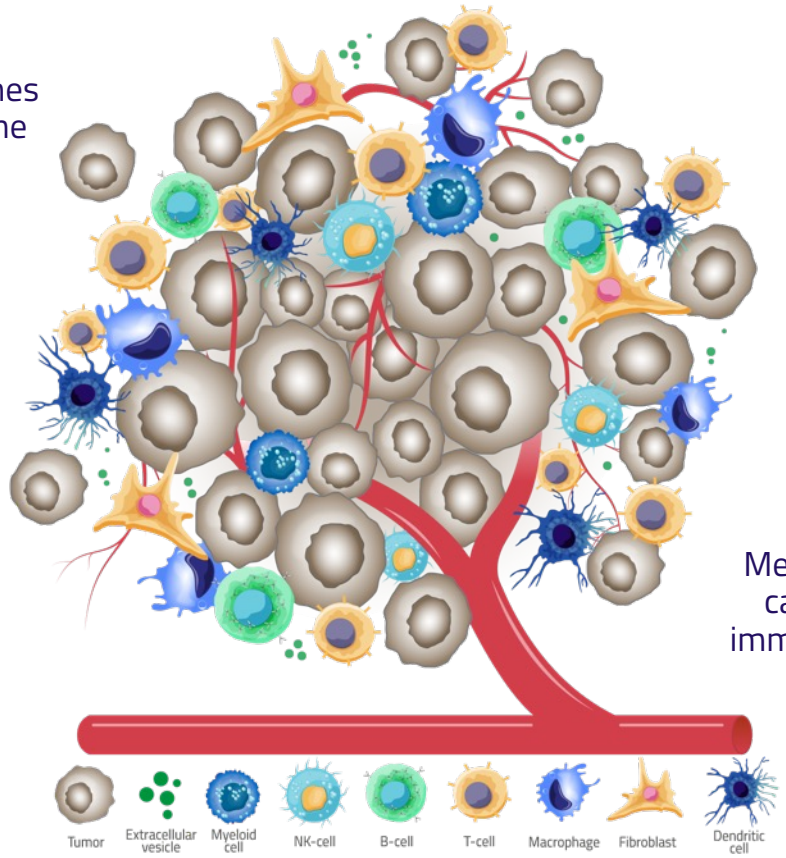
Reduced antigen presentation by cancer cells

Hypoxic & acidic environment

Recruitment of immunosuppressive cells

Promotion of angiogenic environment

Metabolic reprogramming causes the secretion of immunosuppressive factors



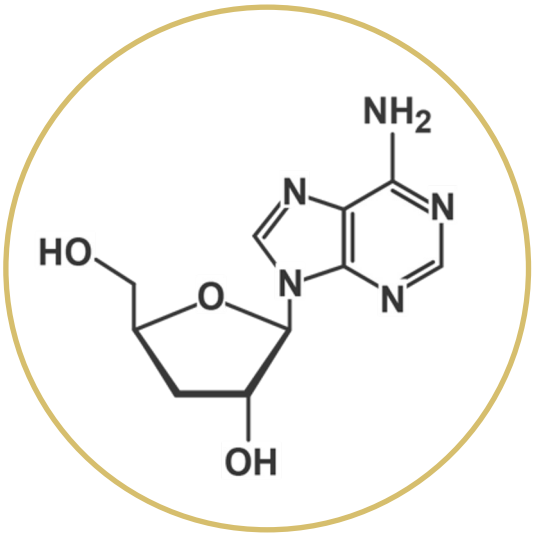
Novel Nucleoside Analog: 3'-deoxyadenosine (3'-dA)

Cordycepin

A Traditional Chinese Medicine found in the Himalayas

3'-dA

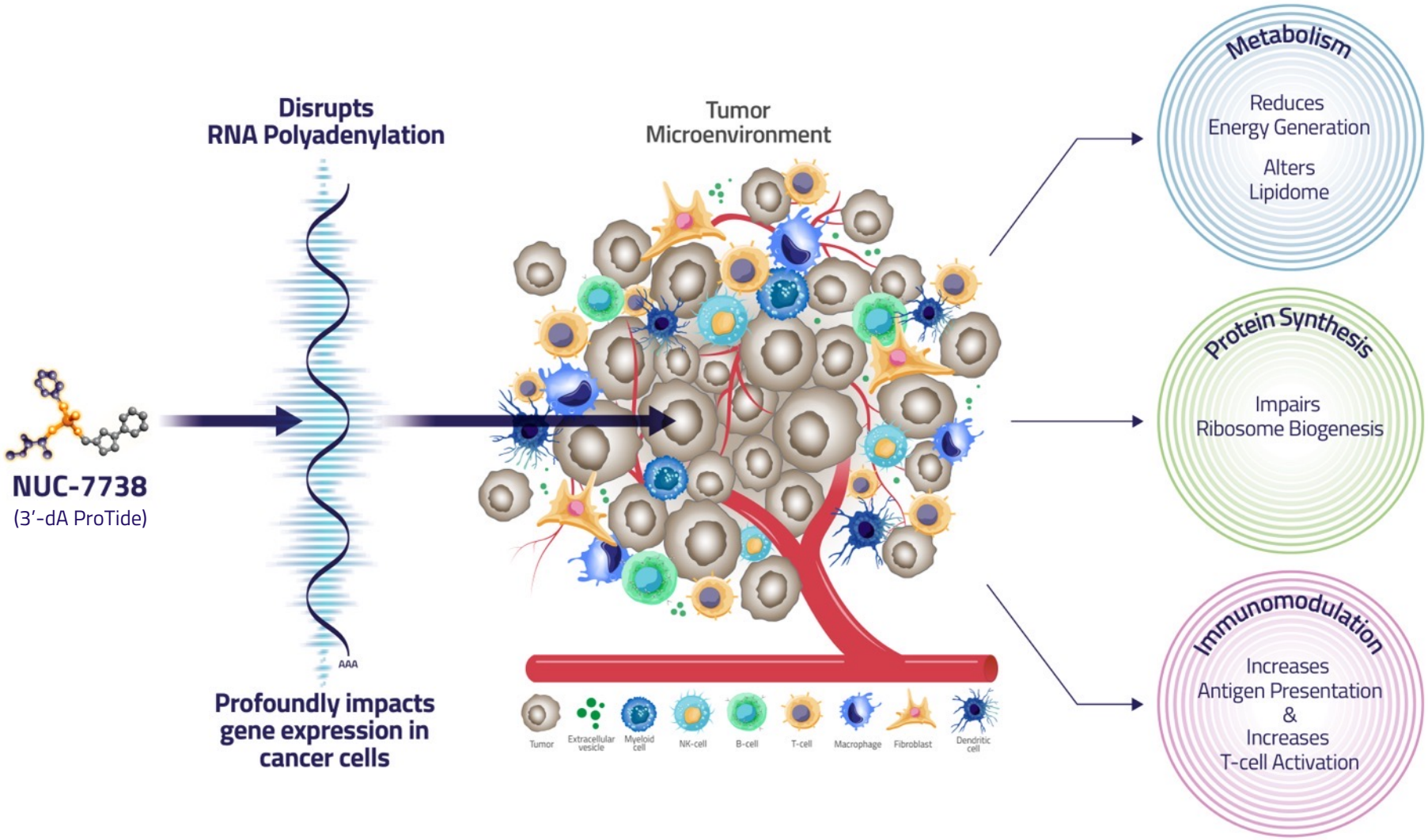
Originally isolated from *Cordyceps sinensis* in1950



3'-dA has potent anti-cancer activity *in vitro* and can modulate components of the TME

Despite this, it has not been successfully developed due to rapid breakdown by adenosine deaminase

NUC-7738 : Targets Multiple Aspects of the Tumor Microenvironment



NUC-7738 transforms PD-1 resistant TME into a therapeutically responsive state

NUC-7738 : Phase 1/2 Study (ongoing)

NuTide:701 Study

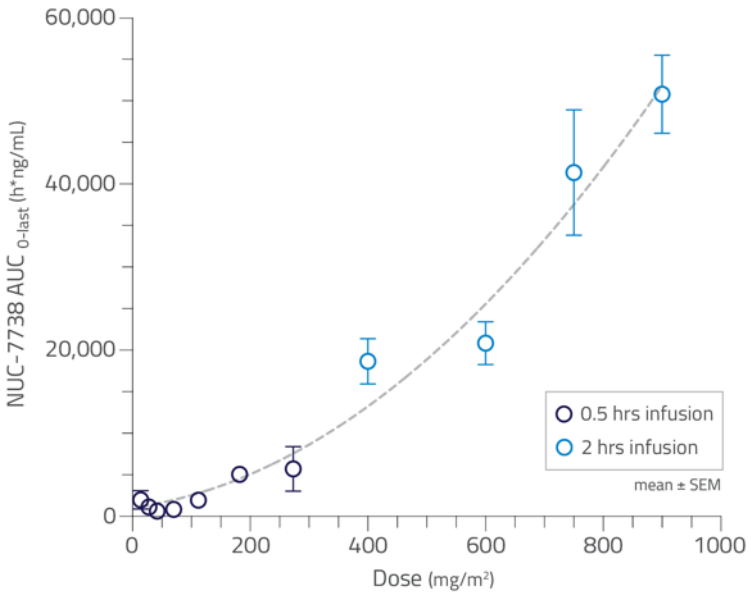
	Phase 1 Dose Escalation (monotherapy)	Phase 2 Dose Optimization (monotherapy)	Phase 2 Dose Confirmation (combination)	Phase 2 Expansion (combination)
	NUC-7738 *completed*	NUC-7738 *completed*	NUC-7738 + pembrolizumab *completed*	NUC-7738 + pembrolizumab *ongoing*
Number of patients	38	11	12	28
Tumor Type	Solid Tumors	Solid Tumors	Cutaneous Melanoma	Cutaneous Melanoma
Patient Population	Exhausted all treatment options	Exhausted all treatment options	PD-1 inhibitor resistant	PD-1 inhibitor resistant

Blagden *et al* (2024) *Ann Oncol*: 35: S482-S535 Abstract ID: 666P (ESMO September 2024). Data cut-off: August 1, 2024

NUC-7738 : Attractive Pharmacokinetic Profile (monotherapy)

Plasma

Dose proportional increase in C_{max} and AUC

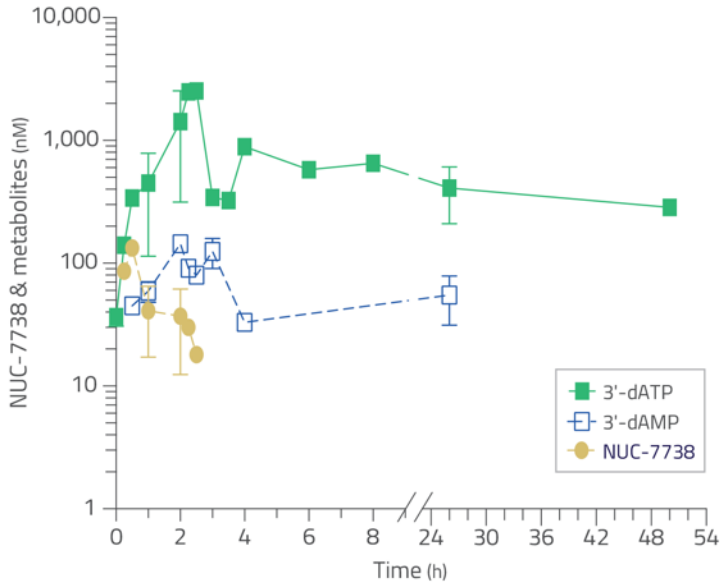


Patients (n=27) dosed at 14 – 900 mg/m²

Intracellular

NUC-7738 efficiently generates active anti-cancer metabolite (3'-dATP)

Long half-life of 3'-dATP (42 hrs)



Patients (n=3) dosed at 900 mg/m²

Symeonides *et al* (2022) *Ann Oncol* 33: S745-S746 Abstract ID: 455MO (ESMO September 2022). Data cut-off: July 7, 2022

NUC-7738 has been well tolerated

- No Grade 4 toxicities
- Low rates of Grade 3 toxicities

Dose AE occurred (mg/m²)	14 n=2	28 n=3	42 n=2	70 n=3	112 n=4	182 n=4	273 n=5	400 n=6	600 n=9	750 n=5	900 n=8	MTD 1350 n=11	2000 n=2	Total* n=38
All Grade Treatment-Related Adverse Events (≥10%)														
Nausea	0	1 (33%)	0	0	0	0	1 (20%)	0	3 (33%)	2 (40%)	3 (38%)	5 (45%)	1 (50%)	16 (42%)
Fatigue	0	1 (33%)	0	0	0	0	0	1 (17%)	3 (33%)	1 (20%)	3 (38%)	7 (64%)	2 (100%)	14 (37%)
Anemia	0	0	0	0	0	0	0	0	0	0	2 (25%)	4 (36%)	2 (100%)	7 (18%)
Diarrhea	0	0	0	0	0	0	1 (20%)	0	0	1 (20%)	1 (13%)	4 (36%)	0	6 (16%)
Vomiting	0	0	0	0	0	0	0	0	0	1 (20%)	1 (13%)	3 (27%)	1 (50%)	6 (16%)
Mucosal inflammation	0	0	0	0	0	0	0	0	1 (11%)	1 (20%)	0	1 (9%)	1 (50%)	4 (11%)
Decreased appetite	0	0	0	1 (33%)	0	1 (25%)	1 (20%)	0	0	0	1 (13%)	0	0	4 (11%)
Grade 3 Treatment-Related Adverse Events (ALL)														
Fatigue	0	0	0	0	0	0	0	0	0	0	0	3 (27%)	2 (100%)	4 (11%)
Anemia	0	0	0	0	0	0	0	0	0	0	1 (13%)	0	0	1 (3%)
Neutropenia	0	0	0	0	0	0	0	0	1 (11%)	0	0	0	0	1 (3%)
Vomiting	0	0	0	0	0	0	0	0	0	0	0	0	1 (50%)	1 (3%)

MTD: maximum tolerated dose
n= number of patients receiving each dose level at any time during the study
*total number of patients who experienced TRAE

Symeonides *et al* (2022) *Ann Oncol* 33: S745-S746 Abstract ID: 455MO (ESMO September 2022). Data cut-off: July 7, 2022

NUC-7738 : Encouraging Signs of Efficacy (monotherapy)

Disease Control Rate: 41% (Efficacy Evaluable Patients)

Metastatic Melanoma

62 years
2 prior lines
1) nivolumab + ipilimumab: discontinued within **1 month**
2) CK7 inhibitor: progressed at **1 month**

NUC-7738 starting dose 14 mg/m^2
Stable Disease: 12 months
14% reduction in tumor volume
Treatment duration: 18 months
- 8 dose escalations

Metastatic Melanoma

65 years
1 prior line
1) nivolumab + ipilimumab: discontinued within **1 month**

NUC-7738 starting dose 400 mg/m^2
Stable Disease: 9 months
NUC-7738 treatment enabled complete resection
patient had diffuse disease that was inoperable
Treatment duration: 11 months
- 1 dose escalation

Metastatic Clival Chordoma

72 years
1 prior line
1) imatinib: progressed at **19 months**

NUC-7738 dose 1,350 mg/m^2
Stable disease: 6 months
45% reduction in mandibular lesion
Complete disappearance of lip lesion
Bleeding from nasal lesion resolved

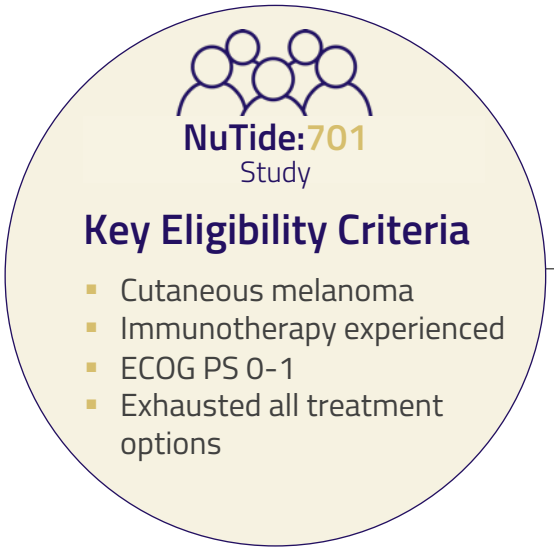
Metastatic Lung Adenocarcinoma

65 years
2 prior lines
1) carboplatin + pemetrexed: progressed at **6 months**
2) docetaxel: progressed at **4 months**

NUC-7738 starting dose 42 mg/m^2
46% reduction in lung lesion 1
Change in character in lung lesion 2 small dense core surrounded by a larger diffuse "ground-glass" periphery
Treatment duration: 6 months
- 4 dose escalations

Symeonides *et al* (2022) *Ann Oncol*: 33: S745-S746 Abstract ID: 455MO (ESMO September 2022). Data cut-off: July 7, 2022

NUC-7738 : Phase 2 Study (combination)



Prior Therapy: median (range)	2 (1-3)
PD-1 inhibitor	12
PD-1 inhibitor (adjuvant)	8
PD-1 inhibitor (non-adjuvant)	8
CTLA-4 inhibitor	11
PD-1 + CTLA-4 inhibitor	9
BRAF + MEK inhibitor	1

*Starting dose was 1125 mg/m² which was escalated to 1350 mg/m² if well tolerated

Blagden *et al* (2024) *Ann Oncol*: 35: S482-S535 Abstract ID: 666P (ESMO September 2024). Data cut-off: August 1, 2024

NUC-7738 : Favorable Safety Profile (combination)

NUC-7738 + pembrolizumab has been well tolerated (n=12)

- Low rates of Grade ≥3 toxicities
- 1 patient experienced Grade 4 transaminitis (ALT/AST increased)

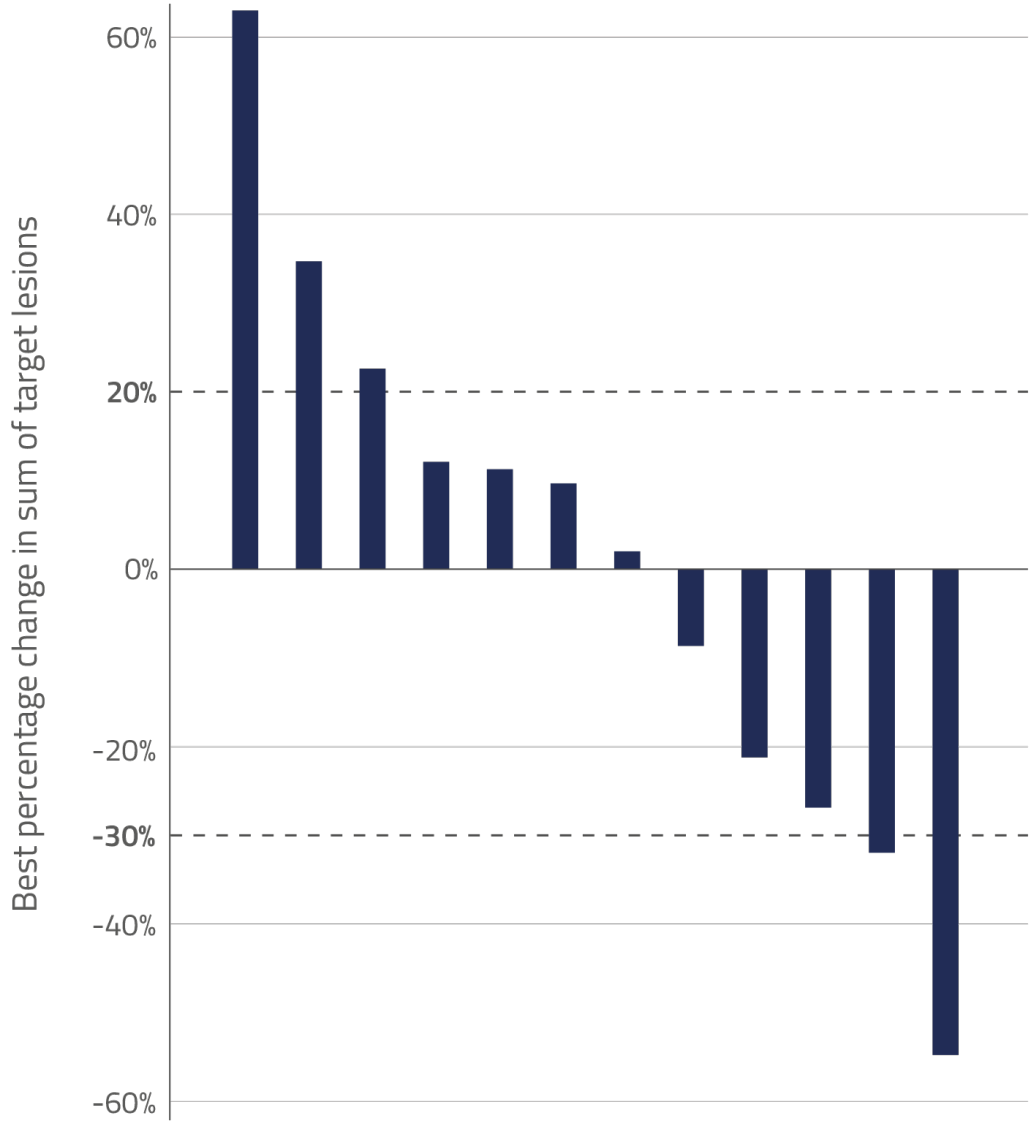
Treatment Related Adverse Events

	All Grades n(%)	Grade 3 n(%)	Grade 4 n(%)
Nausea	9 (75)	0	0
ALT increased	6 (50)	1 (8)	1 (8)
Diarrhea	6 (50)	1 (8)	0
Vomiting	6 (50)	1 (8)	0
Anemia	5 (42)	0	0
AST increased	4 (33)	1 (8)	1 (8)
ALP increased	2 (17)	0	0
Blood magnesium decreased	2 (17)	0	0
Blood sodium decreased	2 (17)	0	0
Decreased appetite	2 (17)	0	0
Fatigue	2 (17)	1 (8)	0
GGT increased	2 (17)	1 (8)	0
Hypophosphatemia	2 (17)	0	0
Rash	2 (17)	0	0

All Grade TRAEs with prevalence ≥10% patients related to NUC-7738, pembrolizumab or both
Additional Grade 3 TRAEs ≤10%: abdominal pain (1 pt); immune-mediated hepatitis (1 pt); adrenal insufficiency, hypercalcemia and hypotension (1 pt). No additional Grade 4 TRAEs

Blagden *et al* (2024) *Ann Oncol*: 35: S482-S535 Abstract ID: 666P (ESMO September 2024). Data cut-off: August 1, 2024

NUC-7738 : Tumor Volume Reductions in PD-1 Inhibitor Resistant Patients (combination)



Patient previously refractory to PD-1 inhibitor (nivolumab) + CTLA-4 inhibitor (ipilimumab) had 55% reduction

Patient with resistance to PD-1 inhibition (pembrolizumab) had 32% reduction

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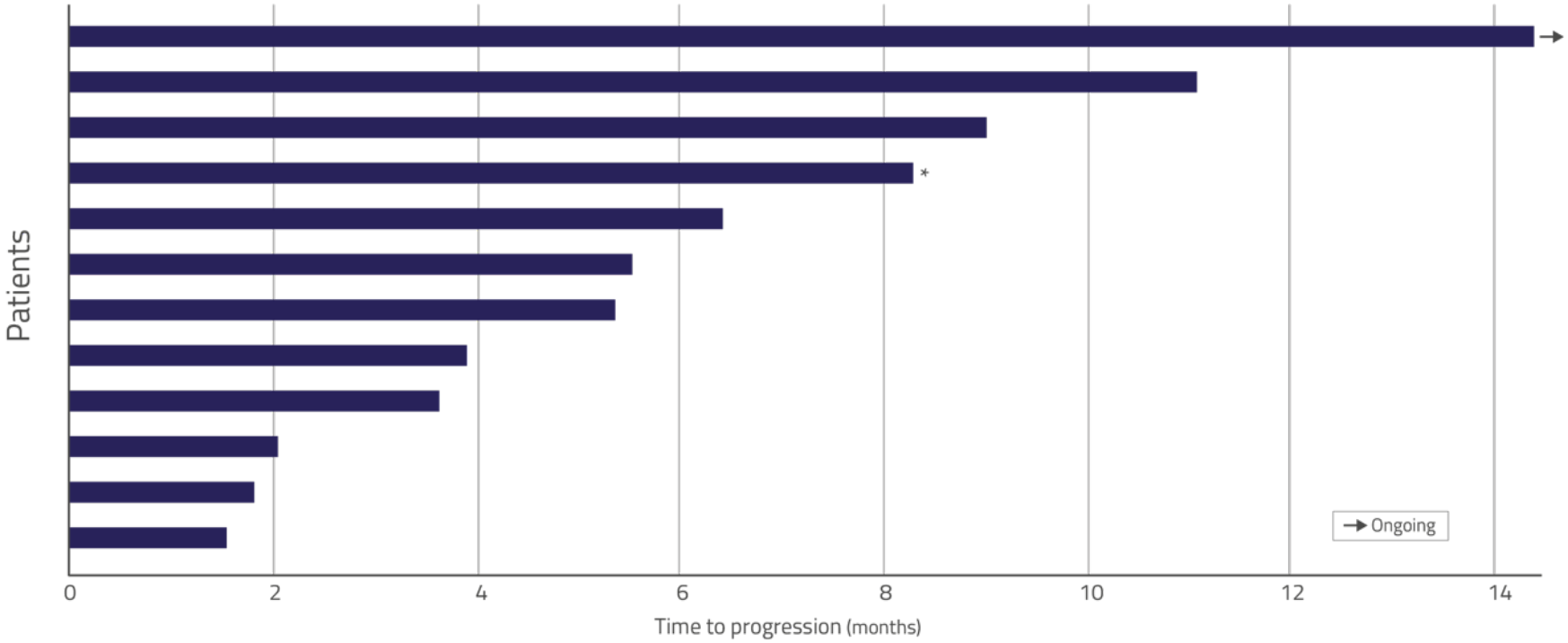
NUC-7738 : Durable PFS in PD-1 Inhibitor Resistant Patients (combination)

PD-1 inhibitor rechallenge typically results in patients progressing at their first scan (2-3 months)



*Patient had mixed response with almost all sub-cutaneous lesions resolved and just two lymph nodes that required RT with resection intended. Patient remains on therapy.

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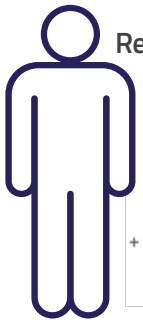
NUC-7738 : Encouraging Efficacy in PD-1 Inhibitor Resistant Patients (combination)

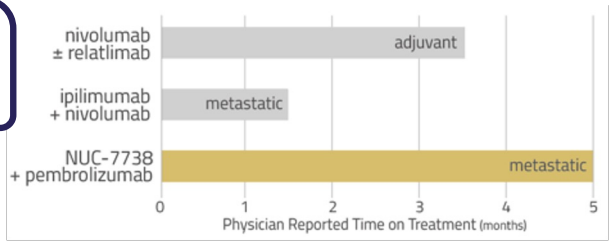
Case Study 1

Partial Response in patient with resistance to PD-1 inhibition



63 years ▪ 2 target lesions (skin) ▪ BRAF wt

Received 2 prior PD-1 inhibitor containing regimens



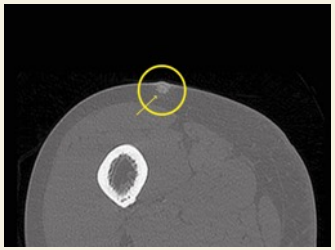
NUC-7738 + pembrolizumab

Partial Response (confirmed): 55% reduction in sum of target lesions

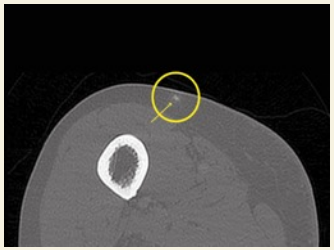
- 42% reduction in target lesion 1
- 70% reduction in target lesion 2 (see scans)

Time to progression 9 months

- 5 months treatment, discontinued due to unrelated SAE
- No further therapy, PR sustained for additional 4 months



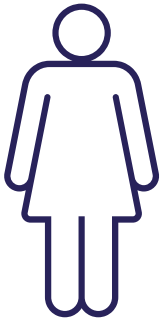
Baseline: 1.0 cm



Week 17: 0.3 cm

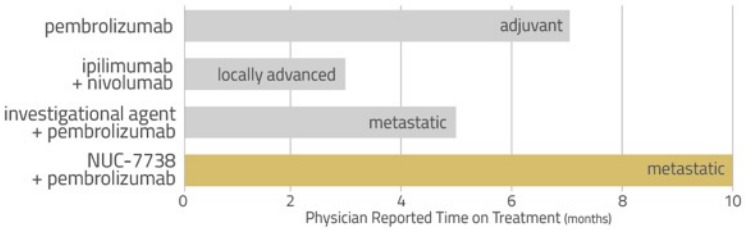
Case Study 2

Evidence of anti-cancer immune response in TME



67 years ▪ 2 target lesions (lymph node) ▪ BRAF wt

Received 3 prior PD-1 inhibitor containing regimens



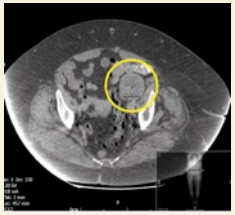
NUC-7738 + pembrolizumab

Partial Response (unconfirmed): 32% reduction in sum of target lesions

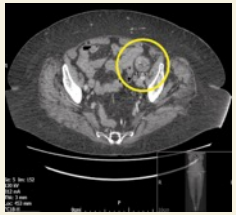
- 22% reduction in target lesion 1
- 45% reduction in target lesion 2 (see scans)

Time to progression 8 months

- Remains on treatment at 10 months due to clinical benefit (mixed response to oligometastatic disease; palliative radiotherapy to progressive lesions)



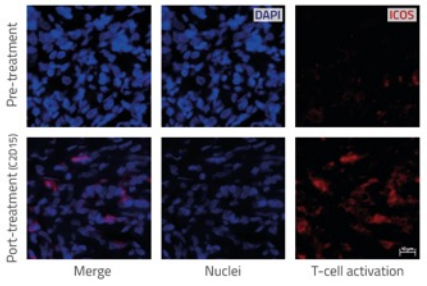
Baseline: 5.53 cm



Week 24: 3.04 cm

T-cell activation post-treatment

Increased expression of ICOS (red) post-treatment indicates T-cell activation



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NUC-7738 : AACR (April 2024)

RNA Regulatory Disruption by 3’-dATP: A Novel Approach to Inhibit Ribosome Biogenesis in Cancer

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Abstract Number: 5650

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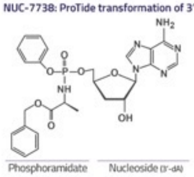
Background

Ribosome Biogenesis & Cancer

- Ribosomes, a complex ensemble of RNA and proteins, play a key role in cell survival, growth, and proliferation
- Ribosome biogenesis (RiBi) is a complex function governed by precise checkpoints and surveillance mechanisms which may become dysregulated in cancer, leading to tumor growth and therapeutic resistance
- Regulation of RNA through polyadenylation is a key post-transcriptional mechanism that influences mRNA metabolism[1]
- Poly(A) tail length of mRNAs, particularly those encoding ribosomal subunits and components of the translational apparatus, is critical for efficient production of proteins
  - These mRNAs are often categorized as 5' terminal oligopyrimidine tract (5'TOP) mRNAs
  - Impairment of 5'TOP mRNA translation can directly impact ribosome biogenesis and function, which in turn can halt cellular proliferation; a hallmark of cancer progression[2,3]
- Targeting polyadenylation machinery to influence the poly(A) tail length of mRNAs encoding ribosomal subunits could be a promising strategy to disrupt the aberrant protein synthesis that supports cancer cell growth
- Ribosome-targeted therapy could provide a promising treatment for cancer

NUC-7738: ProTide transformation of 3'-dA



- Generates sustained intracellular levels of anti-cancer metabolite 3'-dATP
- Influences RNA regulatory processes, including poly(A) tail length
  - Leads to alternative polyadenylation (APA) and splicing
  - Resulting in impaired cellular responses

Figure 1: NUC-7738: 3'-deoxyadenosine (3'-dA) phosphorylated and protected with a phosphoramidate moiety attached at the 5'-position

Aim: to investigate the impact of 3'-dATP on RNA regulation and RiBi utilizing a novel bioinformatic pipeline

Methods

Cell Culture

- Melanoma (A375, MeWo, CHL1, SK-MEL28) and renal cell carcinoma (786O & 769P) cell lines were treated with IC_{50} levels of NUC-7738. Cells were harvested 24 hours post-treatment for RNA-sequencing, for protein analysis whole cell lysates were extracted at 24, 48, 72 and 96 hours. All experiments were carried out on 3 biologically independent replicates.

Paired Biopsies

- Patient paired tumor biopsies were collected from 5 cutaneous melanoma patients treated with 1125 mg/m² NUC-7738 on days 1, 8 & 15 of a 21-day cycle in combination with 200mg pembrolizumab on day 1
- Biopsies were collected pre-(SCR) and post-(OTB) drug infusion (≤6h post infusion) and preserved in Zymo RNA/DNA Shield (Zymo, Cat#R1100)

RNA Extraction

- RNA extracted from cell lines using RNeasy kits (Qiagen 74004)
- Paired patient biopsies, macroscopically assessed for viable tumor, were homogenized using ceramic beads and Precellys 24 homogenizer. RNA isolated using Quick-RNA-Mini kit.

Long-read RNA-Seq library preparation

- Sequencing libraries for PCR-cDNA sequencing were constructed utilizing SQK-PCS111.24 kit from Oxford Nanopore Technologies

RNA seq data processing and analysis

- Sequencing libraries were processed using R9.4.1 flow cells on PromethION P2 sequencer by Nanopore. The sequenced Fast5 files were basecalled using Guppy v6.0.6, employing the settings --fast5_out and --trim_strategy none. Fast5 files were analyzed with the tailfindr package in R4 to estimate poly A tail lengths for each read.
- For gene expression analysis, basecalled FastQ files were aligned to Gencode v41 transcriptome reference using minimap25 v2.17 aligner. Aligned FastQ files were processed through pychopper to identify and orient full-length reads based on their barcodes. Gene-level expression quantification was conducted using Salmon6 in long-read counting mode (-ont). Differential gene expression was assessed with DeSeq2 package in R.

Poly(A) tail analysis

- Tail lengths extracted from tailfindr output together with unique read_id and transcriptome aligned .bam files. Jupyter Notebook environment with Python programming language was employed to process graphs and execute statistical analysis.

JESS capillary Western blots

- Whole cell protein lysates were probed with RPL5, RPL11, RPS3, RPL6 and YAE1 specific antibodies and analyzed by automated JESS Western blot

Multiplexed IF

- Multiplexed immunofluorescence (IF) was carried out on biopsies using the in-house optimized automated methods and the Leica BOND RX autostainer. Each antibody concentration was optimized using a wide range tissue TMA and single staining and multiplexed staining prior to staining biopsies.

Results

NUC-7738 causes a global reduction in poly(A) tail length in cell lines and biopsies

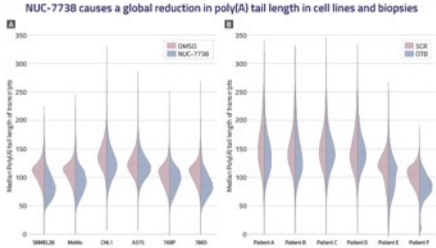


Figure 2: Poly(A) tail changes in cancer cell lines (A) treated with NUC-7738 and paired biopsies from patients (B) treated with NUC-7738 + pembrolizumab. Violin plots depicting the median poly(A) tail length of transcripts from melanoma and renal cell cancer cell lines and paired biopsies. Each plot represents a different cell line or patient paired biopsy, with the median value denoted by a dashed red line. The width of each 'violin' indicates the density of data points at different lengths.

- NUC-7738 caused a shortening of poly(A) tails (blue vs red) in cell lines treated with NUC-7738 and patients' paired biopsies treated with NUC-7738 and pembrolizumab

NUC-7738 shortens poly(A) tails of 5'TOP mRNA and SNHG non-coding RNAs

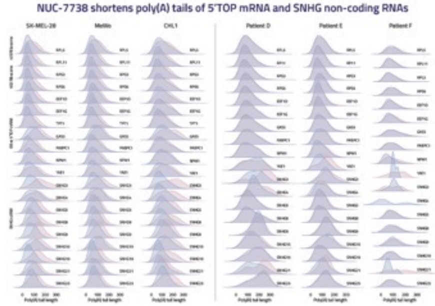


Figure 3A: Ridgeline Plots of Poly(A) Tail Length of genes in cell lines and patients' paired biopsies. The genes are representative of 5'TOP mRNA genes and SNHG non-coding RNA. The x-axis quantifies the poly(A) tail length in nucleotides, while the y-axis represents the kernel density estimation of gene expression.

Results (cont)

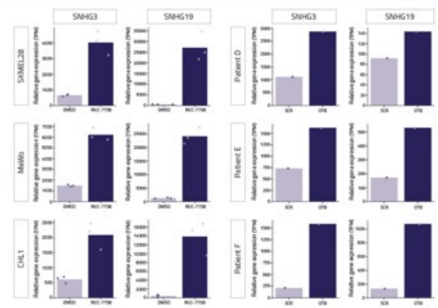


Figure 3B: Transcript levels of SNHG3 and SNHG19 across multiple cell lines and patient paired biopsies. The figure presents bar plots of transcript per million (TPM) measurements for SNHG3 and SNHG19 in a variety of human cell lines treated with NUC-7738 and paired patient biopsies.

- NUC-7738 reduces poly(A) tail length of all 5'TOP genes (median reduction of 15 adenines)
- NUC-7738 reduces poly(A) tail length of SNHG non-coding RNAs (median reduction of 25 adenines)
- NUC-7738 reduces poly(A) tail length of PAXT lncRNAs, SNHG3 and SNHG19, by ~50 adenines, accompanied with an increase in transcript abundance

NUC-7738 reduces the protein levels of ribosomal subunits and YAE1

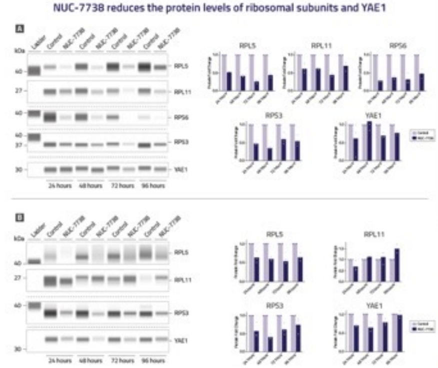


Figure 4: Ribosomal subunit proteins in melanoma cell lines. (A) MeWo and (B) SK-MEL-28 cell lines. Each data point represents an independent biological replicate (n=3).

- NUC-7738 reduces expression of ribosomal subunits proteins by up to 70% across all cell lines
- Expression of YAE1, a regulator of RiBi, also decreased, indicative of impaired RiBi
- These data suggest NUC-7738 targets ribosomal subunits proteins

NUC-7738 + pembrolizumab reduces RPS3 and YAE1 protein

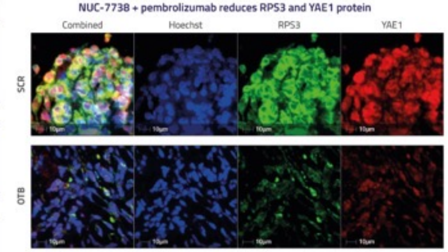


Figure 5: RPS3 and YAE1 protein expression in representative patient paired biopsy treated with NUC-7738 + pembrolizumab. Hoechst staining represents cell nuclei.

- NUC-7738 + pembrolizumab reduces the expression of ribosomal protein RPS3 and YAE1 confirming cell line data

CONCLUSION

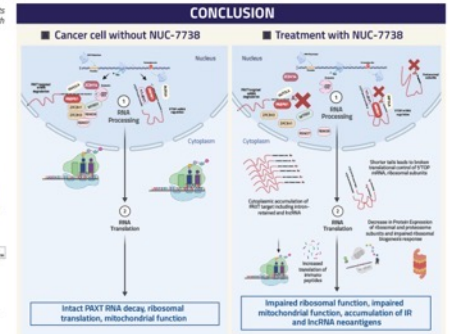

Cancer cell without NUC-7738
Treatment with NUC-7738
Nucleus
Cytoplasm
RNA Processing
RNA Translation
Intact PAXT RNA decay, ribosomal translation, mitochondrial function
Impaired ribosomal function, impaired mitochondrial function, accumulation of IR and lncRNA neoantigens

Figure 6: Proposed mechanisms of action of NUC-7738 contributing to impaired ribosome biogenesis. A) In the absence of NUC-7738, RNA processing and translation proceed regularly, maintaining intact PAXT RNA decay, and ribosomal subunits translation. B) NUC-7738 generates anticancer metabolite 3'-dATP. Incorporation of 3'-dATP by Poly(A) Polymerase results in the premature termination of poly(A) tail elongation. This inhibition disrupts the poly(A) tail exosome targeting (PAXT), causing an accumulation of SNHG gene transcripts and intron-retained transcripts. Reduction of poly(A) tail length impairs the translational control of protein-coding ribosomal mRNA. Consequently, there is a decrease in protein expression of ribosomal subunits, leading to inhibition of ribosomal biogenesis and protein translation, as well as impaired ribosomal function.

- NUC-7738 significantly modulates RNA stability, particularly affecting 5' TOP genes crucial for translational control
- Global poly(A) tail shortening, observed across cancer cell lines, as well as in paired biopsies, indicates broad and targeted mRNA stability impact
- Specific shortening of poly(A) tails within the 5'TOP gene set suggests interference with mRNA regulation, leading to decreased protein translation
- Preliminary data highlight NUC-7738's potential to influence gene regulation, especially in the translational machinery critical for cancer cell growth and survival

Exposing the Heterogeneity of the Lipidome in the TME of Cutaneous Melanoma Following Treatment with NUC-7738 in Combination with anti-PD-1 Therapy

Alison L. Dickson[1,2], Greice M. Zickuhr[1], In Hwa Um[1], Ying Zhang[1], Ruth Plummer[3], Sarah P. Blagden[4], Stefan N. Symeonides[5], Natalie Cook[6], T.R. Jeffry Evans[7], Mustafa Elshani[1,2] & David J. Harrison[1,2]

1) University of St Andrews, St Andrews, UK 2) NuCana plc, Edinburgh, UK 3) Newcastle ECMC, Northern Centre for Cancer Care, Freeman Hospital, Newcastle Upon Tyne, UK 4) Oxford ECMC, Churchill Hospital, University of Oxford, UK 5) Edinburgh ECMC, Western General Hospital, Edinburgh, UK 6) The Christie NHS Trust/University of Manchester, UK 7) The Beatson West of Scotland Cancer Centre/University of Glasgow, UK

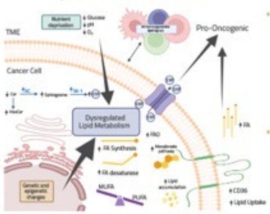
Abstract Number: 6222

Email: alisondickson@nucana.com

Introduction

- Cancer cells adapt to hypoxic and nutrient deprived environments by reprogramming lipid biosynthesis to accelerate malignant behaviour[1]
- Lipids are not only structural components of cell membranes but also serve as signaling molecules within the tumor microenvironment (TME) to modulate immune cells[2]
- Cancer cells transitioning to a malignant phenotype demonstrate marked changes in lipid metabolism including:
  - Changes in the ratio of monounsaturated fatty acids (MUFAs) to polyunsaturated fatty acids (PUFAs)
  - Dysregulation of sphingolipids, crucial for tumor progression and survival
- Reprogramming lipid metabolism within the TME is a recognised strategy for boosting the effects of immunotherapy[3]

Lipid Metabolism in the TME



- NUC-7738 generates sustained intracellular levels of 3' deoxyadenosine triphosphate (3'-dATP), which profoundly alters RNA regulatory processes in tumor cells, resulting in changes in expression of proteins related to lipid metabolism
- Using paired biopsies from patients treated with treated with NUC-7738 ± pembrolizumab, we aim to investigate the potential role of NUC-7738 as a lipid reprogramming agent

Figure 1: In nutrient deprived conditions cancer cells increase lipogenesis, fatty acid uptake via CD36 and fatty acid oxidation (FAO). Cancer cells rich in monounsaturated fatty acids (MUFA) prevent ferroptosis. Alterations in sphingolipid biosynthesis cause accumulation of the pro-tumorigenic and immunosuppressive sphingosine-1-phosphate (S1P) in the TME.

Methods

- Snap frozen and FFPE biopsies were collected from 6 patients (1 x mucosal melanoma and 5x cutaneous melanoma) treated with NUC-7738 ± pembrolizumab. Biopsies were collected pre- and post- drug infusion (≤6h post infusion).
- TIC normalised data was exported into MetaboAnalyst 5.0 for statistical analysis (SAM, PCA, fold-change). LIPID MAPS® Structure Database was used to tentatively assign lipid species.

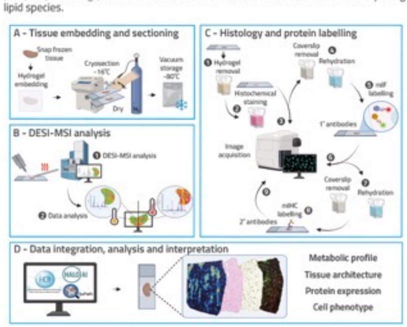

A - Tissue embedding and sectioning
Snap frozen tissue
Hydrogel embedding
Cryosection -16°C
Vacuum storage -80°C
Dry
B - DESI-MSI analysis
DESI-MSI analysis
Data analysis
C - Histology and protein labelling
Hydrogel removal
Coverslip removal
Rehydration
mIF labelling
Histochemical staining
1° antibodies
Image acquisition
Coverslip removal
Rehydration
mIHC labelling
2° antibodies
D - Data integration, analysis and interpretation
Metabolic profile
Tissue architecture
Protein expression
Cell phenotype

Figure 2: Multi-Modal Imaging Workflow for 10µm single-section tissue analysis

Lipid Profiles of Melanoma Cells Change Following Treatment

- The emergence of two distinct classes of lipids suggests NUC-7738 ± pembrolizumab changes the lipidome in the melanoma TME

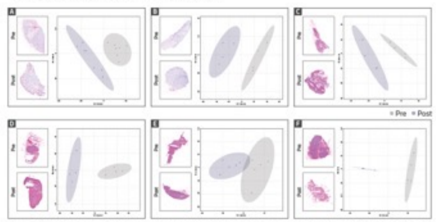


Figure 3: PCA 2D scores plots of lipids in tumor regions from biopsies pre- and post-treatment (A, mucosal and B-F, cutaneous melanoma). ROI were selected by high-magnification analysis of viable tumor regions of H&E-stained sections following DESI-MSI.

Heterogeneity of Lipids and Immune Cells in the TME

- Dimension reduction analysis (UMAP) of data (>1000 lipids) reveals distinct regions within each biopsy

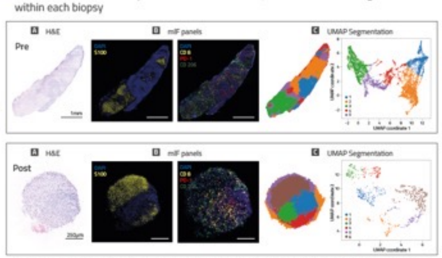


Figure 4: A) Post DESI-MSI H&E section, B) multiplex immunofluorescence (mIF) of DAPI co-localised with; s100 positive cells (indicative of malignant melanoma), CD 8 positive cells (T cells), CD 206 positive cells (macrophages) and PD-1 positive cells. C) UMAP segmentation of single 10µm section based on UMAP cluster data.

Spatial Co-registration for Quantitative Image Analysis

- Quantification of individual cell types within distinct metabolic regions of the tissue show which cell types may be driving changes in lipid signatures

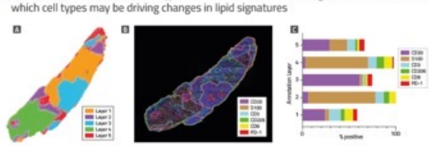


Figure 5: A) UMAP segmentation integrated into HALO AI analysis software as annotation layers. B) annotation layers applied to fused mIF and mIHC images. C) population of cells in each annotation layer determined by random forest classification.

Fatty Acid Reprogramming

- NUC-7738 + pembrolizumab changes the balance of fatty acids in favor of PUFAs, indicative of a shift towards a less aggressive oncogenic phenotype
- MUFAs have been linked to malignant behaviour and resistance to chemotherapy whilst PUFAs have been associated with susceptibility to reactive oxygen species and ferroptosis[4]

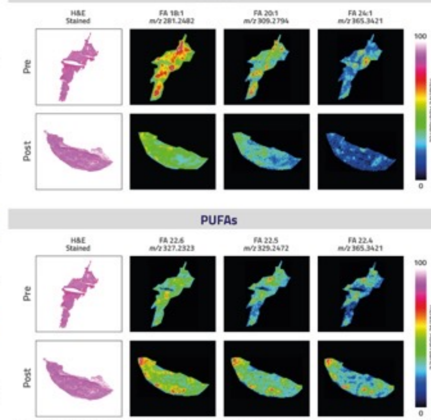


Figure 6: DESI-MSI ion heatmaps show spatial distribution and a decrease in the relative abundance of three MUFA species and increase in three PUFA species in viable tumor regions following NUC-7738 + pembrolizumab treatment

Changes in Apoptotic Related Lipids

- NUC-7738 + pembrolizumab causes a reduction in levels of HexCer species in tumor area
- HexCer, a subclass of sphingolipids are anti-apoptotic and associated with chemoresistance in melanoma patients[5]

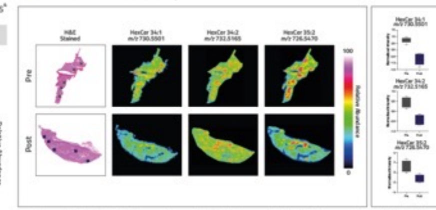


Figure 7: DESI-MSI ion heatmaps show spatial distribution and a significant decrease (|d| < 5) in the relative abundance of three HexCer species in viable tumor regions following NUC-7738 + pembrolizumab treatment

- Pro-apoptotic ceramide (Cer) species increase following treatment

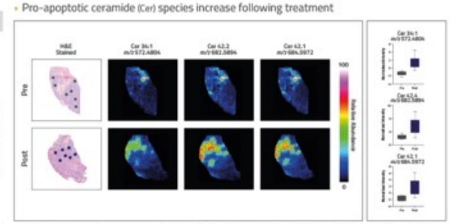
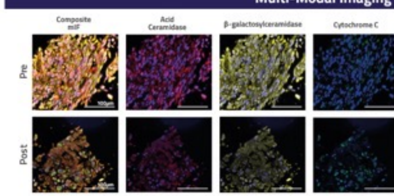


Figure 8: DESI-MSI ion heatmaps show spatial distribution and a significant increase (|d| > 3) in the relative abundance of three Cer species in viable tumor regions following NUC-7738 treatment

Multi-Modal Imaging Confirms Lipidomic Reprogramming



- Pro-apoptotic ceramide (Cer) species increase following treatment with NUC-7738 corresponding with a decrease in protein expression of acid ceramidase (AC) which hydrolyses ceramides into sphingosine and is associated with tumor progression[6]
- β-galactosylceramidase, a key enzyme in sphingolipid metabolism often upregulated in melanoma progression and associated with poor prognosis[7] decreases following NUC-7738 treatment
- Protein expression of Cytochrome C, a marker of apoptosis increases following treatment

Figure 9: mIF of 3µm FFPE section show a decrease in the protein expression of key lipid metabolizing enzymes and an increase in the apoptotic marker cyt c following NUC-7738 treatment

CONCLUSION

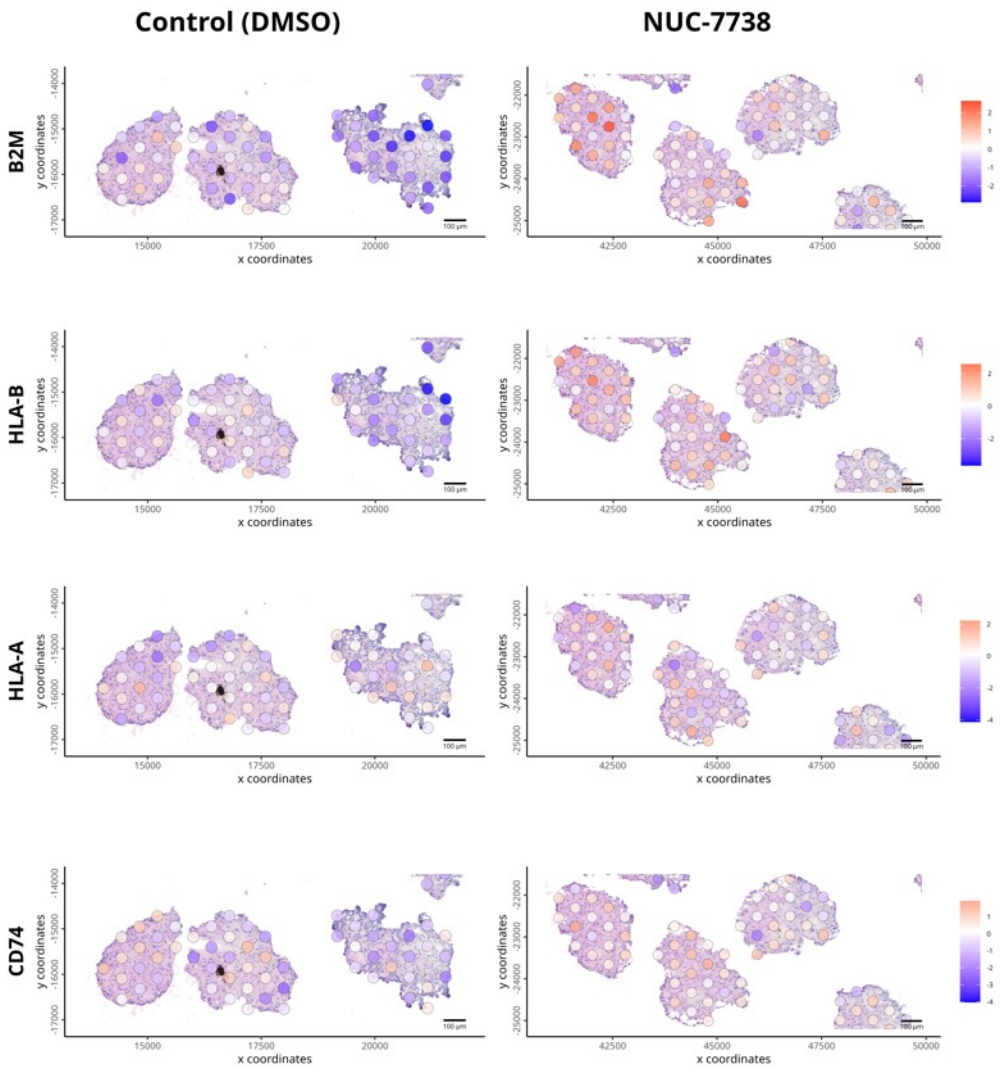
- Novel methodology developed to analyse the spatial relationship between the lipidome and TME of patient biopsies within a single-tissue section
- Preliminary results suggest that NUC-7738 ± pembrolizumab reprograms cancer cell lipid metabolism in the TME
- This platform provides a powerful investigative tool to simultaneously explore the myriad of factors in the TME that contribute to tumorigenesis and therapeutic response

NUC-7738 : Increases Abundance of RNA Transcripts Associated with Immune Presentation in Tumoroids (MHC I & II)

Primary kidney cancer tissue was dissociated & grown for 3 weeks *in vitro* before treatment. Spatial transcriptomic analysis was then performed on harvested tumoroids.

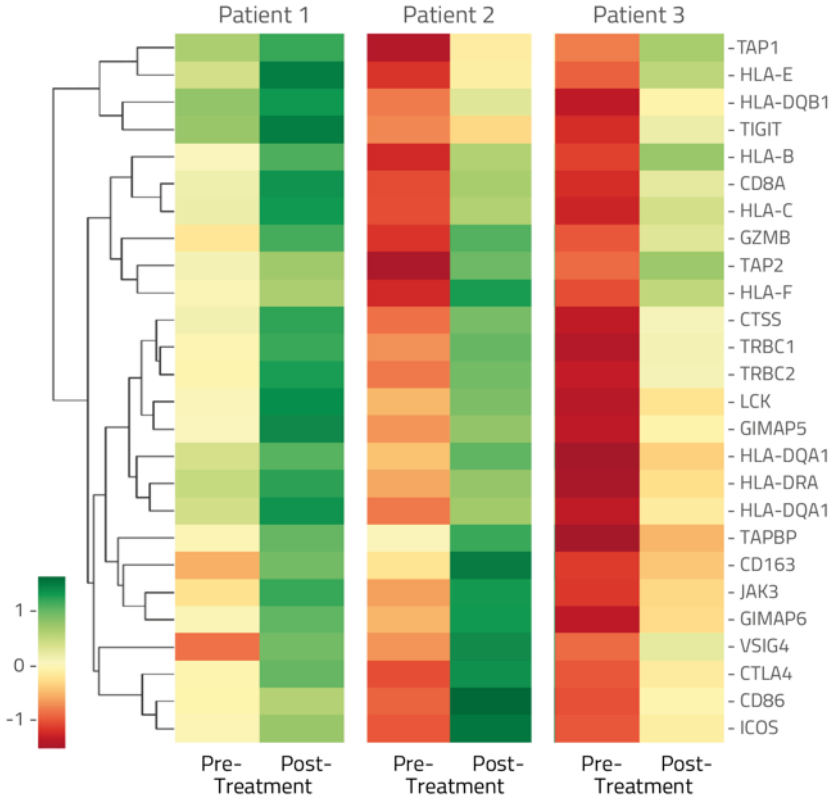
B2M is beta 2 microglobulin, and together with HLA-A and HLA-B form class 1 Major Histocompatibility Complex (MHC). Class I MHC is recognized by CD8+ T lymphocytes.

CD74 (also called HLA-DR antigens-associated invariant chain, part of Class II MHC) helps to transport peptide needed for T-cell activation. Class II MHC is recognized by CD4+ T lymphocytes.

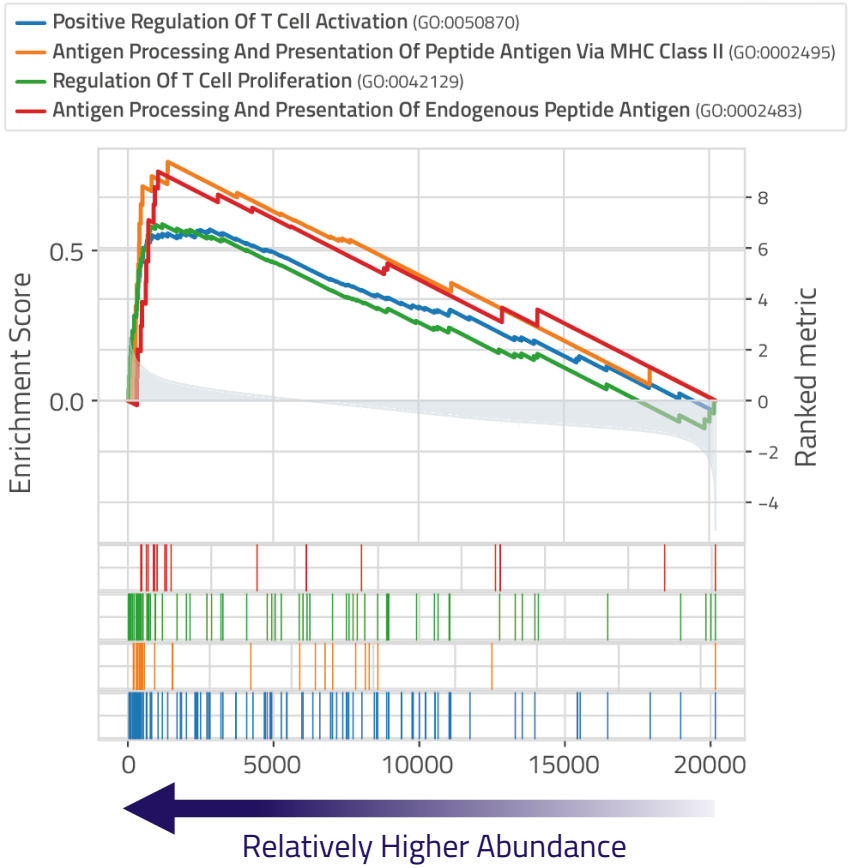


NUC-7738 : Increases Antigen Presentation & T-cell Activation in Patient Biopsies

Heatmaps illustrating RNA expression reveal a relative **increase in mRNA levels of genes associated with antigen transport & presentation and T-cell activation**

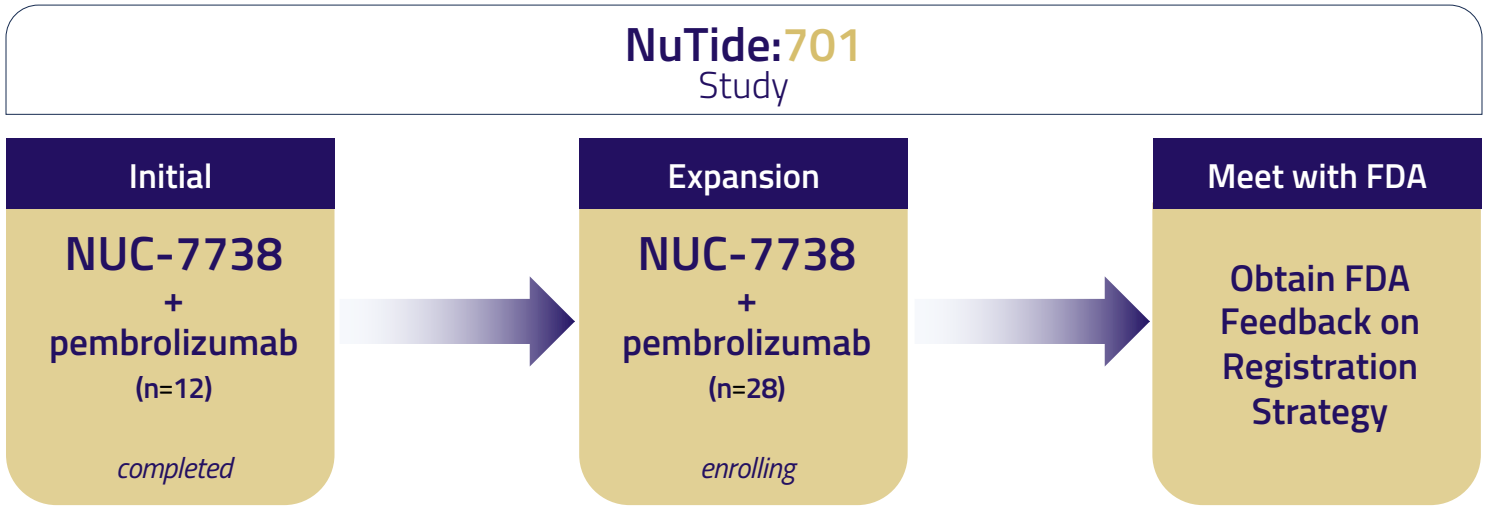


Comparative gene enrichment analysis from biopsies shows **immune pathway activation** related to **antigen processing & presentation, T-cell activation & proliferation**

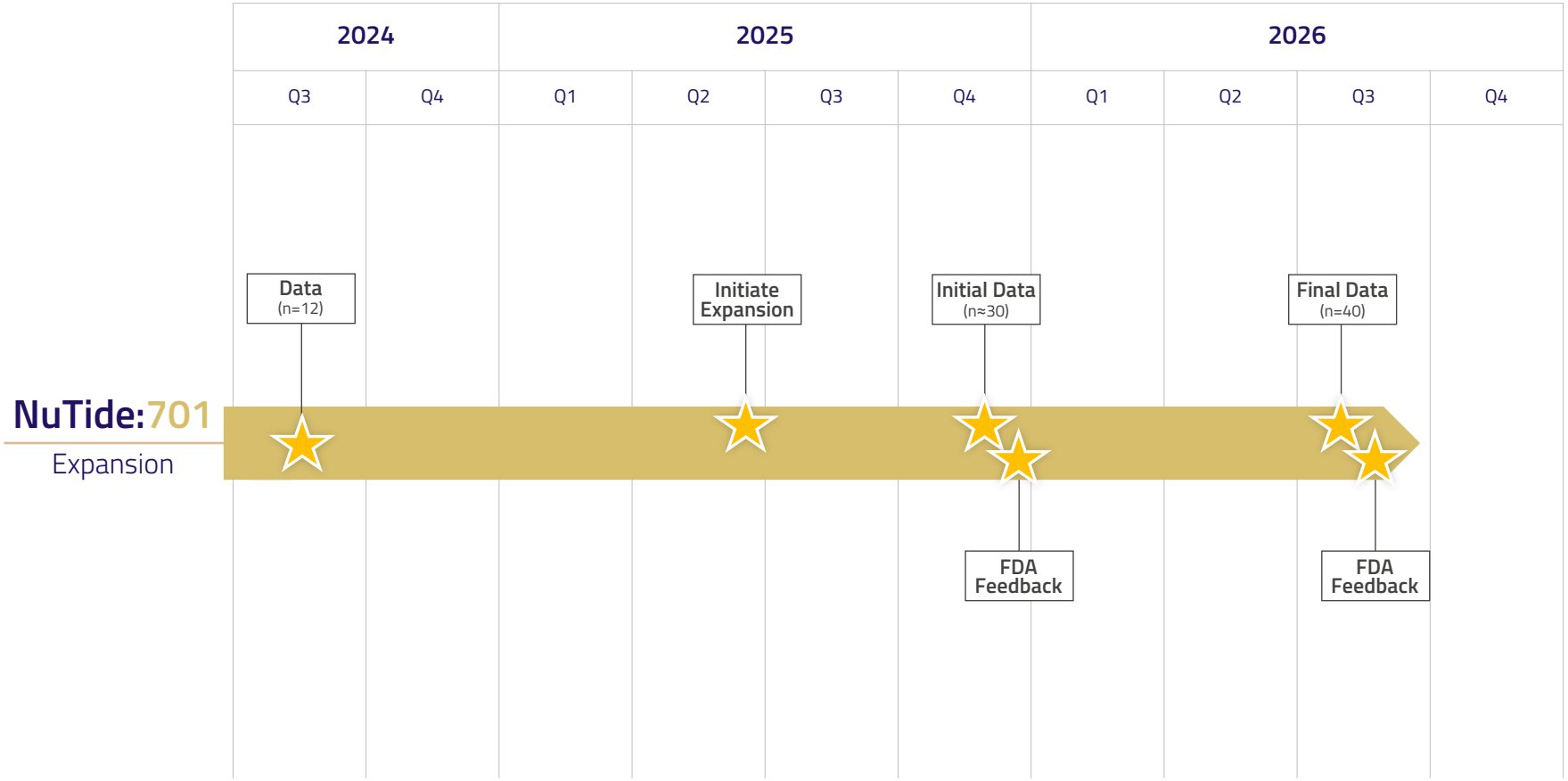


Blagden *et al* (2024) *Ann Oncol*: 35: S482-S535 Abstract ID: 666P (ESMO September 2024). Data cut-off: August 1, 2024

NUC-7738 : Melanoma Development Pathway



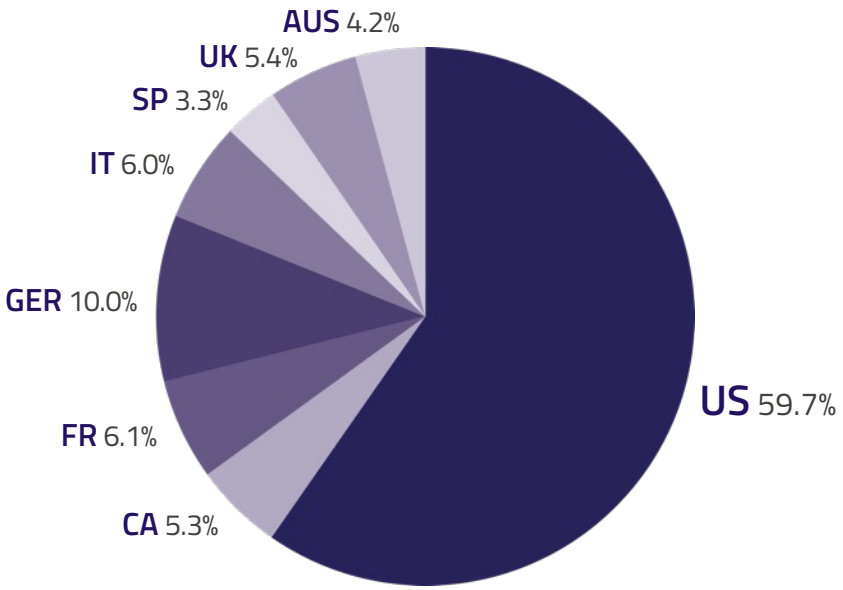
NUC-7738 : Planned Melanoma Development Pathway Milestones & Timelines



NUC-7738 : Melanoma Market Opportunity

$7.4B

Estimated sales in 8 major markets in 2029[2]



331,722 new cases
diagnosed annually[1]

58,667 deaths
annually[1]

13,000 patients
will fail PD-1 inhibitors in US[3]

5-year survival rate: 30%
Stage IV melanoma[4]

1. GLOBOCAN 2022, Cancer Incidence and Mortality Worldwide
2. Global Data Melanoma - Global Drug Forecast and Market Analysis to 2029
3. 2030 estimate based on CancerMPact data and primary market research
4. Melanoma Research Alliance (https://www.curemelanoma.org)

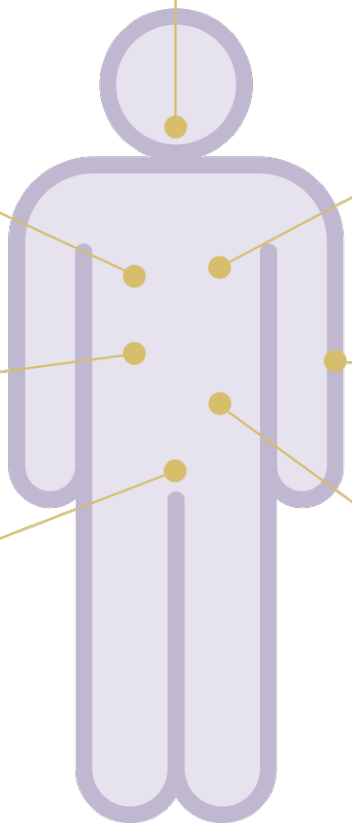
NUC-7738 : Multiple Development Opportunities

Head & Neck cancer
- PD-(L)1 resistant
- Chemo-ineligible

Non-Small Cell Lung cancer
- PD-(L)1 resistant
- Neoadjuvant setting
- Chemo-ineligible

Hepatocellular cancer
- PD-(L)1 resistant
- TKI- treated

Bladder/Urothelial cancer
- PD-(L)1 resistant
- Cisplatin ineligible

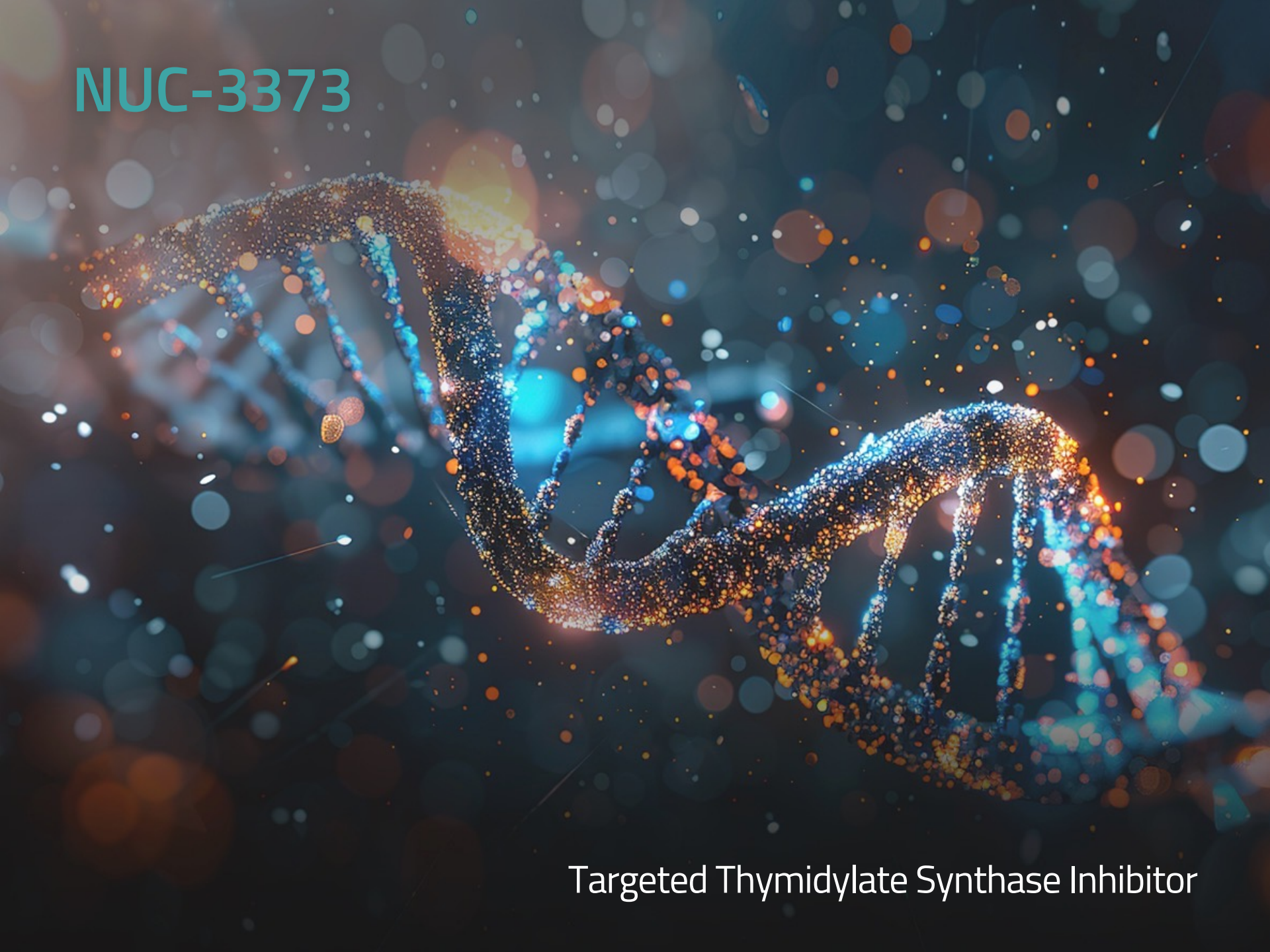


Breast cancer
- PD-(L)1 resistant
- Chemo-ineligible

Melanoma
- PD-(L)1 resistant
- BRAF/MEK refractory
- Neoadjuvant setting

Renal Cell cancer
- PD-(L)1 resistant
- TKI- treated

NUC-3373



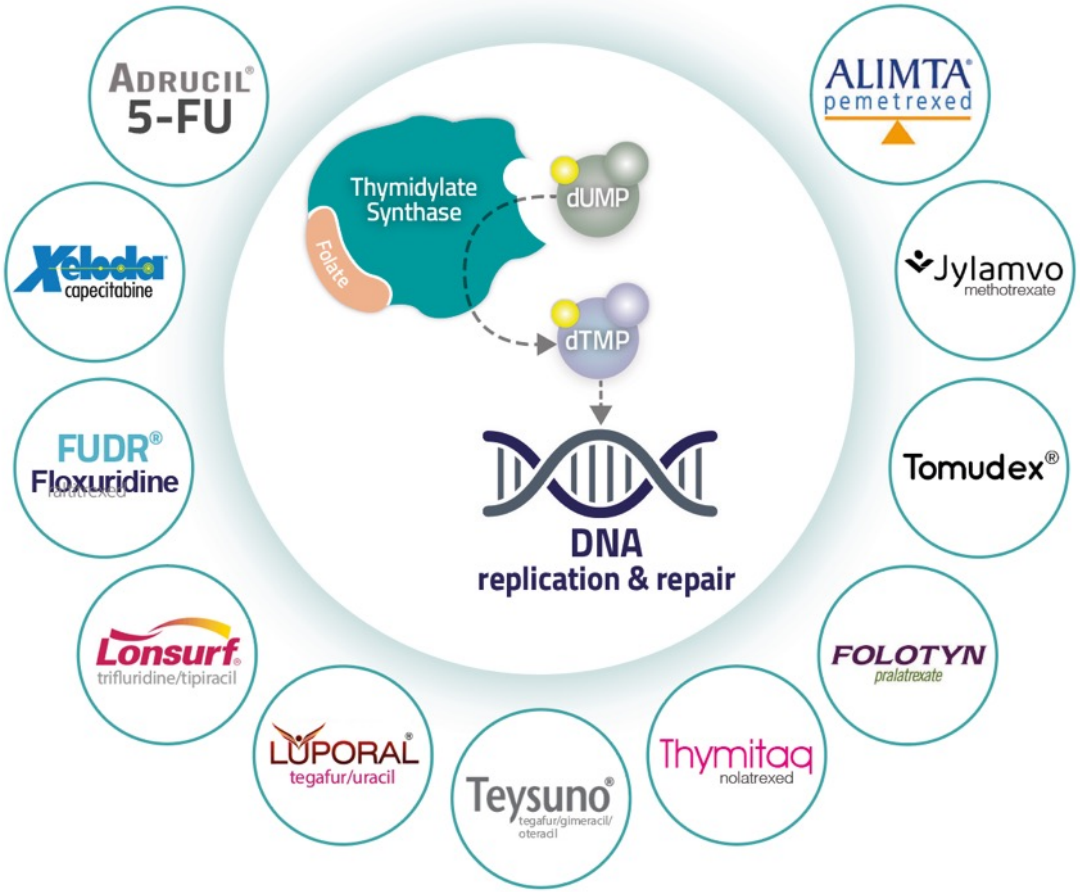
Targeted Thymidylate Synthase Inhibitor

Thymidylate Synthase: An Important Target for Anti-Cancer Therapies

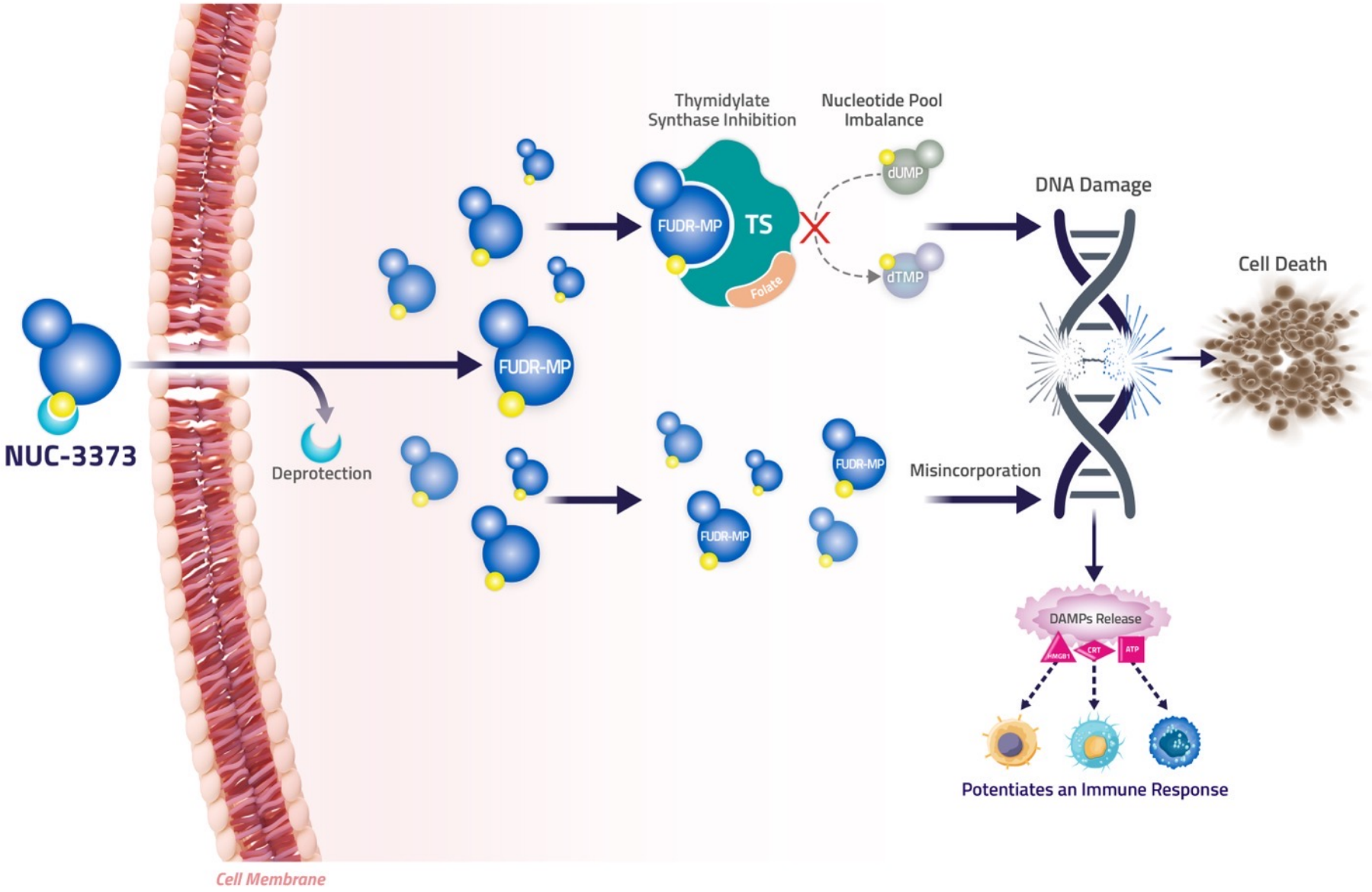
Thymidylate Synthase (TS) is a critical enzyme for nucleotide synthesis

- Converts uridine (dUMP) to thymidine (dTMP)
- Essential for DNA replication and cell proliferation
- Often upregulated in cancer cells

TS inhibitors are widely used despite their insufficient inhibition of the target enzyme



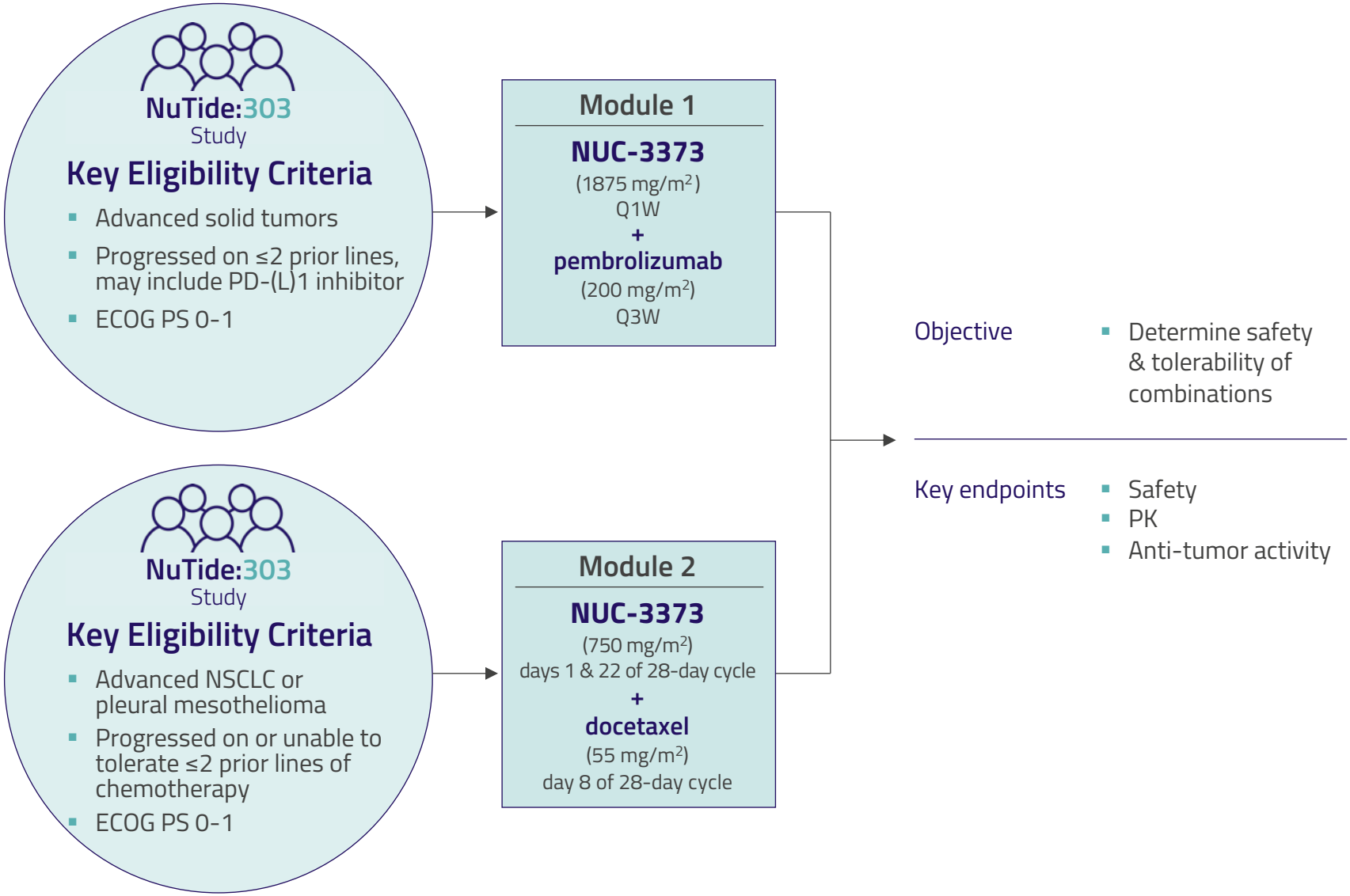
NUC-3373 : Induces DNA Damage & Potentiates an Immune Response



Over 300 patients have received **NUC-3373** across the clinical program

STUDY	COMBINATION	POPULATION	PATIENTS	STATUS
NuTide:301 Phase 1	monotherapy	Solid Tumors (end-stage)	59	Complete
NuTide:302 Phase 1b	leucovorin (LV)	CRC (end-stage)	38	Complete
NuTide:302 Phase 1b	LV + irinotecan	CRC (end-stage)	32	Complete
NuTide:302 Phase 1b	LV + oxaliplatin	CRC (end-stage)	23	Complete
NuTide:302 Phase 2	LV + irinotecan + bevacizumab	CRC (end-stage)	8	Complete
NuTide:302 Phase 2	LV + oxaliplatin + bevacizumab	CRC (end-stage)	6	Complete
NuTide:323 Phase 2 (randomized)	LV + irinotecan + bevacizumab vs. FOLFIRI + bevacizumab	CRC (second-line)	120 (NUC-3373) 57 (5-FU)	Discontinued
NuTide:303 Phase 1b/2	pembrolizumab	Solid Tumors (second/third-line)	13	Ongoing
NuTide:303 Phase 1b/2	docetaxel	Lung Cancer (second/third-line)	4	Ongoing

NUC-3373 : Phase 1b Study (ongoing)



NUC-3373 : Favorable Safety Profile (combination with pembrolizumab)

NUC-3373 + pembrolizumab has been well tolerated (n=13)

- One Grade 3 TRAE: hyponatremia
- No Grade 4 toxicities

Treatment Related Adverse Events

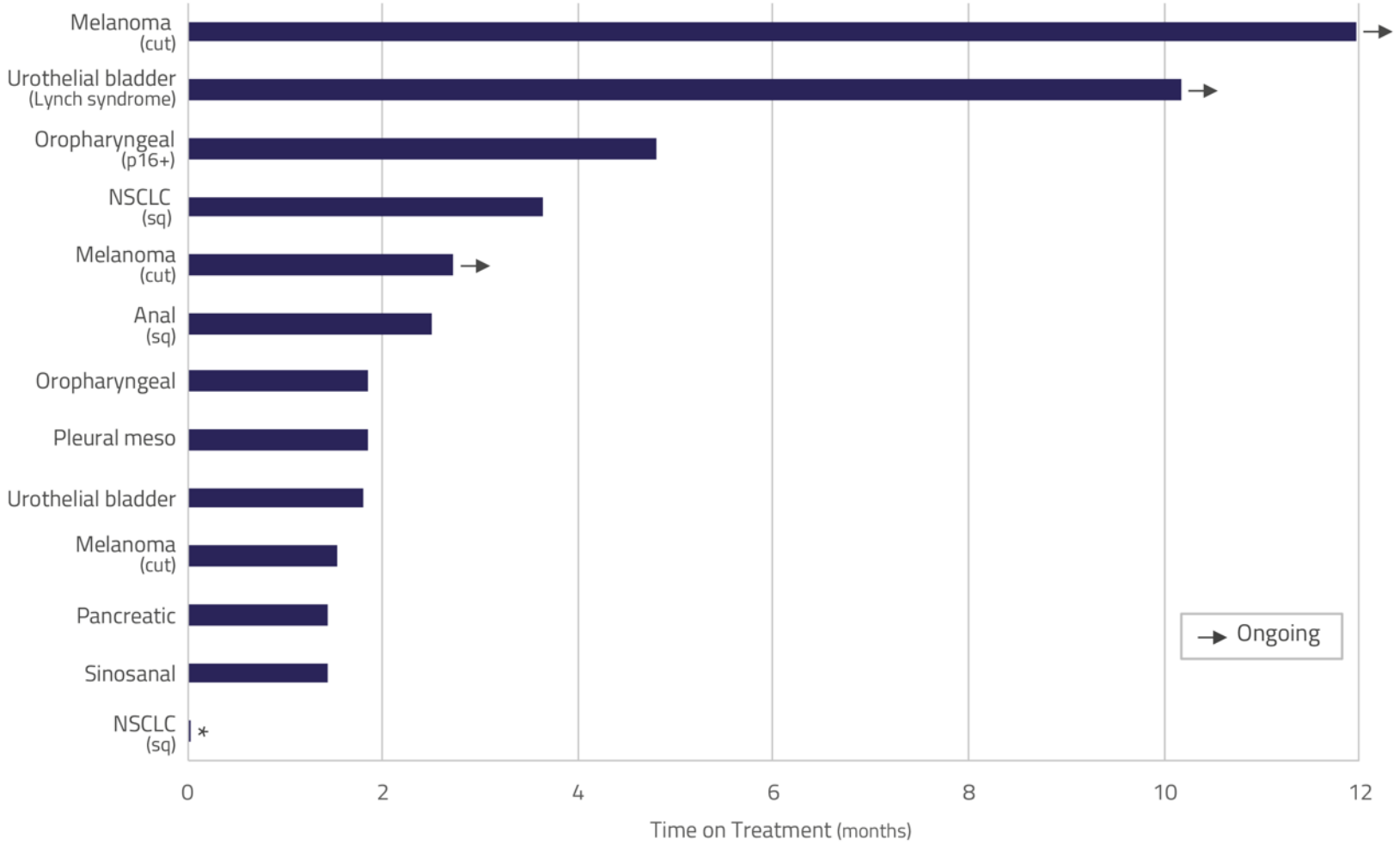
	All Grades n(%)	Grade 3 n(%)	Grade 4 n(%)
Nausea	9 (69)	0	0
Vomiting	9 (69)	0	0
Diarrhea	6 (46)	0	0
Fatigue	5 (38)	0	0
AST increased	4 (31)	0	0
Infusion related reaction	4 (31)	0	0
Anemia	3 (23)	0	0
Constipation	3 (23)	0	0
ALT increased	3 (23)	0	0
Hot flush	3 (23)	0	0
Abdominal pain	2 (15)	0	0
Flushing	2 (15)	0	0

All Grade TRAEs with prevalence ≥15% patients related to NUC-3373, pembrolizumab or both

Middleton *et al* (2024) *medRxiv* doi: 10.1101/2024.11.07.24316829. Data cut-off: October 8, 2024

NUC-3373 : Prolonged Time on Treatment (combination with pembrolizumab)

Encouraging duration of clinical benefit in PD-(L)1 experienced patients



*Patient only received 1 dose of study treatment and was not DLT-evaluable

Middleton *et al* (2024) *medRxiv* doi: 10.1101/2024.11.07.24316829. Data cut-off: October 8, 2024

NUC-3373 : Encouraging Signs of Efficacy (combination with pembrolizumab)

Cutaneous Melanoma

75 years ▪ BRAF mt
2 prior lines

1) pembrolizumab:
progressive disease within **5 months**

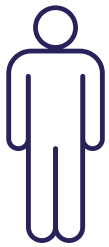
2) trametinib + dabrafenib:
trametinib discontinued after **1 month** (toxicity)
dabrafenib for 7 years (progressive disease)

NUC-3373 1875 mg/m^2 **+ pembrolizumab** 200 mg
- 1 target lesion (bilateral lymph node)

Partial Response (confirmed): 81% reduction in tumor volume

Treatment duration: 12+ months (ongoing)
- No dose reductions

Bladder Cancer



72 years ▪ Lynch Syndrome
2 prior lines

1) gemcitabine + cisplatin (adjuvant):
discontinued due to myelosuppression **2 months**

2) atezolizumab (metastatic):
best response SD, discontinued after **23 months**

NUC-3373 1875 mg/m^2 **+ pembrolizumab** 200 mg
- 1 target lesion (lung)

100% reduction in sum of target lesions

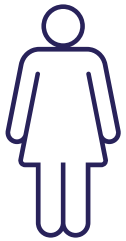
Partial Response (confirmed) due to presence of non-target lesions

Treatment duration: 10+ months (ongoing)
- No dose reductions

Middleton *et al* (2024) *medRxiv* doi: 10.1101/2024.11.07.24316829. Data cut-off: October 8, 2024

NUC-3373 : Encouraging Signs of Efficacy (combination with docetaxel)

Pleural Mesothelioma



60 years
3 prior lines

1) cisplatin + pemetrexed:
progressive disease within **4 months**

2) nivolumab:
progressive disease within **4 months**

3) carboplatin + pemetrexed:
progressive disease within **1 month**

NUC-3373 750 mg/m^2 **+ docetaxel** 55 mg/m^2

- 4 target lesions (2x lymph node, 2x mediastinum)

Stable Disease: 13+ months (ongoing)

Treatment duration: 8.5 months (discontinued due to fatigue)

- NUC-3373 + docetaxel (4 cycles), followed by NUC-3373 (5 cycles)

NSCLC (squamous)

77 years
2 prior lines

1) carboplatin + paclitaxel + pembrolizumab:
stable disease for **2 months**

2) pembrolizumab (maintenance):
progressive disease within **21 months**

NUC-3373 750 mg/m^2 **+ docetaxel** 55 mg/m^2

- 1 target lesion (lung)

Stable Disease: 7 months

Treatment duration: 7 months

- NUC-3373 + docetaxel (6 cycles), followed by NUC-3373 (2 cycles)

Middleton *et al* (2024) *medRxiv* doi: 10.1101/2024.11.07.24316829. Data cut-off: October 8, 2024

Strong Intellectual Property Position

Worldwide exclusive rights for all programs: **395 granted patents** and **83 pending applications***

KEY PATENTS	STATUS	EXPIRATION+ (excluding any extensions)	TERRITORIES
NUC-7738	**95 granted, 44 pending, including:**		
Composition of matter	Granted (US, EP, CN, JP)	2035	 + others
Formulation	Pending	2036	 + others
Manufacturing process	Pending	2038	 + others
Use	Pending	2043	 + others
NUC-3373	**185 granted, 47 pending, including:**		
Composition of matter	Granted (US, EP, CN, JP)	2032	 + others
Formulation	Granted (JP), Pending (US, EP, CN)	2036	 + others
Manufacturing process	Pending	2043	 + others
Use	Pending	2037 / 2038	 + others

*As of February 25, 2025
+Expiration for pending patents if granted

Key Expected Milestones: 2025

	INDICATION	COMBINATION	PHASE	MILESTONE
NUC-7738 NuTide:701 Study	Melanoma	pembrolizumab	Phase 2	Initiate Study Expansion Announce Expansion Data Obtain FDA Feedback on Registration Strategy
NUC-3373 NuTide:303 Study	Solid Tumors	pembrolizumab	Phase 1b/2	Announce Data

Investment Highlights

NUC-7738
Transforms Tumor Microenvironment

Differentiated mode of action: RNA polyadenylation
Encouraging signs of efficacy
Favorable safety profile
Potentiates PD-1 inhibition

NUC-3373
Targeted TS inhibitor

Induces DNA damage
Encouraging signs of efficacy as monotherapy & in combination with PD-1 inhibitor
Favorable safety profile

Experienced Team

Accomplished management team backed by leading biotech investors

Improving Survival Outcomes

Synergy in combination with immune checkpoint inhibitor therapy

Strong IP Protection

Worldwide exclusive rights

Significant Milestones

Numerous value inflection points throughout 2025

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