Adaptive transcriptional and immunomodulatory responses to the novel microtubule destabilizer AUS_001 in pancreatic and colorectal cancer models

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Background

Heterogeneous and therapy-resistant gastrointestinal cancers continue to pose major clinical challenges, calling for novel treatment strategies. Our prior studies revealed that the prenylated hydroxy-stilbene, AUS_001, inhibits tubulin polymerization via its unique binding configuration to the colchicine site of β-tubulin and suppresses cancer cell proliferation both *in vitro* and *in vivo*.

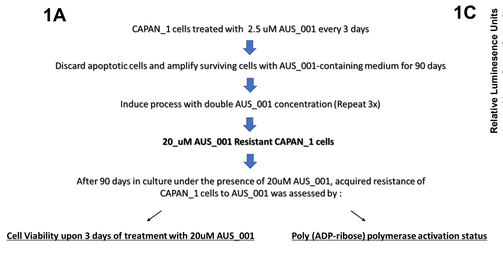
Aim

The goal of the current study was to perform long-term *in vitro* studies that mimic chronic therapy, allowing more predictive insights regarding side effects, chemoresistance and rational drug combinations.

Results

1. CAPAN-1 cells exhibit adaptive resistance upon long-term AUS_001 exposure

Figure 1. (A) Methodology for the establishment of AUS_001 Resistant CAPAN-1 pancreatic cancer cells. The IC50 of the parental cells equals 2.67 μM. **(B)** EVOS™ XL Core Imaging System of CAPAN-1 cells with acquired AUS_001 resistance after 90 days in culture. **(C)** Cell viability assessment and **(D)** semi-quantitative detection of poly (ADP-ribose) deposited by PARP-1 onto immobilized histone proteins in lysates originating from parental (wt) or AUS_001-Resistant CAPAN-1 cells treated with 20μM AUS_001 and the respective vehicle for 72 hours.



2. Gene ontology analysis for DEGs in signaling pathways and molecular processes under prolonged AUS_001 pressure

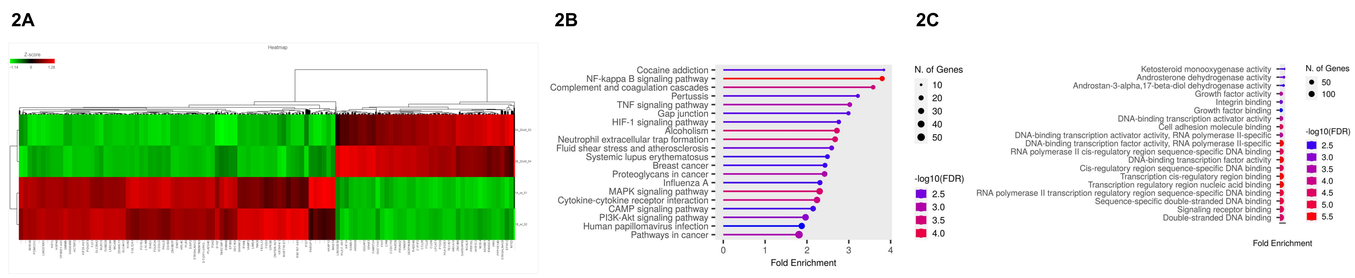


Figure 2. (A) Heatmap with hierarchical clustering of RNA Sequencing–derived differentially expressed genes (DEGs) between parental (wt) and AUS_001 Resistant CAPAN-1 cells. The data is displayed in a grid where each row represents a gene and each column represents a sample. The color and intensity of the boxes is used to represent changes (not absolute values) of gene expression. DEGs are clustered within **(B)** functional categories, including signaling pathways that play critical roles in cellular response to external stimuli, and **(C)** molecular functions linked to transcriptional control.

3. Gene signature of pancreatic cancer cells with acquired AUS_001 resistance

3A

Gene name	Gene Description	P-value	Fold change
TUBB2A	tubulin beta 2A class IIa [Source:HGNC Symbol;Acc:HGNC:20772]	0.0000002257	-4.41
TUBB2B	tubulin beta 2B class IIb [Source:HGNC Symbol;Acc:HGNC:20820]	0.0000015S	-3.83
TUBB3	tubulin beta 3 class III [Source:HGNC Symbol;Acc:HGNC:20772]	0.0000006B2	-2.91
TUBB4A	tubulin beta 4A class IVa [Source:HGNC Symbol;Acc:HGNC:20774]	0.000084	-2.38
TUBD1	tubulin delta 1 [Source:HGNC Symbol;Acc:HGNC:16811]	0.0000237	2.2
MAP10	microtubule associated protein 10 [Source:HGNC Symbol;Acc:HGNC:16810]	2.72E-04	-2.01
MAP9	microtubule associated protein 9 [Source:HGNC Symbol;Acc:HGNC:26538]	4.35E-05	2.22E+00
MAPT	microtubule associated protein tau [Source:HGNC Symbol;Acc:HGNC:6893]	1.47E-06	3.17
MAP2	microtubule associated protein 2 [Source:HGNC Symbol;Acc:HGNC:6839]	0.0000558	3.38
JAKMIP3	janus kinase and microtubule interacting protein 3 [Source:HGNC Symbol;Acc:HGNC:27023]	0.00381	2.22
TTBK2	tau tubulin kinase 2 [Source:HGNC Symbol;Acc:HGNC:30406]	0.0000345	2.23
MACF1	microtubule actin crosslinking factor 1 [Source:HGNC Symbol;Acc:HGNC:13664]	0.000284	-2.03
MICAL3	microtubule associated monooxygenase, calponin and LIM domain containing 3 [Source:HGNC Symbol;Acc:HGNC:29015]	0.000738	-2.1
MAST4	microtubule associated serine/threonine kinase family member 4 [Source:HGNC Symbol;Acc:HGNC:19337]	0.000133	-2.2
TTLL6	tubulin tyrosine ligase like 6 [Source:HGNC Symbol;Acc:HGNC:28864]	0.000017	2.48
TPPP3	tubulin polymerization promoting protein family member 3 [Source:HGNC Symbol;Acc:HGNC:24162]	0.0000182	3.13
ATAT1	alpha tubulin acetyltransferase 1 [Source:HGNC Symbol;Acc:HGNC:21386]	0.00011	-2.09
ACTB	actin beta [Source:HGNC Symbol;Acc:HGNC:132]	0.0000034	-2.37
DNAH12	dynein axonemal heavy chain 12 [Source:HGNC Symbol;Acc:HGNC:2942]	0.0000211	-2.44
DYNC2H1	dynein cytoplasmic 2 heavy chain 1 [Source:HGNC Symbol;Acc:HGNC:2962]	0.000591	2.04
DNAH6	dynein, axonemal, heavy chain 6 [Source:HGNC Symbol;Acc:HGNC:2951]	0.000159	2.47
KIF5C	kinesin family member 5C [Source:HGNC Symbol;Acc:HGNC:6325]	0.0000159	6.03
KIF12	kinesin family member 12 [Source:HGNC Symbol;Acc:HGNC:21493]	0.000914	2.29

3B

Gene name	Gene Description	P-value	Fold change
HLA-DMA	major histocompatibility complex, class II, DM alpha [Source:HGNC Symbol;Acc:HGNC:4934]	1.27E-05	2.46
CIITA	class II major histocompatibility complex transactivator [Source:HGNC Symbol;Acc:HGNC:7067]	3.87E-06	3.6
HLA-DPB1	major histocompatibility complex, class II, DP beta 1 [Source:HGNC Symbol;Acc:HGNC:4940]	0.0000261	4.24
HLA-DMB	major histocompatibility complex, class II, DM beta [Source:HGNC Symbol;Acc:HGNC:4935]	0.000000796	5.49
HLA-DPA1	major histocompatibility complex, class II, DP alpha 1 [Source:HGNC Symbol;Acc:HGNC:4938]	0.000001111	9.11
HLA-DQB1	major histocompatibility complex, class II, DQ beta 1 [Source:HGNC Symbol;Acc:HGNC:4945]	0.000000329	12.7
HLA-DRB6	major histocompatibility complex, class II, DR beta 6 (pseudogene) [Source:HGNC Symbol;Acc:HGNC:4954]	4.77E-08	12.8
HLA-DRA	major histocompatibility complex, class II, DR alpha [Source:HGNC Symbol;Acc:HGNC:4947]	3.75E-09	14
HLA-DRB1	major histocompatibility complex, class II, DR beta 1 [Source:HGNC Symbol;Acc:HGNC:4948]	2.46E-08	16.8
HLA-DRB5	major histocompatibility complex, class II, DR beta 5 [Source:HGNC Symbol;Acc:HGNC:4953]	0.000000138	20.8

Figure 3. (A) Key alterations in genes associated with microtubule cytoskeleton, cytokinesis and cytoskeleton/microtubule interactions were triggered by AUS_001 sustained exposure. **(B)** Dramatic increase in the expression major histocompatibility complex class II genes was observed under AUS_001 pressure.

4. Oral administration of AUS_001 triggers tumor infiltration of lymphocytes *in vivo*

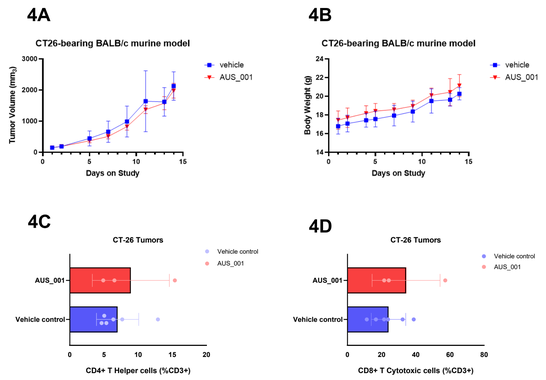


Figure 4. Assessment of **(A)** tumor volume (mm^3), **(B)** body weight, intra-tumoral infiltration by **(C)** CD4+ T Helper cells and **(D)** CD8+ cytotoxic T cells, following daily oral administration of SEDDS-formulated AUS_001. CT26-bearing syngeneic BALB/c mice (n=5) received 900 mg/kg AUS_001 once daily for 4 days, followed by 450 mg/kg daily for an additional 10 days prior to sacrifice. Freshly excised tumors were enzymatically dissociated and analyzed by flow cytometry.

Conclusions

Dose-escalation exposure *to AUS-001 drives transcriptional adaptations characterized by cytoskeletal remodeling and immune modulation across pancreatic and colorectal cancer models. Collectively, these findings provide mechanistic insight into AUS-001 activity and support its further investigation as a potential therapeutic strategy for gastrointestinal cancers.*

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