High-throughput quantitative characterization of cytotoxic antibody-drug conjugates using spheroid models reveals important considerations in potential molecular mechanisms of ADC resistance

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Abstract #57

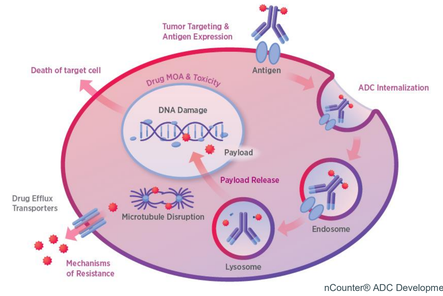
Introduction

Antibody-drug conjugates (ADCs) are a class of cancer therapeutics comprised of a linker-payload conjugated to a monoclonal antibody targeting a tumor-associated antigen (TAA), to enable the delivery of the cytotoxic payload to cancer cells.

Presently, there is a need for improved *in vitro* models that better recapitulate in vivo tumor tissue complexity to aid in the screening and evaluation of novel ADCs during preclinical development. Specifically, we have developed *in vitro* 3D models from cancer cell lines yielding spheroids in a rapid, robust and uniform manner to functionally evaluate the cytotoxic activity of ADCs in vitro.

Further characterization of ADC activity in 3D cell line models utilized the nCounter® ADC Development Panel. In addition to 3D models, we also validated the ADC Development panel for use with FFPE tissue across 5 different cancer types to assess relevant ADC pathways in both pre-clinical and clinical studies.

Methods and nCounter® ADC Development Panel



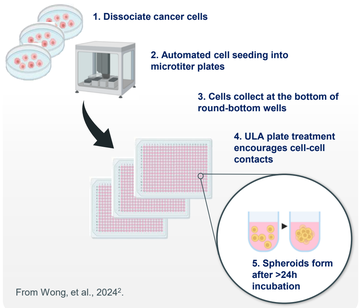
nCounter® ADC Development Panel Product Bulletin[1]

Accuracy, precision, specificity, and RNA input range were assessed using a total of 53 FFPE samples from solid tumor types, including thyroid; colorectal (CRC); endometrial; non-small cell lung cancer (NSCLC); and bladder cancer.

FFPE and *in vitro* cell model derived RNA was analyzed using the nCounter® ADC Development Panel, a 770 gene profiling assay containing genes specifically relevant to ADC activity, including tumor targeting and antigen expression, ADC internalization, payload release; drug mechanism of action, mechanisms of resistance, death of the target cell, and immunogenic cell death.

Cell Culture Methods

Spheroids were generated by seeding the ovarian cancer cell line IGROV-1 into microtiter plates treated with ultra-low attachment coating, using automated liquid-handling robots, followed by two-three days incubation under standard culture conditions[2].



From Wong, et al., 2024[2].

Spheroids were then treated for 4-days with 5 nM of ADC-1 or ADC-2.

In order to generate ADC resistant IGROV-1 cells, standard cell culture conditions were maintained with a 1 nM ADC concentration for a total of 11 weeks.

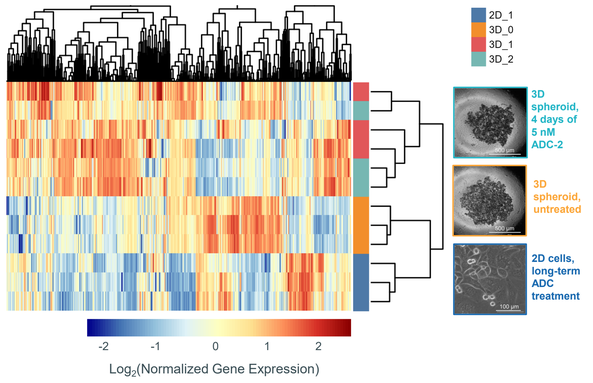
Cells and spheroids were harvested and snap frozen prior to transcriptomic evaluation with the ADC Development Panel.

FFPE Tissue Assessment and RNA Quality

ID	Cancer Type	% Tumor Content	% Necrosis	%Tumor in Circled Area	Total Tumor Surface Area (mm2)	Macro-dissect	RNA Concentration (ng/µL)	RIN	DV200 (%)	260/280
A-17	Bladder Cancer	65	3	75	325	No	191.8	2.4	14	1.91
A-18		80	1	80	240	No	262.3	2.4	16	1.91
A-25		55	2	65	28	No	50.8	1.8	26	1.67
A-26		70	5	70	63	No	59.5	2	36	1.77
A-10	CRC	60	10	70	240	No	211.3	1.8	18	1.86
A-24		45	15	60	135	Yes	120.3	2.5	21	1.8
A-23		40	10	50	160	Yes	113	2.4	21	1.9
A-13		60	10	70	36	No	92.5	1.9	32	1.87
A-21		40	45	65	280	Yes	548.6	1.7	43	1.87
A-16		55	15	70	165	No	254.9	1.4	50	1.99
A-15	Endometrial Cancer	30	3	50	135	Yes	123.7	2.4	26	1.92
A-27		80	10	80	300	No	266.5	2	30	1.89
A-28	NSCLC	40	20	45	80	Yes	118.1	2.2	33	1.94
A-29		40	1	50	120	Yes	158.1	2.7	39	1.85
A-14		45	5	60	108	Yes	186.1	1.2	41	1.89
A-12		45	40	50	108	Yes	256.1	1.5	44	1.94
A-19		60	15	60	78	No	230.4	1.3	55	1.97
A-11	Thyroid Cancer	80	0	90	240	No	51	2.5	10	1.75
A-22		60	0	65	120	No	154.1	2.4	22	1.84
A-20		40	5	65	120	Yes	71.8	2.1	34	1.64

Specimen pathology review determined indication, specimen source, tumor percentage, and tumor surface area, and samples with less than 50% tumor were macro-dissected for total RNA extraction and evaluation with the ADC Development Panel.

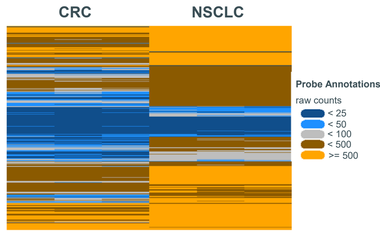
Comparison of ADC Treated *In Vitro* Models



Differential gene expression in 2D long-term ADC-1 treated IGROV-1 cells (**2D_1**) or spheroids that have been treated for 4-days with ADC-1 (**3D_1**) or ADC-2 (**3D_2**) or left untreated (**3D_0**) reveal unsupervised clustering within cell model type and treatment, consistent with observations in functional characterization[2].

Validation of ADC Development Panel

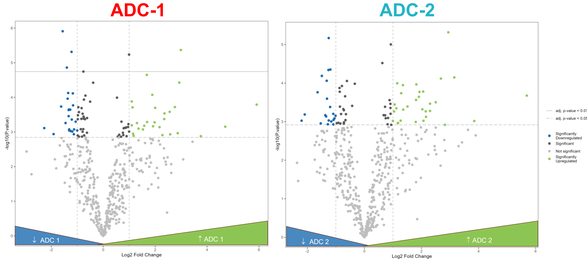
Test Specification	Test Results	Acceptable criteria
Accuracy	100%	≥95%
Repeatability	100%	≥95%
Reproducibility	100%	≥95%



Sample	Average Counts
Water (n=3)	12.0
E-Coli (n=2)	12.1
Tumors (n=53)	692.2

RNA input	Correlation Coefficient (relative to 300ng input)
300ng	1.0
150ng	0.9987
50ng	0.9957

Distinct Pathway Changes with ADC Treatment



Gene	Pathway	ADC 1		ADC 2	
		Fold change	Adj p-value	Fold change	Adj p-value
LAPTM5	Lysosome	60.13	0.0257	51.63	0.0297
CXCL8	Cytokine & Chemokine Signaling Immunogenic Cell Death	25.99	0.0407	14.93	0.0632
PRKCG	Antibody Dependent Cellular Cytotoxicity	10.85	0.0579	14.42	0.0465
TRIM22	Cytokine & Chemokine Signaling Type II Interferon Signaling	6.77	0.0257	8.88	0.0243
HMOX1	Chemical Stress, Inflammasomes Oxidative Stress	7.94	0.00477	7.67	0.0109
EPN3	Endocytosis	7.62	0.0138	6.41	0.0243
PMAIP1	Apoptosis	3.20	0.0105	2.55	0.0243
ABCD1	Drug Efflux Pumps	2.46	0.0361	2.25	0.0471
FEN1	Cell Cycle DNA Replication, Base Excision Repair	-3.07	0.0257	-3.12	0.0271
MCM6	Cell Cycle DNA Replication	-2.64	0.00903	-2.28	0.0243
MKI67	Cell Cycle	-2.25	0.0224	-2.39	0.0243
SEMA3A	Vasculature & Permeability	-2.33	0.00477	-1.73	0.0243

Differentially expressed genes and pathways in spheroids treated with ADC-1 (**3D_1**) or ADC-2 (**3D_2**) vs without ADC (**3D_0**).

Conclusions

- nCounter is a robust and reliable platform to assess the gene expression of RNA samples especially using poor quality, fragmented FFPE-derived samples (DV200 < 30%) across multiple tumor types.
- Results demonstrate the reliability of the nCounter ADC Development panel for use in ADC-focused clinical studies.
- Analysis of *in vitro* cell culture methods (spheroids vs. monolayer), and different ADC treatments show distinct differences in relevant functional pathways, demonstrating its utility for ADC pipeline development.

References

1. nCounter® ADC Development Panel Product Bulletin. 2022.
2. Wong J, Hernández Rojas A., Bissessur A, et al. Abstract 3127: Development of three-dimensional cancer cell line spheroid models for the in vitro functional characterization of cytotoxic antibody-drug conjugates. *Cancer Res.* 2024, 84, 3127.

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