High-throughput quantitative molecular characterization of cytotoxic antibody-drug conjugates in spheroid models for improved functional characterization, screening and candidate selection

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Abstract #1230

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.

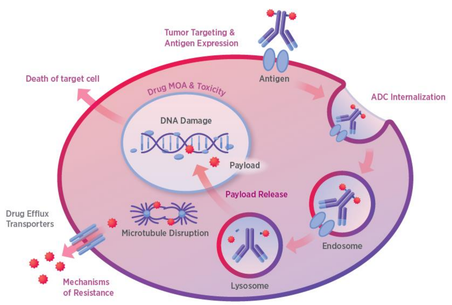
Presently, there is a need for improved in vitro models that better recapitulate in vivo tumor tissue complexity to aid in the screening and evaluation of novel ADCs during preclinical development. Specifically, we have developed in vitro 3D models from cancer cell lines yielding spheroids in a rapid,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

For further comprehensive characterization of ADC activity in 3D cell line models, we utilized the nCounter® ADC Development Panel, a specialized gene expression tool for molecular characterization of biological function, with customizable gene content to address complex questions important for the success of ADCs throughout discovery, pre-clinical and clinical development.

nCounter® ADC Development Panel

The nCounter® ADC Development Panel is a 770 gene profiling panel addressing 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.

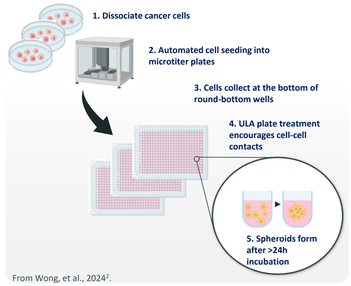
Together with the analytical validation of the panel, in this set of experiments, we focused on the functional theme of mechanisms of resistance.



nCounter® ADC Development Panel Product Bulletin[1] .

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 four days with one of two ADCs at 5 nM concentration.

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 functional transcriptomic evaluation with the ADC Development Panel.

Tissue Assessment and RNA Quality

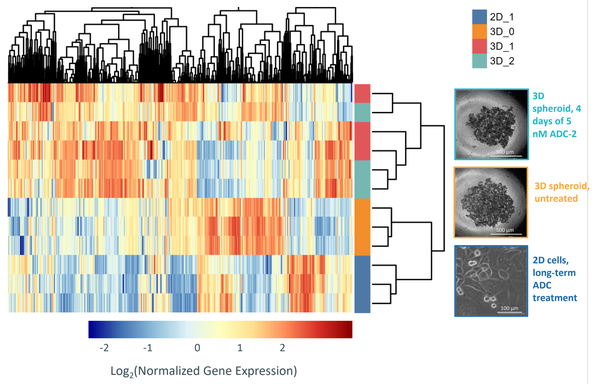
Accuracy, precision, specificity, and RNA input range using the ADC Development Panel were assessed using a total of 53 FFPE samples from solid tumor types, including thyroid; colorectal; endometrial; NSCLC; and bladder.

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 further evaluation.

Accession ID	Cancer Type	% Tumor Content	% Necrosis	%Tumor in Circled Area	Total Tumor Surface Area (mm2)	Macrodissect	RNA Concentration (ng/µL)	RIN	DV200 (%)	260/280
A-00291210	CRC	60	10	70	240	No	211.3	1.8	18	1.86
A-00291211	Thyroid Cancer	80	0	90	240	No	51	2.5	10	1.75
A-00291212	NSCLC	45	40	50	108	Yes	256.1	1.5	44	1.94
A-00291213	CRC	60	10	70	36	No	92.5	1.9	32	1.87
A-00291214	NSCLC	45	5	60	108	Yes	186.1	1.2	41	1.89
A-00291215	Endometrial	30	3	50	135	Yes	123.7	2.4	26	1.92
A-00291216	CRC	55	15	70	165	No	254.9	1.4	50	1.99
A-00291217	Bladder Cancer	65	3	75	325	No	191.8	2.4	14	1.91
A-00291218	Bladder Cancer	80	1	80	240	No	262.3	2.4	16	1.91
A-00291219	NSCLC	60	15	60	78	No	230.4	1.3	55	1.97
A-00291220	Thyroid Cancer	40	5	65	120	Yes	71.8	2.1	34	1.64
A-00291221	CRC	40	45	65	280	Yes	548.6	1.7	43	1.87
A-00291222	Thyroid Cancer	60	0	65	120	No	154.1	2.4	22	1.84
A-00291223	CRC	40	10	50	160	Yes	113	2.4	21	1.90
A-00291224	CRC	45	15	60	135	Yes	120.3	2.5	21	1.80
A-00291225	Bladder Cancer	55	2	65	28	No	50.8	1.8	26	1.67
A-00291226	Bladder Cancer	70	5	70	63	No	59.5	2.0	36	1.77
A-00291227	Endometrial	80	10	80	300	No	266.5	2.0	30	1.89
A-00291228	NSCLC	40	20	45	80	Yes	118.1	2.2	33	1.94
A-00291229	NSCLC	40	1	50	120	Yes	158.1	2.7	39	1.85

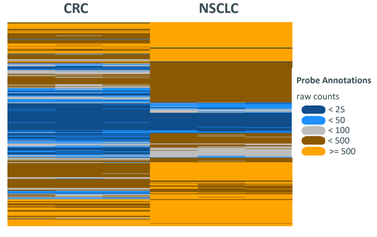
Comparing In Vitro Cell Culture Models With ADC Treatment

Functional genetic comparisons between the 2D long-term ADC-1 treated IGROV-1 cells (**2D_1**) or spheroids that have been treated for four 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 by Wong et al., 2024[2].



Validation of ADC Development Panel

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



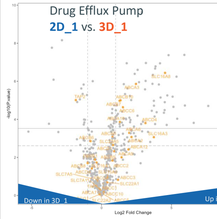
Sample	Average Counts
Water (n=3)	12.0
E-Coli (n=2)	12.1
Tumors (n=53)	692.2

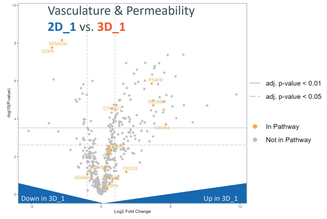
RNA input	Correlation Coefficient (relative to 300ng input)
300ng	1.0
150ng	0.9987
50ng	0.9957

Changes in Mechanisms of Resistance Observed in 2D and 3D Cell Culture Models

One of the functional themes covered by the ADC Development Panel is that of "Mechanisms of Resistance," which includes genes related to drug efflux pumps, tumor stroma, and vasculature & permeability. When comparing the **2D_1** ADC-resistant monolayer (as the baseline) and **3D_1** spheroids treated with ADC-1, several drug efflux pump genes are upregulated in the 3D spheroids compared to the 2D-resistant cells, and multiple changes in vasculature and permeability genes are observed.

The increases observed in genes SLC16A8, PTAFR, and PECAM1 in ovarian cancer spheroids treated with (**3D_1**) or without ADC-1 (**3D_0**) compared to the long-term ADC-1 treated monolayer (**2D_1**) allow us to hypothesize that spheroids have more tumor-like complex structures, providing an improved translational tool between in vitro and in vivo models.





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% demonstrates its robustness and ease of use.
- Results demonstrate the reliability of the nCounter ADC Development panel giving confidence to clients for use in their ADC-focused clinical studies.
- Results from the nCounter ADC Development panel demonstrate consistent genetic changes between cell culture methods (spheroids vs. monolayer), and type of ADC treatment. This will allow further improvement of ADC pipeline development with focus on functional pathway evaluation.

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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