Functional profiling in lung tumor explants show contributions of the tumor microenvironment to response of Dato-DXd ex vivo

Satoru Yasuda[1], Elena Ivanova[2], Minh Ha[2], Sophie Kivlehan[2], Iliana Gjeci[2], Patrick Lizotte[2], Bueno Raphael[3],David A Barbie[2], Cloud P Paweletz[2]

1. Daiichi Sankyo, Baskin Ridge, USA
2. Belfer Center for Applied Cancer Science, Dana Farber Cancer Institute, Boston, USA
3. Brigham and Womens Hospital, Boston, USA

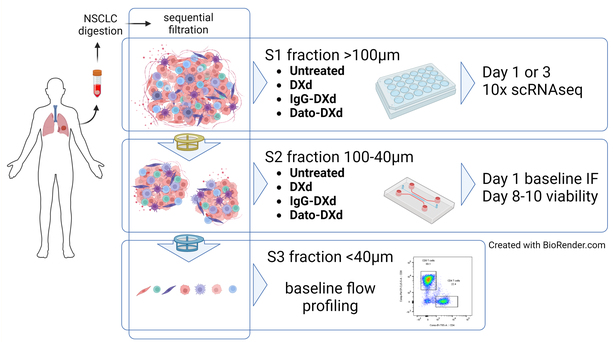
Abstract 5086 / 8

Background

Antibody drug conjugates (ADCs) have demonstrated significant antitumor activity against multiple treatment refractory cancers. While Trastuzumab deruxtecan, a HER2 directed ADC, has shown impressive clinical activity in HER2-expressing cancers, the ability to predict responses of ADCs by IHC has been limited. Dato-DXd is a TROP2 directed ADC that is currently being studied in several registrational phase 3 trial including TROPION-Lung01. While preclinical studies have shown that the density and heterogeneity of the target tumor antigen is critical for efficacy, response to Dato-DXd did not correlate to TROP2 IHC expression in the phase 1b TROPION-Pan tumor 01 trial (TP01) . Here we characterize the response of Dato-DXd in patient-derived organotypic tumor spheroids (PDOTS) grown short term in a 3D microfluidics device by imaging, IF, FCM, and scRNAseq.

Materials and Methods

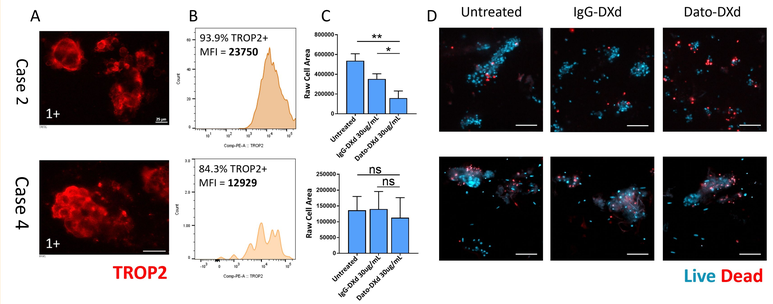
Surgical NSCLC cases collected from Brigham and Women's Hospital and St. Elizabeth's Medical Center under an IRB approved protocol were studied. PDOTS were generated as previously described. *Ex vivo* response was assessed by live/dead imaging, TROP2 antigen density on EPCAM+ cells by flow cytometry (FCM) and by immunofluorescence (IF). T cell and myeloid cell populations were analyzed by FCM of CD45+ cells isolated during tumor preparation. For a subset of samples single cell RNA sequencing (scRNAseq) was performed.



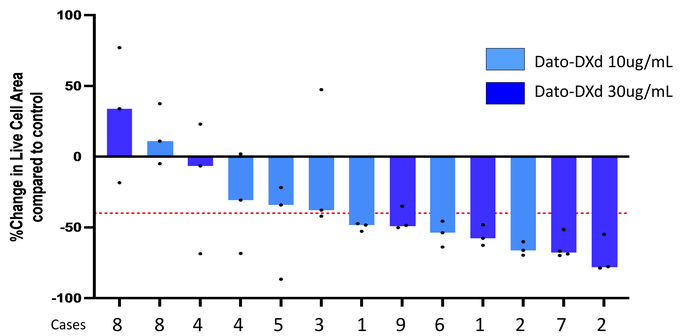
PDOTS Workflow. A tumor specimen is subjected to physical and enzymatic dissociation, yielding tumor tissue containing spheroids, single cells, and macroscopic fragments. This heterogeneous mixture is then sequentially applied to 100 µm and 40 µm filters to obtain three separate fractions, S1 (>100 µm), S2 (40-100 µm), and S3 (<40 µm). The S2 fraction is resuspended in collagen for the subsequent *ex vivo* culture with indicated terminal readouts

Results

TROP2 IF and FCM expression correlation to Dato-DXd response in tumor explants



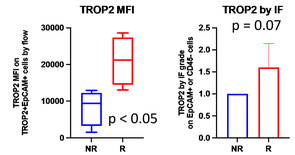
Rapid assessment of ADC killing in NSCLC PDOTS. **A)** IF, **B)** FCM **C)** Reduction in live cell area and **D)** Live-Dead representative staining.



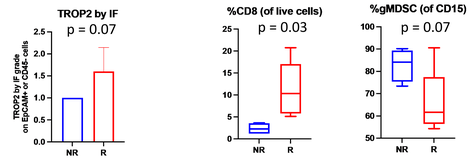
Waterfall plot from 9 patient specimens treated for 8-10 days with 10ug/mL and/or 30 ug / mL Dato-Dxd.

	Response	% change live (10 ug/mL)	% change live (30 ug/mL)	TROP2 by IF	%TROP2 by flow	TROP2 MFI	Histology
1	R	-48.4	-57.7	++	N/A	N/A	Papillary Thyroid Carcinoma
2	R	-66.2	-77.8	+, heterogenous	93.9	23750	N/A
3	NR	-37.8	not evaluated	+, heterogenous	87.4	8370	Adenocarcinoma
4	NR	-30.7	-6.5	+, heterogenous	84.3	12929	Squamous
5	NR	-34.2	not evaluated	+, homogeneous	87.2	10479	Adenocarcinoma
6	R	-53.8	not evaluated	++	99.4	18620	Large Cell
7	R	not evaluated	-67.8	++	99.4	28558	Granuloma
8	NR	11	33.9	+, homogeneous	N/A	N/A	Large Cell Neuroendocrine Carcinoma
9	R	not evaluated	-48.5	+	82.2	13088	Adenocarcinoma
10	n/a	not evaluated	not evaluated	not evaluated	76.3	1549	Adenocarcinoma
11	n/a	not evaluated	not evaluated	++	93.2	31038	Adenocarcinoma

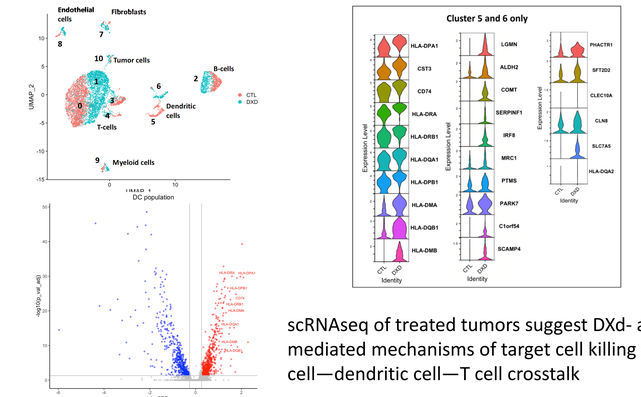
Study summary and patient histology of analyzed specimens. R = responders, NR = non responders, MFI = mean fluorescence intensity. Response is defined as >40% change



TROP2 mean fluorescence intensity by flow cytometry is a better predictor of Dato-DXd response than immunostaining



Flow cytometry of immune cell components in NSCLC PDOTS point to an involvement of the immune tumor microenvironment in Dato-DXd response



scRNAseq of treated tumors suggest DXd- and ADC-mediated mechanisms of target cell killing and tumor cell—dendritic cell—T cell crosstalk

Conclusions

By functionally profiling NSCLC PDOTS that retain immune cells, we correlated multiple parameters with *ex vivo* response to Dato-DXd. Our results have important implications for precision deployment of ADCs and suggest that target tumor antigen density in conjunction with addition immune cell features may refine patient selection strategy.

Acknowledgement

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