Dana-Farber Cancer Institute | Robert and Renée Belfer Center for Applied Cancer Science

#2830

BRIGHAM AND WOMEN'S HOSPITAL | HARVARD MEDICAL SCHOOL

Immunomodulatory response in Dato-DXd-treated non-small cell lung cancer patient-derived organotypic tumor spheroids (pDOTS)

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Background: Antibody-drug conjugates (ADCs) have emerged as promising new platform for medical oncology. Datopotamab deruxtecan (Dato-DXd) is a TROP2-directed ADC that is currently being studied in a number of registrational phase 3 trials, including TROPION-Lung and TROPION-Breast trial series. While the cytotoxic payload-induced apoptotic effect of DXd is well-established, we sought to investigate the less-characterized immunomodulatory effects. Here we analyze tumor cell-intrinsic immunological response to Dato-DXd using short-term microfluidic culture of patient-derived organotypic tumor spheroids (pDOTS) in non-small cell lung cancer (NSCLC) samples.

Methods: Four surgical NSCLC cases collected from Brigham and Women's Hospital under an IRB-approved protocol were studied. TROP2 antigen density on EpCAM+ cells was assessed by quantitative flow cytometry and immunofluorescence; baseline immune profile was assessed by flow. *Ex vivo* response to vehicle, unconjugated Datopotamab, IgG-DXd, and Dato-DXd were assessed by live/dead imaging endpoint analysis. Modulation of tumor cell immunogenicity at 24hr and 72hr was analyzed by 10X single cell RNA sequencing (scRNAseq).

Results: Four NSCLC explants with high tumor cell content and high TROP2 expression (3+ by IF) were studied. Response to drug treatment was measured by change in raw live cell area compared to vehicle treatment. Unexpectedly, EpCAM+TROP2+ tumor cells from all four samples at both timepoints exhibited negative pathway enrichment scores for Hallmark TNF-α Signaling via NFκB and Inflammatory Response when comparing Dato-DXd to vehicle control. Individual gene level analysis revealed significant downregulation of myeloid-associated cytokines (IL-23a, IL-6) and chemokines (CSF1/2, CXCL1/2/3, CCL2, CCL20, IL-8), indicating possible reduced recruitment of myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). Investigation of tumor cell immunogenic cell death in response to Dato-DXd treatment revealed inconsistent induction of pro-inflammatory signaling.

Figure 1. Baseline profiling for immune contexture and TROP2 antigen density in four non-small cell lung cancer cases. EpCAM+ tumor cell content and abundance of major leukocyte lineages were assessed by multiparametric flow cytometry and single cell RNA sequencing. TROP2 antigen density was calculated by quantitative FACS. Tumor cell clusters (circled populations on scRNAseq UMAP plots) were assessed for copy number variation, merged, and further analyzed for differential gene expression between vehicle control, Datopotamab, IgG-DXd, and Dato-DXd conditions.

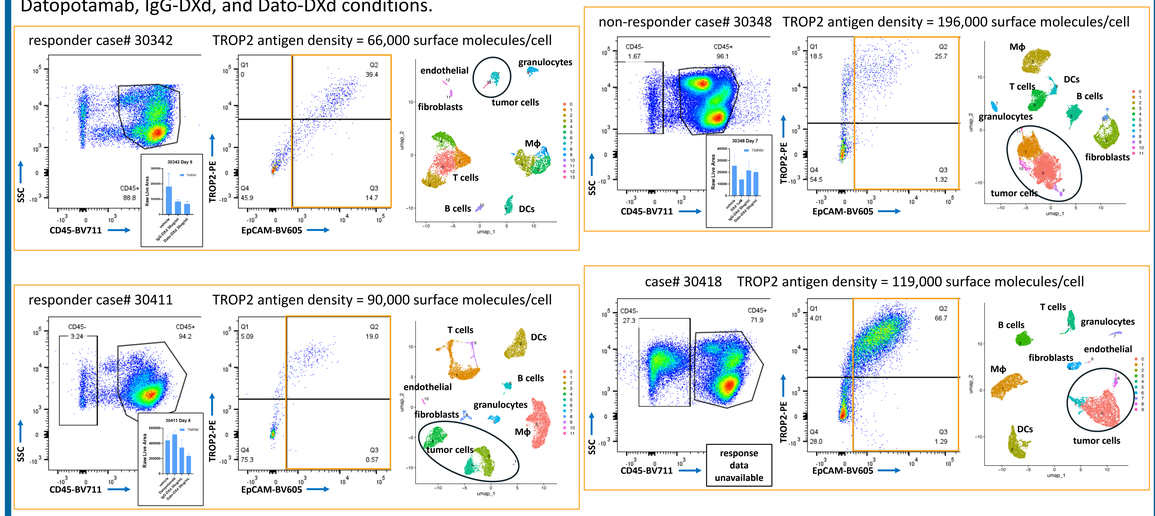
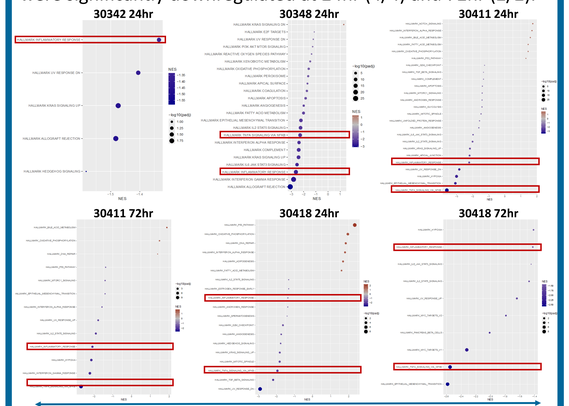


Figure 2. Pathway analysis reveals consistent downregulation of myeloid-associated inflammation in TROP2-positive tumor cells treated with Dato-DXd relative to vehicle control. Myeloid-associated inflammatory Hallmark pathways (red box) were significantly downregulated at 24hr (4/4) and 72hr (2/2).



		30342 24hr	30348 24hr	30411 24hr	30411 72hr	30418 24hr	30418 72hr
NES	HALLMARK_TNFA_SIGNALING_VIA_NFKB	n/a	-2.293	-3.612	-2.696	-1.943	-2.552
	HALLMARK_INFLAMMATORY_RESPONSE	-1.334	-2.637	-2.219	-2.089	-1.367	-1.425
FACS	TROP2 antigen density	66,000	196,000	90,000		119,000	

Figure 3. Expression of individual myeloid-associated cytokines and chemokines is downregulated by tumor cells treated with Dato-DXd. (A) Negative Log2FC genes from representative case #30411 are shown. (B) Leading edge genes aggregated from four NSCLC samples and (C) TNFA_SIGNALING_VIA_NFKB and INFLAMMATORY_RESPONSE pathway scores.

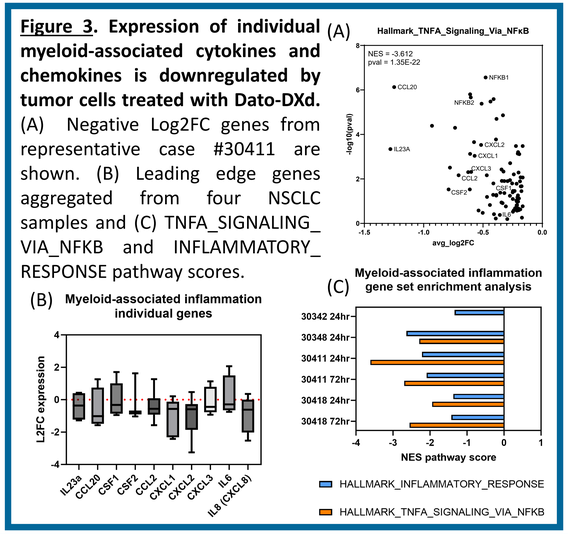
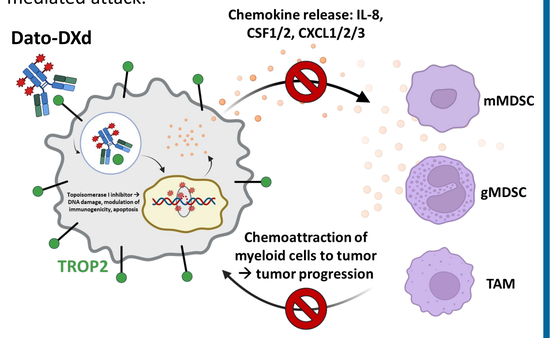


Figure 4. Model for modulation of tumor immune microenvironment in Dato-DXd-treated NSCLC tumor cells. Downregulation of myeloid cell chemoattractants reduces the recruitment of monocytic and granulocytic myeloid-derived suppressor cells, as well as tumor-associated macrophages. As a result, tumor cells become more vulnerable to immune-mediated attack.



Conclusions: Responder NSCLC samples 30342 and 30411 exhibited reduction in live cell area >30% (Dato-DXd vs. vehicle control). 30348 was a non-responder and 30418 data unavailable. Our results indicate immunomodulation of NSCLC tumor cells when treated with Dato-DXd, principally downregulation of myeloid-associated inflammation. We observed consistent results regardless of TROP2 expression, as assessed by quantitative FACS and IF (not shown). Statistically significant differential expression of CSF1/2, CXCL1/2/3, and IL-8 (CXCL8) chemokines reveal a potentially unappreciated mechanism underlying ADC therapeutic efficacy: reduced recruitment of pro-tumoral myeloid cells by tumor cells.

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