Poster 7149

The role of TROP2 in the MoA of Dato-DXd and how it underpins the biologic rationale of the novel AI-guided biomarker TROP2 QCS-NMR

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Objective

- To describe the requirement of TROP2 expression in the mechanism of action of datopotamab deruxtecan (Dato-DXd) and the biological rationale linking TROP2 normalized membrane ratio as measured by quantitative continuous scoring (QCS) (TROP2 QCS-NMR) to its pharmacological mode of action in patients with non-small cell lung cancer (NSCLC).

Conclusions

- TROP2 membrane expression is required, but not sufficient, to predict the antitumor efficacy of Dato-DXd in preclinical tumor models.
- Despite similar levels of Dato-DXd binding across a panel of non-small cell lung cancer (NSCLC) cell lines, differential internalization capacity of Dato-DXd is observed.
- TROP2 QCS-NMR predicts Dato-DXd internalization capacity and Dato-DXd efficacy in NSCLC cell lines.

Plain language summary

Why did we perform this research?

- TROP2 QCS-NMR has recently been described as a potential computational pathology-based biomarker for identifying patients with NSCLC who are most likely to receive benefit from treatment with Dato-DXd.[4]
- Herein, we provide the biological rationale for linking TROP2 QCS-NMR to the mechanism of action (MoA) of Dato-DXd.

How did we perform this research?

Cell line engineering was used to make preclinical models that enabled the evaluation of the requirement of TROP2 in the MoA and pharmacology of Dato-DXd in a preclinical model of NSCLC. A novel, AI-guided image analysis tool (i.e. QCS) was employed to determine the correlation of TROP2 IHC characteristics with key mechanistic features relevant to Dato-DXd efficacy in a panel of NSCLC cell lines.

What were the findings of this research?

- Cell surface TROP2 is required, but not sufficient, for Dato-DXd efficacy in preclinical models of NSCLC.
- Datopotamab binding is not sufficient to predict its internalization capacity in NSCLC cell lines.
- TROP2 QCS-NMR, but not membrane nor cytoplasmic TROP2 as standalone measurements, predict both datopotamab internalization and Dato-DXd efficacy in NSCLC cell lines – providing the biological rationale for this potential novel, AI-guided biomarker for Dato-DXd in patients with NSCLC.

What are the implications of this research?

Key features of antibody-drug conjugate (ADC) target biology may need to be accounted for in the biomarker strategy and approach for identification of patients most likely to receive benefit from ADCs.

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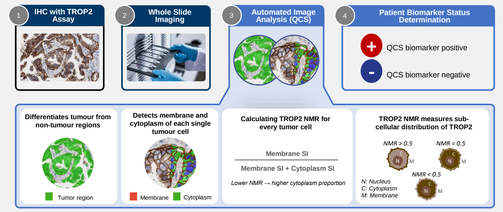
Introduction

- Dato-DXd is an antibody-drug conjugate composed of a humanized trophoblast cell surface antigen 2 (TROP2)–directed monoclonal antibody covalently linked to a topoisomerase I inhibitor payload via a plasma-stable, cleavable linker.[1]
- Dato-DXd is approved for the treatment of patients with unresectable or metastatic 2L+ hormone-receptor positive breast cancer and is being explored in a robust clinical development plan for patients with NSCLC.[2]
- In the phase 3 TROPION-Lung01 study, Dato-DXd monotherapy showed an ORR = 26%, mPFS = 4.4m and mOS = 12.9m in all patients randomized to Dato-DXd (n=299) highlighting the need to identify a biomarker of Dato-DXd efficacy in patients with NSCLC.[3]
- TROP2 QCS-NMR was identified in a retrospective exploratory analysis as a potential novel biomarker that predicted efficacy outcomes in patients receiving Dato-DXd in TROPION-Lung01.[4] Herein, we outline the biological rationale of this potentially novel biomarker for Dato-DXd.

Methods

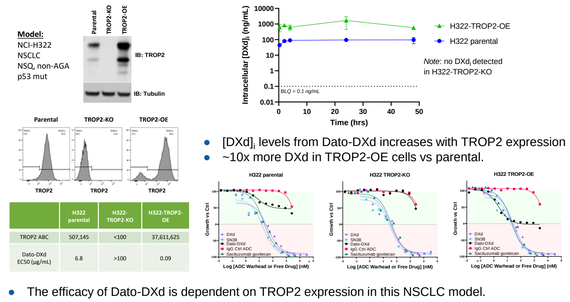
- **Figure 1:** NCI-H322 cells were engineered via a CRISPR guide RNA targeting TACSTD2 to create the 'TROP2-KO'. A lentiviral-based overexpression construct was introduced into the TROP2-KO cells to generate the 'TROP2-OE' cells.
- **Figure 1:** Intracellular payload (DXd) levels were quantified with quantitative mass spectrometry analysis of Dato-DXd treated cell lysates.
- **Figures 1 & 3:** Cell viability experiments were conducted over seven days with Cell-Titer Glo as the endpoint.
- **Figure 2:** Isogenic cell lines were implanted into female CB17 SCID mice. When tumors reached an average volume of ~250mm^3, animals were randomized to receive a single, intravenous dose of vehicle, Control ADC (10 mg/kg) or Dato-DXd (10 mg/kg). Tumor volumes were collected over time and animals were consistently monitored according to AstraZeneca animal welfare policies.
- **Figure 4:** Antibody internalization was measured with the Sartorius Live Cell Internalization Assay (FabFluor-labelled datopotamab) on an Incucyte S3 over 48 hours.
- **Figure 5:** TROP2 immunohistochemistry (IHC) was performed in AstraZeneca pathology labs using a research use only (RUO) TROP2 IHC assay with EPR20043 (rabbit monoclonal antibody) and the Ventana Discovery Ultra auto staining platform.
- **Figure 5 & 6:** Quantitative Continuous Scoring (QCS) is an automated image analysis approach and, when applied to TROP2 IHC stained sections, differentiates tumor cells from non-tumor cells, detects membrane and cytoplasmic regions of each individual tumor cells, measuring TROP2 staining intensity (SI) in each region and calculates the TROP2 normalized membrane ratio (NMR) for each tumor cell.

Figure 6. Workflow of Quantitative Continuous Scoring for TROP2



Results

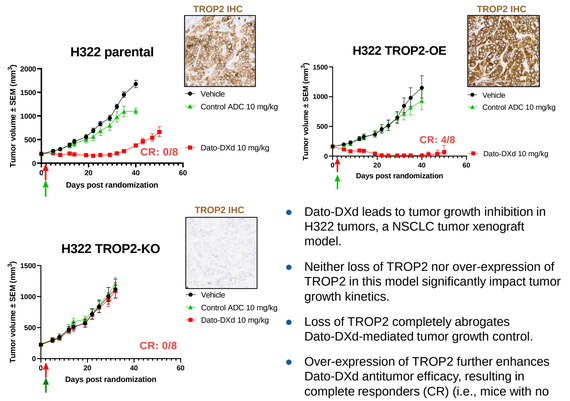
Figure 1. TROP2 expression is required for Dato-DXd binding, intracellular DXd delivery and in vitro efficacy in isogenic NSCLC cell lines



	H322 parental	H322-TROP2-KO	H322-TROP2-OE
TROP2 ABC	507,145	<100	37,611,625
Dato-DXd EC50 (µg/mL)	6.8	>100	0.09

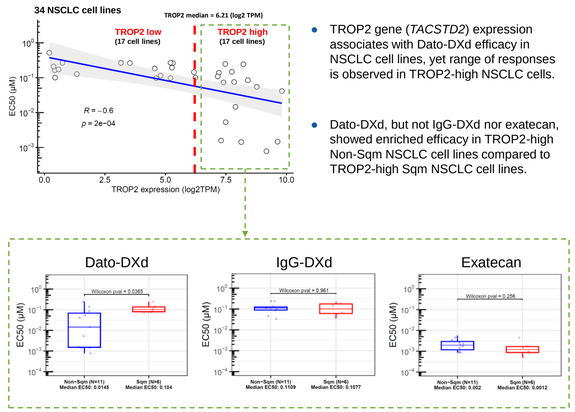
- [DXd]$_i$ levels from Dato-DXd increases with TROP2 expression
- ~10x more DXd in TROP2-OE cells vs parental.
- The efficacy of Dato-DXd is dependent on TROP2 expression in this NSCLC model.
- The efficacy of sacituzumab govitecan* is not dependent on TROP2 expression and may be driven by its more labile linker in this model.

Figure 2. TROP2 expression is required for the antitumor efficacy of Dato-DXd in isogenic NSCLC xenograft system



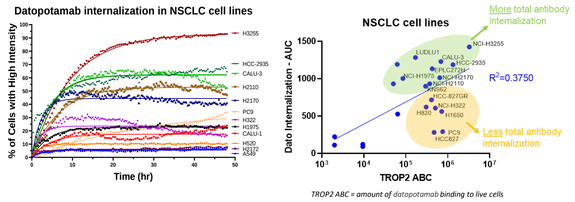
- Dato-DXd leads to tumor growth inhibition in H322 tumors, a NSCLC tumor xenograft model.
- Neither loss of TROP2 nor over-expression of TROP2 in this model significantly impact tumor growth kinetics.
- Loss of TROP2 completely abrogates Dato-DXd-mediated tumor growth control.
- Over-expression of TROP2 further enhances Dato-DXd antitumor efficacy, resulting in complete responders (CR) (i.e., mice with no measurable tumors post-treatment)

Figure 3. TROP2 expression is not sufficient to predict Dato-DXd efficacy in a broad in vitro pharmacology panel



- TROP2 gene (*TACSTD2*) expression associates with Dato-DXd efficacy in NSCLC cell lines, yet range of responses is observed in TROP2-high NSCLC cells.
- Dato-DXd, but not IgG-DXd nor exatecan, showed enriched efficacy in TROP2-high Non-Sqm NSCLC cell lines compared to TROP2-high Sqm NSCLC cell lines.

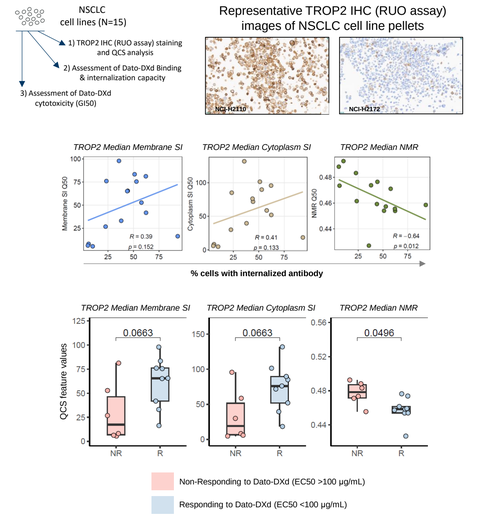
Figure 4. Despite similar levels of binding, differential datopotamab internalization capacity is observed across NSCLC cell lines



TROP2 ABC = amount of datopotamab binding to live cells

- Datopotamab internalization seems to require datopotamab binding, however, differential capacity for internalization post-binding across cell lines is observed.
- Datopotamab binding may not predict datopotamab internalization; thus, a better approach to predict datopotamab internalization in NSCLC models is required.
- This highlights that TROP2 abundance on the cell surface may not be sufficient to predict datopotamab internalization and Dato-DXd activity in preclinical models of NSCLC.

Figure 5. TROP2 QCS-NMR predicts differential Dato-DXd internalization and efficacy across NSCLC cell lines



- TROP2 QCS-NMR shows a significant association with the internalization capacity of datopotamab in a panel of NSCLC cell lines; whereas, there was no significant association with neither median membrane nor cytoplasmic staining intensity (SI) and datopotamab internalization capacity.
- TROP2 QCS-NMR also associates with Dato-DXd response in NSCLC cell lines.

Summary

- TROP2 is required, but not sufficient, for Dato-DXd efficacy in preclinical models of NSCLC.
- Datopotamab binding is not sufficient to predict its internalization capacity in NSCLC cell lines.
- TROP2 QCS-NMR, but not membrane nor cytoplasmic TROP2 as standalone measurements, predict datopotamab internalization and Dato-DXd efficacy in NSCLC cell lines – providing a biological rationale for this potential novel, AI-guided biomarker for Dato-DXd in patients with NSCLC.

Abbreviations

2L, second line; ABC, antigen binding capacity; ADC, antibody-drug conjugate; AGA, actionable genomic alteration; AI, artificial intelligence; AUC, area under the curve; CR, complete responder; CRISPR, clustered regularly interspaced short palindromic repeats; Dato-DXd, datopotamab deruxtecan; EC50, half maximal effective concentration; IgG, immunoglobulin G; IHC, immunohistochemistry; KO, knock-out; MoA, mechanism of action; mOS, median overall survival; mPFS, median progression-free survival; NMR, normalized membrane ratio; NSCLC, non-small cell lung cancer; NSQ, non-squamous; OE, over-expression; ORR, objective response rate; QCS, quantitative continuous scoring; RUO, research use only; SCID, Severe combined immunodeficient; SEM, standard error of mean; SI, staining intensity; Sqm, squamous; TPM, Transcripts Per Million; TROP2, trophoblast cell surface antigen 2.

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Disclosures

All authors are employees and stockholders of AstraZeneca PLC.

Note*

Sacituzumab govitecan was commercially acquired.

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