Poster LB417

Leveraging advanced human lung models to explore mechanisms underlying T-DXd-associated interstitial lung disease (ILD)

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Objective

- Use of human in-vitro lung mucosa models to explore the molecular mechanisms and pathways underlying trastuzumab deruxtecan (T-DXd)-related interstitial lung disease/pneumonitis (ILD/pneumonitis).
- Evaluate the role of target antigen (HER2) in T-DXd-induced lung epithelial changes.

Conclusions

- T-DXd can drive epithelial mechanisms and pathways associated with pulmonary fibrosis, inflammation, and damage, which are dysregulated events observed in ILD/pneumonitis.
- HER2 targeting has limited contribution to these T-DXd-induced lung epithelial changes.
- Topoisomerase I inhibitors (e.g. irinotecan) can cause ILD/pneumonitis[1]. These T-DXd effects on lung epithelia are consistent with known topoisomerase I inhibitor adverse effect (e.g. DNA damage, cellular senescence) on tumor/normal cells[2-4].

Plain language summary

Why did we perform this research?

- Drug-induced interstitial lung disease is a subset of interstitial lung disease/pneumonitis which leads to inflammation and possible scarring in the lung from drugs exposure[1].
- The mechanisms/pathways underlying trastuzumab deruxtecan (T-DXd)-related ILD/pneumonitis are largely unknown.

How did we perform this research?

- Transcriptomic and functional studies were performed in human 3D lung models which mimics the cellular features of bronchial and alveolar epithelium to assess the potential effects of T-DXd on pulmonary epithelial cell homeostasis and function.
- Different treatments were used to evaluate the contribution of HER2 targeting to T-DXd-induced lung epithelial changes: T-DXd, payload (deruxtecan; DXd), IgG control antibody-DXd conjugate (IgG-DXd), and anti-HER2 antibody (trastuzumab).

What were the findings of this research?

- T-DXd was shown to activate the p53 pathway, induce epithelial injury and upregulate pro-senescence, pro-inflammatory and pro-fibrotic mediators in in-vitro lung models.
- Similar effects on lung cells were seen with DXd and IgG-DXd, but trastuzumab did not have any effect.

What are the implications of this research?

- A greater understanding of T-DXd-associated ILD/pneumonitis pathophysiology has the potential to guide alternative interventions to treat or prevent ILD/pneumonitis progression and improve patient care.

Where can I access more information?

- Incidence of adjudicated drug-related ILD/pneumonitis in a pooled analysis of nine T-DXd monotherapy studies[2].

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Poster presented at AACR 2024 by Sonia Terrillon.
Reference: 1. Skeoch S, et al. J Clin Med. 2018;7:356 2. Powell CA, et al. ESMO Open. 2022;4:100554

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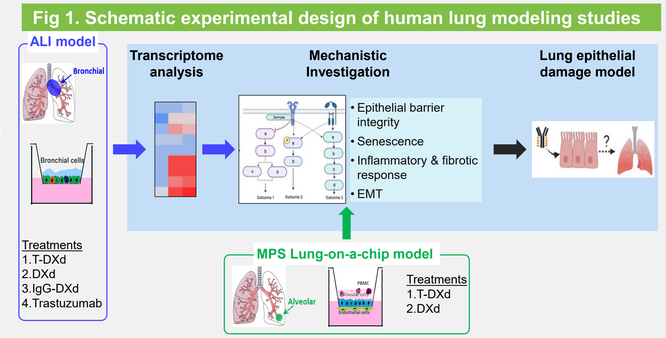
Introduction

- T-DXd is a HER2-directed antibody-drug conjugate approved for several indications in the US, including HER2+ and HER2-low metastatic breast cancer, unresectable or metastatic HER2-mutant non-small cell lung cancer and locally advanced or metastatic HER2+ gastric cancer[5].
- While the safety profile of T-DXd is manageable, ILD/pneumonitis is an important identified risk and considered as an adverse event of special interest[6].
- The therapeutic options for patients that develop ILD/pneumonitis are limited to steroid medications, including prednisone and methylprednisolone[7].
- Hence, a greater understanding of T-DXd-associated ILD/pneumonitis pathophysiology has the potential to guide alternative interventions to treat or prevent ILD/pneumonitis progression and improve patient care.

Methods

- Human bronchial air-liquid interface (ALI) model (internal & Epithelix MucilAir™; n=3-6 donors) and alveolar "lung-on-chip model" microphysiological system (MPS) (AlveoliX; n=2 donors) were used in these studies (**Fig.1**).
- Bronchial ALI were treated with vehicle controls (DMSO, buffer), T-DXd (5, 50, 100, 150 µg/ml), payload (deruxtecan; DXd) (100 ng/ml), IgG control antibody-DXd conjugate (IgG-DXd) (5, 50, 100, 150 µg/ml), and anti-HER2 antibody (trastuzumab) (5, 50, 100, 150 µg/ml). MPS lung-on-a-chip were treated with T-DXd (250 µg/ml) and DXd (150 ng/ml) (**Fig.1**).
- Gene expression was measured using RNA-seq/Gene Set Enrichment Analysis (GSEA), qPCR (**Fig.1**).
- Protein expression was measured using ELISA (GDF-15, CYFRA21-1), MSD (IL-6), Olink targeted proteomics (cytokines/chemokines), western-blot (senescence/epithelial-mesenchymal transition [EMT] events) (**Fig.1**).
- Epithelial barrier integrity was evaluated using barrier function (TEER) and cytotoxicity (LDH) (**Fig.1**).

Fig 1. Schematic experimental design of human lung modeling studies



Results and interpretation

- Transcriptome and GSEA of bronchial ALI revealed that genes and pathways associated with senescence, inflammation, and barrier integrity were dysregulated upon treatment with T-DXd (**Fig.2**).
- Transcriptomic findings linked to senescence and inflammation were confirmed in follow-up ALI experiments where T-DXd was shown to induce DNA damage (γH2AX), activation of p53 pathway (p-p53) (**Fig. 3a**) and upregulate senescence-associated secretory phenotype (SASP) secretion (GDF-15, IL-6) (**Fig. 3b**), pro-inflammatory (e.g. TNF, IL17C) and pro-fibrotic (e.g. CCL3, CCL4) mediators (**Fig. 5**).
- T-DXd induced epithelial injury of ALI cells, which was reflected by loss of barrier function (TEER), increased cytotoxicity (LDH) and release of epithelial damage markers (CYFRA21-1) (**Fig. 4a**).
- These changes were also accompanied by upregulation of mesenchymal cell markers (vimentin, α-SMA), suggesting cells transition to a partial EMT phenotype (**Fig. 4b**).
- T-DXd activated senescence- and inflammation-associated markers (e.g. GDF-15, CCL3, OSM, TNF) in alveolar lung-on-chip model, thus indicating that T-DXd has similar effects in alveoli epithelia (**Fig. 6**).
- Overall, there results suggest DXd showed similar profile with T-DXd but with stronger effects on ALI (**Fig. 3-5**).
- T-DXd and IgG-DXd have similar dose-dependent effects on ALI whereas trastuzumab did not have any effect, suggesting limited contribution of HER2 targeting to T-DXd-induced lung epithelial changes in the experimental models used (**Fig. 3-6**).

Fig 2. T-DXd dysregulates genes and pathways associated with lung fibrosis in ALI model

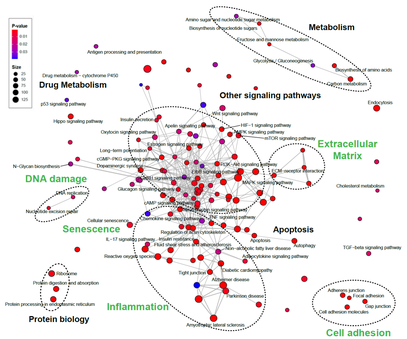


Fig. 2 RNA-Seq was performed after 2 days of treatment with buffer or T-DXd (250 µg/ml) in ALI cells (n=3 donors). Network plot of enriched terms (qvalue < 0.05) from GSEA are shown. Node size indicates the number of significantly dysregulated genes in each pathway and node color indicates the significance of the pathway.

Fig 3. T-DXd promotes cellular senescence and SASP secretion in ALI model

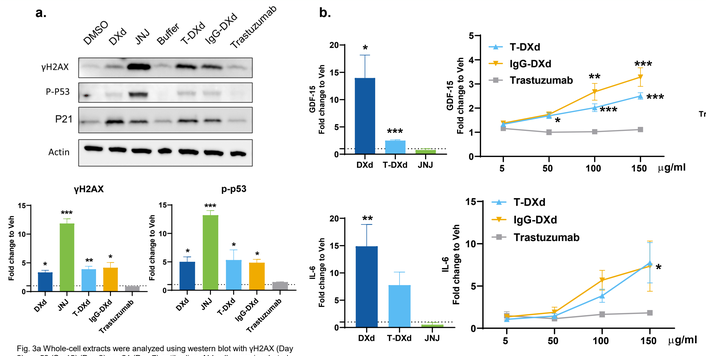


Fig. 3a Whole-cell extracts were analyzed using western blot with γH2AX (Day 2), p-p53 (Ser15) (Day 2) or p21 (Day 7) antibodies. ALI cells were incubated with either DMSO, DXd (100 ng/ml), JNJ (10 µg/ml), buffer, T-DXd (150 µg/ml), IgG-DXd (150 µg/ml) or trastuzumab (150 µg/ml) (n=3-6 donors). JNJ (JNJ26854165) is an inhibitor of MDM2 binding to p53. P values: P < 0.05 (*). P < 0.01 (**), P < 0.001(***).

Fig. 3b Media GDF15 and mucus IL-6 levels were measured in ALI cells treated with either DMSO, DXd (100 ng/ml), JNJ (10 µg/ml), T-DXd (5, 50, 100, 150 µg/ml), IgG-DXd (5, 50, 100, 150 µg/ml) or trastuzumab (5, 50, 100, 150 µg/ml) for 7 days (n=3-6 donors). P values: P < 0.05 (*), P < 0.01 (**), P < 0.001(***).

Fig 4. T-DXd promotes epithelial barrier dysfunction and cell damage in ALI model

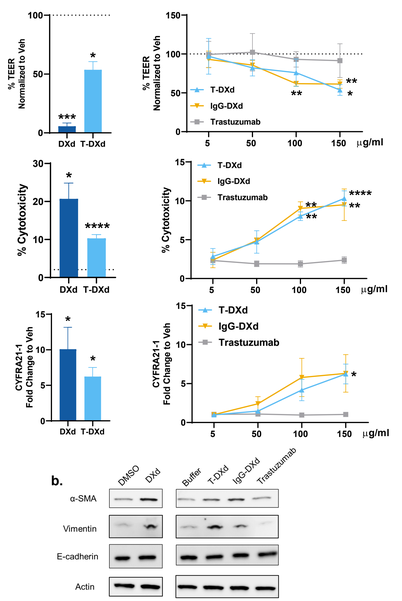


Fig. 4a TEER measurement, LDH and media CYFRA21-1 levels were measured in ALI cells treated with either DMSO, DXd (100 ng/ml), T-DXd (5, 50, 100, 150 µg/ml), IgG-DXd (5, 50, 100, 150 µg/ml) or trastuzumab (5, 50, 100, 150 µg/ml) for 7 days (n=3-6 donors). 4b Whole-cell extracts were analyzed by western blot with antibodies after ALI cells were incubated with either DMSO, DXd (100 ng/ml), buffer, T-DXd (150 µg/ml), IgG-DXd (150 µg/ml) or trastuzumab (150 µg/ml) for 7 days (n=3 donors). P values: P < 0.05 (*), P < 0.01 (**), P < 0.001(***).

Fig 5. T-DXd induces production of pro-inflammatory and pro-fibrotic mediators in ALI model

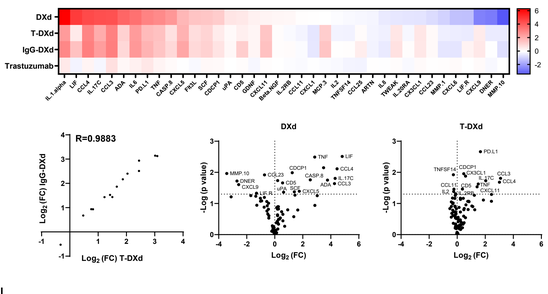


Fig. 5 Targeted proteomics analysis was preformed in mucus samples after ALI cells were incubated with either DMSO, DXd (100 ng/ml), buffer, T-DXd (150 µg/ml), IgG-DXd (150 µg/ml) or trastuzumab (150 µg/ml) for 7 days (n=3 donors). Heatmap of significant proteins (p < 0.05) are shown and Log2-transformed average protein fold changes (FC) are normalized to vehicle controls. Pearson's correlation profile of proteins significantly regulated by both T-DXd and IgG-DXd treatments (p < 0.1). Volcano plots of significant proteins (p < 0.05) are shown and annotated with their names.

Fig 6. T-DXd promotes epithelial changes, senescence and inflammation in MPS alveolar lung-on-a chip model

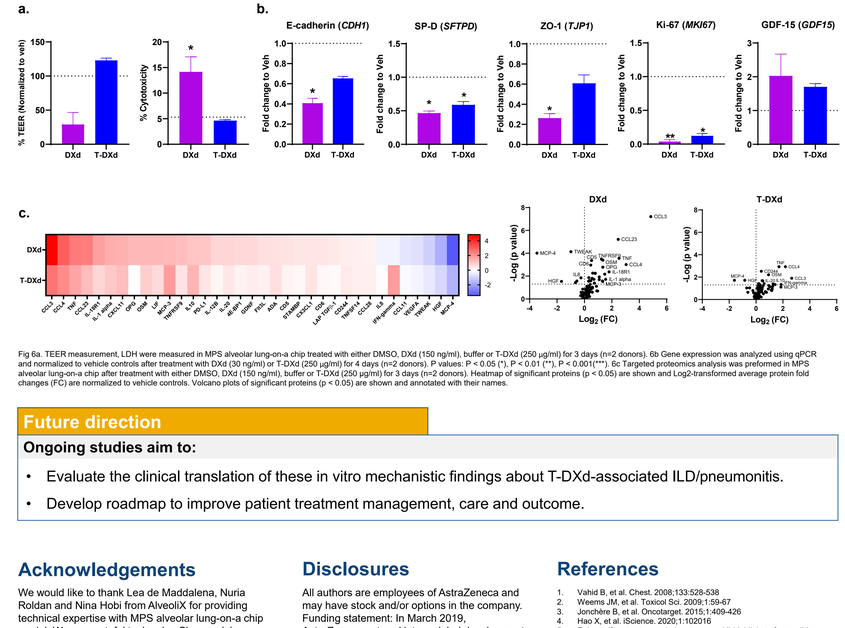


Fig 6a. TEER measurement, LDH were measured in MPS alveolar lung-on-a chip treated with either DMSO, DXd (150 ng/ml), buffer or T-DXd (250 µg/ml) for 3 days (n=2 donors). 6b Gene expression was analyzed using qPCR and normalized to vehicle controls after treatment with DXd (30 ng/ml) or T-DXd (250 µg/ml) for 4 days (n=2 donors). P values: P < 0.05 (*), P < 0.01 (**), P < 0.001(***). 6c Targeted proteomics analysis was preformed in MPS alveolar lung-on-a chip after treatment with either DMSO, DXd (150 ng/ml), buffer or T-DXd (250 µg/ml) for 3 days (n=2 donors). Heatmap of significant proteins (p < 0.05) are shown and Log2-transformed average protein fold changes (FC) are normalized to vehicle controls. Volcano plots of significant proteins (p < 0.05) are shown and annotated with their names.

Future direction

Ongoing studies aim to:

- Evaluate the clinical translation of these in vitro mechanistic findings about T-DXd-associated ILD/pneumonitis.
- Develop roadmap to improve patient treatment management, care and outcome.

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Disclosures

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