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Serum proteomics analysis: potential biomarkers and mechanisms of trastuzumab deruxtecan (T-DXd)–related interstitial lung disease/pneumonitis (ILD)

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Disclosure information

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Charles A. Powell

I have the following relevant financial relationships to disclose:

Consultant for: Daiichi Sankyo, AstraZeneca, BioNTech, Duality, Pfizer, Seagen, Merus, OnCusp, Roche, and Merck

- and -

My additional financial relationship disclosures are:

Support for attending meetings and/or travel from: AstraZeneca, Roche and Duality

Background

- **Trastuzumab deruxtecan (T-DXd)** is a human epidermal growth factor receptor 2 **(HER2)-directed antibody-drug conjugate** approved for the treatment of patients with HER2-positive, HER2-low and HER2-ultralow metastatic breast cancer, HER2-positive metastatic gastric cancer, *HER2*-mutant metastatic non–small cell lung cancer, and HER2-positive (immunohistochemistry 3+) metastatic solid tumors[1,2]

- **Interstitial lung disease (ILD)/pneumonitis is a serious adverse event associated with T-DXd** across various cancer indications and requires regular monitoring in patients[2,3]

- ILD diagnosis is complex and requires physiological, clinical, and radiologic assessments that often lead to delays in diagnosis. Mechanisms driving ILD onset are not well understood; thus, there is a **need for early detection and greater understanding of the pathophysiology of T-DXd–related ILD onset** to identify therapeutic interventions for ILD treatment and/or prevention[3,4]

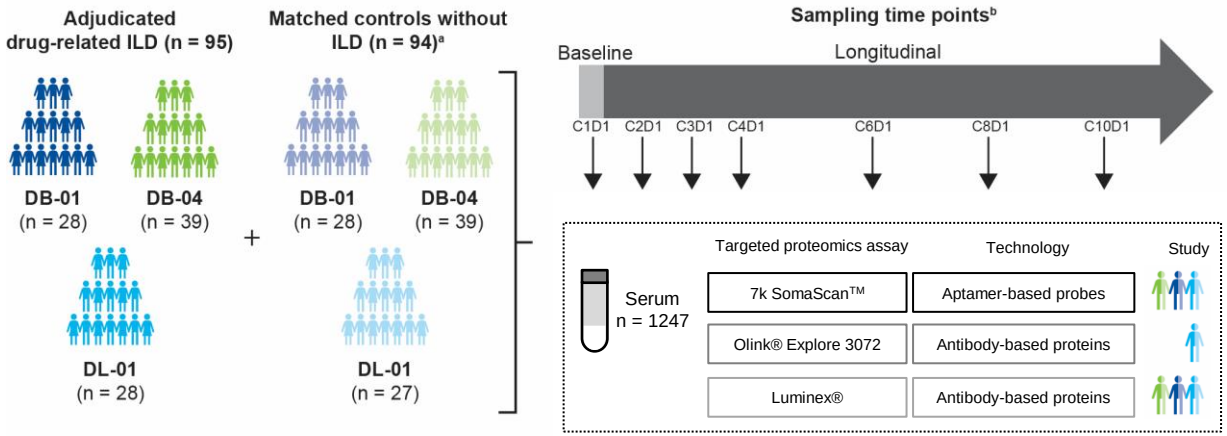
Study objective

To identify risk-monitoring biomarkers and characterize biological mechanisms associated with T-DXd–related ILD via longitudinal proteomics analysis in serum samples of patients with and without ILD who received T-DXd treatment in the DESTINY-Breast01 (DB-01), DESTINY-Breast04 (DB-04), and DESTINY-Lung01 (DL-01) trials

DB, DESTINY-Breast; DL, DESTINY-Lung; HER2, human epidermal growth factor receptor 2; ILD, interstitial lung disease; T-DXd, trastuzumab deruxtecan.

1. AstraZeneca. https://www.astrazeneca.com/media-centre/press-releases/2025/enhertu-approved-in-us-for-breast-cancer-post-et.html. Accessed March 13, 2025. 2. Enhertu. Prescribing information. Daiichi Sankyo, Inc., 2024. 3. Powell CA et al. *ESMO Open*. 2022;7(4):100554. 4. Conte P et al. *ESMO Open*. 2022;7(2):100404.

Study design



1247 baseline and longitudinal serum samples from **189 selected patients** treated with T-DXd across DB-01 (n = 56; NCT03248492), DB-04 (n = 78; NCT03734029), and DL-01 (n = 55; NCT03505710)

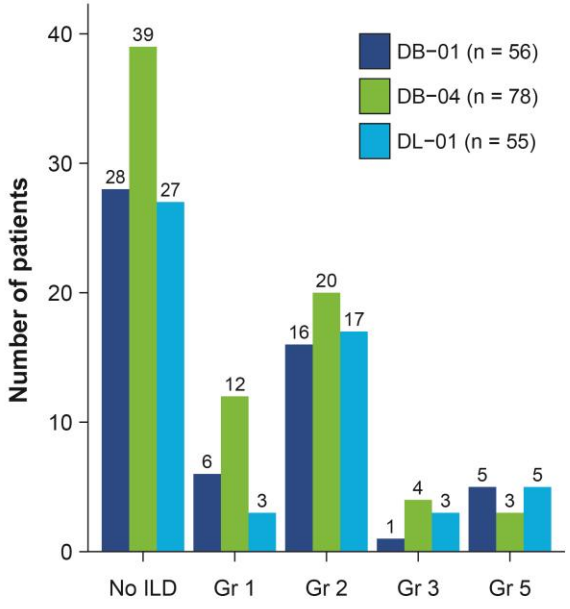
Matched controls were selected based on the following patient characteristics:
DB-01: BOR, age, country, study duration; DB-04: country, age, BOR, sex; DL-01: country, age, BOR, sex, cohort

- **Proteomics** data were generated using the **7k SomaScan™** and **Olink® Explore 3072** assays
- Select proteins of interest were **confirmed** using a quantitative **Luminex®** platform with the custom Luminex Discovery Assay Human Premixed Multi-Analyte Kit
- **Variance decomposition** identified the **main confounding factors** by assessing protein variance explained by each potential covariate
- **Proteins associated with ILD onset**[c] were identified via **longitudinal statistical analysis**, conducted using linear mixed modeling following time alignment of worst ILD diagnosis across patients and adjustment for personal baseline and the top covariates (age, body mass index, and tumor burden)

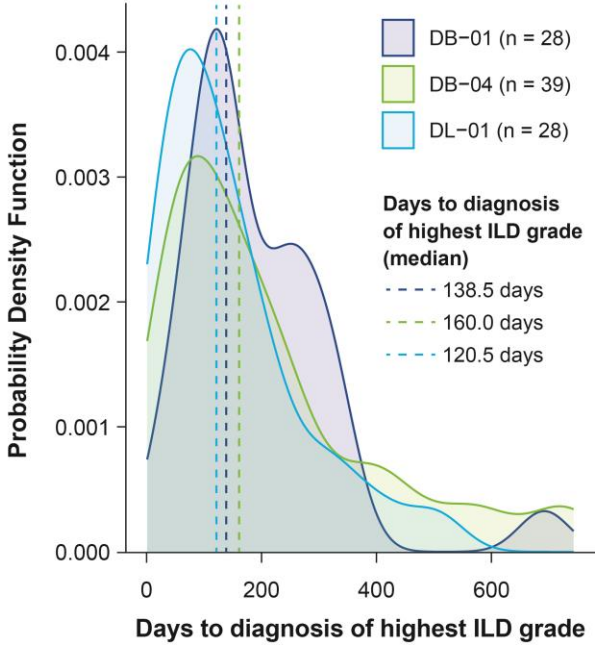
[a]Baseline sample was missing for 1 control; therefore, only 94 matched controls were included.
[b]Baseline and longitudinal serum samples were collected before dosing across cycles 1, 2, 3, and 4, and then every 2 cycles until end of treatment
[c]ILD onset as per the timing adjudicated by the clinician (days since start of treatment).

ILD severity and time to diagnosis

Breakdown by trial of highest ILD grades



Breakdown by trial of time to diagnosis of highest ILD grade



- Of the 95 patients with ILD included in the analysis, 74 developed mild/moderate ILD (grades 1 or 2) and 21 developed severe ILD (grades 3-5)
- **Time to diagnosis of highest ILD grade** was similar between the DB-01, DB-04, and DL-01 cohorts, with a median time of **138.5, 160.0, and 120.5 days**, respectively

DB, DESTINY-Breast; DL, DESTINY-Lung; Gr, grade; ILD, interstitial lung disease.

Differential longitudinal protein discovery

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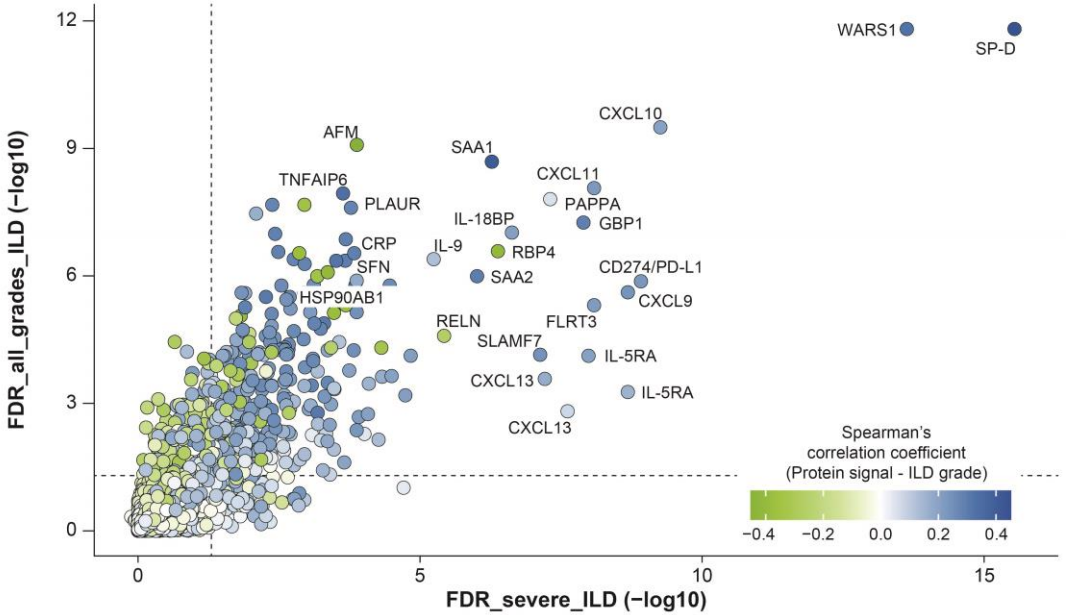
Using linear mixed effect models, **595** and **510** significant proteins were identified in longitudinal samples from patients with **all-grade and severe ILD (grade 3-5)**, respectively (false discovery rate [FDR] < 0.05)

Differentially expressed proteins in patients with all grades of ILD (*y* axis) and severe ILD (*x* axis) (SomaScan)



ILD, interstitial lung disease.

Differential longitudinal proteins at ILD diagnosis

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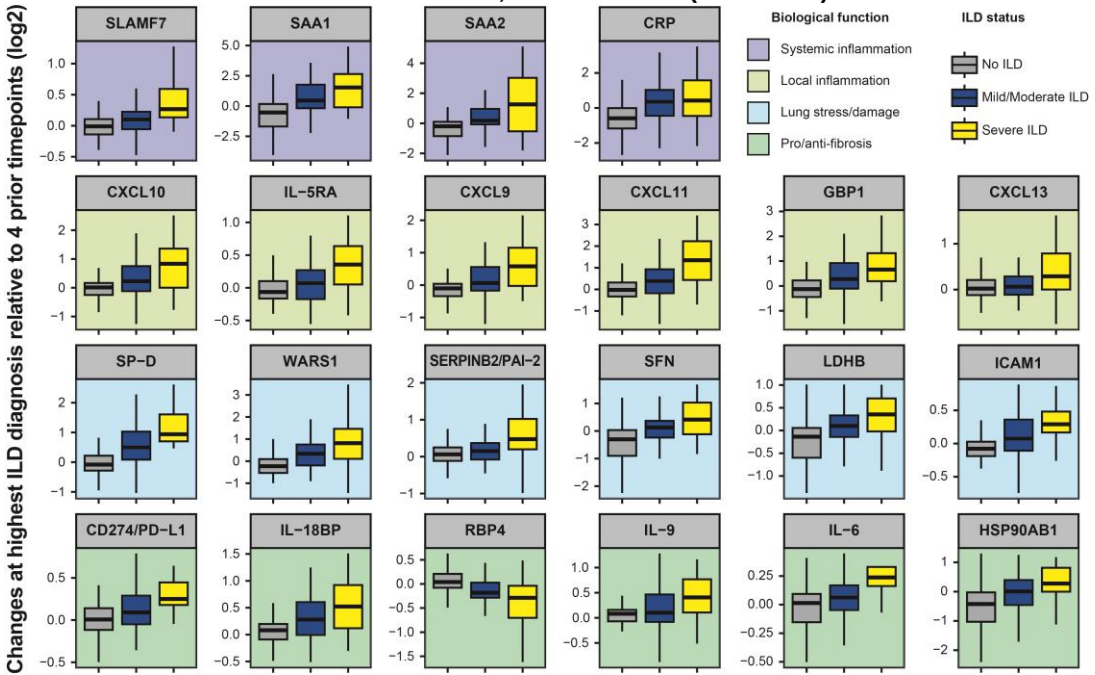
Changes at highest ILD diagnosis relative to 4 prior timepoints between no ILD, mild/moderate ILD, and severe ILD (SomaScan)



Differential proteins were related to **ILD pathophysiology**, including **systemic inflammation** (ie, SAA1, SAA2, CRP), **local inflammation** (ie, IL-5RA, CXCL9, CXCL10, CXCL11), **alveolar damage** (ie, SP-D and SFN), and **fibrosis** (ie, IL-18BP)

Elevation of **CXCL9/10/11** may reflect **immune cell activation** by IFN-gamma, driving T cell recruitment and inflammation in the lungs

Increased **IL5-RA** may indicate heightened **eosinophil activity and recruitment**, exacerbating lung tissue damage and inflammation

Elevated **SP-D** levels suggest **lung epithelial cell injury**, contributing to inflammation and potentially fibrosis

CD274, cluster of differentiation 274; CRP, C-reactive protein; CXCL9/10/11/13, C-X-C motif chemokine ligand 9/10/11/13; GBP1, guanylate binding protein 1; HSP90AB1, heat shock protein HSP 90-beta; ICAM1, intracellular adhesion molecule; IL-18BP, interleukin 18 binding protein; IL-5RA, interleukin-5 receptor subunit alpha; IL-6/9, interleukin 6/9; ILD, interstitial lung disease; LDHB, lactate dehydrogenase subunit B; PAI-2, plasminogen activator inhibitor 2; PD-L1, programmed death ligand 1; RBP4, retinol binding protein 4; SAA1/2, serum amyloid 1/2; SERPINB2, serpin family B member 2; SFN, stratifin; SLAMF7, self-ligand receptor of the signaling lymphocytic activation molecule 7; SP-D, surfactant protein D; WARS1, tryptophanyl-tRNA synthetase 1.

Pathway enrichment analysis

Inflammation
Immune response
(FDR = 4.79E-10)

Lower respiratory tract disease
(FDR = 2.79E-07)

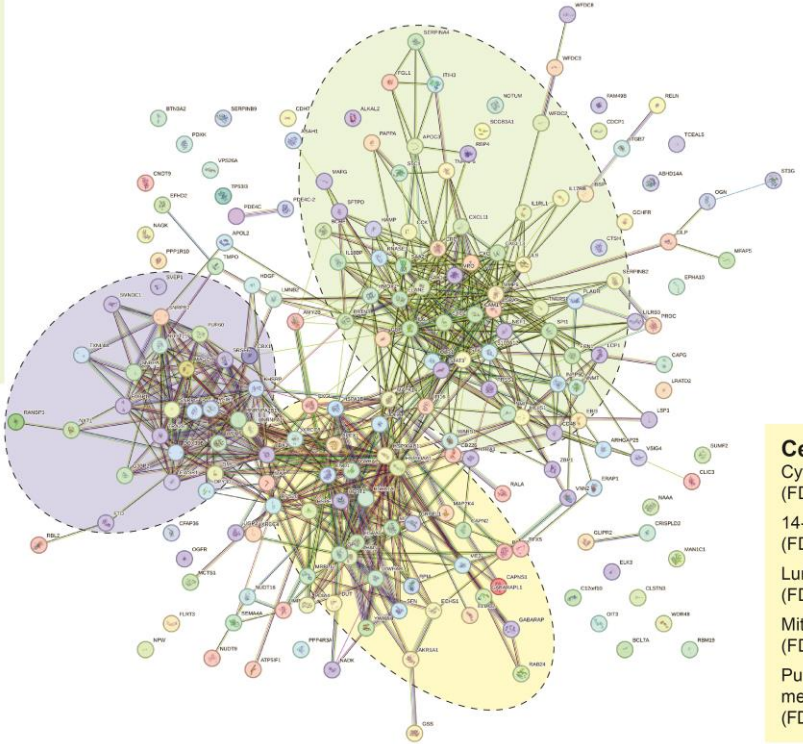
Cytokine signaling in immune system
(FDR = 5.67E-06)

Leukocyte migration
(FDR = 1.6E-04)

Type II interferon signaling
(FDR = 5.7E-04)

CXCR3 chemokine receptor binding
(FDR = 2.6E-03)

Neutrophil degranulation
(FDR = 6.1E-03)



mRNA biology
mRNA processing
(FDR = 5.07E-14)

Cellular damage
Cytosol
(FDR = 2.06E-06)

14-3-3 homologues
(FDR = 1.38E-05)

Lung
(FDR = 3.89E-05)

Mitochondrion
(FDR = 7.57E-05)

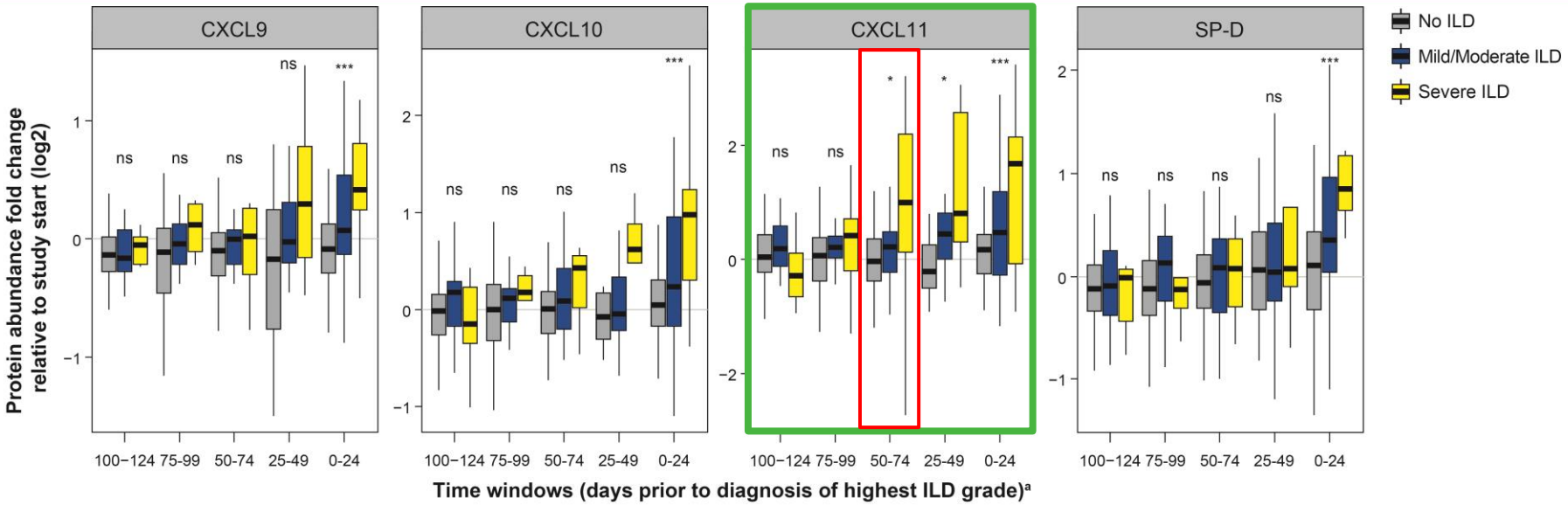
Purine ribonucleotide metabolic process
(FDR = 1.3E-03)

A protein-protein interaction network using significant proteins identified with the linear mixed model in patients with severe ILD showed more interactions than expected (*P* = 2.33E-15)

3 main clusters were identified; ILD onset was associated with biological processes involving **inflammation** (green), **cellular damage** (yellow), and **mRNA biology** (blue)

CXCR, C-X-C chemokine receptor; FDR, false discovery rate; ILD, interstitial lung disease; mRNA, messenger RNA.
Figure generated using the "STRINGdb" package in R. Pathway enrichment analysis was performed across databases, including Gene Ontology, KEGG, and WikiPathways.

Longitudinal trajectory analysis



Longitudinal trajectory analysis identified **CXCL11** as a **promising ILD risk-monitoring biomarker** with **significant abundance increases** observed **50-74 days** (up to 2.5 months) before diagnosis of highest ILD grade in patients with severe ILD

CXCL9, CXCL10, and SP-D abundances increased closer to highest ILD diagnosis (time window, 0-24 days) in patients with severe ILD

CXCL9/10/11, C-X-C motif chemokine ligand 9/10/11; DB, DESTINY-Breast; DL, DESTINY-Lung; ILD, interstitial lung disease; ns, not significant; SP-D, surfactant protein D.
Kruskal-Wallis and Dunn post hoc test, BH correction. *P < 0.05, **P < 0.01, and ***P < 0.001 between no ILD and severe ILD.
[a]For non-ILD controls, 128 days was used as the day of worst ILD diagnosis as this timepoint was within the range of values for median worst ILD diagnosis in the DB-01, DB-04, and DL-01 cohorts.

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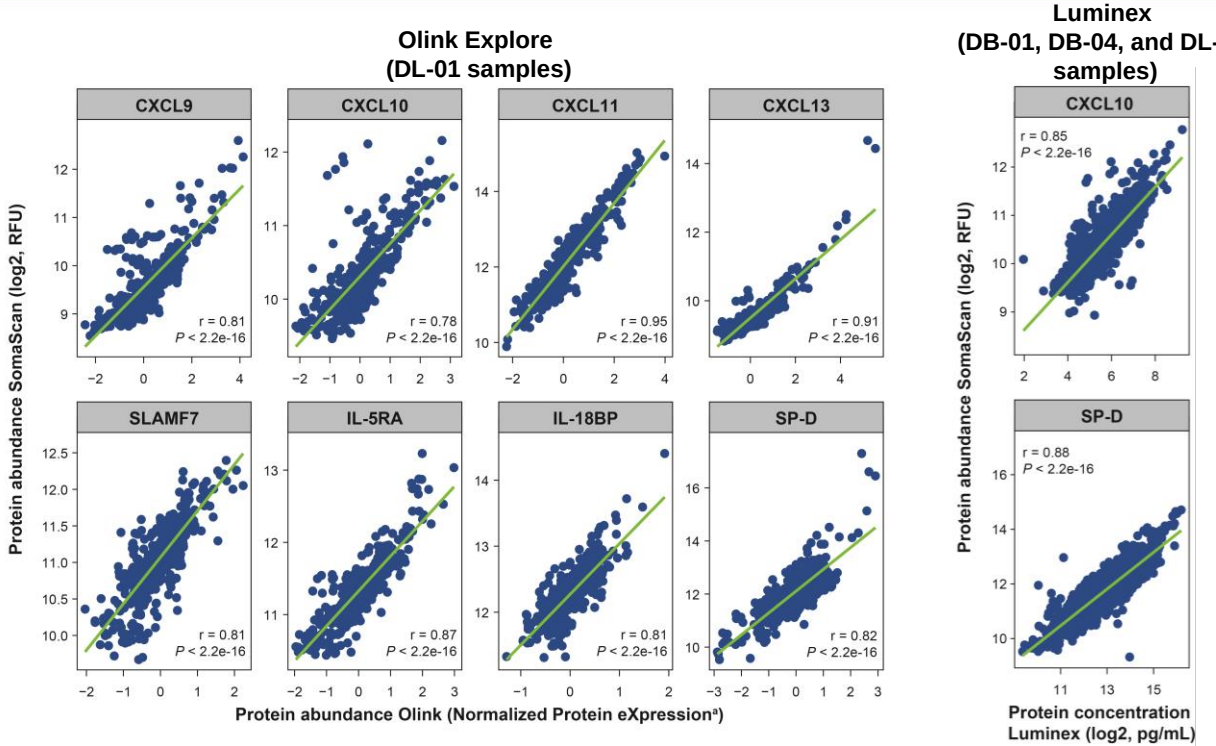
Orthogonal confirmatory analysis

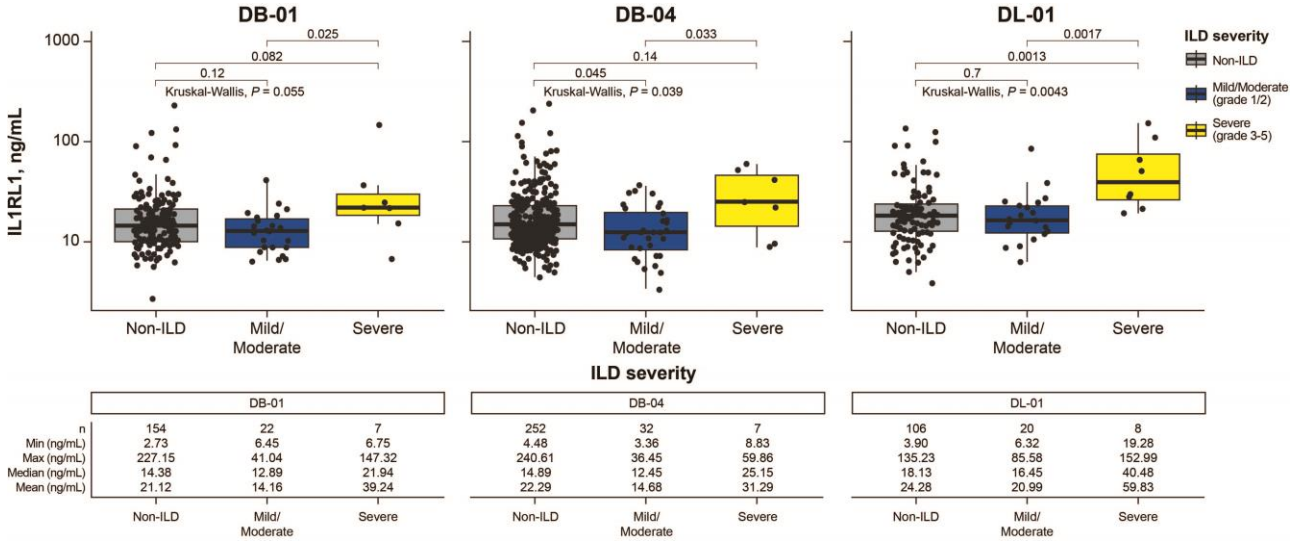


- SomaScan findings were confirmed with orthogonal technologies (Olink and Luminex) showing significant protein abundance correlations
  - Proteins confirmed with Olink technology included CXCL9, CXCL11, IL-5RA, and IL-18BP
  - CXCL10 and SP-D were confirmed with both Olink and Luminex assays

CXCL9/10/11/13, C-X-C motif chemokine ligand 9/10/11/13; DB, DESTINY-Breast; DL, DESTINY-Lung; IL-18BP, interleukin 18 binding protein; IL-5RA, interleukin 5 receptor subunit alpha; SLAMF7, self-ligand receptor of the signaling lymphocytic activation molecule 7; SP-D, surfactant protein D.
[a]Normalized Protein eXpression represents protein abundance in $\log_2$ scale.

Elevated baseline IL1RL1 in severe ILD

- In the baseline samples (n = 189), Luminex **serum IL1RL1** (also known as ST2) was differentially expressed in patients with **severe ILD (n = 22; grade 3-5)**
- Across the 3 studies, median levels of **baseline IL1RL1** were **elevated** in patients with **severe ILD** compared with patients with no ILD and mild/moderate ILD



For additional information on baseline analysis, see poster #5928 (Tsuchihashi et al.)

DB, DESTINY-Breast; DL, DESTINY-Lung; ILD, interstitial lung disease; IL1RL1, interleukin 1 receptor-like 1.
The number of patients with ILD included in each test varies based on the availability of patient samples for specific assays.

Conclusions

- Proteomics analysis of longitudinal serum samples identified **key biological processes** associated with ILD onset including **inflammation, cellular damage** and **mRNA biology**
- **CXCL11 emerged as a candidate risk-monitoring biomarker** due to its early elevated levels prior to ILD onset and potential to identify severe ILD
- **Validation** of potential longitudinal and baseline ILD biomarkers is **underway in additional cohorts** to strengthen the findings
- **These findings could enable early detection of severe ILD in patients who receive T-DXd treatment and help design therapeutic interventions to prevent and/or treat ILD**

CXCL11, C-X-C motif chemokine ligand 11; ILD, interstitial lung disease; T-DXd, trastuzumab deruxtecan.

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