2023.2.20 PSTC Japan
Fujisawa

Novel biomarker exploration using SOMAscan

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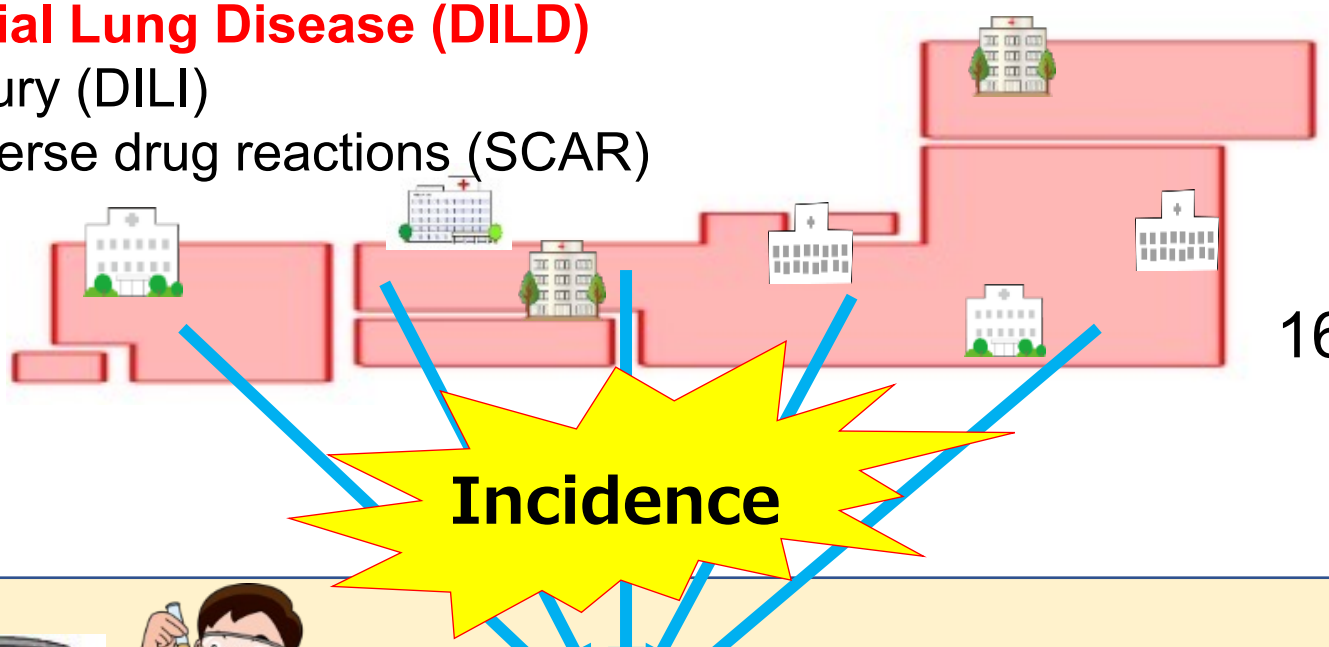
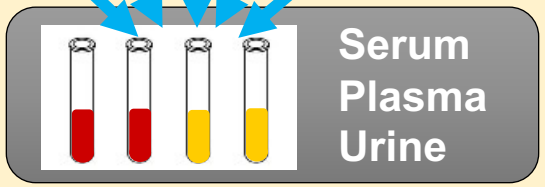
Our ongoing development of biomarkers for serious adverse drug reactions (ADR)

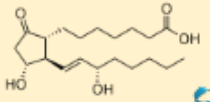
-Drug-induced **Interstitial Lung Disease (DILD)**
-Drug-induced Liver Injury (DILI)
-Severe cutaneous adverse drug reactions (SCAR)



16 hospitals in Japan



- **Biomarker discovery（proteomics**, metabolomics, miRNA）
- **Clinical validation and clinical utility of candidate**

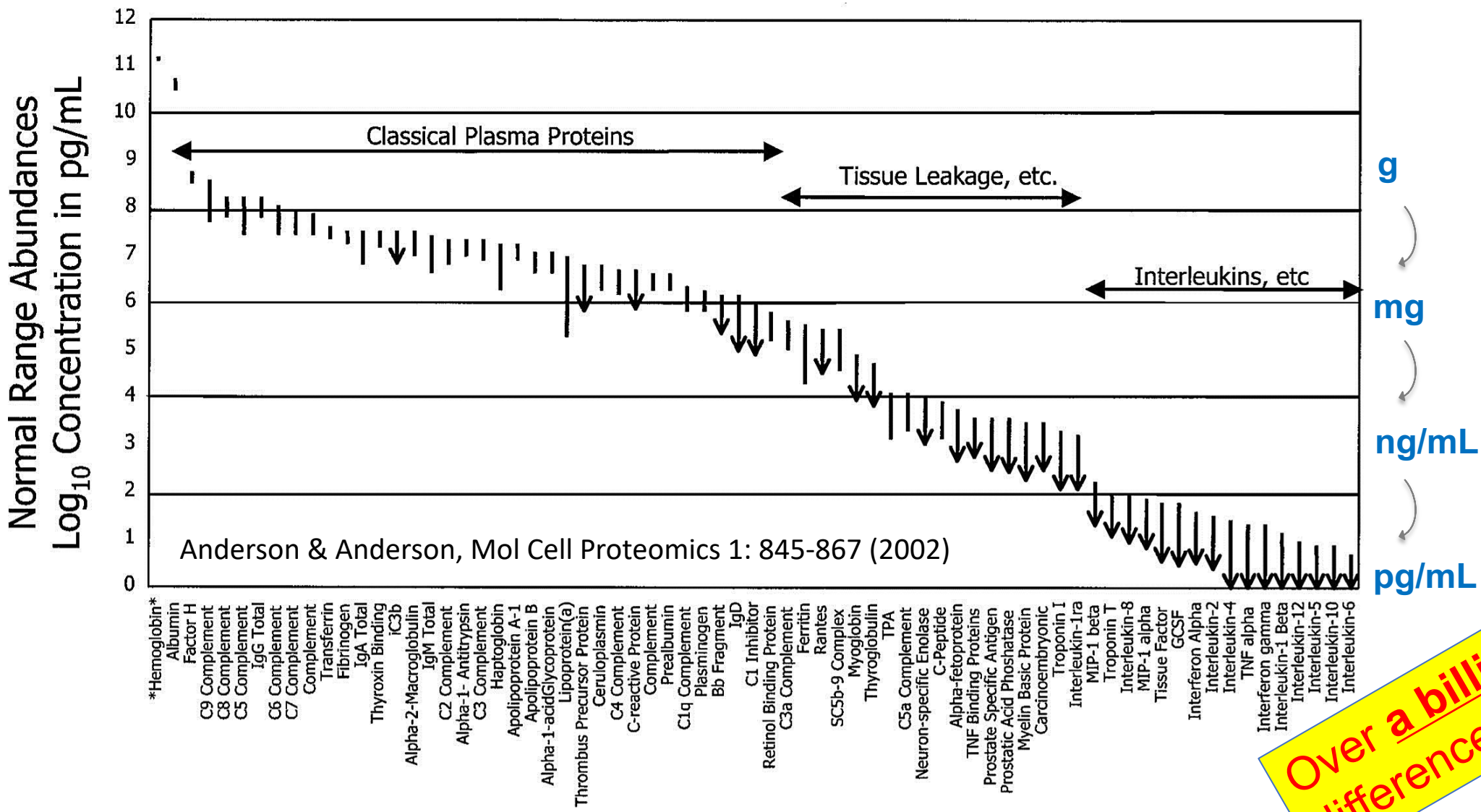


PMDA

qualification

Biomarker use to avoid developing severe ADR

Normal range abundances of blood proteins



Over **a billion-fold** difference in concentration

How much is a billion-fold?

Amount of incinerated garbage per day in Yokohama City

1,000 tons
(= 1 billion grams)

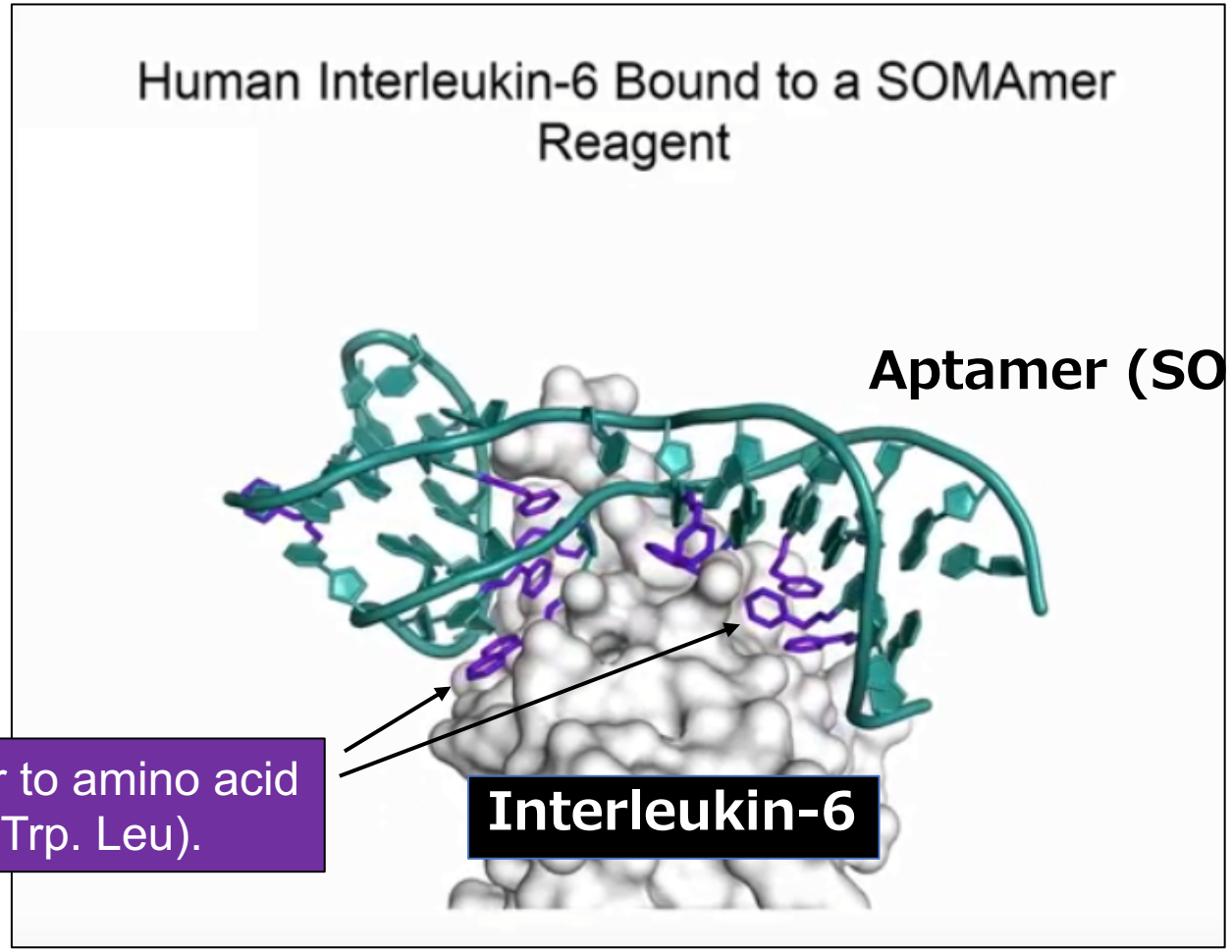
1 grams

City Of Yokohama https://www.youtube.com/watch?v=yaXe5HhyptA

Detecting cytokines in blood by MS/MS technique is like looking for a ring in incinerated garbage.

Affinity proteomics by aptamer technology

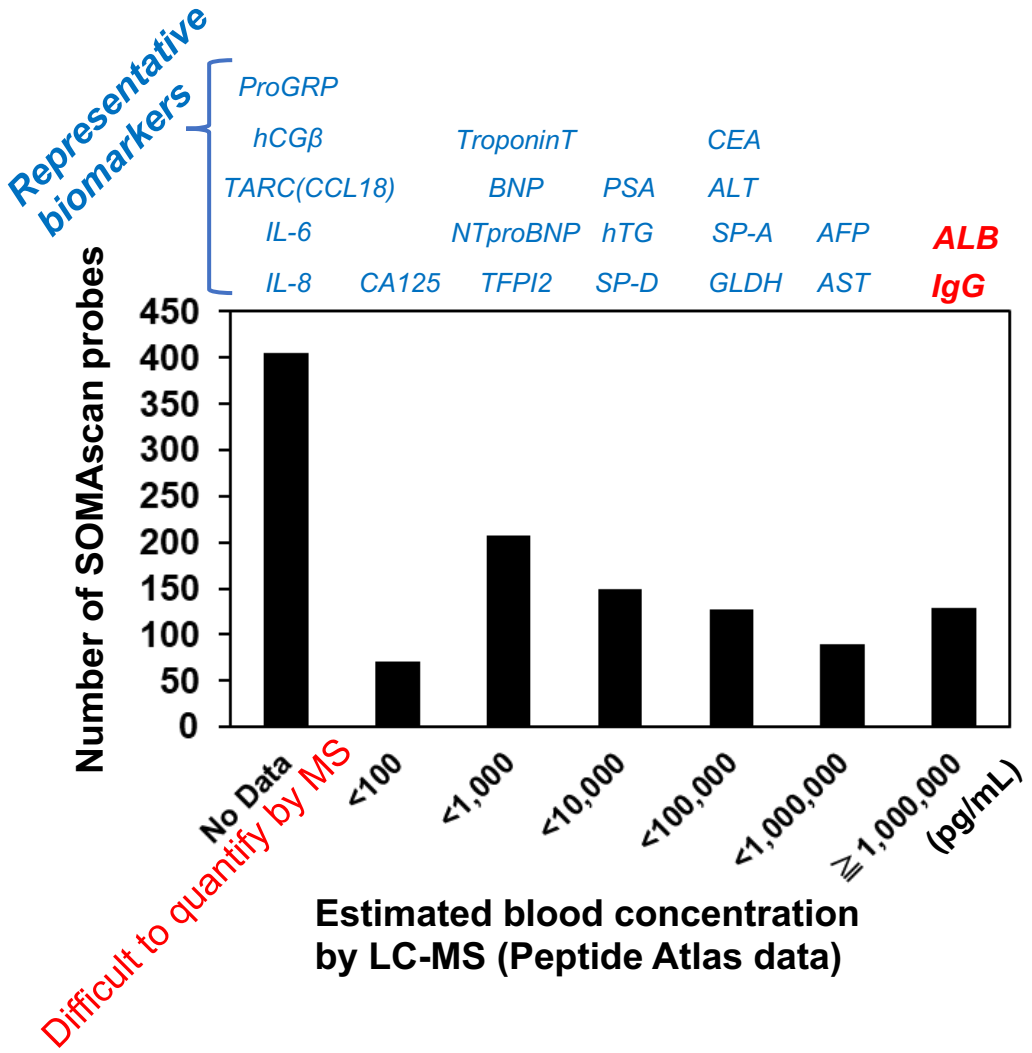
SOMAscan: an affinity proteomics using thousands of artificial DNA aptamers

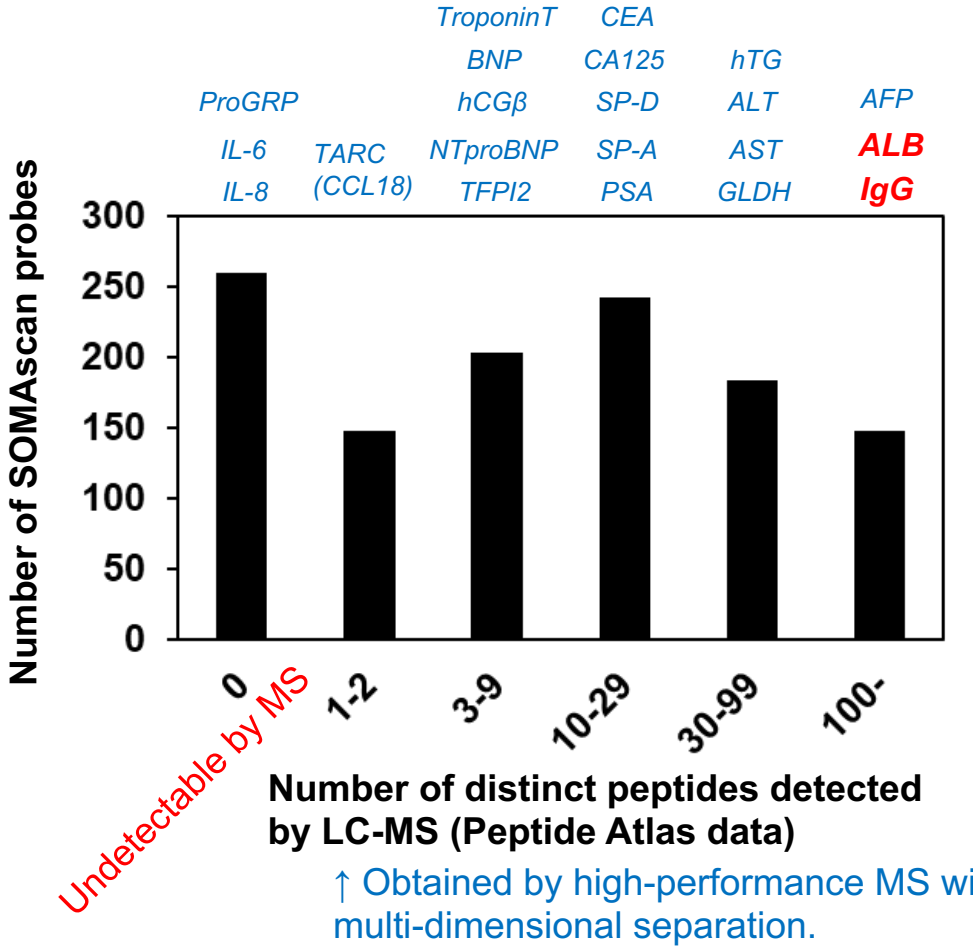


SOMAmer movie (YouTube)

SOMAscan has many probes for low abundant blood proteins

MS/MS-based quantitative information for the SOMAscan-targeting proteins (1st generation: 1310 probes).
Comparison using Peptide Atlas database (Plasma 2021-07, latest data).





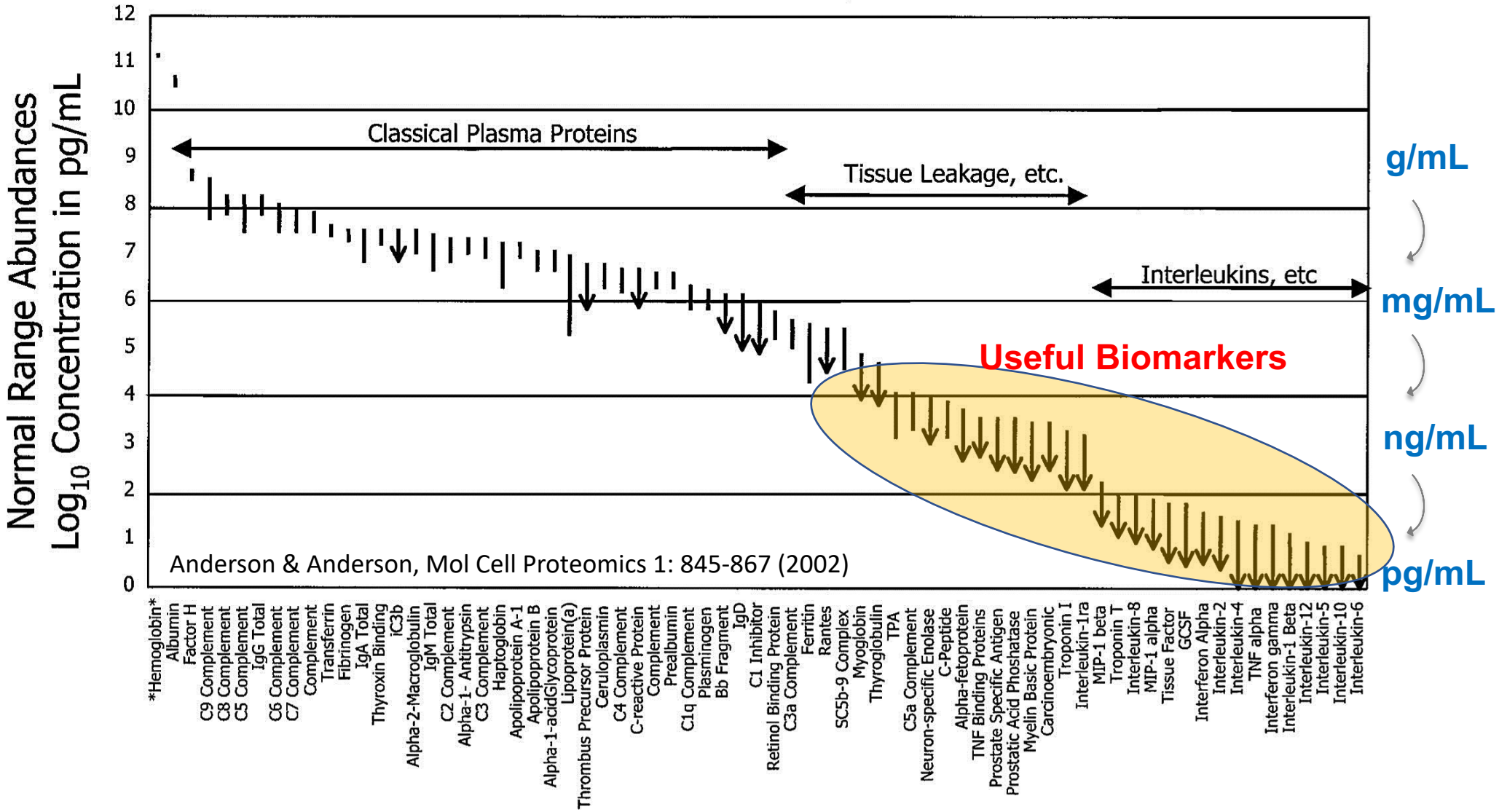
↑ Obtained by high-performance MS with multi-dimensional separation.

Arakawa et al. Unpublished data

Affinity Proteomics (SOMAscan)

LC-MS-based Proteomics

undetectable

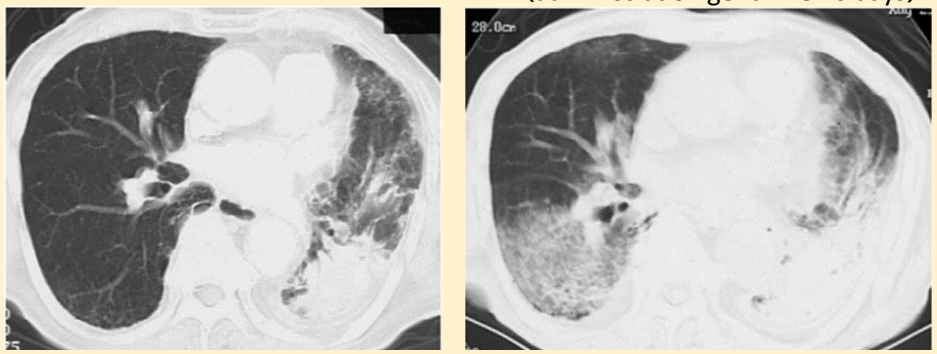


Drug-induced Interstitial Lung Disease (DILD)

Adverse drug reaction with a high number of reported cases in Japanese

Before onset

After onset
(administration gefitinive 20 days)



（厚生労働省　重篤副作用疾患別対応マニュアル）

Causal drugs
-anticancer drugs: gefitinib, erlotinib, bleomycin, 5-FU etc.
-antirheumatic drugs: Leflunomide etc.

Histopathological subtypes

Poor prognosis

- **DAD (diffused alveolar damage)**
  also found in "Acute Exacerbation" of Idiopathic Pulmonary Fibrosis (IPF、特発性肺線維症)
  Acute Respiratory Distress Syndrome (ARDS、急性促迫性症候群)
- NSIP (nonspecific interstitial pneumonia)
- OP (organizing pneumonia)
- HP (hypersensitivity pneumonitis)
- EP (eosinophilic pneumonia)

・・・etc.

When suspecting DILD, it is important to determine whether the disease-type is DAD or not. However, there were no useful biomarkers for the DAD diagnosis.

Sample collection

Collected at four hospitals using a unified protocol.

DILD disease classification :
Final diagnosis was confirmed by consensus in specialists from the four sites.

DAD group

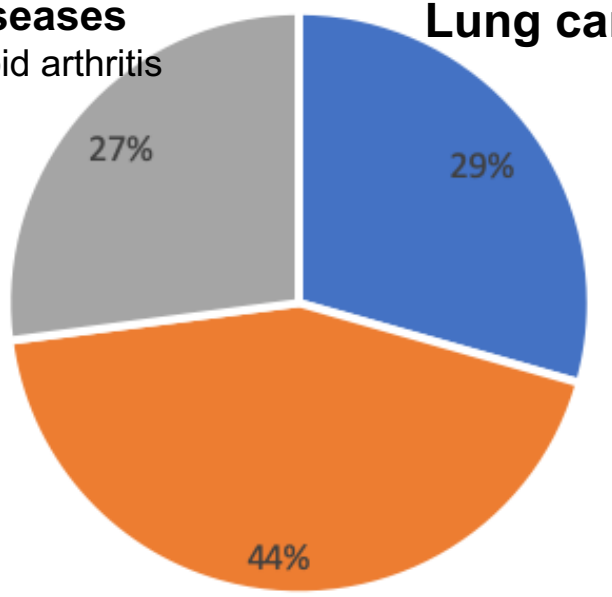
***1, DAD :**
Typical DAD patterns and DAD-dominant patterns
(DAD > HP, DAD > OP)

***2, DAD-mixed :**
Co-presence of DAD and non-DAD patterns, but not DAD-dominant
(OP > DAD, HP > DAD, DAD = HP)

	group	Discovery 2015.4~2016.11	Validation 2016.12~2020.3	Combined
	Total	95	120	432
	Healthy volunteer	24	53	77
DILD	**DAD group**	**10**	**16**	**26**
	DAD*1	6	11	17
	DAD-mixed*2	4	5	9
	non-DAD group	**30**	**28**	**58**
	OP	13	17	30
	NSIP	15	7	22
	Other (HP, EP etc)	2	4	6
	Recovered	31	24	55
	Tolerant control			**31**
Disease controls	Idiopathic ILD			**43**
	Lung Cancer			**58**
	CTD, connective tissue disease			**25**
	COPD			**15**
	NTM, nontuberculous mycobacteria			**14**
	BA, bronchial asthma			**12**
	Infectious bacterial pneumonia.			**19**

Underlying disease and suspected drugs of the DILD patients

Underlying diseases

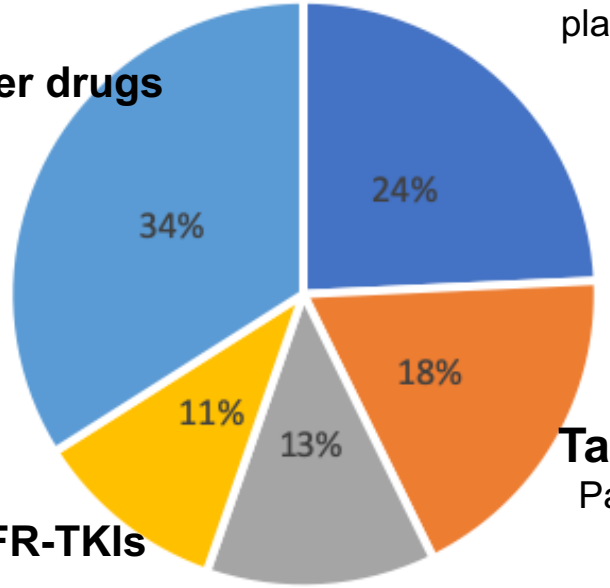


Non cancerous diseases
heart failure, rheumatoid arthritis

Lung cancer

Other tissue cancer
(Pancreatic, esophageal,
breast, renal cancer etc.)

Suspected drugs



DNA-damaging agents
platinum drugs, gemcitabine,
irinotecan, bleomycin etc.

Taxanes
Paclitaxel, docetaxel

Immune checkpoint inhibitors (ICIs)

EGFR-TKIs

Other drugs

Biomarker discovery for DAD by SOMAscan

1310

41

11

Useful biomarker

SOMAscan

Discovery cohort
(n=95)

Selection

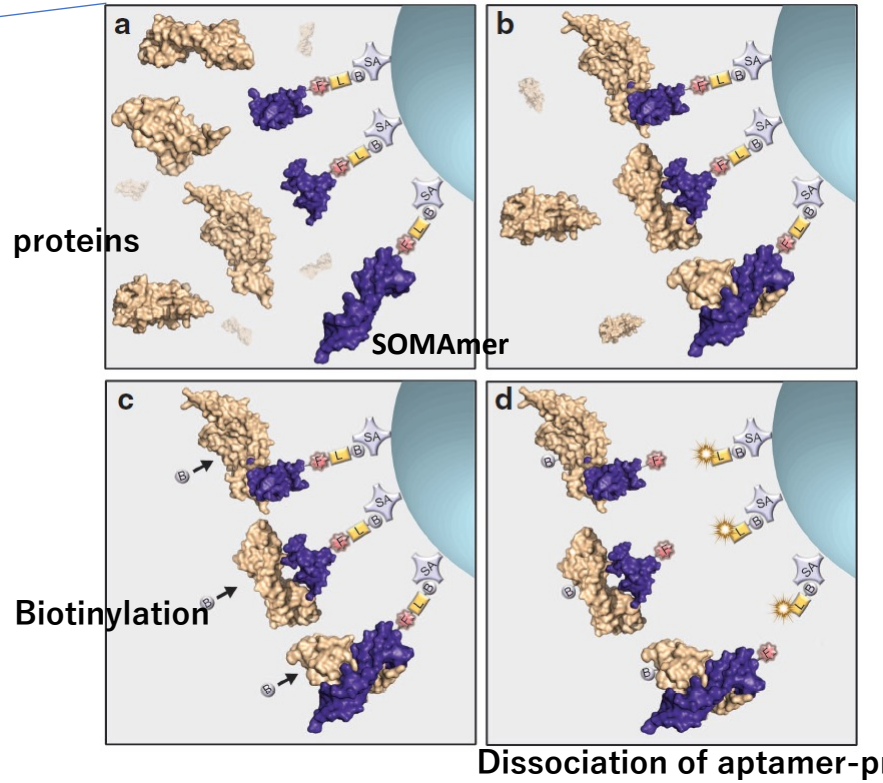
ELISA kit

Analytical Validation

Clinical Validation

Validation cohort
(n=120)

Tolerant control
Disease control
(n=217)



Sample volume needed
Plasma 75 µL

Dissociation of aptamer-protein complexes

Quantification of SOMAmer using a microarray

Protein candidates markedly changed in DAD

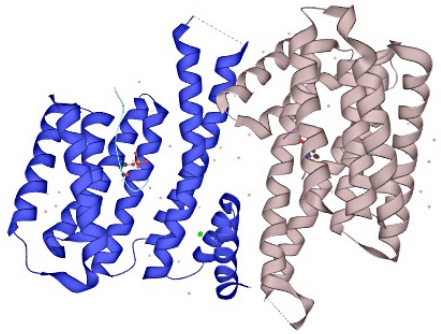
Discovery cohort

[**DAD** n=10]
[**OP** n=13] vs [**Control group** n=55, Healthy Control n=24 + Recovery n=31]
[**NSIP** n=15]

Change	Target	Fold Change (FC)			Effect size (*g* value)		
		DAD	OP	NSIP	DAD	OP	NSIP
up	CAPG	3.7	1.4	1.9	2.2	0.5	1.1
	PARC	3.1	2.0	1.7	2.0	1.2	0.8
	SFN	**2.3**	**1.0**	**1.2**	**2.0**	**0.1**	**0.6**
	IL-1Ra	2.8	1.2	1.4	2.0	0.6	0.9
	sPLA2	5.0	1.5	1.7	1.9	0.5	0.6
	SAA1	15	2.2	4.3	1.8	0.5	0.9
	CRP	5.7	3.8	3.8	1.5	1.2	1.2
	IL-6	2.6	1.1	1.0	1.5	0.2	0.0
down	Carbonic anhydrase 6	0.39	0.89	1.2	2.0	0.9	0.6
	Kallistatin	0.45	0.69	0.69	2.8	1.3	1.2
	Apo-AI	0.42	0.69	0.71	3.1	1.3	1.3

SFN is thought to be as new biomarker candidate for DAD diagnosis.

Arakawa et al., Nat Commun. 2022: 13; 5854

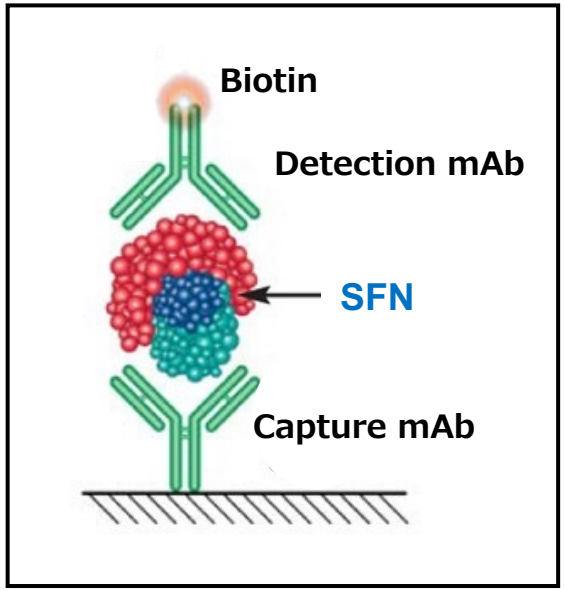


Stratifin (SFN, 14-3-3σ)

248 AA, 28 kDa

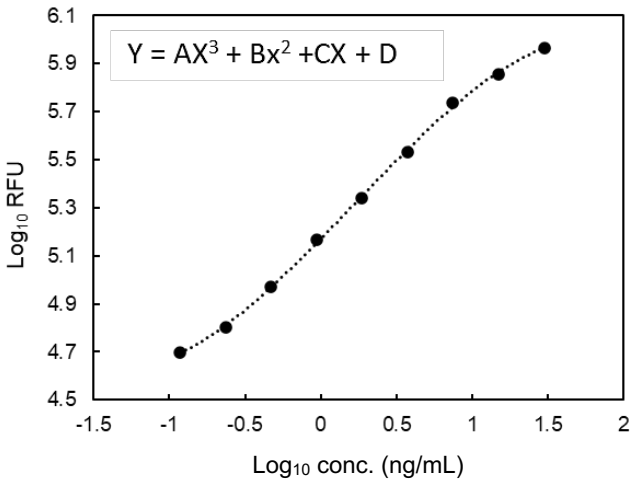
- **Transcriptional regulation by p53.** Cell cycle arrest (G2/M phase) by binding with phosoho-Cdc2.
- Expression : **skin, esophagus** (**epithelial squamous**)
- Localization : cytoplasm, nucleus
- **Highly evolutionarily conserved.** Human SFN is >97% homologous to the homologs in monkey, dog, mouse, rat.
- **No study had reported the relationship with ILD and detailed behavior in blood.**

Establishment and Analytical Validation of in-house ELISA for SFN



Method : Sandwich ELISA
Matrix : Serum
Sample : 25 uL/test

Calibration curve

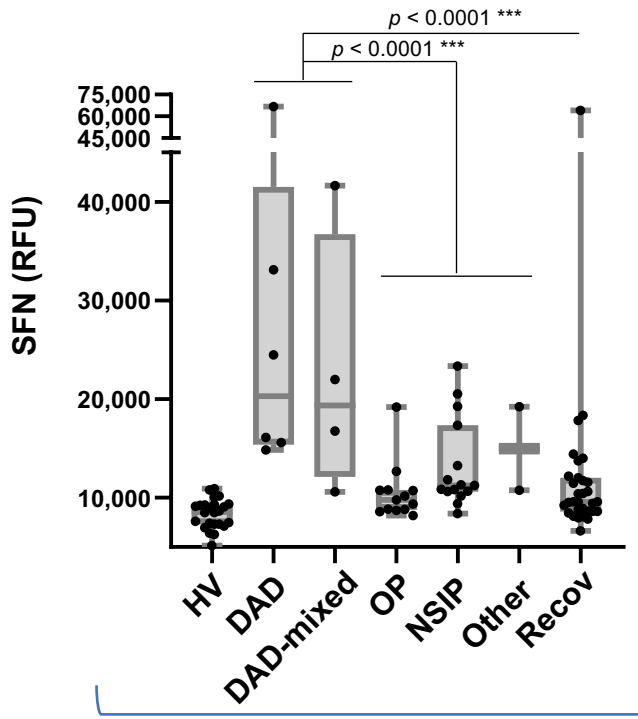


Validation	Performance
Measurement range	0.117 - 30 ng/mL
LLoQ	0.2 ng/mL
Minimum required dilution	1 fold
Dilutional linearity	1 : 1 - 1 : 256
Spike-in recovery	within ± 20%
Within run	within ± 20% (accuracy), CV < 15%
Between runs	within ± 20% (accuracy), CV < 15%
Between days	within ± 20% (accuracy), CV < 15%

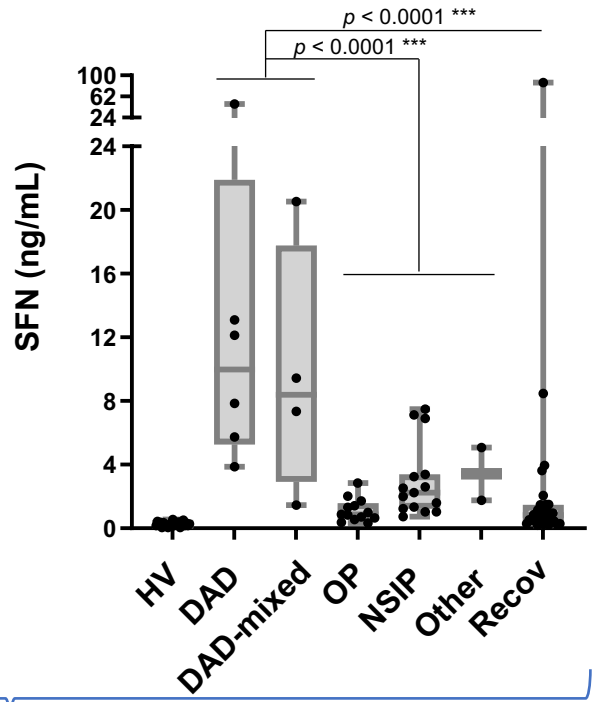
Validation	Performance
Selectivity	Not significantly affected by bilirubin C and F, hemolytic hemoglobin, chyle, ascorbic acid, HAMA, rheumatoid factor, albumin, lipid, or human IgG.
Specificity	Not reacted with human 14-3-3 family proteins except for stratifin
Stability	Short term stability (stable for at least 72 h at 4°C, 48 h at room temperature, and 6 h at 37°C, and for at least 5 freeze-thaw cycles), Long term stability (2 years)

Comparison of SOMAscan and in-house ELISA data

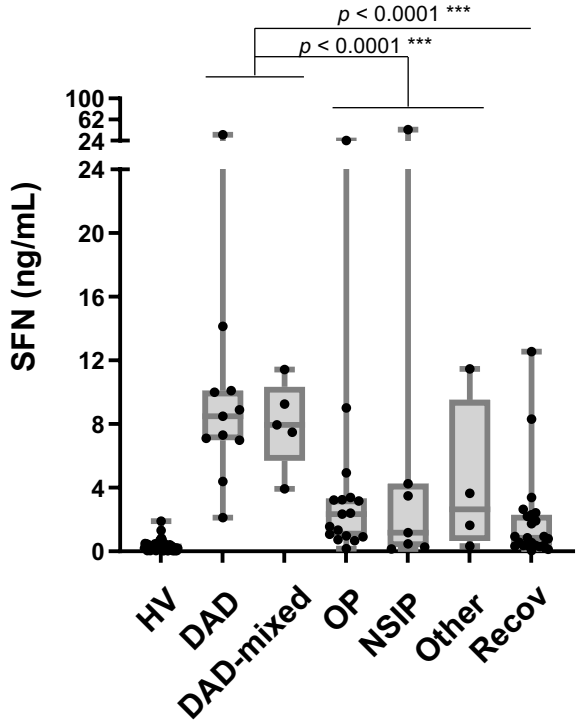
SOMAscan (Discovery cohort)



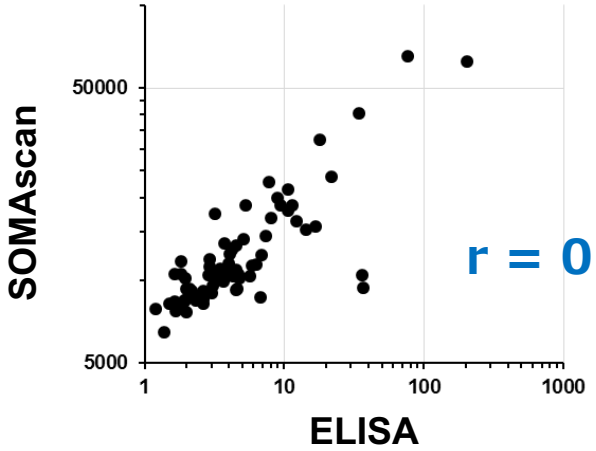
ELISA (Discovery cohort)



ELISA (Validation cohort)



DAD-specific elevation of SFN were reproduced in samples from an independent cohort.

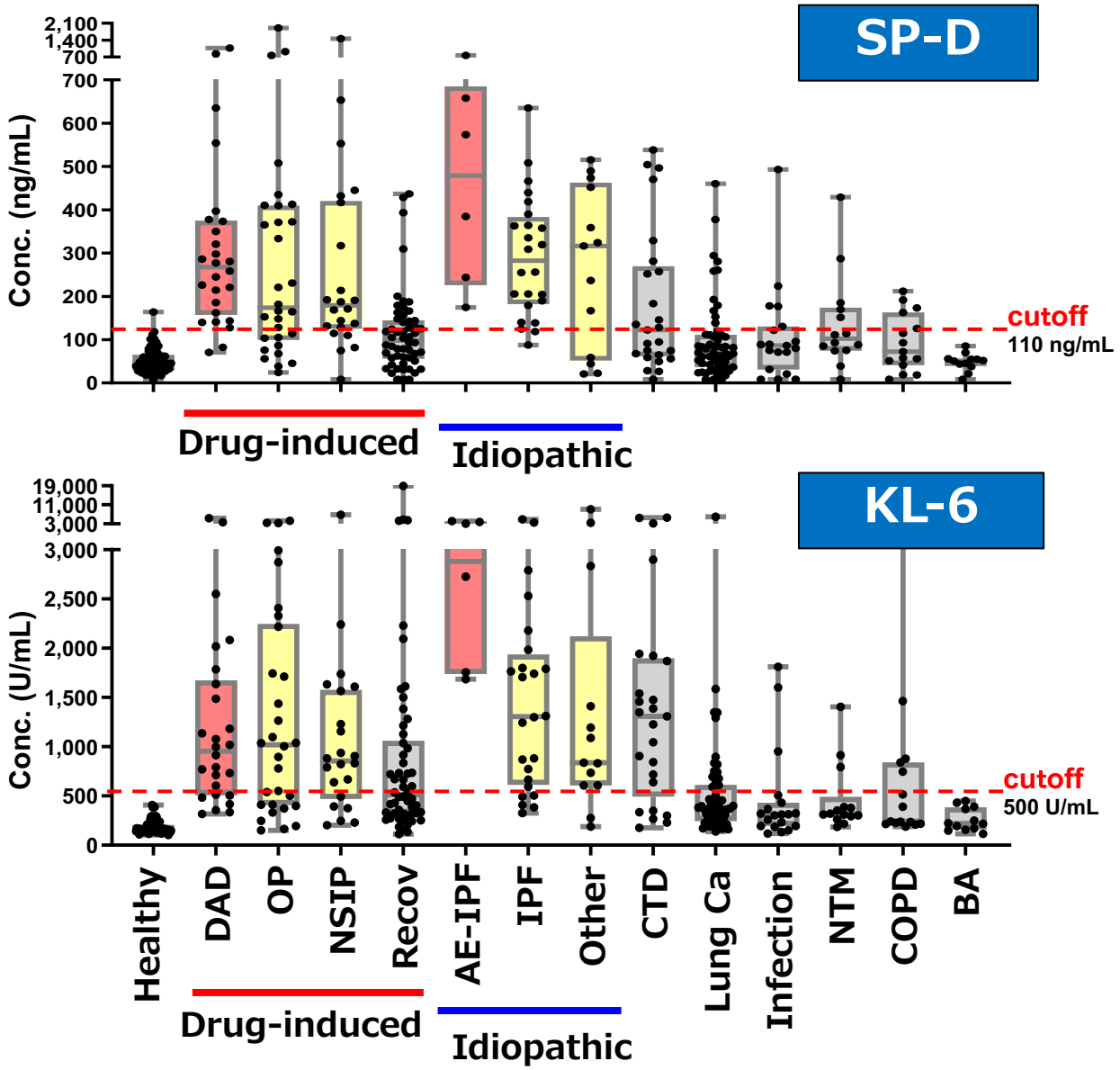


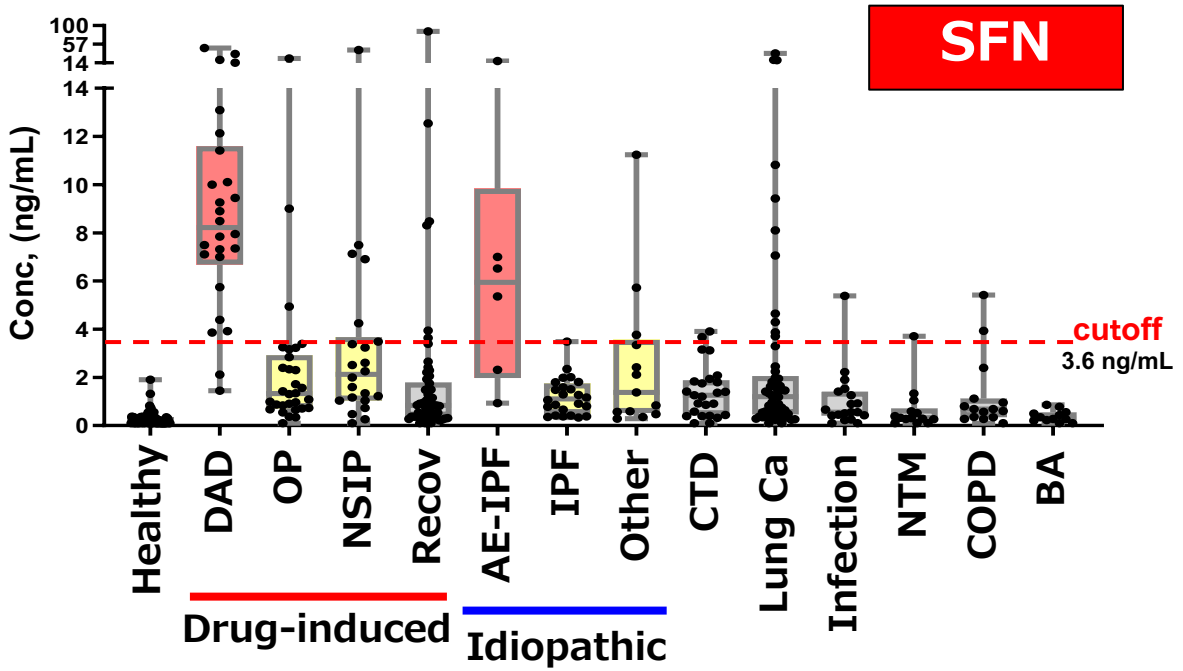
r = 0.852

ELISA data for SFN was strongly correlated with the SOMAscan data.

Distribution of SFN and known biomarkers in patients with various lung diseases

Combined cohort



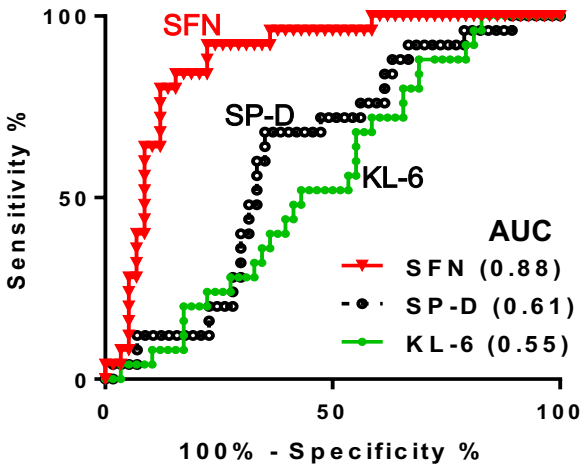


CTD, connective tissue disease
NTM, nontuberculous mycobacteria
COPD, chronic obstructive pulmonary disease
Infection, bacterial pneumonia
BA, bronchial asthma

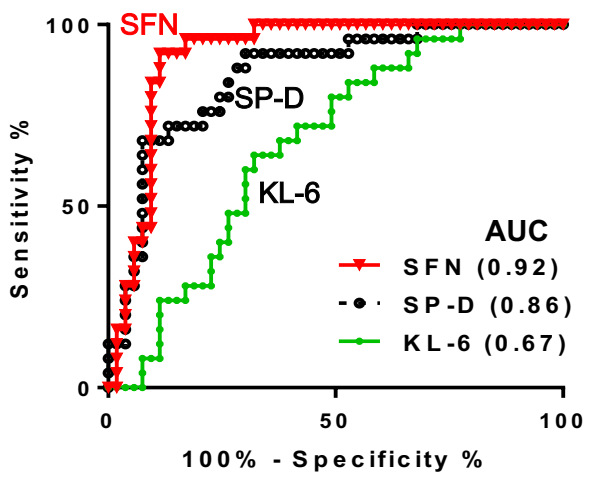
Arakawa et al., Nat Commun. 2022: 13; 5854

SFN has a good DAD-diagnostic performance

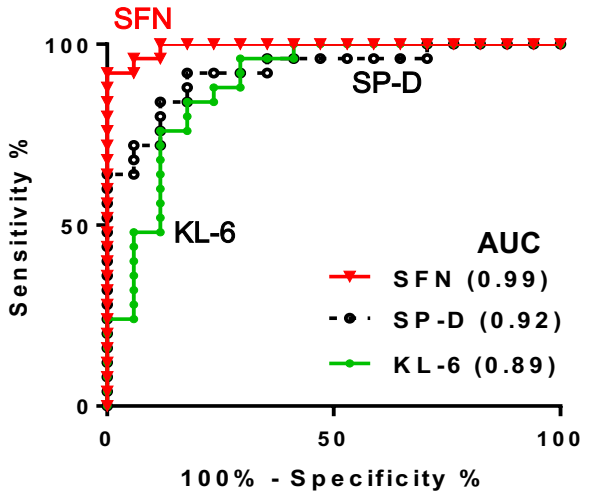
DAD diagnostic performance
ROC（DAD vs non-DAD）



Discrimination of the disease activity
ROC（Acute vs Recovery）



Discrimination with Infectious bacterial pneumonia
ROC（DAD vs Bacterial Infection）



Biomarker performance of SFN for discriminating DAD was superior to those of known biomarkers, KL-6 and SP-D

Arakawa et al., Nat Commun. 2022: 13; 5854

Pathological changes of DAD

The pathological feature of DAD dramatically changes from onset in a time-dependent manner.

Early (Day1-6): Exudative phase

- **Cell death** of type I alveolar epithelial cells
- hyaline membrane formation

Mid (Day7-21): Proliferative (organizing) phase

- **Proliferation and hyperplasia** of type II alveolar epithelial cells

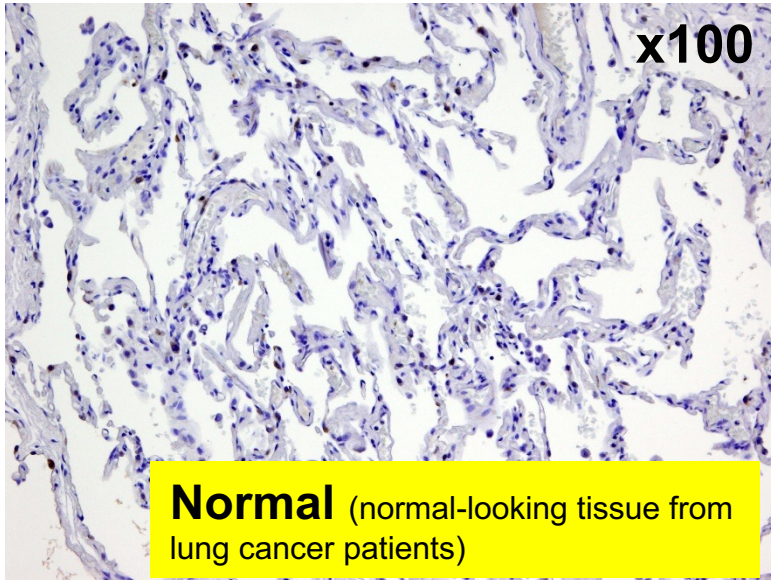
Late (after Day21): Fibrotic phase

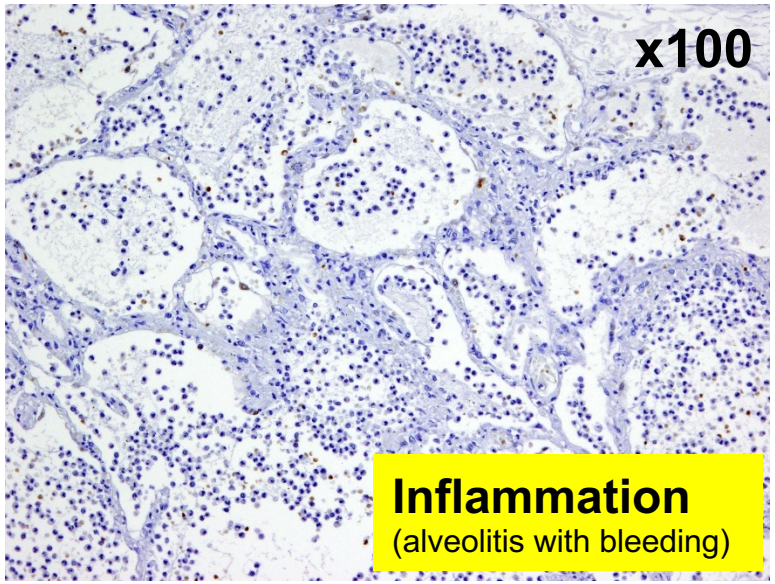
- **Squamous cell metaplasia** of type II alveolar epithelial cells
- **Fibrosis**

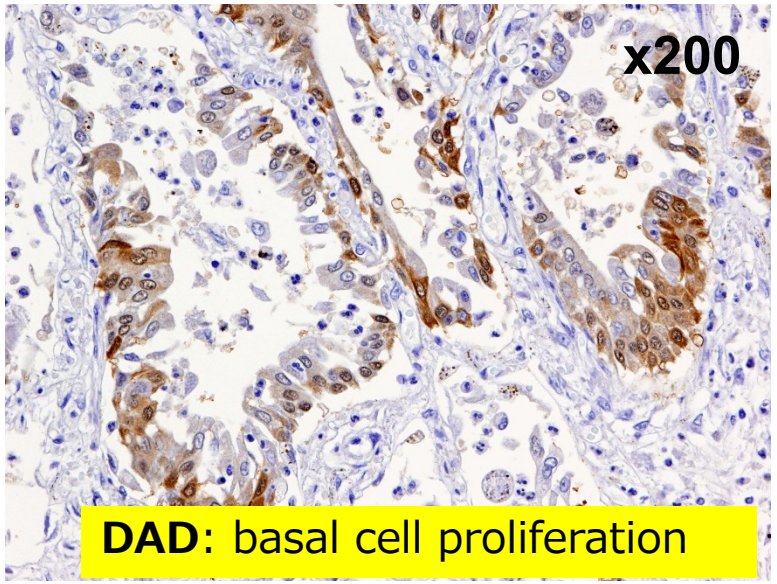
Global mechanisms of wound repair, involved in cell cycle and apoptosis.

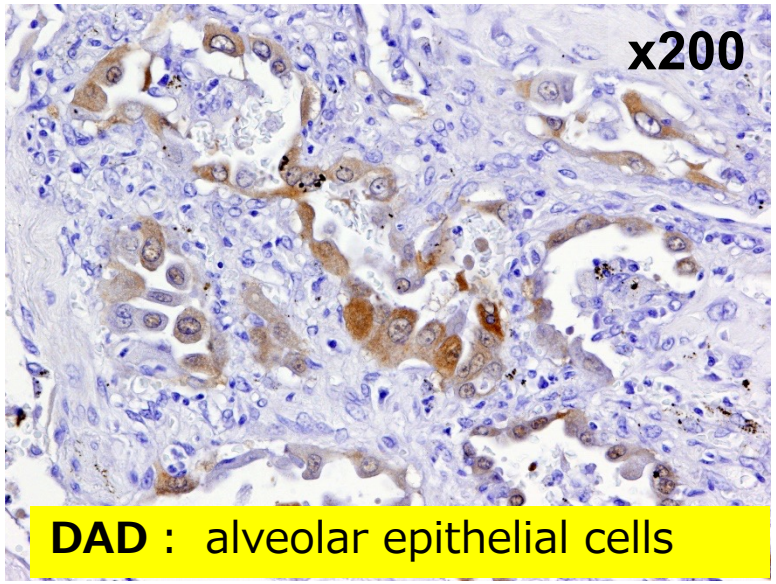
SFN expression in lung tissue

Autopsy specimens from
DILD and idiopathic ILD patients





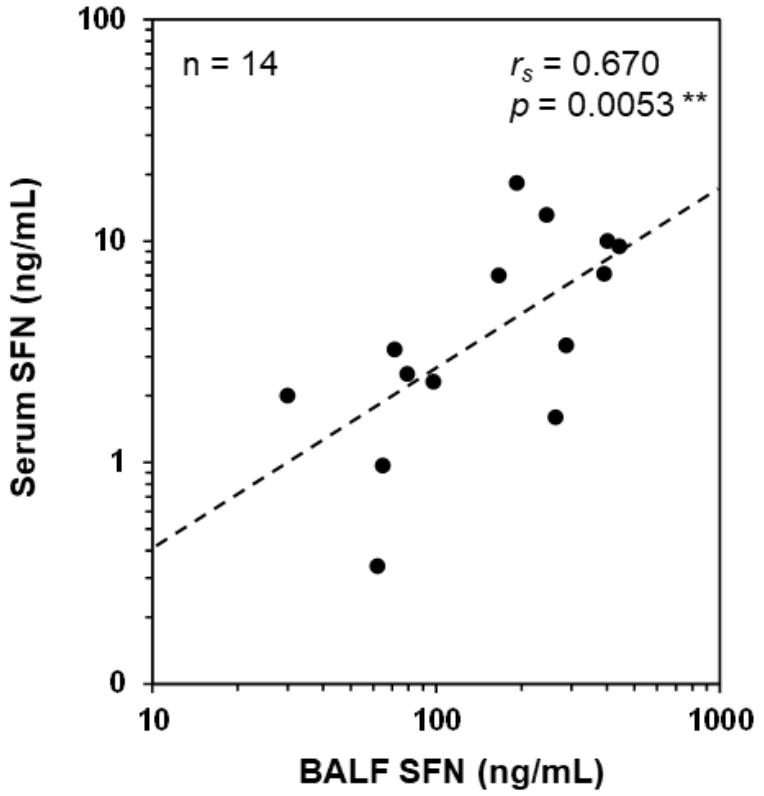




In DAD autopsy cases, SFN expression was observed in bronchioles with a tendency toward basal cell proliferation, which is considered a characteristic of mid- to late-stage DAD, and in proliferated alveolar epithelial cells.

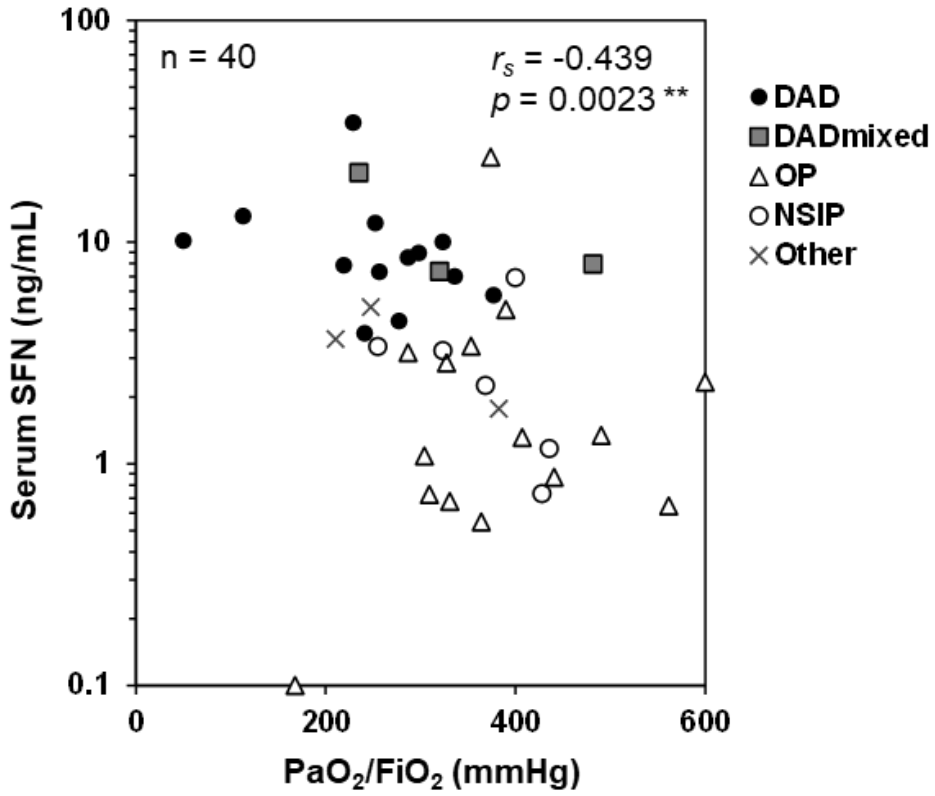
Serum SFN levels were correlated with BALF SFN levels and respiratory parameters

Serum SFN vs. BALF SFN



BALF: Bronchoalveolar lavage fluid

Serum SFN vs PaO_2/FiO_2 ratio



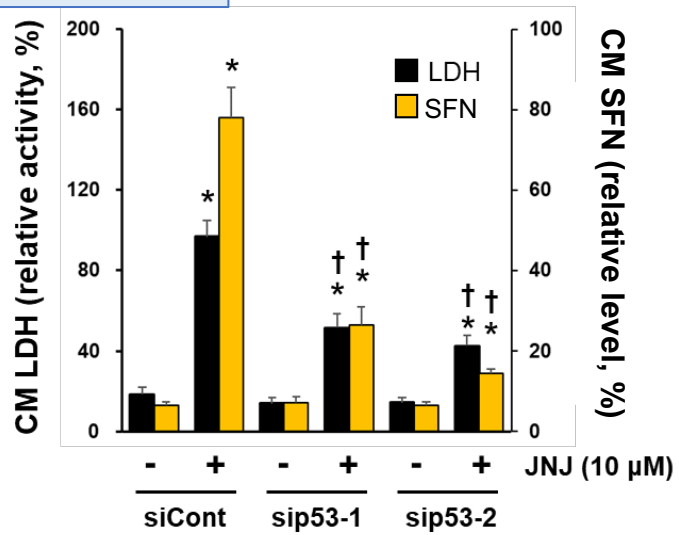
PaO_2: Arterial partial pressure of oxygen
FiO_2: Fractional inspired oxygen

Extracellular release of SFN occurred via p53-dependent apoptosis

A549 cell line

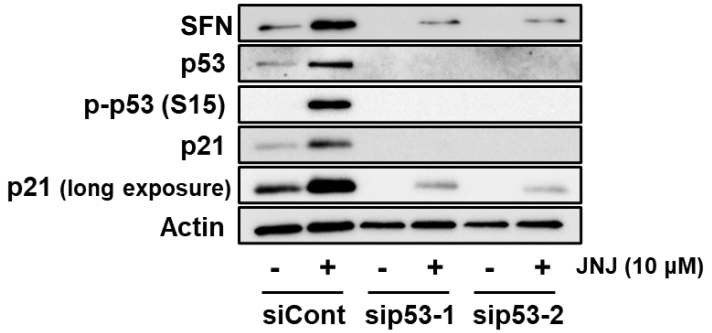
p53-knock down by siRNA

Conditioned medium



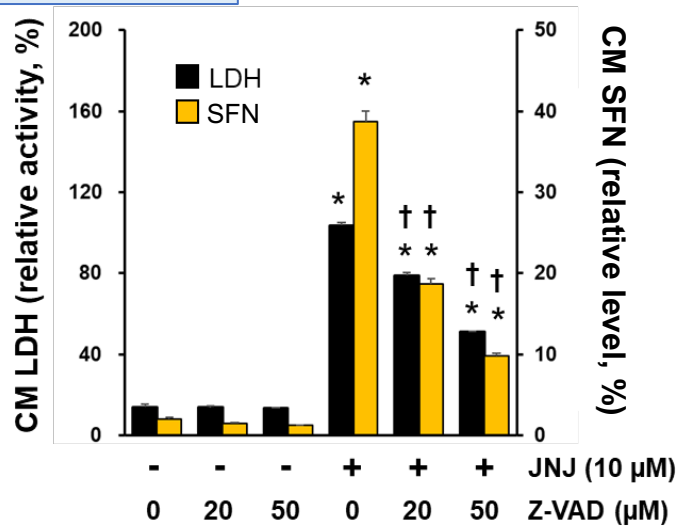
JNJ26854165 (JNJ):
p53 activating reagent
inhibiting p53-MDM2 interaction

Whole cell lysate

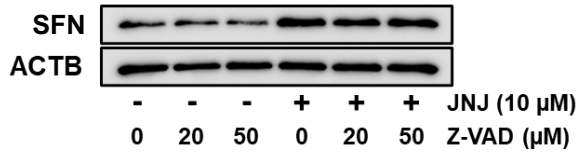


Caspase 3 inhibitor（Z-VAD-FMK）

Conditioned medium



Whole cell lysate

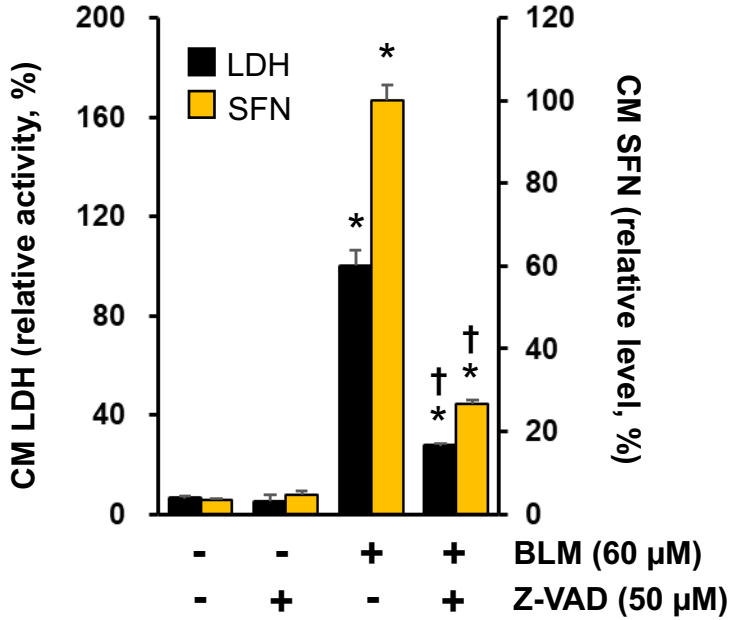


Arakawa N, Saito Y. et al., *Nat Commun.* 2022: 13; 5854

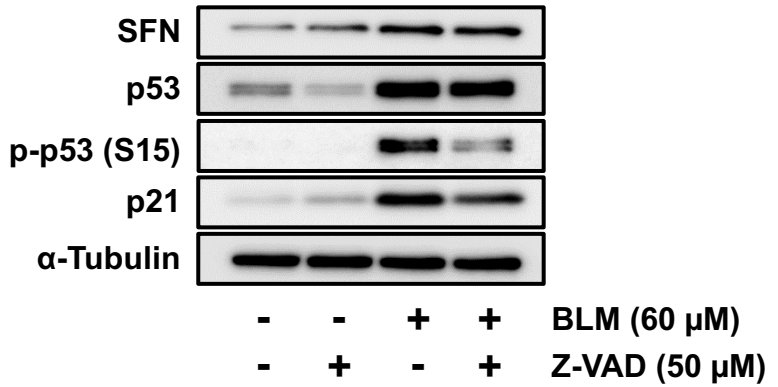
Release/expression of SFN in the primary cultured cells

Conditioned medium

Human Small Airway Epithelial Cells



Whole cell lysate



Relationship between DAD and apoptosis

Bardales RH, et al. Apoptosis is a major pathway responsible for the resolution of type II pneumocytes in acute lung injury. Am J Pathol. 149(3):845-52.1996.

Apoptosis of type II alveolar epithelial cells in Acute Lung Injury

Case	Sex/Age (years)	Diagnosis	% Apoptosis	% PCNA	Clinical history
1	M/63	AIP	<5%	50–60%	ARDS for 3 weeks
2	M/33	DAD	50%	<5%	Smoke inhalation 6 months ago; on respirator for 3 days
3	M/77	DAD	50–70%	<5%	ARDS
4	M/65	DAD	15%	40%	NHL, treated with chemotherapy for 5 weeks and on respirator for 2 days
5	M/64	AIP	30–50%	<5%	ARDS for 10 months and on respirator for 7 days; treated with steroids and cytoxan
6	M/66	AIP	<5%	50%	ARDS for 1 month and on respirator for 14 days
7	M/80	DAD	60–80%	<1%	SCC of lung, treated with high-dose MTX for 1 month

M, male; CHF, congestive heart failure; NHL, non-Hodgkin's lymphoma; MTX, methotrexate; SCC, squamous cell carcinoma.

Apoptosis is more strongly detected in DAD tissues with severe the lung injury.

Apoptosis of type II alveolar epithelial cells in Chronic ILD

Case	Sex/Age (years)	Tissue diagnosis	% Apoptosis	% PCNA
1	M/70	UIP	<5%	10–20%
2	M/40	UIP	<5%	10–20%
3	M/61	UIP	<5%	10%
4	M/46	UIP	<5%	<5%
5	M/67	DIP	<5%	60–70%
6	M/76	UIP	<5%	20–30%
7	M/69	UIP	<5%	40–50%
8	M/81	CIP-NOS	<5%	<5%
9	M/74	UIP	<5%	30–40%
10	M/72	UIP	<5%	<5%
11	M/69	CIP-NOS	<5%	40–50%
12	F/54	UIP	<5%	10%
13	M/71	UIP	<5%	10%
14	M/70	UIP	<5%	20–30%

M, male; F, female; UIP, usual interstitial pneumonia; DIP, desquamative interstitial pneumonia; NOS, not otherwise specified.

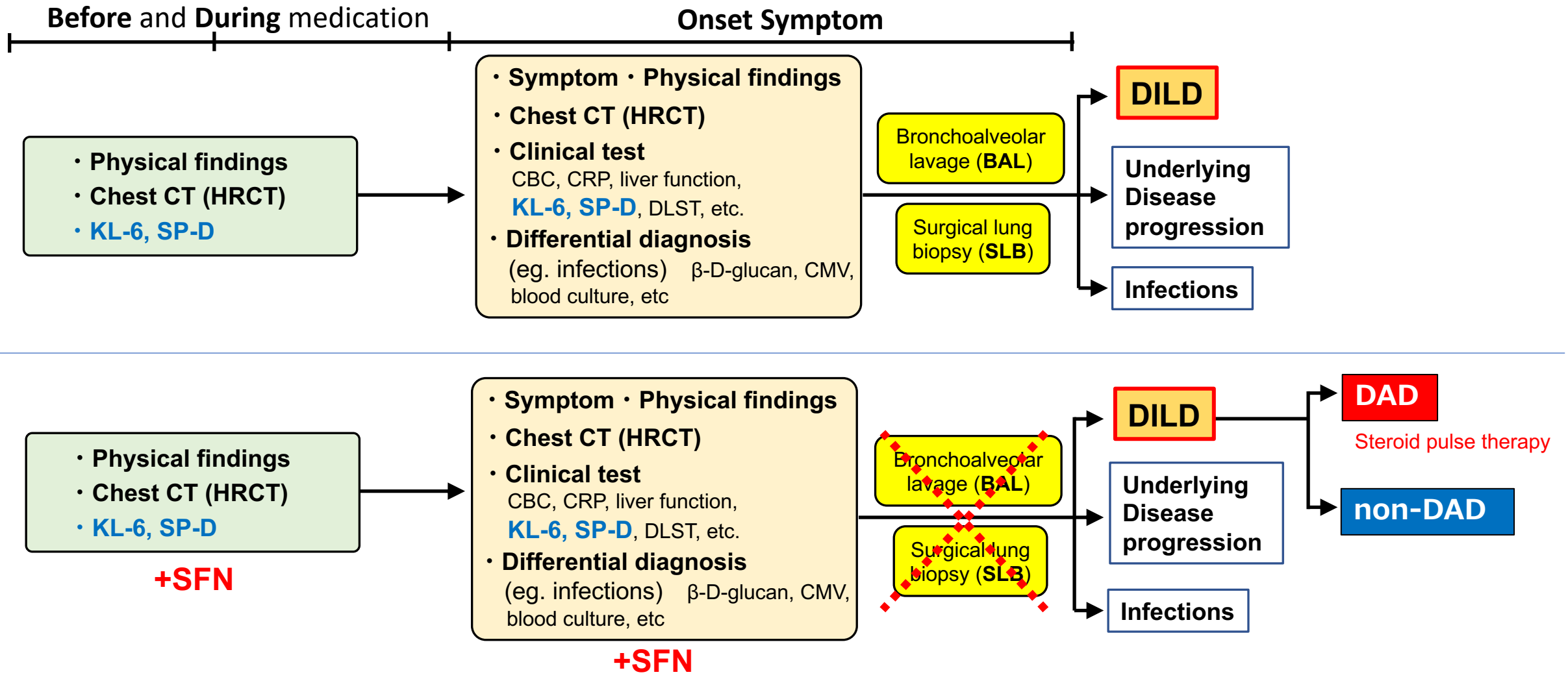
Elevation mechanism of blood SFN (hypo)

(1) Upregulation of intracellular SFN by p53 activation in alveolar epithelium at early DAD.

(2) Apoptosis → Extracellular release of SFN

(3) The event at alveolar epithelium, which is the main field for gas-blood exchange, may contribute to the increase in circulating SFN levels.

Clinical Utility of SFN Assay in DILD diagnosis



SFN assay can provide a supportive information to improve the accuracy of the DILD diagnosis without invasive testing.

Conclusions

- ✓ We found SFN as a new serum biomarker by SOMAscan.
- ✓ SFN is superior to the known biomarkers (KL-6 and SP-D), in discrimination of DAD from other lung diseases.
- ✓ SFN is also increased in patients with idiopathic DAD or severe COVID-19.
- ✓ SFN is also elevated in lung tissues and bronchoalveolar lavage fluid of patients with DAD.
- ✓ Extracellular release of SFN occurs via p53-dependent apoptosis.
- ✓ SFN is thought to be a promising biomarker for DAD.

Collaborators

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Thank you!