Gene expression profiling in patients with idiopathic pulmonary fibrosis (IPF) treated with nintedanib and sildenafil: data from the INSTAGE® trial

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Introduction

- Nintedanib is an intracellular inhibitor of tyrosine kinases approved for the treatment of IPF. Nintedanib reduces the progression of IPF by reducing the rate of decline in forced vital capacity (FVC).[1]
- Sildenafil, a phosphodiesterase-5 inhibitor and pulmonary-selective vasodilator, is an approved treatment for pulmonary arterial hypertension.
- Exploratory analyses of data from the INSTAGE trial, conducted in patients with IPF and severely impaired gas exchange, suggested that treatment with nintedanib plus sildenafil was associated with a numerical reduction in FVC decline over 24 weeks compared to nintedanib alone.[2]

Aim

- To investigate changes in gene expression in patients treated with nintedanib plus sildenafil and nintedanib alone in the INSTAGE trial.

Methods

Trial design[2]

- Subjects with IPF and DLco ≤35% predicted were randomized to receive nintedanib 150 mg bid plus sildenafil 20 mg tid or nintedanib 150 mg bid plus placebo for 24 weeks. Both subjects who were nintedanib-naïve and those who had previously taken nintedanib were eligible to participate.

RNA sequencing

- Analyses were based on total RNA extracted from whole blood samples taken at baseline and week 24.
- RNA quantity and quality were measured using a NanoDrop spectrophotometer.
- Total RNA sequencing, with approximately 50 million reads per sample, was performed using the TruSeq Stranded Total RNA Kit with Ribo-Zero Globin and a HiSeq 4000 (Illumina).

Analyses

- We analyzed changes in gene expression from baseline at week 24 in the two treatment groups:
  - Data were $\log_2$ transformed prior to analysis.
  - p-values were adjusted to control the false discovery rate at 5%.
  - Changes in gene expression over 24 weeks were considered significant if adjusted p≤0.05 and $|\log_2\text{fold change}|\geq 0.5$ (*i.e.*, there was a ≥1.4-fold difference between baseline and week 24).
- In a secondary investigation, we analyzed changes in the expression of nine genes that were downregulated after 12 weeks' treatment with nintedanib in the INMARK trial in subjects with IPF and preserved lung function:[3]
  - Changes were considered significant if unadjusted p≤0.05 and $|\log_2\text{fold change}|\geq 0.5$.
  - Gene set variation analysis assessed the relative enrichment of this set of nine genes. Enrichment scores were tested using a simple linear model and moderated t-statistics.
  - Pathways analyses were performed using EnrichR. The network was generated using Ingenuity Pathway Analysis (QIAGEN, Inc).

Results

Table 1. *Baseline characteristics of subjects in the INSTAGE trial*

	Nintedanib plus sildenafil (n=137)	Nintedanib plus placebo (n=136)
Age, years	70.3 (8.6)	70.0 (7.9)
Male	110 (80.3)	106 (77.9)
Body mass index, kg/m²	26.1 (5.0)	26.5 (4.7)
Years since diagnosis of IPF	2.2 (1.9)	2.1 (1.8)
Emphysema*	51 (37.2)	45 (33.1)
Former/current smoker	104 (75.9)	109 (80.1)
Nintedanib status at randomization		
Naïve	76 (55.5)	87 (64.0)
Currently treated	56 (40.9)	46 (33.8)
Previously treated	5 (3.6)	3 (2.2)
FVC % predicted	67.9 (19.3)	66.1 (18.7)
DLco % predicted†	25.8 (6.8)	25.6 (7.0)

Data are n (%) or mean (SD). *Determined by the investigator based on qualitative assessment of HRCT scans. †Corrected for hemoglobin.

Changes in gene expression

- Data from 224 subjects (112 in each group) were analyzed.
- Of 60,675 genes evaluated, 42,078 had counts per million ≥0.1 in at least half the samples from either treatment group at every time point and were included in the analysis.
- After 24 weeks of treatment:
  - No genes were upregulated in either treatment group. Two genes were downregulated in the nintedanib plus sildenafil group, while none was downregulated in subjects who received nintedanib alone. There were no significant differences in gene expression between treatment groups (Table 2).

Table 2. *Genes downregulated at week 24 (with adjusted p≤0.05 and $|\log_2\text{fold change}|\geq 0.5$) in subjects treated with nintedanib plus sildenafil*

Gene	$\log_2$fold change from baseline at week 24 (adjusted p-value)		Difference in $\log_2$fold change from baseline at week 24 (adjusted p-value)
	Nintedanib plus sildenafil	Nintedanib plus placebo	
TINCR	–0.77 (p=0.043)	0.11 (p=0.82)	–0.88 (p=0.36)
RP11–369K17.2	–0.70 (p=0.016)	0.02 (p=0.98)	–0.71 (p=0.37)

TINCR, tissue differentiation-inducing non-protein coding RNA.

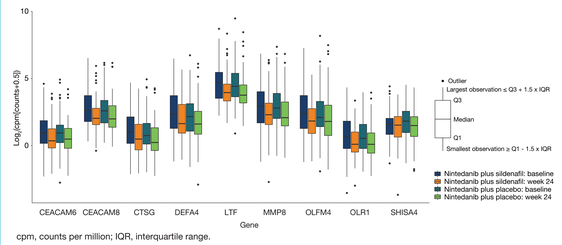
- Of the nine genes downregulated after 12 weeks of nintedanib treatment in the INMARK trial, eight were downregulated at week 24 in the nintedanib plus sildenafil group of the INSTAGE trial, while one was downregulated in subjects receiving nintedanib alone in the INSTAGE trial (Table 3; Figure 1).

Table 3. *Changes in expression in the INSTAGE trial of genes downregulated after 12 weeks' treatment with nintedanib in the INMARK trial*

Gene	$\log_2$fold change from baseline at week 24 (adjusted / unadjusted p-value)		Difference in $\log_2$fold change from baseline at week 24 (adjusted / unadjusted p-value)
	Nintedanib plus sildenafil	Nintedanib plus placebo	
SHISA4	-0.01 (p=0.97 / p=0.94)	-0.31 (p=0.45 / p=0.026)	0.30 (p=0.48 / p=0.13)
DEFA4	-0.50 (p=0.30 / p=0.010)	-0.45 (p=0.45 / p=0.023)	-0.05 (p=0.94 / p=0.85)
OLR1	-0.50 (p=0.29 / p=0.001)	-0.37 (p=0.44 / p=0.020)	-0.13 (p=0.79 / p=0.54)
CEACAM6	-0.50 (p=0.29 / p<0.001)	-0.34 (p=0.45 / p=0.025)	-0.16 (p=0.73 / p=0.45)
LTF	-0.53 (p=0.29 / p=0.001)	-0.48 (p=0.42 / p=0.003)	-0.04 (p=0.94 / p=0.85)
CEACAM8	-0.54 (p=0.29 / p<0.001)	-0.40 (p=0.42 / p=0.010)	-0.14 (p=0.78 / p=0.53)
CTSG	-0.55 (p=0.29 / p=0.001)	-0.39 (p=0.44 / p=0.021)	-0.16 (p=0.77 / p=0.51)
OLFM4	-0.63 (p=0.29 / p=0.002)	-0.38 (p=0.51 / p=0.062)	-0.24 (p=0.70 / p=0.40)
MMP8	-0.64 (p=0.29 / p<0.001)	-0.51 (p=0.42 / p=0.007)	-0.13 (p=0.85 / p=0.64)

SHISA4, shisa family member 4; DEFA4, defensin alpha 4; OLR1, oxidized low density lipoprotein receptor 1; CEACAM6/8, carcinoembryonic antigen related cell adhesion molecule 6/8; LTF, lactotransferrin; CTSG, cathepsin G; OLFM4, olfactomedin 4; MMP8, matrix metalloproteinase 8.
Blue shading: $|\log_2\text{fold change}|\geq 0.5$ and unadjusted p≤0.05.

Figure 1. *Expression at baseline and week 24 in the INSTAGE trial of the nine genes downregulated at week 12 in subjects treated with nintedanib in the INMARK trial*

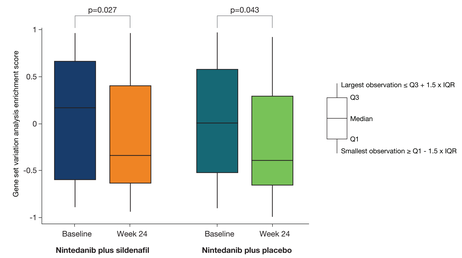


cpm, counts per million; IQR, interquartile range.

Gene set variation analysis

- Gene set variation analysis was performed on the set of nine genes downregulated at week 12 in subjects treated with nintedanib in the INMARK trial.
- Between baseline and week 24 of the INSTAGE trial, genes in this set were positively enriched compared with genes not in this set among subjects treated with nintedanib plus sildenafil and nintedanib alone (Figure 2). The changes in enrichment scores were not significantly different between the treatment groups (p=0.63).

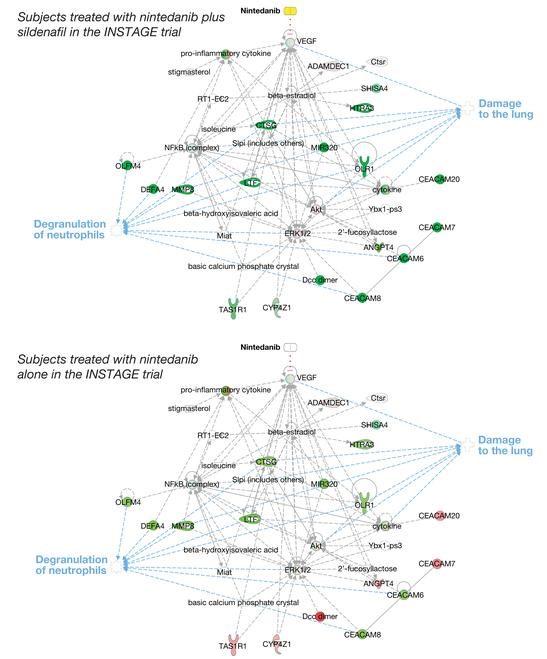
Figure 2. *Gene set variation analysis enrichment scores in the INSTAGE trial for the set of nine genes downregulated in subjects treated with nintedanib in the INMARK trial*



Pathways analyses

- The network of these nine genes showed enrichment of genes related to neutrophil degranulation and lung damage (Figure 3).

Figure 3. *Changes in gene expression at week 24 in the INSTAGE trial based on the network of nine genes downregulated in nintedanib-treated subjects in the INMARK trial*



Conclusions

- **Analyses of gene expression levels in the INSTAGE trial identified a small number of genes that were downregulated after 24 weeks' treatment with nintedanib plus sildenafil or nintedanib alone in subjects with IPF and severely impaired gas exchange.**
- **Some of the genes downregulated in patients treated with nintedanib in the INMARK trial were also found to be downregulated in patients treated with nintedanib plus sildenafil in the INSTAGE trial.**
- **The potential of gene expression profiling as a marker of treatment response in patients with IPF requires further study.**

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Acknowledgements

The INSTAGE trial was funded by Boehringer Ingelheim. Editorial and formatting assistance, supported financially by Boehringer Ingelheim, was provided by Elizabeth Ng and Wendy Morris of FleishmanHillard Fishburn, London, UK during preparation of this poster. The authors were fully responsible for all content and editorial decisions, were involved at all stages of poster development and have approved the final version. Boehringer Ingelheim was given the opportunity to review the poster for medical and scientific accuracy as well as intellectual property considerations. The authors received no direct compensation related to the development of this poster. Eric S White is now an employee of Boehringer Ingelheim Pharmaceuticals, Inc. Francesco Bonella has received honoraria and personal fees from Boehringer Ingelheim. Fernando J Martinez reports being on Steering Committees for ILD studies sponsored by Bayer, Biogen, Gilead, Nitto, Patara/Respivant, ProMedior, ProMetic, and Veracyte; a member of the Data and Safety Monitoring Board for studies supported by Biogen, Boehringer Ingelheim, and Genentech; and a consultant for Boehringer Ingelheim, Genentech, and Zambon.

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Poster developed for the American Thoracic Society International Conference, 2020.