Gene expression profiling in patients with idiopathic pulmonary fibrosis (IPF) in the INMARK® trial

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Introduction

- Nintedanib is an approved treatment for IPF, which reduces the rate of decline in forced vital capacity.[1]
- Nintedanib is an intracellular inhibitor of tyrosine kinases that has antifibrotic effects including inhibition of fibroblast proliferation, migration and differentiation and deposition of extracellular matrix.[2,3]
- The INMARK trial investigated the effect of nintedanib on blood biomarkers that may be associated with the progression of IPF.[4]

Aim

- To investigate changes in gene expression in subjects treated with nintedanib and placebo in the INMARK trial.

Methods

Trial design[4]

- Subjects with IPF and FVC ≥80% predicted were randomized 1:2 to receive nintedanib 150 mg bid or placebo for 12 weeks, followed by an open-label period during which all subjects received nintedanib for 40 weeks.

RNA sequencing

- Analyses were based on total RNA extracted from blood samples taken at baseline and week 12.
- 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 12 in the nintedanib and placebo groups:
  - Data were $\log_2$ transformed prior to analysis.
  - p-values were adjusted to control the false discovery rate (FDR) at 5%.
  - Changes in gene expression over 12 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 12).
- Gene set variation analysis assessed the relative enrichment of differentially expressed 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 INMARK trial*

	Nintedanib (n=116)	Placebo (n=230)
Age, years	70.5 (7.7)	70.2 (7.2)
Male	93 (80.2)	169 (73.5)
Body mass index, kg/m²	27.7 (4.3)	27.2 (4.1)
Race		
White	70 (60.3)	144 (62.6)
Asian	35 (30.2)	68 (29.6)
Missing*	11 (9.5)	18 (7.8)
Years since diagnosis of IPF	0.8 (0.8)	0.9 (1.0)
Former/current smoker	85 (73.3)	167 (72.6)
FVC % predicted	96.6 (15.2)	98.0 (12.6)
DLco % predicted†	60.9 (16.6)	65.5 (21.2)

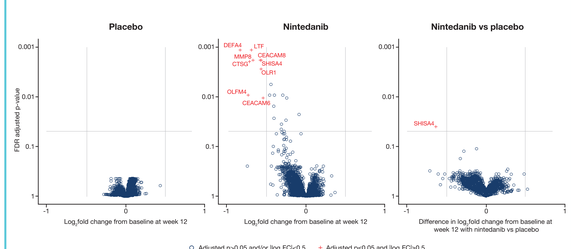
Data are n (%) or mean (SD). *Data on race were not collected in France due to local regulation. †Corrected for hemoglobin.

- Data from 327 subjects (110 randomized to nintedanib, 217 to placebo) were analyzed.
- Of 60,675 genes evaluated, 14,799 had counts per million ≥1 in at least half the samples from either treatment group at every time point and were included in the analysis.

Changes in gene expression

- In adjusted analyses, after 12 weeks of treatment:
  - Nine genes were downregulated in the nintedanib group while none was downregulated in the placebo group (Figure 1).
  - The change at week 12 was significantly different between nintedanib and placebo for one gene (SHISA4).
  - No genes were upregulated in either treatment group.
- In unadjusted analyses, the change in expression at week 12 was significantly different between nintedanib and placebo for five genes: SHISA4, LTF, CTSG, OLFM4, DEFA4 (Table 2).

Figure 1. *Changes in gene expression from baseline at week 12 (adjusted analyses)*



CEACAM6/8, carcinoembryonic antigen related cell adhesion molecule 6/8; SHISA4, shisa family member 4; OLR1, oxidized low density lipoprotein receptor 1; MMP8, matrix metalloproteinase 8; LTF, lactotransferrin; CTSG, cathepsin G; OLFM4, olfactomedin 4; DEFA4, defensin alpha 4.

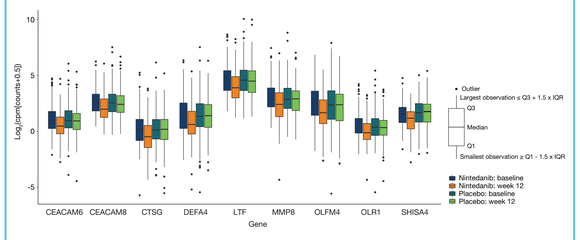
Table 2. *Nine genes downregulated at week 12 (with adjusted p≤0.05 and $|\log_2\text{fold change}|\geq 0.5$) in subjects treated with nintedanib*

Gene	Nintedanib	Placebo	Nintedanib vs placebo
	$\log_2$fold change from baseline at week 12 (adjusted / unadjusted p-value)		Difference in $\log_2$fold change from baseline at week 12 (adjusted / unadjusted p-value)
CEACAM6	**-0.55** (p=0.011 / p<0.001)	**-0.13** (p=0.89 / p=0.12)	**-0.41** (p=0.45 / p=0.006)
SHISA4	**-0.57** (p=0.002 / p<0.001)	**0.07** (p=0.93 / p=0.36)	**-0.64** (p=0.04 / p<0.001)
OLR1	**-0.57** (p=0.003 / p<0.001)	**-0.14** (p=0.88 / p=0.09)	**-0.43** (p=0.42 / p=0.003)
CEACAM8	**-0.58** (p=0.002 / p<0.001)	**-0.15** (p=0.84 / p=0.05)	**-0.43** (p=0.42 / p=0.002)
MMP8	**-0.67** (p=0.002 / p<0.001)	**-0.21** (p=0.73 / p=0.03)	**-0.47** (p=0.45 / p=0.004)
LTF	**-0.69** (p=0.001 / p<0.001)	**-0.17** (p=0.85 / p=0.06)	**-0.52** (p=0.37 / p=0.001)
CTSG	**-0.72** (p=0.002 / p<0.001)	**-0.11** (p=0.93 / p=0.27)	**-0.61** (p=0.37 / p<0.001)
OLFM4	**-0.74** (p=0.009 / p<0.001)	**-0.19** (p=0.88 / p=0.10)	**-0.55** (p=0.45 / p=0.006)
DEFA4	**-0.84** (p=0.001 / p<0.001)	**-0.12** (p=0.93 / p=0.24)	**-0.72** (p=0.32 / p<0.001)

Blue shading: $|\log_2\text{fold change}|\geq 0.5$ and adjusted p≤0.05.
Grey shading: $|\log_2\text{fold change}|\geq 0.5$ and unadjusted p≤0.05.

- Figure 2 shows the expression of the nine genes that were downregulated at week 12 (with adjusted p≤0.05 and $|\log_2\text{fold change}|\geq 0.5$) in subjects treated with nintedanib.

Figure 2. *Expression of the nine genes downregulated at week 12 in subjects treated with nintedanib*

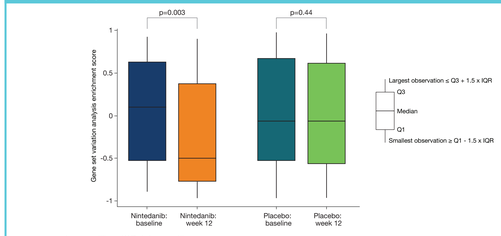


cpm, counts per million; IQR, interquartile range.

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.
- Between baseline and week 12, genes in this set were positively enriched compared with genes not in this set among subjects treated with nintedanib but not among subjects treated with placebo (Figure 3). Changes in enrichment scores between baseline and week 12 were significantly different between nintedanib and placebo (p=0.020).

Figure 3. *Gene set variation analysis enrichment scores for the set of nine genes downregulated at week 12 in subjects treated with nintedanib*

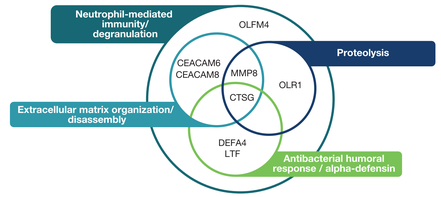


cpm, counts per million; IQR, interquartile range.

Pathways analyses

- Based on EnrichR, of the nine genes downregulated at week 12 in subjects treated with nintedanib, all except SHISA4 are known to be linked to neutrophil function, extracellular matrix organization and/or antibacterial/antiviral immunity (Figure 4). The EnrichR databases did not hold information on SHISA4.

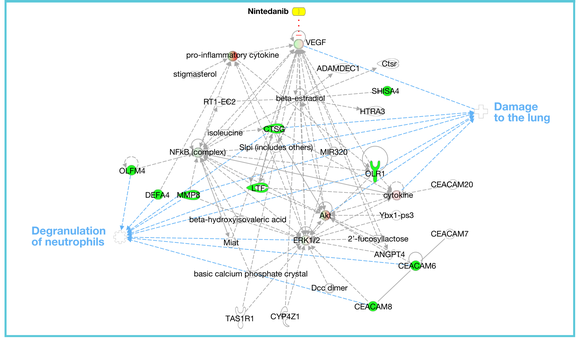
Figure 4. *Functional pathways linked to genes downregulated at week 12 in subjects treated with nintedanib based on EnrichR*



CEACAM6/8, carcinoembryonic antigen related cell adhesion molecule 6/8; CTSG, cathepsin G; DEFA4, defensin alpha 4; LTF, lactotransferrin; MMP8, matrix metalloproteinase 8; OLFM4, olfactomedin 4; OLR1, oxidized low density lipoprotein receptor 1.

- The network of these nine genes created using Ingenuity Pathway Analysis software showed enrichment of genes related to neutrophil degranulation and lung damage (Figure 5).

Figure 5. *Network of the set of nine genes downregulated at week 12 in subjects treated with nintedanib*



Conclusions

- **Genome-wide transcriptome profiling of data from the INMARK trial identified nine genes that were downregulated after 12 weeks of treatment with nintedanib in subjects with IPF and preserved lung function at baseline.**
- **Pathways analysis suggested that the downregulated genes are related to neutrophil function, extracellular matrix organization and antibacterial/antiviral immunity.**
- **The potential of gene expression profiling as a marker of treatment response in patients with IPF requires further study.**

References

1. Richeldi L et al. N Engl J Med 2014;370:2071–82.
2. Wollin L et al. Eur Respir J 2015;45:1434–45.
3. Wollin L et al. Eur Respir J 2019; doi: 10.1183/13993003.00161–2019.
4. Maher TM et al. Lancet Respir Med 2019;7:771–9.

Acknowledgements

The INMARK 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. Moisés Selman has served as a consultant for Boehringer Ingelheim. Toby M Maher reports grants and personal fees from GlaxoSmithKline and UCB, and personal fees from Apellis, Bayer, Biogen Idec, Blade, Boehringer Ingelheim, Bristol-Myers Squibb, Galapagos, Galecto, Indalo, Novartis, Respivent, Roche, and Trevi.

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