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Autotaxin Inhibitor GLPG1690 Affects TGFβ-induced Production of the Pro-Fibrotic Mediators CTGF, IL-6 and ET-1 in Fibroblasts

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Introduction

Autotaxin (ATX), a secreted lysophospholipase, plays a central role in the production of the bioactive lipid lysophosphatidic acid (LPA). LPA signals through multiple LPA receptors and via activation of the Rho family of small GTPases (see Fig. 1). LPA can activate the production of pro-fibrotic genes such as Endothelin-1 (ET-1), Interleukin-6 (IL-6) and Connective Tissue Growth Factor (CTGF) (Stortelers et al, 2008). GLPG1690 is a potent ATX inhibitor (see Fig. 2) that is currently being evaluated in an exploratory Phase IIa study for the treatment of IPF (FLORA; NCT02738801) and has proven anti-fibrotic activity in the preclinical lung bleomycin model. In this study the *in vitro* mode of action of GLPG1690 is further explored in a pro-fibrotic cellular model.

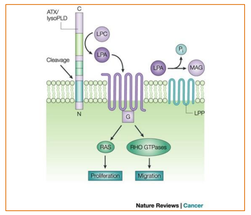

Fig. 1 Regulation of bioactive LPA; Mills & Moolenaar, 2003

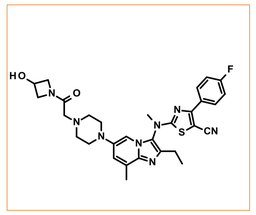

Fig. 2 Chemical structure of GLPG1690

Aims and Objectives

- Evaluate the effect of GLPG1690 on pro-fibrotic triggering by Transforming Growth Factor beta (TGFβ) in two different cellular models:
  - Normal Human Dermal Fibroblasts (NHDF cells)
  - Lung fibroblasts from an Idiopathic Pulmonary Fibrosis patient (IPF cells)
- Evaluate the activity of GLPG1690 and nintedanib on pro-fibrotic gene expression alone and in combination

Methods

- **D0**: NHDF or IPF cells were seeded in 96 well format (see Table 1 for details of seeding medium)
- **D1**: medium was refreshed to 1% charcoal stripped (CS) FBS medium for overnight incubation
- **D2**: cells were pre-treated with compound of interest and treated 1h later with TGFβ (see Table 1 for concentrations)
- **D3/D4**: Supernatant (SN) was harvested and frozen at -20° for later analysis
- **Dx**: SN was analyzed for levels of IL-6 and ET-1 by ELISA and for CTGF by MSD

Table 1: Experimental details regarding the culturing and the treatment of the cells

Matrix	Seeding medium	Medium change	TGFβ trigger	Incubation time
NHDF cells	FGM2 + 2% FBS	FGM2 +1% CS FBS	3 ng/ml	24h
IPF cells	DMEM + 10% FBS	DMEM +1% CS FBS	10 ng/ml	48h

Effect of nintedanib and GLPG1690 on TGFb induced IL-6, CTGF and ET-1

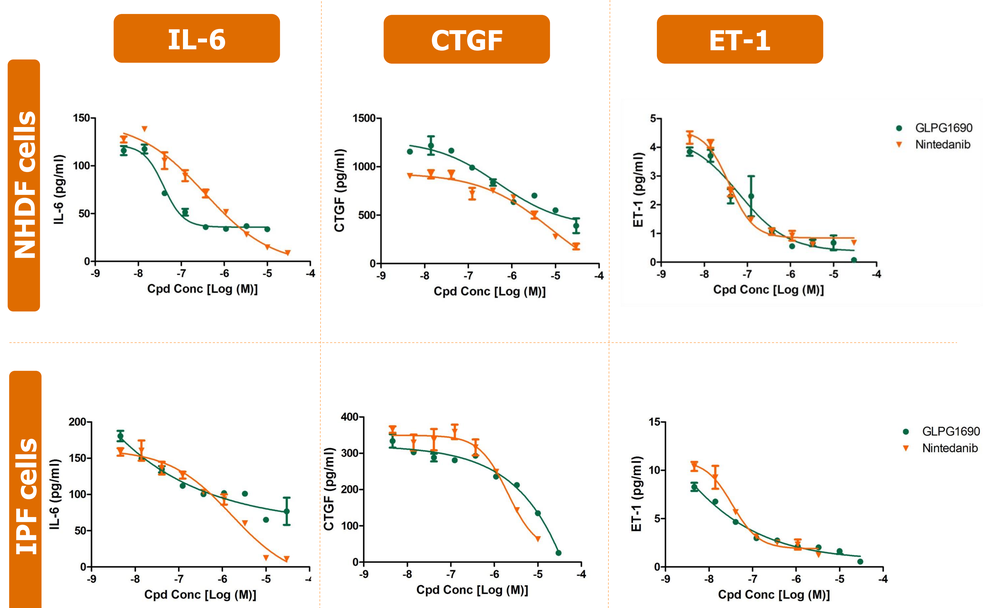

Fig. 3 Representative pictures of the dose-dependent effect of GLPG1690 and nintedanib on three pro-fibrotic read-outs

Combined effects of GLPG1690 and nintedanib

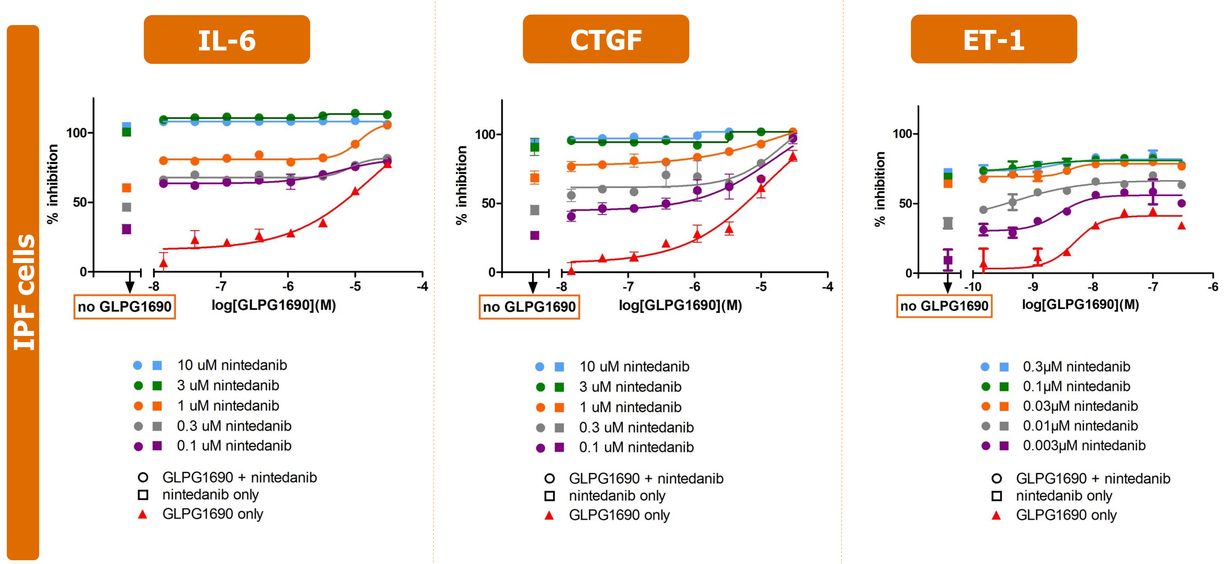

Fig. 4 Representative pictures of the combined effect of GLPG1690 and nintedanib on three pro-fibrotic read-outs

Conclusions

Both GLPG1690 and nintedanib, the current standard of care, dose-dependently reduce the production of several TGFβ induced pro-fibrotic mediators like ET-1, IL-6 and CTGF. The inhibitory effects can be seen in both dermal fibroblasts as well as IPF lung fibroblasts (Fig.3). Also pirfenidone dose-dependently inhibited the production of these cytokines (data not shown). GLPG1690 shows the most potent effect on the TGFβ induced ET-1 production with effects in the lower nM range. When suboptimal doses of GLPG1690 and nintedanib are combined, an additive inhibitory effect is seen on each of the three read-outs (Fig. 4). This suggests a promising outlook for combination therapy in the clinic.

References

Mills, G. B., & Moolenaar, W. H. (2003). The emerging role of lysophosphatidic acid in cancer. TL - 3. *Nature Reviews. Cancer*, *3 VN-re*(8), 582–591

Stortelers, C., Kerkhoven, R., & Moolenaar, W. H. (2008). Multiple actions of lysophosphatidic acid on fibroblasts revealed by transcriptional profiling. *BMC Genomics*, *9*, 387

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