BIO89-100, a novel glycoPEGylated Fibroblast Growth Factor 21 (FGF21) analog activates FGF receptors (FGFR) 1c, 2c and 3c but not FGFR4 in L6 cells transfected with the four different human FGFRs and beta-Klotho (KLB)

M. ROSENSTOCK[1], A. PIERCE[1], A. DI MARCO[2], G. AUCIELLO[2], A. BRESCIANI[2] H. MANSBACH[1] and M. MARGALIT[1]

189bio, Inc., Departments of Preclinical and Clinical Development, Herzliya, Israel and 2IRBM S.p.A., Via Pontina Km 30.600 - 00071 Pomezia, Italy. *Email: moti.rosenstock@89bio.com

INTRODUCTION

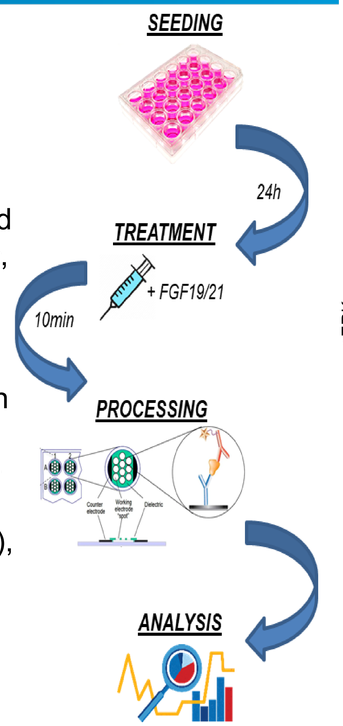
BIO89-100, a novel glycoPEGylated analog of FGF21, is being developed for the treatment of nonalcoholic steatohepatitis (NASH) and severe hypertriglyceridemia (SHTG). FGF21 acts through a cell surface receptor complex comprised of a tyrosine kinase FGF Receptor (FGFR) and the co-receptor, beta-Klotho (KLB). FGFR1c, 2c and 3c activation relate to the beneficial therapeutic effects of FGF21[1], while FGFR4 is expressed in the liver and is associated with bile acid metabolism, hepatocyte proliferation, and hepatocellular carcinoma (HCC)[2].

OBJECTIVES

The study objective was to test activation of the four FGFRs by recombinant human FGF21 (rhFGF21), BIO89-100, FGF19; as well as by FGF23 and EGF served as negative and positive controls, respectively, in L6 cell system transfected with and overexpressing with each of the 4 human FGFRs ± KLB.

METHODS

L6 rat skeletal muscle cells (ATCC CRL1458) were transduced to stably express human KLB, alone or co-expressed with each of the 4 human FGFRs (FGFR1c, 2c, 3c and FGFR4) by lentiviral-mediated transfection. Phosphorylation of extracellular signal regulated kinases (pERK) following ligand-mediated activation of FGFRs was measured using the Mesoscale Discovery platform. BIO89-100-induced activity was compared to the following: FGF21 (endogenous benchmark), FGF19, EGF (broad positive control), and FGF23 (negative control).



RESULTS

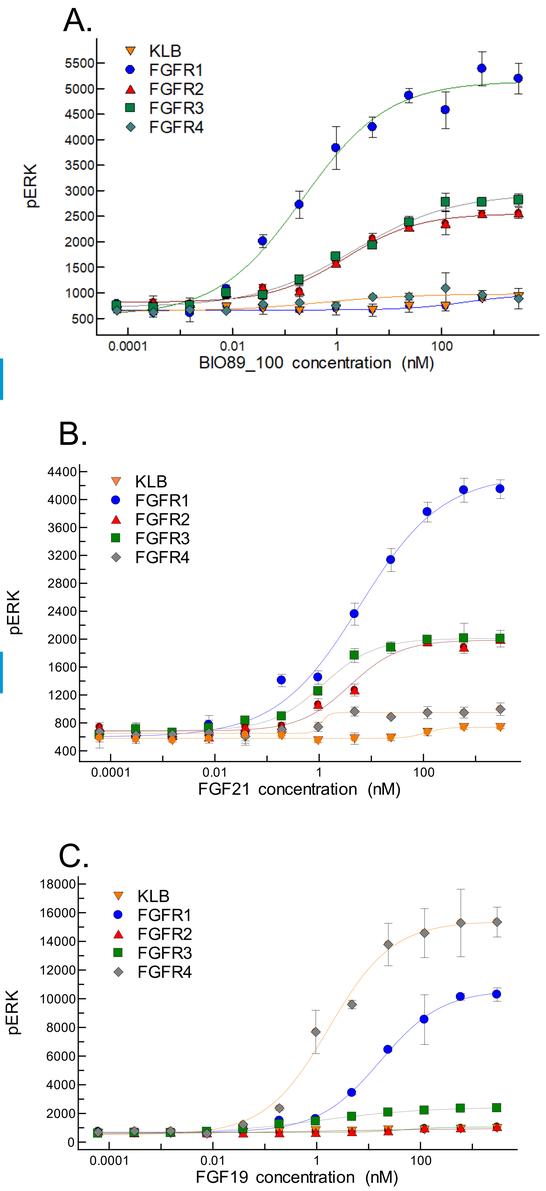


Figure 1: BIO89-100 induced pERK activity at low nanomolar concentrations in cells co-expressing KLB and FGFR1c, FGFR2c or FGF3c, but not FGFR4 (N=3 experiments; panel A). The potency of ERK activation with BIO89-100 was higher that rhFGF21 for KLB-FGFR1c, and comparable for KLB-FGFR2c and KLB-FGFR3c activation (N=3 experiments; panel B). FGF19 induced activation in KLB-FGFR1c and KLB-FGFR4 expressing cells (N=2 experiments, panel C).

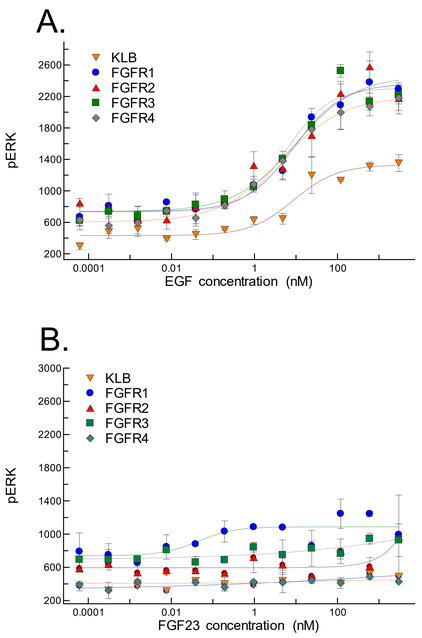


Figure 2: EGF, the positive control, activated each of the four receptors in the with the following EC_{50}/E_{max} values for FGFR1c, 2c, 3c, and 4, respectively: 8.8 ± 4.6/1331, 8.9 ± 2.9/2364, 9.8 ± 6.9/2461, and 4.6 ± 1.0/2198 (N=1 experiment, Panel A). while, FGF23, the negative control, had indeterminately low EC_{50} values for all 4 receptor types was not active in any of the cell lines (N=1 experiment, Panel B).

Table 1: pERK Activity Following FGF21 and BIO89-100

Receptor	FGF21 N = 3		BIO89-100 N = 3	
	EC_{50} (nM) Mean±SD	E_{max} (pERK)	EC_{50} (nM[b]) Mean±SD	E_{max} (pERK)
KLB	ND	726[a]	ND	1089[a]
KLB/FGFR1	4.5 ± 1.0	4277	0.6 ± 0.2	4966
KLB/FGFR2	4.5 ± 0.0	2058	2.2 ± 0.7	2471
KLB/FGFR3	1.8 ± 0.3	2214	2.6 ± 0.8	2770
KLB/FGFR4	ND	969[a]	ND	995[a]

EC_{50}=half maximal effective concentration; E_{max}=maximum effective concentration; FGFR=fibroblast growth factor receptor; KLB=co-receptor β-Klotho; ND=not determined; pERK=protein kinase R-like endoplasmic reticulum kinase; SD=standard deviation.
[a] Low E_{max} (<2-fold basal level)
[b] BIO89-100 weight used for the calculation did not include the 20kD PEG moiety.

- In cells co-expressing KLB and one of each of the receptors FGFR1c, FGFR2c, FGFR3c (but not with FGFR4), rhFGF21 and BIO89-100 induced pERK activity at low nanomolar concentrations.
- In cells expressing KLB alone and in cells co-expressing KLB + FGFR4, rhFGF21 and BIO89-100 were essentially inactive.
- BIO89-100 was more potent than rhFGF21 in cells expressing FGFR1c and was with comparable potency to rhFGF21 in in cells with FGFR2c or FGFR3c.
- FGF19 activated pERK in L6 cells expressing FGFR4 as well as in FGFR1c and FGFR3c.
- EGF, the positive control, activated each of the four receptors.
- FGF23, the negative control was not active in any of the cell lines.

CONCLUSIONS

BIO89-100 induced downstream signal activation in an FGF21-like fashion, with a stronger potency than rhFGF21 to FGFR1c.

Lack of activation of FGFR4 indicates that, in contrast to FGF19, BIO89-100 does not pose hepato-mitogenic risk via activation of this liver receptor2.

Clinical studies are underway to evaluate the safety and biological activity of BIO89-100 in patients with NAFLD/NASH and SHTG.

REFERENCES

1. Adams AC, Coskun T, Rovira AR, et al. PLoS One. 2012;7(5):e38438. doi:10.1371/journal.pone.0038438

2. Hatlen MA, Schmidt-Kittler O, Sherwin CA, et al. Cancer Discov. 2019;9(12):1686-1695. doi:10.1158/2159-8290.CD-19-0367