Agrawal, A1,2., Parlee, SD3, Perez-Tilve, D4, Li, P3, Pan, J3, Mroz, P.A1, Kruse Hansen5, A.M., Andersen, B5, Finan, B3, Kharitonenkov, A3, DiMarchi, R.D.1,2,3

Amino acid scanning identified a common structural basis for this binding despite only partial sequence interaction, and thus biological function. The 25-terminal amino acids of either FGF21 or 19 were determined.

• Produced largely by the liver and upregulated under states of metabolic stress, including fasting. FGF21 is necessary and sufficient within FGF2118-181 to bind KLβ.

• In line with our hypothesis when the enhanced KLB binding potency of 19C26,A26 was incorporated into a FGF21-based analogues based on 21C25,A164 and 21C25,A171 in vivo.

• The selective increase in peptide antagonism identified via Ala-Scan in 19C26,A26 was unexpected. Study of the modified analogs FGF19,A194 and FGF21-19A which used in the subsequent studies (A). Short 25 Amino acid C-terminal of FGF21 and 19 are fully sufficient to support interaction with KLβ.

• In subchronic studies in humans, FGF21-therapy demonstrated clinically meaningful reductions in body weight, insulin sensitivity and energy expenditure.

• Short 25 Amino acid C-terminal peptides are sufficient for KLβ binding and further provide a platform for optimization of the potency of peptide compared to its native counterpart. These regions are therefore thought to be the common functional elements of utmost importance to KLB binding.

• The selective increase in peptide antagonism identified via Ala-Scan in 19C26,A26 was unexpected. Study of the modified analogs FGF19,A194 and FGF21-19A which used in the subsequent studies (A). Short 25 Amino acid C-terminal of FGF21 and 19 are fully sufficient to support interaction with KLβ.

• Comparisons of calculated IC50s in the in vitro activity that was also pharmacologically superior when studied in vivo. This translation of peptide antagonism into super-agonist of the full-length protein is precedent setting.

• Overall our studies identified key regions of FGF21 and 19 that regulate KLβ binding and further provide proof of principal that by optimizing the C-terminus a more potent analog of FGF21 can be identified. What remains to be seen is whether this increase in potency can overcome limitations of previous clinical FGF21 compounds.

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**References**: For complete list of references please see Agrawal, A. et al. 148:774e781.

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