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MASH-TAG 2025

Pleiotropic effects of FGF21 in liver, brain, and adipose tissue

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Chief Scientific Officer

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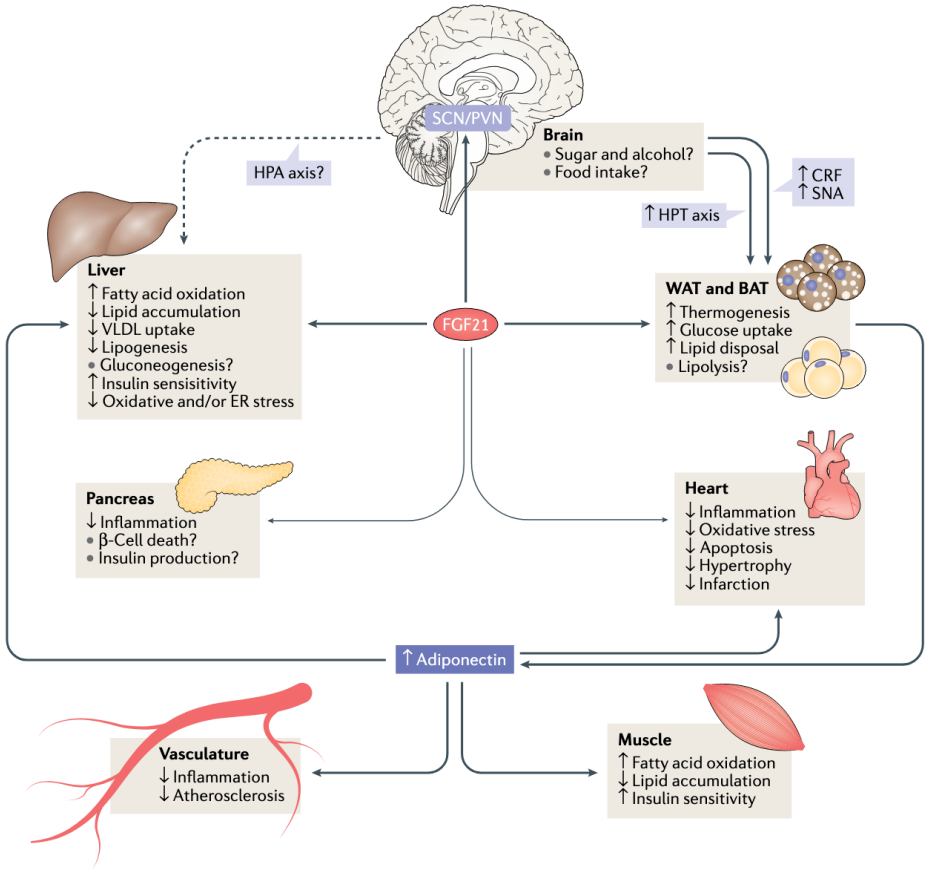
Disclosures

- Akero Therapeutics: co-founder, employee, shareholder
- BioAge Labs: consultant, shareholder

» FGF21 protects cells, organs, and the whole body during periods of stress



Adapted from L. Geng *et al.*, *Nat Rev Endocrinol.* **16**, 654–667 (2020).

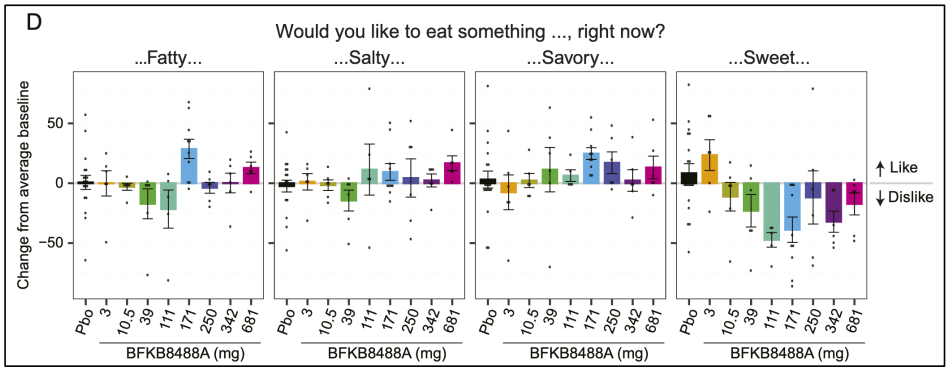
FGF21's potential to treat liver diseases, notably MASH, is supported by its role as an endogenous agent protecting liver against many types of stress

- Stressed hepatocytes are a major source of FGF21
- FGF21 acts on hepatocytes and other liver-resident cells (e.g. Kupffer cells, hepatic stellate cells) [***autocrine/paracrine***], and other tissues/organs [***endocrine***] to alleviate and repair liver injured by stress[1]
- Stressors that induce expression and secretion of FGF21 include:
  - Dietary imbalance, arising from excessive intake of alcohol[2], carbohydrates[3], or fructose[4], or from restriction of protein[5]
  - Exposure to hepatotoxins including acetaminophen[6] and nitrosamines[7]
- Protective actions of FGF21 to mitigate these stressors include:
  - Reducing alcohol[2] and sugar[3,4] consumption
  - Balancing macronutrient intake with whole body needs[3-5]
  - Redirecting excessive dietary energy from liver to adipose tissue by increasing peripheral insulin sensitivity[8]
  - Activating adaptive processes that ameliorate oxidative stress[6] and restore proteostasis via the integrated stress response pathway[9]

[1]E.J. Tillman and T. Rolph, *Front Endocrinol.* **11**, 601290 (2020).
[2]S. Søberg *et al.*, *Mol. Metab.* **11**, 96–103 (2018).
[3]S. Søberg *et al.*, *Cell Metab.* **25**, 1045-53 (2017).
[4]J.R. Dushay *et al.*, *Mol. Metab.* **4**, 51-7 (2015).
[5]Laeger *et al.*, *J. Clin. Investig.* **124**, 3913–22 (2014).
[6]D. Ye *et al.*,*Hepatology* **60**, 977-89 (2014).
[7]P. Xu *et al.*, *Toxicol Appl Pharm.* **290**, 43–53 (2016).
[8]J.P.G. Camporez *et al.*, *Endocrinology*. **154**, 3099–3109 (2013).
[9]S. Jiang *et al.*, *J Biol Chem.* **289**, 29751-65 (2014).

FGF21's potential to treat MASH is supported by phenotypes of humans carrying loss-of-function genetic variants

- Loss-of-function genetic variants in either FGF21 or its receptors (β-Klotho and FGFR1):
  - associate with:
    - obesity and insulin resistance[1]
    - altered preference for alcohol[2], sweet[3] or salty-tasting[4] foods
      - recapitulated by food preference survey from Genentech's BFKB8488A ph1b:
  - do not associate with[5]:
    - lower blood pressure
    - altered skeletal development
    - increased incidence of cancers



A. Baruch *et al.*, *Proc Natl Acad Sci.* **117**, 28992-29000 (2020).

[1]S. Stone *et al.*, *J Endocr Soc.* **4**, bvaa138 (2020). [2]G. Schumann *et al.*, *Proc National Acad Sci.* **113**, 14372-77 (2016). [3]S. Søberg *et al.*, *Cell Metab.* **25**, 1045-53 (2017).
[4]M. Saber-Ayad *et al.*, *J Adv Res.* **24**, 485–494 (2020). [5]T.M. Frayling *et al.*, *Cell Reports.* **23**, 327–336 (2018).

FGF21 signaling is tissue-restricted by β-Klotho expression and transduced by canonical FGFRs

- FGF21 has high affinity for β-Klotho as a cell-surface “trap”, but not for heparan sulfate
- FGF21 signals through a complex of β-Klotho with FGFR 1c/2c/3c, but not with FGFR4 (unlike FGF19)
- β-Klotho expression, unlike heparan sulfate, is tissue-restricted, conferring endocrine and paracrine actions of FGF21
- Co-localization of FGFR1c/2c/3c with β-Klotho required for FGF21’s intracellular signaling
- FGFR1c/2c/3c are receptor tyrosine kinases, in which dimerization and trans-autophosphorylation potentiate signaling

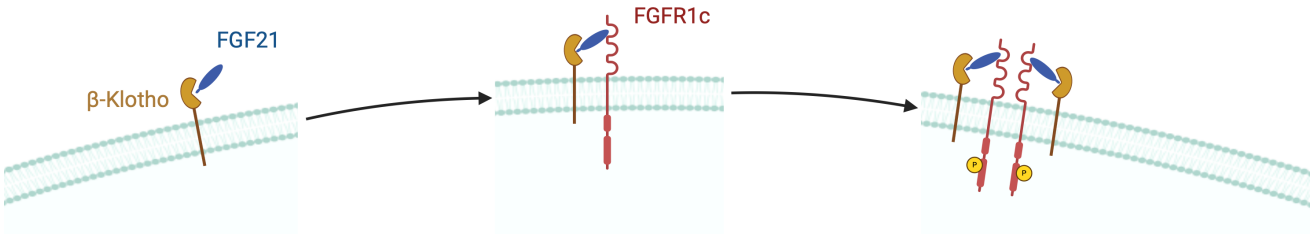
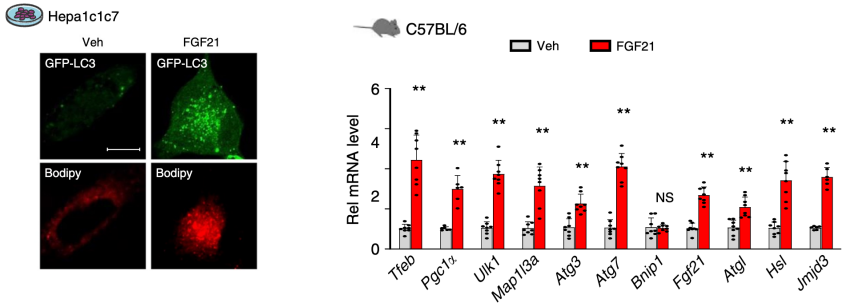


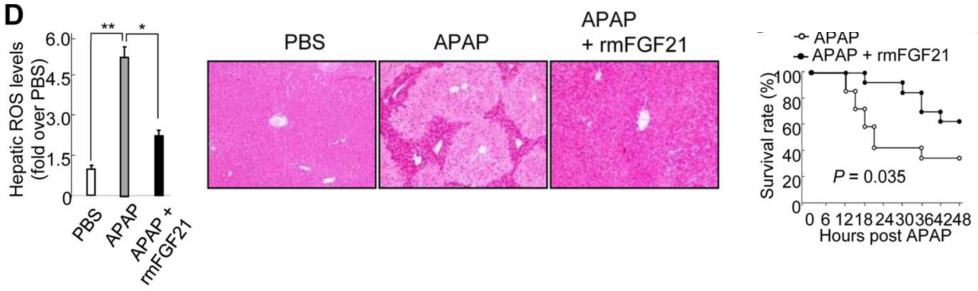
Image generated using BioRender.com

FGF21 enhances lipid breakdown and clearance of damaged proteins, and reduces liver injury in rodents caused by hepatotoxins

FGF21 induces hepatic autophagy and lipophagy *in vitro* and *in vivo*[1]



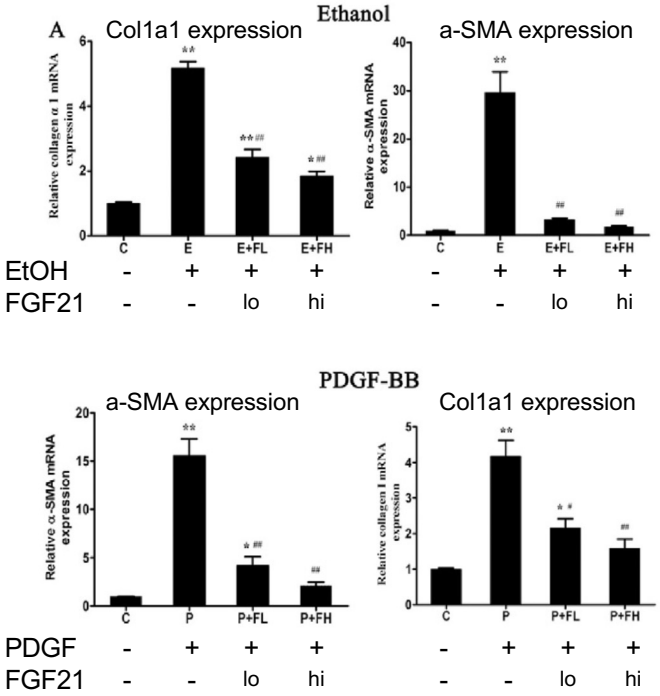
FGF21 reduces acetaminophen-induced oxidative stress, acute liver injury, and mortality *in vivo*[2]



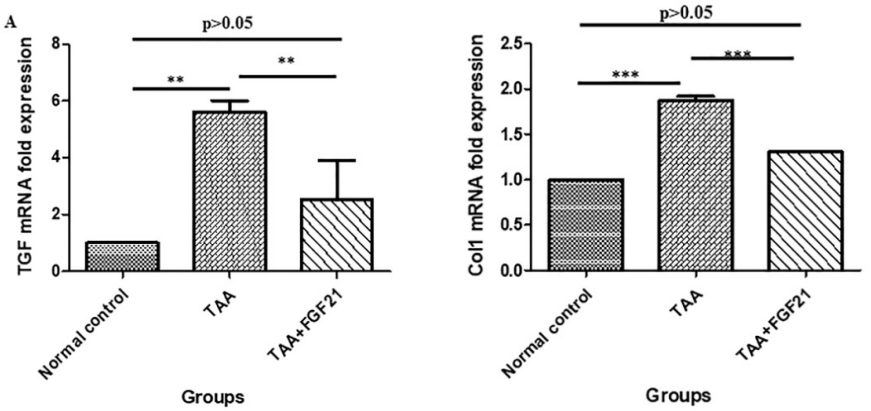
[1]S. Byun *et al.*, *Nat Commun*. **11**, 807 (2020). [2]D. Ye *et al.*, *Hepatology*. **60**, 977–989 (2014).

FGF21 exerts direct anti-fibrotic effects in liver of rodents

FGF21 suppresses HSC fibrogenic gene expression *in vitro*[1]



FGF21 suppresses fibrogenic gene expression in liver in mouse models of chemical (i.e., non-metabolic) liver injury and fibrosis[2]



[1]P. Xu *et al.*, *Toxicol Appl Pharm*. **290**, 43–53 (2016). [2]Y. K. Opoku *et al.*, *Excli J*. **19**, 567–581 (2020).

FGF21 ameliorates fibrosis across diverse organs, independent of any underlying metabolic driver

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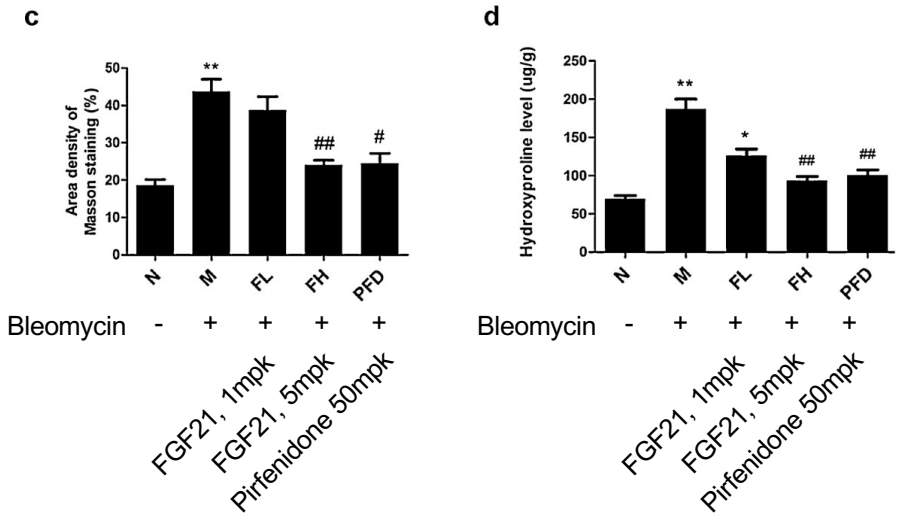
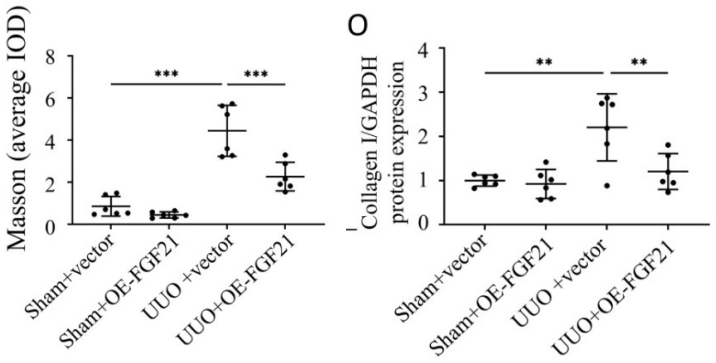
FGF21 reduces bleomycin-induced lung fibrosis *in vivo*[1]



FGF21 reduces unilateral uretral obstruction (UUO)-induced renal fibrosis *in vivo*[2]



[1]S. Zhang *et al.*, *Biomed Pharmacother.* **103**, 1516–1525 (2018). [2]W. Zhong *et al.*, *BBA - Mol. Cell Res.* **1871**, 119620 (2024)

FGF21 enhances adipose tissue capacity to store dietary glucose and lipid safely, and to secrete adiponectin

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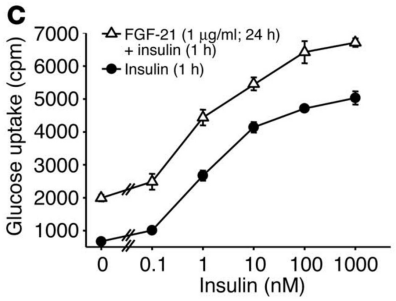
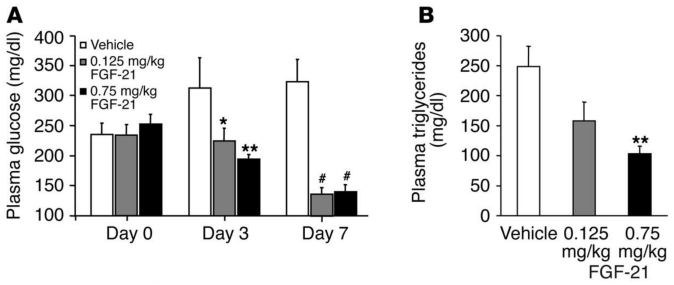
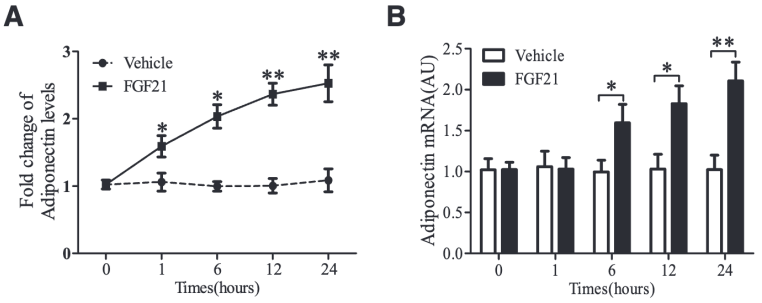
FGF21 enhances insulin-mediated adipocyte glucose uptake *in vitro*[1]



FGF21 reduces plasma glucose and TG *in vivo*[1]



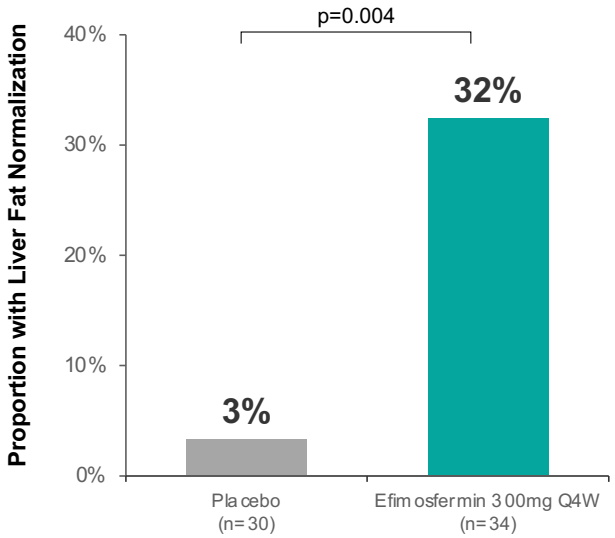
FGF21 induces adiponectin expression and secretion in adipocytes *in vitro*[2]



[1]A. Kharitonenkov *et al.*, *J Clin Invest*. **115**, 1627–1635 (2005). [2]Z. Lin *et al.*, *Cell Metab*. **17**, 779–789 (2013).

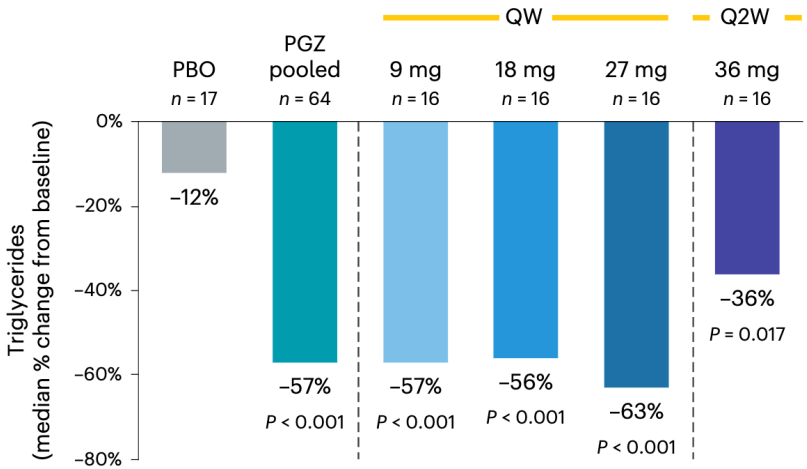
FGF21 analogs consistently improve metabolic health of patients with MASH or severe hypertriglyceridemia (SHTG)

FGF21 (efimosfermin) normalizes liver fat —**24 weeks treatment**



Noureddin *et al.*, *AASLD: The Liver Meeting, LB-5017* (2024).

FGF21 (pegozafermin) reduces TG in SHTG—**8 weeks treatment**

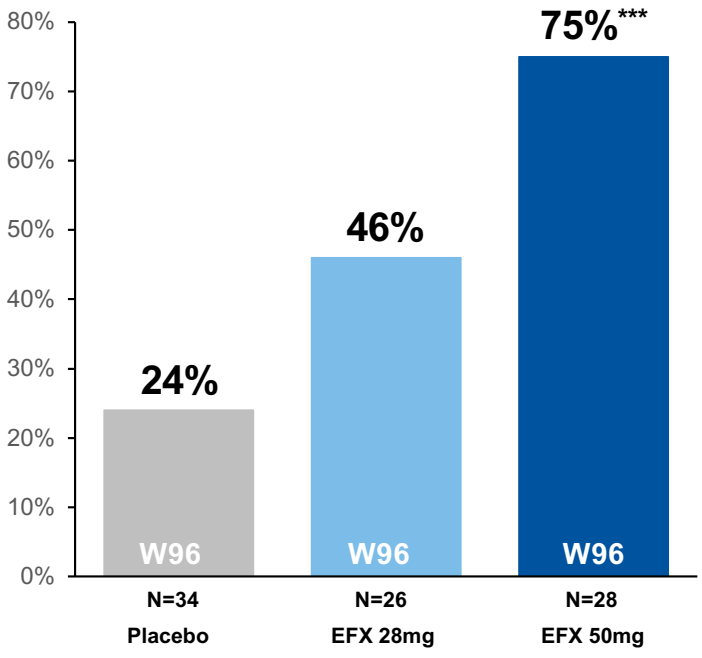


D. L. Bhatt *et al.*, *Nat. Med. 29*, 1782-92 (2023).

FGF21 analogs consistently improve liver histopathology and stiffness in patients with MASH

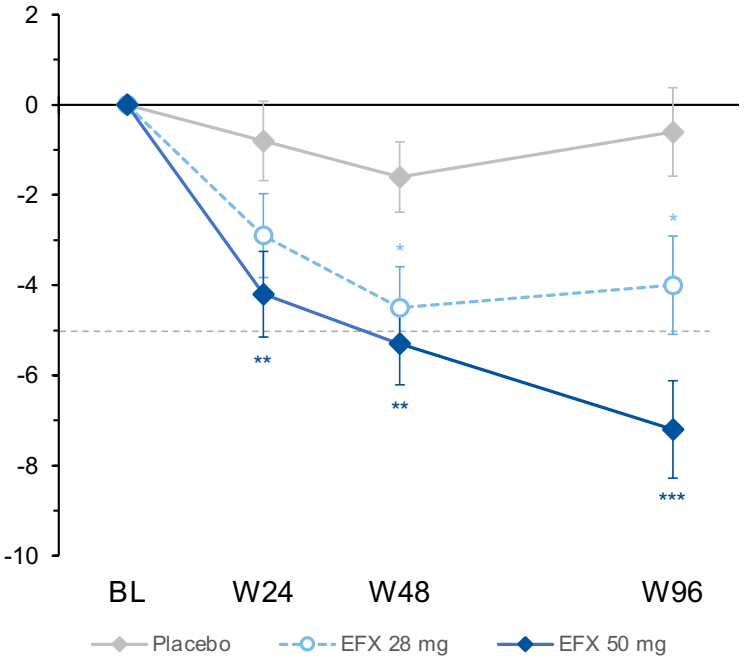
FGF21 (efruxifermin) reverses fibrosis and reduces liver stiffness—**96 weeks treatment**

Fibrosis Improvement ≥1 Stage & No Worsening of MASH[1]



[1] All participants with baseline and week 96 biopsy
*** p<0.001, versus placebo at W96 (Cochran-Mantel-Haenszel Test [CMH])

Change from Baseline in Liver Stiffness (VCTE), kPa

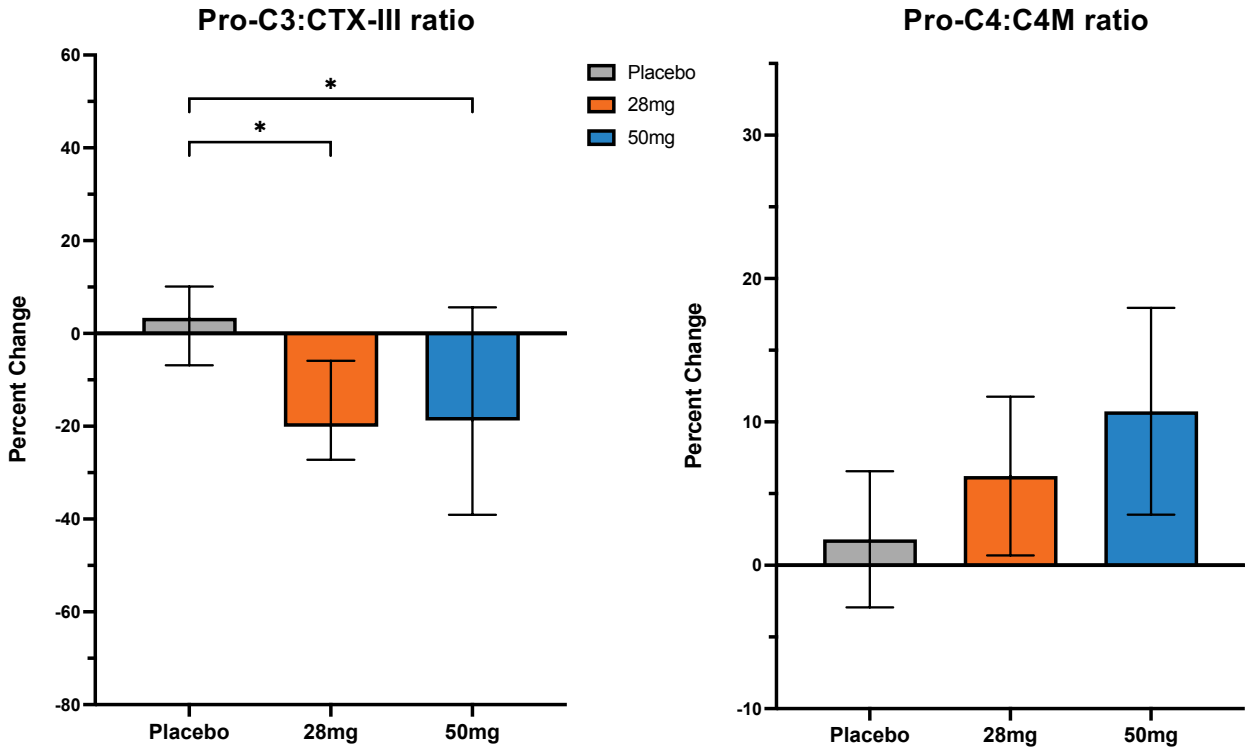


*p<0.05, **p<0.01, ***p<0.001 versus placebo (MMRM)

FGF21 analogs appear to selectively modulate ECM in patients with MASH:
Stimulating degradation of fibrillar collagen and regeneration of basement membrane collagen required for hepatocyte function

FGF21 (efruxifermin) modulates ECM—**24 weeks treatment**

Shift towards **degradation** of pathological, fibrillar type-3 collagen



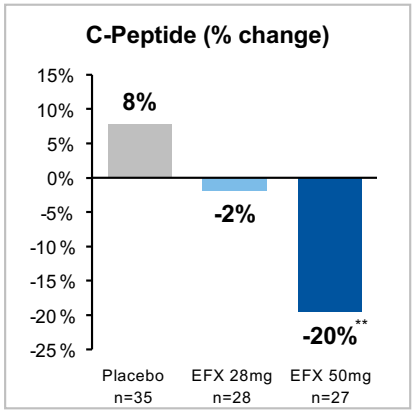
Shift towards **regeneration** of structural, network-forming type-4 collagen

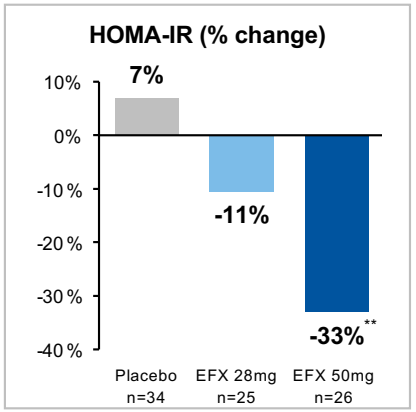
Tillman *et al.*, *Keystone Symposium: MASH and Fibrosis* (2024).

FGF21 analogs redirected energy away from liver to adipose tissue, without increasing body weight in patients with MASH

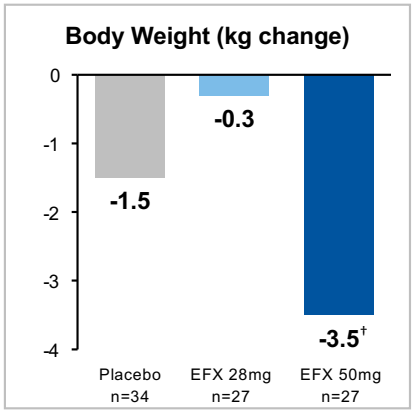
96 weeks treatment

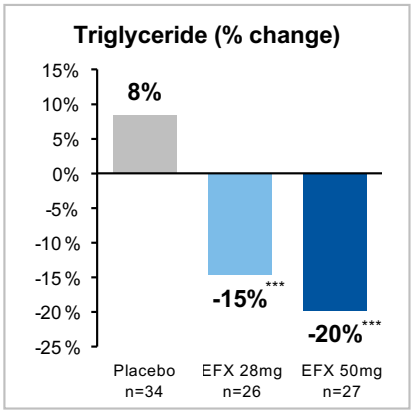
FGF21 (efruxifermin) increases insulin sensitivity





FGF21 (efruxifermin) reduces TG without increasing BW





* p<0.05, ** p<0.01, *** p<0.001, versus placebo (MMRM); † p<0.05, versus baseline (MMRM)

Source Data: FAS, non-missing values only, no imputation

MASH is an FGF21-resistant state: what are the implications for development and dosing of FGF21 analogs?

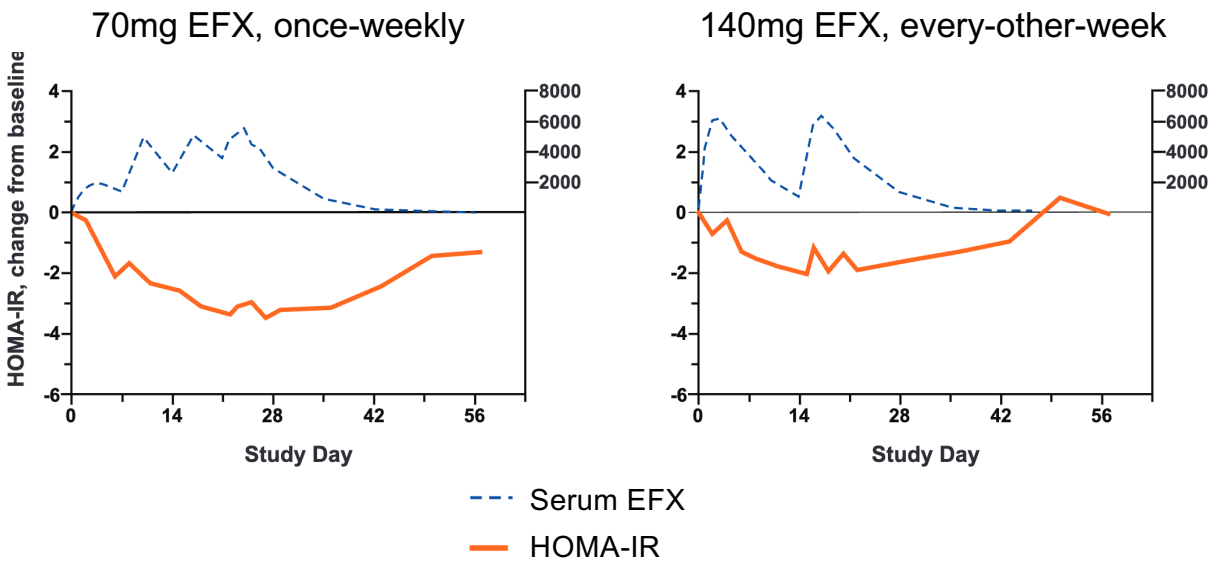
- Continued stress (chronic alcohol intake, obesity, T2D, MASH) appears to desensitize responsiveness to FGF21:
  - Desensitization of receptor tyrosine kinase signaling is an established phenomenon, e.g. insulin receptor in pre-diabetes and diabetes, but it can be “dosed through”
  - Increased serum FGF21[1-3] levels compared to healthy state, analogous to insulin levels in pre-diabetes
  - Decreased adipose *KLB* expression[4]
  - Efficacy of FGF21 analog, pegbelfermin, appeared to diminish with longer dosing[5,6]

How might efficacy of FGF21 analogs be maintained in the FGF21-resistant state?

[1]D. Barb *et al.*, *J Clin Endocrinol Metabolism*. **104**, 3327-36 (2019). [2]J. Dushay *et al.*, *Gastroenterology*. **139**, 456-63 (2010). [3]Y.C. Woo *et al.*, *Clin Endocrinol*. **86**, 37-43 (2017).
[4]f.M. Fisher *et al.*, *Diabetes*. **59**, 2781-9 (2010). [5]R. Loomba *et al.*, *Clin Gastroenterol Hepatol*. **22**, 102-12 (2024). [6]M.F. Abdelmalek *et al., Clin Gastroenterol Hepatol*. **22**, 113-23 (2024)

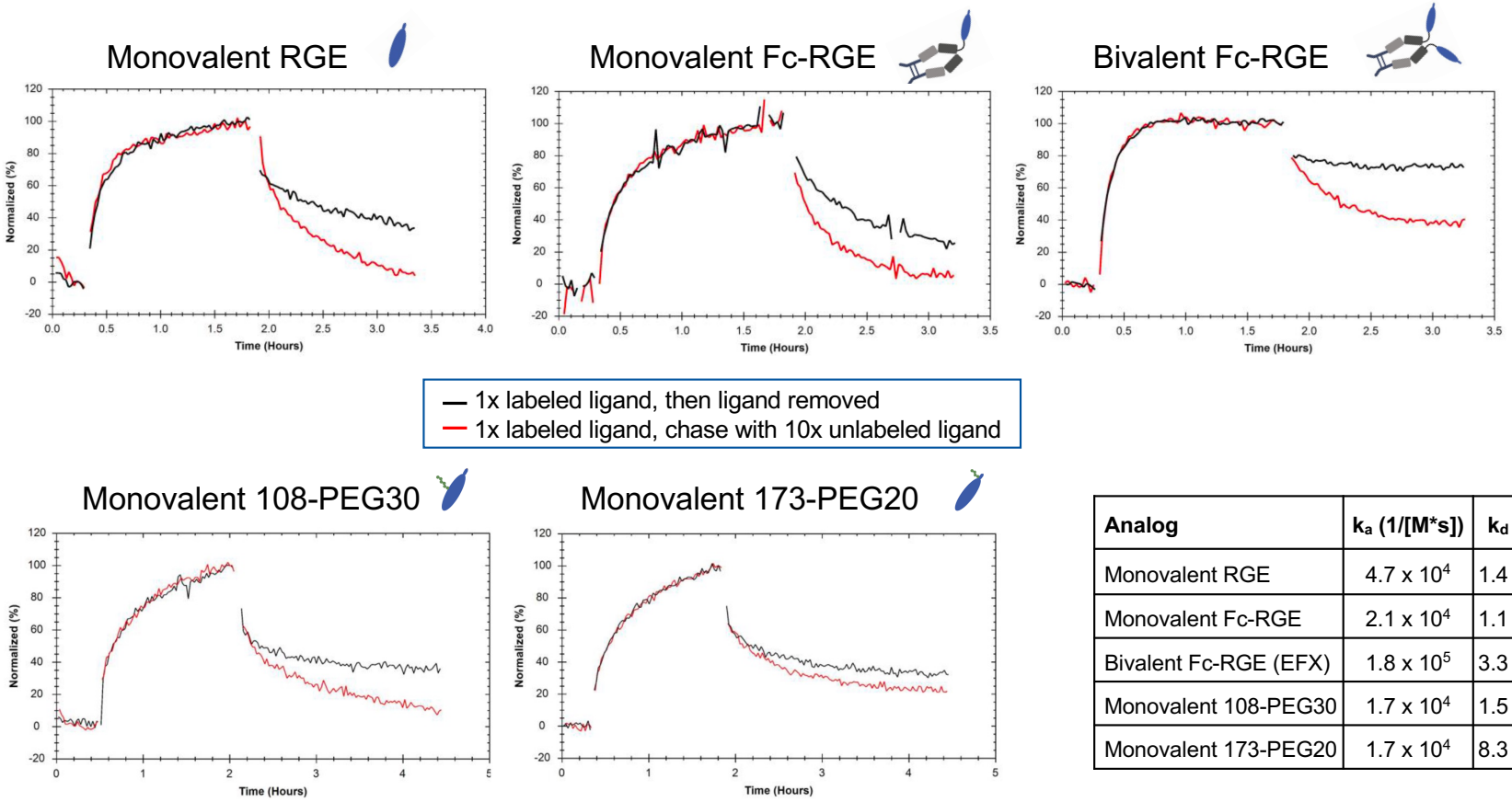
Comparable total exposures with different dosing schedules: Maintaining pharmacology across inter-dose interval appears critical for efficacy

4-week study in patients with T2D



Kaufman *et al.*, *Cell Rep Med*. **1**, 100057 (2020)

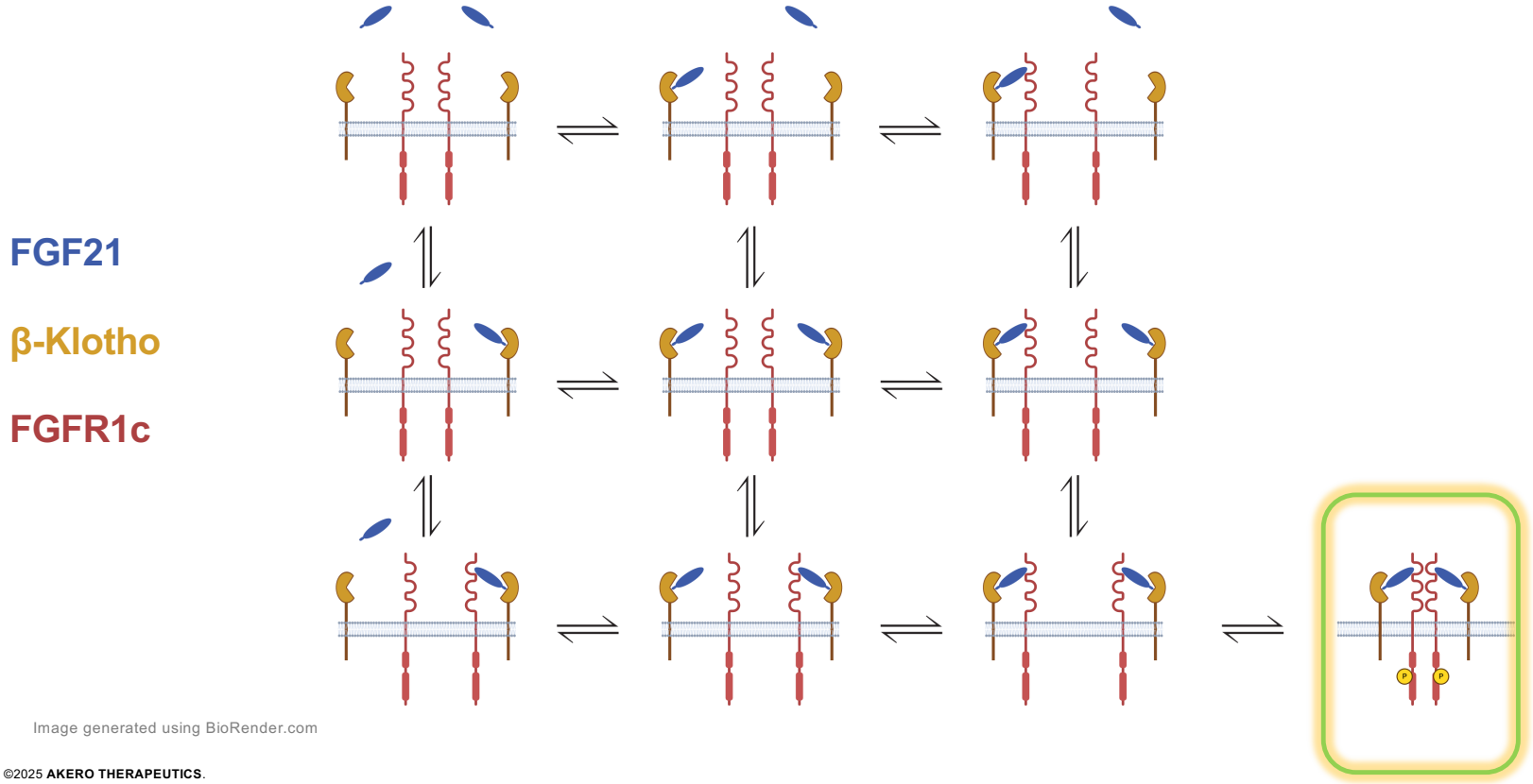
Sustained pharmacodynamic effect may be enabled through continued receptor engagement conferred by a bivalent structure



Analog	k_a (1/[M*s])	k_d (1/s)	K_D (nM)
Monovalent RGE	4.7×10^4	1.4×10^{-4}	3.0
Monovalent Fc-RGE	2.1×10^4	1.1×10^{-4}	5.4
Bivalent Fc-RGE (EFX)	1.8×10^5	3.3×10^{-6}	0.018
Monovalent 108-PEG30	1.7×10^4	1.5×10^{-4}	9.0
Monovalent 173-PEG20	1.7×10^4	8.3×10^{-5}	4.8

Tillman *et al.*, *The Liver Meeting, AASLD.* Poster 5051 (2022).

Independent nature of sequential binding of FGF21 to β-Klotho: β-Klotho expression limits native FGF21 or monovalent analog affinity



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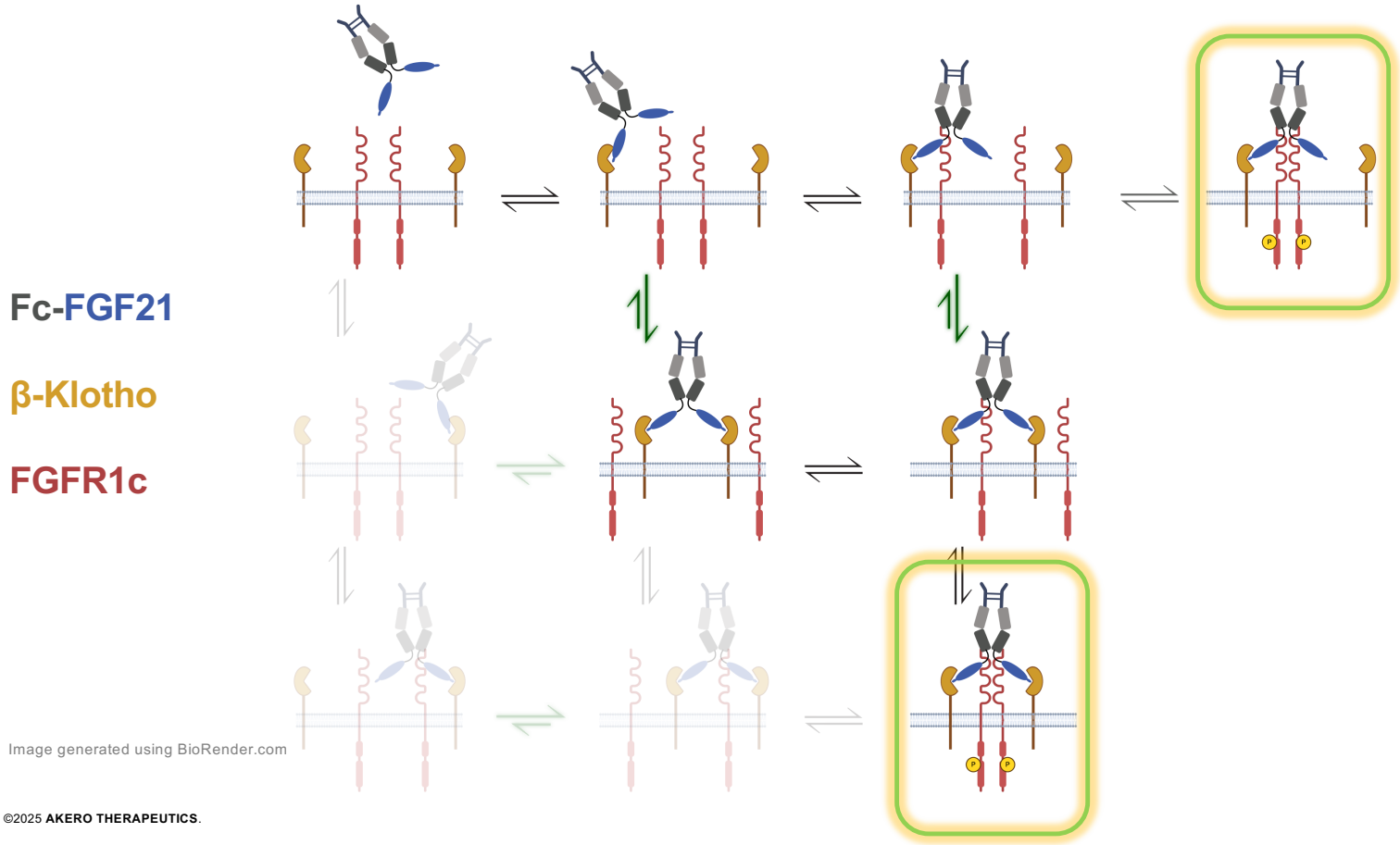
For bivalent FGF21 analogs, sequential β-Klotho and FGFR1c binding events are *not* independent, but rather facilitated by linkage through Fc



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Summary

- FGF21 analogs address both the underlying driver of MASH pathology as well as sustainably arrest or reverse development of liver fibrosis, with potential for:
  - Differentiated efficacy as monotherapy in F2-F3 fibrosis, with potential in F4c
  - Additional efficacy when used with resmetirom or semaglutide
- FGF21's antifibrotic effect appears mediated by two distinct actions:
  - Indirectly, by reducing the underlying profibrotic state of liver fat, lipotoxicity, and injury
  - Directly, by inhibiting synthesis of collagen by liver resident myofibroblasts, while maintaining a natural rate of collagen degradation
- Antifibrotic effects appear quite specific, in reducing pathological fibrillar collagen, while preserving or restoring basement membrane collagen critical for hepatocyte function
- Sustained improvements in overall metabolic health of patients underpins the efficacy of FGF21 analogs in reducing liver injury

Acknowledgments

- Amgen, Inc
- Ridgeview Instruments