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Supplemental Information

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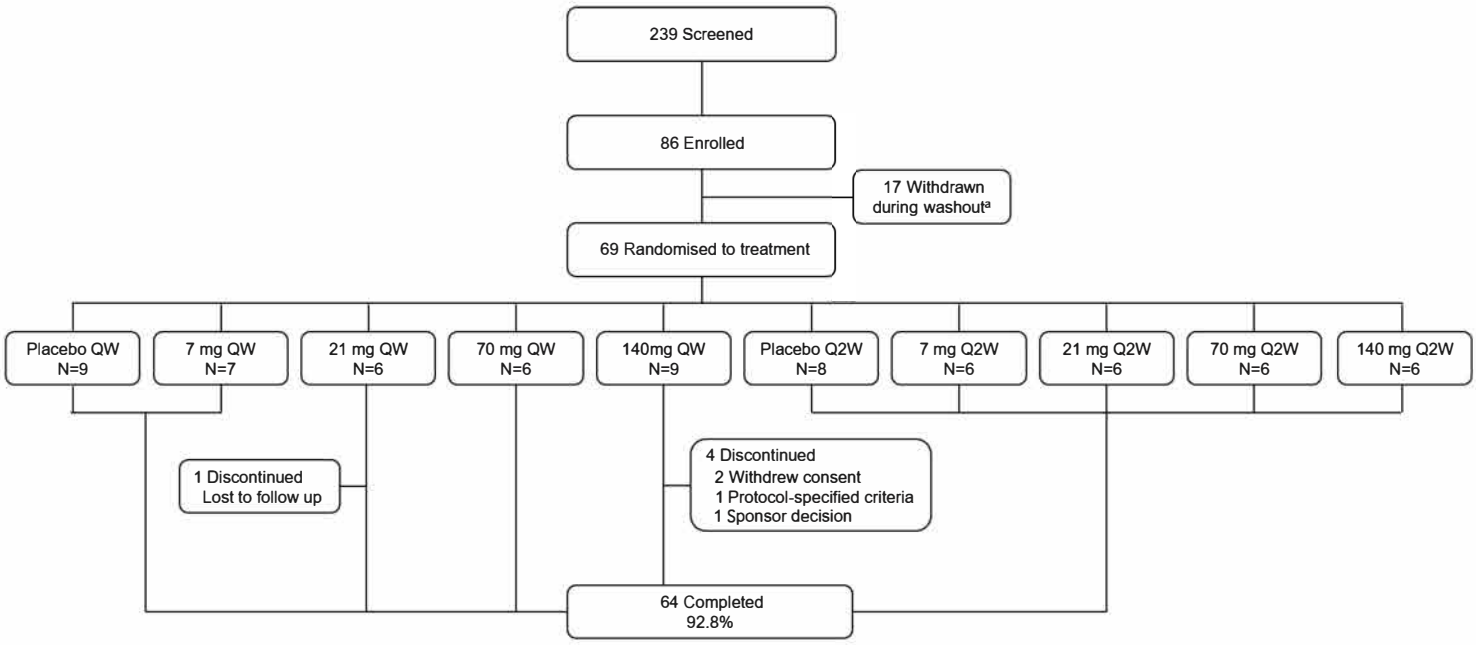

[a]Protocol-specified instructions to withdraw subjects who did not tolerate withdrawal of type 2 diabetes medications

Figure S1. Clinical Study Flow Diagram. Related to Figure 1.

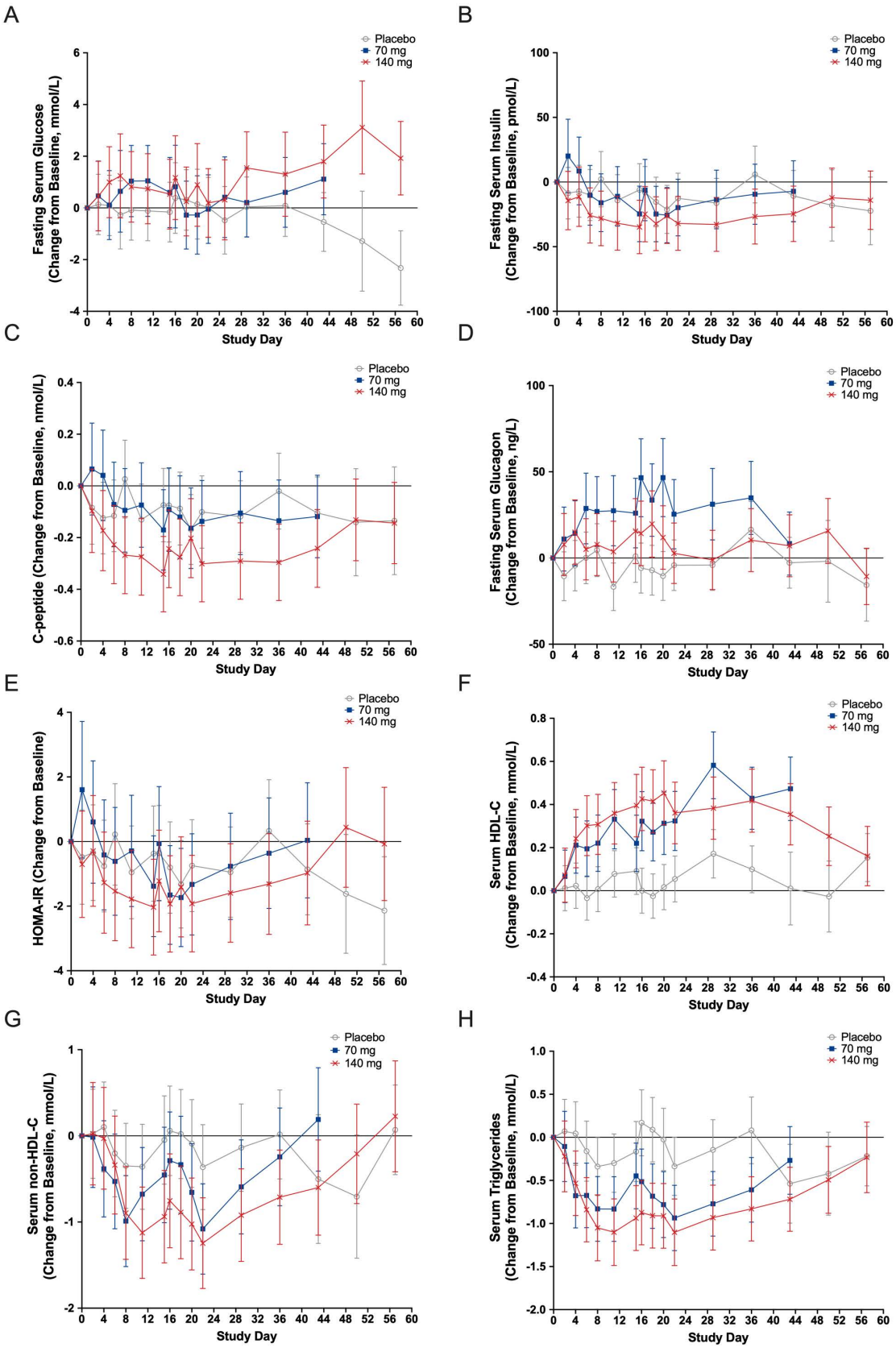

Figure S2. Effect of AKR-001 on markers of glycemic control and lipid metabolism. Related to Figure 3.

Concentration change from baseline of fasted state (A) glucose, (B) insulin, (C) C-peptide, (D) glucagon, (E) HOMA-IR, (F) HDL-cholesterol, (G) non-HDL-cholesterol, (H) triglycerides by study day for placebo (N=8), 70 mg (N=6), and 140 mg (N=6) Q2W dose cohorts. Data are presented as least-squares mean ± 95% confidence interval

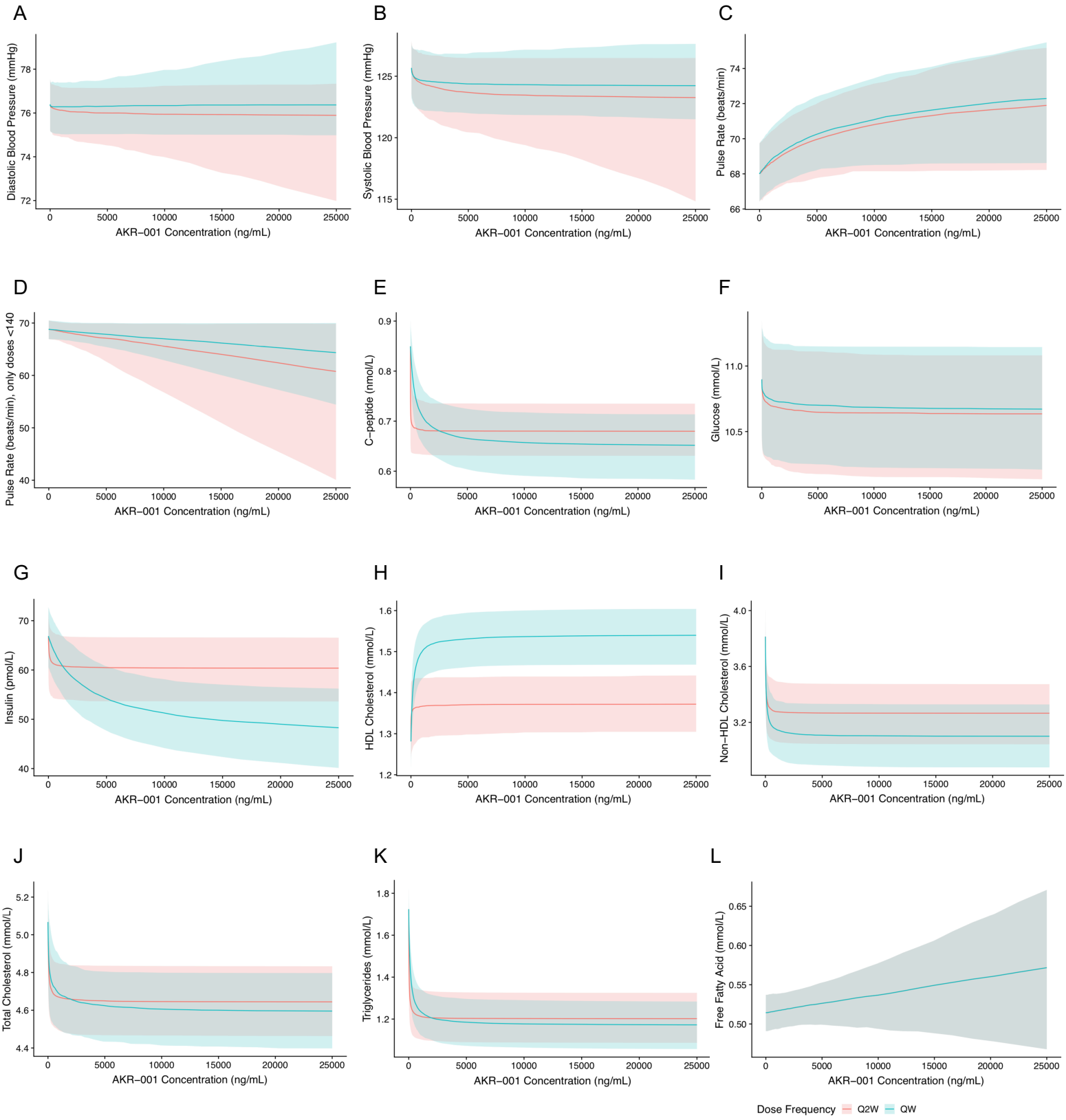

Figure S3. Model-predicted effect of AKR-001 on cardiovascular parameters and markers of glycemic control and lipid metabolism after overnight fast. Related to Table 3.

Model-predicted (A) diastolic blood pressure, (B) systolic blood pressure, (C) pulse rate across all dose groups, (D) pulse rate across 7–70 mg dose groups only, (E) C-peptide, (F) glucose, (G) insulin, (H) HDL-C, (I) non-HDL-C, (J) total cholesterol, (K) triglyceride, (L) free fatty acid, over the range of serum concentrations of AKR-001 at all timepoint measured following administration of 7–140 mg QW and Q2W doses. Shading indicates 95% confidence interval. Blue lines and shading correspond to QW dose groups, red lines and shading correspond to Q2W dose groups.

Table S1. Least-squares mean (95% confidence interval) placebo-corrected percentage change from baseline $AUC_{0\text{-}4hr}$ of metabolic markers following a mixed meal tolerance test (MMTT) on Day 18 (Q2W groups) or Day 25 (QW groups). Related to Table 3.

Treatment group	AKR-001 QW				AKR-001 Q2W			
	7 mg (N=7)	21 mg (N=6)	70 mg (N=6)	140mg (N=5)	7 mg (N=6)	21 mg (N=6)	70 mg (N=6)	140 mg (N=6)
Glucose	-3 (-20, 18)	6 (-13, 28)	-29 (-42, -14)	-30 (-42, -15)	-6 (-23, 15)	14 (-7, 39)	-10 (-26, 10)	-6 (-23, 14)
Insulin	-28 (-44, -8)	-26 (-43, -6)	-24 (-41, -3)	-49 (-60, -35)	-14 (-33, 11)	0 (-22, 29)	-23 (-40, -1)	-28 (-44, -7)
C-peptide	-14 (-27, 1)	-17 (-30, -3)	-15 (-28, 0)	-37 (-46, -26)	2 (-13, 21)	6 (-11, 25)	-7 (-22, 9)	-17 (-30, -2)
Glucagon	34 (14, 58)	3 (-13, 22)	11 (-6, 32)	22 (3, 44)	12 (-6, 32)	-10 (-24, 7)	32 (11, 57)	4 (-13, 23)
FFA	2 (-23, 36)	-10 (-33, 20)	-31 (-49, -8)	-19 (-39, 7)	-18 (-39, 10)	21 (-10, 62)	-4 (-28, 29)	31 (-3, 75)

$AUC_{0\text{-}4hr}$, area under the concentration-time curve in the four hours post-MMTT; QW, once weekly; Q2W, once every two weeks; N, number of subjects per group; FFA, free fatty acids

Table S2. Least-squares mean (95% confidence interval) placebo-corrected percentage change from baseline of *post hoc* biomarkers on Day 29. Related to Table 3.

Treatment group	QW				Q2W			
	7 mg (N=6)	21 mg (N=6)	70 mg (N=6)	140mg (N=5)	7 mg (N=6)	21 mg (N=6)	70 mg (N=6)	140 mg (N=6)
Adiponectin	42 (-13, 131)	62 (-3, 172)	94 (18, 220)	143 (48, 300)	60 (-3, 165)	73 (5, 187)	65 (0, 174)	141 (45, 298)
apoA-1	21 (5, 39)	15 (0, 33)	-7 (-20, 7)	16 (0, 33)	-6 (-19, 10)	0 (-14, 16)	16 (0, 35)	ND
apoB	0 (-19, 23)	-16 (-32, 4)	-42 (-53, -28)	-18 (-34, 1)	-12 (-30, 10)	-15 (-32, 6)	-11 (-29, 11)	ND
IGF-1	26 (-3, 63)	30 (0, 70)	31 (1, 70)	24 (-4, 61)	13 (-13, 48)	7 (-18, 40)	35 (3, 75)	-2 (-25, 29)
FGF19	-30 (-71, 69)	7 (-56, 164)	-8 (-63, 126)	29 (-47, 214)	-61 (-84, -3)	-43 (-77, 41)	-67 (-87, -19)	-75 (-90, -37)
C4	276 (69, 737)	54 (-32, 248)	36 (-40, 206)	47 (-34, 230)	123 (2, 406)	72 (-24, 291)	116 (-5, 391)	56 (-31, 255)
ANGPTL4	-18 (-36, 4)	-30 (-45, -11)	-13 (-32, 11)	-9 (-29, 16)	ND	ND	ND	ND
apoC-2	-48 (-73, -3)	-54 (-75, -14)	-55 (-76, -14)	-56 (-76, -17)	ND	ND	ND	ND
apoC-3	-24 (-49, 14)	-24 (-50, 13)	-46 (-64, -19)	-46 (-64, -19)	ND	ND	ND	ND
Lp(a)	-4 (-30, 33)	-1 (-29, 37)	9 (-22, 50)	11 (-20, 54)	ND	ND	ND	ND

QW, once weekly; Q2W, once every two weeks; N, number of subjects per group; apoA-1, apolipoprotein A; apoB, apolipoprotein B; ND, not determined (insufficient sample numbers); IGF-1, insulin-like growth factor 1; FGF19, Fibroblast Growth Factor-19; C4, 7α-hydroxy-4-cholesten-3-one; ANGPTL4, Angiopoietin-like 4; apoC-2, apolipoprotein C-2; apoC-3, apolipoprotein C-3; Lp(a), Lipoprotein(a)

Table S3. Least-squares mean (95% confidence interval) change from baseline of blood pressure (BP) and pulse rate on Day 18 (Q2W groups) or Day 25 (QW groups). Related to Table 4.

Treatment group	QW					Q2W				
	Placebo (N=8)	7 mg (N=6)	21 mg (N=6)	70 mg (N=6)	140mg (N=5)	Placebo (N=8)	7 mg (N=6)	21 mg (N=6)	70 mg (N=6)	140 mg (N=6)
Systolic BP, mm Hg	-7 (-15, 1)	-3 (-12, 7)	4 (-6, 13)	-8 (-18, 1)	3 (-7, 12)	0 (-9, 8)	0 (-10, 10)	-2 (-11, 8)	-4 (-14, 6)	0 (-10, 10)
Diastolic BP, mm Hg	-2 (-7, 2)	-1 (-6, 5)	-2 (-7, 4)	-6 (-12, -1)	1 (-4, 7)	0 (-5, 4)	0 (-6, 5)	4 (-2, 10)	-2 (-7, 4)	3 (-3, 8)
Pulse rate, bpm	5 (0, 10)	4 (-1, 10)	5 (0, 11)	1 (-4, 7)	7 (2, 13)	-1 (-6, 4)	5 (-1, 11)	1 (-4, 7)	5 (0, 10)	13 (7, 19)

QW, once weekly; Q2W, once every two weeks; N, number of subjects per group; mm Hg, millimeters mercury; bpm, beats per minute

Table S4. Treatment-emergent adverse events by Preferred Term reported in no more than 1 subject in both pooled placebo and pooled all-dose AKR-001 (7–140 mg, QW and Q2W) treatment group. Related to Table 3.

Subjects reporting TEAEs*, n(%)	Placebo QW/Q2W	AKR-001 QW				AKR-001 Q2W				AKR-001 Pooled QW/Q2W	
	(N=17)	7 mg (N=7)	21 mg (N=6)	70 mg (N=6)	140 mg (N=9)	7 mg (N=6)	21 mg (N=6)	70 mg (N=6)	140 mg (N=6)	≤70 mg (N=37)	7–140 mg (N=52)
Abdominal distension	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)
Abdominal pain	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Abdominal tenderness	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)
Anxiety	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Cardiac murmur	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (2.7)	1 (1.9)
Chest pain	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Chills	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (2.7)	1 (1.9)
Cyst	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Decreased appetite	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)
Dermatitis contact	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (1.9)
Dizziness	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)
Epigastric discomfort	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Epistaxis	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)
Eye irritation	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Flushing	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Hot flush	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Hunger	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Hyperglycemia	1 (5.9)	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Hyperhidrosis	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)
Hypoglycemia	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Influenza-like illness	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (1.9)
Injection site bruising	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)
Insomnia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (2.7)	1 (1.9)
Musculoskeletal pain	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Nephrolithiasis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (2.7)	1 (1.9)
Non-cardiac chest pain	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)

Oropharyngeal pain	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Pain	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Parotid gland enlargement	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (2.7)	1 (1.9)
Pruritis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Rash	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (2.7)	1 (1.9)
Sinus headache	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Tachycardia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0.0)	1 (1.9)
Toothache	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	1 (1.9)
Urine output increased	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (2.7)	1 (1.9)
Ventricular extrasystoles	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)
Viral upper respiratory tract infection	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (1.9)
Cough	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dermal cyst	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dysesthesia	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Injection site discoloration	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Injection site hemorrhage	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Myalgia	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oral herpes	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Vertigo positional	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*TEAE by Preferred Term. Coded using Medical Dictionary for Regulatory Activities version 17.0

QW, once weekly; Q2W, once every two weeks; N, number of subjects per group; TEAEs, treatment-emergent adverse events

Table S5. Cross-trial comparison of multiple-dose clinical data of endocrine FGF analogs or mimetics. Related to Figure 2.

Placebo-corrected change from baseline	AKR-001 4 weeks T2D 70mg QW Ph1b, n=6	PF-05231023 25 days obese T2D 100mg BIW Ph1b, n=10	LY2405319 4 weeks obese T2D 20mg QD Ph1b, n=13	Pegbelfermin 16 weeks NASH 20mg QW Ph2a, n=24	Aldafermin 12 weeks NASH 1mg QD[1] Ph2a, n=27	BFKB8488A 12 weeks NAFLD 100mg Q2W Ph1b, n=8
Dose presented	Highest dose being tested in Ph2a	Lowest dose with max. effect	Highest dose tested	Dose selected for further development	Dose selected for further development	Highest dose tolerated in Ph1b
Current stage	Ph2a	discontinued	discontinued	Ph2b	Ph2b	Ph2b
Molecule	Fc-FGF21(RGE)	IgG-FGF21	FGF21 variant, glycosylated	PEG-FGF21	FGF19 variant (+rosuvastatin)	FGFR1c/KLB bispecific mAb
Molecular Weight	~92 kDa	~190 kDa	~20kDa	~50 kDa	~20 kDa	~150 kDa
T1/2 (half-life)	3-3.5 days	7.5-8.5 hours	Short (Dosed QD)	1.5-2 days	Short (Dosed QD)	3-10 days
Body weight	No effect	-4%	-1.5%	No effect	NR	NR
Triglycerides	-69%	-50%	-47%	-8%	-25%	-20%
HDL-C	+61%	+25%	+18%	+14%	0	+20%
Non-HDL-C	-30%	NR	NR	NR	NR	NR
LDL-C	-13%	-15%	-20%	-2%	+55%	NR
Apolipoprotein B	-42%	NR	-27%	NR	NR	NR
Apolipoprotein C3	-46%	NR	-33%	NR	NR	NR
Fasting Insulin	-49%	-5%	-36%	NR	NR	NR
Fasting Glucose	-23%	-5%	-8%	NR	NR	NR
Adiponectin	+94%	+2000%	+72%	+19.2%	NR	+30%
LFC, rel. reduction	NR	NR	NR	-20%	-57%	-35%
Heart Rate	No effect	Increased	NR	No effect	NR	NR
Blood Pressure	No effect	Increased	NR	No effect	NR	NR
Bone turnover	No effect	Increased	NR	No effect	NR	NR
Adverse events	Diarrhea, Nausea	Increased HR, BP	Hypersensitivity	Diarrhea, Nausea	Diarrhea, Nausea	Nausea, Diarrhea
Source	Present study	Talukdar *et al,* 2016 Kim *et al,* 2017	Gaich *et al,* 2013	Sanyal *et al,* 2019	Harrison *et al*, 2018 AASLD	Kunder *et al,* 2019 AASLD

[1] aldafermin 1 mg data is not corrected for placebo, as the trial did not contain a placebo control arm.

T2D, type 2 diabetes; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease; QW, once weekly; QD, once daily; BIW, twice weekly; Q2W, once every two weeks; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LFC, liver fat content; NR, not reported. Data are based on cross-trial comparisons with different populations, study duration, and trial design in the absence of head-to-head trials.