Supplementary Information for

Antibody-mediated activation of the FGFR1/Klothoβ complex corrects metabolic dysfunction and alters food preference in obese humans

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Results

Safety: Additional Details. The frequency of AEs between BFKB8488A- and placebo-treated participants was comparable (87% vs. 89%; Table 2). In the 342-mg and 681-mg cohorts, 75% and 100% of the participants, respectively, experienced gastrointestinal (GI) symptoms, most commonly nausea. All nausea AEs were mild except for one moderate AE in the 342-mg cohort and one severe AE in the 681-mg cohort. Nausea onset occurred within 9 days of drug administration for 19 of 22 (86.4%) BFKB8488A-treated participants compared with 2 of 3 (66.7%) placebo-treated participants. Due to the frequency, severity, and duration of GI events, 681 mg was considered the non-tolerated dose. The 342-mg dose was considered to be the tolerated dose because treatment intervention was not required for the GI symptoms. Other commonly reported AEs included injection-site erythema and upper respiratory tract infection, occurring at comparable rates in placebo- and BFKB8488A-treated participants.

No notable drug-related findings or changes from baseline in hematology laboratory parameters occurred. There were no dose-related trends in either chemistry parameters or ECG findings. Transient changes in urine cortisol were observed in some participants, but these changes appeared to have no clinical significance. Other than weight loss (see Pharmacodynamics section in main text), no notable changes in vital signs occurred.

Nonclinical Study Supplementary Materials and Analyses

Animal Housing and Diet. Animals were housed in individual cages for the duration of the study and were maintained at a temperature of 23±3°C, 50% ± 20% humidity, a light cycle of 12 hours light and 12 hours dark, with air changes of approximately 15–20 per hour. All animals had free access to water and were fed twice per day with a nutritionally balanced normal calorie diet (Beijing Keao Xieli Feed Co., LTD, Beijing, China) enriched with seasonal fruits.

Serum Analysis for Pharmacodynamic (PD) Biomarkers. Blood samples were collected from each animal after a 12-hour fast at specific timepoints on Day -2, Day 0 predose, and Days 1, 2, 3, 5, 7, 14, and 28. Serum was analyzed on a Beckman Coulter AU480 random access chemistry analyzer (Brea, CA) using commercially available kits for NEFAs and BHBA (Randox Laboratories-US, Ltd., Kearneysville, WV). Serum HMW adiponectin was analyzed using the Quantikine® adiponectin enzyme-linked immunosorbent assay (ELISA) kit (Cat. #DHWAD0, R&D Systems, Minneapolis, MN).

Pharmacokinetic Assay for BFKB8488A in Cynomolgus Monkey Serum. A validated generic ELISA sandwich immunoassay was used to quantify BFKB8488A in serum samples. Microtiter plates were coated with sheep anti-human IgG (The Binding Site, San Diego, CA) to capture BFKB8488A. Sheep anti-human IgG, conjugated to horseradish peroxidase (HRP; The Binding Site), was used as the detection agent. The minimum quantifiable concentration (MQC) was 100 ng/mL in cynomolgus monkey serum.

BFKB8488A EC₅₀ Determination in Adipocyte Culture. Human primary subcutaneous pre-adipocytes from five donors (4 female, 1 male) were acquired from Zen-Bio (Research Triangle Park, NC) and underwent checks for quality control at Zen-Bio to verify that pre-adipocytes differentiated into adipocytes, as shown by lipid accumulation, response to lipolytic agents, and leptin and adiponectin secretion. Cells were grown and differentiated according to the supplier’s...
protocol. Briefly, pre-adipocytes were plated at approximately 40,625 cells/cm² in pre-adipocyte medium and incubated for 24 hours at 37°C (5% CO₂). To initiate differentiation, media was aspirated from the wells and replaced with Adipocyte Differentiation Medium (Zen-Bio, Research Triangle Park, NC). After 7 days, cells were fed by removing and replacing half of the media with Adipocyte Medium (Zen-Bio, Research Triangle Park, NC). Two weeks after the initiation of differentiation, cells acquired morphological attributes that were similar to mature adipocytes.

Differentiated human adipocytes were stimulated with BFKB8488A for 15 minutes at 37°C (5% CO₂). Cells were then put on ice, medium was removed, and cells were lysed by adding M-PER Protein Lysis Reagent (Thermo Fisher Scientific, Rockford, IL) + Complete Ultra Protease Inhibitors (Roche, Indianapolis, IN) + Phosphatase Inhibitor Cocktail 3 (Sigma Aldrich, St. Louis, MO). The same volume of protein per sample was loaded onto 4%–12% bis-tris gels (Novex by Life Technologies, Carlsbad, CA). Proteins were separated by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes, and detected using HRP-conjugated anti-ERK 1/2 or anti-phospho-ERK 1/2 (Thr202/Tyr204) primary antibodies (#4348, #8544; Cell Signaling, Danvers, MA). Blots were imaged using the ImageQuant 4000 and quantified with ImageQuant software (GE Healthcare Life Sciences, Pittsburgh, PA). pERK 1/2 protein levels were normalized to total ERK 1/2 protein levels for each sample. The BKF8488A concentration required for a 50% increase (EC50) in pERK 1/2 was calculated by performing a non-linear regression curve fit using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA).

SC Tissue Biopsy and Tissue Collection in Monkey. SC tissue biopsies were collected from overnight-fasted (>12h) animals under sedation with ketamine (10–15 mg/kg, intramuscular (IM)). The area of skin for biopsy on the lateral side of the abdomen was prepared with 10% povidone iodine and chlorhexidine or 70% alcohol scrub prior to biopsy. A 2-cm skin incision was made and overlying skin was isolated in a small area (2x2 cm). A small amount (~150 to 500 mg) of SC fat tissue was collected from the site. The biopsy site was closed with 1–2 skin sutures, and analgesic (buprenophine, IM, 0.01 mg/kg for 2 days) and antibiotic (amoxycillin, SC, 7 mg/kg, for 3 days) were given after biopsy. Collected tissue was roughly trimmed of non-fat tissue and weighed on a precision balance. Each tissue sample was placed into a tube with 2 mL RNAlater (Invitrogen, Carlsbad, CA) and incubated overnight at 4°C. Tissue was removed from the reagent and transferred to −80°C for storage until assays were performed.

mRNA Assays for SPRY and DUSP4 in Adipose Tissue. Adipose tissue from SC biopsies was homogenized in tissue lysis buffer (Roche Molecular Systems, Branchburg, NJ) using a Bertin-Precellys homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France). RNA was prepared using the MagNA Pure Compact RNA isolation kit (Roche Molecular Systems, Branchburg, NJ). The SuperScript VILO cDNA Synthesis Kit (Applied Biosystems; Foster City, CA) was used for cDNA synthesis. Transcripts were quantified as described in the main text.

Dual X-ray Absorptiometry (DEXA) Scan in Monkey. DEXA scans were performed Day 23 on overnight-fasted animals under sedation with ketamine (10–15 mg/kg, IM) using a LUNAR DPX (GE Healthcare, Chicago, IL). Animals were placed face down on a padded table with limbs spread apart and scanned.

Nonclinical Statistical Analysis. For each study parameter, a repeated-measures model was fitted to the change from average baseline data. “Group,” “Timepoint,” and their interaction were included as fixed effects, and “Animal” as the random effect. Treated group means at each postdose timepoint were compared with vehicle using one-way analysis of variance (ANOVA)
with post hoc Dunnett’s t-test at the 5% significance level. All analyses were conducted using SAS, version 9.2 (SAS Institute, Inc., Cary, NC).

**Phase 1 Clinical Study Supplementary Materials and Analyses**

**Additional Study Design Information.** After screening, participants entered a run-in of approximately 1 week, when they followed standard healthy dietary guidelines and avoided any unaccustomed physical activity. Following the run-in, all participants were confined to the study facility from the morning of Day 2 until the morning of Day 8. Study drug was administered on Day 1. Participants received a standardized, weight-adjusted diet during confinement, including breakfast on Day 8 and standardized breakfasts at the study visits on Days 15 and 22. Following confinement, participants could go home and continue normal activities. Participants were asked to follow provided guidelines for healthy eating and avoid any unusual strenuous activity for the study duration. For all study visits, participants were asked to fast overnight, with no fluids except water. For specified visits, water may also have been restricted except for sips to take medications.

**Randomization and Blinding.** Eligible participants received a unique identification number from the master randomization list that was generated using SAS v9.02 or higher (SAS Institute, Cary, NC). Study participants and the site staff remained blinded to treatment assignment at all times, except for pharmacists who prepared and dispensed the study drug and knew the randomization code. Safety Review Team (SRT) members were unblinded to treatment assignment. Personnel performing pharmacokinetic (PK) assays were unblinded to identify the appropriate PK samples for analysis. Other personnel were unblinded only if necessary for data analysis or safety reasons.

**Study Drug.** The starting dose of BFKB8488A (Genentech, Inc., South San Francisco, CA) in humans was 3 mg SC. This dose was expected to result in minimal to no pharmacological activity in humans based on preclinical studies.

BFKB8488A was injected subcutaneously in the abdomen or thigh as a 150 mg/mL solution containing L-histidine/histidine-succinate, L-arginine-succinate, N-acetyl-DL-tryptophan, L-methionine, and polysorbate 20 at pH 5.8. The placebo had the same excipient composition without the study drug.

**Anti-drug antibody assessments.** Incidence of anti-drug antibodies (ADAs) during the study was measured relative to the prevalence of ADAs at baseline. Sampling for ADA assessment was scheduled at baseline and postbaseline on Days 29, 57, 85, and 113. Serum samples were screened for the presence of ADAs using validated bridging screen and confirmatory immunoassays and assay cutpoints statistically determined from individual drug-naive serum samples at 5% and 1% untreated positive rates, respectively. Samples testing positive in the screening assay were further analyzed in the confirmatory assay. Confirmed-positive samples were characterized in a titer assay to determine the relative quantitation of the ADAs. The screening assay was capable of detecting 100 ng/mL of surrogate positive control ADA in the presence of 7.8 µg/ml BFKB8488A.

**Serum triglyceride measurement.** The assay for serum triglycerides was performed at Covance (Princeton, NJ) on Roche Cobas analyzers (Roche Diagnostics, Indianapolis, IN). Lipase hydrolyzes triglycerides to glycerol and fatty acids. Glycerol is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, peroxide reacts with 4-aminophenazone and 4-chlorophenol in a Trinder reaction to a colorimetric
endpoint. The triglyceride reportable range in this assay is 0.23–11.29 mmol/L. If the value was greater than the linearity of the assay, a maximum dilution of X8 was prepared, extending the upper reportable range to 90.32 mmol/L. Conversion factor from mmol/L to mg/dL: X 88.57.

**Serum cholesterol measurement.** Serum cholesterol was measured at Covance (Princeton, NJ) using Roche Cobas analyzers (Roche Diagnostics, Indianapolis, IN). All cholesterol esters present in serum are hydrolyzed quantitatively into free cholesterol and fatty acids by microbial cholesterol esterase. In the presence of oxygen, free cholesterol is oxidized by cholesterol oxidase to Cholest-4-en-3-one. Hydrogen peroxide reacts in the presence of peroxidase with phenol and 4-aminophenazone to form an o-quinone imine dye. The intensity of the color formed is proportional to the cholesterol concentration and is measured photometrically. The reportable range of the assay is 0.26–20.69 mmol/L. If results were greater than 20.69, a maximum dilution of X16 was prepared, extending the upper reportable range to 331.04 mmol/L. Conversion factor from mmol/L to mg/dL = X 38.67.

**Quantification of BFKB8488A effects on appetite.** To quantify BFKB8488A effects on appetite, standardized meals were provided during the confinement period starting Day -2 (morning) to Day 8 (morning). Standardized meals based on daily energy expenditure (DEE) were designed to provide sufficient calories to at least maintain body weight during the confinement period. DEE was determined by calculating the estimated basal metabolic rate (BMR) times 1.5. After the confinement period, a standardized breakfast was also provided at study visits on Days 15 and 22. Food intake was assessed at breakfast following an overnight fast at baseline and on Days 3, 7, 15, and 22 after BFKB8488A administration. Identical meals (same food and amounts) were provided on all food assessment days. Pre- and post-meal weights were measured to determine the amount consumed. Kilocalories (kCals) and macronutrient consumption of the meal were determined using Food Processor Nutrition Analysis software (ESHA Research, Salem, OR).
**Fig. S1.** Determination of BFKB8488A EC$_{50}$ by pERK assay in primary human adipocytes. Graph shows the ratio of pERK to total ERK versus the log BFKB8488A concentration. Values are mean ± SD. EC$_{50}$ = 0.7 µg/mL (4.4 nM).
Fig. S2. BFKB8488A reduces the preference for sweet, but not fatty or salty, food. Percent change from baseline (mean ± SEM) from Day 1 through Day 43, measured by VAS.
Table S1. Body composition measurements by DEXA scan in obese monkeys

|          | Fat (g), mean (SEM) | Lean (g), mean (SEM) | Mean Total Fat (relative to vehicle, %) |
|----------|---------------------|----------------------|----------------------------------------|
|          | Arms                | Legs                 | Trunk                                  | Total Fat                              |
|          |                     |                      |                                        |                                        |
| Vehicle  | 150.8 (15.7)        | 329.8 (98.4)         | 1811.5 (544.5)                         | 2400.5 (659.7)                         |
| 0.6 mg/kg| 123.0 (41.6)        | 247.3 (89.5)         | 1072.8 (295.1)                         | 1534.0 (355)                           |
| 3 mg/kg  | 94.5 (54.7)         | 176.0 (43.1)         | 931.3 (363.2)                          | 1286.0 (471.4)                         |
| 15 mg/kg | 91.5 (30.8)         | 281.0 (80.8)         | 1203.0 (285.5)                         | 1671.8 (409.4)                         |
|          |                     |                      |                                        |                                        |
| Vehicle  | 1059.3 (97.5)       | 1593.5 (172.2)       | 4411.5 (208.9)                         | 7672.3 (394.6)                         |
| 0.6 mg/kg| 998.5 (122.9)       | 1514.0 (249.7)       | 4531.0 (787.6)                         | 7670.5 (1221.5)                        |
| 3 mg/kg  | 974.0 (99.3)        | 1537.3 (102.8)       | 4037.0 (257.9)                         | 7217.3 (425.5)                         |
| 15 mg/kg | 979.0 (103.1)       | 1547.5 (147.4)       | 3965.5 (318.5)                         | 7115.3 (581.2)                         |

DEXA, dual X-ray absorptiometry; SEM, standard error of the mean.
### Table S2. Summary of BFKB8488A serum pharmacokinetic parameters

| Dose | 3 mg | 10.5 mg | 39 mg | 111 mg | 171 mg | 342 mg | 681 mg | 171 mg | 250 mg |
|------|------|---------|-------|--------|--------|--------|-------|--------|-------|
| n    | 3    | 5*      | 6     | 6      | 6      | 6      | 6     | 4      | 6     |
| \(t_{1/2}\) (day) | NE | 7.09 (47.5) | 6.13 (64.6) | 6.79 (58.7) | 6.13 (87.6) | 5.29 (76.9) | 7.55 (53.6) | 6.21 (93.8) | 7.83 (72.7) |
| \(C_{\text{max}}\) (\(\mu\)g/mL) | 0.0291 (29.2) | 0.0878 (106) | 0.673 (150) | 3.08 (55.5) | 5.39 (69.0) | 20.7 (44.8) | 64.8 (14.0) | 7.23 (14.2) | 18.7 (25.4) |
| \(\text{AUC}_{0-\text{inf, obs}}\) (\(\text{day} \cdot \mu\)g/mL) | NE | 1.04 (35.2) | 5.89 (108) | 33.6 (45.0) | 61.1 (68.6) | 287 (49.0) | 1210 (22.4) | 89.9 (26.2) | 282 (24.5) |
| \(\text{CL/F} \) (mL/day) | NE | 10100 (35.2) | 6620 (108) | 3300 (45) | 2800 (68.6) | 1190 (49) | 561 (22.4) | 1900 (26.2) | 887 (24.5) |
| \(t_{\text{max}}\) (day), median (range) | 3.00 (1.00, 5.00) | 3.00 (1.00, 7.00) | 3.00 (3.00, 3.00) | 5.00 (3.00, 7.00) | 4.00 (3.00, 7.00) | 6.00 (3.00, 7.00) | 5.00 (5.00, 7.00) | 5.00 (5.00, 7.00) | 6.00 (5.00, 7.00) |

AUC<sub>0-inf, obs</sub> observed area under the concentration-time curve from time 0 to infinity; CL/F, apparent clearance; \(C_{\text{max}}\), maximum concentration; CV%, percent coefficient of variation of the geometric mean; NE, not estimated due to insufficient data; SC, subcutaneous; \(t_{1/2}\), half-life; \(t_{\text{max}}\), time to maximum concentration.

Data are geometric mean (CV%) unless otherwise indicated.

*For the 10.5-mg dose, 4 participants were included in \(t_{1/2}\), AUC<sub>0-inf</sub>, and CL/F calculations.
**Table S3. Baseline prevalence and postbaseline incidence of ADAs**

|                  | Placebo | All BFKB8488A | Abdomen | Thigh |
|------------------|---------|---------------|---------|-------|
| **BFKB8488A**    | 3 mg    | 10.5 mg       | 39 mg   | 111 mg| 171 mg| 342 mg | 681 mg | 171 mg| 250 mg |
| **Baseline evaluable participants, n** | 18      | 53            | 7       | 6     | 6     | 6    | 6     | 6   | 4     | 6     |
| ADA-positive participants at baseline, n (%) | 0 (0%)  | 0 (0%)        | 0 (0%)  | 0 (0%)| 0 (0%)| 0 (0%)| 0 (0%)| 0 (0%)| 0 (0%)| 0 (0%)|
| **Postbaseline evaluable participants, n** | 18      | 53            | 7       | 6     | 6     | 6    | 6     | 6   | 4     | 6     |
| Participants with treatment-induced ADA, n (%) | 0 (0%)  | 8 (15.0%)     | 0 (0%)  | 0 (0%)| 0 (0%)| 2 (33.3%)| 1 (16.7%)| 2 (33.3%)| 1 (16.7%)| 2 (50.0%)| 0 (0%)|

ADA, anti-drug antibody.
Table S4. Body weight (Days 1–113) and percent change from baseline (Day 1) through Day 8

| Dose       | n  | Baseline (Day 1)   | Day 2    | Day 8    | Day 15   | Day 113   | Mean change from baseline, Days 1–8 | Mean percent change from baseline, Days 1–8 |
|------------|----|-------------------|----------|----------|----------|-----------|-------------------------------------|--------------------------------------------|
| Placebo    | 18 | 101.1 (12.2)      | 101.0 (12.1) | 100.6 (12.5) | 102.9 (12.5) | 98.1 (5.8) | -0.28 (0.73)                        | -0.28 (0.70)                              |
| 3 mg       | 7  | 98.5 (8.5)        | 98.5 (8.2) | 96.2 (7.4) | 97.5 (8.0) | NE (NE)   | -0.30 (0.76)                        | -0.31 (0.78)                              |
| 10.5 mg    | 6  | 98.5 (8.8)        | 98.1 (8.9) | 97.7 (8.5) | 99.8 (8.4) | NE (NE)   | -0.77 (0.76)                        | -0.76 (0.70)                              |
| 39 mg      | 6  | 104.0 (11.9)      | 103.9 (12.0) | 103.2 (12.4) | 105.2 (12.1) | NE (NE)   | -0.83 (0.68)                        | -0.85 (0.78)                              |
| 111 mg     | 6  | 97.9 (15.2)       | 97.8 (14.9) | 96.8 (15.9) | 99.3 (16.1) | NE (NE)   | -1.05 (1.29)                        | -1.17 (1.42)                              |
| 171 mg     | 6  | 100.2 (18.5)      | 100.2 (18.6) | 99.3 (18.4) | 101.4 (19.4) | NE (NE)   | -0.92 (0.85)                        | -0.93 (0.98)                              |
| 342 mg     | 6  | 92.7 (8.1)        | 92.7 (8.0) | 91.2 (7.8) | 93.4 (8.4) | 94.5 (8.4) | -1.53 (0.65)                        | -1.64 (0.61)                              |
| 681 mg     | 6  | 101.9 (11.7)      | 101.8 (11.6) | 99.7 (11.7) | 100.8 (11.5) | 101.2 (11.5) | -2.17 (0.69)                        | -2.15 (0.77)                              |
| 171 mg (thigh)* | 4 | 101.7 (10.2)    | 101.5 (10.2) | 99.3 (9.8) | 100.5 (10.2) | NE (NE)   | -2.35 (0.44)                        | -2.30 (0.25)                              |
| 250 mg (thigh)* | 6 | 86.4 (10.6)    | 85.9 (10.0) | 85.2 (10.5) | 87.4 (10.8) | 90.3 (13.0) | -1.23 (0.86)                        | -1.43 (0.97)                              |

Data are weight in kg, mean (SD), except where indicated.
*All doses were injected into the abdomen, except for the indicated cohorts. NE, not evaluated; SD, standard deviation.