Preclinical pharmacokinetic characterization of an adipose tissue-targeting monoclonal antibody in obese and non-obese animals

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ABSTRACT
Target receptor levels can influence pharmacokinetics (PK) or pharmacodynamics (PD) of monoclonal antibodies (mAbs), and can affect drug development of this class of molecules. We generated an effectorless humanized bispecific antibody that selectively activates fibroblast growth factor receptor (FGFR1) and βKlotho receptor, a FGF21 receptor complex highly expressed in both white and brown adipocytes. The molecule shows cross-species binding with comparable equilibrium binding affinity (Kd) for human, cynomolgus monkey, and mouse FGFR1/βKlotho. To understand the PK/PD relationship in non-obese and obese animals, we evaluated the adipose tissue distribution of the antibody, serum exposures, and an associated PD marker (high-molecular-weight adiponectin), in both non-obese and obese mice and monkeys. Antibody uptake into fat tissue was found to be higher on a per gram basis in non-obese animals compared to obese animals. Since obesity has been reported to be associated with reduced expression of FGRFR and βKlotho receptor in white adipose tissues in mice, our results suggest that the distribution in adipose tissues was influenced by target expression levels. Even so, the overall dose-normalized serum exposures were comparable between non-obese and obese mice and monkeys, suggesting that adipose tissue uptake plays a limited role in overall systemic PK determination. It remains to be determined if and how obesity and receptor expression in humans influence the PK and PD profile of this novel therapeutic candidate.

Abbreviations: ADA, Anti-drug antibody; BAT, Brown adipose tissue; DIO, Diet-induced obese; FGF21, fibroblast growth factor 21; IV, Intravenous; βKlotho, Klotho beta; LLOQ, Lower limit of quantitation; ULOQ, Upper limit of quantitation; mAb, Monoclonal antibody; PK, Pharmacokinetics; PD, Pharmacodynamics; SC, Subcutaneous; WAT, White adipose tissue

Introduction
The pharmacokinetic (PK) properties of a monoclonal antibody (mAb) depend on several factors, including affinity and binding to FcRn and FcγR receptors, target-mediated elimination, charged residues, as described previously.1 When targeting a receptor, the receptor expression levels and the turnover rate of the receptor can influence the PK, pharmacodynamics (PD), or distribution profile of mAbs and may be an important determinant of the PK profile of this class of molecules. For a molecule specifically targeting a receptor complex found abundantly in the adipose tissue, some unique aspects warrant consideration. In particular, inter-subject differences in total receptor levels, overall fat mass, and route of administration of the mAb may affect the overall distribution and serum concentration time profile of the molecule.

We previously generated an effectorless humanized bispecific anti-fibroblast growth factor receptor (FGFR1)/βKlotho antibody designated bFKB1. bFKB1 was designed to selectively activate (FGFR1)/βKlotho, a FGF21 receptor complex, thereby acting as a FGF21 mimetic. FGF21 is a member of the fibroblast growth factor (FGF) superfamily that can stimulate brown adipose tissue (BAT) thermogenesis and increase energy expenditure in rodents.2–5 Supraphysiological exposure to FGF21 also ameliorates obesity, insulin resistance, hyperlipidemia, fatty liver, and hyperglycemia.6,7 Of the seven primary FGF isoforms (1b, 1c, 2b, 2c, 3b, 3c, and 4) expressed in mammals, FGF21 can activate three of these isoforms (1c, 2c, and 3c) when bound to their obligate co-receptor βKlotho to transduce the mitogen-activated-protein-kinase (MAPK) signaling cascade.6,7 βKlotho is expressed in select tissues; most abundantly in liver, pancreas, and adipose tissues.8 The molecule is cross-species selective with comparable single-digit nanomolar equilibrium binding affinity (Kd) for human, cynomolgus monkey, and mouse FGFR1c/βKlotho receptor complex (1.89, 2.55, and 3.92 nM, respectively).

Since bFKB1 targets βKlotho, which is highly expressed in adipose tissue, it provides us a unique opportunity to study the antibody PK in relation to adiposity. In addition, expression
levels of FGFR1c and βKlotho mRNA have been shown to differ in non-obese and obese animals,\(^9,10\) and may affect overall distribution of the molecule in these two distinct populations of animals. We, therefore, performed an adipose tissue distribution study in non-obese and obese mice, to understand if there were differences in molecule uptake in the adipose tissue between these two animal subpopulations.

Concordant with the reported differences in receptor expression in adipose tissue of non-obese and obese mice, we observed a higher uptake of bFKB1 per gram of non-obese mouse adipose tissue mass compared to obese mouse tissue following intravenous (IV) or subcutaneous (SC) administration. The concentration-time profile of the molecule in serum, however, was comparable between both non-obese and obese mice across a wide dose-range, suggesting that the differences in adipose tissue distribution had little impact on overall systemic concentrations. Similarly, comparable PK was observed in non-obese versus obese monkeys. In addition, there was no significant difference in the serum exposures of the molecule after either IV or SC administration in either two species of animals tested, indicating that the overall bioavailability of the molecule was not significantly influenced by the adipose content in the subcutaneous space. This is the first time to our knowledge that the preclinical PK profile of an adipose-targeting mAb has been characterized. Understanding the unique considerations involved in PK characterization of the molecule may help inform development of other molecules in this class.

Results

**PK in non-obese and obese mice**

It has previously been shown that diet-induced obese (DIO) mice have increased endogenous levels of FGF21.\(^10\) Along with the increase in circulating FGF21, obesity was also associated with substantial reduction of FGFR1c and βKlotho expression in white adipose tissue (WAT), which may be an indicator that obesity is primarily a FGF21-resistant state, and that downregulated receptor expression contributes to FGF21 hormone resistance. Similar down-regulated expression of βKlotho receptor has also been previously shown in WAT of obese monkeys.\(^9\) To understand whether differences in receptor expression in mice would affect the distribution of bFKB1 to adipose tissue and overall serum exposures, we administered a single 0.3 mg/kg IV dose of bFKB1 mixed with a tracer dose of radiolabeled bFKB1 to DIO and non-obese mice. A dual radiotracer approach was employed with both non-residualizing (I-125, via tyrosine residues) and residualizing (In-111-DOTA, via lysine residues) probes to capture both intact and total (intact plus catabolized) antibody uptake, respectively. We also dosed DIO mice with a single 0.3 mg/kg IV dose of trastuzumab as a control IgG with no murine-specific antigenic target. Previous reports have demonstrated a comparable PK profile of IgG1 and IgG1 bispecific antibodies.\(^11\) However, by Day 3 post injection, bFKB1 appeared to clear faster than trastuzumab in the serum of obese mice, which is suggestive of target-mediated drug disposition of bFKB1 at these low doses.\(^12\) Nevertheless, as seen in Fig. 1 and Table 1, a comparable serum concentration-time profile of bFKB1 was observed in non-obese and obese mice following an IV dose, indicating that the body mass differences and differential receptor expression had no effect on the short-term serum PK of bFKB1. The short-term PK profile for both In-111(Fig. 1A) and I-125 (Fig. 1B) labeled antibodies were identical, indicating that there was no differential impact of the two radiolabeling methods on exposure.

In addition to serum, levels of radioactivity in adipose tissue were also quantitated on Days 1, 7, and 10 post IV injections (Fig. 2). The 2.8-day decay half-life of In-111 and the limited in vivo stability of the I-125 label limited the ability to perform a longer-term study. The tissue distribution data was plotted as a partition ratio for each tissue to illustrate enrichment that is

![Figure 1. Serum concentration-time profile of (A) In-111 or (B) I-125 labeled bFKB1 or trastuzumab in mice. Two groups of DIO mice and one group of non-obese C57BL/6J mice (n = 9 per group, 3 mice harvested at each time point) were used for this experiment. DIO mice were dosed with 0.3 mg/kg of bFKB1 or trastuzumab. The non-obese C57BL/6J mice were dosed with 0.3 mg/kg of bFKB1. Each dose was a single IV dose, spiked with 5 μCi of (A) I-125 and or (B) In-111 radiolabeled antibodies to study serum concentration profile.](image)

| Table 1. PK parameters of radiolabeled (In-111) bFKB1 after single IV dose of 0.3 mg/kg in obese and non-obese mice. |
| PK Parameter | Obese Mice (n = 9) | Non-Obese Mice (n = 9) |
|-------------|-------------------|----------------------|
| Cmax (%ID/mL) | 52.3 | 67.4 |
| AUClast (%ID/mL·day) | 3160 | 3270 |

AUClast = area under the serum concentration versus time curve from time = 0 to time of the last measurable concentration; Cmax = maximum serum concentration; PK = Pharmacokinetic.

Note: As sparse PK analysis was performed for all mouse PK data, data from individual mice per group was pooled and SD was not reported.
normalized to systemic exposure. The partition ratios were calculated by dividing the %ID/g for each tissue by the %ID/g of systemic antibody detected in the serum at the time of each tissue harvest.

As seen in Fig. 2, distribution to white adipose of intact antibodies was similar in both obese and non-obese animals as represented by I-125 (non-residualizing) signal. At Day 1, the I-125 and In-111 data were largely in agreement, consistent with minimal antibody catabolism, as expected for this short time (Fig. 2A and D, respectively). At Day 7, however, the In-111 (residualizing) signal, an indicator of cumulative antibody uptake (i.e., intact plus degraded) into the tissue showed a 3-fold higher accumulation of total bFKB1 in the non-obese mouse compared to the DIO mouse (Fig. 2E), indicating increased adipose tissue uptake in non-obese mice. Furthermore, the difference between I-125 and In-111 signal in general gave a clear indication that bFKB1 was being internalized and degraded within adipocytes. One animal outlier in the trastuzumab-treated animals contributed to higher variability in partition ratios observed on day 10 for that group (Fig. 2C and F).

Overall, the data were consistent with the previously reported decrease in expression of both FGFR1c and βKlotho in obese mice. Serum levels of both In-111 and I-125 labeled trastuzumab over the 1-week period were within the historical range observed for the molecule. We next set out to understand the PK profile of bFKB1 following repeated doses in mice, which may affect receptor levels or binding saturation, and is relevant to proposed clinical regimens.

We compared the PK parameters of bFKB1 after a total of 5 weekly SC injections of 15 mg/kg in non-obese CD-1 and DIO

Table 2. PK parameters of bFKB1 after five weekly SC injections of 15 mg/kg in non-obese CD-1 mice and diet-induced obese C57BL/6J male.

| PK Parameter                  | Obese Mice (n=12) | Non-Obese Mice (n=12) |
|-------------------------------|-------------------|-----------------------|
| $C_{\text{max}}$ (μg/mL)      | 379               | 314                   |
| $C_{\text{max}, \text{1st dose}}$ (μg/mL) | 137              | 121                   |
| $\text{AUC}_{\text{last}}$ (day/mg/mL) | 11200            | 7720                  |
| $\text{AUC}_{0-7}$ (day/mg/mL) | 815              | 696                   |

$\text{AUC}_{\text{last}}$ = area under the serum concentration versus time curve from time = 0 to time of the last measurable concentration; $\text{AUC}_{0-7}$ = area under the serum concentration versus time curve from Day 0 to Day 7; $C_{\text{max}}$ = maximum serum concentration; PK = Pharmacokinetic.

Note: As sparse PK analysis was performed for all mouse PK data, data from individual mice per group was pooled and SD was not reported.
C57BL/6 male mice (n = 12 per group) (Table 2). Serum samples for PK analysis were collected at various times (n = 3 mice per time point) through the duration of the study (with Day 84 being the last time point), and analyzed by enzyme-linked immunosorbent assay (ELISA) as detailed in the Material and Methods section.

As was previously observed using the single tracer dose of 0.3 mg/kg (Table 1), there was no appreciable difference in overall serum concentrations (within 2-fold difference in all PK parameters studied, which is within the range of variability typically associated with sparse PK analysis using pooled data in mice) after administering bFKB1 at equivalent dose levels between non-obese and obese mice (Table 2). This suggests that adipose mass played a negligible role in overall PK profile of the molecule.

We also studied the effect of body fat on bioavailability of bFKB1. The bioavailability of mAbs depend on several factors, including FcRn affinity, formulation, immunogenicity, and first pass-catabolism at the site of injection.1,14 Since the subcutaneous space includes an adipose cell layer, target-mediated drug disposition at the local site of injection after SC administration can be speculated to affect the overall bioavailability of bFKB1. We compared the overall serum exposures of C57BL/6 mice administered a single IV dose of bFKB1 (3 mg/kg) to a single SC dose of bFKB1 (15 mg/kg) in CD-1 mice. Concentration-time curve and PK parameters including dose-normalized AUCs are shown in Fig. 3 and Table 3.

Dose normalized AUC_{inf-obs} of bFKB1 administered IV at 3 mg/kg were compared to that administered SC at 15 mg/kg. The overall dose normalized AUC_{inf-obs} were comparable between the SC- and IV-administered doses (142.4 and 140 day*C3*mg/mL/(mg/kg), respectively), indicating near complete absorption of the molecule after SC administration at the dose level tested. These data imply that, at the saturating dose-level of 15 mg/kg, target receptors at the local site of injection have a minimal effect on overall absorption of the adipose targeting molecule, bFKB1, after SC administration in mice. As we expect the eventual clinical doses of the molecule to be within the linear PK range, we did not compare bioavailability of bFKB1 at non-saturating dose levels. However, it is possible that bioavailability could be dose dependent.

### PK in non-obese and obese monkeys

Important distinctions exist between monkeys, mice, and humans, with regards to adipose tissue composition, including overall brown fat content.15 In spite of these differences, bFKB1 has been associated with weight loss, and increased high molecular weight (HMW) adiponectin levels in monkeys, similar to observations made previously in mice.16

We characterized the PK of bFKB1 in monkeys with differing adipose tissue mass to understand how the PK profile of the molecule compares in these two populations of animals. After a single IV injection of bFKB1 (3 or 30 mg/kg in non-obese monkeys, and 0.6, 3, or 15 mg/kg in obese monkeys), anti-drug antibodies (ADA) were detected in the serum of all monkeys, and were associated with a significant loss of exposures of the molecule, making determination of some PK parameters, including CL and V difficult (Fig. 4). We therefore compared the exposure of bFKB1 through the first week post injection, prior to the typical onset of ADAs. In both obese and non-obese monkeys, roughly dose proportional exposures were observed through the first 7 days of the study, prior to the development of ADAs. The concentration-time profile of bFKB1 for the first week post single injection at both dose levels tested in non-obese monkeys was consistent with in-house data from a typical, non-specific IgG1 antibody (Fig. 5). The PK parameters for obese and non-obese monkeys are outlined in Table 4.

### Table 3. Pharmacokinetic parameter estimates (mean) after a single IV or SC injection of bFKB1 to mice.

| PK Parameter | 3 mg/kg IV (n = 9) | 15 mg/kg SC (n = 12) |
|--------------|--------------------|----------------------|
| C_{min} (\mu g/mL) | 94.96 | 115 |
| AUC_{inf} (day*\mu g/mL) | 350.06 | 1690 |
| AUC_{last} (day*\mu g/mL) | 419.44 | 2130 |
| CL (CL/F) (mL/kg/day) | 7.15 | 7.03 |
| CL/F (mL/kg/day) | 140 | 142.4 |
| Vss (Vss/F) (mL/kg) | 60.0 | 122 |

AUC_{inf} = area under the serum concentration versus time curve extrapolated to infinity; AUC_{last} = area under the serum concentration versus time curve from time = 0 to time of the last measurable concentration; CL = Clearance; CL/F = apparent clearance; PK = Pharmacokinetic; SC = Subcutaneous; IV = intravenous; DN = Dose normalized; Vss = Volume of distribution at steady state; Vss/F = Apparent volume of distribution at steady state.

Note: As sparse PK analysis was performed for all mouse PK data, data from individual mice per group was pooled and SD was not reported.
Unlike the experiments in mice, in which there was a <2-fold difference in the average weight of obese versus non-obese animals, these two populations of monkeys exhibited an almost 3-fold difference in weight (3.1 kg and 10 kg for the non-obese and obese monkey, respectively). Thus, there was an almost 3-fold increase in overall bFKB1 administered to the obese monkeys relative to the non-obese monkeys at the same mg/kg dose level. When normalizing for the total amount of bFKB1 administered per animal (9 mg, as detailed in the Materials and Methods section), we determined that there was very little difference in the serum concentration-time profile of bFKB1 in obese or non-obese monkeys (Fig. 6). Fixed dose administration in animals (not adjusting dose to body weight), although perhaps informative, was not performed as a follow up to the weight-based dosing paradigm, which has more historical precedence in animals.

Adiponectin is an adipokine predominantly secreted from adipocytes in response to FGF21 activation. Consistent with previous reports, baseline levels of HMW adiponectin were substantially lower in the obese population compared with the lean monkeys. Following administration of bFKB1, there was a marked increase in HMW adiponectin in both obese and non-obese monkeys, indicating activation of FGFR1/bKlotho in adipose tissue (Fig. 7). In addition, the overall decreases in body weight, a downstream effect of activating the FGFR1 pathway, was comparable in both populations of monkeys within the first week after dosing (Fig. 8). As the effect of ADA development on concentrations of bFKB1 and the subsequent modulation of downstream body weight effects are unknown, body weight loss comparisons were restricted to the first week post dosing. Previously, we demonstrated that IV and SC administration resulted in similar exposures in mice, in spite of a hypothetical impact that the SC route could have on the PK of an adipose targeting agent. However, the subcutaneous space properties of monkeys and mice are distinct, and differences in adipocyte level in this region may differ across species. Non-obese male and female monkeys in each of the dose groups (n = 3 per gender) were given SC injections (0.1, 0.75, 2, or 5 mg/kg) of bFKB1 every week for 5 weeks (Table 5). The range of doses for this repeat dose study was determined based on pharmacological observations previously made in this species (data not shown). As observed previously, bFKB1 injection was associated with the development of ADA in 100% of animals, with a significant effect on PK. To evaluate exposures through the first week of dosing (pre-ADA), Cmax after the first dose, and AUC0–7 were evaluated (Table 5).

Table 4. Pharmacokinetic parameter estimates (Mean ± SD) after a single IV injection of bFKB1 to obese or non-obese cynomolgus monkeys.

| PK Parameter | Obese | | | Non-Obese |
|--------------|-------|---|---|---|
| 0.6 mg/kg (n = 4) | 3 mg/kg (n = 4) | 15 mg/kg (n = 4) | 3 mg/kg (n = 3) | 30 mg/kg (n = 3) |
| Cmax(μg/mL) | 28.2 ± 3.22 | 162 ± 12.4 | 1120 ± 353 | 64.1 ± 5.21 | 836 ± 52.5 |
| AUC0–7(μg/mL) | 83.4 ± 10.1 | 458 ± 17.9 | 2690 ± 435 | 203 ± 14.1 | 2900 ± 73.8 |

AUC0–7 = area under the serum concentration versus time curve from Day 0 to Day 7; Cmax = maximum observed concentration; IV = Intravenous; SD = Standard Deviation.
The exposure through the first 7 days after the first dose (AUC0–7) was fairly proportional (with consideration given to the large variability observed) within the dose range tested; with the possible exception of the 0.1–0.75 mg/kg range (Fig. 9, Table 5), indicative of likely target-mediated drug disposition at these very low doses, similar to prior observations in mice. Since the development of ADA had a significant impact on bFKB1 concentrations in all monkeys, we were unable to directly compare the AUC0–inf of bFKB1 in monkeys dosed IV and SC as a measure of bioavailability, as was done in mice. Nevertheless, at comparable dose-levels in non-obese monkeys, the ratio of the dose-normalized AUC values over the first 7 days, (which can serve as cumulative measurements for the initial absorption phase) between monkeys dosed 3 mg/kg IV and 2 mg/kg SC was 76% (68 and 51 μg/mL·day/mg/kg, respectively). Therefore, similar to what was previously observed in mice, the presence of target receptor at site of administration (adipocytes in the subcutaneous space) likely did not have an appreciable impact on expected bFKB1 concentrations post SC injection in cynomolgus monkeys.

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**Discussion**

For full PK characterization of a mAb, several factors need to be taken into account; some of these have been detailed in previously published articles. Factors such as the expression and distribution of the target antigen and the subject population can affect the overall PK profile of a mAb. FGF21, a member of the FGF superfamily, has been found to stimulate BAT thermogenesis and increase energy expenditure in rodents. FGF21 can activate FGFR 1c, 2c, and 3c when bound to their obligate co-receptor βKlotho to transduce the mitogen-activated-protein-kinase (MAPK) signaling cascade. Although recent genetic studies suggest neuron to be the main cell type that mediates the action of FGF21 and anti-FGFR1c/βKlotho, adipose is among the tissues with the highest expression of βKlotho. We generated a bispecific antibody with high affinity binding to βKlotho and relatively weak affinity to FGFR1, conferring βKlotho specificity for FGFR1c signaling. As this is an antibody with an antigenic target abundantly expressed in adipocytes, some unique considerations needed to be taken into account when characterizing the PK of this molecule. It is known that levels of antigenic target can affect clearance of mAbs, and differences in FGFR1c and βKlotho receptor expression levels have been observed in non-obese and obese animals. In both monkeys and mice, down regulation of receptors was observed in WAT of obese animals, consistent with the hypothesis that these animals are in a FGF21-resistant state. We determined whether the distribution of the molecule into...
adipose tissue was affected by the state of obesity in mice. As expected, the overall distribution of bFKB1 per gram of WAT and BAT was higher in non-obese mice compared to obese. This is consistent with higher target antigen expression in adipose tissue of non-obese mice compared to obese mice. Across the dose levels tested, the overall serum PK profile, however, was comparable in non-obese and obese mice. This raises the possibility that, although the uptake of bFKB1 per gram of adipose tissue is higher in non-obese animals, the increased overall fat mass in obese animals results in similar levels of whole-body bFKB1 adipose uptake in non-obese and obese animals, thereby resulting in comparable bFKB1 serum concentrations in the two populations of animals. To this point, in monkeys, the ratio of receptor expression per gram of adipose tissue between non-obese and obese animals is ~4, which is generally comparable to that of overall body weight difference (likely attributed to differences in fat mass) between the two populations of monkeys used in our studies.

More work is needed to establish this hypothesis, including a mass balance study in mice to determine overall body distribution of the molecule. However, in the absence of such data, we relied on serum exposures of the molecule after administration in non-obese and obese animals to understand the effect of fat mass on the systemic PK profile of the molecule. We observed no appreciable difference in serum time-concentration profiles in non-obese and obese mice after single or repeat administration of the molecule. In addition, at saturating dose levels, bFKB1 serum exposures were comparable after SC or IV administration, suggesting that target antigen expression at the local site of injection at these doses plays little role in determination of overall serum PK profile of the molecule in mice.

A significant portion of the extra weight in obese populations is attributed to increased fat mass, and the overall blood volume between lean and obese populations is comparable. This is consistent with our observations that showed the ~3-fold increase in overall bFKB1 administered to obese animals (in proportion to the 3-fold increase in overall weight), resulted in ~3-fold increase in overall systemic exposure of the molecule in these animals. After accounting for differences in total antibody administered to the two populations of monkeys, we observed no difference in overall serum time-concentration profiles after single administration of bFKB1 in non-obese and obese monkeys. This is consistent with previous observations made from a retrospective analysis of 12 mAbs simulated using over 1000 human subjects that showed that both fixed and body weight-based dosing lead to similar variability in drug exposure across the patient population, and that there might be a risk for overdosing subjects with extreme body weight using the weight-based dosing paradigm. Together, these findings support eventual fixed dosing paradigm for bFKB1 in humans.

FGF21 activation induces the biosynthesis of adiponectin, an adipokine predominantly secreted from adipocytes. There was increase in HMW adiponectin post administration in both non-obese and obese monkeys indicating bFKB1 target engagement in the adipose tissue in both of these populations of monkeys. In line with these findings, no differences in body weight loss, a marker of downstream PD activity of bFKB1 was observed between non-obese and obese monkeys.

Overall, we conclude that bFKB1, which is a bispecific antibody that targets receptors that are abundantly present in adipocytes, exhibits a typical IgG1 systemic PK profile, and that the state of obesity or route of administration (IV versus SC) have little influence on overall exposures of this molecule in animals. It remains to be seen how these factors affect the PK profile of the molecule in humans.

Materials and methods

Antibodies

For production of bFKB1, anti-FGFR1 and anti-KLB arms with the knob or the hole mutation were separately purified from transiently or stably transfected CHO cell culture supernatant by affinity purification using Protein A column, and then subjected to an annealing protocol as previously described. Trastuzumab, and an anti-herpes simplex virus glycoprotein D (anti-gD) were obtained from Genentech, Inc.

Radiolabeled PK and tissue distribution study in obese and non-obese mice. Two groups of DIO mice and one group of C57BL/6J mice were purchased from Jackson Laboratory. DIO mice were dosed with 0.3 mg/kg of anti-FGFR/βKlotho antibody bFKB1 or trastuzumab. The lean C57BL6 mice were
dosed with 0.3 mg/kg of bFKB1. Each dose was a single IV
dose, spiked with 5 μCi of I-125 and In-111 radiolabeled anti-
bodies. Each group consisted of 9 mice, so that there were 3
mice harvested at each terminal time point. At 5 min, 2 hr,
6 hr, 24 hr, 1 day, 3 day, 7 day, and 10 days, all mice were bled
retro-orbitally under isoflurane (inhalation to effect). At Days
1, 7, and 10, animals were euthanized under anesthesia of keta-
mine (75 – 80 mg/kg)/xylene (7.5 – 15 mg/kg) by thoracotomy.
The terminal blood sampling was drawn via cardiac puncture
and the following tissues were collected, rinsed in cold phos-
phate-buffered saline (PBS), blotted dry, weighed, and frozen
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**Radiolabeling of DOTA-bFKB1**

**DOTA conjugation**

2.5 mg of bFKB1 was buffer exchanged into sodium borate-
buffered saline, pH 8.5 at 5 mg/mL using a NAP-5 column (17-
0853-02, GE Healthcare). DOTA-NHS-ester (B280, Macrocy-
clicks), diluted to 50 mg/mL in dimethyl sulfoxide, was added at
a 5:1 molar ratio to the antibody and incubated with gentle agi-
tation at 37°C for 1 hr. The DOTA-conjugated antibody was then
purified on a NAP-5 column that was equilibrated with
0.3 M ammonium acetate buffer, pH 7 (Buffer A). DOTA-
bFKB1 was concentrated to 3 mg/mL using a Centricon con-
centrator with a 100 kDa MWCO (Amicon, Millipore).

**Radiolabeling of DOTA-bFKB1 with In-111**

17 μL of DOTA- bFKB1 (3 mg/mL in Buffer A) was reacted
with 3 μL of In-111 (~1 mCi, Nordion) for 1 hr at 37°C for
1 hr with gentle agitation. To quench the reaction, 75 μL of
Buffer A and 5 μL of 0.05 M EDTA was added to the reaction
tube and left at room temperature on the bench for 5 min. The
In-111-DOTA- bFKB1 was then purified on a NAP-5 column
equilibrated with PBS.

**Radiolabeling of bFKB1 with I-125**

3 μL of I-125 (Perkin Elmer) was diluted into 100 μL of PBS
and incubated in an iodogen tube (Pierce) for 5 min with peri-
odic gentle agitation at room temperature. The activated iodine
was then reacted with 75 μg of bFKB1 for 1 min. The iodinated
antibody was purified on a NAP-5 column equilibrated with
phosphate buffered saline.

**Non-radiolabeled PK studies in obese and non-obese mice**

All in-vivo protocols, housing, and anesthesia were approved by
the Institutional Animal Care and Use Committees of Genen-
tech Laboratory Animal Resources, in compliance with the
Association for Assessment and Accreditation of Laboratory
Animal Care (AAALAC) regulations. For studies utilizing DIO
mice, male C57BL/6j mice on 60% high fat diet were obtained
from Jackson Laboratory. The mice were acclimatized to
Genentech housing conditions (for 7 days), and maintained on
a 60% high fat diet (Teklad; TD.06414). Mice were selected for
the study and randomized based on body weight ranging 40–50
gram and between 22–24 weeks of age. For non-obese mice,
males CD-1 mouse were obtained from Charles River. The ani-
mal was 15 to 16 weeks old and weighed 35 gram to 45 gram.
Animals were randomized based on body weight. Only animals
that appeared to be healthy and that were free of obvious
abnormalities were used for any study.

Single dose study in non-obese mice: 12 male CD-1 mice
were administered a single dose of bFKB1 at 15 mg/kg SC.
Blood samples (150–200 μL) were collected via either via retro-
orbital sinus or cardiac puncture under isoflurane anesthesia
on Day 1 (at 5 min, 2hr, and 6 hr), Day 2, Day 4, Day 8 (pre-
dose), Day 10, Day 15, Day 22, Day 29, and Day 36. Samples
were collected into serum separator tubes. The blood was
allowed to clot at ambient temperature for at least 20 minutes.
Clotted samples were maintained at room temperature until
centrifuged, commencing within 1 hr of the collection time.
Each sample was centrifuged at a relative centrifugal force of
1500–2000 × g for 5 minutes at 2°C–8°C. The serum was sepa-
rated from the blood sample within 20 minutes after centrifu-
gation and transferred into labeled 2.0 mL polypropylene,
conical-bottom microcentrifuge tubes.

Single dose study in obese mice: 9 male C57BL/6j mice were
administered a single IV dose of bFKB1 at 3 mg/kg via the tail
vein. Blood samples (150–200 μL) were collected via either via retro-
orbital sinus or cardiac puncture under isoflurane anesthesia
on Day 1 (at 5 min, 2hr, and 6 hr), Day 2, Day 4, Day 8 (pre-
dose), Day 10, Day 15, Day 22, Day 29, and Day 36. Samples
were collected into serum separator tubes. The blood was
allowed to clot at ambient temperature for at least 20 minutes.
Clotted samples were maintained at room temperature until
centrifuged. Each sample was centrifuged at a relative centrifugal force of
1500–2000 × g for 5 minutes at 2°C–8°C. The serum was sepa-
rated from the blood sample within 20 minutes after centrifu-
gation and transferred into labeled 2.0 mL polypropylene,
conical-bottom microcentrifuge tubes.

Multiple dose study: 12 male C57BL/6j mice or CD-1 mice
were given five weekly SC injections of bFKB1at 15 mg/kg. Whole
blood samples (200 μL) were collected from each animal via retro-orbital sinus or cardiac puncture under anesthesia
into serum separator tubes on Days 2, 4, 8, 15, 22, 29, 30, 32, 36,
64, and 85. Samples were collected into serum separator tubes.
The blood was allowed to clot at ambient temperature for at
least 20 minutes. Clotted samples were maintained at room
temperature until centrifuged, commencing within 1 hour of
the collection time. Each sample was centrifuged at a relative centrifugal force of 1500–2000 × g for 5 minutes at
2°C–8°C. The serum was separated from the blood sample within 20 minutes after centrifugation and transferred into labeled 2.0 mL polypropylene,
conical-bottom microcentrifuge tubes.

**PK studies in obese and non-obese monkeys**

Single dose study in non-obese monkeys: Six male purpose-bred, naïve cynomolgus monkeys (Mauritius origin) between
6–7 years old and weighing 2.8–3.4 kg (average 3.1 kg) were
divided into 2 groups of 3 animals each. Animals were
individually housed, and maintained in accordance with the guidelines approved by the AAALAC. Each group was administered a single IV injection of bFKB1 at either 3 or 30 mg/kg. Blood samples (1.2 mL) were collected from each animal via the femoral vein on Day 1 (15 min, 1, 2, 4 and 8 hr), and on Days 2, 4, 8, 11, 15, 22, 28, 36, 43, 50, and 57 post dose. The blood was collected into tubes containing serum separators, and was allowed to clot at ambient temperature for at least 20 min. Clotted samples were maintained at room temperature until centrifuged, commencing within 1 hr of the collection time. Each sample was centrifuged at a relative centrifugal force of 1500–2000 × g for 10–15 minutes at 2°C–8°C. The serum was separated from the blood sample within 20 min after centrifugation and transferred into labeled 2.0 mL polypropylene, conical-bottom microcentrifuge tube.

Single dose study in obese monkey: Sixteen insulin-independent obese male cynomolgus monkeys of Chinese origin (CrownBio), 11–17 years old and weighing 8.54–14.60 kg (average 10 kg), were divided into 3 groups of 4 animals each. All animals were individually housed, had free access to water and were fed twice per day with a nutritionally balanced normal calorie diet, and were and maintained in accordance with the guidelines approved AAALAC. Each group was administered a single IV dose of bFKB1 at 0.6, 3 or 15 mg/kg. Blood (8 mL/per monkey/per time) were collected via a cephalic vein or saphenous vein 4 hr, 1, 2, 3, 7, 14, 28 and 56 days post injection into the appropriately pre-labeled BD Vacutainer® SST II Plus plastic serum tube. Blood were allowed to clot for minimum 30 min and then centrifuged within 1 hr of collection by a refrigerated centrifuge (approximately 2 to 8°C) at 1,500 to 2,000-x g for 10 to 15 minutes. The serum was aliquoted within 20 min (+/-10 min) into pre-labeled plastic serum tube. Animals were returned to their cage immediately after blood collection and supplied with monkey chow.

Weight-based dose normalization: For weight-based dose-normalization comparisons, the 3-mg/kg-dose level (that was tested in both populations of monkeys) was converted to an equivalent flat dose of 9 mg (~9 mg of bFKB1 was administered per lean monkey at this dose level compared to ~30 mg in obese; therefore, serum concentrations in obese monkeys were adjusted by a factor of 3.3 to normalize the amount of bFKB1 administered in both populations of monkeys).

Multiple dose study: 24 Male and female naive cynomolgus monkeys (Mauritius origin) between 6–7 years old, and weighing between 3.8–8.6 kg (average 5 kg) were divided into 4 groups of 6 monkeys each (3 per gender). Each group was given SC injections of bFKB1 at 0.1, 0.75, 2, or 5 mg/kg every week on study Days 1, 8, 15, 22, and 29. Blood (1.5 mL per timepoint) was collected via femoral vein on study Days 1 (predose, 2, 8, 24, and 48 hr), 8 (predose, and 2 hr), 15 (predose, and 2 hr, 22 (predose and 2 hr), 29 (predose, 2, 8, 24, and 48 hr) and 36, 43, 57, 85, and 98. Blood was collected into serum separators, and was allowed to clot at ambient temperature for at least 20 minutes. Clotted samples were maintained at room temperature until centrifuged, commencing within 1 hour of the collection time. Each sample was centrifuged at a relative centrifugal force of 1500–2000 × g for 10–15 minutes at 2°C–8°C. The serum was separated from the blood sample within 20 minutes after centrifugation and transferred into labeled 2.0 mL polypropylene, conical-bottom microcentrifuge tube.

PK assay

An ELISA was developed and validated to quantify bFKB1 in cynomolgus monkey and mouse serum samples. For monkey samples, microtiter plates were coated with sheep anti-human IgG (Binding Site; catalog # AU003.M) to capture bFKB1. Sheep anti-human IgG conjugated to horseradish peroxidase (HRP) (Binding Site; catalog # AP003CUS01) was used as the detection agent. The minimum quantifiable concentration (MQC) was 100 ng/mL in cynomolgus monkey serum. For mouse serum samples, microtiter plates were coated with sheep anti-human IgG to capture bFKB1. Sheep anti-human IgG conjugated to HRP was used as the detection agent. The MQC was 50 ng/mL in mouse serum. The serum concentration-time data from each animal were analyzed using the IV bolus input model (Model [plasma] 200–202; WinNonlin, version 6.4; Pharsight Corporation).

Adiponectin was measured using commercially available assay (R&D systems ELISA DHWAD0 with a lower /upper limit of quantification [LLOQ/ULOQ] of 0.2/2.5 μg/mL).

Disclosure statement

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