

Supplemental Material for 10.1177/0192623318772501

Supplemental text S1. Statistical analysis was performed utilizing SAS Software for Windows, release 9.1 (SAS Institute Inc, Cary, NC, USA). A significance level of 0.05 was used for all statistical tests. Analysis was performed separately for each sex. Data from terminal and recovery animals were combined at each scheduled time point up to the terminal necropsy. During acclimation and dosing periods, body weight, electrocardiogram (ECG) parameters, blood pressure, rectal temperature, hematology, coagulation, serum chemistry, and organ weight (absolute and relative to brain and body weight) parameters were assessed for homogeneity of variance using the Bartlett's test (Bartlett, 1937). When the result was not statistically significant, a one-way analysis of variance (ANOVA) was applied. When the one-way ANOVA was significant, Dunnett's test (Dunnett, 1955) was used to compare the mean values from each dose of the tanezumab with those of the control article. When Bartlett's test yielded a significant result indicating heterogeneity of variance, the data were analyzed by Kruskal-Wallis's H-test (Kruskal and Wallis, 1952). When the Kruskal-Wallis's H-test results were significant, Miller's procedure was performed to compare the dosed tanezumab groups with the control group when sample sizes were equal (Miller, 1981). Dunn's modification to Steel's test was performed when sample sizes were unequal (Dunn, 1964). During the recovery period, body weight, ECG parameters, blood pressure (including rectal temperature), hematology, coagulation, serum chemistry, and organ weight (absolute and relative to brain and body weight) parameters were analyzed by *t*-test because there was only one tanezumab group in recovery phase. Statistical analysis was

conducted on the morphometric and stereologic data using the two-tailed Student's *t*-test.

For the bone biomarker analysis, a nonparametric version of Dunnett's test was used to compare each tanezumab group with the control group. Values recorded as below the lower limit of quantitation (LLOQ) were replaced with the LLOQ for the purposes of summary statistics and statistical analyses. Statistical analyses were performed at each time point, separately for each sex, at the 0.05 and 0.01 levels of significance.

For microcomputed tomography analysis, a parametric one-way ANOVA was used to compare each tanezumab group with the control group. Separate analyses were done for each sex, and the 0.05 level of significance was used for all tests. If the overall test from the ANOVA was significant, then follow-up pairwise comparisons of each tanezumab group vs. control were performed using two-sided Fisher's least significant difference tests. If the overall test was not significant, then no follow-up testing was done.

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Supplemental text S2. In study 2, Levene's test was done to test for variance homogeneity. In the case of heterogeneity of variance at $p < 0.05$, rank transformation was used to stabilize the variance (Levene, 1960). Comparison tests took variance heterogeneity into consideration. One-way analysis of variance (ANOVA) was used to analyze data (Winer, 1971). If ANOVA was significant ($p < 0.05$), Dunnett's *t*-test was used for control vs. treated group comparisons (Dunnett, 1955). For data that exhibited heterogeneous variances after the series of transformations, Dunnett's *t*-test for unequal variances with Welch's degrees of freedom was employed (Welch, 1947).

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Supplemental text S3. In study 1 (cynomolgus monkeys), the C-telopeptide fragments of collagen type 1 (CTX1) assay used was the CrossLaps ELISA (enzyme-linked immunosorbent assay) Kit (Immunodiagnostic Systems, Bolden, Tyne & Wear, UK), the osteocalcin assay used was N-MID[®] Osteocalcin ELISA Kit (Immunodiagnostic Systems) and the type 1 collagen N-terminal propeptide (P1NP) assay used Orion Diagnostica UniQ[™] PINP RIA (Intact N-terminal Propeptide of Type I Procollagen (Immunodiagnostic Systems). In study 2 (Sprague-Dawley rats), the CTX1 assay used was the RatLaps[™] CTx Bone Biomarker Enzyme Linked Immunosorbent Assay (Immunodiagnostic Systems). The P1NP assay was performed by liquid chromatography/mass spectrometry and measures P1NP in rat serum by signature peptide quantification with stable isotope dilution. The Rat Osteocalcin IRMA Kit is a two-site immunoradiometric assay for the measurement of rat osteocalcin. A fit-for-purpose validation was done on the assay.

Supplemental text S4. In study 1 (cyno(22)molgus monkeys), blood samples for pharmacokinetic analysis were collected from all animals at the following times:

- Days 1, 22, 85, and 169: predose, and 3, 12, 24, 48, 72, and 168* hours postdose (*corresponding to predose at the next dose)
- Days 15, 43, 57, 71, 99, 113, 127, 141, and 155 predose
- On the mornings of days 183, 190, 204, 218, and 232 (recovery phase)

Plasma concentrations of tanezumab were determined using a validated enzyme-linked immunosorbent assay (ELISA) for detection and quantitation of tanezumab (Bowman et al., 2015; Zorbas et al., 2011).

For analysis of antidrug antibody (ADA) induction, blood samples were collected from animals once during the first week of acclimation, predose on days 43, 85, 127, and 176, and on days 204 and 232. ADA analysis of serum was conducted using an ELISA validated for detection of anti-tanezumab antibodies (Bowman et al., 2015; Zorbas et al., 2011).

In study 2 (rats), serial blood samples were collected from all main study animals (toxicity animals, dosing and recovery phases) approximately 6 hours postdose on study days 1 and 29. For animals evaluated for recovery, blood samples were collected approximately 672 and 1361 hours postdose after the last dose on study day 29. For toxicokinetic (TK) animals, serial blood samples were collected according to the following sampling schedule (all collections following the last dose were recovery-phase collections):

- Day 1: 0, 1, 6, 24, and 72 hours postdose
- Days 11/12 (males/females): 6 hours postdose

- Day 22 (males only): 0, 1, 6, 24, and 72 hours postdose
- Day 22 (females only): 0, 1, 6, 24, 72, and 96 hours postdose
- Day 25 (males only): 1, 24, and 96 hours postdose
- Day 26 (females only): 1, 24, and 72 hours postdose
- Day 29 (last dose): 168, 336, 504, 672, 840, 1008, 1176, and 1361 hours postdose

For analysis of ADA induction, blood samples were collected from all animals predose on day 1 (all animals) and predose and 672 and 1361 hours postdose on study day 29 (recovery animals only). Blood samples were collected from TK animals according to the following sampling schedule (all collections after the last dose were recovery-phase collections):

- Day 1 (n=11/12, males/females) and day 22: predose
- Day 29 (last dose): predose and 168, 336, 504, 672, 840, 1008, 1176, and 1361 hours postdose

Plasma tanezumab levels and serum ADA analysis were conducted using validated ELISAs similar to those used in study 1.

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Supplemental text S5. At necropsy, the left acetabulum, femur, and tibia, as well as the mandible, were collected immediately after necropsy, cleaned from soft tissues, and perfusion-fixed in paraformaldehyde, transferred to 70% alcohol, and stored at 4°C before being used for x-ray and m-CT analyses. Following x-ray and m-CT evaluations, bone specimens were stored at 4°C in 70% ethanol before they were processed for histology preparation. The bones were radiographed using a digital x-ray system (Faxitron X-Ray LLC, Wheeling, IL, USA) for 12 sec at 35 kV (settings as recommended by manufacturer based on the size of the bone samples). Radiographic images were used to assess status of the growth plates (complete or partial closures of the growth plates) and to determine if fractures or other bone abnormalities were present.

Analyses of bone samples were carried out using the Scanco *VivaCT40* mCT system (Scanco Medical, Bassersdorf, Switzerland) with the following settings for monkeys: 70 kVp at 85 μ A at high resolution (19 μ m) (Bagi et al., 2011; Gasser et al., 2005). Cancellous bone parameters were analyzed at the intertrochanteric region of the proximal femur, femoral neck, and femoral head and at the proximal tibial epiphysis. For consistency in measuring the cancellous bone parameters across the dose groups, the proximal tibia was divided into metaphysis and epiphyses and assessment of the cancellous bone was done and reported separately for these two areas as well as for the medial and lateral halves of the proximal tibial plateau. Mandibular cancellous bone parameters were analyzed only in the area below the first and second molar. Connective tissue surrounding roots of the first and second molar and cortical bone shell was not included in analyses. The following parameters were determined: bone mineral density, bone volume, tissue volume, bone volume/tissue volume ratio,

trabecular number, trabecular thickness, trabecular separation, structural model index, and connective diameter. Cortical bone parameters were analyzed at femoral mid-diaphysis and included bone mineral density, tissue volume, bone volume, bone marrow volume, and cortical thickness. In addition, polar moment of inertia and areal moments of inertia (I_{\max} and I_{\min}) were calculated in the x and y directions in 2D mode.

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Supplemental text S6. Microcomputed tomography (mCT) evaluation of bone mass, structure, and bone mineral density (BMD) was conducted on the right femur collected at necropsy. After dissection, the femur was gently cleaned of soft tissues with a scalpel blade. All bone samples were stored in 10% buffered formalin for a minimum of 48 hours and then transferred to 70% ethanol.

Ex vivo mCT analysis was performed on the whole femur and for the cancellous bone on distal femoral metaphyses utilizing a *viva* mCT-40[®] computed tomography system (Scanco Medical, Bassersdorf, Switzerland), using a previously described technique (Hanson and Bagi, 2004; Laib et al., 2000). Prior to scanning, the distal femur sample was oriented horizontally in a 16.5-mm sample holder with the epiphyseal head facing outward. A control file, or measurement protocol, was created to define scanning parameters such as source energy, sample size, and image resolution desired. Parameters selected for this study included a source voltage of 55 kV and electric current strength of 109 μ A to obtain the best contrast between bone and soft tissues. The sample area selected for three-dimensional structural analysis of cancellous bone was a 2.0-mm length of the metaphyseal secondary spongiosa, originating 0.5 mm below the epiphyseal growth plate and extending cranially. The following cancellous bone parameters were measured in *ex vivo* settings: total volume (TV; bone and bone marrow), bone volume (BV), bone surface, BV/TV, bone surface/bone volume, trabecular number, trabecular thickness, trabecular separation, connective diameter (density), structure model index (for shape), and BMD.

mCT was also conducted on the right tibial epiphysis and metaphysis, and the same cancellous bone parameters described for distal femoral metaphysis were measured as described previously (LaBranche et al., 2017).

mCT of the cortical bone was conducted on the right femur using a μ CT-100[®] computed tomography system (Bagi et al., 2006). The cortical bone parameters were evaluated at mid-diaphysis (midshaft) with a MicroCT 100[®] system using a previously described method. Sample measurements (scans) were performed on 25 slices (1 slice = 10.5 μ m) using high-resolution settings, and the average value was used for the final calculation. The following parameters were evaluated at the cortical mid-diaphysis: BMD, TV, BV, bone marrow volume, cortical thickness, bone area, polar moment of inertia, maximal I value (I_{max}), minimal I value (I_{min}), and I_{max}/C_{max} .

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Supplemental text S7. No animals were found dead. Three animals were euthanized early. One male in the 4 mg/kg group was euthanized on day 13 because of moribund condition combined with skin lesions/multiple abrasions on the right footpad, base of the tail, feet, and ankles. Most of the skin findings (including abrasions, hair loss, and bruising) were present during the acclimation period. Multifocal skin ulcerations were noted at gross and microscopic examination, the cause of which was not determined. One male in the 30-mg/kg iv group was euthanized on day 100 due to a severe necrotic lesion on the base of the tail, that was determined on histopathology to be areas of ulceration—and fibrosis. This and other skin lesions were present during the acclimation and dosing periods. One male in the 30-mg/kg sc group was found lethargic, hunched, and shivering on day 172 and was euthanized on day 173. Gross examination revealed bilaterally enlarged kidneys. Microscopic evaluation revealed marked bilateral suppurative nephritis that was severe enough to be clinically significant and was the likely source of the clinical signs in this animal. The origin of the inflammation was most likely an ascending bacterial infection. These three sacrifices were not considered to be related to tanezumab administration.

Supplemental text S8. Exposure (C_{max} and AUC_{0-72}) in the satellite TK animals was slightly increased on day 22 compared with day 1 of the dosing phase. Exposure (mean plasma concentrations in main study animals) was slightly increased on day 29 vs. day 1 of the dosing phase. With one exception (female, day 29, 6 hours post-dose, 0.0555 $\mu\text{g/ml}$), no quantifiable tanezumab was measured in control main study animals. Quantifiable concentrations of tanezumab were observed throughout the recovery phase in most animals.

Antidrug antibody (ADA) development was observed in 20 of 68 animals administered 0.2 mg/kg/dose, 22 of 68 animals administered 1 mg/kg/dose, and 33 of 68 animals administered 10 mg/kg/dose (endpoint titer results ranged from <4.64 to 23.50. For the ADA analysis on samples collected prior to dose administration on day 1 of the dosing phase, 18/135 males and 17/135 females tested and were confirmed positive for ADA. As none of these animals had been exposed to any dose formulation, these were false-positive results. On day 29 of the dosing phase, 3/33 control males and 31/33 control females (toxicity and toxicokinetic combined) tested and were confirmed positive for ADA. On day 57 of the recovery phase, 2/13 control males and 13/13 control females (toxicity and toxicokinetic combined) tested and were confirmed positive for ADA. There were no quantifiable concentrations of tanezumab in any control males, but there were in 4/33 control females. Although no evidence was found in the dose formulation or dose administration to suggest that female control rats were directly exposed to tanezumab, the combined exposure and ADA data suggest that one of the vehicle control article formulations for the females after day 12 of the dosing phase may have been contaminated with a very low level of tanezumab. Regardless, these findings

do not affect the inferences or conclusions drawn for the ADA or toxicokinetic analysis for this study or the overall study evaluation, as it appears to have been a very low level of tanezumab contamination.

Supplemental text S9. Male rats administered 1 or 10 mg/kg tanezumab had significantly lower mean body weight gain during the second half of the dosing phase (5% and 8%, respectively) and slightly lower mean body weight (2% to 3% lower) by the end of the dosing phase compared with controls. Although tanezumab-related, effects on body weight were minimal, did not correlate with decreased food consumption, and reversed during the recovery phase; therefore, they were not adverse events. Body weights for treated females were comparable with controls during the dosing phase. However, during the recovery phase, tanezumab-treated females had higher body weight gain (29% to 36% higher than controls; not statistically significant) that resulted in higher mean body weight by the end of the recovery phase (5% to 12% higher than controls). The tanezumab-related increase in mean body weight and mean body weight gain correlated with increased food consumption but did not correlate with alterations in clinical pathology parameters and were not adverse events.

Tanezumab administration was associated with several small effects on clinical pathology parameters, some of which were present at all doses and were consistent with a minor inflammatory response and correlated with skin lesions present in these animals. Tanezumab-related effects on hematology and coagulation parameters included minimally lower red blood cell mass for males and females administered 1 or 10 mg/kg/dose, minimally higher white blood cell count for males administered 10 mg/kg/dose and females administered 1 or 10 mg/kg/dose, minimally to mildly higher absolute neutrophil count for males and females at all doses, minimally higher absolute monocyte count for females administered 1 or 10 mg/kg/dose, and minimally higher fibrinogen for males at all doses.

Tanezumab-related effects on clinical chemistry parameters included minimally to mildly lower albumin for males at all doses and females administered 1 or 10 mg/kg/dose, minimally higher globulin levels for males administered 10 mg/kg/dose and females administered 1 or 10 mg/kg/dose, and minimally to mildly lower albumin-to-globulin ratio for males and females administered 1 or 10 mg/kg/dose. All tanezumab-related effects on clinical pathology parameters reversed following recovery. No tanezumab-related deaths occurred.

Supplemental Table S1. Animals and study designs

							Non-dosing 8-week Recovery	
							Treatment	
Dose (mg/kg/wk)	Group	Substance	Route	Male (n)	Female (n)	Duration (weeks)	Male (n)	Female (n)
Study 1 (monkeys)								
0	1	Vehicle ^a	sc	10	10	26	4	4
4	2	Tanezumab	iv	6	6	26		
30	3	Tanezumab	iv	6	6	26		
30	4	Tanezumab	sc	10	10	26	4	4
Study 2, toxicity (rats) ^{b,c}								
0	5	Vehicle ^a	iv	30	30	4	10	10
0.4	6	Tanezumab	iv	30	30	4	10	10
2	7	Tanezumab	iv	30	30	4	10	10
20	8	Tanezumab	iv	30	30	4	10	10
Study 2, toxicokinetic (rats)								
0	9	Vehicle ^a	iv	3	3	4		
0.4	10	Tanezumab	iv	4	4	4		
2	11	Tanezumab	iv	4	4	4		
20	12	Tanezumab	iv	4	4	4		

Study 3 (mice)						
10	13	Mouse IgG1	sc	–	13	12
10	14	muMab911	sc	–	13	12

^aReceived vehicle control article/diluent only.

^bFor the animals on the toxicity arm of study 2, up to 20 animals/sex in Groups 5 to 8, dependent on survival, were designated for euthanasia at the end of the dosing phase. The first 10 animals/sex in Groups 5 to 8, dependent on survival, were assigned to the neural tissue collections subset of animals, and the remaining 10 animals/sex, dependent on survival, were assigned to the bone and standard tissue collections subset of animals.

^cFor the animals on the toxicity arm of study 2, up to 10 animals/sex in Groups 5 to 8, dependent on survival, underwent at least 8 weeks of recovery following the 4-week dosing phase (nine total doses). The first five recovery animals/sex in Groups 5 to 8, dependent on survival, were assigned to the specialized neural tissue collections subset of animals, and the remaining five recovery animals/sex were assigned to the bone and standard tissue collections subset of animals (dependent on survival).

Supplemental Table S2. Monkey biomarker data

Parameter (ng/mL)	Day	Tanezumab Dose (mg/kg/wk), mean ± sd (n)			
		0	4 iv	30 iv	30 sc
Males					
Osteocalcin	-51	6.10 ± 0.00 (3)	6.10 ± 0.00 (3)	6.73 ± 1.10 (3)	7.53 ± 2.48 (3)
	-50	6.27 ± 0.45 (7)	6.50 ± 0.69 (3)	8.20 ± 3.64 (3)	6.47 ± 0.90 (7)
	85	7.09 ± 1.89 (10)	6.88 ± 1.08 (5)	7.45 ± 2.43 (6)	6.92 ± 1.45 (10)
	169	4.31 ± 0.88 (10)	4.46 ± 0.54 (5)	4.22 ± 0.54 (5)	4.89 ± 1.49 (10)
	223	3.70 ± 0.00 (4)	–	–	3.85 ± 0.30 (4)
CTX1	-51	2.73 ± 1.10 (3)	2.20 ± 0.61 (3)	2.30 ± 0.99 (3)	2.70 ± 0.96 (3)
	-50	2.39 ± 0.29 (7)	2.20 ± 0.27 (3)	2.70 ± 0.50 (3)	2.16 ± 0.60 (7)
	85	3.07 ± 0.75 (10)	2.36 ± 0.48 (5)	2.42 ± 0.62 (6)	2.11 ± 0.60(10) ^a
	169	2.54 ± 0.70 (10)	2.88 ± 0.78 (5)	2.40 ± 1.03 (5)	2.13 ± 1.44 (10)
	223	1.73 ± 0.52 (4)	–	–	1.83 ± 1.16 (4)
P1NP	-51	613.0 ± 97.07 (3)	610.3 ± 69.26 (3)	628.0 ± 163.12 (3)	627.0 ± 80.89 (3)
	-50	594.4 ± 163.61 (7)	572.3 ± 262.31 (3)	571.7 ± 190.81 (3)	638.9 ± 174.86 (7)
	85	605.3 ± 193.28 (10)	639.8 ± 181.42 (5)	579.5 ± 201.41 (6)	561.7 ± 275.22 (10)
	169	576.9 ± 111.61 (10)	654.8 ± 72.52 (5)	470.4 ± 163.67 (5)	526.2 ± 209.84 (10)
	223	778.0 ± 225.99 (4)	–	–	656.3 ± 129.41 (4)

Females

Osteocalcin	-55	6.10 ± 0.00 (3)	6.10 ± 0.00 (3)	6.10 ± 0.00 (3)	6.10 ± 0.00 (3)
	-54	6.10 ± 0.00 (7)	6.10 ± 0.00 (3)	6.10 ± 0.00 (3)	6.10 ± 0.00 (7)
	85	6.10 ± 0.00 (10)	6.10 ± 0.00 (6)	6.10 ± 0.00 (6)	6.13 ± 0.10 (10)
	169	3.70 ± 0.00 (10)	3.70 ± 0.00 (6)	3.70 ± 0.00 (6)	3.81 ± 0.26 (10)
	218	3.70 ± 0.00 (4)	–	–	3.70 ± 0.00 (4)
CTX1	-55	1.90 ± 0.61 (3)	2.37 ± 0.61 (3)	2.33 ± 0.71 (3)	2.10 ± 0.27 (3)
	-54	1.81 ± 0.49 (7)	2.27 ± 0.31 (3)	2.07 ± 0.71 (3)	2.33 ± 0.80 (7)
	85	1.60 ± 0.39 (10)	1.78 ± 0.50 (6)	1.85 ± 0.60 (6)	1.51 ± 0.36 (10)
	169	1.37 ± 0.31 (10)	1.38 ± 0.47 (6)	1.55 ± 0.72 (6)	1.24 ± 0.40 (10)
	218	1.03 ± 0.40 (4)	–	–	1.25 ± 0.55 (4)
P1NP	-55	433.0 ± 110.27 (3)	438.7 ± 93.22 (3)	443.3 ± 167.7 (3)	398.7 ± 142.07 (3)
	-54	447.3 ± 93.83 (7)	417.3 ± 66.34 (3)	348.7 ± 76.71 (3)	376.9 ± 88.51 (7)
	85	403.7 ± 93.78 (10)	297.5 ± 104.12 (6)	391.2 ± 172.31 (6)	303.9 ± 99.81 (10)
	169	388.6 ± 107.31 (10)	291.8 ± 84.36 (6)	364.2 ± 113.19 (6)	301.3 ± 126.61 (10)
	218	296.5 ± 76.36 (4)	–	–	325.5 ± 117.93 (4)

*aSignificantly different from control, p≤0.01.

CTX1, C-telopeptide fragments of collagen Type 1; P1NP, procollagen 1 N-terminal peptide; s.d., standard deviation.

Supplemental Table S3. Mean toxicokinetic parameters for tanezumab in monkeys (ADA-positive animals excluded^a) after tanezumab administration

Parameter	Dose (mg/kg)	Day 1		Day 22		Day 85		Day 169	
		Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
T _{max} (h)	4 (iv)	3.0	0.0	3.0	0.0	3.0	0.0	3.0	0.0
	30 (iv)	3.0	0.0	3.0	0.0	3.0	0.0	3.0	0.0
	30 (sc)	68	27	38	21	33	20	48	34
C _{max} (µg/mL) ^b	4 (iv)	101	12.5	184	25.2	232	54.7	233	70.4
	30 (iv)	839	89.1	1310	209	1580	329	1610	277
	30 (sc)	301	47.3	807	357	1150	231	1140	241
AUC ₍₀₋₁₆₈₎ (µg·h/mL)	4 (iv)	8830	1390	20400	4120	26400	8200	26000	10800
	30 (iv)	66900	10300	135000	34100	175000	49100	175000	43400
	30 (sc)	43600	6690	108000	27200	175000	35600	171000	38800

^aThere was one ADA-positive animal in the 30 iv group and one in the 30 sc group. Both animals tested positive starting on day 43, which was the first day of ADA testing post dose administration.

^bLower limit of quantitation was 0.01 µg/ml.

ADA, anti-drug antibodies; AUC₍₀₋₁₆₈₎, area under the plasma drug concentration-time curve from 0 to 168 hours; C_{max}, highest drug concentration observed in plasma; sd, standard deviation; T_{max}, time at which maximum concentration was first observed.

Supplemental Table S4. Mean toxicokinetic parameters for tanezumab in rats (satellite TK animals^a) after tanezumab administration

Dose (mg/kg) ^b	Study Day	Sex	C _{max} ^d (µg/mL)			T _{max} (h)			AUC ₀₋₇₂ (µg·h/mL)			AUC ₀₋₉₆ (µg·h/mL)			AUC ₀₋₁₆₈ (µg·h/mL)		
			Mean	s.d.	n	Mean	s.d.	N	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n
0.2	1	Male	4.59	0.573	4	1.0	0.0	4	161	5.74	4	NA	NA	NA	NA	NA	NA
		Female	4.31	0.194	4	1.0	0.0	4	158	3.86	4	NA	NA	NA	NA	NA	NA
		Overall	4.45	0.424	8	1.0	0.0	8	159	4.76	8	NA	NA	NA	NA	NA	NA
	22	Male	9.25	1.92	4	1.0	0.0	4	377	127	4	NA	NA	NA	NA	NA	NA
		Female ^c	11.1	0.911	4	1.0	0.0	4	529	31.7	4	671	39.0	4	NA	NA	NA
		Overall ^c	10.2	1.70	8	1.0	0.0	8	453	119	8	NA	NA	NA	NA	NA	NA
	25	Male	6.47	3.27	4	6.8	12	4	NA	NA	NA	366	331	4	NA	NA	NA
	26	Female	9.07	1.86	4	6.8	12	4	526	64.9	4	NA	NA	NA	NA	NA	NA
	Wk 4 ^e	Male	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	742	444	4
		Female	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1200	103	4
Overall		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	969	385	8	
1.0	1	Male	18.0	11.9	4	6.8	12	4	652	416	4	NA	NA	NA	NA	NA	NA
		Female	25.4	0.818	4	1.0	0.0	4	856	37.8	4	NA	NA	NA	NA	NA	NA
		Overall	21.7	8.74	8	3.9	8.1	8	754	295	8	NA	NA	NA	NA	NA	NA
	22	Male	38.6	22.8	4	1.0	0.0	4	1840	1260	4	NA	NA	NA	NA	NA	NA
		Female ^c	52.6	8.29	4	1.0	0.0	4	2880	534	4	3650	676	4	NA	NA	NA
		Overall ^c	45.6	17.5	8	1.0	0.0	8	2360	1050	8	NA	NA	NA	NA	NA	NA
	25	Male	35.3	27.4	4	6.8	12	4	NA	NA	NA	2260	1650	4	NA	NA	NA
	26	Female	53.8	7.82	4	1.0	0.0	4	2590	611	4	NA	NA	NA	NA	NA	NA

	Wk 4 ^e	Male	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4100	2900	4
		Female	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6230	835	4
		Overall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5170	2280	8
10	1	Male	247	14.7	4	1.0	0.0	4	8460	583	4	NA	NA	NA	NA	NA	NA
		Female	233	20.9	4	1.0	0.0	4	8160	181	4	NA	NA	NA	NA	NA	NA
		Overall	240	18.5	8	1.0	0.0	8	8310	430	8	NA	NA	NA	NA	NA	NA
	22	Male	363	172	4	1.0	0.0	4	15800	10900	4	NA	NA	NA	NA	NA	NA
		Female ^c	463	124	4	2.3	2.5	4	24100	5120	4	30600	6570	4	NA	NA	NA
		Overall ^c	413	149	8	1.6	1.8	8	20000	9020	8	NA	NA	NA	NA	NA	NA
	25	Male	363	211	4	1.0	0.0	4	NA	NA	NA	21900	15300	4	NA	NA	NA
	26	Female	469	71.6	4	1.0	0.0	4	24000	5290	4	NA	NA	NA	NA	NA	NA
	Wk 4 ^e	Male	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	37700	26100	4
		Female	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	54600	11600	4
		Overall	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	46200	20800	8

^aData from all satellite TK animals (regardless of ADA status).

^bDose was intravenously administered twice weekly for a total of nine doses.

^cFemale and overall mean AUC₍₀₋₇₂₎ and SD manually calculated.

^dLower limit of quantitation was 0.05 µg/mL.

^eCombined data from study day 22 and study days 25/26 (males/females) to create a weekly interval. AUC₀₋₁₆₈ was manually calculated by adding the AUC_{0-t} from study day 22 (AUC₀₋₇₂ for males; AUC₀₋₉₆ for females) and study day 25/26 (AUC₀₋₉₆ for males; AUC₀₋₇₂ for females).

ADA, antidrug antibody; AUC, area under the plasma drug concentration-time curve from 0 to t (72, 96, or 168 hours); C_{max}, highest drug concentration observed in plasma; n, number; NA, not applicable; Overall, combined males plus females; s.d., standard deviation; TK, toxicokinetic; T_{max}, time at which maximum plasma concentration was first observed.

Supplemental Table S5. Incidence of sores/scabs in study 2

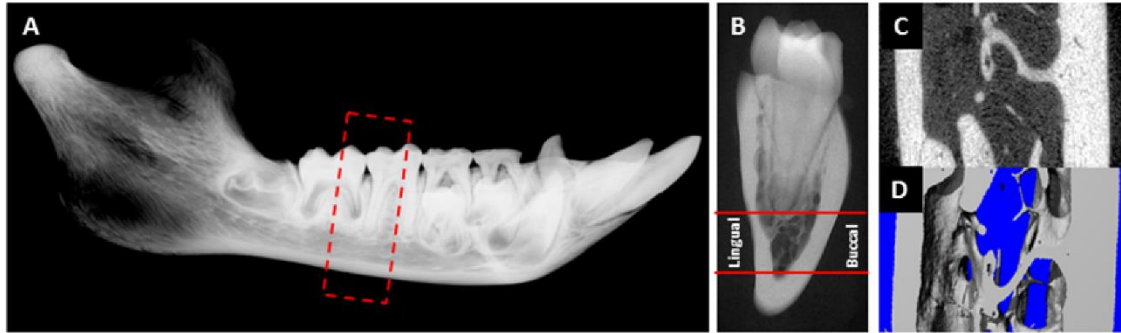
Dose	Dosing Phase	Recovery Phase
(mg/kg/wk)	n (%)	n (%)
Male rats		
Control	0 / 20 (0.0)	0 / 10 (0.0)
0.1	15 / 20 (75.0)	3 / 11 (27.3)
1.0	17 / 20 (85.0)	8 / 10 (80.0)
10.0	18 / 20 (90.0)	9 / 10 (90.0)
Female rats		
Control	0 / 20 (0.0)	0 / 10 (0.0)
0.1	14 / 20 (70.0)	6 / 9 (66.7)
1.0	15 / 20 (75.0)	11 / 11 (100.0)
10.0	19 / 20 (95.0)	9 / 10 (90.0)

Supplemental Figure S1. 2-D and 3-D images of the proximal tibial epiphysis (E) and metaphysis (M) in male and female monkeys. Arrows indicate an open growth plate in some male monkeys

		Group 1 (n=6) No treatment		Group 2 (n=5) 4 mg/kg/wk iv		Group 3 (n=5) 30 mg/kg/wk iv		Group 4 (n=6) 30 mg/kg/week sc	
Male Monkeys, mean ± sd	E								
	M								
	Site								
BMD, g/cm²	Medial	815.61 ± 12.75	815.28 ± 10.03	804.81 ± 9.95	800.08 ± 10.76				
	Lateral	769.48 ± 21.54	768.68 ± 6.43	777.23 ± 11.67	777.83 ± 14.55				
Bone volume, mm³	Medial	9.83 ± 1.58	11.03 ± 1.12	9.83 ± 1.94	8.91 ± 1.65				
	Lateral	10.06 ± 1.62	10.07 ± 1.74	11.21 ± 2.24	12.46 ± 1.19				
Bone surface, mm	Medial	86.22 ± 9.84	98.74 ± 19.60	98.43 ± 14.36	99.52 ± 7.92				
	Lateral	106.66 ± 9.36	114.25 ± 8.49	116.14 ± 15.36	104.18 ± 7.49				
Trabecular number, 1/mm	Medial	1.75 ± 0.17	1.86 ± 0.36	1.87 ± 0.16	1.92 ± 0.15				
	Lateral	1.92 ± 0.14	2.08 ± 0.23	2.08 ± 0.30	1.88 ± 0.08				
Trabecular thickness, μm	Medial	0.23 ± 0.05	0.24 ± 0.08	0.20 ± 0.04	0.18 ± 0.03				
	Lateral	0.19 ± 0.03	0.18 ± 0.03	0.19 ± 0.04	0.24 ± 0.03				
Trabecular separation, μm	Medial	0.35 ± 0.05	0.32 ± 0.05	0.33 ± 0.02	0.34 ± 0.06				
	Lateral	0.33 ± 0.05	0.31 ± 0.06	0.29 ± 0.06	0.29 ± 0.02				
<hr/>									
		Group 1 (n=6) No treatment		Group 2 (n=5) 4 mg/kg/wk iv		Group 3 (n=5) 30 mg/kg/wk iv		Group 4 (n=6) 30 mg/kg/wk sc	
Female Monkeys, mean ± sd	E								
	M								
	Site								
BMD, g/cm²	Medial	775.76 ± 16.29	779.57 ± 13.79	796.29 ± 16.44	793.82 ± 12.29				
	Lateral	805.47 ± 14.55	805.38 ± 17.85	796.15 ± 10.05	798.47 ± 9.48				
Bone volume, mm³	Medial	11.98 ± 1.92	13.57 ± 2.83	14.29 ± 3.25	13.55 ± 2.53				
	Lateral	9.75 ± 1.35	9.66 ± 1.69	8.23 ± 1.11	8.79 ± 1.26				
Bone surface, mm	Medial	106.32 ± 9.07	121.45 ± 12.16	102.01 ± 16.78	103.36 ± 7.99				
	Lateral	91.09 ± 13.17	89.98 ± 9.04	92.01 ± 12.46	98.62 ± 9.58				
Trabecular number, 1/mm	Medial	1.89 ± 0.16	2.01 ± 0.21	1.82 ± 0.24	1.89 ± 0.10				
	Lateral	1.86 ± 0.23	1.78 ± 0.21	1.93 ± 0.24	2.04 ± 0.14				
Trabecular thickness, μm	Medial	0.23 ± 0.04	0.23 ± 0.06	0.20 ± 0.10	0.26 ± 0.04				
	Lateral	0.22 ± 0.04	0.22 ± 0.05	0.18 ± 0.03	0.18 ± 0.03				
Trabecular separation, μm	Medial	0.31 ± 0.06	0.27 ± 0.04	0.27 ± 0.03	0.27 ± 0.03				
	Lateral	0.33 ± 0.05	0.35 ± 0.04	0.34 ± 0.05	0.31 ± 0.05				

2/3-D, two/three-dimensional; BMD, bone mineral density.

Supplemental Figure S2. Example of radiographs of the **male** monkey mandible (**A**) showing area of the first and second molar (dotted red rectangle) that was cut in buccal-lingual plane shown between two red lines in **B**. This area was utilized for mCT assessment of 2-D (**C**) and 3-D (**D**) cancellous bone parameters and BMD.



	Tanezumab Dose (mg/kg/wk), mean \pm sd			
	0	4 iv	30 iv	30 sc
n	6	5	5	6
Cancellous TV, mm ³	41.63 \pm 18.83	41.25 \pm 17.38	52.44 \pm 15.48	44.89 \pm 9.16
Cancellous BV, mm ³	6.85 \pm 3.42	5.54 \pm 2.25	8.12 \pm 2.29	8.88 \pm 2.67
BV/TV ratio	0.18 \pm 0.10	0.17 \pm 0.11	0.16 \pm 0.03	0.20 \pm 0.07
Cancellous bone surface, mm	75.49 \pm 26.99	63.21 \pm 26.67	84.79 \pm 16.86	83.26 \pm 19.90
Bone surface/BV ratio	11.98 \pm 2.72	12.26 \pm 4.25	10.71 \pm 1.83	9.57 \pm 1.23
Trabecular number, 1/mm	0.96 \pm 0.39	0.85 \pm 0.35	0.84 \pm 0.21	0.96 \pm 0.29
Trabecular thickness, μ m	0.18 \pm 0.05	0.18 \pm 0.07	0.19 \pm 0.03	0.21 \pm 0.03
Trabecular separation, μ m	1.04 \pm 0.55	1.25 \pm 0.89	1.05 \pm 0.24	0.92 \pm 0.36
Cancellous BMD, g/cm ²	895.34 \pm 27.78	893.62 \pm 31.99	897.55 \pm 16.13	901.88 \pm 41.59
Connectivity diameter, 1/cm ³	2.69 \pm 1.71	3.49 \pm 1.96	2.96 \pm 1.31	3.11 \pm 1.45
SMI	0.78 \pm 1.17	1.41 \pm 1.59	0.96 \pm 0.36	0.34 \pm 0.71
Cortical BV, mm ³	39.65 \pm 7.30	35.82 \pm 5.21	39.73 \pm 7.16	38.49 \pm 5.83
Cortical BMD, g/cm ²	997.56 \pm 42.01	1022.45 \pm 12.60	1012.03 \pm 35.97	1010.24 \pm 44.08
Cortical buccal thickness, cm	1.60 \pm 0.09	1.67 \pm 0.19	1.66 \pm 0.21	1.61 \pm 0.19
Cortical lingual thickness, cm	1.04 \pm 0.19	1.13 \pm 0.23	0.97 \pm 0.08	0.97 \pm 0.12

2-/3-D, two/three-dimensional; BMD, bone mineral density; BV, bone volume; mCT, microcomputed tomography; SMI, structural model index; TV, tissue volume.

