AIMS
The aims were to 1) develop the pharmacokinetics model to describe and predict observed tanezumab concentrations over time, 2) test possible covariate parameter relationships that could influence clearance and distribution and 3) assess the impact of fixed dosing vs. a dosing regimen adjusted by body weight.

METHODS
Individual concentration–time data were determined from 1608 patients in four phase 3 studies conducted to assess efficacy and safety of intravenous tanezumab. Patients received two or three intravenous doses (2.5, 5 or 10 mg) every 8 weeks. Blood samples for assessment of tanezumab PK were collected at baseline, 1 h post-dose and at weeks 4, 8, 16 and 24 (or early termination) in all studies. Blood samples were collected at week 32 in two studies. Plasma samples were analyzed using a sensitive, specific, validated enzyme-linked immunosorbent assay.

RESULTS
A two compartment model with parallel linear and non-linear elimination processes adequately described the data. Population estimates for clearance (CL), central volume ($V_1$), peripheral volume ($V_2$), inter-compartmental clearance, maximum elimination capacity (VM) and concentration at half-maximum elimination capacity were 0.135 l day$^{-1}$, 2.71 l, 1.98 l, 0.371 l day$^{-1}$, 8.03 μg day$^{-1}$ and 27.7 ng ml$^{-1}$, respectively. Inter-individual variability (IIV) was included on CL, $V_1$, $V_2$ and VM. A mixture model accounted for the distribution of residual error. While gender, dose and creatinine clearance were significant covariates, only body weight as a covariate of CL, $V_1$ and $V_2$ significantly reduced IIV.

CONCLUSIONS
The small increase in variability associated with fixed dosing is consistent with other monoclonal antibodies and does not change risk : benefit.
Introduction

At least 116 million adults in the United States are burdened by chronic pain at some point in their lives, leading to an annual cost of $560–$635 billion for direct medical treatment and lost productivity [1]. Although paracetamol (acetaminophen), non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are considered the gold standard analgesic drugs in clinical practice [2], management of chronic pain is often ineffective or incomplete. Therefore, effective pain relief represents an unmet medical need [3, 4]. The cardiovascular risks and gastrointestinal, hepatic and renal side effects of NSAIDs and paracetamol may limit the use of these medications [4, 5]. Similarly, opioids are associated with a broad spectrum of side effects as well as the potential for loss of effectiveness, constipation, drug diversion or addiction, respiratory depression and accidental death from overdose [2, 4].

Nerve growth factor (NGF) is a mediator of biological mechanisms that cause pain [6]. Elevated NGF levels are associated with inflammation, tissue damage and diseases such as arthritis. NGF administration causes pain, whereas blockade of NGF activity reduces pain [3]. As a result, targeting NGF is a new mechanism being investigated for the treatment of moderate to severe chronic pain. Tanezumab is a humanized immunoglobulin G (IgG) 2 monoclonal antibody that binds to NGF with high affinity and specificity and effectively blocks NGF activity [7]. Tanezumab or its murine precursor is an effective analgesic in animal models of pathological pain, including arthritis and cancer pain [8, 9]. In randomized clinical trials in patients with osteoarthritis (OA) and chronic low back pain, tanezumab has demonstrated consistent efficacy across the domains of pain and physical function [10–12].

An initial population model describing the pharmacokinetics (PK) and pharmacodynamics (PD) of a wide dose range of tanezumab in patients with OA was used to determine the dose and dose regimen for further development [13, 14]. The current analysis characterizes PK across the domains of pain and physical function [10–12].

Methods

Bioanalytical PK assay

Plasma samples were analyzed using a sensitive, specific, validated enzyme-linked immunosorbent assay for tanezumab with a lower limit of quantification (LLOQ) of 12.0 ng ml$^{-1}$ and with a range of 12 to 384 ng ml$^{-1}$ in 100% human plasma (see Supporting Information). Results were analyzed via four parameter logistic curve-fitting programmes and 1/y$^2$ weighting (GenS Secure Software version 1.02 or higher, Winooski, VT, USA). In each of seven analysis runs (performed over 3 days), three replicates of quality control (QC) samples at five concentrations (12.0, 30.0, 150, 300 and 384 ng ml$^{-1}$) were analyzed. Precision, defined by the coefficient of variation (standard deviation/mean × 100), was determined from interpolated (observed) QC sample concentrations and ranged from 2.08% to 11.5% for the inter-runs and ranged from 0.337% to 11.8% for the seven intra-runs. Accuracy was defined by the percent relative error ([observed concentration/nominal concentration] – 1) × 100, and ranged from 0.833% to 7.33% for the inter-runs and from 0% to 15.3% for the intra-runs. To evaluate dilution linearity, six different dilutions were assessed: minimum dilution (1 : 20), 1 : 2000, 1 : 4000, 1 : 6667, 1 : 13 333 and 1 : 133 333. The acceptance criteria were met up to the 13 333-fold dilution with the samples diluted 6667-fold and 13 333-fold showing precision of 0.654% and 1.25%, respectively, and accuracy of 6.00% and 10.7%, respectively, indicating that this assay can accurately measure free tanezumab concentration up to 5 mg ml$^{-1}$.
Final analysis data set
During assembly of the PK data set that was compatible with the modelling software (NONMEM, ICON Development Solutions, Hanover, MD, USA), a number of data issues were identified (for example, unrealistic peak : trough ratios) that were judged likely to have a significant impact on the model building process and covariate identification. To handle the most likely incorrect data which probably originated from either not matching the correct sample with the correct prelabelled collection tube or collecting both pre- and post-dose samples either pre-dose or post-dose, the following cleaning rules were applied:

1) Individuals \( (n = 4) \) with baseline PK observation \( \geq \)750 ng ml\(^{-1} \) and a sample after the first dose in the range of 40–384 ng ml\(^{-1} \) were excluded. The range of 40–384 ng ml\(^{-1} \) was used for the apparent post-sample because this was the range of matrix interference in baseline samples. The cut off value for the apparent baseline sample was set at 750 ng ml\(^{-1} \) since it was two-fold above the maximum baseline background interference and represented 80% of the post first dose 2.5 mg C\(_{\text{max}}\).

2) Each pair of peak/trough observations in which the ratio was \( < 2 \) was excluded (\( n = 188 \)). This exclusion rule addressed specific pre-dose/post-dose (peak/trough) sample pairs that were close in value which could happen if, in error, the post-dose sample was collected pre-dose, or the pre-dose sample collected post-dose. A ratio \( < 2 \) was selected for the cut-off value since this is an unrealistic value considering the expected peak : trough ratio was 10 : 1 based on phase 1 data.

3) The complete PK profile of patients who still had baseline observations \( \geq 0 \) was excluded (\( n = 190 \)). This exclusion criterion was necessary since there were still subjects with detectable levels of drug in the baseline samples despite rules one and two due to lack of a first post-dose sample to help interpret baseline values.

Finally, outlying data points were identified based on initial modelling results and distribution of conditional weighted residuals (CWRES). Data points associated with absolute CWRES \( \geq 5 \) were regarded as outliers and were excluded from further model development. These outlying data points as well as patients excluded based on cleaning rule 3 (\( n = 190 \)) were re-included when the final model was established to assess their impact on parameter estimates.

Pharmacokinetic analysis
All analyses were performed in accordance with appropriate regulatory guidelines [16, 17]. NONMEM version 7.1 was used with Fortran compiler IntelR64 Fortran Professional Compiler version 11.1. The first order conditional estimation method (with or without INTERACTION as appropriate) was used for all analyses. The final model was implemented in ADVAN6. Automated NONMEM run procedures, such as the stepwise covariate model (SCM) building procedure in Perl-speaks-NONMEM (PsN) and visual predictive check (VPC), were implemented using PsN version 3.2.4 [18]. Results were further processed using R Software version 2.10.1. Xpose version 4 was used as an aid in model assessment [19].

Previous work based on phase 1 and 2 data indicated that a two compartment model described tanezumab PK adequately over the clinical dose range. In this two compartment model, WT was an important predictor for interindividual variability in clearance (CL), central volume of distribution (\( V_1 \)) and peripheral volume of distribution (\( V_2 \)). Inter-individual variability (IIV) was included on CL, \( V_1 \) and \( V_2 \) assuming an exponential distribution and variability in residual error was accounted for by log transformation of both sides (i.e. approximately constant coefficient of variation model). This model was used as a starting point for the development of the base PK model for the phase 3 data. Because WT was important in the previous model, different body size measures (WT, body surface area [BSA], body mass index [BMI] and baseline lean body weight [BLBW]) were considered as structural covariates in the form of power functions in the base PK model for the phase 3 data. In addition to body size as a structural covariate, different structural PK models (one and three compartment models, with and without non-linear elimination) were also considered, as well as other IIV and residual error structures. Variability estimates were reported as the coefficient of variation (\%CV, calculated as the square root of variance) estimated by assuming the exponential distribution (IIV) or under the log transformation (residual error).

The appropriateness of the base PK model was assessed by inspection of residual plots and visual predictive checks (VPCs). This basic PK model with the supported IIV terms was used as the starting point for covariate model development. Various patient/disease characteristics were evaluated, including dose, age, gender and race on CL, \( V_1 \) and \( V_2 \) and race, gender and age on maximum elimination capacity (VM) and concentration at half-maximum elimination capacity (KM). In addition, creatinine clearance (CL\(_{\text{cr}}\)) was tested on CL and was computed using the Cockroft–Gault formula utilizing total body weight [20]. Since this formula leads to unreasonably high values for heavy subjects [21], CL\(_{\text{cr}}\) values were truncated at a maximum value of 150 ml min\(^{-1} \). Also, the impact of OA joint (knee or hip) was investigated on all structural model parameters. Missing values for covariates were imputed with the median value or common category during analysis (with
the exception that missing values for OA joint were imputed as knee because missing values occurred only in study A4091011, which only enrolled patients with knee OA). Only a few cases of patients were reported with samples that were positive for anti-drug antibodies (8/1601 patients). Since the PK, clinical pain relief response and safety profile were not different in ADA-positive subjects compared with ADA-negative subjects, ADA status was not included as a variable in the covariate model building.

Covariate model building was performed using the SCM procedure in PsN [18]. The covariate search was not done in one forward and one backward step as is the default in SCM. Instead, to manage the time to complete each run, an initial univariate step was performed to identify the most relevant covariates, followed by two forward/backward searches. More specifically, the search was done as 1) test all parameter-covariate relations on a univariate basis and retain only those covariates that were significant at the $P < 0.05$ level, 2) perform a full forward/backward search with covariates retained from step one with significance levels set to $P < 0.01$ and $P < 0.001$ for the forward and backward step, respectively, and 3) perform a second search with the final model from step two and those covariates excluded from step one with significance levels set to $P < 0.01$ and $P < 0.001$ for the forward and backward step, respectively. Continuous covariate relationships were coded as power models and categorical covariates were coded as a fractional difference to the common category (Equation 1). Reported confidence intervals were computed from the standard error estimates obtained from NONMEM.

$$\text{ParCov}_j = \left( \frac{\text{Cov}_j}{\text{Cov}_{j,\text{median}}} \right)^{\delta_j}$$

$$\text{ParCov}_j = 1 \text{ if most common category or } 1 + \theta_j \text{ if other category}$$

(1)

The parameter for the corresponding typical parameter for the median Cov$_i$ and/or common category is multiplied by ParCov$_j$.

Performance of the final population PK model was evaluated using goodness-of-fit plots, individual plots and diagnostic plots related to the covariate and stochastic models. Predictive performance of the model was assessed through a VPC (with prediction correction), which compares the prediction interval of observed data with the prediction interval of observed data from simulated data using the final population model [22].

The impact of fixed vs. WT-adjusted dosing was performed by simulating the PK profile for the first dosing interval for each patient, assuming either fixed dosing of 2.5, 5 or 10 mg, or WT-adjusted dosing of WT$^\delta$[(2.5, 5 or 10)/median WT] mg. The exposure range (defined as the 5th to 95th percentile) from fixed dosing was compared with predicted exposures from WT-adjusted dosing and the exposure range for WT-adjusted regimen was compared with the predicted fixed dosing.

### Results

After applying all predefined rules, the final analysis data set included 1610 patients with at least one post-dose observation above the LLOQ. After removal of outliers, this number decreased to 1608 patients and 7592 observations (Table 1). Baseline demographics used for evaluating categorical covariates were similar across groups and across the four studies (Table 1).

The base PK model was a two compartment model with parallel linear and non-linear elimination with IVIV on CL, V$_1$, V$_2$, and VM. A correlation term between IVIV in CL and V$_1$ was also included in the model. The mean (%CV) estimates from the base model were CL = 0.135 l day$^{-1}$ (35%), V$_1$ = 2.89 l (27%), V$_2$ = 1.81 l (20%) and VM = 10 μg day$^{-1}$ (37%).

The addition of non-linear PK by including a Michaelis–Menten (MM) component (leading to a decrease in objective function value [$\Delta$OFV] of 359 points) helped account for trends in CWRES vs. predicted concentration and in CWRES vs. time plots. Graphical analysis plots (not shown) demonstrated that a less heavily-tailed distribution of the residual error could be obtained via addition of a second residual error term through a mixture model (Equation 2). The estimate for the mixture probability for the smaller residual error term in the final model was 0.76 and the residual variabilities were estimated to be 13% and 54% for the higher and lower probability, respectively (Table 2).

$$Y_{ij} = \hat{Y}_{ij} + \varepsilon_{1} \text{ if subpopulation 1 or } \varepsilon_{2} \text{ if subpopulation 2}. \quad (2)$$

where $Y_{ij}$ is the ith individual’s jth observation and $\hat{Y}_{ij}$ is the corresponding model prediction.

WT, BSA, BMI and BLBW were tested on CL, V$_1$ and V$_2$ to determine the best measure of body size. Inclusion of WT resulted in the largest $\Delta$OFV when added as a covariate on CL. Whereas BLBW resulted in the largest $\Delta$OFV when included as a covariate on V$_1$ and V$_2$, little difference was noted in fit of the models on CL, V$_1$ and V$_2$. Therefore, WT was selected as the body size measure to include as a structural covariate in the base PK model.

Covariate model development used CL, VM, KM, V$_1$ and V$_2$ as parameters for evaluation of the covariates dose (only on CL, V$_1$ and V$_2$), age, race, gender, site of OA and CLcr (only on CL). The SCM procedure in PsN resulted in a final covariate model with CLcr, gender and dose (2.5 and 5 mg vs. 10 mg) on CL, and gender on V$_1$ in addition to WT as a structural covariate on CL, V$_1$ and V$_2$ (Equations 3–5). Gender on CL was identified as a significant covariate in the second full forward/backward search.
\[ \text{CLWT}_i = \left( \frac{\text{WT}_i}{84.7} \right)^{0.86} \]
\[ \text{CLCLC}_{ri} = \left( \frac{\text{CL}_{CRI}}{93.5} \right)^{0.11} \]
\[ \text{CLDOSE}_i = 1 \text{ if dose } = 10 \text{ mg or } 1 + \theta_{12} \text{ if dose } = 2.5 \text{ or } 5 \text{ mg} \] (3)
\[ \text{CLGENDER}_i = 1 \text{ if female or } 1 + \theta_{14} \text{ if male} \]
\[ \text{TVCL}_i = \theta_1 \cdot \text{CLWT}_i \cdot \text{CLCLC}_{ri} \cdot \text{CLDOSE}_i \cdot \text{CLGENDER}_i \]

TVCL\(_i\) denotes the ith individual’s typical value of CL\(_i\), and \(\theta_{8,11,12,14}\) are parameters of the model that describes the influence of the corresponding covariate. \(\theta_1\) is the CL of a hypothetical female patient weighing 84.7 kg with a CL\(_{CRI}\) of 93.5 ml min\(^{-1}\) after a dose of 10 mg.

\[ V_1\text{WT}_i = \left( \frac{\text{WT}_i}{84.7} \right)^{0.10} \]
\[ V_1\text{GENDER}_i = 1 \text{ if female or } 1 + \theta_{13} \text{ if male} \] (4)
\[ \text{TVV}_1 = \theta_2 \cdot V_1\text{WT}_i \cdot V_1\text{GENDER}_i. \]

TVV\(_1\) denotes the ith individual’s typical value of V\(_1\), and \(\theta_{9,13}\) are parameters of the model that describe the influence of the corresponding covariate. \(\theta_2\) is the V\(_1\) of a hypothetical female patient weighing 84.7 kg.

\[ V_2\text{WT}_i = \left( \frac{\text{WT}_i}{84.7} \right)^{0.10} \]
\[ \text{TVV}_2 = \theta_4 \cdot V_2\text{WT}_i \]

TVV\(_2\) denotes the ith individual’s typical value of V\(_2\), and \(\theta_{10}\) is a parameter of the model that describes the influence of body weight on V\(_2\). \(\theta_4\) is the V\(_2\) of a hypothetical patient weighing 84.7 kg.

Estimated values for the final population PK model parameters of CL, V\(_1\), V\(_2\) and VM for a typical female patient weighing 84.7 kg with a CL\(_{CRI}\) of 93.5 ml min\(^{-1}\) after tanezumab 10 mg were 0.135 l day\(^{-1}\), 2.71 l, 1.98 l and 8.03 \(\mu\)g day\(^{-1}\), respectively (Table 2). Estimates of IIV (as %CV) associated with CL, V\(_1\), V\(_2\) and VM were 26%, 20%, 20% and 41%, respectively. The correlation between IIV of CL (\(\eta_{CL}\)) and V\(_1\) (\(\eta_{V1}\)) was 0.67. The \(\eta\) shrinkages were reasonably low for CL and V\(_1\) (11%/10% and 15%/23%, respectively, for the low/high residual error mixtures) but large for VM and V\(_2\) (66%/71% and 57%/79%, respectively). However, the condition number was low (169) and the correlation between the parameter estimates indicated that the model is not over-parameterized.

All PK parameters were estimated with good precision, as reflected by the narrow range for 95% confidence intervals (CI, Table 2). The final model provided a good fit to the data with good agreement between observed and model predicted (using either

|                  | Tanezumab 2.5 mg n = 289 | Tanezumab 5 mg n = 655 | Tanezumab 10 mg n = 664 | All n = 1608 |
|------------------|--------------------------|------------------------|-------------------------|-------------|
| Age (years)      | Mean ± SD                | Mean ± SD              | Mean ± SD               | Mean ± SD   |
|                  | 61.7 ± 10.0              | 61.4 ± 10.5            | 61.2 ± 10.5             | 61.4 ± 10.4 |
| Range            | 26–88                    | 21–93                  | 32–92                   | 21–93       |
| Weight (kg)      | Mean ± SD                | Mean ± SD              | Mean ± SD               | Mean ± SD   |
|                  | 86.3 ± 18.3              | 86.8 ± 17.7            | 86.6 ± 17.7             | 86.6 ± 17.8 |
| Range            | 44.9–133                 | 44.4–145               | 34–170                  | 34–170      |
| Creatinine clearance (ml min\(^{-1}\)) | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
|                  | 96.0 ± 28.7              | 97.1 ± 31.4            | 98.9 ± 32.2             | 97.7 ± 31.3 |
| Range            | 37.7–197                 | 30.3–301               | 27.5–242                | 27.5–301    |
| Race, n (%)      |                          |                        |                         |             |
| White            | 253 (87.5)               | 575 (87.8)             | 561 (84.5)              | 1389 (86.4) |
| Black            | 30 (10.4)                | 72 (11.0)              | 78 (11.7)               | 180 (11.2)  |
| Asian            | 3 (1.0)                  | 4 (0.6)                | 8 (1.2)                 | 15 (0.9)    |
| Other            | 3 (1.0)                  | 4 (0.6)                | 17 (2.6)                | 24 (1.5)    |
| Gender, n (%)    |                          |                        |                         |             |
| Female           | 173 (59.9)               | 396 (60.5)             | 404 (60.8)              | 973 (60.5)  |
| Male             | 116 (40.1)               | 259 (39.5)             | 260 (39.2)              | 635 (39.5)  |
| Index joint, n (%) |                    |                        |                         |             |
| Knee             | 143 (49.0)               | 479 (73.0)             | 491 (73.9)              | 1113 (69.0) |
| Hip              | 149 (51.0)               | 177 (27.0)             | 173 (26.1)              | 499 (31.0)  |

*Not all percentages sum to 100.0% due to rounding.
population [PRED] or individual [IPRED] predictions) tanezumab concentrations. Also, there were no unacceptable trends in CWRES vs. population predictions or over time to indicate that a more elaborate residual error structure was needed (Figure 1).

When the data points associated with absolute CWRES >5 (n = 110) and those from cleaning rule 3 (n = 190) were re-included in the final model, effects on the model were minimal (<10% change in most fixed parameters with a maximum change of 23% in the estimate of inter-compartmental clearance).

Predictive performance of the model was assessed through a VPC with prediction correction (Figure 2). Overall, the model predicts the observed data well since the median, 5th and 95th data percentiles were adequately captured by the corresponding simulation-based prediction intervals and 95% CI. The simulation to assess the impact of a fixed vs. WT-adjusted dosing regimen shows that fixed dose will lead to a slightly larger variability in exposure (25%–26%) compared with WT-adjusted dosing (19%–20%; Figures 2 and 3).

**Table 2**
The parameter estimates of the final model

| Parameter | Estimate | 95% CI* |
|-----------|----------|---------|
| \( CL_{f} \) (l day\(^{-1}\)) | 0.135 | 0.129, 0.14 |
| \( V_{1} \) (l) | 2.71 | 2.66, 2.76 |
| \( Q \) (l day\(^{-1}\)) | 0.371 | 0.198, 0.545 |
| \( V_{2} \) (l) | 1.98 | 1.72, 2.24 |
| Mixture probability with low RSV | 0.763 | 0.738, 0.789 |
| KM (ng ml\(^{-1}\)) | 27.7 | 7.8, 47.7 |
| VM (μg day\(^{-1}\)) | 8.03 | 5.72, 10.3 |
| WT on CL | 0.77 | 0.682, 0.858 |
| WT on \( V_{1} \) | 0.554 | 0.489, 0.62 |
| WT on \( V_{2} \) | 0.302 | 0.15, 0.454 |
| \( CL_{cr} \), on CL | 0.108 | 0.0738, 0.141 |
| Dose on CL | 0.0669 | 0.0346, 0.0992 |
| Gender on \( V_{1} \) | 0.175 | 0.143, 0.208 |
| Gender on CL | 0.143 | 0.106, 0.181 |
| IV CL, %CV | 26 | 25, 27 |
| IV \( V_{1} \), %CV | 20 | 19, 21 |
| Cov \( CL_{cr}\)–\( V_{1} \) | 0.034 | 0.03, 0.038 |
| IV VM, %CV | 41 | 26, 52 |
| IV \( V_{2} \), %CV | 20 | 15, 24 |
| Low RSV, %CV | 13 | 13, 13 |
| High RSV, %CV | 54 | 52, 55 |

*Confidence interval computed from the standard error estimates obtained from NONMEM. **The estimate is for a female weighing 84.7 kg with a CL\(_{cr}\) of 93.5 ml min\(^{-1}\). **Estimate of the covariance between CL and \( V_{1} \), CI, confidence interval; CL, clearance; \( CL_{cr}\), creatinine clearance; Cov, covariance; %CV, coefficient of variation (calculated by taking the square root of variance estimated by NONMEM); IV, inter-individual variability; KM, concentration at half maximum elimination capacity; Q, inter-compartmental clearance; RSV, residual variability; \( V_{1} \), central volume; \( V_{2} \), peripheral volume; VM, maximum elimination capacity; WT, body weight.

**Discussion**

Population PK of different IgG therapeutic antibodies have been widely published [23]. As is the case for tanezumab, therapeutic antibodies are commonly subject to both linear and non-linear kinetics [24]. Nonetheless, initial population PK modelling indicated a two compartment model with linear elimination adequately characterized the observed PK in phase 1 and 2 studies across a dose range of 3 to 1000 μg kg\(^{-1}\) [13, 14]. Linear elimination of monoclonal antibodies is non-specific because it occurs via catabolism following endocytosis by the reticuloendothelial system. The process is relatively slow because the antibody can be salvaged via binding to the neonatal Fc receptor (FcRn) [25]. The half-life estimated for tanezumab in this population, 21 days [13, 26], is consistent with that of a typical IgG antibody with a long half-life (~23 days) [27]. However, the linear model used to describe the phase 2 data was inadequate for the current, larger phase 3 dataset. A better description of individual PK profiles and residual diagnostics was achieved by including a non-linear component. The similarity of the CL estimates across analyses (0.135 l day\(^{-1}\) for the linear plus non-linear model and 0.207 l day\(^{-1}\) for the linear model only) indicates a relatively small contribution of non-linear CL and is more important for doses <2.5 mg, whereas at doses ≥2.5 mg, the contribution of non-linear CL only accounts for 18%, 10% and 5% of total CL for 2.5, 5 and 10 mg, respectively.

The faster (0.29 l day\(^{-1}\), VM/KM) non-linear pathway is likely related to the target binding (target-mediated drug disposition (TMDD)) which, as for other antibodies, can be adequately described by the MM equation [28, 29]. The inverse correlation of the low non-linear CL contribution to dose suggests that there is an excess of free tanezumab in the systemic circulation over the 8 week dose regimen for these doses. This is consistent with the stable therapeutic effect that has been observed at these doses in phase 3 studies.

Since NGF is a dimer [30], two tanezumab binding sites are on each molecule, at lower tanezumab concentrations a significant amount of NGF would be only half-saturated (i.e. only one of the NGF binding sites occupied, NGF monomer). CL of this NGF monomer could still occur through internalization after binding to the high affinity (tropomyosin-related kinase A, trkA) or low affinity neurotrophin (p75) receptor, which are targets for free NGF [7]. However, assuming that binding to tanezumab does not affect the NGF internalization rate, the non-linear CL associated with this monomer would most likely be faster than the 0.29 l day\(^{-1}\) estimated for the non-linear pathway (VM/KM) and consequently closer to values for free NGF that would have a CL of >100 l day\(^{-1}\) in humans based on a NGF CL of 140–416 ml h\(^{-1}\) kg\(^{-1}\) in animals [31, 32]. Therefore, it is possible that the estimated non-linear CL could be
related to other processes such as formation of small, multimeric (tetramers, hexamers, octamers) complexes [33]. These complexes consisting of NGF and tanezumab could result in slower clearance compared with clearance through NGF receptor internalization but faster than what is measured for antibodies not bound to target. This faster CL for tanezumab in multimeric complexes could be explained by reduced salvage by FcRn due to size-based sorting to lysosomes [34, 35]. The extent of multimeric formation would increase with increasing tanezumab concentration up to a ratio of 1 : 1 (antibody : target) and then decrease as tanezumab concentration increases [33]. Faster clearance due to complexation of therapeutic monoclonal antibody with the soluble target has been postulated for the non-linear CL of omalizumab and denosumab [36, 37].

As is typical for therapeutic antibodies, the initial volume of distribution ($V_d$) for tanezumab was estimated to be 2.71 l (close to plasma volume, 3 l). The volume at steady-state ($V_{ss}$) was approximately double the initial $V_d$ and estimated at 4.69 l ($V_1$ plus $V_2$). This low $V_d$ indicates that distribution of tanezumab was limited to the interstitial space fluid. The extent of this distribution, which probably occurs primarily via convection and transcytosis [35, 38], is similar to what has been reported for other therapeutic antibodies [23].

IIV for CL and volume was moderate (20%–26%) and was as expected considering linear elimination by non-specific, high capacity endocytosis, the limited space distribution for tanezumab with no off-target binding, and with the fact that NGF is not a membrane-bound target. IIV for VM was relatively high at 41% and no covariates were identified that contributed to this variability. Because non-linear CL is most likely mediated via target binding, VM is related to the number of NGF molecules and KM would mostly reflect both the affinity of tanezumab for NGF as well as the affinity of the tanezumab–NGF complex for FcRn.

Covariates were identified that explained some IIV in CL (35%), $V_1$ (27%) and $V_2$ (20%) between patients. WT was the most significant covariate (CL, $V_1$ and $V_2$), followed by gender (CL and $V_1$), $CL_{cr}$ (CL) and dose (CL). Similar covariate effects have been identified for other monoclonal antibodies [23]. The most commonly
reported covariate is body size (weight), which is associated with higher body volume as well as higher capacity to clear the drug, as is typical for both therapeutic antibodies and small molecules. Also, a higher CL and higher $V_d$ are frequently reported in males compared with females [23, 39]. The slower clearance and lower $V_d$ in females compared with males may be due to higher expression of the FcRn receptor in females. This is possibly caused by differences in inflammatory cytokines and/or hormonal expression that are known to modulate FcRn expression [40]. Alternatively, expression of FcRn could be lower in males due to higher muscle mass, where FcRn expression is relatively low [41].

It is less common that a statistically significant effect of CLcr on total body clearance is identified, since renal clearance is not considered important for elimination of monoclonal antibodies due to their large size and inefficient filtration through the glomerulus [38], although expression of FcRn in proximal renal cells suggests possible transfer of IgG in urine by transcytosis [42]. The minor role of the kidney in IgG clearance is confirmed by population analysis results for tanezumab because the percent change in total clearance with changing CLcr (Table 3) represents <15% of total clearance [43]. Similar CLcr effects on total clearance have been reported for ustekinumab and orantuzimab [44, 45].

In this dataset, a 7% lower CL at the highest dose of 10 mg was identified compared with doses of 5 mg and 2.5 mg, independent of concentration-dependent
non-linear CL. Lower CL associated with higher doses that could not be described by saturable non-linear kinetics has been observed for other antibodies as well, such as efalizumab and ING-1 [46, 47].

All of the relationships between covariates described above were highly significant due to the large number of subjects and observations, but WT had the largest influence in reducing IIV in CL from 35% to 27% with an estimated coefficient of 0.77. In contrast, the addition of all other significant covariates only reduced IIV by another 1% (from 27% to 26%). Only a small increase in variability for fixed vs. weight-adjusted dosing would be expected (Figure 3). This is consistent with the predictions from the phase 2 analysis, based on PK data from weight-adjusted doses, which indicated that a fixed-dose strategy would be appropriate for phase 3 [13, 14]. It also aligns with other work exploring how body size-based dosing impacts exposure for monoclonal antibodies in general [48]. It is also likely that the difference between weight adjusted dosing and fixed dosing would be smaller in practice due to rounding and potential errors in weight and dose calculation for the weight-adjusted dosing. Similarly, estimated coefficients for the effect of WT on $V_1$ and $V_2$ (0.554 and 0.302, respectively) are ~0.5 or less, indicating no difference in weight-based and fixed dosing in maximum serum concentration after the initial infusion.

Across the OA programme the general safety and tolerability profile for the weight-adjusted dosing used in phase 2 [49] and fixed dosing used in phase 3 [10, 11, 48] was similar, so there was no indication that the potential small increase in variability was consequential. In particular, while there was an increased risk of rapidly progressive OA between the 5 and 10 mg doses (an adverse event most evident in the long term studies [50]), after accounting for dose, tanezumab concentration was not an additional explanatory factor (unpublished data). This finding is also consistent with the minimal overlap of predicted exposures for 5 mg and 10 mg after either weight-based or fixed dosing (Figure 4).

In conclusion, data were appropriately described by a two compartment model with a parallel linear and non-linear elimination process. The non-linear pathway was significant, but only 18% of the lowest dose was eliminated via this route. Of the covariates identified as significant, only WT had some impact on unexplained variability in CL. Still, the small increase in exposure variability for fixed vs. weight-adjusted dosing was not considered clinically relevant and supports the use of fixed dosing.

### Competing Interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare ENJ was a paid consultant to carry out the data analysis with Pfizer Inc. in the previous 3 years and RX, SFM and

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### Table 3

| Relation     | Impact                                                                 |
|--------------|------------------------------------------------------------------------|
| WT on CL     | 10% change in WT leads to 8% change in CL                              |
| CLcr on CL   | 10% change in CLcr leads to 1% change in CL                             |
| Gender on CL | Males have 14% higher CL than females                                   |
| Dose on CL   | CL is 7% higher with 2.5 and 5 mg compared with 10 mg dose              |
| WT on $V_1$  | 10% change in WT leads to 5% change in $V_1$                           |
| Gender on $V_1$ | Males have 18% higher $V_1$ than females                          |
| WT on $V_2$  | 10% change in body weight leads to 3% change in $V_2$                 |

CL, clearance; CLcr, creatinine clearance; $V_1$, central volume; $V_2$, peripheral volume; WT, body weight.

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### Figure 4

Comparison of the fixed and weight-adjusted dosing regimens. The points represent the predicted AUC for the individuals in the data set assuming a particular dose and dosing regimen. The black vertical bars cover 90% of the exposure ranges. The dark blue points for a given dosing regimen fall within the exposure range of the other regimen at the same dose. The light blue and orange points for one dose and regimen fall above and below the exposure range of the other regimen at the same dose, respectively. AUC, area under the curve. (● below lower limit of the fixed/adjusted dose, ● above upper limit of the fixed/adjusted dose)
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Contributors

E. Niclas Jonsson, Rujia Xie, Scott F. Marshall and Rosalin H. Arens wrote the manuscript, designed the research and performed the research. E. Niclas Jonsson, Scott F. Marshall and Rosalin H. Arens analyzed data.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

In brief, microtitre assay plates were coated with recombinant human NGF overnight, then washed (wash buffer: 1× phosphate buffered saline [PBS]/0.3 M NaCl/0.05% polysorbate-20 or PBS-T/0.3 M NaCl, pH 7.4) to block unadsorbed sites with blocking buffer. Calibration standards (6.0–384 ng ml\(^{-1}\)) and quality control (QC) samples were prepared by diluting the tanezumab reference standard into human serum with diluent buffer 1 : 20. Following incubation (covered and incubated at ambient temperature for at least 60 min with non-vigorous shaking), plates were washed with blocking buffer, and polyclonal donkey anti-human IgG conjugated with horseradish peroxidase was added for binding to captured tanezumab. After the final wash, a chemiluminescent substrate solution (working substrate solution: Supersignal ELISA Femto Maximum Sensitivity Chemiluminescent Substrate System) was added. After 8 to 9 min incubation, relative light units of luminescence (which develops proportionally to the amount of tanezumab) were measured on a luminescence plate reader (Bio-Tek Clarity; Clarity 4.0 Rev. 2 or higher, Winooski, VT, USA).