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Toxicology studies - considerations for design and regulatory requirements

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Foreword

In order to design a toxicology study ready for pharmaceutical development, there are many factors to consider. Despite the referred guidance for the safety evaluation of various pharmaceutical products, detailed design is dependent on such factors like the background of the test article, intended clinical use, previous data and the experience of the team involved in drug discovery. To give an example, the design of a 4-week rodent study may have elements such as dosing route and recovery period adjusted due to the data of pilot studies, proposed clinical design to support and special purposes/parameters included in the toxicology study. For a GLP toxicology study, healthy animals are typically used but in some cases, disease animals can be utilized under specific circumstances.

It is important to avoid conducting unnecessary tests, ensuring appropriate toxicology study design will provide sufficient safety data to help support any proposed clinical trials, in a cost-effective and timely manner. Experienced contract research organizations will work closely with sponsors to help design the study by supplying experience in scientific consideration, strategy planning and regulatory requirements.

In this eBook, we will explore the bioanalytical challenges in reproductive toxicity studies and outline the key translational drug development objectives in oncology.

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Bioanalysis

Bioanalytical assays in support of tanezumab developmental and reproductive toxicity studies: challenges and learnings

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Bioanalytical challenges were encountered during developmental and reproductive toxicity studies of tanezumab in cynomolgus monkeys. Possible changes in breast milk composition over the postpartum period potentially complicated assessment of tanezumab concentration in this matrix, requiring validation of the quantification assay across different time intervals. Immunogenicity assessment in maternal serum was complicated by apparent increases in the incidence of antidrug antibody-positive results in treatment-naive samples as pregnancy progressed that were due to changes in the concentration of nerve growth factor, tanezumab's target protein. This was overcome by employing gestational day-specific cut points throughout pregnancy. Researchers should recognize potential challenges associated with dynamic matrices/physiological conditions and anticipate that assays developed under normal conditions may require adaptation for specialized situations.

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Nerve growth factor (NGF) plays a critical role in neuronal growth and survival during embryogenesis [1,2]. During adulthood, however, NGF acts as a modulator of nociceptive activity [1,2]. As a result, inhibition of NGF activity is an emerging therapeutic approach for the management of chronic pain. Tanezumab is a humanized IgG_2 monoclonal antibody with high selectivity and specificity for NGF [3]. Tanezumab inhibits binding of NGF to its cellular receptors, tropomyosin kinase A and p75 [1,2]. Tanezumab is currently in Phase III development for the treatment of chronic pain and has demonstrated efficacy in clinical studies of patients with conditions such as osteoarthritis and chronic low back pain [4–13].

Extensive nonclinical studies have been conducted as part of tanezumab's development program. Per regulatory guidance, a robust bioanalytical program supported the broader development program by assessing tanezumab pharmacokinetics, toxicokinetics (TK) and immunogenicity. Assessment of tanezumab immunogenicity is of importance since biologic therapies can induce immune responses that may result in production of antidrug antibodies (ADA) [14,15]. The presence and activity of ADAs can alter the pharmacokinetic profile of a drug by increasing clearance from the body, may reduce the efficacy of a drug via binding and neutralization, and can impact safety by inducing potentially harmful generalized immune effects such as hypersensitivity or anaphylaxis [15]. Thus, assessment of ADA is a key component of regulatory filings [14].

The tanezumab development program included studies in cynomolgus monkeys to assess potential developmental and reproductive toxicity (DART) [16,17]. These studies were also supported by the bioanalytical program. However, while there are regulatory guidelines for DART studies in general, there is limited guidance regarding the bioanalytical support required for these types of studies and few examples in the literature [18]. The bioanalytical support of tanezumab DART studies in monkeys ultimately included assessments of tanezumab TK and antitanezumab immune response (presence of ADA) in several matrices, including plasma/serum from pregnant

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and lactating animals, neonatal plasma/serum and milk from lactating animals, with evaluation of milk specifically requested by a regulatory agency. Overall, the bioanalytical support program required standard TK/ADA assays that were developed for use in plasma/serum from normal adult animals to be validated for use in pregnant and neonatal animals and in breast milk.

However, during development of bioanalytical methods required to support DART studies, the program encountered challenges that were attributed to the potentially changing composition of milk over the course of lactation. Unexpected findings in the ADA test across different gestational periods during pregnancy were also observed. The primary findings, including TK and ADA data, have been published previously [16]. However, in hope of informing others who may be involved in or planning bioanalytical support of DART studies, the current manuscript details the specific challenges encountered and illustrates how these challenges were overcome.

Experimental

Validation of assays to assess tanezumab toxicokinetics in plasma & breast milk

Tanezumab was measured in cynomolgus monkey (Indonesian origin) plasma and breast milk using a quantitative enzyme immunoassay method. Recombinant human NGF was diluted in coating buffer (1 × phosphate buffered saline [PBS], pH 7.4) to a concentration of 5 μg/ml, and 100 μl was added to 96-well microtiter plates overnight at 2–8°C. Wells were then washed three-times with approximately 300 μl wash buffer (PBS-T, pH 7.4), and 300 μl block buffer (wash buffer/10% Blocker™ Casein in PBS) was added for 1–3 h at room temperature. Wells were then washed three-times with approximately 300 μl wash buffer. Standards (in duplicate), quality controls (in duplicate, prepared twice) and samples were diluted (1:10 for breast milk samples, 1:100 for plasma samples) with sample diluent buffer (wash buffer, 10% normal goat serum). Subsequent dilutions of each sample were made using 10% (for milk) or 1% (for plasma) matrix in sample diluent buffer to ensure they fell into the linear range of the standard curve. Total 100 μl of standard, control or sample was added to the wells for a approximately 1-h incubation at room temperature. Wells were then washed six-times with approximately 300 μl wash buffer. A 100-μl aliquot of working conjugate solution (goat antihuman IgG peroxidase-conjugated antibody in diluent buffer [wash buffer, 1% bovine serum albumin] at final concentration of 10 [milk and maternal plasma] or 20 ng/ml [neonatal plasma]) was then added to each well and incubated for approximately 1 h at room temperature. Wells were then washed six-times with approximately 300 μl wash buffer. A 100-μl aliquot of working tetramethylbenzidine (TMB) peroxidase substrate solution (50:50 solution of TMB Microwell Peroxidase substrate and Peroxidase substrate solution B) was then added to each well and incubated for approximately 8 (neonatal plasma), approximately 10 (maternal plasma) or approximately 15 min (milk) at room temperature to develop color in proportion to the amount of tanezumab present. A 100-μl aliquot of stop solution (1 M phosphoric acid) was then added. The optical density (OD) of each well was then read within 30 min of adding stop solution using two filters: 450 nm for detection and 620 nm for background. Sample concentrations were determined using a standard curve obtained by plotting OD versus concentration. The calibration curve was generated using a four-parameter logistic fit (with $1/y^2$ weighting for the milk assay). The range for this method in maternal and neonatal monkey plasma was from 100 to 12,800 ng/ml and was from 10.0 to 640 ng/ml in breast milk. A calibrator outside of the validated range of the assay was included to serve as an anchor point to facilitate curve fitting (50.0 ng/ml for plasma; 5.00 ng/ml for breast milk). Maternal plasma was initially collected between gestational weeks 2–15, covering all three trimesters. Maternal plasma was also collected primarily in first trimester (gestational days 40–80), primarily second trimester (gestational days 70–100) and primarily third trimester (gestational days 120–135; see Results section for details). Neonatal plasma was collected between 30 and 90 days after birth. Breast milk was collected in two different time frames, before lactation day 30 and between lactation days 30–90; see Results section for details).

Validation of assays to assess tanezumab antidrug antibodies in serum & breast milk

An ELISA method was used to detect antitanezumab antibodies in cynomolgus monkey serum and breast milk. Tanezumab was diluted in coating buffer (0.05 M sodium carbonate, pH 9.6) to a concentration of 0.1 μg/ml, and 100 μl was added to 96-well microtiter plates overnight at 2–8°C. Wells were then washed three-times with 300 μl wash buffer (PBS-T, pH 7.4 for neonatal serum and milk; PBS, 0.01% polysorbate 20, pH 7.4 for adult serum), and 300 μl block buffer (StartingBlock® [PBS]) was added for at least 1 h at room temperature. Wells were then washed three-times with 300 μl wash buffer. Samples and positive/negative calibrators were diluted 1:50 with diluent buffer, and 100 μl was added to the wells for a 1-h incubation at room temperature to allow any ADA to bind the immobilized tanezumab. Wells were then washed six-times with 300 μl wash buffer. A 100-μl

aliquot of working conjugate antibody solution (biotinylated tanezumab in diluent buffer at a concentration of 500 ng/ml) was added to the wells and incubated for 1 h at room temperature. Wells were then washed six-times with 300 µl wash buffer. A 100-µl aliquot of working streptavidin-horseradish peroxidase conjugate solution (streptavidin-horseradish peroxidase diluted 1:20,000 [for neonatal serum and milk] or 1:32,000 [for adult serum] in diluent buffer) was added to the wells and incubated for 1 h at room temperature. Wells were then washed six-times with 300 µl wash buffer. A 100 µl of working TMB peroxidase substrate solution was added to develop color for approximately 15 min. A 100-µl aliquot of stop solution was then added. The OD of each well was then read within 30 min of adding stop solution using two filters: 450 nm for detection and 620 nm for background. Data were presented as end point titers (log_2; the end point is defined as the reciprocal of the serum/breast milk dilution that is above the cut point of the assay). The positive control (monkey antitanezumab affinity-purified antibody) was analyzed at concentrations of 3.13–1600 ng/ml in 100% adult serum/human breast milk (used as a surrogate for monkey milk). The negative control was pooled 100% adult monkey serum, neonatal monkey serum or breast milk, as appropriate. Adult monkey serum was collected from adult males and/or nonpregnant females, neonatal monkey serum was collected up to 21 days after birth, and breast milk was collected on or before lactation day 30.

Results & discussion

Challenges encountered during validation of tanezumab toxicokinetics & antidrug antibodies assays

TK assays developed for normal adult monkey plasma were validated for use in three separate assays: maternal plasma (collected between gestational days 14 and 105), neonatal plasma (collected between 30 and 90 days after birth) and breast milk (see below for collection times). The composition of breast milk, however, may fluctuate, posing a potential challenge for tanezumab concentration assessment in this matrix. Breast milk consists of water, various fats, proteins and sugars [19]. In humans, the relative composition of breast milk changes over the course of the day and over the course of the postpartum period [19]. Fat content, for example, varies depending on the time of day, and protein levels have been shown to drop approximately 50% by postpartum week 8 in mothers who delivered at term [20,21]. In contrast, such pronounced differentiation of milk composition over time is not observed in rhesus monkeys [22]. In cynomolgus monkeys, milk composition in terms of fat and lactose does not differ from that of rhesus monkeys, although there is no direct evidence available to demonstrate that milk composition does not change over time [22]. Therefore, we had to ensure that the assay in breast milk from cynomolgus monkeys was reliable despite the composition of breast milk potentially changing over the course of time.

To overcome this challenge and assess possible matrix effects, the assay was validated using milk collected at two different time points based on the planned DART study designs. These time points were prior to lactation day 30 (<30 day milk) and between lactation days 30 and 90 (30–90 day milk). Quality control samples at final tanezumab concentrations of 0, 30 and 480 ng/ml were prepared in <30 day milk and in 30–90 day milk. Each of these samples was then evaluated against calibration curves prepared in <30 day milk and again in 30–90 day milk. The pre-established criterion for assay validation was set at eight of ten control sample lots being within ± 20% of each calibration curve. However, only three of ten lots for control samples prepared in 30–90 day milk and only six of ten lots for control samples prepared in <30 day milk had accuracy results <20% when evaluated using the 30–90 day calibration curve. Seven of ten control lots prepared in 30–90 day milk had accuracy results ≤30% when evaluated against the 30–90 day calibration curve, and nine of ten lots had accuracy results ≤30% when evaluated against the <30 day calibration curve. For quality control samples prepared in <30 day milk, nine of ten lots had accuracy results ≤30% when evaluated against the 30–90 day calibration curve, and eight of ten had accuracy results ≤30% when evaluated against the <30 day calibration curve. All unspiked matrix samples (0 ng/ml) had results that were below the limit of quantitation regardless of the milk collection time and the calibration curve used. Thus, in order to accept performance of the assay in milk collected at different times (i.e., <30 day milk and 30–90 day milk), the acceptance range for assay validation was increased from the predetermined criterion of 20% to a criterion of 30% based on the observed matrix effects.

Details regarding the accuracy, precision, stability and other key parameters of the validated ELISA assays in each matrix are shown in Table 1. Based on comparison performed during method development, newborn and adult plasma performed equivalently in the assays.

Assays for the detection of antitanezumab antibodies in adult monkey serum (collected from males and/or nonpregnant females), were validated for use in neonatal monkey serum (collected up to 21 days after birth) and

Table 1. Analytical validation of ELISAs for tanezumab concentration determination.

Assay characteristic	Maternal plasma	Neonatal plasma	Breast milk
Assay range (ng/ml)	100–12,800	100–12,800	10.0–640
Minimum dilution	1:100	1:100	1:10
Inter-run assay accuracy, %RE (QC nominal concentration [(ng/ml]):			
– LLOQ	-13.8 (100)	-4.70 (100)	-2.40 (10)
– Low	10.3 (300)	18.0 (300)	-5.00 (30)
– Mid	-0.92 (4800)	4.40 (4800)	-5.00 (120)
– High	-5.51 (9600)	1.86 (9600)	-7.08 (480)
– ULOQ	-3.59 (12,800)	-9.26 (12,800)	-8.44 (640)
Inter-run assay precision, %CV (QC nominal concentration [ng/ml]):			
– LLOQ	21.2 (100)	10.7 (100)	14.1 (10)
– Low	9.85 (300)	5.11 (300)	6.88 (30)
– Mid	3.93 (4800)	2.43 (4800)	6.00 (120)
– High	3.68 (9600)	2.88 (9600)	4.69 (480)
– ULOQ	3.81 (12,800)	3.11 (12,800)	4.88 (640)
Total error, sum of %CV + absolute value of %RE (ng/ml):			
– LLOQ	35.0 (100)	15.4 (100)	16.5 (10)
– Low	20.2 (300)	23.1 (300)	11.9 (30)
– Mid	4.85 (4800)	6.83 (4800)	11.0 (120)
– High	9.19 (9600)	4.74 (9600)	11.8 (480)
– ULOQ	7.40 (12,800)	12.4 (12,800)	13.3 (640)
Long-term stability (days):			
– -20°C	398	180	187
– -70°C	695	465	632
Benchtop stability	At least 17 h	At least 18 h	At least 24 h
Freeze/thaw stability†	At least four cycles	At least four cycles	At least three cycles
Dilution integrity (%)‡:			
– Precision	4.17	2.24	1.48
– Accuracy	11.5	4.75	-26.9

†Freeze temperature is -70°C; thaw temperature is ambient temperature.
‡Based on 50,000-fold dilution of 100 μg/ml QC for plasma and based on 1:10,000-fold dilution of 100 μg/ml QC for breast milk (1:1000-fold when not including minimum dilution of 1:10).
CV: Coefficient of variation; LLOQ: Lower limit of quantitation; QC: Quality control sample; RE: Relative error; ULOQ: Upper limit of quantitation.

breast milk (collected on or before lactation day 30). Based on comparison performed during method development, newborn and adult serum performed equivalently in the assays. A summary of the design, precision, sensitivity and other key parameters of these assays in each of the matrices can be seen in Table 2.

Challenges encountered during utilization of toxicokinetics & antidrug antibodies assays

Using the ELISA method validated for use in adult serum, an unexpectedly high frequency of ADA-positive samples was reported in maternal serum from both tanezumab-dosed animals and nondosed control animals. Further, the frequency of positive samples increased as pregnancy progressed. This led to the hypothesis that these results were false positives due to physiological change(s) during pregnancy. Thus, further research was performed to identify the cause of these false positives.

It is known that soluble dimeric/multimeric target proteins can interfere with, and produce false positives in, bridging immunoassay platforms such as ELISAs due to their ability to bridge the capture and labeled detection agents [23]. The target protein for tanezumab, NGF, exists naturally as a homodimer but had not previously caused extensive false positives in nonclinical studies at normal physiological levels [24]. It was hypothesized that increased levels of NGF during pregnancy may account for the false positives being reported in the DART studies. This was unexpected because while data were lacking in nonhuman primates, there was no conclusive indication of large increases in NGF levels during pregnancy in humans at the time of assay validation. Therefore, a highly selective and sensitive immunoaffinity LC–MS/MS assay was developed to assess serum NGF levels during pregnancy in

Table 2. Validated ELISAs for antidrug antibodies determination.

Assay characteristic	Adult serum	Neonatal serum	Breast milk
Positive control	Mouse antitanezumab affinity-purified antibody in 100% pooled adult monkey serum	Monkey antitanezumab affinity-purified antibody in 100% pooled adult monkey serum	Monkey antitanezumab affinity-purified antibody in 100% human breast milk
Range (ng/ml)	3.13–1600	3.13–1600	3.13–1600
Minimum dilution	1:50	1:50	1:50
Negative control	Pooled adult monkey serum	Pooled newborn monkey serum	Pooled monkey breast milk
Inter-run assay precision of the positive control end point titer (%CV)	5.78	6.22	3.45
Intra-run assay precision of the positive control (%CV)	1.86	1.91	1.46
Sensitivity: 95% CI (ng/ml)	52.0	43.0	195
Screening cut point factor	1.20	1.14	1.39
Confirmatory cut point (% signal reduction)	36.0	23.9	31.3
Recovery	Not performed	80–125% in 100% of the lots examined	80–125% in 60% of the lots examined
Benchtop stability	At least 7 h	Not performed	At least 21 h
Freeze/thaw stability†	At least five cycles	Not performed	At least six cycles

†Freeze temperature is -20°C for serum and -70°C for breast milk; thaw temperature is ambient temperature.
CV: Coefficient of variation.

cynomolgus monkeys [25]. This assay demonstrated that NGF levels substantially increased in monkeys over the course of pregnancy, with mean concentrations of 541, 1590 and 3560 pg/ml during the middle of the first, second and third trimesters, respectively [25]. This represents a 12-, 34- and 77-fold increase in NGF in the first, second and third trimesters, respectively, relative to nonpregnant controls (46.3 pg/ml). Middle of trimester was defined as 130 ± 21, 58 ± 12 and 17 ± 10 (mean ± standard deviation [SD]) days before birth for the first, second and third trimesters, respectively.

To overcome the issue of increasing NGF over the course of pregnancy during the tanezumab DART studies, ADA assessment utilized gestational day-specific screening cut point factors determined from treatment-naive samples collected at different points throughout pregnancy (day 20 [n = 18], day 97 [n = 16] and day 146 [n = 15]). Samples with a response at or below the screening assay cut point were considered negative, while samples with responses above cut point were considered potentially reactive and retested in a confirmatory assay. To establish these cut points, samples of placebo-dosed monkey serum were analyzed over 3 days (two plates per day, six plates in total). The assay cut point for each plate was defined as the mean response of individual serum samples plus (1.645 × SD) to allow a 5% false-positive rate. Six individual cut point factors (one from each plate) were determined by dividing the assay cut point for that plate by the mean response of the negative controls (blank sera) on each plate. These six cut point factors were then averaged to give a cut point factor for that specific gestational day. The cut point factors were calculated as 0.971 for gestational day 20, 3.50 for day 97 and 11.6 for day 146. Since the cut point factor on day 20 was <1.0 but was not greater than the assay cut point of 1.20 previously determined for nonpregnant monkeys, a cut point of 1.20 was utilized for samples collected on gestational day 20 for the reproductive toxicity study. These gestational day cut point factors were also utilized in a second study of tanezumab reproductive toxicity that included samples collected from gestational days 20–139. The day 20 cut point factor of 1.20 was used for samples collected on gestational days 20–51, and the day 97 cut point factor of 3.50 was used for samples collected on gestational days 93–118. However, a large proportion of samples collected on gestational day 139 from placebo-treated animals resulted in ADA-positive tests when the previously established day 146 cut point factor of 11.6 was applied. It was therefore determined that a new cut point factor was required for gestational day 139 in this particular study. Thus, using ten samples collected from placebo-treated animals on day 139, a cut point factor of 21.7 was calculated and utilized during ADA assessment on gestational day 139 in this study.

The finding that NGF levels increase over the course of pregnancy in monkeys also led us to revisit TK assays in maternal plasma to further refine our assessment of whether gestational age of the plasma impacted quantitation of tanezumab. In the initial TK assay, samples were prepared in pooled plasma collected at gestational weeks 2, 3, 5, 6, 7, 9, 10, 12 and 15 (all n = 1 except week 7, which was n = 2). To ensure the TK assay was valid and reliable across

Table 3. Analytical validation of ELISAs for tanezumab concentration determination in maternal plasma during second and third trimesters.

Assay characteristic	Second trimester	Third trimester
Calibration standard matrix	First trimester pooled plasma	First trimester pooled plasma
QC matrix	Second trimester pooled plasma	Third trimester pooled plasma
Inter-run assay accuracy, %RE (QC nominal concentration [ng/ml]):		
– Low	14.7 (300)	5.00 (300)
– Mid	0.771 (4800)	8.29 (4800)
– High	1.81 (9600)	9.57 (9600)
Inter-run assay precision, %CV (QC nominal concentration [ng/ml]):		
– Low	7.06 (300)	2.60 (300)
– Mid	4.22 (4800)	1.01 (4800)
– High	8.44 (9600)	1.36 (9600)

CV: Coefficient of variation; QC: Quality control sample; RE: Relative error.

all periods of gestation, quality control samples prepared in the second and third trimesters were quantitated using calibrators prepared in plasma collected primarily from the first trimester (gestational day 40 [n = 4], 45 [n = 3], 50 [n = 3], 60 [n = 1] and 80 [n = 1]). Pooled plasma from second trimester was collected at day 70 (n = 1), 71 (n = 1), 80 (n = 1) and 100 (n = 2). Pooled plasma from third trimester was collected at day 120 (n = 3), 130 (n = 1) and 135 (n = 1). Quality control samples prepared in plasma from second and third trimesters quantitated accurately using calibrators prepared in plasma primarily from first trimester, demonstrating that gestational age does not affect the assay's ability to detect tanezumab in maternal plasma. A summary of the accuracy and precision of the assay in the second and third trimesters can be seen in Table 3.

Bioanalytical support of tanezumab developmental and reproductive toxicity studies

The validated assays described above supported two pivotal DART toxicity studies in cynomolgus monkeys [16,17]. The larger of these two studies included 72 pregnant animals (18 per group) receiving weekly intravenous doses of vehicle or tanezumab (0.5, 4 or 30 mg/kg) from gestational day 20 through parturition [16,17]. Tanezumab TK was assessed in maternal plasma regularly from gestational days 20–146 and at postpartum days 30, 60, 90 and 120. Tanezumab concentration was assessed in maternal breast milk once between postpartum days 10–15 and once between postpartum days 30–66. Tanezumab TK was assessed in neonatal plasma at postpartum days 30, 60, 90, 120, 180 and 360. Tanezumab ADA was assessed in maternal serum, using the gestational day-specific cut points described earlier, at gestational days 20, 97 and 146 and at postpartum days 90 and 180. Tanezumab ADA was assessed in maternal breast milk once at postpartum days 10–15 and between postpartum days 50–60. Tanezumab ADA was assessed in neonatal serum at postpartum days 30, 90, 180 and 360.

These bioanalytical assessments demonstrated a few key points [16]. Maternal tanezumab exposure increased with dose (Figure 1) and was consistent with previous findings in male and nonpregnant female monkeys. Despite discontinuation of treatment at parturition, tanezumab was detected in maternal plasma up to 30, 90 and 120 days postpartum in the 0.5, 4 and 30 mg/kg dose groups, respectively. There were also detectable levels of tanezumab in breast milk up to 66, 15 and 60 days postpartum in the 0.5, 4 and 30 mg/kg dose groups, respectively, though at lower concentrations than in maternal plasma (Figure 1). Tanezumab was detectable in neonatal plasma up to 30, 120 and 127 days postpartum in the 0.5, 4 and 30 mg/kg dose groups, respectively (Figure 1). Since concentrations in neonatal plasma were higher than those seen in maternal plasma and breast milk at equivalent time points, neonatal tanezumab exposure is likely a result of placental transfer *in utero*.

Antitanezumab immune response in the form of ADA was detected in all matrices, though not in all dose groups. In maternal serum, the overall incidence of immune response was 28% in the control group and 28, 44 and 17% in the tanezumab 0.5, 4 and 30 mg/kg dose groups, respectively. In the control group, only one of five animals with an immune response had observable levels of drug and it was a trace amount. As described in the previous section, individual gestation day-specific immune response rates were determined using three different gestation time specific cut-point factors after the observation that there were large changes in the NGF during pregnancy in cynomolgus monkeys. These cut point factors were determined based on analysis of gestational day specific individual naïve samples. The ADA incidence values reported are after applying recalculated cut-point factors to

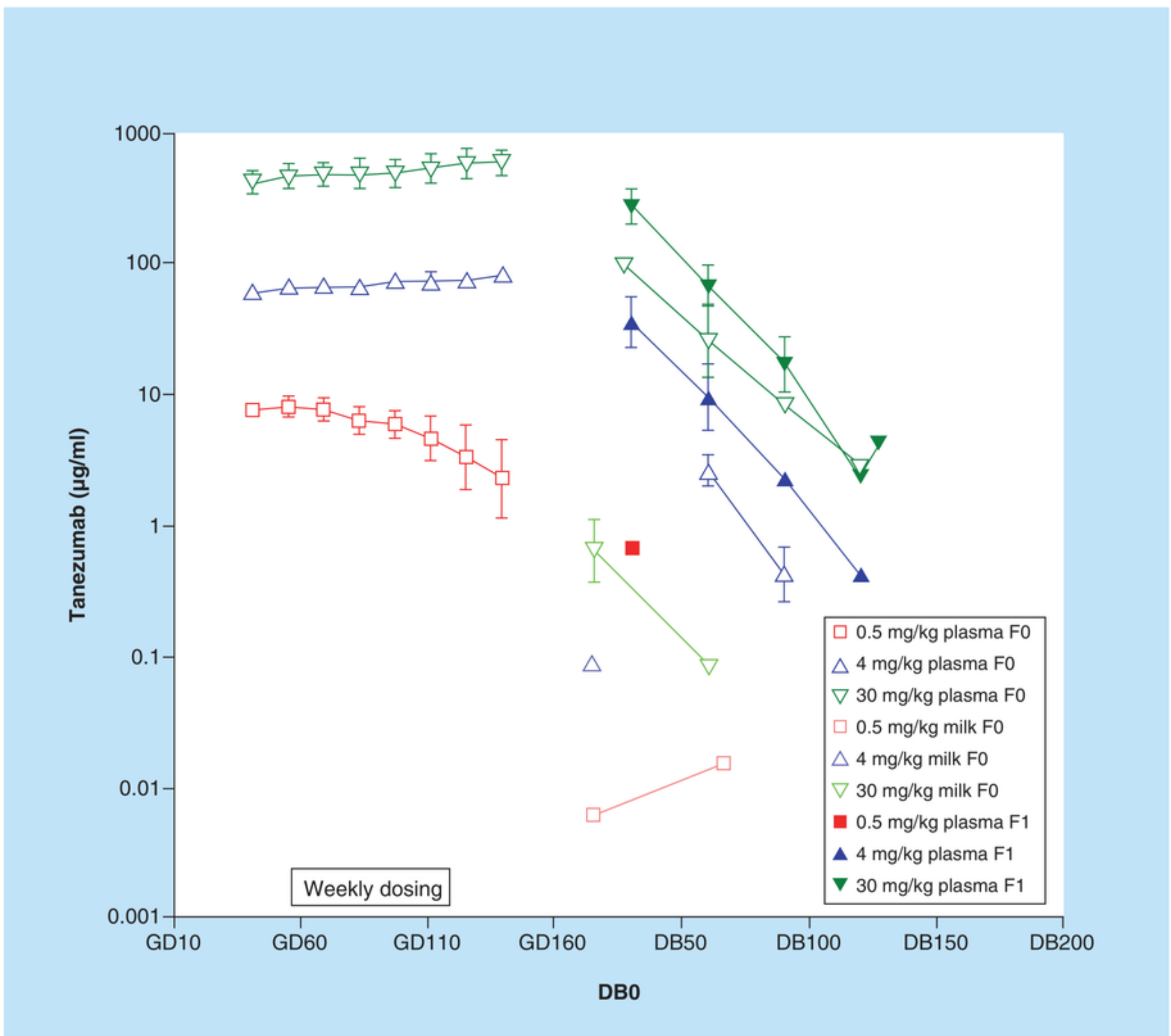

Figure 1. Tanezumab toxicokinetics assessment in maternal plasma, neonatal plasma and breast milk in antidrug antibody-negative animals. Note that maternal plasma concentrations were assessed prior to the next dose. Also note that one neonatal animal in the 30 mg/kg dose group had its sample drawn at day 127 after birth instead of day 120 (solid green triangles).
DB: Day after birth; F0: Maternal animals; F1: Neonatal animals; GD: Gestation day.
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account for target interference in the assay. Therefore the ADA response is considered specific and not due to the target-related interference. The presence of ADA had no marked impact on overall tanezumab exposure in maternal plasma. In breast milk, the overall incidence of immune response was 0% in the control group and 13, 30 and 0% in the tanezumab 0.5, 4 and 30 mg/kg dose groups, respectively. In neonatal serum, the overall incidence of immune response was 17% in the control group and 11, 30 and 0% in the tanezumab 0.5, 4 and 30 mg/kg dose groups, respectively. For each neonate that was ADA positive in the tanezumab-treated group, the associated maternal animal was ADA positive in both serum and milk, demonstrating a correlation between immune response status for the mother and pup. The cut point used for the neonatal serum was 1.14 based on the assay validation data. In the case of cynomolgus neonates, it is unknown if there are changes in the NGF concentration across the study period. However, as noted with the maternal component, increases in the NGF concentration do have the potential to interfere in the ADA assay and cause false positive results.

Conclusion

The tanezumab bioanalytical program supported specialized DART studies in cynomolgus monkeys, which required the validation of assays to assess TK and ADA in maternal plasma/serum, neonatal plasma/serum and breast milk. However, specific challenges were encountered during validation of these assays.

The composition of breast milk can fluctuate over the course of lactation in some species, potentially complicating assessment of tanezumab concentration in this matrix. This potential complication was proactively overcome by validating the TK assay in breast milk across different sampling time intervals (before lactation day 30 and between days 30 and 90). We suggest this approach of validation over different time intervals whenever the composition or characteristics of the particular matrix (i.e., breast milk) have the potential to change over time.

An unanticipated challenge was encountered during tanezumab ADA assessment in maternal serum during pregnancy, which manifested as a high frequency of ADA-positive samples that increased as pregnancy progressed. This spurred further research demonstrating that these false positives were the result of an increase in tanezumab's target protein (NGF) during pregnancy, which was unknown at the time of assay validation. This challenge was overcome by employing gestational day-specific screening cut points at different stages of pregnancy (gestational days 20, 97 and 146). Dynamic physiological conditions, such as pregnancy and lactation, may induce unexpected changes in target proteins, and assays designed for study in normal adult animals may not be appropriate under such conditions. Thus, researchers should assess the potential impact a specific physiological condition may have on their assay and validate that assay across multiple time periods or intervals (according to the specific physiological condition being investigated) to identify any unanticipated complications with the assay.

Future perspective

As regulatory requirements evolve, so too must drug development programs and the bioanalytical studies/approaches that support these programs. In addition to adapting to a changing regulatory environment, bioanalytical programs must also evolve to include newer and better technologies and approaches. In the ELISA-based tanezumab ADA assessments, for example, we handled increasing NGF levels during pregnancy by employing gestational day-specific cut points for samples collected late in pregnancy. This represented the best approach at the time the studies were conducted. However, novel approaches have more recently been developed to reduce the rate of false positives during ADA assessment by utilizing methods that remove or compete with the target protein [23,26]. It should also be noted that, as an example of evolving guidelines, that the current ICH S6(R1) does not require milk analysis [27].

As both regulatory guidance and available technologies evolve, there are bound to be challenges and setbacks. It is imperative that researchers share their learnings from these experiences in order to inform others and advance bioanalytical support of drug development programs.

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This study was sponsored by Pfizer. S Hurst, T Clark and B Gorovits are full-time employees of, and own stock/options in, Pfizer. D Finco was an employee of Pfizer Inc. at the time the studies were conducted. D O'Neil and A Leskovar are employees of ICON plc, the research organization that was contracted by Pfizer to develop and validate the assays described in this manuscript (with the exception of the immunoaffinity lLC–MS/MS assay for NGF). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

Executive summary

- Tanezumab is a humanized monoclonal antibody, directed against nerve growth factor, in development for the treatment of chronic pain.
- This paper describes bioanalytical challenges that were encountered during the design and conduct of tanezumab developmental and reproductive toxicity studies – and how they were overcome.

Experimental

- ELISA-based assays were validated to assess tanezumab toxicokinetics and immunogenicity (presence of antidrug antibodies [ADA]) in cynomolgus monkey maternal plasma/serum, neonatal plasma/serum and breast milk.

Results & discussion

- A challenge was anticipated with the assessment of tanezumab concentration in cynomolgus breast milk because of known changes in the composition of breast milk over the course of lactation in some species. This potential complication was proactively overcome by validating the assay in breast milk across different sampling time intervals.
- An unanticipated challenge was encountered during tanezumab ADA assessment in maternal serum during pregnancy, which manifested as a high frequency of ADA-positive samples that increased as pregnancy progressed. Further research demonstrated that these false positives were the result of an unexpected increase in tanezumab's target protein during pregnancy. This challenge was overcome by employing gestational day-specific screening cut points during the late stages of pregnancy.

Conclusion & practical tips

- Researchers should recognize potential challenges associated with using matrices and physiological conditions whose characteristics have the potential to change over time (such as breast milk and pregnancy), and they should anticipate that assays developed for use under normal conditions may not be appropriate for these more specialized situations.

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60 seconds with Charlene Chen: toxicology studies

Dr Charlene Chen is a Deputy Manager of a QPS Toxicology Center in Taiwan (China). Charlene received her ScD degree from Tulane University in New Orleans (LA, USA). She has more than 20 years of experience in preclinical development in toxicology, PK, PD, pharmacology (including safety pharmacology), immunology, efficacy models and bioavailability in numerous therapeutic areas. Such as: anticancer, anti-infectious/antimicrobial, autoimmunity/immune-related disorder, neurodegeneration and anti-inflammatory.

She also has experience in safety evaluation of materials and products of cosmetics/chemicals including genotoxicity, skin/eye irritation, sensitization, topical and dermal toxicology studies, PDE/OEL establishment and risk assessments. Dr Chen works as the management of GLP compliance laboratory for toxicology and safety evaluation. She also serves as a reviewer, editor and consultant for several Taiwan safety guidance and scientific project funding as well as an adjunct professor. She gives various speeches for conferences, research institutes and universities and is a member of SOT and ACT.

What factors should be considered for the design of toxicology studies?

There are many factors which need to be considered, such as the relevant species, dosing route/frequency, dosing volume, dose levels, age/strain of animals, duration of recovery phase, TK time points and any special parameters to be evaluated. Toxicology studies are not a standard design, they require a detailed understanding of the background and proposed use of test article. Overall, the more information which is known, the better designed and more appropriate the toxicology study will be.

Do the age of animals utilized vary in different types of study?

Yes, basically sexually matured animals should be used for most toxicology studies. However, short term studies, such as those with extended single doses, should consider the use of fully sexually matured animals.

60 seconds with Charlene Chen: toxicology studies

What special considerations should be explored for the safety evaluation of a vaccine?

In addition to routine toxicology evaluation, assays for adjuvant or novel formulation, clinical dose, regimen and patient population are required to be considered. In the toxicology studies, local tolerance and inflammatory biomarkers are usually included as well.

What happens when positive results are found in the genotoxicity studies?

If there is a positive result in the *in vitro* assay, it should be tested in the *in vivo* assay as well. Additional tests are required to access the mode-of-action and to fully evaluate if it possibly is human non-relevant.

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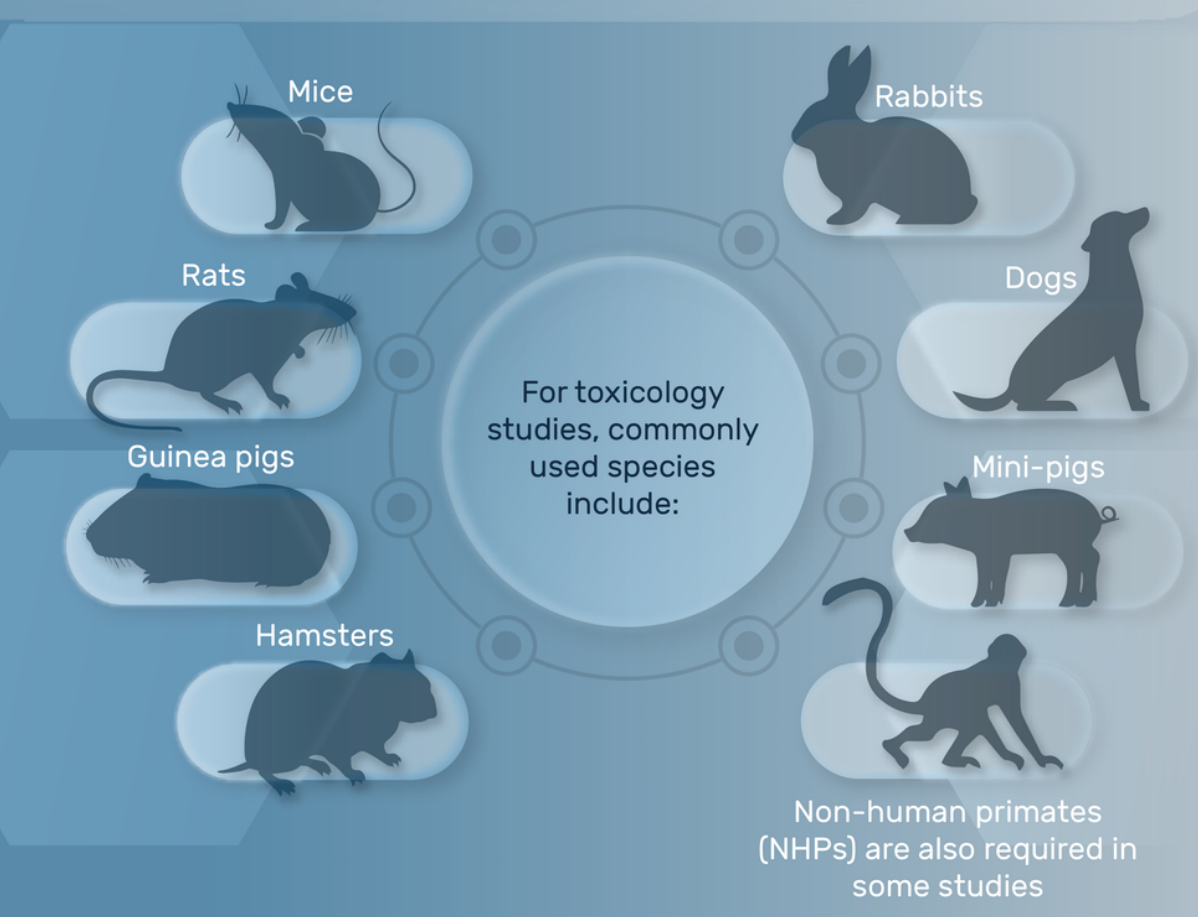
Toxicology studies: design considerations, dosing and instrumentation

How many different types of toxicology studies are there?

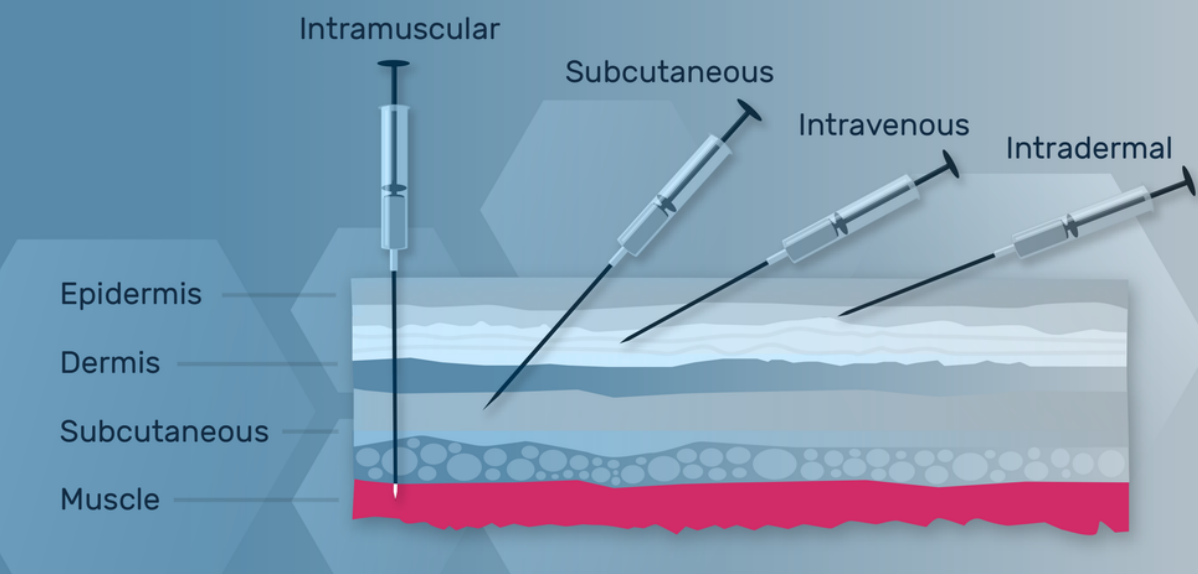
There are many different types of toxicology studies to support drug or medical device development from preclinical to the clinical phase. These include, but are not limited to:

What type of animals are used in toxicology studies?



Which dosing routes are utilized in toxicology studies?

The dosing routes for toxicology animals are usually the same as the proposed human use, however, the related consideration to dose animals in different dosing routes is important for preclinical study design. In addition to the commonly used oral gavage, IV/subcutaneous/intramuscular/intraperitoneal injection, IV infusion, intranasal, instillation, dermal, intra-articular and some specific dosing routes are also applied. The volume of dosing is adjusted to accommodate different species and dosing routes.



What instrumentation is used for clinical pathology and other evaluations?

In order to conduct the required toxicology studies to complete preclinical drug development programs, a full-service toxicology contract research organization is usually equipped with at least four different instruments. These include:

1

Clinical pathology instruments for hematology, serum chemistry, blood coagulation and urine analysis

2

Tissue processors for histology

3

HPLC machines for formulation analysis

4

LC–MS/MS machines for bioanalysis

This infographic has been created as part of a Bioanalysis Zone feature in association with QPS.

Review

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Quantitative translational modeling to facilitate preclinical to clinical efficacy & toxicity translation in oncology

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Significant scientific advances in biomedical research have expanded our knowledge of the molecular basis of carcinogenesis, mechanisms of cancer growth, and the importance of the cancer immunity cycle. However, despite scientific advances in the understanding of cancer biology, the success rate of oncology drug development remains the lowest among all therapeutic areas. In this review, some of the key translational drug development objectives in oncology will be outlined. The literature evidence of how mathematical modeling could be used to build a unifying framework to answer these questions will be summarized with recommendations on the strategies for building such a mathematical framework to facilitate the prediction of clinical efficacy and toxicity of investigational antineoplastic agents. Together, the literature evidence suggests that a rigorous and unifying preclinical to clinical translational framework based on mathematical models is extremely valuable for making go/no-go decisions in preclinical development, and for planning early clinical studies.

Lay abstract: Significant scientific advances in biomedical research have expanded our knowledge of the molecular basis of carcinogenesis, mechanisms of cancer growth and the importance of the cancer immunity cycle. However, despite that in many cases drug treatment can eradicate tumors in animals, treating human tumors remains very difficult. This article describes a mathematical modeling framework to facilitate the prediction of clinical efficacy and toxicity of investigational antineoplastic agents.

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Keywords: cancer growth modeling • drug combination • GRI • myelosuppression • pharmacokinetics • PK/PD • QSP • toxicity • translational • xenograft

Over the last 15 years, scientific advances in biomedical research have expanded our knowledge of the molecular basis of carcinogenesis, mechanisms of cancer growth and the importance of the cancer immunity cycle [1–3]. As a result of those advances, a number of transformative anticancer therapies have been developed, which have brought meaningful declines of mortality and morbidity to patients with this devastating disease. However, despite the scientific advances in the understanding of cancer biology, the success rate of oncology drug development remains the lowest among all therapeutic areas [4]. A recent analysis of 7455 clinical drug development programs between 2006 and 2015 showed that the likelihood of regulatory approval for a Phase I program is the lowest in oncology drug development [4]. Only 5–7% of Phase I clinical oncology programs and <50% of Phase III oncology programs are ultimately approved [5]. A major reason behind this low success rate is the lack of a unifying framework to predict clinical efficacy and toxicity profiles from animal and *in vitro* experiments. A rigorous unifying preclinical to clinical translational framework could facilitate oncology clinical development by better identifying translational strategies, patient selection criteria and appropriate biomarkers to measure [6]. Robust translational research will help with making informed decision early in drug development and improve success rate of late stage programs.

In this review, evidence will be presented to demonstrate that mathematical modeling can be used to build a rigorous and unifying preclinical to clinical translational framework to understand the anticipated exposure–response relationship in humans while considering the tolerability profile. This information can be used to understand the

benefit–risk profile of the investigational drug in different patient populations and maximize the drug's potential in clinical development.

Historically, the development of antineoplastic agents is largely an empirical trial-and-error process. This was particularly true for antineoplastic drugs with cytotoxic mechanisms of action since these drugs are often dosed at the maximum tolerated dose (MTD) in humans and this information was generally obtained experimentally in dose escalation studies.

However, as a result of recent advancement in molecularly targeted therapy, immuno-oncology drugs, cancer vaccines, cell-based therapies, and endless possibilities of drug combinations, obtaining an optimal toxicity–efficacy balance is an increasingly complex task since not all drugs are dosed at MTD. Consequently, the standard empirical approaches used in the past to optimize drug dosing and scheduling in patients are now of limited utility. A more rational dose selection process using mathematical modeling, which is built on a clear understanding of the target biology, to determine the required degree of target engagement would be extremely valuable and could potentially save a lot of time since patient recruitment is challenging for Phase I oncology trials. This mathematical framework can also be easily updated with clinical data and subsequently used to refine drug dosing and scheduling as well as guide go/no-go decisions and trial designs [7].

The scientific and regulatory fields have long recognized the utility of mathematical modeling framework. As early as 2006, the US FDA critical path opportunities report advocated the use of modeling and simulation for decision making in drug development [8]. More recently, the FDA reinforced this idea in their FDA voice blog, which states: "Modeling and simulation play a critical role in organizing diverse datasets and exploring alternate study designs. This enables safe and effective new therapeutics to advance more efficiently through the different stages of clinical trials" [9].

In this review, some of the key translational drug development objectives in oncology will be outlined. Additionally, the literature evidence of how mathematical modeling could be used to build a unifying framework to answer these questions will be summarized. Some recommendations for strategies to build a mathematical modeling framework that facilitates the prediction of clinical efficacy and toxicity of investigational antineoplastic agents will also be discussed.

Key translational objectives of a preclinical oncology drug development program

Generally, there are two key objectives for a standard preclinical oncology drug discovery program. Firstly, the preclinical program needs to provide safety data to support an appropriate starting dose for Phase I clinical programs. This is traditionally achieved using *in vivo* animal toxicology studies. Secondly, the preclinical program needs to provide scientific support for the rationale and biological plausibility of the investigational drug to warrant a clinical study.

A major challenge to achieve these preclinical objectives is to determine the cross-species differences and the relevance of the preclinical efficacy and toxicity data. Mathematical modeling and simulation can be used to account for the species differences and collect all available data to make quantitative predictions about the therapeutic index of the investigational agent in humans. This allows decisions to be made accordingly on whether to advance this drug further into clinical development. The same mathematical model can also be used to determine the appropriate starting dose, the projected human efficacious dose and the appropriate dosing schedule to be evaluated in Phase I trials. This greatly maximizes safety and minimizes toxicity during Phase I studies and align realistic expectations of the drug efficacy. The detailed mathematical approaches to build a translational modeling framework are described in the subsequent sections.

Translating preclinical antitumor activities to clinical efficacy

Due to ethical and practical consideration associated with human clinical studies, animal models have been used extensively in oncology research to evaluate the activities of antineoplastic agents. In contrast to other therapeutic areas such as psychiatry, preclinical oncology research extensively utilizes human tissues [10]. The use of human tissues minimizes the need of accounting for species-specific biology in efficacy translation. In fact, the most commonly used preclinical model in oncology is the mice xenograft model, which comprises subcutaneous implantation of a human cell line/tumor into an immune-compromised host mice [11,12]. The xenograft model represents extreme simplification of human cancer, as it does not account for the complexities of tumor metastasis, host immunity, tumor heterogeneity, and the development of treatment resistance that is routinely observed in cancer patients. [13,14]. Nevertheless, the drug exposure–response relationship derived from these models is still useful for understanding

the degree of antitumor activity associated with the investigational drug, and allows *in vivo* interpretation of tumor growth inhibition data to inform early clinical development [15]. In a typical xenograft experiment, the xenograft tumor volume is measured over time after drug treatments using calipers. The drug-treated tumor volume profile is then compared with that of the vehicle treatment to obtain a quantitative metric of antitumor activity associated with the investigational drug. This information can be used in mathematical models to define efficacy and predict clinical antitumor response.

Due to the variability in the growth rate of human tumors in mice and the small sample sizes of preclinical experiments, rigorous mathematical and statistical analyses are critical in preclinical drug development. There are a number of mathematical approaches to translate the preclinically observed antitumor activity into clinical efficacy. These approaches can generally be categorized into static algebraic approaches and dynamic, differential equation-based approaches. The most commonly used approaches for efficacy translation are discussed in the next section.

Static algebraic approaches of characterizing antitumor activity in preclinical models

There are three static algebraic approaches of antitumor activity that are commonly used in xenograft experiments: tumor volume over control volume (T/C ratio); tumor growth inhibition (TGI); and growth rate inhibition (GRI). Their calculations are summarized in Figure 1. Although there are advantages and disadvantages associated with each algebraic descriptor, GRI is the least dependent on the tumor growth rate and the design of xenograft experiments. Therefore, GRI should be considered as the first choice for translational work. The subsequent paragraphs will introduce each of the three approaches and summarize the advantages of GRI.

T/C ratio is an easy-to-calculate metric of antitumor activity and is often used preclinically to characterize antitumor activity [16–18]. T/C ratio is calculated by dividing the tumor volume of the drug-treated group by the tumor volume of the vehicle-treated group at a predefined time (typically 3 weeks after starting the treatment, illustrated by Figure 1). Although it is easy to calculate, the T/C ratio has some significant limitations since it is heavily influenced by the natural growth rate of the xenograft tumor and the time points at which the T/C ratio is calculated. This limitation is illustrated by the top panel of Figure 1. When treatments of both the slow and fast-growing tumors result in tumor stasis, the T/C ratio generally overestimates the antitumor activity of the fast-growing tumors in comparison to the slow-growing tumors. Due to this undesirable property, the T/C ratio has very limited value for predicting human antitumor efficacy from preclinical data.

TGI is another commonly used static metric to measure antitumor activities (Figure 1, middle panel) [12,19,20]. It is calculated by dividing the tumor volume difference between the vehicle group and the drug-treated group by the tumor volume difference between the vehicle group and the tumor's initial volume (Equation 1). TGI is generally less dependent on the tumor growth rate. Additionally, the TGI obtained from mice subcutaneous tumor models has been show to correlate with human clinical overall response rate for a spectrum of antineoplastic agents [12]. However, it still has a few limitations compared with the GRI.

$$\%TGI = \frac{TV_{vehicle} - TV_{treatment}}{TV_{vehicle} - TV_{initial}} \times 100 \quad \text{(Equation 1)}$$

In the last few years, GRI, a novel rate-based T/C metric, has become more popular for characterizing the preclinical antitumor activity [21]. GRI is calculated by fitting all available tumor volume data first to an exponential growth function. The resulting growth rates under the treatment and control conditions are then used to calculate the percentage of GRI (Equation 2).

$$\%GRI = \frac{GR_{vehicle} - GR_{treatment}}{GR_{vehicle}} \times 100 \quad \text{(Equation 2)}$$

There are a few advantages of using GRI to characterize antitumor activities. Firstly, since all available tumor volume data are used to fit the exponential function, GRI is more efficient than the T/C ratio and TGI; it requires fewer animals to achieve the same statistical power – as little as six animals per group would have sufficient statistical power for translation [21]. Secondly, theoretical simulations have suggested that GRI can tolerate shorter

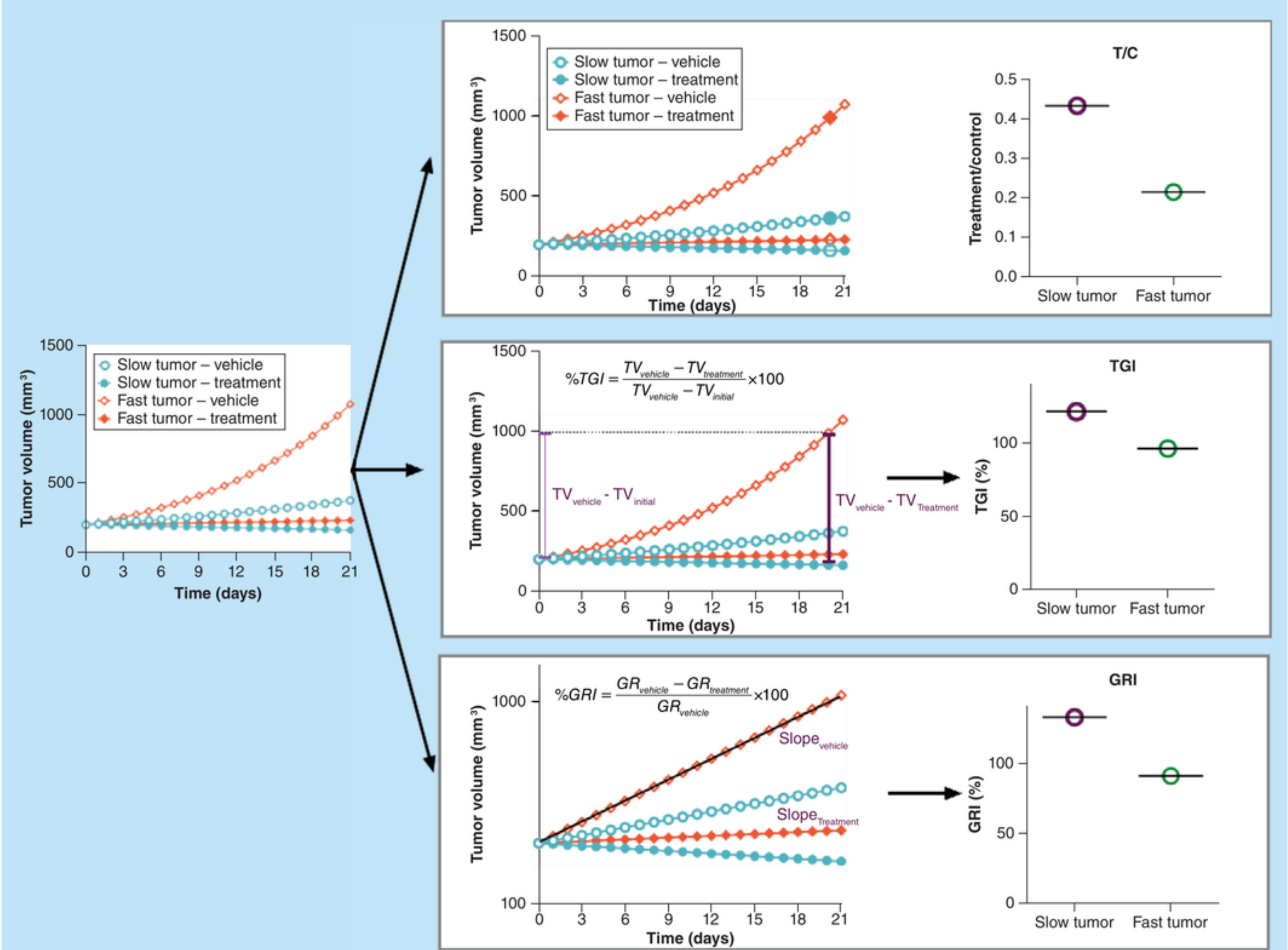

Figure 1. Commonly used antitumor efficacy metrics. Top panel: T/C ratio. Middle panel: TGI. Bottom panel: GRI. T/C overestimates the antitumor activity of the fast-growing tumors compared with the slow-growing tumors, which significantly limits its value for predicting clinical efficacy. TGI is generally less dependent on the tumor growth rate than T/C. GRI, which is calculated by fitting all available tumor volume data first to an exponential growth function, is the least dependent on the intrinsic growth rate of the tumor.
GRI: Growth rate inhibition; T/C: Tumor volume over control volume; TGI: Tumor growth inhibition.

study durations compared with the T/C ratio and TGI [21]. Thirdly, compared with T/C or TGI, GRI is less influenced by the intrinsic growth rate of the xenograft tumor. Therefore, it is ideally suited to compare the drug efficacies across different xenograft models. In particular, GRI has a much more dynamic range compared with TGI in fast-growing tumors. As illustrated by Figure 2, for xenograft tumors which are slower growing (i.e., smaller growth rate), GRI and TGI show a good correlation. However, for xenograft tumors with faster growth rates, GRI has a much more dynamic range compared with TGI, which saturates at around 100% in fast-growing tumors.

Dynamic differential equation-based approaches of characterizing antitumor activity in preclinical models

Although static algebraic approaches are very useful for summarizing the antitumor activity, they often cannot be used to describe complex exposure–response relationships such as sigmoidal or Michaelis–Menten type of dose–response curves. Furthermore, there is often a delay between drug administration and tumor shrinkage (i.e., it takes a while for the drug to kill the tumor cells) which cannot be easily accounted for by static approaches. To overcome these limitations, a number of differential equation-based dynamic approaches have been developed to describe the tumor growth and exposure–response relationship of antineoplastic agents. The common approaches

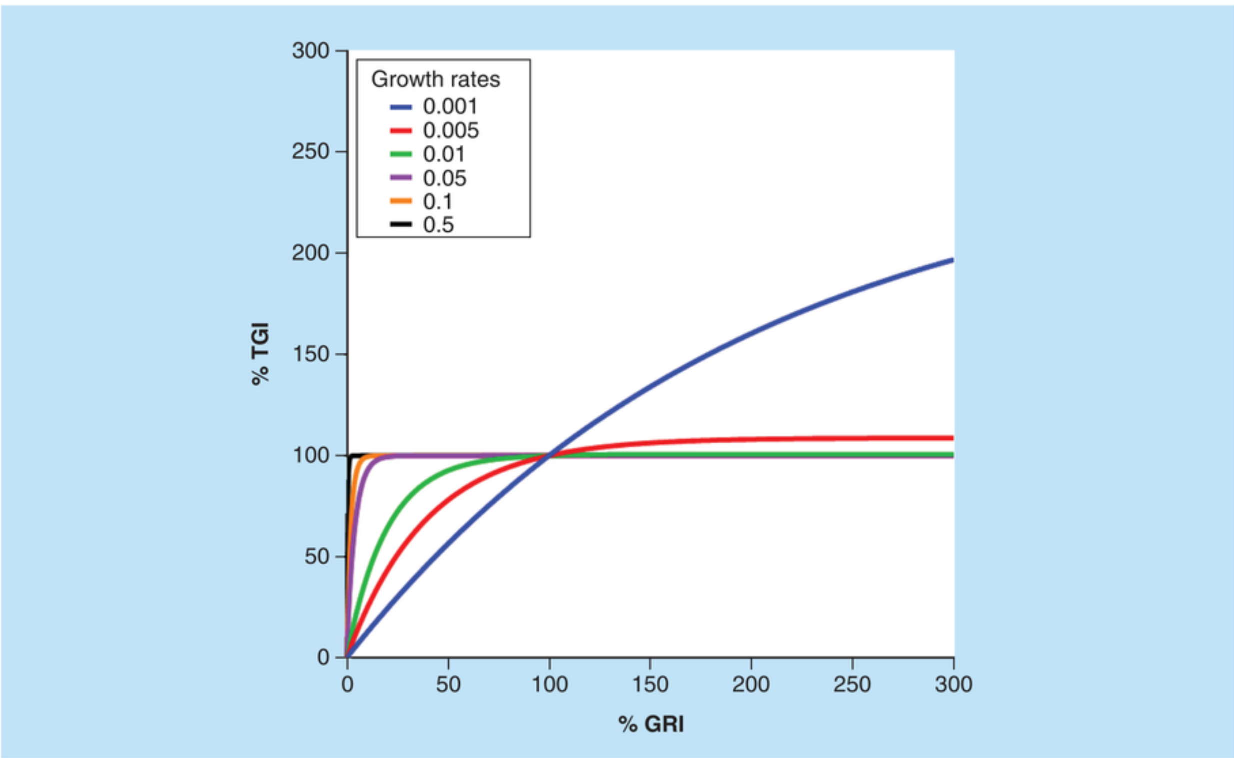

Figure 2. Correlation between tumor growth inhibition and growth rate inhibition for xenograft tumors of different growth rates. Tumor growth inhibition and growth rate inhibition show good correlations for slow-growing xenograft tumors. For fast-growing xenograft tumors, growth rate inhibition has a much more dynamic range compared with the tumor growth inhibition, which saturates at around 100%.
GRI: Growth rate inhibition; TGI: Tumor growth inhibition.

are summarized in Table 1. In general, the dynamic approaches can be categorized into two groups: phase-specific models and phase nonspecific models. The phase-specific models assume that cancer cells are only susceptible to the antineoplastic agents at a specific stage of the cell cycle (for example, antimitotic agents only work on cancer cells that are dividing). These models typically include a series of transit compartments (Table 1) and usually provide a good fitting of observed data [22–25]. In contrast, the phase nonspecific models do not use transit compartments and generally do not fit the observed delay between drug administration and tumor shrinkage well [12,26–28]. Another key aspect of dynamic models is the form of the kill function, which can be either linear, Michaelis–Menten or sigmoidal. The mathematical form of this function dictates whether the model can be used to understand the impact of drug schedule (Table 1). A detailed tutorial has been published to discuss the proper use of dynamic models [29].

Using xenograft antitumor activity to predict antitumor activity in humans

Whether the xenograft transplantation of subcutaneous tumor into immune-deficient mice provides predictive values for discerning clinically efficacious antineoplastic agents has long been debated. Mathematical models of preclinical antitumor data can play an important role in understanding the clinical potential of an investigational antineoplastic agent. The translational modeling of preclinical antitumor activity data usually is a four-step process (Figure 3). Firstly, a robust pharmacokinetic (PK) mathematical model, describing the concentration dynamic of the drug, needs to be constructed based on PK measurements in mice (ideally in the same type of xenograft mice as the efficacy studies). Secondly, using the established mice PK model as a foundation, an exposure–response relationship can be established using xenograft efficacy studies to understand the effects of different doses/schedules on the time course of tumor growth. The exposure–response relationship can be either static (as expressed by a correlation between drug exposure and the resulting GRI on a graph) or dynamic (as expressed using a set of differential equations to account for delayed drug effects and changes in growth rate over time) in nature [23]. A key consideration at this stage is selecting the appropriate xenograft model for clinical translations. This is particularly important for molecularly targeted therapy (such as HER2 or EGFR inhibitors), as it is essential to ensure that

Table 1. Commonly used pharmacokinetic/pharmacodynamics models to describe tumor growth kinetics.

Study (year)	Tumor growth term	Tumor kill term	Transit compartments	Comments	Ref.
Phase nonspecific models					
Kogame *et al.* (2013)	$\frac{dTV}{dt} = k_{ng} \times \left(1 - \frac{TV}{TG_{50} + TV}\right) \times TV_1 - Effect$	$Effect = \frac{K_{max} \times C(t)^n}{KC50^n + C(t)^n}$	0	This model was developed for a potent, selective hedgehog signaling pathway inhibitor. It was used to identify the degree of PD inhibition required to inhibit tumor growth.	[26]
Yamazaki (2008)	$\frac{dTV}{dt} = k_{in} \times \left(1 - \frac{TV}{TG_{50} + TV}\right) \times (1 - Effect) \times TV - k_{out} \times TV$	$Effect = \frac{K_{max} \times C(t)^n}{KC50^n + C(t)^n}$	0	This model was developed to understand the PK/PD relationship of a small molecule cMet inhibitor. The model structure closely resembles an indirect response model.	[28]
Wong *et al.* (2009)	$\frac{dTV}{dt} = k_{ng} \times TV(t) - Effect \times TV$	$Effect = \frac{K_{max} \times C(t)^n}{KC50^n + C(t)^n}$	0	This model was developed to understand the PK/PD relationship of a B-Raf inhibitor. No transit compartments were used.	[30]
Salphati (2010)	$\frac{dTv}{dt} = k_{ng} \times TV(t) - Effect$	$Effect = \frac{K_{max} \times (\%I)^n}{KC50 + C(\%I)^n}$	0	This model was developed understand the PK/PD relationship of a PI3K inhibitor. This model used a hill coefficient on the effect term which is very unique.	[27]
Phase-specific models					
Lobo *et al.* (2002)	$\frac{dTv}{dt} = k_{ng} \times TV(t)$	$Effect = \frac{K_{max} \times C(t)}{KC50 + C(t)}$	3	One of the first PK/PD models utilizing transit compartment to describe the antitumor. It was initially developed for methothrexate.	[22]
Simeoni *et al.* (2004)	$\frac{dTV_1}{dt} = \frac{\lambda_0 \times TV_1}{1 + \left(\frac{\lambda_0}{\lambda 1} \times w(t)\right)^{\psi}} - Effect \times TV_1$	$Effect = K_{kill} \times C(t)$	3	This is one of the most commonly used model structure for modeling preclinical antitumor activity. It was initially validated against a few cytotoxic agents, but the structure has been subsequently applied to many other types of compounds.	[23]
Bueno *et al.* (2008)	$\frac{dTV}{dt} = \frac{k_{ng1} \times (1 - Effect) \times TV}{\left[1 + \left(\frac{k_{ng1}}{k_{ng0}} \times TV\right)^{\gamma}\right]^{1/\gamma}}$	Effect = biomarker	2	It was developed for LY2157299, a new type 1 receptor TGF-β antagonist. Tumor growth inhibition was linked to PD biomarker level.	[24]
Jumbe *et al.* (2010)	$\frac{dTV_1}{dt} = k_{ng} - k_{kill} \times Effect \times dTV_1$	$Effect = \frac{K_{max} \times C(t)}{KC50 + C(t)}$	2	This model was developed for trastuzumab-DM1 (an ADC) in order to determine the optimal dose and dosing schedule for antitumor activity.	[25]

ADC: Antibody–drug conjugate; PK/PD: Pharmacokinetic/pharmacodynamics.

the xenograft model contains the pathway activation phenotype relevant to the drug's mechanism of action. The third step in the translation process is to translate the xenograft exposure–response relationship into humans by substituting the PK portion of the mice model with predicted or observed human PK parameters, depending on whether observed human data are available. A large body of literature exists for human PK predictions of small and large molecules and has been reviewed extensively elsewhere [31–35]. In this step, a key consideration is to incorporate interspecies differences in plasma protein binding to ensure that the translation is based on the free fraction of the drug. After completing this step, a mathematical model can be used to predict the human exposure–response relationship and simulate the clinical dose/schedule effects on tumor growth. The last step is to use a translational exposure-tolerability model to predict the maximum tolerable dose/exposure in humans and evaluate whether the

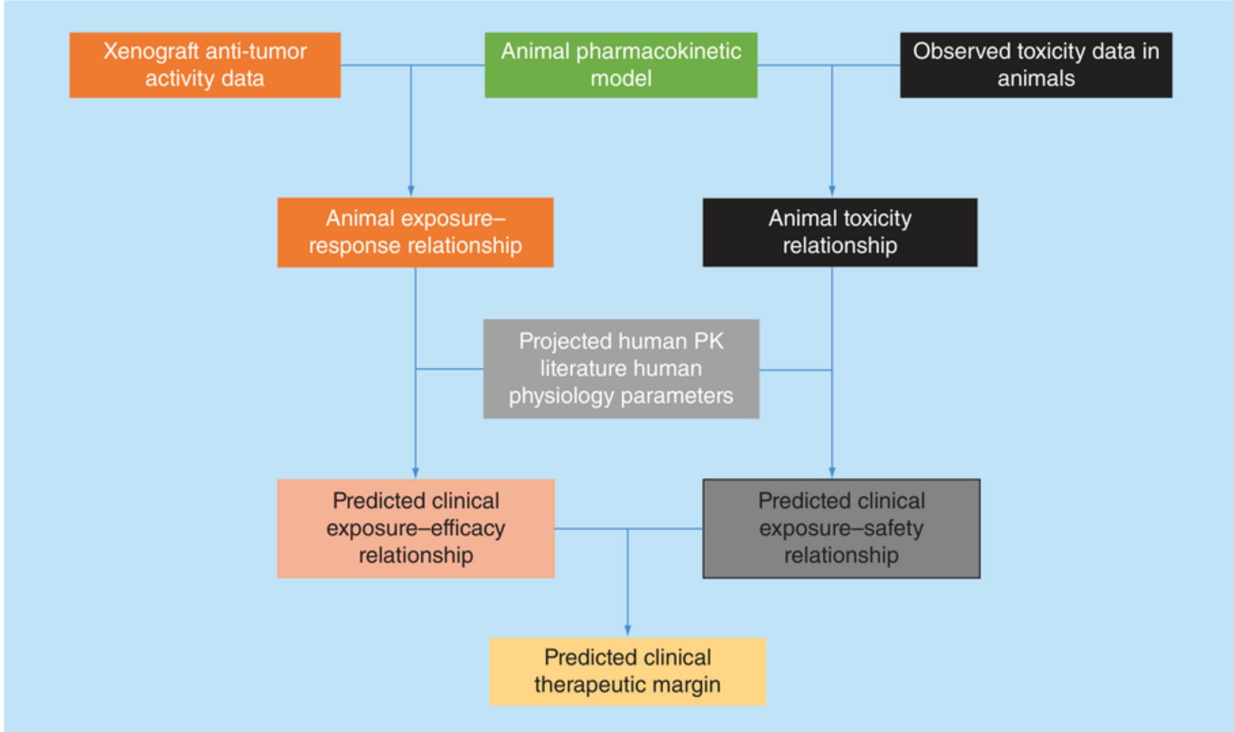

Figure 3. Common translational modeling approach for predicting the efficacy potential of an investigational antineoplastic agent. Firstly, a pharmacokinetic mathematical model needs to be constructed as a foundation, based on pharmacokinetic measurements in mice. Secondly, xenograft efficacy studies are used to establish an exposure–response relationship. Thirdly, the xenograft exposure–response relationship is translated into humans based on human data. Lastly, a translational exposure-tolerability model based on animal and human toxicity data is used to predict whether the drug would have a meaningful tumor regression in humans at a tolerable dose. PK: Pharmacokinetic.

investigational drug would have a meaningful tumor regression in humans at a tolerable dose. This step is also very important since one of the major reasons for the lack of translatability is that mice often can tolerate much higher drug exposure compared with humans. Therefore, even if an antineoplastic agent shows good efficacy in the preclinical model, the efficacious exposure may not be safely achieved in humans. In fact, a poor correlation is observed between the antitumor activity at mice MTD and activity in the clinic, suggesting that proper anticipation of human tolerability is essential for this kind of translational work [12].

More recent research shows that preclinically predicted antitumor activity at human tolerable exposures correlated strongly with clinical response [12]. Figure 4 summarizes the correlation between the preclinical predicted antitumor activity (percentage GRI) and overall clinical response rate for a spectrum of molecularly targeted and cytotoxic agents at clinical maximum tolerable exposure. The data were collected from the literature and percentage GRI was calculated using digitalized data [12]. This analysis suggests that when proper mathematical models are used to account for human tolerable exposures, preclinical antitumor activity is highly predictive of the overall response rate in the clinic. This strongly suggests that xenograft transplantation of subcutaneous tumor into immune-deficient mice can be used to discern the clinical potential of novel antineoplastic agents and highlights the importance of using proper mathematical models for preclinical to clinical translation.

Using the modeling framework summarized in Figure 3, a number of successful preclinical–clinical translation examples have been demonstrated [20,26,36–44]. In the case of cytotoxic agents, Jumbe *et al.* described a cell-cycle phase nonspecific tumor cell kill model which captured the features of tumor growth in trastuzumab-DM1-treated animals under a number of single-dose, multiple-dose, and time–dose–fractionation conditions. This model suggested that the antitumor activity was schedule-independent, and the tumor response was determined by the ratio of drug exposure to a critical tumor stasis concentration [25]. This modeling framework was also used to translate the molecularly targeted agents. For example, Tate *et al.* described a PK–pharmacodynamic-efficacy (PK–PD-E) model for the translation of an investigational cyclin-dependent kinase 4/6 inhibitor. This model dynamically linked drug concentrations to pharmacodynamic effects such as decreased phosphorylation of retinoblastoma protein, cell-cycle

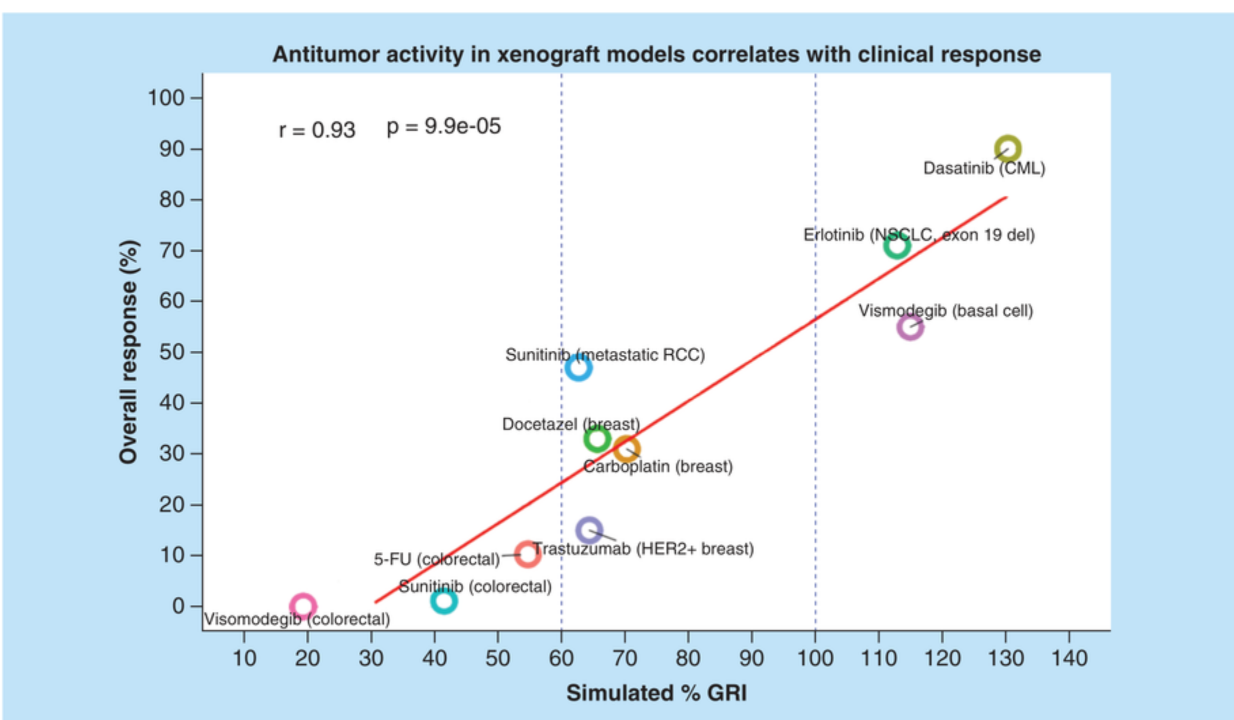

Figure 4. Antitumor activity in xenograft correlates with clinical response. The data were collected from the literature and percentage growth rate inhibition was calculated using digitalized data [12]. Antitumor activity in xenograft correlates with clinical response for a spectrum of molecularly targeted and cytotoxic agents at clinical maximum tolerable exposure.
GRI: Growth rate inhibition; RCC: Renal cell carcinoma.

arrest, and tumor growth inhibition. The model was used to support the clinical chronic dosing regimen for durable cell-cycle inhibition [37].

Although the exposure–response relationship usually translates well for cytotoxic agents and molecularly targeted agents, its utility is more limited in the immuno-oncology field since there are some fundamental differences between human and mice immune systems. In this case, quantitative system pharmacology (QSP) models, which link PK with mechanistic models of biological pathway modulation, become extremely useful. Since these models are built on the understanding of the biological pathways and the model parameters are often generated experimentally rather than fitted empirically, they can be viewed as quantification of the underlying biological processes. QSP models enable the separation of biological and drug specific parameters, and thus have an enhanced interspecies translational ability. This property is ideally suited for translational modeling of immuno-oncology agents since one could re-parameterize the model using human-specific biological parameters while keeping the drug-specific parameters the same between mice and humans since these are properties of the drug.

Several publications investigated the translatability of immuno-oncology agents using this QSP framework. For example, Lindauer *et al.* built a quantitative system pharmacology model to identify the lowest effective dose for evaluation in clinical dose-finding studies for pembrolizumab, a programmed death 1 checkpoint inhibitor. This model linked a compartmental PK model to a published physiologically based tissue compartment and used receptor occupancy as the driver of observed tumor growth inhibition [45]. A similar QSP-based model was also used to predict the safe starting dose and clinical efficacious dose for P-cadherin LP-DART, a bispecific T-cell engager [40]. Overall, although a relatively new concept, the QSP modeling field is growing rapidly and more case examples will become available in the coming years to really explore its full potential.

Methods for predicting clinical efficacy of drug combinations

Another very important aspect of modern oncology research is to understand drug combinations. Mathematical modeling of preclinical data can also provide guidance on the predicted clinical benefit of combination therapies under different dose and scheduling combinations. This is particularly important since combinations can be

extremely complex when all permutations of doses and schedules are considered and testing all combinations in the clinic is simply not possible.

The antitumor activities of combination therapies can be modeled by either static or dynamic approaches. Static translations of antitumor activities are typically done via isobolograms of *in vivo* exposures at various levels of xenograft antitumor activities [46]. This approach is visual and could provide a graphical representation of the exposure–response relationship for drug combinations to guide dose selection and escalation in the clinic. The disadvantage of this isobolograms-based static approach is that it does not consider the time dynamics of drug concentrations and the dynamics of drug effects. A dynamic combination modeling framework has been published to better understand the sequential effect and prioritization of dose pairs (e.g., low-dose drug A + high-dose drug B vs high-dose drug A + low-dose drug B) [47]. This dynamic semimechanistic model allows the optimization of combination dosing schedules and determines which combination schedule would give the highest degree of synergy. The application of combination modeling to guide clinical dosing schedules has been described for combinations of MAPK and PI3K pathway inhibitors [48].

Modeling translation of drug-induced toxicity

In addition to translating antitumor efficacies, mathematical modeling could also facilitate the quantitative prediction of exposure–safety relationship in order to gain a scientific understanding of the tolerability profile. Although antineoplastic agents are associated with many different types of toxicities/adverse events, some of the most common toxicities can be readily described by mathematical models. Properly verified mathematical models can be used to ask the what-if questions related to drug safety. For example, mathematical modeling can be used to assess the impact of intrinsic or extrinsic factors, such as organ impairment, gender, race and drug–drug interaction, on the safety profile. Some of the most mature translational mathematical models are in the areas of myelosuppression, gastrointestinal (GI) toxicity, and cardiac safety. The current state of scientific knowledge is discussed below.

Neutropenia & general myelosuppression models

Antineoplastic agents often have the greatest impact on the growth and survival of rapidly proliferating cells. One of the most notable rapidly proliferating cell types in the body is the hematopoietic stem cell (HSC). In a person's life time, HSCs, which account for just 0.01–0.2% of the total bone marrow mononuclear cells, produce blood cells weighing ten-times more than the bodyweight of that person [49,50]. Due to their high proliferative potential, cells derived from HSC are extremely susceptible to the cytotoxic effects of antineoplastic agents.

As all blood cells, neutrophils are produced by HSC in the bone marrow. Neutrophils are short lived in the blood circulation [51] and their level is dependent on a constant state of production [52]. Thus, neutrophils' homeostasis is particularly susceptible to antineoplastic agents [52]. Neutropenia (low neutrophil counts) and febrile neutropenia increase the risk of hospitalization and complications as a result of an increased susceptibility to infections. Therefore, it has become a primary concern in oncology drug development. A general myelosuppression model, which can be applied to neutropenia, was one of the first mathematical models developed to characterize the time courses of adverse events and has been used to understand the exposure–safety relationships of a huge number of antineoplastic agents [53].

This model mimics myelopoiesis by including a concentration-related drug effect on a proliferating precursor cell compartment. Maturation of the precursor cells in the bone marrow is modeled as a series of transit compartment to explain the delayed effect on circulating neutrophil counts. A homeostatic feedback mechanism is included to stimulate the increase in proliferation of the precursor cells when circulating mature neutrophils counts are low. The attractiveness of this model for translation lies in its ability to separate drug-related versus physiology-related processes, which allows for predictions of untested scenarios in different species. Due to its parsimonious structure, it is frequently applied and has been shown to be highly reproducible and robust [53]. Due to its simplistic nature, this mechanism-based structure also allows for extensions, such as rescue treatments with recombinant granulocyte-colony stimulating factors.

In the context of preclinical to clinical translation of drug safety, this model separates the drug- and physiology-related processes. Since species-dependent parameters can be obtained from the literature, only drug-specific potency parameters are needed to predict the clinical myelosuppression profile from preclinical data. It has been shown that drug-specific potency parameters can be scaled from animal to humans after correcting for species differences in protein binding and *in vitro* sensitivity [54]. Using this kind of translational exposure safety model, the tolerability profile in Phase I studies can be forecasted and updated to increase precision once neutrophil data in humans

become available. This strategy allows more rapid decision making on dosing schedules and dose escalation [55,56]. Furthermore, although neutropenia *per se* is not life threatening, it could result in reduced capability for fighting infections and the development of febrile neutropenia. A body of research has shown that the shape of neutrophil profile is related to the risk of developing febrile neutropenia [57]. A translational understanding of the risk could be invaluable in early clinical developments for identifying patients requiring rescue treatments and maximizing the therapeutic potential of the investigational agent [58,59].

Gastrointestinal toxicity models

In addition to blood cells, enterocytes in the GI tract is another rapidly proliferating cell type in the body. Due to their high proliferative potential, enterocytes are also extremely susceptible to the cytotoxic effects of antineoplastic agents such as DNA-damaging agents, antimitotics, drugs which affect the protein homeostasis pathways and epigenetic regulators [60,61]. In fact, about 50–80% of the patients receiving chemotherapies develop GI-related adverse events [62,63]. The incidences and severity of GI-related adverse events could be potentially mitigated by changing the dosing schedule and employing dosing holidays in order to achieve efficacious exposures while maintaining tolerability [60,64]. The current dosing schedules for many antineoplastic agents are often identified empirically through head to head comparison in clinical trials. However, due to ethical, cost and time duration considerations, only a limited number of schedules could be tested in the clinic. Thus, a mechanistic understanding of GI toxicity using preclinical models and a quantitative translational framework could be extremely beneficial for the management of GI toxicity.

Although the molecular mechanisms regulating the homeostasis of intestinal epithelium are not as well understood as myelopoiesis, the fundamental process maintaining intestinal mucosa integrity has been described. Slowly dividing stem cells located near crypt bottoms produce rapidly dividing progenitor cells, which form mature absorptive cells, enterocytes and secretory cells after lineage commitment [65–67]. Mature epithelial cells then migrate toward the lumen side of the mucosa where they undergo apoptosis and shedding [66]. A number of system biology models have been constructed to investigate this process [68–72]. And more recently, the quantitative translational aspects of gastrointestinal toxicity are investigated using irinotecan [73]. Irinotecan is known to induce gastrointestinal-related adverse events in the clinic [74]. Shankaran *et al.* built a quantitative system pharmacology model to describe the key aspects of intestinal cell dynamics. This model was used to determine the toxicity of the compounds against intestinal crypts in rats and subsequently translated into quantitative predictions of enterocyte loss and recovery kinetics in humans [73]. The model predictions showed good correlation with clinical observed rate of irinotecan-induced gastrointestinal adverse events. The model was then used to simulate a range of clinical schedules to rank the schedules based on the extent of gastrointestinal toxicity [73]. This kind of translational work could be very beneficial for optimizing dosing schedule of cytotoxic agents. For other classes of antineoplastic agents such as immuno-oncology agents, animal models may not be relevant since the mechanism of GI toxicity is often not due to cytotoxicity in humans. For example, for nivolumab, pembrolizumab and ipilimumab, very limited GI toxicity was observed in animals but it is the dose-limiting toxicity in humans. Therefore, at this moment the GI toxicity prediction by mathematical models are more restricted to chemotherapeutic agents.

Other types of toxicity

In addition to myelosuppression and gastrointestinal toxicity, antineoplastic agents, particularly small molecule RTK inhibitors, also cause cardiovascular adverse events including QT prolongation. At a fundamental level, the heart is an electrical organ, and cardiac contractility is a complex interplay between many ion channels. This process can be recapitulated *in silico* through mathematical modeling with a good degree of certainty. In the pharmaceutical industry, significant investments have been made to develop mechanistic-based cardiac models to predict cardiac safety. The largest initiative is the Comprehensive *in vitro* Proarrhythmia Assay. Comprehensive *in vitro* Proarrhythmia Assay proposes to assess a drug's effect on multiple ion channels and integrate the effects in a computer model of the human cardiomyocyte to predict proarrhythmic risks [75]. The *in silico* reconstructions integrate drug effects on multiple human cardiac currents and the results are confirmed with human stem cell-derived cardiomyocytes. The modeling results could guide risk management in clinical trials, and in appropriate cases, potentially obviate the need for a dedicated QT study. Similar efforts are also under way to predict drug-induced liver injury, particularly in the bile acid transporter inhibition area [76,77].

Table 2. The key preclinical to clinical translational differences between cytotoxic antineoplastic agents and immuno-oncology agents.

Key translational differences	Cytotoxic agents	Immuno-oncology agents
Preclinical model	Xenograft	Syngeneic
Target	Tumor	Host immune system
Benchmark for clinical efficacy	At least 60% GRI	–[†]
Efficacy response	Continuous and show dose response	Sometimes binary
Translation	Exposure-based translation	Biomarker-based translation/–[†]
FIH dose	1/6 of HNSTD	MABEL

[†]Unknown.
FIH: First in human; GRI: Growth rate inhibition; HNSTD: Highest nonseverely toxic dose; MABEL: Minimal anticipated biological effect levels.

Conclusion & future perspective

We are in the midst of an evolving paradigm shift for oncology. The growing knowledge of basic molecular and cellular mechanisms underlying carcinogenesis and immuno-oncology as well as the availability of large amount of data will allow mathematical models to adequately describe the processes involved, and make quantitative predictions. The current mathematical modeling knowledge in oncology is largely built on the experience of developing cytotoxic and molecularly targeted agents. For immuno-oncology agents, syngeneic mice with intact immune systems are often used pre-clinically to determine the anti-tumor efficacy since the drug often targets the host immune system rather than the tumor itself. During the mathematical translation work, one would need to consider the species difference between mouse and human immune systems. This cross-species translation makes efficacy and toxicity projection much more difficult (Table 2). Making the cross-species translation even more difficult, the investigational drug often has different binding affinities in different species. Therefore, a key future direction for mathematical modeling in oncology is to build and validate translational immuno-oncology models. The oncology modeling field could do well by learning from the experience of vaccine and inflammation modeling community to solve these problems.

Another future research direction is to understand the heterogeneity of antitumor responses. Due to practical limitations, only a small number of xenograft models could be studied in preclinical developments, and this limits our ability to understand how patients with heterogeneous tumor would respond to the investigational antineoplastic agent. Fortunately, significant advances have been made in the development of genomics technologies and high-throughput preclinical Phase II-like studies where hundreds of patient-derived xenograft tumors could be monitored for drug effects [78]. These data can be combined to determine the preclinical sensitivity differences between different types of molecular signatures [79,80]. Ultimately, this would allow better patient selection in the clinic and lead to more benefits for the patients. Patient-derived xenograft (PDX) models in the context of population pharmacokinetics/pharmacodynamics are a unique way of predicting population distribution of the responders and can be used as a Bayesian prior for clinical development. Furthermore, one of the major problems with current mice xenograft studies is the lack of genomic diversity. In the future, next-generation sequencing in high-throughput Phase II-like PDX studies will provide more information about how genomic diversity will impact drug pharmacodynamics and efficacy. If a predicted PD biomarker can be developed and incorporated into mathematical models, it could facilitate efficacy prediction in tumors of different genomic backgrounds.

Overall, despite many practical challenges, significant advances have been made to establish a mathematical modeling framework to facilitate the efficacy and toxicity translation in oncology. For cytotoxic agents and most of the molecularly targeted antineoplastic agents, mathematical modeling of the preclinical data can now predict the clinical efficacy and toxicity profile with good confidence as demonstrated by the examples discussed in this review. However, translation for immuno-oncology agents remains very difficult. The oncology translational modeling field should look to quantitative system pharmacology fields of inflammation and vaccines for scientific inspiration. The growing use of mathematical modeling in oncology translation will provide a unifying framework for evaluating the potential of an investigational oncology product. Lessons learned from the models could help determine the best clinical development strategy and the kinds of patients who would benefit the most from the new drug. Together, a model-based development paradigm will result in a rational and more efficient oncology drug development process.

Executive summary

- Over the last 15 years, scientific advances in biomedical research have expanded our knowledge of the molecular basis of carcinogenesis, mechanisms of cancer growth and the importance of the cancer immunity cycle. However, despite the scientific advances in the understanding of cancer biology, the success rate of oncology drug development remains the lowest among all therapeutic areas.
- Mathematical modeling can be used to translate preclinical knowledge into clinical predictions to refine drug dosing and scheduling as well as guide go/no-go decisions and trial designs.
- Preclinically, there are a number of mathematical approaches to translate the preclinically observed antitumor activity into clinical efficacy. These approaches can generally be categorized into static algebraic approaches and dynamic, differential equations-based approaches.
- Growth rate inhibition should be considered as the first choice for static algebraic translation of preclinical efficacy data.
- In the case of sigmoidal or Michaelis–Menten type of dose response curves or in a situation there is a delay between drug administration and tumor shrinkage. Dynamic differential equation-based dynamic approaches should be used to describe the exposure–response relationship.
- A four step process can be used to preclinical antitumor activity to clinical efficacy. Once the proper steps are taken, recent research shows that preclinically predicted antitumor activity at human tolerable exposures correlated strongly with clinical response.
- Mathematical modeling of preclinical data can also provide guidance on the predicted clinical benefit of combination therapies under different dose and scheduling combinations. Isobologram-based static approach and differential equation-based dynamic combination modeling framework have been published to better understand the sequential effect and prioritization of dose pairs.
- In addition to translating antitumor efficacies, mathematical modeling could also facilitate the quantitative prediction of exposure–safety relationship. Some of the most mature translational mathematical models are in the areas of myelosuppression, GI toxicity, and cardiac safety. The current state of scientific knowledge is discussed below.
- In summary, the growing use of mathematical modeling in oncology translation will provide a unifying framework for evaluating the potential of an investigational oncology product. Lessons learned from the models could help determine the best clinical development strategy and the kinds of patients who would benefit the most from the new drug. Together, a model-based development paradigm will result in a rational and more efficient oncology drug development process.

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