



Considerations on the Calculation of the Human Equivalent Dose from Toxicology Studies for Biologic Anticancer Agents

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First-in-human (FIH) clinical trials for investigational anticancer agents are often conducted in cancer patients who are resistant and/or refractory to standard therapy or have no other treatment options that would confer clinically relevant benefit. Selecting an appropriate starting dose for these studies is not only important to ensure patient safety but also to enable efficiency in reaching the therapeutically relevant dose range. Starting with a dose that is too low could lead to a lengthy dose escalation and subjecting many terminally ill cancer patients to subtherapeutic doses [1].

The International Conference on Harmonization (ICH) S9 guidance provides recommendations on non-clinical evaluation for anticancer agents, including basic guidelines on determination of the starting dose based on toxicity studies in animal species [2]. The guidance indicates that for most systemically administered small molecules, the starting dose could be determined based on scaling of an appropriate threshold ‘safe dose’ in animal studies to a human equivalent dose (HED), which is then used to determine the starting dose after applying a safety factor [2, 3].

For immune-activating biologics, toxicology studies in animal species may not fully capture the clinically expected immune-related adverse events, and thus may underpredict toxicity in humans. Differences in receptor expression pattern and/or binding affinities between animal species and humans, as well as the potential for eliciting complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC), may result in exaggerated safety findings in humans that may not be observed in

non-clinical toxicology studies [2–4]. Therefore, for biologics with agonistic properties (e.g. those with cellular targets that activate downstream pathways and trigger cytokine release), the starting dose is typically based on the minimally anticipated biologic effect level (MABEL) [2–4]. Data from *in vitro* studies (e.g. target binding affinity, *in vitro* cytotoxicity, or cytokine-release assays) and *in vivo* studies (e.g. tumor growth inhibition studies using relevant preclinical models) are used for MABEL determination [2–4]. It is important to highlight that there is no universal approach for determining MABEL and the cut-offs and assays used are dependent on the therapeutic modality and the intended pharmacological effect. For example, for immune-activating antibodies, e.g. PD-1/PD-L1 inhibitors, starting doses that correspond to 20–80% receptor occupancy (RO) could be associated with acceptable/manageable toxicities [5]. However, the RO approach is not acceptable for T-cell-engaging CD3 bispecifics because doses that correspond to as low as 10% RO were found to be above the human maximum tolerated dose (MTD) or highest human dose [6]. For T-cell-engaging CD3 bispecifics, the first-in-patient starting dose corresponding to concentrations that achieve up to 50% of the maximal effect from the most sensitive *in vitro* activity assay was considered an acceptable approach [6].

Typically, MABEL-based starting dose is lower than that derived based on animal toxicology studies and is considered a more conservative approach for immune-activating biologics [4–6]. However, given the lack of a unified approach for determining MABEL, in some cases the MABEL dose could be similar to or higher than the starting dose calculated based on animal toxicology studies. Therefore, accurate understanding of *in vitro/in vivo* data, exposure–toxicity and exposure–efficacy relationships in preclinical species need to be considered to select an appropriate starting dose. Relevant factors used to convert the animal doses determined in the toxicology studies (e.g. the highest non-severely toxic dose [HNSTD] or the no-observed adverse effect level [NOAEL]) to the HED should be considered [3]. The 2005 US FDA

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guidance on ‘Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers’ recommends using a body surface area (BSA) normalization approach based on the following equation:

$$\text{HED} \left(\frac{\text{mg}}{\text{kg}} \right) = \text{Animal dose} \left(\frac{\text{mg}}{\text{kg}} \right) \times \left(\frac{\text{Animal weight(kg)}}{\text{Human weight(kg)}} \right)^{(1-\text{EXP})}$$

where the animal dose is the HNSTD or NOAEL determined in toxicology studies, HED is the human equivalent dose, and EXP is an allometric scaling exponent.

The FDA guidance used conversion factors that were derived based on a BSA scaling factor of 0.67 [i.e. $\left(\frac{\text{Animal weight(kg)}}{\text{Human weight(kg)}} \right)^{(0.33)}$] [3]. For non-human primates, which are regarded as the most relevant species for preclinical safety assessment of biologics [7], assuming a monkey body weight of 2 kg and human body weight of 60 kg, the conversion factor to calculate the HED is to multiply the animal dose by 0.32 [3]. The allometric scaling exponent of 0.67 was selected as it provides more conservative starting dose estimates compared with a scaling factor of 0.75, despite that 0.75 better correlated animal doses to that in humans and was recommended to be used for scaling between species for doses in carcinogenicity studies [3]. Per the FDA guidance, intravenously administered biologics with a molecular weight > 100 kDa are considered an exception of the BSA scaling approach and should be scaled based on body weight, i.e. setting the HED in mg/kg to be the same as the animal dose, which indicates a scaling exponent of 1 [3]. Despite this exception, the conversion factors derived using a scaling factor of 0.67 are still used in some instances for calculating HED for biologics as a conservative scaling approach, especially for biologics administered via the extravascular route (e.g. subcutaneous administration). Adoption of the conversion factors from the FDA guidance seems to be across therapeutic areas given its simplicity, however it is important to highlight that the guidance is intended for starting dose selection for adult healthy volunteers and not in patients with advanced diseases [3].

While a scaling factor of 0.67 might be appropriate for small molecules, a scaling factor of 0.8–0.9 appears to be more reasonable for biologics, as has been reported in several publications that indicated an exponent ranging from 0.79 to 0.96 would be more appropriate [8–10]. The higher scaling factor is consistent with the FDA guidance, which indicates that biologics >100 kDa administered via the intravenous route should be normalized based on mg/kg, i.e. a scaling factor of 1 [3], and is plausible for biologics where clearance is mainly driven by proteolytic rates rather than by oxidative metabolism, renal clearance, and/or membrane transporters as for small molecules [8]. Furthermore, the conversion factor should also be calculated using monkey body weights that are relevant

to the conducted toxicology studies, and should consider the average body weight of the target patient population in the first-in-patient study rather than assuming standard body weights for monkeys and humans. Figure 1 shows the effect of the allometric scaling exponent and the monkey body weight on the HED calculation. The figure indicates that the use of more adequate scaling factor biologics is expected to be a major determinant for HED calculation.

Figure showing the impact of the allometric scaling exponent and monkey body weight on HED calculations. The allometric scaling exponent is presented on the *x*-axis (0.67, 0.75, 0.81, 0.9), and monkey body weight is presented in different colors. The effect of these parameters is plotted on the *y*-axis as the ratio of the HED using alternative parameters to that using standard parameters (i.e. scaling exponent of 0.67, monkey weight of 2 kg, and 60 kg patient body weight). A ratio of 2.5 indicates a 150% higher HED using alternative versus standard parameters. Simulation using standard parameters is indicated by an arrow and corresponds to a ratio of 1. This simulation assumes a typical patient body weight of 60 kg, which is the body weight used in the FDA guidance [3]. It is important to note that the patient body weight also affects the HED calculation, although of less impact than the scaling exponent and the monkey body weight.

Table 1 presents a hypothetical scenario where HED was determined based on the assumptions outlined in the FDA guidance, and alternative assumptions that considered a scaling factor for biologics and body weights for monkeys and the target patient population. The table shows a 1.56- to 2.36-fold higher HED using the alternative assumptions.

For biologics administered via the subcutaneous or intramuscular routes, the FDA guidance recommends normalizing to the concentration (e.g. mg/area of application) or the amount (mg) of drug at the application site [3]. This may unnecessarily result in a lower HED, especially for biologics that showed no to minimal signs of injection-site reactions in toxicology studies. Therefore, even for biologics administered via alternative routes, the BSA scaling approach using relevant parameters (e.g. scaling exponent = 0.81, monkey weight = 5 kg) and assumptions on the absorption rate and bioavailability could be a reasonable approach for calculating the HED and setting the starting dose for anticancer agents (refer to the example discussed later).

The more recent 2017 European Medicines Agency (EMA) guidance on the topic recommends a holistic approach for selecting starting doses [1]. This guidance advocates for estimation of equivalent exposures to animal doses based on state-of-the-art pharmacokinetic or pharmacokinetic/pharmacodynamic modeling, estimation of MABEL dose, and/or therapeutic range, while considering

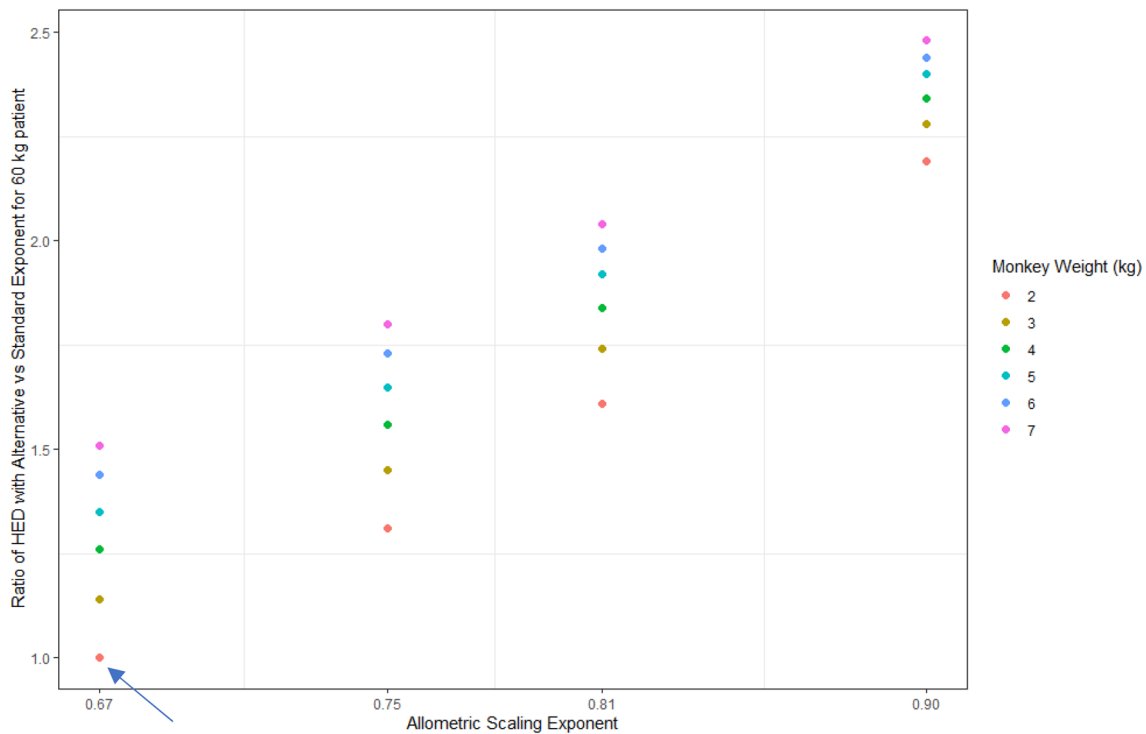


Fig. 1 Effect of allometric scaling exponent and monkey body weight on HED calculations

Table 1 Effect of allometric exponent, monkey, and patient body weight on conversion factor^a

Parameters	Conversion factor	HED (mg/kg)	Starting dose (mg/kg) ^b	Ratio ^c
EXP = 0.67, monkey weight = 2 kg, patient weight = 60 kg	0.325	~ 16	≤ 2.67	–
EXP = 0.75, monkey weight = 5 kg, patient weight = 70 kg	0.517	~ 26	≤ 4.31	1.59
EXP = 0.81 ^d , monkey weight = 5 kg, patient weight = 70 kg	0.606	~ 30	≤ 5	1.88
EXP = 0.81, monkey weight = 2 kg, patient weight = 70 kg	0.509	~ 25	≤ 4.17	1.56
EXP = 0.81, monkey weight = 2 kg, patient weight = 60 kg	0.524	~ 26	≤ 4.33	1.63
EXP = 0.9, monkey weight = 5 kg, patient weight = 70 kg	0.768	~ 38	≤ 6.4	2.36

HNSTD highest non-severely toxic dose, NOAEL no-observed adverse effect level, HED human equivalent dose

^aAssumes HNSTD or NOAEL of 50 mg/kg

^b Starting doses were determined based on a safety factor of 6 (i.e. 1/6 of the HED of the HNSTD)

^cRatio of the HED or starting dose using alternative parameters to those using standard parameters (i.e. exp = 0.67, monkey weight = 2 kg, patient weight = 60 kg)

^dThe 0.81 scaling factor was derived based on a rich dataset including 27 monoclonal antibodies with linear pharmacokinetics [8]

in vitro, preclinical data, and sources of uncertainty in the translational approach from animal to human.

Another important consideration in determining the starting dose is the safety factor that is applied to derive the starting dose from the HED. This factor accounts for uncertainties in predicting the risks of exaggerated pharmacological response, difficulty in detecting certain toxicities in animals, differences in target expression or binding affinities, or uncertainties in pharmacokinetic predictions. Both

FDA and EMA guidances provide general considerations on determining the safety factor [1, 3]. The FDA guidance suggested a default safety factor of 10 for calculating starting doses in healthy volunteer studies. The guidance also provided cases in which the safety factor could be increased, resulting in a lower, more conservative starting dose (e.g. for compounds with novel targets or mechanisms of action, in cases where toxicity is irreversible or difficult to monitor in the clinic, cases where the dose–response curve in animals

has not been adequately characterized in animals or if a steep dose–response curve was observed, and for compounds with non-linear and/or variable pharmacokinetics). On the other hand, the FDA guidance also indicates a lower safety factor could be applied when the uncertainties in predicting the toxicity profiles are minimized by using relevant animal models and well-designed toxicology studies coupled with well characterized dose–response relationship [3].

The following example discusses the starting dose of an antibody, investigated for patients with advanced cancers where the toxicity-based approach would have resulted in a starting dose lower than the MABEL dose. Toxicology studies in monkeys determined the HNSTD to be 3 µg/kg once weekly subcutaneously (i.e. 15 µg for a 5 kg monkey). Using a sixfold safety margin and considering the amount of drug injected in toxicology studies, the starting dose would be <0.04 µg/kg for a 70 kg patient. However, no injection-site reactions were observed in monkeys, therefore the starting dose was not limited to the amount of drug injected. Using the BSA normalization approach with standard parameters and a sixfold safety margin, the starting dose would be 0.16 µg/kg once weekly, whereas a MABEL-based approach indicated a starting dose of 0.6 µg/kg once every 2 weeks. Of note, the toxicology study was designed as a once-weekly regimen; however a once every 2 weeks regimen was later selected for clinical evaluation, which was supported by pharmacokinetic characterization from toxicology studies. The MABEL-based regimen was higher than that based on the BSA normalization approach with standard parameters. Using a scaling factor of 0.81 and a sixfold safety margin also supported the dose of 0.6 µg/kg once every 2 weeks, i.e. $3 \mu\text{g/kg} \times 0.6$ (conversion factor) $\times 1/6 = 0.3 \mu\text{g/kg}$ once weekly, which provides a similar overall exposure as 0.6 µg/kg once every 2 weeks. A scaling factor of 0.81 was selected based on a comprehensive exercise to determine the typical population pharmacokinetic parameters of monoclonal antibodies utilizing a rich dataset including 27 monoclonal antibodies with linear pharmacokinetics [8]. Furthermore, the selected starting dose level was supported by all available data, including (1) non-clinical toxicology studies that showed a safety profile consistent with the target expression and the underlying mechanism of action; (2) similar target binding affinities between humans and monkeys; (3) a similar target expression pattern between humans and monkeys; (4) a well-characterized exposure–safety relationship across a wide range of doses; (5) low expected RO; and (6) low risk of eliciting CDC or ADCC. Therefore, the totality of data suggested a low risk of exaggerated pharmacology and supported the MABEL-based starting dose.

Finally, the starting dose should be viewed as the means, rather than the end, to an efficient FIH study. Efficient dose escalation strategies are an important consideration of the design of efficient FIH studies. Dose escalation strategies

fall into two broad categories, either rule-based (e.g. 3 + 3 design and its variations) or model-based approaches, such as Bayesian-logistic regression models (BLRM) and the continuous reassessment method (CRM). Each of these methods have advantages and disadvantages [11]. The choice of the dose escalation method should consider the novelty of the molecule, the level of uncertainty in the toxicity profile, the starting dose, and the underlying disease.

We call for a balanced approach for selecting starting doses that considers the therapeutic benefit in the target patient population while maintaining adequate safety margins [1]. The starting doses should be determined based on comprehensive evaluation of all available data, including in vitro and in vivo data, in relevant models employing model-informed drug development approaches, the novelty of the mechanism of action, potential differences in the sensitivity of the preclinical species to toxic effects versus humans, relevance of available animal models for predicting efficacy in patients, different receptor density or affinity, differences in systemic bioavailability, and/or drug distribution. If uncertainties in the translation paradigm are too high, resulting in a low starting dose, more efficient dose escalation strategies should be considered, as supported by the emerging clinical data, especially in the initial cohorts.

Declarations

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Conflict of interest Mohamed Elmeliogy, Donghua Yin, and Ken Liao are employees of Pfizer and receive stock and stock options as part of their employment. Chandrasekhar Udata was a Pfizer employee at the time of initiation of this manuscript.

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