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Multi-species toxicology approaches for oncology drugs: the US perspective

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Abstract

The Toxicology and Pharmacology Branch (T&PB) of the National Cancer Institute (NCI) performs pharmacological and toxicological evaluations of new oncology agents according to an agent-directed paradigm in which all studies are tailored to each agent. The United States Food and Drug Administration (US FDA) requires that preclinical toxicology studies be conducted in two species, a rodent and a non-rodent for all small molecules, and T&PB has successfully used this formula. While pharmacokinetic (PK) studies are considered optional, T&PB routinely develops new methods for plasma/tissue drug analysis and employs this methodology throughout development to determine kinetics in various species and toxicokinetics in the toxicity studies. In the current era of molecular target-based development, the T&PB also develops or employs methodology to evaluate effects of the new chemical entity on appropriate biomarkers in tumour and normal tissues. In this comprehensive programme, T&PB is able to correlate safety and toxicity with both plasma drug levels and biomarker modulation in two species for a seamless entry into Phase I. Published by Elsevier Ltd.

Keywords: Antineoplastic agents; Drug evaluation; Preclinical; Toxicology; Pharmacology; Models; Animals

1. Introduction

The recent evolution of the molecularly-targeted approach to the development of new oncology drugs has produced the mistaken impression in some quarters that these newer drugs will be non-toxic unlike their earlier cytotoxic brothers such as doxorubicin (adriamycin), cyclophosphamide (Cytoxan®), cisplatin and paclitaxel (Taxol®) [1] and that these agents will be dosed to biologically-effective doses (BEDs) rather than maximum tolerated doses (MTDs). Unfortunately, based on the toxicity profile of some of these new agents, this is not the case. For example, imatinib mesylate (Gleevec™, Glivec®), which inhibits the Bcr-Abl tyrosine kinase and has shown such dramatic responses in Philadelphia chromosome-positive chronic myeloid leukaemia (CML) patients, can produce severe oedema, liver and kidney toxicity and immunosuppression [2–4]. In the case of farnesyl transferase inhibitors (FTIs), SCH66336 produced dose-limiting gastrointestinal (GI) toxicity and fatigue in addition to renal insufficiency [5]; and R115777 produced dose-limiting neuropathy and fatigue in addition to GI toxicity [6] Both of these FTIs were dosed to MTDs in Phase I rather than to BEDs.

Preclinical toxicology requirements for potential new oncology drugs in the United States are not particularly onerous. As in other therapeutic areas, the development of new chemical entities (NCEs) to treat cancer requires the obligatory, although not unreasonable, step of determining the potential toxicity and safety of these new agents in animal models prior to entry into man. Simply speaking, the Food and Drug Administration (FDA) requests that preclinical toxicology studies should be conducted in two species, a rodent and non-rodent, for small well-defined molecules [7,8]. The development of biotechnology-derived molecules will not be discussed in detail in this paper due to the lack of space, but, more importantly, because many of these development projects require agent-specific special considerations. This requirement for safety/toxicity data results in a variety of studies (pharmacokinetics (PK), pharmacodynamics (PD), range-finding toxicity, Investigational

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New Drug Application (IND)-enabling toxicity studies) usually being conducted in rats and dogs for most NCEs and is illustrated in Table 1. Other species can and are also used, e.g. mice, rabbits, miniature swine, hamsters, guinea pigs, or non-human primates (NHPs; cynomolgus, rhesus, marmosets), when rats and dogs are deemed inappropriate for valid scientific reasons.

Mice, while favoured for many preclinical studies, especially efficacy, because of their small size, cost, ease of use and manipulation, are not typically used as the rodent species for toxicology studies. The National Cancer Institute (NCI) moved away from the use of the mouse in the early 1980s and, prior to that, the mouse was primarily used in lethality studies to define the MELD10 (mouse equivalent lethal dose causing 10% of deaths (LD_{10})), which was used as the basis for the clinical starting dose (SD). Even the Cancer Research UK (CRUK) and European Organisation for Research and Treatment of Cancer (EORTC) have placed more emphasis on the use of the rat in developing toxicity data [9]. There are many reasons for this change in emphasis including the fact that the small size of the mouse precludes serial blood sampling in order to evaluate the effects of the agent on various haematology and clinical chemistry parameters and biomarkers and the fact that mice, in general, tend to be poorer predictors of human toxicity [10,11]. The use of mice to obtain this data rather than the rat also necessitates the use of larger numbers of animals, since additional cohorts of animals are usually necessary, which is contrary to the current thrust within the animal and toxicology community to reduce, refine and replace the use of animals.

Beagle dogs are typically used as the non-rodent rather than other species such as NHPs due to the long history in successfully using this species for pharmaceutical evaluations, the fact that the dog is extremely useful for very intensive procedures (serial bleeding for clinical pathology, pharmacokinetics, biomarkers; long-term continuous intravenous (i.v.) infusion; cardiovascular telemetry, etc.), the difficulty and expense in obtaining and housing NHPs and the widely-held view that NHPs should be reserved for those situations when the beagle is simply not appropriate [12,13]. This practice of using rats and dogs has generally been successful, especially in defining a safe starting dose for the Phase I clinical trial. However, there has been an underlying desire in the oncology community to streamline this process and make it easier to move into Phase I. While adverse effects in man do account for approximately 16% of new drug failures, the overriding reason (46%) why NCEs fail in clinical development, is lack of efficacy [14]. Thus, we really need better predictors of efficacy in man rather than more NCEs in the clinic that will simply fail to be effective. From a toxicity/safety perspective, we need to learn to design and conduct preclinical studies more intelligently so that we can maximise the amount of useful information that we obtain in each animal study. In addition, we need to develop more *in vitro* assays like the bone marrow assay that are actually predictive of human sensitivity.

Table 1
FDA recommended preclinical studies for oncology cytotoxic agents

Studies Considered Important	
	Rodent & Non-rodent. Same schedule and duration as planned clinical trial
	Genetic Toxicity Panel
Studies Considered Useful	
	Pharmacokinetics (PK)
	Pharmacodynamics (PD)
Issues to be Addressed	
	Starting Dose
	End organ toxicities
	Schedule
	Genetic toxicity
	Effective concentrations

Adapted from Ref. [8]. FDA, Food and Drug Administration.

2. Development of small cytostatic molecules

For the toxicological evaluation of small molecules, the FDA seems to rely more heavily on the rat based on the emphasis placed on the determination of what is called the STD_{10} or the severely toxic dose in 10% of the animals for setting the clinical SD [8]. This sounds akin to the old LD10 designation that was used in the 1980s and earlier by the NCI. The Toxicology & Pharmacology Branch (T&PB) of the NCI prefers to use the MTD designation since it is easier to define across species. Studies are not conducted on fixed schedules as they were in the 1980s, i.e. Dx1 and Dx5, but rather are agent-directed and designed specifically for each NCE based on all of the data available such as mechanism of action, efficacy, pharmacodynamics and pharmacokinetics [15–17]. Table 2 illustrates the essential components of this agent-directed philosophy. This agent-directed approach is more flexible and is also espoused

Table 2
Current T&PB drug evaluation philosophy

Agent-directed studies
Pharmacologically (PK/PD)-guided
Integrate with preclinical efficacy data and proposed clinical protocol
Rational evaluation of role of schedule dependence, PD, PK and metabolism in the development of toxicity
Relate drug levels (C_P, AUC, or threshold), biomarkers to safety and to occurrence and severity of toxicity
Extrapolate toxic effects across species

AUC, area under the concentration-time curve; T&PB, the Toxicology & Pharmacology Branch; C_P drug plasma concentration.

by the FDA. Thus, there is no set formula for each and every drug as there was in the past and each development plan as stated above must be specifically designed using all available data in an attempt to maximise success in the clinic. This may be viewed as a stumbling block by some of the smaller, newer biotech firms, with little or no drug development experience since they simply do not know what to do. In this regard, the NCI is available to assist new groups that are not familiar with oncology development.

The European oncology community, led by studies conducted by the former Cancer Research Campaign in the United Kingdom (now know as CRUK), has espoused the use of rodents only for this purpose [9,18]. The latest publication on the success of this approach was released in 1999 [19] and concluded that the use of these rodent only toxicology protocols led to the determination of safe SDs for each of the 25 compounds studied, although other data was relied upon to lower the SD for a number of these agents. Further, if the usual one-tenth of the mouse equivalent MTD/LD10 (expressed on a body surface basis as mg/m^2) had been used in all cases as the basis for the SD, this dose would have been safe.

The agents evaluated in this retrospective study by the CRC were primarily administered *via* the i.v. route on Dx1 or Dx5 schedules. In 22/25 of these studies, the initial schedule in man was the same as the animals, while the other three were similar. The authors from the CRC do caution that the results from this study may not be applicable to newer classes of agents that act by novel mechanisms (such as the newer molecularly-targeted agents) since most of the agents evaluated in this study were conventional cytotoxic drugs. When the animal data was used to predict the human maximum administered dose (MAD), the mouse was able to quantitatively (within a factor of 2) predict this dose for only 12/25 drugs (Human MAD:Mouse MTD/LD10 = 0-2). The ratio ranged as high as 16 for the other 13 drugs, which necessitated long escalation schemes in the clinic.

The mouse studies were only able to predict the human DLT for 11/22 drugs in which DLTs were determined in the corresponding animal studies. The authors stated that only one toxicity was not predicted, hepatotoxicity, in the case of didox, since the other human DLTs were simply not evaluable in the murine model. Thus, due to the limitations of the mouse model, only 50% of the human DLTs were or could be evaluated. In addition to these DLTs, 78 other or non-DLT human toxicities were described for these drugs. The mouse predicted 32% of these toxicities, with approximately 16% not being predicted and once again over 51% not being evaluable in the mouse. This obviously limits the utility of the mouse in predicting human toxicities and reinforces the conclusion stated earlier that the mouse is of limited utility for this purpose.

In an earlier study by the NCI [10], in which the value of the mouse and dog data was evaluated for seven new agents, it was found that the murine data provided a safe SD for 6/7 of the drugs and that the dog data did not provide any additional utility with regard to the SD. However, the dog MTD was a better predictor of the human MTD than was the mouse. When DLTs were evaluated, it was found that the dog was a better prognosticator of human toxicity than the mouse. In 12 drug/schedules which produced 20 human DLTs, the dog predicted 17/20, while the other 3 were not assessed in the dog studies; whereas the mouse only predicted nine of these toxicities, did not predict five others and the final six could not be assessed in the mouse.

In a study reported on a series of Phase I trials conducted between 1993 and 1995 [20], an evaluation of the utility of dog toxicity data was conducted. Many of the SDs were based on 0.1 MELD10, while others were lowered predominantly because of greater sensitivity in the dog. The latter group included six new agents for which data was evaluable for four. The conclusion was that lowering the SD in 3/4 of these agents was warranted since use of the mouse LD10 to set the SD would have resulted in significant human toxicity or the MTD being exceeded.

During an EORTC Workshop [21,22], a preclinical/clinical correlation was conducted on the results of 71 antitumour agents. For most of these, 57, the SD was based on 0.1MELD10, and the human MTD/mouse LD10 was $\leqslant 1$ in 2% of the cases indicating an unsafe SD. In the remainder of the drugs, 14, for which the SD was set based on the more sensitive species, use of the 0.1MELD10 to set the SD would have produced a ratio of $\leqslant 1$ (unsafe SD) for 7% of the agents and $\leqslant 2$ (less than ideal) for 14% of the agents. The conclusion was that a second species, either rat or dog, was necessary to select a safe SD.

More recently, the NCI published the results of a series of preclinical/clinical correlations on seven new agents [17]. When one considers all parameters evaluated, a safe SD, the human to animal MTD ratio and the prediction of human DLTs, the data from the dog was considered more useful and more predictive of human sensitivity than that derived from the mouse or rat in this limited data-set. In a larger study reported last year by the NCI on 37 new agents including 49 drug/schedules [23], the clinical SD when based on the most sensitive of two species utilised was safe 98% of the time.

As has been shown previously, preclinical toxicology data can be most useful when preclinical/clinical correlations are subsequently performed. In a study reported by the FDA in which the toxicity data submitted to the FDA on a series of platinum compounds was evaluated [24], the data indicated that all species utilised in preclinical toxicology studies (mice, rats, and dogs) all had

value in predicting a safe SD and the qualitative toxicities observed in humans. As a result of this study, FDA concluded that toxicity studies in rats should be sufficient for allowing new platinum agents to enter Phase I.

What all of the preceding data-sets show is that it is necessary to use two species for the selection of a safe SD and that the dog has proved very useful, especially to the NCI, for predicting human sensitivity and toxicity. One further point in relation to the utility of the dog beyond this, is that the dog, as stated previously, is a very useful model for conducting intensive procedures such as continuous infusion studies, multiple daily oral doses, serial blood sampling or cardiovascular telemetry studies, etc. since these studies are extremely difficult to perform in rodents, especially large numbers of rodents. What toxicologists need to do in relation to the use of the dog, is to do so judiciously. The T&PB has been using two animals per dose group in range-finding work and four animals per group in definitive studies for the past twenty years and this has proved very satisfactory. Instead of using large numbers of animals so that statistics can be used, dogs can and should be used as their own controls much as patients are in clinical trials.

3. Development of cytostatic, molecularly-targeted therapies

In deciding what animals studies are appropriate for oncology drug development today, one needs to determine exactly what type of information/data is required prior to initiating Phase I trials. If a safe SD is all that is required, then more limited studies may be sufficient. If one wants to know what toxicities may be encountered, then more thorough studies in two species are appropriate. Finally, if one wants to know the impact of the new agent on a target and how this correlates with the kinetics, safety and toxicity of the agent, then a much more comprehensive programme involving PK studies, toxicokinetic determinations and PD biomarker (target modulation) determinations need to be incorporated in appropriately designed studies. The first scenario has the advantages that it costs little in relation to a complete programme and utilises a much smaller amount of compound and fewer animals. However, you get what you pay for, that is, potentially a safe SD with little other information. In the current molecularly-targeted drug development paradigm, this minimalist approach should not work well since it is important to develop PD endpoints to evaluate the impact of this new agent on the molecular target in question, i.e. provide some evidence of purported efficacy. The development of PS-341 (Velcade^{TM}), a proteasome inhibitor, is a perfect example of this scenario.

In mouse efficacy studies, PS-341 treatment clearly suppressed tumour growth at well-tolerated doses. No adverse effects of drug treatment were noted during any of these studies [25]. To determine the effect of PS-341 on proteasome inhibition, several tissues/samples and tumours were collected following PS-341 treatment. Residual 20S proteasome activity was determined in these samples, and results showed that PS-341 elicited a dose-dependent decrease in 20S proteasome activity. In white blood cells (WBCs), for example, 20S proteasome activity was significantly decreased 1 h after treatment and returned to baseline after 24 h. Similar effects were noted in other tissues, except the brain and testes, where no proteasome inhibition was observed. When tumour tissue was evaluated in the same manner, 20S proteasome activity was substantially decreased at the same one hour time point indicating that WBCs could be used as a surrogate for tumour 20S inhibition.

The development of the proteasome bioassay certainly facilitated the conduct of the pharmacology and toxicity studies of PS-341 in animals as a prelude to the clinical evaluation of this agent. It allowed correlation of toxic effects with 20S inhibition in WBCs and provided a stopping point for dose escalation. In all species studied, baseline proteasome activity was restored within 48 to 72 h of PS-341 treatment. Rodents and NHPs exhibited primarily GI side-effects consisting of anorexia, emesis, and diarrhoea. This GI toxicity was dose-related, and overall, treatment with PS-341 was well tolerated until 80% proteasome inhibition was exceeded in WBCs. Above 80% inhibition, GI toxicity became significantly more pronounced, and changes in blood pressure and heart rate occured in NHPs. The *ex vivo* proteasome bioassay became an integral part of the human Phase I and II clinical studies. In this instance, all three species (mouse, rat, NHP) were uniformly equal in predicting the human MTD based on 20S inhibition [25,26].

One final point needs to be considered very carefully in the development of target-based therapeutics since this will make many of the previous discussions moot. While new cytotoxic oncology agents have traditionally been evaluated in terminally-ill cancer patients in Phase I, there is the possibility that some of the newer molecularly-target agents will be evaluated in normal, human volunteers as was the case for marimastat [27]. If single and repeat dose PK/tolerability studies will be the clinical paradigm of choice for the development of target-based agents, we will have to tread more carefully and toxicology programmes will, in all probability, become more comprehensive and expensive.

4. Biotechnology-derived pharmaceuticals

Preclinical toxicology studies for biotechnology-derived pharmaceuticals are an entirely different matter and will not be dealt with in any detail here. The

approach to these studies is governed by the agent under development and selection of the species is paramount. The one factor that is constant with the evaluation of small molecules, is that the proposed clinical formulation, route and schedule should be used as well in the definitive toxicology studies. Since the most appropriate, pharmacologically-relevant animal model available should be used, often only one species can be used in preclinical toxicology studies of biologicals. For this reason, the FDA has not set rigid guidelines for the development of biologicals [28,29]. Many of the studies typically performed for small molecules such as mutagenicity, carcinogenicity, reproductive toxicity are not relevant for the evaluation of biologicals. As a result, the toxicological development of the typical biological is generally abbreviated.

5. Oligonucleotide drugs (ODNs)

The requirements for the evaluation of ODNs is very similar to that for cytotoxic drugs in general. In addition, studies are required to evaluate sequence-related effects and require the use of an ODN with a sequence complimentary to the animal used in the toxicity studies in addition to the human sequence. Due to the activation of the complement cascade by phosphorothioates with resultant cardiovascular effects and death due to bolus administration, it is very important to work out the threshold (PK verus PD) effects very carefully in the NHP prior to entry into the clinic (see Refs. [30,31] for a discussion of these issues). Due to the severity of the toxicities that can be seen with these agents, the only shortcut to the clinic would be to concentrate on the NHP as a model for these studies.

6. Future possibilities

One possible way out of this dilemma for small molecules is to conduct PK, PD and range-finding toxicity studies in both species (the most suitable rodent and non-rodent) and then use the more sensitive species for the IND-enabling study using the clinical route and schedule. In this way, one is able to duplicate the proposed schedule and also obtain toxicological data in two species which helps reinforce the toxicity prediction if both species are in agreement. On the other hand, if PK and PD studies are carefully conducted *in vitro* with tissues such as hepatocytes or liver slices from man and animals, one might be able to determine which species more closely approximates man metabolically and select that species for further toxicological evaluations. If one designs these toxicology studies properly, then additional safety pharmacology studies may not be necessary as stipulated in the ICH Guideline S7A [32]. However, the possibility that a NCE might induce ventricular tachycardia associated with prolongation of the QT interval (Torsades de Pointes) should not be overlooked and appropriate studies should be conducted to evaluate this possibility [33]. Studies longer than 28 days are rarely needed for chronic administration based on the NCI's experience with approximately a dozen different agents over the past 15 years and are generally not recommended by the FDA for cytotoxics and we would advocate that they are probably not necessary for newer molecularly-targeted agents either. Mutagenicity, carcinogenicity and reproductive toxicity studies are not needed prior to Phase I. For agents that are predominantly myelosuppressive, the use of the *in vitro* bone marrow assay, as developed and validated by the NCI [34–37] and more recently validated by the European Centre for the Validation of Alternative Methods (ECVAM) [38] is highly recommended by the T&PB to determine which species more accurately reflects human sensitivity and then this species should be used for the IND-enabling study. These *in vitro* data have been highly predictive of human sensitivity and MTDs. This is why we advocate the development of other *in vitro* assays that will not only rank toxicity among a number of analogues, but also be predictive of human sensitivity in relation to animal models. Since hollow fibres have been used successfully for growing human tumour cells and human immunodeficiency virus (HIV)-infected human lymphocytes and for testing the efficacy of new agents [39,40], perhaps this system can be modified for use with other normal human tissues to establish new toxicity model systems. If we can develop and validate such assays, then maybe one day animal studies may not be necessary and entry into the clinic will be facilitated.

7. Conclusions

Until newer, more predictive assays are developed, studies in animals will be required for the foreseeable future. The objectives of the current NCI agent-directed pharmacology and toxicology development paradigm are listed in Table 3. This programme, which can be used for both traditional cytotoxic and molecular target-based NCEs, can be summarised as follows:

1. Develop sensitive methodology to determine PK in various species, including plasma protein binding.
2. Determine if metabolism is important and identify metabolic pathway. Evaluate metabolism in various species, including man *in vitro*.
3. If possible, select appropriate biomarkers to assess target modulation and develop sensitive methodology to determine the impact of drug treatment on target(s) in tumours and selected

normal tissues. Develop appropriate surrogate for tumour, if possible, e.g. peripheral blood mononuclear cells (PBMCs), skin biopsy, saliva, buccal mucosa cells, etc.

4. Evaluate PK in various species, starting with the mouse since the mouse is typically utilised in efficacy studies. Determine whether peak plasma levels, area under the concentration-time curve (AUC) or time above a threshold is required for efficacy. Utilise data from *in vitro* time *versus* concentration efficacy studies to design appropriate PK and toxicology studies.
5. If routes other than i.v. are required, conduct appropriate bioavailability studies.
6. Evaluate PD, genomics and proteomics, if possible, in conjunction with PK studies.
7. Conduct *in vitro* toxicity assays in relevant target organ systems, if available.
8. Conduct biodistribution studies, if radiolabelled compound is available, to determine possible target organs of toxicity.
9. Determine MTDs and DLTs in single dose studies in both a rodent and non-rodent using abbreviated study designs as a prelude to other repeated dose range-finding studies or definitive IND-enabling studies. Conduct combined range-finding/PK studies in non-rodents rather than separate studies. Determine if efficacious drug concentrations (peak C_P, AUC or threshold) can be safely attained and maintained for the appropriate time period. Determine the impact of these concentrations on selected biomarkers, genomics, proteomics, safety and toxicity. Evaluate reversibility of toxicity or whether toxicity is delayed.
10. Design appropriate IND-enabling studies with the clinical formulation, route and schedule to determine MTDs and DLTs, toxicokinetics and the impact on biomarkers, genomics and proteomics.
11. Determine, if possible, if the modulation of the tumour molecular target is also responsible for toxicity. Adjust, if possible, route and schedule to maximise the therapeutic index while minimising toxicity.

The aforementioned summary represents the ideal drug development scenario and is open to modification, depending on the circumstances of the project. For example, a highly potent compound may not be amenable to the development of an appropriately sensitive assay for plasma drug analysis or the preparation of radiolabelled compound may not be feasible so biodistribution studies could be conducted. While the body of data that is accumulated in these studies described above is very impressive, it will not be very useful and will be a waste of time and effort if we continue to conduct Phase I evaluations in man as we have in the past. We must move away from blindly escalating to MTDs in Phase I by performing real-time PK and PD studies to guide dose escalation in these trials. The knowledge of what the body is doing to the drug and what the drug is doing to the molecular target in the tumour or some surrogate is paramount.

Table 3
Objectives of preclinical pharmacology and toxicology studies of NCEs

Determine in Appropriate Animal Models:
The Maximum Tolerated Dose
Dose-Limiting Toxicities
Schedule-dependent toxicity
Reversibility of adverse effects
A safe Starting Dose
Attain efficacious drug levels *in vivo*
Correlate drug levels and/or biomarker with safety and toxicity across species
Ameliorate toxicity by change in route/schedule
Compare toxicity of NCE with other clinical agents as necessary

NCE, new chemical entities.

It is the opinion of this author, that the pharmacological and toxicological evaluation of new molecularly-targeted agents for the treatment of cancer should not be approached in a simplistic, minimalist fashion. We should try to maximise the amount of useful data that is collected in these studies as described above, while attempting to minimise the number of animals used. We should utilise the most appropriate species whenever possible rather than arbitrarily preselecting the same species for use with each new agent. Since we are dealing with a life-threatening disease and in many cases terminal patients, we should not simply be in a rush to bring new agents into the clinic that will fail, but rather emphasise that we get into the clinic with new agents that have a greater possibility of being effective. Perhaps, we should remember Aesop's fable about the Hare and the Tortoise, "...Slow but steady wins the race", and not rush to bring each and every new chemical entity into the clinic as rapidly as possible. If we are going to take a rational, scientific approach in defining new molecular targets for treatment, we should not follow up with an anti-intellectual approach to pharmacology and toxicology for the sake of expediency.

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