Original Paper

A Perspective on the Use of Two Pharmacologically Relevant Animal Species in the Nonclinical Evaluation of Biotechnology-derived Therapeutics

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ABSTRACT

The requirement of nonclinical studies for the safety evaluation of biotechnology-derived therapeutic drugs using different animal species often depends on the characteristics of the products. Moreover, in practice, the requirement of different types/number of nonclinical studies depends on the regulations and system of national/international drug approval authorities.

This review assessed twenty-four repeat dose toxicology studies of different therapeutic proteins conducted using two different relevant species to determine whether there was any new findings/information on adverse characteristics of therapeutic proteins towards the clinical safe practices and/or whether one species could serve the safety purposes. With this vision, the strategy involved here was the assessment of findings of twenty-four repeated dose toxicity studies conducted in two species for different biosimilar proteins of Reliance Life Sciences Pvt. Ltd., Navi Mumbai.

The critical evaluation of findings of the different therapeutic proteins depicted similar target organs of toxicity and/or secondary responses owing to the exaggerated pharmacological activity in both the species with a difference of dosage levels in a few cases.

This review addressed the significance/implications of these findings at the cost of sacrifice of a second species, and further discussed future options to reduce animal use in biological therapies safety evaluation programs.

Key Words: Nonclinical evaluation; Biotechnology-derived therapeutics

Introduction

The target binding interactions of recombinant therapeutic proteins influence the biopharmaceutical industries to develop novel or biosimilar biological drugs as alternative therapies to small molecules. The cost, time and resources involved in the development of novel therapeutic proteins, stirred many pharmaceutical companies to resort to implement the strategy of development of potential biosimilar products, as a start up effort. The structural complexity and other associated manufacturing processes of biological molecules forced drug approval authorities to regulate follow-on-biologics/biosimilars with the scientific guidelines to assess its efficacy and safety.

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use S6 (ICH S6) and other guidelines related to biotechnology-derived products/biosimilars have given enough flexibility to demonstrate efficacy and safety of biological drug molecules.[1-3]

A pharmacologically active species is very essential for the characterization of efficacy and safety of a biological molecule during drug development programs. The safety evaluation of biological molecules in two species or in a single species has been depicted as a case-by-case approach by ICH S6. In practice, depending on the regulatory authorities (where the product needs approval),

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the requirement of general toxicity studies in two species even for biosimilar product is not uncommon. The significance/relevance of testing in two species when assessing/extrapolating safety to man is also an issue at the cost of animal welfare issues, economical reasons and time involved in the biotechnology-derived drug development.

Considering the future scope of biosimilar drugs, the findings of this manuscript might be significant in making a decision to use two species or one species, reducing the dose levels to one or two at the saturation of exposure instead of three dose levels in a second species, and future options for the reduction of animal usage. This manuscript reviewed the findings of 24 general toxicology studies conducted over the last 6 years with nine different biosimilar therapeutic proteins of Reliance Life Sciences Pvt. Ltd., Navi Mumbai.

Methods and Discussion

The general repeated dose toxicology studies were conducted as a part of regulatory submission. The exposure period and other study details are presented in **Table 1**, and some of the treatment related findings described in the text below are given in **Table 2**. Except studies of recombinant human erythropoietin (r-HuEPO), all other recombinant proteins included minimum of three different dose levels in each study. The rat was used as a rodent species and rabbit as a non-rodent species for all the products except the r-HuEPO (Epostim). Two 90–day repeat dose toxicology studies were conducted using two different species, i.e., rats and dogs for the r-HuEPO. The studies were conducted as per regulatory standards by following the GLP principles. Except two out sourced studies for r-HuEPO, all other general toxicology studies were conducted at Laboratory Animals Research Services, Reliance Life Sciences Pvt. Ltd, Navi Mumbai. This review discusses the important pharmacological and secondary/adverse findings of two species taking into account of species similarities/differences/or predictive information in relation to safety.

Colony Stimulating Factors (rhG-CSF and rhPeg-G-CSF):

Recombinant Human Granulocyte Colony Stimulating Factor (rhG-CSF)

rhG-CSF is the granulocytic growth factor, and induces the differentiation and proliferation of granulocytic cells in the haematopoietic organs. The assessment of 28-day repeated dose toxicity studies of rhG-CSF through subcutaneous (SC) and intramuscular routes confirmed the Sprague Dawley rats and New Zealand white rabbits as relevant species through elevated total white blood cells (WBC) count along with a corresponding increase in the neutrophil count. The dose levels ranged from 50 to 500 mcg/kg during 28-days of repeated administration. Along with the primary pharmacodynamic effects (elevated neutrophils count), other secondary changes such as reduction in cholesterol, extra-medullary granulopoiesis in the spleen and liver, reduced bone formation and injection site reactions with swelling (hind limb) in a few cases were noticed in both the species. Except for consistent increase in the activity of ALP in the rats, no other significant differences were noticed between the species through both the routes of administration. High plasma ALP activity was considered to be due to increased neutrophil proliferation in the bone marrow, though it could be also because of bone changes.[4-6] All changes however, finally contributed to the resultant effects of pharmacological action of G-CSF, or can be classified as secondary effects to exaggerated pharmacological responses. The reflected perturbations were similar in all the four studies.

Recombinant Peg-G-CSF (rhPeg-G-CSF)

Recombinant Peg-G-CSF is the pegylated form of recombinant G-CSF by attaching a 20,000 Dalton methoxypolyethylene glycol propionaldehyde (mPEG-ALD) to the N-terminal amino acid of G-CSF. Its therapeutic indication is similar to G-CSF but has a sustained-duration effect. Dose dependent increases in total WBC count, neutrophil count and M:E ratio were observed in both rats and rabbits as an intended effect. Extramedullary granulopoiesis in the liver and spleen, increased cellularity of granulocytic cells in bone marrow, thinning of trabeculae of sternum and femur, swelling of hind limb and decrease in the values of erythrocyte count (RBC), haemoglobin (Hb) and haematocrit (HCT) were observed both in rats and rabbits, as exaggerated pharmacological responses/adaptive responses. Like G-CSF, peg-GCSF also increased the plasma ALP activity in the rats. However, changes in the liver, spleen and bone were evident in both the species. The observed biochemical difference ultimately reflected the same target organs.

Erythropoiesis Stimulating Proteins (Erythropoietin alfa and Darbepoetin alpha):

Erythropoietin alfa (r-HuEPO)

Erythropoietin is a well-known blockbuster drug, targeted against a variety of primary and secondary anaemic conditions, the wide-therapeutic window also encompassing

Table 1 Study Details and Parameters

Therapeutic protein	Species	Route	Study duration	Parameters
G-CSF	Rat	s.c. & i.m.	28 days treatment and 14 days recovery periods	Daily clinical signs, local reactions, detailed clinical examinations/ behavioural observations, ophthalmoscopy, body weight, food consumption, haematology, clinical chemistry, urinalysis, bone marrow cytology, organ weights, histopathology, plasma erythropoietin estimation (EPO), electrocardiography (dog), blood pressure (dog), respiration (dog), body temperature (dog) and blood gas analysis (dog)
	Rabbit	s.c. & i.m.	28 days treatment and 14 days recovery periods	
Peg-G-CSF	Rat	s.c.	28 days treatment and 14 days recovery periods	
	Rabbit	s.c.	28 days treatment and 14 days recovery periods	
Erythropoietin (EPO)	Rat	s.c.	90 days treatment and 28 days recovery periods	
	Dog	i.v.	90 days treatment and 28 days recovery periods	
Darbepoetin	Rat	i.v. & s.c.	28 days treatment and 14 days recovery periods	
	Rabbit	i.v. & s.c.	28 days treatment and 14 days recovery periods	
rhCG	Rat	s.c.	28 days treatment and 14 days recovery periods	
	Rabbit	s.c.	28 days treatment and 14 days recovery periods	
rhFSH	Rat	i.m.	28 days treatment and 14 days recovery periods	
	Rabbit	i.m.	28 days treatment and 14 days recovery periods	
rhGH	Rat	s.c.	28 days treatment and 14 days recovery periods	
	Rabbit	s.c.	28 days treatment and 14 days recovery periods	
	Rat	s.c.	90 days treatment and 28 days recovery periods	
rhIL-2	Rat	i.v.	28 days treatment and 14 days recovery periods	
	Rabbit	i.v.	28 days treatment and 14 days recovery periods	
	Rat	s.c.	28 days treatment and 14 days recovery periods	
rhTPA	Rat	i.v.	14 days treatment and 14 days recovery periods	
	Rabbit	i.v.	14 days treatment and 14 days recovery periods	

s.c. = Subcutaneous, i.m. = Intramuscular, i.v. = Intravenous, rhCG = Recombinant Human Chorionic Gonadotropin, rhFSH = Recombinant Human Follicle Stimulating Hormone, rhGH = Recombinant Growth Hormone, rhIL-2 = Recombinant Human Interleukin-2, rhTPA = Recombinant Human Tissue Plasminogen Activator

Table 2 Group Mean Values of Some of the Treatment Related Findings in Rats and Rabbits after Therapeutic Proteins Exposure

Therapeutic Protein and Route	Parameter	Species and Sex	Control (n=6 or 3)	Low dose (n=6 or 3)	Mid dose (n=6 or 3)	High dose (n=6 or 3)
rhG-CSF s.c.	WBC counts ($10^3/mm^3$)	Rat Males	8.03 ±1.21	21.82* ±3.56	35.70* ±6.75	54.42* ±11.18
		Rabbit Males	3.93 ±0.60	5.70 ±0.20	9.50 ±6.0	12.07 ±4.81
	ALP (U/L)	Rat Males	192.50 ±15.45	463.80* ±56.59	683.0* ±55.54	1192.2* ±273.45
		Rabbit Males	131 ±60.11	81.33 ±34.82	160.0 ±123.65	119.67 ±27.15
rhPeg-G-CSF s.c.	Neutrophil count (%)	Rat Males	18.17 ±4.83	26.83 ±5.19	40.83* ±11.53	52.00* ±9.21
		Rabbit Males	25.0 ±5.57	34.33 ±6.43	33.0 ±16.46	60.33 ±24.79
	ALP (U/L)	Rat Males	89.00 ±13.19	138.83 ±32.11	159.67 ±36.32	481.83* ±128.96
		Rabbit Males	102.67 ±56.50	76.33 ±22.37	51.00 ±13.08	52.67 ±23.35
Darbepoetin alpha s.c.	HCT (%)	Rat Males	41.40 ±1.29	55.55* ±6.69	61.15* ±2.24	61.53* ±5.92
		Rabbit Males	41.37 ±2.60	59.87 ±0.35	56.93 ±9.52	61.23 ±1.48
rhFSH i.m.	Ovary weight (g)	Rat Females	0.11 ± 0.02	0.2 ± 0.08	0.29* ± 0.14	0.26 ± 0.17
		Rabbit Females	0.39 ± 0.26	1.52 ± 0.86	1.96 ± 0.11	2.65* ± 0.8
rhtPA i.v.	Clotting time (seconds)	Rat Females	87.67 ± 5.28	108.67 ± 11.48	147.83* ± 0.17.6	169.33* ± 21.18
		Rabbit Females	181.67 ± 20.21	283.0* ± 8.54	290.0* ± 10.0	324.33* ± 24.58

* = Significantly increased ($p<0.05$), s.c. = subcutaneous, i.m. = intramuscular, i.v. = intravenous, n = number of animals per group/sex: for rats = 6; for rabbits = 3

prevention of apoptosis and immunomodulatory activities;[7-9] it exists in different structural forms.[10–12] Many a time, the standard *in vivo* toxicology requirements from regulatory bodies include 90-day repeat dose studies in rats and dogs. Secondary toxicity associated with the repeated administration was due to massive erythropoiesis and neutralizing effects of anti-antibodies on endogenous and exogenous erythropoietin, and often the severity depends on the dosage and on the homology between the endogenous and exogenous erythropoietin tested.

Two 90-day studies conducted in two different species, i.e., rats and dogs, for epostim (an erythropoietin product of Reliance Life Science Pvt. Ltd.) along with a comparator drug (eprex) revealed similar responses. Direct and adaptive responses to massive erythropoietic impulse were noticed in several organs along with a dose-related increase in the plasma concentration of erythropoietin in the rat study. The perturbations owing to massive erythropoietic impulse led to mortality in a few rats belonging to both the drugs (epostim and eprex), and discolouration in many organs (adrenals, kidneys, liver, lymph nodes, pancreas, pituitary gland and urinary bladder), enlargement (liver, kidney, heart and spleen), oedema (lungs) and red or dark liquid in the abdominal/or thoracic organs, were also dose-dependent. Dogs treated with eprex or epostim at 300 IU/kg revealed pronounced increase in the erythrocytes and other RBC-associated factors, increase in bile acid concentration, and increased activities of alanine aminotransferase and lactate dehydrogenase. No changes were noticed in the blood pressure, electrocardiography, circulatory functions, respiration, sensorimotor system, and body temperature. Macroscopic observation revealed congestion in the spleen and tightly filled mesenteric vessels. No specific toxicity other than those pharmacologically-mediated were evident either in current rat/dog studies or several published literatures specifying up to 90-days of treatment in rats or dogs.[10–12] Persistent stimulation of the r-HuEPO at higher dose levels resulted in bone marrow fibrosis and/or hyperostosis in many studies of rats or dogs treated for duration of 90-days.[10, 11]

Darbepoetin alpha

Darbepoetin alpha is also an erythropoesis stimulating protein, but with increased sialic acid content compared to recombinat human erythropoetin, and thereby exhibits increase in the biological half-life.[13] To identify its toxic effects, rats and rabbits were administered this protein through two different routes, i.e., intravenous and subcutaneous. Dose levels up to 40 and 20 times more than the intended human exposure were used in the rat and rabbit studies respectively. Both rats and rabbits expressed similar kinds of target actions and secondary changes in the biochemical parameters due to exaggerated pharmacological effects.

The pharmacological activity of darbepoetin alfa resulted in an increase in the production of erythrocytes and associated actions, including widespread extramedullary haemotopoiesis (liver, spleen and bone marrow). Corresponding biochemical changes were also noticed in both the species. Increased potassium with hyponatraemia indicated an increased rate of erythrocyte destruction by splenic activity. Generalized trend of decrease in clotting time was observed as a secondary effect. Increased potential of clot formation was attributed to increased number of RBC in the circulation.[14-16] All changes were well correlated to prolonged stimulation of erythropoiesis. Both the species revealed similar trend of responses through both the routes of administration. The target indications of all the four studies were clearly due to prolonged stimulation of erythropoiesis. Dose levels between the species were adjusted to body surface area, and the secondary changes were similar due to massive erythropoietic responses across the species and routes.

Hormones (Human Chorionic Gonadotropin, Follicle Stimulating Hormone and Growth Hormone): Recombinant Human Chorionic Gonadotropin

Recombinant human chorionic gonadotropin is a glycoprotein hormone, intended for fertility treatment/or enhancement. After 28 days of treatment period, many features of induced hormonal disturbances were noticed in both the species. The major changes included enlarged and increased number of corpus lutea in the ovaries, endometrial atrophy in the uterus, hyperplasia/mucification of vaginal epithelium, hyperplasia of acinar epithelium in the mammary glands and reduction in size, tubular degeneration/atrophy and increased cellularity of Leydig cells in the testes. A few other changes noticed were of haemodilution and hepatocellular hypertrophy. Additionally, rat study showed a slight reduction in size with minimal to mild reversible lymphoid atrophy of thymus in all the treatment groups. This finding again can be correlated to enhanced steroid stimulation.[17]

Recombinant Human Follicle Stimulating Hormone

Recombinant human follicle stimulating hormone is a recombinant form of the original hormone intended to be

used for development of multiple follicles in ovulatory patients participating in an assisted reproduction technology (ART) program and for induction of ovulation and pregnancy in anovulatory infertile females in whom the cause of infertility is functional and not due to primary ovarian failure. The anticipated pharmacodynamic response, increase in the number of maturing follicles in the ovaries, was noticed in both the studied species. Repeated intramuscular administration up to 60 and 47 times of human exposure in rats and rabbits respectively did not indicate any evidence of toxic changes.

Recombinant Human Growth Hormone

Recombinant human growth hormone refers to the growth hormone produced by recombinant DNA technology and is being used to treat children's growth disorders, and in the case of adults with deficiency of growth hormone. The dose levels being used in 28-days studies extended up to 62 and 35 times of human dose in rats and rabbits respectively. Twenty eight days of repeated intramuscular injections exhibited increase in the growth of rats and rabbits. No secondary/toxic manifestations were noticed in either species. Therefore, the 90-day repeat dose study was restricted to only one speces, i.e., rat. No toxic manifestations were noticed even after 90 days of exposure in rats. The lack of adverse findings in all the three studies (two 28-day studies and one 90-day study) also supported the lack of a second species study (rabbit) for 90 days of exposure.

Anticancer Lymphokine:
Recombinant Human Interleukin-2 (rhIL-2)

rhIL-2 therapy possesses the same properties as naturally occurring IL-2 and helps by the activation of immune system to recognize and eliminate certain kinds of cancer cells.[18] The studies have been designed considering the therapeutic dosing cycles through the intravenous route. The selected dose levels in rats ranged from 1.6 to 6.4 times, and for rabbits 0.3 to 2.4 times higher than the intended human exposure based on the body surface area. They both showed similar primary and secondary changes, but rabbits revealed systemic adverse changes at comparatively lower dose levels. Along with the anticipated primary pharmacological effect, i.e., increases in total WBC count and absolute leucocyte count in the animals of treatment groups, a few treatment related clinical signs were observed in both the species during the course of treatment. Histopathology findings revealed lungs, liver, kidneys and spleen as target organs owing to immunomodulation/inflammatory cell infiltration, mediated degenerative/regenerative changes, and/or related changes (**Table 3**). IL-2 mediated proliferation of nonspecific cytotoxic effector cells such as natural killer cells and lymphokine-activated killer cells, and the activation of monocytes and macrophages have been reported earlier. The cause/or mechanism behind the toxic manifestation was determined to be the same, though there were a few differences between the species in the nomenclature of clinical findings during the in-life phase observations. Microscopic findings revealed changes in accordance with the clinical or biochemical findings. Moreover, the dose limiting toxicity of rhIL-2, including mortalities have been quite noticeable in many reported studies.[18–20] Rabbits showed dose-limiting toxicities at lower dose levels when compared with rats, but without any marked differences in the target identification. In addition to intravenous toxicity, a subcutaneous (SC) toxicity was also tested in the rats. Though there were differences in the dose levels between IV and SC routes, no additional toxicity was observed by SC injection in rats, and restricted the SC toxicity study in rats only.

Table 3 Common Microscopic Findings in Rats and Rabbits after rhIL-2 Treatment

Organ	Microscopic Findings
Lungs	Perivascular inflammatory cell infiltration
	Pneumonitis
Liver	Hepatocellular degeneration
	Perivascular/periportal inflammatory cell infiltration
	Bile duct proliferation
Kidneys	Perivascular inflammatory cell infiltration
	Tubular degeneration
	Tubular regeneration
Spleen	Hyperplasia/hypertrophy of the white pulp
	Injection Site Inflammatory cell infiltration

Thrombolytic Protease:
Recombinant Human tissue Plasminogen Activator (rht-PA)

Recombinant human tissue plasminogen activator, a fibrinolytic agent, is intended for diseases which feature blood clots, such as myocardial infarction and stroke. Treatment related changes include anticipated dose-

dependent increase in the clotting time in both the species, and local reactions/haemorrhages at the site of injection. These findings were observed in the rats and rabbits after 14 days of repeated intravenous injection. The dose levels selected extended up to 17 and 12 times higher than the human in rats and rabbits respectively. No additional toxic findings were evident in either species.

Future Options for the Reduction of Animal Usage:

Many a time, structural similarities between human protein and animal species used in preclinical studies contribute to identify differences if any, through primary pharmacodynamic action and/or immune mediated clearance of endogenous or exogenous proteins.[21] The demonstration of safety/efficacy of biotechnology-derived drugs often depend on the relevant species used, design of the study, and the immunogenecity response.[22–24] ICH S6 (R1) addendum provides enough flexibility for the use of two species for the nonclinical evaluation of biological drugs. It emphasizes that if the results are similar in short term toxicology studies, then long term toxicity studies may be done in one species only. The present data on two species revealed similar target organs of toxicity with dose-sensitivity in a few cases. Therefore, it is clear that toxicity study in a second species for most of the biological drugs might not be much informative, when the characterization of toxicity is defined through the first species. It would be probably more meaningful when the mechanism of action is well understood for a particular target/similar therapeutic class; then the omitting of the second species study for a similar class of therapeutics might be scientifically valid.

Considering the existence of a variety of class of biologics, a case-by-case approach for the use of two species can be best practiced when toxicity characterization/identification is not clear. For example, complex compounds such as epoetin containing products can cause adverse reactions due to structural variations in the protein content and the sugar moiety. Depending on characteristics such as neutralizing potential and influence of structural variations to adverse reactions, extended clinical use to novel areas and the new dosing regimen might need additional safety studies through the second relevant preclinical species. Consequences of small changes in the manufacturing processes can potentially perturb the efficacy and safety of biological drugs.[25,26] Un-anticipated clinical consequences were not uncommon owing to structural complexity of epoetin compounds.[27] Otherwise, based on similar target binding, products of similar mode of action and for biosimilar products, restriction of general toxicology studies to one species could be probably scientifically justifiable.

The active ingredients of biological therapies are made up of natural biological compositions; the off-target toxicity is relatively less common.[22,28] Identification of pharmacological responses *in vitro* or *in vivo*, and secondary adverse responses would be very important to identify first-in-human dose. It has been anticipated that the limitations with respect to uncertainties associated with the use of preclinical test systems for biotechnology-derived therapeutic drugs, particularly for monoclonal antibodies, needs different strategies to select first-in-human (FIH) dose rather than the use of conventional no-observed-adverse-effect-level (NOAEL) from a relevant preclinical species.[29–31] It can be well extrapolated to lessons learned from products such as TGN 1412.[32,33] This stark example stimulated the scientific community to think of minimum-anticipated-biological-effect-level (MABEL) as a FIH dose, and is considered as one of the best options after TGN-1412 disaster.[34,35]

However, translational strategies with statistical modeling for various domains of the responses such as receptor induced responses, receptor occupancy, pharmacology and toxicology particularly for biological drugs can minimize most of the uncertainties.[36,38] The aim for toxicological assessment of adverse responses for the 21st century is to develop a Toxicological Factors Analysis and Classification System (TFACS) to reestablish adverse responses and addressing the mechanism of toxicity pathway responses for better prediction.[39] TFACS can be one of the best tools for drug discovery. A large database with statistical modeling/prediction approaches for receptor oriented pharmacological/pharmacodynamic activities can be of future interest since many of the adverse findings were mainly related to pharmacological activities. When non-human primates become the relevant nonclinical species, ethical issues pertaining to the use of large number of primates forms an incompatible factor between animal welfare societies and biological scientists.[40,41] Extensive sacrifice of animals for the purpose of nonclinical testing is as old as 4–8 decades.[42] A standard database of knowledge regarding receptor binding/stimulation, probability of binding to specific and non-specific cells, and their subsequent manifestation of toxicity/responses can help the future of biological drug testing, while preserving the lives of non-human primates/other nonclinical animals species.

When the therapeutic product category is new, comprehensive understanding of molecular mechanism of action and associated adverse events need to be predicted based on the probability of interactions it may pose. Differences between non-human primates or a relevant preclinical species and a human need to be thoroughly studied when there is a difference in the sensitivity of responses, and thereafter FIH dose needs to be adjusted accordingly. Prediction of probability/consequences of receptor interactions, duration of effect, reversibility and species sensitivity, or difference between the human and relevant preclinical species should incorporate translational strategies of standard statistical modeling for better practice. To minimize incidents such as the TGN 1412 catastrophe, differences in sensitivity between the human and the nonclinical species need to be studied with detailed tissue specificity, probability of binding to specific and nonspecific cells, increased precision for the *in vitro* methods, and applying novel approaches such as statistical predictive modeling for the responses, and streamlining further through *in vitro* and *in vivo* studies, if required. Further, more frequent discussions/workshops through international bodies such as the WHO/ICH/EMA can stimulate better scientific practices.[43]

It has been observed that many a time, the high dose is the NOAEL for many of the monoclonal antibodies tested in primates.[28] In the absence of dose response relationship in the first species, selecting one saturating dose as a high dose instead of three dose levels in a second species also might be of future interest, particularly for new biological drugs. When short-term toxicological studies indicate high dose saturation of exposure, or maximum feasible dose as a NOAEL, and FIH dose has been determined from short-term studies, further long-term toxicology can be at one or two dose levels instead of three dose levels, if toxicity is predicted to appear after long-term treatment. This can answer a few ethical and scientific issues related to animals and other scientific issues of drug developmental processes. There is also a need for regulatory approaches towards the acceptance of proper scientific strategies rather than expecting conventional methods for efficacy and safety demonstration.

Considering the ICH S6 option of case-by-case approach, can we eliminate the use of a second nonclinical species through world-wide standard database of similar target bindings/similar therapeutic class/similar receptor mediated actions? Certainly a few challenges need to be solved with the collaboration of private (pharmaceuticals/CROs) and public (regulatory agencies) partnerships.

The translational strategies should accomplish many characteristics such as perturbations caused by the exaggerated pharmacological responses/consequences of receptor mediated actions, tissue/cell specificity, receptor occupancy, nonspecific cell/tissue activation, autoimmunity, hypersensitivity, immune suppression and other immuonogenecity responses. Standard large data base would be one of the primary requirements in this attempt, which can be confidentially accessed or controlled by the government regulated bodies, considering the existence of voluminous standard unpublished data across the globe. In some cases, toxicity cannot be identified when the target is expressed only in the diseased tissues, and therefore receptor-response based statistical model will be of great help. Though manufacturing processes, structural modifications and other associated factors of developmental programs can contribute to off-target activities, the influence of less-costlier, less time-consuming computer prediction can pre-empt a few uncertainties, if not all, along with *in vitro* and *in vivo* responses.

Conclusion

Developments in recombinant therapeutics require continuous scientific examination towards the existing systems/methodologies for the benefit of mankind. Issues such as the requirement of general nonclinical studies through two relevant animal species would need to be reconsidered without compromising safety aspects in an enthusiasm to introduce follow-on/similar products/or new products into practice. Based on the results of first species study, a decision to use two species, and testing one saturating-dose instead of three dose levels for long-term toxicity can be interesting in the future. When there is a defined toxicity from one species for a particular target stimulation/or for a similar class of biological therapeutics, then the need of a second species for the majority of biologically derived therapeutics would be less informative with respect to clinical safety. New scientific strategies should make way for regulatory acceptance. Translational strategies based on the categorized pharmacological profile encompassing *in vivo* and *in vitro* data will certainly increase the sensitivity of preclinical extrapolation to clinical safety.

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