

The Application of ICH S6 to the Preclinical Safety Evaluation of Plasma Derivative Therapeutic Products

Richard M. Lewis, PhD and Joy Cavagnaro, PhD, DABT, RAC

Access BIO, Boyce VA 22620

Summary

The ICH S6 guidance was developed to describe a rational science-based flexible approach to the preclinical evaluation for biotechnology-derived pharmaceutical products. It also suggested that some of the principles described may be suitable for plasma-derived therapeutics. Some of the specific concerns unique to protein-based therapeutics include complexity in structure and potential immunogenicity. Unfortunately S6 has been interpreted incorrectly by some industry and regulatory authorities, often due to lack of experience with these types of products, as encouraging a broader or more conventional toxicology program as is normally conducted for small molecules. The guidance does encourage important and necessary non-clinical evaluations but also recognizes the limitations of studies in animal species which are not relevant, i.e., without pharmacological interaction with the biologic, and which develop an immune response, often limiting useful chronic, reproductive and carcinogenic toxicity evaluations. As such, S6 focuses evaluations on appropriate and well-designed studies in relevant species for reasonable time periods to make the best use of available resources and enable clinical trials without performing studies which can offer little insight into product safety.

Introduction

The International Conference on Harmonization (ICH), comprised of experts from regulatory agencies and experts in the pharmaceutical industries from the United States, Europe and Japan, has made great strides in developing acceptable common policies to address scientific and technical issues related to drug development and registration. The initial goal of this joint effort was to reduce duplicative and unnecessary testing and to enable the gathering of scientific data applicable to registration procedures in all three regions. This global effort established in 1990 continues to provide specific guidance in the areas of quality (CMC), safety (preclinical) and efficacy (clinical) as it applies to the public health.

It is universally recognized that safety is the cornerstone of drug development and that preclinical safety testing is essential to support the initiation and continuance of clinical trials. To establish better guidance to industry for the development of

biopharmaceuticals, the ICH established an Expert Working Group, EWG, in 1995 in order to specifically address preclinical testing for these types of products. The results of such recommended testing would ultimately provide the scientific data to assist sponsors in selecting safe starting doses for first-in-human trials, identifying target organs of toxicity and assisting in the design of clinical trials with appropriate monitoring and dose escalation schemes. The product of the efforts of the EWG was the ICH Guideline S6, Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals (commonly referred to as “S6”) published in 1997.¹ The recommended approach to preclinical safety evaluation was referred to as the “case-by-case” approach, largely based upon product attributes highlighting appropriate species selection with a special attention to immunologically mediated effects and their relevance for patients.”^{2,3}

The scope of S6 primarily addresses biopharmaceuticals which are derived from *in vitro* expression and propagation in various cellular systems such as bacteria, yeast, insect, plant and mammalian cells and is applicable to a wide variety of products and their uses. Although the original intent of the guideline was for biotechnologically-produced biologics, it states in its Scope that among the many products to which this guideline may also be applicable are plasma-derived products. It is the application of S6 to plasma derivatives which is the focus of the current discussion. Although the scope of S6 addresses the possible inclusion of plasma derivatives, the relevancy of specific species as well as the antigenicity of human plasma derivatives in animals makes the application of some methods of evaluation infeasible.

In addition to evaluating the applicability of some provisions of S6, a review of Summary Bases for Approval/Summary Bases for Regulatory Action (SBAs) and European Public Assessment Reports (EPARs) was conducted to help define best practices for the preclinical safety evaluation of currently marketed plasma-derived products. The findings are presented in Table 1., SBAs and Table 2., EPARs.

The products that are encompassed by S6 are predominantly human proteins and as such are expected to exhibit minimal or no antigenicity in humans. However, in animals human-derived proteins can be expected to be immunogenic and, as such, their safety evaluation may have technical limitations. The routine preclinical safety evaluations that are ordinarily applied to small molecule drugs are not applicable to large protein products because of the potentially high immunogenic nature of these molecules and also the high degree of specificity for mode of action which may limit the relevance of the rat and the dog which are commonly used for evaluation. In addition, the long history of clinical safety and efficacy of plasma derivatives in general, and some products in particular, provides confidence in these products which have been manufactured by similar methods by many producers for decades. This confidence often allows scientists, manufacturers and regulators of

plasma derivatives to assure safety by means of limited preclinical toxicology programs.

General Principles of S6

The sponsors of pharmaceutical research are encouraged to use appropriately purified material that is comparable to the intended clinical material to perform early toxicity studies and comparable to the “to be marketed material” for toxicity studies to support later-phase clinical development. The cornerstone of S6 is the recommendation to select a relevant animal species to conduct preclinical assessments and to further emphasize that studies in non-relevant species are not helpful and may be misleading. Ideally toxicity studies to support safety should be conducted according to Good Laboratory Practices (GLPs).

Biological Activity/Pharmacodynamics

In vitro biological assays are used to measure possible clinical activity. The characteristics defined by the various assays determine species reactivity as well as determine the comparative activity across species. This information is then used to select a relevant species providing the necessary information to decide whether standard testing approaches would be useful.

Animal Species/Model Selection

Species selection for initial toxicological studies is thus an essential component of the testing program. S6 suggests the use of two species if both species have been defined as relevant and discusses the option where the use of a single species may be justified if there is only a single relevant species or the biological activity of the product is well understood. Further, if two relevant species are evaluated and demonstrate the same toxicity pattern, it may be possible to limit long-term (e.g., 6 months) studies to a single species.^{3,4}

The species specificity of biological molecules often restricts their use in safety pharmacology studies across species and the antigenicity that they exhibit limits their activity and potential identifiable toxicity when dosed repeatedly in non-homologous species.

It has been recognized that safety evaluation of biopharmaceuticals in animals has limitations that cannot be adequately addressed by the use of standard testing paradigms used for small molecule pharmaceuticals. While S6 was specifically designed for biotechnology-derived products it acknowledges that some aspects of the guideline may be applicable to plasma-derivatives. In the same way, and possibly to a greater extent, because plasma derived products are usually meant to replace or supplement naturally occurring physiological activities, rather than induce a pharmacological effect, they do not readily conform to a routine approach to toxicology testing and some studies may be uninformative and unnecessary.

Selection of Relevant Species

S6 defines relevant species as one in which the product is pharmacologically active. This activity may be expressed through interaction with a receptor, binding to an epitope or interaction in an enzyme cascade such as complement or coagulation factor activation. It should be noted that S6 does not specifically suggest that there be use of the *most relevant* species. This was intentional to avoid the default use of non-human primates especially higher order non human primates.

As previously mentioned, toxicology studies to assess general toxicity are usually performed in two species but in the instance where there is only a single relevant species, a single study may suffice.⁵ In the case of coagulation factors, the molecular structures are conserved across species but may vary in some aspects, so care must be taken in interpretation. When there are no relevant species the development of either transgenic animals (KIs or KOs), animal models of disease or the use of a homologous or surrogate molecule may need to be considered on a “case-by-case” basis. However, S6, carefully points out the limitations of these models. In some cases when there is no relevant species, a study in a single species that is limited to less than 14 days duration might provide some limited toxicity information and may include an evaluation of endpoints such as cardiovascular and respiratory effects. Importantly for protein products the in vitro hERG assay is not relevant thus assessment of cardiovascular endpoints is normally incorporated in repeat dose toxicity studies.⁶

Use of Animal Models of Disease

For some plasma-derived products there are animal models of disease which may be considered relevant. For example, Von Willebrand pig and dog strains have been used to support proof of concept and dose ranging trials for von Willebrand factor products. Similarly, hemophilic dogs have been used to evaluate factor VIII and VIIa products.⁷ These models are useful not only in evaluating the activity and pharmacokinetics of plasma derivatives but they may provide some insight for safety including a recommendation of appropriate safety margins to ensure safe dose estimates. However, the expense and availability of these animals and the antigenicity of human proteins, may limit their utility for classical GLP repeat toxicity studies. As with selection of any species to support preclinical evaluation the use of animal models of disease for toxicity testing should be accompanied by sound scientific justification. It is recognized that animal models may not exactly mirror the human disease and the human molecules may not interact with animal receptors, ligands or enzymes with the same specificity as their human counterparts but as long as there is an understanding of both the human disease and the shortcomings of the animal model, studies may allow a better understanding of the possible toxicities.^{8,9}

Study Design Considerations

In the design of a general toxicity study it is important to determine whether there are any gender-related effects for products that are intended for use in males and females. The numbers of animals per sex per group should be sufficient to be able to recognize and identify a toxicity signal although it is often not possible to sufficiently power studies especially when non human primates are defined as the relevant species. Generally 10-15 animals/sex/group are used for rodent studies (mice and rats) and 4-6 animals/sex/group for non-rodent studies (dogs and monkeys) and 6- 8 animals/sex/group for non-rodent studies (rabbits).

Dose frequency, route should mimic intended parameters planned in the human clinical trials. The pharmacokinetics and practical dose volume administration will be factors in dose selection and frequency. In all cases, appropriate modeling of the level of exposure is most important and the design should be sufficient to gather data that will help to define a toxic dose or a maximum feasible dose and a NOAEL if possible.

Immunogenicity Assessment

The degree of immunogenicity of the product in the animal species chosen is important in designing and interpreting preclinical studies. It should be kept in mind that the formation of antibodies is dependent on multiple factors including the amount of protein injected, the degree that the product is dissimilar to the analogous molecule in the test species and the frequency of injection. The presence of antibodies in response to the product should be measured and characterized for titer, proportion of animals which react, the degree of reaction (titer) and whether the antibodies neutralize activity. (This also suggests the need for an antibody assay of appropriate specificity and sensitivity.) The effects of these antibodies should then be considered when interpreting the results.¹⁰ The potential to alter PK as well as PD should be considered. The detection of neutralizing antibody may be reason for early termination of the study; however, the presence of antibody may not have significance. Because we are discussing human proteins, the potential for antibody formation is greater in animals than in humans and immunogenicity in animals may not be predictive of a human response. Because of the high protein content in plasma derivatives, they are likely to be highly immunogenic and repeat dosing studies beyond antibody development may be difficult to interpret. As presented in Tables 1 and 2, few plasma-derivative products have been tested in repeat dosing studies and those studies performed were of limited duration. In any case, monitoring of the immune response using sensitive, specific and reliable detection methods during repeat dosing in preclinical studies is expected. In general, the ability to assess anti-product antibody is dependent on the quality aspects of the assays developed. The presence, timing and extent of antibodies are important considerations in evaluating toxicity results. When anti-drug antibodies (ADAs) are present, the effects of antibody formation on the PK and

pharmacodynamic parameters, complement activation, and the emergence of pathological changes related to immune complex formation and deposition should be considered when interpreting the data.

Pharmacokinetics and ADME Studies

Pharmacokinetic assessments are conducted to determine exposure and for understanding an appropriate interval of dosing for products that are administered intermittently or chronically. Limited information however will be gained from metabolism studies with most large biological molecules including plasma-derivatives. Metabolism is the consequence of protein degradation or catabolism and the pathways are generally understood.³ If non-natural amino acids are incorporated in the product, metabolism studies may be useful.¹¹ This incorporation of non-natural amino acids however is not anticipated for plasma derivatives. In the cases of complement or coagulation proteins, active molecules may be consumed during functional processes so the physiological state of the recipient animals must also be considered.

Safety Pharmacology

The core battery of safety pharmacology studies that is recommended for small molecules to assess the in vivo effects on cardiovascular, central nervous and respiratory systems prior to human exposure is not generally conducted for biopharmaceuticals. However, incorporation of end points to assess vital organ function is generally included as part of single or repeat dose toxicity studies.

Single Dose Toxicity Studies

Single dose studies are the most informative toxicology studies for evaluation of plasma derivatives. Information can be obtained about possible effects in relation to dose and gender and target organ systems affected. However, for many plasma-derivative products it may be difficult to achieve a toxic dose and the studies would be limited to a maximum feasible dose. In such cases it may be necessary to consider a “maximum sensible dose.”¹² Doses higher than the clinical dose, based on AUC, may not be informative. The specificity of the human-derived biological molecule for the animal system dictates the pharmacological effect. Thus, the multiple of the clinical dose is not as important as the responsiveness to the molecule.¹¹ In animal models for plasma-derivatives, single dose toxicity studies may also incorporate aspects of safety pharmacology. Some authors have suggested that single dose toxicity studies in non-rodents should only be considered when rodents are not a relevant species.¹¹ On the other hand, it has been pointed out that because of the specificity of biopharmaceuticals and the species differences in molecular structure, preclinical safety testing in rodents may have certain limitations.¹³ Often single dose studies are useful in determining parameters for repeat dose toxicity studies for small molecules and some non-plasma derivatives. Data regarding local tolerance can be collected in single dose studies. An important

consideration for plasma-derivatives is the complex nature of the material and the possible unidentified impurities as part of the product or even intended excipients. Single dose studies may also be helpful to characterize the toxicity of those components. In some instances of currently approved products toxicology studies have been conducted with additives to plasma derivatives without testing the actual product. High multiples of additives, such as L-proline and solvent detergent components were studied. The studies without the plasma derivative component (only the additive) may have been performed because preparation of high concentrations or high volumes of the actual product were not feasible.

Repeated Dose Toxicity Studies

S6 provides guidance for the design of repeated dose studies including the route of administration and dosing regimen. Ideally dosing should reflect the intended clinical duration. The duration of dosing is generally 1-3 months and up to 6 months for products intended to be used chronically. The duration of 6 month dosing for chronic studies has been supported by long term post marketing follow-up of approved biopharmaceuticals.¹⁴ For products intended to be used for acute therapy, dosing for 14 days has been adequate to support clinical trials as well as marketing approval which is also consistent with the ICH M3 guideline.⁴ A recovery period is usually a component of the design to determine either recovery or deterioration or delayed manifestation of toxic effects. For some products which exhibit a prolonged recovery, animals should be followed for sufficient time to allow for reversibility. The discussion above regarding the use of relevant species is particularly applicable to the consideration of species to be used for repeat dosing and whether or not it is reasonable or informative to perform such studies for periods of time longer than 14 days. Immunogenicity is a major consideration and potential confounder when administering human-derived proteins to animals. In most cases ADAs may affect overall exposure by altering rate of clearance, neutralizing the function of the protein or inhibiting target binding. If ADAs confound the interpretation of the toxicity study by negatively affecting PK and/or pharmacodynamic activities of the product the studies should be terminated as results will be meaningless. This is particularly true if the immune response is neutralizing.^{8,11} In some cases the immune response may induce reactions that could be misinterpreted as toxicity. In one example, administration of a plasma derivative to NHPs resulted in toxicity but was demonstrated to correlate with the levels of ADA and the reaction resolved after administration was ended. A similar reaction was not observed in clinical trials.¹⁵ As noted in Tables 1 and 2, few products have required repeat-dose studies to support approval. In no cases were chronic studies performed. Those few cases where repeat-doses studies were conducted they were of only 5 - 7 days duration often referred to as sub-acute studies. Since the immune response generally takes about 1-2 weeks this limited number of exposures is probably well within the window where antibodies would develop to confound study interpretation. Thus, it is clear that regulatory authorities and plasma derivative manufacturers recognize

the limitations expressed in S6 and have designed their toxicology testing programs to be consistent with the principles outlined which support the judicious use of animals to obtain information important for the design of the clinical program. In cases where biopharmaceuticals have been uniquely species specific or assessment of toxicity has been precluded by the induction of antibodies, analogous products (also referred to as homologous molecules or surrogate molecules) have been developed (generally murine-derived) and evaluated in the derivative species. The use of analogous products allows for an early understanding of pharmacologic activity in lower order species. However, analogous products are generally derived from a different manufacturing process than the clinical material and they can exhibit differences in potency, pharmacological activity and safety. Therefore, demonstration of comparability would be required and could impact interpretation and extrapolation of the various preclinical studies conducted with the analogous products to the final clinical material. In addition, the manufacture of an analogous product is essentially a parallel development program and thus is often prohibitive for most product developers.⁸ It is also important to note that analogous products can also be immunogenic.⁹ The limitations and challenges in developing analogous products would be the same for plasma-derivatives as they are with biotechnology-derived products.

Immunotoxicity Studies

Many biological products including plasma derivatives may have effects on immunologic function. Therefore, acute studies and repeat dose studies include an evaluation for such possible effects. The need for additional specific testing would be based upon the results obtained.

Reproductive and Developmental Toxicity Studies

The need for reproductive or developmental toxicity studies is dependent on the product, its clinical indication, the patient population for which it is intended and the availability of relevant species. Information regarding the potential for toxic effects may also be indicated by results in acute toxicity studies and the organ systems affected. The decision on the utility of such studies should also be based on accumulated data, information known regarding the particular product class and scientific judgment based on the mode of action. While reproductive/developmental toxicity studies have not been conducted on plasma derivatives, per se, some constituents of final products have been tested. Considering the long historical use of plasma derivatives in males and females without reproductive concerns and a decreased concern for endogenous proteins used as replacement therapies, reproductive/developmental toxicology should be considered when there is a specific scientific reason based on the product's mechanism of action or otherwise cause for concern for a novel additive.

Genotoxicity Studies

S6 is very clear about the lack of relevance of standard pharmaceutical genotoxicity testing as applied to biological molecules. There is no expectation that biological molecules would interact with DNA or other nuclear material that would affect gene structure or expression. In fact, in testing 27 of 44 approved biopharmaceuticals in 85 different assays, no positive results were noted. If needed, a decision tree approach can be applied.¹⁶ The limited cause for concern of genotoxicity is for possible organic linkers that are used in conjugation of more complex protein based products. In such cases a targeted assessment may be needed.¹⁴

Some plasma derivative manufacturers have noted the probable lack of effect as a reason for not testing while others have submitted actual Ames testing results which were predictably negative. Without specific cause, either based on novel formulation components or manufacturing impurities or where there is cause for concern based upon structure activity relationships, there is no utility of genotoxicity testing.

Carcinogenicity Studies

As is the case for genotoxicity testing, standard carcinogenicity testing is generally not appropriate for protein-based therapeutics. In specific circumstances, such as when the product may “induce proliferation of transformed cells,”¹ some assessment may be needed but it should be tailored for the particular product and based on the patient population, dosing anticipated and product activity. For products which intend to be administered for long periods and have been shown to have the potential to be immunosuppressive some methods for evaluation should be considered. Plasma derivatives have not been demonstrated to have any of these effects and such testing would seem irrelevant unless specific product activity suggested there was a cause for concern.

Local Tolerance Studies

Eye or skin irritation studies are generally not performed for biopharmaceuticals although some manufacturers have used them to evaluate topical materials. An evaluation of a possible response at the injection site can be evaluated as a component of single and repeat dose studies. Of those local tolerance studies specifically performed for plasma-derivative products, all are done in rabbits. In the place of specific tests, some manufacturers have noted the absence of irritation at the injection site when performing other toxicity studies.

Update on S6

An ICH expert working group was formed in November 2008 to work on an addendum to ICH S6 with the goal of June 2010 for completion. The scope of products will remain the same but the various sections will be enhanced based upon experience gained over the past decade. Among the topics to be discussed to provide

more specific guidance are the selection of appropriate species, immunogenicity testing, study design including the need for and length of recovery animals, consideration of the use of non-human primates for reproductive/developmental toxicity testing including combined study designs as well as the use of surrogate molecules and transgenic animals for reproductive/developmental toxicity and carcinogenicity assessment.¹⁷

Conclusions

In spite of the encouragement by the S6 document for manufacturers to assess toxicity based on a rational, science-based case-by-case basis, preclinical safety evaluations are occasionally done for the wrong reasons. Industry may perform studies because of perceived regulatory expectations rather than part of a comprehensive scientific strategy; regulatory bodies may ignore scientific strategies and justifications in order to receive what is perceived to be a more complete evaluation (such as a more “classical” toxicology program).¹⁸ Interestingly, the plasma fractionation industry has generally followed the principles described in S6, in spite of the lack of specific references for plasma derivatives within the sections of the document, by applying a case-by-case approach based on science. The programs are an acknowledgement of the effects of immunogenicity on chronic studies and the uninformative data that would be derived from carcinogenicity and genotoxicity studies. Importantly as listed in Table 1 for US approvals and Table 2 for European approvals: repeat dosing studies are rarely performed and if done, are limited to a length of time to avoid neutralizing antibody formation which would confound study interpretation; ADME studies are also not conducted on plasma fractions for the reasons explained; a developmental toxicity study was conducted in rats for C1 esterase inhibitor however interpretation was compromised due to the presence of neutralizing antibodies; and, for most plasma derivatives reproductive/developmental risk is communicated in the label as Pregnancy Category C. As noted in Tables 1 and 2, many products have been approved with very limited toxicological evaluation and some have been approved without animal testing. This is the result of the confidence in the products and the associated manufacturing methods that has been gained over the many years of safe and effective use.

The plasma fractionation industry can be credited with a long history of safe and effective products. Scientific and technical achievements over the years have enabled the extraction and large scale production of important therapeutics from donated plasma. During this time preclinical development programs have been designed and scientifically justified to support not only safe introduction into humans but continued clinical development to confirm safety and efficacy. The principles of S6 further support targeted scientifically justified preclinical programs designed on a case-by-case basis based upon specific product attributes. More importantly S6 discourages toxicity studies in irrelevant species and such studies

provide little to no value to the clinical program with respect to safety and/or communicating risk.

¹ ICH Harmonized Tripartite Guideline, Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, S6, 1997, <http://www.ich.org/LOB/media/MEDIA503.pdf>

² Snodin, D and Ryle, P, 2006, Understanding and Applying Regulatory Guidance on the Nonclinical Development of Biotechnology-Derived Pharmaceuticals *BioDrugs*, 20: 25-52.

³ Cavagnaro, J. 2002, Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals, *Nature Drug Discovery*, 1: 469-475.

⁴ Nakazawa, T., Kai, S., Kawai, M., Maki, E., Sagami, F., Onodera, H., Kitajima, S., and Inoue, T., 2004, "Points to Consider" regarding Safety Assessment of Biotechnology-Derived Pharmaceuticals in Non-Clinical Studies (English Translation). *J. Tox Sci.*, 29: 497-504.

⁵ Dempster, M. Designing Non-Clinical Safety Evaluation Programmes: the view of the toxicologist. In Griffiths, S and Lumley, C. eds. *Safety Evaluation of Biotechnologically-derived Pharmaceuticals: facilitating a scientific approach*, 7-15.

⁶ Vargas, H., et al., 2008, Scientific Review and Recommendations on Preclinical Cardiovascular Safety Evaluation of Biologics, *J. Pharm. Tox Meth.* 58: 72-76.

⁷ Lewis, R., 2008, Preclinical Safety Evaluation of Blood Products, in J. Cavagnaro, ed., *Preclinical Safety Evaluation of Biopharmaceuticals: a science-based approach to facilitating clinical trials*, Wiley, NJ, 669-682.

⁸ Cavagnaro, J., 2008, Implementation of ICH S6 and the Case-by-Case Approach, in J. Cavagnaro, ed., *Preclinical Safety Evaluation of Biopharmaceuticals: a science-based approach to facilitating clinical trials*. Wiley, NJ, 45-65.

⁹ Inoue, T., 1997, Biotechnologically-derived Pharmaceuticals in Japan: Present and future prospects. In Griffiths, S and Lumley, C. eds. *Safety Evaluation of Biotechnologically-derived Pharmaceuticals: facilitating a scientific approach*. 51-63.

¹⁰ Cosenza, M.E., 2008, Implementation of ICH S6: US Perspective, in J. Cavagnaro, ed., *Preclinical Safety Evaluation of Biopharmaceuticals: a science-based approach to facilitating clinical trials*. , in J. Cavagnaro, ed., *Preclinical Safety Evaluation of Biopharmaceuticals: a science-based approach to facilitating clinical trials*, Wiley, NJ, 111-121.

¹¹ Nakazawa, T., 2008, Implementation of ICH S6: Japanese Perspective, in J. Cavagnaro, ed., *Preclinical Safety Evaluation of Biopharmaceuticals: a science-based approach to facilitating clinical trials* Wiley, NJ, 93-109.

¹² Anne Pilaro, personal communication.

¹³ Kawanishi, T, Suzuki, T., Ishii, A., Yamaguchi, T., 2007, 3R's achievement in quality control of recombinant protein drugs, 6th World Congress on Alternatives and Animal Use in the Life Sciences.

¹⁴ Clarke, J., Hurst, C., Martin, P., Vahle, J., Ponce, R., Mounho, B., Heidel, S., Andrews, L., Reynolds, T., Cavagnaro, J., 2006, Duration of Chronic Toxicity Studies for Biotechnology-derived Pharmaceuticals: Is 6 months still appropriate? *Reg. Tox. and Pharmacol.* 50: 2-22.

¹⁵ Romberg, et al., 2002, Utility of Primate Toxicology Studies as a Predictor of Human Safety and Tolerability: Experience with Dry Powder Alpha₁-Antitrypsin Administered to Patients with AAT Deficiency, *Resp. Drug Del.* 8:1-9.

¹⁶ Jacobson-Kram, D and Ghantous, H., 2008, Genetic Toxicology Testing of Biopharmaceuticals, in J. Cavagnaro, ed., *Preclinical Safety Evaluation of Biopharmaceuticals: a science-based approach to facilitating clinical trials* Wiley, NJ

¹⁷ Sims, J., 2008, Rapportuer, Update the guidance of ICH S6 http://www.emea.europa.eu/pdfs/conference/flyers/ICH_regional_meet_brussels/64006708en.pdf

¹⁸ CMR International report, 1998, Unifying Understanding: safety evaluation strategies for biotechnology products. <http://cmr.thomsonreuters.com/pdf/rd18.pdf>

Table 1. Toxicity Studies to Support Licensure of Plasma Derivatives in the US Market^a

Product	Single dose	Repeat dose (days)	Safety Pharmacology	Animal PK/PD ADME	Carcinogenicity, reproductive/ Developmental	In vitro Pharmacology	Thrombogenicity	In vitro characterization	Non-plasma component testing	Immunogenicity Neo-immunogenicity	Manufacturer Year approved
Alpha 1 proteinase inhibitor	Mouse (Local Tolerance) Rabbit	Rat, Rabbit (5 D)				Yes		PMN Elastase inhibition		Rabbit	Aventis 2003
C1 esterase Inhibitor (human)	Rat, Rabbit	Species not stated (7 D)			Rat, repro		Yes				Lev 2008
Fibrin Sealant			Yes								Baxter 1998
Fibrin Sealant				Rabbit PD, Rabbit ADME					Yes		Omrix 2003
Fibrinogen concentrate	Mouse, Rat		Rat Swine	Non-rodent PK						Dog, Rabbit	CSL 2009
Hepatitis B Immune Globulin Intravenous											Cangene 2007

Product	Single dose	Repeat dose (days)	Safety Pharmacology	Animal PK/PD ADME	Carcinogenicity, reproductive/ Developmental	In vitro Pharmacology	Thrombogenicity	In vitro characterization	Non-plasma component testing	Immunogenicity Neo-immunogenicity	Manufacturer Year approved
Immune Globulin Intravenous (Human) 5%										Rat, Dog	Octapharma 2006
Immune Globulin Intravenous (Human) 10%	Mouse, Rat (Local tolerance) Rabbit		Rat, Guinea Pig, Dog	Mice			Rabbit				Baxter 2005
Immune Globulin Subcutaneous	Mice, rats (Local Tolerance) Rabbit		Dog	Rabbit PK					Yes	Rabbit, Guinea Pig	CSL 2006
Protein C	Mouse, Rat (Local Tolerance) Rabbit		Dog, Guinea Pig				Rabbit				Baxter 2007
Thrombin			Non-rodent	Rabbit PK, ADME	Mutagenicity Repro Rat						Omrix 2007

^aBased on Summary Bases of Approval when available

Table 2. Toxicity Studies to Support Approval of Plasma Derivatives by EMEA ^a

Product	Single dose	Repeat dose (days)	Safety Pharmacology	Animal PK	ADME	Carcinogenicity, reproductive/ Developmental	In vitro pharmacology	Thrombogenicity	Neoantigenicity	Non-plasma component testing	Manufacturer Year approved
Protein C	Mouse, Rat (Local Tolerance) Rabbit		Guinea Pig, Dog					Rabbit	Rabbit		Baxter 2001
Human Normal Immunoglobulin	Mouse, Rat			Rat, Rabbit							Grifols 2007
Human Normal Immunoglobulin	Mouse, Rat (Local Tolerance) Rabbit		Mouse, Dog	Rat							Baxter 2006
Human Normal Immunoglobulin	(Local tolerance) Rabbit									Rat	CSL 2008
Blood Coagulation Factor IX								Rabbit, Guinea Pig			Sanguin 2001

^a Based on review of individual EPAR reports.

^b Studies were performed with previous manufacturing process.