

KEY CONSIDERATIONS FOR NON-CLINICAL SAFETY ASSESSMENT OF INHALED BIOPHARMACEUTICALS

In this article Simon Moore, PhD, Kirsty Harper, PhD, and Sylwia Marshall, PhD, all of Envigo, highlight some of the key considerations for the development of inhaled biopharmaceuticals, including non-clinical safety assessment.

Inhalation is the route of choice for the delivery of numerous small molecule drugs, especially for the treatment of respiratory diseases, as it has a number of advantages over parenteral routes. These include faster onset of action due to the large surface area (80–120 m²) and good vascularisation of the lung, improved therapeutic index due to targeted delivery requiring lower doses and improved patient compliance.

Historically, the development of biopharmaceuticals for inhalation has been hindered by challenges such as high drug requirement, manufacturing costs and stability issues. However, technological advances addressing these concerns are now facilitating the development of such modalities. One of the earliest marketed inhaled biopharmaceuticals was Pulmozyme®, which was approved in 1993 for cystic fibrosis. The field has continued to grow since then, and there are an extensive number of inhaled biopharmaceuticals in early development.

The percentage of biopharmaceuticals in the global pipeline has grown from 30% in 2010 to 42% in 2017, and total revenues from their sales increased from 17% of all prescription drugs to 26% over the same period, with the figures expected to reach 30% by 2022.¹ It is highly likely that inhaled biologics will contribute to this projected growth, having already been evaluated for numerous indications (Figure 1).

NON-CLINICAL SAFETY ASSESSMENT OF INHALED BIOPHARMACEUTICALS: GENERAL APPROACH

There are of course numerous differences between biologics and new chemical entities (NCEs) and these heavily influence the development strategy, including non-clinical safety assessment.

Biologics are a heterogeneous group of medicinal products that are generated or derived from biological sources and include biopharmaceuticals (proteins including monoclonal antibodies, peptides and oligonucleotides), vaccines and advanced therapies (gene/cell therapies). Each of these product types has specific features as well as specific biology that must be considered when designing non-clinical safety assessment programmes. Biopharmaceuticals are generally much larger than NCEs with many having complex structures, including secondary and tertiary structures, which are intrinsically linked with their function. Therefore, the physicochemical properties of these products must be taken into consideration when designing delivery systems.

The general approach to safety assessment of biopharmaceuticals is described in the ICH S6 (R1) guideline, “Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals”, where the basic principles of safety assessment in pharmacologically relevant species, and inclusion of appropriate pharmacodynamic (PD) endpoints wherever possible, are specified. This approach translates for assessment of biopharmaceuticals delivered by all routes of administration, inhalation included, and will likely determine the required programme of work for an inhaled biopharmaceutical.

Biopharmaceuticals exert their activities through specific interaction with their targets in the recipient patient, and it is therefore essential that all safety assessment studies replicate the clinical situation as far as possible with regard to target expression, binding and subsequent downstream biology. A comprehensive understanding of the pharmacology of the biomolecule in both humans and the candidate preclinical safety species is therefore required, and studies should only be performed in



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Figure 1: Various indications for which biotherapeutics have been evaluated using the inhalation route.

appropriate species. This may mean that a single species approach is sufficient and there are examples of biopharmaceuticals that received subsequent clinical approval following evaluation in a single species.

Due to the strong emphasis on pharmacology, non-clinical safety programmes are product specific and, unless the biopharmaceutical has a chemical modification, may omit some studies that are routinely found in NCE preclinical safety work packages, such as genetic toxicology studies.

Further, for most biopharmaceuticals, safety pharmacology endpoints are undertaken on a risk-based approach and are often incorporated into the design of pivotal repeat dose toxicity studies, with investigations in a single species commonly being acceptable. Depending on the mechanism of action of the biopharmaceutical, respiratory safety pharmacology may need to be supplemented with investigations of other systems which

may be targeted, such as the central nervous system. The feasibility of such investigations needs to be carefully considered, especially with reference to the selected pharmacologically relevant species.

INHALED BIOPHARMACEUTICAL FORMULATIONS & DEVICES

Inhaled drugs tend to be either liquid formulations administered via a nebuliser in a hospital environment or with the assistance of an experienced carer, or are self-administered as either aerosols or dry powders via handheld inhalers, which are generally acknowledged to be more efficient, stable and convenient for patients.

In general, most biopharmaceuticals show good aqueous solubility, and in the case of repurposing of existing products, a solution formulation is likely to already exist. For powders, however, more novel manufacturing techniques (lyophilisation, spray drying or vacuum foam drying) are

more likely to be used than traditional manufacturing techniques, such as micronisation, as they tend to provide greater stability and ensure structural integrity of the biopharmaceutical. In addition, powders can accommodate the inclusion of various excipients.

There are a number of device types, each associated with their own advantages and disadvantages, that may be used to deliver biopharmaceuticals. Nebulisers can operate with many liquid formulations and are capable of delivering large quantities of drug, which may be needed to ensure sufficient clinical overages for toxicity assessment in the non-clinical setting. Nonetheless, liquid formulations can have limits with viscosity, ionic strength and surface tension which will impact output and drug concentration.

Pressurised metered dose inhalers (pMDIs) are not easily compatible with biopharmaceutical drugs due to the inherent temperature, pressure and excipient aspects, although in some cases there may be viable approaches to stabilise the drug product. An alternative approach to nebulisers and pMDIs are soft-mist devices which provide a pMDI-like dosing experience with an aqueous solution product. However, drawbacks include the requirement for high concentrations and the forces involved in delivering the formulation, which may prove incompatible for drug products where large doses are needed.

In contrast to pMDIs, which require the patient to co-ordinate breathing in with actuating the device by hand, dry powder inhalers (DPIs) generally require little to no hand-breath co-ordination, and they can deliver quite high payloads with a quicker dosing time than nebulisers. However, additional pre-formulation, formulation and device screening is necessary for DPI-based products, to address some of the dry powder formulation and stability characteristics.

AEROSOL SAMPLING & ANALYTICAL METHODOLOGY

Confirmation of the amount of the dosed test material is not only good scientific practice but also a regulatory requirement. To verify the concentration of the delivered dose, samples are collected directly from

the exposure system from locations that are representative of the breathing zone for the animals (generally a facemask or restraint tube attachment position) using methodology that provides optimal trapping of the drug and permits chemical analysis of the active component. For most liquid formulations, this comprises a glass sintered sampling trap using an appropriate trapping solvent. For powder or suspension formulations, a quartz fibre filter is used rather than the standard glass fibre filters for NCEs. This is used in conjunction with silanizing analytical glassware prior to use.

For aerosol concentration and particle size assessment, standard Ultra Performance Liquid Chromatography (UPLC) analysis is normally employed. However, alternative methodology may have to be used depending on the biopharmaceutical. As mentioned earlier, biopharmaceuticals have complex structures and in many cases their activity depends on correct folding and subsequent tertiary structure. The shear forces exerted during the process of aerosol generation can impact the structure and therefore alter the bioactivity of the drug substance, with the worst-case scenario being loss of potency. For feasibility studies, one should consider the inclusion of not only a binding assay, but also a cell-based potency assay, where the pharmacological activity of the test material may be evaluated and any change in potency following aerosolisation noted. Since such assays tend to be product-specific, early dialogue with the selected non-clinical CRO partner is encouraged to ensure smooth transition from exploratory studies to regulatory GLP safety assessment.

BIOANALYTICAL AND BIOMARKER CONSIDERATIONS

Non-clinical safety studies with biopharmaceuticals intended for inhaled delivery have a number of additional considerations that are unique to this method of administration. Although confirmation of drug exposure by comprehensive pharmacokinetic/toxicokinetic (PK/TK) evaluation is expected in all biopharmaceutical safety assessment packages, it is important to consider that for inhaled products systemic exposure may not always be achievable or indeed desired. For instance, there may be limited transport of the delivered biopharmaceutical due to its size (molecules larger than 50 kDa display reduced bioavailability^{2,3}) or targeted delivery, and binding to

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a receptor in the lung or a specific cell population may lead to retention of the drug in the lung.

Therefore, sampling of the local environment by bronchoalveolar lavage (BAL) to confirm that the intended delivery has been achieved as well as establishing systemic exposure should be considered.

The feasibility of obtaining BAL measurements requires careful consideration as, although possible, in-life sampling carries an inherent risk to the animal. For this reason strict sampling limits are imposed and it is highly likely that a full lung TK profile will not be possible in non-rodent species, with rodent studies requiring additional animals for such assessments.

The analytical approaches required for TK assessment of biopharmaceuticals may differ to those more commonly employed for NCEs, with immunoassays based on ligand binding assessment often required, although liquid chromatography-mass spectrometry (LC-MS) or MS based assays can still be utilised if a signature peptide has been identified, or for smaller products such as oligonucleotides.

As mentioned earlier, the safety profile of a biopharmaceutical can only be adequately assessed in a pharmacologically relevant species, ideally where the intended clinical biology can be replicated. As a result, markers to confirm PD activity should be included in safety assessment studies wherever possible. Appropriate markers should be identified based on the expected pharmacological effect and assessment performed at timepoints relevant to its induction.

A detailed understanding of the intended biology is therefore required and this should include any downstream effects in addition to the direct effect of the drug interacting with its target. The relationship of this biology in the non-clinical species to the clinical situation should also be thoroughly investigated so that any differences in the

level or distribution of the target expression can be understood and interpreted.

In addition to PD endpoints, safety biomarkers can also be incorporated into the non-clinical safety studies. These can include markers of immune activation (CRP, cytokines, immune cell activation and/or mobilisation), immunogenicity assessment (discussed later), as well as assessment of “off-target” pathways that have been identified for certain classes of drugs. For example, prolonged coagulation and complement activation have long been associated with oligonucleotides, especially those with a phosphorothioate backbone or products with lipid based formulations.^{4,5} The exact parameters required for analysis are selected based on the biology and risk specific to the individual product, and if this risk is unknown or theoretical it can be assessed in preliminary studies to determine whether further follow up in pivotal studies is required.

IMMUNOGENICITY

One of the considerations specific to biopharmaceuticals is the development of immunogenicity. Administration of a human protein to an animal species can induce an immune response specific to the drug following delivery by any of the main routes of administration. The lung is predisposed to remove foreign material, and populations of the immune system, such as macrophages, specifically support this, so the potential for immunogenicity responses should be explored.

Although it is accepted that immunogenicity in an animal model is not predictive of immunogenicity in the clinical setting, the recognised consequences warrant at least the collection of samples. Blood samples should be collected prior to treatment and following completion of dose administration to assess the presence of systemic anti-

drug antibodies (ADA), should there be any change to the PK/PD relationship during the study. This may be followed-up by more detailed investigations such as assessment of the functionality of the ADAs in neutralising antibody assay and/or immunohistochemistry staining for the presence of immune complexes. Such in depth characterisation is not often needed at the preclinical stage but it should be considered for inclusion in clinical studies.

CONCLUSION

Drug delivery via inhalation is an exciting and growing field of drug development. Despite the additional considerations associated with the inhalation route in the context of biopharmaceuticals, there is considerable research activity in this field. A detailed understanding of the pharmacology and biology of the biopharmaceutical product and careful execution of appropriately designed

non-clinical safety studies, combined with selection of the most appropriate delivery method, can ensure a successful transition from non-clinical to clinical assessment.

ABOUT THE COMPANY

Envigo provides essential products and research services for pharmaceutical, crop protection and chemical companies, as well as universities, governments and other research organisations. With more than 3,300 employees across over 50 locations worldwide, Envigo is committed to helping customers realise the full potential of their research and products, working together to build a healthier and safer world.

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ABOUT THE AUTHORS

Simon Moore, PhD, joined Envigo in 1999 and is now the Director of Inhalation Science and Engineering and Toxicology Operations Inhalation Team Leader. In this role, Dr Moore is responsible for all aerosol technology aspects including the overall interpretation and reporting of the inhalation studies including safety pharmacology and ADME. In addition, he also leads a team of inhalation engineers who design, prototype and manufacture custom inhalation equipment for nonclinical safety assessment studies conducted at Envigo.

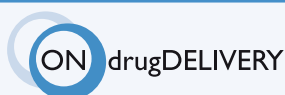
Dr Moore obtained a Chemistry degree from the University of Dundee (UK) in 1996 and gained his PhD in Heterogeneous Catalysis from the University of Glasgow (UK) in 2000 using high-pressure gas flow and chromatography. He lectures at the University of Surrey (UK) as part of the MSc Toxicology course on inhalation dosing, techniques and methodology and is a committee member of the Association of Inhalation Toxicologists and the British Standard Institution on Nanotechnologies.

Kirsty Harper, PhD, joined Envigo in June 2013 in her current role to design safety studies and non-clinical development programmes for biologics in response to customer requests as well as to provide scientific support and advice. Prior to this, she was employed as Principal Scientist at Oxford Immunotec Ltd, where she was responsible for pipeline product development projects and the provision of immunological advice and expertise.

Dr Harper obtained her PhD in Immunology from the University of Bristol (UK) in 2005, after which she completed a post-doctoral position investigating peptide therapy as potential treatment for autoimmune disease. Prior to this she obtained her BSc and MSc in Microbiology at Massey University (New Zealand) and worked at the Malaghan Institute (New Zealand) where she conducted basic research in autoimmune disease.

Sylwia Marshall, PhD, joined Envigo in July 2014 as Director of Biopharmaceutical Development and is responsible for designing safety studies and nonclinical development programmes for biologics, and providing scientific support and advice. Prior to joining Envigo, Dr Marshall held a senior research position at Novartis, where she lead multi-disciplinary biologics projects from early discovery through to clinical development working with external collaborators and CROs.

Dr Marshall received her undergraduate degree (BSc Biomedical Sciences) from the University of Durham (UK) and completed her PhD at University of Manchester (UK) in 2005 where she researched peritoneal wound healing and fibrosis. She then took on post-doctoral research positions at University College London and The Lung Institute of Western Australia which investigated biological processes involved in the development of fibrosis and inflammation.



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