

# Discovery and development of inhaled biopharmaceuticals

*the impact and effective routes of non-clinical safety assessment of inhaled biopharmaceuticals*

With the rapid generation of biological screening data and the potential for innovative selection of compounds for screening, the importance of multi-vendor collaborations together with improvements in automation has never been greater in terms of helping the drug discovery community as a whole.

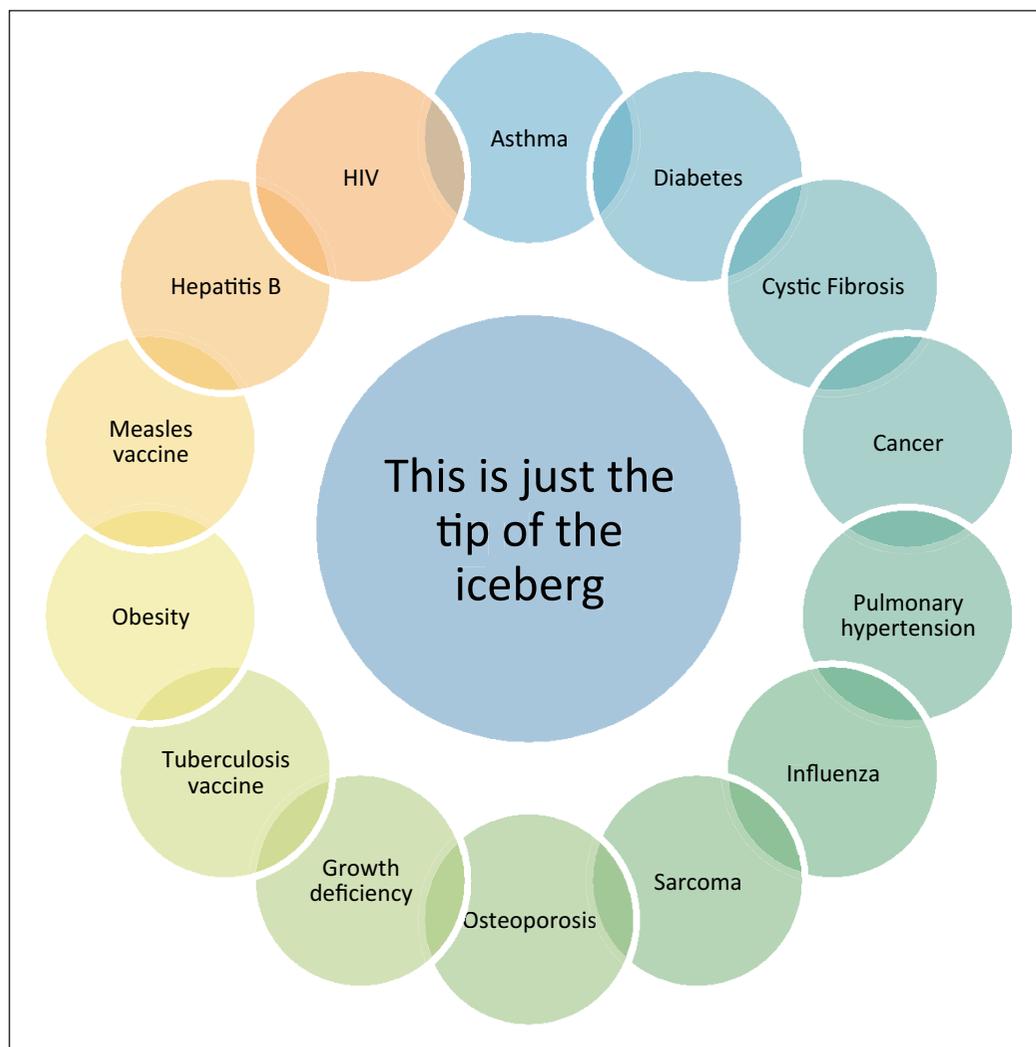
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The creation and subsequent development of inhaled biologics has become highly significant as it is now the route of choice for the delivery of numerous drugs. This is especially the case with biopharmaceuticals developed for the treatment of respiratory diseases. Primary drivers for this include the strong advantages that inhaled biologics have over parenteral routes. These include faster onset of action due to the large surface area (80-120m<sup>2</sup>) and good vascularisation of the lung; avoidance of degradation in the gastrointestinal tract and first pass effect improved therapeutic index due to targeted delivery requiring lower doses (with potentially fewer side-effects); improved patient compliance as it is more convenient, less intrusive and relatively comfortable for the patient; and, in some cases, has improved stability.

Historically, challenges such as high drug requirement, manufacturing costs and stability issues have hindered the development of inhaled biopharmaceuticals. However, technological advances addressing these concerns are now facilitating the development of such modalities. Pulmozyme<sup>®</sup> was one of the initial marketed inhaled biopharmaceuticals to move successfully through discovery and development to obtain

approval in 1993 for use in the treatment of cystic fibrosis. There have been notable developments since then, with the field continuing to grow significantly. Currently, there are in excess of 40 inhaled biopharmaceuticals in the public domain that have passed through the discovery stage and which are now in the early phase of development. The result of these discovery and development successes over the course of the last decade is that the percentage of biopharmaceuticals in the global pipeline has grown from 30% in 2010 to as much as 42% in 2017. Moreover, total revenues from their sale increased from 17% of all prescription drugs in 2010 to 26% in 2017, with the figures expected to reach as high as 30% by 2022. There is also strong likelihood that inhaled biologics will also make a significant contribution to the level of growth that has been projected.

There are a significant number of differences between new chemical entities (NCEs) and biologics (Figure 1) and these heavily influence the overall discovery and development strategies that are established including early-stage non-clinical safety assessment. This article highlights some of the key considerations for the development of inhaled biopharmaceuticals.



**Figure 1**  
Various therapies that have been evaluated using the inhalation delivery route and biopharmaceuticals

**The general approach to the non-clinical safety assessment of inhaled biopharmaceuticals**

Discovered biologics require highly-specialised research in the early pre-clinical phase of development. They 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 highly specific biology that must be considered when designing early stage non-clinical safety assessment programmes. In general, biopharmaceuticals are much larger than NCEs with many having complex structures, including secondary and tertiary structures, which are intrinsically linked with their function. As a consequence, it is crucial to take the physicochemical properties of these products into consideration when setting

about designing delivery methodologies. The general approach to safety assessment of biopharmaceuticals is described in the ICH S6 (R1) guideline Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. In these guidelines, the basic principles of safety assessment in pharmacologically relevant species and inclusion of appropriate pharmacodynamic (PD) end points wherever possible is specified. This approach translates for assessment of biopharmaceuticals delivered by all routes of administration, including inhalation, 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 or animal, 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 induced by the biopharmaceutical in both humans and the candidate preclinical safety species is therefore required, and studies should only be performed in appropriate species. This may mean that a single species approach is sufficient and there are many examples of biopharmaceuticals which received subsequent regulatory approval following evaluation in a single species.

Due to the strong emphasis on pharmacology, non-clinical safety programmes in early-stage development 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. Moreover, for most biopharmaceuticals, safety pharmacology end-points are undertaken on a risk-based approach and are often incorporated into the design of pivotal repeat-dose toxicity studies in pre-clinical research, with investigations in a single species commonly being acceptable. Depending on the mechanism of action of a specific biopharmaceutical, respiratory safety pharmacology may need to be supplemented with investigations of other systems that may be targeted such as the central nervous system. The feasibility of such investigations requires very careful consideration, especially with reference to the selected pharmacologically relevant species.

### Inhaled biopharmaceutical formulations and devices

Inhaled drugs that have progressed through discovery and into development tend to be formulated in one of two ways; either as liquid formulations, where treatments are commonly administered in a hospital environment or with the assistance of an experienced carer, or as a powder, which is generally acknowledged to be more efficient, stable and convenient for patients.

For powders, crystalline forms are more thermodynamically stable and typically more chemically stable than amorphous material. However, amorphous powders are more common than crystalline as they have the ability to wrap round but amorphous powders are very hygroscopic requiring careful handling and stabilisation, for example from dehydration, thermal, shear, oxidation, light and pH. Particle engineering techniques such as lyophilisation, spray drying or vacuum foam drying are often used in preference to traditional manufacturing techniques such as micronisation as they aid with these stability considerations and ensure structural integrity of the biopharmaceutical. The added advantage with these particle engineering techniques is they offer control with parti-

cle density, size, morphology and surface characteristics in order to enhance both the particle population aerodynamic characteristics and preserve the biopharmaceutical chemical structure when formulated with excipients.

Various excipients that have been used include mannitol, trehalose, sucrose, leucine, reffinose, tween-20, sodium chloride, saccharide, surfactant, sodium citrate and citric acid. Importantly, if a novel excipient is required for the formulation then it may be necessary to perform a safety assessment of the excipient alone as well as in the final drug product.

Most biopharmaceuticals show good aqueous solubility for liquid formulations. Nonetheless, liquid formulations can have limits with viscosity, ionic strength and surface tension which will impact output and drug concentration.

Similarly to powder, excipients are added to provide a viable formulation. Common excipients and functions are shown in **Table 1**.

It is not essential to use the nebuliser device proposed for clinical studies for pivotal GLP toxicology studies. Often this not practical or feasible as the generation characteristics of the clinical device are not suitable for delivery of the test material to the non-clinical species in question (eg the need to generate smaller aerosol particle sizes for delivery to rodents) or compromised output as it is important to maximise the delivery of active drug by selecting the most appropriate device(s) as there are a number of device types, each associated with their own advantages and disadvantages.

Device compatibility is crucially important. Though Pressurised Metered Dose Inhalers (pMDI) formulations are not easily compatible with biopharmaceutical drugs due to the inherent temperature, pressure and excipient aspects, 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, one of the drawbacks with these delivery products is the requirement for high concentrations, as well as the forces involved in delivering the formulation, which may prove incompatible for drug products where large doses are needed.

In contrast to MDIs, which require the patient to co-ordinate breathing-in with actuating the dose by hand, dry powder inhalers (DPIs) generally require little or 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.

Furthermore, if the development company is not legally bound to one clinical device, this gives greater flexibility with the product for clinical use to a wider potential human population, however, strategic agreements to a specific device may give product differentiation and extend patent life for the API.

**Aerosol sampling and analytical methodology**

Confirmation of the amount of the dosed test material is not only good scientific practice but also a regulatory requirement.

The precise dose delivered to the animal using a syringe for oral or parenteral routes can be measured exactly, based on the bodyweight of the animal and the concentration of the solution being administered. With inhalation administration it is not possible to calculate the ‘dose’ given to animal in the same way.

The animal is presented with an atmosphere concentration of the test article and spontaneously breathes from that aerosol, effectively self-dosing based on the animal’s own tidal volume and frequency of breathing.

As a consequence the delivered dose needs to be derived based on an estimate of the air volume inhaled during the exposure period as well as the bodyweight and test atmosphere aerosol concentration. Finally, the proportion of inhaled test article that will enter the lungs is dependent on the particle size. The delivered dose is estimated as:

$$DD = \frac{(C \times RMV \times D \times IF)}{W}$$

- DD** Delivered dose
- C** Aerosol concentration of substance in air
- RMV** Respiratory minute volume
- D** Duration of exposure
- IF** Proportion by weight of particles that are inhalable (normally assumed as 1 if >90% of particles are <7 microns)
- W** Bodyweight

To verify the concentration of the delivered dose, samples are collected directly from the exposure system from locations that are representative of the

**Table 1:** Typical excipient types for inhaled biopharmaceuticals (Parry, M et al <http://www.intertek.com/knowledge-education/formulation-biologics-inhaled-nasal-delivery/>)

EXCIPIENT	FUNCTION
Buffers	Control of pH
Salts	Control of ionic strength
Amino acids	Control of ionic strength
Polyols/disaccharides/polysaccharides	Preferential hydration/exclusion to create a stabilising ‘shell’
Surfactants	Protection from hydrophobic interactions, such as at container surfaces
Antioxidants	Protection from oxidation

breathing zone for the animals (generally a facemask or restraint tube attachment position) using methodology that provides optimal trapping of the drug and to permit 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 silanising analytical glassware prior to use.

As well as aerosol concentration determination, the other principle sample collection for any inhalation delivery study is for the assessment of particle size. Similarly, this sample must also be collected from the breathing zone of the non-clinical species being evaluated to ensure a representative and compliant sample and not from the extract of the exposure system. This poor practice will compromise the validity of the study. The Marple or Mercer cascade impactors are the devices of choice for this evaluation.

For aerosol concentration and particle size assessment, standard Ultra Performance Liquid Chromatography analysis is normally employed, however, alternative methodologies may have to be used depending on the biopharmaceutical concerned. As mentioned earlier, biopharmaceutical products 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 in the test system. For feasibility studies, researchers should consider the inclusion of a cell-based potency assay,

where the pharmacological activity of the test material may be evaluated and any change in potency following aerosolisation noted, in addition to the use of a binding assay. Since such assays tend to be product-specific, early dialogue with the selected CRO is encouraged to ensure smooth transition from exploratory studies to regulatory GLP safety assessment.

Depending on the composition of the formulation, the ratio of gravimetric and chemical analysis for most biopharmaceutical powders remains consistent with the original formulation composition due to the type of formulation preparation technique.

The principle reason for a disparity is in case two different particle sizes are used in the preparation with powder formulations and for suspension with liquid formulations.

### **Bioanalytical and biomarker considerations in pre-clinical research**

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 biopharmaceutical products, systemic exposure may not always be achievable or indeed desirable. For instance, there may be limited transport of the delivered biopharmaceutical due to its size (molecules larger than 50kDa display reduced bioavailability) or targeted delivery and binding to 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 LC-MS/MS-based assays can still be utilised if a signature peptide has been identified or for smaller products such as oligonucleotides.

As mentioned earlier in this article, the safety profile of a biopharmaceutical in pre-clinical studies can only be adequately assessed in a pharmacologically-relevant species, ideally where the intended clinical biology can be replicated. As a result, it is imperative that markers to confirm PD activity are 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 properly understood and interpreted.

In addition to PD end-points, 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 phosphorothioate backbone or products with lipid-based formulations. The exact parameters required for analysis are selected based on the biology and the risk specific to an 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 in R&D is the development of immunogenicity. Administration of any human protein to an animal species is essentially delivery of a non-self material to some degree and development of an immune response specific to the drug can be induced 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, are specifically-designed to support this, so the potential for immunogenicity responses should be explored, whether intended biology or not. Although it is accepted that immunogenicity in an animal model in pre-clinical studies is not predictive of immunogenicity in the clinical setting, the recognised consequences of immunogenicity 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 should there be any change to the PK/PD relationship during the study. This may be followed up by more detailed investigations such as an assessment of the functionality of the ADAs in neutralising antibody (NAb) assays and/or immunohistochemistry (IHC) staining for the presence of immune complexes. However, such in-depth characterisation is not often needed at the preclinical stage, although it should definitely be considered for inclusion in clinical studies.

### Conclusion

In the complex field of biopharmaceuticals, inhalation-based drug delivery is an exciting and growing segment in modern drug discovery and development; despite the additional considerations associated with the delivery route and biopharmaceuticals, there is considerable early-stage research activity in this market sector. A detailed understanding of the pharmacology and biology of the biopharmaceutical product moving through discovery and development, careful execution of appropriately designed non-clinical safety studies combined with selection of the most appropriate delivery method, can ensure the successful transition from pre-clinical to clinical assessment. This is critical if biopharmaceutical products are to have the prospect of making it all the way through discovery, all stages of development and onwards to market entry.

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