Accelerating inhalation product development

Choosing between outsourcing and developing internal capabilities

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Introduction

It is inevitable that small- and medium-sized pharmaceutical companies outsource key research and chemistry, manufacturing and control (CMC) functions to contract research organizations (CROs) when it does not make financial sense to build internal capabilities for activities that are infrequent and/or require large capital or headcount investments to establish expertise. Examples of typical outsourced activities include: evaluation of the preclinical efficacy or pharmacokinetic profile of experimental formulations in *in vivo* animal studies, quantitation of a drug and its metabolites from these biological samples, characterization studies such as leachables and extractables testing or aerosol performance testing, manufacturing of drug product, and stability and release testing of that drug product.

For inhalation product development, there is additional complexity in the conduct of preclinical animal studies. Many CROs may not have experience specific to reproducibly delivering an inhaled product to animals to achieve the target lung dose, characterizing the performance of an aerosol delivery system, or providing assurance that the formulation maintains its performance characteristics throughout the duration of the animal exposure study. In addition, multiple CROs may often be needed to conduct various elements of linked activities; e.g., one to manufacture a formulation, a second to evaluate that formulation in an *in vivo* animal study, and a third to validate the bioanalysis method and subsequently quantify the drug in the biological samples from those studies. At the end of the study, if the bioanalytical data does not match expectations, the innovator may wonder if the data is indeed reliable or if an error was made at one of the many steps along the way. Therefore, the quality of the CRO and their procedures and experiments need to be closely monitored to prevent errors. Furthermore, it is essential to establish a relationship with the CRO professionals responsible for the conduct of studies and provide training on methodology that may be novel to them. Periodic assessment and refresher training may also be necessary if the procedures are conducted infrequently.[1]

In this article, we share a few examples of challenges that occurred at Insmed, Inc. (Bridgewater Township, NJ, US) during inhalation pharmaceutical development, how we diagnosed the issues and the steps that we took to improve our confidence in execution of those outsourced studies. For activities where we could forecast a long-term workload demand, we brought those specific functions in-house to improve the quality and timeliness of execution. In other situations, we continued to outsource activities but invested in the relationship with the CRO and provided ongoing training.

Bringing the bioanalytical function in-house

Initially, Insmed did not have its own biological sample analysis laboratory, as this would have required an upfront investment in costly equipment (such as tandem mass spectrometry) and personnel with expertise to develop the analytical methods and operate the equipment. Therefore, our historical preclinical studies were outsourced for analysis to avoid that significant start-up expense.

However, a downside became apparent: the timelines from contract signing with the bioanalytical CRO, to development and qualification of the methodology, followed by subsequent analysis of the biological

samples, to receipt of the final report were often longer than in-house timeframes would have been if we had the internal capability to conduct those activities. For example, if the project team had developed multiple formulations for evaluation in an inhalation pharmacokinetic (PK) study, they would have to wait for one CRO to conduct the *in vivo* study and ship the biological samples to a second CRO, and then wait for the second CRO to quantify the levels of drug in the tissue and blood samples in order to determine whether any of the formulations had the appropriate release profile, or if the release profile of a given formulation would have to be modified before moving into pre-clinical efficacy studies. Also, if the PK results were variable or inconsistent, investigation and troubleshooting would need to be initiated, which would further delay the start of the efficacy study. Therefore, outsourcing the bioanalysis activity would become a bottleneck and result in a delay in the advancement of our research programs.

For these reasons, Insmed decided to develop internal capability in biological sample analysis. Ever since the laboratory was established, sample analysis time has been greatly reduced. When the project team questions the veracity of data, there is immediate and direct contact between the analytical and research team members, allowing for an expedited investigation effort, leading ultimately to resolution. Currently, all biological sample analysis for non-GMP (good manufacturing process) studies are done by our own bioanalytical laboratory. (Figure 1).

In-house benefits

Having internal bioanalytical capability has also resulted in more rapid resolution for issues that were perceived to be associated with the analytical methodology. For example, Insmed is developing novel lipoglycopeptides (LGPs) that may have superior antibiotic properties against planktonic, intracellular or biofilm infections of methicillin-resistant Staphylococcus aureus (MSRA) in the lung.[2] We conducted an inhalation study of one of these novel compounds, LGP-A, in healthy rats to assess the pharmacokinetic profile and we quantified formation of its metabolite. Based on the results from several animals, the concentration of the metabolite generated over the PK time course was higher than the dose of drug initially deposited in the lung, which was an impossible result. The investigation of the methodology provided assurance that the problem was not related to ion suppression or incomplete extraction of LGP-A from the tissue samples. A remaining possibility was that the reference standard for LGP-A and/or its metabolite were unsuitable. This could arise if the reference standard contained impurities or was compromised in some other way. To address this situation, our synthesis group manufactured new, higher-quality, supplies of reference standards of LGP-A and its metabolite and the resulting reanalysis of the PK samples using the new standard curves led to a complete resolution of the mismatch.

Figure 1

Evaluation of bioanalytical samples at Insmed, using a Sciex LC-MS/MS (Concord, Ontario, Canada)

Another example of rapid issue resolution occurred in a study of INS1009, a prodrug of treprostinil that is in development for treatment of pulmonary arterial hypertension.[3] Due to its short half-life, the approved inhaled formulation of treprostinil (Tyvaso®, United Therapeutics) is administered four times daily; moreover, side effects are often dose-limiting. Therefore, a long-acting prostanoid analog like INS1009, which maintains the positive attributes of treprostinil and is amenable to once-daily or twice-daily dosing, but has fewer treprostinil-related side effects, would be of clinical benefit. In a Phase I study in healthy human subjects, a lipid nanoparticle formulation of INS1009 had a lower plasma treprostinil C_{max} and fewer respiratory-related side effects compared to inhaled treprostinil.[4]

We have used an internally developed liquid chromatography–mass spectrometry (LC-MS/MS) method over a two-year period to support the INS1009 research program and the methodology has been robust. However, in a recent study it was observed that the MS signal for a newly prepared standard curve was about 10 times lower than historical observations, resulting in concentrations of INS1009 appearing ten times higher than projected.

We investigated whether the "apparent" loss in signal was due to a reduction in the performance of the mass spectrometer over time by testing the same analyte in solution rather than in the biological matrix. After confirmation that the mass spectrometer was working properly, we evaluated whether the signal loss might be coming from the specific lot of lung tissue used as a matrix for preparing the standards. Ionization enhancement or suppression can occur when compounds that co-elute with the drug interfere with the ionization process in the MS detector and can dramatically affect the sensitivity of the target analytes. We resolved this issue by re-preparing the standard curve in a more appropriate lot of matrix.

The common link between these two "standard curve" examples is that by having our chemistry, analytical and bioanalysis teams together in a single facility, the flow of communication was streamlined and we were able to expedite trouble-shooting efforts and resolution of the issues.

Aerosol exposure studies

To assess the pharmacokinetics in animal studies of a drug intended for deposition in the lung, some of the first questions are which animal model to use and how to deliver the drug.[5] The larger the animal species, the greater the amount of drug required to achieve a target dose relative to the body weight or lung weight. This can be mitigated somewhat by conducting the initial studies in mice or rats. When drug supplies are limited, intratracheal (IT) instillation or insufflation is often the chosen administration method. With IT administration, one has the added benefit of knowing the exact dose delivered to the rodent. However, a major downside to instillation is that "flooding" the lung with the formulation does not replicate the deposition pattern from inhalation of aerosols.

In contrast, the use of nose-only inhalation chambers to deliver aerosols to rodents can better simulate deposition of aerosols in the lung but has the downside of requiring much higher quantities of drug than IT administration. Typically, each rodent may inhale less than 1% of the dose loaded in the nebulizer(s).[5] In addition, most of the inhaled dose deposits in the nasal regions, leading to a lower and less consistent dose to the lung. The regulatory agencies typically utilize assumptions of a 10% deposition fraction (i.e., 10% of the inhaled dose deposits in the lung) for mice and rats.[6] Furthermore, it is important to recognize that differences still exist when trying to bridge to humans. Rodents are obligate nose breathers and the nasal and respiratory tract dimensions in rodents are smaller, leading to greater upper airway deposition. Therefore, the use of smaller particle aerosols can improve lung deposition efficiencies in rodents.[5]

In many of our initial PK and preclinical inhalation efficacy studies, we utilize a nose-only inhalation chamber (Figure 2). In order to estimate the dose delivered to the rodent, the theoretical aerosol concentration can be calculated based on the total drug amount aerosolized, the air flow rate provided to the system and the time of aerosolization. As a confirmation, the actual aerosol concentration at the port can be determined by collecting the aerosol delivered to the chamber on a filter for a specified period of time and quantifying the drug deposited on that filter. The ratio of the measured aerosol concentration to the theoretical aerosol concentration is an indicator of the chamber efficiency. Based on the design of the nose-only chamber and the properties of formulation, the chamber efficiency can vary widely from one set-up to another but never reaches 100%, due to aerosol deposition that occurs in transit prior to reaching the entrance of the nose-only port.[7,8] To provide assurance that the delivery device and methodology achieve the target inhaled dose, we routinely insert a glass fiber filter in one exposure port to assay the deposited drug dose (drawn at a flow rate to mimic the rodent's exposure). In these studies, the concentration of aerosol in the exposure chamber is calculated based on the amount of drug collected on the filter and compared to the expected theoretical target aerosol concentration. In addition, to provide absolute measurements of the deposited dose in the rodent's lung, we sacrifice a subset of the rats immediately after inhalation exposure, homogenize their lungs and assay the amount of deposited drug in the lungs.

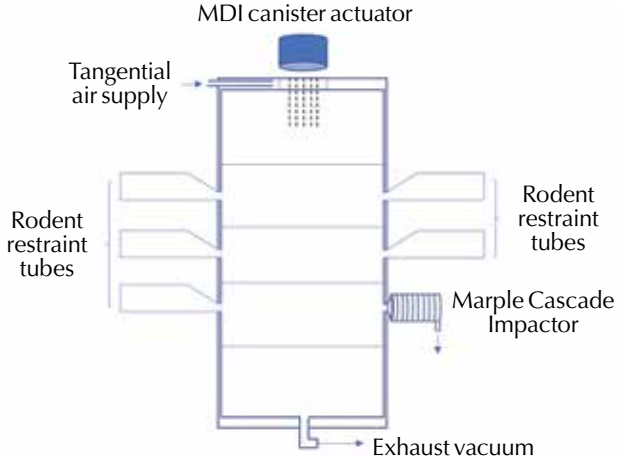
An MDI study at a CRO

We have relationships with CROs and have trained their personnel to conduct nose-only animal inhalation exposure studies in mice, rats and guinea pigs. We typically conduct site visits every 6 to 12 months to discuss any questions or issues with our programs, evaluate their inhalation exposure equipment and provide refresher training. Recently, we asked one CRO to conduct an inhalation exposure and PK study in rats using novel metered dose inhaler (MDI) formulations of INS1009, a drug with which they have experience in both nebulized and dry powder inhaler (DPI) formats. We planned to evaluate two different MDI formulations, one formulated with propellant HFA-227 and one with propellant HFA-134a. The differences in density and vapor pressure of these two propellants, and their interactions with the drug and excipients in the canisters, would be expected to result in changes in aerosol emitted dose, particle size distribution and rates of evaporation.[9]

In the initial study with the first MDI formulation, the chamber efficiency was low; ~15% based on the recovered dose from the filter. The amount of drug recovered from the rat lungs immediately post-exposure was measured and found to be consistent with the dose cal-

Figure 2

A schematic of an MDI nose-only exposure system
(In some experiments, a collection filter is used instead of, or in addition to, a Marple Cascade Impactor.)



culated from the filter. This suggested that the aerosol exposure to the chamber may have been low, possibly due to incorrect aerosol flow rates into the chamber or poor aerosol generation from the MDI. This issue was discussed with the CRO but did not result in an identification of the root cause. Therefore, we conducted a site visit to examine the equipment and procedures. During the visit, we determined that one of the key parameters, the vacuum flow rate of the chamber, was set incorrectly during the preparation of the experiment. After resetting this parameter correctly, we conducted the exposures with the same MDI canisters to provide a comparison with the previous data. The following describes the experimental design and results.

Experimental design

Sprague-Dawley rats were introduced into a nose cone exposure system consisting of a flow-through custom-made inhalation tower. The tower has variable sections comprising of an actuator (for a maximum of six canisters), three levels of exposure ports, with each level having 20 exposure ports for the rats, and a base unit (Figure 2). The aerosols from the MDI were actuated into the tower with a tangential air flow of 2 L/min and vacuum air flow of 20 L/min at the base unit. Using MDI formulation A, two or four MDI canisters (in two separate experiments) were actuated into the exposure system simultaneously, at a frequency of 13.3 seconds per shot for a total of 15 minutes. During the 15-minute exposure time, the aerosol was collected for 10 minutes at a vacuum flow rate of 2 L/min, using a Marple impactor that was connected to one of the unused exposure ports of the inhalation tower. The collection substrate on each stage of the impactor was then placed into an appropriate container and shipped under refrigerated conditions to Insmed for quantitation of drug and determination of the aerosol mass median aerodynamic diameter (MMAD).

After dosing was complete, the MDI actuator was turned off and a 5-minute period was allowed before the rats were removed from the chamber. The MDI canisters were weighed to determine how much formulation was emitted during the experiment. To determine the PK profile following inhalation, rats were sacrificed immediately post-dose (IPD) and at various time points following exposure. Rats were anesthetized with 2% isoflurane and 2.0 mL blood samples were obtained by heart puncture. Blood was then transferred into 2.0 mL K2-EDTA tubes. The tubes were centrifuged to separate the plasma, which was aliquoted into three conical tubes and stored at -80°C for analysis of drug concentrations. Lungs were removed from the thorax, cleaned to remove excess tissue, weighed and stored at -80°C for subsequent analysis of lung drug dose at Insmed.

Experimental results

The key MDI formulation characteristics and the exposure conditions are documented in Table 1. The MDI aerosolization-related results are shown in Table 2. For MDI formulation A, when the number of canisters used in the study was increased from two to four, the measured aerosol concentration doubled, increasing from 7.38 µg/L to 15.1 µg/L (Table 2). The theoretical aerosol concentration, based on the formulation concentration and the amount of formulation aerosolized, was 8.32 µg/L and 22.9 µg/L, respectively. Based on the ratio of actual measured aerosol concentration and the calculated theoretical aerosol concentration, the chamber efficiency was 89% and 66%, respectively, much higher than the initial value of ~15%. This result confirmed that the MDI canisters and aerosol exposure procedures were operating effectively.

The drug concentration measured from the rat lung IPD was 2,795 ng/g and 6,322 ng/g, respectively, for MDI formulation A (Table 2). The amount of drug in the lung increased 2.3-fold when the number of MDI canisters was doubled from two to four, close to the expected value. The aerosol MMAD calculated from the deposition in the impactor stages was 2.14 µm and 2.05 µm, with GSD values of 2.02 and 1.99, respectively, for the two experiments (Table 2 and Figure 3).

A second MDI formulation, MDI-B, was also evaluated and data are reported in Tables 1 and 2. The drug concentration in the formulation was increased from ~0.5 mg/mL to ~1.0 mg/mL. Six canisters were used in this study and the exposure time remained the same, at 15 minutes. Consequently, 6-fold and 3-fold increases in aerosol exposure were expected, compared to experiments 1 and 2. The aerosol concentration increased from 7.38 µg/mL (experiment 1) and 15.1 µg/mL (experiment 2) to 45.0 µg/mL (experiment 3), respectively (Table 2), close to the 6-fold and 3-fold expected results. The aerosol MMAD increased from ~2 µm for MDI formulation A to 3 µm for MDI formulation B.

The drug concentration in the lung for MDI formulation B might also be expected to increase by the same factors of 6-fold or 3-fold, relative to MDI formulation A. However, the drug concentration in the lung increased by a greater amount, from 2,795 ng drug/g and 6,322 ng drug/g lung for MDI-A in experiments 1 and 2, to 32,509 ng/g lung for MDI-B, which were approximately 12-fold and 5-fold increases. Therefore, the lung deposition efficiency was approximately twice as high for MDI formulation B as MDI formulation A. This may be due to a difference in the excipients or choice of propellants: MDI formulation A was prepared in HFA-227 propellant as opposed to MDI formulation B, which utilized HFA-134a. These data show the importance of fully characterizing and qualifying the *in vitro* and *in vivo* methodology, whether those activities are outsourced or conducted in-house.

Conclusions

Development of an inhaled pharmaceutical product can be more challenging than with other dosage forms,

Table 1

Exposure Chamber Conditions for the MDI Experiments

MDI Experiment	MDI Formulation*	Propellant	INS1009 Drug Concentration (mg/mL)	Number of MDI Canisters	Aerosolization Time (min)	Total MDI Weight Loss (g)	Actuation Rate per MDI Canister (g/min)
1	A	HFA-227	0.51	2	15	9.73	0.324
2	A	HFA-227	0.51	4	15	19.05	0.318
3	B	HFA-134a	1.01	6	15	24.62	0.270

*MDI-A equivalent concentration by weight is 0.361 mg/g. MDI-B equivalent concentration by weight is 0.841 mg/g.

as it requires design of a formulation with a specific delivery technology in mind. Furthermore, an inhaled pharmaceutical product has many additional performance tests that it must satisfy; e.g., generation of the specified aerosol emitted dose and aerosol particle size distribution. Therefore, the pharmaceutical development program may be more complex and require an iterative process of developing formulations followed by evaluation in preclinical PK and efficacy studies. Identification of mechanisms to accelerate the generation of that data provides a compounding benefit when the process is iterative. At Insmed, we continue to outsource many preclinical PK and efficacy studies but have brought the bioanalysis function in-house to reduce analysis time and improve communication between research project team members and personnel conducting analyses. We continue to invest in our relationships with CROs and are pleased with the high quality and flexible services they provide.

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Table 2

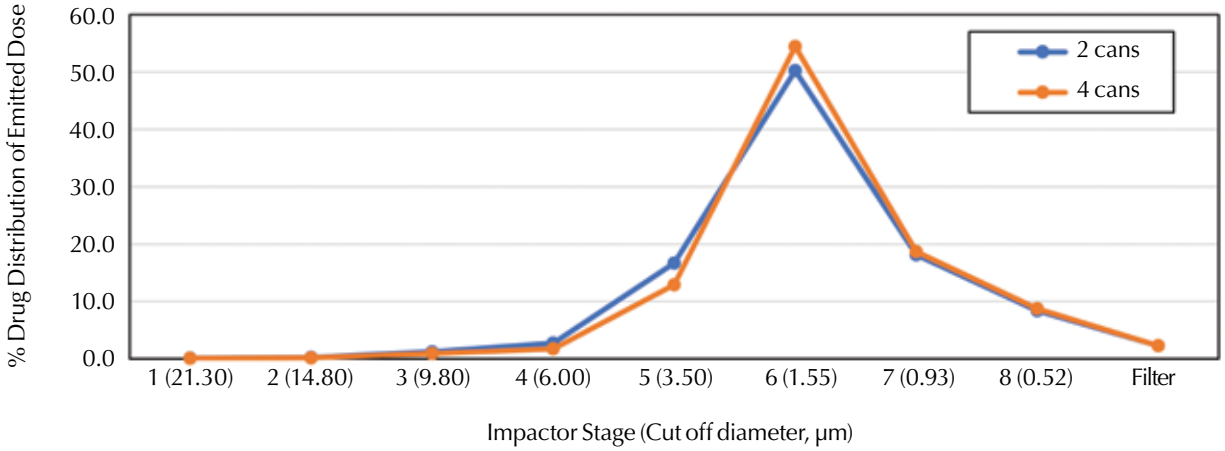
Characterization of the Aerosol Exposure and the Aerosol Particle Size Distribution

MDI Experiment	INS1009 Drug Concentration (mg/mL)	Aerosol Concentration Based on Marple Impactor (µg/L)	Calculated Aerosol Concentration Based on MDI Weight-Actuated (µg/L)	Chamber Efficiency Based on MDI Weight (%)	MMAD (µm)	GSD	INS1009 Drug Concentration in the Lung at IPD* (ng/g)
1	0.51	7.38	8.32	89	2.14	2.02	2,795
2	0.51	15.1	22.9	66	2.05	1.99	6,322
3	1.01	45.0	69.0	65	2.94	1.91	32,509

*IPD = immediate post-aerosol dose

Figure 3

Drug distribution on each impactor stage for two and four canisters of MDI-A



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