Discussion Session Consensus
Dosimetry issues and predicting safety
Understanding PK-PD in the lungs

From workshop held on 10 June 2010
The consensus forming process:

- Six key questions were discussed at the workshop on 10 June 2010;
- Discussion of each question was conducted in 3 parallel workshop sessions allowing all delegates to debate each question in (relatively) small groups;
- The questions were considered by 190 participants (delegates, speakers, workshop organisers) from over 37 organisations (academia, medicine, industry, regulators) from over 10 countries (Europe and USA);

Delegates’ affiliations
Almirall, Apeptico, Applied Research Associates, Argenta, AstraZeneca, Biofocus, Boehringer Ingelheim, Charles River Laboratories, Chiesi Farmaceutici, Covance Laboratories, Domainex, Elpen, Hailam Laboratories, GlaxoSmithKline, Huntingdon Life Sciences, Inhalation Sciences Sweden, Karolinska Institutet, King’s College London, Medicine & Healthcare products Regulatory Agency, National Heart & Lung Institute, Novartis, Nycomed, OINDP Consultant, Pfizer, Philips Respironics, Respiron Consulting, Royal Brompton Hospital, SciLucent, Staffan, St Georges Hospital London, Stewart Erl Associates, University of Athens, University of Bath, University of Bradford, University of Cardiff, University of Nottingham, Vectura.
How are pre-clinical dosing regimens calculated and is this consistent across the industry functions?
1.1 What are the preferred dose metrics?

- There is a terminology issue (and confusion) regarding doses delivered by inhalation, for example: total, nominal, inhaled, deposited, targeted, achieved dose:
  - Nominal (inhaled or delivered) dose is the amount of drug administered,
  - Deposited (achieved) dose is the amount of drug that deposits in the lung;

- It was noted that systemic exposure after inhaled dosing is the sum of the drug absorbed from pulmonary, gastrointestinal and nasal routes dose (i.e. not simply from the dose deposited in the lung)

- There was consensus that the dose delivered to animals in pre-clinical studies and to humans in clinical trials is calculated consistently by:

  \[
  \text{Delivered dose} = (C \times T \times RMV) \div BW \\
  \text{[Equation 1]}
  \]

  \[C = \text{drug concentration, } T = \text{time of exposure, } RMV = \text{respiratory minute volume, } BW = \text{body weight}];\]
Different algorithms for RMV are available, but the one advocated by Alexander and colleagues [Inhal. Tox., 20:1179–1189, 2008] is gaining prominence:

\[
RMV = 0.608 \times BW^{0.852}
\]  

[Equation 2]

Deposited dose is calculated by application of a pulmonary deposition factor (DF) to Equation 1:

\[
\text{Deposited dose} = \frac{(C \times T \times RMV \times DF)}{BW}
\]  

[Equation 3]

Pulmonary deposition factors (DF) are not applicable to all situations, although the FDA use default values (see section 2.1);

Although this represents current standard practice, the use of Equation 3 does not necessarily reflect actual dose deposited:

- There is considerable variability in RMV (up to 40%) which could be caused by either animal variability and/or pharmacology of the inhaled drug,
- The most commonly used DF may be an overestimate (see section 2.1);
Based on current practice and the FDA’s stance on deposition factors, there is sometimes difficulty getting exposure (delivering sufficient drug to pre-clinical species) to cover safety margins (typically 1/6 in dogs and 1/10 in rats);

It was suggested that a maximum dose be introduced for well tolerated compounds to avoid excessive doses. Reasonable upper limits could be based, for example, upon 1 mg/L as an achievable concentration across a number of compounds with maximum dosing times set according to what is appropriate for different species. There is an upper dose limit for orally administered compounds;

Although not required by regulators, a variety of methods were being used to verify dose deposited in the lung (measurement in lung tissue, systemic exposure) and some companies do this routinely. The appropriate method to use depends upon the properties of the compound, such as solubility, absorption rate, oral bioavailability ......;

Lung tissue concentrations were often measured for the purpose of monitoring accumulation during chronic dosing with the aim of using this data to interpret toxicity;
• For studies later in the late discovery phase, aerosol delivery is preferred in both efficacy and toxicity studies as the deposition pattern is more like the clinical situation. Nebulisation is generally used to produce aerosols in these studies;

• As lung concentration will vary with time during dosing (i.e. during the nebulisation period) there was a question regarding when the lung dose should be sampled and how the data should be interpreted, especially for drugs that are rapidly cleared;

• There are limits for aerosol particle size for inhalation safety studies. The FDA expect a mass median aerodynamic diameter (MMAD) of less than 4-5 µM and would consider particles larger than 8-10 µM to be inappropriate for pre-clinical species (i.e. studies are rendered invalid if particle size is too large and therefore unlikely to penetrate and deposit sufficiently in the lung);
• Bolus intra-tracheal dosing (spray or instillation) may be used as an alternative to aerosol delivery for efficacy studies for early safety screens. The advantage of bolus methods is ease of administration and, especially in early studies, the use of small amounts of compound. Bolus methods are also increasingly being used to evaluate the systemic toxicity of pulmonary-administered compounds;

• In bolus dosing, the placement of the dosing device with respect to the major bifurcations produces variation in deposition between lung lobes. Measurement of drug in each lobe immediately after dosing can be used to characterise the delivery pattern (dose/lobe corrected for lobe weight or airway surface area).
1.2 How do aerosol properties and deposition affect the deposited dose?

• Most published deposition data in animals is based on insoluble environmental particulates whereas drug substances can behave differently, e.g. be hygroscopic. The processes of nebulisation, inhalation and deposition on pharmaceutical aerosols may introduce deviations from predictions based on insoluble particles;

• It would be valuable to generate data on pharmaceutical aerosols to develop a better understanding of their dosimetry and deposition in pre-clinical species.
1.3 How do we use preclinical dosimetry data to compare to the clinical dosing?

• Preclinical administration methods do not directly reflect the clinical situation. The exposure in humans is often by bolus with special inhalation manoeuvres, whereas in preclinical studies sustained delivery by nebuliser or dry powder generator and tidal breathing is used.

• However, estimated deposited dose and systemic exposure in pre-clinical studies are used primarily to calculate safety cover for first time in man studies (i.e., pre-clinical data is a prerequisite for first in man studies, but once human exposure data becomes available this can guide further human studies).
1.4 Can we do better?

- The use of better data driven estimates to calculate actual clinical doses would begin to address the problem of over-estimation of deposition during inhaled product development. This includes deposition in pre-clinical species and in man, plus data on the efficiency of delivery equipment and devices, etc;

- Cross industry sharing of deposition data in pre-clinical animal species would provide the data upon which more informed estimates of deposition could be made and negotiated for regulatory acceptance.
1.5 CONCLUSIONS

• There is a terminology issue (and confusion) regarding dose, for example: total, nominal, inhaled, deposited, targeted, achieved dose:
  ➢ Nominal (inhaled or delivered) dose is the amount of drug administered,
  ➢ Deposited (achieved) dose is the amount of drug that deposits in the lung;

• Dose is calculated consistently by: Delivered dose = \((C \times T \times RMV) \div BW\)
  
  \([C = \text{drug concentration}, T = \text{time of exposure}, RMV = \text{respiratory minute volume}, BW = \text{body weight}]\);

• The algorithm for RMV advocated by Alexander and colleagues [Inhal. Tox., 20:1179–1189, 2008] is gaining prominence: \(RMV = 0.608 \times BW^{0.852}\)

• Deposited dose is calculated variously by application of a pulmonary deposition factor (DF): Deposited dose = \((C \times T \times RMV \times DF) \div BW\)
Although not required by regulators, a variety of methods were being used to verify dose deposited in the lung (measurement in lung tissue, systemic exposure) and some companies do this routinely.

- The appropriate method to use depends upon the compound, e.g. solubility, absorption rate, oral bioavailability,
- In multiple dose studies, accumulation in the lung may be detected by measuring lung tissue;

There is a need for agreement on upper dose limits for well-tolerated compounds to avoid excessive doses in pre-clinical species;

There is an opportunity to harmonise how dosimetry is accomplished and produce an industry standard for calculating dose delivered and verifying the dose deposited.
Is there a scientific basis to support a more informed approach to guidelines on dosimetry in safety studies?
2.1 Are estimates of deposition reasonable?

- For human deposition there was a unanimous consensus that the FDA assumption of 100% deposition (DF in Equation 3) does not reflect actual deposition;

- For deposition in pre-clinical species there was a mixed response:
  - some people regarded the FDA assumptions of 10% deposition in rats and 25% in dogs (based on Snipes et al., Health Physics 57 (suppl. I): 69-78, 1989) to be reasonable estimates,
  - others felt that these values potentially underestimate deposition (based on anecdotal data). These observations could be a consequence of particular combinations of compound, formulation, equipment, species effects and other variables (see question 1.2). It is possible that methods are too diverse and there may be benefit from a best-practice platform approach,
  - if deposition data for specific animal models / development projects is generated, alternative deposition values may be justified on a case-by-case basis. This opens up the potential for using higher deposition values other than the FDA default values;
• European regulatory bodies are more amenable to considering evidence for animal deposition values on a case-by-case basis, however the FDA would be likely to consider alternative values to their default position if appropriate supporting evidence were provided;

• Clinical doses include safety margins and are based upon pulmonary and systemic toxicity data from pre-clinical studies. Different safety margins may be applied to monitorable vs non-monitorable toxicity. For rapidly absorbed compounds the maximum plasma concentration (for systemic toxicity) will depend principally on the dose deposited in the lung with the proportion absorbed from the gastrointestinal tract being irrelevant;

• Some companies routinely use particle size, determined using cascade impaction, to predict the deposition factor and have validated this against experimental data. There were some caveats to this approach:
  ➢ Skewed size distributions require adjustments to the deposition factor,
  ➢ The *in vitro* methods for aerosol characterisation were developed for quality control purposes and do not reproduce all the factors affecting drug deposition *in vivo*. 
2.2 What deposition models are available, how can we improve current *in silico* models of dosimetry using data in rat/dog/human?

- The discussion on *in silico* models was limited in depth by the lack of direct experience with these models by many of the workshop attendees;

- There was a high level of interest in the potential of deposition models, but reservations regarding their current status in terms of availability of data and validation for drug development applications and regulatory acceptance (e.g., the models have undergone limited development relating to deposition in pre-clinical species);

- The session speaker (Bahman Asgharian) indicated that the human deposition models have been accepted by the US Environmental Protection Agency for environmental safety assessments and suggested that the applicability of the models for human drug safety studies may not be fully realized by the FDA;
• To develop *in silico* models into useful tools to aid the understanding dosimetry and pharmacokinetics, deposition data to validate and confirm the models is required. As individual datasets are small, precompetitive information could be pooled across the industry for this purpose. This may present an opportunity for *in silico* modellers to generate a specification for the data that would help to improve or validate their models - this data may have already been generated but is currently retained within the pharmaceutical industry.
2.3 Is there an opportunity to build data based on drug-like molecules with different physico-chemical properties?

- There was general recognition that there is an opportunity to generate deposition data on a case-specific basis to potentially support more relevant deposition values in animals (see question 1.2);

- There was a suggestion that deposition factors would not only depend on physico-chemical characteristics, but also differences in deposition between mono- and polydisperse aerosols;

- This area was viewed as providing a positive opportunity to collaborate and pool data.
2.4 What clinical data could you collect to avoid assuming 100% deposition in man?

- Greater use of the available imaging techniques as well as integration of appropriate techniques to characterize lung deposition in man (qualitatively and quantitatively) have the potential to improve our understanding of deposition;

- It was questioned whether data would be sufficient to change regulatory assumptions, which are based primarily on philosophical safety concerns. However, appropriate data will be a prerequisite for opening a dialogue on the current regulatory position, which will otherwise remain unchanged;

- Recovery of drug from the dosing apparatus for subtraction from the nominal dose delivered was suggested. This correction for device efficacy would avoid the non-emitted dose being incorporated into dosimetry calculations;
• It was noted that the impact of reducing the deposition factor in man on clinical dosing would be limited. For example, a 50% deposition factor would only increase the dose 2-fold, i.e. would not have significant impact on efficacy considerations in many projects;

• However, a change in this component combined with other changes in the evaluation of the combined toxicology package (e.g. pre-clinical dosimetry) has the potential to result in a significant cumulative impact on acceptable clinical doses.
2.5 Can estimates of dose based on blood concentration be used?

- Blood concentration is one of the most easily measured surrogates and this data is routinely collected. Systemic pharmacokinetics are used to try to gain understanding around safety and efficacy in the absence of being able to measure concentration directly at the target site;

- The area under the plasma concentration curve (AUC) can provide an estimate of dose. However, a number of caveats related to lung clearance were noted:
  - absorption of swallowed drug via the gastrointestinal tract should be accounted for (or potentially blocked using charcoal);
  - nasal absorption will also occur in pre-clinical species. This cannot be differentiated from pulmonary absorption and can only be prevented by oro-tracheal dosing;
  - mucocilliary cleared particulates will not be 'counted' as being lung deposited using an area under plasma concentration curve approach;
• Pre-clinical doses are often achieved by modifying the duration of exposure (T in Equation 1) and different durations of exposure may lead to variable pharmacokinetics;

• The formulation and solubility of compounds may affect pharmacokinetics; but not the relationship between systemically available dose and area under the plasma concentration curve (unless the formulation affects the oral fraction absorbed);

• Accumulation of soluble dose in the lung can be evaluated as this is mirrored by related increases in blood Cmin (i.e. concentration measured immediately prior to the next dose).
2.6 CONCLUSIONS

- Data-driven estimates of deposited dose provide more rational scaling of doses (taking into account safety margins) between pre-clinical safety studies and first-in-man human safety studies;

- The assumed deposition factor (100%) for human dosing is an overestimate, but a lower value would not increase the dose permitted in clinical trials greatly (e.g. a deposition factor of 50% would only provide a 2-fold increase in dose);

- The deposition factors used by the FDA for pre-clinical studies (10% in rat; 25% in dog) are not entirely unreasonable, but there is scope for using actual data to improve dose estimates across the industry;

- FDA and MHRA approaches to dosimetry in safety studies differ in terms of expectations regarding deposition factors;

- In vitro aerosol data (i.e. aerodynamic diameter) is regarded as helpful for predicting deposited dose and is used by some for this purpose.
QUESTION 3

What measurements can be used consistently across the industry to establish safety or toxicity to allow regulatory bodies to assess new chemical entities?
3.1 What measurements and criteria should be used to establish pulmonary safety or toxicity?

- Histopathology is the primary endpoint of Good Laboratory Practice (GLP) toxicology studies supporting clinical trials. Within these studies, major issues were:
  - Are pathologies consistently defined and classified?
  - What is the background range of normal biological variation?
  - How can an adverse reaction be discriminated from a normal response?

3.1.1 Is there value in sharing control data across the industry?

- The consensus regarding this question was yes. However, this would require consistent measurement and interpretation. The value of sharing control data would be in generating larger data sets to aid evaluation of study data against a background of normal biological variation;

- This would be useful for the regulators and was of interest to industry, although the logistics of such an exercise were viewed as a barrier.
3.1.2 Are measurements consistent across the industry?

- Histopathological procedures are standard. Terminology is the greatest source of variation, which is being addressed (e.g. INHAND, see www.goRENI.org). There should be standard definitions recognised by specialist, non-specialist pathologists and regulators. By their nature, histopathological measurements and severity ratings are semi-quantitative.

3.1.3 Are supplementary endpoints useful?

- These should be considered on a compound by compound basis (and if pharmacodynamic end-points are available these should be used);

- Endpoints being measured included cell infiltrates and cytokines in bronchoalveolar lavage. These were measured more in early, acute and repeat dose studies. Pulmonary function is a safety pharmacology end-point;

- It was agreed that better biomarkers in bronchoalveolar lavage or blood are required.
3.1.4 what are adverse vs non-adverse endpoints?

- Identification of adverse end-points is an area where having better control data would help discern true adverse effects vs normal lung response (although this will also depend upon a common approach to recording findings). The control data includes sham, vehicle and particle controls;

- There was debate regarding what clinical responses to inhaled particles are acceptable;

- Benchmarking to known toxicants (plus currently used inhaled products) was suggested as an approach to discerning what are adverse versus non-adverse effects and providing some validation of these markers;

- It was noted that methods for monitoring of toxicity in the clinic (biomarkers) would enable dose escalation where there are safety issues.
3.2 Can acute studies be used to predict chronic outcomes?

• Acute studies were regarded as useful for toxicity screening;

• However, such studies have limited ability to predict subtle effects over the long term. This is an area where better bronchoalveolar lavage markers and other supplementary end-points would be useful (see question 3.1.3).
3.3 To what extent are lung kinetics measured across the industry?

- Lung kinetics were considered in relation to toxicity testing (different aspects of pharmacokinetics are considered in sections 4-6).

3.3.1 How are such data utilised?

- Lung kinetics require the measurement of drug concentration in lung. This was reported to be measured either (i) not at all, (ii) immediately after dosing or at the end of study, (iii) at various time points;

- Data obtained immediately after dosing is used principally to verify dose. Samples collected at end of the study are used to assess accumulation. For more detailed toxicokinetic analysis, a number of time points are required.
3.3.2 Can we interpret toxicokinetic measurements of lung or plasma concentrations or accumulation?

- Drug quantification in the lung is useful for determining dose proportionality and interpreting histopathology, particularly if there is accumulation in lung.
3.4 CONCLUSIONS

• The terminology used to identify, describe and quantify histopathology requires harmonisation - there are recent initiatives towards doing this;

• Analysis of pooled control data from inhalation safety studies would be useful to establish normal background biological variation (it is recognised that there is also inter-lab and regional variability). Regulators would welcome such a move - there was some interest from industry, but also reservations;

• Various toxicity end-points are being measured. There are issues regarding what changes are adverse and what can be considered non-adverse and there is a lack of validation. Benchmarking using known toxicants and approved products may be useful to define markers of adverse effects;

• In contrast to systemic toxicity, methods for monitoring respiratory toxicity in man are lacking. The development of such methods would enable dose escalation in the clinic when there are safety questions.
How can we study and understand pulmonary pharmacokinetics?
4.1 Are we more interested in formulation effects or intrinsic pharmacokinetics?

- There is interest in both the intrinsic pharmacokinetics and the effect of formulations on pharmacokinetics;

- The effect of formulation and material properties is of great importance for low solubility drugs. Examples were also described where formulation effects were observed for soluble compounds.
4.2 Is the isolated perfused rat lung being used?

• Some use of the isolated perfused lung (IPL) was described, but this was not consistent across either industry or academia. Where the technique was used, it was highly valued for studying mechanistic phenomena;

• The main applications of the IPL were for understanding drug disposition and effects of formulation on pharmacokinetics. More limited use to study drug action was also evident:
  ➢ pharmacokinetics. (i) variation in absorption rate depending on the physicochemical properties of drugs, (ii) effects of formulation and material properties (different salt forms, crystalline polymorphs, suspension vs dry powder) on drug absorption, (iii) evaluating functional impact of drug transporters in lung,
  ➢ efficacy: measures of drug action in different small animal species,
  ➢ pharmacokinetic-pharmacodynamic studies: simultaneous measurement of pharmacology (bronchoconstriction and vasoreactivity) and drug concentrations in perfusate and airway lumen;
Drug administration techniques are critical to mimic clinical dosing for mechanistic studies in the isolated perfused lung:

- respirable aerosols are clearly preferable to instillation procedures,
- concentrated bolus exposures are preferred for better resolved pharmacokinetics;

The use of the single pass perfusion mode was regarded as the most appropriate design for the majority of applications;

Caution may be required in interpreting some pharmacodynamic end-points in the model, which is devoid of innervation and has curtailed bronchial perfusion.

### 4.3 What are the alternative techniques?

- The general method for studying pulmonary pharmacokinetics was *in vivo* delivery to the lung (intra/oro-tracheal administration, nose-only inhalation) with complementary intravenous administration in pre-clinical species;

- The IPL is also used for pharmacokinetic studies (see question 4.2), but other *in vitro* methods such as cell culture were considered less valuable.
4.4 Are transporters relevant for pulmonary PK?

- The consensus was that there is limited data available on functional role of transporters on the lung and their relevance for pulmonary pharmacokinetics is currently unclear;

- In terms of transporter expression, the presence in the lung of different solute carriers (SLC/SLCO) and ATP-binding cassette (ABC) transporters has been demonstrated;

- There is assorted (sometimes inconsistent) functional data for pulmonary transporters different experimental models. It was generally agreed:
  - p-glycoprotein (P-gp) expression is lower in lung than other barriers (e.g. intestine),
  - transporters (especially uptake) appear to be most important for low passive permeability drugs;
• It was suggested that transporters may be of importance for regulating local concentrations of drugs in subcompartments of the lung. More protein and immunohistochemistry data is required to identify transporter distribution in different regions of the lung, different cell types and to discriminate between mucosal and serosal localisation;

• The question “is there is a role of transporters in the compound accumulation in macrophages?” was unanswered;

• There was a clear need to extend our knowledge before consideration of transporters can be factored into respiratory drug discovery and development programmes;

• There was little industry activity looking into the role of transporters in pulmonary pharmacokinetics, but some ongoing interest in academia;
4.5 Is there *in vivo* evidence for the influence of transporters?

- Study design is crucial for pinpointing particular transporter mechanisms. It is difficult to demonstrate activity *in vivo* with all the confounding factors. The use of transgenic animals may be a way forward;

- Due to the rapid absorption for most compounds, efflux transporters are probably of less functional importance for absorption into blood from the lung than in other barriers, although this merits further investigation;

- Functional impact is emerging, but there is little *in vivo* evidence, for example:
  - Cell models: vectorial transport effects attributed to organic cation uptake transporters (OCTNs) have been measured for beta-agonists,
  - *Ex vivo*: rhodamine transport inhibition by P-gp inhibitors in the isolated perfused lung has been reported,
  - *In vivo*: a single undisclosed example of a significant transporter effect in a pre-clinical model during pharmaceutical development was mentioned.
4.6 CONCLUSIONS

- There is interest in the intrinsic pharmacokinetics and the effect of formulations (and delivery vehicles) on pharmacokinetics;

- The isolated perfused lung is being used in industry and academia, but not for routine data collection. The main applications are for mechanistic studies on drug disposition and to investigate the effects of formulation on pharmacokinetics;

- Generally, pharmacokinetic studies are conducted in vivo utilising delivery to the lung (intra/oro-tracheal administration, nose-only inhalation) with complementary intravenous administration in pre-clinical species;

- There is little industry activity looking into the role of transporters; there was a single undisclosed pre-clinical example of the impact of transporters in the lung. The relevance of transporters in the lung is currently unclear.
In which biophase(s) should we measure drug concentration in order to determine pharmacokinetics?
5.1 In what biophases are we measuring drug concentrations for pharmacokinetics and why?

- Drug concentrations are measured in blood / plasma and also, increasingly frequently, in bronchoalveolar fluid and lung tissue. The critical issue is whether the biophase measured reflects the free drug concentration at target.

5.1.1 Blood / plasma

- There was less confidence that blood levels are reflective of those in the lung for drugs delivered by inhalation compared to intravenous or oral administration. For inhaled delivery:
  - the blood is downstream of the lungs and there is the potential for drug loss in the lung by multiple non-absorptive mechanisms.
  - unless blocked, there may be a contribution to systemic exposure arising from the swallowed component of the dose;
• Blood is routinely sampled for drug concentration in pharmacokinetic studies (see section 2.5). Blood concentrations are commonly very different from lung concentrations after inhalation;

• Although pharmacokinetics in blood may reflect lung pharmacokinetics, depending on the properties of the drug, there was no consensus whether this is generally applicable.

5.1.2 Lung tissue

• Lung tissue that is collected for pharmacodynamic evaluations may also be sampled, homogenised and analysed for drug concentration;

• Drug is often quantified for proof of dosing purposes (see section 1.1);

• Lung tissue is sometimes sampled specifically for pharmacokinetic purposes. Concentration of drug in lung tissue over time provides an indication whether the compound is highly retained or disappears rapidly into blood;
• There are a number of issues regarding drug measurements in lung tissue:
  ➢ the measurement provides an average drug concentration in the lung, making it impossible to interpret in terms of localised concentrations,
  ➢ measurements are not necessarily reflective of either free drug concentration or the dose available at the target in the intact tissue,
  ➢ microdissection of lungs to localise deposition has been attempted and may be of more value than an average concentration in lung homogenate - the isolation of individual lobes for assessment of lung concentration was suggested. Imaging techniques, e.g. imaging mass spectrometry, may also provide an insight into the distribution of drugs in different compartments,
  ➢ there was uncertainty regarding how best to use lung tissue data for pharmacokinetic-pharmacodynamic modelling. There is little literature evidence of any work being conducted in this area.

5.1.3 Bronchoalveolar lavage (BAL)

• Routine collection for pharmacodynamic end-points affords the opportunity to measure drug concentrations for pharmacokinetics purposes;
• There were a number of issues regarding drug measurement in BAL:
  ➢ there is no standard methodology, although 3 x 5 ml in rat lung appeared to be used by a number of companies,
  ➢ the fluid used for lavage varies and may affect measurements (e.g., the efficiency with which macrophages are recovered),
  ➢ reproducibility is impaired by an inability to recover completely instilled lavage fluid (although urea can be used as an internal standard),
  ➢ there is a question whether bronchoalveolar lavage concentrations are reflective of the free airway drug levels that are driving pharmacodynamic responses (i.e. BAL may solubilise suspension particles, dry powder; etc);

• The consensus was that collecting BAL drug concentration data is becoming commonplace. Unlike cellular changes in BAL which may be indicative of early lung responses to inhaled material, there is often uncertainty in how to interpret drug concentration which is limiting its use in decision-making;

• Despite its limitations, BAL is obtainable both pre-clinically and clinically and could therefore prove in the future to be a useful translational tool when comparing pre-clinical and clinical pharmacokinetics and PK-PD.
5.2 How is PK being used to assess PK/PD?

- Physiological / pharmacological models were favoured over empiric models;

- Although pharmacokinetic measurements are made routinely, application and interpretation are difficult and therefore limited to date;

- Drug concentrations measured in the terminal samples of pharmacodynamic studies were noted to be of limited value for pharmacokinetic-pharmacodynamic analysis due to the lack of a temporal component;

- Imaging techniques (e.g. gamma scintigraphy, PET, Maldi) are applicable to pharmacokinetic analysis, but are more often used to confirm dose location;

- Microdialysis was regarded as problematic. It is difficult to sample airway fluids (intra-tracheal probe placement, blood contamination, poor reproducibility/contact with fluids) and results may not reflect luminal concentrations.
5.2.1 What about toxicokinetics?

- Drug concentrations are sampled as a proof of dosing rather than to establish relationships to pharmacodynamics or toxicity endpoints (see sections 1.1, 3.3, 5.1.2);

- Drug concentration in the lung is sampled when there is a cause for concern, such as possible inappropriate accumulation, e.g. insoluble particles;

- It is not standard practice to sample lung concentration in toxicokinetic experiments, although blood is routinely collected. The value of sampling lung tissue was questioned as lung toxicokinetics are not a requirement for regulatory submissions. Others felt that such data may help to understand the safety profile of the molecule;

- Where collected, toxicokinetic analysis is performed to try to relate accumulation to toxicity for feedback into the drug discovery & design process.
5.3 Are systemic kinetics a surrogate for lung kinetics?

- Blood is the biological matrix sampled in the clinic and therefore presents an opportunity for data collection if it proves to be a good surrogate for the inhaled compound;

- Theoretically, pharmacokinetic modelling based on blood measurements could be useful for soluble inhaled compounds (see sections 5.1.1);

- To interpret systemic pharmacokinetics following inhaled drug delivery, intravenous dosing data is required and data on oral bioavailability may also be necessary for accurate interpretation.
5.4 CONCLUSIONS

• Measurement of drug concentration in the lung (e.g. in lavage or lung homogenate) is increasing. This is partly for proof of dosing, but also for understanding how well the compound is retained in the lung;

• Broncho-alveolar lavage (BAL) measurements bring technical challenges and the data are difficult to interpret (i.e. assumptions and correction factors needed to relate BAL measurements to drug concentration in epithelial lining fluid);

• Measurement of drug in ex vivo homogenised lungs will not discriminate between undissolved drug and drug in solution. For readily soluble drugs, total lung concentration has been used successfully to build pharmacokinetic-pharmacodynamic relationships (although total lung concentration does not always correspond directly with in vitro estimates of potency);

• In general, pivotal studies still focus on measurement of blood concentrations, whereas drug concentrations in the lung are more typically measured in early studies looking for understanding of inhaled drug disposition.
What approaches are being used to investigate pharmacodynamics in the lung?
6.1 Are pharmacodynamic approaches being used actively in pre-clinical projects?

• There was positive consensus that for inflammation and bronchoconstriction appropriate pharmacodynamic endpoints can and are being evaluated:
  ➢ Inflammation: inflammatory markers in lung lavage,
  ➢ Bronchoconstriction: airway resistance measurements;

• eNO- had been assessed by one group, although the usefulness of the data was open to interpretation;

• Receptor occupancy studies (in vivo or ex vivo) have the potential to provide useful information for lung-targeted drugs;

• It was agreed that there was often no direct link between biomarkers collected in pre-clinical studies versus the clinic. There was concern that measuring different biomarkers pre-clinically versus clinically may not provide appropriate translation and makes it difficult to feed back to pre-clinical studies.
6.1.1 Do they provide decision-making data?

- Pharmacodynamic models provide decision-making data within projects;

- Understanding lung driven efficacy vs systemically driven efficacy of the absorbed dose was generally felt to be achievable. However the methods for this were not described.
6.2 How do we overcome model vs disease state differences for PD and dose prediction?

• The lack of understanding of the disease pathology in acute and chronic pre-clinical models and human disease makes this a difficult question to answer;

• The assumption was that rat and dog were commonly used for precededent approaches, although it is unclear which is most appropriate. However, translation to human is difficult regardless of the pre-clinical model in use;

• For precededent mechanisms there was consensus that dose could be adequately predicted by benchmarking against clinical agents and disease models were less of a concern;

• This situation differs from oral drug delivery approaches where there is more confidence that drug exposure is achieved at the target site.
6.3 Do these approaches enable quantitative translation to human?

- There was some debate whether pharmacodynamic approaches enable quantitative translation from pre-clinical studies to human; one person commented that this had been achieved in a project involving a precedent mechanism;

- In general, translation from pre-clinical studies to human studies is only possible for drugs with precedent rather than non-precedent mechanisms. The best approach to execute projects successfully for drugs with unprecedented mechanisms is unclear;

- It was questioned whether the duration of studies is always sufficient to ensure that the desired outcome is being assessed appropriately;

- The lack of appropriate tools available for pharmacokinetic-pharmacodynamic studies leads projects into clinical studies prematurely.
6.4 How can we best ensure we have tested the pharmacology in the clinic?

• It was accepted this is difficult to achieve;

• Nebulised administration is considered by some to provide reassurance that lack of efficacy is not formulation driven. However, solubility is key limitation of this approach (i.e. poor solubility in nebuliser solutions);

• Benchmarking to comparators may aid dose projection and provide some confidence in clinical efficacy;

• There is a lack of tools / understanding of the best method to ensure that the pharmacology has been tested in clinical studies.
6.5 CONCLUSIONS

• Pharmacokinetic (PK)-pharmacodynamic (PD) experiments are conducted, but mainly on precedent mechanisms (inflammation and bronchoconstriction) and often with a limited temporal component linking PK-PD;

• The development of pharmacodynamic approaches are limited by the limited availability of disease models, biomarkers and short study duration. The lack of appropriate tools leads projects into clinical studies prematurely;

• Translation of projects into man is a major challenge, especially for unprecedented mechanisms;

• A primary concern for studies with negative outcomes is that the pharmacology is not being tested in the clinic (i.e. that insufficient drug concentrations are being delivered to target sites). There are a lack of research tools, such as imaging techniques, available to provide assurance that suitable concentrations are achieved. This is an area that needs development.
What can we do to address common challenges in the discovery and development of inhaled medicines?
7.1 Opportunities for collaborative research

- A number of areas were identified where progress is limited by gaps in understanding or lack of appropriate techniques or models. Collaborative research may help to address these common problems.
  - Benchmarking for toxicology: use known irritants and marketed medicines to discriminate adverse from non adverse findings,
  - Imaging techniques. To measure precededented molecule receptor occupancy and help understand the relevant biophase for pharmacokinetic modelling,
  - Lung fluid: Characterisation to establish solubility parameters for modelling formulation effects on pharmacokinetics,
  - Dosimetry: use of imaging to assess dosimetry, impact of delivery devices on dosimetry with the aim of harmonising dose calculations.

- This will require research funding. This cost may be mitigated by the formation of consortia and the leverage of research council funding.
7.2 Opportunities for pre-competitive data sharing

- A number of areas were identified that may be addressed by the sharing of exiting pre-competitive data without incurring additional research costs:
  - Deposition: sharing of existing deposition data in pre-clinical species (e.g., fluorescent bead data),
  - Dosimetry: provision of data for development of \textit{in silico} models,
  - Safety end-points: sharing of toxicological control data to establish normal biological variation and normal responses to inhalation exposure,
  - Pharmacokinetics: pooling of data to link physicochemical properties and inhaled pharmacokinetic profiles.

7.3 “Drugs in the Lungs” Network

- The organising committee encourage any activity in these areas and will be pleased to provide a platform for reporting progress at future meeting. The committee will also support and facilitate any such activities and invite expressions of interest however preliminary these may be (please contact: ben.forbes@kcl.ac.uk).
The consensus was compiled by:

- Ben Forbes*, King’s College London
- Bahman Asgharian, Applied Research Associates
- Lea Ann Dailey, King’s College London
- Douglas Ferguson, AstraZeneca
- Per Gerde, Karolinska Institutet
- Mark Gumbleton, University of Cardiff
- Colin Hardy, Huntingdon Life Sciences
- David Hassall, GlaxoSmithKline
- Lena Gustavsson, AstraZeneca
- Rhys Jones, Pfizer
- Ruth Lock, Novartis
- Janet Maas, Novartis,
- Tim McGoverin, SciLucent
- Gary Pitcairn, Pfizer
- Graham Somers, GlaxoSmithKline
- Ron Wolff, Novartis

*Communication to: ben.forbes@kcl.ac.uk
Final: 03 August 2010