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INVITED ABSTRACTS

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What is the discriminating measurement actually capable of discriminating?

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Conventional methods of analysis used to discriminate a good lot from a bad lot are focused on the unit dose of the product. Although such a measurement does provide a "yes" or "no" answer in terms of a specification, the analysis, because of the averaging nature of the sampling, provides minimal "product and process understanding." In the emerging era of Design Space, where process understanding is key, the use of the conventional means to discriminate quality from lot to lot has to be questioned. The use of emerging technologies that look at a sample at the micro level, and discriminate lots using the information rich matrix patterns, should be considered as better quality control tools, than those in practice at this time. This paper will seek to illustrate this point with some case examples.

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Spin doctors: meeting clinical need and biological product development

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There is a basic tenet in Endocrinology that optimum hormone replacement should mimic physiology. However, most hormone preparations provide unphysiological hormone profiles. To address this issue our group has undertaken research in two areas: 1) developing glucocorticoid replacement therapy in patients with adrenal insufficiency, and 2) developing long-acting growth hormone agonists for hypopituitary patients. Patients with adrenal insufficiency lack the normal circadian rhythm of cortisol, which rises during the night, peaks in the morning and drops at the time of going to bed. We have shown that current immediate release hydrocortisone can replace cortisol levels during the day but requires thrice daily administration and patients are still missing the night-time rise in cortisol levels (Mah et al 2004). These patients have to take an inconvenient, un-physiological treatment and still have a poor quality of life. We have been addressing this problem through the development of circadian cortisol therapy. Provisional studies using hydrocortisone infusions have demonstrated that circadian cortisol profiles provide better biochemical control of adrenal insufficiency (Merza et al 2006). Based on these studies Diurnal Ltd, a University of Sheffield spinout company, in collaboration with Phoqus Plc have developed a modified release formulation of hydrocortisone that provides a circadian cortisol profile. A clinical development programme is now being undertaken through the orphan drug route. In hypopituitarism patients lack many endocrine hormones including growth hormone. Growth hormone replacement is now recognised to be important in both promoting childhood growth and maintaining normal adult body composition. We routinely replace patients with growth hormone as adults. However, growth hormone replacement requires daily injections, which are both expensive and inconvenient. Developing long acting biologicals has been a major challenge for the pharmaceutical industry. Asterion Ltd, a University of Sheffield spin out company, has been tackling this question by making fusion proteins with growth hormone. A recent molecule looks to have a very attractive pharmacokinetic profile and data will be presented on the possibilities of a 3rd generation of long acting cytokine agonists. From the clinicians perspective the future in hormone replacement therapy is optimising physiological treatment to preserve future health.

Mah, P. M. et al (2004) *Clin. Endocrinol*. **61**: 367–375
Merza, Z. et al (2006) *Clin. Endocrinol. (Oxf)* **65**: 45–50

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Risk mitigation in formulation development

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To exert their effect, biopharmaceuticals require the delivery of the native, biologically active molecule to the site of action. However, their labile nature, poor bioavailability and frequently unfavourable pharmacokinetics often obstructs further development and presents considerable risk to successful commercialisation. To maximise the probability of success, a rational formulation development strategy is required which identifies and addresses the major risk factors. To mitigate risk, the aim should be to develop the simplest, stable formulation possible which meets the target product profile. Freedom to operate and regulatory issues should be considered from the outset and incorporated into the development strategy to reduce the risk in the later stages of development. In designing the formulation development strategy, consideration must be given to the clinical indication, pharmacokinetics, toxicity, physicochemical stability and ultimately the target product profile. Central to this is building a solid foundation of pre-formulation studies. Thorough analytical development, using a range of techniques, is essential to identifying the principle threats to the success of the programme, and addressing them through rational formulation development. The development of a stable formulation of the drug is essential, and the principle degradation pathways and their impact on stability should be identified early on and systematically analysed with the aim of developing the most stable formulation possible. Consideration should be given to the interaction between the API and excipients and what impact, if any, they have on performance, stability and toxicity. In the case of controlled release formulations, an appropriate *in vitro* release model should be selected which is capable of discriminating between formulations with different release characteristics. At the earliest possible opportunity attempts should be made to assess the *in vivo* relevance of the *in vitro* model so that the results can be confidently used to guide formulation development. Early in development, several lead formulations should be identified and optimised as much as possible in order to provide a selection of back ups should any issues arise with a particular composition. Similarly, where at all possible back-up suppliers of key components of the formulation should be sought to minimise risk from external factors. Each of the risk mitigation strategies outlined above will be discussed and illustrated with real life examples with a focus on the development of controlled release formulations of biopharmaceuticals using novel technologies.

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The matrix refolded! Engineering enhanced protein formulation robustness

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Biopharmaceutical development increasingly can succeed or fail on the use of an acceptable formulation and cost of goods. This rapidly expanding and maturing sector demands efficient processes leading to better products and patient compliance and the ability to predict "faster to patient" pathways. Maintaining the chemical and physical stability of a biopharmaceutical therapeutic agent before processing, during processing and during shelf-life storage are major concerns in its formulation development. Whilst an aqueous liquid formulation intrinsically seems advantageous to maintain a protein in its native conformation, chemical instability often dictates that the protein must be dried to obtain a shelf-stable product. The formulation of biopharmaceuticals requires a detailed understanding of how the production process influences the properties of the bioactive component, and how biomolecule/excipient interactions influence biopolmyer stability and condition the solid-state properties of the formulation. For example, the design of particles containing proteins for inhalation must take into account the need to maintain higher order structural stability in addition to the conventional requirements of controlling inter-particle interactions and the optimisation of production/handling properties, transport/mechanical robustness, and physical/chemical stability. The presentation will give an overview of the strategies that have been employed to design and produce protein particles that meet the requirements of modern biopharmaceutical drug delivery.

After a brief introduction to the formulation challenges that proteins present, stabilisation strategies will be discussed. The utility of sugars and polyols will be followed by highlighting our data on the use of novel excipients for solution and solid-state stabilisation. The talk will then address opportunities to improve upon the current methods of producing protein particles. While lyophilisation is typically employed to produce solid protein, the advantages of spray-drying, the use of supercritical fluid technology and protein crystallisation to produce particles in a more controlled manner, such as directing size and shape will be illustrated. Interwoven in the presentation is the third strand to building formulation robustness; namely, through the use of combinations of high-resolution analytical techniques. Hence

a further aim of the presentation is to demonstrate the utility of high sensitivity DSC and FT-Raman spectroscopy amongst others to aid formulation and process optimisation, including excipient selection.

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Understanding the interaction between antigen and adjuvant in an rPA anthrax sub-unit vaccine

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A second generation anthrax vaccine is being currently developed as part of Project Bioshield. This vaccine formulation comprises a recombinant version of the anthrax toxin protein, Protective Antigen (rPA), bound to an aluminium oxyhydroxide colloidal adjuvant (alhydrogel). Although this formulation is highly effective at inducing immune responses in animals and man, how the flexible rPA antigen protein interacts with the highly charged colloidal particles is not fully understood. In order to improve our understanding of the interaction of protein and adjuvant, a series of specialised analytical techniques have been developed. The development of these techniques was initially hampered by the very strong interaction between the colloid and the protein, which meant that standard desorption techniques could not be applied. Certainly, the binding of rPA to the aldhydrogel was not desorbed using high concentrations of citrate/phosphate, but required the further addition of ionic surfactant, demonstrating that a combination of ionic and hydrophobic forces were involved in binding. The difficulty in desorbing the antigen also meant that analytical techniques, which could monitor the protein structure whilst bound to a colloidal particle, were required. A specialised Circular Dichroism (CD) method was developed to investigate the secondary and tertiary structure of the bound protein. rPA binding to the colloidal particles had no significant effect upon the secondary structure, as shown by far UV CD; however an increase of chirality associated with the tertiary structure was detected with near UV CD, indicating that the protein structure had increased rigidity. Differential scanning calorimetry demonstrated an increase in protein melting temperature upon rPA binding, supporting the observed increase in structural rigidity. Similarly, 8-Anilino-1-naphthalene sulphonate (ANS) fluorescence revealed an increase in fluorescence intensity and a blue shift in the λmax upon rPA binding to alhydrogel. This result suggested that binding resulted in a more 'molten globule' type structure, upon interaction with the colloidal particle surface. In conclusion, during the formulation of a second generation anthrax vaccine, the binding of rPA to alhydrogel adjuvant appeared to be the result of ionic and hydrophobic interactions, resulting in very strong binding between protein and colloid. Although this interaction between rPA and colloidal particle had no apparent effect upon the protein secondary structure, effects upon the tertiary structure were observed, indicating an increase in rigidity and the formation of a 'molten globule-like' form. Overall the data demonstrates some distortion of the tertiary rPA protein structure upon binding to the surface of the colloidal particle.

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Mechanism-based PK-PD modeling for prediction of efficacy-safety

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A major challenge in drug discovery and development is the prediction, in a strictly quantitative manner, of drug effects in man on the basis information from *in vitro* bioassays and/or *in vivo* animal studies. Not surprisingly current research in PK-PD modelling focuses on the development of mechanism-based PK-PD models, with much improved properties for extrapolation and prediction. Mechanism-based PK-PD models are based on principles from systems biology and contain specific expressions to characterise processes on the causal path between drug administration and response. This includes a) the biophase distribution, b) the target interaction/activation and c) the homeostatic control mechanisms, which may be operative. The utilisation of these models relies on novel biomarkers characterising, in a quantitative manner, specific processes on the causal path. Another important feature of mechanism-based PK/PD models is the strict distinction between 1) drug-specific and 2) system-specific parameters to describe *in vivo* drug effects (Danhof et al 2007). Due to their anti-parsimonious nature, the utilisation of Bayesian methods and the application of non-linear mixed effects modelling are essential in the development of mechanism-based PK/PD models. Often information on different drugs and/or information on the same drug but obtained under different conditions needs to be simultaneously analysed to derive the *in vivo* transducer function and to obtain estimates of physiological rate constants. Furthermore, the incorporation of information from different sources (i.e. *in vitro* bioassays) may be required. In recent years we have successfully developed mechanism-based PK/PD models for drugs acting at various targets including A_1 Adenosine, μ Opioid, 5-HT_{1A} Serotonin and $GABA_A$ receptors. Our findings show that in general a drug's *in vivo* intrinsic efficacy can be accurately predicted on the basis of *in vitro* bioassays. Prediction of the *in vivo* potency on the other hand appears to be more difficult, presumably as result of complexities at the level of the biophase equilibration. Our results also show that equilibrium concentration-effect relationships can be readily scaled from pre-clinical animal studies to humans. The utility of this approach has been demonstrated recently for semi-synthetic opioids for which a mechanism-based PK/PD model has been developed which can predict, in strictly quantitative manner, the clinical analgesic and respiratory depressant effects on the basis of preclinical *in vitro* and *in vivo* data. In contrast, the scaling of homeostatic feedback mechanisms appears to be more complex. An example of this is our work on allometrical scaling of different biomarkers for 5-HT_{1A} receptor agonists from preclinical *in vitro* and *in vivo* models to man. The latest development in mechanism-based PK/PD modelling has been the application of dynamical systems analysis to characterise drug effects on disease processes and progression. It is concluded that mechanism-based PK/PD models provide a scientific basis for the prediction of efficacy and safety in humans.

Danhof, M. et al (2007) *Annu. Rev. Pharmacol.* **47**: 357–400

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Microdosing in early clinical drug development

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Attrition rates for candidate drugs having entered the clinical drug development process are high. Recent statistics show that more than 90 % of candidates fail.

In analysis of attrition, a major cause of failure seems to be that the preclinical models used to predict outcomes in man have a low predictability. Although modelling and simulation examples have shown improvements in predictability (Gomeni et al 2001), ultimately man should be used a species for candidate selection. Due to the significant investments in preclinical studies that need to be done prior to FIH studies, only a small number of candidates are actually brought to man for the ultimate test.

The microdosing paradigm of using man as a species for candidate selection was proposed in the late 90s and is presently accepted by the regulatory community. Microdosing is defined as a dose $\leq 100\ \mu$g. Microdosing utilises high specificity labelling and Accelerated Mass Spectrometry (AMS) or Positron Emission Tomography (PET) as technologies for estimating systemic pharmacokinetics (AMS) and distribution/binding to receptors/targets and regional pharmacokinetics (PET). In AMS, a candidate drug is labelled with ^{14}C and in PET, the candidate is labelled with an emission tracer (usually ^{11}C) and the actual (intravenous) dose administered is very low (few micrograms). The radioactive burden is very low. Due to the low dose administered, the preclinical requirements will be less cumbersome. Regulatory guidelines for preclinical toxicology required for a microdosing study has been published (EMEA; FDA). Although this is strictly a case-by-case regulatory decision, the basic requirements include an extended single-dose toxicity study in the rat. For compounds with low toxicity, a limit dose approach could be used; allometric scaling from animal to man, using a safety factor of 1000, should be used to set the limit dose. In addition (for Europe), in vitro genotoxicity studies should be performed as recommended in relevant ICH guidance. As a validation of the human microdose data, PET data from primates using higher (clinical) doses should be collected. The site of labelling for PET is crucial since the PET signal could arise from potential metabolites. The short tracer half-life is another limiting factor when using PET. Combining PET data with "cold" analysis using LC/MS-MS or AMS technology is advised.

The limitations of microdosing relate primarily to the low dose and the possibility that the microdose is not predictive of clinical doses. This limitation seems to be less of a problem, since studies both with AMS and PET microdosing have shown that a microdose do mostly predict systemic PK and regional PK/target interactions at clinical doses (Bergström et al 2003; Rowland & Garner 2006).

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Excipients

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Excipients, pharmaceutical ingredients that either have no direct therapeutic value or may influence therapeutic activity by some indirect mechanism, are necessary constituents of medicinal products. For excipient(s) used for the first time in a medicinal product or by a new route of administration, full details of manufacture, characterisation, and controls, with cross references to supporting safety data, both non-clinical and clinical, shall be provided. The toxicology and pharmacokinetics of an excipient used for the first time in the pharmaceutical field shall be investigated (Directive 2001/83 EC, Annex, 4.2 (3) as amended). Article 54 of Directive 2001/83EC requires that all excipients need to be declared on the labelling if the medicinal product is an injectable, a topical, or an eye preparation respectively. Furthermore excipients known to have a recognised action or effect need to be declared on the labelling of all other medicinal products. Therefore the revised Commission Guideline "Excipients in the Labelling and Package Leaflet of Medicinal Products for Human Use" contains warning statements to the presence of certain excipients in medicinal products. The package leaflet must be compatible with the Summary of Product Characteristics (SPC) and shall be drawn up in accordance with the SPC. Therefore consistent information should be stated in both documents. Consensus has existed at the international level for a number of years that the use of Chlorofluorocarbon (CFC) should be reduced to a minimum in view of long term environmental damage. The CPMP has reviewed the toxicology and pharmacokinetics of two new propellants (HFA-134a and HFA-227). The assessment of the two dossiers submitted was similar for both excipients. Whereas the vast majority of adverse drug reactions relate specifically to the drug substance, there is a small but important percentage that are associated with the formulation. Excipients are clearly not consistently inert in their biological activity and, therefore, should not be labelled as such. Safety concerns arise from the European Parliament and of the Council on excipients. The following Commission statement amends the Paediatric Regulation No 1901/2006: In view of the risks of carcinogens, mutagens and substances toxic to reproduction, the Commission will request the Committee for Medicinal Products for Human Use of the European Medicines Agency to draw up an opinion on the use of these categories of substances as excipients of medicinal products for human use. The Commission will transmit the opinion of the Committee for Medicinal Products for Human Use to the European Parliament and the Council. Within six months of the opinion of the Committee for Medicinal Products for Human Use, the Commission will inform the European Parliament and the Council of any necessary action it intends to take to follow-up on this opinion. Examples of the development of new excipients and examples of adverse effects of excipients will be discussed in this session.

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Biopharmaceuticals and immunogenicity

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The number of biologicals (protein based medicines) used in therapy is growing fast. They are large molecules, (glycol) proteins, typically administered by injection. These combination of factors can easily lead to immunogenic reactions that can reduce the therapeutic effect and under some, fortunately rather rare, conditions may lead to severe toxic effects. In a seminal article in *Nature Reviews Drug Discovery*, June 2002, Huub Schellekens discusses the different factors that may contribute to immunogenicity of biologicals. They can be categorized as induced by 'structural properties' and 'other factors'.

Structural properties
Sequence variation
Glycosylation
Other factors
Assays
Contaminants and impurities
Formulation
Downstream processing
Route of application
Dose and length of treatment
Patient characteristics
Unknown factors

In this presentation examples will be given of the listed factors and, in particular, attention will be paid to the evolving approaches to predict the occurrence of immunogenicity.

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Nanoparticulate systems for the delivery of subunit vaccines

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With current gene and protein technology it is now possible to identify specific regions of some whole organisms or cells which are likely to be recognized by the immune system, and to reproduce them synthetically as subunit vaccines. These so called epitopes are very safe because they are non-living but they also tend to be only poorly immune stimulating. To improve the immunogenicity of a poorly immunogenic antigen, our approach is to use nanoparticles as delivery systems. Nanoparticulate delivery systems are thought to enhance the immune response by more closely mimicking a virus or microorganism due to the possibility of multimeric antigen presentation and their large size compared to subunit antigens. Our group has developed and characterised the following colloidal delivery systems:

- functionalised liposomes (mannosylated or including adjuvants such as Quil A),
- immune stimulating complexes (ISCOMs),
- cationic ISCOMs (termed Pluscoms),
- ISCOM implants,
- polymeric nanoparticles on the basis of microemulsions,
- in situ gelling chitosan solutions containing chitosan nanoparticles,
- cubosomes.

In this presentation I will give an overview about the various nanoparticulate delivery systems our group has developed for the delivery of subunit vaccines. I will describe new results in this field, both on physico-chemical characterisation and immunological activity of these nanosystems.

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Fabrications of nano-sized self-assemblies composed of novel amphiphilic graft polymers for protein and drug delivery

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Amphiphilic polymers have been explored in recent years as one of the promising drug delivery systems. An amphiphilic polymer contains both hydrophilic and hydrophobic fragments within the same macromolecule. When the polymer is in the aqueous solution, the hydrophobic fragments aggregate together to form a hydrophobic core that is surrounded by highly hydrated hydrophilic fragments. Unlike low molecular weight surfactants, they can be formed from diverse polymer architectures such as block copolymers or graft polymers. Our research is concerned with the development of novel amphiphilic graft polymers based on water-soluble polymers such as polyallylamine (PAA) for protein and drug delivery. The novel PAA based amphiphilic polymers form nano-sized self-assemblies in aqueous solutions with a hydrodynamic size ranging from 100 nm to 400 nm. Our results showed that the variables in the polymer architecture such as the level of hydrophobic substitution and the addition of hydrophilic moieties have a major influence on the self-assemblies, drug solubilising capability or protein complexation efficiency of these amphiphilic polymers. PAA grafted with a higher level of cholesteryl pendant groups exhibits a lower critical aggregation concentration (0.02 g L^{-1}) compared to PAA modified with a lower cholesteryl substitution (0.08 g L^{-1}). The addition of hydrophilic moieties such as quaternary ammonium moieties increases the overall solubility and stability of the self-assemblies in aqueous solutions. This corresponds to a higher encapsulating efficiency of hydrophobic drugs. PAA grafted with 5%mole cholesteryl groups and 45% mole of quaternary ammonium moieties was shown to be able to enhance the water solubility of propofol and griseofulvin up to 25-fold and 17-fold respectively. Since these polymeric nano-aggregates are able to enhance drug solubilisation at polymer, drug weight ratio as low as 2:1, they are efficient solubilisers compared to conventional formulations, which often require high level of excipients such as low molecular weight surfactants, co-solvents or emulsions. In addition, the ability of these polymers in forming nano-complexes with a protein model drug, insulin, was investigated. Our studies showed these self-assembled polymers spontaneously form nano-complexes with insulin in Tris buffer (pH 7.4), with the complexation efficiency up to 103%. It is hypothesised that they can interact with insulin through electrostatic interactions as well as hydrophobic associations due to their unique architecture that consists of hydrophobic pendant groups and cationic moieties. Apart from the variables in the polymer architecture, the weight ratio of polymer:insulin has an impact on the insulin complexation efficiency of these polymers. Higher levels of complexation were achieved at lower weight ratio of polymer:insulin for non-quaternised polymers but opposite trend was observed for amphiphilic polymers with quaternary ammonium moieties. This is possibly because an increased level of positive charge of quaternised polymers enables the formation of compact nano-complexes with insulin. In conclusion, these results suggest that novel PAA based amphiphilic graft polymers offer considerable potential for the future development of nano-sized delivery systems for hydrophobic drugs and protein and peptide drugs.

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Inhalation nanotoxicology and how it may impact drug delivery to the lung

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Nanoparticle-based vehicles that deliver drugs to or via the lung provide an innovative solution to many deficits currently associated with inhaled therapies. Such systems can improve the lung retention time of a drug, release the drug in a controlled manner or target the drug to specific lung cells resulting in an increased drug efficacy, reduced side effects, improvement of patient compliance and a considerable decrease in the cost of inhaled medicines. Consequently, the focus of scientific effort has been on improving the design and efficacy of such nanoparticle systems for future use as inhaled nanomedicines. However, the aspect of nanoparticle toxicity in the lung has remained largely unexamined. This is astounding given the relatively large body of knowledge already accumulated by environmental scientists investigating the toxicology of airborne nanoparticle systems; systems that are not wholly, but in some key aspects, very similar to many drug delivery vehicles designed for inhalation. In this presentation we will discuss some of the fundamental questions arising from the increased interest in inhaled nanoparticulate drug delivery vehicles. How do nanomedicines interact with the lung? Does this interaction cause an undesired or harmful response? If so, what are the nanoparticle properties responsible for this response? Is it possible to predict which types of nanoparticle materials will be safe for medicinal use? A brief summary of *in vitro* and *in vivo* methodology used to evaluate nanoparticle toxicology in the lung will be provided along with results from our own studies and from the literature highlighting current progress in the field. Future endeavours focusing on the establishment of a nanoparticle library composed of both reference and drug delivery nanoparticles will be discussed. It is envisioned that particles in the library will be subjected to a uniform and systematic evaluation of *in vitro* and *in vivo* toxicological parameters with the results tol be published in an open-access database. Ideally, any new vehicle for inhalation could be analysed under standardised conditions and the information added to the public database. It is envisioned that the ability to evaluate toxicological profiles of new systems at early development stages will be a very powerful tool in the hands of research scientists and pharmaceutical companies trying to bring successful nanomedicines to the market.

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Screening of pharmaceutical materials through nanoscale measurements

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Introduction The quantitative spatial and chemical analysis of pharmaceutical materials and formulations at the micron and sub-micron scale is now relatively routine, using approaches such as infra-red and Raman spectroscopy and imaging. In recent years some nanoscale analyses using the probe microscopes have also become commonplace. This includes, for example the measurement of morphology, surface energy, elastic modulus and adhesion phenomena from nanoscale regions and frequently from single particles. Such probe based studies have also been applied in an imaging mode to determine localized dissolution rates and to identify polymorphic and amorphous forms, again from single particles.

Objective I will present studies demonstrating the potential of incorporating complementary micro and nanoscale analysis data in not only developing a fundamental understanding of the nature of a material or formulation but also in the use of these approaches to provide an early screen for API and excipient selection.

Results The ability of probe microscopes to provide robust quantitative data on surface chemical and material properties will be presented in the context of oral and inhaled delivery formulations as well as polymeric based sustained release systems. Complementary data using spectroscopic and imaging approaches such as Secondary Ion Mass Spectrometry and Micro-CT will be used to validate such novel nanoscale data and to provide a basis to link such results to formulation manufacture and performance.

Conclusions Techniques such as probe microscopy, Raman, Secondary Ion Mass Spectrometry and Micro-CT have matured to become reliable high resolution probes of material chemistry, form and physical properties. This level of sophistication, robustness and increased speed of analysis presents an opportunity to provide a 'smart' screen for materials and formulations much earlier than previously possible.

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What should be identified and measured? (Quality parameters for herbal medicinal products and their ingredients)

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The *European Pharmacopoeia* defines plant materials used in medicinal products as either *herbal drugs* or *herbal drug preparations*. The former describing harvested materials, usually after drying, the latter, products derived from the processing (*eg*. distillation, extraction, milling) of herbal drugs. *Herbal Drugs*: assuring their quality is usually achieved by a series of identification tests including: macroscopy, microscopy and chromatography together with, where relevant, further tests for: moisture, ash, foreign matter and absence of adulterants. A method of quantification is usually included, this may be an assay (*eg*. chromatographic, spectrophotometric, titrametric) or, where an assay is not appropriate, may be achieved by assigning a minimum value to one or more measured parameters (*eg*. extractable matter, swelling index, bitterness value, essential oil content, colour intensity). In addition, there are general quality criteria applicable to herbal drugs depending upon their intended use. Currently, these include tests for: heavy metals, pesticides, aflatoxins and microbial contamination. The introduction of monographs on herbal drugs from other traditions (*eg. Traditional Chinese Medicine, Ayurvedic Medicine*) challenges current definitions for *herbal drugs/herbal drug preparations* and highlights the difference in emphasis which, for example, the *Chinese* and *European Pharmacopoeias* place on certain quality parameters. *Herbal drug preparations* include: *extracts, essential oils, cut herbal drugs for herbal teas* and, more recently, *processed herbal drugs* as used in *Traditional Chinese Medicine. Extracts* are categorised as: (1) *standardised*, where known constituents responsible for the therapeutic activity are controlled within narrow limits (up to ± 10 percent of labeled content); (2) *quantified*, where known constituents responsible for some, but not all, of the therapeutic activity are controlled within a range; (3) so called, *other extracts*, where any therapeutic activity cannot be correlated with known constituents and a minimum value is assigned to the content of constituents which are intended solely as analytical markers. *Processed Herbal Drugs*: the types of processing used in *Traditional Chinese Medicine* tend to retain the integrity of the herbal drug but may alter the physical characteristics and/or chemical constituents either because of the processing method (*eg*. boiling, roasting, stir-baking] and/or because of the addition of processing aids (*eg*. honey, oil, vinegar, wine). As a result, additional tests not normally associated with herbal drugs may need to be introduced (*eg*. herbal drugs processed by stir-baking with oil may require a test for absence of rancidity on storage). *Herbal medicinal products* are consumer products in which the active pharmaceutical ingredients are herbal drugs and/or herbal drug preparations. Wherever possible, with respect to identification and quantification, quality control of herbal medicinal products, at both the time of manufacture and for their shelf life, should correlate with the constituents and analytical methodology applied to the herbal drugs/herbal drug preparations which they contain, particularly when the constituents are either responsible for or contribute to the therapeutic activity of the herbal medicinal product.

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A regulatory perspective

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In the EU, products falling within the definition of a 'medicinal product' must hold marketing authorisations and this includes herbal medicinal products (HMPs). Some 500 or so herbal products on the UK market have product licences. Most of these products were on the market before medicines legislation was introduced in 1968. Since then very few new herbal licences have been granted. This is because the licensing requirements present major challenges both scientifically and financially. The vast majority of herbal products on the UK market are not regulated as medicines but are exempt from licensing requirements under the Medicines Act 1968. This sector of the market has grown exponentially and there are concerns that in contrast to licensed medicinal products, there are no specific safeguards on quality, safety or patient information. There is now substantial evidence that many herbal medicinal products, predominantly in the unregulated sector, are associated with low standards of quality and over the past decade important safety issues associated with the use of herbal products have resulted in regulatory action world-wide. The European Commission has recognised the difficulties faced by herbal manufacturers in fulfilling the regulatory requirements for marketing authorisations and in 2004 adopted a new Directive (2004/24/EC) that provides a simplified registration procedure for traditional herbal medicinal products. Under

the Traditional Herbal Medicinal Products Directive, evidence of efficacy from clinical trials is not needed provided that the product does not require medical supervision and the traditional use of the product is plausible on the basis of long-standing use and experience. However, no derogation is made with regard to the quality aspects of the product and manufacture has to take place in compliance with GMP. Ensuring the quality of HMPs presents a number of unique challenges compared with conventional pharmaceutical formulations. At their simplest HMPs will contain one herbal substance or herbal preparation but this in turn will consist of a complex mixture of phytochemical constituents. More frequently, as is the case with many traditional HMPs, the product contains a mixture of herbal substances/herbal preparations and mixtures of six or more ingredients are not uncommon, especially in Ayurvedic and Traditional Chinese Medicines. The European guidelines on quality of HMPs also apply to traditional HMPs and applicants are required to submit a registration dossier taking account of existing guidelines including stability requirements. Two new guidelines dealing with the declaration of herbal substances/preparations in the Summary of Product Characteristics (SPC) and with quality requirements for combination herbal products have recently been developed. The latter guideline on combination products is of particular relevance to complex Ayurvedic and Traditional Chinese Medicines where, in many instances, identification and quantification of the herbal ingredients in the finished product are not feasible. The UK Traditional Herbal Medicines Registration Scheme came into effect in October 2005 and the first UK product registration was granted earlier this year.

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Development of monographs for herbal medicines

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The *British Pharmacopoeia* is the legal standard for Medicines in the UK. With the introduction of the European Directive on Traditional Herbal Medicinal Products (2004/24/EC), the regulation of traditional herbal medicines has been brought into line with that of pharmaceutical medicines. A consequence of this is that Pharmacopoeial monographs for such medicines have become important to support the simplified registration process being introduced by Competent Authorities such as MHRA. *The need for monographs*: herbal and complementary medicines are widely used within the UK and Europe and assuring their quality is crucial for adequate regulation and ultimately patient safety. It is widely recognised that, currently, traditional medicines can vary widely in quality and there have been a number of well-documented fatalities due to the use of inappropriate of contaminated herbal products. It is also recognised that currently the herbal industry does not have the same degree of scientific and regulatory expertise, or funding as, for example, the pharmaceutical industry. *Balancing the risks and benefits*: In elaborating monographs for traditional herbal medicines it is critical to ensure that the benefits gained are not achieved at the expense of other unintended consequences. For example, monographs that contain restrictive limits may exclude products that have been used safely for many years and monographs that rely on state of the art advanced analytical techniques may be beyond the capability of the industry to implement. Both of these events could lead to an increase in products being supplied illegally outside of the regulatory framework and, clearly this is of no benefit to public health. It is therefore critical that monographs balance science and pragmatism in order to provide an appropriate quality standard that is both robust and accessible to all. *The BP Approach*: The BP approach has been to place the emphasis of the monographs on the *identity* of the herbal medicine and to include tests where appropriate for known *adulterants* or *substituents*. Tests for other *contaminants* (such as heavy metals) and *physical quality* (such as water content or ash) are included and, where possible, a quantitative *assay* for known active and/or marker compounds. Where development of a quantitative assay is not possible, alterative, non-specific tests (such as total saponins or water soluble extractives) are included. The monograph elaboration process mirrors the approach used for pharmaceutical monographs, with the establishment of the Expert Advisory Group on Herbal and Complementary Medicines, with overall responsibility for these monographs. Methods are developed by the BP Laboratory, using existing methods from the literature where possible. The most straightforward analytical techniques are used, except where it is necessary to use more advanced techniques. *Challenges and Solutions*: The main challenges in elaborating herbal monographs are sourcing authentic samples to evaluate, identifying and procuring or developing reference standards and maintaining the balance between a meaningful quality standard and accessibility. To date the BP has elaborated thirteen monographs for traditional/complementary medicines and it is intended to continue to include several new monographs in each future edition.

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Cannabis as a medicine (benefits and risks associated with phytomedicines)

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The benefits of plant medicines, in particular Cannabis, seem to have been known for more than 5,000 years. There are records of its use by inhalation, orally and topically in Egyptian, Judaic, Chinese and South East Asian cultures. Rational use of cannabis dates from Victorian times when O'Shaugnessy investigated its therapeutic use in India, described the preparation of extracts and defined effective dosage. It was widely used in complex prescriptions, and had a history of safe and effective use until it was withdrawn in the mid 1970s because of abuse. Discovery of tetrahydrocannabinol (THC) as the constituent responsible for the characteristic actions of cannabis in 1960 reawakened interest in the mode of action of cannabis, but there was little clinical research until the end of the 20th century. It was assumed that the pharmacology of cannabis was that of THC. Identification and investigation of other cannabinoids (CBs) followed. cannabidiol (CBD), formerly assumed to be an inactive cannabinoid, has very interesting pharmacological properties, some of which sum with, and others antagonise, those of THC. It also has other, non-CB-mediated actions. Recently, the combination of high THC/CBD cannabis extracts in a prescription drug (Sativex) has been shown to be effective treatment for the symptoms of multiple sclerosis and neuropathic pain. It has conditional regulatory approval in Canada, and is available as an unlicensed prescription medicine in the EU. The discovery of endocannabinoids (ECs) has added a new dimension to cannabinoid research. Endogenously produced CBs bind to the same receptors as the plant cannabinoids. ECs appear to be ubiquitous in the animal kingdom, and a study of their generation and activity, at a cellular and receptor level, has enlarged our understanding of their role in maintaining mental, humoral and hormonal homeostasis. The benefits of research into cannabinoids are provision of new medicines for difficult-to-treat diseases, and entry into a whole new area of pharmacological research. Risks associated with growing natural products are well documented; control of infestation, bio-burden, residual levels of pesticides, and heavy metal residues for a start. Growing organically under glass in controlled conditions reduces these risks. The Botanical Raw Material comfortably meets GAP requirements, and when extracted with liquid carbon dioxide as solvent can be used with confidence as the API. Quality has been built into the production process by selection of chemovars characterised by the cannabinoids they produce, rather than morphological characters. The major cannabinoid constituents are well characterised and it is possible to apply to the extracts, many of the analytical procedures normally associated with synthetic APIs. By carefully standardising the cannabis extracts it is possible to use them as APIs in their own right and apply the usual rigour of analytical control. Analysis of the constituents is by HPLC procedures. HPLC-MS has shown that the extract contains a remarkable variety of intermediate compounds in the bio-synthetic pathway—and a number of surprises. It has been found possible to breed varieties in which the bio-synthetic pathway is interrupted to give interesting, largely unstudied, compounds. Benefits of using extracts also accrue from other components, including minor cannabinoids, terpenes and other constituents that synergise the actions of the cannabinoids.

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The right excipients for inhaled products – balancing formulation and regulatory requirements

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For inhaler formulation development, any excipient should ideally be: accepted by regulatory authorities, fully characterisable for parameters known to be crucial for the performance, pure, stable, exhibit no batch or supplier variations and to be available from more than one supplier. It is difficult for any excipient to meet these criteria, and in many cases there are still questions concerning the true physicochemical characteristics, and importantly, their effect on formulation characteristics. Furthermore, these characteristics largely depend on the delivery device. The usual approach by pharmaceutical companies is to design, or to obtain the rights to, a novel inhalation device, which can preferably be used for a wide range of different drugs, concentrations and applications. The products are then typically developed using iterative processes, *i.e.* formulations are prepared based on empirical studies (albeit ones informed by experience) until satisfactory DPI performance and blend characteristics are achieved. One important aspect of this approach is the tailoring of the carrier material to the required formulation and device characteristics. This can ideally be achieved by using 'as supplied' or 'off the shelf' samples of excipient. Alternatively, such

samples could be processed by the user to produce tailor-made excipient mixtures with the required characteristics. On the other hand, an increase of supply with 'customer grade excipients' can be observed, which is produced to a required, tailored specification. This can result in particular supplier/user interface issues. The adoption of The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Quality 8 (ICH Q8) could represent a milestone in the way that pharmaceutical products are developed and Chemistry and Manufacturing Control (CMC) sections are accepted by worldwide regulatory agencies. The text of this guideline states "those aspects of drug substances, *excipients*, and manufacturing processes that are critical and that present a significant risk to product quality, and therefore should be monitored or otherwise controlled, should be identified and discussed". ICH Q8 formalises the ideas of 'Design Space' so that products are better understood and that product failures are less frequent. An important, and welcome, feature of the development paradigms that this guideline formally introduces is that it is the *quality* of the data developed that should be important rather than the quantity of data.

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Controlled release formulations for pulmonary drug delivery

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Objectives This paper describes the use of spray drying of a range of formulations, including solutions, suspensions and double emulsions, to produce respirable powders that exhibit controlled drug release properties.

Methods Formulations for spray-drying were prepared using three methods: Method 1: Aqueous ethanol formulations (Rabbani & Seville 2005) containing lactose, leucine as an aerosolisation enhancer (Seville et al 2007) and low/medium/high molecular weight chitosan (Learoyd et al 2007) ± terbutaline sulfate (TS) ± beclometasone dipropionate (BDP) were spray-dried using a Büchi B-290 mini spray-drier with high performance cyclone. Method 2: Primary emulsions were prepared by vortex-mixing water ± salbutamol sulfate (SS) with Span 80 in a solution of PLGA (50:50) in chloroform ± 80 mg BDP. This primary emulsion was subsequently homogenised with an aqueous ethanol solution of chitosan and leucine ± SS to form w/o/w emulsions containing SS in the inner aqueous phase and/or BDP in the oil phase and/or SS in the outer aqueous phase. The emulsions were then spray-dried. Method 3: The use of a novel two-stage spray-drying process was investigated. Stage 1: an aqueous ethanol solution of lactose ± SS ± BDP was spray-dried to prepare the primary powder. Stage 2: the primary powder was dispersed in a solution of dichloromethane:chloroform (20:80) containing PLGA (50:50) ± SS ± BDP. This organic dispersion was spray-dried using the B-295 inert loop to generate the secondary powder. Following physicochemical characterisation, the aerosolisation performance of the powders was determined using a Spinhaler DPI and a Multi Stage Liquid Impinger (MSLI) at a flow rate of 60 L/min. In vitro powder dissolution studies were performed using USP 2 dissolution apparatus in phosphate buffer (pH 6.8, 37 °C). The emitted dose (ED), deposition at each stage of the MSLI and drug release were determined by HPLC analysis.

Results Method 1: Powders containing low molecular weight chitosan demonstrated fine particle fractions (FPF) of up to 82%; increasing chitosan molecular weight decreased the aerosolisation properties and increased the time taken for drug release (up to 2 and 12 hours for TS and BDP, respectively). Method 2: Emitted doses of up to 97.6% and FPF of 54.7 to 60.3% of the total loaded dose were achieved. In vitro dissolution studies indicated that inclusion of PLGA and chitosan within the formulation resulted in a sustained release profile of over fourteen days for SS incorporated in the inner aqueous phase and BDP incorporated in the oil phase. Salbutamol incorporated in the outer aqueous phase did not undergo sustained release, offering the potential for a dual release profile. Method 3: Powders exhibited an ED of at least 90% of capsule contents and an FPF of over 30% of the total loaded dose. In vitro dissolution studies indicated a sustained release profile of over twenty eight days for SS and BDP.

Conclusions We demonstrate the potential to incorporate different drugs and multiple controlled release entities into a highly respirable particulate system by spray drying a range of formulations. Such systems offer the potential for controlling the release rate of active compounds, thereby aiding the management of respiratory disease.

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Can respiratory epithelial cell lines tell us what is 'particularly' important?

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In order to optimise inhaled drug delivery, particulate formulations are being engineered to possess characteristics that will provide higher respirable fractions, deep lung penetration, modified release or selective targeting. The products of this trend include engineered microparticles, such as porous particles or needles, and nanoparticle-based constructs with evocative epithets such as 'trojan particles' and 'cluster bombs'. The prospect of these formulations improving respiratory targeting for the treatment of lung disease or enhancing the systemic delivery of inhaled drugs is much vaunted. Experience in the field of inhalation particle toxicology, however, provides a note of caution regarding the safety of such formulations. Factors affecting the fate of particles deposited in the lung include dissolution and clearance (biopersistence), particle stability (biodegradability), and uptake/absorption (translocation). The requirement for validated *in vitro* test systems, such as cell culture models of the respiratory epithelium, in which to study these biopharmaceutical (or toxicological) processes and understand them mechanistically has been recognised by both the drug delivery and health protection communities. This raises the question "to what extent can *in vitro* methods provide a guide to the efficacy and safety of novel particle formulations?" Cell culture models of the respiratory epithelium have been developed based on primary cultures of isolated lung epithelial cells and continuous human respiratory epithelial cell lines. These have found application in the study of lung permeability, drug transport mechanisms, drug absorption enhancement, gene delivery and epithelial toxicity. They have also been evaluated for *in vitro-in vivo* correlation to predict respiratory drug absorption. The delivery of respirable particle formulations to respiratory epithelial cell layers in order to study particle-cell interactions has been a logical development. Particles have generally been presented to cell layers in the form of liquid suspensions. The evaluation of nanoparticle (ultrafine particle) disposition has been of particular interest, given the expansion of nanotechnology and the hopes and fears associated with inhaled nanoparticle exposure. Recently, several methods have been reported for delivering aerosolised particles directly to the surface of cultured respiratory epithelial cells *in vitro*. This represents a challenge of dosimetry, assay sensitivity and reproducibility, but allows dissolution-absorption profiles to be measured. These *in vitro* methods are in their infancy, but the development of biological as well as physical techniques to study and characterise novel particle formulations is important.

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Understanding the impact of dry powder formulation characteristics for preclinical models

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Objectives As new chemical entities intended for topical delivery to the lung are being developed with a wide range of physicochemical properties, there is increasing interest in the potential impact of formulation on the deposition, absorption, efficacy and safety of these molecules at the Drug Discovery stage, to help increase confidence in predictions from pre-clinical animal models to clinical data. The Andersen Cascade Impactor and, latterly, the Next Generation Impactor have long been the tools of choice for the formulator producing dry powder formulations for inhalation products to give guidance, from a Quality Assurance perspective, on aerosolisation performance in the human lung. In the investigations described here, we have sought to develop and characterise a number of dry powder formulations intended for delivery to preclinical animal models, using a mini-cascade impactor (In-Tox Products, New Mexico, 7 stages, flow rate 1L/min) and understand the relationship between in-vitro deposition and the apparent aerosolisation performance of these formulations in-vivo.

Methods Initial studies focussed on understanding the deposition characteristics from a 'typical' dry powder formulation in rat airways using a fluorescence technique to produce images of formulations of respirable fluorescent beads following intratracheal delivery using a commercially available dry powder delivery device; the Penn Century DP-4 insufflator. Briefly, 2.0 μm latex fluorescent beads were blended with lactose monohydrate, using a low shear method, and delivered via intratracheal dosing to the airways of male Sprague-Dawley rats using the Penn Century DP-4 device. Frozen whole body sections in the coronal plane were obtained using a cryomacrotome, and scanned for blue/green fluorescence on a STORM860 scanner.

Results Using this technique we demonstrated that the DP-4 device produces a deposition of particles throughout the upper and lower airways in rats. However, aerosolisation data from experiments conducted using the mini-cascade impactor suggested a fairly poor fine particle fraction (FPF) for this formulation (Table 1). Subsequent work involved the development and characterization of formulations of

Table 1 In-vitro and in-vivo deposition in rats of fluorescent beads, fluticasone propionate and budesonide dry powder formulations following delivery into a mini-cascade impactor or intratracheal delivery using the Penn Century DP-4 insufflator

Formulation (binary blend with lactose monohydrate)		In-vitro (%FPF (% <4.6 μm))	In-vivo (approximate % in lung tissue)
API	Blend strength (% w/w)		
2.0 μm Fluorescent beads	20	3.4	Qualitative only. Deposition throughout lung tissues observed
Fluticasone propionate	0.2	7	—
	2	9	—
	20	6	25
Budesonide	0.2	7	—
	2	14	28
	20	13	—

micronised fluticasone propionate and budesonide blended with lactose monohydrate and their delivery to rat airways using the Penn Century DP-4 to investigate the potential impact of formulation on deposition in the lung (Table 1). FPFs were again determined using a mini-cascade impactor and deposition studies in rats were carried out by removing and homogenizing lung tissues following intratracheal dosing and analyzing extracted drug recovered from the tissues beyond the trachea.

Conclusions In accordance with the qualitative fluorescence data, the FPFs calculated here appear to have consistently underestimated the percentage of drug likely to reach the rat lung tissues, which is the opposite of the situation usually encountered with human FPF predictions. Supporting work carried out to investigate the variability in emitted dose from the DP-4 insufflator suggests that differences between in-vitro and in-vivo deposition are likely to be at least partly due to differences between individuals carrying out dosing operations.

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Mass screening versus intelligent design

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Much of recent drug discovery derives from the idea that the only route to new drugs is the reductionist approach of targeting specifically identified biomolecules. This approach has been labelled the rational approach and is analogous to the truly reductionist sciences, such as physics. However, uncritical application of reductionism in biology is fraught with difficulties since evolution creates highly complex pathways of non-linear processes with multiple feedbacks. Perhaps the failure to take account of such complexities accounts in part for the poor record of drug discovery over the last decade where, despite more potential drugs reaching Phase II clinical trials, failures in transition from Phase II to Phase III have grown as a result of a lack of effectiveness in Phase II trials. Thus, it appears that while new clinical candidate drugs are being found more quickly, and are relatively free of metabolic or toxicity deficiencies, they fail because of a lack of clinical effectiveness. This failure has led recently to deconstructionist translational research where studies are performed to validate targets that have already been chosen. Furthermore, molecular targeting by assuming linear processes and singularities in pathways cannot really deal with the complexity of diseases such as those in psychiatry, or the many diseases associated with aging. As an example, this is true for cardiac arrhythmias. There are many types of cardiac arrhythmias, each with its own underlying mechanistic causes, pathways and precipitating causes. Of particular note has been the failure to find preventative treatment for ventricular fibrillation despite the fact this arrhythmia is fatal, unless treated immediately. It is probably the biggest single cause of death in the richer World. A drug for such a condition would be a block-buster. Most ventricular fibrillation arises from sustained myocardial ischaemia due to blockade of a coronary artery. There is no single molecular mechanism underlying this arrhythmia neither in terms of arrhythmogens, nor malfunctioning cardiac ion channels. Fibrillation occurs as ischaemic tissue dies, and electrical activity is severely impaired before becoming quiescent. Therapeutic approaches are to rescue such dying tissue, or render it electrically quiet. The former is current therapy, while the later has been shown experimentally with multi-channel blockers activated by conditions found in ischaemic tissue. This required a directed functional drug discovery approach and not high throughput screening and combinatorial chemistry. Thus, the old and tried approach of functional and complex screens was useful. Perhaps similar approaches are still needed in mechanistically complex diseases? Thus reductionist techno-fixes will not be effective in finding new drugs for all existing diseases. Should we keep our drug discovery options to many different techniques and approaches?

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Preclinical evaluation

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Despite the enormous increase in R&D expenditure by the pharmaceutical industry over the past 10 years, including a heavy investment, for example, in high throughput screening, the number of new molecular entities being introduced each year has declined by 60% over the same time period. The risks associated with drug development are enormous with a new drug costing around $1 billion over 12 years to develop and with a very limited time to recover the investment because of patent life. Statistically, for every 5000–10 000 compounds screened, only 250 will enter pre-clinical evaluation and only one of these will reach the market. Moreover, over the years we have seen many high profile drugs fail soon after marketing, almost always due to some adverse reaction, rofecoxib (in 2004) and troglitazone (in 1997) being two relatively recent casualties. Innovation may be inhibited by the high costs, the prospect of failure and the ever increasing regulatory burdens driven by safety. Yet the potential benefits are also huge both to patients and to the companies. For example, in the developed countries, thanks to the advent of antiretroviral agents, HIV is now regarded as a chronic illness rather an inevitably terminal illness. The technology for screening very large numbers of compounds from huge chemical libraries to generate 'hits' is ever more sophisticated. However, a high percentage of compounds fail subsequently during clinical trial because of poor activity or unfavourable absorption, distribution, excretion, metabolism and toxicity (ADMET) profiles. We need more and better validated targets and better ways of predicting adverse drug reactions at an early stage and, of course, better animal models to predict efficacy in human disease and to evaluate the potential to cause adverse effects. Currently, the effectiveness of many drugs is well-predicted by animal models, whereas others that were predicted to be effective from animal studies turn out to be ineffective in man. The latter case is exemplified by tiralazad, which was neuroprotective in models of ischaemic stroke in animals but tended to worsen outcome of stroke patients in clinical trials (Bath et al 2001). Where there is poor agreement between animal studies and clinical trials, this may reflect factors other than the inappropriateness of the particular model. This presentation will focus on the most effective methods for preclinical evaluation of drugs with emphasis on the appropriateness of in vivo and in vitro methods, with examples drawn from rheumatoid arthritis, schizophrenia, stroke, diabetes and obesity.

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EPR-effect, its mechanism, and further extension towards more tumour selective cancer therapy

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Advances in molecular and cell biology of cancer cells have been remarkable in the past. However, advances of pathophysiology and vascular biology of cancer mass have been relatively slow in view of cancer treatment. Research on cancer chemotherapy has been more focused at molecular level in vitro. We studied vascular leakage of macromolecules in solid tumour tissue in vivo and found that it is uniquely different from that of normal tissue. This principle of enhanced permeability and retention (EPR) effect of solid tumour tissue for macromolecules can thus be utilized for development of new class of macromolecular anticancer drugs. Indeed we observe many polymeric drugs or nanomedecine in recent years are shown to have much improved pharmacokinetic profile, far more tumour targeting, less toxic, patient friendly and cost effective. In addition to the EPR-effect based drug-design, vulnerability of cancer cells to oxystress is not fully appreciated in cancer therapy. Namely, tumour cells in vivo exhibit suppressed capacity for oxygen radical scavenging potential in general. For example, catalase, glutathione peroxidase, SOD are highly down regulated; instead, heme oxygenase (HO-1) is highly upregulated to replace this; consequently HO-1 can produce antioxidant [billirubin] to cope with oxystress in cancer. To utilize two of these unique factors above (i.e. tumour selective accumulation and vulnerability to oxygen free radicals in tumour tissues), I found macromolecular inhibitor of HO-1 and delivery of D-amino acid oxidase

(DAO) can extert more cancer selective chemotherapy. The generation and surge of H_2O_2 by DAO in tumour can be controlled by exogenous administration of D-amino acid such as D-proline. To achieve this goal, we prepared recombinant porcine DAO (DAO) in *E.coli*, and examined antitumour effect against tumour cell in vitro and in vivo. DAO is first pegylated with succinimidyl PEG 5000. Most tumour cells are 5–10 times more sensitive to H_2O_2 than normal fibroblasts, renal cells, and lymphocytes in culture. When PEG-DAO (MW 80k Da) is injected iv in mice, plasma AUC is about 4 times higher than native DAO (MW 39k Da) and it progressively accumulated in tumour, a great contrast to native DAO. This phenomenon (EPR-effect) was not observed in other normal organs. Antitumour effect of PEG-DAO against S-180 and colon 38 tumour was evaluated after iv injection, 2 times in first week only, each time followed by D-proline injection ip 6–12 hrs after DAO iv. The result showed almost complete inhibition of tumour during 5 weeks observation. DAO/D-proline system has superiority in the tumour selective mode of action: (a) DAO is preferentially accumulated in tumour, (b) Only the targeted tumour is vulnerable to H_2O_2 because most normal cells can scavenge H_2O_2 toxicity by catalase or peroxidase. (c) There is no need for drug release (active principle) from micelles as in liposomes. (d) DAO is an endogenous enzyme found in large amount in the kidney, liver and other normal organs. (e) H_2O_2, if over produced, can be decomposed by catalase in red blood cells. Thus, this therapeutic tactic will yield more selective damage to tumour than normal cells.

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Endocytic pathways: gateways for intracellular drug delivery

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The unravelling of the human genome together with proteomic and genomic databases of specific diseases identifies a wealth of new therapeutic targets. The target is often intracellular, the therapeutic a macromolecule or nanomedicine and therefore breaching barriers posed by the plasma membrane and intracellular membranes presents a major challenge to drug delivery research. Cells internalise macromolecules using endocytic pathways; these are efficient multifarious and dynamic tubular and vesicular networks originating from the plasma membrane. They also represent vital avenues for intracellular delivery of therapeutic macromolecules. The effectiveness of using endocytosis for drug delivery may be constrained by the fact that the fate of the therapeutic within one of these pathways is predetermined by the dynamics of that pathway and the barriers posed by the plasma and endolysosomal membranes. Thus, more fragile molecules such as proteins and genes may be rapidly delivered to biologically hostile environments such as lysosomes and inactivated before they reach their intended targets. Improved intracellular delivery of nanomedicines is therefore dependent on attaining an equal high level of understanding of specific endocytic pathways that are inherent in the target cell, the traffic and fate of the therapeutic within endocytic organelles, the effect of the macromolecule on the dynamics of endocytic pathways and finally the downstream effects of these on the integrity of the cell (Jones et al 2003; Watson et al 2005). Drug delivery researchers therefore search for molecules that promote endocytosis and the escape of therapeutics from early stages of the endocytic pathway. Described systems include bioresponsive polymers to domains of proteins that have been shown to interact with and promote their own internalisation and escape of associated cargo across membrane bilayers. These include the hundereds of defined sequences classified as cell penetrating peptides (CPP) or protein transduction domains. The most intensely studied include those derived from proteins such as the HIV TAT protein and the *Drosophila melanogaster* homeobox protein Antennapedia (penetratin), to simpler arginine repeats such as octaarginine. Extensive research has focused on attempting to understand how and where they traverse biological membranes; information vital to furthering their promise as effective drug delivery vectors. Here, focus will be given to techniques that have allowed us to better understand endocytic pathways of a number of different cell types and how these were then used study endocytosis and intracellular dynamics of candidate drug delivery vectors such as CPPs (Watson et al 2005; Fretz et al 2007; Jones 2007).

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Design, synthesis, and characterization of biorecognizable polymers for drug delivery

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The concept of targeted polymer-drug conjugates was developed to address the lack of specificity of low-molecular weight drugs for malignant cells. Features needed to design an effective conjugate include: a polymer-drug linker that is stable during transport and able to release the drug in the lysosomal compartment of the target cell at a predetermined rate, adequate physicochemical properties of the conjugate (solubility, conformation in the biological environment), and the capability to target the diseased cell or tissue by an active or a passive mechanism. The advantages of polymer-bound drugs (when compared to low-molecular weight drugs) comprise (Kopeček 1977; Cuchelkar & Kopeček 2006): a) active uptake by fluid-phase pinocytosis (non-targeted polymer-bound drug) or receptor-mediated endocytosis (targeted polymer-bound drug), b) increased *active* accumulation of the drug at the tumor site by targeting, c) increased *passive* accumulation of the drug at the tumor site by the enhanced permeability and retention effect, d) long-lasting circulation in the bloodstream, e) decreased non-specific toxicity of the conjugated drug, f) decreased immunogenicity of the targeting moiety, g) immunoprotecting and immunomobilizing activities, and h) and modulation of the cell signaling and apoptotic pathways. The state-of-the-art in the development of water-soluble polymeric drugs (macromolecular therapeutics) will be demonstrated through the example of water-soluble *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–drug conjugates. HPMA copolymer-based polymeric drug delivery systems have proved to be effective for chemotherapy, photodynamic therapy, combination therapy, and imaging of cancer. They have been also used in the design of bone-targeting macromolecular therapeutics (Pan et al 2006). Whereas the targetability of macromolecular therapeutics to cell surface antigens/receptors is well established, the manipulation of their subcellular fate needs to be studied (Nori & Kopeček 2005). The conjugates of the future will have a double-targeting capability. They will be recognized by diseased cells and internalized by endocytic or other pathways. Once in the cytoplasm, the drug will be specifically targeted to a subcellular organelle. Examples of subcellular targeting to the nucleus and to mitochondria will be presented. Finally, the design of water-soluble polymers, whose self-assembly into precisely organized three-dimensional structures (hydrogels) is mediated by coiled-coil domains (Wang et al 1999; Yang et al 2006), will be presented. These tailored supramolecular structures possess an application potential in drug delivery, tissue engineering, and other biomedical applications.

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Delivery of poorly water-soluble drug using NanoCrystal Technology—clinical challenges, commercial opportunities

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Many new chemical entities (NCE's) are poorly water soluble. For this reason, a considerable number of NCE's are discarded at the discovery stage and throughout development due to formulation problems. For poorly water-soluble compounds, Elan's

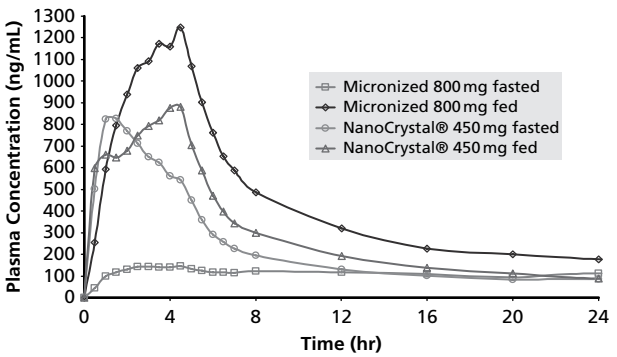

Figure 1 Pharmacokinetic profile comparing NanoCrystal Megestrol Acetate dispersion versus micronised Megestrol Acetate.

Table 1 Cmax and AUC pharmacokinetic comparison data presented in Figure 1

Formulation	Cmax(fed)/ Cmax(fasted)	$AUC_{0\text{-inf}}$ (fed)/ $AUC_{0\text{-inf}}$ (fasted)
NanoCrystal form	1.13	1.23
Micronised form	7.29	2.08

NanoCrystal Technology can enable formulation and improve compound bioavailability and final product characteristics. Particle size distribution is recognised as one of the most critical physical parameters for consistent therapeutic performance. Particle size therefore has a direct impact on formulation/product performance (e.g. drug release/ dissolution, bioavailability, efficacy, safety). By achieving a particle diameter fifty times smaller than conventional micronisation, NanoCrystal particles are reproducibly produced by a proprietary milling technique and stabilised against agglomeration to create a suspension that can have solution-like properties. A number of pharmaceutical products that incorporate NanoCrystal Technology have been successfully commercialised (e.g TriCor 145 mg, Rapamune, Emend and Megace ES). NanoCrystal particles are defined as small particles with an approximate average diameter size of 80–400 nanometres. These particles are made by high energy wet milling of the drug substance in an aqueous continuous phase. Particles in dispersion/suspension are isolated most of the time, but can be subjected to interparticulate forces (aggregation) and/or interfacial tension (coalescence or Ostwald ripening). It is therefore critical during formulation development to impart colloidal stability by specific absorption of stabilisers to the particle surface as means to provide both steric and/or electrostatic stabilisation. Stabilisers used in the NanoCrystal Technology process consist of pharmaceutically acceptable excipients which are generally regarded as safe (GRAS). For poorly water-soluble compounds, Elan's proprietary NanoCrystal Technology can facilitate formulation development and improve compound activity and final product characteristics. The NanoCrystal Technology can be incorporated into a large range of dosage forms including parenterals, oral solid, liquid, fast-melt, pulsed release and controlled release dosage forms. NanoCrystal Technology has been proven commercially to provide a problem solving opportunity for delivery of poorly water soluble drugs which may be impacted by poor bioavailability, fed versus fasted variability, sub-optimal efficacy, variability in biologic response and slow onset. Product compliance issues can also be addressed using NanoCrystal Technology (e.g. Rapamune and Megace). Figure 1 shows the pharmacokinetic profile of Megestrol Acetate (Megace) in both the NanoCrystal form and the micronised form. The data clearly shows substantially similar pharmacokinetic profiles of the NanoCrystal Megestrol Acetate compositions when administered in the fed versus the fasted state. The NanoCrystal formulation dramatically minimised fed/fasted variability compared to the micronised Megestrol Acetate composition (Table 1). The data further indicates that a reduced amount of drug can be used in the final dosage form to obtain the same pharmacological effect. This data emphasises the significant benefits offered by the development of a Nanoparticulate colloidal dispersion using Elan's property enhancing NanoCrystal Technology.

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Monitoring and control of roller compaction

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Quality by Design (QbD) is a much discussed topic in pharmaceutical development these days. Though it would seem a simple goal as it only requires the application of good science to our processes (as is done in many other sectors), there are significant barriers to full implementation of the concepts. One such barrier is the lack of sufficient integrating information management and informatics, another is the gap in our understanding of molecular organic phases, and finally is the paucity of first engineering principles models of our unit operations. Modeling and monitoring for understanding and control is the "desired state" of manufacturing which means that even for unit operations and materials commonly in use, much work is left undone. Roller compaction or dry granulation for solid oral dosage form development is a case in point. This technique has been used for many years to process materials with unsuitable micromeritic or mechanical characteristics for direct compression or capsule filling and/or for compounds which are moisture/heat labile. Despite the obvious advantages, the technique is arguably underused for our products. The approach employed to address the problem was to focus on the ribbon density as the critical response variable for the process. The ribbon density was found to predict tensile strength of the ribbon and the post milled particle size distribution for a given set of mill conditions. The slopes of the diffuse NIR spectra collected on the ribbons was also found to respond to density changes (as reported for tablets by e.g., Drennen). This combination of factors allowed real-time monitoring of ribbon density for ultimate process control. Systems of microcrystalline cellulose and paracetamol or tolmetin sodium dehydrate were used as model systems. As important is the use of the techniques to provide data to test multi-scale modeling of the compaction process. Results of the monitoring and modeling activities will be discussed with emphasis on the sensitivity of the process to variation in the raw materials. The multi-scale modeling activities which are part of the NSF-ERC-SOPS will be introduced.

Contributors Josephine Soh, Ryan McCann, Steef Boerrigter, Saly Romero (now Schering Plough), Abhay Gupta (now U.S. FDA), Purdue University, West Lafayette, IN, USA; Rodolfo Romanach, University of Puerto Rico, Mayaguez, USA; Alberto Cuitino, Rutgers University, New Brunswick, NJ, USA

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The application of Quality by Design principles in drug product development

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Introduction Quality by Design has been described by FDA (Moheb Nasr, 2007) as: a scientific, risk based, holistic and proactive approach to pharmaceutical development; a deliberate design effort from product conception through commercialization; a full understanding of how product attributes and process relate to product performance. The application of these principles in an industrial setting will be illustrated by means of a detailed case study on the drug product development of a new chemical entity (NCE).

Methods The development started with a Target Product Profile for the NCE which required an immediate release tablet with reproducible PK properties (clinical quality), comprising 100 and 300mg of active ingredient with acceptable physical and chemical stability and minimized tablet size. Formal risk assessment processes using Failure Mode Effect Analysis (FMEA) were then used to direct activities throughout the development. The compound is BCS Class II (poorly soluble) and the initial risk assessment identified process parameters and tablet attributes with the highest risk of impacting on clinical performance (i.e. safety and efficacy). These were: drug substance particle size, extent of granulation and changes in formulation (level of binder and disintegrant). Tablet variants incorporating these highest risk variables were manufactured and evaluated in a human PK study with the aim of developing an In Vitro–In Vivo Relationship (IVIVR). The outcome of this study enabled a biorelevant, discriminating dissolution method to be developed as a surrogate for clinical quality and this was used to evaluate all other process and product variables in the establishment of the Design Space. A wide range of process parameters were evaluated using DoE methodologies. Detailed cause and effect relationships between process parameters and tablet quality attributes were established and the resultant tablets were tested against the discriminating dissolution method.

Results The tablet variants incorporating the highest risk variables demonstrated equivalent PK performance in the human study indicating that the product is robust with respect to clinical quality. The dissolution profile of the slowest releasing tablet in the study gave a limit above which all other future variants could be considered to be bioequivalent. All tablets manufactured at the extremes of the potential process parameter ranges gave dissolution profiles above that of the slowest variant tested in the human study (i.e. at the extremes of the parameter ranges tested all products were bioequivalent). A reassessment of the FMEA demonstrated that the risks to clinical quality had been reduced to an acceptable level. Having demonstrated the robustness of the product with respect to clinical quality, it was then possible to optimize the manufacturing process for the less important attributes. For example, an understanding of the impact of granule size distribution and tablet physical attributes enabled an at-line NIR model to be established and provide a mechanism for feed-forward and feed-back control of the manufacturing process to ensure consistently high quality material is produced.

Conclusions The IVIVR study enabled a surrogate test of clinical quality to be established (i.e. the discriminatory dissolution test). This test was used to evaluate the impact of the most relevant process parameters and tablet attributes on clinical quality and thereby define the drug product Design Space. Detailed process understanding, confirmed at the manufacturing site, will ensure that tablets of a consistently high quality can be manufactured. The flexibility afforded by the establishment of the Design Space will allow continuous improvement of the manufacturing process throughout the life cycle of the product.

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Counterfeiting of medicines—an industry insight

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The counterfeiting of medicines is both an opportunistic and premeditated criminal activity. The perpetrators take advantage of market opportunities such as authentic stock not being available, and will use the 'high quality' reputation of branded products from certain countries to disguise counterfeits as parallel imports. The industry surrounding the production of counterfeits is a 'grey shadow' of the legitimate pharmaceutical industry, with most of the same business units. The key aspects missing will be the rigorous control and attention to good manufacturing practice which governs the legitimate pharmaceutical industry. The types of counterfeits and their frequency was reviewed by WHO in relation to reports received between January 1999 and October 2000 and demonstrated that the make up of counterfeit medicines varies widely between:
Products with no active ingredients
Products with the wrong amount of active ingredients
The wrong active ingredients
The correct active ingredients and fake packaging
Products with high levels of impurities

It is essential when suspect counterfeit product is discovered that a rapid and co-ordinated response is made. Key personnel must be identified with the responsibility for taking appropriate actions. A specialist forensic packaging service is invaluable for a speedy and accurate conclusion to be drawn about the authenticity of the suspect samples. The examination of packaging is just as important as analysing the product, in order to establish the exact nature of the suspect pack. A counterfeit carton may contain a genuine primary container; the motives for this may become clear during the pack examination, and the findings help to build a complete picture of the circumstances surrounding the incident. The results of the packaging examination enable the precise objectives of laboratory analysis to be set; for example the first objective should be to confirm the presence of the stated active ingredient or identify any substitute actives present. Depending on the results, it will be important to establish the bioavailability and efficacy of the product and the presence of impurities. Each of these factors is important in establishing the level of patient risk. Factors such as the amount of sample available will affect decisions on which tests to conduct, or the need for reduced scale testing. It is essential that a concise and descriptive report be produced, to provide a record of key evidence to support any likely legal action. This information will also enable investigators or field personnel to more effectively spot similar counterfeits still on sale. The proactive remit of a Pack Security unit is to evaluate the information gained from the examination of counterfeit packs and establish the capabilities of counterfeiters. As a result, technologies and strategies can be identified to provide the best pack protection measures. Intelligence gained from a review of company gathered statistics and trends gives great insight into the highest risk territories and affected therapeutic areas. From this, an effective risk mitigation strategy can be developed to protect packs from the risk of counterfeiting and ensure fast and effective detection of suspect packs. GlaxoSmithKline has a Public Policy Position on the Counterfeiting of Healthcare Products, as well as a Corporate Policy and Procedure on dealing with counterfeit incidents. In addition to this, a Global Quality Policy outlines an effective strategy for pack protection measures.

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The regulatory challenge of counterfeit medicines

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When managing the incidences of counterfeit medicines that have penetrated the legal regulated supply chain, regulators need to balance the risk posed to public health by the counterfeit against the need to maintain public confidence in the quality of the medicinal products they are receiving so that they continue with their treatment. Counterfeit medicines present a risk to public health for the following reasons:
they may contain no active ingredient;
they may contain the wrong active ingredient;
they may contain an unapproved active ingredient;
the content of the active ingredient may be below/above specification;
the product is badly manufactured; the product quality is poor.
When counterfeit medicines are found in the regulated supply chain they must be withdrawn from the market. In managing the recall, the regulator must ensure that the public can be informed of the potential health risks from having taken the counterfeit product. Health professionals also need to be advised on the medical consequences so that they can communicate these to their patients accordingly. In order to achieve these objectives the regulator needs to know the qualitative and quantitative details of the composition of the product, the pharmaceutical and medical characteristics of the product, specific health risks associated with the product, and the distribution of the product in the supply chain. These assessments take place in parallel with enforcement investigations to find the illegal source of the product. Following the discovery of a counterfeit medicine the work of the regulator is by its very nature reactive. It would be preferable to proactively detect counterfeit medicines earlier in the distribution process before it is supplied to the public. Surveillance programme for counterfeit medicines present different challenges from product quality surveillance projects, due to the infrequent intermittent occurrence of counterfeits in the regulated supply chain and the low numbers involved. Sampling programmes in such circumstances are not easy to devise. The presentation will use case studies of recent cases of counterfeit medicines to demonstrate the MHRA process for the assessment and management of the public health risk of counterfeit medicines and will also discuss the outcomes of analytical surveillance projects for the detection of counterfeit medicines.

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Pharmacogenomic applications in drug development

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The development of targeted therapeutics requires diagnostic tools to predict individual response to therapy to maximize drug efficacy and minimize the risk or severity of adverse events. These diagnostic tools include novel pharmacodynamic (PD) markers for exploration of PK/PD relationships and to adjust dose and schedule, predictive markers for drug efficacy and risk of on- or off-target adverse events, and prognostic markers to predict the likely course of disease progression and adjust therapies appropriately. New molecular profiling methodologies allow comprehensive analysis of single nucleotide polymorphisms (SNPs), gene expression, and protein profiles for the discovery of novel genomic and dynamic biomarkers. Application of these technologies in pre-clinical and exploratory clinical development permits discovery of candidate markers in Phase I and IIa that can be independently replicated in Phase IIb, III and IV studies. In this presentation, examples will be given of gene expression profiles to enrich efficacy of selected oncology drugs, and for SNPs or haplotypes to predict increased risk of adverse events in patients treated with highly active antiretroviral therapy (HAART).

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Pharmacogenetics: a SNPshot into the future of personalized (psychotropic) prescribing

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Pharmacogenetics aims to predict treatment response and vulnerability to side-effects by identifying genetic variation associated with these clinical variables. The majority of psychotropic drugs have unknown mechanisms of action, have severe side-effects and a proportion of patients fail to respond to treatment. Therefore the discovery of genetic biomarkers will not only lead to better targeting of drugs to patients likely to benefit from treatment, but will also help to elucidate the pathophysiology of psychiatric disease, leading to the development of improved medication. A growing body of evidence implicates genes within the serotonergic system, dopaminergic system and also genes encoding the liver cytochrome P450 isoenzymes in the prediction of response or side-effects after treatment with antipsychotics or antidepressants. Combinations of genetic variants have been used to predict treatment response but these data require replication. In our recent studies, we have examined single nucleotide polymorphisms (SNPs) of the genes encoding the serotonin (5-HT) receptor subtypes 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C}, in schizophrenia and schizoaffective disorder patients. Subjects were assessed for levels of psychopathology at baseline and after treatment with the atypical antipsychotic drug, clozapine, using clinical measures such as the Global Assessment Scale and the Brief Psychiatric Rating Scale. Of the SNPs tested, the 5-HT_{2A} 452 His/Tyr was associated with severity of anxiety and improvement in depressed mood after clozapine treatment. The 5-HT_{2C} 23 cys/ser polymorphism was associated with overall response to clozapine, while 5-HT_{2C} −759 C/T has been associated with antipsychotic-induced weight gain. Genetically engineered constructs of the receptor isoforms which have been associated with treatment response have been examined to elucidate their functional significance *in vitro*. When cells expressing the 5-HT_{2A} Tyr isoform were compared with 5-HT_{2A} His, it was found that the 5-HT_{2A} Tyr form of the receptor produced lower levels of signal transduction. Additional experiments indicate that clozapine reduces the constitutive activity of 5-HT_{2A} His and 5-HT_{2A} Tyr isoforms similarly. The specific 5-HT_{2A} antagonist, MDL-100907, also eliminated the PI response of both isoforms equally. By contrast the functional consequences of the 5-HT_{2C} 23 cys/ser polymorphism are not clear, but the 5-HT_{2C} −759 C/T polymorphism, which occurs in the gene promotor, has been shown to alter the expression levels of 5-HT_{2C} transcript. In

summary, our data suggest that the 5- T_{2A} SNPs are markers for anxiety and improvement in depressed mood, while the 5-HT_{2C} SNPs are associated with dimensions of response to clozapine and also some side-effects of the drug. These and other published pharmacogenetic data indicate that the 5-HT_{2A} and 5-HT_{2C} receptor subtypes are important targets for antipsychotic and antidepressant drugs.

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Applications of pharmacogenomics to mental health

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Pharmacogenomics is the study of associations between data available at a genomic level and pharmacological outcomes. This talk will give examples of the application of such methodology in mental health. GENDEP is a European multicentre pharmacogenomic study of two representative antidepressants (escitalopram and nortriptyline), including a clinical component, *in vitro* and *in vivo*. The *in vitro* part includes microarray analysis of mRNA from a cell line treated with the above two antidepressants, and qPCR analysis. In a separate microarray study of cerebral mRNA from juvenile rats treated with antidepressants, we have found a number of genes to be significantly differentially expressed. In a proof of concept study using the Affymetrix 500K array, in subjects with a psychotic illness and physical anomalies, we have demonstrated that the same technology can be used for both whole genome association studies and copy number analysis. Such technology could be readily applied to any large-scale clinical trial in which DNA is available.

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Pharmacogenetics of drug-induced blistering skin reactions

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Toxicogenomics is a discipline by which the activity of a particular chemical substance on living tissue is identified based upon profiling its interactions with genetic material. It may be of use as a preventative measure to predict adverse side effects of pharmaceutical drugs on susceptible individuals. Studies of single nucleotide polymorphisms that are correlated to toxicological events in clinical trials (pharmacogenetics) may allow the development of diagnostic markers (measurable signs) for these adverse effects. Using such methods, it could be theoretically possible to test an individual patient for his or her likely susceptibility to these adverse events before administering a drug. Patients that show the marker could be switched to a different drug. While such individualisation is some way off, it has great potential. Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) are rare severe blistering skin diseases which are mainly caused by drugs. The two idiosyncratic conditions are distinguished on the basis of the degree of blistering, possibly representing diseases at different ends of the same spectrum. A genetic predisposition has been postulated. In a large international collaboration, GSK identified a heterogeneous group of USA patients with SJS and TEN induced by a number of marketed drugs and evaluated candidate genetic predisposition. Predisposition varied according to ethnicity. There was a correlation with the MHC ancestral 57.1 haplotype and its constituents in self-reported Caucasians which did not differ with gender. The HLA-DRB and -DRQ genetic predisposition for SJS/TEN appears distinct, but further work is needed for both conditions to identify the causal variants. No conclusion concerning correlations with different drugs could be made. Possession of 57.1 does not diagnose nor predict SJS/TEN with good ROC characteristics. Relevance and relationship to the immunogenetic basis of other type 2 idiosyncratic adverse events (rash, hypersensitivity etc) induced by drug exposure will be outlined. I stress the importance of accurate clinical phenotyping; exemplify a novel analysis method to dissect complicated samples; and call for prospective studies.

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Drug delivery and big pharma

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One of today's dilemmas for drug developers is how much effort should be invested at what point (and at how much cost) in delivery systems for the development of New Chemical Entities (NCE's). There has been a trend towards 'front loaded' development to give a molecule the best chance of becoming a medicine as soon as possible. Success in this arena could have major advantages for both the patient and the company but it is a risky business. Planning for success with every NCE is a fool's paradise as, across the industry, attrition rates remain high. Novel delivery systems have a habit of 'looking for an application' and often live or die as a result of being too closely associated with the wrong molecule. So should novel delivery systems be the reserve of line extensions or can it ever be right to carry the high risk of a novel delivery system with a promising drug asset?

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New approaches to formulate hydrophobic drugs

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Objectives To develop methodologies that can (1) be utilised to aid in the solubilisation of hydrophobic APIs and (2) enhance the properties of existing APIs. Often pre-clinical candidates are burdened by low solubility and by toxicity issues. There is also a significant number of existing APIs that could be formulated to display improved dissolution profiles. Many new and evolving methodologies are being developed to improve the pharmaceutical properties of APIs. Our approach involves two different strategies: (1) association of an API to a water-soluble polymer and (2) the use of spray drying homogeneous solutions of an API with two matched excipients as a means to prolong the existence of the amorphous form of the API in the resulting dispersion.

Methods *Strategy (1).* A water-soluble complex of poly(methaacrylic acid) amphotericin B (AmB) was reproducibly prepared from a narrow molecular weight active ester precursor polymer. AmB was present while the precursor polymer underwent a mild hydrolysis to give, after lyophilisation, a yellow/orange solid that was water-soluble. Haemolysis was determined using human red blood cells and activity was determined using *Leishmania* pro- and amastigotes. *Strategy (2).* Solid dispersions were prepared by spray-drying a homogeneous aqueous organic solution of griseofulvin (40 and 60% w/w), PVP and a second polymeric excipient. The solution was spray-dried in a nitrogen atmosphere using a Niro SD Micro spray drier.

Results The AmB complex displayed little haemolysis compared with clinical grade AmB. *In vitro* and *in vivo* activity was maintained against Leishmania as compared with clinical grade AmB. The solubility (up to 20 mg/ml with AmB loading up to 50%) and activity were maintained over several months for different batches of the AmB complex. This was accomplished in the absence of haemolytic toxicity. It is thought that low toxicity is possible when AmB does not aggregate. AmB aggregation can be estimated by the ratio of the UV intensities at 328 to 406 nm. We observed that low haemolytic activity occurred for ratios of less then 2 between these two peaks. Stability studies of the griseofulvin dispersions over a 13 week period at 50 °C at both 0 and 30% relative humidity (RH) indicated that the incorporation of poly(hydroxypropyl methaacrylate) as the second polymeric excipient prolonged the time until griseofulvin crystallisation was observed.

Conclusions Two strategies are being developed to address issues of API solubility and in some cases to moderate API toxicity while maintaining activity. While the first approach is being developed for a specific parenteral formulation of AmB, both strategies can be used to fabricate solid dosage forms for oral administration.

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The influence of alcohol on drug release from modified release oral dosage forms

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Unintended, rapid drug release from modified release (MR) oral dosage forms, commonly referred to as "dose dumping", can pose a significant risk to patients and is usually caused by a compromise of the release rate-controlling mechanism. The potential impact of concomitant alcohol consumption on drug release from MR oral dosage forms *in vivo* has not been thoroughly investigated. However, following the suspended marketing of a hydromorphone product (FDA Alert 2005) due to a potentially fatal interaction between the modified release capsule formulation and alcohol, the current aim within the pharmaceutical industry is to ensure resistance to alcohol induced dose dumping when developing new MR formulations and also to assess the robustness of existing MR products. *In vivo* pharmacokinetic studies involving co-administration of an MR product and significant amounts of alcohol pose ethical and operational challenges. Consequently, *in vitro* studies, providing insight on release mechanisms in hydro-alcoholic media are required to guide formulation programs such that the potential for alcohol related dose dumping is avoided. Recent research (Roberts et al 2007) has shown that hydro-alcoholic media can affect the kinetics and

mechanism of drug release from matrix-based hypromellose formulations in relation to the ethanol content. Ethanol interaction with hypromellose, particularly in the initial period of contact, resulted in moderated drug release but did not result in a dose dumping effect. The aim of this presentation is to provide an overview of the risks of alcohol induced dose dumping from MR oral dosage forms and of the on-going research into drug dissolution in hydro-alcoholic media.

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US Food and Drug Administration alert for healthcare professionals (2005) *Hydromorphone hydrochloride extended-release capsules* (marketed as Palladone)

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Drug delivery from polymeric scaffolds for tissue engineering

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The field of regenerative medicine offers great promise for the treatment of diseases and injuries that are currently untreatable. Examples of using stem cells and growth factors to regenerate human tissue in applications such as heart attack recovery, Type 1 diabetes and stroke are beginning to generate encouraging clinical results. However, the field faces many challenges in the development of robust and commercially viable products. Delivery systems for growth factors and stem cells will be a key component in successful regenerative medicine products. This talk will discuss the challenges in designing such delivery systems. In particular, the difficulty of combining the properties of high material porosity, controlled delivery of biopharmaceuticals, maintenance of cell viability and scaleable manufacturing will be explored. To address these challenges we have invented a series of porous scaffolds that are administered by implantation or injection. These materials must possess pores with diameters in the size range of 50–400 microns and approximately 50–90% overall porosity. Controlled release of biopharmaceuticals from such materials is difficult became of the high surface area of the materials and heterogeneity of the pore structure. Additionally, manufacturing of these products requires new techniques to allow delicate drugs to be incorporated into thru complex composites. New approaches using supercritical carbon dioxide and miscible polymer blinds will be presented.

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Steroid sulfatase inhibitors: from concept to clinic

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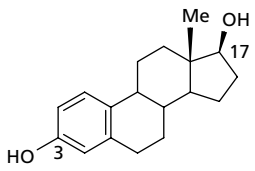
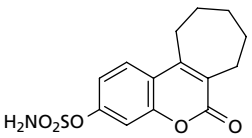
Background Many breast tumours are hormone-dependent (17ß-estradiol) with estrogens playing a key role in their growth and development. There is increasing evidence that inhibition of steroid sulfatase (STS), which converts oestrone (E1) sulfate to E1 and also dehydroepiandrosterone (DHEA) sulfate to DHEA, will attenuate estrogenic stimulation in hormone dependent breast cancer (HDBC). We discovered E1–3-*O*-sulfamate (EMATE) as the first potent, orally active, irreversible active site-directed STS inhibitor (Howarth et al 1994) and this compound reached multiple phase I/II clinical trials for a non-oncology indication. We subsequently designed non-steroidal compounds with even superior potency to EMATE and a series of tricyclic sulfamate candidates led to the clinical drug candidate STX64 (Woo et al 2000).

Results *In vivo*, STX64 was non-estrogenic, inhibited STS potently and caused regression of E1S-stimulated growth in an NMU-induced tumour model and in nude mouse tumour xenografts. STX64 had a very high rodent oral bioavailability of 95%, attributed to the protection of STX64 from metabolic degradation through sequestration into red blood cells by binding to carbonic anhydrase II (hCAII). We recently solved the crystal structure of STX64 bound to hCAII (Lloyd 2005). STX64 is the first STS inhibitor to enter clinical trial and, in postmenopausal women with HDBC, is well tolerated orally and very potent, as measured in peripheral blood lymphocytes or even directly in tumour tissue samples, with *ca* 100% targeted enzyme inhibition even at doses as low as 5–20 mg and with an elimination half-life of ca 30 h. The recently concluded Phase I clinical trial in women with locally advanced or metastatic breast cancer, who had already been heavily pre-treated with other agents including tamoxifen and aromatase inhibitors, showed evidence of 5/8 evaluable women exhibiting stable disease for up to 7 months, indicating that inhibition of STS constitutes a promising novel form of anti-endocrine therapy for the treatment of HDBC (Stanway et al 2006). Further clinical trials are in progress during 2007. Extension of the concept to dual sulfatase-aromatase inhibition *via* a single molecule (Woo et al 2007) and the potential of other novel sulfamate-based agents targeted against *hormone-independent* cancers, and now in preclinical development, will also be discussed (Leese et al 2006).

Conclusions Steroid sulfatase is an attractive novel oncology target for clinical intervention. The aryl sulfamate pharmacophore is a powerful new motif for anticancer drug design.



17ß-estradiol



STX64

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A chemical map of the PI3-kinase family

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Phosphoinositide 3-kinases (PI3-kinases) are activated by receptor tyrosine kinases and other cell surface receptors to produce the lipid second messenger PIP_3. PIP_3 in turn recruits to the plasma membrane and activates numerous downstream effectors that promote cell growth, stimulate nutrient uptake, and suppress apoptosis. Cancer cells frequently co-opt this PI3-kinase signaling pathway in order to promote their survival and proliferation, and the PI3-kinase p110alpha is the most frequently mutated kinase in primary tumors. For this reason, there is now intense interest in the PI3-kinase family of enzymes as potential cancer drug targets, and the first PI3-kinase inhibitors entered clinical trials this year. Nonetheless, there are still many unanswered questions surrounding efforts to drug these enzymes. The PI3-kinase family consists of 15 proteins with overlapping substrate specificities, expression patterns, and modes of regulation. In many cases, it is unclear which specific PI3-kinase isoform is responsible for each of the known physiological functions of PI3-kinase. It is likewise unclear which PI3-kinase(s) should be optimally targeted for different diseases. Moreover, there is limiting understanding of the chemical rules that should guide the design of potent and selective PI3-kinase inhibitors, as the first such compounds have only been reported in the past few years. I will give an overview of efforts to identify selective small molecule inhibitors of PI3-kinase and describe how these compounds have been used to understand signaling by PI3-kinase isoforms. Through the synthesis of a chemically diverse panel of PI3-kinase inhibitors and the biochemical enumeration of their target selectivity, we have identified trends in the sensitivity of different PI3-kinase isoforms to small molecule inhibitors. By comparing this biochemical data with crystal structures of these drugs bound to PI3-kinase, we have been able to identify structural determinants that control inhibitor potency and isoform selectivity. Finally, we have used these isoform selective inhibitors to probe the role of different PI3-kinase isoforms in normal physiology and disease. Through these experiments, we discovered that the PI3-kinase p110alpha is uniquely required for the metabolic effects of insulin, and, more generally, plays a dominant role in mediating signaling downstream of numerous growth factor receptors. This ubiquitous coupling of p110alpha to growth factor signals suggests a likely rationale for the frequent mutation of this isoform in cancer.

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Discovery and preclinical development of kinase inhibitors as targeted agents for oncology indications

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Imatinib mesylate (Gleevec, STI-571) offers an effective treatment for Philadelphia chromosome positive (Ph+) human chronic myelogenous leukemia (CML), a hematological stem cell disorder driven by the constitutively active protein tyrosine kinase (PTK) Bcr-Abl. Emerging evidence indicates that a substantial patient population, particularly those with advanced stages of the disease (accelerated and blast crisis), is either refractory to imatinib therapy or has developed resistance to imatinib following an initial sensitive phase and thus, an unmet medical need still exists.

Various mechanisms have been proposed to account for imatinib resistance, such as: (1) point mutations in the kinase domain of Bcr-Abl, (2) overexpression of the Bcr-Abl PTK, and (3) activation of alternative oncogenes, including members of the Src-family of non-receptor PTKs. In addition, Src activation has been linked to tumorigenesis and metastasis in a wide array of human cancers. We recently identified a novel class of substituted 2-(aminopyridyl)- and 2-(aminopyrimidinyl)-thiazole-5-carboxamides as potent inhibitors of the Src-family of kinases (Chen et al 2004). Several analogues in the series also demonstrated robust biochemical activity against Bcr-Abl and provided broad spectrum antiproliferative effects against hematological and solid tumor cell lines (Lombardo et al 2004). Dasatinib, an analogue in the series, was identified as a highly potent, ATP-competitive inhibitor of both Src ($K_i = 16 \pm 1.0$ pM) and Bcr-Abl ($K_i = 30 \pm 22$ pM) PTKs. In cell culture, dasatinib is considerably more potent than imatinib in Bcr-Abl dependent leukemia cell lines (K562, KU-812, MEG-01, SUP-B15) and several preclinically- and clinically-derived imatinib resistant human cell lines (Shah et al 2004). Dasatinib also demonstrated significant activity against a panel of human solid tumor cell lines (PC3 prostate, MDA-MB-231 breast and WiDr colon). Structural evidence based on X-ray crystallographic analysis of dasatinib bound to the catalytic domain of Abl supports the binding of the inhibitor to many of the imatinib-resistant Bcr-Abl isoforms. In both imatinib-sensitive and imatinib-resistant *in vivo* tumor models of CML, oral administration of dasatinib provided complete tumor regressions and low toxicity at multiple dose levels. Moreover, the dual Src/Abl inhibitor provided significant growth inhibition in multiple solid tumor xenograft models. On the basis of its robust *in vivo* efficacy and favorable pharmacokinetic profile, dasatinib was advanced into clinical trials in cancer patients. The synthesis, structure-activity relationships, and pharmcological profiles leading to the identification of dasatinib will be presented.

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Fragment based drug discovery—from crystal to clinic

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Fragment-based screening is becoming increasingly popular due to its ability to identify ligand-efficient, low molecular weight hits that can be rapidly optimised to potent lead molecules with the use of structural information. Astex-Therapeutics uses its proprietary technology, Pyramid, which utilises high-throughput X-ray crystallography and other biophysical techniques to identify high quality fragment hits for specific drug targets. In particular, we have successfully used this approach against a number of key oncology targets, including the cyclin dependent kinases (CDKs), aurora kinases and the molecular chaperone HSP90. Structural information generated during screening has been used to design further compounds and so rapidly optimise fragment hits against each target into distinct series of potent lead molecules with corresponding cellular activity. Optimisation by these methods has led to preclinical candidates for all the above targets. In each case these compounds were characterised in a number of assays and show potent activity against specific targets, clear mechanism of action in cellular assays and *in vivo* efficacy in mouse xenograft models. Efficacy is coupled with a clear pharmacodynamic response showing inhibition of the specific target within the xenograft tissue. Two compounds identified in this way, AT7519 (CDK inhibitor) and AT9283 (Aurora inhibitor) have already progressed into Phase I clinical trials. AT7519 progressed from first synthesis to first patient in 18 months, exemplifying the success of the fragment based approach to cancer drug discovery.

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The mysterious charm of homoeopathy

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Homoeopathy has recently been in the news, partly as a result of a letter sent by a group of biomedical scientists and clinicians, under the umbrella of Sense About Science, to Directors of Commissioning in Primary Care Trusts (PCTs) urging that they should not commit NHS resources to an unproven approach to treatment. A recently published survey of PCTs' spending plans, using data obtained under the Freedom of Information Act, revealed that already over half had withdrawn, or were planning to withdraw, funding for homoeopathic services. Given the lack of evidence for the effectiveness of homoeopathy and its biological, indeed physical, implausibility, its popularity remains perplexing as does the fact that some PCTs are still funding its use. The talk will review the outcomes of detailed reviews of trials of homoeopathy that show that, despite very large numbers of studies, evidence of its effectiveness over and above the placebo effect, either in general, or in specific conditions, remains at best equivocal and well below the standard that would be required for an allopathic medicine to be licensed or reimbursed under the NHS. The theoretical basis for homoeopathy—and in particular the notion of the 'memory' of the water solvent in which the remedies are dissolved and then removed by dilution—is examined critically. It is noted that water is about 14 000 000 000 years old and will have had many encounters with substances other than the homoeopathic brew. If it really did have a memory, there is no reason why it should have selective recall of one experience over another. The final part of the talk will be devoted to explaining the continuing attraction of homoeopathy and, indeed, many alternative remedies. This will focus on the need patients sometimes have to be offered treatments that make intuitive sense, even if this is at odds with scientific fact, and the allure of the rhetoric of 'holism' and 'healing' that accompanies such treatments. There is the additional advantage of many alternative remedies: since they do not have any biological effects, they are also without side effects, other than financial ones.

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Cleansing the system, or just clearing out your wallet? The truth about detox

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The detox business is said to be worth tens of millions of pounds. Products, including diet plans, tablets and drinks, are sold on the basis that they flush out the toxins that accumulate in our bodies as a result of modern lifestyle and dietary patterns. Detox diets frequently involve cutting out certain foods and substances for a specified time and eating only a limited range of fresh, unprocessed foods. Advocates of such diets insist that a period of detox will aid the liver and kidneys, help the body to cleanse itself from the inside and boost the immune system. Indeed detox diets have been said to banish various ailments and symptoms, from sluggishness and lethargy to cellulite, headaches, bad breath, spots, allergies and other aches and pains. But we know that the body is more than capable of detoxing itself. Everyday, the body is exposed to many toxins, such as caffeine and alcohol as well as the naturally occurring toxins in some fruit and vegetables. The liver is the organ responsible for the detoxification of the xenobiotics (or toxins) that enter the body from the environment, food and medicines and most toxins are broken down by liver enzymes in what is known as "first pass metabolism"—that is the first time blood containing nutrients or xenobiotics pass through the liver. There is some evidence to suggest that there are compounds in fruits, vegetables and other plant foods, known as phytochemicals, which have the capacity to modulate enzymes involved in detoxification, in laboratory experiments. However, whether the compounds have the same effect in the human body remains to be seen. Following a 'detox' diet is unlikely to be harmful in the short term and some individuals may find that it can help to kick-start them into better eating habits. However, such diets should not be followed in the long term as they involve cutting out whole food groups from the diet. No single food can provide all the nutrients that the body needs, and therefore it is important to consume a balanced and varied diet in order to obtain adequate amounts of energy, protein, vitamins, minerals and fibre required for good health. However, following a restricted detox diet for more than a few days can cause headaches, lethargy, dizziness, weakness and irritability. Some diets may be lacking in fibre, which can lead to constipation. A healthy and varied diet is more likely to help maintain a healthy body weight, and to enhance general wellbeing, and will be far friendlier on your wallet.

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Genotoxic impurities: the EMEA guideline

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Unknown/undetermined low levels of genotoxic impurities may be present in an active pharmaceutical ingredient (API). These impurities may have a genotoxic effect even at very low levels, far below ICH identification and qualification thresholds. Which levels of genotoxic impurities may be acceptable for clinical trial material or marketed products? The guideline on the limits of genotoxic impurities (CPMP/SWP/5199/02) has come into effect since January 2007. In the presentation the guideline and the principles in this guideline are discussed. Crucial issues such as the scope, the principle of the Threshold of Toxicological Concern (TTC) and the justification of acceptable limits are addressed. Recent papers from industry in which the principles are further elaborated and practical guidance is proposed (Dobo et al 2006; Müller et al 2006) are considered as well. Based on current insight a strategy for

defining acceptable limits is presented. Pharmaceutical evaluation should include consideration of alternatives avoiding the genotoxic impurity and—in case of unavoidability—application of the 'As Low As Reasonaby Practicable' (ALARP) principle. For genotoxic impurities discussion should include an evaluation of the starting materials, the routes of synthesis, the fate of impurities during further synthesis and degradation processes, in order to compile a list of impurities that can reasonably be expected to be present in the drug substance or product. From this list, compounds known to be genotoxic or with structural alerts should be identified. Where needed, potentially genotoxic impurities should be tested as isolated impurities or in spiked batches at a sufficiently high concentration, as these impurities are usually present below the detection level of the genotoxicity assay when the drug substance is used in the assay. The toxicological acceptability of the level of the genotoxic impurity should be assessed, taking into consideration the mechanism of genotoxicity, the availability of existing genotoxicity and carcinogenicity data, and—if no or little data exist—the application of the TTC. Acceptability of a limit would be influenced by factors such as the profile of genotoxicity results, the duration of the exposure, the stage of the development, the indication of the drug, the life-expectancy of the patient and the extent of exposure from additional sources.

Dobo, K. L., et al (2006) *Reg. Tox. Pharm.* **44**: 282–293

Guideline on the Limits of Genotoxic Impurities, Committee for Medicinal Products (CHMP), European Medicines Agency, London, 28 June 2006 (CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006)

Müller, L., et al (2006) *Reg. Tox. Pharm.* **44**: 198–211

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How realistic is the avoidance of risk? The challenges of genotoxic impurities

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Genotoxic impurities are currently receiving world-wide regulatory focus. ICH Q3A/ICH Q3 B limits do not apply when impurities present a probable potent risk. The objective is to provide understanding of the nature and impact of genotoxic impurities. In particular, structural motifs of concern, current regulatory environments, environmental exposure risks and the risk/benefits of controlling genotoxic impurities to the ppm level, will be discussed. The CHMP guidance on Genotoxic Impurities (2006) divided genotoxins into threshold and non-threshold classes. The former do not directly interfere with DNA, but induce DNA damage *via* interference with a cellular activity (e.g. topoisomerase inhibitors, spindle poisons). In contrast, the latter directly interfere with DNA e.g. alkylating agents. The former are addressed using a PDE approach (ICH Q3C); whereas, the latter would be addressed using the Toxicological Threshold of Concern (TTC). The risk is based on exposure, dose and probability. CHMP proposed that a TTC of 1.5 micrograms/day for a particular genotoxin represented a 1×10^{-5} increased risk of cancer following lifetime exposure. FDA is believed to be in favour of similar 'risk based' approach, with equivalent limits for life time exposure, but slightly elevated levels for shorter duration of exposure (providing meaningful cover for single and repeat dose early human clinical studies). In parallel, EMEA also drafted guidelines for herbal products (EMEA, 2006). However, CHMP highlighted that the growth in use of herbal medicines for self-treatment is unlikely to be impacted by this guidance. EMEA stressed the need to develop robust risk-benefit assessments for herbal products. Interestingly, the chemical reactivity of these intermediates can be often utilized to develop strategies for control. The approach of Dobo et al (2006) on the impurity fate mapping of several alkylating agents together with evaluation of genotoxic impurities in drug substance, are discussed. The typical western lifestyle results in the daily uptake of 1.5 g of genotoxic materials per day. Most of these agents deemed to be genotoxic *in vitro* either do not have adverse effects *in vivo* or are present in too small a quantity to have an *in vivo* effect. The formation of genotoxins is common place in nature. Some examples of genotoxins in common vegetables and the impact of over-cooking red meat will be discussed. The impact on human health is mitigated by antimutagens in food and the induction of metabolic responses; the so-called Electrophile Attack Response (e.g. epoxide hydrolase, glutathione conjugation). In conclusion, does the current focus on ppm levels of genotoxic impurities materially improve patient safety?

Concept Paper on the Development of a Guideline on the Assessment of Genotoxic Constituents in Herbal Substances/Preparations, Committee for Medicinal Products (CHMP), European Medicines Agency, London, 25 October 2006 (EMEA/HPPC/413271/2006)

Dobo, K. L., et al (2006), *Reg. Tox. Pharm.* **44**: 282–293

Guideline on the Limits of Genotoxic Impurities, Committee for Medicinal Products (CHMP), European Medicines Agency, London, 28 June 2006 (CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006)

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Sulphonate esters: a real or imagined risk? PQRI studies to determine actual risk

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Since 2000 there has been an increased level of concern about the potential presence of genotoxic impurities within drug substances. In 2004, the EMEA Committee for Medicinal Products for Human Use (CHMP) released a revised draft Guideline on the Limits of Genotoxic Impurities (EMEA Guidelines 2004), which established the need to restrict levels of such impurities to a limit of 1.5 ug/day (threshold of toxicological concern (TTC). This became effective on 1st January 2007. FDA have also shown considerable interest in this area and participated in the DIA meeting 'Regulation and Control of Genotoxic Impurities in Pharmaceuticals' held in November 2005. Within the context of this issue, of particular concern has been the presence of sulphonate esters. There is experimental data suggesting that a number of sulphonate esters (e.g. methyl methanesulphonate) are potential human carcinogens. This has lead to concerns over their levels and driven regulatory authorities to ask for these to be tightly controlled in line with the limits defined within the aforementioned EMEA guideline. Sulphonic acids are widely used in synthetic processes to manufacture drug substances (in the form of catalyst (e.g. tosic acid), and are also formed as a by-product of their use as leaving groups) and in salt formation; sulphonic acids are widely used as they form very effective (highly crystalline) and safe salts. Elimination of the use of sulphonic acids is highly undesirable either from a synthetic process or salt formation perspective. Sulphonate esters can potentially be formed through the interaction of sulphonic acids and alcohols. The problem of formation could be eliminated by the avoidance of alcoholic solvents. However in many instances substituting alcohols for other solvents is either impractical, impacts upon the quality of drug substance, and/or could have environmental consequences. What is unclear at present is the level of such impurities formed under synthetically relevant conditions. The purpose of the Product Quality Research Institute (PQRI) studies is to is to understand the kinetics that relate to the formation and decomposition of sulphonate esters, under synthetically relevant conditions. This will be achieved through the implementation of a robust and thorough QbD-based approach to the studies performed. Once this fundamental understanding has been established it should be possible to understand the absolute levels of such impurities that can form under any given set of reaction conditions. This will allow both the selection of the optimum process conditions to minimize the levels formed initially plus the ability to develop effective purge processes based on the thorough knowledge of the actual levels formed.

Guideline on the Limits of Genotoxic Impurities, Committee for Medicinal Products (CHMP), European Medicines Agency, London, 28 June 2006 (CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006)

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Genotoxic impurities in excipients: regulatory implications for International Pharmaceutical Excipients Council

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The Committee for Medicinal Products for Human Use (CHMP) recently issued a guideline (EMEA/CHMP/QWP/251344/2006; effective 01 January 2007) on limiting genotoxic impurities in pharmaceuticals to an effective dose of 1.5 micrograms per day (μg/d). This value is derived from mathematical models of a variety of experimental carcinogens indicating an association between a dose of 0.15 μg/d and an excess cancer risk of 1×10^{-6}, and the recognition that the benefit provided by most pharmaceuticals should allow an increase of excess cancer risk of 1×10^{-5}. There is controversy throughout the industry about the approach taken in the CHMP guideline and its real benefit to patients. Although the CHMP guideline currently is only applicable to Active Pharmaceutical Ingredients (APIs), some regulators have discussed its possible applicability to excipients. The risks related to excipients are quite different to those associated with APIs, and many experts do not feel that extension of this guideline to excipients would be appropriate. It is important that this be carefully considered since random application of this guideline to excipients could result in the elimination of many common excipients from drug formulations in Europe. This could cause significant drug availability problems for patients with no real safety benefit. The International Pharmaceutical Excipients Council (IPEC) has maintained that excipients comprise a regulatory category distinct from the Active Pharmaceutical Ingredients (APIs) for the following reasons:

Unlike APIs, which are usually developed for new therapeutic applications and do not have significant human exposure history, excipients are typically used in many existing drug applications and have a long history of safe use in humans.

Excipients in oral dosage forms are often food ingredients for which general human population exposure is already substantial. Applying standards to potential genotoxic impurities in a material used as an excipient, but not to the same material used as a food ingredient would be inconsistent.

Excipients are well known chemicals or mixtures which have been substantially characterized over the years and evaluated extensively using various non-clinical toxicology studies that have shown the excipient to be safe for its intended use.

Excipients typically are not "pure" substances, in contrast to many APIs. They contain a number of major and minor components which are related to the raw material origin and manufacturing processes used to produce a quality product. These components may be necessary for excipient performance.

The composition profiles for many excipients may sometimes contain some potential genotoxic impurities at levels which have already been determined to be safe by studies performed on the excipient as a whole and therefore do not represent a significant risk. These major and minor components are often necessary for excipient performance and their presence can be important.

While recognizing the need to control exposure to toxic impurities and degradants, IPEC maintains that the data used to conclude a genotoxic hazard may not be applicable to excipients in the same manner as APIs. Providing safe excipients to the patient is a key requirement to our industry; IPEC has published a number of guidance documents to that effect, specific to excipients on subjects such as non-clinical Safety Evaluation, GMP, change control, and certificates of analysis that are distinctly different from existing regulatory guidances for APIs. In summary, IPEC proposes that composition profiles (including potential genotoxic impurities) continue to be monitored in pharmaceutical excipients as specified under current pharmacopoeial and IPEC guidance, but that establishing specific procedures for identifying and limiting potential genotoxic impurities in excipients such as is required in the CHMP genotoxicity guidance for APIs is unnecessary. Indeed this could be misleading and affect excipient availability of patient life-saving medicines. Consequently, IPEC recommends that excipients should not be considered in the future for inclusion in the CHMP guideline on potential genotoxic impurities.

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Safety of herbal medicines: where are we now and what needs to be done?

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The increase in popularity of the use of herbal products in the UK over the last decade has been consumer driven, and has been based partly on disillusionment with conventional medicine and also a misguided belief that all things natural are safe. It is likely that this surge in popularity in the next decade will continue unabated. The growing UK herbals market is largely unregulated and this has led to safety concerns among healthcare professionals and regulators (Barnes 2003; Medicines and Healthcare Products Regulatory Agency 2007). Lack of herbal regulation has resulted in variable quality and manufacturing standards of herbal products, often accompanied by inappropriate use through ignorance or disingenuous promotion by some manufacturers. These issues have been compounded by the general ignorance of healthcare professionals about herbal products, at a time when patients and customers require accurate and appropriate advice about these products from reliable sources. The implementation of the European Traditional Herbal Medicinal Products Directive (THMPD), enacted in April 2004, is pivotal to enabling wide access of high quality and "safe" OTC herbal products to the consumer in the future. Such products will be accompanied by appropriate and reliable consumer information. Herbal products to be registered under this new Directive will be assessed by the MHRA for quality, safety and the ability to prove traditional use of the herb for at least thirty years in an EU member state for the approved indication. Post-marketing elements of medicines regulation which are associated with patient safety, such as pharmacovigilance and good distribution practice, will also form part of the menu of safety requirements for these registered products, which will be identical to existing licensed medicines. Although registration applications have been accepted by the MHRA for assessment from November 2005, unlicensed herbal medicines will be allowed to continue to be sold until April 2011, providing they were on the market in April 2004. This lengthy transitional period has been allowed in order to enable manufacturers sufficient time to make material changes to their working practices and manufacture, where necessary, and submit and obtain registration approval from the MHRA for their unlicensed products. Registered products approved by the MHRA are beginning to emerge onto the UK market place under the Directive, and as the number of these products increases in the run-up to April 2011, so the market will move towards a regulated and safer environment for consumers.

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Safety also depends on quality: examples from TCM

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The Problem Increasing uses of Chinese medicinal materials (CMM) and proprietary Chinese medicinal products (PCM) in the West have created both skepticism and support of traditional Chinese medicine (CM) practice. A major debate on this probably started consequential to the successful randomised clinical trial on using the 10-CM herb prescription decoction for treatment of atopic eczema published in 1992 (Atherton 2002). Available in the market are PCM adulterated with pharmaceutical drugs and crude CMM wrongly supplied or substituted with materials possessing liver and kidney toxicity. Reviews on the use of CMM indicate the lack of information on how best to use them safely or their uses are related to adverse reactions when taken as complementary or alternative treatments (Ernst 2005). Yet increases are observed in setting up of CM clinics in major cities in Austria, Finland, Germany, the UK and USA. Existing problems in some regions have been probably the lack of knowledge, recognition, qualified practitioners, quality CMM products and evidence-based studies of these products compared with the cumulative experience in integrative practice and research & development of CM in China over the past 10 years (Chan 2005). In traditional CM practice, qualified CM practitioners prescribe CMM (based on cumulative written experience) as mixture of herbs to individual patients. The herbal mixture is taken as freshly prepared decoction. PCM may be used, though not frequently, as convenient substitutes. The efficacy of CM treatment depends very much on the quality of CMM used.

The Solution Hence the quality of supplied CMM and PCM products is an important issue to guarantee safe use by the public (Chan 2003, 2005). However, these products contain numerous chemical compounds whose identities, structures and bioactivities are often unknown. The quality control (QC) and assurance (QA), safety and efficacy assessment of herbal products present several difficulties (Liang et al 2004). Good practices should be put into action, including good agricultural practice (GAP), good supply practice (GSP as traceability), good laboratory practice (GLP) and good manufacturing practice (GMP), and regulations implemented. Regulatory agencies producing monographs to ascertain standards should be a global effort. This paper addresses the recent progress with case studies in setting standards for CMM from source of raw materials to the market as means of providing the QC and QA through regulatory control.

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Interactions between herbal medicines and prescription drugs: the role of CYP enzymes and P-glycoprotein

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The true extent of the incidence and significance of herb-drug interactions (H-DIs) is still largely unknown, since monitoring the use and sales of herbal medicines, and therefore assessing H-DIs, is difficult (Williamson 2005). The use of herbal medicinal products is increasing, and it has been estimated that in the US, 24% of the general population regularly take herbal products (Kauffmann et al 2002). In the UK in 2000–2001, about 20% of the general population, so probably even more of the patient population, used complementary or alternative medicine (CAM) (Koh et al 2003), and it is not limited to the lay public: according to one study in Singapore in 2003, 84% of pharmacists have tried it themselves (Ernst 2003). At such levels of usage, it is likely that some clinically significant H-DIs will occur. Consumers regard CAM highly, as demonstrated by the volume of sales of these products, and believing them to be safe, or not even 'drugs', may not inform their general practitioner

that they are taking herbal or nutritional supplements. However, the metabolism of herbal medicines occurs via the same pathways as any other drug—namely, the hepatic cytochrome P-450 enzyme system and P-glycoprotein—so some form of interaction may be inevitable. Reports show that the main prescription drugs involved to date are those which are already susceptible to interactions with many others, such as cyclosporin, digoxin, warfarin, protease inhibitors and anti-cancer drugs (Williamson 2005). It is also apparent that only a few herbal drugs have been cited in reports so far: for example in 2003 (the last time this was investigated) only 22 true H-DIs were identified from the world's medical literature with substantial evidence (defined as involving more than one person, and not just an animal study, or suggestion) (Brazier & Levine 2003). Most reports concerned St John's wort, Ginkgo biloba, Dan Shen, liquorice, ginseng and garlic. It is crucial that accurate information is available as there is a real danger that over-reaction on the part of health professionals, for example by scaremongering or exaggerating of safety concerns, may cause the patient to ignore even sound advice on the subject. This presentation will discuss the mechanisms of herb-drug interactions and their importance.

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Drug interactions with *Echinacea*: does it matter how the product is made?

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Echinacea preparations are one of the best selling herbal medicinal products (HMPs) with a well established therapeutic use in the prophylaxis of upper respiratory tract infections. These are licensed herbal medicines in many Continental European countries, but currently unlicensed in the UK. Because of their importance, there is a need to evaluate the safety of HMPs containing *Echinacea* spp. Using the supersome assay we recently showed that commercially available *Echinacea* tinctures prepared from fresh plant and fresh pressed juice preparations vary widely in their inhibitory activity on CYP3A4 (IC50 values: 12.71 μg/mL −1812 μg/mL) (Modarai et al 2007a). Further analysis revealed that the inhibitory activity of the extracts resided mostly in the ethanolic fraction (with IC50 values ten fold lower than the original extract e.g. Echinaforce IC50: 22 μg/mL, vs ethanolic fraction 2 μg/mL) and that these inhibitory values co-vary with the preparations' content of alkylamides (Modarai et al 2007b). These data raise a series of essential questions for the practice of pharmacy. While Echinacea has an excellent safety record and while our data point to a low probability that Echinacea preparation will produce clinically relevant interactions with the CYP-system, there clearly is a need to evaluate individual products. It is problematic to extrapolate from one preparation to another one (even if similar, but not identical extraction and formulation processes are used) and therefore strictly literature-based safety evaluations are problematic. The example of Echinacea highlights the complexity of such a safety assessment and that there are intrinsic links between the production process and quality monitoring on the one side and the composition of a product (and thus its pharmacological and toxicological profile) on the other. This is of particular importance most notably in case of extracts where the active constituents are not known ('other extracts' which are—according to the Eur Ph. (2004)—defined by their production process and not their chemical composition). In these cases there may be a need to ascertain experimentally that the individual products do not cause herbal drug-drug interactions. Since this has important cost implications for producers, an assessment of what experimental evidence may be needed for individual preparations will be essential. Herbal medicines are coming under much more rigorous regulation and this offers novel opportunities to community pharmacists, but also puts additional responsibilities on the profession.

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Looking ahead: a new era in AMD care

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Age-related macula degeneration (AMD) is a leading cause of registerable sight loss in the developed world and hitherto has been considered untreatable. New information on the genetics of AMD, assisted by the human genome project, has led to insights into pathogenesis and fresh data on genetic risk for the more severe forms of the disease. In addition, the importance of smoking cessation and diet have been emphasised by several landmark studies of particular relevance when patients seek advice from their pharmacist. Novel approaches to treating choroidal neovascular or "wet" disease using VEGFI (vascular endothelial growth factor inhibitors) by direct intravitreal injection have been a major milestone in AMD management. Issues of VEGFI drug safety and efficacy will be discussed. There is an urgent need to integrate community and hospital-based services targeting early intervention for wet disease, and low vision rehabilitation for individuals with sight loss from AMD.