Session I-3: Biological and biotechnology-based therapeutics

Session I-3: Oral presentations

I-3-066

Consideration of alternative approaches for the purpose of reducing animal numbers in the preclinical development of biotherapeutic products

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As the development of biotherapeutics becomes a more advanced science-based challenge, the selection of relevant animal models, utility of traditional species and alternatives to traditional safety approaches are becoming more accepted and in fact, necessary. The last ten years has seen a significant advancement of our knowledge and development of biotechnology-derived products for the treatment of debilitating, life-threatening diseases. As the therapies being developed are more sophisticated and generally more specific, the need to establish safety in relevant models has become more and more of a challenge. Alternatives to the traditional safety approach include the use of homologous proteins, transgenic animals, animal models of disease as well as state of the art non-invasive, non-terminal technologies, such as high resolution imaging and scanning methods. In addition, a science-based approach to rational study design has allowed for a better use of animals through the development process. Study design considerations must be addressed in order to most effectively utilize animals and wherever possible reduce the need for large numbers and multiple studies. The opportunities and challenges for these approaches as well as the approach to implementing these areas to help reduce animal use and advance the science of biotechnology drugs will be discussed.

I-3-656

Minimising non-human primate use in monoclonal antibody (mAb) development

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The changing environment of monoclonal antibody (mAb) development is impacting on the cost of drug development and the use of experimental animals, particularly non-human primates (NHPs). The drive to reduce these costs is huge and involves rethinking and improving nonclinical studies to make them more efficient and more predictive of man. As our knowledge base on mAbs expands, the information can be used to improve drug development and maximise the output of experimental data. Cross-company data-sharing of nonclinical study decisions and designs in a non-competitive way can establish an evidence base to influence regulatory change. This presentation will focus on the data collected and analysed by an NC3Rs/industry

consortium of 21 pharmaceutical and biotechnology companies, contract research organisations and regulatory bodies on mAbs currently in development. Analysis from two rounds of data collection has shown that there are opportunities to design novel studies that use rodents for chronic studies, fewer dose groups and less recovery animals. These opportunities have been developed into practical guidance and recommendations on the use of science-based rationale to design studies using fewer animals. The aim is to give an overview of approaches that companies are currently using to develop mAbs and how novel approaches can be translated into practice.

I-3-480

Informal communication with US FDA: pre-preIND approach to reduction of animal use in translational research and product development

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In research and development for cell therapy (CT) and gene therapy (GT) products, preclinical studies are essential for supporting safe clinical trials and eventually effective use in humans. According to 21 CFR 312.23 (a)(8), adequate information derived from pharmacology and toxicology studies is needed to support a trial that is reasonably safe and scientifically feasible. The Pharmacology/Toxicology Branch in OCTGT/CBER/FDA has established an informal pre-preIND process to provide an opportunity for sponsors to engage in a scientific discussion with OCTGT pharmacology/toxicology reviewers regarding development of appropriate preclinical paradigms to evaluate the safety and rationale for administration of novel CT and GT products prior to a formal preIND meeting with the CBER/FDA. The goal of this pre-preIND process is to provide guidance in preclinical study designs that is based on advanced science and technology, as well as applicable regulations, and to ensure judicious use of animals in assessment of these novel products. The current thinking and approaches applied toward the reduction of animal use in the preclinical assessment of CT and GT products are described. This process is one example of the FDA's support and application of the principle of the 3Rs to regulation of investigational products.

I-3-356

Better prediction of immunogenicity of biopharmaceuticals in humans: is it possible?

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Due to the exponential growth of biotechnology, the total number of new biopharmaceuticals has increased rapidly. A major drawback of these products is the possible induction of immunogenicity upon clinical use that may result in a safety issue and/or a reduction of drug efficacy. Current preclinical models have proven difficulties with correlation to predict clinical immunogenicity. Therefore, there is a need for better methods to predict which drugs are likely to induce immunogenicity in clinical trials without the use of non-human primates (NHP).

TNO is developing a multidisciplinary toolbox for prediction of relative immunogenicity based on historical data of structurally, therapeutically and/or "mode-of-action" similar compounds. Since there is not one validated model available and various assays are being used, the toolbox will be an integrated approach including different *in silico*, *in vitro* and *in vivo* tools, preferably including human data. Data will be gathered and combined in an (self-learning) algorithm to perform a structured analysis of the potential immunogenicity. Therefore it is cost and time efficient to eliminate candidates that present a high risk of provoking anti-product immune response.

Since the use of NHP is under increasing societal pressure, the second approach is an alternative animal model for safety evaluation of biopharmaceuticals, the minipig. We explored a.o. the possibilities for immunogenicity testing of 3 known biopharmaceuticals and it was concluded that comparable results were obtained in respect to the immunogenicity in minipigs and NHP. These approaches enable us to use minimal testing, reduce the use of animals, reduce costs and provide good predictive data for induction of potential immunogenicity.

I-3-380

In vitro MABEL approach for nonclinical safety assessment of MEDI-565 (MT111), a novel CEA/CD3-bispecific single-chain BiTE antibody

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MEDI-565 (MT111) is a novel bispecific single-chain antibody of the BiTE® (bispecific T cell engager) class that transiently links carcinoembryonic antigen (CEA; also called CEACAM5, CD66e) on cancer cells with CD3 on T cells. Binding of MEDI-565 to CEA and CD3 results in T-cell-mediated killing of cancer cells expressing CEA. MEDI-565 specifically binds to human and cynomolgus monkey CEA with high affinity but not to any other member of the CEACAM family; rodents do not express CEA. MEDI-565 binds to human CD3, but does not bind to cynomolgus monkey or mouse CD3. Consequently, no pharmacologically relevant animal species exists for testing the toxicity of MEDI-565. In an effort to introduce a pharmacologically relevant model, two surrogate antibodies were made, cyS111 and hyS111, with specificity to monkey or mouse CD3, respectively. However, the characteristics of these two antibodies were different from those of MEDI-565 to an extent that it was determined that hyS111 and cyS111 would not have utility in nonclinical toxicity studies. Hence, no *in vivo* toxicology studies were conducted in a relevant animal model with either MEDI-565 or with the two surrogate antibodies. Rather, MedImmune implemented a strategy which utilized an *in vitro* approach to assess nonclinical safety instead of performing *in vivo* toxicity studies which would have required the use of nonhuman primates. Results from these studies were used to select an appropriate starting dose for Phase 1 clinical studies of MEDI-565 for the treatment of patients with cancers expressing CEA.

I-3-620

Pharmaceutical testing of follicle stimulating hormone (FSH): a new cell-based assay for the replacement of the Steelman-Pohley *in vivo* assay

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We will present a new cell based assay (CBA) for the replacement of the animal based Steelman-Pohley *in vivo* assay for pharmaceutical testing of follicle stimulating hormone (FSH). The Steelman-Pohley *in vivo* assay (SPA), first published in 1953, is mandatory for pharmaceutical batch release testing according to the European (Ph. Eur.) and US Pharmacopoeia (USP). The *in vivo* assay is based on rat treatment. During 3 days of treatment the immature female rats are exposed 3 times to FSH. Then the animals are killed and the ovaries are prepared and weighed. The FSH bioactivity is related to the ovarial growth. The animal test is designed as a confirmatory assay to verify an expected FSH sample bioactivity. The SPA is also applied to analytics in R&D, lead optimisation, process development and in-process controls.

For a single batch release testing, 3 different dosages have to be tested on at least 5 animals each. In combination with the application of an additional 15 rats for reference material testing and 5 rats for negative control, 35 animals are required in total.

In addition to the ethical concerns, the *in vivo* assay has several scientific and technical limitations: no full-dose-response analysis, few doses only, limited relevance for recombinant human FSH products, concerns about reproducibility of ovary preparation and weighing and extensive logistics, e.g. sample shipment to animal facility and prearrangement of animal breeding and treatment.

The new cell based assay (CBA) is based on the FSH-sensitive human granulosa cell line KGN. In KGN cells the progesterone production and secretion is induced specifically by FSH.

The progesterone concentration in culture supernatants is quantified by diagnostic-grade ELISA. The CBA is designed in the 96-well format for screening and full-dose-response analyses. Up to 10 different sample dilutions (1:3 steps) can be applied in quadruplicates. The assay is successfully validated for pharmaceutical batch release testing according to the USP (Chapter 1033; Biological assay validation) and was part of a ring test for the evaluation of a new FSH WHO standard coordinated by the NIBSC (2010; data unpublished).

The new CBA for pharmaceutical FSH testing will help to reduce the number of test animals and is intended to replace the Steelman-Pohley *in vivo* assay. The *in vitro* assay is improving experimental data by full dose-response curves and is suitable for comparability testing of biosimilars. The assay could also be used for clinical monitoring of immunogenicity (formation of drug neutralising antibodies, ADA). The new format reduces experimental costs and time.

Session I-3: Poster presentations

I-3-050

Critical selection of reliable reference genes for gene expression study in the HepaRG cell line

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The human HepaRG cell line has proven to be a valuable *in vitro* tool for repeated exposure to chemical compounds and to evaluate their potential toxic outcome. Seeing the importance given by the current EU legislation of cosmetics and chemical substances to the use of *in vitro* methods in human safety evaluation, one can expect that HepaRG cells will gain importance as a human-relevant cell source. At the transcriptional level, RT-qPCR assays are often used to obtain quantitative results. The choice of internal control is important as it may affect the study outcome. Indeed, it is well known that expression levels of traditional reference genes can vary across tissue types and across experimental settings within one specific tissue type. As limited information exists with respect to S18, often used as internal control in HepaRG cell experiments, we aimed to select the most optimal reference genes for gene expression studies in HepaRG cells and to check whether S18 is a suitable reference gene. The expression stability level of 12 candidate genes was analyzed by three algorithms (geNorm, BestKeeper, Normfinder). These identified TBP as the optimal single reference gene and TBP, UBC, SDHA, RLP13, YHWAZ, HMBS, B2M and HPRT1 as the most suitable set of reference genes for HepaRG transcriptional profiling. This study provides a new set of reference genes to be tested whenever RT-qPCR data for HepaRG cells are generated. The most stable ones can then be selected for further normalization.

I-3-178

The effect of three different types of extract of *Viscum album* in two squamous cell carcinoma cell lines of the tongue

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Head and neck squamous cell carcinoma is the 6th most frequent malignant neoplasia worldwide. The carcinogenesis is a multifactorial process in which respective proteins are the result of several molecular events from oncogenes or tumor suppressors. The treatment of these tumors is mostly surgical excision. Occasionally radiotherapy or chemotherapy can also be used. Even with advances in adjuvant therapies, the survival rate has not significantly changed over the last 20 years. Extracts of *Viscum album* (VA), a *Loranthaceace* family plant, have been used in adjuvant cancer therapy in European countries with promising results.

Three VA extracts (Iscador Qu Spezial, Iscador P and Iscador M) in two squamous cell carcinoma cell lines of the tongue (SCC9 and SCC25) were investigated and compared. The VA extracts at concentrations of 0.3 mg/ml (IC_{50}) were added into the culture medium, and after 24 and 48 h the Annexin V and FITC/propidium iodide assay was performed to evaluate apoptosis rate. A Western blot was also performed to verify the expression levels of pAkt, PTEN and Cyclin D1. The proteins for the Western blot analyses were obtained after the cells had been incubated for 24 and 48 h with the respective drugs.

The VA extracts presented positive results in apoptosis induction. Both cell lines presented different behavior in the presence of the drug. The quantitative protein analysis by SDS-PAGE showed different expression levels, especially of Cyclin D1. The Iscador Qu Spezial and Iscador M possess higher cytotoxic potential on both cell lines compared to Iscador P.

I-3-219

Reduction in the number of animals needed for immunogenicity studies by improved analysis of biopharmaceutical-specific antibody responses

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Immunogenicity is a continuous efficacy and safety issue in the development of biopharmaceuticals. Preclinical models for prediction of immunogenicity in humans, as well as new biomarkers to reveal potential anti-drug reactions, are still needed. A sensitive, robust and specific immunogenicity assay has therefore been developed that can detect anti-drug antibodies of all five Ig classes in plasma or serum. The production of drug-induced antibodies in mice injected with a recombinant human protein has been measured by using a modified version of a multi-parametric bead analysis technique (Luminex). Competitive binding was included to verify drug-specificity of the antibodies. Additionally, validation was performed to evaluate reproducibility and specificity.

Results showed that the murine response against the recombinant human protein was IgG1- and IgG2b-specific, suggesting that the drug-induced response was driven by both Th1/Th2 cells, a finding confirmed by results from cytokine profiles. The assay requires considerably lower volumes of plasma or serum in order to screen for the presence of drug-specific antibodies of different classes and sub-classes in one single sample. Altogether, 1 μl plasma from each animal was required for the analysis, while a conventional ELISA measurement would have required 20 times more material for the same analysis. Thus, this refined test system allows for a reduced number of animals needed, due to the possibility of "piggy-backing" on the analyses of other studies.

With the described assay, anti-drug antibody class and subclass screening may be executed in one step, potentially facilitating immunogenicity screening in clinical trials also.

I-3-364

Correlation of *Erythrina velutina* biological activities: behavior *in vivo* and toxicity *in vitro*

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Erythrina velutina is used for its effects on the central nervous system as an anxiolytic and sedative. The aim of this study was to obtain *Erythrina velutina* (bark and leaves) powder samples of different particle sizes and evaluate its biological activity in animals and cells. The behavioral studies were conducted through the elevated plus maze assay, using four groups of adult Swiss male mice, weighing 25-40 g. The cytotoxicity studies were conducted using the neutral red uptake assay using NCTC clone 929 cell culture. The particle size of the obtained powders was in the range of 710.0 to 355.0 μm and 150.0 to 75 μm, which were determined by granulometry test and scanning electron microscope. The samples (1000 mg/kg) from infusion (MUC01 and MUC02) and infusion (MUF01 and MUF02) of *Erythrina velutina* showed different behavioral activity in the number of visits and time spent in the open and closed arms of the maze. The DL_{50} values obtained for MUC01 and MUC02 were: 72.47 mg/ml and 53.01 mg/ml, respectively; and for MUF01 and MUF02 were 50.08 mg/ml and 37.38 mg/ml, respectively. The data derived from the *in vivo* behavioral assay for *Erythrina velutina* herbal medicine powder samples with larger and smaller particle size showed considerable correlation. The *Erythrina velutina* leaves powder with smaller particle size showed good correlation between the *in vivo* and *in vitro* data.

I-3-412

A single dose subcutaneous injection efficacy study followed by a 14-day observation period for reduction of subcutaneous fat in female Göttingen minipigs

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The objective of the study was to determine the efficacy of parenteral formulations containing different Merz test articles following a single occasion of dosing by subcutaneous injection in the fat tissue of female Göttingen minipigs followed by a 14 day observation period.

The test and control/vehicle articles were administered by subcutaneous injection in the fat tissue in pre-determined regions on the dorsal and ventro-lateral side of the pig. The dorsal region of the pig was divided into 6 treatment areas, while the ventral region was divided into 2 regions, for a total of 8 treatment regions per pig. A permanent skin marker was used to delineate a 3 cm x 3 cm square (for a total surface area of 9 cm^2) in each of the 8 treatment areas. The different treatment areas were separated by at least 6 cm from each other to be able to separate the effects of the different products. Each treatment region received five 1 ml subcutaneous injections using a 27 G needle attached to a syringe. The injections were so that a square pattern was formed (the 5 injections formed the four points of the square and a center point). Subcutaneous fat thickness was measured using ultrasonography prior to treatment, and on days 1, 3, 7, 10 and 14. A 5 Mhz linear probe was used to obtain an ultrasonographic image perpendicular to the skin surface including the subcutaneous fat layer. The ultrasound images was used to measure the thickness of the subcutaneous fat layer and the screen image with anatomic markers and fat thickness values were printed and kept in the raw data.

I-3-420

The value of non-human primates in the development of therapeutic monoclonal antibodies

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The pharmaceutical industry is increasingly focusing on the development of biological therapeutics. These molecules generally cause no off-target toxicity and are highly species specific. Therefore, non-human primates (NHPs) are often the only relevant species in which to conduct regulatory safety testing to support clinical trials. However, species specificity and immunogenicity may negatively impact the predictive value of these ethically contentious animals and thus limits their value as a test species for drug development.

To study what the value has been of 30 years of NHP testing in drug development, we investigated the drug registration files of all therapeutic monoclonal antibodies (mAbs) which were approved in the European Union to date. We analysed 30 mAbs of which 5 were diagnostic agents. As the industry moved towards the development of more human proteins, we observed that the average use of NHPs also increased. 16 registration files described studies in which anti-drug-antibodies caused increased clearance of the therapeutic and potentially confounded the study. Post mortem analysis in repeated-dose toxicity studies rarely revealed new or unexpected findings nor did embryo-fetal and peri-postnatal developmental toxicity studies. These issues limited the value of NHPs in the safety assessment of new monoclonal antibodies. To reduce the use of less relevant NHP studies in the development of new biological drugs, regulatory demands might be decreased, and manufacturers should be given incentives for successfully evaluating the safety of biological therapeutics using alternative technologies.