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Review

Drug safety Africa: An overview of safety pharmacology & toxicology in South Africa



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ABSTRACT

This meeting report is based on presentations given at the first Drug Safety Africa Meeting in Potchefstroom, South Africa from November 20–22, 2018 at the North-West University campus. There were 134 attendees (including 26 speakers and 34 students) from the pharmaceutical industry, academia, regulatory agencies as well as 6 exhibitors. These meeting proceedings are designed to inform the content that was presented in terms of Safety Pharmacology (SP) and Toxicology methods and models that are used by the pharmaceutical industry to characterize the safety profile of novel small chemical or biological molecules. The first part of this report includes an overview of the core battery studies defined by cardiovascular, central nervous system (CNS) and respiratory studies. Approaches to evaluating drug effects on the renal and gastrointestinal systems and murine phenotyping were also discussed. Subsequently, toxicological approaches were presented including standard strategies and options for early identification and characterization of risks associated with a novel therapeutic, the types of toxicology studies conducted and relevance to risk assessment supporting first-in-human (FIH) clinical trials and target organ toxicity. Biopharmaceutical development and principles of immunotoxicology were discussed as well as emerging technologies. An additional poster session was held that included 18 posters on advanced studies and topics by South African researchers, postgraduate students and postdoctoral fellows.

1. Introduction: drug safety in Africa

New drugs are beginning to make inroads against some of the most important and debilitating diseases threatening mankind. The need is still great in western countries, in which ageing populations face the onset of diseases of the central nervous system (e.g., Alzheimer's disease, Parkinson's disease and psychiatric diseases), the cardiovascular system (e.g., arterial disease with associated stroke and myocardial infarction), Diabetes (and associated long-term complications including cardiovascular and peripheral arterial disease and stroke) and cancer. Africa is further challenged with the still high incidence of HIV and

related autoimmune diseases, as well as carrier-mediated infectious diseases such as malaria. Furthermore, the high incidence of autoimmune disease has led to a reemergence of secondary diseases thought to be eradicated in most Western cultures, with tuberculosis being an important example. Thus, the medical need in Southern Africa goes beyond those diseases that are the primary focus of major pharmaceutical development in Europe, North America and Japan, and the financial incentive for large pharma to become active to address these important needs is lacking.

The hallmark of any new, successful medicine is the clinical proof of efficacy and proof of its safety with clinical use. Thus, drug safety and

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tolerability are a prerequisite for dealing with the current medical needs of the world, including those of Africa. Most of this work has not been conducted in Africa but times are changing and new infrastructure is being put into place to fill this void (Guth & Grobler, 2018). It is time to bring drug safety into Africa for the benefit of the population. For the first time, an international conference was held to bring together African experts with others across the world to discuss current approaches and challenges to drug safety testing.

Drug Safety Africa 2018 was a first for Africa. The conference presented state-of-the-art lectures from academic and industry scientists involved in drug safety testing. African scientists originating from Ghana, Cameroon, Nigeria, Lesotho, Mauritius and South Africa met with experts from Europe (Germany, Switzerland, France, and the Netherlands), UK, USA and South America over three days in a concentrated, interactive lecture series. Four main areas of drug safety were addressed at this conference: safety pharmacology, toxicology, clinical drug safety and precision medicine. Each day included plenary lectures by international experts describing newly developed methods and approaches to enhance the prediction of drug safety with their ultimate use in a diverse patient population. Of particular interest and a dedicated focus point for the plenary lectures, was the degree of translation between both *in vitro* and *in vivo* preclinical studies to first-in-man clinical trials and to further, larger clinical trials.

The conference thus provided a forum for South African scientists, postgraduate students and postdoctoral fellows to learn about new developments in drug safety testing. The conference also provided a forum for identifying new partnerships and collaborations devoted to finding new, safe drugs to address unmet medical need in Africa and world-wide. In addition, the meeting showcased the newly established drug safety testing infrastructure within Southern Africa.

2. Safety pharmacology in drug development

The central nervous, cardiovascular and respiratory systems are designated as vital-for-life and the tests that address possible drug-induced effects on these physiological systems have been designated the 'Safety Pharmacology Core Battery' of studies (Pugsley, Authier, & Curtis, 2008). These are outlined in the ICHS7A and ICH S7B regulatory guidance documents that were first published by the US FDA in 2001 (Anon, 2001) and 2005 (Anon, 2005a, Anon, 2005b), respectively.

2.1. An overview of safety pharmacology and the safety pharmacology society (Michael K. Pugsley, safety pharmacology society, Fairfield, CT, USA)

Dr. Pugsley opened the conference by providing an overview of the discipline of SP and the mandate of the Safety Pharmacology Society (SPS). Safety Pharmacology studies are conducted to predict whether novel drugs (new chemical entities (NCE)) are safe for human use. Non-clinical safety pharmacology studies aim to detect and characterize potentially undesirable pharmacodynamic activities using an array of *in silico*, *in vitro* and *in vivo* animal models (Pugsley et al., 2008; Pugsley et al., 2018). Methodological innovation and advancement of drug safety science is driven through the hard work of members of SPS. The society also focuses on important partnerships with both regulatory authorities and technology providers and facilitates interaction with organizations of common interest such as primary pharmacology and toxicology. Education is a priority of the SPS and this is driven by content at both regional e.g., US Northeast and European meetings and the Annual SPS general meeting (https://safetypharmacology.org/meetings_sps.asp). The society also generates added educational content through the conduct of monthly, highly topical webinars and the publication of manuscripts derived from studies presented at the annual SPS meeting. This publication occurs as a focused issue in the *Journal of Pharmacological & Toxicological Methods*. Ultimately, however, all educational material is made available to inform the general drug safety

and regulatory community regarding progress made by members (or groups) within the society (Pugsley et al., 2018). To advance the discipline, the SPS sets achievable goals, determining actions to successfully achieve those goals, and mobilizing society resources to support those actions. As the discipline is evolving rapidly, the SPS provides coverage of novel methods and models for use in drug safety testing and this is certain to expand to provide better guidance for more types of test systems (Pugsley, Harter, et al., 2018). Dr. Pugsley's lecture provided key information regarding the establishment of the discipline of Safety Pharmacology which remains distinct from related medical disciplines including pharmacology, biochemistry, physiology and toxicology; however, conveyed its reliance on these fundamental fields to evaluate the safety profile of an NCE.

2.2. Drug-induced effects on the respiratory system (Michael K. Pugsley, safety pharmacology society, Fairfield, CT, USA)

Dr. Pugsley outlined methods used to investigate drug-induced effects on the respiratory system during the evaluation of a NCE or biological molecule (*i.e.*, a protein, peptide or monoclonal antibody). All use standard endpoints such as respiratory rate and pulmonary tidal volume (Murphy, 2014). While anesthetized animals may be used to conduct these studies, the use of anesthetized animal models is strongly discouraged in SP studies since cardio-pulmonary reflexes are damped by anesthetics (Anon, 2001). Alternatively, conscious animals can be studied using whole body plethysmography or through the placement of a pressure-sensitive catheter (attached to a radiotelemetry transmitter) beneath the pleural surface (Murphy, Renninger, & Coatney, 2001; Murphy, Renninger, & Gossett, 1998). A similar method has been developed for use in the conscious non-human primate (NHP) which may be useful in the evaluation of biological molecules that may require the NHP as a test species (Murphy et al., 2001). Numerous endpoints are accessible, including ventilatory flow rates (*i.e.*, flow volume and time), arterial blood gases, blood pH, hemoglobin-oxygen saturation and pulmonary receptor function. Similarly, these methods can also measure lung mechanics including changes in pulmonary pressure, pulmonary resistance and lung dynamic compliance (Murphy, 2014). Recently, Murphy (2016) showed that a quantitative measurement of apnea or hyperventilation can also be made in telemetered animals that ensure accurate monitoring at both low and high respiration rates. The ability of a drug to cause ventilatory instability should also be considered because chronic disruption can result in a multitude of adverse events due to intermittent hypoxia. Ventilatory instability is easily identified by the presence of prolonged end-expiratory pauses or apneic periods. Thus, safety pharmacologists continue to refine respiratory methods for use in the preclinical detection of potential NCE and biological molecule adverse event liability. However, a re-evaluation of standard respiratory models and novel study endpoints is long overdue by the safety pharmacology community.

2.3. Drug-induced effects on the cardiovascular system (focus on telemetry based CV models in non-rodents) (Michael Markert, Boehringer Ingelheim Pharma GmbH + Co KG, Biberach, Germany)

Drug-induced effects on the cardiovascular (CV) system are of particular importance and can be investigated in conscious, unrestrained animals using telemetry-based technology (Markert et al., 2004; Klump et al., 2006). This methodology allows for continuous monitoring of CV parameters but produces very large data sets. Thus, a cloud-based transmission and real-time analysis of the recorded physiological signal system was developed (Markert et al., 2017). This system allowed for an assessment of physiological signals despite a substantial geographic separation between the instrumented animals and the evaluating home laboratory. Mr. Markert described an approach for handling and evaluating such data sets. Animals (dogs, minipigs or cynomolgus monkey) were instrumented with a newly designed

full-implanted telemetry based device allowing for measurement of aortic blood pressure, left ventricular pressure, ECG and body temperature. After recovery, animals were randomly assigned to cross-over design studies testing drugs at three different oral doses together with the vehicle used. The data were acquired on a beat-to-beat basis, continuously throughout the observation period. To allow for data visualization and statistical analysis, the data was binned as 10 min median values and stored in an Oracle database. Spotfire® was used for visualization of the data and all statistical analyses were performed using SAS®. Excellent signal quality was obtained and stable hemodynamic parameters were measured in all species. An example of this high signal quality and stable parameters with low variability can be found for the minipig in the report from Markert, et al. (Markert et al., 2009). Between 120,000 (dogs) and 400,000 (cynomolgus) heart beats are typically analyzed per study. Access to the stored data was fast using the SAS® and Spotfire® systems and resulted in both a graphic illustration and statistical analysis within a short time after finishing the data acquisition. NOTOCORD-sense® was used to perform the cloud-based data collection and transmission and real-time data analysis. Large data sets generated in CV studies present a challenge for data reduction and evaluation. The approach described offers convenient data handling and a flexible graphic illustration of the summarized data to enhance and speed study evaluation. The implants used were well tolerated and the animals recovered rapidly from the instrumentation procedure. Excellent signal quality was obtained and stable hemodynamic and electrophysiological parameters could be measured. We successfully showed that a remote cloud-based data acquisition for real-time, beat-to-beat (cardiovascular) data collection by telemetry is feasible.

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showed that a remote cloud-based data acquisition for real-time, beat-to-beat (cardiovascular) data collection by telemetry is feasible.

2.4. The minipig as test species for safety pharmacology studies (Andrea Greiter-Wilke, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland)

Dr. Greiter-Wilke discussed the use of the minipig in SP studies designed to detect potential cardiovascular effects of new drugs. The Göttingen minipig was originally bred at the University of Göttingen by crossing breeding the Minnesota minipig with the Vietnamese potbelly pig and the German landrace pig in order to obtain a relatively small and gentle breed. Its use as a non-rodent species in both toxicity and safety pharmacology studies is gaining acceptance within the pharmaceutical industry and by different regulatory agencies (Forster, Bode, Ellegaard, & van der Laan, 2010). Minipigs reach sexual maturity at 4 to 5 months of age and weigh approximately 10 kg at this time (McAnulty, Dayan, Ganderup, & Hastings, 2012). If kept long-term for use in telemetry studies, their weight can remain below 35 kg with strict food control. Minipigs can easily be trained and group housed. The common routes of administration can be performed similarly as in the dog and the monkey; however, more manpower may be needed for restraint. The use of telemetry-based data acquisition and the needed instrumentation is comparable to other species, but the ECG leads of the implant should be of sufficient length to account for the growth of the animal. The resting heart rate of minipigs is comparable to dogs at 60–80 beats/min (Kuwahara et al., 2004; Markert et al., 2009) and therefore lower than the monkey. When being fed, the heart rate of the minipig will increase for several hours, which needs to be taken into account in the study design (Stubhan et al., 2008; Yuan et al., 2014). In minipigs the QT interval of the ECG is longer compared to other species and it is also advised to correct QT for heart rate changes with an individual correction formula (Holzgreffe et al., 2014). Moxifloxacin, which is commonly used as a positive control drug for prolongation of the QT interval, has been shown to prolong the QT interval in the minipig (Markert et al., 2009) even to a greater extent than in other species at comparable doses and plasma drug levels (Holzgreffe et al., 2014). Minipigs can be rather sensitive to drug-induced body temperature decreases, which will lead to increases in the QT interval and this needs to be interpreted accordingly. The characterization of pharmacokinetic drug profiles in the Göttingen minipig, when reference human drugs are used, revealed comparable PK data to dog or monkey (Lignet et al., 2016). Major metabolic pathways (Phases I and II) are present in the minipig and are consistent with human and other non-clinical toxicology species, with the exception of an increased amide hydrolysis which might lead to reduced human metabolic coverage (Jones et al., 2016). Gastric emptying can be prolonged, especially when animals are kept on straw (Suenderhauf et al., 2014). The use of minipigs in CNS and respiratory studies is described in the literature (Willens, Cox, Braue, Myers, & Wegner, 2014; Zhong et al., 2017); however, these study types have not become as routine as cardiovascular assessments. Thus, the minipig is well suited as an alternative species to the dog and the monkey for drug safety testing, raises less ethical concerns and is without the potential issue of limited animal supply.

2.5. Drug-induced effects on the gastrointestinal and renal systems (Brian D. Guth, Boehringer Ingelheim Pharma GmbH + Co KG, Biberach, Germany)

In designing and conducting SP studies, most attention is paid to the core battery studies that are intended to detect drug-induced effects that are possibly life threatening even with just a single administration. Nevertheless, other effects on physiological function can be highly relevant to the overall success of a new drug and effects on both gastrointestinal and renal function can either lead to drug failure in clinical

trials or to restricted use based on poor tolerability. Thus, there is a strong rationale to examine drug candidates for such effects early during preclinical development. Animal models for this type of testing have been used successfully for many years (Pairet et al., 1997) and have been shown to be predictive for effects seen with their clinical use (Pestel, Martin, Maier, & Guth, 2006). Thus, there are reliable tools available to make these assessments. The presentation of Dr. Guth focused on two such rat models, one examining gastrointestinal effects and the other testing for possible drug-induced effects on renal and metabolic function.

The first model entails the determination of the rate of gastric emptying and the extent of intestinal transit in the conscious rat. This has been used as a screening model in multiple drug discovery projects and has been used as an optimization test in some projects that have been associated with gastrointestinal effects. It is a simple test in which fasted rats are given a test meal of known volume (barium sulfate or charcoal are typically used) at a predetermined time following the administration of a test article. The timing of the test meal is usually chosen to correspond to the expected maximal drug concentration. Thirty minutes following the administration of the test meal the animals are euthanized and the extent of emptying of the stomach is measured as well as the distance that the test meal has travelled into the upper intestine. Minor modifications of the study protocol make it also suitable for use in mice.

Effects of new drugs on renal function can be assessed through urine analysis and the measurement of serum-based parameters. The evaluation includes an assessment of the excretory function of the kidney, including electrolytes and metabolites. Additionally, markers of potential drug-induced acute renal injury are measured in urine. The study entails keeping the animals in metabolic cages for the collection of urine into temperature-controlled collection vessels. During predetermined time intervals, urine is collected and analyzed for urinary volume, electrolyte concentrations, selected metabolites and markers of renal epithelial stress. At the end of the protocol, blood is drawn and a variety of parameters are measured in serum to detect possible metabolic effects as well as to measure hepatic enzyme levels as an indication of acute hepatic stress or possible toxicity.

3. Toxicology in drug development

Nonclinical toxicology has historically been a leading contributor to attrition of candidate drug compounds (Waring, Arrowsmith, Leach, et al., 2015). However, there is considerable benefit to patients and drug discovery companies in identifying and eliminating risks early in pre-clinical studies rather than in clinical efficacy trials. The toxicology session was introduced by James Smith (Boehringer-Ingelheim, Ridgefield, CT) with a review of the strengths and weaknesses in translational risk characterization by standard toxicity study screening (Butler, Guzzie-Peck, Hartke, et al., 2017; Valentin, Guillon, Jenkinson, et al., 2018) with a particular focus on toxicities commonly observed in specific organ systems.

3.1. Safety assessment from new therapeutic concept to candidate selection (James Smith, Boehringer-Ingelheim, Ridgefield, CT)

In the first presentation of the toxicology session, James Smith outlined standard strategies and customized options for early identification and characterization of risks associated with a novel therapeutic. For both small and large molecules, these activities include *in silico*, *in vitro* and *in vivo* experimental approaches and are most efficiently undertaken in parallel to confirmation of efficacy that ensures swift and smooth progression to a candidate molecule. The use of a comprehensive, literature-based assessment of risk (hazard and exposure) associated with a therapeutic concept against a specific target and the scope and timing of such an assessment was presented. Such an assessment considers the strength of evidence for the risk, its severity,

monitorability and the ability to investigate the risk in preclinical toxicological studies that is translatable to patients in a specific therapeutic indication and duration of treatment (Brennan, 2017; Butler et al., 2017). In this presentation, emphasis was placed on the benefit of such an assessment before the identification of a lead molecule for any drug discovery program and the accessibility of predominantly publicly available sources of information needed for such an evaluation.

A summary of standard safety screening methods mandated by ICH guidelines for new chemicals (ICH.org; Anon, 2007) was laid out and included: genotoxic screening (*in silico*, bacterial (Ames), *in vitro* mammalian cell and *in vivo* (micronucleus) assays), cytotoxicity, phospholipidosis and safety pharmacology studies (see above). In addition, for small molecules there is considerable evidence for the association of the physiochemical properties, presence of specific structures, target binding promiscuity and efficacious dose with preclinical and clinical toxicities (Bowes, Brown, Hamon, et al., 2012; Greene, Aleo, Louise-May, et al., 2010; Stepan, Walker, Bauman, et al., 2011; Sutherland, Raymond, Stevens, et al., 2012; Brennan & Kiessling, 2017). The optimum timing and scope of standard and custom property-based safety screens for candidate compounds was discussed as well as the technical feasibility, due to the requirement for relatively small amount of compounds, and the significant reduction in the use of animals. Despite these benefits, established screens do not predict off target toxicities or those that are influenced by metabolism or bioavailability. More predictive, emerging safety screening approaches for small molecules using advanced computational approaches, custom *in vitro* systems or novel *in vivo* analyses were briefly introduced (Lamore, Ahlberg, Boyer, et al., 2017; Liu, Patlewicz, Williams, et al., 2017; Passini, Britton, Lu, et al., 2017; Peters, Landry, Pin, et al., 2018). These tools hold great promise for smaller drug discovery organizations due to high predictivity with minimal investment. The standard safety screening strategy for large molecule biological therapeutics includes evaluations of immunogenicity and tissue cross-reactivity, cytokine release, complement and antibody mediated cytotoxicity (Brennan, 2017). These were introduced briefly but covered in more detail in the Biologics Development and Immunotoxicology presentation (see below). Attention was given to novel *in silico* and custom *in vitro* and *in vivo* assays for de-risking therapeutic candidates that are being developed to support this highly dynamic and promising class of therapeutics (Brennan, 2017). In summary, significant savings in time and resources as well as improved prediction of patient safety is achieved by applying selected safety assessments in the drug discovery and development process well before identification of a lead molecule.

3.2. General toxicology programs to support first in human trials for small molecules and toxicology beyond first in human trials (Danuta Herzyk, Merck Sharp & Dohme Corp., a subsidiary of Merck & co., Inc., Kenilworth, NJ, USA)

Dr. Herzyk initially discussed preclinical drug development with an emphasis on toxicology studies, their objectives, types and relevance to risk assessment supporting first-in-human (FIH) clinical trials with new drug candidates. In a second presentation (below), she described the role of toxicology studies in the progress to later phases of clinical evaluation that ultimately lead to the approval and marketing authorization for a new drug by regulatory agencies.

The development of a new drug is a very long and complex process. A multitude of activities must be completed before a new medicinal entity is approved for human use and can be applied to the patient. Many different preclinical studies focused on safety of a new drug candidate are required to be conducted prior to progression to clinical trials (Kramer, Sagartz, & Morris, 2007). In addition to early screening of drug candidates using *in vitro* and *in vivo* pharmacology test systems, comprehensive and highly regulated toxicology studies in animals are conducted to enable identification of potential toxicity on one hand and prediction of safe exposure in humans on the other. Toxicology

programs are planned according to international regulatory guidelines, primarily implemented by ICH (Anon, 2007) to promote uniformity in technical requirements for registration of pharmaceuticals for human use, and to ensure that safe, effective, and high-quality medicines are developed and registered in the most efficient and cost-effective manner.

Preclinical toxicology studies are conducted based on some general assumptions, including two key premises: 1) that animal models generally will predict human safety, and 2) the use of high doses in animal studies will maximize model sensitivity to detect toxic effects. The objectives of toxicology studies are to identify the target organ of toxicity, to examine potential gender differences in response to a drug candidate and to understand if observed effects are expected based on pharmacology and mechanism of action (on-target) or unexpected (off-target). When toxicity is observed in animals, it needs to be characterized in relationship to the systemic exposure to a drug candidate, mainly to establish if there is a linear dose-response. The next step is to evaluate if the observed toxicity is reversible or progressive, and if it can be monitored clinically. Toxicology studies are categorized as two types, pivotal safety studies and exploratory studies. Safety studies are highly regulated by laws and need to be compliant with Good Laboratory Practice (GLP) rules to be acceptable by regulatory agencies that review preclinical data prior to permitting a safe conduct of clinical trials and registration of new drugs. Exploratory (non-GLP) studies involve investigative work in early screening phases of drug candidates and/or follow-up studies to characterize the mechanism of toxicity found in GLP studies.

The quality of toxicology studies is highly dependent on the study design taking in to account some important considerations. These considerations include animal species selection, route of administration, frequency and duration of the drug candidate administration to animals, and study parameters to be evaluated. Species selection is based on pharmacokinetic and metabolic profile of a compound, its pharmacologic activity or other scientific basis. For chemical drugs two species, one rodent (rat or mouse) and one non-rodent (typically dog or monkey), are required. Route and frequency of animal dosing needs to be consistent with route of administration utilized in the clinic. Chemical drugs are routinely given to study animals by oral (gavage) administration on a daily basis. However, intravenous, subcutaneous, intramuscular, inhalation, or dermal administration routes are used when needed. Duration of toxicology studies ought to cover or exceed the number of days used for dosing in the clinic. Standard endpoints of a toxicology study include a battery of antemortem endpoints, such as clinical observations and body weight, hematology, serum chemistry, urine analysis, and ophthalmology examination, followed by post-mortem histopathology examination of all major organs and tissues. In addition, concentration of a drug in blood plasma is evaluated over the course of the study. Interpretation of results from a toxicology study involves the determination of adverse and non-adverse effect levels. A no-observed-adverse-effect-level (NOAEL) is a dose (or level of exposure) determined by empirical study at which adverse effects (*i.e.*, harmful anatomical, biochemical, or functional changes) were not induced by test article administration in that study. A NOAEL is typically used as push-off point for selection of a starting dose and dose escalation scheme in FIH studies. Clinical doses of novel drug are limited to exposure that does not exceed the NOAEL in animal toxicology studies. This rule, however, may not apply to anticancer drugs to be given to patients with late-stage cancer disease where potential benefit *versus* risk from a drug candidate is an important factor. To minimize exposure to low doses that are unlikely to exhibit expected pharmacological activity in cancer patients, the starting dose may represent a severely toxic dose in 10% (STD) in rodents or 1/6th of highest non-severely toxic dose (HNSTD) in non-rodents, whichever is lower (Anon, 2010). In addition to the safety margin determined in toxicology studies based on the systemic exposure observed in animals *versus* predicted exposure in humans, important deliberations in risk assessment include the

nature of the dose-limiting adverse events (*e.g.*, findings in liver vs. brain), therapeutic indication being sought (*e.g.*, more tolerance for life threatening cancer), intended patient population (*e.g.*, no reproductive concerns for post-menopausal women), potential for reversibility of the observed toxicity, and the ability to monitor the toxicity in the clinic using biomarkers. Efforts should be made to understand the mechanism of the observed toxicity. The goal of risk assessment is to establish if the uncovered adverse effects may be relevant to human health or represent animal-specific findings.

After a new drug candidate has entered the clinic, toxicology evaluation of the drug candidate in animal studies continues in parallel with the clinical studies during the drug development process. A proof of therapeutic concept between phase 1 and phase 2 clinical trials, leads to further efficacy studies in a large population of patients in phase 3 clinical trials. Animal studies supporting these clinical trials include chronic toxicology, reproductive and developmental toxicity (DART), and evaluation of carcinogenicity potential. The goal of chronic toxicity studies is to find out if potential and/or identified toxicity profiles will change upon prolonged administration of the drug. To support clinical trials, where patient populations include women of child-bearing potential, DART studies in rats and rabbits are required to evaluate potential pregnancy risk and fetal toxicity upon exposure to the drug. When the treatment of a disease requires a duration of 6 months or longer, the new drug is evaluated for carcinogenicity risk. For chemical drugs, typical animal carcinogenicity studies are conducted in rodents for life time, *i.e.*, 2-year duration in rats and mice. The 2-year mouse study may be substituted by a 6-month duration in Tg-rasH2 transgenic mice, which are genetically altered as a tumor sensitive strain. Selection of doses for these studies as well as predictive interpretation of results and relevance to human risk are the key components of drug safety assessment as described above. Also, it is vital to keep in mind that the potential benefit is just as important as the potential risk associated with a novel therapeutic.

3.3. A pathologist's perspective on target organ toxicity (Ken Frazier, GlaxoSmithKline, King of Prussia, PA, USA)

Dr. Ken Frazier presented a lecture on target organ toxicity and the critical role that the pathologist assumes in preclinical toxicology studies. The pathologist helps develop protocols regarding which tissues to evaluate, assigns and evaluates clinical pathology parameters, determines appropriate recovery phases if necessary, and supervises necropsy. Most importantly the pathologist identifies lesions, interprets treatment-related macroscopic and microscopic findings, and determines cause of death by assessing target organ toxicity. Multiple examples of important and common lesions noted in animal studies were presented, with their clinical implications, in liver, kidney, heart, bone and the blood (Frazier et al., 2012). Common spontaneous background changes lacking clinical relevance were also discussed, such as chronic progressive nephropathy, rodent cardiomyopathy and amyloidosis. While liver, kidney and heart are the most common toxicologic target organs for candidate drugs, it was stressed that any organ may be affected by drug treatment and a complete list of tissues are examined macroscopically and microscopically in GLP animal studies to support first-in-human and later clinical trials (Frazier & Seely, 2018). Pre-clinical drug-related findings do not necessarily prohibit the initiation of clinical trials, but are instead utilized to set starting doses in the clinic and to provide important information for clinicians for monitoring patients. Clinical margins are extremely important as is the nature of the finding, so the dose and exposure at which changes occur are equally important in gauging the potential toxicity of a candidate drug or compound. The role of the pathologist in tumor diagnosis in carcinogenicity studies and clinical relevance of findings were also discussed.

3.4. A pathologist's perspective on adverse vs non-adverse (Ken Frazier, GlaxoSmithKline, King of Prussia, PA, USA)

In Dr. Frazier's second lecture, he discussed the importance of assessing adversity in preclinical toxicity studies and how adversity calls are determined. The "no observed adverse effect level" (NOAEL), as discussed in earlier lectures, is expected on reports describing good laboratory practice (GLP) preclinical toxicity studies by the Food and Drug Administration, European Medicines Agency, and all other governing bodies, but adversity can be difficult to apply in practice to many drug-related effects (Kerlin et al., 2016). Adversity calls in a preclinical report can highlight potential safety issues that may need to be addressed in the clinical program by clinical safety personnel and these adversity calls can impact clinical starting doses and clinical dosing limitations. There are many nuances and challenges in determining and interpreting the NOAEL with consistency. A summary definition for NOAEL and guidelines for its components have been agreed upon by the toxicology community to facilitate consistency and compatibility across institutions and among individual pathologists (Kerlin et al., 2016). The NOAEL as described above (see section 3.2) is a key component of risk assessment that needs to be established for each drug candidate. To evaluate the potential for risk in humans, one or more multi-dose animal studies are used to establish the highest level that does not produce harmful (*i.e.*, adverse) effects. The benchmark dose (BMD), which employs data modeling to examine the dose response, is used in some settings as an alternative approach to the NOAEL. Study NOAELs are established at the level of the overall study report, but each finding in sub-reports (pathology contribution or clinical pathology contribution, *etc.*) can be classified as adverse or not, at a particular dose level. A finding may be non-adverse at a low dose, but at the high dose may be of sufficient severity to be considered adverse. Test article-related effects should be described on their own merits within a study, and not on whether they may progress with increased dose or longer duration treatment. Organ effects in a study which are not related to test article are not evidence for adversity. Adversity does not depend on reversibility and thus whether a drug effect recovers from injury is considered separately and is not an aspect of the adversity call. In preclinical summary documents, NOAELs from multiple studies are considered together to define the most important adverse responses in the most sensitive species. Some effects that can be modulated by drug treatment represent exacerbations of incidental background changes that are irrelevant and/or have no known human counterparts. When combined with other information on mechanism, species sensitivity, reversibility and progression, kinetics and metabolism, data on important adverse effects in preclinical species can best inform human risk assessment (Palazzi et al., 2016). A case example was presented involving hyaline droplets in the kidney to allow congress participants a chance to practice in adversity assignment of a specific target organ toxicity.

3.5. Biologics development and immunotoxicology (Danuta Herzyk, Merck Sharp & Dohme Corp., a subsidiary of Merck & co., Inc., Kenilworth, NJ, USA)

This lecture by Dr. Herzyk was focused on biopharmaceuticals. Biopharmaceuticals (also called 'biologics') are protein-based human-specific large molecules that differ in many respects from chemical drugs and their development requires many considerations that are unique to this group of medicines. Biopharmaceuticals are made in living cells from plants, yeast, bacteria, insects or mammalian organs (*e.g.*, Chinese hamster ovary) *via* DNA recombinant technology. They are human protein analogs of large size (molecular weight, MW, of 5000 Da – 150,000 Da) and complexity with a highly ordered structure (primary, secondary and tertiary) as well as being markedly heterogeneous in nature. Because of their large size, biologics (in contrast to chemical drugs with MW of 200–500 Da) do not enter intracellular organelles, thus do not interact with genetic material, and do not

interact with ion channels that play a crucial role in cardiac electrophysiology. Because complex protein engineering is applied in constructing novel biologics, the production process of a biologic drug is difficult to duplicate by others or create a copy of the same molecule using different cell lines, expression systems, *etc.* In the world of biologics, "the process is the product". Highly specialized analytical methods need to be developed to measure and control the stability and other physicochemical properties of biologics. In addition, their detection in blood for pharmacokinetic evaluation often requires unique, molecule-specific techniques, *e.g.*, immunoassays or cell-based assays.

As protein-based molecules, biopharmaceuticals are sensitive to proteolytic enzymes present in the digestive system, therefore, they cannot be administered orally and need to be injected, typically intravenously or subcutaneously. As analogs to human native proteins, after administration biopharmaceuticals are catabolized and excreted (mainly *via* kidney) like other physiologic proteins. Also, biopharmaceuticals typically have longer half-life in the blood circulation (*i.e.*, days to weeks), which allows for less frequent administration compared to chemical drugs. Most importantly, biologic drugs bind to desired therapeutic targets, *i.e.*, soluble or cell surface receptors, with high specificity, therefore do not exhibit off-target activity or unexpected toxicity and have very good safety profiles. There are multiple types and classes of biopharmaceuticals, which include such constructs as monoclonal antibodies and their derivatives, *e.g.*, fragments or fusion proteins; recombinant proteins, *e.g.*, physiologically deficient hormones, enzymes or cytokines; multi-specific antibodies; and nanobodies. Other types of complex biopharmaceuticals include vaccines and viral vectors as well as emerging cell therapies and gene therapies. The type of biopharmaceutical candidate often determines the design and approaches to its safety testing and toxicology studies. Particularly, in-depth understanding of the biology of a modulated target by a novel biopharmaceutical plays a crucial role in the selection of appropriate and relevant animal species for the evaluation of its safety. Also, biologic drugs, being large proteins with some "unnatural" modifications, have a potential to be recognized by a treated host's immune system and result in production of anti-drug antibodies. Such anti-drug antibody responses to the treatment, often referred to as immunogenicity, needs to be considered in toxicology studies in animals. During the lecture, Dr. Herzyk provided reference to the key regulatory guidance, ICH S6(R1) (Anon, 2011) and its salient points that give directions to animal species selection as well as immunogenicity evaluation in preclinical studies with biologic molecules (Ponce et al., 2009). Many unique features of biologic drug candidates and recommendations for their safety testing are also addressed in published literature (Cavagnaro, 2008; Plitnick & Herzyk, 2013). Those aspects were discussed using "compare and contrast" summary of approaches and studies done with biologics vs chemical drug candidates. The last part of the lecture highlighted the evaluation of potential immunotoxicity of different types of drug candidates. There is a specific ICH S8 guidance addressing this topic (Anon, 2005b), which is focused on drugs that are not intended to affect the immune system. However, the real challenge in drug safety assessment is to evaluate the immunotoxicity potential of drug candidates (both chemical and biologics) that modulate immune function by design. While we have a "tool box" with multiple *in vitro*, *ex vivo* and *in vivo* models to study the immune system and its responses to a drug treatment, we need to better understand inherent risks of immunomodulatory therapeutics during early non-clinical stages of drug development, particularly for novel drugs to treat immuno-oncology and immuno-inflammatory diseases.

3.6. Emerging technologies and novel therapeutic modalities

Rapid technological advances have a big impact on pharmaceutical drug development. Novel technology is being adapted to methods and models used both as *in vitro* and *in vivo* systems. Particularly, availability of transgenic mice, novel imaging techniques and reagents

enabling the testing of materials and tissues at the molecular and genetic level all play increasing roles in methodologies applied in drug safety evaluation. In addition, we are facing an increasing number of modalities as potential medicines, which include highly engineered biochemical molecules, vaccines, human cells and genetic elements, viral components, and hybrids of the above. Emergence of such unconventional therapeutic modalities brings many challenges into pharmaceutical drug development, including approaches to safety assessment. Dr. Herzyk presented a comprehensive survey of both these challenges and the unprecedented therapeutic potential of these novel approaches.

3.7. Carcinogenicity case study (James Smith, Boehringer-Ingelheim, Ridgefield, CT)

Mr. James Smith brought together many of the concepts presented during the toxicology session in the final presentation of the session: a case study of the investigation undertaken to de-risk a promising new therapeutic after unforeseen identification of a carcinogenicity risk late in clinical development.

Empagliflozin, a sodium glucose co-transporter 2 (SGLT2) inhibitor, indicated to lower blood glucose in the treatment of type 2 diabetes, had successfully passed Phase 1 studies when a histopathology finding of increased renal tumor incidence in male mice in a 2-year carcinogenicity study necessitated comprehensive mechanistic studies to de-risk the compound and enable further clinical progression. This finding was unexpected, as it was not directly attributable to suprapharmacology, was not observed in rats or dogs (Bogdanffy, Stachlewitz, van Tongeren, et al., 2014; Knight, Yuan, Kogler, et al., 2018) and empagliflozin was not shown to be genotoxic in standard assays.

Due to the type of finding, a definition of the mechanism of action leading to the lesion was deemed more important than safety margins. Establishment of a chain of pathological events resulting in tumor formation was undertaken as well as the characterization of the human relevance *versus* mouse specificity of each step. The key events, which are all necessary but not individually sufficient, were: 1) a predisposing cystogenic background in mice, 2) added SGLT2 pharmacology-related renal stress, 3) non-pharmacology-related renal stress, 4) exhaustion of stress handling reserves, and 4) conversion to constitutive cell growth. Experimental investigations of the key events were done using *in vivo* and *in vitro* studies. A 13-week investigative pathogenesis study in mice identified gender differences in gene expression patterns, renal tubular flow and non-neoplastic degenerative/regenerative renal histopathology preceding neoplasia (Knight et al., 2018). In addition to suprapharmacology-related osmotic diuresis, these findings support a non-genotoxic mode of action. The weight of evidence was increased by the *in vitro* identification of a male mouse-predominant renal metabolite that is not present in humans and which leads to the generation of a highly reactive aldehyde (Taub, Ludwig-Schwellinger, Ishiguro, et al., 2015). Further characterization of this metabolite confirmed that it was cytotoxic but not genotoxic (Smith, Huang, Escobar, et al., 2017), while empagliflozin was neither. Finally, renal *in vivo* gene expression data confirmed the temporal profile of exhaustion or stress handling reserves and the switch to constitutive cell growth (Knight et al., 2018). In summary, empagliflozin induces pharmacology-related renal tubular injury in the male mouse, exacerbating a genetic background predisposing cystogenic changes in males. Further renal stress is imposed by nephrotoxic effects of a male mouse-predominant renal aldehydic metabolite and oxidative stress facilitated by a predominantly oxidative pathway of empagliflozin metabolism, which differs from the human metabolic pathway. The weight of evidence on genotoxicity, gender and mouse strain specificity, and the dose-response and temporal relationships is consistent with a non-genotoxic mode-of-action having no relevance to humans. This approach was used to enable successful product launch.

4. Animal models for use in Safety Pharmacology & Toxicology

4.1. Modeling human disease for preclinical testing (Karen Svenson, The Jackson Laboratory, USA)

Dr. Svenson presented a brief overview of The Jackson Laboratory past and present and highlighted important considerations for building and testing preclinical mouse models.

The Jackson Laboratory (JAX) is often perceived as only a distributor of research mice and its activities as a non-profit research institute are not as well known. The Laboratory was established in 1929 by scientists who believed in the genetic etiology of cancer. Now with four campuses in the US, JAX comprises more than 50 Principal Investigator-lead programs in a broad range of disciplines and supports graduate and postgraduate training programs. The Laboratory has been a National Cancer Institute-designated Cancer Center since 1983. Campuses are defined by their focus areas: Mammalian Genetics; Genomic and Precision Medicine; *In vivo*, cancer and stem cell services; and Production and Distribution of mice. In addition to a diversity of inbred, hybrid and outbred strains with various features of numerous human diseases, the laboratory offers highly specialized engineered mouse models and serves as a repository for externally-derived models. Of particular use in studying cancer, inflammatory and infectious disease and hematopoiesis are a series of humanized immunodeficient models, popularly used for checkpoint inhibitor development and efficacy testing of novel immunotherapies. A large library of patient-derived xenograft (PDX) models are available to guide clinical approaches, and can be used by investigator-initiated projects or in JAX-directed preclinical efficacy studies. Often working with Foundations to import and distribute models with minimal licensing barriers, numerous preclinical mouse models have been developed by enhancing and refining existing disease models. Examples presented include improved models for Spinal Muscular Atrophy (SMA) and Duchenne Muscular Dystrophy. By partnering with Foundations, pharmaceutical companies and biotechnology firms, JAX has established the Rare and Orphan Disease Center to establish relevant models for use in pre-clinical testing platforms including performing dosing studies.

Development of a robust preclinical mouse model can be approached in more than one way. A gene-centric approach starts with single mouse or human target genes identified as having relevance to human disease and presents them in well-defined genetic backgrounds. Relevant genes can be ascertained using epidemiological data, data from genome-wide association studies, or from basic research-driven strategies revealing candidate genes involved in the etiology of disease processes. Because mice and humans share many of the same diseases, another approach is to survey existing inbred and engineered strains for phenotypes that recapitulate key features of human diseases under standard or manipulated environmental conditions, such as dietary challenges. Models should follow an optimal timeline for disease progression that closely follows relative progression in humans; not too fast and not too slow, so that interventions can be made and responses can be tracked. Models must be phenotypically robust and highly penetrant. Females and males must be used, unless the disease of interest is clearly sex-specific. Using only one sex can lead to clinical trial and market failures by not considering effects of treatments in both sexes. Different mouse strains generally have very different genetic backgrounds, and therefore it is imperative that a survey of at least a limited panel of strains is performed to assess the disease of interest and how a genetic manipulation behaves under varied backgrounds. Humans also have varied genetic makeup and one size does not fit all. An example of the diversity in side effects of chemotherapy drugs was presented to demonstrate that using just one mouse strain for drug testing would be comparable to testing a drug only in identical twins. The utility of a recently developed outbred strain was presented as a better system to model variation in toxicity (Svenson et al., 2012). Finally, given the important revelations emerging about the role of the gut microbiome in

disease, Dr. Svenson urged the inclusion of microbial profiling in evaluating preclinical models, both before and after drug treatment. Mouse resources, investigator profiles and educational opportunities can be further queried at <http://www.jax.org>.

4.2. Designing new genetically altered (GA) mouse lines for preclinical research (Sara Wells, MRC Harwell Institute, UK)

For many years' geneticists have been altering the mouse genome, initially in individual laboratories and over the last decade in larger global consortia (www.mousephenotype.org) providing large catalogues of genetically altered (GA) strains for the biomedical research community. More recently the prolific use of genome editing tools has accelerated the number of novel strains available. These truly are exciting times for genetic research as accessibility to GA models (not just of mice) becomes easier and their inclusion in more scientific programs accelerates. The speed and rapid expansion in the use of genetic engineering techniques also comes with the responsibility of rigorous quality control at all levels from the wild type strains used for modifications to stringent analysis of the structure of novel alleles generated and well-designed, controlled characterizations of new strains. CRISPR/Cas 9 and other technologies are opening up a whole new range of possibilities in terms of altering the genome and refining animal models. This technology significantly shortened the period of time (less than 6 months) taken to generate mutations in the mouse genome that can faithfully recapitulate those in human genomic studies. However, these molecular tools are still in development and a significant amount of quality control is required to ensure that unintended alterations at the targeted loci or elsewhere in the genome have not occurred as these pose a significant risk to the integrity of the experiment (Mianné et al., 2017). Lastly, as our ability to hop and chop around the genome increases, this must come with a concurrent increase in the sophistication in the technologies we are using to analyze the GA strains and a deepening of the ethical discussions ensuring that using a live animal is the only option. Automation and non-subjective phenotyping will play a pivotal role in the robustness and reproducibility of data delivered in the future (Bains et al., 2016). We are in extraordinary times of huge advancements in genetic science providing the opportunity to close both the molecular and physiological gap between model organisms and humans. Rigorous control of genetic integrity and experimental design will be essential if this prospect is to be realized.

4.3. The INFRAFRONTIER Research Infrastructure – resources and services to advance the understanding of human health and disease using mammalian models (Asrar Ali Khan, INFRAFRONTIER GmbH, Germany)

Dr. Ali Khan provided an overview of INFRAFRONTIER, the European Research Infrastructure for the development, phenotyping, archiving and distribution of model mammalian genomes. The INFRAFRONTIER Research Infrastructure provides access to first-class tools and data for biomedical research, and thereby contributes to improving the understanding of gene function in human health and disease using mice (Raess et al., 2016). The INFRAFRONTIER network currently consists of 29 partners that are engaged in several European Community funded projects, such as INFRAFRONTIER2020, Research Infrastructure for Phenotyping, Archiving and Distribution of Mouse Diseases Models (IPAD-MD) and CORBEL, and contributes to the International Mouse Phenotyping Consortium (IMPC). The core services of INFRAFRONTIER comprises of model generation, specialized phenotyping services, systemic phenotyping of mouse mutants in the participating mouse clinics, as well as archiving and distribution of mouse mutant lines by the European Mouse Mutant Archive (EMMA). INFRAFRONTIER also offers specialized services, such as the generation of germ-free mice (axenic service) and training in state-of-the-art cryopreservation and phenotyping technologies. Reduction and refinement to improve animal welfare are among the major goals of INFRAFRONTIER'S

technology development programme. To promote international co-operation and facilitate access to the global biomedical research community, INFRAFRONTIER offers trans-national access calls for projects and provides funding based on proposal merit (INFRAFRONTIER Consortium, 2015). So far 25 open calls have funded 378 user projects with several high impact publications. The EMMA branch of INFRAFRONTIER offers the worldwide scientific community a free archiving service for its mutant mouse lines and access to a wide range of disease models and other research tools. EMMA currently holds nearly 7000 mutant mouse strains, half of which have been produced from the International Mouse Knockout Consortium (IKMC) resource. The EMMA network is comprised of 16 partners from 13 countries that operate as the primary mouse repository in Europe. The afore-mentioned IPAD-MD project addresses global cooperation and coordination between the pan-European INFRAFRONTIER Research Infrastructure and complementary research infrastructures in America, Asia and Australia contributing to the global effort of the IMPC. The IPAD-MD project also reaches out to complementary infrastructures and user communities in Africa.

4.4. The International Mouse Phenotyping Consortium (IMPC) - Phenotyping mice for drug development (Tertius Hough, MRC Harwell Institute, UK)

The International Mouse Phenotyping Consortium (IMPC) is currently composed of 19 research institutions and 5 national funders from 11 countries. The consortium is building a catalogue of mammalian gene function by producing and phenotyping a knockout line for every protein-coding gene in the mouse genome (Dickinson, Flenniken, Ji, et al., 2017). This involves the creation of 20,000 knockout mouse strains on an isogenic C57BL/6N background and characterizing each strain through a standardized phenotyping protocol. Embryonic, Adult and Terminal phenotyping pipelines were designed to assess embryonic, neuromuscular, sensory, cardiovascular, metabolic, hematological, and neurological parameters (Brown, Holmes, Mallon, et al., 2018). The associated phenotyping protocols were established by specialist working groups are continually refined and are freely available from The International Mouse Phenotyping Resource of Standardized Screens (IMPRESS, see: www.mousephenotype.org/impress). Teams of dedicated data wranglers perform rigorous quality control and statistical analysis on the data submitted to a central database. Further analysis of these data includes disease / phenotype associations and visualizations. All data are freely available via the IMPC portal (see: www.mousephenotype.org). To date, the IMPC has generated and characterized over 5200 mutant lines. One-third of the lines have been found to be non-viable and over 300 new mouse models of human disease have been identified so far (Meehan, Conte, West, et al., 2017)). The mouse strains that have been generated are deposited in the KOMP repository and the European Mutant Mouse Archive (EMMA). The integration of this valuable resource of phenotyping data with data from the human genome is a powerful approach for the interpretation of human genetic variation and its relationship to disease. In this way, the IMPC is aiding in the identification of candidate genes for studying human disease conditions and delivering information about the underlying mechanisms (Rozman, Rathkolb, Oestereicher, et al., 2018)). Access to the KO mouse strains and the freely available phenotyping data produced by the IMPC consortium also represents a valuable resource for drug discovery and target validation.

5. Drug safety and the preclinical drug development platform (PCDDP)

The PCDDP is a state-of-the-art facility situated on the Potchefstroom campus of the North-West University in South Africa and was established in 2011. This platform is meant to function as a national preclinical testing platform to conduct qualitative and

quantitative preclinical studies for companies and research institutes in Africa ultimately enabling South Africa to play a significant role in the production of drugs and phyto-medicines. The scientists at the PCDDP conduct safety pharmacology, pharmacokinetic and selected toxicology studies for new compounds and/or formulations. The facility also develops, establishes and maintains preclinical animal models related to the treatment of many disorders including infectious disease, neurobiology and other chronic disease states. The PCDDP includes a state of the art rodent vivarium that can breed rats and mice for use in studies and permits the training of laboratory scientists to conduct of a wide range of drug efficacy and safety studies. The vivarium is the first Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International-accredited animal facility in sub-Saharan Africa and is also qualified for the conduct of Good Laboratory Practice (GLP) studies. The PCDDP has prioritized the establishment of *in vitro* electrophysiological and *in vivo* SP models to conduct core battery (*i.e.*, CNS, CV and respiratory) evaluations of NCEs in development by academics and pharmaceutical/biotechnology companies in South Africa. Thus, this meeting drew preclinical drug development experts from across the globe to South Africa to assist in enhancing the capability of South Africa to participate in cutting-edge research and drug development.

6. Abstracts overview

There were 18 posters presented at the meeting consisting of topics ranging from selective targeting of cancer cells by using novel drug delivery systems to the use of cytokines as indicators for drug safety. These were presented primarily by students from across Africa. These abstracts can be found elsewhere in this edition of the *Journal of Pharmacological & Toxicological Methods*.

7. Conclusions

The first Drug Safety Africa Meeting in Potchefstroom, SA offered the attendees a wide range of topics covering safety pharmacology and toxicology study designs, models and issues that may be encountered. Both sessions were marked with many highlights since both provoked important questions and exciting discussions by meeting participants. Overall, attendees (student, faculty and industry scientists) generally felt this to be a highly successful meeting with the establishment of many new scientific relationships. The establishment of a network of scientists in Southern Africa with an interest in drug safety and with connections to international scientists with similar interests is a cornerstone for advancing drug safety in Africa. Given the success of this meeting, plans for a subsequent meeting are already ongoing.

Disclaimer

The opinions presented here are those of the authors. No official support or endorsement by participating companies is intended or should be inferred.

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Conflict of interest

None of the authors have any conflicts of interest, other than their employment in commercial pharmaceutical companies, academic institutions, or contract research organizations (CROs). No information is presented that advocates for or promotes commercial products from any organization.

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