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EMERGING TECHNOLOGIES AND THEIR ROLE IN REGULATORY REVIEW

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1.1 INTRODUCTION

The sequencing of the human genome and the emergence of other omics-based technologies have provided drug discoverers with powerful new tools that can be used as a framework for understanding disease mechanisms and predicting patient outcomes (Venter, 2000; Venter et al., 2001; Castle et al., 2002; Kennedy, 2002; Goodsaid, 2003; Guerreiro et al., 2003; Witzmann and Grant, 2003; Walgren and Thompson, 2004; Robertson, 2005; Kell, 2006; Lindon et al., 2007; Clarke and Haselden, 2008). Since the turn of the century, pharmaceutical scientists have been able to incorporate these approaches into their work: to identify specific molecular targets involved in disease initiation and progression; to establish links between animal models and clinical activity at the level of genes, proteins, and pathways; and to devise new ways of measuring and monitoring drug response. In contrast to finding drugs that act at proven drug targets and behaved "correctly" in established preclinical tests, discovery efforts were directed toward screening against sets of novel and sometimes closely related molecular targets that had not yet been thoroughly validated in medical practice, using new preclinical models and assays to confirm therapeutic benefits and define potential toxicities, and streamlined development strategies to obtain early proof of concept in clinical trials (Food Drug Administration, 2006a; Sarapa, 2007; Butz and Morelli, 2008; Takimoto, 2008). Importantly, the vast multidimensional data sets generated by genomics, proteomics, metabolomics, and other reductionist approaches were accompanied by the development of new computational methods needed to cut through the noise and variability associated with in these complex measurements and to assign therapeutic significance to the data. The emergence of systems biology provided an organizational framework that attempted to address the need to reconstitute these data sets into a functioning organic whole (Butcher et al., 2004; Hood and Perlmutter, 2004; Fischer, 2005; Edwards and Preston, 2008).

Not surprisingly, as more innovation and opportunity entered the drug discovery process, the risk of clinical failure did not always go down, except perhaps in cases where disease or toxicity was found to have a relatively straightforward etiology involving a single gene or a well-characterized and understood biochemical process. Despite impressive technological advances, late-stage attrition remained a problem in drug development, and serious and sometimes rare or unexpected adverse events continued to be seen during clinical investigations or postapproval (Arrowsmith, 2011a, b; Arrowsmith and Miller, 2013). Regulatory agencies interpreted this unexpected attrition to indicate that critical

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gaps still existed in the preclinical testing pathway and the translation of preclinical toxicology findings to clinical outcomes of interest. Some of these critical gaps can be traced to how regulatory toxicology studies are currently conducted. These studies tend to use healthy animals and are designed to identify robust toxicities that depend on dose and exposure rather than conditional effects triggered by individual susceptibilities or interactions with disease and disease comorbidities. Toxicology studies are also designed to characterize the possibility and type of toxicity and to suggest an initial "safe" human dose range rather than to determine the expected clinical prevalence and magnitude of the effect. In some cases, species differences in basic physiology and how a drug may be transported or biotransformed will confound the translation of preclinical findings to human patients. As a result, while preclinical safety data can reasonably predict clinical risk under appropriate testing conditions (Ewart et al., 2014; Holzgrefe et al., 2014), a lack of concordance can sometimes be found between preclinical and clinical findings, including the observation of toxicities in animal models that have no observed correlate in clinical experience (Olson et al., 1998, 2000; Alden et al., 2011; Wang and Gray, 2014).

To help address these issues and promote the advancement of new technologies, the FDA has issued several documents that define key regulatory science priorities as well as a process for introducing new tools into drug development. Beginning with the publication of the FDA's Critical Path Initiative and Opportunities List in 2004, these documents highlight the need for new methods in toxicology, including the evaluation and development of more predictive models and assays; the identification and performance characterization of more reliable biomarkers; and the application of *in silico* approaches and large data sets to organize and interpret diverse safety data (Food Drug Administration, 2004a, b, 2006b, 2011; Woodcock, 2007). In parallel and in response, the pace of scientific innovation has accelerated, with numerous emerging technologies being positioned as transformative new drug development tools with the potential to improve safety assessment and reduce the possibility of late-stage attrition. Recent attempts to "humanize" animal models (Cheung and Gonzalez, 2008; Zhang et al., 2009; Shultz et al., 2012) and to replicate human response *in vitro* using organotypic cultures (Schmeichel and Bissell, 2003; Huh et al., 2011; Mathur et al., 2013; Sung et al., 2013; Abaci and Shuler, 2015) and induced pluripotent stem cells (iPSCs) (Sirenko et al., 2013, 2014a, b; Kolaja, 2014; Doherty et al., 2015) have opened additional avenues for assessing human drug safety and efficacy. New *in silico* and *in vitro* approaches are being proposed to assess the risk of drug-induced proarrhythmia (Mirams et al., 2011, 2012; Johannesen et al., 2014; Sager et al., 2014) and to strengthen safety signals detected during postmarket pharmacovigilance (Szarfman et al., 2004; Harpaz et al., 2013; Liu et al., 2013; White et al., 2013).

In some cases, new regulatory pathways have been developed to improve the prediction of clinical risk based on fresh insights into toxicity mechanisms. One example is using assays based on the human ether-a-go-go-related gene (hERG) channel, which is believed to encode the native cardiac potassium channel responsible for generating the rapid delayed rectifier potassium current (IKr) in the human heart (Kiehn et al., 1995; Sanguinetti et al., 1995). The recognition that some drugs can trigger torsade de pointes (TdP), a serious and usually fatal cardiac arrhythmia, by excessively prolonging ventricular repolarization through block of IKr led to the development of a new approach for assessing cardiac safety, currently embodied in the International Council on Harmonisation (ICH) S7B and E14 guidelines (FDA, 2005a, b; ICH, 2005). This new pathway involves testing drug effects on the hERG channel in a clonal cell line expression system (Hammond and Pollard, 2005), with confirmation of any notable findings in the clinical Thorough QT (TQT) study, which measures changes in the electrocardiographic QT interval (Darpo et al., 2006).

The purpose of this chapter is to identify specific questions that may arise when evaluating the potential regulatory impact of a new technology as well as the type of criteria that can be used to determine whether a new tool has general applicability as a basis for regulatory decision-making in drug development.

1.2 SAFETY ASSESSMENT IN DRUG DEVELOPMENT AND REVIEW

1.2.1 Drug Discovery

The likelihood that a new chemical will become a safe and effective therapeutic product is typically assessed at multiple stages in the drug development process. In the discovery phase, potential drug candidates are screened broadly for toxicity issues to eliminate those with obvious liabilities, using a variety of methods including computational analyses based on chemical structures or the evaluation of possible off-target effects in comprehensive panels of *in vitro* assays covering a wide range of pharmacological targets and activity endpoints. It is important to recognize that there are no specific regulatory recommendations governing how early assessments of drug safety should be made. It is up to the sponsor to determine the specific technologies and acceptance criteria needed to support advancing a candidate to the next decision point. The scope and thoroughness of the testing done at this stage of development are intended to provide comfort to the sponsor that the candidate drug warrants further investment. Early adopters of emerging technologies may use novel data sets to complement and support the results obtained in more traditional studies, but the weight given these additional data will depend on the level of comfort that management has in the credibility of the assay and the degree to which the technology has been validated. In all cases, the decisions made during the discovery phase will be

company specific and shaped by current knowledge about the molecular target and concerns about the pharmacologic class or therapeutic indication, some of which may be known publicly but much of which may be proprietary to the company and contained in its base of institutional knowledge. For example, structural alerts generated by quantitative structure–activity relationship ((Q)SAR) models are commonly used during lead optimization to flag potential drug candidates based on their predicted safety profiles (Kruhlak et al., 2012). Measuring the transcriptional changes generated by a drug candidate and comparing them to a reference database of standard known toxicants is another example of exploratory research that can be conducted on to assess and reduce risk in candidate selection (Ganter et al., 2006; Judson et al., 2012; Bouhifd et al., 2015). These types of early evaluation typically combine the use of commercial assay kits, models, and analytical tools integrated with unique methods and data sets developed internally by each company.

1.2.2 Preclinical Development

As a drug candidate advance from lead selection into preclinical development, the safety studies conducted take on increasing importance in shaping the downstream development program and its probability of success. Rather than supporting the feasibility of a particular lead candidate within a company's larger research and development portfolio, study results now become the basis for a series of regulatory decisions that will inform the design, cost, and duration of the clinical development program. The appearance of organ toxicities in animal studies will define the dose ranges expected to be safely tolerated in humans and the drug concentrations that can be targeted to explore compound efficacy as fully as possible. While some toxicology studies are typically done later in development (e.g., carcinogenicity, reproductive toxicology), the earliest toxicology studies are intended to select a safe starting dose for humans and address the following specific questions: (i) Is there one or more target organ toxicities and are these toxicities reversible? (ii) What is the margin of safety between a clinical and a toxic dose? (iii) Can the relationship between critical pharmacodynamic–toxicodynamic endpoints and pharmacokinetic parameters be predicted?

Regulatory guidelines currently exist for the conduct of the toxicology and safety pharmacology studies intended to characterize the toxicities that might be expected to occur under the conditions of the proposed clinical trials (International Council on Harmonization, 2001, 2010; Food Drug Administration, 2005a). Safety pharmacology studies evaluate the functional effects of a candidate drug on a core battery of key organ systems (cardiovascular, central nervous system, respiratory) using therapeutic plasma concentrations and above. In designing a safety pharmacology program to support a new regulatory submission, the ICH S7A Tripartite Guideline encourages the use of new technologies and methodologies, as long as they are relevant, sound, and scientifically valid (International Council on Harmonization, 2010). Sponsors may select from a wide range of *in vivo* and *in vitro* test systems to identify adverse pharmacodynamic and/or pathophysiological effects and the mechanism(s) by which these effects are produced. Supplemental safety data can also be generated as needed for other organ systems, including renal/urinary, the autonomic nervous system, the gastrointestinal system, and others, when there may be reasons for concern. Compliance with the principles of good laboratory practices (GLP) is generally required in the conduct of these studies, to ensure the reliability and quality of the data obtained, with justification for any safety pharmacology and follow-up studies not conducted under GLP. However, studies intended to characterize the primary and secondary pharmacologic effects of a new drug candidate can be conducted under non-GLP conditions.

In conjunction with the series of core battery and supplemental safety pharmacology studies, and prior to the initiation of clinical trials, sponsors must also characterize the concentrations of drug achieved over a range of doses considered to be therapeutic and toxicological. In addition, information on how a drug is metabolized is important, to allow for a comparison of human and animal metabolites and their associated risk of producing toxicity. These data will be used to support the selection of the most appropriate species and dose regimen for the nonclinical toxicology studies and ultimately to relate exposure levels to toxicity findings.

Information on acute toxicity is used to predict human tolerability and the possible consequences of drug overdose. Typically, acute toxicity is assessed in a single-dose toxicology study conducted in two mammalian species (rodent and nonrodent) using the intended clinical route of administration as well as parenteral dosing, but other approaches can also be considered (e.g., dose escalation studies, short-duration dose-ranging studies, or studies that achieve large or maximal exposures). The need for repeat dose toxicology studies is determined by the expected duration of treatment, the therapeutic indication, and the nature of the clinical trials described in the clinical development plan. As a general rule, repeat dose studies are also conducted in two species with durations that are equal to or exceed the duration of the human clinical trials up to a maximum of 6 months (rodent species) and 9 months (nonrodent species), with a minimum of 2 weeks.

These preclinical toxicology studies provide an estimate of the first dose that can be used in human trials. The no observed adverse effect level (NOAEL) is defined by the FDA as "the highest dose tested in an animal species that does not produce a significant increase in adverse effects in comparison to the control group" (Food Drug Administration, 2005c). It is important to note that any observed adverse event that can be considered biologically significant will determine the NOAEL; there is no need to demonstrate that the observation is statistical significance. The findings that determine the NOAEL may include the observation of overt toxicity (e.g., clinical signs, gross and histopathology

lesions), changes in the levels of toxicity biomarkers (e.g., hepatic enzyme levels as surrogates for liver injury), and exaggerated pharmacodynamic effects. Once a NOAEL is determined, it is converted to a human equivalent dose (HED) using scaling techniques based on differences in body surface area between animals and humans. The lowest HED is obtained in the most sensitive animal species and usually informs the decision on initial clinical dosing, but in some cases, sponsors can justify using data from a less sensitive species and a higher HED if it can be argued as being more relevant in the assessment of human risk.

1.3 THE ROLE OF NEW TECHNOLOGIES IN REGULATORY SAFETY ASSESSMENT

Regulatory agencies have made a long-standing commitment to identify and promote the application of new technologies to drug, with the goal of reducing or replacing the need for animal studies and improving the prediction of clinical risk. However, before any advanced scientific method can be adopted as a basis for regulatory decision-making, it must be considered scientifically valid and be available to sponsors as a viable option for generating reliable and reproducible data. To assist researchers in gaining regulatory acceptance for new drug development tools, the FDA has established a formal qualification process that considers the requirements for establishing an assay as technically valid, as well as the process for generating the supporting data needed to define the specific utility of the measurement and the type of regulatory decisions it will be able to support (Food Drug Administration, 2014). Currently, the FDA's drug development tool process has centered on the qualification of three different types of tools: (i) new biomarkers intended for use in assessing drug safety and efficacy, (ii) patient reported outcome (PRO) rating instruments intended for use in clinical trials, and (iii) animal models intended to support product approval under the Animal Rule. However, the FDA's drug development tool process can also support other approaches as they become available. For example, *in vitro* assays may be determined to fall within the scope of the current process if they generate biomarkers used to predict drug safety. So far, it has been reported that five drug development tools have been qualified with ~80 applications being considered in the three qualification program areas noted earlier (Parekh et al., 2015), including biomarkers for monitoring renal and cardiac toxicity with better performance characteristics than conventional surrogates (Dieterle et al., 2010; Harpur et al., 2011; Hausner et al., 2013; Ennulat and Adler, 2015). Research within the FDA has focused on collaborating in the collection of the qualification data sets and on evaluating and setting standards for data quality and the analytical methods used to anchor biomarker performance to the endpoints of interest (Rouse et al., 2011, 2012, 2014; Goodwin et al., 2014; Shea et al., 2014; Amur et al., 2015; Rouse, 2015).

While the FDA's formal drug development tool qualification process currently does not extend beyond biomarkers, PROs, and animals models, the agency is considering other ways of recognizing the regulatory utility of an emerging technology and expressing confidence in its regulatory use. This includes issuing a "Letter of Acceptance" that deems a new tool "fit for purpose," such as was done to support the use of a simulation tool developed by the Critical Path Institute's Coalition Against Major Diseases (CAMD) as an aid in the design and interpretation of clinical trials for drugs intended to treat mild to moderate Alzheimer's disease (Rogers et al., 2012; Ito et al., 2013; Panegyres et al., 2014; Romero et al., 2015). By using this clinical trial simulation tool, which has been made available as a public resource, researchers can explore different outcomes in "virtual" Alzheimer's disease trials that build on knowledge about anonymized placebo responses extracted from prior clinical studies.

A key concept in the drug development tool qualification process is that of "context of use." The context of use is a clear and concise statement that specifies how and when the tool will be used in drug development and the conditional boundaries for its use as justified by the data submitted to support its qualification. The context of use is described in terms of its general area of use (e.g., nonclinical or clinical, pharmacodynamics, disease, or toxicology), its specific area of use (e.g., in clinical trial design, disease monitoring, dose or patient selection, assessment of drug effects including efficacy and toxicity), the critical parameters governing its use (e.g., drug or drug class specific, prognostic or diagnostic, type of assay platform), and the specific regulatory decision it is intended to inform. For the qualification of animal models, the context of use statement must include those details needed to replicate the model, including a description of the animals and challenge agent to be used, treatment information, descriptions of the primary and secondary endpoints, and the value ranges for the quality criteria determining successful implementation of the model in other labs.

1.3.1 *In Silico* Models for Toxicity Prediction

Drug developers and regulatory agencies already rely heavily on the use of modeling and simulation technologies to guide decision-making and to predict clinical outcomes. *In silico* models are used throughout drug development, early on in discovery to help identify and validate new drug targets, later in development to select appropriate doses for first-in-human trials and to estimate doses in special populations, and in all phases to set boundaries on the types of drug product manufacturing changes permitted under quality by design. However, unlike the assays and biomarkers considered under the FDA's drug development tool guidance, computational models are viewed as dynamic and in need of revision as

soon as new knowledge becomes available about the chemical and biological process they are intended to represent. Consequently, modeling and simulation in drug development are seen as "fit for purpose" and tightly constrained by the specific data sets used to calibrate and validate model performance.

While the current drug development tool qualification process does not extend to the use of *in silico* models, the recent ICH M7 guidelines issued for the use of (Q)SAR models to assess the genotoxicity of drugs, metabolites, and product contaminants/impurities refer to a set of principles for model validation developed by the Organisation for Economic Co-operation and Development (OECD) (International Council on Harmonization, 2014). The OECD principles state that, to be considered valid for regulatory use, a (Q)SAR model should be associated with the following information: a defined endpoint; an unambiguous algorithm; a defined domain of applicability (i.e., context of use); appropriate measures of goodness of fit, robustness, and predictivity; and a mechanistic interpretation, if possible. There are clear parallels between these requirements and those applied to the technical validation and qualification of new drug development tools as currently implemented by the FDA. This may be useful to consider as a framework for evaluating the general regulatory utility of an *in silico* model.

1.3.2 Cell-Based Assays for Toxicity Prediction

As noted previously, the purpose of preclinical toxicology testing is to identify potential organ toxicities and the drug levels at which they occur so that a safe starting dose in human trials can be determined. New technologies intended to replace or supplement existing safety assessment pathways should have this as their ultimate goal. In cases where *in vitro* assays using human cells or cell lines are used, including iPSC-derived organotypic cells, initial questions to be asked include: (i) How closely does the assay replicate or predict the human outcome of interest? (ii) Can the assay provide knowledge about the drug concentration ranges producing the effect? (iii) Are the results sufficiently robust and reproducible across laboratories and studies to support a regulatory (vs. company internal) decision on product safety? In addition, it will be important to demonstrate that the relevant drug effects on the specific endpoints of interest can be distinguished from changes seen solely due to experimental constraints and conditions. Finally, concordance should be demonstrated with current approaches before new technologies are adopted for regulatory use.

One example of a cell-based assay that has been successfully incorporated into the safety assessment pathway is the assessment of drug-induced proarrhythmia risk based on block of the cardiac repolarization current IKr and the clinical assessment of the electrocardiographic QT interval, as discussed in the ICH S7B and ICH E14 harmonized guidelines. While the regulatory recommendations for assessing IKr pharmacology are quite broad and allow for the use of either native or expressed channels as systems for the study of IKr pharmacology, heterologous expression of the hERG channel in a clonal cell line is widely used as a readily accessible human test system that meets the basic requirements for accepted regulatory use: it is scientifically valid and robust, assay protocols can be standardized, the results are reasonably reproducible, and the measured endpoint is considered relevant for assessing human risk. The assay is also attractive for drug developers because it can be performed using either manual or high-throughput automated patch clamp methods, making it possible to screen larger compound libraries in the drug discovery phase prior to candidate selection. The hERG assay is most often conducted at room temperature using a hERG channel assembled as 1a subunits due to improved expression and ease of measurement (see, e.g., Chen et al., 2007), even though in the adult human heart the IKr channel appears to exist as the combination of 1a/1b subunits (London et al., 1997; Jones et al., 2004, 2014). Studies have shown that heteromeric hERG 1a/1b currents are much larger in magnitude and exhibit faster gating kinetics than channels composed of hERG 1a subunits only (Sale et al., 2008), and also exhibit different drug sensitivities (Abi-Gerges et al., 2011), potentially confounding the assessment of clinical risk. The use of room temperature in the hERG assay represents an additional factor to consider when evaluating predictivity of the assay, as raising the temperature increases current magnitude and also speeds the kinetics of channel gating (Milnes et al., 2010). Finally, drug effects have been typically measured in terms of IC_{50} values, despite the recognition that channel block is dynamic with a marked dependence on transmembrane voltage, channel state, and the frequency of stimulation. A final challenge is in relating the concentration used *in vitro* to the drug concentrations predicted for efficacy, taking into account protein binding, to provide a window between therapeutic and toxic levels.

Despite these apparent limitations, the hERG assay and the subsequent clinical TQT study have been able to identify potentially torsadogenic drugs early on and prevent their entry into the market. However, some clinically important drugs have been found to block IKr and prolong the QT interval at therapeutic plasma concentrations, but not to be proarrhythmic. The almost decade of experience with the regulatory pathways outlined in ICH S7B and ICH E14 has indicated that while the hERG–TQT paradigm may be highly sensitive to potentially torsadogenic drugs, it is not very accurate in predicting actual clinical risk. Consequently, there is a concern that a number of new drugs with interesting and therapeutically important profiles may have been terminated early in development due to a positive hERG result. Ventricular repolarization in the heart is a complex process that depends on the time- and voltage-dependent

interactions of a variety of ion channels and membrane transport mechanisms. In many cases, drugs that block hERG also have activity at other ion channels that can exacerbate or mask the effect of a reduction of IKr on QT prolongation and the appearance of ventricular arrhythmia.

To address this limitation, the FDA's Center for Drug Evaluation and Research is collaborating with a wide range of scientists representing industry, academic, and nonprofit groups, including the Cardiovascular Safety Research Consortium, the Safety Pharmacology Society, and ILSI-HESI, to develop and characterize a new way of approaching the prediction of drug-induced proarrhythmia. The Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) initiative is proposing to integrate measurements of drug effects on multiple cardiac ion channels with *in silico* models of the human ventricular myocyte and the results from studies using iPSC-derived cardiomyocytes to create a mechanism-based ranking of torsadogenic risk for investigational drugs while eliminating the need for the clinical TQT study and concerns about its potential false positives (Sager et al., 2014; Fermini et al., 2015).

1.4 CONCLUSIONS

The effort needed to advance a drug from discovery through development to approval remains time and resource intensive, and despite best efforts, unanticipated adverse events leading to late-stage attrition or market withdrawal can still occur. As scientific advances continue to yield with new tools and technologies with better performance characteristics and predictive power than the traditional assays and biomarkers used in drug development, it will become increasingly important to see that these approaches are thoroughly tested and rigorously validated and find their way into regulatory decision-making.

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