A Real-time Evaluation of Human-based Approaches to Safety Testing: What We Can Do Now

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Abstract

Despite ever-increasing efforts in early safety assessment in all industries, there are still many drugs that prove toxic in humans. While greater use of human in vitro test methods may serve to reduce this problem, the formal validation process applied to such tests represents a major hurdle to their adoption. We contend that what is really needed to justify the adoption of any new test is not a demonstration of an ability to identify all potential safety issues in one approach, but a clear demonstration that it is adding value or is superior to whatever is currently in use – 'pragmatic validation'. A study based on such pragmatic validation, comparing the value of a range of human-based in vitro test methods with established regulatory tests, is currently underway and preliminary data will be presented. Importantly, all the in vitro tests have undergone significant evaluation already through two phases of the EPA ToxCast Program and are already used to various degrees by many pharmaceutical, agrichemical and cosmetic companies internally. The pragmatic validation approach we are testing now is designed to reduce to practice the application of these tests, bring them to a common denominator and provide the guidance to industry and regulators on the appropriate context of use. The study employs a range of marketed drugs that passed regulatory safety testing but were subsequently withdrawn, having caused serious toxicity in human subjects. Each of these drugs is paired with a negative control, i.e. a structurally and/or functionally similar marketed drug that does not exhibit such toxicity. This study is now being conducted as a distinct part of US EPA's Phase 3 ToxCast in vitro profiling program. Data are to be made publicly available when testing is completed, at such time they will be submitted for detailed analysis to compare the performance of the new in vitro tests with the regulatory regime that secured the original marketing approval. On completion, the outcome of this unique study will be presented to the regulatory authorities with the aim of developing appropriate documents for use by the industry and published in peer-reviewed media. *This poster does not necessarily reflect US EPA policy.*

Introduction

It is commonly accepted that the currently required pre-clinical tests need to be improved. Many advances in predictive toxicology offer the potential to improve safety, while also reducing time and costs of development of medicines. But are they sufficiently validated to be accepted in the industry and by the regulatory authorities, which are responsible for ensuring patient safety? While there are several important studies underway to investigate the value of novel approaches, they are unlikely to produce a clear outcome for some years due to large scale and other factors. In the meantime, those responsible for developing new medicines will continue to test their products in line with the current regulatory guidelines, and any serious attempts to move towards more human-based testing are likely to remain marginal.

We therefore proposed to test a new step-wise validation approach to compare new human *in vitro* approaches with the current regime of regulatory tests in order to bring the possible advantages of such approaches to the attention of both those responsible for pharma R&D, preclinical development and the regulatory authorities.

Pilot Study Design

We have selected a small number of compound that passed the pre-clinical safety hurdles and obtained approval for clinical use, but have subsequently been withdrawn for a variety of safety reasons, following adverse effects in patients (Table 1). This selection of drugs is being submitted, blinded, to a range of *in vitro* tests selected by US EPA as part of the phase 3 testing in the ToxCast program. In order to adequately control the study, each 'failed' drug is paired with a chemically similar drug that does not share its specific toxicity. The assays were chosen by EPA from over 300 that were part of ToxCast phases 1 and 2 and based on providing the broadest range of coverage of bioactivity. Initial data from the study is presented here.

Positive control	Indication	Mode of Action	Off-target effects	Negative control
Cerivastatin	CV disease	HMG-CoA reductase inhibitor	Muscle damage (rhabdomyolysis)	pitavastatin
Troglitazone	Diabetes	PPARγ agonist	liver damage	Roziglitazone*
Astemizole	Allergy	H_1 receptor blocker	prolonged QT interval	mizolastine
Rofecoxib	inflammatory pain	COX-2 inhibitor	heart attack (PG-related thrombogenesis ?)	diclofenac
Dexfenfluramine	Obesity	serotonin reuptake inhibitor	endocardial fibrosis	levfenfluramine

Table 1. Compounds selected for the pilot study

Assay Platform	Status
Attagene Factorial Transcription Factors	Completed
Perkin-Elmer/Novascreen Core Biochemical Assays	Single concentration completed
Zebrafish Development Assays	In progress
BioSeek Primary Human Cell Systems	Requested
ACEA Cell Growth Kinetics	2015
Vala Complex Cell Systems	2015
Cyprotex HepG2 High-Content Imaging Assays	2015

Table 2. Assays selected for the pilot study

Preliminary Data

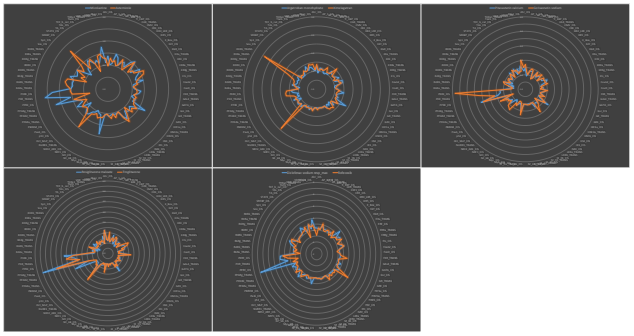

Fig. 1 Paired drugs (currently marketed-blue; withdrawn-orange) were compared for maximal responses to the Attagene Factorial cis and trans assays. Values plotted are log2 Emax.

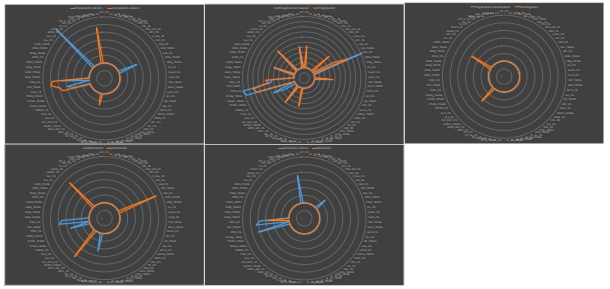

Fig.2 Paired drugs (currently marketed-blue; withdrawn-orange) were compared for maximal responses to the Attagene Factorial *cis* and *trans* assays. Values plotted are -logAC50. Inactives were set to an AC50 of 1000 μM.

Discussion

Preliminary results were shown from testing of five drug pairs, marketed versus withdrawn for safety concerns, for the Attagene Factorial transcription factor *cis* and *trans* systems. One pair, troglitazone and rosiglitazone, had therapeutic targets (PPARg) represented in the assay endpoints measured and these targets showed the highest response of all endpoints tested. Other drugs lacked direct therapeutic targets in the assays and responses seen could be considered as potential off-target activities or possibly indirect effects of the intended mechanism of action. A number of the withdrawn drugs activated the metal response element (MRE), a major stress pathway response, and would be considered an off-target effect. For diclofenac, PPARg was activated, consistent with its known partial agonist activity for PPARg, but would not necessarily be considered an adverse event. These results are only the initial piece of the larger data set will need to be considered in trying to identify signatures associated with serious human health effects.

Next Steps

The full data set from this study will be analyzed by selected independent third party analysis groups (FDA NCTR computational group (Dr. Weida Tong) and FDA Critical Path Institute) and will be presented to FDA and PSTC (Pharmaceutical Safety Testing Consortium) for feedback and advice. If proven successful, this approach is going to be used as a foundation for series of studies of similar design that will become the foundation of the new qualification/validation paradigm. The outcome of these studies is going to be a Guidance to Industries issued by EPA and FDA on the use of these technologies in safety evaluation of unknown chemicals.