HESI®
H E S I ILSI Health and Environmental Sciences Institute

# PROJECT PROPOSAL

**Topic:** Validation of A New In Vitro Testing Paradigm for Detecting Chemical Carcinogenicity and Development of Biomarkers for Chemical Carcinogenesis Applicable to Risk Assessment

**Submitted by:** Jiri Aubrecht, Pharm.D., Ph.D., Pfizer, Groton CT; Albert Fornace Jr, MD, Georgetown University, Washington, DC; Robert H. Schiestl, Ph.D., UCLA, Los Angeles, CA

The assessment of cancer risk associated with exposure to chemicals relies on the genotoxicity testing battery followed by the 2-year rodent carcinogenicity bioassay. In this paradigm, the genotoxicity testing battery enables relatively simple, rapid and inexpensive hazard identification and the 2-year rodent carcinogenicity bioassay provides mainly an assessment of a cancer risk in vivo. Because of the long term nature of the carcinogenicity testing and significant cost limitations, the genotoxicity testing battery is relied on as a surrogate marker of carcinogenicity in early drug development and evaluation of chemicals. Although the link between genotoxicity and carcinogenicity is well documented, this relationship is complicated due to the impact of non-genotoxic mechanisms of carcinogenesis and by nature of the *in vitro* genotoxicity assays and endpoints. Therefore, the predictivity of the current in vitro testing paradigm for carcinogenicity is challenging. Thus several positions and guidance have been published [1-3] resulting in the development of a mode-of-action framework [4]. Improving the predictivity of the genetox battery is also a subject of current discussions on the ICH testing guidelines and the crucial need for development of appropriate scientific approaches is fully recognized by the EU REACH initiative [5].

Here we propose to evaluate a new in vitro testing paradigm published by Ku et al 2007[6] consisting of a single test that addresses the relevant principal genetic lesions covered in the current test battery (DEL recombination assays, [7]) followed by toxicogenomic analysis to differentiate genotoxic and carcinogenic mechanisms [8, 9]. The advantage of the DEL assay resides in its ability to detect cancer-relevant changes in a single test system amenable to higher throughput and automation. In fact, recently the EPA in their ToxCast testing scheme as well as the NTP proposed incorporating the automated version of the DEL assay into their HTS screening. Currently available data indicate that the DEL assay has accuracy for predicting carcinogenicity of 92% compared to 62% with the Salmonella assay. Furthermore, the shape of the dose response for DEL recombination induction in relation to cytotoxicity could be used as a first criterion to differentiate direct (DNA reactive) from indirect genotoxic mechanisms, and thereby serve to prioritize the need for further toxicogenomic evaluation. The subsequent toxicogenomic analysis is directed to investigate the nature and biological relevance of DEL recombination induction in mammalian cells in vitro (possibly even in animals *in vivo*), thus

enabling a more mechanism-based risk assessment as a follow-up in place of conducting the conventional secondary genotoxicity tests that may have questionable relevance to evaluating carcinogenic risks. Recent data from our laboratories and literature provide the evidence for differentiating mechanisms of genotoxic and carcinogenic mechanisms via toxicogenomics [8, 10-12] and this approach is also pursued by EPA, NTP and by Carcinogenomic initiative in Europe. The major advantage of toxicogenomics is in providing mechanistic information applicable to risk assessment and assessing carcinogenic agents that are not genotoxic. The genomic approach would make an important step in developing the application of systems biology for use in risk estimation including development of relevant biomarkers.