Guidance for Industry and Review Staff

Recommended Approaches to Integration of Genetic Toxicology Study Results

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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Pharmacology and Toxicology

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I. INTRODUCTION

The purpose of this guidance is to inform industry and the review staff in the Center for Drug Evaluation and Research (CDER) on how CDER views positive findings in genetic toxicology assays during drug development. The guidance provides recommendations on how to proceed with clinical studies while ensuring the safety of study participants when results in genotoxicity studies suggest a potential cancer or genetic hazard. This guidance pertains to pharmaceuticals administered through oral, intravenous, topical, and other routes, as appropriate.

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II. BACKGROUND

The timing and conduct of genetic toxicology studies have been described in the ICH guidelines M3, S2A, and S2B.[2] We recommend that these guidances be consulted and that this document be considered an adjunct guidance.

[1] This guidance has been prepared by the Pharmacology Toxicology Coordinating Committee (PTCC) in the Office of New Drugs (OND) in the Center for Drug Evaluation and Research (CDER) of the Food and Drug Administration.

[2] ICH guidance for industry *M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals*, ICH guidance for industry *S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals*, and ICH guidance for industry *S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals*. (http://www.fda.gov/cder/guidance/index.htm)

Risk for carcinogenesis is usually determined in rodent assays, either 2-year studies or shorter-term studies using alternative models.[3] A core battery of genetic toxicology studies has been accepted by industry and regulators through the International Conference on Harmonisation (ICH) consultative process. These studies, which are designed to identify genotoxic hazard, include:

- A test for gene mutation in bacteria;
- An in vitro assessment of chromosomal damage using mammalian cells or an in vitro mouse lymphoma $tk^{+/-}$ assay; and
- An in vivo test for chromosomal damage using rodent hematopoietic cells.

The following discussion is based on current guidance documents.[4] We recommend that results from in vitro genetic toxicology studies be available before the initiation of phase 1 trials.

III. INTEGRATION OF GENETIC TOXICOLOGY STUDY RESULTS

The Agency takes into account the totality of safety data when considering whether it is safe to proceed with a clinical trial when there are positive genetic toxicology study results. This consideration includes a thorough evaluation of all the genetic toxicology data and the nature of the proposed trial. If the results of the genetic toxicology tests indicate a lack of genotoxic potential, then clinical trials can generally be undertaken in healthy subjects or patient populations with the proposed medical indication.

Pharmaceuticals that give positive results in genetic toxicology assays but do not directly interact with DNA do not always present a significant in vivo risk. In such cases, we recommend providing evidence of the mechanism of genotoxicity and relevance of the mechanism to anticipated in vivo exposure. Alternatively, it is also appropriate to rule out mechanisms involving direct interaction with DNA (e.g., demonstration that a drug does not cause DNA alkylation or DNA strand breakage).

Drugs known to directly damage DNA may be permitted to be used in patients with debilitating or life-threatening diseases, such as cancer, but should not be administered to healthy subjects.[5]

If any of the three assays in the ICH genotoxicity standard battery are positive, then we recommend completing the fourth test in the ICH battery. Equivocal studies should be repeated to determine the reproducibility of the results. If a positive response is seen in one or more assays, sponsors should consider choosing from one or more of the following options.

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[3] ICH guidance for industry *S1B Testing for Carcinogenicity of Pharmaceuticals*. (http://www.fda.gov/cder/guidance/index.htm)

[4] We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance Web page at http://www.fda.gov/cder/guidance/index.htm.

[5] ICH guidance for industry *S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals*. (http://www.fda.gov/cder/guidance/index.htm)

A. Weight-of-Evidence Approach

In some instances, after evaluation of all available data, the weight of evidence (WOE) suggests a lack of genotoxic hazard. For example, a positive response is observed in one exposure regimen of an in vitro cytogenetics assay. The positive result is seen only at the high dose, and the increase is within or just outside the range for historical control values for the solvent and cell line employed. The WOE approach could indicate that although a small increase in the frequency of chromosomal aberrations is statistically significant, it lacks biological relevance. Contributing considerations could include (1) the level of cytotoxicity at which the response was seen, and (2) corroborating data from the same or complementary assays. For example, a positive response seen with a short-term exposure without metabolic activation but not corroborated with the longer exposure at comparable levels of cytotoxicities would argue against the biological significance of the positive result. Similarly, such a positive finding in an in vitro chromosomal aberration assay that is not corroborated by the matching exposure regimen of the mouse lymphoma assay could also call into question the significance of the positive finding. If the WOE approach indicates a lack of genotoxic hazard, clinical studies could proceed provided the positive response is described in the investigator's brochure and the informed consent form.

B. Mechanism of Action

Positive results are sometimes satisfactorily explained by knowledge of the mechanism of action. For example, it has been demonstrated that in vitro clastogenic effects can result from excessively high osmolarity or low pH. Positive responses elicited under such nonphysiologic exposure conditions are not relevant to human risk. In addition, certain genotoxic responses are thought to have thresholds below which a hazard does not exist. Agents that induce effects by indirect mechanisms (e.g., interference with metabolism of nucleotides and their precursors, damage to spindle proteins, inhibition of DNA synthesis, or inhibition of topoisomerase) can have thresholds for genotoxic effects. In such cases, we recommend presenting evidence of the existence of a threshold that would not be attained during the proposed clinical exposure or presenting evidence of a mechanism not expected to be operative in vivo. Positive responses that are satisfactorily explained by an MOA may allow clinical studies in normal volunteers or in patients to proceed without additional studies.

C. Additional Supportive Studies

On occasion, results from in vitro studies demonstrate a reproducible positive dose-response. Results from bone marrow cytogenetic studies are frequently negative, even for those compounds giving positive results in in vitro genetic toxicology assays. This discrepancy can result from a number of differences between cultured cells and intact animals: differing metabolic pathways occurring in vitro and in vivo, metabolic inactivation in the intact animal, failure of the parent compound or active metabolite to reach the target cell, or simply, an inability to achieve plasma levels in vivo comparable to concentrations that generated positive responses in the in vitro assays.

Additional in vivo assays can be useful in clarifying in vitro positive results. For example, peripheral blood smears from repeat-dose toxicity studies in mice can be evaluated for

micronucleus induction, and peripheral blood lymphocytes from repeat-dose studies in rats or monkeys can be cultured and assessed for chromosome damage in metaphase spreads. DNA damage can be assessed in potential target tissues (e.g., DNA adducts or DNA strand breakage using the Comet or alkaline elution assay), or transgenic rats or mice can be used to assess mutagenicity in potential target tissues.[6]

The Syrian hamster embryo cell (SHE) transformation assay has been suggested as a follow-up assay in the face of positive in vitro genotoxicity results. Data in the literature suggest that the SHE assay correlates well with rodent carcinogenicity results for chemicals in general (Isfort et al. 1996). Results from an International Life Sciences Institute (ILSI) validation effort on human pharmaceuticals, although smaller in scope, suggest that the SHE assay is less predictive for human carcinogenic risk (Mauthe et al. 2001). With respect to human pharmaceuticals, the ILSI study found that the SHE assay had high sensitivity (83 percent) for detection of human carcinogens. However, its low specificity (15 percent) for prediction of *putative human noncarcinogens* led to a poor overall concordance of 37 percent. Although transformation assays measure endpoints more akin to the health effect of concern (cancer) and can be useful in making a WOE judgment, they also have inherent limitations. Many pharmaceuticals that give positive responses in 2-year rodent carcinogenicity studies do so through exaggerated pharmacological effects, immune suppression, or hormonal disequilibrium. It is unclear how an in vitro assay could be responsive to these mechanisms.

In the last several years, a number of transgenic mouse strains have become available for use in short-term carcinogenicity studies. The p53 haplo insufficient mouse has been found to be useful in the identification of mutagenic carcinogens (MacDonald et al. 2004). Negative results in a p53 carcinogenicity study are considered evidence that a genotoxic agent does not present a carcinogenic hazard to humans through a p53-mediated mechanism.

Supportive studies contribute to the WOE determination as to whether a drug giving a positive response in one of the ICH-specified assays presents a risk of genetic damage to subjects involved in clinical trials. The decision as to whether early assessment of oncogenic potential will be needed will, out of necessity, be on a case-by-case basis. Factors influencing the decision include target population, disease indication, duration of exposure, and safety profile of other drugs in the class or other drugs serving the same medical need.

[6] ICH guidance for industry *S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals*. (http://www.fda.gov/cder/guidance/index.htm)

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