DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Silver Spring, MD 20993

September 22, 2010

ATTN: Syril D Pettit
Associate Director, Scientific Outreach
HESI[1]
One Thomas Circle, NW, 9th Fl
Washington, DC 20005

RE: Biomarker Qualification Decision

Dear Dr. Pettit:

Please refer to your Biomarker Qualification Letter of Intent dated April 7, 2008. This letter communicates our qualification decision, review conclusions, and recommendations for future development for your three proposed urinary biomarkers of drug-induced nephrotoxicity in rats.

I. Qualification Decision and Context of Use

We have completed our review of this submission and conclude that:

- Urinary Clusterin and Renal Papillary Antigen (RPA-1) are qualified biomarkers for the context of use described below.
- Alpha-glutathione S-transferase (α-GST) is not qualified at this time.

Urinary Clusterin

Urinary Clusterin was previously qualified by FDA April 14, 2008. The data from this submission support the prior conclusions and clarify the context of use[1] as follows:

> **Urinary Clusterin** is a qualified biomarker for voluntary use in the detection of acute drug-induced renal tubule alterations, particularly when regeneration is present, in male rats when used in conjunction with traditional clinical chemistry markers and histopathology in GLP toxicology studies for drugs for which there is previous preclinical evidence of drug induced nephrotoxicity or where it is likely given the experience with other members of the pharmacologic class.

[1] Previous context of use statement containing Clusterin: "KIM-1, Albumin, Clusterin and Trefoil Factor-3 can be included as biomarkers of drug-induced acute tubular alterations in Good Laboratory Practice (GLP) rat studies to support clinical trials."

Renal Papillary Antigen-1 (RPA-1)

Urinary RPA-1 is a novel biomarker not previously qualified. The data from this submission support the context of use as follows:

> **Urinary RPA-1** is a qualified biomarker for voluntary use in detecting acute drug-induced renal tubule alterations, particularly in the collecting duct, in male rats when used in conjunction with traditional clinical chemistry markers and histopathology in GLP toxicology studies for drugs for which there is previous preclinical evidence of drug induced nephrotoxicity or where it is likely given the experience with other members of the pharmacologic class.

Please note that these biomarkers are not currently qualified for routine monitoring of drug-induced nephrotoxicity in the clinical setting. Although use of these biomarkers in the qualified context is voluntary, all collected biomarker data must be submitted to FDA along with other data from GLP toxicology studies conducted as part of an investigational new drug (IND) development program.

II. Review Conclusions

1. When tested with a limited number of nephrotoxic compounds, the Receiver Operating Characteristic (ROC) analyses showed that urinary clusterin and renal papillary antigen-1 (RPA-1) have better sensitivity and specificity than BUN and creatinine for the detection of specific kidney pathologies in male rats. Clusterin and RPA-1 provide additional and complementary information to BUN, serum creatinine (sCr), and histopathology for the detection of acute drug-induced nephrotoxicity in safety assessment studies. Sponsors may use these biomarkers to determine more conservative NOAELs for estimating starting doses in the initial human clinical trial of a drug that displays nonclinical nephrotoxicity as determined by histopathology.

2. Alpha-glutathione S-transferase behaved differently depending on the location of renal injury. Increases in urinary α-GST showed greater sensitivity than sCr and BUN for the detection of proximal tubule injury. In contrast, decreases in urinary α-GST showed greater sensitivity than BUN and sCr for the detection of collecting duct injury. The opposite behavior of urinary α-GST in response to proximal tubule and collecting duct injury may confound the interpretation of α-GST levels, particularly for compounds for which there is limited mechanistic information. Therefore, urinary α-GST is not qualified at this time.

III. Recommendations for Future Development

1. Additional testing of these biomarkers should be done in the female rat and other animal species to determine whether the context of use might be extended to female rats and other animal species when appropriate assays become available.

2. Additional nonclinical studies comparing the performance of each biomarker to that of sCr and BUN against the reference standard of histopathology should be done with a wider array of nephrotoxicants and non-nephrotoxicants to confirm the findings from the HESI submission, to aid in the determination of optimal biomarker thresholds for acute drug-induced renal tubule alterations, and to assess the presence of false positives (i.e., positive findings with non-nephrotoxicants).

3. Additional non-clinical studies should be conducted to characterize more fully the correlation of drug-induced injury (as determined by histology) with changes in biomarker levels by testing throughout the evolution of injury. Specifically, it is recommended that studies be conducted:

- to demonstrate that the biomarkers can be used to detect early drug-induced renal injury (i.e., before histopathology changes).
- to assess whether reversibility and recovery of injury (determined by histopathology) after drug cessation can be related to timing, extent, or duration of biomarker changes.

4. Prospectively designed, hypothesis driven, nonclinical studies are valuable to address the correlation between biomarker levels and evolution of lesions and any claims concerning localization of injury. Immunohistochemistry or other appropriate techniques should be used to define the temporal relationship between changes in histopathology, changes in tissue levels of the biomarkers, and changes in urinary biomarker levels. Such studies will strengthen the utility of the biomarkers and may enable expansion of the qualified context of use.

5. Given the limited amount of data on the specificity of the α-GST biomarker assay, future studies should address the effect of potential interfering substances, dilutional effects, and cross-reactivity of other GST isoforms as possible explanations for the decrease in urinary α-GST observed with collecting duct injury. Studies utilizing immunohistochemistry to localize the expression of various GST isoforms before and after collecting duct injury should be conducted to clarify the response of α-GST to different areas of renal injury and provide a better understanding of the mechanistic basis for the observed decreases following collecting duct injury. Additional nephrotoxicants should also be studied to explore the effect of isolated collecting duct injury as well as the effect of concomitant proximal tubule and collecting duct injury on α-GST levels.

6. Future studies should address the issue of the minimum number of tissue sections needed in biomarker qualification studies to detect adequately the presence or absence of renal injury, particularly low levels of injury. Such studies will be needed to support any claims concerning the ability of these biomarkers to detect injury prior to histopathology changes.

7. Blinded assessment of histopathology should be the standard in future biomarker qualification studies.

8. With respect to the clinical use, urinary clusterin and RPA-1 can be explored when and if sufficiently validated assays become available. At present, urinary clusterin and RPA-1 are not currently qualified as primary renal injury monitoring tests or to define dose-stopping criteria in clinical drug development studies. For the time being, sponsors and regulatory divisions should decide on a case-by-case basis how best to explore and/or make use of these biomarkers in a clinical development program.

We consider the qualification of novel biomarkers an incremental process and welcome the submission of additional animal and human data to support further application contexts for these biomarkers.

Sincerely,

Janet Woodcock, M.D.
Director, CDER
U.S. Food and Drug Administration