EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 October 2010
EMA/CHMP/SAWP/283298/2010
Committee for Medicinal Products for Human Use (CHMP)

Qualification Opinion ILSI/HESI Submission of Novel Renal Biomarkers for Toxicity

Agreed by Scientific Advice Working Party	February 2010
Adoption by CHMP for release for consultation	18 March 2010
End of consultation (deadline for comments)	31 July 2010
Adoption by CHMP	21 October 2010

Keywords	*Non-clinical, renal biomarkers, nephrotoxicity*

7 Westferry Circus • Canary Wharf • London E14 4HB • United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

Table 9 - AUC_{ROC} estimates (standard error) for Sprague-Dawley animals

Pathology	BUN	SCr	NAG	Protein	α-GST	μ-GST	RPA	Clust
PT degeneration or necrosis	0.78 (0.05)	0.75 (0.06)	0.93 (0.02)	0.86 (0.04)	0.83 (0.04)	0.91 (0.03)	0.80 (0.04)	0.88 (0.04)
PT deg/nec with no regen	0.67 (0.09)	0.54 (0.12)	0.72 (0.05)	0.70 (0.08)	0.69 (0.11)	0.72 (0.07)	0.60 (0.05)	0.60 (0.08)
PT deg/nec with regen	0.79 (0.06)	0.84 (0.06)	0.97 (0.02)	0.88 (0.05)	0.85 (0.04)	0.94 (0.04)	0.84 (0.04)	0.95 (0.03)
Cortical tubular regeneration/basophilia	0.63 (0.05)	0.74 (0.04)	0.56 (0.06)	0.59 (0.05)	0.53 (0.06)	0.56 (0.06)	0.92 (0.03)	0.84 (0.04)
DT degeneration or necrosis	0.53 (0.07)	0.59 (0.05)	0.87 (0.04)	0.74 (0.05)	0.94 (0.03)	0.85 (0.04)	0.93 (0.02)	0.67 (0.06)
CD degeneration or necrosis	0.57 (0.06)	0.59 (0.05)	0.91 (0.03)	0.68 (0.06)	0.96 (0.02)	0.89 (0.03)	0.89 (0.03)	0.65 (0.05)
CD deg/nec with no regen	0.60 (0.09)	0.52 (0.11)	0.94 (0.02)	0.61 (0.14)	0.91 (0.02)	0.90 (0.02)	0.79 (0.09)	0.65 (0.09)
CD deg/nec with regen	0.63 (0.06)	0.62 (0.05)	0.87 (0.04)	0.69 (0.06)	0.94 (0.03)	0.86 (0.04)	0.90 (0.03)	0.64 (0.06)
Regeneration NOS with no degeneration	0.62 (0.11)	0.53 (0.08)	0.70 (0.11)	0.74 (0.07)	0.65 (0.13)	0.71 (0.11)	0.70 (0.12)	0.63 (0.09)
Intratubular casts, granular, cortex	0.93 (0.03)	0.77 (0.14)	0.98 (0.01)	0.89 (0.06)	0.92 (0.03)	0.96 (0.01)	0.86 (0.03)	0.96 (0.02)
Intratubular casts, hyaline, cortex	0.69 (0.09)	0.76 (0.08)	0.72 (0.09)	0.69 (0.08)	0.55 (0.10)	0.69 (0.09)	0.81 (0.07)	0.86 (0.05)
Inflammation, interstitial, chronic, cortex	0.64 (0.04)	0.62 (0.05)	0.70 (0.04)	0.68 (0.04)	0.65 (0.04)	0.72 (0.04)	0.63 (0.04)	0.65 (0.04)

Table 10 - AUC_{ROC} estimates (standard error) for Wistar animals

Pathology	BUN	SCr	NAG	Protein	α-GST	μ-GST	RPA	Clust
PT degeneration or necrosis	0.53 (0.05)	0.59 (0.05)	0.54 (0.04)	0.64 (0.05)	0.85 (0.03)	0.67 (0.05)	0.54 (0.04)	0.54 (0.05)
PT deg/nec with no regen	0.58 (0.06)	0.53 (0.05)	0.67 (0.04)	0.52 (0.06)	0.73 (0.05)	0.59 (0.05)	0.62 (0.04)	0.66 (0.04)
PT deg/nec with regen	0.76 (0.09)	0.79 (0.08)	0.74 (0.04)	0.92 (0.04)	0.93 (0.03)	0.75 (0.06)	0.65 (0.06)	0.93 (0.02)
Cortical tubular regeneration/basophilia	0.61 (0.05)	0.51 (0.06)	0.68 (0.05)	0.66 (0.05)	0.59 (0.05)	0.64 (0.04)	0.63 (0.05)	0.79 (0.04)
CD degeneration or necrosis	0.65 (0.05)	0.74 (0.05)	0.94 (0.03)	0.53 (0.05)	0.89 (0.03)	0.57 (0.06)	0.96 (0.03)	0.86 (0.03)
CD deg/nec with no regen	0.68 (0.05)	0.62 (0.09)	0.88 (0.07)	0.51 (0.07)	0.87 (0.04)	0.65 (0.09)	0.87 (0.07)	0.80 (0.07)
CD deg/nec with regen	0.60 (0.07)	0.79 (0.06)	0.92 (0.02)	0.55 (0.06)	0.84 (0.04)	0.50 (0.07)	0.95 (0.01)	0.84 (0.03)
Medullary tubular regeneration/basophilia	0.63 (0.07)	0.61 (0.08)	0.76 (0.08)	0.54 (0.06)	0.79 (0.05)	0.56 (0.07)	0.82 (0.07)	0.76 (0.05)
Regeneration NOS with no degeneration	0.54 (0.06)	0.56 (0.07)	0.58 (0.07)	0.52 (0.05)	0.51 (0.06)	0.50 (0.06)	0.63 (0.08)	0.51 (0.06)
Intratubular casts, granular, cortex	0.56 (0.07)	0.54 (0.07)	0.80 (0.06)	0.57 (0.11)	0.69 (0.10)	0.79 (0.06)	0.73 (0.09)	0.60 (0.11)
Intratubular casts, hyaline, cortex	0.88 (0.06)	0.88 (0.06)	0.66 (0.06)	0.88 (0.07)	0.84 (0.07)	0.86 (0.06)	0.62 (0.07)	0.81 (0.08)
Inflammation, interstitial, chronic, cortex	0.58 (0.18)	0.50 (0.16)	0.62 (0.10)	0.74 (0.14)	0.83 (0.08)	0.51 (0.12)	0.61 (0.14)	0.71 (0.12)

Table 11 - AUC_{ROC} estimates (standard error) for pooled data

Pathology	BUN	SCr	NAG	Protein	α-GST	μ-GST	RPA	Clust
PT degeneration or necrosis	0.62 (0.04)	0.62 (0.04)	0.69 (0.03)	0.73 (0.04)	0.84 (0.03)	0.77 (0.03)	0.59 (0.03)	0.69 (0.03)
PT deg/nec with no regen	0.56 (0.05)	0.58 (0.04)	0.52 (0.04)	0.53 (0.06)	0.74 (0.04)	0.62 (0.04)	0.57 (0.04)	0.57 (0.04)
PT deg/nec with regen	0.79 (0.05)	0.82 (0.05)	0.87 (0.02)	0.89 (0.03)	0.87 (0.03)	0.87 (0.03)	0.76 (0.04)	0.94 (0.02)
Cortical tubular regeneration/basophilia	0.62 (0.04)	0.64 (0.04)	0.63 (0.04)	0.63 (0.03)	0.52 (0.04)	0.59 (0.04)	0.77 (0.03)	0.81 (0.03)
DT degeneration or necrosis	0.52 (0.06)	0.67 (0.04)	0.89 (0.03)	0.73 (0.05)	0.94 (0.03)	0.87 (0.04)	0.85 (0.02)	0.63 (0.06)
CD degeneration or necrosis	0.54 (0.04)	0.57 (0.04)	0.56 (0.06)	0.58 (0.04)	0.92 (0.02)	0.72 (0.04)	0.93 (0.02)	0.76 (0.03)
CD deg/nec with no regen	0.64 (0.05)	0.60 (0.07)	0.63 (0.11)	0.52 (0.06)	0.88 (0.03)	0.72 (0.06)	0.85 (0.06)	0.76 (0.06)
CD deg/nec with regen	0.51 (0.05)	0.55 (0.05)	0.52 (0.07)	0.61 (0.04)	0.90 (0.02)	0.70 (0.05)	0.92 (0.02)	0.73 (0.04)
Medullary tubular regeneration/basophilia	0.59 (0.07)	0.66 (0.07)	0.81 (0.07)	0.51 (0.06)	0.77 (0.05)	0.54 (0.06)	0.84 (0.07)	0.77 (0.04)
Regeneration NOS with no degeneration	0.52 (0.05)	0.58 (0.05)	0.57 (0.06)	0.52 (0.05)	0.52 (0.06)	0.56 (0.05)	0.53 (0.07)	0.56 (0.05)
Intratubular casts, granular, cortex	0.62 (0.09)	0.59 (0.08)	0.54 (0.11)	0.71 (0.08)	0.79 (0.07)	0.56 (0.11)	0.51 (0.10)	0.64 (0.09)
Intratubular casts, hyaline, cortex	0.79 (0.06)	0.82 (0.05)	0.70 (0.06)	0.78 (0.05)	0.69 (0.07)	0.76 (0.06)	0.71 (0.05)	0.83 (0.05)
Inflammation, interstitial, chronic, cortex	0.63 (0.04)	0.64 (0.04)	0.59 (0.04)	0.63 (0.04)	0.62 (0.04)	0.67 (0.04)	0.56 (0.04)	0.61 (0.04)

Based on these results and additional pairwise statistical comparisons of AUCROC for the novel BMs vs. reference BMs the HESI concluded:

- These results indicate the diagnostic value of an increase of urinary α-GST as a BM for PT injury
- The data set is insufficient to support any conclusion about the diagnostic value of μ-GST.
- The results shown clearly indicate both the specificity of RPA-1 for CD and its superior performance over all of the reference BMs for detection of degeneration/necrosis in the CD, particularly when regeneration is also present.
- The diagnostic value of clusterin for tubular regeneration is evident.

The incremental diagnostic value of each novel marker (α-GST, μ-GST, RPA-1, clusterin), when used in conjunction with reference markers, was assessed. Despite finding that some combinations of the novel markers with traditional markers enhanced diagnostic performance of the traditional markers for a given diagnosis, HESI concluded that the magnitude of the added value was minimal. This conclusion was based on comparison between AUCROC value for the combination of reference markers with novel biomarker to the AUCROC value for the novel marker alone.

Regulatory Data Assessment

The FDA and the European Medicines Agency contributed to the evaluation via the ad hoc appointed Biomarkers Qualification Teams (QTs) providing (via written procedures and Joint Teleconferences/ meetings with the HESI representatives) elements for gap analysis, questions on the statistical evaluations and drafting the conclusions.

Gaps identified by CHMP in the current qualification exercise

The QT assessed the data presented by the Applicant and identified some gaps in the qualification exercise. The Applicant is encouraged to address the gaps in future investigations.

Analytical methods

- Some of the results of interference testing are missing [Hb, bilirubin and high salt for clusterin assay and metals (mercury, cadmium, lead, lithium, gadolinium) for all assays].

- The impact of the criteria for repeatability, intermediate precision and reproducibility on the diagnostic performance of the biomarkers was not evaluated.

Limitations of the studies to address specificity of the biomarkers for injury at a particular site

- The specificity of these BMs to kidney injury needs to be further investigated since other possible target organs were not investigated (i.e. α-GST present in the liver also, clusterin in the cytoplasm of interstitial macrophages within stomach, skeletal muscle, heart, tongue, as well as macrophages within the medulla of thymus and lymph node of an untreated control rat). Liver toxicity could potentially interfere in the evaluation of the diagnostic performance of these novel biomarkers. In the current exercise the examination of liver was not standardised between studies and the statements that the liver is not affected by the three compounds cannot be supported. In this context the assessment of the liver should be standardised in future dose finding and definitive studies. Furthermore the testing of an additional intermediate (between clearly toxic and non toxic) doses in the future dose finding studies could help define a more appropriate control group and possibly increase the power of the definitive studies to identify specific biomarkers of renal vs. liver toxicity.

- The number and type of nephrotoxic compounds in the studies was limited.

- There were no studies conducted with non-nephrotoxic compounds (e.g. hepatotoxins).

Reproducibility of experiments

The QT notices the inconsistency between dose-finding and definitive studies for gentamicin and NPAA which makes the interpretation difficult.

Difference between strains and inference

The possibility of strain dependent sensitivity to nephrotoxicants and differential BMs response should be further investigated. The QT notices differences in the histopathological finding between the two rat strains. Based on the descriptive statistics strain differences in the BMs response are observed. For α-GST the correlation between severity of histopathological findings and BM fold change is evident for Wistar but not for Sprague Dawley, likewise (though perhaps not as clearly) for RPA-1. For clusterin the correlation is more evident for Sprague Dawley. Inconsistency is also observed between strains in the AUCROC values. Consequently pooling together the results from the two strains is not considered optimal and complicates inference.

Extrapolation of findings to female rats is not possible

Only male animals were used which limits the scope of the qualification.

Unexpected findings

-Consistent with the immunohistochemistry localisation of α-GST to the proximal tubule, increases in urinary α-GST were seen with PT injury in the absence of CD injury. However when isolated CD injury was induced by NPAA, α-GST values were consistently decreased in urine in both strains and α-GST was superior to all the reference BMs for the diagnosis of CD injury in the absence of PT injury. The opposing effects of the proximal and collecting duct injury on α-GST levels are not adequately understood and their impact on the diagnostic performance of the BM is not evaluated.

-It could be useful to revisit samples to understand elevation of biomarker levels in the absence of histopathological changes.

CHMP Qualification Opinion

Clusterin was previously qualified by the FDA and the European Medicines agency after review of the PSTC submission. (published report: http://www.emea.europa.eu/pdfs/human/sciadvice/67971908en.pdf):

"The urinary kidney BMs (Kim-1, Albumin, Total Protein, β2-Microglobulin, Urinary Clusterin, Urinary Trefoil Factor 3 and Urinary Cystatin C) are considered acceptable in the context of non-clinical drug development for the detection of acute drug-induced nephrotoxicity, either tubular or glomerular with associated tubular involvement.

They provide additional and complementary information to BUN and Serum Creatinine to correlate with histo-pathological alterations considered to be the gold standard.

Additional data on the correlation between the BMs and the evolution and reversibility, of acute kidney injury are needed. Also, further knowledge on species-specificity is required."

The findings of the current HESI submission increase the level of evidence supporting the use of Urinary Clusterin. Urinary Clusterin is a biomarker that may be used by Applicants to detect acute drug-induced renal tubule alterations, particularly when regeneration is present, in male rats and can be included along with traditional clinical chemistry markers and histopathology in GLP toxicology studies which are used to support renal safety in clinical trials.

In addition the HESI data indicate that urinary RPA-1 is a biomarker that may be used to detect acute drug-induced renal tubular alterations, particularly in the collecting duct, in male rats and can be included along with traditional clinical chemistry markers and histopathology in GLP toxicology studies which are used to support renal safety in clinical trials.

The QT acknowledges that the HESI data may support the use of urinary α-GST in detecting proximal tubule injury in male rats. However the opposing effects of proximal and collecting duct injury on α-GST levels raise uncertainty about the usefulness of this biomarker for detecting early mild renal injury. Therefore before α-GST is qualified in this context further studies will be needed to evaluate the mechanistic basis and usefulness of this BM.

CHMP Recommendations towards future qualification experiments

Methodological Considerations

- Replication of evidence: the conclusions drawn can be made more robust if replicated evidence is available from another, similar series of experiments.

- Biomarkers Normalisation: For all urinary markers, analyte concentrations for all animals were first normalised by dividing by the corresponding urine creatinine concentration. All individual animal marker values (normalised to creatinine in the case of urinary markers) were divided by the mean of the values in the concurrent control (i.e. vehicle-dosed) animals. Thus, all marker values were expressed as a fold-change versus the time-matched control group mean. Urine creatinine normalisation of BMs values is a standard practice and is considered acceptable. However normalisation of the urinary BMs by the mean of the values in the concurrent control is not recommended. It is acknowledged that this is done to minimise the impact of inter-study variability in the BMs performance. However, the BMs should be normalised to the individual baseline BMs values. Since urine baseline data was not collected in this experiment it is recommended to conduct this normalisation in future studies. The Applicant argues that intra-animal variability is greater than inter-animal, suggesting that baseline data may be of limited value with respect to variance reduction. However both applicant and QT agree that collection of baseline data in future studies will be beneficial

to characterize the dynamic range for each marker and the effects of age, gender, diet and circadian rhythm.

- Histopathology reading was not fully blinded. This is considered acceptable for the proposed qualification context. Depending on the qualification claims a fully blinded histopathology reading might be required. In any case methods to avoid bias in evaluating the standard of truth and the BM results should be implemented and justified in all BMs' submission.

- For the specific context of use a single section per kidney is adequate. However multiple sections from each kidney could help elucidate the cases of BM elevation in absence of histopathology findings or support a prodromal submission. The sponsor is encouraged to discuss the number of sections needed in future experiments.

Recommendation for future claims

- BMs to report injury to the other parts of the nephron.

- Extension of work to non rodent species.

- Combinations of novel and/or conventional BMs to optimize diagnostic performance.

- Prodromal claims (BM to detect injury prior to histopathology changes).

- Claims on the reversibility.

- Claims following the chronic administration of nephrotoxicants.

- Investigation of site specific rather than aetiology specific BMs. These BMs could detect a lesion regardless of the aetiology and manifestation.

HESI is encouraged to seek a qualification advice on these claims.

The extension of this exercise into the evaluation of use of novel BMs for renal injury in the translational and clinical context is of great importance and could be also the topic of a future qualification advice.