(Attachment 1)

Record of the Consultation on Pharmacogenomics/Biomarkers

May 31, 2010
Pharmaceuticals and Medical Devices Agency

Concerning the following consultation on pharmacogenomics/biomarkers requested, the documents submitted by the applicant of consultation (hereinafter referred to as "applicant") and the brief summary of assessment by Pharmaceuticals and Medical Devices Agency (PMDA) are as follows.

It should be noted that decisions in this document were made on the scientific level at the time of face-to-face consultation based on the data submitted by the applicant. Interpretation for the validity of the decisions may vary based on possible new findings and scientific advances, etc.

Date and No. of receipt:	August 12, 2009, #P-BM1
Biomarkers for consultation:	Urinary kidney injury molecule (Kim-1), urinary clusterin, urinary albumin, urinary trefoil factor-3 (TFF3), urinary cystatin C, urinary β2-microglobulin, urinary total protein
Category of consultation:	Consultation on Pharmacogenomics/Biomarkers
Consultation applicant:	Critical Path Institute's Predictive Safety Testing Consortium (PSTC)
Office (team) in charge:	Omics Project Team

1. Summary of the submitted documents

1.(1) Background

Early diagnosis of drug-induced acute kidney injury is important for early decision of discontinuation of causative drugs or therapeutic strategy for the injury, etc. At present, serum creatinine (sCr) and blood urea nitrogen (BUN), etc. are used as clinical and non-clinical biomarkers (BM) for kidney injury, but they are not sufficient in specificity and sensitivity.

Therefore, to investigate novel BMs superior in specificity and sensitivity to these existent BMs for acute kidney injury, the applicant considered 23 urinary BMs in total (albumin, β2-microglobulin, calbindin d28, clusterin, cystatin C, EGF, GST α, GST μ, kidney injury molecule [Kim-1], lipocalin2 [NGAL], macrophage migration inhibitory factor, monokine induced by interferon γ, interferon γ induced 10Kda protein, NAG, osteoactivin, osteopontin, podocin, RPA1, Timp1, trefoil factor-3 [TFF3], total protein, uromodulin [Tamm-Horsfall] and VEGF). As the result, the applicant concluded that usefulness as BM for drug-induced acute kidney injury has been proved and specific context of use has been qualified for 7 novel BMs (urinary Kim-1, urinary clusterin, urinary albumin, urinary TFF3, urinary cystatin C, urinary β2-microglobulin and urinary total protein; the 7 BMs may be expressed without "urinary" in the following text unless noted) at present, and requested this consultation to confirm the appropriateness. The applicant explained that similar documents had been already submitted to FDA and EMEA (EMA at present) for qualification of the 7 novel BMs in 2008, and that these were judged to be qualified as BMs to detect acute kidney injury in rats in non-clinical studies.

1.(2) Consultation items

The following 3 items were presented by the applicant as the objectives for submission of documents for qualification of BMs in the consultation:

1) To report results obtained by the applicant concerning several BMs for drug-induced acute kidney injury.
2) To seek agreement of PMDA with the applicant's claim that the submitted non-clinical study data on the 7 novel BMs as well as clinical study data in literature support the regulatory qualification of each novel BM.
3) To explain the strategy for subsequent studies and qualification proposed and to seek

agreement of PMDA to that matter so that broader acceptance and better understanding of the 7 novel BMs and other promising BMs for drug-induced kidney injury for use in regulatory decision making.

1.(3) Summary of the studies performed by the applicant

In the present consultation, the applicant considered that the 7 novel BMs (Kim-1, clusterin, albumin, TFF3, cystatin C, β2-microglobulin and total protein) were confirmed for their usefulness as BMs for drug-induced acute kidney injury, and submitted the documents in Attachment 2. The content included the results of short term (3 weeks) rat toxicity studies using existent chemicals known to cause acute renal injury, and the studies were performed by 3 parties including Merck (20 studies), Novartis Pharma (10 studies) and FDA (4 studies). In addition, assessment results of published literatures concerning clinical studies were also submitted.

Two different strains of rats were used in the rat toxicity studies. Han Wistar rats were used in the studies in Novartis Pharma, and Sprague-Dawley rats were used in the studies in Merck and FDA. Brief summary of the rat toxicity studies performed is shown in Table 1.

Table 1: Outline of the studies

Testing facility	Merck	Novartis Pharma	**FDA**
Strain of rats	Sprague-Dawley	Han Wistar	Sprague-Dawley
Sex	Male[a]	Male	Male
No. of animals per group	4-6	6	3-6
No. of nephrotoxicants[b]	11	8	4
No. of non-nephrotoxicants[c]	9	2	0
BMs measured	Kim-1, albumin, TFF3, sCr, BUN	Kim-1, clusterin, cystatin C, β2-microglobulin, total protein, sCr, BUN	Kim-1, sCr, BUN

a: Only 1 study using carbapenem included females.
b: gentamicin, vancomycin, doxorubicin, furosemide, lithium carbonate, cisplatin, puromycin, tacrolimus, carbapenem, cyclosporine, thioacetamide, hexachlorobutadiene, allopurinol, phenylanthranilic acid, D-serine, propyleneimine, mercuric chloride, sodium dichromate
c: α-naphtyl-isothiocyanate, methapyrilene, isoproterenol, furan, genipin, cerivastatin, carbon tetrachloride, trichlorobromomethane, water, 2% sodium chloride aqueous solution, 4% sucrose aqueous solution

Following treatment with nephrotoxicants and non-nephrotoxicants, histopathology data, hematology and clinical chemistry data and data of 7 novel BMs were collected, and accumulated into a shared database. ROC (Receiver Operating Characteristic) analysis was performed to compare usefulness of these 7 novel BMs with that of sCr and BUN, the present standard BMs for kidney injury. When a change of BM is observed without a change in histopathology, it is difficult to judge exactly whether it is a change of BM preceding histopathological change or a false positive change. Therefore, in the ROC analysis, an exclusion analysis in which data of nephrotoxicant dose groups without histopathological changes are excluded from analysis was performed, and the results were presented as the main results. In addition, separately, an inclusion analysis with all test samples was also performed, in which above mentioned tested animal data were included. Furthermore, measured values of BMs for kidney injury were normalized with urinary creatinine (UCr) value to minimize effects of experimental artifacts (leakage from water bottles, water consumption behavior due to pharmacological or toxicological effects of test substance and effects on urine volume, etc.).

Major results of the ROC analysis are shown in Figure 1 and Table 2 (renal tubular injury), and Figure 2 and Table 3 (glomerular injury).

1.(3).1) Renal tubular injury

Table 2: Results of ROC analysis (exclusion) in each testing facility

Testing facility	BM	AUC[a]	Threshold[b]	Relative sensitivity[b]	No. of test animals (control group[c]/ disease group[d])	*p* value[e]
Merck	Kim-1	0.99 (0.00)	1.88	99	46/77	0.00001
	Albumin	0.90 (0.01)	2.23	71	246/224	9.99E-10
	TFF3 (UCr)	0.90 (0.02)	2.01	78	105/134	0.70557
	TFF3 (excreted amount)	0.92 (0.02)	2.15	77	106/111	0.27375
	TFF3 (concentration)	0.93 (0.02)	2.47	87	117/135	0.07381
	sCr	0.77 (0.02)	1.22	48	246/224	-
	BUN	0.82 (0.02)	1.26	61	246/224	-
Novartis Pharma	Kim-1	0.91 (0.02)	1.87	79	283/132	3.02E-07
	Clusterin	0.88 (0.02)	1.85	70	289/132	1.16E-04
	sCr	0.73 (0.03)	1.15	40	289/132	-
	BUN	0.79 (0.03)	1.20	51	289/132	-
FDA	Kim-1 (not normalized)	0.77 (0.04)	1.39	64	28/131	3.62E-01
	Kim-1	0.84 (0.03)	1.77	68	28/129	9.53E-03
	sCr	0.72 (0.05)	1.42	34	28/134	-
	BUN	0.76 (0.04)	1.22	62	28/133	-

a: Values in parenthesis are standard errors, b: Values corresponding to specificity of 95-97%
c: Animals without kidney-specific lesions/histopathological changes
d: Animals with kidney-specific lesions/histopathological changes
e: $p<0.05$ (DeLong test: AUC of each novel BM vs AUC of sCr) is shown with underline.

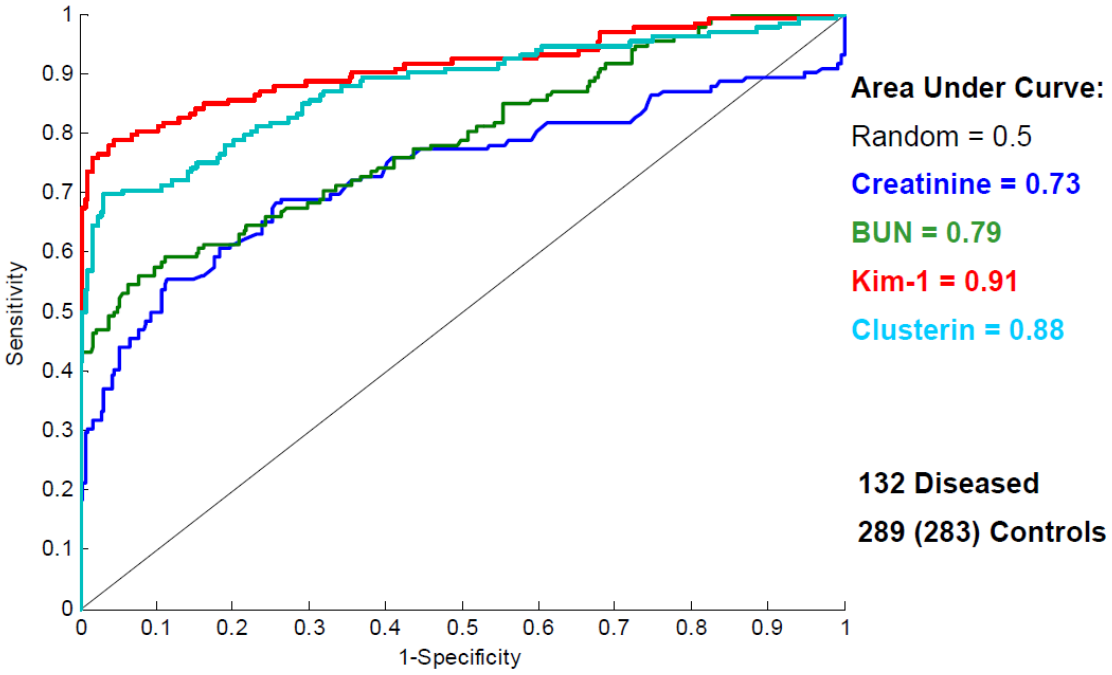

Figure 1: Assessment of renal tubular injury (degeneration, necrosis, apoptosis and cell sloughing) by Novartis Pharma

Determination of Kim-1 was performed in 18 studies in total including those performed by Merck (n=4), Novartis Pharma (n=10) and FDA (n=4), and the results were evaluated using the same analytical methods. As indicated in Table 2 and Figure 1, Kim-1 showed the highest values both in AUC and sensitivity from results of analysis performed in any of the facilities. The diagnostic threshold of Kim-1 to specificity 97%, 95% and 96% (in order of Merck, Novartis Pharma and FDA) was 1.88, 1.87 and 1.77, respectively, indicating consistent results obtained from independent assessment for detection performance with Kim-1 in multiple testing facilities. In addition, from the results of ROC analysis for individual histopathological findings of degeneration, necrosis, dilatation and regeneration performed by Merck in parallel with the above mentioned ROC analysis, Kim-1 was considered to outperform sCr, BUN and other novel BMs (albumin, TFF3), regardless of type of renal tubular injury.

Furthermore, from the results of ROC analysis performed by Merck and Novartis Pharma, both albumin and clusterin were shown to significantly outperform sCr and BUN in detection of drug-induced acute renal tubular injury. Although TFF3 did not show significant difference from sCr and BUN in detection of renal tubular injury, it was considered that TFF3 was indicated to have usefulness superior to sCr and BUN in detection of drug-induced acute renal tubular regeneration and dilatation, from the results of ROC analysis for individual histopathological findings performed by Merck.

1.(3).2) Glomerular injury

Table 3: Results of ROC analysis (exclusion) in Novartis Pharma

BM	AUC[a]	Threshold[b]	Relative sensitivity[b]	No. of test animals (control group[c]/ disease group[d])	*p* value[e]
Cystatin C	0.91 (0.03)	3.11	65	291/40	1.47E-06
β2-microglobulin	0.89 (0.03)	3.59	73	291/40	1.72E-05
Total protein	0.86 (0.04)	1.90	78	291/40	1.12E-05
sCr	0.53 (0.05)	0.91	30	291/40	-
BUN	0.80 (0.04)	1.29	48	291/40	-

a: Values in parenthesis are standard errors, b Values corresponding to specificity of 99%
c: Animals without kidney-specific lesions/histopathological changes
d: Animals with kidney-specific lesions/histopathological changes
e: $p<0.05$ (DeLong test: AUC of each novel BM vs AUC of sCr) is shown with underline.

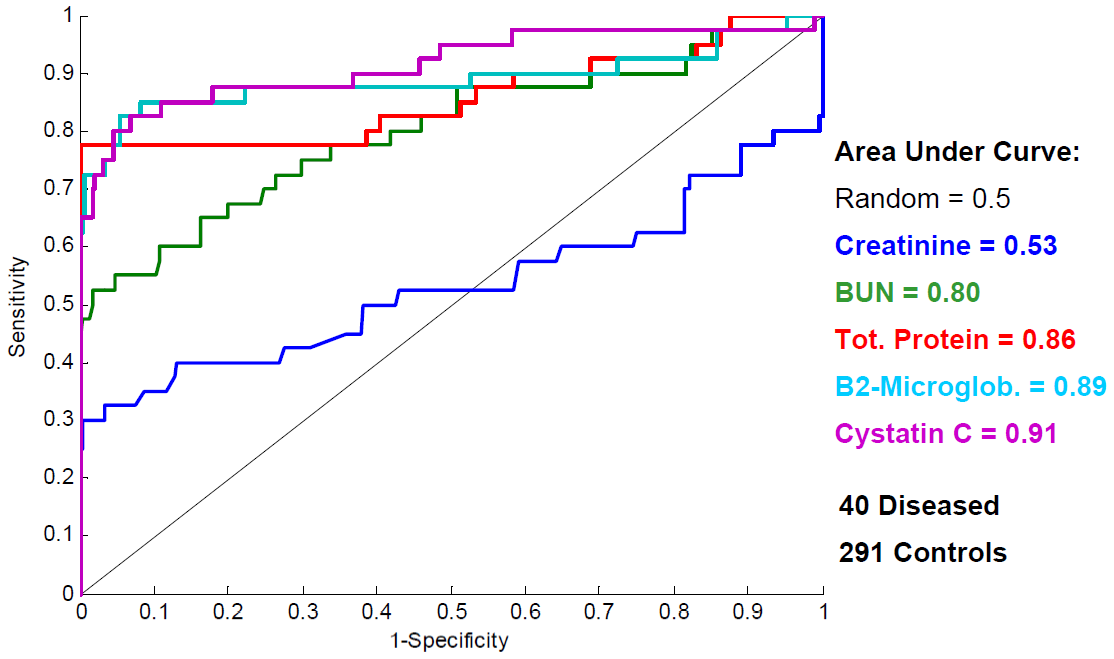

Figure 2: Assessment of glomerular injury by Novartis Pharma

Cystatin C, β2-microglobulin and total protein were indicated to significantly outperform sCr and to outperform BUN in detection of glomerular injury and following urinary tubular reabsorption disorder.

Furthermore, relative sensitivity (value corresponding to 99% specificity) and relative specificity (specificity corresponding to 85% sensitivity) calculated on the basis of the results of ROC analysis were 65% and 92% in cystatin C, 73% and 89% in β2-microglobulin, 78% and 49% in total protein, 30% and 0% in sCr and 48% and 49% in BUN, respectively, indicating that cystatin C and β2-microglobulin are with high sensitivity in detection of mild glomerular injury and following renal tubular reabsorption impairment and that total protein is with high specificity in detection of glomerular injury and following renal tubular reabsorption impairment.

1.(4) Assessment of clinical study results in published literatures

Results of assessment of published literatures reporting clinical study results which support clinical use of Kim-1, albumin, cystatin C, β2-microglobulin and total protein were presented for each BM, and a discussion that all of the 5 novel BMs represent kidney injury BMs with high-sensitivity in humans was shown (Attachment 2, Document No. 5). In addition, the applicant claimed that, based on the results of assessment of literatures as well as the results of non-clinical study outlined in the former section, BMs which demonstrated changes among the novel 5 BMs in animal studies with sufficient sensitivity after treatment with specific test drugs are appropriate to be used in early clinical studies.

1.(5) Future plan of the applicant

1.(5).1) Experimental strategy

As a next step after the present consultation, the following examination objectives were presented by the applicant.

a. Conduct of additional assessment for the 7 novel BMs as well as sCr and BUN using remnant samples from the rat toxicity studies performed before this consultation.
b. Assessment of other BM candidates (NAG, GST-α, GST-μ, osteopontin, lipocalin-2, uromodulin, RPA-1, osteoactivin and calbindin d28) for performance on detection of kidney injury using the remnant samples shown in a..

c. Further assessment for specificity of the 7 novel BMs in detection of kidney injury using samples obtained from the studies with non-nephrotoxicants.
d. Conduct of a few additional studies in rats and humans to investigate additional claims concerning usage of the 7 novel BMs.

1.(5).2) Potential gaps

The following items were presented by the applicant as potential gaps to be examined for the next qualification of the 7 novel BMs:

a. As the present consultation is focused on acute toxicity studies, it is also necessary to perform assessment for changes in the novel BMs against chronic kidney injury in studies in which dose levels of nephrotoxicants, treatment period and observation period are appropriately selected.
b. Changes in the novel BMs up to appearance of renal lesions and up to disappearance or recovery of the lesions are also necessary to be assessed.
c. Assessment of the novel BMs should be performed also in animal species other than rats, to confirm biological significance of changes of the novel BMs.
d. When the novel BMs are used for safety monitoring in early clinical studies, historical control (normal) values in the targeted clinical population should be established.
e. For BMs which are other than the 7 novel BMs and in which usefulness in detection of drug-induced acute kidney injury has been confirmed in non-clinical studies but no clinical experience has been obtained, assessment with small scale clinical studies will be performed to justify the use in clinical studies.

2. Brief summary of assessment

Brief summary of opinion of the applicant and assessment by PMDA concerning qualification of each novel BM is as follows:

2.(1) Consultation item 2. The opinion of the applicant concerning qualification of each novel BM

The applicant explained that the 7 novel BMs were considered qualified for following context of use 1)-3) based on the submitted documents (Table 4).

1) In rat toxicity studies, 6 out of the 7 novel BMs excluding TFF3 (Kim-1, clusterin, albumin, cystatin C, β2-microglobulin and total protein) can outperform and add information to BUN and sCr as early diagnostic BMs for drug-induced acute renal tubular alterations or drug-induced acute glomerular alterations/damage. In addition, although TFF3 could not outperform BUN and sCr, it provides additional information as early diagnostic BMs for drug-induced acute renal tubular alterations to those obtained from BUN and sCr.
2) The applicant considers that these 7 novel BMs are qualified for regulatory decision making[1)] as BMs that may be used by sponsors on a voluntary basis to demonstrate that drug-induced acute renal tubular alteration or drug-induced acute glomerular alterations/damage are monitorable in GLP rat studies which are used to support safe conduct of clinical trials.
3) The applicant considers that 5 novel BMs (Kim-1, albumin, cystatin C, β2-microglobulin and total protein) out of the 7 novel BMs excluding clusterin and TFF3 are qualified for regulatory decision making[1)] as BMs monitoring kidney safety to support further testing of drugs in clinical development (for example, phase I and phase II clinical trials), when animal toxicology findings generate a concern for renal tubular alterations or glomerular alterations/damage with associated tubular impairment or, when such animal studies demonstrate early detection of reversible renal injury.

[1)] Data on the novel BMs submitted this time may be used for safety assessment in reviewing protocols of early clinical study in PMDA.

Table 4: Claims of the applicant for context of use of the novel BMs

BM	Changes in measured values on kidney injury	Claims for use of novel BMs			Published data supporting claims of clinical usefulness
		Outperform BUN and/or sCr	Measurement in addition to BUN and/or sCr	Type of kidney injury to be monitored [a]	
Kim-1	Increase	Outperform BUN and sCr	Valuable	Renal tubule	Exist
Clusterin	Increase	Outperform BUN and sCr	Valuable	Renal tubule	Not exist
Albumin	Increase	Outperform BUN and sCr	Valuable	Renal tubule	Exist
TFF 3 (UCr-normalized)	Decrease	Not outperform	Valuable	Renal tubule	Not exist
Cystatin C	Increase	Outperform sCr	Valuable	Glomerulus	Exist
β2-microglobulin	Increase	Outperform sCr	Valuable	Glomerulus	Exist
Total protein	Increase	Outperform sCr	Valuable	Glomerulus	Exist

a: "Renal tubule" means drug-induced acute renal tubular alterations and "Glomerulus" means drug-induced acute glomerular alterations/damage with associated kidney tubular reabsorption impairment.

2.(1).1) Context of use of the novel BMs presented

a. Albumin

PMDA asked the applicant to explain whether it is appropriate that albumin is positioned as one of valid BMs for "renal tubular alterations", similar to Kim-1, clusterin or TFF3, (or whether albumin should be a BM with more limited context of use), because, based on the results of ROC analysis (inclusion) for albumin with renal tubular dilatation and regeneration among urinary tubular alterations detected by histopathology, AUC and sensitivity values of albumin lower than those of sCr and BUN were observed. AUC values for renal tubular dilatation were 0.88 ± 0.05, 0.95 ± 0.03 and 0.86 ± 0.05 for albumin, sCr and BUN, respectively. Sensitivity values for renal tubular dilatation were 0.50, 0.71 and 0.63 for albumin, sCr and BUN, respectively. AUC values for renal tubular regeneration were 0.78 ± 0.05, 0.84 ± 0.05 and 0.85 ± 0.05 for albumin, sCr and BUN, respectively. Sensitivity values for renal tubular regeneration were 0.41, 0.59 and 0.55 for albumin, sCr and BUN, respectively.

The answer of the applicant was as follows:

First, each of the 7 novel BMs presented in this consultation is not intended to replace sCr and BUN but is supposed to be used together with sCr and BUN, and albumin is considered to have been qualified based on information available at present. Next, from data of Merck showing scores for each of 3 renal tubular changes consisting of "renal tubular degeneration or necrosis", "renal tubular regeneration" and "renal tubular dilatation" among "urinary tubular impairment", albumin is considered to be most useful when pathologic changes to the tubules are noted, especially degeneration or necrosis (but not exclusively) that impair reabsorption by the tubular epithelium of albumin from the lumen. Although data analysis of each change does not include statistical test against sCr and BUN in order to avoid multiplicity, those are not considered to mean that albumin is inferior to other BMs in detection of changes other than urinary tubular degeneration or necrosis. On the other hand, an overall conclusion that albumin can outperform sCr and BUN assays in detection of drug-induced acute renal tubular alterations according to a statistical test (DeLong's test) has been obtained by analysis using Maximum Composite score [2)]. Therefore, at present we

[2)] Highest score among 3 scores for histopathological findings including renal tubular degeneration/necrosis, renal tubular regeneration and others (renal tubular dilatation, etc.).

consider that it is not necessary to limit the claim of use of albumin to renal tubular degeneration or necrosis.

Based on the answer from the applicant, PMDA asked the applicant to explain how to differentiate only functional changes without structural or pathological changes and those with structural changes including renal tubular degeneration or necrosis when albumin elevation is observed, after showing the ratio of urine samples from non-clinical studies with increased urinary albumin, between those with structural changes including renal tubular degeneration or necrosis and those lacking those changes. In addition, PMDA asked the applicant to examine for appropriateness to use albumin in combination with other novel BMs (Kim-1, TFF3, etc.), because it has been indicated that albumin is not suitable for detection of renal tubular regeneration and dilatation when used as a BM of renal structural changes.

The answer of the applicant was as follows:

Among the total 700 cases from the total 20 studies performed by Merck, the number of cases with structural changes or functional changes was counted. As the result, increased albumin exceeding the threshold of 95% specificity (exclusion analysis: 2.23-fold) was observed in 240 cases, including 158 cases with "degeneration/necrosis", 15 cases lacking "degeneration/necrosis" but with "dilatation" or "regeneration" and 67 cases lacking "degeneration/necrosis", "dilatation" or "regeneration". Therefore, the ratio of those lacking histopathological renal changes to the samples with increased albumin was 28% (67/240 cases). Since increased albumin was observed also in samples lacking histopathological renal changes, a possibility that increased albumin may occasionally be more sensitive than histopathological findings was suggested as well as a possibility that urinary protein may occur in cases without histopathological renal changes due to direct drug-induced inhibition of functional protein uptake by proximal tubular epithelium (a mechanism without cellular injury, for example by specific competitive inhibitor of the megalin-cubilin transporter complex). In cases in which functional protein uptake by proximal tubular epithelium is directly inhibited by a mechanism without cellular injury, urinary protein is considered to elevate without increases in BMs released into urine with cellular injury such as Kim-1. Therefore, simultaneous determination of albumin and Kim-1 may identify cases with only functional changes without structural changes. However, the utility is not considered to have been clarified at present. For possibility to distinguish specific cases by combination of multiple specific BMs, further examination in carefully designed studies is necessary, and this is considered to be a future challenge.

PMDA agrees to the explanation of the applicant that combination of multiple specific BMs to distinguish specific cases is a future challenge. However, PMDA considers that albumin is not recommended to be used alone, and it is desirable to be used in combination with Kim-1 and TFF3, etc. as far as possible in measurement in future because a possibility that albumin is influenced by a mechanism without cellular injury cannot be excluded.

b. TFF3

PMDA asked the applicant to explain the appropriateness of the proposed context of use of TFF3, since it is the only BM with which results of Delong's test did not outperform sCr and BUN assays among the novel BMs proposed this time (Kim-1, clusterin, albumin and TFF3) (Tables 2 and 4). In addition, PMDA also asked the applicant to explain which parameter is considered to be appropriate to normalize TFF3, because analysis and examination have been performed using data of TFF3 normalized to "UCr", "excretion" and "concentration."

The answer of the applicant was as follows:

There has been no evidence that TFF3, if used alone, would outperform sCr or BUN, but to assess the contribution of TFF3, the applicant analyzed the additional information that TFF3 provides within the context of a statistical model. As the result, by addition of TFF3 to a model of sCr and BUN (a binary logistic regression model with sCr and BUN as explanatory variables for histopathological response of the kidney), the likelihood ratio statistic calculated from the model improved from 187.3 to 224.4. Therefore, TFF3 is considered to be a novel BM providing useful information for detection of acute renal damage, and it is considered to be appropriate to use TFF3 in combination with current standard BMs. On the other hand, concerning normalization of TFF3, at present it is considered to be best to normalize with UCr to guard against false-positive conclusions when decreases in TFF3 concentrations are measured as a result of diuresis in the absence of injury or perhaps as a result of a leakage from water bottles during overnight urine collection. However, for appropriateness of normalization to UCr, examination based on further experience and accumulation of data is considered to be necessary, because TFF3 is the only BM among the 7 novel BMs which decreases in response to kidney injury and sufficient experience has not been accumulated. Therefore, this time the applicant decided it appropriate to present data normalized to the 3 parameters.

On the basis of the answer from the applicant, PMDA asked the applicant to explain how to differentiate decrease in TFF3 due to kidney injury and decrease due to insufficient detection ability, since TFF3 is the only BM that uses decrease in urine as an indicator among the 7 novel BMs evaluated this time.

The answer of the applicant was as follows:

For analytical performance of TFF3, validation (determination of lower limit of quantification, and confirmation of lack of interference by urine, test substance and known contaminants) was performed first. In addition, a positive control in which recombinant TFF3 was spiked into buffer was set for each analysis and replicate analysis was performed as an additional validation.

PMDA considers that having set a positive control in performing additional validation is valuable. However, for TFF3, since it is difficult to differentiate decrease due to kidney injury and decrease due to sensitivity for detection, setting a positive control group in each analysis in principle is considered to be desirable in the studies performed in future.

Furthermore, the statistical analytical results did not demonstrate superiority of TFF3 to sCr and BUN among proposed renal tubular injury BMs this time, and TFF3 is the only BM which decreases in response to kidney injury among the presently proposed 7 novel BMs, and in absence of "positive control" it is difficult to differentiate effects of kidney injury and reduction in detective ability when TFF3 is used alone. Therefore, PMDA considers it is not recommended to use TFF3 alone, as with albumin, but it is desirable to use TFF3 in combination with Kim-1 or albumin, etc. as far as possible in future examinations.

2.(1).2) Normalization of measured values of the novel BMs with UCr values

In the submitted literature (Han WK et al, *J. Am. Soc. Nephrol.* 16: 1126-1134. 2005), it is reported that normalization of Kim-1 values with UCr has a problem due to unstable creatinine balance in acute kidney injury patients, and that there was no significant difference between values before normalization and after normalization. In addition, normalization of the novel BMs with UCr had been prescribed beforehand, while both normalized and non-normalized data of Kim-1 and TFF3 were presented. Thus, assessment was performed in mixed 2 different methods. Moreover, assessment methods and appropriateness of the normalization was empirically determined according to the obtained study results. Therefore, PMDA asked the applicant to explain the appropriateness

to perform the normalization with UCr for all of the novel BMs to keep scientific integrity of analytical methods.

The answer of the applicant was as follows:

It is considered that experimental artifacts (possibility of changes in urinary BM levels not related to kidney injury but related to some other factors, for examples, due to leakage from water bottles in animal studies, influence on water consumption behavior due to pharmacological or toxicological effects, and pharmacological effects on urine volume) can be avoided by normalization of urinary BM with UCr, and that it will enable precise assessment of changes due to treatment-related kidney injury. However, as reported by Han, et al., the applicant recognizes that normalization to UCr levels may not appropriately work during the period up to reaching to stable equilibrium between sCr and UCr excretion after acute marked changes in glomerular filtration and urinary excretion of creatinine. Therefore, on normalization with UCr, it should be carefully performed together with an assessment of changes in UCr levels, in cases in which UCr decrease before increase of sCr may occur, such as acute renal dysfunction within 24 hours after onset. In cases where the results are suspected to be anomalous, repeated urine collection and measurement seem to be necessary. Based on above matters, at present, the best assessment method is considered to be to normalize values of the 7 novel BMs including TFF3 to UCr.

PMDA considers that "it should be carefully performed together with an assessment of changes in UCr levels" in explanation by the applicant is important on correction with UCr, and that compliance with this point should be a premise for all assessment of BMs proposed in this consultation. In addition, PMDA considers that detailed examination results for changes in UCr levels also should be presented for evaluation in future.

2.(1).3) Blinding of histopathological assessment

The histopathological assessment initially performed in Merck and Novartis Pharma was not blinded. Based on the discussion in the FDA/EMEA VXDS joint meeting, a blinded histopathological assessment was performed again in Merck, Novartis Pharma and SRI International (hereinafter referred to as "SRI").

The ROC analysis results from Merck and Novartis Pharma based on these blinded histopathological reassessment results showed higher AUC values in comparison to the results from SRI, in most of the 7 novel BMs. PMDA asked the applicant to explain the reason of these matters.

The answer of the applicant was as follows:

The main reason why the results from Merck and Novartis Pharma showed higher AUC than that in the results from SRI is considered to be the difference in criteria for assessment between each assessment facility (difference in terminology, difference in severity grading systems, different thresholds between pathologists, etc.). For example, a finding of renal tubular injury was graded very slight by the pathologist in SRI and considered to be pseudo-negative. On the other hand, the same finding was considered by the pathologist in Merck to be below the threshold of diagnosis and considered to be normal variation. However, as demonstrated in the results of ROC analysis (exclusion) in these 3 facilities shown in Table 5-1 and Table 5-2, although lower AUC was obtained in SRI assessment, relative position of each BM seems to be almost similar with minimal variation. Considering the difference in assessment criteria between the facilities, the difference of analytical results observed between the facilities is not considered to largely affect the proposed appropriateness of context of use for the 7 novel BMs.

Table 5-1: Results of ROC analysis (exclusion) using BM values obtained in Merck

BM	AUC (Merck) [a]	AUC (SRI) [b]
Kim-1	1.00 (NA)	0.98 (0.02)
Albumin	0.99 (0.01)	0.96 (0.02)
TFF3 (UCr)	1.00 (0.01)	0.97 (0.02)
sCr	0.95 (0.03)	0.90 (0.04)
BUN	0.90 (0.04)	0.91 (0.04)

a : AUC obtained by ROC analysis based on blinded histopathological assessment performed in Merck; the value in parenthesis is the standard error.
b: AUC obtained by ROC analysis based on blinded histopathological assessment performed in SRI; the value in parenthesis is the standard error.

Table 5-2 : Results of ROC analysis (exclusion) using BM values obtained in Novartis Pharma

BM	AUC (Novartis Pharma) [a]	AUC (SRI) [b]
Kim-1	0.95 (0.02)	0.82 (0.04)
Clusterin	0.93 (0.03)	0.84 (0.04)
sCr	0.66 (0.06)	0.62 (0.06)
BUN	0.54 (0.06)	0.53 (0.07)

a: AUC obtained by ROC analysis based on blinded histopathological assessment performed in Novartis Pharma; the value in parenthesis is the standard error.
b: AUC obtained by ROC analysis based on blinded histopathological assessment performed in SRI; the value in parenthesis is the standard error.

PMDA considers that the applicant has not clarified the reason why different terminology and different severity grading systems were used among the assessment facilities on blinded histopathological reassessment, and that strict comparison of the results between facilities is difficult. Albeit these matters, PMDA does not consider that the AUC values obtained from ROC analysis based on the histopathological reassessment performed by Merck and Novartis Pharma are largely different from those by SRI. In addition, the relative position of each BM obtained by the ROC analysis (exclusion) was also not largely different between Merck/Novartis Pharma and SRI. Thus, PMDA does not consider that the difference in the reassessment results may largely affect the assessment of context of use of the 7 novel BMs proposed by the applicant. On qualification of novel BMs performed in future, PMDA considers that histopathological assessment should be performed under blinded condition to appropriately guarantee reliability of assessment results.

2.(1).4) Site specificity of the novel BMs in kidney injury

PMDA asked the applicant to explain the possibility that the values of the proposed novel BMs for glomerular injury (β2-microglobulin, cystatin C, total protein) may increase not only in samples in which both glomeruli and renal tubules are injured but also in samples in which only renal tubules are injured.

The answer of the applicant was as follows:

In most cases, values of the novel BMs for glomerular injury increased in samples in which glomerular injury associated with renal tubular reabsorption imparment was observed, and did not increase in samples in which only renal tubules were injured. However, since values of the novel BMs for glomerular injury increased in the group receiving gentamicin which is not considered to induce glomerular injury, a possibility that values of these glomerular injury BMs may increase due to other factors affecting renal tubular reabsorption complex cannot be excluded.

PMDA considers that usefulness of β2-microglobulin, cystatin C and total protein as novel BMs detecting drug-induced glomerular injury with renal tubular reabsorption impairment has been suggested. However, PMDA considers that a possibility that these novel BMs values for glomerular injury may increase in cases without glomerular injury cannot be excluded, and that it is necessary to further examine factors affecting these BM values.

2.(1).5) Quantification method of Kim-1

Since most of Kim-1 values presented in this consultation were obtained with the newly developed Luminex micro-bead method, PMDA asked the applicant to explain the reliability of quantification of Kim-1 with Luminex micro-bead method, with comparison to other quantification methods including ELISA.

The answer of the applicant was as follows:

Using a subset of the same samples from 4 studies performed by Merck, concentrations of Kim-1 were determined using Luminex micro-bead method (performed in the laboratory of J. Bonventre, Brigham & Women's Hospital) and Mesoscale Discovery chemiluminescent ELISA method (hereinafter referred to as "Mesoscale method") (performed in the laboratory of Z. Erdos, Merck & Co., Inc.), and correlation coefficient (R^2) was calculated. As the result, the mean value of R^2 was about 0.8 (0.83, 0.80, 0.64, 0.78), indicating correlation between the 2 quantification methods. On the other hand, the slope of each regression line showed variation at 0.48, 0.50, 0.55 and 1.8. This variation was considered to be due to the very early stage in development of Mesoscale method at the time point of determination, and difference in the Kim-1 standard samples used in Mesoscale method from the standard samples used in Bonventre laboratory, etc. However, as Kim-1 has been assessed based on fold-change relative to concurrent control values or pre-treatment values, and are not considered to depend on absolute concentrations obtained using different standard samples. Therefore, the present determination results are considered to have sufficient reliability.

PMDA accepted the above explanation of the applicant.

2.(2) Consultation item 3. Further qualification of the novel BMs

2.(2).1) The criteria for selection and combination of BMs in further qualification of novel BMs

PMDA asked the applicant to explain the criteria for selection and combination of BMs on use of the 7 novel BMs submitted this time for drug-induced acute kidney injury.

The answer of the applicant was as follows:

It is considered that the choice of a single novel BM or combinations of multiple novel BMs for a particular target group depends on the objectives of investigation of BMs intended by the drug development sponsor, and that it is necessary to use each BM taking the known information about its usefulness, threshold and limitations into account as much as possible. Thus, in order to qualify these novel BMs for clinical use, the applicant will use as many BMs as possible in the future clinical studies, and from the results advantages and disadvantages of each BM will be understood. In addition, relative relationship between the BMs will be evaluated by collecting BM values determined in same samples.

PMDA accepted the view of the applicant.

2.(2).2) Study plan to solve potential gaps and issues raised in the opinion concerning context of use of the novel BMs

The applicant pointed out potential gaps in qualifying the novel BMs, including the necessity of time-course assessment of kinetic profiles of BMs in relation to lesion development and recovery after exposure to diverse nephrotoxicants. Concerning this matter, PMDA asked the applicant to explain the following points:

a. For plans to conduct non-clinical and clinical studies to investigate relationship between progression or recovery of renal functional impairments and time-course change of the levels of the novel BMs
b. For details of time-course assessment of BM level in studies presented as a solution for potential gaps

The answer of the applicant was as follows:

a. Multiple non-clinical studies in which several nephrotoxicants (for example, gentamicin or carbapenem A) are administered to rats and reversibility of toxicity is evaluated are ongoing, and in these studies, time-course assessment in individual animals and sacrifice of group of animals at various time points following completion of dosing are planned. In addition, additional rat

recovery studies are planned or already underway using gentamicin, adriamycin, bacitracin, cisplatin and puromycin, for example. On the other hand, the applicant is considering the conduct of clinical trials to examine the time-course change of BM levels during and after drug administration in patients with head and neck cancer receiving standard of care treatment with cisplatin, and in patients with cystic fibrosis receiving standard of care treatment with aminoglycoside.

b. The novel BMs are planed to be assessed over time (for example, values on days 3, 7 and 14) in a part of non-clinical studies described in the above a. In these studies, the applicant plans to examine other BMs in addition to the novel BMs, and carefully assess precise relationship between BM levels at each time point and histopathologies of active lesions. Furthermore, non-clinical studies of longer duration with continuous exposure to renal toxicants at dose levels confirmed to be well tolerated in short term studies are also planned.

PMDA accepted the view of the applicant.

3. Opinion of PMDA

3.(1) Consultation item 1. Reporting of results concerning BMs for drug-induced acute kidney injury

PMDA considers as follows:

Use of novel BMs related to drug response in development of medicines is expected to enhance and to realize creation of medicines with higher efficacy and less adverse effects. However, use of novel BMs in development of medicines without sufficient evaluation may cause false judgment. Therefore, it is an important process to qualify novel BMs for the objective and context of use and so on at the stage before wide use of novel BMs in development of medicines. Although the analytical results submitted by the applicant this time were limited to non-clinical data, such a positive evaluation performed is meaningful, and the results obtained this time constitute useful basic data in future development of medicines. In addition, PMDA expects positive conduct of continuous non-clinical and clinical evaluations for further qualification of the novel BMs in future, and it is necessary to perform assessments for qualification again when new results are obtained.

3. (2) Consultation item 2. The claim of the applicant concerning qualification of each novel BM

PMDA considers as follows:

Concerning the opinions 1)-3) on context of use of the 7 novel BMs submitted by the applicant (see the section of “2. (1) Consultation item 2. The opinion of the applicant concerning qualification of each novel BM”), based on the submitted study results and literatures on clinical findings of the 7 novel BMs for detection of drug-induced acute kidney injury submitted this time, use of the 7 novel BMs is acceptable as BMs that provide additional information, given that these BMs are used for the purpose to detect drug-induced acute urinary tubular changes or acute glomerular alterations/damage in rat GLP studies, and they are used in combination with existent BMs (sCr and BUN).

However, these 7 novel BMs has not been sufficiently qualified for general wide use in early clinical studies (phase I study, etc.) for detecting drug-induced acute kidney injury, and in such cases, utility of these BMs should be judged on a case-by-case basis. Examination of these renal BMs in clinical trials is expected in future clinical development of drugs or a future BM qualification in Japan and other countries in order to gather further data.

Based on the above considerations and the documents submitted this time, it is desirable to proactively perform further evaluation in future for at least the following non-clinical issues.

1) The results of non-clinical studies submitted this time are based on short term rat toxicity studies, and changes of BMs over time during long term treatment (changes or consistency over time) and its reversibility (whether the BMs correlate with regression of lesions) are not considered to have been clarified.
2) As all of the 34 studies excluding 1 study were performed only in males, evaluation for sex difference is considered to be insufficient.

3) Information on organ-specificity of the test substances (effects of the used nephrotoxicants on organs other than the kidney and effects of non-nephrotoxicants on the kidney) and site-specificity of the substances to urinary tubules or glomerulus have not been sufficiently accumulated, and how the novel BMs are affected by injuries in organs other than the kidney and in specific site of the kidney are not considered to have been clarified.

In addition, with the fact that there were some examination items for which different statistical analytical results were obtained between exclusion analysis (in which data of test animals without histopathological changes are excluded from analysis) and inclusion analysis (all samples are included in analysis), the reliability of exclusion analysis should be continuously examined in future.

3.(3) Consultation item 3. Further qualifications of the novel BMs

For further qualifications of the novel BMs and other drug-induced kidney injury BMs with expected utility, PMDA considers that the planned subsequent non-clinical studies presented by the applicant are useful to collect data concerning the future issues highlighted by PMDA in the above 3.(2). On the other hand, although clinical use of the 7 novel BMs submitted this time may add information to those from current BMs when used together, PMDA considers that a number of further clinical studies for extensive evaluation is needed before widespread use of the BMs for detection of drug-induced kidney injury in humans. Thus, the utility, etc. of the 7 novel BMs should be continuously evaluated in future clinical studies, including exploratory use of the 7 novel BMs together with existent BMs. PMDA expects further vigorous evaluation.