14 December 2016
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Executive Director

Letter of support for drug-induced renal tubular injury Biomarker(s)

On 12 August 2016 the Applicant Firalis SAS (on behalf of Safer and Faster Evidence-based Translation (SAFE-T) consortium) requested follow up qualification advice for novel biomarkers to assess DIKI pursuant to Article 57(1)(n) of Regulation (EC) 726/2004 of the European Parliament and of the Council. The procedure started during the SAWP meeting held on 30 August – 02 September 2016 and the advice was given in the framework of a joint EMA-FDA parallel advice procedure. The discussion meeting with the consortium took place on 26 October 2016.

During its meeting held on 24 – 27 October 2016, the SAWP agreed on the advice to be given to the Applicant. During its meeting held on 07 – 10 November 2016, the CHMP adopted the advice to be given to the Applicant. On the basis of the qualification advice Letter EMA issues this Letter of Support to the Safer and Faster Evidence-based Translation (SAFE-T) Consortium and Critical Path Institute's (C-Path) Predictive Safety Testing Consortium (PSTC) to encourage the further development and exploratory use of urinary alpha-glutathione S-transferase (α-GST), urinary clusterin (CLU), urinary cystatin C (CysC), urinary Kidney Injury Molecule-1 (KIM-1), urinary neutrophil gelatinase-associated lipocalin (NGAL), urinary osteopontin (OPN), urinary albumin (ALB) and urinary total protein (TPRO), as well as serum cystatin C, as biomarkers of drug-induced renal tubular injury in early clinical trials[1]. This is an expanded list of promising renal toxicity biomarkers from the list included in the Letter of Support issued on 6 November 2014 that encouraged the development of urinary OPN and NGAL as biomarkers of renal toxicity for use in early clinical drug development. This is also an expanded use for urinary ALB, TPRO and serum CysC, which are traditionally used to monitor for glomerular injury in early clinical trials.

Current clinical monitoring for nephrotoxicity with serum creatinine (sCr) lacks adequate sensitivity for early detection of clinically relevant kidney injury. Unlike sCr, which is a marker of function, urinary α-GST, CLU, CysC, KIM-1, NGAL, and OPN are thought to be markers of cellular injury and/or stress in the kidney. Because these biomarkers are localized in different regions of the nephron, the panel is expected to respond to a diversity of nephrotoxicants. It is anticipated that the candidate biomarkers will have the most utility when combined with traditional biomarkers.

[1] Reference numbers for all biomarkers listed in the letter are provided in the appendix to ensure clarity and allow for consistency in future studies.

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OPN is reported to be constitutively expressed in the thick ascending limb of the loop of Henle and distal convoluted tubules in both rodents and humans. OPN has been reported to be upregulated in the kidney in response to certain kinds of tissue stress and during tubular epithelial regeneration. NGAL has been reported to increase within the thick ascending limb of the loop of Henle, distal tubule, and collecting duct in response to nephrotoxic injury in rodents and humans. CLU is expressed in response to kidney injury in the proximal and distal tubules, glomerulus, and collecting duct. KIM-1 mRNA levels are elevated more than any other known gene across multiple species after initiation of kidney injury. α-GST is localized to the proximal renal tubule and is readily released into the urine during injury. CysC is freely filtered at the glomerulus and then reabsorbed by the renal tubular epithelium. Upon kidney injury, impairment of re-absorption in proximal tubules can lead to a several hundred-fold increase in urinary levels of CysC. Both urinary total protein and urinary albumin are established markers of renal injury of unspecified aetiology with total protein broadly reflective of glomerular and tubular injuries, and albumin more specifically reflecting glomerular injury. As clinically-accepted indicators of onset and progression of kidney damage whose aetiology may include nephrotoxic drug exposure, quantitative changes in urinary total protein / urinary creatinine ratio and urinary albumin / creatinine ratio contextualise changes in standard serum clinical biomarkers and novel urinary biomarkers.

The consortium evaluated the performance of the novel kidney biomarkers using data from the studies shown below. The original plan discussed in previous qualification procedures was to conduct also a confirmatory cisplatin and a confirmatory contrast study. Due to limitations of funding the cisplatin confirmatory study has not been carried out. Due to iohexol standardization methodologic issues, GFR measurements could not be validated by a reference laboratory for the confirmatory contrast media study.

Overview of clinical studies providing data for the letter of support

Brief study title	**Study population**	**Drug administered**	**N of subjects completed**	**N of subjects in final data package**
Exploratory cisplatin study	Cancer patients receiving high dose cisplatin	Cisplatin	114 cisplatin-treated patients; 21 control patients	105 cisplatin-treated patients; 20 control patients
Exploratory contrast study	Patients undergoing coronary angiography	Iodinated contrast medium	167 contrast patients; 20 control patients	121 contrast patients; 18 control patients
Healthy volunteers	Healthy male and female volunteers	None	55 healthy subjects	25

The exploratory human data available suggest that the candidate drug-induced renal tubular injury biomarkers may be more sensitive and specific for the detection of acute kidney injury, especially when used in combination, than traditional means of monitoring for nephrotoxicity. In addition, the consortium observed a rise in most of the candidate biomarkers (urinary α-GST, CLU, KIM-1, NGAL, CysC, OPN and serum cystatin C) that preceded a clinically-relevant rise in sCr. Since every biomarker in the proposed panel did not respond to each specific nephrotoxicant, quantitation of changes in a broader urinary biomarker panel will allow drug developers to evaluate potential drug-induced renal tubular injury caused by drugs with different targets for tubular toxicity. To date, these urinary biomarkers have not been definitively demonstrated to detect nephrotoxicity reliably across multiple

classes of drugs whose mechanism of drug-induced renal tubular injury span a variety of mechanisms of toxicity. Greater experience in the clinical setting with urinary α- GST, CLU, CysC, KIM-1, NGAL, OPN, ALB, TPRO is needed to understand the sensitivity and specificity of these urinary biomarkers, when used in conjunction with other traditional biomarkers, for drug-induced renal tubular injury. EMA are aware that there are several efforts to qualify these urinary biomarkers formally for use in clinical trials and support these development initiatives.

When including novel urinary biomarkers in early clinical studies for the evaluation of a new compound, traditional means of monitoring for nephrotoxicity (e.g. serum creatinine (sCr), serum CysC (sCysC), blood urea nitrogen (BUN), and urinalysis) should also be used. Furthermore, the novel urinary biomarkers could be included in preclinical safety studies in addition to clinical testing to expand the knowledge base.

No specific test system or assay validation process is endorsed for the above listed biomarkers. The analytical assay performance characteristics (e.g. quantitative range, limits of the detection, precision, reproducibility, linearity, and interference) should be established in advance of use. The sample stability for each of the biomarkers proposed herein should be validated for its intended storage, shipping and use conditions.

EMA encourage the exploratory use of these urinary biomarkers (α-GST, CLU, CysC, KIM-1, NGAL, OPN, ALB, TPRO) and of serum CysC as biomarkers of renal tubule injury in early clinical trials. The performance characteristics of these biomarkers have not been fully determined and, therefore, biomarker findings should be interpreted in the context of results for traditional biomarkers and clinical and nonclinical findings. We support data sharing and integration of these novel biomarkers across multiple clinical trials. If sponsors intend to include analyses of this panel of urinary biomarkers to support regulatory decision-making for a given development program, they should prospectively discuss the approach to these analyses with the European National Authorities responsible for clinical trial authorisation, and with SAWP/CHMP.

Any groups (academia, industry, government) that would like to join in this effort or contribute information or data that may be useful to this qualification effort can contact Drs. Gary Steven Friedman (GarySteven.Friedman@pfizer.com), Stefan Sultana (stefan.sultana@novartis.com), Jean-Charles Gautier (Jean-Charles.Gautier@sanofi.com), John-Michael Sauer (jsauer@c-path.org) or via the IMI SAFE-T Website www.imi-safe-t.eu or Critical Path Institute Website (https://c-path.org).

Sincerely,

Guido Rasi
Executive Director

I. **Appendix**

Reference Libraries

1. UniProt (Universal Protein Resource) is a catalog of information on proteins: http://www.uniprot.org/
2. HGNC (HUGO Gene Nomenclature Committee) is responsible for approving unique symbols and names for human loci: http://www.genenames.org/
3. No entries in the following libraries

- EC (Enzyme Commission) number is a numerical classification system for enzymes: http://www.chem.qmul.ac.uk/iubmb/enzyme/
- CAS (Chemical Abstracts Service) number is a unique identifier for chemical substances: https:/ /www.cas.org/

Reference Numbers

Alpha Glutathione S-Transferase

- UniProtKB: - P08263 (GSTA1_HUMAN)
- Gene: GSTA1
- Alternative names: Ligandin, “Glutathione S-Transferase alpha 1”,
- HGNC ID: HGNC:4626

Kidney Injury Molecule 1

- UniProtKB: - Q96D42 (HAVR1_HUMAN)
- Gene: HAVCR1
- Alternative names: "Hepatitis A virus cellular receptor 1, CD365, HAVCR, HAVCR-1, KIM1, "T-cell immunoglobulin mucin family member 1", TIM-1, TIM1, TIMD1
- HGNC ID: - HGNC:17866

Clusterin

- UniProtKB: - P10909 (CLUS_HUMAN)
- Gene: CLU
- Alternative names: “Aging-associated gene 4 protein” “Apolipoprotein J” “Apo-J” “Complement cytolysis inhibitor”, CLI, “Complement-associated protein SP-40”, “Ku70-binding protein 1” NA1/NA2, “Testosterone-repressed prostate message 2”, “TRPM-2”, “Sulfated glycoprotein 2”
- HGNC ID: - HGNC:2095

Cystatin C

- UniProtKB: - P01034 (CYTC_HUMAN)
- Gene: CST3

- Alternative names: “Cystatin-3”, “Gamma-trace”, “Neuroendocrine basic polypeptide”, “Post-gamma-globulin”
- HGNC ID: - HGNC:2475

Neutrophil Gelatinase-Associated Lipocalin

- UniProtKB: - P80188 (NGAL_HUMAN)
- Gene: LCN2
- Alternative names: NGAL, “25 kDa alpha-2-microglobulin-related subunit of MMP-9”, “Lipocalin-2”, “Oncogene 24p3”, “Siderocalin LCN2”, p25
- HGNC ID: - HGNC:6526

Osteopontin

- UniProtKB: - P10451 (OSTP_HUMAN)
- Gene: SPP1
- Alternative names: “Bone sialoprotein 1”, BSPI, Nephropontin, “Secreted phosphoprotein 1”, SPP-1, “Urinary stone protein”, Uropontin, "Early T-lymphocyte activation 1", ETA-1
- HGNC ID: - HGNC:11255

(Micro)albumin

- UniProtKB: - P02768 (ALBU_HUMAN)
- Gene: ALB
- HGNC ID: - HGNC:399