Food and Drug Administration
Center for Drug Evaluation and Research
10903 New Hampshire Avenue
Silver Spring, MD 20993

Date: July 25, 2016

ATTN: Safer and Faster Evidence-based Translation (SAFE-T) Consortium
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               Gerd Kullak-Ublick, Novartis
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Subject: Letter of Support for Drug-Induced Liver Injury (DILI) Biomarker(s)

Dear Safe-T Consortium,

We are issuing this Letter of Support to the SAFE-T Consortium to encourage the further development and exploratory use of[1]:

- Cytokeratin 18 (CK-18)
- Total and hyperacetylated high mobility group protein B1 (HMGB1)
- Osteopontin
- Macrophage colony-stimulating factor 1 receptor (CSF1R)

alone or in combination as soluble monitoring biomarkers to assess the risk of progression of drug-induced liver injury (DILI) in patients in whom an initial DILI diagnosis has been established based on elevations of the standard biomarkers alanine aminotransferase (ALT) alone or in combination with total bilirubin (TBIL) as a clinical safety assessment in clinical trials in a drug development context.

Due to the rarity and severity of idiosyncratic DILI, this adverse drug reaction remains an important cause of drug development late stage failures and post-marketing withdrawals. Current standard biochemical detection and assessment of DILI includes measuring serum enzyme activities of ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) as measures of hepatocellular or cholestatic injury. In addition, TBIL concentrations, serum albumin and prothrombin time are used as functional measures of liver activity. Some of these standard biomarker measures have been used in combination via Hy's Law[2] to identify liver dysfunction and patients with DILI. However, the sensitivity and specificity of Hy's Law are challenged by commonly observed mild elevations of bilirubin and inadequate early detection of injury. For a Hy's Law assessment to be positive, a significant amount of liver damage has already occurred. In contrast, changes in aminotransferase activities, particularly ALT, without bilirubin elevations are more sensitive, but not sufficiently specific for drug-related liver injury due

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[1] Reference numbers for all entities listed in the letter are provided in the appendix to ensure clarity and allow for consistency in future studies.
[2] Defined biochemically as elevation of ALT > 3x upper limit of normal range with concomitant elevation of serum total bilirubin > 2x upper limit of normal range.

to extra-hepatic sources of ALT. Furthermore, mild elevations of ALT can be observed with drugs that do not have the potential to cause severe DILI and in many cases these elevations will resolve despite continued treatment (i.e. liver adaptation). Therefore, there is a clear need for new biomarkers, especially for monitoring purposes, once a diagnosis of DILI has been made according to current methods.

Published information and unpublished data suggest that the above listed biomarkers may serve to address the above need. These biomarkers were assessed for their performance in detecting DILI and predicting the outcome of acute DILI. Biomarker levels at the time of liver injury were correlated with clinical outcomes of liver-related death/transplantation and recovery in samples provided by the US DILI Network (DILIN). The ability of the biomarkers to differentiate between these two groups was determined by ROC analysis. Other factors such as the pathophysiological significance of the biomarkers in the context of DILI and previously generated evidence from published literature were also taken into account.

Greater experience in the nonclinical and clinical setting is needed to better understand the applicability of each of these biomarkers for assessing drug-induced liver injury, including the time course of the biomarkers. As the above listed biomarkers are further developed as potential monitoring biomarkers in the drug development context described, the following deserve careful consideration:

- DILI processes of necrosis, apoptosis and immune activation identified by the studied biomarkers are likely in some instances to be overlapping. In addition, biomarker profiles for these processes may be impacted by the particular hepatotoxic drug, the dose of the drug, the inciting mechanism of injury (e.g. reactive metabolites, mitochondrial toxicity, immuno-allergic hypersensitivity, adaptive immune injury, and/or drug-induced autoimmunity), as well as the character of the injury (acute vs chronic), the liver cell type(s) that is affected, and whether cholestasis is present. Exploring the impact of these variables will likely be informative.

- The characterization of DILI biomarkers that are monitoring for injury progression may require the enrollment of study subjects at a uniformly early phase of liver injury prior to progression, as well as consistent longitudinal case follow-up to correlate these markers reliably with outcomes.

- To optimally manage patients in the future, biomarkers that predict DILI progression must be easily and quickly measurable. This principle would also pertain to the practical utility of routine mass spectrometric testing of early DILI patients for hyper-acetylated HMGB1.

- Due to adaptation, the expression of sensitive markers of early liver injury may have poor specificity as identifiers of the subset of individuals who are destined to develop clinically serious DILI with continued exposure to a study drug.

- In addition, the biomarkers micro RNA-122 (miR-122) and glutamate dehydrogenase (GLDH) could be studied further as biomarkers of liver specific injury based on their performance in patients with acute DILI, including patients with DILI due to acetaminophen overdose, compared to non-DILI controls.

No specific serum or plasma test system or assay validation process is endorsed for the above listed biomarkers. Good scientific and laboratory practices for quality control of the assay test system are imperative. The analytical assay performance characteristics (e.g. quantitative range, limits of the detection, precision, reproducibility, linearity, interference, etc.) 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.

We support the collaborative initiative of both the SAFE-T consortium and PSTC to encourage the voluntary and complementary use of these biomarkers to better understand their potential role as monitoring

biomarkers in the context of clinical trials where DILI has been diagnosed by the standard methods described above. We will consider data collection on these biomarkers to be exploratory in nature at the current state of clinical knowledge. If sponsors intend to include analysis of these biomarkers to support regulatory decision making for a given investigational new drug (IND) development program, they should actively discuss with the appropriate CDER regulatory review division.

Any groups (academia, industry, government) that would like to join in this effort or have information or data that may be useful can contact Drs. Gerd Kullak-Ublick (gerd.kullak-ublick@novartis.com), Sif Ormarsdottir (Sif.Ormarsdottir@astrazeneca.com), John-Michael Sauer (jsauer@c-path.org) or Douglas Keller (Douglas.Keller@sanofi.com) or view either the Critical Path Institute Website (https://c-path.org) or the IMI SAFE-T Consortium Website (http://www.imi-safe-t.eu/).

Sincerely,

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

I. Appendix

Reference Libraries

1. UniProt (Universal Protein Resource) is a catalog of information on proteins: http://www.uniprot.org/

2. HGNC (HUGO Gene Nomenclautre Committee) is responsible for approving unique symbols and names for human loci: http://www.genenames.org/

3. EC (Enzyme Commission) number is a numerical classification system for enzymes: http://www.chem.qmul.ac.uk/iubmb/enzyme/

4. CAS (Chemical Abstracts Service) number is a unique identifier for chemical substances: https://www.cas.org/

Reference Numbers

Alanine aminotransferase (ALT):
- EC: 2.6.1.2

Alkaline phosphatase (ALP)
- EC: 3.1.3.1

Aspartate aminotransferase (AST):
- EC: 2.6.1.1

Cytokeratin 18 (CK-18), also called Keratin, type I cytoskeletal 18:
- UniProt: P05783; KRT18
- HGNC: 6430

Gamma glutamyl transferase (GGT):
- EC: 2.3.2.2

Glutamate dehydrogenase (GLDH):
- EC: 1.4.1.3

Macrophage colony-stimulating factor 1 receptor:
- UniProt: P07333; CSF1R

- o HGNC: 2433

Micro RNA-122 (miR-122):
- o HGCN: 31501

Osteopontin:
- o UniProt: P10451; SPP1
- o HGNC: 11255

Total and hyperacetylated high mobility group protein B1:
- o UniProt: P09429; HMGB1
- o HGNC: 4983

Total bilirubin (TBIL):
- o CAS: 635-65-4