Biomarker Qualification Letter of Intent (LOI)

Administrative Information

Requesting Organization

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If there is a prior, current, or planned submission to other regulatory agencies, list the agencies and dates as appropriate

The Predictive Safety Testing Consortium (PSTC) Skeletal Muscle Working Group and Duchenne Regulatory Science Consortium (D-RSC) would like to make this biomarker qualification a parallel submission with US FDA and EMA as soon as possible and envisions that a tripartite meeting to discuss the qualification would be requested if a Qualification Plan is accepted.

Drug Development Need

Describe the drug development needs that the biomarker is intended to address, including (if applicable) the proposed benefit over currently used biomarkers for similar COUs (limited to 1,500 *characters*).

Skeletal muscle (SKM) injury, defined as myocyte degeneration/necrosis, is currently monitored in clinical drug development trials and in muscular and neuromuscular diseases using circulating concentrations of creatine kinase (CK) and aspartate amino transferase (AST). However, these biomarkers lack the desired sensitivity and tissue specificity. Furthermore, clinical symptoms of SKM injury are difficult to interpret, due to the subjective nature of self-reporting. As a result, drug-induced SKM injury (DIMI) remains a poorly understood and difficult to predict side-effect of new and existing medications.

The proposed novel biomarkers, in conjunction with standard endpoints, can sensitively and specifically measure SKM injury (myocyte degeneration/necrosis) regardless of the etiology of the injury in humans.

Biomarker Information

Biomarker name:

If composite, please list the biomarker components. For molecular biomarkers, please provide a unique ID

Scheme: UniProt (http://uniprot.org/)

1. Skeletal troponin I fast-twitch (Type II) (TNNI2 P48788) – a component of myofilaments with expression restricted to SKM
2. Myosin light chain 3 (MYL3 P08590) – a component of myofilaments found predominantly in slow-twitch SKM and cardiac muscle
3. Fatty-acid binding protein 3 (FABP3 P05413) – a cytosolic lipid transport protein, abundant in SKM and cardiac muscle, also present in brain, liver, and small intestine
4. Creatine kinase muscle type (CKM P06732) – a cytosolic enzyme involved in utilization and generation of adenosine triphosphate (ATP); highly abundant in skeletal muscle

Biomarker Description:

(Source, composition and decision process) If biomarker is an index/scoring system, please provide information on how the index is derived (e.g. algorithm), the biologic rationale for inclusion of each of the components, the rationale for any differential weighting of the elements, and the meaning/interpretation of the index/score (limited to 1,500 characters) Primary biomarker category (See BEST Glossary). Select one:

The biomarkers are **molecular** biomarkers in the plasma. The primary biomarker category is as a **monitoring** biomarker.

The biomarkers are proposed as a panel to be used in monitoring SKM injury in humans. The initial exploratory assessment will determine the performance of each biomarker individually for SKM injury, and statistical approaches will then be utilized to determine if a combination of individual biomarkers (composite) provides additional value.

Biological Rationale:

(underlying biological process) Describe the mechanistic rationale or biologic plausibility to support the biomarker and its associated COU (limited to 1,500 *characters)*

The 4 SKM injury biomarkers indicate myocyte degeneration/necrosis irrespective of mechanism or cause of injury, and their presence in the plasma indicates compromise of myocyte membrane integrity. Further information on each biomarker can be found in Appendix 2.

- Skeletal troponin I fast-twitch (Type II): Troponin is a protein trimer consisting of three subunits, calcium-responsive (C), inhibitory (I), and tropomyosin-binding (T), which regulate the interaction of myosin with actin necessary for the control of muscle contraction. Expression of troponin subunit isoforms is specific to cardiac muscle, slow-twitch SKM or fast-twitch SKM.
- Myosin light chain 3 (MYL3): MYL3 is an essential light chain of the myosin molecule released into the blood stream following muscle damage. MYL3 is expressed predominantly in cardiac muscle and SKM.
- Fatty-acid binding protein 3 (FABP3): FABP3 is a small (14.5 kDa) cytoplasmic protein that has been shown to increase in plasma in response to physiological conditions such as SKM/cardiac damage that increase fatty acid demand/availability.
- Creatine kinase M (CKM): The cytosolic form of the creatine kinase (CK) enzyme is a dimer of subunits encoded by the CK-M and CK-B genes. CKM is responsible for the regeneration of adenosine triphosphate (ATP) from phosphocreatine and adenosine diphosphate (ADP), which is critical to contraction of cardiac muscle and SKM. While measuring CK activity does not discriminate between isoforms, measuring CKM protein (i.e. CK-MM homodimer) is highly selective for SKM CK.

Additional Considerations for Radiographic Biomarkers

Not applicable

Context of Use

Proposed Context of Use (COU) (limited to 500 *characters*)

A safety biomarker panel to aid in the detection of acute drug induced skeletal muscle injury in phase 1 trials in healthy volunteers in conjunction with aspartate transaminase (AST) and total creatine kinase (CK) enzymatic activity when there is an a priori concern that a drug may cause skeletal muscle injury in humans.

Biomarker Measurement Information (Analytical)

Provide a general description of what aspect of the biomarker is being measured and by what methodology (e.g. radiologic findings such as lesion number, specific measure of organ size, serum level of an analyte, change in the biomarker level relative to a reference such as baseline) (limited to 1,500 characters).

The proposed biomarkers are cellular leakage biomarkers measured in plasma to detect SKM degeneration/necrosis. Circulating levels of these biomarkers in rats and dogs increase over baseline in response to various SKM toxicants and correlate with the severity of muscle injury as determined by histopathology (myocyte degeneration/necrosis). The biomarkers return to baseline on recovery from SKM injury as determined by histopathology (Burch, 2015 and Vlasakova, 2017). We infer that the biomarkers correlate to the same histopathology endpoint in humans irrespective of mechanism of toxicity or disease pathogenesis as defined by standard endpoints of imaging, functional tests, established circulating biomarkers, and/or standard clinical chemistry tests.

The biomarkers are measured using sandwich ELISA procedures on an electrochemiluminescent platform (Meso Scale Discovery platform, Meso Scale Diagnostics, LLC, www.mesoscale.com); which are Research Use Only assays and therefore not under review. There are no standard operating procedures for sample collection, storage, or assay conduct established at this time, and assays will not be performed in a Clinical Laboratory Improvement Amendments certified laboratory. For humans, a fit for purpose validation of the assays has been conducted, examining intra- and inter-assay precision, dilutional linearity, limit of blank (analytical sensitivity), limits of quantitation (upper and lower), matrix/recovery, and sample-freeze/thaw stability.

Biomarker Measurement (Clinical)

Description of Clinical Decision Process and Tool:

- **Characterization of Biomarker for COU: Describe the Clinical Decision Criteria and How the Biomarker Measurement Is Used**
- **Calculation/Modeling/Construction of Biomarker into a Decision Tool**
- **Expected Distribution of Decision Criteria for COU**

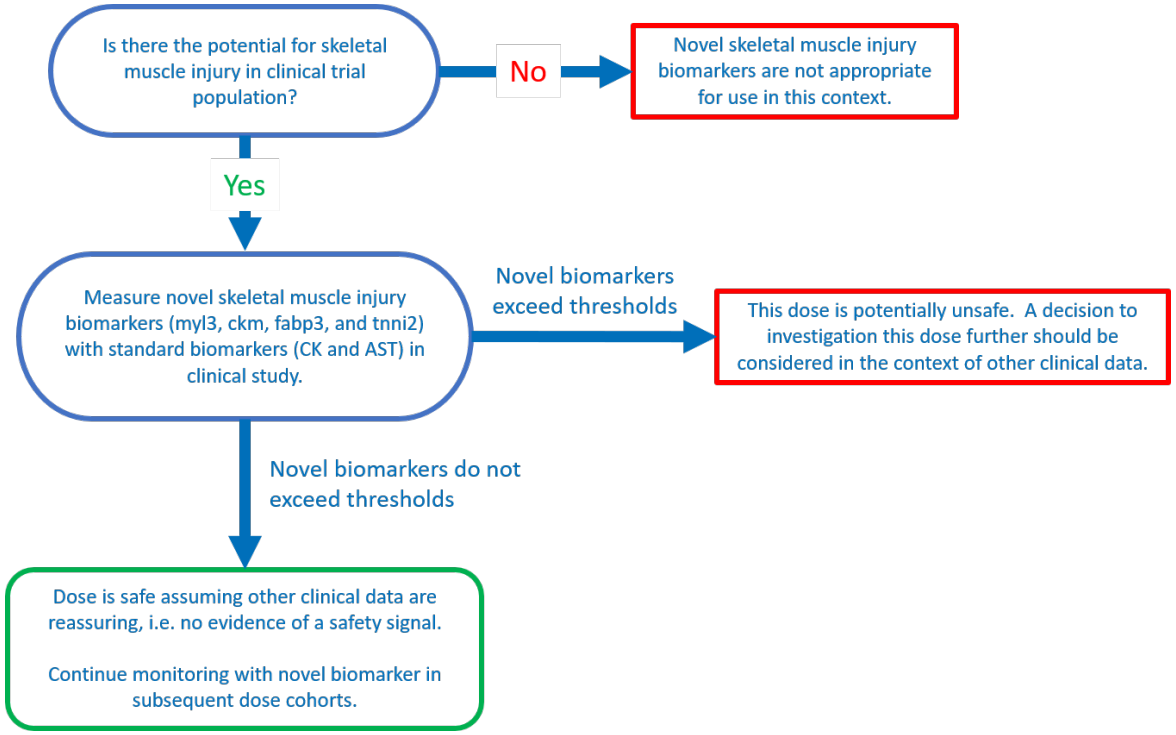
- **Decision Criteria Limits/Cut-offs and Application to COU**
- **Clinical Validation**
- **Benefits and Risks of Applying Clinical Decision Tool**
- **Describe Knowledge Gaps, Limitations and Assumptions**

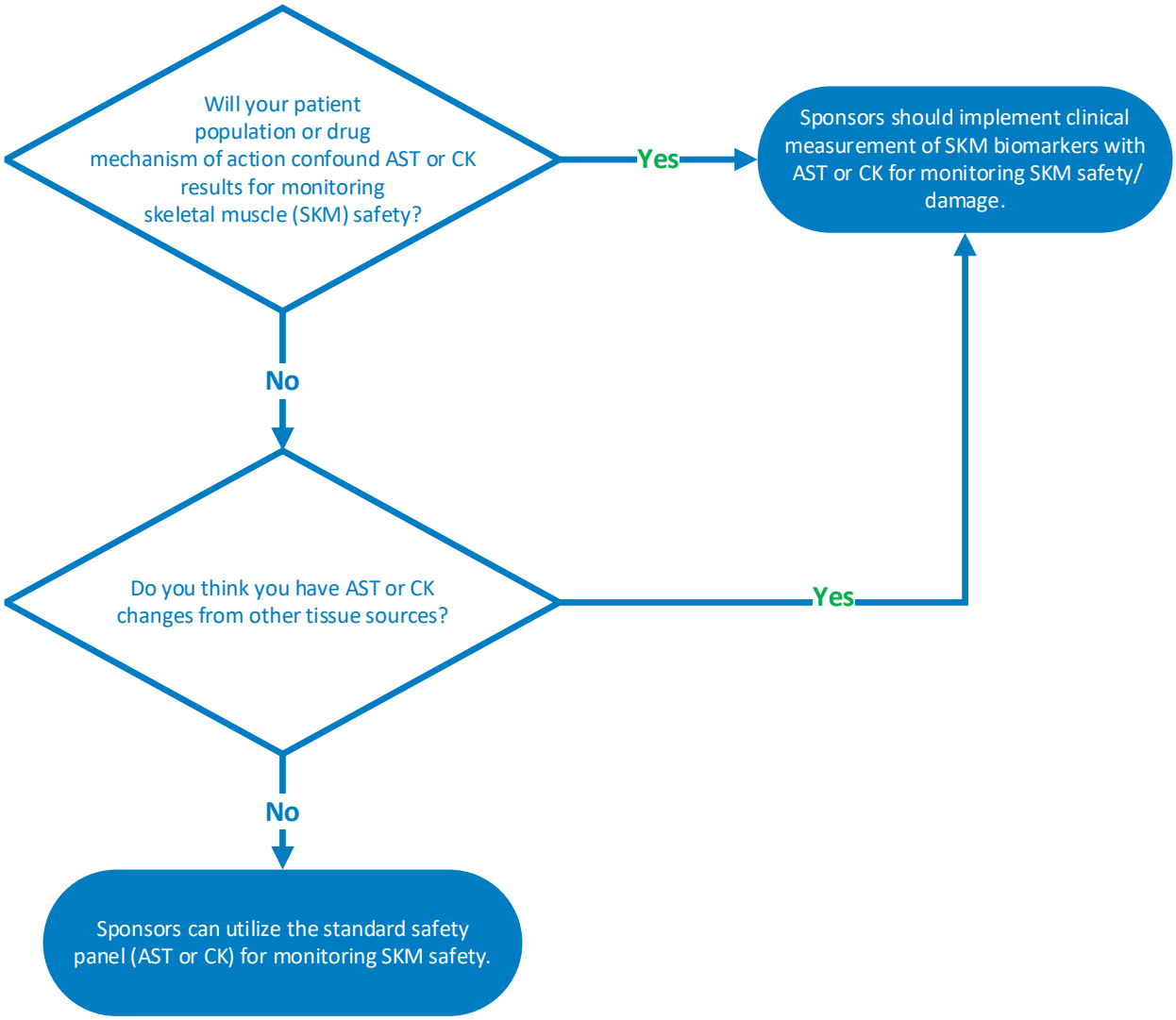
The novel biomarkers are proposed to be applied in early clinical drug development (phase I). The flow chart of the clinical decision criteria and how the biomarker data will be used to make decisions is shown in Figure 1. To date, a study in normal human volunteers conducted by PSTC established baseline levels of the four biomarkers. In addition, data on 74 Duchenne muscular dystrophy (DMD) patients, 38 Becker muscular dystrophy (BMD) patients, and 49 limb-girdle muscular dystrophy type 2B (LGMD2B) patients were compared to data from 32 healthy controls (Burch 2015) indicating that serum levels of these proteins were significantly elevated in muscular dystrophy patient populations. These studies are described in Appendix 3 and these data will serve as a foundation for further work. As data are collected on the four biomarkers of interest, an analysis will allow further construction of the data into a decision tool, considering each biomarker individually and potentially as a composite.

The clinical use of the biomarkers is envisioned as a supplement to AST and total CK enzymatic activity to sensitively and specifically detect and monitor SKM degeneration/necrosis. In situations where AST elevations could be caused by liver damage for example, the biomarkers would enable differentiation of SKM due to disease processes or drug exposure from liver derived elevations. Resolution or progression of SKM injury apart from other disease or drug induced damage could be monitored and clinical decisions made in the context of AST and CK data as well as other standard endpoints as appropriate.

Because these biomarkers would be used in conjunction with, and not instead of, the routinely used biomarkers, specificity and sensitivity will be increased without increased risk. The studies described in the attachments and the Qualification Plan (QP) will address clinical validation and examine specificity, sensitivity, and correlation with standard biomarkers of SKM. It is assumed that the four biomarkers will translate from preclinical to clinical, based on the clinical data already generated and the fact that nonclinical performance is correlated with histopathology rather than mechanism of toxicity or pathogenesis of disease.

Figure 1: Decision tree for clinical use of SKM biomarkers





Supporting Information

Please summarize existing preclinical or clinical data to support the biomarker in its COU (e.g. summaries of literature findings, previously conducted studies) (limited to 2,000 characters):

Extensive nonclinical data correlating the biomarker response to SKM histopathology has been generated resulting in the award of a Letter of Support (LOS, January 22, 2015). Data in the literature, from observational studies, randomized control trials, natural history studies, and non-clinical studies (see Appendix 3), further support the correlation between the biomarker response and SKM histopathology. In addition, clinical samples have been prospectively collected in support of these biomarkers (see Appendix 3) and will be analyzed retrospectively for the proposed SKM biomarkers. A study in normal healthy volunteers conducted by PSTC established baseline levels of the four biomarkers. In addition, data on 74 DMD patients, 38 BMD patients, and 49 LGMD2B patients were compared to data from 32 healthy controls (Burch 2015) and demonstrated that serum levels of these biomarkers were significantly elevated

in the muscular dystrophy patient populations. These increases correlated to early damage and muscle mass present in these patients.

Please summarize any planned studies to support the biomarker and COU. How will these studies address any current knowledge gaps (limited to 2,000 characters)?

The four biomarkers will be measured in several populations to:

1. Establish a reference range for each biomarker in healthy subjects
2. Evaluate the effect of age, gender, and ethnicity, for each biomarker and characterize inter- and intra-subject variability in healthy subjects
3. Establish the performance of each biomarker in the presence of SKM injury and determine the correlation of each biomarker with AST and total enzymatic CK using:
    a. Cross sectional and longitudinal studies in patients with drug-induced SKM injury
    b. Cross sectional and longitudinal studies in patients with muscular and neuromuscular diseases associated with myocyte degeneration/necrosis
    c. Rat toxicity studies with histopathology as a gold standard using drugs that cause injury to SKM and heart muscle to analyze sensitivity
    d. Rat toxicity studies with histopathology using drugs that cause injury to kidney, liver, vasculature, and gastrointestinal tract to analyze specificity
4. Establish thresholds (cut-points) in plasma that indicate medically important SKM injury for each biomarker in a population with SKM injury associated with myocyte degeneration/necrosis
5. Assess the capability of each biomarker to differentiate liver, kidney and/or heart injury from SKM injury in human subjects with myocyte degeneration/necrosis; and in rats with various drug-induced end organ injuries confirmed by histopathology

Previous Qualification Interactions and Other Approvals

Select all that apply

All prior regulatory interactions are summarized in Appendix 4.

Letter of Support (Date 1/22/2015):

https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/UCM605348.pdf

Attachments

Appendix 1: References

Appendix 2: Summary of Biomarkers*

Appendix 3: Summary of Studies*

Appendix 4: Summary of Regulatory Interactions*

***Optional information will not be posted publicly.**

Please refer to the Biomarker Qualification Contacts and Submitting Procedures for the mailing address and other important submission-related instructions. If you have any questions about submission procedures, please contact CDERBiomarkerQualificationProgram@fda.hhs.gov.