Biomarkers for Muscle Diseases – Data Supporting Glutamate Dehydrogenase as a Specific Biomarker of Liver Damage

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The Critical Path Institute (C-Path) is a non-profit organization set up to foster development of new evaluation tools to inform medical product development through responding to the needs of the Food and Drug Administration's (FDA) Critical Path Initiative. C- Path's Consortia:

- Act as a trusted, neutral third party working with industry, academia, non-profit groups and regulators
- Enable iterative EMA/FDA/PMDA participation in developing new methods to assess the safety and efficacy of medical products
- Seek regulatory endorsement of novel methodologies and drug development tools – biomarkers, clinical outcome assessments, models and *in vitro* tools.
- Develop and share data standards and databases.

The Predictive Safety Testing Consortium's (PSTC) goal is to find improved safety testing methods. PSTC brings together pharmaceutical companies to share and validate innovative safety testing methods under advisement of the FDA, EMA, and PMDA. PSTC utilizes a translational approach to qualify safety biomarkers in six working groups: kidney injury, liver injury, pancreatic injury, skeletal muscle, testicular toxicity, and vascular injury. All biomarker programs have a strong translational focus to select new safety tools that are applicable across the drug development spectrum. PSTC has qualified 7 biomarkers with FDA, EMA, and PMDA, and has received 11 letters of support (LOS).

The Duchenne Regulatory Science Consortium (D-RSC) develops tools to accelerate therapy development for Duchenne Muscular Dystrophy (DMD). D-RSC's focus is on understanding natural history of the disease and disease progression [see poster #45]. D-RSC is also interested in biomarker development

D-RSC has published a CDISC Therapeutic Area User Guide to describe database structure for Duchenne data, developed an integrated database of nearly 1000 patients' data and is working on developing a clinical trial enrichment platform for the disease.

Liver Toxicity Biomarkers in Patients with Muscle Damage

Drug-induced liver injury (DILI) remains the single greatest cause for termination of development of drug candidates and withdrawal of approved drugs from the market.[1,2] Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum activity measurements are used as gold standard biomarkers for the identification of liver injury in clinical practice, and ALT is commonly used to assess risk of liver injury during drug development. However, both biomarkers are present in muscle as well as liver.

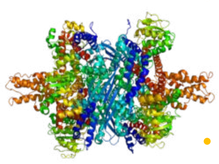
- As ALT and AST are present in myofibers, serum ALT and AST activities increase in subjects with muscle injury
- This severely limits the utility of ALT/AST as markers of liver damage in subjects with underlying muscle impairments such as those with muscular dystrophies or myositis
- Increased levels of ALT/AST due to underlying muscle damage may potentially mask a hepatotoxic signal, creating a diagnostic challenge for clinicians
- Persistent transaminasemia is frequently misdiagnosed as liver injury in patients with inherited muscle disorders such as DMD and idiopathic inflammatory myopathies[3,4,5]

There is a need for a biomarker that detects liver damage in patient with muscle disease to:

1. **Demonstrate if a new drug is causing liver damage**
2. **Demonstrate if a patient has liver damage in a clinical setting**
3. **Reduce misdiagnosis of liver injury in patients with muscle disorders; potentially accelerate diagnosis.**

Glutamate Dehydrogenase (GLDH)

GLDH



- Converts glutamate to ketoglutarate in mitochondria
- Tissue distribution:
  - Liver>>kidney, pancreas and intestinal mucosa. Only trace amount present in muscle, lymphocyte and other tissues.
- Utility of GLDH as liver specific biomarker of liver injury shown in preclinical species

GLDH correlates with liver damage

Table 1: Liver safety biomarkers ALT and AST are high in boys with DMD even when there is no liver injury. GLDH levels increase with liver injury (APAP toxicity), but not in patients with only muscle disease.

	GLDH (u/L)	ALT (u/L)	AST (u/L)	CK (u/L)
Healthy adults (364)	3 ± 2	20 ± 6	22 ± 4	104 ± 57
Healthy boys (3)	3 ± 0	21 ± 10	29 ± 2	131 ± 42
DMD boys (41)	**5 ± 2**	**378 ± 214**	**235 ± 145**	**11,162 ± 7,977**
APAP tox adults (8)	963 ± 1,000	3,788 ± 1,730	3,614± 2,824	884 ± 1,456

Figure 1: Correlation of DILI biomarkers with muscle damage. ALT levels increase with creatine kinase (A); GLDH levels do not (B).

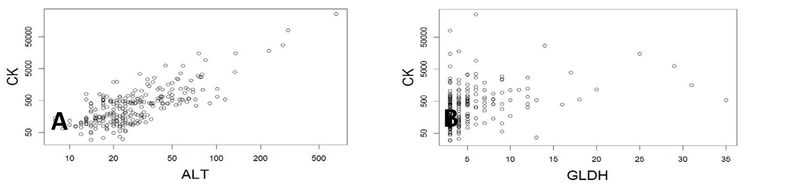


Figure 2: GLDH as a DILI biomarker in patients with underlying muscle damage. GLDH is low prior to liver damage, then increases in a patient with rhabdomyolosis.

Assay Characteristics

The Randox GLDH assay was validated according to Clinical Laboratory Improvement Amendments (CLIA) guidelines for Laboratory Developed Tests (LDT). It has been tested for: accuracy, precision, analytical sensitivity, long term stability, freeze/thaw stability, analytical specificity to include interfering substances, reportable range, and reference interval.

GLDH threshold levels

Figure 3: Correlation of GLDH and ALT. Serum GLDH cutoff levels are 25 U/L and 48 U/L, which can also be expressed at 2.5x and 5x ULN GLDH. Confidence intervals (95%) for these cut-offs are (2.1, 3.4) and (3.9, 6.6), respectively.

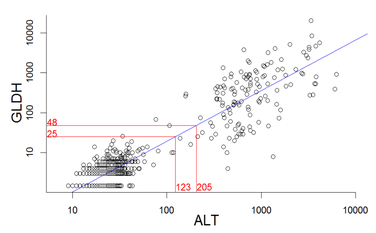


Table 2: Comparable diagnostic performance of GLDH and ALT. Contingency tables classifying subjects as above/below the cutoffs for each marker. Here, using the 2.5x ULN threshold for GLDH (25 U/L), there is 98% agreement with the 3x ULN threshold for ALT (123 U/L). Sensitivity and specificity of GLDH at this threshold are 92% and 99.5%, respectively. Similarly, there is 97% agreement for the 5x ULN cutoffs.

	ALT ≤ 3x	ALT > 3x	Total		ALT ≤ 5x	ALT > 5x	Total
GLDH ≤ 2.5x	618	11	629	GLDH ≤ 5x	622	23	645
GLDH > 2.5x	3	126	129	GLDH > 5x	3	110	113
Total	621	137	758	Total	625	133	758
Concordance:	98%			Concordance:	97%		
Sensitivity:	92%			Sensitivity:	82.7%		
Specificity:	99.5%			Specificity:	99.5%		

For these analyses, subjects selected had both AST and ALT levels greater than two times normal healthy levels with a diagnosed disease resulting in impaired liver function (including, but not limited to, liver transplant, hepatic carcinoma, cirrhosis or liver impairment).

Studies supporting qualification

Table 3: Studies completed in support of GLDH as a DILI biomarker. More confirmation is ongoing.

Objective(s)	Description
Establish GLDH reference range for healthy subjects and evaluate influence of sex, ethnicity, and age.	Analysis of serum from healthy volunteers meeting recruitment criteria for Phase I trials (n = 186)
Establish GLDH reference range for healthy subjects and evaluate influence of sex, ethnicity, and age.	Analysis of serum from healthy subjects with normal liver function (n = 364).
Establish GLDH reference range and evaluate influence of sex, ethnicity, and age, as well as intra- and inter-subject variability.	Analysis of serum from healthy volunteers meeting recruitment criteria for PSTC biomarker study (n = 81)
1) Confirm correlation between GLDH and ALT. 2) Establish specificity and sensitivity of GLDH for liver injury using Expert Working Group definition (Aithal et al., 2011): ≥ 5x ALT or ≥ 2x ALP or [≥ 3x ALT and ≥ 2x TBil]. 3) Plot ALT and GLDH values in APAP overdose patients over a time course to observe kinetics of biomarker change. 4) Establish GLDH threshold values for liver injury through linear regression model with log GLDH and log ALT, then calculate GLDH levels that correspond to 3x and 5xULN ALT.	Serum samples from subjects with AST and ALT > 2xULN and with diagnosed disease or injury resulting in increased liver enzymes (total n = 479). All healthy subjects used in reference range study were included in analysis.
1) Examine specificity of GLDH for liver injury in human subjects with muscle impairment. 2) Determine if GLDH can detect liver injury onset in rhabdomyolysis patients.	Serum samples from (a) healthy subjects (n = 125; 3-64 years of age, and (b) subjects with muscle injury (n = 131; 2-78 years of age).
Determine GLDH specificity for liver and characterize levels in DMD subjects.	Serum samples from DMD patients (n=40; 5-14 years of age).
Determine whether GLDH changes with exercise.	Observational study in subjects (n=12) participating in extreme adventure race. Samples taken pre- and post-race.
Confirm correlation of ALT and GLDH for hepatocellular injury, and specificity of GLDH for hepatocellular injury when other organ toxicities present.	Histopathology and biomarker data for rat toxicology studies with multiple toxicants (n=30), including those targeting liver, kidney, heart, and pancreas.
Examine specificity of GLDH for liver injury.	Histopathology and biomarker data (ALT, AST, and GLDH) collected from rats treated with acetaminophen (APAP) and of 2,3,5,6-Tetramethyl-p-phenylenediamine (TMPD)

Use of GLDH as a DILI biomarker

When GLDH is utilized in clinical studies:

1. GLDH activity should be utilized as a complement to standard methods for assessing drug induced liver injury (DILI).
2. The mechanism by which GLDH and ALT appear in serum following hepatocellular injury is similar; their enzymatic activity is highly correlated in humans and animals with a diversity of liver injuries and diseases;
3. GLDH activity levels 2.5x and 5x above upper limit of normal (ULN) are estimated to correspond to 3x and 5x above ULN for ALT, though this still requires further confirmation. These fold changes of GLDH could be utilized, along with the standard hepatic injury monitoring panel, for the assessment of DILI, in the same manner as 3x and 5x ULN ALT

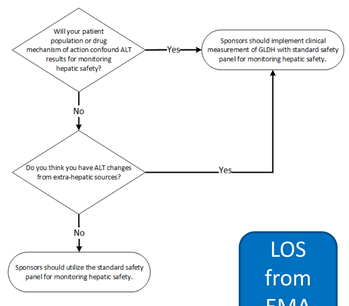
GLDH qualification

PSTC and D-RSC are seeking qualification of GLDH as a biomarker of liver toxicity independent of muscle damage.

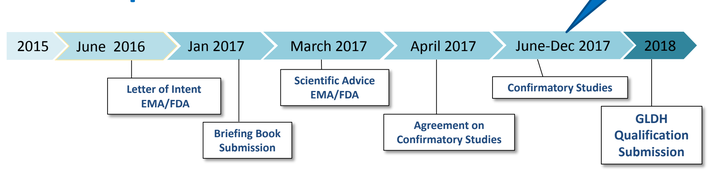
Context of use:

Elevated serum enzymatic activity of GLDH is a measure of hepatocellular injury, and can be used in healthy subjects and patients as an adjunct to ALT in all stages of drug development trials. When ALT increases are observed from suspected extrahepatic sources such as muscle, GLDH can lend weight of evidence to confirm or rule out hepatocellular injury.

Figure 4: Decision scheme for GLDH use in drug development



Timeline for qualification




EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH
Letter of support for glutamate dehydrogenase, a biomarker of hepatocellular liver injury

http://www.ema.europa.eu/docs/en_GB/document_library/Other/2017/11/WC500239388.pdf

References:

1) Yuan and Kaplowitz, 2013; 2) Kaplowitz, 2005, 3) Rutledge et al., 1985, 4) Begum et al., 2000, 5) Nathwani et al., 2005

Next Steps: We will complete analyses for qualification, submit the final qualification package, and work towards *in vitro* diagnostic status for a GLDH assay.

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