Hepatotoxicity and Drug-Induced Liver Injury Detection

Detecting potential hepatotoxicity is crucial during drug development and drug safety testing in order to reduce the risk of drug candidate withdrawal, and pharmaceuticals showing unwanted hepatic side effects in future surveillance studies.

Standard hepatic injury biomarkers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), show inadequate sensitivity and specificity with limited predictive value. During preclinical drug development, AST/ALT elevations are often observed in the absence of hepatic injury. Conversely, transaminases may not increase even when tissue injury occurs. Around 40 percent of research subjects with drug-induced liver injury (DILI) are not detected in safety studies, and therefore new biomarkers are very much needed. Ximelagatran was the first orally available direct thrombin inhibitor under development that also reached the market in numerous countries. The drug was tested in an extensive preclinical and clinical program before it was approved and subsequently withdrawn from the market due to hepatotoxicity reports. The preclinical toxicology program in a variety of models, and later short-term use in phase III clinical trials, did not show this hepatotoxic effect. It wasn’t until long-term use of ximelagatran when increasing hepatic laboratory tests, such as ALT elevations, were observed. The standard preclinical toxicological studies at the time provided no indication that ximelagatran affected hepatic function. How much money was spent? It may not be clear but the average cost of taking a new drug from the bench to the shelf now exceeds $4 billion by some estimates. Many other stories exist, such as the tragic troglitazone story that made national headlines in the late 1990s. Clearly there is a need for better biomarkers in drug development.

DILI is the most frequent cause of acute liver failure. Today’s methods for detecting such liver damages are imperfect, and these adverse side effects often surface in a late stage, after market launch. This late detection may result in withdrawal of the pharmaceutical from the market, which costs the industry hundred millions of dollars, and years of research and development lost.

The Predictive Safety Testing Consortium (PSTC) and Safer and Faster Evidence-based Translation

To mitigate the issues with standard liver tests, the PSTC, in collaboration with the SAFE-T, is validating novel liver injury biomarkers. Among the assays of investigation are caspase-cleaved (ccK18) and total keratin 18 (K18), which are studied for application in the regulatory decision making process for use in drug development and as potential drug safety tests.
Clinical Research Hepatotoxicity

Paracetamol (acetaminophen, APAP) is a common analgesic pharmaceutical which is safe when administered in recommended doses, while acute liver failure (ALF) due to DILI is observed in about 50 percent of APAP overdose cases. Antoine et al. evaluated APAP overdose in a research study and showed that during the acute phase of APAP hepatotoxicity, both ccK18 (M30) and K18 (M65) concentrations were elevated, but necrosis was the dominant form of cell death. Current standard liver tests remained normal in this early phase.

Recently, Ku et al. described novel K8/K18 variants in subjects that had fatal DILI, further highlighting the critical role these intermediate filaments play in the liver. K18 is found only in simple epithelial tissues and is among the most abundant proteins in the liver. K18 is not found in muscle or blood (source for false positive aminotransferase levels).

While rigorous exercise or overeating in seemingly healthy subjects may cause aminotransferase elevations in early phase research studies, K18 does not increase. In a recent study, Thulin et al. showed in an extreme adventure race study that ALT, AST, and CK were highly elevated while M30 ccK18 (M30) and K18 (M65) remained unchanged.

This study also showed that M65 (K18) was the more sensitive liver marker in drug studies. M65 levels increased significantly at an earlier time point than ALT. In addition, M65 decreased towards baseline when the study ended, while ALT levels remained elevated. The advantage of a faster turn-over is a quicker determination of the drug effect.

For assessing the safety of a new drug candidate, it is of similarly high importance that novel mechanism-based biomarkers such, as ccK18 and K18, show no increases with treatments that are known not to cause liver damages.

Harrill et al. investigated heparin, an anticoagulant drug known to be safe for the liver while leading to significant increases of AST and ALT. Research subjects receiving heparin had asymptomatic elevations in serum ALT and AST in more than 90 percent of subjects.

While levels of K18 (M65) also increased with treatment, ccK18 (M30) concentrations remained unchanged, suggesting that heparins may cause mild hepatocyte necrosis, but not apoptosis. This study clearly shows that ccK18 and K18 hold potential as mechanism-based prognostic biomarkers when performing research studies in assessing the safety of a new drug candidate.

K18 Biomarker Features
Outperforms ALT in specificity when defined as organ of origin
Highly abundant in liver tissue
Minimal day-to-day fluctuations in healthy subjects
Remarkably stable in serum/plasma after sampling
Relatively short half-life in the circulation for examining effects of a drug
Provides informative mechanistic information on hepatocyte injury (apoptosis and/or necrosis)

Apoptosis Products
M30 Apoptosense® ELISA Prod. No. 10011
M30 CytoDeath™ ELISA Prod. No. 10900

Total Cell Death Products
M65® ELISA Prod. No. 10020
M65 EpiDeath® ELISA Prod. No. 10040