



APOPTOSIS & NECROSIS:

Biomarkers ccK18 and K18 in hepatotoxicity

Detection and quantification of liver damage with M30 and M65

Peviva Products M30/M65 are for Research Use in the US and Canada



Defend the safety of your new drug with new biomarkers

- Reduce risk of unnecessary costs by preventing large clinical trials with indeterminate liver safety concerns
- Prevent unnecessary withdrawal or clinical hold for promising new drug candidates
- Most importantly, prevent delay of new medications to the market

The use of new biomarkers and new models can:

- Increase liver specificity
- Provide mechanistic information
- Predict liver injury at earlier time points than ALT

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) have been used for over half a century and 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 The drug was tested in an extensive preclinical and clinical program before it was approved and subsequently withdrawn from the market due to hepatotoxicity reports.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. 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 average cost of taking a new drug from the bench to the shelf now exceeds \$4 billion by some estimates. Cases of idiosyncratic DILI have led to tragic results, such as with troglitazone story in the late 1990s that had resulted in liver-related deaths before its withdrawal from the market. Clearly there is a need for better DILI biomarkers in drug development.

The Predictive Safety Testing Consortium (PSCT) and International Safety Program (IMI)

Collaborative efforts are ongoing to identify valuable biomarkers that can help overcome the hurdles in drug development. The SAFE-T consortium consists of a unique partnership between the European Communities and the pharmaceutical industry. Their goal is to address the problems associated with withdrawal of drugs in a late stages and patient safety. To mitigate the issues with standard liver tests, the PSTC, in collaboration with the SAFE-T, is validating novel liver injury biomarkers, and a subset of biomarkers have received letters of support from both the EMA and FDA for use in an exploratory development setting. Among the assays being investigated are cckK18 and total K18, which are studied process for use in drug development and as potential drug safety tests.

K18 in Clinical Research Hepatotoxicity

Keratin 18 (K18) (also referred to as cytokeratin 18 [CK18]), measured by the M65 EpiDeath® ELISA, and caspase-cleaved K18 (cckK18), measured by the M30 Apoptosense® ELISA, are among the select biomarkers in preliminary investigations. K18 biomarkers were assessed for their performance and potential among a variety of candidate DILI biomarkers, and further research on K18 to address the need of more sensitive and specific biomarkers in detecting and predicting the outcome of DILI is encouraged.

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 cckK18 (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, novel K8/K18 variants were described in subjects that had fatal DILI, further highlighting the critical role these intermediate filaments play in the liver. While rigorous exercise or overeating in seemingly healthy subjects may cause aminotransferase elevations in early phase research studies, K18 does not increase. K18 is present only in epithelial cells, such as hepatocytes where it is one of the most abundant proteins. For example, in an extreme adventure race study that ALT, AST, and CK were highly elevated while M30 cckK18 (M30) and K18 (M65) remained unchanged.



For assessing the safety of a new drug candidate, it is of similarly high importance that novel mechanism-based biomarkers such, as cckK18 and K18, show no increases with treatments that are known not to cause liver damages. Heparin, an anticoagulant drug known to be safe for the liver and used for decades, can lead 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, cckK18 (M30) concentrations remained unchanged, suggesting that heparins may cause mild hepatocyte necrosis, but not apoptosis. This study clearly shows that cckK18 and K18 hold potential as mechanism-based biomarkers for DILI.



More recently, this theory on whether the use of new biomarkers and mathematical modeling could improve liver safety risk assessment was examined. Cimaglermin alfa was being studied for heart failure in a phase I clinical trial but met the stopping criteria outlined by the FDA for potential hepatotoxicity. With use of caspase-cleaved cckK18 and K18 biomarkers, along with additional biomarker measurement and subsequent mathematical modeling, it was determined that the loss of hepatocytes may not have indicated serious liver injury. Use of mechanistic biomarkers together with mathematical modeling can improve interpretation of potential hepatotoxicity in clinical trials.

K18 Biomarker Features

- Increased before ALT
- Increased liver specificity
- Highly abundant in liver tissue
- Minimal day-to-day fluctuations in healthy subjects
- Remarkably stable in serum/plasma after sampling
- Provides mechanistic information on hepatocyte injury (apoptosis and/or necrosis; apoptotic index)

Apoptosis Products

M30 Apoptosense® ELISA (P10011)
M30 CytoDeath™ ELISA (P10900)

Total Cell Death Products

M65® ELISA (P10020)
M65 EpiDeath® ELISA (P10040)