COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

DRAFT

NON-CLINICAL GUIDELINE ON DRUG-INDUCED HEPATOTOXICITY

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EXECUTIVE SUMMARY

During the last decade liver toxicity has been one of the most frequent reasons for pharmacovigilance safety reports and the withdrawal from the market of an approved medicinal product. This guideline introduces a stepwise (Step I - III) approach in detecting hepatotoxicity signals and conducting mechanistic studies to assess the clinical relevance of non-clinical hepatotoxicity. The main emphasis in this guideline is to optimise the use of data obtained in standard non-clinical studies (Step I). Moreover, based on the findings in standard non-clinical studies additional, mechanism-oriented, studies are proposed (Step II - III). Key elements in the evaluation of hepatotoxic signals are:

1) The identification of compound-related effects in clinical pathology parameters in relevant species and determination of the magnitude of that effect through the comparison of individual animal and group mean data with concurrent controls. Historical control data can be used to place the magnitude of the changes in perspective but are not the sole determinant of whether or not a change is compound-related.

2) For non-rodent studies, attention should be focused on the comparison of individual animal data prior to the treatment and at different experimental time points rather than using the group mean data for such comparisons. Despite the fact that hepatotoxicity findings may not be statistically significant due to the low number of non-rodents used in non-clinical studies, relevant hepatotoxicity signals observed in non-rodent studies should be thoroughly investigated.

3) The use of data obtained from mechanistic in vitro and/or in vivo models.

The risk assessment should take into consideration all available data. The collection of hepatotoxic signals is not restricted to any specific phase of the development programme of a medicinal product; rather it is a continuous process covering all phases. The principles of the present guideline can also be applied in resolving hepatotoxic signals that have been picked up in pharmacovigilance safety monitoring. These principles can also be applied when assessing bibliographic information. Improved detection and prediction of drug-induced hepatotoxicity could be achieved by the use of new predictive biomarkers and in vitro and/or in vivo models, not yet available and/or validated.

1. INTRODUCTION

One of the most frequent reasons for the withdrawal from the market of an approved drug during the last decade is liver toxicity. Critical assessment of these cases has led to the observation that the non-clinical data available at the time of marketing application sometimes contained signals that (in retrospect) could have predicted hepatotoxicity. Therefore, there is a need for guidance on how to act when non-clinical and/or clinical signals of hepatotoxicity are detected in order to improve human safety.

2. SCOPE

The objective of this document is to provide guidance on how to identify, collect and report non-clinical signs of drug-induced hepatotoxicity in order to decrease the risk of clinical adverse liver reactions. This guideline applies to medicinal products that are systemically absorbed. DNA-reactive (cytotoxic) anti-cancer medicinal products are exempted from the scope of this guideline. The principles as introduced in the present guideline would also help to identify mechanisms underlying drug-related adverse liver reactions of a medicinal product already on the market.

3. LEGAL BASIS

This document should be read in conjunction with Directive 2001/83/EC (as amended) and all relevant non-clinical guidelines. The following guidance documents are especially relevant:

- Note for guidance on Duration of Chronic Toxicity Testing in Animals (Rodent and non Rodent Toxicity Testing) (CPMP/ICH/300/95),
4. GENERAL CONSIDERATIONS
Drug-induced hepatotoxic reactions are of multiple types and origin and the time to onset varies from being very short to exhibiting a long latency. Clinically, the most relevant reactions include liver necrosis, hepatitis, cholestasis, vascular changes and steatosis. It is worth emphasizing that species differences in drug metabolism, target molecule and pathobiology must be taken into account in the interpretation of findings and in assessing the relevance of such findings to humans. For example, steatosis has significant implications to clinicians (i.e., non-alcoholic steato hepatitis; NASH), yet it is generally a less important finding non-clinically, particularly if observed in rodents.

A drug can cause liver toxicity via several mechanisms. For instance, it can be directly acting or indirectly through reactive metabolites. The drug or its metabolites may cause liver toxicity after specific receptor binding, or reactive metabolites can react with hepatic macromolecules leading to direct cytotoxicity. On the other hand, liver toxicity can be mediated via an immunological cascade. Development of liver tumours cannot be considered as a signal for hepatotoxicity and, is therefore, not addressed in this guideline.

Increases in the levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum, in combination with increased bilirubin levels, are actually considered to be the most relevant signal of liver toxicity. Macroscopic and in particular histopathological observations will allow confirmation of the occurrence of liver toxicity and will provide further evidence of the type of liver toxicity. However, the absence of histological findings does not exclude liver toxicity.

Assessment of hepatotoxicity should:

i) identify the changes, and their magnitude e.g. clinical pathology and histopathology, in-life observations, etc.

ii) provide a NOAEL (no observed adverse effect level) for liver toxicity in light of either the dose level or systemic exposure and to determine safety margins in relation to exposure to humans,

iii) determine a mechanism/pathogenesis for the toxicity if it can improve prediction of clinical safety.

Based upon the non-clinical data, if feasible, the selection of an appropriate biomarker(s) could be considered. These biomarkers could be used to monitor potential hepatotoxic clinical events.

In the future and when available, -omics data may be useful to study hepatotoxicity.

5. PROTOCOL DESIGN
The collection of hepatotoxic signals is a continuous process starting from non-clinical findings, and continuing to clinical trials (phase I-III) and post-marketing experiences. The observation of prolonged unresolved abnormal liver function in humans could be linked to the drug (medicinal product) used. Such findings may trigger a re-evaluation of the non-clinical data and, possibly, conduct of ancillary non-clinical studies.
In the detection and evaluation of hepatotoxicity the main focus is on how to use the data obtained from the existing non-clinical studies optimally. Moreover, there might be a need for further investigations. Therefore, the detection and evaluation of hepatotoxicity will be a stepwise procedure;

i) **Evaluation of hepatotoxic effects**: to identify signals indicating liver toxicity in standard pharmacological and toxicological studies;

ii) **Follow-up studies to characterise hepatotoxic effects**: to identify possible mechanism(s) of liver toxicity using *in vitro* and/or *in vivo* tests, including possible identification of an appropriate biomarker based upon non-clinical results;

iii) **Additional studies**: to develop new mechanistic *in vitro* methods and/or *in vivo* models.

With respect to the animal species used in standard non-clinical studies, a general assumption is that: the higher the animal species (rodent, non-rodent, non-human primate) that demonstrates signs of liver toxicity or histopathological adverse responses, the greater the relevance of clarifying the mechanism(s) responsible for liver toxicity. In a comprehensive retrospective examination of published data, non-clinical concordance to a clinical hepatotoxic event was lowest with a rodent finding only, increased when the finding was in non-rodent only, and was greatest when a finding was found in both species.

### 5.1 Hepatotoxic end points

In the early steps of the drug development programme any hepatotoxic signal should be assessed critically. In non-clinical studies, especially in non-rodents, statistical analysis of changes in hepatotoxicity parameters is complicated by inter- and intra-animal variability. Due to the low number of non-rodents used in non-clinical studies, a relevant hepatotoxic signal may be biologically significant despite the fact that the finding may not be statistically significant. Historical control data are important for evaluating the relevance of sporadic hepatotoxicity findings. The biological significance of relevant hepatotoxic signals in non-rodents and rodents should be clarified.

#### 5.2 Step 1: Evaluation of hepatotoxic effects:

Hepatotoxic signals including reversibility may be identified and evaluated from standard non-clinical studies. Important signals may be obtained from:

i) clinical chemistry,

ii) histopathology and ultrastructural pathology,

iii) bioaccumulation, expression of xenobiotic metabolising enzymes, and generation of reactive metabolites,

iv) immune-related hepatotoxicity.

Based upon these hepatotoxic signals, an integrated risk assessment can be carried out.

##### 5.2.1 Clinical chemistry

Various clinical chemistry variables may be used to obtain information on liver toxicity. Parameters for identification of liver toxicity in non-clinical studies (rodents and non-rodents) could consist of the following:

**Table 1 Clinical chemistry variables that are considered useful in identifying liver toxicity.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hepatocellular</th>
<th>Hepatobiliary</th>
<th>Mitochondrial</th>
</tr>
</thead>
<tbody>
<tr>
<td>alanine aminotransferase (ALT)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aspartate aminotransferase (AST)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sorbitol dehydrogenase (SDH)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### glutamate dehydrogenase (GLDH)  X  
### total bile acids (TBA)  X  X  
### alkaline phosphatase (ALP)  X  
### gamma glutamyltransferase (GGT)  X  
### 5’-nucleotidase (5-NT)  X  
### total bilirubin (TBILI)  X  

#### Potential ancillary markers

<table>
<thead>
<tr>
<th>marker</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>lactate</td>
<td>X</td>
</tr>
<tr>
<td>lactate dehydrogenase (LDH)</td>
<td>X</td>
</tr>
<tr>
<td>ornithine carbamyltransferase (OCT)</td>
<td>X</td>
</tr>
<tr>
<td>unconjugated bilirubin (UBILI)</td>
<td>X</td>
</tr>
</tbody>
</table>

Total protein, albumin, triglycerides, cholesterol, glucose and blood urea nitrogen, activated partial thromboplastin time (APTT) and prothrombin time (PT) can be used as supplementary tests for hepatic synthetic functions.

#### 5.2.1.1 Hepatocellular toxicity:

Among the above-mentioned marker activities, ALT and AST alone or in combination with total bilirubin are primarily recommended for the assessment of hepatocellular injury in rodents and non-rodents in non-clinical studies. ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST. An increase of ALT activity in the range of 2-4 fold and higher compared to concurrent control average or individual pre-treatment values in non-rodents, should raise concern as an indication of potential hepatic injury unless a clear alternative explanation is present. The potential ancillary markers of Table 1 are useful when additional indicators of hepatic pathology are desired.

#### 5.2.1.2 Hepatobiliary toxicity:

GGT is specific for cholestasis and is commonly used to assess cholestasis in non-rodents. ALP is more sensitive but much less specific than GGT. Increases in total bilirubin (TBILI) or total bile acids (TBA) concentrations in individual or group mean data, compared to concurrent controls, should be critically evaluated in the context of other study data, to exclude extrahepatic factors that are known to influence their concentrations. In the absence of changes in other hepatic parameters or in the presence of evidence of haemolysis, increases in bilirubin alone are unlikely to reflect adverse liver reactions.

#### 5.2.2 Histopathology and ultrastructural pathology

Liver histopathology should serve as the most important tool for identifying and characterizing liver injury whether or not clinical chemistry changes are also identified. The presence of significant apoptosis/necrosis should be addressed (the pattern of cellular damage, the presence of cellular infiltrates, and the presence of necrotic and/or apoptotic cells).

Ultrastructural pathology can provide evidence for enzyme induction, mitochondrial changes, drug accumulation, and early indications of cholestasis, necrosis, steatose etc.
5.2.3 Bioaccumulation, Expression of Xenobiotic Metabolising Enzymes and Generation of Reactive Metabolites:

In the absence of other signals of hepatotoxicity, accumulation of the drug in the liver is not a cause of concern.

Investigation of metabolic pathways provides clues to the production of (possible reactive) metabolites. At therapeutic dose levels, drugs may affect expression of xenobiotic metabolising enzymes (induction or inhibition) and potentially increase the concentration of metabolites.

In the presence of hepatotoxicity signals, possible reactive metabolites should be identified in Step II.

5.2.4 Immune-based hepatotoxicity

Signals for an immune-based liver damage may be obtained from histopathological analysis (e.g. an increase in parenchymal eosinophils, mild hepatic inflammation or granulomas).

Additionally, haematological findings should be considered together with histopathology of immunological organs (spleen, thymus, lymph nodes).

If hepatotoxicity and inflammatory reactions are observed, it is important to address potential causes of these signals before deciding if additional studies (Step II, 5.3.5) are needed.

5.2.5 Integrated risk assessment

The initiation of mechanistic studies (Step II) is dependent on the outcome of an integrated risk assessment of all available data obtained in Step I. This integrated assessment should consider:

1. dose dependency and safety margins obtained in different species
2. reversibility of the toxic effect
3. the ability to use a premonitory clinical biomarker
4. intended patient population and disease status
5. clinical safety data, if available

If this integrated risk assessment identifies a toxicologically/biologically significant effect and thus indicates that Step II studies are warranted, these studies should be hypothesis-based and focused on further understanding the clinical relevance of the animal findings.

5.3 Step II; follow-up studies to characterise hepatotoxic effects

The testing strategy depends on:

- the signals arising from the standard non-clinical studies (Step I)
- whether the test substance belongs to a class of chemically or structurally similar compounds known to pose a risk for hepatotoxicity, or whether signals are described in literature. For further in vitro investigations, the inclusion of positive controls from the chemical class is recommended. For in vivo studies the inclusion of positive controls should be considered.

Step II studies are mechanism-oriented. These may include:

i) testing using in vitro liver models,
ii) studies to further investigate potential cholestatic injury,
iii) functional and morphological mitochondrial tests ,
iv) additional histopathology,
v) immunology-based tests.
5.3.1 Testing of parent compound or metabolites in in vitro liver models:

If hepatotoxic signals occur in vivo (Step I) or signals are described in literature, parent compound or (reactive) metabolites if feasible, should be screened in in vitro mechanistic tests for potential hepatotoxicity.

Conventional cytotoxicity assays rely on measuring one or more cytotoxic indicators, including loss of membrane integrity or cytolysis (e.g. LDH release, membrane-impermeable DNA stain), apoptosis (e.g. increase of caspase-1- or caspase-3-like activities), loss of critical macromolecules or small molecule scavengers (e.g. ATP or glutathione (GSH)), increased formation of reactive oxygen species, mitochondrial effects (e.g. tetrazolium salt assays, Alamar blue assay) or anti-proliferative effects (e.g. inhibition of DNA or protein synthesis), and excretion of pro-inflammatory cytokines (i.e. IL-1). In addition, adaptive cell responses - such as changes in cell division, up-regulated detoxification and enhanced expression of survival factors could be investigated. For this purpose; several in vitro human and animal liver models are available ranging from short-term to long-term cell or tissue culture systems. The use of metabolically-competent in vitro liver models (that express relevant phase I and phase II biotransformation enzymes) is recommended. However, since each in vitro liver model suffers from specific limitations (e.g. loss of liver-specific functions), these should be taken into account.

Signals indicating increased cell toxicity at doses close to therapeutic concentrations should be considered for their relevance and if needed initiate more studies.

5.3.2 Indicators of cholestatic injury:

When cholestatic injury has been identified in Step I (see 5.2.1 Hepatobiliary toxicity), potential drug interactions with transporters (e.g. P-glycoprotein, bile salt export pump, MRPs) expressed in the liver may be considered. In the case that a relevant interaction can be predicted for future clinical use of a medicinal product, relevant in vitro/in vivo non-clinical interaction studies should be prospectively carried out.

If inhibition or suppression of bile flow (increase in conjugated bilirubin or bile acids) and an increase in serum ALP activity are seen in Step I in rats, a concomitant increase in 5-NT levels could help the identification of cholestatic injury.

Hepatotoxicity and in particular cholestatic damage is more prevalent in older patients. Therefore, studies on older animals and on cholestatic models of damage should be considered.

5.3.3 Changes indicative of mitochondrial toxicity (in vitro/in vivo):

Mitochondria are important targets for virtually all types of injurious stimuli, including hypoxia and toxins.

Standard non-clinical animal studies are insufficient for the identification of a mitochondrial-based hepatotoxicity.

The following clinical chemistry signals, may be indicative:

- An increase of blood lactate levels.
- An increase in OCT levels: OCT is an urea cycle enzyme found in mitochondria, very liver specific and inducible in rat liver.

An increase in GLDH levels: GLDH is normally present in liver mitochondria but can be released in plasma following liver dysfunction.

In vivo, microvesicular steatosis can be observed with compounds that have been associated with decreased mitochondrial fatty acid β oxidation, mitochondrial dysfunction and/or oxidative stress in preclinical species and in humans. Therefore, because of the clinical implication of this finding, compounds associated with microscopic evidence of microvesicular steatosis (see 5.2.2) should be tested in vitro for these endpoints.

Immunohistochemistry and histochemistry provide an accurate index of mitochondrial dysfunction and can be sensitive to the point of detecting mitochondrial dysfunction in absence of pathology. Thorough histopathological evaluation of the liver can give indications of potential mitochondrial
effects of medicinal products. Electron microscopy might be used to check morphologic changes in mitochondria, which frequently accompany cell injury (e.g. swelling, appearance of small phospholipid-rich amorphous densities in swollen mitochondria).

Mitochondrial functional parameters should be determined from isolated mitochondria and include respiration, membrane potential, Reactive Oxygen Species (ROS) production, and mitochondrial complex activity (activity of the mitochondrial enzymes of the respiratory chain). If changes in these parameters occur further studies may be considered (e.g. measurement of mitochondrial enzyme activities or mitochondrial oxygen consumption in cell cultures).

Several agents exert their mitochondrial toxic effects through depletion of mtDNA content. Therefore, quantification of mtDNA could be used as a marker of mitochondrial toxicity (in vitro, in vivo). However, the need for these types of investigations should be determined on a case-by-case basis taking into account the potential liability for mitochondrial injury based on specific drug-related mechanisms of action and/or association with a specific histological presentation.

Possible mitochondrial damage induced by secondary mechanisms - such as the formation of reactive metabolites that may cause either liver cell necrosis or apoptosis - should be potentially analysed by investigating DNA damage and cellular accumulation of p53 and expression of p53 targets that indirectly affect mitochondrial functioning as determined by quantitative real-time-PCR, glutathione depletion, protein thiol oxidation and increased cytosolic Ca2+. All of these alterations can lead to the opening of mitochondrial permeability transition pore (MPTP) and induction of apoptosis (see Step III).

5.3.4 Histopathology/electron microscopy

If signs of histopathological alteration occur in step I, samples obtained in vivo could be further studied (e.g. analysis using specific fluorescent techniques, immunohistochemistry, and electron microscopy). For mitochondrial toxicity see 5.3.3.

5.3.5 Immune-based hepatotoxicity

Follow-up studies should be conducted with species in which immune-based hepatotoxicity is identified (Step I) and may include evaluation of cytokine profile and cellular activation upon in vivo and/or ex vivo challenge with a drug. However, it should be considered that there are important species differences, i.e., Kupffer cell phagocytic properties vary across species.

It should be noted that presently there are no validated models for immune-mediated hepatotoxicity testing. In light of broad ongoing efforts to investigate and validate various approaches and methods, exploratory testing may be recommended for consideration.

For example, a more potent immune response upon re-administering drug to previously exposed animals compared to naïve animals would indicate a drug hypersensitivity reaction as a potential mechanism for immune-based liver injury.

Ex vivo assays demonstrating drug-induced lymphocyte activation in sensitized animals and/or humans could be clinically applied for monitoring potential immune sensitization and associated drug allergy reactions. However, it should be pointed out that although the lymphocyte transformation test is used clinically and appears to be somewhat predictive, this has been primarily linked with drug-induced skin hypersensitivity reactions. There are only a few studies that have evaluated the lymphocyte transformation test for adaptive immune-mediated DILI.

5.3.6 Integrated risk assessment

Integrated risk assessment from Step I should be updated with the data obtained in Step II studies. Insight in to the mechanistic background of the hepatotoxic effect will guide the decision making process.

5.4 Step III – Annex 1

If product-induced hepatotoxicity is identified as a safety issue in steps I-II with an unfavourable benefit/risk assessment and unknown mechanism of action, further investigations might be necessary. In this context the experimental methods in Annex 1 (not a comprehensive list) could be considered.
However the selection of studies and endpoints should be dependant upon state-of-the-art scientific progress. Consultation with competent authorities on testing strategies is recommended.

6. TIMING OF TOXICOLOGICAL DATA PROCESSING IN RELATION TO CLINICAL DEVELOPMENT

Non-clinical hepatotoxicity data accumulates with the development programme. The final risk estimation will be done at the stage of compiling the marketing authorisation application. However, in order to evaluate the risk of drug-induced hepatotoxicity, all cumulative non-clinical and clinical data should be summarised especially before the first-in-human study, and prior to entering each phase of the clinical programme (I, II, or III).

In the case that liver toxicity emerges after marketing authorisation, the sponsor should immediately revisit their non-clinical documentation including post-marketing studies and start non-clinical mechanistic studies if indicated as outlined in this document.

7. PRESENTATION OF DATA

Relevant non-clinical hepatotoxic data should be presented in module 4 and should be discussed in the Non-clinical Summary and Overview in an integrated approach considering all available data.
ANNEX I: SUGGESTIONS FOR FURTHER INVESTIGATING THE MECHANISM(S) OF ACTION OF HEPATOTOXICITY

1. General considerations:

Additional studies may be necessary to clarify the mechanism(s) involved and to estimate the degree of risk. In this respect duration, reversibility and time to hepatotoxicity in relation to exposure is crucial.

It is also anticipated that there will be a considerable variation in the testing protocols depending on such factors as clinical and non-clinical findings, known class effects, proposed indication for the product, medical need etc. In some cases, new methods may have to be developed and validated. Consequently, no firm recommendations can be made for this step but some suggestions are given below. Development and investigation of new liver injury-detecting biomarkers is encouraged (e.g. alpha-GST, osteopontin, paraoxonase-1, malate dehydrogenase).

2. Specific animal models to assess Immuno-related hepatotoxicity:

The search for immunologically-linked hepatic biomarkers is encouraged

Several animal models are in use and under development for drug-induced immunologically linked hepatotoxicity. However the molecular mechanisms and predictivity are uncertain.

Evidence is accumulating that an (underlying) inflammatory reaction e.g. of viral/bacterial or host-dependent basis reduces the hepatotoxicity threshold. For example, if hepatotoxicity has been observed in Steps I and II and if the medicinal product is indicated in diseases such as rheumatoid arthritis, infectious diseases, diabetes etc, then hepatotoxic effects might be investigated in vivo under a modest lipopolysaccharide (LPS) induced inflammatory reaction. However, this model has not been validated.

Levels of inflammatory cytokines can be monitored as these have been reported to correspond with clinical features that are predictive of hepatocellular injury. Inflammation - or injury-linked immunomodulators include interferon gamma, and tumour necrosis factor. Protective factors include IL-10, IL-6, monocyte chemoattractant protein 1, macrophage inflammatory protein and IP-10.

The potential role of cytokines in the drug-induced hepatotoxicity could also be studied in knock-out mice. For example, IL-4 and IL-10 knock out mice and mice lacking inducible nitric oxide synthase appear to be more susceptible to acetaminophen induced liver toxicity than wild type mice. However, this model has not been validated.

3. Apoptosis/necrosis:

Disturbances in mitochondrial energy production and uncoupling potency and mitochondrial permeability leading to transient opening of the MPTP are signals of mitochondrial injury. Consequently, swelling and rupture of the outer mitochondrial membrane that leads to the release of cytochrome c, which then activates the caspase cascade and initiates apoptosis, could serve as a mechanistic marker for liver responses. The release of cytochrome c into the cytosol or of caspase-cleaved product of cytokeratin 18 (CK18) are considered to be potential biomarkers of mitochondrial perturbations.

Potential signals for the extrinsic pathway begins with the engagement of a death receptor at the cell surface. The principal hepatic death receptors are tumor necrosis factor receptor (TNF-R1) and Fas, which are activated when bound by tumor necrosis factor and Fas ligand. Therefore, Fas ligand should be evaluated.

4. New technologies

Improved detection of drug-induced hepatotoxicity will likely be facilitated by the future availability of new predictive biomarkers and in vitro and/or in vivo models of drug-induced hepatotoxicity. Omics technologies may be of use to study mechanism(s) of action(s) or interspecies differences in terms of sensitivity to hepatotoxicants and in the development of appropriate biomarkers for liver toxicity.
Currently these technologies are not yet suitable for understanding detailed mechanisms of hepatotoxicity.

Gene chip technology can be used to investigate patterns of gene expression that are associated with drug-induced hepatotoxic potential. This strategy has the advantage that it does not require prior knowledge of the mechanisms and can examine many different genes. In addition, transcriptional and proteomic profiling of hepatotoxic medicinal products and non-hepatotoxic counterparts might result in the discovery of new biomarkers for liver toxicity.
ABBREVIATIONS:

5-NT  5'-nucleotidase  
ALP  alkaline phosphatase  
ALT  alanine aminotransferase  
APTT  activated partial thromboplastin time  
AST  aspartate aminotransferase  
CK 18  cytokeratin  
Co-A  co-enzyme A  
CRP  C-reactive protein  
GGT  gamma glutamyltransferase  
GLDH  glutamate dehydrogenase  
GST  Glutathione S-Transferase  
IL-1  interleukin-1  
INFγ  interferon gamma  
LDH  lactate dehydrogenase  
LPS  lipopolysaccharide  
MPTP  mitochondrial permeability transition pore  
MRP  Multi-Drug Resistant Associated Proteins  
NASH  Non-Alcoholic Steato-Hepatitis  
NK  natural killer cells  
NKT  natural killer T cells  
NO  nitric oxide  
OCT  ornithine carbamyltransferase;  
PT  prothrombin time;  
SDH  sorbitol dehydrogenase  
TBA  total bile acids  
TBILI  total bilirubin  
TNFα  tumor necrosis factor alpha  
UBILI  unconjugated bilirubin  
DILI  Drug-Induced Liver Injury
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