**miR-122 and other microRNAs As Potential Circulating Biomarkers of Drug-Induced Liver Injury**

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**Abstract**

**Introduction:** Drug-induced liver injury (DILI) is a severe adverse drug reaction which is of major concern to patients, clinicians and the pharmaceutical industry. Accurate and rapid detection of DILI is important for patient stratification and treatment in the clinic and benefits pre-clinical drug design and risk assessment. microRNAs (miRNAs) offer a potential new and improved class of circulating biomarkers of DILI over the current gold standard biomarkers. **Areas covered:** This review highlights the shortcomings of the currently used panel of biomarkers, and how miRNAs, primarily miR-122, show an improved level of specificity and sensitivity in the prediction of DILI. Furthermore, the use of miRNAs as potential markers of progression of DILI and specific zonated damage within the liver is discussed. **Expert commentary:** miRNAs, especially miR-122, offer a more sensitive and specific marker over the current biomarkers for DILI. Combinations of different miRNAs may be able to relay the location of DILI and the progression of disease. More studies using different hepatotoxins apart from acetaminophen will ultimately strengthen the case for the clinical introduction of miRNAs as biomarkers of DILI.

**Keywords**

microRNA, biomarker, DILI, hepatotoxicity, zonation, specificity, sensitivity

1. **Introduction**
   1. **Drug Induced Liver Injury**

Adverse drug reactions (ADR) are a major concern for healthcare systems and the pharmaceutical industry with an estimated annual cost to the UK of £1 billion and $4 billion to the USA (1). Although drug-induced liver injury (DILI) is an infrequent disease within the general population, it is a common ADR. It is the leading cause of acute liver failure (ALF) in the western world, with relatively poor prognosis (2). Six months after initial DILI onset, 10% of patients have died or undergone liver transplantation. Of the remaining patients, 20% present with persistent hepatocellular injury requiring further medical attention (3). Furthermore, it is one of the major causes of drug attrition from throughout all stages the drug discovery process. In early development, 50% of all pre-clinical candidate drugs display effects upon the liver at supra-therapeutic doses (4) and over a 50 year period, DILI was responsible for 18% of all drugs retracted post-marketing which represents the largest single reason for withdrawal (5).

DILI is categorised as either intrinsic or idiosyncratic. Intrinsic reactions are those which are perceived as an extension of the pharmacology of the drug. These are predictable in nature and show a clear dose-response relationship, such as liver toxicity associated with supra-therapeutic doses of acetaminophen (APAP) (6). Idiosyncratic reactions are complex to model pre-clinically and are unpredictable. This is due to patient genetic variation, alongside complex pathophysiological mechanisms which includes both metabolic and immune-mediated responses. These reactions can be very severe in nature and are commonly undiscovered until post-marketing distribution, where the drug becomes widely available to susceptible individuals (4, 7).

To help identify and accurately diagnose the onset of DILI in both preclinical and clinical settings, there is a need for sensitive and specific biomarker(s) that could aid drug design and modification, as well as patient diagnosis, stratification and treatment. A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention” (8).

When evaluating candidate biomarkers for DILI, the ideal biomarker would show complete liver specificity as well as an explicit response to DILI and no other hepatic type injury. It should also be sensitive enough that it can be detected at the early stages of disease with the use of non-specialised equipment to aid with early diagnosis and prognosis within the clinic or point-of-care environment. Early diagnosis would also be greatly facilitated by biomarkers easily obtained from bio-fluids by a non-invasive procedure, such as those found in the circulation. They should be conserved and translational between humans and preclinical models, allowing the transition of candidate markers in preclinical studies to be clinically viable in humans.

* 1. **Current biomarkers of DILI**

Currently, there is no single biomarker that is suitable for the diagnosis of DILI, due to the multi-factorial character of its pathophysiology. Consequently, current diagnosis is one based upon exclusion of conditions that commonly mimic DILI symptomatically in order to create a causal link between a drug and any potential liver injury. These causality valuations into drug mediated hepatotoxicity are made using methods such as Roussel-Uclaf Causality Assessment Method (RUCAM) and combined expert opinion, among others (9).

A major component of diagnosis relies on a liver function test, which examines biochemical perturbation within patient with the use of a panel of serum biomarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (TBIL). Elevations of ALT/AST in the serum correlate with hepatocyte necrosis and subsequent release of these markers into the circulation, elevations in ALP are reflects damage to the biliary epithelial cells or canalicular membrane and TBIL is indicative of whole liver function (10, 11).

These panels have been clinically relevant for many years and remain the gold standard in DILI diagnosis. As an extension of these biomarker panels, the FDA endorses the use of Hy’s law, which is typically used pre-clinically to identify hepatotoxicity. Hy’s law is based on the observations of Dr Hyman Zimmerman and it states that “drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, leads to death or liver transplantation in >10% of cases”. This has been refined to mean that a drug which causes elevations of ALT/AST >x3 and TBIL >x2 upper limit of normal (ULN) in the absence of other cholestatic/hepatic co-morbidities is likely to cause hepatotoxicity. The use of Hy’s law has been validated in various DILI consorts (12-14). However, as the calculation only considers ALT/AST and TBIL, Hy’s law has focused on the more serious hepatocellular damage, with the understanding that cholestatic injury will resolve itself upon removal of the drug. Recently, modification of Hy’s law has shown that inclusion of patients with elevated ALP can detect DILI with both greater sensitivity and specificity than the standard criteria in patients who later developed ALF (15).

The current diagnostic biomarkers of DILI are not without their limitations. None of the aforementioned markers offer true mechanistic insight into the basis of DILI and are detectable in other forms of liver disease, such as viral hepatitis and ischaemic injury (10). Furthermore, they are not liver-specific and injuries in other tissue types cause elevations of these markers which may cause false-positive diagnosis in patients with multiple co-morbidities. Both ALT and AST are present in skeletal muscle and have shown to be elevated in the serum of patients of polymyositis and extreme exercise (16). ALP is also present in bone tissue and is increased in response to osteoblast activity. Elevated serum levels have been detected in metabolic bone disease, downstream effects of hyperthyroidism and in post-menopausal women, who incidentally represent a high-risk group for DILI (17, 18). Serum TBIL can also appear elevated by the processing of erythrocytes and subsequent degradation of haemoglobin or by alteration of bilirubin transporters (19). Furthermore, TBIL is detected later than the aminotransferases when liver injury is already advanced, limiting potential treatment options. As TBIL is used as a measurement of total liver function, the assumption is that after sufficient loss of hepatocytes detected by elevated ALT/AST, total liver function therefore deteriorates leading to elevations of circulating TBIL.

Although Hy’s law has relatively high sensitivity for detecting DILI, it lacks specificity as many incidences that match the criteria for hepatotoxicity will fully recover without progression to ALF (20). These shortcomings of Hy’s law are highlighted by the introduction of *in-silico* methods to assist in the determination of DILI, shown by a recent suspension of GGF2 from phase I clinical trials due to two patients meeting the criteria for Hy’s law. However, it was noted in these patients that TBIL was elevated co-currently with the aminotransferases. Following serum sampling and mathematical modelling from the phase I patients, it was predicted that there was <13% hepatocyte death, which would not be sufficient to raise circulating TBIL <2x ULN. This indicates a different mechanism of elevated TBIL than reduced liver function mediated via hepatotoxicity (21).

The detection of circulating ALT/AST is purely diagnostic as these markers are released into the serum after the initial injury, representing a defined period of latency in the recognition of DILI following exclusion of other diseases. Relatively large elevations in ALT/AST can also be observed in response to drug treatment that do not cause overt progressive DILI such as statins and heparins, which may only serve to remove safe candidate drugs during pre-clinical testing in the future (22, 23).

Despite these inadequacies, the combination of ALT/AST, ALP and TBIL remain the current gold standard in DILI detection in man. Any novel biomarkers must therefore provide added value to the pre-existing ones in order to be deemed clinically useful. Novel markers would therefore have to show increased specificity and sensitivity, while demonstrating prognostic and mechanistic attributes.

The evaluation of novel circulating biomarkers of DILI is dependent upon serum or plasma samples from pre-clinical and clinical studies. The majority of published data on DILI is from cohorts assessing APAP-induced DILI, which is reflected in the fact that APAP overdose is the most common cause of ALF in northern Europe and the United States (2). APAP is a commonly used analgesic and anti-pyretic drug with a safe therapeutic dose of <4g per day for an adult. Pre-clinically, it is the most common model hepatotoxin in the assessment of DILI, as it is readily available to the public, predictable in nature with respect to hepatotoxicity and is clinically relevant.

The mechanisms of APAP toxicity are well documented. In supra-therapeutic doses, the glucuronidation and sulphation pathways are overloaded and metabolism is prominently mediated by CYP2E1 into the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). NAPQI brings about a rapid depletion of glutathione and the formation of reactive oxygen species, protein adducts and release of inflammatory cytokines. Critically these adducts can also form within the mitochondria, disrupting the electron transport chain, which ultimately leads to necrotic cell death (24).

Consequently, there has been recent interest in the development of novel circulating biomarkers which are able to detect mitochondrial damage (GLDH, mitochondrial and nuclear DNA fragments), inflammation (acetylated HMGB1), cell death (total HMGB1, full and caspase cleaved keratin-18) and APAP-protein adducts in APAP-DILI patient cohorts. Many of these circulating biomarkers show improvement over the existing set in terms of specificity, prognostic and mechanism based utility and are reviewed elsewhere (19, 25). However, a major limitation of these biomarkers is that they are not liver specific.

* 1. **MicroRNAs as biomarkers**

MicroRNAs (miRNAs) have been shown to be a capable source of biomarkers for a wide range of pathological states across many organs. They are small (18-25 nucleotides long) non-coding RNA molecules that regulate post-transcriptional gene expression (26). The production and processing of miRNA is a tightly regulated process, whereby the hairpin pri-miRNA structure is initially transcribed from miRNA genes or introns. These pre-cursor structures are subsequently cleaved into pre-miRNA by the type 3 RNAse Drosha and translocated into the cytosol by an Exportin-5 complex (27). The hairpin structure is then processed into a double strand RNA molecule by another type 3 RNAse DICER, and mature single strand miRNA is produced by association with the RNA-inducing silencing complex (RISC). Within the RISC complex, mature miRNA can associate and bind to target mRNA, preventing its translation and ultimately repressing protein expression (28).

miRNAs make for an attractive non-invasive biomarker as following organ damage, they are released from the cell into easily obtained bio-fluids, such as whole blood and to a lesser extent, urine. Within the bloodstream, they are incorporated into extracellular vesicles (e.g. microparticles, exosomes or apoptotic bodies) or are protein-bound (e.g. lipoproteins or argonaute2) which protects them from endogenous RNase degradation, ensuring their stability (29). Furthermore, miRNAs are very highly conserved across mammalian genomes, which greatly aids a translational approach from pre-clinical studies to the clinic (30).

Many miRNAs have been shown to be highly enriched or tissue specific. Of those miRNAs that are liver enriched, the highlight candidate biomarker for DILI is miR-122. miR-122 is one of the most abundant adult hepatic miRNAs, accounting for approximately 70% of the total liver miRNAome (31). Due to its prevalence within the liver, it is of no surprise that it regulates a wide range of key gene networks such as hepatic circadian rhythm, lipid metabolism and cell differentiation (32-34). Figure 1 details the initial cellular processing and ultimate release of miRNAs and other biomarkers into the circulation following toxicity.

The role of circulating miRNAs in DILI was first demonstrated back in 2009, where elevated levels of liver-enriched miRNAs, including miR-122 and miR-192, were discovered in mouse plasma following toxic APAP exposure. It was further noted that also in this study that changes in the serum levels of these miRNAs could be detected significantly earlier than the amino transferases (35). The translational aspect of miRNAs as potential biomarkers of DILI was subsequently highlighted in a cohort of 53 APAP-overdose humans, where circulating levels of miR-122 were found to be around 100 times higher than control samples (36). Since then, there have been several studies showing the release of liver enriched miRNAs in response to toxic injury in both human and rodents, but also in cynomolgus monkeys (37) and zebrafish (38). The release of miRNAs into cell culture medium by *in vitro* modelling of drug induced non-alcoholic fatty liver disease has also been shown to correlate to circulating miRNAs in patient serum samples, highlighting the potential use of miRNAs at the multiple stages of pre-clinical testing (39).

One of the major limitations of the aminotransferases is the relatively low sensitivity and delayed release from the cell following DILI. Furthermore, they have an extended half-life in circulation; although biomarker stability is necessary, ALT has a half-life of approximately 47h and AST has a half-life of 17h (10). This may be undesirable as the DILI may have resolved and the liver be in repair, whereas ALT/AST measurements would not reflect this. Conversely, it has been shown in clinical case studies that miR-122 is detectable up to 8h before ALT and only 4h after initial toxic insult, thus proving a more rapid approach in diagnosing DILI (40). Furthermore, in serum samples of patients with APAP toxicity with early presentation to hospital, Receiver Operating Characteristic (ROC) analysis of miR-122 showed greater sensitivity in predicting APAP toxicity compared to aminotransferases (41).

This is important as it allows miR-122 to be used independently or alongside the aminotransferases in the diagnosis of DILI, especially at early hospital presentation where ALT and AST are not detectable. Ultimately, this reduces clinical burden by achieving an earlier diagnosis and appropriate treatment plans. This was highlighted in a case report where at first presentation a patient’s circulating ALT was not indicative of DILI after ingestion of 30g of APAP and was discharged without NAC treatment. The patient returned to hospital after deteriorating, when APAP poisoning was confirmed by a second ALT test. Subsequent analysis on the patients serum at first presentation revealed a 50-fold increase of miR-122 compared to ULN (40). The half-life of miR-122 is also shorter than either ALT or AST, returning to baseline after 3-7 days, which may be representative of the progression of liver injury (36).

miRNAs also offer some prognostic value in severe acute DILI. Serum samples extracted from 78 patients within 2 weeks of initial DILI showed that those with low levels of miR-122, 4463 and 4270 were associated with fatalities within a 6-month period. This was further shown to correlate with low levels of serum albumin, whereby no patient with miR-122 levels above the median value and serum albumin levels >2.8 g/dL died within 6 months of DILI injury. The combination of miRNA and albumin had sensitivity, specificity, positive and negative predictive values were 100%, 81%, 38%, and 100% with performed better at predicting terminal outcome than the MELD score (Model for end-stage liver disease) (42).

miRNAs are detectable in many bio-fluids such as blood, urine, faeces which are all easily obtainable, non-invasive and do not require any significant medical input. Much of the published work regarding circulating miRNAs is performed with serum or plasma, which required centrifugation of whole blood (43). Ideally, to hasten point of care and diagnosis, bio-fluids would not require any additional processing. However, it has recently been shown that levels of miR-122 was detectable in capillary blood obtained by finger venepuncture in both healthy and DILI patients. Basal levels of miR-122 in capillary blood correlated to levels in venous blood and plasma samples in the same patient and finger venepuncture of DILI patients showed a fold increase in miR-122 of 86 when compared to control patients (44). The use of whole blood is possible as miR-122 is highly liver specific and not expressed in cell types found in whole blood. Rapid collection methods, using miRNA containing matrixes, allows a rapid point of care and stratified treatment approach in suspected DILI cases. The main caveats of using miRNAs as biomarkers are that the detection and validation of results is often time consuming, expensive and requires specialist equipment, such as amplification for PCR. Consequently, there has been a drive to develop rapid, cheap and easy to use assays to determine miRNA release in potential DILI cases. Recent and promising examples include pH- responsive paper assay (45), portable PCR-microarray machines (46) biosensors (47, 48).

A lack of specificity in diagnostic biomarkers is problematic due to the occurrences of false-positive results. Current biomarkers suffer from a lack of liver specificity, whereas the use of liver specific miRNAs such as miR-122 would overcome this. This was shown by Thulin *et al*, who demonstrated that serum ALT/AST were significantly elevated in healthy volunteers after extreme exercise with fold changes of 2.5 and 5.5 respectively when compared to pre-exercise serum samples. On the other hand, levels of miR-122 were actually significantly reduced by 0.3 fold change before and after exercise (49).

Although liver specific, miR-122 is not unique to DILI profiles. It is differentially processed and elevated in serum in response to other hepatic injury types of differing aetiology. In hepatocellular carcinoma, levels of miR-122 are significantly reduced and are associated with poor prognosis and loss of hepatocyte phenotype (50). Whereas in hepatitis C infection, miR-122 is an essential host factor in binding to viral genomic RNA, facilitating viral replication (51). Circulating levels of miR-122 have been shown to increase in bile duct ligated mice (52), reperfusion injury and acute rejection of liver transplantation (53) and in non-alcoholic fatty liver disease (NAFLD) (54). Therefore, it is important to exclude these other hepatic pathological conditions before using individual miRNAs to diagnose DILI. This has led to the idea that a panel of serum miRNA profiles may be a more accurate method to diagnose liver injury. Ward et al showed that a panel of 11 miRNAs, including miR-122, could discriminate a diagnosis of APAP-induced DILI against patients with ischaemic hepatitis (55). This work has recently been advanced by examining serum samples from 72 patients from patients with APAP-DILI, hepatitis B infection, liver cirrhosis and type II diabetes with next generation sequencing. It was found that patients of each disease group presented with elevated ALT levels, indicative of liver damage. However, each disease state presented with a specific “signature” of circulating miRNA which could be used to identify the individual underlying cause of liver injury (56).

* 1. **Mechanistic miRNA**

As previously stated, APAP is the most widely used model hepatotoxin, because of its predictable dose dependent perivenous toxicity in animal models and its clinically relevant nature (24, 57). Consequently, the majority of research has been focused on miR-122, due to its abundance and release into the circulation as a response to APAP-induced hepatocellular toxicity. There has been emerging research into whether other microRNAs offer mechanistic insight into DILI with respect to progression of the disease

Such mechanistic markers include miR-155 and miR-21, which have been postulated to be markers of inflammation and hepatic regeneration respectively. As inflammation is a key adaptive response to acute DILI, any involvement of miRNAs in the recruitment of the immune system could potentially be indicative of disease progression. For example, exosome-bound miR-122 from alcohol-treated human hepatocytes has been shown to transfer into human monocytes, altering their immune phenotype. Such mechanisms may also be at play in DILI (58). Following APAP administration into a mouse model, it was noted there was a time-dependant elevation of the inflammatory markers miR-155, 146a and 125b parallel to elevations in serum miR-122 (59). Elevated levels of miR-155 were also detected in the perivenous area as well as the plasma of APAP treated rats, indicating an immune response (60).

Markers of regeneration would also be of clinical use, as patients presenting at the hospital who already express regenerative markers would show favourable outcome that could resolve without medical intervention, saving money and bed space. Colony-stimulating factor (CSF1) is a promising regenerative marker that has shown to be elevated following APAP administration and partial hepatectomy (61). For a miRNA equivalent, miR-21 was shown to be overexpressed 48 hours post-partial hepatectomy in rodent models, driving hepatocyte proliferation and liver regeneration (62, 63).

However, the literature is conflicted on the potential utility of these biomarkers. Park *et al* noted that following APAP treatment, levels of both miR-155 and miR-21 remained stable, even though hepatotoxicity was confirmed by elevated miR-122 and ALT (64). Furthermore, inhibition of miR-21 in rats that underwent partial hepatectomy did not affect cell cycle progression in liver regeneration, dampening enthusiasm for this miRNA as a standalone mechanistic marker (65). Further investigation into these miRNAs should be performed to understand differences in published work, alongside their utility as potential mechanistic markers in DILI

* 1. **Zonated miRNA**

Hepatocytes within the liver exist as a heterogeneous population between the periportal and perivenous areas due to environmental and genetic factors, including miRNA regulation (66). Though the majority of biomarker research focuses on APAP which exerts its toxicity in the perivenous area, there are many drug classes that produce periportal DILI (67).

Identification of DILI in either zone I or zone III of the liver by a circulating biomarker would be advantageous for a number or reasons. Cholangiocytes are found within the periportal region and play many important roles within the liver, such as regulating of bile composition. They are targets of toxicity for numerous commonly prescribed drugs, such as the penicillins (68). Serious damage of these cells may ultimately lead to vanish bile duct syndrome, which requires liver transplantation. Diagnosis is dependent upon liver biopsies, whereas a panel of miRNA biomarkers selective for cholangiocyte or generalised periportal injury would ensure a non-invasive, early diagnosis (69). Liver zonation causes a marked gradient of key physiological processes across the liver, such as the expression of key drug metabolising phase I and II enzymes. Detection of zone-specific miRNAs in pre-clinical testing may ultimately aid future drug design by linking the drugs chemical properties to the area of DILI.

Investigations into miRNA serum profiles of other zonal hepatotoxins are in their infancy, yet may prove to be very important in the future search for novel mechanistic biomarkers. Figure 2 demonstrates some of the key findings of recent papers, though more studies and a translational approach will be needed in the future to validate these findings.

Yamaura *et al* induced hepatocellular necrosis in the perivenous and periportal regions by dosing rats with APAP and methapyrilene respectively. They noted large upregulations of miRNA in the serum of rats between the two dosing groups, such as increases in miR-122, but also uniquely upregulated and downregulated miRNAs (APAP: 8 upregulated, 4 downregulated, methapyrilene: 6 upregulated, 5 down regulated), which may indicate a unique zonated biomarker (70). They noted in a follow-up paper that the miRNAs that are upregulated in serum do not necessarily correlate to miRNA that are downregulated in liver tissue following DILI, indicating a potentially more complex method of miRNA release than a simple deposition of highly abundant miRNA into biofluids. (60).

Church *et al* recently used the hepatobiliary toxins alpha-naphthylisothiocyanate (ANIT) and FP004BA to cause periportal injury in rats. Aside from detecting previously identified liver-enriched miRNAs following DILI, they also identified several miRNAs that were consistently elevated in blood samples from the two drugs, indicating possible novel and unique hepatobiliary injury markers. Furthermore, they noted miR-182-5p remained elevated 14 days after injury, whereas miR-122-5p had returned to baseline, potentially identifying a candidate for monitoring hepatobiliary injury (71).

1. **Expert commentary**

DILI is a major concern within the clinic and remains the focus on pre-clinical drug safety testing. The currently approved circulating biomarkers ALT/AST, ALP and TBIL offer a non-invasive diagnostic tool in the diagnosis of DILI and play an important role in disease diagnosis. However, their utility in predicting and diagnosing DILI is lacking. Elevations of these biomarkers are seen in diseases of other organs and general liver injury and in the case of DILI, are only elevated and detectable after the progression of liver damage. The development of novel, translational biomarkers that exhibit increased sensitivity and specificity for use in both pre-clinical and clinical settings are therefore desirable.

MiRNAs have recently emerged as a group of potential biomarkers for a variety of diseases. Of particular relevance to DILI, is miR-122, which has shown enhanced specificity and sensitivity over ALT/AST in a number of preclinical and patient cohort studies. The merging use of panels of biomarkers to more accurately diagnose is also particularly promising, as it compensates for the fact that miR-122, as an individual biomarker, is seen in a range of other hepatic conditions. The use of multiple biomarkers will also aid diagnosis of DILI as it may remove the need for excluding other hepatic injury as the cause of miRNA release.

While the use of APAP is attractive to researchers to the clinical prevalence and predictable and translational response in pre-clinical studies, it is imperative to remember that there are approximately 1,000 drugs that will cause liver injury. Currently in large patient cohorts of hepatotoxicity, it is not uncommon to see APAP vs non-APAP sub-populations, which represents a grouping of toxins with potentially very different mechanisms of action. However, there currently still remains the need to fully understand the kinetics and how miRNAs are released following toxic insult, whereby a model toxin such as APAP retains its utility. Future work dictating the expansion of miRNAs as circulating biomarkers should continue the testing of drugs with differing mechanisms of toxicity to evaluate specific miRNA release profiles, which will ultimately lead to improved drug design and risk management.

1. **5 Year review**

Due to the advances in the detection of miRNAs, in the coming years, we may see the addition of miRNAs to the existing clinical biomarkers, for example, miR-122 as an earlier predictor of hepatotoxicity compared to ALT/AST. Although beyond the scope of this review, other novel non-miRNA biomarkers of DILI, such as GLDH and HMGB1 also show promise for clinical implementation due their mechanistic basis. Further work will be required in the validation of these new candidate biomarkers before they can be applied to the clinical setting, alongside examining if mechanistic miRNA such as miR-21 provide additional benefit. The future of DILI assessment is very unlikely to be predicted from one biomarker and will likely be a combination of biomarkers old and new, assisted with *in silico* modelling.

1. **Key points**

* Drug induced liver injury (DILI) is a serious adverse drug reaction encountered in both pre-clinical and clinical settings.
* The current gold standard biomarkers for DILI detection have limitations. Additional novel biomarkers that improve on these biomarkers are needed to aid clinical diagnosis and pre-clinical testing.
* miRNAs offer a potential robust newer class of biomarkers, which show increased specificity, sensitivity and diagnostic potential.
* Individual or panels of miRNAs may be able to diagnose specific liver disease and defined areas of damage within the liver.

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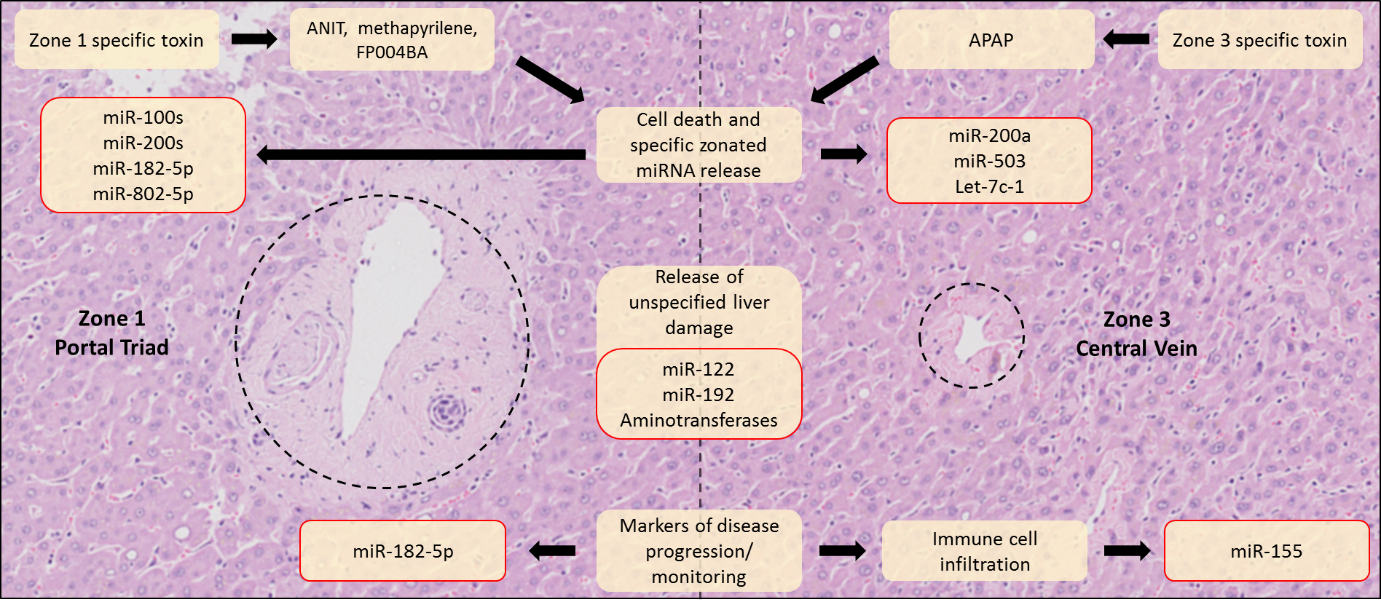
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**M:\PhD 2015-2018\miRNA as potential biomarkers review\Final Submission\Figure 1 FINAL.tifFigures-**

**Figure 1- Production and processing of miRNAs in response to drug-induced liver injury.**

The cell on the left shows how miRNAs are produced within the nucleus and translocated into the cytoplasm as immature pre-miRNA under basal conditions. These pre-miRNAs are then processed into mature miRNA whereby then can alter mRNA transcription through a series of ribonucleases and associations with protein complexes such as RISC. They then further associate with proteins (e.g. lipoproteins, argonaute2) or incorporate into extracellular vesicles (e.g. microparticles, exosomes).

Upon delivery to the liver, liver toxins are taken up by the cells and are processed into reactive metabolites. Both the parent compound and metabolites may cause acute toxicity to the cell, which causes the early (<4h) release of liver-enriched miRNAs into the blood stream as a detectable biomarker. As DILI progresses into later stages, miRNAs may also be detected in apoptotic bodies. Aminotransferases are the current gold standard biomarker for hepatocellular injury, but their use is limited as they are released at these later time points (8h) compared to miRNAs. They can also be detected once DILI has resolved and progressed into hepatic recovery.

**Figure 2– Potential novel biomarkers of zonated drug-induced liver injury.**

Hepatocytes within the liver are heterogeneous due to zonation and may express different miRNAs depending upon their cellular location. Recent interest in recognising potential miRNA biomarkers of liver zonation has the potential to aid clinical diagnosis and improve pre-clinical drug design.

In response to the use of specific periportal or perivenous toxins, there is a release of markers of generalised liver toxicity (miR-122 and ALT/AST). However, some potential zone-specific circulating miRNA biomarkers have been identified in response to initial injury and its progression, offering diagnostic and mechanistic insight to DILI (Church 2016, Yamaura 2012, 2014).