

Title: Circulating microRNAs as biomarkers of toxicity

Traditionally, biomarker studies have focused on the identification of a single factor that indicates the presence of a given disease. However, recent studies have shown that combined signatures of multiple biomarkers (multiplex biomarker profiling) can better account for patient heterogeneity, and provide a more accurate indication of patient health [1].

Organ toxicity is one of the leading causes of compound failure during pre-clinical and clinical trials. Therefore, it is essential to identify biomarkers that can be used in early stages of drug safety assessment. Moreover, early and reliable detection and monitoring of adverse events is essential to understand toxic mechanisms.

MicroRNAs with tissue specific expression patterns are a novel class of biomarkers of toxicity. Increasing levels of tissue-specific microRNAs can be detected in the circulation as a consequence of local cell damage or transformation due to exposure to toxicants. We have developed the toxomiR® panel which combines a panel of carefully selected microRNAs for early diagnosis of adverse effects on 8 target tissues as well as endocrine disruptive activity [2].

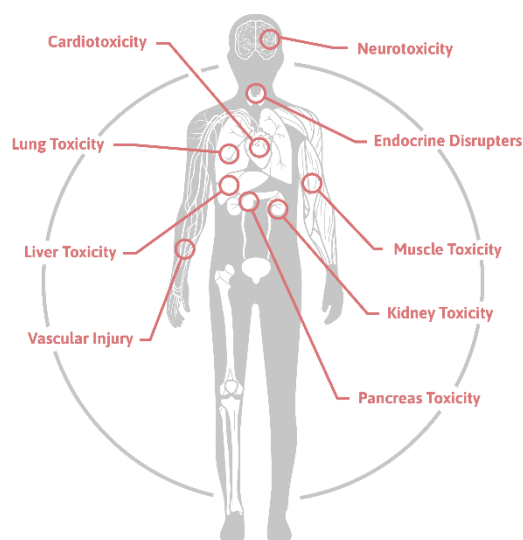
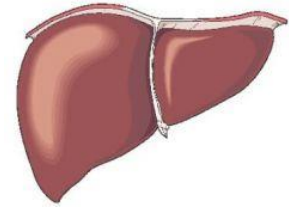


Figure 1: TAmiRNAs toxomiR® panel

This technical note gives an overview of the toxomiR® panel with brief description including the most important literature.

Liver toxicity:

Liver injury caused by drugs, alcohol or chemicals constitutes a significant threat to patient health and has an enormous impact on health care expenditures. Elevated serum levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutamate dehydrogenase (GDH) are often used to assess liver injury. However, these enzyme markers cannot differentiate between drug-induced and non-drug-induced liver injuries [3]. Like the ALT levels, miRNA species showed dose- and time dependent changes in plasma after acetaminophen exposure [4].



miR-122-5p is one of the most abundant miRNAs in the liver, accounting for 70% of all miRNAs and regulate several functions, including cell cycle, differentiation and apoptosis [5]. miR-122-5p exhibits dose- and exposure duration-dependent changes in the plasma that correlate with ALT serum levels and with histopathological changes during liver degeneration [6] and changes are noted before increased ALT activity. Circulating miR-122-5p represents a potential novel early, predictive and reliable blood marker for viral-, alcohol-, and chemical-induced liver injury [6,7].

miR-192-5p is enriched in the liver tissue and, similar to miR-122-5p, exhibits dose and exposure duration-dependent changes in the plasma that correlate with ALT serum levels and with histopathological changes during liver degeneration [4].

miR-29 is the most widely studied miRNA in liver fibrosis is miR-29 family that is known to have strong anti-fibrotic role in clinical specimen and animal models. The first instance showing the downregulation of miR-29 in cardiac fibrosis with the altered expression of collagens indicates a therapeutic target for tissue fibrosis [8].

Neurotoxicity:

The brain takes up and retains toxins very effectively. Toxins can build up and damage the brain, causing symptoms such as personality changes, memory loss, inability to focus, physical tics, fatigue, seizures and depression. NSE (Neuron specific enolase) and S100B belong among the so-called structural proteins of the central nervous system. These two proteins are considered as quite established and specific markers of the CNS tissue damage [9]. But there



seem to be limitations in marker sensitivity, depending on the age of the patients [10]. In the nervous system, miRNAs play important roles in growth, development, differentiation, function, and pathogenesis of neurodegenerative diseases [11].

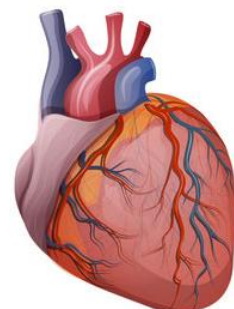
miR-150-5p is a putative novel biomarker of active inflammatory disease with the potential to be used for early diagnosis of multiple sclerosis (MS). Levels of miR-150-5p are elevated in patients with MS and patients with clinically isolated syndrome (CIS) who convert to MS. It can be a challenge for doctors to diagnose MS because so far no test enables definitive diagnosis, and many other conditions lead to similar symptoms [12]. miR-150-5p might be able to fill this gap.

miR-9-5p is highly enriched in the nervous system. Serum levels of miR-9-5p are elevated after a single administration of trimethyltin (TMT) chloride in rats. TMT is an organotin compound used in plastic stabilizers, chemostabilizers, and fungicides. It induces nervous symptoms (such as vocalization, seizures, hyperactivity, and tremor), learning and memory deficits, and hearing loss in humans and experimental animals [11].

miR-124-3p is expressed specifically in brain tissue and could be used to monitor ischemia-related brain injury. The observed increase in brain-specific miR-124-3p after ischemic occlusion demonstrates the utility of miRNAs as accessible biomarkers for monitoring tissue injury originating from a specific organ [13].

Cardiotoxicity:

Historically myoglobin and the creatine kinase (CK)-MB isoform served as myocardial markers despite limited cardiac specificity and relatively short serum half-life. Currently, cardiac troponins (cTn)-I and -T are becoming markers of choice for detection of cardiotoxicity and cardiac ischemic necrosis based on their cardiac specificity and their longer serum half-life [14,15]. Studies in human and rodent samples have identified miRNA signatures for monitoring cardiac injury after myocardial infarction (MI), as well as drug-induced cardiac toxicity a relatively frequent and potentially serious complication of anti-tumor treatment.



Circulating **miR-34a-5p** levels are enhanced in doxorubicin induced cardiotoxicity. Plasma and exosomes from rats affected by DOXO-induced cardiomyopathy were highly enriched in miR-34a-5p compared to control animals. MiR-34a-5p levels increased 4.7-fold in plasma and 3.5-

fold in the exosome fraction, indicating that it is mainly released into the blood within exosomes [16].

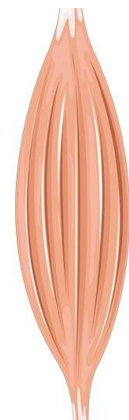
Plasma **miR-208a-3p** was robustly increased from the first sampling point through 24 h after dosing with isoproterenol treatment. In contrast, there was no significant change in the plasma miR-208a-3p level after dosing with doxorubicin [17].

miR-499a-5p is significantly elevated in patients diagnosed with AMI. Plasma levels are already detectable as early as 1 h after onset of chest pain and continue to increase gradually within 9 hours. miR-499a-5p is highly positively correlated with serum creatine kinase-MB and cTnl [18].

Muscle toxicity:

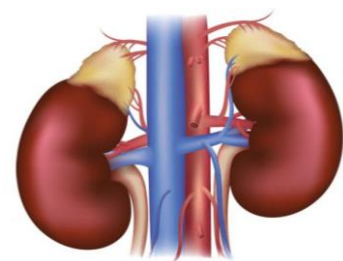
Aspartate aminotransferase activity (AST) and creatine kinase (CK; serum CK activity), the traditional biomarkers of skeletal muscle toxicity, lack both specificity and sensitivity [15].

miR-133a-3p is a specific markers of muscle toxicity. In combination with miR-208a-3p, miR-133a-3p can be used to differentiate cardiac from skeletal muscle toxicity. Increases in miR-133a-3p and miR-208a-3p were observed at 24 h following AAM administration, which is consistent with the delayed onset of heart and skeletal muscle toxicity noted by histopathology for this treatment group. Across all groups, increases in serum miR-208a-3p were only observed in rats with evidence of cardiac injury as assessed by histopathology. There were no significant increases in serum miR-208a-3p in the 2 rats from the AAM-treated group which had no cardiac lesions [19].



Nephrotoxicity:

Drug-induced kidney injury (DKI) is usually identified in a clinical setting through changes of the glomerular filtration rate (GFR). Yet GFR is only indirectly linked to kidney injury and changes in GFR are only seen as a late consequence of a primary nephrotoxic insult [20]. Clinicians have used serum creatinine in diagnostic testing for acute kidney injury for decades, despite its imperfect



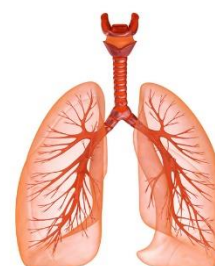
sensitivity and specificity [21]. Nephrotoxicity occurs when kidney-specific detoxification and excretion do not work properly due to the damage or destruction of kidney function by exogenous or endogenous toxicants. As the kidneys represent the major control system for homeostasis maintenance, they are especially susceptible to xenobiotics, and exposure to drugs often results in kidney toxicity. Approximately 20% of nephrotoxicity cases are induced by drugs.

miR-146b-5p is a promising biomarker for acute kidney injury and an indicator for its recovery after treatment with mesenchymal stem cells (MSCs). In acute kidney injury induced by cisplatin, miR-146b-5p serum levels respond more quickly to kidney injury than currently used biomarkers (serum creatinine level and blood urea nitrogen (BUN) levels), and decreased upon restoration of MSCs [22].

miR-30a-5p and miR-30a-5p expression levels can be used to distinguish diabetic nephropathy (DN) from other conditions [23].

Lung toxicity:

The protein Clara cell protein (CC16) is a homodimer of 15.8 kDA. Several lines of evidence indicate that CC16 is a natural immunoregulator protecting the respiratory tract from unwanted inflammatory reactions. CC16 secreted in the respiratory tract diffuses passively by transudation into plasma from where it is rapidly eliminated by glomerular filtration before being taken up and catabolized in proximal tubule cells. Studies suggest that CC16 in BAL fluid or serum is a sensitive indicator of acute or chronic bronchial epithelium injury [24,25]. Aldehydes are a group of volatile organic compounds (VOCs) that are associated with health risks. The major sources of aldehydes are building materials, furniture, paints, carpets, fabrics, detergents, cleaning products, cooking fumes, and fried foods. Aldehydes can cause lung- and respiratory diseases, exert mutagenic and carcinogenic effects, and play a critical role in airway inflammatory disorders such as asthma and COPD. Smoking is by far the leading risk factor for lung cancer. About 80% of lung cancer deaths are thought to result from smoking. The risk for lung cancer among smokers is many times higher than among non-smokers, and correlates with the duration and the extent of tobacco consumption. Secondhand smoke is thought to cause more than 7,000 deaths from lung cancer each year in the USA [26].



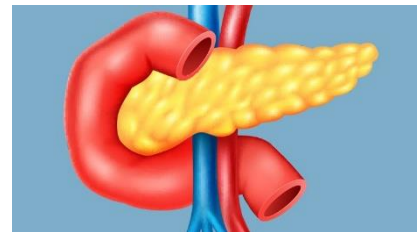
miR-210-3p is commonly upregulated by exposure to three aldehydes, propanal, butanal and pentanal [27].

Circulating **miR-422a** may provide a potential target for therapeutic approaches in the management of lung cancer and could be used to diagnose lymphatic metastasis [28].

miR-155-5p: The main risk factor for COPD is cigarette smoking. MiRNA-155-5p may be relevant in cigarette smoke induced inflammation and the pathogenesis of COPD [26].

Pancreas toxicity

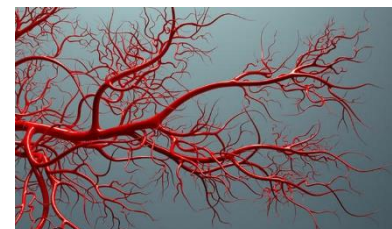
The diagnosis of drug-induced acute pancreatitis first requires a diagnosis of acute pancreatitis. In patients with pancreatitis, serum amylase levels vary depending on the severity of the disease. Serum lipase levels increase within hours of onset of clinical symptoms and peak at about 24 h. However, a variety of non-pancreatic conditions cause increased amylase or lipase levels. Based on sensitivity and specificity, an elevated trypsin level has a higher likelihood for detecting acute pancreatitis [29].



miR-216-5p is pancreas specific, leak into the circulation from the injured pancreatic cells and might serve as a good biomarker for pancreatic injury. MiR-216a-5p may be more specific than the currently used amylase d lipase as a biomarker for acute pancreatitis [30].

Vascular Injury

Drug-induced vascular injury (DIVI) is a common preclinical readout for toxicity and is usually characterized by hemorrhage, vascular endothelial and smooth muscle damage, and inflammation. DIVI due to change in vascular tone develops acutely (hours to days) in response to drug administration and often progresses to vascular inflammation. Most currently used biomarkers of vascular inflammation in the clinic are non-specific and include markers of endothelial and smooth muscle cell damage (IL-6, VCAM, VEGF, ...)



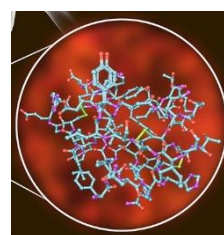
miR-126-3p is specifically expressed in the endothelium and is involved in vascular remodeling in response to laminar shear stress in HUVEC cells. MiR-126-3p levels are deregulated in murine and human serum in the course of experimental chronic kidney disease and in human diabetic patients [31].

miR-29b regulates the phenotypic switch of VSMC, which is mediated by PDGF-BB through SIRT1 and is a potential target in treatment of vascular diseases [32].

miR-145 is selectively expressed by pericytes and is a regulator of Fli1. Increased or decreased expression of miR-145 leads to reduced cell migration in response to growth factor gradients [33].

Endocrine-disrupting chemicals (EDCs):

EDCs are diverse natural and synthetic chemicals that may alter various mechanisms of the endocrine system and produce adverse developmental, reproductive, metabolic, and neurological effects in both humans and wildlife. To date, around 800 chemicals are known or suspected to interfere with hormone receptors and/or hormone synthesis and may play a larger role in the causation of many endocrine diseases and disorders.



In humans, it has been shown that several EDCs such as DDT or BPA decreased the expression of **miR-21-5p** which has a key role in cancer especially in breast cancer development. In addition, decreased expression of **let-7f-5p** is also associated with breast cancer [34].

miR-146a-5p is involved in Alzheimer's disease; miR-146a was strongly induced by BPA treatment; miR-146a could be used as biomarker for Alzheimer's disease after EDCs exposure.

Hepatic miRNAs (miR-22b, miR-140, miR-210a, miR-301, miR-457b, and let-7d) are increased in fluoxetine (the active ingredient in Prozac) exposed female zebrafish [35].

We at TAmiRNA are specialized in technologies for profiling levels of blood-circulating microRNAs and developing multi-parametric classification algorithms ("signatures"). TAmiRNA uses these technologies to develop minimal-invasive diagnostic tests for drug development, early diagnosis and prognosis of disease, and as companion diagnostic tests to support treatment decisions.

If you have further questions, do not hesitate to [contact](#) us. We are happy to help you successfully complete your microRNA project.

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