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Biomarkers & Applications

Editorial

Ahuja V. Biomark Applic: BMAP-121.
DOI: 10.29011/BMAP-121. 100021

Biomarkers in Pharmaceutical Preclinical Safety Testing: An Update

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Citation: Varun Ahuja (2018) Biomarkers in Pharmaceutical Preclinical Safety Testing: An Update. Biomark Applic: BMAP-121. DOI: 10.29011/BMAP-121. 100021

Received Date: 18 February, 2018; **Accepted Date:** 01 March, 2018; **Published Date:** 13 March, 2018

Abstract

Macacine herpesvirus-1 (B virus) belongs to the Herpes group of viruses and occurs naturally in Macaques. B virus infection is very mild in monkeys; however, it is fatal in 80% of untreated humans. Fatality is related to the upper spinal cord and brainstem. The Initial stage of infection is characterized by flu like symptoms and the final stage of infection is characterized by an ascending transverse myelitis. There are very few infected human cases reported so far and all of them were detected positive for B virus by serological testing and not by their symptoms. If the symptoms progress to late stage it results in fatality during acute infection, however, in latent infection timely treatment can prevent the progression of disease. As such, it is important to regularly monitor the positive patients and determine the levels of B virus specific IgG in their serum. Here, we demonstrate that B virus specific IgG3 subclass serves as a biomarker for reactivation in a patient with long-term B virus infection.

Keywords: Biomarker; Exploratory; Mechanistic; Safety; Translation

Introduction

In the pharmaceutical industry, safety biomarkers are applied pre-clinically, for early detection of toxicity, selection of the safest drug candidate, sensitive safety monitoring in regulatory toxicity studies, and selection of dosing regimens. Modern high-throughput technologies for transcripts, proteins, and endogenous metabolites offer a major opportunity to systematically identify sensitive and specific safety biomarkers which could serve as an index of damage specific to particular tissues and organs. Biomarkers can be critical to preclinical drug research and development phase, where a greater understanding of the molecular basis of toxicity and its influence on disease and disease progression can play a major role in drug development outcomes, including cost and overall success of new drugs. In this early phase of research, biomarkers support the mechanistic characterization of toxicity, show an early indication of toxicity, and help define the maximal tolerated dose. When relevant, safety biomarkers are studied in each preclinical species used in the development of a drug, allowing for refinement of drug dosing, administration, and formulation through the interspecies correlation of pharmacokinetic and Pharmacodynamics data. Safety biomarkers can also play an important role in deciding if candidate drugs are transferred from the preclinical to the clinical phase in the case where traditional clinical markers would not detect early-onset organ toxicity. If a pharmaceutical company can clearly show in preclinical studies that the novel biomarkers can be used to detect early toxicity, monitor onset and reversibility, and manage any potential adverse effect of a new drug with significant therapeutic potential, a clinical implementation strategy with these biomarkers can enable a clinical development program on a case-by-case basis. Safety biomarkers can play an important role in the progression of certain highly promising drugs from pre-clinical into human studies-drugs that, in the past, would otherwise have been abandoned because of the lack of performance of traditional markers in detecting early-onset organ injury.

Translational safety biomarkers that are minimally invasive and are specific and sensitive markers of early clinical injury are urgently needed to assess whether toxicities observed in preclinical toxicology studies are relevant to humans at therapeutic doses. Exploratory biomarkers are used with the goal of arriving at a suitable panel that can subsequently be tested and validated, for use as an endpoint in future clinical trials. Mechanistic biomarkers, a subtype of actionable biomarker, are embedded in disease pathogenesis and, therefore, represents a superior biomarker. In recognition of the importance of mechanistic biomarkers in drug development, increasing effort is put into integration of molecular diagnostics with therapeutics technologies. In this article, we discuss various types of these biomarkers.

Qualified safety biomarkers

An important safety biomarker success story is the recent recognition of kidney safety biomarkers for pre-clinical and limited translational contexts by FDA (Food and Drug Administration, USA) and EMA (European Medicines Agency). This knowledge acquired for kidney biomarkers is being transferred to other organ toxicities, namely liver, heart, and vascular system [1]. Some of the approved safety biomarkers are enlisted in (Table 1). Some biomarkers are under qualification process and are listed in (Table 2).

Organ	Biomarker	Pathology monitored	Qualification level	Reference
Heart	Troponins: cardiac troponin T (cTnT), and I (cTnI)	Necrosis of heart muscle	For safety assessment studies in rats and dogs for following context of use: • When there is previous indication of cardiac structural damage with a particular drug, cardiac troponin testing can help estimate a lowest toxic dose or a highest non-toxic dose to help choose doses for human testing • When there is known cardiac structural damage with a particular pharmacologic class of a drug and histopathologic analyses do not reveal structural damage, circulating cardiac troponins may be used to support or refute the inference of low cardio toxic potential • When unexpected cardiac structural toxicity is found in a nonclinical study, the retroactive examination of serum or plasma from that study for cardiac troponins can be used to help determine a No Observed Adverse Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL).	[2]
Kidney	Kidney injury molecule-1 (Kim-1)	Acute tubular alteration	• Can be included as biomarkers of drug induced acute kidney tubular alterations in GLP rat studies to support clinical trials	[2-5]
	β2-microglobulin (B2M)	Acute glomerular alteration	• Can be included as biomarkers of acute drug induced glomerular alteration/damage and/or impairment of kidney tubular reabsorption in GLP rat studies used to support clinical trials	[2-4]
	Cystatin-C (CysC)	Acute glomerular alteration	• Can be included as biomarkers of acute drug induced glomerular alteration/damage and/or impairment of kidney tubular reabsorption in GLP rat studies used to support clinical trials	[2-4]
	Clusterin (CLU)	Acute tubular alteration	• Can be included as biomarkers of drug induced acute kidney tubular alterations in GLP rat studies to support clinical trials	[2-4]
	Trefoil Factor-3 (TFF3)	Acute tubular alteration	• Can be included as biomarkers of drug induced acute kidney tubular alterations in GLP rat studies to support clinical trials	[2-4]
	Renal Papillary Antigen (RPA-1)	Acute tubular alteration	• Can be included as biomarkers of drug induced acute kidney tubular alterations, particularly in the collecting duct, in male rats	[2]

Table 1: Qualified Pre-clinical safety biomarkers.

Biomarker	Reference
Genomic Biomarker Approach for Positive Findings in the In vitro Chromosome Damage Assays in Mammalian Cells	[6,7]
Drug-Induced Non-Clinical Kidney Injury Biomarkers (NGAL, OPN)	[6,8]
Serum Glutamate Dehydrogenase (GLDH)	[6,9]
Drug-Induced Skeletal Muscle Injury Biomarkers (Myl3, sTnI, FABP3, CK-M)	[6,10]

Table 2: Biomarkers under qualification process.

Emerging Biomarkers: the microRNAs

In recent years, microRNAs (miRNAs) have been evaluated as potential candidate biomarkers of tissue injury. There are 788 known miRNAs in rats, 1899 in mice and 2585 in humans [11]. MiRNAs are endogenous, small (21-22 nucleotides), single-stranded, noncoding RNAs that regulate gene expression at the post-transcriptional level by binding to the 3′Untranslated Regions (UTRs) of their target mRNAs leading either to degradation or translational repression [12]. Studies have shown that miRNAs are involved in multiple biological processes such as proliferation, differentiation, development and cell death. The complementarity between miRNA and mRNA does not have to be perfect for translational inhibition, therefore one miRNA regulates several hundred mRNAs and likewise, one mRNA is regulated by several miRNAs [13]. In fact, it is estimated that over 50% of all protein-coding genes are regulated by miRNAs in mammals [14] revealing their overall involvement in diverse physiological as well as pathological processes [15]. Many miRNAs are found to be highly enriched in particular organs or at a particular stage of development or disease progression in human body [16,17] (Figure 1, Table 3)

Organ	Biomarker	Specific purpose	Reference
Kidney	miR-192	Kidney cortex	[18]
Liver	miR-122	Early liver injury	[19]
Heart	miR-21-5p	Cardiac inflammation	[20]
	miR-208a	Cardiac injury	[21]
Skeletal muscle	miR-133a/b	Skeletal muscle injury	[22]

Table 3: Micro RNAs.

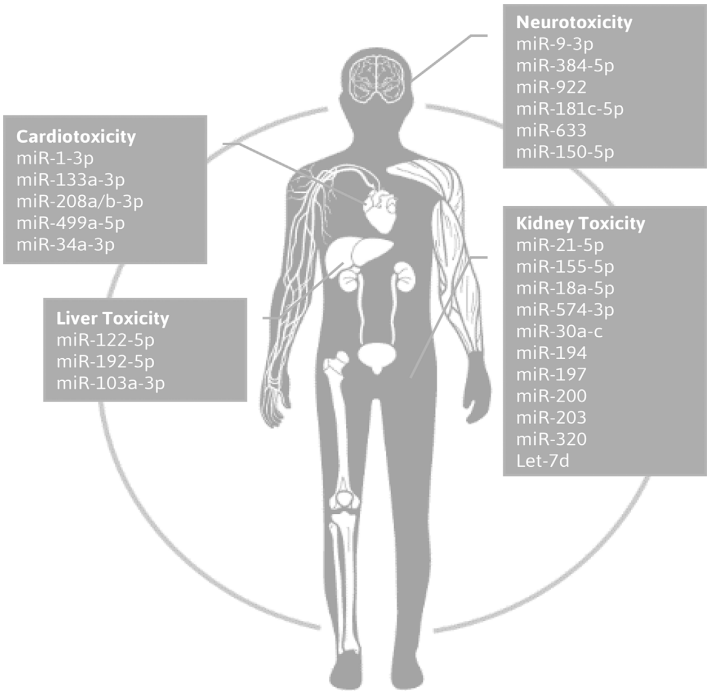

Figure 1: MicroRNAs altered by toxicants in target organs. (Reprinted from [23]: Schraml E. at al. 2017).

Outside the cell, miRNAs were discovered for the first time in serum/plasma from cancer patients [24] and afterward in other body fluids like urine, breast milk, saliva and cerebral fluid [25]. Extracellular miRNAs are very stable and resistant to degradation even with long-time storage at room temperature, exogenous RNAse treatment, pH variability and multiple freeze-thaw cycles [26,27]. Their stability is probably due to an association with RNA-binding

proteins or being packed into vesicles [28-30]. MicroRNAs show a highly evolutionary conservation; they are stable in various body fluids, and can therefore easily be measured in clinical samples [31]. MiRNAs can be readily detected in small sample volumes using Quantitative Real-Time PCR (qRT-PCR) techniques and are known to circulate in a stable, exosomal form [32]. Although the exact biological functions of many miRNAs are not fully understood, the tissue- or cell-specific distribution of certain miRNAs may make them promising candidates as biomarkers of target organ toxicity. Importantly, both the sequences and tissue expression patterns are highly conserved between species, suggesting they may be translational biomarkers that can be used in both experimental animals and humans. miRNAs are implicated in a range of diseases, including cancer, autoimmune diseases, neurobiological disease and cardiovascular pathologies [33].

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