THE SOONER THE BETTER

utilising biomarkers to eliminate drug candidates with cardiotoxicity in preclinical development

The rapidly escalating costs of drug development is causing the biopharmaceutical industry to focus its R&D efforts on identifying new technologies and methods that can predict the safety and efficacy of new compounds as early as possible in the drug development process. Today, only one in 11 compounds advance from first-in-man studies to regulatory approval and these late-stage failures – mainly caused by safety issues – exact a heavy toll on the biopharmaceutical industry as the cost of clinical trials is extremely expensive, legal liability is of concern and companies are under great scrutiny by investors

By Dr Federica Crivellente

Cardiotoxicity, or drug-induced cardiac injury, is one of the leading causes of drug withdrawals (eg, rofecoxib (Vioxx®), terfenadine), labelling changes restricting drug use ((rosiglitazone (Avandia®), delays in regulatory approval and late-stage compound failures. Drugs that cause heart muscle or valve damage or potentially fatal arrhythmias in patients have been implicated in 28% of drug withdrawals in the United States over the past 30 years. The discovery of drug-induced cardiotoxicity holds enormous consequences for pharmaceutical companies: if cardiotoxicity is discovered during drug development, then the programme will be terminated; if discovered after launch, then the drug may be withdrawn or restricted, and the companies can possibly be sued for negligence.

The growing concern surrounding cardiotoxicity is not restricted to a specific therapeutic area. Almost every therapeutic class of drugs has produced unanticipated cardiotoxicities (eg, torsade de pointe, progressive cardiomyopathy), including anthracyclines and other anticancer agents, antiretrovirals, antibacterials, antifungals, psychotropics and antihistamines. A recent high-profile case of drug-induced cardiotoxicity is trastuzumab (Herceptin®), which has been successfully used for the treatment of advanced breast carcinoma with overexpression of the HER2 protein. Trastuzumab did not reveal cardiotoxicity in preclinical animal studies; however, this antibody demonstrated an unexpectedly high incidence of left ventricular (LV) dysfunction in subsequent clinical trials. Although the cardiac damage is reversible, it frequently results in discontinuation of treatment.

Cardiotoxicity is becoming particularly concerning for chronically administered drugs such as neurologic/psychiatric agents and chemotherapeutic agents because toxicity may only become evident after long-term accumulation of the drug or its metabolites in the heart. Additionally, some cardiac events may be so rare that they can only be identified many years post-launch and only after many thousands or millions of patients have been

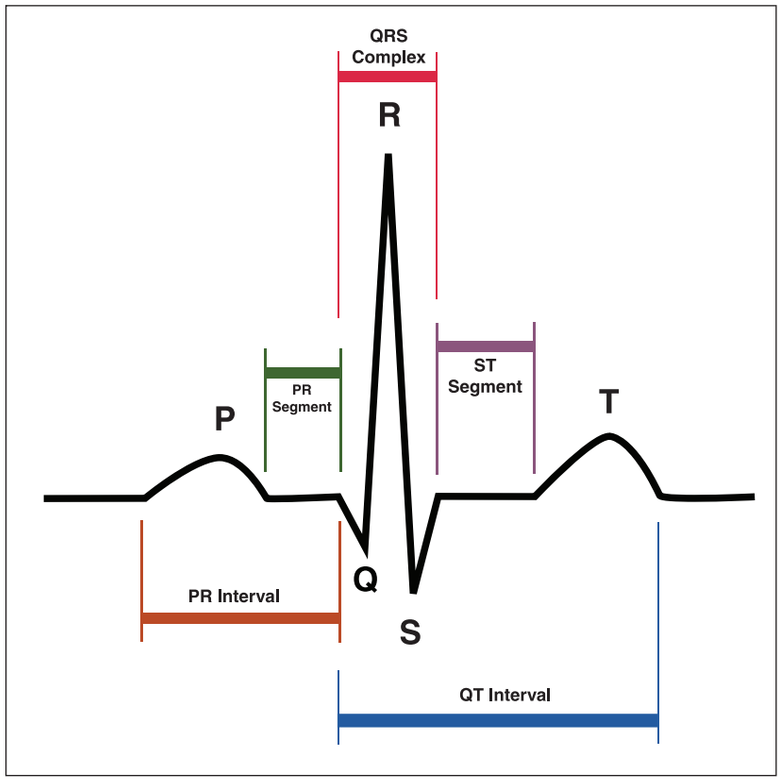

Figure 1
Schematic representation of normal ECG trace (sinus rhythm), with waves, segments, and intervals labelled

treated as was the case with the antihistamine, terfenadine. For these reasons, identifying a new drug's cardiac safety profile as early as possible in drug development remains one of the greatest challenges facing the industry.

Predicting success

Scientific innovation being made in the fields of genomics, proteomics, bioinformatics and imaging technologies is revealing the molecular basis of disease while rapidly transforming the drug development process – and even starting to change the way we diagnose and treat patients. For example, we now know that non-small cell lung cancer (NSCLC) can be further sub-classified into EGFR-positive, KRAS-positive, or ALK-positive NSCLC based on mutations within each of these genes, and each type of tumour is treated differently. Such advancements are the result of translational research, which involves expediting the translation of scientific discoveries made at the bench into clinical practice – and then bringing clinical findings back to the bench again. One important outcome of translational research is the identification of biomarkers that can be used to diagnose and measure the progress of disease and/or the effects of treatment.

A biomarker is any non-invasive, measurable factor that can predict or signal a disease state or a response to therapy. Historically, a biomarker referred to a physiological measurement, such as blood pressure, but today the term also refers to molecular or biological markers, including genes and genetic variants, RNA, proteins and metabolites. Genes and genetic aberrations can provide information on the likelihood of a patient developing a certain disease or how a patient may respond to treatment, while proteins and their metabolites can diagnose what is happening in a patient in real time (eg, is disease present?). Proteins and metabolites are more dynamic and reflective of physiology and they carry more diagnostic information compared to DNA and RNA. This is because each gene can give rise to many different proteins depending on splicing and post-translational modifications; proteins and metabolites, on the other hand, are the end-product.

An increased emphasis is being placed on the development of predictive biomarkers and developing evidentiary standards for their use. Predictive biomarkers can provide early evidence of safety and efficacy – while a molecule is still in preclinical development – and can provide assurance that lead candidates will have a high probability of success in subsequent milestones, potentially reducing the time and cost of drug development. The search for non-invasive biomarkers that can be objectively linked to adverse effects and cardiovascular toxicities, and can then be translated from preclinical to clinical development, is a high priority for federal agencies and biopharmaceutical companies. In fact, a 2010 survey conducted by the Tufts Center for the Study of Drug Development (Tufts CSDD) found that of the 25 pharmaceutical companies surveyed, 100% are using biomarkers to evaluate compounds in discovery and 58%, 50% and 34% are using biomarkers and/or targeted therapies in preclinical, early clinical (Phase I/IIa), and late clinical (Phase IIb/IV) development, respectively.

There are several biomarkers that may be used to mitigate the risk of potential cardiovascular toxicities at the early phases of preclinical drug development. These include, but are not limited to, heart rate, blood pressure, QT interval prolongation, lipids, troponins, natriuretic peptides (BNP and ANP), creatine kinase MB, C – reactive protein (CRP), lactate dehydrogenase isoenzymes, creatine kinase isoenzymes, aspartate aminotransferase, myoglobin, fatty acid binding protein, *ex-vivo* platelet aggregation and the use of imaging biomarkers.

Biomarkers of cardiotoxicity

Non-invasive or systemic biomarkers that can predict or track cardiotoxicity are greatly needed, both for clinical monitoring and as surrogate endpoints in drug development. Ideally, biomarkers of cardiotoxicity should be able to distinguish myocardial from skeletal muscle or other tissue damage, be present in serum or plasma, and be translatable from animal to human studies. It remains unlikely that there will ever be a single biomarker that can predict all drug-induced cardiotoxicities, but rather an optimal panel of biomarkers will be developed as a multiplex test for use in the laboratory and in the clinic. Selection of the appropriate biomarkers, time of sample collection and interpretation of test results will all be critical factors in go/no-go candidate decision making.

The QT interval is one of the oldest and most widely used safety biomarkers in drug development. There are also specific circulating molecular biomarkers that may be used to signal potential drug-induced cardiotoxicity. The cardiac troponins and natriuretic peptides may represent early and specific signals of cardiac damage or functional impairment – and could greatly complement QT interval in the cardiac screening of new compounds early in development.

QT interval

The QT interval remains one of the most widely used safety biomarkers in drug development. A great deal is known about the molecular mechanisms that link QT interval to clinical outcomes, namely cardiotoxicity. Ion channels present in cardiac cell membranes are responsible for generating the electrical currents that lead to cardiac muscle contraction, enabling the heart to pump blood. These currents have a distinct pattern of voltage change known as an action potential, which can be measured at the skin surface using an electrocardiogram (ECG). The length of the interval between two well-defined points on the ECG – the QRS complex and the T wave (the QT interval) – is commonly measured to identify the cardiotoxicity potential of a drug (**Figure 1**). Deviation from a predictable ECG pattern signals potential cardiac problems such as the potentially fatal cardiac arrhythmia torsades de pointes (TdP). Ion channels, such as the human Ether-à-go-go Related Gene (hERG), are the most likely molecular targets by which drugs prolong the QT interval.

In the early 1990s, there was a significant increase in the number of TdP adverse events being reported in post-marketing data, which led

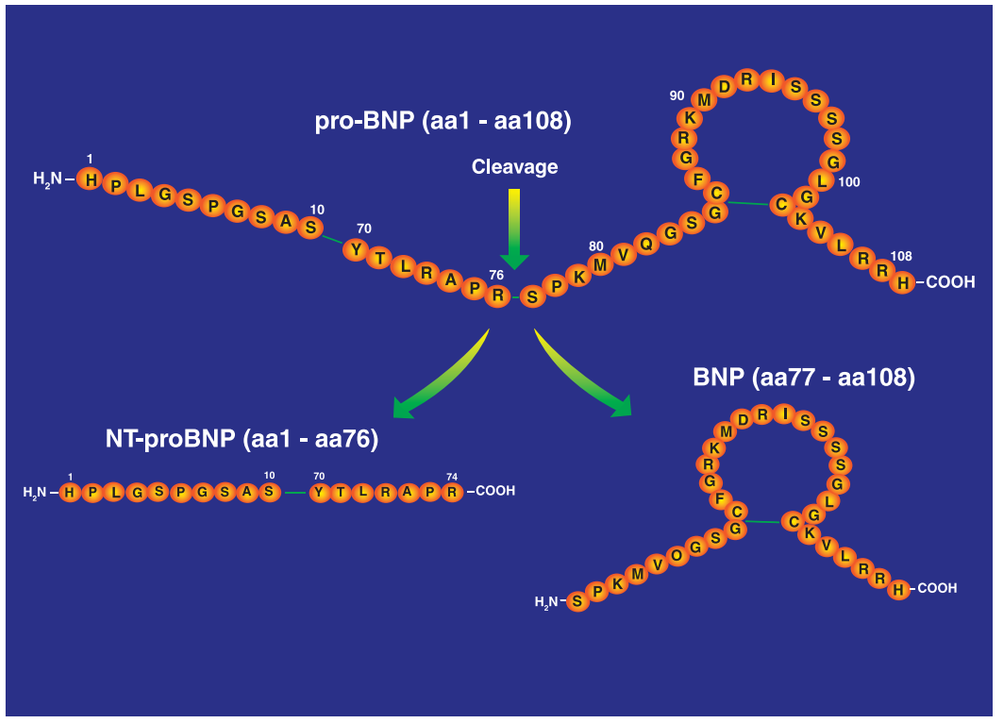

Figure 2
Cleavage of proBNP into N-terminal-proBNP (NT-proBNP), a biologically inert molecule and BNP, the biologically active counterpart

to the removal of a number of drugs from the market, including terfenadine, astemazole, grepafloxacin and cisapride. Terfenadine was withdrawn with an incidence of TdP of 1/28,500 prescriptions; grepafloxacin was withdrawn due to seven cardiac-related deaths and three cases of TdP out of 2.7 million prescriptions. Lidoflazine was rejected by regulatory authorities because of QT interval effects.

The effect of a drug on the QT interval – a surrogate marker for TdP – has become a critically important factor in regulatory decision making, is used clinically by physicians and impacts how pharmaceutical companies design and prioritise their drug development programmes. However, the use of the QT interval as a surrogate does have its limitations: drugs that cause TdP prolong the QT interval but not all drugs that prolong the QT interval cause TdP. Therefore, there remains the risk that drugs that are perfectly safe and effective in patients could be discontinued. To overcome this issue, a high-throughput screening assay is greatly needed that is capable of distinguishing between drugs that safely interact with hERG and those that are most likely to cause TdP.

Cardiac troponins

Cardiac troponins are well-established biomarkers of ischaemic heart disease and are preferred tests for suspected myocardial infarction. Their role as biomarkers of drug-induced cardiotoxicity, however, is still being explored. Cardiac troponins are part of a three-unit complex (troponin I, T and C) located on the actin filament that is integral to cardiac muscle contraction. Cardiac troponin I and T (cTnI, cTnT) are sensitive, specific biomarkers of myocardial damage; troponin C (cTnC) is not cardiac-specific—it is shared by slow-twitch skeletal muscles—and is therefore not used to diagnose cardiac injury.

Cardiac troponins are a marker of subclinical myocardial injury. cTnI and cTnT are released into the serum soon after cardiac damage and reflect the extent of irreversible drug-induced cardiac injury. It is important to note that while these proteins report active irreversible myocardial cell injury, they do not predict damage. Evidence suggests that the troponins are potentially useful translational biomarkers as they can effectively assess cardiac injury in both laboratory animals and humans.

Brain Natriuretic Peptide

Hypertrophy is an adaptive cardiac response to increased hemodynamic load, characterised by increased cardiomyocyte volume. Hypertrophy initially helps to maintain cardiac output in the presence of increased demand, but can quickly become

dysfunctional if left untreated. The natriuretic peptides are cardiac neurohormones that are released from the atrial and ventricular myocardium in response to increased wall stress: Atrial natriuretic peptide (ANP) and its amino-terminal fragments (NT-proANP) are released primarily from the atria; Brain natriuretic peptide (BNP) and its N-terminal fragments (NT-proBNP), are predominantly released from the ventricular cardiomyocytes (**Figure 2**). BNP is gaining acceptance within the industry as a biomarker for diagnosing congestive heart failure.

BNP is involved in many physiologic functions including natriuresis, diuresis, vasodilation, inhibition of sympathetic tone, inhibition of the renin-angiotensin-aldosterone system (RAAS), and inhibition of endothelin-1. The release of BNP directly correlates with the degree of ventricular wall tension, reflecting disease severity and prognosis. It has been suggested, however, that measuring NT-proBNP may be of greater clinical utility because its half-life is much longer than that of BNP (120 minutes versus 22 minutes) in humans. This also holds true in veterinary medicine, and is supported by recent studies showing that the assessment of NT-proBNP may be useful in identifying cardiac disease in dogs and grading disease severity.

An integrative approach

The ability to determine the serum levels of troponins and natriuretic peptides (NT-proBNP and NT-proANP) in a preclinical assessment would allow for the early identification of cardiovascular liability and would prevent unexpected drug-induced adverse effects in clinical trials during the late stages of drug development. The use of these biomarkers, which have been widely accepted in the clinical setting, could reduce the health risks for patients and healthy volunteers.

One approach to the issue of cardiotoxicity early in preclinical development is through the integration of several investigational tools, including the measurement of serum biomarkers (troponins and natriuretic peptides) in conjunction with other heart parameters such as heart weight relative to body weight (standard evaluation of cardiac hypertrophy), standard examinations (ie, histopathology) and non-standard endpoints (ie, echocardiography and macroscopic morphometry).

Casopitant is a potent and selective antagonist of the human neurokinin 1 (NK1) receptor which was developed for the chronic treatment of depression and anxiety, and was under investigation (and later withdrawn) for the prevention of chemotherapy-induced and post-operative nausea and vomiting. Casopitant was found to cause cardiotoxicity after long-term toxicity studies in late-stage drug development. In a 39-week dog study, oral administration of casopitant caused increased heart weight and myocardial necrosis, degeneration, inflammation and phospholipidosis. Previous short-term studies up to 13 weeks in duration suggested that casopitant was extensively metabolised and retained for longer periods in tissues, but cardiac changes were limited to a minimal increase of heart weight. Thus, drug tissue accumulation with chronic administration was responsible for late-onset toxicity, and the cardiac changes were only evident following long-term observation in dogs.

An integrated assessment of the onset and progression of the cardiotoxicity was conducted to provide insights for risk assessment, to aid in the identification of early translational biomarkers of cardiotoxicity, and to support decision making. The integration of different disciplines and multiple tools across various early time points constituted a powerful approach for the characterisation of casopitant-induced cardiotoxicity in preclinical development.

Together with transmission electron microscopy (TEM) and metabolite tissue accumulation, serum cTnI provided early evidence of cardiac cell necrosis starting from six weeks of treatment and these lesions increased in severity over time, suggesting a progressive impairment of the cardiomyocytes. An increase in heart weight, left ventricular (LV) mass, wall thickening, heart rate and prolongation of the QT interval were also observed after six weeks of treatment. Serum levels of NT-proBNP, on the other hand, were increased after only two weeks of casopitant treatment (**Figure 3**), preceding all of the anatomical and functional changes observed. In addition, LV mass, QTc interval and heart rate all recovered following treatment withdrawal; NT-proBNP did not return to normal values even after 22 weeks of withdrawal. This finding supports the sensitivity of NT-proBNP as an early prognostic biomarker of cardiotoxicity, indicating drug-induced cardiac mass changes and hemodynamic stress, and represents a potentially useful tool for clinical risk mitigation.

This case highlights the limitations in terms of sensitivity of the standard regulatory toxicity studies, and suggests that the increased use of non-conventional tools could better characterise new drug candidates. NT-proBNP changes preceded all anatomical and functional changes and served as an early biomarker of cardiac hypertrophy even in the absence of evidence of functional impairment. The results support the use of

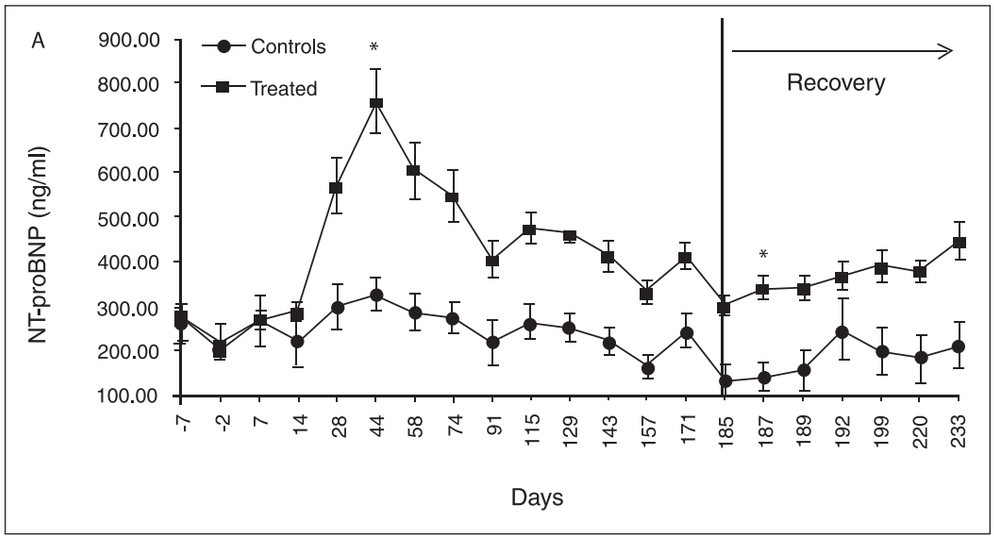

Figure 3
NT-proBNP levels (expressed as ng/ml) measured in control (●) and treated dogs (■) at all time-points of the study and during recovery. Results are expressed as mean±SEM (n=4). *p<0.05 in Casopitant-treated animals compared to controls

NT-proBNP in preclinical toxicological studies as a reliable, sensitive, non-invasive marker for the early detection of drug-induced hypertrophy.

Conclusion

Pharmaceutical companies are beginning to regularly incorporate biomarker platforms into the drug discovery and development process as far upstream as possible. The earlier candidate efficacy and safety profiles can be established, the earlier go/no-go decisions can be made, leading to a more efficient process. The integration of predictive biomarkers in preclinical studies with other data may reduce the high level of late attrition so common in drug development and optimise selection of the best agents for further clinical evaluation. DDW

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