Biomarkers for safety and toxicology: Drug induced cardiac injury and dysfunction

Paul A. Duffy, Graham Betton, Steve Horner, Judith Horner, Peter Cotton, Nick McMahon, Chris Lawrence, Helen Prior, Duncan Armstrong, Karen Philp, Ruth A. Roberts*

Safety Assessment, AstraZeneca R & D, Macclesfield, UK

Abstract

Cancer chemotherapeutic drugs, by their very nature of targeting robust and often rapidly dividing cells, inherently carry more toxicological burden and risk than drugs aimed at treating more benign disease. However, clinical oncologists have a continual need to utilise every therapeutic tool in their armoury in their attempts to treat this debilitating and often fatal disease. It is important that the physician has suitable biomarkers not only for efficacy, but also for drug related toxicity, in order to exploit maximally what is often a narrow therapeutic margin between benefit and unacceptable toxicity. Here we present data on a novel anticancer agent intended for combination therapy. The drug caused impaired cardiac functionality in pre-clinical studies at high doses, potentially leading to restrictive exclusion criteria in early clinical trials. To assist in clinical development, we generated biomarker data to be used in a weight of evidence approach to increase confidence in safe clinical progression.

Introduction

Biomarkers have been used for many years as indicators of biological change. In the course of drug development, biomarkers enjoy utility both as predictors of possible efficacy and also as warning signs of potential toxicity. Although these two types of biomarker serve diverse purposes, the parameters dictating their identification, development and utility are very similar. However, there is one key aspect in which safety biomarkers differ; safety biomarkers are most useful when they predict rather than report damage. For example, an efficacy biomarker can provide rapid confirmation that the drug has hit its target perhaps by confirmation of growth factor receptor inhibition in a skin biopsy. However, a safety biomarker of liver damage would ideally precede the damage (either in dose or time) to be of maximum use to the clinician. Despite this, biomarkers that report rather than predict can still be useful if they prevent further damage or permit cessation of treatment and subsequent recovery. In this paper, we discuss potential safety issues encountered in developing a new anti-cancer drug and the biomarker-based strategies under development to overcome these issues, facilitating clinical progression.

Biomarkers are used in pre-clinical and clinical drug development as indicators of likely response to drug. The conceptual framework for their use and some harmonising definitions were captured in the output of the Biomarkers Definitions Working Group [1] where biomarkers are defined as ‘a characteristic that is objectively measured and evaluated as in an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention’. The driving force for better clinical biomarkers of potential adverse events is to monitor patient safety and many such biomarkers will be discovered and developed within the context of human medicine. However, from a non-clinical perspective the discovery and development of toxicological biomarkers facilitates the translation to the clinic of knowledge that will avoid or minimise the risk of occurrence of a potential unwanted outcome identified in animal studies. Thus, it is key to identify pre-clinical biomarkers of the adverse response of animals to potential new drugs and to evaluate fully their relevance and applicability to humans. One argument against this approach is that findings in animals may not be relevant for human safety; however the job of the clinician is to manage the best possible therapy whilst minimising the risk of adverse reactions. Thus, such data would form part of an integrated assessment of safety and efficacy (Fig. 1) that can inform pre-clinical decisions.

There are several characteristics required of a good biomarker; it should be measurable in a way that

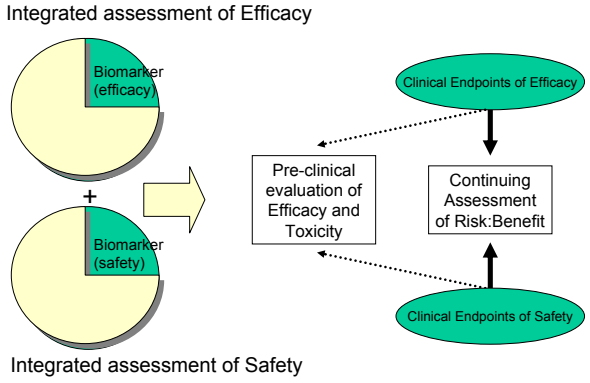

Fig. 1. The place of biomarkers of safety and efficacy in an integrated pre-clinical and clinical risk-benefit analysis. Adapted from the 'Biomarkers Definitions Working Group' [1].

is reliable, accurate and reproducible. In addition, it should exhibit sufficient sensitivity (not too many false negatives) and specificity (not too many false positives) to underpin clinical decisions. However, there are additional practical considerations to enhance appropriateness and usability. First, a biomarker needs to be accessible and ideally could be non-invasive. If this is not possible, a biomarker could be measurable in blood and other body fluids obtained with minimal additional impact on the subject. If this cannot be achieved, biomarkers may be detectable with increasing impact on the patient via a peripheral or central biopsy. Finally, biomarker research for the advancement of medical knowledge can be conducted after autopsy. Examples of non-invasive methods would include standard physiological measures of cardiovascular parameters like blood pressure, heart rate and electrocardiogram or could be ophthalmological. Fluids could include faeces, saliva and urine in addition to standard blood sampling. Skin and hair follicle would be examples of peripheral biopsy and muscle and liver needle samples provide the most common route of more invasive biopsy.

An important consideration in the use of biomarkers is the practicalities of the subsequent analysis which is likely to be far more successful and robust if based on an uncomplicated physiological or biochemical assay that can be carried out with minimal training and pre-existing equipment. Nonetheless, the need for specialist techniques or expertise should not exclude a potential biomarker especially where it contributes to advancing therapies for serious or unmet medical need. Other practical considerations include the need for assays to be transferable across international boundaries, supported by low background and variability of the measured parameter coupled with a robust increase/decrease signifying the biological endpoint of interest. Indeed lower and upper limits for normal values would be better if minimally influenced by age, sex or race within the human population. Similarly, a biomarker of drug induced toxicity would be better if it was largely unaffected by disease states expected in the patient population of interest or indeed any co-medication that might be present or recently administered. However, this is often not the case and the best that can be expected is some understanding of these relationships to mitigate against difficult or erroneous interpretation.

Biomarkers of safety

One key aspect where biomarkers of safety differ from those of efficacy is that they preferably *predict* rather than *report* the biological effect they are flagging. Thus the biomarker should ideally precede that biological effect either in dose (that is, be detectable at doses below where the damage occurs) or in time (be detectable perhaps after one dose when the damage requires repeated dosing). These biomarker characteristics would provide support and confidence to the clinician during dose escalation in early clinical trials or during the transition from single to multiple doses. During later clinical development, a predictive biomarker could monitor for onset in a patient who is beginning to develop toxicity perhaps after several months of drug use. In the absence of evidence that a biomarker can predict onset, an ideal biomarker of drug-induced toxicity would characteristically show detectable changes prior to any serious or irreversible tissue injury and would also show a reversibility of the biomarker signal upon removal of the toxicological insult.

The use of safety biomarkers in cancer drug development

Traditionally, anti-cancer therapies aim to kill dividing cells either by direct DNA damage or by impairing the cell replication machinery. Although this approach has a firm mechanistic basis, many cancers are resistant; several mechanisms have been implicated in this resistance [2]. It has become apparent that one key parameter in dictating success is a dependence upon cell cycle parameters, with some stages of the cell cycle being more susceptible than others [3]. In addition, cancer cells can escape death by cell cycle arrest, pending repair. In order to exploit this increasing knowledge of the response of cells to DNA damage, much has been invested in developing drugs for combination

Table 1
CV/Haemodynamic assessment in the anaesthetised dog

Study	Dose			Mean max decrease (%)			Mean CO (μM)	Margin based on mg/m^2
	μmol/kg	mg/kg	mg/m^2	SBP	DBP	contractility		
15-min inf	0.75	0.3	5	NAD	NAD	9	0.8	0.49
	1.50	0.5	11	4	NAD	21	1.6	0.98
	5.00	1.8	36	20	9	42	5.0	3.26
	25.00	9.1	181	55	46	76	28.0	16.31
	50.00	18.1	362	>50	>50	>50	59.0	32.62
	100.00	36.2	724	>50	>50	>50	126.0	65.24

SBP: systolic blood pressure; DBP: diastolic blood pressure; CO: plasma concentrations at end of infusion.

therapy that can sensitise cancer cells to DNA damaging agents, overcoming these resistance mechanisms. AstraZeneca has developed an agent (AZDX) that sensitises tumour cells to the anticancer effects of conventional DNA damaging agents. As described below, biomarker-based strategies were employed to assist with the potential safety issues encountered in developing the drug, facilitating clinical progression.

Defining the pre-clinical toxicological profile

During the earlier phases of pre-clinical development, AZDX was evaluated for potential toxicity first in standard *in vitro* genetic toxicology studies, then in a series of single and repeat dose studies by intravenous infusion, designed to match the intended clinical plan. Both rats and dogs were evaluated in order to provide the standard two-species package required to support drug registration. In addition, a package of safety pharmacology studies was conducted to evaluate any potential cardiovascular, respiratory and central nervous system (CNS) effects.

The data generated in this standard test cascade indicated that AZDX caused adverse toxicological effects in the cardiovascular system of both rats and dogs. Specifically, in the dog there was impairment of normal physiological function expressed as reduced cardiac contractility and output coupled with significant hypotension. In the rat, the hypotension was associated with lethality at high doses and high exposures and was often accompanied by drug related histopathological change in the ventricular myocardium.

Follow-up analyses of the adverse cardiac effects: further definition of the physiology

In order to understand further the observed cardiac effects, further studies on AZDX were carried out in both rats and dogs. In a telemetry study in conscious rats, the clinical signs indicative of a hypotensive effect noted previously was confirmed as hypotension and was found to be associated with a bradycardia, decreased core temperature and a respiratory depressant effect. The response of the dog to AZDX was studied first in an anaesthetised haemodynamic model where the drug produced a clear dose related decrease in cardiac ventricular contractility and blood pressure which was associated with increased heart rate and decreased femoral blood flow (see Table 1). The suppression of these parameters was very marked and was non-reversible, resulting in termination of the animals. Understandably, this represented a significant clinical concern, as the margins to the anticipated therapeutic dose of AZDX were not large. In order to confirm this effect in a more physiologically relevant model, these studies were repeated in conscious, telemetered dogs. Fortunately, in conscious animals the effects, although still present, were less marked and were reversible.

Follow-up analyses of the adverse cardiac effects: mechanistic analyses

As a result of the decrease in cardiac contractility and arterial blood pressure seen at low doses in the anaesthetised dog cardiovascular study, several additional investigations were undertaken. First, in a study in the isolated rat heart, a significant reduction was seen in ventricular contractility. Next, we investigated the potential for adenosine receptor and calcium channel effects since these can be associated with the observed physiology. These follow-up studies did not conclusively demonstrate that AZDX is an agonist/antagonist of adenosine A1 receptor nor did they demonstrate consistent effects on the L-type calcium channel. Therefore the putative cellular, receptor or ion

Table 2
Cardiac function and monitoring

Cardiac function exclusion criteria	Cardiac monitoring
Stage II, III, or IV cardiac status, according to NYHA classification; recent history (i.e. within 6 months) of coronary artery disease or arteriosclerotic cardiovascular disease (angina, myocardial infarction [MI]). May widen inclusion to allow Stage II in the expansion phase if contractility issues are not seen during dose-escalation phase.	Blood pressure and heart rate will be closely monitored for 4 h after the first AZDX infusion alone and in combination with the chemotherapy.
Any prior anthracycline treatment.	MUGA/Echo cardiography for 2 to 6 h after AZDX.
Resting LVEF <55% measured by MUGA/ECHO. If no contractility issues are seen during the dose-escalation phase, this exclusion criteria may be changed for patients participating in the expansion phase to: resting LVEF $\leqslant$40.	If decrease in ejection fraction to <50%, repeat MUGA/Echo cardiography at 24 h.
Second degree heart block. This requirement may be eliminated pending review of dose escalation safety data.	Repeat cardiac troponin (cTnT) and MUGA after three treatment doses, and every other month thereafter.
PR interval greater than 217 ms.	PR interval measurements derived from ECG.
Use of any known potent negative inotropic drug – calcium channel blockers: verapamil, diltiazem; beta-blockers: metoprolol, propranolol, atenolol, bisoprolol, carvedilol, timolol, sotalol, esmolol; anti-arrythmics (Class I): disopyramide, procainamide, mexiletine; (Class III): amiodarone; (Class IV): adenosine; miscellaneous: acetylcholine, propofol, nitric oxide, TNF, interleukin-1, interleukin-2.	Blood pressure and heart rate will be closely monitored for 4 h after the first AZDX infusion alone and in combination with the chemotherapy.

channel mediated mechanism for these changes was not conclusively identified.

Clinical progression: managing the potential risk

The cardiovascular and haemodynamic changes seen in the pre-clinical safety pharmacology and toxicology studies posed a real concern for the safety of patients. However, since the effects observed in pre-clinical studies manifest themselves as a measurable physiological change, standard clinical approaches were thought more than adequate to monitor the potential for onset of a comparable toxicological event in patients. Consequently, it was considered safe to progress with the early clinical trial programme but with restrictive exclusion criteria relating to cardiac function coupled with extensive monitoring (see Table 2).

Myocarditis

Although it was considered safe to progress with the early clinical programme, it was clear that the pre-clinical observations of impaired cardiac function potentially associated with cardiac myocarditis could be restrictive to clinical development. To address this, we initiated a plan of work to identify potential biomarkers that could predict the onset of the pathological lesion and therefore protect patients during dose escalation or during repeated dose cycles. As mentioned earlier, ventricular myocarditis was observed in the pre-clinical rat studies of AZDX. This myocardial lesion is an inflammatory mediated response (Fig. 2) to what is considered to be degenerative and cumulative cellular injury, which with time progresses to a fibrosis.

Studies of drug-induced ventricular myocarditis are confounded by a relatively high background incidence in the rat [4]. This is exacerbated when the putative drug-induced lesion occurs at a low level and with a histological appearance comparable to the background lesion. Furthermore, standard practice in the histological assessment of rodent regulatory toxicity studies typically uses only one vertical longitudinal section. Such an evaluation in the pivotal rat study suggested a lesion that was predominately expressed in males and with an equivocal dose response relationship, although there was a slight increase in the severity of the lesion with increasing dose (Table 3). Although this study concluded that the myocarditis was compound-related, the evidence for this was weak and required clarification.

Before embarking on further studies, we conducted a retrospective evaluation of all rat studies con-

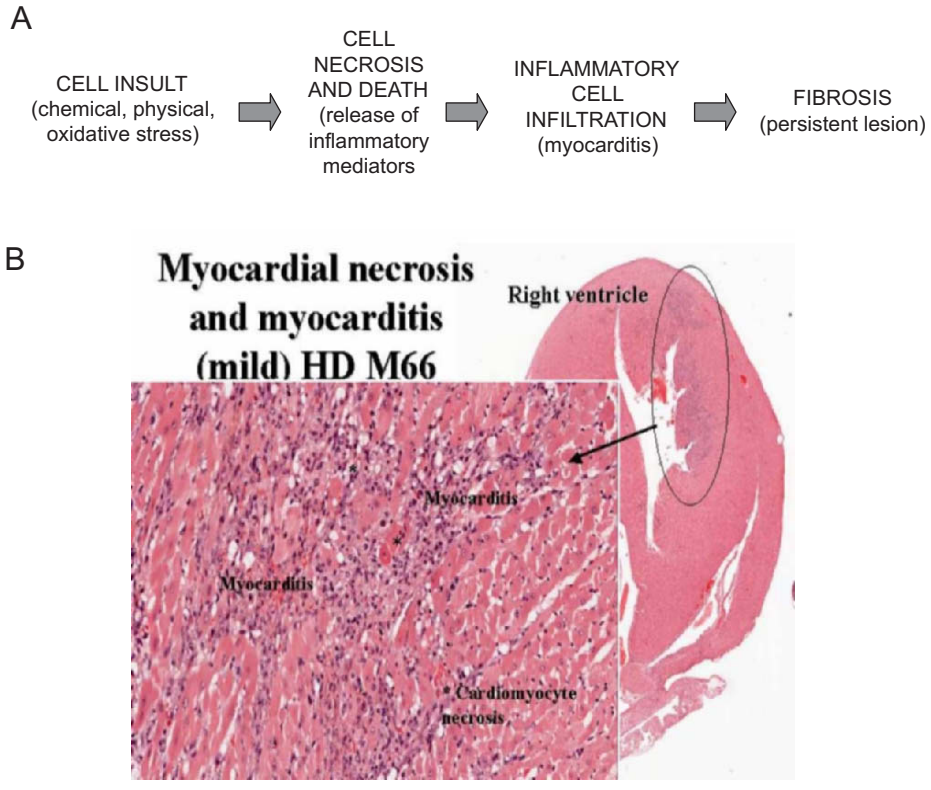

Fig. 2. Aetiology (A) and pathology (B) of myocarditis in the rat ventricle. Although the initiating event in molecular terms remains unclear, it is thought that the lesion is triggered by various sources of cellular stress leading to cell death and inflammatory cell inflammation.

Table 3
Incidence and dose response of myocarditis in the pivotal rat toxicology study

10 rats/group	M	M	M	M	F	F	F	F
Dose, mg/m^2 1 h infusion weekly $\times 3$	0	43	130	326	0	43	130	326
Myocarditis								
Minimal		4	1	1				
Mild			1	2				1

Minimal = $\leqslant$ than approximately 10% of the tissue section affected; Mild = between 10 and 25% of the tissue affected.

ducted to date with this potential drug candidate; unfortunately the data were not helpful in providing a definitive answer on causality and dose response (Table 4). To provide a clearer picture, it was necessary to conduct a more detailed analysis with an increased number of cardiac sections to provide enhanced sensitivity. In addition, to assist in clinical development, we wished to identify a suitable biomarker to use in conjunction with physiological measurements to mitigate against the occurrence of unwanted cardiac side effects.

Potential biomarkers: the troponins

There are several biomarkers of cardiac cellular injury used in animal studies [5]. Amongst these is a contractile protein termed troponin [6], an isoform of which is used as a diagnostic marker of myocardial infarct in humans and a biomarker of cardiac injury in animals [7]. Troponins are components of muscle contraction proteins along with actin and myosin. Troponin is made up of three proteins (I, T and C) and is essential for normal contractility in skeletal and cardiac muscle tissue (see [8] for review). Troponin is attached to another protein, tropomyosin, situated between actin filaments. In the absence of a contactile stimulus tropomyosin acts as a barrier to the attachment of myosin cross bridges thus helping to keep the muscle relaxed. The release of calcium following an action potential allows calcium to bind to the troponin molecule which in turn alters the conformation of tropomyosin and allows myosin to create the necessary cross bridges for contraction (Fig. 3).

The three types of troponin (I, T and C) are found in all muscle types, but there are cardiac muscle specific isoforms of I and T (cTnI and cTnT respectively) that are useful as diagnostic markers since they are released

Table 4
Comparison of the incidence of cardiac ventricular myocarditis across several rat studies

Study	Single dose	5d repeat dose	DRF weekly ×3	Pivotal weekly ×3
Animal number per group	3M	4M, 4F	3M, 3F	10M, 10F
Strain	Han Wistar	Han Wistar	Han Wistar	Han Wistar
Route	iv bolus	iv bolus	1 h inf	1 h inf
Formulation	Saline	Saline	Phosphate manitol	Phosphate manitol
Doses mg/m^2	130, 196, 282	65, 130, 196	293, 391, 489	43, 130, 326
Doses µmol/kg	60, 90, 130	30, 60, 90	135, 180, 225	20,60,150
PM	6d post dose	1d post the last dose	Up to 7 d	3 d post the last dose
Myocarditis	1/3 M high dose	1 control	1/1 M (only high dose evaluated)	Low 4/10 M, Mid 2/10 M High 2/10 M, /10 F

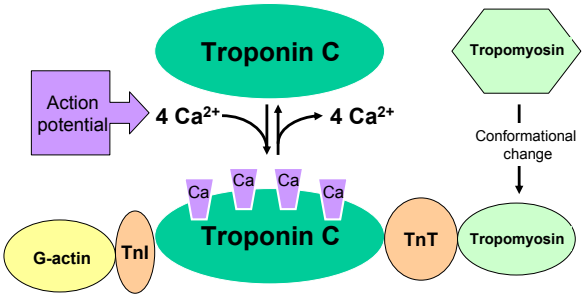

Fig. 3. A schematic of troponin biology. Troponins I, T and C form part of a complex with tropomyosin. Tropomyosin is situated between actin filaments where it acts as a barrier to the attachment of myosin cross bridges, maintaining the muscle in a relaxed state. Calcium released following an action potential binds to the troponin molecule which in turn alters the conformation of tropomyosin and allows myosin to create the necessary cross bridges for contraction.

following cellular injury. Current clinical grading is provided by the NIH Common Technical Criteria publications [9] as illustrated in Table 5. This clearly shows that for clinical myocardial injury in humans, the levels of elevated cTnT are between 0.03 for asymptomatic injury up to 0.2 ng/ml for a myocardial infarct against a low background circulating level of <0.03 ng/ml. Based on these clinical data, cTnT was identified as a potential biomarker for AZDX induced myocardial injury.

Table 5
Grading of troponin T as a clinical marker of myocardial injury

Common Toxicity Criteria		Troponin T range (ng/ml)
CTC grade 1	Asymptomatic arterial narrowing without ischemia	0.03–<0.05
CTC grade 2	Asymptomatic testing suggesting ischemia; stable angina	0.05–<0.1
CTC grade 3	Symptomatic and testing consistent with ischemia; unstable angina	0.1–<0.2
CTC grade 4	Acute myocardial infarction	0.2 ng/ml

Troponins in drug-induced myocarditis

In order to understand the relationship between intravenous administration of AZDX and myocardial injury in the rat, we first had to establish background plasma levels of cTnT and to define the time course of maximal elevation in cTnT release following administration of AZDX. The study design involved a single high dose of AZDX, previously shown to be associated with cardiac injury. Cardiac specific troponin T (cTnT) in plasma was determined using the Roche Elecsys® Troponin T STAT immunoassay. Sampling was extensive over a period of 72 h and included both blood and timed terminations to provide histopathological examination of the heart. Heart sections were increased over the routine single section as discussed previously to a total of 21 sections per heart, providing increased resolution and sensitivity. These data showed that, at least for this strain of rat, the optimal time for the detection of elevations in plasma cTnT resulting from compound related ventricular myocyte injury was 8 h. Elevations in plasma cTnT were found predominately in rats treated with AZDX, although there were incidences in control animals (Fig. 4). The 8-h time point was selected for use in the subsequent study utilising the clinical regimen of once weekly by 1-h infusion for 3 weeks with measurements being taken after each dose. Analysis of the results from the 3 week study indicated that, for this strain of rat under the conditions of 1-h infusion and a clinical regimen, chronic ventricular

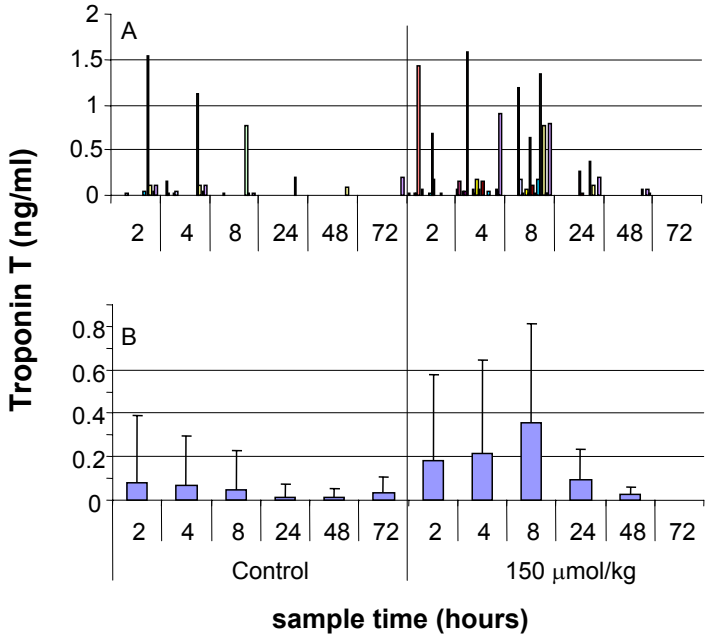

Fig. 4. Time course of AZDX-induced elevation in rat plasma troponin either as individual data (A) or as mean ±SD (B). Cardiac specific troponin T (cTnT) in plasma was determined using the Roche Elecsys® Troponin T STAT immunoassay, a highly specific chemiluminescent assay with an analytical sensitivity (lower detection limit) of 0.01 ng/ml.

Table 6
A comparison of incidence of cardiac lesions and elevated troponins in the rats (6/group) over a timecourse

Dose, μmol/kg	4 h		8 h		24 h		72 h	
	0	150	0	150	0	150	0	150
Necrosis								
Minimal								2
Mild				1				1
Moderate						2		
Myocarditis								
Minimal	2	1	3				2	3
Mild					1		2	1
Moderate							1	2
Increased troponin T	2	5	2	6	1	6	4	6

myocarditis was present both in control and treated animals. All the data from the original pivotal study, the time course study and the 3 week investigative study were combined to permit comparative analysis (Table 6).

Troponins and early events in myocardial injury

The data on incidence and severity of chronic ventricular myocarditis did not permit easy delineation of treatment related effects specifically or with respect to time of administration of AZDX. Despite this, it was evident that ventricular myocardial necrosis (an early predecessor to the myocarditis) was chronologically associated with administration of AZDX and was also associated with clear elevations in plasma cTnT. This useful observation will be discussed later.

In considering the data on cTnT and the incidence of myocarditis in the rat, several conclusions can be drawn that may assist in future work. At the start of these experiments in the rat, it was assumed that the clear correlation between cTnT and clinical grading of myocarditis as defined in humans would be applicable to a pre-clinical species such as the rat. However this was not the case for several reasons: the data show that rats exhibit a high normal control range of cTnT up to 0.2 ng/ml, making the clinical gradings of myocarditis (starts with a grading of CTC1 at 0.03–<0.05 ng/ml; see Table 5) based on cTnT inapplicable to pre-clinical data. In addition, minimal myocarditis appears to be within the background range in this strain and was present in some animals without detectable increase in cTnT. Plausible explanations for these observations include chronology where we may have missed a cTnT associated causative event in the development of myocarditis in the rat or the myocarditis could be attributable to a non-specific stress associated with the dosing procedure.

Support for clinical progression

Although the data set on mycocarditis were not as anticipated, several useful observations were made to assist in clinical progression. An increase in cTnT was clearly associated with myocardial damage (necrosis), a lesion that precedes the myocarditis (see Fig. 2). Therefore the data supported cTnT as a useful and predictive biomarker of cardiac injury, even if the correlation was not with myocarditis *per se*.

Clinical biomarkers

As described above for the physiological effects on the heart, progression in to efficacy studies in cancer patients was only permissible with clearly defined biomarkers of cardiovascular dysfunction. These included ECG, BP, HR and ejection fraction (contractility measure) assessments. Taking the same approach to protecting patients from an unacceptable degree of cardiac injury, cTnT was employed as a clinical biomarker of myocardial injury as a prerequisite for progression of the clinical trials for AZDX in cancer patients. The investigative biomarker work in animal studies supported this choice of an appropriate clinical biomarker and generated clearly defined stop criteria to minimised risk for cardiovascular adverse events in patients. These are based upon limits in elevation of cTnT relative to the dosing regime and therapeutic window (Table 7). Built into this is the need for cessation of enrolment and clinical consultation if any patient exhibits Grade 3 elevations in cTnT. Ultimately, how well did these biomarkers of potential cardiac dysfunction and injury map against the 'ideal characteristics' that one might pursue in the development of a good clinical biomarker. The standard cardiovascular measurements plus ejection fraction could certainly be considered as non-invasive sensitive, specific geographically robust and have a very large database to assess deviation from normal. However, these assessments are often complex and require the input of an expert cardiologist to make meaningful interpretations of any observed changes. Cardiac functionality can certainly be influenced by both cardiac disease and other co-morbidities although pre-trial evaluations for individual patients would go some way to mitigating against this when evaluating trial data. In terms of biomarker changes that might precede irreversible or tissue injury, these measures, if of small magnitude and relatively transient in nature, would often be considered clinically to precede such event. Nevertheless, that is not to ignore tissue injury that could occur if significant or prolonged changes were observed (e.g. potential ischemia and necrosis due to low cardiac perfusion pressure or inadequately supported increased myocardial demand) or indeed that the parameter changes may be as a consequence of tissue injury that had already taken place.

The use of troponin T as a biomarker of myocardial injury is less well defined. Although widely recognised diagnostic for myocardial infarct there is relatively little published information on its use as a biomarker for drug induced myocardial injury. It involves a degree of invasiveness as blood samples are required and although an uncomplicated assay does require specialist kit/equipment, but new assays are being developed. It is considered a more specific and sensitive assay than other markers of cardiac muscle damage such as creatine kinase, but has the significant drawback of being an early and transient event making sampling times very critical. Background levels and variability in rats are less well understood and therefore problematical, but this appears less of an issue in the clinical setting. Non-drug related cardiac disease or cardiotoxic co-medications would certainly complicate the picture. Yet despite being a good clinical biomarker of cardiac injury, and a supplement if not an improvement on existing methods, the fact remains that when elevated troponins are observed in relation to drug administration, irreversible tissue damage has already occurred. At best all the clinician can do is to withdraw treatment to minimise and prevent further injury. It is not surprising that many drugs with the potential for myocardial injury are confined to use in patients with serious life threatening disease.

Table 7
Stopping criteria based on grading of cTnT

Grade 1 cTnT (0.03 to 0.05 ng/ml) is a DLT
• If one patient has grade 1, dosing reverts to the prior dosing cohort in the absence of a well defined concurrent event (such as sepsis or PE).
• Do not retreat patients with cTnT elevation.
Establish a therapeutic window twice dose (and/or Cp at 24 h) needed to inhibit the cellular target that does not produce *even grade 1* increase in cTnT
• For instance, if 20 mg/m^2 produces plasma concentration at 24 h of 20 nM, and that this represented the ED90, we would like to see 0/6 patients at 40 mg/m^2 with NO increase in troponin, even grade 1.
• Even grade 1 elevations could produce cumulative cardiotoxicity – would not be acceptable for a modulator
If any patient has grade 3 cTnT, enrollment ceases temporarily and cardiac consultation is undertaken internally and externally

Conclusion

The development of targeted and novel anti-cancer agents presents unique challenges to both the toxicology and the medical community. Clinically useful biomarkers of toxicity can be derived from appropriately designed preclinical experiments and such biomarkers can significantly assist safe progression into the clinic with an approach design to minimise patient risk. These biomarkers can be derived from mode of action studies in pre-clinical species then translated to potential clinical use or, as we presented here, could already been established in clinical practice. This translational science approach requires close collaboration between the clinical and the pre-clinical scientists. The development of potential biomarkers is now a key component of drug development at AstraZeneca, and is now included in programmes of pre-clinical drug development.

Conflict of interest statement

None declared.

References

1 Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001, **69**, 89–95.
2 Luqmani, YA. Mechanisms of drug resistance in cancer chemotherapy. *Med Principles Pract* 2005, **14**(Suppl. 1), 35–48.
3 Baguley B, Marshall ES. In vitro modelling of human tumour behaviour in drug discovery programmes. *Eur J Cancer* 2004, **40**, 794–801.
4 Tucker MJ, ed. *Diseases of the Wistar Rat*. London. Taylor and Francis, 2007.
5 O'Brien PJ, Smith DE, Knechtel TJ, *et al.* Cardiac troponin I is a sensitive, specific biomarker of cardiac injury in laboratory animals. Laboratory Animals 2006, **40**(2), 153–171.
6 Stein H, Giannitsis E, Futterer S, Merten C, Juenger C, Katus H. Cardiac troponin T at 96 hours after acute myocardial infarction correlates with infarct size and cardiac function. J Am Coll Cardio 2006, **48**, 2192–2194.
7 O'Brien P. Blood cardiac troponin in toxic myocardial injury: archetype of a translational safety biomarker. Exp Rev Mol Diag 2006, **6**(5), 685–702.
8 Wikipedia. Wikipedia, the free encyclopedia: Troponin. http://en.wikipedia.org/wiki/Troponin, 2007.
9 nci.nih.gov.webapps.ctep.nci.nih.gov/webobjs/ctc/webhelp/Welcome_to_CTCAE.htm#CARDIAC_GENERAL_CATEGORY.htm).nci.nih.gov, 2007.